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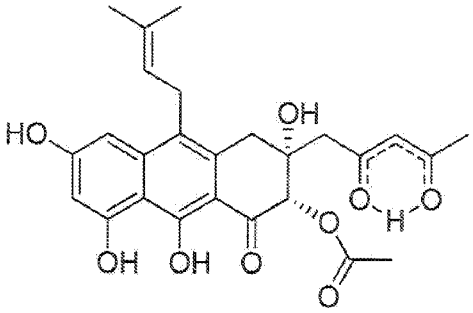
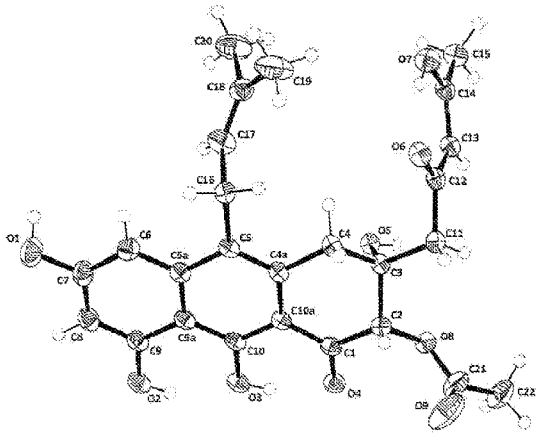
(54) Title: A PRENYLATED ANTHRACENONE POLYKETIDE AS AN IMMUNOSUPPRESSANT

(57) Abstract: The present invention provides a neosartoricin compound and method of making and using the same.



neosartoricin, I
Chemical Formula: $C_{26}H_{28}O_9$



X-ray crystal structure of I

Figure 1

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A PRENYLATED ANTHRACENONE POLYKETIDE AS AN IMMUNOSUPPRESSANT

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Yi Tang
Pin Wang

CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit of U.S. provisional application No. 61/641,276, filed on May 1, 2012, the teaching of which is incorporated herein by reference in its entirety.

STATEMENT OF GOVERNMENT SUPPORT

This invention was made with Government support under Grant No. GM085128 awarded by the National Institutes of Health and Grant No. W81XWH-11-1-0372 awarded by the United States Army, Medical Research and Materiel Command. The Government has certain rights in this invention.

FIELD OF THE INVENTION

The present invention generally relates to an immunosuppressant and methods of making and using the same.

BACKGROUND OF THE INVENTION

The current widely used immunosuppressive agent Cyclosporin A, a fungal metabolite, is associated with the development of hypertension and hyperlipidemia in patients undergoing organ transplantation or treatment for immunologically mediated diseases. Cyclosporin A induces vascular contraction, attenuates vascular relaxation (Huang et al., 1987; Xue et al., 1987; Rego et al., 1990) induces hypertriglyceridemia (Jarowenko et al., 1987; Vathsala et al., 1989), and causes numerous other adverse reactions. Thus, there remains a need for alternative immunosuppressive agents.

The embodiments described below address the above identified need and issues.

SUMMARY OF THE INVENTION

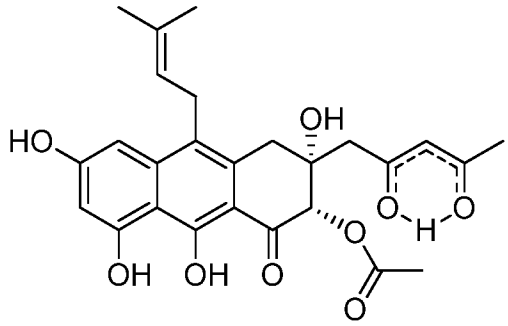
The present invention provides, among others, 1) a method for production of a compound (named neosartoricin) having the structure compound (I) (Figure 1), and 2) its application as immunosuppresant drugs, and 3) methods for discovering and synthesis of structurally related compounds.

In one aspect of the present invention, it is provided an isolated neosartoricin compound in a substantially purified form.

In some embodiments of the invention, optionally in combination with any or all the various embodiments disclosed herein, the isolated neosartoricin compound is of about 80% or above purity , e.g., of about 85% or above purity, of about 90% or above purity, of about 95% or above purity, or of about 99% or above purity.

In another aspect, it is provided a composition, comprising a neosartoricin compound of invention or a pharmaceutically acceptable salt, solvate, hydrate, clathrate, or prodrug thereof.

In some embodiments of the invention composition, the neosartoricin compound has a structure of



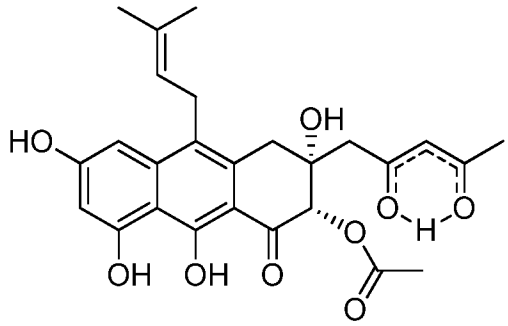
neosartoricin, **I**

In another aspect, it is provided a method of forming a composition comprising a neosartoricin compound of invention or a pharmaceutically acceptable salt, solvate, hydrate, clathrate, or prodrug thereof, which method comprising:

providing the neosartoricin compound in an amount effective as an immunosuppressant, and

forming the composition.

In some embodiments of the method, optionally in combination with any or all the various embodiments disclosed herein, the neosartoricin compound has a structure of

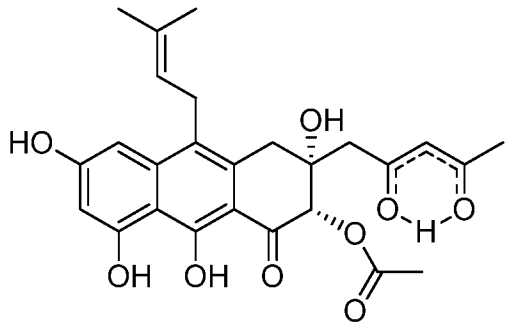


neosartoricin, **I**

In a further embodiments of the present invention, it is provided a method of treating or ameliorating a medical condition in a subject in need thereof, comprising administering to the subject a neosartoricin compound of invention or a pharmaceutically acceptable salt, solvate, hydrate, clathrate, or prodrug thereof in an amount effective for treating or ameliorating the medical condition.

In some embodiments of the invention method, optionally in combination with any or all the various embodiments disclosed herein, the neosartoricin compound, the pharmaceutically acceptable salt thereof, or the prodrug thereof is included in a composition in an amount effective for treating or ameliorating the medical condition.

In some embodiments of the invention method, optionally in combination with any or all the various embodiments disclosed herein, the neosartoricin compound has a structure of



neosartoricin, **I**

In some embodiments of the invention method, optionally in combination with any or all the various above embodiments, the medical condition is a condition related to organ transplant or to implantation of a medical device.

In some embodiments of the invention method, optionally in combination with any or all the various embodiments disclosed herein, the medical device is a stent.

In a further aspect of the present invention, it is provided a method of producing compound (I), comprising overexpressing the pathway specific Zn(II)2Cys6-type regulator nscR in N. fischeri and the nscR homolog in A. fumugatus.

In some embodiments of the invention method, optionally in combination with any or all the various above embodiments, the nscR is overexpressed under the control of an Aspergillus nidulans gpdA promoter by cloning the NscR gene into a pBARGPE1 plasmid.

In some embodiments of the invention method, optionally in combination with any or all the various above embodiments, the plasmid comprises a fungal selection marker for glufosinate resistance, wherein the nscR gene is amplified using polymerase chain reaction (PCR) and cloned into pBARGPE1 by restriction digest to generate plasmid pBARGPE1-NscR, and wherein the resultant plasmid pBARGPE1-NscR is transformed into N. fischeri using polyethylene glycol (PEG)-mediated transformation.

In a further aspect of the present invention, it is provided a method of identifying and making immunosuppressive compounds, comprising:

a) genome mining for fungal strains harboring gene clusters capable of producing compounds which are identical or structurally related to compound (I) by searching for homologs of the polycyclic prenyltansferase (pcPTase) gene nscD, which homologs exhibits 60%-100% protein identify to NscD, or, for fungal strains where their genomes are not sequenced, amplifying the pcPTase gene by degenerate primer PCR using a primer pair pcPT-F (SEQ ID NO:1) and pcPT-R (SEQ ID NO:2),

b) expressing or overexpressing the homologs of the pcPTase gene or the amlified pcPTase gene to produce candidate immunosupressive compounds, and

c) subjecting the candidate immunosupressive compounds to an immunosuppressive study, and

d) designating a candidate immunosuppressive compound as an immunosuppressive compound if result of the immunosuppressive study is positive (immunosuppressive).

In some embodiments of the invention method, optionally in combination with any or all the various above embodiments, the homologs of the pcPTase gene nscD are found in the genome of dermatophytic fungi that belongs to the family Arthrodermataceae with 60-65% protein identity to NscD.

In some embodiments of the invention method, optionally in combination with any or all the various above embodiments, the homologs comprise non-reducing polyketide synthase (NRPKS) genes similar to nscA, metallo-beta-lactamase (MbL) genes similar to nscB and flavin-dependent monooxygenase (FMO) genes similar to nscC.

In some embodiments of the invention method, optionally in combination with any or all the various above embodiments, the immunosuppressive study is an in vitro study against human T-cells or in vivo study using a mouse tumor rejection model.

In some embodiments of the invention method, optionally in combination with any or all the various above embodiments, the immunosuppressive compound is produced using pcPTase gene-containing gene clusters in the athrodemataceous dermatophytes selected from the group consisting of Microsporum canis, Microsporum gypseum, Athroderma benhamiae, Trichophyton tonsurans, Trichophyton equinum, Trichophyton rubrum and Trichophyton verrucosum.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the chemical formula and x-ray crystal structure of neosartoricin.

Figure 2 shows the gene cluster that encodes neosartoricin.

Figure 3 shows a gene construct for producing neosartoricin.

Figure 4 shows the results of a cell-based assay against anti-CD3/anti-CD28-activated murine T-cells where neosartoricin exhibits antiproliferative activity at IC50 of 3 μM (1.452 μg/mL).

Figure 5 shows results of a cell-based assay against anti-CD3/anti-CD28-activated murine T-cells where neosartoricin exhibits a dossage-dependent inhibitory activity against IL-2 expression by the T-cells.

Figure 6 shows the results of an evolutionary analysis which show pcPTases are highly related to NscD and formed a distinct clade on phylogenetic tree.

DETAILED DESCRIPTION OF THE INVENTION

In general, the present invention provides, among others, 1) a method for production of a compound (named neosartoricin) having the structure compound (I) (Figure 1), and 2) its application as immunosuppresant drugs, and 3) methods for discovering and synthesis of structurally related compounds.

In one aspect of the present invention, it is provided an isolated neosartoricin compound in a substantially purified form.

In some embodiments of the invention, optionally in combination with any or all the various embodiments disclosed herein, the isolated neosartoricin compound is of about 80% or above purity , e.g., of about 85% or above purity, of about 90% or above purity, of about 95% or above purity, or of about 99% or above purity.

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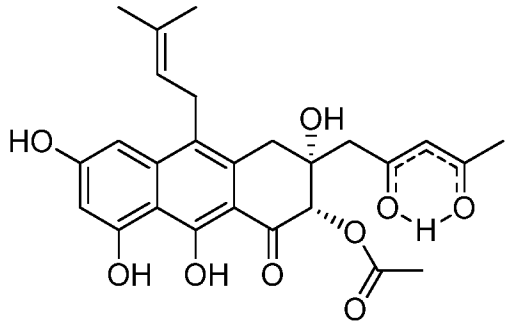

neosartoricin, **I**

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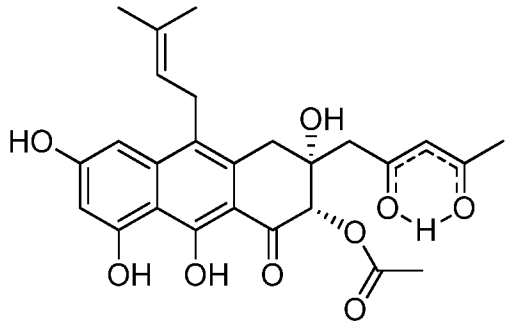

neosartoricin, **I**

In a further embodiments of the present invention, it is provided a method of treating or ameliorating a medical condition in a subject in need thereof, comprising administering to the subject a neosartoricin compound of invention or a pharmaceutically acceptable salt, solvate, hydrate, clathrate, or prodrug thereof in an amount effective for treating or ameliorating the medical condition.

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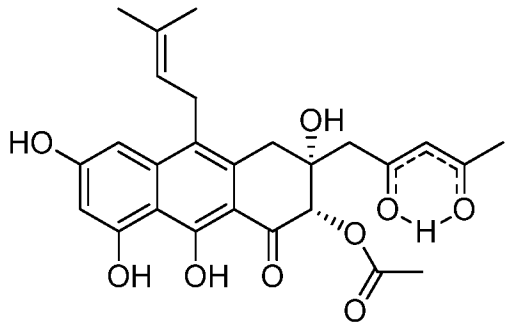

neosartoricin, **I**

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In some embodiments of the invention method, optionally in combination with any or all the various above embodiments, the nscR is overexpressed under the control of an Aspergillus nidulans gpdA promoter by cloning the NscR gene into a pBARGPE1 plasmid.

In some embodiments of the invention method, optionally in combination with any or all the various above embodiments, the plasmid comprises a fungal selection marker for glufosinate resistance, wherein the nscR gene is amplified using polymerase chain reaction (PCR) and cloned into pBARGPE1 by restriction digest to generate plasmid pBARGPE1-NscR, and wherein the resultant plasmid pBARGPE1-NscR is transformed into N. fischeri using polyethylene glycol (PEG)-mediated transformation.

In a further aspect of the present invention, it is provided a method of identifying and making immunosuppressive compounds, comprising:

a) genome mining for fungal strains harboring gene clusters capable of producing compounds which are identical or structurally related to compound (I) by searching for homologs of the polycyclic prenyltansferase (pcPTase) gene nscD, which homologs exhibits 60%-100% protein identify to NscD, or, for fungal strains where their genomes are not sequenced, amplifying the pcPTase gene by degenerate primer PCR using a primer pair pcPT-F (SEQ ID NO:1) and pcPT-R (SEQ ID NO:2),

b) expressing or overexpressing the homologs of the pcPTase gene or the amlified pcPTase gene to produce candidate immunosupressive compounds, and

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In some embodiments of the invention method, optionally in combination with any or all the various above embodiments, the homologs of the pcPTase gene nscD are found in the genome of dermatophytic fungi that belongs to the family Arthrodermataceae with 60-65% protein identity to NscD.

In some embodiments of the invention method, optionally in combination with any or all the various above embodiments, the homologs comprise non-reducing polyketide synthase (NRPKS) genes similar to nscA, metallo-beta-lactamase (MbL) genes similar to nscB and flavin-dependent monooxygenase (FMO) genes similar to nscC.

In some embodiments of the invention method, optionally in combination with any or all the various above embodiments, the immunosuppressive study is an in vitro study against human T-cells or in vivo study using a mouse tumor rejection model.

In some embodiments of the invention method, optionally in combination with any or all the various above embodiments, the immunosuppressive compound is produced using pcPTase gene-containing gene clusters in the athrodemataceous dermatophytes selected from the group consisting of Microsporum canis, Microsporum gypseum, Athroderma benhamiae, Trichophyton tonsurans, Trichophyton equinum, Trichophyton rubrum and Trichophyton verrucosum.

Definitions

As used herein, the term substantially purified form means that the compound of invention is in a state of existence different from its state of natural existence and is of about 80% or above purity, e.g., of about 85% or above purity, of about 90% or above purity, of about 95% or above purity, or of about 99% or above purity.

A prodrug is a medication that is administered as an inactive (or less than fully active) chemical derivative that is subsequently converted to an active pharmacological agent in the body, often through normal metabolic processes. A prodrug serves as a type of precursor to the intended drug.

As used herein, the term “a pharmaceutically acceptable salt” refers to a salt of the compound of invention suitable for use in the art of medicine. Such salts can be salts of an organic acid or inorganic acid, e.g., acetate, oxylate, hydrochloride, sulfate, phosphate, and other acids that generate a salt useable as an active ingredient in a pharmaceutical composition.

As used herein, the term “solvate” means a solvent adduct of a compound of invention.

As used herein, the term “hydrate” means a water adduct of a compound of invention.

As used herein, the term “clathrate” means a compound of invention having a lattice that traps certain molecules. Generally, such trapped molecules are pharmaceutically

acceptable or inert.

As used herein, the term "controlled release" means release of a compound of invention in a controlled manner. The term is further described in more detail below.

Method production of neosartoricin (I).

An embodiment of making neosartoricin (I) is described below:

The gene cluster encodes for production of compound (I) is identified in the genome of Neosartorya fischeri NRRL 181 and Aspergillus fumigatus Af293 (Figure 2). The expression of the this gene cluster (named nsc cluster) is silent in N. fischeri NRRL 181. The production of the compound can be enabled by overexpression of the pathway specific Zn(II)2Cys6-type regulator nscR in N. fischeri and the nscR homolog in A. fumigatus. For example, nscR can be overexpressed under the control of Aspergillus nidulans gpdA promoter by cloning the NscR gene into the pBARGPE1 plasmid (Figure 3). The plasmid pBARGPE1 contain the fungal selection marker for glufosinate resistance (bar gene) and is available at Fungal Genetics Stock Center (http://www.fgsc.net/fgn/pall.html). The nscR gene is amplified using polymerase chain reaction (PCR) and cloned into pBARGPE1 by restriction digest.The resultant plasmid pBARGPE1-NscR can be transformed into N. fischeri using polyethylene glycol (PEG)-mediated transformation. Briefly, the spores are harvested from N. fischeri culture growing on solid agar medium. The spores are inoculated into a liquid minimal medium for overnight growth. The mycelia are harvested by filtering and subjected to cell wall digestion using cell wall-digesting enzyme to generate protoplast. After several washing, the protoplasts are concentrated by centrifugation, in which about the plasmid DNA of pBARGPE1-NscR is added to the protoplasts. Finally, three times volume of 60% PEG was added to the protoplasts/DNA mixture to induce the transfer of the plasmid DNA into the N. fischeri protoplasts. Selection of the N. fischeri transformants carrying additional copies of nscR genes is carried out on solid agar medium containing glufosinate for selection. Integration of the additional nscR gene with gpdA promoter can be verified by PCR. The resulting N. fischeri PgpdA::nscR strain will be able to produce compound (I) at high concentration (35 mg per liter and above) when grown on medium containing glucose.

A number of exemplary embodiments of the production of neosartoricin (I) are described below:

1) Production of compound (I) can be achieved by using the N. fischeri strain overexpressing nscR gene. Large scale production can be achieved by using industrial-scale

fermentor at optimized medium and growth condition. Ability to obtain compound (I) in large amount will permit further pharmacological and clinical studies.

2) The efficacy of compound (I) as an immunosuppressive agent in vivo can be evaluated by using a mouse tumor rejection model (e.g. as described in Hammond-McKibben et al. 2001).

3) The protein sequences of NscD can be used to search for pcPTase homologs in newly sequenced fungal genomes. The degenerate primers pcPT-F/pcPT-R can be used to PCR amplify pcPTase genes from genomic DNA of fungal strains potentially produces compounds similar to compound (I).

Method for identification and synthesis of structurally related compounds.

Genome mining for fungal strains harboring gene clusters that can produce compounds which are identical or structurally related to compound (I) can be achieved by searching for homologs of the polycyclic prenyltransferase (pcPTase) gene nscD. The pcPTases are a distinct group of prenyltransferases that are only moderately related (~25 % protein identity) to the more common indole prenyltransferases in fungi. They can catalyze the transfer of a dimethylallyl group to linearly fused polycyclic ring systems and typically exhibit 60-100% protein identity to NscD. For sequenced fungal genome, the genome mining of gene clusters that can produce compound structurally related to compound (I) can be achieved by simple BLAST search (e.g. at http://blast.ncbi.nlm.nih.gov). For example, a group of such pcPTases can be found in the genome of dermatophytic fungi belongs to the family Arthrodermataceae with 60-65% protein identity to NscD. In an evolutionary analysis, these pcPTases are highly related to NscD and formed a distinct clade on phylogenetic tree (Figure 6). The presence of non-reducing polyketide synthase (NRPKS) genes similar to nscA, metallo-beta-lactamase (MbL) genes similar to nscB and flavin-dependent monooxygenase (FMO) genes similar to nscC, are additional signature features of gene cluster encoding for production of compounds that are structurally related to compound (I) (Figure 6). For fungal strains where their genomes are not sequenced, the pcPTase gene can be amplified by degenerate primer PCR using the primer pair pcPT-F and pcPT-R, which consist of the nucleotide sequences in Table 1. As an example, the pcPT-F and pcPT-R primers were successfully employed to amplify the pcPTase gene in Hypomyces aurantius known to produce the antifungal hypomycetin, which contain a linearly fused tetracyclic ring system and a dimethylallyl group (Breinholt et al. 1997).

Table 1

pcPT-F	Catggcccgcatgatgywngargc (SEQ ID NO:1)
pcPT-R	Cgcgtaccgaggctggatrtcrwarta (SEQ ID NO:2)

Compound (I) is also noted to be relatively specific as it does not exhibit growth inhibitory activity against bacteria and yeasts, as well as human cell lines HeLA and HFF.

Studies on the activity of compound (I) in vitro against human T-cells and in vivo can be performed using a mouse tumor rejection model. Studies on the mechanism of action of compound (I) can be performed by quantification of various cell-surface receptors of T-cells, such as CD4, CD8, CD62l, CD69, etc. Compound (I) can also be tested against other immune cell targets, such as B-lymphocytes and macrophages.

Compounds similar to neosartoricin (I) can be produced by fungal strains harboring gene clusters that can produced compounds similar to compound (I). For example, the pcPTase gene-containing gene clusters in the athrodemataceous dermatophytes (Microsporum canis, Microsporum gypseum, Athroderma benhamiae, Trichophyton tonsurans, Trichophyton equinum, Trichophyton rubrum and Trichophyton verrucosum). The compounds successfully isolated from these fungal strains can be subjected to similar immunosuppressive studies whereby immunosuppressive drugs are indentified.

Chemical synthesis of compounds of invention

In general methods for synthesizing compounds of invention can be found, for example, in March, Advanced Organic Chemistry, third edition, (1985), John Wiley & Sons, the entire teachings of which are incorporated herein by reference.

Methods of Treatment and Prevention

In accordance with the invention, an effective amount of a compound of invention or a pharmaceutically acceptable salt, solvate, clathrate, and prodrug thereof, or a pharmaceutical composition comprising a compound of invention or a pharmaceutically acceptable salt, solvate, clathrate, and prodrug thereof, is administered to a patient in need of immunosuppression or in need of treatment or prevention of an immunoreaction associated with organ transplant or implantation of a device such as stent, an inflammatory condition, an immune disorder, or an allergic disorder. Such patients may be treatment naive or may experience partial or no response to conventional therapies.

Responsiveness of a particular immunoreaction, inflammatory condition, immunoreaction, immune disorder, or allergic disorder in a subject can be measured directly

after administration of a compound of this invention or can be inferred based on an understanding of disease etiology and progression according to established methods, which are generally known to a person of ordinary skill in the art. The compounds of invention or pharmaceutically acceptable salts, solvates, clathrates, and prodrugs thereof can be assayed in vitro or in vivo, for the desired therapeutic or prophylactic activity, prior to use in humans. For example, known animal models of inflammatory conditions, immune disorders, or allergic disorders can be used to demonstrate the safety and efficacy of compounds of this invention.

Pharmaceutical Compositions and Dosage Forms

Pharmaceutical compositions and dosage forms of the invention comprise one or more active ingredients in relative amounts and formulated in such a way that a given pharmaceutical composition or dosage form can be used for immunosuppression or to treat or prevent inflammatory conditions, immune disorders, and allergic disorders. Preferred pharmaceutical compositions and dosage forms comprise compound (I), or a pharmaceutically acceptable prodrug, salt, solvate, or clathrate thereof, optionally in combination with one or more additional active agents.

Single unit dosage forms of the invention are suitable for oral, mucosal (e.g., nasal, sublingual, vaginal, buccal, or rectal), parenteral (e.g., subcutaneous, intravenous, bolus injection, intramuscular, or intraarterial), or transdermal administration to a patient. Examples of dosage forms include, but are not limited to: tablets; caplets; capsules, such as soft elastic gelatin capsules; cachets; troches; lozenges; dispersions; suppositories; ointments; cataplasms (poultices); pastes; powders; dressings; creams; plasters; solutions; patches; aerosols (e.g., nasal sprays or inhalers); gels; liquid dosage forms suitable for oral or mucosal administration to a patient, including suspensions (e.g., aqueous or non-aqueous liquid suspensions, oil-in-water emulsions, or a water-in-oil liquid emulsions), solutions, and elixirs; liquid dosage forms suitable for parenteral administration to a patient; and sterile solids (e.g., crystalline or amorphous solids) that can be reconstituted to provide liquid dosage forms suitable for parenteral administration to a patient.

The composition, shape, and type of dosage forms of the invention will typically vary depending on their use. For example, a dosage form suitable for mucosal administration may contain a smaller amount of active ingredient(s) than an oral dosage form used to treat the same indication. This aspect of the invention will be readily apparent to those skilled in the

art. See, e.g., Remington's Pharmaceutical Sciences (1990) 18th ed., Mack Publishing, Easton Pa.

Typical pharmaceutical compositions and dosage forms comprise one or more excipients. Suitable excipients are well known to those skilled in the art of pharmacy, and non-limiting examples of suitable excipients are provided herein. Whether a particular excipient is suitable for incorporation into a pharmaceutical composition or dosage form depends on a variety of factors well known in the art including, but not limited to, the way in which the dosage form will be administered to a patient. For example, oral dosage forms such as tablets may contain excipients not suited for use in parenteral dosage forms.

The suitability of a particular excipient may also depend on the specific active ingredients in the dosage form. For example, the decomposition of some active ingredients can be accelerated by some excipients such as lactose, or when exposed to water. Active ingredients that comprise primary or secondary amines (e.g., N-desmethylvenlafaxine and N,N-didesmethylvenlafaxine) are particularly susceptible to such accelerated decomposition. Consequently, this invention encompasses pharmaceutical compositions and dosage forms that contain little, if any, lactose. As used herein, the term "lactose-free" means that the amount of lactose present, if any, is insufficient to substantially increase the degradation rate of an active ingredient. Lactose-free compositions of the invention can comprise excipients that are well known in the art and are listed, for example, in the U.S. Pharmocopia (USP) SP (XXI)/NF (XVI). In general, lactose-free compositions comprise active ingredients, a binder/filler, and a lubricant in pharmaceutically compatible and pharmaceutically acceptable amounts. Preferred lactose-free dosage forms comprise active ingredients, microcrystalline cellulose, pre-gelatinized starch, and magnesium stearate.

This invention further encompasses anhydrous pharmaceutical compositions and dosage forms comprising active ingredients, since water can facilitate the degradation of some compounds. For example, the addition of water (e.g., 5%) is widely accepted in the pharmaceutical arts as a means of simulating long-term storage in order to determine characteristics such as shelf-life or the stability of formulations over time. See, e.g., Jens T. Carstensen (1995) Drug Stability: Principles & Practice, 2d. Ed., Marcel Dekker, NY, N.Y., 379-80. In effect, water and heat accelerate the decomposition of some compounds. Thus, the effect of water on a formulation can be of great significance since moisture and/or humidity

are commonly encountered during manufacture, handling, packaging, storage, shipment, and use of formulations.

Anhydrous pharmaceutical compositions and dosage forms of the invention can be prepared using anhydrous or low moisture containing ingredients and low moisture or low humidity conditions. Pharmaceutical compositions and dosage forms that comprise lactose and at least one active ingredient that comprises a primary or secondary amine are preferably anhydrous if substantial contact with moisture and/or humidity during manufacturing, packaging, and/or storage is expected.

An anhydrous pharmaceutical composition should be prepared and stored such that its anhydrous nature is maintained. Accordingly, anhydrous compositions are preferably packaged using materials known to prevent exposure to water such that they can be included in suitable formulary kits. Examples of suitable packaging include, but are not limited to, hermetically sealed foils, plastics, unit dose containers (e.g., vials), blister packs, and strip packs.

The invention further encompasses pharmaceutical compositions and dosage forms that comprise one or more compounds that reduce the rate by which an active ingredient will decompose. Such compounds, which are referred to herein as "stabilizer" include, but are not limited to, antioxidants such as ascorbic acid, pH buffers, or salt buffers.

Like the amounts and types of excipients, the amounts and specific types of active ingredients in a dosage form may differ depending on factors such as, but not limited to, the route by which it is to be administered to patients. However, typical dosage forms of the invention comprise a compound of invention or a pharmaceutically acceptable salt, solvate, clathrate, or prodrug thereof in an amount of from about 1 mg to about 1000 mg, preferably in an amount of from about 50 mg to about 500 mg, and most preferably in an amount of from about 75 mg to about 350 mg. The typical total daily dosage of a compound of invention, or a pharmaceutically acceptable salt, solvate, clathrate, or prodrug thereof can range from about 1 mg to about 5000 mg per day, preferably in an amount from about 50 mg to about 1500 mg per day, more preferably from about 75 mg to about 1000 mg per day. It is within the skill of the art to determine the appropriate dose and dosage form for a given patient.

Oral Dosage Forms

Pharmaceutical compositions of the invention that are suitable for oral administration can be presented as discrete dosage forms, such as, but are not limited to, tablets (e.g., chewable tablets), caplets, capsules, and liquids (e.g., flavored syrups). Such dosage forms contain predetermined amounts of active ingredients, and may be prepared by methods of pharmacy well known to those skilled in the art. See generally, Remington's Pharmaceutical Sciences (1990) 18th ed., Mack Publishing, Easton Pa.

Typical oral dosage forms of the invention are prepared by combining the active ingredient(s) in an admixture with at least one excipient according to conventional pharmaceutical compounding techniques. Excipients can take a wide variety of forms depending on the form of preparation desired for administration. For example, excipients suitable for use in oral liquid or aerosol dosage forms include, but are not limited to, water, glycols, oils, alcohols, flavoring agents, preservatives, and coloring agents. Examples of excipients suitable for use in solid oral dosage forms (e.g., powders, tablets, capsules, and caplets) include, but are not limited to, starches, sugars, micro-crystalline cellulose, diluents, granulating agents, lubricants, binders, and disintegrating agents.

Because of their ease of administration, tablets and capsules represent the most advantageous oral dosage unit forms, in which case solid excipients are employed. If desired, tablets can be coated by standard aqueous or nonaqueous techniques. Such dosage forms can be prepared by any of the methods of pharmacy. In general, pharmaceutical compositions and dosage forms are prepared by uniformly and intimately admixing the active ingredients with liquid carriers, finely divided solid carriers, or both, and then shaping the product into the desired presentation if necessary.

For example, a tablet can be prepared by compression or molding. Compressed tablets can be prepared by compressing in a suitable machine the active ingredients in a free-flowing form such as powder or granules, optionally mixed with an excipient. Molded tablets can be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

Examples of excipients that can be used in oral dosage forms of the invention include, but are not limited to, binders, fillers, disintegrants, and lubricants. Binders suitable for use in pharmaceutical compositions and dosage forms include, but are not limited to, corn starch, potato starch, or other starches, gelatin, natural and synthetic gums such as acacia, sodium

alginate, alginic acid, other alginates, powdered tragacanth, guar gum, cellulose and its derivatives (e.g., ethyl cellulose, cellulose acetate, carboxymethyl cellulose calcium, sodium carboxymethyl cellulose), polyvinyl pyrrolidone, methyl cellulose, pre-gelatinized starch, hydroxypropyl methyl cellulose, (e.g., Nos. 2208, 2906, 2910), microcrystalline cellulose, and mixtures thereof.

Suitable forms of microcrystalline cellulose include, but are not limited to, the materials sold as AVICEL-PH-101, AVICEL-PH-103 AVICEL RC-581, AVICEL-PH-105 (available from FMC Corporation, American Viscose Division, Avicel Sales, Marcus Hook, Pa.), and mixtures thereof. One specific binder is a mixture of microcrystalline cellulose and sodium carboxymethyl cellulose sold as AVICEL RC-581. Suitable anhydrous or low moisture excipients or additives include AVICEL-PH-103J and Starch 1500 LM.

Examples of fillers suitable for use in the pharmaceutical compositions and dosage forms disclosed herein include, but are not limited to, talc, calcium carbonate (e.g., granules or powder), microcrystalline cellulose, powdered cellulose, dextrates, kaolin, mannitol, silicic acid, sorbitol, starch, pre-gelatinized starch, and mixtures thereof. The binder or filler in pharmaceutical compositions of the invention is typically present in from about 50 to about 99 weight percent of the pharmaceutical composition or dosage form. Disintegrants are used in the compositions of the invention to provide tablets that disintegrate when exposed to an aqueous environment. Tablets that contain too much disintegrant may disintegrate in storage, while those that contain too little may not disintegrate at a desired rate or under the desired conditions. Thus, a sufficient amount of disintegrant that is neither too much nor too little to detrimentally alter the release of the active ingredients should be used to form solid oral dosage forms of the invention. The amount of disintegrant used varies based upon the type of formulation, and is readily discernible to those of ordinary skill in the art. Typical pharmaceutical compositions comprise from about 0.5 to about 15 weight percent of disintegrant, preferably from about 1 to about 5 weight percent of disintegrant.

Disintegrants that can be used in pharmaceutical compositions and dosage forms of the invention include, but are not limited to, agar-agar, alginic acid, calcium carbonate, microcrystalline cellulose, croscarmellose sodium, crospovidone, polacrilin potassium, sodium starch glycolate, potato or tapioca starch, other starches, pre-gelatinized starch, other starches, clays, other algins, other celluloses, gums, and mixtures thereof.

Lubricants that can be used in pharmaceutical compositions and dosage forms of the invention include, but are not limited to, calcium stearate, magnesium stearate, mineral oil, light mineral oil, glycerin, sorbitol, mannitol, polyethylene glycol, other glycols, stearic acid, sodium lauryl sulfate, talc, hydrogenated vegetable oil (e.g., peanut oil, cottonseed oil, sunflower oil, sesame oil, olive oil, corn oil, and soybean oil), zinc stearate, ethyl oleate, ethyl laureate, agar, and mixtures thereof. Additional lubricants include, for example, a syloid silica gel (AEROSIL 200, manufactured by W.R. Grace Co. of Baltimore, Md.), a coagulated aerosol of synthetic silica (marketed by Degussa Co. of Plano, Tex.), CAB-O-SIL (a pyrogenic silicon dioxide product sold by Cabot Co. of Boston, Mass.), and mixtures thereof. If used at all, lubricants are typically used in an amount of less than about 1 weight percent of the pharmaceutical compositions or dosage forms into which they are incorporated.

Controlled Release Dosage Forms

Active ingredients of the invention can be administered by controlled release means or by delivery devices that are well known to those of ordinary skill in the art. Examples include, but are not limited to, those described in U.S. Pat. Nos. 3,845,770; 3,916,899; 3,536,809; 3,598,123; and 4,008,719, 5,674,533, 5,059,595, 5,591,767, 5,120,548, 5,073,543, 5,639,476, 5,354,556, and 5,733,566, each of which is incorporated herein by reference. Such dosage forms can be used to provide slow or controlled-release of one or more active ingredients using, for example, hydropropylmethyl cellulose, other polymer matrices, gels, permeable membranes, osmotic systems, multilayer coatings, microparticles, liposomes, microspheres, or a combination thereof to provide the desired release profile in varying proportions. Suitable controlled-release formulations known to those of ordinary skill in the art, including those described herein, can be readily selected for use with the active ingredients of the invention. The invention thus encompasses single unit dosage forms suitable for oral administration such as, but not limited to, tablets, capsules, gelcaps, and caplets that are adapted for controlled-release.

All controlled-release pharmaceutical products have a common goal of improving drug therapy over that achieved by their non-controlled counterparts. Ideally, the use of an optimally designed controlled-release preparation in medical treatment is characterized by a minimum of drug substance being employed to cure or control the condition in a minimum amount of time. Advantages of controlled-release formulations include extended activity of the drug, reduced dosage frequency, and increased patient compliance. In addition,

controlled-release formulations can be used to affect the time of onset of action or other characteristics, such as blood levels of the drug, and can thus affect the occurrence of side (e.g., adverse) effects.

Most controlled-release formulations are designed to initially release an amount of drug (active ingredient) that promptly produces the desired therapeutic effect, and gradually and continually release of other amounts of drug to maintain this level of therapeutic or prophylactic effect over an extended period of time. In order to maintain this constant level of drug in the body, the drug must be released from the dosage form at a rate that will replace the amount of drug being metabolized and excreted from the body. Controlled-release of an active ingredient can be stimulated by various conditions including, but not limited to, pH, temperature, enzymes, water, or other physiological conditions or compounds.

A particular extended release formulation of this invention comprises a therapeutically or prophylactically effective amount of a compound of invention or a pharmaceutically acceptable salt, solvate, hydrate, clathrate, or prodrug thereof, in spheroids which further comprise microcrystalline cellulose and, optionally, hydroxypropylmethyl-cellulose coated with a mixture of ethyl cellulose and hydroxypropylmethylcellulose. Such extended release formulations can be prepared according to U.S. Pat. No. 6,274,171, the entire teachings of which are incorporated herein by reference.

A specific controlled-release formulation of this invention comprises from about 6% to about 40% a compound of invention by weight, about 50% to about 94% microcrystalline cellulose, NF, by weight, and optionally from about 0.25% to about 1% by weight of hydroxypropyl-methylcellulose, USP, wherein the spheroids are coated with a film coating composition comprised of ethyl cellulose and hydroxypropylmethylcellulose.

Parenteral Dosage Forms

Parenteral dosage forms can be administered to patients by various routes including, but not limited to, subcutaneous, intravenous (including bolus injection), intramuscular, and intraarterial. Because their administration typically bypasses patients' natural defenses against contaminants, parenteral dosage forms are preferably sterile or capable of being sterilized prior to administration to a patient. Examples of parenteral dosage forms include, but are not limited to, solutions ready for injection, dry products ready to be dissolved or suspended in a pharmaceutically acceptable vehicle for injection, suspensions ready for injection, and emulsions.

Suitable vehicles that can be used to provide parenteral dosage forms of the invention are well known to those skilled in the art. Examples include, but are not limited to: Water for Injection USP; aqueous vehicles such as, but not limited to, Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, and Lactated Ringer's Injection; water-miscible vehicles such as, but not limited to, ethyl alcohol, polyethylene glycol, and polypropylene glycol; and non-aqueous vehicles such as, but not limited to, corn oil, cottonseed oil, peanut oil, sesame oil, ethyl oleate, isopropyl myristate, and benzyl benzoate.

Compounds that increase the solubility of one or more of the active ingredients disclosed herein can also be incorporated into the parenteral dosage forms of the invention.

Transdermal, Topical, and Mucosal Dosage Forms

Transdermal, topical, and mucosal dosage forms of the invention include, but are not limited to, ophthalmic solutions, sprays, aerosols, creams, lotions, ointments, gels, solutions, emulsions, suspensions, or other forms known to one of skill in the art. See, e.g., Remington's Pharmaceutical Sciences (1980 & 1990) 16th and 18th eds., Mack Publishing, Easton Pa. and Introduction to Pharmaceutical Dosage Forms (1985) 4th ed., Lea & Febiger, Philadelphia. Dosage forms suitable for treating mucosal tissues within the oral cavity can be formulated as mouthwashes or as oral gels. Further, transdermal dosage forms include "reservoir type" or "matrix type" patches, which can be applied to the skin and worn for a specific period of time to permit the penetration of a desired amount of active ingredients.

Suitable excipients (e.g., carriers and diluents) and other materials that can be used to provide transdermal, topical, and mucosal dosage forms encompassed by this invention are well known to those skilled in the pharmaceutical arts, and depend on the particular tissue to which a given pharmaceutical composition or dosage form will be applied. With that fact in mind, typical excipients include, but are not limited to, water, acetone, ethanol, ethylene glycol, propylene glycol, butane-1,3-diol, isopropyl myristate, isopropyl palmitate, mineral oil, and mixtures thereof to form lotions, tinctures, creams, emulsions, gels or ointments, which are non-toxic and pharmaceutically acceptable. Moisturizers or humectants can also be added to pharmaceutical compositions and dosage forms if desired. Examples of such additional ingredients are well known in the art. See, e.g., Remington's Pharmaceutical Sciences (1980 & 1990) 16th and 18th eds., Mack Publishing, Easton Pa.

Depending on the specific tissue to be treated, additional components may be used prior to, in conjunction with, or subsequent to treatment with active ingredients of the invention. For example, penetration enhancers can be used to assist in delivering the active ingredients to the tissue. Suitable penetration enhancers include, but are not limited to: acetone; various alcohols such as ethanol, oleyl, and tetrahydrofuryl; alkyl sulfoxides such as dimethyl sulfoxide; dimethyl acetamide; dimethyl formamide; polyethylene glycol; pyrrolidones such as polyvinylpyrrolidone; Kollidon grades (Povidone, Polyvidone); urea; and various water-soluble or insoluble sugar esters such as Tween 80 (polysorbate 80) and Span 60 (sorbitan monostearate).

The pH of a pharmaceutical composition or dosage form, or of the tissue to which the pharmaceutical composition or dosage form is applied, may also be adjusted to improve delivery of one or more active ingredients. Similarly, the polarity of a solvent carrier, its ionic strength, or tonicity can be adjusted to improve delivery. Compounds such as stearates can also be added to pharmaceutical compositions or dosage forms to advantageously alter the hydrophilicity or lipophilicity of one or more active ingredients so as to improve delivery. In this regard, stearates can serve as a lipid vehicle for the formulation, as an emulsifying agent or surfactant, and as a delivery-enhancing or penetration-enhancing agent. Different salts, hydrates or solvates of the active ingredients can be used to further adjust the properties of the resulting composition.

Combination Therapy

The methods for immunosuppression or for treating or preventing inflammatory conditions and immune disorders in a patient in need thereof can further comprise administering to the patient being administered a compound of this invention, an effective amount of one or more other active agents. Such active agents may include those used conventionally for immunosuppression or for inflammatory conditions or immune disorders. These other active agents may also be those that provide other benefits when administered in combination with the compounds of this invention. For example, other therapeutic agents may include, without limitation, steroids, non-steroidal anti-inflammatory agents, antihistamines, analgesics, immunosuppressive agents and suitable mixtures thereof. In such combination therapy treatment, both the compounds of this invention and the other drug agent(s) are administered to a subject (e.g., humans, male or female) by conventional methods. The agents may be administered in a single dosage form or in separate dosage

forms. Effective amounts of the other therapeutic agents and dosage forms are well known to those skilled in the art. It is well within the skilled artisan's purview to determine the other therapeutic agent's optimal effective-amount range.

In one embodiment of the invention where another therapeutic agent is administered to a subject, the effective amount of the compound of this invention is less than its effective amount when the other therapeutic agent is not administered. In another embodiment, the effective amount of the conventional agent is less than its effective amount when the compound of this invention is not administered. In this way, undesired side effects associated with high doses of either agent may be minimized. Other potential advantages (including without limitation improved dosing regimens and/or reduced drug cost) will be apparent to those of skill in the art.

In one embodiment relating to autoimmune and inflammatory conditions, the other therapeutic agent may be a steroid or a non-steroidal anti-inflammatory agent. Particularly useful non-steroidal anti-inflammatory agents, include, but are not limited to, aspirin, ibuprofen, diclofenac, naproxen, benoxaprofen, flurbiprofen, fenoprofen, flubufen, ketoprofen, indoprofen, piroprofen, carprofen, oxaprozin, pramoprofen, muroprofen, trioxaprofen, suprofen, aminoprofen, tiaprofenic acid, fluprofen, bucloxic acid, indomethacin, sulindac, tolmetin, zomepirac, tiopinac, zidometacin, acemetacin, fentiazac, clidanac, oxpinac, mefenamic acid, meclofenamic acid, flufenamic acid, niflumic acid, tolfenamic acid, diflurisal, flufenisal, piroxicam, sudoxicam, isoxicam; salicylic acid derivatives, including aspirin, sodium salicylate, choline magnesium trisalicylate, salsalate, diflunisal, salicylsalicylic acid, sulfasalazine, and olsalazin; para-aminophennol derivatives including acetaminophen and phenacetin; indole and indene acetic acids, including indomethacin, sulindac, and etodolac; heteroaryl acetic acids, including tolmetin, diclofenac, and ketorolac; anthranilic acids (fenamates), including mefenamic acid, and meclofenamic acid; enolic acids, including oxicams (piroxicam, tenoxicam), and pyrazolidinediones (phenylbutazone, oxyphenthartazone); and alkanones, including nabumetone and pharmaceutically acceptable salts thereof and mixtures thereof. For a more detailed description of the NSAIDs, see Paul A. Insel, Analgesic-Antipyretic and Antiinflammatory Agents and Drugs Employed in the Treatment of Gout, in Goodman & Gilman's The Pharmacological Basis of Therapeutics 617-57 (Perry B. Molinhoff and Raymond W. Ruddon eds., 9.sup.th ed 1996) and Glen R. Hanson, Analgesic, Antipyretic and Anti-Inflammatory Drugs in Remington: The Science and

Practice of Pharmacy Vol II 1196-1221 (A. R. Gennaro ed. 19th ed. 1995) which are hereby incorporated by reference in their entireties.

Of particular relevance to allergic disorders, the other therapeutic agent may be an anthihistamine. Useful antihistamines include, but are not limited to, loratadine, cetirizine, fexofenadine, desloratadine, diphenhydramine, chlorpheniramine, chlorcyclizine, pyrilamine, promethazine, terfenadine, doxepin, carbinoxamine, clemastine, tripelennamine, brompheniramine, hydroxyzine, cyclizine, meclizine, cyproheptadine, phenindamine, acrivastine, azelastine, levocabastine, and mixtures thereof. For a more detailed description of anthihistamines, see Goodman & Gilman's The Pharmacological Basis of Therapeutics (2001) 651-57, 10.sup.th ed).

Immunosuppressive agents include glucocorticoids, corticosteroids (such as Prednisone or Solumedrol), T cell blockers (such as cyclosporin A and FK506), purine analogs (such as azathioprine (Imuran)), pyrimidine analogs (such as cytosine arabinoside), alkylating agents (such as nitrogen mustard, phenylalanine mustard, buslfan, and cyclophosphamide), folic acid antagonsists (such as aminopterin and methotrexate), antibiotics (such as rapamycin, actinomycin D, mitomycin C, puramycin, and chloramphenicol), human IgG, antilymphocyte globulin (ALG), and antibodies (such as anti-CD3 (OKT3), anti-CD4 (OKT4), anti-CD5, anti-CD7, anti-IL-2 receptor, anti-alpha/beta TCR, anti-ICAM-1, anti-CD20 (Rituxan), anti-IL-12 and antibodies to immunotoxins).

The foregoing and other useful combination therapies will be understood and appreciated by those of skill in the art. Potential advantages of such combination therapies include a different efficacy profile, the ability to use less of each of the individual active ingredients to minimize toxic side effects, synergistic improvements in efficacy, improved ease of administration or use and/or reduced overall expense of compound preparation or formulation.

The invention is further defined by reference to the following examples describing in detail the preparation of compounds of the invention. It will be apparent to those skilled in the art that many modifications, both to materials and methods, may be practiced without departing from the purpose and interest of this invention. The following examples are set forth to assist in understanding the invention and should not be construed as specifically limiting the invention described and claimed herein. Such variations of the invention, including the substitution of all equivalents now known or later developed, which would be

within the purview of those skilled in the art, and changes in formulation or minor changes in experimental design, are to be considered to fall within the scope of the invention incorporated herein.

The compounds of the invention and the other therapeutically active agents can be administered at the recommended maximum clinical dosage or at lower doses. Dosage levels of the active compounds in the compositions of the invention may be varied so as to obtain a desired therapeutic response depending on the route of administration, severity of the disease and the response of the patient. When administered in combination with other therapeutic agents, the therapeutic agents can be formulated as separate compositions that are given at the same time or different times, or the therapeutic agents can be given as a single composition.

Examples

The following examples illustrate the present invention and shall not be construe to limit the present invention.

Example 1. Studies on use of compound (I) as an immunosuppressive drug.

T-cells assays were performed. The results are summarized in Figure 4 and Figure 5.

In a cell-based assay against anti-CD3/anti-CD28-activated murine T-cells, compound (I) exhibits antiproliferative activity at IC50 of 3 μM (1.452 μg/mL) (Figure 4). Compound (I) also exhibits a dossage-dependent inhibitory activity against IL-2 expression by the T-cells (Figure 5).

Materials and methods

The materials and methods of the murin T-cells assays are briefly described as follow: spleen cells (5×105 cells/well) isolated from C57BL/6 mice (The Jackson Laboratory, Bar Harbor, Maine) were cultured in 96-well round-bottom plates. Plates were incubated with anti-CD3 (Clone 145-2C11, 2 μg/mL, Biolegend, San Diego, CA) and anti-CD28 (Clone E18, 2 μg/mL, Biolegend) antibodies for 2 hrs at 37 °C and washed with phosphate buffer saline (PBS) before seeded with the spleen cells. Compound (I) dissolved in 1 mg/mL DMSO is further diluted in culture medium and added to wells before cells were plated (final DMSO concentration not exceed 0.5%). After 48 hr of culturing, 50 μL media were taken out from each well for the measurement of IL-2 production by ELISA. Fresh medium containing [3H]-thymidine (1 μCi/well; Perkin Elmer Life Science, Waltham, MA) were added and [3H]-thymidine in-corporation was measured 12 hr later using a Wallac Microbeta Trilux scintillation counter (Perkin Elmer Life Science). For the analysis of IL-2 production,

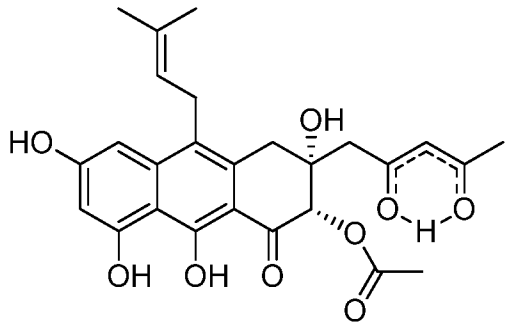
supernatant from the 48 hr culture were assayed for IL-2 by ELISA (BD Biosciences, San Jose, CA). Compound (I) relatively specific as it does not exhibit growth inhibitory activity against yeast and bacteria, as well as human cell lines HeLA and HFF.

We claim:

1. An isolated neosartoricin compound in a substantially purified form.

2. The neosartoricin compound of claim 1, which is about 80% or above purity.

3. The neosartoricin compound of claim 1, having a structure of



neosartoricin, **I**

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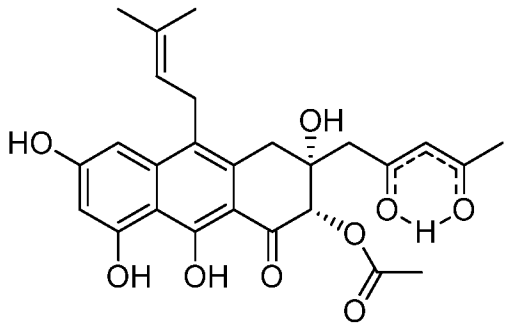
4. A composition, comprising a neosartoricin compound according to any of claims 1-3.

5. A method of forming a composition comprising a neosartoricin compound of invention or a pharmaceutically acceptable salt, solvate, hydrate, clathrate, or prodrug thereof, which method comprising:

providing the neosartoricin compound in an amount effective as an immunosuppressant, and forming the composition.

6. The method according to claim 5, wherein the neosartoricin compound has a structure



of neosartoricin, **I**

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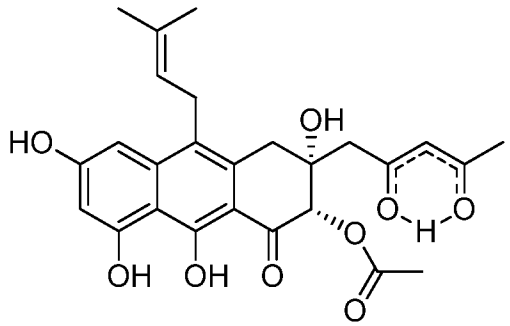
7. A method of treating or ameliorating a medical condition in a subject in need thereof, comprising administering to the subject a neosartoricin compound of invention or a pharmaceutically acceptable salt, solvate, hydrate, clathrate, or prodrug thereof in an amount effective for treating or ameliorating the medical condition.

8. The method of claim 7, wherein the neosartoricin compound, the pharmaceutically acceptable salt thereof, or the prodrug thereof is included in a composition in an amount effective for treating or ameliorating the medical condition.

9. The method of claim 7, wherein the neosartoricin compound has a structure of



neosartoricin, **I**

10. The method of claim 7, wherein the medical condition is a condition related to organ transplant or to implantation of a medical device.

11. The method of claim 10, wherein the medical device is a stent.

12. A method of producing compound (I), comprising overexpressing the pathway specific Zn(II)2Cys6-type regulator nscR in N. fischeri and the nscR homolog in A. fumugatus.

13. The method of claim 12, wherein nscR is overexpressed under the control of an Aspergillus nidulans gpdA promoter by cloning the NscR gene into a pBARGPE1 plasmid.

14. The method of claim 13, wherein the plasmid comprises a fungal selection marker for glufosinate resistance, wherein the nscR gene is amplified using polymerase chain reaction (PCR) and cloned into pBARGPE1 by restriction digest to generate plasmid pBARGPE1-NscR, and wherein the resultant plasmid pBARGPE1-NscR is transformed into N. fischeri using polyethylene glycol (PEG)-mediated transformation.

15. A method of identifying and making immunosuppressive compounds, comprising:

a) genome mining for fungal strains harboring gene clusters capable of producing compounds which are identical or structurally related to compound (I) by searching for homologs of the polycyclic prenyltansferase (pcPTase) gene nscD, which homologs exhibits 60%-100% protein identify to NscD, or, for fungal strains where their genomes are not sequenced, amplifying the pcPTase gene by degenerate primer PCR using a primer pair pcPT-F (SEQ ID NO:1) and pcPT-R (SEQ ID NO:2),

b) expressing or overexpressing the homologs of the pcPTase gene or the amlified pcPTase gene to produce candidate immunosupressive compounds, and

c) subjecting the candidate immunosupressive compounds to an immunosuppressive study, and

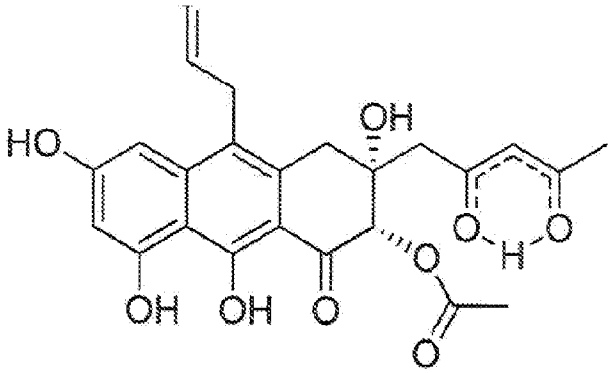
d) designating a candidate immunosuppressive compound as an immunosuppressive compound if result of the immunosuppressive study is positive (immunosuppressive).

16. The method of claim 15, wherein the homologs of the pcPTase gene nscD are found in the genome of dermatophytic fungi that belongs to the family Arthrodermataceae with 60-65% protein identity to NscD.

17. The method of claim 16, wherein the homologs comprise non-reducing polyketide synthase (NRPKS) genes similar to nscA, metallo-beta-lactamase (MbL) genes similar to nscB and flavin-dependent monooxygenase (FMO) genes similar to nscC.

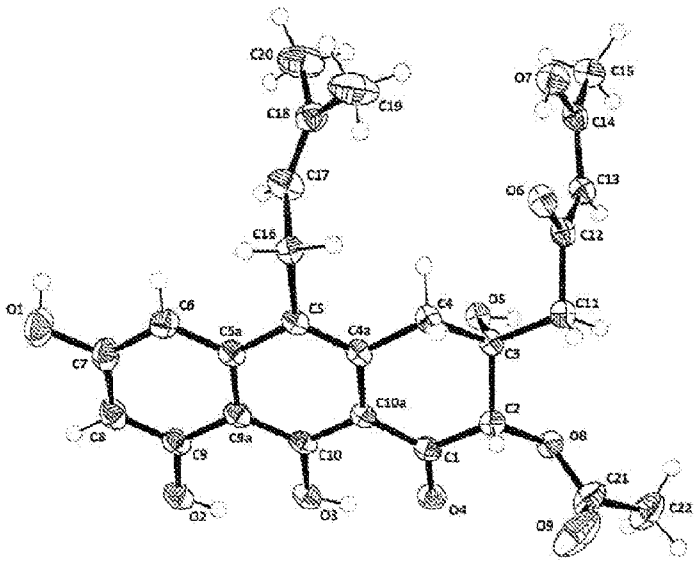
18. The method of claim 15, wherein the immunosuppressive study is an in vitro study against human T-cells or in vivo study using a mouse tumor rejection model.

19. The method of claim 15, wherein the immunosuppressive compound is produced using pcPTase gene-containing gene clusters in the athrodemataceous dermatophytes selected from the group consisting of Microsporum canis, Microsporum gypseum, Athroderma benhamiae, Trichophyton tonsurans, Trichophyton equinum, Trichophyton rubrum and Trichophyton verrucosum.



neosartoricin, I

Chemical Formula: $C_{26}H_{28}O_9$



X-ray crystal structure of I

Figure 1

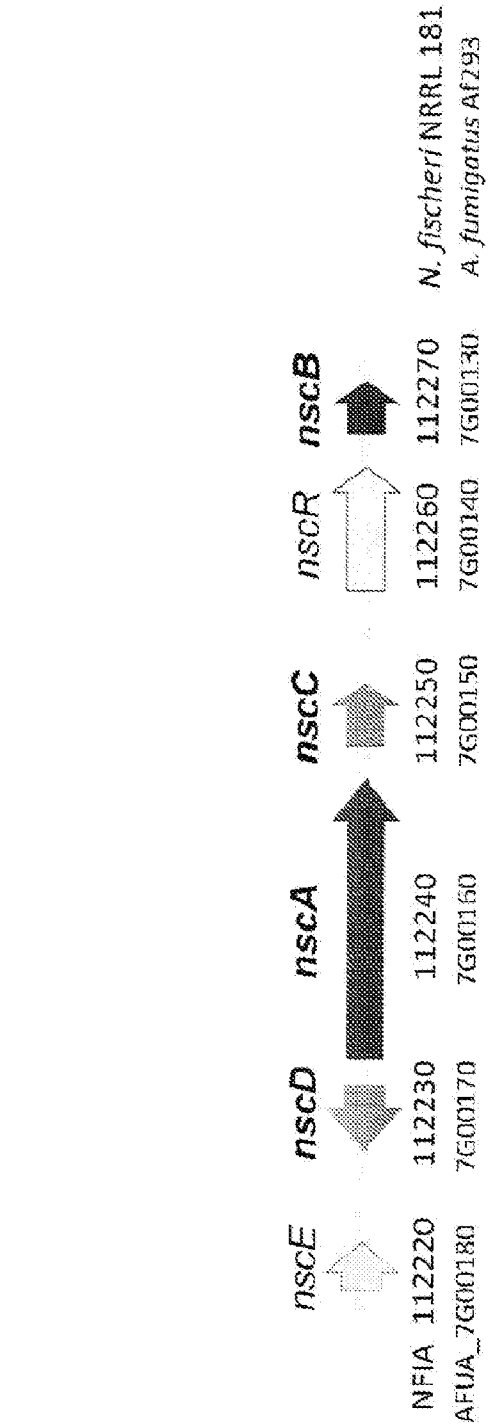

nscE
nscD
nscA
nscC
nscR
nscB
NFIA_112220 112230 112240 112250 112260 112270 N. fischeri NRRL 181
AFUA_7G00180 7G00170 7G00160 7G00150 7G00140 7G00130 A. fumigatus Af293

Figure 2

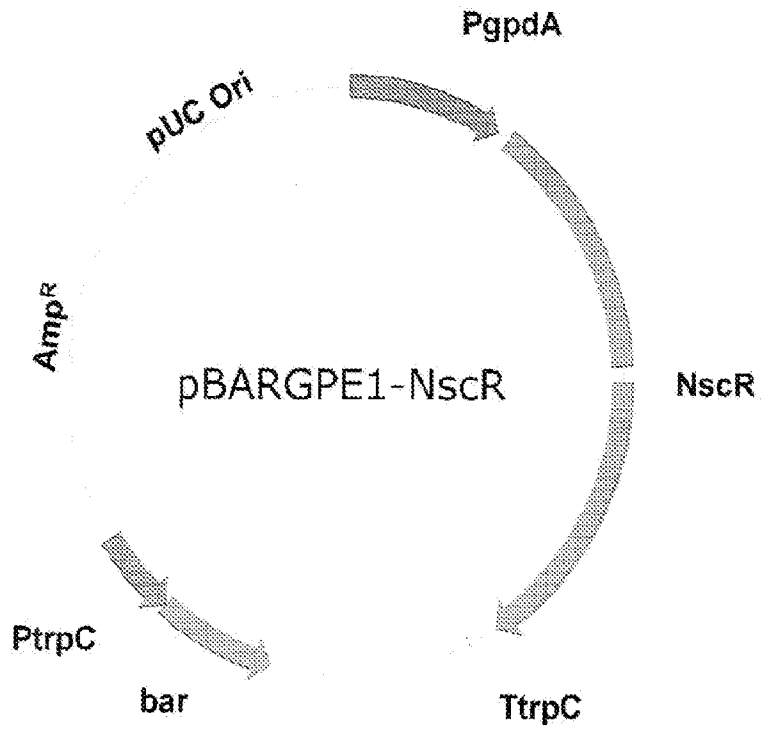


Figure 3

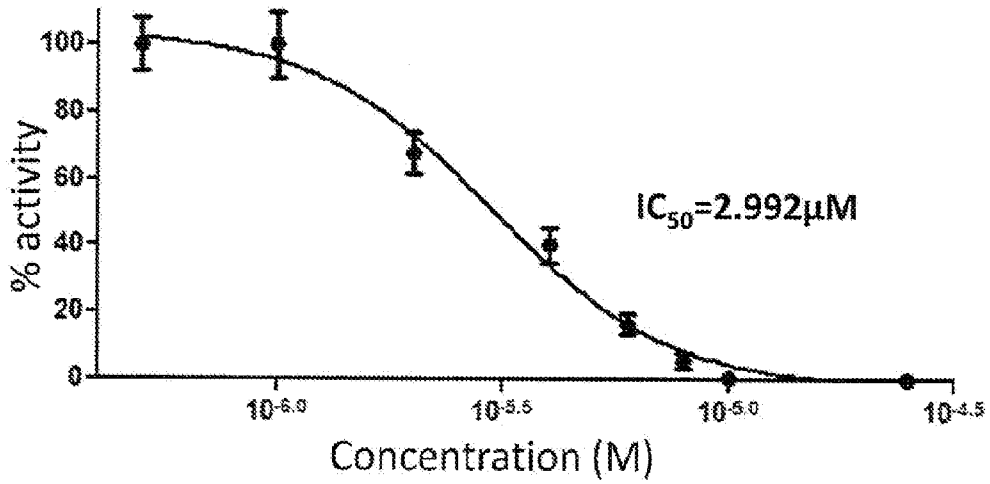


Figure 4

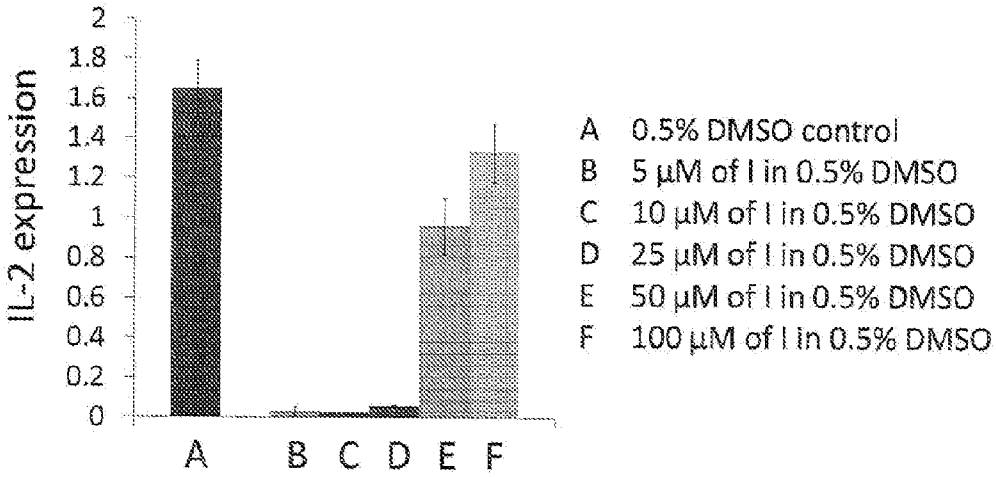
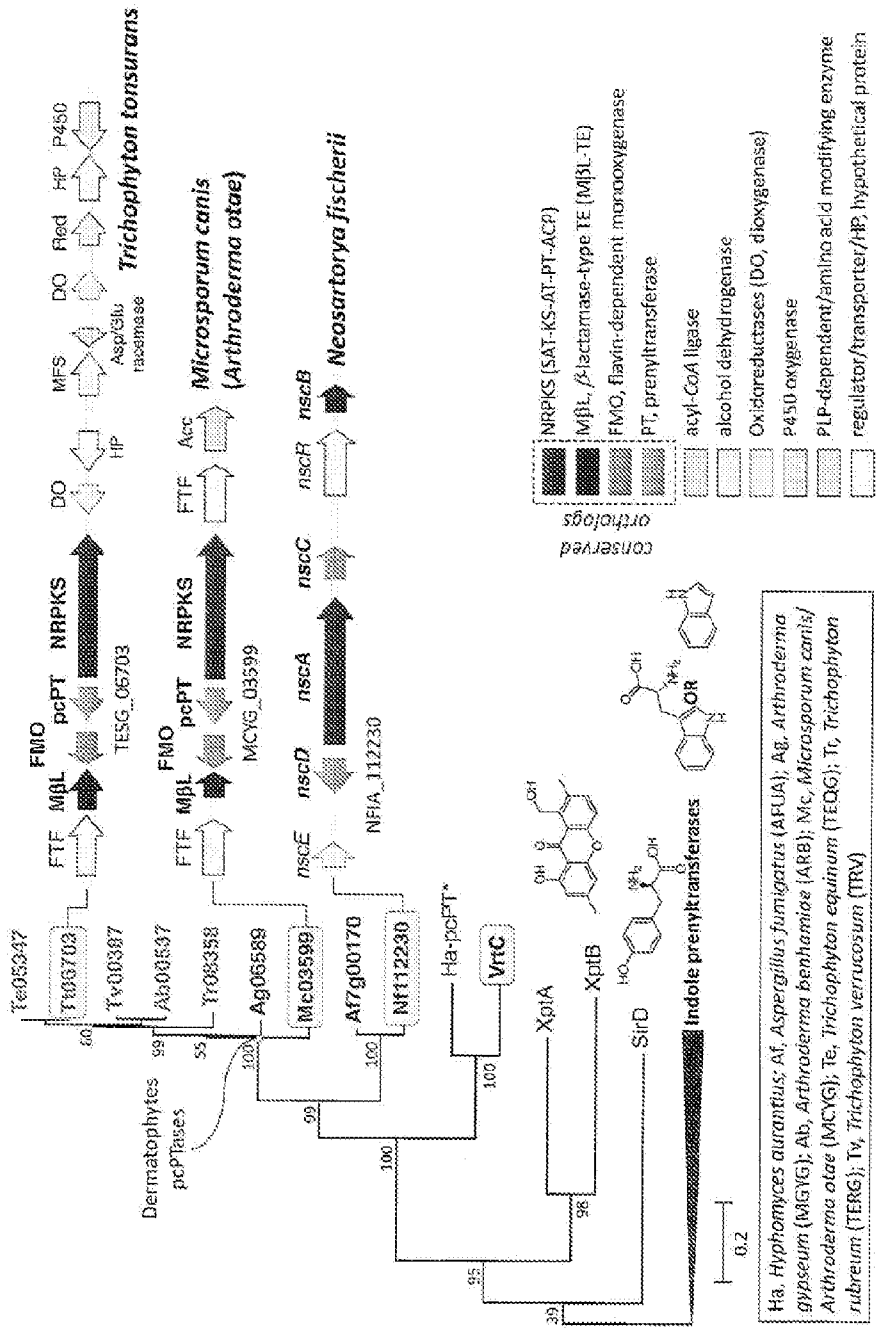


Figure 5



Ha, *Hypomyces aurantius*; Af, *Aspergillus fumigatus* (AFUA); Ag, *Arthroderma gypseum* (MGYG); Ab, *Arthroderma benhamiae* (ARB); Mc, *Microsporum canis*/*Arthroderma otae* (MCYG); Te, *Trichophyton equinum* (TEQG); Tr, *Trichophyton rubreum* (TERG); Tv, *Trichophyton verrucosum* (TRV)

Figure 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/039146

A. CLASSIFICATION OF SUBJECT MATTER

A61K 9/20(2006.01)i, A61K 9/48(2006.01)i, A61K 31/357(2006.01)i, A61K 31/122(2006.01)i, A61P 35/00(2006.01)i

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K 9/20; A61K 9/48; A61K 31/357; A61K 31/122; A61P 35/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKOMPASS(KIPO internal) & Keywords: neosartoricin, (2S,3R)-3,6,8,9-tetrahydroxy-3-[(3Z)-4-hydroxy-2-oxopent-3-en-1-yl]-10-(3-methylbut-2-en-1-yl)-1-oxo-1,2,3,4-tetrahydroanthracen-2-yl acetate, immunosuppressant

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WATANABE, A. et al., `Immunosuppressive substances in Aspergillus fumigatus culture filtrate`, Journal of Infection and Chemotherapy, 2003, Vol. 9, pages 114-121. See abstract; and pages 114, 119 and 120.	1-6,12-19
A	GILSENAN, J. E. M. et al., `Aspergillus genomes and the Aspergillus cloud`, Nucleic Acids Research, 2009, Vol. 37, pages D509-D514. See abstract; and pages D509-D511.	1-6,12-19
A	TAJIMA, K. et al., `Immunomodulatory effects of cyclosporin A on human peripheral blood dendritic cell subsets`, Immunology, 2003, Vol. 108, pages 321-328. See abstract; and pages 321 and 322.	1-6,12-19
PX	CHOOI, Y.-H. et al., `Genome mining of a prenylated and immunosuppressive polyketide from pathogenic fungi`, Organic Letters, 31 January 2013 (E-pub.), Vol. 15, No. 4, pages 780-783. See the whole document.	1-6

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

☒ See patent family annex.

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

Date of the actual completion of the international search	Date of mailing of the international search report
21 August 2013 (21.08.2013)	**21 August 2013 (21.08.2013)**

Name and mailing address of the ISA/KR
Korean Intellectual Property Office
189 Cheongsa-ro, Seo-gu, Daejeon Metropolitan City, 302-701, Republic of Korea
Facsimile No. +82-42-472-7140

Authorized officer
CHOI Sung Hee
Telephone No. +82-42-481-8740

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/039146

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

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

1. ☒ Claims Nos.: 7-11
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 7-11 pertain to methods for treatment of the human body by therapy, and thus relate to a subject matter which this International Searching Authority is not required, under Article 17(2)(a)(i) of the PCT and Rule 39.1(iv) of the Regulations under the PCT, to search.

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

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

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

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

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

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

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

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

Remark on Protest

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

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/US2013/039146

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
None			