PCT

WORLD INTELECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6:
C07K 5/08, 5/06, A61K 31/41

(11) International Publication Number:
WO 98/25949

(43) International Publication Date:
18 June 1998 (18.06.98)

(21) International Application Number:
PCT/US97/22534

(22) International Filing Date:
9 December 1997 (09.12.97)

(30) Priority Data:
08/762,503 9 December 1996 (09.12.96) US


(72) Inventors; and


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: SUBSTITUTED 5-AMINO-1,3,4-THIADIAZOLE-2-THIONES

(57) Abstract

The use of N5-substituted 5-amino-1,3,4-thiadiazole-2-thiols as metalloproteinases to inhibit matrix metalloproteinase enzymes and cartilage degradation is disclosed. Methods of treating diseases caused by over-activity of matrix metalloproteinases, such as osteoarthritis and rheumatoid arthritis, are also disclosed.
### 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.

<table>
<thead>
<tr>
<th>Code</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>AL</td>
<td>Albania</td>
</tr>
<tr>
<td>AM</td>
<td>Armenia</td>
</tr>
<tr>
<td>AT</td>
<td>Austria</td>
</tr>
<tr>
<td>AU</td>
<td>Australia</td>
</tr>
<tr>
<td>AZ</td>
<td>Azerbaijan</td>
</tr>
<tr>
<td>BA</td>
<td>Bosnia and Herzegovina</td>
</tr>
<tr>
<td>BB</td>
<td>Barbados</td>
</tr>
<tr>
<td>BE</td>
<td>Belgium</td>
</tr>
<tr>
<td>BF</td>
<td>Burkina Faso</td>
</tr>
<tr>
<td>BG</td>
<td>Bulgaria</td>
</tr>
<tr>
<td>RJ</td>
<td>Benin</td>
</tr>
<tr>
<td>BR</td>
<td>Brazil</td>
</tr>
<tr>
<td>BY</td>
<td>Belarus</td>
</tr>
<tr>
<td>CA</td>
<td>Canada</td>
</tr>
<tr>
<td>CF</td>
<td>Central African Republic</td>
</tr>
<tr>
<td>CG</td>
<td>Congo</td>
</tr>
<tr>
<td>CH</td>
<td>Switzerland</td>
</tr>
<tr>
<td>CI</td>
<td>Côte d'Ivoire</td>
</tr>
<tr>
<td>CM</td>
<td>Cameroon</td>
</tr>
<tr>
<td>CN</td>
<td>China</td>
</tr>
<tr>
<td>CU</td>
<td>Cuba</td>
</tr>
<tr>
<td>CZ</td>
<td>Czech Republic</td>
</tr>
<tr>
<td>DE</td>
<td>Germany</td>
</tr>
<tr>
<td>DK</td>
<td>Denmark</td>
</tr>
<tr>
<td>EE</td>
<td>Estonia</td>
</tr>
<tr>
<td>ES</td>
<td>Spain</td>
</tr>
<tr>
<td>FI</td>
<td>Finland</td>
</tr>
<tr>
<td>FR</td>
<td>France</td>
</tr>
<tr>
<td>GA</td>
<td>Gabon</td>
</tr>
<tr>
<td>GB</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>GE</td>
<td>Georgia</td>
</tr>
<tr>
<td>GH</td>
<td>Ghana</td>
</tr>
<tr>
<td>GN</td>
<td>Guinea</td>
</tr>
<tr>
<td>GR</td>
<td>Greece</td>
</tr>
<tr>
<td>HU</td>
<td>Hungary</td>
</tr>
<tr>
<td>IE</td>
<td>Ireland</td>
</tr>
<tr>
<td>IL</td>
<td>Israel</td>
</tr>
<tr>
<td>IS</td>
<td>Iceland</td>
</tr>
<tr>
<td>IT</td>
<td>Italy</td>
</tr>
<tr>
<td>JP</td>
<td>Japan</td>
</tr>
<tr>
<td>KE</td>
<td>Kenya</td>
</tr>
<tr>
<td>KG</td>
<td>Kyrgyzstan</td>
</tr>
<tr>
<td>KP</td>
<td>Democratic People's Republic of Korea</td>
</tr>
<tr>
<td>CM</td>
<td>Cameroon</td>
</tr>
<tr>
<td>CN</td>
<td>China</td>
</tr>
<tr>
<td>KZ</td>
<td>Kazakhstan</td>
</tr>
<tr>
<td>LC</td>
<td>Saint Lucia</td>
</tr>
<tr>
<td>LI</td>
<td>Liechtenstein</td>
</tr>
<tr>
<td>LK</td>
<td>Sri Lanka</td>
</tr>
<tr>
<td>LR</td>
<td>Liberia</td>
</tr>
<tr>
<td>LS</td>
<td>Lesotho</td>
</tr>
<tr>
<td>LT</td>
<td>Lithuania</td>
</tr>
<tr>
<td>LU</td>
<td>Luxembourg</td>
</tr>
<tr>
<td>LV</td>
<td>Latvia</td>
</tr>
<tr>
<td>MC</td>
<td>Monaco</td>
</tr>
<tr>
<td>MD</td>
<td>Republic of Moldova</td>
</tr>
<tr>
<td>MG</td>
<td>Madagascar</td>
</tr>
<tr>
<td>MK</td>
<td>The former Yugoslav Republic of Macedonia</td>
</tr>
<tr>
<td>ML</td>
<td>Mali</td>
</tr>
<tr>
<td>MN</td>
<td>Mongolia</td>
</tr>
<tr>
<td>MR</td>
<td>Mauritania</td>
</tr>
<tr>
<td>MW</td>
<td>Malawi</td>
</tr>
<tr>
<td>MX</td>
<td>Mexico</td>
</tr>
<tr>
<td>NE</td>
<td>Niger</td>
</tr>
<tr>
<td>NL</td>
<td>Netherlands</td>
</tr>
<tr>
<td>NO</td>
<td>Norway</td>
</tr>
<tr>
<td>NZ</td>
<td>New Zealand</td>
</tr>
<tr>
<td>PL</td>
<td>Poland</td>
</tr>
<tr>
<td>PT</td>
<td>Portugal</td>
</tr>
<tr>
<td>RO</td>
<td>Romania</td>
</tr>
<tr>
<td>RU</td>
<td>Russian Federation</td>
</tr>
<tr>
<td>SD</td>
<td>Sudan</td>
</tr>
<tr>
<td>SE</td>
<td>Sweden</td>
</tr>
<tr>
<td>SG</td>
<td>Singapore</td>
</tr>
<tr>
<td>SI</td>
<td>Slovenia</td>
</tr>
<tr>
<td>SK</td>
<td>Slovakia</td>
</tr>
<tr>
<td>SN</td>
<td>Senegal</td>
</tr>
<tr>
<td>SZ</td>
<td>Swaziland</td>
</tr>
<tr>
<td>TD</td>
<td>Chad</td>
</tr>
<tr>
<td>TG</td>
<td>Togo</td>
</tr>
<tr>
<td>TJ</td>
<td>Tajikistan</td>
</tr>
<tr>
<td>TM</td>
<td>Turkmenistan</td>
</tr>
<tr>
<td>TR</td>
<td>Turkey</td>
</tr>
<tr>
<td>TT</td>
<td>Trinidad and Tobago</td>
</tr>
<tr>
<td>UA</td>
<td>Ukraine</td>
</tr>
<tr>
<td>UG</td>
<td>Uganda</td>
</tr>
<tr>
<td>US</td>
<td>United States of America</td>
</tr>
<tr>
<td>UZ</td>
<td>Uzbekistan</td>
</tr>
<tr>
<td>VN</td>
<td>Viet Nam</td>
</tr>
<tr>
<td>YU</td>
<td>Yugoslavia</td>
</tr>
<tr>
<td>ZW</td>
<td>Zimbabwe</td>
</tr>
</tbody>
</table>
Substituted 5-Amino-1,3,4-Thiadiazole-2-Thiones

Background of the Invention

Matrix metalloproteinases are a class of zinc-dependent, proteolytic enzymes. These enzymes play a role in a number of disease processes.

Increased levels of collagenase and stromelysin have been observed in synovium and cartilage in several arthritic diseases (Dean, et al., J. Clin. Invest., 84:678 (1989) and the levels correlate with the severity and advancement of the disease (Blanckaert, et al., Clin. Chim. Acta, 185:73 and Valakovits, et al., Arthr. Rheum., 35:35 (1992)). They have also been linked to cartilage matrix degradation (Brown, et al., J. Med. Chem., 37:674 (1994) and Gordon, et al. Clin. Exp. Rheumatol. 11 (Supplement 8):S91 (1993)). Matrix metalloproteinases also contribute to cartilage degradation by cleaving α1-antiproteinase inhibitor-1, thereby removing its ability to inactivate human neutrophil elastase. Furthermore, it has been shown in vivo that inhibitors of matrix metalloproteinases are able to inhibit angiogenesis (Garlardy, et al., Cancer Research, 54:4715 (1994)), i.e. the formation of new blood vessels. Although angiogenesis occurs in normal processes, such as ovulation, placental development and wound healing, it is also involved in pathological processes such as arthritis and inflammation (D’Armore, et al., Ann. Rev. Physiol., 49:453 (1987)).

Many members of the metalloproteinase family were originally described in malignant cell lines and appear to play a role in tumor metastasis (Liotta, and Rao, Lab Invest., 49:636-649 (1983)). For example, certain small molecular weight inhibitors of metalloproteinases inhibit the growth of human tumor cells in nude mice (Naito et al., Int. J. Cancer, 58:730-735 (1994)). In addition, angiogenesis is also involved in tumor malignancy (D’Armore, et al.).

Many of the pathological processes associated with the diseases could be slowed, arrested or even reversed if the activity of the matrix metalloproteinases responsible for the pathological processes could be inhibited. Although there are known inhibitors of matrix metalloproteinases, e.g. peptidyl hydroxamates, they exhibit poor bioavailability and are therefore unable to significantly modify the progression of osteoarthritis and other diseases. Consequently, there is a need for new inhibitors of matrix metalloproteinases which can be used as therapeutics.

**Summary of the Invention**

The present invention is broadly directed to a method for inhibiting matrix metalloproteinases by contacting a matrix metalloproteinase with an effective amount of an N5-substituted 5-amino-1,3,4-thiadiazole-2-thione, 5-amino-1,3,4-thiadiazole-2-one or 5-amino-1,3,4-oxadiazole-2-thione having Formula I, as described herein. Investigation has shown that the 5-amino position of one of 5-amino-1,3,4-thiadiazole-2-thione, 5-amino-1,3,4-thiadiazole-2-one, or 5-amino-1,3,4-oxadiazole-2-thiol can be covalently attached to a variety of organic moieties and the resulting compounds have the ability to bind to and inhibit matrix metalloproteinases.

Another aspect of the present invention is directed to compositions useful for inhibiting a matrix metalloproteinase. The compositions comprise a compound having the Formula I, and a carrier or diluent.

Yet another aspect of the present invention is a method of treating a disease in an individual or animal which can be ameliorated by inhibiting at least one matrix metalloproteinase. The method comprising administering to the individual or animal a therapeutically effective amount of a compound having the structure of Formula I.

Substituted 5-amino-1,3,4-thiadiazole-2-thiones and their analogs of the present invention are useful for treating individuals and animals with diseases resulting from over activity of matrix metalloproteinases, such as osteoarthritis,
rheumatoid arthritis, cancer and the inflammation associated with many of these diseases. The thiazole derivatives of the present invention have other in vivo uses, such as aiding in identifying the location of matrix metalloproteinases in an individual or animal. These thiazole compounds are also useful in vitro for preventing the degradation of tissue and proteins present in biological samples containing matrix metalloproteinases, as an aid in identifying new drug targets for the treatment of these diseases and in isolating matrix metalloproteinases.

**Detailed Description of the Preferred Embodiments**

The present invention relates to novel compositions comprising inhibitors of matrix metalloproteinases. Known inhibitors of matrix metalloproteinases comprise an oligopeptide bound to a functional group such as a hydroxamic acid or thiol which can chelate the zinc atom in the active site of the matrix metalloproteinase. It has now been found that 5-substituted 1,3,4-thiadiazol-2-thiones and their analogs, as defined by Formula I which are capable of chelating zinc can also inhibit matrix metalloproteinases.

The 5-amino position of one of 5-amino-1,3,4-thiadiazole-2-thione, 5-amino-1,3,4-thiadiazole-2-one, or 5-amino-1,3,4-oxadiazole-2-thione can be covalently attached to a variety of organic moieties and the resulting compounds have the ability to bind to and inhibit matrix metalloproteinases. Additionally, Applicants have discovered that the 2-thiol substituent of the tautomeric N5-substituted, 5-amino-1,3,4-thiadiazole-2-thiols of the present invention can form disulfide linkages with one another, thereby forming dimers. These disulfide linked dimers also exhibit metalloproteinase inhibition activity when screened in the assays described herein. Without wishing to be bound by theory, it is postulated that the 1,3,4-thiadiazole-2-thione, and/or its tautomers interact with the zinc atom that is present at the active site of metalloproteinases. Thus, diverse organic radicals can be attached to the 5-amino-1,3,4-thiadiazole-2-thione, as long as the radical does not diminish the ability of the 1,3,4-thiadiazole-2-thione
to interact with the active site of matrix metalloproteinases. Applicants have discovered that the structural requirements to bind to the active site of matrix metalloproteinases are not very stringent.

Thus, a first aspect of the present invention is directed to a method for inhibiting matrix metalloproteinases, comprising contacting a matrix metalloproteinase with a compound of Formula I:

\[
\begin{align*}
Z &\quad \text{N} \\
&\quad \text{A} \\
&\quad \text{Q}
\end{align*}
\]

or a pharmaceutically acceptable salt thereof, wherein

Q and A are each independently selected from the group consisting of sulfur and oxygen and one of Q and A is sulfur;

R1 is one of hydrogen, lower alkyl or acyl; and

Z represents an organic radical that does not substantially interfere with the ability of the group

\[
\begin{align*}
&\quad \text{N} \\
&\quad \text{A} \\
&\quad \text{Q}
\end{align*}
\]

(IV) to inhibit a matrix metalloproteinase, provided that said compound is other than a compound of Formula II herein, where R4 is hydrogen. Preferred compounds include compounds where A and Q are both sulfur.

A further aspect of the present invention provides pharmaceutical compositions, comprising a pharmaceutically acceptable carrier or diluent, and an amount effective to inhibit a matrix metalloproteinase of a compound of Formula I wherein A, Q, Z and R1 are defined as above; provided that said compound is not a compound of Formula II herein, where R4 is hydrogen; and
further provided that said compound is not a compound of Formula \textit{VI} or Formula \textit{VII} herein.

The present invention provides the use of a compound of Formula \textit{I}, or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for the treatment and/or prevention of conditions, in particular disorders, such as collagen degradation or abnormal angiogenesis, which require the administration of a selective inhibitor of matrix metalloproteinases.

The language "does not substantially interfere with the ability of the group

\[
\begin{align*}
\text{N} & \quad \text{N} \\
\text{A} & \quad \text{Q}
\end{align*}
\]  

(\textit{IV}) to inhibit a matrix metalloproteinase," for purposes of the present invention means that a compound resulting from the attachment of a particular organic radical to an oxa- or thia-diazole ring (Formula \textit{IV}) exhibits matrix metalloproteinase inhibition activity, as measured in one of the biological assays described hereinbelow (Examples 38-41). Preferably, the compounds of the invention have an IC\textsubscript{50} of 50 \(\mu\text{M}\) or less, more preferably 5 \(\mu\text{M}\) or less, as measured in one of the assays described in Examples 38-41.

Useful organic radicals "\textit{Z}" include straight-chained, branched and cyclic groups, including heterocyclic groups, containing up to 25 carbon atoms, suitably up to 15 carbon atoms, and conveniently up to 12 carbon atoms, wherein said heterocyclic groups can include from 1 to 5, preferably, 1 to 3 heteroatoms independently selected from the group consisting of oxygen, nitrogen, sulfur, and wherein said radicals can be optionally substituted. Suitable organic radicals include \(\text{C}_{1-12}\) alkyl, \(\text{C}_{2-10}\) alkenyl, \(\text{C}_{2-10}\) alkynyl, \(\text{C}_{3-8}\) cycloalkyl, \(\text{C}_{3-7}\) cycloalkyl(\(\text{C}_{1-6}\))alkyl, \(\text{C}_{3-8}\) cycloalkyl(\(\text{C}_{2-6}\))alkenyl, aryl, aryl(\(\text{C}_{2-6}\))alkyl, aryl(\(\text{C}_{2-6}\))alkynyl, \(\text{C}_{3-7}\) heterocycloalkyl, \(\text{C}_{3-8}\) heterocycloalkyl(\(\text{C}_{1-6}\))alkyl, heteroaryl, heteroaryl(\(\text{C}_{1-6}\))alkyl,
heteroaryl(C<sub>2</sub>-<sub>6</sub>)alkenyl. The cyclic and heterocyclic groups can also be part of a fused or spiro ring system having from one to four rings.

The organic radical, may in turn, be optionally substituted by one or more groups selected from hydroxy, nitro, trifluoromethyl, halogen,

halo(C<sub>1</sub>-<sub>4</sub>)alkyl, C<sub>6</sub>-alkoxy, C<sub>4</sub>-alko(C<sub>6</sub>-alkoxy, adamantyl, aryl, benzyl, aryloxy, heteroaryl, heterocycloalkyl, keto, C<sub>1</sub>-<sub>3</sub> alkylenedioxo, cyano, carboxy, C<sub>2</sub>-<sub>6</sub> alkoxycarbonyl, C<sub>2</sub>-<sub>6</sub> alkoxycarbonyl(C<sub>6</sub>-alkyl, C<sub>2</sub>-<sub>6</sub> alky carbonyl, optionally substituted arylcarbonyl, C<sub>1</sub>-<sub>6</sub> alklythiol, C<sub>1</sub>-<sub>6</sub> alky sulfonyl, arythio, amino, mono- or di(C<sub>1</sub>-<sub>6</sub>)alkylamino, C<sub>2</sub>-<sub>6</sub>
alquilcarbonylamino, C<sub>2</sub>-<sub>6</sub> alkoxy carbonylamino, C<sub>2</sub>-<sub>6</sub>
alkoxy carbonylamino(C<sub>1</sub>-<sub>6</sub>)alkyl, amino(C<sub>1</sub>-<sub>6</sub>) alk oxy, amino(C<sub>1</sub>-<sub>6</sub>)alkyl, hydroxy(C<sub>1</sub>-<sub>6</sub>)alkyl, hydrox y(C<sub>1</sub>-<sub>6</sub>)alkoxy, C<sub>2</sub>-<sub>6</sub> carboxyalkyl, C<sub>2</sub>-<sub>6</sub>
carboxyalkoxy, mono((C<sub>1</sub>-<sub>6</sub>)hydroxyalkyl)amino, di((C<sub>1</sub>-<sub>6</sub>)hydroxyalkyl)amino, mono(carboxy(C<sub>1</sub>-<sub>6</sub>)alkyl)amino, di(carboxy(C<sub>1</sub>-<sub>6</sub>)alkyl)amino, C<sub>1</sub>-<sub>6</sub>
alkoxy carbonylamino, C<sub>1</sub>-<sub>6</sub> alkoxy carbonyl, aryl(C<sub>2</sub>-<sub>6</sub>)al koxy carbonyl, C<sub>3</sub>-<sub>6</sub> alkenyl carbonyl, C<sub>3</sub>-<sub>6</sub> al kynyl carbonyl, carboxy(C<sub>2</sub>-<sub>6</sub>)alkoxy carbonyl, C<sub>2</sub>-<sub>6</sub>
alkanoyl, C<sub>2</sub>-<sub>6</sub> alkoxycarbonyl, phenyl(C<sub>2</sub>-<sub>6</sub>)ALKOXY CARBONYL, carbamoyl, C<sub>2</sub>-<sub>6</sub>
alkylcarbamoyl, hydroxy(C<sub>2</sub>-<sub>6</sub>)alkylcarbamoyl, di((C<sub>2</sub>-<sub>6</sub>)alkyl)carbamoyl, amino(C<sub>2</sub>-<sub>6</sub>)alkylcarbamoyl, cyc loalkylcarbamoyl,
cycloalkyl(C<sub>2</sub>-<sub>6</sub>)alkyl car bamoyl, N-hydroxy carbamoyl, N-(C<sub>2</sub>-<sub>6</sub>)alk enyloxy carbamoyl, phosphono, C<sub>1</sub>-<sub>6</sub> alkylphosphono, di((C<sub>1</sub>-<sub>6</sub>)alkyl)phosphono, tri((C<sub>1</sub>-<sub>6</sub>)alkyl)alkylphosphono, C<sub>1</sub>-<sub>6</sub> alky sulfonyl, C<sub>2</sub>-<sub>6</sub> alkenylsulfonyl, C<sub>2</sub>-<sub>6</sub> alkynyl sulfonyl, C<sub>1</sub>-<sub>6</sub> alky sulfinyl, arythio, C<sub>1</sub>-<sub>6</sub> al kylthio, C<sub>1</sub>-<sub>6</sub> alkyl sulfonamido, amidino, guanidino, C<sub>1</sub>-<sub>6</sub> alkyliminoamino,
formyliminoamino, trifluoromethoxy or perfluoroethoxy, and when said radical is other than alkyl, said radical may also be substituted with one or two lower alkyl moieties.

The organic radical can be covalently linked to the 5-amino nitrogen through an amide, amine, sulfonamide, urea, thiourea, carbamate,
thiocarbamate, sulfinamide (-S(O)NR<sub>1</sub> -), sulfenamide (-SNR<sub>1</sub> -),
phosphonamide or phosphinamide bond. Preferred compounds have an amide, amine, sulfonamide, carbamate, thiocarbamate, sulfinamide, phosphonamide or phosphinamide with an amide linkage between the 5-amino moiety of the heterocyclic thiadiazole group and the organic radical being most preferred. Thus, most preferred organic radicals include C_{1-12} alkanoyl, C_{3-18} alkenoyl, C_{3-18} alkynoyl, C_{3-7} cycloalkyl(C_{1-6})alkanoyl, aryl(C_{2-6})alkanoyl, C_{3-8} heterocycloalkyl(C_{1-6})alkanoyl, and heteroaryl(C_{1-6})alkanoyl. The cyclic and heterocyclic groups can also be part of a fused or spiro ring system having from one to four rings.

Examples of useful radicals that can be employed as "Z" include: steroids, amino acids, oligopeptides, and moieties commonly referred to as amino protecting groups. Examples of amino protecting groups include, but are not limited to, 9-fluorenyl methoxycarbonyl, t-butoxycarbonyl, (4-phenyl)phenylacetyl, 8-quinolinesulfonyl, 2-methylthionicotyl, xanthene-9-carbonyl, hydrocinamoyl, phenylbenzoyl, nonanoyl, (4-benzyloxy)benzoyl, acetyl and (4-(4-t-butylnaphthylsulfonamino)benzoyl. Amino protecting groups are more fully described in Greene, T.W. and Wuts, P.G.M., *Protective Groups in Organic Synthesis*, 2nd edition, John Wiley and Sons, Inc. New York (1991).

In a preferred embodiment, compounds of Formula I are other than thiadiazolyl(thio)urea derivatives represented by Formula VI:

\[
\begin{align*}
\text{VI} & \\
\end{align*}
\]

or pharmaceutical acceptable salts thereof wherein:

X is O or S;
R₁ is H, C₁₋₅ alkyl, -(CH₂)ₓ-aryl, -(CH₂)ₓ-cycloalkyl, -(C₁₋₅ alkyl)-O-R₄, -(C₁₋₅ alkyl)-S-R₄, -(CH₂)ₓ-Het, -C(=O)-O-R₄, -C(=O)-NR₃R₅, or -(CH₂)ₓ-O-Si(R₄)ₓ;  
R₂ is -O-R₅, or -NR₆R₇;  
R₃ is H, C₁₋₅ alkyl, -(CH₂)ₓ-aryl, -(CH₂)ₓ-cycloalkyl, -(C₁₋₅ alkyl)-O-R₄, -(C₁₋₅ alkyl)-S-R₄, or -OR₆;  
R₄ is H, C₁₋₅ alkyl, or -(CH₂)ₓ-aryl;  
R₅ is H, C₁₋₅ alkyl, or aryl;  
R₆ and R₇ may be the same or differently, H, C₁₋₅ alkyl, C₁₋₅ OR₄, -(CH₂)ₓ-aryl, -(CH₂)ₓ-cycloalkyl, -(CH₂)ₓ-Het, -(CH₂)ₓ-Q, -(CH₂)ₓ-C(=O)-OR₄, -(CH₂)ₓ-C(=O)-NR₃R₅, 5-(((5-(dimethylamino)-1-naphthalenyl)sulfonyl)amino)pentyl, or R₆ and R₇ taken together with the linking N-atom to form azetidinyl, pyrrolidinyl, piperidinyl, morpholino, 4-thiomorpholiny, or

\[
\text{\textbullet-N-N-R₈}
\]

R₈ is H, C₁₋₅ alkyl, -(CH₂)ₓ-aryl, benzhydryl, or -(CH₂)ₓ-Het;  
aryl is phenyl, biphenyl, or naphthalene, optionally substituted with one to five of the following: C₁₋₅ alkyl, -OR₄, halogen, -NR₃R₅, -C(=O)-NR₃R₅, -NHC(=O)R₆, -SO₂NR₃R₅, -NHSO₂R₅, -NO₂, -CF₃, or -O-Si(R₄)ₓ;  
Het is a 5-, 6-, 9-, or 10-membered heteroaromatic moiety having one or more atoms selected from the group consisting of N, O, and S;  
Q is a saturated 5-, or 6-membered heterocyclic moiety having 1-2 atoms selected from the group consisting of N, O, and S;  
i is 0, 1, 2, 3, or 4; j is 1, 2, 3, or 4; n is 0, or 1.  
In another preferred embodiment, compounds of Formula I are other than thiadiazolyl(thio)urea derivatives compounds of Formula VII:
wherein R₉ is benzyl or 2-phenylethyl.

In a most preferred embodiment, compounds of Formula I are other than compounds of Formulae VI and VII.

The published application discloses that the thiadiazole derivatives are matrix metalloproteinase inhibitors that are useful as preventatives and therapeutics for diseases related to connective tissue degradation.

The present invention further provides a composition for inhibiting matrix metalloproteinases, comprising a compound of Formula I, wherein said compound is present in an amount effective to inhibit matrix metalloproteinases; and a carrier or diluent. Preferably, the carrier or diluent is a pharmaceutically acceptable carrier or diluent.

The matrix metalloproteinase inhibitors of the present invention include amino acid amides of 5-amino-1,3,4-thiadiazole-2-thiol represented by Structural Formula II:

Alternatively, the matrix metalloproteinase inhibitors of the present invention can be represented by Formula III:
In Formulae II and III:

Q and A are each independently selected from the group consisting of sulfur and oxygen and one of Q and A is sulfur. It is preferred that Q and A are both sulfur.

n is a positive integer and is chosen such that the compound inhibits a matrix metallocproteinase. Preferably, n is an integer from 1 to about 10. More preferably, n is an integer from 1 to about 4. In a separate embodiment of the invention, the value of n can be zero for compounds of Formula III.

R1 is selected from the group consisting of -H, lower alkyl and acyl. Lower alkyl includes C1 to about C6 straight or branched chain hydrocarbons. The hydrocarbon can be saturated or can have one or more units of unsaturation. Suitable acyl groups include -CO-(lower alkyl), wherein lower alkyl is defined above. Preferably, R1 is -H.

Each R4 is hydrogen, or is taken together with the R2 on the carbon atom adjacent to the nitrogen to which R4 is bound to form a 3 or 4 carbon atom carbocyclic bridge, that is optionally substituted or optionally fused to a benzene ring. Preferred groups formed by the combination of R4 and R2 include proline (azacyclo-pentane) and hydroxyproline. In Formula II, R4 is preferably other than hydrogen.

Each R2, when not taken with R4 to form a carbocyclic bridge, is independently selected from the group consisting of C1-C10 straight or branched alkyl, C1-C10 straight or branched substituted alkyl, C3-C8 cyclic alkyl, substituted C3-C8 cyclic alkyl, C1-C10 straight or branched alkenyl, C1-C10 straight or branched substituted alkenyl, C1-C10 straight or branched substituted alkenyl, C1-C10 straight or branched
alkynyl, C1-C10 straight or branched substituted alkynyl, aryl, substituted aryl, heteroaryl and substituted heteroaryl. Thus, the R2 in each amino acid subunit in Formulae II and III can be the same or different.

Suitable substituents on a substituted alkyl, alkenyl or alkynyl group include halo, -COOH, -COO(X), -CHO, -OH, -CN, -NO2, -NH2, -O(M), -SH, -S(M), -NH(M), -N(M2), -NH-C(=NH)-NH2, -NH-C(=NH)-NH(M), lower alkyl, aryl, substituted aryl, heteroaryl and substituted heteroaryl. A substituted alkyl, alkenyl and alkynyl group can optionally have more than one substituent. An alkyl, alkenyl or alkynyl group can also be completely substituted, e.g. perfluorinated. An alkenyl or alkynyl group can have more than one double or triple bond.

M is selected from the group consisting of -X, X-CO-, X-CS-, X-SO2-, X-O-CO- and X-O-CS-. X is selected from the group consisting of C1-C10 alkyl, C1-C10 substituted alkyl, aryl, substituted aryl, heteroaryl and substituted heteroaryl. X can also be heterocycloalkyl and substituted heterocycloalkyl.

An aryl group can be monocyclic (e.g. phenyl) or polycyclic. A polycyclic aromatic group includes fused polycyclic structures, e.g. naphthyl, tetrahydronaphthyl or anthracyl. A polycyclic aromatic group also includes structures with two or more aromatic rings connected by a linker containing one or more single bonds, carbon atoms, and/or heteroatoms, e.g. biphenyl, xanthene and fluorenyl. Suitable aryl substituents include halo, -COOH, -COO(M), -CHO, -OH, -CN, -NO2, -NH2, -O(M), -SH, -S(M), -NH(M), -N(M2), aryl, substituted aryl, heteroaryl and substituted heteroaryl. M is as defined above. A substituted aryl group can optionally have more than one substituent.

Suitable heteroaryl groups include monocyclic or polycyclic aromatic groups containing one or more heteroatoms such as oxygen, nitrogen or sulfur. Suitable monocyclic heterocyclic groups include imidazolyl, thieryl, pyridyl, furanyl, oxazoyl, pyrrollyl, pyrimidinyl, furanyl, pyrazolyl, pyrrolyl, thiazolyl
and the like. A polycyclic heteroaryl group includes fused structures such as quinonyl, indoyl, benzimidazoyl, benzothiazoyl, benzothiophenyl, benzofuranyl and benzopyranyl. A polycyclic heteroaromatic group can also include structures with a heteroaromatic ring and one or more aromatic or heteroaromatic rings connected by a linker containing one or more single bonds. Examples include phenylthienyl, thiienylthienyl, phenylfuranyl, phenyloxazoyl, thiényloxazoyl and the like. Suitable heteroaryl substituents include halo, -COOH, -COO(alkyl), -OH, -CN, -NO₂, -NH₂, -O(M), -SH, -S(M), -NH(M), -N(M₂), aryl, substituted aryl heteroaryl and substituted heteroaryl. M is as defined above. A substituted heteroaryl group can optionally have more than one substituent.

Preferably, R₂ is selected from the group consisting of cyclohexyl, cyclopentyl, (substituted phenyl)-CH₂-, naphthyl, naphthyl-CH₂-, the side chain of a naturally occurring amino acid, and the side chain of a naturally occurring amino acid having a derivatized heteroatom-containing functional group. R₂ is also preferably phenyl.

A substituted phenyl can have the same substituents as described above for aryl. 2-Fluoro, 3,4-diido, 4-nitro 4-benzylxycarbonylamino 4-dibenzylamino and 4-fluoro are examples. Naphthyl can be either 1-naphthyl or 2-naphthyl.

An amino acid has the general structure NH₂-CHR-COOH, wherein R is the side chain. Naturally occurring amino acids include alanine, valine, leucine, isoleucine, proline, methionine, phenylalanine, homophenylalanine, tryptophan, glycine, serine, homoserine, threonine, cysteine, homocysteine, tyrosine, aminoacidipic acid, asparagine, glutamine, aspartic acid, glutamic acid, lysine, histidine, proline, ornithine, homocysteine, hydroxyproline, phenylglycine and tryptophan.

The side chains of many naturally occurring amino acids have heteroatom-containing functional groups which can be derivatized. Examples of such heteroatom-containing functional groups include the thiol of cysteine,
the hydroxyl of serine, hydroxyproline and threonine, the carboxylic acid of glutamic acid, adipic acid and aspartic acid, the phenol of tyrosine, the amine of lysine, ornithine, arginine and histidine and the amide of asparagine and glutamine. Suitable derivatizing groups include -X, X-CO-, X-CS-, X-SO₂-, X-O-CO- and X-O-CS-, wherein X is as described above.

Specific examples of suitable derivatizing groups include O-benzyl for tryosyl, seryl, glutamoyl; S-benzyl for cysteinyl; N-trityl for glutamoyl; O-methylene-2-naphthyl for tryosyl; N-trityl for glutamyl; N,N-dibenzy for glutamyl; ε-N-t-butoxycarbonyl for lysyl; and N-2-phenylethyl for glutamyl.

R³ is an amine derivatizing group such as an amine protecting group. An "amine protecting group" is a functional group which can be bonded to a primary amine, which can be cleaved from the primary amine without causing undesired side reactions in other parts of the molecule and which results in a matrix metalloproteinase inhibitor. Other examples of suitable amine derivatizing groups include X-CO-, X-CS-, X-SO₂-, X-O-CO- and X-O-CS-, wherein X is as defined above.

Preferred amine derivatizing groups include 9-fluorenylmethoxycarbonyl, t-butoxycarbonyl, (4-phenyl)phenylacetyl, 8-quinolinesulfonyl, 2-methylthionicotyl, xanthene-9-carbonyl, hydrocinnamoyl, phenylbenzoyl, nonanoyl, (4-benzyloxy)benzoyl, acetyl and (4-(4-t-butylyphenylsulfonamino)benzoyl. 4-Phenylbenzoyl, nonanoyl, benzlyoxybenzoyl and (4-(4-t-butylyphenylsulfonamino)benzoyl are more preferred.

A subgenus within the scope of Formula I encompasses compounds other than the amino acid amides of 5-amino-1,3,4-thiadiazole-2-thione represented by Formula II where R₄ is -H.

A preferred subgenus within the scope of Formula II which encompasses compounds that exhibit potent and selective activity as matrix metalloproteinases includes compounds of Formula V:
and tautomers and pharmaceutically acceptable salts thereof, wherein

Y is one of -CO- or -SO₂-;

Q and A are each independently selected from the group consisting of

sulfur and oxygen and one of Q and A is sulfur;

R²¹ is one of hydrogen, lower alkyl or acyl;

R²² is one of C₁-C₁₀ straight or branched alkyl, C₁-C₁₀ straight or
branched substituted alkyl, C₃-C₈ cyclic alkyl, substituted C₃-C₈ cyclic alkyl,
C₂-C₁₀ straight or branched alkenyl, C₂-C₁₀ straight or branched substituted
alkenyl, C₂-C₁₀ straight or branched alkynyl, C₂-C₁₀ straight or branched
substituted alkynyl, aryl, substituted aryl, heteroaryl and substituted heteroaryl;
and

R²₃ is C₁-C₁₀ alkyl, C₁-C₁₀ substituted alkyl, aryl, substituted aryl,
heteroaryl or substituted heteroaryl;

Preferably, R²¹ is hydrogen.

Preferably, R²² is selected from the group consisting of cyclohexyl,
cyclopentyl, phenyl, (substituted phenyl)-CH₂-, naphthyl, naphthyl-CH₂-, the
side chain of a naturally occurring amino acid, and the side chain of a naturally
occurring amino acid having a derivatized heteroatom-containing functional
group.  R²² is also preferably phenyl.

A substituted phenyl can have the same substituents as described above
for aryl.  2-Fluoro, 3,4-diiodo, 4-nitro 4-benzylloxycarbonylamino
4-dibenzylamino and 4-fluoro are examples.  Naphthyl can be either
1-naphthyl or 2-naphthyl.
An amino acid has the general structure NH₂-CHR-COOH, as defined above for R₂. The side chains of many of these naturally occurring amino acids have heteroatom-containing functional groups which can be derivatized as described above for R₂.

Preferred combinations of \( R^{23} - Y \) include 9-fluorenylmethoxycarbonyl, \( t \)-butoxycarbonyl, (4-phenyl)phenylacetyl, 8-quinolinesulfonyl, 2-methylthionicotyl, xanthene-9-carbonyl, hydrocinamoyl, phenylbenzoyl, nonanoyl, (4-benzyloxy)benzoyl, acetyl and (4-(4-\( t \)-butylphenylsulfonamino)benzoyl. 4-Phenylbenzoyl, nonanoyl, benzyloxybenzoyl and (4-(4-\( t \)-butylphenylsulfonamino)benzoyl are more preferred.

The compounds of the present invention will in general exist in equilibrium with its other tautomeric forms. For example, compounds of generic Formula I, will exist as tautomers, including the structures A and B:

\[
\begin{align*}
A & \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quan
5-(N-(9-fluorenylmethoxycarbonyl)tryptonylamino)-1,3,4-thiadiazole-2-thione,
5-(N-(9-fluorenylmethoxycarbonyl)leucylamino)-1,3,4-thiadiazole-2-thione,
5-(N-(9-fluorenylmethoxycarbonyl)methionylamino)-1,3,4-thiadiazole-2-thione,
5-(N-(9-fluorenylmethoxycarbonyl)homophenylalanlamino)-1,3,4-thiadiazole-2-thione,
5-(N-(4-phenyl)phenylacetyl)valylamino)-1,3,4-thiadiazole-2-thione,
5-(N-benzyloxy carbonyl-(€-fluoro)phenylalanlamino)-1,3,4-thiadiazole-2-thione,
5-(N-(8-quinolinesulfonyl)phenylalanlvalylamino)-1,3,4-thiadiazole-2-thione,
5-(N-(2-methylthionicotyl)phenylalanlyvalylamino)-1,3,4-thiadiazole-2-thione,
5-(N-(xanthene-9-carbonyl)glycylphenylalanlamino)-1,3,4-thiadiazole-2-thione,
5-(N-hydrocinamoyl-phenylalanlyvalylamino)-1,3,4-thiadiazole-2-thione,
5-(N-(4-phenylbenzoyl)phenylalanlyvalylamino)-1,3,4-thiadiazole-2-thione,
5-(N-nonanoyl-phenylalanly-valylamino)-1,3,4-thiadiazole-2-thione,
5-(N-(4-phenyl)phenylacetyl-phenylalanly-valylamino)-1,3,4-thiadiazole-2-thione,
5-(N-(4-benzyloxy)benzoyl-phenylalanly-valylamino)-1,3,4-thiadiazole-2-thione,
5-(N-(4-phenoxy)benzoyl-phenylalanly-valylamino)-1,3,4-thiadiazole-2-thione,
5-(N-(4-(4-t-butylphenylsulfonyarn)benzoyl)-phenylalanly-valylamino)-1,3,4-thiadiazole-2-thione,
5-(N-benzyloxy carbonyl-phenylalanly-leucylamino)-1,3,4-thiadiazole-2-thione,
5-(N-benzyloxy carbonyl-phenylalanly-valylamino)-1,3,4-thiadiazole-2-thione,
5-(N-benzyloxy carbonyl-tryptol-valylamino)-1,3,4-thiadiazole-2-thione,
5-(N-benzyloxy carbonyl-tryptol-phenylalanlyamino)-1,3,4-thiadiazole-2-thione,
5-(N-benzyloxy carbonyl-tryptol-phenylalanlyamino)-1,3,4-thiadiazole-2-thione,
5-(N-benzyloxy carbonyl-leucylmethionylamino)-1,3,4-thiadiazole-2-thione,
5-(N-benzyloxy carbonyl-(2-(1-napthy))alanly-valylamino)-1,3,4-thiadiazole-2-thione,
5-(N-benzyloxy carbonyl-(2-(2-napthy))alanly-valylamino)-1,3,4-thiadiazole-2-thione,
5-(N-benzyloxy carbonyl-(O-benzyl)tyrosyl-valylamino)-1,3,4-thiadiazole-2-thione,
5-(N-benzyloxy carbonyl-(p-fluoro)phenylalanly-valylamino)-1,3,4-thiadiazole-2-thione,
5-(N-benzyloxy carbonyl-leucyl-valylamino)-1,3,4-thiadiazole-2-thione,
5-(N-benzyloxy carbonyl-cyclohexylglycyl-valylamino)-1,3,4-thiadiazole-2-thione,
5-(N-benzyloxy carbonyl-isoleucyl-valylamino)-1,3,4-thiadiazole-2-thione,
5-(N-benzyloxy carbonyl-(O-benzyl)glutamoyl-valylamino)-1,3,4-thiadiazole-2-thione,
5-(N-benzyloxy carbonyl-(p-nitro)phenylalanly-valylamino)-1,3,4-thiadiazole-2-thione,
5-(N-benzyloxy carbonyl-(p-benzyloxy carbonylaminophenylalanly)-valylamino)-1,3,4-thiadiazole-2-thione,
5-(N-benzyloxy carbonyl-(3,4-diiodo)phenylalanly)-valylamino)-1,3,4-thiadiazole-2-thione,
5-(N-benzyloxycarbonyl-((S-benzyl)cysteinyl)-valylamino)-1,3,4-thiadiazole-2-thione,
5-(N-benzyloxycarbonyl-((ortho-fluoro)phenylalanyl)-valylamino)-1,3,4-thiadiazole-2-thione,
5-(N-benzyloxycarbonyl-((O-benzyl)seryl)-valylamino)-1,3,4-thiadiazole-2-thione,
5-(N-benzyloxycarbonyl-((N-trityl)glutamyl)-valylamino)-1,3,4-thiadiazole-2-thione,
5-(N-benzyloxycarbonyl-((O-benzyl)tyrosyl)-aminoisobutyrolamino)-1,3,4-thiadiazole-2-thione,
5-(N-benzyloxycarbonyl-((O-benzyl)tyrosyl)-phenylglycylamino)-1,3,4-thiadiazole-2-thione,
5-(N-benzyloxycarbonyl-((O-methylene-2-naphthyl)tyrosyl)-valylamino)-1,3,4-thiadiazole-2-thione,
5-(N-benzyloxycarbonyl-((O-benzyl)tyrosyl)-glycylamino)-1,3,4-thiadiazole-2-thione,
5-(N-benzyloxycarbonyl-((O-benzyl)tyrosyl)-(t-butyl)glycylamino)-1,3,4-thiadiazole-2-thione,
5-(N-benzyloxycarbonyl-((N-trityl)glutamyl)-phenylglycylamino)-1,3,4-thiadiazole-2-thione,
5-(N-benzyloxycarbonyl-((O-benzyl)tyrosyl)-cyclohexylglycylamino)-1,3,4-thiadiazole-2-thione,
5-(N-t-butyloxycarbonyl-((O-benzyl)tyrosyl)-phenylglycylamino)-1,3,4-thiadiazole-2-thione,
5-(N-benzyloxycarbonyl-((N,N-dibenzyl)glutamyl)-phenylglycylamino)-1,3,4-thiadiazole-2-thione,
5-(N-benzyloxycarbonyl-((p-N,N-dibenzylamino)phenylalanyl)-phenylglycylamino)-1,3,4-thiadiazole-2-thione,
5-(N-benzyloxycarbonyl-((O-benzyl)tyrosyl)-leucylamino)-1,3,4-thiadiazole-2-thione,
5-(N-benzyloxycarbonyl-((N-2-phenylethyl)glutamyl-phenylglycylamino)-1,3,4-thiadiazole-2-thione,
5-(N-benzyloxycarbonyl-phenylalanyl-leucyl-tryptophylamino)-1,3,4-thiadiazole-2-thione,
5-(N-benzyloxycarbonyl-phenylalanyl-valyl-tryptophylamino)-1,3,4-thiadiazole-2-thione,
5-(N-benzyloxycarbonyl-phenylalanyl-tryptophyl-valylamino)-1,3,4-thiadiazole-2-thione,
5-(N-benzyloxycarbonyl-lysyl(N-epsilon-t-butyloxycarbonyl)-tyrosyl(O-benzyl)-phenylglycylamino)-1,3,4-thiadiazole-2-thione.

The compounds of the present invention are formed by reacting (A) 5-amino-1,3,4-thiadiazole-2-thione, 5-amino-1,3,4-thiadiazole-2-one, or 5-amino-1,3,4-oxadiazole-2-thione with (B) an organic molecule possessing a functional group capable of reacting with the amino group of (A). Suitable functional groups capable of reacting with the 5-amino group of (A) include: carboxylic acids, acid halides and other activated acid groups as are known in the art;

The compounds of Formula I are generally synthesized by reacting 5-amino-1,3,4-thiadiazole-2-thione, or an analog thereof (where one of Q or A is oxygen), with an organic compound having a functional group capable of reacting with the 5-amino group. 5-Amino-1,3,4-thiadiazole-2-thione is prepared according to known methods (Cho and Kim, *J. Heterocyclic Chem.*, 30:397 (1993)). The 5-amino group may be reacted with a carboxylic acid, an activated carboxylic acid or acid chloride to form an amide linkage to the 1,3,4-thiadiazole-2-thione. Direct reaction of the 5-amino group with a carboxylic acid is suitably carried out by methods that are typically employed for peptide synthesis, for example by dicyclohexylcarbodiimide mediated coupling in the presence of 1-hydroxybenzotriazole (see Examples 1-37). Alternatively, the 5-amino group can be reacted with a sulfonil chloride to form a sulfonamide linkage to the 1,3,4-thiadiazole-2-thione. The 5-amino group can also be reacted with an isocyanate to form a urea or with a thioisocyanate to form a thiourea. In instances when the organic molecule that is to be reacted with the 5-amino-1,3,4-thiadiazole-2-thione has more than one reactive functional group, the extra functional groups are first selectively reacted with an appropriate protecting group. Protecting groups are well known in the art. See, Greene and Wuts, *Protecting Groups in Organic Synthesis*, John Wiley and Sons, (1991).

For a number of useful compounds, the 5-amino group is first derivatized to form another reactive functional group, that is capable of further reaction. For example, the 5-amino group is converted to an isocyanate or isothiocyanate group. Thiourea compounds can be formed by reacting 5-amino-1,3,4-thiadiazole-2-thione with thiophosgene and triethylamine to give a 5-isothiocyanate group. Reaction of the isothiocyanate with an amine will give a
thiourea compound. Urea compounds can be prepared by reacting 5-amino-1,3,4-
thiadiazole-2-thione with triphosgene and triethylamine to give the 5-isocyanate
derivative, followed by reaction with an appropriate amine. Carbamate
compounds can be prepared by reacting the isocyanate intermediate formed above
with an appropriate anhydrous alcohol.

The compounds of Formulae II, III and V are synthesized by coupling 5-
amino-1,3,4-thiadiazole-2-thione with an N-terminus protected amino acid or
oligopeptide. 5-Amino-1,3,4-thiadiazole-2-thione is prepared according to
known methods (Cho and Kim, J. Heterocyclic Chem., 30:397 (1993)). Methods
of protecting the N-terminus of amino acids or oligopeptides are also well known.
See, Greene and Wuts, Protecting Groups in Organic Synthesis, John Wiley and
Sons, (1991). Coupling is carried out by known methods of peptide synthesis, for
example by dicyclohexylcarbodiimide mediated coupling in the presence of 1-
hydroxybenzotriazole (see Examples 1-36). Synthesis of compounds having
formula V is further illustrated in Example 37. Additional amino acids or
oligopeptides can be added to the N-terminus by cleavage of the amino protecting
group and then performing a second coupling with an N-terminus protected
amino acid or oligopeptide. The process can be repeated as often as required to
synthesize a thiadiazole having an oligopeptide of desired length and sequence
which is bound to the 5-amino group of the thiadiazole.

The methods of the present invention comprise contacting the matrix
metalloproteinase with an inhibitory amount of a compound represented by
Formulae I, II, III or V.

Matrix metalloproteinases are a class of zinc-dependent, proteolytic
enzymes which bind and cleave peptides having a specific amino acid sequence.
Examples of enzymes in this class of proteins include stromelysin (MMP-3),
human fibroblast collagenase (MMP-1), human 72-kDa latent gelatinase (MMP-2),
human neutrophil collagenase (MMP-8), human 92-kDa gelatinase (MMP-9) and
matrilysin (MMP-10).
An inhibitory amount of the compound is the quantity of the compound which results in reduced cleavage of matrix metalloproteinase substrates in the presence of the compound compared with in its absence. An inhibitory amount depends on several factors, including the inhibitor used, the pH of the solution, other constituents in the solution and temperature. The skilled artisan is able to vary the amount of inhibitor used, depending on the application. Typically, a concentration from about 1 nanomolar or less to about 10,000 nanomolar is used, preferably about 1 nanomolar or less to about 1000 nanomolar and more preferably about 1 nanomolar or less to about 500 nanomolar.

Specific examples where at least one matrix metalloproteinase is inhibited in vitro with an amino acid amide of 5-amino-1,3,4-thiadiazole-2-thione are provided in Examples 38-41. In these examples amino acid amides of 5-amino-1,3,4-thiadiazole-2-thiones are tested in vitro for their ability to inhibit stromelysin, 92 kDa human gelatinase, 72 kDa human gelatinase and human neutrophil collagenase. Inhibition data are provided in Tables I-IV and VI as the IC$_{50}$.

Another embodiment of the present invention is a method of treating an individual with a disease that can be ameliorated by inhibiting at least one matrix metalloproteinase enzyme. The method comprises administering a therapeutically effective amount of a compound of Formulae I, II, III or V.

The method can also be used to treat an animal with a disease that can be ameliorated by inhibiting at least one matrix metalloproteinase enzyme. Animals which can be treated by this method include, dogs, cats, farm animals, guinea pigs and the like.

A disease is "ameliorated" when the development or progression of a disease process associated with the disease is slowed, arrested or reversed as a result of a treatment. For example, osteoarthritis and rheumatoid arthritis can be ameliorated by slowing the cartilage degradation that occurs as a result of the disease. Alternatively, "amelioration" can include alleviating pain and inflammation in the afflicted joints of an individual with osteoarthritis or
rheumatoid arthritis. Another example of disease "amelioration" includes increasing the life expectancy of individual with the disease, for example an individual with cancer, or increasing the quality of life of the individual, e.g. by increasing the mobility of an individual with osteoarthritis.

Specific examples of where a disease process is ameliorated by the administration of amino acid amides of 5-amino-1,3,4-thiadiazole-2-thione are provided in Example 41. In this example, compounds are tested for their ability to inhibit the degradation of extracellular matrix in tissue culture. Extracellular cartilage degradation occurs in osteoarthritis and rheumatoid arthritis. Inhibition data for the compounds tested in the tissue culture assay are provided in Table V as the percent inhibition of cartilage degradation at the given concentration.

Other diseases which can be treated with the metalloproteinase inhibitors of the present invention include tumor cell metastasis in cancer, ulcerations and infections resulting from periodontal disease or epidermolysis bullosa. In addition, these compounds can be used to treat inflammation in diseases in which inflammation is caused by the overactivity of at least one matrix metalloproteinase enzyme.

A therapeutically effective amount of the compound is the quantity which brings about an amelioration of the disease without causing unacceptable side effects. The amount of compound which is administered to the individual or animal depends on many factors, including the age, sex, weight and general health of the individual as well the severity of the disease with which the individual is afflicted. The skilled artisan will be able to vary the amount of compound administered to the individual, depending on these and other factors. Typically, a therapeutically effective amount ranges from about 0.1 mg/kg per day or less to about 100 mg/kg per day, preferably from about 0.1 mg/kg per day or less to about 20.0 mg/kg per day.

The compound can be administered orally, for example, in capsules, suspensions or tablets. Other modes of administration which can be used include systemic administration, such as by intramuscular, intravenous, subcutaneous, or
intraperitoneal injection. When treating osteoarthritis, the compound is preferably administered intraarticularly into the afflicted joint, for example by intraarticular injection.

The compound can be administered to the individual in conjunction with an acceptable pharmaceutical carrier as part of a pharmaceutical composition for treating osteoarthritis. Suitable pharmaceutical carriers may contain inert ingredients which do not interact with the compound. Standard pharmaceutical formulation techniques may be employed such as those described in Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA. Suitable pharmaceutical carriers for intraarticular and other parenteral administration include, for example, sterile water, physiological saline, bacteriostatic saline (saline containing about 0.9% mg/ml benzyl alcohol), phosphate-buffered saline, Hank's solution, Ringer's-lactate and the like. Methods for encapsulating compositions (such as in a coating of hard gelatin of cyclodextran) are known in the art (Baker, et al., Controlled Release of Biological Active Agents, John Wiley and Sons, 1986).

In another embodiment of the present invention the composition, in addition to the compound, additionally comprises another pharmacologically active agent. Osteoarthritis and rheumatoid arthritis are characterized by pain in the afflicted joints. Individuals with cancer often suffer from pain resulting from tumors contacting organs or other body tissue. Consequently, when treating osteoarthritis, rheumatoid arthritis or cancer it can be advantageous to co-administer the compound with an analgesic or other pain-killing medication. Suitable analgesics include acetominophen, acetyl salicylic acid and the like. Osteoarthritis and rheumatoid arthritis are also characterized by inflammation in the afflicted joints. Consequently, it can be advantageous to administer the compound together with an anti-inflammatory agent such as a non-steroidal anti-inflammatory drug or steroid (e.g. triamcinolone, aminodide and the like) when treating osteoarthritis and rheumatoid arthritis.
The metalloproteinase inhibitors of the present invention have useful applications *in vitro*. Because matrix metalloproteinases have protease activity and are present in a wide variety of tissue, the isolation of useful tissue and biological fluids is often hampered by undesired proteolysis of useful proteins by these enzymes. Destruction of useful tissue and proteins by these matrix metalloproteinases can be prevented by adding an inhibitory amount of the thia diazoles of the present invention. Matrix metalloproteinases, as discussed earlier, are involved in a wide variety of disease processes. Consequently, inhibitors of matrix metalloproteinase are useful in disease research, for example to study the structure activity requirements for designing new and better inhibitors of these enzymes.

A substituted 1,3,4-thiadiazole-2-thione, or analog thereof of the present invention can be coupled to a radiolabel, such as the Te⁹⁹ or I¹³¹ scintigraphic labels, using standard coupling methods. A radiolabeled amino acid amide of a 1,3,4-thiadiazole-2-thione is then administered to a subject to determine any locations of excess amounts of one or more metalloproteinase *in vivo*. The ability of a thiadiazole compound to selectively bind to a metalloproteinase is then used to map the distribution of these enzymes *in situ*. The techniques can also, of course, be employed in the histological procedures, and the labeled compounds can be used in competitive immunoassays.

At least one amino acid amide of a 1,3,4-thiadiazole-2-thione can also be coupled to a solid support, such as a separation membrane, a chromatographic support, for example agarose, sepharose, polyacrylamide, or the like, or to a microtiter plate to provide an affinity support which is useful in purifying a matrix metalloproteinase enzyme. The selective binding of the matrix metalloproteinase to the thiadiazole compound permits the adsorption of the desired enzyme and its subsequent elution using, for example, altered ionic strength and/or pH conditions.

The invention will now be further and specifically described by the following examples.
Example 1

5-(N-(9-fluorenlymethoxycarbonyl)tryptonylamino)-1,3,4-thiadiazole-2-thione

Dicyclohexylcarbodiimide (0.42 grams) was dissolved in 5 mL of anhydrous dimethylformamide (DMF), followed by the addition of N-fluorenlymethoxycarbonyl-tryptophan (N-(9-fluorenlymethoxycarbonyl is referred to as "Fmoc") (0.85 grams) and 1-hydroxybenzotriazole (HBT) (0.36 grams). The solution was kept at room temperature until dicyclohexylurea precipitation was completed (about 40 minutes).

5-Amino-1,3,4-thiadiazole-2-thione (0.3 grams) was added and the reaction mixture was allowed to stir for 48 hours. An excess of ethyl acetate was the added (100 mL) and the resulting solution was washed three times with 5% aqueous sodium bicarbonate, 10% aqueous citric acid and water. The ethyl acetate layer was dried with magnesium sulfate and evaporated to dryness. The resulting oily residue was recrystallized from ethanol-pentane. The resulting white solid was filtered off and air-dried. M.P. 162-163°C. NMR spectrum (d6-DMSO) 14.05 (s, 1H), 12.75 (s, 1H), 10.85 (s, 1H), (6.9-8.0, m, 13 H; NH+aromatics), 4.5 (m,1H), 4.1 (m, 3H), 2.9-3.5 (m, 2H).

Example 2

5-(N-(9-fluorenlymethoxycarbonyl)valylamino)-1,3,4-thiadiazole-2-thione

Fmoc-Valine (0.68 grams), 1-hydroxybenzotriazole (0.36 grams), dicyclohexylcarbodiimide (0.42 grams) and 5-amino-1,3,4-thiadiazole-2-thione (0.4 grams) were reacted according to the procedures described in Example 1. The resulting product was recrystallized from ethanol-pentane. M.P. 141-144°C.

NMR spectrum (d6-DMSO) 14.06 (s, 1H), 12.56 (s,1H), 8.00-7.60;7.5-7.2 (m, 9H; NH+aromatics) 4.4-4.0 (m, 4H) 2.2-2.0 (m, 1H), 0.98 (bs, 6H).

Example 3

5-(N-benzyloxycarbonyl-tryptonylamino)-1,3,4-thiadiazole-2-thione
N-Benzylloxycarbonyl-tryptophan (1.7 grams), 1-hydroxybenzotriazole (1 gram), dicyclohexylcarbodiimide (1.1 grams) and 5-amino-1,3,4-thiadiazole-2-thione (2.5 grams) were reacted according to the procedure described in Example 1. The resulting product was recrystallized from ethanol/ethyl acetate/pentane to give a white powder. M.P. 128-132°C. NMR spectrum (d$_6$-DMSO) 14.08 (s, 1H), 12.77 (s, 1H), 10.84 (s, 1H), 7.8-7.6, 7.4-6.9 (M, 11H, NH+aromatics), 4.95 (s, 2H), 4.5 (m, 1H) 3.3-2.9 (m, 2H).

**Example 4**

5-((N-9-fluorenylmethoxycarbonyl)methionylamino)-1,3,4-thiadiazole-2-thione

Fmoc-Methionine (0.371 grams), 1-hydroxybenzotriazole (0.2 grams), dicyclohexylcarbodiimide (0.21 grams) and 5-amino-1,3,4-thiadiazole-2-thione (0.25 grams) were reacted according to the procedure described in Example 1. The resulting product was recrystallized from ethanol/petroleum ether to give the product as white powder. M.P. 166-167°C. NMR spectrum (d$_6$-DMSO) 14.1 (s, 1H), 12.62 (s, 1H), 7.9-7.2 (m, 9H, NH+aromatics), 4.4-4.1 (M, 4H), 2.1 (s, 3H), 2.2-1.8 (m, 4H).

**Example 5**

5-((N-9-fluorenylmethoxycarbonyl)homophenylalanylamino)-1,3,4-thiadiazole-2-thione

Fmoc-phenylalanine (0.8 grams), 1-hydroxybenzotriazole (0.38 grams), dicyclohexylcarbodiimide (0.4 grams) and 5-amino-1,3,4-thiadiazole-2-thione (0.4 grams) were reacted according to the procedure described in Example 1. The resulting product was recrystallized from ethanol/pentane to give a white powder. M.P. 186-187°C. NMR spectrum (d$_6$-DMSO) 14.08 (s, 1H), 12.6 (s, 1H), 8.00-7.1(m, 14 H, NH+aromatics), 4.4-4.1 (m, 4H), 2.8-2.3 (m, 2H), 2.1-1.8 (m, 2H).

**Example 6**

5-((N-((4-phenylphenylacetyl) valylamino)-1,3,4-thiadiazole-2-thione
(4-Phenyl)phenylacetlyl)-valine (0.98 grams), 1-hydroxybenzotriazole (0.6 grams), dicyclohexylcarbodiimde (0.64 grams) and 5-amino-1,3,4-thiadiazole-2-thione (1.2 grams) were reacted according to the procedure described in Example 1. The resulting product was recrystallized from ethanol/pentane to give a white solid. M.P. 232-236°C. NMR spectrum (d$_2$-DMSO) 14.1 (s, 1H), 12.6 (s, 1H), 8.42 (d, 1H, NH), 7.8-7.2 (m, 9H, aromatics), 4.35 (m, 1H), 3.75-3.4 (m, 2H), 2.05 (m, 1H), 0.9 (d, 6H).

**Example 7**

5-(N-benzyloxycarbonyl-phenylalanlyl-valylamino)-1,3,4-thiadiazole-2-thione

Cbz-Phenylalanine-valine-OH (0.8 grams), 1-hydroxybenzotriazole (0.35 grams), dicyclohexylcarbodiimide (0.41 grams) and 5-amino-1,3,4-thiadiazole-2-thione (0.9 grams) were reacted according to the procedure described in Example 1. The resulting product was recrystallized from ethyl acetate/petroleum ether to give a white solid. M.P. 170-174°C. NMR spectrum (d$_2$-DMSO) 14.1 (s, 1H), 12.6 (s, 1H), 8.22 (d, 1H), 7.52 (d, 1H), 7.1-7.4 (m, 10 H, aromatics), 4.95 (s, 2H), 4.38 (m, 2H), 2.9 (m, 1H), 2.75 (m, 1H), 2.05 (m, 1H), 0.9 (bs, 6H).

**Example 8**

5-(N-(4-(4-t-Butylphenylsulfonylamino)benzoyl)-phenylalanlyl-valylamino)-1,3,4-thiadiazole-2-thione

(4-(4-t-Butylphenylsulfonylamino)benzoyl)-phenylalanine-valine, (0.48 grams) 1-hydroxybenzotriaole (0.15 grams), dicyclohexylcarbodiimide (0.17 grams) and 5-amino-1,3,4-thiadiazole-2-thione (0.5 grams) were reacted according to the procedure described in Example 1. The resulting product was recrystallized from ethanol/pentane to give a white solid. M.P. 140-144°C. NMR spectrum (d$_2$-DMSO) 14.1 (s, 1H), 12.6,12.5 (s, 1H), 10.8 (s, 1H), 8.6-8.2, 7.8-7.4, 7.4-7.0 (m, 15 H, NH+aromatics), 4.8 (m, 1H), 4.35 (m, 1H), 3.5-2.8 (m, 2H), 2.05 (m, 1H), 1.2 (s, 9H), 0.85 (bs, 6H).
Example 9

5-(N-benzyloxycarbonyl-(2-(1-naphthyl))alanin-valylamino)-
1,3,4-thiadiazole-2-thione

tert-Butyloxycarbonyl-1-naphthalanine (Boc-1-Naphthalanine) (500 mg, 1.6
mmol), valine methyl ester hydrochloride (292 mg, 1.1 equivalent),
diisopropylethylamine (305 mL, 1.1 eq) and 1-hydroxybenzotriazole (242 mg, 1.0
eq) were added to 15 mL of CH₂Cl₂. The solution was allowed to equilibrate 15
minutes at room temperature, followed by the addition of
dicyclohexylcarbodiimide (360 mg, 1.1 eq). The reaction was then stirred at
room temperature overnight. The precipitated dicyclohexylurea was removed by
filtration. The resulting solution was washed with 5% HCl (2 x 30 mL), 10%
NaHCO₃ (2 x 30 mL) and brine (2 x 30 mL). The organic layer was then dried
(Na₂SO₄) and evaporated to dryness. The resulting material was used without
further purification.

Deprotection of the Boc group was carried out as reported in literature
(Bodanszky and Bodanszky "The Practice of Peptide Synthesis") using neat
trifluoroacetic acid at 0°C for 15 minutes. Excess trifluoroacetic acid was
removed under reduced pressure and the trifluoroacetic acid salt was dried
overnight over a bed of NaOH.

The free N-terminus was acetylated with benzylchloroformate (Cbz-Cl)
by dissolving the trifluoroacetic acid salt in CH₂Cl₂ at 0°C.
Dissopropylethylamine (695 mL, 2.5 eq) was added, followed by the dropwise
addition of benzyl chloroformate (250 mL, 1.2 eq). The reaction was then
allowed to warm to room temperature over 1 hour. The reaction mixture was
washed with 5% HCl (2 x 30 mL), 10% NaHCO₃ (2 x 30 mL) and brine (2 x 30
mL). The organic layer was dried (Na₂SO₄), evaporated to dryness, and used
without further purification.

Saponification of the methyl ester was accomplished by dissolving the
ester in 2 mL methanol (MeOH), adding 2 mL of 1 N NaOH and allowing the
reaction to stir at room temperature. The reaction is conveniently monitored by
TLC and complete reaction is usually seen after about 1 hour. The MeOH:H₂O
mix is diluted out with H₂O and washed with ether (2 x 15 mL). The aqueous phase is carefully acidified (1N HCl) and washed with ethyl acetate (5 x 10 mL). The organic layers are combined, washed once with 5% HCl and brine, dried (Na₂SO₄) and evaporated to dryness. TLC showed one spot and this was used without further purification. Yield of the crude acid was 500 mg (69%).

Coupling with the thiadiazole (5-amino-1,3,4-thiadiazole-2-thiol) was accomplished by dissolving the above acid in 5 mL DMF under a stream of argon. The thiaidazole, (330 mg, 2.2 mmol, 2 eq), 1-hydroxybenzotriazole (HOBt) (180 mg) and dicyclohexylycarbodiimide (253 mg, 1.1 eq) were then added to the reaction mixture. The reaction mixture was thoroughly flushed with argon, stoppered tightly, and allowed to stir at room temperature 72 hrs. Workup was as described in Examples 1-3.

An analytically pure sample was obtained by preparative TLC using CH₂Cl₂:MeOH (95:5), Rₜ=0.47. Melting point 176.8-180°C. ¹H-NMR (δ, ppm, 1H DMSO, internal ref.) 14.1 (br s, NH thiaidazole) 12.5 (br s, NH amide thiaidazole) 8.4-7.0 (m, 14H, aromatic + amides) 4.9 (s, 2H, CH₂ CBZ) 4.5 (m, 1H, chiral) 4.3 (m, 1H, chiral) 3.5 (m, 2H, CH₂ benzylc) 2.0 (m, 1H, CH valine) 0.8 (m, 6H, CH₃).

**Example 10**

5-(N-benzyloxy carbonyl-(2-(2-phenyl)alanin-valylamino)-1,3,4-thiadiazole-2-thione

The synthesis was carried out according to the procedure described in Example 9, except that Boc-2-naphthalanine was used in place of Boc-naphthalanine.

An analytically pure sample was obtained by preparative TLC using CH₂Cl₂:MeOH (95:5), Rₜ=0.41. Melting point 125.2-132.5°C. ¹H-NMR (δ, ppm, 1H DMSO, int. std.) 14.1 (br s, 1/3H, NH thiaidazole) 12.6 (br s, 1/3H, NH amide thiaidazole) 8.3 (d, 14H, amide) 7.9-7.0 (m, 13H, aromatic + 1 amide) 4.9 (s, 2H, CH₂ CBZ) 4.5 (m, 1H, chiral) 4.3 (m, 1H, chiral) 0.8 (m, 6H, CH₃).
Example 11

5-(N-benzyloxy carbonyl-(O-benzyl)tyrosyl-valylamino)-1,3,4-thiadiazole-2-thione

N-Benzyl oxycarbonyl-(O-benzyl) tyrosine (6.35, 1.5 mmol), valine methyl ester hydrochloride (250 mg, 1.1 eq), diisopropylethylamine (260 mL, 1.1 eq) and HOBt (230 mg) were added to 15 mL of DMF. The reaction was then allowed to equilibrate for 15 minutes, followed by the addition of dicyclohexyl carbodiimide (340 mg 1.1 eq). The reaction was allowed to stir overnight at room temperature, after which the precipitated dicyclohexylurea was filtered off. The supernatant was extracted with 5% HCl (2 x 30 mL), 10% NaHCO₃ (2 x 30 mL) and brine (2 x 30 mL). The organic layer was dried, (Na₂SO₄) evaporated to dryness and used without further purification.

Saponification of the methyl ester was accomplished by dissolving the ester in 2 mL methanol and adding 2 mL of 1 N NaOH and allowing the reaction to stir at room temperature. The reaction is conveniently monitored by TLC and complete reaction is usually seen after about 1 hour. The methanol H₂O mixture is diluted out with H₂O and washed with ether (2 x 15 mL). The aqueous phase is carefully acidified (1 N HCl) and washed with ethyl acetate (5 x 10 mL). The organic layer is combined, washed once with 5% HCl and brine, dried (Na₂SO₄) and evaporated to dryness. TLC showed one spot. This product was used without further purification. Yield of the crude acid was 530 mg (70%).

Coupling with the 5-amino-1,3,4-thiadiazole-2-thiole was performed as described in Example 9.

An analytical sample was prepared by preparative TLC using CH₂Cl₂:MeOH (95:5), Rₜ=0.39. Melting point 111.7-119.4°C. ¹H-NMR (d₆-DMSO, int. std.) 14.1 (br s, 1/3H, NH thiadiazole) 12.6 (br s, 1/3H, NH amide thiadiazole) 8.2 (d, 1H, amide) 7.6-6.8 (m, 15H, aromatic + 1 amide) 5.1 (s, 2H, CH₂ benzyl) 4.9 (s, 2H, CH₂ CBZ) 4.3 (m, 2H, 2 x chiral) 0.9 (m, 6H, CH₃).
Example 12

5-(N-benzylxycarbonyl-(1,2,3,4-tetrahydroisoquinoline-3-carboxy)-(valylamino)-1,3,4-thiadiazole-2-thione

The synthesis was carried out as described in Example 9 except that Boc-(1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid) (Advanced ChemTech, Louisville, KY) was used in place of Boc-1-naphthalanilne.

An analytical sample was prepared by preparative TLC using CH₂Cl₂:MeOH (95:5), Rf = 0.34. Melting point 128.5-133.5°C. ¹H-NMR (d6-DMSO, int. std.) 14.1 (br s, 1/3H, NH thiadiazole) 12.5 (br s, 1/3H, NH amide thiadiazole) 8.1 (d, 1H, amide valine) 7.5-7.1 (m, 9H, aromatic) 5.2 (m, 2H, CH₂ CBZ) 4.6 (m, 3H, tetrahydroisoquinoline) 4.2 (q, 1H, chiral) 3.1 (m, 2H, tetrahydroisoquinoline) 0.7 (m, 6H, CH₃).

Example 13

5-(N-benzylxycarbonyl(para-fluoro)phenylalanin-valylamino)-1,3,4-thiadiazole-2-thione

The synthesis was carried out as described in Example 9 except that Boc-4-fluoro-phenylalanine was used in place of Boc-1-naphthalanilne.

An analytical sample was prepared by preparative TLC using CH₂Cl₂:MeOH (95:5), Rf = 0.26. Melting point 127.6-130.2°C. ¹H-NMR (d₆-DMSO, int. std.) 14.1 (br s, 1/2H, NH thiadiazole) 12.6 (br s, 1/2H, NH amide thiadiazole) 8.2 (d, 1H, amide valine) 7.5 (d, 1H, amide) 7.4-6.9 (m, 9H, aromatic) 4.9 (s, 2H, CH₂, CBZ) 4.3 (m, 2H, 2 x chiral) 2.9-2.7 (m, 2H, CH₂, phenyl) 2.0 (app q, 1H, CH val, app J = 0.9 (app s, 6H, CH₃).

Example 14

5-(N-benzylxycarbonyl-cyclohexylglycyl-valylamino)-1,3,4-thiadiazole-2-thione

Synthesis of the N-benzylxycarbonyl-cyclohexylglycine (Cbz-CHG) was accomplished using Schotten-Baumann conditions (Bodanszky, "Principles of Peptide Synthesis"). (L)-Cyclohexylglycine (Advanced ChemTech, Louisville,
Kentucky) was dissolved in a water/dioxane mixture with benzyl chloroformate (Cbz-Cl). 5 N NaOH was used to maintain the pH at about 10. After the reaction was complete, the mixture was adjusted to pH 7, concentrated to half-volume, diluted with water, acidified to pH 3 and extracted with ethyl acetate. The combined organic washes were dried (Na$_2$SO$_4$) and evaporated to dryness. The crude product, Cbz-CHG, was used without further purification. The procedure described in Example 11, using Cbz-CHG in place of N-benzylloxycarbonyl-(O-benzyl)tyrosine, was used to complete the synthesis.

An analytical sample was prepared by preparative TLC using CH$_2$Cl$_2$:MeOH (95:5), R$_f$=0.25. Melting point 190-193.8°C. $^1$H-NMR (d$_6$-DMSO, int. std.) 14.1 (s, 1/3H, NH thiadiazole) 12.5 (s, 1/3H, NH amide thiadiazole) 8.1 (d, 1H, amide) 7.3 (apps, 6H, aromatic + 1 amide) 5.0 (s, 2H, CH, CBZ) 4.3 (q, 1H, chiral) 3.9 (m, 1H, chiral) 3.6 (br m, 6H, cyclohexane) 1.0 (br m, 11H, cyclohexane + CH$_3$).

**Example 15**

5-(benzylloxycarbonyl-(p-nitro)phenylalanyl-valylamino)-1,3,4-thiadiazole-2-thione

Synthesis of the requisite N-benzylloxycarbonyl-(p-nitro)phenylalanine was accomplished using the Schotten-Baumann conditions (Bodanszky, "Principles of Peptide Synthesis"). (L)-4-NO$_2$-Phenylalanine (Bachem Bioscience, Inc., King of Prussia, PA) was dissolved in a water/dioxane mixture with Cbz-Cl. 5 N NaOH was used to maintain the pH at about 10. After the reaction was complete, the mixture was adjusted to pH 7, concentrated to half-volume, diluted with water, acidified to pH 3 and extracted with ethyl acetate. The combined organic washes were dried (Na$_2$SO$_4$) and evaporated to dryness. The crude product, Cbz-4-nitrophenylalanine, was used without further purification.

The procedure described in Example 11, using Cbz-4-nitrophenylalanine in place of Cbz-(O-benzyl)tyrosine, was used to complete the synthesis.
An analytical sample was prepared by preparative TLC using CH₂Cl₂:MeOH (95:5), Rf=0.22. Melting point 137.6-51.5°C. ¹H-NMR (d₇-
DMSO, int. std.) 14.1 (br s, 1/2H, NH thiazole) 12.6 (br s, 1/2H, NH amide thiazole) 8.3 (d, 1H, amide) 8.1 (d 2H, aromatic α to NO₂) 7.6 = 7.2 (m 8H, aromatic + 1 amide) 4.9 (s, 2H, CH₂ CBZ) 4.4 (m, 2H, 2 x chiral) 2.0 (m, 1H, CH valine) 0.9 (app s, 6H, CH₃).

Example 16

5-(N-benzyloxy carbonyl-((p-benzyloxy carbonylamino)
phenyl-alanyl)-valylamino)-1,3,4-thiadiazole-2-thione

N-Benzyl oxy carbonyl-(p-benzyloxy carbonylamino)-phenyl alanine compound was prepared using Schotten-Baumann conditions (Bodanszky, "Principles of Peptide Synthesis") as described in Examples 14 and 15 starting with (L)-4-amino-phenylalanine (Bachem Bioscience, King of Prussia, PA) and using two equivalents of Cbz-Cl. The procedure described in Example 11, using N-benzyloxy carbonyl-(p-benzyloxy carbonylamino)phenyl alanine in place of N-benzyloxy carbonyl-(O-benzyl) tyrosine, was used to complete the synthesis.

An analytical sample was prepared by preparative TLC using CH₂Cl₂:MeOH (95:5), Rf=0.22. Melting point 195.2-203.4°C. ¹H-NMR (d₇-
DMSO, int. std.) 14.1 (br s, 1/2H, NH thiazole) 12.6 (br s, 1/2H, NH amide thiazole) 9.7(d, 1H, amide) 8.2 (d 1H, amide) 7.3 (m 15H, aromatic + amide) 5.2 (s, 2H, CH₂ CBZ on para-amino group) 4.9 (s, 2H, CH₂-CBZ on α nitrogen) 4.3 (m, 2H, 2 x chiral) 2.0 (app s, 1H, CH valine) 0.8 (m, 6H, CH₃).

Example 17

5-(N-benzyloxy carbonyl-(O-benzyl)-glutamoyl-valylamino)-
1,3,4-thiadiazole-2-thione

(N-t-butoxycarbonyl)valine (750 mg, 3.4 mmol) was added to 5 mL of anhydrous DMF in a reaction flask that had been flushed with argon. 5-Amino-1,3,4-thiadiazole-2-thiol (920 mg, 2 equivalents), HOBt (530 mg) and dicyclohexylcarbodiimide (782 mg, 1.1 equivalents) were then added. The
reaction vessel was tightly stoppered and the reaction allowed to stir at room temperature for 72 hours. The reaction mixture was then diluted with ethyl acetate (50 mL). The precipitated dicyclohexylurea was filtered off and the supernatant washed with 10% citric acid (3 x 20 mL), 10% NaHCO₃ (3 x 20 mL) and brine (3 x 20 mL). The organic layer was dried (Na₂SO₄) and evaporated to dryness. The crude product was crystallized from CH₂Cl₂/petroleum ether (50 - 110°C). Yield of the Boc-Val-X was 670 mg, 59%. Deprotection of the t-butoxycarbonyl group was preformed as reported in literature (Bodanszky and Bodanszky "The Practice of Peptide Synthesis") by stirring in 4N HCl:dioxane overnight under argon. The solvent was removed under reduced pressure and the hydrochloride salt was dried overnight over a bed of NaOH. The product was used without further purification.

N-Benzylxycarboxyl-(O-benzyl)glutamate (200 mg, 0.54 mmol) was added to 5 mL of anhydrous DMF under argon. To this mixture was added 5-valyamino-1,3,4-thiadiazole-2-thione (144 mg, 1.1 eq), diisopropylethylamine (DIEA) (100 mL, 1.1 eq), HOBt (82 mg) and dicyclohexylcarbodiimide (122 mg, 1.1 eq). The reaction vessel was flushed with argon, tightly stoppered and allowed to stir overnight at room temperature. After diluting the reaction mixture with ethyl acetate (50 mL), the dicyclohexylurea was filtered off. The supernatant was then washed 10% citric acid (3 x 20 mL), 10% NaHCO₃ (3 x 20 mL) and brine (3 x 20 mL). The organic layer was dried (Na₂SO₄) and evaporated to dryness. An analytical sample was prepared by preparative TLC using CH₂Cl₂:MeOH (95:5), Rf=0.24. Melting point 97.6-102.3°C. ¹H-NMR (d₆-DMSO, int. std.) 14.1 (br s, 1H, NH thiadiazole) 12.7 (br s, 1H, NH amide thiadiazole) 8.2 (d, 1H, amide) 7.5 (d 1H, amide) 7.4 (app s, 10H, aromatic) 5.1 (s, 2H, CH₂ benzyl) 5.0 (s, 2H, CH₂ CBZ) 4.3 (t, 1H, chiral, J +) 4.1 (m, 1H, chiral) 1.9 (m, 4H, CH₂ Glu 0.9 (app s, 6H, CH₃).

Example 18

5-(N-benzylxycarbonyl-((3,4-diiodophenylalanyl)-valylamino)-1,3,4-thiadiazole-2-thione
This compound was prepared according to the procedure described in Example 9, using Boc-(3,5-diiodo)-phenylalanine in place of Boc-1-naphthalanine.

An analytical sample was prepared by preparative TLC using CH₂Cl₂:MeOH (95:5), R_f=0.38. Melting point 178.4-181.2°C. ¹H-NMR (d₆-DMSO, int. std.) 14.1 (br s, 1H, NH thiadiazole) 12.6 (br s, 1H, NH amide thiadiazole) 9.3 (s, 1H, OH Tyr) 8.3 (s 1H, amide) 7.7 (s, 2H, aromatic Tyr) 7.5 (d, 1H, amide) 7.2 (m, 5H, aromatic CBZ) 4.9 (s, 2H, CH₂ CBZ) 4.3 (m, 2H, 2 x chiral) 2.0 (br d, 1H, CH Val) 0.9 (app s, 6H, CH₃).

Example 19

5-(N-benzylxocarbonyl-(O-benzyl)seryl-valylamino)-1,3,4-thiadiazole-2-thione

This compound was prepared according the method described in Example 11 using N-benzylxocarbonyl-(O-benzyl)sereine in place of N-benzylxocarbonyl-(O-benzyltyrosine).

An analytical sample was prepared by preparative TLC using CH₂Cl₂:MeOH (95:5), R_f=0.33. Melting point 122.6-128.9°C. ¹H-NMR (d₆-DMSO, int. std.) 14.1 (br s, 1H, NH thiadiazole) 12.5 (br s, NH amide thiadiazole) 8.2 (d, 1H, amide) 7.3 (m 10H, aromatic) 5.0 (s, 2H, CH₂ benzyl) 4.5 (s, 2H, CH₂ CBZ) 4.4 (m, 2H, 2 x chiral) 2.6 (br app s, CH₂ Ser) 2.0 (br app s, CH Val) 0.9 (app d, 6H, CH₃).

Example 20

5-(N-benzylxocarbonyl-(γ-N-trityl)glutamyl-valylamino)-1,3,4-thiadiazole-2-thione

This compound was prepared according the method described in Example 11 using N-benzylxocarbonyl-(N-trityl)glutamine in place of N-benzylxocarbonyl-(O-benzyltyrosine).

An analytical sample was prepared by preparative TLC using CH₂Cl₂:MeOH (95:5), R_f=0.30. Melting point 146.8-155.7°C. ¹H-NMR (d₆-DMSO, int. std.) 14.1 (br s, NH thiadiazole) 12.6 (br s, NH amide thiadiazole) 8.5
(s, 1H, amide) 8.0 (s, 1H, amide) 7.2 (m 20H, aromatic) 5.0 (s, 2H, CH₂ CBZ) 4.4 (m, 1H, chiral) 4.1 (m, 1H, chiral) 2.4-1.6 (m, 5H) 0.8 (app s, 6H, CH₃).

Example 21

5-(N-benzyloxycarbonyl-((O-benzyl)tyrosyl)-aminoisobutyrolamino)-1,3,4-thiadiazole-2-thione

The synthesis of methyl 2-aminoisobutyrate (AibOMe) was accomplished by esterifying 2-aminoisobutyric acid in methanolic HCl. The HCl salt was crystallized from methanol/ether. The remainder of the synthesis was carried out according to the procedure described in Example 11, using AibOMe in place of valine methyl ester hydrochloride.

An analytical sample was prepared by preparative TLC using CH₂Cl₂:MeOH (95:5), Rf=0.47. Melting point 106.5-114.5°C. ¹H-NMR (d₆-DMSO, int. std.) 14.0 (br s, NH thiadiazole) 11.8 (br s, NH amide thiadiazole) 8.4 (s, 1H, amide) 7.5-6.9 (m, 15H, aromatic + 1 amide) 5.1 (s 2H, CH₂ benzyl) 4.9 (dd, 2H, CH₂ CBZ) 4.2 (m, 1H, chiral) 1.4 (s, 6H, CH₃).

Example 22

5-(N-benzyloxycarbonyl-((O-benzyl)tyrosyl)-phenylglycylamino)-1,3,4-thiadiazole-2-thione

This compound was prepared according to the procedure described in Example 11 using phenylglycine methyl ester hydrochloride in place of valine methyl ester hydrochloride.

An analytical sample was prepared by preparative TLC using CH₂Cl₂:MeOH (95:5), Rf=0.42. Melting point 123.0-127.8°C. ¹H-NMR (d₆-DMSO, int. std.) 14.1-12.7 (br hump, NH and NH amide of thiadiazole) 9.0 (d, 1H, amide) 7.6 - 6.8 (m, 20H, aromatics + 1 amide) 5.6 (d, 1H, chiral phenylglycyl) 5.1 (s 2H, CH₂ benzyl) 4.9 (s, 2H, CH₂ CBZ) 4.4 (br app s, 1H, chiral Tyr) 3.0 - 2.6 (m, 2H, CH₂ Tyr).
Example 23

5-(N-benzyloxycarbonyl-((O-methylene-2-naphthyl)tyrosyl)-valylamino)-1,3,4-thiadiazole-2-thione

The synthesis of O-methylene-2-naphthyl-tyrosine was accomplished using standard procedures through the copper chelate (Bodanszky and Bodanszky "The Practice of Peptide Synthesis") and crystallized from aqueous acetic acid. Acylation of O-methylene-2-naphthyl-tyrosine was accomplished using Schotten-Baumann conditions (Bodanszky, "Principles of Peptide Synthesis") in a water dioxane mixture with Cbz-Cl. 5 N NaOH was used to maintain the pH at about 10. After the reaction was complete, the mixture was adjusted to pH 7, concentrated to half-volume, diluted with water, acidified to pH 3 and extracted with ethyl acetate. The combined organic washes were dried (Na₂SO₄) and evaporated to dryness. The crude product, N-benzyloxycarbonyl-(O-methylene-2-naphthyl)tyrosine (Cbz-Tyr(Naph)), was used without further purification.

The remainder of the synthesis was carried out according to the procedure described in Example 11, using Cbz-Tyr(Naph) in place of N-benzyloxycarbonyl-(O-benzyl) tyrosine.

An analytical sample was prepared by preparative TLC using CH₂Cl₂:MeOH (95:5), Rf=0.45. Melting point 104.8-111.4°C. ¹H-NMR (δ, DMSO, int. std.) 14.1 (s, 1H, NH thiadiazole) 12.6 (s, 1H, NH amide thiadiazole) 8.2 (d, 1H, amide) 8.0-6.9 (m, 17H, aromatics + 1 amide) 5.2 (s, 2H, CH₂ benzyllic) 4.9 (s, 2H, CH₂ CBZ) 4.3 (m, 2H, 2 x chiral) 0.9 (m, 6H, CH₃).

Example 24

5-(N-benzyloxycarbonyl-((γ-N-trityl)glutamyl)-phenylglycylamino)-1,3,4-thiadiazole-2-thione

This compound was prepared according to the procedure described in Example 11 using N-benzyloxycarbonyl-((γ-N-trityl)glutamine and phenylglycine methyl ester hydrochloride in place of N-benzyloxycarbonyl-(O-benzyl)tyrosine) and valine methyl ester hydrochloride.
An analytical sample was prepared by preparative TLC using CH₂Cl₂:MeOH (95:5), Rᵣ=0.46. Melting point 158.4-167.4°C. H-NMR (d ₇ DMSO, int. std.) 14.0-12.6 (br hump, 1.5H, NH and NH amide thiadiazole) 8.7 (d, 1H, amide) 8.5 (s, 1H, amide) 7.6-6.9 (m, 25H, aromatics) 5.6 (app dd, 1H, chiral phenylglycyl) 5.0 (s, 2H, CH₂ CBZ) 4.1 (m, 1H, chiral Gln) 2.4-1.6 (m, 4H).

**Example 25**

5-(N-benzylxycarbonyl-(γ-N,N-dibenzyl)glutamylphenylglycylamino)-1,3,4-thiadiazole-2-thione

Dicyclohexylcarbodiimide (0.22 grams), then N-benzylxycarbonyl-(γ-N,N,N-dibenzyl)glutamine-phenylglycine (0.48 grams) and the 1-hydroxybenzotriazole (0.15 grams) were dissolved in 5 mL of anhydrous DMF. The solution was kept in room temperature until dicyclohexylurea precipitation was completed (about 40 minutes). 5-Amino-1,3,4- thiadiazole-2-thione (0.39 grams) was added and solution was left for 2 days. An excess of ethyl acetate was added (50mL) and dicyclohexylurea was filtered off. The supernatant was washed several times washed with 10% aqueous citric acid (3 x 20 mL), 5% aqueous sodium bicarbonate (3 x 20 mL) and brine (3 x 20 mL). The ethyl acetate layer was dried with sodium sulfate and evaporated to dryness. The light yellow solid was purified by preparative TLC. The resulting as white solid was dried at 50°C under vacuum. M.P. 140°C. Rᵣ 0.37; eluent: methanol:methylene-chloride 5:95. NMR spectrum (d₆-DMSO) 14.1 (s, 1H), 12.8 (s, 1H), 8.8 (s, 1H), 7.3 (m, 21H), 5.6 (d, 1H), 5.0 (d, 2H), 4.4 (m, 4H), 4.2 (m, 1H), 2.5 (m, 2H), 2.0 (m, 2H).

**Example 26**

5-(N-benzylxycarbonyl-(γ-N-2-phenylethyl)glutamylphenylglycylamino)-1,3,4-thiadiazole-2-thione

This compound was prepared according to the procedure described in Example 25 using N-benzylxycarbonyl-(γ-N-phenylethyl)glutamine-
phenylglycine in place of N-benzyloxy carbonyl-((γ-N,N-dibenzyl)glutamine-phenylglycine. M.P. 145°C. Rf 0.40; eluent: methanol: methylene-chloride 10:100. NMR spectrum (d₆-DMSO) 14.1 (s, 1H), 12.8 (s, 1H), 8.8 (s, 1H), 7.9 (m, 1H), 7.2 (m, 16H), 5.7 (d, 2H), 4.1 (m, 1H), 3.2 (m, 2H), 2.6 (m, 2H), 2.5 (m 2H), 2.0 (m, 2H).

**Example 27**

5-((N-benzyloxy carbonyl-leucyl-valylamino)-1,3,4-thiadiazole-2-thione

N-Benzyl oxycarbonyl-leucyl-valine (0.186 grams) and 5-amino-1,3,4-thiadiazole-2-thione (0.497 grams) were added to 6 mL of anhydrous DMF, followed by dicyclohexylcarbodiimide (0.277 grams). The reaction mixture was allowed to stir for three days at room temperature. The DMF was partially removed on a rotary evaporator and the residue was diluted with 100 mL of ethyl acetate. The dicyclohexylurea was filtered off and the filtrate was extracted with 10% citric acid (3 x 30 mL), 10% sodium bicarbonate (3 x 30 mL), and brine solution (3 x 30 mL). The ethyl acetate solution was dried over sodium sulfate, filtered and evaporated to dryness on a rotary evaporator. The final product was purified from preparative TLC and gave one spot material on TLC (Rf 0.67: eluent; methylene chloride: methanol 95:5). M.P. 146°C. NMR spectrum (d₆-DMSO) 14.1 (s, 1H), 12.70 (s, 1H), 8.1 (s, 1H), 7.4 (s, 6H), 5.0 (s, 2H), 4.3 (s, 1H), 4.1 (s, 1H), 1.8 (m, 1H), 1.4 (m, 2H), 0.90 (s, 12H).

**Example 28**

5-(N-Benzyl oxycarbonyl-isoleucyl-valylamino)-1,3,4-thiadiazole-2-thione

This compound was prepared by the procedure described in Example 28 except that (benzyloxy carbonyl)isoleucyl-valine was used in place of N-(benzyloxy carbonyl)leucyl-valine. The final product was purified by preparative TLC and gave one spot by TLC analysis (Rf 0.55: eluent; Methylene chloride:
methanol 95:5). M.P. 197-1980°C. NMR spectrum (d$_6$-DMSO) 14.1 (s, 1H), 12.70 (s, 1H), 8.1 (s, 1H), 7.7 (s, 1H), 7.3 (s, 6H), 5.0 (s, 2H), 4.1 (s, 1H), 1.8 (m, 2H), 1.4 (m, 1H), 1.2 (m, 3H), 0.80 (s, 9H).

**Example 29**

5-((N-Benzylloxycarbonyl)-(2-fluoro)phenyl-valylamino)-1,3,4-thiadiazole-2-thione

This compound was prepared by the procedure described in Example 27 except that N-(benzylloxycarbonyl)-(2-fluoro)phenylalanyl-valine was used in place of N-(benzylloxycarbonyl)leucyl-valine. The final product was purified from preparative TLC and gave one spot by TLC analysis (Rf 0.81: eluent; Methylene chloride: methanol 95:5). M.P. 148°C. NMR spectrum (d$_6$-DMSO) 14.1 (s, 1H), 12.70 (s, 1H), 7.9 (s, 1H), 7.3 (m, 10H), 5.0 (s, 2H), 4.5 (s, 1H).

**Example 30**

5-((N-benzylloxycarbonyl)-(O-Benzyl)tyrosyl-glycylamino)-1,3,4-thiadiazole-2-thione

This compound was prepared by the procedure described in Example 27 except that N-(benzylloxycarbonyl)-(O-benzyl)tyrosyl-glycine was used in place of N-(benzylloxycarbonyl)leucyl-valine and the reaction was stirred for six days. The final product was purified from preparative TLC and gave one spot by TLC analysis (Rf 0.65: eluent; Methylene chloride: methanol 95:5). M.P. 201-202°C. NMR spectrum (d$_6$-DMSO) 14.1 (s, 1H), 12.5 (s, 1H), 8.5 (s, 1H), 7.9-6.6 (m, 14H), 5.1 (s, 2H), 4.9 (s, 2H), 4.2 (s, 1H), 4.0 (s, 2H).

**Example 31**

5-((N-benzylloxycarbonyl)-(O-Benzyl)tyrosyl-(t-buty)glycylamino)-1,3,4-thiadiazole-2-thione

This compound was prepared by the procedure described in Example 27 except that N-(benzylloxycarbonyl)-(O-benzyl)tyrosyl-(t-buty)glycine was used in place of N-(benzylloxycarbonyl)leucyl-valine and the reaction was stirred for
six days. The final product was purified from preparative TLC and gave one spot by TLC analysis (Rf 0.84: eluent; Methylene chloride: methanol 95:5). M.P.146°C. NMR spectrum (d6-DMSO) 14.05 (s, 1H), 12.3 (s, 1H) 7.6-6.9 (m, 14H), 5.6 (s, 1H), 5.1 (s, 2H), 4.9 (s, 2H), 4.4 (m, 2H), 1.0 (m, 14H).

Example 32

5-(N-benzyloxy carbonyl-(O-Benzyl)tyrosyl-(cyclohexyl)glycylamino)-1,3,4-thiadiazole-2-thione

This compound was prepared by the procedure described in Example 27 except that N-(benzyloxy carbonyl)-(O-benzyl)tyrosyl-(cyclohexyl)glycine was used in place of N-(benzyloxy carbonyl)leucyl-valine and the reaction was stirred for four days. The final product was purified from preparative TLC and gave one spot by TLC analysis (Rf 0.64: eluent; Methylene chloride: methanol 95:5). M.P.150°C. NMR spectrum (d6-DMSO) 14.1 (s, 1H), 12.6 (s, 1H), 7.7-6.8 (m, 14H), 5.0 (s, 2H), 4.9 (s, 2H), 4.3 (broad s, 2H), 1.6 (m, 6H), 1.1 (m, 6H).

Example 33

5-(N-benzyloxy carbonyl-(N,N'-dibenzylamino)phenylalanine-phenylglycylamino)-1,3,4-thiadiazole-2-thione

N-Benzyloxy carbonyl-(p-N,N'-dibenzylamino)phenylalanine (0.294 grams), 1-hydroxybenzotriazole (0.89 grams), and 5-phenylalaninylamino-1,3,4-thiadiazole-2-thione hydrochloride (0.193 grams) were added to 5 mL methylene chloride, followed by dicyclohexylcarbodiimide (0.132 grams). The reaction mixture was allowed to stir for 24 hours. The methylene chloride was partially removed on the rotary evaporator and the residue was diluted with 100 mL of ethyl acetate. The dicyclohexylurea was filtered off and the filtrate was extracted with 10% citric acid (3 x 20 mL), 10% sodium carbonate (3 x 20 mL), and brine solution (3 x 20 mL). The ethyl acetate was dried over sodium sulfate and evaporated to dryness on the rotary evaporator. The final product was purified by preparative TLC and gave one spot by TLC (Rf 0.80: eluent; Methylene chloride: methanol 95:5). M.P. 197-199°C. NMR spectrum
(d$_6$-DMSO) 14.1 (s, 1H), 12.9 (s, 1H), 8.9 (d, 1H), 7.7-6.6 (m, 24H), 5.8 (d, 1H), 4.9 (dd, 2H), 4.6 (s, 4H), 4.2 (s 1H).

**Example 34**

5-\((N\text{-benzyloxy carbonyl-phenylalanyl-tryptonyl-valylamino)}\)\-1,3,4-thiadiazole-2-thione

Dicyclohexylcarbodiimide (0.47 grams) was dissolved in 5 mL of anhydrous DMF, followed by N-(benzyloxy carbonyl)phenylalanyl-tryptonyl-valine (1.34 grams) and 1-hydroxybenzotriazole (0.45 grams). The solution was kept at room temperature until dicyclohexylurea was completely precipitated (about 1 hour). 5-Amino-1,3,4-thiadiazole-2-thione (1.1 grams) was added and the mixture was allowed to stir for 2 days. An excess of ethyl acetate was added (150 mL) and the organic layer was washed with 5% aqueous sodium bicarbonate (3 x 50 mL), 10% aqueous citric acid (3 x 50 mL) and water (3 x 50 mL). The organic layer was separated, dried over sodium sulfate and evaporated to dryness. The residue was recrystallized from ethyl acetate/pentane to give a white solid. M.P. 203-206°C. NMR spectrum (d$_6$-DMSO) 14.1 (s, 1H), 12.6 (s, 1H), 10.8 (s, 1H), 8.25 (d, 1H), 8.15 (d, 1H), 7.6-6.8 (m, 16 H, NH+aromatics), 4.9 (s, 2H), 4.65 (m, 1H), 4.35 (m, 1H), 4.25 (m, 1H), 3.2-2.6 (m, 4H), 2.0 (m, 1H), 0.9 (bs, 4H).

**Example 35**

5-\((N\text{-Benzyloxy carbonyl-glycyl-tyrosyl-}()-(O\text{-benzyl) tyrosyl)}\text{-phenylglycyl amino)}\)-1,3,4-thiadiazole-2-thione

\textit{N-t-Butoxycarbonyl-(O-benzyl)tyrosyl-phenylglycyl-methylester}

Phenylglycine methyl ester hydrochloride (1.8 grams), triethylamine (1.3 mL) and HBT (1.09 grams) were dissolved in 30 mL CH$_2$Cl$_2$. N-t-Boc-(O-benzyl)tyrosine (3.0 grams) was then added and the solution was stirred at room temperature for 15 minutes. Dicyclohexylcarbodiimide (1.83 grams) was then added and the solution stirred for 6 hours. An excess of methylene chloride (250
mL) was added and the resulting solution was washed until TLC analysis showed
one spot. (Rf 0.71: eluent; methanol:chloroform 5:95). The organic layer was
separated, dried over sodium sulfate and evaporated to dryness. The product was
obtained as an off white powder. M.P. 130-132°C. NMR spectrum (d_6-DMSO) 8.6
(d, 1H), 6.7-7.6 (m, 16H, NH + aromatics), 5.5 (m, 1H), 5 (s, 2H), 4.3 (m, 1H),
3.7 (s, 3H) 2.9 (m, 2H), 1.3 (s, 9H).

**N-t-Butoxycarbonyl-(O-benzyl)tyrosyl-phenylglycine**

2N NaOH was dissolved in 10 mL of dioxane, followed by the addition
of N-t-boc-(O-benzyl)tyrosyl-phenylglycine methyl ester. The solution was
stirred at room temperature for 45 minutes. An excess of water (60 mL) was
added to the reaction mixture and the resulting solution was washed with ethyl
acetate (2 x 30 mL). The aqueous layer was separated and acidified to pH 3. The
resulting solution was extracted with ethyl acetate (5x 50 mL), dried over sodium
sulfate and evaporated to dryness. The product was obtained as an off white
powder. M.P. 158-161°C. NMR spectrum (d_6-DMSO) 8.6 (d, 1H), 6.7-7.5 (m,
16H, NH+ aromatics), 5.3 (m, 1H), 5 (s, 2H), 4.2 (m, 1H), 2.9 (m, 2H), 1.3 (s,
9H).

**5-(N-t-Butoxycarbonyl-(O-benzyl)tyrosyl-phenylglycineamino)-1,3,4-
thiadiazole-2-thione**

5-Amino-1,3,4-thiadiazole-2-thione (0.875 grams) was dissolved under
a nitrogen atmosphere in 8 mL of anhydrous DMF. N-t-Boc-(O-benzyl)tyrosyl-
phenylglycine (1.0 grams) and HBT (0.33 grams) were then added and the
solution was stirred at room temperature for about 30 minutes. Dicyclohexylcarbodiimide (0.5 grams) was then added and the reaction was
stirred for 3 days. An excess of ethyl acetate was added (100 mL) and the
resulting solution was washed several times with 5% aqueous sodium bicarbonate, 10% aqueous citric acid and water until TLC analysis showed one
spot (Rf 0.45: eluent; methanol: chloroform 5:95). The organic layer was
separated, dried over sodium sulfate and evaporated to dryness. The product was
obtained as a yellow residue which was recrystallized from methylene chloride-petroleum. M.P. 118-120.5°C. NMR spectrum (d_6-DMSO) 14.1 (s, 1H), 12.9 (s, 1H), 8.8 (d, 1H), 6.8-7.8 (m, 19H, NH+ aromatics), 5.6 (m, 1H), 5.0 (d, 4H), 4.3 (m, 1H), 2.9 (m, 2H), 1.3 (s, 9H).

5-((O-Benzyl)tyrosyl-phenylglycylamino)-1,3,4-thiadiazole-2-thione hydrochloride

4N HCl (5 mL) followed by 5-(N-t-butoxycarbonyl-(O-benzyl)tyrosyl-phenylglycylamino)-1,3,4-thiadiazole-2-thione (1.0 grams) were dissolved in 5 mL of dioxane. Nitrogen was bubbled through the reaction mixture for 15 minutes and the reaction was then allowed to stir at room temperature overnight. The reaction mixture was evaporated to dryness to give a yellow powder as the product. NMR spectrum (d_6-DMSO) 14.1 (s, 1H), 12.9 (s, 1H), 8.8 (d, 1H), 6.8-7.8 (m, 19H, NH+ aromatics), 5.6 (m, 1H), 5.0 (d, 4H), 4.3 (m, 1H), 2.9 (m, 2H).

5-((N-benzylxocarbonyl-glycyl-tyrosyl-(O-benzyl)tyrosyl)-phenylglycylamino)-1,3,4-thiadiazole-2-thione hydrochloride (135 mg), triethylamine (35 µL) and HBT (33 mg) were dissolved in 2 mL ethyl acetate, followed by the addition of N-(t-boc-glycyl-tyrosine) (100 mg). The solution was stirred at room temperature for 15 minutes. Dicyclohexylcarbodiimide (55 mg) was added to the reaction mixture, which was then stirred for 24 hours. An excess of ethyl acetate (50 mL) was added and the resulting solution was washed several times with 5% aqueous sodium bicarbonate, 10% aqueous citric acid and brine until TLC analysis showed one spot. (RF:0.4, eluent; methanol:chloroform 5:95). The organic layer was separated, dried over sodium sulfate and evaporated to dryness. The product was recrystallized from methylene chloride-petroleum to give an off white powder. M.P. 143-154°C. NMR spectrum (d_6-DMSO) 14.1(s, 1H), 12.9 (s, 1H), 8.8 (m,
1H), 8.1 (m, 1H), 7.8 (m, 1H), 6.6-7.6 (m, 25H, NH + aromatics), 5.5 (m, 1H), 5.0 (s, 4H), 4.3-4.7 (m, 2H), 3.7 (s, 2H), 2.8 (m, 4H).

Example 36
5-(N-benzylxocarbonyl-proyl-phenylalanyl-((O-benzyl)tyrosyl)-phenylglycylamino)-1,3,4-thiadiazole-2-thione

5-((O-Benzyl)tyrosyl-phenylglycylamino)-1,3,4-thiadiazole-2-thione (159.4 mg), triethylamine (42 µl) and HBT (38 mg) were dissolved in 2 ml ethyl acetate, followed by the addition of N-boc-proyl-phenylalanine (125 mg), triethylamine (42 µL) and HBT (38 mg). The solution was stirred at room temperature for 15 minutes. Dicyclohexylcarbodiimide (65 mg) was then added and the reaction stirred for 24 hours. An excess of ethyl acetate (50 mL) was added and the resulting solution was washed several times with 5% aqueous sodium bicarbonate, 10% aqueous citric acid and brine until TLC analysis showed one spot. (Rf : 0.38, eluent; methanol:chloroform 5:95). The organic layer was separated, dried over sodium sulfate and evaporated to dryness. The product was recrystallized from methylene chloride-petroleum to give an off white powder. M.P.144.7 - 149.2°C. NMR spectrum (δ-DMSO) 14.05 (s, 1H), 12.8 (s, 1H), 9 (m, 1H), 8 (m, 2H) 6.8-7.6 (m, 24H, aromatics), 5.5 (m, 1H), 5.0 (m, 4H), 4.8 (m, 1H), 4.5 (m, 1H), 4.1 (m, 1H), 2.6-3.0 (m, 4H), 1.4 -1.8 (m, 6H).

Example 37
5-(N-4-(4-tert-butylbenzamido)benzoylphenylglycinylamino)-1,3,4-thiadiazole-2-thione

To a solution of N-t-BOC-L-phenylglycine (2 g) in DMF (25 mL) was added 5-amino-1,3,4-thiadiazole-2-thione (3.2 g) and 1-hydroxybenzotriazole (1.18 g). After 15 min, dicyclohexylcarbodiimide (1.8 g) was added. The reaction mixture was allowed to stir under a nitrogen atmosphere for 2 days. The reaction mixture was filtered and the filtrate was diluted with ethyl acetate. The resultant solution was washed with 5% aqueous sodium bicarbonate, 10% aqueous citric acid, and water. The organic layer was dried over sodium sulfate
and evaporated to dryness. The residue was redissolved in ethyl acetate and
filtered to remove dicyclohexylurea. The filtrate was concentrated to dryness, and
the residue was crystallized from ethyl acetate-petroleum ether to afford 5-(\(N\text{-}t\text{-}
BOC\)-L-phenylglycinylamino-1,3,4-thiadiazole-2-thione.

5(N\text{-}t\text{-}BOC\text{-}L\text{-}Phenylglycinylamino-1,3,4-thiadiazole-2-thione (3 g) was
dissolved in dioxane (30 mL) and nitrogen was bubbled through the resultant
solution for 20 min. A solution of 4N HCl (30 mL) was added dropwise over a
period of 20 min, and the resultant mixture was stirred at room temperature
overnight. The reaction mixture was concentrated to dryness, and the residue was
crystallized from ethanol-ether to produce 2.4 g of 5-(L-phenylglycinylamino-
1,3,4-thiadiazole-2-thione hydrochloride as white crystals.

To a solution of 4-(4-\text{-}tert\text{-}butylbenzamido)benzoic acid (2.1 g; prepared
by reaction of methyl 4-aminobenzoate with 4-\text{-}tert\text{-}butylbenzoyl chloride,
followed by base hydrolysis) in a mixture of ethyl acetate and DMF was added
5-(L-phenylglycinylamino-1,3,4-thiadiazole-2-thione hydrochloride (2 g),
followed by triethylamine (0.95 mL). \text{1-Hydroxybenzotriazole (1.08 g) was
added and the resultant mixture was stirred under nitrogen for 15 min.
Dicyclohexylcarbodiimide (1.65 g) was added and the reaction mixture was
stirred at room temperature overnight. The reaction mixture was filtered and the
filtrate was diluted with ethyl acetate. The resultant solution was washed with
5% aqueous sodium bicarbonate, 10% aqueous citric acid, and water. The
organic layer was dried over sodium sulfate and evaporated to dryness. The
residue was purified by column chromatography (elution with 30-50% ethyl
acetate-hexane). The product was recrystallized from ethyl acetate-petroleum
ether to afford 2.2 g of the title compound. \(R_f = 0.47\) (5% \text{MeOH-CH}_2\text{Cl}_2).
Example 38

Assay of Stromelysin Inhibition Activity

Stromelysin was first activated by trypsin. This was done by preparing a reaction mixture in H-150 (H-150 consists of 10 mM CaCl₂, 150 mM NaCl and 100 mM HEPES at pH 7.4) containing a final concentration of 25 μg/mL trypsin and 2.2 μM of stromelysin (Marcy et al., Biochemistry, 30:6476 (1991); Koklititis et al., Biochem. J., 376:217 (1991). The reaction was incubated for 30 minutes at 37°C and then quenched by adding trypsin inhibitor agarose to the reaction mixture at a 20-fold excess with respect to trypsin.

The reaction mixture was centrifuged at 14,000 rpm (16,000 x g) for 30 minutes using an Eppendorf Centrifuge 5415C. The supernatant, which contains activated stromelysin, was concentrated with a Centricon 10 (5000 g, 1 hour). A sample was analyzed by Bradford total protein assay and 12% SDS polyacrylamide gel electrophoresis.

All steps of the stromelysin inhibition assay were performed at room temperature. Assay solutions were prepared for each inhibitor tested. Activated stromelysin was added to a stirred cuvette to a final concentration of 2 nM in 2 mL of H-150 buffer. 4 μL 5 mM coumarin peptide substrate was dissolved in DMSO and the initial rate of hydrolysis was measured for 100-200 seconds.

Substrate hydrolysis was assessed by fluorescence using a slit width of 10:10, excitation at 328 nm and emission at 393 nm. Ki was calculated based on the assumption of simple competitive inhibition and a substrate concentration much less than Km. The results of these assays are listed in Table I as the IC₅₀. 2 μL of 10 mM inhibitor in DMSO was added and the reduction in hydrolysis rate measured. The addition of 2 μL of inhibitor was continued until a 30-70% inhibition was observed.
<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; [nM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-(N-benzoyloxycarbonyl-tryptylamino)-1,3,4-thiadiazole-2-thione</td>
<td>3370</td>
</tr>
<tr>
<td>5-(N-(9-fluorenlymethoxycarbonyl)valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>128</td>
</tr>
<tr>
<td>5-(N-benzoyloxycarbonyl-phenylalanylamino)-1,3,4-thiadiazole-2-thione</td>
<td>2360</td>
</tr>
<tr>
<td>5-(N-(9-fluorenlymethoxycarbonyl-norvalyl)-1,3,4-thiadiazole-2-thione</td>
<td>509</td>
</tr>
<tr>
<td>5-(N-(9-fluorenlymethoxycarbonyl)tryptylamino)-1,3,4-thiadiazole-2-thione</td>
<td>341</td>
</tr>
<tr>
<td>5-(N-(9-fluorenlymethoxycarbonyl)leucylamino)-1,3,4-thiadiazole-2-thione</td>
<td>184</td>
</tr>
<tr>
<td>5-(N-(9-fluorenlymethoxycarbonyl)methionylamino)-1,3,4-thiadiazole-2-thione</td>
<td>195</td>
</tr>
<tr>
<td>5-(N-(9-fluorenlymethoxycarbonyl)homophenylalanylamino)-1,3,4-thiadiazole-2-thione</td>
<td>436</td>
</tr>
<tr>
<td>5-(N-t-butoxycarbonyl-leucylamino)-1,3,4-thiadiazole-2-thione</td>
<td>10200</td>
</tr>
<tr>
<td>5-(N-t-butoxycarbonyl-homophenylalanylamino)-1,3,4-thiadiazole-2-thione</td>
<td>11900</td>
</tr>
<tr>
<td>5-(N-((4-phenyl)phenylacetyl)valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>100</td>
</tr>
<tr>
<td>5-(N-benzoyloxycarbonyl-(ortho-fluoro)phenylalanylamino)-1,3,4-thiadiazole-2-thione</td>
<td>630</td>
</tr>
<tr>
<td>5-(N-(8-quinolinesulfonil)phenylalanyl-valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>531</td>
</tr>
<tr>
<td>5-(N-(2-methylthionicotyl)phenylalanyl-valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>917</td>
</tr>
<tr>
<td>5-(N-hydrocinamoyl-phenylalanyl-valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>267</td>
</tr>
<tr>
<td>5-(N-(4-phenylbenzoyl)phenylalanyl-valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>82</td>
</tr>
<tr>
<td>5-(N-nonanoyl-phenylalanyl-valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>83</td>
</tr>
<tr>
<td>5-(N-(4-phenyl)phenylacetyl-phenylalanyl-valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>135</td>
</tr>
<tr>
<td>5-(N-(4-benzoylox)benzoyl-phenylalanyl-valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>61</td>
</tr>
<tr>
<td>5-(N-(4-phenoxy)benzoyl-phenylalanyl-valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>101</td>
</tr>
<tr>
<td>5-(N-acetyl-leucyl-leucylamino)-1,3,4-thiadiazole-2-thione</td>
<td>6440</td>
</tr>
<tr>
<td>Compounds</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; [nM]</td>
</tr>
<tr>
<td>--------------------------------------------------------------------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>5-((N-(4-((4-t-butylphenyl)sulfonamino)benzoyl)-phenylalanyl-valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>44</td>
</tr>
<tr>
<td>5-((N-benzylxycarbonyl-phenylalanyl-leucylamino)-1,3,4-thiadiazole-2-thione</td>
<td>169</td>
</tr>
<tr>
<td>5-((N-benzylxycarbonyl-leucyl-tyrosylamino)-1,3,4-thiadiazole-2-thione</td>
<td>1300</td>
</tr>
<tr>
<td>5-((N-benzylxycarbonyl-phenylalanyl-alanylamino)-1,3,4-thiadiazole-2-thione</td>
<td>1400</td>
</tr>
<tr>
<td>5-((N-benzylxycarbonyl-phenylalanyl-glycylamino)-1,3,4-thiadiazole-2-thione</td>
<td>3950</td>
</tr>
<tr>
<td>5-((N-benzylxycarbonyl-phenylalanyl-methionylamino)-1,3,4-thiadiazole-2-thione</td>
<td>1121</td>
</tr>
<tr>
<td>5-((N-benzylxycarbonyl-phenylalanyl-valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>279</td>
</tr>
<tr>
<td>5-((N-benzylxycarbonyl-tryptoyl-valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>741</td>
</tr>
<tr>
<td>5-((N-benzylxycarbonyl-tryptoyl-phenylalanylamino)-1,3,4-thiadiazole-2-thione</td>
<td>225</td>
</tr>
<tr>
<td>5-((N-benzylxycarbonyl-leucyl-methionylamino)-1,3,4-thiadiazole-2-thione</td>
<td>490</td>
</tr>
<tr>
<td>5-((N-benzylxycarbonyl-(2-(1-naphtyl))alanyl-valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>190</td>
</tr>
<tr>
<td>5-((N-benzylxycarbonyl-(2-(2-naphtyl))alanyl-valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>221</td>
</tr>
<tr>
<td>5-((N-benzylxycarbonyl-(O-benzyl))tryosyl-valylamino-1,3,4-thiadiazole-2-thione</td>
<td>80</td>
</tr>
<tr>
<td>5-((N-benzylxycarbonyl-tic-valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>200</td>
</tr>
<tr>
<td>5-((N-benzylxycarbonyl-(para-fluoro)phenylalanyl-valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>156</td>
</tr>
<tr>
<td>5-((N-benzylxycarbonyl-leucyl-valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>660</td>
</tr>
<tr>
<td>5-((N-benzylxycarbonyl-cyclohexylglycyl-valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>180</td>
</tr>
<tr>
<td>5-((N-benzylxycarbonyl-isoleucyl-valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>940</td>
</tr>
<tr>
<td>5-((N-benzylxycarbonyl-(O-benzyl)glutamoyl-valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>146</td>
</tr>
<tr>
<td>Compounds</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; [nM]</td>
</tr>
<tr>
<td>--------------------------------------------------------------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>5-((N-benzyloxycarbonyl)-(p-nitro)phenylalanyl-valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>180</td>
</tr>
<tr>
<td>5-((N-benzyloxycarbonyl)-((p-benzyloxycarbonylamino)phenylalanyl)-valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>62</td>
</tr>
<tr>
<td>5-((N-benzyloxycarbonyl)-((3,4-diiodophenylalanyl)-valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>97</td>
</tr>
<tr>
<td>5-((N-benzyloxycarbonyl)-((S-benzyl)cysteinyl)-valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>202</td>
</tr>
<tr>
<td>5-((N-benzyloxycarbonyl)-((ortho-flouro)phenylalanyl)-valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>633</td>
</tr>
<tr>
<td>5-((N-benzyloxycarbonyl)-(O-benzyl)seryl)-valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>236</td>
</tr>
<tr>
<td>5-((N-benzyloxycarbonyl)-((N-trityl)glutamyl)-valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>91</td>
</tr>
<tr>
<td>5-((N-benzyloxycarbonyl)-((O-benzyl)tyrosyl)-aminoisobutyrolylamo)-1,3,4-thiadiazole-2-thione</td>
<td>237</td>
</tr>
<tr>
<td>5-((N-benzyloxycarbonyl)-((O-benzyl)tyrosyl)-phenylglycylamino)-1,3,4-thiadiazole-2-thione</td>
<td>39</td>
</tr>
<tr>
<td>5-((N-benzyloxycarbonyl)-((O-methylene-2-naphtyl)tyrosyl)-valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>62</td>
</tr>
<tr>
<td>5-((N-benzyloxycarbonyl)-((O-benzyl)tyrosyl-(t-butyl)glycylamino)-1,3,4-thiadiazole-2-thione</td>
<td>89</td>
</tr>
<tr>
<td>5-((N-benzyloxycarbonyl)-((N-trityl)glutamyl)-phenylglycylamino)-1,3,4-thiadiazole-2-thione</td>
<td>50</td>
</tr>
<tr>
<td>Compounds</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; [nM]</td>
</tr>
<tr>
<td>---------------------------------------------------------------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>5-((N-benzyloxycarbonyl-((O-benzyl)tyrosyl)-cyclohexylglycylamino)-1,3,4-thiadiazole-2-thione</td>
<td>71</td>
</tr>
<tr>
<td>5-((N-t-butyloxycarbonyl-((O-benzyl)tyrosyl)-phenylglycylamino)-1,3,4-thiadiazole-2-thione</td>
<td>64</td>
</tr>
<tr>
<td>5-((N-benzyloxycarbonyl-((N,N-dibenzyl)glutamyl)-phenylglycylamino)-1,3,4-thiadiazole-2-thione</td>
<td>19</td>
</tr>
<tr>
<td>5-((N-benzyloxycarbonyl-((p-N,N-dibenzylamino)phenylalanyl)-phenylglycylamino)-1,3,4-thiadiazole-2-thione</td>
<td>205</td>
</tr>
<tr>
<td>5-((N-benzyloxycarbonyl-((O-benzyl)tyrosyl)-leucylamino)-1,3,4-thiadiazole-2-thione</td>
<td>96</td>
</tr>
<tr>
<td>5-((N-benzyloxycarbonyl-(N-2-phenylethyl)glutamyl-phenylglycylamino)-1,3,4-thiadiazole-2-thione</td>
<td>57</td>
</tr>
<tr>
<td>5-((N-benzyloxycarbonyl-phenylalanyl-leucyl-tryptonylamino)-1,3,4-thiadiazole-2-thione</td>
<td>246</td>
</tr>
<tr>
<td>5-((N-benzyloxycarbonyl-phenylalanyl-valyl-tryptonylamino)-1,3,4-thiadiazole-2-thione</td>
<td>273</td>
</tr>
<tr>
<td>5-((N-benzyloxycarbonyl-prolyl-leucyl-alanylamino)-1,3,4-thiadiazole-2-thione</td>
<td>1740</td>
</tr>
<tr>
<td>5-((N-benzyloxycarbonyl-phenylalanyl-tryptonyl-valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>60</td>
</tr>
<tr>
<td>5-((N-benzyloxycarbonyl-lysyl(N-s-t-butyloxycarbonyl)-tyrosyl(0-benzyl)-phenylglycylamino)-1,3,4-thiadiazole-2-thione</td>
<td>148</td>
</tr>
<tr>
<td>5-((N-benzyloxycarbonyl-lysyl-((O-benzyl)tyrosyl)-phenylglycylamino)-1,3,4-thiadiazole-2-thione</td>
<td>1890</td>
</tr>
<tr>
<td>5-((N-benzyloxycarbonyl-glycyl-tyrosyl-((O-benzyl)tyrosyl)-phenylglycylamino)-1,3,4-thiadiazole-2-thione</td>
<td>110</td>
</tr>
</tbody>
</table>
Example 39

Assay of Human Neutrophil Collagenase Inhibition Activity

H-150 buffer, pH 7.4, was prepared by adding CaCl$_2$$\cdot$H$_2$O (1.47 grams), NaCl (8.77 grams) and N-[2-hydroxyethyl]piperazine-N’-[2-ethanesulfonic acid] (23.83 grams) to distilled and deionized water and then bringing the final volume of the solution to 1 liter.

A reaction mixture with a final volume of 2.0 mL was prepared from H-150 buffer, human neutrophil collagenase (Schnierer, et al., Biochem. Biophys. Res. Comm., 191:319 (1993); Knight et al., Federation of European Biochemical Societies 296:263 (1992)) (2 nM). Coumarin peptide substrate (4 µL of a 5 mM solution in dimethyl sulfoxide) was added to the reaction mixture. The rate of hydrolysis was determined for about 100-200 seconds by measuring the fluorescence of the reaction at ex328 nanometers and em329 nanometers using a Hitachi F-2000 Fluorescence Spectrophotometer.

2 µL of a 10 mM inhibitor stock solution (in dimethyl sulfoxide) is added and the reduction in the rate of hydrolysis is determined. Additional 2 µL aliquots of the stock inhibitor solution is added until a 30% to 70% inhibition is observed. $K_i$ is calculated on the assumption of simple competitive inhibition and that the substrate concentration is much less than $K_m$. The results are shown in Table II.
### TABLE II
INHIBITION DATA FOR HUMAN NEUTROPHIL COLLAGENASE

<table>
<thead>
<tr>
<th>Compounds</th>
<th>$K_{i(app)}$ [µM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-(N-t-butyloxy carbonyl-((O-benzyl) tyrosyl-phenylglycylamino)-1,3,4-thiadiazole-2-thione</td>
<td>0.99</td>
</tr>
<tr>
<td>5-(N-(4-(4-t-butylyphenylsulfonylamino)benzoyl)phenylalanyl-valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>1.04</td>
</tr>
<tr>
<td>5-(N-benzyloxy carbonyl-((p-benzyloxy carbonylamino)phenylalanyl)-valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>1.17</td>
</tr>
<tr>
<td>5-(N-benzyloxy carbonyl-phenylalanyl-leucyl-tryptonylamino)-1,3,4-thiadiazole-2-thione</td>
<td>1.45</td>
</tr>
<tr>
<td>5-(N-benzyloxy carbonyl-((O-benzyl) tyrosyl)phenylglycylamino)-1,3,4-thiadiazole-2-thione</td>
<td>1.57</td>
</tr>
<tr>
<td>5-(N-(4-phenoxy)benzoyl-phenylalanyl-valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>1.67</td>
</tr>
<tr>
<td>5-(N-(4-phenylbenzoyl)phenylalanyl-valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>2.6</td>
</tr>
<tr>
<td>5-(N-benzyloxy carbonyl-phenylalanyl-valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>4.8</td>
</tr>
<tr>
<td>5-(N-benzyloxy carbonyl-((O-benzyl) tyrosyl)valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>2.15</td>
</tr>
<tr>
<td>5-(N-benzyloxy carbonyl-((O-benzyl) tyrosyl-leucylamino)-1,3,4-thiadiazole-2-thione</td>
<td>1.83</td>
</tr>
<tr>
<td>5-(N-benzyloxy carbonyl-((S-benzyl) cysteiny lamino)-1,3,4-thiadiazole-2-thione</td>
<td>3.2</td>
</tr>
<tr>
<td>5-(N-benzyloxy carbonyl-phenylalanyl-valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>7.8</td>
</tr>
<tr>
<td>5-(N-benzyloxy carbonyl-phenylalanyl-methionylamino)-1,3,4-thiadiazole-2-thione</td>
<td>7.8</td>
</tr>
<tr>
<td>5-(N-benzyloxy carbonyl-((γ-N-2-phenylethyl)glutamyl)-phenylglycylamino)-1,3,4-thiadiazole-2-thione</td>
<td>0.54</td>
</tr>
<tr>
<td>5-(N-benzyloxy carbonyl-phenylalanyl-tryptoyl-valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>2.4</td>
</tr>
<tr>
<td>5-(N-benzyloxy carbonyl-phenylalanyl-homophenylalanyl amino)-1,3,4-thiadiazole-2-thione</td>
<td>2.9</td>
</tr>
</tbody>
</table>
Example 40

Assay of 72 KD Gelatinase Inhibition Activity

H-150 buffer, pH 7.4, was prepared by CaCl₂·H₂O (1.47 grams), NaCl (8.77 grams) and N-[2-hydroxyethyl]piperazine-N’-[2-ethanesulfonic acid] (23.83 grams) to distilled and deionized water and then bring the final volume of the solution to 1 liter. An reaction mixture having a final volume of 250 µL was prepared with H-150 buffer, 40 µ grams of Pro-72 KD gelatinase (Strongin, et al., J. Biol. Chem. 268:14033 (1993); Goldberg, et al., J. Biol. Chem., 267:4583 (1992); Kleiner, et al., Biochemistry 32:1583 (1993)) and p-aminophenyl mercuric acetate (Sigma Chemical Co., St. Louis, MO) (1 mM). The reaction mixture was then incubated for 3 hours at 25°C.

The reaction mixture was applied to a NAP-5 column (G-25 SEPHADEX™, Pharmacia). Fractions containing 92KD gelatinase were identified using 12% SDS polyacrylamide gel electrophoresis and concentrated to about 100-200 µliters by centrifugation (5000 g, 15 minutes).

A solution containing H-150 buffer (2.0 mL), 72KD gelatinase (180 pM) and ethylphenolpoly(ethylene-glycolether)ₙ (Boehringer Mannheim) (1.3 µM) was prepared in a stirred cuvette. Coumarin peptide substrate (2 µL of a 1 mM solution in dimethyl sulfoxide) was added to the reaction mixture. The rate of hydrolysis was determined for about 100-200 seconds by measuring the fluorescence of the reaction at ex328 nanometers and em329 nanometers using a Hitachi F-2000 Fluorescence Spectrophotometer.

2 µL of a 10 mM inhibitor stock solution (in dimethyl sulfoxide) is added and the reduction in the rate of hydrolysis is determined. Additional 2 µL aliquots of the stock inhibitor solution is added until a 30% to 70% inhibition is observed. Kᵢ is calculated on the assumption of simple competitive inhibition and that the substrate concentration is much less than Kₘ. The results are shown in Table III.
<table>
<thead>
<tr>
<th>Compounds</th>
<th>$K_{i(app)}$ [µM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-((N-t-butyloxycarbonyl-((O-benzyl)tyrosyl)-phenylglycylamino)-1,3,4-thiadiazole-2-thione</td>
<td>0.51</td>
</tr>
<tr>
<td>5-((N-4-((4-t-butylyphenylsulfonylamino)benzoyl)phenylalanyl-valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>0.68</td>
</tr>
<tr>
<td>5-((N-benzyloxycarbonyl-((p-benzyloxycarbonylamino)phenylalanyl)-valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>0.29</td>
</tr>
<tr>
<td>5-((N-benzyloxycarbonyl-phenylalanyl-methionyl-((O-benzyl)tyrosyl)-phenylglycylamino)-1,3,4-thiadiazole-2-thione</td>
<td>0.2</td>
</tr>
<tr>
<td>5-((N-benzyloxycarbonyl-((O-benzyl)tyrosyl)-phenylglycylamino)-1,3,4-thiadiazole-2-thione</td>
<td>0.145</td>
</tr>
<tr>
<td>5-((N-benzyloxycarbonyl-((O-benzyl)tyrosyl)-glycylamino)-1,3,4-thiadiazole-2-thione</td>
<td>0.38</td>
</tr>
<tr>
<td>5-((N-(4-phenylbenzoyl)phenylalanyl-valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>0.8</td>
</tr>
<tr>
<td>5-((N-benzyloxycarbonyl-phenylalanyl-leucylamino)-1,3,4-thiadiazole-2-thione</td>
<td>1.95</td>
</tr>
<tr>
<td>5-((N-benzyloxycarbonyl-glycyl-tyrosyl-((O-benzyl)tyrosyl)-phenylglycylamino)-1,3,4-thiadiazole-2-thione</td>
<td>0.073</td>
</tr>
<tr>
<td>5-((N-benzyloxycarbonyl-(O-benzyl)tyrosyl-leucylamino)-1,3,4-thiadiazole-2-thione</td>
<td>0.24</td>
</tr>
<tr>
<td>5-((N-benzyloxycarbonyl-(S-benzyl)cysteiny1-valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>1.1</td>
</tr>
<tr>
<td>5-((N-benzyloxycarbonyl-phenylalanyl-valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>4.6</td>
</tr>
<tr>
<td>5-((N-benzyloxycarbonyl-phenylalanyl-methionylamino)-1,3,4-thiadiazole-2-thione</td>
<td>1.7</td>
</tr>
<tr>
<td>5-((N-benzyloxycarbonyl-((γ-N-2-phenylethyl)glutamyl)-phenylglycylamino)-1,3,4-thiadiazole-2-thione</td>
<td>0.52</td>
</tr>
<tr>
<td>5-((N-benzyloxycarbonyl-phenylalanyl-tryptoy1-valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>0.71</td>
</tr>
<tr>
<td>5-((N-benzyloxycarbonyl-phenylalanyl-homophenylalanylamino)-1,3,4-thiadiazole-2-thione</td>
<td>0.95</td>
</tr>
</tbody>
</table>
**Example 41**

*Assay of 92 KD Gelatinase Inhibition Activity*

H-150 buffer, pH 7.4, was prepared by CaCl₂•H₂O (1.47 grams), NaCl (8.77 grams) and N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid] (23.83 grams) to distilled and deionized water and then bring the final volume of the solution to 1 liter. A reaction mixture having a final volume of 500 µL was prepared with H-150 buffer, 200 µgrams of Pro-92KD gelatinase (Strongin et al., *J. Biol. Chem.*, 268:14033 (1993); Goldberg, et al., *J. Biol. Chem.*, 267:4583 (1992); Okada et al., *J. Biol. Chem. 267:21712* (1992)), p-aminophenyl mercuric acetate (Sigma Chemical Co., St. Louis, MO) (1 mM) and Brij (dodecylpoly (oxyethylene glycolether))(0.008% w/v). The reaction mixture was then incubated for 24 hours at 37°C.

The reaction mixture was applied to a NAP-5 column (G-25 SEPHADEX™, Pharmacia). Fractions containing 92KD gelatinase were identified using 12% polyacrylamide gel electrophoresis and concentrated to about 100-200 µliters by centrifugation (5000 g, 1 hour).

A solution containing H-150 buffer (2.0 mL), 92KD gelatinase (25 pM) and ethylphenolpoly(ethylene-glycolether)₉ (Boehringer Mannheim) (4.0 µM) was prepared in a stirred cuvette. Coumarin peptide substrate (4 µL of a 5 mM solution in dimethyl sulfoxide) was added to the reaction mixture. The rate of hydrolysis was determined for about 100-200 seconds by measuring the fluorescence of the reaction at ex328 nanometers and em329 nanometers using a Hitachi F-2000 Fluorescence Spectrophotometer.

2 µL of a 10 mM inhibitor stock solution (in dimethyl sulfoxide) is added and the reduction in the rate of hydrolysis is determined. Additional 2 µL aliquots of the stock inhibitor solution is added until a 30% to 70% inhibition is observed. Kᵢ is calculated on the assumption of simple competitive inhibition and that the substrate concentration is much less than Kₘ. The results are shown in Table IV.
<table>
<thead>
<tr>
<th>Compounds</th>
<th>$K_{i(app)}$ [µM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-[(N-t-butyloxy carbonyl)-(O-benzyl) tyrosyl]-phenylglycylamino]-1,3,4-thiadiazole-2-thione</td>
<td>0.245</td>
</tr>
<tr>
<td>5-[(N-4-(4-t-butyloxy sulfonamido) benzoyl) phenylalanyl-valylamino]-1,3,4-thiadiazole-2-thione</td>
<td>0.31</td>
</tr>
<tr>
<td>5-[(N-benzyloxy carbonyl)-(p-benzyloxy carbonylamino phenylalanyl)-valylamino]-1,3,4-thiadiazole-2-thione</td>
<td>0.145</td>
</tr>
<tr>
<td>5-[(N-benzyloxy carbonyl)-phenylalanyl-alanylamino]-1,3,4-thiadiazole-2-thione</td>
<td>3.6</td>
</tr>
<tr>
<td>5-[(N-benzyloxy carbonyl)-(O-benzyl) tyrosyl phenylalanylamino]-1,3,4-thiadiazole-2-thione</td>
<td>0.15</td>
</tr>
<tr>
<td>5-[(N-benzyloxy carbonyl)-phenylalanyl-tryptyl-valylamino]-1,3,4-thiadiazole-2-thione</td>
<td>0.36</td>
</tr>
<tr>
<td>5-[(N-(4-phenylbenzoyl) phenylalanyl-valylamino]-1,3,4-thiadiazole-2-thione</td>
<td>0.31</td>
</tr>
<tr>
<td>5-[(N-benzyloxy carbonyl)-phenylulcylamino]-1,3,4-thiadiazole-2-thione</td>
<td>2.3</td>
</tr>
<tr>
<td>5-[(N-(9-fluorenylmethoxy carbonyl)-phenylalanylamino]-1,3,4-thiadiazole-2-thione</td>
<td>0.23</td>
</tr>
<tr>
<td>5-[(N-benzyloxy carbonyl)-(O-benzyl) tyrosyl-leucylamino]-1,3,4-thiadiazole-2-thione</td>
<td>0.29</td>
</tr>
<tr>
<td>5-[(N-benzyloxy carbonyl)-(S-benzyl) cysteinyln-valylamino]-1,3,4-thiadiazole-2-thione</td>
<td>2.1</td>
</tr>
<tr>
<td>5-[(N-benzyloxy carbonyl)-phenylalanyl-valylamino]-1,3,4-thiadiazole-2-thione</td>
<td>1.5</td>
</tr>
<tr>
<td>5-[(N-benzyloxy carbonyl)-phenylalanyl-methionylamino]-1-3,4-thiadiazole-2-thione</td>
<td>3.6</td>
</tr>
<tr>
<td>5-[(N-benzyloxy carbonyl) -((γ-N-2-phenylethyl) glutamyl)-phenylglycylamino]-1,3,4-thiadiazole-2-thione</td>
<td>0.17</td>
</tr>
<tr>
<td>5-[(N-benzyloxy carbonyl)-prolyl-(O-benzyl) tyrosyl]-phenylglycylamino]-1,3,4-thiadiazole-2-thione</td>
<td>0.76</td>
</tr>
<tr>
<td>5-[(N-(9-fluorenylmethoxy carbonyl)-homophenylalanlamino]-1,3,4-thiadiazole-2-thione</td>
<td>0.35</td>
</tr>
</tbody>
</table>
Example 42

Inhibition of Cartilage Degradation in the Bovine Cartilage Explant Assay

A tissue culture assay was used to measure the ability of the compounds of the present invention to slow the degradation of the extracellular matrix by metalloproteinases. This assay measured the amount of $^{35}$S-glycosaminoglycan ($^{35}$S-GAG) released from labeled bovine cartilage explants.

Knee joints from a 1 to 3 week old calf were obtained immediately after sacrifice from the Abattoir and then transported on ice. The intact joints were washed well with tap water and soaked in 50% (v/v) Povidine iodine solution, obtained from Burre National, Inc., Baltimore, MD. All subsequent steps were performed in a laminar flow tissue culture hood using standard sterile technique. The joint was immobilized in a shank holder and the joint capsule was cut open to expose the articular cartilage. Cartilage explant plugs, approximately 15 mg wet weight, were removed from the flat articulating surfaces in the lower-most region of the knee joint using a sterile steel cork-borer and collected in a 250 mL roller bottle containing about 100 mL fresh Delbecco's minimum essential medium (DMEM), obtained from Gibco BRC, Life Technologies, Gaithersburg, MD, containing 4.5 g/l (D)-glucose and (L)-glutamine, without sodium pyruvate. The fresh media also contained enough Hepes buffer and sodium bicarbonate such that the pH was about 7.4. The media was then further supplemented just before use with 100 units Penicillin, 100 µg Streptomycin, and 50 µg (L)-ascorbic acid per mL of medium.

Once collected, the explant plugs were washed four times with 50 mL fresh DMEM. The plugs were then placed in the incubator for a minimum of 1 hour to equilibrate, before proceeding to make disks from the articulating surface of each plug. A 1 mm thick disk was sliced from individual plugs from the end that was the articulating surface of the joint. The plug was held steady in the sterile template (4 mm diameter x 1.5 mm deep) using sterile forceps. A scalpel blade was used to carefully slice off the disk. Only the superficial articulating surface responded well in culture.
Individual disks obtained were transferred to a tissue culture flask containing about 100 mL fresh media. The flask containing the disks was placed in an incubator at 37°C (with 5% CO₂, 95% air) and allowed to equilibrate overnight and at least one additional day before labeling. When ready to label, the old media was replaced with 50 mL fresh media containing about 500 μCi ³⁵S-Sodium Sulfate. The plugs were labeled in bulk for about 48 hours. The next morning, the "hot" media was removed and replaced with fresh "cold" media. The disks were again allowed to equilibrate overnight before being used for actual experiments.

The media in which the disks were stored was changed immediately prior to performing the assay. The disks were then returned to the incubator until the test media and the two control media had been prepared. The test media consisted of the desired concentration of a compound being tested for its ability to inhibit extracellular matrix degradation and concomitant recombinant human Interleukin rhIL-1α (5 ng/mL) in fresh DMEM solution. The control media were identical to the test media, except that the first control media lacked rhIL-1α and the second control media lacked the test compound. 250 μL of each of the test and control media were transferred to separate 96-well TC plates. Flamed forceps were used to transfer a disk from the incubator to each 96-well TC plate that had been filled with either the test media or one of the two control media.

The TC plates were then placed in the incubator and cultured for 3-4 days (initial incubation with rhIL-1α alpha takes at least 3 days to stimulate endogenous metalloproteinases). A 50 μL aliquot of media from each TC plate was saved and counted. The rest of the media was removed with a suction device.

The cartilage disks from each TC plate were also saved for counting. The disks were removed with forceps and placed in microcentrifuge tubes and then dissolved in 250 μL of full strength formic acid. The tubes were capped and placed at 65-70°C in a block-heater for 4-6 hours. A 50 μL aliquot was then counted.
The percent $^{35}$S-GAG release is calculated as follows:

$$\% \, ^{35}\text{S-GAG} \, \text{release} = \left( \frac{\text{cpm}_{\text{medium}}}{\text{cpm}_{\text{medium}} + \text{cpm}_{\text{explant}}} \right) \times 100\%$$

The percent inhibition of extracellular matrix damage in cartilage explant was calculated as follows:

$$\% \, \text{Inhibition} = \left( \frac{A - B - (C - B)}{A - B} \right) \times 100,$$

wherein

A = $\%$ GAG release induced by rhIL-1$\alpha$;
B = $\%$ GAG release in the absence of rhIL-1$\alpha$; and
C = $\%$ GAG release in the presence of rhIL-1$\alpha$ plus the compound being tested.

The percent inhibition of amino acid amides of 5-amino-1,3,4-thiadiazole-2-thione and the concentrations at which they were tested are given in Table V below.
TABLE V.
INHIBITION OF PLASMINOGEN/IL-1 STIMULATED CARTILAGE DEGRADATION

<table>
<thead>
<tr>
<th>Compound</th>
<th>% inhibition (concentration [µM])</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-((N-benzyloxy carbonyl-phenylalanyl-leucylamino)-1,3,4-thiadiazole-2-thione</td>
<td>33% (50)</td>
</tr>
<tr>
<td>5-((N-(9-fluorenlymethoxycarbonyl)valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>42% (20)</td>
</tr>
<tr>
<td>5-((N-benzyloxy carbonyl-phenylalanyl-valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>40% (10)</td>
</tr>
<tr>
<td>5-((N-benzyloxy carbonyl-tryptolyl-phenylalanylamino)-1,3,4-thiadiazole-2-thione</td>
<td>35% (50)</td>
</tr>
<tr>
<td>5-((N-benzyloxy carbonyl-(O-benzyl)tyrosyl)-valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>59% (50)</td>
</tr>
<tr>
<td>5-((N-benzyloxy carbonyl-(O-benzyl)glutamoyl)-valylamino-1,3,4-thiadiazole-2-thione</td>
<td>33% (25)</td>
</tr>
<tr>
<td>5-((N-benzyloxy carbonyl-D-homophenylalanylamino)-1,3,4-thiadiazole-2-thione</td>
<td>22% (25)</td>
</tr>
<tr>
<td>5-((N-(4-phenyl)phenylacetyl-phenylalanyl-valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>37% (25)</td>
</tr>
<tr>
<td>5-((N-benzyloxy carbonyl-((NH-trityl)glutamyl)-valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>56% (25)</td>
</tr>
<tr>
<td>5-((N-(4-phenyl)phenylacetyl-valylamino)-1,3,4-thiadiazole-2-thione</td>
<td>47% (25)</td>
</tr>
<tr>
<td>5-((N-benzyloxy carbonyl-((O-benzyl)tyrosyl)-phenylglycylamino)-1,3,4-thiadiazole-2-thione</td>
<td>20% (25)</td>
</tr>
<tr>
<td>5-((N-(9-fluorenlymethoxycarbonyl-homophenylalanylamino)-1,3,4-thiadiazole-2-thione</td>
<td>65% (50)</td>
</tr>
</tbody>
</table>
Example 43

Assay of Additional Compounds for Stromelysin Inhibition Activity, Human Neutrophil Collagenase and 72 KD Gelatinase

A number of additional compounds within the scope of the present invention were assayed according to the procedures outlined in Examples 38, 39 and 40 herein. IC₅₀ values for these compounds for each of the listed enzymes are reported in Table VI.
<table>
<thead>
<tr>
<th>Table VI. Inhibition Data for Additional Compounds of the Present Invention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound</td>
</tr>
<tr>
<td>----</td>
</tr>
<tr>
<td>![Compound 1]</td>
</tr>
<tr>
<td>![Compound 2]</td>
</tr>
</tbody>
</table>
| Compound | \[\text{Collagenase (MMP-1)} \]
<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>(72\text{kDa Gelatinase (MMP-2)} )</td>
</tr>
<tr>
<td>(\text{IC}_{50} ) ((\mu\text{M}))</td>
</tr>
</tbody>
</table>

| Compound | \[\text{Stromelysin (MMP-3)} \]
<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

**SUBSTITUTE SHEET (RULE 26)**
<table>
<thead>
<tr>
<th>Compound</th>
<th>Stromelysin (MMP-3) IC$_{50}$ (µM)</th>
<th>72kDa Gelatinase (MMP-2) IC$_{50}$ (µM)</th>
<th>Collagenase (MMP-1) IC$_{50}$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>![Chemical Structure 1]</td>
<td>0.063 (w/o det)</td>
<td>3.913 (39 µM NP40/tris) 0.868 (w/o det)</td>
<td>1.547</td>
</tr>
<tr>
<td>![Chemical Structure 2]</td>
<td>0.047 (w/o det)</td>
<td>1.924 1.374 (w/o det)</td>
<td>2.257</td>
</tr>
<tr>
<td>![Chemical Structure 3]</td>
<td>0.350 (w/o det)</td>
<td>4.519 3.106 (w/o det)</td>
<td>9.912</td>
</tr>
<tr>
<td>Compound</td>
<td>72kDa Gelatinase (MMP-2) IC₅₀ (μM)</td>
<td>Stromelysin (MMP-3) IC₅₀ (μM)</td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>---------------------------------</td>
<td>-----------------------------</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.195 (w/o det)</td>
<td>0.500 (w/o det)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.171 (w/o det)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collagenase (MMP-1) IC₅₀ (μM)</td>
<td>0.207</td>
<td>1.665</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collagenase (MMP-1)</td>
<td>Gelatinase (MMP-2)</td>
<td>Stromelysin (MMP-3)</td>
<td></td>
</tr>
<tr>
<td>---------------------</td>
<td>---------------------</td>
<td>---------------------</td>
<td></td>
</tr>
<tr>
<td>IC&lt;sub&gt;50&lt;/sub&gt; (µM)</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; (µM)</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; (µM)</td>
<td></td>
</tr>
<tr>
<td>0.736</td>
<td>0.736</td>
<td>0.335</td>
<td></td>
</tr>
<tr>
<td>72kDa</td>
<td>1.572</td>
<td>0.439 (w/o det)</td>
<td></td>
</tr>
<tr>
<td>0.439 (w/o det)</td>
<td>0.908</td>
<td>0.345 (w/o det)</td>
<td></td>
</tr>
<tr>
<td>14.995 (w/o det)</td>
<td>0.132 (w/o det)</td>
<td>0.522 (w/o det)</td>
<td></td>
</tr>
<tr>
<td>0.132 (w/o det)</td>
<td>0.121 (w/o det)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Compound**

![Chemical Structures]
<table>
<thead>
<tr>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Chemical Structure 1" /></td>
</tr>
<tr>
<td><img src="image2.png" alt="Chemical Structure 2" /></td>
</tr>
<tr>
<td><img src="image3.png" alt="Chemical Structure 3" /></td>
</tr>
</tbody>
</table>

| Collagenase (MMP-1) $IC_{50}$ (µM) | 0.513 | 15.525 | 33.406 |
| 72kDa Gelatinase (MMP-2) $IC_{50}$ (µM) | 0.569 (w/o det) | 8.422 (w/o det) | 1.619 |
| Stromelysin (MMP-3) $IC_{50}$ (µM) | 0.099 (w/o det) | 5.245 (w/o det) | 1.543 |

**SUBSTITUTE SHEET (RULE 26)**
<table>
<thead>
<tr>
<th>Compound</th>
<th>72kDa Gelatinase (MMP-2) IC₅₀ (µM)</th>
<th>Collagenase (MMP-1) IC₅₀ (µM)</th>
<th>Stromelysin (MMP-3) IC₅₀ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Compound Image" /></td>
<td>1.108</td>
<td>7.034</td>
<td>0.229</td>
</tr>
<tr>
<td><img src="image2" alt="Compound Image" /></td>
<td>0.329</td>
<td>1.462</td>
<td>0.242</td>
</tr>
<tr>
<td><img src="image3" alt="Compound Image" /></td>
<td>36.714</td>
<td>0.000</td>
<td>5.838</td>
</tr>
<tr>
<td>Compound</td>
<td>Stromelysin (MMP-3) IC\textsubscript{50} (\mu M)</td>
<td>72kDa Gelatinase (MMP-2) IC\textsubscript{50} (\mu M)</td>
<td>Collagenase (MMP-1) IC\textsubscript{50} (\mu M)</td>
</tr>
<tr>
<td>----------</td>
<td>-----------------------------------------------</td>
<td>--------------------------------------------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td><img src="image1.png" alt="Chemical Structure 1" /></td>
<td>16.078</td>
<td>1.017</td>
<td>40.192</td>
</tr>
<tr>
<td><img src="image2.png" alt="Chemical Structure 2" /></td>
<td>0.176</td>
<td>0.161</td>
<td>0.845</td>
</tr>
<tr>
<td></td>
<td>Collagenase (MMP-1) $IC_{50}$ (µM)</td>
<td>72kDa Gelatinase (MMP-2) $IC_{50}$ (µM)</td>
<td>Stromelysin (MMP-3) $IC_{50}$ (µM)</td>
</tr>
<tr>
<td>---------------------</td>
<td>-----------------------------------</td>
<td>---------------------------------------</td>
<td>-------------------------------------</td>
</tr>
<tr>
<td></td>
<td>0.336</td>
<td>0.122</td>
<td>0.111</td>
</tr>
<tr>
<td>Compound 1</td>
<td><img src="image1" alt="Compound 1" /></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compound 2</td>
<td><img src="image2" alt="Compound 2" /></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Collagenase (MMP-1) IC_{50} (µM)</td>
<td>Gelatinase (MMP-2) IC_{50} (µM)</td>
<td>Stromelysin (MMP-3) IC_{50} (µM)</td>
</tr>
<tr>
<td>---------------------</td>
<td>---------------------------------</td>
<td>---------------------------------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>72 kDa</td>
<td>0.145</td>
<td>0.248</td>
<td>1.241</td>
</tr>
<tr>
<td></td>
<td>0.0075</td>
<td>0.177</td>
<td>0.259</td>
</tr>
<tr>
<td></td>
<td>0.027</td>
<td>0.020</td>
<td>0.028</td>
</tr>
</tbody>
</table>

<p>| Compound            | ![Compound 1]                  | ![Compound 2]                  | ![Compound 3]                    |</p>
<table>
<thead>
<tr>
<th>Compound</th>
<th>Collagenase (MMP-1) IC₅₀ (nM)</th>
<th>72kDa Gelatinase (MMP-2) IC₅₀ (µM)</th>
<th>Stromelysin (MMP-3) IC₅₀ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.976</td>
<td>0.435</td>
<td>0.581</td>
</tr>
<tr>
<td></td>
<td>1.893</td>
<td>0.321</td>
<td>0.287</td>
</tr>
<tr>
<td></td>
<td>1.384</td>
<td>0.570</td>
<td>0.125</td>
</tr>
</tbody>
</table>

[Chemical structures of compounds]
<table>
<thead>
<tr>
<th>Compound</th>
<th>IC_{50} (μM)</th>
<th>Compound</th>
<th>IC_{50} (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagenase (MMP-1)</td>
<td>1.194</td>
<td>72kDa Gelatinase (MMP-2)</td>
<td>0.150</td>
</tr>
<tr>
<td>Stromelysin (MMP-3)</td>
<td>0.244</td>
<td></td>
<td>0.308</td>
</tr>
</tbody>
</table>

The table above shows the IC_{50} values for different enzymes with the indicated compounds.
<table>
<thead>
<tr>
<th>Compound</th>
<th>72kDa Gelatinase (IC_{50} [µM])</th>
<th>Stromelysin (IC_{50} [µM])</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagenase (MMP-1) IC_{50} [µM]</td>
<td>0.240</td>
<td>6.741</td>
</tr>
<tr>
<td>72kDa Gelatinase (MMP-2) IC_{50} [µM]</td>
<td>0.303</td>
<td>0.085</td>
</tr>
<tr>
<td>Stromelysin (MMP-3) IC_{50} [µM]</td>
<td>0.124</td>
<td>0.647</td>
</tr>
</tbody>
</table>

![Chemical structures of compounds](image)
<table>
<thead>
<tr>
<th>Compound</th>
<th>72kDa Gelatinase (MMP-2) IC₅₀ (µM)</th>
<th>Stromelysin (MMP-3) IC₅₀ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.314</td>
<td>0.454</td>
</tr>
<tr>
<td></td>
<td>1.087</td>
<td>1.586</td>
</tr>
</tbody>
</table>

**Formula Images:**

- [Chemical Structure 1](image)
- [Chemical Structure 2](image)
<table>
<thead>
<tr>
<th>Compound</th>
<th>Collagenase (MMP-1) IC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>72kDa Gelatinase (MMP-2) IC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>Stromelysin (MMP-3) IC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20.149</td>
<td>0.165</td>
<td>0.965</td>
</tr>
</tbody>
</table>

![Compound Structures](image)
<table>
<thead>
<tr>
<th>Compound</th>
<th>IC₅₀ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagenase (MMP-1)</td>
<td>0.228</td>
</tr>
<tr>
<td>Gelatinase (MMP-2)</td>
<td>0.178</td>
</tr>
<tr>
<td>Stromelysin (MMP-3)</td>
<td>0.388</td>
</tr>
<tr>
<td></td>
<td>6.375</td>
</tr>
</tbody>
</table>

[Chemical structures for each compound are shown in the diagram.]

<table>
<thead>
<tr>
<th>Compound</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>72kDa Gelatinase (MMP-2)</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; (μM)</td>
<td>3.035</td>
</tr>
<tr>
<td>Stromelysin (MMP-3)</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; (μM)</td>
<td>0.199 (w/o det)</td>
</tr>
<tr>
<td>Collagenase (MMP-1)</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; (μM)</td>
<td>7.852</td>
</tr>
</tbody>
</table>

The table includes the IC<sub>50</sub> values for different enzymes in micromolar (μM) units.
<table>
<thead>
<tr>
<th>Compound</th>
<th>Stromelysin (MMP-3) IC&lt;sub&gt;50&lt;/sub&gt; (μM)</th>
<th>72kDa Gelatinase (MMP-2) IC&lt;sub&gt;50&lt;/sub&gt; (μM)</th>
<th>Collagenase (MMP-1) IC&lt;sub&gt;50&lt;/sub&gt; (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Compound 1" /></td>
<td>1.669</td>
<td>0.216</td>
<td>0.526</td>
</tr>
<tr>
<td><img src="image2" alt="Compound 2" /></td>
<td>0.019 (w/o det)</td>
<td>0.216</td>
<td>0.526</td>
</tr>
<tr>
<td>Compound</td>
<td>72kDa Gelatinase (MMP-2) IC₅₀ (µM)</td>
<td>11.336</td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>---------------------------------</td>
<td>--------</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.853</td>
<td>19,808</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.706</td>
<td>6.088</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.306</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In the chemical diagrams, the abbreviation "Bz" refers to a benzyl group.

1: "w/o det" refers to without detergent. In certain instances, a particular detergent and concentration will appear in parentheses.
Equivalents

Those skilled in the art will know, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. These and all other equivalents are intended to be encompassed by the following claims.
What Is Claimed Is:

1. A method of inhibiting matrix metalloproteinases, comprising contacting a matrix metalloproteinase with a compound that binds at the active site of said metalloproteinase thereby inhibiting the proteolytic activity of said metalloproteinase, wherein said compound has the Formula I:

\[
\begin{array}{c}
\text{Z} \rightarrow \text{N} \rightarrow \text{A} \\
\text{R1} \\
\end{array}
\]

or a pharmaceutically acceptable salt thereof, wherein

- Q and A are independently selected from the group consisting of sulfur and oxygen and one of Q and A is sulfur;
- R1 is one of hydrogen, lower alkyl or acyl; and

Z represents an organic radical that does not substantially interfere with the ability of the group

\[
\begin{array}{c}
\text{N} \rightarrow \text{A} \\
\text{R1} \\
\end{array}
\]

(IV) to inhibit a matrix metalloproteinase; provided that said compound is other than a compound of Formula II herein where R4 is hydrogen.

2. The method of claim 1, wherein Z is covalently linked to the 5-amino nitrogen through an amide, amine, sulfonamide, urea, thiourea, carbamate, thiocarbamate, sulfinamide, sulfenamide, phosphonamide or phosphinamide bond.
3. The method of claim 2, wherein Z is covalently linked to the 5-amino nitrogen through an amide, amine, sulfonamide, carbamate, thiocarbamate, sulfinamide, phosphonamide or phosphinamide bond.

4. The method of claim 1, wherein Z is a steroid, amino acid, oligopeptide, or amino protecting group.

5. The method of claim 1, wherein the compound of Formula I is other than a compound of Formula VI or Formula VII.

6. The method of any one of claims 1-5, wherein A and Q are both sulfur.

7. The method of any one of claims 1-5, wherein R1 is hydrogen.

8. A pharmaceutical composition, which comprises:
   a) a pharmaceutically acceptable carrier or diluent; and
   b) an amount of a compound effective for treating a mammal
      with a disease, wherein said disease is ameliorated by inhibiting at least one
      matrix metalloproteinase enzyme to whom said composition is administered, and
      wherein said compound has the Formula I:

      \[
      \begin{align*}
      \text{Z} & \quad \text{N} \quad \text{A} \quad \text{Q} \\
      \text{R1} &
      \end{align*}
      \]

      or a pharmaceutically acceptable salt thereof, wherein
      Q and A are independently selected from the group consisting of sulfur
      and oxygen and one of Q and A is sulfur;
R1 is one of hydrogen, lower alkyl or acyl; and

Z represents an organic radical that does not substantially interfere with

the ability of the group

\[
\begin{array}{c}
\text{N} \\
\text{A}
\end{array}
\xrightarrow{\text{Q}}
\begin{array}{c}
\text{N} \\
\text{R1}
\end{array}
\]  \(IV\)

(IV) to inhibit a matrix metalloproteinase;

provided that said compound is other than a compound of Formula II herein, where R4 is hydrogen.

9. The pharmaceutical composition of 8, wherein Z is covalently linked to the 5-amino nitrogen through an amide, amine, sulfonamide, urea, thiourea, carbamate, thiocarbamate, sulfinamide, sulfenamide, phosphonamide or phosphinamide bond.

10. The pharmaceutical composition of 9, wherein Z is covalently linked to the 5-amino nitrogen through an amide, amine, sulfonamide, carbamate, thiocarbamate, sulfinamide, phosphonamide or phosphinamide bond.

11. The pharmaceutical composition of 8, wherein Z is a steroid, amino acid, oligopeptide, or amino protecting group.

12. The pharmaceutical composition of 8, wherein the compound of Formula I is other than a compound of Formula VI or Formula VII.

13. The pharmaceutical composition of any one of claims 8-12, wherein A and Q are both sulfur.

14. The pharmaceutical composition of any one of claims 8-12, wherein R1 is hydrogen.
15. The pharmaceutical composition any one of claims 8-12, wherein the disease is osteoarthritis.

16. The pharmaceutical composition any one of claims 8-12, wherein the disease is rheumatoid arthritis.

17. The pharmaceutical composition any one of claims 8-12, wherein the disease is cancer.

18. The pharmaceutical composition any one of claims 8-12, wherein the inhibition of at least one matrix metalloproteinase enzyme results in a decrease in inflammation caused by the disease.

19. A method of treating an individual with a disease, wherein said disease is ameliorated by inhibiting at least one matrix metalloproteinase enzyme, comprising administering a therapeutically effective amount of a composition any one of claims 8-12.

20. The method of claim 19, wherein the disease is osteoarthritis.

21. A compound of Formula II:

\[
\begin{align*}
R3 - (R4)N & \quad \text{O} \\
R2 & \quad \cdots \\
\text{n-1} & \\
\end{align*}
\]

\[
\begin{align*}
\text{HN} & \quad \text{O} \\
R2 & \quad \text{N} \quad \text{A} \quad \text{Q} \\
R1 & \quad \text{NH} \\
\end{align*}
\]

wherein:
Q and A are each independently selected from the group consisting of sulfur and oxygen and one of Q and A is sulfur;
n is a positive integer which results in a matrix metalloproteinase inhibitor;
R1 is selected from the group consisting of -H, lower alkyl and acyl;
R4 is taken together with the R2 on the carbon atom adjacent to the nitrogen to which R4 is bound to form a 3 or 4 carbon atom carbocyclic bridge, that is optionally substituted or optionally fused to a benzene ring;
each R2, when not taken with R4 to form a carbocyclic bridge, is independently selected from the group consisting of C1-C10 straight or branched alkyl, C1-C10 straight or branched substituted alkyl, C3-C8 cyclic alkyl, substituted C3-C8 cyclic alkyl, C1-C10 straight or branched alkenyl, C1-C10 straight or branched substituted alkenyl, C1-C10 straight or branched substituted alkynyl, C1-C10 straight or branched substituted alkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl;
R3 is selected from the group consisting of an amine protecting group X-CO-, X-CS-, X-SO₂-, X-O-CO- and X-O-CS-;
X is selected from the group consisting of C1-C10 alkyl, C1-C10 substituted alkyl, aryl, substituted aryl, heteroaryl and substituted heteroaryl; or a physiologically active salt thereof.

22. The compound of claim 21, wherein:
n is an integer from 1-10;
R1 is -H; and
R3 is selected from the group consisting of X-CO-, X-CS-, X-SO₂-, X-O-CO- and X-O-CS-.

23. The compound of claim 22, wherein:
A and Q are each sulfur; and
each R2 that is not on the carbon atom adjacent to the nitrogen to which R4 is bound is selected from the group consisting of a side chain of a naturally occurring amino acid, (substituted phenyl)-CH₂-, naphtyl-CH₂-, (O-substituted)tyrosyl, cycloalkyl, (O-substituted)glutamoyl, (S-substituted) cysteiny1, (O-substituted)seryl, (N-substituted)glutamyl, (N,N-disubstituted) glutamyl, (N-ε-substituted)lysyl, aryl and substituted aryl.

24. The compound of claim 23, wherein:

n is an integer from 1-4; and

R3 is benzylxocarbonyl, 9-fluorenylmethoxycarbonyl, t-butoxycarbonyl, (4-phenyl)phenylacetyl, 8-quinolinesulfonyl, 2-methylthionicotyl, xanthe-9-carbonyl, hydrocinamoyl, phenylbenzoyl, nonanoyl, (4-benzylxoy)benzoyl, acetyl and (4-(4-t-buty1phenylsulfonamino) benzoyl.

25. The compound of claim 24, wherein:

each R2 that is not on the carbon atom adjacent to the nitrogen to which R4 is bound is selected from the group consisting of phenyl, cyclohexyl and the side chain of (O-benzyl)tyrosine, (O-methy1ene-2-naphtyl)tyrosyl, (N-trityl) glutamyl, (N,N-dibenzyl)glutamyl, (N-2-phenylethyl)glutamyl, phenylalanine, valine and tryptophan; and

R3 is selected from the group consisting of 4-phenylbenzoyl, nonanoyl, benzylxobenzoyl and (4-(4-t-buty1phenylsulfonamino)benzoyl.

26. A method of inhibiting a matrix metalloproteinase, comprising contacting a matrix metalloproteinase with an inhibitory amount of a compound of any one of claims 21-25 or a physiologically active salt thereof.
27. The method of claim 26, wherein the matrix metalloproteinase is selected from the group consisting of interstitial collagenase, stromelysin, gelatinases and human neutrophil collagenase.

28. A pharmaceutical composition for treating an individual with a disease, wherein said disease is ameliorated by inhibiting at least one matrix metalloproteinase enzyme, wherein the composition comprises:

a) a pharmaceutically acceptable carrier or diluent; and

b) a compound of Formula II:

\[
\begin{align*}
\text{R3} & \quad (\text{R4})N \quad \text{O} \\
\text{R2} & \quad \text{n-1} \\
\text{R1} & \quad \text{n-1}
\end{align*}
\]

\[
\begin{align*}
\text{R2} & \quad \text{Q} \\
\text{R1} & \quad \text{A} \\
\text{Q} & \quad \text{NH}
\end{align*}
\]

wherein:

Q and A are each independently selected from the group consisting of sulfur and oxygen and one of Q and A is sulfur;

n is a positive integer which results in a matrix metalloproteinase inhibitor;

R1 is selected from the group consisting of -H, lower alkyl and acyl;

R4 is taken together with the R2 on the carbon atom adjacent to the nitrogen to which R4 is bound to form a 3 or 4 carbon atom carbocyclic bridge, that is optionally substituted or optionally fused to a benzene ring;

each R2, when not taken with R4 to form a carbocyclic bridge, is independently selected from the group consisting of C1-C10 straight or branched alkyl, C1-C10 straight or branched substituted alkyl, C3-C8 cyclic alkyl, substituted C3-C8 cyclic alkyl, C1-C10 straight or branched alkenyl, C1-C10
straight or branched substituted alkenyl, C1-C10 straight or branched alkynyl, C1-C10 straight or branched substituted alkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl;

R3 is selected from the group consisting of an amine protecting group X-CO-, X-CS-, X-SO₂-, X-O-CO- and X-O-CS-;

X is selected from the group consisting of C1-C10 alkyl, C1-C10 substituted alkyl, aryl, substituted aryl, heteroaryl and substituted heteroaryl; or a physiologically active salt thereof.

29. The pharmaceutical composition of claim 28, wherein the disease is osteoarthritis.

30. The pharmaceutical composition of claim 28, wherein the disease is rheumatoid arthritis.

31. The pharmaceutical composition of claim 28, wherein the disease is cancer.

32. The pharmaceutical composition of claim 28, wherein the inhibition of at least one matrix metalloproteinase enzyme results in a decrease in inflammation caused by the disease.

33. The pharmaceutical composition of claim 28, wherein:

- n is an integer from 1-10;
- R1 and R4 are -H;

R3 is selected from the group consisting of X-CO-, X-CS-, X-SO₂-, X-O-CO- and X-O-CS-.

34. The pharmaceutical composition of claim 33, wherein:

- n is an integer from 1-4;
each R2 is selected from the group consisting of a side chain of a naturally occurring amino acid, (substituted phenyl)-CH₂-, napthyl-CH₂-, (O-substituted)tyrosyl, cycloalkyl, (O-substituted)glutamoyl, (S-substituted) cysteinyl, (O-substituted)seryl, (N-substituted)glutamyl, (N,N-disubstituted) glutamyl, (N-ε-substituted)lysyl, aryl and substituted aryl; and 

R₃ is benzylloxycarbonyl, 9-fluorenylmethoxycarbonyl, t-butoxycarbonyl, (4-phenyl)phenylacetyl, 8-quinolinesulfonyl, 2-methylthionicotyl, xanthene-9-carbonyl, hydrocinamoyl, phenylbenzoyl, nonanoyl, (4-benzylxoy)benzoyl, acetyl and (4-(4-t-butylphenyl)sulfonamino)benzoyl.

35. A method of treating an individual with a disease, wherein said disease is ameliorated by inhibiting at least one matrix metalloproteinase enzyme, comprising administering a therapeutically effective amount of a composition of any one of claims 28-34.

36. The method of claim 35, wherein the disease is osteoarthritis.

37. A compound of Formula V:

\[
\begin{align*}
\text{or a pharmaceutically acceptable salt thereof, wherein} \\
Y & \text{ is one of } -\text{CO-} \text{ or } -\text{SO}_2^-; \\
Q & \text{ and } A \text{ are each independently selected from the group consisting of sulfur and oxygen and one of } Q \text{ and } A \text{ is sulfur;}
\end{align*}
\]

R²¹ is one of hydrogen, lower alkyl or acyl;
R\textsuperscript{22} is one of C1-C10 straight or branched alkyl, C1-C10 straight or branched substituted alkyl, C3-C8 cyclic alkyl, substituted C3-C8 cyclic alkyl, C2-C10 straight or branched alkenyl, C2-C10 straight or branched substituted alkenyl, C2-C10 straight or branched alkynyl, C2-C10 straight or branched substituted alkynyl, aryl, substituted aryl, heteroaryl, and substituted heteroaryl; and

R\textsuperscript{23} is C1-C10 alkyl, C1-C10 substituted alkyl, aryl, substituted aryl, heteroaryl or substituted heteroaryl.

38. A compound of claim 37, wherein:

Y is \text{-CO-};

Q and A are each sulfur;

R\textsuperscript{21} is hydrogen;

R\textsuperscript{22} is one of a side chain of a naturally occurring amino acid, (substituted phenyl)-CH\textsubscript{2}-, napthyl-CH\textsubscript{2}-, (O-substituted)tyrosyl, cycloalkyl, (O-substituted)glutamoyl, (S-substituted)cysteiny1, (O-substituted)seryl, (N-substituted)glutamyl, (N,N-disubstituted)glutamyl, (N-ε-substituted)lysyl, aryl and substituted aryl; and

R\textsuperscript{23} is C1-C10 alkyl, C1-C10 substituted alkyl, aryl, substituted aryl, heteroaryl or substituted heteroaryl.

39. A compound of claim 37, wherein R\textsuperscript{23} is benzyloxy carbonyl, 9-fluorenylmethoxy carbonyl, \textit{t}-butoxy carbonyl, (4-phenyl)phenylacetyl, 8-quino line sulfonyl, 2-methylthionicotyl, xanthene-9-carbonyl, hydrocinamoyl, phenylbenzoyl, nonanoyl, (4-benzyloxy)benzoyl, acetyl, (4-(4-\textit{t}-butylphenylsulfonamino)benzoyl and (biphenyl)carbonyl.

40. A compound of claim 37, which is one of:
41. A pharmaceutical composition for treating an individual with a disease, wherein said disease is ameliorated by inhibiting at least one matrix metalloproteinase enzyme, wherein the composition comprises:

a) a pharmaceutically acceptable carrier or diluent; and

b) a compound of any one of claims 37-40.

42. A method of inhibiting a matrix metalloproteinase, comprising contacting the matrix metalloproteinase with an inhibitory amount of a compound of any one of claims 37-40, or a physiologically active salt thereof.

43. A method of treating an individual with a disease, wherein said disease is ameliorated by inhibiting at least one matrix metalloproteinase enzyme, comprising administering a therapeutically effective amount of a composition of claim 41.
44. A compound of Formula \textit{III}:

\[
\begin{array}{c}
\text{III} \\
\begin{array}{c}
\text{R3} \\
(\text{R4})_n
\end{array}
\end{array}
\]

or a pharmaceutically acceptable salt thereof; wherein

Q and A are independently selected from the group consisting of sulfur and oxygen and one of Q and A is sulfur;

n is zero, or from 1 to 4;

R1 is selected from the group consisting of -H, lower alkyl and acyl;

each R4 is hydrogen, or is taken together with the R2 on the carbon atom adjacent to the nitrogen to which R4 is bound to form a 3 or 4 carbon atom carbocyclic bridge, that is optionally substituted or optionally fused to a benzene ring;

each R2, when not taken with R4 to form a carbocyclic bridge, is independently selected from the group consisting of C1-C10 straight or branched alkyl, C1-C10 straight or branched substituted alkyl, C3-C8 cyclic alkyl, substituted C3-C8 cyclic alkyl, C1-C10 straight or branched alkenyl, C1-C10 straight or branched substituted alkenyl, C1-C10 straight or branched alkynyl, C1-C10 straight or branched substituted alkynyl, aryl, substituted aryl, heteroaryl and substituted heteroaryl;

R3 is an amine derivatizing group.

45. A compound of claim 44, wherein R3 is one of X-CO-, X-CS-, X-SO\(_2\)-, X-O-CO- or X-O-CS-,
wherein X is selected from the group consisting of C1-C10 alkyl, C1-C10 substituted alkyl, aryl, substituted aryl, heteroaryl and substituted heteroaryl.

46. A pharmaceutical composition for treating an individual with a disease, wherein said disease is ameliorated by inhibiting at least one matrix metalloproteinase enzyme, wherein the composition comprises:
   a) a pharmaceutically acceptable carrier or diluent; and
   b) a compound of claim 44 or claim 45.

47. A method of inhibiting a matrix metalloproteinase, comprising contacting the matrix metalloproteinase with an inhibitory amount of a compound of claim 44 or claim 45, or a physiologically active salt thereof.

48. A method of treating an individual with a disease, wherein said disease is ameliorated by inhibiting at least one matrix metalloproteinase enzyme, comprising administering a therapeutically effective amount of a composition of claim 46.
INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/22534

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :C07K 5/08 5/06; A61K 31/41
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)

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 practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>DE 3407505A (Uhlandorf et al) 5 september 1985, ex. 3</td>
<td>21-48</td>
</tr>
<tr>
<td>X</td>
<td>JP61-161281A, 21 July 1986(Kikazawa et al) , column 6 line 12</td>
<td>21-48</td>
</tr>
</tbody>
</table>

Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:
A* document defining the general state of the art which is not considered to be of particular relevance
E* earlier document 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

Date of the actual completion of the international search
01 APRIL 1998

Date of mailing of the international search report
2 APR 1998

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
Robert Gross
Telephone No. (703) 308-1235

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