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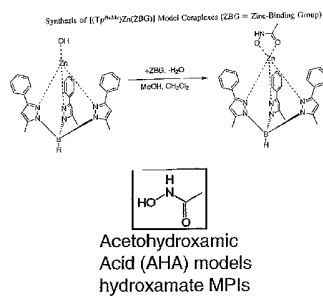
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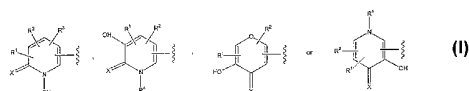
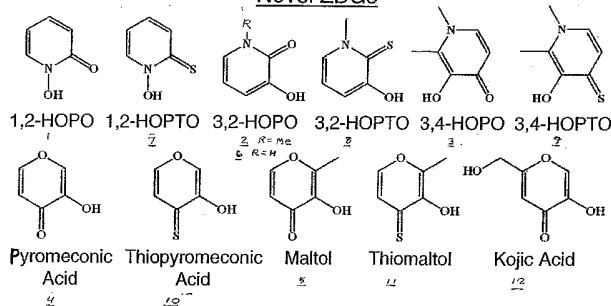
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(54) Title: ZINC-BINDING GROUPS FOR METALLOPROTEIN INHIBITORS

FIGURE 1.



Novel ZBGs



(57) Abstract: The present invention relates to metalloprotein inhibitors comprising: a. an organic backbone molecule (Pep) and at least one zinc binding group (ZBG) covalently attached thereto; or b. at least one ZBG substituted by a side chain that comprises one or more amido and/or amino moieties, wherein the ZBG is of formula (I): wherein the wavy line represents a Pep molecule or a side chain that is an R³ or an R⁴ group which comprises one or more amido and/or amino moieties, and wherein X is O or S and each R¹, R², R³, and R⁴ is individually hydrogen or an organic radical. The metalloprotein inhibitors are useful for preventing or treating a pathological disease, condition, or symptom that is associated with pathological metalloprotein activity and/or that is alleviated by inhibition of said activity.



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ZINC-BINDING GROUPS FOR METALLOPROTEIN INHIBITORS

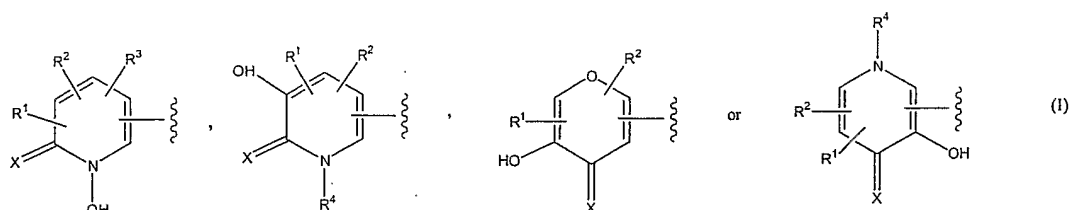
Background of the Invention

Matrix metalloproteinases (MMPs) are zinc(II)-containing hydrolytic enzymes that are involved in the breakdown of the extracellular matrix. The activity of these enzymes is often associated with illnesses such as cancer, arthritis, and inflammatory disease.¹⁻³ The correlation between MMP activity and these debilitating diseases has made these hydrolytic enzymes targets of drug-based inhibition. However, despite the evaluation of thousands of compounds, no matrix metalloproteinase inhibitors (MPIs) have completed phase III clinical trials for cancer.⁴

Many factors must be considered in designing an effective and selective drug. In the case of MPIs, the drug typically consists of two parts, a peptidomimetic backbone and a zinc-binding group (ZBG). The backbone serves as a substrate analogue, allowing the inhibitor to fit in the active-site cleft of the enzyme. The ZBG is crucial for binding to the catalytic zinc(II) ion, thereby rendering the MMP inactive. The vast majority of MPI investigations have focused on improving the backbone interactions of MPIs while opting to use a well-known ZBG, namely a hydroxamic acid moiety, that has been in regular use for more than 20 years.¹ Although extensive efforts have been made to improve MPIs by manipulating the substrate-like backbone of the drug, significantly fewer efforts have concentrated on improving the ZBG. Thus, the identification of more potent and selective ZBGs is required to take MPIs toward a more productive second generation of development.

Summary of the Invention

The present invention provides a metalloprotein inhibitor, such as a matrix metalloproteinase inhibitor (MPI), comprising an organic backbone molecule (Pep) and at least one zinc binding group (ZBG) covalently attached thereto, or at least one ZBG substituted by a side chain that comprises one or more amido and/or amino moieties, wherein the ZBG is of formula (I):



wherein the wavy line represents a Pep molecule or a side chain that is an R³ or an R⁴ group which comprises one or more amido and/or amino moieties, and

- 5 wherein each X is O or S, and each R¹, R², R³ and R⁴ is individually hydrogen or an organic radical, including halo, sulfonamido, nitro, CN, and amino radicals.

The invention also provides a pharmaceutical composition, such as a unit dosage form, comprising a metalloprotein inhibitor, such as an MPI compound of the invention, or a pharmaceutically acceptable salt thereof, in combination with a pharmaceutically acceptable
 10 diluent or carrier, that can optionally include stabilizers, preservatives, buffers, and absorption control agents.

Additionally, the invention provides a therapeutic method for preventing or treating a pathological disease, condition or symptom in a mammal, such as a human, that is associated with pathological metalloprotein activity, such as a matrix metalloproteinase (MMP) activity
 15 or histone deacetylase activity and/or that is alleviated by inhibition of said activity, comprising administering to a mammal in need of such therapy, an effective amount of a metalloprotein inhibitor, such as an MPI of the invention, including a pharmaceutically acceptable salt thereof.

Since they bind to both natural MMPs and organometallic models therefore, the
 20 present MPIs are also useful as *in vitro* screening agents to identify and develop additional MPIs.

Also within the scope of the invention is a method of preparing a metalloprotein inhibitor, such as an MPI, by covalently attaching a ZBG of formula (I) to a backbone molecule.

25 Additionally, many of the MPIs of the invention can be used as intermediates to prepare other MPIs of the invention.

Summary of the Figures

Figure 1. Synthesis of $[(\text{Tp}^{\text{Ph,Me}})\text{Zn}(\text{ZBG})]$ Model Complexes (ZBG = Zinc-Binding Group), structure of acetohydroxamic acid (AHA) and representative ZBGs of the invention.

Figure 2. Structural diagrams of $[(\text{Tp}^{\text{Ph,Me}})\text{Zn}(1\text{-hydroxy-2(1H)-pyridinone})]$ (top) and $[(\text{Tp}^{\text{Ph,Me}})\text{Zn}(3\text{-hydroxy-2(1H)-pyridinone})]$ (bottom) with partial atom numbering schemes (ORTEP, 50% probability ellipsoids). Hydrogen atoms, solvent, and partial occupancy disorder (for $[(\text{Tp}^{\text{Ph,Me}})\text{Zn}(3\text{-hydroxy-2(1H)-pyridinone})]$) have been omitted for clarity.

Figure 3. Structural diagrams of $[(\text{Tp}^{\text{Ph,Me}})\text{Zn}(3\text{-hydroxy-1-methyl-2(1H)-pyridinone})]$ (top) and $[(\text{Tp}^{\text{Ph,Me}})\text{Zn}(3\text{-hydroxy-1,2-dimethyl-4(1H)-pyridinone})]$ (bottom) with partial atom numbering schemes (ORTEP, 50% probability ellipsoids). Hydrogen atoms and solvent molecules have been omitted for clarity.

Figure 4. Structural diagrams of $[(\text{Tp}^{\text{Ph,Me}})\text{Zn}(1\text{-hydroxy-2(1H)-pyridinethione})]$ (top) and $[(\text{Tp}^{\text{Ph,Me}})\text{Zn}(3\text{-hydroxy-2-methyl-4-pyrone})]$ (bottom) with partial atom numbering schemes (ORTEP, 50% probability ellipsoids). Hydrogen atoms and solvent molecules have been omitted for clarity.

Figure 5. Images of model with insertion of 3-hydroxy-2-methyl-4-pyrone into the active site of MMP-3. Orientation A displays an obvious steric clash between the ring of the ZBG with the protein. Orientation B displays no steric clashes between the ZBG and protein; however, it also suggests that addition of a peptidomimetic "backbone" from the 2-methyl group would result in a steric clash. Orientation C demonstrates the least-steric obstacles for both the ZBG and a requisite drug "backbone." For clarity, the protein is shown in blue (space filling), zinc(II) in green (space filling), and His 201, 205, 211, and the ZBG are shown colored by element (stick models).

Figure 6. Synthetic routes to ZBGs of the invention.

Figure 7. Synthetic routes to ZBGs of the invention.

Figure 8. Depicts structural diagrams of ZBGs 8, 9 and 11 bound to $(\text{Tp}^{\text{Ph,Me}})\text{Zn}$.

Figure 9. Schematically depicts the MMP-3 binding assay and the relative potencies of ZBGs of the invention.

Figure 10 is a graphical depiction of the raw data set for a thiopyromeconic acid MMP assay trial.

Figure 11 is a graph depicted averaged uncorrected data for one of the MMP assays.

Figure 12 is a graph depicting fluorescence control experiments for an MMP assay.

5 Figure 13 is a graph depicting the fluorescence correction for a ZBG of the invention.

Figure 14 is a reaction scheme for the titration of a ZBG complex of the invention.

Figure 15 is a graph of the UV-Vis absorbance spectrum of the titration of a ZBG complex of the invention with AHA.

Figure 16 is the absorbance plot used to determine the relative binding affinities.

10 Figure 17 is a table of the relative binding affinities.

Figure 18 is a scheme for the synthesis of MPIs of the invention.

Figure 19 summarizes MMP inhibition/toxicity studies in rat fibroblasts.

Detailed Description of the Invention

Connective tissue, extracellular matrix constituents and basement membranes are required components of all mammals. These components are the biological materials that provide rigidity, differentiation, attachments and, in some cases, elasticity to biological systems including human beings and other mammals. Connective tissues components include, for example, collagen, elastin, proteoglycans, fibronectin and laminin. These biochemicals makeup, or are components of structures, such as skin, bone, teeth, tendon, cartilage, basement membrane, blood vessels, cornea and vitreous humor.

Under normal conditions, connective tissue turnover and/or repair processes are controlled and in equilibrium. The loss of this balance for whatever reason leads to a number of disease states. Inhibition of the enzymes responsible for loss of equilibrium provides a control mechanism for this tissue decomposition and, therefore, a treatment for these diseases.

Degradation of connective tissue or connective tissue components is carried out by the action of proteinase enzymes released from resident tissue cells and/or invading inflammatory or tumor cells. A major class of enzymes involved in this function is the zinc metalloproteinases (metalloproteases).

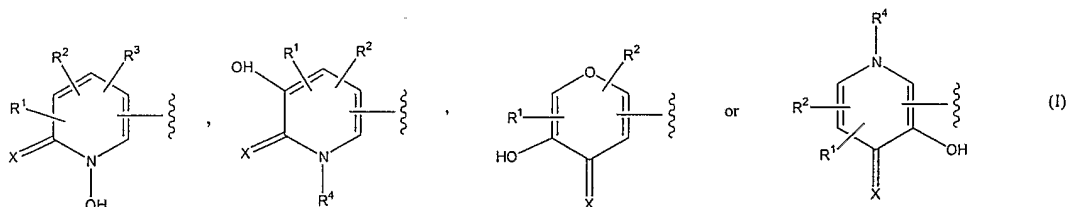
The metalloproteinase enzymes are divided into classes with some members having several different names in common use. Examples are: collagenase I (MMP-1, fibroblast collagenase; EC 3.4.24.3); collagenase II (MMP-8, neutrophil collagenase; EC 3.4.24.34), collagenase III (MMP-13), stromelysin 1 (MMP-3; EC 3.4.24.17), stromelysin 2 (MMP-10; EC 3.4.24.22), proteoglycanase, matrilysin (MMP-7), gelatinase A (MMP-2, 72 kDa gelatinase, basement membrane collagenase; EC 3.4.24.24), gelatinase B (MMP-9, 92 kDa gelatinase; EC 3.4.24.35), stromelysin 3 (MMP-11), metalloelastase (MMP-12, HME, human macrophage elastase) and membrane MMP (MMP-14). MMP is an abbreviation or acronym representing the term matrix metalloproteinase with the attached numerals providing differentiation between specific members of the MMP group.

The present invention provides metalloprotein inhibitors, such as matrix metalloproteinase inhibitors (MPIs) comprising:

- a. an organic backbone molecule (Pep) and at least one zinc binding group (ZBG)

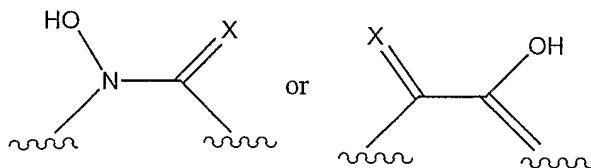
covalently attached thereto; or

b. at least one ZBG substituted by a side chain that comprises one or more amido and/or amino moieties, wherein the ZBG is of formula (I):



- 5 wherein the wavy line represents a Pep molecule or a side chain that is an R³ or an R⁴ group which comprises one or more amido and/or amino moieties, and wherein X is O or S and each R¹, R², R³, and R⁴ is individually hydrogen or an organic radical, including halo, sulfonamido, nitro, CN, and amino radicals.

10 Preferably the organic radical(s) do(es) not substantially interfere with the ability of the moiety



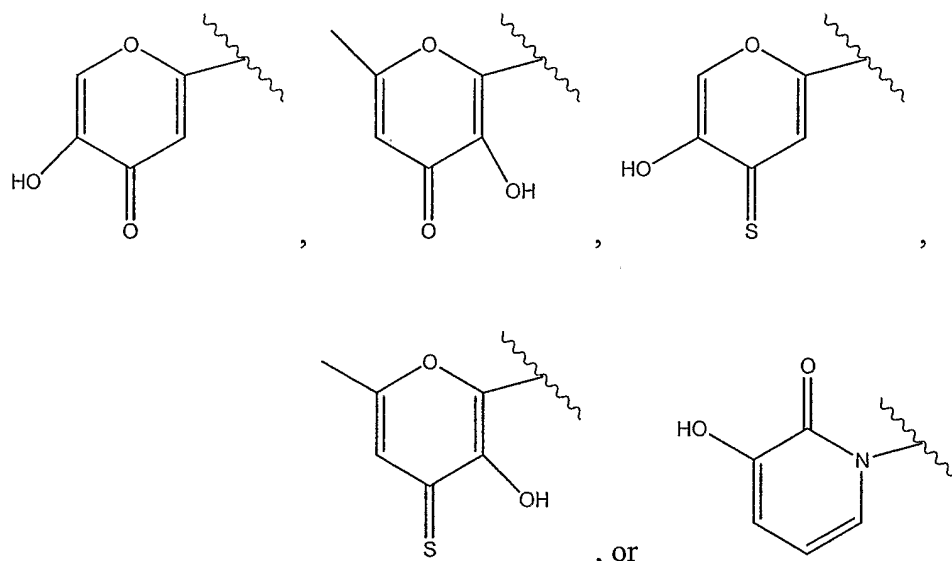
to bind to the metal ion of the target metalloprotein, such as the Zn(II) of a matrix metalloproteinase (MMP). Preferably, the organic radical(s) enhance(s) the ability of said moieties to bind to said Zn(II), or other metal ion.

- 15 Generally, R¹, R², R³ and R⁴ are less bulky than the organic backbone moiety (Pep), although 1-2 of R¹, R², or R³ can be a second or third backbone moiety in some instances. Thus, R¹, R², R³, and R⁴ are individually H, halo, CN, nitro, carboxyl, amino, sulfonamido, (C₁-C₆)alkyl, (C₁-C₆)alkoxy, (C₃-C₆)cycloalkyl, (C₃-C₆)cycloalkyl((C₁-C₆)alkyl), (C₆-C₁₀)aryl, (C₆-C₁₀)aryl(C₂-C₁₀)alkyl, (C₃-C₆)heterocycloalkyl, (C₃-C₆)heterocycloalkyl(C₁-C₆)alkyl, (C₂-C₆)alkenyl, (C₂-C₆)alkynyl, (C₁-C₆)alkanoyl, halo(C₁-C₆)alkyl, hydroxy(C₁-C₆)alkyl, (C₁-C₆)alkoxycarbonyl, (C₁-C₆)alkylthio, thio(C₁-C₆)alkyl, (C₁-C₆)alkanoyloxy, biphenylcarbonyl, biphenylcarbonyl- (C₁-C₆)alkyl, biphenyl(C₁-C₆)alkylcarbonyl, biphenyl(C₁-C₆)alkylcarbonyl (C₁-C₆)alkyl, phenoxyphenylcarbonyl, (C₆-C₁₀)aryl(C₁-C₆)alkylamino(C₁-C₆)alkyl, biphenyl(C₁-C₆)alkylamino(C₁-C₆)alkyl, (C₆-C₁₀)arylcarbonylamino(C₁-C₆)alkyl, (C₆-C₁₀)aryl(C₁-C₆)alkylcarbonylamino(C₁-C₆)alkyl,

biphenyloxy(C₁-C₆)alkylcarbonylamino(C₁-C₆)alkyl, phenoxyphenylcarbonyl (C₁-C₆)alkyl, N(R⁶)(R⁷) or SO₂N(R⁶)(R⁷), wherein R⁶ and R⁷ are individually H, =O, -OH, (C₁-C₆)alkyl, (C₃-C₆)cycloalkyl, (C₃-C₆)cycloalkyl(C₁-C₆)alkyl, phenyl or benzyl, or R⁶ and R⁷, together with the N to which they are attached, form a 5- or 6-membered ring which may optionally
 5 contain 1-2 S, N(R⁶) or nonperoxide O; or R¹ and R² together are methylenedioxy; optionally any of R¹, R², R³, and R⁴ is substituted with one to four R¹.

In one embodiment, one of R³ or R⁴ is $[(C_6-C_{10})\text{aryl}]_q-[O]_q-[(C_6-C_{10})\text{aryl}]-[O]_q-[(C_1-C_6)\text{alkyl}]_q-[C(O)]_q-[N(R)]-[C(O)]_q-[(C_1-C_6)\text{alkyl}]_q$ wherein q is 0-1 and R is H, (C₁-C₄)alkyl, phenyl, or benzyl.

10 In another embodiment, one of R³ or R⁴ is $[(C_6-C_{10})\text{aryl}]_q-[O]_q-[(C_6-C_{10})\text{aryl}]-[O]_q-[(C_1-C_6)\text{alkyl}]_q-[C(O)]_q-[N(R)]-[C(O)]_q-[(C_1-C_6)\text{alkyl}]_q$ wherein q is 0-1 and R is H, (C₁-C₄)alkyl, phenyl, or benzyl, and the ZBG is



In a further embodiment, R³ and R⁴ are side chains that are individually biphenylcarbonyl, biphenylcarbonyl- (C₁-C₆)alkyl, biphenyl(C₁-C₆)alkylcarbonyl, biphenyl(C₁-C₆)alkylcarbonyl (C₁-C₆)alkyl, phenoxyphenylcarbonyl, (C₆-C₁₀)aryl(C₁-C₆)alkylamino(C₁-C₆)alkyl, biphenyl(C₁-C₆)alkylamino(C₁-C₆)alkyl, (C₆-C₁₀)arylcarbonylamino(C₁-C₆)alkyl, (C₆-C₁₀)aryl(C₁-C₆)alkylcarbonylamino(C₁-C₆)alkyl, biphenyloxy(C₁-C₆)alkylcarbonylamino(C₁-C₆)alkyl, phenoxyphenylcarbonyl(C₁-C₆)alkyl, wherein the phenyl or aryl group(s) in the side chains may be optionally substituted with one
 20

to four R¹.

Exemplary side chains include biphenylmethylcarbamyl, phenoxyphenylcarbamyl, biphenylcarbamyl, benzylaminomethyl, phenethylaminomethyl, benzoylaminomethyl, benzylcarbonylaminomethyl, phenethylcarbonylaminomethyl, phenylpropylcarbonylaminomethyl, biphenylmethylcarbamylmethyl, phenoxyphenylcarbamylmethyl, biphenylcarbamylmethyl, and biphenyloxyethylcarbonylaminomethyl, wherein the phenyl groups are optionally substituted with one to four R¹.

Further embodiments of the invention include MPI compounds comprising ZBGs of formula (I) wherein the dangling valence is substituted by R⁷, wherein R⁷ is selected from R¹, R², or R³.

As used herein, the term "treatment" of a metalloprotein-associated pathology, e.g., an MMP-associated pathology, includes inhibiting metalloprotein activity such as histone deacetylase or MMP activity in a subject exhibiting at least one of the symptoms of the onset of a metalloprotein-associated pathology or who is likely to develop such a pathology as well as the ability to halt or slow the progression of a metalloprotein-associated pathology or to reduce or alleviate at least one of the symptoms of said pathology.

A "therapeutic effect", "effective amount," or "therapeutic effective amount" is intended to qualify the amount of an anticancer agent according to the present invention required to relieve to some extent one or more of the symptoms and/or conditions of cancer, including, but is not limited to: 1) reduction in the number of cancer cells; 2) reduction in tumor size; 3) inhibition (i.e., slowing to some extent, preferably stopping) of cancer cell infiltration into peripheral organs; 3) inhibition (i.e., slowing to some extent, preferably stopping) of tumor metastasis; 4) inhibition, to some extent, of tumor growth; 5) relieving or reducing to some extent one or more of the symptoms associated with cancer; and/or 6) relieving or reducing the side effects associated with the administration of anticancer agents. The terms also are intended to qualify the amounts of anti-inflammatory agents or anti-anthrax lethal factor agents according to the present invention required to relieve to some extent one or more of the symptoms and/or conditions of diseases including arthritis (e.g., RA), restenosis, aortic aneurism, IBD, glomerular nephritis, MS, stroke, diabetes, bacterial

meningitis, and graft vs. host disease. The terms also are intended to qualify the amounts of agents according to the present invention required to relieve to some extent one or more of the symptoms and/or conditions of diseases include epidermal scars, myocardial infarction, and periodontal disease.

5 The use of the term "about" in the present disclosure means "approximately," and encompasses variations in parameters that would arise during practice of the relevant art. Illustratively, the use of the term "about" indicates that dosages outside the cited ranges may also be effective and safe, and such dosages are also encompassed by the scope of the present claims.

10 The term "pharmaceutically acceptable" is used adjectivally herein to mean that the modified noun is appropriate for use in a pharmaceutical product.

 The phrase "matrix metalloproteinase inhibitor" or "MPI" includes agents that specifically inhibit a class of enzymes, the zinc metalloproteinases (metalloproteases). The zinc metalloproteinases are involved in the degradation of connective tissue or connective
15 tissue components. These enzymes are released from resident tissue cells and/or invading inflammatory or tumor cells. Blocking the action of zinc metalloproteinases interferes with the creation of paths for newly forming blood vessels to follow. Examples of MMP inhibitors are described in Golub, L M, Inhibition of Matrix Metalloproteinases: Therapeutic Applications (Annals of the New York Academy of Science, Vol 878). Robert A. Greenwald
20 and Stanley Zucker (Eds.), June 1999), and is hereby incorporated by reference.

 The following definitions are used, unless otherwise described: halo is fluoro, chloro, bromo, or iodo. Alkyl, alkoxy, alkenyl, alkynyl, etc. denote both straight and branched groups; but reference to an individual radical such as "propyl" embraces only the straight chain radical, a branched chain isomer such as "isopropyl" being specifically referred to.

25 Aryl denotes a phenyl radical or an ortho-fused bicyclic carbocyclic radical having about nine to ten ring atoms in which at least one ring is aromatic. Heteroaryl encompasses a radical attached via a ring carbon of a monocyclic aromatic ring containing about 5 or 6 ring atoms consisting of carbon and one to four heteroatoms each selected from the group consisting of non-peroxide oxygen, sulfur, and N(R⁷) wherein R⁷ is absent or is as defined
30 above; as well as a radical of an ortho-fused bicyclic heterocycle of about eight to ten ring

atoms derived therefrom, particularly a benz-derivative or one derived by fusing a propylene, trimethylene, or tetramethylene diradical thereto.

Specific and preferred values listed below for radicals, substituents, and ranges, are for illustration only; they do not exclude other defined values or other values within defined ranges for the radicals and substituents.

Specifically, (C₁-C₆)alkyl can be methyl, ethyl, propyl, isopropyl, butyl, iso-butyl, sec-butyl, pentyl, 3-pentyl, or hexyl; (C₃-C₁₂)cycloalkyl can be monocyclic, bicyclic or tricyclic and includes cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl,

bicyclo[2.2.2]octanyl, norbornyl, adamantyl as well as various terpene and terpenoid structures. (C₃-C₁₂)cycloalkyl(C₁-C₆)alkyl includes the foregoing cycloalkyl and can be cyclopropylmethyl, cyclobutylmethyl, cyclopentylmethyl, cyclohexylmethyl, 2-cyclopropylethyl, 2-cyclobutylethyl, 2-cyclopentylethyl, or 2-cyclohexylethyl.

Heterocycloalkyl and (heterocycloalkyl)alkyl include the foregoing cycloalkyl wherein the cycloalkyl ring system is monocyclic, bicyclic or tricyclic and optionally comprises 1-2 S, non-peroxide O or N(R⁷) as well as 2-12 ring carbon atoms; such as morpholinyl, piperidinyl, piperazinyl, indanyl, 1,3-dithian-2-yl, and the like; the cycloalkyl ring system optionally includes 1-3 double bonds or epoxy moieties and optionally is substituted with 1-3 OH, (C₁-C₆)alkanoyloxy, (CO), (C₁-C₆)alkyl or (C₂-C₆)alkynyl. (C₁-C₆)alkoxy can be methoxy, ethoxy, propoxy, isopropoxy, butoxy, iso-butoxy, sec-butoxy, pentoxy, 3-pentoxy, or

hexyloxy; (C₂-C₆)alkenyl can be vinyl, allyl, 1-propenyl, 2-propenyl, 1-butenyl, 2-butenyl, 3-butenyl, 1-pentenyl, 2-pentenyl, 3-pentenyl, 4-pentenyl, 1-hexenyl, 2-hexenyl, 3-hexenyl, 4-hexenyl, or 5-hexenyl; (C₂-C₆)alkynyl can be ethynyl, 1-propynyl, 2-propynyl, 1-butylnyl, 2-butylnyl, 3-butylnyl, 1-pentylnyl, 2-pentylnyl, 3-pentylnyl, 4-pentylnyl, 1-hexynyl, 2-hexynyl, 3-hexynyl, 4-hexynyl, or 5-hexynyl; (C₁-C₆)alkanoyl can be formyl, acetyl, propanoyl or

butanoyl; halo(C₁-C₆)alkyl can be iodomethyl, bromomethyl, chloromethyl, fluoromethyl, trifluoromethyl, 2-chloroethyl, 2-fluoroethyl, 2,2,2-trifluoroethyl, or pentafluoroethyl; hydroxy(C₁-C₆)alkyl can be alkyl substituted with 1 or 2 OH groups, such as hydroxymethyl, 1-hydroxyethyl, 2-hydroxyethyl, 1-hydroxypropyl, 2-hydroxypropyl, 3-hydroxypropyl, 1-hydroxybutyl, 4-hydroxybutyl, 3, 4-dihydroxybutyl, 1-hydroxypentyl, 5-hydroxypentyl, 1-hydroxyhexyl, or 6-hydroxyhexyl; (C₁-C₆)alkoxycarbonyl can be methoxycarbonyl,

ethoxycarbonyl, propoxycarbonyl, isopropoxycarbonyl, butoxycarbonyl, pentoxycarbonyl, or hexyloxycarbonyl; (C₁-C₆)alkylthio can be methylthio, ethylthio, propylthio, isopropylthio, butylthio, isobutylthio, pentylthio, or hexylthio; (C₂-C₆)alkanoyloxy can be acetoxy, propanoyloxy, butanoyloxy, isobutanoyloxy, pentanoyloxy, or hexanoyloxy; aryl can be phenyl, indenyl, indanyl, or naphthyl; and heteroaryl can be furyl, imidazolyl, triazolyl, triazinyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, pyrazolyl, pyrrolyl, pyrazinyl, tetrazolyl, pyridyl, (or its N-oxide), thienyl, pyrimidinyl (or its N-oxide), 1H-indolyl, isoquinolyl (or its N-oxide) or quinolyl (or its N-oxide).

Optionally, any of R¹, R², or R³ can be substituted by one to three R¹ (except for H, halo or CN).

Preferably, one of R¹, R², or R³ is (C₁-C₃)alkyl.

Preferably, one of R¹, R², or R³ is H.

Preferably, R⁴ is (C₁-C₃)alkyl, benzyl, t-Boc, (C₃-C₆)cycloalkyl(C₁-C₃)alkyl, or H.

Preferred backbone molecules comprise the structural features depicted in Fig. 5 and Fig. 6 of Whittaker et al., cited above, wherein the moiety HON(H)-C(O)-CH(R_a)- has been replaced with a ZBG of formula (I), or the structural features depicted in the claims of EPA 126,974, wherein at least the moiety COR¹ has been replaced by a ZBG of formula (I).

The structure of the organic backbone molecule (Pep) would preferably not interfere with and, would preferably enhance the ability of the MPI to direct the ZBG toward one or more complexed metal ions, such as Zn(II) atoms of the MMP. For example, Pep can be any of the organic radicals derived from the structures shown on Scheme 1 of the Whittaker M. et. Al., *Chem. Rev.*, **1999**, *99*, 2735-2776, after removal of the C(O)NH(OH) group, or in the claims of published European patent application No. 126,974, after removal of COR¹.

Pep can be a naturally-occurring peptide, a synthetic peptide or a peptide analog (peptidomimetic). Such groups may comprise one or more amido moieties (-C(O)NH-), which can be or comprise, peptidyl bonds, e.g., amide bonds formed by reaction of the amino group of an *alpha*-aminocarboxylic acid with the carboxy group of a second amino acid.

For example, embodiments of the compound of formula (I) can be represented by [ZBG]-C(R⁵)(R⁶)-C(O)NH-, [ZBG]-C(R⁸)-C(O)N(H)-CH(R⁹)-C(O)NH(R¹⁰), or

[ZBG-C(R⁵)(R⁶)-C(O)N(H)-CH(R⁹)-C(O)NH(R¹⁰), wherein R⁸, R⁹ and R¹⁰ correspond to R¹,

R² and R³ respectively, and wherein R⁵ is H and R⁶ is (C₁-C₂₂)alkyl, (C₂-C₆)alkenyl, (C₆-C₁₀)aryl, (C₁-C₆)alkyl, (C₆-C₁₀)heteroaryl, (C₆-C₁₀)heteroaryl(C₁-C₆)alkyl, (C₃-C₆)cycloalkyl, (C₃-C₆)cycloalkyl(C₁-C₆)alkyl, or R⁵ and R⁶ together with the carbon atom to which they are attached can be (C₄-C₆) spiroalkyl or spiroheterocycloalkyl. In certain embodiments of the invention, peptidyl or peptidomimetic Pep is terminated by a ZBG of formula (I), and optionally, the other terminus is C(O)N(R⁶)(R⁷).

It will be appreciated by those skilled in the art that compounds of the invention having a chiral center may exist in and be isolated in optically active and racemic forms. Some compounds may exhibit polymorphism. It is to be understood that the present invention encompasses any racemic, optically-active, polymorphic, or stereoisomeric form, or mixtures thereof, of a compound of the invention, which possess the useful properties described herein, it being well known in the art how to prepare optically active forms (for example, by resolution of the racemic form by recrystallization techniques, by synthesis from optically-active starting materials, by chiral synthesis, or by chromatographic separation using a chiral stationary phase) and how to determine anti-toxin activity using the standard tests described herein, or using other similar tests which are well known in the art.

In cases where compounds are sufficiently basic or acidic to form stable nontoxic acid or base salts, administration of the compounds as salts may be appropriate. Pharmaceutically acceptable salts may be obtained using standard procedures well known in the art, for example by reacting a sufficiently basic compound such as an amine with a suitable acid affording a physiologically acceptable anion.

Pharmaceutically acceptable salts include metallic ions and organic ions. More preferred metallic ions include, but are not limited to appropriate alkali metal (Group Ia) salts, for example, sodium potassium, or lithium, and alkaline earth metal (Group IIa) salts, for example, calcium, and other physiological acceptable metal ions. Exemplary ions include aluminum, calcium, lithium, magnesium, potassium, sodium and zinc in their usual valences. Preferred organic ions include protonated tertiary amines and quaternary ammonium cations, including in part, trimethylamine, diethylamine, N,N'-dibenzylethylenediamine, chloroprocaine, choline, diethanolamine, ethylenediamine, meglumine (N-methylglucamine) and procaine.

Illustrative pharmaceutically acceptable salts are prepared from hydrochloric, hydrobromic, phosphoric, sulfuric, formic, acetic, propionic, succinic, glycolic, gluconic, lactic, malic, tartaric, citric, ascorbic, glucuronic, maleic, fumaric, pyruvic, aspartic, glutamic, benzoic, anthranilic, mesylic, stearic, salicylic, p-hydroxybenzoic, phenylacetic, mandelic, embonic (pamoic), methanesulfonic, ethanesulfonic, benzenesulfonic, pantothenic, 5 toluenesulfonic, 2-hydroxy-ethanesulfonic, sulfanilic, cyclohexylaminosulfonic, algenic, β -hydroxybutyric, galactaric and galacturonic acids.

The compositions of the present invention are usually administered in the form of pharmaceutical compositions. These compositions can be administered by any appropriate 10 route including, but not limited to, oral, nasogastric, rectal, transdermal, parenteral (for example, subcutaneous, intramuscular, intravenous, intramedullary, intrasternal, and intradermal injections, or infusion techniques), intranasal, transmucosal, implantation, vaginal, topical, buccal, and sublingual administration. Such preparations may routinely contain buffering agents, preservatives, penetration enhancers, compatible carriers, and other 15 therapeutic or non-therapeutic ingredients.

Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions can be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation can also be a sterile injectable solution or suspension in a nontoxic parenterally acceptable diluent or solvent, for 20 example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that can be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of 25 injectables. Dimethyl acetamide, surfactants including ionic and non-ionic detergents, or polyethylene glycols can be used. Mixtures of solvents and wetting agents such as those discussed above are also useful.

Suppositories for rectal administration of the drug can be prepared by mixing the drug with a suitable nonirritating excipient such as cocoa butter, synthetic mono- di- or

triglycerides, fatty acids and polyethylene glycols that are solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum and release the drug.

Solid dosage forms for oral administration can include capsules, ingestible tablets, buccal tablets, troches, dragées, pills, powders, granules, and wafers. In such solid dosage

5 forms, the compounds of this invention are ordinarily combined with one or more adjuvants appropriate to the indicated route of administration. The tablets, troches, pills, capsules, and the like may also contain the following: binders such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a

10 sweetening agent such as sucrose, fructose, lactose or aspartame or a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring may be added. When the unit dosage form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier, such as a vegetable oil or a polyethylene glycol. Various other materials may be present as coatings or to otherwise modify the physical form of the solid unit dosage form. For

15 instance, tablets, pills, granules, or capsules may be coated with gelatin, wax, shellac or sugar and the like. The tablets, pills, granules, or capsules comprising the inventive compositions may be film coated or enteric-coated.

Liquid dosage forms for oral administration can include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs containing inert diluents commonly

20 used in the art, such as water. Such compositions can also comprise adjuvants, such as wetting agents, emulsifying and suspending agents, and sweetening, flavoring, and perfuming agents. A syrup or elixir may contain the active compound, sucrose or fructose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring such as cherry or orange flavor. Of course, any material used in preparing any unit dosage form

25 should be pharmaceutically acceptable and substantially non-toxic in the amounts employed. In addition, the active compound may be incorporated into sustained-release preparations and devices.

For therapeutic purposes, formulations for parenteral administration can be in the form of aqueous or non-aqueous isotonic sterile injection solutions or suspensions. These

30 solutions and suspensions can be prepared from sterile powders or granules having one or

more of the carriers or diluents mentioned for use in the formulations for oral administration. Solutions of the active compound or its salts can be prepared in water, optionally mixed with a nontoxic surfactant. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, triacetin, and mixtures thereof and in oils. Under ordinary conditions of storage and
5 use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical dosage forms suitable for injection or infusion can include sterile aqueous solutions or dispersions or sterile powders comprising the active ingredient which are adapted for the extemporaneous preparation of sterile injectable or infusible solutions or dispersions, optionally encapsulated in liposomes. In all cases, the ultimate dosage form
10 must be sterile, fluid and stable under the conditions of manufacture and storage. The liquid carrier or vehicle can be a solvent or liquid dispersion medium comprising, for example, water, ethanol, a polyol (for example, glycerol, propylene glycol, liquid polyethylene glycols, and the like), vegetable oils, nontoxic glyceryl esters, and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the formation of liposomes, by the
15 maintenance of the required particle size in the case of dispersions or by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, buffers or sodium chloride. Prolonged absorption of the injectable
20 compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compound in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filter sterilization. In the case of sterile powders for the
25 preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze drying techniques, which yield a powder of the active ingredient plus any additional desired ingredient present in the previously sterile-filtered solutions.

For topical administration, the present compounds may be applied in pure form, i.e., when they are liquids. However, it will generally be desirable to administer them to the skin

as compositions or formulations, in combination with a dermatologically acceptable carrier, which may be a solid or a liquid.

Useful solid carriers include finely divided solids such as talc, clay, microcrystalline cellulose, silica, alumina, and the like. Useful liquid carriers include water, alcohols or glycols, or water-alcohol/glycol blends, in which the present compounds can be dissolved or dispersed at effective levels, optionally with the aid of non-toxic surfactants. Adjuvants such as fragrances and additional antimicrobial agents can be added to optimize the properties for a given use. The resultant liquid compositions can be applied from absorbent pads, used to impregnate bandages and other dressings, or sprayed onto the affected area using pump-type or aerosol sprayers.

Thickeners such as synthetic polymers, fatty acids, fatty acid salts and esters, fatty alcohols, modified celluloses or modified mineral materials can also be employed with liquid carriers to form spreadable pastes, gels, ointments, soaps, and the like, for application directly to the skin of the user.

Examples of useful dermatological compositions which can be used to deliver the compounds of formula I to the skin are known to the art; for example, see Jacquet et al. (U.S. Pat. No. 4,608,392), Geria (U.S. Pat. No. 4,992,478), Smith et al. (U.S. Pat. No. 4,559,157) and Wortzman (U.S. Pat. No. 4,820,508).

Useful dosages of the compounds of formula I can be determined by comparing their *in vitro* activity, and *in vivo* activity in animal models. Methods for the extrapolation of effective dosages in mice, and other animals, to humans are known to the art; for example, see U.S. Pat. No. 4,938,949.

The amount of active ingredient that can be combined with the carrier materials to produce a single dosage form varies depending upon the mammalian host treated and the particular mode of administration.

The present invention also includes methods employing a pharmaceutical composition that contains the composition of the present invention associated with pharmaceutically acceptable carriers or excipients. As used herein, the terms "pharmaceutically acceptable carrier" or "pharmaceutically acceptable excipients" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic

and absorption delaying agents, and the like. The use of such media and agents for ingestible substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the compositions, its use is contemplated. Supplementary active ingredients can also be incorporated into the compositions. In making the compositions of the present invention, the compositions(s) can be mixed with a pharmaceutically acceptable excipient, diluted by the excipient or enclosed within such a carrier, which can be in the form of a capsule, sachet, or other container. The carrier materials that can be employed in making the composition of the present invention are any of those commonly used excipients in pharmaceuticals and should be selected on the basis of compatibility with the active drug and the release profile properties of the desired dosage form.

Illustratively, pharmaceutical excipients are chosen below as examples:

(a) Binders such as acacia, alginic acid and salts thereof, cellulose derivatives, methylcellulose, hydroxyethyl cellulose, hydroxypropyl cellulose, magnesium aluminum silicate, polyethylene glycol, gums, polysaccharide acids, bentonites, hydroxypropyl methylcellulose, gelatin, polyvinylpyrrolidone, polyvinylpyrrolidone/vinyl acetate copolymer, crospovidone, povidone, polymethacrylates, hydroxypropylmethylcellulose, hydroxypropylcellulose, starch, pregelatinized starch, ethylcellulose, tragacanth, dextrin, microcrystalline cellulose, sucrose, or glucose, and the like.

(b) Disintegration agents such as starches, pregelatinized corn starch, pregelatinized starch, celluloses, cross-linked carboxymethylcellulose, sodium starch glycolate, crospovidone, cross-linked polyvinylpyrrolidone, croscarmellose sodium, microcrystalline cellulose, a calcium, a sodium alginate complex, clays, alginates, gums, or sodium starch glycolate, and any disintegration agents used in tablet preparations.

(c) Filling agents such as lactose, calcium carbonate, calcium phosphate, dibasic calcium phosphate, calcium sulfate, microcrystalline cellulose, cellulose powder, dextrose, dextrates, dextran, starches, pregelatinized starch, sucrose, xylitol, lactitol, mannitol, sorbitol, sodium chloride, polyethylene glycol, and the like.

(d) Surfactants such as sodium lauryl sulfate, sorbitan monooleate, polyoxyethylene sorbitan monooleate, polysorbates, polaxomers, bile salts, glyceryl monostearate, PluronicTM line (BASF), and the like.

(e) Solubilizer such as citric acid, succinic acid, fumaric acid, malic acid, tartaric acid, maleic acid, glutaric acid sodium bicarbonate and sodium carbonate and the like.

(f) Stabilizers such as any antioxidation agents, buffers, or acids, and the like, can also be utilized.

5 (g) Lubricants such as magnesium stearate, calcium hydroxide, talc, sodium stearyl fumarate, hydrogenated vegetable oil, stearic acid, glyceryl behapate, magnesium, calcium and sodium stearates, stearic acid, talc, waxes, Stearowet, boric acid, sodium benzoate, sodium acetate, sodium chloride, DL-leucine, polyethylene glycols, sodium oleate, or sodium lauryl sulfate, and the like.

10 (h) Wetting agents such as oleic acid, glyceryl monostearate, sorbitan monooleate, sorbitan monolaurate, triethanolamine oleate, polyoxyethylene sorbitan monooleate, polyoxyethylene sorbitan monolaurate, sodium oleate, or sodium lauryl sulfate, and the like.

(i) Diluents such lactose, starch, mannitol, sorbitol, dextrose, microcrystalline cellulose, dibasic calcium phosphate, sucrose-based diluents, confectioner's sugar, monobasic
15 calcium sulfate monohydrate, calcium sulfate dihydrate, calcium lactate trihydrate, dextrates, inositol, hydrolyzed cereal solids, amylose, powdered cellulose, calcium carbonate, glycine, or bentonite, and the like.

(j) Anti-adherents or glidants such as talc, corn starch, DL-leucine, sodium lauryl sulfate, and magnesium, calcium, or sodium stearates, and the like.

20 (k) Pharmaceutically compatible carrier comprises acacia, gelatin, colloidal silicon dioxide, calcium glycerophosphate, calcium lactate, maltodextrin, glycerine, magnesium silicate, sodium caseinate, soy lecithin, sodium chloride, tricalcium phosphate, dipotassium phosphate, sodium stearyl lactylate, carrageenan, monoglyceride, diglyceride, or pregelatinized starch, and the like.

25 Additionally, drug formulations are discussed in, for example, Remington's The Science and Practice of Pharmacy (2000). Another discussion of drug formulations can be found in Liberman, H.A. and Lachman, L., Eds., Pharmaceutical Dosage Forms, Marcel Decker, New York, N.Y., 1980.

Besides being useful for human treatment, the present invention is also useful for
30 other subjects including veterinary animals, reptiles, birds, exotic animals, and farm animals,

including mammals, rodents, and the like. Mammal includes a primate, for example, a monkey, or a lemur, a horse, a dog, a pig, or a cat. A rodent includes a rat, a mouse, a squirrel, or a guinea pig.

Additionally, the invention provides a therapeutic method for preventing or treating a pathological disease, condition or symptom in a mammal, such as a human, that is associated with pathological metalloprotein activity such as a matrix metalloproteinase (MMP) activity or histone deacetylase activity and/or that is alleviated by inhibition of said activity, comprising administering to a mammal in need of such therapy, an effective amount of a metalloprotein inhibitor, such as an MPI of the invention, including a pharmaceutically acceptable salt thereof.

Such conditions, disease or symptoms include cancer, anthrax pathogenesis associated with anthrax lethal factor, and the inflammatory pathologies set forth in Whitaker, et al. or EPA 126,974, cited above, including arthritis (e.g., RA), restenosis, aortic aneurism, IBD, glomerular nephritis, MS, stroke, diabetes, bacterial meningitis, and graft vs. host disease. Cancers amenable to treatment include leukemia, myeloma, lymphoma, metastatic breast or metastatic prostate cancer, Hodgkin's lymphoma, non-Hodgkin's lymphoma, osteosarcoma, germ cell tumor, lung cancer, ovarian cancer, pancreatic cancer, renal cell carcinoma, melanoma, myelodysplastic syndrome, Ewing's sarcoma, and Paget's disease. Other conditions or diseases amenable to treatment include epidermal scars, myocardial infarction, and periodontal disease.

For treatment of a pathological disease, condition, or symptom associated with pathological metalloprotein activity, such as a matrix metalloproteinase (MMP) activity or histone deacetylase activity and/or that is alleviated by inhibition of said activity, compositions of the invention can be used to provide a dose of a compound of the present invention in an amount sufficient to elicit a therapeutic response, e.g., inhibition of tumor growth, for example a dose of about 5 ng to about 1000 mg, or about 100 ng to about 600 mg, or about 1 mg to about 500 mg, or about 20 mg to about 400 mg. Typically a dosage effective amount will range from about 0.0001 mg/kg to 1500 mg/kg, more preferably 1 to 1000 mg/kg, more preferably from about 1 to 150 mg/kg of body weight, and most preferably about 50 to 100 mg/kg of body weight. A dose can be administered in one to

about four doses per day, or in as many doses per day to elicit a therapeutic effect.

Illustratively, a dosage unit of a composition of the present invention can typically contain, for example, about 5 ng, 50 ng, 100 ng, 500 ng, 1 mg, 10 mg, 20 mg, 40 mg, 80 mg, 100 mg, 125 mg, 150 mg, 200 mg, 250 mg, 300 mg, 350 mg, 400 mg, 450 mg, 500 mg, 550 mg, 600 mg, 700 mg, 800 mg, 900 mg, or 1000 mg of a compound of the present invention. The dosage form can be selected to accommodate the desired frequency of administration used to achieve the specified dosage. The amount of the unit dosage form of the composition that is administered and the dosage regimen for treating the condition or disorder depends on a variety of factors, including, the age, weight, sex and medical condition, of the subject, the severity of the condition or disorder, the route and frequency of administration, and this can vary widely, as is well known.

In one embodiment of the present invention, the composition is administered to a subject in an effective amount, that is, the composition is administered in an amount that achieves a therapeutically effective dose of a compound of the present invention in the blood serum of a subject for a period of time to elicit a desired therapeutic effect. Illustratively, in a fasting adult human (fasting for generally at least 10 hours) the composition is administered to achieve a therapeutically effective dose of a compound of the present invention in the blood serum of a subject from about 5 minutes after administration of the composition. In another embodiment of the present invention, a therapeutically effective dose of the compound of the present invention is achieved in the blood serum of a subject at about 10 minutes from the time of administration of the composition to the subject. In another embodiment of the present invention, a therapeutically effective dose of the compound of the present invention is achieved in the blood serum of a subject at about 20 minutes from the time of administration of the composition to the subject. In yet another embodiment of the present invention, a therapeutically effective dose of the compound of the present invention is achieved in the blood serum of a subject at about 30 minutes from the time of administration of the composition to the subject. In still another embodiment of the present invention, a therapeutically effective dose of the compound of the present invention is achieved in the blood serum of a subject at about 40 minutes from the time of administration of the composition to the subject. In one embodiment of the present invention, a

therapeutically effective dose of the compound of the present invention is achieved in the blood serum of a subject at about 20 minutes to about 12 hours from the time of administration of the composition to the subject. In another embodiment of the present invention, a therapeutically effective dose of the compound of the present invention is achieved in the blood serum of a subject at about 20 minutes to about 6 hours from the time of administration of the composition to the subject. In yet another embodiment of the present invention, a therapeutically effective dose of the compound of the present invention is achieved in the blood serum of a subject at about 20 minutes to about 2 hours from the time of administration of the composition to the subject. In still another embodiment of the present invention, a therapeutically effective dose of the compound of the present invention is achieved in the blood serum of a subject at about 40 minutes to about 2 hours from the time of administration of the composition to the subject. And in yet another embodiment of the present invention, a therapeutically effective dose of the compound of the present invention is achieved in the blood serum of a subject at about 40 minutes to about 1 hour from the time of administration of the composition to the subject.

In one embodiment of the present invention, a composition of the present invention is administered at a dose suitable to provide a blood serum concentration with a half maximum dose of a compound of the present invention. Illustratively, a blood serum concentration of about 0.01 to about 1000 nM, or about 0.1 to about 750 nM, or about 1 to about 500 nM, or about 20 to about 1000 nM, or about 100 to about 500 nM, or about 200 to about 400 nM is achieved in a subject after administration of a composition of the present invention.

Contemplated compositions of the present invention provide a therapeutic effect as compound of the present invention medications over an interval of about 5 minutes to about 24 hours after administration, enabling once-a-day or twice-a-day administration if desired.

In one embodiment of the present invention, the composition is administered at a dose suitable to provide an average blood serum concentration with a half maximum dose of a compound of the present invention of at least about 1 $\mu\text{g/ml}$, or at least about 5 $\mu\text{g/ml}$, or at least about 10 $\mu\text{g/ml}$, or at least about 50 $\mu\text{g/ml}$, or at least about 100 $\mu\text{g/ml}$, or at least about 500 $\mu\text{g/ml}$, or at least about 1000 $\mu\text{g/ml}$ in a subject about 10, 20, 30, or 40 minutes after administration of the composition to the subject.

The amount of therapeutic agent necessary to elicit a therapeutic effect can be experimentally determined based on, for example, the absorption rate of the agent into the blood serum, the bioavailability of the agent, and the potency for treating the disorder. It is understood, however, that specific dose levels of the therapeutic agents of the present invention for any particular subject depends upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, and diet of the subject (including, for example, whether the subject is in a fasting or fed state), the time of administration, the rate of excretion, the drug combination, and the severity of the particular disorder being treated and form of administration. Treatment dosages generally may be titrated to optimize safety and efficacy. Typically, dosage-effect relationships from *in vitro* and/or *in vivo* tests initially can provide useful guidance on the proper doses for subject administration. Studies in animal models generally may be used for guidance regarding effective dosages for treatment of diseases in accordance with the present invention. In terms of treatment protocols, it should be appreciated that the dosage to be administered will depend on several factors, including the particular agent that is administered, the route administered, the condition of the particular subject, etc. Generally speaking, one will desire to administer an amount of the compound that is effective to achieve a serum level commensurate with the concentrations found to be effective *in vitro* for a period of time effective to elicit a therapeutic effect. Thus, where a compound is found to demonstrate *in vitro* activity at, for example, a half-maximum effective dose of 200 nM, one will desire to administer an amount of the drug that is effective to provide about a half-maximum effective dose of 200 nM concentration *in vivo* for a period of time that elicits a desired therapeutic effect, for example, treating a disorder related to high beta-amyloid-induced neurotoxicity and other indicators as are selected as appropriate measures by those skilled in the art. Determination of these parameters is well within the skill of the art. These considerations are well known in the art and are described in standard textbooks.

In order to measure and determine the effective amount of a compound of the present invention to be delivered to a subject, serum concentrations of a compound of the present invention can be measured using standard assay techniques.

Contemplated compositions of the present invention provide a therapeutic effect over an interval of about 30 minutes to about 24 hours after administration to a subject. In one embodiment compositions provide such therapeutic effect in about 30 minutes. In another embodiment compositions provide therapeutic effect over about 24 hours, enabling once-a-day administration to improve patient compliance.

The present methods and compositions can also be used in combination ("combination therapy") with another pharmaceutical agent that is indicated for treating cancer, anthrax pathogenesis associated with anthrax lethal factor, and the inflammatory pathologies and other conditions or diseases set forth above.

Previous work has shown that tris(pyrazolyl)borate complexes of zinc provide an accurate model for the tris(histidine) active site of several metalloproteins including MMPs.⁵⁻⁸ In addition, studies on some of these model compounds have shown that acetohydroxamic acid forms a complex (Figure 1) that is structurally identical to the coordination environment of hydroxamate-based drugs bound to the catalytic zinc(II) ion in MMPs.^{9,10} Using this same model, the interaction of ZBGs from identified inhibitors was evaluated where the mode of binding was unknown.¹¹⁻¹³ This study proved to be very informative, because a direct correlation between the inhibitory activity and mode of binding was found.¹⁰ These observations further validate the use of model complexes as an effective strategy for determining, at a molecular level, the interactions between inhibitors and MMPs.

The complexes that formed also demonstrate that tris(pyrazolyl)borate complexes of zinc(II) can be used as an initial screen for ZBGs by providing structural and qualitative binding information without the need for sophisticated drug synthesis or protein structure determination. Six ligands were selected to demonstrate the range of compounds that might serve as effective ZBGs. These ligands share several features in common with the regularly utilized hydroxamate ZBG because these compounds are monoanionic ligands that were anticipated to bind the zinc ion in a chelating bidentate fashion. The compounds studied were also selected because of their potential biocompatibility based on their presently known uses in biological systems (vide infra). The data herein demonstrate that the ZBGs depicted in Fig. 1, 1-hydroxy-2(1H)-pyridinone (1), 3-hydroxy-2(1H)-pyridinone (6), 3-hydroxy-1-methyl-2(1H)-pyridinone (2), 3-hydroxy-1,2-dimethyl-4(1H)-pyridinone (3), 1-hydroxy-

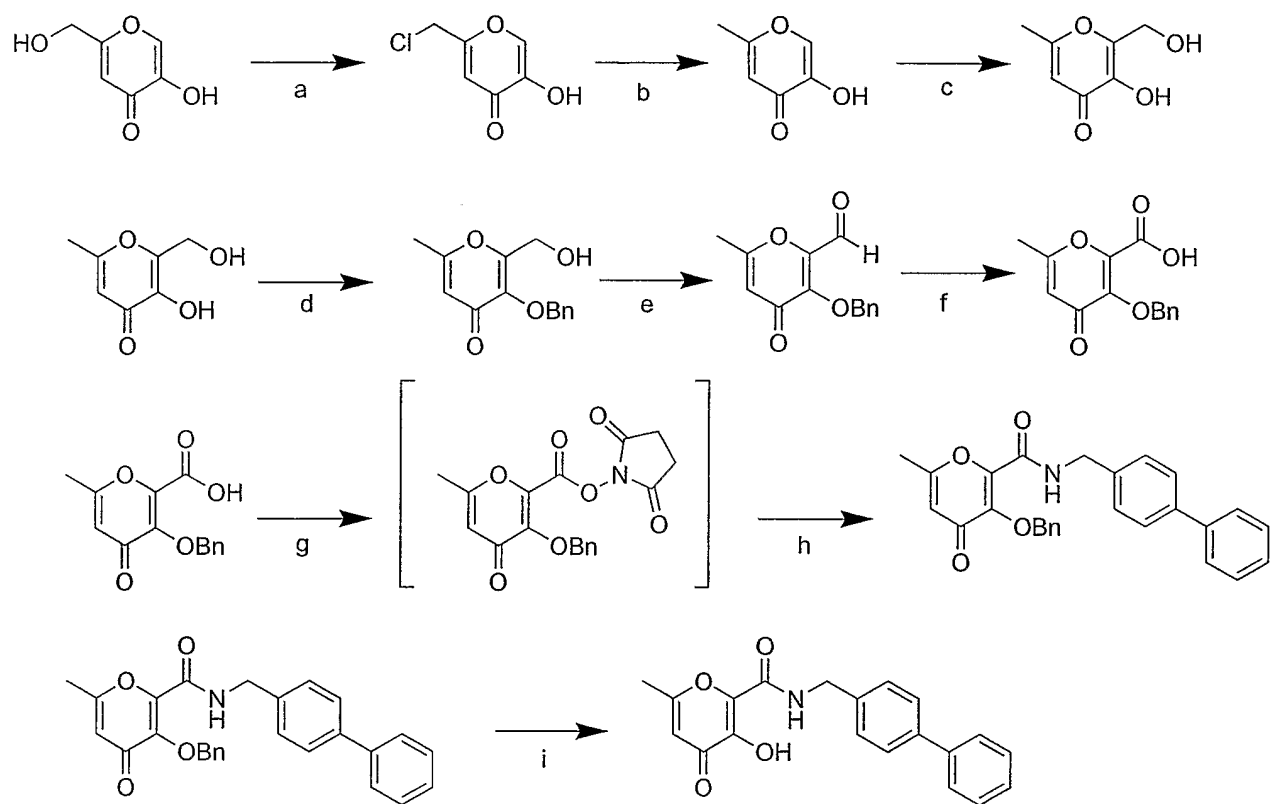
2(1H)-pyridinethione (7), and 3-hydroxy-2-methyl-4-pyrone (5) each displace the hydroxide ligand in $[(\text{Tp}^{\text{Ph,Me}})\text{ZnOH}]$ and coordinate the zinc(II) ion in a bidentate fashion. The metal-ligand bond lengths are compared to those found in the corresponding acetohydroxamate complex.¹⁰ The model-based approach described here is believed to provide an effective route toward second-generation MPI design.

The present invention further provides processes for making the compounds of formula I.

Unless otherwise noted, starting materials were obtained from commercial suppliers (e.g., Aldrich), have been reported in the scientific literature, or can be prepared from readily available starting materials using procedures known in the field. Commercially available starting materials were used without further purification. The abbreviation DMAP stands for 4-dimethylaminopyridine; TBSCl stands for tert-butyldimethylsilyl chloride; HMDO stands for hexamethyldisiloxane. $[(\text{Tp}^{\text{Ph,Me}})\text{ZnOH}]^5$ and 3-hydroxy-1-methyl-2(1H)-pyridinone¹⁴⁻¹⁶ were synthesized as previously described. Elemental analysis was performed at the University of California, Berkeley Analytical Facility. $1\text{H}/^{13}\text{C}$ NMR spectra were recorded on a Varian FT-NMR spectrometer running at 300 or 400 MHz at the Department of Chemistry and Biochemistry, University of California, San Diego. Infrared spectra were collected on a Nicolet AVATAR 320 FT-IR instrument at the Department of Chemistry and Biochemistry, University of California, San Diego. Illustrative syntheses are disclosed hereinbelow:

[2-(biphenyl-4-ylmethyl)-carbamyl-3-hydroxy-6-methyl-4-pyrone] (BLT-1)

BLT-1 was synthesized according to synthetic scheme 1 below:

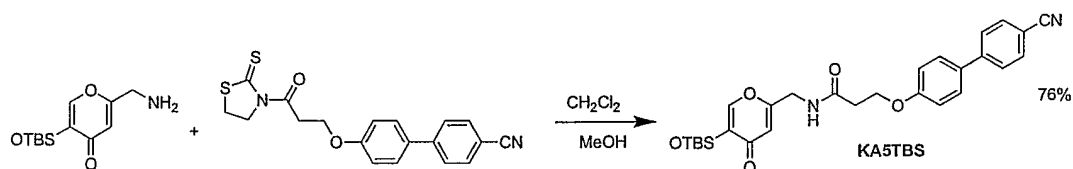
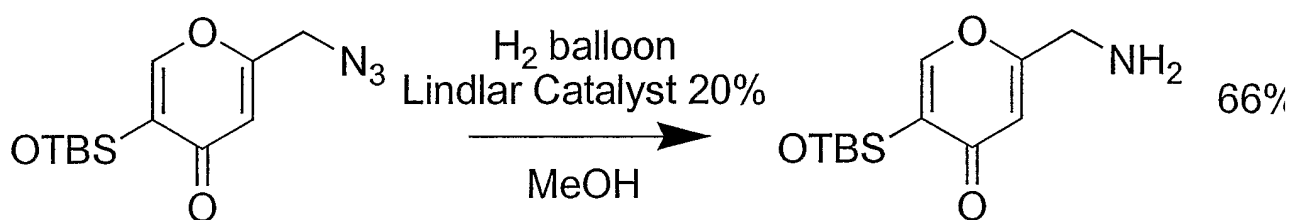
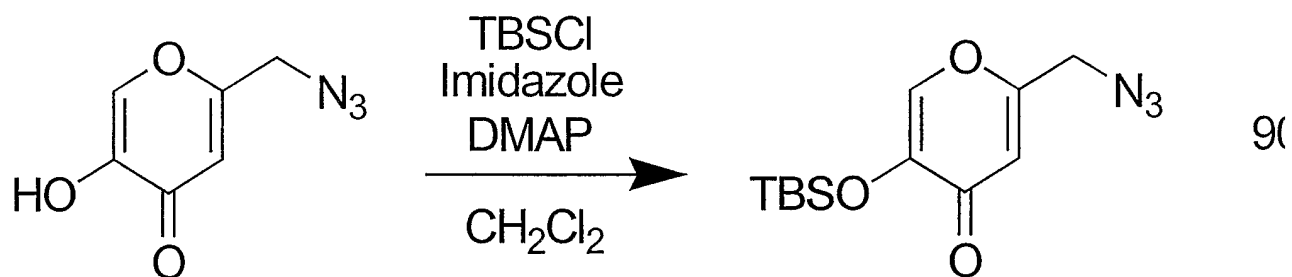


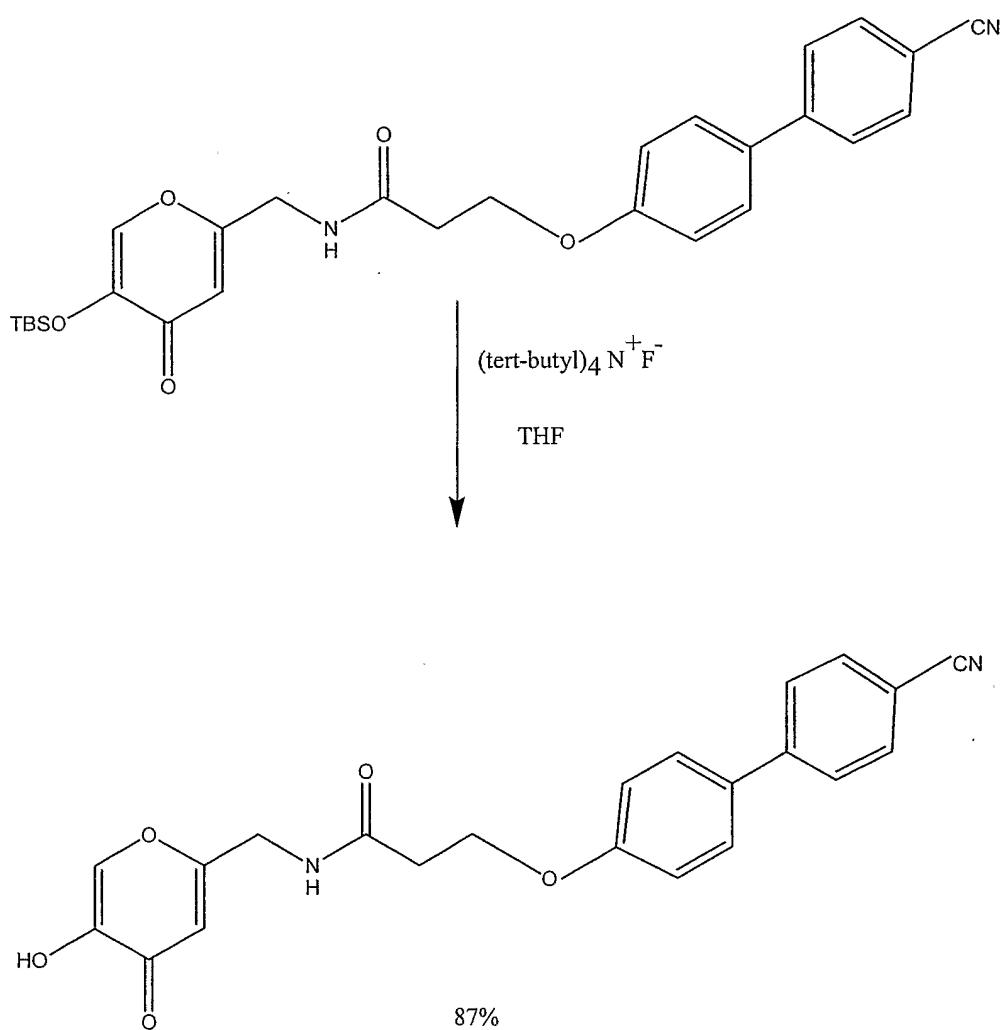
Scheme 1. Synthesis of BLT-1. a) SOCl_2 , dry CH_2Cl_2 , RT, 88%; b) Zn/HCl , H_2O , 70°C, 90%; c) HCHO , NaOH , H_2O , RT, 57%; d) BnBr , NaOH(aq) , MeOH , 75°C, 83%; e) SO_3 : pyridine, Et_3N , DMSO , CHCl_3 , RT, 89%; f) NaClO_2 , $\text{NH}_2\text{SO}_3\text{H}$, $\text{H}_2\text{O/acetone}$, RT, 81%; g) NHS , DCC , dry THF , RT; h) phenylbenzylamine, dry THF , 88% (2 steps); i) Pd/C 10%, H_2 35psi, MeOH , RT, 60%.

BLT-1 may be thionated with the thionating agent $\text{P}_4\text{S}_{10}/\text{HMDO}$ in benzene at 90° to produce the corresponding pyran-4-thione in 50% yield.

[3-(4'-cyanobiphenyl-4-oxy)-1-ketopropylaminomethyl]-5-hydroxy-4-pyrone (KA5)

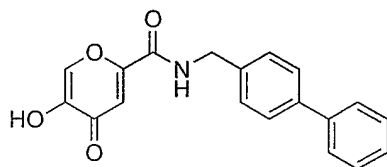
KA5 was synthesized according to scheme 2 below:





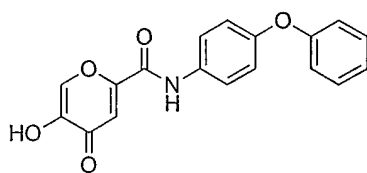
10 Scheme 2. Synthesis of KA5

The following hydroxypyrrone derivatives were prepared similarly:

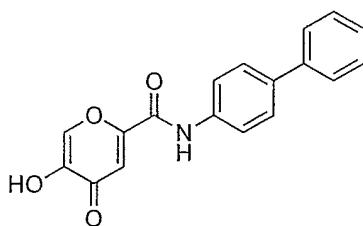


CA1

5

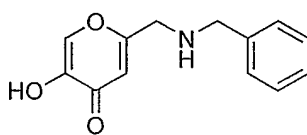


CA2



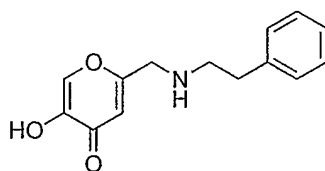
CA3

10

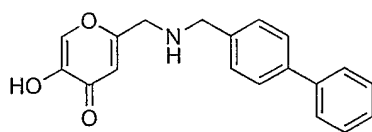


CK1

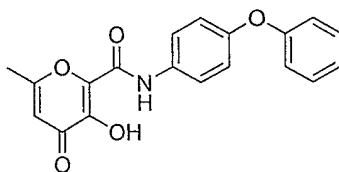
15



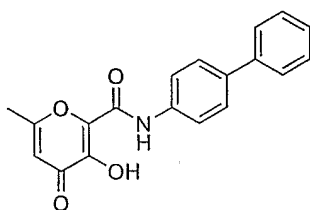
CK2



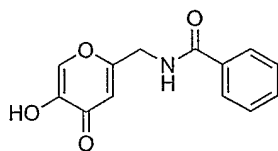
CK3



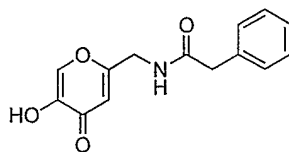
BLT-2



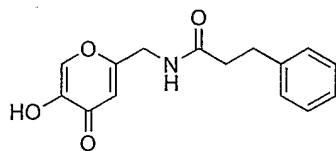
BLT-3



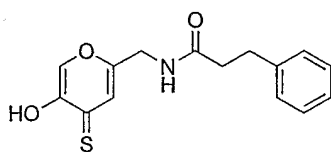
KA1



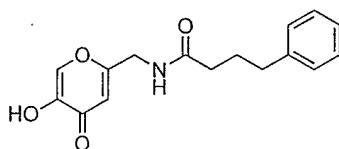
KA2



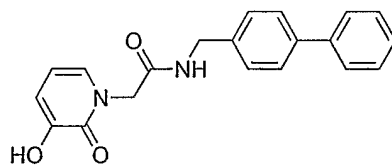
KA3



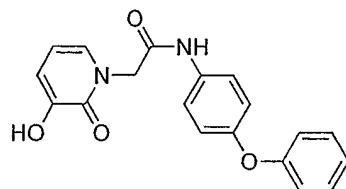
TKA3



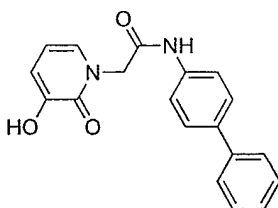
KA4



JAL-1



JAL-2



JAL-3

$[(\text{Tp}^{\text{Ph,Me}})\text{Zn}(1\text{-hydroxy-2(1H)-pyridinone})]$. In a 100-mL round-bottom flask,

- 5 $[(\text{Tp}^{\text{Ph,Me}})\text{ZnOH}]$ (145 mg, 0.26 mmol) was added to 10 mL of CH_2Cl_2 . To this solution was added 1.0 equiv of 1-hydroxy-2(1H)-pyridinone (29 mg, 0.26 mmol) dissolved in 15 mL of MeOH. The mixture was stirred at room temperature overnight under a nitrogen atmosphere. After stirring, the turbid solution was evaporated to dryness on a rotary evaporator to give a white solid. The solid was dissolved in a minimum amount of benzene (~15 mL) and filtered
- 10 to remove any insoluble material, and the filtrate was recrystallized by diffusion of the solution with pentane. Yield: 94%. ^1H NMR (CDCl_3 , 400 MHz, 25°C): 2.51 (s, 9H, pyrazole- CH_3), 5.78 (d, $J = 8.0$ Hz, 1H, pyridinone-H), 6.10 (t, $J = 6.4$ Hz, 1H, pyridinone-H), 6.17 (s, 3H, pyrazole-H), 6.84 (t, $J = 7.0$ Hz, 1H, pyridinone-H), 7.10 (m, 9H, phenyl-H), 7.58 (d, $J = 6.8$ Hz, 6H, phenyl-H), 7.67 (d, $J = 8.0$ Hz, 1H, pyridinone-H). ^{13}C NMR
- 15 (CDCl_3 , 100 MHz, 25°C): 13.0, 104.4, 106.8, 113.8, 127.2, 127.3, 127.6, 131.4, 132.8, 134.0, 144.7, 152.6, 159.9 (C=O). IR (film from CHCl_3): 1370, 1537, 1625, 2546 (B-H) cm^{-1} . Anal. Calcd for $\text{C}_{35}\text{H}_{32}\text{BN}_7\text{O}_2\text{Zn}$: C, 63.80; H, 4.90; N, 14.88. Found C, 63.69; H, 4.91; N, 15.14.

- $[(\text{Tp}^{\text{Ph,Me}})\text{Zn}(3\text{-hydroxy-2(1H)-pyridinone})]$. The same procedure was used as in the
- 20 synthesis of $[(\text{Tp}^{\text{Ph,Me}})\text{Zn}(1\text{-hydroxy-2(1H)-pyridinone})]$. Yield: 76%. ^1H NMR (CDCl_3 , 400 MHz, 25°C): 2.51 (s, 9H, CH_3 , pyrazole- CH_3), 6.00 (m, 1H, pyridinone-H), 6.08 (t, $J = 7.4$ Hz, 1H, pyridinone-H), 6.18 (s, 3H, pyrazole-H), 6.39 (d, $J = 6.8$ Hz, 1H, pyridinone-H), 7.10 (t, $J = 6.0$ Hz, 9H, phenyl-H), 7.61 (m, 6H, phenyl-H). ^{13}C NMR (CDCl_3 , 100 MHz, 25°C): 13.0, 104.2, 110.2, 113.6, 113.8, 127.1, 127.4, 127.5, 132.4, 144.7, 152.5, 155.8,
- 25 162.6 (C=O). IR (film from CHCl_3): 1305, 1546, 1619, 2547 (B-H) cm^{-1} . Anal. Calcd for $\text{C}_{35}\text{H}_{32}\text{BN}_7\text{O}_2\text{Zn}$: C, 63.80; H, 4.90; N, 14.88. Found C, 63.61; H, 5.03; N, 14.91.

[(Tp^{Ph,Me})Zn(3-hydroxy-1-methyl-2(1H)-pyridinone)]. The same procedure was used as in the synthesis of [(Tp^{Ph,Me})Zn(1-hydroxy-2(1H)-pyridinone)]. Yield: 80%. ¹H NMR (CDCl₃, 400 MHz, 25°C): 2.55 (s, 9H, pyrazole-CH₃), 3.50 (s, 3H, pyridinone-CH₃), 6.01 (t, J = 6.8 Hz, 1H, pyridinone-H), 6.24 (s, 3H, pyrazole-H), 6.68 (t, J = 5.6 Hz, 1H pyridinone-H), 7.22 (m, 9H, phenyl-H), 7.45 (d, J = 5.6 Hz, 1H pyridinone-H), 7.56 (d, J = 6.8 Hz, 6H, phenyl-H). ¹³C NMR (CDCl₃, 100 MHz, 25°C): 13.0, 37.7, 104.0, 105.3, 128.1, 128.2, 128.6, 128.9, 130.6, 135.8, 145.8, 153.9. IR (film from CDCl₃): 1301, 1371, 1573, 2544 (B-H) cm⁻¹. Anal. Calcd for C₃₆H₃₄N₇O₂BZn·C₆H₆·H₂O: C, 65.60; H, 5.50; N, 12.75. Found C, 65.65; H, 5.41; N, 12.84.

[(Tp^{Ph,Me})Zn(3-hydroxy-1,2-dimethyl-4(1H)-pyridinone)]. The same procedure was used as in the synthesis of [(Tp^{Ph,Me})Zn(1-hydroxy-2(1H)-pyridinone)]. Yield: 22%. ¹H NMR (CDCl₃, 400 MHz, 25°C): 2.00 (s, 3H, pyridinone-CH₃), 2.51 (s, 9H, pyrazole-CH₃), 3.57 (s, 3H, pyridinone-CH₃), 5.47 (d, J = 6.4 Hz, 1H, pyridinone-H), 6.16 (s, 3H, pyrazole-H), 6.66 (d, J = 6.0 Hz, 1H, pyridinone-H), 7.04 (m, 9H, phenyl-H), 7.61 (d, J = 5.6 Hz, 6H, phenyl-H). ¹³C NMR (CDCl₃, 100 MHz, 25 C): 12.4, 13.0, 42.4, 104.4, 107.4, 126.8, 127.3, 127.6, 128.2, 132.9, 144.6, 152.5. IR (film from CHCl₃): 1367, 1553, 1594, 2547 (B-H) cm⁻¹. Anal. Calcd for C₃₇H₃₆N₇O₂Zn·H₂O: C, 63.04; H, 5.43; N, 13.91. Found C, 62.89; H, 5.39; N, 13.66.

[(Tp^{Ph,Me})Zn(1-hydroxy-2(1H)-pyridinethione)]. The same procedure was used as in the synthesis of [(Tp^{Ph,Me})Zn(1-hydroxy-2(1H)-pyridinone)]. Yield: 70%. ¹H NMR (d₆-benzene, 400 MHz, 25°C): 2.26 (s, 9H, pyrazole-CH₃), 5.50 (t, J = 7.0 Hz, 1H, pyridinethione-H), 6.01 (s, 3H, pyrazole-H), 6.03 (t, J = 4.8 Hz, 1H, pyridinethione-H), 6.67 (d, J = 8.0 Hz, 1H, pyridinethione-H), 6.90 (t, J = 6.2 Hz, 3H, phenyl-H), 7.01 (t, J = 6.8 Hz, 6H, phenyl-H), 7.18 (m, 1H, pyridinethione-H), 7.81 (d, J = 8.0 Hz, 6H, phenyl-H). ¹³C NMR (CDCl₃, 100 MHz, 25°C): 13.0, 104.9, 116.0, 125.6, 127.1, 127.4, 128.0, 128.8, 132.9, 135.2, 144.4, 152.9. IR (film from CHCl₃): 1456, 1546, 1596, 2551 (B-H) cm⁻¹. Anal. Calcd for C₃₅H₃₂N₇OSBZn: C, 62.28; H, 4.78; N, 14.53. Found C, 62.17; H, 4.89; N, 14.79.

[(Tp^{Ph,Me})Zn(3-hydroxy-2-methyl-4-pyrone)]. The same procedure was used as in the synthesis of [(Tp^{Ph,Me})Zn(1-hydroxy-2(1H)-pyridinone)]. Yield: 61%. ¹H NMR (CDCl₃, 400

MHz, 25°C): 2.23 (s, 3H, pyrone-CH₃), 2.51 (s, 9H, pyrazole-CH₃), 5.29 (d, J = 5.2 Hz, 1H, pyrone-H), 6.17 (s, 3H, pyrazole-H), 7.10 (m, 9H, phenyl-H), 7.18 (d, 1H, J = 5.2 Hz, pyrone-H), 7.59 (d, J = 4.0 Hz, 6H, phenyl-H). ¹³C NMR (CDCl₃, 100 MHz, 25°C): 13.0, 14.7, 104.3, 109.2, 127.0, 127.4, 127.5, 128.2, 132.7, 144.7, 150.4, 152.5. IR (film from
5 CHCl₃): 1282, 1455, 1597, 2544 (B-H) cm⁻¹. Anal. Calcd for C₃₆H₃₃N₆O₃BZn: C, 64.16; H, 4.94; N, 12.47. Found C, 64.74; H, 5.03; N, 12.23.

It may be desirable optionally to use a protecting group during all or portions of the above described synthetic procedures. Such protecting groups and methods for their introduction and removal are well known in the art. See Greene, T.W.; Wutz, P.G.M.

10 "Protecting Groups In Organic Synthesis" second edition, 1991, New York, John Wiley & Sons, Inc.

X-ray Crystallographic Analysis. Data were collected on a Bruker AXS area detector diffractometer. Crystals were mounted on quartz capillaries by using Paratone oil and were
15 cooled in a nitrogen stream (Kryo-flex controlled) on the diffractometer (-173°C). Peak integrations were performed with the Siemens SAINT software package. Absorption corrections were applied using the program SADABS. Space group determinations were performed by the program XPREP. The structures were solved by direct or Patterson methods and refined with the SHELXTL software package.¹⁷ Unless noted otherwise, all
20 hydrogen atoms were fixed at calculated positions with isotropic thermal parameters; all non-hydrogen atoms were refined anisotropically.

[(Tp^{Ph,Me})Zn(1-hydroxy-2(1H)-pyridinone)]. Colorless blocks were grown out of a solution of the complex in benzene diffused with pentane. The hydrogen atom on the boron was found in the difference map, and the position was refined.

25 [(Tp^{Ph,Me})Zn(3-hydroxy-2(1H)-pyridinone)]. Colorless blocks were grown within a few minutes from a solution of the complex in benzene diluted with pentane. The hydrogen atom on the boron was found in the difference map, and its position was refined. The complex cocrystallized with one molecule of benzene that was refined to half occupancy in the asymmetric unit. The complex also contained a disordered phenyl ring on one of the
30 pyrazole arms that was refined in two orientations (partial occupancy 55:45 split). There was

no disorder observed in the coordination environment. No hydrogen atoms were calculated or refined for the disordered phenyl ring.

$[(\text{Tp}^{\text{Ph,Me}})\text{Zn}(3\text{-hydroxy-1-methyl-2(1H)-pyridinone})]$. Colorless blocks were grown out of a solution of the complex in benzene diffused with pentane. The hydrogen atom on the boron was found in the difference map, and the position was refined. The complex

5 cocrystallized with one disordered molecule of benzene in the asymmetric unit. No hydrogen atoms were calculated or refined for the disordered benzene solvent molecule.

$[(\text{Tp}^{\text{Ph,Me}})\text{Zn}(3\text{-hydroxy-1,2-dimethyl-4(1H)-pyridinone})]$. Colorless blocks were grown out of a solution of the complex in benzene diffused with pentane. The hydrogen atom

10 on the boron was found in the difference map, and the position was refined.

$[(\text{Tp}^{\text{Ph,Me}})\text{Zn}(1\text{-hydroxy-2(1H)-pyridinethione})]$. Colorless blocks were grown out of a solution of the complex in benzene diffused with pentane. The hydrogen atoms on the boron atoms were found in the difference map, and their positions were refined. The asymmetric unit contains four molecules of the complex.

$[(\text{Tp}^{\text{Ph,Me}})\text{Zn}(3\text{-hydroxy-2-methyl-4-pyrone})]$. Colorless blocks were grown out of a solution of the complex in benzene diffused with pentane. The hydrogen atom on the boron was found in the difference map, and the position was refined. The complex cocrystallized with one-half molecule of benzene in the asymmetric unit.

Computer Modeling Analysis. Computer analysis was performed on PC workstations running a Linux (Red Hat) operating system. Superpositions were performed on the structure of human stromelysin-1 (MMP-3) by using coordinates from the Protein Data Bank (entry 1CQR, Chain A)^{18,19}. The coordinating pyrazole nitrogen atoms were directly superimposed onto the N2 atoms of the coordinating histidine residues in the protein. The superpositions

25 were executed using a custom-written script²⁰ that overlaid the small-molecule X-ray coordinates onto the protein structure by using a least-squares fitting of the corresponding nitrogen atoms. Three different orientations were constructed for each analysis (vide infra). The resulting structures were then examined by using Rasmol (v. 2.7.2.1, April 2001) and were visually inspected for steric clashes with spacefilling models based on van der Waals

radii. Superpositions where the ZBG occupied the same space as the protein were determined to be in steric conflict.

Utilizing $[(\text{Tp}^{\text{Ph,Me}})\text{ZnOH}]$ as a starting point, a number of complexes were synthesized to serve as models of novel ZBGs bound to the active site of MMPs. Ternary
5 complexes of $[(\text{Tp}^{\text{Ph,Me}})\text{ZnOH}]$ have proven to be an accurate structural model for MMP active-site inhibition.^{9,10} The series of ZBGs presented here can be separated into three groups: hydroxypyridinonate-based, N-methylated-hydroxypyridinonate-based, and hydroxypyridinonate derivatives. The complexes of $[(\text{Tp}^{\text{Ph,Me}})\text{Zn}(\text{ZBG})]$ have all been characterized by X-ray crystallography, $1\text{H}/^{13}\text{C}$ NMR, IR, and elemental analysis.

10 The X-ray structures of hydroxypyridinonate-based ligands bound to the model complex show that these chelators coordinate in a bidentate fashion to the catalytic zinc(II) center (Figure 1). The ZBG 1-hydroxy-2(1H)-pyridinone is a cyclic analogue of hydroxamic acid and was therefore anticipated to bind in a chelating manner. In the structure of $[(\text{Tp}^{\text{Ph,Me}})\text{Zn}(1\text{-hydroxy-2(1H)-pyridinone})]$, the zinc center can be described as distorted
15 trigonal bipyramidal ($T = 0.64$)²¹ with the 2-hydroxy oxygen atom and one of the pyrazole ring nitrogen atoms occupying the axial positions of the coordination sphere. The coordinating oxygen atoms in this complex have Zn-O bond lengths of 1.97 Å (O1) and 2.09 Å (O2). These bond lengths are similar to the corresponding bond lengths in $[(\text{Tp}^{\text{Ph,Me}})\text{Zn}(\text{acetohydroxamate})]$, suggesting that this ZBG may bind with an affinity
20 comparable to the hydroxamic acid ZBG.¹⁰

In the structure of $[(\text{Tp}^{\text{Ph,Me}})\text{Zn}(3\text{-hydroxy-2(1H)-pyridinone})]$, the zinc center can also be described as distorted trigonal bipyramidal ($T = 0.63$, Figure 2) with one oxygen donor and one of the pyrazole rings occupying the axial positions of the coordination sphere. This isomer of 1-hydroxy-2(1H)-pyridinone has Zn-O bond lengths of 1.92 Å (O1) and 2.23
25 Å (O2), demonstrating strong bidentate coordination to the metal center.

The second group of ZBGs examined was the N-methylated-hydroxypyridinonate group. These ZBGs show similar binding to the unsubstituted hydroxypyridinonates (Figure 3). In the complex $[(\text{Tp}^{\text{Ph,Me}})\text{Zn}(3\text{-hydroxy-1-methyl-2(1H)-pyridinone})]$, the coordination environment around the zinc center can be described as distorted trigonal bipyramidal ($T =$
30 0.52). The coordinating oxygen atoms are bonded to the model complex in a bidentate

fashion with a Zn-O1 bond length of 1.94 Å and a Zn-O2 bond length of 2.21 Å. The ZBG 3-hydroxy-1,2-dimethyl-4(1H)-pyridinone has the shortest averaged phenolic and carbonyl Zn-O bond lengths of all the novel ligands presented herein. The X-ray structure clearly demonstrates the tight binding of this ZBG with bond lengths of 1.96 Å (Zn-O1) and 2.05 Å (Zn-O2). The coordination environment is best described as distorted tetragonal ($T = 0.44$) and is more distorted from trigonal bipyramidal than the other ZBGs described here.

The final group of novel ZBGs examined is classified as hydroxypyridinonate derivatives. This group includes 1-hydroxy-2(1H)-pyridinethione and 3-hydroxy-2-methyl-4-pyrone. The complex $[(\text{Tp}^{\text{Ph,Me}})\text{Zn}(1\text{-hydroxy-2(1H)-pyridinethione})]$ reveals that 1-hydroxy-2(1H)-pyridinethione binds in the expected bidentate fashion (Figure 4). The coordination environment around the zinc center can be described as distorted trigonal bipyramidal ($T = 0.54$). The bond lengths of the coordinating sulfur and oxygen are longer than the coordinating oxygen atoms in the corresponding acetohydroxamic acid complex¹⁰ because of the larger sulfur atom. In addition to the comparably longer Zn-S (2.32 Å) distance, the sulfur binding also affects the binding of the adjacent oxygen atom, increasing the Zn-O bond length to 2.08 Å.

3-Hydroxy-2-methyl-4-pyrone binds to $[(\text{Tp}^{\text{Ph,Me}})\text{ZnOH}]$, resulting in a complex (Figure 3) with comparable bond lengths as in $[(\text{Tp}^{\text{Ph,Me}})\text{Zn}(\text{acetohydroxamate})]$.¹⁰ The Zn-O1 distance is 1.94 Å, and the Zn-O3 distance is 2.18 Å. The coordination environment is similar to that of the hydroxypyridinonate ZBGs described above. The coordination sphere can be described as distorted trigonal bipyramidal ($T = 0.69$) with one oxygen and one nitrogen donor occupying the axial positions.

The solid-state structures of these ZBGs clearly demonstrate strong bidentate chelation of the zinc(II) metal centers. NMR spectra were acquired for all of the metal complexes to confirm ZBG coordination in solution. Large changes in the ¹H NMR spectra between the free and bound ZBGs support the stability of these complexes in solution. In the complex $[(\text{Tp}^{\text{Ph,Me}})\text{Zn}(3\text{-hydroxy-1-methyl-2(1H)-pyridinone})]$, significant shifts are observed in the ¹H NMR spectra. An unambiguous upfield shift is noticed for all protons in the ZBG bound to the metal center. These shifts are most likely the result of both the direct interaction of the chelator with the zinc(II) ion and the inclusion of the ZBG into the

aromatic, hydrophobic pocket created by the $\text{Tp}^{\text{Ph,Me}}$ ligand. For example, the proton para to the carbonyl group shifts from 6.24 ppm in the free ZBG to 6.01 ppm in the complex. In the complex $[(\text{Tp}^{\text{Ph,Me}})\text{Zn}(3\text{-hydroxy-2-methyl-4-pyrone})]$, the ^1H NMR also demonstrates from 0.5 to greater than 1.0 ppm changes in the proton resonances of the ZBG. Significant changes are also observed in the spectra of $[(\text{Tp}^{\text{Ph,Me}})\text{Zn}(1\text{-hydroxy-2(1H)-pyridinethione})]$ relative to the free ZBG where again, in the ^1H NMR spectrum, notable shifts in the protons of the ZBG are observed. The ^1H NMR data suggest that the ligand binding observed in the solid state is preserved in solution at room temperature.

To confirm further the mode of binding, IR spectra of the free ZBGs were examined and compared to the corresponding zinc complexes. Cast films from CHCl_3 solutions onto NaCl plates of the ZBGs and complexes allowed for measurement of the carbonyl stretching frequencies. Appropriate B-H stretches were observed for the $[(\text{Tp}^{\text{Ph,Me}})\text{Zn}(\text{ZBG})]$ complexes. All six of the ZBGs showed shifts in the $\text{C}=\text{O}$ stretch to lower energy when complexed as $[(\text{Tp}^{\text{Ph,Me}})\text{Zn}(\text{ZBG})]$. In the free ZBG 3-hydroxy-2-methyl-4-pyrone, the carbonyl (1624 cm^{-1}) exhibits a 27 cm^{-1} shift to lower wavenumbers in the metal complex. Similarly, comparison of the spectra of 1-hydroxy-2(1H)-pyridinone and of the complex $[(\text{Tp}^{\text{Ph,Me}})\text{Zn}(1\text{-hydroxy-2(1H)-pyridinone})]$ demonstrates a carbonyl shift from 1643 cm^{-1} for the free ZBG to 1625 cm^{-1} for $[(\text{Tp}^{\text{Ph,Me}})\text{Zn}(1\text{-hydroxy-2(1H)-pyridinone})]$. The data here are consistent with other reports of changes in carbonyl stretching frequencies upon metal coordination.^{22,23} All three ZBG classes (as defined above) demonstrate shifts in the carbonyl vibration consistent with the bidentate mode of binding found in the X-ray structures.

Computer modeling of these new ZBGs with human MMP-3 was performed to determine whether these chelators could fit within the MMP active site. The ZBG, zinc(II) ion, and coordinating nitrogen atoms from the model complexes were superimposed into a crystal structure of enzyme MMP-3¹⁸. The nitrogen atoms of the pyrazole model complexes do not uniquely correspond to any of the three histidine residues bound to the zinc(II) ion in the protein active site. Therefore, three orientations were scrutinized to reveal which superpositions allowed for the ZBG to reside in the active site without colliding with the protein surface. These studies generally yielded similar results regardless of the ZBG. Upon

insertion into the protein crystal structure, the ZBGs either showed severe steric clashes with the protein, no steric clashes but with little or no room for attachment of a peptidomimetic "backbone" (required for MPI design),^{1,24} or a binding conformation that showed no steric clashes and ample space for both the ZBG and a requisite drug "backbone". As a
5 representative case, the three different conformations for the superposition of 3-hydroxy-2-methyl-4-pyrone are shown in Figure 5.

Since MMPs were first linked to diseases such as inflammatory conditions such as arthritis and cancer, hydroxamate-derived drugs have been the staple for MPI design.¹ Hydroxamate-based inhibitors display good activity, and there are thousands of potential
10 MMP inhibitors currently in some stage of the design process. It is believed that greater than 90% of these inhibitors utilize the hydroxamic acid-based ZBG.¹ There has been much effort placed to improve the design of better peptidomimetic "backbones" of MMP inhibitors, with the effort focused on enhancing the ZBG being relatively miniscule in comparison.

It has been shown that tris(pyrazolyl)borate complexes are good structural models for
15 the active site of MMPs.⁵⁻⁸ Using this model of the MMP active site, a number of complexes were synthesized to examine new routes to inhibitor design. All six complexes show that the novel ZBGs of the invention bind in a bidentate fashion. The bond lengths of the ZBGs to the zinc center are comparable to those in $[(\text{Tp}^{\text{Ph,Me}})\text{Zn}(\text{acetohydroxamate})]$.¹⁰ A better understanding of the interactions between the zinc(II) ion and the inhibitor is a recognized
20 component of MPI design.^{1,25,26}

Using the successful hydroxamate ZBG as a starting point, six new chelators were initially selected that were expected to bind as well as or better than hydroxamates. Hydroxypyridinones (HOPOs) were selected as lead compounds for several reasons. HOPOs have a high structural homology to hydroxamic acids and are known to be strong metal
25 chelators.^{14,27-30} In addition, the cyclic structure of hydroxypyridinones reduces the degrees of freedom in the ligand, preventing the *cis* to *trans* isomerization that can occur in hydroxamic acids, which ultimately detracts from the thermodynamic affinity of the metal-ligand interaction. The basicity of hydroxypyridinones varies between isomers, which potentially allows for tuning the protonation state of the ligand to accommodate possible
30 hydrogen-bonding interactions in the protein active site.²⁴ Finally, many hydroxypyridinones

and related compounds have been or are used in medical and food industry applications,^{14,27,31} suggesting a reasonable level of biological tolerance for these chemical moieties.

Several hydroxypyridinone and hydroxypyrrone derivatives were also developed, including N-methylated hydroxypyridinones, a hydroxypyridinethione, and 3-hydroxy-2-methyl-4-pyrone (see above and also see Fig. 1). These ligands share many of the same features as the hydroxypyridinones, but demonstrate other interesting features as well. The N-methylated hydroxypyridinones, 3-hydroxy-1-methyl-2(1H)-pyridinone(2), and 3-hydroxy-1,2-dimethyl-4(1H)-pyridinone (3) were examined to show that substitutions on the hydroxypyridinone ring did not affect the binding or present steric problems toward zinc binding. This is important to demonstrate because these ZBGs are appended with backbones such as peptidomimetic backbones to prepare fully functional inhibitors. The hydroxypyridinethione, 1-hydroxy-2(1H)-pyridinethione (6), was examined to show that sulfur derivatives also bind in a bidentate fashion to the zinc center. Sulfur-containing ligands of this sort may be very good ZBGs because of the apparent thiophilicity of zinc(II).^{32,33} Similarly, other thiol-based MPIs have been studied¹⁰ and have shown reasonably good activity when compared to hydroxamate-based inhibitors.^{1,11,12} Combining the best features of both hydroxamates and thiol inhibitors into a single ZBG such as 1-hydroxy-2(1H)-pyridinethione provides the basis for inhibitors of formula I. Finally, 3-hydroxy-2-methyl-4-pyrone (5) or "Maltol" (an FDA-approved food additive) was examined as a ZBG. Maltol also binds in a bidentate fashion with bond lengths similar to those of the other ZBGs. Maltol is an attractive ZBG because of its particularly good biological tolerance as evidenced by use as a food additive^{34,35} and potential therapeutic applications as an insulin mimetic when complexed with vanadate.^{31,36} These novel ZBGs demonstrate strong bidentate binding similar to that found in the complex [(Tp^{Ph,Me})Zn(acetohydroxamate)], which has been shown to be an accurate model for the MMP-inhibitor complex.¹⁰

In addition to crystallographic, NMR, and IR data, computer modeling has been performed to explore the binding of the new ZBGs inside the active-site pocket of an MMP. This experiment was used to determine if any of the ZBGs would encounter steric problems upon binding. The structures of the complexes [(Tp^{Ph,Me})Zn(ZBG)] were fixed into the crystal

structure of MMP-3,¹⁸ with the histidine nitrogen atoms and the zinc(II) ion in the protein crystal structure aligned with the corresponding atoms from the model complex.²⁰ When placed in two of three possible orientations, the ZBGs were found to have no steric conflicts with the protein active site, and one of the three conformations appeared to be preferred for the development of inhibitors with a peptidomimetic substrate backbone (vide supra). These modeling studies are an essential first step toward performing computer-aided drug discovery using novel ZBGs.²⁰

To address the problems of hydroxamate-based MPIs, the present invention is based on the use of molecules like maltol, which is a common food additive, and its derivatives as novel ZBGs that are expected to have better oral and bioavailability. Sulfur-containing ZBGs were developed because of the higher affinity of sulfur for the Zn^{2+} ion compared to oxygen. Furthermore, ZBGs with rigid ring structures that lock the O,S-donor atoms in a *cis* conformation, can bind Zn^{2+} ion in a bidentate fashion, to minimize oxidation and disulfide bond formation, which can be problems for thiol MPIs, in biological systems. Figure 1 discloses the small molecules which were evaluated as models for more complex MPIs. However, compounds 1-12 can also be used directly as inhibitors.

Sulfur-Containing MPIs

As shown in Figure 6, various thionation routes were explored to optimize reaction conditions. Neat P_4S_{10} reactions at 200°C led to decomposition and low yields. The Lawesson's Reagent route had expensive reagents, undesired byproducts, product volatility during deprotection, and low product yields. Facile, high-yielding reactions for novel sulfur-containing ligands have been developed (Figure 6) See, e.g., Lewis, J.A.; Puerta, D.T.; Cohen, S.M.; *Inorg. Chem.*, **2003**, *42*, 7455; Scarrow, R.C. et al., *Inorg. Chem.*, **1985**, *24*, 954; Abu-Dari, K. et al., *Inorg. Chem.*, **1993**, *32*, 3052.

As demonstrated above for oxygen-containing ZBGs, the sulfur-containing ZBGs **11**, **8**, and **9** bind the Zn^{2+} metal center in $(\text{Tp}^{\text{Ph,Me}})\text{Zn}$ in a bidentate fashion similar to acetohydroxamic acid, which makes them promising ZBGs. In the trigonal bipyramidal complex structures, the O,S ligands have the sulfur atom axial to the Zn^{2+} ion while the oxygen is equatorial to the Zn^{2+} ion. (Figure 8).

MMP Assays with ZBGs of the Present Invention

Biological assays were used to test the potency of the ZBGs with respect to binding the MMP enzyme. Knight C.G., et al., FEBS Lett., **1992**, 296, 263. The assay plate was incubated with buffer, various concentrations of ZBG, and enzyme MMP-3 at 37°C for 1 h.

5 The reaction was started by adding fluorescent substrate, as shown in Figure 9. Increasing fluorescence was measured over time at 37°C.

As shown on the Table on Figure 9, all of the ZBGs tested in the biological assay were more potent than acetohydroxamic acid (AHA). The O,S mixed donor ligands (7-11) were all about two orders of magnitude more potent than AHA, with 7 showing low
10 micromolar activity. The raw data from the assays (Figure 10) were converted to percentages of the control activity (Figure 11) by comparing the slopes of the data sets to the control slope. Control activity was determined from wells with enzyme, buffer and substrate. The amount of fluorescence over time correlated with MMP-3 enzymatic activity, wherein, more fluorescence over shorter time periods indicated a less potent ZBG. When evaluated as
15 controls, some of the ZBGs quenched fluorescence, and one exhibited autofluorescence. The fluorescence quenching controls showed that the assays were still valid to assess the relative binding affinities of the various ZBGs. (Figure 12). After correcting for fluorescence quenching, the activity trend was still linear. The uncorrected IC₅₀ values were not due to fluorescence quenching. The point corrected IC₅₀ value for thiopyromeconic acid was found
20 to be 0.118 mM. (Figure 13). The IC₅₀ value for BLT-1 was 270 μM. BLT-1 inhibited MMP-1 at an IC₅₀ value >50000 nM and inhibited MMP-2 at an IC₅₀ value of 9300 nM. Additional representative IC₅₀ values for MMP-3 inhibition include BLT-3: 90 μM; KA2: 200 μM; KA3: 930 μM; TKA3: 90 μM; KA4: 400 μM; and CA1: 25% inhibition @ 50 μM.

In order to confirm the values obtained fluorescence-based assays, additional
25 experiments were performed on some ZBGs using a widely-used colorimetric-based assay. Weingarten, H. et al., J. Biochemistry, **1985**, 24, 6730. The IC₅₀ values from the colorimetric assays (Fig. 9) are in good agreement with those obtained by fluorescence measurements, with the exception of compound 5 that shows approximately 3-fold lower potency when determined colorimetrically.

[(Tp^{Ph,Me})Zn(ZBG)] vs. AHA Titrations

The poor aqueous solubility of compound **3** precluded evaluation of this ligand in MMP assays. This prompted using the aforementioned complexes as thermodynamic models of MMP inhibition. [(Tp^{Ph,Me})Zn(ZBG)] complexes of various ZBGs in organic solvents
 5 were titrated with increasing amounts of AHA in order to obtain equilibrium constants that represent relative binding constants between the bound ZBG and AHA.

The competition experiment scheme shown in Figure 14 demonstrates the displacement of thiomaltol by AHA in the [(Tp^{Ph,Me})Zn(ZBG)] complex. Parkin, G., *Chem. Rev.*, **2004**, *104*, 903. Although there are two equilibria in solution, the situation can be
 10 reduced to the displacement of the ZBG by AHA in the (Tp^{Ph,Me})Zn complex as shown in Figure 14, wherein a 1:1 binding isotherm fits the data well. This means that the association constant is related to the apparent binding affinity as follows:

$$K_a = K_{app} = K_{AHA}/K_{ZBG}$$

The 1:1 binding isotherm data fits give efficiency against AHA. Specifically, 60 μ M
 15 [(Tp^{Ph,Me})Zn(Thiomaltol)] in 1:250 DMF:MeOH was titrated with 10 M AHA. The solutions were prepared from crystalline material, and the cuvette was incubated for 1.5 minutes between AHA additions.

The graph in Figure 15 shows absorbance spectra of [(Tp^{Ph,Me})Zn(thiomaltolato)] titrated with AHA in 1:250 DMF:MeOH to give [(Tp^{Ph,Me})Zn(AHA)] and thiomaltol. The
 20 plot in Figure 16 shows the absorbance of thiomaltol (λ_{max} = 360 nm, K_a = 0.0685) as circles and the absorbance of [(Tp^{Ph,Me})Zn(thiomaltolato)] (λ_{max} = 398 nm, K_a = 0.0785) as squares as a function of acetohydroxamic acid concentration.

The relative potencies of the ZBGs in the MMP assays and the UV-visible titrations do not agree completely. This may reflect limitations of the model complexes to mimic
 25 biology. However, the fluorescence assay uses active enzyme in biologically relevant conditions (Figure 9), while the titration experiments used model complexes in organic solvents. 3,4-HOPO (**3**) was not soluble enough to be used in the fluorescence assay. The relative rankings of the ZBGs shown in Figure 17 demonstrate that the present ZBGs can be used as substituents for MPIs. Both the titration experiments and the biological assays show

that all of the novel ZBGs tested were more potent than AHA. Both the experiments show that the O,S-donor ligands were better inhibitors than their O,O-donor ligand counterparts.

The design of potent and selective metalloprotein inhibitors such as matrix metalloproteinase inhibitors presents the possibility to treat many diseases, including those disclosed in Refs. 1-3, incorporated by reference herein. The present invention provides the synthesis and characterization of at least eleven ZBG-binding moieties and at least nine [(Tp^{Ph,Me})Zn(ZBG)] complexes to provide new ZBGs for incorporation into MPIs of formula I.

The cyclic ZBGs shown in Figure 1 can readily be elaborated to afford a wide variety of substituents to the open ring positions, particularly peptidomimetic backbones, such as those shown in Reference 1 and in EPA 126,974. Figure 18 summarizes the synthetic route to organic backbones comprising amido moiety and the ZBG maltol (or thiomaltol). Compounds A1-A4 have been prepared by this route (Figure 18).

Biological Evaluation of MMP Inhibitors

The following models were used to evaluate anti-cancer activity of the MPIs of the present invention:

1. Pancreatic Cell (PC-3) Model

In this study, the test groups were a vehicle control and an MPI according to the present invention with n=10 for each group. The tumors were measured with a caliper and the volume calculated using the formula for the volume of an ellipsoid. The MPI was first administered about 6:00 pm the evening of the same day that the tumor cells were injected in the morning. The same dose of MPI was administered BID for each following day. Tumor volume (mm.sup.3) was measured on day 25.

2. Breast Tumor Model

This study was carried out essentially as the PC-3 model. MX-1 breast tumor pieces were implanted (with a trocar) into nude mice with n=10 per group. Dosing with an MPI according to the present invention (10 mpk or 50 mpk, PO BID) was initiated when the tumors reached a size of 60-120 mg. Dosing was continued for 26 days. The tumors were

measured using a caliper and the volume calculated using the formula for the volume of an ellipsoid.

3. MX-1 Adjuvant Model

- 5 Mice were implanted with MX-1 tumors and allowed to grow to 50-100 mm³. The animals were dosed with cyclophosphamide (100 or 80 mpk). This was considered Day 1. Two weeks later the animals were pair matched after tumor regression and dosing BID with the MPI was begun until the end of the experiment. Tumors were measured weekly. The endpoint for the study was a final tumor size of 1.5 g.

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All publications, patents, and patent documents are incorporated by reference herein, as though individually incorporated by reference. The invention has been described with reference to various specific and preferred embodiments and techniques. However, it should be understood that many variations and modifications may be made while remaining within
5 the spirit and scope of the invention.

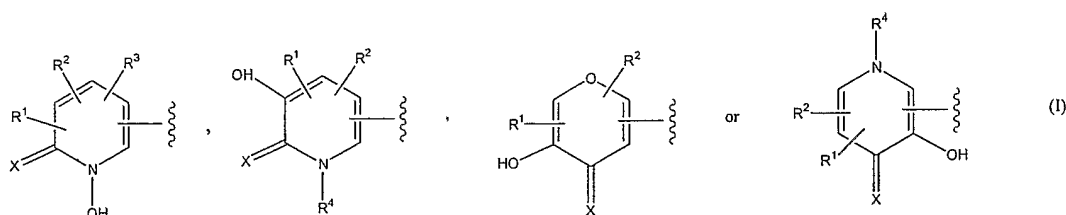
What is claimed is:

1. A metalloprotein inhibitor comprising:

a. an organic backbone molecule (Pep) and at least one zinc binding group (ZBG)

5 covalently attached thereto; or

b. at least one ZBG substituted by a side chain that comprises one or more amido and/or amino moieties, wherein the ZBG is of formula (I):



wherein the wavy line represents a Pep molecule or a side chain that is an R³ or an R⁴ group

10 which comprises one or more amido and/or amino moieties, and

wherein X is O or S and each R¹, R², R³, and R⁴ is individually hydrogen or an organic radical.

2. The metalloprotein inhibitor of claim 1 which is a matrix metalloprotein inhibitor.

15

3. The metalloprotein inhibitor of claim 1 which is a histone deacetylase.

4. The inhibitor of any one of claims 1-3 wherein R¹, R², R³, and R⁴ are individually H,

halo, CN, nitro, amino, sulfonamido, (C₁-C₆)alkyl, (C₁-C₆)alkoxy, (C₃-C₆)cycloalkyl, (C₃-

20 C₆)cycloalkyl((C₁-C₆)alkyl), (C₆-C₁₀)aryl, (C₆-C₁₀)aryl(C₂-C₁₀)alkyl, (C₃-

C₆)heterocycloalkyl, (C₃-C₆)heterocycloalkyl(C₁-C₆)alkyl, (C₂-C₆)alkenyl, (C₂-C₆)alkynyl,

(C₁-C₆)alkanoyl, halo(C₁-C₆)alkyl, hydroxy(C₁-C₆)alkyl, (C₁-C₆)alkoxycarbonyl, (C₁-

C₆)alkylthio, thio(C₁-C₆)alkyl, (C₁-C₆)alkanoyloxy, biphenylcarbamyl, biphenylcarbamyl-

(C₁-C₆)alkyl, biphenyl(C₁-C₆)alkylcarbamyl, biphenyl(C₁-C₆)alkylcarbamyl (C₁-C₆)alkyl,

25 phenoxyphenylcarbamyl, (C₆-C₁₀)aryl(C₁-C₆)alkylamino(C₁-C₆)alkyl, biphenyl(C₁-

C₆)alkylamino(C₁-C₆)alkyl, (C₆-C₁₀)arylcarbonylamino(C₁-C₆)alkyl, (C₆-C₁₀)aryl(C₁-

C₆)alkylcarbonylamino(C₁-C₆)alkyl, biphenyloxy(C₁-C₆)alkylcarbonylamino(C₁-C₆)alkyl,

phenoxyphenylcarbamyl(C₁-C₆)alkyl, N(R⁶)(R⁷), or SO₂N(R⁶)(R⁷), wherein R⁶ and R⁷ are individually H, =O, -OH, (C₁-C₆)alkyl, (C₃-C₆)cycloalkyl, (C₃-C₆)cycloalkyl(C₁-C₆)alkyl, phenyl or benzyl, or R⁶ and R⁷, together with the N to which they are attached, form a 5- or 6-membered ring which may optionally contain 1-2 S, N(R⁶) or nonperoxide O; or R¹ and R² together are methylenedioxy; optionally any of R¹, R², R³, and R⁴ is substituted with one to four R¹; or a pharmaceutically-acceptable salt thereof.

5. The inhibitor of any one of claims 1-4 wherein Pep is a naturally-occurring peptide, a synthetic peptide, or a peptide analog (peptidomimetic).

6. The inhibitor of claim 5 wherein Pep comprises one or more amido moieties (-C(O)NH-).

7. The inhibitor of claim 6 which comprises the moiety [ZBG]-C(R⁵)(R⁶)-C(O)NH- wherein R⁵ is H and R⁶ is (C₁-C₂₂)alkyl, (C₂-C₆)alkenyl, (C₆-C₁₀)aryl, (C₁-C₆)alkyl, (C₆-C₁₀)heteroaryl, (C₆-C₁₀)heteroaryl(C₁-C₆)alkyl, (C₃-C₆)cycloalkyl, (C₃-C₆)cycloalkyl(C₁-C₆)alkyl, or R⁵ and R⁶ together with the carbon atom to which they are attached can be (C₄-C₆) spiroalkyl or spiroheterocycloalkyl.

8. The inhibitor of any one of claims 1-7 wherein Pep is terminated by a ZBG of formula (I) at one terminus and by -C(O)N(R⁶)(R⁷) at the other terminus.

9. The inhibitor of any one of claims 1-7 which is of the formula:

[ZBG]-C(R⁸)-C(O)N(H)-CH(R⁹)-C(O)NH(R¹⁰) or

[ZBG-C(R⁵)(R⁶)-C(O)N(H)-CH(R⁹)-C(O)NH(R¹⁰)

wherein R⁸, R⁹, and R¹⁰ correspond to R¹, R², and R³ respectively.

10. The inhibitor of any one of claims 1-9 where one of R¹, R², R³, or R⁴ is (C₁-C₃)alkyl.

11. The inhibitor of any one of claims 1-10 wherein one of R¹, R², or R³ is H.

12. The inhibitor of any one of claims 1-11 wherein R^4 is (C_1-C_3) alkyl, benzyl, t-Boc, (C_3-C_6) cycloalkyl (C_1-C_3) alkyl, or H.

13. The inhibitor of any one of claims 1-4 wherein R^1 , R^2 , R^3 , and R^4 comprise one or two aryl groups that are optionally substituted with one to four R^1 .

14. The metalloprotein inhibitor of claim 1 wherein the organic radical is selected from the group consisting of halo, loweralkyl, sulfonamido, amino, NO_2 , and CN radicals.

15. A therapeutic method of treating a mammal threatened or afflicted with pathology associated with elevated metalloprotein activity comprising administering to said mammal an effective amount of the inhibitor of any one of claims 1-14.

16. The method of claim 15 wherein the pathology is MMP activity or histone deacetylase activity.

17. The method of claim 15 wherein the inhibitor is administered orally.

18. The method of claim 15 wherein the inhibitor is administered parenterally.

19. The method of claim 15 wherein the pathology is cancer, anthrax pathogenesis associated with anthrax lethal factor, or a pathology comprising inflammation.

20. The method of claim 15 wherein the pathology is myocardial infarction.

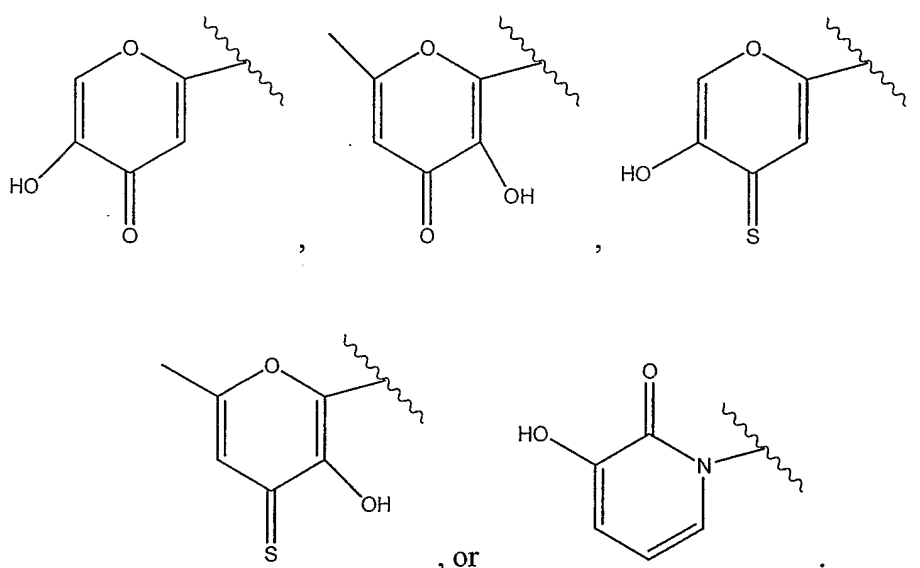
21. A method of inhibiting metalloprotein activity in a mammal in need of therapy for a pathological disease, condition, or symptom associated with metalloprotein activity, which comprises administering an effective amount of the inhibitor of any one of claims 1-14.

22. The method of claim 21 wherein the metalloprotein activity is a matrix metalloproteinase activity or histone deacetylase activity.

23. A composition comprising an inhibitor of any one of claims 1-14 in combination with a solid or liquid carrier or diluent.

24. The inhibitor of any one of claims 1-3 wherein one of R^3 or R^4 is $[[C_6-C_{10}aryl]_q-[O]_q-[(C_1-C_6)alkyl]_q-[C(O)]_q-[N(R)]-[C(O)]_q-[(C_1-C_6)alkyl]_q-]$ wherein q is 0-1 and R is H, $(C_1-C_4)alkyl$, phenyl, or benzyl.

25. The inhibitor of claim 24 wherein the ZBG is



26. The inhibitor of any one of claims 1-3 wherein R^3 and R^4 are side chains that are individually biphenylcarbamyl, biphenylcarbamyl $(C_1-C_6)alkyl$, biphenyl $(C_1-C_6)alkylcarbamyl$, biphenyl $(C_1-C_6)alkylcarbamyl(C_1-C_6)alkyl$, biphenyl $(C_1-C_6)alkylcarbamyl(C_1-C_6)alkyl$, phenoxyphenylcarbamyl, $(C_6-C_{10})aryl(C_1-C_6)alkylamino(C_1-C_6)alkyl$, biphenyl $(C_1-C_6)alkylamino(C_1-C_6)alkyl$, $(C_6-C_{10})arylcarbonylamino(C_1-C_6)alkyl$, $(C_6-C_{10})aryl(C_1-C_6)alkylcarbonylamino(C_1-C_6)alkyl$, biphenyloxy $(C_1-C_6)alkylcarbonylamino(C_1-C_6)alkyl$, or phenoxyphenylcarbamyl $(C_1-C_6)alkyl$, wherein, the phenyl or aryl group(s) in the side chains may be optionally

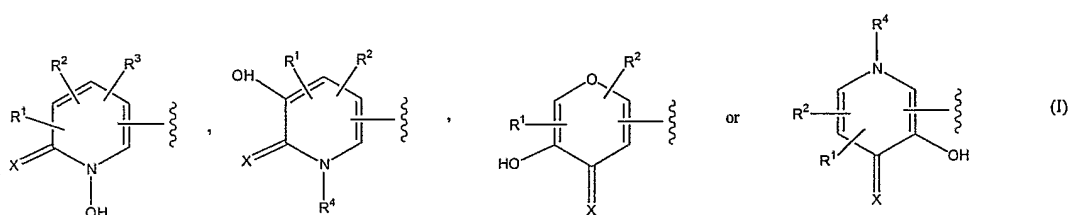
substituted with one to four R¹.

27. The inhibitor of claim 26 wherein the side chains are selected from the group consisting of biphenylmethylcarbamyl, phenoxyphenylcarbamyl, biphenylcarbamyl, benzylaminomethyl, phenethylaminomethyl, benzoylaminomethyl, benzylcarbonylaminomethyl, phenethylcarbonylaminomethyl, phenylpropylcarbonylaminomethyl, biphenylmethylcarbamylmethyl, phenoxyphenylcarbamylmethyl, biphenylcarbamylmethyl, and biphenyloxyethylcarbonylaminomethyl, wherein the phenyl group(s) in the side chains may be optionally substituted with one to four R¹.

28. A composition comprising an inhibitor of claim 26 in combination with a solid or liquid carrier or diluent.

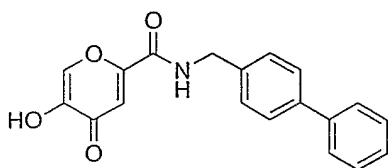
29. A therapeutic method of treating a mammal threatened or afflicted with pathology associated with elevated metalloprotein activity comprising administering to said mammal an effective amount of the inhibitor of claim 26.

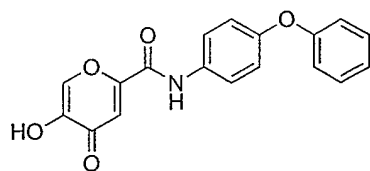
30. A composition comprising a compound of formula I



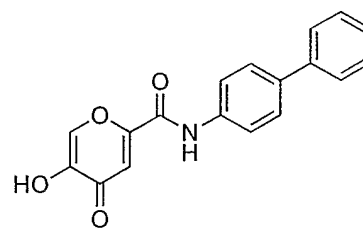
in combination with a pharmaceutically acceptable carrier, wherein the wavy line, R¹, R², R³, and R⁴ are as defined in claims 1, 4, or 26.

31. A metalloprotein inhibitor selected from the group consisting of

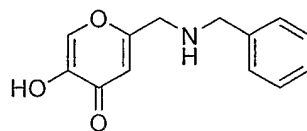




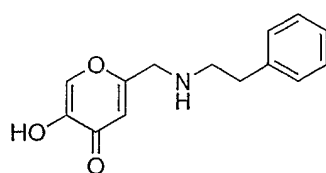
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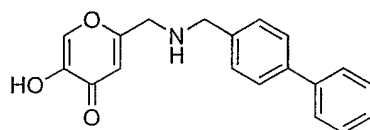
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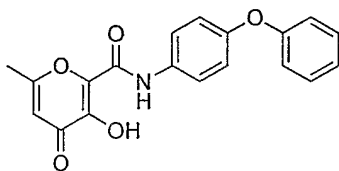
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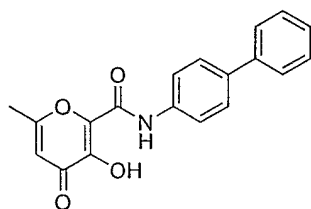


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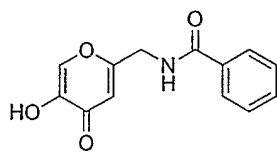
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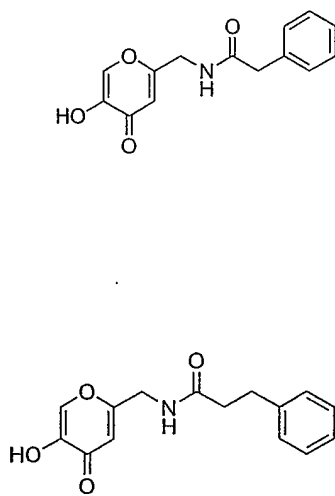
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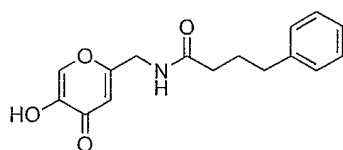
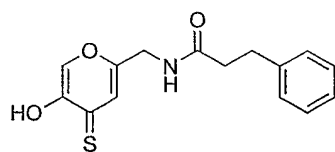


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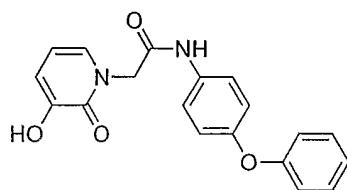
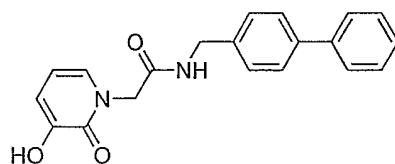
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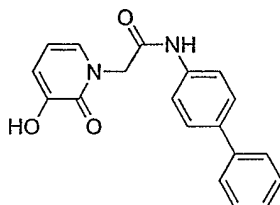
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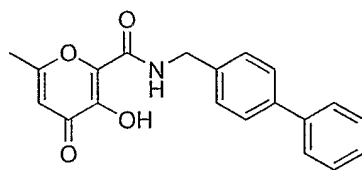
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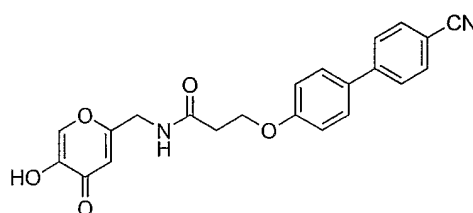
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, and



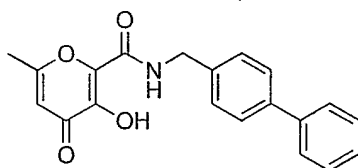
5

32. A composition comprising an inhibitor of claim 31 in combination with a solid or liquid carrier or diluent.

10 33. A method of inhibiting metalloprotein activity in a mammal in need of therapy for a pathological disease, condition, or symptom associated with metalloprotein activity, which comprises administering an effective amount of an inhibitor of claim 31.

34. The inhibitor of claim 1 having the formula:

15



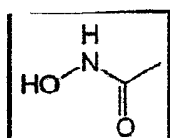
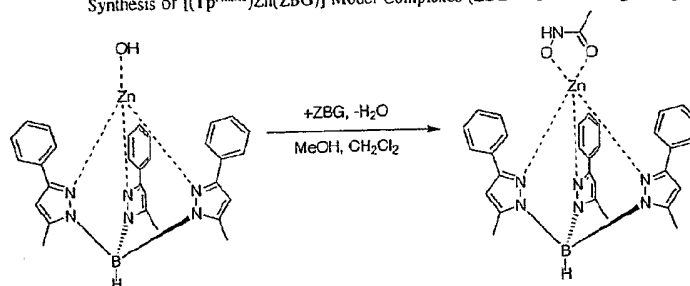
35. A composition comprising an inhibitor of claim 34 in combination with a solid or liquid carrier or diluent.

20

36. A method of inhibiting metalloprotein activity in a mammal in need of therapy for a pathological disease, condition, or symptom associated with metalloprotein activity, which

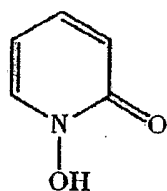
comprises administering an effective amount of the inhibitor of claim 34.

FIGURE 1.

Synthesis of $[(Tp^{Ph,Me})Zn(ZBG)]$ Model Complexes (ZBG = Zinc-Binding Group)

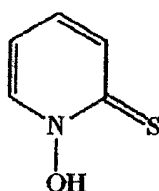
Acetohydroxamic
Acid (AHA) models
hydroxamate MPis

Novel ZBGs



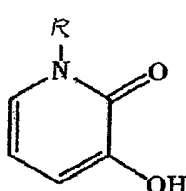
1,2-HOPO

1



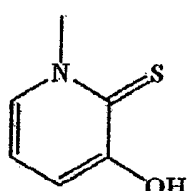
1,2-HOPTO

2



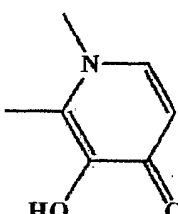
3,2-HOPO

3 R = Me
4 R = H



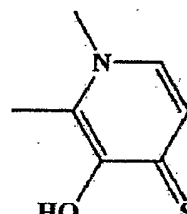
3,2-HOPTO

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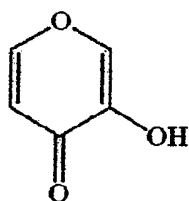
3,4-HOPO

3

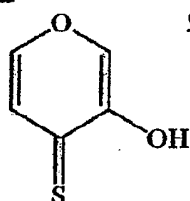


3,4-HOPTO

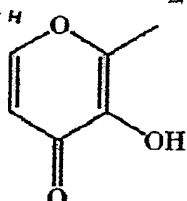
7

Pyromeconic
Acid

4

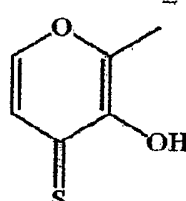
Thiopyromeconic
Acid

10



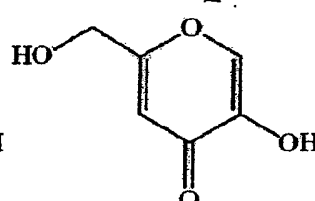
Maltol

5



Thiomaltol

11



Kojic Acid

12

FIGURE 2.

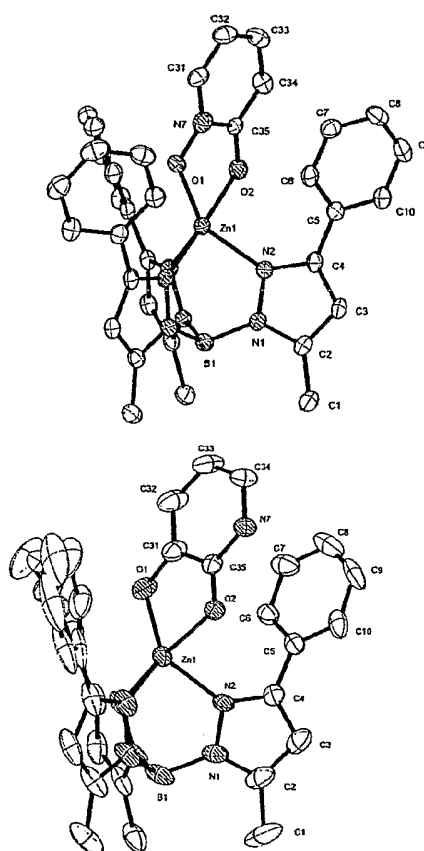


FIGURE 3.

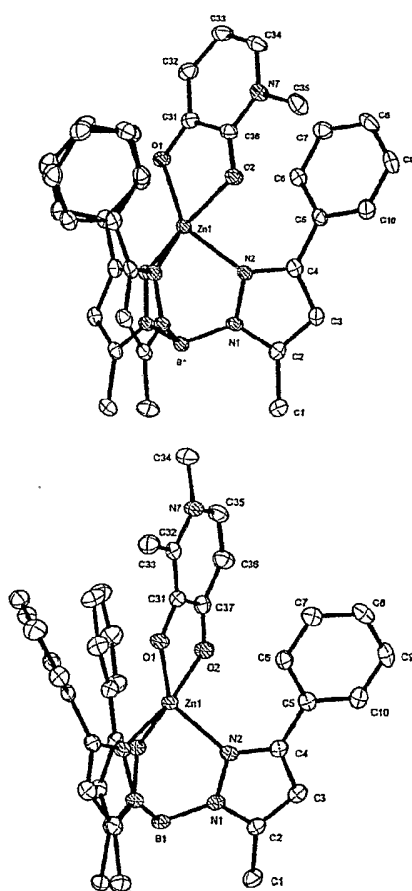


Figure 4.

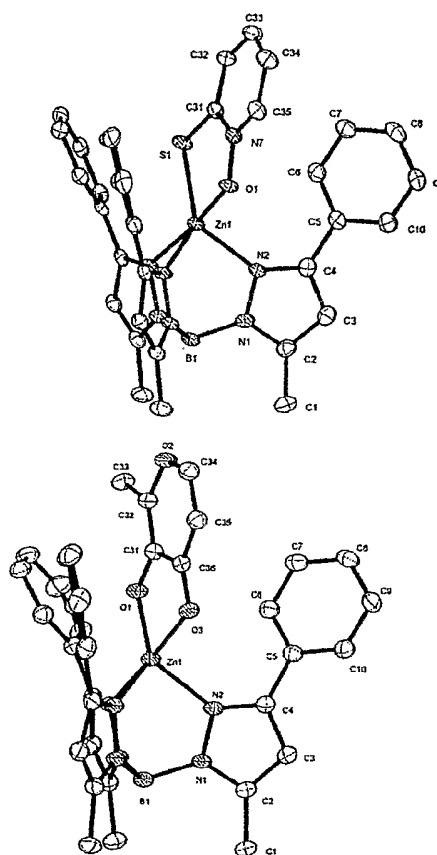


FIGURE 5

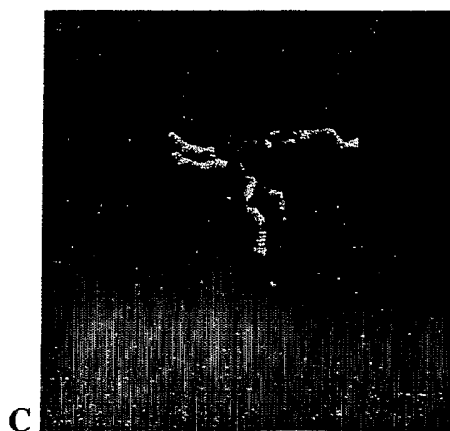
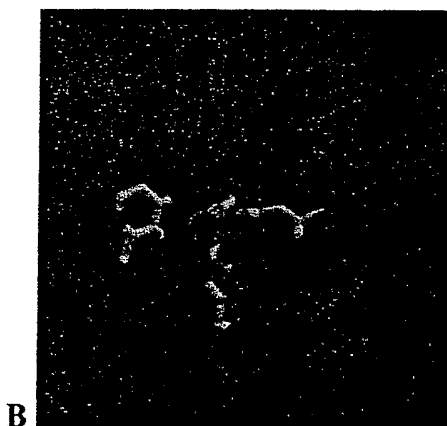
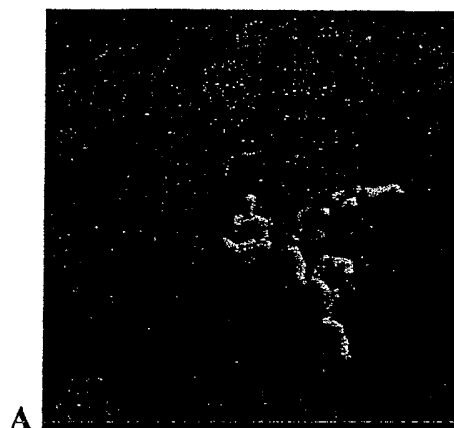


FIGURE 6.

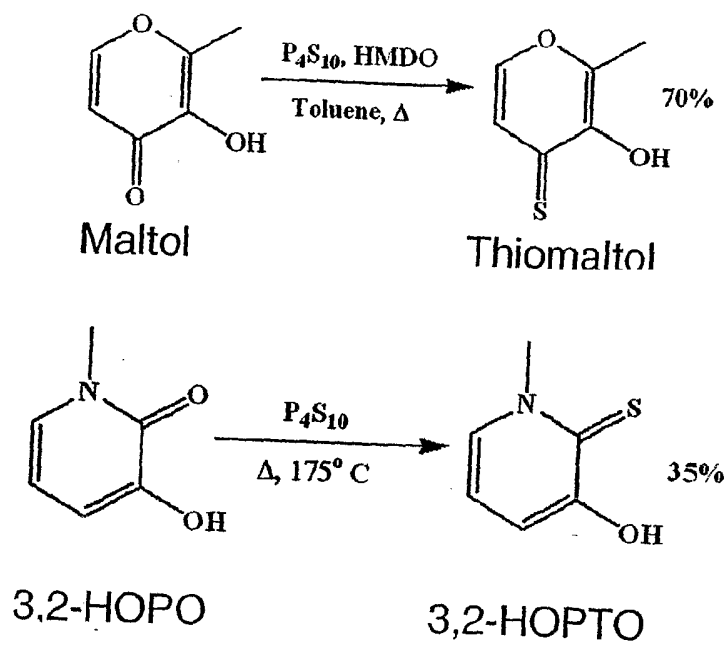


Figure 7.

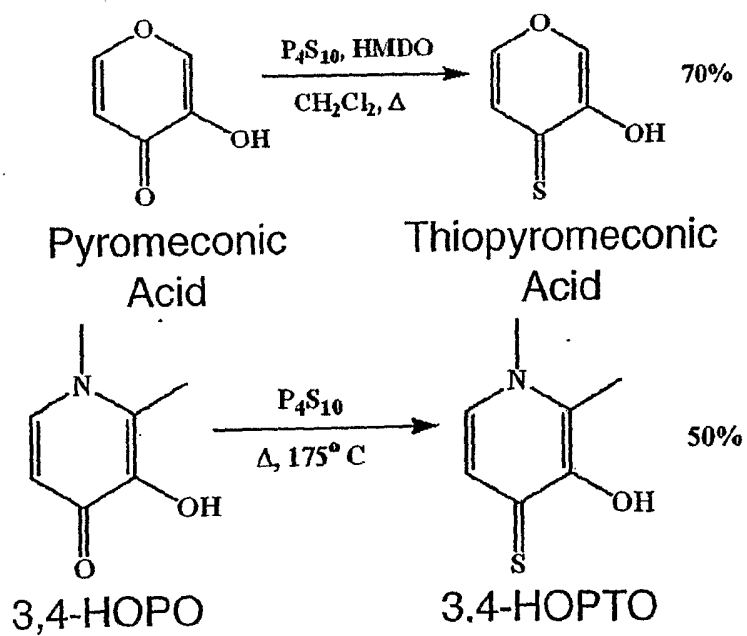


Fig. 8

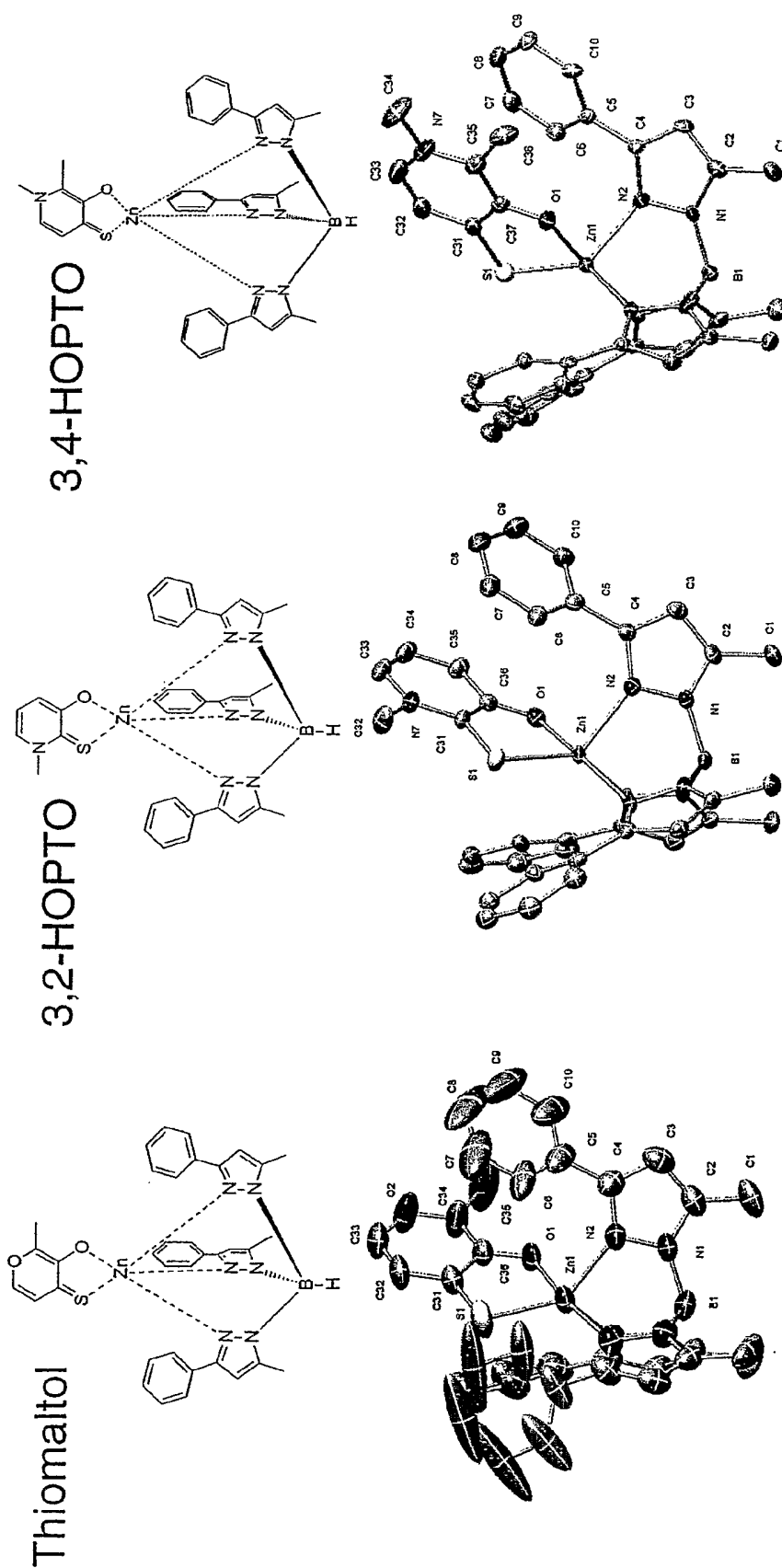
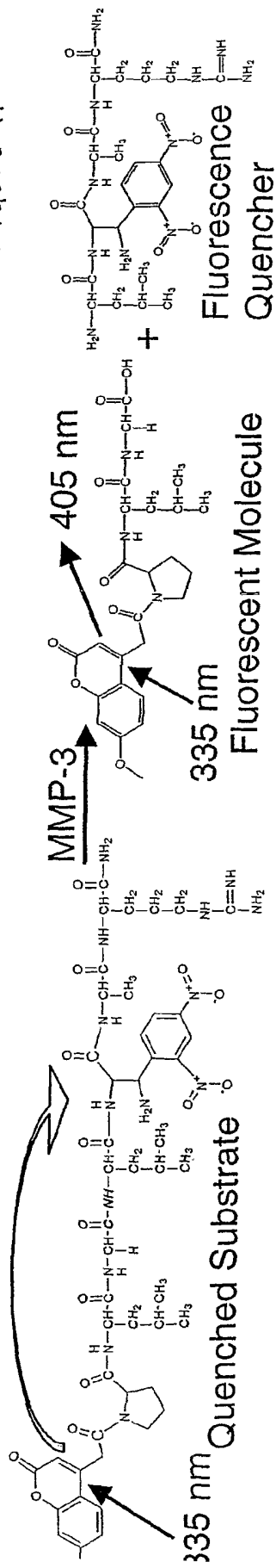


Figure 9.



ZBG	Fluorescence IC ₅₀ (μM) ^{a,b}	Colorimetric IC ₅₀ (μM) ^a	Potency AHA ^c	v.
AHA	25100 (±4000)	—	n/a	
1	1600 (±100)	1500 (±10)	16-fold	
2	5100 (±200)	—	4.9-fold	
4	7200 (±1200)	8300 (±900)	3.5-fold	
5	5700 (±100)	16000 (±2000)	4.4-fold	
6	5700 (±200)	5000 (±1000)	4.4-fold	
7	35 (±3)	20 (±4)	717-fold	
8	362 (±3)	—	69-fold	
9	137 (±20)	—	183-fold	
10	118 (±40)	—	213-fold	
11	210 (±20)	—	120-fold	

^a Obtained from at least three independent experiments; ^b Corrected for fluorescence quenching (see Supporting Information); ^c Based on IC₅₀ value from fluorescence assay

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FIGURE 10.

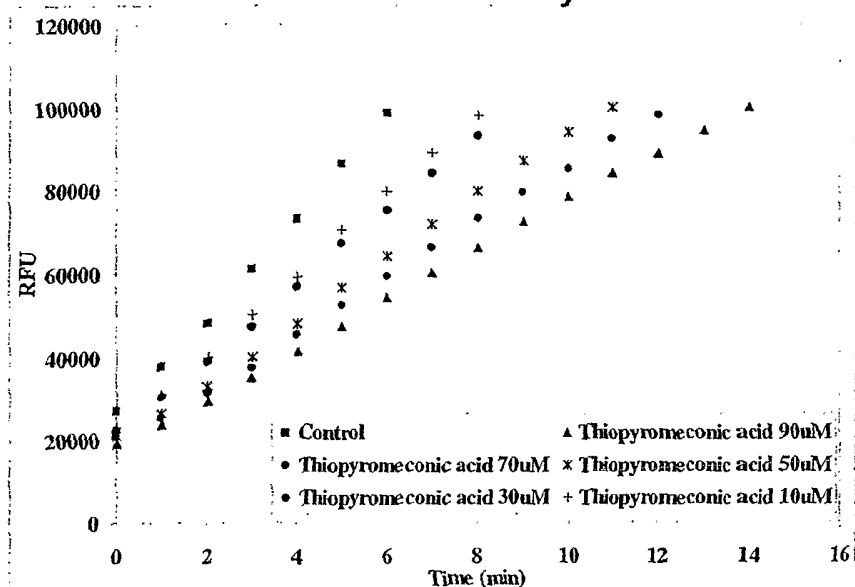
Raw Data Set for a Thiopyromeconic
Acid MMP Assay Trial

FIGURE 11.

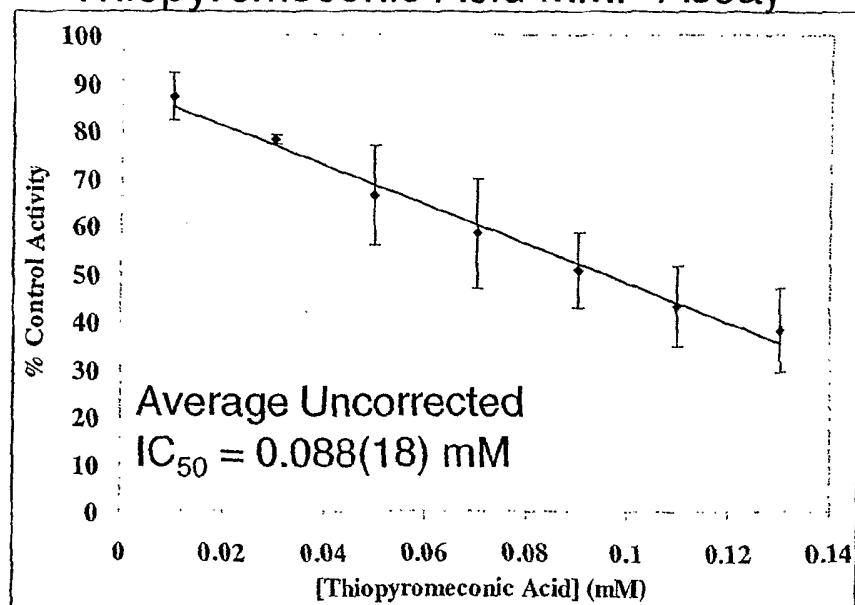
Averaged Uncorrected Data for
Thiopyromeconic Acid MMP Assay

FIGURE 12.

Fluorescence Control Experiments for Thiopyromeconic Acid MMP Assays

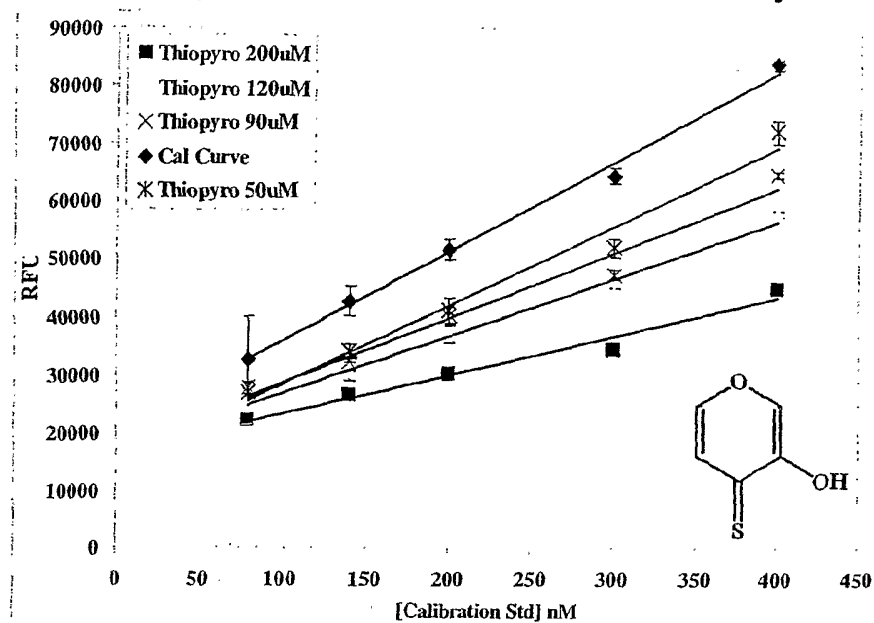


FIGURE 13.

Fluorescence Correction for Thiopyromeconic Acid

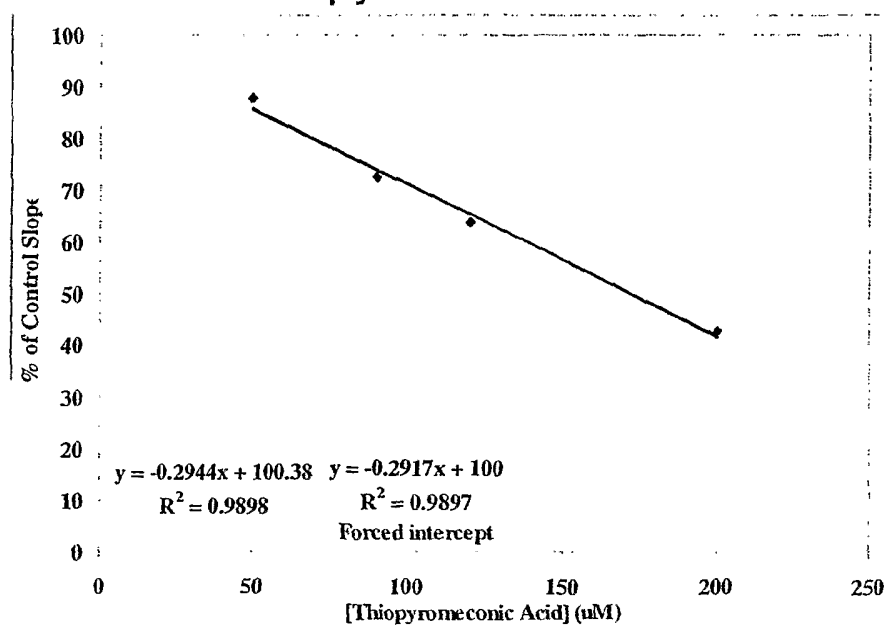


FIGURE 14.

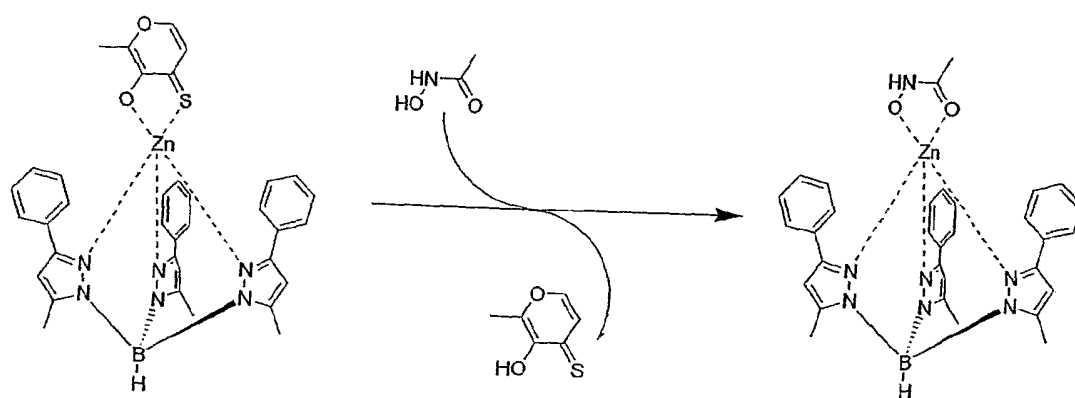
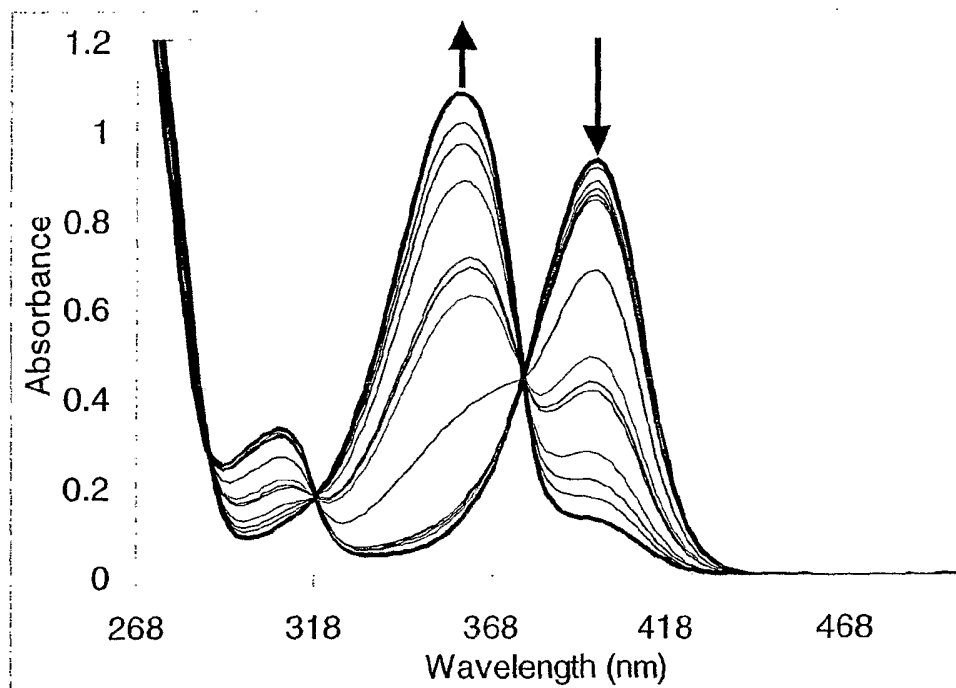


FIGURE 15.

UV-Visible Absorbance Spectra of Titration of
[(Tp^{Ph,Me})Zn(Thiomaltolato)] with AHA



Absorbance Plot to Determine
Relative Binding Affinities

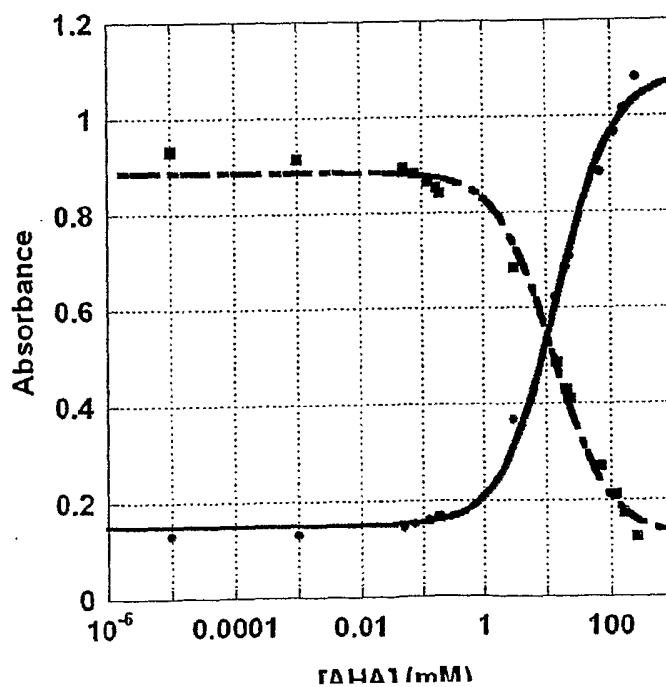


FIG. 16

FIGURE 17.

Relative binding affinities of several novel ZBGs for a tris(pyrazolyl)borate model complex.

$[(Tp^{Ph,Me})Zn(ZBG)]$	K_{app}^a	Affinity v. AHA
2	0.32($\pm$ 0.01)	3.1-fold
3	0.54($\pm$ 0.06)	1.9-fold

5	0.46($\pm$ 0.16)	2.2-fold
8	0.072($\pm$ 0.010)	14-fold
9	0.078($\pm$ 0.006)	13-fold
11	0.067($\pm$ 0.009)	15-fold

^a Obtained from at least two independent experiments

Figure 18.

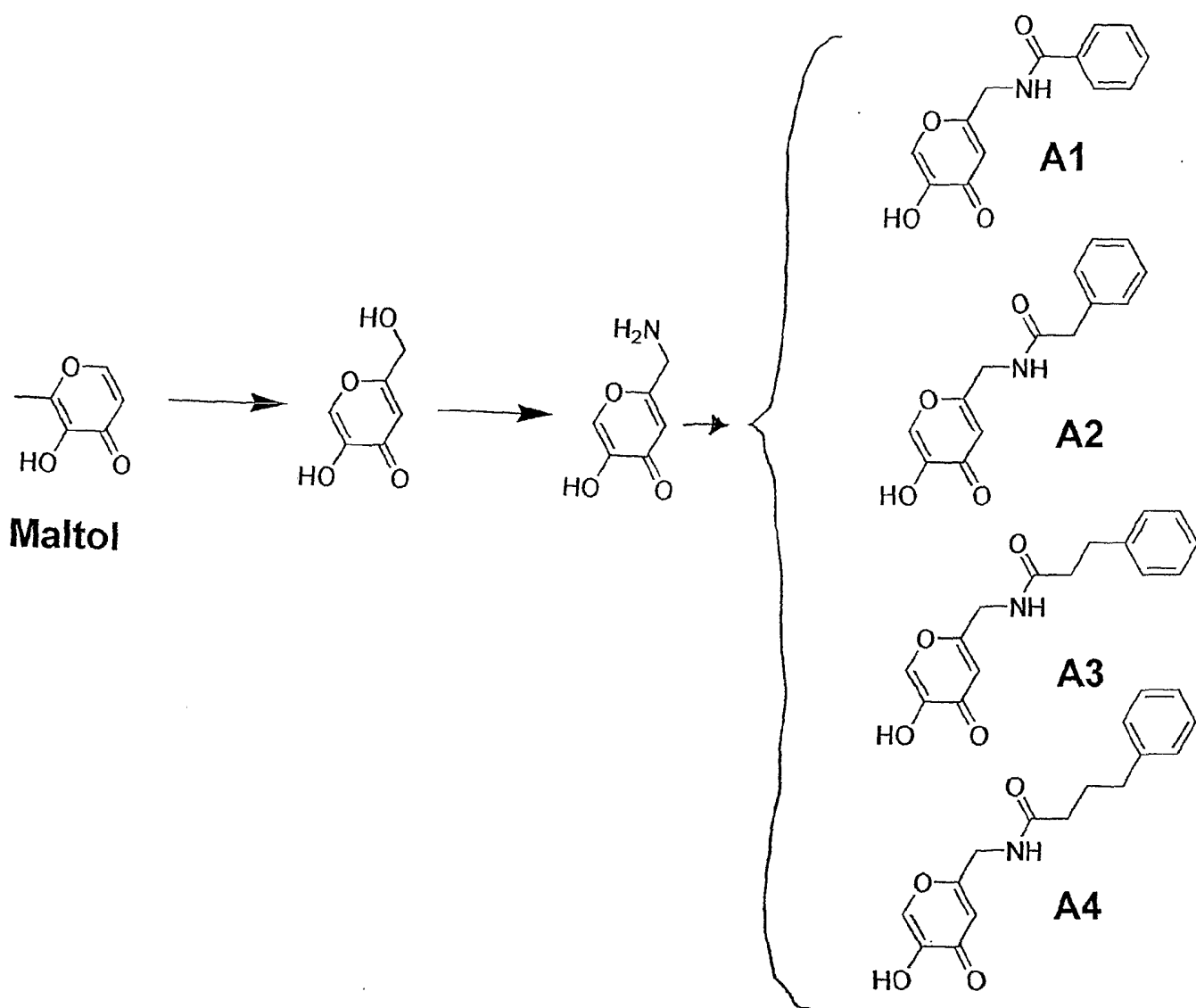
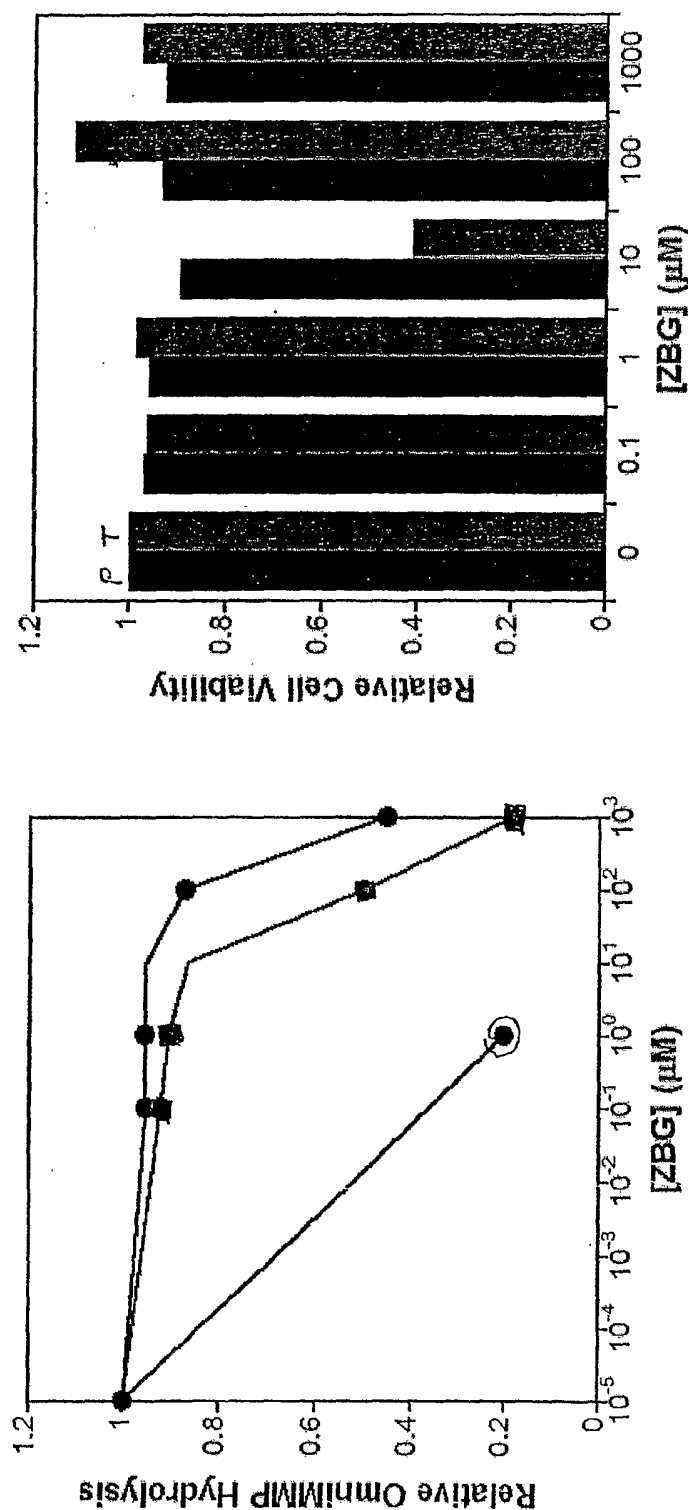


Fig. 19

Fibroblast Studies



- Neonatal rat ventricular fibroblasts (NRVFs) were activated with 60 μg/mL bovine plasminogen for 24 h.
- Assays show the O,S ligand thiomaltol (■, τ) is more potent than the O,O ligand pyromeconic acid (●, p^-) – a control with hydroxamate GM6001 (○, ϕ) is shown.
- Toxicity of these compounds appears to be low (MTT assay).