(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
16 January 2003 (16.01.2003)

PCT

(10) International Publication Number
WO 03/004098 A1

(51) International Patent Classification[7]: A61P 37/06, 17/00, A61K 31/435, 31/495, 31/535, 38/13

(21) International Application Number: PCT/JP02/06670

(22) International Filing Date: 2 July 2002 (02.07.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/303,148 6 July 2001 (06.07.2001) US

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/004098 A1

(54) Title: COMPOSITION FOR TOPICAL ADMINISTRATION

(57) Abstract: The present invention provides a composition for topical administration comprising an interleukin 2 inhibitor and an antimicrobial agent as active ingredients thereof, wherein said interleukin 2 inhibitor contains a tricyclo compound as shown by the general formula (I) or pharmaceutically acceptable salt thereof. The present invention further provides a method for treating inflammations and/or infections comprising topical administration of an effective amount of an interleukin 2 inhibitor and an antimicrobial agent to a subject in need of the treatment of inflammations and/or infections.

DESCRIPTION

COMPOSITION FOR TOPICAL ADMINISTRATION

TECHNICAL FIELD

The present invention relates to a composition for topical administration comprising an interleukin 2 inhibitor and an antimicrobial agent as active ingredients thereof. More particularly, the present invention relates a composition for topical administration comprising an interleukin 2 inhibitor and an antimicrobial agent as active ingredients thereof for the treatment of inflammations and/or infections.

BACKGROUND ART

Interleukin 2 inhibitor is a substance having interleukin 2 inhibitory activity. Known as examples of such substance are interleukin 2 production inhibitory substance and interleukin 2 signal transduction inhibitory substance. Interleukin 2 is necessary for activating T cells to proliferate. Interleukin 2 inhibitor shows an immunosuppressive effect through the T cell activating mechanism.

In recent years, interleukin 2 inhibitors have been tried for the treatment of various inflammations or diseases accompanying inflammations. For example, macrolide compounds such as FK506 and cyclosporins are known to be effective for the treatment of allergic conjunctivitis, allergic dermatitis and allergic rhinitis (U.S. Patent Nos. 5,514,686, 5,385,907, etc.). Prior to this, the present inventor has reported that interleukin 2 inhibitor(s) comprising macrolide compound(s) such as FK506 are effective for the treatment of dry eye (WO00/66122) and topical ophthalmic treatment of ocular inflammations (U.S. Provisional Application No. 60/283,169, now PCT/JP02/03664).

However, in the topical treatment of various inflammations using interleukin 2 inhibitor, the

immunosuppressive effect caused by interleukin 2 inhibitor may result in such side effects as infections. Accordingly, it is desired to develop anti-inflammatory agents reducing such side effects without adversely affecting main effects of interleukin 2 inhibitor.

Meanwhile, antimicrobial agents have been used for the ocular or dermal infection and the prevention of postoperative infection. In treating such infections, it is very important to prevent the proliferation and spread of microorganism at pathologically changed locations. Likewise, it is very important to inhibit the accompanying inflammations or an excessive immuno-inflammatory reaction following phylaxis. In order to meet such requirements, steroid drugs are mainly used as additional anti-inflammatory agents. However, using steroid drugs has the risk of causing such side effects as accentuation of infections by microorganism at pathologically changed locations; thinning of skin and pilosebaceous abnormal activation; weakened vascular wall in the air duct or nasal cavity; and steroidal glaucoma in the eye. Therefore, it is desired to develop safe and effective antimicrobial agent for the treatment of infections and their accompanying inflammations or infections showing an excessive immuno-inflammatory reaction following phylaxis.

DISCLOSURE OF THE INVENTION

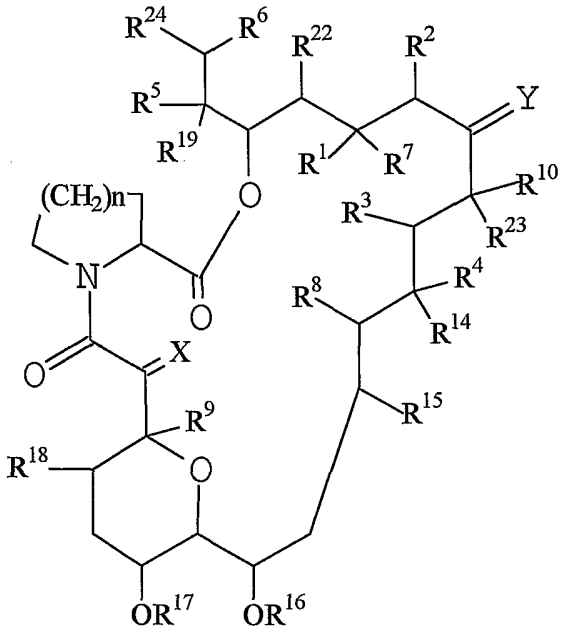
The present inventor has conducted intensive studies and found that in the topical treatment of inflammations and/or infections, the combined interleukin 2 (hereinafter, may be simply referred to as IL-2) inhibitor and antimicrobial agent will inhibit inflammations and/or infections without adversely affecting main effects of each agent, which has resulted in the completion of the present invention.

Accordingly, the present invention provides the following.

(1) A composition for topical administration comprising an interleukin 2 inhibitor and an antimicrobial agent as active ingredients thereof.

(2) The composition of (1), wherein said interleukin 2 inhibitor is a macrolide compound or cyclosporin.

(3) The composition of (2), wherein said macrolide compound is a tricyclo compound as shown by the following general formula (I) or pharmaceutically acceptable salt thereof.



(I)

wherein adjacent pairs of R^1 and R^2, R^3 and R^4, and R^5 and R^6 each independently

a) show two adjacent hydrogen atoms, wherein R^2 is optionally alkyl, or

b) form another bond optionally between carbon atoms binding with the members of said pairs;

R^7 is hydrogen atom, hydroxy, alkyloxy or protected hydroxy, or may form oxo with R^1;

R^8 and R^9 each independently show hydrogen atom or hydroxy;

R^{10} is hydrogen atom, alkyl, alkenyl, alkyl substituted by one or more hydroxy, alkenyl substituted by one or more hydroxy or alkyl substituted by oxo;

X is oxo, (hydrogen atom, hydroxy), (hydrogen atom,

hydrogen atom), or a group of the formula $-CH_2O-$;

Y is oxo, (hydrogen atom, hydroxy), (hydrogen atom, hydrogen atom), or a group of the formula $N-NR^{11}R^{12}$ or $N-OR^{13}$;

R^{11} and R^{12} each independently show hydrogen atom, alkyl, aryl or tosyl;

R^{13}, R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19}, R^{22} and R^{23} each independently show hydrogen atom or alkyl;

R^{24} is an optionally substituted ring that may contain one or more heteroatom(s); and

n is 1 or 2.

In addition to the meaning noted above, Y, R^{10} and R^{23} may show, together with the carbon atom they bind with, a saturated or unsaturated 5 or 6-membered heterocyclic group containing nitrogen atom, sulfur atom and/or oxygen atom, wherein the heterocyclic group may be optionally substituted by one or more group(s) selected from the group consisting of alkyl, hydroxy, alkyloxy, benzyl, a group of the formula $-CH_2Se(C_6H_5)$, and alkyl substituted by one or more hydroxy.

(4) The composition of (3), wherein said tricyclo compound is FK506.

(5) The composition of any of (1) to (4), wherein said antimicrobial agent is quinolone antimicrobial agent.

(6) The composition of (5), wherein said quinolone antimicrobial agent is nalidixic acid, pipemidic acid, piromidic acid, norfloxacin, ofloxacin, levofloxacin, ciprofloxacin, lomefloxacin, tosufloxacin, fleroxacin, sparfloxacin, enrofloxacin and enoxacin or a mixture thereof.

(7) The composition of (6), wherein said quinolone antimicrobial agent is ofloxacin.

(8) A method for treating inflammations and/or infections comprising topical administration of an effective amount of an interleukin 2 inhibitor and an effective amount of an antimicrobial agent to a subject in need of the treatment of inflammations and/or infections.

(9) A use of an interleukin 2 inhibitor and an antimicrobial agent for manufacturing a composition for topical administration for the treatment of inflammations and/or infections.

(10) The use of (9), wherein said interleukin 2 inhibitor is a macrolide compound or cyclosporin.

(11) The use of (10), wherein said macrolide compound is a tricyclo compound as shown by the general formula (I) or pharmaceutically acceptable salt thereof.

(12) The use of (11), wherein said tricyclo compound is FK506.

(13) The use of any of (9) to (12), wherein said antimicrobial agent is quinolone antimicrobial agent.

(14) The use of (13), wherein said quinolone antimicrobial agent is nalidixic acid, pipemidic acid, piromidic acid, norfloxacin, ofloxacin, levofloxacin, ciprofloxacin, lomefloxacin, tosufloxacin, fleroxacin, sparfloxacin, enrofloxacin and enoxacin or a mixture thereof.

(15) The use of (14), wherein said quinolone antimicrobial agent is ofloxacin.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a composition for topical administration comprising an interleukin 2 inhibitor and an antimicrobial agent as active ingredients thereof.

The present invention further relates to a method for treating inflammations and/or infections comprising topical administration of an effective amount of an interleukin 2 inhibitor and an antimicrobial agent to a subject in need of the treatment of inflammations and/or infections.

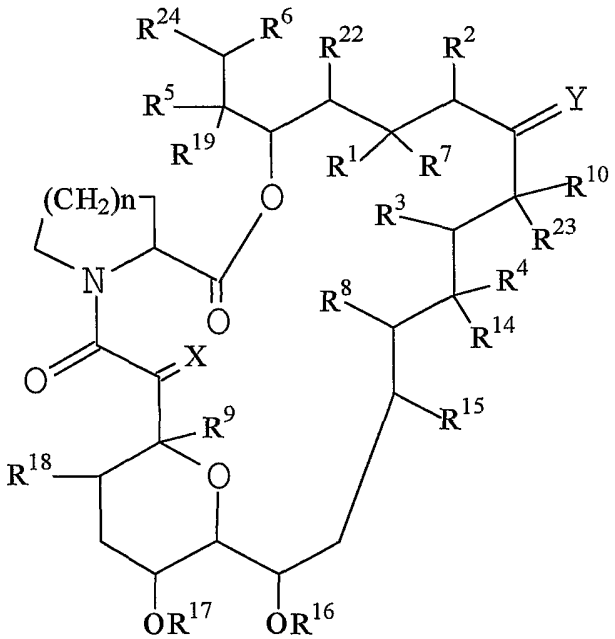
Moreover, the present invention relates to a use of an interleukin 2 inhibitor and an antimicrobial agent for manufacturing a composition for topical administration for the treatment of inflammations and/or infections.

The present IL-2 inhibitor should not be particularly limited, as far as they have IL-2 inhibitory activity. A

specific example of such agents is IL-2 production inhibitory substance. Another specific example of such agent is IL-2 signal transduction inhibitory substance. Preferred specific examples of the above are macrolide compounds (e.g., FK506, ascomycin derivative and rapamycin derivative) and cyclosporins. A single or a combination of two or more IL-2 inhibitors may be used.

The present invention encompasses an embodiment wherein an IL-2 inhibitor and an antimicrobial agent are contained in a single pharmaceutical preparation and an embodiment wherein they are separately formed into pharmaceutical preparations and topically administered simultaneously, which is what is called a combination use.

A specific example of the macrolide compound is a tricyclo compound as shown by the following general formula (I) or pharmaceutically acceptable salt thereof.



(I)

wherein adjacent pairs of R^1 and R^2, R^3 and R^4, and R^5 and R^6 each independently

a) consist of two adjacent hydrogen atoms, wherein R^2 is optionally alkyl, or

b) form another bond optionally between carbon atoms

binding with the members of said pairs;

R^7 is hydrogen atom, hydroxy, alkyloxy or protected hydroxy, or may form oxo with R^1;

R^8 and R^9 each independently show hydrogen atom or hydroxy;

R^{10} is hydrogen atom, alkyl, alkenyl, alkyl substituted by one or more hydroxy, alkenyl substituted by one or more hydroxy or alkyl substituted by oxo;

X is oxo, (hydrogen atom, hydroxy), (hydrogen atom, hydrogen atom), or a group of the formula $-CH_2O-$;

Y is oxo, (hydrogen atom, hydroxy), (hydrogen atom, hydrogen atom), or a group of the formula $N-NR^{11}R^{12}$ or $N-OR^{13}$;

R^{11} and R^{12} each independently show hydrogen atom, alkyl, aryl or tosyl;

R^{13}, R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19}, R^{22} and R^{23} each independently show hydrogen atom or alkyl;

R^{24} is an optionally substituted ring that may contain one or more heteroatom(s); and

n is 1 or 2.

In addition to the meaning noted above, Y, R^{10} and R^{23} may show, together with the carbon atom they bind with, a saturated or unsaturated 5 or 6-membered heterocyclic group containing nitrogen atom, sulfur atom and/or oxygen atom, wherein the heterocyclic group may be optionally substituted by one or more group(s) selected from the group consisting of alkyl, hydroxy, alkyloxy, benzyl, a group of the formula $-CH_2Se(C_6H_5)$, and alkyl substituted by one or more hydroxy, or its pharmaceutically acceptable salt.

In the general formula (I), preferable R^{24} is, for example, cyclo(C_5-C_7)alkyl optionally having suitable substituent, such as the following.

(a) 3,4-dioxocyclohexyl;

(b) 3-R^{20}-4-R^{21}-cyclohexyl,

wherein R^{20} is hydroxy, alkyloxy or $-OCH_2OCH_2CH_2OCH_3$, and R^{21} is hydroxy, -OCN, alkyloxy, heteroaryloxy optionally

having suitable substituent, $-OCH_2OCH_2CH_2OCH_3$, protected hydroxy, chloro, bromo, iodo, aminooxalyloxy, azide, p-tolyloxythiocarbonyloxy, or $R^{25}R^{26}CHCOO-$ (wherein R^{25} is hydroxy optionally protected where desired or protected amino, and R^{26} is hydrogen atom or methyl, or R^{20} and R^{21} in combination form an oxygen atom of epoxide ring); or

(c) cyclopentyl wherein cyclopentyl is substituted by methoxymethyl, optionally protected hydroxymethyl where desired, acyloxymethyl (wherein acyl moiety is optionally quaternized dimethylamino or optionally esterified carboxy), one or more optionally protected amino and/or hydroxy, or aminooxalyloxymethyl. Preferable examples include 2-formylcyclopentyl.

The definition of each symbol used in the formula (I), specific examples thereof and preferable embodiments thereof will be explained in detail in the following.

"Lower" means a group having 1 to 6 carbon atoms unless otherwise indicated.

Preferable examples of the alkyl moiety of "alkyl" and "alkyloxy" include linear or branched aliphatic hydrocarbon residue, such as lower alkyl (e.g., methyl, ethyl, propyl, isopropyl, butyl, isobutyl, pentyl, neopentyl, hexyl and the like).

Preferable examples of "alkenyl" include linear or branched aliphatic hydrocarbon residue having one double bond, such as lower alkenyl (e.g., vinyl, propenyl (e.g., allyl and the like), butenyl, methylpropenyl, pentenyl, hexenyl and the like).

Preferable examples of "aryl" include phenyl, tolyl, xylyl, cumenyl, mesityl, naphthyl and the like.

Preferable examples of the protective group for "protected hydroxy" and "protected amino" include 1-(loweralkylthio)(lower)alkyl such as lower alkylthiomethyl

(e.g., methylthiomethyl, ethylthiomethyl, propylthiomethyl, isopropylthiomethyl, butylthiomethyl, isobutylthiomethyl, hexylthiomethyl and the like), with more preference given to C_1 - C_4 alkylthiomethyl and most preference given to methylthiomethyl;

tri-substituted silyl such as tri(lower)alkylsilyl (e.g., trimethylsilyl, triethylsilyl, tributylsilyl, tert-butyl dimethylsilyl, tri-tert-butylsilyl and the like), and lower alkyldiarylsilyl (e.g., methyldiphenylsilyl, ethyldiphenylsilyl, propyldiphenylsilyl, tert-butyldiphenylsilyl and the like), with more preference given to tri(C_1 - C_4)alkylsilyl and C_1 - C_4 alkyldiphenylsilyl, and most preference given to tert-butyl-dimethylsilyl and tert-butyldiphenylsilyl;

acyl such as aliphatic acyl, aromatic acyl and aliphatic acyl substituted by aromatic group, which are derived from carboxylic acid, sulfonic acid and carbamic acid; and the like.

The aliphatic acyl is exemplified by lower alkanoyl optionally having one or more suitable substituent(s) (e.g., carboxy) such as formyl, acetyl, propionyl, butyryl, isobutyryl, valeryl, isovaleryl, pivaloyl, hexanoyl, carboxyacetyl, carboxypropionyl, carboxybutyryl, carboxyhexanoyl and the like;

cyclo(lower)alkyloxy(lower)alkanoyl optionally having one or more suitable substituent(s) (e.g., lower alkyl) such as cyclopropyloxyacetyl, cyclobutyloxypropionyl, cycloheptyloxybutyryl, mentyloxyacetyl, mentyloxypropionyl, mentyloxybutyryl, mentyloxypentanoyl, mentyloxyhexanoyl and the like;

camphorsulfonyl;

lower alkylcarbamoyl having one or more suitable substituent(s) such as carboxy or protected carboxy and the like, such as carboxy(lower)alkylcarbamoyl (e.g.,

carboxymethylcarbamoyl, carboxyethylcarbamoyl, carboxypropylcarbamoyl, carboxybutylcarbamoyl, carboxypentylcarbamoyl, carboxyhexylcarbamoyl) and tri(lower)alkylsilyl(lower)alkyloxycarbonyl(lower)alkyl-carbamoyl (e.g., trimethylsilylmethoxycarbonylethylcarbamoyl, trimethylsilylethoxycarbonylpropylcarbamoyl, triethylsilylethoxycarbonylpropylcarbamoyl, tert-butyl dimethylsilylethoxycarbonylpropylcarbamoyl, trimethylsilylpropoxycarbonylbutylcarbamoyl); and the like.

Aromatic acyl is exemplified by aroyl optionally having one or more suitable substituent(s) (e.g., nitro), such as benzoyl, toluoyl, xyloyl, naphthoyl, nitrobenzoyl, dinitrobenzoyl, nitronaphthoyl and the like; and arenesulfonyl optionally having one or more suitable substituent(s) (e.g., halogen), such as benzenesulfonyl, toluenesulfonyl, xylenesulfonyl, naphthalenesulfonyl, fluorobenzenesulfonyl, chlorobenzenesulfonyl, bromobenzenesulfonyl, iodobenzenesulfonyl and the like.

The aliphatic acyl substituted by aromatic group may be, for example, ar(lower)alkanoyl optionally having one or more suitable substituent(s) (e.g., lower alkyloxy or trihalo(lower)alkyl and the like), wherein specific examples are phenylacetyl, phenylpropionyl, phenylbutyryl, 2-trifluoromethyl-2-methoxy-2-phenylacetyl, 2-ethyl-2-trifluoromethyl-2-phenylacetyl, 2-trifluoromethyl-2-propoxy-2-phenylacetyl and the like.

Of the above-mentioned acyl, more preferable acyl includes C_1 - C_4 alkanoyl optionally having carboxy, cyclo(C_5 - C_6)alkyloxy(C_1 - C_4)alkanoyl having two (C_1 - C_4)alkyl in the cycloalkyl moiety, camphorsulfonyl, carboxy (C_1 - C_4)alkylcarbamoyl, tri(C_1 - C_4)alkylsilyl(C_1 - C_4)alkyloxycarbonyl(C_1 - C_4)alkylcarbamoyl, benzoyl optionally having one or two nitro groups, and benzenesulfonyl having halogen, phenyl(C_1 - C_4)alkanoyl having C_1 - C_4 alkyloxy and

trihalo(C_1 - C_4)alkyl. Of these, most preferred are acetyl, carboxypropionyl, mentyloxyacetyl, camphorsulfonyl, benzoyl, nitrobenzoyl, dinitrobenzoyl, iodobenzenesulfonyl, 2-trifluoromethyl-2-methoxy-2-phenylacetyl and the like.

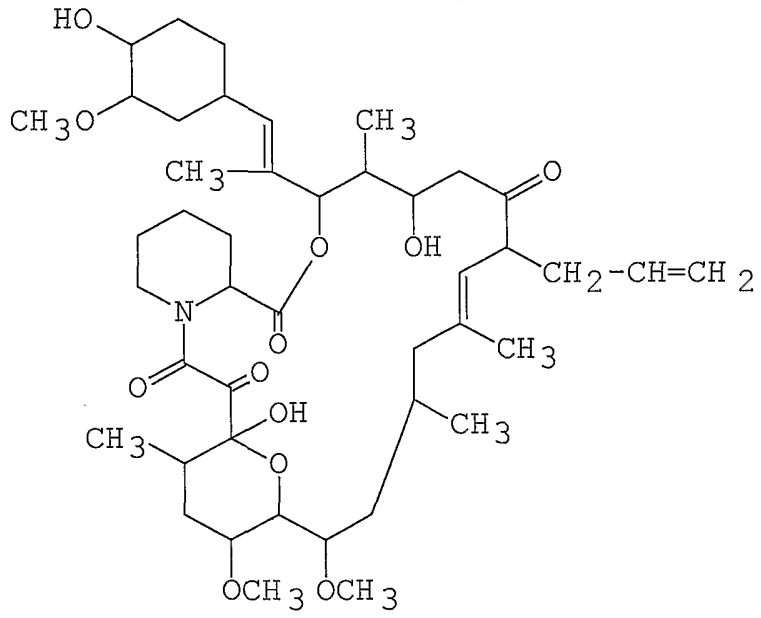
Preferable examples of the "heterocyclic group consisting of saturated or unsaturated 5 or 6-membered ring having nitrogen atom, sulfur atom and/or oxygen atom" are pyrrolyl, tetrahydrofuryl and the like.

The "heteroaryl optionally having a suitable substituent" moiety of the "heteroaryloxy optionally having a suitable substituent" is that exemplified for R^1 of the compound of the formula I of EP-A-532,088, with preference given to 1-hydroxyethylindol-5-yl. The disclosure is incorporated herein by reference.

The tricyclo compound (I) used in the present invention is described in the publications EP-A-184162, EP-A-323042, EP-A-423714, EP-A-427680, EP-A-465426, EP-A-480623, EP-A-532088, EP-A-532089, EP-A-569337, EP-A-626385, WO89/05303, WO93/05058, WO96/31514, WO91/13889, WO91/19495, WO93/5059 and the like. The disclosures of these publications are incorporated herein by reference.

In particular, the compounds called FR900506 (=FK506), FR900520 (Ascomycin), FR900523 and FR900525 are produced by the genus *Streptomyces*, such as *Streptomyces tsukubaensis*, No. 9993 (depository: National Institute of Advanced Industrial Science and Technology, International Patent Organism Depositary, Central 6, 1-1, Higashi 1-chome, Tsukuba-shi, Ibaraki-ken, Japan (formerly: Fermentation Research Institute, Agency of Industrial Science and Technology, the Ministry of International Trade and Industry), date of deposit: October 5, 1984, deposit number: FERM BP-927) or *Streptomyces hygroscopicus subsp. Yakushimaensis*, No. 7238 (depository: National Institute of Advanced Industrial Science and Technology, International Patent Organism Depositary, Central

6, 1-1, Higashi 1-chome, Tsukuba-shi, Ibaraki-ken, Japan (formerly: National Institute of Bioscience and Human-Technology Agency of Industrial Science and Technology, the Ministry of International Trade and Industry), date of deposit: January 12, 1985, deposit number: FERM BP-928 (EP-A-0184162)), and the compound of the following formula, FK506 (general name: Tacrolimus) is a representative compound.



Chemical name: 17-allyl-1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone

Of the tricyclo compounds (I), more preferred is a compound wherein adjacent pairs of R^3 and R^4, and R^5 and R^6 each independently form another bond optionally between carbon atoms binding with the members of said pairs;

R^8 and R^{23} each independently show hydrogen atom;

R^9 is hydroxy;

R^{10} is methyl, ethyl, propyl or allyl;

X is (hydrogen atom, hydrogen atom) or oxo;

Y is oxo;

R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19} and R^{22} each independently show methyl;

R^{24} is 3-R^{20}-4-R^{21}-cyclohexyl,

wherein R^{20} is hydroxy, alkyloxy or $-OCH_2OCH_2CH_2OCH_3$, and R^{21} is hydroxy,-OCN, alkyloxy, heteroaryloxy optionally having suitable substituent, $-OCH_2OCH_2CH_2OCH_3$, protected hydroxy, chloro, bromo, iodo, aminooxalyloxy, azide, p-tolyloxythiocarbonyloxy

or $R^{25}R^{26}CHCOO-$ (wherein R^{25} is optionally protected hydroxy as desired, or protected amino, and R^{26} is hydrogen atom or methyl), or R^{20} and R^{21} in combination form an oxygen atom of epoxide ring; and

n is 1 or 2.

Particularly preferable tricyclo compounds (I) include, besides FK506, Ascomycin derivatives such as halogenated derivative of 33-epi-chloro-33-desoxy Ascomycin described in Example 66a of EP-A-427,680 and the like.

Other preferable IL-2 inhibitor (macrolide compound) include Rapamycin described in MERCK INDEX, 12 edition, No. 8288 and derivatives thereof. Preferable examples thereof include O-substituted derivative described at page 1 of WO95/16691, formula A, wherein the 40th hydroxy is $-OR_1$ (wherein R_1 is hydroxyalkyl, hydroalkyloxyalkyl, acylaminoalkyl and aminoalkyl), such as 40-O-(2-hydroxy)ethyl Rapamycin, 40-O-(3-hydroxy)propyl Rapamycin, 40-O-[2-(2-hydroxy)ethoxy]ethyl Rapamycin and 40-O-(2-acetaminoethyl)-Rapamycin. These O-substituted derivatives can be produced by reacting, under appropriate conditions, Rapamycin (or dihydro or deoxo Rapamycin) and an organic radical bound with leaving group (e.g., RX wherein R is an organic radical desirable as O-substituent, such as alkyl, allyl and benzyl moiety, and X is a leaving group such as $CCl_3C(NH)O$ and CF_3SO_3)). The conditions may be: when X is $CCl_3C(NH)O$, acidic or neutral

conditions, such as in the presence of trifluoromethanesulfonic acid, camphorsulfonic acid, p-toluenesulfonic acid or their corresponding pyridinium or substituted pyridinium salt, and when X is CF_3SO_3, in the presence of a base such as pyridine, substituted pyridine, diisopropylethylamine and pentamethylpiperidine. The most preferable Rapamycin derivative is 40-O-(2-hydroxy)ethyl Rapamycin as disclosed in WO94/09010, which is hereby incorporated into the specification by reference.

The pharmaceutically acceptable salt of tricyclo compound (I), Rapamycin and derivatives thereof are nontoxic and pharmaceutically acceptable conventional salts, which are exemplified by salts with inorganic or organic base such as alkali metal salt (e.g., sodium salt, potassium salt and the like), alkaline earth metal salt (e.g., calcium salt, magnesium salt and the like), ammonium salt, and amine salt (e.g., triethylamine salt, N-benzyl-N-methylamine salt and the like).

In the IL-2 inhibitor of the present invention, particularly macrolide compound, conformer or one or more pairs of stereoisomers, such as optical isomers and geometric isomers due to asymmetric carbon atom and double bond may be present. Such conformers and isomers are also encompassed in the present invention. In addition, macrolide compounds can form solvates, which case is also encompassed in the present invention. Preferable solvate is exemplified by hydrates and ethanolates.

Other IL-2 inhibitors are known from MERCK INDEX, 12th ed., No. 2821, US Patent Nos. 4,117,118, 4,215,199, 4,288,431, 4,388,307, Helv. Chim. Acta, *60*, 1568 (1977) and *65*, 1655 (1982) and Transplant. Proc. *17*, 1362 (1985) and the like. Specifically, they are cyclosporins such as cyclosporin A, B, C, D, E, F and G and derivatives thereof. Particularly preferred is cyclosporin A. The disclosures of these

publications are incorporated into the specification by reference.

The tricyclo compound (I), pharmaceutically acceptable salt thereof, cyclosporins and derivatives thereof can be classified as "IL-2 production inhibitor" that inhibits production of IL-2. Rapamycin and derivative thereof can be classified as "IL-2 signal transduction inhibitor" that inhibit transmission of IL-2 signal.

The tricyclo compound (I) and its pharmaceutically acceptable salt are nontoxic and pharmaceutically acceptable conventional salts, which are exemplified by salts with inorganic or organic base such as alkali metal salt (e.g., sodium salt, potassium salt and the like), alkaline earth metal salt (e.g., calcium salt, magnesium salt and the like), ammonium salt, and amine salt (e.g., triethylamine salt, N-benzyl-N-methylamine salt and the like).

In the tricyclo compound of the present invention, conformers or one or more pairs of stereoisomers such as optical isomers and geometric isomers due to asymmetric carbon atom and double bond may be present. Such conformers and isomers are also encompassed in the present invention. In addition, the tricyclo compound can form solvates, which case is also encompassed in the present invention. Examples of preferable solvates include hydrates and ethanolates.

The present antimicrobial agents are not particularly limited, unless they adversely affect the IL-2 inhibitor's inhibitory activity, and preference is given to those antimicrobial agents having no IL-2 inhibitory action. Further preference is given to those antimicrobial agents having a different structure from the macrolide compound (especially the one as shown by the formula (I)) and cyclosporins. Preferred examples of such antimicrobial agents are as follows: penicillins (e.g., benzylpenicillin, methicillin, oxacillin, cloxacillin, ampicillin, hetacillin,

carbenicillin, sulbenicillin, bacampicillin, amoxicillin, ticarcillin, piperacillin and aspoxicillin); cephalosporins (e.g., cephalothin, cefazolin, cefotiam, cefotaxime, cefoperazone, ceftizoxime, cefmenoxime, cefpiramide, ceftazidime, cefodizime, cefpiome, cefepime, cefozopran, cefsulodin and cefoselis); cephamycins (e.g., cefoxitin, cefmetazole and cefminox); oxacephems (e.g., latamoxef and flomoxef); monobactams (e.g., aztreonam); carbapenems (e.g., meropenem); penems (e.g., faropenem); aminoglycosides (e.g., amikacin, tobramycin, dibekacin, arbekacin, gentamicin and isepamicin); lincomycins (e.g., lincomycin and clindamycin); tetracyclines (e.g., oxytetracycline, doxycycline and minocycline); chloramphenicols (e.g., chloramphenicol and thiamphenicol); quinolones (nalidixic acid, pipemidic acid, piromidic acid, norfloxacin, ofloxacin, levofloxacin, ciprofloxacin, lomefloxacin, tosufloxacin, fleroxacin, sparfloxacin, enrofloxacin and enoxacin); and glycopeptides (e.g., vancomycin and teicoplanin). Preference is given to quinolone antimicrobial agent, with special preference given to ofloxacin. A single or a combination of two or more antimicrobial agents may be used.

The term "treatment" used herein includes any means of control such as prevention, care, relief of the condition, attenuation of the condition and arrest of progression.

The present composition is topically administered to such location as eye, skin, air duct, nasal cavity, labial, pubis and pudenda.

In the case of administering a formulation, the formulation manufactured by conventional methods may be administered, which includes all the formulations for topical ocular administration used in the field of ophthalmology (e.g., eye drops and eye ointment) and all the formulations for external use in the fields of dermatology and otolaryngology (e.g., ointment, cream, lotion and spray).

The eye drops are prepared by dissolving the active ingredient in a sterile aqueous solution such as physiological saline, buffering solution, etc., or by combining powder compositions to be dissolved before use. Eye drops such as the ones as described in EP-A-0406791 are preferred. If desired, additives ordinarily used in the eye drops can be added. Such additives include isotonizing agents (e.g., sodium chloride, etc.), buffer agent (e.g., boric acid, sodium monohydrogen phosphate, sodium dihydrogen phosphate, etc.), preservatives (e.g., benzalkonium chloride, benzethonium chloride, chlorobutanol, etc.), thickeners (e.g., saccharide such as lactose, mannitol, maltose, etc.; e.g., hyaluronic acid or its salt such as sodium hyaluronate, potassium hyaluronate, etc.; e.g., mucopolysaccharide such as chondroitin sulfate, etc.; e.g., sodium polyacrylate, carboxyvinyl polymer, crosslinked polyacrylate, etc.). The disclosure of the above publication is incorporated herein by reference.

The ointment (including eye ointment) is prepared by mixing the active ingredient with the base. The formulation can be prepared according to the ordinary method. For example, mixing the active ingredient into the base ordinarily used for the ointment and formulating it according to ordinary methods can sterilely prepare the ointment. Examples of the base for the ointment include petrolatum, selen 50, Plastibase, macrogol, etc., but not limited thereto. Further, in order to increase the hydrophilicity, a surface-active agent can be added. Regarding the ointment, the above-mentioned additives such as the preservatives, etc. can be combined, if necessary.

The present composition can be formulated as a sterile unit dose type containing no preservatives.

The amount of administration and the number of administration of the active ingredient used in the present invention vary according to sex, age and body weight of patient, symptoms to be treated, desirable therapeutic effects,

administration routes and period of treatment. Ordinarily, in the case of using as eye drops for an adult, the formulations containing IL-2 inhibitor of 0.0001-10.0 W/V%, preferably 0.005-5.0 W/V% and the antimicrobial agent of 0.0001-50.0 W/V%, preferably 0.005-10.0 W/V% may be administered several times a day per eye, preferably one to six times, more preferably one to four times, several drops per time, preferably one to four drops. In using for ointment, cream, lotion or spray, the formulations containing IL-2 inhibitor of 0.0001-10.0 W/V%, preferably 0.005-5.0 W/V% and the antimicrobial agent of 0.0001-50.0 W/V%, preferably 0.005-10.0 W/V% may be applied or sprayed several times a day, preferably one to six times, more preferably one to four times. The compounding ratio of each ingredient may be suitably increased or decreased based on the degree of inflammations or infections.

In the present invention, the formulation can include a single IL-2 inhibitor and antimicrobial agent as active ingredients thereof or a combination of two or more of these agents. In a combination of plural active ingredients, their respective contents may be suitably increased or decreased in consideration of their effects and safety.

When an IL-2 inhibitor and an antimicrobial agent are separately formed into pharmaceutical preparations and topically administered simultaneously according to the present invention, the dose of the active ingredient and administration frequency can be appropriately determined in consideration of sex, age and body weight of patient, symptoms to be treated, desirable therapeutic effects, administration routes and period of treatment.

The present formulation can further include other pharmacological active ingredients as far as they do not contradict the purpose of the present invention.

The inflammations and/or infections in the present invention are not particularly limited, as far as they are the

diseases topically treated at eye, skin, air duct, nasal cavity, labial, pubis, pudenda, etc. Examples of such diseases are as follows: infections caused by microorganism such as bacteria (e.g., Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus, gonococcus and Syphilis) and fungi (trichophyton, Malassezia and Candida); diseases generically called dermatitis such as allergic dermatitis (e.g., atopic dermatitis and contact dermatitis) and dermatitis seborrheica; diseases accompanying ocular inflammations such as uveitis, conjunctivitis, cyclitis, scleritis, episcleritis, optic neuritis, retrobulbar optic neuritis, keratitis, blepharitis, corneal ulcer and conjunctival ulcer; diseases generically called rhinitis such as allergic rhinitis and vasomoter rhinitis; diseases accompanying air duct inflammations such as bronchial asthma, infantile asthma, acute bronchitis and chronic bronchitis. The present invention also includes inflammations and/or infections at locations undergoing ophthalmic operations (e.g., operation for cataract) or surgical operations.

The present invention enables to inhibit inflammations and/or infections by topically treating with a combination of interleukin 2 inhibitor and antimicrobial agent without adversely affecting main effects of each ingredient. The present invention also enables to reduce the dosage of active ingredients, as compared with a single use of each ingredient, and to obtain strong anti-inflammatory and/or antimicrobiral activities with a small dosage, thus providing a drug with reduced side effects. Accordingly, the present composition may be effectively and safely administered for the treatment of infections and their accompanying inflammations or infections of a subject showing the excessive immuno-inflammatory reaction following phylaxis. Further, the present composition may be effectively and safely administered for the treatment of inflammations of a subject showing signs

of infections or spreading infections due to the immunosuppressant effect caused by IL-2 inhibitor or some causes (injuries or operation) other than IL-2 inhibitor.

The present invention will be described in more detail with reference to the following examples, which are not intended to limit the present invention.

Example 1

A pharmaceutical composition for topical treatment of the present invention was prepared.

Example 1		
FK-506	0.3 mg	0.03%
ofloxacin	3 mg	0.3%
Benzalkonium Chloride	0.1 mg	0.01%
Sodium Chloride	8.56 mg	0.856%
Disodium hydrogen phosphate	0.05 mg	0.005%
Sodium dihydrogen phosphate	0.76 mg	0.076%
Phosphoric acid and/or Sodium Hydroxide	q.s. for pH adjustment to 5.0 ± 0.5	
Purified Water	q.s. to 1 mL	q.s. to 100%

Test Example 1 (Anti-microbial test)

Single colony isolate of *Pseudomonas aeruginosa* IID-1210 (provided by Department of Ophthalmology, Yokohama City University School of Medicine, Japan) on NAC agar plate was inoculated in 2 mL of heart infusion broth, and kept for overnight at 37°C. One hundred microliters of the overnight culture was inoculated in 10 mL of heart infusion broth, and then grown for about 12 hours at 37°C with shaking.

Twelve Japanese white rabbits (13 weeks old, Std: JW/CSK, Japan SLC, Inc.) were anesthetized by intravenous injection of pentobarbital sodium (25 mg/kg), and then topical anesthesia was made by instillation of 0.4% oxybuprocaine hydrochloride to both eyes. The corneal wound was produced bilaterally using a 6-mm trephine and 27-gauge needle according to the method described by Hatano H *et al.* (Japanese Review of Clinical Ophthalmology 79: 1153, 1985). Forty microliters of the bacterial suspension prepared above was

instilled onto the wounded cornea twice (Day 1, 21:00). After bacterial inoculation, the rabbits were divided into 4 groups (three rabbits-six eyes/group). Fifty microliters of each test substance or vehicle was topically applied to both eyes of each animal once 12 hours after bacterial inoculation (Day 2, 9:00).

As test substances, 0.06% FK-506 (group 2), 0.03% ofloxacin (group 3), a mixture containing 0.06% FK-506 and 0.03% ofloxacin (group 4) and a vehicle (group 1) were used.

Four hours after administration of test substances, the animals were sacrificed with an intravenous overdose of pentobarbital sodium, eyes were enucleated, and then corneas were excised using a 6-mm trephine. After weighing, each cornea was homogenized in 1 mL of sterile physiological saline. Aliquot (0.1 mL) of each homogenate was plated on NAC agar, and incubated for 24 hours at 37°C. The colonies were then counted. All quantitative cultures were run in triplicate, and the arithmetic mean of three measurements was determined for each cornea. Results were expressed as the number of organisms (determined by measures of colony forming units [CFU]) per gram of corneal weight.

Table 1 shows the colony forming units of each group. Treatment with 0.06% FK-506 decreased the viable *Pseudomonas* counts as compared with vehicle treatment, but not significantly. Treatment with 0.3% ofloxacin eradicated the bacteria. Treatment with mixture containing 0.06% FK-506 and 0.3% ofloxacin also eradicated the bacteria. The results indicated that FK-506 had no effect on the inhibition of infection by ofloxacin.

It has been also clarified that a combined use with an antimicrobial agent, such as ofloxacin, obviates the risk of bacterial infection associated with single administration of an IL-2 inhibitor having an immunosuppressive action, such as FK-506.

Table 1

Group	Treatment	Number of eyes	Colony forming units per gram of cornea Mean ± SE
1.	Vehicle (Control)	6	43382 ± 16081
2.	0.06% FK-506	6	31225 ± 7204
3.	0.3% ofloxacin	6	0[a),b)]
4.	0.06% FK-506 0.3% ofloxacin	6	0[a),b)]

a) $p<0.05$ Significantly different from group 1 (Tukey test)

b) $p<0.01$ Significantly different from group 2 (Tukey test)

Test Example 2 (Anti-inflammatory test)

Experimental *Pseudomonas* keratitis was induced for 12 Japanese white rabbits (13 weeks old, Std: JW/CSK) as described in Test Experiment 1. After bacterial inoculation, the rabbits were divided into 4 groups (three rabbits-six eyes/group). Fifty microliters of each test substance or vehicle was topically applied to both eyes of each animal 4 times a day at intervals of 4 hours beginning 12 hours after the bacterial inoculation. The test substance and vehicle were the same as those used in Test Example 1.

Rabbit eyes were examined with a slit lamp biomicroscope, and severity of conjunctival inflammation at 48 hours after bacterial inoculation and corneal inflammation at 60 hours after bacterial inoculation were evaluated by assigning a numerical value to the following signs based on the method described by Kuriyama H *et al*. Corneal inflammation was evaluated by sums of the scores of corneal opacity (score 0 - 8) and corneal ulcer (score 0 - 3). Conjunctival inflammation was evaluated by sums of the scores of redness of palpebral conjunctival inflammation (score 0 - 4), edema of palpebral conjunctiva (score 0 - 4), nictitating membrane status (score 0 - 3) and discharge (score 0 - 3).

As shown in Table 2, the treatment with 0.06% FK-506 or

0.3% ofloxacin tended to decrease the conjunctival inflammation. Treatment with the mixture containing 0.06% FK-506 and 0.3% ofloxacin decreased significantly the conjunctival inflammation as compared with vehicle treatment.

Table 2

Group	Treatment	Number of eyes	Score (conjunctiva) Mean ± SE
1.	Vehicle (Control)	6	7.3 ± 0.6
2.	0.06% FK-506	6	6.7 ± 0.4
3.	0.3% ofloxacin	6	6.0 ± 0.5
4.	0.06% FK-506 0.3% ofloxacin	6	5.5 ± 0.3[a)]

a) $p<0.05$ Significantly different from group 1 (Tukey test)

As shown in Table 3, treatments with 0.06% FK-506 tended to decrease the corneal inflammation. Treatment with 0.3% ofloxacin or the mixture containing 0.06% FK-506 and 0.3% ofloxacin decreased significantly the corneal inflammation as compared with vehicle treatment. The mixture containing 0.06% FK-506 and 0.3% ofloxacin decreased significantly the corneal inflammation as compared with 0.06% FK-506 treatment.

Table 3

Group	Treatment	Number of eyes	Score (cornea) Mean ± SE
1.	Vehicle (Control)	6	7.3 ± 1.0
2.	0.06% FK-506	6	6.0 ± 0.0
3.	0.3% ofloxacin	6	2.1 ± 0.4[a)]
4.	0.06% FK-506 0.3% ofloxacin	6	1.6 ± 0.3[a),b)]

a) $p<0.01$ Significantly different from group 1 (Tukey test)

b) $p<0.05$ Significantly different from group 2 (Tukey test)

The above results indicated that topical treatment with

a combination of FK-506 and ofloxacin inhibited inflammation and/or infection without adversely affecting main effects of each ingredient. The results further indicate that FK-506 and ofloxacin showed an addictive effect and/or synergistic effect on inflammation when used in combination.

Industrial applicability

A composition for topical administration comprising an interleukin 2 inhibitor and an antimicrobial agent as active ingredients shows an antiinflammatory effect while suppressing side effects, such as infectious diseases and the like. The composition for topical administration of the present invention affords prevention or treatment of infectious diseases, therewith-associated inflammation or infectious diseases accompanying excessive immunoinflammatory response due to phylaxis, while suppressing inflammation. Therefore, the composition for topical administration of the present invention is useful for the treatment of inflammation and/or infectious diseases.

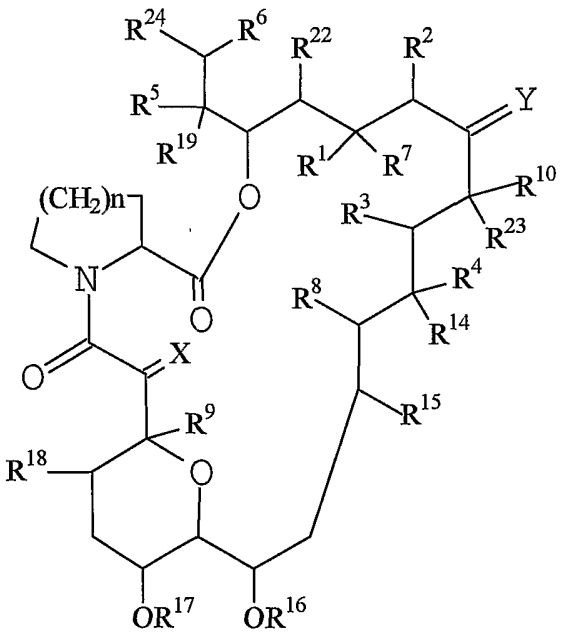
This application is based on application No. 60/303,148 filed in United States of America, the content of which is incorporated hereinto by reference.

CLAIMS

1. A composition for topical administration comprising an interleukin 2 inhibitor and an antimicrobial agent as active ingredients thereof.

2. The composition of Claim 1, wherein said interleukin 2 inhibitor is a macrolide compound or cyclosporin.

3. The composition of Claim 2, wherein said macrolide compound is a tricyclo compound as shown by the following general formula (I) or pharmaceutically acceptable salt thereof:



(I)

wherein adjacent pairs of R^1 and R^2, R^3 and R^4, and R^5 and R^6 each independently

a) show two adjacent hydrogen atoms, wherein R^2 is optionally alkyl, or

b) form another bond optionally between carbon atoms binding with the members of said pairs;

R^7 is hydrogen atom, hydroxy, alkyloxy or protected hydroxy, or may form oxo with R^1;

R^8 and R^9 each independently show hydrogen atom or hydroxy;

R^{10} is hydrogen atom, alkyl, alkenyl, alkyl substituted by one or more hydroxy, alkenyl substituted by one or more hydroxy or alkyl substituted by oxo;

X is oxo, (hydrogen atom, hydroxy), (hydrogen atom, hydrogen atom), or a group of the formula $-CH_2O-$;

Y is oxo, (hydrogen atom, hydroxy), (hydrogen atom, hydrogen atom), or a group of the formula $N-NR^{11}R^{12}$ or $N-OR^{13}$;

R^{11} and R^{12} each independently show hydrogen atom, alkyl, aryl or tosyl;

R^{13}, R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19}, R^{22} and R^{23} each independently show hydrogen atom or alkyl;

R^{24} is an optionally substituted ring that may contain one or more heteroatom(s);

n is 1 or 2;

wherein

Y, R^{10} and R^{23} may show, together with the carbon atom they bind with, a saturated or unsaturated 5 or 6-membered heterocyclic group containing nitrogen atom, sulfur atom and/or oxygen atom, wherein the heterocyclic group may be optionally substituted by one or more group(s) selected from the group consisting of alkyl, hydroxy, alkyloxy, benzyl, a group of the formula $-CH_2Se(C_6H_5)$, and alkyl substituted by one or more hydroxy.

4. The composition of Claim 3, wherein said tricyclo compound is FK506.

5. The composition of any of Claims 1 to 4, wherein said antimicrobial agent is quinolone antimicrobial agent.

6. The composition of Claim 5, wherein said quinolone antimicrobial agent is nalidixic acid, pipemidic acid, piromidic acid, norfloxacin, ofloxacin, levofloxacin,

ciprofloxacin, lomefloxacin, tosufloxacin, fleroxacin, sparfloxacin, enrofloxacin and enoxacin or a mixture thereof.

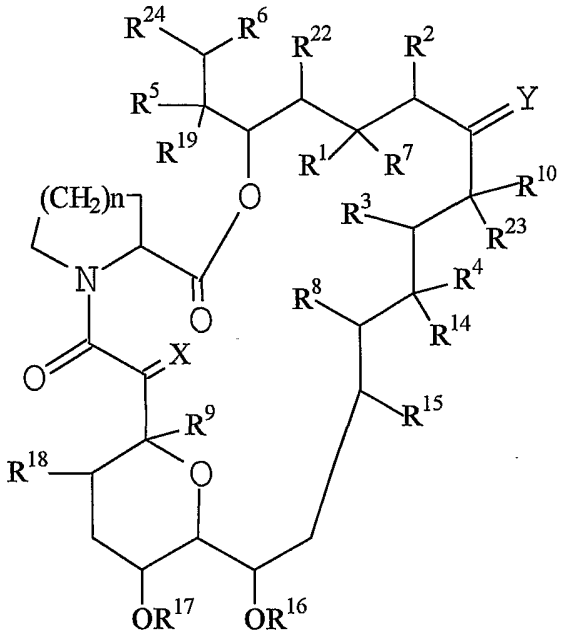
7. The composition of Claim 6, wherein said quinolone antimicrobial agent is ofloxacin.

8. A method for treating inflammations and/or infections comprising topical administration of an effective amount of an interleukin 2 inhibitor and an effective amount of an antimicrobial agent to a subject in need of the treatment of inflammations and/or infections.

9. A use of an interleukin 2 inhibitor and an antimicrobial agent for manufacturing a composition for topical administration for the treatment of inflammations and/or infections.

10. The use of Claim 9, wherein said interleukin 2 inhibitor is a macrolide compound or cyclosporin.

11. The use of claim 10, wherein said macrolide compound is a tricyclo compound as shown by the following general formula (I) or pharmaceutically acceptable salt thereof:



(I)

wherein adjacent pairs of R^1 and R^2, R^3 and R^4, and R^5 and R^6 each independently

a) show two adjacent hydrogen atoms, wherein R^2 is optionally alkyl, or

b) form another bond optionally between carbon atoms binding with the members of said pairs;

R^7 is hydrogen atom, hydroxy, alkyloxy or protected hydroxy, or may form oxo with R^1;

R^8 and R^9 each independently show hydrogen atom or hydroxy;

R^{10} is hydrogen atom, alkyl, alkenyl, alkyl substituted by one or more hydroxy, alkenyl substituted by one or more hydroxy or alkyl substituted by oxo;

X is oxo, (hydrogen atom, hydroxy), (hydrogen atom, hydrogen atom), or a group of the formula $-CH_2O-$;

Y is oxo, (hydrogen atom, hydroxy), (hydrogen atom, hydrogen atom), or a group of the formula $N-NR^{11}R^{12}$ or $N-OR^{13}$;

R^{11} and R^{12} each independently show hydrogen atom, alkyl, aryl or tosyl;

R^{13}, R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19}, R^{22} and R^{23} each independently show hydrogen atom or alkyl;

R^{24} is an optionally substituted ring that may contain one

or more heteroatom(s);

n is 1 or 2;

wherein

Y, R^{10} and R^{23} may show, together with the carbon atom they bind with, a saturated or unsaturated 5 or 6-membered heterocyclic group containing nitrogen atom, sulfur atom and/or oxygen atom, wherein the heterocyclic group may be optionally substituted by one or more group(s) selected from the group consisting of alkyl, hydroxy, alkyloxy, benzyl, a group of the formula

$-CH_2Se(C_6H_5)$, and alkyl substituted by one or more hydroxy.

12. The use of claim 11, wherein said tricyclo compound is FK506.

13. The use of any of claims 9 to 12, wherein said antimicrobial agent is quinolone antimicrobial agent.

14. The use of claim 13, wherein said quinolone antimicrobial agent is nalidixic acid, pipemidic acid, piromidic acid, norfloxacin, ofloxacin, levofloxacin, ciprofloxacin, lomefloxacin, tosufloxacin, fleroxacin, sparfloxacin, enrofloxacin and enoxacin or a mixture thereof.

15. The use of claim 14, wherein said quinolone antimicrobial agent is ofloxacin.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/JP 02/06670

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61P37/06 A61P17/00 A61K31/435 A61K31/495 A61K31/535
A61K38/13

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)
IPC 7 A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, EPO-Internal, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00 50007 A (LIPOCINE INC) 31 August 2000 (2000-08-31) claims 44,45,114,115 ---	1-7
X	US 6 248 776 B1 (HARRIS JAMES W) 19 June 2001 (2001-06-19) column 36, line 49 -column 38, line 9 ---	1,2,5-7
X	US 6 312 715 B1 (CANTOR ADAM S ET AL) 6 November 2001 (2001-11-06) column 7, line 27 -column 8, line 32 --- -/--	1,2,5-7

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in 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 document 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 another 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
15 November 2002	27/11/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Felder, C

INTERNATIONAL SEARCH REPORT

International Application No
PCT/JP 02/06670

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	GILLUM J G ET AL: "PHARMACOKINETIC DRUG INTERACTIONS WITH ANTIMICROBIAL AGENTS" CLINICAL PHARMACOKINETICS, LEA & FEBIGER, PHILADELPHIA, PA, US, vol. 25, no. 6, 1993, pages 450-482, XP001028138 page 456, right-hand column, last paragraph -page 457, left-hand column, paragraph 2 table III	1,2,5-7
X	MCLELLAN ROMAN A ET AL: "Norfloxacin interferes with cyclosporine disposition in pediatric patients undergoing renal transplantation." CLINICAL PHARMACOLOGY & THERAPEUTICS, vol. 58, no. 3, 1995, pages 322-327, XP001118735 ISSN: 0009-9236 abstract page 326, left-hand column, paragraph 4 -page 326, right-hand column, last paragraph	1,2,5-7
X	PATERSON DAVID L ET AL: "Interactions between tacrolimus and antimicrobial agents." CLINICAL INFECTIOUS DISEASES, vol. 25, no. 6, December 1997 (1997-12), pages 1430-1440, XP001118720 ISSN: 1058-4838 page 1430, left-hand column, paragraph 4 -page 1430, right-hand column, paragraph 2 abstract page 1435, left-hand column, paragraph 1 -page 1435, left-hand column, last paragraph page 1438, right-hand column, paragraph 2 -page 1438, right-hand column, last paragraph	1-7
X	IWASAKI KAZUHIDE ET AL: "Effects of twenty-three drugs on the metabolism of FK506 by human liver microsomes." RESEARCH COMMUNICATIONS IN CHEMICAL PATHOLOGY AND PHARMACOLOGY, vol. 82, no. 2, 1993, pages 209-216, XP001118085 ISSN: 0034-5164 page 209, paragraph 1 page 215, paragraph 2 -page 215, last paragraph	1-7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP 02/06670

Box I Observations where certain claims were found unsearchable (Continuation of item 1 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. [X] Claims Nos.: –
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [X] 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:

see FURTHER INFORMATION sheet PCT/ISA/210

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 II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

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

1. [ ] As all required additional 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.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claim 8 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.1

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

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Continuation of Box I.2

The subject-matter of present claims 1-15 is defined by means of the functional features:

-compound being an interleukin-2 inhibitor
-compound being an antimicrobial agent

Because of the character of the functional features, it cannot be guaranteed that the performed search is complete.

It cannot be excluded that compounds fulfilling the requirements of the functional feature have not been identified as doing so in the prior art. If such compounds have not been identified in the application either, they have not been covered by the search.

The search has been carried out, based on the functional features per se as well as the examples given in the application.

It is further pointed out that the substantive examination can only be carried out to the same extent as the search.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/JP 02/06670

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0050007 A	31-08-2000	US 6294192 B1	25-09-2001
		AU 2224200 A	14-09-2000
		EP 1158959 A1	05-12-2001
		NZ 513810 A	28-09-2001
		WO 0050007 A1	31-08-2000
		US 2002012680 A1	31-01-2002
US 6248776 B1	19-06-2001	US 6063809 A	16-05-2000
		AU 3997700 A	04-10-2000
		WO 0054768 A1	21-09-2000
		US 6476066 B1	05-11-2002
		AU 750224 B2	11-07-2002
		AU 9028898 A	16-03-1999
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