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(54) Title: COMPOSITIONS AND METHODS FOR TREATING DIABETIC ULCERS

(57) Abstract: The disclosures herein provide pharmaceutical compositions that may contain various combinations of lipoic acid, aminoguanidine, aminoguanidium, antimicrobial agents, and L-arginine, or a pharmaceutically acceptable salt thereof. In certain embodiments, the pharmaceutical compositions may be formulated for topical, transdermal, or subdermal administration, for example, as a patch or a wound dressing such as a hydrogel. These compositions may be used for treating skin ulcers, such as diabetic foot ulcers and venous ulcers.

WO 2009/137074 A9

COMPOSITIONS AND METHODS FOR TREATING DIABETIC ULCERS

RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application 61/126,760 filed May 6, 2008, the specification of which is hereby incorporated by reference in its entirety.

BACKGROUND

Diabetes is one of the foremost causes of death in many countries and a leading cause of blindness, renal failure, and non-traumatic amputations. Foot disorders are a major source of morbidity and a leading cause of hospitalization for persons with diabetes. Ulceration, infection, gangrene, and amputation are the significant complications of the disease, estimated to cost billions of dollars each year.

Compelling evidence from animal and human studies clearly suggest that nitric oxide (NO) is vital to the wound healing process. It has regulatory functions on various cell types involved in inflammation and proliferation. NO is synthesized from L-arginine, a substrate for both nitric oxide synthase (NOS) and arginase. In wounds, inducible nitric oxide synthase (iNOS) catalyzes arginine to citrulline and nitric oxide which is produced in micromolar amount, whereas arginase converts arginine to ornithine and urea. Ornithine is a precursor for proline and polyamines, which are essential for wound healing.

An important hallmark of diabetes is chronically elevated blood glucose levels. Hyperglycemia stimulates NADPH oxidase and superoxide formation. In addition, it leads to the production of toxic advanced glycation end-products (AGE). It also increases nitric oxide synthase (NOS) activity. As a consequence, there is overproduction of NO and of superoxide. High levels of NO produced by inducible nitric oxide synthase (iNOS) interact with oxygen free radicals derived from polymorphonuclear (PMN) macrophages to produce peroxynitrite responsible for apoptosis and necrosis at the ulcer site. Increased oxidative stress is also responsible for stimulation of NFkB and the inflammatory reaction. Diabetic ulcers, including diabetic foot ulcers, are therefore characterized by oxidative and nitrosative stress.

Infections are common in diabetic patients and are often more severe than infections found in non-diabetic patients. Persons with diabetes have an increased risk for developing an infection of any kind and a several fold risk for developing osteomyelitis. It is well documented that diabetic foot infections are frequently polymicrobial in nature. Hyperglycemia, impaired immunologic responses, neuropathy, and peripheral arterial disease are the major predisposing factors leading to limb-threatening diabetic foot infections. There is a need in the art for compositions that promote optimal NO levels in wounds such as diabetic foot ulcers.

SUMMARY

The disclosures herein provide pharmaceutical compositions that may contain various combinations of lipoic acid, aminoguanidine, aminoguanidium, antimicrobial agents, and L-arginine, or a pharmaceutically acceptable salt thereof. In certain embodiments, the pharmaceutical compositions may be formulated for topical, transdermal, or subdermal administration, for example, as a patch or a wound dressing such as a hydrogel.

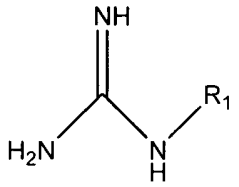
In certain aspects, this disclosure provides a pharmaceutical composition comprising:

(i) at least one of an antimicrobial agent and L-arginine; and

(ii) a salt, wherein the salt comprises:

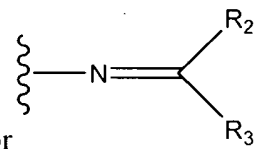
(a) lipoic acid enriched for the R-(+) enantiomer, and

(b) a compound having the structure of Formula (I):

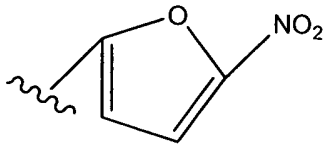
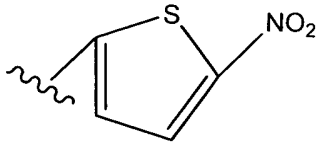
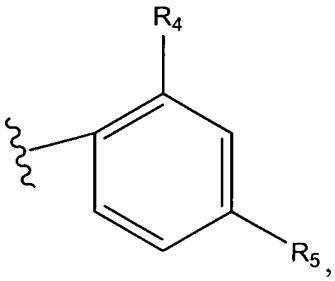
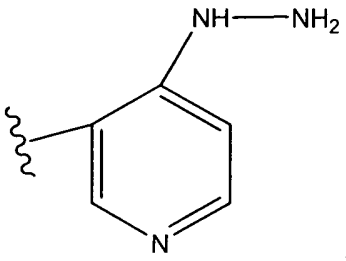
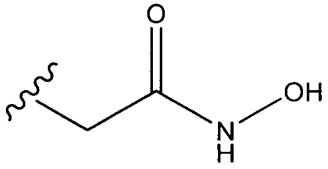


(I)

wherein



R_1 is: $-NH_2$ or ;

R_2 and R_3 are each independently selected from: CH_3,  ,  ,  ,  ,  and ;

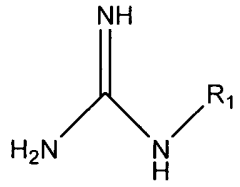
and R_4 and R_5 are each independently selected from Cl and NO_2.

In some aspects, this disclosure provides a pharmaceutical composition comprising:

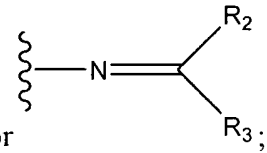
(i) an antimicrobial agent

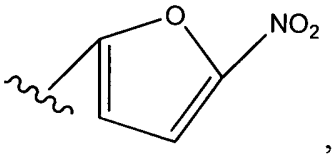
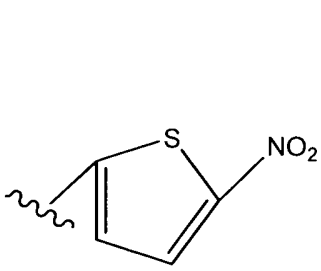
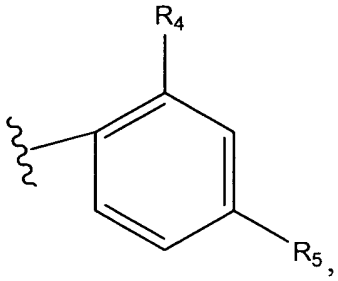
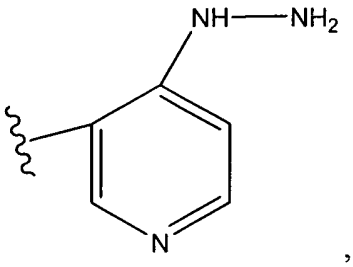
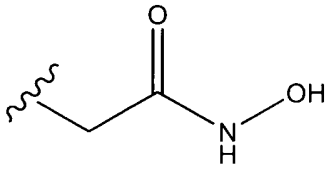
(ii) at least one of L-arginine and lipoic acid enriched for the R-(+) enantiomer; and

(iii) a compound having the structure of Formula (I):

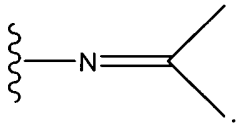


(I)

wherein R_1 is: $-NH_2$ or  ;

R_2 and R_3 are each independently selected from: CH_3,  ,  ,  ,  , and  ;

and R_4 and R_5 are each independently selected from Cl and NO_2.

In some embodiments, R_1 is: $-NH_2$ or  .

In certain embodiments, the pharmaceutical composition comprises L-arginine and an antimicrobial agent.

In certain aspects, this application provides a pharmaceutical composition comprising: (a) an antimicrobial agent, (b) L-arginine, and (c) lipoic acid enriched for the R-(+) enantiomer.

In certain embodiments, the antimicrobial agent is iodine or silver.

In some embodiments, the pharmaceutical composition further comprises hydroxamate or a hydroxamate analogue. The hydroxamate analogue may be, for example, hydroxamate covalently bound to PEG, silicone, guanidine, or aminoguanidine.

In certain embodiments, the salt is in crystalline form.

In some embodiments, the composition is substantially free of the S-enantiomer of lipoate.

In certain embodiments, the pharmaceutical composition further comprises a pharmaceutically acceptable carrier. In certain embodiments, the pharmaceutical composition is

formulated for subdermal, transdermal, or topical administration. The pharmaceutical composition may, in certain embodiments, further comprise at least one of a pharmaceutically acceptable stabilizer, diluent, surfactant, filler, binder, and lubricant.

In certain aspects, the invention relates to a patch comprising any of the pharmaceutical compositions described herein. The patch may be, for example, active or passive.

In some aspects, this disclosure provides a wound dressing comprising any of the pharmaceutical compositions herein. In certain embodiments, the wound dressing is a hydrogel.

In other embodiments, the invention relates to a method of treating a diabetic ulcer comprising administering to a patient in need thereof a therapeutically effective amount of any of the pharmaceutical compositions described herein. The diabetic ulcer may be, for example, a diabetic foot ulcer or a venous ulcer. In certain embodiments, the pharmaceutical composition is administered topically, subdermally, or transdermally. In some embodiments, the pharmaceutical composition is administered using an active patch, passive patch, or wound dressing such as a hydrogel, for example.

Furthermore, in certain aspects the invention relates to a kit comprising a pharmaceutical preparation that includes the any of pharmaceutical compositions disclosed herein.

In certain embodiments, the pharmaceutical composition further comprises L-arginine and/or a antimicrobial agent. In certain embodiments, the antimicrobial agent may be iodine or silver.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts NMR data showing the ^{1}H and ^{13}C chemical shifts of the lipoic acid salt of a compound having a structure of Formula (I), in DMSO-$_{d6}$, in graphical and table form.

Figure 2 depicts the ^{1}H NMR spectra of the lipoic acid salt of a compound having a structure of Formula (I), in DMSO-$_{d6}$.

Figure 3 depicts the ^{13}C NMR spectra of the lipoic acid salt of a compound having a structure of Formula (I), in DMSO-$_{d6}$.

Figure 4 depicts the infra-red spectra of the lipoic acid salt of a compound having a structure of Formula (I), in a deuteriated form.

Figure 5 depicts the crystal structure of the lipoic acid salt of a compound having a structure of Formula (I).

Figure 6 is a table depicting structural data derived from the crystal structure of the lipoic acid salt of a compound having a structure of Formula (I).

Figure 7 is a graph representing the Power XRD pattern of the lipoic acid salt of a compound having a structure of Formula (I).

Figure 8 is a chart summarizing the Power XRD pattern of Figure 7.

Figure 9 depicts Differential Scanning Calorimetry (DSC) of the lipoic acid salt of a compound having a structure of Formula (I). The DSC thermogram indicates that: 1. The crystals undergo an endothermic phase transition at 88°C; 2. The crystals show a sharp melting point at 188.7°C; and 3. The compound decomposes soon after melting. The decomposition endotherm is broad and spans the temperature range 190 – 290°C.

Figure 10 is a graph showing the Thermogravimetric Analysis (TGA) analysis of the lipoic acid salt of a compound having a structure of Formula (I). The TGA analysis indicates that: 1. In the open pan the complete decomposition of the compound begins at 150 °C and ends at 250 °C; 2. No other transitions were associated with the compound; from this one may infer that there is no solvent loss at all; and 3. The compound totally decomposes by the end of the run within experimental error.

DETAILED DESCRIPTION

1. Definitions

As used herein, the following terms and phrases shall have the meanings set forth below. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art.

As used herein, the term "antimicrobial agent" refers to a substance that treats, reduces the severity of, or prevents, a microbial infection in a patient. Antimicrobial agents include antiseptics, disinfectants, antibiotics, anti-fungal agents, and the like. Antibiotics include, but are not limited to, amikacin, gentamicin, kanamycin, neomycin, netilmicin, streptomycin, tobramycin, paromomycin, loracarbef, ertapenem, doripenem, imipenem/cilastatin, meropenem, cefadroxil, cefazolin, cefalotin, cefalothin, cefalexin, cefaclor, cefamandole, cefoxitin, cefprozil, cefuroxime, cefixime, cefdinir, cefditoren, cefoperazone, cefotaxime, cefpodoxime, ceftazidime, ceftibuten, ceftizoxime, ceftriaxone, cefepime, ceftobiprole, teicoplanin, vancomycin, azithromycin, clarithromycin, dirithromycin, erythromycin, roxithromycin, troleandomycin, telithromycin, spectinomycin, aztreonam, amoxicillin, ampicillin, azlocillin, carbenicillin, cloxacillin, dicloxacillin, flucloxacillin, mezlocillin, meticillin, nafcillin, oxacillin, penicillin, piperacillin, ticarcillin, bacitracin, colistin, polymyxin b, ciprofloxacin, enoxacin, gatifloxacin, levofloxacin, lomefloxacin, moxifloxacin, norfloxacin, ofloxacin, trovafloxacin, mafenide, prontosil, sulfacetamide, sulfamethizole, sulfanilimide, sulfasalazine, sulfisoxazole, trimethoprim, trimethoprim-sulfamethoxazole (co-trimoxazole) (TMP-SMX), demeclocycline, doxycycline, minocycline, oxytetracycline, tetracycline, arsphenamine, chloramphenicol, clindamycin, lincomycin, ethambutol, fosfomycin, fusidic acid, furazolidone, isoniazid, linezolid, metronidazole, mupirocin, nitrofurantoin, platensimycin, pyrazinamide, quinupristin/dalfopristin, rifampin, rifampicin, and tinidazole. The term "antibiotic" includes, for example, bacteriocidal and bacteriostatic agents. Disinfectants and antiseptics are substances that may be used topically or transdermally to treat a microbial infection. Disinfectants and antiseptics include alcohols, quaternary ammonium compounds, boric acid, chlorhexidine gluconate, hydrogen peroxide, iodine, iodine-generating compositions, octenidine dihydrochloride, phenol (carbolic acid) compounds, sodium chloride, and sodium hypochlorite.

As used herein, the term “hydrogel” refers to a polymeric material which exhibits the ability to swell in water and to retain a significant portion of water within its structure without dissolution. These materials are generally constructed of one or more hydrophilic polymer molecules, although copolymerization with hydrophobic monomers may also lead to the formation of a hydrogel. These materials are generally elastic, and exhibit a three-dimensional network that is either crosslinked directly by chemical bonds or indirectly through cohesive forces such as ionic or hydrogen bonding.

As used herein, the term "lipoic acid" also encompasses its conjugate base, lipoate. The term "lipoic acid" also includes both stereoisomers (the R and S forms) of lipoic acid and lipoate, as well as all the particular salts of the lipoic acid, such as, for example, the potassium, sodium, or ammonium salt. In certain embodiments, the term "lipoic acid" also encompasses lipoic acid in its free form as well as in a form bound to macromolecules such as the polypeptides ACP, AMP, and an E2 domain containing protein.

A "patient," "subject," or "host" to be treated by the subject method may mean either a human or non-human animal, such as primates, mammals, and vertebrates.

The phrase "pharmaceutically acceptable" is art-recognized. In certain embodiments, the term includes compositions, polymers and other materials and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

The phrase "pharmaceutically acceptable carrier" is art-recognized, and includes, for example, pharmaceutically acceptable materials, compositions or vehicles, such as a liquid or solid filler, diluent, solvent or encapsulating material involved in carrying or transporting any subject composition, from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of a subject composition and not injurious to the patient. In certain embodiments, a pharmaceutically acceptable carrier is non-pyrogenic. Some examples of materials which may serve as pharmaceutically acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, sunflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) phosphate buffer

solutions; and (21) other non-toxic compatible substances employed in pharmaceutical formulations.

The term "polymorph" as used herein is art-recognized and refers to one crystal structure of a given compound.

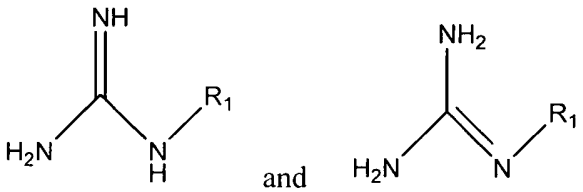
The term "preventing" is art-recognized, and when used in relation to a condition, such as a local recurrence (e.g., pain), a disease such as cancer, a syndrome complex such as heart failure or any other medical condition, is well understood in the art, and includes administration of a composition which reduces the frequency of, or delays the onset of, symptoms of a medical condition in a subject relative to a subject which does not receive the composition. Thus, prevention of cancer includes, for example, reducing the number of detectable cancerous growths in a population of patients receiving a prophylactic treatment relative to an untreated control population, and/or delaying the appearance of detectable cancerous growths in a treated population versus an untreated control population, e.g., by a statistically and/or clinically significant amount. Prevention of an infection includes, for example, reducing the number of diagnoses of the infection in a treated population versus an untreated control population, and/or delaying the onset of symptoms of the infection in a treated population versus an untreated control population. Prevention of pain includes, for example, reducing the magnitude of, or alternatively delaying, pain sensations experienced by subjects in a treated population versus an untreated control population.

A skin ulcer is a local loss of the epidermis and at least part of the dermis, sometimes accompanied by the disintegration of tissue and/or the formation of pus. Skin ulcers are often marked by loss of integrity of the affected area, a secondary infection of the site by bacteria, fungus or virus, generalized weakness of the patient, and delayed healing. Causes of skin ulcers include physical trauma, acute bacterial infection, chronic bacterial infections, fungal infections, peripheral vascular diseases, neuropathies, systemic scleroderma, and tumors. A diabetic ulcer is a skin ulcer in a diabetic patient, where there is a likelihood that diabetes contributed to the formation and/or persistence of the ulcer. The major predisposing cause of diabetic ulcers is diabetic polyneuropathy. The resulting sensory denervation impairs the perception of trauma, which promotes the formation and persistence of skin ulcers in diabetic patients. Diabetic foot ulcers are diabetic ulcers found on the foot. Another example of a skin ulcer is a venous ulcer.

A venous ulcer is a shallow wound that develops when the veins do not move blood back toward the heart normally. Sometimes, venous ulcers affect the skin but also extend beneath the skin, e.g. into muscle.

When used with respect to a pharmaceutical composition or other material, the term "sustained release" is art-recognized. For example, a subject composition which releases a substance over time may exhibit sustained release characteristics, in contrast to a bolus type administration in which the entire amount of the substance is made biologically available at one time. For example, in particular embodiments, upon contact with body fluids including blood, spinal fluid, mucus secretions, lymph or the like, one or more of the pharmaceutically acceptable excipients may undergo gradual or delayed degradation (e.g., through hydrolysis) with concomitant release of any material incorporated therein, e.g., an therapeutic and/or biologically active salt and/or composition, for a sustained or extended period (as compared to the release from a bolus). This release may result in prolonged delivery of therapeutically effective amounts of any of the therapeutic agents disclosed herein.

As used herein, the term "tautomers" refers to isomeric compounds which differ only in the migration of a proton and movement of a double bond or more than one conjugated double bonds. For example, a compound drawn as Formula (I) may exist as its tautomeric forms (I) or (Ia):



(I) (Ia).

For the purposes of the present application, Formula (I) should be understood to encompass the tautomers indicated by Formulae (I) and (Ia).

The phrase "therapeutically effective amount" is an art-recognized term. In certain embodiments, the term refers to an amount of a salt or composition disclosed herein that produces some desired effect at a reasonable benefit/risk ratio applicable to any medical treatment. In certain embodiments, the term refers to that amount necessary or sufficient to

eliminate or reduce medical symptoms for a period of time. The effective amount may vary depending on such factors as the disease or condition being treated, the particular targeted constructs being administered, the size of the subject, or the severity of the disease or condition. One of ordinary skill in the art may empirically determine the effective amount of a particular composition without necessitating undue experimentation.

The term "treating" is art-recognized and includes preventing a disease, disorder or condition from occurring in an animal which may be predisposed to the disease, disorder and/or condition but has not yet been diagnosed as having it; inhibiting the disease, disorder or condition, e.g., impeding its progress; and relieving the disease, disorder, or condition, e.g., causing regression of the disease, disorder and/or condition. Treating the disease or condition includes ameliorating at least one symptom of the particular disease or condition, even if the underlying pathophysiology is not affected, such as treating the pain of a subject by administration of an analgesic agent even though such agent does not treat the cause of the pain. The term "treating", "treat" or "treatment" as used herein includes curative, preventative (e.g., prophylactic), adjunct and palliative treatment.

"Wound dressings" as referred to herein comprise a wound dressing carrier and a therapeutic agent. The wound dressing carrier may be, for example, a gauze pad, transparent film, hydrogel, foam, a hydrocolloid, calcium alginate, or a collagen dressing.

2. Introduction

The subject compositions contain two or more of the following therapeutic agents: lipoic acid, a compound having a structure of Formula (I), an antimicrobial agent, hydroxamine or a hydroxamine analogue, and L-arginine, or a pharmaceutically acceptable salt thereof. Lipoic acid, or a pharmaceutically acceptable salt thereof, is known in the art to be therapeutically useful in treating diabetes. The compound having a structure of Formula (I) is known in the art to be therapeutically useful in treating diabetes. L-arginine is another known therapy for diabetes. Hydroxamine is a matrix metalloprotease inhibitor (MMPs) that promotes wound healing when administered to ulcers. Finally, antimicrobial agents may be administered to kill or prevent the growth of bacteria or other pathogens in an ulcer.

3. Compositions for treating diabetic ulcers, and synthesis thereof

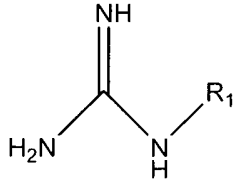
The compositions as described herein may contain various combinations of lipoic acid, aminoguanidine, aminoguanidium, antimicrobial agents, disinfectants, hydroxamine or a hydroxamine analogue, and L-arginine, or a pharmaceutically acceptable salt thereof. The pharmaceutical compositions may be formulated for topical, transdermal, or subdermal administration. For example, the pharmaceutical compositions may be administered via a patch or a wound dressing.

Precursors for the compositions described herein may be obtained or produced readily as set forth below. Aminoguanidine hydrochloride may be prepared as disclosed in *Journal of American Chemistry Society 57: 2730 (1935)*. Lipoic acid is commercially available and its synthesis is reported in, for example, *Chem. Commun., 1986, 1408*. L-arginine is commercially available, for example, from Sigma-Aldrich, Iodine is commercially available, for example, from Sigma-Aldrich.

3.1 Compounds of Formula (I)

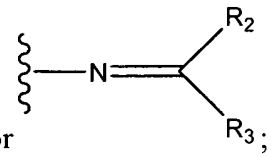
Aminoguanidine is a prototype therapeutic agent for the inhibition of AGE formation *Jour. Carbo. Chem., 12(6): 731-742; Diabetes 41:26-29; U.S Patent Nos: 5,128,360 and 5,238,963*; and is also an inhibitor of NOS, including iNOS *Eur. Jour. Pharma., 233, 119-125*. However, aminoguanidine has significant safety/tolerability issues that limit its utility. The side effects of aminoguanidine may be reduced by co-administration with lipoic acid (see International Application PCT/US2009/001401).

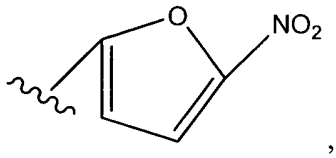
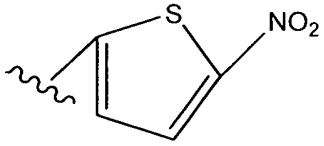
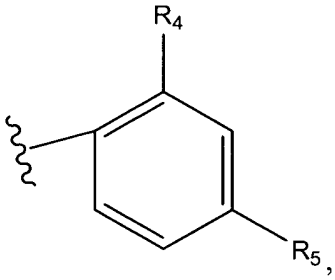
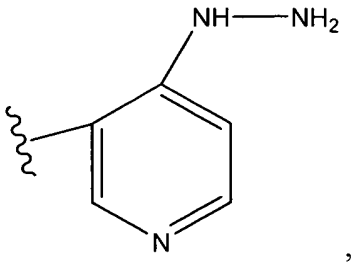
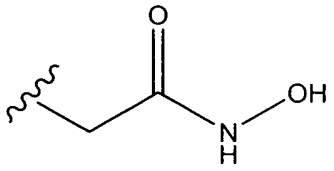
In certain embodiments, the invention relates to a compound having the structure of Formula (I) or a pharmaceutically acceptable salt thereof:



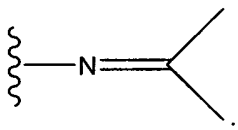
(I)

wherein

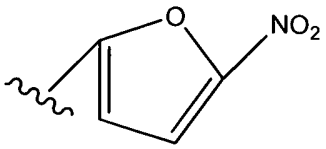
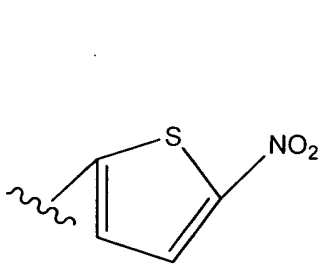
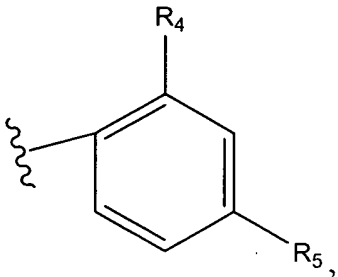
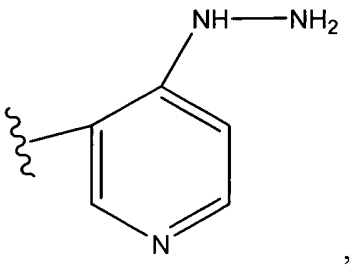
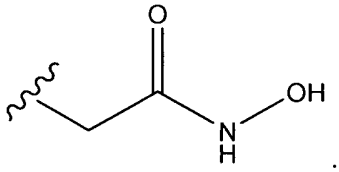
R_1 is: $-NH_2$ or  ;

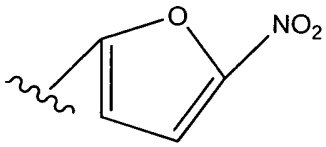
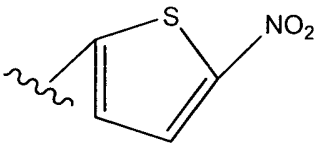
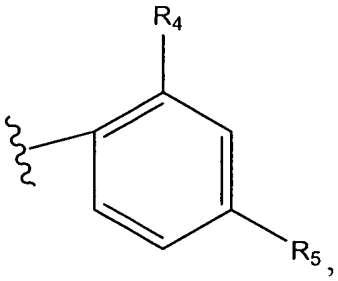
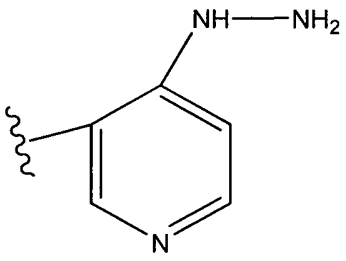
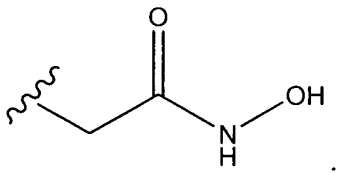
R_2 and R_3 are each independently selected from: CH_3,  ,  ,  ,  ,  and ; and

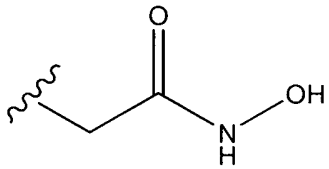
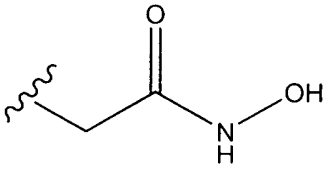
R_4 and R_5 are each independently selected from Cl and NO_2.

In some embodiments, R_1 is: $-NH_2$ or  .

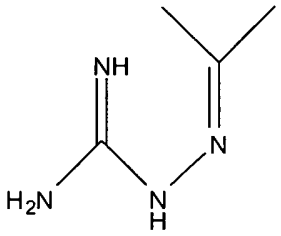
In certain embodiments, both R_4 and R_5 are Cl. In certain embodiments, both R_4 and R_5 are NO_2.

In certain embodiments, R_2 is CH_3 and R_3 is selected from:  ,  ,  ,  , and  .

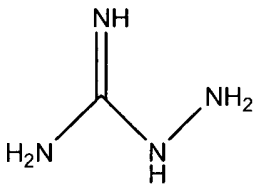
In some embodiments, R_2 and R_3 are identical to each other, and R_2 and R_3 are selected from:  ,  ,  ,  , and  .

In some embodiments, R_2 is  and R_3 is  or CH_3.

In certain embodiments, the compositions described herein are prodrugs that are metabolized into an active form in a subject's body. For example, according to the non-limiting theory herein, when ingested, the compound having the structure of Formula (II) may be converted to the compound having the structure of Formula (III).



(II)

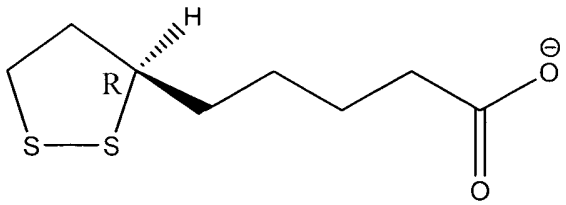


(III)

3.2 Lipoic acid and lipoic acid salts

Alpha-lipoic acid has a variety of names. In addition to being known as α -lipoic acid and thioctic acid, it is also known as lipoic acid, 1,2-dithiolane-3-pentanoic acid; 1,2-ditholane-3-valeric acid; 6,8-thioctic acid; 5-[3-C1,2-dithiolanyl)]-pentanoic acid; delta-[3-(1,2-dithiacyclopentyl)] pentanoic acid; acetate replacing factor and pyruvate oxidation factor. Lipoic acid has an asymmetric carbon atom and is usually employed in the form of a racemic mixture of its (R)- and (S)-enantiomers. It is commercially available (e.g. from Sigma Aldrich). Lipoic acid administration has been shown to be active in oxidative stress models including in ischemia-reperfusion injury model. In certain embodiments, the lipoic acid is enriched for the R-(+) enantiomer.

In certain embodiments, R-(+)-lipoate, which is the conjugate base of R-(+)-lipoic acid, has the structure:



In certain embodiments, the salts described herein are crystalline.

In certain embodiments, the invention relates to a crystalline salt of lipoic acid and a compound having a structure of Formula (I). In certain embodiments, the crystalline salt is in the form of a polymorph designated herein after as polymorph A. This crystal structure is shown in Figure 5. Polymorph A has high purity and stability, including thermodynamic stability and resistance to moisture in the air (hygroscopicity), as well as high bioavailability. In addition, polymorph A has high solubility, and high solubility typically leads to high bioavailability. Another advantage of polymorph A is that it is better suitable for the manufacture of pharmaceutical formulations in large scale than said salt in amorphous form because of better handling properties.

The lipoic acid salt of a compounds having a structure of Formula (I) are readily prepared as set forth below.

A salt of a compound having a structure of Formula (I) (for example, aminoguanidine hydrochloride) and lipoic acid may be dissolved in an appropriate inert solvent. As used herein, the expression "inert solvent" refers to a solvent or mixture of solvents, which does not interact with starting materials, reagents, intermediates or products in a manner, which adversely affects the yield of the desired product. Appropriate solvents include methanol, ethanol, n-propanol, isopropanol, butanols, acetonitrile, acetone, ethyl methyl ketone, diethyl ketone and methyl isobutyl ketone. When aminoguanidine salts are employed, a non-reacting base may be used to sufficiently neutralize the salts. Non-reacting bases include alkali and alkali metal hydroxides, alkali and alkali metal carbonates and bicarbonates and tertiary amines. Also included are resin bases. Examples of these non-reacting bases include sodium methoxide, sodium hydroxide, potassium hydroxide, sodium carbonate, potassium carbonate, sodium bicarbonate, potassium bicarbonate, triethyl amine, N-methyl isopropyl amine, and the ion exchange AMBERLYST™ resins.

The reaction mixture is stirred at about ambient temperature to about the refluxing temperature of the solvent being used for about two hours to about six hours, for instance at ambient temperature for about two hours. The reaction mixture may be stirred using any appropriate stirring device. The salts may be isolated from the reaction mixture by methods well known to those skilled in the art and crystallized from an appropriate solvent or mixture of

solvents, including, but not limited to, alcohols (including methanol, ethanol, and isopropanol), acetonitrile, and acetone.

Hydrates and solvates of lipoic acid salts of the compounds having a structure of Formula (I) are also encompassed in the scope of the disclosures herein.

3.3 Antimicrobial agents

In the disclosed compositions, various antimicrobial agents may be included. In some aspects, the antimicrobial agent is an antiseptic or disinfectant. In other aspects, the antimicrobial agent is an antibiotic. In yet other aspects, the antimicrobial agent is an anti-fungal agent. In other aspects, the antimicrobial agent is an antiprotozoal agent. The antimicrobial agent may have broad-spectrum activity, and may be active against one or more types of microorganism. For example, the antimicrobial agent may act against bacteria and fungi.

Examples of suitable antibiotics include amikacin, gentamicin, kanamycin, neomycin, netilmicin, streptomycin, tobramycin, paromomycin, loracarbef, ertapenem, doripenem, imipenem/cilastatin, meropenem, cefadroxil, cefazolin, cefalotin, cefalothin, cefalexin, cefaclor, cefamandole, cefoxitin, cefprozil, cefuroxime, cefixime, cefdinir, cefditoren, cefoperazone, cefotaxime, cefpodoxime, ceftazidime, ceftibuten, ceftizoxime, ceftriaxone, cefepime, ceftobiprole, teicoplanin, vancomycin, azithromycin, clarithromycin, dirithromycin, erythromycin, roxithromycin, troleandomycin, telithromycin, spectinomycin, aztreonam, amoxicillin, ampicillin, azlocillin, carbenicillin, cloxacillin, dicloxacillin, flucloxacillin, mezlocillin, meticillin, nafcillin, oxacillin, penicillin, piperacillin, ticarcillin, bacitracin, colistin, polymyxin b, ciprofloxacin, enoxacin, gatifloxacin, levofloxacin, lomefloxacin, moxifloxacin, norfloxacin, ofloxacin, trovafloxacin, mafenide, prontosil, sulfacetamide, sulfamethizole, sulfanilimide, sulfasalazine, sulfisoxazole, trimethoprim, trimethoprim-sulfamethoxazole (co-trimoxazole) (TMP-SMX), demeclocycline, doxycycline, minocycline, oxytetracycline, tetracycline, arsphenamine, chloramphenicol, clindamycin, lincomycin, ethambutol, fosfomycin, fusidic acid, furazolidone, isoniazid, linezolid, metronidazole, mupirocin, nitrofurantoin, platensimycin, pyrazinamide, quinupristin/dalfopristin, rifampin, rifampicin, and tinidazole.

Other appropriate microbial agents include topical disinfectants such as alcohols, quaternary ammonium compounds, boric acid, chlorhexidine gluconate, chloroxylenol, hydrogen

peroxide, iodine, octenidine dihydrochloride, phenol (carbolic acid) compounds, sodium chloride, sodium hypochlorite, silver, and silver compounds.

Commonly used alcohols include ethanol (usually at 60-90%), 1-propanol (usually at 60-70%) and 2-propanol/isopropanol (usually at 70-80%) or mixtures of these alcohols. They may be given in combination with iodine (tincture of iodine) or some cationic surfactants (benzalkonium chloride 0.05 - 0.5%, chlorhexidine 0.2 - 4.0% or octenidine dihydrochloride 0.1 - 2.0%).

Quaternary ammonium compounds include benzalkonium chloride (BAC), cetyl trimethylammonium bromide (CTMB), cetylpyridinium chloride (Cetrim, CPC) and benzethonium chloride (BZT). Related disinfectants include chlorhexidine and octenidine.

Chlorhexidine gluconate is typically used in concentrations of 0.5 - 4.0% alone, or in lower concentrations in combination with other compounds, such as alcohols.

Hydrogen peroxide is generally used as a 6% aqueous solution to clean wounds and ulcers. 1% or 2% solutions of hydrogen peroxide are also used for minor wounds.

Iodine is often used in an alcoholic solution (called tincture of iodine) or as Lugol's iodine solution. Certain other iodine disinfectants such as povidone-iodine may also be used. According to a non-limiting theory, povidone-iodine may leave a depot of active iodine at the site of administration, creating a remanent or persistent effect. Another iodine disinfectant is cadexomer iodine.

Iodine-generating compositions may also be used as disinfectants. Various iodine-generating compositions are known in the art. Certain iodine-generating compositions comprise an oxidized iodine in combination with a reducing agent at an appropriate pH. The oxidized iodine can be a material such as potassium iodate or iodine pentoxide. The iodine generating composition can also be formulated by combining a compound such as an alkali metal iodide with a suitable oxidizing agent such as persulfate, perborate and a additional source of protons such as citric acid. Other oxidizing agents which can be used in making an iodine-generating composition include hydroqen peroxide, tertary butyl peroxide, alkali metal periodate, hypochlorite salts and free hypochlorous acid as well as halogen amines such as chloramine.

Octenidine dihydrochloride is cationic surfactant and bis-(dihydropyridinyl)-decane derivative, often used in concentrations of 0.1 - 2.0%. In aqueous formulations, it is often potentiated with addition of 2-phenoxyethanol.

Phenol (carbolic acid) compounds may also be used as topical disinfectants. Phenol may be used in the form of a powder. Certain phenolic antiseptics include triclosan and sodium 3,5-dibromo-4-hydroxybenzenesulfonate (Dibromol).

Silver compositions, including silver sulfadiazine, silver nitrate, silver chloride, silver iodine, silver lactate, and colloidal silver, may also be used as topical disinfectants.

3.4 L-arginine

L-Arginine is known to have several modulatory functions in the endocrine and immune system. Supplemental L-arginine improves wound healing in healthy and elderly humans, as well as in rodents. L-arginine is the unique substrate for nitric oxide synthesis, which is involved in many regulatory mechanisms relevant to wound healing such as angiogenesis, cell proliferation, collagen synthesis, epithelialization by its inducible NOS.

In certain embodiments, the L-arginine is free L-arginine, i.e. not polymerized and not part of a polypeptide or oligopeptide. In certain embodiments, the L-arginine is substantially free of D-arginine. It should be understood that the term "L-arginine" as used herein encompasses all tautomers of L-arginine.

3.5 Hydroxamate and hydroxamate analogues

Hydroxamate has metal chelating activity, and in particular is useful for chelating zinc ions. This makes hydroxamate a potent inhibitor of matrix metalloproteases (MMPs). MMP levels are often elevated in diabetic ulcers, leading to collagen breakdown, and preventing wound healing.

In certain embodiments, pharmaceutical compositions as disclosed herein may comprise hydroxamate. The pharmaceutical compositions may also include hydroxamate analogues. Hydroxamate analogues include hydroxamate covalently bonded to PEG, silicone, guanidine, or aminoguanidine.

3.6 Compositions

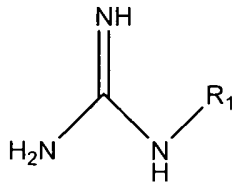
In certain aspects, this disclosure provides a pharmaceutical composition comprising:

(i) at least one of an antimicrobial agent and L-arginine; and

(ii) a salt, wherein the salt comprises:

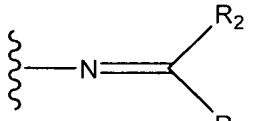
(a) lipoic acid enriched for the R-(+) enantiomer, and

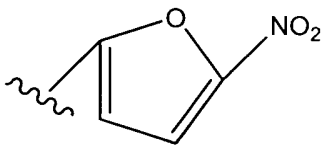
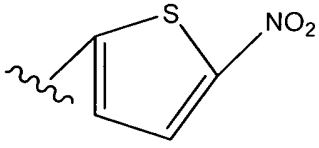
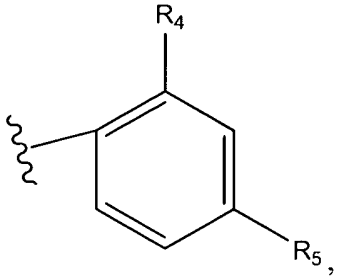
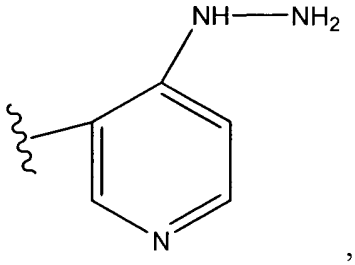
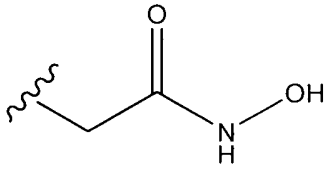
(b) a compound having the structure of Formula (I):



(I)

wherein

R_1 is: $-NH_2$ or  ;

R_2 and R_3 are each independently selected from: CH_3,  ,  ,  ,  ,  and ;

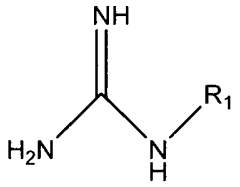
and R_4 and R_5 are each independently selected from Cl and NO_2.

In some aspects, this disclosure provides a pharmaceutical composition comprising:

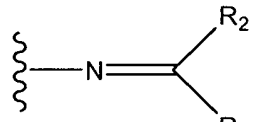
(i) an antimicrobial agent

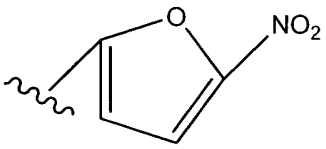
(ii) at least one of L-arginine and lipoic acid enriched for the R-(+) enantiomer; and

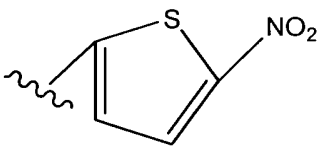
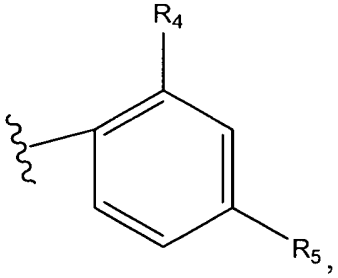
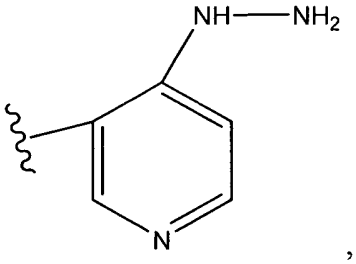
(iii) a compound having the structure of Formula (I):

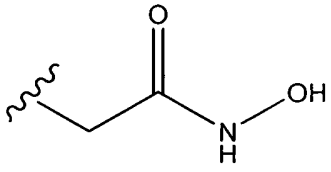


(I)

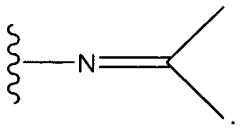
wherein R_1 is: $-NH_2$ or  ;

R_2 and R_3 are each independently selected from: CH_3,  ,

 ,  ,  ,

and  ;

and R_4 and R_5 are each independently selected from Cl and NO_2.

In some embodiments, R_1 is: $-NH_2$ or  .

In certain embodiment, the pharmaceutical composition comprises L-arginine and an antimicrobial agent.

In certain aspects, this application provides a pharmaceutical composition comprising: (a) an antimicrobial agent, (b) L-arginine, and (c) lipoic acid enriched for the R-(+) enantiomer.

In certain embodiments, the antimicrobial agent is iodine or silver.

In some embodiments, the pharmaceutical composition further comprises hydroxamate or a hydroxamate analogue. The hydroxamate analogue may be, for example, hydroxamate covalently bonded to PEG, silicone, guanidine, or aminoguanidine.

In certain embodiments, the salt is in crystalline form.

In some embodiments, the composition is substantially free of the S enantiomer of lipoate.

In certain embodiments, the pharmaceutical composition further comprises a pharmaceutically acceptable carrier. In certain embodiments, the pharmaceutical composition is formulated for subdermal, transdermal, or topical administration. The pharmaceutical composition may, in certain embodiments, further comprise at least one of a pharmaceutically acceptable stabilizer, diluent, surfactant, filler, binder, and lubricant.

This application provides, *inter alia,* certain compositions comprising lipoic acid, compounds having a structure of Formula (I), and L-arginine. Additionally, herein is provided a composition comprising the lipoic acid salt of L-arginine (or the conjugate base of L-arginine), and a compound having a structure of Formula (I). Furthermore, the present application also provides a composition comprising: a) a lipoic acid salt of L-arginine, or the conjugate base of L-arginine, and b) a lipoic acid salt of a compound having a structure of Formula (I).

4. Dosages of the subject compositions

In certain embodiments, the pharmaceutical compositions described herein are formulated in a manner such that said compositions will be delivered to a patient in a therapeutically effective amount, as part of a prophylactic or therapeutic treatment. The desired amount of the composition to be administered to a patient will depend on absorption, inactivation, and excretion rates of the drug as well as the delivery rate of the salts and compositions from the subject compositions. It is to be noted that dosage values may also vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the

individual need and the professional judgment of the person administering or supervising the administration of the compositions. Typically, dosing will be determined using techniques known to one skilled in the art.

Additionally, the optimal concentration and/or quantities or amounts of any particular salt or composition may be adjusted to accommodate variations in the treatment parameters. Such treatment parameters include the clinical use to which the preparation is put, e.g., the site treated, the type of patient, e.g., human or non-human, adult or child, and the nature of the disease or condition.

In certain embodiments, the dosage of the subject compositions provided herein may be determined by reference to the plasma concentrations of the therapeutic composition or other encapsulated materials. For example, the maximum plasma concentration (C_{max}) and the area under the plasma concentration-time curve from time 0 to infinity may be used.

An effective amount of the compositions described herein refers to the amount of one of said salts or compositions which is capable of inhibiting or preventing a skin ulcer such as a diabetic ulcer. An effective amount may be sufficient to prohibit, treat, alleviate, ameliorate, halt, restrain, slow or reverse the progression, or reduce the severity of a diabetic ulcer. In certain embodiments, the diabetic ulcer is one that arises from elevated advanced glycation end products (AGE) and/or elevated reactive oxidative-nitrosative species and/or elevated nitric oxide synthase (NOS) activity. As such, these methods include both medical therapeutic (acute) and/or prophylactic (prevention) administration as appropriate. The amount and timing of compositions administered will, of course, be dependent on the subject being treated, on the severity of the affliction, on the manner of administration and on the judgment of the prescribing physician. Thus, because of patient-to-patient variability, the dosages given above are a guideline and the physician may titrate doses of the drug to achieve the treatment that the physician considers appropriate for the patient. In considering the degree of treatment desired, the physician must balance a variety of factors such as age of the patient, presence of preexisting disease, as well as presence of other diseases.

The subject compositions may be administered once, or may be divided into a number of smaller doses to be administered at varying intervals of time, depending in part on the release rate of the compositions and the desired dosage. For example, a patch or wound dressing may

be replaced once per day, once every two days, once every 4 days, once every week, once every two weeks, or once every month.

5. Pharmaceutical additives and excipients

The pharmaceutical compositions described herein may be formulated for topical, dermal, or subdermal administration. The pharmaceutical composition may further comprise at least one of a pharmaceutically acceptable carrier, stabilizer, diluent, surfactant, filler, binder, and lubricant.

Dosage forms for administration of the compositions described herein include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, and patches (for example, active or passive patches). A subject composition may be mixed under sterile conditions with a pharmaceutically acceptable carrier, and with any preservatives, buffers, or propellants that may be required. For transdermal administration, the complexes may include lipophilic and hydrophilic groups to achieve the desired water solubility and transport properties.

Ointments, as is well known in the art of pharmaceutical formulation, are semisolid preparations that are typically based on petrolatum or other petroleum derivatives. The specific ointment base to be used, as will be appreciated by those skilled in the art, is one that will provide for optimum drug delivery, and, preferably, will provide for other desired characteristics as well, e.g., emolliency or the like. As with other carriers or vehicles, an ointment base should be inert, stable, nonirritating and nonsensitizing. As explained in *Remington: The Science and Practice of Pharmacy, 19th Ed.* (Easton, Pa.: Mack Publishing Co., 1995), at pages 1399-1404, ointment bases may be grouped in four classes: oleaginous bases; emulsifiable bases; emulsion bases; and water-soluble bases. Oleaginous ointment bases include, for example, vegetable oils, fats obtained from animals, and semisolid hydrocarbons obtained from petroleum. Emulsifiable ointment bases, also known as absorbent ointment bases, contain little or no water and include, for example, hydroxystearin sulfate, anhydrous lanolin and hydrophilic petrolatum. Emulsion ointment bases are either water-in-oil (W/O) emulsions or oil-in-water (O/W) emulsions, and include, for example, cetyl alcohol, glyceryl monostearate, lanolin and stearic acid. Appropriate water-soluble ointment bases are prepared from polyethylene glycols of varying molecular weight; again, see *Remington: The Science and Practice of Pharmacy* for further information.

Creams, as also well known in the art, are viscous liquids or semisolid emulsions, either oil-in-water or water-in-oil. Cream bases are water-washable, and contain an oil phase, an emulsifier and an aqueous phase. The oil phase, also called the "internal" phase, is generally comprised of petrolatum and a fatty alcohol such as cetyl or stearyl alcohol. The aqueous phase usually, although not necessarily, exceeds the oil phase in volume, and generally contains a humectant. The emulsifier in a cream formulation is generally a nonionic, anionic, cationic or amphoteric surfactant.

As will be appreciated by those working in the field of pharmaceutical formulation, gels are semisolid, suspension-type systems. Single-phase gels contain organic macromolecules distributed substantially uniformly throughout the carrier liquid, which is typically aqueous, but also, may contain an alcohol and, optionally, an oil. Certain "organic macromolecules" that may be used in the disclosed formulations include gelling agents, crosslinked acrylic acid polymers such as the "carbomer" family of polymers, e.g., carboxypolyalkylenes that may be obtained commercially under the Carbopol™ trademark. Also available are hydrophilic polymers such as polyethylene oxides, polyoxyethylene-polyoxypropylene copolymers and polyvinylalcohol; cellulosic polymers such as hydroxypropyl cellulose, hydroxyethyl cellulose, hydroxypropyl methylcellulose, hydroxypropyl methylcellulose phthalate, and methyl cellulose; gums such as tragacanth and xanthan gum; sodium alginate; and gelatin. In order to prepare a uniform gel, dispersing agents such as alcohol or glycerin can be added, or the gelling agent can be dispersed by trituration, mechanical mixing or stirring, or combinations thereof.

Lotions are preparations to be applied to the skin surface without friction, and are typically liquid or semiliquid preparations in which solid particles, including the active agent, are present in a water or alcohol base. Lotions are usually suspensions of solids, and optionally comprise a liquid oily emulsion of the oil-in-water type. Lotions are one type of appropriate formulation for treating large body areas, because of the ease of applying a more fluid composition. Lotions will typically contain suspending agents to produce better dispersions as well as compounds useful for localizing and holding the active agent in contact with the skin, e.g., methylcellulose, sodium carboxymethyl-cellulose, or the like.

Pastes are semisolid dosage forms in which the active agent is suspended in a suitable base. Depending on the nature of the base, pastes are divided between fatty pastes or those made

from a single-phase aqueous gels. The base in a fatty paste is generally petrolatum or hydrophilic petrolatum or the like. The pastes made from single-phase aqueous gels generally incorporate carboxymethylcellulose or the like as a base.

Formulations may also be prepared with liposomes, micelles, and microspheres. Liposomes are microscopic vesicles having a lipid wall comprising a lipid bilayer, and can be used as drug delivery systems herein as well. Generally, liposome formulations are appropriate for poorly soluble or insoluble pharmaceutical agents. Liposomal preparations for use with the disclosed compositions include cationic (positively charged), anionic (negatively charged) and neutral preparations. Cationic liposomes are readily available. For example, N[1-2,3-dioleyloxy)propyl]-N,N,N-triethylammonium (DOTMA) liposomes are available under the tradename Lipofectin.RTM. (GIBCO BRL, Grand Island, N.Y.). Similarly, anionic and neutral liposomes are readily available as well, e.g., from Avanti Polar Lipids (Birmingham, Ala.), or can be easily prepared using readily available materials. Such materials include phosphatidyl choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphoshatidyl ethanolamine (DOPE), among others. These materials can also be mixed with DOTMA in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

Micelles are known in the art as comprised of surfactant molecules arranged so that their polar headgroups form an outer spherical shell, while the hydrophobic, hydrocarbon chains are oriented towards the center of the sphere, forming a core. Micelles form in an aqueous solution containing surfactant at a high enough concentration so that micelles naturally result. Surfactants useful for forming micelles include, but are not limited to, potassium laurate, sodium octane sulfonate, sodium decane sulfonate, sodium dodecane sulfonate, sodium lauryl sulfate, docusate sodium, decyltrimethylammonium bromide, dodecyltrimethylammonium bromide, tetradecyltrimethylammonium bromide, tetradecyltrimethylammonium chloride, dodecylammonium chloride, polyoxyl 8 dodecyl ether, polyoxyl 12 dodecyl ether, nonoxynol 10 and nonoxynol 30. Micelle formulations can be used in conjunction with the compositions herein either by incorporation into the reservoir of a topical or transdermal delivery system, or into a formulation to be applied to the body surface.

Microspheres, similarly, may be incorporated into the present formulations and drug delivery systems. Like liposomes and micelles, microspheres essentially encapsulate a drug or drug-containing formulation. They are generally although not necessarily formed from lipids, such as charged lipids such as phospholipids. Preparation of lipidic microspheres is well known in the art and described in the pertinent texts and literature.

Various additives, known to those skilled in the art, may be included in the topical formulations. For example, solvents, including relatively small amounts of alcohol, may be used to solubilize certain drug substances. Other optional additives include opacifiers, antioxidants, fragrance, colorant, gelling agents, thickening agents, stabilizers, surfactants and the like. Other agents may also be added, such as antimicrobial agents, to prevent spoilage upon storage, i.e., to inhibit growth of microbes such as yeasts and molds. Suitable antimicrobial agents are typically selected from the group consisting of the methyl and propyl esters of p-hydroxybenzoic acid (i.e., methyl and propyl paraben), sodium benzoate, sorbic acid, imidurea, and combinations thereof.

For those drugs having an unusually low rate of permeation through the skin, it may be desirable to include a permeation enhancer in the formulation. Any enhancers should minimize the possibility of skin damage, irritation, and systemic toxicity. Examples of suitable enhancers include, but are not limited to: ethers such as diethylene glycol monoethyl ether (available commercially as Transcutol™) and diethylene glycol monomethyl ether; surfactants such as sodium laurate, sodium lauryl sulfate, cetyltrimethylammonium bromide, benzalkonium chloride, Poloxamer (231, 182, 184), Tween (20, 40, 60, 80) and lecithin (U.S. Pat. No. 4,783,450); alcohols such as ethanol, propanol, octanol, benzyl alcohol, and the like; fatty acids such as lauric acid, oleic acid and valeric acid; fatty acid esters such as isopropyl myristate, isopropyl palmitate, methylpropionate, and ethyl oleate; polyols and esters thereof such as polyethylene glycol, and polyethylene glycol monolaurate (PEGML; see, e.g., U.S. Pat. No. 4,568,343); amides and other nitrogenous compounds such as urea, dimethylacetamide (DMA), dimethylformamide (DMF), 2-pyrrolidone, 1-methyl-2-pyrrolidone, ethanolamine, diethanolamine and triethanolamine; terpenes; alkanones; and organic acids, particularly citric acid and succinic acid. Azone™ and sulfoxides such as DMSO and C10MSO may also be used. "Percutaneous Penetration Enhancers" eds. Smith *et al.* (CRC Press, 1995) provides an excellent

overview of the field and further information concerning possible secondary enhancers for use in conjunction with the compositions disclosed herein.

The formulation may also contain irritation-mitigating additives to minimize or eliminate the possibility of skin irritation or skin damage resulting from the drug, the enhancer, or other components of the formulation. Suitable irritation-mitigating additives include, for example: α-tocopherol; monoamine oxidase inhibitors, particularly phenyl alcohols such as 2-phenyl-1-ethanol; glycerin; salicylic acids and salicylates; ascorbic acids and ascorbates; ionophores such as monensin; amphiphilic amines; ammonium chloride; N-acetylcysteine; cis-urocanic acid; capsaicin; and chloroquine. The irritant-mitigating additive, if present, may be incorporated into the present formulations at a concentration effective to mitigate irritation or skin damage, typically representing not more than about 20 wt %, more typically not more than about 5 wt %, of the formulations.

The ointments, pastes, creams and gels may contain, in addition to subject compositions, other carriers, such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof. Powders and sprays may contain, in addition to a subject composition, excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of such substances. Sprays may additionally contain customary propellants, such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane.

For purposes of transdermal and/or topical administration, dilute sterile, aqueous or partially aqueous solutions (usually in about 0.1% to 5% concentration) may be prepared. These solutions may be prepared in sesame or peanut oil, aqueous propylene glycol, or in sterile aqueous solutions may be employed. Such aqueous solutions should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. In this connection, the sterile aqueous media employed are all readily available by standard techniques known to those skilled in the art. Examples of suitable aqueous and non-aqueous carriers which may be employed in the pharmaceutical compositions include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper

fluidity may be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

Examples of pharmaceutically acceptable binders include, but are not limited to, starches; celluloses and derivatives thereof, e.g., microcrystalline cellulose, hydroxypropyl cellulose hydroxylethyl cellulose and hydroxylpropylmethyl cellulose; sucrose; dextrose; corn syrup; polysaccharides; and gelatin. The binder, e.g., may be present in an amount from about 1 % to about 40% by weight of the composition such as 1 % to 30% or 1 % to 25% or 1 % to 20%.

Optionally, one, two, three or more diluents can be added to the formulations disclosed herein. Examples of pharmaceutically acceptable fillers and pharmaceutically acceptable diluents include, but are not limited to, confectioner's sugar, compressible sugar, dextrates, dextrin, dextrose, lactose, mannitol, microcrystalline cellulose, powdered cellulose, sorbitol, sucrose and talc. The filler and/or diluent, e.g., may be present in an amount from about 15% to about 40% by weight of the composition. In certain embodiments, diluents are microcrystalline cellulose which is manufactured by the controlled hydrolysis of alpha-cellulose, obtained as a pulp from fibrous plant materials, with dilute mineral acid solutions. Following hydrolysis, the hydrocellulose is purified by filtration and the aqueous slurry is spray dried to form dry, porous particles of a broad size distribution. Suitable microcrystalline cellulose will have an average particle size of from about 20 nm to about 200 nm. Microcrystalline cellulose is available from several suppliers. Suitable microcrystalline cellulose includes Avicel PH 101 , Avicel PH 102, Avicel PH 103, Avicel PH 105 and Avicel PH 200, manufactured by FMC Corporation. The microcrystalline cellulose may be present in a tablet formulation in an amount of from about 25% to about 70% by weight. Another appropriate range of this material is from about 30% to about 35% by weight; yet another appropriate range of from about 30% to about 32% by weight. Another diluent is lactose. The lactose may be ground to have an average particle size of between about 50 μm and about 500 μm prior to formulating. The lactose may be present in the tablet formulation in an amount of from about 5% to about 40% by weight, and can be from about 18% to about 35% by weight, for example, can be from about 20% to about 25% by weight.

Other conventional solid fillers or carriers, such as, cornstarch, calcium phosphate, calcium sulfate, calcium stearate, magnesium stearate, stearic acid, glyceryl mono- and

distearate, sorbitol, mannitol, gelatin, natural or synthetic gums, such as carboxymethyl cellulose, methyl cellulose, alginate, dextran, acacia gum, karaya gum, locust bean gum, tragacanth and the like, diluents, binders, lubricants, disintegrators, coloring and flavoring agents could optionally be employed.

Methods of preparing these formulations or compositions include the step of bringing into association subject compositions with the carrier and, optionally, one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association a subject composition with liquid carriers, or finely divided solid carriers, or both, and then, if necessary, shaping the product. In addition, in certain embodiments, subject compositions of the present application maybe lyophilized or subjected to another appropriate drying technique such as spray drying.

Additional examples of useful excipients which can optionally be added to the composition are described in the Handbook of Pharmaceutical Excipients, 3rd edition , Edited by A.H.Kibbe, Published by: American Pharmaceutical Association, Washington DC, ISBN: 0-917330-96-X, or Handbook of Pharmaceutical Excipients (4th edition), Edited by Raymond C Rowe - Publisher: Science and Practice.

5.1 Dosage forms: administration via a patch

A patch is a composition with an at least partially adhesive backing, and a pharmaceutical composition. A patch is applied to the skin, for delivery of the pharmaceutical composition. A patch may deliver a pharmaceutical composition topically or transdermally.

Transdermal patches may be passive or active. A passive patch is one that simply applies the pharmaceutical composition to the skin, and does not actively assist one or more components of the pharmaceutical composition in crossing the skin. Passive transdermal drug delivery systems currently available, such as the nicotine, estrogen and nitroglycerine patches, typically deliver small-molecule drugs.

Active patches, in contrast, actively promote passage of one or more components of the pharmaceutical composition through the skin, achieving a rate of passage faster than that of a passive patch delivering the same pharmaceutical composition. While passive methods rely on

natural forces and pressures alone such as diffusion or concentration gradients, active methods use externally applied forces, for example, electrical potential or hydraulic forces, to force one or more components of a pharmaceutical composition into the skin.

Many of the newly developed proteins and peptide drugs are too large to be delivered optimally through passive transdermal patches, and may be delivered using technology such as electrical assist (iontophoresis) for large-molecule drugs. Iontophoresis is a technique employed for enhancing the flux of ionized substances through membranes by application of electric current. One example of an iontophoretic membrane is given in U.S. Pat. No. 5,080,646 to Theeuwes. The principal mechanisms by which iontophoresis enhances molecular transport across the skin are (a) repelling a charged ion from an electrode of the same charge, (b) electroosmosis, the convective movement of solvent that occurs through a charged pore in response the preferential passage of counter-ions when an electric field is applied or (c) increase skin permeability due to application of electrical current.

Methods of delivering a composition or compositions via a transdermal patch are known in the art. Exemplary patches and methods of patch delivery are described in US Patent Nos. 6,974,588, 6,564,093, 6,312,716, 6,440,454, 6,267,983, 6,239,180, and 6,103,275.

In one embodiment, a transdermal patch may comprise an outer backing foil, a matrix and a protective liner wherein a) the composition or compositions are present in the matrix in a solution (which may be oversaturated), b) the matrix may contain 1 to 5% activated SiO_2, and c) the matrix may have a moisture content of less than 0.7%. Moisture-free matrix patches which contain activated silicon dioxide in the matrix show an enhanced drug release into the skin.

In another embodiment, a transdermal patch may comprise: a substrate sheet comprising a composite film formed of a resin composition comprising 100 parts by weight of a polyvinyl chloride-polyurethane composite and 2-10 parts by weight of a styrene-ethylene-butylene-styrene copolymer, a first adhesive layer on the one side of the composite film, and a polyalkylene terephthalate film adhered to the one side of the composite film by means of the first adhesive layer, a primer layer which comprises a saturated polyester resin and is formed on the surface of the polyalkylene terephthalate film; and a second adhesive layer comprising a styrene-diene-styrene block copolymer containing a pharmaceutical agent layered on the primer layer. A method for the manufacture of the above-mentioned substrate sheet comprises preparing

the above resin composition molding the resin composition into a composite film by a calendar process, and then adhering a polyalkylene terephthalate film on one side of the composite film by means of an adhesive layer thereby forming the substrate sheet, and forming a primer layer comprising a saturated polyester resin on the outer surface of the polyalkylene terephthalate film.

The pharmaceutical compositions herein can be packaged to produce a "reservoir type" transdermal patch with or without a rate-limiting patch membrane. The size of the patch and or the rate limiting membrane can be chosen to deliver the transdermal flux rates desired. Such a transdermal patch can consist of a polypropylene/polyester impervious backing member heat-sealed to a polypropylene porous/permeable membrane with a reservoir therebetween. The patch can include a pharmaceutically acceptable adhesive (such as a acrylate, silicone or rubber adhesive) on the membrane layer to adhere the patch to the skin of the host, e.g., a mammal such as a human. A release liner such as a polyester release liner can also be provided to cover the adhesive layer prior to application of the patch to the skin as is conventional in the art. This patch assembly can be packaged in an aluminum foil or other suitable pouch, again as is conventional in the art.

Alternatively, the compositions herein can be formulated into a "matrix-type" transdermal patch. Drug Delivery Systems Characteristics and Biomedical Application, R. L Juliano, ed., Oxford University Press. N.Y. (1980); and Controlled Drug Delivery, Vol. I Basic Concepts, Stephen D. Bruck (1983) describe the theory and application of methods useful for transdermal delivery systems. The drug-matrix could be formed utilizing various polymers, e.g. silicone, polyvinyl alcohol. The "drug matrix" may then be packaged into an appropriate transdermal patch.

Another type of patch comprises incorporating the drug directly in a pharmaceutically acceptable adhesive and laminating the drug-containing adhesive onto a suitable backing member, e.g. a polyester backing membrane. The drug should be present at a concentration which will not affect the adhesive properties, and at the same time deliver the required clinical dose.

In certain embodiments, a patch delivers a sustained-release formulation of a pharmaceutical composition.

5.2 Dosage forms: administration via a wound dressing

Wound dressings comprise a wound dressing carrier and a therapeutic agent. The wound dressing carrier may be, for example, a gauze pad, film, hydrogel, foam, a hydrocolloid, calcium alginate, collagen dressing, low adherent absorbent pads, hydrofibre, impregnated mesh dressing/contact layers, and secondary absorbent pads.

Gauze pads may be made from, for example, sterile gauze or sterile cotton. Wound dressings such as gauze pads may be made from a material having elasticity.

Films may be polyurathane, optionally having a drainage adhesive layer, and may be semipermeable. In some embodiments, the film is transparent. Examples of appropriate films include Opsite films and Tegaderm films, both of which comprise a thin polyurethane membrane coated with a layer of an acrylic adhesive.

Hydrogels may be in the form of a gel, a sheet, or a gauze, and often include glycerin. Hydrogels may be made from hyaluronic acid, polyurethene, intrasitegel, PEG, or silicone.

Foams may be made from polyurathane. Examples of foams include Allevyn, Biatain, Lyofoam, and Mepilex.

Hydrocolloids may be made, for example, from carbosymethylcellulose, pectin, or gelatin. Exemplary hydrocolloids include DueDerm and Comfeel.

Calcium alginates may be fiber pads derived from seaweed. Alginates include Algisite M and Kaltostat.

Collagen dressings may be particles or composite pads having collagen components. The collagen may be derived from, for example, bovine collagen preparations or collagen synthesized by transgenic cells.

Low adherent absorbent pads include Melolin and Cutilin.

Hydrofibres include Aquacel.

Impregnated mesh dressing/contact layers include Adaptic, Mepitel, and Urgotul.

Secondary absorbent pads include Combine, Zetuvit plus, Mesorb, and Adsorb plus.

Dressings may include a number of active agents such as iodine (e.g. Inadine, Iodosorb), silver (e.g. Atrauman Ag, Acticoat, and Aquacel Ag), honey (e.g. Medihoney/Combita), odor control agents, hydroxamates, hydroxamate analogues, matrix metalloproteinase inhibitors (including zinc ion chelators such as phosphinyls).

Optionally, the wound dressing may be an occlusive dressing; it may seal the wound against new infections.

6. Methods of treating skin ulcers

In some embodiments, this application provides a method of treating a diabetic ulcer comprising administering to a patient in need thereof a therapeutically effective amount of any of the pharmaceutical compositions described herein. The diabetic ulcer may be, for example, a diabetic foot ulcer or a venous ulcer. In certain embodiments, the pharmaceutical composition is administered topically, subdermally, or transdermally. In some embodiments, the pharmaceutical composition is administered using an active patch, passive patch, or wound dressing such as a hydrogel, for example.

The identification and diagnosis of skin ulcers is well known in the art. For example, diabetic ulcers are often diagnosed by a primary care provider. The primary care provider takes into account major risk factors such as diabetic neuropathy, structural foot deformity and peripheral arterial occlusive disease. He or she may then perform a physical exam. In addition, monofilament testing for neuropathy and noninvasive testing for arterial insufficiency may be performed to identify patients having diabetic ulcers and those at risk for diabetic ulcers. Furthermore, specimens may be taken and tested for the presence of bacteria. Once a patient has been diagnosed as having a diabetic ulcer, the patient may be treated with the compositions and methods described herein.

The compositions described herein may be used to treat a number of conditions including diabetic ulcers (such as diabetic foot ulcers, ulcers on the limbs, and ulcers on the digits), diabetic chronic wounds, and poorly-healing wounds. At least some of these conditions are not mutually exclusive.

In certain embodiments, the pharmaceutical composition is administered topically, subdermally, or transdermally. In certain embodiments, the pharmaceutical composition is administered using an active patch, passive patch, or wound dressing. The pharmaceutical composition may also be administered using a hydrogel.

Topical administration of a pharmaceutical composition refers to the delivery of a composition by application to the skin. Formulations like creams, lotions, and ointments may achieve topical administration. Also, formulations may be delivered in the form of a patch or wound dressing to achieve topical administration.

Subdermal administration refers to administration of a pharmaceutical composition such that it is situated under the skin, and acts substantially locally. Capsules containing an active agent may be implanted to achieve subdermal administration. Also, a composition may be injected subdermally.

Transdermal administration refers to administration of a pharmaceutical composition that is applied to the skin for delivery to the adjacent and nearby tissue including the bloodstream. Patches and wound dressings may be used to administer a composition transdermally.

EXAMPLES

Example 1: Preparation of a salt of lipoic acid and a compound having a structure of Formula (I)

Sodium methoxide (2.4 g) was dissolved in 10 mL methanol and to this solution was added 5.0 g aminoguanidine hydrochloride while stirring. The stirring was continued for an additional 20 min, after which 200 mL acetone was added. The mixture was stirred for 30 min, and then filtered. To the filtrate, 9.3g R-(+)-lipoic acid dissolved in 100 mL acetone was added dropwise with constant stirring resulting in the precipitation of a pale yellow solid. The mixture was stirred for an additional 20 min. and filtered. The light yellow solid was washed with 30 mL acetone, filtered, and dried to yield the compound having the structure shown in Figure 1 (yield: 95%).

Figures 1 through 3 illustrate NMR data used to identify the resulting salt as the lipoic acid salt of the compound of Formula (I).

Liquid chromatography-mass spectrometry (LC-MS) was also used to identify the resulting salt as the lipoic acid salt of the compound of Formula (I). LC-MS showed two peaks corresponding to:

- R-(+)-alpha lipoic acid: Retention time 10.59 min, molecular weight 207 (m+H)
- Schiff's base adduct of aminoguanidine: Retention time 1.20 min, molecular weight 114 (m+H)

Furthermore, the optical rotation of the resulting salt was determined to be $[\alpha]_D^{25} = 64.5$ to 67.5 (c=1, methanol).

The melting point of the resulting salt was determined to be 163 - 176 °C.

Example 2: Recrystallization of a salt of lipoic acid and a compound having a structure of Formula (I)

The salt produced in Example 1 (50 mg) was dissolved in 1:1 water-methanol (800 µl) with stirring and heating in water bath at 60 °C. This solution was centrifuged for 10 min. Acetonitrile was added slowly to the supernatant with a continuous vortex. Acetonitrile (~ 20 mL) was added until a semi- permanent turbidity turns to a clear solution by stirring. The solution was left undisturbed in the refrigerator at 4 °C for 10-15 hours. Fine crystalline needles were formed, which were filtered and dried.

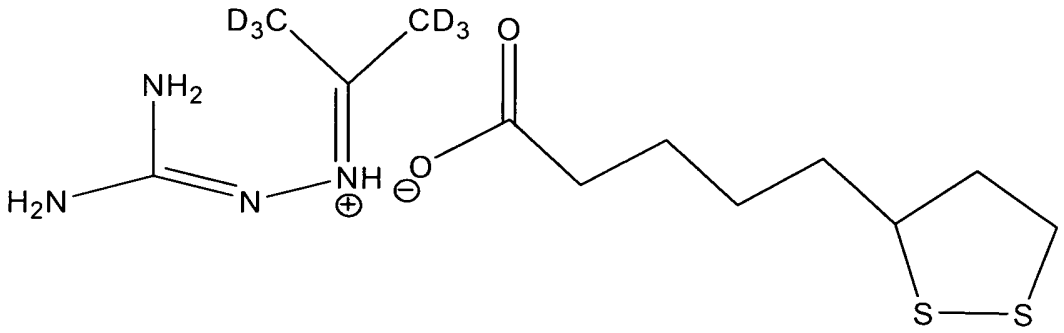
In addition, elemental analysis was performed. The theoretical values were determined to be: %C 44.97; %H 7.55; %N 17.48; %S 20.01. The experimental values were determined to be:

- Salt: %C 44.63; %H 7.57; %N 17.36; %S 20.06
- Re-crystallized salt: %C 44.96; %H 7.70; %N 17.43; %S 20.21

The re-crystallized salt was found to have the same melting point, optical rotation, and NMR spectra as the salt prior to re-crystallization.

Example 3: Preparation of a salt of lipoic acid and a deuteriated compound having a structure of Formula (I)

Sodium methoxide (2.4 g) was dissolved in 10 mL methanol, and to this solution was added 5.0 g aminoguanidine hydrochloride while stirring. The stirring was continued for an additional 20 min. 200 mL deuteriated acetone was then added, stirred for 30 min, and the mixture was filtered through a Whatman 0. 45μm PVDF filter. To the filtrate, 9.3g R-(+)-lipoic acid dissolved in 100 mL deuteriated acetone was added dropwise with constant stirring, resulting in the precipitation of a pale yellow solid. The mixture was stirred for an additional 20 minutes and filtered. The light yellow solid was washed with 30 mL deuteriated acetone, filtered, and dried to yield the salt having a structure as shown below (yield: 95%).



The infra-red spectra of the compound was measured, and is shown in Figure 4.

Example 4: Crystal structure of the lipoic acid salt of a compound having the structure of Formula (I)

The crystal structure of the re-crystallized salt of Example 2 was determined using standard methods. The crystal structure is shown in Figure 5. The structural characteristics are enumerated in the table of Figure 6.

Example 5: Power XRD analysis of the lipoic acid salt of a compound having the structure of Formula (I)

The Power XRD (X-ray diffraction) pattern of the recrystallized salt of Example 2 was determined using standard methods, and is shown in Figure 7. The peak assignments and the absolute and relative intensities in the powder XRD are shown in Figure 8.

Example 6: Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TGA) of the lipoic acid salt of a compound having the structure of Formula (I)

Figure 9 depicts Differential Scanning Calorimetry (DSC) of the recrystallized salt of Example 2. From the DSC thermogram, it can be seen that the crystals undergo an endothermic phase transition at 88°C. In addition, the crystals show a sharp melting point at 188.7°C. The compound decomposes soon after melting. The decomposition endotherm is broad and spans the temperature range 190 – 290°C.

Figure 10 is a graph showing the Thermogravimetric Analysis (TGA) analysis of the recrystallized salt of Example 2. The TGA analysis indicates that, in the open pan, the complete decomposition of the compound begins at 150 °C and ends at 250 °C. Furthermore, no other transitions were associated with the compound. From this one may infer that there is no solvent loss at all. Finally, it was observed that the compound totally decomposes by the end of the run, within experimental error.

Example 7: In vitro pharmacology analysis of the lipoic acid salt of a compound having the structure of Formula (I)

The IC_{50} of the recrystallized lipoic acid salt of Example 2 was determined for each of three assays.

First, an inducible NOS activity assay was performed on the recrystallized salt of Example 2. This assay measures the formation of nitrite from arginine using an enzyme isolated from LPS- + INFγ-treated mouse macrophages. In this assay, the test salt (as a 10-fold concentrated solution in H_2O), reference compound or water (control) are incubated for 180 min at 37°C with the enzyme (0.5 U) in a buffer containing 40 mM Tris-HCl (pH 8.0), 0.5 mM NADPH, 4 μM FAD, 12 μM BH_4, 3 mM DTT and 0.1 mM L-arginine. For basal control measurements, the enzyme is omitted from the incubation medium. Following incubation, Griess reagent containing 0.05% naphtylene diamine, 0.5% sulfanilamide and 2.5% orthophosphoric acid is added and the samples are incubated for 10 min at 22°C. The amount of nitrite produced is then quantified with a microplate reader (Spectrafluorplus, Tecan) by measuring the absorbance at λ = 550 nm. The results are expressed as IC_{50} in M. This assay may be performed using the standard inhibitory reference compound,1400W, which may be

tested in each experiment at several concentrations to obtain an inhibition curve from which its IC_{50} value is calculated. Further information regarding this protocol may be found in Tayeh and Marletta (1989), Macrophage oxidation of L-arginine to nitric oxide, nitrite, and nitrate, J. Biol. Chem., 264 : 19654. In this assay, the salt displayed an IC_{50} of 3.7E-05 M and an n_H of 0.9.

In addition, the effect on superoxide O_2 secretion was measured. This assay quantifies the secretion of superoxide O_2^- from phorbol 12-mysirate 13-acetate (PMA)-stimulated human HL-60 cells, by the measurement of cytochrome C reduction. The test salt (as a 10-fold concentrated solution in H_2O), reference compound or water (control) are pre-incubated for 15 min at 37°C with HL-60 cells ($5x10^5$ cells) suspended in a buffer containing 137 mM NaCl, 2.68 mM KCl, 0.9 mM $CaCl_2$, 0.5 mM $MgCl_2$, 8.1 mM Na_2HPO_4, 1.47 mM KH_2PO_4 (pH 7.4) and 19 µM cytochrome C. The absorbance is then measured at λ=550 nm using a spectrophotometer to detect any compound interference with the photometric detection at this wavelength. Thereafter, the reaction is initiated by the addition of 30 nM PMA and the mixture is incubated for 15 min at 37°C in the dark. For basal control measurements, the incubation medium also contains 275 U/ml superoxyde dismutase (SOD) to catalyze the destruction of superoxide O_2^-.. Following incubation, the mixture is cooled to 4°C, centrifuged at 250 g for 5 min and the supernatants are collected. The absorbance is then measured at λ=550 nm and the activity is determined by subtracting signal measured in the presence of SOD from that measured in its absence. This assay may be performed using the standard inhibitory reference compound, diphenyleneiodonium, which may be tested in each experiment at several concentrations to obtain an inhibition curve from which its IC_{50} value is calculated. Further information about the protocol may be found in Lorico *et al.* (1986), Gentisic acid : an aspirin metabolite with multiple effects on human blood polymorphonuclear leukocytes, Biochem. Pharmacol., 35 : 2443. In this assay, the salt displayed an IC_{50} of 3.1E-04 M.

Furthermore, the effect on lipid peroxidation quantified by the measurement of ascorbic acid-induced production of malonaldehyde in rat liver microsomes. Specifically, homogenates of liver microsomes (150 µg) are pre-incubated for 5 min at 37°C with the test salt (as a 10-fold concentrated solution in H_2O), reference compound or water (control) in a buffer containing 300 mM NaCl, 0.1 mM $FeCl_3$ and 8 mM NaH_2PO_4/Na_2HPO_4 (pH 7.4). Thereafter, the reaction is initiated by the addition of 0.1 mM ascorbic acid and the mixture is incubated for 20 min at 37°C. For basal control measurements, ascorbic acid is omitted from the incubation medium.

These measurements are also used to detect any compound interference with the photometric detection at the selected wavelength. Following incubation, the reaction is stopped by the addition of 5 mM EDTA/NaOH. Lipid peroxides are extracted by the addition of 1% 2-thiobarbituric acid and 2.8% trichloroacetic acid followed by heating to 100°C for 15 min then cooling to 4°C, addition of n-butanol-1 and centrifugation at 1200 x g for 5 min. The amount of lipid peroxides present in the supernatant is quantified by measuring the absorbance at λ=532 nm using a spectrophotometer. This assay may be performed using the standard inhibitory reference compound N-propyl gallate, which may be tested in each experiment at several concentrations to obtain an inhibition curve from which its IC_{50} value is calculated. Additional details on this assay may be found in Aruoma *et al.* (1990), An evaluation of the antioxidant and potential pro-oxidant properties of food additives and of trolox C, vitamin E and probucol, Free Rad. Res. Commun., 10 : 143. In this assay, the salt displayed an IC_{50} of 1.8E-03 M.

EQUIVALENTS

The present disclosure provides among other things compositions and methods for treating NOS-associated diseases. While specific embodiments of the subject disclosure have been discussed, the above specification is illustrative and not restrictive. Many variations of the systems and methods herein will become apparent to those skilled in the art upon review of this specification. The full scope of the claimed systems and methods should be determined by reference to the claims, along with their full scope of equivalents, and the specification, along with such variations.

INCORPORATION BY REFERENCE

All publications and patents mentioned herein, including those items listed below, are hereby incorporated by reference in their entirety as if each individual publication or patent was specifically and individually indicated to be incorporated by reference. In case of conflict, the present application, including any definitions herein, will control.

Also incorporated by reference in their entirety are any polynucleotide and polypeptide sequences which reference an accession number correlating to an entry in a public database, such as those maintained by The Institute for Genomic Research (TIGR) (www.tigr.org) and/or the National Center for Biotechnology Information (NCBI) (www.ncbi.nlm.nih.gov).

CLAIMS:

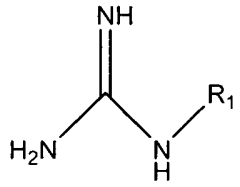
1. A pharmaceutical composition comprising:

(i) at least one of an antimicrobial agent and L-arginine; and

(ii) a salt, wherein the salt comprises:

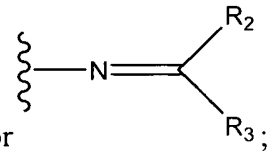
(a) lipoic acid enriched for the R-(+) enantiomer, and

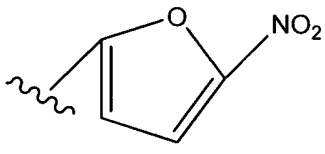
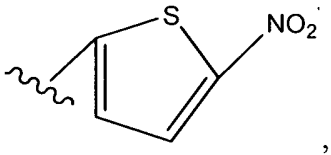
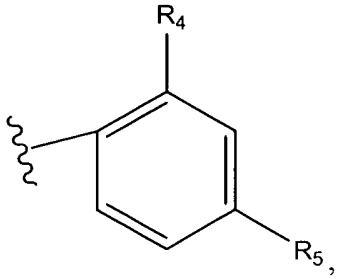
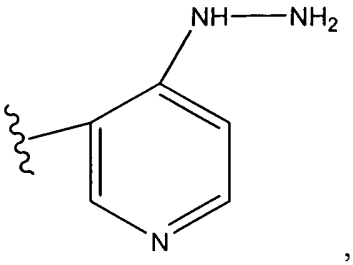
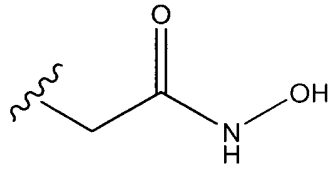
(b) a compound having the structure of Formula (I):



(I)

wherein

R_1 is: $-NH_2$ or  ;

R_2 and R_3 are each independently selected from: CH_3,  ,  ,  ,  , and  ;

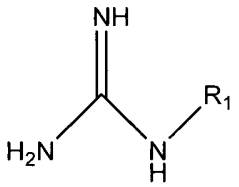
and R_4 and R_5 are each independently selected from Cl and NO_2.

2. A pharmaceutical composition comprising:

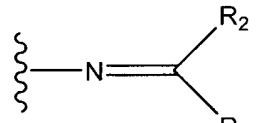
(i) an antimicrobial agent

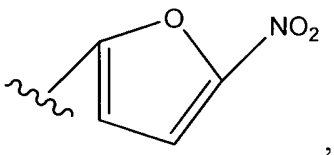
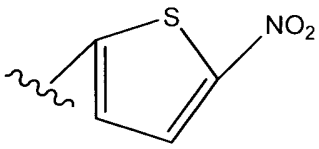
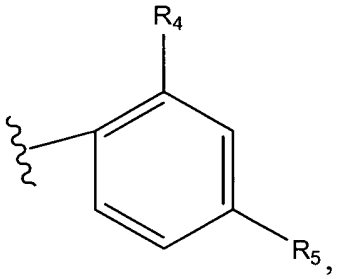
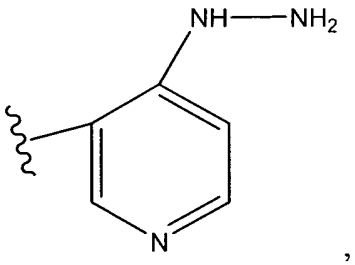
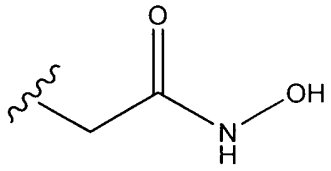
(ii) at least one of L-arginine and lipoic acid enriched for the R-(+) enantiomer; and

(iii) a compound having the structure of Formula (I):

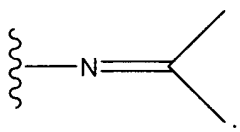


(I)

wherein R_1 is: $-NH_2$ or  ;

R_2 and R_3 are each independently selected from: CH_3,  ,  ,  ,  , and  ;

and R_4 and R_5 are each independently selected from Cl and NO_2.

3. The pharmaceutical composition of either of claims 1 or 2, wherein R_1 is: $-NH_2$ or  .

4. The pharmaceutical composition of any one of claims 1-3, comprising L-arginine and an antimicrobial agent.

5. A pharmaceutical composition comprising:

(a) an antimicrobial agent,

(b) L-arginine, and

(c) lipoic acid enriched for the R-(+) enantiomer.

6. The pharmaceutical composition of any one of claims 1-5, wherein the antimicrobial agent is iodine or silver.

7. The pharmaceutical composition of any one of claims 1-6, further comprising hydroxamate or a hydroxamate analogue.

8. The pharmaceutical composition of claim 7, wherein the hydroxamate analogue is hydroxamate covalently bonded to PEG, silicone, guanidine, or aminoguanidine.

9. The pharmaceutical composition of claim 1, wherein the salt is in crystalline form.

10. The pharmaceutical composition of any one of claims 1-4, wherein the composition is substantially free of the S-enantiomer of lipoate.

11. The pharmaceutical composition of any one of claims 1-4, further comprising a pharmaceutically acceptable carrier.

12. The pharmaceutical composition of any one of claims 1-4, which is formulated for subdermal or transdermal administration.

13. The pharmaceutical composition of any one of claims 1-4, which is formulated for topical administration.

14. The pharmaceutical composition of any one of claims 1-4, further comprising at least one of a pharmaceutically acceptable stabilizer, diluent, surfactant, filler, binder, and lubricant.

15. A patch comprising the pharmaceutical composition of any one of claims 1-14.

16. The patch of claim 15, which is active or passive.

17. A wound dressing comprising the pharmaceutical composition of any one of claims 1-13.

18. The wound dressing of claim 17, wherein the wound dressing is a hydrogel.

19. A method of treating a diabetic ulcer comprising administering to a patient in need thereof a therapeutically effective amount of the pharmaceutical composition of any of claims 1-14.

20. The method of claim 19, wherein the diabetic ulcer is a diabetic foot ulcer.

21. The method of claim 19, wherein the diabetic ulcer is a venous ulcer.

22. The method of claim 19, wherein the pharmaceutical composition is administered topically, subdermally, or transdermally.

23. The method of claim 22, wherein the pharmaceutical composition is administered using an active patch, passive patch, or wound dressing.

24. The method of claim 22, wherein the pharmaceutical composition is administered using a hydrogel.

25. A kit comprising a pharmaceutical preparation that includes the pharmaceutical composition of any of claims 1-14.

26. A composition comprising:
    (a) the pharmaceutical composition of claims 19, and
    (b) a hydrogel.

27. The pharmaceutical composition of any of claims 1-14 for treating diabetic ulcers.

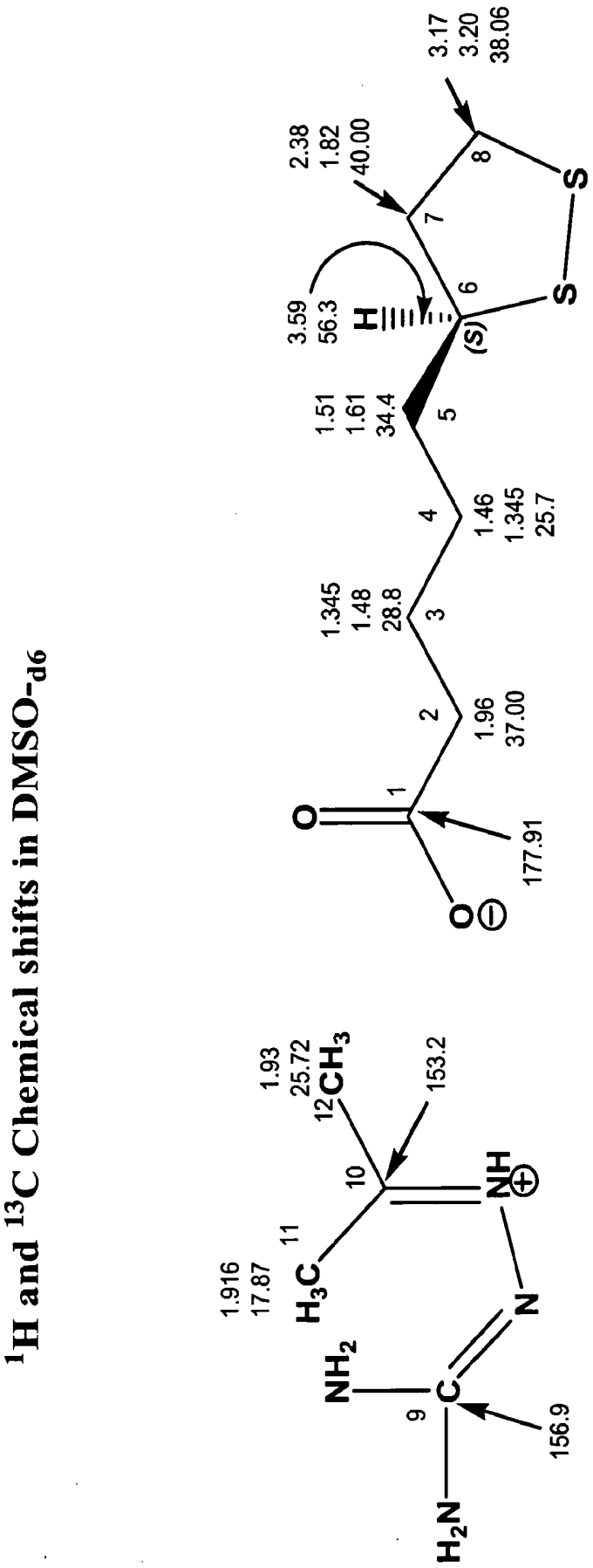


Figure 1 (part 1)

	^{1}H NMR		^{13}C NMR
No	(δ, ppm)	Multiplicity	(δ, ppm)
1	-	-	177.91
2	1.96	t, J= 7.3 Hz	37.00
3	1.35, 1.48	m	28.8
4	1.46, 1.35	m	25.7
5	1.51, 1.61	m	34.4
6	3.59	m	56.3
7	2.38, 1.82	m	40.00
8	3.17, 3.20	m	38.06
9	-	-	156.9
10	-	-	153.2
11	1.92	s, 3H	17.87
12	1.93	s, 3H	25.72

Figure 1 (part 2)

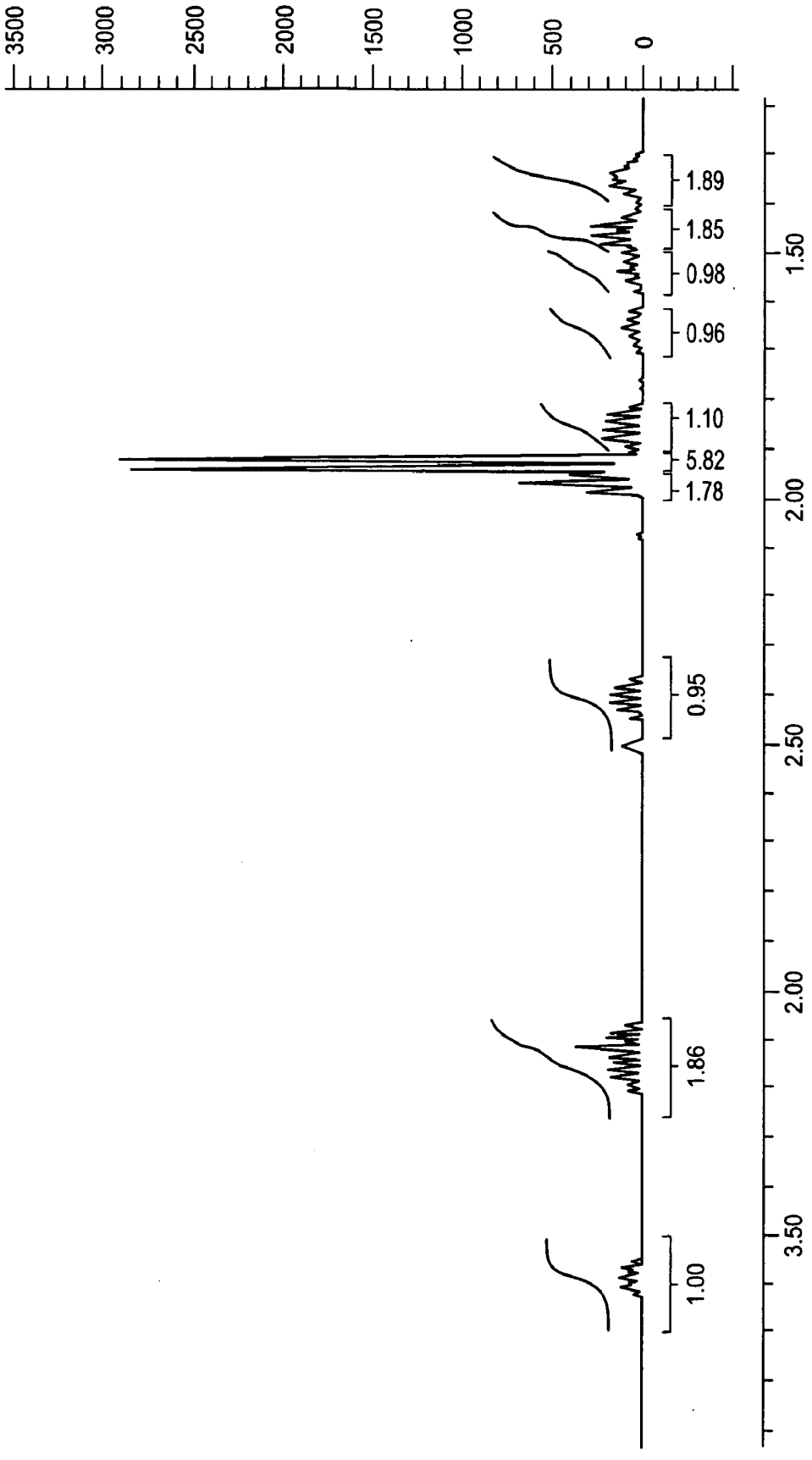

3500
3000
2500
2000
1500
1000
500
0
1.89
1.85
0.98
0.96
1.10
5.82
1.78
0.95
1.86
1.00
1.50
2.00
2.50
2.00
3.50
ppm(t1)

Figure 2

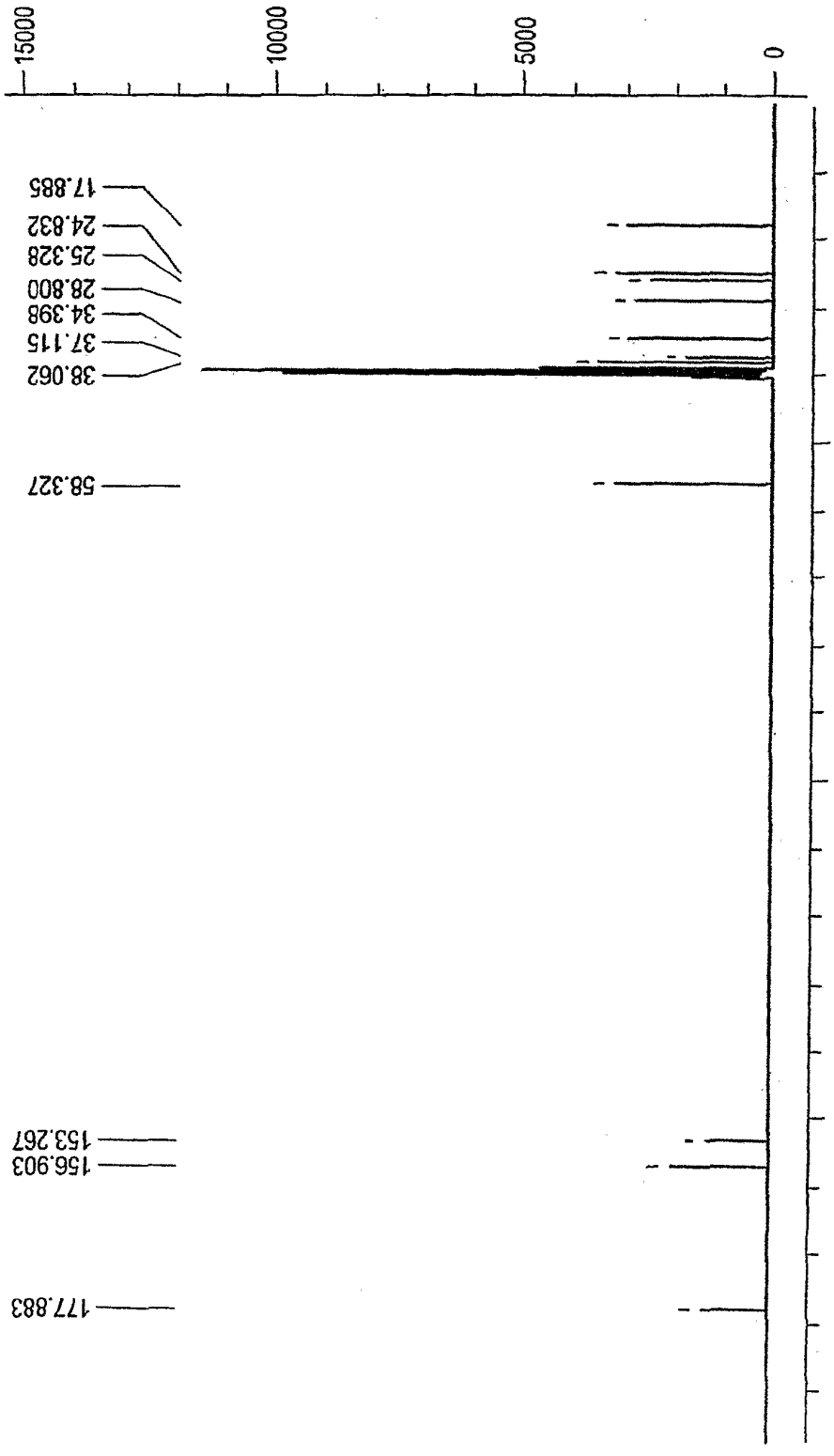

15000
10000
5000
0
17.885
24.832
25.328
28.800
34.398
37.115
38.062
58.327
153.267
156.903
177.883
50
100
150
ppm(t1)

Figure 3

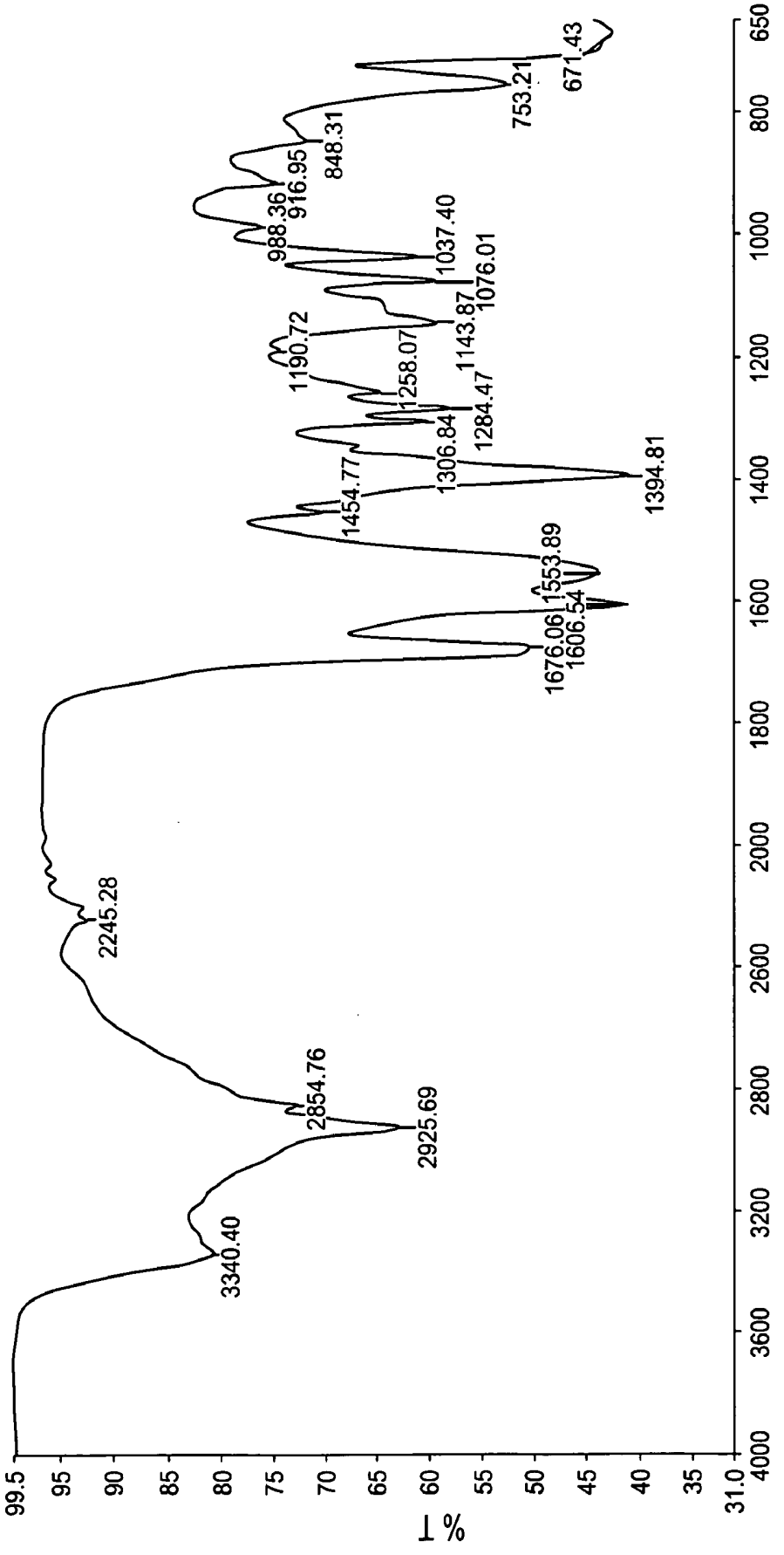

T %
99.5
95
90
85
80
75
70
65
60
55
50
45
40
35
31.0
4000
3600
3200
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
650
cm-1
3340.40
2925.69
2854.76
2245.28
1676.06
1606.54
1553.89
1454.77
1394.81
1306.84
1284.47
1258.07
1190.72
1143.87
1076.01
1037.40
988.36
916.95
848.31
753.21
671.43

Figure 4

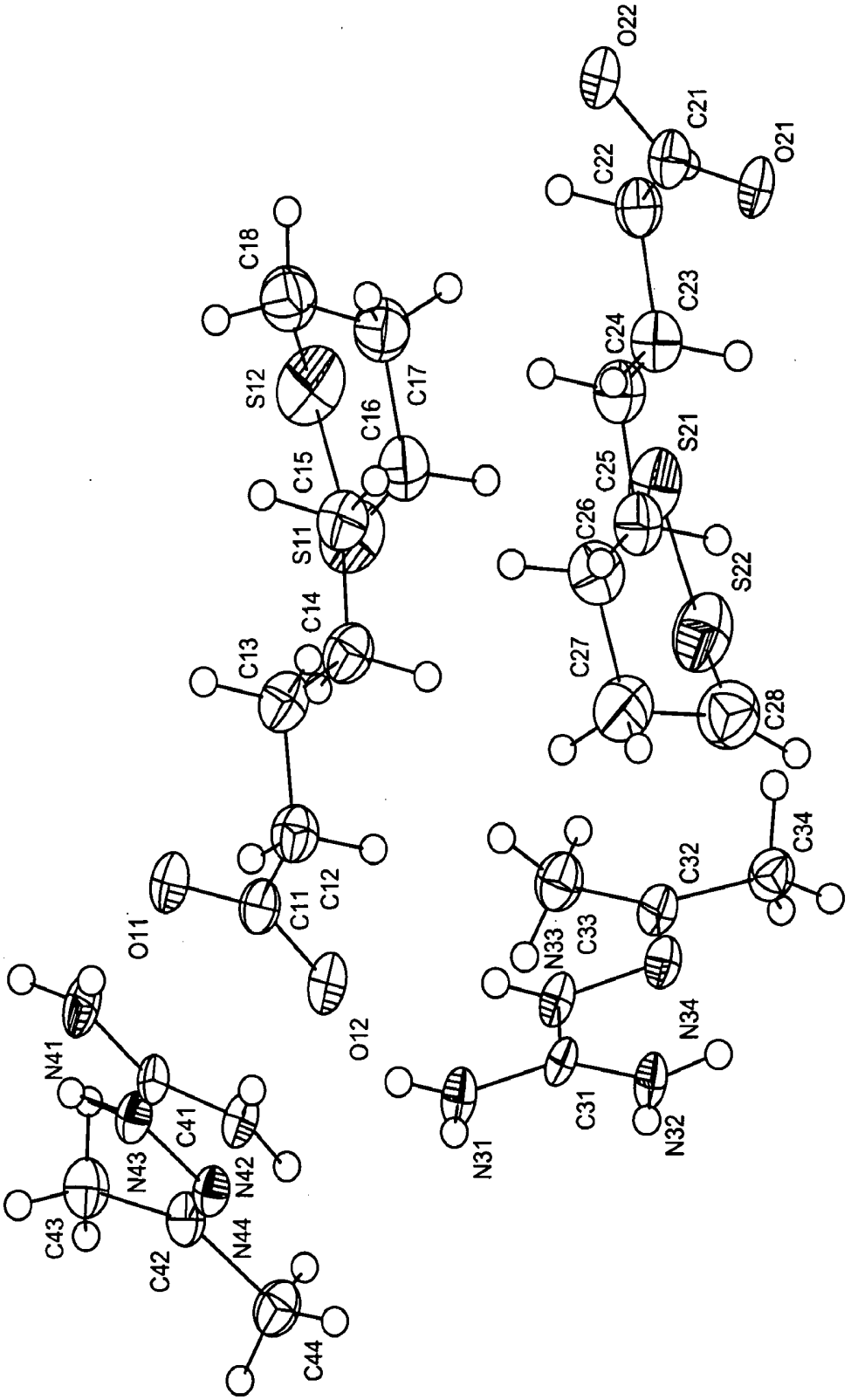

O22
C21
O21
C22
C23
C24
S21
C25
C26
S22
C27
C28
C34
C32
C33
N33
N34
C31
N31
N32
C18
S12
C16
C17
C15
S11
C14
C13
C11
C12
O11
O12
N41
C41
N43
N42
C43
N44
C42
C44

Figure 5

Empirical formula	C12 H23.50 N4 O2 S2
Formula weight	319.97
Temperature	100(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	$P2_1$
Unit cell dimensions	a = 7.5542(8) Å α = 90°. b = 24.850(3) Å β = 98.241(9)°. c = 9.0789(11) Å γ = 90°.
Volume	1686.7(3) $Å^3$
Z	4
Density (calculated)	1.260 Mg/m^3
Absorption coefficient	0.323 mm^{-1}
F(000)	686
Crystal size	0.96 x 0.20 x 0.01 mm^3
Theta range for data collection	1.64 to 22.50°.
Index ranges	-8<=h<=8, -26<=k<=26, -9<=l<=9
Reflections collected	10373
Independent reflections	4333 [R(int) = 0.0839]
Completeness to theta = 22.50°	99.9 %
Max. and min. transmission	0.9984 and 0.7471
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	4333 / 515 / 365
Goodness-of-fit on F2	1.055
Final R indices [I>2sigma(I)]	R1 = 0.1284, wR2 = 0.3231
R indices (all data)	R1 = 0.1769, wR2 = 0.3522
Absolute structure parameter	-0.1(3)
Largest diff. peak and hole	0.767 and -0.646 $e.Å^{-3}$

Figure 6

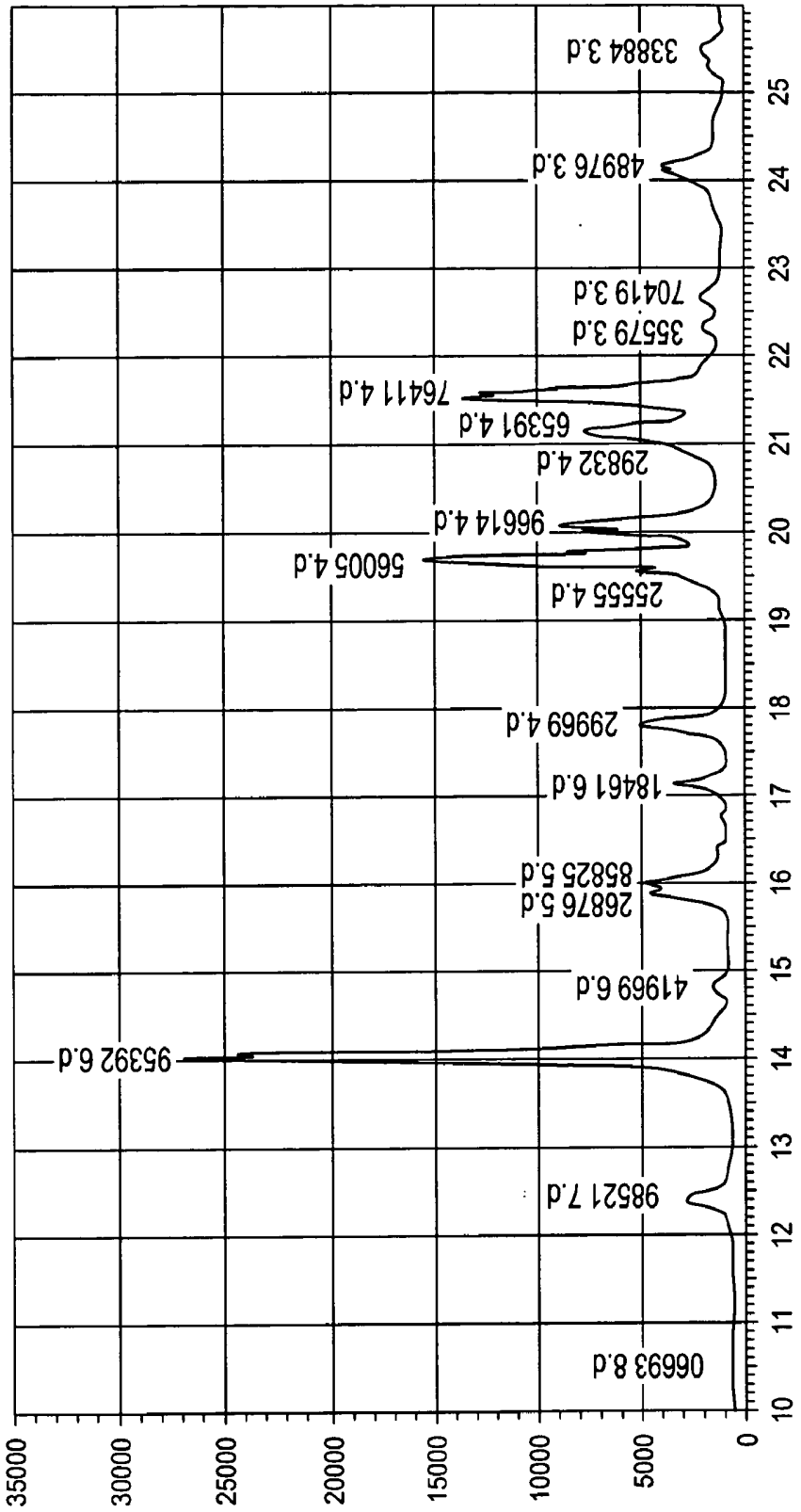

33884 3.d
48976 3.d
70419 3.d
35579 3.d
76411 4.d
65391 4.d
29832 4.d
96614 4.d
56005 4.d
25555 4.d
29969 4.d
18461 6.d
85825 5.d
26876 5.d
41969 6.d
95392 6.d
98521 7.d
06693 8.d
35000
30000
25000
20000
15000
10000
5000
0
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
2-Theta-Scale

Figure 7

2θ(°)	d SPACING (Å)	INTENSITY (CPS)	INTENSITY %
4.76	18.57	851	3.1
7.06	12.51	1332	4.9
10.48	8.44	614	2.2
12.42	7.12	2815	10.3
14.03	6.31	27362	100
14.79	5.98	1356	5
15.95	5.55	4000	14.6
17.14	5.17	2354	8.6
17.82	4.97	4879	17.8
19.73	4.50	14008	51.2
20.25	4.38	2315	8.5
21.06	4.22	4806	17.6
21.57	4.12	13352	48.8
22.55	3.94	1316	4.8
24.12	3.69	3487	12.7
25.47	3.49	1716	6.3
26.70	3.34	2399	8.8
27.32	3.26	2928	10.7
28.92	3.08	1227	4.5
29.64	3.01	1589	5.8
30.60	2.92	3404	12.4
32.30	2.77	1336	4.9
33.70	2.66	1069	3.9
24.67	2.59	952	3.5

Figure 8

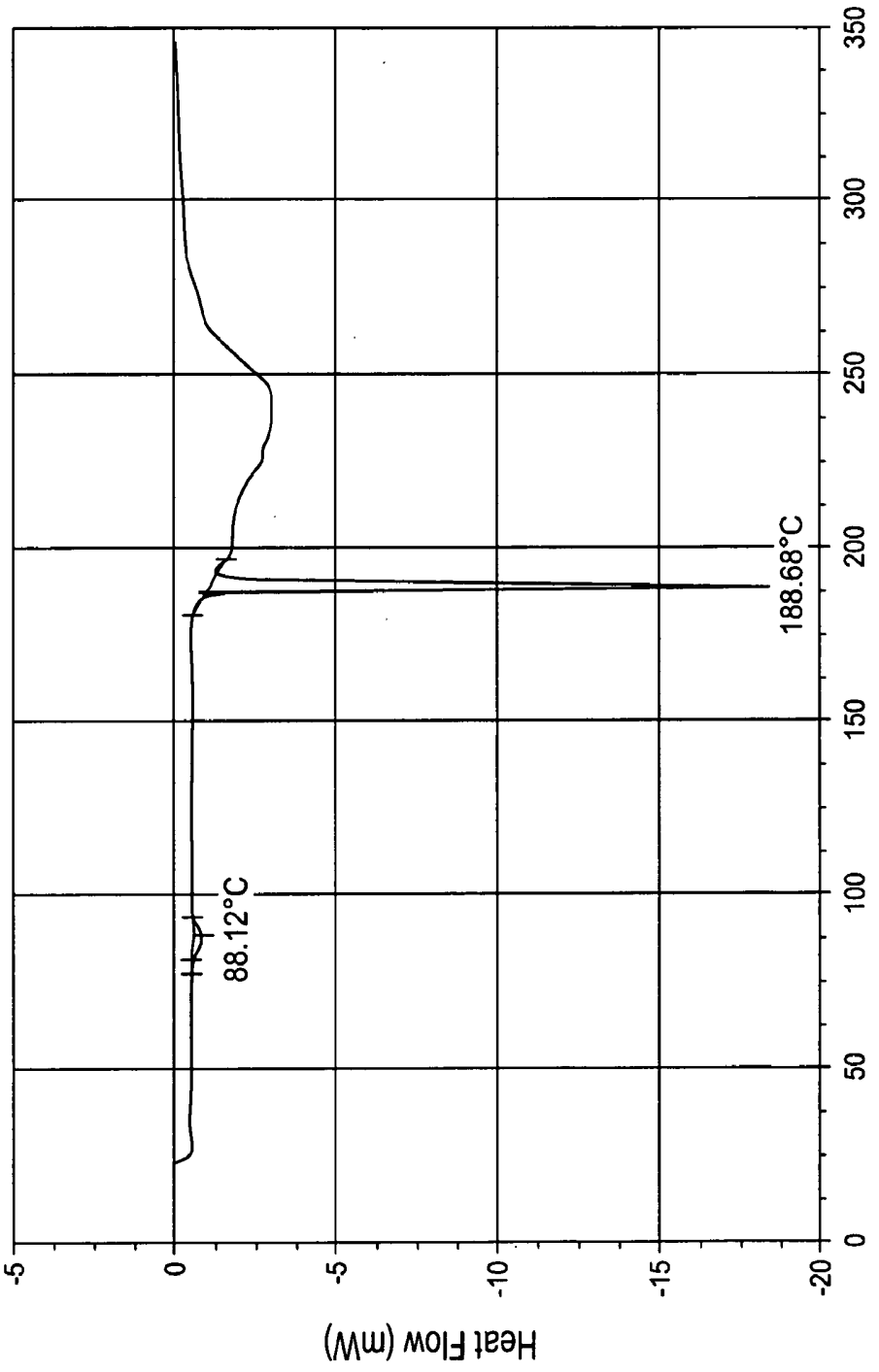

Heat Flow (mW)
-5
0
-5
-10
-15
-20
Temperature (°C)
0
50
100
150
200
250
300
350
88.12°C
188.68°C

Figure 9

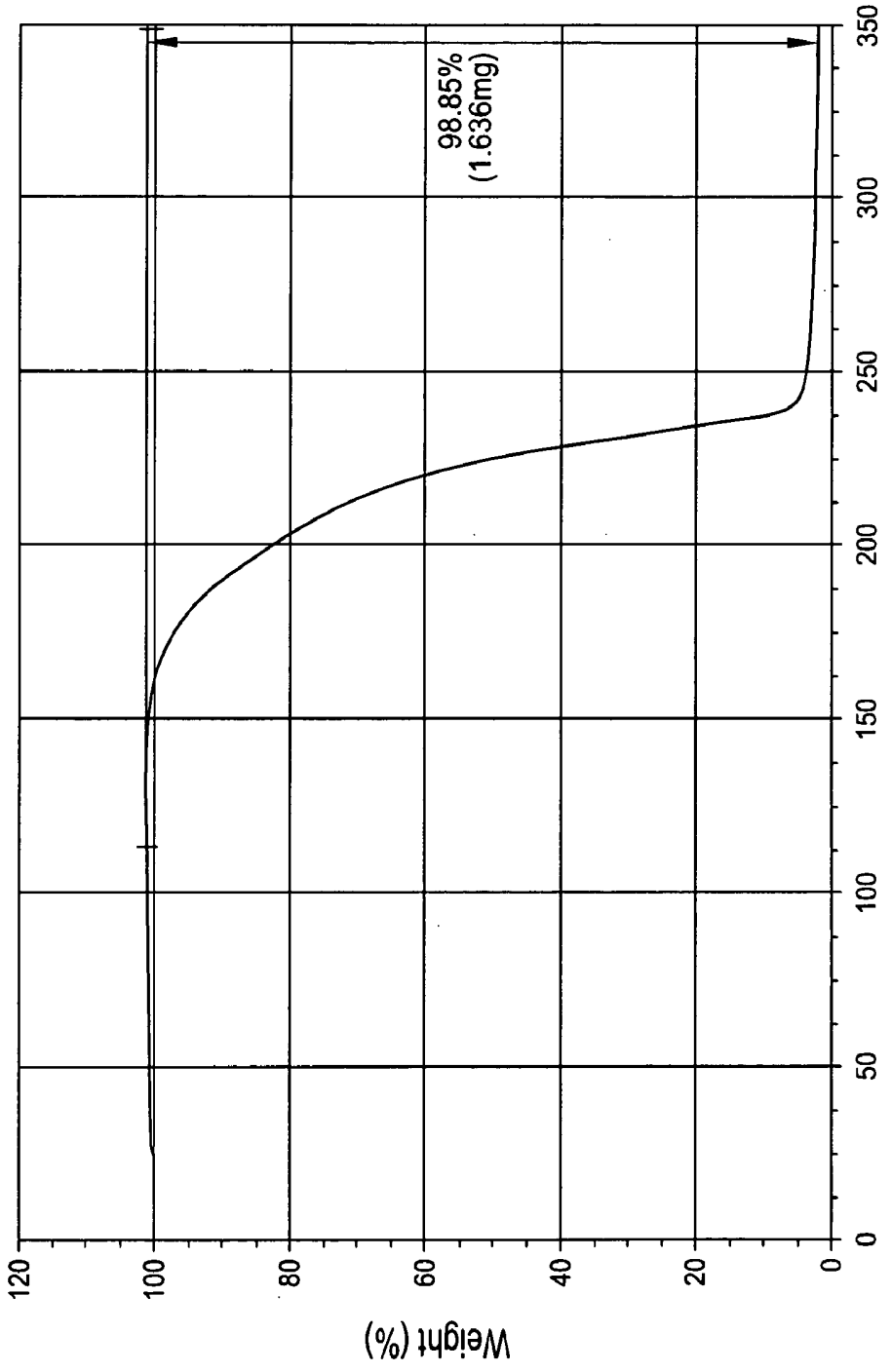

98.85%
(1.636mg)
Weight (%)
120
100
80
60
40
20
0
0
50
100
150
200
250
300
350
Temperature (°C)

Figure 10

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2009/002831

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/155 A61K31/198 A61K31/385 A61K45/06 A61P17/02

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 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)
EPO-Internal, WPI Data, EMBASE, BIOSIS, BEILSTEIN Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2001/010826 A1 (USALA ANTON-LEWIS [US]) 2 August 2001 (2001-08-02) abstract paragraphs [0052], [0055] claims 1,2,13-16	1-4,6-27
Y	US 2002/077277 A1 (USALA ANTON-LEWIS [US]) 20 June 2002 (2002-06-20) abstract paragraphs [0032], [0038] claims 32-44	1-4,6-27
Y	US 2007/264354 A1 (HERMAN RICHARD M [US]) 15 November 2007 (2007-11-15) abstract claims 1,4,8,9	5-8, 15-27

-/--

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

[X] See patent family annex.

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"P" document published prior to the international filing date but later than the priority date claimed

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"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

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 July 2009	18/08/2009

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, Fax: (+31-70) 340-3016	Garabatos-Perera, J

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2009/002831

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	HUMAIRA LATEEF ET AL: "Pretreatment of diabetic rats with lipoic acid improves healing of subsequently-induced abrasion wounds" ARCHIVES OF DERMATOLOGICAL RESEARCH ; FOUNDED IN 1869 AS ARCHIV FÜR DERMATOLOGIE UND SYPHILIS, SPRINGER, BERLIN, DE, vol. 297, no. 2, 1 August 2005 (2005-08-01), pages 75-83, XP019341150 ISSN: 1432-069X abstract page 75, right-hand column, lines 3-6 page 78; figure 1 page 81, left-hand column, lines 15-21	1-27
Y	ARANA V ET AL: "Healing of diabetic foot ulcers in l-arginine-treated patients" BIOMEDICINE AND PHARMACOTHERAPY, ELSEVIER, PARIS, FR, vol. 58, no. 10, 1 December 2004 (2004-12-01), pages 588-597, XP004672231 ISSN: 0753-3322 abstract	1-27
Y	FRANK CHRISTOPHER ET AL: "Approach to infected skin ulcers." CANADIAN FAMILY PHYSICIAN MÉDECIN DE FAMILLE CANADIEN OCT 2005, vol. 51, October 2005 (2005-10), pages 1352-1359, XP002536890 ISSN: 0008-350X abstract page 1355, right-hand column, line 5 - page 1356, left-hand column, line 7	5

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2009/002831

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2001010826 A1	02-08-2001	NONE	
US 2002077277 A1	20-06-2002	NONE	
US 2007264354 A1	15-11-2007	WO 2007134123 A2	22-11-2007