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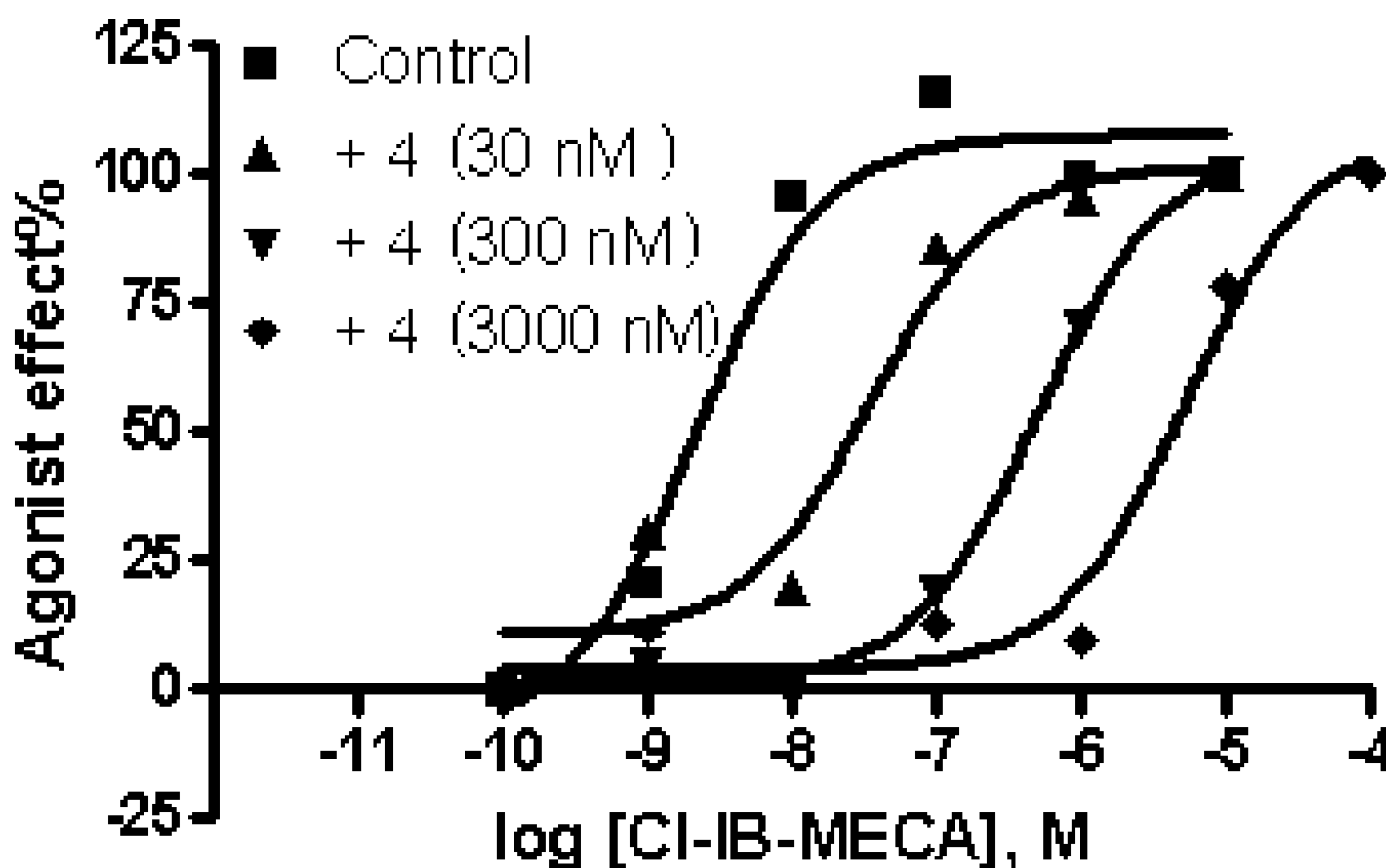
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(54) Titre : DERIVES D'ADENOSINE, PROCEDE POUR LA SYNTHESE DE CES DERNIERS, ET COMPOSITIONS
PHARMACEUTIQUES POUR LA PREVENTION ET LE TRAITEMENT DE MALADIES INFLAMMATOIRES
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(54) Title: ADENOSINE DERIVATIVES, METHOD FOR THE SYNTHESIS THEREOF, AND THE PHARMACEUTICAL
COMPOSITIONS FOR THE PREVENTION AND TREATMENT OF THE INFLAMMATORY DISEASES CONTAINING
THE SAME AS AN ACTIVE INGREDIENT

[Fig. 1]



(57) Abrégé/Abstract:

Disclosed are adenosine derivatives, methods for the synthesis thereof, and pharmaceutical compositions for the prevention and treatment of inflammatory diseases, comprising the same as an active ingredient. The adenosine derivatives have high binding

(57) **Abrégé(suite)/Abstract(continued):**

affinity and selectivity for adenosine receptors, especially for A3 adenosine receptors and act as A3 adenosine receptor antagonists, and exhibit anti-inflammatory activity. Thus, the adenosine derivatives are useful in the prevention and treatment of inflammatory diseases.

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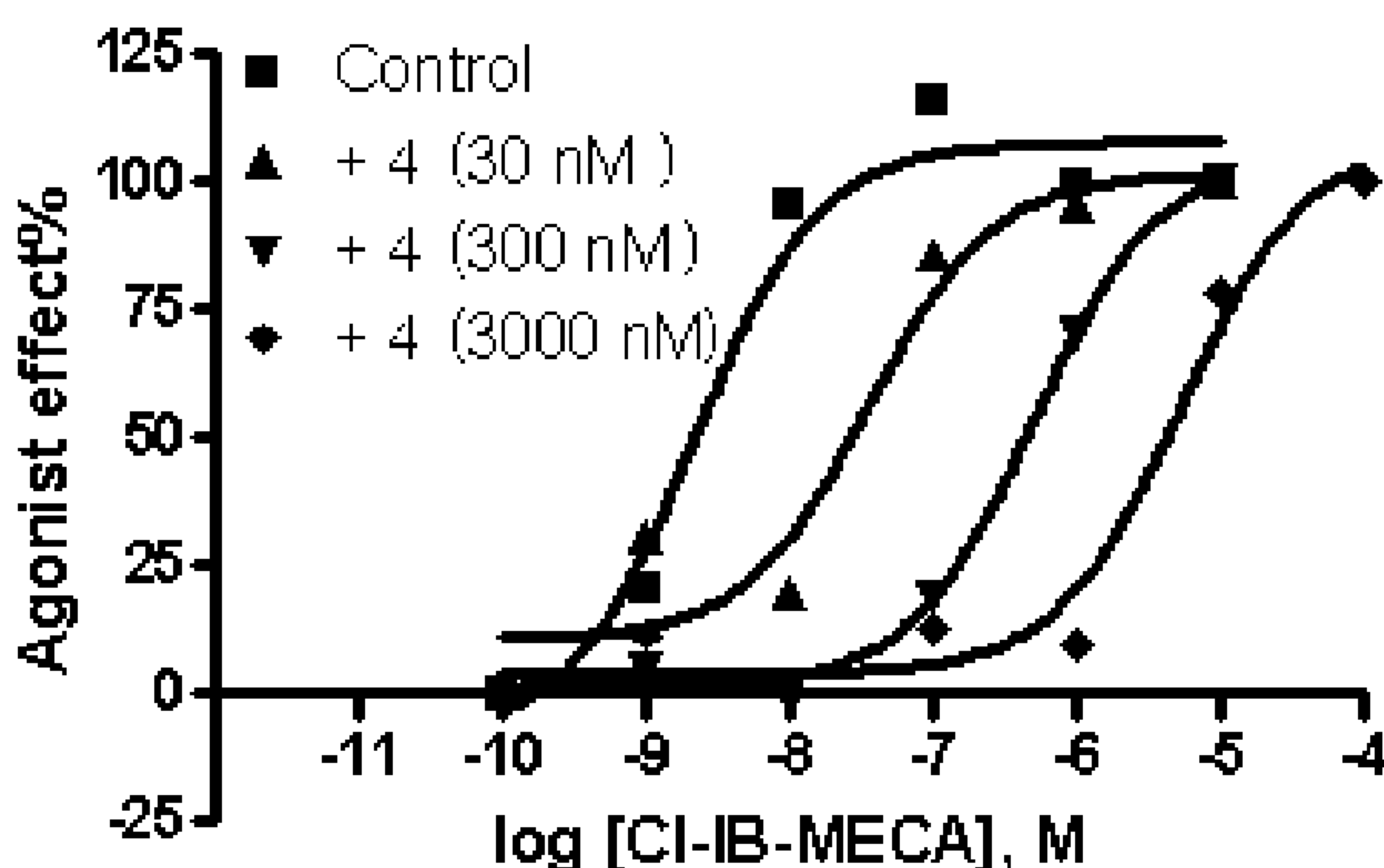
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(54) Title: ADENOSINE DERIVATIVES, METHOD FOR THE SYNTHESIS THEREOF, AND THE PHARMACEUTICAL COMPOSITIONS FOR THE PREVENTION AND TREATMENT OF THE INFLAMMATORY DISEASES CONTAINING THE SAME AS AN ACTIVE INGREDIENT

[Fig. 1]



(57) Abstract: Disclosed are adenosine derivatives, methods for the synthesis thereof, and pharmaceutical compositions for the prevention and treatment of inflammatory diseases, comprising the same as an active ingredient. The adenosine derivatives have high binding affinity and selectivity for adenosine receptors, especially for A3 adenosine receptors and act as A3 adenosine receptor antagonists, and exhibit anti-inflammatory activity. Thus, the adenosine derivatives are useful in the prevention and treatment of inflammatory diseases.

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**ADENOSINE DERIVATIVES, METHOD FOR THE SYNTHESIS THEREOF,
AND THE PHARMACEUTICAL COMPOSITIONS FOR THE PREVENTION AND
TREATMENT OF THE INFLAMMATORY DISEASES CONTAINING THE SAME AS
AN ACTIVE INGREDIENT**

5

Technical Field

The present invention relates to adenosine derivatives. More particularly, the present invention relates to adenosine derivatives and pharmaceutically acceptable salts thereof
10 which show high binding affinity and selectivity for specific A_3 adenosine receptors. Also, the present invention relates to a method for synthesizing the adenosine derivatives and a pharmaceutical composition for the prevention and treatment of inflammatory diseases, comprising the adenosine derivatives or
15 pharmaceutically acceptable salts thereof.

Background Art

Adenosine is a ligand which regulates cell signaling, which accounts for various physiological functions through
20 specific adenosine receptors located in the cell membrane. Adenosine, an extracellular substance, acts as a neurotransmitter in a variety of physical systems, typically functioning to compensate for overactivity of certain organs and protect the body from the harmful effects of stress
25 (Jacobson, K. A. et al., J. Med. Chem., 35, 407-422,

1992). These functions are based on a part of the negative feedback loop in which adenosine, formed through the dephosphorylation of endocellular or extracellular ATP (adenosine triphosphate), decreases the cellular energy and increases oxygen supply. Adenosine plays an important role in maintaining the homeostasis of organs such as the brain, the heart and the kidneys. For example, when externally administered to the brain, an adenosine agonist was proven to show neuroprotective effects and was also found to be involved in pain, recognition, exercise and sleep.

Pharmacological research and molecular cloning studies have thus far revealed two classes (P1 and P2) of adenosine receptors. In mediating signal transduction, P1 receptors are adapted for adenosine while P2 receptors are adapted for ATP, ADP, UTP and UDP. Four subtypes of P1 receptors have been identified. They can be divided into A_1 , A_2 and A_3 according to ligand affinity, distribution within the body, and functional pathway, and A_2 further into A_{2A} and A_{2B} . These adenosine receptors are members of the G-protein-coupled receptor family. Pharmacological functions of adenosine A_1 , A_{2A} and A_{2B} receptors have been revealed using various selective ligands. As for the A_3 receptor, it was first identified in 1992 (Zhou, Q. Y, et al., Proc. Natl. Acad. Sci., U.S.A., 89, 7432-7436, 1992) and its pathophysiological functions have been extensively studied.

Adenosine A₁ and A₂ receptor agonists, most derived from adenosine, have been intensively studied for use as hypotensive agents, therapeutics for mental illness and arrhythmia, lipid metabolism suppressant (therapeutics for 5 diabetes) and neuroprotectives. On the other hand, their antagonists, derived from xanthine or in the form of two or more fused heterocyclic compounds, are developed as anti-asthmatics, anti-depressants, anti-arrhythmics, renal protectants, drugs for Parkinson's disease, and intelligence 10 enhancers. Despite extensive study, only a few commercial products have been developed, including adenosine itself for the treatment of supraventricular tachycardia, and dipyridamole, the adenosine transfer inhibiting drug, which is used as a supplemental drug for warfarin in preventing blood 15 coagulation after cardiectomy. The reason why little progress toward the commercialization of adenosine derivatives has been made is that because adenosine receptors are distributed throughout the body, and the activation thereof is accompanied by various pharmaceutical activities. In brief, there are no 20 compounds that are able to activate only the adenosine receptors of a desired tissue.

The function of the adenosine A₃ receptor, the most recently identified, remains unknown, in contrast to the A₁ and A₂ receptors, the functions of which are well known. 25 Extensive research has been conducted to develop selective

ligands of the adenosine A₃ receptor. In this regard, three radiolabeled ligands [¹²⁵I]ABA (N⁶-(4-amino-3-[¹²⁵I]iodobenzyl)-adenosine), [¹²⁵I]APNEA (N⁶-2-(4-amino-3-[¹²⁵I]iodophenyl)-ethyl adenosine) and [¹²⁵I]AB-MECA (N⁶-(4-amino-3-[¹²⁵I]iodobenzyl)-adenosine-5'-N-methylcarboxamide) are currently used for the pharmacological study of adenosine A₃ receptor. For example, it was found through research on the radiolabeled ligands that when expressed in Chinese Hamster Ovary (CHO) cells, the A₃ receptor inhibited adenylyl cyclase, an enzyme that produces cAMP from ATP. Also, when activated by agonists, the A₃ receptor was proven to mediate the activation of guanosine triphosphate-dependent phospholipase C, an enzyme which catalyzes the degradation of phosphatidyl inositol into inositol triphosphate and diacylglycerol (DAG) in the brain (Ramkumar, V. et al., J. Biol. Chem., 268, 168871-168890, 1993; Abbracchio, M. P. et al., Mol. Pharmacol., 48, 1038-1045, 1995). These findings indicate the possibility that there is a reaction pathway mediated by the A₃ receptor in cerebral ischemia when it is activated. The reason is that this second messenger system acts as a reaction pathway leading to nerve injury in cerebral ischemia. Also, A₃ receptor agonists are known to prevent cerebral diseases, such as epilepsy, and to protect the heart as well as inhibiting the release of TNF- α (tumor necrosis factor), an inflammation mediator, and the production of MIP-1 α ,

interleukin-12 and interferon- γ , all acting as inflammation mediators. On the other hand, the inactivation of A_3 adenosine receptor causes the release of inflammation factors, such as histamine, from mast cells, bronchoconstriction, and
5 the apoptosis of immune cells. Accordingly, A_3 adenosine antagonists have the possibility of being candidates as anti-inflammatory agents and anti-asthmatics. Therefore, compounds with pharmacological selectivity are believed to be drugs useful in the treatment of various diseases, including asthma,
10 inflammation, cerebral ischemia, heart diseases, cancer, etc.

The nucleoside based compounds N^6 -(3-iodobenzyl)-5'-(N-methylcarbamoyl)-adenosine (IB-MECA) and N^6 -(3-iodobenzyl)-2-chloro-5'-(N-methylcarbamoyl)-adenosine (CI-IB-MECA) are representative human adenosine A_3 agonists, and exhibit higher
15 affinity and selectivity for the A_3 adenosine receptor than for the A_1 and A_2 adenosine receptors. On the other hand, most potent and selective human A_3 adenosine receptor antagonists possess non-purinerbic heterocyclic skeleton compounds. However, nearly all of the non-purinerbic
20 heterocyclic human A_3 adenosine antagonists are found to induce weak or ineffective activity through rat A_3 adenosine receptor and thus were unsuitable for evaluation in small animal models, which is indispensable to the development of drugs for clinical application (Baraldi, P. G. et al., Curr.
25 Med. Chem., 12, 1319-1329, 2005).

However, since A_3 AR antagonists with nucleoside skeletons, in contrast to non-purinergic heterocyclic antagonists, exhibit high affinity and selectivity independent of species, the applicability thereof for animal test makes
5 the nucleoside skeleton-based A_3 AR antagonists preferred drug candidates. Accordingly, there is a need for the development of selective A_3 antagonists based on nucleoside compounds.

Through various previous research, the present inventors discovered that nucleoside compounds must have an N-
10 methylcarbamoyl group at position 5 of the sugar moiety and a base substituted with an arylamino group or alkylamino group at position 6 of the purine moiety for A_3 adenosine receptor agonism, as in the representative materials IB-MECA and Cl-IB-MECA. Since, since the N-methylcarbamoyl group at position 5
15 of the sugar moiety forms a hydrogen bond to cause a conformational change essential for the agonism of the receptors (Kim, S-K. et al., J. Mol. Graph. Model., 25, 562-577, 2006), compounds devoid of an N-methylcarbamoyl at position 5 of the sugar moiety are thought to be strong
20 candidates for A_3 adenosine receptor antagonists.

Leading to the present invention, thorough and intensive research into A_3 adenosine receptor ligands and pharmaceutical effects, resulted in the finding that specific adenosine
25 derivatives selected on the basis of the structure-activity

relationship thereof have high binding affinity and selectivity for A₃ adenosine receptors compared to A₁ or A₂ adenosine receptors and can act as selective antagonists on A₃ adenosine receptors, thus showing high therapeutic effects on
5 inflammatory diseases.

Disclosure

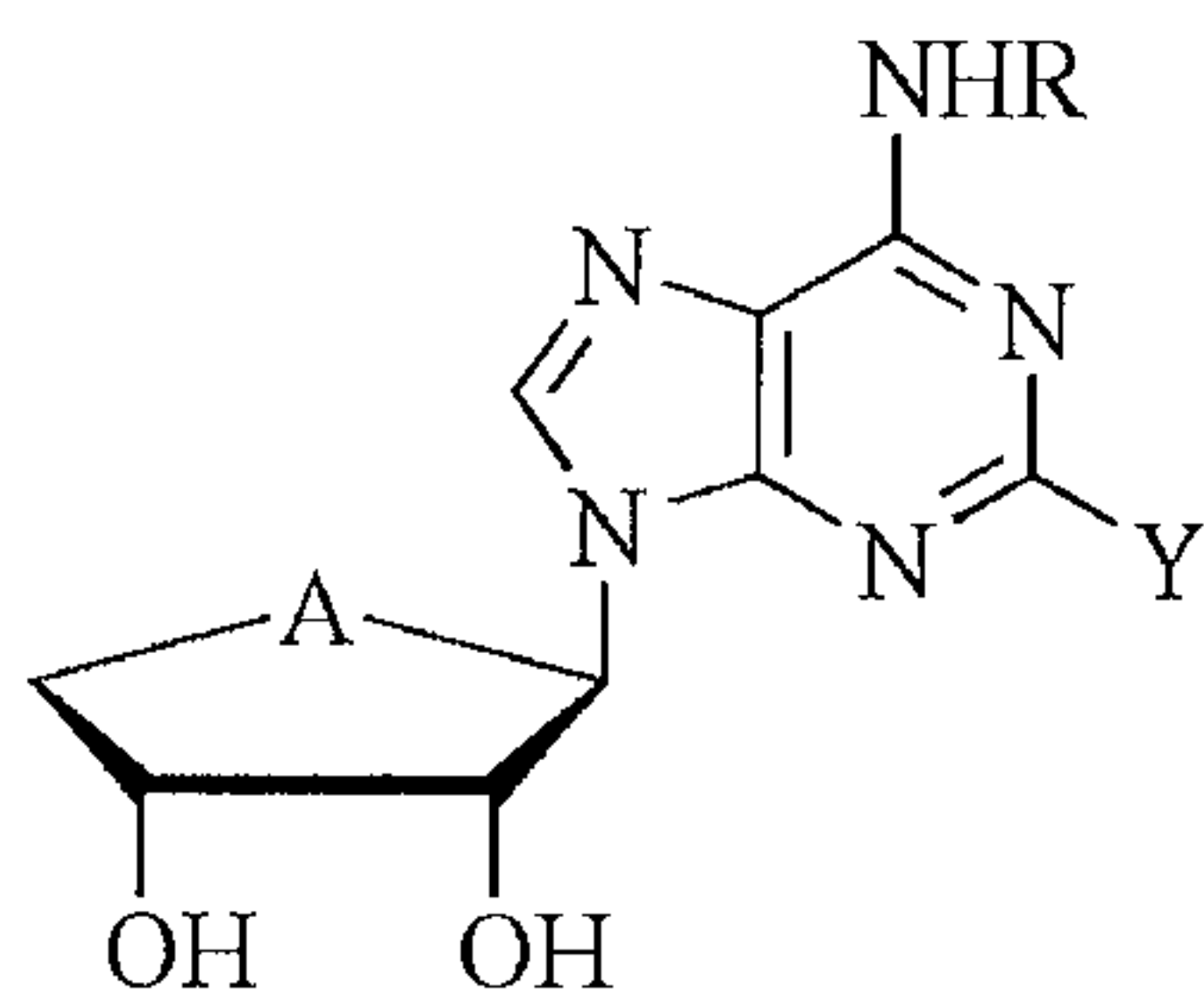
Technical Problem

It is an object of the present invention to provide a
10 novel adenosine derivative which acts as a selective A₃ adenosine receptor antagonist to show therapeutic activity on inflammatory diseases, a method for the synthesis thereof, and a pharmaceutical composition for the prevention and treatment of inflammatory diseases, comprising the same as an active
15 ingredient.

Technical Solution

In accordance with an aspect of the present invention, there is provided a novel adenosine derivative, represented by
20 the following Chemical formula 1, or a pharmaceutically acceptable salt thereof.

[Chemical formula 1]



wherein, A is O or S; R is a linear or branched C₁~C₅ alkyl which is non-substituted or is independently or
 5 selectively substituted with one or more C₆~C₁₀ aryl groups, a benzyl which is non-substituted or is independently or selectively substituted with halogen or one or more linear or branched C₁~C₄ alkoxy groups, or a hydroxycarbonyl-substituted benzyl; and Y is H or a halogen atom.

10 In accordance with another aspect of the present invention, there is provided a method for the synthesis of the novel adenosine derivative or the pharmaceutically acceptable salt thereof.

In accordance with a further aspect of the present
 15 invention, there is provided an A₃ adenosine receptor antagonist comprising the adenosine derivative or pharmaceutically acceptable salt thereof.

In accordance with still a further aspect of the present invention, there is provided a pharmaceutical composition for
 20 the prevention and treatment of inflammatory diseases, comprising the adenosine derivative or the pharmaceutically

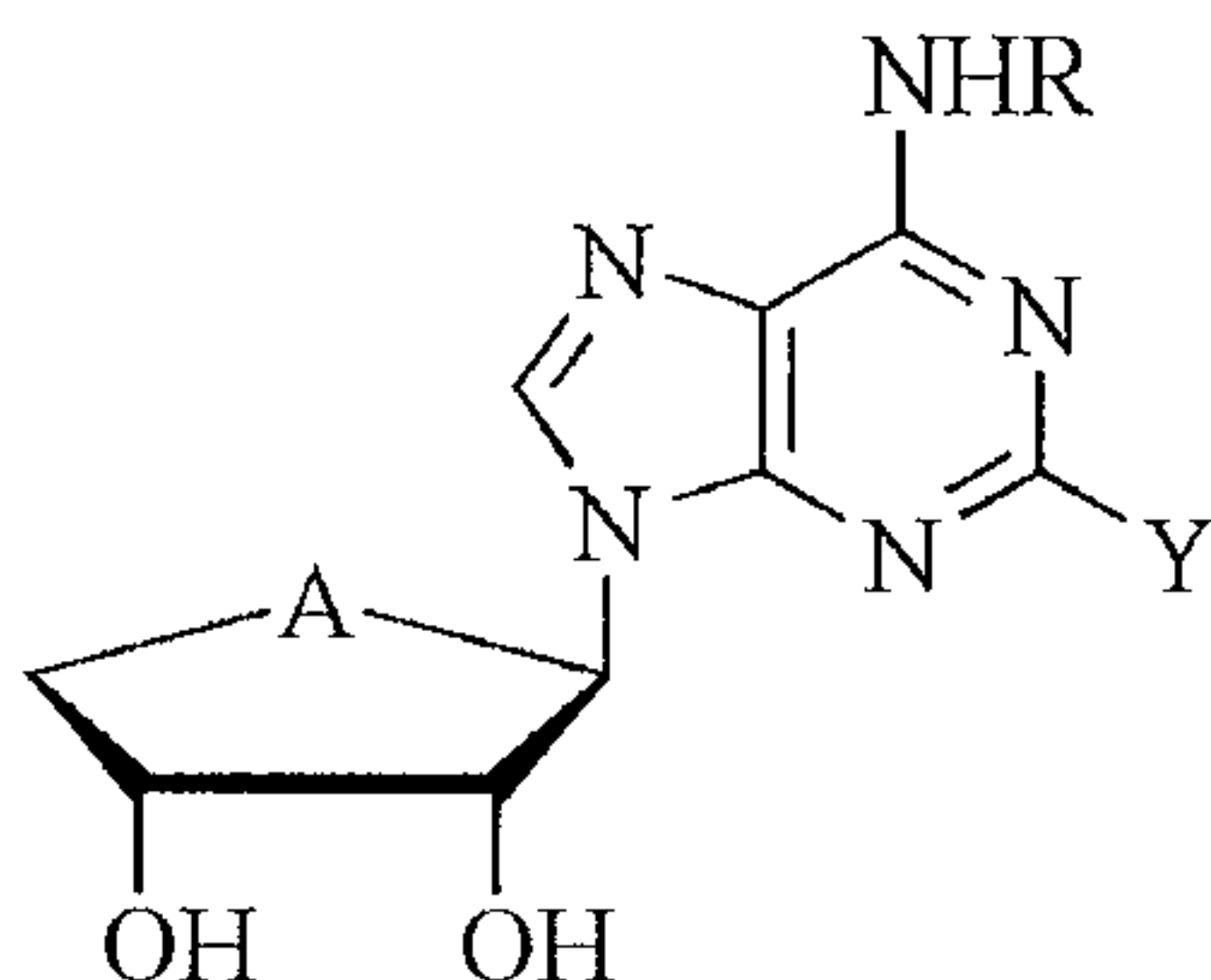
acceptable salt thereof as an active ingredient.

Below, a detailed description is given of the present invention.

5 In accordance with an aspect thereof, the present invention pertains to an adenosine derivative compound represented by the following Chemical formula 1, a pharmaceutically acceptable salt thereof, and a composition comprising the compound or the salt as an active ingredient.

10

【Chemical formula 1】



wherein,

15 A is O or S,

R is a linear or branched C₁~C₅ alkyl which is non-substituted or is independently or selectively substituted with one or more C₆~C₁₀ aryl groups, a benzyl which is non-substituted or is independently or selectively substituted
20 with halogen or one or more linear or branched C₁~C₄ alkoxy groups, or a hydroxycarbonyl-substituted benzyl; and

Y is H or a halogen atom.

In a preferable compound of Chemical formula 1,

A is O or S,

R is methyl, ethyl, propyl, naphthylmethyl, benzyl,
5 benzyl independently or selectively substituted with a
substituent selected from a group consisting of F, Cl, Br, I,
C₁~C₃ alkoxy and combinations thereof, or toluic acid, and

Y is H or Cl.

In a more preferable embodiment,

10 A is O or S,

R is methyl, ethyl, 1-naphthylmethyl, benzyl, 2-
chlorobenzyl, 3-fluorobenzyl, 3-chlorobenzyl, 3-bromobenzyl,
3-iodobenzyl, 2-methoxy-5-chlorobenzyl, 2-methoxybenzyl, or 3-
toluic acid, and

15 Y is H or Cl.

Concrete examples of the novel adenosine derivatives
according to a preferred embodiment of the present invention
include:

20 1) (2*R*, 3*R*, 4*S*)-2-(2-chloro-6-(3-fluorobenzylamino)-9*H*-
purin-9-yl)tetrahydrothiophen-3,4-diol;

2) (2*R*, 3*R*, 4*S*)-2-(2-chloro-6-(3-chlorobenzylamino)-9*H*-
purin-9-yl)tetrahydrothiophen-3,4-diol;

3) (2*R*, 3*R*, 4*S*)-2-(6-(3-bromobenzylamino)-2-chloro-9*H*-
25 purin-9-yl)tetrahydrothiophen-3,4-diol;

- 4) (2*R*, 3*R*, 4*S*)-2-(2-chloro-6-(3-iodobenzylamino)-9*H*-
purin-9-yl)tetrahydrothiophen-3,4-diol;
- 5) (2*R*, 3*R*, 4*S*)-2-(2-chloro-6-(2-chlorobenzylamino)-9*H*-
purin-9-yl)tetrahydrothiophen-3,4-diol;
- 5 6) (2*R*, 3*R*, 4*S*)-2-(2-chloro-6-(5-chloro-2-
methoxybenzylamino)-9*H*-purin-9-yl)tetrahydrothiophen-3,4-diol;
- 7) (2*R*, 3*R*, 4*S*)-2-(2-chloro-6-(2-methoxybenzylamino)-9*H*-
purin-9-yl)tetrahydrothiophen-3,4-diol;
- 8) (2*R*, 3*R*, 4*S*)-2-(2-chloro-6-(naphthalen-1-
10 ylmethylamino)-9*H*-purin-9-yl)tetrahydrothiophen-3,4-diol;
- 9) 3-((2-chloro-9-((2*R*, 3*R*, 4*S*)-3,4-
dihydroxytetrahydrothiophen-2-yl)-9*H*-purine-6-
ylamino)methyl)benzoic acid;
- 10) 2-(2-chloro-6-methylamino-purin-9-
15 yl)tetrahydrothiophen-3,4-diol;
- 11) (2*R*, 3*R*, 4*S*)-2-(6-(3-fluorobenzylamino)-9*H*-purin-9-
yl)tetrahydrothiophen-3,4-diol;
- 12) (2*R*, 3*R*, 4*S*)-2-(6-(3-chlorobenzylamino)-9*H*-purin-9-
yl)tetrahydrothiophen-3,4-diol;
- 20 13) (2*R*, 3*R*, 4*S*)-2-(6-(3-bromobenzylamino)-9*H*-purin-9-
yl)tetrahydrothiophen-3,4-diol;
- 14) (2*R*, 3*R*, 4*S*)-2-(6-(3-iodobenzylamino)-9*H*-purin-9-
yl)tetrahydrothiophen-3,4-diol;
- 15) (2*R*, 3*R*, 4*R*)-2-(6-(3-bromobenzylamino)-2-chloro-9*H*-
25 purin-9-yl)tetrahydrofuran-3,4-diol; and

16) (2*R*, 3*R*, 4*R*)-2-(2-chloro-6-(3-iodobenzylamino)-9*H*-
purin-9-yl)tetrahydrofuran-3,4-diol.

The novel adenosine derivative, represented by Chemical
5 formula 1, in accordance with the present invention may be in
the form of pharmaceutically acceptable salts. Useful are
acid addition salts formed with a variety of pharmaceutically
acceptable organic acids or inorganic acids. Examples of
suitable organic acids include carboxylic acid, phosphonic
10 acid, sulfonic acid, acetic acid, propionic acid, octanoic
acid, decanic acid, glycolic acid, lactic acid, fumaric acid,
succinic acid, adipic acid, malic acid, tartaric acid, citric
acid, glutamic acid, aspartic acid, maleic acid, benzoic acid,
salicylic acid, phthalic acid, phenylacetic acid, benzene
15 sulfonic acid, 2-naphthalene sulfonic acid, methyl sulfonic
acid, ethyl sulfonic acid, and dodecyl sulfonic acid.
Suitable inorganic acids may be exemplified by hydrochloric
acid, sulfuric acid, halogen acid, and phosphoric acid.

It should be noted that the adenosine derivatives
20 represented by Chemical formula 1 are intended to include all
salts, hydrates and solvates thereof as long as they can be
prepared using conventional methods, as well as
pharmaceutically acceptable salts thereof.

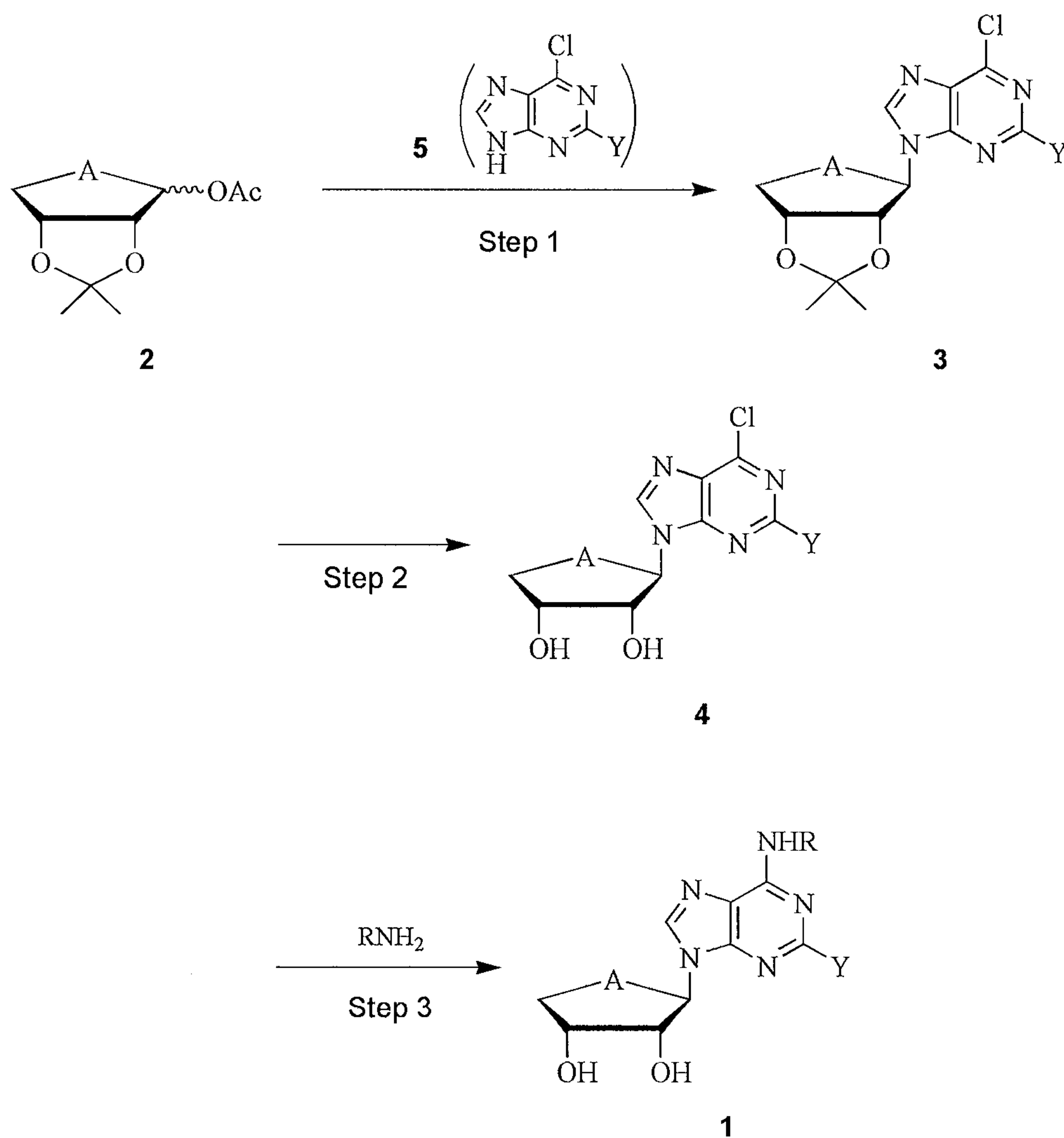
In accordance with another aspect thereof, the present
25 invention pertains to a method for preparing the novel

adenosine derivative represented by Chemical formula 1.

In an embodiment of this aspect, the adenosine derivative may be synthesized according to the following Scheme 1.

5 The method comprises reacting a compound of Chemical formula 2 with a silylated purine compound in the presence of a Lewis acid catalyst to produce a β -anomer compound of Chemical formula 3 (Step 1); adding hydrochloric acid to the β -anomer compound of Chemical formula 3 to produce a diol
10 compound of Chemical formula 4 (Step 2); and reacting the diol compound of Chemical formula 4 with an amine compound in the presence of a base as a catalyst to produce the adenosine derivative (Step 3).

[Scheme 1]



wherein, A, R and Y are as defined above.

5 The method will be stepwise explained in detail.

In Step 1, the synthesis of the novel adenosine derivative starts with the compound of Chemical formula 2. In the presence of a Lewis acid as a catalyst, this starting material is reacted with a silylated purine compound to give
 10 the β -anomer compound of Chemical formula 3. Trimethylsilyl

trifluoromethanesulfonate (TMSOTf) may be used as the Lewis acid catalyst. Dichloroethane, chloroform, acetonitrile, or dichloromethane is preferably used as the solvent in Step 1, with higher preference for dichloroethane. The silylated
5 purine compound can be obtained by reaction between the purine compound of Chemical formula 5 and hexamethyldisilazane (HMDS) in the presence of ammonium sulfate as a catalyst.

In Step 2, acetonide is removed from the compound of Chemical formula 3 with HCl to give a diol compound of
10 Chemical formula 4. Instead of HCl, acetic acid, sulfuric acid or p-toluene sulfonic acid may be used.

In Step 3, the diol compound of Chemical formula 4 obtained in Step 2 is reacted with an amine compound in the presence of a base as a catalyst to give the adenosine
15 derivative.

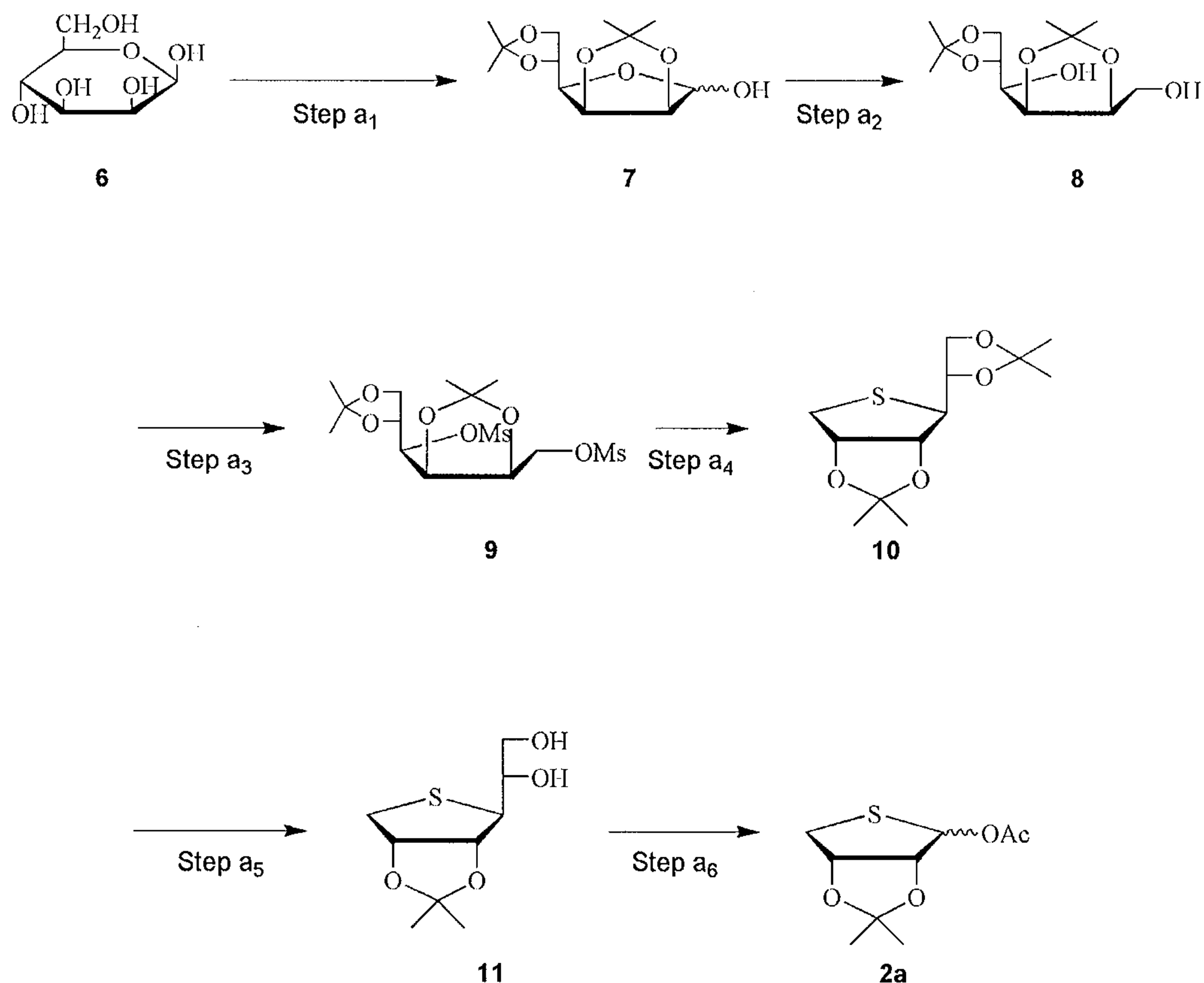
Examples of the base catalyst useful in Step 3 include triethylamine, pyridine, N,N-dimethylaminopyridine, and 1,4-dioxane with preference for triethylamine. In addition, the reaction may be preferably conducted in a solvent selected
20 from among lower alcohols such as methanol and ethanol, 1,4-dioxane, tetrahydrofuran and chloroform.

Depending on the kinds of the substituent A, the compound of Chemical formula 2 used as the starting material for the synthesis of the adenosine derivative according to the
25 present invention may be synthesized through the reaction

route of either Scheme 2 or 3.

When the substituent A is sulfur (S), Scheme 2 is taken for the synthesis of the starting material. As seen in Scheme 2, the synthesis of the starting material is accomplished by
5 reacting the D-mannose compound of Chemical formula 6 with 2,2-dimethoxypropane in the presence of an acid as a catalyst to give the diacetonide compound of Chemical formula 7 (Step a₁); opening the compound of Chemical formula 7 obtained in Step a₁ in the presence of a reducing agent to afford the diol
10 compound of Chemical formula 8 (Step a₂); mesylating the diol compound of Chemical formula 8 obtained in Step a₂ to afford the dimesyl compound of Chemical formula 9 (Step a₃); cyclizing the compound of Chemical formula 9 obtained in Step a₃ to afford the thiosugar compound of Chemical formula 10
15 (Step a₄); selectively hydrolyzing the compound of Chemical formula 10 obtained in Step a₄ to afford the diol compound of Chemical formula 11 (Step a₅); and converting the compound of Chemical formula 11 obtained in Step a₅ into an acetate compound of Chemical formula 2a in the presence of a catalyst
20 (Step a₆).

[Scheme 2]



Compound 2a in Scheme 2 is identical to the compound of Chemical formula 2.

Below, the synthesis of Compound 2a will be further explained stepwise in greater detail.

As in Step a₁, the synthesis of Compound 2 starts from the D-mannose of Chemical formula 6. D-Mannose is reacted with 2,2-dimethoxypropane in the presence of an acid as a catalyst to give diacetonide compound of Chemical formula 7.

An acid in combination with anhydrous acetic acid, functioning to catalyze the conversion of D-mannose of Chemical formula 6 into the compound of Chemical formula 7,

may be an inorganic acid, such as conc. sulfuric acid or hydrochloric acid, or an organic acid, such as p-toluenesulfonic acid.

In Step a₂, the compound of Chemical formula 7 is ring-
5 opened in the presence of a reducing agent to afford the diol compound of Chemical formula 8.

The treatment of the compound of Chemical formula 7 with the reducing agent sodium borohydride produces the compound of Chemical formula 8. In lieu of sodium borohydride, a metal
10 hydride, such as lithium aluminum hydride, or sodium sulfite may be used.

In Step a₃, the compound of Chemical formula 8 obtained in Step a₂ is mesylated into the dimesyl compound of Chemical formula 9.

15 The mesylation of the compound of Chemical formula 8 with methanesulfonylchloride (MsCl) into the compound of Chemical formula 9 is preferably conducted in an inert solvent, such as ethyl ether, petroleum ether, dichloromethane, tetrahydrofuran and N,N-
20 dimethylformamideformamide.

In Step a₄, the compound of Chemical formula 9 obtained in Step a₃ is cyclized into a thiosugar compound of Chemical formula 10.

The compound of Chemical formula 10 can be obtained by
25 reacting the compound of Chemical formula 9 with sodium

sulfide. Alternatively, the compound of Chemical formula 10 can be achieved by substitution with a thio ester, such as methyl thioacetate, followed by reaction with sodium alkoxide. N,N-dimethylformamide or dimethylsulfoxide may be used as the
5 solvent for Step a₄.

In Step a₅, the compound of Chemical formula 10 obtained in Step a₄ is selectively hydrolyzed into the diol compound of Chemical formula 11.

For the hydrolysis of the compound of Chemical formula
10 10 into the compound of Chemical formula 11, acetic acid may be used. In place of acetic acid, sulfuric acid, hydrochloric acid or p-toluene sulfonic acid may be used.

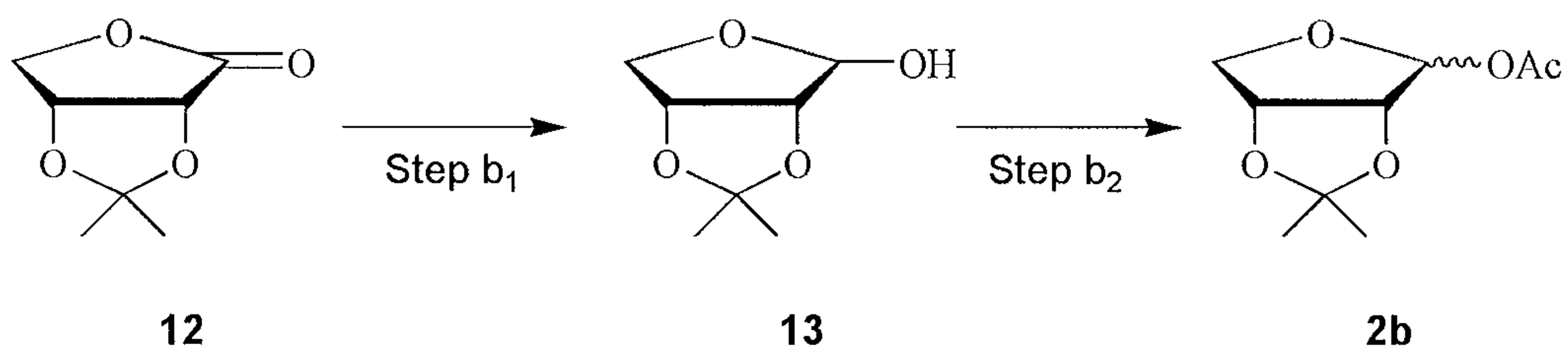
In Step a₆, the compound of Chemical formula 11 obtained
15 in Step a₅ is converted into the acetate compound of Chemical formula 2a in the presence of a catalyst.

Conversion into the compound of the Chemical formula 2a is accomplished by reacting the compound of Chemical formula 11 with red tetraacetate (Pd(OAc)₄).
20

When the substituent A is oxygen (O), Scheme 3 is taken for the synthesis of the starting material 2. As seen in Scheme 3, the synthesis of the starting material is accomplished by reacting the compound of Chemical formula 12
25 with a reducing agent to afford the lactol compound of

Chemical formula 13 (Step b₁); and reacting the compound of Chemical formula 13 obtained in Step b₁ with anhydrous acetic acid to afford an acetate compound of Chemical formula 2b (Step b₂).

5 [Scheme 3]



Compound 2b of Scheme 3 is identical to the compound of Chemical formula 2.

10 Below, the synthesis of Compound 2b will be further explained stepwise in greater detail.

In Step b₁, the compound of Chemical formula 12 is reduced into the lactol compound of Chemical formula 13.

For the reduction of the compound of Chemical formula 12, an easily synthesizable compound, into the compound of Chemical formula 13, diisobutylammonium hydride (DIBAL) may be used as a catalyst.

In Step b₂, the compound of Chemical formula 13 is reacted with anhydrous acetate to afford the acetate compound of Chemical formula 2b.

Thus, the compound of Chemical formula 2b can be

obtained by reacting the lactol compound of Chemical formula 13 with acetate.

In accordance with a further aspect thereof, the present invention pertains to an A₃ adenosine receptor antagonist comprising the adenosine derivative represented by Chemical formula 1 or a pharmaceutically acceptable salt thereof as an active ingredient.

In accordance with still a further aspect thereof, the present invention pertains to a pharmaceutical composition for the prevention and treatment of inflammatory diseases, comprising the adenosine derivative represented by Chemical formula 1 or a pharmaceutically acceptable salt thereof as an active ingredient.

When expressed in Chinese Hamster Ovary (CHO) cells, A₃ adenosine receptors were found to inhibit adenylyl cyclase, an enzyme that produces cAMP from ATP. Also, when activated by agonists, the A₃ adenosine receptor was proven to mediate the activation of guanosine triphosphate-dependent phospholipase C, an enzyme which catalyzes the degradation of phosphatidyl inositol into inositol triphosphate and DAG in the brain (Ramkumar, V. et al., J. Biol. Chem., 268, 168871-168890, 1993; Abbracchio, M. P. et al., Mol. Pharmacol., 48, 1038-

1045, 1995). These findings account for the possibility that there is a reaction pathway mediated by the A₃ adenosine receptor in cerebral ischemia when it is activated because this second messenger system serves as a reaction pathway for
5 nerve injury in cerebral ischemia. Also, A₃ receptor agonists are known to prevent cerebral diseases, such as epilepsy, and to protect the heart as well as inhibiting the release of TNF- α (tumor necrosis factor), an inflammation mediator, and the production of MIP-1 α , interleukin-12 and interferon- γ , all
10 of which act as inflammation mediators. On the other hand, the inactivation of A₃ adenosine receptor causes the release of inflammation factors, such as histamine, from mast cells, bronchoconstriction, and the apoptosis of immune cells. Accordingly, A₃ adenosine antagonists have the possibility of
15 being candidates as anti-inflammatory agents and anti-asthmatics.

The adenosine derivatives of the present invention were assayed for human adenosine receptor (hAR)-binding affinity and selectivity. In an assay for binding affinity (refer to
20 Experimental Example 1), the adenosine derivatives of the present invention were found to have high binding affinity for human A₃ adenosine receptors (hA₃ AR), but low affinity for A₁ and A_{2A} adenosine receptors, thereby showing high selectivity. Particularly, the compound of Example 12 shows the highest
25 binding affinity for hA₃ AR with K_i determined at 1.50 ± 0.40

nM, followed by the compound of Example 2 ($K_i = 1.66 \pm 0.90$ nM), the compound of Example 14 ($K_i = 2.50 \pm 1.00$ nM), the compound of Example 10 ($K_i = 3.69 \pm 0.25$ nM) and the compound of Example 4 ($K_i = 4.16 \pm 0.50$ nM) in decreasing order of binding affinity. Also, the compound of Example 4 was measured to have high binding affinity for the rat A_3 adenosine receptor expressed in CHO cells ($K_i = 3.89 \pm 1.15$ nM). In addition, the compounds of Examples 15 and 16, both adenosine derivatives in the form of 4'-O oxonucleoside, show high binding affinity and selectivity (see Table 1).

In assays for anti-inflammatory activity (refer to Experimental Examples 3~6), the adenosine derivatives of the present invention were found to have anti-inflammatory activity, although this was low compared to that of the control hydrocortisone.

When administered to mice treated with TPA in the ears, the compounds of Examples 2 to 4, diluted in acetone, were observed to decrease inflammation of the ears to some degree (see FIG. 2). In addition, the compounds of Examples 1 and 6 were found to have anti-inflammatory activity four or more times that of the compounds of Examples 2 to 4, as measured on the basis of inhibition percentage (FIG. 3).

In an assay for anti-inflammatory activity, the compounds of Examples 5, 7 and 8, diluted at a concentration

of 0.5% in a mixture of distilled water and acetone (1:4), were measured to have percentages of inflammation inhibition of 17%, 34% and 53%, respectively (FIG. 4). The compounds of Examples 15 and 16, diluted at a concentration of 0.5% in a mixture of DMSO and acetone (1:9), were measured to have percentages of inflammation inhibition of 59% and 79%, respectively (FIG. 5). Based on the observations in the assay, the adenosine derivatives of the present invention were proven to have anti-inflammatory activity.

10

Having high binding affinity and selectivity for A₃ adenosine receptors, thus, the adenosine derivatives, represented by Chemical formula 1, according to the present invention, can be effectively used as A₃ adenosine receptor antagonists. Further, the adenosine derivatives of the present invention exert antagonism on A₃ adenosine receptors, showing anti-inflammatory activity, and thus are useful in the prevention and treatment of inflammatory diseases.

The inflammatory disease to which the adenosine derivatives of the present invention can be effectively applied include acute and chronic inflammatory diseases, such as ulcerative inflammation, exudative inflammation, purulent inflammation, hemorrhagic inflammation, and hyperplastic inflammation.

25

With regard to pharmaceutical compositions comprising the adenosine derivative of the present invention or pharmaceutically acceptable salts thereof, they are formulated into dosage forms with expedients, as will be explained with the following examples, which are illustrative only, and are not intended to limit the present invention. The compositions of the present invention may be administered systemically or topically.

The compound of the present invention may be clinically administered in oral or non-oral forms. It is usually formulated in combination with a diluent or excipient, such as a filler, a thickening agent, a binder, a wetting agent, a disintegrant, a surfactant, etc. Solid agents intended for oral administration of the compound of the present invention may be in the form of tablets, pills, powders, granules, capsules, and the like. These solid agents are formulated in combination with at least one excipient such as starch, calcium carbonate, sucrose, lactose, or gelatine. Besides, a lubricant, such as magnesium stearate, talc and the like, may be added, as well. Liquid agents intended for oral administration include suspensions, internal use solutions, emulsion, syrups, and the like. In addition to a simple diluent such as water or liquid paraffin, various excipients, such as wetting agents, sweetening agents, aromatics, preservatives, and the like may be contained in the liquid

agents for the oral administration of the compound of the present invention.

Also, non-oral dosage forms of the compound of the present invention include injections, emulsions, inhalations, and suppositories. For injections, sterile aqueous solutions, non-aqueous solvents, and suspensions made from propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and esters such as ethylolate may be used. The basic materials of suppositories include witepsol, macrogol, tween 61, cacao paper, laurin paper, glycerol, and gelatine. The compound of the present invention may be formulated into ointments or cream for topical application.

Depending on the conditions of patients, including age, body weight, sex, administration route, and disease severity, the administration dose of the compound of the present invention to humans may vary. Typically, the compound of the present invention is administered at a dose from 0.001 to 100 mg per kg of body weight a day and preferably at a dose from 0.01 to 30 mg per kg of body weight a day. The compound may be administered in a single dose or in divided doses per day. The compound of the present invention is contained in an amount from 0.0001 to 10% by weight based on the total weight of the composition and preferably in an amount from 0.001 to 1% by weight. Also, the administration route is dependent on patient's health state and disease severity.

Description of Drawings

The application of the preferred embodiments of the present invention is best understood with reference to the accompanying drawings, in which like reference numerals are used for like and corresponding parts, wherein:

FIG. 1 is a graph showing the antagonist effect of the compound of Example 4 on the CHO cells treated with the agonist Cl-IB-MECA;

FIG. 2 is a graph showing the anti-inflammatory activity of the compounds of the present invention (Examples 2, 3 and 4) in animal tests;

FIG. 3 is a graph showing the anti-inflammatory activity of the compounds of the present invention (Examples 1 and 6) in animal tests;

FIG. 4 is a graph showing the anti-inflammatory activity of the compounds of the present invention (Examples 5, 7 and 8) in animal tests; and

FIG. 5 is a graph showing the anti-inflammatory activity of the compounds of the present invention (Examples 15 and 16) in animal tests.

Mode for Invention

A better understanding of the present invention may be

obtained in light of the following examples which are set forth to illustrate, but are not to be construed to limit the present invention.

5 Synthesis of the Starting Material

<PREPARATION EXAMPLE 1> Preparation of (3aR,4R,6aS)-2,2-Ddimethyltetrahydrothieno[3,4-d][1,3]dioxol-4-yl acetate

Step a₁. Preparation of (3aR,4R,6R,6aR)-6-(2,2-dimethyl-1,3-dioxolan-4-yl)-2,2-dimethyl-tetrahydrofuro[3,4-
10 d][1,3]dioxol-4-ol

 To acetone (50 ml) were added D-mannose(1.74 g, 6.52 mmol) and 2,2-dimethoxypropane (2.45 ml, 19.55 mmol) with stirring, followed by cooling the solution to 0°C. To the solution was dropwise added conc. sulfuric acid (0.45 g, 1.96
15 mmol). The resulting reaction mixture was stirred at room temperature for 24 hrs, followed by neutralization with triethyl amine and concentration in a vacuum. The concentrate was purified by silica gel column chromatography using a mixture of hexane:ethylacetate (1:1, v/v) as an elution
20 solvent to afford the object compound as a white solid (1.61 g, 95%).

 m.p. 120.3-120.5°C

¹H-NMR (CDCl₃) δ 5.34(s, 1 H), 4.76-4.79(m, 1 H), 4.58(d, 1 H, J = 6.0 Hz), 4.34-4.39(m, 1 H), 4.15(dd, 1 H, J = 3.6,
25 7.2 Hz), 4.00-4.08(m, 2 H);

$[\alpha]^{25}_D$ 11.71 (c 0.11, CH_2Cl_2);

FAB-MS m/z 261 $[\text{M} + \text{H}]^+$.

Step a₂. Preparation of (1*R*)-(2,2-dimethyl-1,3-dioxolan-4-yl) ((4*R*,5*S*)-5-(hydroxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methanol

(3*aR*,4*R*,6*R*,6*aR*)-6-(2,2-dimethyl-1,3-dioxolan-4-yl)-2,2-dimethyl-tetrahydrofuro[3,4-*d*][1,3]dioxol-4-ol (1.50 g, 5.76 mmol), prepared in Step a₁, was carefully added to ethanol (25 ml) and the solution was cooled to 0°C. To the solution was added sodium borohydride (NaBH_4 , 440 mg, 11.53 mmol), followed by stirring the solution at room temperature for 2 hrs. The reaction mixture was neutralized with acetic acid and concentrated in a vacuum. The concentrate was extracted with ethylacetate and water. The organic layer was dried over anhydrous magnesium sulfate (MgSO_4), filtered and concentrated in a vacuum. The concentrate was purified by silica gel column chromatography using a mixture of hexane:ethylacetate (1:1, v/v) as an elution solvent to afford the object compound in a syrup form (1.38 g, 92%).

$^1\text{H-NMR}$ (CDCl_3) δ 4.33 (dd, 1 H, $J = 1.6, 7.2$ Hz), 4.24-4.28 (m, 1 H), 4.06-4.13 (m, 2 H), 3.92-3.97 (m, 1 H), 3.76-3.85 (m, 2 H), 3.59-3.61 (m, 1 H), 1.48 (s, 3 H), 1.38 (s, 3 H), 1.36 (s, 3 H), 1.33 (s, 3 H);

$[\alpha]^{25}_D$ -3.88 (c 0.44, CH_2Cl_2);

FAB-MS m/z 263 $[M + H]^+$.

Step a₃. Preparation of (1*R*)-(2,2-dimethyl-1,3-dioxolan-4-yl)((4*S*,5*S*)-2,2-dimethyl-5-
5 ((methylsulfonyloxy)methyl)-1,3-dioxolan-4-yl)methylmethanesulfonate

(1*R*)-(2,2-dimethyl-1,3-dioxolan-4-yl)((4*R*,5*S*)-5-(hydroxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methanol (38.52 g, 146.85 mmol), prepared in Step a₂, and 4-dimethylaminopyridine (4-DMAP, 5.38 mg, 44.06 mmol) were added
10 to a mixture of dichloromethane (300 ml) and triethylamine (163.75 ml, 1.17 mol), and the solution was stirred and cooled to 0°C. To this was dropwise added dimethanesulfonyl chloride (47.59 ml, 587.42 mmol). After stirring at room temperature
15 for 1 hr, the reaction mixture was extracted with dichloromethane and washed with a saturated sodium hydrogen carbonate (NaHCO₃) solution. The organic layer thus obtained was dried over anhydrous magnesium sulfate (MgSO₄), filtered and concentrated in a vacuum. The dimethyl compound thus
20 produced, having the form of a brown syrup, was purified through silica gel column chromatography using a mixture of hexane:ethylacetate (5:1, v/v) as an elution solvent to afford the object compound in syrup form (57.83 g, 94%).

¹H-NMR (CDCl₃) δ 4.75(pseudo t, 1 H, J = 7.4 Hz), 4.33-
25 4.45(m, 4 H), 4.06-4.20(m, 3 H), 3.12(s, 3 H), 3.07(s, 3 H),

1.51(s, 3 H), 1.43(s, 3 H), 1.37(s, 3 H), 1.33(s, 3 H);

$[\alpha]^{25}_{\text{D}}$ 38.32 (c 0.29, CH₂Cl₂);

FAB-MS m/z 419 [M + H]⁺.

5 Step a₄. Preparation of (3aR,4S,6aS)-4-(2,2-dimethyl-1,3-dioxolan-4-yl)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxol

(1R)-(2,2-dimethyl-1,3-dioxolan-4-yl)((4S,5S)-2,2-dimethyl-5-((methylsulfonyloxy)methyl)-1,3-dioxolan-4-yl)methyl
10 methanesulfonate (993.80 g, 2.23 mmol), prepared in Step a₃, was dissolved in DMF (50 ml). Following the addition of sodium sulfide (348.30 g, 4.46 mmol) thereto, the solution was stirred at 80°C under a reflux condition overnight. Thereafter, the solvent was removed in a vacuum and the
15 residue was extracted with ethyl acetate and water. The organic layer was dried over anhydrous magnesium sulfate (MgSO₄), filtered, and concentrated in a vacuum. The concentrate was purified through silica gel column chromatography using a mixture of hexane:ethylacetate (8:1,
20 v/v) as an elution solvent to afford the object compound in a syrup form (453.0 mg, 78%).

¹H-NMR (CDCl₃) δ 4.92(dt, 1 H, J = 1.8, 5.6 Hz), 4.72(dd, 1 H, J = 2.0, 6.0 Hz), 4.26-4.30(m, 1 H), 4.04(s, 1 H), 3.79(t, 1 H, J = 3.8 Hz), 3.31-3.32(m, 1 H), 3.19(dd, 1 H, J =
25 5.4, 12.0 Hz), 2.84(dd, 1 H, J = 1.6, 12.0 Hz), 1.51(s, 3 H),

1.43(s, 3 H), 1.32(dd, 6 H, $J = 8.4$ Hz);

$[\alpha]^{25}_D -96.04$ (c 0.20, CH_2Cl_2);

FAB-MS m/z 261 $[\text{M} + \text{H}]^+$.

5 Step a₅. Preparation of 1-((3aR,4S,6aS)-2,2-
dimethyltetrahydrothieno[3,4-d][1,3]dioxol-4-yl)ethan-1,2-diol
 (3aR,4S,6aS)-4-(2,2-dimethyl-1,3-dioxolan-4-yl)-2,2-
 dimethyltetrahydrothieno[3,4-d][1,3]dioxol (21.78 g, 83.66
 mmol), prepared in Step a₄, was dissolved in a 60% aqueous
 10 acetic acid solution (250 ml), followed by stirring the
 solution at room temperature for 2 hrs. The reaction mixture
 was concentrated in a vacuum and the concentrate was purified
 through silica gel column chromatography using a mixture of
 hexane:ethylacetate (1:2, v/v) as an elution solvent to afford
 15 the object compound as a white solid (14.85 g, 81%).

$^1\text{H-NMR}$ (CDCl_3) δ 4.92(dt, 1 H, $J = 1.8, 5.6$ Hz), 4.72(dd,
 1 H, $J = 2.0, 6.0$ Hz), 4.26-4.30(m, 1 H), 4.04(s, 1 H),
 3.79(t, 1 H, $J = 3.8$ Hz), 3.31-3.32(m, 1 H), 3.19(dd, 1 H, $J =$
 5.4, 12.0 Hz), 2.84(dd, 1 H, $J = 1.6, 12.0$ Hz), 1.51(s, 3 H),
 20 1.43(s, 3 H), 1.32(dd, 6 H, $J = 8.4$ Hz);

$[\alpha]^{25}_D -96.04$ (c 0.20, CH_2Cl_2);

FAB-MS m/z 261 $[\text{M} + \text{H}]^+$.

Step a₆. Preparation of (3aR,4R,6aS)-2,2-
 25 dimethyltetrahydrothieno[3,4-d][1,3]dioxol-4-yl acetate

1-((3aR,4S,6aS)-2,2-dimethyltetrahydrothieno[3,4-
d][1,3]dioxol-4-yl)ethan-1,2-diol (14.85 g, 67.41 mmol),
prepared in Step a₅, was dissolved in ethylacetate (300 ml)
and cooled to 0°C. To the solution was added red tetraacetate
5 (Pb(OAc)₄, 157.31 g, 337.06 mmol), followed by stirring at
room temperature overnight. The reaction mixture was filtered
through a Celite filter and the filtrate was diluted in ethyl
acetate. The organic layer was diluted in dichloromethane,
washed with a saturated aqueous sodium hydrogen carbonate
10 (NaHCO₃) solution, dried over anhydrous magnesium sulfate, and
concentrated in a vacuum. The concentrate was purified
through silica gel column chromatography using a mixture of
hexane:ethylacetate (8:1, v/v) as an elution solvent to afford
the object compound in a syrup form (8.82 g, 60%).

15 ¹H-NMR (CDCl₃) δ 5.03(dd, 1 H, J = 5.6, 9.6 Hz), 4.79(dd,
1 H, J = 5.6, 8.8 Hz), 3.21-3.27(m, 2 H), 3.01(dt, 2 H, J =
0.8, 12.8 Hz), 2.05(s, 3 H), 1.50(s, 3 H), 1.31(s, 3 H);

[α]_D²⁵ -258.15 (c 0.18, CH₂Cl₂);

FAB-MS m/z 218 [M]⁺.

20

<PREPARATION EXAMPLE 2> Preparation of (3aS,4S,6aS)-2,2-
Dimethyl-tetrahydrofuro[3,4-d][1,3]dioxol-4-yl acetate

Step b₁. Preparation (3aR,4R,6aR)-2,2-dimethyl-
tetrahydrofuro[3,4-d][1,3]dioxol-4-ol

25 2,3-O-isopropylidene-D-erythrone (1.04 g, 6.42

mmol) was dissolved in toluene (20 ml), followed by the addition of 1 M diisobutylammonium hydride (DIBAL)/THF to the solution at -78°C. The reaction mixture was stirred at the same temperature for 30 min and methanol was slowly added
5 until the reaction terminated. The suspension was filtered through a Celite filter and the filtrate was extracted with ethyl acetate and water, followed by silica gel column chromatography using a mixture of hexane:ethylacetate (3:1, v/v) to give the object compound in syrup form (1.94 g, 96%).

10 $^1\text{H-NMR}$ (CDCl_3) δ 5.39(s, 1 H), 4.82(dd, 1 H, $J = 3.6, 6.0$ Hz), 4.55(d, 1 H, $J = 6.0$ Hz), 4.05(dd, 1 H, $J = 3.6, 10.2$ Hz), 4.00(d, 1 H, $J = 10.0$ Hz), 1.45(s, 3 H), 1.30(s, 3 H).

Step b₂. Preparation of (3aS,4S,6aS)-2,2-dimethyl-
15 tetrahydrofuro[3,4-d][1,3]dioxol-4-yl acetate

To a solution of the lactol compound (875.9 mg, 5.47 mmol), prepared in Step b₁, in pyridine (10 ml) was added anhydrous acetic acid (0.67 ml, 6.56 mmol) at 0°C. The reaction mixture was stirred at room temperature for 3 hrs and
20 concentrated in a vacuum. The concentrate was extracted with ethylacetate and water and the organic layer was dried over anhydrous magnesium sulfate and concentrated in a vacuum. The residue was purified by silica gel column chromatography using a mixture of hexane:ethylacetate (8:1, v/v) to give the object
25 compound in a syrup form (702.1 mg, 65%).

^1H -NMR (CDCl_3) δ 6.16(s, 1 H), 4.86(dd, 1 H, $J = 3.6, 6.0$ Hz), 4.66(d, 1 H, $J = 6.0$ Hz), 4.12(d, 1 H, $J = 6.4$ Hz), 3.99(dd, 1 H, $J = 3.6, 10.8$ Hz), 2.05(s, 3 H), 1.48(s, 3 H), 1.33(s, 3 H).

5

<EXAMPLE 1> Synthesis of (2R,3R,4S)-2-(2-Chloro-6-(3-fluorobenzylamino)-9H-purin-9-yl)tetrahydrothiophen-3,4-diol

Step 1. Preparation of 2,6-dichloro-9-((3aR,4R,6aS)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxol-4-yl)-9H-purine

10 A solution of 2,6-dichloropurine (2.29 g, 22.12 mmol) and ammonium sulfate (438 mg, 3.32 mmol) in hexamethyldisilazane (HMDS, 50 ml) was fluxed overnight under inert, dry conditions. The resulting reaction mixture was concentrated in a vacuum and the solid mixture thus formed was
15 re-dissolved in cold 1, 2-dichloroethene (20 ml). To this solution were dropwise added a solution of (3aR, 4R, 6aS)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxol-4-yl acetate (1.41 g, 11.06 mmol), obtained in Preparation Example 1, in 1,2-dichloroethane (20 ml), and then trimethylsilyl
20 trifluoromethanesulfonate (TMSOTf, 4.0 ml, 22.12 mmol). The resulting solution was stirred at 0°C for 30 min and then at room temperature for 1 hr, and heated at 80°C for 2 hrs with stirring. The reaction mixture was cooled, diluted in dichloromethane and washed with a saturated aqueous sodium
25 hydrogen carbonate (NaHCO_3) solution. The organic solvent was

dried over anhydrous magnesium sulfate (MgSO_4) and concentrated in a vacuum to give a residue in the form of a yellow syrup. The residue was purified through silica gel column chromatography using a mixture of
5 dichloromethane:methanol(50:1, v/v) as an elution solvent to afford the object compound in the form of a foam (3.03 g, 79%).

UV (CH_2Cl_2) λ_{max} 275.0 nm;

^1H -NMR (CDCl_3) δ 8.17(s, 1 H), 5.87(s, 1 H), 5.32(pseudo
10 t, 1 H, $J = 4.8$ Hz), 5.21(d, 1 H, $J = 5.6$ Hz), 3.79(dd, 1 H, J
= 4.4, 12.8 Hz), 3.26(d, 1 H, $J = 13.2$ Hz), 1.59(s, 3 H),
1.36(s, 3 H);

$[\alpha]^{25}_{\text{D}} -42.04$ (c 0.16, CH_2Cl_2);

FAB-MS m/z 347 $[\text{M} + \text{H}]^+$.

15

Step 2. Preparation of (2R,3S,4S)-2-(2,6-dichloro-9H-purin-9-yl)tetrahydrothiophen-3,4-diol

To a solution of 2,6-dichloro-9-((3aR,4R,6aS)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxol-4-yl)-9H-purine,
20 prepared in Step 1, in tetrahydrofuran (20 ml) was added 2 N HCl, followed by stirring the solution overnight. The reaction mixture was neutralized with 1 N sodium hydroxide and carefully concentrated in a vacuum. The concentrate was purified through silica gel column chromatography using a
25 mixture of dichloromethane:methanol(20:1, v/v) as an elution

solvent to afford the object compound as a white solid (1.94 g, 96%).

m.p. 198.3-200.3°C;

UV (MeOH) $\lambda_{\max}$ 275. nm;

5 $^1\text{H-NMR}$ (CD_3OD) δ 8.87(s, 1 H), 6.08(d, 1 H, $J = 6.8$ Hz), 4.69(q, 1 H, $J = 3.2$ Hz), 4.48(q, 1 H, $J = 3.6$ Hz), 3.56(dd, 1 H, $J = 4.4, 11.2$ Hz), 2.97(dd, 1 H, $J = 3.4, 11.2$ Hz);

$[\alpha]^{25}_{\text{D}} -50.43$ (c 0.12, DMSO);

FAB-MS m/z 307 $[\text{M} + \text{H}]^+$.

10

Step 3. Preparation of (2*R*,3*R*,4*S*)-2-(2-chloro-6-(3-fluorobenzylamino)-9*H*-purin-9-yl)tetrahydrothiophen-3,4-diol

(2*R*,3*S*,4*S*)-2-(2,6-dichloro-9*H*-purin-9-yl)tetrahydrothiophen-3,4-diol (1 equivalent), prepared in
15 Step 2, and 3-fluorobenzylamine (1.5 equivalents) were dissolved in ethanol (5 ml) at room temperature for 2~3 hrs with stirring. The reaction mixture was concentrated in a vacuum and the concentrate was purified through silica gel column chromatography using a mixture of
20 dichloromethane:methanol (20:1, v/v) as an elution solvent to afford the object compound (0.10 g, 80%).

m.p. 183.2-183.5°C;

UV (MeOH) $\lambda_{\max}$ 275.0 nm;

$^1\text{H-NMR}$ (DMSO-d_6) δ 8.91(t, 1 H-NH, $J = 5.8$ Hz), 8.51(s, 1 H), 7.33-7.39(m, 1 H), 7.13-7.18(m, 2 H), 7.06(dt, 1 H, $J =$

2.8, 11.6 Hz), 5.82(d, 1 H, $J = 7.2$ Hz), 5.56(d, 1 H-OH, $J = 6.0$ Hz), 5.37(d, 1 H-OH, $J = 4.4$ Hz), 4.65(d, 1 H, $J = 6.0$ Hz), 4.60(m, 1 H), 4.33-4.35(m, 1 H), 3.41(dd, 1 H, $J = 4.0, 10.8$ Hz), 2.79(dd, 1 H, $J = 2.8, 10.8$ Hz);

5 $[\alpha]_D^{25} -96.21$ (c 0.12, DMSO);

FAB-MS m/z 396 $[M + H]^+$.

<EXAMPLE 2> Synthesis of (2*R*,3*R*,4*S*)-2-(2-Chloro-6-(3-chlorobenzylamino)-9*H*-purin-9-yl)tetrahydrothiophen-3,4-diol

10 Step 1. Preparation of 2,6-dichloro-9-((3*aR*,4*R*,6*aS*)-2,2-dimethyltetrahydrothieno[3,4-*d*][1,3]dioxol-4-yl)-9*H*-purine

The same procedure as in Step 1 of Example 1 was conducted to give the object compound in foam form.

15 Step 2. Preparation of (2*R*,3*S*,4*S*)-2-(2,6-dichloro-9*H*-purin-9-yl)tetrahydrothiophen-3,4-diol

The same procedure as in Step 2 of Example 1 was conducted to give the object compound as a white solid.

20 Step 3. Preparation of (2*R*,3*R*,4*S*)-2-(2-chloro-6-(3-chlorobenzylamino)-9*H*-purin-9-yl)tetrahydrothiophen-3,4-diol

A procedure similar to that of Step 3 of Example 1 was conducted, with the exception that 3-chlorobenzylamine was used instead of 3-fluorobenzylamine, to give the object
25 compound (0.11 g, 83%).

m.p. 163.3–165.3°C;

UV (MeOH) $\lambda_{\max}$ 274.5 nm;

$^1\text{H-NMR}$ (CD_3OD) δ 8.34 (s, 1 H), 7.41 (s, 1 H), 7.24–7.34 (m, 3 H), 5.94 (d, 1 H, $J = 6.4$ Hz), 4.75 (brs, 2 H), 4.61 (q, 1 H, $J = 3.2$ Hz), 4.45 (q, 1 H, $J = 4.0$ Hz), 3.51 (dd, 1 H, $J = 4.8$, 11.2 Hz), 2.95 (dd, 1 H, $J = 3.6$, 10.8 Hz);

FAB-MS m/z 411 $[\text{M}]^+$.

<EXAMPLE 3> Synthesis of (2*R*,3*R*,4*S*)-2-(2-Chloro-6-(3-bromobenzylamino)-9*H*-purin-9-yl)tetrahydrothiophen-3,4-diol

Step 1. Preparation of 2,6-dichloro-9-((3*aR*,4*R*,6*aS*)-2,2-dimethyltetrahydrothieno[3,4-*d*][1,3]dioxol-4-yl)-9*H*-purine

The same procedure as in Step 1 of Example 1 was conducted to give the object compound in foam form.

Step 2. Preparation of (2*R*,3*S*,4*S*)-2-(2,6-dichloro-9*H*-purin-9-yl)tetrahydrothiophen-3,4-diol

The same procedure as in Step 2 of Example 2 was conducted to give the object compound as a white solid.

Step 3. Preparation of (2*R*,3*R*,4*S*)-2-(2-chloro-6-(3-bromobenzylamino)-9*H*-purin-9-yl)tetrahydrothiophen-3,4-diol

A procedure similar to that of Step 3 of Example 1 was conducted, with the exception that 3-bromobenzylamine was used instead of 3-fluorobenzylamine, to give the object compound

(0.12 g, 83%).

m.p. 184.0–185.0°C;

UV (MeOH) $\lambda_{\max}$ 274.0 nm;

$^1\text{H-NMR}$ (DMSO- d_6) δ 8.91 (brs, 1 H-NH), 8.51 (s, 1 H),
5 7.55 (s, 1 H), 7.43 (d, 1 H, $J = 7.6$ Hz), 7.33–7.35 (m, 1 H),
7.26–7.30 (m, 1 H), 5.82 (d, 1 H, $J = 7.2$ Hz), 5.57 (d, 1 H-OH, J
= 6.0 Hz), 5.38 (d, 1 H-OH, $J = 4.0$ Hz), 4.60–4.63 (m, 3 H),
4.34 (s, 1 H), 3.41 (dd, 1 H, $J = 4.4, 11.2$ Hz), 2.80 (dd, 1 H, J
= 2.8, 10.8 Hz);

10 FAB-MS m/z 456 $[\text{M} + \text{H}]^+$.

<EXAMPLE 4> Synthesis of (2*R*,3*R*,4*S*)-2-(2-Chloro-6-(3-iodobenzylamino)-9*H*-purin-9-yl)tetrahydrothiophen-3,4-diol

Step 1. Preparation of 2,6-dichloro-9-((3*aR*,4*R*,6*aS*)-2,2-dimethyltetrahydrothieno[3,4-*d*][1,3]dioxol-4-yl)-9*H*-purine

The same procedure as in Step 1 of Example 1 was conducted to give the object compound in foam form.

Step 2. Preparation of (2*R*,3*S*,4*S*)-2-(2,6-dichloro-9*H*-purin-9-yl)tetrahydrothiophen-3,4-diol

The same procedure as in Step 2 of Example 1 was conducted to give the object compound as a white solid.

Step 3. Preparation of (2*R*,3*R*,4*S*)-2-(2-chloro-6-(3-iodobenzylamino)-9*H*-purin-9-yl)tetrahydrothiophen-3,4-diol

A procedure similar to that of Step 3 of Example 1 was conducted, with the exception that 3-iodobenzylamine was used instead of 3-fluorobenzylamine, to give the object compound (0.14 g, 84%).

5 m.p. 198.7–199.9°C;

UV (MeOH) $\lambda_{\max}$ 274.0 nm;

$^1\text{H-NMR}$ (DMSO- d_6) δ 8.90 (t, 1 H-NH, $J = 6.4$ Hz), 8.51 (s, 1 H), 7.74 (s, 1 H), 7.60 (d, 1 H, $J = 7.6$ Hz), 7.35 (d, 1 H, $J = 7.6$ Hz), 7.13 (t, 1 H, $J = 8.0$ Hz), 5.82 (d, 1 H, $J = 7.6$ Hz),
10 5.56 (d, 1 H, $J = 6.4$ Hz), 5.37 (d, 1 H, $J = 4.0$ Hz), 4.60 (d, 3 H, $J = 4.4$ Hz), 4.34 (brs, 1 H), 3.38 (dd, 1 H, $J = 4.0, 10.8$ Hz), 2.80 (dd, 1 H, $J = 4.0, 10.8$ Hz);

$[\alpha]^{25}_D -78.91$ (c 0.13, DMSO);

FAB-MS m/z 504 $[\text{M} + \text{H}]^+$.

15

<EXAMPLE 5> Synthesis of (2R,3R,4S)-2-(2-Chloro-6-(2-chlorobenzylamino)-9H-purin-9-yl)tetrahydrothiophen-3,4-diol

Step 1. Preparation of 2,6-dichloro-9-((3aR,4R,6aS)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxol-4-yl)-9H-purine

20 The same procedure as in Step 1 of Example 1 was conducted to give the object compound in foam form.

Step 2. Preparation of (2R,3S,4S)-2-(2,6-dichloro-9H-purin-9-yl)tetrahydrothiophen-3,4-diol

25 The same procedure as in Step 2 of Example 1 was

conducted to give the object compound as a white solid.

Step 3. Preparation of (2*R*,3*R*,4*S*)-2-(2-chloro-6-(2-chlorobenzylamino)-9*H*-purin-9-yl)tetrahydrothiophen-3,4-diol

5 A procedure similar to that of Step 3 of Example 1, with the exception that 2-chlorobenzylamine was used instead of 3-fluorobenzylamine, was conducted to give the object compound (0.11 g, 81%).

m.p. 198.7-199.7°C;

10 UV (MeOH) $\lambda_{\max}$ 273.5 nm;

$^1\text{H-NMR}$ (CD_3OD) δ 8.35 (brs, 1 H), 7.45-7.47 (m, 1 H), 7.39-7.43 (m, 1 H), 7.25-7.29 (m, 2 H), 5.95 (d, 1 H, $J = 6.4$ Hz), 4.60-4.63 (m, 1 H), 4.45 (dd, 1 H, $J = 3.6, 8.0$ Hz), 3.51 (dd, 1 H, $J = 4.8, 10.8$ Hz), 2.95 (dd, 1 H, $J = 4.0, 10.8$ Hz);

15 $[\alpha]^{25}_{\text{D}} -96.21$ (c 0.12, DMSO);

FAB-MS m/z 412 $[\text{M} + \text{H}]^+$.

<EXAMPLE 6> Synthesis of (2*R*,3*R*,4*S*)-2-(2-Chloro-6-(5-chloro-2-methoxybenzylamino)-9*H*-purin-9-yl)tetrahydrothiophen-
20 3,4-diol

Step 1. Preparation of 2,6-dichloro-9-((3*aR*,4*R*,6*aS*)-2,2-dimethyltetrahydrothieno[3,4-*d*][1,3]dioxol-4-yl)-9*H*-purine

The same procedure as in Step 1 of Example 1 was conducted to give the object compound in a foam form.

25

Step 2. Preparation of (2*R*,3*S*,4*S*)-2-(2,6-dichloro-9*H*-purin-9-yl)tetrahydrothiophen-3,4-diol

The same procedure as in Step 2 of Example 1 was conducted to give the object compound as a white solid.

5

Step 3. Preparation of (2*R*,3*R*,4*S*)-2-(2-chloro-6-(5-chloro-2-methoxybenzylamino)-9*H*-purin-9-yl)tetrahydrothiophen-3,4-diol

A procedure similar to that of Step 3 of Example 1 was
10 conducted, with the exception that 5-chloro-2-methoxybenzylamine was used instead of 3-fluorobenzylamine, to give the object compound (0.11 g, 78%).

m.p. 188.8-189.8°C;

UV (MeOH) $\lambda_{\max}$ 275.5 nm;

15 $^1\text{H-NMR}$ (DMSO- d_6) δ 8.64(t, 1 H-NH, $J = 6.0$ Hz), 8.51(s, 1 H), 7.21-7.25(m, 1 H), 7.12(d, 1 H, $J = 7.2$ Hz), 7.00(d, 1 H, $J = 8.0$ Hz), 6.85-6.89(m, 1 H), 5.82(d, 1 H, $J = 7.6$ Hz), 5.57(d, 1 H-OH, $J = 6.4$ Hz), 5.37(d, 1 H-OH, $J = 4.0$ Hz), 4.61-4.63(m, 2 H), 4.35(m, 1 H), 3.84(s, 3 H), 3.71(dd, 1 H, $J = 3.6, 10.4$ Hz), 2.80(dd, 1 H, $J = 2, 4, 10.8$ Hz);

$[\alpha]^{25}_{\text{D}} -96.10$ (c 0.21, DMSO);

FAB-MS m/z 442 $[\text{M} + \text{H}]^+$.

<EXAMPLE 7> Preparation of (2*R*,3*R*,4*S*)-2-(2-chloro-6-(2-methoxybenzylamino)-9*H*-purin-9-yl)tetrahydrothiophen-3,4-diol
25

Step 1. Preparation of 2, 6-dichloro-9-((3a*R*, 4*R*, 6a*S*)-
2,2-dimethyltetrahydrothieno[3,4-*d*][1,3]dioxol-4-yl)-9*H*-purine

The same procedure as in Step 1 of Example 1 was
conducted to give the object compound in a foam form.

5

Step 2. Preparation of (2*R*, 3*S*, 4*S*)-2-(2,6-dichloro-9*H*-
purin-9-yl)tetrahydrothiophen-3,4-diol

The same procedure as in Step 2 of Example 1 was
conducted to give the object compound as a white solid.

10

Step 3. Preparation of (2*R*, 3*R*, 4*S*)-2-(2-chloro-6-(2-
methoxybenzylamino)-9*H*-purin-9-yl)tetrahydrothiophen-3,4-diol

A procedure similar to that of Step 3 of Example 1 was
conducted, with the exception that 2-methoxybenzylamine was
15 used instead of 3-fluorobenzylamine, to give the object
compound (0.12 g, 88%).

m.p. 188.0°C;

UV (MeOH) $\lambda_{\max}$ 276.5 nm;

$^1\text{H-NMR}$ (DMSO- d_6) δ 8.65 (t, 1 H-NH, $J = 6.0$ Hz), 8.51 (s, 1
20 H), 7.21-7.25 (m, 1 H), 7.12 (d, 1 H, $J = 7.2$ Hz), 7.00 (d, 1 H,
 $J = 8.0$ Hz), 6.85-6.89 (m, 1 H), 5.83 (d, 1 H, $J = 6.8$ Hz),
5.58 (d, 1 H-OH, $J = 6.4$ Hz), 5.39 (d, 1 H-OH, $J = 3.6$ Hz),
4.62-4.64 (m, 2 H), 4.35 (s, 1 H), 3.84 (s, 1 H), 3.42 (dd, 1 H, J
= 3.6, 10.4 Hz), 2.79-2.82 (m, 1 H);

25 $[\alpha]^{25}_D -93.53$ (c 0.17, DMSO);

FAB-MS m/z 407 $[M + H]^+$.

<EXAMPLE 8> Synthesis of (2R,3R,4S)-2-(2-Chloro-6-(naphthalen-1-ylmethylbenzylamino)-9H-purin-9-yl)tetrahydrothiophen-3,4-diol

Step 1. Preparation of 2,6-dichloro-9-((3aR,4R,6aS)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxol-4-yl)-9H-purine

The same procedure as in Step 1 of Example 1 was conducted to give the object compound in foam form.

Step 2. Preparation of (2R,3S,4S)-2-(2,6-dichloro-9H-purin-9-yl)tetrahydrothiophen-3,4-diol

The same procedure as in Step 2 of Example 1 was conducted to give the object compound as a white solid.

Step 3. Preparation of (2R,3R,4S)-2-(2-chloro-6-(naphthalen-1-ylmethylbenzylamino)-9H-purin-9-yl)tetrahydrothiophen-3,4-diol

A procedure similar to that of Step 3 of Example 1 was conducted, with the exception that naphthalen-1-ylmethylbenzylamine was used instead of 3-fluorobenzylamine, to give the object compound (0.13 g, 90%).

m.p. 226.3°C (decomp);

UV (MeOH) $\lambda_{\max}$ 281.0 nm;

$^1\text{H-NMR}$ (DMSO- d_6) δ 8.96 (t, 1 H-NH, $J = 6.0$ Hz), 8.51 (s,

1 H), 8.25(d, 1 H, $J = 8.0$ Hz), 7.95-7.97(m, 1 H), 7.83-
7.85(m, 1 H), 7.53-7.61(m, 2 H), 7.43-7.46(m, 2 H), 5.82(d, 1
H, $J = 7.6$ Hz), 5.56(d, 1 H, $J = 6.4$ Hz), 5.38(d, 1 H, $J = 4.0$
Hz), 5.12(d, 1 H, $J = 6.0$ Hz), 4.59-4.61(m, 1 H), 4.34-4.35(m,
5 1 H), 3.40-3.44(m, 1 H), 2.80(dd, 1 H, $J = 2.4, 6.8$ Hz);

FAB-MS m/z 428 $[M + H]^+$.

<EXAMPLE 9> Synthesis of 3-((2-chloro-9-((2*R*,3*S*,4*R*)-3,4-
dihydroxytetrahydrothiophen-2-yl)-9*H*-purine-6-
10 ylamino)methyl)benzoic acid

Step 1. Preparation of 2,6-dichloro-9-((3*aR*,4*R*,6*aS*)-2,2-
dimethyltetrahydrothieno[3,4-*d*][1,3]dioxol-4-yl)-9*H*-purine

The same procedure as in Step 1 of Example 1 was
conducted to give the object compound in foam form.

15

Step 2. Preparation of (2*R*,3*S*,4*S*)-2-(2,6-dichloro-9*H*-
purin-9-yl)tetrahydrothiophen-3,4-diol

The same procedure as in Step 2 of Example 1 was
conducted to give the object compound as a white solid.

20

Step 3. Preparation of 3-((2-chloro-9-((2*R*,3*S*,4*R*)-3,4-
dihydroxytetrahydrothiophen-2-yl)-9*H*-purine-6-
ylamino)methyl)benzoic acid

A procedure similar to that of Step 3 of Example 1 was
25 conducted, with the exception that 3-(aminomethyl)benzoic acid

was used instead of 3-fluorobenzylamine, to give the object compound (0.12 g, 84%).

mp 254.0-256.9°C;

UV (MeOH) $\lambda_{\max}$ 275.5 nm;

5 $^1\text{H-NMR}$ (DMSO- d_6) δ 8.95 (t, 1 H-NH, $J = 6.0$ Hz), 8.52 (s, 1 H), 7.89 (d, 1 H, $J = 8.4$ Hz), 7.43 (d, 1 H, $J = 8.0$ Hz), 5.82 (d, 1 H, $J = 7.6$ Hz), 5.57 (brs, 1 H), 5.38 (brs, 1 H), 4.71 (d, 1 H, $J = 6.0$ Hz), 4.60 (brs, 1 H), 4.34 (brs, 1 H), 3.41 (dd, 1 H, $J = 4.0, 10.8$ Hz), 2.80 (dd, 1 H, $J = 2.8, 10.8$ Hz);

$[\alpha]^{25}_D -94.55$ (c 0.11, DMSO);

FAB-MS m/z 422 $[\text{M} + \text{H}]^+$.

<EXAMPLE 10> Synthesis of 2-(2-Chloro-6-methylamino-
15 purin-9-yl) (2R,3S,4R)-tetrahydrothiophen-3,4-diol

Step 1. Preparation of 2,6-dichloro-9-((3aR,4R,6aS)-2,2-
dimethyltetrahydrothieno[3,4-d][1,3]dioxol-4-yl)-9H-purine

The same procedure as in Step 1 of Example 1 was conducted to give the object compound in foam form.

20

Step 2. Preparation of (2R,3S,4S)-2-(2,6-dichloro-9H-
purin-9-yl)tetrahydrothiophen-3,4-diol

The same procedure as in Step 2 of Example 1 was conducted to give the object compound as a white solid.

25

Step 3. Preparation of 2-(2-chloro-6-methylamino-purin-9-yl) (2R,3S,4R)-tetrahydrothiophen-3,4-diol

A procedure similar to that of Step 3 of Example 1 was conducted, with the exception that methylamine was used instead of 3-fluorobenzylamine, to give the object compound (0.89 g, 90%).

UV (MeOH) $\lambda_{\max}$ 269.5 nm (pH 7);

$^1\text{H-NMR}$ (CDCl_3) δ 2.99 (1H, dd, 4'-CH, $J = 4.4, 10.8$ Hz), 3.12 (3H, brs, NH-CH₃), 3.44 (1H, dd, 4'-CH, $J = 4, 10.8$ Hz), 4.41 (1H, m, 2'-CH, $J = 5.6$ Hz), 4.47 (1H, m, 3'-CH), 5.89 (1H, d, 1'-CH, $J = 5.6$ Hz), 8.40 (s, 1H, 8-CH);

$[\alpha]^{25}_{\text{D}} -34.8$ (c 0.115, DMSO);

FAB-MS m/z 302.3 $[\text{M} + \text{H}]^+$.

15 <EXAMPLE 11> Synthesis of (2R,3R,4S)-2-(6-(3-fluorobenzylamino)-9H-purin-9-yl)tetrahydrothiophen-3,4-diol

Step 1. Preparation of 6-chloro-9-((3aR, 4R, 6aS)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxol-4-yl)-9H-purine

A procedure similar to that of Step 1 of Example 1 was conducted, with the exception that 6-chloropurine (2.29 g, 22.12 mmol) was used instead of 2,6-chloropurine, to give the object compound in foam form (1.84 g, 91%).

UV (CH_2Cl_2) $\lambda_{\max}$ 265.0 nm;

$^1\text{H-NMR}$ (CDCl_3) δ 8.67 (pseudo t, 1 H, $J = 1.4$ Hz), 8.23 (s, 1 H), 5.88 (s, 1 H), 5.23 (m, 2 H), 3.69 (dd, 1 H, $J = 4.0$,

13.2 Hz), 3.18(d, 1 H, $J = 12.8$ Hz), 1.52(s, 3 H), 1.29(s, 3 H);

^{13}C -NMR (CDCl_3) δ 152.05, 151.39, 151.09, 144.34, 132.56, 111.90, 89.60, 84.31, 70.30, 40.76, 26.40, 24.63;

5 $[\alpha]^{25}_{\text{D}} -157.64$ (c 0.15, MeOH);

FAB-MS m/z 313 $[\text{M} + \text{H}]^+$.

Step 2. Preparation of (2*R*,3*S*,4*S*)-2-(6-chloro-9*H*-purin-9-yl)tetrahydrothiophen-3,4-diol

10 Synthesis was conducted from 6-chloro-9-((3*aR*,4*R*,6*aS*)-2,2-dimethyltetrahydrothieno[3,4-*d*][1,3]dioxol-4-yl)-9*H*-purine (1.84 g, 5.88 mmol), prepared in Step 1, in a manner similar to that of Step 2 of Example 1 to afford the object compound as a white solid (1.27 g, 79%).

15 m.p. 192.3-192.8°C;

UV (MeOH) λ_{max} 264.5 nm;

^1H -NMR ($\text{DMSO}-d_6$) δ 9.02(s, 1 H), 8.82(s, 1 H), 6.02(d, 1 H, $J = 7.6$ Hz), 5.62(d, 1 H-OH, $J = 6.0$ Hz), 5.43(d, 1 H-OH, $J = 4.0$ Hz), 4.70-4.74(m, 1 H), 4.36-4.40(m, 1 H), 3.47(dd, 1 H, $J = 4.0, 10.8$ Hz), 3.17(d, 1 H, $J = 5.2$ Hz), 2.84(dd, 1 H, $J = 2.8, 11.2$ Hz);

$[\alpha]^{25}_{\text{D}} -109.15$ (c 0.16, DMSO);

FAB-MS m/z 273 $[\text{M} + \text{H}]^+$.

25 Step 3. Preparation of (2*R*,3*R*,4*S*)-2-(6-(3-

fluorobenzylamino)-9H-purin-9-yl)tetrahydrothiophen-3,4-diol

(2*R*, 3*S*, 4*S*)-2-(6-chloro-9*H*-purin-9-yl)tetrahydrothiophen-3,4-diol (1 equivalent), prepared in Step 2, and 3-fluorobenzylamine (1.5 equivalents) were dissolved in ethanol (5 ml) at room temperature for 2~3 hrs with stirring. The reaction mixture was concentrated in a vacuum and the concentrate was purified through silica gel column chromatography using a mixture of dichloromethane:methanol (20:1, v/v) as an elution solvent to afford the object compound (0.11 g, 82%).

m.p. 180.5-180.7°C;

UV (MeOH) $\lambda_{\max}$ 273.5 nm;

¹H-NMR (DMSO-*d*₆) δ 8.46(s, 1 H), 8.22(s, 1 H), 7.31-7.39(m, 1 H), 7.12-7.18(m, 2 H), 7.01-7.05(m, 1 H), 5.90(d, 1 H, *J* = 7.2 Hz), 5.53(d, 1 H-OH, *J* = 6.4 Hz), 5.35(d, 1 H-OH, *J* = 4.0 Hz), 4.67-4.71(m, 2 H), 4.35-4.37(m, 1 H), 3.39-3.43(m, 1 H), 3.17(d, 1 H, *J* = 5.2 Hz), 2.80(dd, 1 H, *J* = 3.2, 11.2 Hz);

$[\alpha]^{25}_{\text{D}}$ -141.2 (c 0.11, DMSO);

FAB-MS *m/z* 362 [M + H]⁺.

<EXAMPLE 12> Synthesis of (2*R*, 3*R*, 4*S*)-2-(6-(3-Chlorobenzylamino)-9*H*-purin-9-yl)tetrahydrothiophen-3,4-diol

Step 1. Preparation of 6-chloro-9-((3*aR*, 4*R*, 6*aS*)-2,2-dimethyltetrahydrothieno[3,4-*d*][1,3]dioxol-4-yl)-9*H*-purine

The same procedure as in Step 1 of Example 11 was conducted to give the object compound in foam form.

Step 2. Preparation of (2*R*,3*S*,4*S*)-2-(6-chloro-9*H*-purin-9-yl)tetrahydrothiophen-3,4-diol

The same procedure as in Step 2 of Example 11 was conducted to give the object compound as a white solid.

Step 3. Preparation of (2*R*,3*R*,4*S*)-2-(6-(3-chlorobenzylamino)-9*H*-purin-9-yl)tetrahydrothiophen-3,4-diol

A procedure similar to that of Step 3 of Example 11 was conducted, with the exception that 3-chlorobenzylamine was used instead of 3-fluorobenzylamine, to give the object compound (0.12 g, 85%).

m.p. 165.0-165.3°C;

UV (MeOH) $\lambda_{\max}$ 274.5 nm;

¹H-NMR (DMSO-*d*₆) δ 8.47(s, 1 H), 8.22(s, 1 H), 7.39(s, 1 H), 7.26-7.35(m, 3 H), 5.91(d, 1 H, *J* = 7.2 Hz), 5.53(d, 1 H-OH, *J* = 6.4 Hz), 5.35(d, 1 H-OH, *J* = 4.0 Hz), 4.67-4.71(m, 2 H), 4.33-4.37(m, 1 H), 3.40-3.48(m, 2 H), 2.80(dd, 1 H, *J* = 3.2, 10.4 Hz);

$[\alpha]^{25}_{\text{D}} -162.5$ (*c* 0.10, DMSO);

FAB-MS *m/z* 378 [M + H]⁺.

<EXAMPLE 13> Synthesis of (2*R*,3*R*,4*S*)-2-(6-(3-

bromobenzylamino)-9H-purin-9-yl)tetrahydrothiophen-3,4-diol

Step 1. Preparation of 6-chloro-9-((3aR,4R,6aS)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxol-4-yl)-9H-purine

The same procedure as in Step 1 of Example 11 was
5 conducted to give the object compound in foam form.

Step 2. Preparation of (2R,3S,4S)-2-(6-chloro-9H-purin-9-yl)tetrahydrothiophen-3,4-diol

The same procedure as in Step 2 of Example 11 was
10 conducted to give the object compound as a white solid.

Step 3. Preparation of (2R,3R,4S)-2-(6-(3-bromobenzylamino)-9H-purin-9-yl)tetrahydrothiophen-3,4-diol

A procedure similar to that of Step 3 of Example 11 was
15 conducted, with the exception that 3-bromobenzylamine was used instead of 3-fluorobenzylamine, to give the object compound (0.11 g, 70%).

m.p. 183.0-184.0°C;

UV (MeOH) $\lambda_{\max}$ 270.0 nm;

20 $^1\text{H-NMR}$ (DMSO- d_6) δ 8.46(s, 1 H), 8.22(s, 1 H), 7.53(s, 1 H), 7.39-7.42(m, 1 H), 7.34-7.35(m, 1 H), 7.24-7.28(m, 1 H), 5.90(d, 1 H, $J = 7.2$ Hz), 5.53(d, 1 H-OH, $J = 6.4$ Hz), 5.35(d, 1 H-OH, $J = 4.0$ Hz), 4.67-4.71(m, 2 H), 4.35-4.37(m, 1 H), 3.41(dd, 1 H, $J = 4.0, 10.8$ Hz), 3.06(q, 1 H, $J = 7.2$ Hz),
25 2.80(dd, 1 H, $J = 2.8, 10.8$ Hz);

$[\alpha]_D^{25} -100.72$ (c 0.14, DMSO);

FAB-MS m/z 422 $[M + H]^+$.

<EXAMPLE 14> Synthesis of (2*R*, 3*R*, 4*S*)-2-(6-(3-iodobenzylamino)-9*H*-purin-9-yl)tetrahydrothiophen-3,4-diol

Step 1. Preparation of 6-chloro-9-((3*aR*, 4*R*, 6*aS*)-2,2-dimethyltetrahydrothieno[3,4-*d*][1,3]dioxol-4-yl)-9*H*-purine

The same procedure as in Step 1 of Example 11 was conducted to give the object compound in foam form.

Step 2. Preparation of (2*R*, 3*S*, 4*S*)-2-(6-chloro-9*H*-purin-9-yl)tetrahydrothiophen-3,4-diol

The same procedure as in Step 2 of Example 11 was conducted to give the object compound as a white solid.

Step 3. Preparation of (2*R*, 3*R*, 4*S*)-2-(6-(3-iodobenzylamino)-9*H*-purin-9-yl)tetrahydrothiophen-3,4-diol

A procedure similar to that of Step 3 of Example 11 was conducted, with the exception that 3-iodobenzylamine was used instead of 3-fluorobenzylamine, to give the object compound (0.12 g, 72%).

m.p. 198.8-199.8°C;

UV (MeOH) $\lambda_{\max}$ 271.5 nm;

$^1\text{H-NMR}$ (DMSO- d_6) δ 8.46(s, 1 H), 8.22(s, 1 H), 7.72(s, 1 H), 7.56-7.59(m, 1 H), 7.35-7.36(d, 1 H, $J = 7.6$ Hz), 7.01-

7.12 (m, 1 H), 5.90 (d, 1 H, $J = 7.2$ Hz), 5.53 (d, 1 H-OH, $J = 6.4$ Hz), 5.35 (d, 1 H-OH, $J = 4.4$ Hz), 4.67-4.71 (m, 2 H), 4.34-4.38 (m, 1 H), 3.41 (dd, 1 H, $J = 4.0, 10.8$ Hz), 3.16 (d, 1 H, $J = 7.2$ Hz), 2.80 (dd, 1 H, $J = 2.8, 10.8$ Hz);

5 $[\alpha]^{25}_{\text{D}} -97.08$ (c 0.14, DMSO);

FAB-MS m/z 470 $[M + H]^+$.

<EXAMPLE 15> Synthesis of (2*R*,3*R*,4*R*)-2-(6-(3-Bromobenzylamino)-2-chloro-9*H*-purin-9-yl)tetrahydrofuran-3,4-
10 diol

Step 1. Preparation of 2, 6-dichloro-9-((3*aR*,4*R*,6*aR*)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)-9*H*-purine

Synthesis was conducted using (3*aR*,4*R*,6*aR*)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-ol (702.1 g, 3.472
15 mmol), prepared in Preparation Example 2, in the same manner as in Step 1 of Example 1 to afford the object compound in foam form (793.0 mg, 69%).

UV (MeOH) λ_{max} 276.5 nm;

$^1\text{H-NMR}$ (CDCl_3) δ 8.15 (s, 1 H), 6.07 (s, 1 H), 5.41 (d, 1 H, $J = 6.0$ Hz), 5.26-5.29 (m, 1 H), 4.25-4.31 (m, 2 H), 1.57 (s, 3
20 H), 1.41 (s, 3 H);

$[\alpha]^{25}_{\text{D}} -21.00$ (c 0.10, DMSO);

FAB-MS m/z 331 $[M + H]^+$.

25 Step 2. Preparation of (2*R*,3*R*,4*R*)-2-(2,6-dichloro-9*H*-

purin-9-yl)tetrahydrofuro-3,4-diol

Synthesis was conducted using 2,6-dichloro-9-
 ((3*aR*,4*R*,6*aS*)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-
 yl)-9*H*-purine (900 mg, 2.0 mmol), prepared in Step 1, in the
 5 same manner as in Step 2 of Example 1 to give the object
 compound as a white solid (0.46 g, 80%).

m.p. 122.7-123.4°C;

UV (MeOH) $\lambda_{\max}$ 276.5 nm;

¹H-NMR (DMSO-*d*₆) δ 8.98 (s, 1 H), 5.96 (d, 1 H, *J* = 6.4
 10 Hz), 5.57 (d, 1 H-OH, *J* = 6.0 Hz), 5.32 (d, 1 H-OH, *J* = 4.0 Hz),
 4.69-4.74 (m, 1 H), 4.41 (dd, 1 H, *J* = 3.6, 9.2 Hz), 4.29-
 4.32 (m, 1 H), 3.87 (dd, 1 H, *J* = 2.0, 9.6 Hz);

$[\alpha]^{25}_{\text{D}}$ -68.09 (*c* 0.14, DMSO);

FAB-MS *m/z* 291 [M + H]⁺.

15

Step 3. Preparation of (2*R*,3*R*,4*R*)-2-(6-(3-
 bromobenzylamino)-2-chloro-9*H*-purin-9-yl)tetrahydrofuro-3,4-
 diol

(2*R*,3*S*,4*S*)-2-(2,6-dichloro-9*H*-purin-9-yl)tetrahydrofuro-
 20 3,4-diol (1 equivalent), prepared in Step 2, and 3-
 bromobenzylamine (1.5 equivalents) were dissolved in ethanol
 (5 ml) at room temperature for 2~3 hrs with stirring. The
 reaction mixture was concentrated in a vacuum and the
 concentrate was purified through silica gel column
 25 chromatography using a mixture of dichloromethane:methanol

(20:1, v/v) as an elution solvent to afford the object compound (0.12 g, 82%).

m.p. 181.5-181.7°C;

UV (MeOH) $\lambda_{\max}$ 274.5 nm;

5 $^1\text{H-NMR}$ (DMSO- d_6) δ 8.92 (t, 1 H-NH, $J = 6.0$ Hz), 8.43 (s, 1 H), 7.55 (s, 1 H), 7.44 (d, 1 H, $J = 8.0$ Hz), 7.33-7.35 (m, 1 H), 7.26-7.30 (m, 1 H), 5.81 (d, 1 H, $J = 6.4$ Hz), 5.47 (d, 1 H, $J = 6.4$ Hz), 5.22 (d, 1 H, $J = 4.0$ Hz), 4.66-4.69 (m, 1 H), 4.62 (s, 2 H), 4.32 (dd, 1 H, $J = 3.6, 9.2$ Hz), 4.25 (brs, 1 H), 3.80 (dd,
10 1 H, $J = 1.6, 9.2$ Hz);

$[\alpha]^{25}_D -62.75$ (c 0.10, DMSO);

FAB-MS m/z 440 $[\text{M} + \text{H}]^+$.

<EXAMPLE 16> Synthesis of (2R,3R,4R)-2-(6-(3-iodobenzylamino)-2-chloro-9H-purin-9-yl)tetrahydrofuro-3,4-
15 diol

Step 1. Preparation of 2,6-dichloro-9-((3aR,4R,6aR)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-9H-purine

The same procedure as in Step 1 of Example 15 was
20 conducted to give the object compound in foam form.

Step 2. Preparation of (2R,3R,4R)-2-(2,6-dichloro-9H-purin-9-yl)tetrahydrofuro-3,4-diol

The same procedure as in Step 2 of Example 15 was
25 conducted to give the object compound as a white solid in

syrup form.

Step 3. Preparation of (2*R*, 3*R*, 4*R*)-2-(6-(3-iodobenzylamino)-2-chloro-9*H*-purin-9-yl)tetrahydrofuro-3,4-
5 diol

A procedure similar to that of Step 3 of Example 15 was conducted, with the exception that 3-iodobenzylamine was used instead of 3-bromobenzylamine to give the object compound (0.13 g, 78%).

10 m.p. 195.5-195.8°C;

UV (MeOH) $\lambda_{\max}$ 274.0 nm;

$^1\text{H-NMR}$ (DMSO- d_6) δ 8.91 (t, 1 H-NH, $J = 6.4$ Hz), 8.44 (s, 1 H), 7.75 (s, 1 H), 7.61 (d, 1 H, $J = 8.0$ Hz), 7.36 (d, 1 H, $J = 7.6$ Hz), 7.13 (t, 1 H, $J = 4.0$ Hz), 5.81 (d, 1 H, $J = 6.8$ Hz),
15 5.47 (d, 1 H-OH, $J = 6.8$ Hz), 5.23 (d, 1 H-OH, $J = 4.0$ Hz), 4.72 (dd, 1 H, $J = 6.4, 10.8$ Hz), 4.61 (d, 1 H, $J = 6.0$ Hz), 4.34 (dd, 1 H, $J = 3.6, 9.2$ Hz), 3.81 (dd, 1 H, $J = 1.2, 9.2$ Hz);

$[\alpha]^{25}_{\text{D}} -68.07$ (c 0.12, DMSO);

20 FAB-MS m/z 488 $[\text{M} + \text{H}]^+$.

<EXPERIMENTAL EXAMPLE 1> Assay for Binding Affinity for Adenosine Receptors

The adenosine derivatives of the present invention were
25 assayed for binding affinity for human A_1 , A_{2A} and A_3 adenosine

receptors as follows.

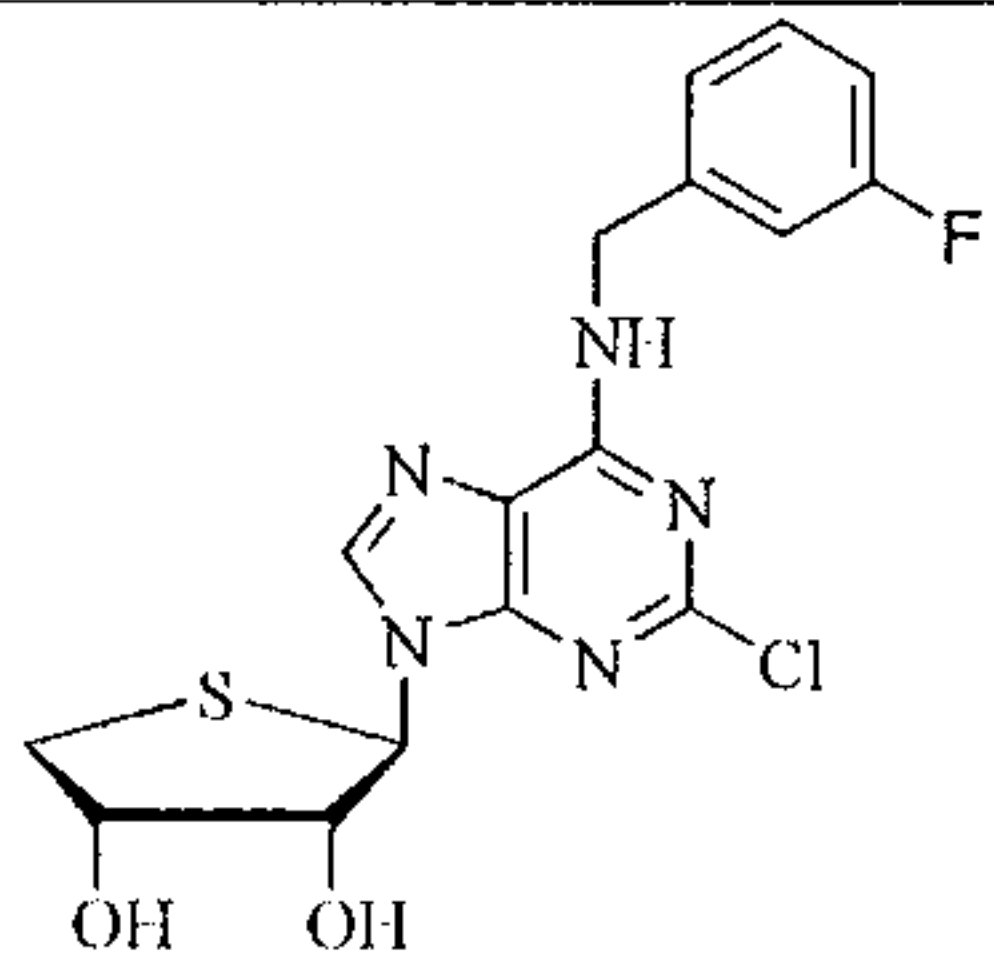
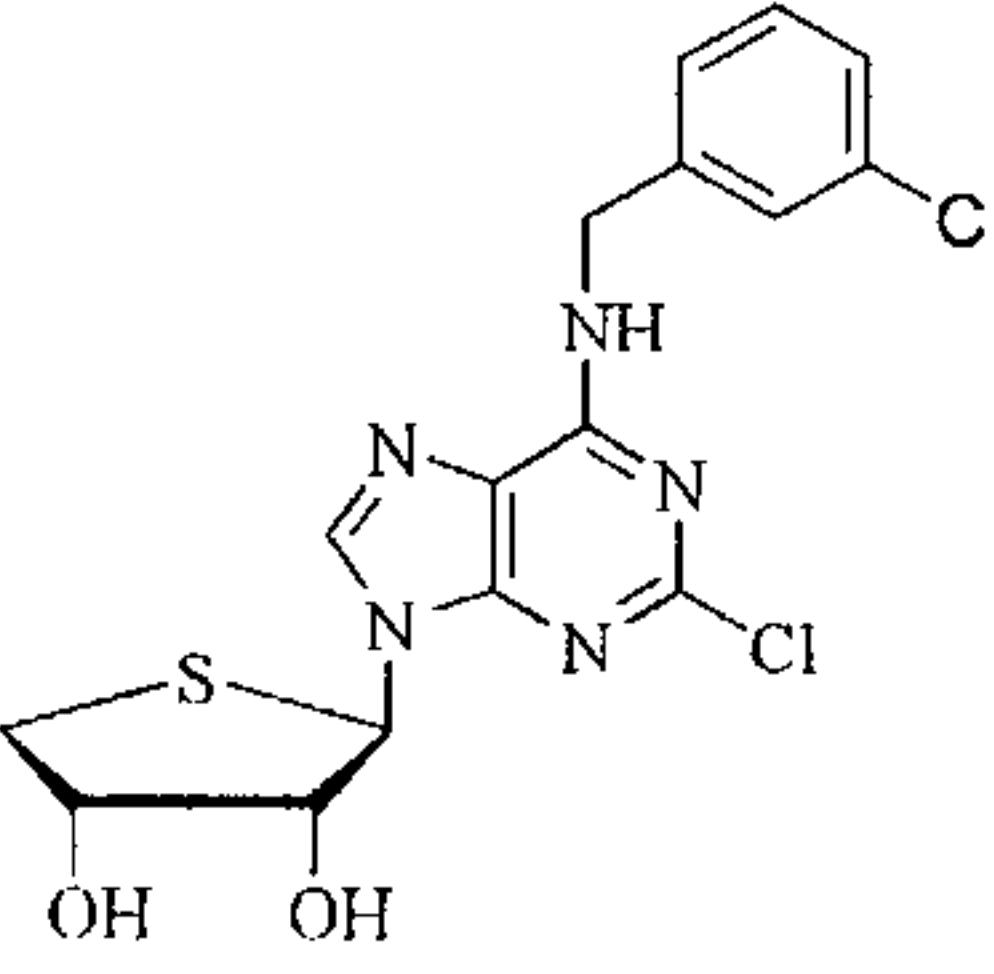
CHO cells (ATCC No. CCL-61), in which A₁ and A₃ adenosine receptors were expressed, were cultured in F-12 media (Gibco, U.S.A.) supplemented with 10% fetal bovine serum (FBS) and penicillin/streptomycin (100 units/ml and 100 µg/ml), at 37°C in a 5% CO₂ atmosphere. A predetermined amount of suitable hAR-expressed CHO cells was mixed with labeled ligands (1 nM [³H]CCPA and 0.5 nM [¹²⁵I]AB-MECA) specifically binding to A₁ and A₃ adenosine receptors in a 50/10/1 buffer in test tubes. The derivatives of the present invention were dissolved at various concentrations in dimethylsulfoxide (DMSO) and diluted in the buffer, taking care that the final concentration of DMSO did not exceed 1%. Incubation for 1 hr in a 37°C incubator was followed by rapid filtration in a vacuum using a cell collector (TOMTEC, U.S.A.). Subsequently, the test tubes were washed three times with 3 ml of the buffer before radioactivity was measured using a γ-counter. In the same condition as that for total binding, the equilibrium constant K_i for non-specific binding was determined in the presence of 10 µM of 5'-N-ethylcarboxamide adenosine (NECA) as a non-labeled ligand. The equilibrium constant K_i was calculated according to the Cheng-Prusoff equation on the assumption that [¹²⁵I]AB-MECA has a K_d value of 1.48 nM. K_i for binding affinity was determined by subtracting the non-specific binding value from the total binding value. On the

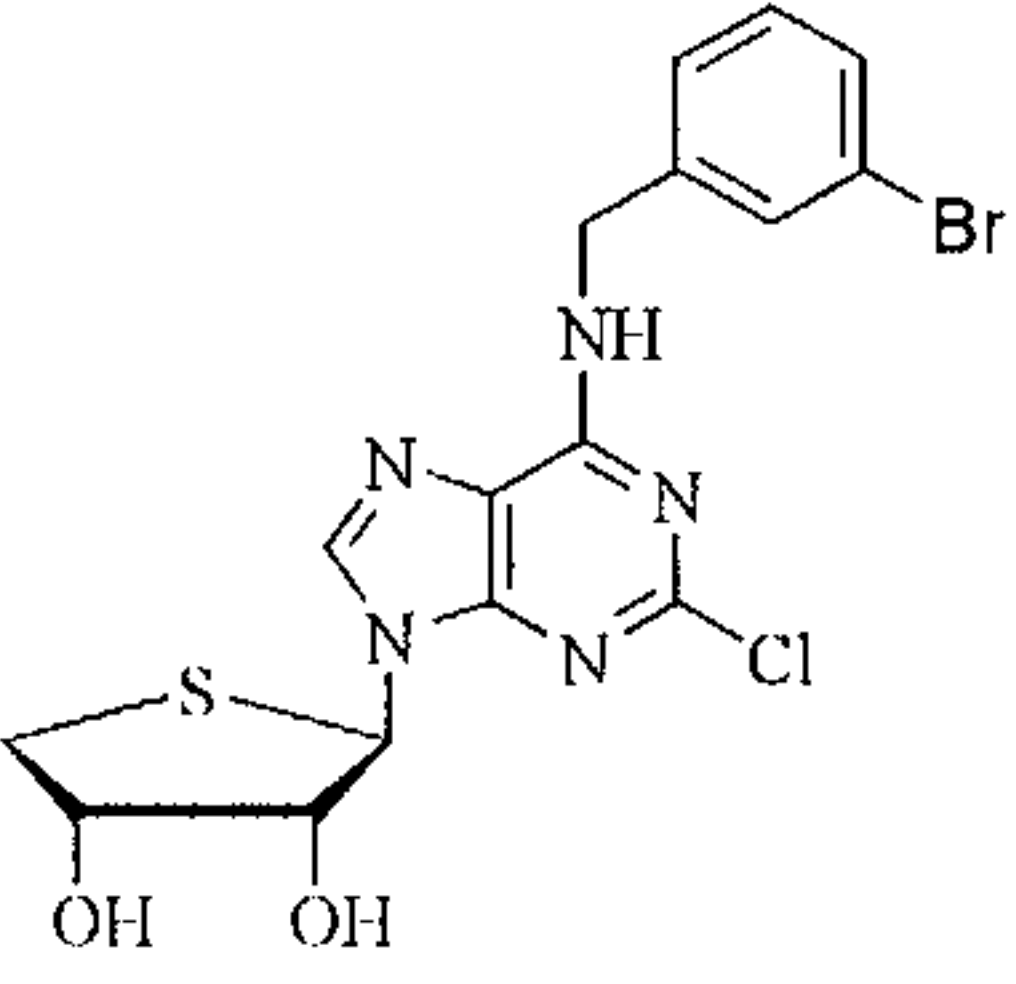
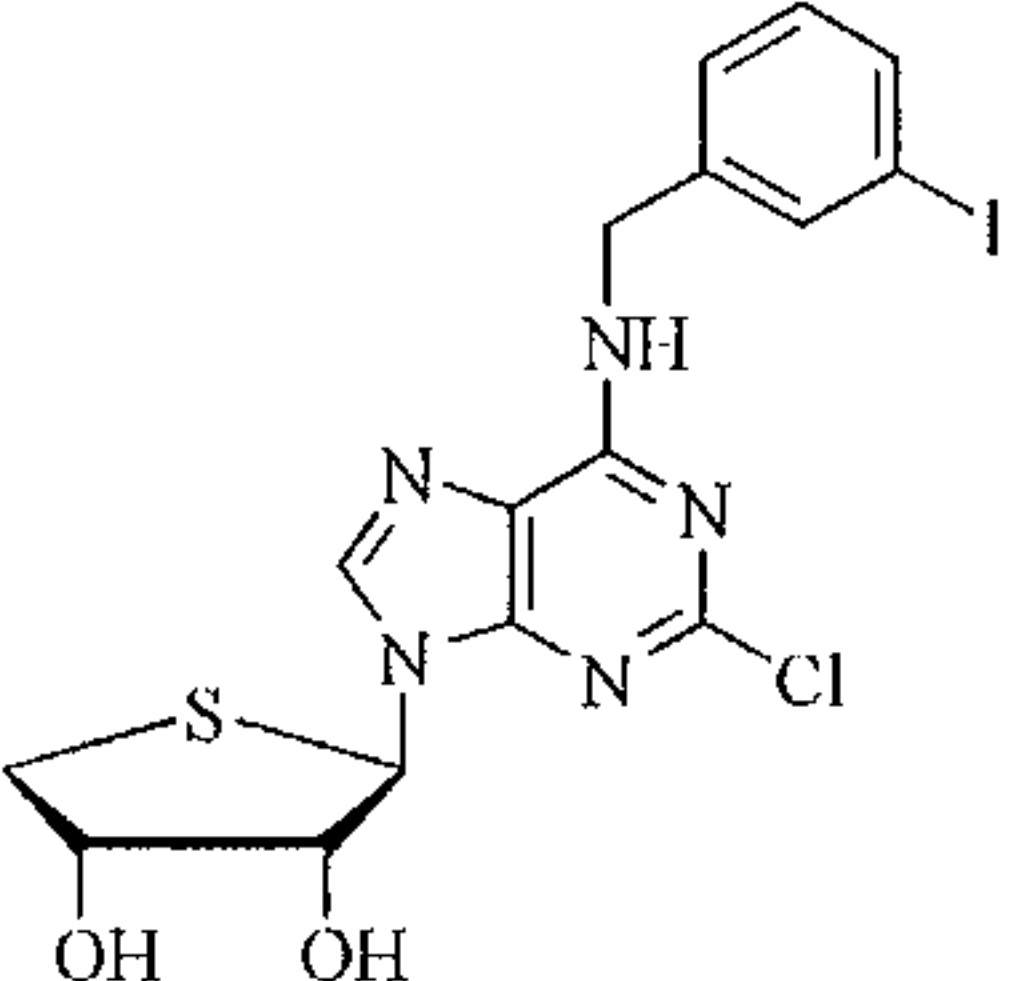
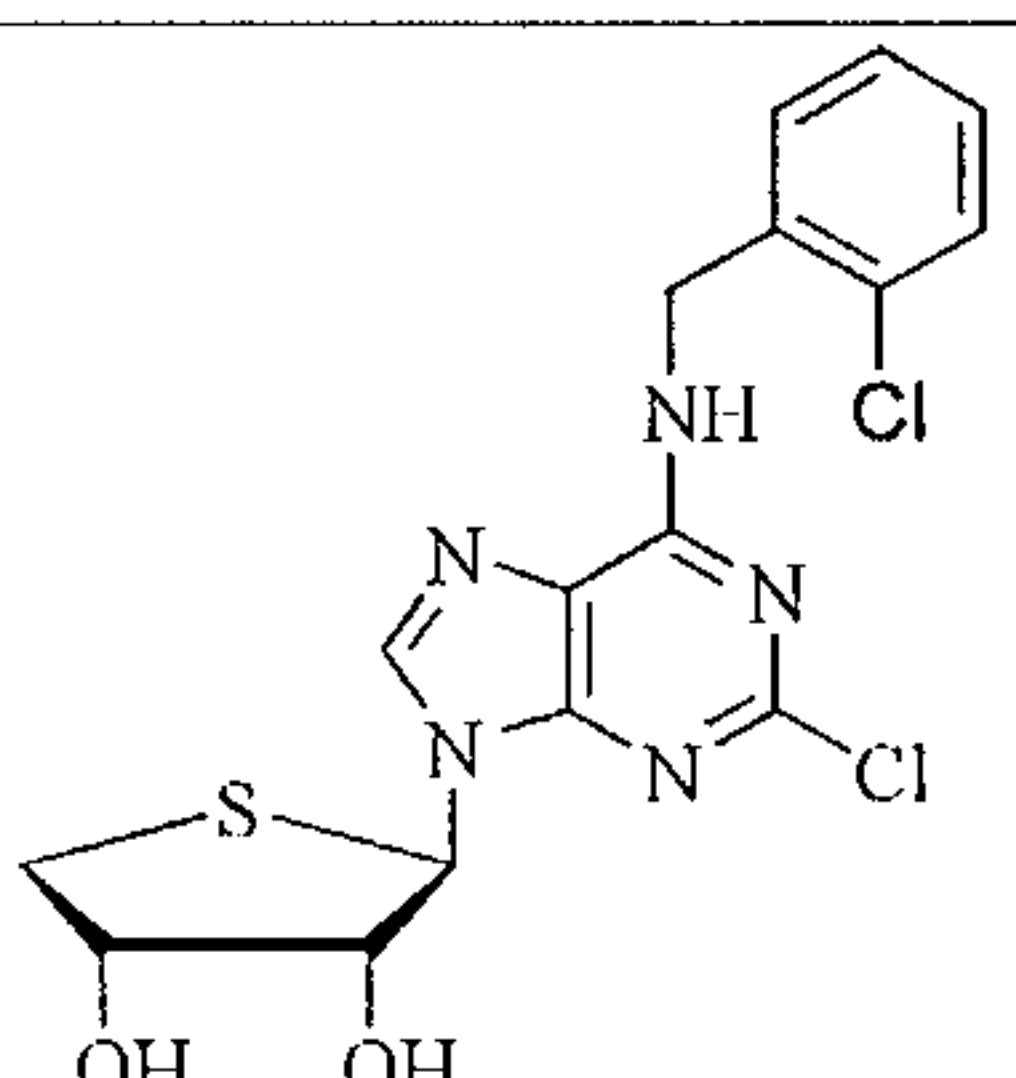
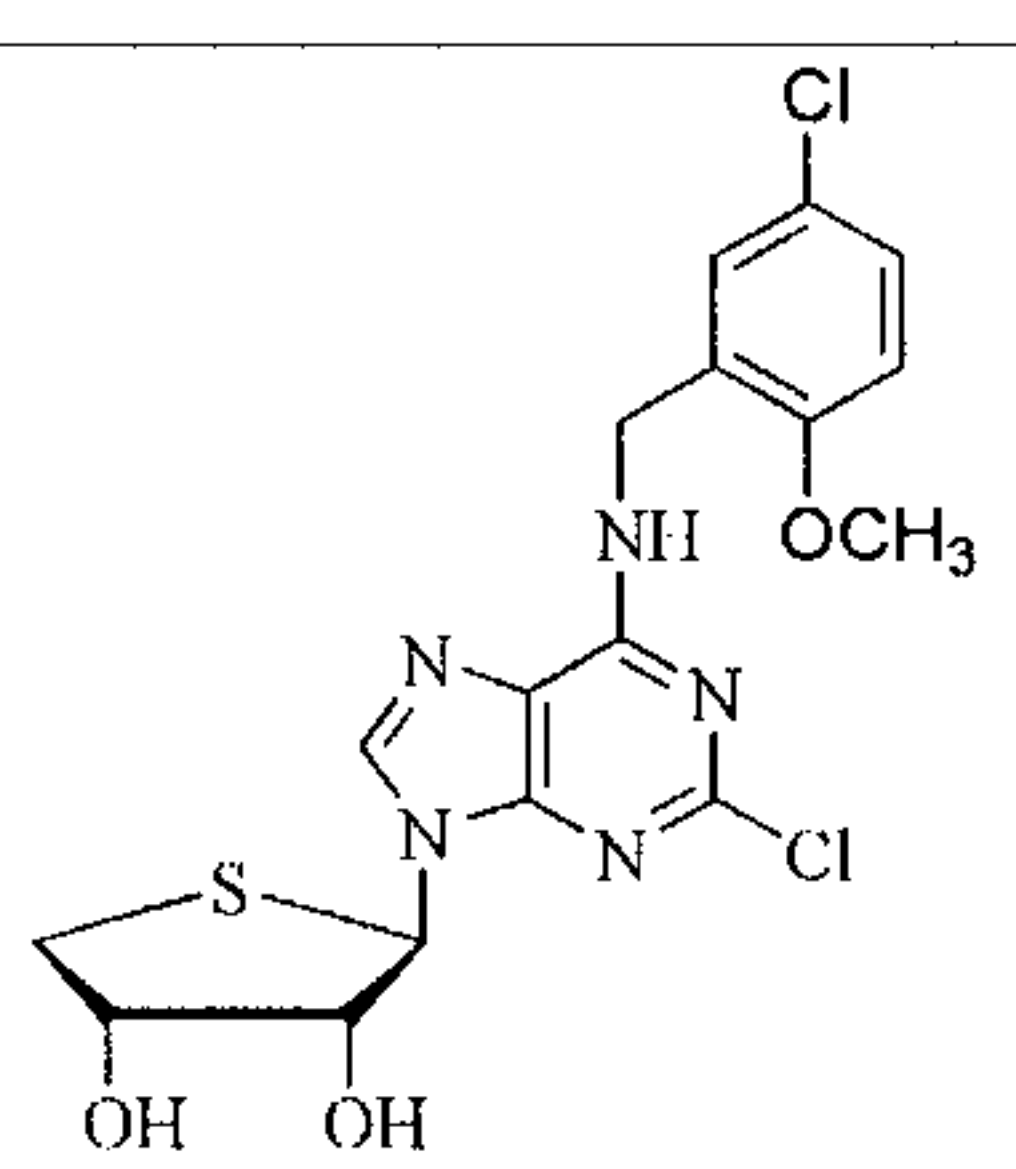
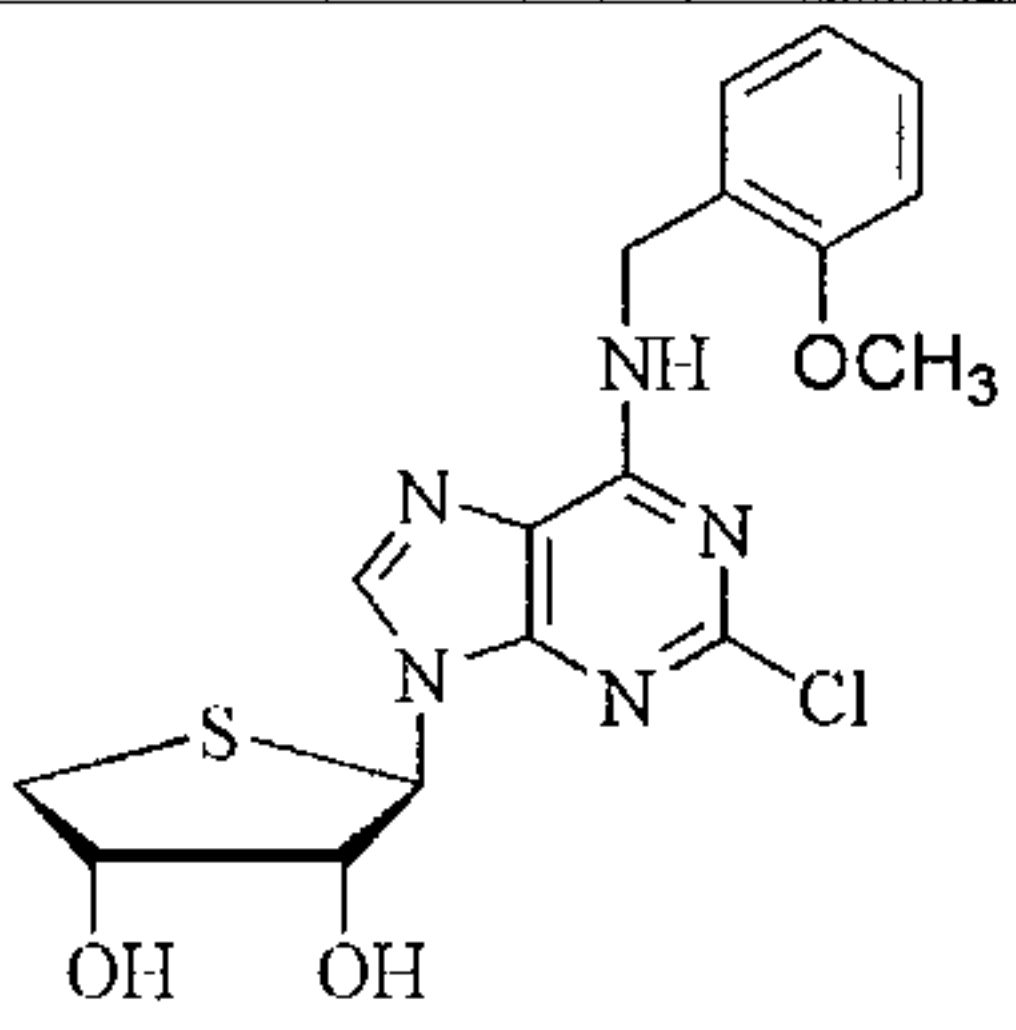
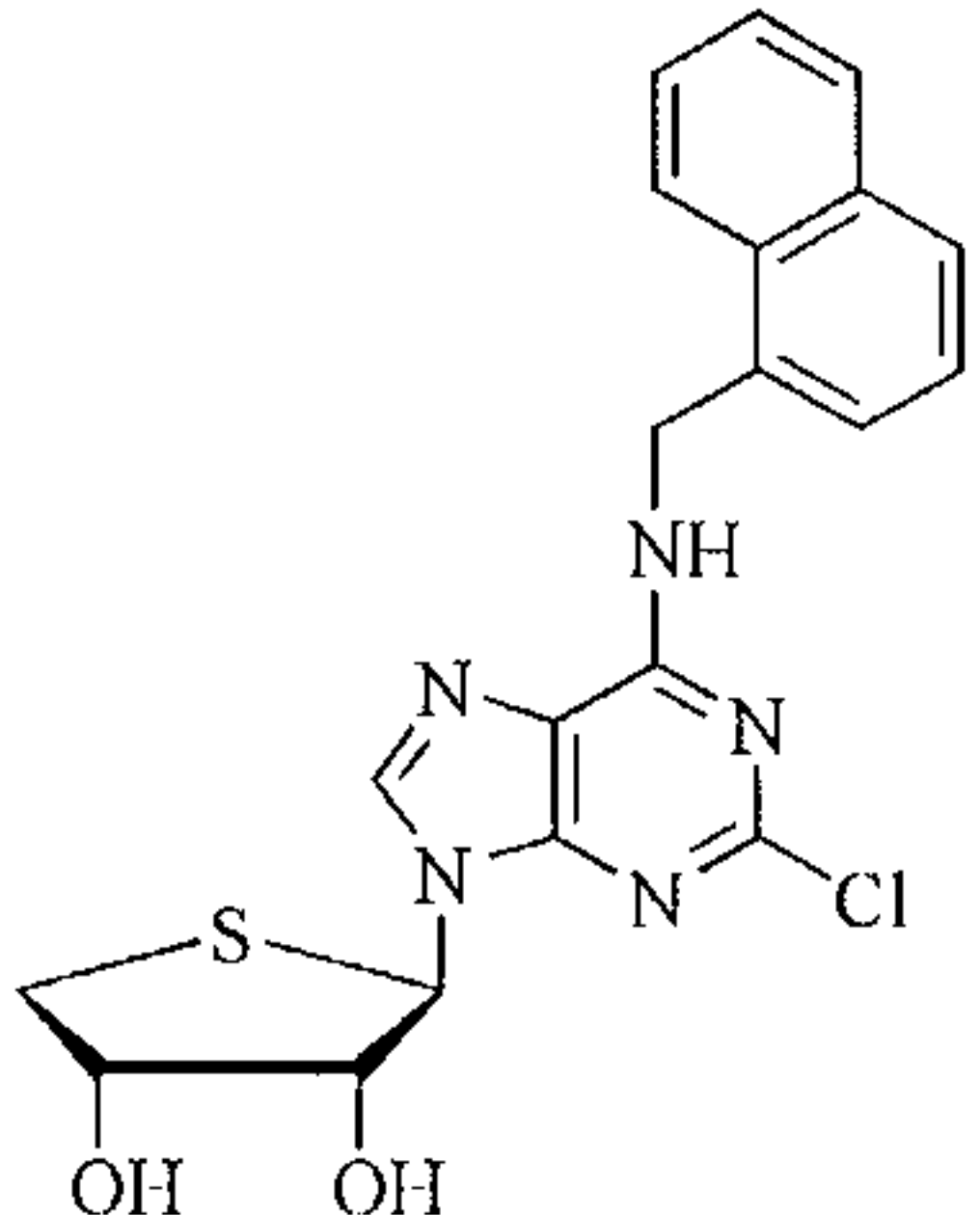
basis of the specific binding values, the samples were analyzed for binding affinity to various adenosine receptors.

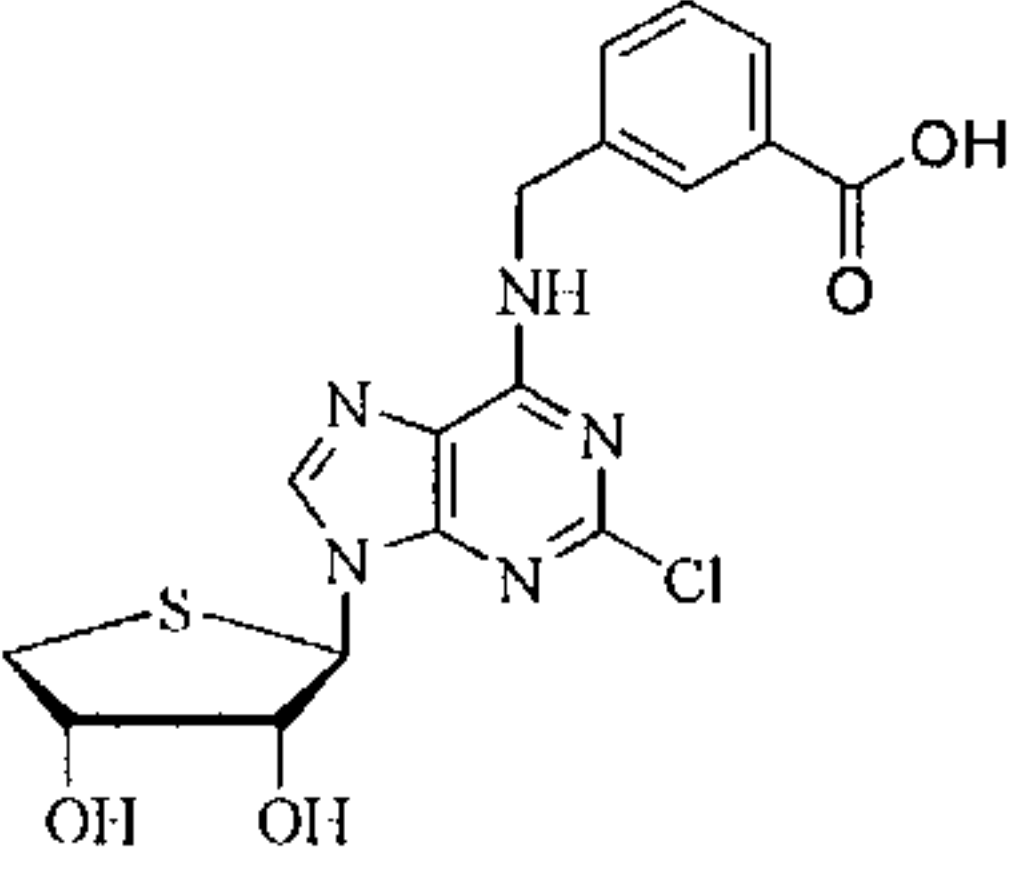
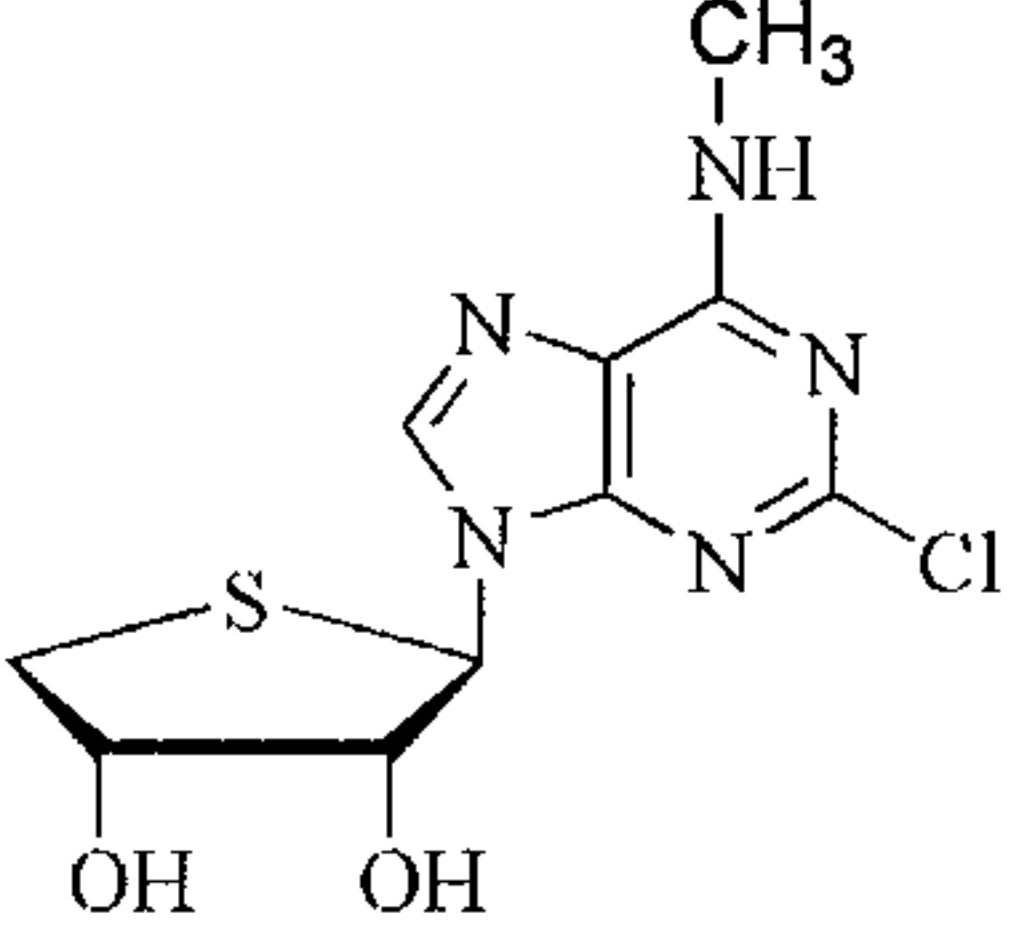
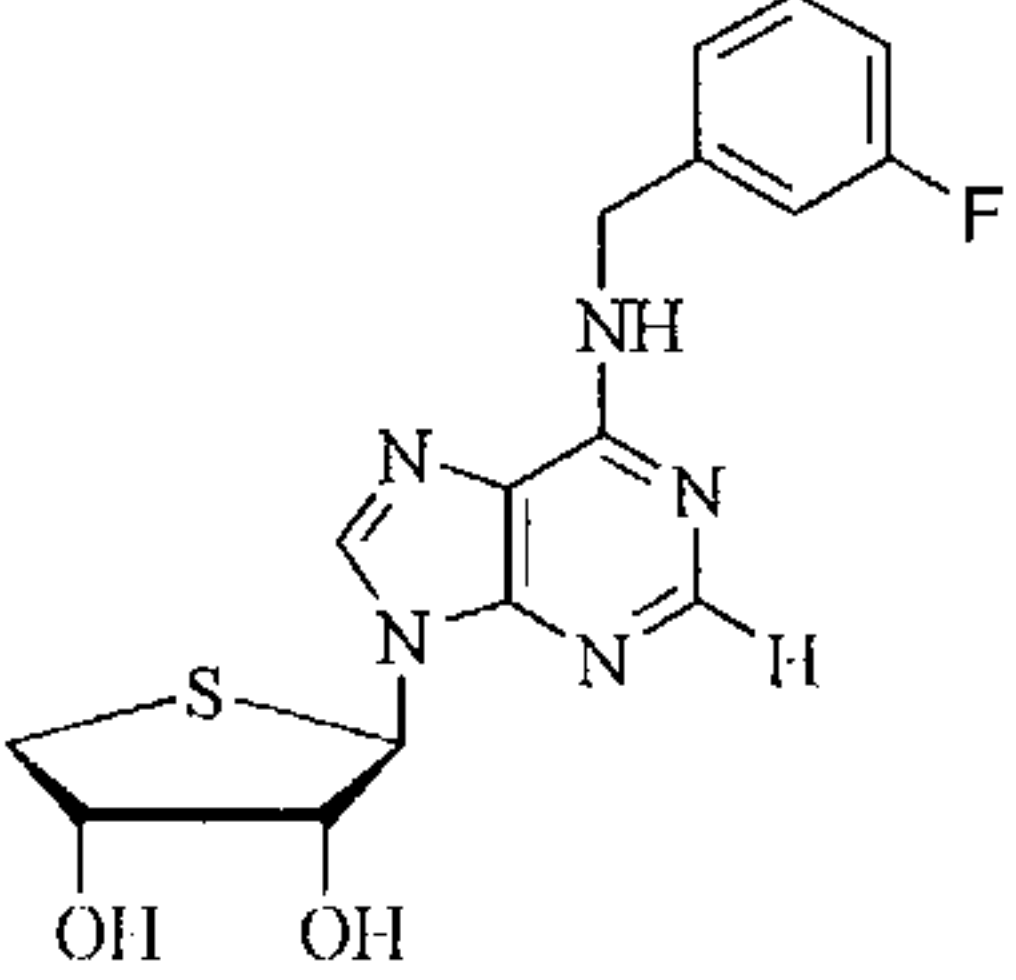
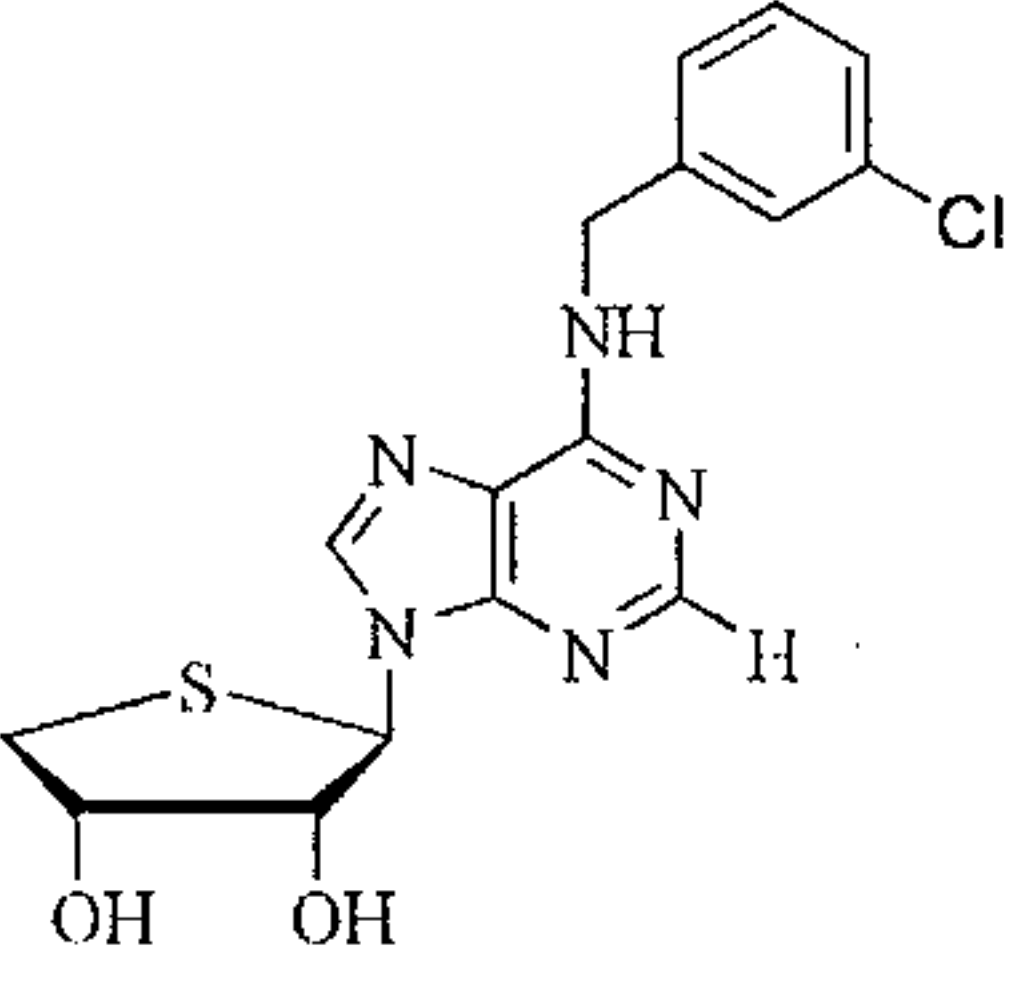
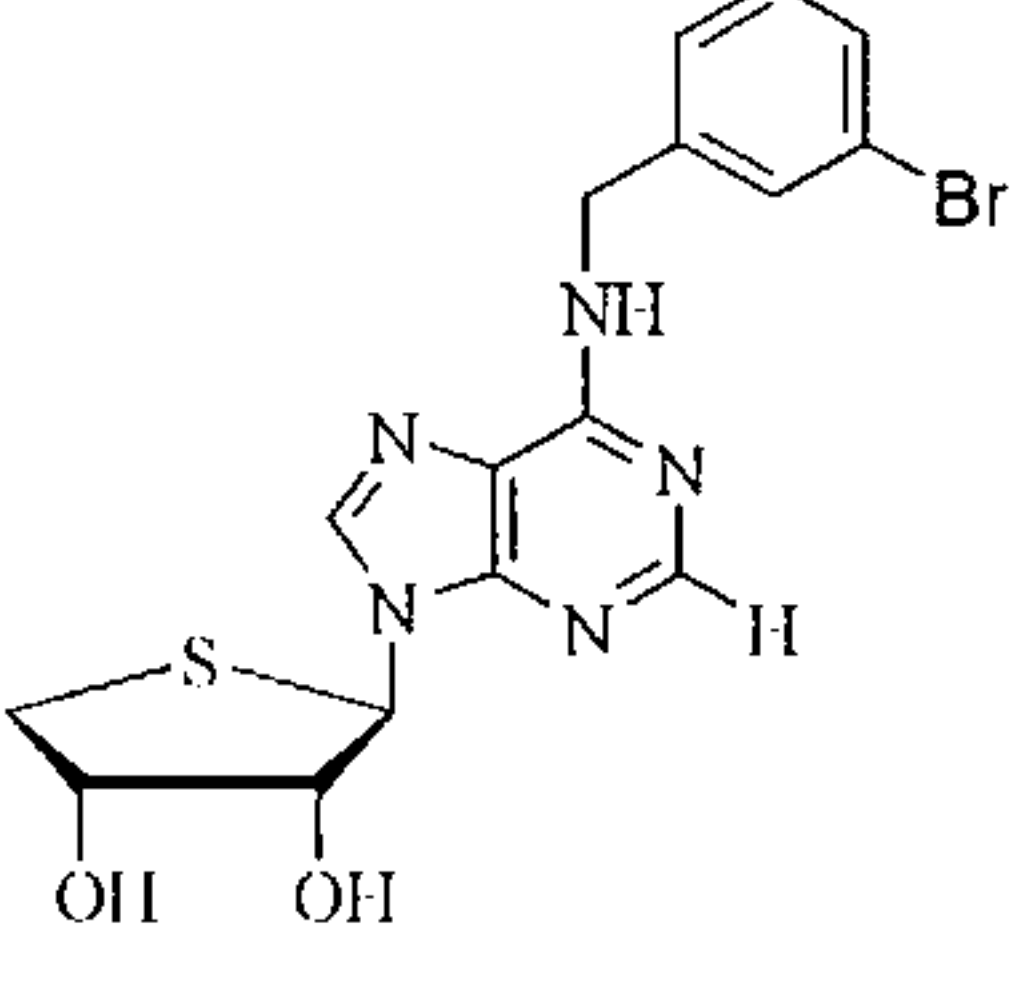
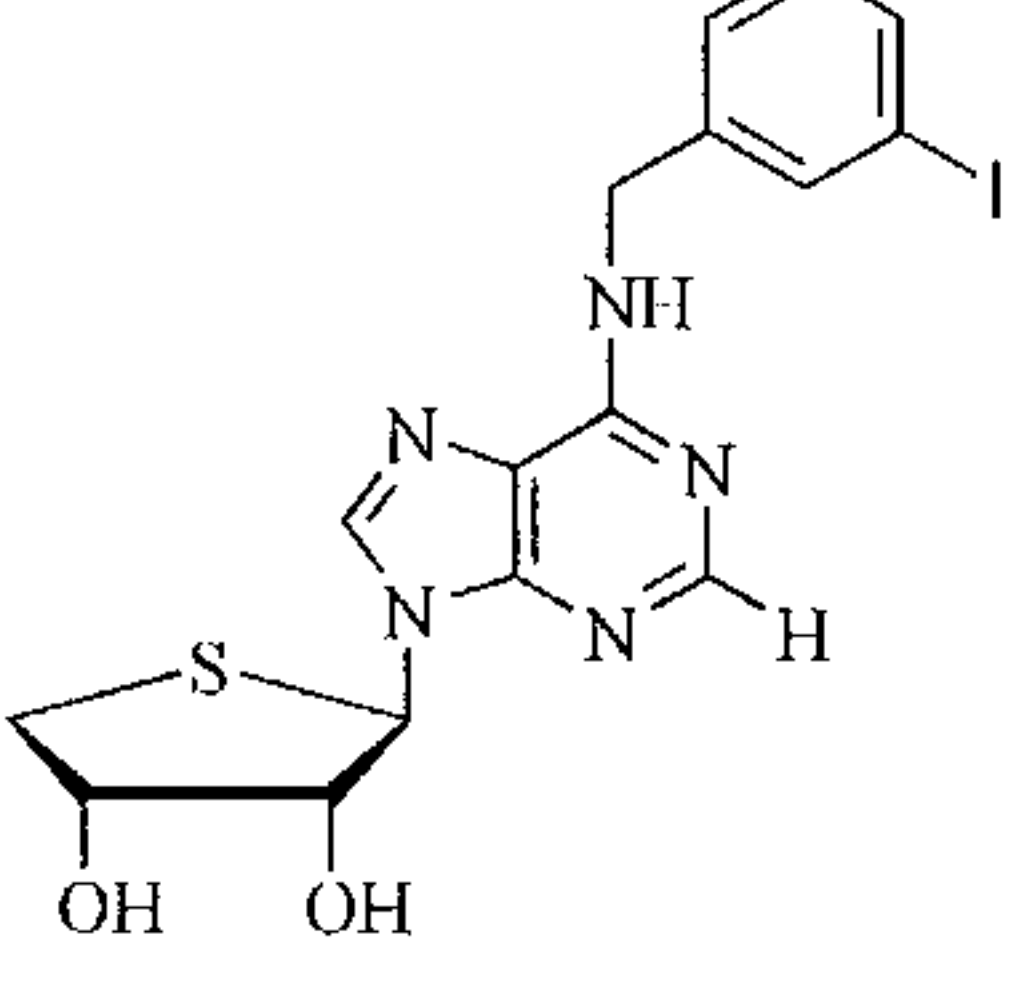
In addition, the binding of the labeled ligand [³H]CGS-21680 (2-(((4-(2-carboxyethyl)phenyl)ethylamino)-5'-N-ethylcarbamoyl)adenosine) to the A_{2A} adenosine receptor expressed on HEK 293 cell was assayed as follows. Adenosine deaminase was added alone or in combination with a radioactive ligand when cerebral meninges were incubated at 30°C for 30 min. Each of the compounds synthesized in the examples was measured for IC₅₀ at at least 6 different concentrations, and the measurements were analyzed using PLAT? software to determine K_i values. Chemical Structures of the compounds synthesized in the examples, substituents, and K_i values for binding affinity are summarized in Table 1, below.

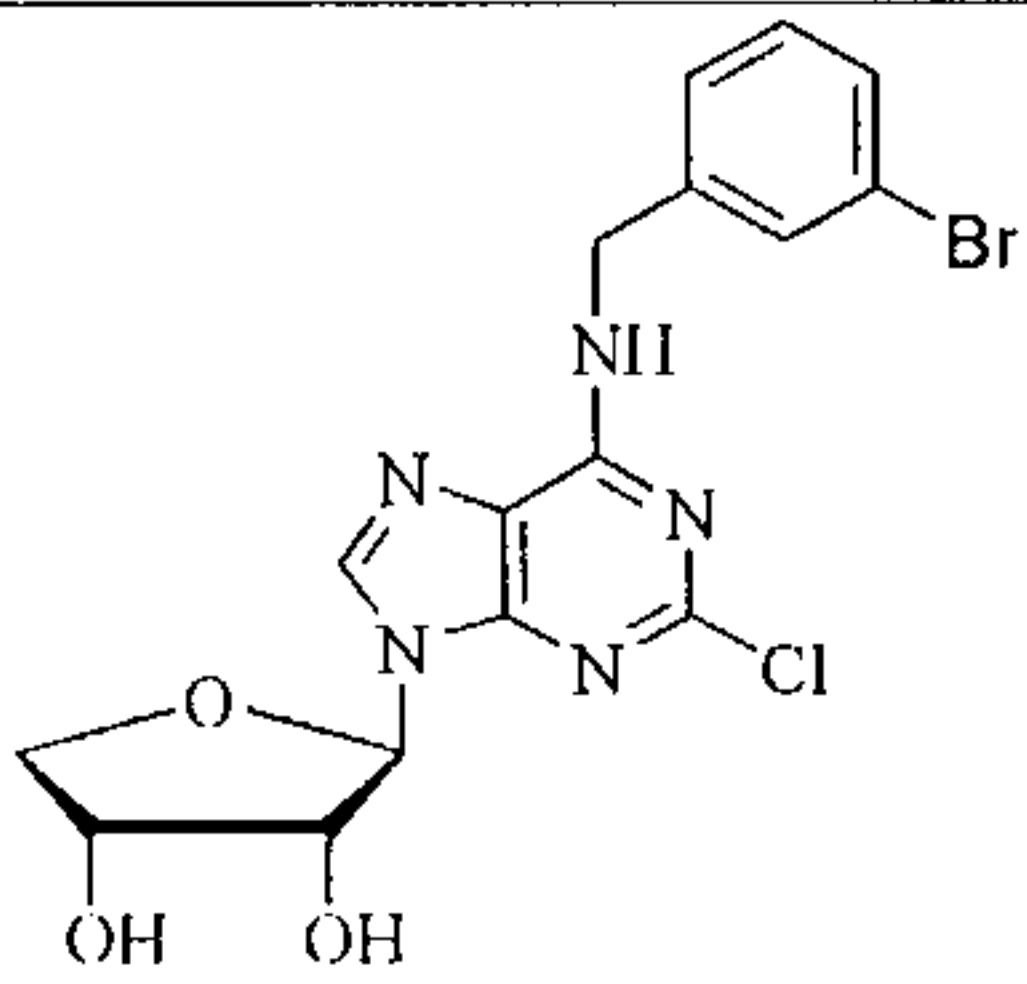
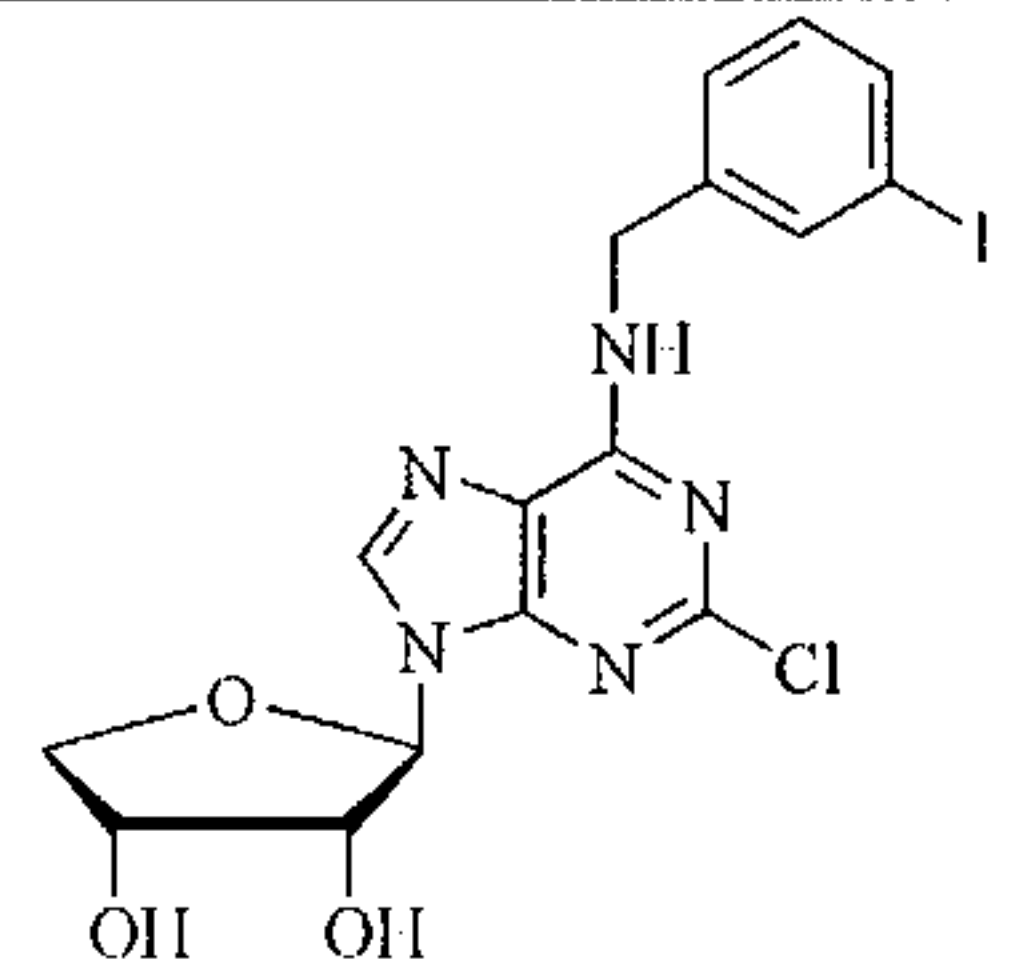
15

<TABLE 1>

Ex. No.	Structures	Substituents			K _i (nM) or %		
		A	R	Y	hA ₁	hA _{2A}	hA ₃
1		S	3-fluorobenzyl	Cl	19.8%	47.6%	7.4±1.3
2		S	3-chlorobenzyl	Cl	37.9%	17.7%	1.66±0.90

3		S	3-bromobenzyl	Cl	34.2%	18.4%	8.99±5.17
4		S	3-iodobenzyl	Cl	2490 ± 940	341 ± 75	4.16±0.50
5		S	2-chlorobenzyl	Cl	12.8%	1600 ± 135	25.8±6.3
6		S	5-chloro-2-methoxybenzyl	Cl	23.8%	4020 ± 1750	12.7±3.7
7		S	2-methoxybenzyl	Cl	9.4%	17.5%	19.9±7.1
8		S	1-naphthylmethyl	Cl	22.0%	-8.3%	24.8±8.1

9		S	3-toluic acid	Cl	13.1%	-0.18%	41.5%
10		S	methyl	Cl	55.4±1.8%	45.0±1.4%	3.69±0.25
11		S	3-fluorobenzyl	H	1430±420	1260±330	7.3±0.6
12		S	3-chlorobenzyl	H	860±210	440±110	1.5±0.4
13		S	3-bromobenzyl	H	790±190	420±32	6.8±3.4
14		S	3-iodobenzyl	H	530±97	230±65	2.5±1.0

15		O	3-bromobenzyl	Cl	39.8%	22.8%	13.0±6.9
16		O	3-iodobenzyl	Cl	37.7%	28.6%	42.9±8.9

Unit: nM ± SEM

"%" represents percentage inhibition of specific binding of 10 μM labeled ligand in the presence of 10 μM of the unlabeled ligand NECA.

As can be understood from the data of Table 1, the compounds synthesized in the examples of the present invention were found to have high binding affinity for human A₃ adenosine receptors (hA₃ AR), but low affinity for A₁ and A_{2A} adenosine receptors, thereby showing high selectivity. Particularly, the compound of Example 12 shows the highest binding affinity for hA₃ AR, with K_i determined to be 1.50±0.40 nM, followed by the compound of Example 2 (K_i = 1.66±0.90 nM), the compound of Example 14 (K_i = 2.50±1.00 nM), the compound of Example 10 (K_i = 3.69±0.25 nM) and the compound of Example 4 (K_i = 4.16±0.50 nM) in decreasing order of binding affinity. Also, the compound of Example 4 was measured to have high binding affinity for the rat A₃ adenosine receptor expressed in CHO cells (K_i = 3.89±1.15 nM)

and was not observed as an agonist or antagonist on human A_{2B} adenosine receptor.

In addition, the compounds having halobenzyl
5 substituents were found to have binding affinity in decreasing order of Cl > I > F > Br. The compound of Example 2, having 3-chlorobenzyl, had higher binding affinity for hA₃ adenosine receptor than the compound of Example 5, having 2-chlorobenzyl ($K_i = 25.8 \pm 6.3$ nM). In addition, the adenosine derivatives
10 having a substituent at the 3-position of the benzene ring in accordance with the present invention had stronger binding affinity for hA₃ AR than the adenosine derivatives, having a substituent at the 2- or 4-position, or two substituents at the 2- and 5-position. The compounds of Examples 15 and 16,
15 both adenosine derivatives having 4'-O oxonucleoside forms, also had high binding affinity and selectivity, which were, however, not superior to those of the corresponding 4'-S thionucleoside forms, such as those of Examples 3 and 4. The compounds of Examples 10 to 14, in which the chloro group at
20 the 2-position of the purine base was substituted with a hydrogen atom, were observed to exceed the 2-chloro compounds with regard to binding affinity and selectivity.

<EXPERIMENTAL EXAMPLE 2> Antagonist Effect of Adenosine
25 Derivatives on A₃ Adenosine Receptors and cAMP Inhibition

In order to examine whether the derivatives of the present invention are effective as human A₃ adenosine receptor antagonists, an assay for antagonism and cAMP inhibition was conducted by treating CHO cells with the compound of Example 4
5 and Cl-IB-MECA.

When CHO cells, in which human A₃ adenosine receptor was expressed, were treated with various concentrations of the compound of Example 4, as seen in FIG. 1, the agonist effect
10 of the 100% pure agonist Cl-IB-MECA was observed to be inhibited in a dose-dependent manner, indicating that the compound of the present invention competes with Cl-IB-MECA for the same receptor binding site. Results of a test for human A₃ adenosine receptor-mediated cAMP inhibition in the CHO
15 cells demonstrates that the compounds synthesized in the examples of the present invention are 100% pure A₃ adenosine receptor antagonists. Thus, the compounds synthesized according to the present invention are found to exhibit a dissociation constant K_B of 1.92 nM, as measured using Schild
20 analysis.

<EXPERIMENTAL EXAMPLES 3 TO 6> Anti-Inflammatory
Activity of Adenosine Derivatives

The adenosine derivatives of the present invention were
25 examined for anti-inflammatory activity in the following

animal test. Seven-week-old male ICR mice were treated with TPA (12-O-tetradecanoylphorbol 13-acetate, 20 µl) in the right ear. Within 15 minutes, the compounds of Examples 1 to 16 were diluted at a concentration of 0.5% in acetone (20 µl),
 5 distilled water, or mixtures of DMSO and acetone (compositions shown in Tables 2 to 5) before being administered to the mice. Hydrocortisone was used at the same concentration as a control.

6 hrs after treatment with TPA, the mice were
 10 secondarily treated with the adenosine derivatives of the present invention. 24 hrs after TPA treatment, test animals were euthanized using a cervical dislocation method. Samples were obtained from the right ear using a 6 mm diameter punch. The activity was observed by measuring the ear sample using a
 15 microbalance. Percentages of inhibition were calculated using the following Equation 1. The compositions and amounts used in these experiments are summarized in Tables 2 to 5 and the anti-inflammatory activities thereof are shown in FIGS. 2 to 5.

20

【Equation 1】

$$\% \text{ Inhibition} = \frac{1 - \text{Rt.Ear (Test - Non treated)}}{\text{Rt.Ear (TPA only - Non treated)}}$$

<TABLE 2>

Exp. Ex.3	Compositions	Amounts
3-1	Non-treated	-
3-2	TPA alone	20 μ l
3-3	TPA + acetone	20 μ l + 20 μ l
3-4	TPA + acetone + Cpd. Of Ex. 2	20 μ l + 0.5%/20 μ l
3-5	TPA + acetone + Cpd. Of Ex. 3	20 μ l + 0.5%/20 μ l
3-6	TPA + acetone + Cpd. Of Ex. 4	20 μ l + 0.5%/20 μ l
3-7	TPA + acetone + hydrocortisone	20 μ l + 0.5%/20 μ l

<TABLE 3>

Exp. Ex.4	Compositions	Amounts
4-1	Non-treated	-
4-2	TPA alone	20 μ l
4-3	TPA + acetone	20 μ l + 20 μ l
4-4	TPA + acetone + Cpd. Of Ex. 1	20 μ l + 0.5%/20 μ l
4-5	TPA + acetone + Cpd. Of Ex. 6	20 μ l + 0.5%/20 μ l
4-6	TPA + acetone + hydrocortisone	20 μ l + 0.5%/20 μ l

5

<TABLE 4>

Exp. Ex.5	Compositions	Amounts
5-1	Non-treated	-
5-2	TPA alone	20 μ l
5-3	TPA + solvent mix (water:acetone 1:4)	20 μ l + 20 μ l
5-4	TPA + solvent mix + Cpd. Of Ex. 5	20 μ l + 0.5%/20 μ l
5-5	TPA + solvent mix + Cpd. Of Ex. 7	20 μ l + 0.5%/20 μ l
5-6	TPA + solvent mix + Cpd. Of Ex. 8	20 μ l + 0.5%/20 μ l
5-7	TPA + solvent mix + hydrocortisone	20 μ l + 0.5%/20 μ l

<TABLE 5>

Exp. Ex. 6	Compositions	Amounts
6-1	Non-treated	-
6-2	TPA alone	20 µl
6-3	TPA + solvent mix (DMSO:acetone 1:9)	20 µl + 20 µl
6-4	TPA + solvent mix + Cpd. Of Ex. 15	20 µl + 0.5%/20 µl
6-5	TPA + solvent mix + Cpd. Of Ex. 16	20 µl + 0.5%/20 µl
6-6	TPA + solvent mix + hydrocortisone	20 µl + 0.5%/20 µl

When applied to the mice, as seen in FIG. 2, dilutions of the compounds of Examples 2 to 4 were found to inhibit the TPA-induced inflammation of the mouse ear to some degree, although this anti-inflammatory activity was very small compared to that of the control hydrocortisone.

The anti-inflammatory activity of the compounds of Examples 1 and 6, as shown in FIG. 3, was measured to be four or more times that of the compounds of Examples 2 to 4.

As seen in FIG. 4, the compounds of Examples 5, 7 and 8, diluted at a concentration of 0.5% in a mixture of distilled water and acetone (1:4), were measured to have percentages of inflammation inhibition of 17%, 34% and 53%, respectively.

As shown in FIG. 5, the compounds of Examples 15 and 16, diluted at a concentration of 0.5% in a mixture of DMSO and acetone (1:9), were measured to have percentages of inflammation inhibition of 59% and 79%, respectively. Based

on the observations in this test, the adenosine derivatives of the present invention were proven to have anti-inflammatory activity.

5 <EXPERIMENTAL EXAMPLE 7> Toxicity Assay

The compounds synthesized in the examples of the present invention were assayed for cytotoxicity in animals. Three test groups of three 25±5 g ICR rats (Central Experiment Animal, Korea) and three test groups of three 235±10 g
10 specific pathogen-free (SPF) Sprague Dawley rats (Central Experiment Animal, Korea) were abdominally administered with the compound of Example 2 at doses of 20 mg/kg, 10 mg/kg, and 1 mg/kg, respectively, followed by observation for 24 hrs.

None of the members of the three groups died. No
15 difference in weight gain or feed intake was detected between the control group and the test groups. Therefore, the derivative compounds of the present invention were proven as being safe.

20 The adenosine compounds of the present invention may be administered in the following dosage forms and the following Formulation Examples are set forth to illustrate, but not limit, the present invention.

25 <FORMULATION EXAMPLE 1> Preparation of Powder

Adenosine Derivative	500 mg
Corn Starch	100 mg
Lactose	100 mg
Talc	10 mg

5 The ingredients were mixed and filled in an airtight bag.

<FORMULATION EXAMPLE 2> Preparation of Tablet

Adenosine Derivative	100 mg
10 Corn Starch	100 mg
Lactose	100 mg
Mg Stearate	2 mg

 The ingredients were mixed and compressed into tablets according to a conventional method.

15

<FORMULATION EXAMPLE 3> Preparation of Capsule

Adenosine Derivative	50 mg
Lactose	50 mg
Mg Stearate	1 mg

20 The ingredients were mixed and filled in gelatin capsules according to a conventional method.

<FORMULATION EXAMPLE 4> Preparation of Injection

Adenosine Derivative	10 mg
25 Sterile Water for injection	suitable amount

pH Adjuster

Suitable amount

The pH of a solution of the active ingredient in distilled water was adjusted to 7.5 and the solution was diluted in sterile water to a volume 2 ml and loaded into 5 ampules before sterilization.

<FORMULATION EXAMPLE 5> Preparation of Liquid Form

	Adenosine Derivative	1 g
	Isomerized Sugar	10 g
10	Sucrose	10 g
	Lemon Flavor	suitable amount
	Pure water	suitable amount

A liquid dosage form was prepared by dissolving the ingredients in pure water, adding a suitable amount of lemon 15 flavor, increasing the volume to 100 ml with pure water, loading the volume into a brown vial, and sterilizing.

Industrial Applicability

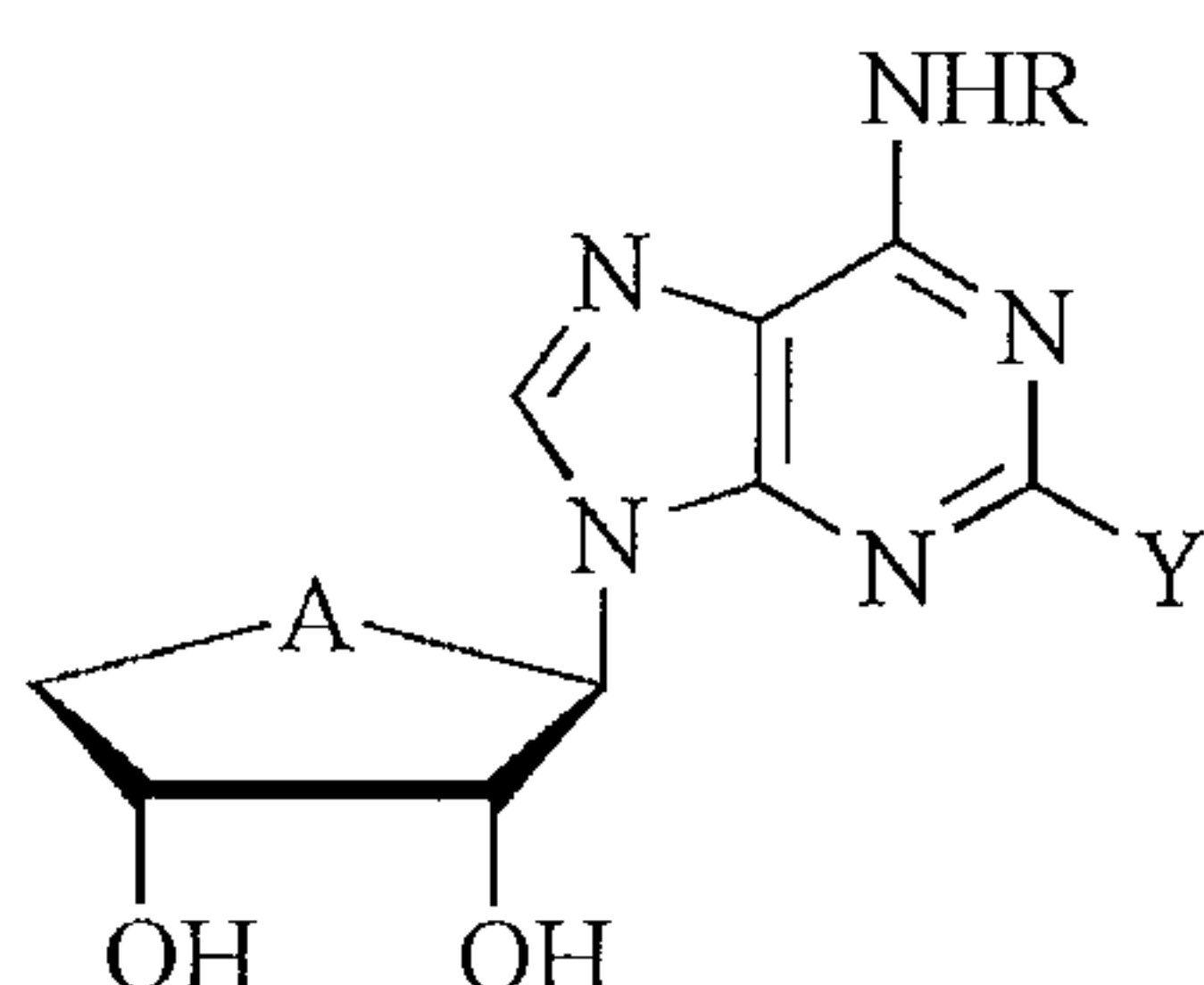
As described hitherto, the adenosine derivatives of the 20 present invention have high binding affinity and selectivity for adenosine receptors, especially for A₃ adenosine receptors, and act as A₃ adenosine receptor antagonists, showing anti-inflammatory activity. Therefore, the adenosine derivatives of the present invention are useful in the 25 prevention and treatment of inflammatory diseases.

Examples are described in terms of the preferred embodiment of present invention. However, it should not be understood that such disclosure is not limited to explicit description of present invention. The description and the
5 claims of present invention are to be interpreted as covering all alterations and modifications within the true scope of this invention.

CLAIMS**【Claim 1】**

An adenosine derivative, represented by the following
Chemical formula 1, or a pharmaceutically acceptable salt
5 thereof:

[Chemical formula 1]



wherein,

10 A is O or S,

R is a linear or branched C₁~C₅ alkyl which is non-substituted or is independently or selectively substituted with one or more C₆~C₁₀ aryl groups, a benzyl which is non-substituted or is independently or selectively substituted
15 with halogen or one or more linear or branched C₁~C₄ alkoxy groups, or a hydroxycarbonyl-substituted benzyl; and

Y is H or a halogen atom.

【Claim 2】

20 The adenosine derivative or pharmaceutically acceptable salt according to claim 1, wherein

A is O or S,

R is methyl, ethyl, propyl, naphthylmethyl, benzyl, benzyl independently or selectively substituted with a substituent selected from a group consisting of F, Cl, Br, I, C₁~C₃ alkoxy and combinations thereof, or toluic acid, and

Y is H or Cl.

【Claim 3】

The adenosine derivative or a pharmaceutically acceptable salt according to claim 1, wherein

A is O or S,

R is methyl, ethyl, 1-naphthylmethyl, benzyl, 2-chlorobenzyl, 3-fluorobenzyl, 3-chlorobenzyl, 3-bromobenzyl, 3-iodobenzyl, 2-methoxy-5-chlorobenzyl, 2-methoxybenzyl, or 3-toluic acid, and

Y is H or Cl.

【Claim 4】

The adenosine derivative or pharmaceutically acceptable salt according to claim 1 or 3, wherein the adenosine derivative is selected from a group consisting of:

- 1) (2*R*, 3*R*, 4*S*)-2-(2-chloro-6-(3-fluorobenzylamino)-9*H*-purin-9-yl)tetrahydrothiophen-3,4-diol;
- 2) (2*R*, 3*R*, 4*S*)-2-(2-chloro-6-(3-chlorobenzylamino)-9*H*-purin-9-yl)tetrahydrothiophen-3,4-diol;

- 3) (2*R*, 3*R*, 4*S*)-2-(6-(3-bromobenzylamino)-2-chloro-9*H*-
purin-9-yl)tetrahydrothiophen-3,4-diol;
- 4) (2*R*, 3*R*, 4*S*)-2-(2-chloro-6-(3-iodobenzylamino)-9*H*-
purin-9-yl)tetrahydrothiophen-3,4-diol;
- 5) (2*R*, 3*R*, 4*S*)-2-(2-chloro-6-(2-chlorobenzylamino)-9*H*-
purin-9-yl)tetrahydrothiophen-3,4-diol;
- 6) (2*R*, 3*R*, 4*S*)-2-(2-chloro-6-(5-chloro-2-
methoxybenzylamino)-9*H*-purin-9-yl)tetrahydrothiophen-3,4-diol;
- 7) (2*R*, 3*R*, 4*S*)-2-(2-chloro-6-(2-methoxybenzylamino)-9*H*-
purin-9-yl)tetrahydrothiophen-3,4-diol;
- 8) (2*R*, 3*R*, 4*S*)-2-(2-chloro-6-(naphthalen-1-
ylmethylamino)-9*H*-purin-9-yl)tetrahydrothiophen-3,4-diol;
- 9) 3-((2-chloro-9-((2*R*, 3*R*, 4*S*)-3,4-
dihydroxytetrahydrothiophen-2-yl)-9*H*-purine-6-
ylamino)methyl)benzoic acid;
- 10) 2-(2-chloro-6-methylamino-purin-9-
yl)tetrahydrothiophen-3,4-diol;
- 11) (2*R*, 3*R*, 4*S*)-2-(6-(3-fluorobenzylamino)-9*H*-purin-9-
yl)tetrahydrothiophen-3,4-diol;
- 12) (2*R*, 3*R*, 4*S*)-2-(6-(3-chlorobenzylamino)-9*H*-purin-9-
yl)tetrahydrothiophen-3,4-diol;
- 13) (2*R*, 3*R*, 4*S*)-2-(6-(3-bromobenzylamino)-9*H*-purin-9-
yl)tetrahydrothiophen-3,4-diol;
- 14) (2*R*, 3*R*, 4*S*)-2-(6-(3-iodobenzylamino)-9*H*-purin-9-
yl)tetrahydrothiophen-3,4-diol;

15) (2*R*, 3*R*, 4*R*)-2-(6-(3-bromobenzylamino)-2-chloro-9*H*-
purin-9-yl)tetrahydrofuran-3,4-diol; and

16) (2*R*, 3*R*, 4*R*)-2-(2-chloro-6-(3-iodobenzylamino)-9*H*-
purin-9-yl)tetrahydrofuran-3,4-diol.

5

【Claim 5】

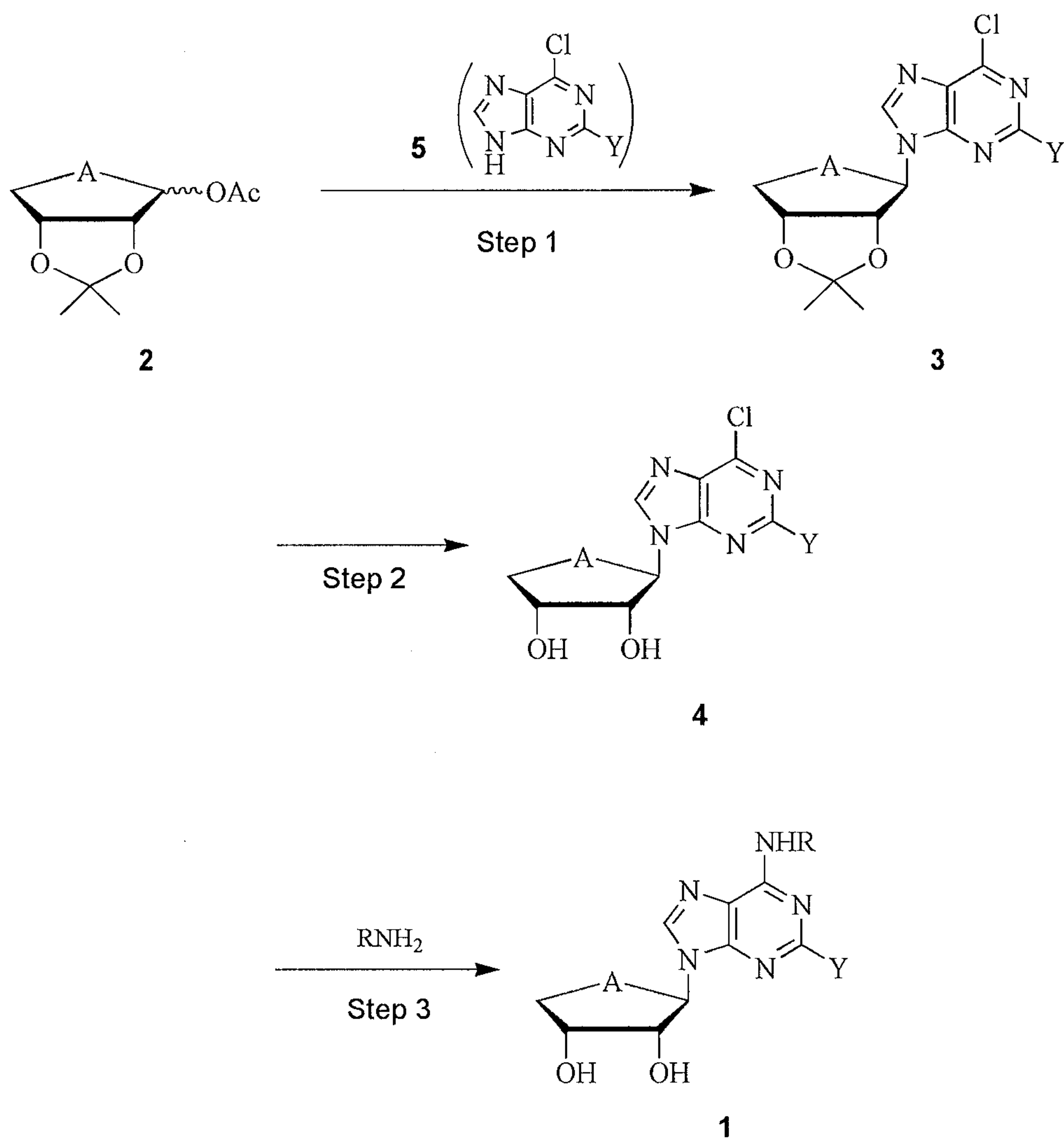
A method for synthesizing the adenosine derivative of
one of claims 1 to 4 or the pharmaceutically acceptable salt
thereof, as represented by the following Scheme 1, comprising:

10 (1) reacting a starting material of Chemical formula 2
with a silylated purine compound in the presence of a Lewis
acid as a catalyst to give a β -anomer;

(2) adding HCl to the compound of Chemical formula 1
obtained in Step (1) to give a diol compound 4 of Chemical
15 formula 4; and

(3) reacting the diol compound of Chemical formula 4
obtained in Step (2) with an amine compound in the presence of
a base as a catalyst to give an adenosine compound of Chemical
formula 1.

20 [Scheme 1]



(wherein, A, R and Y are as defined in Chemical formula 1.)

5 【Claim 6】

The method according to claim 5, wherein Step (1) is conducted in a solvent selected from a group consisting of dichloroethane, chloroform, acetonitrile and dichloromethane.

10 【Claim 7】

The method according to claim 5, wherein the Lewis acid of Step (1) is trimethylsilyl trifluoromethanesulfonate.

【Claim 8】

5 The method according to claim 5, wherein the silylated purine compound of Step 1 is prepared by reacting a purine compound of Chemical formula 5 with hexamethylsilazane in the presence of ammonium sulfate as a catalyst.

10 【Claim 9】

The method according to claim 5, wherein the catalyst of Step (3) is selected from a group consisting of triethylamine, pyridine, N,N-dimethylaminopyridine and 1,4-dioxane.

15 【Claim 10】

The method according to claim 5, wherein the starting material of Chemical formula 2 is prepared, as represented by the following Scheme 2, by:

(a₁) reacting D-mannose of Chemical formula 6 with 2,2-dimethoxypropane in the presence of an acid as catalyst to
20 give a diacetonide compound of Chemical formula 7;

(a₂) subjecting the compound of Chemical formula 7 obtained in step (a₁) to ring opening in the presence of a reducing agent to give a diol compound of Chemical formula 8;

25 (a₃) mesylating the compound of Chemical formula 8

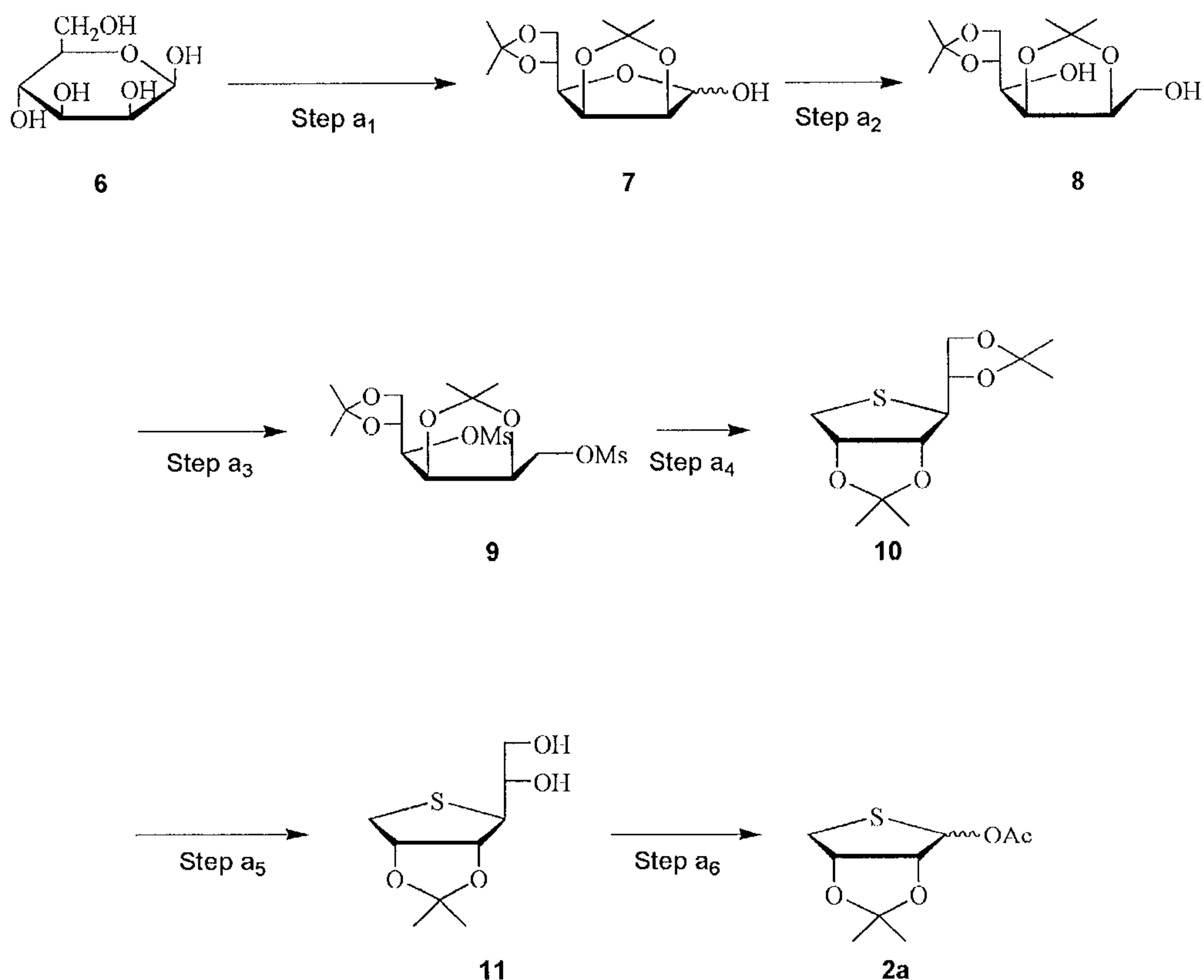
obtained in step (a₂) into a dimesyl compound of Chemical formula 9;

(a₄) cyclizing the compound of Chemical formula 9 obtained in step (a₃) into a thiosugar compound of Chemical formula 10;

(a₅) selectively hydrolyzing the compound of Chemical formula 10 obtained in step (a₄) into a diol compound of Chemical formula 11; and

(a₆) converting the compound of Chemical formula 11 obtained in step (a₅) in the presence of a catalyst into an acetate compound of Chemical formula 2a.

[Scheme 2]



(wherein compound 2a is identical to the compound of Chemical formula 2.)

【Claim 11】

5 The method according to claim 10, wherein the catalyst of step (a₁) is selected from a group consisting of conc. sulfuric acid, hydrogen chloride gas, and p-toluene sulfonic acid.

10 **【Claim 12】**

 The method according to claim 10, wherein the reducing agent of step (a₂) is selected from a group consisting of sodium borohydride, lithium aluminum hydride and sodium sulfite.

15

【Claim 13】

 The method according to claim 10, wherein the step (a₃) is conducted in the presence of methanesulfonyl chloride as a methylating agent.

20

【Claim 14】

 The method according to claim 10, wherein the step (a₄) is conducted in a solvent selected from a group consisting of ethyl ether, petroleum ether, dichloromethane, tetrahydrofuran
25 and N,N-dimethylformamide.

【Claim 15】

The method according to claim 10, wherein the step (a₅) is conducted in the presence of an acid selected from a group consisting of acetic acid, sulfuric acid, hydrochloric acid, and p-toluene sulfonic acid.

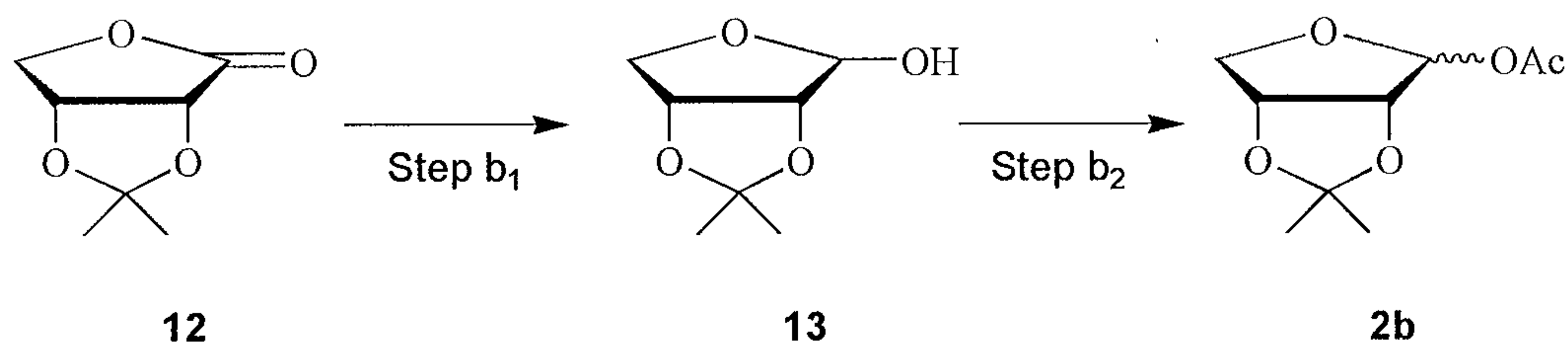
【Claim 16】

The method according to claim 5, wherein the starting material of Chemical formula 2 is prepared, as represented by the following Scheme 3, by:

(b₁) reacting a compound of Chemical formula 12 with a reducing agent into a lactol compound of Chemical formula 13; and

(b₂) reacting the compound of Chemical formula 13, obtained in step (b₁), with anhydrous acetic acid to give an acetate compound of Chemical formula 2b.

[Scheme 3]



(wherein Compound 2b is the compound of Chemical formula 2.)

【Claim 17】

The method according to claim 16, wherein the reducing agent of step (b₁) is diisobutylammonium hydride.

5 **【Claim 18】**

An A₃ adenosine receptor antagonist, comprising the adenosine derivative, represented by Chemical formula 1, of claim 1 or a pharmaceutically acceptable salt thereof.

10 **【Claim 19】**

A pharmaceutical composition for prevention and treatment of an inflammatory disease, comprising the adenosine derivative, represented by Chemical formula 1, of claim 1, or a pharmaceutically acceptable salt thereof.

15

【Claim 20】

The pharmaceutical composition according to claim 19, wherein the inflammatory disease is selected from a group of inflammation, exudative inflammation, purulent inflammation, hemorrhagic inflammation, hyperplastic inflammation, and combinations thereof.

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FIGURES

Fig. 1

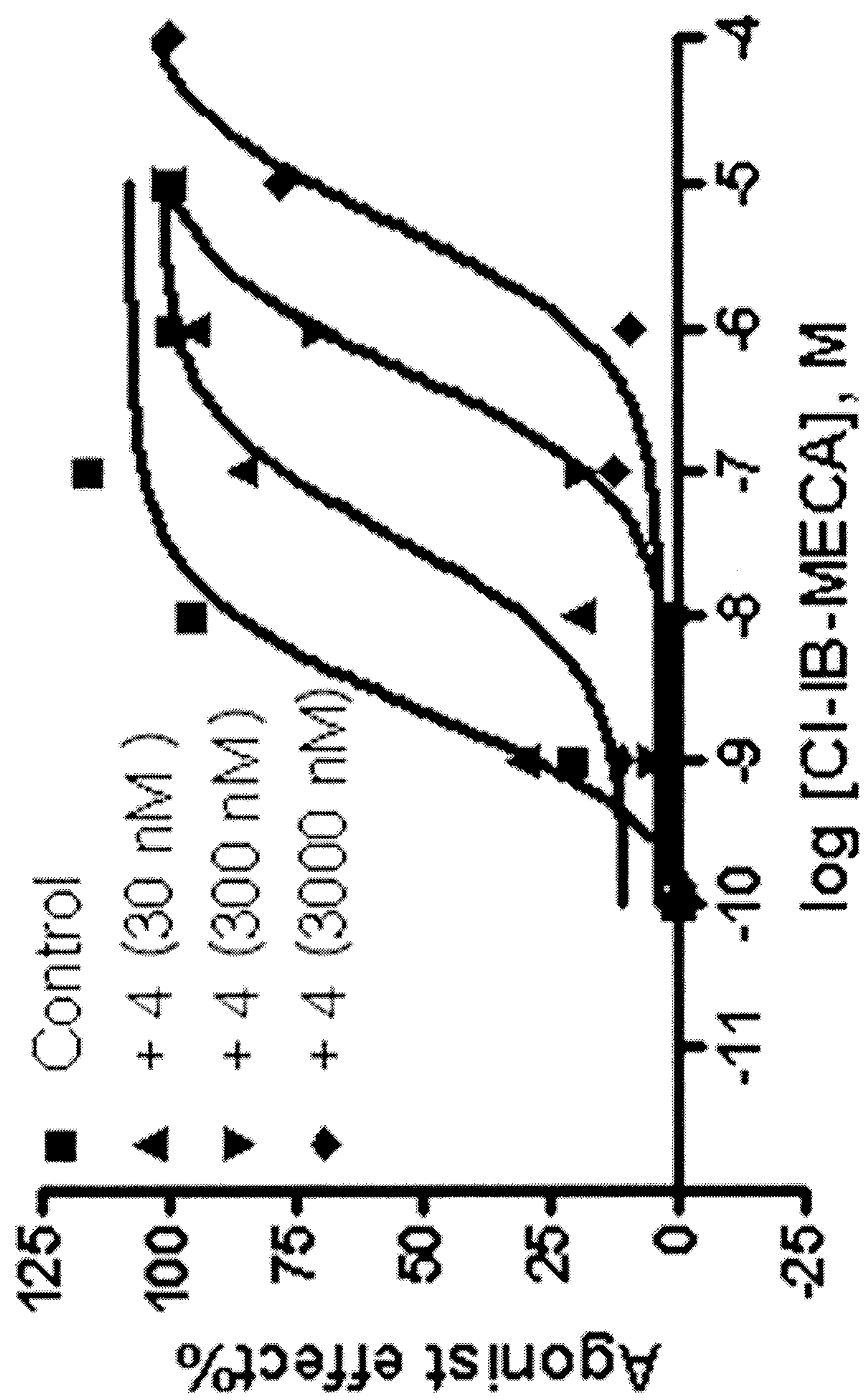
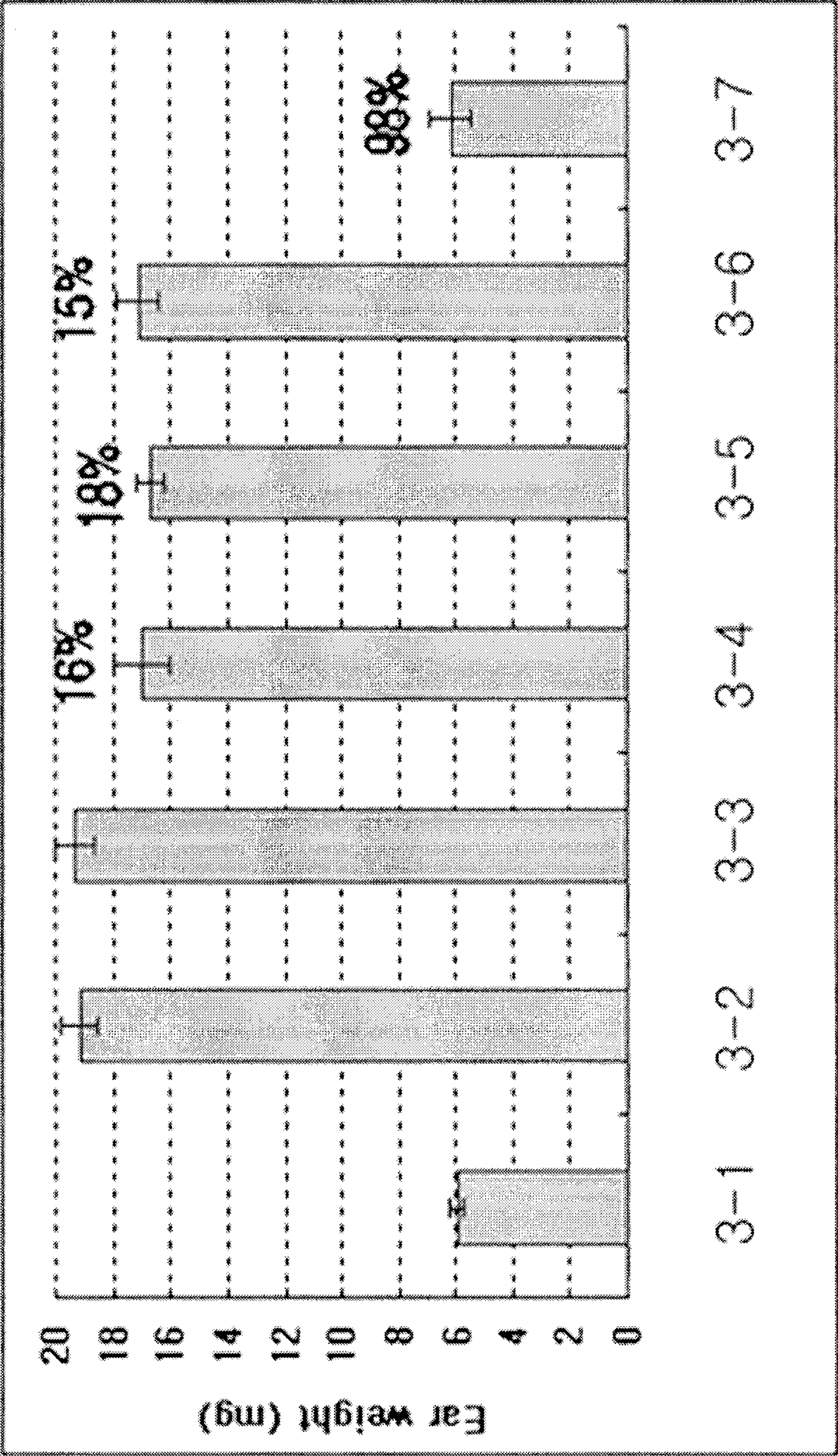
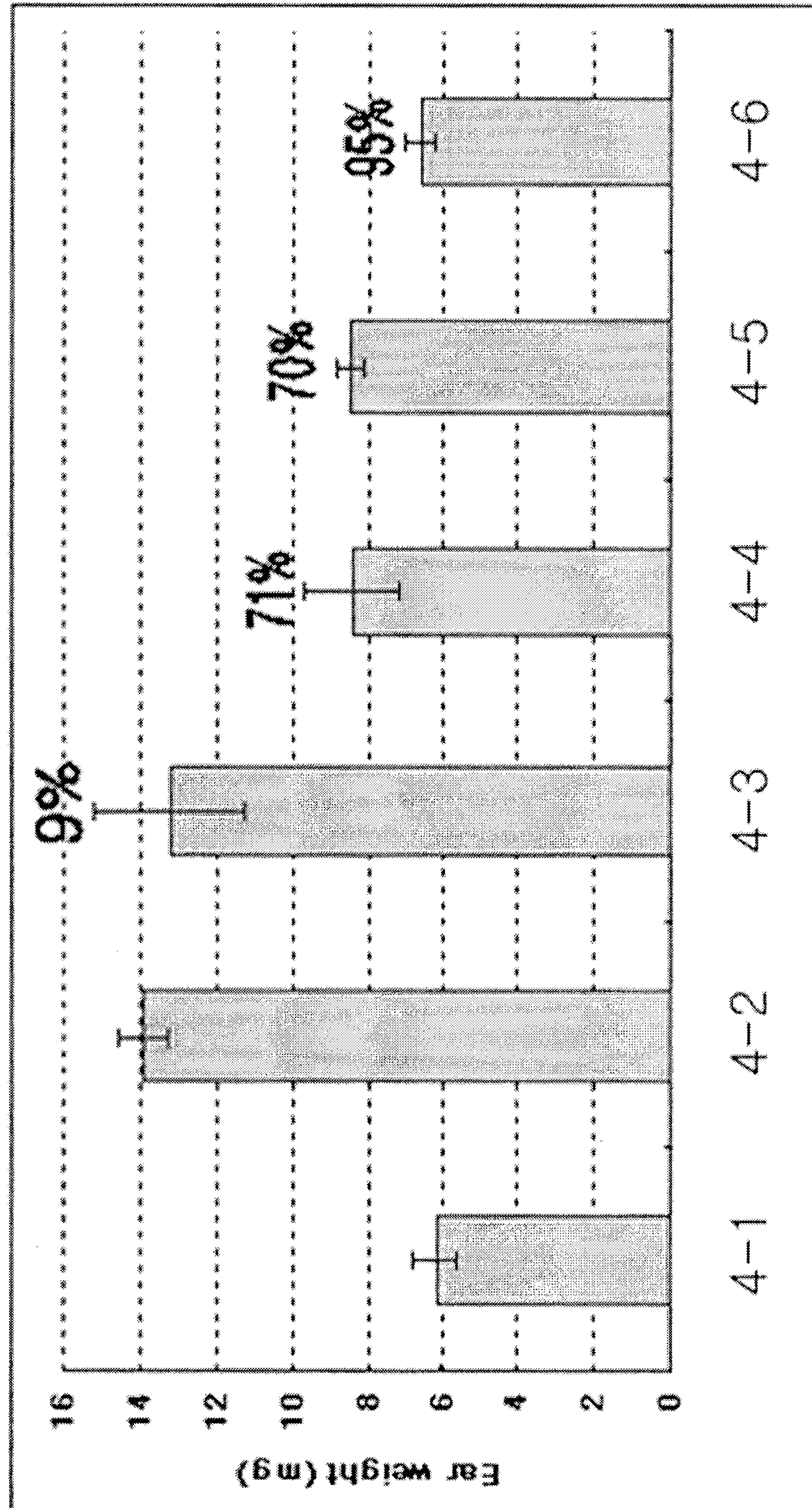


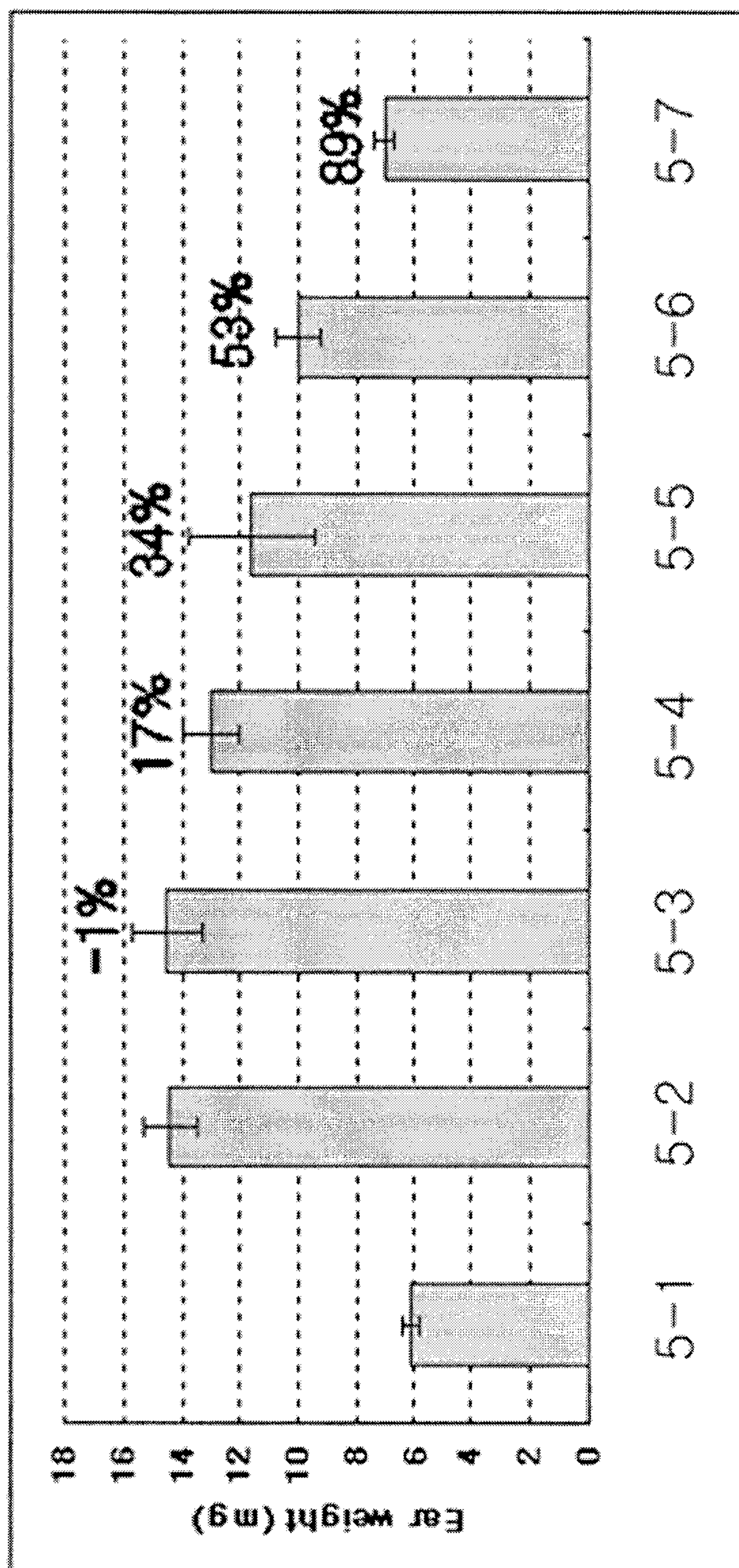
Fig. 2



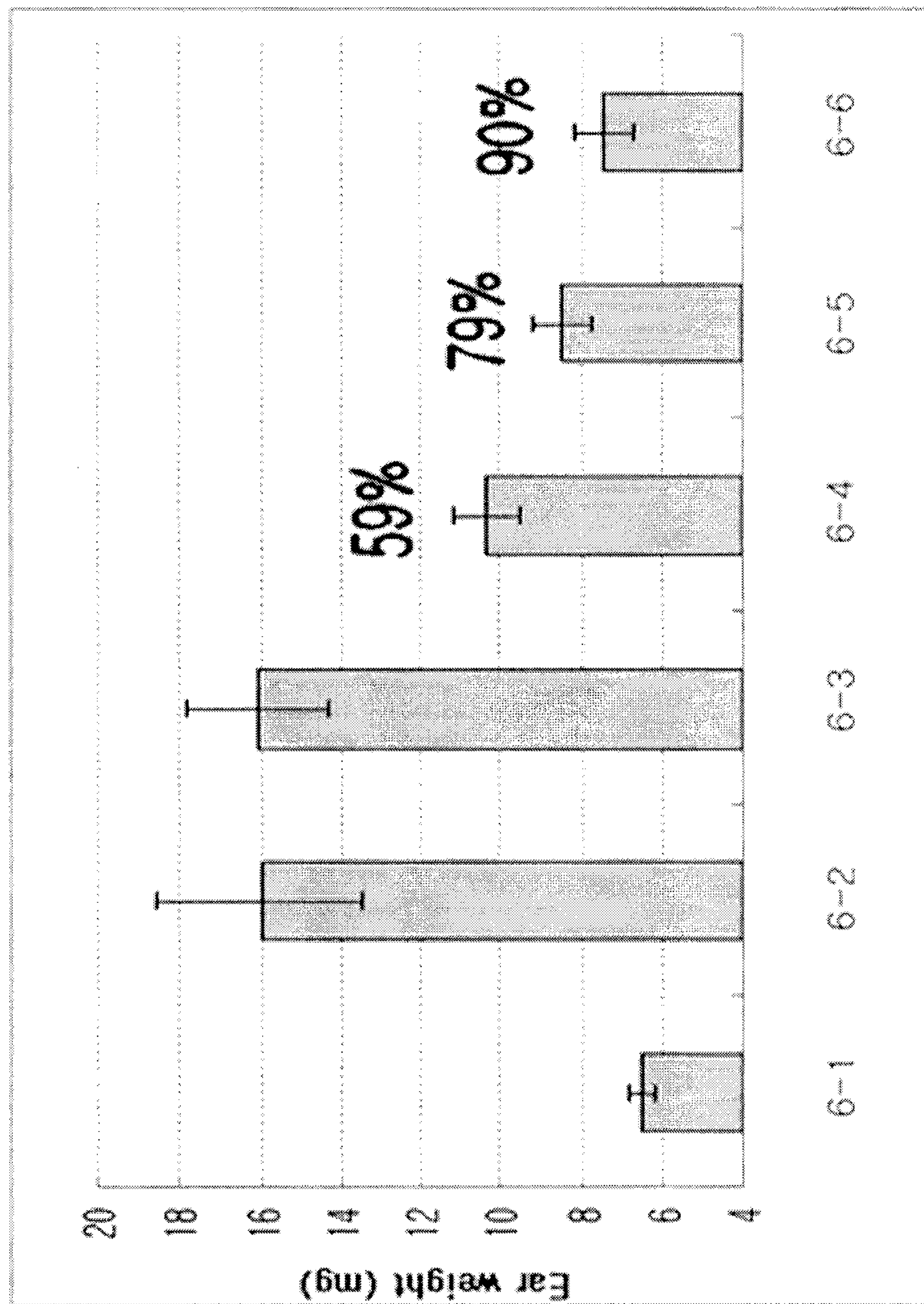
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Fig. 3

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Fig.4

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Fig. 5

[Fig. 1]

