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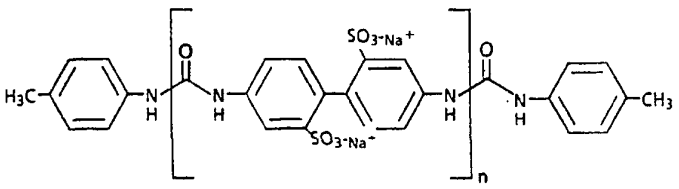
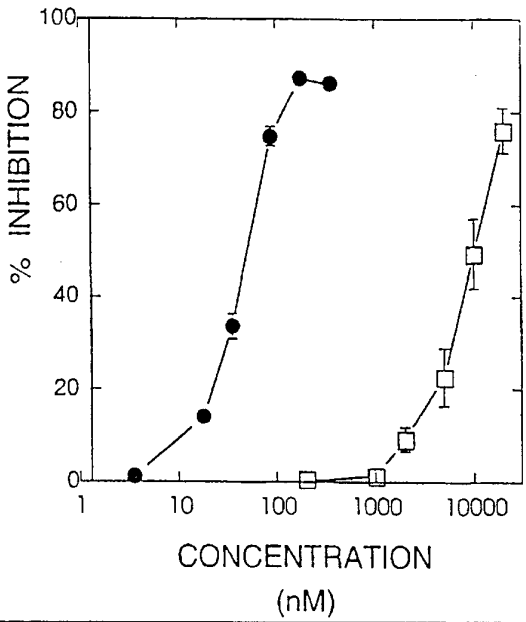
(54) Title: METHOD OF PREVENTING NEUTROPHIL MEDIATED CONNECTIVE TISSUE DAMAGE

(57) Abstract

Oligomers of formula (I) have demonstrated effectiveness in preventing connective tissue damage.



(I)



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METHOD OF PREVENTING NEUTROPHIL MEDIATED CONNECTIVE TISSUE DAMAGE

BACKGROUND OF THE INVENTION

The Diamino-biphenyl-disulfonic acid oligomers of the present invention are able to inhibit enzymes that are actively involved in the degradation of connective tissues. Specifically, the diamino-biphenyl-disulfonic acid oligomers of the present invention are able to inhibit the activity of elastase and cathepsin G.

Elastase and cathepsin G are serine proteases found in the primary granules of human neutrophils that have the capacity to degrade numerous connective tissue macromolecules including elastin, fibronectin, collagen and proteoglycan. Because large numbers of neutrophils are present at sites of inflammation, neutrophil elastase and cathepsin G have been implicated in the tissue destruction associated with a number of diseases including adult respiratory distress syndrome, cystic fibrosis, acute bronchitis, emphysema and arthritis.

Normally the large quantities of endogenous protease inhibitors present in plasma and mucous secretions provide ample protection against connective tissue damage mediated by neutrophil proteolytic enzymes. However, when the balance between inhibitors and proteases is disturbed tissue damage may occur. When connective tissue proteases

are left unchecked the effect is a deterioration of the underlying connective tissue. This effect may result from a host of initial causes of connective tissue destruction, including direct physical injury, aging, and genetic imbalances. For instance, individuals with a genetic deficiency resulting in reduced levels of the endogenous inhibitor, α-1-antitrypsin, have a strong tendency to develop emphysema at an early age thus further supporting the enzyme imbalance theory in connective tissue diseases.

An object of this invention is the use of diamino-biphenyl-disulfonic acid oligomers as inhibitors of elastase and cathepsin G. These inhibitors thereby provides an opportunity to control disease states associated with the degradation of these tissues.

DESCRIPTION OF THE PRIOR ART

The diamino-biphenyl-disulfonic acid oligomers of this invention are described in detail in the European Patent Application published January 22, 1992, under Publication No. 0467185 A2, wherein they were described as having utility in the diagnosis and/or treatment of AIDS and AIDS related complex.

The major focus of therapeutic intervention targeted at neutrophil proteolytic enzymes has centered on elastase. Free granulocyte elastase has been detected in the broncholavage fluids from patients with adult respiratory distress syndrome (ARDS)*(McGuire, et al., J. Clin. Invest., 69:543-553, 1982; Lee, et al., N. Engl. J. Med. 304:192-196, 1981)* and in the sputum of patients with cystic fibrosis *(Suter, et al, J. Inf. Dis. 153:902-909, 1986; Goldstein & Doring, Am. Rev. Respir. Dis. 134:49-56, 1986).* Instillation of elastase into the lungs of hamsters induces acute damage as measured by hemorrhage *(Bonney, et al., J. Cellular Biochem. 39:47-53, 1989; Fletcher, et al., Am Rev. Respir. Dis, 141:672-677, 1990; Hassall, et al., FEBS Lett, 1983:201-205, 1985)* and long term

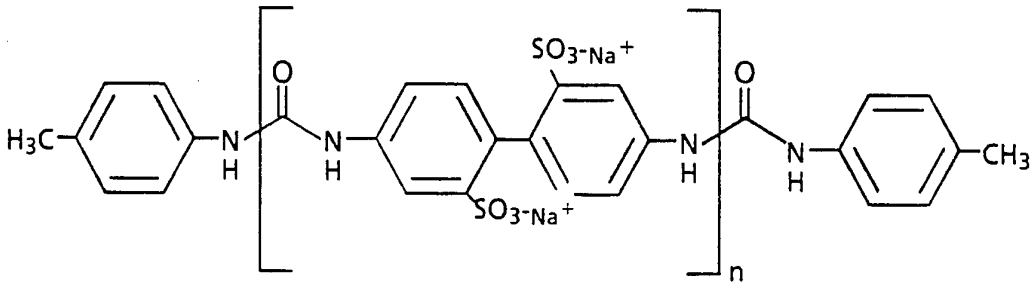
changes resembling those seen in emphysema *(Stone, et al., Am. Rev. Respir. Dis 141:47-52, 1990)*. The role of cathepsin G in disease states is unknown. However, elastase and cathepsin G are present in the neutrophil in approximately equal amounts and free cathepsin G has been detected along with elastase in certain pathological states. Both elastase and cathepsin G have been shown to stimulate secretion from bovine submucosal serous cells suggesting a role for these enzymes in submucosal gland hypersecretion in pulmonary disorders associated with cigarette smoking, chronic bronchitis, and cystic fibrosis *(Sommerhoff, et al., J. Clin. Invest. 85:682-689, 1990)*. Recently, cathepsin G has been shown to induce platelet aggregation, calcium influx and serotonin release from platelets *(Renesto et al., Lab. Invest. 62:409-416 1990)* and to be responsible for the platelet activation stimulated by tumor necrosis factor treated neutrophils *(Renesto et al., J. Immunol. 146:2305-2309 1991)*. In addition, cathepsin G has been shown to activate complement component C3 on the plasma membranes or U-937 cells *(Maison et al., J. Immunol. 147:921-926 1991)*.

Previously, the degradation of cartilage matrix proteoglycan by stimulated neutrophils or neutrophil proteases was used as a model for connective tissue degradation. Using specific synthetic inhibitors of elastase or cathepsin G alone and in combination, connective tissue matrix degradation (mediated by free neutrophil proteases or breakdown occurring in the serum-antiprotease protected pericellular region between neutrophils and substrate) was found to be blocked only by inhibiting both elastase and cathepsin G *(Janusz, et al., J. Immunol. 146:3922-3928 1991)*. Recently, several polyanionic polysaccharides have been shown to inhibit elastase and cathepsin G *(Baici, et al., Biochem. Pharmacol. 29:1723-1727 1980 and Redini, et al., Biochem. J. 252:515-519 1988)*. The inhibitory capacity of these anionic polymers against the cationic elastase and

cathepsin G was found to increase with polymer size and degree of sulfation.

SUMMARY OF THE INVENTION

The invention herein described discloses a method of inhibiting the activity of elastase and cathepsin G by administering a compound of the formula:



wherein n is a whole number selected from the range of 5 to 10 inclusive and the pharmaceutically acceptable salts thereof (herein also referred to as "compounds"). A preferred embodiment of the claimed compounds are those compounds wherein n is equal to 6.

An object of the invention is the use of compounds of the present invention, or the pharmaceutically acceptable salts thereof, to treat pulmonary diseases including adult respiratory distress syndrome (ARDS), chronic bronchitis, cystic fibrosis, emphysema and to treat inflammatory diseases of the joint tissues such as arthritis. These disease have in common the degradation of connective tissues.

An advantage of the present invention is that the described compounds inhibit elastase and cathepsin G, which are both known to actively contribute to, or are present during, the progression of ARDS, cystic fibrosis, acute bronchitis, emphysema, and arthritis. Specifically, the forementioned compounds have been shown to directly inhibit

the human forms of elastase and cathepsin G. Because these compounds are able to inhibit human elastase and human cathepsin G, these compounds afford a mode of intervention and therapy.

Another object of the present invention is the treatment of arthritis. These compounds are advantageous by their ability to protect cartilage matrix proteoglycan (CMP) from destructive processes involving elastase and cathepsin G. Connective tissue protection of CMP by these compounds thereby affords a mode of intervention and therapy to arthritis.

Use of the compounds of the present invention may also be used to protect from acute lung injury or to protect from further damage to the lung tissues caused by elastases and cathepsin G. The present compounds can protect from connective tissue injury occurring from neutrophil elastase, and thereby, affords a means of invention and treatment of ARDS, cystic fibrosis, acute bronchitis, and emphysema.

DETAILED DESCRIPTION OF THE INVENTION

This disclosure shows that a low molecular weight sulfated polymers can block the action of elastase and cathepsin G against synthetic peptide substrates and against macromolecular connective tissue molecules. An exemplary compound within the scope of this invention was shown to inhibit elastase induced lung hemorrhage when tested in vivo. These results suggest that anionic polymers may be effective in treating neutrophil mediated connective tissue damage.

MATERIALS AND METHODS

Elastase activity is assayed using the specific substrate, N-methoxysuccinyl-Ala-Ala-Pro-Val-p-nitroanalide, and cathepsin G activity was measured using N-succinyl-Ala-Pro-Phe-p-nitroanalide. The assay buffer, 0.1 M HEPES with 0.5 M NaCl and 0.1% Brij-35, is used to dilute the elastase substrate to a final concentration of 0.2 mM and the cathepsin G substrate to 1 mM. The appropriate substrate in 2 ml of assay buffer is incubated at 37°C in a heated spectrophotometer cell and the reaction is started by the addition of enzyme. In experiments where inhibitors were tested, the inhibitors were incubated with the enzyme for 5 min before the addition of substrate. Cleavage of enzyme was continuously monitored at 410 nm using a diode array spectrophotometer. Kinetic constants were determined using the kinetics software of the spectrophotometer.

Human leukocytes were isolated from citrated blood by dextran sedimentation of erythrocytes, washed free of plasma and platelets in Hank's Balanced Salt Solution (HBSS), pH 7.4, that lacked phenol red, calcium, and magnesium, and purified by gradient centrifugation. Neutrophils were collected and washed once in HBSS, incubated with distilled water for 30 seconds to lyse contaminating erythrocytes, washed twice in HBSS, resuspended in serumless media containing 10% heat inactivated fetal calf serum (FCS), glutamine (2mM), HEPES (20mM) and gentamycin (50 µM) and counted in a Coulter Counter. Differential staining with Diff-Quik revealed more than 95% neutrophils with 1-5% eosinophils. Viability was more than 98% as assessed by trypan blue exclusion.

Human neutrophil granule lysates were prepared by resuspending 2.5 X 10^8 in 4 ml of 0.34 N sucrose and

sonicating for 30 seconds using a microtip probe. Microscopic examination showed virtually complete breakage of the neutrophils with release of the granules. The neutrophil lysate was centrifuged at 1000 Xg for 10 min. at 4°C, the supernatant was collected and centrifuged at 30,000 Xg for 30 min. at 4°C and the pellet containing neutrophil granules was resuspended in 2 ml of 0.05 M sodium acetate buffer, pH 5.5, containing 1 M NaCl and 0.1% Brij-35 and kept on ice for 30 min. with several brief sonications of approximately 30 seconds during the incubation period. Microscopic examination showed disruption of the granules. The granule lysate was centrifuged at 30,000 Xg for 30 min. at 4°C and the supernatant was collected, tested for elastase and cathepsin G content using synthetic substrates as described above, aliquoted and stored at 70°C until used.

PREPARATION OF RADIOLABELED BOVINE CARTILAGE

Fresh bovine nasal septa were obtained from a local abattoir and 4 mm cartilage cylinders were prepared using a No. 2 cork borer. The cylinders were sliced into 1 mm discs. The top and bottom discs were discarded and the remaining cartilage discs were pooled, washed three times in HBSS and resuspended in Dulbecco's Modified Eagles Medium (DMEM) with antibiotics and supplements. The sulfated sugars that constitute the glycosaminoglycan side chains of cartilage proteoglycan were radiolabeled by the addition of 10 μ Ci/ml of $Na_2{}^{35}SO_4$ followed by incubation overnight at 37°C with gentle rocking. The radioactive medium was removed and the discs were washed ten times with HBSS to remove unincorporated label. The radiolabeled cartilage discs were freeze-thawed five times and heated at 65°C for 15 min. to kill chondrocytes and inactivate endogenous enzymes. This treatment eliminated autolysis of the cartilage but did not significantly alter the sensitivity of the proteoglycan to exogenous proteases.

The majority of the discs used in these studies contained 5.0 X 10^4 to 2.0 X 10^5 dpm of incorporated ^{35}S.

Neutrophil Protease Degradation Of Connective Tissue Matrix

The ability of neutrophil proteases to degrade connective tissue matrix was measured by incubating ^{35}S-radiolabeled cartilage discs with neutrophil lysate, purified elastase or cathepsin G in serumless media for 4 hours at 37°C and collecting the supernatants and counting the radiolabeled material in the supernatants and discs. The radioactivity remaining in the cartilage discs after removal of the supernatant was assayed by completely digesting the discs in 6 M HCl at 100°C for 1 hour, removing the HCl by vacuum evaporation and resuspending the dried material in 0.5 ml HBSS. Supernatants and processed discs were counted in a Beckman LS 3801 scintillation counter. The data were expressed as the percent at ^{35}S label released into the supernatant.

Connective Tissue Degradation By Human Neutrophils

Radiolabeled cartilage discs were placed into the wells of 96-well microtiter plates and overlayed with human neutrophils suspended in DMEM containing 10% FCS. The neutrophils were stimulated by the addition of 1 mg/ml of opsonized zymosan and incubated for 4 hours at 37°C with 5% CO_2. Connective tissue matrix degradation was quantitated by the amount of radiolabel released. In experiments using protease inhibitors, the inhibitors were added immediately after the neutrophils. The viability of the neutrophils incubated with the protease inhibitors was determined by assaying the supernatants for lactic dehydrogenase (LDH) and by neutrophil exclusion of trypan blue. In addition, the effect of protease inhibitors on primary granule enzyme secretion was monitored by measuring the release of myeloperoxidase.

Elastase Induced Pulmonary Hemorrhage

Male Sprague Dawley rats were anesthetized with chloral hydrate (300 mg/kg) and either saline or human neutrophil elastase was administered intratracheally. Briefly, rats were placed on a surgical board, ventral side up, and their mouths held open using a rubber band placed over their incisors. The tongue was held to the side and 100 micro liters of elastase solution (1 mg/ml) was administered into the trachea with the aid of a bright light source and a 3 inch, 22 gauge blunt-end needle. One hour after elastase administration, the rats were sacrificed using CO_2 and the trachea was surgically exposed and cannulated. The lungs were lavaged with 10 ml of saline and the hemoglobin content was determined using the cyanmethemoglobin assay. In experiments where inhibitors were tested, the compounds were given intratracheally in 100 µl of saline at various times before elastase administration.

RESULTS

Inhibition Of Elastase And Cathepsin G Cleavage Of Synthetic Substrates By Sulfated Polymers

The ability of several sulfated polymers to inhibit elastase and cathepsin G degradation of their respective substrates was examined. MDL-101,028, a compound falling within the scope of the claimed invention, where the value of n is 6, and 4,4'-Diisothiocyanatostilbene-2,2'-disulfonic acid, internally identified as MDL-101,114 (DIDS) inhibited both elastase and cathepsin G in a dose-dependent manner. The IC_{50} for the inhibition of 2 µg of elastase was 40 nM for MDL-101,028(•-•) and 1100 nM for DIDS (□-□) (Fig. 1). Slightly higher concentrations of compounds were required for the inhibition of 2 micrograms of cathepsin G with an IC_{50} of 80 nM and 1400 nM for MDL-101,028(•-•) and DIDS (□-□) respectively (Fig. 2).

Effect Of Sulfated Polymers On Elastase And Cathepsin G Degradation Of Cartilage Matrix Proteoglycan

Cartilage matrix proteoglycan (CPG) was used as a model system for the degradation of macromolecular connective tissue substrate. Both purified human neutrophil elastase and cathepsin G degrade CPG in a dose and time-dependent manner. MDL-101,028 inhibited both elastase (Fig. 3) and cathepsin G (Fig. 4) mediated CPG degradation in a dose-dependent manner with an IC_{50} of approximately 8 and 9 μM, respectively. MDL 101,114 also inhibited elastase (Fig. 3) and cathepsin G (Fig. 4) mediated cartilage degradation with $IC_{50's}$ of 38 and 50 μm, respectively.

Effect Of Sulfated Polymers On Human Neutrophil Lysate Mediated Degradation Of CPG

Human neutrophil lysate at a 1:100 dilution in serumless media degraded CPG by 34% ± 2% (mean + S.E.M., n=3) after a 4 hour incubation at 37°C (Fig. 5). MDL-101,028 inhibited CPG degradation by 54%, 70%, and 79%, at concentrations of 4, 10, and 25 μmolar respectively (Fig. 5).

Effect Of Sulfated Polymers On CPG Degradation Mediated By Human Neutrophils

Human neutrophils in media containing 10% FCS were allowed to settle onto cartilage discs in the wells of microtiter plates and were stimulated with 1 mg/ml of opsonized zymosan and incubated at 37°C for 4 hours in the presence and absence of sulfated polymers. Stimulated neutrophils in the presence of serum antiproteases degraded CPG by 23% plus or minus 2% (± S.E.M., n=4) (Fig. 6). Incubation of stimulated human neutrophils with MDL-101,028 inhibited CPG degradation by 31%, 47%, and 73% at concentrations of 4, 10 and 25 μmolar, respectively.

The viability of the neutrophils was not diminished by treatment with MDL-101,028 as assessed by trypan blue

exclusion and by lactic dehydrogenase release. The percentage of neutrophils stimulated with zymosan in media for 4 hours that excluded trypan blue was 85% compared to 77%-87% for neutrophils incubated with MDL-101,028 at doses of 4, 10, and 25 μM, respectively. This correlated with LDH release which was 9% for stimulated neutrophils treated with media, compared to 7-11% for neutrophils incubated with MDL-101,028 at concentrations of 4-25 μM. MDL-101,028 which did not inhibit CPG degradation by blocking enzyme release from neutrophils as myeloperoxidase release was not inhibited.

MDL-101,028 Inhibits Elastase Induced Lung Hemorrhage In Rats

Intratracheal instillation of human leukocyte elastase produced acute lung injury as assessed by hemorrhage (Fig. 7). The hemorrhage was quantified by measuring the hemoglobin content of the pulmonary lavage fluid. Instillation of 100 μg of neutrophil elastase intratracheally into rats resulted in a bright red hemorrhagic lung lavage fluid that contained 1.7 ±.3 (mean ± SEM, n=12) mg/ml hemoglobin of (Fig. 7). When MDL-101,028 was administered intratracheally immediately before elastase (within 30 seconds) at doses of 280 μg, 700 μg, or 2800 μg, lung hemorrhage was inhibited by 48%, 90%, and 90% respectively (Fig. 7).

MDL-101,028 was administered to rats at various time intervals before instillation of elastase to determine the duration of action. Administration of elastase intratracheally to rats resulted in hemorrhagic lung fluid that contained 1.5 ± .2 (mean ± S.E.M., n=20) mg/ml of hemoglobin (Fig. 8). Administration of MDL-101,028 1, 2, or 4 hours before elastase resulted in the inhibition of lung hemorrhage of 61%, 63%, and 20% respectively (Fig. 8). Therefore, the duration of action by the intratracheal route was greater than two hours and less than four hours.

The compounds of the present invention may be administered by a variety of routes. The compounds may also be administered orally or parenterally (i.e. subcutaneously, intravenously, intramuscularly, intraperitoneally, or intratracheally). More specifically, the present invention provides a method for the treatment of a patient afflicted with pulmonary diseases including adult respiratory distress syndrome (ARDS), chronic bronchitis, cystic fibrosis, emphysema and inflammatory diseases of joint tissues such as arthritis, wherein such disease states may be treated by the administration of an effective inhibitory amount of a compound of the present invention to a patient in need thereof.

A therapeutically effective inhibitory amount of a compound of the present invention refers to an amount which is effective in controlling connective tissue degradation that may be associated with adult respiratory distress syndrome, cystic fibrosis, acute bronchitis, emphysema and arthritis. The term "controlling" is intended to refer to all processes wherein there may be a slowing, interrupting, arresting, or stopping of the progression of the disease and does not necessarily indicate a total elimination of all disease symptoms.

A therapeutically effective inhibitory amount of the compounds used in the treatment described herein can be readily determined by the attending diagnostician, as one skilled in the art, by the use of conventional techniques and by observing results obtained under analogous circumstances. In determining the therapeutically effective dose, a number of factors are considered by the attending diagnostician, including, but not limited to: the species of mammal; its size, age, and general health; the specific disease involved; the degree of or involvement or the severity of the disease; the response of the individual

patient; the particular compound administered; the mode of administration; the bioavailability characteristic of the preparation administered; the dose regimen selected; the use of concomitant medication; and other relevant circumstances.

A therapeutically effective amount of a compound of formula (1) is expected to vary from about 0.1 milligram per kilogram of body weight per day (mg/kg/day) to about 100 mg/kg/day. Preferred amounts are able to be determined by one skilled in the art. One skilled in the art of preparing formulations can readily select the proper form and mode of administration depending upon the particular characteristics of the compound selected the disease state to be treated, the stage of the disease, and other relevant circumstances (Remington's Pharmaceutical Sciences, 18th Edition, Mack Publishing Co. (1990)).

Pharmaceutical compositions can be manufactured utilizing techniques known in the art. Typically a protective amount of the compound will be admixed with a pharmaceutically acceptable carrier.

For oral administration, the compounds can be formulated into solid or liquid preparations such as capsules, pills, tablets, lozenges, melts, powders, suspensions, or emulsions. Solid unit dosage forms can be capsules of the ordinary gelatin type containing, for example, surfactants, lubricants and inert fillers such as lactose, sucrose, and cornstarch or they can be sustained release preparations.

In another embodiment, the compounds of this invention can be tableted with conventional tablet bases such as lactose, sucrose, and cornstarch in combination with binders, such as acacia, cornstarch, or gelatin, disintegrating agents such as potato starch or alginic acid, and a lubricant such as stearic acid or magnesium

stearate. Liquid preparations are prepared by dissolving the active ingredient in an aqueous or non-aqueous pharmaceutically acceptable solvent which may also contain suspending agents, sweetening agents, flavoring agents, and preservative agents as are known in the art.

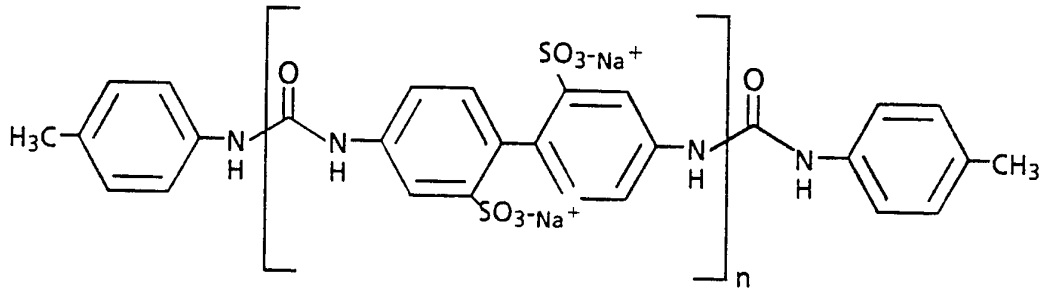
For parenteral administration the compounds may be dissolved in a physiologically acceptable pharmaceutical carrier and administered as either a solution or a suspension. Illustrative of suitable pharmaceutical carriers are water, saline, dextrose solutions, fructose solutions, ethanol, or oils of animal, vegetative, or synthetic origin. The pharmaceutical carrier may also contain preservatives, buffers, etc., as are known in the art.

The compounds of this invention can also be administered topically. This can be accomplished by simply preparing a solution of the compound to be administered, preferably using a solvent known to promote transdermal absorption such as ethanol or dimethyl sulfoxide (DMSO) with or without other excipients. Preferably topical administration will be accomplished using a patch either of the reservoir and porous membrane type or of a solid matrix variety.

As used herein, the term "patient" refers to a warm blooded animal such as a mammal which is afflicted with a particular inflammatory disease state. It is understood that guinea pigs, dogs, cats, rats, mice, horses, cattle, sheep, and humans are examples of animals within the scope of the meaning of the term.

WHAT IS CLAIMED IS:

1. A method of controlling connective tissue degradation by administering to a patient in need thereof a therapeutically effective amount of a compound of the formula:



wherein n is a whole number within the range of 5 to 10 inclusive and the pharmaceutically acceptable salts thereof.

2. A method of controlling connective tissue degradation of claim 1 with said compound wherein n is 6.

3. A method of one of claims 1-2 for controlling connective tissue degradation occurring in adult respiratory distress syndrome.

4. A method of one of claims 1-2 for controlling connective tissue degradation occurring in cystic fibrosis.

5. A method of one of claims 1-2 for controlling connective tissue degradation occurring in acute bronchitis,

6. A method of one of claims 1-2 for controlling connective tissue degradation occurring in emphysema.

7. A method of one of claims 1-2 for controlling connective tissue degradation occurring in arthritis.

8. A method of one of claims 1-2 for controlling connective tissue damage by inhibiting the damage induced by neutrophils

9. A method of one of claims 1-2 for controlling connective tissue damage by inhibiting the damage induced by elastase.

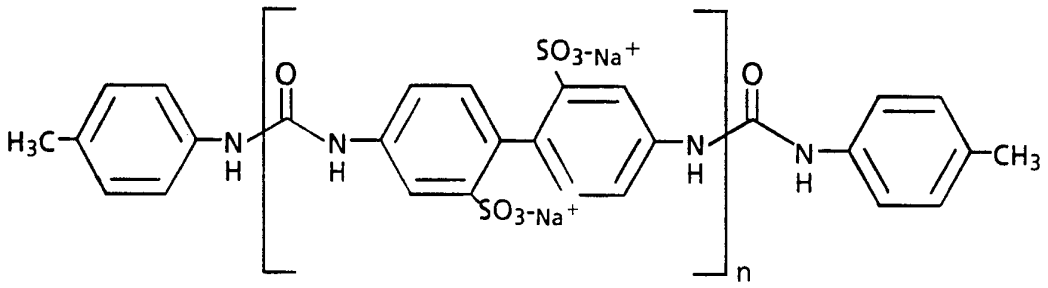
10. A method of one of claims 1-2 for controlling connective tissue damage by inhibiting the damage induced by cathepsin G.

11. A method of one of claims 1-2 for controlling connective tissue degradation using said compounds with pharmaceutically acceptable carrier.

AMENDED CLAIMS

[received by the International Bureau on 19 December 1994(19.12.94); original claims 1-11 replaced by amended claims 1-10 (2 pages)]

1. A pharmaceutical composition for controlling connective tissue degradation comprising a therapeutically effective amount of a compound of the formula:



wherein n is a whole number within the range of 5 to 10 inclusive or pharmaceutically acceptable salts thereof and a pharmaceutically acceptable carrier.

2. A pharmaceutical composition for controlling connective tissue degradation of claim 1 with said compound wherein n is 6.

3. A pharmaceutical composition for controlling connective tissue degradation of claims 1-2 occurring in adult respiratory distress syndrome.

4. A pharmaceutical composition for controlling connective tissue degradation of claims 1-2 occurring in adult respiratory distress syndrome occurring in cystic fibrosis.

5. A pharmaceutical composition for controlling connective tissue degradation of claims 1-2 occurring in acute bronchitis.

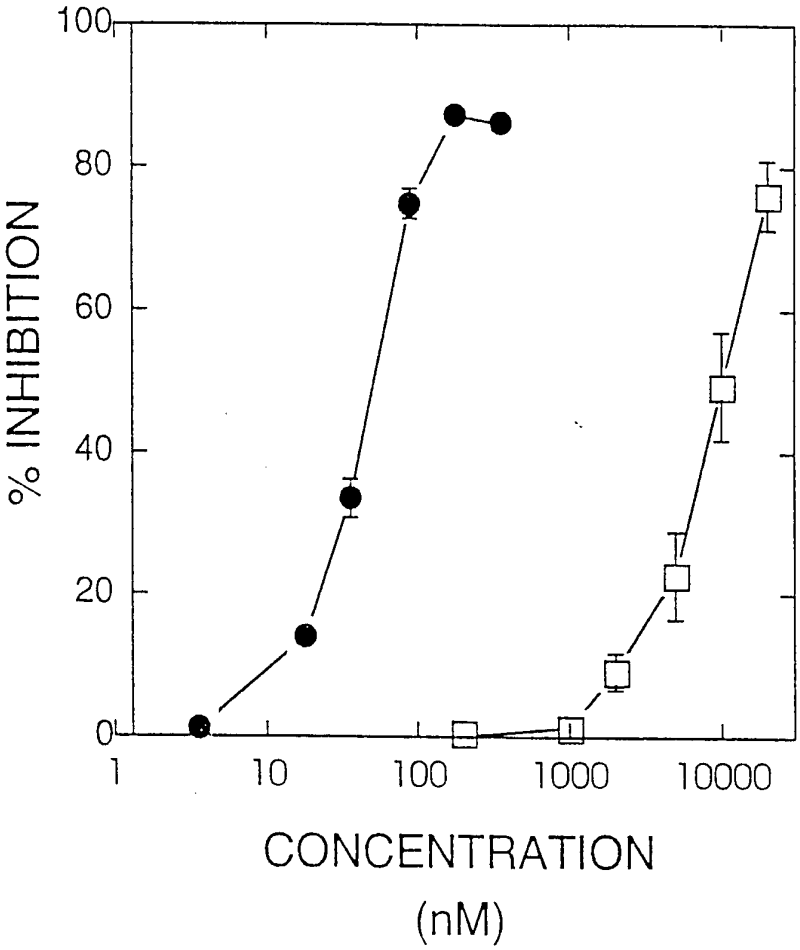
6. A pharmaceutical composition for controlling connective tissue degradation of claims 1-2 occurring in emphysema.

7. A pharmaceutical composition for controlling connective tissue degradation of claims 1-2 occurring in arthritis.

8. A pharmaceutical composition for controlling connective tissue degradation of claims 1-2 by inhibiting the damage induced by neutrophils

9. A pharmaceutical composition for controlling connective tissue degradation of claims 1-2 by inhibiting the damage induced by elastase.

10. A pharmaceutical composition for controlling connective tissue degradation of claims 1-2 by inhibiting the damage induced by cathepsin G.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CONCENTRATION
(nM)

FIGURE 1

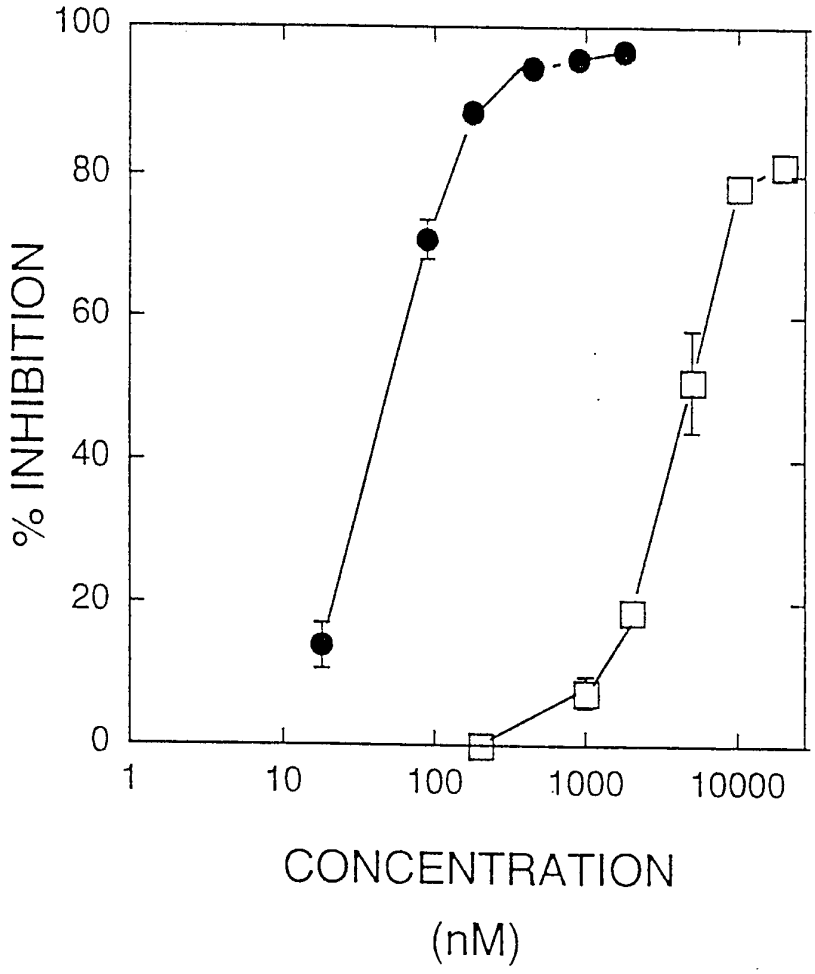
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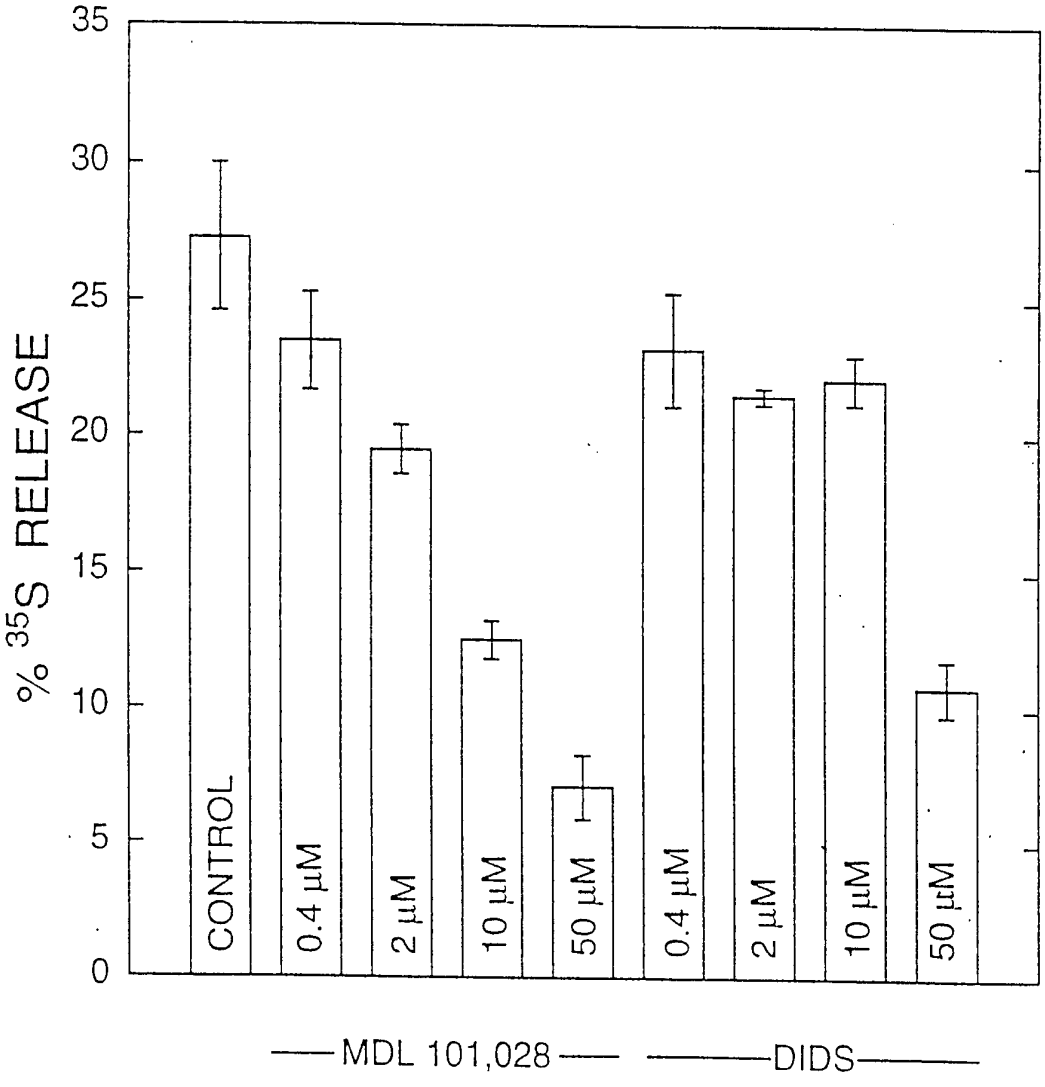
FIGURE 2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0
% ^{35}S RELEASE
CONTROL
0.4 µM
2 µM
10 µM
50 µM
0.4 µM
2 µM
10 µM
50 µM
MDL 101,028
DIDS

FIGURE 3

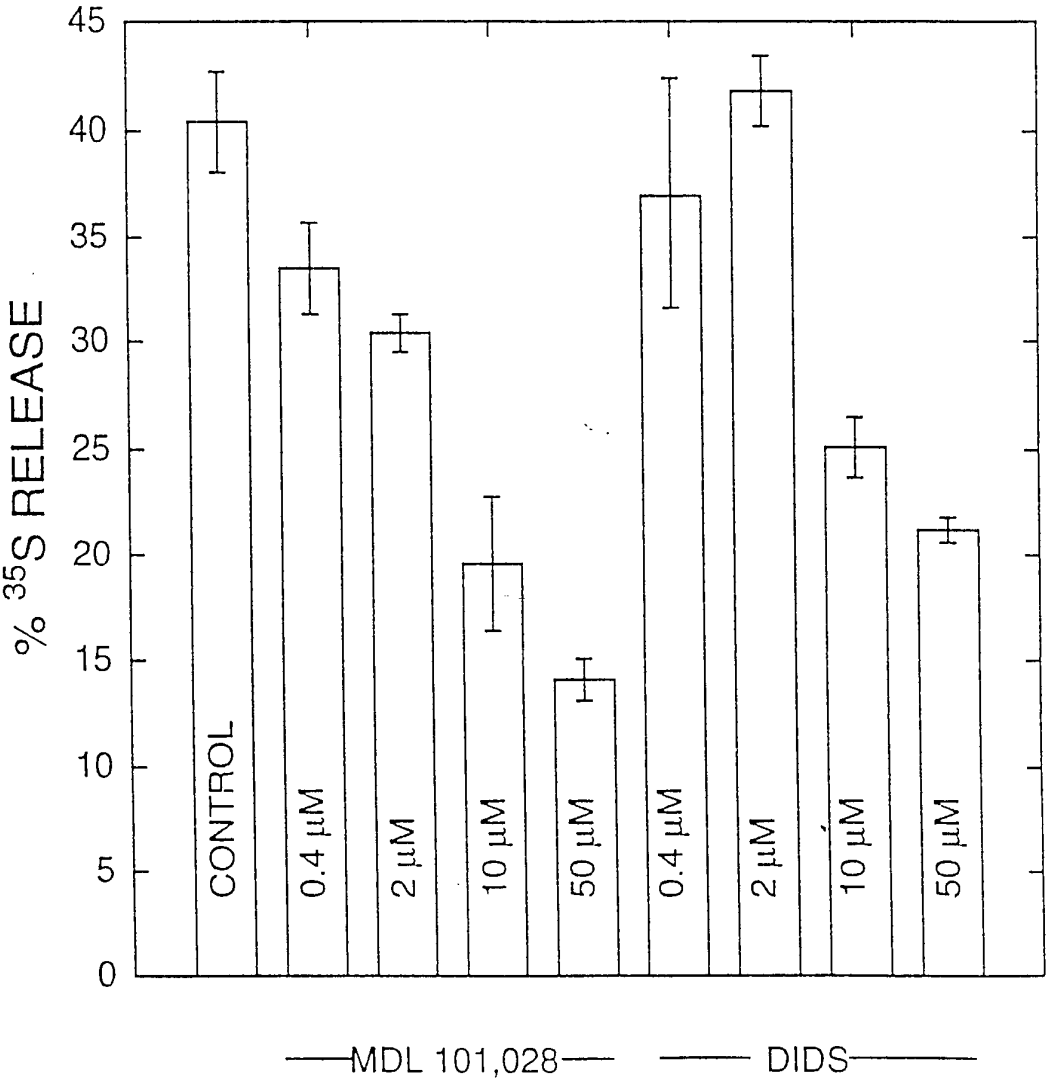
45
40
35
30
25
20
15
10
5
0
% ^{35}S RELEASE
CONTROL
0.4 µM
2 µM
10 µM
50 µM
0.4 µM
2 µM
10 µM
50 µM
MDL 101,028
DIDS

FIGURE 4

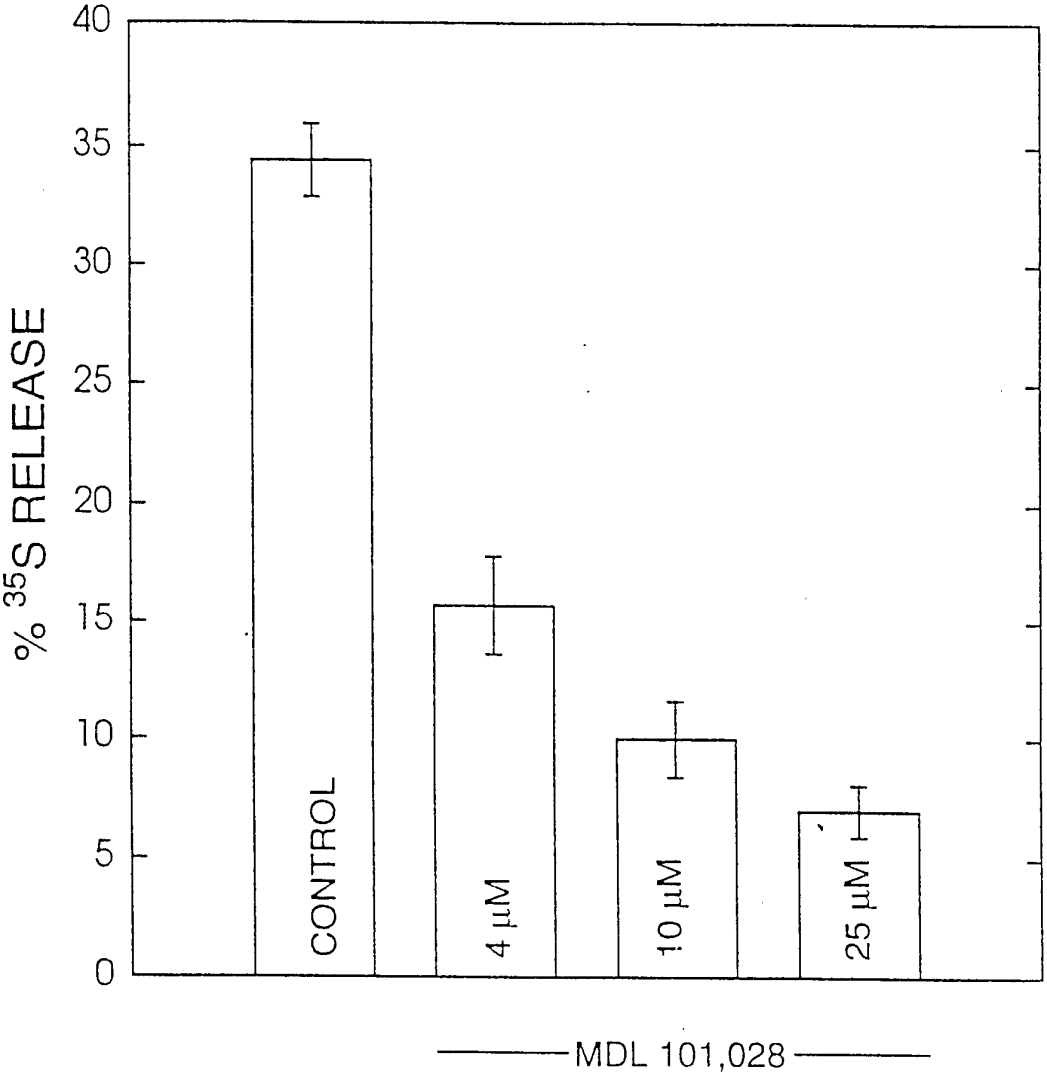
40
35
30
25
20
15
10
5
0
% ^{35}S RELEASE
CONTROL
4 µM
10 µM
25 µM
MDL 101,028

FIGURE 5

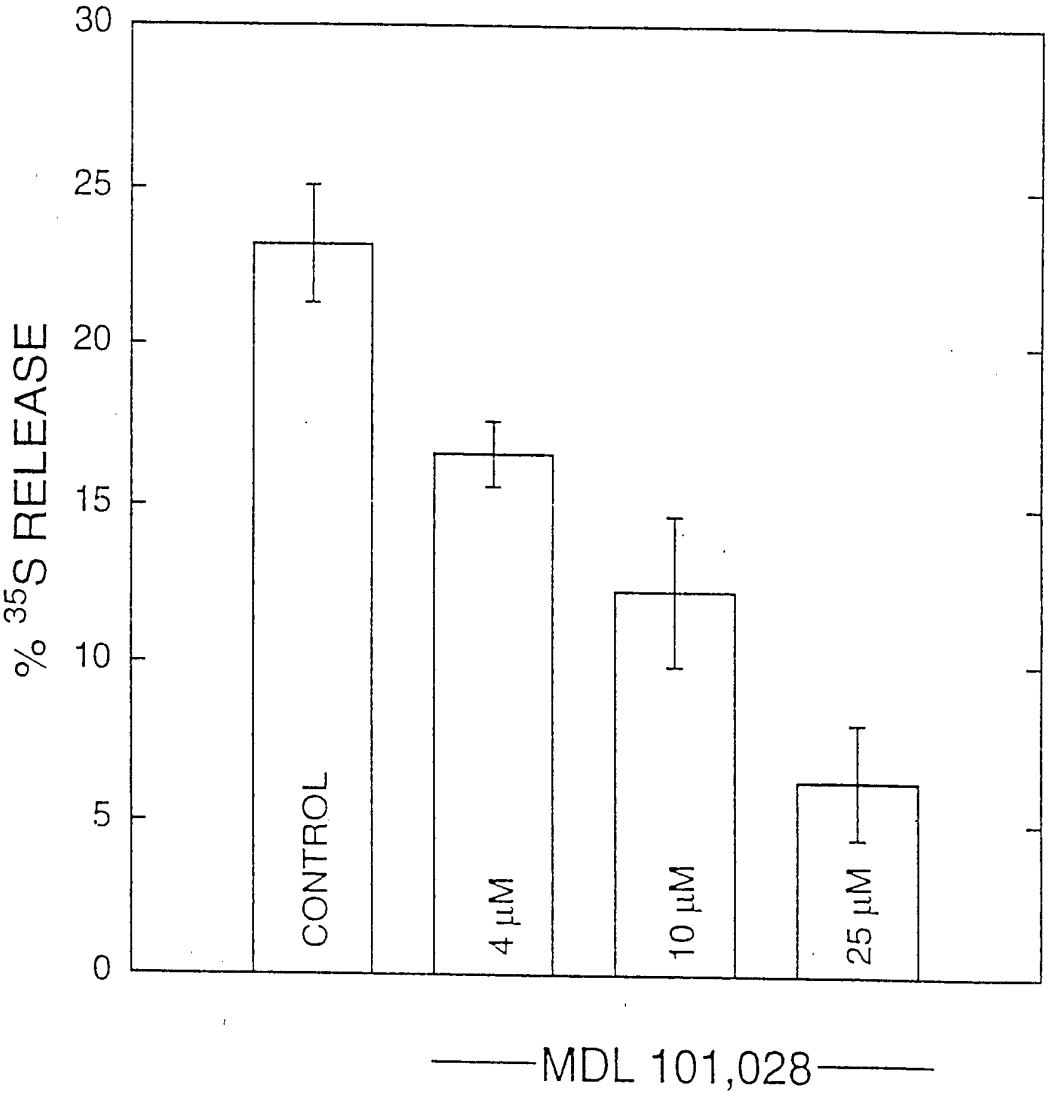

FIGURE 6

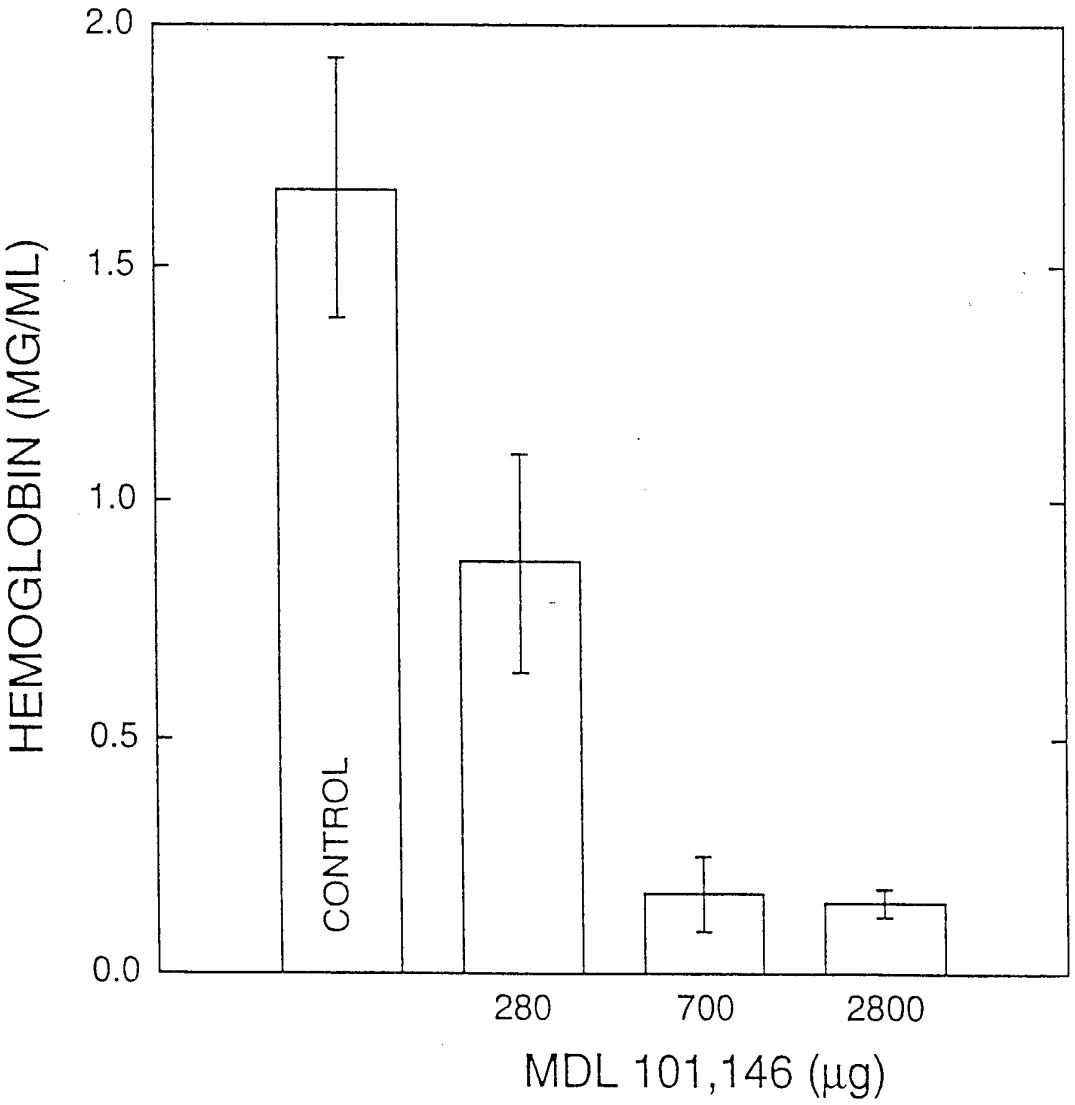
2.0
1.5
1.0
0.5
0.0
HEMOGLOBIN (MG/ML)
CONTROL
280
700
2800
MDL 101,146 (µg)

FIGURE 7

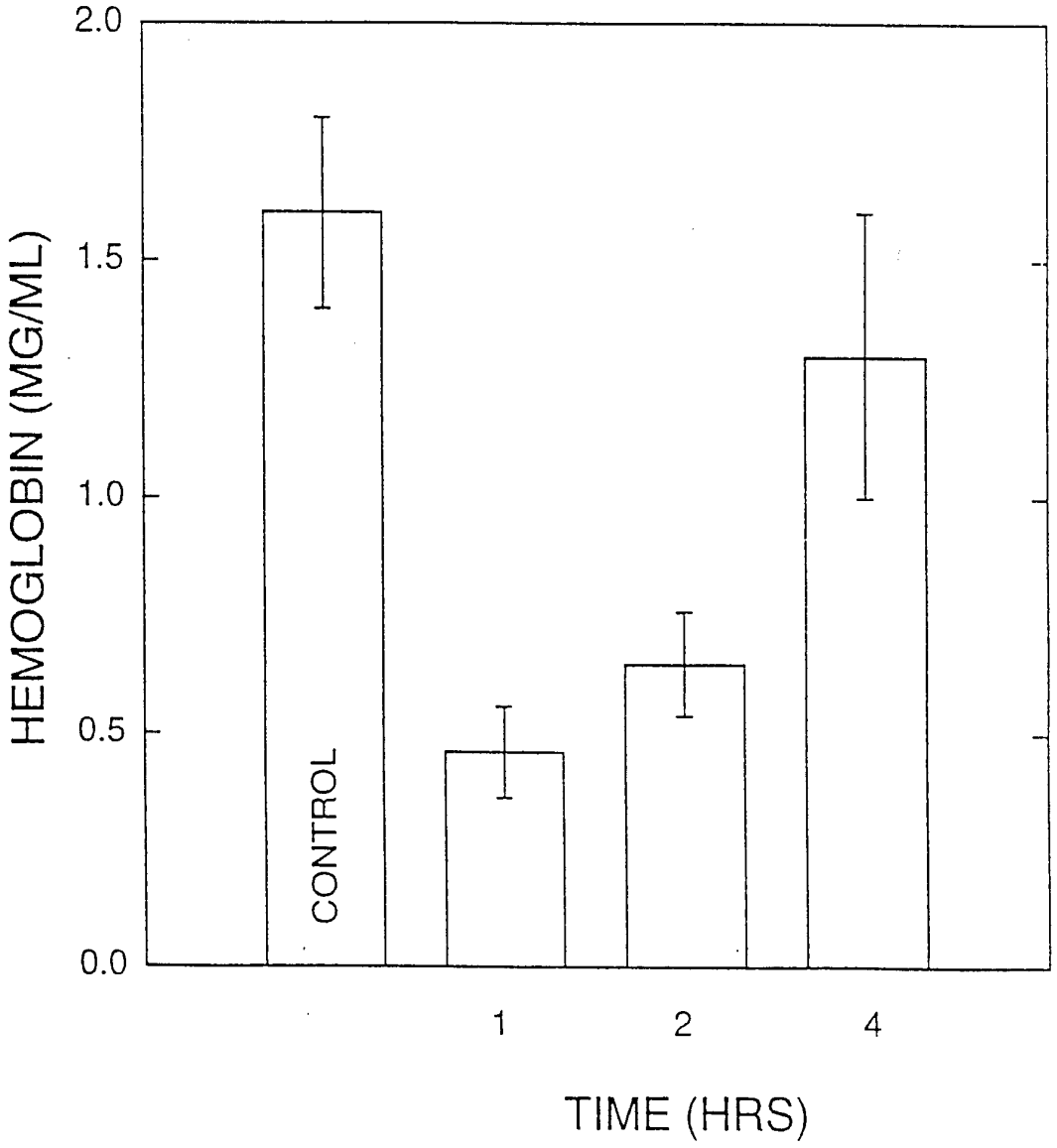

FIGURE 8

INTERNATIONAL SEARCH REPORT

Inter ›nal Application No
PCT/US 94/07696

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/795

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 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.
T	INTERNATIONAL JOURNAL OF IMMUNOPHARMACOLOGY, vol.16, no.8, 1994 pages 623 - 632 M.J. JANUSZ ET AL. 'INHIBITION OF HUMAN NEUTROPHIL ELASTASE AND CATHEPSIN G BY A BIPHENYL DISULFONIC ACID COPOLYMER' see the whole document ---	1-11
Y	J. CELL. BIOCHEM., vol.SUPPL., January 1993 D. TAYLOR,S. TYMS ET AL. see page 365 see page 383 --- -/--	1-11

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

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
14 October 1994	26. 10. 94

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	Hoff, P

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	THE BIOCHEMICAL JOURNAL, vol.252, no.2, 1988 pages 515 - 519 F. REDINI ET AL. 'INHIBITION OF LEUCOCYTE ELASTASE BY HEPARIN AND ITS DERIVATIVE' cited in the application see the whole document	1-11
Y	AMERICAN REVIEW OF RESPIRATORY DISEASE, vol.142, no.2, 1990 pages 407 - 412 N.V. RAO ET AL. 'SULFATED POLYSACCHARIDES PREVENT HUMAN LEUKOCYTE ELASTASE-INDUCED ACUTE LUNG INJURY AND EMPHYSEMA IN HAMSTERS' see the whole document	1-11
Y	EP,A,0 208 623 (SANOFI) 14 January 1987 see abstract see page 1, line 1 - page 5, line 12 see page 12, line 13 - line 36; claims; example 7	1-11
A	EP,A,0 467 185 (THE DOW CHEMICAL COMPANY) 22 January 1992 cited in the application see abstract; claims 1-11,18-21,38-46; example 1; tables I-II,IV-VIII	1-11
A	EP,A,0 465 895 (FUJISAWA) 15 January 1992 see abstract see page 3, line 1 - line 4 see page 10, line 57 - page 13, line 11; claims 1,8-15	1-11

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/07696

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

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

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark : Although claims 1-11 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

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

Inter nal Application No
PCT/US 94/07696

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0208623	14-01-87	FR-A-	2584606	16-01-87
		JP-A-	62018401	27-01-87
EP-A-0467185	22-01-92	US-A-	5276182	04-01-94
		AU-B-	635850	01-04-93
		AU-A-	8024291	09-01-92
		AU-B-	650281	16-06-94
		AU-A-	8286791	04-02-92
		CA-A-	2046491	10-01-92
		CN-A-	1058959	26-02-92
		EP-A-	0538373	28-04-93
		JP-A-	4226521	17-08-92
		JP-T-	6500535	20-01-94
		WO-A-	9200749	23-01-92
EP-A-0465895	15-01-92	AU-B-	640241	19-08-93
		AU-A-	7939491	09-01-92
		CN-A-	1057655	08-01-92
		JP-A-	4279600	05-10-92
		US-A-	5292510	08-03-94