

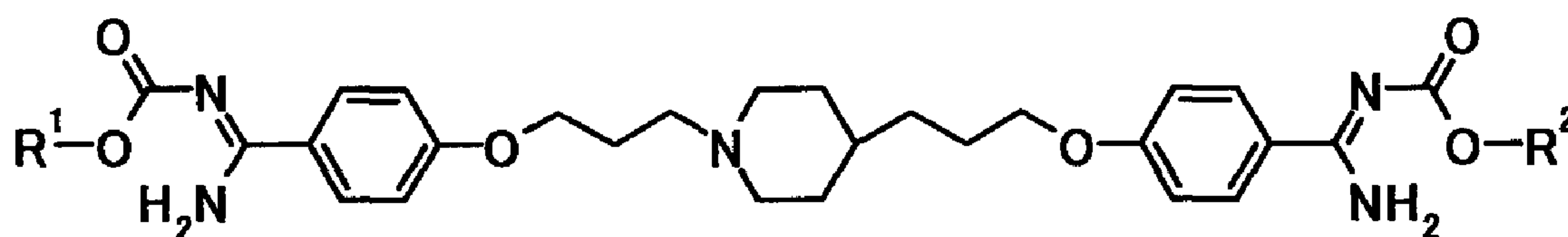


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(54) **Titre :** COMPOSITION PHARMACEUTIQUE CONTENANT UN DERIVE DE PHENYLAMIDINE ET PROCEDE D'UTILISATION DE LA COMPOSITION PHARMACEUTIQUE COMBINEE A UN AGENT ANTIFONGIQUE
(54) **Title:** PHARMACEUTICAL COMPOSITION COMPRISING PHENYLAMIDINE DERIVATIVE AND METHOD OF USING THE PHARMACEUTICAL COMPOSITION IN COMBINATION WITH ANTIFUNGAL AGENT

[CHEMICAL FORMULA 1]



(57) **Abrégé/Abstract:**

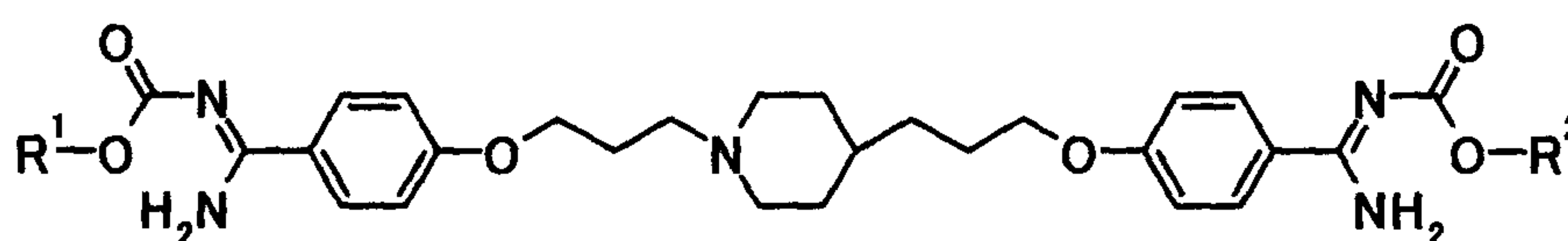
A pharmaceutical composition comprising a phenylamide derivative or a salt thereof, represented by a general formula, (see formula 1) wherein R¹ and R² may be same or different, and represent an optionally substituted C₃₋₄ alkyl group; and one or more agents selected from azole antifungal agents, polyene antifungal agents, candin antifungal agents and fluoropyrimidine antifungal agents, and a method for combination use of the phenylamide derivative or a salt thereof and the agents are useful for treating fungal infections caused by a fungal pathogen.



ABSTRACT

A pharmaceutical composition comprising a phenylamidine derivative or a salt thereof, represented by a general formula,

[CHEMICAL FORMULA 1]



wherein R^1 and R^2 may be same or different, and represent an optionally substituted C_{3-4} alkyl group; and one or more agents selected from azole antifungal agents, polyene antifungal agents, candin antifungal agents and fluoropyrimidine antifungal agents, and a method for combination use of the phenylamidine derivative or a salt thereof and the agents are useful for treating fungal infections caused by a fungal pathogen.

DESCRIPTION

PHARMACEUTICAL COMPOSITION COMPRISING PHENYLAMIDINE
DERIVATIVE AND METHOD OF USING THE PHARMACEUTICAL
COMPOSITION IN COMBINATION WITH ANTIFUNGAL AGENT

FIELD OF THE INVENTION

[0001]

The present invention relates to a
pharmaceutical composition which is useful for the
5 treatment of fungal infections caused by fungal
pathogens and which comprises a new phenylamidine
derivative or a salt thereof, and one or more agents
selected from azole antifungal agents, polyene
antifungal agents, candin antifungal agents and
10 fluoropyrimidine antifungal agents. The present
invention also relates to a method of using the new
phenylamidine derivative or a salt in combination with
the agents for treating fungal infections.

BACKGROUND ART

15 [0002]

Serious deep mycosis such as invasive
candidiasis can often be a fatal disease. In the past,
it has been considered that the principal protective
mechanism on the side of a host organism against fungi
20 such as Candida is nonspecific immunization by
neutrophils. When this protective mechanism functions

normally there is little risk of becoming infected with fungi. However, in recent years, the risk of suffering from deep mycosis has been boosted because of the increased number of patients with underlying diseases decreasing the immunological function of the body, such as malignant tumors (in particular, hemopoietic malignant tumors such as acute leukemia or malignant lymphoma) and AIDS, frequent use of anticancer agents or immunosuppressants, heavy use of antibacterial antibiotics or steroid hormones, long-term use of central venous hyperalimentation or venous catheterization and the like (Non-Patent Document 1).

Agents used for the treatment of such deep mycosis are very few, when compared to antibacterial agents used, and include only amphotericin B, flucytosine, miconazole, fluconazole, fosfluconazole, itraconazole, voriconazole, micafungin and the like. [0003]

On the other hand, there is an increasing need for safe and effective agents against opportunistic fungal infections caused by fungal pathogens such as Candida, Cryptococcus and Aspergillus.

While the agents that are used at present, for example, amphotericin B, have an extremely strong fungicidal action, they have a problem regarding side effects such as nephrotoxicity, so that their clinical usage is limited. Flucytosine has problems with the

development of resistance. Micafungin has a low activity against the Cryptococcus. Azoles such as fluconazole and voriconazole are most frequently used at present due to their balance between effectiveness and safety, although their fungicidal action is inferior to that of amphotericin B (Non-Patent Documents 2 and 3).

Methods for combination use of antifungal agents are being used for purposes such as to boost treatment effects (Non-Patent Document 4). Research is also progressing into the combination of antifungal agents (Patent Documents 1, 2 and 3). However, the number of agents being combined is limited, meaning that satisfactory treatment effects cannot be guaranteed.

[0004]

Further, phenylamidine derivatives having antifungal activity are known (Patent Document 4).

[0005]

Patent Document 1: Japanese Patent No. 3288051

Patent Document 2: JP-A-11-504931

Patent Document 3: JP-A-2003-527314

Patent Document 4: International Patent Publication No. WO2006/003881

Non-Patent Document 1: Rinsho to Biseibutsu (Clinics and Microorganisms), Vol. 17, pp. 265-266, 1990

Non-Patent Document 2: Rinsho to Biseibutsu (Clinics and Microorganisms), Vol. 21, pp. 277-283, 1994

Non-Patent Document 3: Rinsho to Biseibutsu (Clinics and Microorganisms), Vol. 30, pp. 595-614, 2003

Non-Patent Document 4: Diagnosis and treatment guideline of deep mycosis, PP. 20, 29, Ishiyaku

5 Publishers, Inc., 2003

DISCLOSURE OF THE INVENTION

PROBLEM TO BE SOLVED BY THE INVENTION

[0006]

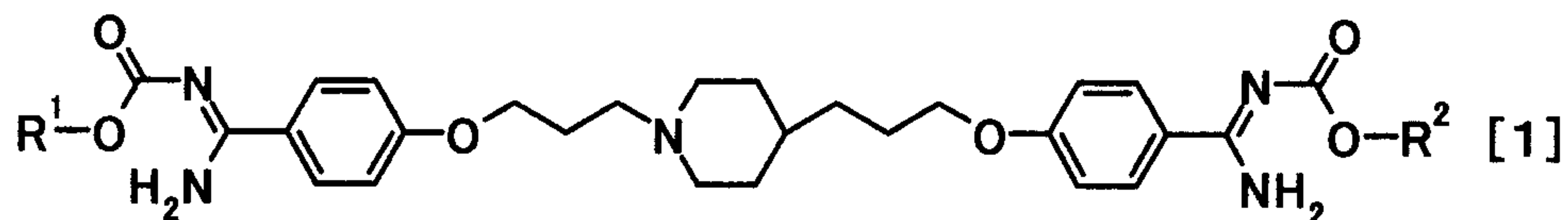
Desirable are a pharmaceutical composition
10 which is useful for treating fungal infections and which has strong antifungal activity yet few side effects, and a method for combination use of antifungal agents.

MEANS TO SOLVE THE PROBLEM

15 [0007]

Under such circumstances, as a result of intensive study, the present inventors discovered that a pharmaceutical composition comprising a phenylamidine derivative or a salt thereof, represented by the
20 general formula [1] which is a new compound:

[Chemical Formula 1]



wherein R¹ and R² may be same or different, and represent an optionally substituted C₃₋₄alkyl group; and

one or more agents selected from azole antifungal agents, polyene antifungal agents, candin antifungal agents and fluoropyrimidine antifungal agents, has a strong antifungal activity and is useful for treating fungal infections, and that a method for combination use of these antifungal agents is useful for treating fungal infections, thereby arriving at the present invention.

EFFECT OF THE INVENTION

10 [0008]

The pharmaceutical composition comprising the new phenylamide derivative or a salt thereof, and one or more agents selected from azole antifungal agents, polyene antifungal agents, candin antifungal agents and fluoropyrimidine antifungal agents, has strong antifungal activity and is useful for treating fungal infections. The method for combination use of these antifungal agents is useful as an excellent treatment method of fungal infections.

20 BEST MODE FOR CARRYING OUT THE INVENTION

[0009]

The present invention will now be described in more detail.

In the present specification, unless otherwise noted, a halogen atom refers to a fluorine atom, a chlorine atom, a bromine atom, or an iodine

atom; and a C₃₋₄alkyl group means propyl, isopropyl, n-butyl, sec-butyl, isobutyl and tert-butyl.

[0010]

Examples of the salts of the compound

5 represented by general formula [1] include the salts of mineral acids, such as hydrochloric acid, hydrobromic acid, phosphoric acid and sulfuric acid; the salts of organic carboxylic acids, such as succinic acid, maleic acid and fumaric acid; the salts of sulfonic acids such
10 as methanesulfonic acid, benzenesulfonic acid, p-toluenesulfonic acid, mesitylenesulfonic acid and naphthalenesulfonic acid.

Preferred salts of the compound represented by the general formula [1] include pharmacologically
15 acceptable salts.

[0011]

For the substituent of unsubstituted or substituted C₃₋₄alkyl group in R¹ and R², halogen atom, hydroxyl group and carboxyl group are given.

20 [0012]

In compounds of the general formula [1] used for the present invention, the preferred compounds are the compounds given below.

Compounds in which R¹ is a C₃₋₄alkyl group are
25 preferred, compounds in which R¹ is a propyl, isopropyl or n-butyl group are more preferred, and compounds in which R¹ is n-butyl group are furthermore preferred.

Compounds in which R² is a C₃₋₄alkyl group are

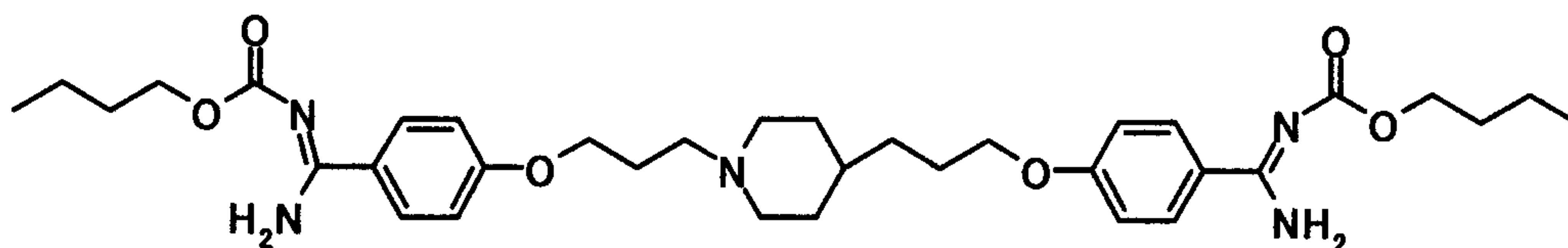
preferred, compounds in which R^2 is a propyl, isopropyl or n-butyl group are more preferred, and compounds in which R^2 is n-butyl group are furthermore preferred.

Compounds in which R^1 and R^2 are a same group
5 are preferred.

Concretely, for the compound of the general formula [1], the following compound is preferred.

[0013]

[Chemical Formula 2]



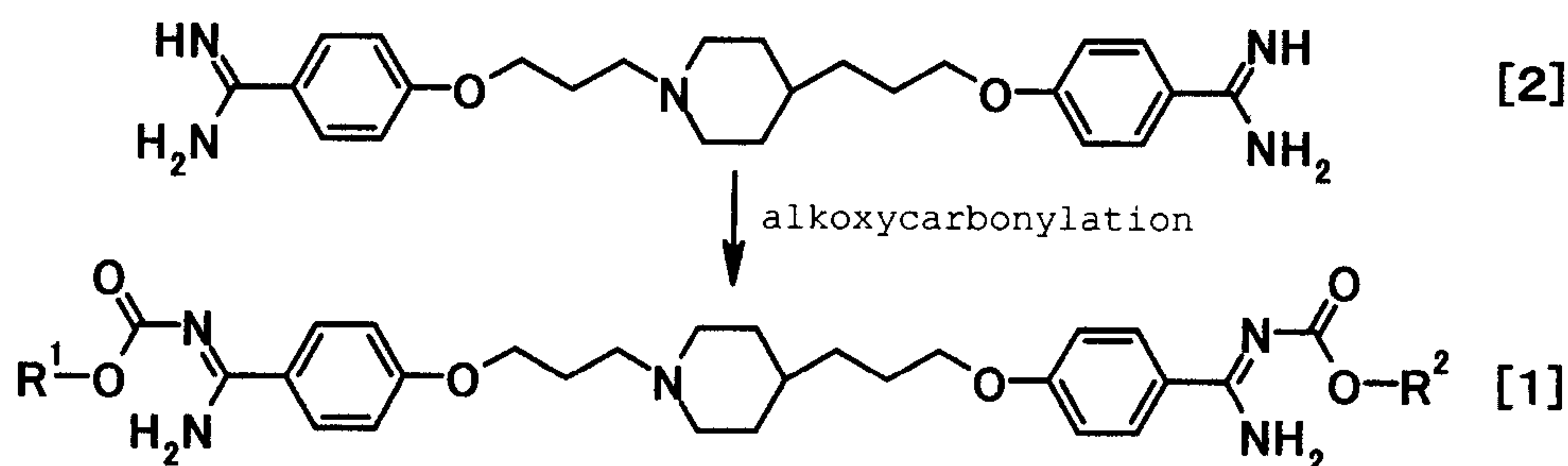
10 [0014]

Next, manufacturing methods of present invention compounds are explained.

The compounds of the present invention are produced by the combination of conventional methods per
15 se, for example, can be manufactured by the methods shown in next.

[0015]

[Manufacturing method]



wherein R^1 and R^2 have the same meanings as the above.

[0016]

The compound of the general formula [1] can be manufactured by subjecting the compound of formula
5 [2] and the reactive derivative to alkoxycarbonylation reaction in the presence or absence of base.

For a solvent used in this reaction, it is not limited particularly as long as it does not affect the reaction adversely, for example, amides such as
10 N,N-dimethylformamide, N,N-dimethylacetamide and 1-methyl-2-pyrrolidone; halogenated hydrocarbons such as dichloromethane, chloroform and dichloroethane; aromatic hydrocarbons such as benzene, toluene and xylene; ethers such as dioxane, tetrahydrofuran,
15 anisole, di(ethylene glycol) dimethyl ether, di(ethylene glycol) diethyl ether and ethylene glycol monomethyl ether; nitriles such as acetonitrile; sulfoxides such as dimethyl sulfoxide; ketones such as acetone and 2-butanone; esters such as ethyl acetate;
20 carboxylic acids such as acetic acid; heteroaromatics such as pyridine and water are given, and these may be mixed and the mixture may be used.

[0017]

For a reactive derivative used in this
25 reaction, for example, chlorocarbonic esters such as propyl chloroformate, isopropyl chloroformate, butyl chloroformate and isobutyl chloroformate; active esters such as 4-nitrophenyl propylcarbonate, 4-nitrophenyl

isopropylcarbonate, butyl 4-nitrophenylcarbonate and isobutyl 4-nitrophenylcarbonate are given. These reactive derivatives may be used after preparation in situ without isolating.

5 [0018]

For a base used in this reaction by a wish, for example, metal alkoxides such as sodium methoxide, sodium ethoxide, potassium tert-butoxide and sodium tert-butoxide; inorganic bases such as sodium
10 hydroxide, potassium hydroxide, sodium hydrogen carbonate, sodium carbonate, potassium carbonate, sodium hydride and potassium hydride and organic bases such as triethylamine, N,N-diisopropylethylamine, 1,8-diazabicyclo[5.4.0]undec-7-ene(DBU) and pyridine are
15 given.

The amount of the reactive derivative and the base used are 2-100 times mole per that of the compound of the formula [2], and are preferably 2-10 times mole.

This reaction may be carried out at -20 to
20 100°C, preferably at 0 to 50°C for 1 minute to 24 hours.
[0019]

For a compound in the manufacturing process described above, solvates, hydrates and various kinds of crystals can be used.

25 [0020]

The compound of the formula [2] which is a raw material of production of the present invention is manufactured by the combination of conventional methods

per se, for example, it can be manufactured by the methods as described in patent document 4.

[0021]

Examples of the azole antifungal agents used
5 in the present invention include triazole antifungal agents, such as fluconazole, fosfluconazole, itraconazole, voriconazole, posaconazole, ravuconazole, BMS-379224, BAL-8557 and CS-758, as well as imidazole antifungal agents, such as ketoconazole, miconazole,
10 bifonazole, laniconazole and luliconazole.

Preferred examples of the azole antifungal agents include triazole antifungal agents, such as fluconazole, fosfluconazole, itraconazole, voriconazole, posaconazole, ravuconazole, BMS-379224,
15 BAL-8557 and CS-758. More preferred are fluconazole, fosfluconazole, voriconazole and itraconazole, and even more preferred are fluconazole and voriconazole.

[0022]

Examples of the polyene antifungal agents
20 used in the present invention include, for example, amphotericin B and liposomal formulations thereof (e.g., Abelcet (trade name) or AmBisome (trade name)), nystatin, trichomycin, SPK-843 and pimaricin.

Preferred examples of the polyene antifungal
25 agents include amphotericin B and liposomal formulations thereof.

[0023]

Examples of the candin antifungal agents used

in the present invention include, for example, micafungin, caspofungin, anidulafungin and aminocandin.

Preferred examples of the candin antifungal agents used include micafungin.

5 [0024]

Examples of the fluoropyrimidine antifungal agents used in the present invention include, for example, flucytosine.

[0025]

10 The administration route of the phenylamidine derivative or the salt thereof represented by the general formula [1] is not especially limited, and the phenylamidine derivative or the salt thereof can be administered intravenously, orally, intramuscularly,
15 subcutaneously or by some other administration route. Further, the phenylamidine derivative or the salt thereof represented by the general formula [1] can also be administered simultaneously, separately, or in a specific order, with the azole antifungal agents,
20 polyene antifungal agents, candin antifungal agents and fluoropyrimidine antifungal agents.

[0026]

The pharmaceutical composition of the present invention exhibits excellent action against fungi such
25 as *Candida*, *Cryptococcus* and *Aspergillus*. The pharmaceutical composition of the present invention exhibits especially excellent action against *Candida* such as *Candida albicans*, *Candida glabrata*, *Candida*

guilliermondii, Candida kefyr, Candida krusei, Candida parapsilosis, Candida stellatoidea, Candida tropicalis and Candida lusitaniae; Cryptococcus such as Cryptococcus neoformans; Aspergillus such as Aspergillus clavatus, Aspergillus flavus, Aspergillus fumigatus, Aspergillus nidulans, Aspergillus niger, Aspergillus terreus, Aspergillus versicolor and Aspergillus restrictus.

[0027]

10 The pharmaceutical composition of the present invention is effective in the prevention and treatment of a variety of fungal infections, such as candidosis, cryptococcosis and aspergillosis.

 With the pharmaceutical composition of the present invention, more serious fungal infections can be treated. In addition, since a strong antifungal action is exhibited even if the amount of each of the agents that are administered is lowered, the side effects of the respective agents can be reduced.

20 [0028]

 When pharmaceutical compositions of the present invention are used, pharmaceutical aids such as excipients, carrier and diluting agents, those of which are used by pharmaceutical preparations, may be usually mixed appropriately, and these can be administered to conventional methods in oral or parenteral in morphology such as tablets, encapsulated formulations, powders, syrups, granules, pills, suspending agents,

emulsions, liquid drugs, powder formulations, suppositories, ophthalmic washes, nose drops, ear drops, patches, ointments or injections. In addition, medication methods, dosages and the number of
5 medication can be selected appropriately according to an age, body weight and symptom of a patient. Usually, for an adult, dosage of 0.01 to 1000mg/kg may be divided into several portions and administered once a day from one to several times by oral or parenteral
10 administration (for example, injection, continuous infusion and administration to rectum locus).

EXAMPLE

[0029]

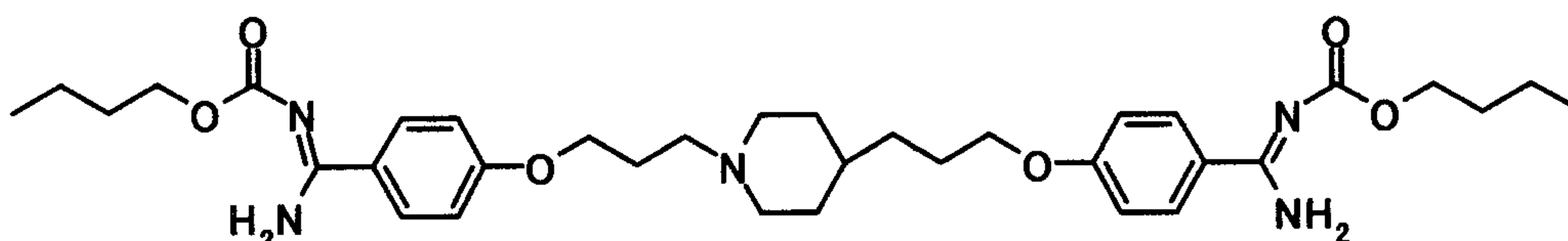
The present invention will now be described
15 in more detail with Test examples. However, the present invention is not limited to these examples.

The respective abbreviations have the following meaning.

FLCZ: fluconazole; MCFG: micafungin; AMPH-B:
20 amphotericin B; 5-FC: flucytosine; VCZ: voriconazole
[0030]

The following compound was selected as the test compound. The chemical structural formula of this compound is illustrated below.

[Chemical Formula 3]



[0031]

As the agents, fluconazole, micafungin, amphotericin B, voriconazole and flucytosine were
5 selected.

[0032]

Test example 1: in vivo test (Candida)

In vivo activity was evaluated in murine systemic infection caused by *Candida albicans*.

10 Mice (4-week old (at infection) male ICR mice, 5 mice per group) were intraperitoneally administered 200 mg/kg of cyclophosphamide 4 days before infection and 100 mg/kg on the following day after infection. *Candida albicans* TIMM1623, which were
15 prepared from overnight culture on a Sabouraud Dextrose Agar plate at 35°C, were suspended in sterile physiological saline solution. After counting cell number of the suspension with a biological microscope, the suspension was diluted with sterile physiological
20 saline solution to give the inoculum solution. Systemic infection was induced in mice by intravenous inoculation of 0.2 mL of the inoculum solution into the tail vein (2.6×10^4 CFU/mouse).

The test compound was dissolved in a small

amount of 0.1 mol/L hydrochloric acid, and the solution was diluted with sterilized water to prepare predetermined concentrations.

Fluconazole (a commercial name: FLANOS
5 intravenous drip solution 100mg, made by Toyama Chemical Co., Ltd.) was diluted with sterilized water to prepare fluconazole solutions of predetermined concentrations.

Micafungin sodium (a commercial name:
10 Funguard 50mg for infusion, made by Astellas Pharma Inc.) was dissolved in sterile physiological saline solution to prepare micafungin solutions of predetermined concentrations.

Amphotericin B for injection (a commercial
15 name: FUNGIZONE, made by Bristol pharmaceutical Ltd.) was dissolved in 5% glucose to prepare an amphotericin B solution of a predetermined concentration.

The test compound (0.25 and 0.5 mg/kg) and fluconazole (0.25 and 0.5 mg/kg) were orally
20 administered. Micafungin (0.125 and 0.25 mg/kg) and amphotericin B (0.1 mg/kg) were subcutaneously administered. These administrations were conducted once 2 hours after the infection and then once daily for the following 6 days, totaling 7 times.

25 On one hand each of the agents was administered singly, on the other hand each of the agents was administered immediately after the test compound was administered.

The efficacy was evaluated on the basis of the survival rate on day 21 after infection.

[0033]

The results of the combination therapy of the test compound and fluconazole against infection of *Candida albicans* are shown in Table 1, the results of the combination therapy of the test compound and micafungin are shown in Table 2 and the results of the combination therapy of the test compound and amphotericin B are shown in Table 3.

[0034]

[TABLE 1]

Administration composition	Test compound 0.5mg/kg	FLCZ 0.25mg/kg	FLCZ 0.5mg/kg	Test compound 0.25mg/kg FLCZ 0.25mg/kg
Survival rate	0	20	40	60

[0035]

[TABLE 2]

Administration composition	Test compound 0.5mg/kg	MCFG 0.125mg/kg	MCFG 0.25mg/kg	Test compound 0.5mg/kg MCFG 0.125mg/kg
Survival rate	0	0	40	80

[0036]

[TABLE 3]

Administration composition	Test compound 0.5mg/kg	AMPH-B 0.1mg/kg	Test compound 0.25mg/kg AMPH-B 0.1mg/kg
Survival rate	0	20	80

[0037]

In murine systemic infection caused by *Candida albicans*, the combined administration of the test compound and fluconazole, the test compound and micafungin, and the test compound and amphotericin B exhibited excellent therapeutic effects.

[0038]

Test example 2: in vivo test (*Aspergillus*)

In vivo activity was evaluated in murine systemic infection caused by *Aspergillus fumigatus*.

Mice (4-week old (at infection) male ICR mice, 5 mice per group) were intraperitoneally administered 200 mg/kg of cyclophosphamide 4 days before infection and 100 mg/kg on the following day after infection. Conidia suspension of *Aspergillus fumigatus* IFM46895 was diluted with sterile physiological saline solution containing 0.05% Tween 80 (manufactured by Difco Laboratories) in sterile physiological saline solution to give the inoculum solution. Systemic infection was induced in mice by intravenous inoculation of 0.2mL of the inoculum solution into the tail vein (1.6×10^5 CFU/mouse).

The test compound was dissolved in a small amount of 0.1 mol/L hydrochloric acid, and the solution was diluted with sterilized water to prepare predetermined concentrations.

Flucytosine (made by Sigma Company) was suspended in 0.5% methyl cellulose liquid to prepare

predetermined concentrations for administration.

Voriconazole (a commercial name: Vfend 200 mg for intravenous use, made by Pfizer Inc.) was diluted with sterilized water to prepare voriconazole solutions of predetermined concentrations for administration.

The test compound (1 and 3 mg/kg), flucytosine (50 and 250 mg/kg) and voriconazole (5 and 10 mg/kg) were orally administered. The administrations were conducted once 2 hours after the infection and then once daily for the following 6 days, totaling 7 times.

On one hand each of the agents was administered singly, on the other hand each of the agents was administered immediately after the test compound was administered. The efficacy was evaluated on the basis of the survival rate on day 21 after infection.

[0039]

The results of the combination therapy of the test compound and flucytosine against infection of *Aspergillus fumigatus* are shown in Table 4, and the results of the combination therapy of the test compound and voriconazole are shown in Table 5.

[0040]

[TABLE 4]

Administration composition	Test compound 3mg/kg	5-FC 250mg/kg	Test compound 3mg/kg 5-FC 50mg/kg
Survival rate	20	0	80

[0041]

[TABLE 5]

Administration composition	Test compound 1mg/kg	Test compound 3mg/kg	VCZ 10mg/kg	Test compound 1mg/kg VCZ 5mg/kg
Survival rate	0	20	0	40

[0042]

In murine systemic infection caused by *Aspergillus fumigatus*, the combined administration of the test compound and flucytosine, the test compound and voriconazole exhibited excellent therapeutic effects. Especially, flucytosine, as usual, has almost no effect against *Aspergillus fumigatus*. In the above test, 250mg/kg administration of flucytosine had no therapeutic effect. However, the combined administration of one fifth of 250mg/kg of flucytosine and the test compound showed remarkably excellent antifungal effect.

[0043]

15 Test example 3: in vivo test (*Cryptococcus*)

In vivo activity was evaluated in murine systematic infection caused by *Cryptococcus neoformans*.

Mice (4-week old (at infection) male ICR mice, 5 mice per group) were intraperitoneally administered 200 mg/kg of cyclophosphamide 4 days before infection and 100 mg/kg on the following day after infection. Cryptococcus neoformans ATCC90112 cells, which were prepared from overnight culture on a Sabouraud Dextrose Agar plate at 35°C, were suspended in sterile physiological saline solution. After counting cell number of the suspension with a biological microscope, the suspension was diluted with sterile physiological saline solution to give the inoculum solution. Systematic infection was induced in mice by intravenous injection of 0.2mL of the inoculum solution into the tail vein (8.5×10^4 CFU/mouse).

The test compound was dissolved in a small amount of 0.1 mol/L hydrochloric acid, and the solution was diluted with sterilized water to prepare predetermined concentrations.

Fluconazole (a commercial name: FLANOS intravenous drip solution 100 mg, made by Toyama Chemical Co., Ltd.) was diluted with sterilized water to prepare fluconazole solutions of predetermined concentrations.

The test compound (0.5 and 1 mg/kg) and fluconazole (20 mg/kg) were orally administered. The administrations were conducted once 2 hours after the infection and then once daily for the following 6 days, totaling 7 times.

21

On one hand each of the agents was administered singly, on the other hand each of the agents was administered immediately after the test compound was administered. The efficacy was evaluated
5 on the basis of the survival rate on day 21 after infection.

[0044]

The results of the combination of the test compound and fluconazole against infection caused by
10 *Cryptococcus neoformans* are shown in Table 6.

[0045]

[TABLE 6]

Administration composition	Test compound 1mg/kg	FLCZ 20mg/kg	Test compound 0.5mg/kg FCZ 20mg/kg
Survival rate	0	20	60

[0046]

In murine systematic infection caused by *Cryptococcus neoformans*, the combined administration of
15 the test compound and fluconazole exhibited excellent therapeutic effects.

[0047]

In addition, the test compound did not show toxicity at all when it was orally administered
20 consecutively to mice, which were used the above tests, at 25mg/kg for 2 weeks, and the test compound had high safety.

[0048]

It is clear from the above results that the combination of the phenylamidine derivative or the salt thereof represented by general formula [1] with various antifungal agents or the like exhibits synergistic
5 antifungal activity and treatment effects, and is effective in the treatment of fungal infections caused by fungal pathogens.

[Preparation]

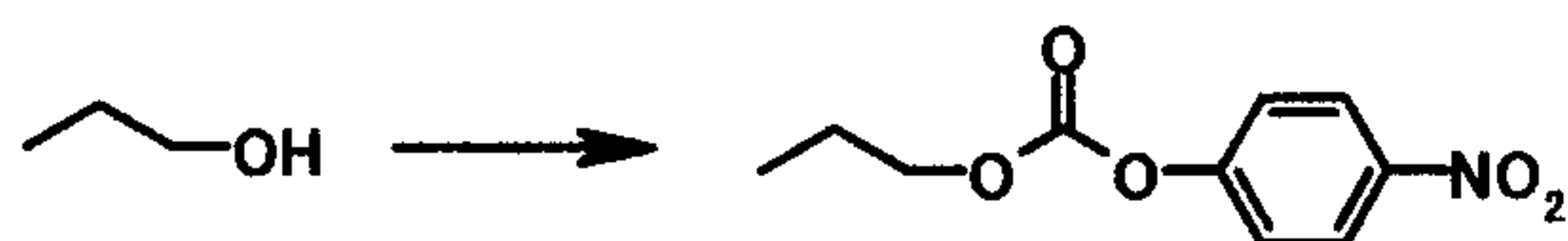
[0049]

10 Next, the present invention will now be described in more detail with Reference examples and Examples. However, the present invention is not limited to these examples.

 The mixing ratio in the eluant is by capacity
15 ratio and the carrier for the silica gel column chromatography is B.W. silica gel, BW-127ZH (Fujisilysia Chemical Ltd).

[0050]

Reference example 1



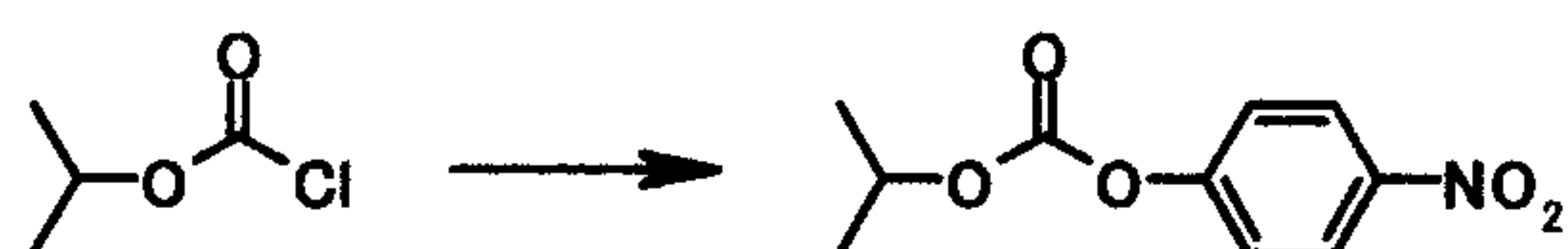
20 To tetrahydrofuran 10mL solution of propanol 0.75g and triethylamine 1.90mL, a tetrahydrofuran 15mL solution of 4-nitrophenyl chloroformate 2.50g was dropped under ice-cooling. Ethyl acetate and water were added to the reaction mixture after stirring at

room temperature for 20 minutes. After organic layer was separated, collected and washed with water and a saturated aqueous sodium chloride solution sequentially, the organic layer was dried over anhydrous magnesium sulfate, and followed by distilling off the solvent. After hexane was added to the residue, filtration of insolubles and removal of the solvent under reduced pressure yielded 4-nitrophenyl propylcarbonate 2.59g as light yellow oil.

¹H-NMR(CDCl₃) δ value: 1.03(3H,t,J=7.4Hz), 1.71-1.85(2H,m), 4.26(2H,t,J=6.7Hz), 7.39(2H,d,J=9.0Hz), 8.28(2H,d,J=9.0Hz)

[0051]

Reference example 2



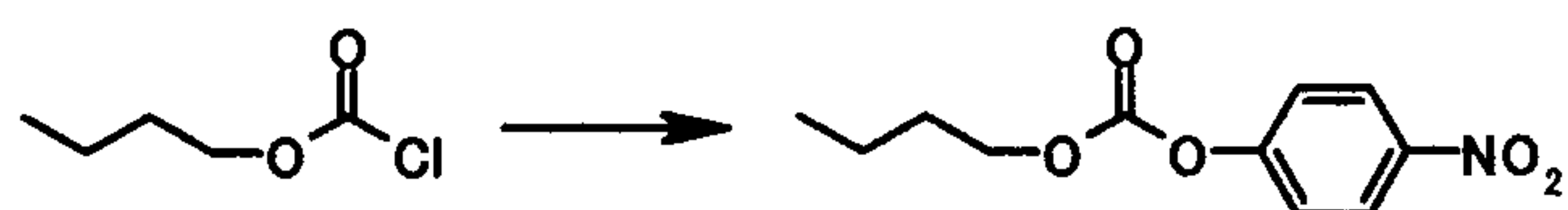
To tetrahydrofuran 30mL solution of 4-nitrophenol 3.00g and triethylamine 3.31mL, isopropyl chloroformate 2.46mL was dropped under ice-cooling. Ethyl acetate and water were added to the reaction mixture after stirring at the same temperature for 10 minutes. After organic layer was separated, collected and washed with a saturated aqueous sodium chloride solution, the organic layer was dried over anhydrous magnesium sulfate, followed by distilling off the solvent under reduced pressure. After the residue was dissolved in 50ml of ethyl acetate and washed with 5%

aqueous solution of potassium carbonate and a saturated aqueous sodium chloride solution sequentially, the organic layer was dried over anhydrous magnesium sulfate and followed by distilling off the solvent under reduced pressure to yield 4-nitrophenyl isopropylcarbonate 3.00g as a light yellow solid.

$^1\text{H-NMR}(\text{CDCl}_3)$ δ value: 1.41(6H,d,J=6.3Hz), 4.96-5.07(1H,m), 7.36-7.41(2H,m), 8.25-8.30(2H,m)

[0052]

10 Reference example 3

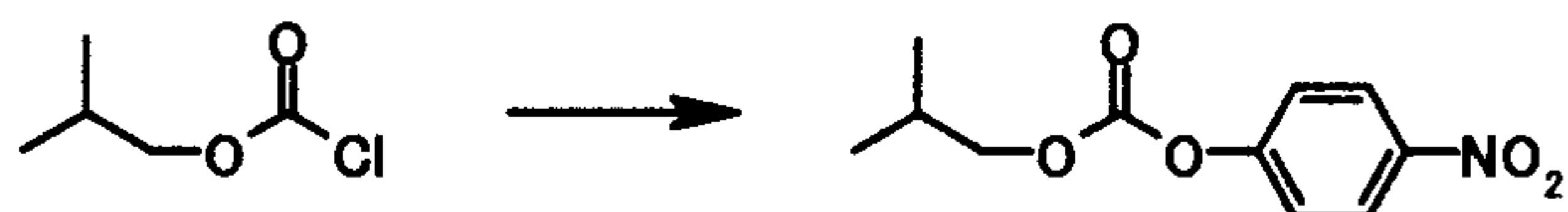


To tetrahydrofuran 30mL solution of 4-nitrophenol 3.00g and triethylamine 3.31mL, butyl chloroformate 2.75mL was dropped under ice-cooling. Ethyl acetate and water were added to the reaction mixture after stirring at the same temperature for 10 minutes. After organic layer was separated, collected and washed with a saturated aqueous sodium chloride solution, the organic layer was dried over anhydrous magnesium sulfate and followed by distilling off the solvent to yield 4-nitrophenyl butylcarbonate 4.60g as light yellow oil.

$^1\text{H-NMR}(\text{CDCl}_3)$ δ value: 0.99(3H,t,J=7.4Hz), 1.41-1.52(2H,m), 1.70-1.80(2H,m), 4.30(2H,t,J=6.6Hz), 7.36-7.41(2H,m), 8.26-8.31(2H,m)

25 [0053]

Reference example 4

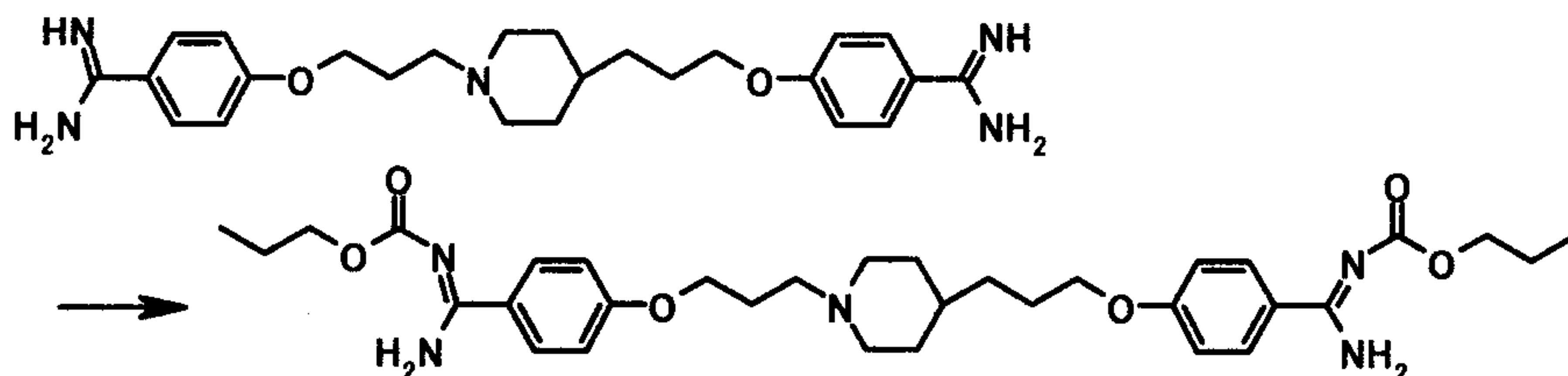


Similarly to the reference example 3,
isobutyl 4-nitrophenyl carbonate 5.63g was obtained as
light yellow oil from 4-nitrophenol 3.00g and isobutyl
5 chloroformate 2.80mL.

¹H-NMR(CDCl₃) δ value: 1.02(6H,d,J=6.6Hz), 2.02-
2.13(1H,m), 4.08(2H,d,J=6.6Hz), 7.39(2H,d,J=9.1Hz),
8.28(2H,d,J=9.1Hz)

[0054]

10 Example 1



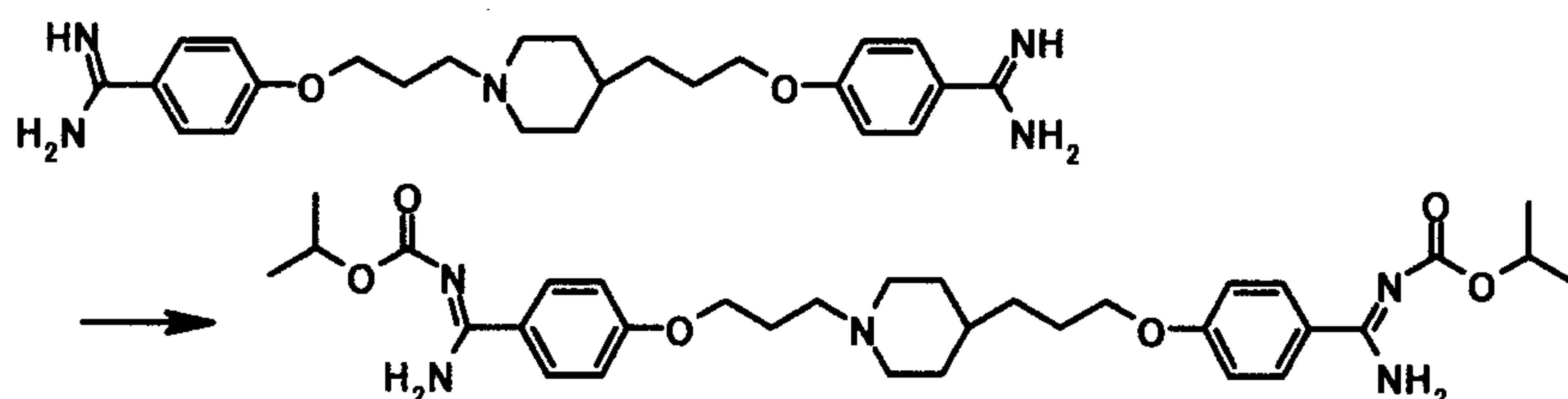
To N,N-dimethylformamide 15mL solution of 4-
nitrophenylpropyl carbonate 1.71g, 4-{3-[4-(3-{4-
[amino(imino)methyl]phenoxy}propyl)-1-
piperidinyl]propoxy}benzamidine 1.50g was added at room
15 temperature, and the solution was stirred at the same
temperature for 4 hours. Chloroform and water were
added to the reaction mixture. After organic layer was
separated, collected and washed with water, 5% aqueous
solution of potassium carbonate twice and a saturated

aqueous sodium chloride solution sequentially, the organic layer was dried over anhydrous magnesium sulfate, and followed by distilling off the solvent. The residue obtained was purified with silica gel column chromatography [eluant, chloroform:methanol=4:1]. The solid obtained was dissolved in chloroform, after the solution was washed with 5% aqueous solution of potassium carbonate and a saturated aqueous sodium chloride solution sequentially, the solution was dried over anhydrous magnesium sulfate, and followed by distilling off the solvent to provide 4-{3-[4-(3-{4-[amino(propoxycarbonylimino)methyl]phenoxy}propyl)-1-piperidinyl]propoxy}-N'-(propoxycarbonyl)benzamidine 1.25g as a white solid.

$^1\text{H-NMR}(\text{CDCl}_3)$ δ value: 0.99(6H,t,J=7.4Hz), 1.22-1.45(5H,m), 1.66-1.86(8H,m), 1.90-2.04(4H,m), 2.46-2.54(2H,m), 2.90-2.98(2H,m), 3.99(2H,t,J=6.5Hz), 4.06(2H,t,J=6.3Hz), 4.11(4H,t,J=7.0Hz), 6.88-6.96(4H,m), 7.82-7.88(4H,m)

[0055]

Example 2



Similarly to the example 1, 4-{3-[4-(3-{4-

[amino(isopropoxycarbonylimino)methyl]phenoxy}propyl)-
1-piperidinyl] propoxy}-N'-

(isopropoxycarbonyl)benzamidine 1.35g of a white solid
was obtained from 4-nitrophenyl isopropyl carbonate

5 1.71g and 4-{3-[4-(3-{4-

[amino(imino)methyl]phenoxy}propyl)-1-
piperidinyl]propoxy}benzamidine 1.50g.

$^1\text{H-NMR}(\text{CDCl}_3)$ δ value: 1.20-1.46(5H,m),

1.34(12H,d,J=6.3Hz), 1.56-1.86(4H,m), 1.88-2.04(4H,m),

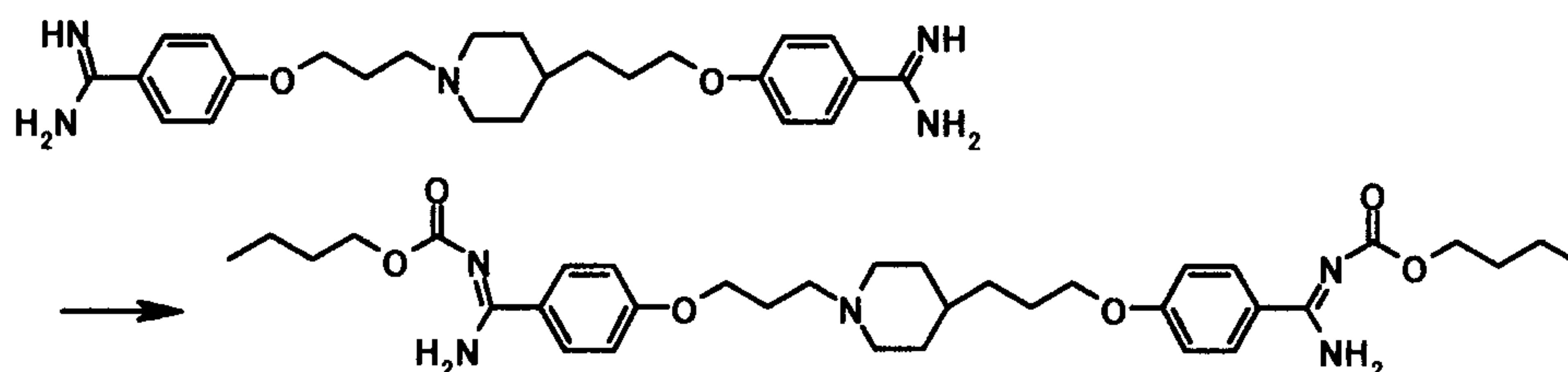
10 2.46-2.54(2H,m), 2.90-2.98(2H,m), 3.99(2H,t,J=6.5Hz),

4.06(2H,t,J=6.3Hz), 4.94-5.04(2H,m), 6.88-6.96(4H,m),

7.80-7.88(4H,m)

[0056]

Example 3



15 Similarly to the example 1, 4-{3-[4-(3-{4-
[amino(butoxycarbonylimino)methyl]phenoxy}propyl)-1-
piperidinyl]propoxy}-N'-(butoxycarbonyl)benzamidine

1.39g of a white solid was obtained from butyl 4-
nitrophenyl carbonate 1.82g and 4-{3-[4-(3-{4-

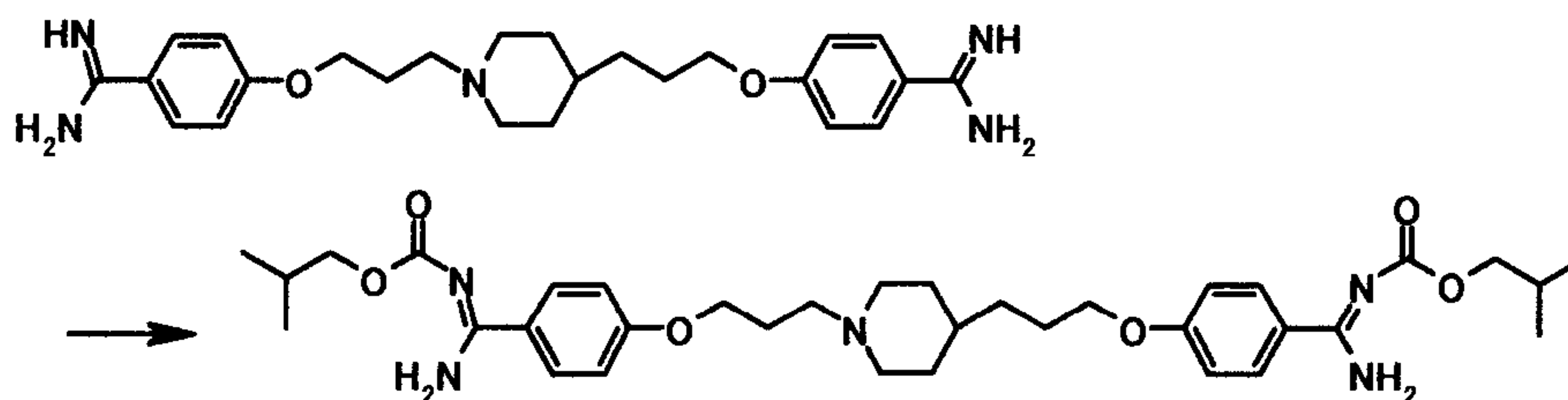
20 [amino(imino)methyl]phenoxy}propyl)-1-
piperidinyl]propoxy}benzamidine 1.50g.

$^1\text{H-NMR}(\text{CDCl}_3)$ δ value: 0.95(6H,t,J=7.3Hz), 1.20-

1.50(9H,m), 1.60-2.05(12H,m), 2.45-2.54(2H,m), 2.90-

3.00 (2H, m), 3.99 (2H, t, J=6.6 Hz), 4.06 (2H, t, J=6.3 Hz),
 4.16 (4H, t, J=6.8 Hz), 6.88-6.96 (4H, m), 7.82-7.88 (4H, m)
 [0057]

Example 4



5 To N,N-dimethylformamide 15mL solution of
 isobutyl 4-nitrophenyl carbonate 1.82g, 4-{3-[4-(3-{4-
 [amino(imino)methyl]phenoxy}propyl)-1-
 piperidinyl]propoxy}benzamidinium 1.50g was added at room
 temperature, and the solution was reacted at the same
 10 temperature for 17 hours. Chloroform and water were
 added to the reaction mixture. After organic layer was
 separated, collected and washed with water, 5% aqueous
 solution of potassium carbonate and a saturated aqueous
 sodium chloride solution sequentially, the organic
 15 layer was dried over anhydrous magnesium sulfate, and
 followed by distilling off the solvent. The residue
 obtained was purified with silica gel column
 chromatography [eluant, chloroform:methanol=4:1]. The
 residue obtained was dissolved in chloroform, after the
 20 solution was washed with 5% aqueous solution of
 potassium carbonate and a saturated aqueous sodium
 chloride solution sequentially, the solution was dried
 over anhydrous magnesium sulfate, and followed by

distilling off the solvent to provide 4-{3-[4-(3-{4-[amino(isobutoxycarbonylimino)methyl]phenoxy}propyl)-1-piperidinyl]propoxy}-N'-(isobutoxycarbonyl)benzamidine 1.43g as a white solid.

5 ¹H-NMR(CDCl₃) δ value: 0.99(12H,d,J=6.8Hz), 1.20-1.45(5H,m), 1.55-2.12(10H,m), 2.46-2.53(2H,m), 2.90-3.00(2H,m), 3.94(4H,d,J=6.8Hz), 3.99(2H,t,J=6.5Hz), 4.06(2H,t,J=6.3Hz), 6.88-6.96(4H,m), 7.80-7.90(4H,m).
[0058]

10 Pharmaceutical example 1

The compound 100mg obtained in the example 3 and sodium chloride 18g were added to water for injection 1.8L. It was adjusted to pH 4 with hydrochloric acid and dissolved, and diluted to 2L of
15 total volume with water for injection. The dissolved solution was filtered through a membrane filter of 0.22 μm, and the obtained pharmaceutical solution 100mL was packed and sealed into an ampule to give injections.
[0059]

20 Pharmaceutical example 2

The compound 500mg obtained in the example 3, lactose 350mg, corn starch 250mg and crystalline cellulose [a commercial name: CEOLUS PH101: Asahi Kasei Chemicals Corporation] 400mg were mixed, 5%
25 hydroxypropylcellulose aqueous solution 0.6mL and water were added to the mixture and the mixture was kneaded. After the mixture obtained was dried at 60°C, cross povidone [a commercial name: Kollidon CL, BASF] 100mg,

light anhydrous silicic acid 100mg and magnesium stearate 20mg were added to the mixture and the mixture was mixed. The mixture 175mg was formulated into circular tablets having a diameter of 8mm to give 5 tablets.

[0060]

Pharmaceutical example 3

The compound 500mg obtained in the example 3, lactose 200mg and corn starch 530mg were mixed, 5% hydroxypropylcellulose aqueous solution 0.6mL and water were added to the mixture and the mixture was kneaded. After the mixture obtained was dried at 60°C, cross povidone [a commercial name: Kollidon CL, BASF] 70mg, crystalline cellulose [a commercial name: CEOLUS PH302, Asahi Kasei Chemicals Corporation] 180mg and magnesium stearate 20mg were added to the mixture, and the resulting mixture was mixed. The mixture 150mg was packed into 3-type gelatin capsule to give capsules.

[Industrial applicability]

20 [0061]

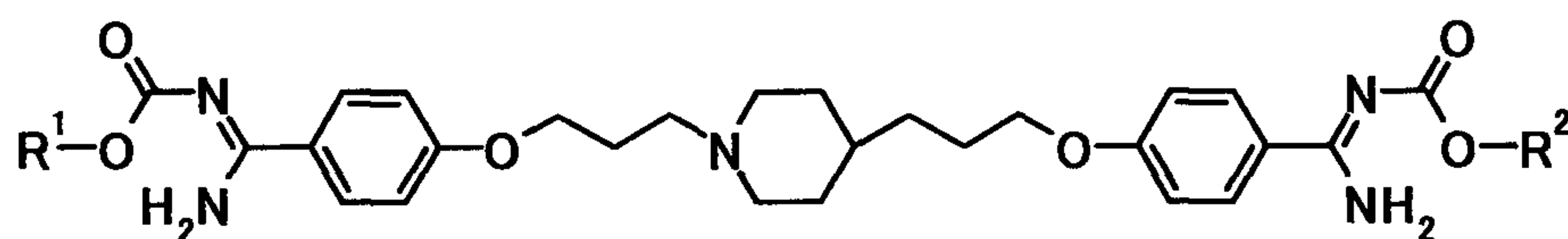
The pharmaceutical composition comprising the new phenylamidine derivative or a salt thereof, and one or more antifungal agents selected from azole antifungal agents, polyene antifungal agents, candin antifungal agents and fluoropyrimidine antifungal agents, has strong antifungal activity and is useful for treating fungal infections. The method for combination use of these antifungal agents is useful as

an excellent treatment method of fungal infections.

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A pharmaceutical composition for treating a fungal infection, comprising:

a phenylamidine derivative or a salt thereof,
represented by a general formula,
[CHEMICAL FORMULA 1]



wherein R^1 and R^2 are the same or different, and represent an optionally substituted C_{3-4} alkyl group; and at least one antifungal agent which is an azole antifungal agent, polyene antifungal agent, candin antifungal agent or fluoropyrimidine antifungal agent, or any combination thereof.

2. The pharmaceutical composition according to claim 1, wherein R^1 and R^2 are the same, and are a C_{3-4} alkyl group.

3. The pharmaceutical composition according to claim 1 or 2, wherein the at least one agent is an azole antifungal agent.

4. The pharmaceutical composition according to claim

3, wherein the azole antifungal agent is a triazole antifungal agent.

5. The pharmaceutical composition according to claim 4, wherein the triazole antifungal agent is fluconazole, fosfluconazole, voriconazole or itraconazole.

6. The pharmaceutical composition according to claim 1 or 2, wherein the at least one agent is a polyene antifungal agent.

7. The pharmaceutical composition according to claim 6, wherein the polyene antifungal agent is amphotericin B or a liposomal formulation thereof.

8. The pharmaceutical composition according to claim 1 or 2, wherein the at least one agent is a candin antifungal agent.

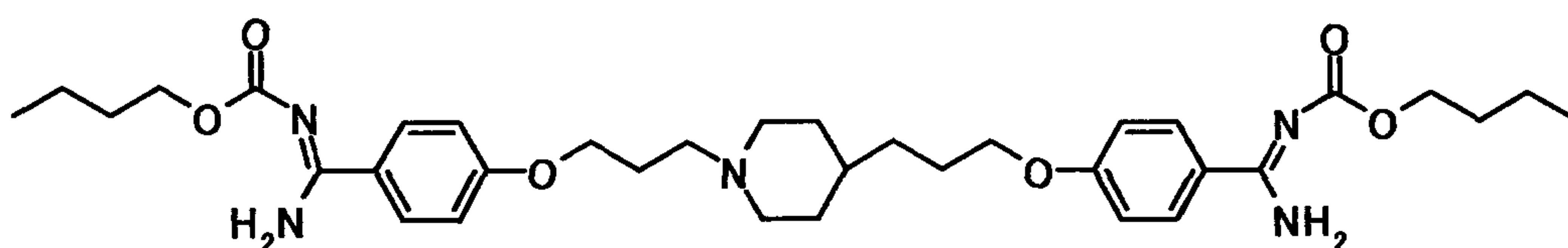
9. The pharmaceutical composition according to claim 8, wherein the candin antifungal agent is micafungin.

10. The pharmaceutical composition according to claim 1 or 2, wherein the at least one agent is a fluoropyrimidine antifungal agent.

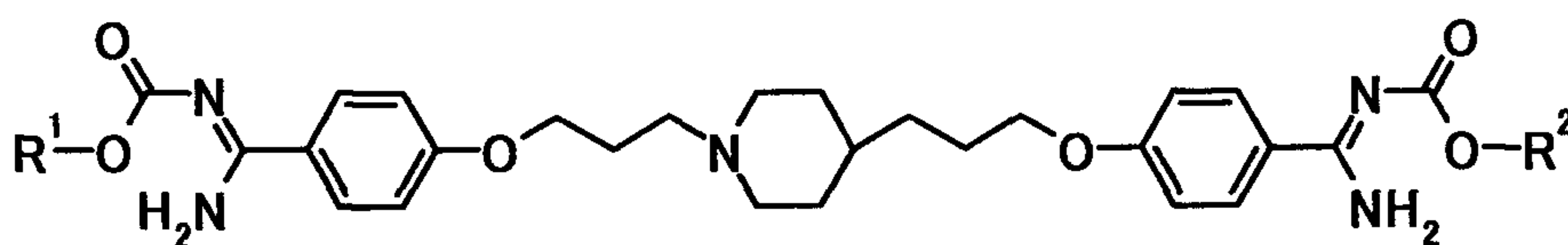
11. The pharmaceutical composition according to any one of claims 1 to 10, wherein the fungal infection is

caused by a fungal pathogen which is Candida, Cryptococcus or Aspergillus.

12. The pharmaceutical composition according to any one of claims 1 to 11, wherein the compound of Formula 1 is [CHEMICAL FORMULA 2]



13. Use of a phenylamidine derivative or a salt thereof, represented by a general formula, [CHEMICAL FORMULA 1]



wherein R^1 and R^2 are the same or different, and represent an optionally substituted C_{3-4} alkyl group; and at least one antifungal agent which is an azole antifungal agent, polyene antifungal agent, candin antifungal agent or fluoropyrimidine antifungal agent, or any combination thereof;

in the manufacture of a medicament for treating a fungal infection caused by a fungal pathogen.

14. The use according to claim 13, wherein R^1 and R^2 are the same, and are a C_{3-4} alkyl group.

15. The use according to claim 13 or 14, wherein the at least one agent is an azole antifungal agent.

16. The use according to claim 15, wherein the azole antifungal agent is a triazole antifungal agent.

17. The use according to claim 16, wherein the triazole antifungal agent is fluconazole, fosfluconazole, voriconazole or itraconazole.

18. The use according to claim 13 or 14, wherein the at least one agent is a polyene antifungal agent.

19. The use according to claim 18, wherein the polyene antifungal agent is amphotericin B or liposomal formulations thereof.

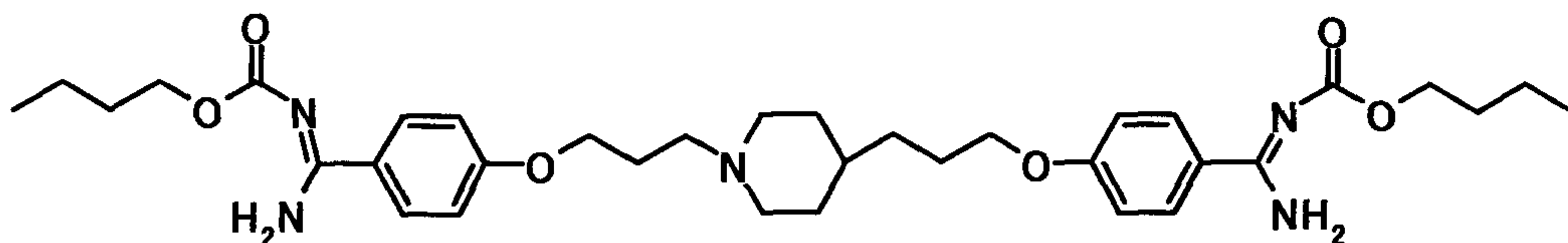
20. The use according to claim 13 or 14, wherein the at least one agent is a candin antifungal agent.

21. The use according to claim 20, wherein the candin antifungal agent is micafungin.

22. The use according to claim 13 or 14, wherein the at least one agent is a fluoropyrimidine antifungal agent.

23. The use according to any one of claims 13 to 22, wherein the fungal infection is caused by a fungal pathogen which is Candida, Cryptococcus or Aspergillus.

24. The use according to any one of claims 13 to 23, wherein the compound of Formula 1 is
[CHEMICAL FORMULA 2]



25. A kit comprising:
a phenylamidine derivative or a salt thereof, as defined in any one of claims 1 to 12;
at least one antifungal agent as defined in any one of claims 1 to 12;
together with instructions for their use in combination for treating a fungal infection caused by a fungal pathogen.

26. The kit according to claim 25, wherein the fungal pathogen is Candida, Cryptococcus or Aspergillus.

[CHEMICAL FORMULA 1]

