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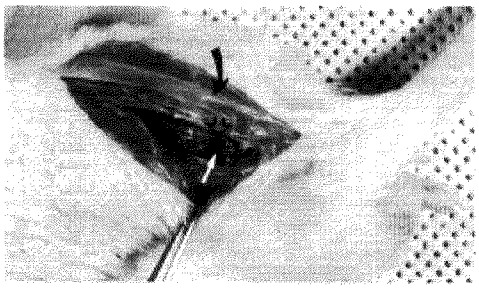
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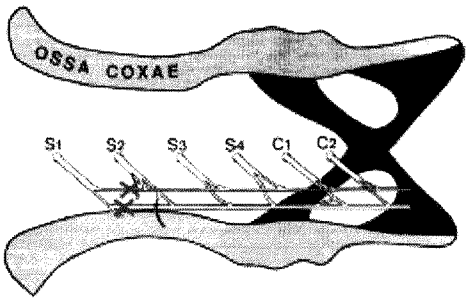
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(54) **Title:** PAIN-RELIEVING COMPOSITION





(57) **Abstract:** This invention relates to a composition for relieving pain, comprising the compounds of formula (I) or pharmaceutically acceptable salts thereof as an effective substance. The composition of the present invention relieves pain, particularly neuropathic pain and inflammatory pain, and so is useful for preventing or treating pain not controlled by previous analgesics.

WO 2007/108566 A1

PAIN-RELIEVING COMPOSITION

TECHNICAL FIELD

This invention relates to a composition for relieving pain.

BACKGROUND ART

Pain is defined as unpleasant sensory and emotional experience related to real or potential injury to tissues. Pain is a main symptom in various diseases, but its perception is very subjective. Thus, pain is one of the most difficult pathologies that are difficult to diagnose and treat effectively. Pain gives severe damage to functional ability, and threatens the patient's work, social life, and family life.

Pain is classified into acute pain, and chronic pain such as neuropathic pain.

Acute pain is generally caused from inflammation and soft tissues damage. Neuronic mechanism causing acute pain is well known in the art, and so the pain can be treated by analgesics such as nonsteroidal anti-inflammatory drug (NSAID), opium products, etc., currently in market.

Neuropathic pain is caused by injury or infection to peripheral sensory nerves, and is very difficult to treat because the degree of pain is extremely severe, and the pain frequently recurs despite use of common analgesics like opium products.

Neuropathic pain may be caused by various diseases, such as herpes virus infection, diabetes mellitus, causalgia, plexus avulsion, neuroma, limb amputation, and vasculitis, in addition to peripheral sensory nerves damage.

And, neuropathic pain is also caused by nerve damage from chronic alcoholism, human immunodeficiency virus infection, hypothyroidism, uremia, or vitamin deficiencies.

Neuropathic pain may be classified into three types; hyperalgesia, allodynia, and

spontaneous pain.

"Hyperalgesia" is an excessive pain above a normal level. "Allodynia" is a pain caused by delicate response to such a slight extent of stimuli that normally cannot induce pain. And, "spontaneous pain" is a pain felt though there is no stimulus.

For example, allodynia includes mechanical allodynia that is a pain felt by weak mechanical stimuli, and cold/warm allodynia that is a pain felt by a certain range of temperature change that normally does not feel unpleasantness. There have been continuous studies to develop a substance that can prevent, control, and treat these various pains.

Analgesics currently in market are classified into two kinds: one is non steroidal anti-inflammatory drug (NSAID) and COX-2 inhibitors related thereto, and the other is opium products like morphine. These analgesics are effective for controlling common pain. But, they are little effective against a certain type of pain like neuropathic pain. Only, some pain-relieving effect may be achieved by administrating a high dose of opiates, which could cause serious side effects and addiction. In particular, NSAID has less pain-relieving effect, and so should be used by a higher dose than opiates, to be effective. Also, in the case, the irritation to gastro-intestinal tract peculiarly caused by NASID may be accompanied.

Therefore, new compounds capable of controlling neuropathic pain by different mechanism from analgesics known in the art, or uses thereof have been urgently needed.

A representative example of target materials for inventions of such compounds is bradykinin that is a physiological active substance inducing pain, blood vessel expansion, and increase of blood vessel permeability.

Bradykinin is an endogenous peptide hormone consisting of nine amino acids, produced from kininogen, a precursor, by kallikrein that is a group of proteolytic enzyme.

Bradykinin is secreted by inflammation, trauma, burns, shock, allergy, and certain

cardiovascular disease. Once secreted, it initiates or increases secretion of mediums stimulating pain-sensible afferent peripheral nerve from white blood cells. Various pathophysiological responses induced by bradykinin are septic shock, hemorrhagic shock, anaphylaxis, arthritis, rhinitis, asthma, inflammatory bowel disease, acute pancreatitis, postgastrectomy dropping syndrome, carcinoid syndrome, migraine headache, genetic angioedema, etc.

Bradykinin receptor is classified into B1 type and B2 type according to biological effects of bradykinin analogue. The functions of bradykinin in B1 receptor is mainly contraction of arteries and veins, but also may relax peripheral resistance blood vessels. However, many important functions of bradykinin are controlled by B2 receptor. Bradykinin in B2 receptor induces low blood pressure and inflammation, and also induces edemas and pain by increasing permeability of capillary. Thus, bradykinin is known to play an important role in various diseases such as inflammation, cardiovascular diseases, pain, and cold. Nevertheless, the antagonist of bradykinin has never used as pain controller until now.

The present inventors invented new compounds having antagonistic action against bradykinin patented as Korean Patent No.0446711. This patent disclosed inhibition effects of visceral muscle contraction activity, but did not describe any pain-relieving effect.

The present inventors discovered use of bradykinin as antagonists for relieving pain to complete this invention.

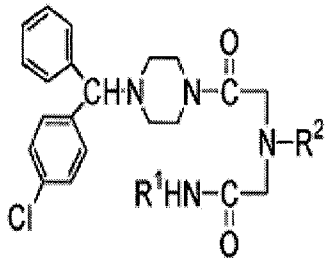
DISCLOSURE OF INVENTION

TECHNICAL PROBLEM

The object of the present invention is to provide a composition for relieving pain, comprising a non-peptide compound as an effective substance, a method of preparing the same, and a method for relieving pain by using the same.

TECHNICAL SOLUTION

The present invention provides a composition for relieving pain, comprising a compound of general formula (I) or pharmaceutically acceptable salts thereof, as an effective substance.



(I)

wherein

R^1 is isopropyl, benzyl, 4-methylbenzyl, 4-methoxybenzyl, 1-methyl-3-phenyl propyl, 3,4,5-trimethoxybenzyl, 2-(4-(4-chlorophenyl)thiazole), 3-(5-t-butylisoxazole), 2-(4-(4-bromophenyl)thiazole), cyclohexyl, 5-(3-methylisoxazole) or 3,4-dibenzyloxypenethyl;

R^2 is H or R^3CO; and

$R^3$is methyl, phenyl, benzyl, naphthyl($C_{10}H_7$) or $C_{10}H_7CH_2$.

Preferably, the compound (I) is N-(3,4,5-trimethoxybenzyl)-N'-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid diamide (N-(3,4,5-trimethoxybenzyl)-N'-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid diamide) or N-phenylacetyl-N'-(4-methoxybenzyl)-N"-1-(4-chlorobenzhydryl) piperazine iminodiacetic acid triamide((N-phenylacetyl-N'-(4-methoxybenzyl)-N"-1-(4-chlorobenzhydryl)-piperazine iminodiacetic triamide).

The composition of the present invention relieves various pains, particularly, neuropathic pain such as cold and warm allodynia. Also, the present composition relieves inflammatory pain. Thus, the present composition can be used to relieve pains that have not been easily controlled by prior analgesics.

The method of preparing the present composition is described below.

Compound (I) can be prepared by using a known method in the art, a solution-phase combination synthesis method that iminodiacetic acid anhydrous compound as a template reacts with amine, 1-(4-chlorobenzhydryl)piperazine, and carboxylic acid in sequence.

The composition of the present invention is prepared by mixing the compound as prepared above or pharmaceutically acceptable salts thereof as an effective substance with pharmaceutically acceptable carriers (excipients).

Pharmaceutically acceptable carriers (excipients) used in this invention may be ones well known to a skilled person in the art. Pharmaceutically acceptable carriers for liquid-solution formulation include, for example, saline solution and sterile water. If necessary, antioxidants, butter solutions, bacteriostatic agents, and other common additive agents may be used together.

Also, the composition of the present invention can be formulated as pills, capsules, granules, and tablets, by additionally containing diluents, dispersing agents, detergents, binders, and lubricants. A person skilled in the art can formulate the present composition by a well recognized method known in the art such as one disclosed in Remington's Pharmaceutical Sciences, Gennaro, Ed., Mack Publishing Co., Easton, PA 1990. Moreover, the present composition may be prepared as patches for transdermal application.

Further, the present invention provides a method of relieving pain by using the composition of the present invention.

The present composition can be prepared to various formulations, and so can be administrated orally or parenterally. For example, pharmaceutical compositions can be prepared by mixing the present compound with inert and pharmaceutically acceptable solid or solution carriers. Solid formulations include powder, tablet, spray granule, capsule, cachets, patch, and suppository. Other formulations of solids and liquids can be prepared by a method known in the art. The present composition may be administrated orally by proper formulations, parenterally by intravenous injection, intramuscular injection, or transdermal

injection by liquid formulations, and parenterally by patches.

In the unit dose of formulation, the dose of effective substance according to the present invention can be 1-100 mg/kg per patient per day. This dose may be changed depending on conditions of patient, severity of injury, and compound used. Therefore, the concentration and dose (dosage) may be properly changed by a skilled person in the art. Patches having a dose of 1-100μg as effective substance may be used by local transdermal administration.

The following Examples are intended to further illustrate the present invention, but the scope of the present invention are not intended to be limited thereby in any way.

DESCRIPTION OF THE DRAWINGS

Fig. 1 is a schematic drawing illustrating a preparation process of animal model having neuropathic pain.

Figs. 2a, 2b, and 2c are schematic drawings illustrating the relieving effect of mechanical allodynia by the composition of the present invention.

Figs. 3a and 3b are schematic drawings illustrating the relieving effect of cold allodynia by the composition of the present invention.

Fig. 4 is a schematic drawing illustrating the relieving effect of warm allodynia by the composition of the present invention.

Fig. 5 is a schematic drawing illustrating the relieving effect of inflammatory pain by administrating the present composition to abdominal cavity.

Fig. 6 is a schematic drawing illustrating the relieving effect of inflammatory pain according to transdermal administration of the present composition.

MODE FOR INVENTION

[Preparation Example 1] Preparation of the Composition 1 of this invention comprising N-(3,4,5-trimethoxybenzyl)-N'-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid diamide

1) Preparation of N-((t-butyloxy)carbonyl)iminodiacetic acid

To obtain N-phenylacetyl-N'-(4-methoxybenzyl)-N''-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide, N-((t-butyloxy)carbonyl)iminodiacetic acid as a template was prepared by the following method.

Iminodiacetic acid (13.3g, 100mmol), dioxane (200mℓ), and NaOH (8g, 200mmol) dissolved in 200mℓ of water were added into a 1ℓflask, and stirred until the mixture formed a uniform solution. Di-t-butyldicarbonate (25mℓ, 110mmol) was added into the solution, which was stirred for 72h at room temperature. Then, the reaction mixture was washed with ether (2×100mℓ).

The aqueous layer was acidified with 10% aqueous HCl (100mℓ), and extracted with ethylacetate (3×150mℓ). The collected organic layer was washed with saturated aqueous NaCl (2×150mℓ), from which moisture was removed with Na_2SO4. And, the resulting mixture was recrystallized (ethylacetate : hexane = 1 : 2) to obtain colorless crystal.

Yield: 10.33g (44%)

^{1}H-NMR(Acetone-d_6, 400㎒) : 4.12 and 4.08(two s, 4H), 1.4(s, 9H)

2) Preparation of N-((t-butyloxy)carbonyl)-N'-(3,4,5-trimethoxybenzyl)iminodiacetic acid monoamide

The title compound of combining the first functional group of N-((t-butyloxy)carbonyl)iminodiacetic acid prepared in the above 1) and 3,4,5-trimethoxybenzylamine was prepared by the following method.

N-((t-butyloxy)carbonyl)iminodiacetic acid (0.5g, 2.14mmol) prepared in the above 1) and EDCI (0.41g, 2.14mmol) in DMF (6.4mℓ) were added to a flask, and stirred for 1h at room temperature. 3,4,5-trimethoxybenzylamine(0.42g, 2.14mmol) was slowly added to the solution by a syringe, and stirred for 20h at room temperature. The solution was diluted with ethylacetate (60mℓ), and washed with 10% aqueous HCl (2×40mℓ), and saturated with aqueous NaCl (40mℓ). Then, moisture was removed from the mixture with anhydrous Na_2SO_4. And, solvent was removed from the mixture under a vacuum to obtain pale yellow oil.

Yield: 0.66g (75%)

1H-NMR(Acetone-d6, 400MHz) 6.5(2H, d), 4.4(2H, dd), 4.0(4H, d), 3.8(9H, dd), 1.4(9H, s)

FABMS m/z 413.18

3) Preparation of N-((t-butyloxy)carbonyl)-N'-(3,4,5-trimethoxybenzyl)-N"-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid diamide

The title compound of combining the compound prepared in the above 2) and 1-(4-chlorobenzhydryl)piperazine was prepared by the following method.

The compound prepared in the above 2) (0.5g, 1.21mmol), 4-chloro-benzhydrylpiperazine (0.35g, 1.21mmol), and benzotriazole-1-yl-oxy-trispyrrolidino phosphonium hexafluorophosphate(PyBOP)(0.72g, 1.33mmol) were added in DMF 15ml. And, N,N-diisopropylethylamine(0.62mL, 2.42mmol) was added into the solution. The solution was stirred for 16h at 25℃, and moved to a separate funnel.

Then, the solution was diluted with ethylacetate (100mL), washed with 10% aqueous HCl(50mL) three times, and washed with saturated aqueous $NaHCO_3$ (50mL) twice. After that, the mixture was washed with saturated aqueous NaCl, and dried with anhydrous Na2SO4. And, solvent was removed from the mixture under a vacuum to obtain a pale yellow solid.

Yield: 0.67g (81%)

^{1}H-NMR(Acetone-d_6, 400MHz) 7.2-7.5(9H, m), 6.6(2H, d), 4.4(1H, d), 4.3(2H, d), 4.2(2H, d), 4.1(2H, d), 3.8(9H, d), 3.6(4H, d), 1.3(9H, d)

FABMS m/z 681.30

4) Preparation of N-(3,4,5-trimethoxybenzyl)-N'-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid diamide

The compound prepared in the above 3) (0.3g, 0.44mmol) was dissolved in chloroform (5mL) and 4M HCl-dioxane (5mL), and stirred for 3 h. After the reaction was completed, solvents and remaining acids were removed from the mixture under a vacuum, which was washed with aqueous $NaHCO_3$. After removing the solvents, the mixture was subjected to column chromatography (EtOAc: MeOH = 10:1) to obtain a yellow solid.

Yield: 138mg (54%)

1H-NMR(Acetone-d6, 400MHz) 7.2-7.5(9H, m), 6.6(2H, d), 4.4(1H, d), 4.3(2H, d), 4.2(2H, d), 4.1(2H, d), 3.8(9H, d), 3.6(4H, d), 2.4(4H, d)

FABMS m/z 581.30.

5) Preparation of Composition 1 of the present invention

Composition 1 of the present invention was prepared by dissolving N-(3,4,5-trimethoxybenzyl)-N'-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid diamide prepared above in a solution of mixing Tween 80: 100% ethanol: a saline solution by the ratio of 8:1:1 (shown as sub 1 in each of the figures).

[Preparation Example 2] Preparation of the Composition 2 of the present invention comprising N-phenylacetyl-N'-(4-methoxybenzyl)-N"-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide

1) Preparation of N-((t-butyloxy)carbonyl)iminodiacetic acid

To obtain N-phenylacetyl-N'-(4-methoxybenzyl)-N''-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide, N-((t-butyloxy)carbonyl)iminodiacetic acid as a template was prepared by the following method.

Iminodiacetic acid (13.3g, 100mmol), dioxane (200mℓ) and NaOH (8g, 200mmol)in 200mℓ of water were added to a 1ℓflask, and stirred until the mixture formed a uniform solution. Di-t-butyldicarbonate (25mℓ, 110mmol) was added into the solution, and stirred for 72h at room temperature. Then, the reaction mixture was washed with ether (2×100mℓ). The aqueous layer was acidified with 10% aqueous HCl (100mℓ), and extracted with ethylacetate (3×150mℓ). The collected organic layer was washed with saturated aqueous NaCl (2×150mℓ). Moisture was removed from the mixture with Na_2SO_4, and the mixture was recrystallized (ethylacetate : hexane = 1 : 2) to obtain colorless crystal.

Yield: 10.33g (44%)

1H-NMR(Acetone-d6, 400㎒) : 4.12 and 4.08(two s, 4H), 1.4(s, 9H)

2) Preparation of N-((t-butyloxy)carbonyl)-N'-(4-methoxybenzyl)iminodiacetic acid monoamide

The title compound of combining the first functional group of N-((t-butyloxy)carbonyl)iminodiacetic acid prepared in the above 1) and 4-methoxybenzylamine was prepared by the following method.

N-((t-butyloxy)carbonyl)iminodiacetic acid (0.5g, 2.14mmol) prepared in the above 1) and EDCI (0.41g, 2.14mmol)in DMF (6.4mℓ) were added to a flask, and stirred for 1h at room temperature. 4-methoxybenzylamine (0.23g, 2.14mmol) was added slowly to the solution by a syringe, and stirred for 20h at room temperature. The solution was diluted with ethylacetate (60mℓ), and washed with 10% aqueous HCl (2×40mℓ) and saturated aqueous NaCl(40mℓ). And then, moisture was removed from the solution with anhydrous Na_2SO_4. Solvents were removed from the mixture under a vacuum to obtain pale yellow oil.

Yield: 0.43g (55%)

1H-NMR(Acetone-d6, 400㎒) : 6.8(2H,dt), 7.2 and 7.3(2H,t), 4.4(2H,d), 4.2(2H,d), 4.0(2H,d), 3.8(3H,s), 1.4(9H, two s)

FABMS m/z : 353.16

3) Preparation of N-((t-butyloxy)carbonyl)-N'-(4-methoxybenzyl)-N"-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid diamide

The title compound of combining the compound prepared in the above 2) and 1-(4-chlorobenzhydryl)piperazine was prepared by the following method.

N-((t-butyloxy)carbonyl)-iminodiacetic acid monoamide (0.48g, 1.31㎜ol), 1-(4-chlorobenzhydryl)piperazine (0.41g, 1.441㎜ol), benzotriazole-1-yl-oxy-trispyrrolidino phosphonium hexafluorophosphate(PyBOP) (0.75g, 1.44㎜ol) were added into a 30㎖ vial. DMF (15㎖), and N,N-diisopropylethylamine(i-Pr_2NEt; 0.5㎖, 2.62㎜ol) are added to the mixture, which was stirred for 16 h at room temperature. Then, the mixture was diluted with ethylacetate (100mL), and washed three times with 10% aqueous HCl (50mL), and then washed twice with saturated aqueous NaHCO3 (50mL). After that, the mixture was washed with saturated aqueous NaCl (50ml), and dried with anhydrous Na2SO4. Solvents were removed from the mixture under a vacuum to obtain an orange color solid.

Yield: 0.67g (89%)

1H-NMR(Acetone-d6, 400㎒) : 7.2-7.6(11H,m), 6.8(2H,t), 4.4(1H,s), 4.3(2H,d), 4.2(2H,d), 3.8(2H,d), 3.7(3H,s), 3.6(4H,m), 2.4(4H,m), 1.4(9H,d)

FABMS m/z : 621.28

4) Prepartion of N-phenylacetyl-N'-(4-methoxybenzyl)-N"-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide

The title compound was prepared by having the compound prepared in the above 3)

reacted with carboxylic acid by the following method.

The compound prepared in the above 3) (0.031g, 0.050mmol), chloroform (1mL), and 4M HCl-dioxane (1mL) were added into a 4㎖ vial, and stirred for 3 h. Solvents and acids remaining in the mixture were evaporated and removed. Then, other residues were dissolved in 1㎖ DMF. To the mixture, i-Pr_2NEt 26.1㎕ (0.15mmol) phenylacetic acid 7.49㎎ (0.055mmol), and PyBrOP 25.64㎎ (0.055mmol) were added and stirred for 16h. After the stirring was completed, the mixture was diluted with ethylacetate (40mL), and washed three times with 10% aqueous HCl (30mL), and then washed twice with saturated aqueous NaHCO3 (30mL). After that, the mixture was washed with saturated aquoeus NaCl (30ml), and dried with anhydrous Na2SO4. Solvents were removed from the mixture under a vacuum to obtain brown yellow oil.

Yield: 25㎎ (77%)

1H-NMR(Acetone-d_6, 400㎒) : 7.2-7.6(11H,m), 7.0-7.2(5H,m) 6.8(2H,t), 4.4(1H,s), 4.3(2H,d), 4.2(2H,d), 3.8(2H,d), 3.7(3H,s), 3.6(4H,m), 3.4(2H,s), 2.4(4H,m)

FABMS m/z : 639.10

5) Preparation of Composition 2 of the present invention

Composition 2 of the present invention was prepared by dissolving N-phenylacetyl-N'-(4-methoxybenzyl)-N"-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide prepared above in a solution of mixing Tween 80: 100% ethanol: a saline solution by the ratio of 8:1:1 (Composition 2 was shown as sub 2 in each of the figures).

[Experimental Example 1] Pain-relieving effect for chronic neuropathic pain of the composition of the present invention

1. Preparation of tail nerve injury subject inducing chronic neuropathic pain

Test subjects for chronic neuropathic pain were prepared in the following method.

Eight-week-old sprague-Dawley rats were caged, four a cage. And, they were freely bred by providing enough feed and water, with maintaining 22-25℃ and a 12-hour interval of light-dark cycle (providing a light into the cages from 7 a.m.).

After anesthetizing the rats with enflurane (2% for induction and 0.5% for maintenance) to induce neuropathic pain, the inferior/superior caudal trunks distributed at their tails were cut between the S1 and S2 spinal nerves as shown in Fig. 1. To prevent reconnection of the proximal and distal ends of the severed trunk, the proximal end of the trunk was cut more by about 1 mm.

The test subjects prepared as shown above feel chronic neurophthic pain since their S1 spinal nerves of controlling nerves around the tail are injured.

2. Neuropathic pain-relieving effect of the composition according to the present invention

1) Behavioral assessment

To confirm the neuropathic pain-relieving effect of the composition according to the present invention, the present composition was injected into the abdominal cavity of the subject rats as prepared in the above 1 in 14 days from the S1 spinal nerve injury, when allodynia of the subjects is at its peak.

Composition 1 prepared in the above Preparation Example 1, comprising N-(3,4,5-trimethoxybenzyl)-N'-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid diamide (6mg/kg or 60mg/kg) or Composition 2 prepared in the above Preparation Example 2, comprising N-phenylacetyl-N'-(4-methoxybenzyl)-N''-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide(3mg/kg) was injected to the abdominal cavities of the subjects. Or, Composition 1 (60μg) was injected hypodermally. Here, a solvent used for preparing the above Composition 1 was used as control group(vehicle).

To examine the neuropathic pain in the tails, the behavioral tests were carried out 1 day before the surgery, and 1, 7 and 14 day(s) after the nerve surgery. To give mechanical, cold, and warm stimuli to the tails, the subject rats were restrained in a transparent plastic tube (4.5×2, 5.5×5, 6.5×8㎝ in diameter×length). Prior to the behavioral tests, the subject rats were adapted to the test environment for 1h.

At the 14^{th} day after the nerve surgery, the present composition was injected to each of the subject rats. The behavioral tests were conducted at 1, 3 and 5h after the injection, to measure the anti-allodynia effect of the present composition.

The results were shown in each of the figures where the drug-injected times were described in vertical dotted lines, and "n" is the number of rat used in the tests.

2) Mechanical allodynia test

To confirm the mechanical allodynia-relieving effect of the composition according to the present invention, the mechanical allodynia test was conducted by the following methods.

To assess mechanical sensitivity at the tails of the subject rats, withdrawal threshold was measured by using the up-down method. By using a set of von Frey filaments (0.4, 0.6, 1.0, 2.0, 4.0, 6.0, 8.0, 15.0 g, Stoelting, Wood Dale, IL, USA), a brisk tail flick to von Frey application was regarded as withdrawal response. The first stimulus was made by 2.0 g filament. When there was a withdrawal response, the next weaker filament was used. When no response was shown, the next stronger filament was administered. Interpolation was carried out to 50% threshold according to the Dixon method (1980).

In Fig. 2, Composition 1 (sub1) and Composition 2 (sub2) of the present invention showed a pain-relieving effect, compared with the control group (vehicle).

3) Cold and warm allodynia tests

To confirm the cold and warm allodynia-relieving effects of the composition according to the present invention, withdrawal latency was measured to assess cold and warm sensitivity in the following methods.

Withdrawal latency was measured by a time to show withdrawal response after immersing the tails of the subject rats in cold (4 ℃) and warm (40 ℃) water baths. If the response was not shown till 15 sec, 15 sec was recorded as the result. This test was repeated five times at intervals of 5 min, and the results were averaged. As a result, it was confirmed that as the response time is shorter, cold and warm allodynia of the subject rats is more severe.

Composition 1 (sub 1) of the present invention showed a significant level of pain relieving effect to a stimulus causing cold allodynia at 1 h after the injection, as shown in Figs. 3a and 3b.

Composition 1 (sub 1) of the present invention showed a significant level of pain relieving effect to a stimulus causing warm allodynia at 1 h after the injection, as shown in Fig. 4.

[Experimental Example 2] Inflammatory pain-relieving effect of the composition according to the present invention

1) In case of an abdominal cavity-injection

To confirm the inflammatory pain-relieving effect of the composition according to the present invention, a test was conducted by using formalin that is an inflammation-inducing material.

Composition 1 (60 mg/kg) was injected into an abdominal cavity of the subject rats 10 min before the formalin injection. Formalin (5%, 50 ul) was injected subcutaneously into the dorsal surfaces of one-side hindpaws of the subject white rats, which were then placed into an observation chamber (16 x 14 cm each) for observing licking and biting their paws at intervals

of 5 min. A mirror positioned behind the observation chamber gave an unobstructed view of their hindpaws. Immediately after the formalin injection, the time spent for licking and biting the injected paws was recorded for 60 min. Phase I was defined as first 10 min after the formalin injection, and phase II was the following 50 min.

Composition 1 (sub 1) of the present invention showed a pain-relieving effect, compared with the control group (vehicle) in Phase II (from 10 min to 60 min after the injection), as shown in Fig. 5. However, no pain-relieving effect was observed in Phase I (from the injection to 10 min after the injection).

2) In case of a transdermal injection

To confirm the inflammatory pain-relieving effect of the composition according to the present invention, a test was conducted by using formalin that is an inflammation-inducing material.

Formalin (5%, 50 μl) and Composition 1 of the present invention (60μg/kg) were injected subcutaneously into the dorsal surfaces of one-side hindpaws of the subject rats. Formalin (5%, 50 μl) was injected subcutaneously into the dorsal surfaces of one-side hindpaws of the control group, which were then placed into an observation chamber (16 x 14 cm each) to observe licking and biting their paws at intervals of 5 min for 60 min. A mirror positioned behind the observation chamber gave an unobstructed view of their hindpaws. Immediately after the formalin injection, the time spent for licking and biting the injected paws was recorded for 60 min. Phase I was defined as first 10 min after the formalin injection, and phase II was the following 50 min.

Composition 1 (sub 1) of the present invention showed a superior pain-relieving effect to the control group (vehicle) in Phase II (from 10 min to 60 min after the injection), as shown in Fig. 6.

From the above experiments, it can be concluded that the composition of the present

invention has an inflammatory pain- relieving effect.

INDUSTRIAL APPLICABILITY

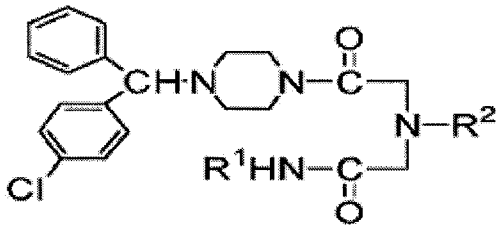
The composition of the present invention has a pain-relieving effect, particularly neuropathic pain such as cold allodynia or warm allodynia.

Also, the present composition effectively relieves inflammatory pain.

Therefore, the present composition is useful for preventing or treating pain not controlled by previous analgesics.

WHAT IS CLAIMED IS:

1. A composition for relieving pain, comprising a compound of the following general formula (I) or pharmaceutically acceptable salts thereof as an effective substance,



(I)

wherein

R^1 is isopropyl, benzyl, 4-methylbenzyl, 4-methoxybenzyl, 1-methyl-3-phenyl propyl, 3,4,5-trimethoxybenzyl, 2-(4-(4-chlorophenyl)thiazole), 3-(5-t-butylisoxazole), 2-(4-(4-bromophenyl)thiazole), cyclohexyl, 5-(3-methylisoxazole) or 3,4-dibenzyloxypenethyl;

R^2 is H or R_3CO; and

R^3 is methyl, phenyl, benzyl, naphthyl ($C_{10}H_7$) or $C_{10}H_7CH_2$.

2. The composition of Claim 1, wherein the compound of general formula (I) is N-(3,4,5-trimethoxybenzyl)-N'-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid diamide or pharmaceutically acceptable salts thereof.

3. The composition of Claim 1, wherein the compound of general formula (I) is N-phenylacetyl-N'-(4-methoxybenzyl)-N"-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide or pharmaceutically acceptable salts thereof.

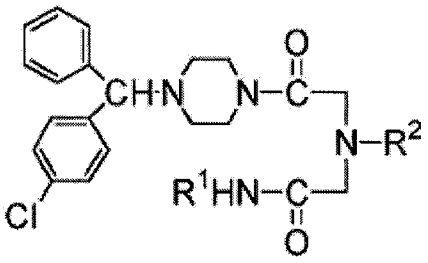
4. The composition of any of claim 1, 2, or 3, wherein the pain is neuropathic pain.

5. The composition of claim 4, wherein the neuropathic pain is cold allodynia or warm allodynia.

6. The composition of any of claim 1, 2 or 3, wherein the pain is inflammatory pain.

7. Patches comprising the composition of any of claim 1, 2 or 3 as an effective substance.

8. A method for relieving pain, comprising administering a compound of the following formula (I) or pharmaceutically acceptable salt thereof to a subject,



(I)

wherein

R^1 is isopropyl, benzyl, 4-methylbenzyl, 4-methoxybenzyl, 1-methyl-3-phenyl propyl, 3,4,5-trimethoxybenzyl, 2-(4-(4-chlorophenyl)thiazole), 3-(5-t-butylisoxazole), 2-(4-(4-bromophenyl)thiazole), cyclohexyl, 5-(3-methylisoxazole) or 3,4-dibenzyloxypenethyl;

R^2 is H or R^3CO; and

R^3 is methyl, phenyl, benzyl, naphthyl ($C_{10}H_7$) or $C_{10}H_7CH_2$.

9. The method of claim 8, wherein the compound of formula (I) is N-(3,4,5-trimethoxybenzyl)-N'-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid diamide or pharmaceutically acceptable salts thereof.

10. The method of claim 8, wherein the compound of formula (I) is N-phenylacetyl-N'-(4-methoxybenzyl)-N"-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide or pharmaceutically acceptable salts thereof.

11. The method of any of claim 8, 9 or 10, wherein the pain is neuropathic pain.

12. The method of claim 11, wherein the neuropathic pain is cold allodynia or warm allodynia.

13. The method of any of claim 8, 9 or 10, wherein the pain is inflammatory pain.

FIGURES

Fig. 1

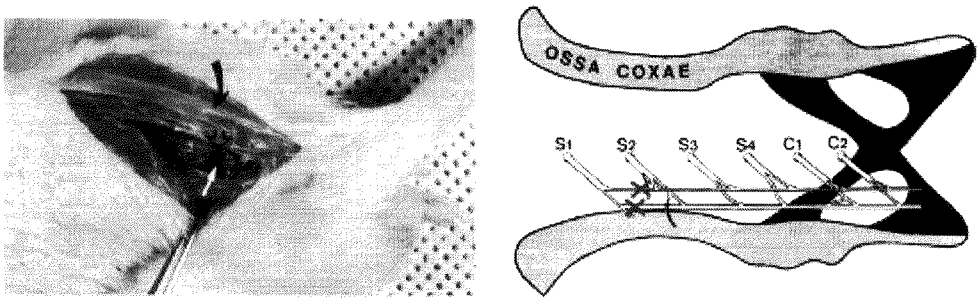


Fig. 2

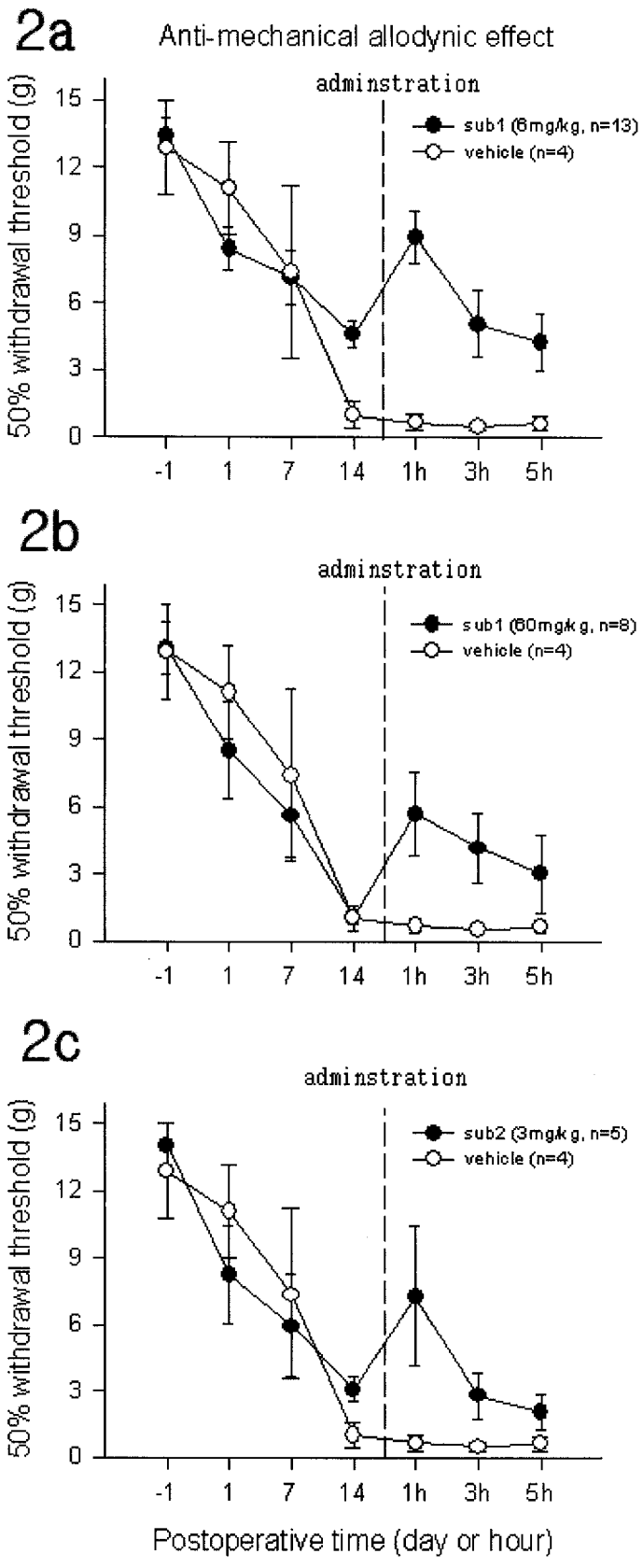

2a
Anti-mechanical allodynic effect
adminstration
sub1 (6mg/kg, n=13)
vehicle (n=4)
50% withdrawal threshold (g)
15
12
9
6
3
0
-1
1
7
14
1h
3h
5h
2b
adminstration
sub1 (60mg/kg, n=8)
vehicle (n=4)
50% withdrawal threshold (g)
2c
adminstration
sub2 (3mg/kg, n=5)
vehicle (n=4)
50% withdrawal threshold (g)
Postoperative time (day or hour)

Fig. 3

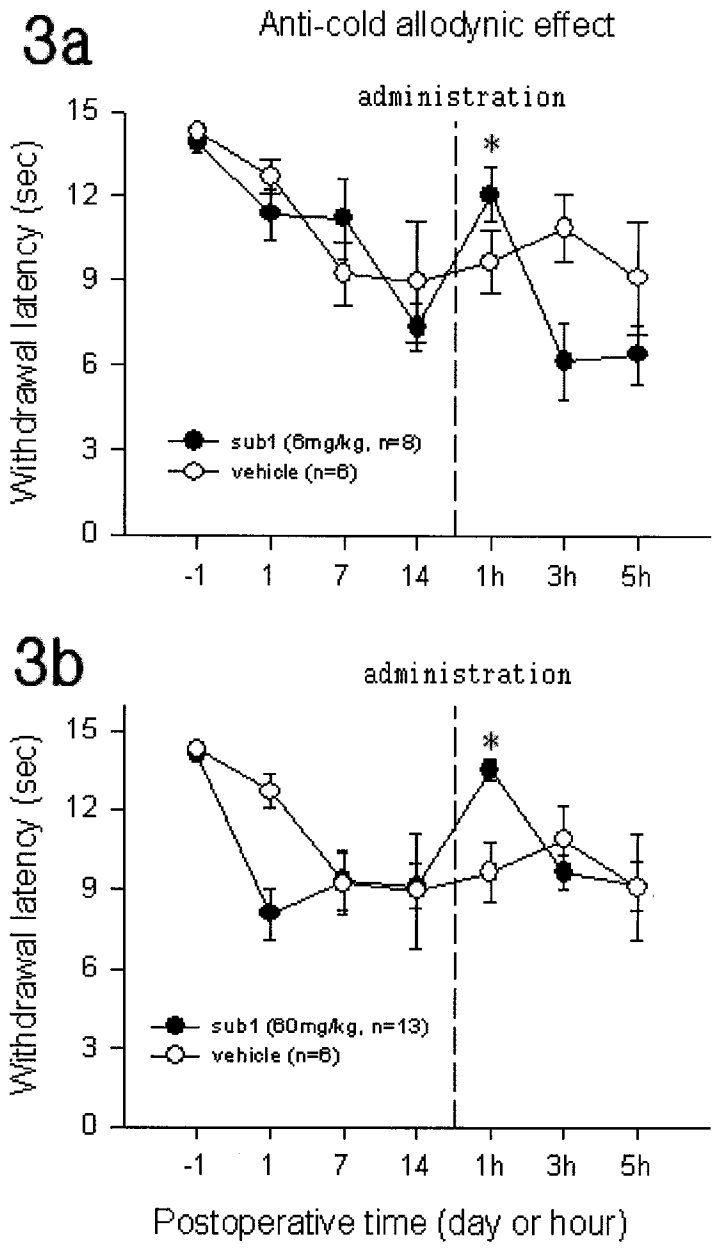

3a
Anti-cold allodynic effect
administration
Withdrawal latency (sec)
15
12
9
6
3
0
*
sub1 (6mg/kg, n=8)
vehicle (n=6)
-1
1
7
14
1h
3h
5h
3b
administration
Withdrawal latency (sec)
15
12
9
6
3
0
*
sub1 (60mg/kg, n=13)
vehicle (n=6)
-1
1
7
14
1h
3h
5h
Postoperative time (day or hour)

Fig. 4

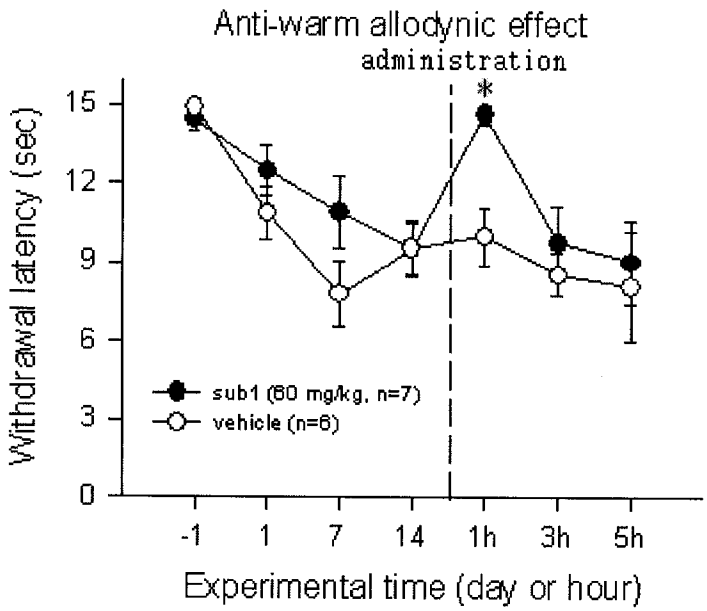

Anti-warm allodynic effect
administration
Withdrawal latency (sec)
15
12
9
6
3
0
sub1 (60 mg/kg, n=7)
vehicle (n=6)
-1
1
7
14
1h
3h
5h
Experimental time (day or hour)

Fig. 5

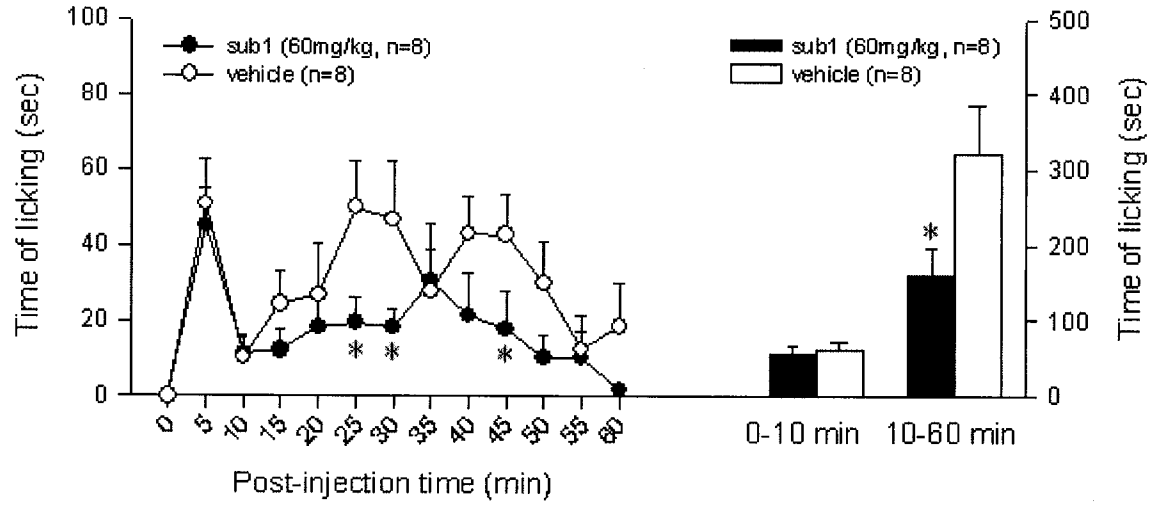
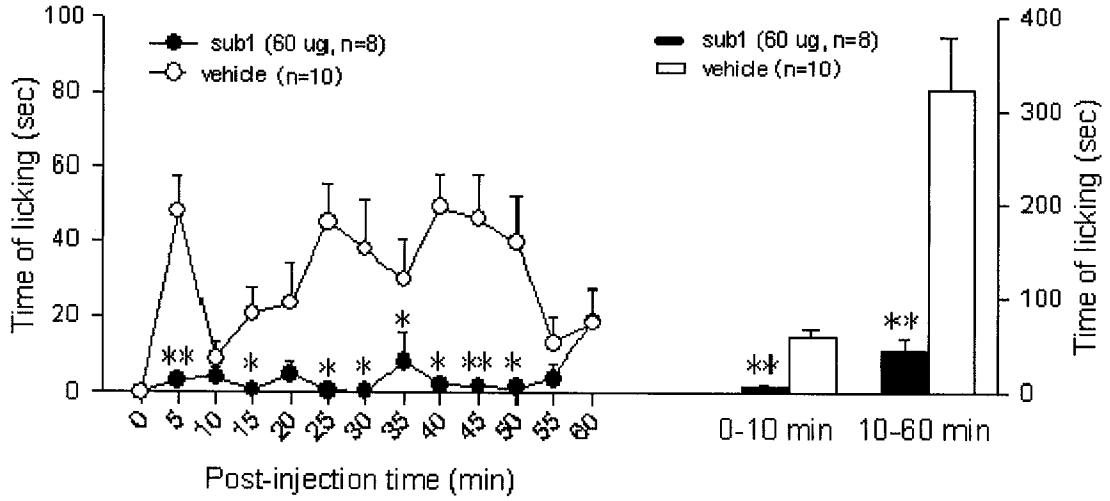

Time of licking (sec)
100
80
60
40
20
0
sub1 (60mg/kg, n=8)
vehicle (n=8)
0 5 10 15 20 25 30 35 40 45 50 55 60
Post-injection time (min)
sub1 (60mg/kg, n=8)
vehicle (n=8)
500
400
300
200
100
0
Time of licking (sec)
0-10 min
10-60 min

Fig. 6


sub1 (60 ug, n=8)
vehicle (n=10)
Time of licking (sec)
Post-injection time (min)
0-10 min
10-60 min

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2006/000997

A. CLASSIFICATION OF SUBJECT MATTER

A61K 31/496(2006.01)i, A61P 29/00(2006.01)i

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K 31/496

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean Patents and Applications for Inventions since 1975

Electronic data base consulted during the intertnational search (name of data base and, where practicable, search terms used)
NCBI pubmed database, NCBI blastn database, Delphion Research Intellectual Property network database, CA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	European Journal of Pharmacology 2005, Vol. 523, pp143-50 See Fig. 1	1-7
Y	Clin Exp Pharmacol Physiol. 1977, Vol.4, No.5, pp443-52 See abstract	1-7
A	J. Physiol, 1967, Vol. 193, pp.255-267 See entire document	1-7

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
08 DECEMBER 2006 (08.12.2006)	**11 DECEMBER 2006 (11.12.2006)**

Name and mailing address of the ISA/KR
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Republic of Korea
Facsimile No. 82-42-472-7140

Authorized officer
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Telephone No. 82-42-481-5600

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2006/000997

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 8-13
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 8-13 pertain to methods for treatment of human or animal body by therapy, as well as diagnostic methods, and thus relate to a subject matter which this International Searching Authority I not required, under article 17(2)(a)(i) of the PCT and Rule 39.1(iv) of the Regulations under the PCT, to search.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required addtional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
☐ No protest accompanied the payment of additional search fees.