.....

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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
19 May 2005 (19.05.2005)

PCT

(10) International Publication Number
WO 2005/044234 A2

(51) International Patent Classification[7]: A61K 9/14

(21) International Application Number:
PCT/US2004/036337

(22) International Filing Date:
2 November 2004 (02.11.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/517,106 5 November 2003 (05.11.2003) US

(71) Applicant *(for all designated States except US)*: **ELAN PHARMA INTERNATIONAL, LTD.** [IE/IE]; WIL House, Shannon Business Park, Shannon, County Clare (IE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **CUNNINGHAM, James** [US/US]; 903 Charleston Greene, Malvern, PA 19355 (US). **LIVERSIDGE, Elaine, Merisko** [US/US]; 258 Colwyn Terrace, West Chester, PA 19380 (US).

(74) Agents: **SIMKIN, Michele, M.** et al.; Foley & Lardner LLP, Washington Harbour, 3000 K Street, N.W. Suite 500, Washington, DC 20007-5101 (US).

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

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

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ,*

[Continued on next page]

(54) Title: NANOPARTICULATE COMPOSITIONS HAVING A PEPTIDE AS A SURFACE STABILIZER

A B



(57) Abstract: The present invention is directed to nanoparticulate active agent compositions comprising at least one peptide as a surface stabilizer. Also encompassed by the invention are pharmaceutical compositions comprising a nanoparticulate active agent composition of the invention and methods of making and using such nanoparticulate and pharmaceutical compositions.

WO 2005/044234 A2

CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)*

Published:

— *without international search report and to be republished upon receipt of that report*

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

NANOPARTICULATE COMPOSITIONS HAVING A PEPTIDE AS A SURFACE STABILIZER

FIELD OF THE INVENTION

The present invention is directed to nanoparticulate active agent compositions having a peptide adsorbed onto or associated with the surface of the active agent as a surface stabilizer, and methods of making and using such compositions.

BACKGROUND OF THE INVENTION

Nanoparticulate active agent compositions, first described in U.S. Patent No. 5,145,684 ("the '684 patent"), are particles consisting of a poorly soluble therapeutic or diagnostic agent having adsorbed onto, or associated with, the surface thereof a non-crosslinked surface stabilizer. The '684 patent describes the use of a variety of surface stabilizers for nanoparticulate compositions. The use of a peptide as a surface stabilizer for nanoparticulate active agent compositions is not described by the '684 patent.

The '684 patent describes a method of screening active agents to identify useful surface stabilizers that enable the production of a nanoparticulate composition. Not all surface stabilizers will function to produce a stable, non-agglomerated nanoparticulate composition for all active agents. Moreover, known surface stabilizers may be unable to produce a stable, non-agglomerated nanoparticulate composition for certain active agents. Thus, there is a need in the art to identify new surface stabilizers useful in making nanoparticulate active agent compositions. Additionally, such new surface stabilizers may have superior properties over prior known surface stabilizers.

Methods of making nanoparticulate active agent compositions are described, for example, in U.S. Patent Nos. 5,518,187 and 5,862,999, both for "Method of Grinding Pharmaceutical Substances;" U.S. Patent No. 5,718,388, for "Continuous Method of Grinding Pharmaceutical Substances;" and U.S. Patent No. 5,510,118 for "Process of Preparing Therapeutic Compositions Containing Nanoparticles."

Nanoparticulate active agent compositions are also described, for example, in U.S. Patent Nos. 5,298,262 for "Use of Ionic Cloud Point Modifiers to Prevent Particle Aggregation During Sterilization;" 5,302,401 for "Method to Reduce Particle Size Growth During Lyophilization;" 5,318,767 for "X-Ray Contrast Compositions Useful in Medical Imaging;" 5,326,552 for "Novel Formulation For Nanoparticulate X-Ray Blood Pool Contrast Agents Using High Molecular Weight Non-ionic Surfactants;" 5,328,404 for "Method of X-Ray Imaging Using Iodinated Aromatic Propanedioates;" 5,336,507 for "Use of Charged Phospholipids to Reduce Nanoparticle Aggregation;" 5,340,564 for "Formulations Comprising Olin 10-G to Prevent Particle Aggregation and Increase Stability;" 5,346,702 for "Use of Non-Ionic Cloud Point Modifiers to Minimize Nanoparticulate Aggregation During Sterilization;" 5,349,957 for "Preparation and Magnetic Properties of Very Small Magnetic-Dextran Particles;" 5,352,459 for "Use of Purified Surface Modifiers to Prevent Particle Aggregation During Sterilization;" 5,399,363 and 5,494,683, both for "Surface Modified Anticancer Nanoparticles;" 5,401,492 for "Water Insoluble Non-Magnetic Manganese Particles as Magnetic Resonance Enhancement Agents;" 5,429,824 for "Use of Tyloxapol as a Nanoparticulate Stabilizer;" 5,447,710 for "Method for Making Nanoparticulate X-Ray Blood Pool Contrast Agents Using High Molecular Weight Non-ionic Surfactants;" 5,451,393 for "X-Ray Contrast Compositions Useful in Medical Imaging;" 5,466,440 for "Formulations of Oral Gastrointestinal Diagnostic X-Ray Contrast Agents in Combination with Pharmaceutically Acceptable Clays;" 5,470,583 for "Method of Preparing Nanoparticle Compositions Containing Charged Phospholipids to Reduce Aggregation;" 5,472,683 for "Nanoparticulate Diagnostic Mixed Carbamic Anhydrides as X-Ray Contrast Agents for Blood Pool and Lymphatic System Imaging;" 5,500,204 for "Nanoparticulate Diagnostic Dimers as X-Ray Contrast Agents for Blood Pool and Lymphatic System Imaging;" 5,518,738 for "Nanoparticulate NSAID Formulations;" 5,521,218 for "Nanoparticulate Iododipamide Derivatives for Use as X-Ray Contrast Agents;" 5,525,328 for "Nanoparticulate Diagnostic Diatrizoxy Ester X-Ray Contrast Agents for Blood Pool

and Lymphatic System Imaging;" 5,543,133 for "Process of Preparing X-Ray Contrast Compositions Containing Nanoparticles;" 5,552,160 for "Surface Modified NSAID Nanoparticles;" 5,560,931 for "Formulations of Compounds as Nanoparticulate Dispersions in Digestible Oils or Fatty Acids;" 5,565,188 for "Polyalkylene Block Copolymers as Surface Modifiers for Nanoparticles;" 5,569,448 for "Sulfated Non-ionic Block Copolymer Surfactant as Stabilizer Coatings for Nanoparticle Compositions;" 5,571,536 for "Formulations of Compounds as Nanoparticulate Dispersions in Digestible Oils or Fatty Acids;" 5,573,749 for "Nanoparticulate Diagnostic Mixed Carboxylic Anydrides as X-Ray Contrast Agents for Blood Pool and Lymphatic System Imaging;" 5,573,750 for "Diagnostic Imaging X-Ray Contrast Agents;" 5,573,783 for "Redispersible Nanoparticulate Film Matrices With Protective Overcoats;" 5,580,579 for "Site-specific Adhesion Within the GI Tract Using Nanoparticles Stabilized by High Molecular Weight, Linear Poly(ethylene Oxide) Polymers;" 5,585,108 for "Formulations of Oral Gastrointestinal Therapeutic Agents in Combination with Pharmaceutically Acceptable Clays;" 5,587,143 for "Butylene Oxide-Ethylene Oxide Block Copolymers Surfactants as Stabilizer Coatings for Nanoparticulate Compositions;" 5,591,456 for "Milled Naproxen with Hydroxypropyl Cellulose as Dispersion Stabilizer;" 5,593,657 for "Novel Barium Salt Formulations Stabilized by Non-ionic and Anionic Stabilizers;" 5,622,938 for "Sugar Based Surfactant for Nanocrystals;" 5,628,981 for "Improved Formulations of Oral Gastrointestinal Diagnostic X-Ray Contrast Agents and Oral Gastrointestinal Therapeutic Agents;" 5,643,552 for "Nanoparticulate Diagnostic Mixed Carbonic Anhydrides as X-Ray Contrast Agents for Blood Pool and Lymphatic System Imaging;" 5,718,388 for "Continuous Method of Grinding Pharmaceutical Substances;" 5,718,919 for "Nanoparticles Containing the R(-)Enantiomer of Ibuprofen;" 5,747,001 for "Aerosols Containing Beclomethasone Nanoparticle Dispersions;" 5,834,025 for "Reduction of Intravenously Administered Nanoparticulate Formulation Induced Adverse Physiological Reactions;" 6,045,829 "Nanocrystalline Formulations of Human Immunodeficiency Virus (HIV) Protease Inhibitors Using Cellulosic Surface Stabilizers;" 6,068,858 for "Methods of Making

Nanocrystalline Formulations of Human Immunodeficiency Virus (HIV) Protease Inhibitors Using Cellulosic Surface Stabilizers;" 6,153,225 for "Injectable Formulations of Nanoparticulate Naproxen;" 6,165,506 for "New Solid Dose Form of Nanoparticulate Naproxen;" 6,221,400 for "Methods of Treating Mammals Using Nanocrystalline Formulations of Human Immunodeficiency Virus (HIV) Protease Inhibitors;" 6,264,922 for "Nebulized Aerosols Containing Nanoparticle Dispersions;" 6,267,989 for "Methods for Preventing Crystal Growth and Particle Aggregation in Nanoparticle Compositions;" 6,270,806 for "Use of PEG-Derivatized Lipids as Surface Stabilizers for Nanoparticulate Compositions;" 6,316,029 for "Rapidly Disintegrating Solid Oral Dosage Form," 6,375,986 for "Solid Dose Nanoparticulate Compositions Comprising a Synergistic Combination of a Polymeric Surface Stabilizer and Dioctyl Sodium Sulfosuccinate," 6,428,814 for "Bioadhesive nanoparticulate compositions having cationic surface stabilizers;" 6,431,478 for "Small Scale Mill;" 6,432,381 for "Methods for Targeting Drug Delivery to the Upper and/or Lower Gastrointestinal Tract," Patent No. 6,582,285 for "Apparatus for Sanitary Wet Milling;" 6,592,903 for "Nanoparticulate Dispersions Comprising a Synergistic Combination of a Polymeric Surface Stabilizer and Dioctyl Sodium Sulfosuccinate," 6,742,734 for "System and Method for Milling Materials," and 6,745,962 for "Small Scale Mill and Method Thereof," all of which are specifically incorporated by reference. In addition, U.S. Patent Application No. 20020012675 A1, published on January 31, 2002, for "Controlled Release Nanoparticulate Compositions," and WO 02/098565 for "System and Method for Milling Materials," describe nanoparticulate active agent compositions, and are specifically incorporated by reference. None of these references describe nanoparticulate active agent compositions comprising a peptide surface stabilizer.

Amorphous small particle compositions are described, for example, in U.S. Patent Nos. 4,783,484 for "Particulate Composition and Use Thereof as Antimicrobial Agent;" 4,826,689 for "Method for Making Uniformly Sized Particles from Water-Insoluble Organic Compounds;" 4,997,454 for "Method for Making Uniformly-Sized Particles From Insoluble Compounds;" 5,741,522 for "Ultrasmall, Non-aggregated

Porous Particles of Uniform Size for Entrapping Gas Bubbles Within and Methods;" and 5,776,496, for "Ultrasmall Porous Particles for Enhancing Ultrasound Back Scatter."

There is a need in the art for new surface stabilizers useful in preparing nanoparticulate active agent compositions. The present invention satisfies this need.

SUMMARY OF THE INVENTION

The present invention is directed to nanoparticulate compositions comprising at least one active agent and at least one peptide as a surface stabilizer adsorbed on to, or associated with, the surface of the active agent.

Another aspect of the invention is directed to pharmaceutical compositions comprising a nanoparticulate active agent composition of the invention. The pharmaceutical compositions preferably comprise at least one active agent, at least one peptide, and a pharmaceutically acceptable carrier, as well as any desired excipients.

In yet another embodiment, the invention is directed to bioadhesive nanoparticulate active agent compositions comprising at least one cationic peptide as a surface stabilizer, or at least one non-cationic peptide surface stabilizer in combination with at least one secondary cationic surface stabilizer. Such compositions can coat the gut, or the desired site of application, and be retained for a period of time, thereby increasing the efficacy of the active agent as well as eliminating or decreasing the frequency of dosing.

This invention further discloses a method of making a nanoparticulate active agent composition having a peptide surface stabilizer adsorbed on or associated with the surface of the active agent. Such a method comprises contacting an active agent with at least one peptide for a time and under conditions sufficient to provide a Nanoparticle active agent/peptide composition. The peptide surface stabilizer can be contacted with the active agent either before, preferably during, or after size reduction of the active agent.

The present invention is further directed to a method of treatment comprising administering to a mammal a therapeutically effective amount of a nanoparticulate active agent/peptide composition according to the invention.

Both the foregoing general description and the following detailed description are exemplary and explanatory and are intended to provide further explanation of the invention as claimed. Other objects, advantages, and novel features will be readily apparent to those skilled in the art from the following detailed description of the invention.

BRIEF DESCRIPTION OF THE FIGURES

FIGURE 1: Shows representative photomicrographs of nystatin crystals before (Fig. 1A) and after (Fig. 1B) milling;

FIGURE 2: Shows the results of monitoring the particle size stability over time at 5°C (solid line), 25°C (dashed line), and 40°C (dotted line) for a nanoparticulate nystatin composition comprising the peptide poly(Lysine, Tryptophan) 4:1 hydrobromide as a surface stabilizer; and

FIGURE 3: Shows representative micrographs of cells with anionic particles (Fig. 3A) and cationic particles (Fig. 3B).

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to compositions comprising nanoparticulate active agents having at least one peptide as a surface stabilizer adsorbed on or associated with the surface thereof, and methods of making and using such nanoparticulate compositions.

As taught in the '684 patent, not every combination of surface stabilizer and active agent will result in a stable nanoparticulate composition. The discovery of the present invention is surprising in that peptides are biological compounds having secondary and tertiary structures which are critical to the activity of the peptide. It was surprising that such a compound could be successfully used to stabilize a

nanoparticulate active agent. Moreover, it was even more surprising that milling of a peptide surface stabilizer did not change the activity or function of the peptide.

A "peptide" is defined as any compound consisting of two or more amino acids where the alpha carboxyl group of one is bound to the alpha amino group of another. A polypeptide is a long peptide chain. A protein is a large macromolecule composed of one or more polypeptide chains. In the context of the present invention, "peptide" refers to a peptide or a polypeptide, but not a protein.

A striking characteristic of peptides is that they have well-defined three dimensional structures. Peptides fold into compact structures with nominal bond lengths. The strong tendency of hydrophobic amino acid residues to flee from water drives the folding of soluble peptides.

A stretched-out or randomly arranged polypeptide chain is devoid of biological activity. This is because the function of a peptide arises from conformation, which is the three dimensional arrangement of atoms in a structure. *See e.g.*, L. Stryer, *Biochemistry*, 3rd Edition, p. 1-41 (W.H. Freeman & Co., NY, 1988). Amino acid sequences are important because they specify the conformation of peptides. *Id.*

Peptides have several different defined structures, including a primary, secondary, and tertiary structure. The primary structure of a peptide is generally the amino acid sequence of the peptide and the location of disulfides. *See e.g.*, L. Stryer, *Biochemistry*, 3rd Edition, p. 31 (W.H. Freeman & Co., NY, 1988). Secondary structure refers to the spatial arrangement of amino acid residues that are near one another in the linear sequence. Examples of these steric relationships are structures known as an alpha helix, a beta pleated sheet, and a collagen helix. *Id.* Tertiary structure refers to the spatial arrangement of amino acid residues in a peptide or polypeptide that are far apart in the linear sequence.

Proteins, comprising multiple polypeptide chains, also have a quaternary structure, which refers to the spatial arrangement of the polypeptide subunits and the nature of their contacts. *Id.*

It was very surprising that such complex compounds as peptides and polypeptides could be successfully utilized as a surface stabilizer for a nanoparticulate active agent. In addition to enabling the use of a new class of surface stabilizers for nanoparticulate active agents, this discovery is significant as the peptide surface stabilizer in the compositions of the invention may also have therapeutic or diagnostic properties. This is in contrast to prior art nanoparticulate active agent compositions, in which the surface stabilizer is generally a surfactant, which lacks such therapeutic or diagnostic properties.

The nanoparticulate active agent compositions of the invention may also offer the following advantages as compared to prior conventional or non-nanoparticulate active agent compositions: (1) faster onset of action; (2) a potential decrease in the frequency of dosing; (3) smaller doses of active agent required to obtain the same pharmacological effect; (4) increased bioavailability; (5) an increased rate of dissolution; (6) improved performance characteristics for oral, intravenous, subcutaneous, or intramuscular injection, such as higher active agent dose loading and smaller tablet or liquid dose volumes; (7) improved pharmacokinetic profiles, such as improved T_{max}, C_{max}, and AUC profiles; (8) substantially similar or bioequivalent pharmacokinetic profiles of the nanoparticulate active agent compositions when administered in the fed versus the fasted state; (9) bioadhesive active agent compositions, which can coat the gut or the desired site of application and be retained for a period of time, thereby increasing the efficacy of the active agent as well as eliminating or decreasing the frequency of dosing; (10) high redispersibility of the nanoparticulate active agent particles present in the compositions of the invention following administration; (11) the nanoparticulate active agent compositions can be formulated in a dried form which readily redisperses; (12) low viscosity liquid nanoparticulate active agent dosage forms can be made; (13) for liquid nanoparticulate active agent compositions having a low viscosity - better subject compliance due to the perception of a lighter formulation which is easier to consume and digest; (14) for liquid nanoparticulate active agent compositions having a low viscosity - ease of dispensing because one can use a cup or a syringe; (15) the nanoparticulate active

agent compositions can be used in conjunction with other active agents; (16) the nanoparticulate active agent compositions can be sterile filtered; (17) the nanoparticulate active agent compositions are suitable for parenteral administration; and (18) the nanoparticulate active agent compositions do not require organic solvents or pH extremes.

A preferred dosage form of the invention is a solid dosage form, although any pharmaceutically acceptable dosage form can be utilized. Exemplary dosage forms include, but are not limited to, tablets, capsules, sachets, lozenges, powders, pills, granules, liquid dispersions, oral suspensions, gels, aerosols (including nasal and pulmonary), ointments, and creams.

The dosage form of the invention can be, for example, a fast melt dosage form, controlled release dosage form, lyophilized dosage form, delayed release dosage form, extended release dosage form, pulsatile release dosage form, mixed immediate release and controlled release dosage form, or a combination thereof.

In addition, the compositions of the invention can be formulated for any suitable administration route, such as oral, pulmonary, rectal, opthalmic, colonic, parenteral, intracisternal, intravaginal, intraperitoneal, local, buccal, nasal, or topical administration.

The present invention is described herein using several definitions, as set forth below and throughout the application.

As used herein, "about" will be understood by persons of ordinary skill in the art and will vary to some extent on the context in which it is used. If there are uses of the term which are not clear to persons of ordinary skill in the art given the context in which it is used, "about" will mean up to plus or minus 10% of the particular term.

"Conventional" or "non-nanoparticulate active agent" shall mean an active agent which is solubilized or which has an effective average particle size of greater than about 2 microns. Nanoparticulate active agents as defined herein have an effective average particle size of less than about 2 microns.

"Pharmaceutically acceptable" as used herein refers to those compounds, materials, compositions, 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.

"Pharmaceutically acceptable salts" as used herein refers to derivatives wherein the parent compound is modified by making acid or base salts thereof. Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as carboxylic acids; and the like. The pharmaceutically acceptable salts include the conventional non-toxic salts or the quaternary ammonium salts of the parent compound formed, for example, from non-toxic inorganic or organic acids. For example, such conventional non-toxic salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, nitric, and the like; and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pamoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isethionic, and the like.

"Poorly water soluble drugs" as used herein means those having a solubility of less than about 30 mg/ml, preferably less than about 20 mg/ml, preferably less than about 10 mg/ml, or preferably less than about 1 mg/ml. Such drugs tend to be eliminated from the gastrointestinal tract before being absorbed into the circulation.

As used herein with reference to stable drug particles, "stable" includes, but is not limited to, one or more of the following parameters: (1) that the active agent particles do not appreciably flocculate or agglomerate due to interparticle attractive forces, or otherwise significantly increase in particle size over time; (2) that the physical structure of the active agent particles is not altered over time, such as by conversion from an amorphous phase to crystalline phase; (3) that the active agent

particles are chemically stable; and/or (4) where the active agent has not been subject to a heating step at or above the melting point of the active agent in the preparation of the nanoparticles of the invention.

"Therapeutically effective amount" as used herein with respect to an active agent dosage, shall mean that dosage that provides the specific pharmacological response for which the active agent is administered in a significant number of subjects in need of such treatment. It is emphasized that "therapeutically effective amount," administered to a particular subject in a particular instance will not always be effective in treating the diseases described herein, even though such dosage is deemed a 'therapeutically effective amount' by those skilled in the art. It is to be further understood that active agent dosages are, in particular instances, measured as oral dosages, or with reference to active agent levels as measured in blood.

I. Preferred Characteristics of the Nanoparticulate Active Agent Compositions of the Invention

A. Increased Bioavailability, Frequency of Dosing, and Dosage Quantity

The nanoparticulate active agent compositions of the invention, having at least one peptide as a surface stabilizer, may preferably exhibit increased bioavailability and require smaller doses as compared to prior non-nanoparticulate compositions of the same active agent administered at the same dose.

Any active agent can have adverse side effects. Thus, lower doses of an active agent that can achieve the same or better therapeutic effects as those observed with larger doses of a non-nanoparticulate composition of the same active agent are desired. Such lower doses may be realized with the nanoparticulate active agent compositions of the invention because the nanoparticulate active agent compositions may exhibit greater bioavailability as compared to non-nanoparticulate compositions of the same active agent, which means that smaller doses of the active agent are likely required to obtain the desired therapeutic effect.

The nanoparticulate active agent compositions of the invention may be administered less frequently and at lower doses, as compared to conventional non-nanoparticulate compositions of the same active agent, in dosage forms such as liquid dispersions, powders, sprays, aerosols (pulmonary and nasal), solid re-dispersable dosage forms, gels, ointments, creams, *etc.* of the nanoparticulate active agent. Lower dosages can be used because the small particle size of the active agent particles ensure greater absorption, and in the case of bioadhesive nanoparticulate active agent compositions, the active agent is retained at the desired site of application for a longer period of time as compared to conventional, non-nanoparticulate active agent dosage forms.

In one embodiment of the invention, the therapeutically effective amount of the nanoparticulate active agent compositions is 1/6, 1/5, ¼, 1/3rd, or ½ of the therapeutically effective amount of a non-nanoparticulate composition of the same active agent.

Such lower doses are preferred as they may decrease or eliminate adverse effects of the active agent. In addition, such lower doses decrease the cost of the dosage form and may increase patient compliance.

B. Pharmacokinetic Profiles of the Nanoparticulate Active Agent Compositions of the Invention

The invention also preferably provides nanoparticulate active agent compositions, having at least one peptide as a surface stabilizer, and having a desirable pharmacokinetic profile when administered to mammalian subjects. The desirable pharmacokinetic profile of the active agent compositions preferably includes, but is not limited to: (1) a T_{max} for an active agent, when assayed in the plasma of a mammalian subject following administration, that is preferably less than the T_{max} for a non-nanoparticulate composition of the same active agent, administered at the same dosage; (2) a C_{max} for an active agent, when assayed in the plasma of a mammalian subject following administration, that is preferably greater than the C_{max} for a non-nanoparticulate composition of the same active agent, administered at the same dosage; and/or (3) an AUC for an active agent, when assayed in the plasma of a

mammalian subject following administration, that is preferably greater than the AUC for a non-nanoparticulate composition of the same active agent, administered at the same dosage.

The desirable pharmacokinetic profile, as used herein, is the pharmacokinetic profile measured after the initial dose of the active agent. The compositions can be formulated in any way as described herein and as known to those of skill in the art.

A preferred active agent composition of the invention, comprising at least one peptide as a surface stabilizer, exhibits in comparative pharmacokinetic testing with a non-nanoparticulate composition of the same active agent, administered at the same dosage, a T_{max} not greater than about 100%, not greater than about 90%, not greater than about 80%, not greater than about 70%, not greater than about 60%, not greater than about 50%, not greater than about 40%, not greater than about 30%, not greater than about 25%, not greater than about 20%, not greater than about 15%, not greater than about 10%, or not greater than about 5% of the T_{max} exhibited by the non-nanoparticulate active agent composition. This shorter T_{max} translates into a faster onset of therapeutic activity.

A preferred active agent composition of the invention, comprising at least one peptide as a surface stabilizer, exhibits in comparative pharmacokinetic testing with a non-nanoparticulate composition of the same active agent, administered at the same dosage, a C_{max} which is at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, at least about 110%, at least about 120%, at least about 130%, at least about 140%, at least about 150%, at least about 160%, at least about 170%, at least about 180%, at least about 190%, or at least about 200% greater than the C_{max} exhibited by the non-nanoparticulate active agent composition.

A preferred active agent composition of the invention, comprising at least one peptide as a surface stabilizer, exhibits in comparative pharmacokinetic testing with a non-nanoparticulate composition of the same active agent, administered at the same

dosage, an AUC which is at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, at least about 110%, at least about 120%, at least about 130%, at least about 140%, at least about 150%, at least about 160%, at least about 170%, at least about 180%, at least about 190%, or at least about 200% greater than the AUC exhibited by the non-nanoparticulate active agent formulation.

Any formulation giving the desired pharmacokinetic profile is suitable for administration according to the present methods.

C. The Pharmacokinetic Profiles of the Nanoparticulate Active Agent Compositions of the Invention are Preferably not Substantially Affected by the Fed or Fasted State of the Subject Ingesting the Compositions

The invention encompasses nanoparticulate active agent compositions, comprising at least one peptide as a surface stabilizer, wherein preferably the pharmacokinetic profile of the active agent is not substantially affected by the fed or fasted state of a subject ingesting the composition. This means that there is no substantial difference in the quantity of active agent absorbed or the rate of active agent absorption when the nanoparticulate active agent compositions are administered in the fed versus the fasted state. Thus, the nanoparticulate active agent compositions of the invention can preferably substantially eliminate the effect of food on the pharmacokinetics of the active agent.

In another embodiment of the invention, the pharmacokinetic profile of the active agent compositions of the invention, comprising at least one peptide as a surface stabilizer, when administered to a mammal in a fasted state, is bioequivalent to the pharmacokinetic profile of the same nanoparticulate active agent composition administered at the same dosage, when administered to a mammal in a fed state.

"Bioequivalency" is preferably established by a 90% Confidence Interval (CI) of between 0.80 and 1.25 for both C_{max} and AUC under U.S. Food and Drug Administration (USFDA) regulatory guidelines, or a 90% CI for AUC of between

0.80 to 1.25 and a 90% CI for C_{max} of between 0.70 to 1.43 under the European Medicines Evaluation Agency (EMEA) regulatory guidelines (T_{max} is not relevant for bioequivalency determinations under USFDA and EMEA regulatory guidelines).

Preferably the difference in AUC (*e.g.*, absorption) of the nanoparticulate active agent composition of the invention, comprising at least one peptide as a surface stabilizer, when administered in the fed versus the fasted state, is less than about 100%, less than about 90%, less than about 80%, less than about 70%, less than about 60%, less than about 50%, less than about 40%, less than about 35%, less than about 30%, less than about 25%, less than about 20%, less than about 15%, less than about 10%, less than about 5%, or less than about 3%.

In addition, preferably the difference in C_{max} of the nanoparticulate active agent composition of the invention, comprising at least one peptide as a surface stabilizer, when administered in the fed versus the fasted state, is less than about 100%, less than about 90%, less than about 80%, less than about 70%, less than about 60%, less than about 50%, less than about 40%, less than about 35%, less than about 30%, less than about 25%, less than about 20%, less than about 15%, less than about 10%, less than about 5%, or less than about 3%.

Finally, preferably the difference in the T_{max} of the nanoparticulate active agent compositions of the invention, comprising at least one peptide as a surface stabilizer, when administered in the fed versus the fasted state, is less than about 100%, less than about 90%, less than about 80%, less than about 70%, less than about 60%, less than about 50%, less than about 40%, less than about 30%, less than about 20%, less than about 15%, less than about 10%, less than about 5%, less than about 3%, or essentially no difference.

Benefits of a dosage form that substantially eliminates the effect of food include an increase in subject convenience, thereby increasing subject compliance, as the subject does not need to ensure that they are taking a dose either with or without food.

D. Redispersibility Profiles of the Nanoparticulate Active Agent Compositions of the Invention

An additional feature of the nanoparticulate active agent compositions of the invention, comprising at least one peptide as a surface stabilizer, comprising at least one peptide as a surface stabilizer, is that the compositions redisperse such that the effective average particle size of the redispersed active agent particles is less than about 2 microns. This is significant, as if upon administration the nanoparticulate active agent particles present in the compositions of the invention did not redisperse to a substantially nanoparticulate particle size, then the dosage form may lose the benefits afforded by formulating the active agent into a nanoparticulate particle size.

This is because the nanoparticulate active agent compositions of the invention benefit from the small particle size of the active agent; if the nanoparticulate active agent particles do not redisperse into the small particle sizes upon administration, then "clumps" or agglomerated active agent particles are formed. With the formation of such agglomerated particles, the bioavailability of the dosage form may fall.

Moreover, the nanoparticulate active agent compositions of the invention exhibit dramatic redispersion of the active agent particles upon administration to a mammal, such as a human or animal, as demonstrated by reconstitution in a biorelevant aqueous media. Such biorelevant aqueous media can be any aqueous media that exhibit the desired ionic strength and pH, which form the basis for the biorelevance of the media. The desired pH and ionic strength are those that are representative of physiological conditions found in the human body. Such biorelevant aqueous media can be, for example, aqueous electrolyte solutions or aqueous solutions of any salt, acid, or base, or a combination thereof, which exhibit the desired pH and ionic strength.

Biorelevant pH is well known in the art. For example, in the stomach, the pH ranges from slightly less than 2 (but typically greater than 1) up to 4 or 5. In the small intestine the pH can range from 4 to 6, and in the colon it can range from 6 to 8. Biorelevant ionic strength is also well known in the art. Fasted state gastric fluid has an ionic strength of about 0.1M while fasted state intestinal fluid has an ionic strength

of about 0.14. *See e.g.*, Lindahl et al., "Characterization of Fluids from the Stomach and Proximal Jejunum in Men and Women," *Pharm. Res., 14* (4): 497-502 (1997).

It is believed that the pH and ionic strength of the test solution is more critical than the specific chemical content. Accordingly, appropriate pH and ionic strength values can be obtained through numerous combinations of strong acids, strong bases, salts, single or multiple conjugate acid-base pairs (*i.e.*, weak acids and corresponding salts of that acid), monoprotic and polyprotic electrolytes, *etc.*

Representative electrolyte solutions can be, but are not limited to, HCl solutions, ranging in concentration from about 0.001 to about 0.1 M, and NaCl solutions, ranging in concentration from about 0.001 to about 0.1 M, and mixtures thereof. For example, electrolyte solutions can be, but are not limited to, about 0.1 M HCl or less, about 0.01 M HCl or less, about 0.001 M HCl or less, about 0.1 M NaCl or less, about 0.01 M NaCl or less, about 0.001 M NaCl or less, and mixtures thereof. Of these electrolyte solutions, 0.01 M HCl and/or 0.1 M NaCl, are most representative of fasted human physiological conditions, owing to the pH and ionic strength conditions of the proximal gastrointestinal tract.

Electrolyte concentrations of 0.001 M HCl, 0.01 M HCl, and 0.1 M HCl correspond to pH 3, pH 2, and pH 1, respectively. Thus, a 0.01 M HCl solution simulates typical acidic conditions found in the stomach. A solution of 0.1 M NaCl provides a reasonable approximation of the ionic strength conditions found throughout the body, including the gastrointestinal fluids, although concentrations higher than 0.1 M may be employed to simulate fed conditions within the human GI tract.

Exemplary solutions of salts, acids, bases or combinations thereof, which exhibit the desired pH and ionic strength, include but are not limited to phosphoric acid/phosphate salts + sodium, potassium and calcium salts of chloride, acetic acid/acetate salts + sodium, potassium and calcium salts of chloride, carbonic acid/bicarbonate salts + sodium, potassium and calcium salts of chloride, and citric acid/citrate salts + sodium, potassium and calcium salts of chloride.

In other embodiments of the invention, the redispersed active agent particles of the invention (redispersed in an aqueous, biorelevant, or any other suitable media) have an effective average particle size of less than about 1900 nm, less than about 1800 nm, less than about 1700 nm, less than about 1600 nm, less than about 1500 nm, less than about 1400 nm, less than about 1300 nm, less than about 1200 nm, less than about 1100 nm, less than about 1000 nm, less than about 900 nm, less than about 800 nm, less than about 700 nm, less than about 600 nm, less than about 500 nm, less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 200 nm, less than about 150 nm, less than about 100 nm, less than about 75 nm, or less than about 50 nm, as measured by light-scattering methods, microscopy, or other appropriate methods.

Redispersibility can be tested using any suitable means known in the art. *See e.g.,* the example sections of U.S. Patent No. 6,375,986 for "Solid Dose Nanoparticulate Compositions Comprising a Synergistic Combination of a Polymeric Surface Stabilizer and Dioctyl Sodium Sulfosuccinate."

E. Bioadhesive Nanoparticulate Active Agent Compositions

Bioadhesive nanoparticulate active agent compositions of the invention comprise at least one cationic peptide surface stabilizer, or in addition to at least one non-cationic peptide as a surface stabilizer, at least one secondary non-peptide cationic surface stabilizer. Exemplary non-peptide cationic surface stabilizers are described in more detail below. Bioadhesive formulations of active agents exhibit exceptional bioadhesion to biological surfaces, such as mucous and skin.

Cationic surface stabilizers generally confer relatively large, positive zeta potentials to particles on which they adsorb or associate. To increase the bioadhesive properties of a nanoparticulate composition, two or more cationic surface stabilizers can be utilized.

In the case of bioadhesive nanoparticulate active agent compositions, the term "bioadhesion" is used to describe the adhesion between the nanoparticulate active agent compositions and a biological substrate (*i.e.*, gastrointestinal mucin, lung tissue,

nasal mucosa, *etc.*). *See e.g.*, U.S. Patent No. 6,428,814 for "Bioadhesive Nanoparticulate Compositions Having Cationic Surface Stabilizers," which is specifically incorporated by reference.

There are basically two mechanisms which may be responsible for this bioadhesion phenomena: mechanical or physical interactions and chemical interactions. The first of these, mechanical or physical mechanisms, involves the physical interlocking or interpenetration between a bioadhesive entity and the receptor tissue, resulting from a good wetting of the bioadhesive surface, swelling of the bioadhesive polymer, penetration of the bioadhesive entity into a crevice of the tissue surface, or interpenetration of bioadhesive composition chains with those of the mucous or other such related tissues. The second possible mechanism of bioadhesion incorporates forces such as ionic attraction, dipolar forces, van der Waals interactions, and hydrogen bonds. It is this form of bioadhesion which is primarily responsible for the bioadhesive properties of the nanoparticulate active agent compositions of the invention. However, physical and mechanical interactions may also play a secondary role in the bioadhesion of such nanoparticulate active agent compositions.

The bioadhesive active agent compositions of the invention are useful in any situation in which it is desirable to apply the compositions to a biological surface. The bioadhesive active agent compositions preferably coat the targeted surface in a continuous and uniform film that is invisible to the naked human eye.

A bioadhesive nanoparticulate active agent composition slows the transit of the composition, and some active agent particles would also most likely adhere to tissue other than the mucous cells and therefore give a prolonged exposure to the active agent, thereby increasing absorption and the bioavailability of the administered dosage.

The adhesion exhibited by the inventive compositions means that nanoparticulate active agent particles are not easily washed off, rubbed off, or otherwise removed from the biological surface for an extended period of time. The period of time in which a biological cell surface is replaced is the factor that limits

retention of the bioadhesive nanoparticulate active agent particles to that biological surface.

F. Low Viscosity Active Agent Dosage Forms

A liquid dosage form of a conventional microcrystalline or non-nanoparticulate active agent composition would be expected to be a relatively large volume, highly viscous substance which would not be well accepted by patient populations. Moreover, viscous solutions can be problematic in parenteral administration because these solutions require a slow syringe push and can stick to tubing. In addition, conventional formulations of poorly water-soluble active agents tend to be unsafe for intravenous administration techniques, which are used primarily in conjunction with highly water-soluble substances.

Liquid dosage forms of the nanoparticulate active agent compositions of the invention, comprising at least one peptide as a surface stabilizer, provide significant advantages over a liquid dosage form of a conventional microcrystalline or solubilized active agent composition. The low viscosity and silky texture of liquid dosage forms of the nanoparticulate active agent compositions of the invention result in advantages in both preparation and use. These advantages include, for example: (1) better subject compliance due to the perception of a lighter formulation which is easier to consume and digest; (2) ease of dispensing because one can use a cup or a syringe; (3) potential for formulating a higher concentration of active agent resulting in a smaller dosage volume and thus less volume for the subject to consume; and (4) easier overall formulation concerns.

Liquid active agent dosage forms that are easier to consume are especially important when considering juvenile patients, terminally ill patients, and elderly patients. Viscous or gritty formulations, and those that require a relatively large dosage volume, are not well tolerated by these patient populations. Liquid oral dosage forms can be particularly preferably for patient populations who have difficulty consuming tablets, such as infants and the elderly.

The viscosities of liquid dosage forms of a nanoparticulate active agent according to the invention are preferably less than about 1/200, less than about 1/175, less than about 1/150, less than about 1/125, less than about 1/100, less than about 1/75, less than about 1/50, or less than about 1/25 of a liquid oral dosage form of a non-nanoparticulate composition of the same active agent, at about the same concentration per ml of active agent.

Typically liquid nanoparticulate active agent dosage forms of the invention, comprising at least one peptide as a surface stabilizer, have a viscosity at a shear rate of 0.1 (1/s) measured at 20°C, is from about 2000 mPa s to about 1 mPa s, from about 1900 mPa·s to about 1 mPa·s, from about 1800 mPa·s to about 1 mPa·s, from about 1700 mPa·s to about 1 mPa·s, from about 1600 mPa·s to about 1 mPa·s, from about 1500 mPa·s to about 1 mPa·s, from about 1400 mPa·s to about 1 mPa·s, from about 1300 mPa·s to about 1 mPa·s, from about 1200 mPa·s to about 1 mPa·s, from about 1100 mPa·s to about 1 mPa·s, from about 1000 mPa·s to about 1 mPa·s, from about 900 mPa·s to about 1 mPa·s, from about 800 mPa·s to about 1 mPa·s, from about 700 mPa·s to about 1 mPa·s, from about 600 mPa·s to about 1 mPa·s, from about 500 mPa·s to about 1 mPa·s, from about 400 mPa·s to about 1 mPa·s, from about 300 mPa·s to about 1 mPa·s, from about 200 mPa·s to about 1 mPa·s, from about 175 mPa·s to about 1 mPa·s, from about 150 mPa·s to about 1 mPa·s, from about 125 mPa·s to about 1 mPa·s, from about 100 mPa·s to about 1 mPa·s, from about 75 mPa·s to about 1 mPa·s, from about 50 mPa·s to about 1 mPa·s, from about 25 mPa·s to about 1 mPa·s, from about 15 mPa·s to about 1 mPa·s, from about 10 mPa·s to about 1 mPa·s, or from about 5 mPa·s to about 1 mPa·s. Such a viscosity is much more attractive for subject consumption and may lead to better overall subject compliance.

Viscosity is concentration and temperature dependent. Typically, a higher concentration results in a higher viscosity, while a higher temperature results in a lower viscosity. Viscosity as defined above refers to measurements taken at about 20°C. (The viscosity of water at 20°C is 1 mPa s.) The invention encompasses equivalent viscosities measured at different temperatures.

Another important aspect of the invention is that the nanoparticulate active agent compositions of the invention, formulated into a liquid dosage form, are not turbid. "Turbid," as used herein refers to the property of particulate matter that can be seen with the naked eye or that which can be felt as "gritty." The nanoparticulate active agent compositions of the invention, formulated into a liquid dosage form, can be poured out of or extracted from a container as easily as water, whereas a liquid dosage form of a non-nanoparticulate or solubilized composition of the same active agent is expected to exhibit notably more "sluggish" characteristics.

The liquid formulations of this invention can be formulated for dosages in any volume but preferably equivalent or smaller volumes than a liquid dosage form of a non-nanoparticulate composition of the same active agent.

G. Sterile Filtered Nanoparticulate Active Agent Compositions

The nanoparticulate active agent compositions of the invention can be sterile filtered. This obviates the need for heat sterilization, which can harm or degrade an active agent, as well as result in crystal growth and particle aggregation of the active agent.

Sterile filtration can be difficult because of the required small particle size of the composition. Filtration is an effective method for sterilizing homogeneous solutions when the membrane filter pore size is less than or equal to about 0.2 microns (200 nm) because a 0.2 micron filter is sufficient to remove essentially all bacteria. Sterile filtration is normally not used to sterilize suspensions of micron-sized active agents because the active agent particles are too large to pass through the membrane pores.

A sterile nanoparticulate active agent dosage form is particularly useful in treating immunocompromised patients, infants or juvenile patients, and the elderly, as these patient groups are the most susceptible to infection caused by a non-sterile liquid dosage form.

Because the nanoparticulate active agent compositions of the invention, comprising at least one peptide as a surface stabilizer and formulated into a liquid dosage form, can be sterile filtered, and because the compositions can have a very small active agent effective average particle size, the compositions are suitable for parenteral administration.

H. Combination Pharmacokinetic Profile Compositions

In yet another embodiment of the invention, a first nanoparticulate active agent composition providing a desired pharmacokinetic profile is co-administered, sequentially administered, or combined with at least one other active agent composition that generates a desired different pharmacokinetic profile. More than two active agent compositions can be co-administered, sequentially administered, or combined. While the first active agent composition has a nanoparticulate particle size, the additional one or more active agent compositions can be nanoparticulate, solubilized, or have a microparticulate particle size.

The second, third, fourth, *etc.*, active agent compositions can differ from the first, and from each other, for example: (1) in the identity of the active agent; (2) in the effective average particle sizes of the active agent; or (3) in the dosage of the active agent. Such a combination composition can reduce the dose frequency required.

For example, a first active agent composition can have a nanoparticulate particle size, conferring a short T_{max} and typically a higher C_{max}. This first active agent composition can be combined, co-administered, or sequentially administered with a second composition comprising: (1) the same active agent having a larger (but still nanoparticulate as defined herein) particle size, and therefore exhibiting slower absorption, a longer T_{max}, and typically a lower C_{max}; or (2) a microparticulate or solubilized composition of the same active agent, exhibiting a longer T_{max}, and typically a lower C_{max}.

If the second active agent composition has a nanoparticulate particle size, then preferably the active agent particles of the second composition have at least one

surface stabilizer associated with the surface of the active agent particles. The one or more surface stabilizers can be the same as or different from the surface stabilizer(s) present in the first active agent composition.

Preferably where co-administration of a "fast-acting" formulation and a "longer-lasting" formulation is desired, the two formulations are combined within a single composition, for example a dual-release composition.

I. Miscellaneous Benefits of the Nanoparticulate Active Agent Compositions of the Invention

The nanoparticulate active agent compositions of the invention, comprising at least one peptide as a surface stabilizer, preferably exhibit an increased rate of dissolution as compared to microcrystalline or non-nanoparticulate forms of the same active agent. In addition, the nanoparticulate active agent compositions preferably exhibit improved performance characteristics for oral, intravenous, subcutaneous, or intramuscular injection, such as higher dose loading and smaller tablet or liquid dose volumes. Moreover, the nanoparticulate active agent compositions of the invention do not require organic solvents or pH extremes.

II. Compositions

The compositions of the invention comprise a nanoparticulate active agent and at least one peptide as a surface stabilizer adsorbed to or associated with the surface of the active agent. In addition, the compositions can comprise one or more secondary surface stabilizers. Surface stabilizers useful herein physically adhere to or associate with the surface of the nanoparticulate active agent but do not chemically react with the active agent or itself. Individual molecules of the surface stabilizer are essentially free of intermolecular cross-linkages.

The present invention also includes nanoparticulate active agent compositions, having at least one peptide as a surface stabilizer, formulated into compositions together with one or more non-toxic physiologically acceptable carriers, adjuvants, or vehicles, collectively referred to as carriers.

A. Peptide Surface Stabilizer

The choice of a surface stabilizer is non-trivial and usually requires extensive experimentation to realize a desirable formulation. Accordingly, the present invention is directed to the surprising discovery that a peptide, used as a nanoparticulate surface stabilizer, yields stable nanoparticulate active agent compositions that exhibit low degrees of aggregation.

A "peptide" is defined as any compound consisting of two or more amino acids, which are the basic structural units or "building blocks" of peptides. All peptides in all species, from bacteria to humans, are constructed from the same set of twenty commonly occurring, genetically encoded amino acids, as shown in the table below.

Each amino acid contains an "amine" group (NH_3), a "carboxy" group (COOH), a hydrogen atom, and a distinctive R group, or sidechain, bonded to a carbon atom. The amino acids vary in their sidechains, with variations in size, shape, charge, hydrogen-bonding capacity, and chemical reactivity. *See e.g.*, L. Stryer, *Biochemistry,* 3rd Edition, 1-40 (W.H. Freeman & Co., NY, 1988).

Amino Acid	3 Letter Abbreviation	1 Letter Abbreviation
alanine	ALA	A
asparagine	ASN	N
aspartic acid	ASP	D
arginine	ARG	R
cysteine	CYS	C
glutamic acid	GLU	E
glutamine	GLN	Q
glycine	GLY	G
histidine	HIS	H
isoleucine	ILE	I
leucine	LEU	L

Amino Acid	3 Letter Abbreviation	1 Letter Abbreviation
lysine	LYS	K
methionine	MET	M
phenylalanine	PHE	F
proline	PRO	P
serine	SER	S
threonine	THR	T
tryptophan	TRP	W
tyrosine	TYR	Y
valine	VAL	V
aspartic acid or asparagines	ASX	
glutamic acid or glutamine	GLX	
Unknown or other	Xaa	X

Peptides useful in the present invention can also comprise substituents other than amino acids. There are also naturally occurring chemical modifications of these twenty genetically encoded amino acids, such as hydroxylation of proline, addition of carbohydrates and lipids, and phosphorylation of serine and tyrosine. In addition, D-isomers of the amino acids, as opposed to the L-isomers found in naturally-occuring peptides and proteins, have been synthesized.

The amino acids of a peptide are connected by a amide, covalent linkage between the alpha carboxyl group of one amino acid and the alpha amino group of another amino acid. Many amino acids are joined by peptide bonds to form a polypeptide chain, which is unbranched. A polypeptide chain is a long peptide chain, consisting of a regularly repeating part, called the main chain, and a variable part, comprising the distinctive sidechains. Disulfide cross-links can be formed by cysteine residues in polypeptides. Most natural polypeptide chains contain between 50 and

2000 amino acids residues. The mean molecular weight of an amino acid residue is about 110 daltons, and so the molecular weights of most polypeptide chains are between 5500 and 220,000. *See e.g.*, L. Stryer, *Biochemistry*, 3rd Edition, p. 22 (W.H. Freeman & Co., NY, 1988).

A protein is a large macromolecule composed of one or more polypeptide chains. In the context of the present invention, a "peptide" refers to a peptide or a polypeptide, but not a protein.

Preferably, the peptide surface stabilizers of the invention are water soluble. By "water soluble," it is meant that the peptide has a water solubility of greater than about 1 mg/mL, greater than about 10 mg/mL, greater than about 20 mg/mL, or greater than about 30 mg/mL. This is in contrast to prior art compositions teaching the use of a peptide as an active agent in a nanoparticulate active agent composition. *See e.g.*, U.S. Patent Nos. 6,270,806; 6,592,903; 6,428,814; and 6,375,986. In such prior art references, when a peptide is utilized as an active agent in a nanoparticulate composition, the peptide is *poorly water soluble.*

There is an extensive catalog of commercially available peptides that can be used in the compositions of the invention. For example, the on-line peptide catalog http://www.peptide-catalog.com/PC/Peptides provides a list of hundreds of commercially available peptides, along with their structure and molecular weight. In addition, to the many commercially available peptides, custom peptides can be made and utilized in the compositions of the invention.

A preferred peptide surface stabilizer is poly(Lysine, Tryptophan)) 4:1 hydrobromide.

B. Secondary or Auxiliary Surface Stabilizers

The compositions of the invention can also include one or more auxiliary non-peptide surface stabilizers in addition to the at least one peptide surface stabilizer.

The auxiliary surface stabilizers of the invention are preferably adsorbed on, or associated with, the surface of the active agent particles. The auxiliary surface

stabilizers especially useful herein preferably do not chemically react with the active agent particles or itself. Preferably, individual molecules of the auxiliary surface stabilizer are essentially free of intermolecular cross-linkages.

Two or more auxiliary surface stabilizers can be employed in the compositions and methods of the invention.

Suitable surface stabilizers can preferably be selected from known organic and inorganic pharmaceutical excipients. Such excipients include various polymers, low molecular weight oligomers, natural products, and surfactants. Preferred auxiliary surface stabilizers include nonionic, anionic, cationic, zwitterionic, and ionic surfactants.

Representative examples of secondary surface stabilizers include gelatin, casein, lecithin (phosphatides), dextran, gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glycerol monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers (*e.g.*, macrogol ethers such as cetomacrogol 1000), polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters (*e.g.*, the commercially available Tweens® such as *e.g.*, Tween 20® and Tween 80® (ICI Speciality Chemicals)); polyethylene glycols (*e.g.*, Carbowaxs 3550® and 934® (Union Carbide)), polyoxyethylene stearates, colloidal silicon dioxide, phosphates, sodium dodecylsulfate, carboxymethylcellulose calcium, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hydroxypropyl celluloses (e.g., HPC, HPC-SL, and HPC-L), hydroxypropyl methylcellulose (HPMC), hydroxypropylmethyl-cellulose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol (PVA), polyvinylpyrrolidone (PVP), 4-(1,1,3,3-tetramethylbutyl)-phenol polymer with ethylene oxide and formaldehyde (also known as tyloxapol, superione, and triton), poloxamers (*e.g.*, Pluronics F68® and F108®, which are block copolymers of ethylene oxide and propylene oxide); poloxamines (*e.g.*, Tetronic 908®, also known as Poloxamine 908®, which is a tetrafunctional block copolymer derived from sequential addition of propylene oxide and ethylene oxide to

ethylenediamine (BASF Wyandotte Corporation, Parsippany, N.J.)); Tetronic 1508® (T-1508) (BASF Wyandotte Corporation), dialkylesters of sodium sulfosuccinic acid (*e.g.*, Aerosol OT®, which is a dioctyl ester of sodium sulfosuccinic acid (DOSS) (American Cyanamid)); Duponol P®, which is a sodium lauryl sulfate (DuPont); Tritons X-200®, which is an alkyl aryl polyether sulfonate (Rohm and Haas); Crodestas F-110®, which is a mixture of sucrose stearate and sucrose distearate (Croda Inc.); p-isononylphenoxypoly-(glycidol), also known as Olin-10G® or Surfactant 10-G® (Olin Chemicals, Stamford, CT); Crodestas SL-40® (Croda, Inc.); and SA9OHCO, which is $C_{18}H_{37}CH_2C(O)N(CH_3)\text{-}CH_2(CHOH)_4(CH_2OH)_2$ (Eastman Kodak Co.); decanoyl-N-methylglucamide; n-decyl β-D-glucopyranoside; n-decyl β-D-maltopyranoside; n-dodecyl β-D-glucopyranoside; n-dodecyl β-D-maltoside; heptanoyl-N-methylglucamide; n-heptyl-β-D-glucopyranoside; n-heptyl β-D-thioglucoside; n-hexyl β-D-glucopyranoside; nonanoyl-N-methylglucamide; n-noyl β-D-glucopyranoside; octanoyl-N-methylglucamide; n-octyl-β-D-glucopyranoside; octyl β-D-thioglucopyranoside; lysozyme, PEG-derivatized phospholipid, PEG-derivatized cholesterol, PEG- derivatized cholesterol derivative, PEG-derivatized vitamin A, PEG-derivatized vitamin E, random copolymers of vinyl pyrrolidone and vinyl acetate, and the like.

Examples of useful cationic surface stabilizers include but are not limited to polymers, biopolymers, polysaccharides, cellulosics, alginates, phospholipids, and nonpolymeric compounds, such as zwitterionic stabilizers, poly-n-methylpyridinium, anthryul pyridinium chloride, cationic phospholipids, a charged phospholipid such as dimyristoyl phophatidyl glycerol, chitosan, polylysine, polyvinylimidazole, polybrene, polymethylmethacrylate trimethylammoniumbromide bromide (PMMTMABr), hexyldesyltrimethylammonium bromide (HDMAB), and polyvinylpyrrolidone-2-dimethylaminoethyl methacrylate dimethyl sulfate.

Other useful cationic stabilizers include, but are not limited to, cationic lipids, sulfonium, phosphonium, and quarternary ammonium compounds, such as stearyltrimethylammonium chloride, benzyl-di(2-chloroethyl)ethylammonium bromide, coconut trimethyl ammonium chloride or bromide, coconut methyl

dihydroxyethyl ammonium chloride or bromide, dodecyl trimethyl ammonium bromide, decyl triethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium chloride or bromide, C_{12-15}dimethyl hydroxyethyl ammonium chloride or bromide, coconut dimethyl hydroxyethyl ammonium chloride or bromide, myristyl trimethyl ammonium methyl sulphate, lauryl dimethyl benzyl ammonium chloride or bromide, lauryl dimethyl (ethenoxy)$_4$ ammonium chloride or bromide, N-alkyl (C_{12-18})dimethylbenzyl ammonium chloride, N-alkyl (C_{14-18})dimethyl-benzyl ammonium chloride, N-tetradecylidmethylbenzyl ammonium chloride monohydrate, dimethyl didecyl ammonium chloride, N-alkyl and (C_{12-14}) dimethyl 1-napthylmethyl ammonium chloride, trimethylammonium halide, alkyl-trimethylammonium salts and dialkyl-dimethylammonium salts, lauryl trimethyl ammonium chloride, ethoxylated alkyamidoalkyldialkylammonium salt and/or an ethoxylated trialkyl ammonium salt, dialkylbenzene dialkylammonium chloride, N-didecyldimethyl ammonium chloride, N-tetradecyldimethylbenzyl ammonium, chloride monohydrate, N-alkyl(C_{12-14}) dimethyl 1-naphthylmethyl ammonium chloride and dodecyldimethylbenzyl ammonium chloride, dialkyl benzenealkyl ammonium chloride, lauryl trimethyl ammonium chloride, alkylbenzyl methyl ammonium chloride, alkyl benzyl dimethyl ammonium bromide, C_{12}, C_{15}, C_{17} trimethyl ammonium bromides, dodecylbenzyl triethyl ammonium chloride, poly-diallyldimethylammonium chloride (DADMAC), dimethyl ammonium chlorides, alkyldimethylammonium halogenides, tricetyl methyl ammonium chloride, decyltrimethylammonium bromide, dodecyltriethylammonium bromide, tetradecyltrimethylammonium bromide, methyl trioctylammonium chloride (ALIQUAT 336™), POLYQUAT 10™, tetrabutylammonium bromide, benzyl trimethylammonium bromide, choline esters (such as choline esters of fatty acids), benzalkonium chloride, stearalkonium chloride compounds (such as stearyltrimonium chloride and Di-stearyldimonium chloride), cetyl pyridinium bromide or chloride, halide salts of quaternized polyoxyethylalkylamines, MIRAPOL™ and ALKAQUAT™ (Alkaril Chemical Company), alkyl pyridinium salts; amines, such as alkylamines, dialkylamines, alkanolamines, polyethylenepolyamines, N,N-dialkylaminoalkyl acrylates, and vinyl pyridine, amine salts, such as lauryl amine

acetate, stearyl amine acetate, alkylpyridinium salt, and alkylimidazolium salt, and amine oxides; imide azolinium salts; protonated quaternary acrylamides; methylated quaternary polymers, such as poly[diallyl dimethylammonium chloride] and poly-[N-methyl vinyl pyridinium chloride]; and cationic guar.

Such exemplary cationic surface stabilizers and other useful cationic surface stabilizers are described in J. Cross and E. Singer, *Cationic Surfactants: Analytical and Biological Evaluation* (Marcel Dekker, 1994); P. and D. Rubingh (Editor), *Cationic Surfactants: Physical Chemistry* (Marcel Dekker, 1991); and J. Richmond, *Cationic Surfactants: Organic Chemistry,* (Marcel Dekker, 1990).

Particularly preferred nonpolymeric primary stabilizers are any nonpolymeric compound, such benzalkonium chloride, a carbonium compound, a phosphonium compound, an oxonium compound, a halonium compound, a cationic organometallic compound, a quarternary phosphorous compound, a pyridinium compound, an anilinium compound, an immonium compound, a hydroxylammonium compound, a primary ammonium compound, a secondary ammonium compound, a tertiary ammonium compound, and quarternary ammonium compounds of the formula $NR_1R_2R_3R_4^{(+)}$. For compounds of the formula $NR_1R_2R_3R_4^{(+)}$:

(i) none of R_1-R_4 are CH_3;

(ii) one of R_1-R_4 is CH_3;

(iii) three of R_1-R_4 are CH_3;

(iv) all of R_1-R_4 are CH_3;

(v) two of R_1-R_4 are CH_3, one of R_1-R_4 is $C_6H_5CH_2$, and one of R_1-R_4 is an alkyl chain of seven carbon atoms or less;

(vi) two of R_1-R_4 are CH_3, one of R_1-R_4 is $C_6H_5CH_2$, and one of R_1-R_4 is an alkyl chain of nineteen carbon atoms or more;

(vii) two of R_1-R_4 are CH_3 and one of R_1-R_4 is the group $C_6H_5(CH_2)_n$, where $n>1$;

(viii) two of R_1-R_4 are CH_3, one of R_1-R_4 is $C_6H_5CH_2$, and one of R_1-R_4 comprises at least one heteroatom;

(ix) two of R_1-R_4 are CH_3, one of R_1-R_4 is $C_6H_5CH_2$, and one of R_1-R_4 comprises at least one halogen;

(x) two of R_1-R_4 are CH_3, one of R_1-R_4 is $C_6H_5CH_2$, and one of R_1-R_4 comprises at least one cyclic fragment;

(xi) two of R_1-R_4 are CH_3 and one of R_1-R_4 is a phenyl ring; or

(xii) two of R_1-R_4 are CH_3 and two of R_1-R_4 are purely aliphatic fragments.

Such compounds include, but are not limited to, behenalkonium chloride, benzethonium chloride, cetylpyridinium chloride, behentrimonium chloride, lauralkonium chloride, cetalkonium chloride, cetrimonium bromide, cetrimonium chloride, cethylamine hydrofluoride, chlorallylmethenamine chloride (Quaternium-15), distearyldimonium chloride (Quaternium-5), dodecyl dimethyl ethylbenzyl ammonium chloride(Quaternium-14), Quaternium-22, Quaternium-26, Quaternium-18 hectorite, dimethylaminoethylchloride hydrochloride, cysteine hydrochloride, diethanolammonium POE (10) oletyl ether phosphate, diethanolammonium POE (3)oleyl ether phosphate, tallow alkonium chloride, dimethyl dioctadecylammoniumbentonite, stearalkonium chloride, domiphen bromide, denatonium benzoate, myristalkonium chloride, laurtrimonium chloride, ethylenediamine dihydrochloride, guanidine hydrochloride, pyridoxine HCl, iofetamine hydrochloride, meglumine hydrochloride, methylbenzethonium chloride, myrtrimonium bromide, oleyltrimonium chloride, polyquaternium-1, procainehydrochloride, cocobetaine, stearalkonium bentonite, stearalkoniumhectonite, stearyl trihydroxyethyl propylenediamine dihydrofluoride, tallowtrimonium chloride, and hexadecyltrimethyl ammonium bromide.

Most of these surface stabilizers are known pharmaceutical excipients and are described in detail in the *Handbook of Pharmaceutical Excipients*, published jointly by the American Pharmaceutical Association and The Pharmaceutical Society of Great Britain (The Pharmaceutical Press, 1986), specifically incorporated by reference. The surface stabilizers are commercially available and/or can be prepared by techniques known in the art.

C. Active Agents

The nanoparticles of the invention comprise at least one active, therapeutic, or diagnostic agent, collectively referred to as a "drug." A therapeutic agent can be a pharmaceutical agent, including biologics such as proteins, peptides, and nucleotides, or a diagnostic agent, such as a contrast agent, including x-ray contrast agents.

The active agent exists as a crystalline phase, an amorphous phase, a semi-amorphous phase, a semi-crystalline phase, or mixtures thereof. The crystalline phase differs from a non-crystalline or amorphous phase which results from precipitation techniques, such as those described in EP Patent No. 275,796.

The invention can be practiced with a wide variety of active agents. The active agent is preferably present in an essentially pure form, is poorly soluble, and is dispersible in at least one liquid dispersion media. By "poorly soluble" it is meant that the active agent has a solubility in a liquid dispersion media of less than about 30 mg/mL, less than about 20 mg/mL, less than about 10 mg/mL, or less than about 1 mg/mL. Useful liquid dispersion medias include, but are not limited to, water, aqueous salt solutions, safflower oil, and solvents such as ethanol, t-butanol, hexane, and glycol. A preferred liquid dispersion media is water.

Two or more active agents can be used in combination.

1. Active Agents Generally

The active agent can be selected from a variety of known classes of drugs, including, for example, nutraceuticals, COX-2 inhibitors, retinoids, anticancer agents, NSAIDS, proteins, peptides, nucleotides, anti-obesity drugs, nutraceuticals, dietary supplements, carotenoids, corticosteroids, elastase inhibitors, anti-fungals, oncology therapies, anti-emetics, analgesics, cardiovascular agents, anti-inflammatory agents, anthelmintics, anti-arrhythmic agents, antibiotics (including penicillins), anticoagulants, antidepressants, antidiabetic agents, antiepileptics, antihistamines, antihypertensive agents, antimuscarinic agents, antimycobacterial agents, antineoplastic agents, immunosuppressants, antithyroid agents, antiviral agents,

anxiolytics, sedatives (hypnotics and neuroleptics), astringents, beta-adrenoceptor blocking agents, blood products and substitutes, cardiac inotropic agents, contrast media, corticosteroids, cough suppressants (expectorants and mucolytics), diagnostic agents, diagnostic imaging agents, diuretics, dopaminergics (antiparkinsonian agents), haemostatics, immunological agents, lipid regulating agents, muscle relaxants, parasympathomimetics, parathyroid calcitonin and biphosphonates, prostaglandins, radio- pharmaceuticals, sex hormones (including steroids), anti-allergic agents, stimulants and anoretics, sympathomimetics, thyroid agents, vasodilators, and xanthines.

Examples of representative active agents useful in this invention include, but are not limited to, acyclovir, alprazolam, altretamine, amiloride, amiodarone, benztropine mesylate, bupropion, cabergoline, candesartan, cerivastatin, chlorpromazine, ciprofloxacin, cisapride, clarithromycin, clonidine, clopidogrel, cyclobenzaprine, cyproheptadine, delavirdine, desmopressin, diltiazem, dipyridamole, dolasetron, enalapril maleate, enalaprilat, famotidine, felodipine, furazolidone, glipizide, irbesartan, ketoconazole, lansoprazole, loratadine, loxapine, mebendazole, mercaptopurine, milrinone lactate, minocycline, mitoxantrone, nelfinavir mesylate, nimodipine, norfloxacin, olanzapine, omeprazole, penciclovir, pimozide, tacolimus, quazepam, raloxifene, rifabutin, rifampin, risperidone, rizatriptan, saquinavir, sertraline, sildenafil, acetyl-sulfisoxazole, temazepam, thiabendazole, thioguanine, trandolapril, triamterene, trimetrexate, troglitazone, trovafloxacin, verapamil, vinblastine sulfate, mycophenolate, atovaquone, atovaquone, proguanil, ceftazidime, cefuroxime, etoposide, terbinafine, thalidomide, fluconazole, amsacrine, dacarbazine, teniposide, and acetylsalicylate.

Exemplary nutraceuticals and dietary supplements are disclosed, for example, in Roberts et al., *Nutraceuticals: The Complete Encyclopedia of Supplements, Herbs, Vitamins, and Healing Foods* (American Nutraceutical Association, 2001), which is specifically incorporated by reference. A nutraceutical or dietary supplement, also known as a phytochemical or functional food, is generally any one of a class of dietary supplements, vitamins, minerals, herbs, or healing foods that have medical or

pharmaceutical effects on the body. Exemplary nutraceuticals or dietary supplements include, but are not limited to, lutein, folic acid, fatty acids (*e.g.*, DHA and ARA), fruit and vegetable extracts, vitamin and mineral supplements, phosphatidylserine, lipoic acid, melatonin, glucosamine/chondroitin, Aloe Vera, Guggul, glutamine, amino acids (*e.g.*, iso-leucine, leucine, lysine, methionine, phenylanine, threonine, tryptophan, and valine), green tea, lycopene, whole foods, food additives, herbs, phytonutrients, antioxidants, flavonoid constituents of fruits, evening primrose oil, flax seeds, fish and marine animal oils, and probiotics. Nutraceuticals and dietary supplements also include bio-engineered foods genetically engineered to have a desired property, also known as "pharmafoods."

Active agents to be administered in an aerosol formulation are preferably selected from the group consisting of proteins, peptide, bronchodilators, corticosteroids, elastase inhibitors, analgesics, anti-fungals, cystic-fibrosis therapies, asthma therapies, emphysema therapies, respiratory distress syndrome therapies, chronic bronchitis therapies, chronic obstructive pulmonary disease therapies, organ-transplant rejection therapies, therapies for tuberculosis and other infections of the lung, fungal infection therapies, respiratory illness therapies associated with acquired immune deficiency syndrome, an oncology drug, an anti-emetic, an analgesic, and a cardiovascular agent.

2. Anticancer Active Agents

Useful anticancer agents are preferably selected from alkylating agents, antimetabolites, natural products, hormones and antagonists, and miscellaneous agents, such as radiosensitizers.

Examples of alkylating agents include: (1) alkylating agents having the bis-(2-chloroethyl)-amine group such as, for example, chlormethine, chlorambucile, melphalan, uramustine, mannomustine, extramustinephoshate, mechlore-thaminoxide, cyclophosphamide, ifosfamide, and trifosfamide; (2) alkylating agents having a substituted aziridine group such as, for example, tretamine, thiotepa, triaziquone, and mitomycine; (3) alkylating agents of the alkyl sulfonate type, such as, for example,

busulfan, piposulfan, and piposulfam; (4) alkylating N-alkyl-N-nitrosourea derivatives, such as, for example, carmustine, lomustine, semustine, or streptozotocine; and (5) alkylating agents of the mitobronitole, dacarbazine and procarbazine type.

Examples of antimetabolites include: (1) folic acid analogs, such as, for example, methotrexate; (2) pyrimidine analogs such as, for example, fluorouracil, floxuridine, tegafur, cytarabine, idoxuridine, and flucytosine; and (3) purine derivatives such as, for example, mercaptopurine, thioguanine, azathioprine, tiamiprine, vidarabine, pentostatin, and puromycine.

Examples of natural products include: (1) vinca alkaloids, such as, for example, vinblastine and vincristine; (2) epipodophylotoxins, such as, for example, etoposide and teniposide; (3) antibiotics, such as, for example, adriamycine, daunomycine, doctinomycin, daunorubicin, doxorubicin, mithramycin, bleomycin, and mitomycin; (4) enzymes, such as, for example, L-asparaginase; (5) biological response modifiers, such as, for example, alpha-interferon; (6) camptothecin; (7) taxol; and (8) retinoids, such as retinoic acid.

Examples of hormones and antagonists include: (1) adrenocorticosteroids, such as, for example, prednisone; (2) progestins, such as, for example, hydroxyprogesterone caproate, medroxyprogesterone acetate, and megestrol acetate; (3) estrogens, such as, for example, diethylstilbestrol and ethinyl estradiol; (4) antiestrogens, such as, for example, tamoxifen; (5) androgens, such as, for example, testosterone propionate and fluoxymesterone; (6) antiandrogens, such as, for example, flutamide; and (7) gonadotropin-releasing hormone analogs, such as, for example, leuprolide.

Examples of miscellaneous agents include: (1) radiosensitizers, such as, for example, 1,2,4-benzotriazin-3-amine 1,4-dioxide (SR 4889) and 1,2,4-benzotriazine-7-amine 1,4-dioxide (WIN 59075); (2) platinum coordination complexes such as cisplatin and carboplatin; (3) anthracenediones, such as, for example, mitoxantrone;

(4) substituted ureas, such as, for example, hydroxyurea; and (5) adrenocortical suppressants, such as, for example, mitotane and aminoglutethimide.

In addition, the anticancer agent can be an immunosuppressive drug, such as, for example, cyclosporine, azathioprine, sulfasalazine, methoxsalen, and thalidomide.

The anticancer agent can also be a COX-2 inhibitor.

3. Analgesic Active Agents

An analgesic can be, for example, an NSAID or a COX-2 inhibitor.

Exemplary NSAIDS that can be formulated in compositions of the invention include, but are not limited to, suitable nonacidic and acidic compounds. Suitable nonacidic compounds include, for example, nabumetone, tiaramide, proquazone, bufexamac, flumizole, epirazole, tinoridine, timegadine, and dapsone. Suitable acidic compounds include, for example, carboxylic acids and enolic acids. Suitable carboxylic acid NSAIDs include, for example: (1) salicylic acids and esters thereof, such as aspirin, diflunisal, benorylate, and fosfosal; (2) acetic acids, such as phenylacetic acids, including diclofenac, alclofenac, and fenclofenac; (3) carbo- and heterocyclic acetic acids such as etodolac, indomethacin, sulindac, tolmetin, fentiazac, and tilomisole; (4) propionic acids, such as carprofen, fenbufen, flurbiprofen, ketoprofen, oxaprozin, suprofen, tiaprofenic acid, ibuprofen, naproxen, fenoprofen, indoprofen, and pirprofen; and (5) fenamic acids, such as flufenamic, mefenamic, meclofenamic, and niflumic. Suitable enolic acid NSAIDs include, for example: (1) pyrazolones such as oxyphenbutazone, phenylbutazone, apazone, and feprazone; and (2) oxicams such as piroxicam, sudoxicam, isoxicam, and tenoxicam.

Exemplary COX-2 inhibitors that can be formulated in combination with the nanoparticulate nimesulide composition of the invention include, but are not limited to, celecoxib (SC-58635, CELEBREX®, Pharmacia/Searle & Co.), rofecoxib (MK-966, L-748731, VIOXX®, Merck & Co.), meloxicam (MOBIC®, co-marketed by Abbott Laboratories, Chicago, IL, and Boehringer Ingelheim Pharmaceuticals), valdecoxib (BEXTRA®, G.D. Searle & Co.), parecoxib (G.D. Searle & Co.),

etoricoxib (MK-663; Merck), SC-236 (chemical name of 4-[5-(4-chlorophenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl)] benzenesulfonamide; G.D. Searle & Co., Skokie, IL); NS-398 (N-(2-cyclohexyloxy-4-nitrophenyl)methane sulfonamide; Taisho Pharmaceutical Co., Ltd., Japan); SC-58125 (methyl sulfone spiro(2.4)hept-5-ene I; Pharmacia/Searle & Co.); SC-57666 (Pharmacia/Searle & Co.); SC-558 (Pharmacia/Searle & Co.); SC-560 (Pharmacia/Searle & Co.); etodolac (Lodine®, Wyeth-Ayerst Laboratories, Inc.); DFU (5,5-dimethyl-3-(3-fluorophenyl)-4-(4-methylsulfonyl)phenyl 2(5H)-furanone); monteleukast (MK-476), L-745337 ((5-methanesulphonamide-6-(2,4-difluorothio-phenyl)-1-indanone), L-761066, L-761000, L-748780 (all Merck & Co.); DUP-697 (5-Bromo-2-(4-fluorophenyl)-3-(4-(methylsulfonyl)phenyl; DuPont Merck Pharmaceutical Co.); PGV 20229 (1-(7-tert.-butyl-2,3-dihydro-3,3-dimethylbenzo(b)furan-5-yl)-4-cyclopropylbutan-1-one; Procter & Gamble Pharmaceuticals); iguratimod (T-614; 3-formylamino-7-methylsulfonylamino-6-phenoxy-4H-1- benzopyran-4-one; Toyama Corp., Japan); BF 389 (Biofor, USA); CL 1004 (PD 136095), PD 136005, PD 142893, PD 138387, and PD 145065 (all Parke-Davis/Warner-Lambert Co.); flurbiprofen (ANSAID®; Pharmacia & Upjohn); nabumetone (FELAFEN®; SmithKline Beecham, plc); flosulide (CGP 28238; Novartis/Ciba Geigy); piroxicam (FELDANE®; Pfizer); diclofenac (VOLTAREN® and CATAFLAM®, Novartis); lumiracoxib (COX-189; Novartis); D 1367 (Celltech Chiroscience, plc); R 807 (3 benzoyldifluoromethane sulfonanilide, diflumidone); JTE-522 (Japan Tobacco, Japan); FK-3311 (4'-Acetyl-2'-(2,4-difluorophenoxy)methanesulfonanilide), FK 867, FR 140423, and FR 115068 (all Fujisawa, Japan); GR 253035 (Glaxo Wellcome); RWJ 63556 (Johnson & Johnson); RWJ 20485 (Johnson & Johnson); ZK 38997 (Schering); S 2474 ((E)-(5)-(3,5-di-tert-butyl-4-hydroxybenzylidene)-2-ethyl-1,2-isothiazolidine-1,1-dioxide indomethacin; Shionogi & Co., Ltd., Japan); zomepirac analogs, such as RS 57067 and RS 104897 (Hoffmann La Roche); RS 104894 (Hoffmann La Roche); SC 41930 (Monsanto); pranlukast (SB 205312, Ono-1078, ONON®, ULTAIR®; SmithKline Beecham); SB 209670 (SmithKline Beecham); and APHS (heptinylsulfide).

D. Nanoparticulate Active Agent Particle Size

The compositions of the invention contain nanoparticulate active agent particles which have an effective average particle size of less than about 2000 nm (*i.e.*, 2 microns). In other embodiments of the invention, the active agent particles have a size of less than about 1900 nm, less than about 1800 nm, less than about 1700 nm, less than about 1600 nm, less than about 1500 nm, less than about 1400 nm, less than about 1300 nm, less than about 1200 nm, less than about 1100 nm, less than about 1000 nm, less than about 900 nm, less than about 800 nm, less than about 700 nm, less than about 600 nm, less than about 500 nm, less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 200 nm, less than about 150 nm, less than about 100 nm, less than about 75 nm, or less than about 50 nm, as measured by light-scattering methods, microscopy, or other appropriate methods.

By "an effective average particle size of less than about 2000 nm" it is meant that at least 50% by weight of the active agent particles have a particle size less than the effective average, *i.e.*, less than about 2000 nm, 1900 nm, 1800 nm, *etc.*, when measured by the above-noted techniques. In other embodiments of the invention, at least about 70%, at least about 90%, at least about 95%, or at least about 99% of the active agent particles have a particle size less than the effective average, *i.e.*, less than about 2000 nm, 1900 nm, 1800 nm, *etc.*

If the nanoparticulate active agent composition is combined with a conventional active agent composition, then such a composition is either solubilized or has an effective average particle size greater than about 2 microns. By "an effective average particle size of greater than about 2 microns" it is meant that at least 50% of the microparticulate active agent particles have a particle size greater than about 2 microns, by weight, when measured by the above-noted techniques. In other embodiments of the invention, at least about 70%, at least about 90%, at least about 95%, or at least about 99%, by weight, of the microparticulate active agent particles have a particle size greater than about 2 microns.

In the present invention, the value for D50 of a nanoparticulate active agent composition is the particle size below which 50% of the active agent particles fall, by weight. Similarly, D90 and D99 are the particle sizes below which 90% and 99%, respectively, of the active agent particles fall, by weight.

5. Concentration of Nanoparticulate Active Agent and Peptide Stabilizer

The relative amounts of active agent and peptide surface stabilizer, and optionally one or more secondary surface stabilizers, can vary widely. The optimal amount of the individual components can depend, for example, upon the particular active agent selected, the hydrophilic lipophilic balance (HLB), melting point, and the surface tension of water solutions of the stabilizer, *etc.*

The concentration of the peptide surface stabilizer can vary from about 0.5% to about 99.999%, from about 5.0% to about 99.9%, or from about 10% to about 99.5%, by weight, based on the total combined dry weight of the at least one active agent and at least one peptide surface stabilizer, not including other excipients.

The concentration of the at least one active agent can vary from about 99.5% to about 0.001%, from about 95% to about 0.1%, or from about 90% to about 0.5%, by weight, based on the total combined dry weight of the active agent and at least one peptide surface stabilizer, not including other excipients.

B. Methods of Making Nanoparticulate Active Agent Formulations

The nanoparticulate active agent compositions of the invention, comprising at least one peptide as a surface stabilizer, can be made using, for example, milling, homogenization, or precipitation techniques. Exemplary methods of making nanoparticulate compositions are described in the '684 patent. Methods of making nanoparticulate active agent compositions are also described in U.S. Patent No. 5,518,187 for "Method of Grinding Pharmaceutical Substances;" U.S. Patent No. 5,718,388 for "Continuous Method of Grinding Pharmaceutical Substances;" U.S. Patent No. 5,862,999 for "Method of Grinding Pharmaceutical Substances;" U.S.

Patent No. 5,665,331 for "Co-Microprecipitation of Nanoparticulate Pharmaceutical Agents with Crystal Growth Modifiers;" U.S. Patent No. 5,662,883 for "Co-Microprecipitation of Nanoparticulate Pharmaceutical Agents with Crystal Growth Modifiers;" U.S. Patent No. 5,560,932 for "Microprecipitation of Nanoparticulate Pharmaceutical Agents;" U.S. Patent No. 5,543,133 for "Process of Preparing X-Ray Contrast Compositions Containing Nanoparticles;" U.S. Patent No. 5,534,270 for "Method of Preparing Stable Drug Nanoparticles;" U.S. Patent No. 5,510,118 for "Process of Preparing Therapeutic Compositions Containing Nanoparticles;" and U.S. Patent No. 5,470,583 for "Method of Preparing Nanoparticle Compositions Containing Charged Phospholipids to Reduce Aggregation," all of which are specifically incorporated by reference.

The resultant nanoparticulate active agent compositions can be utilized in any desired dosage form.

1. Milling to obtain Nanoparticulate Active Agent Dispersions

Milling the active agent to obtain a nanoparticulate dispersion comprises dispersing active agent particles in a liquid dispersion media in which the active agent is poorly soluble, followed by applying mechanical means in the presence of grinding media to reduce the particle size of the active agent to the desired effective average particle size. The dispersion media can be, for example, water, safflower oil, ethanol, t-butanol, glycerin, polyethylene glycol (PEG), hexane, or glycol. Water is a preferred dispersion media.

The active agent particles are preferably reduced in size in the presence of at least one peptide surface stabilizer. Alternatively, the active agent particles can be contacted with at least one peptide surface stabilizer either during or after attrition. One or more secondary surface stabilizers may also be added before, during, or after attrition. Other compounds, such as a diluent, can be added to the active agent/peptide surface stabilizer composition before, during, or after the size reduction process. Dispersions can be manufactured continuously or in a batch mode.

2. Precipitation to Obtain Nanoparticulate Active Agent Compositions

Another method of forming the desired nanoparticulate active agent composition is by microprecipitation. This is a method of preparing stable dispersions of poorly soluble active agents in the presence of one or more peptide surface stabilizers and one or more colloid stability enhancing surface active agents free of any trace toxic solvents or solubilized heavy metal impurities. Such a method comprises, for example: (1) dissolving the poorly soluble active agent in a suitable solvent; (2) adding the formulation from step (1) to a solution comprising at least one peptide surface stabilizer and optionally one or more secondary surface stabilizers, to form a clear solution; and (3) precipitating the formulation from step (2) using an appropriate non-solvent. The method can be followed by removal of any formed salt, if present, by dialysis or diafiltration and concentration of the dispersion by conventional means.

3. Homogenization to Obtain Nanoparticulate Active Agent Compositions

Exemplary homogenization methods of preparing active agent nanoparticulate compositions are described in U.S. Patent No. 5,510,118, for "Process of Preparing Therapeutic Compositions Containing Nanoparticles."

Such a method comprises dispersing active agent particles in a liquid dispersion media in which the active agent is poorly soluble, followed by subjecting the dispersion to homogenization to reduce the particle size of the active agent to the desired effective average particle size. The active agent particles can be reduced in size in the presence of at least one peptide surface stabilizer and, if desired, one or more additional surface stabilizers. Alternatively, the active agent particles can be contacted with at least one peptide surface stabilizer and, if desired, one or more additional surface stabilizers, either during or after attrition. Other compounds, such as a diluent, can be added to the active agent/peptide surface stabilizer composition either before, during, or after the size reduction process. Dispersions can be manufactured continuously or in a batch mode.

C. Methods of Using Nanoparticulate Active Agent Formulations

The nanoparticulate active agent compositions of the present invention can be administered to humans and animals via any conventional means including, but not limited to, orally, rectally, ocularly, parenterally (intravenous, intramuscular, or subcutaneous), intracisternally, pulmonary, intravaginally, intraperitoneally, locally (powders, ointments or drops), or as a buccal or nasal spray.

Compositions suitable for parenteral injection may comprise physiologically acceptable sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions and sterile powders for reconstitution into sterile injectable solutions or dispersions. Examples of suitable aqueous and nonaqueous carriers, diluents, solvents, or vehicles including water, ethanol, polyols (propyleneglycol, polyethylene-glycol, glycerol, and the like), suitable mixtures thereof, vegetable oils (such as olive oil) and injectable organic esters such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

The nanoparticulate active agent compositions may also contain adjuvants such as preserving, wetting, emulsifying, and dispensing agents. Prevention of the growth of microorganisms can be ensured by various antibacterial and antifungal agents, such as parabens, chlorobutanol, phenol, sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, such as aluminum monostearate and gelatin.

Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active agent is admixed with at least one of the following: (a) one or more inert excipients (or carrier), such as sodium citrate or dicalcium phosphate; (b) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and silicic acid; (c) binders, such as carboxymethylcellulose, alignates, gelatin, polyvinylpyrrolidone, sucrose and acacia; (d) humectants, such as glycerol; (e) disintegrating agents, such as agar-agar, calcium

carbonate, potato or tapioca starch, alginic acid, certain complex silicates, and sodium carbonate; (f) solution retarders, such as paraffin; (g) absorption accelerators, such as quaternary ammonium compounds; (h) wetting agents, such as cetyl alcohol and glycerol monostearate; (i) adsorbents, such as kaolin and bentonite; and (j) lubricants, such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, or mixtures thereof. For capsules, tablets, and pills, the dosage forms may also comprise buffering agents.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs. In addition to the active agent, the liquid dosage forms may comprise inert diluents commonly used in the art, such as water or other solvents, solubilizing agents, and emulsifiers. Exemplary emulsifiers are ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propyleneglycol, 1,3-butyleneglycol, dimethylformamide, oils, such as cottonseed oil, groundnut oil, corn germ oil, olive oil, castor oil, and sesame oil, glycerol, tetrahydrofurfuryl alcohol, polyethyleneglycols, fatty acid esters of sorbitan, or mixtures of these substances, and the like.

Besides such inert diluents, the composition can also include adjuvants, such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents.

Actual dosage levels of active agent in the nanoparticulate compositions of the invention may be varied to obtain an amount of active agent that is effective to obtain a desired therapeutic response for a particular composition and method of administration. The selected dosage level therefore depends upon the desired therapeutic effect, the route of administration, the potency of the administered active agent, the desired duration of treatment, and other factors.

Dosage unit compositions may contain such amounts of such submultiples thereof as may be used to make up the daily dose. It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors

including the body weight, general health, sex, diet, time and route of administration, potency of the administered active agent, rates of absorption and excretion, combination with other active agents, and the severity of the particular disease being treated.

* * * * *

The following examples are given to illustrate the present invention. It should be understood, however, that the invention is not to be limited to the specific conditions or details described in these examples. Throughout the specification, any and all references to a publicly available document, including a U.S. patent, are specifically incorporated by reference.

The formulations in the examples that follow were also investigated using a light microscope. Here, "stable" nanoparticulate dispersions (uniform Brownian motion) were readily distinguishable from "aggregated" dispersions (relatively large, nonuniform particles without motion).

Example 1

The purpose of this example was to prepare a nanoparticulate nystatin composition having a peptide surface stabilizer.

Nystatin is a poorly water-soluble antimycotic polyene antibiotic obtained from *Streptomyces noursei.* It is an antifungal agent indicated for oral, gastrointestinal, and vaginal candidiasis. Oral candidiasis, in particular, is a common affliction of immunocompromised patients. Nystatin is indicated in the therapy of all infections caused by susceptible microorganisms in those patients in whom candidal (monilial) infections are most likely to complicate therapy.

A slurry of 2% (w/w) nystatin (Sigma-Aldrich Co.) and 1% (w/w) poly(Lysine, Tryptophan) 4:1 hydrobromide ("Poly(Lys,Trp)") (Sigma; St. Louris, MO), which is a cationic random co-polyamino acid having a molecular weight of 38,000, in water was

milled for 1 day using low energy (ball milling) techniques in the presence of ceramic YTZ grinding media.

The mean size of the nystatin particles following milling was 149 nm, with a D90 of 270 nm, as determined by static light scattering using a Horiba LA-910 light-scattering particle size analyzer (Horiba Instruments, Irvine, CA). The composition had a zeta potential of 47.7 mV, as measured by electrophoresis in $5x10^{-4}$ M NaCl (Malvern ZetaSizer). Dispersibility was verified by phase contrast microscopy.

Figure 1 shows representative photomicrographs of the nystatin crystals before (Fig. 1A) and after (Fig. 1B) milling.

Particle size stability under controlled conditions was monitored over time. Figure 2 shows the results of monitoring the nystatin particle size stability over time at 5°C (solid line), 25°C (dashed line), and 40°C (dotted line) for the nanoparticulate nystatin/peptide composition.

These results demonstrate that a peptide surface stabilizer can be successfully used to stabilize an active agent at a nanoparticulate particle size. Moreover, such a peptide surface stabilizer may confer additional therapeutic advantages to the final formulation. For example, the peptide surface stabilizer Poly(Lys,Trp) is cationic and, therefore, nanoparticulate active agent compositions utilizing this surface stabilizer will be bioadhesive.

The resultant composition exhibited a mean particle sizes of 149 nm and were free of agglomeration. Moreover, the nanoparticulate nystatin/peptide composition exhibited virtually no particle size growth at all three temperatures tested.

Example 2

The purpose of this example was to determine whether a cationic surface charge, such as that obtained with the use of a cationic peptide surface stabilizer, enhances the adhesion of small particles to cells.

Cell-binding experiments were performed with polystyrene latex microspheres as a model. A positive surface charge would be expected to enhance the interaction of particles with cell-surface macromolecules, which have a net negative charge.

Cationic microspheres with a mean zeta-potential (51.5 mV) comparable to the nanoparticulate nystatin/peptide composition of Example 1 were tested against anionic microspheres (mean zeta-potential = -50.9 mV). The microspheres were incubated with NIH/3T3 fibroblasts, washed thoroughly, fixed, and subjected to SEM analysis.

Figure 3 shows representative micrographs of cells with anionic particles (Fig. 3A) and cationic particles (Fig. 3B).

The results indicate that positively-charged particles interact more strongly with the cell surface than negatively-charged particles, and it is believed that nanoparticulate active agent compositions having a cationic peptide as a surface stabilizer with comparable zeta potentials will follow the same trend.

Example 3

The purpose of this example was to determine if milling of an active agent, such as nystatin, having a peptide surface stabilizer affects the active agent's activity.

The minimum inhibitory concentration (MIC) of a milled nystatin composition having as a peptide surface stabilizer Poly(Lys, Trp) was compared to the MIC of two unmilled nystatin compositions. Nystatin for the milled nanoparticulate composition was obtained from Sigma-Aldrich Co. and the two unmilled nystatin compositions were obtained from Sigma-Aldrich Co. and Paddock Laboratories, Inc. Details regarding the milled and unmilled nystatin compositions are given in Table 1 below, including particle size of the milled nanoparticulate nystatin/Poly(Lys, Trp) composition and the potency (USP U/ml) and MIC for each nystatin composition.

TABLE 1				
Nystatin Concentration	**Surface Stabilizer and Concentration**	**Mean Particle Size (nm)**	**Potency (USP U/ml)**	**MIC**
2% (Sigma)	1% Poly(Lys, Trp)[1]	129	101,200	1:10,000
5% (Sigma)	N/A – unmilled	N/A	253,000	1:10,000
4% (Paddock)	N/A – unmilled	N/A	253,000	1:100,000

[1]Poly(Lysine, Tryptophan) is a cationic random co-polyamino acid.

The nanoparticulate sample was ball milled for 26 hours with ceramic YTZ milling media.

The minimum inhibitory concentration (MIC) of the milled nystatin/peptide composition and the two unmilled samples were determined in cultures of *C. albicans*. MIC as reported here is the maximum dilution of formulation in culture broth which inhibits growth of *C albicans*. As shown in Table 1, above, the milled nystatin/peptide composition did not exhibit any significant differences in MIC, and surprisingly, was more active than at least one of the unmilled nystatin samples. These data confirm that the milling process does not decrease the activity of nystatin.

Example 4

The purpose of this example was to prepare a nanoparticulate composition of a diuretic, Compound A, utilizing a peptide surface stabilizer. Diuretics can be used to reduce the swelling and fluid retention caused by various medical problems, including heart or liver disease. They are also is used to treat high blood pressure.

A slurry of 2% (w/w) Compound A and 1% (w/w) poly(Lysine, Tryptophan) 4:1 hydrobromide as a peptide surface stabilizer in water was milled for 3 days in an aqueous environment in a low energy mill, in the presence of 0.8 mm yttrium-stabilized ceramic media.

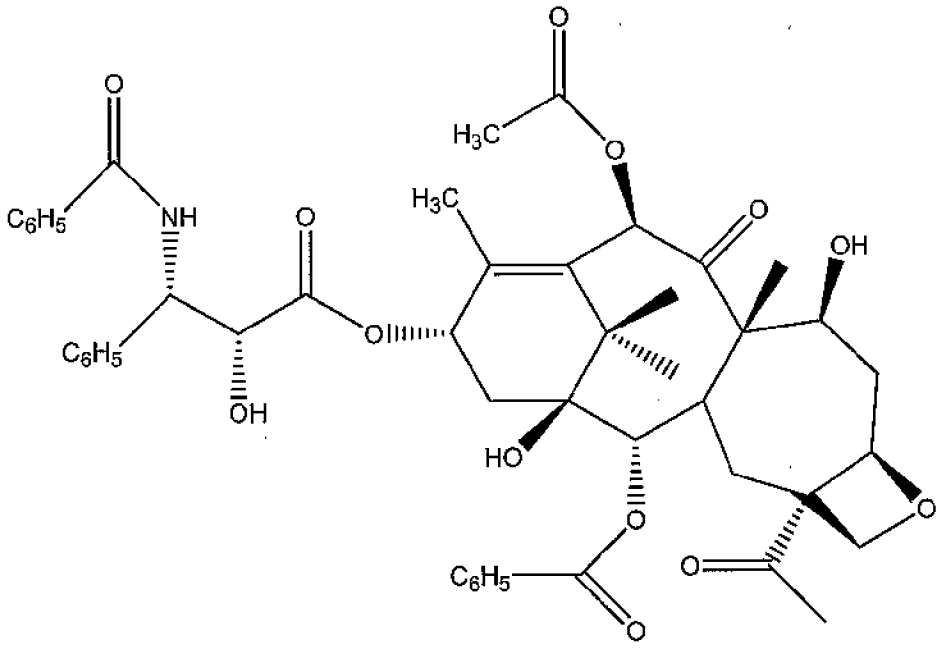
Particle size analysis of the resulting Compound A dispersion was conducted via laser light diffraction using the Horiba LA 910 particle size analyzer (Horiba Instruments, Irvine, CA) and water as a diluent. The mean particle size of the milled

Compound A dispersion was 99 nm, with a D90 of 138 nm. The composition was stable.

Example 5

The purpose of this example was to prepare a nanoparticulate composition of paclitaxel utilizing a peptide surface stabilizer. Paclitaxel belongs to the group of medicines called antineoplastics. It is used to treat cancer of the ovaries, breast, certain types of lung cancer, and a cancer of the skin and mucous membranes more commonly found in patients with acquired immunodeficiency syndrome (AIDS). It may also be used to treat other kinds of cancer.

Paclitaxel has the following chemical structure:



A slurry of 2% (w/w) paclitaxel and 1% (w/w) poly(Lysine, Tryptophan) 4:1 hydrobromide as a peptide surface stabilizer in water was milled for 3 days in an aqueous environment in a low energy mill, in the presence of 0.8 mm yttrium-stabilized ceramic media.

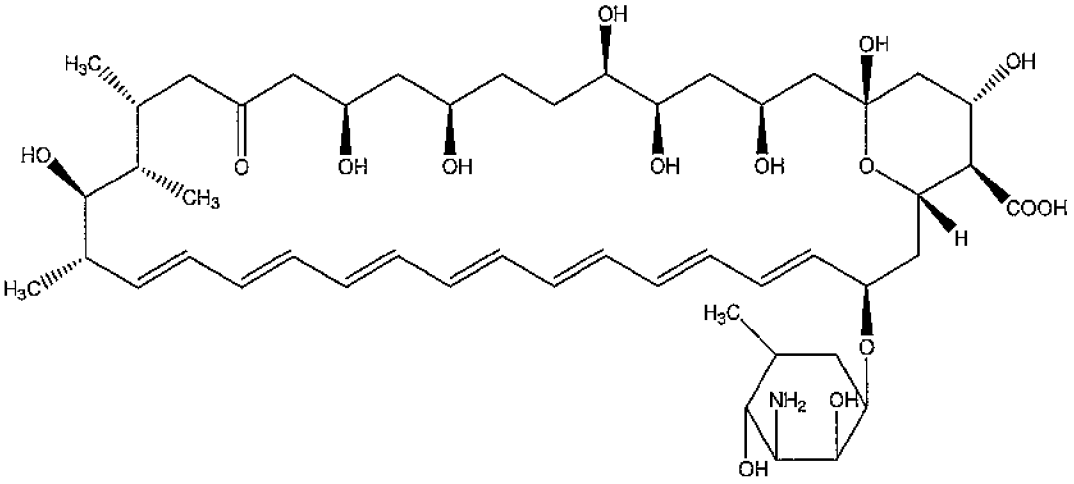
Particle size analysis of the resulting paclitaxel dispersion was conducted via laser light diffraction using the Horiba LA 910 particle size analyzer (Horiba Instruments, Irvine, CA) and water as a diluent. The mean particle size of the milled

paclitaxel dispersion was 139 nm, with a D90 of 185 nm. The composition was stable.

Example 6

The purpose of this example was to prepare a nanoparticulate composition of amphotericin B utilizing a peptide surface stabilizer. Amphotericin B is a poorly water soluble antifungal agent. Topically, it is used to treat skin yeast infections; intravenously, it is used to treat a variety of life-threatening fungal infections.

Amphotericin B has the following chemical structure:



In this experiment, amphotericin B was milled with Poly (Lys, Trp) 4:1 Hydrobromide as a peptide surface stabilizer. A 2% (w/w) slurry of amphotericin B (Sigma) in water was prepared with 1% (w/w) poly (Lys, Trp) (Sigma). The composition was ball-milled for 24 hours with 0.8 mm ceramic YTZ milling media. The particle size of the resulting amphotericin B dispersion was characterized by static laser light scattering on a Horiba LA-910 particle size distribution analyzer. The results are shown in Table 2, below.

TABLE 2

Drug and Concentration	Surface Stabilizer and Concentration	Mean Particle Size (nm)	D50 (nm)	D90 (nm)
2% Amphotericin B	1% Poly(Lys, Trp)	121	96	230

These results demonstrate that amphotericin B dispersions can be successfully stabilized by a peptide surface stabilizer, such as the random copolypeptide poly (Lys, Trp) 4:1 Hydrobromide.

* * * *

It will be apparent to those skilled in the art that various modifications and variations can be made in the methods and compositions of the present invention without departing from the spirit or scope of the invention. Thus, it is intended that the present invention cover the modifications and variations of this invention provided they come within the scope of the appended claims and their equivalents.

We claim:

1. A composition comprising:

(a) particles of at least one active agent having an effective average particle size of less than about 2000 nm; and

(b) at least one water soluble peptide surface stabilizer.

2. The composition of claim 1, wherein the peptide surface stabilizer is poly(Lysine, Tryptophan) 4:1 hydrobromide.

3. The composition of claim 1 or claim 2, further comprising at least one secondary surface stabilizer.

4. The composition of any one of claims 1 to 3, wherein the secondary surface stabilizer is selected from the group consisting of an anionic surface stabilizer, a cationic surface stabilizer, a zwitterionic surface stabilizer, and an ionic surface stabilizer.

5. The composition of claim 3 or claim 4, wherein the secondary surface stabilizer is selected from the group consisting of cetyl pyridinium chloride, gelatin, casein, phosphatides, dextran, glycerol, gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glycerol monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, polyethylene glycols, dodecyl trimethyl ammonium bromide, polyoxyethylene stearates, colloidal silicon dioxide, phosphates, sodium dodecylsulfate, carboxymethylcellulose calcium, hydroxypropyl celluloses, hypromellose, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hypromellose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol, polyvinylpyrrolidone, 4-(1,1,3,3-tetramethylbutyl)-

phenol polymer with ethylene oxide and formaldehyde, poloxamers; poloxamines, a charged phospholipid, dioctylsulfosuccinate, dialkylesters of sodium sulfosuccinic acid, sodium lauryl sulfate, alkyl aryl polyether sulfonates, mixtures of sucrose stearate and sucrose distearate, p-isononylphenoxypoly-(glycidol), decanoyl-N-methylglucamide; n-decyl β-D-glucopyranoside; n-decyl β-D-maltopyranoside; n-dodecyl β-D-glucopyranoside; n-dodecyl β-D-maltoside; heptanoyl-N-methylglucamide; n-heptyl-β-D-glucopyranoside; n-heptyl β-D-thioglucoside; n-hexyl β-D-glucopyranoside; nonanoyl-N-methylglucamide; n-noyl β-D-glucopyranoside; octanoyl-N-methylglucamide; n-octyl-β-D-glucopyranoside; octyl β-D-thioglucopyranoside; lysozyme, PEG-phospholipid, PEG-cholesterol, PEG-cholesterol derivative, PEG-vitamin A, and random copolymers of vinyl acetate and vinyl pyrrolidone, a cationic polymer, a cationic biopolymer, a cationic polysaccharide, a cationic cellulosic, a cationic alginate, a cationic nonpolymeric compound, a cationic phospholipids, cationic lipids, polymethylmethacrylate trimethylammonium bromide, sulfonium compounds, polyvinylpyrrolidone-2-dimethylaminoethyl methacrylate dimethyl sulfate, hexadecyltrimethyl ammonium bromide, phosphonium compounds, quarternary ammonium compounds, benzyl-di(2-chloroethyl)ethylammonium bromide, coconut trimethyl ammonium chloride, coconut trimethyl ammonium bromide, coconut methyl dihydroxyethyl ammonium chloride, coconut methyl dihydroxyethyl ammonium bromide, decyl triethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium chloride bromide, C_{12-15}dimethyl hydroxyethyl ammonium chloride, C_{12-15}dimethyl hydroxyethyl ammonium chloride bromide, coconut dimethyl hydroxyethyl ammonium chloride, coconut dimethyl hydroxyethyl ammonium bromide, myristyl trimethyl ammonium methyl sulphate, lauryl dimethyl benzyl ammonium chloride, lauryl dimethyl benzyl ammonium bromide, lauryl dimethyl $(ethenoxy)_4$ ammonium chloride, lauryl dimethyl $(ethenoxy)_4$ ammonium bromide, N-alkyl (C_{12-18})dimethylbenzyl ammonium chloride, N-alkyl (C_{14-18})dimethyl-benzyl ammonium chloride, N-tetradecylidmethylbenzyl ammonium chloride monohydrate, dimethyl didecyl ammonium chloride, N-alkyl and (C_{12-14}) dimethyl 1-napthylmethyl

ammonium chloride, trimethylammonium halide, alkyl-trimethylammonium salts, dialkyl-dimethylammonium salts, lauryl trimethyl ammonium chloride, ethoxylated alkyamidoalkyldialkylammonium salt, an ethoxylated trialkyl ammonium salt, dialkylbenzene dialkylammonium chloride, N-didecyldimethyl ammonium chloride, N-tetradecyldimethylbenzyl ammonium, chloride monohydrate, N-alkyl(C_{12-14}) dimethyl 1-naphthylmethyl ammonium chloride, dodecyldimethylbenzyl ammonium chloride, dialkyl benzenealkyl ammonium chloride, lauryl trimethyl ammonium chloride, alkylbenzyl methyl ammonium chloride, alkyl benzyl dimethyl ammonium bromide, C_{12} trimethyl ammonium bromides, C_{15} trimethyl ammonium bromides, C_{17} trimethyl ammonium bromides, dodecylbenzyl triethyl ammonium chloride, poly-diallyldimethylammonium chloride (DADMAC), dimethyl ammonium chlorides, alkyldimethylammonium halogenides, tricetyl methyl ammonium chloride, decyltrimethylammonium bromide, dodecyltriethylammonium bromide, tetradecyltrimethylammonium bromide, methyl trioctylammonium chloride, POLYQUAT 10™, tetrabutylammonium bromide, benzyl trimethylammonium bromide, choline esters, benzalkonium chloride, stearalkonium chloride compounds, cetyl pyridinium bromide, cetyl pyridinium chloride, halide salts of quaternized polyoxyethylalkylamines, MIRAPOL™, ALKAQUAT™, alkyl pyridinium salts; amines, amine salts, amine oxides, imide azolinium salts, protonated quaternary acrylamides, methylated quaternary polymers, and cationic guar.

6. The composition of any one of claims 1 to 5, wherein the composition is formulated for administration selected from the group consisting of oral, pulmonary, rectal, opthalmic, colonic, parenteral, intracisternal, intravaginal, intraperitoneal, local, buccal, nasal, and topical administration.

7. The composition of any one of claims 1 to 6 formulated into a dosage form selected from the group consisting of liquid dispersions, oral suspensions, gels, aerosols, ointments, creams, tablets, capsules, sachets, lozenges, powders, pills, granules, controlled release formulations, fast melt formulations, lyophilized

formulations, delayed release formulations, extended release formulations, pulsatile release formulations, and mixed immediate release and controlled release formulations.

8. The composition of any one of claims 1 to 7, wherein:

(a) the active agent is present in an amount selected from the group consisting of from about 99.5% to about 0.001%, from about 95% to about 0.1%, and from about 90% to about 0.5%, by weight, based on the total combined dry weight of the active agent and at least one peptide surface stabilizer, not including other excipients; or

(b) the at least one surface stabilizer is present in an amount selected from the group consisting of from about 0.5% to about 99.999% by weight, from about 5.0% to about 99.9% by weight, and from about 10% to about 99.5% by weight, based on the total combined dry weight of the active agent and at least one peptide surface stabilizer, not including other excipients.

9. The composition of any one of claims 1 to 8, wherein the active agent is selected from the group consisting of a crystalline phase, an amorphous phase, a semi-crystalline phase, a semi-amorphous phase, and mixtures thereof.

10. The composition of any one of claims 1 to 10, wherein the effective average particle size of the active agent particles is selected from the group consisting of less than about 1900 nm, less than about 1800 nm, less than about 1700 nm, less than about 1600 nm, less than about 1500 nm, less than about 1400 nm, less than about 1300 nm, less than about 1200 nm, less than about 1100 nm, less than about 1000 nm, less than about 900 nm, less than about 800 nm, less than about 700 nm, less than about 600 nm, less than about 500 nm, less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 200 nm, less than about 100 nm, less than about 75 nm, and less than about 50 nm.

11. The composition of any one of claims 1 to 10, wherein at least about 70%, at least about 90%, at least about 95%, or at least about 99% of the active agent particles have a particle size less than the effective average particle size.

12. The composition of any one of claims 1 to 11, further comprising at least one additional active agent composition having an effective average particle size which is different that the effective average particle size of the active agent composition of claim 1.

13. The composition of any one of claims 1 to 12, wherein the active agent is selected from the group consisting of nystatin, paclitaxel, amphotericin B, a diuretic, a dermal agent, nutraceuticals, COX-2 inhibitors, retinoids, anticancer agents, NSAIDS, proteins, peptides, nucleotides, anti-obesity drugs, dietary supplements, carotenoids, corticosteroids, elastase inhibitors, anti-fungals, oncology therapies, anti-emetics, analgesics, cardiovascular agents, anti-inflammatory agents, anthelmintics, anti-arrhythmic agents, antibiotics, anticoagulants, antidepressants, antidiabetic agents, antiepileptics, antihistamines, antihypertensive agents, antimuscarinic agents, antimycobacterial agents, antineoplastic agents, immunosuppressants, antithyroid agents, antiviral agents, anxiolytics, sedatives, astringents, beta-adrenoceptor blocking agents, blood products and substitutes, cardiac inotropic agents, contrast media, corticosteroids, cough suppressants, diagnostic agents, diagnostic imaging agents, diuretics, dopaminergics, haemostatics, immunological agents, lipid regulating agents, muscle relaxants, parasympathomimetics, parathyroid calcitonin and biphosphonates, prostaglandins, radio-pharmaceuticals, sex hormones, anti-allergic agents, stimulants, anoretics, sympathomimetics, thyroid agents, vasodilators, xanthines, acyclovir, alprazolam, altretamine, amiloride, amiodarone, benztropine mesylate, bupropion, cabergoline, candesartan, cerivastatin, chlorpromazine, ciprofloxacin, cisapride, clarithromycin, clonidine, clopidogrel, cyclobenzaprine, cyproheptadine, delavirdine, desmopressin, diltiazem, dipyridamole, dolasetron, enalapril maleate, enalaprilat, famotidine, felodipine, furazolidone, glipizide, irbesartan, ketoconazole, lansoprazole,

loratadine, loxapine, mebendazole, mercaptopurine, milrinone lactate, minocycline, mitoxantrone, nelfinavir mesylate, nimodipine, norfloxacin, olanzapine, omeprazole, penciclovir, pimozide, tacolimus, quazepam, raloxifene, rifabutin, rifampin, risperidone, rizatriptan, saquinavir, sertraline, sildenafil, acetyl-sulfisoxazole, temazepam, thiabendazole, thioguanine, trandolapril, triamterene, trimetrexate, troglitazone, trovafloxacin, verapamil, vinblastine sulfate, mycophenolate, atovaquone, atovaquone, proguanil, ceftazidime, cefuroxime, etoposide, terbinafine, thalidomide, fluconazole, amsacrine, dacarbazine, teniposide, and acetylsalicylate.

14. The composition of claim 13, wherein the nutraceutical is selected from the group consisting of dietary supplements, vitamins, minerals, herbs, lutein, folic acid, fatty acids, fruit extracts, vegetable extracts, phosphatidylserine, lipoic acid, melatonin, glucosamine/chondroitin, Aloe Vera, Guggul, glutamine, amino acids, green tea, lycopene, whole foods, food additives, herbs, phytonutrients, antioxidants, flavonoid constituents of fruits, evening primrose oil, flax seeds, fish oils, marine animal oils, and probiotics.

15. The composition of claim 13, wherein the anticancer agent is selected from the group consisting of alkylating agents, antimetabolites, anthracenediones, natural products, hormones, antagonists, radiosensitizers, platinum coordination complexes, adrenocortical suppressants, immunosuppressive agent, substituted ureas, and COX-2 inhibitors.

16. The composition of claim 15, wherein:

(a) the alkylating agent is selected from the group consisting of chlormethine, chlorambucile, melphalan, uramustine, mannomustine, extramustinephoshate, mechlore-thaminoxide, cyclophosphamide, ifosfamide, trifosfamide, tretamine, thiotepa, triaziquone, mitomycine, busulfan, piposulfan, piposulfam, carmustine, lomustine, semustine, streptozotocine, mitobronitole, dacarbazine and procarbazinc; or

(b) the antimetabolite is selected from the group consisting of methotrexate, fluorouracil, floxuridine, tegafur, cytarabine, idoxuridine, flucytosine, mercaptopurine, thioguanine, azathioprine, tiamiprine, vidarabine, pentostatin, and puromycine; or

(c) the natural product is selected from the group consisting of vinblastine, vincristine, etoposide, teniposide, adriamycine, daunomycine, doctinomycin, daunorubicin, doxorubicin, mithramycin, bleomycin, mitomycin, L-asparaginase, alpha-interferon, camptothecin, taxol, and retinoic acid; or

(d) the hormone or antagonist is selected from the group consisting of prednisone, hydroxyprogesterone caproate, medroxyprogesterone acetate, megestrol acetate, diethylstilbestrol, ethinyl estradiol, tamoxifen, testosterone propionate, fluoxymesterone, flutamide, leuprolide; or

(e) the anticancer agent is selected from the group consisting of cisplatin, carboplatin, mitoxantrone, hydroxyurea, mitotane, aminoglutethimide, cyclosporine, azathioprine, sulfasalazine, methoxsalen, and thalidomide.

17. The composition of claim 13, wherein the NSAID is selected from the group consisting of nabumetone, tiaramide, proquazone, bufexamac, flumizole, epirazole, tinoridine, timegadine, dapsone, aspirin, diflunisal, benorylate, fosfosal, diclofenac, alclofenac, fenclofenac, etodolac, indomethacin, sulindac, tolmetin, fentiazac, tilomisole, carprofen, fenbufen, flurbiprofen, ketoprofen, oxaprozin, suprofen, tiaprofenic acid, ibuprofen, naproxen, fenoprofen, indoprofen, pirprofen, flufenamic, mefenamic, meclofenamic, niflumic, oxyphenbutazone, phenylbutazone, apazone, feprazone, piroxicam, sudoxicam, isoxicam, and tenoxicam.

18. The composition of claim 13, wherein the COX-2 inhibitor is selected from the group consisting of nimesulide, celecoxib, rofecoxib, meloxicam, valdecoxib, parecoxib, etoricoxib, flurbiprofen, nabumetone, etodolac, iguratimod, flosulide, piroxicam, diclofenac, lumiracoxib, monteleukast, pranlukast, heptinylsulfide, SC-236, SC-58125, SC-57666, SC-558, SC-560, SC 41930, NS-398, DFU, L-745337, L-

761066, L-761000, L-748780, DUP-697, PGV 20229, BF 389, CL 1004, PD 136005, PD 142893, PD 138387, PD 145065, D 1367, R 807, JTE-522, FK-3311, FK 867, FR 140423, FR 115068, GR 253035, RWJ 63556, RWJ 20485, ZK 38997, S 2474, RS 57067, RS 104897, RS 104894, and SB 209670.

19. The composition of any one of claims 1 to 18, wherein upon administration to a mammal the active agent particles redisperse such that the particles have an effective average particle size selected from the group consisting of less than about 2 microns, less than about 1900 nm, less than about 1800 nm, less than about 1700 nm, less than about 1600 nm, less than about 1500 nm, less than about 1400 nm, less than about 1300 nm, less than about 1200 nm, less than about 1100 nm, less than about 1000 nm, less than about 900 nm, less than about 800 nm, less than about 700 nm, less than about 600 nm, less than about 500 nm, less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 200 nm, less than about 150 nm, less than about 100 nm, less than about 75 nm, and less than about 50 nm.

20. The composition of any one of claims 1 to 19, wherein the composition redisperses in a biorelevant media such that the active agent particles have an effective average particle size selected from the group consisting of less than about 2 microns, less than about 1900 nm, less than about 1800 nm, less than about 1700 nm, less than about 1600 nm, less than about 1500 nm, less than about 1400 nm, less than about 1300 nm, less than about 1200 nm, less than about 1100 nm, less than about 1000 nm, less than about 900 nm, less than about 800 nm, less than about 700 nm, less than about 600 nm, less than about 500 nm, less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 200 nm, less than about 150 nm, less than about 100 nm, less than about 75 nm, and less than about 50 nm.

21. The composition of claim 20, wherein the biorelevant media is selected from the group consisting of water, aqueous electrolyte solutions, aqueous solutions of a

salt, aqueous solutions of an acid, aqueous solutions of a base, and combinations thereof.

22. The composition of any one of claims 1 to 21, wherein:

(a) the T_{max} of the active agent, when assayed in the plasma of a mammalian subject following administration, is less than the T_{max} for a non-nanoparticulate composition of the same active agent, administered at the same dosage; or

(b) the C_{max} of the active agent, when assayed in the plasma of a mammalian subject following administration, is greater than the C_{max} for a non-nanoparticulate composition of the same active agent, administered at the same dosage; or

(c) the AUC of the active agent, when assayed in the plasma of a mammalian subject following administration, is greater than the AUC for a non-nanoparticulate composition of the same active agent, administered at the same dosage.

23. The composition of claim 22, wherein the T_{max} is selected from the group consisting of not greater than about 90%, not greater than about 80%, not greater than about 70%, not greater than about 60%, not greater than about 50%, not greater than about 30%, not greater than about 25%, not greater than about 20%, not greater than about 15%, not greater than about 10%, and not greater than about 5% of the T_{max} exhibited by a non-nanoparticulate composition of the same active agent, administered at the same dosage.

24. The composition of claim 22, wherein the C_{max} is selected from the group consisting of at least about 50%, at least about 100%, at least about 200%, at least about 300%, at least about 400%, at least about 500%, at least about 600%, at least about 700%, at least about 800%, at least about 900%, at least about 1000%, at least about 1100%, at least about 1200%, at least about 1300%, at least about 1400%, at

least about 1500%, at least about 1600%, at least about 1700%, at least about 1800%, or at least about 1900% greater than the C_{max} exhibited by a non-nanoparticulate composition of the same active agent, administered at the same dosage.

25. The composition of claim 22, wherein the AUC is selected from the group consisting of at least about 25%, at least about 50%, at least about 75%, at least about 100%, at least about 125%, at least about 150%, at least about 175%, at least about 200%, at least about 225%, at least about 250%, at least about 275%, at least about 300%, at least about 350%, at least about 400%, at least about 450%, at least about 500%, at least about 550%, at least about 600%, at least about 750%, at least about 700%, at least about 750%, at least about 800%, at least about 850%, at least about 900%, at least about 950%, at least about 1000%, at least about 1050%, at least about 1100%, at least about 1150%, or at least about 1200% greater than the AUC exhibited by the non-nanoparticulate composition of the same active agent, administered at the same dosage.

26. The composition of any one of claims 1 to 25 which does not produce significantly different absorption levels when administered under fed as compared to fasting conditions.

27. The composition of claim 26, wherein the difference in absorption of the active agent composition of the invention, when administered in the fed versus the fasted state, is selected from the group consisting of less than about 100%, less than about 90%, less than about 80%, less than about 70%, less than about 60%, less than about 50%, less than about 40%, less than about 30%, less than about 25%, less than about 20%, less than about 15%, less than about 10%, less than about 5%, and less than about 3%.

28. The composition of any one of claims 1 to 27, wherein administration of the composition to a human in a fasted state is bioequivalent to administration of the composition to a subject in a fed state.

29. The composition of claim 28, wherein "bioequivalency" is established by:

(a) a 90% Confidence Interval of between 0.80 and 1.25 for both C_{max} and AUC; or

(b) a 90% Confidence Interval of between 0.80 and 1.25 for AUC and a 90% Confidence Interval of between 0.70 to 1.43 for C_{max}.

30. The composition of any one of claims 1 to 29, formulated into a liquid dosage form and having a viscosity at a shear rate of 0.1 (1/s), measured at 20°C, selected from the group consisting of less than about 2000 mPa·s, from about 2000 mPa·s to about 1 mPa·s, from about 1900 mPa·s to about 1 mPa·s, from about 1800 mPa·s to about 1 mPa·s, from about 1700 mPa·s to about 1 mPa·s, from about 1600 mPa·s to about 1 mPa·s, from about 1500 mPa·s to about 1 mPa·s, from about 1400 mPa·s to about 1 mPa·s, from about 1300 mPa·s to about 1 mPa·s, from about 1200 mPa·s to about 1 mPa·s, from about 1100 mPa·s to about 1 mPa·s, from about 1000 mPa·s to about 1 mPa·s, from about 900 mPa·s to about 1 mPa·s, from about 800 mPa·s to about 1 mPa·s, from about 700 mPa·s to about 1 mPa·s, from about 600 mPa·s to about 1 mPa·s, from about 500 mPa·s to about 1 mPa·s, from about 400 mPa·s to about 1 mPa·s, from about 300 mPa·s to about 1 mPa·s, from about 200 mPa·s to about 1 mPa·s, from about 175 mPa·s to about 1 mPa·s, from about 150 mPa·s to about 1 mPa·s, from about 125 mPa·s to about 1 mPa·s, from about 100 mPa·s to about 1 mPa·s, from about 75 mPa·s to about 1 mPa·s, from about 50 mPa·s to about 1 mPa·s, from about 25 mPa·s to about 1 mPa·s, from about 15 mPa·s to about 1 mPa·s, from about 10 mPa·s to about 1 mPa·s, and from about 5 mPa·s to about 1 mPa·s.

31. The composition of claim 30, wherein the viscosity of the dosage form is:

(a) selected from the group consisting of less than about 1/200, less than

about 1/100, less than about 1/50, less than about 1/25, and less than about 1/10 of the viscosity of a liquid dosage form of a non-nanoparticulate composition of the same active agent, at about the same concentration per ml of active agent; or

(b) selected from the group consisting of less than about 5%, less than about 10%, less than about 15%, less than about 20%, less than about 25%, less than about 30%, less than about 35%, less than about 40%, less than about 45%, less than about 50%, less than about 55%, less than about 60%, less than about 65%, less than about 70%, less than about 75%, less than about 80%, less than about 85%, and less than about 90% of the viscosity of a liquid dosage form of a non-nanoparticulate composition of the same active agent, at about the same concentration per ml of active agent.

32. The composition of any one of claims 1 to 31, further comprising one or more pharmaceutically acceptable excipients, carriers, or a combination thereof.

33. The composition according to any one of claims 1 to 32, wherein the composition is bioadhesive.

34. The use of a composition according to any one of claims 1 to 33 for the manufacture of a pharmaceutical medicament.

35. A method of making a composition according to any one of claims 1 to 33, comprising contacting particles of at least one active agent with at least one water-soluble peptide surface stabilizer for a time and under conditions sufficient to provide an active agent composition having an effective average particle size of less than about 2000 nm.

FIGURE 1

A B

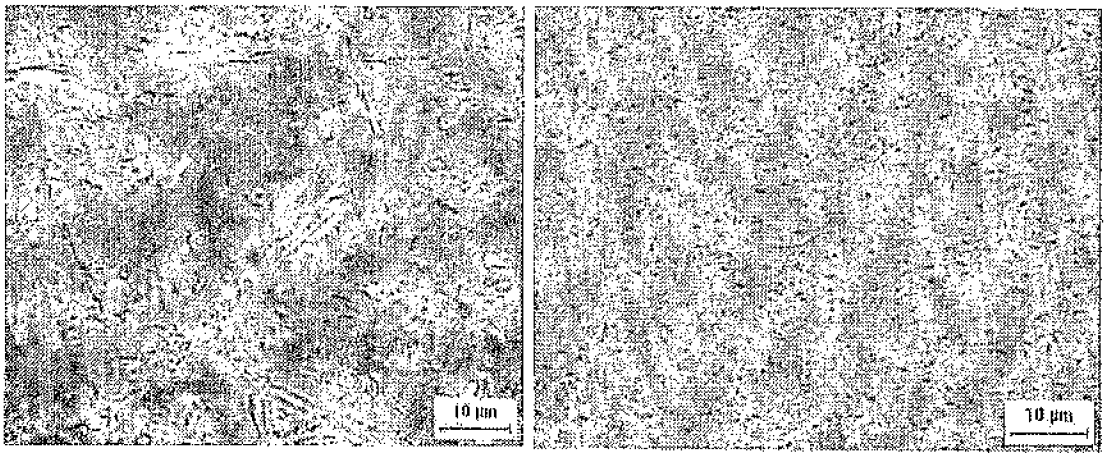


FIGURE 2

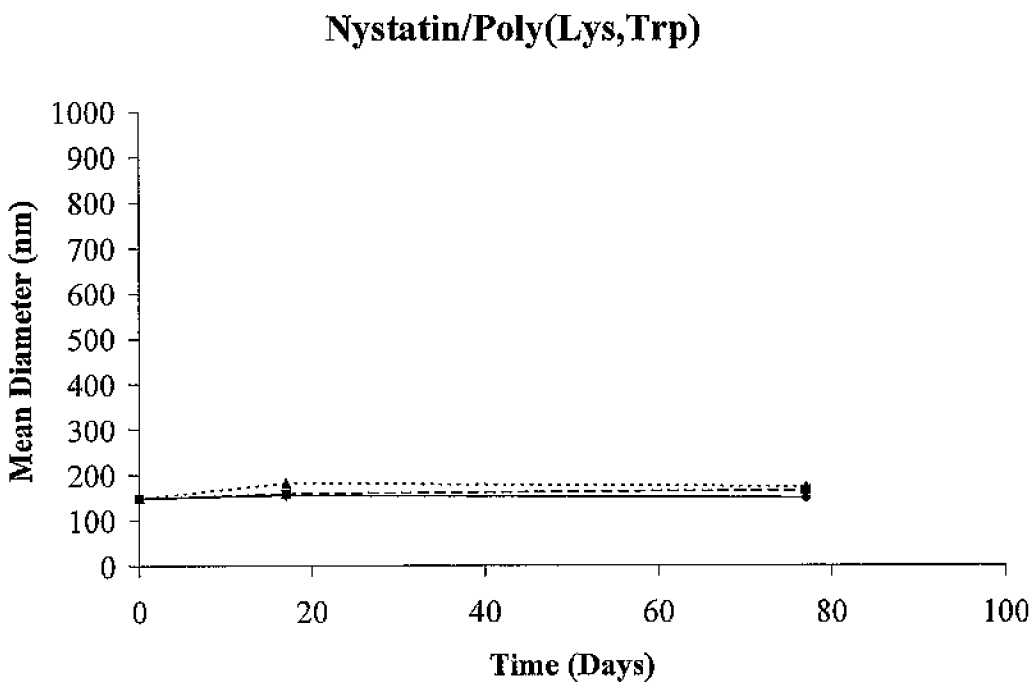
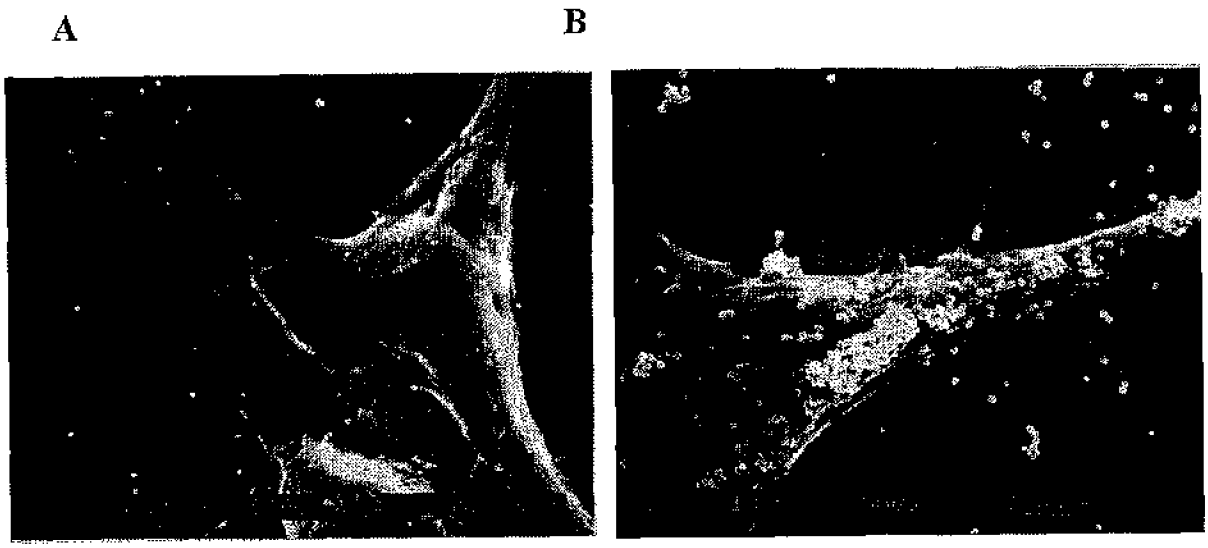

Nystatin/Poly(Lys,Trp)
Mean Diameter (nm)
1000
900
800
700
600
500
400
300
200
100
0
0
20
40
60
80
100
Time (Days)

FIGURE 3



PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : **C07C 323/62, 323/60, C07D 333/34 C07C 327/32, 317/50, 313/48 A61K 31/13, 31/38**	**A1**	**(11) International Publication Number:** **WO 90/05719** **(43) International Publication Date:** 31 May 1990 (31.05.90)

(21) International Application Number: PCT/GB89/01399

(22) International Filing Date: 23 November 1989 (23.11.89)

(30) Priority data:
8827305.7 23 November 1988 (23.11.88) GB

(71) Applicant *(for all designated States except US):* BRITISH BIO-TECHNOLOGY LIMITED [GB/GB]; Watlington Road, Cowley, Oxford OX4 5LY (GB).

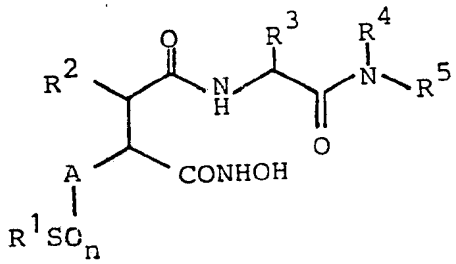
(72) Inventors; and
(75) Inventors/Applicants *(for US only) :* CAMPION, Colin [GB/GB]; 3 Howe Close, Wheatley, Oxon OX4 5LY (GB). DAVIDSON, Alan, Hornsby [GB/GB]; 27 Newland Mill, Witney, Oxon OX8 6HH (GB). DICKENS, Jonathan, Philip [GB/GB]; Burton House, Park Farm Road, High Wycombe, Bucks HP12 4AF (GB). CRIMMIN, Michael, John [GB/GB]; Oaklea, 64 Fernbank Road, Ascot SL5 8HE (GB).

(74) Agents: SHEARD, Andrew, Gregory et al.; Kilburn & Strode, 30 John Street, London WC1N 2DD (GB).

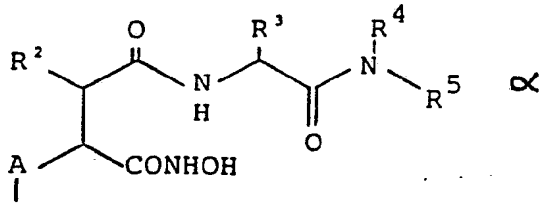
(81) Designated States: AT (European patent), AU, BE (European patent), CH (European patent), DE (European patent), DK, ES (European patent), FI, FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), NO, SE (European patent), US.

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: HYDROXAMIC ACID BASED COLLAGENASE INHIBITORS



(I)



(57) Abstract

Compounds of general formula (I), wherein R^1 represents hydrogen or an alkyl, phenyl, thiophenyl, substituted phenyl, phenylalkyl, heterocyclyl, alkylcarbonyl phenacyl or substituted phenacyl group; or, when n = 0, R^1 represents SR^X, wherein R^X represents a group (α); R^2 represents a hydrogen atom or an alkyl, alkenyl, phenylalkyl, cycloalkylalkyl or cycloalkenylalkyl group; R^3 represents an amino acid residue with R or S stereochemistry or an alkyl, benzyl, (C_1-C_6 alkoxy) benzyl or benzyloxy(C_1-C_6 alkyl) group; R^4 represents a hydrogen atom or an alkyl group; R^5 represents a hydrogen atom or a methyl group; n is an integer having the value 0, 1 or 2; and A represents a hydrocarbon chain optionally substituted with one or more alkyl, phenyl or substituted phenyl groups; and their salts and N-oxides are collagenase inhibitors and are useful in the management of disease involving tissue degradation and/or the promotion of wound healing. Diseases involving tissue degradation include arthropathy (particularly rheumatoid arthritis), inflammation, dermatological diseases, bone resorption diseases and tumour invasion.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	ES	Spain	MG	Madagascar
AU	Australia	FI	Finland	ML	Mali
BB	Barbados	FR	France	MR	Mauritania
BE	Belgium	GA	Gabon	MW	Malawi
BF	Burkina Faso	GB	United Kingdom	NL	Netherlands
BG	Bulgaria	HU	Hungary	NO	Norway
BJ	Benin	IT	Italy	RO	Romania
BR	Brazil	JP	Japan	SD	Sudan
CA	Canada	KP	Democratic People's Republic of Korea	SE	Sweden
CF	Central African Republic			SN	Senegal
CG	Congo	KR	Republic of Korea	SU	Soviet Union
CH	Switzerland	LI	Liechtenstein	TD	Chad
CM	Cameroon	LK	Sri Lanka	TG	Togo
DE	Germany, Federal Republic of	LU	Luxembourg	US	United States of America
DK	Denmark	MC	Monaco		

HYDROXAMIC ACID BASED COLLAGENASE INHIBITORS.

This invention relates to pharmaceutically and veterinarily active compounds, which are derivatives of hydroxamic acid.

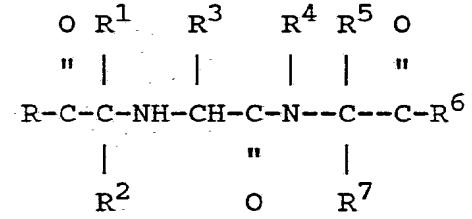
The compounds of the present invention act as inhibitors of metalloproteases involved in tissue degradation, such as collagenase, which initiates collagen breakdown, stromelysin (protoglycanase), gelatinase and collagenase (IV). There is evidence implicating collagenase as one of the key enzymes in the breakdown of articular cartilage and bone in rheumatoid arthritis (Arthritis and Rheumatism, **20**, 1231 - 1239, 1977). Potent inhibitors of collagenase and other metalloproteases involved in tissue degradation are useful in the treatment of rheumatoid arthritis and related diseases in which collagenolytic activity is important. Inhibitors of metalloproteases of this type can therefore be used in treating or preventing conditions which involve tissue breakdown; they are therefore useful in the treatment of arthropathy, dermatological conditions, bone resorption, inflammatory diseases and tumour invasion and in the promotion of wound healing. Specifically, compounds of the present invention may be useful in the treatment of osteopenias such as osteoporosis, rheumatoid arthritis, osteoarthritis, periodontitis, gingivitis, corneal ulceration and tumour invasion.

A number of small peptide like compounds which inhibit metalloproteases have been described. Perhaps the most notable of these are those relating to the

angiotensin converting enzyme (ACE) where such agents act to block the conversion of the decapeptide angiotensin I to angiotensin II a potent pressor substance. Compounds of this type are described in EP-A-0012401.

Certain hydroxamic acids have been suggested as collagenase inhibitors as in US-A-4599361 and EP-A-0236872. Other hydroxamic acids have been prepared as ACE inhibitors, for example in US-A-4105789, while still others have been described as enkephalinase inhibitors as in US-A-4496540.

EP-A-0012401 discloses antihypertensive compounds of the formula:



wherein

R and R^6 are the same or different and are hydroxy, alkoxy, alkenoxy, dialkylamino alkoxy, acylamino alkoxy, acyloxy alkoxy, aryloxy, alkyloxy, substituted aryloxy or substituted aralkoxy wherein the substituent is methyl, halo, or methoxy, amino, alkylamino, dialkylamino, aralkylamino or hydroxyamino;

R^1 is hydrogen, alkyl of from 1 to 20 carbon atoms, including branched, cyclic and unsaturated alkyl groups;

substituted alkyl wherein the substituent is halo, hydroxy, alkoxy, aryloxy amino, alkylamino, dialkylamino, acrylamino, arylamino, guanidino, imidazolyl, indolyl, mercapto, alkylthio, arylthio, carboxy, carboxamido, carbalkoxy, phenyl, substituted phenyl wherein the substituent is alkyl, alkoxy or halo; aralkyl or heteroaralkyl, aralkenyl or heteroaralkenyl, substituted aralkyl, substituted heteroaralkyl, substituted aralkenyl or substituted hetereoaralkenyl, wherein the substituent is halor or dihalo, alkyl, hydroxy, alkoxy, amino, aminomethyl, acrylamino, dialkylamino, alkylamino, carboxyl, haloalkyl, cyano or sulphonamido, aralkyl or hetereoaralkyl substituted on the alkyl portion by amino or acylamino;

R^2 and R^7 are hydrogen or alkyl;

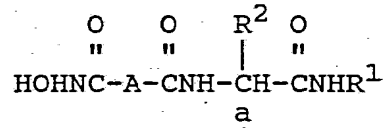
R^3 is hydrogen, alkyl, phenylalkyl, aminomethylphenylalkyl, hydroxyphenylalkyl, hydroxyalkyl, acetylaminoalkyl, acylaminoalkyl, acylaminoalkyl aminoalkyl, dimethylaminoalkyl, haloalkyl, guanidinoalkyl, imidazolylalkyl, indolylalkyl, mercaptoalkyl and alkylthioalkyl;

R^4 is hydrogen or alkyl;

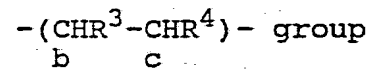
R^5 is hydrogen, alkyl, phenyl, phenylalkyl, hydroxyphenylalkyl, hydroxyalkyl, aminoalkyl, guanidinoalkyl, imidazolylalkyl, indolylalkyl, mercaptoalkyl or alkylthioalkyl;

R^4 and R^5 may be connected together to form an alkylene bridge of from 2 to 4 carbon atoms, an alkylene bridge of from 2 to 3 carbon atoms and one sulphur atom, an alkylene bridge of from 3 to 4 carbon atoms containing a double bond or an alkylene bridge as above, substituted with hydroxy, alkoxy or alkyl and the pharmaceutically acceptable salts thereof.

US-A-4599361 discloses compounds of the formula:



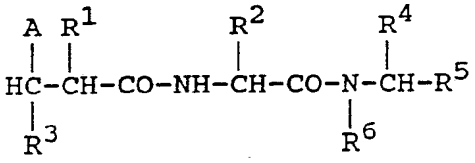
wherein
R^1 is C_1-C_6 alkyl;
R^2 is C_1-C_6 alkyl, benzyl, benzyloxybenzyl, (C_1-C_6 alkoxy)benzyl or benzyloxy(C_1-C_6 alkyl);
a is a chiral centre with optional R or S stereochemistry;
A is a



or a $-(CR^3=CR^4)-$ group wherein b and c are chiral centres with optional R or S stereochemistry;

R^3 is hydrogen, C_1-C_6 alkyl, phenyl or phenyl(C_1-C_6 alkyl) and R^4 is hydrogen, C_1-C_6 alkyl, phenyl(C_1-C_6 alkyl), cycloalkyl or cycloalkyl(C_1-C_6 alkyl).

EP-A-0236872 discloses generically compounds of the formula



wherein

A represents a group of the formula HN(OH)-CO- or HCO-N(OH)-;

R^1 represents a C_2-C_5 alkyl group;

R^2 represents the characterising group of a natural alpha-amino acid in which the functional group can be protected, amino groups may be acylated and carboxyl groups can be amidated, with the proviso that R^2 can not represent hydrogen or a methyl group;

R^3 represents hydrogen or an amino, hydroxy, mercapto, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_1-C_6 acylamino, C_1-C_6-alkylthio, aryl-(C_1-C_6 alkyl)-, amino-(C_1-C_6-alkyl)-, hydroxy(C_1-C_6-alkyl)-, mercapto(C_1-C_6 alkyl) or carboxy(C_1-C_6 alkyl) group,

wherein the amino, hydroxy, mercapto or carboxyl groups can be protected and the amino groups may be acylated or the carboxyl groups may be amidated;

R^4 represents hydrogen or a methyl group;

R^5 represents hydrogen or a C_1-C_6 acyl, C_1-C_6 alkoxy-C_1-C_6 alkyl, di(C_1-C_6-alkoxy)methylene, carboxy, (C_1-C_6 alkyl)carbinyl, (C_1-C_6 alkoxy)carbinyl, arylmethoxy carbinyl, (C_1-C_6 alkyl)amino carbinyl or arylamino carbinyl group; and

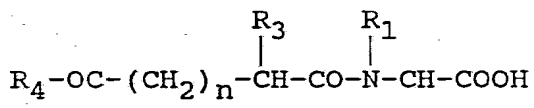
R^6 represents hydroxy or a methylene group; or

R^2 and R^4 together represent a group-$(CH_2)_n$-, wherein n represents a number from 4 to 11; or

R^4 and R^5 together represent a trimethylene group;

and pharmaceutically acceptable salts of such compounds, which are acid or basic.

US-A-4105789 generically discloses compounds which have the general formula



and salts thereof, wherein

R_1 is hydrogen, lower alkyl, phenyl lower alkylene, hydroxy-lower alkylene, hydroxyphenyl lower alkylene, amino-lower alkylene, guanidine lower

alkylene, mercapto-lower alkylene, lower alkyl-mercapto-lower alkylene, imidazolyl lower alkylene, indolyl-lower alkylene or carbamoyl lower alkylene;

R_2 is hydrogen or lower alkyl;

R_3 is lower alkyl or phenyl lower alkylene;

R_4 is hydroxy, lower alkoxy or hydroxyamino; and

n is 1 or 2.

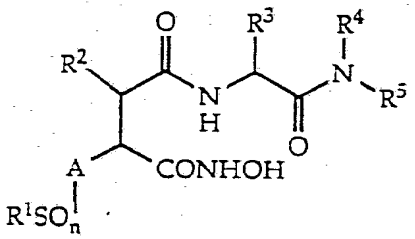
US-A-4496540 discloses compounds of the general formula:

A-B-NHOH

wherein A is one of the aromatic group-containing amino acid residues L-tryptophyl, D-tryptophyl, L-tyrosyl, D-tyrosyl, L-phenylalanyl, or D-phenylalanyl, and B is one of the amino acids glycine, L-alanine, D-alanine, L-leucine, D-leucine, L-isoleucine, or D-isoleucine; and pharmaceutically acceptable salts thereof.

It would however be desirable to improve on the solubility of known collagenase inhibitors and/or stomelysin inhibitors (whether as the free base or the salt) and, furthermore, increases in activity have also been sought. It is not a simple matter, however, to predict what variations in known compounds would be desirable to increase or even retain activity; certain modifications of known hydroxamic acid derivatives have been found to lead to loss of activity.

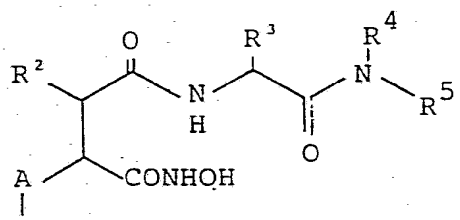
According to a first aspect of the invention, there is provided a compound of general formula I:



(I)

wherein:

R^1 represents a C_1-C_6 alkyl, phenyl, thiophenyl, substituted phenyl, phenyl(C_1-C_6)alkyl, heterocyclyl, (C_1-C_6)alkylcarbonyl, phenacyl or substituted phenacyl group; or, when n = 0, R^1 represents SR^X, wherein R^X represents a group:



;

R^2 represents a hydrogen atom or a C_1-C_6 alkyl, C_1-C_6 alkenyl, phenyl(C_1-C_6)alkyl, cycloalkyl(C_1-C_6)alkyl or cycloalkenyl(C_1-C_6)alkyl group;

R^3 represents an amino acid side chain or a C_1-C_6 alkyl, benzyl, (C_1-C_6 alkoxy)benzyl, benzyloxy(C_1-C_6 alkyl) or benzyloxybenzyl group;

R^4 represents a hydrogen atom or a C_1-C_6 alkyl group;

R^5 represents a hydrogen atom or a methyl group;

n is an integer having the value 0, 1 or 2; and

A represents a C_1-C_6 hydrocarbon chain, optionaly substituted with one or more C_1-C_6 alkyl, phenyl or substituted phenyl groups;

or a salt thereof.

Hereafter in this specification, the term "compound" includes "salt" unless the context requires otherwise.

As used herein the term "C_1-C_6 alkyl" refers to a straight or branched chain alkyl moiety having from one to six carbon atoms, including for example, methyl, ethyl, propyl, isopropyl, butyl, t-butyl, pentyl and hexyl, and cognate terms (such as "C^1-C^6 alkoxy") are to be construed accordingly.

The term "C_1-C_6 alkenyl" refers to a straight or branched chain alkyl moiety having one to six carbons and having in addition one double bond, of either E or Z stereochemistry where applicable. This term would include, for example, an alpha, beta-unsaturated methylene group, vinyl, 1-propenyl, 1- and 2-butenyl and 2-methyl-2-propenyl.

The term "cycloalkyl" refers to a saturated alicyclic moiety having from 3 to 8 carbon atoms and includes for example, cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl.

The term "cycloalkenyl" refers to an unsaturated alicycle having from 3 to 8 carbon atoms and includes cyclopropenyl, cyclobutenyl and cyclopentenyl, cyclohexenyl.

The term "substituted", as applied to a phenyl or other aromatic ring, means substituted with up to four substituents each of which independently may be C_1-C_6 alkyl, C_1-C_6 alkoxy, hydroxy, thiol, C_1-C_6 alkylthiol, amino, halo (including fluoro, chloro, bromo and iodo), triflouromethyl or nitro.

The term "amino acid side chain" means a characteristic side chain attached to the $-CH(NH_2)(COOH)$ moiety in the following R or S amino acids: glycine, alanine, valine, leucine, isoleucine, phenylalanine, tyrosine, tryptophan, serine, threonine, cysteine, methionine, asparagine, glutamine, lysine, histidine, arginine, glutamic acid and aspartic acid.

The term "hydrocarbon chain" includes alkylene, alkenylene and alkynylene chains of from 1 to 6 carbon atoms. Preferably the carbon atom of the hydrocarbon chain nearest to the hydroxamic acid group is a methylene carbon atom.

There are several chiral centres in the compounds according to the invention because of the presence of asymmetric carbon atoms. The presence of several asymmetreic carbon atoms gives rise to a number of diastereomers with the appropriate R or S stereochemistry at each chiral centre. General formula I and, where apprpriate, all other formulae in this

specification are to be understood to include all such stereoisomers and mixtures (for example racemic mixtures) thereof. Compounds in which the chiral centre adjacent the substituent R^3 has S stereochemistry and/or the chiral centre adjacent the substituent R^2 has R stereochemistry are preferred.

Further or other preferred compounds include those in which, independently or in any combination:

R^1 represents a hydrogen atom or a C_1-C_4 alkyl, phenyl, thiophenyl, benzyl, acetyl or benzoyl group;

R^2 represents a C_3-C_6 alkyl (for example isobutyl) group;

R^3 represents a benzyl or 4-(C_1-C_6)alkoxyphenylmethyl or benzyloxybenzyl group;

R^4 represents a C_1-C_4 alkyl (for example methyl) group; and

R^5 represents a hydrogen atom.

Particularly preferred compounds include:

1. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(phenylthio-methyl)-succinyl]-L-phenylalanine-N-methylamide,

2. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(thiophenyl-thio-methyl)succinyl]-L-phenylalanine-N-methylamide,

3. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(benzylthiomethyl) succinyl]-L-phenylalanine-N-methylamide,

4. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(acetylthiomethyl)succinyl]-L-phenylalanine-N-methylamide and

5. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(thiolmethyl) succinyl]-L-phenylalanine-N-methylamide

6. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(benzoylthiomethyl)succinyl]-L-phenylalanine-N-methylamide

7. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(pivaloylthiomethyl)succinyl]-L-phenylalanine-N-methylamide

8. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(phenylthiomethyl)succinyl]-L-phenylalanine-N-methylamide sodium salt

9. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-methoxyphenyl-thiomethyl)succinyl]-L-phenylalanine-N-methylamide

10. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-hydroxyphenylthiomethyl)succinyl]-L-phenylalanine-N-methylamide

11 [4-(N-Hydroxyamino)-2R-isobutyl-3S-(2-thiophenethiomethyl)succinyl]-L-phenylalanine-N-methylamide sodium salt

12. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-methoxyphenylthiomethyl)succinyl]-L-phenylalanine-N-methylamide sodium salt

13. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-tert-butylphenylthiomethyl)succinyl]-L-phenylalanine-N-methylamide

14. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(2,4-dimethylphenylthiomethyl)succinyl]-L-phenylalanine-N-methylamide

15. bis-S,S'-{[4(N-Hydroxyamino-2R-isobutyl-3S-(thiomethyl)succinyl]-L-phenylalanine-N-methylamide) disulphide

16. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(3-bromophenylthio-methyl)succinyl]-L-phenylalanine-N-methylamide

17. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(3-chlorophenylthiomethyl)succinyl]-L-phenylalanine-N-methylamide

18. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(3-methylphenylthiomethyl)succinyl]-L-phenylalanine-N-methylamide

19. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-(N-acetyl)-aminophenylthiomethyl)succinyl]-L-phenylalanine-N-methylamide

20. [4-(N-Hydroxyamino)-2R-isobutyl-3S-phenyl-sulphinylmethylsuccinyl]-L-phenylalanine-N-methyl-amide

21. [4-(N-Hydroxyamino)-2R-isobutyl-3S-phenyl-sulphonylmethylsuccinyl]-L-phenylalanine-N-methyl-amide

22. [4-(N-Hydroxyamino)-2R-isobutyl-3S-thiophenyl-sulphinylmethyl-succinyl]-L-phenylalanine-N-methylamide

23. [4-(N-Hydroxyamino)-2R-isobutyl-3S-thiophenyl-sulphonylmethyl-succinyl]-L-phenylalanine-N-methylamide

24. [4-(N-Hydroxyamino)-2R-isobutyl-3S-phenyl-sulphonylmethyl-succinyl]-L-phenylalanine-N-methylamide sodium salt

25. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-(isobutyl-oxycarbonylamino)phenyl)thiomethyl-succinyl]-L-phenylalanine-N-methylamide

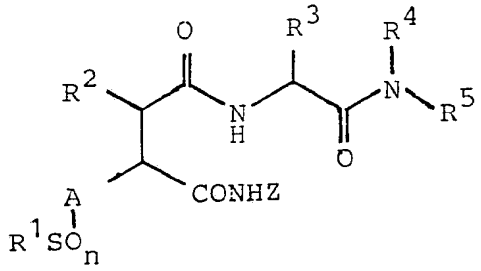
26. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-(N-methyl-N-(tert-butoxycarbonyl)-glycylamino)phenyl)thio-methylsuccinyl]-L-phenylalanine-N-methylamide

and, where appropriate, their salts. Compounds 2 and 5 are especially preferred and compound 2 is the most preferred, because of its good collagenase-inhibiting and protoglycanase-inhibiting activities.

Compounds of general formula I may be prepared by any suitable method known in the art and/or by the following process, which itself forms part of the invention.

According to a second aspect of the invention, there is provided a process for preparing a compound of general formula I as defined above, the process comprising:

(a) deprotecting a compound of general formula II

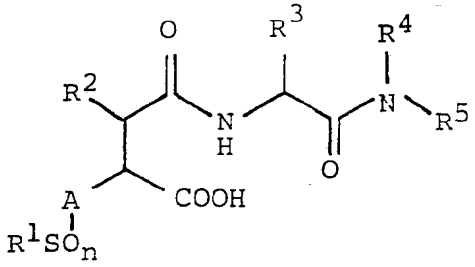


(II)

wherein:

R^1, R^2, R^3, R^4, R^5, A and n are as defined in general formula I and Z represents a protective group such as a benzyl group; or

(b) reacting a compound of general formula III



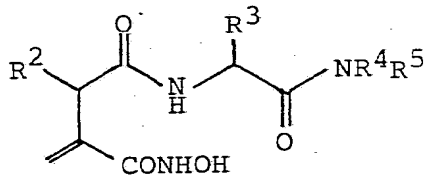
(III)

wherein:

R^1, R^2, R^3, R^4, R^5, A and n are as defined in general formula I,

with hydroxylamine or a salt thereof; or

(c) reacting a compound of general formula VIA



(VIA)

wherein

R^2, R^3, R^4 and R^5 are as defined in general formula I,

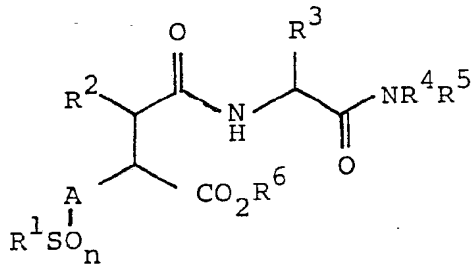
either with a thiol of the general formula R^1S, wherein R^1 is as defined in general formula I to give a compound of general formula I in which A represents a methylene group and n is 0,

or with a cuprate of the general formula $(R^1S\text{-}A^1)_2CuLi$, wherein R^1 is as defined in general formula I and A^1 is such that $-A^1-CH_2-$ is identical to -A-, as defined in general formula I.

(d) optionally after step (a), step (b) or step (c) converting a compound of general formula I into another compound of general formula I.

Compounds of general formula I which are sulphoxides or sulphones can be derived from thiol compounds of general formula I by oxidation. Alternatively, thiols of general formula II or III may be oxidised. Compounds of general formula I which are disulphides (ie compounds wherein R^1 represents SR^X) may be derived from thiol esters of general formula I by milk oxidation, for example in air.

A compound of general formula II may be prepared from a compound of general formula III by reaction with an O-protected (such as benzyl) hydroxylamine. A compound of general formula III may be prepared by desterification (such as hydrolysis) of an ester of the general formula IV

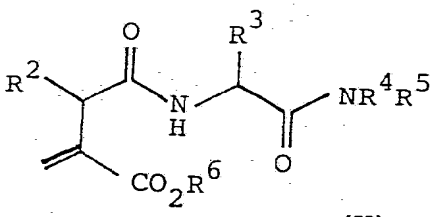


(IV)

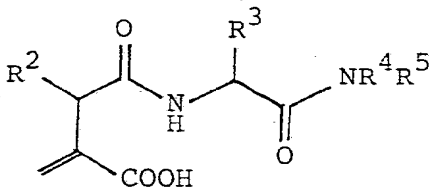
wherein:

R^1, R^2, R^3, R^4, R^5, A and n are as defined in general formula I and R^6 represents C_1-C_6 alkyl, phenyl C_1-C_6 alkyl or substituted phenyl C_1-C_6 alkyl.

A compound of general formula IV can be prepared from an ester of general formula V or an acid of general formula VI



(V)



(VI)

wherein:

R^2, R^3, R^4 and R^5 are as defined in general formula I and R^6 represents C_1-C_6 alkyl, phenyl C_1-C_6 alkyl or substituted phenyl C_1-C_6 alkyl

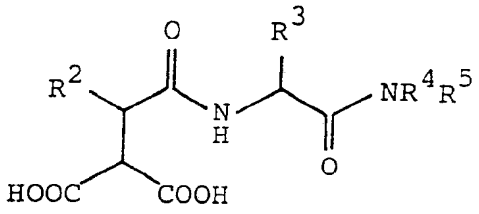
by reaction with a thiol R^1SH, wherein R^1 is as defined in general formula I, to give compounds wherein A represents a methylene group,

or by reaction with a cuprate of the general formula $(R^1S\text{-}A^1)_2CuLi$, wherein R^1 is as defined in general formula I and A^1 is such that $-A^1-CH_2-$ is identical to -A-, as defined in general formula I.

Esters of general formula V can be prepared by esterifying acids of general formula VI with an appropriate alcohol R^6OH or other esterifying agent.

Compounds of general formula VIA can be prepared by reacting compounds of general formula VI with hydroxylamine or a salt thereof.

An acid of general formula VI can be prepared by reacting a malonic acid derivative of general formula VII



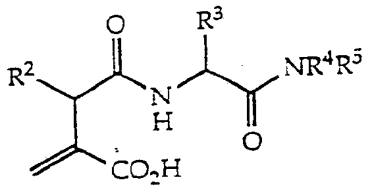
(VII)

wherein:

R^2, R^3, R^4 and R^5 are as defined in general formula I

with formaldehyde in the presence of pyridine.

An acid of general formula VII can in turn be prepared by desterifying (for example hydrolysing) a compound of general formula VIII

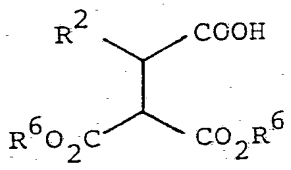


(VIII)

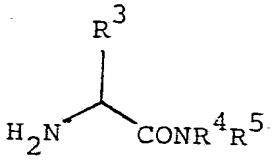
wherein:

R^2, R^3, R^4 and R^5 are as defined in general formula I and R^6 represents C_1-C_6 alkyl, phenyl C_1-C_6 alkyl or substituted phenyl C_1-C_6 alkyl.

A compound of general formula VIII can be prepared by reacting a compound of general formula IX with a compound of general formula X



(IX)



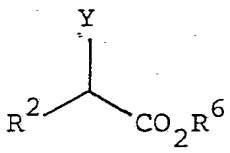
(X)

wherein:

R^2, R^3, R^4 and R^5 are as defined in general formula I and R^6 represents C_1-C_6 alkyl, phenyl C_1-C_6 alkyl or substituted phenyl C_1-C_6 alkyl.

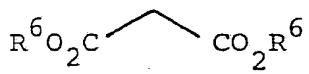
The starting materials and other reagents are either available commercially or can be synthesised by simple chemical procedures.

For example, a substituted acid of general formula IX may be prepared by reacting an ester of the general formula XI



(XI)

wherein Y represents halo and R^5 is as defined above and R^2 and R^6 as defined above, with a malonate derivative of the general formula XII



(XII)

wherein R^6 is as defined above with the proviso that when R^6 is aromatic in general formula XI it is aliphatic in general formula XII or vice versa, and selectively de-esterifying.

Compounds of general formula XI can simply be derived from amino acids, which can be obtained in enantiomerically pure form, enabling a choice of optically active compounds of general formula I to be prepared.

Compounds of general formulae II and III are valuable intermediates in the preparation of compounds of general formula I. According to a third aspect of the invention, there is therefore provided a compound of general formula II. According to a fourth aspect of the invention, there is provided a compound of general formula III.

As mentioned above, compounds of general formula I are useful in human or veterinary medicine as they are active inhibitors, of metalloproteases involved in tissue degradation.

According to a fifth aspect of the invention, there is provided a compound of general formula I for use in human or veterinary medicine, particularly in the management (by which is meant treatment of prophylaxis) of disease involving tissue degradation, in particular rheumatoid arthritis, and/or in the promotion of wound healing.

According to a sixth aspect of the invention, there is provided the use of a compound of general formula I in the preparation of an agent for the management of disease involving tissue degradation, particularly rheumatoid arthritis, and/or in the promotion of wound healing. Compounds of general formula I can therefore be used in a method of treating disease involving tissue degradation, particularly rheumatoid arthritis, and/or in a method of promoting wound healing, the method in either case comprising administering to a human or animal patient an effective amount of a compound of general formula I.

The potency of compounds of general formula I to act as inhibitors of collagenase (a metalloprotease involved in tissue degradation) was determined by the procedure of Cawston and Barrett, (Anal. Biochem., **99**, 340-345, 1979) and their potency to act as inhibitors of stromelysin was determined using the procedure of Cawston et al (Biochem. J., **195**, 159-165 1981), both of which techniques are to be described more fully in the examples and are incorporated by reference herein so far as the law allows.

According to a seventh aspect of the invention, there is provided a pharmaceutical or veterinary formulation comprising a compound of general formula I and a pharmaceutically and/or veterinarily acceptable carrier. One or more compounds of general formula I may be present in association with one or more non-toxic pharmaceutically and/or veterinarily acceptible carriers and/or diluents and/or adjuvents and if desired other active ingredients.

According to an eighth aspect of the invention, there is provided a process for the preparation of a pharmaceutical or veterinary formulation in accordance with the seventh aspect, the process comprising admixing a compound of general formula I and a pharmaceutically and/or veterinarily acceptable carrier.

Compounds of general formula I may be formulated for administration by any route and would depend on the disease being treated. The compositions may be in the form of tablets, capsules, powders, granules, lozenges, liquid or gel preparations, such as oral, topical, or sterile parental solutions or suspensions.

Tablets and capsules for oral administration may be in unit dose presentation form, and may contain conventional excipients such as binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, or polyvinyl-pyrollidone; fillers for example lactose, sugar, maize-starch, calcium phosphate,sorbitol or glycine; tabletting lubricant, for example magnesium sterate, talc, polyethylene glycol or silica; disintegrants, for example potato starch, or acceptable wetting agents such as sodium lauryl sulphate. The tablets may be coated according to methods well known in normal pharmaceutical practice. Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid

preparations may contain coventional additives such as suspending agents, for example sorbitol, syrup, methyl cellulose, glucose syrup, gelatin, hydrogenated edible fats; emulsifiying agents, for example lecithin, sorbitan monooleate, or acacia; non-aqujeous vehicles (which may include edible oils), for example almond oil, fractionated coconut oil, oily esters such as glycerine, propylene glycol, or ethyl alcohol; preservatives, for example methyl or propyl p-hydroxybenzoate or sorbic acid, and if desired conventional flavouring or colouring agents.

The dosage unit involved in oral administration may contain from about 1 to 250 mg, preferably from about 25 to 250 mg of a compound of general formula I. A suitable daily dose for a mammal may vary widely depending on the condition of the patient. However, a dose of a compound of general formula I of about 0.1 to 300mg/kg body weight, particularly from about 1 to 100 mg/kg body weight may be appropriate.

For topical application to the skin the drug may be made up into a cream, lotion or ointment. Cream or ointment formulations that may be used for the drug are conventional fomulations well known in the art, for example, as described in standard text books of pharmaceutics such as the British Pharmacopoeia.

For topical applications to the eye, the drug may be made up into a solution or suspension in a suitable sterile aqueous or non-aqueous vehicle. Additives, for instance buffers such as sodium metabisulphite or disodium edeate; preservatives including bactericidal

and fungicidal agents, such as phenyl mercuric acetate or nitrate, benzalkonium chloride or chlorohexidine, and thickening agents such as hypromellose may also be included.

The dosage employed for the topical administration will, of course, depend on the size of the area being treated. For the eyes each dose will be typically in the range from 10 to 100 mg of the compound of general formula I.

The active ingredient may also be administered parenterally in a sterile medium. The drug depending on the vehicle and concentration used, can either be suspended or dissolved in the vehicle. Advantageously, adjuvants such as a local anasthetic, preservative and buffering agents can be dissolved in the vehicle.

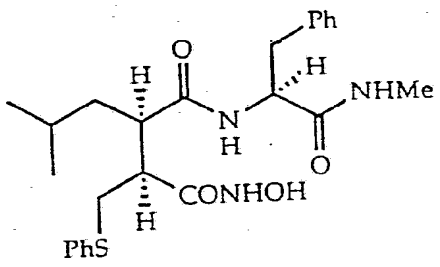
For use in the treatment of rheumatoid arthritis the compounds of this invention can be administered by the oral route or by injection intra-articularly into the affected joint. The daily dosage for a 70 kg mammal will be in the range of 10 mgs to 1 gram of a compound of general formula I.

The following examples illustrate the invention, but are not intended to limit the scope in any way. The following abbreviations have been used in the Examples:-

DCC - Dicyclohexylcarbodiimide
DCM - Dichloromethane
DCU - Dicyclohexylurea
DIPE - Diisopropyl ether
DMF - N,N-dimethylformamide
HOBT - Hydroxybenztriazole
NMM - N-Methylmorpholine
TFA - Trifluoroacetic acid
THF - Tetrahydrofuran
WSCDI - N-(Dimethylaminoethyl)-N'-ethylcarbodiimide

Example 1

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(phenylthiomethyl)-succinyl]-L-phenylalanine-N-methylamide



a) 2R-Bromo-5-methylpentanoic acid.

D-Leucine (100g, 0.76 mol) and potassium bromide (317.5g, 2.67 mol) were dissolved in aqueous acid (150ml concentrated sulphuric acid in 500ml of water). The solution was cooled to -2° and sodium nitrite (69.6g, 0.95 mol in water) was added over 1h taking care to maintain the temperature between -1 and -2°. After addition was complete the mixture was kept at 0° for a further hour, then DCM was added and the mixture stirred for a few minutes. The layers were separated

and the aqeous phase was washed with further portions of DCM (5 x 250ml). The combined organic layers were dried over magnesium sulphate then the solvent removed to give the acid as a pale yellow oil (123.1g, 0.63 mol, 83%)

$[alpha]_D$ = +38.0° (c = 2, methanol)

$delta_H$ (250 MHz, $CDCl_3$) 4.29 (1H, t, J= 6.5Hz, $BrC\underline{H}CO_2H$), 1.91 (2H, t, J= 7Hz, $CHC\underline{H}_2CH$), 1.83 (1H, m, $Me_2C\underline{H}$), and 0.94 (6H, 2xd, J= 7Hz, $(C\underline{H}_3)_2CH$)

b) tert-Butyl 2R-Bromo-5-methylpentanoate.

2R-Bromo-5-methylpentanoic acid (123g, 0.63 mol) was dissolved in DCM (400ml) and the solution cooled to -40° while isobutene was condensed in to roughly double the volume. Maintaining the temperature at -40° concentrated sulphuric acid (4ml) was added dropwise. When the addition was complete the reaction was allowed to warm to room temperature overnight. The resultant solution was concentrated to half the volume by removing the solvent at reduced pressure, then the DCM was washed twice with an equal volume of 10% sodium bicarbonate solution. The organic layer was dried over magnesium sulphate and the solvent removed under reduced pressure to leave the title compound as a yellow oil (148.0g, 0.59 mol, 94%).

$[alpha]_D$ = +23.0° (c = 2, methanol)

delta$_H$ (250 MHz, $CDCl_3$) 4.18 (1H, t, J= 6.5Hz, $BrC\underline{H}CO_2H$), 1.89 (2H, m, $CHC\underline{H}_2CH$), 1.78 (1H, m, $Me_2C\underline{H}$), 1.49 (9H, s, $(CH_3)_3C$) and 0.94 (6H, 2xd, J= 7Hz, $(CH_3)_2CH$)

delta$_C$ (63.9 MHz, $CDCl_3$) 167.0, 82.0, 46.3, 43.4, 27.6, 26.3, 22.2, and 21.6.

c) Benzyl (2-benzloxycarbonyl-3R-(tert-butoxycarbonyl)-5-methylhexanoate.

Dibenzyl malonate (124.5g, 0.44 mol) was taken up in dry DMF and potassium tert-butoxide (49.2g, 0.44 mol) was added portionwise with stirring and cooling. When a homogeneous solution had formed it was cooled to 0° then tert-butyl-2R-bromo-5-methylpentanoate (110.0g, 0.44 mol) in DMF (200 ml) was added dropwise over 1h. When addition was complete the reaction was transfered to a cold room at $<5^o$ and left for 4 days. The reaction mixture was partitioned between ethyl acetate and saturated ammonium chloride then the aqueous layer extracted with further ethyl acetate (4x500ml), drying and solvent removal left an oil (228g) heavily contaminated with DMF. This oil was taken into ether (1 litre) and washed with brine (2x1l) then the organic layer dried (magnesium sulphate), solvent removed under reduced pressure to leave the desired material (179g) contaminated with a small amount of dibenzyl malonate.

$[alpha]_D = +22.5^o$ (c = 2, methanol)

delta$_H$ (250 MHz, $CDCl_3$) 7.40 - 7.25 (10H, m, Aromatic H), 5.14 (4H, 2xABq, $C\underline{H}_2Ph$), 3.77 (1H, d, J= 10Hz, $BnO_2CC\underline{H}CO_2Bn$), 3.09 (1H, dt, J= 10,6Hz, $CH_2C\underline{H}CO_2tBu$), 1.50 (3H, m, CH_2 + $C\underline{H}Me_2$)1.41 (9H, s, $C(C\underline{H}_3)_3$) and 0.88 (6H, 2xd, J= 7Hz).

d) [4-Benzyloxy-3-benzyloxycarbonyl-2R-isobutyl-succinyl]-L-phenylalanine-N-methylamide

Benzyl(2-benzyloxycarbonyl-5-methyl-3R-tert-butoxycarbonyl)-hexanoate (281.4g, 0.56 mol) was taken up in 5% water in TFA (410 ml) and allowed to stand at 5° overnight. After this time the TFA was evaporated under reduced pressure then the residue partitioned between DCM (1l) and brine (200ml). Solvent removal left an oil which crystallised on standing (230g).

The crude acid from this reaction was dissolved in DMF (1l), then HOBT (95.3g, 0.64 mol), NMM (64g, 0.64 mol) and phenylalanine-N-methylamide (113.0g, 0.64 mol) were added at room temperature. The mixture was cooled to 0° before dropwise addition of DCC (131.0g, 0.64 mol) in THF (1l). This solution was stirred to room temperature over the weekend. The precipitated DCU was removed by filtration then the solvents were removed from the filtrate under reduced pressure to leave an oil. This oily residue was dissolved in ethyl acetate then washed with 10% citric acid, 10% sodium bicarbonate and saturated brine. The organic layer was dried (magnesium sulphate), filtered then the solvent removed under reduced pressure to give the title compound as an oil (400g). This material was columned on silica using gradient elution (0 - 50% ethyl

acetate in hexane) to remove impurities and separate a small amount of the minor diastereoisomer. The material from the column (195g) was recrystallised from DIPE to give the title compound as a white crystalline solid (140.2g, 0.25 mol, 47%)

m.p. 98 -99°
Analysis calculated for $C_{33}H_{38}N_2O_6$
Requires C 70.95 H 6.86 N 5.01
Found C 70.56 H 6.89 N 5.06

$delta_H$ (250MHz, $CDCl_3$) 7.42 - 7.13 (15H ,m, Aromatic H), 6.58 (1H, d, J=7.7Hz, CONH), 5.75 (1H, m, CONHMe), 5.20 - 5.05 (4H, m, OCH_2Ph), 4.50 (1H, dt, J= 6.9,7.7Hz, $CHCH_2Ph$), 3.79 (1H, d, J= 9.1Hz, $CH(CO_2Bn)$), 3.15 - 2.91 (2H, m, CH_2Ph), 2.65 (3H, d, J= 4.8Hz, $CONHCH_3$), 1.52 (1H, m, $CHCH_2CH$), 1.32 (1H, m, $CH(CH_3)$), 1.05 (1H, m, $CHCH_2CH$), and 0.74 (6H, 2xd, J= 6.5Hz, $CH(CH_3)_2$)

e) [4-Hydroxy-2R-isobutyl-3-ethenylsuccinyl]-L-phenylalanine-N-methylamide.

[4-Benzyloxy-3-benzyloxycarbonyl-2R-isobutylsuccinyl]-L-phenylalanine-N-methylamide (29.6g, 53mmol) was taken up in ethanol, ammonium formate (16.7g, 265mmol) added followed by 10% palladium on charcoal (6g) as a slurry in isopropyl alcohol. After 30 minutes at room temperature the catalyst was removed by filtration, then washed with ethanol to give a solution of the crude diacid. To this was added piperidine (5.0g) and the mixture stirred at room temperature for 15 minutes before addition of aqueous formaldehyde (40%

solution, 25ml). After 18 hours at room temperature the mixture was refluxed for 1 h. Solvents were removed under reduced pressure and the residue partitioned between ethyl acetate and citric acid. The acid layer was extracted with further portions of ethyl acetate (2x250ml), the combined organic layers were extracted with potassium carbonate (3x200ml). These base extracts were acidified to pH 4 and re-extracted with DCM then the organic layer dried over magnesium sulphate. Solvent removal under reduced pressure gave the desired product as a white solid (9.35g, 27.0mmol, 51%).

m.p. 149-151°C

delta$_H$ (250MHz, $CDCl_3$) 8.37 (2H, d, J= 9.0Hz, CONH), 7.39 (1H, m, CONHMe), 7.27 - 7.06 (5H, m, Aromatic H), 6.40 (1H, s, CH_2CHCO_2H), 5.78 (1H, s, CH_2CHCO_2H), 4.93 (1H, q, J= 7Hz, $CHCH_2Ph$), 3.92 (1H, m, $CH_2CHCONH$), 2.95 (2H, m, CH_2Ph), 2.71 (3H, d, J= 4.1Hz, $NHCH_3$), 1.68 (1H, m), 1.45 (2H, m), and 0.86 (6H, 2xd, J= 5.8Hz, $CH(CH_3)_2$).

delta$_C$ (63.9Hz, $CDCl_3$) 173.3, 172.8, 169.6, 139.1, 136.3, 129.2, 128.3, 127.0, 126.6, 54.4, 43.5, 41.4, 39.1, 26.2, 25.7, 22.5 and 22.4

f) [4-Hydroxy-2R-isobutyl-3S-(phenylthiomethyl)-succinyl]-L-phenylalanine-N-methylamide

[4-Hydroxy-2R-isobuty-3-ethenylsuccinyl]-L-phenyl-alanine-N-methylamide (15.0g, 44mmol) was dissolved in thiophenol

(150ml) and the mixture stirred in the dark under nitrogen at 60° for 2 days. Ether was added to the cooled reaction mixture and the precipitated product collected by filtration. The solid was washed with large volumes of ether and dried under vacuum to give the title compound (13.1g, 28.7mmol, 65%).

m.p. 199-201°C
Analysis calculated for $C_{25}H_{32}N_2O_4S$
Requires C 65.76 H 7.06 N 6.14 S 7.02
Found C 65.69 H 7.06 N 6.07 S 7.05

$delta_H$ (250MHz, D_6-DMSO) 8.40 (1H, d, J= 9Hz, CONH), 7.82 (1H, m, CONHMe), 7.35 - 7.10 (7H, m, Aromatic H), 7.04 (3H, m, Aromatic H), 4.62 (1H, m, $CHCH_2Ph$), 2.94 (1H, dd, J= 14,5Hz, $CHCH_2Ph$), 2.89 (1H, dd, J= 14,9Hz, $CHCH_2Ph$), 2.62 (3H, d, J= 4.5Hz, $CONHCH_3$), 2.41 (3H, m, 2xCH + CH_2SPh), 2.23 (1H, d, J= 12Hz, CH_2SPh), 1.43 (1H, m, $CHCH_2CH$), 1.30 (1H, bm, $CH(CH_3)_2$), 0.90 (1H, m, $CHCH_2CH$) and 0.78 (6H, 2xd, J= 6.5Hz, $CH(CH_3)_2$.

g) [4-(N-Hydroxyamino)-2R-isobutyl-3S-(phenylthiomethyl) succinyl]-L-phenylalanine-N-methylamide

[4-Hydroxy-2R-isobutyl-3S-(phenylthiomethyl)succinyl]-L-phenylalanine-N-methylamide (16.8g, 37 mmol) and HOBT (6.6g, 44 mmol) were dissolved in DCM / DMF (4:1) and the mixture cooled to 0° before adding WSCDI (8.5g, 44 mmol) and NMM (4.5g, 44 mmol). The mixture was stirred at 0° for 1h to ensure complete formation of the activated ester. Hydroxylamine hydrochloride (3.8g, 55 mmol) and NMM (5.6g, 55 mmol) were dissolved in DMF then this mixture added dropwise to the cooled

solution of the activated ester. After 1h the reaction was poured into ether / water (1:1) whereupon the desired product precipitated as white crystals. These were collected by filtration, further washed with ether and water then dried under vacuum at 50°. This material was recrystallised from methanol / water (1:1) to remove a trace of the minor diastereomer (9.03g, 19.2 mmol, 52%).

m.p. 227-229°C

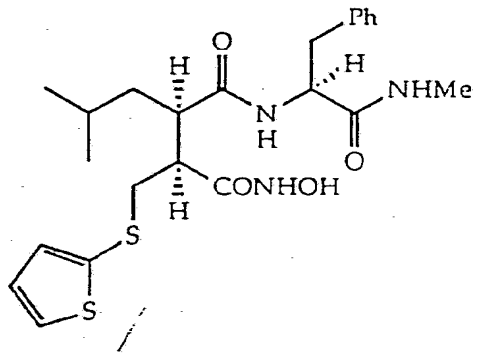
$[\alpha]_D$ = -88° (c = 10 , methanol)

δ_H (250MHz, D_6-DMSO) 8.84 (1H, d, J= 1.5Hz, NHOH), 8.35 (1H, d, J= 8.7Hz, CONH), 7.87 (1H, m, CONHMe), 7.29 - 6.92 (11H, m, Aromatic H + NHOH), 4.60 (1H, m, $CHCH_2Ph$), 2.94 (1H, dd, J= 13.5,4.3, $CHCH_2Ph$), 2.77 (1H, dd, J= 13.5,10, $CHCH_2Ph$), 2.60 (3H, d,J= 4.6Hz), 2.53 (1H, m), 2.41 (1H, m), 2.20 (1H, dd, J= 13.4,2.2Hz, CH_2SPh), 2.09 (1H, dd, J=13.4,2.4Hz, CH_2SPh), 1.38 (2H, m, $CHMe_2$ + $CHCH_2CH$), 0.88 (1H, m, $CHCH_2CH$), 0.82 (3H, d, J= 6.4Hz, $CH(CH_3)_2$), and 0.74 (3H, d, J+ 6.4Hz, $CH(CH_3)_2$).

δ_C (63.9MHz, D_6-DMSO) 172.9, 171.6, 166.3, 138.1, 136.7, 129.1, 128.9, 128.0, 127.3, 126.4, 125.2, 54.2, 46.4, 46.0, 37.7, 32.4, 25.6, 25.2, 24.2, and 21.7.

Example 2

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(thiophenylthiomethyl) succinyl]-L-phenylalanine-N-methylamide



a) [4-N-Hydroxy-2R-isobutyl-3S-(thiophenylthiomethyl) succinyl]-L-phenylalanine-N-methylamide

The title compound was prepared from [4-Hydroxy-2R-isobutyl-3-ethenylsuccinyl]-L-phenylalanine-N-methylamide (400mg, 1.16mmol) by the method described in example 1f, substituting thiophenethiol in the place of thiophenol to give a material (320mg, 0.73mmol, 63%) with the following characteristics.

m.p. 184-186°C

delta$_H$ (250MHz, D_6-DMSO) 8.29 (1H, d, J= 8.1Hz, CONH), 7.84 (1H, m, CONHMe), 7.57 (1H, d, J= 5.1Hz, Thiophene H), 5H, m, Aromatic H), 7.00 (2H, m, Thiophene H), 4.50 (1H, m, $CHCH_2Ph$), 2.91 (1H, m, $CHCH_2Ph$), 2.75 (1H, m, $CHCH_2Ph$), 2.56 (3H, d, J= 4.0Hz, $CONHCH_3$), 2.34 (3H, m), 1.99 (1H, d, J= 9.3Hz,

CH_2SHet), 1.42 (1H, m, $CHCH_2CH$), 1.29 (1H, bm, $CH(CH_3)_2$), 0.87 (1H, m, $CHCH_2CH$), 0.79 (3H, d, J= 6.4Hz, $CH(CH_3)_2$), and 0.72 (3H, d, J= 6.4Hz, $CH(CH_3)_2$).

b) [4-(N-Hydroxyamino)-2R-isobutyl-3S-(thiophenylthiomethyl)succinyl]-L-phenylalanine-N-methylamide

Prepared by the method described in example 1g to give material with the following characteristics

m.p. 236-238°C

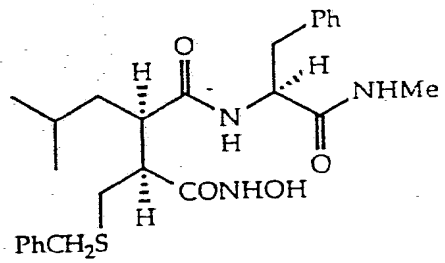
Analysis calculated for $C_{23}H_{30}N_2O_4S_2$
Requires C 57.84 H 6.54 N 8.80
Found C 57.64 H 6.48 N 8.85

$delta_H$ (250MHz, D_6-DMSO) 8.80 (1H, s, CONHOH), 8.08 (1H, d, J=8Hz, CONH), 7.52 (1H, m, CONHMe), 7.32 (1H, dd, J= 4.6,2.9Hz, Thiophene H), 7.17 - 6.95 (5H, m, Aromatic H), 6.89 (2H, m, Thiophene H), 4.46 (1H, m, $CHCH_2Ph$), 2.89 (1H, dd, J=13.6,4.4Hz, $CHCH_2Ph$), 2.72 (1H, dd, J= 13.6,10.5Hz, $CHCH_2Ph$), 2.54 (3H, d, J= 4.3Hz, $CONHCH_3$), 2.46 (1H, d, J= 12.1Hz, CH_2S), 2.35 (1H, bt, J= 10.2Hz), 2.14 (1H, bt, J= 10.2Hz), 1.98 (1H, dd, J=12.7,2.5Hz, $CHCH_2Ph$), 1.35 (1H, bt, J= 11.4Hz, $CHCH_2CH$), 1.22 (1H, bm, $CH(CH_3)_2$), 0.86 (1H, bt, J=12.6Hz, $CHCH_2CH$), 0.74 (3H, d, J= 6.3Hz, $CH(CH_3)_2$), and 0.68 (3H, d, J= 6.4Hz, $CH(CH_3)_2$).

$delta_C$ (63.9MHz, D_6-DMSO) 172.5, 171.6, 166.1, 138.0, 133.8, 132.7, 129.4, 129.2, 128.1, 127.8, 126.5, 54.2, 46.2, 46.0, 38.5, 37.6, 25.8, 25.2, 24.2, and 21.7.

Example 3

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(benzylthiomethyl) succinyl]-L-phenylalanine-N-methylamide



Prepared by the method described in example 1g to give material with the following characteristics

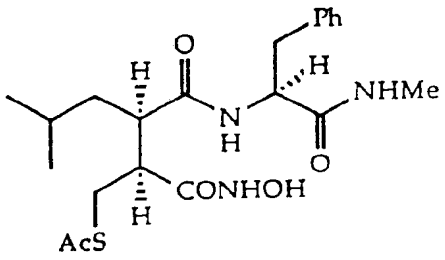
m.p. °

Analysis calculated for $C_{27}H_{37}N_3O_5S.0.5H_2O$
Requires C 61.81 H 7.30 N 8.00
Found C 61.85 H 7.15 N 7.45

$delta_H$ (250MHz, D_6-DMSO) 8.40 (1H, s, CONHOH), 8.22 (1H, m, NHMe), 7.20 (5H, m, Aromatic H), 6.58 (4H, m), 4.10 (1H, m, $CHCH_2Ph$), 3.22 (3H, s, OCH_3), 3.04 - 2.45 (4H, m, $2xCH_2Ar$), 2.42 (3H, d, J= 6Hz, $NHCH_3$), 2.32 - 2.08 (4H, m), 0.78 (2H, m, $CHCH_2CH$), and 0.40 - 0.18 (7H, m, $(CH_3)_2CH$).

Example 4

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(acetylthiomethyl) succinyl]-L-phenylalanine-N-methylamide



Prepared by the method described in example 1g to give material with the following characteristics

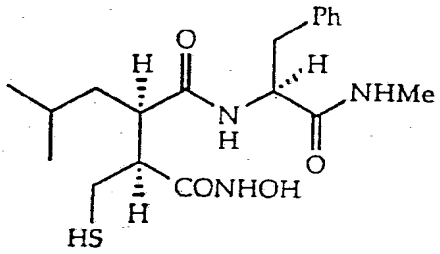
m.p. 226-227°C

Analysis calculated for $C_{21}H_{31}N_3O_5S.H_2O$
Requires C 55.37 H 7.30 N 9.22
Found C 55.57 H 6.99 N 9.53

$delta_H$ (250MHz, D_6-DMSO) 8.84 (1H, s, NHOH), 8.36 (1H, d, J= 8Hz, CONH), 7.80 (1H, d, J= 6Hz, NHMe), 7.20 (%h, m, Aromatic H), 4.58 (1H, m, $CHCH_2Ph$), 3.16 - 2.62 (2H, m, $CHCH_2Ph$), 2.54 (3H, d, J= 4Hz, $NHCH_3$), 2.22 (3H, s, CH_3COS), 2.36 - 2.10 (4H, m, $CHCHCH_2S$), 1.36 (2H, m, $CHCH_2CH$), and 0.98 - 0.66 (7H, m, $CH(CH_3)_2$).

Example 5

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(thiolmethyl) succinyl]-L-phenylalanine-N-methylamide



[4-(N-Hydroxyamino)-2R-isobutyl-3S-(acetylthiomethyl) succinyl]-L-phenylalanine-N-methylamide (30mg, 0.06mmol) was stirred in methanol (3ml) with methylamine (1ml methanolic solution) at room temperature. After 30 minutes the crystalline product (20mg, 0.05mmol, 74%) was filtered off and dried.

m.p. 234°C

Analysis calculated for $C_{19}H_{39}N_3O_4S.1.5H_2O$

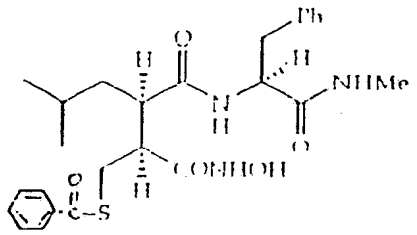
Requires C 54.10 H 7.63 N 9.94 S 7.60

Found C 54.28 H 7.16 N 10.43 S 7.80

$delta_H$ (250MHz, D_6-DMSO) 8.28 (1H, d, J= 9Hz, NHOH), 7.80 (1H, m, NHMe), 7.22 (5H, m, Aromatic H), 4.60 (1H, m, $CHCH_2Ph$), 3.08 - 2.56 (2H, m, $CHCH_2Ph$), 2.50 (3H, d, J= 4Hz, $NHCH_3$), 2.40 - 2.02 (4H, m, $CHCHCH_2SH$), 1.44 - 1.22 (2H, m, $CHCH_2CH$) and 0.98 - 0.72 (7H, m, $CH(CH_3)_2$).

Example 6

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(benzoylthiomethyl)-succinyl]-L-phenylalanine-N-methylamide



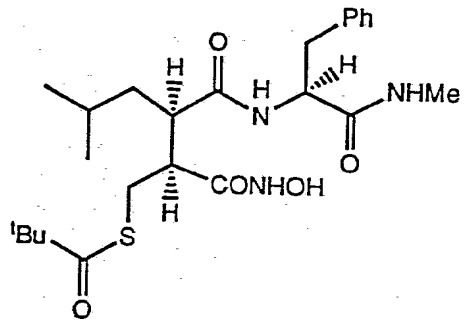
The title compound was prepared by the method described in Example 1g to give material with the following characteristics

m.p. 227 - 228°
Analysis calculated for $C_{21}H_{31}N_3O_5S$
Requires C 62.50 H 6.66 N 8.41
Found C 62.32 H 6.67 N 8.40

$delta_H$ (250 MHz, $CDCl_3$:D_6DMSO (1:1)) 8.82 (1H, s, NHOH), 8.25 (1H, d, J=8.4Hz, NHOH), 7.87 (2H, dd, J=8.5, 1.1Hz), 7.60 (2H, m, Ar-H and CONH), 7.50 (2H, t, J=8.2Hz), 7.28 (2H, d, J=8.4Hz), 7.16 (2H, t, J=7.2Hz), 7.04 (1H, t, J=8.5Hz), 4.65 (1H, m, $CHCH_2Ph$), 3.06 (1H, dd, J=14.1, 5.0Hz, $CHCH_2Ph$), 2.90 (1H, dd, J=13.9, 10Hz, $CHCH_2Ph$), 2.73 (2H, m SCH_2Ph), 2.65 (3H, d, J=4.7Hz, NHMe), 2.33 (1H, dt, J=11.0, 4.7Hz), 1.51 (1H, t, J=7Hz, CH_2CHMe_2), 1.24 (1H, m, $CHMe_2$), 0.97 (1H, t, J=7Hz, CH_2CHMe_2), 0.84 (3H, d, J=6.5Hz, $CHMe_2$) and 0.79 (3H, d, J=6.5Hz, $CHMe_2$).

Example 7

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(pivaloylthiomethyl) succinyl]-L-phenylalanine-N-methylamide



[4-Hydroxy-2R-isobutyl-3S-(pivaloylthiomethyl) succinyl]-L-phenylalanine-N-methylamide (0,8g, 1.7 mmol) and HOBT (0.31g, 2.1 mmol) were dissolved in 1:1 DCM/DMF and the mixture cooled to 0°C before adding WSDCI (0.4g, 2.1mmol) and NMM (0.21g, 2.1mmol). The mixture was stirred at 0°C for 1h to ensure complete formation of the activated ester. Hydroxylamine hydrochloride (0.18g, 2.6mmol) and NMM (0.26g, 2.6mmol) were dissolved in DMF then this mixture was added dropwise to the cooled solution of the activated ester. After 1h the reaction was poured into ether/water (1:1) whereupon the desired product precipitated as white crystals. These were collected by filtration, further washed with ether and water, then dried under vacuum at 50°C. This material was recrystallised from methanol/water (1:1) to remove a trace of the minor diastereomer (0.38g, 0.7mmol, 45%).

m.p. 225°C

[alpha]$_D$ = -3.5° (c=2, methanol)

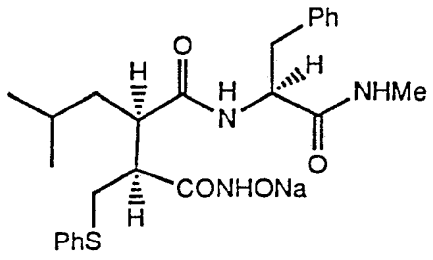
Analysis calculated for $C_{24}H_{39}N_3O_5S.0.5\ H_2O$
Requires: C58.99 H7.84 N8.60
Found: C58.96 H7.63 N8.55

delta$_H$ (250MHz, D_6-DMSO) 8.81 (1H, s, J = 1.5Hz, NHOH), 8.30 (1H, d, J=8Hz, CONH), 7.78 (1H, d, J=6Hz, CONHMe), 7.27-7.03 (5H, m, aromatic H), 4.54 (1H, m, $CHCH_2Ph$), 2.94 (1H, dd, J = 12,5Hz, $CHCH_2Ph$), 2.79 (1H, dd, J = 13,10Hz, $CHCH_2Ph$) 2.56 (3H, d, J = 4.5Hz, $NHCH_3$), 2.44 (2H, m), 2.20 (1H, dd, J = 13,3Hz, CH_2S), 2.07 (1H, dt), 1.36 (2H, m), 1.13 (9H, s, $C(CH_3)_3$), 0.87 (1H, m, $CH_2CH(CH_3)_2$), 0.79 (3H, d, J = 6Hz, $CH(CH_3)_2$), and 0.74 (3H, d, J = 6Hz, $CH(CH_3)_2$).

delta$_C$ (63.9MHz, D_6-DMSO) 172.55, 171.59, 168.24, 138.03, 129.18, 128.00, 126.24, 54.21, 46.48, 45.84, 45.55, 37.61, 28.30, 27.13, 25.64, 25.25, 24.24, and 21.63.

Example 8

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(phenylthiomethyl) succinyl]-L-phenylalanine-N-methylamide sodium salt



[4-(N-Hydroxyamino)-2R-isobutyl-3S-(phenylthiomethyl) succinyl]-L-phenylalanine-N-methylamide (0,2g, 0.4 mmol) was dissolved in 20ml of methanol and 1eq of 0.1N NaOH(aq) added. The solvent was removed in vacuo and the residue dissolved in water and freeze-dried (0.21g,0.4 mmol,100%).

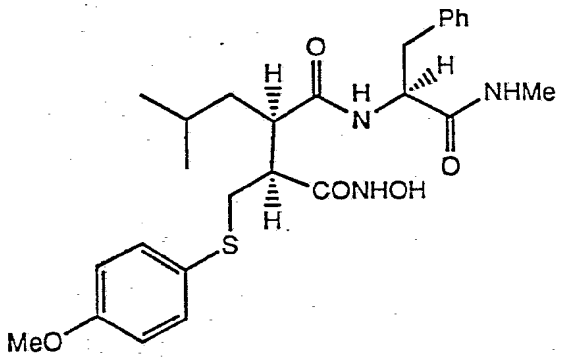
m.p. 184°C

$[\alpha]_D$ = -7.7° (c=2, methanol)

δ_H (250MHz, D_6-DMSO) 8.62 (1H, s, J = 1.5Hz, NHOH), 8.28 (1H, d, J = 8Hz, CONH), 7.26 - 7.04 (10H, m, aromatic H), 4.43 (1H, m, $CHCH_2Ph$), 3.00 (1H, dd, J = 14,4Hz, $CHCH_2Ph$), 2.84 (1H, dd, J = 14,10Hz, $CHCH_2Ph$), 2.55 (3H, d, J = 4.5Hz, $NHCH_3$), 2.46 (3H, m), 2.21 (1H, m), 1.39 (1H, m), 1.14 (1H, m), 1.00 (1H,m), and 0.70 (6H, d , J = 5.7Hz)

Example 9

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-methoxyphenyl-thiomethyl)



succinyl]-L-phenylalanine-N-methylamide[4-Hydroxy-2R-isobutyl-3S-(4-methoxyphenylthiomethyl)succinyl]-L-phenylalanine-N-methylamide (0,5g, 1 mmol) and HOBT (0.18g, 1.2 mmol) were dissolved in 1:1 DCM/DMF and the mixture cooled to 0°C before adding WSDCI (0.23g, 1.2mmol) and NMM (0.12g, 1.2mmol). The mixture was stirred at 0°C for 1h to ensure complete formation of the activated ester. Hydroxylamine hydrochloride (0.1g, 1.5mmol) and NMM (0.15g, 1.5mmol) were dissolved in DMF then this mixture was added dropwise to the cooled solution of the activated ester. After 1h the reaction was poured into ether/water (1:1) whereupon the desired product precipitated as white crystals. These were collected by filtration, further washed with ether and water, then dried under vacuum at 50°C. This material was recrystallised from methanol/water (1:1) to remove a trace of the minor diastereomer (0.36g, 0.7mmol, 72%).

m.p. 225°C

$[\alpha]_D$ = +8° (c=0.5, methanol)

Analysis calculated for $C_{26}H_{35}N_3O_5S$
Requires: C62.25 H7.04 N8.38
Found: C62.43 H7.09 N8.37

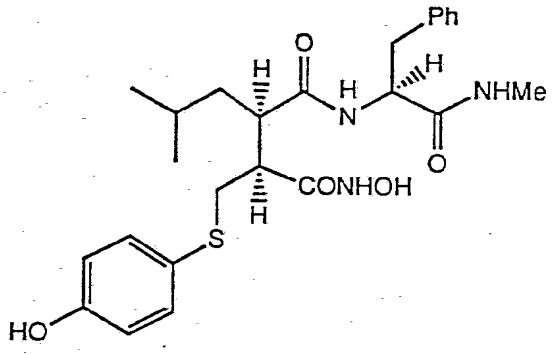
δ_H (250MHz, D_6-DMSO) 8.83 (1H, s, J = 1.5Hz, NHOH), 8.28 (1H, d, J = 8Hz, CONH), 7.83 (1H, d, J = 6Hz, CONHMe), 7.28 - 6.86 (9H, m, aromatic H), 4.52 (1H, m, CHCH$_2$Ph), 3.73 (3H, s, OCH3), 2.91 (1H, dd, J = 14,4Hz, CHCH$_2$Ph), 2.75 (1H, dd, J = 14,10Hz, CHCH$_2$Ph), 2.57 (3H, d, J = 4.5Hz, NHCH$_3$), 2.50 - 2.34 (2H,m), 2.16 -

1.99 (2H, m, $CH_2CH(CH3)_2$) 1.36 (2H, m), 0.88 (1H, m, $C\underline{H}_2CH(CH_3)_2$), 0.80 (3H, d, J = 6Hz, $CH(C\underline{H}_3)_2$), and 0.73 (3H, d, J = 6Hz, $CH(C\underline{H}_3)_2$).

$delta_C$ (63.9MHz, D_6-DMSO) 172.79, 171.62, 168.39, 138.14, 131.34, 129.19, 128.00, 126.44, 114.59, 55.32, 54.20, 38.68, 25.63, 25.17, 24.26, and 21.70.

Example 10

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-hydroxyphenyl-thiomethyl) succinyl]-L-phenylalanine-N-methylamide



[4-Hydroxy-2R-isobutyl-3S-(4-hydroxyphenylthiomethyl) succinyl]-L-phenylalanine-N-methylamide (0,4g, 0.8 mmol) and HOBT (0.15g, 1.0 mmol) were dissolved in 1:1 DCM/DMF and the mixture cooled to 0°C before adding WSDCI (0.20g, 1.0mmol) and NMM (0.1g, 1.0mmol). The mixture was stirred at 0°C for 1h to ensure complete formation of the activated ester. Hydroxylamine hydrochloride (0.09g, 1.3mmol) and NMM (0.13g,1.3mmol) were dissolved in DMF then this mixture was added dropwise to the cooled solution of the activated ester. After 1h the reaction was poured into ether/water (1:1)

whereupon the desired product precipitated as white crystals. These were collected by filtration, further washed with ether and water, then dried under vacuum at 50°C. This material was recrystallised from methanol/water (1:1) to remove a trace of the minor diastereomer (0.13g, 0.2mmol, 31%).

m.p. 216°C

$[\alpha]_D$ = -65° (c=0.5, methanol)

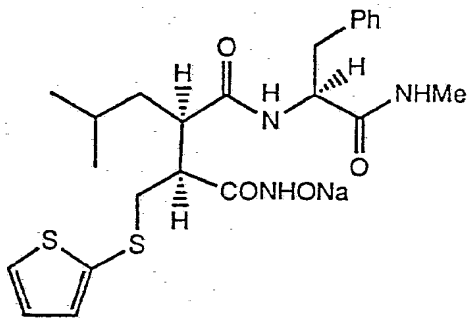
Analysis calculated for $C_{25}H_{33}N_3O_5S$
Requires: C61.58 H6.82 N8.62
Found: C61.43 H6.81 N8.08

δ_H (250MHz, D_6-DMSO) 8.82 (1H, s, J = 1.5Hz, NHOH), 8.26 (1H, d, J = 8Hz, CONH), 7.81 (1H, d, J = 6Hz, CONHMe), 7.27 - 6.64 (9H, m, aromatic H), 4.49 (1H, m, $CHCH_2Ph$), 2.90 (1H, dd, J=14,4Hz, $CHCH_2Ph$), 2.74 (1H, dd, J=14,10Hz, $CHCH_2Ph$), 2.57 (3H, d, J = 4.5Hz, $NHCH_3$), 2.54 - 2.29 (2H, m), 2.14 - 1.98 (2H, m, $CH_2CH(CH3)_2$), 1.35 (2H, m), 0.88 (1H, m, $CH_2CH(CH_3)_2$), 0.80 (3H, d, J = 6Hz, $CH(CH_3)_2$), and 0.73 (3H, d, J = 6Hz, $CH(CH_3)_2$).

δ_C (63.9MHz, D_6-DMSO) 172.81, 171.66, 168.46, 156.50, 133.02, 132.17, 129.17, 128.02, 126.44, 124.17, 116.00, 54.20, 46.35, 46.13, 37.59, 35.40, 25.62, 25.16, 24.27, and 21.69.

Example 11

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(2-thiophenethiomethyl)succinyl]-L-phenylalanine-N-methylamide sodium salt



[4-Hydroxyamino)-2R-isobutyl-3S-(2-thiophenethiomethyl) succinyl]-L-phenylalanine-N-methylamide (0,2g, 0.4 mmol) was dissolved in 20ml of methanol and 1eq of 0.1N-NaOH(aq) added. The solvent was removed in vacuo and the residue dissolved in water and freeze-dried (0.21g,0.4 mmol,100%).

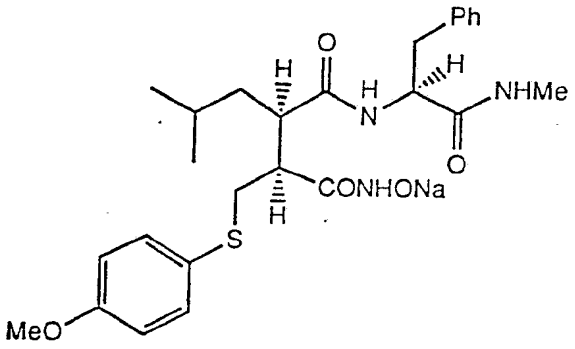
m.p. 170°C

$[\alpha]_D$ = -67° (c=1, methanol)

δ_H (250MHz, d_6-DMSO), 7.51 (1H, d), 7.19 - 6.97 (8H, m, aromatic H), 4.32 (1H, m, $C\underline{H}CH_2Ph$), 3.00 (1H, dd, J = 14,4Hz, $CHC\underline{H}_2Ph$), 2.84 (1H, dd, J = 14,10Hz, $CHC\underline{H}_2Ph$) 2.53 (3H, d, J = 4.5Hz, $NHC\underline{H}_3$), 2.46 2.19 (3H, m), 1.37 (1H, m), 1.09 (1H, m), 0.93 (1H, m), and 0.67 (6H, m)

Example 12

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-methoxyphenyl-thiomethyl)succinyl]-L-phenylalanine-N-methylamide sodium salt



[4-Hydroxyamino)-2R-isobutyl-3S-(4-methoxyphenylthio-methyl)succinyl]-L-phenylalanine-N-methylamide (0,1g, 0.2 mmol) was dissolved in 20ml of methanol and 1eq of 0.1N NaOH(aq) added. The solvent was removed in vacuo and the residue dissolved in water and freeze-dried (0.1g,0.2 mmol,100%).

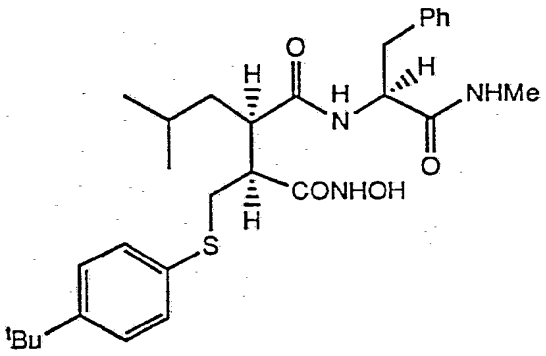
m.p. 174°C

$[\alpha]_D$ = -58° (c=1, methanol)

δ_H (250MHz, D_6-DMSO 7.26 - 7.04 (10H, m, aromatic H), 4.31 (1H, m, $C\underline{H}CH_2Ph$), 3.73 (3H, s, $OC\underline{H}_3$), 3.25 - 2.72 (2H, m, $CHCH_2Ph$), 2.50 (3H, s, $NHC\underline{H}_3$), 2.36 (1H, m), 2.15 (1H, m), 1.37 (1H, m), 0.95 (1H, m), and 0.69 (6H, d, $CHCH_2(C\underline{H}_3)_2$).

Example 13

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-tertbutylphenyl-thiomethyl) succinyl]-L-phenylalanine-N-methylamide



[4-Hydroxy-2R-isobutyl-3S-(4-tertbutylphenylthiomethyl) succinyl]-L-phenylalanine-N-methylamide (5.0g, 10 mmol) and HOBT (1.76g, 12 mmol) were dissolved in 1:1 DCM/DMF and the mixture cooled to 0°C before adding WSDCI (2.3g, 12mmol) and NMM (1.2g, 12mmol). The mixture was stirred at 0°C for 1h to ensure complete formation of the activated ester. Hydroxylamine hydrochloride (1.0g, 15mmol) and NMM (1.2g, 15mmol) were dissolved in DMF then this mixture was added dropwise to the cooled solution of the activated ester. After 1h the reaction was poured into ether/water (1:1) whereupon the desired product precipitated as white crystals. These were collected by filtration, further washed with ether and water, then dried under vacuum at 50°C. This material was repeatedly recrystallised from methanol/water (1:1) to remove a trace of the minor diastereomer (0.7g, 1.3mmol, 14%).

M.p. 188.5 -190°C

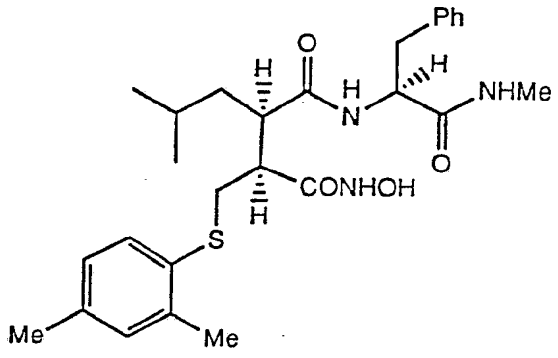
Analysis calculated for $C_{29}H_{41}N_3O_4S$
Requires: C66.00 H7.83 N7.96
Found: C65.80 H7.81 N7.76

$delta_H$ (250MHz, D_6-DMSO) 8.83 (1H, s, NHOH), 8.33 (1H, d, J = 8Hz, CONH), 7.86 (1H, d, J = 6Hz, CONHMe), 7.28 - 6.90 (9H, m, aromatic H), 4.60 (1H, m, $CHCH_2Ph$), 2.94 (1H, dd, J = 14,4Hz, $CHCH_2Ph$), 2.77 (1H, dd, J = 14,10Hz, $CHCH_2Ph$), 2.58 (3H, d, J = 4.5Hz, $NHCH_3$), 2.55 - 2.37 (2H, m), 2.22 - 2.08 (2H, m, $CH_2CH(CH3)_2$), 1.37 (2H, m), 1.26 (9H, s, $C(CH_3)_3$), 0.88 (1H, m, $CH_2CH(CH_3)_2$), 0.81 (3H, d, J = 6Hz, $CH(CH_3)_2$), and 0.74 (3H, d, J = 6Hz, $CH(CH_3)_2$).

$delta_C$ (63.9MHz, D_6-DMSO) 172.88, 171.59, 168.34, 147.87, 138.10, 133.09, 129.13, 127.95, 127.45, 126.36, 125.70, 54.19, 54.20, 46.38, 46.06, 37.70, 34.20, 32.79 31.24, 25.64, 25.19, 24.25, and 21.72.

Example 14

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(2,4-dimethylphenylthiomethyl) succinyl]-L-phenylalanine-N-methylamide



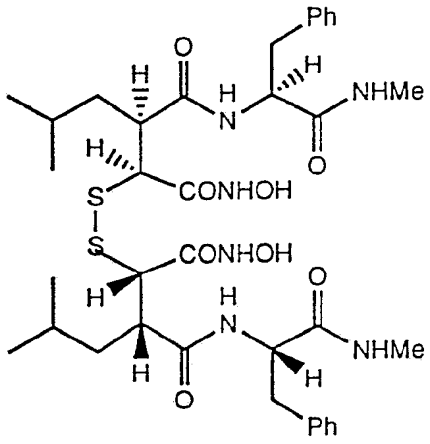
[4-Hydroxy-2R-isobutyl-3S-(2,4-dimethylphenylthiomethyl)succinyl]-L-phenylalanine-N-methylamide (1.8g, 3.7 mmol) and HOBT (0.67g, 12 mmol) were dissolved in 1:1 DCM/DMF and the mixture cooled to 0°C before adding WSDCI (0.86g, 4.5mmol) and NMM (0.45g, 4.5mmol). The mixture was stirred at 0°C for 1h to ensure complete formation of the activated ester. Hydroxylamine hydrochloride (0.39g, 5.6mmol) and NMM (0.56g, 5.6mmol) were dissolved in DMF then this mixture was added dropwise to the cooled solution of the activated ester. After 1h the reaction was poured into ether/water (1:1) whereupon the desired product precipitated as white crystals. These were collected by filtration, further washed with ether and water, then dried under vacuum at 50°C. This material was repeatedly recrystallised from methanol/water (1:1) to remove a trace of the minor diastereomer (1.08g, 2.2mmol, 58%).

m.p. 226°C (dec.)

Analysis calculated for $C_{27}H_{37}N_3O_4S$
Requires: C64.90 H7.46 N8.41
Found: C65.15 H7.48 N8.40

$delta_H$ (250MHz, D_6-DMSO) 8.83 (1H, s, NHOH), 8.32 (1H, d, J = 8Hz, CONH), 7.85 (1H, d, J = 6Hz, CONHMe), 7.30 - 6.71 (9H, m, aromatic H), 4.56 (1H, m, $CHCH_2Ph$), 2.91 (1H, dd, J = 14,4Hz, $CHCH_2Ph$), 2.76 (1H, dd, J = 14,10Hz, $CHCH_2Ph$), 2.57 (3H, d, J = 4.5Hz, $NHCH_3$), 2.53 - 2.38 (2H, m), 2.23 (3H, s, $C_6H_5(CH_3)2$), 2.13 (3H, s, $C_6H_5(CH_3)$), 1.30 (2H, m), 0.89 (1H, m, $CH_2CH(CH_3)_2$), 0.81 (3H, d, J = 6Hz, $CH(CH_3)_2$), and 0.74 (3H, d, J = 6Hz, $CH(CH_3)_2$).

Example 15

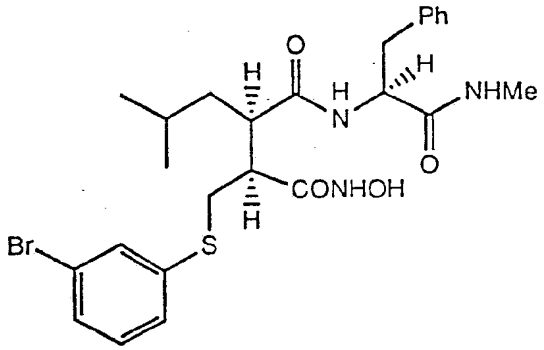


[4(N-Hydroxyamino-2R-isobutyl-3S-(acetylthiomethyl) succinyl]-L-phenylalanine-N-methylamide (1.0g, 2.4 mmol) was dissolved in 750ml methanol and 350ml pH 7 buffer added. Left to stand overnight and solvent removed in vacuo to 2/3 volume,left to crystallise for a further two hours. Filtered and dried to give 0.87g off-white crystals

Analysis calculated for $C_{38}H_{56}N_6O_8S_2.1.9H2O$
Requires: C55.34 H6.93 N9.88
Found: C55.44 H7.32 N10.21

Example 16

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(3-bromophenyl-thiomethyl) succinyl]-L-phenylalanine-N-methylamide



Prepared by the method described in example 1g to give material with the following characteristics.

m.p. 225 -229°C

$[alpha]_D$ = -164.8°

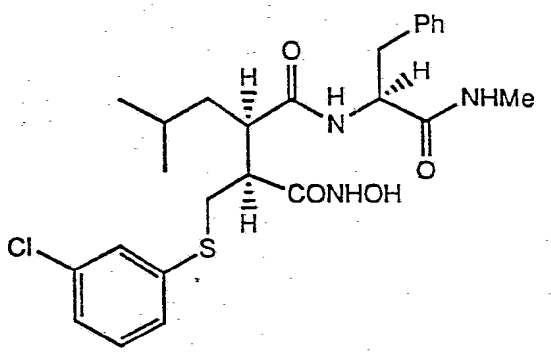
Analysis calculated for $C_{25}H_{32}BrN_3O_4S$
Requires: C54.40 H5.89 N7.40
Found: C54.54 H5.86 N7.63

$delta_H$ (250MHz, D_6-DMSO) 8.83 (1H, s, NHOH), 8.35 (1H, d, J = 8Hz, CONH), 7.90 (1H, q, J = 6Hz, CONHMe), 7.35 - 6.87 (9H, m, aromatic H), 4.64 (1H, m, $CHCH_2Ph$), 2.94 (1H, dd, J = 14,4Hz, $CHCH_2Ph$), 2.76 (1H, t, J = 13Hz, $CHCH_2Ph$) 2.60 (3H, d, J = 5Hz, $NHCH_3$), 2.55 - 2.35 (2H, m, CH_2S), 2.15 (1H, t, J = 10Hz, CHCO), 2.01 (1H, d, J = 11.5Hz, CHCO), 1.37 (2H, m), 0.88 (1H, m, $CH_2CH(CH_3)_2$), 0.81 (3H, d, J = 6Hz, $CH(CH_3)_2$), and 0.74 (3H, d,J = 6Hz,$CH(CH_3)_2$).

$delta_C$ (63.9MHz, D_6-DMSO) 173.0, 171.0, 168.8, 139.8, 138.0, 130.5, 129.0, 128.5, 127.5, 125.8, 125.5, 54.2, 46.0, 45.5, 38.0, 31.5, 25.5, 25.2, 24.7, and 21.0.

Example 17

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(3-chlorophenylthio-methyl) succinyl]-L-phenylalanine-N-methylamide



Prepared by the method described in example 1g to give material with the following characteristics.

m.p. 231-234°C

$[\alpha]_D = -96.5°$

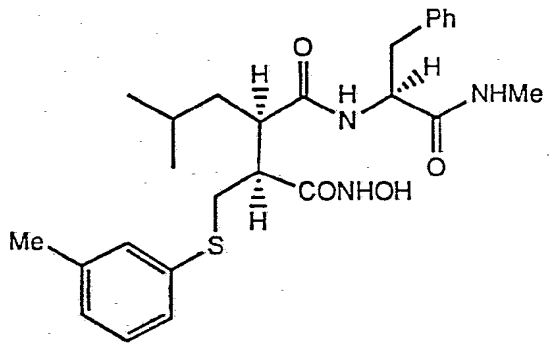
Analysis calculated for $C_{25}H_{32}ClN_3O_4S$
Requires: C59.34 H6.37 N8.30
Found: C59.51 H6.43 N8.24

δ_H (250MHz, D_6-DMSO) 8.85 (1H, s, NHOH), 8.37 (1H, d, J = 8.5Hz, CONH), 7.90 (1H, m, CONHMe), 7.30 - 6.88 (9H, m, aromatic H), 4.66 (1H, m, CHCH$_2$Ph), 2.96 (1H, bd, J = 14Hz, CHCH$_2$Ph), 2.76 (1H, bt, J = 13Hz, CHCH$_2$Ph) 2.60 (3H, d, J = 5Hz, NHCH$_3$), 2.55 - 2.40 (2H, m, CH$_2$S), 2.16 (1H, m, CHCO), 2.01 (1H, d, J = 14Hz, CHCO), 1.37 (2H, m), 0.91 (1H, m, CH$_2$CH(CH$_3$)$_2$), 0.81 (3H, d, J = 6Hz, CH(CH$_3$)$_2$), and 0.74 (3H, d, J = 6Hz,CH(CH$_3$)$_2$).

δ_C (63.9MHz, D_6-DMSO) 172.7, 171.6, 168.1, 139.2, 138.1, 130.3, 129.2, 127.9, 126.2, 125.9, 125.5, 125.0, 54.1, 46.3, 45.8, 37.8, 32.0, 25.7, 25.2, 24.2, and 21.7.

Example 18

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(3-methylphenylthiomethyl) succinyl]-L-phenylalanine-N-methylamide



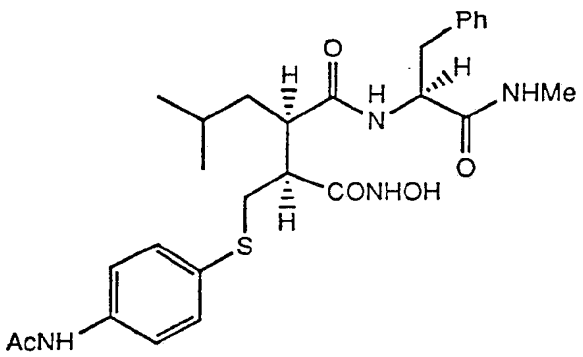
Prepared by the method described in example 1g to give material with the following characteristics.

Analysis calculated for $C_{26}H_{35}N_3O_4S$
Requires: C64.30 H7.26 N8.65
Found: C63.81 H7.21 N8.48

$delta_H$ (250MHz, D_6-DMSO) 8.83 (1H, s, NHOH), 8.35 (1H, d, J = 8.5Hz, CONH), 7.86 (1H, m, CONHMe), 7.28 - 6.77 (9H, m, aromatic H), 4.66 (1H, m, $CHCH_2Ph$), 2.96 (1H, dd, J = 14,4Hz, $CHCH_2Ph$), 2.80 (1H, bt, J = 13Hz, $CHCH_2Ph$) 2.59 (3H, d, J = 5Hz, $NHCH_3$), 2.55 - 2.37 (2H, m, CH_2S), 2.16 (2H, m, 2x$CHCO$), 1.38 (2H, m), 0.91 (1H, m, $CH_2CH(CH_3)_2$), 0.81 (3H, d, J = 6Hz, $CH(CH_3)_2$), and 0.74 (3H, d, J = 6Hz, $CH(CH_3)_2$).

Example 19

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-(N-acetyl)-aminophenylthiomethyl)succinyl]-L-phenylalanine-N-methylamide.



A) [2R-isobutyl-3S-(4-aminophenylthiomethyl)succinyl]-L-phenylalanine -N-methylamide.

Prepared by the method described in example 1f to give material with the following characteristics.

delta$_H$ (250MHz, D_6-DMSO) 8.27 (1H, d, J = 8.5Hz, CONH), 7.81 (1H, m, CONHMe), 7.30 - 7.00 (5H, m, phenyl H), 6.86 (2H, d, J = 8.5Hz, aromatic H), 6.45 (2H, d, J = 8.5Hz, aromatic H), 5.25 (1H, bs, CO_2H), 4.48 (1H, m, $CHCH_2Ph$), 2.91 (1H, dd, J = 14,4Hz, $CHCH_2Ph$), 2.88 (1H, dd, J = 14,10Hz, $CHCH_2Ph$) 2.56 (3H, d, J = 5Hz, $NHCH_3$), 2.43 - 2.24 (3H, m, CH_2S and CHCO), 2.03 (1H, d, J = 10Hz, CHCO), 1.41 (1H, t, J = 11Hz, $CH_2CH(CH_3)_2$), 1.26 (1H, m, $CH_2CH(CH_3)_2$), 0.85 (1H, m, $CH_2CH(CH_3)_2$), 0.81 (3H, d, J = 6Hz, $CH(CH_3)_2$), and 0.74 (3H, d, J=6Hz, $CH(CH_3)_2$).

B) [2R-isobutyl-3S-(4-(N-acetyl)aminophenyl-thiomethyl)- succinyl]-Lphenylalanine-N-methylamide.

The product from above (350mg, 0.74 mmol) was dissolved in DCM (5 ml) cooled in an ice bath then triethylamine (75mg, 0.74 mmol), DMAP (91mg, 7.4 mmol) and finally acetic anhydride (83mg, 8.2 mmol) were added and the solution stirred at RT for 90 minutes. The mixture was partitioned between ethyl acetate and citric acid then the organic layer washed with water and finally dried over magnesium sulphate. Solvent removal gave the crude product as pale yellow crystals (160mg, 0.31 mmol, 42%).

$delta_H$ (250MHz, D_6-DMSO) 9.94 (1H, s, CO_2H), 8.34 (1H, d, J = 8.5Hz, CONH), 7.90 (1H, m, CONHMe), 7.46 (2H, d, J = 8.5Hz, aromatic H) 7.30 - 7.00 (5H, m, phenyl H), 6.96 (2H, d, J = 8.5Hz, aromatic H), 4.57 (1H, m, $CHCH_2Ph$), 2.91 (1H, dd, J = 14,4Hz, $CHCH_2Ph$), 2.88 (1H, bt, J = 13Hz, $CHCH_2Ph$), 2.58 (3H, d, J = 5Hz, $NHCH_3$), 2.43 - 2.16 (3H, m, CH_2S and CHCO), 2.10 (1H, d, J = 14Hz, CHCO), 1.35 (1H, t, J = 14Hz, $CH_2CH(CH_3)_2$), 1.26 (1H, m, $CH_2CH(CH_3)_2$), 0.86 (1H, m, $CH_2CH(CH_3)_2$), 0.81 (3H, d, J = 6Hz, $CH(CH_3)2$), and 0.74 (3H, d, J = 6Hz,$CH(CH_3)_2$).

C) [4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-(N-acetyl)-aminophenylthiomethyl)succinyl]-L-phenylalanine-N-methylamide.

Prepared by the method described in example 1g to give material with the following characteristics.

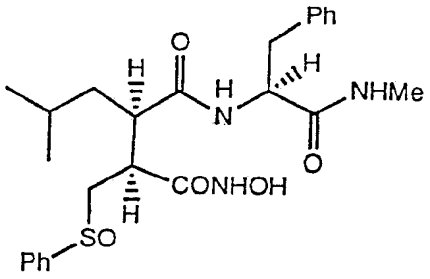
m.p. 201 -202°C (dec.)

$[alpha]_D$ = -7.5° (c=1.0, methanol)

$delta_H$ (250MHz, D_6-DMSO) 9.90 (1H, s, NHOH), 8.82 (1H, s, NHOH), 8.30 (1H, d, J = 8.5Hz, CONH), 7.85 (1H, m, CONHMe), 7.45 (2H, d, J = 8.5Hz, aromatic H), 7.28 - 6.94 (5H, m, phenyl H), 6.90 (2H, d, J = 8.5Hz, aromatic H), 4.66 (1H, m, $CHCH_2Ph$), 2.90 (1H, dd, J = 14,4Hz, $CHCH_2Ph$), 2.76 (1H, bt, J = 13Hz, $CHCH_2Ph$), 2.50 (3H, d, J = 5Hz, $NHCH_3$), 2.49 - 2.35 (2H, m, CH_2S), 2.14 (1H, m, CHCO), 2.03 (4H, s + m, $COCH_3$ and CHCO), 1.35 (2H, m), 0.86 (1H, m, $CH_2CH(CH_3)_2$), 0.81 (3H, d, J = 6Hz, $CH(CH_3)_2$), and 0.74 (3H, d, J = 6Hz, $CH(CH_3)_2$).

Example 20

[4-(N-Hydroxyamino)-2R-isobutyl-3S-phenylsulfinyl-methylsuccinyl]-L-phenylalanine-N-methylamide.



[4-(N-Hydroxyamino)-2R-isobutyl-3S-phenylthiomethyl-succinyl]-L-phenylalanine-N-methylamide (250mg, 0.53mmol) was dissolved in methanol (50 ml) and meta-

chloroperbenzoic acid (100mg, 0.58 mmol) was added. After stirring for 1h at room temperature ether was added and the mixture filtered. Solvent removal gave the crude white solid which was recrystallised from methanol / water then slurried in ether to remove final traces of meta-chlorobenzoic acid to give the desired material (70 mg, 0.014 mmol, 27%).

m.p. 186 -188°C

$[\alpha]_D$ = -13.6° (c=0.5, methanol)

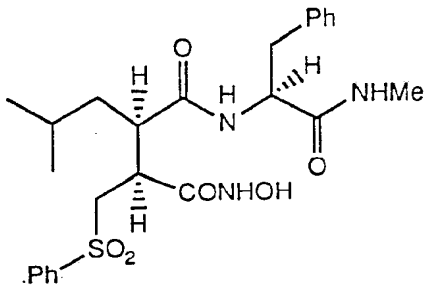
Analysis calculated for $C_{25}H_{33}N_3O_5S.0.5H_2O$
Requires: C60.46 H6.90 N8.46
Found: C60.58 H6.69 N8.29

δ_H (250MHz, D_6-DMSO, mixture of diastereomers) 9.04 + 8.93 (1H, 2xs, NHOH), 8.29 + 8.16 (1H, 2xd, J = 8.5 Hz, CONH), 7.79 (1H, m, CONHMe), 7.90 - 7.40 (8H, m, aromatic H), 7.06 + 6.82 (2H, 2xm, SO-Aromatic), 4.37 (1H, m, $CHCH_2Ph$), 2.93 - 2.58 (3H, m, containing $CHCH_2Ph$), 2.52 (3H, m, $NHCH_3$), 2.49 + 2.37 (1H, 2xm), 1.49 - 1.25 (2H, m, $CH_2CH(CH_3)_2$ and $CH2CH(CH_3)_2$), 0.95 (1H, m, $CH_2CH(CH_3)_2$), 0.81 (3H, d, J = 6Hz, $CH(CH_3)_2$), and 0.74 (3H, d, J=6Hz, $CH(CH_3)_2$).

δ_C (63.9MHz, D_6-DMSO, mixture of diastereomers) 172.2, 171.4, 171.3, 167.7, 144.5, 138.0, 137.9, 131.3, 130.9, 129.6, 129.3, 129.1, 128.8, 128.3, 127.8, 126.5, 126.2, 124.3, 123.6, 59.8, 58.1, 54.3, 54.0, 46.2, 45.8, 41.6, 40.9, 37.6, 37.4, 25.6, 25.0, 24.3, 24.2, 21.7, and 21.6.

Example 21

[4-(N-Hydroxyamino)-2R-isobutyl-3S-phenylsulfonyl-methylsuccinyl]-L-phenylalanine-N-methylamide.



[4-(N-Hydroxyamino)-2R-isobutyl-3S-phenylthiomethyl-succinyl]-L-phenylalanine-N-methylamide (50mg, 0.11mmol) was dissolved in methanol (12 ml) and meta-chloroperbenzoic acid (40mg, 0.23 mmol) was added. After stirring for 3h at room temperature ether was added and the mixture filtered. Solvent removal gave the crude white solid which was slurried in ether to remove final traces of meta-chlorobenzoic acid to give the desired material.

m.p. 228 - 231°C

$[\alpha]_D$ = 16.8° (c=0.5, methanol)

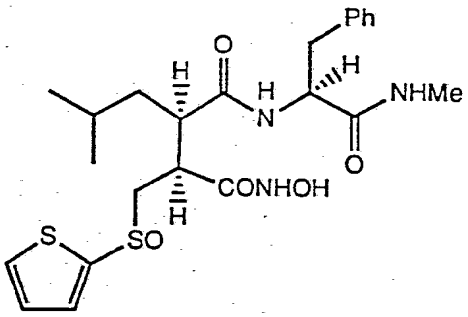
Analysis calculated for $C_{25}H_{33}N_3O_6S.0.3H_2O$
Requires: C58.99 H6.65 N8.25
Found: C58.92 H6.51 N8.05

δ_H (250MHz, D_6-DMSO) 8.66 (1H, s, NHOH), 8.25 (1H, d, J = 8.5 Hz, CONH), 7.83 (1H, m, CONHMe), 7.75 - 7.50 (5H, m, aromatic H), 7.30 7.05 (5H, m, aromatic H),

4.36 (1H, m, C<u>H</u>CH$_2$Ph), 2.86 (1H, dd, J = 14,5 Hz, CHC<u>H</u>$_2$Ph), 2.75 (1H, dd, J = 14,10 Hz, CHC<u>H</u>$_2$Ph), 2.54 (3H, d, J = 4.5 Hz, NHC<u>H</u>$_3$), 2.54 (2H, m), 1.30 (2H, m, C<u>H</u>$_2$CH(CH$_3$)$_2$ and CH$_2$C<u>H</u>(CH$_3$)$_2$), 0.86 (1H, m, C<u>H</u>$_2$CH(CH$_3$)$_2$), 0.75 (3H, d, J = 6Hz, CH(C<u>H</u>$_3$)$_2$), and 0.71 (3H, d, J = 6Hz,CH(C<u>H</u>$_3$)$_2$).

<u>Example 22</u>

[4-(N-Hydroxyamino)-2R-isobutyl-3S-thiophenylsulphinylmethyl-succinyl] -L-phenylalanine-N-methylamide



[4-(N-Hydroxyamino)-2R-isobutyl-3S-thiophenylthio-methyl-succinyl]-L-phenylalanine-N-methylamide (50mg, 0.11mmol) was treated as described in example 21 to yield the title compound (16mg, 0.03 mmol, 29%) as a mixture of diastereomer with the following characteristics:

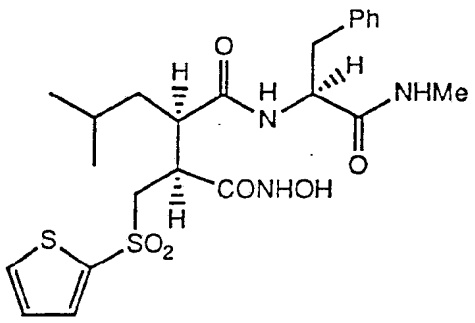
m.p. 195 -197°C (dec.)

Analysis calculated for $C_{23}H_{31}N_3O_5S_2.0.5H_2O$
Requires: C54.96 H6.42 N8.36
Found: C54.91 H6.23 N8.23

delta$_H$ (250MHz, D_6-DMSO, mixture of diastereomers) 9.04 + 8.96 (1H, 2xs, NHOH), 8.34 + 8.29 (1H, 2xd, J = 8.5 Hz, CONH), 8.02 + 7.98 (1H, 2xm, CONHMe), 7.81 (1H, bs, thiophene-H), 7.42 (1H, s, thiophene-H), 7.25 - 7.15 (5H, m, phenyl), 7.03 (1H, bs, thiophene-H), 4.43 (1H, m, $CHCH_2Ph$), 3.0 - 2.6 (4H, m, containing $CHCH_2Ph$), 2.52 (7H, m, containing $NHCH_3$), 2.05 (1H, m), 1.6 - 1.2 (2H, m, $CH_2CH(CH_3)_2$ and $CH_2CH(CH_3)_2$), 0.87 (1H, m, $CH_2CH(CH_3)_2$), and 0.85 - 0.71 (6H, m, $CH(CH_3)_2$).

Example 23

[4-(N-Hydroxyamino)-2R-isobutyl-3S-thiophenylsulphonylmethyl-succinyl] -L-phenylalanine-N-methylamide.



[4-(N-Hydroxyamino)-2R-isobutyl-3S-thiophenylthio-methyl-succinyl]-L-phenylalanine-N-methylamide (75mg, 0.16mmol) was treated as described in example 22 to yield the title compound (40mg, 0.08 mmol, 49%) with the following characteristics:

m.p. 215 - 216°C

Analysis calculated for $C_{23}H_{31}N_3O_6S_2$

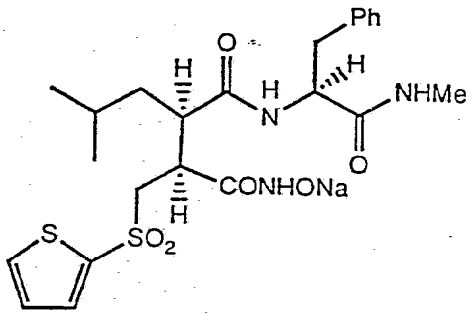
Requires: C54.21 H6.13 N8.24
Found: C54.07 H6.19 N8.04

delta$_H$ (250MHz, D_6-DMSO) 887 (1H, s, NHOH), 8.25 (1H, d, J = 8.5 Hz, CONH), 8.09 (1H, d, J = 4.7 Hz, thiophene-H), 7.83 (1H, m, CONHMe), 7.53 (1H, d, J = 3 Hz, thiophene H), 7.25 - 7.12 (6H, m, phenyl and thiophene-H), 4.36 (1H, m, $CHCH_2Ph$), 3.38 (1H, dd, J = 14,11 Hz, SCH_2), 2.87 (1H, dd, J = 14,5 Hz, $CHCH_2Ph$), 2.75 (1H, dd, J = 14,10 Hz, $CHCH_2Ph$), 2.70 - 2.36 (6H, m, containing $NHCH_3$), 1.20 (2H, m, $CH_2CH(CH_3)_2$ and $CH_2CH(CH_3)_2$), 0.89 (1H,m, $CH_2CH(CH_3)_2$), and 0.75 (6H, m, $CH(CH_3)_2$).

delta$_C$ (63.9MHz, D_6-DMSO) 172.0, 171.2, 166.5, 140.0, 138.0, 135.4, 134.6, 129.0, 128.4, 128.2, 126.6, 54.3, 45.6, 37.5, 25.6, 25.0, 24.2, and 21.7.

Example 24

[4-(N-Hydroxyamino)-2R-isobutyl-3S-phenylsulfonyl-methylsuccinyl]-L-phenylalanine-N-methylamide sodium salt.



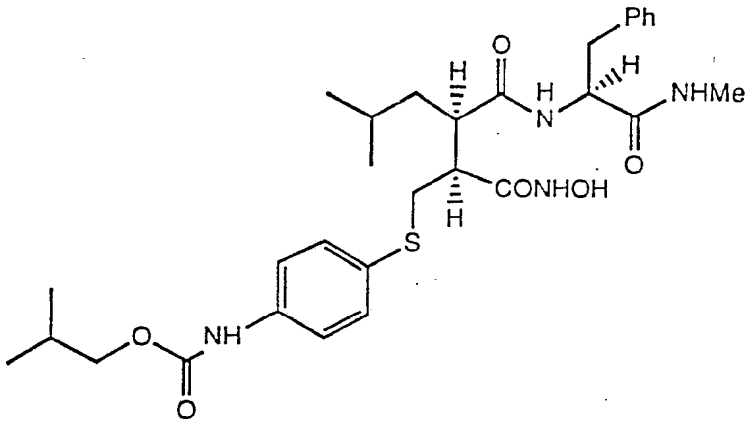
[4-(N-Hydroxyamino)-2R-isobutyl-3S-phenylsulfonyl-

methylsuccinyl]-L-phenylalanine-N-methylamide (50mg, 0.1mmol) was dissolved in methanol (10ml) and sodium hydroxide solution (0.1M, 1.0ml) added to give a homogeneous solution. The methanol was removed under reduced pressure then the residual aqueous solution freeze dried to give the title compound (40mg).

delta$_H$ (250MHz, D_6-DMSO) 8.66 (1H, s, NHOH), 8.25 (1H, d, J = 8.5 Hz, CONH), 7.83 (1H, m, CONHMe), 7.75 - 7.50 (5H, m, aromatic H), 7.30 7.05 (5H, m, aromatic H), 4.36 (1H, m, $CHCH_2Ph$), 2.86 (1H, dd, J = 14,5 Hz, $CHCH_2Ph$), 2.75 (1H, dd, J = 14,10 Hz, $CHCH_2Ph$), 2.54 (3H, d, J=4.5 Hz, $NHCH_3$), 2.54 (2H, m), 1.30 (2H, m, $CH_2CH(CH_3)_2$ and $CH_2CH(CH_3)_2$), 0.86 (1H, m, $CH_2CH(CH_3)_2$), 0.75 (3H, d, J = 6Hz, $CH(CH_3)_2$), and 0.71 (3H, d, J = 6Hz, $CH(CH_3)_2$).

Example 25

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-(isobutyloxy-carbonylamino)phenyl)thiomethyl-succinyl]-L-phenyl-alanine-N-methylamide



a) [4-Hydroxy-2R-isobutyl-3S-(4-aminophenyl)thio-

methylsuccinyl]-L-phenylalanine-N-methylamide was prepared by the method described in example 1f to give a compound with the following characteristics.

delta$_H$ (250MHz, D_6-DMSO) 8.26 (1H, d, J = 8.5 Hz, CONH), 7.81 (1H, m, CONHMe), 7.27 - 7.15 (5H, m, phenyl H), 6.85 (2H, d, J = 8.5Hz, aromatic H), 6.46 (2H, d, J = 8.5Hz, aromatic H), 5.2 (1H, bs, CO_2H), 4.48 (1H, m, $CHCH_2Ph$), 2.90 (1H, dd, J = 13.5,4.3 Hz, $CHCH_2Ph$), 2.75 (1H, dd, J = 13.6, 10 Hz, $CHCH_2Ph$), 2.56 (3H, d, J = 4.5 Hz, $NHCH_3$), 2.50 - 2.25 (3H, m), 2.03 (1H, d, J = 10 Hz), 1.41 (1H, m, $CH_2CH(CH_3)_2$), 1.26 (1H, m, $CH_2CH(CH_3)_2$), 0.86 (1H, m, $CH_2CH(CH_3)_2$), 0.75 (3H, d, J = 6Hz, $CH(CH_3)_2$), and 0.71 (3H, d, J = 6Hz,$CH(CH_3)_2$).

b) N,N-Dimethylglycine (100mg, 0.97 mmol) was stirred in dry THF (50ml) and triethylamine (108mg, 1.1mmol) and isobutylchloroformate (146mg, 1.1mmol) were added. After 1h the product from example 26a (500mg, 1.1mmol) was addedand the mixture stirred for a further 1h. The reaction was worked up by partitioning between citric acid and ethyl acetate, drying the organic layer and solvent removal to give the crude product (1g). Solution of the crude solid in ethyl acetate then precipitation with ether resulted in white crystals of the isobutylchloroformate derivative.

c) [4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-(isobutyloxy-carbonylamino) phenyl)thiomethyl-succinyl]-L-phenyl-alanine-N-methylamide

The product from example 26b was converted to the hydroxamic acid as described in example 1g. to give a compound with the following characteristics.

m.p. 198 - 200°C

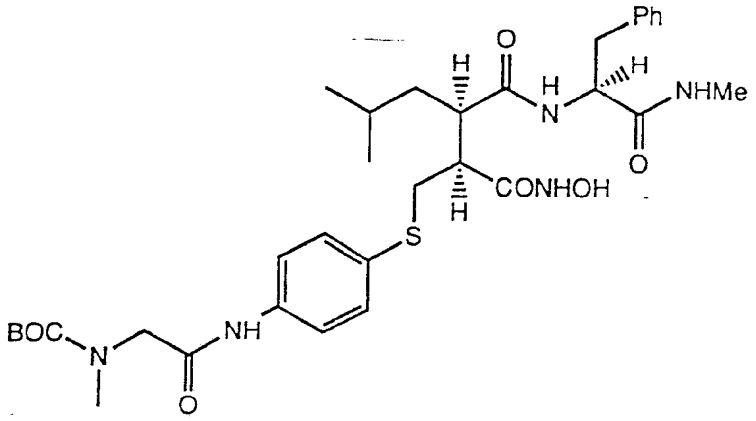
$[alpha]_D$ = -8.5° (c=1, methanol)

Analysis calculated for $C_{30}H_{42}N_4O_6S$
Requires: C61.41 H7.22 N9.55
Found: C62.04 H7.32 N9.67

$delta_H$ (250MHz, D_6-DMSO) 9.60 (1H, s, NHOH), 8.83 (1H, s, NHOH), 8.31 (1H, d, J = 8.5 Hz, CONH), 7.85 (1H, m, CONHMe), 7.36 - 7.25 (4H, m, aromatic H), 7.14 - 7.05 (3H, m, aromatic H), 6.91 (2H, d, J = 8.5Hz, aromatic H), 4.56 (1H, m, $CHCH_2Ph$), 3.87 (2H, d, J = 7Hz, $OCH_2CH(CH_3)_2$), 2.92 (1H, dd, J = 13.7,4.0 Hz, $CHCH_2Ph$), 2.76 (1H, dd, J = 13.6,10 Hz, $CHCH_2Ph$), 2.58 (3H, d, J = 4.5 Hz, $NHCH_3$), 2.50 - 2.34 (2H, m), 2.16 - 1.87 (3H, m), 1.35 (2H, m, $CH_2CH(CH_3)_2$ and $CH_2CH(CH_3)_2$), 0.93 (6H, d, J = 6.6Hz, $OCH_2CH(CH_3)_2$), 0.87 (1H,m, $CH_2CH(CH_3)_2$), 0.75 (3H, d, J = 6Hz, $CH(CH_3)_2$), and 0.71 (3H, d, J = 6Hz, $CH(CH_3)_2$).

Example 26

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-(N-methyl-N-(tertbutoxycarbonyl)-glycylamino) phenyl)thiomethyl-succinyl]-Lphenylalanine-N-methylamide.



a) [4-Hydroxy-2R-isobutyl-3S-(4-(N-methyl-N-(tert-butoxycarbonyl)glycylamino) phenyl)thiomethyl-succinyl]-L-phenylalanine-N-methylamide was prepared as described in example 26b by substitution of N-BOC sarcosine for the acid component.

$delta_H$ (250MHz, D_6-DMSO) 9.97 (1H, s, $CO_2\underline{H}$), 8.36 (1H, d, J = 8.5 Hz, CON$\underline{H}$), 7.91 (1H, m, CON$\underline{H}$Me), 7.48 (2H, d, J = 8.5Hz, aromatic H), 7.40 - 7.05 (5H, m, aromatic H), 6.97 (2H, d, J = 8.5Hz, aromatic H), 4.58 (1H, m, C$\underline{H}CH_2$Ph), 3.95 (2H, d, J = 9Hz, NC$\underline{H}_2$CO), 2.92 (4H, m+d, CHC$\underline{H}_2$Ph and BOCNC$\underline{H}_3$), 2.76 (1H, dd, J = 13,10 Hz, CHC$\underline{H}_2$Ph), 2.58 (3H, d, J = 4.5 Hz, NHC$\underline{H}_3$), 2.50 - 2.09 (4H, m), 1.46 - 1.33 (11H, m + 2xs, $(C\underline{H}_3)_3C$, $C\underline{H}_2CH(CH_3)_2$ and $CH_2C\underline{H}(CH_3)_2$), 0.87 (1H, m, $C\underline{H}_2CH(CH_3)_2$), 0.75 (3H, d, J = 6Hz, $CH(C\underline{H}_3)_2$), and 0.71 (3H, d, J = 6Hz, $CH(C\underline{H}_3)_2$).

b) [4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-(N-methyl- N-(tertbutoxycarbonyl)-glycylamino)phenyl)- thiomethyl-succinyl]-Lphenylalanine-N-methylamide was prepared from the material produced in example 27a as described in example 1g.

$delta_H$ (250MHz, D_6-DMSO) 9.97 (1H, s, CONHO$\underline{H}$), 8.83 (1H, s, N$\underline{H}$OH), 8.32 (1H, d, J = 8.5 Hz, CON$\underline{H}$), 7.86 (1H, m, CON$\underline{H}$Me), 7.46 (2H, d, J = 8.5Hz, aromatic H), 7.28 - 7.00 (5H, m, aromatic H), 6.97 (2H, d, J = 8.5Hz, aromatic H), 4.56 (1H, m, C$\underline{H}CH_2$Ph), 3.94 (2H, d, J = 9Hz, NC$\underline{H}_2$CO), 2.87 (4H, m+d, CHC$\underline{H}_2$Ph and BOCNC$\underline{H}_3$), 2.76 (1H, m, CHC$\underline{H}_2$Ph), 2.57 (3H, d, J = 4.5 Hz, NHC$\underline{H}_3$), 2.25 - 1.91 (2H, m), 1.42 - 1.30 (11H, m + 2xs, $(C\underline{H}_3)_3C$, $C\underline{H}_2CH(CH_3)_2$ and $CH_2C\underline{H}(CH_3)_2$), 0.92 (1H, m, $C\underline{H}_2CH(CH_3)_2$), 0.80 (3H, d, J = 6Hz, $CH(C\underline{H}_3)_2$), and 0.73 (3H, d, J=6Hz, $CH(C\underline{H}_3)_2$).

Example 27

Collagenase inhibition activity

The potency of compounds of general formula I to act as inhibitors of collagenase (a metalloproteas involved in tissue degradation) was determined by the procedure of Cawston and Barrett, (Anal. Biochem., 99, 340-345, 1979), hereby incorporated by reference, whereby a 1mM solution of the inhibitor being tested or dilutions thereof was incubated at 37° for 16 hours with collagen and collagenase (buffered with 25mM Hepes, pH 7.5 containing 5mM $CaCl_2$, 0.05% Brij 35 and 0.02% NaN_3). The collagen was acetylated ^{14}C collagen prepared by the method of Cawston and Murphy (Methods in Enzymology, **80**, 711, 1981), hereby incorporated by reference. The samples were centrifuged to sediment undigested collagen and an aliquot of the radioactive supernatant removed for assay on a scintillation counter as a measure of hydrolysis. The collagenase activity in the presence of 1 mM inhibitor, or a dilution thereof, was compared to activity in a control devoid of inhibitor and the results reported below as that inhibitor concentration effecting 50% inhibition of the collagenase (IC_{50}).

Compound of Example No.	IC_{50}
1	20 nM
2	8 nM
5	3 nM
6	(50% @ 1 mcM)

Example 28

Stromelysin inhibition activity

The potency of compounds of general formula I to act as inhibitors of stromelysin was determined using the procedure of Cawston et al (Biochem. J., 195, 159-165 1981), hereby incorporated by reference, whereby a 1mM solution of the inhibitor being tested or dilutions thereof was incubated at 37°C for 16 hours with stromelysin and ^{14}C acetylate casein (buffered with 25mM Hepes, pH 7.5 containing 5mM $CaCl_2$, 0.05% Brij 35 and 0.02% NaN_3. The casein was ^{14}C acetylated according to the method described in Cawston et al (Biochem. J., 195, 159-165, 1981), hereby incorporated by reference. The stromelysin activity in the presence of 1mM, or a dilution thereof, was composed to activity in a control devoid of inhibitor and the results reported below as that inhibitor concentration effecting 50% inhibition of the stromelysin (IC_{50}).

Compound of Example No.	IC_{50}
1	10 nM
2	20 nM

Examples of unit dosage compositions are as follows:

Example 29

Capsules:

Ingredients	Per Capsule	Per 10,000 Capsules
1. Active ingredient Cpd. of Form. I	40.0 mg	400 g
2. Lactose	150.0 mg	1500 g
3. Magnesium stearate	4.0 mg	40 g
	194.0 mg	1940 g

Procedure for capsules:

Step 1. Blend ingredients No. 1 and No. 2 in a suitable blender.

Step 2. Pass blend from Step 1 through a No. 30 mesh (0.59 mm) screen.

Step 3. Place screened blend from Step 2 in a suitable blender with ingredient No. 3 and blend until the mixture is lubricated.

Step 4. Fill into No. 1 hard gelatin capsule shells on a capsule machine.

Example 30

Tablets:

	Ingredients	Per Tablet	Per 10,000 Tablets
1.	Active ingredient Cpd. of Form. I	40.0 mg	400 g
2.	Corn Starch	20.0 mg	200 g
3.	Alginic acid	20.0 mg	200 g
4.	Sodium alginate	20.0 mg	200 g
5.	Magnesium stearate	1.3 mg	13 g
		101.3 mg	1013 g

Procedure for tablets:

Step 1. Blend ingredients No. 1, No. 2, No. 3 and No. 4 in a suitable mixer/blender.

Step 2. Add sufficient water portionwise to the blend from Step 1 with careful mixing after each addition. Such additions of water and mixing until the mass is of a consistency to permit its conversion to wet granules.

Step 3. The wet mass is converted to granules by passing it through an oscillating granulator using a No. 8 mesh (2.38) screen.

Step 4. The wet granules are then dried in an oven at 140°F (60°C) until dry.

Step 5. The dry granules are lubricated with ingredient No. 5.

Step 6. The lubricated granules are compressed on a suitable tablet press.

Example 31

Intramuscular Injection:

Ingredient	Per ml.	Per liter
1. Compound of Formula I Active ingredient	10.0 mg	10 g
2. Istonic buffer solution pH 4.0.	q.s.	q.s.

Procedure:

Step 1. Dissolve the active ingredient in the buffer solution.

Step 2. Aseptically filter the solution from Step 1.

Step 3. The sterile solution is now aseptically filled into sterile ampoules.

Step 4. The ampoules are sealed under aspetic conditions.

Example 32

Suppositories:

Ingredients	Per Supp.	Per 1,000 Supp
1. Compound of Form. I Active ingredient	40.0 mg	40 g
2. Polyethylene Glycol 1000	1350.0 mg	1,350 g
3. Polyethylene Glycol 4000	450.0 mg	450 g
	1840.0 mg	1,840 g

Procedure:

Step 1. Melt ingredient No. 2 and No. 3 together and stir until uniform.

Step 2. Dissolve ingredient No. 1 in the molten mass from Step 1 and stir until uniform.

Step 3. Pour the molten mass from Step 2 into suppository moulds and chill.

Step 4. Remove the suppositories from moulds and wrap.

Example 33

Eye Ointment

An appropriate amount of a compound of general formula I is formulated into an eye ointment base having the following composition:

Liquid paraffin	10%
Wool fat	10%
Yellow soft paraffin	80%

Example 34

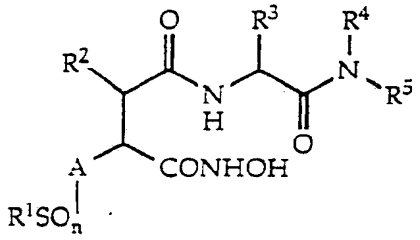
Topical skin ointment

An appropriate amount of a compound of general formula I is formulated into a topical skin ointment base having the following composition:

Emulsifying wax	30%
White soft paraffin	50%
Liquid paraffin	20%

CLAIMS

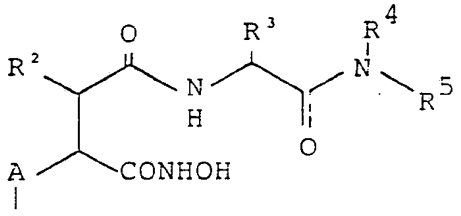
1. A compound of general formula I:



(I)

wherein:

R^1 represents a C_1-C_6 alkyl, phenyl, thiophenyl, substituted phenyl, phenyl(C_1-C_6)alkyl, heterocyclyl, (C_1-C_6)alkylcarbonyl or phenacyl or substituted phenacyl group; or when n = 0, R^1 represents SR^x, wherein R^x represents a group:



;

R^2 represents a hydrogen atom or a C_1-C_6 alkyl, C_1-C_6 alkenyl, phenyl(C_1-C_6)alkyl, cycloalkyl(C_1-C_6)alkyl or cycloalkenyl(C_1-C_6)alkyl group;

R^3 represents an amino acid side chain or a C_1-C_6 alkyl, benzyl, (C_1-C_6 alkoxy)benzyl or benzyloxy(C_1-C_6 alkyl) or benzyloxy benzyl group;

R^4 represents a hydrogen atom or a C_1-C_6 alkyl group;

R^5 represents a hydrogen atom or a methyl group;

n is an integer having the value 0, 1 or 2; and

A represents a C_1-C_6 hydrocarbon chain, optionaly substituted with one or more C_1-C_6 alkyl, phenyl or substituted phenyl groups;

or a salt thereof.

2. A compound as claimed in Claim 1, in which the chiral centre adjacent the substituent R^3 has S stereochemistry.

3. A compound as claimed in Claim 1 or 2, wherein the chiral centre adjacent the substituent R^2 has R stereochemistry.

4. A compound as claimed in Claim 1, 2 or 3, in which R^1 represents a hydrogen atom or a C_1-C_4 alkyl, phenyl, thiophenyl, benzyl, acetyl or phenacyl group.

5. A compound as claimed in any one of Claims 1 to 4, wherein R^2 represents a C_3-C_6 alkyl group.

6. A compound as claimed in any one of Claims 1 to 5, wherein R^3 represents a benzyl or 4-(C_1-C_6)alkoxyphenylmethyl or benzyloxybenzyl group.

7. A compound as claimed in any one of Claims 1 to 6, wherein R^4 represents a C_1-C_4 alkyl group.

8. A compound as claimed in any one of Claims 1 to 7, wherein R^5 represents a hydrogen atom.

9. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(phenylthiomethyl)-succinyl]-L-phenylalanine-N-methylamide,

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(thiophenylthiomethyl) succinyl]-L-phenylalanine-N-methylamide,

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(benzylthiomethyl) succinyl]-L-phenylalanine-N-methylamide,

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(acetylthiomethyl) succinyl]-L-phenylalanine-N-methylamide or

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(thiolmethyl) succinyl]-L-phenylalanine-N-methylamide

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(pivaloylthiomethyl) succinyl]-L-phenylalanine-N-methylamide

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(phenylthiomethyl) succinyl]-L-phenylalanine-N-methylamide sodium salt

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-methoxyphenylthiomethyl)succinyl]-L-phenylalanine-N-methylamide

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-hydroxyphenylthiomethyl)succinyl]-L-phenylalanine-N-methylamide

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(2-thiophenethiomethyl)succinyl]-L-phenylalanine-N-methylamide sodium salt

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-methoxyphenyl-thiomethyl)succinyl]-L-phenylalanine-N-methylamide sodium salt

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-tertbutylphenyl-thiomethyl)succinyl]-L-phenylalanine-N-methylamide

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(2,4-dimethylphenyl-thiomethyl) succinyl]-L-phenylalanine-N-methylamide

bis-S,S'-{[4(N-Hydroxyamino-2R-isobutyl-3S-(thiomethyl) succinyl]-L-phenylalanine-N-methylamide) disulphide

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(3-bromophenylthio-methyl) succinyl]-L-phenylalanine-N-methylamide

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(3-chlorophenylthio-methyl) succinyl]-L-phenylalanine-N-methylamide

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(3-methylphenylthio-methyl) succinyl]-L-phenylalanine-N-methylamide

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-(N-acetyl)-amino-phenylthiomethyl)succinyl]-L-phenylalanine-N-methyl-amide

[4-(N-Hydroxyamino)-2R-isobutyl-3S-phenylsulphinyl-methylsuccinyl]-L-phenylalanine-N-methylamide

[4-(N-Hydroxyamino)-2R-isobutyl-3S-phenylsulphonyl-methylsuccinyl]-L-phenylalanine-N-methylamide

[4-(N-Hydroxyamino)-2R-isobutyl-3S-thiophenylsulphinyl-methyl-succinyl]-L-phenylalanine-N-methylamide

[4-(N-Hydroxyamino)-2R-isobutyl-3S-thiophenylsulphonyl-methyl-succinyl]-L-phenylalanine-N-methylamide

[4-(N-Hydroxyamino)-2R-isobutyl-3S-phenylsulphonyl-methyl-succinyl]-L-phenylalanine-N-methylamide sodium salt

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-(isobutyloxy-carbonylamino)phenyl)thiomethyl-succinyl]-L-phenyl-alanine-N-methylamide

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-(N-methyl-N-(tert-butoxycarbonyl)-glycylamino)phenyl)thiomethyl-succinyl]-L-phenylalanine-N-methylamide

or, where appropriate, a salt of such a compound.

10. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(thiophenyl-thiomethyl) succinyl]-L-phenylalanine-N-methylamide, or

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(thiolmethyl) succinyl]-L-phenylalanine-N-methylamide

or a salt thereof.

11. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(thiophenyl-thiomethyl)succinyl]-L-phenylalanine-N-methylamide or a salt thereof.

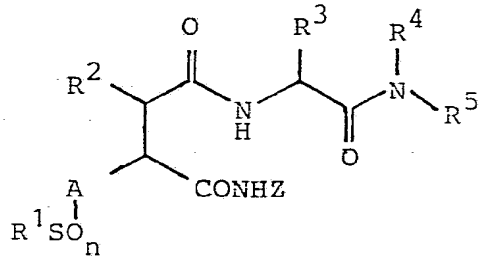
12. A compound as claimed in any one of claims 1 to 11 for use in human or veterinary medicine.

13. The use of a compound as claimed in any one of claims 1 to 11 in the preparation of an agent for use in the management of disease involving tissue degradation and/or in the promotion of wound healing.

14. A pharmaceutical or veterinary formulation comprising a compound as claimed in any one of claims 1 to 11 and a pharmaceutically and/or veterinarily acceptable carrier.

15. A process for preparing a compound of general formula I as defined in claim 1, the process comprising:

(a) deprotecting a compound of general formula II

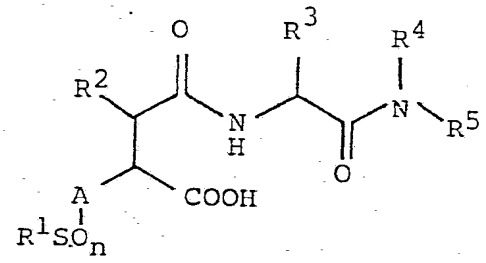


(II)

wherein:

R^1, R^2, R^3, R^4, R^5, A and n are as defined in general formula I and Bn represents a benzyloxycarbonyl group; or

(b) reacting a compound of general formula III



(III)

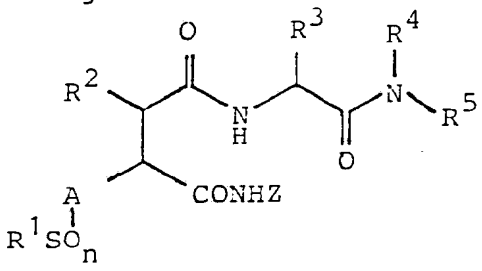
wherein:

R^1, R^2, R^3, R^4, R^5, A and n are as defined in general formula I,

with hydroxylamine or a salt thereof; and

(c) optionally after step (a) or step (b) converting a compound of general formula I into another compound of general formula I.

16. A compound of general formula II

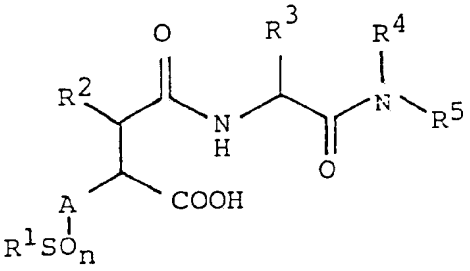


(II)

wherein:

R^1, R^2, R^3, R^4, R^5, A and n are as defined in general formula I and Z represents a protecting group.

17. A compound of general formula III



(III)

wherein:

R^1, R^2, R^3, R^4, R^5, A and n are as defined in general formula I.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 89/01399

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[5]: C 07 C 323/62, 323/60, C 07 D 333/34, C 07 C 327/32, 317/50, 313/48, A 61 K 31/13, 31/38

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[5]	C 07 C 259/00, 323/00, C 07 D 333/00, C 07 C 327/00, 317/00, 313/00

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A	EP, A, 0236872 (F. HOFFMANN-LA ROCHE) 16 September 1987 see claim 1 cited in the application	1-17
A	EP, A, 0012401 (MERCK & CO. INC.) 25 June 1980 see claim 1 cited in the application	1-17
A	DE, A, 2720996 (E.R. SQUIBB & SONS) 24 November 1977 see claim 1 cited in the application & US, A, 4105789	1-17
A	EP, A, 0274453 (LABORATOIRE ROGER BELLON) 13 July 1988 see claim 1	1-17
A	EP, A, 0214639 (G.D. SEARLE) 18 March 1987 see claim 1	1-17

./.

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

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

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
8th March 1990	17 AVR. 1990
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	MISS T. TAZELAAR

Form PCT/ISA/210 (second sheet) (January 1985)

International Application No. PCT/GB 89/01399

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
	cited in the application & US, A, 4599361 --	
A	Chemical Abstracts, volume 83, no. 7, 18 August 1975, (Columbus, Ohio, US), J.P. Devlin et al.: "Antibiotic actinonin. III. Synthesis of structural analogs of actinonin by the anhydride-imide method", see page 549, abstract 59249e, & J. Chem. Soc., Perkin Trans. I, 1975, (9), 830-41	1-17

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 8901399 SA 33118

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 04/04/90
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0236872	16-09-87	AU-B- 588437	14-09-89
		AU-A- 6990287	17-09-87
		JP-A- 62230757	09-10-87
EP-A- 0012401	25-06-80	AT-T- E6503	15-03-84
		AU-B- 530380	14-07-83
		AU-A- 5346179	19-06-80
		CA-C- 1262684	07-11-89
		JP-A- 55081845	20-06-80
		US-A- 4374829	22-02-83
DE-A- 2720996	24-11-77	US-A- 4105789	08-08-78
		CA-A- 1103259	16-06-81
		FR-A,B 2421874	02-11-79
		GB-A- 1575850	01-10-80
		JP-A- 52136121	14-11-77
		US-A- 4146639	27-03-79
		US-A- 4228184	14-10-80
		US-A- 4153725	08-05-79
		US-A- 4192882	11-03-80
		US-A- 4146641	27-03-79
		US-A- 4207342	10-06-80
		US-A- 4200649	29-04-80
		US-A- 4206232	03-06-80
		US-A- 4192881	11-03-80
		US-A- 4207336	10-06-80
		US-A- 4207337	10-06-80
EP-A- 0274453	13-07-88	FR-A- 2609289	08-07-88
		JP-A- 63258449	25-10-88
EP-A- 0214639	18-03-87	US-A- 4599361	08-07-86
		US-A- 4743587	10-05-88
		AU-B- 588362	14-09-89
		AU-A- 6240886	12-03-87
		JP-A- 62103052	13-05-87

EPO FORM P0479

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C07C 311/29, C07D 295/18, 213/56, A61K 31/535, 31/44, 31/495, 31/40, 31/18

A1

(11) International Publication Number: **WO 96/27583**

(43) International Publication Date: 12 September 1996 (12.09.96)

(21) International Application Number: PCT/US96/02679

(22) International Filing Date: 7 March 1996 (07.03.96)

(30) Priority Data:
08/401,049 8 March 1995 (08.03.95) US

(60) Parent Application or Grant
(63) Related by Continuation
US 08/401,049 (CON)
Filed on 8 March 1995 (08.03.95)

(71) Applicant *(for all designated States except US)*: PFIZER INC. [US/US]; 235 East 42nd Street, New York, NY 10017-5755 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: ROBINSON, Ralph, P. [US/US]; 30 Friartuck Drive, Gales Ferry, CT 06335 (US). RIZZI, James, P. [US/US]; 34 Devonshire Drive, Waterford, CT 06335 (US).

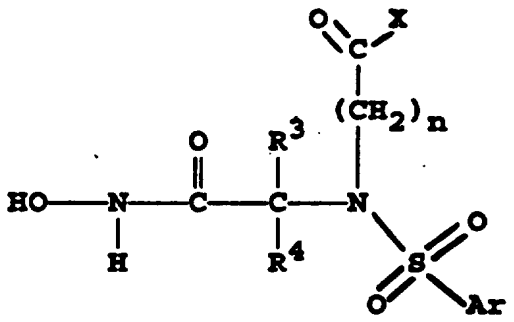
(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: ARYLSULFONYLAMINO HYDROXAMIC ACID DERIVATIVES

(57) Abstract

A compound of formula (I), wherein n, X, R^3, R^4 and Ar are as defined above, useful in the treatment of a condition selected from the group consisting of arthritis, cancer, tissue ulceration, restenosis, periodontal disease, epidermolysis bullosa, scleritis and other diseases characterized by matrix metalloproteinase activity, AIDS, sepsis, septic shock and other diseases involving the production of TNF.



(I)

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	**GB**	United Kingdom	**MW**	Malawi
AT	Austria	**GE**	Georgia	**MX**	Mexico
AU	Australia	**GN**	Guinea	**NE**	Niger
BB	Barbados	**GR**	Greece	**NL**	Netherlands
BE	Belgium	**HU**	Hungary	**NO**	Norway
BF	Burkina Faso	**IE**	Ireland	**NZ**	New Zealand
BG	Bulgaria	**IT**	Italy	**PL**	Poland
BJ	Benin	**JP**	Japan	**PT**	Portugal
BR	Brazil	**KE**	Kenya	**RO**	Romania
BY	Belarus	**KG**	Kyrgystan	**RU**	Russian Federation
CA	Canada	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CF	Central African Republic			**SE**	Sweden
CG	Congo	**KR**	Republic of Korea	**SG**	Singapore
CH	Switzerland	**KZ**	Kazakhstan	**SI**	Slovenia
CI	Côte d'Ivoire	**LI**	Liechtenstein	**SK**	Slovakia
CM	Cameroon	**LK**	Sri Lanka	**SN**	Senegal
CN	China	**LR**	Liberia	**SZ**	Swaziland
CS	Czechoslovakia	**LT**	Lithuania	**TD**	Chad
CZ	Czech Republic	**LU**	Luxembourg	**TG**	Togo
DE	Germany	**LV**	Latvia	**TJ**	Tajikistan
DK	Denmark	**MC**	Monaco	**TT**	Trinidad and Tobago
EE	Estonia	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**UG**	Uganda
FI	Finland	**ML**	Mali	**US**	United States of America
FR	France	**MN**	Mongolia	**UZ**	Uzbekistan
GA	Gabon	**MR**	Mauritania	**VN**	Viet Nam

ARYLSULFONYLAMINO HYDROXAMIC ACID DERIVATIVES

Background of the Invention

The present invention relates to arylsulfonylamino hydroxamic acid derivatives which are inhibitors of matrix metalloproteinases or the production of tumor necrosis factor (hereinafter also referred to as TNF) and as such are useful in the treatment of a condition selected from the group consisting of arthritis, cancer, tissue ulceration, restenosis, periodontal disease, epidermolysis bullosa, scleritis and other diseases characterized by matrix metalloproteinase activity, AIDS, sepsis, septic shock and other diseases involving the production of TNF.

This invention also relates to a method of using such compounds in the treatment of the above diseases in mammals, especially humans, and to the pharmaceutical compositions useful therefor.

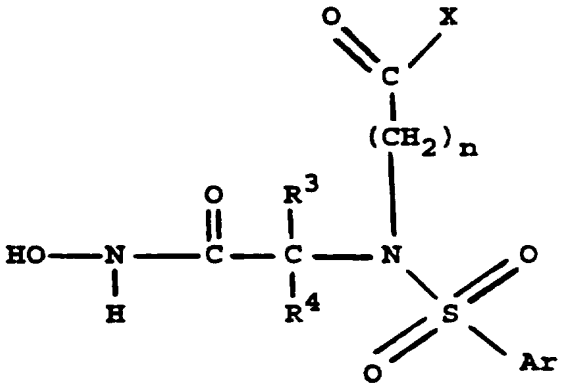
There are a number of enzymes which effect the breakdown of structural proteins and which are structurally related metalloproteases. Matrix-degrading metalloproteinases, such as gelatinase, stromelysin and collagenase, are involved in tissue matrix degradation (e.g. collagen collapse) and have been implicated in many pathological conditions involving abnormal connective tissue and basement membrane matrix metabolism, such as arthritis (e.g. osteoarthritis and rheumatoid arthritis), tissue ulceration (e.g. corneal, epidermal and gastric ulceration), abnormal wound healing, periodontal

disease, bone disease (e.g. Paget's disease and osteoporosis), tumor metastasis or invasion, as well as HIV-infection (J. Leuk. Biol., 52 (2):244-248, 1992).

Tumor necrosis factor is recognized to be involved in many infectious and auto-immune diseases (W. Friers, FEBS Letters, 1991, 285, 199). Furthermore, it has been shown that TNF is the prime mediator of the inflammatory response seen in sepsis and septic shock (C.E. Spooner et al., Clinical Immunology and Immunopathology, 1992, 62 S11).

Summary of the Invention

The present invention relates to a compound of the formula



I

or the pharmaceutically acceptable salts thereof, wherein

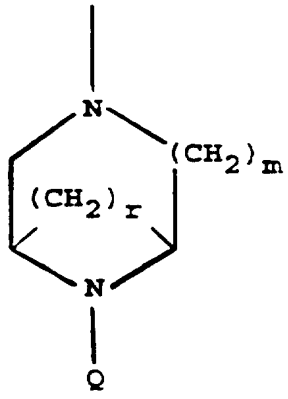
n is 1 to 6;

X is hydroxy, (C_1-C_6)alkoxy or NR^1R^2 wherein R^1 and R^2 are each independently selected from the group consisting of hydrogen, (C_1-C_6)alkyl, piperidyl, (C_1-C_6)alkylpiperidyl, (C_6-C_{10})arylpiperidyl, (C_5-C_9)heteroarylpiperidyl, (C_6-C_{10})aryl(C_1-C_6)alkylpiperidyl, (C_5-C_9)heteroaryl(C_1-C_6)alkylpiperidyl, (C_1-C_6)acylpiperidyl, (C_6-C_{10})aryl, (C_5-C_9)heteroaryl, (C_6-C_{10})aryl(C_1-C_6)alkyl, (C_5-C_9)heteroaryl(C_1-C_6)alkyl, (C_6-C_{10})aryl(C_6-C_{10})aryl, (C_6-C_{10})aryl(C_6-C_{10})aryl(C_1-C_6)alkyl, (C_3-C_6)cycloalkyl, (C_3-C_6)cycloalkyl(C_1-C_6)alkyl, $R^5(C_2-$

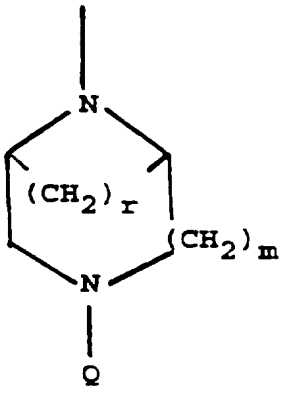
C_6)alkyl, (C_1-C_5)alkyl(CHR^5)(C_1-C_6)alkyl wherein R^5 is hydroxy, (C_1-C_6)acyloxy, (C_1-C_6)alkoxy, piperazino, (C_1-C_6)alkylamino, (C_1-C_6)alkylthio, (C_6-C_{10})arylthio, (C_1-C_6)alkylsulfinyl, (C_6-C_{10})arylsulfinyl, (C_1-C_6)alkylsulfoxyl, (C_6-C_{10})arylsulfoxyl, amino, (C_1-C_6)alkylamino, ((C_1-C_6)alkyl)$_2$amino, (C_1-C_6)acylpiperazino, (C_1-C_6)alkylpiperazino, (C_6-C_{10})aryl(C_1-C_6)alkylpiperazino, (C_5-C_9)heteroaryl(C_1-C_6)alkylpiperazino, morpholino, thiomorpholino, piperidino or pyrrolidino; R^6 (C_1-C_6)alkyl, (C_1-C_5)alkyl(CHR^6)(C_1-C_6)alkyl wherein R^6 is piperidyl, (C_1-C_6)alkylpiperidyl, (C_6-C_{10})arylpiperidyl, (C_6-C_{10})aryl(C_1-C_6)alkylpiperidyl, (C_5-C_9)heteroarylpiperidyl or (C_5-C_9)heteroaryl(C_1-C_6)alkylpiperidyl; and CH(R^7)COR^8 wherein R^7 is hydrogen, (C_1-C_6)alkyl, (C_6-C_{10})aryl(C_1-C_6)alkyl, (C_5-C_9)heteroaryl(C_1-C_6)alkyl, (C_1-C_6)alkylthio(C_1-C_6)alkyl, (C_6-C_{10})arylthio(C_1-C_6)alkyl, (C_1-C_6)alkylsulfinyl(C_1-C_6)alkyl, (C_6-C_{10})arylsulfinyl(C_1-C_6)alkyl, (C_1-C_6)alkylsulfonyl(C_1-C_6)alkyl, (C_6-C_{10})arylsulfonyl(C_1-C_6)alkyl, hydroxy(C_1-C_6)alkyl, amino(C_1-C_6)alkyl, (C_1-C_6)alkylamino(C_1-C_6)alkyl, ((C_1-C_6)alkylamino)$_2$(C_1-C_6)alkyl, R^{9}R^{10}NCO(C_1-C_6)alkyl or R^{9}OCO(C_1-C_6)alkyl wherein R^9 and R^{10} are each independently selected from the group consisting of hydrogen, (C_1-C_6)alkyl, (C_6-C_{10})aryl(C_1-C_6)alkyl and (C_5-C_9)heteroaryl(C_1-C_6)alkyl; and R^8 is R^{11}O or R^{11}R^{12}N wherein R^{11} and R^{12} are each independently selected from the group consisting of hydrogen, (C_1-C_6)alkyl, (C_6-C_{10})aryl(C_1-C_6)alkyl and (C_5-C_9)heteroaryl(C_1-C_6)alkyl;

or R^1 and R^2, or R^9 and R^{10}, or R^{11} and R^{12} may be taken together to form an azetidinyl, pyrrolidinyl, morpholinyl, thiomorpholinyl, indolinyl, isoindolinyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, (C_1-C_6)acylpiperazinyl, (C_1-C_6)alkylpiperazinyl, (C_6-C_{10})arylpiperazinyl, (C_5-

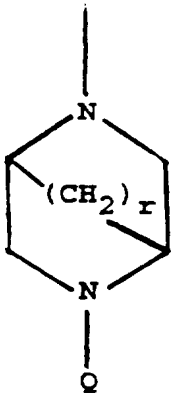
C_9)heteroarylpiperazinyl or a bridged diazabicycloalkyl ring selected from the group consisting of



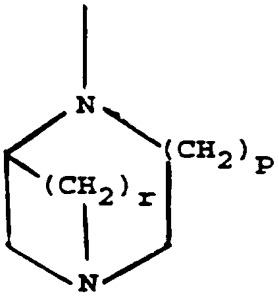
a



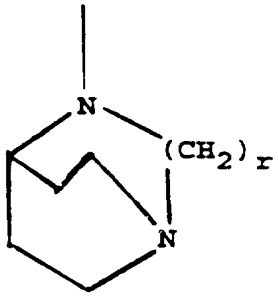
b



c



d



e

wherein r is 1, 2 or 3;

m is 1 or 2;

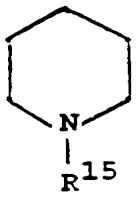
p is 0 or 1; and

Q is hydrogen, (C_1-C_3)alkyl or (C_1-C_6)acyl;

R^3 and R^4 are each independently selected from the group consisting of hydrogen, (C_1-C_6)alkyl, trifluoromethyl, trifluoromethyl(C_1-C_6)alkyl, (C_1-C_6)alkyl (difluoromethylene), (C_1-C_3)alkyl(difluoromethylene)(C_1-C_3)alkyl, (C_6-C_{10})aryl, (C_5-C_9)heteroaryl, (C_6-C_{10})aryl(C_1-C_6)alkyl, (C_5-C_9)heteroaryl(C_1-C_6)alkyl, (C_6-C_{10})aryl(C_6-C_{10})aryl, (C_6-C_{10})aryl(C_6-C_{10})aryl(C_1-C_6)alkyl, (C_3-C_6)cycloalkyl, (C_3-C_6)cycloalkyl(C_1-C_6)alkyl, hydroxy(C_1-C_6)alkyl, (C_1-C_6)acyloxy(C_1-C_6)alkyl, (C_1-C_6)alkoxy(C_1-C_6)alkyl, piperazinyl(C_1-C_6)alkyl, (C_1-

C_6)acylamino(C_1-C_6)alkyl, piperidyl, (C_1-C_6)alkylpiperidyl, (C_6-C_{10})aryl(C_1-C_6)alkoxy(C_1-C_6)alkyl, (C_5-C_9)heteroaryl(C_1-C_6)alkoxy(C_1-C_6)alkyl, (C_1-C_6)alkylthio(C_1-C_6)alkyl, (C_6-C_{10})arylthio(C_1-C_6)alkyl, (C_1-C_6)alkylsulfinyl(C_1-C_6)alkyl, (C_6-C_{10})arylsulfinyl(C_1-C_6)alkyl, (C_1-C_6)alkylsulfonyl(C_1-C_6)alkyl, (C_6-C_{10})arylsulfonyl(C_1-C_6)alkyl, amino(C_1-C_6)alkyl, (C_1-C_6)alkylamino(C_1-C_6)alkyl, ((C_1-C_6)alkylamino)$_2$(C_1-C_6)alkyl, $R^{13}CO$(C_1-C_6)alkyl wherein R^{13} is $R^{20}O$ or $R^{20}R^{21}N$ wherein R^{20} and R^{21} are each independently selected from the group consisting of hydrogen, (C_1-C_6)alkyl, (C_6-C_{10})aryl(C_1-C_6)alkyl or (C_5-C_9)heteroaryl(C_1-C_6)alkyl; or R^{14}(C_1-C_6)alkyl wherein R^{14} is (C_1-C_6)acylpiperazino, (C_6-C_{10})arylpiperazino, (C_5-C_9)heteroarylpiperazino, (C_1-C_6)alkylpiperazino, (C_6-C_{10})aryl(C_1-C_6)alkylpiperazino, (C_5-C_9)heteroaryl(C_1-C_6)alkylpiperazino, morpholino, thiomorpholino, piperidino, pyrrolidino, piperidyl, (C_1-C_6)alkylpiperidyl, (C_6-C_{10})arylpiperidyl, (C_5-C_9)heteroarylpiperidyl, (C_6-C_{10})aryl(C_1-C_6)alkylpiperidyl, (C_5-C_9)heteroaryl(C_1-C_6)alkylpiperidyl or (C_1-C_6)acylpiperidyl;

or R^3 and R^4, or R^{20} and R^{21} may be taken together to form a (C_3-C_6)cycloalkyl, oxacyclohexyl, thiocyclohexyl, indanyl or tetralinyl ring or a group of the formula



wherein R^{15} is hydrogen, (C_1-C_6)acyl, (C_1-C_6)alkyl, (C_6-C_{10})aryl(C_1-C_6)alkyl, (C_5-C_9)heteroaryl(C_1-C_6)alkyl or (C_1-C_6)alkylsulfonyl; and

Ar is (C_6-C_{10})aryl, (C_5-C_9)heteroaryl, (C_1-C_6)alkyl(C_6-C_{10})aryl, (C_1-C_6)alkoxy(C_6-C_{10})aryl, ((C_1-

C_6)alkoxy)$_2$(C_6-C_{10})aryl, (C_6-C_{10})aryloxy(C_6-C_{10})aryl, (C_5-C_9)heteroaryloxy(C_6-C_{10})aryl, (C_1-C_6)alkyl(C_5-C_9)heteroaryl, (C_1-C_6)alkoxy(C_5-C_9)heteroaryl, ((C_1-C_6)alkoxy)$_2$(C_5-C_9)heteroaryl, (C_6-C_{10})aryloxy(C_5-C_9)heteroaryl, (C_5-C_9)heteroaryloxy(C_5-C_9)heteroaryl;

with the proviso that when either R^1 or R^2 is $CH(R^7)COR^8$ wherein R^7 and R^8 are as defined above, the other of R^1 or R^2 is hydrogen, (C_1-C_6)alkyl or benzyl.

The term "alkyl", as used herein, unless otherwise indicated, includes saturated monovalent hydrocarbon radicals having straight, branched or cyclic moieties or combinations thereof.

The term "alkoxy", as used herein, includes O-alkyl groups wherein "alkyl" is defined above.

The term "aryl", as used herein, unless otherwise indicated, includes an organic radical derived from an aromatic hydrocarbon by removal of one hydrogen, such as phenyl or naphthyl, optionally substituted by 1 to 3 substituents selected from the group consisting of fluoro, chloro, trifluoromethyl, (C_1-C_6)alkoxy, (C_6-C_{10})aryloxy, trifluoromethoxy, difluoromethoxy and (C_1-C_6)alkyl.

The term "heteroaryl", as used herein, unless otherwise indicated, includes an organic radical derived from an aromatic heterocyclic compound by removal of one hydrogen, such as pyridyl, furyl, pyroyl, thienyl, isothiazolyl, imidazolyl, benzimidazolyl, terazolyl, pyrazinyl, pyrimidyl, quinolyl, isoquinolyl, benzofuryl, isobenzofuryl, benzothienyl, pyrazolyl, indolyl, isoindolyl, purinyl, carbazolyl, isoxazolyl, thiazolyl, oxazolyl, benzthiazolyl or benzoxazolyl, optionally substituted by 1 to 2 substituents selected from the group consisting of fluoro, chloro, trifluoromethyl, (C_1-C_6)alkoxy, (C_6-C_{10})aryloxy, trifluoromethoxy, difluoromethoxy and (C_1-C_6)alkyl.

The term "acyl", as used herein, unless

otherwise indicated, includes a radical of the general formula RCO wherein R is alkyl, alkoxy, aryl, arylalkyl or aryalkyloxy and the terms "alkyl" or "aryl" are as defined above.

The term "acyloxy", as used herein, includes O-acyl groups wherein "acyl" is defined above.

The compound of formula I may have chiral centers and therefore exist in different enantiomeric forms. This invention relates to all optical isomers and stereoisomers of the compounds of formula I and mixtures thereof.

Preferred compounds of formula I include those wherein n is 2.

Other preferred compounds of formula I include those wherein Ar is 4-methoxyphenyl or 4-phenoxyphenyl.

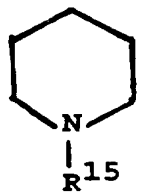
Other preferred compounds of formula I include those wherein either R^3 or R^4 is not hydrogen.

Other preferred compounds of formula I include those wherein n is 1 and either R^1 or R^2 is hydrogen.

Other preferred compounds of formula I include those wherein X is hydroxy, Ar is 4-methoxyphenyl or 4-phenoxyphenyl and either R^3 or R^4 is not hydrogen.

Other preferred compounds of formula I include those wherein X is alkoxy, Ar is 4-methoxyphenyl or 4-phenoxyphenyl and either R^3 or R^4 is not hydrogen.

Other preferred compounds of formula I include those wherein Ar is 4-methoxyphenyl or 4-phenoxyphenyl and R^3 and R^4 are taken together to form (C_3-C_6)cycloalkanyl, oxacyclohexanyl, thiocyclohexanyl, indanyl or a group of the formula



wherein R^{15} is (C_1-C_6)acyl, (C_1-C_6)alkyl, (C_6-C_{10})aryl(C_1-C_6)alkyl, (C_5-C_9)heteroaryl(C_1-C_6)alkyl or (C_1-C_6)alkylsulfonyl.

More preferred compounds of formula I are those wherein n is 2, Ar is 4-methoxyphenyl or 4-phenoxyphenyl, R^1 and R^2 are taken together to form piperazinyl, (C_1-C_6)alkylpiperazinyl, (C_6-C_{10})aryl piperazinyl or (C_5-C_9)heteroaryl(C_1-C_6)alkylpiperazinyl, and either R^3 or R^4 is not hydrogen or both R^3 and R^4 are not hydrogen.

More preferred compounds of formula I are those wherein n is 2, Ar is 4-methoxyphenyl or 4-phenoxyphenyl, R^1 is hydrogen or (C_1-C_6)alkyl, R^2 is 2-pyridylmethyl, 3-pyridylmethyl or 4-pyridylmethyl, and either R^3 or R^4 is not hydrogen or both R^3 and R^4 are not hydrogen.

More preferred compounds of formula I are those wherein n is 1, Ar is 4-methoxyphenyl or 4-phenoxyphenyl, R^1 is hydrogen, R^2 is 2-pyridylmethyl, 3-pyridylmethyl or 4-pyridylmethyl, and either R^3 or R^4 is not hydrogen or both R^3 and R^4 are not hydrogen.

More preferred compounds of formula I are those wherein n is 2, Ar is 4-methoxyphenyl, R^1 is hydrogen or (C_1-C_6)alkyl and R^2 is R^5 (C_2-C_6)alkyl wherein R^5 is morpholino, thiomorpholino, piperidino, pyrrolidino, (C_1-C_6)acylpiperazino, (C_1-C_6)alkylpiperazino, (C_6-C_{10})arylpiperazino, (C_5-C_9)heteroarylpiperazino, (C_6-C_{10})aryl(C_1-C_6)alkylpiperazino or (C_5-C_9)heteroaryl(C_1-C_6)alkylpiperazino and either R^3 or R^4 is not hydrogen or both R^3 and R^4 are not hydrogen.

More preferred compounds of formula I are

those wherein n is 1, Ar is 4-methoxyphenyl or 4-phenoxyphenyl, R^1 is hydrogen, R^2 is R^5 (C_2-C_6)alkyl wherein R^5 is morpholino, thiomorpholino, piperidino, pyrrolidino (C_1-C_6)acylpiperazino, (C_1-C_6)alkylpiperazino, (C_6-C_{10})arylpiperazino, (C_5-C_9)heteroarylpiperazino, (C_6-C_{10})aryl(C_1-C_6)alkylpiperazino or (C_5-C_9)heteroaryl(C_1-C_6)alkylpiperazino and either R^3 or R^4 is not hydrogen or both R^3 and R^4 are not hydrogen.

Specific preferred compounds of formula I include the following:

2-(R)-N-Hydroxy-2-[(4-methoxybenzenesulfonyl)(3-morpholin-4-yl-3-oxopropyl)amino]-3-methylbutyramide;

2-(R)-2-[(2-Benzylcarbamoylethyl)(4-methoxybenzenesulfonyl)amino]-N-hydroxy-3-methylbutyramide;

2-(R)-N-Hydroxy-2-((4-methoxybenzenesulfonyl)(2-[(pyridin-3-ylmethyl)-carbamoyl]ethyl)amino)-3-methylbutyramide;

2-(R)-N-Hydroxy-2-([4-methoxybenzenesulfonyl][2-(methylpyridin-3-ylmethylcarbamoyl)ethyl]amino)-3-methylbutyramide;

4-(3-[1-(R)-1-Hydroxycarbamoyl-2-methylpropyl)(4-methoxybenzenesulfonyl)amino]propionyl)piperazine-1-carboxylic acid, tert-butyl ester;

2-(R)-N-Hydroxy-2-[(4-methoxybenzenesulfonyl)(3-oxo-3-piperazin-1-ylpropyl)amino)-3-methylbutyramide hydrochloride;

2-(R)-2-[(Benzylcarbamoylmethyl)(4-methoxybenzenesulfonyl)amino]N-hydroxy-3-methylbutyramide;

2-(R)-N-Hydroxy-2-([4-methoxybenzenesulfonyl]-[(2-morpholin-4-ylethylcarbamoyl)methyl]amino)-3-methylbutyramide; and

2-(R)-N-Hydroxy-2-((4-

methoxybenzenesulfonyl)([(pyridin-3-ylmethyl)carbamoyl]methyl)amino)-3-methylbutyramide.

Other specific compounds of formula I include the following:

2-(R)-3,3,3-Trifluoro-N-hydroxy-2-[(methoxybenzenesulfonyl)(3-morpholin-4-yl-3-oxopropyl)amino]propionamide;

2-(R)-N-Hydroxy-2-((4-phenoxybenzenesulfonyl)[2-(methylpyridin-4-ylmethylcarbamoyl)ether]amino)-3-methylbutyramide;

4-[4-Methoxybenzenesulfonyl)(3-morpholin-4-yl-3-oxopropyl)amino]-1-methylpiperidene-4-carboxylic acid hydroxyamide;

2-(R)-N-Hydroxy-2-((4-methoxybenzenesulfonyl)-[3-(4-methylpiperazin-1-yl)-3-oxopropyl]amino)-3-methylbutyramide;

2-(R)-2-[(2-Carboxyethyl)(4-methoxybenzenesulfonyl)amino]-N-hydroxy-3-methylbutyramide;

[(2-Carboxyethyl)(3,4-dimethoxybenzenesulfonyl)amino]-N-hydroxy-acetamide;

2-(R)-2-[(2-Carbamoylethyl)(4-methoxybenzenesulfonyl)amino]-N-hydroxy-3-methylbutyramide;

2-(R),3-(R)-3,N-Dihydroxy-2-[(4-methoxybenzenesulfonyl)(3-oxo-3-piperidin-1-ylpropyl)amino]-butyramide;

2-(R)-N-Hydroxy-2-((4-methoxybenzenesulfonyl)[3-(methylpyridin-3-ylmethylcarbamoyl)propyl]amino)-3-methylbutyramide;

2-(R)-N-Hydroxy-2-((4-methoxybenzenesulfonyl)[2-(methylcarboxymethylcarbamoyl)ethyl]amino)-3-methylbutyramide;

2-(R)-N-Hydroxy-2-((4-methoxybenzenesulfonyl)-[(1-methylpiperidin-4-ylcarbamoyl)methyl]amino)-3-methylbutyramide;

2-(R)-2-Cyclohexyl-N-hydroxy-2-((4-methoxybenzenesulfonyl)-[3-(4-methylpiperazin-1-yl)-3-oxopropyl]amino)-acetamide;

2-(R)-N-Hydroxy-2-[(methoxybenzenesulfonyl)(3-morpholin-4-yl-3-oxopropyl)amino]-4-(morpholin-4-yl)butyramide.

The present invention also relates to a pharmaceutical composition for (a) the treatment of a condition selected from the group consisting of arthritis, cancer, tissue ulceration, restenosis, periodontal disease, epidermolysis bullosa, scleritis and other diseases characterized by matrix metalloproteinase activity, AIDS, sepsis, septic shock and other diseases involving the production of tumor necrosis factor (TNF) or (b) the inhibition of matrix metalloproteinases or the production of tumor necrosis factor (TNF) in a mammal, including a human, comprising an amount of a compound of claim 1 or a pharmaceutically acceptable salt thereof, effective in such treatments and a pharmaceutically acceptable carrier.

The present invention also relates to a method for the inhibition of (a) matrix metalloproteinases or (b) the production of tumor necrosis factor (TNF) in a mammal, including a human, comprising administering to said mammal an effective amount of a compound of claim 1 or a pharmaceutically acceptable salt thereof.

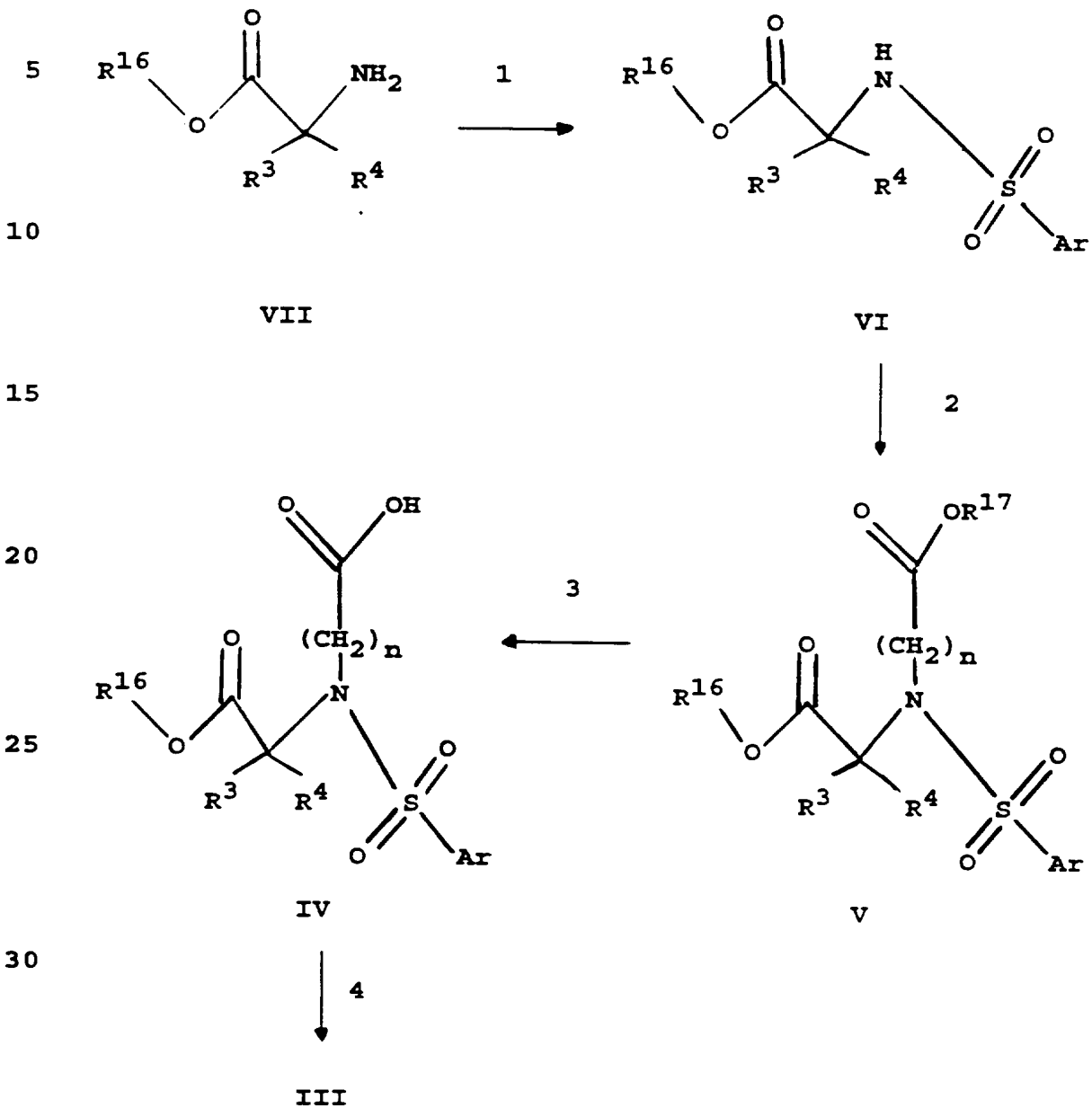
The present invention also relates to a method for treating a condition selected from the group consisting of arthritis, cancer, tissue ulceration, restenosis, periodontal disease, epidermolysis bullosa, scleritis and other diseases characterized by matrix metalloproteinase activity, AIDS, sepsis, septic shock and other diseases involving the production of tumor necrosis factor (TNF) in a mammal, including a human, comprising administering to said mammal an amount of a

compound of claim 1 or a pharmaceutically acceptable salt thereof, effective in treating such a condition.

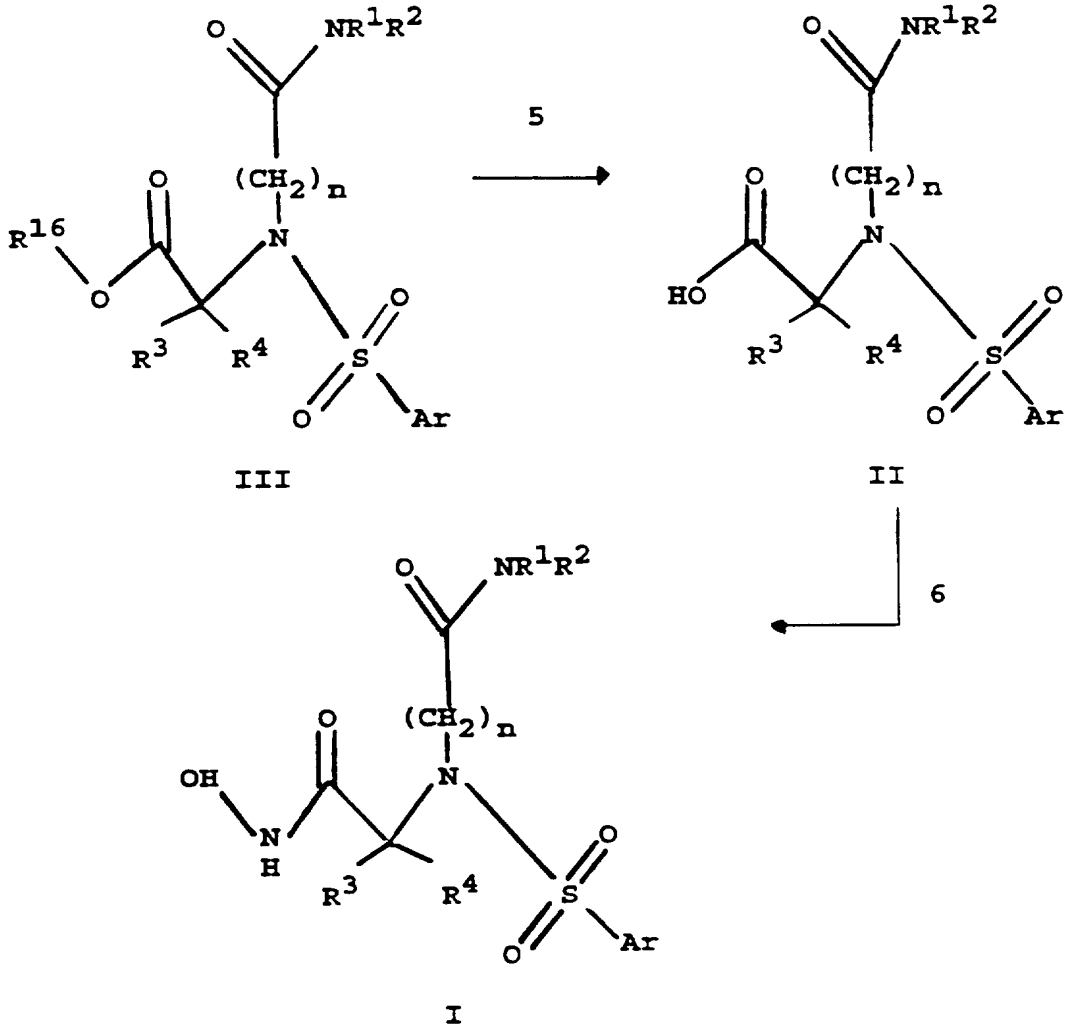
Detailed Description of the Invention

The following reaction Schemes illustrate the preparation of the compounds of the present invention. Unless otherwise indicated R^1, R^2, R^3, R^4, n and Ar in the reaction Schemes and the discussion that follow are defined as above.

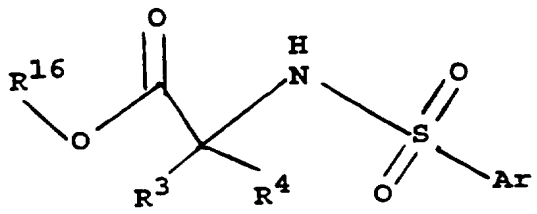
Scheme 1



Scheme 1 cont'd

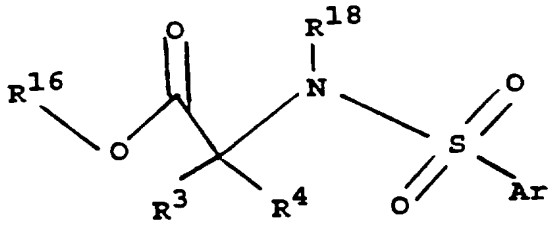


Scheme 2



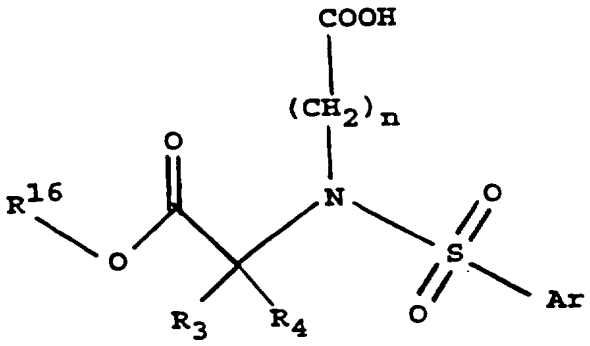
VI

1



VIII

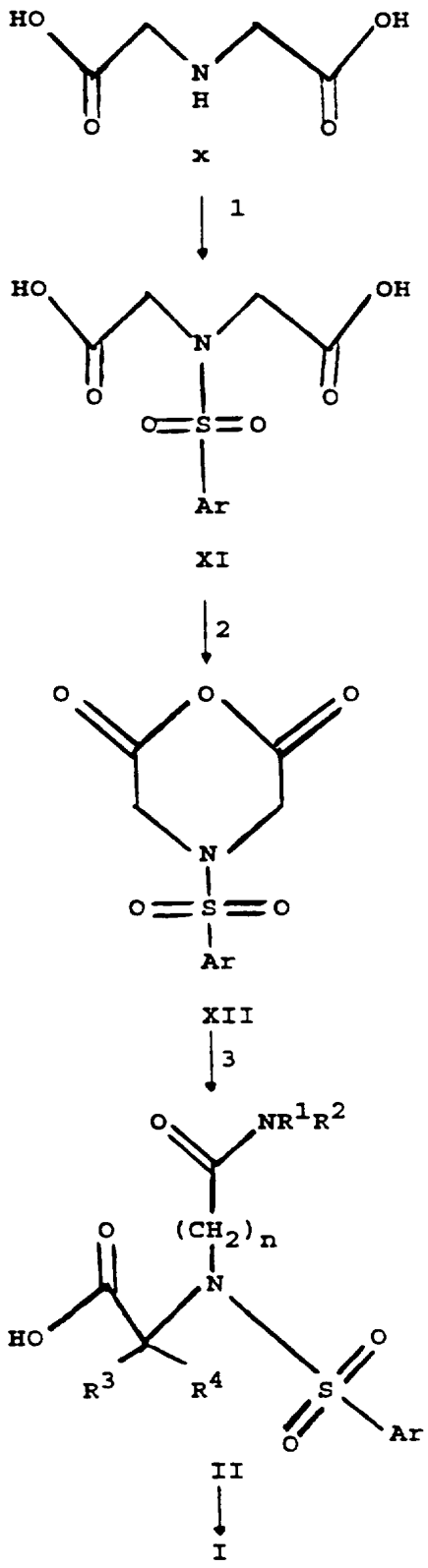
2



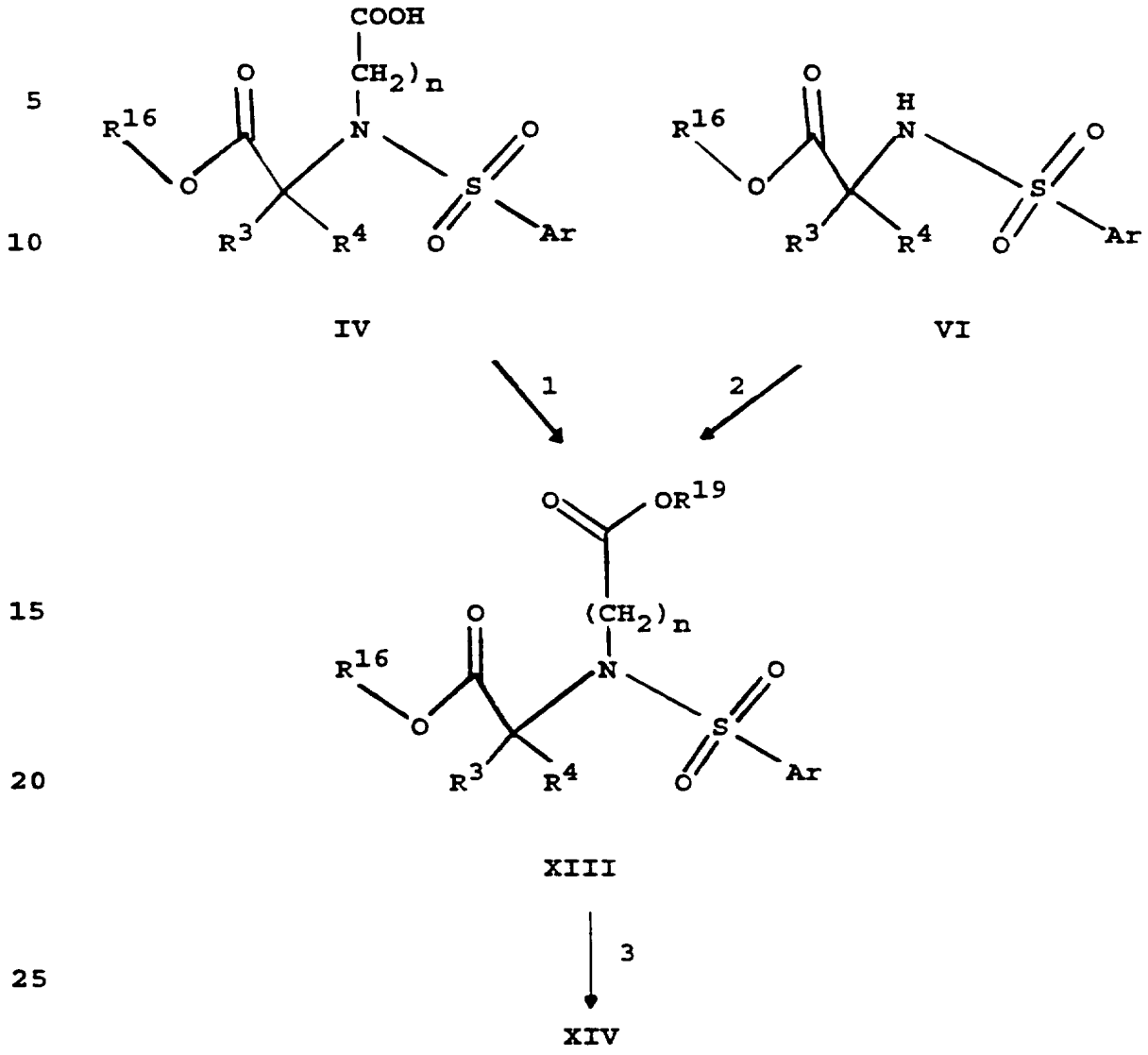
IV

I

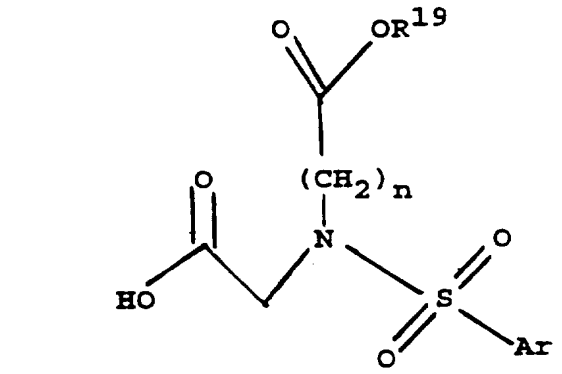
Scheme 3


HO
OH
N
H
O
O
x
1
HO
OH
N
O
O
O=S=O
Ar
XI
2
O
O
O
N
O=S=O
Ar
XII
3
O
NR1R2
(CH2)n
O
N
HO
R3
R4
S
O
O
Ar
II
I

Scheme 4

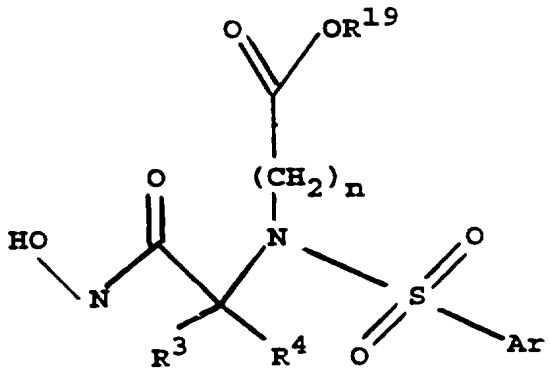


Scheme 4 cont'd



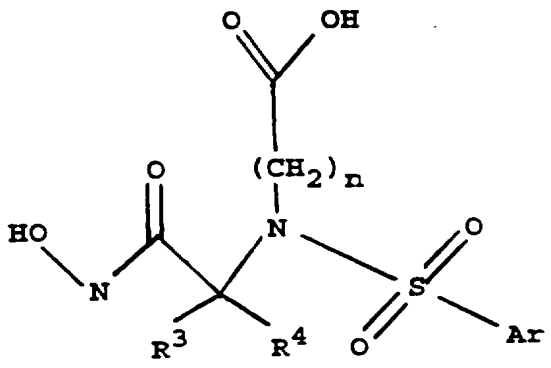
XIV

4



XV

5



XVI

In reaction 1 of Scheme 1, the amino acid compound of formula VII, wherein R^{16} is (C_1-C_6)alkyl, benzyl, allyl or tert-butyl, is converted to the corresponding compound of formula VI by reacting VII with a reactive functional derivative of an arylsulfonic acid compound, such as an arylsulfonyl chloride, in the presence of a base, such as triethylamine, and a polar solvent, such as tetrahydrofuran, dioxane, water or acetonitrile, preferably a mixture of dioxane and water. The reaction mixture is stirred, at room temperature, for time period between about 10 minutes to about 24 hours, preferably about 60 minutes.

In reaction 2 of Scheme 1, the arylsulfonyl amino compound of formula VI, wherein R^{16} is (C_1-C_6)alkyl, benzyl, allyl or tert-butyl, is converted to the corresponding compound of formula V, wherein n is 1, 3, 4, 5 or 6, by reacting VI with a reactive derivative of an alcohol of the formula

$$R^{17}\text{-O-}\overset{\overset{O}{\|}}{C}\text{-}(CH_2)_n\text{-OH}$$

such as the chloride, bromide or iodide derivative, preferably the bromide derivative, wherein the R^{17} protecting group is (C_1-C_6)alkyl, benzyl, allyl or tert-butyl, in the presence of a base such as potassium carbonate or sodium hydride, preferably sodium hydride, and a polar solvent, such as dimethylformamide. The reaction mixture is stirred, at room temperature, for a time period between about 60 minutes to about 48 hours, preferably about 18 hours. The R^{17} protecting group is chosen such that it may be selectively removed in the presence of and without loss of the R^{16} protecting group, therefore, R^{17} cannot be the same as R^{16}. Removal of the R^{17} protecting group from the compound of formula V to give the corresponding carboxylic acid of formula IV, in reaction 3 of Scheme 1, is carried out under conditions appropriate for that particular

R^{17} protecting group in use which will not affect the R^{16} protecting group. Such conditions include; (a) saponification where R^{17} is (C_1-C_6)alkyl and R^{16} is tert-butyl, (b) hydrogenolysis where R^{17} is benzyl and R^{16} is tert-butyl or (C_1-C_6)alkyl, (c) treatment with a strong acid such as trifluoroacetic acid or hydrochloric acid where R^{17} is tert-butyl and R^{16} is (C_1-C_6)alkyl, benzyl or allyl, or (d) treatment with tributyltinhydride and acetic acid in the presence of catalytic bis(triphenylphosphine) palladium (II) chloride where R^{17} is allyl and R^{16} is (C_1-C_6)alkyl, benzyl or tert-butyl.

In reaction 4 of Scheme 1, the carboxylic acid of formula IV is condensed with an amine, R^1R^2NH, or the salt thereof, to give the corresponding amide compound of formula III. The formation of amides from primary or secondary amines or ammonia and carboxylic acids is achieved by conversion of the carboxylic acid to an activated functional derivative which subsequently undergoes reaction with a primary or secondary amine or ammonia to form the amide. The activated functional derivative may be isolated prior to reaction with the primary or secondary amine or ammonia. Alternatively, the carboxylic acid may be treated with oxalyl chloride or thionyl chloride, neat or in an inert solvent, such as chloroform, at a temperature between about 25°C. to about 80°C., preferably about 50°C., to give the corresponding acid chloride functional derivative. The inert solvent and any remaining oxalyl chloride or thionyl chloride is then removed by evaporation under vacuum. The remaining acid chloride functional derivative is then reacted with the primary or secondary amine or ammonia in an inert solvent, such as methylene chloride, to form the amide. The preferred method for the condensation of the carboxylic acid of formula IV with an amine to provide the corresponding amide compound of

formula III is the treatment of IV with (benzotriazol-1-yloxy)tris(dimethylamino) phosphonium hexafluorophosphate in the presence of a base, such as triethylamine, to provide the benzotriazol-1-oxy ester in situ which, in turn, reacts with the amine, R^1R^2N, in an inert solvent, such as methylene chloride, at room temperature to give the amide compound of formula III.

Removal of the R^{16} protecting group from the compound of formula III to give the corresponding carboxylic acid of formula II, in reaction 5 of Scheme 1, is carried out under conditions appropriate for the particular R^{16} protecting group in use. Such conditions include; (a) saponification where R^{16} is lower alkyl, (b) hydrogenolysis where R^{16} is benzyl, (c) treatment with a strong acid, such as trifluoroacetic acid or hydrochloric acid, where R^{16} is tert-butyl, or (d) treatment with tributyltinhydride and acetic acid in the presence of catalytic bis(triphenylphosphine) palladium (II) chloride where R^{16} is allyl.

In reaction 6 of Scheme 1, the carboxylic acid compound of formula II is converted to the hydroxamic acid compound of formula I by treating II with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide and 1-hydroxybenztriazole in a polar solvent, such as dimethylformamide, followed by the addition of hydroxylamine to the reaction mixture after a time period between about 15 minutes to about 1 hour, preferably about 30 minutes. The hydroxylamine is preferably generated in situ from a salt form, such as hydroxylamine hydrochloride, in the presence of a base, such as N-methylmorpholine. Alternatively, a protected derivative of hydroxylamine or its salt form, where the hydroxyl group is protected as a tert-butyl, benzyl or allyl ether, may be used in the presence of (benzotriazol-1-yloxy)tris(dimethylamino) phosphonium

hexafluorophosphate and a base, such as N-methylmorpholine. Removal of the hydroxylamine protecting group is carried out by hydrogenolysis for a benzyl protecting group or treatment with a strong acid, such as trifluoroacetic acid, for a tert-butyl protecting group. The allyl protecting group may be removed by treatment with tributyltinhydride and acetic acid in the presence of catalytic bis(triphenylphosphine) palladium (II) chloride. N,O-bis(4-methoxybenzyl)hydroxylamine may also be used as the protected hydroxylamine derivative where deprotection is achieved using a mixture of methanesulfonic acid and trifluoroacetic acid.

In reaction 1 of Scheme 2, the arylsulfonylamino compound of formula VI, wherein R^{16} is (C_1-C_6)alkyl, benzyl or tert-butyl, is converted to the corresponding compound of formula VIII, wherein R^{18} is 2-propenyl or 3-butenyl, by reacting IX with a reactive functional derivative, such as the halide, preferably the iodide derivative, of 2-propen-1-ol when R^{18} is 2-propenyl or 3-buten-1-ol when R^{18} is 3-butenyl, in the presence of a base, such as potassium carbonate, cesium carbonate or sodium hydride, preferably sodium hydride when R^{18} is 2-propenyl or cesium carbonate when R^{18} is 3-butenyl. The reaction is stirred in a polar solvent, such as dimethylformamide, at room temperature, for a time period between about 2 hours to about 48 hours, preferably about 18 hours.

In reaction 2 of Scheme 2, the compound of formula VIII is converted to the carboxylic acid compound of formula IV, wherein n is 2. The compound of formula VIII, wherein R^{18} is 2-propenyl, is converted to the compound of formula IV, wherein n is 2, by reacting VIII with borane-dimethylsulfide complex, followed by immediate oxidation using chromium trioxide in aqueous acetic acid. The oxidative

cleavage of terminal olefins to carboxylic acids can be achieved by several methods known in the art. The preferred method for the oxidative cleavage of the compound of formula VIII, wherein R^{18} is 3-butenyl, to obtain the carboxylic acid compound of formula IV is to react VIII with sodium periodate in the presence of a catalytic amount of ruthenium (III) chloride in a mixture of carbon tetrachloride, acetonitrile and water.

The compound of formula IV, wherein n is 2, is further reacted to provide the hydroxamic acid compound of formula I, wherein n is 2, according to the procedure described above in reactions 4, 5 and 6 of Scheme 1.

An alternative method for the synthesis of the hydroxamic acid compound of formula I, wherein n is 1 and R^3 and R^4 are both hydrogen, is shown in reaction 1 of Scheme 3, beginning with reacting iminoacetic acid or a metal or ammonium salt of iminoacetic acid of formula X with a functional derivative of an arylsulfonic acid compound, such as an arylsulfonyl chloride, at room temperature, in the presence of a suitable base, such as triethylamine, and a polar solvent such as tetrahydrofuran, dioxane, water or acetonitrile, preferably a mixture of dioxane and water, to give the corresponding dicarboxylic acid compound of formula XI.

In reaction 2 of Scheme 3, the dicarboxylic acid compound of formula XI is dehydrated to give a cyclic anhydride compound of formula XII. The formation of cyclic anhydrides by dehydration of dicarboxylic acids may be achieved by a variety of means. The preferred method for the dehydration of the dicarboxylic acid compound of formula XI to give a cyclic anhydride compound of formula XII is to treat XI with an excess of acetic anhydride at a temperature between about 25°C. to about 80°C., preferably about

60°C. Excess acetic anhydride and acetic acid, a by-product of the reaction, are removed by evaporation under reduced pressure leaving the cyclic anhydride compound of formula XII.

In reaction 3 of Scheme 3, the cyclic anhydride compound of formula XII is reacted, at room temperature, with an amine, NR^1R^2, or a salt of the amine, such as the hydrochloride, in the presence of a base, such as triethylamine, to give the carboxylic acid of formula II, wherein n is 1 and R^3 and R^4 are both hydrogen. Suitable solvents for the reaction are those that will not react with the starting materials, which include chloroform, methylene chloride and dimethylformamide, preferably methylene chloride.

The compound of formula II is further reacted to give the hydroxamic acid compound of formula I, wherein n is 1 and R^3 and R^4 are both hydrogen, according to the procedure described above in reaction 6 of Scheme 1.

In reaction 1 of Scheme 4, the carboxylic acid compound of formula IV, wherein n is 2, is converted to the corresponding compound of formula V, wherein R^{19} is (C_1-C_6)alkyl or tert-butyl, by reacting IV with a compound of the formula

$$(R^{19}O)_2CHN(CH_3)_2$$

wherein R^{19} is (C_1-C_6)alkyl or tert-butyl, in an inert solvent, such as toluene, at a temperature between about 60°C. to about 100°C., preferably about 100°C., for a time period between about 1 hour to about 3 hours, preferably 2 hours. In reaction 2 of Scheme 4, the arylsulfonyl amino compound of formula VI wherein n is 1, 3, 4, 5 or 6 and R^{16} is (C_1-C_6)alkyl, benzyl, allyl or tert-butyl, is converted to the corresponding compound of formula XIII, wherein R^{19} is (C_1-C_6)alkyl or tert-butyl, by reacting VI with a reactive derivative of an alcohol of the formula

$$R^{19}\text{-O-}\overset{O}{\overset{\|}{C}}\text{-}(CH_2)_n\text{-OH}$$

such as the chloride, bromide or iodide derivative, preferably the bromide derivative, wherein R^{19} is (C_1-C_6)alkyl or tert-butyl, in the presence of a base such as potassium carbonate or sodium hydride, preferably sodium hydride, and a polar solvent, such as dimethylformamide. The reaction is stirred, at room temperature, for a time period between about 60 minutes to about 48 hours, preferably about 18 hours. The R^{16} protecting group, of the compounds of formulas IV and VI, is chosen such that it may be selectively removed in the presence of and without loss of the R^{19} (C_1-C_6)alkyl or tert-butyl group, therefore, R^{16} cannot be the same as R^{19}. Removal of the R^{16} protecting group from the compound of formula XIII to give the corresponding carboxylic acid of formula XIV, wherein n is 1 to 6, in reaction 3 of Scheme 4, is carried out under conditions appropriate for that particular R^{16} protecting group in use which will not affect the R^{19} (C_1-C_6)alkyl or tert-butyl group. Such conditions include; (a) saponification where R^{16} is (C_1-C_6)alkyl and R^{19} is tert-butyl, (b) hydrogenolysis where R^{16} is benzyl and R^{19} is tert-butyl or (C_1-C_6)alkyl, (c) treatment with a strong acid such as trifluoroacetic acid or hydrochloric acid where R^{16} is tert-butyl and R^{19} is (C_1-C_6)alkyl, or (d) treatment with tributyltinhydride and acetic acid in the presence of catalytic bis(triphenylphosphine) palladium (II) chloride where R^{16} is allyl and R^{19} is (C_1-C_6)alkyl or tert-butyl.

In reaction 4 of Scheme 4, the carboxylic acid of formula XIV is converted to the hydroxamic acid compound of formula XV, wherein n is 1 to 6, by treating XIV with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide and 1-hydroxybenztriazole in a polar solvent, such as dimethylformamide, followed by the

addition of hydroxylamine to the reaction mixture after a time period between about 15 minutes to about 1 hour, preferably about 30 minutes. The hydroxylamine is preferably generated in situ from a salt form, such as hydroxylamine hydrochloride, in the presence of a base, such as N-methylmorpholine. Alternatively, a protected derivative of hydroxylamine or its salt form, where the hydroxyl group is protected as a tert-butyl, benzyl or allyl ether, may be used in the presence of (benzotriazol-1-yloxy)tris(dimethylamino) phosphonium hexafluorophosphate and a base, such as N-methylmorpholine. Removal of the hydroxylamine protecting groups is carried out by hydrogenolysis for a benzyl protecting group or treatment with a strong acid, such as trifluoroacetic acid, for a tert-butyl protecting group. The allyl protecting group may be removed by treatment with tributyltinhydride and acetic acid in the presence of catalytic bis(triphenylphosphine) palladium (II) chloride. N,O-bis(4-methoxybenzyl)hydroxylamine may also be used, when R^{19} is (C_1-C_6)alkyl, as the protected hydroxylamine derivative where deprotection is achieved using a mixture of methanesulfonic acid and trifluoroacetic acid.

In reaction 5 of Scheme 4, the amide formula of formula XV is, if desired, converted to the corresponding carboxylic acid compound of formula XVI by (a) saponification where R^{19} is lower alkyl or (b) treatment with a strong acid, such as trifluoroacetic acid or hydrochloric acid, where R^{19} is tert-butyl.

Pharmaceutically acceptable salts of the acidic compounds of the invention are salts formed with bases, namely cationic salts such as alkali and alkaline earth metal salts, such as sodium, lithium, potassium, calcium, magnesium, as well as ammonium salts, such as ammonium, trimethyl-ammonium, diethylammonium, and tris-(hydroxymethyl)-

methylammonium salts.

Similarly acid addition salts, such as of mineral acids, organic carboxylic and organic sulfonic acids e.g. hydrochloric acid, methanesulfonic acid, maleic acid, are also possible provided a basic group, such as pyridyl, constitutes part of the structure.

The ability of the compounds of formula I or their pharmaceutically acceptable salts (hereinafter also referred to as the compounds of the present invention) to inhibit matrix metalloproteinases or the production of tumor necrosis factor (TNF) and, consequently, demonstrate their effectiveness for treating diseases characterized by matrix metalloproteinase or the production of tumor necrosis factor is shown by the following in vitro assay tests.

Biological Assay

Inhibition of Human Collagenase (MMP-1)

Human recombinant collagenase is activated with trypsin using the following ratio: 10 μg trypsin per 100 μg of collagenase. The trypsin and collagenase are incubated at room temperature for 10 minutes then a five fold excess (50 μg/10 μg trypsin) of soybean trypsin inhibitor is added.

10 mM stock solutions of inhibitors are made up in dimethyl sulfoxide and then diluted using the following Scheme:

10 mM ---> 120 μM ---> 12 μM ---> 1.2 μM ---> 0.12 μM

Twenty-five microliters of each concentration is then added in triplicate to appropriate wells of a 96 well microfluor plate. The final concentration of inhibitor will be a 1:4 dilution after addition of enzyme and substrate. Positive controls (enzyme, no inhibitor) are set up in wells D1-D6 and blanks (no enzyme, no inhibitors) are set in wells D7-D12.

Collagenase is diluted to 400 ng/ml and 25 μl is then added to appropriate wells of the microfluor plate. Final concentration of collagenase in the assay

is 100 ng/ml.

Substrate (DNP-Pro-Cha-Gly-Cys(Me)-His-Ala-Lys(NMA)-NH_2) is made as a 5 mM stock in dimethyl sulfoxide and then diluted to 20 μM in assay buffer. The assay is initiated by the addition of 50 μl substrate per well of the microfluor plate to give a final concentration of 10 μM.

Fluorescence readings (360 nM excitation, 460 nm emission) were taken at time 0 and then at 20 minute intervals. The assay is conducted at room temperature with a typical assay time of 3 hours.

Fluorescence vs time is then plotted for both the blank and collagenase containing samples (data from triplicate determinations is averaged). A time point that provides a good signal (the blank) and that is on a linear part of the curve (usually around 120 minutes) is chosen to determine IC_{50} values. The zero time is used as a blank for each compound at each concentration and these values are subtracted from the 120 minute data. Data is plotted as inhibitor concentration vs % control (inhibitor fluorescence divided by fluorescence of collagenase alone x 100). IC_{50}'s are determined from the concentration of inhibitor that gives a signal that is 50% of the control.

If IC_{50}'s are reported to be <0.03 μM then the inhibitors are assayed at concentrations of 0.3 μM, 0.03 μM, 0.03 μM and 0.003 μM.

Inhibition of Gelatinase (MMP-2)

Inhibition of gelatinase activity is assayed using the Dnp-Pro-Cha-Gly-Cys(Me)-His-Ala-Lys(NMA)-NH_2 substrate (10 μM) under the same conditions as inhibition of human collagenase (MMP-1).

72kD gelatinase is activated with 1 mM APMA (p-aminophenyl mercuric acetate) for 15 hours at 4°C. and is diluted to give a final concentration in the assay of 100 mg/ml. Inhibitors are diluted as for inhibition of human collagenase (MMP-1) to give final

concentrations in the assay of 30 μM, 3 μM, 0.3 μM and 0.03 μM. Each concentration is done in triplicate.

Fluorescence readings (360 nm excitation, 460 emission) are taken at time zero and then at 20 minutes intervals for 4 hours.

IC_{50}'s are determined as per inhibition of human collagenase (MMP-1). If IC_{50}'s are reported to be less than 0.03 μM, then the inhibitors are assayed at final concentrations of 0.3 μM, 0.03 μM, 0.003 μM and 0.003 μM.

Inhibition of Stromelysin Activity (MMP-3)

Inhibition of stromelysin activity is based on a modified spectrophotometric assay described by Weingarten and Feder (Weingarten, H. and Feder, J., Spectrophotometric Assay for Vertebrate Collagenase, Anal. Biochem. 147, 437-440 (1985)). Hydrolysis of the thio peptolide substrate [Ac-Pro-Leu-Gly-$SCH[CH_2CH(CH_3)_2]CO$-Leu-Gly-OC_2H_5] yields a mercaptan fragment that can be monitored in the presence of Ellman's reagent.

Human recombinant prostromelysin is activated with trypsin using a ratio of 1 μl of a 10 mg/ml trypsin stock per 26 μg of stromelysin. The trypsin and stromelysin are incubated at 37°C. for 15 minutes followed by 10 μl of 10 mg/ml soybean trypsin inhibitor for 10 minutes at 37°C. to quench trypsin activity.

Assays are conducted in a total volume of 250 μl of assay buffer (200 mM sodium chloride, 50 mM MES, and 10 mM calcium chloride, pH 6.0) in 96-well microliter plates. Activated stromelysin is diluted in assay buffer to 25 μg/ml. Ellman's reagent (3-Carboxy-4-nitrophenyl disulfide) is made as a 1M stock in dimethyl formamide and diluted to 5 mM in assay buffer with 50 μl per well yielding at 1 mM final concentration.

10 mM stock solutions of inhibitors are made in dimethyl sulfoxide and diluted serially in assay

buffer such that addition of 50 μL to the appropriate wells yields final concentrations of 3 μM, 0.3 μM, 0.003 μM, and 0.0003 μM. All conditions are completed in triplicate.

A 300 mM dimethyl sulfoxide stock solution of the peptide substrate is diluted to 15 mM in assay buffer and the assay is initiated by addition of 50 μl to each well to give a final concentration of 3 mM substrate. Blanks consist of the peptide substrate and Ellman's reagent without the enzyme. Product formation was monitored at 405 nm with a Molecular Devices UVmax plate reader.

IC_{50} values were determined in the same manner as for collagenase.

Inhibition of MMP-13

Human recombinant MMP-13 is activated with 2mM APMA (p-aminophenyl mercuric acetate) for 1.5 hours, at 37°C. and is diluted to 400 mg/ml in assay buffer (50 mM Tris, pH 7.5, 200 mM sodium chloride, 5mM calcium chloride, 20μM zinc chloride, 0.02% brij). Twenty-five microliters of diluted enzyme is added per well of a 96 well microfluor plate. The enzyme is then diluted in a 1:4 ratio in the assay by the addition of inhibitor and substrate to give a final concentration in the assay of 100 mg/ml.

10 mM stock solutions of inhibitors are made up in dimethyl sulfoxide and then diluted in assay buffer as per the inhibitor dilution scheme for inhibition of human collagenase (MMP-1): Twenty-five microliters of each concentration is added in triplicate to the microfluor plate. The final concentrations in the assay are 30 μM, 3 μM, 0.3 μM and 0.03 μM.

Substrate (Dnp-Pro-Cha-Gly-Cys(Me)-His-Ala-Lys(NMA)-NH_2) is prepared as for inhibition of human collagenase (MMP-1) and 50 μl is added to each well to give a final assay concentration of 10 μM.

Fluorescence readings (360 nM excitation; 450 emission) are taken at time 0 and every 5 minutes for 1 hour.

Positive controls consist of enzyme and substrate with no inhibitor and blanks consist of substrate only.

IC_{50}'s are determined as per inhibition of human collagenase (MMP-1). If IC_{50}'s are reported to be less than 0.03 μM, inhibitors are then assayed at final concentrations of 0.3 μM, 0.03 μM, 0.003 μM and 0.0003 μM.

Inhibition of TNF Production

The ability of the compounds or the pharmaceutically acceptable salts thereof to inhibit the production of TNF and, consequently, demonstrate their effectiveness for treating diseases involving the production of TNF is shown by the following in vitro assay:

Human mononuclear cells were isolated from anti-coagulated human blood using a one-step Ficoll-hypaque separation technique. The mononuclear cells were washed three times in Hanks balanced salt solution (HBSS) with divalent cations and resuspended to a density of 2 x 10^6/ml in HBSS containing 1% BSA. Differential counts determined using the Abbott Cell Dyn 3500 analyzer indicated that monocytes ranged from 17 to 24% of the total cells in these preparations.

180μ of the cell suspension was aliquoted into flate bottom 96 well plates (Costar). Additions of compounds and LPS (100ng/ml final concentration) gave a final volume of 200μl. All conditions were performed in triplicate. After a four hour incubation at 37°C. in an humidified CO_2 incubator, plates were removed and centrifuged (10 minutes at approximately 250 x g) and the supernatants removed and assayed for TNFα using the R&D ELISA Kit.

For administration to humans for the inhibition of matrix metalloproteinases or the

production of tumor necrosis factor (TNF), a variety of conventional routes may be used including orally, parenterally and topically. In general, the active compound will be administered orally or parenterally at dosages between about 0.1 and 25 mg/kg body weight of the subject to be treated per day, preferably from about 0.3 to 5 mg/kg. However, some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject.

The compounds of the present invention can be administered in a wide variety of different dosage forms, in general, the therapeutically effective compounds of this invention are present in such dosage forms at concentration levels ranging from about 5.0% to about 70% by weight.

For oral administration, tablets containing various excipients such as microcrystalline cellulose, sodium citrate, calcium carbonate, dicalcium phosphate and glycine may be employed along with various disintegrants such as starch (and preferably corn, potato or tapioca starch), alginic acid and certain complex silicates, together with granulation binders like polyvinylpyrrolidone, sucrose, gelation and acacia. Additionally, lubricating agents such as magnesium stearate, sodium lauryl sulfate and talc are often very useful for tabletting purposes. Solid compositions of a similar type may also be employed as fillers in gelatin capsules; preferred materials in this connection also include lactose or milk sugar as well as high molecular weight polyethylene glycols. When aqueous suspensions and/or elixirs are desired for oral administration, the active ingredient may be combined with various sweetening or flavoring agents, coloring matter or dyes, and, if so desired,

emulsifying and/or suspending agents as well, together with such diluents as water, ethanol, propylene glycol, glycerin and various like combinations thereof.

For parenteral administration (intramuscular, intraperitoneal, subcutaneous and intravenous use) a sterile injectable solution of the active ingredient is usually prepared. Solutions of a therapeutic compound of the present invention in either sesame or peanut oil or in aqueous propylene glycol may be employed. The aqueous solutions should be suitably adjusted and buffered, preferably at a pH of greater than 8, if necessary and the liquid diluent first rendered isotonic. These aqueous solutions are suitable intravenous injection purposes. The oily solutions are suitable for intraarticular, intramuscular and subcutaneous injection purposes. The preparation of all these solutions under sterile conditions is readily accomplished by standard pharmaceutical techniques well known to those skilled in the art.

The present invention is illustrated by the following examples, but it is not limited to the details thereof.

EXAMPLE 1

2-(R)-N-Hydroxy-2-[(-methoxybenzenesulfonyl)(2-morpholin-4-yl-2-oxoethyl)amino]-3-methylbutyramide

To a solution of D-valine benzyl ester hydrochloride (2.4 grams, 10 mmol) and triethylamine (2.5 grams, 3.5 mL, 25 mmol) in water (50 mL) and 1,4-dioxane (50 mL) is added 4-methoxybenzenesulfonyl chloride (2.3 grams, 11 mmol). The mixture was stirred at room temperature for 1 hour and then most of the solvent was removed by evaporation under vacuum. The mixture was diluted with ethyl acetate and was washed successively with dilute hydrochloric acid solution, water and brine. The organic solution was dried over magnesium sulfate and concentrated to leave N-(4-methoxybenzenesulfonyl)-D-valine benzyl ester as a

white solid, 3.6 grams (97%); m.p. 92-94°C.

N-(4-Methoxybenzenesulfonyl)-D-valine benzyl ester (1.50 grams, 4.0 mmol) was added to a suspension of sodium hydride (0.1 grams, 4.2 mmol) in dry dimethylformamide (20 mL) and, after 30 minutes, tert-butyl bromoacetate (0.8 mL, 4.2 mmol) was added. The resulting mixture was stirred overnight at room temperature and was then quenched by addition of saturated ammonium chloride solution (3 mL). The dimethylformamide was removed by evaporation under vacuum. The residue was taken up in ethyl acetate and washed with water and brine. After drying over magnesium sulfate, ethyl acetate was evaporated to leave an oil from which 2-(R)-2-[tert-butoxycarbonylmethyl(4-methoxybenzenesulfonyl)amino]-3-methylbutyric acid benzyl ester, a clear oil (1.92 grams, 98%), was isolated using flash chromatography on silica gel eluting with 15% ethyl acetate in hexane.

To a cold (0°C.) solution of 2-(R)-2-[tert-butoxycarbonylmethyl(4-methoxybenzenesulfonyl)amino]-3-methylbutyric acid benzyl ester (1.92 grams, 3.9 mmol) in methylene chloride (28 mL) was added trifluoroacetic acid (7 mL). The resulting solution was allowed to warm to room temperature and was stirred overnight. The methylene chloride and trifluoroacetic acid were evaporated under vacuum leaving 2-(R)-2-[carboxymethyl(4-methoxybenzenesulfonyl)amino)]-3-methylbutyric acid benzyl ester as a clear oil, 1.70 grams (100%).

To a solution of 2-(R)-2-[carboxymethyl(4-methoxybenzenesulfonyl)amino]-3-methylbutyric acid benzyl ester (573 mg, 1.32 mmol) in methylene chloride (12 mL) were added sequentially triethylamine (0.46 mL, 3.28 mmol), morpholine (0.127 mL, 1.46 mmol) and (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (646 mg, 1.46 mmol). The mixture was stirred at room temperature overnight and then

diluted with ethyl acetate. The solution was washed with 0.5 N hydrochloric acid solution and brine, dried over magnesium sulfate and concentrated under vacuum. The residue was chromatographed on silica gel using 40% ethyl acetate in hexane affording 2-(R)-2-[(4-methoxybenzenesulfonyl)(2-morpholin-4-yl-oxoethyl)amino]-3-methylbutyric acid benzyl ester as a clear oil, 590 mg (89%).

To a solution of 2-(R)-2-[(4-methoxybenzenesulfonyl)(2-morpholin-4-yl-2-oxoethyl)amino]-3-methylbutyric acid benzyl ester (590 mg, 1.17 mmol) in ethanol (50 mL) was added 10% palladium on activated carbon (200 mg). The mixture was agitated under 3 atmospheres hydrogen in a Parr shaker for 2 hours. The catalyst was removed by filtration through nylon (pore size 0.45 μm) and the solvent was evaporated leaving 2-(R)-2-[(4-methoxybenzenesulfonyl)(2-morpholin-4-yl-2-oxoethyl)amino]-3-methylbutyric acid as a white foam, 485 mg (100%).

To a solution of 2-(R)-2-[(4-methoxybenzenesulfonyl)(2-morpholin-4-yl-2-oxoethyl)amino]-3-methylbutyric acid (485 mg, 1.17 mmol) in methylene chloride (12 mL) were added sequentially triethylamine (0.52 mL, 3.71 mmol), O-benzylhydroxylamine hydrochloride (205 mg, 1.28 mmol) and (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (570 mg, 1.29 mmol). The mixture was stirred at room temperature overnight and then diluted with ethyl acetate. The solution was washed sequentially with 0.5 N hydrochloric acid solution, water, saturated sodium hydrogen carbonate solution and brine, dried over magnesium sulfate and concentrated under vacuum. The residue was chromatographed on silica gel using 20% hexane in ethyl acetate to afford 2-(R)-N-benzyloxy-2-[(4-methoxybenzenesulfonyl)(2-

morpholin-4-yl-2-oxoethyl)amino]-3-methylbutyramide as a white foam, 510 mg (84%).

To a solution of 2-(R)-N-benzyloxy-2-[(4-methoxybenzenesulfonyl)(2-morpholin-4-yl-2-oxoethyl)amino]-3-methylbutyramide (510 mg, 0.98 mmol) in methanol (50 mL) was added 5% palladium on activated carbon (120 mg). The mixture was agitated under 2 atmospheres hydrogen in a Parr shaker for 2 hours. The catalyst was removed by filtration through nylon (pore size 0.45 μm) and the solvent was evaporated leaving 2-(R)-N-hydroxy-2-[(-methoxybenzenesulfonyl)(2-morpholin-4-yl-2-oxoethyl)amino]-3-methylbutyramide as a white solid, 418 mg (99%); ^{1}H NMR ($CDCl_3$): δ 10.3 (br s, 1H), 7.90 (br s, 1H, overlapped), 7.86 (d, J = 8.8 Hz, 2H, overlapped), 6.94 (d, J = 8.8 Hz, 2H), 4.39 (d, J = 17.1 Hz, 1H), 4.09 (d, J = 17.1, 1H), 3.84 (s, 3H), 3.80-3.48 (m, 9H), 2.20-1.95 (m, 1H), 0.82 (d, J = 6.5 Hz, 3H), 0.45 (d, J = 6.5 Hz, 3H); MS (LSIMS): m/z 430 (M+H).

EXAMPLE 2

2-(R)-N-Hydroxy-2-[(4-methoxybenzenesulfonyl)(3-morpholin-4-yl-3-oxopropyl)amino]-3-methylbutyramide

To a solution of N-(4-methoxybenzenesulfonyl)-D-valine benzyl ester (2.2 grams, 5.83 mmol) in dry dimethylformamide (40 mL) were added cesium carbonate (2.3 grams, 7.1 mmol) and 1-iodo-3-butene (1.3 grams, 7.1 mmol). The mixture was stirred at room temperature overnight and was then poured into water. The mixture was extracted twice with ether and the combined ether extracts were washed with brine, dried over magnesium sulfate and concentrated under reduced pressure. The residue was taken up in 20% ethyl acetate/hexane; starting material N-(4-methoxybenzenesulfonyl)-D-valine benzyl ester (1.5 g) crystallized from the mixture and was recovered by filtration. The filtrate was concentrated under vacuum and the residue was chromatographed on silica

gel using 20% ethyl acetate/hexane as eluent to provide 2-(R)-2-[but-3-enyl(4-methoxybenzenesulfonyl)amino]-3-methylbutyric acid benzyl ester as a clear oil, 404 mg (16%).

To a mixture of 2-(R)-2-[but-3-enyl(4-methoxybenzenesulfonyl)amino]-3-methylbutyric acid benzyl ester (780 mg, 1.81 mmol) and ruthenium (III) chloride hydrate (10 mg, 0.048 mmol) in acetonitrile (6 mL), carbon tetrachloride (6 mL) and water (8 mL) was added sodium periodate (1.7 grams, 7.9 mmol). After stirring at room temperature for 2 hours, the mixture was diluted with methylene chloride and filtered through diatomaceous earth. The organic layer was separated, washed with dilute hydrochloric acid solution and brine, dried over magnesium sulfate and concentrated to leave 2-(R)-2-[2-carboxyethyl(4-methoxybenzenesulfonyl)amino]-3-methylbutyric acid benzyl ester as an oil, 710 mg (87%).

Alternatively, the intermediate 2-(R)-2-[2-carboxyethyl(4-methoxybenzenesulfonyl)amino]-3-methylbutyric acid benzyl ester was prepared by the following higher yielding procedure:

N-(4-Methoxybenzenesulfonyl)-D-valine benzyl ester (18.8 grams, 49.8 mmol) was added to a suspension of sodium hydride (1.3 grams, 54 mmol) in dry dimethylformamide (200 mL) and, after 1.5 hours, a solution of allyl bromide (4.7 mL, 54 mmol) was added. The resulting mixture was stirred overnight at room temperature and was then quenched by addition of saturated ammonium chloride solution. The dimethylformamide was removed by evaporation under vacuum. The residue was taken up in ether and washed with water and brine. After drying over magnesium sulfate, ether was evaporated to leave an oil from which 2-(R)-2-[(4-methoxybenzenesulfonyl)prop-2-enylamino]-3-methylbutyric acid benzyl ester, a clear oil (18.1 grams, 87%), was isolated using flash

chromatography on silica gel eluting with 10% ethyl acetate in hexane and then 20% ethyl acetate in hexane.

To a 1 M solution of borane/disulfide complex in methylene chloride (1.45 mL, 2.9 mmol) was added a solution of 2-(R)-2-[(4-methoxybenzenesulfonyl)prop-2-enylamino]-3-methylbutyric acid benzyl ester (3.6 grams, 8.6 mmol) in methylene chloride (8 mL). The solution was stirred at room temperature for 4 hours at which time more 1 M solution of borane/disulfide complex in methylene chloride (2.0 mL, 4.0 mmol) was added. The mixture was stirred at room temperature overnight and was then added dropwise to a mechanically stirred solution of chromium trioxide (5.1 grams, 51.6 mole) in acetic acid (31 mL) and water (3.5 mL) while keeping the internal temperature between -5°C. and 10°C. After stirring at room temperature overnight, the mixture was diluted with water and extracted with methylene chloride. The extract was washed with brine, dried (magnesium sulfate) and concentrated. The residue was chromatographed on silica gel eluting successively with chloroform and 2% methanol in chloroform to afford 2-(R)-2-[2-carboxyethyl(4-methoxybenzenesulfonyl)amino]-3-methylbutyric acid benzyl as an oil (2.42 grams, 63%).

To a solution of 2-(R)-2-[2-carboxyethyl(4-methoxybenzenesulfonyl)amino]-3-methylbutyric acid benzyl ester (710 mg, 1.58 mmol) in methylene chloride (15 mL) were added sequentially triethylamine (0.47 mL, 3.35 mmol), morpholine (0.15 mL, 1.72 mmol) and (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (769 mg, 1.74 mmol). The mixture was stirred at room temperature overnight and then diluted with methylene chloride. The solution was washed with 0.5 N hydrochloric acid solution and brine, dried over magnesium sulfate and concentrated under vacuum. The solid residue was chromatographed on silica gel using 20% hexane in ethyl acetate affording

2-(R)-2-[(4-methoxybenzenesulfonyl)(3-morpholin-4-yl-3-oxopropyl)amino]-3-methylbutyric acid benzyl ester as a clear oil, 725 mg (88%).

To a solution of 2-(R)-2-[(4-methoxybenzenesulfonyl)(3-morpholin-4-yl-3-oxopropyl)amino]-3-methylbutyric acid benzyl ester (725 mg, 1.40 mmol) in ethanol (35 mL) was added 10% palladium on activated carbon (50 mg). The mixture was agitated under 3 atmospheres hydrogen in a Parr shaker for 3 hours. The catalyst was removed by filtration through nylon (pore size 0.45 μm) and the solvent was evaporated leaving 2-(R)-(2)-[(4-methoxybenzenesulfonyl)(3-morpholin-4-yl-3-oxopropyl)amino]-3-methyl-butyric acid as a white solid, 540 mg (90%).

To a solution of 2-(R)-2-[(4-methoxybenzenesulfonyl)(3-morpholin-4-yl-3-oxopropyl)amino]-3-methylbutyric acid (540 mg, 1.26 mmol) and 1-hydroxybenztriazole hydrate (205 mg, 1.33 mmol) in dry dimethylformamide (12 mL) was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (289 mg, 1.51 mmol). After stirring for 30 minutes, hydroxylamine hydrochloride (350 mg, 5.04 mmol) and then triethylamine (1.0 mL, 7.17 mmol) were added. The mixture was stirred at room temperature overnight and then diluted with ethyl acetate. The solution was washed sequentially with water, 0.5 N hydrochloric acid solution and brine. The solution was then dried over magnesium sulfate and concentrated under vacuum to leave a white foam. The material was dissolved in toluene, filtered and concentrated. The residue was triturated with ether to afford 2-(R)-N-hydroxy-2[(4-methoxybenzenesulfonyl)(3-morpholin-4-yl-3-oxopropyl)amino]-3-methylbutyramide as a solid, 200 mg (36%); ^{1}H NMR ($CDCl_3$): δ 9.35 (br s, 1H), 7.73 (d, J = 8.9 Hz, 2H), 6.95 (d, J = 8.9 Hz, 2H), 3.86 (s, 3H), 3.83-3.73 (m, 1H), 3.70-3.52 (m, 7H), 3.46-3.43 (m,

2H), 3.41-3.29 (m, 1H), 2.92-2.69 (m, 2H), 2.30-2.17 (m, 1H), 0.84 (d, J = 6.5 Hz, 3H), 0.41 (d, J = 6.5 Hz, 3H); MS (particle beam): m/z 444 (M+H), 428, 383, 329; HRMS calculated for $C_{19}H_{30}N_3O_7S$ (M+H): 444.1804, Found: 464.1818.

The title compounds of Examples 3-6 were prepared by a method analogous to that described in Example 2 using 2-(R)-2-[2-carboxyethyl(4-methoxybenzenesulfonyl)amino]-3-methylbutyric acid benzyl ester as the starting material which is coupled with the amine indicated.

EXAMPLE 3

2-(R)-2-[(2-Benzylcarbamoylethyl)(4-methoxybenzenesulfonyl)amino]-N-hydroxy-3-methylbutyramide

Coupled with benzylamine; ^{1}H NMR (DMSO-d_6): δ 10.72 (s, 1H), 8.89 (s, 1H), 8.39 (m, 1H), 7.74 (d, J = 9.0 Hz, 2H), 7.32-7.21 (m, 5H), 7.05 (d, J = 9.0 Hz, 2H), 4.21 (d, J = 5.9 Hz, 2H), 4.02-3.87 (m, 1H), 3.82 (s, 3H), 3.63 (d, J = 10.8 Hz, 1H), 3.29-3.17 (m, 1H), 2.71-2.57 (m, 1H), 2.52-2.40 (m, 1H), 2.06-1.94 (m, 1H), 0.77 (d, J = 6.6 Hz, 3H), 0.74 (d, J = 6.5 Hz, 3H); MS (LSIMS): m/z 464 (M+H); HRMS calculated for $C_{22}H_{30}N_3O_6S$ (M+H): 464.1855. Found: 464.1832.

EXAMPLE 4

2-(R)-N-Hydroxy-2-((4-methoxybenzenesulfonyl)(2-[(pyridin-3-ylmethyl)carbamoyl]ethyl)amino)-3-methylbutyramide

Coupled with 3-pyridylmethylamine: ^{1}H NMR (DMSO-d_6): δ 10.72 (s, 1H), 8.89 (s, 1H), 8.49-8.42 (m, 3H), 7.73 (d, J = 8.9 Hz, 2H), 7.63-7.60 (m, 1H), 7.32 (dd, J = 4.8, 7.8 Hz, 1H), 7.05 (d, J = 8.9 Hz, 2H), 4.23 (d, J = 5.8 Hz, 2H), 4.00-3.88 (m, 1H), 3.81 (s, 3H), 3.62 (d, J = 10.8 Hz, 1H), 3.27-3.17 (m, 1H), 2.69-2.58 (m, 1H), 2.52-2.41 (m, 1H), 2.07-1.94 (m, 1H), 0.76 (d, J = 6.5 Hz, 3H), 0.72 (d, J = 6.4 Hz, 3H); MS (LSIMS): m/z 465 (M+H).

EXAMPLE 5

2-(R)-N-Hydroxy-2-([4-methoxybenzenesulfonyl][2-(methylpyridin-3-ylmethylcarbamoyl)ethyl]amino)-3-methylbutyramide

Coupled with 3-(N-methylaminomethyl)pyridine: ^{1}H NMR (DMSO-d_6): δ 10.75 (br s, 1H), 8.92 (s, 1H), 8.52-8.29 (m, 2H), 7.75 (d, J = 8.8 Hz, 1.4 H), 7.67 (d, J = 8.8 Hz, 0.6 H), 7.62-7.58 (m, 1H), 7.42-7.32 (m, 1H), 7.06 (d, J = 8.8 Hz, 1.4 H), 7.01 (d, J = 8.8 Hz, 0.6 H), 4.55-4.41 (m, 2H), 3.94-3.82 (m, 1H), 3.81 (s, 2.1 H), 3.80 (s, 0.9 H), 3.68-3.60 (m, 1H), 3.33-3.19 (m, 1H), 2.90-2.50 (m, 2H), 2.88 (s, 2.1 H overlapped), 2.79 (s, 0.9 H), 2.05-1.80 (m, 1H), 0.79-0.63 (m, 6H): MS (thermospray): m/z 479 (M+H), 364.

EXAMPLE 6

4-(3-[(1-(R)-1-Hydroxycarbamoyl-2-methylpropyl)(4-methoxybenzenesulfonyl)amino]propionyl)piperazine-1-carboxylic acid, tert-butyl ester

Coupled with tert-butyl-1-piperazinecarboxylate: ^{1}H NMR (DMSO-d_6): δ 10.77 (br s, 1H), 8.93 (s, 1H), 7.74 (d, J = 8.9 Hz, 2H), 7.06 (d, J = 8.9 Hz, 2H), 3.90-3.80 (m, 1H), 3.82 (s, 3H, overlapped), 3.64 (d, J = 10.8 Hz, 1H), 3.60-3.16 (m, 9H), 2.80-2.71 (m, 1H), 2.59-2.47 (m, 1H), 2.03-1.91 (m, 1H), 1.39 (s, 9H), 0.77 (d, J = 6.5 Hz, 3H), 0.71 (d, J = 6.5, 3H); MS (thermospray): m/z 543 (M+H), 443, 382, 328.

EXAMPLE 7

2-(R)-N-Hydroxy-2-[(4-methoxybenzenesulfonyl)(3-oxo-3-piperazin-1-ylpropyl)amino]-3-methylbutyramide hydrocholoride

A solution of 4-(3-[(1-(R)-1-hydroxycarbamoyl-2-methylpropyl)(4-methoxybenzenesulfonyl)amino]propionyl)piperazine-1-carboxylic acid, tert-butylester [Example 6] (430 mg, 0.79 mmol) in methylene chloride (11 mL) was cooled to

0°C. Hydrogen chloride gas was then bubbled through the solution for about 0.5 minute. The solution was allowed to warm to room temperature with stirring over 1 hour. Volatiles were removed by evaporation and the residue was filtered washing with methylene chloride to collect solid 2-(R)-N-hydroxy-2-[(4-methoxybenzenesulfonyl)(3-oxo-3-piperazin-1-ylpropyl)amino]-3-methylbutyramide hydrochloride, 375 mg (99%). ^{1}H NMR (DMSO-d_6): δ 10.78 (br s, 1H), 9.16 (br s, 1H), 7.74 (d, J = 8.8 Hz, 2H), 7.07 (d, J = 8.9 Hz, 2H), 3.82 (s, 3H), 3.62 (br s, 4H), 3.38-3.18 (m, 1H), 3.16-3.07 (br s, 2H), 3.07-2.98 (br s, 2H), 2.83-2.73 (m, 1H), 2.65-2.53 (m, 1H), 2.06-1.90 (m, 1H), 0.76 (d, J = 6.5 Hz, 3H), 0.72 (d, J = 6.5 Hz, 3H). A broad water peak between δ 4.0 and 3.5 obscured some signals from this compound; MS (thermospray): m/z 443 (M+H), 382, 328.

EXAMPLE 8

2-(R)-2-[(Benzylcarbamoylmethyl)(4-methoxybenzenesulfonyl)amino]-N-hydroxy-3-methyl-butyramide

To a solution of 2-(R)-2-[carboxymethyl(4-methoxybenzenesulfonyl)amino]-3-methylbutyric acid benzyl ester (example 1) (905 mg, 2.08 mmol) in methylene chloride (18 mL) were added sequentially triethylamine (0.72 mL, 5.14 mmol), benzylamine (0.25 mL, 2.29 mmol) and (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluoro-phosphate (1.01 grams, 2.28 mmol). The mixture was stirred at room temperature overnight and then diluted with ethyl acetate. The solution was washed with 0.5 N hydrochloric acid solution and brine, dried over magnesium sulfate and concentrated under vacuum. The residue was chromatographed on silica gel using a 2:5:16 ratio of methylene chloride/ethyl acetate/hexane affording 2-(R)-2-[(benzylcarbamoylmethyl)(4-methoxybenzenesulfonyl)amino]-3-methylbutyric acid

benzyl ester as a clear oil, 933 mg (86%).

To a solution of 2-(R)-2-[(benzylcarbamoylmethyl)(4-methoxybenzenesulfonyl)-amino]-3-methylbutyric acid benzyl ester (933 mg, 1.17 mmol) in ethanol (50 mL) was added 10% palladium on activated carbon (85 mg). The mixture was agitated under 3 atmospheres hydrogen in a Parr shaker for 4 hours. The catalyst was removed by filtration through nylon (pore size 0.45 μm) and the solvent was evaporated leaving 2-(R)-2-[(benzylcarbamoylmethyl)(4-methoxybenzenesulfonyl)amino]-3-methylbutyric acid as a white foam, 755 mg (98%).

To a solution of 2-(R)-2-[(benzylcarbamoylmethyl)(4-methoxybenzenesulfonyl)amino]-3-methylbutyric acid (655 mg, 1.51 mmol) and 1-hydroxybenztriazole hydrate (224 mg, 1.46 mmol) in dry dimethylformamide (15 mL) was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (316 mg, 1.65 mmol). After stirring for 30 minutes, hydroxylamine hydrochloride (416 mg, 6.0 mmol) and then N-methylmorpholine (0.99 mL, 9.0 mmol) were added. The mixture was stirred at room temperature overnight and then diluted with ethyl acetate. The solution was washed sequentially with water, 0.5 N hydrochloric acid solution and brine. The solution was then dried over magnesium sulfate and concentrated under vacuum to leave a white foam which was chromatographed on silica gel eluting with ethyl acetate to afford 2-(R)-2-[(benzylcarbamoylmethyl)(4-methoxybenzene-sulfonyl)amino]-N-hydroxy-3-methylbutyramide as a white foam, 570 mg (84%); ^{1}H NMR (DMSO-d_6): δ 10.75 (br s, 1H), 8.90 (s, 1H), 8.47 (m, 1H), 7.85 (d, J = 8.9 Hz, 2H), 7.83-7.19 (m, 5H), 7.04 (d, J = 8.9 Hz, 2H), 4.37 (d, J = 11.4 Hz, 1H), 4.28 (d, J = 5.9 Hz, 2H), 3.89 (d, J = 11.4 Hz, 1H), 3.82 (s, 3H), 3.45 (d, J = 10.3 Hz, 1H), 1.90-1.79 (m, 1H), 0.73 (d, J = 6.3 Hz, 6H); MS (LSIMS): m/z 450 (M+H).

EXAMPLE 9

2-(R)-2-[(Benzylmethylcarbamoylmethyl)(4-methoxybenzenesulfonyl)amino]-N-hydroxy-3-methylbutyramide

To a solution of 2-(R)-2-[carboxymethyl(4-methoxybenzenesulfonyl)amino]-3-methylbutyric acid benzyl ester (Example 1) (1.05 grams, 2.41 mmol) in methylene chloride (20 mL) were added sequentially triethylamine (0.84 mL, 6.0 mmol), N-benzylmethylamine (0.34 mL, 2.63 mmol) and (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (1.17 grams, 2.69 mmol). The mixture was stirred at room temperature overnight and then diluted with ethyl acetate. The solution was washed with 0.5 N hydrochloric acid solution and brine, dried over magnesium sulfate and concentrated under vacuum. The residue was chromatographed on silica gel using 35% ethyl acetate in hexane (plus a small amount of methylene chloride to load the sample on the column) affording 2-(R)-2-[benzylmethylcarbamoylmethyl)(4-methoxybenzenesulfonyl)amino]-3-methylbutyric acid benzyl ester as a clear oil, 1.14 grams (88%).

To a solution of 2-(R)-2-[(benzylmethylcarbamoylmethyl)(4-methoxybenzenesulfonyl)amino]-3-methylbutyric acid benzyl ester (1.14 grams, 2.12 mmol) in ethanol (100 mL) was added 10% palladium on activated carbon (400 mg). The mixture was agitated under 3 atmospheres hydrogen in a Parr shaker for 3 hours. The catalyst was removed by filtration through nylon (pore size 0.45 μm) and the solvent was evaporated leaving 2-(R)-2-[(benzylmethylcarbamoylmethyl)(4-methoxybenzenesulfonyl)amino]-3-methylbutyric acid as a white foam, 902 mg (95%).

To a solution of 2-(R)-2-[(benzylmethylcarbamoylmethyl)(4-methoxybenzenesulfonyl)amino]-3-methylbutyric acid (902

mg, 2.01 mmol) in methylene chloride (20 mL) were added sequentially triethylamine (0.90 mL, 6.42 mmol), O-allylhydroxylamine hydrochloride (242 mg, 2.21 mmol) and (benzotriazol-1-yloxy)tris-(dimethylamino)phosphonium hexafluorophosphate (978 mg, 2.21 mmol). The mixture was stirred at room temperature overnight and then diluted with ethyl acetate. The solution was washed with 0.5 N hydrochloric acid solution and brine, dried over magnesium sulfate and concentrated under vacuum. The residue was chromatographed on silica gel using 40% hexane in ethyl acetate to afford 2-(R)-N-allyloxy-2-[(benzylmethylcarbamoylmethyl)(4-methoxy-benzenesulfonyl)amino]-3-methylbutyramide as an oil, 1.008 grams (100%).

To a solution of 2-(R)-N-allyloxy-2-[(benzylmethyl-carbamoylmethyl)(4-methoxybenzenesulfonyl)amino]-3-methylbutyramide (500 mg, 0.99 mmol) in methylene chloride (40 mL) was added bis(triphenylphosphine)palladium (II) chloride (280 mg, 0.4 mmol) and then, dropwise, tributyltinhydride (0.43 mL, 2.2 mmol). The solution was stirred at room temperature for 1 hour, diluted with methylene chloride, washed with 1 N hydrochloric acid solution, dried over magnesium sulfate and concentrated. The residue was taken up in ethyl acetate and filtered to remove a solid. After concentration, the filtrate was chromatographed on silica gel eluting with chloroform and then 2% methanol in chloroform to afford 2-(R)-2-[(benzylmethylcarbamoylmethyl)(4-methoxybenzene-sulfonyl)amino]-N-hydroxy-3-methylbutyramide as a white solid (340 mg, 74%). ^{1}H NMR (DMSO-d_6): δ 10.66 (br s, 1H), 8.87 (br s, 0.6 H), 8.84 (s, 0.4 H), 7.91 (d, J = 8.9 Hz, 1.2 H), 7.78 (d, J = 8.9 Hz, 0.8 H), 7.43-7.21 (m, 5H), 7.05 (d, J = 8.9 Hz, 1.2 H), 7.00 (d, J = 8.9 Hz, 0.8 H) 4.72 (d, J = 17.7 Hz, 0.4 H), 4.70 (d, J = 17.7 Hz, 0.6 H), 4.59-4.42 (m, 1H), 4.25 (d, J = 17.8

Hz, 0.6H), 4.07 (d, J = 17.7 Hz, 0.4H), 3.82 (s, 3H), 3.46-3.40 (m, 1H), 2.91 (s, 1.8H), 2.83 (s, 1.2 H), 1.92-1.70 (m, 1H), 0.75-0.69 (m, 6H); MS (thermospray): m/z 464 (M+H), 307, 239.

The title compounds of Examples 10-11 were prepared by a method analogous to that described in Example 9 using 2-(R)-2-[carboxymethyl(4-methoxybenzenesulfonyl)amino]-3-methylbutyric acid benzyl ester (Example 1) as the starting material which is coupled with the amine indicated.

EXAMPLE 10

2-(R)-N-Hydroxy-2-([4-methoxybenzenesulfonyl]-[(2-morpholin-4-ylethylcarbamoyl)methyl]amino)-3-methylbutyramide

Coupled with 4-(2-aminoethyl)morpholine: ^{1}H NMR (DMSO-d_6): δ 10.74 (br s, 1H), 8.90 (br s, 1H), 7.84 (br s, 1H, overlapped), 7.84 (d, J = 8.8 Hz, 2H), 7.06 (d, J = 8.8 Hz, 2H), 4.33 (d, J = 17.5 Hz, 1H), 3.83 (s, 3H), 3.78 (d, J = 17.5 Hz, 1H), 3.57-3.54 (m, 4H), 3.49 (d, J = 10.2 Hz, 1H), 3.28-3.06 (m, 2H), 2.34-2.30 (m, 6H), 1.93-1.77 (m, 1H), 0.77-0.74 (m, 6H).

EXAMPLE 11

2-(R)-N-Hydroxy-2-[(4-methoxybenzenesulfonyl)(2-oxo-2-pyrrolidin-1-ylethyl)amino]-3-methylbutyramide

Coupled with pyrrolidine: ^{1}H NMR (CD_3OD): δ 7.90 (d, J = 8.9 Hz, 2H), 7.04 (d, J = 8.9 Hz, 2H), 4.50 (d, J = 17.6 Hz, 1H), 4.15 (d, J = 17.6 Hz, 1H), 3.87 (s, 3H), 3.56-3.39 (m, 5H), 2.07-1.82 (m, 5H), 0.83 (d, J = 6.6 Hz, 3H), 0.73 (d, J = 6.6 Hz, 3H); MS (thermospray): m/z 414 (M+1); HRMS calculated for $C_{18}H_{28}N_3O_6S$ (M+H): 414.1699. Found 414.1703.

EXAMPLE 12

2-[Dimethylcarbamoylmethyl(4-methoxybenzenesulfonyl)amino]-N-hydroxy-3-methyl-butyramide

A solution of 2-(R)-2-[carboxymethyl(4-methoxybenzenesulfonyl)amino]-3-methylbutyric acid benzyl ester (Example 1) (1.89 grams, 4.34 mmol) in thionyl chloride (25 mL) was heated at reflux for 1 hour. After cooling, the excess thionyl chloride was evaporated. The residue was taken up in methylene chloride (50 mL) and the solution was cooled in an ice bath. Dimethylamine gas was slowly bubbled through the solution for 1 hour. After evaporation of the solvent, the residue was taken up in ethyl acetate, washed with 1 N hydrochloric acid solution, water and brine, dried over magnesium sulfate and concentrated to leave dimethylcarbamoylmethyl(4-methoxybenzenesulfonyl)amino-3-methylbutyric acid benzyl ester as an oil, 1.77 grams (88%).

To a solution of dimethylcarbamoylmethyl(4-methoxybenzenesulfonyl)amino-3-methylbutyric acid benzyl ester (1.77 grams, 3.83 mmol) in ethanol (100 mL) was added 10% palladium on activated carbon (644 mg). The mixture was agitated under 3 atmospheres hydrogen in a Parr shaker for 1.5 hours. The catalyst was removed by filtration through nylon (pore size 0.45 μm) and the solvent was evaporated leaving dimethylcarbamoylmethyl(4-methoxybenzenesulfonyl)amino-3-methylbutyric acid as a white foam, 1.42 grams (100%).

To a solution of dimethylcarbamoylmethyl(4-methoxybenzenesulfonyl)amino-3-methylbutyric acid (1.42 grams, 3.81 mmol) and 1-hydroxybenztriazole hydrate (687 mg, 4.48 mmol) in dry dimethylformamide (7 mL) was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (974 mg, 5.08 mmol). After stirring for 30 minutes, hydroxylamine hydrochloride (1.17 grams, 16.8 mmol) and then N-methylmorpholine (2.8 mL, 25.5

mmol) were added. The mixture was stirred at room temperature overnight and then concentrated under vacuum. The residue was taken up in ethyl acetate and the resulting solution was washed sequentially with water, 0.5 N hydrochloric acid solution and brine. The solution was then dried over magnesium sulfate and concentrated under vacuum to leave an oil which was chromatographed on silica gel eluting successively with ethyl acetate, 5% methanol in chloroform and 10% methanol in chloroform to afford 2-[dimethylcarbamoylmethyl(4-methoxybenzenesulfonyl)amino]-N-hydroxy-3-methylbutyramide as a white solid, 390 mg (26%). ^{1}H NMR (DMSO-d_6): δ 10.70 (br s, 1H), 8.89 (s, 1H), 7.80 (d, J = 8.9 Hz, 2H), 7.10 (d, J = 8.9 Hz, 2H), 4.62 (d, J = 17.7 Hz, 1H), 4.14 (d, J = 17.7 Hz, 1H), 3.84 (s, 3H), 3.40 (d, J = 10.4 Hz, 1H), 2.97 (s, 3H), 2.82 (s, 3H), 1.88-1.72 (m, 1H), 0.72 (d, J = 6.5 Hz, 6H); MS (thermospray): m/z 388 (M+1); HRMS calculated for $C_{16}H_{26}N_3O_6S$ (M+H): 388.1542 Found: 388.1592.

EXAMPLE 13

2-(R)-2-N-Hydroxy-2-((4-methoxybenzenesulfonyl)([(pyridin-3-ylmethyl)carbamoyl]methyl)amino)-3-methylbutyramide

2-(R)-N-Hydroxy-2-((4-methoxybenzenesulfonyl)([(pyridin-3-ylmethyl)carbamoyl]methyl)amino)-3-methylbutyramide was prepared by a procedure similar to that of Example 12 starting with 2-(R)-2-[carboxymethyl(4-methoxybenzenesulfonyl)amino]-3-methylbutyric acid benzyl ester (Example 1) and coupling this to 3-pyridylmethylamine via the acid chloride. ^{1}H NMR (CD_3OD): δ 8.55-8.53 (m, 1H), 8.43-8.40 (m, 1H), 7.90-7.82 (m, 1H, overlapped), 7.86 (d, J = 8.9 Hz, 2H), 7.40 (dd, J = 4.8, 7.8 Hz, 1H), 7.04 (d, J = 8.9 Hz, 2H), 4.50 (d, J = 17.5 Hz, 1H), 4.39 (d, J = 17.5 Hz, 1H), 4.32 (d, J = 17.7 Hz, 1H), 4.02 (d, J = 17.7 Hz,

1H), 3.87 (s, 3H), 3.60 (d, J = 10.3 Hz, 1H), 2.08-1.93 (m, 1H), 0.85 (d, J = 6.5 Hz, 3H), 0.70 (d, J = 6.5 Hz, 3H); MS (thermospray): m/z 451 (M+H), 336, 320.

EXAMPLE 14

N-Hydroxy-[(4-methoxybenzenesulfonyl)(2-morpholin-4-yl-2-oxoethyl)amino]acetamide

To a solution of iminoacetic acid disodium salt monohydrate (5.0 grams, 25.6 mmol) in dioxane (50 ml) and water (50 ml) was added triethylamine (5.3 ml, 38 mmol) followed by 4-methoxybenzenesulfonyl chloride (5.8 grams, 28.0 mmol). The mixture was stirred overnight at room temperature and diluted with methylene chloride. The solution was washed with 1 N hydrochloric acid solution, water and brine, dried over magnesium sulfate and concentrated under vacuum leaving [carboxymethyl(4-methoxybenzenesulfonyl)amino]acetic acid as a white solid, 3.83 grams (49%).

[Carboxymethyl(4-methoxybenzenesulfonyl)amino]acetic acid (0.5 grams, 1.65 mmol) in acetic anhydride (15 mL) was dissolved in acetic anhydride by gentle warming. The resulting solution was stirred at room temperature overnight. The acetic anhydride was removed by evaporation under vacuum; the residue was dissolved in methylene chloride and morpholine (0.16 mL, 1.82 mmol) was added. The mixture was stirred overnight at room temperature and then concentrated under vacuum. The residue was dissolved in ethyl acetate, washed with 1 N hydrochloric acid solution, water and brine, dried over magnesium sulfate and concentrated to afford [(4-methoxybenzenesulfonyl)(2-morpholin-4-yl-2-oxoethyl)amino]acetic acid as an oil, 0.33 grams (54%).

To a solution of [(4-methoxybenzenesulfonyl)(2-morpholin-4-yl-2-oxoethyl)-amino]acetic acid (0.33 grams, 0.89 mmol) in methylene chloride (10 mL) were added sequentially triethylamine (0.43 mL, 3.1 mmol), O-benzylhydroxylamine

hydrochloride (0.15 grams, 0.94 mmol) and (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (0.43 grams, 0.97 mmol). The mixture was stirred at room temperature overnight and then diluted with ethyl acetate. The solution was washed sequentially with 0.5 N hydrochloric acid solution, water and brine, dried over magnesium sulfate and concentrated under vacuum. The residue was chromatographed on silica gel using ethyl acetate to afford N-benzyloxy-[(4-methoxybenzenesulfonyl)(2-morpholin-4-yl-2-oxoethyl)amino]acetamide as a white solid, 0.33 grams (78%).

To a solution of N-benzyloxy-[(4-methoxybenzenesulfonyl)(2-morpholin-4-yl-2-oxoethyl)amino]acetamide (0.33 grams, 0.69 mmol) in methanol (35 mL) was added 5% palladium on activated carbon (85 mg). The mixture was agitated under 2 atmospheres hydrogen in a Parr shaker for 1.5 hours. The catalyst was removed by filtration through nylon (pore size 0.45 μm) and the solvent was evaporated. The residue was chromatographed on silica gel eluting with 5% methanol in methylene chloride to afford N-methoxy-[(4-methoxybenzenesulfonyl)(2-morpholin-4-yl-2-oxoethyl)amino]acetamide as a white solid, 65 mg (24%); ^{1}H NMR (CD_3OD): δ 7.82 (d, J = 9.0 Hz, 2H), 7.08 (d, J = 9.0 Hz, 2H), 4.24 (s, 2H), 3.88 (s, 3H), 3.84 (s, 2H), 3.68-3.64 (m, 4H), 3.58-3.50 (m, 4H); MS (thermospray): m/z 388 (M+1), 387 (M); HRMS calculated for $C_{16}H_{22}N_3O_7S$ (M+H): 388.1178, Found 338.1180.

The title compounds of Examples 15-16 were prepared by a method analogous to that described in Example 14 using [carboxymethyl(4-methoxybenzenesulfonyl)amino]acetic acid as the starting material which, after treatment with acetic anhydride, is coupled with the amine indicated.

EXAMPLE 15

N-Hydroxy-[(4-methoxybenzenesulfonyl)(2-oxo-2-pyrrolidin-1-ylethyl)amino]acetamide

Coupled with pyrrolidine: ^{1}H NMR (DMSO-d_6): δ 11.26 (br s, 1H), 8.89 (s, 1H), 7.81 (d, J = 8.9 Hz, 2H), 7.10 (d, J = 8.9 Hz, 2H), 4.09 (s, 2H), 3.85 (s, 3H), 3.74 (s, 2H), 3.45-3.25 (m, 4H), 1.93-1.72 (m, 4H); MS (thermospray): m/z 372 (M+1): Analysis calculated for $C_{15}H_{21}N_3O_6S$: C, 48.51; H, 5.70; N, 11.31. Found: C, 48.51; H, 5.82; N, 11.24.

EXAMPLE 16

2-[Dimethylcarbamoylmethyl(4-methoxybenzenesulfonyl)amino]-N-hydroxyacetamide

Coupled with dimethylamine: mp: 170°C. (dec.); ^{1}H NMR (DMSO-d_6): δ 10.69 (br s, 1H), 8.88 (s, 1H), 7.91 (d, J = 8.9 Hz, 2H), 7.06 (d, J = 8.9 Hz, 2H), 4.19 (s, 2H), 3.85 (s, 3H), 3.73 (s, 2H), 2.94 (s, 3H), 2.84 (s, 3H); MS (thermospray): m/z 346 (M+1); Analysis calculated for $C_{13}H_{19}N_3O_6S$: C, 45.21; H, 5.55 N, 12.17. Found: C, 44.93, H, 5.61; N, 12.03.

EXAMPLE 17

2-(R)-2-[(2-Carbamoylethyl)(4-methoxybenzenesulfonyl)amino]-N-hydroxy-3-methylbutyramide

To a solution of 2-(R)-2-[(2-carboxyethyl(4-methoxybenzenesulfonyl)amino]-3-methylbutyric acid benzyl ester (example 2) (900 mg., 2.0 mmol) in methylene chloride (10 mL) was added thionyl chloride (0.16 mL, 2.2 mmol). The reaction mixture was stirred for 1.5 hours at room temperature and then concentrated in vacuo. After dissolving the residue in methylene chloride (10 mL), ammonia gas was bubbled through the solution for 0.5 minutes. The solution was stirred at room temperature overnight and was concentrated under vacuum. Flash chromatography of the residue on silica gel eluting with 2% methanol in chloroform provided 2-(R)-2-[(2-carbamoylethyl)(4-

methoxybenzenesulfonyl)amino]-N-hydroxy-3-methylbutyric acid benzyl ester as a clear oil (275 mg, 31%).

To a solution of 2-(R)-2-[(2-carbamoylethyl)(4-methoxybenzenesulfonyl)amino]-N-hydroxy-3-methylbutyric acid benzyl ester (275 mg, 0.61 mmol) in ethanol (15 mL) was added 10% palladium on activated carbon (30 mg). The mixture was agitated under 3 atmospheres hydrogen in a Parr shaker for 5 hours. The catalyst was removed by filtration through diatomaceous earth and the solvent was evaporated leaving 2-(R)-2-[(2-carbamoylethyl)(4-methoxybenzenesulfonyl)amino]-N-hydroxy-3-methylbutyric acid as a white foam, 211 mg (96%).

To a solution of 2-(R)-2-[(2-carbamoylethyl)(4-methoxybenzenesulfonyl)amino]-N-hydroxy-3-methylbutyric acid (205 mg, 0.57 mmol) and 1-hydroxybenztriazole hydrate (85 mg, 0.55 mmol) in dry dimethylformamide (5 mL) was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (120 mg, 0.63 mmol). After stirring for 30 minutes, hydroxylamine hydrochloride (158 mg, 2.3 mmol) and then N-methylmorpholine (0.37 mL, 3.4 mmol) were added. The mixture was stirred at room temperature overnight and then diluted with ethyl acetate. The solution was washed with water and brine. The solution was then dried over magnesium sulfate and concentrated under vacuum to leave an oil which was chromatographed on silica gel eluting with 2% methanol in chloroform to afford 2-(R)-2-[(2-carbamoylethyl)(4-methoxybenzenesulfonyl)amino]-N-hydroxy-3-methylbutyramide as a white solid, 45 mg (21%); ^{1}H NMR (DMSO-d_6): δ 10.74 (br s, 1H), 8.91 (br s, 1H), 7.74 (d, J = 8.8 Hz, 2H), 7.33 (br s, 1H), 7.07 (d, J = 8.8 Hz, 2H), 6.79 (br s, 1H), 3.93-3.82 (m, 1H, overlapped), 3.83 (s, 3H), 3.64 (d, J = 10.7 Hz, 1H), 3.25-3.12 (m, 1H), 2.62-2.48 (m, 1H), 2.42-2.30 (m, 1H), 2.06-1.94 (m, 1H), 0.79 (d, J = 6.6 Hz, 3H), 0.76

(d, J = 6.6 Hz, 3H); MS (thermospray): m/z 374 (M+H).

EXAMPLE 18

2-(R)-2-[(2-tert-Butoxycarbonylethyl)(4-methoxybenzenesulfonyl)-amino]-N-hydroxy-3-methylbutyramide

A solution of N,N-dimethylformamide di-tert-butyl acetal (1.9 mL, 7.9 mmol) in toluene (15 mL) was added dropwise to a solution of 2-(R)-2-[(2-carboxyethyl(4-methoxybenzenesulfonyl)amino]-3-methylbutyric acid benzyl ester (example 2) 900 mg, 2.0 mmol) in toluene at 80°C. After heating for 2 hours at 80°C., the mixture was cooled and concentrated to leave an amber oil which was chromatographed on silica gel eluting with chloroform to afford (2-(R)-2-[(2-tert-butoxycarbonylethyl)(4-methoxybenzenesulfonyl)amino]-3-methylbutyric acid benzyl ester as an oil, 3.75 mg (37%).

To a solution of 2-(R)-2-[(2-tert-butoxycarbonylethyl)(4-methoxybenzenesulfonyl)amino]-3-methylbutyric acid benzyl ester (370 mg, 0.73 mmol) in ethanol (20 mL) was added 10% palladium on activated carbon (40 mg). The mixture was agitated under 3 atmospheres hydrogen in a Parr shaker for 5 hours. The catalyst was removed by filtration through diatomaceous earth and the solvent was evaporated leaving 2-(R)-2-[(2-tert-butoxycarbonylethyl)(4-methoxybenzenesulfonyl)amino]-3-methylbutyric acid as a white foam, 30 mg (100%).

To a solution of 2-(R)-2-[(2-tert-butoxycarbonylethyl)(4-methoxybenzenesulfonyl)amino]-3-methylbutyric acid (303 mg, 0.73 mmol) and 1-hydroxybenztriazole hydrate (108 mg, 0.70 mmol) in dry dimethylformamide (10 mL) was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (153 mg, 0.80 mmol). After stirring for 45 minutes, hydroxylamine hydrochloride (203 mg, 2.9 mmol) and then N-methylmorpholine (0.48 mL, 4.4 mmol) were added. The

mixture was stirred at room temperature overnight and then concentrated under vacuum. The residue was chromatographed on silica gel eluting with 2% methanol in chloroform and again with 10% ethyl acetate in hexane to afford 2-(R)-2-[(2-tert-butoxycarbonylethyl)(4-methoxybenzenesulfonyl)amino]-N-hydroxy-3-methylbutyramide as a white foam, 135 mg (43%); ^{1}H NMR (DMSO-d_6): δ 10.77 (br s 1H), 7.74 (d, J = 8.9 Hz, 2H), 7.08 (d, J = 8.9 Hz, 2H), 3.93-3.82 (m, 1H, overlapped), 3.83 (s, 3H), 3.64 (d, J = 10.8 Hz, 1H), 3.26-3.14 (m, 1H), 2.70-2.60 (m, 1H), 2.50-2.38 (m, 1H), 2.04-1.91 (m, 1H), 1.38 (s, 9H), 0.78 (d, J = 6.5 Hz, 3H), 0.72 (d, J = 6.5 Hz, 3H); MS (thermospray): m/z 431 (M+H), 375, 314.

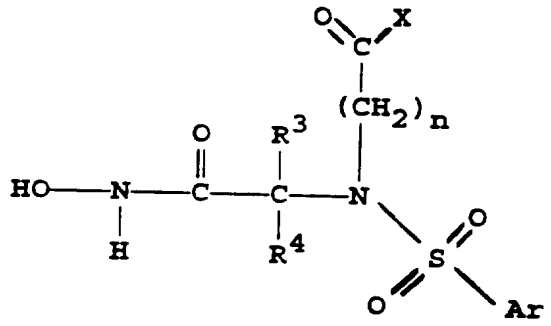
EXAMPLE 19

2-(R)-2-[2-Carboxyethyl)(4-methoxybenzenesulfonyl)amino]-N-hydroxy-3-methylbutyramide

To a solution of 2-(R)-2-[2-tert-butoxycarbonylethyl)(4-methoxybenzenesulfonyl)amino]-N-hydroxy-3-methylbutyramide (example 18) (100 mg, 0.23 mmol) in methylene chloride (1 mL) at 0°C. was added trifluoroacetic acid (1 mL). The mixture was allowed to warm to room temperature while stirring overnight. After evaporation of the trifluoroacetic acid and methylene chloride, the residue was chromatographed on silica gel eluting with 5% methanol in chloroform. Concentration of the appropriate fractions afforded 2-(R)-2-[2-carboxyethyl(4-methoxybenzenesulfonyl)amino]-N-hydroxy-3-methylbutyramide as a white solid, 35 mg (41%). ^{1}H NMR (DMSO-d_6): δ 10.79 (br s,1H), 8.97 (br s, 1H), 7.76 (d, J = 8.9 Hz, 2H), 7.09 (d, J = 8.9 Hz, 2H), 3.95-3.82 (m, 1H, overlapped), 3.84 (s, 3H), 3.66 (d, J = 10.8 Hz, 1H), 3.30-3.20 (m, 1H), 2.73-2.62 (m, 1H), 2.50-2.40 (m, 1H), 2.07-1.94 (m, 1H), 0.80 (d, J = 6.5 Hz, 3H), 0.74 (d, J = 6.5 Hz, 3H); MS (thermospray): m/z 375 (M+H), 314.

C L A I M S

1. A compound of the formula



I

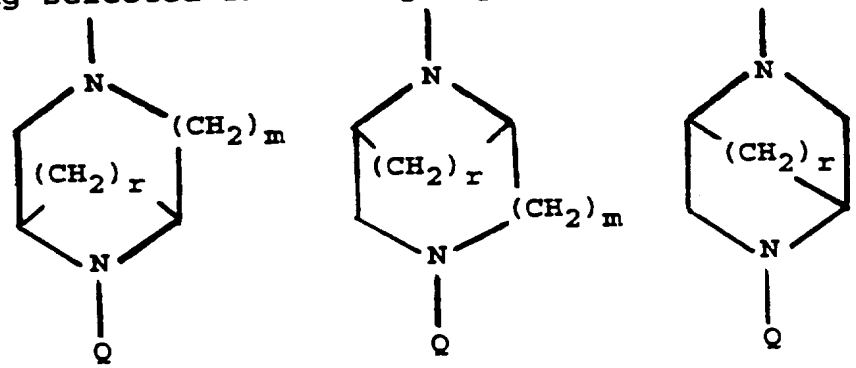
or the pharmaceutically acceptable salts thereof, wherein

n is 1 to 6;

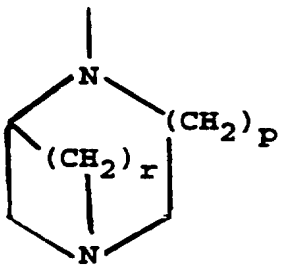
X is hydroxy, (C_1-C_6)alkoxy or NR^1R^2 wherein R^1 and R^2 are each independently selected from the group consisting of hydrogen, (C_1-C_6)alkyl, piperidyl, (C_1-C_6)alkylpiperidyl, (C_6-C_{10})arylpiperidyl, (C_5-C_9)heteroarylpiperidyl, (C_6-C_{10})aryl(C_1-C_6)alkylpiperidyl, (C_5-C_9)heteroaryl(C_1-C_6)alkylpiperidyl, (C_1-C_6)acylpiperidyl, (C_6-C_{10})aryl, (C_5-C_9)heteroaryl, (C_6-C_{10})aryl(C_1-C_6)alkyl, (C_5-C_9)heteroaryl(C_1-C_6)alkyl, (C_6-C_{10})aryl(C_6-C_{10})aryl, (C_6-C_{10})aryl(C_6-C_{10})aryl(C_1-C_6)alkyl, (C_3-C_6)cycloalkyl, (C_3-C_6)cycloalkyl(C_1-C_6)alkyl, $R^5(C_2-C_6)$alkyl, (C_1-C_5)alkyl$(CHR^5)(C_1-C_6)$alkyl wherein R^5 is hydroxy, (C_1-C_6)acyloxy, (C_1-C_6)alkoxy, piperazino, (C_1-C_6)acylamino, (C_1-C_6)alkylthio, (C_6-C_{10})arylthio, (C_1-C_6)alkylsulfinyl, (C_6-C_{10})arylsulfinyl, (C_1-C_6)alkylsulfoxyl, (C_6-C_{10})arylsulfoxyl, amino, (C_1-C_6)alkylamino, $((C_1-C_6)$alkyl$)_2$amino, (C_1-C_6)acylpiperazino, (C_1-C_6)alkylpiperazino, (C_6-C_{10})aryl(C_1-C_6)alkylpiperazino, (C_5-C_9)heteroaryl(C_1-C_6)alkylpiperazino, morpholino, thiomorpholino, piperidino or pyrrolidino; $R^6(C_1-C_6)$alkyl, (C_1-C_5)alkyl$(CHR^6)(C_1-C_6)$alkyl wherein R^6 is piperidyl, (C_1-C_6)alkylpiperidyl, (C_6-C_{10})arylpiperidyl, (C_6-C_{10})aryl(C_1-C_6)alkylpiperidyl, $(C_5-$

C_9)heteroarylpiperidyl or (C_5-C_9)heteroaryl(C_1-C_6)alkylpiperidyl; and CH(R^7)COR^8 wherein R^7 is hydrogen, (C_1-C_6)alkyl, (C_6-C_{10})aryl(C_1-C_6)alkyl, (C_5-C_9)heteroaryl(C_1-C_6)alkyl, (C_1-C_6)alkylthio(C_1-C_6)alkyl, (C_6-C_{10})arylthio(C_1-C_6)alkyl, (C_1-C_6)alkylsulfinyl(C_1-C_6)alkyl, (C_6-C_{10})arylsulfinyl(C_1-C_6)alkyl, (C_1-C_6)alkylsulfonyl(C_1-C_6)alkyl, (C_6-C_{10})arylsulfonyl(C_1-C_6)alkyl, hydroxy(C_1-C_6)alkyl, amino(C_1-C_6)alkyl, (C_1-C_6)alkylamino(C_1-C_6)alkyl, ((C_1-C_6)alkylamino)$_2$(C_1-C_6)alkyl, $R^9R^{10}NCO$(C_1-C_6)alkyl or R^9OCO(C_1-C_6)alkyl wherein R^9 and R^{10} are each independently selected from the group consisting of hydrogen, (C_1-C_6)alkyl, (C_6-C_{10})aryl(C_1-C_6)alkyl and (C_5-C_9)heteroaryl(C_1-C_6)alkyl; and R^8 is $R^{11}O$ or $R^{11}R^{12}N$ wherein R^{11} and R^{12} are each independently selected from the group consisting of hydrogen, (C_1-C_6)alkyl, (C_6-C_{10})aryl(C_1-C_6)alkyl and (C_5-C_9)heteroaryl(C_1-C_6)alkyl;

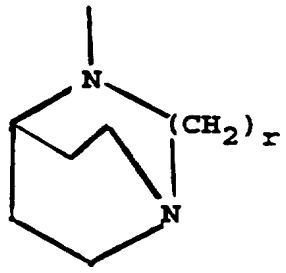
or R^1 and R^2, or R^9 and R^{10}, or R^{11} and R^{12} may be taken together to form an azetidinyl, pyrrolidinyl, morpholinyl, thiomorpholinyl, indolinyl, isoindolinyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, (C_1-C_6)acylpiperazinyl, (C_1-C_6)alkylpiperazinyl, (C_6-C_{10})arylpiperazinyl, (C_5-C_9)heteroarylpiperazinyl or a bridged diazabicycloalkyl ring selected from the group consisting of



a b c



d



e

wherein r is 1, 2 or 3;

m is 1 or 2;

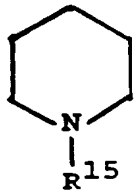
p is 0 or 1; and

Q is hydrogen, (C_1-C_3)alkyl or (C_1-C_6)acyl;

R^3 and R^4 are each independently selected from the group consisting of hydrogen, (C_1-C_6)alkyl, trifluoromethyl, trifluoromethyl(C_1-C_6)alkyl, (C_1-C_6)alkyl (difluoromethylene), (C_1-C_3)alkyl(difluoromethylene)(C_1-C_3)alkyl, (C_6-C_{10})aryl, (C_5-C_9)heteroaryl, (C_6-C_{10})aryl(C_1-C_6)alkyl, (C_5-C_9)heteroaryl(C_1-C_6)alkyl, (C_6-C_{10})aryl(C_6-C_{10})aryl, (C_6-C_{10})aryl(C_6-C_{10})aryl(C_1-C_6)alkyl, (C_3-C_6)cycloalkyl, (C_3-C_6)cycloalkyl(C_1-C_6)alkyl, hydroxy(C_1-C_6)alkyl, (C_1-C_6)acyloxy(C_1-C_6)alkyl, (C_1-C_6)alkoxy(C_1-C_6)alkyl, piperazinyl(C_1-C_6)alkyl, (C_1-C_6)acylamino(C_1-C_6)alkyl, piperidyl, (C_1-C_6)alkylpiperidyl, (C_6-C_{10})aryl(C_1-C_6)alkoxy(C_1-C_6)alkyl, (C_5-C_9)heteroaryl(C_1-C_6)alkoxy(C_1-C_6)alkyl, (C_1-C_6)alkylthio(C_1-C_6)alkyl, (C_6-C_{10})arylthio(C_1-C_6)alkyl, (C_1-C_6)alkylsulfinyl(C_1-C_6)alkyl, (C_6-C_{10})arylsulfinyl(C_1-C_6)alkyl, (C_1-C_6)alkylsulfonyl(C_1-C_6)alkyl, (C_6-C_{10})arylsulfonyl(C_1-C_6)alkyl, amino(C_1-C_6)alkyl, (C_1-C_6)alkylamino(C_1-C_6)alkyl, $((C_1-C_6)$alkylamino$)_2(C_1-C_6)$alkyl, $R^{13}CO(C_1-C_6)$alkyl wherein R^{13} is $R^{20}O$ or $R^{20}R^{21}N$ wherein R^{20} and R^{21} are each independently selected from the group consisting of hydrogen, (C_1-C_6)alkyl, (C_6-C_{10})aryl(C_1-C_6)alkyl or (C_5-C_9)heteroaryl(C_1-C_6)alkyl; or $R^{14}(C_1-C_6)$alkyl wherein R^{14} is (C_1-C_6)acylpiperazino, $(C_6-$

C_{10})arylpiperazino, (C_5-C_9)heteroarylpiperazino, (C_1-C_6)alkylpiperazino, (C_6-C_{10})aryl(C_1-C_6)alkylpiperazino, (C_5-C_9)heteroaryl(C_1-C_6)alkylpiperazino, morpholino, thiomorpholino, piperidino, pyrrolidino, piperidyl, (C_1-C_6)alkylpiperidyl, (C_6-C_{10})arylpiperidyl, (C_5-C_9)heteroarylpiperidyl, (C_6-C_{10})aryl(C_1-C_6)alkylpiperidyl, (C_5-C_9)heteroaryl(C_1-C_6)alkylpiperidyl or (C_1-C_6)acylpiperidyl;

or R^3 and R^4, or R^{20} and R^{21} may be taken together to form a (C_3-C_6)cycloalkyl, oxacyclohexyl, thiocyclohexyl, indanyl or tetralinyl ring or a group of the formula



wherein R^{15} is hydrogen, (C_1-C_6)acyl, (C_1-C_6)alkyl, (C_6-C_{10})aryl(C_1-C_6)alkyl, (C_5-C_9)heteroaryl(C_1-C_6)alkyl or (C_1-C_6)alkylsulfonyl; and

Ar is (C_6-C_{10})aryl, (C_5-C_9)heteroaryl, (C_1-C_6)alkyl(C_6-C_{10})aryl, (C_1-C_6)alkoxy(C_6-C_{10})aryl, ((C_1-C_6)alkoxy)$_2$(C_6-C_{10})aryl, (C_6-C_{10})aryloxy(C_6-C_{10})aryl, (C_5-C_9)heteroaryloxy(C_6-C_{10})aryl, (C_1-C_6)alkyl(C_5-C_9)heteroaryl, (C_1-C_6)alkoxy(C_5-C_9)heteroaryl, ((C_1-C_6)alkoxy)$_2$(C_5-C_9)heteroaryl, (C_6-C_{10})aryloxy(C_5-C_9)heteroaryl, (C_5-C_9)heteroaryloxy(C_5-C_9)heteroaryl;

with the proviso that when either R^1 or R^2 is $CH(R^7)COR^8$ wherein R^7 and R^8 are as defined above, the other of R^1 or R^2 is hydrogen, (C_1-C_6)alkyl or benzyl.

2. A compound according to claim 1, wherein n is 2.

3. A compound according to claim 1, wherein Ar is 4-methoxyphenyl or 4-phenoxyphenyl.

4. A compound according to claim 1, 2 or 3, wherein either R^3 or R^4 is not hydrogen.

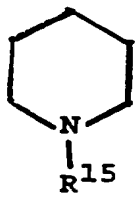
5. A compound according to claim 1, wherein n is

1 and either R^1 or R^2 is hydrogen.

6. A compound according to claim 4, wherein X is hydroxy, Ar is 4-methoxyphenyl or 4-phenoxyphenyl.

7. A compound according to claim 4, wherein X is alkoxy, Ar is 4-methoxyphenyl or 4-phenoxyphenyl.

8. A compound according to claim 1, wherein Ar is 4-methoxyphenyl or 4-phenoxyphenyl and R^3 and R^4 are taken together to form (C_3-C_6)cycloalkanyl, oxacyclohexanyl, thiocyclohexanyl, indanyl or a group of the formula



wherein R^{15} is (C_1-C_6)acyl, (C_1-C_6)alkyl, (C_6-C_{10})aryl(C_1-C_6)alkyl, (C_5-C_9)heteroaryl(C_1-C_6)alkyl or (C_1-C_6)alkylsulfonyl.

9. A compound according to claim 1, wherein n is 2, Ar is 4-methoxyphenyl or 4-phenoxyphenyl, R^1 and R^2 are taken together to form piperazinyl, (C_1-C_6)alkylpiperazinyl, (C_6-C_{10})aryl piperazinyl or (C_5-C_9)heteroaryl(C_1-C_6)alkylpiperazinyl, and either R^3 or R^4 is not hydrogen or both R^3 and R^4 are not hydrogen.

10. A compound according to claim 1, wherein n is 2, Ar is 4-methoxyphenyl or 4-phenoxyphenyl, R^1 is hydrogen or (C_1-C_6)alkyl, R^2 is 2-pyridylmethyl, 3-pyridylmethyl or 4-pyridylmethyl, and either R^3 or R^4 is not hydrogen or both R^3 and R^4 are not hydrogen.

11. A compound according to claim 1, wherein n is 1, Ar is 4-methoxyphenyl or 4-phenoxyphenyl, R^1 is hydrogen, R^2 is 2-pyridylmethyl, 3-pyridylmethyl or 4-pyridylmethyl, and either R^3 or R^4 is not hydrogen or both R^3 and R^4 are not hydrogen.

12. A compound according to claim 2, wherein Ar is 4-methoxyphenyl, R^1 is hydrogen or (C_1-C_6)alkyl and R^2 is $R^5(C_2-C_6)$alkyl wherein R^5 is morpholino,

thiomorpholino, piperidino, pyrrolidino, (C_1-C_6)acylpiperazino, (C_1-C_6)alkylpiperazino, (C_6-C_{10})arylpiperazino, (C_5-C_9)heteroarylpiperazino, (C_6-C_{10})aryl(C_1-C_6)alkylpiperazino or (C_5-C_9)heteroaryl(C_1-C_6)alkylpiperazino and either R^3 or R^4 is not hydrogen or both R^3 and R^4 are not hydrogen.

13. A compound according to claim 1, wherein n is 1, Ar is 4-methoxyphenyl or 4-phenoxyphenyl, R^1 is hydrogen, R^2 is R^5(C_2-C_6)alkyl wherein R^5 is morpholino, thiomorpholino, piperidino, pyrrolidino, (C_1-C_6)acylpiperazino, (C_1-C_6)alkylpiperazino, (C_6-C_{10})arylpiperazino, (C_5-C_9)heteroarylpiperazino, (C_6-C_{10})aryl(C_1-C_6)alkylpiperazino or (C_5-C_9)heteroaryl(C_1-C_6)alkylpiperazino and either R^3 or R^4 is not hydrogen or both R^3 and R^4 are not hydrogen.

14. A compound according to claim 1, wherein said compound is selected from:

2-(R)-N-Hydroxy-2-[(4-methoxybenzenesulfonyl)(3-morpholin-4-yl-3-oxopropyl)amino]-3-methylbutyramide;

2-(R)-2-[(2-Benzylcarbamoylethyl)(4-methoxybenzenesulfonyl)amino]-N-hydroxy-3-methylbutyramide;

2-(R)-N-Hydroxy-2-((4-methoxybenzenesulfonyl)(2-[(pyridin-3-ylmethyl)carbamoyl]ethyl)amino)-3-methylbutyramide;

2-(R)-N-Hydroxy-2-([4-methoxybenzenesulfonyl][2-(methylpyridin-3-ylmethylcarbamoyl)ethyl]amino)-3-methylbutyramide;

4-(3-[1-(R)-1-Hydroxycarbamoyl-2-methylpropyl)(4-methoxybenzenesulfonyl)amino]propionyl)piperazine-1-carboxylic acid, tert-butyl ester;

2-(R)-N-Hydroxy-2-[(4-methoxybenzenesulfonyl)(3-oxo-3-piperazin-1-ylpropyl)amino)-3-methylbutyramide hydrochloride;

2-(R)-2-[(Benzylcarbamoylmethyl)(4-

methoxybenzenesulfonyl)amino]N-hydroxy-3-methylbutyramide;

2-(R)-N-Hydroxy-2-[(4-methoxybenzenesulfonyl]-[(2-morpholin-4-ylethylcarbamoyl)methyl]amino)-3-methylbutyramide;

2-(R)-N-Hydroxy-2-((4-methoxybenzenesulfonyl)([(pyridin-3-ylmethyl)carbamoyl]methyl)amino)-3-methylbutyramide;

2-(R)-3,3,3-Trifluoro-N-hydroxy-2-[(methoxybenzenesulfonyl)(3-morpholin-4-yl-3-oxopropyl)amino]propionamide;

2-(R)-N-Hydroxy-2-((4-phenoxybenzenesulfonyl)[2-(methylpyridin-4-ylmethylcarbamoyl)ether]amino)-3-methylbutyramide;

4-[4-Methoxybenzenesulfonyl)(3-morpholin-4-yl-3-oxopropyl)amino]-1-methylpiperidene-4-carboxylic acid hydroxyamide;

2-(R)-N-Hydroxy-2-((4-methoxybenzenesulfonyl)-[3-(4-methylpiperazin-1-yl)-3-oxopropyl]amino)-3-methylbutyramide;

2-(R)-2-[(2-Carboxyethyl)(4-methoxybenzenesulfonyl)amino]-N-hydroxy-3-methylbutyramide;

[(2-Carboxyethyl)(3,4-dimethoxybenzenesulfonyl)amino]-N-hydroxy-acetamide;

2-(R)-2-[(2-Carbamoylethyl)(4-methoxybenzenesulfonyl)amino]-N-hydroxy-3-methylbutyramide;

2-(R),3-(R)-3,N-Dihydroxy-2-[(4-methoxybenzenesulfonyl)(3-oxo-3-piperidin-1-ylpropyl)amino]-butyramide;

2-(R)-N-Hydroxy-2-((4-methoxybenzenesulfonyl)[3-(methylpyridin-3-ylmethylcarbamoyl)propyl]amino)-3-methylbutyramide;

2-(R)-N-Hydroxy-2-((4-methoxybenzenesulfonyl)[2-(methylcarboxymethylcarbamoyl)ethyl]amino)-3-

methylbutyramide;

2-(R)-N-Hydroxy-2-((4-methoxybenzenesulfonyl)-[(1-methylpiperidin-4-ylcarbamoyl)methyl]amino)-3-methylbutyramide;

2-(R)-2-Clyclohexyl-N-hydroxy-2-((4-methoxybenzenesulfonyl)-[3-(4-methylpiperazin-1-yl)-3-oxopropyl]amino)-acetamide; and

2-(R)-N-Hydroxy-2-[(methoxybenzenesulfonyl)(3-morpholin-4-yl-3-oxopropyl)amino]-4-(morpholin-4-yl)butyramide.

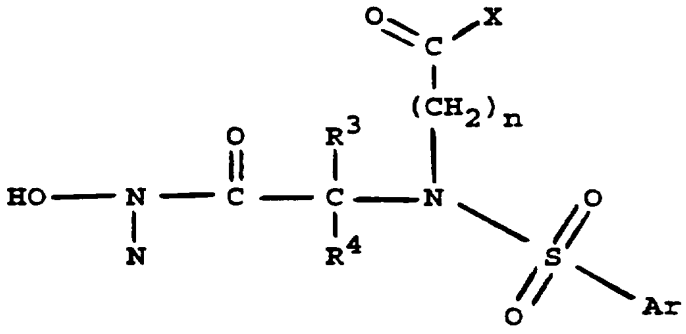
15. A pharmaceutical composition for (a) the treatment of a condition selected from the group consisting of arthritis, cancer, tissue ulceration, restenosis, periodontal disease, epidermolysis bullosa, scleritis and other diseases characterized by matrix metalloproteinase activity, AIDS, sepsis, septic shock and other diseases involving the production of tumor necrosis factor (TNF) or (b) the inhibition of matrix metalloproteinases or the production of tumor necrosis factor (TNF) in a mammal, including a human, comprising an amount of a compound of claim 1 or a pharmaceutically acceptable salt thereof, effective in such treatments and a pharmaceutically acceptable carrier.

16. A method for the inhibition of (a) matrix metalloproteinases or (b) the production of tumor necrosis factor (TNF) in a mammal, including a human, comprising administering to said mammal an effective amount of a compound of claim 1 or a pharmaceutically acceptable salt thereof.

17. A method for treating a condition selected from the group consisting of arthritis, cancer, tissue ulceration, restenosis, periodontal disease, epidermolysis bullosa, scleritis and other diseases characterized by matrix metalloproteinase activity, AIDS, sepsis, septic shock and other diseases involving the production of tumor necrosis factor (TNF) in a

mammal, including a human, comprising administering to said mammal an amount of a compound of claim 1 or a pharmaceutically acceptable salt thereof, effective in treating such a condition.

18. A method of preparing a compound of the formula



I

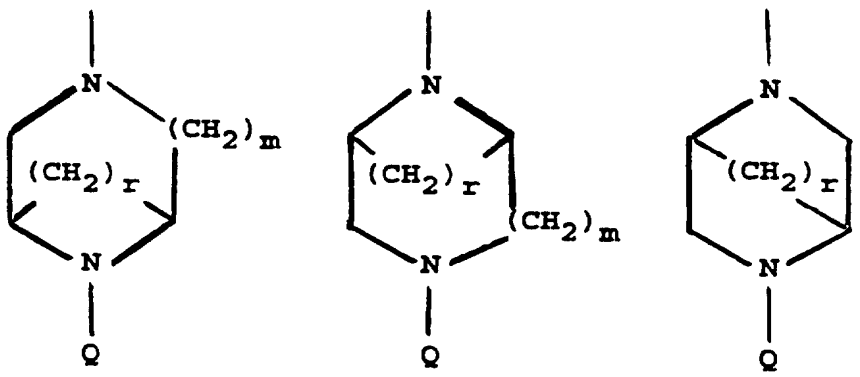
or the pharmaceutically acceptable salts thereof, wherein

n is 1 to 6;

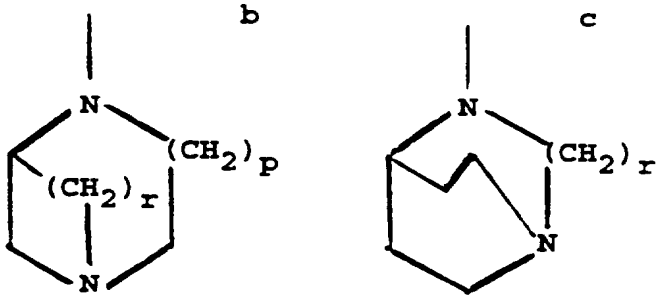
X is hydroxy, (C_1-C_6)alkoxy or NR^1R^2 wherein R^1 and R^2 are each independently selected from the group consisting of hydrogen, (C_1-C_6)alkyl, piperidyl, (C_1-C_6)alkylpiperidyl, (C_6-C_{10})arylpiperidyl, (C_5-C_9)heteroarylpiperidyl, (C_6-C_{10})aryl(C_1-C_6)alkylpiperidyl, (C_5-C_9)heteroaryl(C_1-C_6)alkylpiperidyl, (C_1-C_6)acylpiperidyl, (C_6-C_{10})aryl, (C_5-C_9)heteroaryl, (C_6-C_{10})aryl(C_1-C_6)alkyl, (C_5-C_9)heteroaryl(C_1-C_6)alkyl, (C_6-C_{10})aryl(C_6-C_{10})aryl, (C_6-C_{10})aryl(C_6-C_{10})aryl(C_1-C_6)alkyl, (C_3-C_6)cycloalkyl, (C_3-C_6)cycloalkyl(C_1-C_6)alkyl, $R^5(C_2-C_6)$alkyl, (C_1-C_5)alkyl$(CHR^5)(C_1-C_6)$alkyl wherein R^5 is hydroxy, (C_1-C_6)acyloxy, (C_1-C_6)alkoxy, piperazino, (C_1-C_6)acylamino, (C_1-C_6)alkylthio, (C_6-C_{10})arylthio, (C_1-C_6)alkylsulfinyl, (C_6-C_{10})arylsulfinyl, (C_1-C_6)alkylsulfoxyl, (C_6-C_{10})arylsulfoxyl, amino, (C_1-C_6)alkylamino, $((C_1-C_6)\text{alkyl})_2$amino, (C_1-C_6)acylpiperazino, (C_1-C_6)alkylpiperazino, (C_6-C_{10})aryl(C_1-C_6)alkylpiperazino, (C_5-C_9)heteroaryl$(C_1-$

C_6)alkylpiperazino, morpholino, thiomorpholino, piperidino or pyrrolidino; R^6 (C_1-C_6)alkyl, (C_1-C_5)alkyl(CHR^6)(C_1-C_6)alkyl wherein R^6 is piperidyl, (C_1-C_6)alkylpiperidyl, (C_6-C_{10})arylpiperidyl, (C_6-C_{10})aryl(C_1-C_6)alkylpiperidyl, (C_5-C_9)heteroarylpiperidyl or (C_5-C_9)heteroaryl(C_1-C_6)alkylpiperidyl; and $CH(R^7)COR^8$ wherein R^7 is hydrogen, (C_1-C_6)alkyl, (C_6-C_{10})aryl(C_1-C_6)alkyl, (C_5-C_9)heteroaryl(C_1-C_6)alkyl, (C_1-C_6)alkylthio(C_1-C_6)alkyl, (C_6-C_{10})arylthio(C_1-C_6)alkyl, (C_1-C_6)alkylsulfinyl(C_1-C_6)alkyl, (C_6-C_{10})arylsulfinyl(C_1-C_6)alkyl, (C_1-C_6)alkylsulfonyl(C_1-C_6)alkyl, (C_6-C_{10})arylsulfonyl(C_1-C_6)alkyl, hydroxy(C_1-C_6)alkyl, amino(C_1-C_6)alkyl, (C_1-C_6)alkylamino(C_1-C_6)alkyl, ((C_1-C_6)alkylamino)$_2$(C_1-C_6)alkyl, $R^9R^{10}NCO(C_1$-C_6)alkyl or $R^9OCO(C_1$-C_6)alkyl wherein R^9 and R^{10} are each independently selected from the group consisting of hydrogen, (C_1-C_6)alkyl, (C_6-C_{10})aryl(C_1-C_6)alkyl and (C_5-C_9)heteroaryl(C_1-C_6)alkyl; and R^8 is $R^{11}O$ or $R^{11}R^{12}N$ wherein R^{11} and R^{12} are each independently selected from the group consisting of hydrogen, (C_1-C_6)alkyl, (C_6-C_{10})aryl(C_1-C_6)alkyl and (C_5-C_9)heteroaryl(C_1-C_6)alkyl;

or R^1 and R^2, or R^9 and R^{10}, or R^{11} and R^{12} may be taken together to form an azetidinyl, pyrrolidinyl, morpholinyl, thiomorpholinyl, indolinyl, isoindolinyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, (C_1-C_6)acylpiperazinyl, (C_1-C_6)alkylpiperazinyl, (C_6-C_{10})arylpiperazinyl, (C_5-C_9)heteroarylpiperazinyl or a bridged diazabicycloalkyl ring selected from the group consisting of



a b c



d e

wherein r is 1, 2 or 3;

m is 1 or 2;

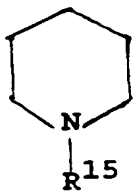
p is 0 or 1; and

Q is hydrogen, (C_1-C_3)alkyl or (C_1-C_6)acyl;

R^3 and R^4 are each independently selected from the group consisting of hydrogen, (C_1-C_6)alkyl, trifluoromethyl, trifluoromethyl(C_1-C_6)alkyl, (C_1-C_6)alkyl (difluoromethylene), (C_1-C_3)alkyl(difluoromethylene)(C_1-C_3)alkyl, (C_6-C_{10})aryl, (C_5-C_9)heteroaryl, (C_6-C_{10})aryl(C_1-C_6)alkyl, (C_5-C_9)heteroaryl(C_1-C_6)alkyl, (C_6-C_{10})aryl(C_6-C_{10})aryl, (C_6-C_{10})aryl(C_6-C_{10})aryl(C_1-C_6)alkyl, (C_3-C_6)cycloalkyl, (C_3-C_6)cycloalkyl(C_1-C_6)alkyl, hydroxy(C_1-C_6)alkyl, (C_1-C_6)acyloxy(C_1-C_6)alkyl, (C_1-C_6)alkoxy(C_1-C_6)alkyl, piperazinyl(C_1-C_6)alkyl, (C_1-C_6)acylamino(C_1-C_6)alkyl, piperidyl, (C_1-C_6)alkylpiperidyl, (C_6-C_{10})aryl(C_1-C_6)alkoxy(C_1-C_6)alkyl, $C_5-C_9)$heteroaryl(C_1-C_6)alkoxy(C_1-C_6)alkyl, (C_1-C_6)alkylthio(C_1-C_6)alkyl, (C_6-C_{10})arylthio$(C_1-$

C_6)alkyl, (C_1-C_6)alkylsulfinyl(C_1-C_6)alkyl, (C_6-C_{10})arylsulfinyl(C_1-C_6)alkyl, (C_1-C_6)alkylsulfonyl(C_1-C_6)alkyl, (C_6-C_{10})arylsulfonyl(C_1-C_6)alkyl, amino(C_1-C_6)alkyl, (C_1-C_6)alkylamino(C_1-C_6)alkyl, ((C_1-C_6)alkylamino)$_2$(C_1-C_6)alkyl, $R^{13}CO$(C_1-C_6)alkyl wherein R^{13} is $R^{20}O$ or $R^{20}R^{21}N$ wherein R^{20} and R^{21} are each independently selected from the group consisting of hydrogen, (C_1-C_6)alkyl, (C_6-C_{10})aryl(C_1-C_6)alkyl or (C_5-C_9)heteroaryl(C_1-C_6)alkyl; or R^{14}(C_1-C_6)alkyl wherein R^{14} is (C_1-C_6)acylpiperazino, (C_6-C_{10})arylpiperazino, (C_5-C_9)heteroarylpiperazino, (C_1-C_6)alkylpiperazino, (C_6-C_{10})aryl(C_1-C_6)alkylpiperazino, (C_5-C_9)heteroaryl(C_1-C_6)alkylpiperazino, morpholino, thiomorpholino, piperidino, pyrrolidino, piperidyl, (C_1-C_6)alkylpiperidyl, (C_6-C_{10})arylpiperidyl, (C_5-C_9)heteroarylpiperidyl, (C_6-C_{10})aryl(C_1-C_6)alkylpiperidyl, (C_5-C_9)heteroaryl(C_1-C_6)alkylpiperidyl or (C_1-C_6)acylpiperidyl;

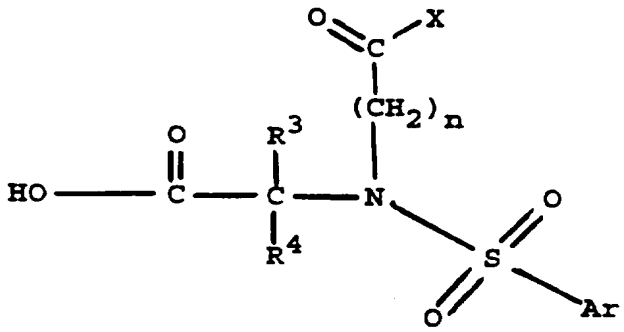
or R^3 and R^4, or R^{20} and R^{21} may be taken together to form a (C_3-C_6)cycloalkyl, oxacyclohexyl, thiocyclohexyl, indanyl or tetralinyl ring or a group of the formula



wherein R^{15} is hydrogen, (C_1-C_6)acyl, (C_1-C_6)alkyl, (C_6-C_{10})aryl(C_1-C_6)alkyl, (C_5-C_9)heteroaryl(C_1-C_6)alkyl or (C_1-C_6)alkylsulfonyl; and

Ar is (C_6-C_{10})aryl, (C_5-C_9)heteroaryl, (C_1-C_6)alkyl(C_6-C_{10})aryl, (C_1-C_6)alkoxy(C_6-C_{10})aryl, ((C_1-C_6)alkoxy)$_2$(C_6-C_{10})aryl, (C_6-C_{10})aryloxy(C_6-C_{10})aryl, (C_5-C_9)heteroaryloxy(C_6-C_{10})aryl, (C_1-C_6)alkyl(C_5-C_9)heteroaryl, (C_1-C_6)alkoxy(C_5-C_9)heteroaryl, ((C_1-C_6)alkoxy)$_2$(C_5-C_9)heteroaryl, (C_6-C_{10})aryloxy(C_5-C_9)heteroaryl, (C_5-C_9)heteroaryloxy(C_5-C_9)heteroaryl;

with the proviso that when either R^1 or R^2 is $CH(R^7)COR^8$ wherein R^7 and R^8 are as defined above, the other of R^1 or R^2 is hydrogen, (C_1-C_6)alkyl or benzyl; comprising reacting a compound of the formula



wherein n, X, R^3, R^4 and Ar are as defined above with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, 1-hydroxybenztriazole and hydroxylamine.

INTERNATIONAL SEARCH REPORT

In onal Application No
PCT/US 96/02679

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07C311/29 C07D295/18 C07D213/56 A61K31/535 A61K31/44
A61K31/495 A61K31/40 A61K31/18

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07C C07D

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)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 606 046 (CIBA-GEIGY AG) 13 July 1994 see the whole document ---	1-15,18
A	WO,A,90 05719 (BRITISH BIOTECHNOLOGY LTD) 31 May 1990 see the whole document -----	1-15,18

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

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

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

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
15 May 1996	04.06.96

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

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Ir ional Application No
PCT/US 96/02679

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-606046	13-07-94	US-A-	5455258	03-10-95
		AU-B-	5265593	04-05-95
		CA-A-	2112779	07-07-94
		FI-A-	940012	07-07-94
		HU-A-	70536	30-10-95
		JP-A-	6256293	13-09-94
		NO-A-	940038	07-07-94
		NZ-A-	250517	26-10-95
		US-A-	5506242	09-04-96
		ZA-A-	9400048	11-08-94
WO-A-9005719	31-05-90	AU-B-	644064	02-12-93
		AU-B-	4800390	12-06-90
		CA-A-	2003718	23-05-90
		DE-D-	68914687	19-05-94
		DE-T-	68914687	08-09-94
		EP-A-	0446267	18-09-91
		ES-T-	2055409	16-08-94
		JP-T-	4502008	09-04-92
		NO-B-	177701	31-07-95
		US-A-	5310763	10-05-94
		US-A-	5240958	31-08-93

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C07D 211/96, A61K 31/445, C07D 241/04, 241/08

A1

(11) International Publication Number: **WO 96/33172**

(43) International Publication Date: 24 October 1996 (24.10.96)

(21) International Application Number: PCT/IB95/00279

(22) International Filing Date: 20 April 1995 (20.04.95)

(71) Applicant *(for all designated States except US)*: PFIZER INC. [US/US]; 235 East 42nd Street, New York, NY 10017 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: PISCOPIO, Anthony, D. [US/US]; 196 Payer Lane, Mystic, CT 06355 (US). RIZZI, James, P. [US/US]; 34 Devonshire Drive, Waterford, CT 06385 (US).

(74) Agents: SPIEGEL, Allen, J. et al.; Pfizer Inc., 235 East 42nd Street, New York, NY 10017 (US).

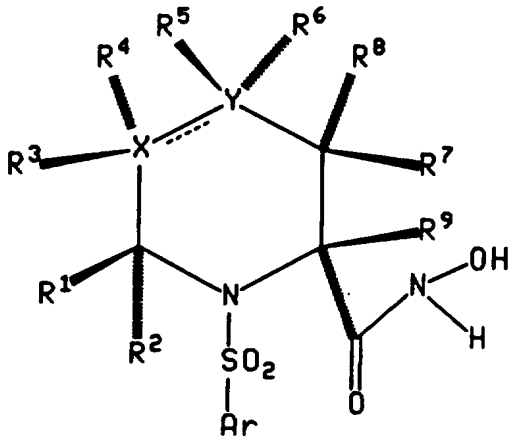
(81) Designated States: CA, FI, JP, MX, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: ARYLSULFONYL HYDROXAMIC ACID DERIVATIVES AS MMP AND TNF INHIBITORS

(57) Abstract

A compound of formula (I) wherein R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9 and Ar are as defined above, useful in the treatment of a condition selected from the group consisting of arthritis, cancer, tissue ulceration, restenosis, periodontal disease, epidermolysis bullosa, scleritis and other diseases characterized by matrix metalloproteinase activity, as well as AIDS, sepsis, septic shock and other diseases involving the production of TNF.



(I)

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia
AT	Austria
AU	Australia
BB	Barbados
BE	Belgium
BF	Burkina Faso
BG	Bulgaria
BJ	Benin
BR	Brazil
BY	Belarus
CA	Canada
CF	Central African Republic
CG	Congo
CH	Switzerland
CI	Côte d'Ivoire
CM	Cameroon
CN	China
CS	Czechoslovakia
CZ	Czech Republic
DE	Germany
DK	Denmark
EE	Estonia
ES	Spain
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
GE	Georgia
GN	Guinea
GR	Greece
HU	Hungary
IE	Ireland
IT	Italy
JP	Japan
KE	Kenya
KG	Kyrgystan
KP	Democratic People's Republic of Korea
KR	Republic of Korea
KZ	Kazakhstan
LI	Liechtenstein
LK	Sri Lanka
LR	Liberia
LT	Lithuania
LU	Luxembourg
LV	Latvia
MC	Monaco
MD	Republic of Moldova
MG	Madagascar
ML	Mali
MN	Mongolia
MR	Mauritania
MW	Malawi
MX	Mexico
NE	Niger
NL	Netherlands
NO	Norway
NZ	New Zealand
PL	Poland
PT	Portugal
RO	Romania
RU	Russian Federation
SD	Sudan
SE	Sweden
SG	Singapore
SI	Slovenia
SK	Slovakia
SN	Senegal
SZ	Swaziland
TD	Chad
TG	Togo
TJ	Tajikistan
TT	Trinidad and Tobago
UA	Ukraine
UG	Uganda
US	United States of America
UZ	Uzbekistan
VN	Viet Nam

ARYLSULFONYL HYDROXAMIC ACID DERIVATIVES AS MMP AND TNF INHIBITORS

Background of the Invention

The present invention relates to arylsulfonyl hydroxamic acid derivatives which are inhibitors of matrix metalloproteinases or the production of tumor necrosis factor (hereinafter also referred to as TNF) and as such are useful in the treatment of a condition selected from the group consisting of arthritis, cancer, tissue ulceration, restenosis, periodontal disease, epidermolysis bullosa, scleritis and other diseases characterized by matrix metalloproteinase activity, as well as AIDS, sepsis, septic shock and other diseases involving the production of TNF.

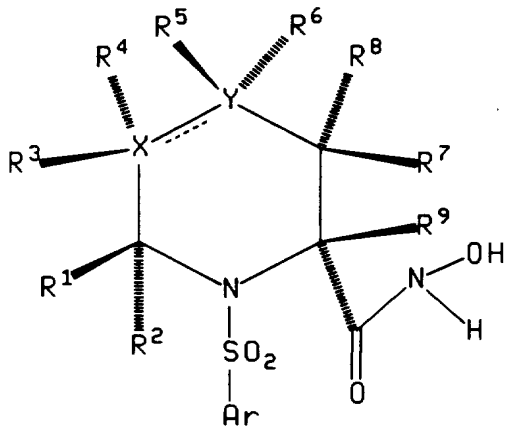
This invention also relates to a method of using such compounds in the treatment of the above diseases in mammals, especially humans, and to the pharmaceutical compositions useful therefor.

There are a number of enzymes which effect the breakdown of structural proteins and which are structurally related metalloproteases. Matrix-degrading metalloproteinases, such as gelatinase, stromelysin and collagenase, are involved in tissue matrix degradation (e.g. collagen collapse) and have been implicated in many pathological conditions involving abnormal connective tissue and basement membrane matrix metabolism, such as arthritis (e.g. osteoarthritis and rheumatoid arthritis), tissue ulceration (e.g. corneal, epidermal and gastric ulceration), abnormal wound healing, periodontal disease, bone disease (e.g. Paget's disease and osteoporosis), tumor metastasis or invasion, as well as HIV-infection (J. Leuk. Biol., 52 (2): 244-248, 1992).

Tumor necrosis factor is recognized to be involved in many infectious and auto-immune diseases (W. Friers, FEBS Letters, 1991, 285, 199). Furthermore, it has been shown that TNF is the prime mediator of the inflammatory response seen in sepsis and septic shock (C.E. Spooner et al., Clinical Immunology and Immunopathology, 1992, 62 S11).

Summary of the Invention

The present invention relates to a compound of the formula



I

or the pharmaceutically acceptable salt thereof, wherein the broken line represents an optional double bond;

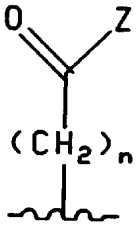
X is carbon, oxygen or sulfur;

Y is carbon, oxygen, sulfur, sulfoxide, sulfone or nitrogen;

R^1, R^2 R^3, R^4 R^5, R^6, R^7, R^8 and R^9 are selected from the group consisting of hydrogen, (C_1-C_6)alkyl optionally substituted by (C_1-C_6)alkylamino, (C_1-C_6)alkylthio, (C_1-C_6)alkoxy, trifluoromethyl, (C_6-C_{10})aryl, (C_5-C_9)heteroaryl, (C_6-C_{10})arylamino, (C_6-C_{10})arylthio, (C_6-C_{10})aryloxy, (C_5-C_9)heteroarylamino, (C_5-C_9)heteroarylthio, (C_5-C_9)heteroaryloxy, (C_6-C_{10})aryl(C_6-C_{10})aryl, (C_3-C_6)cycloalkyl, hydroxy(C_1-C_6)alkyl, (C_1-C_6)alkyl(hydroxymethylene),piperazinyl,(C_6-C_{10})aryl(C_1-C_6)alkoxy,(C_5-C_9)heteroaryl(C_1-C_6)alkoxy, (C_1-C_6)acylamino, (C_1-C_6)acylthio, (C_1-C_6)acyloxy, (C_1-C_6)alkylsulfinyl, (C_6-C_{10})arylsulfinyl, (C_1-C_6)alkylsulfonyl, (C_6-C_{10})arylsulfonyl, amino, (C_1-C_6)alkylamino or ((C_1-C_6)alkylamino)$_2$; (C_2-C_6)alkenyl, (C_6-C_{10})aryl(C_2-C_6)alkenyl, (C_5-C_9)heteroaryl(C_2-C_6)alkenyl, (C_2-C_6)alkynyl, (C_6-C_{10})aryl(C_2-C_6)alkynyl, (C_5-C_9)heteroaryl(C_2-C_6)alkynyl, (C_1-C_6)alkylamino, (C_1-C_6)alkylthio, (C_1-C_6)alkoxy, trifluoromethyl, (C_1-C_6)alkyl (difluoromethylene), (C_1-C_3)alkyl(difluoromethylene)(C_1-C_3)alkyl, (C_6-C_{10})aryl, (C_5-C_9)heteroaryl, (C_6-C_{10})arylamino, (C_6-C_{10})arylthio, (C_6-C_{10})aryloxy, (C_5-C_9)heteroarylamino, (C_5-C_9)heteroarylthio, (C_5-C_9)heteroaryloxy, (C_3-C_6)cycloalkyl, (C_1-C_6)alkyl(hydroxymethylene), piperidyl, (C_1-C_6)alkylpiperidyl, (C_1-C_6)acylamino, (C_1-

C_6)acylthio, (C_1-C_6)acyloxy, R^{13}(C_1-C_6)alkyl wherein R^{13} is (C_1-C_6)acylpiperazino, (C_6-C_{10})arylpiperazino, (C_5-C_9)heteroarylpiperazino, (C_1-C_6)alkylpiperazino, (C_6-C_{10})aryl(C_1-C_6)alkylpiperazino,(C_5-C_9)heteroaryl(C_1-C_6)alkylpiperazino,morpholino,thiomorpholino, piperidino, pyrrolidino, piperidyl, (C_1-C_6)alkylpiperidyl, (C_6-C_{10})arylpiperidyl, (C_5-C_9)heteroarylpiperidyl,(C_1-C_6)alkylpiperidyl(C_1-C_6)alkyl,(C_6-C_{10})arylpiperidyl(C_1-C_6)alkyl, (C_5-C_9)heteroarylpiperidyl(C_1-C_6)alkyl or (C_1-C_6)acylpiperidyl;

or a group of the formula

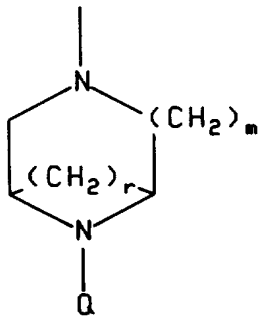


wherein n is 0 to 6;

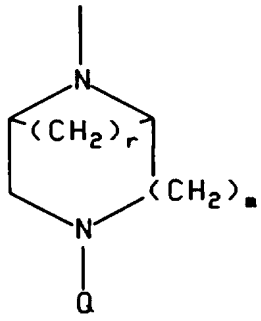
Z is hydroxy, (C_1-C_6)alkoxy or $NR^{14}R^{15}$ wherein R^{14} and R^{15} are each independently selected from the group consisting of hydrogen, (C_1-C_6)alkyl optionally substituted by (C_1-C_6)alkylpiperidyl, (C_6-C_{10})arylpiperidyl, (C_5-C_9)heteroarylpiperidyl, (C_6-C_{10})aryl, (C_5-C_9)heteroaryl, (C_6-C_{10})aryl(C_6-C_{10})aryl or (C_3-C_6)cycloalkyl; piperidyl, (C_1-C_6)alkylpiperidyl, (C_6-C_{10})arylpiperidyl, (C_5-C_9)heteroarylpiperidyl, (C_1-C_6)acylpiperidyl, (C_6-C_{10})aryl,(C_5-C_9)heteroaryl,(C_6-C_{10})aryl(C_6-C_{10})aryl,(C_3-C_6)cycloalkyl,R^{16}(C_2-C_6)alkyl, (C_1-C_5)alkyl(CHR^{16})(C_1-C_6)alkyl wherein R^{16} is hydroxy, (C_1-C_6)acyloxy, (C_1-C_6)alkoxy, piperazino, (C_1-C_6)acylamino, (C_1-C_6)alkylthio, (C_6-C_{10})arylthio, (C_1-C_6)alkylsulfinyl, (C_6-C_{10})arylsulfinyl, (C_1-C_6)alkylsulfoxyl, (C_6-C_{10})arylsulfoxyl, amino, (C_1-C_6)alkylamino, ((C_1-C_6)alkyl)$_2$ amino, (C_1-C_6)acylpiperazino, (C_1-C_6)alkylpiperazino, (C_6-C_{10})aryl(C_1-C_6)alkylpiperazino,(C_5-C_9)heteroaryl(C_1-C_6)alkylpiperazino,morpholino,thiomorpholino, piperidino or pyrrolidino; R^{17}(C_1-C_6)alkyl, (C_1-C_5)alkyl(CHR^{17})(C_1-C_6)alkyl wherein R^{17} is piperidyl or (C_1-C_6)alkylpiperidyl; and CH(R^{18})COR^{19} wherein R^{18} is hydrogen, (C_1-C_6)alkyl, (C_6-C_{10})aryl(C_1-C_6)alkyl, (C_5-C_9)heteroaryl(C_1-C_6)alkyl, (C_1-C_6)alkylthio(C_1-C_6)alkyl, (C_6-C_{10})arylthio(C_1-C_6)alkyl, (C_1-C_6)alkylsulfinyl(C_1-C_6)alkyl, (C_6-C_{10})arylsulfinyl(C_1-C_6)alkyl, (C_1-C_6)alkylsulfonyl(C_1-C_6)alkyl, (C_6-C_{10})arylsulfonyl(C_1-C_6)alkyl, hydroxy(C_1-C_6)alkyl, amino(C_1-C_6)alkyl, (C_1-C_6)alkylamino(C_1-C_6)alkyl, ((C_1-C_6)alkylamino)$_2$(C_1-C_6)alkyl, $R^{20}R^{21}$NCO(C_1-C_6)alkyl or R^{20}OCO(C_1-C_6)alkyl wherein R^{20} and R^{21} are each independently selected from the group consisting of hydrogen, (C_1-

C_6)alkyl, (C_6-C_{10})aryl(C_1-C_6)alkyl and (C_5-C_9)heteroaryl(C_1-C_6)alkyl; and R^{19} is $R^{22}O$ or $R^{22}R^{23}N$ wherein R^{22} and R^{23} are each independently selected from the group consisting of hydrogen, (C_1-C_6)alkyl, (C_6-C_{10})aryl(C_1-C_6)alkyl and (C_5-C_9)heteroaryl(C_1-C_6)alkyl;

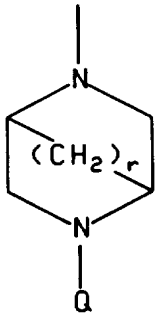
or R^{14} and R^{15}, or R^{20} and R^{21}, or R^{22} and R^{23} may be taken together to form an azetidinyl, pyrrolidinyl, morpholinyl, thiomorpholinyl, indolinyl, isoindolinyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, (C_1-C_6)acylpiperazinyl, (C_1-C_6)alkylpiperazinyl, (C_6-C_{10})arylpiperazinyl, (C_5-C_9)heteroarylpiperazinyl or a bridged diazabicycloalkyl ring selected from the group consisting of



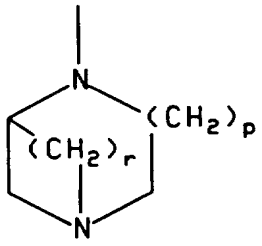
a



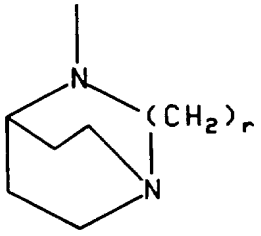
b



c



d



e

wherein r is 1, 2 or 3;

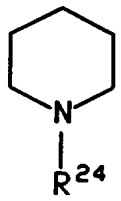
m is 1 or 2;

p is 0 or 1; and

Q is hydrogen, (C_1-C_3)alkyl, (C_1-C_6)acyl or (C_1-C_6)alkoxy carbamoyl;

or R^1 and R^2, or R^3 and R^4, or R^5 and R^6 may be taken together to form a carbonyl;

or R^1 and R^2, or R^3 and R^4, or R^5 and R^6, or R^7 and R^8 may be taken together to form a $(C_3$-$C_6)$cycloalkyl, oxacyclohexyl, thiocyclohexyl, indanyl or tetralinyl ring or a group of the formula



wherein R^{24} is hydrogen, $(C_1$-$C_6)$acyl, $(C_1$-$C_6)$alkyl, $(C_6$-$C_{10})$aryl$(C_1$-$C_6)$alkyl, $(C_5$-$C_9)$heteroaryl$(C_1$-$C_6)$alkyl or $(C_1$-$C_6)$alkylsulfonyl; and

Ar is $(C_6$-$C_{10})$aryl or $(C_5$-$C_9)$heteroaryl, each of which may be optionally substituted by $(C_1$-$C_6)$alkyl, one or two $(C_1$-$C_6)$alkoxy, $(C_6$-$C_{10})$aryloxy or $(C_5$-$C_9)$heteroaryloxy;

with the proviso that R^7 is other than hydrogen only when R^8 is other than hydrogen;

with the proviso that R^6 is other than hydrogen only when R^5 is other than hydrogen;

with the proviso that R^3 is other than hydrogen only when R^4 is other than hydrogen;

with the proviso that R^2 is other than hydrogen only when R^1 is other than hydrogen;

with the provisio that when R^1, R^2 and R^9 are a substituent comprising a heteroatom, the heteroatom cannot be directly bonded to the 2- or 6- positions;

with the proviso that when X is nitrogen, R^4 is not present;

with the proviso that when X is oxygen, sulfur, sulfoxide, sulfone or nitrogen and when one or more of the group consisting of R^1, R^2, R^5 and R^6, is a substituent comprising a heteroatom, the heteroatom cannot be directly bonded to the 4- or 6- positions;

with the proviso that when Y is oxygen, sulfur, sulfoxide, sulfone or nitrogen and when one or more of the group consisting of R^3, R^4, R^7 and R^8, are independently a

substituent comprising a heteroatom, the heteroatom cannot be directly bonded to the 3- or 5- positions;

with the proviso that when X is oxygen, sulfur, sulfoxide or sulfone, R^3 and R^4 are not present;

with the proviso that when Y is nitrogen, R^4 is not present;

with the proviso that when Y is oxygen, sulfur, sulfoxide or sulfone, R^5 and R^6 are not present;

with the proviso that when Y is nitrogen, R^6 is not present;

with the proviso that when the broken line represents a double bond, R^4 and R^5 are not present;

with the proviso that when R^3 and R^5 are independently a substituent comprising a heteroatom when the broken line represents a double bond, the heteroatom cannot be directly bonded to positions X and Y;

with the proviso that when either the X or Y position is oxygen, sulfur, sulfoxide, sulfone or nitrogen, the other of X or Y is carbon;

with the proviso that when X or Y is defined by a heteroatom, the broken line does not represent a double bond;

with the proviso that when R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8 and R^9 are all defined by hydrogen or $(C_1\text{-}C_6)$alkyl, either X or Y is oxygen, sulfur, sulfoxide, sulfone or nitrogen, or the broken line represents a double bond.

The term "alkyl", as used herein, unless otherwise indicated, includes saturated monovalent hydrocarbon radicals having straight, branched or cyclic moieties or combinations thereof.

The term "alkoxy", as used herein, includes O-alkyl groups wherein "alkyl" is defined above.

The term "aryl", as used herein, unless otherwise indicated, includes an organic radical derived from an aromatic hydrocarbon by removal of one hydrogen, such as phenyl or naphthyl, optionally substituted by 1 to 3 substituents independently selected from the group consisting of fluoro, chloro, cyano, nitro, trifluoromethyl, $(C_1\text{-}C_6)$alkoxy, $(C_6\text{-}C_{10})$aryloxy, trifluoromethoxy, difluoromethoxy and $(C_1\text{-}C_6)$alkyl.

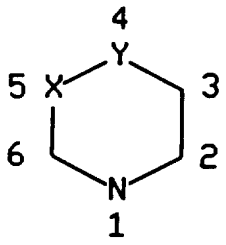
The term "heteroaryl", as used herein, unless otherwise indicated, includes an organic radical derived from an aromatic heterocyclic compound by removal of one hydrogen, such as pyridyl, furyl, pyroyl, thienyl, isothiazolyl, imidazolyl, benzimidazolyl,

tetrazolyl, pyrazinyl, pyrimidyl, quinolyl, isoquinolyl, benzofuryl, isobenzofuryl, benzothienyl, pyrazolyl, indolyl, isoindolyl, purinyl, carbazolyl, isoxazolyl, thiazolyl, oxazolyl, benzthiazolyl or benzoxazolyl, optionally substituted by 1 to 2 substituents independently selected from the group consisting of fluoro, chloro, trifluoromethyl, (C_1-C_6)alkoxy, (C_6-C_{10})aryloxy, trifluoromethoxy, difluoromethoxy and (C_1-C_6)alkyl.

The term "acyl", as used herein, unless otherwise indicated, includes a radical of the general formula RCO wherein R is alkyl, alkoxy, aryl, arylalkyl or arylalkyloxy and the terms "alkyl" or "aryl" are as defined above.

The term "acyloxy", as used herein, includes O-acyl groups wherein "acyl" is defined above.

The positions on the ring of formula I, as used herein, are defined as follows:



The preferred conformation of the compound of formula I includes hydroxamic acid axially disposed in the 2-position.

The compound of formula I may have chiral centers and therefore exist in different enantiomeric forms. This invention relates to all optical isomers and stereoisomers of the compounds of formula I and mixtures thereof.

Preferred compounds of formula I include those wherein Y is oxygen, nitrogen or sulfur.

Other preferred compounds of formula I include those wherein Ar is 4-methoxyphenyl or 4-phenoxyphenyl.

Other preferred compounds of formula I include those wherein R^8 is (C_6-C_{10})aryl, (C_5-C_9)heteroaryl, (C_6-C_{10})aryl(C_1-C_6)alkyl, (C_5-C_9)heteroaryl(C_1-C_6)alkyl, carboxylic acid or carboxylic acid (C_1-C_6)alkyl.

Other preferred compounds of formula I include those wherein R^2, R^3, R^6, R^7 and R^9 are hydrogen.

More preferred compounds of formula I include those wherein Y is carbon, Ar is 4-methoxyphenyl or 4-phenoxyphenyl and R^8 is (C_6-C_{10})arylalkynyl or (C_5-C_9)heteroarylalkynyl.

More preferred compounds of formula I include those wherein Y is oxygen, Ar is 4-methoxyphenyl or 4-phenoxyphenyl and R^8 is (C_6-C_{10})arylalkynyl or (C_5-C_9)heteroarylalkynyl.

More preferred compounds of formula I include those wherein Y is carbon, Ar is 4-methoxyphenyl or 4-phenoxyphenyl and R^8 is carboxylic acid or carboxylic acid (C_1-C_6)alkyl.

More preferred compounds of formula I include those wherein Y is oxygen, Ar is 4-methoxyphenyl or 4-phenoxyphenyl and R^8 is carboxylic acid or carboxylic acid (C_1-C_6)alkyl.

More preferred compounds of formula I include those wherein Y is carbon, Ar is 4-methoxyphenyl or 4-phenoxyphenyl and R^5 is (C_6-C_{10})arylalkynyl or (C_5-C_9)heteroarylalkynyl.

More preferred compounds of formula I include those wherein Y is oxygen, Ar is 4-methoxyphenyl or 4-phenoxyphenyl and R^5 is (C_6-C_{10})arylalkynyl or (C_5-C_9)heteroarylalkynyl.

More preferred compounds of formula I include those wherein Y is carbon, Ar is 4-methoxyphenyl or 4-phenoxyphenyl and R^5 is carboxylic acid or carboxylic acid (C_1-C_6)alkyl.

More preferred compounds of formula I include those wherein Y is oxygen, Ar is 4-methoxyphenyl or 4-phenoxyphenyl and R^5 is carboxylic acid or carboxylic acid (C_1-C_6)alkyl.

More preferred compounds of formula I include those wherein Y is carbon, Ar is 4-methoxyphenyl or 4-phenoxyphenyl and R^5 is (C_1-C_6)alkylamino.

More preferred compounds of formula I include those wherein Y is oxygen, Ar is 4-methoxyphenyl or 4-phenoxyphenyl and R^8 is (C_1-C_6)alkylamino.

Specific preferred compounds of formula I include the following:

(2R,3S)-N-hydroxy-3-ethynyl-1-(4-methoxybenzenesulfonyl)-piperidine-2-carboxamide;

(2R,3S)-N-hydroxy-l-(4-methoxybenzenesulfonyl)-3-(5-methoxythiophene-2-yl-ethynyl)-piperidine-2-carboxamide;

(2R,3R)-N-hydroxy-1-(4-methoxybenzenesulfonyl)-3-(3-pyridin-3-yl-prop-2-ynyl)-piperidine-2-carboxamide;

(2S,3R)-N-hydroxy-4-(4-methoxybenzenesulfonyl)-2-pyridine-3-yl-morpholine-3-carboxamide;

(2S,3R)-N-hydroxy-2-hydroxycarbamoyl-4-(4-methoxybenzenesulfonyl)-morpholine-3-carboxamide;

(2R,3R)-N-hydroxy-2-hydroxycarbamoyl-4-(4-methoxybenzenesulfonyl)-piperidine-2-carboxamide;

(2R,3S)-N-hydroxy-1-(4-methoxybenzenesulfonyl)-3-(4-phenylpyridine-2-yl)-piperidine-2-carboxamide;

(2S,3R)-N-hydroxy-1-(4-methoxybenzenesulfonyl)-2-(4-phenylpyridine-2-yl)-morpholine-2-carboxamide;

(2R,3S)-N-hydroxy-3-(2-chloro-4-fluorophenyl)-1-(4-methoxybenzenesulfonyl)-piperidine-2-carboxamide; and

(2S,3R)-N-hydroxy-2-(2-chloro-4-fluorophenyl)-1-(4-methoxybenzenesulfonyl)-piperidine-3-carboxamide.

The present invention also relates to a pharmaceutical composition for (a) the treatment of a condition selected from the group consisting of arthritis, cancer, tissue ulceration, restenosis, periodontal disease, epidermolysis bullosa, scleritis and other diseases characterized by matrix metalloproteinase activity, AIDS, sepsis, septic shock and other diseases involving the production of tumor necrosis factor (TNF) or (b) the inhibition of matrix metalloproteinases or the production of tumor necrosis factor (TNF) in a mammal, including a human, comprising an amount of a compound of claim 1 or a pharmaceutically acceptable salt thereof, effective in such treatments or inhibition and a pharmaceutically acceptable carrier.

The present invention also relates to a method for the inhibition of (a) matrix metalloproteinases or (b) the production of tumor necrosis factor (TNF) in a mammal, including a human, comprising administering to said mammal an effective amount of a compound of claim 1 or a pharmaceutically acceptable salt thereof.

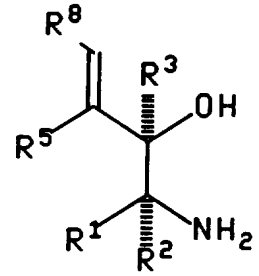
The present invention also relates to a method for treating a condition selected from the group consisting of arthritis, cancer, tissue ulceration, restenosis, periodontal disease, epidermolysis bullosa, scleritis and other diseases characterized by matrix metalloproteinase activity, AIDS, sepsis, septic shock and other diseases involving the

production of tumor necrosis factor (TNF) in a mammal, including a human, comprising administering to said mammal an amount of a compound of claim 1 or a pharmaceutically acceptable salt thereof, effective in treating such a condition.

Detailed Description of the Invention

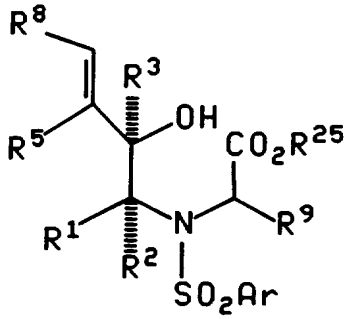
The following reaction Schemes illustrate the preparation of the compounds of the present invention. Unless otherwise indicated R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, n and Ar in the reaction Schemes and the discussion that follow are defined as above.

Preparation 1



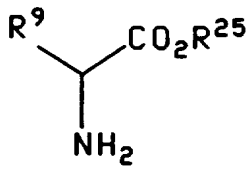
XVI

1



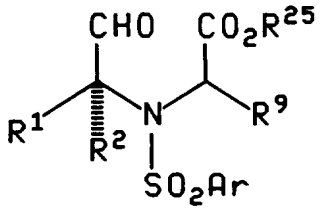
VI

Preparation 2



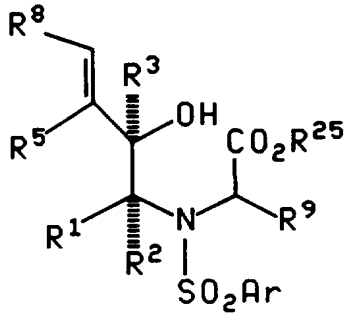
XVIII

1



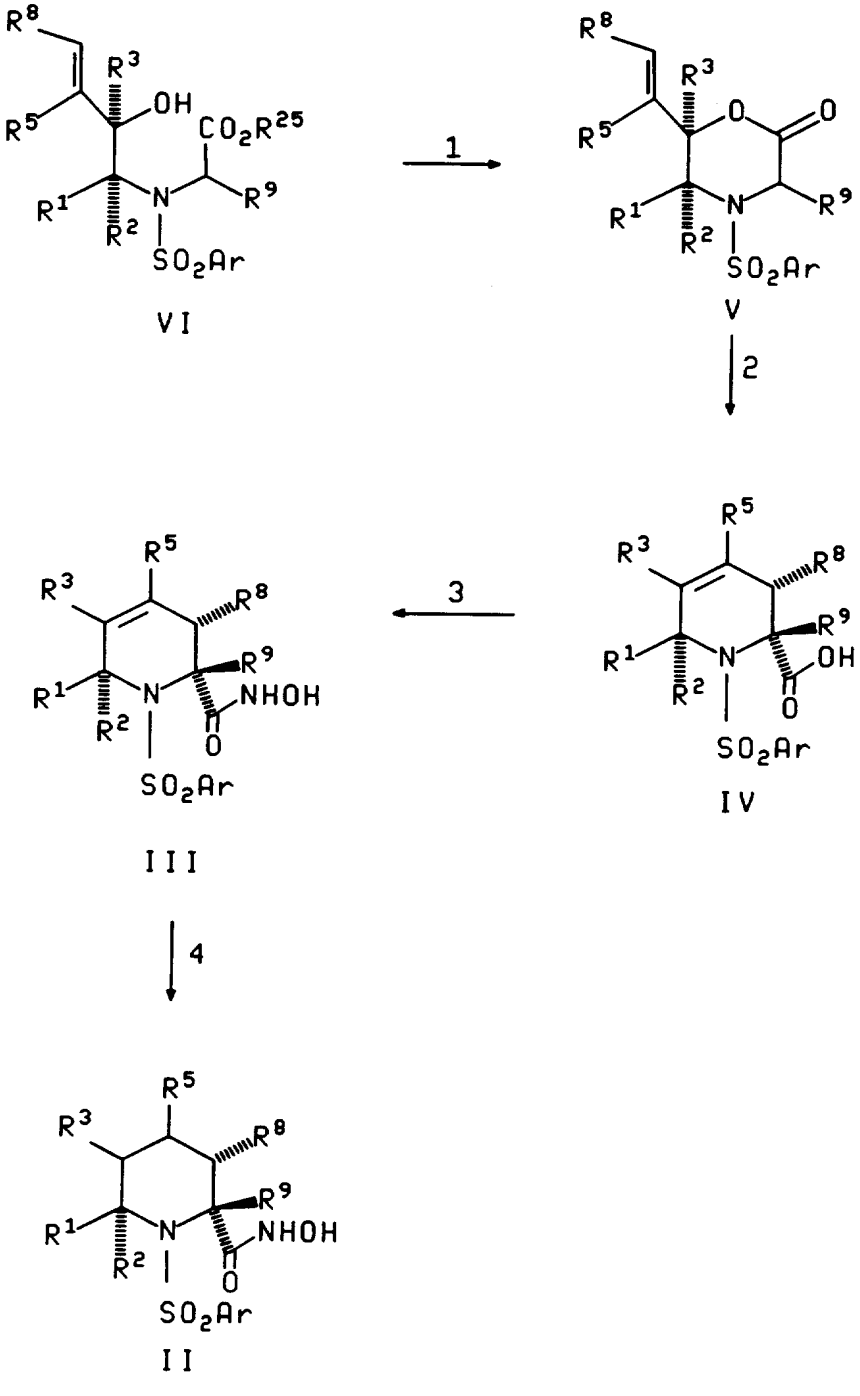
XVII

2

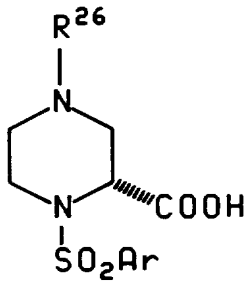


VI

Scheme 1

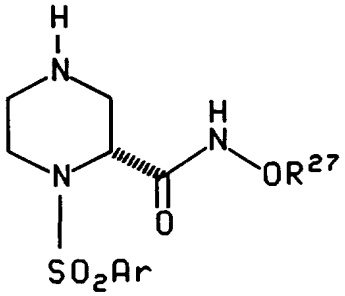


Scheme 2



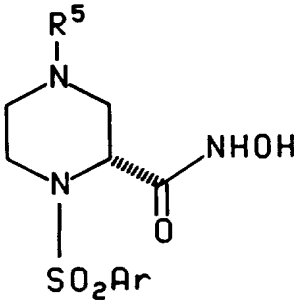
IX

1



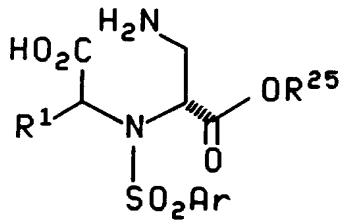
VIII

2



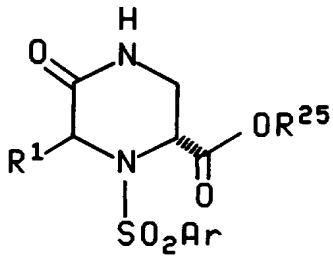
VII

Scheme 3



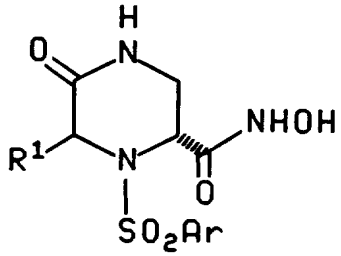
XII

1



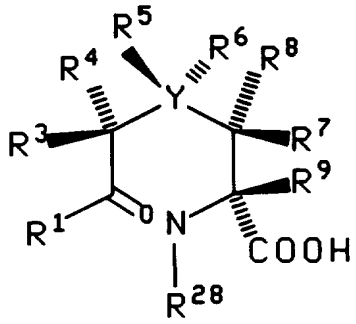
XI

2



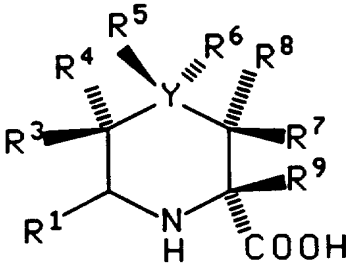
X

Scheme 4



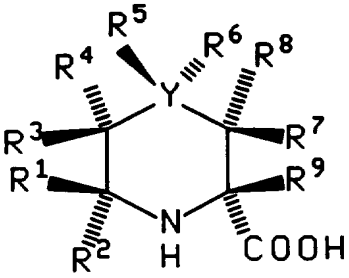
XXII

1



XXI

2

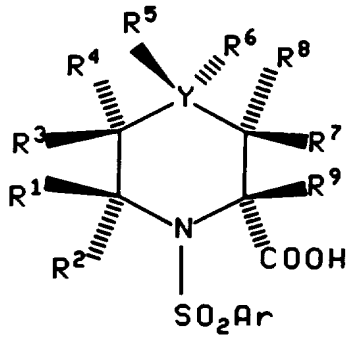


XX

Scheme 4 continued

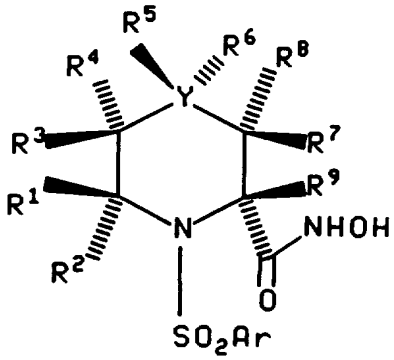
XX

3



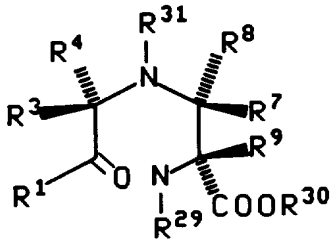
XIX

4



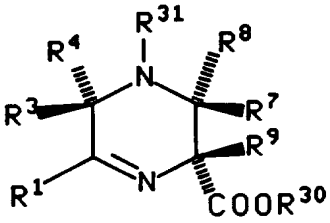
XIII

Scheme 5



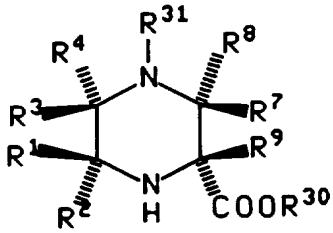
XXVI

1



XXV

2

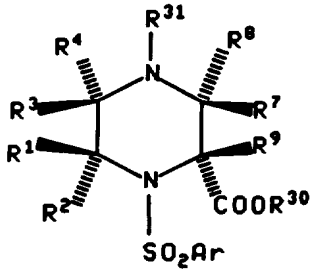


XXIV

Scheme 5 continued

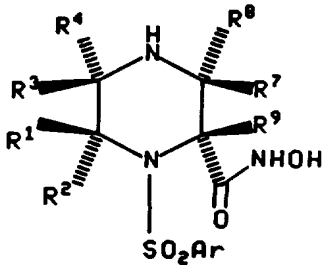
XXIV

3



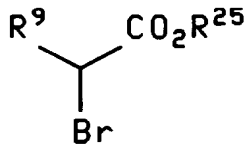
XXIII

4



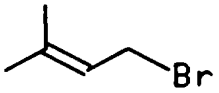
XIV

In reaction 1 of Preparation 1, the compound of formula **XVI** is converted to the corresponding hydroxy ester compound of formula **VI** by first reacting **XVI** with an arylsulfonylhalide in the presence of triethylamine and an aprotic solvent, such as methylene chloride, tetrahydrofuran or dioxane, at a temperature between about 20°C to about 30°C, preferably at room temperature. The compound so formed is further reacted with a compound of the formula

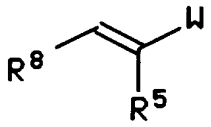


wherein R^{25} is carbobenzyloxy, (C_1-C_6)alkyl, benzyl, allyl or tert-butyl, in the presence of sodium hexamethyldisilazane and a tetrahydrofuran-dimethylformamide solvent mixture at a temperature between about -20°C to about 20°C, preferably about 0°C, to form the hydroxy ester compound of formula **VI**.

In reaction 1 of Preparation 2, the amine compound of formula **XVIII**, wherein R^{25} is as defined above, is converted to the corresponding arylsulfonyl amine compound of formula **XVII** by (1) reacting **XVIII** with an arylsulfonylhalide in the presence of triethylamine and an aprotic solvent, such as methylene chloride, tetrahydrofuran, or dioxane, at a temperature between about 20°C to about 30°C, preferably at room temperature, (2) reacting the compound so formed with a compound of the formula



in the presence of sodium hexamethyldisilazane and a tetrahydrofuran-dimethylformamide solvent mixture at a temperature between about -20°C to about 20°C, preferably about 0°C, and (3) further reacting the compound so formed with ozone in a methylene chloride-methanol solution at a temperature between about -90°C to about -70°C, preferably about -78°C. The unstable ozonide compound so formed is then reacted with triphenylphosphine to form the arylsulfonyl amine compound formula **XVII**. In Reaction 2 of Preparation 2, the arylsulfonyl amine compound of formula **XVII** is converted to the corresponding hydroxy ester compound of formula **VI** by reacting **XVII** with a compound of the formula



wherein W is lithium, magnesium, copper or chromium.

In reaction 1 of Scheme 1, the compound of formula VI, wherein the R^{25} protecting group is carbobenzyloxy, (C_1-C_6) alkyl, benzyl, allyl or tert-butyl, is converted to the corresponding morpholinone compound of formula V by lactonization and subsequent Claisen rearrangement of the compound of formula VI. The reaction is facilitated by the removal of the R^{25} protecting group from the compound of formula VI is carried out under conditions appropriate for that particular R^{25} protecting group in use. Such conditions include: (a) treatment with hydrogen and a hydrogenation catalyst, such as 10% palladium on carbon, where R^{25} is carbobenzyloxy, (b) saponification where R^{25} is lower alkyl, (c) hydrogenolysis where R^{25} is benzyl, (d) treatment with a strong acid, such as trifluoroacetic acid or hydrochloric acid, where R^{25} is tert-butyl, or (e) treatment with tributyltinhydride and acetic acid in the presence of catalytic bis(triphenylphosphine) palladium (II) chloride where R^{25} is allyl.

In reaction 2 of Scheme 1, the morpholinone compound of formula V is converted to the carboxylic acid compound of formula IV by reacting V with lithium hexamethyldisilazane in an aprotic solvent, such as tetrahydrofuran, at a temperature between about -90°C to about -70°C, preferably about -78°C. Trimethylsilyl chloride is then added to the reaction mixture and the solvent, tetrahydrofuran, is removed in vacuo and replaced with toluene. The resuling reaction mixture is heated to a temperature between about 100°C to about 120°C, preferably about 110°C, and treated with hydrochloric acid to form the carboxylic acid compound of formula IV.

In reaction 3 of Scheme 1, the carboxylic acid compound of formula IV is converted to the corresponding hydroxamic acid compound of formula III by treating IV with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide and 1-hydroxybenztriazole in a polar solvent, such as dimethylformamide, followed by the addition of hydroxylamine to the reaction mixture after a time period between about 15 minutes to about 1 hour, preferably about 30 minutes. The hydroxylamine is preferably generated in situ from a salt form, such as hydroxylamine hydrochloride, in the presence of a base, such as N-methylmorpholine. Alternatively, a protected derivative of hydroxylamine or its salt

form, where the hydroxyl group is protected as a tert-butyl, benzyl or allyl ether, may be used in the presence of (benzotriazol-1-yloxy)tris(dimethylamino) phosphonium hexafluorphosphate and a base, such as N-methylmorpholine. Removal of the hydroxylamine protecting group is carried out by hydrogenolysis for a benzyl protecting group or treatment with a strong acid, such as trifluoroacetic acid, for a tert-butyl protecting group. The allyl protecting group may be removed by treatment with tributyltinhydride and acetic acid in the presence of catalytic bis(triphenylphosphine) palladium (II) chloride. N,O-bis(4-methoxybenzyl)hydroxylamine may also be used as the protected hydroxylamine derivative where deprotection is achieved using a mixture of methanesulfonic acid and trifluoroacetic acid.

In reaction 4 of Scheme 1, the hydroxamic acid compound of formula III is converted, if desired, to the corresponding piperidine compound of formula II by treating III with hydrogen and a hydrogenation catayst, such a 10% palladium on carbon.

In reaction 1 of Scheme 2, the arylsulfonylpiperazine compound of formula IX, wherein R^{26} is carbobenzyloxy, benzyl or carbotertbutyloxy, is converted to the compound of formula VIII by reacting IX with a protected derivative of hydroxylamine of the formula

$$R^{27}ONH_2 \bullet HCl$$

wherein R^{27} is tertbutyl, benzyl or allyl, in the presence of dicyclohexylcarbodiimide, dimethylaminopyridine and an aprotic solvent, such as methylene chloride. The R^{26} protecting group is chosen such that it may be selectively removed in the presence of an without loss of the R^{27} protecting group, therefore, R^{26} cannot be the same as R^{27}. Removal of the R^{26} protecting group from the compound of formula IX is carried out under conditions appropriate for that particular R^{26} protecting group in use. Such conditions include; (a) treatment with a hydrogen and a hydrogenation catalyst, such as 10% palladium on carbon, where R^{26} is carbobenzyloxy, (b) hydrogenolysis where R^{26} is benzyl or (c) treatment with a strong acid, such as trifluoroacetic acid or hydrochloric acid where R^{26} is carbotertbutyloxy.

In reaction 2 of Scheme 2, the compound of formula VIII is converted to the corresponding hydroxamic acid compound of formula VII, wherein R^5 is hydrogen or $(C_1\text{-}C_6)$alkyl, by reacting, if desired, VIII with an alkylhalide when R^5 is $(C_1\text{-}C_6)$alkyl. Subsequent removal of the R^{27} hydroxylamine protecting group is carried out by

hydrogenolysis for a benzyl protecting group or treatment with a strong acid, such as trifluoroacetic acid, for a tert-butyl protecting group. The allyl protecting group may be removed by treatment with tributyltinhydride and acetic acid in the presence of catalytic bis(triphenylphosphine) palladium (II) chloride.

In reaction 1 of Scheme 3, the arylsulfonylamine compound of formula **XII**, wherein R^{25} is as defined above, is converted to the corresponding piperizine compound of formula **XI** by reacting **XII** with a carbodiimide and a base, such as triethylamine. The compound of formula **XI** is further reacted to give the hydroxamic acid compound of formula **X** according to the procedure described above in reaction 3 of Scheme 1.

In reaction 1 of Scheme 4, removal of the R^{28} protecting group and subsequent reductive amination of the compound of formula **XXII**, wherein Y is oxygen, sulfur or carbon, to give the corresponding imine compound of formula **XXI** is carried out under conditions appropriate for that particular R^{28} protecting group in use. Such conditions include those used above for removal of the R^{26} protecting group in reaction 1 of Scheme 2.

In reaction 2 of Scheme 4, the imine compound of formula **XXI** is converted to the corresponding piperidine compound of formula **XX** by reacting **XXI** with a nucleophile of the formula R^2M wherein M is lithium, magnesium halide or cerium halide. The reaction is carried out in ether solvents, such as diethyl ether or tetrahydrofuran, at a temperature between about -78°C to about 0°C, preferably about -70°C.

In reaction 3 of Scheme 4, the sulfonation of the piperidine compound of formula **XX** to given the corresponding arylsulfonylpiperidine compound of formula **XIX** is carried out by reacting **XX** with an arylsulfonylhalide in the presence of triethylamine and an aprotic solvent, such as metherone chloride, tetrahydrofuran or dioxane, at a temperature between about 20°C to about 30°C, preferably at room temperature.

In reaction 4 of Scheme 4, the arylsulfonylpiperidine compound of formula **XIX** is converted to the hydroxamic acid compound of formula **XIX** according to the procedure described above in reaction 3 of Scheme 1.

In reaction 1 of Scheme 5, the compound of formula **XXVI**, wherein the R^{29} and R^{31} protecting groups are each independently selected from the group consisting of carbobenzyloxy, benzyl and carbotertbutyloxy and R^{30} is carbobenzyloxy, $(C_1\text{-}C_6)$alkyl,

benzyl, allyl or tert-butyl, is converted to the corresponding imine compound of formula **XXV** by the removal of the R^{29} protecting group and subsequent reductive amination of the compound of formula **XXVI**. The R^{29} protecting group is chosen such that it may be selectively removed in the presence of and without loss of the R^{31} protecting group. Removal of the R^{29} protecting group from the compound of formula **XXVI** is carried out under conditions appropriate for that particular R^{29} protecting group in use which will not affect the R^{31} protecting group. Such conditions include; (a) treatment with hydrogen and a hydrogenation catalyst, such as 10% palladium on carbon, where R^{29} is carbobenzyloxy and R^{31} is tert-butyl, (b) saponification where R^{29} is $(C_1\text{-}C_6)$alkyl and R^{31} is tert-butyl, (c) hydrogenolysis where R^{29} is benzyl and R^{31} is $(C_1\text{-}C_6)$ alkyl or tert-butyl, (d) treatment with a strong acid such as trifluoroacetic acid or hydrochloric acid where R^{29} is tert-butyl and R^{31} is $(C_1\text{-}C_6)$alkyl, benzyl or allyl, or (e) treatment with tributyltinhydride and acetic acid in the presence of catalytic bis(triphenylphosphine) palladium (II) chloride where R^{29} is allyl and R^{31} is $(C_1\text{-}C_6)$alkyl, benzyl or tert-butyl. The R^{30} protective group may be selected such that it is removed in the same reaction step as the R^{29} protecting group.

In reaction 2 of Scheme 5, the imine compound of formula **XXV** is converted to the corresponding compound of formula **XXIV** by reacting **XXV** with a nucleophile of the formula R^2M wherein M is lithium, magnesium halide or calcium halide. The reaction is carried out in ether solvents, such as diethyl ether or tetrahydrofuran, at a temperature between about -78°C to about 0°C, preferably about -70°C.

In reaction 3 of Scheme 5, the sulfonation of the piperidine compound of formula **XXIV** to give the corresponding arylsulfonylpiperidine compound of formula **III** is carried out according to the procedure described above in reaction 3 of Scheme 4.

In reaction 4 of Scheme 5, the arylsulfonylpiperidine compound of formula **XXIII** is converted to the hydroxamic acid compound of formula **XIV** by (1) removing the R^{30}, if needed, and R^{31} protecting groups from **XXIII** followed by (2) reacting **XXIII** according to the procedure described above in reaction 3 of Scheme 1. Removal of the R^{30} and R^{31} protecting groups from the compound of formula **XXIII** is carried out under conditions appropriate for that particular R^{30} and R^{31} protecting group in use. Such conditions include those used above for removal of the R^{25} protecting group in reaction 1 of Scheme 1.

Pharmaceutically acceptable salts of the acidic compounds of the invention are salts formed with bases, namely cationic salts such as alkali and alkaline earth metal salts, such as sodium, lithium, potassium, calcium, magnesium, as well as ammonium slats, such as ammonium, trimethyl-ammonium, diethylammonium, and tris-(hydroxymethyl)-methylammonium slats.

Similarly acid addition salts, such as of mineral acids, organic carboxylic and organic sulfonic acids e.g. hydrochloric acid, methanesulfonic acid, maleic acid, are also possible provided a basic group, such as pyridyl, constitutes part of the structure.

The ability of the compounds of formula I or their pharmaceutically acceptable salts (hereinafter also referred to as the compounds of the present invention) to inhibit matrix metalloproteinases or the production of tumor necrosis factor (TNF) and, consequently, demonstrate their effectiveness for treating diseases characterized by matrix metalloproteinase or the production of tumor necrosis factor is shown by the following in vitro assay tests.

Biological Assay

Inhibition of Human Collagenase (MMP-1)

Human recombinant collagenase is activated with trypsin using the following ratio: 10 μg trypsin per 100 μg of collagenase. The trypsin and collagenase are incubated at room temperature for 10 minutes then a five fold excess (50 μg/10 μg trypsin) of soybean trypsin inhibitor is added.

10 mM stock solutions of inhibitors are made up in dimethyl sulfoxide and then diluted using the following Scheme:

10 mM ——> 120 μM ——> 12 μM ——> 1.2 μM ——> 0.12 μM

Twenty-five microliters of each concentration is then added in triplicate to appropriate wells of a 96 well microfluor plate. The final concentration of inhibitor will be a 1:4 dilution after addition of enzyme and substrate. Positive controls (enzyme, no inhibitor) are set up in wells D1-D6 and blanks (no enzyme, no inhibitors) are set in wells D7-D12.

Collagenase is diluted to 400 ng/ml and 25 μl is then added to appropriate wells of the microfluor plate. Final concentration of collagenase in the assay is 100 ng/ml.

Substrate (DNP-Pro-Cha-Gly-Cys(Me)-His-Ala-Lys(NMA)-NH_2) is made as a 5 mM stock in dimethyl sulfoxide and then diluted to 20 μM in assay buffer. The assay is

initiated by the addition of 50 μl substrate per well of the microfluor plate to give a final concentration of 10 μM.

Fluorescence readings (360 nM excitation, 460 nm emission) were taken at time 0 and then at 20 minute intervals. The assay is conducted at room temperature with a typical assay time of 3 hours.

Fluorescence vs time is then plotted for both the blank and collagenase containing samples (data from triplicate determinations is averaged). A time point that provides a good signal (the blank) and that is on a linear part of the curve (usually around 120 minutes) is chosen to determine IC_{50} values. The zero time is used as a blank for each compound at each concentration and these values are subtracted from the 120 minute data. Data is plotted as inhibitor concentration vs % control (inhibitor fluorescence divided by fluorescence of collagenase alone x 100). IC_{50}'s are determined from the concentration of inhibitor that gives a signal that is 50% of the control.

If IC_{50}'s are reported to be <0.03 μM then the inhibitors are assayed at concentrations of 0.3 μM, 0.03 μM, 0.03 μM and 0.003 μM.

Inhibition of Gelatinase (MMP-2)

Inhibition of gelatinase activity is assayed using the Dnp-Pro-Cha-Gly-Cys(Me)-His-Ala-Lys(NMA)-NH_2 substrate (10 μM) under the same conditions as inhibition of human collagenase (MMP-1).

72kD gelatinase is activated with 1 mM APMA (p-aminophenyl mercuric acetate) for 15 hours at 4°C and is diluted to give a final concentration in the assay of 100 mg/ml. Inhibitors are diluted as for inhibition of human collagenase (MMP-1) to give final concentrations in the assay of 30 μM, 3 μM, 0.3 μM and 0.03 μM. Each concentration is done in triplicate.

Fluorescence readings (360 nm excitation, 460 emission) are taken at time zero and then at 20 minutes intervals for 4 hours.

IC_{50}'s are determined as per inhibition of human collagenase (MMP-1). If IC_{50}'s are reported to be less than 0.03 μM, then the inhibitors are assayed at final concentrations of 0.3 μM, 0.03 μM, 0.003 μM and 0.003 μM.

Inhibition of Stromelysin Activity (MMP-3)

Inhibition of stromelysin activity is based on a modified spectrophotometric assay described by Weingarten and Feder (Weingarten, H. and Feder, J., Spectrophotometric Assay for Vertebrate Collagenase, Anal. Biochem. 147, 437-440 (1985)). Hydrolysis of the thio peptolide substrate [Ac-Pro-Leu-Gly-$SCH[CH_2CH(CH_3)_2]CO$-Leu-Gly-OC_2H_5] yields a mercaptan fragment that can be monitored in the presence of Ellman's reagent.

Human recombinant prostromelysin is activated with trypsin using a ratio of 1 μl of a 10 mg/ml trypsin stock per 26 μg of stromelysin. The trypsin and stromelysin are incubated at 37°C for 15 minutes followed by 10 μl of 10 mg/ml soybean trypsin inhibitor for 10 minutes at 37°C for 10 minutes at 37°C to quench trypsin activity.

Assays are conducted in a total volume of 250 μl of assay buffer (200 mM sodium chloride, 50 mM MES, and 10 mM calcium chloride, pH 6.0) in 96-well microliter plates. Activated stromelysin is diluted in assay buffer to 25 μg/ml. Ellman's reagent (3-Carboxy-4-nitrophenyl disulfide) is made as a 1M stock in dimethyl formamide and diluted to 5 mM in assay buffer with 50 μl per well yielding at 1 mM final concentration.

10 mM stock solutions of inhibitors are made in dimethyl sulfoxide and diluted serially in assay buffer such that addition of 50 μL to the appropriate wells yields final concentrations of 3 μM, 0.3 μM, 0.003 μM, and 0.0003 μM. All conditions are completed in triplicate.

A 300 mM dimethyl sulfoxide stock solution of the peptide substrate is diluted to 15 mM in assay buffer and the assay is initiated by addition of 50 μl to each well to give a final concentration of 3 mM substrate. Blanks consist of the peptide substrate and Ellman's reagent without the enzyme. Product formation was monitored at 405 nm with a Molecular Devices UVmax plate reader.

IC_{50} values were determined in the same manner as for collagenase.

Inhibition of MMP-13

Human recombinant MMP-13 is activated with 2mM APMA (p-aminophenyl mercuric acetate) for 1.5 hours, at 37°C and is diluted to 400 mg/ml in assay buffer (50 mM Tris, pH 7.5, 200 mM sodium chloride, 5mM calcium chloride, 20μM zinc chloride, 0.02% brij). Twenty-five microliters of diluted enzyme is added per well of a 96 well microfluor plate. The enzyme is then diluted in a 1:4 ratio in the assay by the addition of inhibitor and substrate to give a final concentration in the assay of 100 mg/ml.

10 mM stock solutions of inhibitors are made up in dimethyl sulfoxide and then diluted in assay buffer as per the inhibitor dilution scheme for inhibition of human collagenase (MMP-1): Twenty-five microliters of each concentration is added in triplicate to the microfluor plate. The final concentrations in the assay are 30 μM, 3μM, 0.3 μM, and 0.03 μM.

Substrate (Dnp-Pro-Cha-Gly-Cys(Me)-His-Ala-Lys(NMA)-NH_2) is prepared as for inhibition of human collagenase (MMP-1) and 50 μl is added to each well to give a final assay concentration of 10 μM. Fluorescence readings (360 nM excitation; 450 emission) are taken at time 0 and every 5 minutes for 1 hour.

Positive controls consist of enzyme and substrate with no inhibitor and blanks consist of substrate only.

IC_{50}'s are determined as per inhibition of human collagenase (MMP-1). If IC_{50}'s are reported to be less than 0.03 μM, inhibitors are then assayed at final concentrations of 0.3 μM, 0.03 μM, 0.003 μM and 0.0003 μM.

Inhibition of TNF Production

The ability of the compounds or the pharmaceutically acceptable salts thereof to inhibit the production of TNF and, consequently, demonstrate their effectiveness for treating diseases involving the production of TNF is shown by the following in vitro assay:

Human mononuclear cells were isolated from anti-coagulated human blood using a one-step Ficoll-hypaque separation technique. (2) The mononuclear cells were washed three times in Hanks balanced salt solution (HBSS) with divalent cations and resuspended to a density of 2 x 10^6 /ml in HBSS containing 1% BSA. Differential counts determined using the Abbott Cell Dyn 3500 analyzer indicated that monocytes ranged from 17 to 24% of the total cells in these preparations.

180μ of the cell suspension was aliquoted into flate bottom 96 well plates (Costar). Additions of compounds and LPS (100ng/ml final concentration) gave a final volume of 200μl. All conditions were performed in triplicate. After a four hour incubation at 37°C in an humidified CO_2 incubator, plates were removed and centrifuged (10 minutes at approximately 250 x g) and the supernatants removed and assayed for TNFα using the R&D ELISA Kit.

For administration to humans for the inhibition of matrix metalloproteinases or the production of tumor necrosis factor (TNF), a variety of conventional routes may be

used including orally, parenterally and topically. In general, the active compound will be administered orally or parenterally at dosages between about 0.1 and 25 mg/kg body weight of the subject to be treated per day, preferably from about 0.3 to 5 mg/kg. However, some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject.

The compounds of the present invention can be administered in a wide variety of different dosage forms, in general, the therapeutically effective compounds of this invention are present in such dosage forms at concentration levels ranging from about 5.0% to about 70% by weight.

For oral administration, tablets containing various excipients such as microcrystalline cellulose, sodium citrate, calcium carbonate, dicalcium phosphate and glycine may be employed along with various disintegrants such as starch (and preferably corn, potato or tapioca starch), alginic acid and certain complex silicates, together with granulation binders like polyvinylpyrrolidone, sucrose, gelation and acacia. Additionally, lubricating agents such as magnesium stearate, sodium lauryl sulfate and talc are often very useful for tabletting purposes. Solid compositions of a similar type may also be employed as fillers in gelatin capsules; preferred materials in this connection also include lactose or milk sugar as well as high molecular weight polyethylene glycols. When aqueous suspensions and/or elixirs are desired for oral administration, the active ingredient may be combined with various sweetening or flavoring agents, coloring matter or dyes, and, if so desired, emulsifying and/or suspending agents as well, together with such diluents as water, ethanol, propylene glycol, glycerin and various like combinations thereof.

For parenteral administration (intramuscular, intraperitoneal, subcutaneous and intravenous use) a sterile injectable solution of the active ingredient is usually prepared. Solutions of a therapeutic compound of the present invention in either sesame or peanut oil or in aqueous propylene glycol may be employed. The aqueous solutions should be suitably adjusted and buffered, preferably at a pH of greater than 8, if necessary and the liquid diluent first rendered isotonic. These aqueous solutions are suitable intravenous injection purposes. The oily solutions are suitable for intraarticular, intramuscular and subcutaneous injection purposes. The preparation of all these

solutions under sterile conditions is readily accomplished by standard pharmaceutical techniques well known to those skilled in the art.

Additionally, it is possible to administer the compounds of the present invention topically, e.g., when treating inflammatory conditions of the skin and this may be done by way of creams, jellies, gels, pastes, and ointments, in accordance with standard pharmaceutical practice.

The present invention is illustrated by the following examples, but it is not limited to the details thereof.

EXAMPLE 1

(+)-(2R*,3R*)-(N-hydroxy)-1-(4-methoxy-benzenesulfonyl)-3-methyl-1,2,3,6-tetrahydropyridine-2-carboxamide.

(a) To a solution of (E)-1-amino-3-pentent-2-ol (2.0 grams, 10.0 mmol) in methylene chloride (50 ml) is added triethylamine (160 μL, 11.0 mmol) followed by 4-methoxybenzenesulfonyl chloride (2.07 grams, 10.0 mmol). The mixture is stirred at room temperature for 12 hours and diluted with ethyl acetate. The mixture is washed with water, 10% citric acid, dried (sodium sulfate), filtered and concentrated. The crude product is purified by silica gel chromatography (elution with 2:1 ethyl acetate-hexanes) to provide (N-(2-hydroxy-pent-3-enyl)-4-methoxybenzenesulfonamide.

(b) To a solution of (±)-(E)-N-(2-hydroxy-pent-3-enyl)-4-methoxybenzenesulfonamide (1.2 grams, 4.42 mmol) in tetrahydrofuran-dimethylformamide (10 mL, ca. 3:1) at 0°C is added sodium bis(trimethylsilyl)amide (4.9 mL, 1.0 M solution in tetrahydrofuran). After 10 minutes, t-butylbromoacetate (786 mL, 4.83 mmol) is added. The mixture is warmed to room temperature, stirred for 1 hour and quenched with saturated ammonium chloride solution. The mixture is extracted with ethyl acetate and the combined extracts are dried (sodium sulfate), filtered and concentrated. The crude product is purified by silica gel chromatography (elution with 1:1 ethyl acetate-hexanes) to provide [(2-hydroxy-pent-3-enyl)-(4-methoxybenzenesulfonyl)-amino]-acetic acid t-butyl ester.

(c) To a solution of (±)-(E)-N-(2-hydroxy-pent-3-enyl)-4-methoxybenzenesulfonyl)-amino]-acetic acid t-butyl ester (900 mg, 2.43 mmol) in benzene (10 ml) is added trifluoroacetic acid (56 μL, 0.73 mmol). The solution is heated at 80°C for 3 hours, cooled to room temperature and concentrated to provide

(±)-(E)-4-(4-methoxybenzenesulfonyl)-6-propenylmorpholin-2-one which is used without further purification.

(d) To a solution of lithium bis(trimethylsilyl)amide (2.67 mmol, 1.0 M in tetrahydrofuran) in tetrahydrofuran (5.0 ml) at -78°C is added a solution of (±)-(E)-4-(4-methoxybenzenesulfonyl)-6-propenylmorpholine-2-one crude from the previous step. After 15 minutes, trimethylsilyl chloride (1.53 ml, 12.15 mmol) is added and the mixture warmed to room temperature. The solvent is removed (in vacuo) and replaced with toluene (10 ml). The resulting mixture is heated at 110°C for 3 hours, cooled to room temperature and treated with 1N hydrochloric acid solution. After stirring for 10 minutes, the mixture is extracted with ethyl acetate and the combined extracts are dried (sodium sulfate), filtered and concentrated. The crude product is purified by silica gel chromatography (elution with 2:1 ethyl acetate-hexanes with 1% acetic acid) to provide (±)-(2R*, 3R*)-1-(4-methoxy-benzenesulfonyl)-3-methyl-1,2,3,6-tetrahydropyridine-2-carboxylic acid.

(e) To a sodium of (±)-(2R*,3R*)-1-(4-methoxy-benzensulfonyl)-3-methyl-1,2,3,6-tetrahydropyridine-2-carboxylic acid (100 mg, 0.36 mmol) in dimethylformamide (5 ml.) is added hydroxybentriazole (53 mg, 0.39 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (75 mg, 0.39 mmol). After 1 hour, hydroxylamine hydrochloride (75 mg, 1.08 mmol) is added followed by triethylamine (150 μL, 1.08 mmol). After stirring overnight, the mixture is diluted with water and extracted with ethyl acetate. The combined extracts are dried, filtered and concentrated. The crude product is purified by silica gel chromatography (elution with 2:1 ethyl acetate-hexanes with 1% acetic acid) to provide (±)-(2R*,3R*)-(N-hydroxy)-1-(4-methoxy-benzenesulfonyl)-3-methyl-1,2,3,6-tetrahydropyridine-2-carboxamide as a white solid. Melting point 173°C (dec.). Mass spectrum (thermospray): m/Z 326 (m-C(O)N(H)OH, 100%, (m, 7%), (m+H, 30%), (m+NH_4, 10%). ^{1}H NMR ($CDCl_3$, 250 MHz, ppm): δ 7,72 (d, J = 8.9 Hz, 2H), 7.03 (d, J=8.9 Hz, 2H), 5.66 (dq, J=13.0, 2.7 Hz, 1H), 5.45 (dd, 13.0, 1.9 Hz), 4.37 (d, 7.0 Hz, 1H), 4.06-3.82 (m, 2H), 3.82 (s, 3H), 3.43-3.30 (m, 1H), 2.62-231 (m, 1H), 0.97 (d, 7.5 Hz, 3H).

EXAMPLE 2

N-hydroxy-1-(4-methoxybenzenesulfonyl)-3-phenyl-1,2,3,6-tetrahydropyridine-2-carboxamide

(a) To a solution of glycine t-butyl ester (5.0 grams, 29.82 mmol) in methylene chloride (50 ml) is added triethylamine (6.65 ml, 62.63 mmol) followed by 4-methoxybenzenesulfonyl chloride (29.82 mmol, 6.2 grams). The solution is stirred for 24 hours, diluted with water and extracted with ethyl acetate. The combined extracts are dried (sodium sulfate), filtered and concentrated. The crude product is purified by silica gel chromatography (elution with 6:1 hexane-ethyl acetate) to provide (4-methoxybenzenesulfonylamino) acetic acid t-butyl ester.

(b) To a solution of (4-methoxybenzenesulfonylamino) acetic acid t-butyl ester (3.0 grams, 10 mmol) in tetrahydrofuran-dimethylformamide (mL, ca. 3:1) at 0°C is added sodium bis(trimethylsilyl)amide (10.0 mL, 1.0 M solution in tetrahydrofuran). After 10 minutes, 4-bromo-2-methyl-2-butene (1.27 μL, 11.0 mmol) is added. The mixture is warmed to room temperature, stirred for 1 hour and quenched with saturated ammonium chloride solution. The mixture is extracted with ethyl acetate and the combined extracts are dried (sodium sulfate), filtered and concentrated. The crude product is purified by silica gel chromatography (elution with 1:1 ethyl acetate-hexanes) to provide [(4-methoxybenzenesulfonyl)-(3-methyl-but-2-enyl)-amino]-acetic acid t-butyl ester.

(c) Ozone is passed through a solution of [(4-methoxybenzenesulfonyl)-(3-methyl-but-2-enyl)-amino]-acetic acid t-butyl ester (2.0 grams, 5.4 mmol) in methylene chloride-methanol (50 mL, ca. 1:1) at -78°C until a blue color persisted. Triphenylphosphine (4.24 grams, 16.2 mmol) is added and the resulting solution is stirred at room temperature for 3 hours. Concentration provided the crude product which is purified by silica gel chromatography (elution with 1:1 ethyl acetate-hexanes) to provide [(4-methoxybenzenesulfonyl)-(2-oxo-ethyl)-amino]-acetic acid t-butyl ester.

(d) To a slurry of chromium (II) chloride (1.3 grams, 10.49 mmol) in dimethylformamide (20 ml) is added a suspension of nickel (II) chloride (0.026 mmol, 1 mg) in dimethylformamide (1 ml) followed by a mixture of (trans)-ß-iodostyrene (1.20 grams, 5.24 mmol) and [(4-methoxybenzenesulfonyl)-2-oxo-athyl)-amino]acetic acid t-butyl ester (900 mg, 2.62 mmol) in dimethylformamide (5 ml). The resulting solution is stirred for three hours, diluted with water and extracted with ethyl acetate. The

combined extracts are washed with brine, dried (sodium sulfate), filtered and concentrated. The crude product is purified by silica gel chromatography (elution with 3:2 hexane-ethyl acetate) to provide (±)-(E)-[(2-hydroxy-4-phenyl-but-3-enyl)-(4-methoxybenzenesulphonyl)-amino]-acetic acid t-butyl ester.

(e) (±)-(E)-[(2-hydroxy-4-phenyl-but-3-enyl)-(4-methoxybenzenesulphonyl)-amino]-acetic acid t-butyl ester is subjected to the conditions described in Example 1c. The crude product is recrystalized from chloroform to provide (±)-(E)-4-(4-methoxybenzenesulfonyl)-6-styryl-morpholin-2-one.

(f) (±)-(E)-4-(4-methoxybenzenesulfonyl)-6-styryl-morpholin-2-one is subjected to the conditions described in Example 1d. The crude product is purified by silica gel chromatography (elution with 2:1 hexane-ethyl acetate with 1% acetic acid) to provide (±)-(2R*-3R*)-1-(4-methoxybenzenesulfonyl)-3-phenyl-1,2,3,6-tetrahydropyridine-2-carboxylic acid.

(g) (±)-(2R*-3R*)-1-(4-methoxybenzenesulfonyl)-3-phenyl-1,2,3,6-tetrahydropyridine-2-carboxylic acid is subject to the conditions described in Example 1e. The crude product is purified by silica gel chromatography (elution with 1:1 hexane-ethyl acetate with 1% acetic acid) to provide N-hydroxy-1-(4-methoxybenzenesulfonyl)-3-phenyl-1,2,3,6-tetrahydropyridine-2-carboxamide as a white solid. Melting point 151-154°C (dec.). Mass spectrum [PBMS w/C.I. (NH_3)]: m/Z 388 (m+NH_4, 100%). ^{1}H NMR (CD_3OD) δ 7.75 (d, J = 8.5 Hz, 2H), 7.38-7.12 (m, 5H), 7.04 (d, J = 8.5 Hz, 2H), 5.91 (d, J = 8.9 Hz, 1H), 5.28 (d, J = 9.9 Hz, 1H), 4.89 (s, H_2O), 4.57 (d, 6.8 Hz, 1H), 4.07 (ABq, JAB = 18.0 Hz, $\Delta\nu$ AB = 39.1 Hz, 2H), 3.85 (o, 3H), 3.39 (bs, CD_3OD).

EXAMPLE 3

(±)-(2R*-3R*)-N-hydroxy-1-(4-methoxybenzenesulfonyl)-3-phenyl-piperidine-2-carboxamide

(a) To a solution of (±)-(2R*-3R*)-1-(4-methoxybenzenesulfonyl)-3-phenyl-1,2,3,6-tetrahydropyridine-2-carboxylic acid (65 mg, 0.17 mmol) (from Example 20), is added benzylhydroxylamine hydrochloride (32 mg, 0.20 mmol), dicyclohexylcarbodiimide (41 mg, 0.20 mmol) and dimethylaminopyridine (27 mg, 0.22 mmol). The resulting mixture is stirred overnight, diluted with ethyl acetate and filtered through Celite™ and evaporated. The crude product is purified by chromatography elution with 1:1 hexane-ethyl acetate to provide (±)-(2R*-3R*)-N-benzyloxy-1-(4-methoxybenzenesulfonyl)-3-phenyl-1,2,3,6-tetrahydropyridine-2-carboxamide.

(b) To a solution of (±)-(2R*-3R*)-N-benzyloxy-1-(4-methoxybenzenesulfonyl)-3-phenyl-1,2,3,6-tetrahydropyridine-2-carboxamide (35 mg, 0.073 mmol) in ethanol (5 ml) is added 10% palladium on carbon (10 mg, 5 mol) . The flask is evacuated and backfilled with hydrogen (repeated two times). The reaction mixture is then stirred for 1 hour at which time it is filtered through Celite™ and concentrated. The product (±)-2R*-3R*)-N-hydroxy-1-(4-methoxybenzenesulfonyl)-3-phenylpiperidine-2-carboxamide was collected as a white solid. Melting point 163°C (dec). Mass spectrum [PBMS w/C.I. (NH_3)]: m/Z 390 (m+H_2), (m+NH_4). ^{1}H NMR (CD_3OD) δ 7.73 (d, J = 8.9 Hz, 2H), 7.31-737 (m, 5H), 7.04 (d, 8.9 Hz, 2H0, 4.89 (s, H_2O), 4.34 (d, J = 5.4 Hz, 1H), 3.86 (s, 3H), 3.74-3.63 (m, 2H), 3.31 (bs, CD_3OD), 2.99-2.90 (m, 1H), 2.58-2.52 (m, 1H), 1.94-1.88 (m, 1H), 1.67-160 (m, 2H).

EXAMPLE 4

(+)-N-hydroxy-1-(4-methoxybenzenesulfonyl)-2-piperazinecarboxamide hydrochloride

(a) To a solution of (±)-4-benzyloxycarbonyl-2-piperazinecarboxylic acid (1.90 grams, 7.2 mmol) in dioxane-water (10 ml, ca. 1:1) is added 1N sodium hydroxide solution (15 ml, 15 mmol) followed by 4-methoxybenzenesulfonyl chloride. The solution is stirred for 1 hour, acidified with 1N hydrochloric acid and extracted with ethyl acetate. The combined extracts are dried (sodium sulfate), filtered and concentrated. The crude product is purified by silica gel chromatography (elution with 2:1 ethyl acetate-hexanes with 1% acetic acid) to provide (±)-1-(4-methoxybenzenesulfonyl)-4-benzyloxycarbonyl-2-piperazinecarboxylic acid.

(b) To a solution of (±)-1-(4-methoxybenzenesulfonyl)-4-benzyloxycarbonyl-2-piperazinecarboxylic acid (100 mg, 0.23 mmol) in methylene chloride (5 ml) is added O-t-butylhydroxylamine hydrochloride (35 mg, 0.28 mmol), dimethylaminopyridine (37 mg, 0.30 mmol), and dicyclohexycarbodiimide (57 mg, 0.28 mmol). After stirring overnight, the reaction is diluted with hexanes and the precipitated solid filtered off. The solution is concentrated and the crude product is purified by silica gel chromatography (elution with 2:1 ethyl acetate-hexanes with 1% acetic acid) to provide (±)-N-(t-butyloxy)-1-(4-methoxybenzenesulfonyl)-4-benzyloxycarbonyl-2-piperazinecarboxamide.

(c) To a solution of (±)-N-(t-butyloxy)-1-(4-methoxybenzenesulfonyl)-4-benzyloxycarbonyl-2-piperazinecarboxamide (68 mg, 0.134 mmol), in methanol (6 ml)

is added 10% palladium on carbon (7 mg). The flask is evacuated and backfilled with hydrogen (repeated 2 times). The reaction mixture is then stirred for 1 hour at which time it is filtered through Celite™ and concentrated. The product (±)-N-(t-butyloxy)-1-(4-methoxybenzenesulfonyl)-2-piperazinecarboxamide is used without any further purification.

(d) To a solution of (±)-N-(t-butyloxy)-1-(4-methoxybenzenesulfonyl)-2-piperazinecarboxamide (30 mg, in dichloroethane is added ethanol (1 drop). The solution is cooled to -10°C and hydrogen chloride gase is bubbled through for 5 minutes. The reaction is then sealed and stirred for 24 hours at which time the volume is reduced to 1/3 by evaporation and the precipitated solids are filtered and dried (in vacuo) to give (±)-N-hydroxy-1-(4-methoxybenzenesulfonyl)-2-piperazinecarboxamide hydrochloride as a white solid. Melting point 167 °C. (dec.). Mass spectrum (thermospray): m/Z 343 (m + 1 100%). ^{1}H NMR (CD_3OD, 250 MHz, ppm): δ 7.76 (d, J = 8.9 Hz, 2H), 7.07 (d, J = 8.9 Hz, 2H), 3.87 (bs, H_2O), 4.19 (d, J = 3.3 Hz, 1H), 3.87 (s, 3H), 3.58 (bd, J = 6.2 Hz, 1H), 3.42 (bd, J = 6.1 Hz, 1H), 3.30 (bs, CD_3OD), 3.16 (d, J = 13.5 Hz, 1H), 2.87 (bd, J = 13.3 Hz, 1H), 2.69 (dd, J = 13.3, 3.0 Hz, 1H), 2.51 (dt, J = 12.5, 3.8 Hz, 1H).

EXAMPLE 5

N-hydroxy-1-(4-methoxybenzenesulfonyl)-5-oxo-piperazine-2-carboxamide

(a) To a solution of (±)-benzyloxycarbonylamino-2-t-butoxycarbonyl aminopropionate (2.8 grams, 7.9 mmol) in methylene chloride (25 ml) at 0°C is added a solution of hydrochloric acid (g) dissolved in dioxane (25 ml). The solution is stirred at O°C for 4 hours and then concentrated. The crude product 3-benzyloxycarbonylamino-2-amino-propionic acid methyl ester hydrochloride is used without further purification.

(b) 3-benzyloxycarbonylamino-2-amino-propionic acid methyl ester hydrochloride is subjected to the conditions described in Example 1a. The crude product is purified by silica gel chromatography (elution with 1:1 hexane-ethyl acetate) to provide (±)-3-benzyloxycarbonylamino-2-(4-methoxybenzenesulfonylamino)-propionic acid methyl ester.

(c) (±)-3-benzyloxycarbonylamino-2-(4-methoxybenzene sulfonylamino)-propionic acid methyl ester is subjected to the conditions described in Example 1. The crude product is purified by silica gel chromatography (elution with 3:2 ethyl acetate-

hexane) to provide (±)-3-benzyloxycarbonylamino-2-[t-butoxycarbonylmethyl-(4-methoxybenzenesulfonyl)-amino]-propionic acid methyl ester.

(d) (±)-3-benzyloxycarbonylamino-2-[t-butoxycarbonylmethyl-(4-methoxybenzenesulfonyl)-amino]-propionic acid methyl ester is subjected to the conditions described in Example 4c. The product 3-amino-2-[t-butoxycarbonylmethyl-(4-methoxybenzene-sulfonyl)-amino]-propionic acid methyl ester is used without further purification.

(e) To a solution of 3-amino-2-[t-butoxycarbonylmethyl-(4-methoxybenzenesulfonyl)-amino]-propionic acid methyl ester (2.46 grams, 6.1 mmol) in methylene chloride (20 ml) at 0°C is added trifluoroacetic acid (5 ml). The solution is stirred at 0°C for 12 hours and then concentrated. The crude product 3-amino-2-[carboxymethyl-(4-methoxybenzenesulfonyl)-amino]-propionic acid methyl ester trifluoroacetic acid salt is used without further purification.

(f) To a solution of 3-amino-2-[carboxymethyl-(4-methoxybenzenesulfonyl)-amino]-propionic acid methyl ester trifluoracetic acid salt (2.11 grams, 6.1 mmol) in methylene chloride (5 ml) is added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1.76 grams, 9.2 mmol) and triethyamine (3.4 ml, 24.4 mmol). The resulting mixture is stirred overnight, diluted with ethyl acetate and washed with 1N hydrochlori acid. The organic layer is dried (sodium sulfate), filtered and concentrated. The crude product is purified by silica gel chromatography (elution with ethyl acetate) to provide 1-(4-methoxybenzenesulfonyl)-5-oxo-piperazine-2-carboxylic acid methyl ester.

(g) To a solution of 1-(4-methoxybenzenesulfonyl)-5-oxo-piperazine-2-carboxylic acid methyl ester. (200 mg, 0.61 mmol) in methanol-tetrahydrofuran-water (5 ml, ca. 6:2:1) at 0°C is added lithium hydroxide (64 mg, 1.53 mmol). The resulting mixture is stirred for 30 minutes, acidified with 1N hydrochloric acid and extracted with ethyl acetate. The combined extracts are dried (sodium sulfate), filtered and concentrated. The crude product 1-(4-methoxybenzenesulfonyl)-5-oxo-piperazine-2-carboxylic acid is used without furtehr purification.

(h) To a solution of 1-(4-methoxybenzenesulfonyl)-5-oxo-piperazine-2-carboxylic acid (166 mg, 0.53 mmol) in methylene chloride (5 ml) is added 0-benzyl hydroxylamine hydrochloride (255 mg, 1.6 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (153 mg, 0.8 mmol) and triethylamine (370 μL 2.65

mmol). The resulting mixture is stirred overnight, diluted with ethyl acetate and washed with 1N hydrocloric acid. The organic layer is dried (sodium sulfate), filtered and concentrated. The crude product is purified by silica gel chromatography (elution with 5% methanol in methylene chloride) to provide N-(benzyloxy)-1-(4-methoxybenzenesulfonyl)-5-oxo-piperazine-2-carboxamide.

(i) N-(benzyloxy)-1-(4-methocybenzenesulfonyl)-5-oxo-piperazine-2-carboxamide is subjected to the conditions described in Example 4c to give N-hydroxy-1-(4-methoxybenzenesulfonyl)-5-oxo-piperazine-2-carboxamide as a white solid. Mass spectrum (thermospray): m/Z 343 (m+H, 60%), (m+NH_4, 17%). ^{1}H NMR (CD_3OD), 250 MHz, ppm) δ7.79 (d, J = 8.9 Hz, 2H), 4.90 (s, H_2O), 4.47 (dd, J = 5.0, 3.2 Hz, 1H), (4.03, s, 2H), 3.88 (s, 3H), 3.47 (dd, J = 13.4, 3.2 Hz, 1H), 3.35-3.30 (m, 1H), 3.30 (s, CD_3OD)

EXAMPLE 6

N-hydroxy-1-(4-methoxybenzenesulfonyl)-morpholin-2-carboxamide

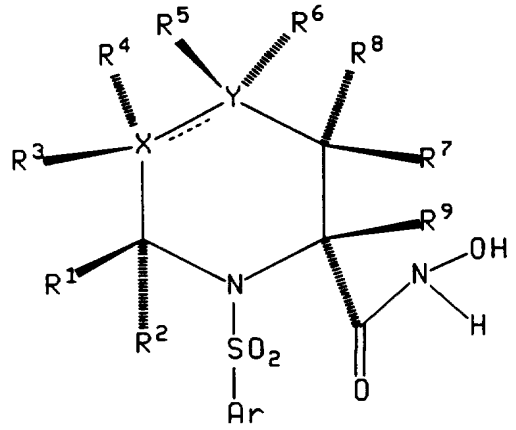
(a) morpholine-2-carboxylic acid is subjected to the conditions described in Example 4a to give 1-(4-methoxybenzenesulfonyl)-morpholin-2-carboxylic acid.

(b) 1-(4-methoxybenzenesulfonyl)-morpholin-2-carboxylic acid is subjected to the conditions described in example 5h to give N-benzyloxy-1-(4-methoxybenzenesulfonyl)-morpholin-2-carboxamide.

(c) N-benzyloxy-1-(4-methoxybenzenesulfonyl)-morpholin-2-carboxamide is subjected to the conditions described in Example 4c to give N-hydroxy-1-(4-methoxybenzenesulfonyl)-morpholin-2-carboxamide as a white foam. Mass spectrum (thermospray): m/Z 343 (m+H, 100%), $[\alpha]_D$: + 57° (c = 0.60, $CHCl_3$. ^{1}H NMR ($CDCL_3$), 250 MHz, ppm) δ7.78 (bd, J = 8.0 Hz, 2H), 7.38 (bs, 1H), 7.01 (bd, J = 8.0 Hz, 2H), (4.34 (bs, J = 2H), 3.87 (s, 3H), 3.85-3.30 (m, 3H), 3.30-3.15 (m, 2H).

CLAIMS

1. A compound of the formula



I

or the pharmaceutically acceptable salt thereof, wherein the broken line represents an optional double bond;

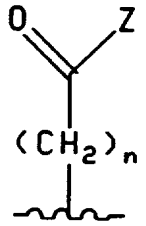
X is carbon, oxygen or sulfur;

Y is carbon, oxygen, sulfur, sulfoxide, sulfone or nitrogen;

R^1, R^2 R^3, R^4 R^5, R^6, R^7, R^8 and R^9 are selected from the group consisting of hydrogen, (C_1-C_6)alkyl optionally substituted by (C_1-C_6)alkylamino, (C_1-C_6)alkylthio, (C_1-C_6)alkoxy, trifluoromethyl, (C_6-C_{10})aryl, (C_5-C_9)heteroaryl, (C_6-C_{10})arylamino, (C_6-C_{10})arylthio, (C_6-C_{10})aryloxy, (C_5-C_9)heteroarylamino, (C_5-C_9)heteroarylthio, (C_5-C_9)heteroaryloxy, (C_6-C_{10})aryl(C_6-C_{10})aryl, (C_3-C_6)cycloalkyl, hydroxy(C_1-C_6)alkyl, (C_1-C_6)alkyl(hydroxymethylene),piperazinyl,(C_6-C_{10})aryl(C_1-C_6)alkoxy,(C_5-C_9)heteroaryl(C_1-C_6)alkoxy, (C_1-C_6)acylamino, (C_1-C_6)acylthio, (C_1-C_6)acyloxy, (C_1-C_6)alkylsulfinyl, (C_6-C_{10})arylsulfinyl, (C_1-C_6)alkylsulfonyl, (C_6-C_{10})arylsulfonyl, amino, (C_1-C_6)alkylamino or ((C_1-C_6)alkylamino)$_2$; (C_2-C_6)alkenyl, (C_6-C_{10})aryl(C_2-C_6)alkenyl, (C_5-C_9)heteroaryl(C_2-C_6)alkenyl, (C_2-C_6)alkynyl, (C_6-C_{10})aryl(C_2-C_6)alkynyl, (C_5-C_9)heteroaryl(C_2-C_6)alkynyl, (C_1-C_6)alkylamino, (C_1-C_6)alkylthio, (C_1-C_6)alkoxy, trifluoromethyl, (C_1-C_6)alkyl (difluoromethylene), (C_1-C_3)alkyl(difluoromethylene)(C_1-C_3)alkyl, (C_6-C_{10})aryl, (C_5-C_9)heteroaryl, (C_6-C_{10})arylamino, (C_6-C_{10})arylthio, (C_6-C_{10})aryloxy, (C_5-C_9)heteroarylamino, (C_5-C_9)heteroarylthio, (C_5-C_9)heteroaryloxy, (C_3-C_6)cycloalkyl, (C_1-C_6)alkyl(hydroxymethylene), piperidyl, (C_1-C_6)alkylpiperidyl, (C_1-C_6)acylamino, (C_1-

C_6)acylthio, (C_1-C_6)acyloxy, R^{13}(C_1-C_6)alkyl wherein R^{13} is (C_1-C_6)acylpiperazino, (C_6-C_{10})arylpiperazino, (C_5-C_9)heteroarylpiperazino, (C_1-C_6)alkylpiperazino, (C_6-C_{10})aryl(C_1-C_6)alkylpiperazino,(C_5-C_9)heteroaryl(C_1-C_6)alkylpiperazino,morpholino,thiomorpholino, piperidino, pyrrolidino, piperidyl, (C_1-C_6)alkylpiperidyl, (C_6-C_{10})arylpiperidyl, (C_5-C_9)heteroarylpiperidyl,(C_1-C_6)alkylpiperidyl(C_1-C_6)alkyl,(C_6-C_{10})arylpiperidyl(C_1-C_6)alkyl, (C_5-C_9)heteroarylpiperidyl(C_1-C_6)alkyl or (C_1-C_6)acylpiperidyl;

or a group of the formula

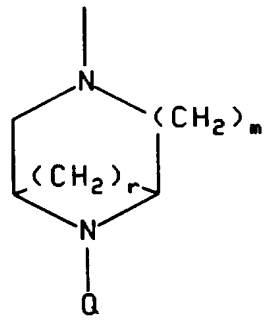


wherein n is 0 to 6;

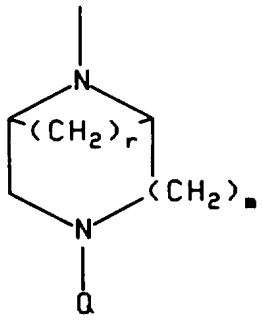
Z is hydroxy, (C_1-C_6)alkoxy or $NR^{14}R^{15}$ wherein R^{14} and R^{15} are each independently selected from the group consisting of hydrogen, (C_1-C_6)alkyl optionally substituted by (C_1-C_6)alkylpiperidyl, (C_6-C_{10})arylpiperidyl, (C_5-C_9)heteroarylpiperidyl, (C_6-C_{10})aryl, (C_5-C_9)heteroaryl, (C_6-C_{10})aryl(C_6-C_{10})aryl or (C_3-C_6)cycloalkyl; piperidyl, (C_1-C_6)alkylpiperidyl, (C_6-C_{10})arylpiperidyl, (C_5-C_9)heteroarylpiperidyl, (C_1-C_6)acylpiperidyl, (C_6-C_{10})aryl,(C_5-C_9)heteroaryl,(C_6-C_{10})aryl(C_6-C_{10})aryl,(C_3-C_6)cycloalkyl,R^{16}(C_2-C_6)alkyl, (C_1-C_5)alkyl(CHR^{16})(C_1-C_6)alkyl wherein R^{16} is hydroxy, (C_1-C_6)acyloxy, (C_1-C_6)alkoxy, piperazino, (C_1-C_6)acylamino, (C_1-C_6)alkylthio, (C_6-C_{10})arylthio, (C_1-C_6)alkylsulfinyl, (C_6-C_{10})arylsulfinyl, (C_1-C_6)alkylsulfoxyl, (C_6-C_{10})arylsulfoxyl, amino, (C_1-C_6)alkylamino, ((C_1-C_6)alkyl)$_2$ amino, (C_1-C_6)acylpiperazino, (C_1-C_6)alkylpiperazino, (C_6-C_{10})aryl(C_1-C_6)alkylpiperazino,(C_5-C_9)heteroaryl(C_1-C_6)alkylpiperazino,morpholino,thiomorpholino, piperidino or pyrrolidino; R^{17}(C_1-C_6)alkyl, (C_1-C_5)alkyl(CHR^{17})(C_1-C_6)alkyl wherein R^{17} is piperidyl or (C_1-C_6)alkylpiperidyl; and $CH(R^{18})COR^{19}$ wherein R^{18} is hydrogen, (C_1-C_6)alkyl, (C_6-C_{10})aryl(C_1-C_6)alkyl, (C_5-C_9)heteroaryl(C_1-C_6)alkyl, (C_1-C_6)alkylthio(C_1-C_6)alkyl, (C_6-C_{10})arylthio(C_1-C_6)alkyl, (C_1-C_6)alkylsulfinyl(C_1-C_6)alkyl, (C_6-C_{10})arylsulfinyl(C_1-C_6)alkyl, (C_1-C_6)alkylsulfonyl(C_1-C_6)alkyl, (C_6-C_{10})arylsulfonyl(C_1-C_6)alkyl, hydroxy(C_1-C_6)alkyl, amino(C_1-C_6)alkyl, (C_1-C_6)alkylamino(C_1-C_6)alkyl, ((C_1-C_6)alkylamino)$_2$(C_1-C_6)alkyl, $R^{20}R^{21}NCO$(C_1-C_6)alkyl or $R^{20}OCO$(C_1-C_6)alkyl wherein R^{20} and R^{21} are each independently selected from the group consisting of hydrogen, (C_1-

C_6)alkyl, (C_6-C_{10})aryl(C_1-C_6)alkyl and (C_5-C_9)heteroaryl(C_1-C_6)alkyl; and R^{19} is $R^{22}O$ or $R^{22}R^{23}N$ wherein R^{22} and R^{23} are each independently selected from the group consisting of hydrogen, (C_1-C_6)alkyl, (C_6-C_{10})aryl(C_1-C_6)alkyl and (C_5-C_9)heteroaryl(C_1-C_6)alkyl;

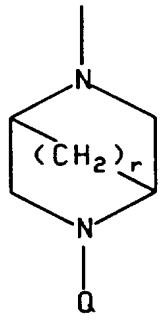
or R^{14} and R^{15}, or R^{20} and R^{21}, or R^{22} and R^{23} may be taken together to form an azetidinyl, pyrrolidinyl, morpholinyl, thiomorpholinyl, indolinyl, isoindolinyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, (C_1-C_6)acylpiperazinyl, (C_1-C_6)alkylpiperazinyl, (C_6-C_{10})arylpiperazinyl, (C_5-C_9)heteroarylpiperazinyl or a bridged diazabicycloalkyl ring selected from the group consisting of



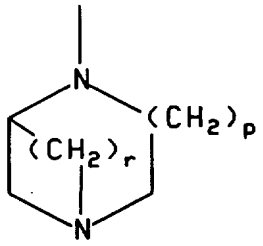
a



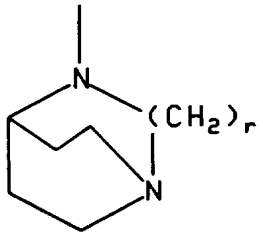
b



c



d



e

wherein r is 1, 2 or 3;

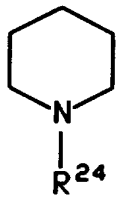
m is 1 or 2;

p is 0 or 1; and

Q is hydrogen, (C_1-C_3)alkyl, (C_1-C_6)acyl or (C_1-C_6)alkoxy carbamoyl;

or R^1 and R^2, or R^3 and R^4, or R^5 and R^6 may be taken together to form a carbonyl;

or R^1 and R^2, or R^3 and R^4, or R^5 and R^6, or R^7 and R^8 may be taken together to form a (C_3-C_6)cycloalkyl, oxacyclohexyl, thiocyclohexyl, indanyl or tetralinyl ring or a group of the formula



wherein R^{24} is hydrogen, (C_1-C_6)acyl, (C_1-C_6)alkyl, (C_6-C_{10})aryl(C_1-C_6)alkyl, (C_5-C_9)heteroaryl(C_1-C_6)alkyl or (C_1-C_6)alkylsulfonyl; and

Ar is (C_6-C_{10})aryl or (C_5-C_9)heteroaryl, each of which may be optionally substituted by (C_1-C_6)alkyl, one or two (C_1-C_6)alkoxy, (C_6-C_{10})aryloxy or (C_5-C_9)heteroaryloxy;

with the proviso that R^7 is other than hydrogen only when R^8 is other than hydrogen;

with the proviso that R^6 is other than hydrogen only when R^5 is other than hydrogen;

with the proviso that R^3 is other than hydrogen only when R^4 is other than hydrogen;

with the proviso that R^2 is other than hydrogen only when R^1 is other than hydrogen;

with the provisio that when R^1, R^2 and R^9 are a substituent comprising a heteroatom, the heteroatom cannot be directly bonded to the 2- or 6- positions;

with the proviso that when X is nitrogen, R^4 is not present;

with the proviso that when X is oxygen, sulfur, sulfoxide, sulfone or nitrogen and when one or more of the group consisting of R^1, R^2, R^5 and R^6, is a substituent comprising a heteroatom, the heteroatom cannot be directly bonded to the 4- or 6- positions;

with the proviso that when Y is oxygen, sulfur, sulfoxide, sulfone or nitrogen and when one or more of the group consisting of R^3, R^4, R^7 and R^8, are independently a

substituent comprising a heteroatom, the heteroatom cannot be directly bonded to the 3- or 5- positions;

with the proviso that when X is oxygen, sulfur, sulfoxide or sulfone, R^3 and R^4 are not present;

with the proviso that when Y is nitrogen, R^4 is not present;

with the proviso that when Y is oxygen, sulfur, sulfoxide or sulfone, R^5 and R^6 are not present;

with the proviso that when Y is nitrogen, R^6 is not present;

with the proviso that when the broken line represents a double bond, R^4 and R^5 are not present;

with the proviso that when R^3 and R^5 are independently a substituent comprising a heteroatom when the broken line represents a double bond, the heteroatom cannot be directly bonded to positions X and Y;

with the proviso that when either the X or Y position is oxygen, sulfur, sulfoxide, sulfone or nitrogen, the other of X or Y is carbon;

with the proviso that when X or Y is defined by a heteroatom, the broken line does not represent a double bond;

with the proviso that when R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8 and R^9 are all defined by hydrogen or $(C_1\text{-}C_6)$alkyl, either X or Y is oxygen, sulfur, sulfoxide, sulfone or nitrogen, or the broken line represents a double bond.

2. A compound according to claim 1, wherein Y is oxygen, nitrogen or sulfur.

3. A compound according to claim 1, wherein Ar is 4-methoxyphenyl or 4-phenoxyphenyl.

4. A compound according to claim 1, wherein R^8 is $(C_6\text{-}C_{10})$aryl, $(C_5\text{-}C_9)$heteroaryl, $(C_6\text{-}C_{10})$aryl$(C_1\text{-}C_6)$alkyl, $(C_5\text{-}C_9)$heteroaryl$(C_1\text{-}C_6)$alkyl, carboxylic acid or carboxylic acid $(C_1\text{-}C_6)$alkyl.

5. A compound according to claim 1, wherein R^2, R^3, R^6, R^7 and R^9 are hydrogen.

6. A compound according to claim 1, wherein Y is carbon, Ar is 4-methoxyphenyl or 4-phenoxyphenyl and R^8 is $(C_6\text{-}C_{10})$arylalkynyl or $(C_5\text{-}C_9)$heteroarylalkynyl.

7. A compound according to claim 1, wherein Y is oxygen, Ar is 4-methoxyphenyl or 4-phenoxyphenyl and R^8 is (C_6-C_{10})arylalkynyl or (C_5-C_9)heteroarylalkynyl.

8. A compound according to claim 1, wherein Y is carbon, Ar is 4-methoxyphenyl or 4-phenoxyphenyl and R^8 is carboxylic acid or carboxylic acid (C_1-C_6)alkyl.

9. A compound according to claim 1, wherein Y is oxygen, Ar is 4-methoxyphenyl or 4-phenoxyphenyl and R^8 is carboxylic acid or carboxylic acid (C_1-C_6)alkyl.

10. A compound according to claim 1, wherein Y is carbon, Ar is 4-methoxyphenyl or 4-phenoxyphenyl and R^5 is (C_6-C_{10})arylalkynyl or (C_5-C_9)heteroarylalkynyl.

11. A compound according to claim 1, wherein Y is oxygen, Ar is 4-methoxyphenyl or 4-phenoxyphenyl and R^5 is (C_6-C_{10})arylalkynyl or (C_5-C_9)heteroarylalkynyl.

12. A compound according to claim 1, wherein Y is carbon, Ar is 4-methoxyphenyl or 4-phenoxyphenyl and R^5 is carboxylic acid or carboxylic acid (C_1-C_6)alkyl.

13. A compound according to claim 1, wherein Y is oxygen, Ar is 4-methoxyphenyl or 4-phenoxyphenyl and R^5 is carboxylic acid or carboxylic acid (C_1-C_6)alkyl.

14. A compound according to claim 1, wherein Y is carbon, Ar is 4-methoxyphenyl or 4-phenoxyphenyl and R^5 is (C_1-C_6)alkylamino.

15. A compound according to claim 1, wherein Y is oxygen, Ar is 4-methoxyphenyl or 4-phenoxyphenyl and R^8 is (C_1-C_6)alkylamino.

16. A compound according to claim 1, wherein said compound is selected from the group consisting of:

(2R,3S)-N-hydroxy-3-ethynyl-1-(4-methoxybenzenesulfonyl)-piperidine-2-carboxamide;

(2R,3S)-N-hydroxy-l-(4-methoxybenzenesulfonyl)-3-(5-methoxythiophene-2-yl-ethynyl)-piperidine-2-carboxamide;

(2R,3R)-N-hydroxy-1-(4-methoxybenzenesulfonyl)-3-(3-pyridin-3-yl-prop-2-ynyl)-piperidine-2-carboxamide;

(2S,3R)-N-hydroxy-4-(4-methoxybenzenesulfonyl)-2-pyridine-3-yl-morpholine-3-carboxamide;

(2S,3R)-N-hydroxy-2-hydroxycarbamoyl-4-(4-methoxybenzenesulfonyl)-morpholine-3-carboxamide;

(2R,3R)-N-hydroxy-2-hydroxycarbamoyl-4-(4-methoxybenzenesulfonyl)-piperidine-2-carboxamide;

(2R,3S)-N-hydroxy-1-(4-methoxybenzenesulfonyl)-3-(4-phenylpyridine-2-yl)-piperidine-2-carboxamide;

(2S,3R)-N-hydroxy-1-(4-methoxybenzenesulfonyl)-2-(4-phenylpyridine-2-yl)-morpholine-2-carboxamide;

(2R,3S)-N-hydroxy-3-(2-chloro-4-fluorophenyl)-1-(4-methoxybenzenesulfonyl)-piperidine-2-carboxamide; and

(2S,3R)-N-hydroxy-2-(2-chloro-4-fluorophenyl)-1-(4-methoxybenzenesulfonyl)-piperidine-3-carboxamide.

17. A pharmaceutical composition for (a) the treatment of a condition selected from the group consisting of arthritis, cancer, tissue ulceration, restenosis, periodontal disease, epidermolysis bullosa, scleritis and other diseases characterized by matrix metalloproteinase activity, AIDS, sepsis, septic shock and other diseases involving the production of tumor necrosis factor (TNF) or (b) the inhibition of matrix metalloproteinases or the production of tumor necrosis factor (TNF) in a mammal, including a human, comprising an amount of a compound of claim 1 or a pharmaceutically acceptable salt thereof, effective in such treatments or inhibition and a pharmaceutically acceptable carrier.

18. A method for the inhibition of (a) matrix metalloproteinases or (b) the production of tumor necrosis factor (TNF) in a mammal, including a human, comprising administering to said mammal an effective amount of a compound of claim 1 or a pharmaceutically acceptable salt thereof.

19. A method for treating a condition selected from the group consisting of arthritis, cancer, tissue ulceration, restenosis, periodontal disease, epidermolysis bullosa, scleritis and other diseases characterized by matrix metalloproteinase activity, AIDS, sepsis, septic shock and other diseases involving the production of tumor necrosis factor (TNF) in a mammal, including a human, comprising administering to

said mammal an amount of a compound of claim 1 or a pharmaceutically acceptable salt thereof, effective in treating such a condition.

INTERNATIONAL SEARCH REPORT

Internat. Application No
PCT/IB 95/00279

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D211/96 A61K31/445 C07D241/04 C07D241/08

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D

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)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 606 046 (CIBA GEIGY AG) 13 July 1994 see claims 1,2; example 6	

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

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

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

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

2

Date of the actual completion of the international search	Date of mailing of the international search report
12 December 1995	20.12.95

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

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Int. ional application No.
PCT/IB 95/00279

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 18 and 19 are directed to a method of treatment of (diagnostic method practised on) the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

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

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

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

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

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

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

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

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

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internat Application No
PCT/IB 95/00279

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0606046	13-07-94	US-A-	5455258	03-10-95
		AU-B-	5265593	04-05-95
		CA-A-	2112779	07-07-94
		FI-A-	940012	07-07-94
		HU-A-	70536	30-10-95
		JP-A-	6256293	13-09-94
		NO-A-	940038	07-07-94
		NZ-A-	250517	26-10-95

Form PCT/ISA/210 (patent family annex) (July 1992)

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C07F 9/30, A61K 31/66, C07F 9/58, 9/6553, 9/655

A1

(11) International Publication Number: **WO 98/03516**

(43) International Publication Date: 29 January 1998 (29.01.98)

(21) International Application Number: PCT/IB97/00800

(22) International Filing Date: 30 June 1997 (30.06.97)

(30) Priority Data:
60/021,959 18 July 1996 (18.07.96) US

(71) Applicant *(for all designated States except US)*: PFIZER INC. [US/US]; 235 East 42nd Street, New York, NY 10017 (US).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: REITER, Lawrence, Alan [US/US]; 32 West Mystic Avenue, Mystic, CT 06355 (US).

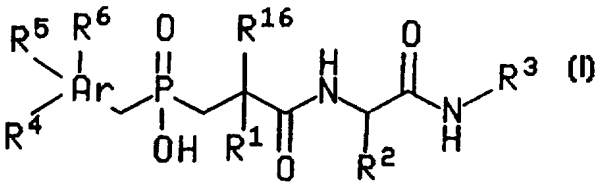
(74) Agents: SPIEGEL, Allen, J. et al.; Pfizer Inc., Patent Dept., 235 East 42nd Street, New York, NY 10017 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: PHOSPHINATE BASED INHIBITORS OF MATRIX METALLOPROTEASES

(57) **Abstract**



A compound of the formula (I) wherein R^1, R^2, R^3, R^4, R^5, R^6 and Ar are as defined above, useful in the treatment of a condition selected from the group consisting of arthritis, cancer, synergy with cytotoxic anticancer agents, tissue ulceration, macular degeneration, restenosis, periodontal disease, epidermolysis bullosa, scleritis, in combination with standard NSAID'S and analgesics and other diseases characterized by matrix metalloproteinase activity, AIDS, sepsis, septic shock and other diseases involving the production of TNF. In addition, the compounds of the present invention may be used in combination therapy with standard non-steroidal anti-inflammatory drugs (NSAID'S) and analgesics, and in combination with cytotoxic drugs such as adriamycin, daunomycin, cis-platinim, etoposide, taxol, taxotere and other alkaloids, such as vincristine, in the treatment of cancer.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

PHOSPHINATE BASED INHIBITORS OF MATRIX METALLOPROTEASES

Background of the Invention

The present invention relates to phosphinate based derivatives which are inhibitors of matrix metalloproteinases or the production of tumor necrosis factor (TNF) and as such are useful in the treatment of a condition selected from the group consisting of arthritis, cancer, tissue ulceration, restenosis, periodontal disease, epidermolysis bullosa, scleritis and other diseases characterized by matrix metalloproteinase activity, AIDS, sepsis, septic shock and other diseases involving the production of TNF. In addition, the compounds of the present invention may be used in combination therapy with standard non-steroidal anti-inflammatory drugs (hereinafter NSAID'S) and analgesics, and in combination with cytotoxic drugs such as adriamycin, daunomycin, cis-platinim, etoposide, taxol, taxotere and other alkaloids, such as vincristine, in the treatment of cancer.

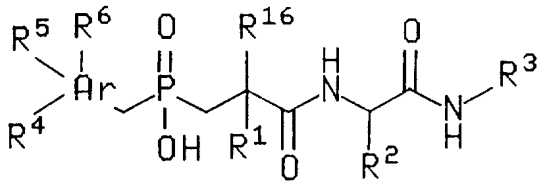
This invention also relates to a method of using such compounds in the treatment of the above diseases in mammals, especially humans, and to pharmaceutical compositions useful therefor.

There are a number of enzymes which effect the breakdown of structural proteins and which are structurally related metalloproteases. Matrix-degrading metalloproteinases, such as gelatinase, stromelysin and collagenase, are involved in tissue matrix degradation (e.g. collagen collapse) and have been implicated in many pathological conditions involving abnormal connective tissue and basement membrane matrix metabolism, such as arthritis (e.g. osteoarthritis and rheumatoid arthritis), tissue ulceration (e.g. corneal, epidermal and gastric ulceration), abnormal wound healing, periodontal disease, bone disease (e.g. Paget's disease and osteoporosis), tumor metastasis or invasion, as well as HIV-infection (J. Leuk. Biol., 52 (2): 244-248, 1992).

Tumor necrosis factor is recognized to be involved in many infectious and auto-immune diseases (W. Friers, FEBS Letters, 1991, 285, 199). Furthermore, it has been shown that TNF is the prime mediator of the inflammatory response seen in sepsis and septic shock (C.E. Spooner et al., Clinical Immunology and Immunopathology, 1992, 62 S11).

Summary of the Invention

The present invention relates to a compound of the formula



I

or a pharmaceutically acceptable salt thereof; wherein

Ar is phenyl, pyridyl, pyrimidinyl, pyrazinyl, pyridazinyl, thienyl, furyl, pyrrolyl, oxazolyl, thiazolyl, isoxazolyl, isothiazolyl or imidazolyl;

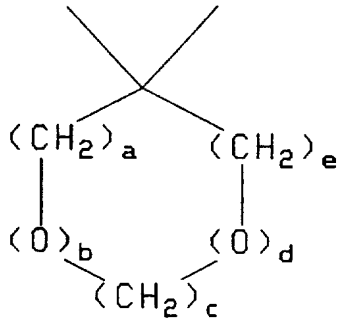
R^1 and R^{16} are each independently hydrogen, $(C_1\text{-}C_6)$alkyl, (trifluoromethyl)$_2(C_1\text{-}C_6)$alkyl, perfluoro$(C_1\text{-}C_6)$alkyl, perfluoro$(C_1\text{-}C_6)$alkyl$(C_1\text{-}C_6)$alkyl, difluoromethoxy, trifluoromethoxy, $(C_3\text{-}C_7)$cycloalkyl$(C_1\text{-}C_6)$alkyl, $(C_6\text{-}C_{10})$aryl$(C_1\text{-}C_6)$alkyl, $(C_6\text{-}C_{10})$aryloxy$(C_1\text{-}C_6)$alkyl or $(C_6\text{-}C_{10})$aryl$(C_1\text{-}C_6)$alkoxy$(C_1\text{-}C_6)$alkyl;

R^2 is $(C_1\text{-}C_6)$alkyl or $(C_6\text{-}C_{10})$aryl$(C_1\text{-}C_6)$alkyl optionally substituted by hydroxy, amino, halo, $(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_6)$alkoxy, (trifluoromethyl)$_2(C_1\text{-}C_6)$alkyl, perfluoro$(C_1\text{-}C_6)$alkyl, perfluoro$(C_1\text{-}C_6)$alkyl$(C_1\text{-}C_6)$alkyl, difluoromethoxy, trifluoromethoxy, carboxy or carboxamoyl;

R^3 is $(C_1\text{-}C_6)$alkyl or $(C_6\text{-}C_{10})$aryl;

R^4 is hydrogen, $(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_6)$alkoxy, $(C_3\text{-}C_7)$cycloalkyl$(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_6)$alkylsulfonyl, $(C_6\text{-}C_{10})$aryl, $(C_6\text{-}C_{10})$aryloxy, $(C_6\text{-}C_{10})$arylsulfonyl, $(C_6\text{-}C_{10})$aryl$(C_1\text{-}C_6)$alkyl, $(C_6\text{-}C_{10})$aryl $(C_1\text{-}C_6)$alkoxy, $(C_6\text{-}C_{10})$aryl$(C_1\text{-}C_6)$alkylsulfonyl, N-phthalimido, $(C_6\text{-}C_{10})$arylNHCO, $(C_6\text{-}C_{10})$arylNHSO$_2$, R^7OOC, R^7R^8NCO, $R^7R^8NSO_2$ wherein R^7 and R^8 are each independently hydrogen, $(C_1\text{-}C_6)$alkyl or $(C_6\text{-}C_{10})$aryl$(C_1\text{-}C_6)$alkyl; $(C_1\text{-}C_6)$alkyl CR^9R^{10}, $(C_6\text{-}C_{10})$aryl CR^9R^{10}, $(C_6\text{-}C_{10})$aryl$(C_1\text{-}C_6)$alkylCR^9R^{10} wherein R^9 and R^{10} are each independently fluoro, $(C_1\text{-}C_6)$alkyl or $(C_1\text{-}C_6)$alkoxy;

or R^9 and R^{10} may be taken together with the carbon to which they are attached to form a group of the formula



wherein a is 0, 1 or 2;

b is 0 or 1;

c is 1, 2, or 3;

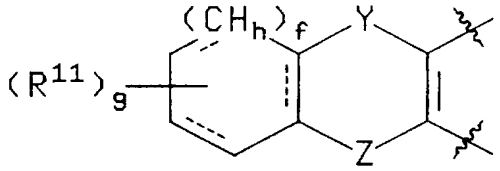
d is 0 or 1; and

e is 0, 1 or 2;

R^5 and R^6 are each independently hydrogen, $(C_1$-$C_6)$alkyl, $(C_1$-$C_6)$alkoxy, halo, (trifluoromethyl)$_2(C_1$-$C_6)$alkyl, perfluoro$(C_1$-$C_6)$alkyl, perfluoro$(C_1$-$C_6)$alkyl$(C_1$-$C_6)$alkyl, difluoromethoxy, trifluoromethoxy, $(C_1$-$C_6)$alkylthio, $(C_1$-$C_6)$alkylsulfinyl or $(C_1$-$C_6)$alkylsulfonyl;

or R^1 and R^{16} may be taken together with the carbon to which they are attached to form a $(C_3$-$C_7)$cycloalkyl group optionally substituted by $(C_1$-$C_6)$alkyl, $(C_1$-$C_6)$alkoxy, $(C_6$-$C_{10})$aryl$(C_1$-$C_6)$alkyl, $(C_6$-$C_{10})$aryl$(C_1$-$C_6)$alkyl or $(C_6$-$C_{10})$aryloxy;

or R^5 and R^6, when attached to adjacent carbon positions, may be taken together to form a group of the formula



wherein the broken lines represent optional double bonds;

h is 1 or 2;

f and g are each independently 0, 1 or 2;

Y and Z are each independently CH_2, O, CO, SO_2, CH_2CH_2, CH_2O, CH_2S, CH_2NH, CH_2CO, CH_2SO_2, NHCO or $NHSO_2$; and

R^{11} is hydrogen, halo, (C_1-C_6)alkyl, (C_1-C_6)alkoxy, (trifluoromethyl)$_2(C_1-C_6)$alkyl, perfluoro(C_1-C_6)alkyl, perfluoro(C_1-C_6)alkyl(C_1-C_6)alkyl, difluoromethoxy or trifluoromethoxy;

with the proviso that when either a or e is 0, the other must be 1;

with the proviso that when b and d are 1, the sum of a, c and e cannot be 5, 6 or 7;

with the proviso that when b and d are 0, the sum of a, c and e cannot be 7;

with the proviso that the methyene carbon attached to the phosphorus atom must be attached to a carbon atom of the Ar ring; and

with the proviso that R^5 and R^6 must be attached to carbon atoms of the Ar ring.

The term "alkyl", as used herein, unless otherwise indicated, includes saturated monovalent hydrocarbon radicals having straight, branched or cyclic moieties or combinations thereof.

The term "alkoxy", as used herein, includes O-alkyl groups wherein "alkyl" is defined above.

The term "aryl", as used herein, unless otherwise indicated, includes an organic radical derived from an aromatic hydrocarbon by removal of one hydrogen, such as phenyl or naphthyl, optionally substituted by 1 to 3 substituents selected from the group consisting of fluoro, chloro, trifluoromethyl, (C_1-C_6)alkoxy, (C_6-C_{10})aryloxy, trifluoromethoxy, difluoromethoxy and (C_1-C_6)alkyl.

The compound of formula I may have chiral centers and therefore exist in different enantiomeric forms. This invention relates to all optical isomers and stereoisomers of the compounds of formula I and mixtures thereof.

Preferred compounds of formula I include those wherein Ar is phenyl or thienyl.

Other preferred compounds of formula I include those wherein R^1 is 2-methylpropyl, trifluoromethylethyl, cyclopropylmethyl, cyclobutylmethyl, phenoxybutyl, cyclohexylmethyl, or phenylethyl.

Other preferred compounds of formula I include those wherein R^2 is (C_1-C_6)alkyl or 4-methoxybenzyl.

Other preferred compounds of formula I include those wherein R^3 is methyl.

Other preferred compounds of formula I include those wherein R^4 is benzyl, 2-chlorobenzyl, 2-fluorobenzyl, 3-fluorobenzyl or 4-fluorobenzyl.

More preferred compounds of formula I include those wherein Ar is phenyl or thienyl; R^1 is 2-methylpropyl, trifluoromethylethyl, cyclopropylmethyl, cyclobutylmethyl, phenoxybutyl, cyclohexylmethyl or phenylethyl; R^2 is (C_1-C_6)alkyl or 4-methoxybenzyl; R^3 is methyl and R^4 is benzyl, 2-chlorobenzyl, 2-fluorobenzyl, 3-fluorobenzyl or 4-fluorobenzyl.

Specific preferred compounds of formula I include the following:

(4-Benzylbenzyl)-[2-(2,2-dimethyl-1-methylcarbamoyl-propylcarbamoyl)-4-methyl-pentyl]-phosphinic acid;

(4-Benzylbenzyl-[2-(2,2-dimethyl-1-methylcarbamoyl-propylcarbamoyl)-5,5,5-trifluoropentyl]-phosphinic acid;

[2-(2,2-Dimethyl-1-methylcarbamoyl-propylcarbamoyl)-4-methylpentyl]-[4-(3-fluorobenzyl)-benzyl]-phosphinic acid;

Benzyl-{2-[2-(4-methoxyphenyl)-1-methylcarbamoyl-ethylcarbamoyl]-6-phenoxy-hexyl}-phosphinic acid;

(4-Benzylbenzyl)-{2-[2-(4-methoxyphenyl)-1-methylcarbamoyl-ethylcarbamoyl]-6-phenoxyhexyl}-phosphinic acid;

(4-Benzylbenzyl)-{3-cyclohexyl-2-[2-(4-methoxyphenyl)-1-methylcarbamoyl-ethylcarbamoyl]-propyl}-phosphinic acid;

(4-Benzylbenzyl)-[3-cyclohexyl-2-(2,2-dimethyl-1-methylcarbamoyl-propylcarbamoyl)-propyl]-phosphinic acid;

(4-Benzylbenzyl)-[2-(2,2-dimethyl-1-methylcarbamoyl-propylcarbamoyl)-4-phenyl-butyl]-phosphinic acid;

(4-Cyclohexylmethylbenzyl)-[2-(2,2-dimethyl-1-methylcarbamoyl-propylcarbamoyl)-4-methyl-pentyl]-phosphinic acid;

[2-(2,2-Dimethyl-1-methylcarbamoyl-propylcarbamoyl)-4-methylpentyl]-(4-isobutylbenzyl)-phosphinic acid;

[2-(2,2-Dimethyl-1-methylcarbamoyl-propylcarbamoyl)-4-methylpentyl]-[4-(4-fluoro-benzyl)-benzyl]-phosphinic acid;

[2-(2,2-Dimethyl-1-methylcarbamoyl-propylcarbamoyl)-4-methylpentyl]-[4-(2-fluoro-benzyl)-benzyl]phophinic acid;

(4-Benzylbenzyl)-{2-[2-(4-methoxyphenyl)-1-methylcarbamoyl-ethylcarbamoyl]-4-methyl-pentyl}-phosphinic acid;

[4-(2-Chlorobenzyl)benzyl]-[2-(2,2-dimethyl-1-methylcarbamoyl-1-propylcarbamoyl)-4-methylpentyl]phosphinic acid;

(5-Benzyl-pyridin-2-ylmethyl)-[2-(2,2-dimethyl-1-methylcarbamoyl-propylcarbamoyl)-4-methyl-pentyl]phosphinic acid;

[2-(2,2-Dimethyl-1-methylcarbamoyl-propylcarbamoyl)-5,5,5-trifluoro-pentyl]-[4-(2-fluoro-benzyl)-benzyl]phosphinic acid;

[3-Cyclopropyl-2-(2,2-dimethyl-1-methylcarbamoyl-propylcarbamoyl)-propyl]-[4-(2-fluoro-benzyl)-benzyl]phosphinic acid;

[3-Cyclobutyl-2-(2,2-dimethyl-1-methylcarbamoyl-propylcarbamoyl)-propyl]-[4-(2-fluoro-benzyl)-benzyl]-phosphinic acid; and

(5-Benzyl-thiophen-2-ylmethyl)-[2-(2,2-dimethyl-1-methylcarbamoyl-propylcarbamoyl)-4-methylpentyl]-phosphinic acid.

The present invention also relates to a pharmaceutical composition for (a) the treatment of a condition selected from the group consisting of arthritis, cancer, synergy with cytotoxic anticancer agents, tissue ulceration, macular degeneration, restenosis, periodontal disease, epidermolysis bullosa, scleritis, in combination with standard NSAID'S and analgesics and other diseases characterized by matrix metalloproteinase activity, AIDS, sepsis, septic shock and other diseases involving the production of tumor necrosis factor (TNF) or (b) the inhibition of matrix metalloproteinases or the production of tumor necrosis factor (TNF) in a mammal, including a human, comprising an amount of a compound of formula I or a pharmaceutically acceptable salt thereof effective in such treatments and a pharmaceutically acceptable carrier.

The present invention also relates to a method for the inhibition of (a) matrix metalloproteinases or (b) the production of tumor necrosis factor (TNF) in a mammal, including a human, comprising administering to said mammal an effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof.

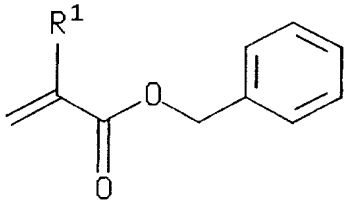
The present invention also relates to a method for treating a condition selected from the group consisting of arthritis, cancer, synergy with cytotoxic anticancer agents, tissue ulceration, macular degeneration, restenosis, periodontal disease, epidermolysis bullosa, scleritis, in combination with standard NSAID'S and analgesics and other diseases characterized by matrix metalloproteinase activity, AIDS, sepsis, septic shock and other diseases involving the production of tumor necrosis factor (TNF) in a mammal, including a human, comprising administering to said mammal an amount of

a compound of formula I or a pharmaceutically acceptable salt thereof effective in treating such a condition.

Detailed Description of the Invention

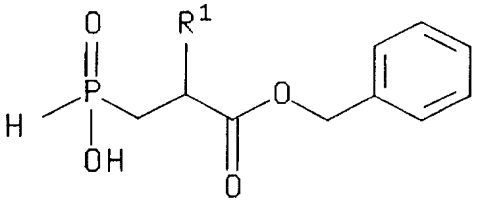
The following reaction Schemes illustrate the preparation of the compounds of the present invention. Unless otherwise indicated R^1, R^2, R^3, R^4, R^5, R^6 and Ar in the reaction Schemes and the discussion that follow are defined as above.

SCHEME 1



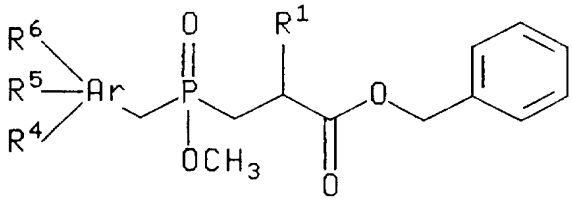
VI

1



V

2

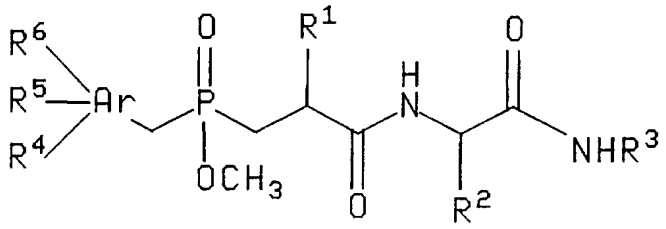


IV

3

III

SCHEME 1 (continued)

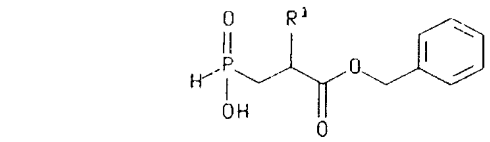


III

4

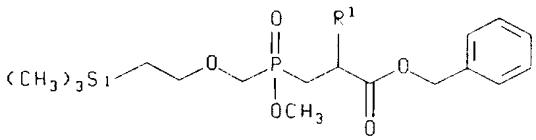
I

SCHEME 2



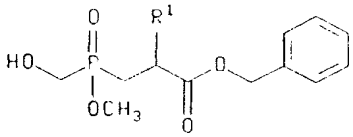
V

1



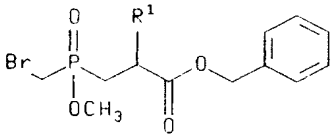
VIII

2



VII

3



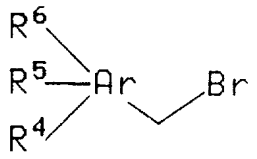
VI

4

IV

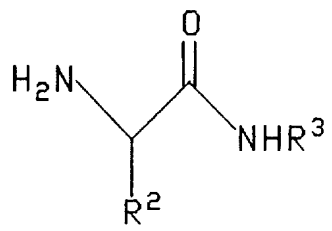
In reaction 1 of Scheme 1, the compound of formula VI is converted to the corresponding (2-benzyloxycarbonyl)phosphinic acid compound of formula **V** by reacting **VI** with bis-trimethylsilylphosphonite in an aprotic solvent, such as methylene chloride. The reaction mixture is stirred at room temperature for a time period between about 8 hours to about 48 hours, preferably about 18 hours.

In reaction 2 of Scheme 1, the compound of formula **V** is converted to the corresponding compound of formula **IV** by reacting **V** with an arylmethylhalide of the formula



and N,O-bis(trimethylsilyl)acetamide in an inert aprotic solvent, such a methylene chloride. The reaction mixture is stirred at room temperature or heated to reflux for a time period between about 18 hours to about 72 hours, preferably about 24 hours. An excess of trimethylsilyldiazomethane in a 7:3 ratio mixture of toluene and methanol is then added to the crude reaction product so formed for a time period between about 15 minutes to about 2 hours, preferably about 30 minutes.

In reaction 3 of Scheme 1, the compound of formula **IV** is converted to the corresponding compound of formula **III** by (1) hydrogenating **IV** in the presence of a catalyst, such 5% palladium on barium sulfate, and a protic solvent, such as methanol, under a pressure between about 30 psi to about 60 psi, preferably about 45 psi, for a time period between about 15 minutes to about 3 hours, preferably about 1 hour, (2) reacting the intermediate so formed with hydroxysuccinimide and 2-diethylaminoethyl propyl carbodiimide hydrochloride in a polar aprotic solvent, such as dimethylformamide, at room temperature, for a time period between about 8 hours to about 48 hours, preferably about 20 hours, and (3) reacting the 2,5-dioxo-pyrrolidin-1-yl intermediate so formed with an amine of the formula



In an aprotic solvent, such as methylene chloride, at room temperature, for a time period between about 16 hours to about 48 hours, preferably about 18 hours.

In reaction 4 of Scheme 1, the compound of formula **III** is converted to the corresponding compound of formula **I** by treating **III** with 10% aqueous trifluoroacetic acid. The reaction mixture is stirred, at room temperature, for a time period between about 30 minutes to about 24 hours, preferably about 2 hours.

Scheme 2 presents an alternative method for preparing a compound of formula **IV**.

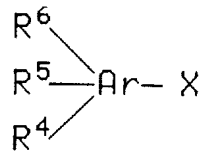
In reaction 1 of Scheme 2, the compound of formula **V** is converted to the corresponding compound of formula **VIII** by reacting **V** with 2-(trimethylsilyl) ethoxymethyl chloride and N,O-bis(trimethylsilyl)acetamide in an inert aprotic solvent, such as methylene chloride. The reaction mixture is stirred at a temperature between about 20°C to about 40°C, preferably about 25°C, for a time period between about 8 hours to about 48 hours, preferably about 18 hours. An excess of trimethylsilyl-diazomethane in a 7:3 ratio mixture of toluene and methanol is then added to the crude reaction product so formed for a time period between about 15 minutes to about 2 hours, preferably about 30 minutes.

In reaction 2 of Scheme 2, the compound of formula VIII is converted to the corresponding compound of formula **VII** by reacting **VIII** with boron trifluoride diethyl etherate in a inert aprotic solvent, such as methylene chloride. The reaction mixture is stirred at a temperature between about 0°C to about 40°C, preferably about 25°C, for a time period between about 1 hour to about 8 hours, preferably about 3 hours.

In reaction 3 of Scheme 2, the compound of formula **VII** is converted to the corresponding compound of formula **VI** by reacting **VII** with carbon tetrabromide in the presence of triphenylphosphine and diethyl azodicarboxylate in an inert aprotic solvent, such as methylene chloride. The reaction mixture is stirred at a temperature between

about 0°C to about 40°C, preferably about 25°C, for a time period between about 2 hours to about 24 hours, preferably about 4 hours.

In reaction 4 of Scheme 2, the compound of formula **VI** is converted to the corresponding compound of formula **IV** by reacting **VI** with an arylhalide of the formula



wherein X is bromo or iodo, in the presence of n-butyl lithium and copper (1) iodide in an inert aprotic solvent, such as tetrahydrofuran. The reaction mixture is stirred at a temperature between about -70°C to about 60°C, preferably about 0°C, for a time period between about 1 hour to about 48 hours, preferably about 18 hours.

Pharmaceutically acceptable salts of the acidic compounds of the invention are salts formed with bases, namely cationic salts such as alkali and alkaline earth metal salts, such as sodium, lithium, potassium, calcium, magnesium, as well as ammonium salts, such as ammonium, trimethyl-ammonium, diethylammonium, and tris-(hydroxymethyl)-methylammonium salts.

Similarly acid addition salts, such as of mineral acids, organic carboxylic and organic sulfonic acids e.g. hydrochloric acid, methanesulfonic acid, maleic acid, are also possible provided a basic group, such as pyridyl, constitutes part of the structure.

The ability of the compounds of formula **I** or their pharmaceutically acceptable salts (hereinafter also referred to as the compounds of the present invention) to inhibit matrix metalloproteinases or the production of tumor necrosis factor (TNF) and, consequently, demonstrate their effectiveness for treating diseases characterized by matrix metalloproteinase or the production of tumor necrosis factor is shown by the following in vitro assay tests.

Biological Assay

Inhibition of Human Collagenase (MMP-1)

Human recombinant collagenase is activated with trypsin using the following ratio: 10 μg trypsin per 100 μg of collagenase. The trypsin and collagenase are incubated at room temperature for 10 minutes then a five fold excess (50 μg/10 μg trypsin) of soybean trypsin inhibitor is added.

10 mM stock solutions of inhibitors are made up in dimethyl sulfoxide and then diluted using the following Scheme:

10 mM ------> 120 μM ------> 12 μM ------> 1.2 μM ------> 0.12 μM

Twenty-five microliters of each concentration is then added in triplicate to appropriate wells of a 96 well microfluor plate. The final concentration of inhibitor will be a 1:4 dilution after addition of enzyme and substrate. Positive controls (enzyme, no inhibitor) are set up in wells D1-D6 and blanks (no enzyme, no inhibitors) are set in wells D7-D12.

Collagenase is diluted to 400 ng/ml and 25 μl is then added to appropriate wells of the microfluor plate. Final concentration of collagenase in the assay is 100 ng/ml.

Substrate (DNP-Pro-Cha-Gly-Cys(Me)-His-Ala-Lys(NMA)-NH_2) is made as a 5 mM stock in dimethyl sulfoxide and then diluted to 20 μM in assay buffer. The assay is initiated by the addition of 50 μl substrate per well of the microfluor plate to give a final concentration of 10 μM.

Fluorescence readings (360 nM excitation, 460 nm emission) were taken at time 0 and then at 20 minute intervals. The assay is conducted at room temperature with a typical assay time of 3 hours.

Fluorescence vs time is then plotted for both the blank and collagenase containing samples (data from triplicate determinations is averaged). A time point that provides a good signal (the blank) and that is on a linear part of the curve (usually around 120 minutes) is chosen to determine IC_{50} values. The zero time is used as a blank for each compound at each concentration and these values are subtracted from the 120 minute data. Data is plotted as inhibitor concentration vs % control (inhibitor fluorescence divided by fluorescence of collagenase alone x 100). IC_{50}'s are determined from the concentration of inhibitor that gives a signal that is 50% of the control.

If IC_{50}'s are reported to be <0.03 μM then the inhibitors are assayed at concentrations of 0.3 μM, 0.03 μM, 0.03 μM and 0.003 μM.

Inhibition of Gelatinase (MMP-2)

Inhibition of gelatinase activity is assayed using the Dnp-Pro-Cha-Gly-Cys(Me)-His-Ala-Lys(NMA)-NH_2 substrate (10 μM) under the same conditions as inhibition of human collagenase (MMP-1).

72kD gelatinase is activated with 1 mM APMA (p-aminophenyl mercuric acetate) for 15 hours at 4°C and is diluted to give a final concentration in the assay of 100 ng/ml. Inhibitors are diluted as for inhibition of human collagenase (MMP-1) to give final concentrations in the assay of 30 μM, 3 μM, 0.3 μM and 0.03 μM. Each concentration is done in triplicate.

Fluorescence readings (360 nm excitation, 460 emission) are taken at time zero and then at 20 minutes intervals for 4 hours.

IC_{50}'s are determined as per inhibition of human collagenase (MMP-1). If IC_{50}'s are reported to be less than 0.03 μM, then the inhibitors are assayed at final concentrations of 0.3 μM, 0.03 μM, 0.003 μM and 0.003 μM.

Inhibition of Stromelysin Activity (MMP-3)

Inhibition of stromelysin activity is based on a modified spectrophotometric assay described by Weingarten and Feder (Weingarten, H. and Feder, J., Spectrophotometric Assay for Vertebrate Collagenase, Anal. Biochem. 147, 437-440 (1985)). Hydrolysis of the thio peptolide substrate [Ac-Pro-Leu-Gly-$SCH[CH_2CH(CH_3)_2]CO$-Leu-Gly-OC_2H_5] yields a mercaptan fragment that can be monitored in the presence of Ellman's reagent.

Human recombinant prostromelysin is activated with trypsin using a ratio of 1 μl of a 10 mg/ml trypsin stock per 26 μg of stromelysin. The trypsin and stromelysin are incubated at 37°C for 15 minutes followed by 10 μl of 10 mg/ml soybean trypsin inhibitor for 10 minutes at 37°C for 10 minutes at 37°C to quench trypsin activity.

Assays are conducted in a total volume of 250 μl of assay buffer (200 mM sodium chloride, 50 mM MES, and 10 mM calcium chloride, pH 6.0) in 96-well microliter plates. Activated stromelysin is diluted in assay buffer to 25 μg/ml. Ellman's reagent (3-Carboxy-4-nitrophenyl disulfide) is made as a 1M stock in dimethyl formamide and diluted to 5 mM in assay buffer with 50 μl per well yielding at 1 mM final concentration.

10 mM stock solutions of inhibitors are made in dimethyl sulfoxide and diluted serially in assay buffer such that addition of 50 μL to the appropriate wells yields final concentrations of 3 μM, 0.3 μM, 0.003 μM, and 0.0003 μM. All conditions are completed in triplicate.

A 300 mM dimethyl sulfoxide stock solution of the peptide substrate is diluted to 15 mM in assay buffer and the assay is initiated by addition of 50 μl to each well to give a final concentration of 3 mM substrate. Blanks consist of the peptide substrate and Ellman's reagent without the enzyme. Product formation was monitored at 405 nm with a Molecular Devices UVmax plate reader.

IC_{50} values were determined in the same manner as for collagenase.

Inhibition of MMP-13

Human recombinant MMP-13 is activated with 2mM APMA (p-aminophenyl mercuric acetate) for 1.5 hours, at 37°C and is diluted to 400 ng/ml in assay buffer (50 mM Tris, pH 7.5, 200 mM sodium chloride, 5mM calcium chloride, 20μM zinc chloride, 0.02% brij). Twenty-five microliters of diluted enzyme is added per well of a 96 well microfluor plate. The enzyme is then diluted in a 1:4 ratio in the assay by the addition of inhibitor and substrate to give a final concentration in the assay of 100 ng/ml.

10 mM stock solutions of inhibitors are made up in dimethyl sulfoxide and then diluted in assay buffer as per the inhibitor dilution scheme for inhibition of human collagenase (MMP-1): Twenty-five microliters of each concentration is added in triplicate to the microfluor plate. The final concentrations in the assay are 30 μM, 3μM, 0.3 μM, and 0.03 μM.

Substrate (Dnp-Pro-Cha-Gly-Cys(Me)-His-Ala-Lys(NMA)-NH_2) is prepared as for inhibition of human collagenase (MMP-1) and 50 μl is added to each well to give a final assay concentration of 10 μM. Fluorescence readings (360 nM excitation; 450 emission) are taken at time 0 and every 5 minutes for 1 hour.

Positive controls consist of enzyme and substrate with no inhibitor and blanks consist of substrate only.

IC_{50}'s are determined as per inhibition of human collagenase (MMP-1). If IC_{50}'s are reported to be less than 0.03 μM, inhibitors are then assayed at final concentrations of 0.3 μM, 0.03 μM, 0.003 μM and 0.0003 μM.

Inhibition of TNF Production

The ability of the compounds or the pharmaceutically acceptable salts thereof to inhibit the production of TNF and, consequently, demonstrate their effectiveness for treating diseases involving the production of TNF is shown by the following in vitro assay:

Human mononuclear cells were isolated from anti-coagulated human blood using a one-step Ficoll-hypaque separation technique. (2) The mononuclear cells were washed three times in Hanks balanced salt solution (HBSS) with divalent cations and resuspended to a density of 2×10^6 /ml in HBSS containing 1% BSA. Differential counts determined using the Abbott Cell Dyn 3500 analyzer indicated that monocytes ranged from 17 to 24% of the total cells in these preparations.

180μl of the cell suspension was aliquoted into flate bottom 96 well plates (Costar). Additions of compounds and LPS (100ng/ml final concentration) gave a final volume of 200μl. All conditions were performed in triplicate. After a four hour incubation at 37°C in an humidified CO_2 incubator, plates were removed and centrifuged (10 minutes at approximately 250 x g) and the supernatants removed and assayed for TNFα using the R&D ELISA Kit.

For administration to mammals, including humans, for the inhibition of matrix metalloproteinases or the production of tumor necrosis factor (TNF), a variety of conventional routes may be used including orally, parenterally and topically. In general, the active compound will be administered orally or parenterally at dosages between about 0.1 and 25 mg/kg body weight of the subject to be treated per day, preferably from about 0.3 to 5 mg/kg. However, some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject.

The compounds of the present invention can be administered in a wide variety of different dosage forms. In general, the therapeutically effective compounds of this invention are present in such dosage forms at concentration levels ranging from about 5.0% to about 70% by weight.

For oral administration, tablets containing various excipients such as microcrystalline cellulose, sodium citrate, calcium carbonate, dicalcium phosphate and glycine may be employed along with various disintegrants such as starch (and

preferably corn, potato or tapioca starch), alginic acid and certain complex silicates, together with granulation binders like polyvinylpyrrolidone, sucrose, gelation and acacia. Additionally, lubricating agents such as magnesium stearate, sodium lauryl sulfate and talc are often very useful for tabletting purposes. Solid compositions of a similar type may also be employed as fillers in gelatin capsules; preferred materials in this connection also include lactose or milk sugar as well as high molecular weight polyethylene glycols. When aqueous suspensions and/or elixirs are desired for oral administration, the active ingredient may be combined with various sweetening or flavoring agents, coloring matter or dyes, and, if so desired, emulsifying and/or suspending agents as well, together with such diluents as water, ethanol, propylene glycol, glycerin and various like combinations thereof. In the case of animals, they are advantageously contained in an animal feed or drinking water in a concentration of 5-5000 ppm, preferably 25 to 500 ppm.

For parenteral administration, e.g., for intramuscular, intraperitoneal, subcutaneous and intravenous use, a sterile injectable solution of the active ingredient is usually prepared. Solutions of a therapeutic compound of the present invention in either sesame or peanut oil or in aqueous propylene glycol may be employed. The aqueous solutions should be suitably adjusted and buffered, preferably at a pH of greater than 8, if necessary and the liquid diluent first rendered isotonic. These aqueous solutions are suitable for intravenous injection purposes. The oily solutions are suitable for intraarticular, intramuscular and subcutaneous injection purposes. The preparation of all these solutions under sterile conditions is readily accomplished by standard pharmaceutical techniques well known to those skilled in the art. In the case of animals, compounds can be administered intramuscularly or subcutaneously at dosage levels of about 0.1 to 50 mg/kg/day, advantageously 0.2 to 10 mg/kg/day given in a single dose or up to 3 divided doses.

The present invention is illustrated by the following examples, but it is not limited to the details thereof.

Example 1

S,S and R,S (4-Benzylbenzyl)[2-(2,2-dimethyl-1-methylcarbamoyl propylcarbamoyl)-4-methylpentyl]phosphinic acid

Step A: 4-Benzoylbenzyl bromide (2.75 grams, 10.0 mmole) and triethylsilane (2.33 grams, 20 mmole) in trifluoroacetic acid (4.56 grams, 40 mmole)

were warmed to 60°C for 18 hours. The cooled mixture was diluted with ethyl acetate (50 ml) and carefully washed with saturated sodium bicarbonate solution (2 x 50 ml). After drying with magnesium sulfate, the extract was filtered and concentrated. The residue was chromatographed (0.5:99.5 to 2:98 - ethyl acetate:hexane) to give 1.37 grams (52%) of 4-benzylbenzyl bromide as a colorless oil.

Step B: (2-Benzyloxycarbonyl-4 -methylpentyl)phosphinic acid (1.14 grams, 4.0 mmole), 4-benzylbenzyl bromide (1.31 grams, 5.0 mmole) and N,O-bis(trimethylsilyl) acetamide (2.44 grams, 12 mmole) were combined in dry methylene chloride (40 ml); the mixture was degassed with a stream of dry nitrogen, then stirred at room temperature for 18 hours and refluxed for 24 hours. The cooled solution was quenched with 1N hydrochloric acid (25 ml). The methylene chloride layer was separated and washed with 1N hydrochloric acid (2 x 25 ml), dried with magnesium sulfate, filtered and concentrated to a turbid oil. This was dissolved in methanol (10 ml) / toluene (40 ml) and treated with excess trimethylsilyldiazomethane (commercial hexane solution). After 30 minutes the excess trimethylsilyldiazo-methane was destroyed with acetic acid. The solution was concentrated to an oil which was chromatographed (75:25 - ethyl acetate:hexane) to give 1.18 grams (62%) of 2-[(4-benzylbenzyl)methoxyphosphinoylmethyl]-4-methylpentanoic acid benzyl ester as a colorless oil.

Step C: 2-[(4-Benzyl benzyl)methoxyphosphinoylmethyl]- 4-methylpentanoic acid benzyl ester (650 mg, 1.36 mmole) was hydrogenated at 45 psi at room temperature in methanol (50 ml) over 5% palladium on barium sulfate (650 mg) for 1 hour. The catalyst was filtered off and washed with methanol. The filtrate was concentrated and traces of methanol removed by twice diluting the sample with methylene chloride and reconcentrating. The intermediate 2-[(4-benzyl benzyl)methoxyphosphinoylmethyl]-4-methylpentanoic acid was dissolved in dry dimethylformamide (14 ml) and hydroxysuccinimide (235 mg, 2.04 mmole) and dimethylaminopropylethylcarbodiimide hydrochloride (391 mg, 2.04 mmol) added. After stirring at room temperature for 20 hours the solution was diluted with ether (50 ml) and washed with 1N hydrochloric acid (50 ml, 2 x 25 ml) and saturated sodium bicarbonate solution (25 ml) and dried with magnesium sulfate. After

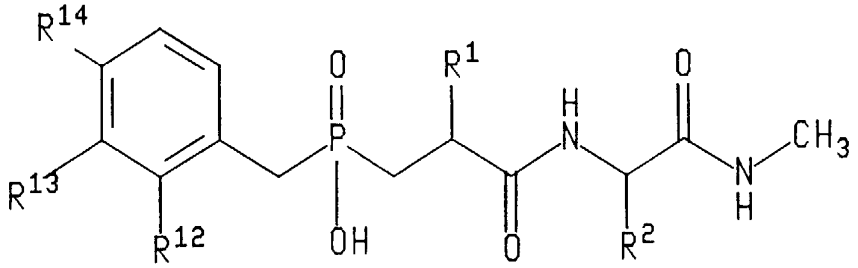
filtration and concentration 566 mg (86%) of 2-[(4-Benzylbenzyl) methoxyphosphinoylmethyl]-4- methyl-pentanoic acid 2,5-dioxo-pyrrolidin-1-yl ester was obtained as an oil.

Step D: 2-[(4-Benzylbenzyl)methoxyphosphinoylmethyl]-4-methylpentanoic acid 2,5-dioxo-pyrrolidin-1-yl ester (120 mg, 0.25 mmole), (S)-2-amino-3,3,N-trimethylbutyramide hydrochloride (25 mg, 0.30 mmole) and diisopropylethylamine (39 mg, 0.30 mmole) were combined and stirred together for 18 hours at room temperature in dry methylene chloride (10 ml). Additional (S)-2-amino-3,3,N-trimethylbutyramide hydrochloride (25 mg, 0.30 mmole) and diisopropylethylamine (39 mg, 0.30 mmole) were added to the reaction mixture. After four days the solution was washed with 1N hydrochloric acid (2 x 10 ml) and saturated sodium bicarbonate solution (2 x 10 ml) and dried with magnesium sulfate. After filtration and concentration the residue was chromatographed (3:97 - methanol:chloroform) to give 77 mg (60%) of (4-Benzylbenzyl)-[2-(2,2-dimethyl-1-methyl carbamoylpropylcarbamoyl)-4-methylpentyl]-phosphinic acid methyl ester.

Step E: (4-Benzylbenzyl)-[2-(2,2-dimethyl-1-methyl carbamoylpropylcarbamoyl)-4-methylpentyl]-phosphinic acid methyl ester (77 mg, 0.15 mmole) was dissolved in 10% aqueous trifluoroacetic acid (6 ml). After 4 hours at room temperature the reaction mixture was concentrated. Residual water was removed by twice diluting the sample with toluene and reconcentrating to give 75 mg (100%) of the title compound as a hard glass which was a 63:37 mixture of S,S and R,S isomers, respectively. Mass spectrum *m/e*: M^++1 501, M^++Na^+ 523, M^++K^+ 540, M^++2Na^+ 555. HPLC retention times: 13.00/15.90 minutes.

The compounds in Tables 1-4 were prepared by a method analogous to that described in in Example 1.

Table 1



EX	R^1	R^2	R^{12}	R^{13}	R^{14}	R^1 S/R	Ret. Time	MS
2	isobutyl	4-methoxybenzyl	H	phenyl	H	50/50	16.27/17.52	LSIMS: 551 M^++HP+
3	isobutyl	4-methoxybenzyl	phenyl	H	H	52/48	16.48/17.74	LSIMS: 551 M^++Na^+ 573 M^++Na^+ 589 M^++K^+
4	isobutyl	4-methoxybenzyl	H	H	phenyl	51/49	13.70/15.13	CI: 551 M^++H^+ 573 M^++Na^+
5	isobutyl	4-methoxybenzyl	H	H	methoxy	59/41	5.91/8.36	CI: 505 M^++H^+

EX	R^1	R^2	R^{12}	R^{13}	R^{14}	R^1 S/R	Ret. Time	MS
6	isobutyl	4-methoxybenzyl	H	H	H	49/51	7.03/9.42	LSIMS: 475 M^++H^+ 497 M^++Na^+
7	isobutyl	4-methoxybenzyl	H	H	benzyl	98/2	15.41/16.83	LSIMS: 565 M^++H^+
8	isobutyl	4-methoxybenzyl	H	H	benzyl	17/83	14.88/16.22	LSIMS: 565 M^++H^+
9	isobutyl	4-methoxybenzyl	H	1-phenyl-ethyl	H	51/49	16.45/17.64	LSIMS: 579 M^++H^+
10	phenoxybutyl	4-methoxybenzyl	H	H	H	49/51	13.10/14.34	LSIMS: 567 M^++H^+ 589 M^++Na+
11	phenoxybutyl	4-methoxybenzyl	H	H	benzyl	53/47	18.59/19.65	LSIMS: 657 M^++H^+
12	isobutyl	4-methoxybenzyl	H	H	benzyl	53/47	15.52/16.94	
13	isobutyl	4-methoxybenzyl	H	H	phenyl-sulfonyl	50/50	10.36/11.94	LSIMS: 615 M^++H^+
14	isobutyl	4-methoxybenzyl	H	H	phenoxy	50/50	14.58/15.98	LSIMS: 567 M^++H^+
15	isobutyl	methyl	H	H	benzyl	51/49	10.65/12.57	LSIMS: 459 M^++H^+ 481 M^++Na+

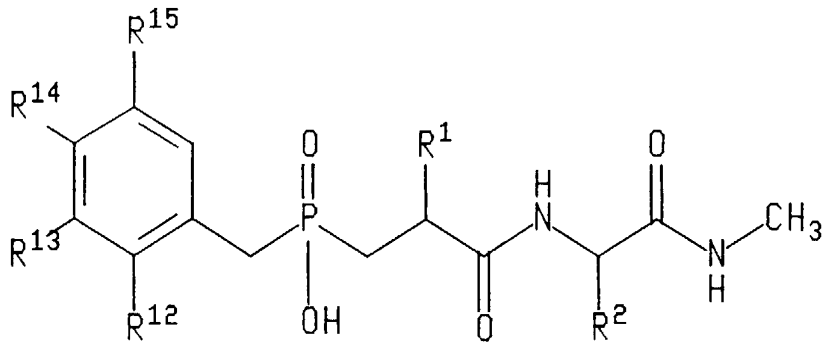
EX	R^1	R^2	R^{12}	R^{13}	R^{14}	R^1 S/R	Ret. Time	MS
16	cyclohexylmethyl	4-methoxybenzyl	H	H	benzyl	100:0	18.61/-	LSIMS: 605 M^++H^+ 627 M^++Na+
17	cyclohexylmethyl	4-methoxybenzyl	H	H	benzyl	19/81	18/55/19.92	LSIMS: 605 M^++H^+ 627 M^++H^+
18	isobutyl	tert-butyl	H	H	benzyl	63/37	13.00/15.90	LSIMS: 501 M^++H^+ 523 M^++Na+
19	cyclohexylmethyl	4-methoxybenzyl	H	H	H	50/50	12.00/13/59	LSIMS: 515 M^++H^+
20	cyclohexylmethyl	tert-butyl	H	H	benzyl	56/44	16.48/19.64	LSIMS: 541 M^++H^+ 563 M^++Na+
21	cyclohexylmethyl	tert-butyl	H	H	H	66/34	9.11/13/08	LSIMS: 451 M^++H^+
22	cyclohexylmethyl	4-methoxybenzyl	H	H	phenyl-sulfonyl	49/51	1395/15/21	LSIMS: 677 M^++Na+
23	cyclohexylmethyl	tert-butyl	H	H	phenyl-sulfonyl	52/48	11.63/14/71	LSIMS: 591 M^++H^+
24	cyclohexylmethyl	methyl	H	H	phenyl-sulfonyl	47/53	8.99/10.90	LSIMS: 549 M^++H^+ 571 M^++Na+

EX	R^1	R^2	R^{12}	R^{13}	R^{14}	R^1 S/R	Ret. Time	MS
25	isobutyl	methyl	H	H	H	48/52	2.22/3.10	CI: 369 M^+ 370 M^++H^+
26	isobutyl	tert-butyl	H	H	H	51/49	10.05/11.63	CI: 411 M^++H^+ 428 M^++NH_4+$^+$
27	phenethyl	tert-butyl	H	H	H	62/38	6.91/10/51	CI: 459 M^+ 460 M^++NH_4^+
28	trans 4-methyl-cyclohexylmethyl	tert-butyl	H	H	H	50/50	16.08/17.54	CI: 465 M^++H^+ 466 M^++$2H_4^+$
29	trans 4-methyl cyclohexylmethyl	4-methoxybenzyl	H	H	H	50/50	14.54/15/91	CI: 529 M^++H^+
30	trans 4-methyl-cyclohexylmethyl	methyl	H	H	H	100/0	11.59/-	CI: 530 M^++$2H^+$
31	isobutyl	4-methoxybenzyl	H	H	isobutyl	50/50	15.85/17/45	LSIMS: 423 M^++H^+
32	isobutyl	tert-butyl	H	H	isobutyl	50/50	13/46/16.64	LSIMS: 531 M^++H^+
33	isobutyl	methyl	H	H	isobutyl	45/55	11.31/13.34	LSIMS: 425 M^++H^+ 447 M^++Na+

EX	R^1	R^2	R^{12}	R^{13}	R^{14}	R^1 S/R	Ret. Time	MS
34	isobutyl	tert-butyl	H	H	cyclohexyl methyl	52/48	18.36/21.46	LSIMS: 507 M^++H^+
35	isobutyl	4-methoxybenzyl	H	H	cyclohexyl methyl	42/58	20.24/21.81	LSIMS: 571 M^++H^+
36	phenethyl	tert-butyl	H	H	benzyl	50/50	15.30/17.53	CI: 549 M^++H^+ 550 M^++$2H^+$
37	phenethyl	4-methoxybenzyl	H	H	benzyl	62/38	16.00/17.86	CI: 613 M^++H^+ 614 M^++$2H^+$
38	phenethyl	methyl	H	H	benzyl	3/97	12.94/14.34	CI: 507 M^++H^+ 508 M^++$2H^+$
39	isopentyl	tert-butyl	H	H	benzyl	53/47	14.92/17.69	CI: 515 M^++H^+ 516 M^++$2H^+$
40	isopentyl	4-methoxybenzyl	H	H	benzyl	54/46	16.69/18.09	CI: 579 M^++H^+ 580 M^++$2H^+$
41	cyclohexylethyl	tert-butyl	H	H	benzyl	53/47	18.44/21.66	CI: 555 M^++H^+
42	cyclohexylethyl	4-methoxybenzyl	H	H	benzyl	53/47	20.28/21.55	CI: 619 M^++H^+

EX	R^1	R^2	R^{12}	R^{13}	R^{14}	R^1 S/R	Ret. Time	MS
43	3,3,3-trifluoropropyl	tert-butyl	H	H	benzyl	47/53	13.01/15.03	CI: 540 M^+ 541 M^++H^+
44	isobutyl	tert-butyl	H	H	3-fluoro-benzyl	53/47	12.67/15.56	CI: 519 M^++H^+
45	isobutyl	tert-butyl	H	H	phenyl-CO-	54/46	8.06/11.58	CI: 515 M^++H^+ 516 M^++2H^+
46	propyl	tert-butyl	H	H	benzyl	50/50	10.13/13.15	CI: 487 M^++H^+
47	isobutyl	tert-butyl	H	H	4-fluoro-benzyl	48/52	12.85/15.59	CI: 519 M^++H^+
48	isobutyl	tert-butyl	H	H	2-fluoro-benzyl	43/57	12.95/15.85	CI: 519 M^++H^+

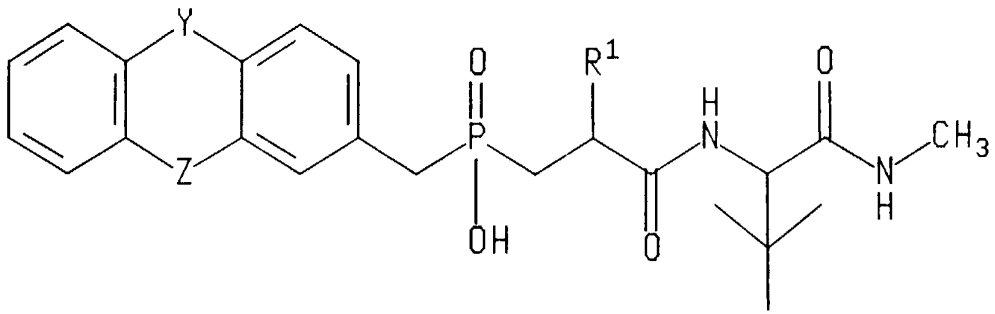
Table 2



EX	R^1	R^2	R^{12}	R^{13}	R^{14}	R^{15}	R^1 S/R	Ret. Time	MS
49	isobutyl	4-methoxy-benzyl	H	H	benzylamino-carbonyl	H	57/43	9.24/10.76	LSIMS: 608 M^++H^+
50	isobutyl	4-methoxy-benzyl	H	H	methylamino-carbonyl	H	48/52	15.35/16.68	CI: 532 M^++H^+ 538 M^++Li^+
51	isobutyl	4-methoxy-benzyl	benzylamino-carbonyl	H	H	H	49/51	11.42/13.4	LSIMS: 608 M^++H^+ 630 M^++Na^+
52	isobutyl	4-methoxy-benzyl	H	benzylamino-carbonyl	H	H	47/53	09.74/11.44	LSIMS: 608 M^++H^+ 630 M^++Na^+

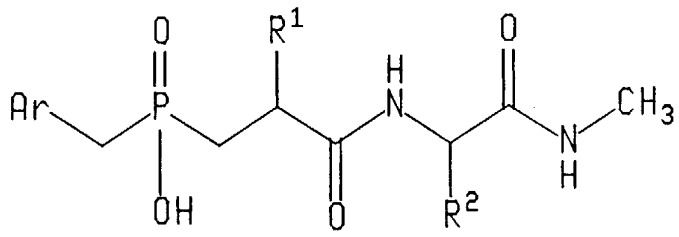
EX	R^1	R^2	R^{12}	R^{13}	R^{14}	R^{15}	R^1 S/R	Ret. Time	MS
53	isobutyl	4-methoxy-benzyl	H	H	dimethylamino-carbonyl	H	34/66	10.77/12.64	LSIMS: 546 M^++H^+
54	isobutyl	4-methoxy-benzyl	H	dimethylamino-carbonyl	H	H	47/53	11.66/13.49	LSIMS: 546 M^++H^+
55	isobutyl	4-methoxy-benzyl	H	H	benzyl(methyl) amino-carbonyl	H	45/55	10.64/12.16	LSIMS: 622 M^++H^+
56	isobutyl	4-methoxy-benzyl	H	benzyl(methyl) amino-carbonyl	H	H	50/50	11.55/13.20	LSIMS: 622 M^++H^+ 644 M^++Na^+
57	isobutyl	4-methoxy-benzyl	H	methoxy	benzylamino-carbonyl	CH_3O	42/58	9.63/11.15	LSIMS: 690 M^++Na^+ 712 M^++2Na^+
58	isobutyl	4-methoxy-benzyl	dimethylamino-carbonyl	H	H	H	45/55	13.54/15.44	LSIMS: 546 M^++H^+ 568 M^++2Na^+
59	isobutyl	4-methoxy-benzyl	benzyl(methyl) amino-carbonyl	H	H	H	53/47	13.11/14.83	LSIMS: 622 M^++H^+
60	isobutyl	4-methoxy-benzyl	H	methylamino-carbonyl	H	H	46/54	10.02/12.09	532 M^++H^+
61	isobutyl	tert-butyl	H	H	benzylamino-carbonyl	H	50/50	3.88/6.54	566 M^++2Na^+

Table 3



Ex	Y	Z	R^1	R^1 S/R	Ret. Time	MS
62	$-CH_2-$	$-CH_2CH_2-$	isobutyl	49/51	14.15/17.08	CI: 527 $M^+ + H^+$
63	$-CH_2-$	$-CH_2O-$	isobutyl	53/47	11.19/14.23	CI: 529 $M^+ + H^+$

Table 4



5

EX	R^1	R^2	Ar	R^1 S/R	Ret. Time	MS
64	isobutyl	4-methoxybenzyl	3-carbethoxy-2-pyridyl	50/50	3.36/4.30	LSIMS: 548 M^++H^+

Example 65

S,S and R,S (4-Benzoylaminobenzyl)-{2-[2-(4-methoxyphenyl)-1-methylcarbamoylethylcarbamoyl] -4-methylpentyl}phosphinic acid.

Step A: 2-[Methoxy(4-nitrobenzyl) phosphinoylmethyl]-4-methylpentanoic acid benzyl ester (prepared from 4-nitrobenzyl bromide and (2-benzyloxycarbonyl-4-methylpentyl)phosphinic acid by the procedure described in Example 1/Step B) (900 mg, 2.08 mmole) in a mixture of ethanol (25 ml) and water (6 ml) was treated with concentrated hydrochloric acid (3 drops) and iron powder (1.14 grams, 20 mmole) at reflux. After 2 hours the cooled mixture was filtered through diatomaceous earth. The filtrate was concentrated and the residue chromatographed (ethyl acetate) to give 444 mg (53%) of 2-[(4-Aminobenzyl) methoxyphosphinoylmethyl]-4-methylpentanoic acid benzyl ester as a yellow oil.

Step B: 2-[(4-Aminobenzyl)methoxyphosphinoylmethyl]-4-methylpentanoic acid benzyl ester (230 mg, 0.57 mmole), benzoyl chloride (96 mg, 0.68 mmole), and triethylamine (69 mg, 0.68 mmole) were combined in cold (ice bath) chloroform (10 ml). After stirring for 1 hour at ice bath temperature the reaction mixture was diluted with chloroform (150 ml) and washed with water (20 ml), 1N hydrochloric acid (2 x 20 ml) and saturated sodium bicarbonate solution (2 x 20 ml) and dried with magnesium sulfate. After filtration and concentration the yellow residue was chromatographed (ethyl acetate) to give 190 mg (66%) of 2-[(4-Benzoylamino-benzyl)methoxy phosphinoylmethyl]-4- methylpentanoic acid benzyl ester as a light yellow oil.

Step C: 2-[(4-Benzoylaminobenzyl)methoxy phosphinoylmethyl]-4-methylpentanoic acid benzyl ester (226 mg, 0.44 mmole) was hydrogenated hydrogenated at 50 psi at room temperature in methanol (20 ml) over 5% palladium on carbon (300 mg) for 2 hours. The catalyst was filtered off and washed with methanol. The filtrate was concentrated to give 154 mg (83%) of 2-[(4-benzoyl-aminobenzyl)methoxyphosphinoylmethyl]-4-methylpentanoic acid as an oil.

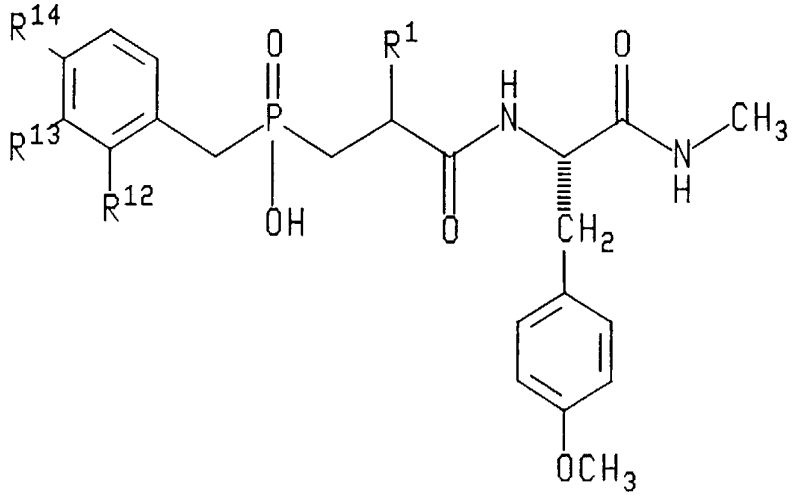
Step D: 2-[(4-Benzoylaminobenzyl)methoxyphosphinoyl methyl]-4-methylpentanoic acid (154 mg, 0.37 mmole), (S)-2-amino-3-(4-methoxyphenyl)-N- methylpropionamide (100 mg, 0.41 mmole), benzotriazol-1-yloxy -tris(dimethylamino)phosphonium hexafluorophosphate (180 mg, 0.41 mmole) and diisopropylethylamine (238 mg, 1.85 mmole) were stirred together in dry methylene chloride (10 ml) for 18 hours. The reaction mixture was

concentrated and diluted with ethyl acetate (100 ml). This solution was washed with 1N hydrochloric acid (20 ml) and saturated sodium bicarbonate solution (20 ml) and dried with magnesium sulfate. Filtration and concentration gave the crude product which was purified by chromatography (10:90 - methanol:methylene chloride) yielding 153 mg (68%) of (4-Benzoylamino benzyl){2-[2-(4-methoxyphenyl) -1-methylcarbamoylethylcarbamoyl]-4-methylpentyl} phosphinic acid methyl ester as a white solid.

Step E: By the procedure described in Example 1/Step E (4-Benzoylamino benzyl){2-[2-(4-methoxyphenyl) -1-methylcarbamoylethylcarbamoyl]-4-methylpentyl} phosphinic acid methyl ester (153 mg, 0.25 mmole) was converted to 100 mg (67%) the title compound, a white solid which was a 50:50 mixture of S,S and R,S isomers, respectively. Mass spectrum *m/e*: M^++H^+ 594, M^++Na^+ 616. HPLC retention times: 8.32/10.33 minutes.

The compounds in Table 5 were prepared by a method analogous to that described in Example 65.

Table 5



EX	R^1	R^{12}	R^{13}	R^{14}	R^1 S/R	Ret. Time	MS
66	isobutyl	H	H	benzamido	50/50	8.32/10.33	LSIMS 594 M^++H^+ 616 M^++Na^+
67	isobutyl	H	H	acetamido	45/55	9.93/11.81	532 M^++H^+ 554 M^++Na^+
68	isobutyl	H	benzamido	H	48/52	9.95/11.64	594 M^++H^+ 616 M^++Na^+
69	isobutyl	H	acetamido	H	43/57	11.16/12.96	532 M^++H^+ 554 M^++Na^+

EX	R^1	R^{12}	R^{13}	R^{14}	R^1 S/R	Ret. Time	MS
70	isobutyl	benzamido	H	H	66/34	8.80/11.30	594 M^++H^+ 616 M^++Na^+
71	isobutyl	acetamido	H	H	51/49	11.98/13.82	594 M^++H^+ 616 M^++Na^+
72	isobutyl	H	phenylsulfonyl-amino	H	51/49	16.38/17.35	652 M^++Na^+

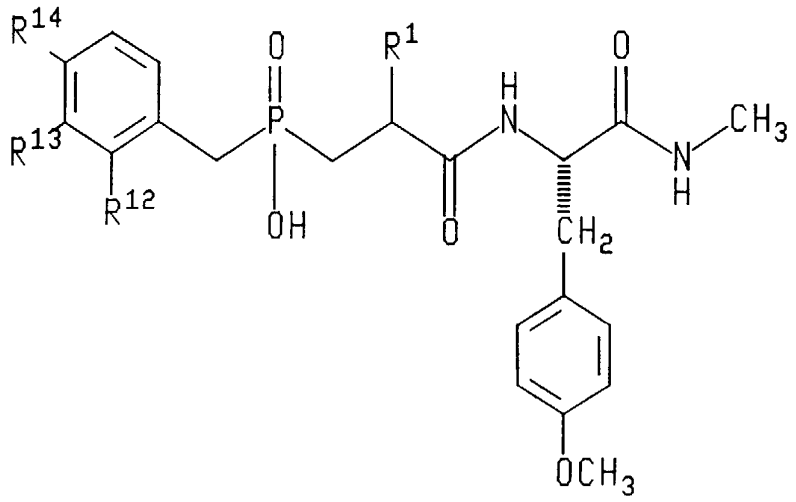
Example 73

S,S and R,S [4-(1,3-Dioxo-1,3-dihydroisoindol-2-yl)benzyl] {2-[2-(4-methoxyphenyl)-1-methyl carbamoylethylcarbamoyl]-4-methylpentyl} phosphinic acid

Step A: 2-[(4-Aminobenzyl)methoxyphosphinoylmethyl]-4-methylpentanoic acid benzyl ester (prepared as described in Example 2/Step A) (242 mg, 0.60 mmole) and phthalic anhydride (133 mg, 0.90 mmole) in acetic acid (10 ml) were refluxed for 1 hour. The cooled reaction mixture was concentrated and the residue dissolved in ethyl acetate (100 ml). This solution was washed with saturated sodium bicarbonate solution (3 x 20 ml) and dried with magnesium sulfate. Filtration and concentration gave a light yellow oil which was purified by chromatography (ethyl acetate) yielding 162 mg (51%) of 2-{[4-(1,3-dioxo-1,3-dihydroisoindol-2-yl)benzyl] methoxyphosphinoyl methyl}-4-methylpentanoic acid benzyl ester as a yellow solid. Step B: By the procedures described in Example 2/Steps C-E 2-{[4-(1,3-dioxo-1,3-dihydroisoindol-2-yl)benzyl] methoxyphosphinoyl methyl}-4-methylpentanoic acid benzyl ester (269 mg, 0.50 mmole) was converted to 61 mg (20% - 3 steps) of the title compound, a white solid which was a 50:50 mixture of S,S and R,S isomers, respectively. Mass spectrum *m/e*: $M^+ + H^+$ 620, $M^+ + Na^+$ 642. HPLC retention times: 10.12/11.92 minutes.

The compounds in Table 6 were prepared by a method analogous to that described in Example 73.

Table 6



EX	R^1	R^{12}	R^{13}	R^{14}	R^1 S/R	Ret. Time	MS
74	isobutyl	H	H	phthalimide	50/50	10.12/11.92	LSIMS: 620 M^++H^+ 642 M^++Na^+
75	isobutyl	H	phthalimide	H	46/54	10.58/12.65	LSIMS: 620 M^++H^+ 642 M^++Na^+
76	isobutyl	phthalimide	H	H	54/46	11.44/14.67	LSIMS: 620 M^++H^+ 642 M^++Na^+

Example 77

S,S and R,S (3-Aminobenzyl)-{2-[2-(4-methoxyphenyl)-1-methylcarbamoylethylcarbamoyl]-4-methylpentyl}phosphinic acid.

Step A: {2-[2-(4-Methoxyphenyl)-1-methylcarbamoylethylcarbamoyl]-4-methylpentyl}-[3-(2,2,2-trifluoroacetylamino)benzyl]phosphinic acid methyl ester (prepared from the appropriate starting materials using the procedures described in Example 2/Steps A-D) (105 mg, 0.18 mmole) was treated with potassium carbonate (242 mg, 1.75 mmole) in 10% aqueous methanol (10 ml) for 18 hours. 1N Sodium hydroxide (1 ml) was added and after 3 hours the reaction mixture was concentrated and ethyl acetate (25 ml) and water (5 ml) added. The ethyl acetate layer was removed and the water extracted with ethyl acetate (3 x 20 ml). The combined ethyl acetate extracts were dried with magnesium sulfate and filtered. The filtrate was concentrated to give 56 mg (64%) of (3-aminobenzyl){2-[2-(4-methoxy phenyl)-1-methylcarbamoylethyl carbamoyl]-4-methylpentyl}phosphinic acid methyl ester as a light yellow oil.

Step B: By the procedure described in Example 1/Step E (3-aminobenzyl){2-[2-(4-methoxy phenyl)-1-methylcarbamoylethyl carbamoyl]-4-methylpentyl}phosphinic acid methyl ester (56 mg, 0.11 mmole) was converted to 40 mg (74%) of the title compound, a white solid which was a 44:56 mixture of S,S and R,S isomers, respectively. Mass spectrum *m/e*: $M^+ + H^+$ 490. HPLC retention times (20% to 80% gradient): 6.17/8.94 minutes.

Example 78

S,S and R,S (3-Benzylaminobenzyl)-{2-[2- (4-methoxyphenyl)-1-methylcarbamoylethylcarbamoyl]-4-methylpentyl}phosphinic acid.

Step A: (3-Aminobenzyl){2-[2-(4-methoxyphenyl)-1-methylcarbamoyl ethylcarbamoyl]-4-methylpentyl}phosphinic acid methyl ester (prepared as described in Example 4/Step A) (150 mg, 0.30 mmole), benzaldehyde (38 mg, 0.36 mmole), sodium cyanoborohydride (23 mg, 0.357 mmole) and acetic acid (1 drop) in methanol were stirred at room temperature for 3 hours. The reaction was quenched with 1N hydrochloric acid (few ml's) and the reaction mixture concentrated. The residue was dissolved in ethyl acetate (20 ml) and washed with 1N hydrochloric acid (20 ml), saturated sodium bicarbonate solution (20 ml) and dried with magnesium sulfate. Filtration and concentration gave the crude product which was purified by chromatography (3:97 - methanol:methylene chloride) yielding 133 mg (75%) of

(3-Benzylamino benzyl)-{2-[2-(4-methoxyphenyl)-1- methylcarbamoylethylcarbamoyl] -4-methylpentyl} phosphinic acid methyl ester as an oil.

Step B: By the procedure described in Example 1/Step E (3-Benzylamino benzyl)-{2-[2-(4-methoxyphenyl)-1- methylcarbamoylethylcarbamoyl]-4-methylpentyl} phosphinic acid methyl ester (133 mg, 0.22 mmole) was converted to 100 mg (64%) of the title compound, a white solid which was a 67:33 mixture of S,S and R,S isomers, respectively. Mass spectrum *m/e*: M^++H^+ 580, M^++Na^+ 602. HPLC retention times: 7.29/9.61 minutes.

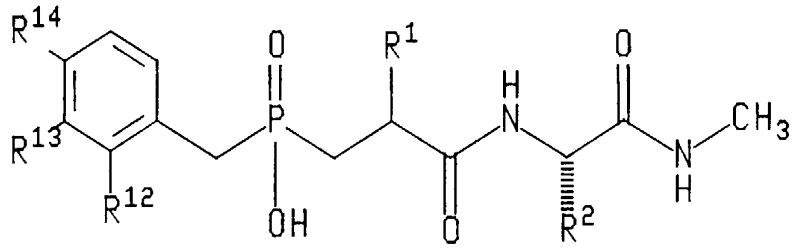
Example 79

Separation of S,S and R,S (4-benzylbenzyl)[2-(2,2-dimethyl -1-methylcarbamoylpropylcarbamoyl)-4- methylpentyl]phosphinic acid

A mixture of S,S and R,S (4-benzylbenzyl)[2-(2,2-dimethyl-1-methylcarbamoylpropylcarbamoyl)-4-methylpentyl]phosphinic acid (prepared as described in Example 1) (609 mg) was chromatographed on a preparative reverse phase (C-18) column eluting first with 40% aqueous acetonitrile containing 0.1% trifluoroacetic acid and then with 50% aqueous acetonitrile containing 0.1% trifluoroacetic acid. This gave nearly complete separation of the two diastereomers. Concentration of the fractions containing the two pure components gave 304 mg of S,S (4-benzylbenzyl)[2- (2,2-dimethyl-1-methylcarbamoyl propylcarbamoyl)-4-methylpentyl] phosphinic acid as a white solid: ^{1}HNMR (CD_3OD) d 0.83 (d,3H,J=6.9 Hz), 0.89 (d,3H,J=6.9 Hz), 1.02 (s,9H), 1.32 (m,1H), 1.42 (m,1H), 1.53 (m,1H), 1.67 (m,1H), 1.99 (m,1H), 2.69 (s,3H), 2.81 (m,1H), 3.10 (d,2H,J=17.1 Hz), 3.94 (s,2H), 4.08 (s,1H), 7.1-7.3 (m,9H); mass spectrum *m/e*: 501 M^++H^+; HPLC retention time: 12.96 minutes; and 208 mg of R,S (4-benzylbenzyl)[2-(2,2-dimethyl-1-methyl carbamoylpropylcarbamoyl)-4-methylpentyl]phosphinic acid as a white solid: ^{1}HNMR (CD_3OD) d 0.86 (d,3H,J=6.9 Hz), 0.91 (d,3H,J=6.9 Hz), 1.02 (s,9H), 1.22 (m,1H), 1.4-1.7 (m,3H), 2.00 (m,1H), 2.64 (s,3H), 2.85 (m,1H), 3.10 (d,2H,J=17.1 Hz), 3.94 (s,2H), 4.13 (s,1H), 7.1-7.3 (m,9H); mass spectrum *m/e*: 501 M^++H^+; HPLC retention time: 15.84 minutes.

The compounds in Table 7 were separated by a method analogous to that described in Example 79.

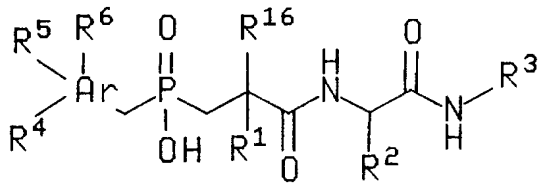
Table 7



EX	R^1	R^2	R^{12}	R^{13}	R^{14}	R^1 S/R	Ret. Time	MS
80	isobutyl	tert-butyl	H	H	benzyl	0/100	-/15.84	CI: 501 M^++H^+
81	isobutyl	tert-butyl	H	H	benzyl	100/0	12.96/-	CI: 501 M^++H^+
82	isobutyl	tert-butyl	H	H	3-fluorobenzyl	100/0	13.54/-	CI: 519 M^++H^+
83	isobutyl	tert-butyl	H	H	3-fluorobenzyl	0/100	-/16.20	CI: 519 M^++H^+
84	3,3,3-trifluoropropyl	tert-butyl	H	H	benzyl	100/0	13.38/-	CI: 540 M^+ 541 M^++H^+
85	3,3,3-trifluoropropyl	tert-butyl	H	H	benzyl	0/100	-/15.16	CI: 540 M^+ 541 M^++H^+

CLAIMS

1. A compound of the formula



I

or a pharmaceutically acceptable salt thereof; wherein

Ar is phenyl, pyridyl, pyrimidinyl, pyrazinyl, pyridazinyl, thienyl, furyl, pyrrolyl, oxazolyl, thiazolyl, isoxazolyl, isothiazolyl or imidazolyl;

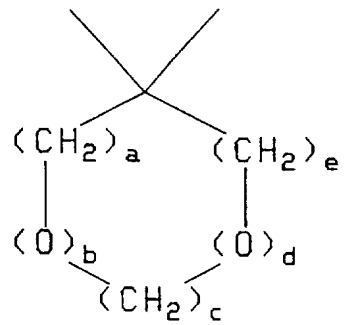
R^1 and R^{16} are each independently hydrogen, $(C_1\text{-}C_6)$alkyl, $(\text{trifluoromethyl})_2(C_1\text{-}C_6)$alkyl, perfluoro$(C_1\text{-}C_6)$alkyl, perfluoro$(C_1\text{-}C_6)$alkyl$(C_1\text{-}C_6)$alkyl, difluoromethoxy, trifluoromethoxy, $(C_3\text{-}C_7)$cycloalkyl$(C_1\text{-}C_6)$alkyl, $(C_6\text{-}C_{10})$aryl$(C_1\text{-}C_6)$alkyl, $(C_6\text{-}C_{10})$aryloxy$(C_1\text{-}C_6)$alkyl or $(C_6\text{-}C_{10})$aryl$(C_1\text{-}C_6)$alkoxy$(C_1\text{-}C_6)$alkyl;

R^2 is $(C_1\text{-}C_6)$alkyl or $(C_6\text{-}C_{10})$aryl$(C_1\text{-}C_6)$alkyl optionally substituted by hydroxy, amino, halo, $(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_6)$alkoxy, $(\text{trifluoromethyl})_2(C_1\text{-}C_6)$alkyl, perfluoro$(C_1\text{-}C_6)$alkyl, perfluoro$(C_1\text{-}C_6)$alkyl$(C_1\text{-}C_6)$alkyl, difluoromethoxy, trifluoromethoxy, carboxy or carboxamoyl;

R^3 is $(C_1\text{-}C_6)$alkyl or $(C_6\text{-}C_{10})$aryl;

R^4 is hydrogen, $(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_6)$alkoxy, $(C_3\text{-}C_7)$cycloalkyl$(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_6)$alkylsulfonyl, $(C_6\text{-}C_{10})$aryl, $(C_6\text{-}C_{10})$aryloxy, $(C_6\text{-}C_{10})$arylsulfonyl, $(C_6\text{-}C_{10})$aryl$(C_1\text{-}C_6)$alkyl, $(C_6\text{-}C_{10})$aryl $(C_1\text{-}C_6)$alkoxy, $(C_6\text{-}C_{10})$aryl$(C_1\text{-}C_6)$alkylsulfonyl, N-phthalimido, $(C_6\text{-}C_{10})$arylNHCO, $(C_6\text{-}C_{10})$arylNHSO$_2$, R^7OOC, R^7R^8NCO, $R^7R^8NSO_2$ wherein R^7 and R^8 are each independently hydrogen, $(C_1\text{-}C_6)$alkyl or $(C_6\text{-}C_{10})$aryl$(C_1\text{-}C_6)$alkyl; $(C_1\text{-}C_6)$alkyl CR^9R^{10}, $(C_6\text{-}C_{10})$aryl CR^9R^{10}, $(C_6\text{-}C_{10})$aryl$(C_1\text{-}C_6)$alkylCR^9R^{10} wherein R^9 and R^{10} are each independently fluoro, $(C_1\text{-}C_6)$alkyl or $(C_1\text{-}C_6)$alkoxy;

or R^9 and R^{10} may be taken together with the carbon to which they are attached to form a group of the formula



wherein a is 0, 1 or 2;

b is 0 or 1;

c is 1, 2, or 3;

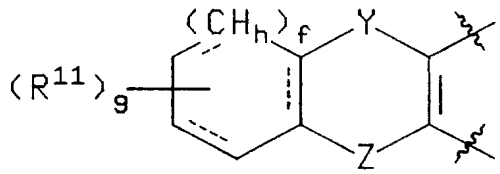
d is 0 or 1; and

e is 0, 1 or 2;

R^5 and R^6 are each independently hydrogen, $(C_1$-$C_6)$alkyl, $(C_1$-$C_6)$alkoxy, halo, (trifluoromethyl)$_2(C_1$-$C_6)$alkyl, perfluoro$(C_1$-$C_6)$alkyl, perfluoro$(C_1$-$C_6)$alkyl$(C_1$-$C_6)$alkyl, difluoromethoxy, trifluoromethoxy, $(C_1$-$C_6)$alkylthio, $(C_1$-$C_6)$alkylsulfinyl or $(C_1$-$C_6)$alkylsulfonyl;

or R^1 and R^{16} may be taken together with the carbon to which they are attached to form a $(C_3$-$C_7)$cycloalkyl group optionally substituted by $(C_1$-$C_6)$alkyl, $(C_1$-$C_6)$alkoxy, $(C_6$-$C_{10})$aryl$(C_1$-$C_6)$alkyl, $(C_6$-$C_{10})$aryl$(C_1$-$C_6)$alkyl or $(C_6$-$C_{10})$aryloxy;

or R^5 and R^6, when attached to adjacent carbon positions, may be taken together to form a group of the formula



wherein the broken lines represent optional double bonds;

h is 1 or 2;

f and g are each independently 0, 1 or 2;

Y and Z are each independently CH_2, O, CO, SO_2, CH_2CH_2, CH_2O, CH_2S, CH_2NH, CH_2CO, CH_2SO_2, NHCO or $NHSO_2$; and

R^{11} is hydrogen, halo, $(C_1$-$C_6)$alkyl, $(C_1$-$C_6)$alkoxy, (trifluoromethyl)$_2(C_1$-$C_6)$alkyl, perfluoro$(C_1$-$C_6)$alkyl, perfluoro$(C_1$-$C_6)$alkyl$(C_1$-$C_6)$alkyl, difluoromethoxy or trifluoromethoxy;

with the proviso that when either a or e is 0, the other must be 1;

with the proviso that when b and d are 1, the sum of a, c and e cannot be 5, 6 or 7;

with the proviso that when b and d are 0, the sum of a, c and e cannot be 7;

with the proviso that the methyene carbon attached to the phosphorus atom must be attached to a carbon atom of the Ar ring; and

with the proviso that R^5 and R^6 must be attached to carbon atoms of the Ar ring.

2. A compound according to claim 1, wherein Ar is phenyl or thienyl.

3. A compound according to claim 1, wherein R^1 is 2-methylpropyl, trifluoromethylethyl, cyclopropylmethyl, cyclobutylmethyl, phenoxybutyl, cyclohexylmethyl or phenylethyl.

4. A compound according to claim 1, wherein R^2 is (C_1-C_6)alkyl or 4-methoxybenzyl.

5. A compound according to claim 1, wherein R^3 is methyl.

6. A compound according to claim 1, wherein R^4 is hydrogen, benzyl, 2-chlorobenzyl, 2-fluorobenzyl, 3-fluorobenzyl or 4-fluorobenzyl.

7. A compound according to claim 1, wherein Ar is phenyl or thienyl; R^1 is 2-methylpropyl, trifluoromethylethyl, cyclopropylmethyl, cyclobutylmethyl, phenoxybutyl, cyclohexylmethyl or phenylethyl; R^2 is (C_1-C_6)alkyl or 4-methoxybenzyl; R^3 is methyl and R^4 is hydrogen, benzyl, 2-chlorobenzyl, 2-fluorobenzyl, 3-fluorobenzyl or 4-fluorobenzyl.

8. A compound according to claim 1, wherein said compound is selected from the group consisting of:

(4-Benzylbenzyl)-[2-(2,2-dimethyl-1-methylcarbamoyl-propylcarbamoyl)-4-methyl-pentyl]-phosphinic acid;

(4-Benzylbenzyl-[2-(2,2-dimethyl-1-methylcarbamoyl-propylcarbamoyl)-5,5,5-trifluoropentyl]-phosphinic acid;

[2-(2,2-Dimethyl-1-methylcarbamoyl-propylcarbamoyl)-4-methylpentyl]-[4-(3-fluorobenzyl)-benzyl]-phosphinic acid;

Benzyl-{2-[2-(4-methoxyphenyl)-1-methylcarbamoyl-ethylcarbamoyl]-6-phenoxy-hexyl}-phosphinic acid;

(4-Benzylbenzyl)-{2-[2-(4-methoxyphenyl)-1-methylcarbamoyl-ethylcarbamoyl]-6-phenoxyhexyl}-phosphinic acid;

(4-Benzylbenzyl)-{3-cyclohexyl-2-[2-(4-methoxyphenyl)-1-methylcarbamoyl-ethylcarbamoyl]-propyl}-phosphinic acid;

(4-Benzylbenzyl)-[3-cyclohexyl-2-(2,2-dimethyl-1-methylcarbamoyl-propylcarbamoyl)-propyl]-phosphinic acid;

(4-Benzylbenzyl)-[2-(2,2-dimethyl-1-methylcarbamoyl-propylcarbamoyl)-4-phenyl-butyl]-phosphinic acid;

(4-Cyclohexylmethylbenzyl)-[2-(2,2-dimethyl-1-methylcarbamoyl-propylcarbamoyl)-4-methyl-pentyl]-phosphinic acid;

[2-(2,2-Dimethyl-1-methylcarbamoyl-propylcarbamoyl)-4-methylpentyl]-(4-isobutylbenzyl)-phosphinic acid;

[2-(2,2-Dimethyl-1-methylcarbamoyl-propylcarbamoyl)-4-methylpentyl]-[4-(4-fluoro-benzyl)-benzyl]-phosphinic acid;

[2-(2,2-Dimethyl-1-methylcarbamoyl-propylcarbamoyl)-4-methylpentyl]-[4-(2-fluoro-benzyl)-benzyl]phophinic acid;

(4-Benzylbenzyl)-{2-[2-(4-methoxyphenyl)-1-methylcarbamoyl-ethylcarbamoyl]-4-methyl-pentyl}-phosphinic acid;

[4-(2-Chlorobenzyl)benzyl]-[2-(2,2-dimethyl-1-methylcarbamoyl-1-propylcarbamoyl)-4-methylpentyl]phosphinic acid;

(5-Benzyl-pyridin-2-ylmethyl)-[2-(2,2-dimethyl-1-methylcarbamoyl-propylcarbamoyl)-4-methyl-pentyl]phosphinic acid;

[2-(2,2-Dimethyl-1-methylcarbamoyl-propylcarbamoyl)-5,5,5-trifluoro-pentyl]-[4-(2-fluoro-benzyl)-benzyl]phosphinic acid;

[3-Cyclopropyl-2-(2,2-dimethyl-1-methylcarbamoyl-propylcarbamoyl)-propyl]-[4-(2-fluoro-benzyl)-benzyl]phosphinic acid;

[3-Cyclobutyl-2-(2,2-dimethyl-1-methylcarbamoyl-propylcarbamoyl)-propyl]-[4-(2-fluoro-benzyl)-benzyl]-phosphinic acid; and

(5-Benzyl-thiophen-2-ylmethyl)-[2-(2,2-dimethyl-1-methylcarbamoyl-propylcarbamoyl)-4-methylpentyl]-phosphinic acid.

9. A pharmaceutical composition for (a) the treatment of a condition selected from the group consisting of arthritis, cancer, synergy with cytotoxic anticancer agents, tissue ulceration, mucular degeneration, restenosis, periodontal disease, epidermolysis bullosa, scleritis, in combination with standard NSAID'S and analgesics and other diseases characterized by matrix metalloproteinase activity, AIDS, sepsis, septic shock and other diseases involving the production of tumor necrosis factor (TNF) or (b) the inhibition of matrix metalloproteinases or the production of tumor necrosis factor (TNF) in a mammal, including a human, comprising an amount of a compound of claim 1 effective in such treatment and a pharmaceutically acceptable carrier.

10. A method for the inhibition of (a) matrix metalloproteinases or (b) the production of tumor necrosis factor (TNF) in a mammal, including a human, comprising administering to said mammal an effective amount of a compound of claim 1.

11. A method for treating a condition selected from the group consisting of arthritis, cancer, synergy with cytotoxic anticaner agents, tissue ulceration, macular degeneration, restenosis, periodontal disease, epidermolysis bullosa, scleritis, in combination with standard NSAID'S and analgesics and other diseases characterized by matrix metalloproteinase activity, AIDS, sepsis, septic shock and other diseases involving the production of tumor necrosis factor (TNF) in a mammal, including a human, comprising administering to said mammal an amount of a compound of claim 1, effective in treating such a condition.

INTERNATIONAL SEARCH REPORT

In' ational Application No
PCT/IB 97/00800

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07F9/30 A61K31/66 C07F9/58 C07F9/6553 C07F9/655

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07F A61K

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)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 93 14112 A (MERCK & CO.) 22 July 1993 see the whole document ---	1-11
Y	WO 95 12603 A (SYNTEX INC.) 11 May 1995 see the whole document -----	1-11

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

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

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

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search
15 September 1997

Date of mailing of the international search report
30 -09- 1997

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

Authorized officer
Beslier, L

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

In' tional Application No
PCT/IB 97/00800

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9314112 A	22-07-93	AU 3475393 A	03-08-93
		CA 2126687 A	22-07-93
		EP 0623143 A	09-11-94
		JP 7503016 T	30-03-95
WO 9512603 A	11-05-95	AU 8089794 A	23-05-95
		BR 9407960 A	26-11-96
		CA 2175667 A	11-05-95
		CN 1134153 A	23-10-96
		CZ 9601260 A	13-11-96
		DE 69404324 D	21-08-97
		EP 0726903 A	21-08-96
		FI 961857 A	01-07-96
		HU 74730 A	28-02-97
		NO 961780 A	03-07-96
		NZ 275315 A	27-07-97
		PL 314134 A	19-08-96
		ZA 9408691 A	03-05-96

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C07D 211/58, 211/46, 211/62, 295/18, 211/34, C07C 311/29, A61K 31/445, 31/495, 31/18

A1

(11) International Publication Number: **WO 98/07697**

(43) International Publication Date: 26 February 1998 (26.02.98)

(21) International Application Number: PCT/IB97/00924

(22) International Filing Date: 25 July 1997 (25.07.97)

(30) Priority Data:
60/024,675 23 August 1996 (23.08.96) US

(71) Applicant *(for all designated States except US)*: PFIZER INC. [US/US]; 235 East 42nd Street, New York, NY 10017 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: BLUMENKOPF, Todd, A. [US/US]; 9 Fairway Lane, Old Lyme, CT 06371 (US). ROBINSON, Ralph, P. [US/US]; 30 Friar Tuck Drive, Gales Ferry, CT 06335 (US).

(74) Agents: SPIEGEL, Allen, J. et al.; Pfizer Inc., 235 East 42nd Street, New York, NY 10017 (US).

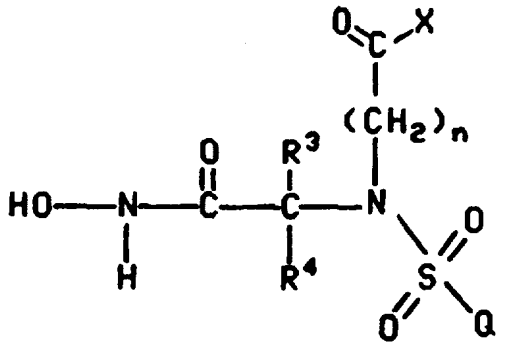
(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: ARYLSULFONYLAMINO HYDROXAMIC ACID DERIVATIVES

(57) Abstract

A compound of formula (I) wherein n, X, R^3, R^4 and Q are as defined above, useful in the treatment of a condition selected from the group consisting of arthritis, cancer, tissue ulceration, macular degeneration, restenosis, periodontal disease, epidermolysis bullosa, scleritis, and other diseases characterized by matrix metalloproteinase activity, AIDS, sepsis, septic shock and other diseases involving the production of TNF. In addition, the compounds of the present invention may be used in combination therapy with standard non-steroidal anti-inflammatory drugs (NSAID'S) and analgesics, and in combination with cytotoxic drugs such as adriamycin, daunomycin, cis-platinum, etoposide, taxol, taxotere and other alkaloids, such as vincristine, in the treatment of cancer.



(I)

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

ARYLSULFONYLAMINO HYDROXAMIC ACID DERIVATIVES

Background of the Invention

The present invention relates to arylsulfonylamino hydroxamic acid derivatives which are inhibitors of matrix metalloproteinases or the production of tumor necrosis factor (TNF) and as such are useful in the treatment of a condition selected from the group consisting of arthritis, cancer, tissue ulceration, restenosis, periodontal disease, epidermolysis bullosa, scleritis and other diseases characterized by matrix metalloproteinase activity, AIDS, sepsis, septic shock and other diseases involving the production of TNF. In addition, the compounds of the present invention may be used in combination therapy with standard non-steroidal anti-inflammatory drugs (hereinafter NSAID'S) and analgesics for the treatment of arthritis, and in combination with cytotoxic drugs such as adriamycin, daunomycin, cis-platinum, etoposide, taxol, taxotere and alkaloids, such as vincristine, in the treatment of cancer.

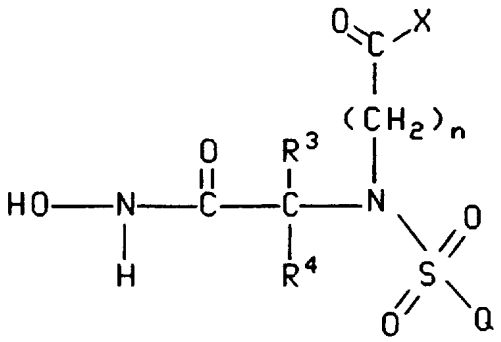
This invention also relates to a method of using such compounds in the treatment of the above diseases in mammals, especially humans, and to pharmaceutical compositions useful therefor.

There are a number of enzymes which effect the breakdown of structural proteins and which are structurally related metalloproteases. Matrix-degrading metalloproteinases, such as gelatinase, stromelysin and collagenase, are involved in tissue matrix degradation (e.g. collagen collapse) and have been implicated in many pathological conditions involving abnormal connective tissue and basement membrane matrix metabolism, such as arthritis (e.g. osteoarthritis and rheumatoid arthritis), tissue ulceration (e.g. corneal, epidermal and gastric ulceration), abnormal wound healing, periodontal disease, bone disease (e.g. Paget's disease and osteoporosis), tumor metastasis or invasion, as well as HIV-infection (J. Leuk. Biol., 52 (2): 244-248, 1992).

Tumor necrosis factor is recognized to be involved in many infectious and auto-immune diseases (W. Fiers, FEBS Letters, 1991, 285, 199). Furthermore, it has been shown that TNF is the prime mediator of the inflammatory response seen in sepsis and septic shock (C.E. Spooner et al., Clinical Immunology and Immunopathology, 1992, 62 S11).

Summary of the Invention

The present invention relates to a compound of the formula

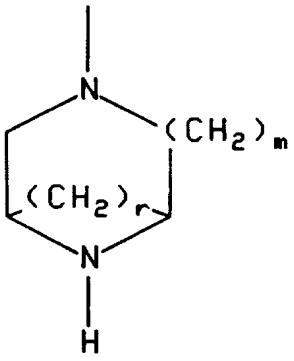


I

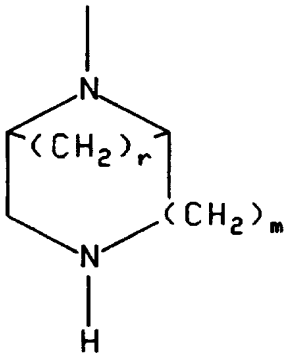
or the pharmaceutically acceptable salts thereof, wherein

n is 1 to 6;

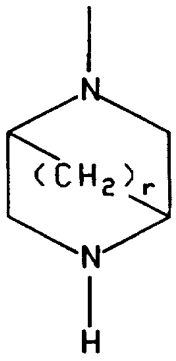
X is OR^1 wherein R^1 is as defined below; azetidinyl, pyrrolidinyl, piperidinyl, morpholinyl, thiomorpholinyl, indolinyl, isoindolinyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, piperazinyl or a bridged diazabicycloalkyl ring selected from the group consisting of



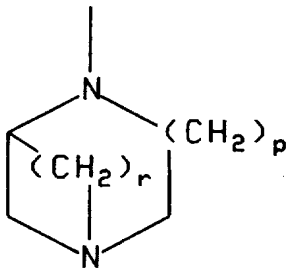
a



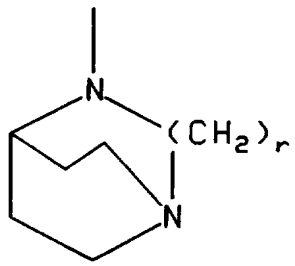
b



c



d



e

wherein r is 1, 2 or 3;

m is 1 or 2; and

p is 0 or 1;

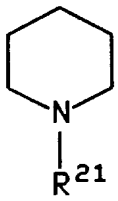
wherein each heterocyclic group may optionally be substituted by one or two groups selected from hydroxy, (C_1-C_6)alkyl, (C_1-C_6)alkoxy, (C_1-C_{10})acyl, (C_1-C_{10})acyloxy, (C_6-C_{10})aryl, (C_5-C_9)heteroaryl, (C_6-C_{10})aryl(C_1-C_6)alkyl, (C_5-C_9)heteroaryl (C_1-C_6)alkyl, hydroxy (C_1-C_6)alkyl, (C_1-C_6)alkoxy (C_1-C_6)alkyl, (C_1-C_6)acyloxy(C_1-C_6)alkyl, (C_1-C_6)alkylthio, (C_1-C_6)alkylthio (C_1-C_6)alkyl, (C_6-C_{10})arylthio, (C_6-C_{10}) arylthio(C_1-C_6)alkyl, $R^9R^{10}N$, $R^9R^{10}NSO_2$, $R^9R^{10}NCO$, $R^9R^{10}NCO(C_1$-$C_6)$alkyl wherein R^9 and R^{10} are each independently hydrogen, (C_1-C_6)alkyl, (C_6-C_{10})aryl, (C_5-C_9)heteroaryl, (C_6-C_{10})aryl (C_1-C_6)alkyl or (C_5-C_9)heteroaryl (C_1-C_6)alkyl or R^9 and R^{10} may be taken together with the nitrogen to which they are attached to form an azetidinyl, pyrrolidinyl, piperidinyl, morpholinyl or thiomorpolinyl ring; $R^{12}SO_2$, $R^{12}SO_2NH$ wherein R^{12} is trifluoromethyl, (C_1-C_6)alkyl, (C_6-C_{10})aryl, (C_5-C_9)heteroaryl, (C_6-C_{10})aryl(C_1-C_6)alkyl or (C_5-C_9)heteroaryl (C_1-C_6)alkyl; $R^{13}CONR^9$ wherein R^9 is as defined above and R^{13} is hydrogen, (C_1-C_6)alkyl, (C_1-C_6)alkoxy, (C_6-C_{10})aryl, (C_5-C_9)heteroaryl, (C_1-C_6)aryl(C_1-C_6)alkyl(C_6-C_{10})aryl(C_1-C_6)alkoxy or (C_5-C_9)heteroaryl(C_1-C_6)alkyl; $R^{14}OOC$, $R^{14}OOC(C_1$-$C_6)$alkyl wherein R^{14} is (C_1-C_6)alkyl, (C_6-C_{10})aryl, (C_5-C_9)heteroaryl, (C_6-C_{10})aryl (C_1-C_6)alkyl, 5-indanyl, CHR^5OCOR^6 wherein R^5 is hydrogen or (C_1-C_6)alkyl and R^6 is (C_1-C_6)alkyl, (C_1-C_6)alkoxy or (C_6-C_{10})aryl; $CH_2CONR^7R^8$ wherein R^7 and R^8 are each independently hydrogen or (C_1-C_6)alkyl or may be taken together with the nitrogen to which they are attached to form an azetidinyl, pyrrolidinyl, piperidinyl, morpholinyl or thiomorpholinyl ring; or $R^{15}O$

(C_1C_6)alkyl wherein R^{15} is $H_2N(CHR^{16})CO$ wherein R^{16} is the side chain of a natural D- or L-amino acid;

R^1 is (C_6-C_{10})aryl, (C_5-C_9)heteroaryl, (C_6-C_{10})aryl(C_1-C_6)alkyl, 5-indanyl, CHR^5OCOR^6 or $CH_2CONR^7R^8$ wherein R^5, R^6, R^7 and R^8 are as defined above;

R^3 and R^4 are each independently selected from the group consisting of hydrogen, (C_1-C_6)alkyl, trifluoromethyl, trifluoromethyl(C_1-C_6)alkyl, (C_1-C_6)alkyl (difluoromethylene), (C_1-C_3)alkyl(difluoromethylene)(C_1-C_3)alkyl, (C_6-C_{10})aryl, (C_5-C_9)heteroaryl, (C_6-C_{10})aryl(C_1-C_6)alkyl, (C_5-C_9)heteroaryl(C_1-C_6)alkyl, (C_6-C_{10})aryl(C_6-C_{10})aryl, (C_6-C_{10})aryl(C_6-C_{10})aryl(C_1-C_6)alkyl, (C_3-C_6)cycloalkyl, (C_3-C_6)cycloalkyl(C_1-C_6)alkyl, hydroxy(C_1-C_6)alkyl, (C_1-C_{10})acyloxy(C_1-C_6)alkyl, (C_1-C_6)alkoxy(C_1-C_6)alkyl, (C_1-C_{10})acylamino(C_1-C_6)alkyl, piperidyl, (C_1-C_6)alkylpiperidyl, (C_6-C_{10})aryl(C_1-C_6)alkoxy(C_1-C_6)alkyl, (C_5-C_9)heteroaryl(C_1-C_6)alkoxy(C_1-C_6)alkyl, (C_1-C_6)alkylthio(C_1-C_6)alkyl, (C_6-C_{10})arylthio(C_1-C_6)alkyl, (C_1-C_6)alkylsulfinyl(C_1-C_6)alkyl, (C_6-C_{10})arylsulfinyl(C_1-C_6)alkyl, (C_1-C_6)alkylsulfonyl(C_1-C_6)alkyl, (C_6-C_{10})arylsulfonyl(C_1-C_6)alkyl, amino(C_1-C_6)alkyl, (C_1-C_6)alkylamino(C_1-C_6)alkyl, ((C_1-C_6)alkylamino)$_2$(C_1-C_6)alkyl, $R^{17}CO(C_1$-C_6)alkyl wherein R^{17} is $R^{14}O$ or R^7R^8N wherein R^7, R^8 and R^{14} are as defined above; or $R^{18}(C_1$-C_6)alkyl wherein R^{18} is piperazinyl, (C_1-C_{10})acylpiperazinyl, (C_6-C_{10})arylpiperazinyl, (C_5-C_9)heteroarylpiperazinyl, (C_1-C_6)alkylpiperazinyl, (C_6-C_{10})aryl(C_1-C_6)alkylpiperazinyl, (C_5-C_9)heteroaryl(C_1-C_6)alkylpiperazinyl, morpholinyl, thiomorpholinyl, piperidinyl, pyrrolidinyl, piperidyl, (C_1-C_6)alkylpiperidyl, (C_6-C_{10})arylpiperidyl, (C_5-C_9)heteroarylpiperidyl, (C_6-C_{10})aryl(C_1-C_6)alkylpiperidyl, (C_5-C_9)heteroaryl(C_1-C_6)alkylpiperidyl or (C_1-C_{10})acylpiperidyl;

or R^3 and R^4 may be taken together to form a (C_3-C_6)cycloalkyl, oxacyclohexyl, thiocyclohexyl, indanyl or tetralinyl ring or a group of the formula



wherein R^{21} is hydrogen, (C_1-C_{10})acyl, (C_1-C_6)alkyl, (C_6-C_{10})aryl(C_1-C_6)alkyl, (C_5-C_9)heteroaryl(C_1-C_6)alkyl or (C_1-C_6)alkylsulfonyl; and

Qis (C_1-C_6)alkyl, (C_6-C_{10})aryl, (C_6-C_{10})aryloxy(C_6-C_{10})aryl, (C_6-C_{10})aryl(C_6-C_{10})aryl, (C_6-C_{10})aryl(C_6-C_{10})aryl(C_1-C_6)alkyl, (C_6-C_{10})aryloxy(C_5-C_9)heteroaryl, (C_5-C_9)heteroaryl, (C_1-C_6)alkyl(C_6-C_{10})aryl, (C_1-C_6)alkoxy(C_6-C_{10})aryl, (C_6-C_{10})aryl(C_1-C_6)alkoxy(C_6-C_{10})aryl, (C_6-C_{10})aryl(C_1-C_6)alkoxy(C_1-C_6)alkyl, (C_5-C_9)heteroaryloxy(C_6-C_{10})aryl, (C_1-C_6)alkyl(C_5-C_9)heteroaryl, (C_1-C_6)alkoxy(C_5-C_9)heteroaryl, (C_6-C_{10})aryl(C_1-C_6)alkoxy(C_5-C_9)heteroaryl, (C_5-C_9)heteroaryloxy(C_5-C_9)heteroaryl, (C_6-C_{10})aryloxy(C_1-C_6)alkyl, (C_5-C_9)heteroaryloxy(C_1-C_6)alkyl, (C_1-C_6)alkyl(C_6-C_{10})aryloxy(C_6-C_{10})aryl, (C_1-C_6)alkyl(C_5-C_9)heteroaryloxy(C_6-C_{10})aryl, (C_1-C_6)alkyl(C_6-C_{10})aryloxy(C_5-C_9)heteroaryl, (C_1-C_6)alkoxy(C_6-C_{10})aryloxy(C_6-C_{10})aryl, (C_1-C_6)alkoxy(C_5-C_9)heteroaryloxy(C_6-C_{10})aryl or (C_1-C_6)alkoxy(C_6-C_{10})aryloxy(C_5-C_9)heteroaryl wherein each aryl group is optionally substituted by fluoro, chloro, bromo, (C_1-C_6)alkyl, (C_1-C_6)alkoxy or perfluoro(C_1-C_3)alkyl;

with the proviso that X must be substituted when defined as azetidinyl, pyrrolidinyl, morpholinyl, thiomorpholinyl, indolinyl, isoindolinyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, piperazinyl, (C_1-C_{10})acylpiperazinyl, (C_1-C_6)alkylpiperazinyl, (C_6-C_{10})arylpiperazinyl, (C_5-C_9)heteroarylpiperazinyl or a bridged diazabicycloalkyl ring.

The term "alkyl", as used herein, unless otherwise indicated, includes saturated monovalent hydrocarbon radicals having straight, branched or cyclic moieties or combinations thereof.

The term "alkoxy", as used herein, includes O-alkyl groups wherein "alkyl" is defined above.

The term "aryl", as used herein, unless otherwise indicated, includes an organic radical derived from an aromatic hydrocarbon by removal of one hydrogen, such as phenyl or naphthyl, optionally substituted by 1 to 3 substituents selected from the group consisting of fluoro, chloro, trifluoromethyl, (C_1-C_6)alkoxy, (C_6-C_{10})aryloxy, trifluoromethoxy, difluoromethoxy and (C_1-C_6)alkyl.

The term "heteroaryl", as used herein, unless otherwise indicated, includes an organic radical derived from an aromatic heterocyclic compound by removal of one hydrogen, such as pyridyl, furyl, pyroyl, thienyl, isothiazolyl, imidazolyl, benzimidazolyl, tetrazolyl, pyrazinyl, pyrimidyl, quinolyl, isoquinolyl, benzofuryl, isobenzofuryl, benzothienyl, pyrazolyl, indolyl, isoindolyl, purinyl, carbazolyl, isoxazolyl, thiazolyl, oxazolyl, benzthiazolyl or benzoxazolyl, optionally substituted by 1 to 2 substituents selected from the group consisting of fluoro, chloro, trifluoromethyl, (C_1-C_6)alkoxy, (C_6-C_{10})aryloxy, trifluoromethoxy, difluoromethoxy and (C_1-C_6)alkyl.

The term "acyl", as used herein, unless otherwise indicated, includes a radical of the general formula RCO wherein R is alkyl, alkoxy, aryl, arylalkyl or arylalkyloxy and the terms "alkyl" or "aryl" are as defined above.

The term "acyloxy", as used herein, includes O-acyl groups wherein "acyl" is defined above.

The term "D- or L-amino acid", as used herein, unless otherwise indicated, includes glycine, alanine, valine, leucine, isoleucine, phenylalanine, asparagine, glutamine, tryptophan, proline, serine, threonine, tyrosine, hydroxyproline, cysteine, cystine, methionine, aspartic acid, glutamic acid, lysine, arginine or histidine.

The compound of formula I may have chiral centers and therefore exist in different enantiomeric forms. This invention relates to all optical isomers and stereoisomers of the compounds of formula I and mixtures thereof.

Preferred compounds of formula I include those wherein n is 2.

Other preferred compounds of formula I include those wherein either R^3 or R^4 is not hydrogen.

Other preferred compounds of formula I include those wherein Ar is $(C_1$-$C_6)$alkoxy$(C_6$-$C_{10})$aryl,$(C_6$-$C_{10})$aryl$(C_1$-$C_6)$alkoxy$(C_6$-$C_{10})$aryl,4-fluorophenoxy$(C_6$-$C_{10})$aryl, 4-fluorobenzyloxy$(C_6$-$C_{10})$aryl or $(C_1$-$C_6)$alkyl$(C_6$-$C_{10})$aryloxy$(C_6$-$C_{10})$aryl.

Other preferred compounds of formula I include those wherein X is indolinyl or piperidinyl.

More preferred compounds of formula I include those wherein n is 2; either R^3 or R^4 is not hydrogen; Ar is $(C_1$-$C_6)$alkoxy$(C_6$-$C_{10})$aryl, $(C_6$-$C_{10})$aryl$(C_1$-$C_6)$alkoxy$(C_6$-$C_{10})$aryl, 4-fluorophenoxy$(C_6$-$C_{10})$aryl, 4-fluorobenzyloxy$(C_6$-$C_{10})$aryl or $(C_1$-$C_6)$alkyl$(C_6$-$C_{10})$aryloxy$(C_6$-$C_{10})$aryl; and X is indolinyl or piperidinyl.

Specific preferred compounds of formula I include the following:

3-[(Cyclohexylhydroxycarbamoylmethyl)-(4-methoxybenzenesulfonyl)-amino]-propionic acid indan-5-yl ester;

Acetic acid 1-{3-[(1-hydroxycarbamoyl-2-methylpropyl)-(4-methoxy-benzene-sulfonyl)-amino]propionyl}piperidin-4-yl ester;

2-Cyclohexyl-N-hydroxy-2-[[3-(4-hydroxypiperidin-1-yl)-3-oxo-propyl]-(4-methoxy-benzenesulfonyl)amino]acetamide;

Benzoic acid 1-{3-[(1-hydroxycarbamoyl-2-methylpropyl)-(4-methoxy-benzene-sulfonyl)amino]propionyl}piperidin-4-yl ester;

N-Hydroxy-2-[[3-(4-hydroxypiperidin-1-yl)-3-oxopropyl]-(4-methoxybenzenesulfonyl)amino]-3-methylbutyramide;

1-{3-[(Cyclohexylhydroxycarbamoylmethyl)-(4-methoxybenzenesulfonyl)-amino]propionyl}piperidine-4-carboxylic acid;

1-{3-[(Cyclohexylhydroxycarbamoylmethyl)-(4-methoxybenzenesulfonyl)-amino]propionyl}piperidine-4-carboxylic acid ethyl ester;

2-Cyclohexyl-N-hydroxy-2-{(4-methoxybenzenesulfonyl)-[3-(4-methylaminopiperidin-1-yl)-3-oxopropyl]amino}acetamide;

3-(4-Chlorophenyl)-N-hydroxy-2-{(4-methoxybenzenesulfonyl)-[3-(4-methylaminopiperidin-1-yl)-3-oxopropyl]amino}propionamide;

3-Cyclohexyl-N-hydroxy-2-{(4-methoxybenzenesulfonyl)-[3-(4-methylaminopiperidin-1-yl)-3-oxopropyl]amino}propionamide;

N-Hydroxy-2-[{3-[4-(2-hydroxy-2-methylpropyl)piperazin-1-yl]-3-oxopropyl}-(4-methoxy-benzenesulfonyl)amino]-3-methylbutyramide;

2,2-Dimethylpropionic acid 2-(4-{3-[(1-hydroxycarbamoyl-2-methylpropyl)-(4-methoxy-benzenesulfonyl)amino]propionyl}piperazin-1-yl)ethyl ester; and

Benzoicacid2-(4-{3-[(1-hydroxycarbamoyl-2-methylpropyl)-(4-methoxybenzenesulfonyl)-amino]propionyl}piperazin-1-yl)-ethyl ester.

Other specific compounds of formula I include the following:

2-Cyclohexyl-N-hydroxy-2-[{3-[4-(2-hydroxyethyl)piperazin-1-yl]-3-oxopropyl}-(4-methoxybenzenesulfonyl)amino]acetamide;

N-Hydroxy-2-[{3-[5-(2-hydroxyethyl)-2,5-diazabicyclo[2.2.1]-hept-2-yl]-3-oxopropyl}-(4-methoxybenzenesulfonyl)amino]-3-methylbutyramide;

2-{(4-Benzyloxybenzenesulfonyl)-[3-(4-hydroxypiperidin-1-yl)-3-oxopropyl]amino}-N-hydroxy-3-methylbutyramide;

2-Cyclohexyl-2-{[4-(4-fluorophenoxy)benzenesulfonyl]-[3-(4-hydroxypiperidin-1-yl)-3-oxopropyl]-amino}-N-hydroxyacetamide;

2-{[4-(4-Butylphenoxy)benzenesulfonyl]-[3-(4-hydroxypiperidin-1-yl)-3-oxopropyl]-amino}-N-hydroxy-3-methylbutyramide;

1-{(4-Methoxybenzenesulfonyl)-[3-(4-methylaminopiperidin-1-yl)-3-oxopropyl]amino}-cyclopentanecarboxylic acid hydroxyamide;

4-{3-[(1-Hydroxycarbamoyl-2-methylpropyl)-(4-methoxybenzenesulfonyl)amino]-propionyl}piperazine-2-carboxylic acid ethyl ester;

3-[(Cyclohexylhydroxycarbamoylmethyl)-(4-methoxybenzene-sulfonyl)amino]propionic acid ethoxycarbonyloxymethyl ester;

3-[(1-Hydroxycarbamoylpentyl)-(4-methoxybenzenesulfonyl)amino]propionic acid ethoxycarbonyloxymethyl ester;

3-[[4-(4-Fluorobenzyloxy)-benzenesulfonyl]-(1-hydroxy-carbamoyl-2-methyl-propyl)-amino]-propionic acid ethoxycarbonyloxymethyl ester; and

3-[[4-(4-Fluorophenoxy)-benzenesulfonyl]-(1-hydroxycarbamoyl-2-methyl-propyl)-amino]-propionic acid ethoxycarbonyloxymethyl ester.

The present invention also relates to a pharmaceutical composition for (a) the treatment of a condition selected from the group consisting of arthritis, cancer, synergy with cytotoxic anticancer agents, tissue ulceration, macular degeneration, restenosis, periodontal disease, epidermolysis bullosa, scleritis, in combination with standard NSAID'S and analgesics and other diseases characterized by matrix metalloproteinase activity, AIDS, sepsis, septic shock and other diseases involving the production of tumor necrosis factor (TNF) or (b) the inhibition of matrix metalloproteinases or the production of tumor necrosis factor (TNF) in a mammal, including a human, comprising an amount of a compound of formula I or a pharmaceutically acceptable salt thereof effective in such treatments and a pharmaceutically acceptable carrier.

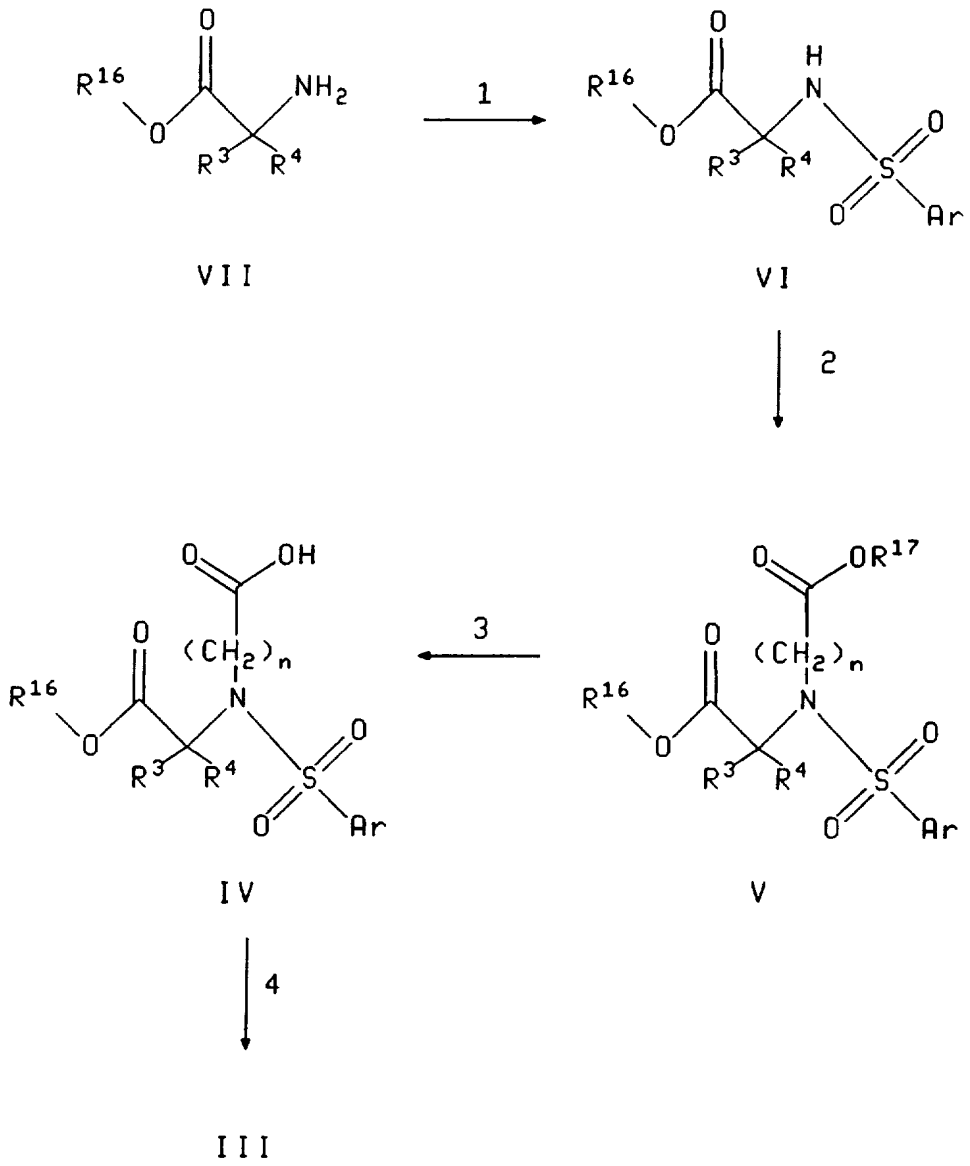
The present invention also relates to a method for the inhibition of (a) matrix metalloproteinases or (b) the production of tumor necrosis factor (TNF) in a mammal, including a human, comprising administering to said mammal an effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof.

The present invention also relates to a method for treating a condition selected from the group consisting of arthritis, cancer, tissue ulceration, macular degeneration, restenosis, periodontal disease, epidermolysis bullosa, scleritis, compounds of formula I may be used in combination with standard NSAID'S and analgesics and in combination with cytotoxic anticancer agents, and other diseases characterized by matrix metalloproteinase activity, AIDS, sepsis, septic shock and other diseases involving the production of tumor necrosis factor (TNF) in a mammal, including a human, comprising administering to said mammal an amount of a compound of formula I or a pharmaceutically acceptable salt thereof effective in treating such a condition.

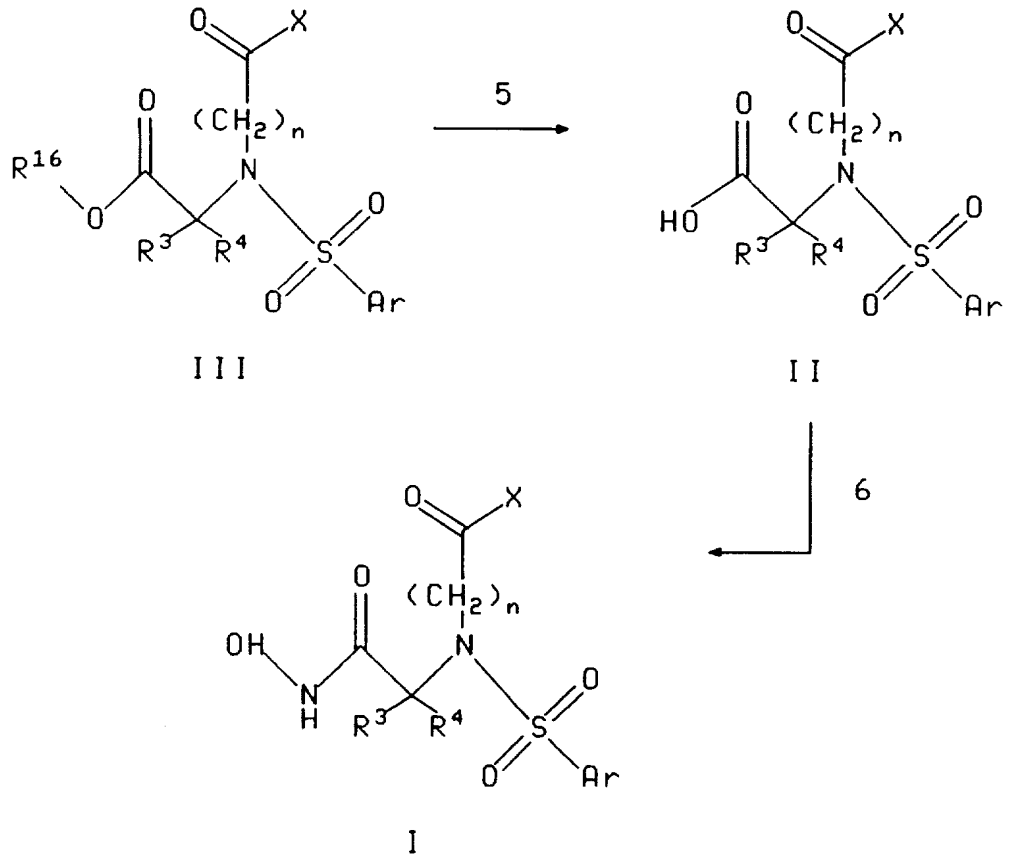
Detailed Description of the Invention

The following reaction Schemes illustrate the preparation of the compounds of the present invention. Unless otherwise indicated n, R^3, R^4, X and Ar in the reaction Schemes and the discussion that follow are defined as above.

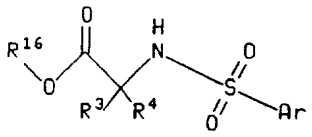
Scheme 1



Scheme 1 cont'd

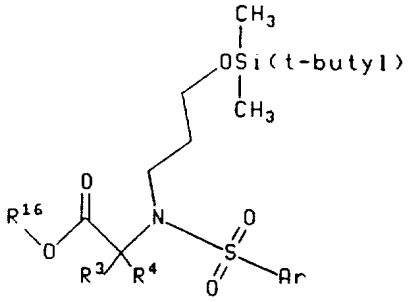


Scheme 2



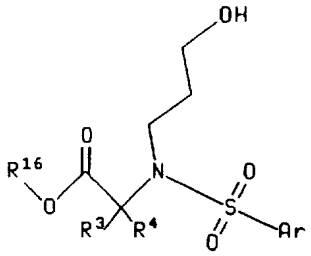
VI

1



VIII

2

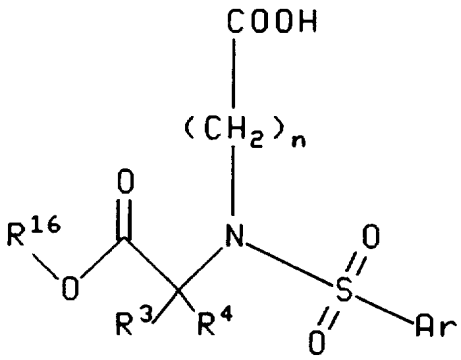


IX

3

IV

Scheme 2 (continued)



IV

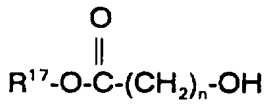
↓

↓

I

In reaction 1 of Scheme 1, the amino acid compound of formula **VII**, wherein R^{16} is $(C_1\text{-}C_6)$alkyl, benzyl, allyl or tert-butyl, is converted to the corresponding compound of formula **VI** by reacting **VII** with a reactive functional derivative of an arylsulfonic acid compound, such as an arylsulfonyl chloride, in the presence of a base, such as triethylamine, and a polar solvent, such as tetrahydrofuran, dioxane, water or acetonitrile, preferably a mixture of dioxane and water. The reaction mixture is stirred, at room temperature, for a time period between about 10 minutes to about 24 hours, preferably about 60 minutes.

In reaction 2 of Scheme 1, the arylsulfonyl amino compound of formula **VI**, wherein R^{16} is $(C_1\text{-}C_6)$alkyl, benzyl, allyl or tert-butyl, is converted to the corresponding compound of formula **V**, wherein n is 1, 3, 4, 5 or 6, by reacting **VI** with a reactive derivative of an alcohol of the formula



such as the chloride, bromide or iodide derivative, preferably the iodide derivative, wherein the R^{17} protecting group is $(C_1\text{-}C_6)$alkyl, benzyl, allyl or tert-butyl, in the presence of a base such as potassium carbonate or sodium hydride, preferably sodium hydride, and a polar solvent, such as dimethylformamide. The reaction mixture is stirred, at room temperature, for a time period between about 60 minutes to about 48 hours, preferably about 18 hours. The R^{17} protecting group is chosen such that it may be selectively removed in the presence of and without loss of the R^{16} protecting group, therefore, R^{17} cannot be the same as R^{16}. Removal of the R^{17} protecting group from the compound of formula **V** to give the corresponding carboxylic acid of formula **IV**, in reaction 3 of Scheme 1, is carried out under conditions appropriate for that particular R^{17} protecting group in use which will not affect the R^{16} protecting group. Such conditions include; (a) saponification where R^{17} is $(C_1\text{-}C_6)$alkyl and R^{16} is tert-butyl, (b) hydrogenolysis where R^{17} is benzyl and R^{16} is tert-butyl or $(C_1\text{-}C_6)$alkyl, (c) treatment with a strong acid, such as trifluoroacetic acid or hydrochloric acid where R^{17} is tert-butyl and R^{16} is $(C_1\text{-}C_6)$alkyl, benzyl or allyl, or (d) treatment with tributyltinhydride and acetic acid in the presence of catalytic bis(triphenylphosphine) palladium (II) chloride where R^{17} is allyl and R^{16} is $(C_1\text{-}C_6)$alkyl, benzyl or tert-butyl.

In reaction 4 of Scheme 1, the carboxylic acid of formula **IV** is condensed with a compound of the formula HX or the salt thereof, wherein X is as defined above, to

give the corresponding amide compound of formula III. The formation of amides from primary or secondary amines or ammonia and carboxylic acids is achieved by conversion of the carboxylic acid to an activated functional derivative which subsequently undergoes reaction with a primary or secondary amine or ammonia to form the amide. The activated functional derivative may be isolated prior to reaction with the primary or secondary amine or ammonia. Alternatively, the carboxylic acid may be treated with oxalyl chloride or thionyl chloride, neat or in an inert solvent, such as chloroform, at a temperature between about 25°C to about 80°C, preferably about 50°C, to give the corresponding acid chloride functional derivative. The inert solvent and any remaining oxalyl chloride or thionyl chloride is then removed by evaporation under vacuum. The remaining acid chloride functional derivative is then reacted with the primary or secondary amine or ammonia in an inert solvent, such as methylene chloride, to form the amide. The preferred method for the condensation of the carboxylic acid of formula IV with a compound of the formula HX, wherein X is as defined above, to provide the corresponding compound of formula III is the treatment of IV with (benzotriazol-1-yloxy)tris(dimethylamino) phosphonium hexafluorophosphate in the presence of a base, such as triethylamine, to provide the benzotriazol-1-oxy ester in situ which, in turn, reacts with the compound of the formula HX, in an inert solvent, such as methylene chloride, at room temperature to give the compound of formula III.

Removal of the R^{16} protecting group from the compound of formula III to give the corresponding carboxylic acid of formula II, in reaction 5 of Scheme 1, is carried out under conditions appropriate for the particular R^{16} protecting group in use. Such conditions include; (a) saponification where R^{16} is lower alkyl, (b) hydrogenolysis where R^{16} is benzyl, (c) treatment with a strong acid, such as trifluoroacetic acid or hydrochloric acid, where R^{16} is tert-butyl, or (d) treatment with tributyltinhydride and acetic acid in the presence of catalytic bis(triphenylphosphine) palladium (II) chloride where R^{16} is allyl.

In reaction 6 of Scheme 1, the carboxylic acid compound of formula II is converted to the hydroxamic acid compound of formula I by treating II with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide and 1-hydroxybenztriazole in a polar solvent, such as dimethylformamide, followed by the addition of hydroxylamine to the reaction mixture after a time period between about 15 minutes to about 1 hour, preferably about 30 minutes. The hydroxylamine is preferably generated in situ from a salt form, such

as hydroxylamine hydrochloride, in the presence of a base, such as N-methylmorpholine. Alternatively, a protected derivative of hydroxylamine or its salt form, where the hydroxyl group is protected as a tert-butyl, benzyl, allyl or trimethylsilylether, may be used in the presence of (benzotriazol-1-yloxy)tris-(dimethylamino) phosphonium hexafluorophosphate and a base, such as N-methylmorpholine. Removal of the hydroxylamine protecting group is carried out by hydrogenolysis for a benzyl protecting group or treatment with a strong acid, such as trifluoroacetic acid, for a tert-butyl protecting group. The allyl protecting group may be removed by treatment with tributyltinhydride and acetic acid in the presence of catalytic bis(triphenylphosphine) palladium (II) chloride. The 2-trimethylsilylethyl ether may be removed by reaction with a strong acid, such as trifluoroacetic acid or by reaction with a fluoride source such as boron trifluoride etherate. N,O-bis(4-methoxybenzyl)hydroxyl-amine may also be used as the protected hydroxylamine derivative where deprotection is achieved using a mixture of methanesulfonic acid and trifluoroacetic acid.

In reaction 1 of Scheme 2, the arylsulfonylamino compound of formula **VI**, wherein R^{16} is $(C_1\text{-}C_6)$alkyl, benzyl or tert-butyl, is converted to the corresponding compound of formula **VIII** by reacting **VI** with a reactive functional derivative, such as the halide, preferably the iodide derivative, of 3-(tert-butyldimethylsilyloxy)-1-propanol in the presence of a base, such as sodium hydride. The reaction is stirred in a polar solvent, such as dimethylformamide, at room temperature, for a time period between about 2 hours to about 48 hours, preferably about 18 hours.

In reaction 2 of Scheme 2, the compound of formula **VIII** is converted to the alcohol compound of formula **IX** by treatment of **VIII** with an excess of an acid, such as acetic acid, or an excess of a Lewis acid, such as boron trifluoride etherate. When using an acid, such as acetic acid, water is added and a water-soluble cosolvent, such as tetrahydrofuran, can be added to promote solubility. The reaction is stirred for a time period between about 18 hours to about 72 hours, preferably about 24 hours, at a temperature between about room temperature to about 60°C, preferably about 50°C. When using a Lewis acid, such as boron trifluoride etherate, the reaction is stirred in a solvent, such as methylene chloride, for a time period between about 10 minutes to about 6 hours, preferably about 20 minutes, at a temperature between about -20°C to about room temperature, preferably about room temperature.

In reaction 3 of Scheme 2, the alcohol compound of formula **IX** is oxidized to the carboxylic acid compound of formula **IV**, wherein n is 2, by reacting **IX** with an excess of sodium periodate and a catalytic amount of ruthenium trichloride in a solvent mixture consisting of acetonitrile, water and carbon tetrachloride, at room temperature, for a time period between about 1 hour to about 24 hours, preferably about 4 hours.

The compound of formula **IV**, wherein n is 2, is further reacted to provide the hydroxamic acid compound of formula **I**, wherein n is 2, according to the procedure described above in reactions 4, 5 and 6 of Scheme 1.

Pharmaceutically acceptable salts of the acidic compounds of the invention are salts formed with bases, namely cationic salts such as alkali and alkaline earth metal salts, such as sodium, lithium, potassium, calcium, magnesium, as well as ammonium salts, such as ammonium, trimethyl-ammonium, diethylammonium, and tris-(hydroxymethyl)-methylammonium slats.

Similarly acid addition salts, such as of mineral acids, organic carboxylic and organic sulfonic acids e.g. hydrochloric acid, methanesulfonic acid, maleic acid, are also possible provided a basic group, such as pyridyl, constitutes part of the structure.

The ability of the compounds of formula **I** or their pharmaceutically acceptable salts (hereinafter also referred to as the compounds of the present invention) to inhibit matrix metalloproteinases or the production of tumor necrosis factor (TNF) and, consequently, demonstrate their effectiveness for treating diseases characterized by matrix metalloproteinase or the production of tumor necrosis factor is shown by the following in vitro assay tests.

Biological Assay

Inhibition of Human Collagenase (MMP-1)

Human recombinant collagenase is activated with trypsin using the following ratio: 10 μg trypsin per 100 μg of collagenase. The trypsin and collagenase are incubated at room temperature for 10 minutes then a five fold excess (50 μg/10 μg trypsin) of soybean trypsin inhibitor is added.

10 mM stock solutions of inhibitors are made up in dimethyl sulfoxide and then diluted using the following Scheme:

10 mM ------> 120 μM ------> 12 μM ------> 1.2 μM ------> 0.12 μM

Twenty-five microliters of each concentration is then added in triplicate to appropriate wells of a 96 well microfluor plate. The final concentration of inhibitor will

be a 1:4 dilution after addition of enzyme and substrate. Positive controls (enzyme, no inhibitor) are set up in wells D1-D6 and blanks (no enzyme, no inhibitors) are set in wells D7-D12.

Collagenase is diluted to 400 ng/ml and 25 μl is then added to appropriate wells of the microfluor plate. Final concentration of collagenase in the assay is 100 ng/ml.

Substrate (DNP-Pro-Cha-Gly-Cys(Me)-His-Ala-Lys(NMA)-NH_2) is made as a 5 mM stock in dimethyl sulfoxide and then diluted to 20 μM in assay buffer. The assay is initiated by the addition of 50 μl substrate per well of the microfluor plate to give a final concentration of 10 μM.

Fluorescence readings (360 nM excitation, 460 nm emission) were taken at time 0 and then at 20 minute intervals. The assay is conducted at room temperature with a typical assay time of 3 hours.

Fluorescence vs time is then plotted for both the blank and collagenase containing samples (data from triplicate determinations is averaged). A time point that provides a good signal (the blank) and that is on a linear part of the curve (usually around 120 minutes) is chosen to determine IC_{50} values. The zero time is used as a blank for each compound at each concentration and these values are subtracted from the 120 minute data. Data is plotted as inhibitor concentration vs % control (inhibitor fluorescence divided by fluorescence of collagenase alone x 100). IC_{50}'s are determined from the concentration of inhibitor that gives a signal that is 50% of the control.

If IC_{50}'s are reported to be <0.03 μM then the inhibitors are assayed at concentrations of 0.3 μM, 0.03 μM, 0.03 μM and 0.003 μM.

<u>Inhibition of Gelatinase (MMP-2)</u>

Inhibition of gelatinase activity is assayed using the Dnp-Pro-Cha-Gly-Cys(Me)-His-Ala-Lys(NMA)-NH_2 substrate (10 μM) under the same conditions as inhibition of *human collagenase* (MMP-1).

72kD gelatinase is activated with 1 mM APMA (p-aminophenyl mercuric acetate) for 15 hours at 4°C and is diluted to give a final concentration in the assay of 100 mg/ml. Inhibitors are diluted as for inhibition of human collagenase (MMP-1) to give final concentrations in the assay of 30 μM, 3 μM, 0.3 μM and 0.03 μM. Each concentration is done in triplicate.

Fluorescence readings (360 nm excitation, 460 emission) are taken at time zero and then at 20 minutes intervals for 4 hours.

IC_{50}'s are determined as per inhibition of human collagenase (MMP-1). If IC_{50}'s are reported to be less than 0.03 μM, then the inhibitors are assayed at final concentrations of 0.3 μM, 0.03 μM, 0.003 μM and 0.003 μM.

Inhibition of Stromelysin Activity (MMP-3)

Inhibition of stromelysin activity is based on a modified spectrophotometric assay described by Weingarten and Feder (Weingarten, H. and Feder, J., Spectrophotometric Assay for Vertebrate Collagenase, Anal. Biochem. 147, 437-440 (1985)). Hydrolysis of the thio peptolide substrate [Ac-Pro-Leu-Gly-$SCH[CH_2CH(CH_3)_2]CO$-Leu-Gly-OC_2H_5] yields a mercaptan fragment that can be monitored in the presence of Ellman's reagent.

Human recombinant prostromelysin is activated with trypsin using a ratio of 1 μl of a 10 mg/ml trypsin stock per 26 μg of stromelysin. The trypsin and stromelysin are incubated at 37°C for 15 minutes followed by 10 μl of 10 mg/ml soybean trypsin inhibitor for 10 minutes at 37°C for 10 minutes at 37°C to quench trypsin activity.

Assays are conducted in a total volume of 250 μl of assay buffer (200 mM sodium chloride, 50 mM MES, and 10 mM calcium chloride, pH 6.0) in 96-well microliter plates. Activated stromelysin is diluted in assay buffer to 25 μg/ml. Ellman's reagent (3-Carboxy-4-nitrophenyl disulfide) is made as a 1M stock in dimethyl formamide and diluted to 5 mM in assay buffer with 50 μl per well yielding at 1 mM final concentration.

10 mM stock solutions of inhibitors are made in dimethyl sulfoxide and diluted serially in assay buffer such that addition of 50 μL to the appropriate wells yields final concentrations of 3 μM, 0.3 μM, 0.003 μM, and 0.0003 μM. All conditions are completed in triplicate.

A 300 mM dimethyl sulfoxide stock solution of the peptide substrate is diluted to 15 mM in assay buffer and the assay is initiated by addition of 50 μl to each well to give a final concentration of 3 mM substrate. Blanks consist of the peptide substrate and Ellman's reagent without the enzyme. Product formation was monitored at 405 nm with a Molecular Devices UVmax plate reader.

IC_{50} values were determined in the same manner as for collagenase.

Inhibition of MMP-13

Human recombinant MMP-13 is activated with 2mM APMA (p-aminophenyl mercuric acetate) for 1.5 hours, at 37°C and is diluted to 400 mg/ml in assay buffer (50 mM Tris, pH 7.5, 200 mM sodium chloride, 5mM calcium chloride, 20μM zinc chloride, 0.02% brij). Twenty-five microliters of diluted enzyme is added per well of a 96 well microfluor plate. The enzyme is then diluted in a 1:4 ratio in the assay by the addition of inhibitor and substrate to give a final concentration in the assay of 100 mg/ml.

10 mM stock solutions of inhibitors are made up in dimethyl sulfoxide and then diluted in assay buffer as per the inhibitor dilution scheme for inhibition of human collagenase (MMP-1): Twenty-five microliters of each concentration is added in triplicate to the microfluor plate. The final concentrations in the assay are 30 μM, 3μM, 0.3 μM, and 0.03 μM.

Substrate (Dnp-Pro-Cha-Gly-Cys(Me)-His-Ala-Lys(NMA)-NH_2) is prepared as for inhibition of human collagenase (MMP-1) and 50 μl is added to each well to give a final assay concentration of 10 μM. Fluorescence readings (360 nM excitation; 450 emission) are taken at time 0 and every 5 minutes for 1 hour.

Positive controls consist of enzyme and substrate with no inhibitor and blanks consist of substrate only.

IC_{50}'s are determined as per inhibition of human collagenase (MMP-1). If IC_{50}'s are reported to be less than 0.03 μM, inhibitors are then assayed at final concentrations of 0.3 μM, 0.03 μM, 0.003 μM and 0.0003 μM.

Inhibition of TNF Production

The ability of the compounds or the pharmaceutically acceptable salts thereof to inhibit the production of TNF and, consequently, demonstrate their effectiveness for treating diseases involving the production of TNF is shown by the following in vitro assay:

Human mononuclear cells were isolated from anti-coagulated human blood using a one-step Ficoll-hypaque separation technique. (2) The mononuclear cells were washed three times in Hanks balanced salt solution (HBSS) with divalent cations and resuspended to a density of 2 x 10^6 /ml in HBSS containing 1% BSA. Differential counts determined using the Abbott Cell Dyn 3500 analyzer indicated that monocytes ranged from 17 to 24% of the total cells in these preparations.

180μ of the cell suspension was aliquoted into flate bottom 96 well plates (Costar). Additions of compounds and LPS (100ng/ml final concentration) gave a final volume of 200μl. All conditions were performed in triplicate. After a four hour incubation at 37°C in an humidified CO_2 incubator, plates were removed and centrifuged (10 minutes at approximately 250 x g) and the supernatants removed and assayed for TNFα using the R&D ELISA Kit.

For administration to mammals, including humans, for the inhibition of matrix metalloproteinases or the production of tumor necrosis factor (TNF), a variety of conventional routes may be used including orally, parenterally and topically. In general, the active compound will be administered orally or parenterally at dosages between about 0.1 and 25 mg/kg body weight of the subject to be treated per day, preferably from about 0.3 to 5 mg/kg. However, some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject.

The compounds of the present invention can be administered in a wide variety of different dosage forms, in general, the therapeutically effective compounds of this invention are present in such dosage forms at concentration levels ranging from about 5.0% to about 70% by weight.

For oral administration, tablets containing various excipients such as microcrystalline cellulose, sodium citrate, calcium carbonate, dicalcium phosphate and glycine may be employed along with various disintegrants such as starch (and preferably corn, potato or tapioca starch), alginic acid and certain complex silicates, together with granulation binders like polyvinylpyrrolidone, sucrose, gelation and acacia. Additionally, lubricating agents such as magnesium stearate, sodium lauryl sulfate and talc are often very useful for tabletting purposes. Solid compositions of a similar type may also be employed as fillers in gelatin capsules; preferred materials in this connection also include lactose or milk sugar as well as high molecular weight polyethylene glycols. When aqueous suspensions and/or elixirs are desired for oral administration, the active ingredient may be combined with various sweetening or flavoring agents, coloring matter or dyes, and, if so desired, emulsifying and/or suspending agents as well, together with such diluents as water, ethanol, propylene glycol, glycerin and various like combinations thereof. In the case of animals, they are

advantageously contained in an animal feed or drinking water in a concentration of 5-5000 ppm, preferably 25 to 500 ppm.

For parenteral administration (intramuscular, intraperitoneal, subcutaneous and intravenous use) a sterile injectable solution of the active ingredient is usually prepared. Solutions of a therapeutic compound of the present invention in either sesame or peanut oil or in aqueous propylene glycol may be employed. The aqueous solutions should be suitably adjusted and buffered, preferably at a pH of greater than 8, if necessary and the liquid diluent first rendered isotonic. These aqueous solutions are suitable intravenous injection purposes. The oily solutions are suitable for intraarticular, intramuscular and subcutaneous injection purposes. The preparation of all these solutions under sterile conditions is readily accomplished by standard pharmaceutical techniques well known to those skilled in the art. In the case of animals, compounds can be administered intramuscularly or subcutaneously at dosage levels of about 0.1 to 50 mg/kg/day, advantageously 0.2 to 10 mg/kg/day given in a single dose or up to 3 divided doses.

The present invention is illustrated by the following examples, but it is not limited to the details thereof.

EXAMPLE 1

2-Cyclohexyl-N-hydroxy-2-{(4-methoxybenzenesulfonyl)-[3-(4-methyl-aminopiperidin-1-yl)-3-oxopropyl]amino}acetamide

(A) To a solution of D-cyclohexylgycine benzyl ester hydrochloride (17.0 grams, 59.9 mmol) and triethylamine (17.6 mL, 126.3 mmol) in water (60 mL) and 1,4-dioxane (100 mL) was added 4-methoxybenzenesulfonyl chloride (13.0 grams, 62.9 mmol). The mixture was stirred at room temperature for 16 hours and then most of the solvent was removed by evaporation under vacuum. The mixture was diluted with ethyl acetate and was washed successively with dilute hydrochloric acid solution, water, saturated sodium bicarbonate solution, and brine. The organic solution was dried over magnesium sulfate and concentrated to leave N-(4-methoxybenzenesulfonyl)-D-cyclohexylglycine benzyl ester as a white solid, 24.51 grams (99%).

(B) N-(4-Methoxybenzenesulfonyl)-D-cyclohexylglycine benzyl ester (12.0 grams, 29.16 mmol) was added to a suspension of sodium hydride (0.78 grams, 32.5 mmol) in dry N,N-dimethylformamide (100 ml) and, after 20 minutes, tert-butyl-(3-iodopropoxy)-dimethylsilane(9.2 grams, 30.6 mmol) was added. The resulting mixture was stirred at

room temperature for 16 hours and was then quenched by addition of saturated ammonium chloride solution. The N,N-dimethylformamide was then removed by evaporation under vacuum. The residue was taken up in diethyl ether and washed successively with dilute hydrochloric acid solution, water and brine. After drying over magnesium sulfate, the diethyl ether was evaporated under vacuum to afford a yellow oil from which [[3-(tert-butyldimethylsilanyloxy)propyl](4-methoxy-benzenesulfonyl)-amino]cyclohexylacetic acid benzyl ester, a clear oil (13.67 grams, 79%), was isolated by flash chromatography on silica gel eluting with 10% ethyl acetate in hexane.

(C) To a solution of [[3-(tert-butyldimethylsilanyloxy)propyl](4-methoxybenzene-sulfonyl)amino]cyclohexylacetic acid benzyl ester (13.67 grams, 23.2 mmol) in methylene chloride (60 mL) at room temperature was added boron trifluoride etherate (21 mL, 171 mmol). After 20 minutes, the reaction was quenched by addition of saturated ammonium chloride solution and subsequent addition of ethyl acetate and water. The organic phase was separated, washed with brine and dried over magnesium sulfate. Evaporation of the solvent under vacuum gave an oil from which cyclohexyl[(3-hydroxypropyl)(4-methoxy-benzenesulfonyl)amino]acetic acid benzyl ester, a clear oil (11.25 grams, 100%), was isolated by flash chromatography on silica gel eluting with 20% ethyl acetate in hexane and then 40% ethyl acetate in hexane.

(D) Cyclohexyl[(3-hydroxypropyl)(4-methoxybenzenesulfonyl)amino]acetic acid benzyl ester (45.8 grams, 96 mmol) and sodium periodate (92.6 grams, 433 mmol) were dissolved in a mixture of acetonitrile (345 mL), carbon tetrachloride (345 mL) and water (460 mL). While cooling in an ice bath, ruthenium trichloride monohydrate (4.4 grams, 21 mmol) was then added. The resulting mixture was mechanically stirred with ice bath cooling for 30 minutes. The bath was removed and stirring was continued at room temperature for 4 hours. The reaction mixture was diluted with ethyl acetate and filtered through diatomaceous earth. The organic layer was separated and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with water and saturated brine. After drying over magnesium sulfate, the solvents were evaporated to give a dark oil from which 3-[(benzyloxycarbonylcyclohexylmethyl)-(4-methoxybenzenesulfonyl)amino]propionic acid, a white foam (28.1 grams, 60%), was isolated by flash chromatography on silica gel eluting sequentially with chloroform and 1% methanol in chloroform.

(E) To a solution of 3-[(benzyloxycarbonylcyclohexylmethyl)(4-methoxybenzenesulfonyl)-amino]propionic acid (1.57 grams, 3.21 mmol) in methylene chloride (45 mL) were added sequentially triethylamine (1.12 mL, 8.04 mmol), methylpiperidin-4-ylcarbamic acid tert-butyl ester (0.89 grams, 4.15 mmol) and (benzotriazol-1-yloxy)tris(dimethylamino)-phosphonium hexafluoroborate (1.56 grams, 3.53 mmol). The resulting mixture was stirred for 16 hours at room temperature and then diluted with methylene chloride. The solution was washed successively with 0.5 M hydrochloric acid solution, saturated sodium bicarbonate solution and brine. The solution was dried over magnesium sulfate and concentrated to yield an oil which was chromatographed on silica gel eluting with 50% ethyl acetate in hexane to afford [{3-[4-(tert-butoxycarbonylmethylamino)piperidin-1-yl]-3-oxopropyl}(4-methoxybenzenesulfonyl)amino]cyclohexylacetic acid benzyl ester as an oil (1.89 grams, 86%).

(F) To a solution of [{3-[4-(tert-butoxycarbonylmethylamino)piperidin-1-yl]-3-oxopropyl}(4-methoxybenzenesulfonyl)amino]cyclohexylacetic acid benzyl ester (1.89 grams, 2.76 mmol) in ethanol (90 mL) was added 10% palladium on activated carbon (0.32 grams). The mixture was agitated under 3 atmospheres hydrogen in a Parr shaker for 2 hours. The catalyst was removed by filtration through nylon (pore size 0.45 μm) and the solvent was evaporated leaving [{3-[4-(tert-butoxycarbonylmethylamino)piperidin-1-yl]-3-oxo-propyl}(4-methoxybenzenesulfonyl)amino]cyclohexylacetic acid as a white foam (1.65 grams, 100%).

(G) To a solution of [{3-[4-(tert-butoxycarbonylmethylamino)piperidin-1-yl]-3-oxopropyl}(4-methoxybenzenesulfonyl)amino]cyclohexylacetic acid (1.65 grams, 2.76 mmol) in methylene chloride (30 mL) were added sequentially O-benzylhydroxylamine hydrochloride (0.47 grams, 2.94 mmol), triethylamine (1.25 mL, 9.0 mmol) and (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluoroborate (1.36 grams, 3.07 mmol). The resulting mixture was stirred for 24 hours at room temperature and then concentrated in vacuo. The residue was taken up in ethyl acetate and washed successively with 0.5 M hydrochloric acid solution, water, saturated sodium bicarbonate solution and brine. The solution was dried over magnesium sulfate and concentrated to yield an oil which was chromatographed on silica gel eluting with 40% hexane in ethylacetatetoafford(1-{3-[(benzyloxycarbamoylcyclohexylmethyl)(4-methoxybenzenesulfonyl)amino]-propionyl}piperidin-4-yl)methylcarbamic acid tert-butyl esteras a clear oil (1.86 grams, 96%).

(H) To a solution of (1-{3-[(benzyloxycarbamoylcyclohexylmethyl)(4-methoxy-benzenesulfonyl)amino]propionyl}piperidin-4-yl)methylcarbamic acid tert-butyl ester (1.86 grams, 2.65 mmol) in methanol (80 mL) was added 5% palladium on barium sulfate (0.85 grams). The mixture was agitated under 3 atmospheres hydrogen in a Parr shaker for 2.5 hours. The catalyst was removed by filtration through nylon (pore size 0.45 μm) and the solvent was evaporated leaving (1-{3-[(cyclohexylhydroxycarbamoylmethyl)(4-methoxybenzene-sulfonyl)amino]propionyl}piperidin-4-yl)methylcarbamic acid tert-butyl esteras a white foam (1.53 grams, 95%).

The title compounds of examples 2-8 were prepared analogously to that described in Example 1 using D-valine benzyl ester as the starting material in step A and the indicated amine in step E.

EXAMPLE 2

Acetic acid 1-{3-[(1-hydroxycarbamoyl-2-methylpropyl)(4-methoxybenzene-sulfonyl)-amino]propionyl}piperidin-4-yl ester

Coupled with acetic acid piperidin-4-yl ester. MS: 500 (M+1).

EXAMPLE 3

Butyric acid 1-{3-[(1-hydroxycarbamoyl-2-methylpropyl)-(4-methoxy-benzenesulfonyl)-amino]propionyl}piperidin-4-yl ester

Coupled with butyric acid piperidin-4-yl ester. MS: 528 (M+1).

EXAMPLE 4

Benzoic acid 1-{3-[(1-hydroxycarbamoyl-2-methylpropyl)(4-methoxy-benzene-sulfonyl)amino]propionyl}piperidin-4-yl ester

Coupled with benzoic acid piperidin-4-yl ester. MS: 562 (M+1). Analysis Calculated for $C_{27}H_{35}N_3O_8S \cdot 1.75\ H_2O$: C, 54.67; H, 6.54; N, 7.08. Found: C, 54.52, H, 6.14; N, 7.85.

Example 5

N-Hydroxy-2-[[3-(4-hydroxypiperidin-1-yl)-3-oxopropyl]-(4-methoxy-benzene-sulfonyl)amino]-3-methylbutyramide

Coupled with 4-hydroxypiperidine. MS: 458 (M+1). Analysis calculated for $C_{20}H_{31}N_3O_7S \cdot H_2O$: C, 50.51; H, 6.99; N, 8.84. Found: C, 50.04; H, 6.84; N, 9.14.

EXAMPLE 6

(1-{3-[(1-Hydroxycarbamoyl-2-methylpropyl)(4-methoxybenzenesulfonyl)-amino]-propionyl}piperidin-4-yl)-methylcarbamic acid tert-butyl ester

Coupled with methyl-piperidin-4-ylcarbamic acid tert-butyl ester.

EXAMPLE 7

1-{3-[(1-Hydroxycarbamoyl-2-methylpropyl)(4-methoxybenzenesulfonyl)-amino]-propionyl}piperidine-4-carboxylic acid ethyl ester

Coupled with piperidine-4-carboxylic acid ethyl ester. MS: 513 (M+1).

EXAMPLE 8

(4-{3-[(1-Hydroxycarbamoyl-2-methylpropyl)(4-methoxybenzenesulfonyl)-amino]-propionyl}piperazin-1-yl)-acetic acid ethyl ester

Coupled with piperazin-1-ylacetic acid ethyl ester. HRMS calculated for $C_{23}H_{37}N_4O_8S$(M+1): 529.2332. Found: 529.2366.

The title compounds of Examples 9-10 were prepared analogously to that described in Example 1 using D-leucine benzyl ester as the starting material in step A and the indicated amine in step E.

EXAMPLE 9

(1-{3-[(1-Hydroxycarbamoyl-3-methylbutyl)(4-methoxybenzenesulfonyl)-amino]-propionyl}piperidin-4-yl)methylcarbamic acid tert-butyl ester

Coupled with methyl-piperidin-4-ylcarbamic acid tert-butyl ester. MS: 585 (M+1).

EXAMPLE 10

1-{3-[(1-Hydroxycarbamoyl-3-methylbutyl)-(4-methoxybenzenesulfonyl)-amino]-propionyl}piperidine-4-carboxylic acid ethyl ester

Coupled with piperidine-4-carboxylic acid ethyl ester. Melting pont 78-80°C. MS: 528 (M+1).

The title compounds of Examples 11-13 were prepared analogously to that described in Example 1 using D-norleucine benzyl ester as the starting material in step A and the indicated amine or alcohol in step E.

EXAMPLE 11

(1-{3-[(1-Hydroxycarbamoylpentyl)(4-methoxybenzenesulfonyl)amino]-propionyl}piperidin-4-yl)methylcarbamic acid tert-butyl ester

Coupled with methyl-piperidin-4-ylcarbamic acid tert-butyl ester.

EXAMPLE 12

1-{3-[(1-Hydroxycarbamoylpentyl)(4-methoxybenzenesulfonyl)amino]-propionyl}piperidine-4-carboxylic acid ethyl ester

Coupled with piperidine-4-carboxylic acid ethyl ester. MS: 528 (M+1).

EXAMPLE 13

3-[(1-Hydroxycarbamoylpentyl)(4-methoxybenzenesulfonyl)amino]-propionic acid indan-5-yl ester

Coupled with 5-indanol. MS: 505 (M+1).

The title compounds of Examples 14-15 were prepared analogously to that described in Example 1 using D-tert-butylalanine benzyl ester as the starting material in step A and the indicated amine in step E.

EXAMPLE 14

(1-{3-[(1-Hydroxycarbamoyl-3,3-dimethylbutyl)-(4-methoxybenzene-sulfonyl)-amino]propionyl}piperidin-4-yl)methylcarbamic acid tert-butyl ester

Coupled with methyl-piperidin-4-ylcarbamic acid tert-butyl ester. MS: 599 (M+1).

EXAMPLE 15

1-{3-[(1-Hydroxycarbamoyl-3,3-dimethylbutyl)(4-methoxy-benzenesulfonyl)-amino]propionyl}piperidine-4-carboxylic acid ethyl ester

Coupled with piperidine-4-carboxylic acid ethyl ester. MS: 542 (M+1).

The title compounds of Examples 16-18 were prepared analogously to that described in Example 1 using D-cyclohexylglycine benzyl ester as the starting material in step A and the indicated amine or alcohol in step E.

EXAMPLE 16

2-Cyclohexyl-N-hydroxy-2-[[3-(4-hydroxypiperidin-1-yl)-3-oxopropyl]-(4-methoxy-benzenesulfonyl)amino]acetamide

Coupled with 4-hydroxypiperidine. MS: 498 (M+1). Analysis calculated for $C_{23}H_{35}N_3O_7S \bullet 0.5H_2O$: C, 54.53; H, 7.16; N, 8.29. Found: C, 54.21; H, 6.98; N, 8.21.

EXAMPLE 17

1-{3-[(Cyclohexylhydroxycarbamoylmethyl)(4-methoxybenzenesulfonyl)-amino]propionyl}piperidine-4-carboxylic acid ethyl ester

Coupled with piperidine-4-carboxylic acid ethyl ester. MS: 554 (M+1). Analysis calculated for $C_{26}H_{39}N_3O_8S \bullet 0.5H_2O$: C, 55.59; H, 7.16; N, 7.47. Found: C, 55.53; H, 7.18; N, 7.57.

EXAMPLE 18

3-[(Cyclohexylhydroxycarbamoylmethyl)-(4-methoxybenzenesulfonyl)-amino]-propionic acid indan-5-yl ester

Coupled with 5-indanol. MS: 531 (M+1). Analysis calculated for $C_{27}H_{34}N_2O_7S \bullet H_2O$: C, 59.11; H, 6.61; N, 5.10. Found: C, 59.40; H, 6.17; N, 5.06.

The title compounds of Examples 19-20 were prepared analogously to that described in Example 1 using D-phenylalanine benzyl ester as the starting material in step A and the indicated amine in step E.

EXAMPLE 19

(1-{3-[(1-Hydroxycarbamoyl-2-phenylethyl)(4-methoxybenzenesulfonyl)-amino]-propionyl}piperidin-4-yl)methylcarbamic acid tert-butyl ester

Coupled with methyl-piperidin-4-ylcarbamic acid tert-butyl ester. MS: 619 (M+1).

EXAMPLE 20

1-{3-[(1-Hydroxycarbamoyl-2-phenylethyl)-(4-methoxybenzenesulfonyl)-amino]-propionyl}piperidine-4-carboxylic acid ethyl ester

Coupled with piperidine-4-carboxylic acid ethyl ester. MS: 561 (M+1).

The title compounds of Examples 21-22 were prepared analogously to that described in Example 1 using D-4-fluorophenylalanine benzyl ester as the starting material in step A and the indicated amine in step E.

EXAMPLE 21

(1-{3-[[2-(4-Fluorophenyl)-1-hydroxycarbamoylethyl]-(4-methoxy-benzene-sulfonyl)amino]propionyl}piperidin-4-yl)methylcarbamic acid tert-butyl ester

Coupled with methyl-piperidin-4-ylcarbamic acid tert-butyl ester.

EXAMPLE 22

1-{3-[[2-(4-Fluorophenyl)-1-hydroxycarbamoylethyl](4-methoxy-benzenesulfonyl) amino]propionyl}piperidine-4-carboxylic acid ethyl ester

Coupled with piperidine-4-carboxylic acid ethyl ester. MS: 580 (M+1). Analysis calculated for $C_{27}H_{34}FN_3O_8S$: C, 55.95; H, 5.91; N, 7.25. Found: C, 55.72; H, 5.79; N, 7.08.

The title compounds of Examples 23-24 were prepared analogously to that described in Example 1 using D-4-homophenylalanine benzyl ester as the starting material in step A and the indicated amine in step E.

EXAMPLE 23

(1-{3-[(1-Hydroxycarbamoyl-3-phenylpropyl)-(4-methoxybenzene-sulfonyl)-amino]propionyl}piperidin-4-yl)methylcarbamic acid tert-butyl ester

Coupled with tert-butyl ester using methyl-piperidin-4-ylcarbamic acid tert-butyl ester. MS: 633 (M+1).

EXAMPLE 24

1-{3-[(1-Hydroxycarbamoyl-3-phenylpropyl)-(4-methoxybenzene-sulfonyl)amino]-propionyl}piperidine-4-carboxylic acid ethyl ester

Coupled with piperidine-4-carboxylic acid ethyl ester. MS: 576 (M+1).

The title compounds of Examples 27-28 were prepared analogously to that described in Example 1 using D-O-tert-butylserine benzyl ester as the starting material in step A and the indicated amine in step E.

EXAMPLE 25

(1-{3-[(2-tert-Butoxy-1-hydroxycarbamoylethyl)(4-methoxybenzene-sulfonyl)-amino]propionyl}piperidin-4-yl)methylcarbamic acid tert-butyl ester

Coupled with methyl-piperidin-4-ylcarbamic acid tert-butyl ester. MS: 615 (M+1).

EXAMPLE 26

1-{3-[(2-tert-Butoxy-1-hydroxycarbamoylethyl)(4-methoxy-benzenesulfonyl)-amino]propionyl}piperidine-4-carboxylic acid ethyl ester

Coupled with piperidine-4-carboxylic acid ethyl ester. MS: 558 (M+1).

The title compounds of Examples 27-28 were prepared analogously to that described in Example 1 using D-cyclohexylalanine benzyl ester as the starting material in step A and the indicated amine in step E.

EXAMPLE 27

(1-{3-[(2-Cyclohexyl-1-hydroxycarbamoylethyl)-(4-methoxy-benzene-sulfonyl)-amino]propionyl}piperidin-4-yl)methylcarbamic acid tert-butyl ester

Coupled with methyl-piperidin-4-ylcarbamic acid tert-butyl ester. MS: 625 (M+1).

EXAMPLE 28

1-{3-[(2-Cyclohexyl-1-hydroxycarbamoylethyl)(4-methoxy-benzenesulfonyl)-amino]propionyl}piperidine-4-carboxylic acid ethyl ester

Coupled with piperidine-4-carboxylic acid ethyl ester. MS: 568 (M+1).

The title compounds of Examples 29-30 were prepared analogously to that described in Example 1 using D-1-naphthylalanine benzyl ester as the starting material in step A and the indicated amine in step E.

EXAMPLE 29

(1-{3-[(1-Hydroxycarbamoyl-2-naphthalen-1-ylethyl)-(4-methoxy-benzenesulfonyl)amino]propionyl}piperidin-4-yl)methylcarbamic acid tert-butyl ester

Coupled with methylpiperidin-4-ylcarbamic acid tert-butyl ester.

EXAMPLE 30

1-{3-[(1-Hydroxycarbamoyl-2-naphthalen-1-ylethyl)(4-methoxybenzene-sulfonyl)amino]propionyl}piperidine-4-carboxylic acidethyl ester

Coupled with piperidine-4-carboxylic acid ethyl ester. MS: 611 (M+1).

EXAMPLE 31

2-Cyclohexyl-N-hydroxy-2-{(4-methoxybenzenesulfonyl)-[3-(4-methyl-amino-piperidin-1-yl)-3-oxopropyl]-amino}acetamide

A solution of 1-{3-[(cyclohexylhydroxycarbamoylmethyl)(4-methoxybenzene-sulfonyl)-amino]-propionyl}piperidin-4-yl)methylcarbamic acid tert-butyl ester (1.53 grams, 2.50 mmol) in methylene chloride (70 mL) was bubbled with hydrochloric acid gas for 2 minutes. The ice bath was removed and the reaction mixture was allowed to stir at room temperature for 1 hour. The solvent was evaporated and twice methanol was added to the residue and evaporated leaving 2-cyclohexyl-N-hydroxy-2-{(4-methoxybenzenesulfonyl)-[3-(4-methylaminopiperidin-1-yl)-3-oxopropyl]-amino}acetamide hydrochloride dihydrate as a white solid (1.22 grams, 90%). MS: 511 (M+1). Analysis calculated for $C_{24}H_{39}ClN_4O_6S \cdot 2H_2O$: C, 49.43; H, 7.43; N, 9.61. Found: C, 49.86; H, 7.23; N, 9.69.

The title compounds of Examples 32-41 were prepared analogously to that described in Example 33 using the starting material indicated.

EXAMPLE 32

N-Hydroxy-2-{(4-methoxybenzenesulfonyl)[3-(4-methylaminopiperidin-1-yl)-3-oxopropyl]amino}-3-methylbutyramide hydrochloride

Starting material: (1-{3-[(1-hydroxycarbamoyl-2-methylpropyl)(4-methoxybenzene-sulfonyl)-amino]propionyl}piperidin-4-yl)-methylcarbamic acid tert-butyl ester using methyl-piperidin-4-ylcarbamic acid tert-butyl. MS: 471 (M+1).

EXAMPLE 33

2-{(4-Methoxybenzenesulfonyl)-[3-(4-methylaminopiperidin-1-yl)-3-oxo-propyl]-amino}4-methylpentanoic acid hydroxyamide hydrochloride

Starting material: (1-{3-[(1-hydroxycarbamoyl-3-methylbutyl)(4-methoxybenzene-sulfonyl)amino]propionyl}piperidin-4-yl)methylcarbamic acid tert-butyl ester. Melting Point 170-173°C. MS: 485 (M+1).

EXAMPLE 34

2-{(4-Methoxybenzenesulfonyl)-[3-(4-methylaminopiperidin-1-yl)-3-oxo-propyl] amino}hexanoic acid hydroxyamide hydrochloride

Starting material: (1-{3-[(1-hydroxycarbamoylpentyl)-(4-methoxybenzenesulfonyl)-amino]-propionyl}piperidin-4-yl)methyl-carbamic acid tert-butyl ester. MS: 485 (M+1). Analysis calculated for $C_{21}H_{34}N_4O_6S \bullet HCl \bullet 4H_2O$: C, 43.5; H, 7.48; N, 9.67. Found: C, 43.65; H, 7.03; N, 9.79.

EXAMPLE 35

2-{(4-Methoxybenzenesulfonyl)-[3-(4-methylaminopiperidin-1-yl)-3-oxo-propyl] amino}-4,4-dimethylpentanoic acid hydroxyamide hydrochloride

Starting material: (1-{3-[(1-hydroxy-carbamoyl-3,3-dimethylbutyl)(4-methoxy-benzenesulfonyl)amino]propionyl}piperidin-4-yl)methylcarbamic acid tert-butyl ester. MS: 499 (M+1).

EXAMPLE 36

N-Hydroxy-2-{(4-methoxybenzenesulfonyl)-[3-(4-methylaminopiperidin-1-yl)-3-oxopropyllamino}-3-phenylpropionamide hydrochloride

Starting material: (1-{3-[(1-hydroxycarbamoyl-2-phenylethyl)(4-methoxybenzene-sulfonyl)-amino]propionyl}piperidin-4-yl)methylcarbamic acid tert-butyl ester. MS: 519 (M+1).

EXAMPLE 37

3-(4-Fluorophenyl)-N-hydroxy-2-{(4-methoxybenzenesulfonyl)-[3-(4-methylamino piperidin-1-yl)-3-oxo-propyllamino}propionamide hydrochloride

Starting material: (1-{3-[[2-(4-fluorophenyl)-1-hydroxycarbamoylethyl]-(4-methoxy-benzenesulfonyl)amino]propionyl}-piperidin-4-yl)methylcarbamic acid tert-butyl ester (Example 21). MS: 537 (M+1). Analysis calculated for

$C_{25}H_{33}FN_4O_6S \bullet HCl \bullet 2H_2O$: C, 49.30; H, 6.29; N, 9.20. Found: C, 49.14; H, 5.82; N, 9.24.

EXAMPLE 38

N-Hydroxy-2-{(4-methoxybenzenesulfonyl)-[3-(4-methylaminopiperidin-1-yl)-3-oxopropyllamino}-4-phenylbutyramide hydrochloride

Starting material: (1-{3-[(1-hydroxycarbamoyl-3-phenylpropyl)(4-methoxy-benzenesulfonyl)amino]propionyl}piperidin-4-yl)methylcarbamic acid tert-butyl ester. Melting Point 160-170°C. MS: 533 (M+1). Analysis calculated for $C_{26}H_{36}N_4O_6S \bullet HCl \bullet 1.5H_2O$: C, 52.38; H, 6.76; N, 9.40. Found: C, 52.25; H, 6.40; N, 9.00.

EXAMPLE 39

3-tert-Butoxy-N-hydroxy-2-{(4-methoxybenzenesulfonyl)-[3-(4-methyl-amino-piperidin-1-yl)-3-oxopropyl]-amino}propionamide hydrochloride

Starting material: (1-{3-[(2-tert-butoxy-1-hydroxycarbamoylethyl)(4-methoxy-benzenesulfonyl)amino]propionyl}piperidin-4-yl)methylcarbamic acid tert-butyl ester. MS: 515 (M+1).

EXAMPLE 40

3-Cyclohexyl-N-hydroxy-2-{(4-methoxybenzenesulfonyl)-[3-(4-methyl-amino-piperidin-1-yl)-3-oxopropyllamino}propionamide hydrochloride

Starting material: (1-{3-[(2-cyclohexyl-1-hydroxycarbamoylethyl)-(4-methoxy-benzenesulfonyl)amino]propionyl}piperidin-4-yl)methylcarbamic acid tert-butyl ester. MS: 525 (M+1).

EXAMPLE 41

N-Hydroxy-2-{(4-methoxybenzenesulfonyl)-[3-(4-methylaminopiperidin-1-yl)-3-oxopropyllamino}-3-naphthalen-1-ylpropionamide hydrochloride

Starting material: (1-{3-[(1-hydroxy-carbamoyl-2-naphthalen-1-ylethyl)-(4-methoxy-benzenesulfonyl)amino]propionyl}-piperidin-4-yl)methylcarbamic acid tert-butyl ester. MS: 569 (M+1).

EXAMPLE 42

1-{3-[(Cyclohexylhydroxycarbamoylmethyl)-(4-methoxybenzenesulfonyl)-amino]-propionyl}piperidine-4-carboxylic acid

To a solution of 1-{3-[(cyclohexylhydroxycarbamoylmethyl)(4-methoxy-benzenesulfonyl)amino]propionyl}piperidine-4-carboxylic acid ethyl ester (0.62 grams, 1.16 mmol) (Example 17) in ethanol (45 mL) and water (5 mL) was added lithium hydroxide monohydrate (0.24 grams, 5.72 mmol). After stirring for 3 hours at room temperature ethanol-washed Amberlite IR-120 plus ion exchange resin (6 grams) was added. Stirring was continued for 15 minutes and then the mixture was filtered. The filtrate was concentrated in vacuo to give 1-{3-[(cyclohexylhydroxycarbamoylmethyl)-(4-methoxy-benzenesulfonyl)amino]propionyl}-piperidine-4-carboxylic acid monohydrate as a white solid (0.52 grams, 88%). MS: 526 (M+1). Analysis calculated for $C_{24}H_{35}N_3O_8S \bullet H_2O$: C, 53.03; H, 6.86; N, 7.73. Found: C, 53.53; H, 7.15; N, 7.70.

The title compounds of Examples 43-53 were prepared analogously to that described in Example 45 using the starting material indicated.

EXAMPLE 43

1-{3-[(1-Hydroxycarbamoyl-2-methylpropyl)(4-methoxybenzene-sulfonyl)amino]propionyl}piperidine-4-carboxylic acid

Starting material: 1-{3-[(1-hydroxycarbamoyl-2-methylpropyl)(4-methoxybenzene-sulfonyl)amino]propionyl}piperidine-4-carboxylic acid ethyl ester. MS: 486 (M+1).

EXAMPLE 44

(4-{3-[(1-Hydroxycarbamoyl-2-methylpropyl)(4-methoxybenzene-sulfonyl)amino]propionyl}piperazin-1-yl)acetic acid

Starting material: (4-{3-[(1-hydroxycarbamoyl-2-methylpropyl)(4-methoxybenzene-sulfonyl)amino]-propionyl}piperazin-1-yl)acetic acid ethyl ester (Example 8). MS: 500 (M+1).

EXAMPLE 45

1-{3-[(1-Hydroxycarbamoyl-3-methylbutyl)-(4-methoxybenzenesulfonyl)-amino]-propionyl}piperidine-4-carboxylic acid

Starting material: 1-{3-[(1-hydroxycarbamoyl-3-methylbutyl)(4-methoxybenzene-sulfonyl)-amino]propionyl}piperidine-4-carboxylic acid ethyl ester. Melting Point 118-120°C. MS: 500 (M+1).

EXAMPLE 46

1-{3-[(1-Hydroxycarbamoylpentyl)(4-methoxybenzenesulfonyl)amino]-propionyl}piperidine-4-carboxylic acid

Starting material: 1-{3-[(1-hydroxycarbamoylpentyl)(4-methoxybenzenesulfonyl)-amino]propionyl}piperidine-4-carboxylic acid ethyl ester. MS: 500 (M+1).

EXAMPLE 47

1-{3-[(1-Hydroxycarbamoyl-3,3-dimethylbutyl)(4-methoxy-benzene-sulfonyl)-amino]propionyl}piperidine-4-carboxylic acid

Starting material: 1-{3-[(1-hydroxycarbamoyl-3,3-dimethylbutyl)(4-methoxybenzene-sulfonyl)-amino]propionyl}piperidine-4-carboxylic acid ethyl ester. MS: 514 (M+1).

EXAMPLE 48

1-{3-[(1-Hydroxycarbamoyl-2-phenylethyl)-(4-methoxybenzenesulfonyl)-amino]-propionyl}piperidine-4-carboxylic acid

Starting material: 1-{3-[(1-hydroxycarbamoyl-2-phenyl-ethyl)(4-methoxybenzene-sulfonyl)-amino]propionyl}piperidine-4-carboxylic acid ethyl ester. MS: 534 (M+1).

EXAMPLE 49

1-{3-[[2-(4-Fluorophenyl)-1-hydroxycarbamoylethyl](4-methoxybenzene-sulfonyl)amino]propionyl}piperidine-4-carboxylic acid

Starting material: 1-{3-[[2-(4-fluorophenyl)-1-hydroxycarbamoylethyl](4-methoxy-benzenesulfonyl)amino]propionyl}piperidine-4-carboxylic acid ethyl ester.
MS: 552 (M+1). Analysis calculated form $C_{25}H_{30}FN_3O_8S \cdot 0.5H_2O$: C, 53.56; H, 5.57; N, 7.50. Found: C, 53.53; H, 5.39; N, 7.28.

EXAMPLE 50

1-{3-[(1-Hydroxycarbamoyl-3-phenylpropyl)(4-methoxybenzenesulfonyl)-amino]-propionyl}piperidine-4-carboxylic acid

Starting material: 1-{3-[(1-hydroxycarbamoyl-3-phenyl-propyl)-(4-methoxybenzene-sulfonyl)-amino]propionyl}piperidine-4-carboxylic acid ethyl ester. Melting Point 85-92°C. MS: 598 (M+1).

EXAMPLE 51

1-{3-[(2-tert-Butoxy-1-hydroxycarbamoylethyl)(4-methoxybenzene-sulfonyl)-amino]propionyl}piperidine-4-carboxylic acid

Starting material: 1-{3-[(2-tert-butoxy-1-hydroxycarbamoylethyl)(4-methoxy-benzenesulfonyl)-amino]propionyl}piperidine-4-carboxylic acid ethyl ester.
MS: 529 (M+1).

EXAMPLE 52

1-{3-[(2-Cyclohexyl-1-hydroxycarbamoylethyl)(4-methoxybenzene-sulfonyl)-amino]propionyl}piperidine-4-carboxylic acid

Starting material: 1-{3-[(2-cyclohexyl-1-hydroxycarbamoylethyl)(4-methoxy-benzenesulfonyl)amino]propionyl}piperidine-4-carboxylic acid ethyl ester.
MS: 540 (M+1).

EXAMPLE 53

1-{3-[(1-Hydroxycarbamoyl-2-naphthalen-1-ylethyl)(4-methoxybenzene-sulfonyl) amino]propionyl}piperidine-4-carboxylic acid

Starting material: 1-{3-[(1-hydroxycarbamoyl-2-naphthalen-1-ylethyl)(4-methoxy-benzenesulfonyl)amino]propionyl}piperidine-4-carboxylic acidethyl ester.
MS: 584 (M+1).

EXAMPLE 54

N-Hydroxy-2-[{3-[4-(2-hydroxyethyl)piperazin-1-yl]-3-oxopropyl}-(4-methoxyben zenesulfonyl)amino]-3-methylbutyramide

(A) To a solution of 2-[(2-carboxyethyl)-(4-methoxybenzenesulfonyl)amino]-3-methylbutyric acid benzyl ester(prepared staring from D-valine benzyl ester according to the procedure of Example 1, steps A to D) (1.35 grams, 3.0 mmol) in methylene chloride (45 mL) were added sequentially triethylamine (0.92 mL, 6.9 mmol),

2-piperazin-1-ylethanol (0.43 grams, 3.3 mmol) and (benzotriazol-1-yloxy)tris-(dimethylamino)-phosphonium hexafluoroborate (1.53 grams, 3.45 mmol). The resulting mixture was stirred for 16 hours at room temperature and then concentrated in vacuo. The residue was taken up in ethyl acetate and washed with saturated sodium bicarbonate solution and brine. The solution was dried over magnesium sulfate and concentrated to yield an oil which was chromatographed on silica gel eluting with 5% methanol in chloroform to afford 2-[{3-[4-(2-hydroxyethyl)piperazin-1-yl]-3-oxo-propyl}(4-methoxybenzenesulfonyl)amino]-3-methylbutyric acid benzyl esteras an oil (1.40 grams, 83%). Conversion to the hydrochloride salt was subsequently carried out using anhydrous hydrochloric acid in cold (0°C) methylene chloride.

(B) To a solution of 2-[{3-[4-(2-hydroxyethyl)piperazin-1-yl]-3-oxopropyl}-(4-methoxy-benzenesulfonyl)amino]-3-methylbutyric acid benzyl ester hydrochloride (1.49 grams, 2.49 mmol) in ethanol (80 mL) was added 10% palladium on activated carbon (0.11 grams). The mixture was agitated under 3 atmospheres hydrogen in a Parr shaker for 16 hours. The catalyst was removed by filtration through nylon (pore size 0.45 μm) and the solvent was evaporated leaving 2-[{3-[4-(2-hydroxyethyl)piperazin-1-yl]-3-oxo-propyl}(4-methoxybenzenesulfonyl)amino]-3-methylbutyric acid hydrochloride as a white solid (1.16 grams, 92%).

(C) To a solution of 2-[{3-[4-(2-hydroxyethyl)piperazin-1-yl]-3-oxo-propyl}(4-methoxy-benzenesulfonyl)amino]-3-methylbutyric acid hydrochloride (1.10 grams, 2.17 mmol) in methylene chloride (50 mL) and N,N-dimethylformamide (0.5 mL) were added sequentially O-benzylhydroxylamine hydrochloride (0.41 grams, 2.60 mmol), triethylamine (0.91 mL, 6.5 mmol) and (benzotriazol-1-yloxy)tris-(dimethylamino)-phosphonium hexafluoroborate (1.20 grams, 2.71 mmol). The resulting mixture was stirred for 16 hours at room temperature and then concentrated in vacuo. The residue was taken up in ethyl acetate and washed successively with saturated sodium bicarbonate solution, water and brine. The solution was dried over magnesium sulfate and concentrated to yield an oil which was chromatographed on silica gel eluting with 3% methanol in chloroform to afford N-benzyloxy-2-[{3-[4-(2-hydroxyethyl)piperazin-1-yl]-3-oxopropyl}(4-methoxybenzenesulfonyl)am

ino]-3-methylbutyramide as a clear oil (0.85 grams, 68%). Conversion to the hydrochloride salt was subsequently carried out using anhydrous hydrochloric acid in cold (0°C) methylene chloride.

(D) To a solution of N-benzyloxy-2-[{3-[4-(2-hydroxyethyl)piperazin-1-yl]-3-oxopropyl}-(4-methoxybenzenesulfonyl)amino]-3-methylbutyramide hydrochloride (0.39 grams, 0.63 mmol) in methanol (30 mL) was added 5% palladium on barium sulfate (0.19 grams). The mixture was agitated under 3 atmospheres hydrogen in a Parr shaker for 2.25 hours. The catalyst was removed by filtration through nylon (pore size 0.45 μm) and the solvent was evaporated to a tan foam which was chromatographed on silica gel eluting with 15% methanol in chloroform containing 0.5% ammonium hydroxide. Clean fractions containing the desired product were taken up in saturated sodium bicarbonate solution. The resulting mixture was extracted several times with ethyl acetate and the combined extracts were concentrated to afford N-hydroxy-2-[{3-[4-(2-hydroxyethyl)piperazin-1-yl]-3-oxopropyl}-(4-methoxybenzenesulfonyl)amino]-3-methyl-butyramide as an oil. The hydrochloride salt (0.20 grams, 61%) was formed using anhydrous hydrochloric acid in cold (0°C) methanol. MS: 487 (M+1). Analysis calculated for $C_{21}H_{34}N_4O_7S \cdot HCl \cdot 0.5H_2O$: C, 47.41; H, 6.82; N, 10.53. Found: C, 47.41; H, 7.11; N, 9.91.

The title compounds of Examples 55-57 were prepared analogously to that described in Example 58 using the indicated amine in step A.

EXAMPLE 55

2-[[3-(4-Dimethylaminopiperidin-1-yl)-3-oxopropyl](4-methoxybenzene-sulfonyl)amino]-N-hydroxy-3-methylbutyramide

Coupled with dimethylpiperidin-4-ylamine. MS: 485 (M+1).

EXAMPLE 56

N-Hydroxy-2-[{3-[4-(3-hydroxypropyl)piperazin-1-yl]-3-oxopropyl}-(4-methoxybenzenesulfonyl)amino]-3-methylbutyramide

Coupled with 3-piperazin-1-ylpropan-1-ol. MS: 500 (M+1).

EXAMPLE 57

2-[(3-[1,4']Bipiperidinyl-1'-yl-3-oxopropyl)-(4-methoxybenzenesulfonyl)-amino]-N-hydroxy-3-methylbutyramide

Coupled with using [1,4']bipiperidinyl. MS: 525 (M+1). Analysis calculated for $C_{25}H_{40}N_4O_6S \bullet HCl \bullet 1.5H_2O$: C, 51.05; H, 7.54; N, 9.52. Found: C, 50.80; H, 7.45; N, 9.36.

EXAMPLE 58

1-{3-[(1-Hydroxycarbamoyl-2-methylpropyl)-(4-phenoxybenzenesulfonyl) amino] propionyl] piperidine-4-carboxylic acid ethyl ester

The title compound was prepared analogously to that described in Example 1 using D-valine benzyl ester and 4-phenoxybenzenesulfonyl chloride as the starting materials in step A and piperidine-4-carboxylic acid ethyl ester in step E. Analysis calculated for $C_{28}H_{37}N_3O_8S$. $0.1CH_2Cl_2$: C, 57.78; H, 6.42; N, 7.19. Found: C, 57.46; H, 6.41; N, 7.11.

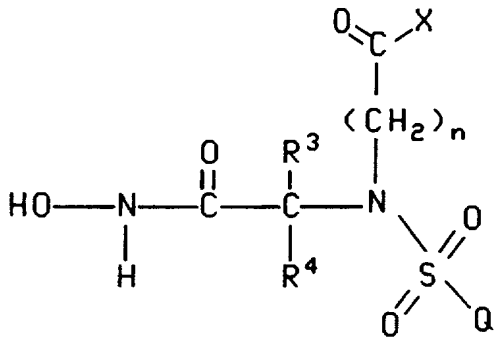
EXAMPLE 59

1-{3-[(1-Hydroxycarbamoyl-2-methylpropyl)-(4-phenoxybenzenesulfonyl) amino] propionyl]piperidine-4-carboxylic acid

The title compound was prepared analogously to that described in Example 42 using 1-{3-[(1-hydroxycarbamoyl-2-methylpropyl)-(4-phenoxybenzenesulfonyl)amino] propionyl]piperidine-4-carboxylic acid ethyl ester (Example 58) as the starting material. MS: 548 (M+1). Analysis calculated for $C_{26}H_{33}N_3O_8S$. $0.5H_2O$: C, 56.10; H, 6.16; N, 7.75. Found: C, 55.99; H, 6.06; N, 7.43.

CLAIMS

1. A compound of the formula

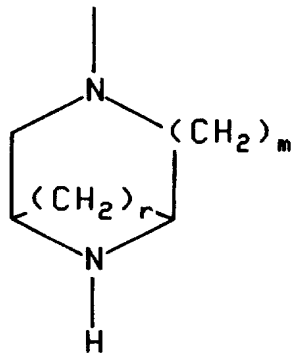


I

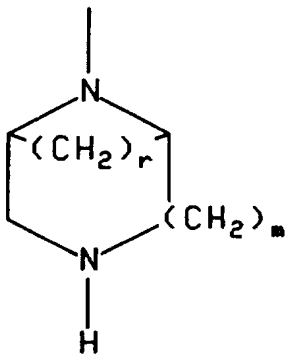
or the pharmaceutically acceptable salts thereof, wherein

n is 1 to 6;

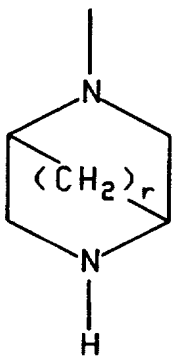
X is OR^1 wherein R^1 is as defined below; azetidinyl, pyrrolidinyl, piperidinyl, morpholinyl, thiomorpholinyl, indolinyl, isoindolinyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, piperazinyl or a bridged diazabicycloalkyl ring selected from the group consisting of



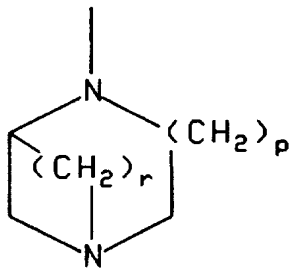
a



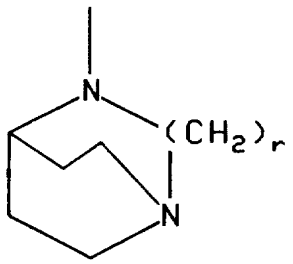
b



c



d



e

wherein r is 1, 2 or 3;

m is 1 or 2; and

p is 0 or 1;

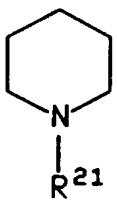
wherein each heterocyclic group may optionally be substituted by one or two groups selected from hydroxy, (C_1-C_6)alkyl, (C_1-C_6)alkoxy, (C_1-C_{10})acyl, (C_1-C_{10})acyloxy, (C_6-C_{10})aryl, (C_5-C_9)heteroaryl, (C_6-C_{10})aryl(C_1-C_6)alkyl, (C_5-C_9)heteroaryl (C_1-C_6)alkyl, hydroxy (C_1-C_6)alkyl, (C_1-C_6)alkoxy (C_1-C_6)alkyl, (C_1-C_6)acyloxy(C_1-C_6)alkyl, (C_1-C_6)alkylthio, (C_1-C_6)alkylthio (C_1-C_6)alkyl, (C_6-C_{10})arylthio, (C_6-C_{10}) arylthio(C_1-C_6)alkyl, $R^9R^{10}N$, $R^9R^{10}NSO_2$, $R^9R^{10}NCO$, $R^9R^{10}NCO(C_1$-$C_6)$alkyl wherein R^9 and R^{10} are each independently hydrogen, (C_1-C_6)alkyl, (C_6-C_{10})aryl, (C_5-C_9)heteroaryl, (C_6-C_{10})aryl (C_1-C_6)alkyl or (C_5-C_9)heteroaryl (C_1-C_6)alkyl or R^9 and R^{10} may be taken together with the nitrogen to which they are attached to form an azetidinyl, pyrrolidinyl, piperidinyl, morpholinyl or thiomorpolinyl ring; $R^{12}SO_2$, $R^{12}SO_2NH$ wherein R^{12} is trifluoromethyl, (C_1-C_6)alkyl, (C_6-C_{10})aryl, (C_5-C_9)heteroaryl, (C_6-C_{10})aryl(C_1-C_6)alkyl or (C_5-C_9)heteroaryl (C_1-C_6)alkyl; $R^{13}CONR^9$ wherein R^9 is as defined above and R^{13} is hydrogen, (C_1-C_6)alkyl, (C_1-C_6)alkoxy, (C_6-C_{10})aryl, (C_5-C_9)heteroaryl, (C_1-C_6)aryl(C_1-C_6)alkyl(C_6-C_{10})aryl(C_1-C_6)alkoxy or (C_5-C_9)heteroaryl(C_1-C_6)alkyl; $R^{14}OOC$, $R^{14}OOC(C_1$-$C_6)$alkyl wherein R^{14} is (C_1-C_6)alkyl, (C_6-C_{10})aryl, (C_5-C_9)heteroaryl, (C_6-C_{10})aryl (C_1-C_6)alkyl, 5-indanyl, CHR^5OCOR^6 wherein R^5 is hydrogen or (C_1-C_6)alkyl and R^6 is (C_1-C_6)alkyl, (C_1-C_6)alkoxy or (C_6-C_{10})aryl; $CH_2CONR^7R^8$ wherein R^7 and R^8 are each independently hydrogen or (C_1-C_6)alkyl or may be taken together with the nitrogen to which they are attached to form an

azetidinyl, pyrrolidinyl, piperidinyl, morpholinyl or thiomorpholinyl ring; or $R^{15}O(C_1C_6)$alkyl wherein R^{15} is $H_2N(CHR^{16})CO$ wherein R^{16} is the side chain of a natural D- or L-amino acid;

R^1 is $(C_6\text{-}C_{10})$aryl, $(C_5\text{-}C_9)$heteroaryl, $(C_6\text{-}C_{10})$aryl$(C_1\text{-}C_6)$alkyl, 5-indanyl, CHR^5OCOR^6 or $CH_2CONR^7R^8$ wherein R^5, R^6, R^7 and R^8 are as defined above;

R^3 and R^4 are each independently selected from the group consisting of hydrogen, $(C_1\text{-}C_6)$alkyl, trifluoromethyl, trifluoromethyl$(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_6)$alkyl (difluoromethylene), $(C_1\text{-}C_3)$alkyl(difluoromethylene)$(C_1\text{-}C_3)$alkyl, $(C_6\text{-}C_{10})$aryl, $(C_5\text{-}C_9)$heteroaryl, $(C_6\text{-}C_{10})$aryl$(C_1\text{-}C_6)$alkyl, $(C_5\text{-}C_9)$heteroaryl$(C_1\text{-}C_6)$alkyl, $(C_6\text{-}C_{10})$aryl$(C_6\text{-}C_{10})$aryl, $(C_6\text{-}C_{10})$aryl$(C_6\text{-}C_{10})$aryl$(C_1\text{-}C_6)$alkyl, $(C_3\text{-}C_6)$cycloalkyl, $(C_3\text{-}C_6)$cycloalkyl$(C_1\text{-}C_6)$alkyl, hydroxy$(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_{10})$acyloxy$(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_6)$alkoxy$(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_{10})$acylamino$(C_1\text{-}C_6)$alkyl, piperidyl, $(C_1\text{-}C_6)$alkylpiperidyl, $(C_6\text{-}C_{10})$aryl$(C_1\text{-}C_6)$alkoxy$(C_1\text{-}C_6)$alkyl, $(C_5\text{-}C_9)$heteroaryl$(C_1\text{-}C_6)$alkoxy$(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_6)$alkylthio$(C_1\text{-}C_6)$alkyl, $(C_6\text{-}C_{10})$arylthio$(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_6)$alkylsulfinyl$(C_1\text{-}C_6)$alkyl, $(C_6\text{-}C_{10})$arylsulfinyl$(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_6)$alkylsulfonyl$(C_1\text{-}C_6)$alkyl, $(C_6\text{-}C_{10})$arylsulfonyl$(C_1\text{-}C_6)$alkyl, amino$(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_6)$alkylamino$(C_1\text{-}C_6)$alkyl, $((C_1\text{-}C_6)\text{alkylamino})_2(C_1\text{-}C_6)$alkyl, $R^{17}CO(C_1\text{-}C_6)$alkyl wherein R^{17} is $R^{14}O$ or R^7R^8N wherein R^7, R^8 and R^{14} are as defined above; or $R^{18}(C_1\text{-}C_6)$alkyl wherein R^{18} is piperazinyl, $(C_1\text{-}C_{10})$acylpiperazinyl, $(C_6\text{-}C_{10})$arylpiperazinyl, $(C_5\text{-}C_9)$heteroarylpiperazinyl, $(C_1\text{-}C_6)$alkylpiperazinyl, $(C_6\text{-}C_{10})$aryl$(C_1\text{-}C_6)$alkylpiperazinyl, $(C_5\text{-}C_9)$heteroaryl$(C_1\text{-}C_6)$alkylpiperazinyl, morpholinyl, thiomorpholinyl, piperidinyl, pyrrolidinyl, piperidyl, $(C_1\text{-}C_6)$alkylpiperidyl, $(C_6\text{-}C_{10})$arylpiperidyl, $(C_5\text{-}C_9)$heteroarylpiperidyl, $(C_6\text{-}C_{10})$aryl$(C_1\text{-}C_6)$alkylpiperidyl, $(C_5\text{-}C_9)$heteroaryl$(C_1\text{-}C_6)$alkylpiperidyl or $(C_1\text{-}C_{10})$acylpiperidyl;

or R^3 and R^4 may be taken together to form a $(C_3\text{-}C_6)$cycloalkyl, oxacyclohexyl, thiocyclohexyl, indanyl or tetralinyl ring or a group of the formula



wherein R^{21} is hydrogen, (C_1-C_{10})acyl, (C_1-C_6)alkyl, (C_6-C_{10})aryl(C_1-C_6)alkyl, (C_5-C_9)heteroaryl(C_1-C_6)alkyl or (C_1-C_6)alkylsulfonyl; and

Q is (C_1-C_6)alkyl, (C_6-C_{10})aryl, (C_6-C_{10})aryloxy(C_6-C_{10})aryl, (C_6-C_{10})aryl(C_6-C_{10})aryl, (C_6-C_{10})aryl(C_6-C_{10})aryl(C_1-C_6)alkyl, (C_6-C_{10})aryloxy(C_5-C_9)heteroaryl, (C_5-C_9)heteroaryl, (C_1-C_6)alkyl(C_6-C_{10})aryl, (C_1-C_6)alkoxy(C_6-C_{10})aryl, (C_6-C_{10})aryl(C_1-C_6)alkoxy(C_6-C_{10})aryl,(C_6-C_{10})aryl(C_1-C_6)alkoxy(C_1-C_6)alkyl,(C_5-C_9)heteroaryloxy(C_6-C_{10})aryl, (C_1-C_6)alkyl(C_5-C_9)heteroaryl, (C_1-C_6)alkoxy(C_5-C_9)heteroaryl, (C_6-C_{10})aryl(C_1-C_6)alkoxy(C_5-C_9)heteroaryl, (C_5-C_9)heteroaryloxy(C_5-C_9)heteroaryl, (C_6-C_{10})aryloxy(C_1-C_6)alkyl, (C_5-C_9)heteroaryloxy(C_1-C_6)alkyl, (C_1-C_6)alkyl(C_6-C_{10})aryloxy(C_6-C_{10})aryl,(C_1-C_6)alkyl(C_5-C_9)heteroaryloxy(C_6-C_{10})aryl,(C_1-C_6)alkyl(C_6-C_{10})aryloxy(C_5-C_9)heteroaryl, (C_1-C_6)alkoxy(C_6-C_{10})aryloxy(C_6-C_{10})aryl, (C_1-C_6)alkoxy(C_5-C_9)heteroaryloxy(C_6-C_{10})aryl or (C_1-C_6)alkoxy(C_6-C_{10})aryloxy(C_5-C_9)heteroaryl wherein each aryl group is optionally substituted by fluoro, chloro, bromo, (C_1-C_6)alkyl, (C_1-C_6)alkoxy or perfluoro(C_1-C_3)alkyl;

with the proviso that X must be substituted when defined as azetidinyl, pyrrolidinyl, morpholinyl, thiomorpholinyl, indolinyl, isoindolinyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, piperazinyl, (C_1-C_{10})acylpiperazinyl, (C_1-C_6)alkylpiperazinyl, (C_6-C_{10})arylpiperazinyl, (C_5-C_9)heteroarylpiperazinyl or a bridged diazabicycloalkyl ring.

2. A compound according to claim 1, wherein n is 2.

3. A compound according to claim 1, wherein either R^3 or R^4 is not hydrogen.

4. A compound according to claim 1, wherein Q is (C_1-C_6)alkoxy(C_6-C_{10})aryl, (C_6-C_{10})aryl(C_1-C_6)alkoxy(C_6-C_{10})aryl, phenoxy(C_6-C_{10})aryl, 4-fluorophenoxy(C_6-C_{10})aryl, 4-fluorobenzyloxy(C_6-C_{10})aryl or (C_1-C_6)alkyl(C_6-C_{10})aryloxy(C_6-C_{10})aryl.

5. A compound according to claim 1, wherein X is indolinyl or piperidinyl.

6. A compound according to claim 1, wherein n is 2; either R^3 or R^4 is not hydrogen; Q is (C_1-C_6)alkoxy(C_6-C_{10})aryl, (C_6-C_{10})aryl(C_1-C_6)alkoxy(C_6-C_{10})aryl, 4-fluorophenoxy(C_6-C_{10})aryl, phenoxy(C_6-C_{10})aryl, 4-fluorobenzyloxy(C_6-C_{10})aryl or (C_1-C_6)alkyl(C_6-C_{10})aryloxy(C_6-C_{10})aryl; and X is indolinyl or piperidinyl.

7. A compound according to claim 1, wherein said compound is selected from the group consisting of:

3-[(Cyclohexylhydroxycarbamoylmethyl)-(4-methoxybenzenesulfonyl)-amino]-propionic acid indan-5-yl ester;

Acetic acid 1-{3-[(1-hydroxycarbamoyl-2-methylpropyl)-(4-methoxy-benzenesulfonyl)-amino]propionyl}piperidin-4-yl ester;

2-Cyclohexyl-N-hydroxy-2-[[3-(4-hydroxypiperidin-1-yl)-3-oxopropyl]-(4-methoxy-benzenesulfonyl)amino]acetamide;

Benzoic acid 1-{3-[(1-hydroxycarbamoyl-2-methylpropyl)-(4-methoxy-benzenesulfonyl)amino]propionyl}piperidin-4-yl ester;

N-Hydroxy-2-[[3-(4-hydroxypiperidin-1-yl)-3-oxopropyl]-(4-methoxybenzenesulfonyl)amino]-3-methylbutyramide;

1-{3-[(Cyclohexylhydroxycarbamoylmethyl)-(4-methoxybenzenesulfonyl)-amino]propionyl}piperidine-4-carboxylic acid;

1-{3-[(Cyclohexylhydroxycarbamoylmethyl)-(4-methoxybenzenesulfonyl)-amino]propionyl}piperidine-4-carboxylic acid ethyl ester;

2-Cyclohexyl-N-hydroxy-2-{(4-methoxybenzenesulfonyl)-[3-(4-methylaminopiperidin-1-yl)-3-oxopropyl]amino}acetamide;

3-(4-Chlorophenyl)-N-hydroxy-2-{(4-methoxybenzenesulfonyl)-[3-(4-methylaminopiperidin-1-yl)-3-oxopropyl]amino}propionamide;

3-Cyclohexyl-N-hydroxy-2-{(4-methoxybenzenesulfonyl)-[3-(4-methylaminopiperidin-1-yl)-3-oxopropyl]amino}propionamide;

N-Hydroxy-2-[{3-[4-(2-hydroxy-2-methylpropyl)piperazin-1-yl]-3-oxopropyl}-(4-methoxy-benzenesulfonyl)amino]-3-methylbutyramide;

2,2-Dimethylpropionic acid 2-(4-{3-[(1-hydroxycarbamoyl-2-methylpropyl)-(4-methoxy-benzenesulfonyl)amino]propionyl}piperazin-1-yl)ethyl ester;

Benzoic acid 2-(4-{3-[(1-hydroxycarbamoyl-2-methylpropyl)-(4-methoxybenzenesulfonyl)-amino]propionyl}piperazin-1-yl)-ethyl ester;

2-Cyclohexyl-N-hydroxy-2-[{3-[4-(2-hydroxyethyl)piperazin-1-yl]-3-oxopropyl}-(4-methoxybenzenesulfonyl)amino]acetamide;

2-Hydroxy-2-[{3-[5-(2-hydroxyethyl)-2,5-diazabicyclo[2.2.1]-hept-2-yl]-3-oxopropyl}-(4-methoxybenzenesulfonyl)amino]-3-methylbutyramide;

2-{(4-Benzyloxybenzenesulfonyl)-[3-(4-hydroxypiperidin-1-yl)-3-oxopropyl]amino}-N-hydroxy-3-methylbutyramide;

2-Cyclohexyl-2-{[4-(4-fluorophenoxy)benzenesulfonyl]-[3-(4-hydroxy-piperidin-1-yl)-3-oxopropyl]-amino}-N-hydroxyacetamide;

2-{[4-(4-Butylphenoxy)benzenesulfonyl]-[3-(4-hydroxypiperidin-1-yl)-3-oxopropyl]-amino}-N-hydroxy-3-methylbutyramide;

1-{(4-Methoxybenzenesulfonyl)-[3-(4-methylaminopiperidin-1-yl)-3-oxo-propyl]amino}-cyclopentanecarboxylic acid hydroxyamide;

4-{3-[(1-Hydroxycarbamoyl-2-methylpropyl)-(4-methoxybenzene-sulfonyl)amino]-propionyl}piperazine-2-carboxylic acid ethyl ester;

3-[(Cyclohexylhydroxycarbamoylmethyl)-(4-methoxybenzene-sulfonyl)amino]propionic acid ethoxycarbonyloxymethyl ester;

3-[(1-Hydroxycarbamoylpentyl)-(4-methoxybenzenesulfonyl)amino]propionic acid ethoxycarbonyloxymethyl ester;

1-{3-[(1-Hydroxycarbamoyl-2-methylpropyl)-(4-phenoxybenzenesulfonyl)amino] propionyl] piperidine-4-carboxylic acid.

3-[[4-(4-Fluorobenzyloxy)-benzenesulfonyl]-(1-hydroxy-carbamoyl-2-methyl-propyl)-amino]-propionic acid ethoxycarbonyloxymethyl ester; and

3-[[4-(4-Fluorophenoxy)-benzenesulfonyl]-(1-hydroxycarbamoyl-2-methyl-propyl)-amino]-propionic acid ethoxycarbonyloxymethyl ester.

8. A pharmaceutical composition for (a) the treatment of a condition selected from the group consisting of arthritis, cancer, tissue ulceration, mucular degeneration, restenosis, periodontal disease, epidermolysis bullosa, scleritis, in combination with standard NSAID'S and analgesics and in combination with cytotoxic anticancer agents, and other diseases characterized by matrix metalloproteinase activity, AIDS, sepsis, septic shock and other diseases involving the production of tumor necrosis factor (TNF) or (b) the inhibition of matrix metalloproteinases or the production of tumor necrosis factor (TNF) in a mammal, including a human,