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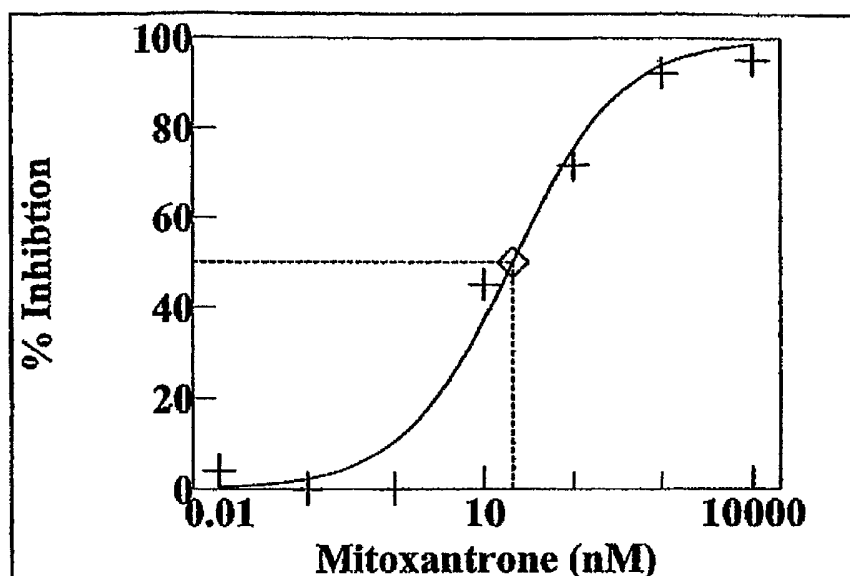
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(54) Title: DRUG DELIVERY FROM RAPID GELLING POLYMER COMPOSITION



(57) Abstract: Compositions are disclosed that afford drug delivery from two-part polymer compositions that rapidly form covalent linkages when mixed together. Such compositions are particularly well suited for use in a variety of tissue related applications when rapid adhesion to the tissue and gel formation is desired along with drug delivery. For example, the compositions are useful as tissue sealants, in promoting hemostasis, in effecting tissue adhesion, in providing tissue augmentation, and in the prevention of surgical adhesions.



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DRUG DELIVERY FROM RAPID GELLING POLYMER COMPOSITION

BACKGROUND OF THE INVENTION

Field of the Invention

This invention relates generally to compositions that afford drug
5 delivery from two-part polymer compositions that rapidly form covalent linkages
when mixed together. Such compositions are particularly well suited for use in
a variety of tissue related applications when rapid adhesion to the tissue and
gel formation is desired along with drug delivery. For example, the
compositions are useful as tissue sealants, in promoting hemostasis, in
10 effecting tissue adhesion, in providing tissue augmentation, and in the
prevention of surgical adhesions.

Description of the Related Art

The use of polymer compositions in tissue engineering is now
widely recognized, particularly those consisting of synthetic polymers. In
15 contrast to many naturally derived compositions, synthetic polymer
compositions can be formulated to exhibit predetermined physical
characteristics such as gel strength, as well as biological characteristics such
as degradability.

In a variety of tissue engineering applications, it is desirable to
20 use compositions that can be administered as liquids, but subsequently form
hydrogels at the site of administration. Such in situ hydrogel forming
compositions are more convenient to use since they can be administered as
liquids from a variety of different devices, and are more adaptable for
administration to any site, since they are not preformed. Many different
25 mechanisms have been described that can be used to promote hydrogel
formation in situ. For example, photoactivatable mixtures of water-soluble co-
polyester prepolymers and polyethylene glycol have been described to create
hydrogel barriers, as well as drug release matrices. In another approach, block

copolymers of polyalkylene oxide polymers (e.g., PLURONIC compounds from BASF Corporation, Mount Olive, NJ) and poloxamers have been designed that are soluble in cold water, but form insoluble hydrogels that adhere to tissues at body temperature (Leach, et al., Am. J. Obstet. Gynecol. 162:1317-1319 (1990)). Polymerizable cyanoacrylates have also been described for use as tissue adhesives (Ellis, et al., J. Otolaryngol. 19:68-72 (1990)). In yet another approach, two-part synthetic polymer compositions have been described that, when mixed together, form covalent bonds with one another, as well as with exposed tissue surfaces. (PCT WO 97/22371, which corresponds to U.S. application Ser. No. 08/769,806 U.S. Pat. No. 5,874,500.) In a similar approach involving a two-part composition, a mixture of protein and a bifunctional crosslinking agent has been described for use as a tissue adhesive (U.S. Pat. No. 5,583,114.)

One difficulty encountered when designing in situ hydrogel forming compositions is that optimizing the composition to enhance gel formation may worsen tissue inflammation at the site of administration. A possible explanation for this effect is that highly reactive composition components that are capable of rapid gel formation may adversely affect tissue surfaces.

The compositions of the present invention have been formulated to provide for rapid gelation, and also cause less tissue inflammation at the site of administration than previously described compositions.

BRIEF SUMMARY OF THE INVENTION

Briefly stated, the present invention provides compositions and methods for drug delivery, including precursors to said compositions.

For example, in one aspect, the present invention provides a biocompatible gel-forming drug-delivering composition for *in vivo* administration, comprising:

a drug;

a first component comprising at least one sulfhydryl group-containing compound in a liquid medium having an alkaline pH, wherein said sulfhydryl group-containing compound is given by the formula Compound₁ - (SH)_m, wherein $m \geq 2$; and

5 a second component comprising at least one sulfhydryl reactive group-containing compound in either a liquid medium having a neutral or acidic pH or in powder form, wherein said sulfhydryl reactive group-containing compound is given by the formula Compound₂ -Y_n, wherein Y is a sulfhydryl reactive group and wherein $n \geq 2$;

10 wherein at least one of the first or second components is a polyalkylene oxide and wherein the sulfhydryl groups and the sulfhydryl reactive groups react with one another to form covalent bonds therebetween when said components are mixed together. Preferably, the covalent bonds form a gel in less than one minute after mixing.

15 The invention also provides a method for treating tissues, comprising the steps of:

administering to a tissue site a first component comprising at least one sulfhydryl group-containing compound in liquid medium having an alkaline pH, wherein said sulfhydryl group-containing compound is given by the formula
20 Compound₁ -(SH)_m, wherein $m \geq 2$; and

simultaneously or subsequently administering to the tissue site a second component comprising at least one sulfhydryl reactive group-containing compound either a liquid medium having a neutral or acidic pH or in powder form, wherein said sulfhydryl reactive group-containing compound is given by
25 the formula Compound₂ -Y_n, wherein Y is a sulfhydryl reactive group and wherein $n \geq 2$, and wherein at least one of the first or second components is a polyalkylene oxide; and

simultaneously or subsequently administering to the tissue site a drug; and

allowing the sulfhydryl groups and the sulfhydryl reactive groups to react with one another to form covalent bonds therebetween to form a gel in less than one minute.

In another aspect, the invention provides a biocompatible gel-forming drug-delivering composition for *in vivo* administration with a gel time of less than one minute, comprising:

polyalkylene oxide-(SH)₄ and drug in a liquid medium having a pH of between 8 and 10.5; and

polyalkylene oxide-Y₄, wherein Y is succinimidyl, in a liquid medium having an acidic pH.

In another aspect, the invention provides a biocompatible gel-forming drug-delivering composition for *in vivo* administration with a gel time of less than one minute, comprising:

polyalkylene oxide-(SH)₁₂ and drug in a liquid medium having an alkaline pH; and

polyalkylene oxide-Y₁₂ in a liquid medium having an acidic pH, wherein Y is a succinimidyl or maleimidyl group.

In another aspect, the invention provides a biocompatible gel-forming composition for *in vivo* administration, comprising:

a sulfhydryl group-containing polyalkylene oxide in a liquid medium having an acidic pH, wherein said sulfhydryl group-containing polyalkylene oxide is given by the formula Core-(SH)_m, wherein $m \geq 2$;

a buffer solution with an alkaline pH; and

drug in admixture with the polyalkylene oxide and/or the buffer solution;

wherein the sulfhydryl groups react with one another to form covalent bonds therebetween when said components are mixed together to form a gel in less than one minute.

In another aspect, the present invention provides a biocompatible gel-forming drug-delivering composition for *in vivo* administration, comprising:

at least one sulfhydryl group-containing compound in a liquid medium having an alkaline pH, wherein said sulfhydryl group-containing compound is given by the formula Compound₁ -(SH)_m, wherein $m \geq 2$;

at least one sulfhydryl reactive group-containing compound either
5 a liquid medium having a neutral or acidic pH or in powder form, wherein said sulfhydryl reactive group-containing compound is given by the formula Compound₂ -Y_n, wherein Y is a sulfhydryl reactive group and wherein $n \geq 2$;

at least one drug in admixture with either or both of the at least one sulfhydryl group-containing compound and the at least one sulfhydryl
10 reactive group-containing compound; and
collagen;

wherein at least one of either the sulfhydryl group-containing compound or the sulfhydryl reactive group-containing compound is a polyalkylene oxide, and wherein the sulfhydryl groups and the sulfhydryl
15 reactive groups are capable of reacting with one another to form covalent bonds therebetween.

In another aspect, the present invention provides a biocompatible gel-forming drug-delivering composition for *in vivo* administration, comprising:

(a) a first component in a liquid medium having an acidic pH
20 comprising:

(i) at least one sulfhydryl group-containing compound given by the formula Compound₁ -(SH)_m, wherein $m \geq 2$;

(ii) at least one sulfhydryl reactive group-containing compound given by the formula Compound₂ -Y_n, wherein Y is a sulfhydryl reactive group
25 and wherein $n \geq 2$; and

(iii) collagen; and

(b) a second component comprising a buffer having a pH of between 8 and 10.5;

wherein a drug is present in admixture with either or both of the
30 first component or the second component; and

wherein at least one of either the sulfhydryl group containing compound or the sulfhydryl reactive group containing compound is a polyalkylene oxide.

Optionally, in each of these and other aspects of the invention as disclosed herein, the drug is a hydrophobic drug in admixture with a secondary carrier to provide drug/carrier, the drug/carrier being in admixture with either or both of the at least one sulfhydryl group-containing compound and the at least one sulfhydryl reactive group-containing compound.

Furthermore, the present invention provides various methods that are useful in preparing drug-containing delivery vehicles. For example, in one aspect the invention provides a method for forming a drug delivery composition, comprising

a) selecting a first component, a second component and a drug, wherein

the first component comprises at least one sulfhydryl group-containing compound in a liquid medium having an alkaline pH, wherein said sulfhydryl group-containing compound is given by the formula Compound₁-(SH)_m, wherein $m \geq 2$; and

the second component comprises at least one sulfhydryl reactive group-containing compound in either a liquid medium having a neutral or acidic pH or in powder form, wherein said sulfhydryl reactive group-containing compound is given by the formula Compound₂-Y_n, wherein Y is a sulfhydryl reactive group and wherein $n \geq 2$;

at least one of the first or second components is a polyalkylene oxide;

the sulfhydryl groups and the sulfhydryl reactive groups react with one another to form covalent bonds therebetween when said components are mixed together to form a gel in less than one minute;

b) combining the first and second components in the presence of the drug, under conditions where the first component reacts with

the second component. The invention also provides a product produced by this method.

In another aspect, the invention provides a method for forming a drug delivery composition, comprising

- 5 a) forming an admixture of polyalkylene oxide-(SH)₄ and drug in a liquid medium having a pH of between 8 and 10.5; and
- b) forming an admixture of polyalkylene oxide-Y₄, wherein Y is succinimidyl and liquid medium, the admixture having an acidic pH. The invention may further include the step of combining the admixtures of steps a)
- 10 and b), and in addition the invention provides the product produced by this method.

In another aspect, the invention provides a method for forming a biocompatible gel-forming drug-delivering composition for *in vivo* administration, preferably having a gel time of less than one minute, comprising:

- 15 a) preparing an admixture of polyalkylene oxide-(SH)₁₂ and drug in a liquid medium having an alkaline pH; and
- b) preparing polyalkylene oxide-Y₁₂ in a liquid medium having an acidic pH, wherein Y is a succinimidyl or maleimidyl group. In one aspect, this method further includes the step of combining a) and b), while in a related
- 20 aspect the invention provides the product produced by this method.

In another aspect, the present invention provides a method for forming a biocompatible gel-forming composition for *in vivo* administration, the method comprising:

- a) preparing a sulfhydryl group-containing polyalkylene oxide
- 25 in a liquid medium having an acidic pH, wherein said sulfhydryl group-containing polyalkylene oxide is given by the formula Core-(SH)_m, wherein $m \geq 2$;
- b) providing a buffer solution with an alkaline pH; and
- c) adding drug to either or both of a) and b);
- wherein the sulfhydryl groups react with one another to form
- 30 covalent bonds therebetween when said components are mixed together to form a gel in less than one minute. Optionally, the method includes combining

a) and b), while in a related aspect the invention provides the product produced by this method.

In another aspect, the present invention provides a method for forming a biocompatible gel-forming drug-delivering composition for *in vivo*

5 administration, comprising:

a) providing an at least one sulfhydryl group-containing compound in a liquid medium having an alkaline pH, wherein said sulfhydryl group-containing compound is given by the formula Compound₁ -(SH)_m, wherein $m \geq 2$;

10 b) providing an at least one sulfhydryl reactive group-containing compound either in a liquid medium having a neutral or acidic pH or in powder form, wherein said sulfhydryl reactive group-containing compound is given by the formula Compound₂ -Y_n, wherein Y is a sulfhydryl reactive group and wherein $n \geq 2$;

15 c) combining a drug with either or both of the at least one sulfhydryl group-containing compound and the at least one sulfhydryl reactive group-containing compound; and

d) providing collagen;

20 wherein at least one of either the sulfhydryl group-containing compound or the sulfhydryl reactive group-containing compound is a polyalkylene oxide; and

wherein the sulfhydryl groups and the sulfhydryl reactive groups are capable of reacting with one another to form covalent bonds therebetween.

Optionally, the method includes the step of combining a), b) and d), and in a

25 related aspect the invention provides the product produced by this method.

A variety of drugs may be included in the compositions of the present invention, and used in the methods of the present invention. These drugs are set forth in detail below. The following are specific aspects of the present invention, which are exemplary only: in one aspect, the compositions and methods of the invention employ (*i.e.*, include in a composition, or use in a

30 method) a cell cycle inhibitor; in one aspect, the compositions and methods of

the invention employ paclitaxel; in one aspect, the compositions and methods of the invention employ doxorubicin; in one aspect, the compositions and methods of the invention employ mitoxantrone; in one aspect, the compositions and methods of the invention employ podophyllotoxin (e.g., etoposide); in one
5 aspect, the compositions and methods of the invention employ an immunomodulatory agents; in one aspect, the compositions and methods of the invention employ rapamycin; in one aspect, the compositions and methods of the invention employ everolimus; in one aspect, the compositions and methods of the invention employ tacrolimus; in one aspect, the compositions and
10 methods of the invention employ biolimus; in one aspect, the compositions and methods of the invention employ a heat shock protein 90 antagonist; in one aspect, the compositions and methods of the invention employ geldanamycin; in one aspect, the compositions and methods of the invention employ a HMG CoA Reductase inhibitor; in one aspect, the compositions and methods of the
15 invention employ simvastatin; in one aspect, the compositions and methods of the invention employ an IMPDH Inhibitor; in one aspect, the compositions and methods of the invention employ mycophenolic acid; in one aspect, the compositions and methods of the invention employ 1-alpha-25 dihydroxy vitamin D3; in one aspect, the compositions and methods of the invention
20 employ an antimycotic agent; in one aspect, the compositions and methods of the invention employ sulconazole; in one aspect, the compositions and methods of the invention employ a P38 MAP kinase inhibitor; in one aspect, the compositions and methods of the invention employ SB220025; in one aspect, the compositions and method of the invention employ talcum powder; in one
25 aspect, the compositions and method of the invention employ metallic beryllium and oxides thereof; in one aspect, the compositions and method of the invention employ copper; in one aspect, the compositions and method of the invention employ silk; in one aspect, the compositions and method of the invention employ silica; in one aspect, the compositions and method of the
30 invention employ crystalline silicates; in one aspect, the compositions and method of the invention employ talc; in one aspect, the compositions and

method of the invention employ quartz dust; in one aspect, the compositions and method of the invention employ ethanol; in one aspect, the compositions and method of the invention employ a component of extracellular matrix; in one aspect, the compositions and method of the invention employ fibronectin; in one aspect, the compositions and method of the invention employ collagen; in one aspect, the compositions and method of the invention employ fibrin; in one aspect, the compositions and method of the invention employ fibrinogen; in one aspect, the compositions and method of the invention employ polylysine; in one aspect, the compositions and method of the invention employ poly(ethylene-co-vinylacetate); in one aspect, the compositions and method of the invention employ chitosan; in one aspect, the compositions and method of the invention employ N-carboxybutylchitosan; in one aspect, the compositions and method of the invention employ a RGD protein; in one aspect, the compositions and method of the invention employ vinyl chloride; in one aspect, the compositions and method of the invention employ a polymer formed from vinyl chloride; in one aspect, the compositions and method of the invention employ a cyanoacrylate adhesive; in one aspect, the compositions and method of the invention employ an adhesive comprising crosslinked poly(ethylene glycol) derived material and methylated collagen; in one aspect, the compositions and method of the invention employ an inflammatory cytokine; in one aspect, the compositions and method of the invention employ an inflammatory cytokine selected from the group consisting of TGF β , PDGF, VEGF, bFGF, TNF α , NGF, GM-CSF, IGF-a, IL-1, IL-8, IL-6, and growth hormone; in one aspect, the compositions and method of the invention employ a connective tissue growth factor (CTGF); in one aspect, the compositions and method of the invention employ a bone morphogenic protein (BMP); in one aspect, the compositions and method of the invention employ a BMP selected from BMP-2, BMP-3, BMP-4, BMP-5, BMP-6, or BMP-7; in one aspect, the compositions and method of the invention employ bleomycin; in one aspect, the compositions and method of the invention employ an analogue or derivative of bleomycin; in one aspect, the compositions and method of the invention employ a proliferative agent that

stimulates cellular proliferation; in one aspect, the compositions and method of the invention employ dexamethasone and analogues and derivatives thereof; in one aspect, the compositions and method of the invention employ isotretinoin and analogues and derivatives thereof; in one aspect, the compositions and method of the invention employ 17- β -estradiol and analogues and derivatives thereof; in one aspect, the compositions and method of the invention employ estradiol and analogues and derivatives thereof; in one aspect, the compositions and method of the invention employ diethylstilbestrol and analogues and derivatives thereof; in one aspect, the compositions and method of the invention employ cyclosporine A and analogues and derivatives thereof; in one aspect, the compositions and method of the invention employ All-trans retinoic acid (ATRA) and analogues and derivatives thereof. Additional drugs that may be employed in the present invention are set forth below.

These and other aspects of the present invention will become evident upon reference to the following detailed description. In addition, various references are set forth herein. Each of these references is incorporated herein by reference in its entirety as if each were individually noted for incorporation.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWING(S)

Figure 1 is a tetrafunctionally activated PEG succinimidyl glutarate (ester linkage) (SG-PEG).

Figure 2 depicts the structure of various sulfhydryl-reactive groups, with "R" representing the chemical structure to which the reactive group is attached.

Figure 3 is a schematic illustration showing sites of action within a biological pathway where Cell Cycle Inhibitors may act to inhibit the cell cycle.

Figure 4 depicts the rheometric measurements of gelation of a mixture of reactive tetrafunctional polyethylene glycols.

Figure 5 depicts the formation of two "12-arm" PEG compounds from "4-arm" intermediates.

Figure 6 is a graph showing % inhibition of human fibroblast cell proliferation as a function of Mitoxantrone concentration.

Figure 7 is a graph showing % inhibition of nitric oxide production in RAW 264.7 cells as a function of Mitoxantrone concentration.

5 Figure 8 is a graph showing % inhibition of $\text{TNF}\alpha$ production by THP-1 cells as a function of Bay 11-7082 concentration.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to drug delivery via a two-part polymer composition that forms a matrix when mixed together. Each
10 component of the composition is generally administered separately to the tissue site, and the drug may be delivered with either component, or may be delivered separately. Then, within a very short time after being mixed together at the site of administration, the composition forms a gel with sufficient adhesive and cohesive strength to become anchored in place, and allow delivery of the drug
15 to this location.

The components can be mixed prior to application to the tissue with the drug being mixed with the components prior to gelation or added after gelation has occurred.

Definitions

20 The following definitions are provided to further describe various aspects of the preferred embodiments of the present invention.

The term "gel" refers to the state of matter between liquid and solid. As such, a "gel" has some of the properties of a liquid (*i.e.*, the shape is resilient and deformable) and some of the properties of a solid (*i.e.*, the shape
25 is discrete enough to maintain three dimensions on a two dimensional surface.) Accordingly, "gelation time", also referred to herein as "gel time", refers to the time it takes for a composition to become non-flowable under modest stress. This is generally exhibited as achieving a gel strength, G' , of greater than or equal to 10^2 dynes/cm² in less than 1 minute.

The term "cohesive strength" refers to the ability of the compositions of the present invention to remain intact, *i.e.*, not rupture, tear or crack, when subjected to physical stresses or environmental conditions. Cohesive strength is sometimes measured as a function of "burst strength".

5 The term "adhesive strength" refers to the ability of the compositions of the present invention to be able to remain attached to the tissues at the site of administration when subjected to physical stresses or environmental conditions.

10 The term "polymer" refers to a molecule consisting of individual chemical moieties, which may be the same or different, but are preferably the same, that are joined together. As used herein, the term "polymer" refers to individual chemical moieties that are joined end-to-end to form a linear molecule, as well as individual chemical moieties joined together in the form of a branched (*e.g.*, a "multi-arm" or "star-shaped") structure.

15 The term "biocompatible" refers to the ability of the compositions of the present invention to be applied to tissues without eliciting significant inflammation and fibrosis or other adverse tissue responses.

20 The term "synthetic polymer" refers to polymers that are not naturally occurring and that are produced by chemical or recombinant synthesis. As such, naturally occurring proteins such as collagen and naturally occurring polysaccharides such as hyaluronic acid are specifically excluded. Proteins such as synthetic collagen, and carbohydrates such as synthetic hyaluronic acid, and their derivatives, are included.

25 The term "activated synthetic polymers" refers to synthetic polymers that have or have been chemically modified to have at least one functional group (*e.g.*, a sulfhydryl group) that is capable of reacting with a corresponding reaction partner (*e.g.*, a sulfhydryl-reactive group) to form a covalent bond. The term "multifunctionally activated" refers to synthetic polymers having two or more nucleophilic or electrophilic groups. Types of
30 multifunctionally activated synthetic polymers include di-functionally activated,

tri-functionally activated, tetra-functionally activated, and star-shaped activated polymers (that have four or more functional groups).

"Fibrosis" or "Scarring" refers to the formation of fibrous tissue in response to injury or medical intervention. Fibrosis or scarring is defined to

5 involve biological processes which include an increase in one or more of the following: inflammation including production and release of cytokines and/or chemokines, angiogenesis, cellular proliferation (typically fibroblasts and/or smooth muscle cells), cell migration, ECM (extracellular matrix) production, tissue remodeling and cell adhesion.

10 Therapeutic agents which inhibit fibrosis or scarring can do so through one or more mechanisms including: inhibiting inflammatory processes such as production of cytokines and chemokines, inhibiting angiogenesis, inhibiting migration or proliferation of connective tissue cells (such as fibroblasts, and smooth muscle cells), reducing ECM production and/or

15 inhibiting tissue remodeling. In addition, numerous therapeutic agents described in this invention will have the additional benefit of also reducing tissue regeneration (the replacement of injured cells by cells of the same type) when appropriate. An agent that modulates any of these events is referred to herein as an anti-scarring or a fibrosis-inhibiting agent.

20 Therapeutic agents which increase fibrosis or scarring can do so through an increase in one or more of the following processes: inflammation including production and release of cytokines and/or chemokines, angiogenesis, cellular proliferation (typically fibroblasts and/or smooth muscle cells), cell migration, ECM (extracellular matrix) production, tissue remodeling,

25 cell adhesion and/or free radical production and release. Numerous therapeutic agents described in this invention are capable of inducing fibrosis or scarring and are referred to herein as fibrosing or scarring agents.

Composition Components

The compositions of the present invention comprise two or more

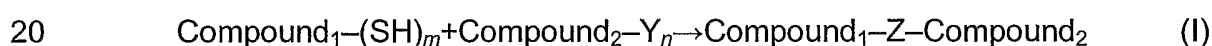
30 different compounds, and at least one of which is a polymer, that react with one

another to form a covalently crosslinked gel matrix. Depending on the reactivity of the compounds towards each other, the different compounds can be in separate parts of the starting compositions, or they can be in the same part of the starting composition. As such, they can easily be administered separately or
5 simultaneously, and rapidly form gels at the site of administration. The compositions can also be formed into gels prior to application to the desired site. The compositions also include a drug that will be contained with the gel and delivered to the tissue at the site of gel administration.

In one aspect of the compositions of the present invention, each
10 component is present in one of the two separate parts, or "components", of the composition, along with other optional ingredients as described elsewhere herein. In total, at least three components are delivered, namely, two reactive components that together form a gel, and a drug.

In another aspect of the compositions of the present invention, the
15 components are mixed together under conditions such that they do not form a gel immediately. These components can be mixed with an activating solution (e.g., buffer, peroxide, etc.) such that a gel is rapidly formed.

The two reactive compounds and the gel matrix that forms when they are mixed together can be represented by Formula I as follows:



Compound₁ has multiple ($m \geq 2$) sulfhydryl groups (SH) that react with Compound₂, which has multiple ($n \geq 2$) sulfhydryl-reactive groups (Y). It should be understood that sulfhydryl groups are also "sulfhydryl reactive groups", since it is well known that sulfhydryl groups will react with one another
25 under certain conditions. When mixed together, the two compounds become interconnected via a covalent bond (Z). However, when $m+n \geq 5$, and appropriate ratios of the two components are utilized as described elsewhere herein, Compound₁ and/or Compound₂ can form multiple attachments to Compound₁ and/or Compound₂, resulting in an interconnected three-
30 dimensional matrix. Preferably, both compounds contain four or more functional groups, since such multifunctionality results in a gel matrix with

greater overall cohesive strength. In a particularly preferred embodiment, each of the compounds is tetrafunctionally activated.

In another preferred embodiment, the compounds each have 12 functional groups. Such compounds are formed from reacting a first
5 tetrafunctionally activated polymer with a second tetrafunctionally activated polymer, wherein the functional groups of each of the two compounds are a reaction pair, and react together to form "12-arm" functionally activated polymers. An example of such a "12-arm" compound is dodeca-sulfhydryl-PEG, 50,000 mol. wt., which is constructed from a core tetra-functional
10 succinimide ester PEG coupled to four (exterior) tetra-functional sulfhydryl-PEG molecules. Such polymers range in size from over 10,000 mol. wt. to greater than 100,000 mol. wt. depending on the molecular weight of the tetra-functionally activated polymer starting materials.

Other types of multifunctional polymers can easily be synthesized
15 using routine synthesis. However, care should be taken to produce multi-arm products with consistent arm lengths to avoid steric hindrance of the reactive groups.

Accordingly, activated polymers that are suitable for use in the present invention may have a variety of geometric shapes and configurations.
20 Exemplary polymers according to the present invention, as well as methods of their manufacture and use, are described in U.S. Patent Nos. 5,874,500; 6,051,648; 6,166,130; 6,312,725; 6,323,278; and 6,458,889.

Compound Core

As described above, each of the compounds has multiple
25 functional groups, either sulfhydryl groups or sulfhydryl-reactive groups. The non-reactive remainder of the compound is considered to be its "core". At least one of the two compounds must have a polymer core in order to form an efficient gel matrix. When one of the compounds contains a polymer core, the other compound can be a small organic molecule with multiple sulfhydryl-

reactive groups. However, for most applications, it is preferred for both compounds to have the same or a different polymer core.

The polymer core may be a synthetic polyamino acid, a polysaccharide, or a synthetic polymer. A preferred polymer core material is a synthetic hydrophilic polymer. Suitable synthetic hydrophilic polymers include, inter alia, polyalkylene oxide, such as polyethylene oxide $((\text{CH}_2\text{CH}_2\text{O})_n)$, polypropylene oxide $((\text{CH}(\text{CH}_3)\text{CH}_2\text{O})_n)$ or a polyethylene/polypropylene oxide mixture $((\text{CH}_2\text{CH}_2\text{O})_n - (\text{CH}(\text{CH}_3)\text{CH}_2\text{O})_n)$. A particularly preferred synthetic hydrophilic polymer is a polyethylene glycol (PEG) having a molecular weight within the range of about 100 to about 100,000 mol. wt., more preferably about 1,000 to about 20,000 mol. wt. More preferably still, when the polymer core is polyethylene glycol, it generally has a molecular weight within the range of about 7,500 to about 20,000 mol. wt. Most preferably, the polyethylene glycol has a molecular weight of approximately 10,000 mol. wt.

Multifunctionally activated polyalkylene oxides, such as polyethylene glycol, are commercially available, and are also easily prepared using known methods. For example, see Chapter 22 of Poly(ethylene Glycol) Chemistry: Biotechnical and Biomedical Applications, J. Milton Harris, ed., Plenum Press, NY (1992); and Shearwater Polymers, Inc. Catalog, Polyethylene Glycol Derivatives, Huntsville, Ala. (1997-1998). For use as a tissue sealant, the preferred combination of activated polymers is as follows: the sulfhydry-reactive group-containing compound is the tetrafunctional PEG, pentaerythritol poly(ethylene glycol) ether tetra-succinimidyl glutarate (10,000 mol. wt.); and the sulfhydryl group-containing compound is the tetrafunctional PEG, pentaerythritol poly(ethylene glycol) ether tetra-sulfhydryl (10,000 mol. wt.). In both cases, these "four-arm" PEGs are formed by ethoxylation of pentaerythritol, where each of the four chains is approximately 2,500 mol. wt., and then derivatized to introduce the functional groups onto each of the four arms. Also preferred are analogous poly(ethylene glycol)-like compounds polymerized from di-glycerol instead of pentaerythritol.

When only one of the reactive compounds comprises a polymer core, the other reactive compound is a multifunctionally active small organic molecule. Such compounds include the di-functional di-succinimidyl esters and di-maleimidyl compounds, as well as other well known commercially available compounds (Pierce Chemical Co., Rockford, IL). In addition, one of skill in the art could easily synthesize a low molecular weight multi-functional reactive compound using routine organic chemistry techniques. On such compound is shown in FIG. 1, which is a penta-erythritol coupled to four glutarates, with each arm capped with N-hydroxy-succinimidyl esters (NHS). Analogous compounds can be synthesized from inositol (radiating 6 arm), lactitol (9 arm) or sorbitol (linear 6-arm). The end-capped reactive group can just as easily be sulfhydryl, maleimidyl, vinyl-sulfone, vinyl, acrylate, acrylamide, *etc.*, instead of NHS. The polymer or the small molecule can carry either reactive end group as long as there are reactive pairs in the composition such as NHS and SH, maleimidyl and SH, *etc.*

Reactive Groups and Matrix Linkages

In the present invention, the linkage, Z, comprises a covalent bond between the sulfur atom in the sulfhydryl group-containing compound and, the carbon or sulfur atom in the sulfhydryl-reactive group-containing compound. Accordingly, the linkage may be a thioester, a thioether, a disulfide, or the like. A wide variety of sulfhydryl-reactive groups and the types of linkages they form when reacted with sulfhydryl groups are well known in the scientific literature. For example, see Bodanszky, M., Principles of Peptide Synthesis, 2nd ed., pages 21 to 37, Springer-Verlog, Berlin (1993); and Lundbland, R. L., Chemical Reagents for Protein Modification, 2nd ed., Chapter 6, CRC Press, Boca Raton, Fla. (1991).

For most applications, sulfhydryl reactive groups that react with sulfhydryl groups to form thioester linkages are preferred. Such compounds are depicted in FIG. 2 and include, inter alia, the following compounds, with the numbers in parentheses corresponding to the structures shown in FIG. 2: mixed

anhydrides, such as PEG-glutaryl-acetyl-anhydride (1), PEG-glutaryl-isovaleryl-anhydride (2), PEG-glutaryl-pivalyl-anhydride (3) and related compounds as presented in Bodanszky, p. 23; Ester derivatives of phosphorus, such as structures (4) and (5); ester derivatives of p-nitrophenol (6) of p-nitrothiophenol (7), of pentafluorophenol (8), of structure (9) and related active esters as presented by Bodanszky, pp. 31-32, and Table 2; esters of substituted hydroxylamines, such as those of N-hydroxy-phthalimide (10), N-hydroxy-succinimide (11), and N-hydroxy-glutarimide (12), as well as related structures in Bodanszky; Table 3; esters of 1-hydroxybenzotriazole (13), 3-hydroxy-3,4-dihydro-benzotriazine-4-one (14) and 3-hydroxy-3,4-dihydro-quinazoline-4-one; derivatives of carbonylimidazole; and isocyanates. With these compounds, auxiliary reagents can also be used to facilitate bond formation. For example, reagents such as 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide can be used to facilitate coupling of carboxyl groups (*i.e.*, glutarate and succinate) with sulfhydryl groups.

In addition to the sulfhydryl reactive compounds that form thioester linkages, various other compounds can be utilized that form other types of linkages. For example, compounds that contain methyl imidate derivatives form imido-thioester linkages with sulfhydryl groups. Alternatively, sulfhydryl reactive groups can be employed that form disulfide bonds with sulfhydryl groups, such as ortho pyridyl disulfide, 3-nitro-2-pyridenesulfonyl, 2-nitro-5-thiocyanobenzoic acid, 5,5'-dithio-bis(2-nitrobenzoic acid), derivatives of methane-thiosulfate, and 2,4-dinitrophenyl cysteinyl disulfides. In such instances, auxiliary reagents, such as the hydrogen peroxide or di-tert-butyl ester of azodicarboxylic acid, can be used to facilitate disulfide bond formation.

Other classes of sulfhydryl reactive groups that form thioether bonds with sulfhydryl groups include, *inter alia*, iodoacetamide, N-ethylmaleimide and other maleimides, including dextran maleimides, mono-bromo-bimane and related compounds, vinylsulfones, epoxides, derivatives of O-methyl-isourea, ethyleneimines, aziridines, vinyl derivatives, acrylate

derivatives, acrylamide derivatives and 4-(aminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole.

Chain Extenders

Functional groups may be directly attached to the compound core,
5 or they may be indirectly attached through a chain extender. Such chain
extenders are well known in the art. See, for example, PCT WO 97/22371,
which describes "linking groups" that would be suitable for use as chain
extenders in the compositions of the present invention. Chain extenders are
useful to avoid steric hindrance problems that are sometimes associated with
10 the formation of direct linkages between molecules. Alternatively, chain
extenders may be used to link several multifunctionally activated compounds
together to make larger molecules. In a particularly preferred embodiment, the
chain extender can also be used to alter the degradative properties of the
compositions after administration and resultant gel formation. For example,
15 chain extenders can be incorporated into one or both of the multifunctionally
activated polymers to promote hydrolysis, to discourage hydrolysis, or to
provide a site for enzymatic degradation. Chain extenders can also activate or
suppress activity of sulfhydryl and sulfhydryl-reactive groups. For example,
electron-withdrawing groups within one or two carbons of the sulfhydryl group
20 would be expected to diminish its effectiveness in coupling, due to a lowering of
nucleophilicity. Double-bond carbon and carbonyl carbon would be anticipated
to have this effect. Bulky nearby groups for either partner are anticipated to
diminish coupling rates, due to steric hindrance. Electron-withdrawing groups
adjacent to the reactive carbonyl of glutaryl-N-hydroxysuccinimidyl would be
25 anticipated to make this carbonyl carbon even more reactive with the sulfhydryl
partner.

Chain extenders may provide sites for degradation, *i.e.*,
hydrolysable sites. Examples of hydrolysable chain extenders include, inter
alia, alpha-hydroxy acids such as lactic acid and glycolic acid; poly(lactones)
30 such as caprolactone, valerolactone, gamma butyl lactone and p-dioxanone;

poly(amino acids); poly(anhydrides) such as glutarate and succinate;
poly(orthoesters); poly(orthocarbonates) such as trimethylene carbonate;
poly(phosphoesters), as well as polymers and copolymers comprising one or
more of the units of the monomers lactic acid, glycolic acid, D-lactide, L-lactide,
5 D,L-lactide, glycolide, ϵ -caprolactone, trimethylene carbonate, 1,4-dioxane-2-
one or 1,5-dioxepan-2-one. Examples of non-degradable chain extenders
include, inter alia, succinimide, propionic acid and carboxymethylate. See, for
example, PCT WO 99/07417. Examples of enzymatically degradable chain
extenders include Leu-Gly-Pro-Ala, which is degraded by collagenase; and Gly-
10 Pro-Lys, which is degraded by plasmin.

Gel Strength and Gel Time

The compositions of the present invention are formulated to
exhibit adequate strength and rapid gel time. The elastic modulus, G' , is the
preferred measure of gel strength. Preferred compositions for use as tissue
15 sealants can achieve a gel strength of about 10^3 to 10^8 dynes/cm², and more
preferably 10^4 to 10^7 dynes/cm². Preferred compositions for use as hemostatic
agents or for adhesion prevention have a gel strength of at least 10^2 to 10^4
dynes/cm² if a soft gel is desired, or 10^5 to 10^8 dynes/cm² if a harder matrix is
desired.

20 The gel time of preferred formulations is less than 60 seconds,
more preferably less than 30 seconds, and most preferably less than 15
seconds. The fast gel time ensures maximum material at the site to be treated
and sufficient mechanical properties.

Drug

25 In addition to the reactive compounds described above, the
compositions of the present invention include a drug. As used herein, the term
"drug" refers to an organic molecule that exerts biological effects *in vivo*. In one
aspect, the drug is in combination with Compound₁. In another aspect, the drug
is in combination with Compound₂. Suitable drugs are described below. In one

aspect, the drug is hydrophobic. In another aspect, the drug is hydrophyllic. One aspect of the invention involves pharmacological alteration of cellular and/or non-cellular processes involved in the development and/or maintenance of surgical adhesions. Another aspect of this invention involves pharmacological
5 alteration of cellular and/or non-cellular processes involved in the development and/or maintenance of restenosis. Thus, pharmacological agents (*i.e.*, drugs) within the scope of this invention include but are not limited to those which inhibit one or a combination of processes including but not limited to cell division, cell secretion, cell migration, cell adhesion, cytokine, chemokine (or
10 other inflammatory activator) production and/or release, angiogenesis, and/or free radical formation and/or release. Drugs within the scope of this invention may inhibit or affect other processes involved in the scarring process.

In addition, an aspect of this invention involves pharmacological alteration of cellular and/or non-cellular processes which increase the
15 development of fibrosis. Thus, pharmacological agents (*i.e.*, drugs) within the scope of this invention include but are not limited to those which increase one or a combination of processes including but not limited to cell division, cell secretion, cell migration, cell adhesion, cytokine, chemokine (or other inflammatory activator) production and/or release, angiogenesis, and/or free
20 radical formation and/or release. Drugs within the scope of this invention may increase or affect other processes involved in the scarring process.

Thus, while the non-drug loaded formulation can act as a sealant and/or hemostatic agent and/or adhesion prevention agent, the addition of a drug can effect an increase or decrease in fibrosis, and/or result in tissue
25 augmentation and/or increase or reduction in surgical adhesions depending on the drug mechanism. For example, a drug which decreases fibrosis will be expected to reduce surgical adhesions. Furthermore, the drug-loaded formulation may increase the sealant and/or hemostatic properties of the formulation, especially when the agent acts to increase fibrosis.

30 One aspect of the invention involves pharmacological alteration of cellular and/or non-cellular processes involved in the development and/or

maintenance of surgical adhesions or restenosis or in more general terms inhibit one or more processes involved in fibrosis. Thus, pharmacological agents within the scope of this invention include but are not limited to those which inhibit one or a combination of processes such as cell division, cell

5 secretion, cell migration, cell adhesion, extracellular matrix production, cytokine (e.g., TNF alpha, IL-1, IL-6), or other inflammatory activator, e.g., chemokines (e.g., MCP-1 or IL-8)) production and/or release, angiogenesis, and/or free radical formation and/or release .

Suitable fibrosis, adhesion or stenosis-inhibiting agents may be

10 readily determined based upon the *in vitro* and *in vivo* (animal) models such as those provided in Examples 29-33. Numerous fibrosis, adhesion and/or stenosis-inhibiting therapeutic compounds have been identified that are of utility in the invention including:

1. Angiogenesis Inhibitors

15 In one embodiment, the pharmacologically active compound is an angiogenesis inhibitor (e.g., 2-ME (NSC-659853), PI-88 (D-Mannose, O-6-O-phosphono-Alpha-D-mannopyranosyl-(1-3)-O-Alpha-D-mannopyranosyl-(1-3)-O-Alpha-D-mannopyranosyl-(1-3)-O-Alpha-D-mannopyranosyl-(1-2)- hydrogen sulphate [CAS]), thalidomide (1H-Isoindole-1,3(2H)-dione, 2-(2,6-dioxo-3-

20 piperidinyl)- [CAS]), CDC-394, CC-5079, ENMD-0995 (S-3-amino-phthalidoglutarimide), AVE-8062A, Vatalanib, SH-268, Halofuginone hydrobromide)) or an analogue or derivative thereof.

2. 5-Lipoxygenase Inhibitors & Antagonists

25 In another embodiment, the pharmacologically active compound is a 5-lipoxygenase inhibitor or antagonist (e.g., licofelone (ML3000), 2-uredo thiophene/2 amino thiophene, 15-deoxy-Prostaglandin J2, Wy-50295 (2-Naphthaleneacetic acid, Alpha-methyl-6-(2-quinolinylmethoxy)-, (S)-[CAS]), ONO-LP-269 (2,11,14-Eicosatrienamide, N-[4-hydroxy-2-(1H-tetrazol-5-yl)-8-quinolinyl]-, (E,Z,Z)-[CAS]), licofelone (1H-Pyrrolizine-5-acetic acid, 6-(4-

chlorophenyl)-2,3-dihydro-2,2-dimethyl-7-phenyl- [CAS]), CMI-568 (Urea, N-butyl-N-hydroxy-N'-[4-[3-(methylsulfonyl)-2-propoxy-5-[tetrahydro-5-(3,4,5-trimethoxyphenyl)-2-furanyl]phenoxy]butyl]-,trans- [CAS]), IP-751 ((3R,4R)-(delta6)-THC-DMH-11-oic acid), PF-5901 (Benzenemethanol, Alpha-pentyl-3-

5 (2-quinolinylmethoxy)- [CAS]), LY-293111 (Benzoic acid, 2-[3-[3-[(5-ethyl-4'-fluoro-2-hydroxy[1,1'-biphenyl]-4-yl)oxy]propoxy]-2-propylphenoxy]- [CAS]), RG-5901-A (Benzenemethanol, Alpha-pentyl-3-(2-quinolinylmethoxy)-, hydrochloride [CAS]), rilopirox (2(1H)-Pyridinone, 6-[[4-(4-chlorophenoxy)phenoxy]methyl]-1-hydroxy-4-methyl- [CAS]), L-674636 (Acetic

10 acid, ((4-(4-chlorophenyl)-1-(4-(2-quinolinylmethoxy)phenyl)butyl)thio)-AS)), 7-[[3-(4-methoxy-tetrahydro-2H-pyran-4-yl)phenyl]methoxy]-4-phenylnaphtho[2,3-c]furan-1(3H)-one, MK-886 (1H-Indole-2-propanoic acid, 1-[(4-chlorophenyl)methyl]-3-[(1,1-dimethylethyl)thio]-Alpha,Alpha-dimethyl-5-(1-methylethyl)- [CAS]), quiflapon (1H-Indole-2-propanoic acid, 1-[(4-

15 chlorophenyl)methyl]-3-[(1,1-dimethylethyl)thio]-Alpha,Alpha-dimethyl-5-(2-quinolinylmethoxy)- [CAS]), quiflapon (1H-Indole-2-propanoic acid, 1-[(4-chlorophenyl)methyl]-3-[(1,1-dimethylethyl)thio]-Alpha,Alpha-dimethyl-5-(2-quinolinylmethoxy)- [CAS]), docebenone (2,5-Cyclohexadiene-1,4-dione, 2-(12-hydroxy-5,10-dodecadiynyl)-3,5,6-trimethyl- [CAS]), zileuton (Urea, N-(1-

20 benzo[b]thien-2-ylethyl)-N-hydroxy- [CAS])) or an analogue or derivative thereof.

3. Chemokine Receptor Antagonists CCR (1, 3, & 5)

In another embodiment, the pharmacologically active compound is a chemokine receptor antagonist (e.g., AMD-3100 (Anormed), ONO-4128

25 (1,4,9-Triazaspiro(5.5)undecane-2,5-dione, 1-butyl-3-(cyclohexylmethyl)-9-((2,3-dihydro-1,4-benzodioxin-6-yl)methyl)- [CAS]), L-381, CT-112 (L-Arginine, L-threonyl-L-threonyl-L-seryl-L-glutaminy-L-valyl-L-arginyl-L-prolyl- [CAS]), AS-900004, SCH-C, ZK-811752, PD-172084, UK-427857, SB-380732, vMIP II, SB-265610, DPC-168, TAK-779 (N, N-Dimethyl-N-[4-[2-(4-methylphenyl)-6,7-

dihydro-5H-benzocyclohepten-8-ylcarboxamido]benzyl]tetrahydro-2H-pyran-4-aminium chloride), TAK-220, KRH-1120) or an analogue or derivative thereof.

4. Cell Cycle Inhibitors

In another embodiment, the pharmacologically active compound is a cell cycle inhibitor or an analogue or derivative thereof. In related embodiments, the cell-cycle inhibitor is a taxane (e.g., paclitaxel, or an analogue or derivative thereof), an antimetabolite, an alkylating agent, or a vinca alkaloid. In another embodiment, the cell-cycle inhibitor is camptothecin or an analogue or derivative thereof. Other suitable compounds include mitoxantrone, etoposide, 5-fluorouracil, doxorubicin, methotrexate, Peloruside A - a microtubule stabilizing agent, Mitomycin-C, and CDK-2 inhibitors.

"Cell Cycle Inhibitor" as used herein refers to any protein, peptide, chemical or other molecule which delays or impairs a dividing cell's ability to progress through the cell cycle and replicate. A wide variety of methods may be utilized to determine the ability of a compound to inhibit the cell cycle including univariate analysis of cellular DNA content and multiparameter analysis. A Cell Cycle Inhibitor may act to inhibit the cell cycle at any of the steps of the biological pathways shown in FIG. 3, as well as at other possible steps in other biological pathways. In addition, it should be understood that while a single cell cycle agent is often referred to, that this in fact should be understood to include two or more cell cycle agents, as more than one cell cycle agent may be utilized within the compositions, methods and/or devices described herein (e.g., two cell-cycle inhibitors may be selected that act on different steps shown in FIG. 3).

A wide variety of cell cycle inhibitory agents can be utilized, either with or without a carrier (e.g., a polymer or ointment or vector), within the context of the present invention. Representative examples of such agents include taxanes (e.g., paclitaxel (discussed in more detail below) and docetaxel) (Schiff *et al.*, *Nature* 277:665-667, 1979; Long and Fairchild, *Cancer Research* 54:4355-4361, 1994; Ringel and Horwitz, *J. Nat'l Cancer Inst.* 83(4):288-291, 1991; Pazdur *et al.*, *Cancer Treat. Rev.* 19(40):351-386, 1993),

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- 25 methotrexate derivatives (Webber *et al.*, *Chem. Biol. Pteridines, Pteridines Folic Acid Deriv., Proc. Int. Symp. Pteridines Folic Acid Deriv.: Chem., Biol. Clin. Aspects:* 659-62, 1986), iodoacetyl lysine methotrexate analogue (Delcamp *et al.*, *Chem. Biol. Pteridines, Pteridines Folic Acid Deriv., Proc. Int.*
- 30 *Symp. Pteridines Folic Acid Deriv.: Chem., Biol. Clin. Aspects:* 807-9, 1986), 2,omega.-diaminoalkanoic acid-containing methotrexate analogues (McGuire

- et al.*, *Biochem. Pharmacol.* 35(15):2607-13, 1986), polyglutamate methotrexate derivatives (Kamen & Winick, *Methods Enzymol.* 122(Vitam. Coenzymes, Pt. G):339-46, 1986), 5-methyl-5-deaza analogues (Piper *et al.*, *J. Med. Chem.* 29(6):1080-7, 1986), quinazoline methotrexate analogue
- 5 (Mastropaolo *et al.*, *J. Med. Chem.* 29(1):155-8, 1986), pyrazine methotrexate analogue (Lever & Vestal, *J. Heterocycl. Chem.* 22(1):5-6, 1985), cysteic acid and homocysteic acid methotrexate analogues (4,490,529), γ -tert-butyl methotrexate esters (Rosowsky *et al.*, *J. Med. Chem.* 28(5):660-7, 1985), fluorinated methotrexate analogues (Tsushima *et al.*, *Heterocycles* 23(1):45-9,
- 10 1985), folate methotrexate analogue (Trombe, *J. Bacteriol.* 160(3):849-53, 1984), phosphonoglutamic acid analogues (Sturtz & Guillaumot, *Eur. J. Med. Chem.-Chim. Ther.* 19(3):267-73, 1984), poly (L-lysine) methotrexate conjugates (Rosowsky *et al.*, *J. Med. Chem.* 27(7):888-93, 1984), dilysine and trilycine methotrexate derivatives (Forsch & Rosowsky, *J. Org. Chem.*
- 15 49(7):1305-9, 1984), 7-hydroxymethotrexate (Fabre *et al.*, *Cancer Res.* 43(10):4648-52, 1983), poly- γ -glutamyl methotrexate analogues (Piper & Montgomery, *Adv. Exp. Med. Biol.*, 163(*Folyl Antifolyl Polyglutamates*):95-100, 1983), 3',5'-dichloromethotrexate (Rosowsky & Yu, *J. Med. Chem.* 26(10):1448-52, 1983), diazoketone and chloromethylketone methotrexate analogues
- 20 (Gangjee *et al.*, *J. Pharm. Sci.* 71(6):717-19, 1982), 10-propargylaminopterin and alkyl methotrexate homologs (Piper *et al.*, *J. Med. Chem.* 25(7):877-80, 1982), lectin derivatives of methotrexate (Lin *et al.*, *JNCI* 66(3):523-8, 1981), polyglutamate methotrexate derivatives (Galivan, *Mol. Pharmacol.* 17(1):105-10, 1980), halogenated methotrexate derivatives (Fox, *JNCI* 58(4):J955-8,
- 25 1977), 8-alkyl-7,8-dihydro analogues (Chaykovsky *et al.*, *J. Med. Chem.* 20(10):J1323-7, 1977), 7-methyl methotrexate derivatives and dichloromethotrexate (Rosowsky & Chen, *J. Med. Chem.* 17(12):J1308-11, 1974), lipophilic methotrexate derivatives and 3',5'-dichloromethotrexate (Rosowsky, *J. Med. Chem.* 16(10):J1190-3, 1973), deaza amethopterin
- 30 analogues (Montgomery *et al.*, *Ann. N.Y. Acad. Sci.* 186:J227-34, 1971), MX068 (Pharma Japan, 1658:18, 1999) and cysteic acid and homocysteic acid

- methotrexate analogues (EPA 0142220); N3-alkylated analogues of 5-fluorouracil (Kozai *et al.*, *J. Chem. Soc., Perkin Trans. 1*(19):3145-3146, 1998), 5-fluorouracil derivatives with 1,4-oxaheteroepane moieties (Gomez *et al.*, *Tetrahedron* 54(43):13295-13312, 1998), 5-fluorouracil and nucleoside
- 5 analogues (Li, *Anticancer Res.* 17(1A):21-27, 1997), cis- and trans-5-fluoro-5,6-dihydro-6-alkoxyuracil (Van der Wilt *et al.*, *Br. J. Cancer* 68(4):702-7, 1993), cyclopentane 5-fluorouracil analogues (Hronowski & Szarek, *Can. J. Chem.* 70(4):1162-9, 1992), A-OT-fluorouracil (Zhang *et al.*, *Zongguo Yiyao Gongye Zazhi* 20(11):513-15, 1989), N4-trimethoxybenzoyl-5'-deoxy-5-fluorocytidine
- 10 and 5'-deoxy-5-fluorouridine (Miwa *et al.*, *Chem. Pharm. Bull.* 38(4):998-1003, 1990), 1-hexylcarbamoyl-5-fluorouracil (Hoshi *et al.*, *J. Pharmacobio-Dun.* 3(9):478-81, 1980; Maehara *et al.*, *Chemotherapy (Basel)* 34(6):484-9, 1988), B-3839 (Prajda *et al.*, *In Vivo* 2(2):151-4, 1988), uracil-1-(2-tetrahydrofuryl)-5-fluorouracil (Anai *et al.*, *Oncology* 45(3):144-7, 1988), 1-(2'-deoxy-2'-fluoro- β -D-
- 15 arabinofuranosyl)-5-fluorouracil (Suzuko *et al.*, *Mol. Pharmacol.* 31(3):301-6, 1987), doxifluridine (Matuura *et al.*, *Oyo Yakuri* 29(5):803-31, 1985), 5'-deoxy-5-fluorouridine (Bollag & Hartmann, *Eur. J. Cancer* 16(4):427-32, 1980), 1-acetyl-3-O-toluy-5-fluorouracil (Okada, *Hiroshima J. Med. Sci.* 28(1):49-66, 1979), 5-fluorouracil-m-formylbenzene-sulfonate (JP 55059173), N'-(2-furanidyl)-5-
- 20 fluorouracil (JP 53149985) and 1-(2-tetrahydrofuryl)-5-fluorouracil (JP 52089680); 4'-epidoxorubicin (Lanius, *Adv. Chemother. Gastrointest. Cancer*, (Int. Symp.), 159-67, 1984); N-substituted deacetylvinblastine amide (vindesine) sulfates (Conrad *et al.*, *J. Med. Chem.* 22(4):391-400, 1979); and Cu(II)-VP-16 (etoposide) complex (Tawa *et al.*, *Bioorg. Med. Chem.* 6(7):1003-1008, 1998),
- 25 pyrrolecarboxamidino-bearing etoposide analogues (Ji *et al.*, *Bioorg. Med. Chem. Lett.* 7(5):607-612, 1997), 4 β -amino etoposide analogues (Hu, University of North Carolina Dissertation, 1992), γ -lactone ring-modified arylamino etoposide analogues (Zhou *et al.*, *J. Med. Chem.* 37(2):287-92, 1994), N-glucosyl etoposide analogue (Allevi *et al.*, *Tetrahedron Lett.*
- 30 34(45):7313-16, 1993), etoposide A-ring analogues (Kadow *et al.*, *Bioorg. Med. Chem. Lett.* 2(1):17-22, 1992), 4'-deshydroxy-4'-methyl etoposide (Saulnier *et*

al., *Bioorg. Med. Chem. Lett.* 2(10):1213-18, 1992), pendulum ring etoposide analogues (Sinha *et al.*, *Eur. J. Cancer* 26(5):590-3, 1990) and E-ring desoxy etoposide analogues (Saulnier *et al.*, *J. Med. Chem.* 32(7):1418-20, 1989).

Within one preferred embodiment of the invention, the cell cycle
5 inhibitor is paclitaxel, a compound which disrupts mitosis (M-phase) by binding to tubulin to form abnormal mitotic spindles or an analogue or derivative thereof. Briefly, paclitaxel is a highly derivatized diterpenoid (Wani *et al.*, *J. Am. Chem. Soc.* 93:2325, 1971) which has been obtained from the harvested and dried bark of *Taxus brevifolia* (Pacific Yew) and *Taxomyces Andreanae* and
10 *Endophytic Fungus* of the Pacific Yew (Stierle *et al.*, *Science* 60:214-216, 1993). "Paclitaxel" (which should be understood herein to include formulations, prodrugs, analogues and derivatives such as, for example, TAXOL[®] (Bristol-Myers Squibb Company, New York, NY), TAXOTERE[®] (Aventis Pharmaceuticals, France), docetaxel, 10-desacetyl analogues of paclitaxel and
15 3'-N-desbenzoyl-3'-N-t-butoxy carbonyl analogues of paclitaxel) may be readily prepared utilizing techniques known to those skilled in the art (see, e.g., Schiff *et al.*, *Nature* 277:665-667, 1979; Long and Fairchild, *Cancer Research* 54:4355-4361, 1994; Ringel and Horwitz, *J. Nat'l Cancer Inst.* 83(4):288-291, 1991; Pazdur *et al.*, *Cancer Treat. Rev.* 19(4):351-386, 1993; WO 94/07882; WO 94/07881; WO 94/07880; WO 94/07876; WO 93/23555; WO 93/10076; WO 94/00156; WO 93/24476; EP 590267; WO 94/20089; U.S. Patent Nos. 5,294,637; 5,283,253; 5,279,949; 5,274,137; 5,202,448; 5,200,534; 5,229,529; 5,254,580; 5,412,092; 5,395,850; 5,380,751; 5,350,866; 4,857,653; 5,272,171; 5,411,984; 5,248,796; 5,248,796; 5,422,364; 5,300,638; 5,294,637; 5,362,831;
20 5,440,056; 4,814,470; 5,278,324; 5,352,805; 5,411,984; 5,059,699; 4,942,184; *Tetrahedron Letters* 35(52):9709-9712, 1994; *J. Med. Chem.* 35:4230-4237, 1992; *J. Med. Chem.* 34:992-998, 1991; *J. Natural Prod.* 57(10):1404-1410, 1994; *J. Natural Prod.* 57(11):1580-1583, 1994; *J. Am. Chem. Soc.* 110:6558-6560, 1988), or obtained from a variety of commercial sources, including for
30 example, Sigma Chemical Co., St. Louis, Missouri (T7402 – from *Taxus brevifolia*).

Representative examples of paclitaxel derivatives or analogues include 7-deoxy-docetaxol, 7,8-cyclopropataxanes, N-substituted 2-azetidones, 6,7-epoxy paclitaxels, 6,7-modified paclitaxels, 10-desacetoxytaxol, 10-deacetyltaxol (from 10-deacetylbaaccatin III), phosphonoxy and carbonate derivatives of taxol, taxol 2',7-di(sodium 1,2-benzenedicarboxylate, 10-desacetoxy-11,12-dihydrotaxol-10,12(18)-diene derivatives, 10-desacetoxytaxol, Protaxol (2'-and/or 7-O-ester derivatives), (2'-and/or 7-O-carbonate derivatives), asymmetric synthesis of taxol side chain, fluoro taxols, 9-deoxotaxane, (13-acetyl-9-deoxobaccatine III, 9-deoxotaxol, 7-deoxy-9-deoxotaxol, 10-desacetoxy-7-deoxy-9-deoxotaxol, Derivatives containing hydrogen or acetyl group and a hydroxy and tert-butoxycarbonylamino, sulfonated 2'-acryloyltaxol and sulfonated 2'-O-acyl acid taxol derivatives, succinyltaxol, 2'- γ -aminobutyryltaxol formate, 2'-acetyl taxol, 7-acetyl taxol, 7-glycine carbamate taxol, 2'-OH-7-PEG(5000) carbamate taxol, 2'-benzoyl and 2',7-dibenzoyl taxol derivatives, other prodrugs (2'-acetyltaxol; 2',7-diacetyltaxol; 2'succinyltaxol; 2'-(beta-alanyl)-taxol); 2'gamma-aminobutyryltaxol formate; ethylene glycol derivatives of 2'-succinyltaxol; 2'-glutaryltaxol; 2'-(N,N-dimethylglycyl) taxol; 2'-(2-(N,N-dimethylamino)propionyl)taxol; 2'orthocarboxybenzoyl taxol; 2'aliphatic carboxylic acid derivatives of taxol, Prodrugs {2'(N,N-diethylaminopropionyl)taxol, 2'(N,N-dimethylglycyl)taxol, 7(N,N-dimethylglycyl)taxol, 2',7-di-(N,N-dimethylglycyl)taxol, 7(N,N-diethylaminopropionyl)taxol, 2',7-di(N,N-diethylaminopropionyl)taxol, 2'-(L-glycyl)taxol, 7-(L-glycyl)taxol, 2',7-di(L-glycyl)taxol, 2'-(L-alanyl)taxol, 7-(L-alanyl)taxol, 2',7-di(L-alanyl)taxol, 2'-(L-leucyl)taxol, 7-(L-leucyl)taxol, 2',7-di(L-leucyl)taxol, 2'-(L-isoleucyl)taxol, 7-(L-isoleucyl)taxol, 2',7-di(L-isoleucyl)taxol, 2'-(L-valyl)taxol, 7-(L-valyl)taxol, 2',7-di(L-valyl)taxol, 2'-(L-phenylalanyl)taxol, 7-(L-phenylalanyl)taxol, 2',7-di(L-phenylalanyl)taxol, 2'-(L-prolyl)taxol, 7-(L-prolyl)taxol, 2',7-di(L-prolyl)taxol, 2'-(L-lysyl)taxol, 7-(L-lysyl)taxol, 2',7-di(L-lysyl)taxol, 2'-(L-glutamyl)taxol, 7-(L-glutamyl)taxol, 2',7-di(L-glutamyl)taxol, 2'-(L-arginyl)taxol, 7-(L-arginyl)taxol, 2',7-di(L-arginyl)taxol}, Taxol analogues with

modified phenylisoserine side chains, taxotere, (N-debenzoyl-N-tert-(butoxycaronyl)-10-deacetyltaxol, and taxanes (e.g., baccatin III, cephalomannine, 10-deacetylbaccatin III, brevifolol, yunantaxusin and taxusin); and other taxane analogues and derivatives, including 14-beta-hydroxy-10

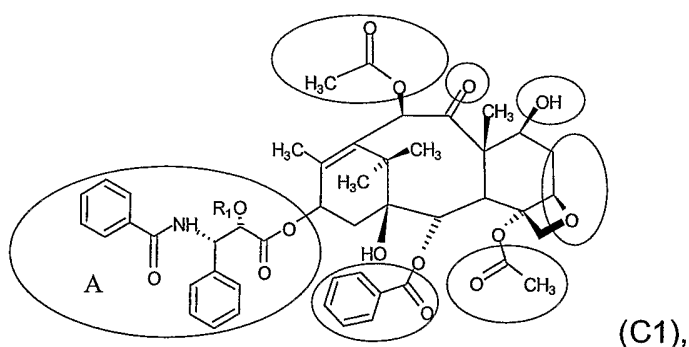
5 deacetylbaccatin III, debenzoyl-2-acyl paclitaxel derivatives, benzoate paclitaxel derivatives, phosphonoxy and carbonate paclitaxel derivatives, sulfonated 2'-acryloyltaxol; sulfonated 2'-O-acyl acid paclitaxel derivatives, 18-site-substituted paclitaxel derivatives, chlorinated paclitaxel analogues, C4 methoxy ether paclitaxel derivatives, sulfenamide taxane derivatives, brominated paclitaxel

10 analogues, Girard taxane derivatives, nitrophenyl paclitaxel, 10-deacetylated substituted paclitaxel derivatives, 14- beta -hydroxy-10 deacetylbaccatin III taxane derivatives, C7 taxane derivatives, C10 taxane derivatives, 2-debenzoyl-2-acyl taxane derivatives, 2-debenzoyl and -2-acyl paclitaxel derivatives, taxane and baccatin III analogues bearing new C2 and C4 functional groups, n-acyl

15 paclitaxel analogues, 10-deacetylbaccatin III and 7-protected-10-deacetylbaccatin III derivatives from 10-deacetyl taxol A, 10-deacetyl taxol B, and 10-deacetyl taxol, benzoate derivatives of taxol, 2-aroyle-4-acyl paclitaxel analogues, ortho-ester paclitaxel analogues, 2-aroyle-4-acyl paclitaxel analogues and 1-deoxy paclitaxel and

20 1-deoxy paclitaxel analogues.

In one aspect, the Cell Cycle Inhibitor is a taxane having the formula (C1):



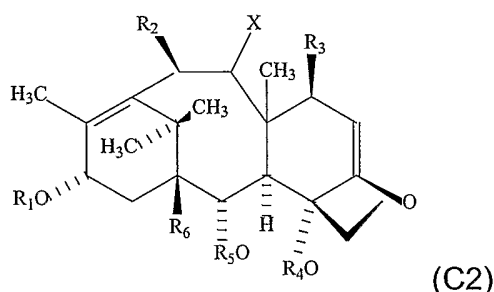
where the gray-highlighted portions may be substituted and the non-highlighted

25 portion is the taxane core. A side-chain (labeled "A" in the diagram) is

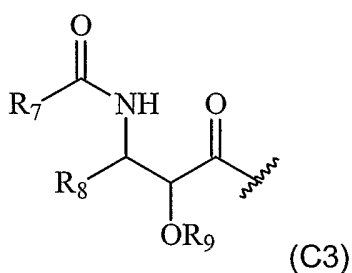
desirably present in order for the compound to have good activity as a Cell Cycle Inhibitor. Examples of compounds having this structure include paclitaxel (Merck Index entry 7117), docetaxol (TAXOTERE, Merck Index entry 3458), and 3'-desphenyl-3'-(4-nitrophenyl)-N-debenzoyl-N-(t-butoxycarbonyl)-10-

5 deacetyltaxol.

In one aspect, suitable taxanes such as paclitaxel and its analogues and derivatives are disclosed in U.S. Patent No. 5,440,056 as having the structure (C2):



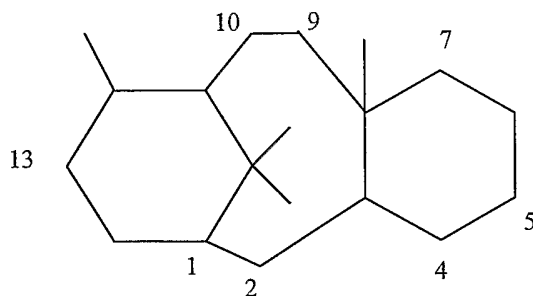
10 wherein X may be oxygen (paclitaxel), hydrogen (9-deoxy derivatives), thioacyl, or dihydroxyl precursors; R₁ is selected from paclitaxel or taxotere side chains or alkanoyl of the formula (C3)



wherein R₇ is selected from hydrogen, alkyl, phenyl, alkoxy, amino, phenoxy
 15 (substituted or unsubstituted); R₈ is selected from hydrogen, alkyl, hydroxyalkyl, alkoxyalkyl, aminoalkyl, phenyl (substituted or unsubstituted), alpha or beta-naphthyl; and R₉ is selected from hydrogen, alkanoyl, substituted alkanoyl, and aminoalkanoyl; where substitutions refer to hydroxyl, sulfhydryl, allalkoxyl, carboxyl, halogen, thioalkoxyl, N,N-dimethylamino, alkylamino, dialkylamino,
 20 nitro, and -OSO₃H, and/or may refer to groups containing such substitutions; R₂ is selected from hydrogen or oxygen-containing groups, such as hydroxyl,

alkoyl, alkanoyloxy, aminoalkanoyloxy, and peptidyalkanoyloxy; R_3 is selected from hydrogen or oxygen-containing groups, such as hydroxyl, alkoyl, alkanoyloxy, aminoalkanoyloxy, and peptidyalkanoyloxy, and may further be a silyl containing group or a sulphur containing group; R_4 is selected from acyl, alkyl, alkanoyl, aminoalkanoyl, peptidylalkanoyl and aroyl; R_5 is selected from acyl, alkyl, alkanoyl, aminoalkanoyl, peptidylalkanoyl and aroyl; R_6 is selected from hydrogen or oxygen-containing groups, such as hydroxyl alkoyl, alkanoyloxy, aminoalkanoyloxy, and peptidyalkanoyloxy.

In one aspect, the paclitaxel analogues and derivatives useful as Cell Cycle Inhibitors in the present invention are disclosed in PCT International Patent Application No. WO 93/10076. As disclosed in this publication, the analogue or derivative should have a side chain attached to the taxane nucleus at C_{13} , as shown in the structure below (formula C4), in order to confer antitumor activity to the taxane.



15

(C4)

WO 93/10076 discloses that the taxane nucleus may be substituted at any position with the exception of the existing methyl groups. The substitutions may include, for example, hydrogen, alkanoyloxy, alkenoyloxy, aryloxy. In addition, oxo groups may be attached to carbons labeled 2, 4, 9, 10. As well, an oxetane ring may be attached at carbons 4 and 5. As well, an oxirane ring may be attached to the carbon labeled 4.

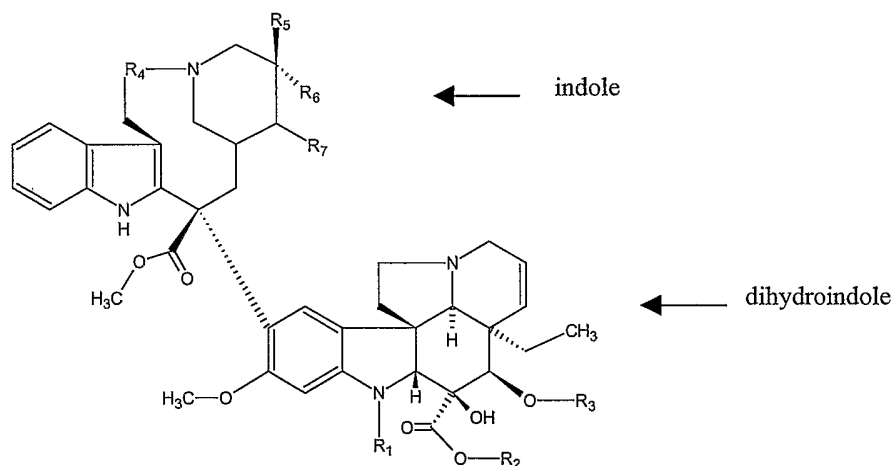
In one aspect, the taxane-based Cell Cycle Inhibitor useful in the present invention is disclosed in U.S. Patent No. 5,440,056, which discloses 9-deoxy taxanes. These are compounds lacking an oxo group at the carbon labeled 9 in the taxane structure shown above (formula C4). The taxane ring

25

may be substituted at the carbons labeled 1, 7 and 10 (independently) with H, OH, O-R, or O-CO-R where R is an alkyl or an aminoalkyl. As well, it may be substituted at carbons labeled 2 and 4 (independently) with arylol, alkanoyl, aminoalkanoyl or alkyl groups. The side chain of formula (C3) may be substituted at R₇ and R₈ (independently) with phenyl rings, substituted phenyl rings, linear alkanes/alkenes, and groups containing H, O or N. R₉ may be substituted with H, or a substituted or unsubstituted alkanoyl group.

Taxanes in general, and paclitaxel is particular, is considered to function as a Cell Cycle Inhibitor by acting as an anti-microtubule agent, and more specifically as a stabilizer. These compounds have been shown useful in the treatment of proliferative disorders, including: non-small cell (NSC) lung; small cell lung; breast; prostate; cervical; endometrial; head and neck cancers.

In another aspect, the Cell Cycle Inhibitor is a Vinca Alkaloid. Vinca alkaloids have the following general structure. They are indole-dihydroindole dimers.

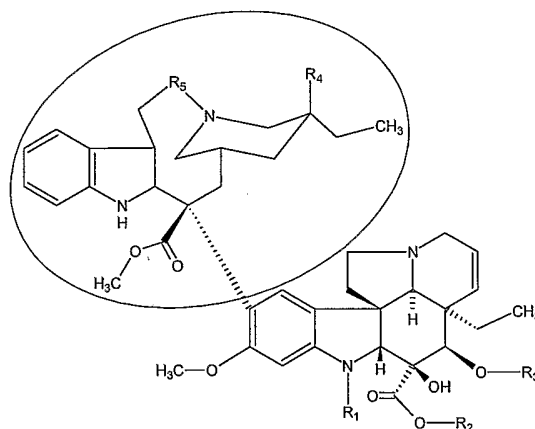


As disclosed in U.S. Patent Nos. 4,841,045 and 5,030,620, R₁ can be a formyl or methyl group or alternately H. R₁ could also be an alkyl group or an aldehyde-substituted alkyl (e.g., CH₂CHO). R₂ is typically a CH₃ or NH₂ group. However it can be alternately substituted with a lower alkyl ester or the ester linking to the dihydroindole core may be substituted with C(O)-R where R is NH₂, an amino acid ester or a peptide ester. R₃ is typically C(O)CH₃, CH₃ or H. Alternately, a protein fragment may be linked by a bifunctional group such

as maleoyl amino acid. R_3 could also be substituted to form an alkyl ester which may be further substituted. R_4 may be $-\text{CH}_2-$ or a single bond. R_5 and R_6 may be H, OH or a lower alkyl, typically $-\text{CH}_2\text{CH}_3$. Alternatively R_6 and R_7 may together form an oxetane ring. R_7 may alternately be H. Further

- 5 substitutions include molecules wherein methyl groups are substituted with other alkyl groups, and whereby unsaturated rings may be derivatized by the addition of a side group such as an alkane, alkene, alkyne, halogen, ester, amide or amino group.

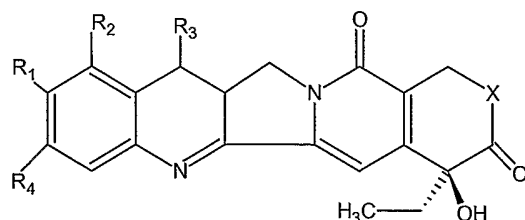
Exemplary Vinca Alkaloids are vinblastine, vincristine, vincristine
10 sulfate, vindesine, and vinorelbine, having the structures:



	R_1	R_2	R_3	R_4	R_5
Vinblastine:	CH_3	CH_3	$\text{C}(\text{O})\text{CH}_3$	OH	CH_2
Vincristine:	CH_2O	CH_3	$\text{C}(\text{O})\text{CH}_3$	OH	CH_2
Vindesine:	CH_3	NH_2	H	OH	CH_2
Vinorelbine:	CH_3	CH_3	CH_3	H	single bond

Analogues typically require the side group (shaded area) in order to have activity. These compounds are thought to act as Cell Cycle Inhibitors by functioning as anti-microtubule agents, and more specifically to inhibit
15 polymerization. These compounds have been shown useful in treating proliferative disorders, including NSC lung; small cell lung; breast; prostate; brain; head and neck; retinoblastoma; bladder; and penile cancers; and soft tissue sarcoma.

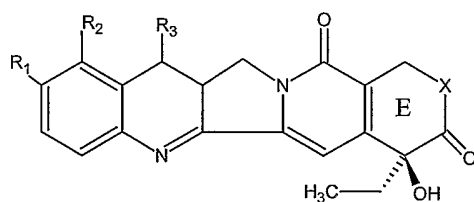
In another aspect, the Cell Cycle Inhibitor is Camptothecin, or an analogue or derivative thereof. Camptothecins have the following general structure.



- 5 In this structure, X is typically O, but can be other groups, *e.g.*, NH in the case of 21-lactam derivatives. R₁ is typically H or OH, but may be other groups, *e.g.*, a terminally hydroxylated C₁₋₃ alkane. R₂ is typically H or an amino containing group such as (CH₃)₂NHCH₂, but may be other groups *e.g.*, NO₂, NH₂, halogen (as disclosed in, *e.g.*, U.S. Patent No. 5,552,156) or a short
- 10 alkane containing these groups. R₃ is typically H or a short alkyl such as C₂H₅. R₄ is typically H but may be other groups, *e.g.*, a methylenedioxy group with R₁.

Exemplary camptothecin compounds include topotecan, irinotecan (CPT-11), 9-aminocamptothecin, 21-lactam-20(S)-camptothecin, 10,11-methylenedioxycamptothecin, SN-38, 9-nitrocamptothecin, 10-

15 hydroxycamptothecin. Exemplary compounds have the structures:



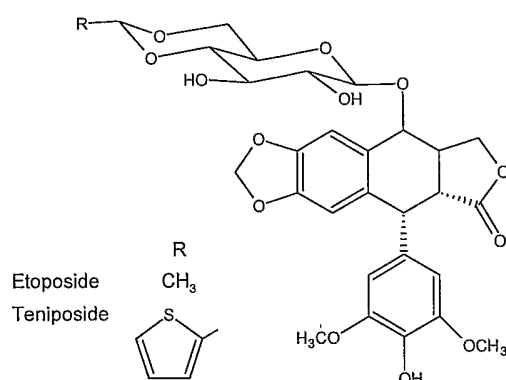
	R ₁	R ₂	R ₃
Camptothecin:	H	H	H
Topotecan:	OH	(CH ₃) ₂ NHCH ₂	H
SN-38:	OH	H	C ₂ H ₅

X: O for most analogs, NH for 21-lactam analogs

Camptothecins have the five rings shown here. The ring labeled E must be intact (the lactone rather than carboxylate form) for maximum activity and minimum toxicity. These compounds are useful to as Cell Cycle Inhibitors,

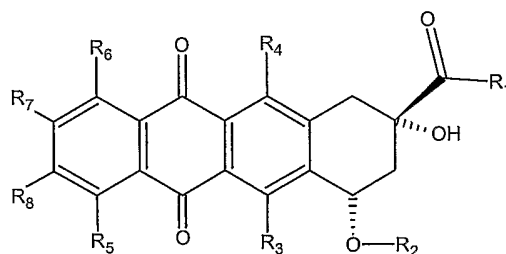
where they function as Topoisomerase I Inhibitors and/or DNA cleavage agents. They have been shown useful in the treatment of proliferative disorders, including, for example, NSC lung; small cell lung; and cervical cancers.

- 5 In another aspect, the Cell Cycle Inhibitor is a Podophyllotoxin, or a derivative or an analogue thereof. Exemplary compounds of this type are Etoposide or Teniposide, which have the following structures:



- These compounds are thought to function as Cell Cycle Inhibitors by being Topoisomerase II Inhibitors and/or by DNA cleaving agents. They have been shown useful as antiproliferative agents in, e.g., small cell lung, prostate, and brain cancers, and in retinoblastoma.

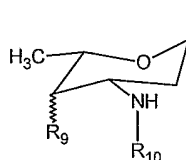
- In another aspect, the Cell Cycle Inhibitor is an Anthracycline. Anthracyclines have the following general structure, where the R groups may be a variety of organic groups:



According to U.S. Patent No. 5,594,158, suitable R groups are: R₁ is CH₃ or CH₂OH; R₂ is daunosamine or H; R₃ and R₄ are independently one of OH, NO₂, NH₂, F, Cl, Br, I, CN, H or groups derived from these; R₅₋₇ are all H

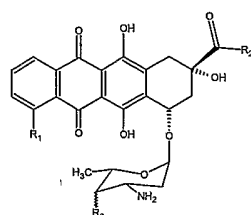
or R_5 and R_6 are H and R_7 and R_8 are alkyl or halogen, or vice versa: R_7 and R_8 are H and R_5 and R_6 are alkyl or halogen.

According to U.S. Patent No. 5,843,903, R_2 may be a conjugated peptide. According to U.S. Patent Nos. 4,215,062 and 4,296,105, R_5 may be
 5 OH or an ether linked alkyl group. R_1 may also be linked to the anthracycline ring by a group other than C(O), such as an alkyl or branched alkyl group having the C(O) linking moiety at its end, such as $-\text{CH}_2\text{CH}(\text{CH}_2\text{-X})\text{C(O)-R}_1$, wherein X is H or an alkyl group (see, e.g., U.S. Patent No. 4,215,062). R_2 may alternately be a group linked by the functional group $=\text{N-NHC(O)-Y}$, where Y is
 10 a group such as a phenyl or substituted phenyl ring. Alternately R_3 may have the following structure:

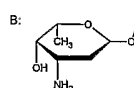
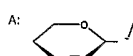


in which R_9 is OH either in or out of the plane of the ring, or is a second sugar moiety such as R_3 . R_{10} may be H or form a secondary amine with a group such
 15 as an aromatic group, saturated or partially saturated 5 or 6 membered heterocyclic having at least one ring nitrogen (see U.S. Patent No. 5,843,903). Alternately, R_{10} may be derived from an amino acid, having the structure – $\text{C(O)CH(NHR}_{11})(\text{R}_{12})$, in which R_{11} is H, or forms a C_{3-4} membered alkylene with R_{12} . R_{12} may be H, alkyl, aminoalkyl, amino, hydroxy, mercapto, phenyl, benzyl
 20 or methylthio (see U.S. Patent No. 4,296,105).

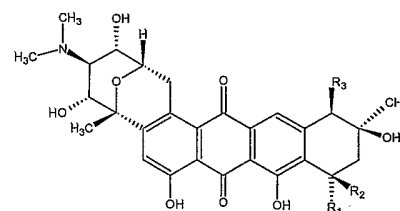
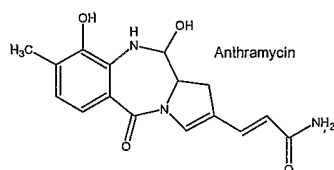
Exemplary Anthracyclines are Doxorubicin, Daunorubicin, Idarubicin, Epirubicin, Pirarubicin, Zorubicin, and Carubicin. Suitable compounds can have the structures:



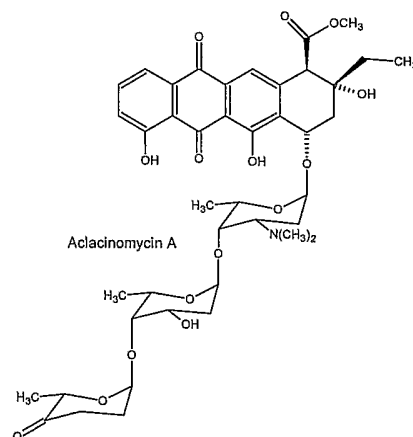
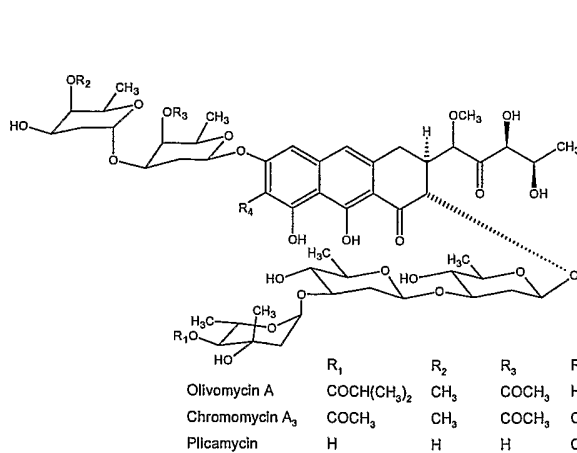
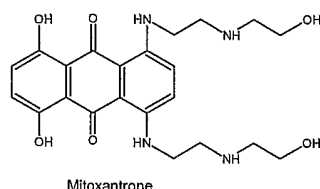
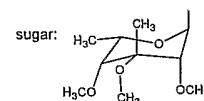
	R ₁	R ₂	R ₃
Doxorubicin:	OCH ₃	CH ₂ OH	OH out of ring plane
Epirubicin: (4' epimer of doxorubicin)	OCH ₃	CH ₂ OH	OH in ring plane
Daunorubicin:	OCH ₃	CH ₃	OH out of ring plane
Idarubicin:	H	CH ₃	OH out of ring plane
Pirarubicin	OCH ₃	OH	A
Zorubicin	OCH ₃	=N-NHC(O)C ₆ H ₅	B
Carubicin	OH	CH ₃	B



Other suitable Anthracyclines are Anthramycin, Mitoxantrone, Menogaril, Nogalamycin, Aclacinomycin A, Olivomycin A, Chromomycin A₃, and Plicamycin having the structures:

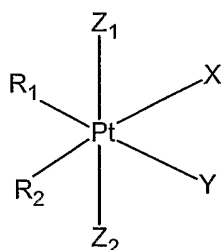


	R ₁	R ₂	R ₃
Menogaril	H	OCH ₃	H
Nogalamycin	O-sugar	H	COOCH ₃



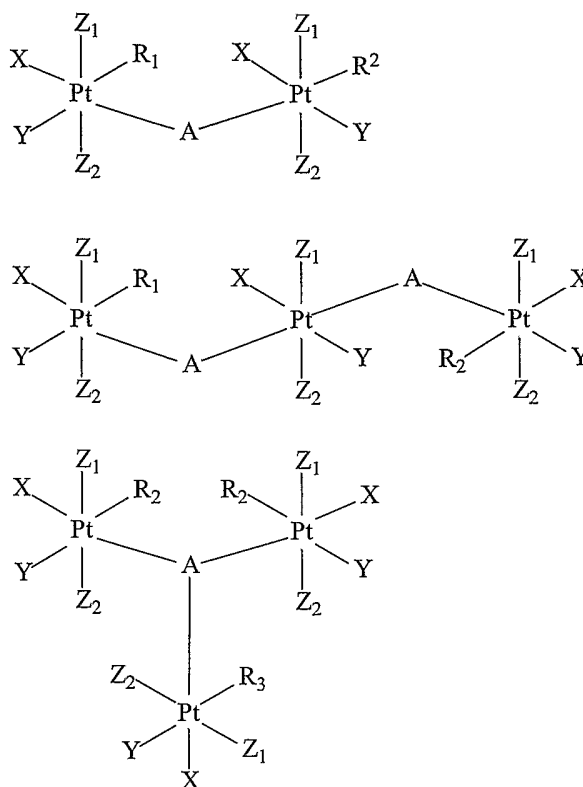
- These compounds are thought to function as Cell Cycle Inhibitors by being Topoisomerase Inhibitors and/or by DNA cleaving agents. They have been shown useful in the treatment of proliferative disorders, including small cell lung; breast; endometrial; head and neck; retinoblastoma; liver; bile duct;
- 5 islet cell; and bladder cancers; and soft tissue sarcoma.

In another aspect, the Cell Cycle Inhibitor is a Platinum compound. In general, suitable platinum complexes may be of Pt(II) or Pt(IV) and have this basic structure:

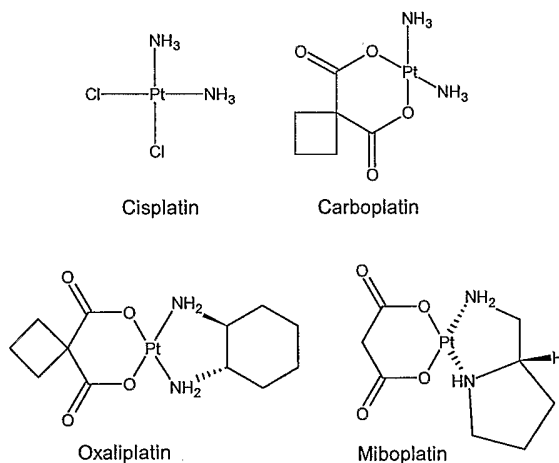


- 10 wherein X and Y are anionic leaving groups such as sulfate, phosphate, carboxylate, and halogen; R₁ and R₂ are alkyl, amine, amino alkyl any may be further substituted, and are basically inert or bridging groups. For Pt(II) complexes Z₁ and Z₂ are non-existent. For Pt(IV) Z₁ and Z₂ may be anionic groups such as halogen, hydroxy, carboxylate, ester, sulfate or phosphate.
- 15 See, e.g., U.S. Patent Nos. 4,588,831 and 4,250,189.

Suitable platinum complexes may contain multiple Pt atoms. See, e.g., U.S. Patent Nos. 5,409,915 and 5,380,897. For example bisplatinum and triplatinum complexes of the type:



Exemplary Platinum compounds are Cisplatin, Carboplatin, Oxaliplatin, and Miboplatin having the structures:



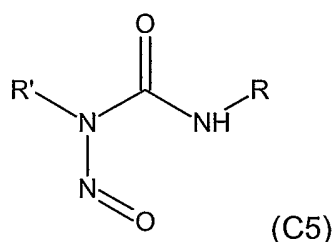
- 5 These compounds are thought to function as Cell Cycle Inhibitors by binding to DNA, *i.e.*, acting as alkylating agents of DNA. These compounds have been shown useful in the treatment of cell proliferative disorders, including, *e.g.*, NSC lung; small cell lung; breast; cervical; brain; head and neck;

esophageal; retinoblastom; liver; bile duct; bladder; penile; and vulvar cancers; and soft tissue sarcoma.

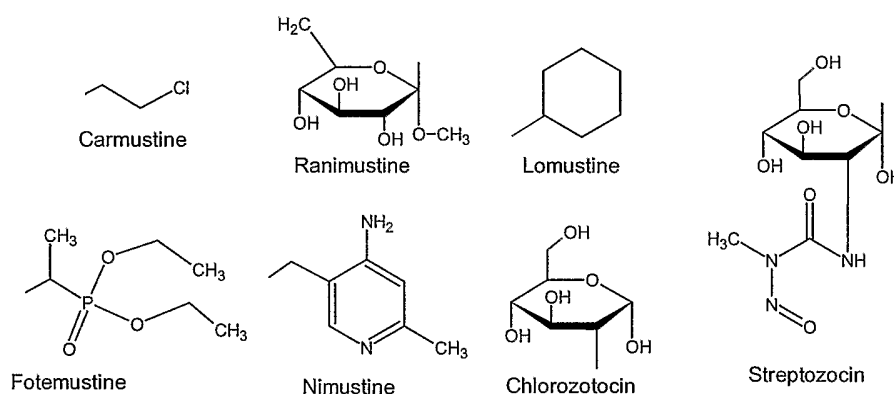
In another aspect, the Cell Cycle Inhibitor is a Nitrosourea.

Nitrosoureae have the following general structure (C5), where typical R groups

5 are shown below.



R Group:

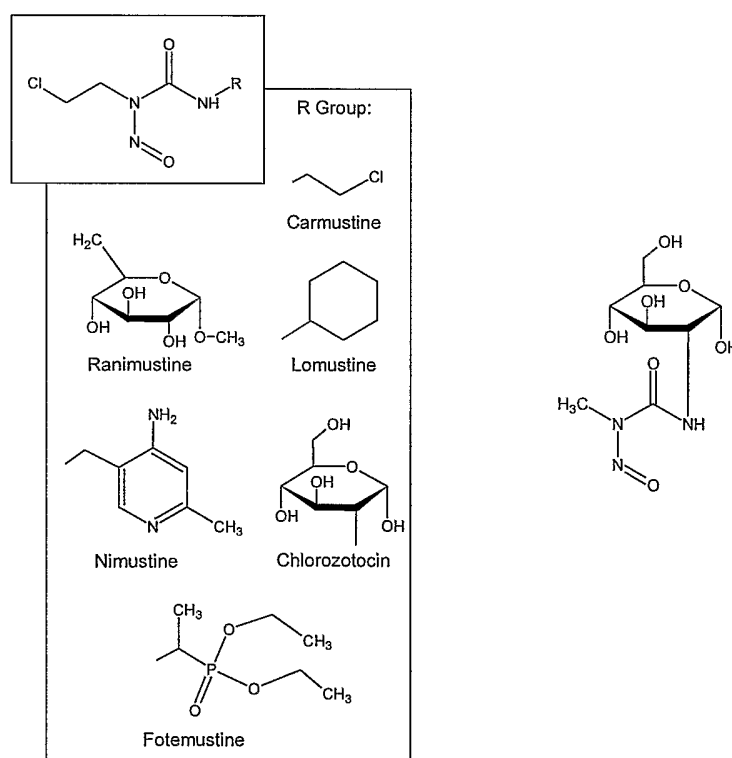


Other suitable R groups include cyclic alkanes, alkanes, halogen substituted groups, sugars, aryl and heteroaryl groups, phosphonyl and sulfonyl groups. As disclosed in U.S. Patent No. 4,367,239, R may suitably be CH₂-C(X)(Y)(Z), wherein X and Y may be the same or different members of the following groups: phenyl, cyclohexyl, or a phenyl or cyclohexyl group substituted with groups such as halogen, lower alkyl (C₁₋₄), trifluore methyl, cyano, phenyl, cyclohexyl, lower alkyloxy (C₁₋₄). Z has the following structure:

15 -alkylene-N-R₁R₂, where R₁ and R₂ may be the same or different members of the following group: lower alkyl (C₁₋₄) and benzyl, or together R₁ and R₂ may form a saturated 5 or 6 membered heterocyclic such as pyrrolidine, piperidine, morfoline, thiomorfoline, N-lower alkyl piperazine, where the heterocyclic may be optionally substituted with lower alkyl groups.

As disclosed in U.S. Patent No. 6,096,923, R and R' of formula (C5) may be the same or different, where each may be a substituted or unsubstituted hydrocarbon having 1-10 carbons. Substitutions may include hydrocarbyl, halo, ester, amide, carboxylic acid, ether, thioether and alcohol groups. As disclosed in U.S. Patent No. 4,472,379, R of formula (C5) may be an amide bond and a pyranose structure (e.g., Methyl 2'-[N-[N-(2-chloroethyl)-N-nitroso-carbamoyl]-glycyl]amino-2'-deoxy- α -D-glucopyranoside). As disclosed in U.S. Patent No. 4,150,146, R of formula (C5) may be an alkyl group of 2 to 6 carbons and may be substituted with an ester, sulfonyl, or hydroxyl group. It may also be substituted with a carboxylic acid or CONH₂ group.

Exemplary Nitrosoureas are BCNU (Carmustine), Methyl-CCNU (Semustine), CCNU (Lomustine), Ranimustine, Nimustine, Chlorozotocin, Fotemustine, Streptozocin, and Streptozocin, having the structures:



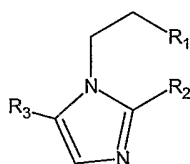
15

These nitrosourea compounds are thought to function as Cell Cycle Inhibitor by binding to DNA, that is, by functioning as DNA alkylating agents. These Cell Cycle Inhibitors have been shown useful in treating cell

proliferative disorders such as, for example, islet cell; small cell lung; melanoma; and brain cancers.

In another aspect, the Cell Cycle Inhibitor is a Nitroimidazole, where exemplary Nitroimidazoles are Metronidazole, Benznidazole,

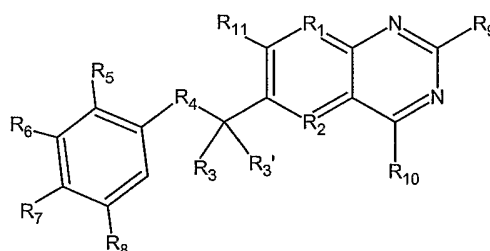
- 5 Etanidazole, and Misonidazole, having the structures:



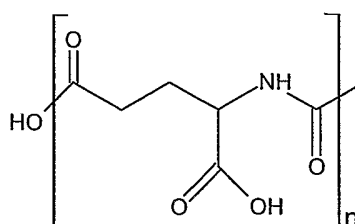
	R ₁	R ₂	R ₃
Metronidazole	OH	CH ₃	NO ₂
Benznidazole	C(O)NHCH ₂ -benzyl	NO ₂	H
Etanidazole	CONHCH ₂ CH ₂ OH	NO ₂	H

Suitable nitroimidazole compounds are disclosed in, *e.g.*, U.S. Patent Nos. 4,371,540 and 4,462,992.

- In another aspect, the Cell Cycle Inhibitor is a Folic acid antagonist, such as Methotrexate or derivatives or analogues thereof, including
- 10 Edatrexate, Trimetrexate, Raltitrexed, Piritrexim, Denopterin, Tomudex, and Pteropterin. Methotrexate analogues have the following general structure:

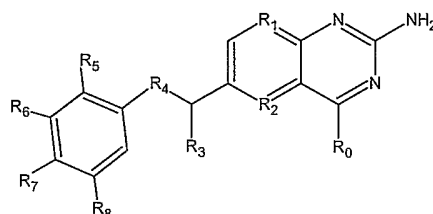


- The identity of the R group may be selected from organic groups, particularly
- 15 those groups set forth in U.S. Patent Nos. 5,166,149 and 5,382,582. For example, R₁ may be N, R₂ may be N or C(CH₃), R₃ and R_{3'} may H or alkyl, *e.g.*, CH₃, R₄ may be a single bond or NR, where R is H or alkyl group. R_{5,6,8} may be H, OCH₃, or alternately they can be halogens or hydro groups. R₇ is a side chain of the general structure:

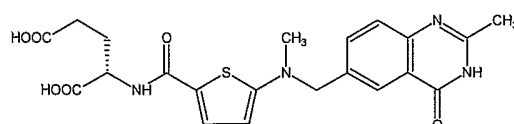
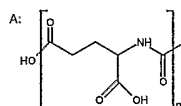


wherein $n = 1$ for methotrexate, $n = 3$ for pteropterin. The carboxyl groups in the side chain may be esterified or form a salt such as a Zn^{2+} salt. R_9 and R_{10} can be NH_2 or may be alkyl substituted.

5 Exemplary folic acid antagonist compounds have the structures:



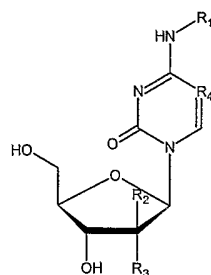
	R_9	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
Methotrexate	NH_2	N	N	H	$N(CH_3)$	H	H	A ($n=1$)	H
Edatrexate	NH_2	N	N	H	$N(CH_2CH_3)$	H	H	A ($n=1$)	H
Trimetrexate	NH_2	N	$C(CH_3)_3$	H	NH	H	OCH_3	OCH_3	OCH_3
Pteropterin	NH_2	N	N	H	$N(CH_3)$	H	H	A ($n=3$)	H
Denopterin	OH	N	N	CH_3	$N(CH_3)$	H	H	A ($n=1$)	H
Piritrexim	NH_2	N	$C(CH_3)_3$	single bond	OCH_3	H	H	OCH_3	H



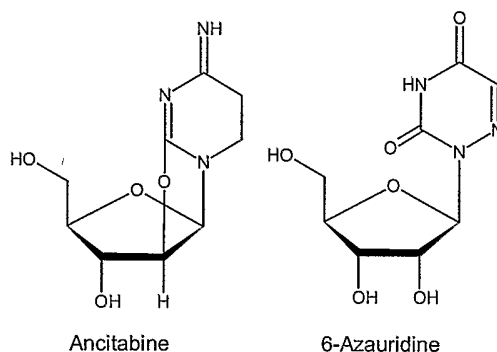
Tomudex

These compounds are thought to function as Cell Cycle Inhibitors by serving as antimetabolites of folic acid. They have been shown useful in the treatment of cell proliferative disorders including, for example, soft tissue sarcoma, small cell lung, breast, brain, head and neck, bladder, and penile cancers.

In another aspect, the Cell Cycle Inhibitor is a Cytidine analogue, such as Cytarabine or derivatives or analogues thereof, including Enocitabine, FMdC ((E)-2'-deoxy-2'-(fluoromethylene)cytidine), Gemcitabine, 5-Azacitidine, Ancitabine, and 6-Azaauridine. Exemplary compounds have the structures:

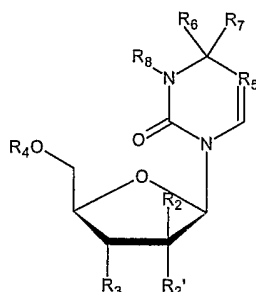


	R ₁	R ₂	R ₃	R ₄
Cytarabine	H	OH	H	CH
Enocitabine	C(O)(CH ₂) ₂₀ CH ₃	OH	H	CH
Gemcitabine	H	F	F	CH
Azacitidine	H	H	OH	N
FMdC	H	CH ₂ F	H	CH



These compounds are thought to function as Cell Cycle Inhibitors as acting as antimetabolites of pyrimidine. These compounds have been shown useful in the treatment of cell proliferative disorders including, for example, pancreatic, breast, cervical, NSC lung, and bile duct cancers.

In another aspect, the Cell Cycle Inhibitor is a Pyrimidine analogue. In one aspect, the Pyrimidine analogues have the general structure:

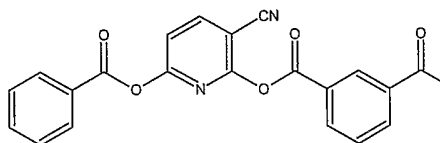


wherein positions 2', 3' and 5' on the sugar ring (R₂, R₃ and R₄, respectively) can be H, hydroxyl, phosphoryl (see, *e.g.*, U.S. Patent No. 4,086,417) or ester (see, *e.g.*, U.S. Patent No. 3,894,000). Esters can be of alkyl, cycloalkyl, aryl or

heterocyclo/aryl types. The 2' carbon can be hydroxylated at either R₂ or R₂', the other group is H. Alternately, the 2' carbon can be substituted with halogens *e.g.*, fluoro or difluoro cytidines such as Gemcytabine. Alternately, the sugar can be substituted for another heterocyclic group such as a furyl

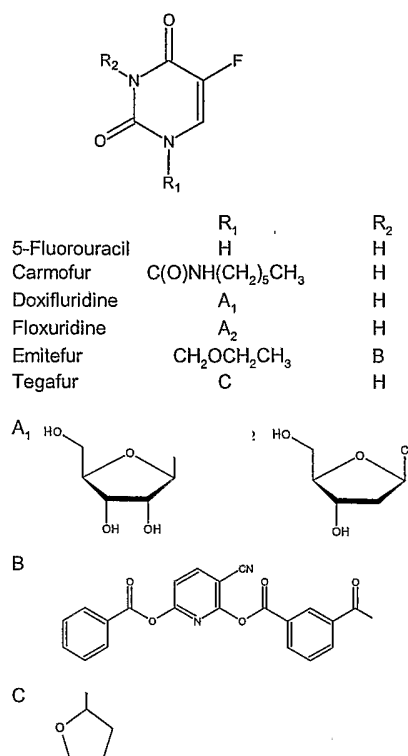
5 group or for an alkane, an alkyl ether or an amide linked alkane such as C(O)NH(CH₂)₅CH₃. The 2° amine can be substituted with an aliphatic acyl (R₁) linked with an amide (*see, e.g.*, U.S. Patent No. 3,991,045) or urethane (*see, e.g.*, U.S. Patent No. 3,894,000) bond. It can also be further substituted to form a quaternary ammonium salt. R₅ in the pyrimidine ring may be N or CR, where

10 R is H, halogen containing groups, or alkyl (*see, e.g.*, U.S. Patent No. 4,086,417). R₆ and R₇ can together can form an oxo group or R₆ = -NH-R₁ and R₇ = H. R₈ is H or R₇ and R₈ together can form a double bond or R₈ can be X, where X is:

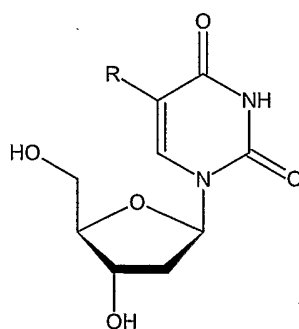


15 Specific pyrimidine analogues are disclosed in U.S. Patent No. 3,894,000 (*see, e.g.*, 2'-O-palmitoyl-ara-cytidine, 3'-O-benzoyl-ara-cytidine, and more than 10 other examples); U.S. Patent No. 3,991,045 (*see, e.g.*, N4-acyl-1-β-D-arabinofuranosylcytosine, and numerous acyl groups derivatives as listed therein, such as palmitoyl).

20 In another aspect, the Cell Cycle Inhibitor is a Fluoro-pyrimidine Analog, such as 5-Fluorouracil, or an analogue or derivative thereof, including Carmofur, Doxifluridine, Emitefur, Tegafur, and Floxuridine. Exemplary compounds have the structures:



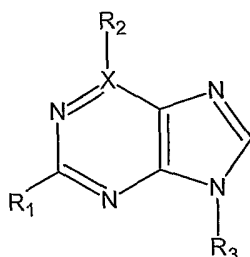
Other suitable Fluoropyrimidine Analogues include 5-FudR (5-fluoro-deoxyuridine), or an analogue or derivative thereof, including 5-iododeoxyuridine (5-IudR), 5-bromodeoxyuridine (5-BudR), Fluorouridine triphosphate (5-FUTP), and Fluorodeoxyuridine monophosphate (5-dFUMP). Exemplary compounds have the structures:



5-Fluoro-2'-deoxyuridine: R = F
 5-Bromo-2'-deoxyuridine: R = Br
 5-Iodo-2'-deoxyuridine: R = I

These compounds are thought to function as Cell Cycle Inhibitors by serving as antimetabolites of pyrimidine.

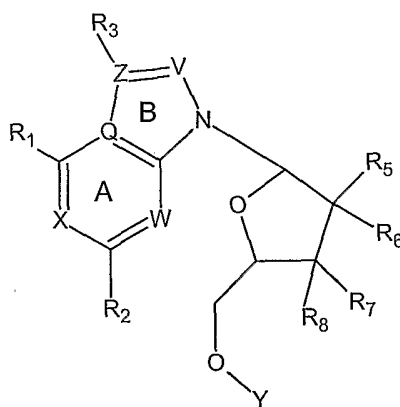
10 In another aspect, the Cell Cycle Inhibitor is a Purine Analogue. Purine analogues have the following general structure:



wherein X is typically carbon; R₁ is H, halogen, amine or a substituted phenyl;
 R₂ is H, a primary, secondary or tertiary amine, a sulfur containing group,
 typically –SH, an alkane, a cyclic alkane, a heterocyclic or a sugar;
 R₃ is H, a
 5 sugar (typically a furanose or pyranose structure), a substituted sugar or a
 cyclic or heterocyclic alkane or aryl group. See, e.g., U.S. Patent No.
 5,602,140 for compounds of this type.

In the case of pentostatin, X-R₂ is –CH₂CH(OH)–. In this case a
 second carbon atom is inserted in the ring between X and the adjacent nitrogen
 10 atom. The X-N double bond becomes a single bond.

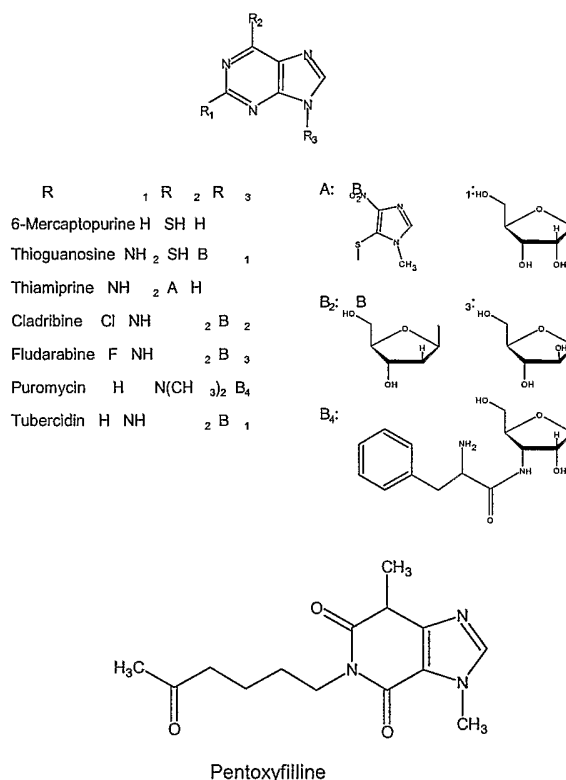
U.S. Patent No. 5,446,139 describes suitable purine analogues of
 the type shown in the following formula:



wherein N signifies nitrogen and V, W, X, Z can be either carbon or nitrogen
 15 with the following provisos. Ring A may have 0 to 3 nitrogen atoms in its
 structure. If two nitrogens are present in ring A, one must be in the W position.
 If only one is present, it must not be in the Q position. V and Q must not be
 simultaneously nitrogen. Z and Q must not be simultaneously nitrogen. If Z is
 nitrogen, R₃ is not present. Furthermore, R₁₋₃ are independently one of H,
 20 halogen, C₁₋₇ alkyl, C₁₋₇ alkenyl, hydroxyl, mercapto, C₁₋₇ alkylthio, C₁₋₇ alkoxy,

C₂₋₇ alkenyloxy, aryl oxy, nitro, primary, secondary or tertiary amine containing group. R₅₋₈ are H or up to two of the positions may contain independently one of OH, halogen, cyano, azido, substituted amino, R₅ and R₇ can together form a double bond. Y is H, a C₁₋₇ alkylcarbonyl, or a mono- di or tri phosphate.

- 5 Exemplary suitable purine analogues include 6-Mercaptopurine, Thioguanosine, Thiamiprine, Cladribine, Fludarabine, Tubercidin, Puromycin, Pentoxifylline; where these compounds may optionally be phosphorylated. Exemplary compounds have the structures:

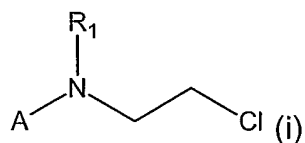


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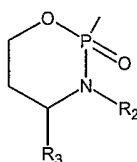
These compounds are thought to function as Cell Cycle Inhibitors by serving as antimetabolites of purine.

- In another aspect, the Cell Cycle Inhibitor is a Nitrogen Mustard. Many suitable Nitrogen Mustards are known and are suitably used as a Cell Cycle Inhibitor in the present invention. Suitable Nitrogen Mustards are also known as cyclophosphamides.
- 15

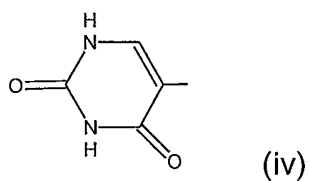
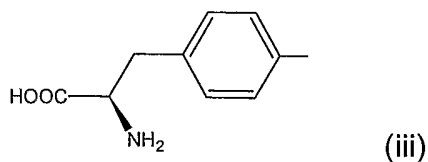
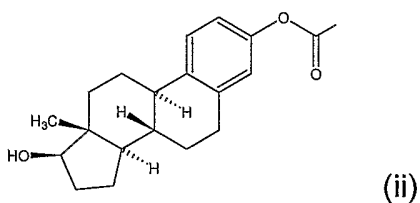
A preferred Nitrogen Mustard has the general structure:



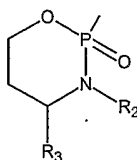
Where A is:



or $-\text{CH}_3$ or other alkane, or chlorinated alkane, typically $\text{CH}_2\text{CH}(\text{CH}_3)\text{Cl}$, or a
 5 polycyclic group such as B, or a substituted phenyl such as C or a heterocyclic
 group such as D.

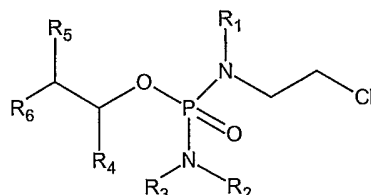


10 Suitable Nitrogen Mustards are disclosed in U.S. Patent No.
 3,808,297, wherein A is:



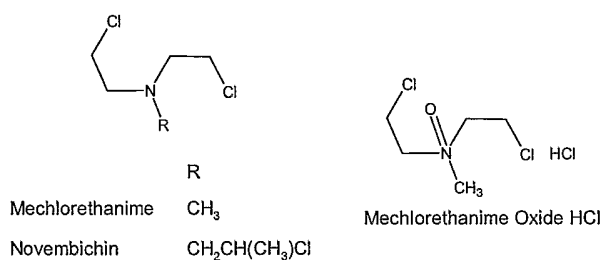
R_{1-2} are H or $\text{CH}_2\text{CH}_2\text{Cl}$; R_3 is H or oxygen-containing groups such as
 15 hydroperoxy; and R_4 can be alkyl, aryl, heterocyclic.

The cyclic moiety need not be intact. See, e.g., U.S. Patent Nos. 5,472,956, 4,908,356, 4,841,085 that describe the following type of structure:

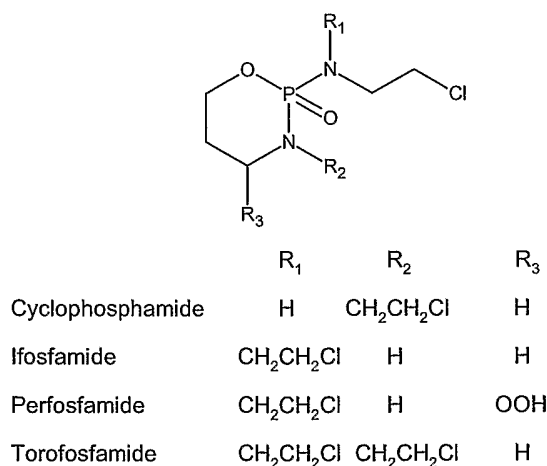


wherein R_1 is H or $\text{CH}_2\text{CH}_2\text{Cl}$, and R_{2-6} are various substituent groups.

- 5 Exemplary Nitrogen Mustards include methylchloroethamine, and analogues or derivatives thereof, including methylchloroethamine oxide hydrochloride, Novembichin, and Mannomustine (a halogenated sugar). Exemplary compounds have the structures:

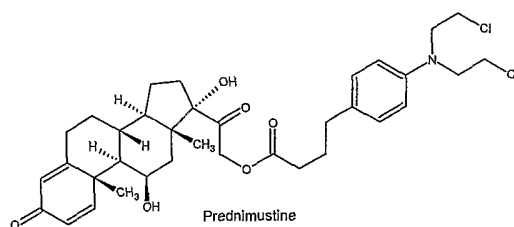
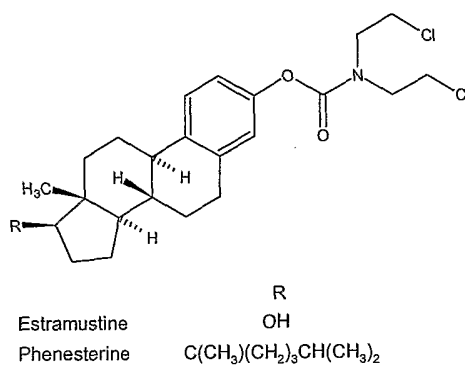


- 10 The Nitrogen Mustard may be Cyclophosphamide, Ifosfamide, Perfosfamide, or Torofosfamide, where these compounds have the structures:

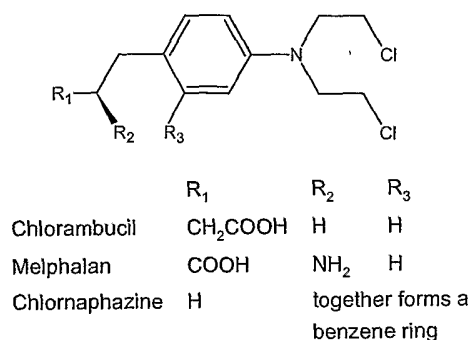


The Nitrogen Mustard may be Estramustine, or an analogue or derivative thereof, including Phenesterine, Prednimustine, and Estramustine

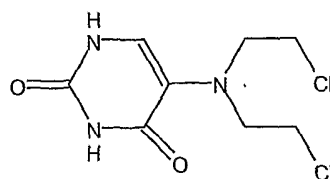
PO₄. Thus, suitable Nitrogen Mustard type Cell Cycle Inhibitors of the present invention have the structures:



5 The Nitrogen Mustard may be Chlorambucil, or an analogue or derivative thereof, including Melphalan and Chlormaphazine. Thus, suitable Nitrogen Mustard type Cell Cycle Inhibitors of the present invention have the structures:

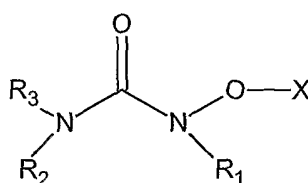


10 The Nitrogen Mustard may be uracil mustard, which has the structure:

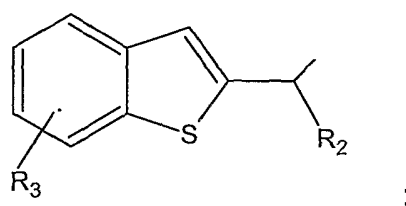


The Nitrogen Mustards are thought to function as cell cycle inhibitors by serving as alkylating agents for DNA.

The cell cycle inhibitor of the present invention may be a hydroxyurea. Hydroxyureas have the following general structure:



Suitable hydroxyureas are disclosed in, for example, U.S. Patent No. 6,080,874, wherein R_1 is:

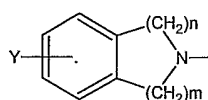


10 and R_2 is an alkyl group having 1-4 carbons and R_3 is one of H, acyl, methyl, ethyl, and mixtures thereof, such as a methylether.

Other suitable hydroxyureas are disclosed in, e.g., U.S. Patent No. 5,665,768, wherein R_1 is a cycloalkenyl group, for example N-[3-[5-(4-fluorophenylthio)-furyl]-2-cyclopenten-1-yl]N-hydroxyurea; R_2 is H or an alkyl
15 group having 1 to 4 carbons and R_3 is H; X is H or a cation.

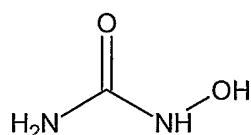
Other suitable hydroxyureas are disclosed in, e.g., U.S. Patent No. 4,299,778, wherein R_1 is a phenyl group substituted with on or more fluorine atoms; R_2 is a cyclopropyl group; and R_3 and X is H.

Other suitable hydroxyureas are disclosed in, e.g., U.S. Patent
20 No. 5,066,658, wherein R_2 and R_3 together with the adjacent nitrogen form:



wherein m is 1 or 2, n is 0-2 and Y is an alkyl group.

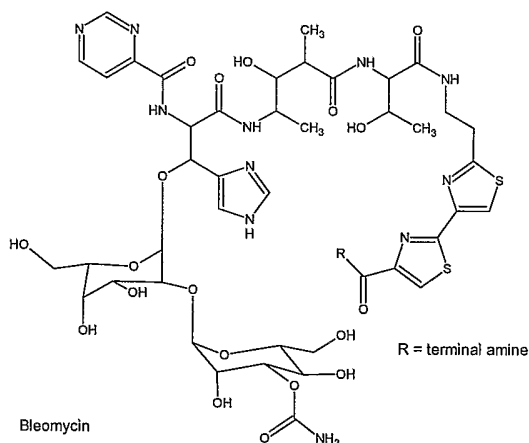
In one aspect, the hydroxy urea has the structure:



Hydroxyurea

- 5 Hydroxyureas are thought to function as Cell Cycle Inhibitors by serving to inhibit DNA synthesis.

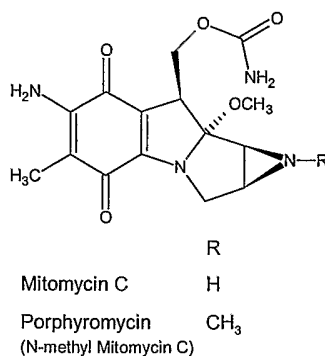
In another aspect, the Cell Cycle Inhibitor is a Belomycin, such as Bleomycin A₂, which have the structures:



- 10 Bleomycin A₂: R = (CH₃)₂S⁺(CH₂)₃NH-

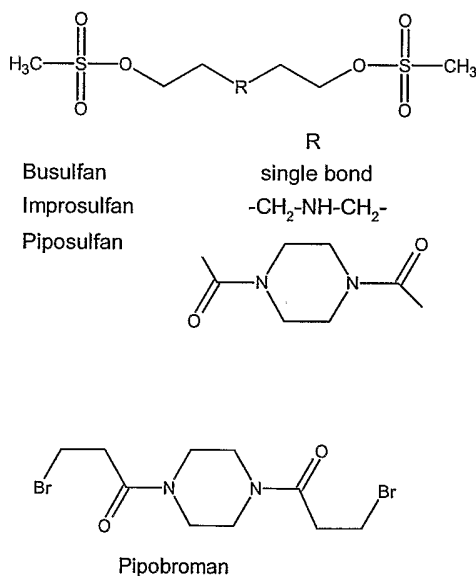
Belomycins are thought to function as Cell Cycle Inhibitors by cleaving DNA. They have been shown useful in the treatment of cell proliferative disorder such as, e.g., penile cancer.

- In another aspect, the Cell Cycle Inhibitor is a Mytomycin, such as
 15 Mitomycin C, or an analogue or derivative thereof, such as Porphyromycin.
 Suitable compounds have the structures:



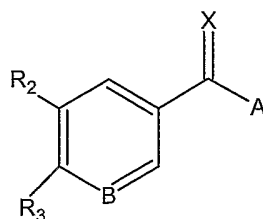
These compounds are thought to function as Cell Cycle Inhibitors by serving as DNA alkylating agents.

In another aspect, the Cell Cycle Inhibitor is an Alkyl Sulfonate, such as Busulfan, or an analogue or derivative thereof, such as Treosulfan, Improsulfan, Pipsulfan, and Pipobroman. Exemplary compounds have the structures:



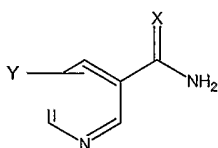
10 These compounds are thought to function as Cell Cycle Inhibitors by serving as DNA alkylating agents.

In another aspect, the Cell Cycle Inhibitor is a Benzamide. In yet another aspect, the Cell Cycle Inhibitor is a Nicotinamide. These compounds have the basic structure:



wherein X is either O or S; A is commonly NH₂ or it can be OH or an alkoxy group; B is N or C-R₄, where R₄ is H or an ether-linked hydroxylated alkane such as OCH₂CH₂OH, the alkane may be linear or branched and may contain
 5 one or more hydroxyl groups. Alternately, B may be N-R₅ in which case the double bond in the ring involving B is a single bond. R₅ may be H, and alkyl or an aryl group (see, e.g., U.S. Patent No. 4,258,052); R₂ is H, OR₆, SR₆ or NHR₆, where R₆ is an alkyl group; and R₃ is H, a lower alkyl, an ether linked lower alkyl such as -O-Me or -O-Ethyl (see, e.g., U.S. Patent No. 5,215,738).

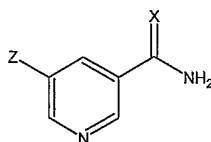
10 Suitable Benzamide compounds have the structures:



Benzamides
 X = O or S
 Y = H, OR, CH₃, acetoxy
 Z = H, OR, SR, NHR
 R = alkyl group

where additional compounds are disclosed in U.S. Patent No. 5,215,738, (listing some 32 compounds).

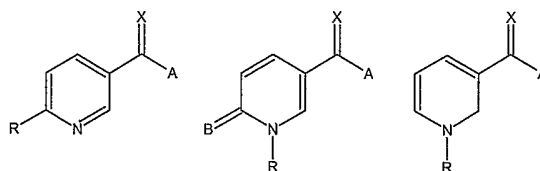
Suitable Nicotinamide compounds have the structures:



Nicotinamides
 X = O or S
 Z = H, OR, SR, NHR
 R = alkyl group

15

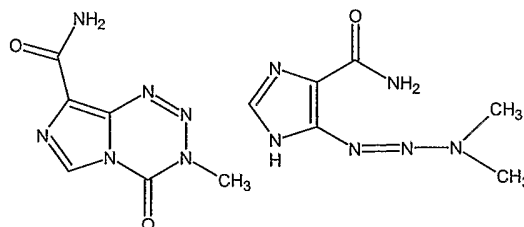
where additional compounds are disclosed in U.S. Patent No. 5,215,738 (listing some 58 compounds, e.g., 5-OH nicotinamide, 5-aminonicotinamide, 5-(2,3-dihydroxypropoxy) nicotinamide), and compounds having the structures:



Nicotinamides
 X = O or S (only O is described)
 A = OH, NH₂, alkoxy
 B = O
 R = alkyl or aryl group

- 5 and U.S. Patent No. 4,258,052 (listing some 46 compounds, e.g., 1-methyl-6-keto-1,6-dihydronicotinic acid).

In one aspect, the Cell Cycle Inhibitor is a Tetrazine compound, such as Temozolomide, or an analogue or derivative thereof, including Dacarbazine. Suitable compounds have the structures:

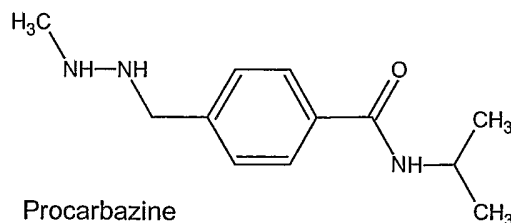


Temozolomide

Dacarbazine

10

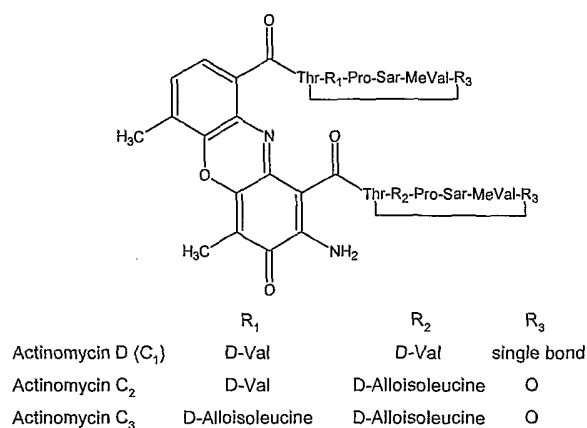
Another suitable Tetrazine Compound is Procarbazine, including HCl and HBr salts, having the structure:



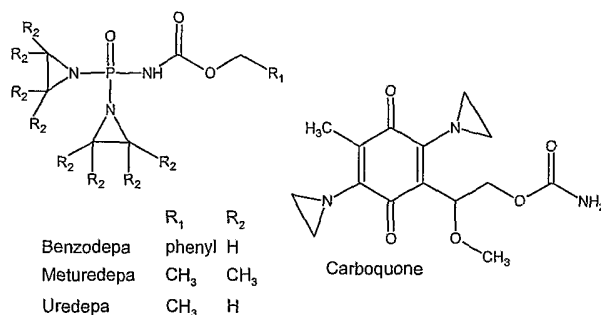
Procarbazine

- In another aspect, the Cell Cycle Inhibitor is Actinomycin D, or
 15 other members of this family, including Dactinomycin, Actinomycin C₁,

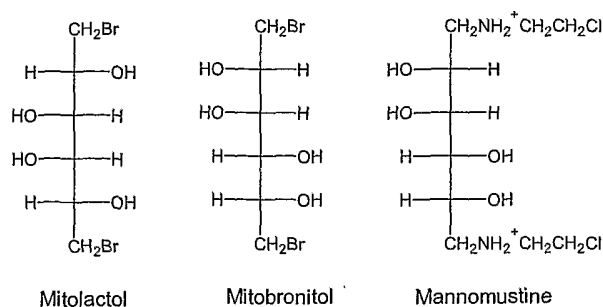
Actinomycin C₂, Actinomycin C₃, and Actinomycin F₁. Suitable compounds have the structures:



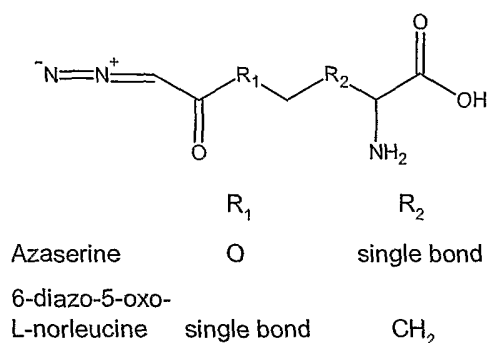
In another aspect, the Cell Cycle Inhibitor is an Aziridine compound, such as Benzodepa, or an analogue or derivative thereof, including Meturedepa, Uredepa, and Carboquone. Suitable compounds have the structures:



In another aspect, the Cell Cycle Inhibitor is a Halogenated Sugar, such as Mitolactol, or an analogue or derivative thereof, including Mitobronitol and Mannomustine. Suitable compounds have the structures:



In another aspect, the Cell Cycle Inhibitor is a Diazo compound, such as Azaserine, or an analogue or derivative thereof, including 6-diazo-5-oxo-L-norleucine and 5-diazouracil (also a pyrimidine analog). Suitable compounds have the structures:



5

Other compounds that may serve as Cell Cycle Inhibitors according to the present invention are Pazelliptine; Wortmannin; Metoclopramide; RSU; Buthionine sulfoxime; Tumeric; Curcumin; AG337, a thymidylate synthase inhibitor; Levamisole; Lentinan, a polysaccharide;

10 Razoxane, an EDTA analog; Indomethacin; Chlorpromazine; α and β interferon; MnBOPP; Gadolinium texaphyrin; 4-amino-1,8-naphthalimide; Staurosporine derivative of CGP; and SR-2508.

Thus, in one aspect, the Cell Cycle Inhibitor is a DNA alkylating agent. In another aspect, the Cell Cycle Inhibitor is an anti-microtubule agent.

15 In another aspect, the Cell Cycle Inhibitor is a Topoisomerase inhibitor. In another aspect, the Cell Cycle Inhibitor is a DNA cleaving agent. In another aspect, the Cell Cycle Inhibitor is an antimetabolite. In another aspect, the Cell Cycle Inhibitor functions by inhibiting adenosine deaminase (e.g., as a purine analog). In another aspect, the Cell Cycle Inhibitor functions by inhibiting

20 purine ring synthesis and/or as a nucleotide interconversion inhibitor (e.g., as a purine analogue such as mercaptopurine). In another aspect, the Cell Cycle Inhibitor functions by inhibiting dihydrofolate reduction and/or as a thymidine monophosphate block (e.g., methotrexate). In another aspect, the Cell Cycle Inhibitor functions by causing DNA damage (e.g., Bleomycin). In another

25 aspect, the Cell Cycle Inhibitor functions as a DNA intercalation agent and/or

RNA synthesis inhibition (e.g., Doxorubicin). In another aspect, the Cell Cycle Inhibitor functions by inhibiting pyrimidine synthesis (e.g., N-phosphonoacetyl-L-Aspartate). In another aspect, the Cell Cycle Inhibitor functions by inhibiting ribonucleotides (e.g., hydroxyurea). In another aspect, the Cell Cycle Inhibitor
5 functions by inhibiting thymidine monophosphate (e.g., 5-fluorouracil). In another aspect, the Cell Cycle Inhibitor functions by inhibiting DNA synthesis (e.g., Cytarabine). In another aspect, the Cell Cycle Inhibitor functions by causing DNA adduct formation (e.g., platinum compounds). In another aspect, the Cell Cycle Inhibitor functions by inhibiting protein synthesis (e.g., L-
10 Asparaginase). In another aspect, the Cell Cycle Inhibitor functions by inhibiting microtubule function (e.g., taxanes). In another aspect, the Cell Cycle Inhibitors acts at one or more of the steps in the biological pathway shown in FIG. 3.

Additional Cell Cycle Inhibitors useful in the present invention, as well as a discussion of their mechanisms of action, may be found in Hardman
15 J.G., Limbird L.E. Molinoff R.B., Ruddon R. W., Gilman A.G. editors, Chemotherapy of Neoplastic Diseases in Goodman and Gilman's The Pharmacological Basis of Therapeutics Ninth Edition, McGraw-Hill Health Professions Division, New York, 1996, pages 1225-1287. See also U.S. Patent Nos. 3,387,001; 3,808,297; 3,894,000; 3,991,045; 4,012,390; 4,057,548;
20 4,086,417; 4,144,237; 4,150,146; 4,210,584; 4,215,062; 4,250,189; 4,258,052; 4,259,242; 4,296,105; 4,299,778; 4,367,239; 4,374,414; 4,375,432; 4,472,379; 4,588,831; 4,639,456; 4,767,855; 4,828,831; 4,841,045; 4,841,085; 4,908,356; 4,923,876; 5,030,620; 5,034,320; 5,047,528; 5,066,658; 5,166,149; 5,190,929; 5,215,738; 5,292,731; 5,380,897; 5,382,582; 5,409,915; 5,440,056; 5,446,139;
25 5,472,956; 5,527,905; 5,552,156; 5,594,158; 5,602,140; 5,665,768; 5,843,903; 6,080,874; 6,096,923; and RE030561.

Numerous polypeptides, proteins and peptides, as well as nucleic acids that encode such proteins, can also be used therapeutically as cell cycle inhibitors. This is accomplished by delivery by a suitable vector or gene
30 delivery vehicle which encodes a cell cycle inhibitor (Walther & Stein, Drugs 60(2):249-71, Aug 2000; Kim *et al.*, Archives of Pharmacol Res. 24(1):1-15,

Feb 2001; and Anwer *et al.*, Critical Reviews in Therapeutic Drug Carrier Systems 17(4):377-424, 2000. Genes encoding proteins that modulate cell cycle include the INK4 family of genes (US 5,889,169; US 6,033,847), ARF-p19 (US 5,723,313), p21^{WAF1/CIP1} and p27^{KIP1} (WO 95/13375; WO 98/35022),
 5 p27^{KIP1} (WO 97/38091), p57^{KIP2} (US 6,025,480), ATM/ATR (WO 99/04266), Gadd 45 (US 5,858,679), Myt1 (US 5,744,349), Wee1 (WO 99/49061) smad 3 and smad 4 (US 6,100,032), 14-3-3 σ (WO 99/31240), GSK3 β (Stambolic, V. and Woodgett, J. R., Biochem Journal 303: 701-704, 1994), HDAC-1 (Furukawa, Y. *et al.*, Cytogenet. Cell Genet. 73: 130-133, 1996; Taunton, J. *et al.*, Science 272: 408-411, 1996), PTEN (WO 99/02704), p53 (U.S. Patent No.
 10 5,532,220), p33^{ING1} (U.S. Patent No. 5,986,078), Retinoblastoma (EPO 390530), and NF-1 (WO 92/00387).

A wide variety of gene delivery vehicles may be utilized to deliver and express the proteins described herein, including for example, viral vectors such as
 15 retroviral vectors (*e.g.*, U.S. Patent Nos. 5,591,624, 5,716,832, 5,817,491, 5,856,185, 5,888,502, 6,013,517, and 6,133,029; as well as subclasses of retroviral vectors such as lentiviral vectors (*e.g.*, PCT Publication Nos. WO 00/66759, WO 00/00600, WO 99/24465, WO 98/51810, WO 99/51754, WO 99/31251, WO 99/30742, and WO 99/15641)), alphavirus based vector systems
 20 (*e.g.*, U.S. Patent Nos. 5,789,245, 5,814,482, 5,843,723, and 6,015,686), adeno-associated virus-based system (*e.g.*, U.S. Patent Nos. 6,221,646, 6,180,613, 6,165,781, 6,156,303, 6,153,436, 6,093,570, 6,040,183, 5,989,540, 5,856,152, and 5,587,308) and adenovirus-based systems (*e.g.*, U.S. Patent Nos. 6,210,939, 6,210,922, 6,203,975, 6,194,191, 6,140,087, 6,113,913, 6,080,569, 6,063,622,
 25 6,040,174, 6,033,908, 6,033,885, 6,020,191, 6,020,172, 5,994,128, and 5,994,106), herpesvirus based or 'amplicon' systems (*e.g.*, U.S. Patent Nos. 5,928,913, 5,501,979, 5,830,727, 5,661,033, 4,996,152 and 5,965,441) and, "naked DNA" based systems (*e.g.*, U.S. Patent Nos. 5,580,859 and 5,910,488) (all of which are, as noted above, incorporated by reference in their entirety).

30 Within one aspect of the invention, ribozymes or antisense sequences (as well as gene therapy vehicles which can deliver such sequences)

can be utilized as cell cycle inhibitors. One representative example of such inhibitors is disclosed in PCT Publication No. WO 00/32765 (which, as noted above, is incorporated by reference in its entirety).

5. Cyclin Dependent Protein Kinase Inhibitors

5 In another embodiment, the pharmacologically active compound is a cyclin dependent protein kinase inhibitor (e.g., R-roscovitine, CYC-101, CYC-103, CYC-400, MX-7065, alvocidib (4H-1-Benzopyran-4-one, 2-(2-chlorophenyl)-5,7-dihydroxy-8-(3-hydroxy-1-methyl-4-piperidiny)-, cis-(-)- [CAS]), SU-9516, AG-12275, PD-0166285, CGP-79807, fascaplysin, GW-8510
10 (Benzenesulfonamide, 4-[[[(Z)-(6,7-dihydro-7-oxo-8H-pyrrolo[2,3-g]benzothiazol-8-ylidene)methyl]amino]-N-(3-hydroxy-2,2-dimethylpropyl)- [CAS]), GW-491619, Indirubin 3' monoxime, GW8510) or an analogue or derivative thereof.

6. EGF (Epidermal Growth Factor) Receptor Kinase Inhibitors

In another embodiment, the pharmacologically active compound
15 is an EGF (epidermal growth factor) kinase inhibitor (e.g., erlotinib (4-Quinazolinamine, N-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy)-, monohydrochloride [CAS]), VIATRIS (Viatris GMBH & Co., Germany), erbstatin, BIBX-1382, gefitinib (4-Quinazolinamine, N-(3-chloro-4-fluorophenyl)-7-methoxy-6-(3-(4-morpholinyl)propoxy) [CAS])) or an analogue or derivative
20 thereof.

7. Elastase Inhibitors

In another embodiment, the pharmacologically active compound is an elastase inhibitor (e.g., ONO-6818, sivelestat sodium hydrate (Glycine, N-[2-[[[4-(2,2-dimethyl-1-oxopropoxy)phenyl]sulfonyl]amino]benzoyl]- [CAS]),
25 erdosteine (Acetic acid, [[2-oxo-2-[(tetrahydro-2-oxo-3-thienyl)amino]ethyl]thio]- [CAS]), MDL-100948A, MDL-104238 (N-[4-(4-morpholinylcarbonyl)benzoyl]-L-valyl-N'-[3,3,4,4,4-pentafluoro-1-(1-methylethyl)-2-oxobutyl]-L-2-azetamide), MDL-27324 (L-Prolinamide, N-[[5-(dimethylamino)-1-naphthalenyl]sulfonyl]-L-

alanyl-L-alanyl-N-[3,3,3-trifluoro-1-(1-methylethyl)-2-oxopropyl]-, (S)- [CAS]),
 SR-26831 (Thieno[3,2-c]pyridinium, 5-[(2-chlorophenyl)methyl]-2-(2,2-dimethyl-
 1-oxopropoxy)-4,5,6,7-tetrahydro-5-hydroxy- [CAS]), Win-68794, Win-63110,
 SSR-69071 (2-(9(2-Piperidinoethoxy)-4-oxo-4H-pyrido[1,2-a]pyrimidin-2-
 5 yloxymethyl)-4-(1-methylethyl)-6-methoxy-1,2-benzisothiazol-3(2H)-one-1,1-
 dioxide), (N(Alpha)-(1-adamantylsulfonyl)N(epsilon)-succinyl-L-lysyl-L-prolyl-L-
 valinal), Ro-31-3537 (N(Alpha)-(1-adamantanesulphonyl)-N-(4-carboxybenzoyl)-
 L-lysyl-alanyl-L-valinal), R-665, FCE-28204, ((6R,7R)-2-(Benzoyloxy)-7-
 methoxy-3-methyl-4-pivaloyl-3-cephem 1,1-dioxide), 1,2-Benzisothiazol-3(2H)-
 10 one, 2-(2,4-dinitrophenyl)-, 1,1-dioxide [CAS], L-658758 (L-Proline, 1-[[3-
 [(acetyloxy)methyl]-7-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-2-
 yl]carbonyl]-, S,S-dioxide, (6R-cis)- [CAS]), L-659286 (Pyrrolidine, 1-[[7-
 methoxy-8-oxo-3-[[[(1,2,5,6-tetrahydro-2-methyl-5,6-dioxo-1,2,4-triazin-3-
 yl)thio]methyl]-5-thia-1-azabicyclo[4.2.0]oct-2-en-2-yl]carbonyl]-, S,S-dioxide,
 15 (6R-cis)- [CAS]), L-680833 (Benzeneacetic acid, 4-[[[3,3-diethyl-1-[[[1-(4-
 methylphenyl)butyl]amino]carbonyl]-4-oxo-2-azetidinyloxy]-, [S-(R*,S*)]- [CAS])
) or an analogue or derivative thereof.

8. Factor Xa Inhibitors

In another embodiment, the pharmacologically active compound
 20 is a factor Xa inhibitor (e.g., CY-222, fondaparinux sodium (Alpha-D-
 Glucopyranoside, methyl O-2-deoxy-6-O-sulfo-2-(sulfoamino)-Alpha-D-
 glucopyranosyl-(1-4)-O-β-D-glucopyranuronosyl-(1-4)-O-2-deoxy-3,6-di-O-
 sulfo-2-(sulfoamino)-Alpha-D-glucopyranosyl-(1-4)-O-2-O-sulfo-Alpha-L-
 idopyranuronosyl-(1-4)-2-deoxy-2-(sulfoamino)-, 6-(hydrogen sulfate) [CAS]),
 25 danaparoid sodium) or an analogue or derivative thereof.

9. Farnesyltransferase Inhibitors

In another embodiment, the pharmacologically active compound
 is a farnesyltransferase inhibitor (e.g., dichlorobenzoprim (2,4-diamino-5-[4-
 (3,4-dichlorobenzylamino)-3-nitrophenyl]-6-ethylpyrimidine), B-581, B-956 (N-

[8(R)-Amino-2(S)-benzyl-5(S)-isopropyl-9-sulfanyl-3(Z),6(E)-nonadienoyl]-L-methionine), OSI-754, perillyl alcohol (1-Cyclohexene-1-methanol, 4-(1-methylethenyl)- [CAS], RPR-114334, Ionafarnib (1-Piperidinecarboxamide, 4-[2-[4-[(11R)-3,10-dibromo-8-chloro-6,11-dihydro-5H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-yl]-1-piperidinyl]-2-oxoethyl]- [CAS]), Sch-48755, Sch-226374, (7,8-Dichloro-5H-dibenzo[b,e][1,4]diazepin-11-yl)-pyridin-3-ylmethylamine, J-104126, L-639749, L-731734 (Pentanamide, 2-[[2-[(2-amino-3-mercaptopropyl)amino]-3-methylpentyl]amino]-3-methyl-N-(tetrahydro-2-oxo-3-furanyl)-, [3S-[3R*[2R*[2R*(S*),3S*],3R*]]]- [CAS]), L-744832 (Butanoic acid, 2-((2-((2-((2-amino-3-mercaptopropyl)amino)-3-methylpentyl)oxy)-1-oxo-3-phenylpropyl)amino)-4-(methylsulfonyl)-, 1-methylethyl ester, (2S-(1(R*(R*)),2R*(S*),3R*))- [CAS]), L-745631 (1-Piperazinepropanethiol, β-amino-2-(2-methoxyethyl)-4-(1-naphthalenylcarbonyl)-, (βR,2S)- [CAS]), N-acetyl-N-naphthylmethyl-2(S)-[(1-(4-cyanobenzyl)-1H-imidazol-5-yl)acetyl]amino-3(S)-methylpentamine, (2Alpha)-2-hydroxy-24,25-dihydroxylanost-8-en-3-one, BMS-316810, UCF-1-C (2,4-Decadienamide, N-(5-hydroxy-5-(7-((2-hydroxy-5-oxo-1-cyclopenten-1-yl)amino-oxo-1,3,5-heptatrienyl)-2-oxo-7-oxabicyclo(4.1.0)hept-3-en-3-yl)-2,4,6-trimethyl-, (1S-(1Alpha,3(2E,4E,6S*),5Alpha,5(1E,3E,5E),6Alpha))- [CAS]), UCF-116-B) or an analogue or derivative thereof.

10. Fibrinogen Antagonists

In another embodiment, the pharmacologically active compound is a fibrinogen antagonist (e.g., 2(S)-[(p-Toluenesulfonyl)amino]-3-[[[5,6,7,8-tetrahydro-4-oxo-5-[2-(piperidin-4-yl)ethyl]-4H-pyrazolo-[1,5-a][1,4]diazepin-2-yl]carbonyl]-amino]propionic acid, streptokinase (Kinase (enzyme-activating), strepto- [CAS]), urokinase (Kinase (enzyme-activating), uro- [CAS]), plasminogen activator, pamiteplase, monteplase, heberkinase, anistreplase, alteplase, pro-urokinase, picotamide (1,3-Benzenedicarboxamide, 4-methoxy-N,N'-bis(3-pyridinylmethyl)- [CAS])) or an analogue or derivative thereof.

11. Guanylate Cyclase Stimulants

In another embodiment, the pharmacologically active compound is a guanylate cyclase stimulant (e.g., isosorbide-5-mononitrate (D-Glucitol, 1,4:3,6-dianhydro-, 5-nitrate [CAS])) or an analogue or derivative thereof.

12. Heat Shock Protein 90 Antagonists

In another embodiment, the pharmacologically active compound is a heat shock protein 90 antagonist (e.g., geldanamycin; NSC-33050 (17-Allylaminogeldanamycin), rifabutin (Rifamycin XIV, 1',4-didehydro-1-deoxy-1,4-dihydro-5'-(2-methylpropyl)-1-oxo-[CAS]), 17AAG), or an analogue or derivative thereof.

13. HMGCoA Reductase Inhibitors

In another embodiment, the pharmacologically active compound is an HMGCoA reductase inhibitor (e.g., BCP-671, BB-476, fluvastatin (6-Heptenoic acid, 7-[3-(4-fluorophenyl)-1-(1-methylethyl)-1H-indol-2-yl]-3,5-dihydroxy-, monosodium salt, [R*,S*-(E)]-(±) [CAS]), dalvastatin (2H-Pyran-2-one, 6-(2-(2-(2-(4-fluoro-3-methylphenyl)-4,4,6,6-tetramethyl-1-cyclohexen-1-yl)ethenyl)tetrahydro)-4-hydroxy-, (4Alpha,6β(E))-(+/-) [CAS]), glenvastatin (2H-Pyran-2-one, 6-[2-[4-(4-fluorophenyl)-2-(1-methylethyl)-6-phenyl-3-pyridinyl]ethenyl]tetrahydro-4-hydroxy-, [4R-[4Alpha,6β(E)]]- [CAS]), S-2468, N-(1-oxododecyl)-4Alpha,10-dimethyl-8-aza-trans-decal-3β-ol, atorvastatin calcium (1H-Pyrrole-1-heptanoic acid, 2-(4-fluorophenyl)-β,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-, calcium salt [R-(R*,R*)]- [CAS]), CP-83101 (6,8-Nonadienoic acid, 3,5-dihydroxy-9,9-diphenyl-, methyl ester, [R*,S*-(E)]-(+/-) [CAS]), pravastatin (1-Naphthaleneheptanoic acid, 1,2,6,7,8,8a-hexahydro-β,δ,6-trihydroxy-2-methyl-8-(2-methyl-1-oxobutoxy)-, monosodium salt, [1S-[1Alpha(βS*,δS*),2Alpha,6Alpha,8β(R*),8aAlpha]]- [CAS]), U-20685, pitavastatin (6-Heptenoic acid, 7-[2-cyclopropyl-4-(4-fluorophenyl)-3-quinolinyl]-3,5-dihydroxy-, calcium salt (2:1), [S-[R*,S*-(E)]]- [CAS]), N-((1-methylpropyl)carbonyl)-8-[2-(tetrahydro-4-hydroxy-6-oxo-2H-

- pyran-2-yl)ethyl]-perhydro-isoquinoline, dihydromevinolin (Butanoic acid, 2-methyl-, 1,2,3,4,4a,7,8,8a-octahydro-3,7-dimethyl-8-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)ethyl]-1-naphthalenylester[1Alpha(R*),3Alpha,4aAlpha,7β,8β(2S*,4S*),8aβ]]- [CAS]), HBS-107, dihydromevinolin (Butanoic acid, 2-methyl-, 1,2,3,4,4a,7,8,8a-octahydro-3,7-dimethyl-8-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)ethyl]-1-naphthalenyl ester[1Alpha(R*),3Alpha,4aAlpha,7β,8β(2S*,4S*),8aβ]]- [CAS]), L-669262 (Butanoic acid, 2,2-dimethyl-, 1,2,6,7,8,8a-hexahydro-3,7-dimethyl-6-oxo-8-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)ethyl]-1-naphthalenyl[1S-[1Alpha,7β,8β(2S*,4S*),8aβ]]- [CAS]), simvastatin (Butanoic acid, 2,2-dimethyl-, 1,2,3,7,8,8a-hexahydro-3,7-dimethyl-8-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)ethyl]-1-naphthalenyl ester, [1S-[1Alpha,3Alpha,7β,8β(2S*,4S*),8aβ]]- [CAS]), rosuvastatin calcium (6-Heptenoic acid, 7-(4-(4-fluorophenyl)-6-(1-methylethyl)-2-(methyl(methylsulfonyl)amino)-5-pyrimidinyl)-3,5-dihydroxy- calcium salt (2:1) (S-(R*, S*-(E))) [CAS]), meglutol (2-hydroxy-2-methyl-1,3-propanedicarboxylic acid), lovastatin (Butanoic acid, 2-methyl-, 1,2,3,7,8,8a-hexahydro-3,7-dimethyl-8-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)ethyl]-1-naphthalenyl ester, [1S-[1.alpha.(R*),3Alpha,7β,8β(2S*,4S*),8aβ]]- [CAS])) or an analogue or derivative thereof.

20 14. Hydroorotate Dehydrogenase Inhibitors

- In another embodiment, the pharmacologically active compound is a hydroorotate dehydrogenase inhibitor (e.g., leflunomide (4-Isoxazolecarboxamide, 5-methyl-N-[4-(trifluoromethyl)phenyl]- [CAS]), laflunimus (2-Propenamide, 2-cyano-3-cyclopropyl-3-hydroxy-N-(3-methyl-4(trifluoromethyl)phenyl)-, (Z)-[CAS])) or an analogue or derivative thereof.

15. IKK2 Inhibitors

In another embodiment, the pharmacologically active compound is an IKK2 inhibitor (e.g., MLN-120B, SPC-839) or an analogue or derivative thereof.

16. IL-1, ICE, and IRAK Antagonists

In another embodiment, the pharmacologically active compound is an IL-1, ICE ((aryl)acyloxymethyl ketone) & IRAK antagonist (e.g., VX-765 (Vertex Pharmaceuticals, Cambridge, MA), VX-740 (Vertex Pharmaceuticals),

5 E-5090 (2-Propenoic acid, 3-(5-ethyl-4-hydroxy-3-methoxy-1-naphthalenyl)-2-methyl-, (Z)- [CAS]), CH-164, CH-172, CH-490, AMG-719, iguratimod (N-[3-(Formylamino)-4-oxo-6-phenoxy-4H-chromen-7-yl] methanesulfonamide), AV94-88, pralnacasan (6H-Pyridazino(1,2-a)(1,2)diazepine-1-carboxamide, N-((2R,3S)-2-ethoxytetrahydro-5-oxo-3-furanyl)octahydro-9-((1-

10 isoquinolinylcarbonyl)amino)-6,10-dioxo-, (1S,9S)- [CAS]), (2S-cis)-5-[Benzyloxycarbonylamino-1,2,4,5,6,7-hexahydro-4-(oxoazepino[3,2,1-hi]indole-2-carbonyl)-amino]-4-oxobutanoic acid, AVE-9488, ESONARIMOD (Taisho Pharmaceutical Co., Ltd., Japan) (Benzenebutanoic acid, Alpha-[(acetylthio)methyl]-4-methyl-Gamma-oxo- [CAS]), pralnacasan (6H-

15 Pyridazino(1,2-a)(1,2)diazepine-1-carboxamide, N-((2R,3S)-2-ethoxytetrahydro-5-oxo-3-furanyl)octahydro-9-((1-isoquinolinylcarbonyl)amino)-6,10-dioxo-, (1S,9S)- [CAS]), tranexamic acid (Cyclohexanecarboxylic acid, 4-(aminomethyl)-, trans- [CAS]), Win-72052, Tomazarit (Ro-31-3948) (Propanoic acid, 2-[[2-(4-chlorophenyl)-4-methyl-5-oxazolyl]methoxy]-2-methyl-[CAS]), PD-

20 163594, SDZ-224-015 (L-Alaninamide N-((phenylmethoxy)carbonyl)-L-valyl-N-((1S)-3-((2,6-dichlorobenzoyl)oxy)-1-(2-ethoxy-2-oxoethyl)-2-oxopropyl)-[CAS]), L-709049 (L-Alaninamide, N-acetyl-L-tyrosyl-L-valyl-N-(2-carboxy-1-formylethyl)-, (S)- [CAS]), TA-383 (1H-Imidazole, 2-(4-chlorophenyl)-4,5-dihydro-4,5-diphenyl-, monohydrochloride, cis- [CAS]), EI-1507-1 (6a,12a-

25 Epoxybenz[a]anthracen-1,12(2H,7H)-dione, 3,4-dihydro-3,7-dihydroxy-8-methoxy-3-methyl- [CAS]), Ethyl 4-(3,4-dimethoxyphenyl)-6,7-dimethoxy-2-(1,2,4-triazol-1-yl methyl)quinoline-3-carboxylate, EI-1941-1, TJ-114, anakinra (Interleukin 1 receptor antagonist (human isoform x reduced), N2-L-methionyl-[CAS])) or an analogue or derivative thereof.

17. IL-4 Agonists

In another embodiment, the pharmacologically active compound is an IL-4 agonist (e.g., glatiramer acetate (L-Glutamic acid, polymer with L-alanine, L-lysine and L-tyrosine, acetate (salt) [CAS])) or an analogue or
 5 derivative thereof.

18. Immunomodulatory Agents

In another embodiment, the pharmacologically active compound is an immunomodulatory agent (e.g. Biolimus, leflunamide, ABT-578, methylsulfamic acid 3-(2-methoxyphenoxy)-2-
 10 [[[(methylamino)sulfonyl]oxy]propyl ester, sirolimus, CCI-779 (Rapamycin 42-(3-hydroxy-2-(hydroxymethyl)-2-methylpropanoate) [CAS]), LF-15-0195, NPC15669 (L-Leucine, N-[[[(2,7-dimethyl-9H-fluoren-9-yl)methoxy]carbonyl]-[CAS]), NPC-15670 (L-Leucine, N-[[[(4,5-dimethyl-9H-fluoren-9-yl)methoxy]carbonyl]-[CAS]), NPC-16570 (4-[2-(Fluoren-9-yl)ethoxy-
 15 carbonyl]aminobenzoic acid), sufosfamide (Ethanol, 2-[[[3-(2-chloroethyl)tetrahydro-2H-1,3,2-oxazaphosphorin-2-yl]amino]-, methanesulfonate (ester), P-oxide [CAS]), tresperimus (2-[N-[4-(3-Aminopropylamino)butyl]carbamoyloxy]-N-(6-guanidinoethyl)acetamide), 4-[2-(Fluoren-9-yl)ethoxycarbonylamino]-benzo-hydroxamic acid, laquinimod, PBI-
 20 1411, azathioprine (6-[(1-Methyl-4-nitro-1H-imidazol-5-yl)thio]-1H-purine), PBI0032, beclometasone, MDL-28842 (9H-Purin-6-amine, 9-(5-deoxy-5-fluoro-β-D-threo-pent-4-enofuranosyl)-, (Z)- [CAS]), FK-788, AVE-1726, ZK-90695, ZK-90695, Ro-54864, didemnin-B, Illinois (Didemnin A, N-[1-(2-hydroxy-1-oxopropyl)-L-prolyl]-, (S)- [CAS]), SDZ-62-826 (Ethanaminium, 2-[[[hydroxy[[1-
 25 [(octadecyloxy)carbonyl]-3-piperidinyl]methoxy]phosphinyl]oxy]-N,N,N-trimethyl-, inner salt [CAS]), argyris B ((4S,7S,13R,22R)-13-Ethyl-4-(1H-indol-3-ylmethyl)-7-(4-methoxy-1H-indol-3-ylmethyl)-18,22-dimethyl-16-methyl-ene-24-thia-3,6,9,12,15,18,21,26-octaazabicyclo[21.2.1]-hexacosa-1(25),23(26)-diene-2,5,8,11,14,17,20-heptaone [CAS]), everolimus (Rapamycin, 42-O-(2-
 30 hydroxyethyl)- [CAS]), SAR-943, L-687795, 6-[(4-Chlorophenyl)sulfinyl]-2,3-

- dihydro-2-(4-methoxy-phenyl)-5-methyl-3-oxo-4-pyridazinecarbonitrile, 91Y78 (1H-Imidazo[4,5-c]pyridin-4-amine, 1-β-D-ribofuranosyl- [CAS]), auranofin (Gold, (1-thio-β-D-glucopyranose 2,3,4,6-tetraacetato-S)(triethylphosphine)- [CAS]), 27-0-Demethylrapamycin, tipredane (Androsta-1,4-dien-3-one, 17-
- 5 (ethylthio)-9-fluoro-11-hydroxy-17-(methylthio)-, (11β,17Alpha)- [CAS]), AI-402, LY-178002 (4-Thiazolidinone, 5-[[3,5-bis(1,1-dimethylethyl)-4-hydroxyphenyl]methylene]-[CAS]), SM-8849 (2-Thiazolamine, 4-[1-(2-fluoro[1,1'-biphenyl]-4-yl)ethyl]-N-methyl- [CAS]), piceatannol, resveratrol, triamcinolone acetonide (Pregna-1,4-diene-3,20-dione, 9-fluoro-11,21-
- 10 dihydroxy-16,17-[(1-methylethylidene)bis(oxy)]-, (11β,16Alpha)- [CAS]), ciclosporin (Cyclosporin A- [CAS]), tacrolimus (15,19-Epoxy-3H-pyrido(2,1-c)(1,4)oxaazacyclotricosine-1,7,20,21(4H,23H)-tetrone, 5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-(2-(4-hydroxy-3-methoxycyclohexyl)-1-methylethenyl)-14,16-dimethoxy-
- 15 4,10,12,18-tetramethyl-8-(2-propenyl)-, (3S-(3R*(E(1S*,3S*,4S*)),4S*,5R*,8S*,9E,12R*,14R*,15S*,16R*,18S*,19S*,26aR*)) - [CAS]), gusperimus (Heptanamide, 7-[(aminoiminomethyl)amino]-N-[2-[[4-[(3-aminopropyl)amino]butyl]amino]-1-hydroxy-2-oxoethyl]-, (+/-)- [CAS]), tixocortol pivalate (Pregn-4-ene-3,20-dione, 21-[(2,2-dimethyl-1-oxopropyl)thio]-11,17-
- 20 dihydroxy-, (11β)- [CAS]), alefacept (1-92 LFA-3 (Antigen) (human) fusion protein with immunoglobulin G1 (human hinge-CH2-CH3 Gamma1-chain), dimmer), halobetasol propionate (Pregna-1,4-diene-3,20-dione, 21-chloro-6,9-difluoro-11-hydroxy-16-methyl-17-(1-oxopropoxy)-, (6Alpha,11β,16β)- [CAS]), iloprost trometamol (Pentanoic acid, 5-[hexahydro-5-hydroxy-4-(3-hydroxy-4-methyl-1-octen-6-ynyl)-2(1H)-pentalenylidene]- [CAS]), beraprost (1H-
- 25 Cyclopenta[b]benzofuran-5-butanoic acid, 2,3,3a,8b-tetrahydro-2-hydroxy-1-(3-hydroxy-4-methyl-1-octen-6-ynyl)- [CAS]), rimexolone (Androsta-1,4-dien-3-one,11-hydroxy-16,17-dimethyl-17-(1-oxopropyl)-, (11β,16Alpha,17β)- [CAS]), dexamethasone (Pregna-1,4-diene-3,20-dione,9-fluoro-11,17,21-trihydroxy-16-
- 30 methyl-, (11β,16Alpha)- [CAS]), sulindac (cis-5-fluoro-2-methyl-1-[(p-methylsulfinyl)benzylidene]indene-3-acetic acid), proglumetacin (1H-Indole-3-

- acetic acid, 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-, 2-(4-(3-((4-(benzoylamino)-5-(dipropylamino)-1,5-dioxopentyl)oxy)propyl)-1-piperazinyl)ethylester, (+/-)- [CAS]), alclometasone dipropionate (Pregna-1,4-diene-3,20-dione, 7-chloro-11-hydroxy-16-methyl-17,21-bis(1-oxopropoxy)-, 5 (7Alpha,11Beta,16Alpha)- [CAS]), pimecrolimus (15,19-Epoxy-3H-pyrido(2,1-c)(1,4)oxaazacyclotricosine-1,7,20,21(4H,23H)-tetrone, 3-(2-(4-chloro-3-methoxycyclohexyl)-1-methylethenyl)-8-ethyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-14,16-dimethoxy-4,10,12,18-tetramethyl-, (3S-
- 10 (3R*(E(1S*,3S*,4R*)),4S*,5R*,8S*,9E,12R*,14R*,15S*,16R*,18S*,19S*,26aR*)) - [CAS]), hydrocortisone-17-butyrate (Pregn-4-ene-3,20-dione, 11,21-dihydroxy-17-(1-oxobutoxy)-, (11Beta)- [CAS]), mitoxantrone (9,10-Anthracenedione, 1,4-dihydroxy-5,8-bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]- [CAS]), mizoribine (1H-Imidazole-4-carboxamide, 5-hydroxy-1-Beta-D-ribofuranosyl- [CAS]),
- 15 prednicarbate (Pregna-1,4-diene-3,20-dione, 17-[(ethoxycarbonyl)oxy]-11-hydroxy-21-(1-oxopropoxy)-, (11Beta)- [CAS]), lobenzarit (Benzoic acid, 2-[(2-carboxyphenyl)amino]-4-chloro- [CAS]), glucametacin (D-Glucose, 2-[[[1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl]acetyl]amino]-2-deoxy- [CAS]), fluocortolone monohydrate ((6Alpha)-fluoro-16Alpha-methylpregna-1,4-
- 20 dien-11Beta,21-diol-3,20-dione), fluocortin butyl (Pregna-1,4-dien-21-oic acid, 6-fluoro-11-hydroxy-16-methyl-3,20-dioxo-, butyl ester, (6Alpha,11Beta,16Alpha)- [CAS]), difluprednate (Pregna-1,4-diene-3,20-dione, 21-(acetyloxy)-6,9-difluoro-11-hydroxy-17-(1-oxobutoxy)-, (6Alpha,11Beta)- [CAS]), diflorasone diacetate (Pregna-1,4-diene-3,20-dione, 17,21-bis(acetyloxy)-6,9-difluoro-11-hydroxy-16-
- 25 methyl-, (6Alpha,11Beta,16Beta)- [CAS]), dexamethasone valerate (Pregna-1,4-diene-3,20-dione, 9-fluoro-11,21-dihydroxy-16-methyl-17-[(1-oxopentyl)oxy]-, (11Beta,16Alpha)- [CAS]), methylprednisolone, deprodone propionate (Pregna-1,4-diene-3,20-dione, 11-hydroxy-17-(1-oxopropoxy)-, (11.beta.)- [CAS]), bucillamine (L-Cysteine, N-(2-mercapto-2-methyl-1-oxopropyl)- [CAS]),
- 30 amcinonide (Benzeneacetic acid, 2-amino-3-benzoyl-, monosodium salt, monohydrate [CAS]), acemetacin (1H-Indole-3-acetic acid, 1-(4-chlorobenzoyl)-

5-methoxy-2-methyl-, carboxymethyl ester [CAS])) or an analogue or derivative thereof. Further, analogues of rapamycin include tacrolimus and derivatives thereof (e.g., EP0184162B1 and U.S. Patent No. 6,258,823) everolimus and derivatives thereof (e.g., U.S. Patent No. 5,665,772). Further representative

5 examples of sirolimus analogues and derivatives include ABT-578 and those found in PCT Publication Nos. WO 97/10502, WO 96/41807, WO 96/35423, WO 96/03430, WO 96/00282, WO 95/16691, WO 95/15328, WO 95/07468, WO 95/04738, WO 95/04060, WO 94/25022, WO 94/21644, WO 94/18207, WO 94/10843, WO 94/09010, WO 94/04540, WO 94/02485, WO 94/02137,

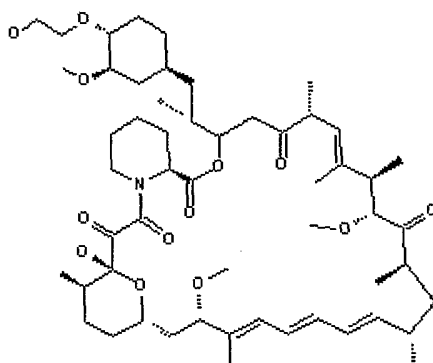
10 WO 94/02136, WO 93/25533, WO 93/18043, WO 93/13663, WO 93/11130, WO 93/10122, WO 93/04680, WO 92/14737, and WO 92/05179. Representative U.S. patents include U.S. Patent Nos. 6,342,507; 5,985,890; 5,604,234; 5,597,715; 5,583,139; 5,563,172; 5,561,228; 5,561,137; 5,541,193; 5,541,189; 5,534,632; 5,527,907; 5,484,799; 5,457,194; 5,457,182; 5,362,735; 5,324,644;

15 5,318,895; 5,310,903; 5,310,901; 5,258,389; 5,252,732; 5,247,076; 5,225,403; 5,221,625; 5,210,030; 5,208,241; 5,200,411; 5,198,421; 5,147,877; 5,140,018; 5,116,756; 5,109,112; 5,093,338; and 5,091,389.

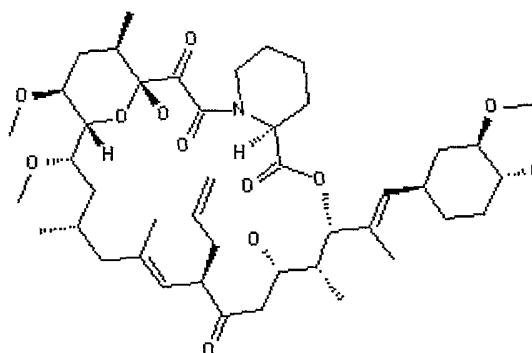
The structures of sirolimus, everolimus, and tacrolimus are provided below:

20

Name	Code Name	Company	Structure
Everolimus	SAR-943	Novartis	See below
Sirolimus	AY-22989	Wyeth	See below
Rapamune	NSC-226080		
Rapamycin			
Tacrolimus	FK506	Fujusawa	See below

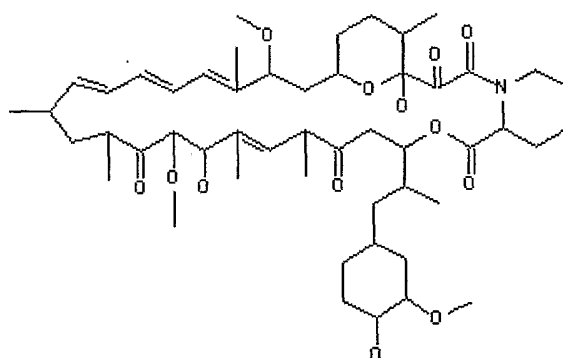


Everolimus



Tacrolimus

5



Sirolimus

19. Inosine monophosphate dehydrogenase inhibitors

In another embodiment, the pharmacologically active compound is an inosine monophosphate dehydrogenase inhibitor (e.g., Mycophenolate Mofetil (4-Hexenoic acid, 6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-

5-isobenzofuranyl)-4-methyl-, 2-(4-morpholinyl)ethyl ester, (E)- [CAS]), ribavirin (1H-1,2,4-Triazole-3-carboxamide, 1-β-D-ribofuranosyl- [CAS]), tiazofurin (4-Thiazolecarboxamide, 2-β-D-ribofuranosyl- [CAS]), viramidine, aminothiadiaazole, thiophenfurin, tiazofurin) or an analogue or derivative thereof.

5 Additional representative examples are included in U.S. Patent Nos. 5,536,747; 5,807,876; 5,932,600; 6,054,472, 6,128,582; 6,344,465; 6,395,763; 6,399,773; 6,420,403; 6,479,628; 6,498,178; 6,514,979; 6,518,291; 6,541,496; 6,596,747; 6,617,323; and 6,624,184, U.S. Publication Nos. 2002/0040022A1, 2002/0052513A1, 2002/0055483A1, 2002/0068346A1, 2002/0111378A1, 10 2002/0111495A1, 2002/0123520A1, 2002/0143176A1, 2002/0147160A1, 2002/0161038A1, 2002/0173491A1, 2002/0183315A1, 2002/0193612A1, 2003/0027845A1, 2003/0068302A1, 2003/0105073A1, 2003/0130254A1, 2003/0143197A1, 2003/0144300A1, 2003/0166201A1, 2003/0181497A1, 2003/0186974A1, 2003/0186989A1, and 2003/0195202A1, and PCT 15 Publication Nos. WO 00/24725A1, WO 00/25780A1, WO 00/26197A1, WO 00/51615A1, WO 0056331A1, WO 00/73288A1, WO 01/00622A1, WO 01/66706A1, WO 01/79246A2, WO 01/81340A2, WO 01/85952A2, WO 02/16382A1, WO 02/18369A2, WO 02/51814A1, WO 02/57287A2, WO 02/57425A2, WO 02/60875A1, WO 02/60896A1, WO 02/60898A1, WO 20 02/68058A2, WO 03/20298A1, WO 03/37349A1, WO 03/39548A1, WO 03/45901A2, WO 03/47512A2, WO 03/53958A1, WO 03/55447A2, WO 03/59269A2, WO 03/63573A2, WO 03/87071A1, WO 90/01545A1, WO 97/40028A1, WO 97/41211A1, WO 98/40381A1, and WO 99/55663A1.

20. Leukotriene Inhibitors

25 In another embodiment, the pharmacologically active compound is a leukotriene inhibitor (e.g., DTI-0026, ONO-4057(Benzenepropanoic acid, 2-(4-carboxybutoxy)-6-[[6-(4-methoxyphenyl)-5-hexenyl]oxy]-, (E)- [CAS]), ONO-LB-448, pirodomast 1,8-Naphthyridin-2(1H)-one, 4-hydroxy-1-phenyl-3-(1-pyrrolidinyl)- [CAS], Sch-40120 (Benzo[b][1,8]naphthyridin-5(7H)-one, 10-(3-chlorophenyl)-6,8,9,10-tetrahydro- [CAS]), L-656224 (4-Benzofuranol, 7-chloro-

30

- 2-[(4-methoxyphenyl)methyl]-3-methyl-5-propyl- [CAS]), MAFP (methyl arachidonyl fluorophosphonate), ontazolast (2-Benzoxazamine, N-[2-cyclohexyl-1-(2-pyridinyl)ethyl]-5-methyl-, (S)- [CAS]), amelubant (Carbamic acid, ((4-((3-((4-(1-(4-hydroxyphenyl)-1-methylethyl)phenoxy)methyl)phenyl)methoxy) phenyl)iminomethyl)- ethyl ester [CAS]), SB-201993 (Benzoic acid, 3-[[[6-[(1E)-2-carboxyethenyl]-5-[[8-(4-methoxyphenyl)octyl]oxy]-2-pyridinyl]methyl]thio]methyl]-[CAS]), LY-203647 (Ethanone, 1-[2-hydroxy-3-propyl-4-[4-[2-[4-(1H-tetrazol-5-yl)butyl]-2H-tetrazol-5-yl]butoxy]phenyl]- [CAS]), LY-210073, LY-223982 (Benzenepropanoic acid, 5-(3-carboxybenzoyl)-2-[[6-(4-methoxyphenyl)-5-hexenyl]oxy]-, (E)- [CAS]), LY-293111 (Benzoic acid, 2-[3-[3-[(5-ethyl-4'-fluoro-2-hydroxy[1,1'-biphenyl]-4-yl)oxy]propoxy]-2-propylphenoxy]- [CAS]), SM-9064 (Pyrrolidine, 1-[4,11-dihydroxy-13-(4-methoxyphenyl)-1-oxo-5,7,9-tridecatrienyl]-, (E,E,E)- [CAS]), T-0757 (2,6-Octadienamide, N-(4-hydroxy-3,5-dimethylphenyl)-3,7-dimethyl-, (2E)- [CAS])) or an analogue or derivative thereof.

21. MCP-1 Antagonists

- In another embodiment, the pharmacologically active compound is a MCP-1 antagonist (e.g., nitronaproxen (2-Napthaleneacetic acid, 6-methoxy-Alpha-methyl 4-(nitrooxy)butyl ester (AlphaS)- [CAS]), Bindarit (2-(1-benzylindazol-3-ylmethoxy)-2-methylpropanoic acid), 1-alpha-25 dihydroxy vitamin D₃) or an analogue or derivative thereof.

22. MMP Inhibitors

- In another embodiment, the pharmacologically active compound is a MMP inhibitor (e.g., D-9120, doxycycline (2-Naphthacenecarboxamide, 4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo- [4S-(4Alpha,4aAlpha,5Alpha,5aAlpha,6Alpha,12aAlpha)]-[CAS]), BB-2827, BB-1101 (2S-allyl-N1-hydroxy-3R-isobutyl-N4-(1S-methylcarbamoyl-2-phenylethyl)-succinamide), BB-2983, solimastat (N'-[2,2-Dimethyl-1(S)-[N-(2-pyridyl)carbamoyl]propyl]-N4-hydroxy-2(R)-isobutyl-3(S)-

- methoxysuccinamide), BATIMASTAT (Butanediamide, N4-hydroxy-N1-[2-(methylamino)-2-oxo-1-(phenylmethyl)ethyl]-2-(2-methylpropyl)-3-[(2-thienylthio)methyl]-, [2R-[1(S*),2R*,3S*]]-[CAS]; British Biotech, UK), CH-138, CH-5902, D-1927, D-5410, EF-13 (Gamma-linolenic acid lithium salt), CMT-3
- 5 (2-Naphthacenecarboxamide, 1,4,4a,5,5a,6,11,12a-octahydro-3,10,12,12a-tetrahydroxy-1,11-dioxo-, (4aS,5aR,12aS)- [CAS]), MARIMASTAT (N-[2,2-Dimethyl-1(S)-(N-methylcarbamoyl)propyl]-N,3(S)-dihydroxy-2(R)-isobutylsuccinamide, British Biotech, UK), TIMP'S, ONO-4817, rebimastat (L-Valinamide, N-((2S)-2-mercapto-1-oxo-4-(3,4,4-trimethyl-2,5-dioxo-1-
- 10 imidazolidinyl)butyl)-L-leucyl-N,3-dimethyl- [CAS]), PS-508, CH-715, nimesulide (Methanesulfonamide, N-(4-nitro-2-phenoxyphenyl)- [CAS]), hexahydro-2-[2(R)-[1(RS)-(hydroxycarbamoyl)-4-phenylbutyl]nonanoyl]-N-(2,2,6,6-tetramethyl-4-piperidinyl)-3(S)-pyridazine carboxamide, Rs-113-080, Ro-1130830, Cipemastat (1-Piperidinebutanamide, β -(cyclopentylmethyl)-N-hydroxy-
- 15 Gamma-oxo-Alpha-[(3,4,4-trimethyl-2,5-dioxo-1-imidazolidinyl)methyl]-, (AlphaR, β R)- [CAS]), 5-(4'-biphenyl)-5-[N-(4-nitrophenyl)piperazinyl]barbituric acid, 6-methoxy-1,2,3,4-tetrahydro-norharman-1-carboxylic acid, Ro-31-4724 (L-Alanine, N-[2-[2-(hydroxyamino)-2-oxoethyl]-4-methyl-1-oxopentyl]-L-leucyl-ethyl ester[CAS]), prinomastat (3-Thiomorpholinecarboxamide, N-hydroxy-2,2-
- 20 dimethyl-4-((4-(4-pyridinyloxy) phenyl)sulfonyl)-, (3R)- [CAS]), AG-3433 (1H-Pyrrole-3-propanic acid, 1-(4'-cyano[1,1'-biphenyl]-4-yl)-b-[[[(3S)-tetrahydro-4,4-dimethyl-2-oxo-3-furanyl]amino]carbonyl]-, phenylmethyl ester, (bS)- [CAS]), PNU-142769 (2H-Isoindole-2-butanamide, 1,3-dihydro-N-hydroxy-Alpha-[(3S)-3-(2-methylpropyl)-2-oxo-1-(2-phenylethyl)-3-pyrrolidinyl]-1,3-dioxo-, (AlphaR)-
- 25 [CAS]), (S)-1-[2-[[[(4,5-Dihydro-5-thioxo-1,3,4-thiadiazol-2-yl)amino]-carbonyl]amino]-1-oxo-3-(pentafluorophenyl)propyl]-4-(2-pyridinyl)piperazine, SU-5402 (1H-Pyrrole-3-propanoic acid, 2-[(1,2-dihydro-2-oxo-3H-indol-3-ylidene)methyl]-4-methyl- [CAS]), SC-77964, PNU-171829, CGS-27023A, N-
- 30 hydroxy-2(R)-[(4-methoxybenzene-sulfonyl)(4-picolyl)amino]-2-(2-tetrahydrofuranyl)-acetamide, L-758354 ((1,1'-Biphenyl)-4-hexanoic acid, Alpha-butyl-Gamma-(((2,2-dimethyl-1-

((methylamino)carbonyl)propyl)amino)carbonyl)-4'-fluoro-, (AlphaS-(AlphaR*,GammaS*(R*)))- [CAS]], GI-155704A, CPA-926 or an analogue or derivative thereof. Additional representative examples are included in U.S. Patent Nos. 5,665,777; 5,985,911; 6,288,261; 5,952,320; 6,441,189; 6,235,786; 5 6,294,573; 6,294,539; 6,563,002; 6,071,903; 6,358,980; 5,852,213; 6,124,502; 6,160,132; 6,197,791; 6,172,057; 6,288,086; 6,342,508; 6,228,869; 5,977,408; 5,929,097; 6,498,167; 6,534,491; 6,548,524; 5,962,481; 6,197,795; 6,162,814; 6,441,023; 6,444,704; 6,462,073; 6,162,821; 6,444,639; 6,262,080; 6,486,193; 6,329,550; 6,544,980; 6,352,976; 5,968,795; 5,789,434; 5,932,763; 6,500,847; 10 5,925,637; 6,225,314; 5,804,581; 5,863,915; 5,859,047; 5,861,428; 5,886,043; 6,288,063; 5,939,583; 6,166,082; 5,874,473; 5,886,022; 5,932,577; 5,854,277; 5,886,024; 6,495,565; 6,642,255; 6,495,548; 6,479,502; 5,696,082; 5,700,838; 6,444,639; 6,262,080; 6,486,193; 6,329,550; 6,544,980; 6,352,976; 5,968,795; 5,789,434; 5,932,763; 6,500,847; 5,925,637; 6,225,314; 5,804,581; 5,863,915; 15 5,859,047; 5,861,428; 5,886,043; 6,288,063; 5,939,583; 6,166,082; 5,874,473; 5,886,022; 5,932,577; 5,854,277; 5,886,024; 6,495,565; 6,642,255; 6,495,548; 6,479,502; 5,696,082; 5,700,838; 5,861,436; 5,691,382; 5,763,621; 5,866,717; 5,902,791; 5,962,529; 6,017,889; 6,022,873; 6,022,898; 6,103,739; 6,127,427; 6,258,851; 6,310,084; 6,358,987; 5,872,152; 5,917,090; 6,124,329; 6,329,373; 20 6,344,457; 5,698,706; 5,872,146; 5,853,623; 6,624,144; 6,462,042; 5,981,491; 5,955,435; 6,090,840; 6,114,372; 6,566,384; 5,994,293; 6,063,786; 6,469,020; 6,118,001; 6,187,924; 6,310,088; 5,994,312; 6,180,611; 6,110,896; 6,380,253; 5,455,262; 5,470,834; 6,147,114; 6,333,324; 6,489,324; 6,362,183; 6,372,758; 6,448,250; 6,492,367; 6,380,258; 6,583,299; 5,239,078; 5,892,112; 5,773,438; 25 5,696,147; 6,066,662; 6,600,057; 5,990,158; 5,731,293; 6,277,876; 6,521,606; 6,168,807; 6,506,414; 6,620,813; 5,684,152; 6,451,791; 6,476,027; 6,013,649; 6,503,892; 6,420,427; 6,300,514; 6,403,644; 6,177,466; 6,569,899; 5,594,006; 6,417,229; 5,861,510; 6,156,798; 6,387,931; 6,350,907; 6,090,852; 6,458,822; 6,509,337; 6,147,061; 6,114,568; 6,118,016; 5,804,593; 5,847,153; 5,859,061; 30 6,194,451; 6,482,827; 6,638,952; 5,677,282; 6,365,630; 6,130,254; 6,455,569; 6,057,369; 6,576,628; 6,110,924; 6,472,396; 6,548,667; 5,618,844; 6,495,578;

6,627,411; 5,514,716; 5,256,657; 5,773,428; 6,037,472; 6,579,890; 5,932,595;
 6,013,792; 6,420,415; 5,532,265; 5,691,381; 5,639,746; 5,672,598; 5,830,915;
 6,630,516; 5,324,634; 6,277,061; 6,140,099; 6,455,570; 5,595,885; 6,093,398;
 6,379,667; 5,641,636; 5,698,404; 6,448,058; 6,008,220; 6,265,432; 6,169,103;
 5 6,133,304; 6,541,521; 6,624,196; 6,307,089; 6,239,288; 5,756,545; 6,020,366;
 6,117,869; 6,294,674; 6,037,361; 6,399,612; 6,495,568; 6,624,177; 5,948,780;
 6,620,835; 6,284,513; 5,977,141; 6,153,612; 6,297,247; 6,559,142; 6,555,535;
 6,350,885; 5,627,206; 5,665,764; 5,958,972; 6,420,408; 6,492,422; 6,340,709;
 6,022,948; 6,274,703; 6,294,694; 6,531,499; 6,465,508; 6,437,177; 6,376,665;
 10 5,268,384; 5,183,900; 5,189,178; 6,511,993; 6,617,354; 6,331,563; 5,962,466;
 5,861,427; 5,830,869; and 6,087,359.

23. NF kappa B Inhibitors

In another embodiment, the pharmacologically active compound is a NF kappa B inhibitor (*e.g.*, Celgene (SP100030, SP100207, SP100393),
 15 AVE-0545, Oxi-104 (Benzamide, 4-amino-3-chloro-N-(2-(diethylamino)ethyl)-
 [CAS]), dexlipotam, INDRA, R-flurbiprofen ([1,1'-Biphenyl]-4-acetic acid, 2-
 fluoro-Alpha-methyl), SP100030 (2-chloro-N-[3,5-di(trifluoromethyl)phenyl]-4-
 (trifluoromethyl)pyrimidine-5-carboxamide), AVE-0545, VIATRIS, AVE-0547,
 Bay 11-7082, Bay 11-7085, 15 deoxy-prostaylandin J2, bortezomib (Boronic
 20 acid, [(1R)-3-methyl-1-[[[(2S)-1-oxo-3-phenyl-2-
 [(pyrazinylcarbonyl)amino]propyl]amino]butyl]- [CAS]) or an analogue or
 derivative thereof.

24. NO Agonists

In another embodiment, the pharmacologically active compound
 25 is a NO antagonist (*e.g.*, NCX-4016 (Benzoic acid, 2-(acetyloxy)-, 3-
 ((nitrooxy)methyl)phenyl ester [CAS]), NCX-2216, L-arginine or an analogue or
 derivative thereof.

25. P38 MAP Kinase Inhibitors

In another embodiment, the pharmacologically active compound is a P38 MAP kinase inhibitor (e.g., VX-745 (Vertex Pharmaceuticals, Inc., Cambridge, MA), GW-2286, SK86002, CGP-52411, BIRB-798, SB220025, RO-
5 320-1195, RWJ-67657, RWJ-68354, SCIO-469, SCIO-323, AMG-548, CMC-146, SD-31145, CC-8866, Ro-320-1195, Roche (3853,4507, 6145, 8464,0945, 6257, 3391, 3470, 1151634,5274, 5161, 4194, 1195), BIX 983 (Boehringer Ingelheim), PD-98059 (4H-1-Benzopyran-4-one, 2-(2-amino-3-methoxyphenyl)- [CAS]), CGH-2466, doramapimod, SB-203580 (Pyridine, 4-[5-(4-fluorophenyl)-
10 2-[4-(methylsulfinyl)phenyl]-1H-imidazol-4-yl]- [CAS]), SB-220025 ((5-(2-Amino-4-pyrimidinyl)-4-(4-fluorophenyl)-1-(4-piperidinyl)imidazole)), SB-281832, PD169316, SB202190 or an analogue or derivative thereof. Additional representative examples are included in U.S. Patent Nos. 6,300,347; 6,316,464; 6,316,466; 6,376,527; 6,444,696; 6,479,507; 6,509,361; 6,579,874; 15 and 6,630,485, U.S. Publication Nos. 2001/0044538A1; 2002/0013354A1; 2002/0049220A1; 2002/0103245A1; 2002/0151491A1; 2002/0156114A1; 2003/0018051A1; 2003/0073832A1; 2003/0130257A1; 2003/0130273A1; 2003/0130319A1; 2003/0139388A1; 2003/0139462A1; 2003/0149031A1; 2003/0166647A1; and 2003/0181411A1; and PCT Publication Nos. WO
20 00/63204A2, WO 01/21591A1, WO 01/35959A1, WO 01/74811A2, WO 02/18379A2, WO 02/064594A2, WO 02/083622A2, WO 02/094842A2, WO 02/096426A1, WO 02/101015A2, WO 02/103000A2, WO 03/008413A1, WO 03/016248A2, WO 03/020715A1, WO 03/024899A2, WO 03/031431A1, WO 03/040103A1, WO 03/053940A1, WO 03/053941A2, WO 03/063799A2, WO
25 03/079986A2, WO 03/080024A2, WO 03/082287A1, WO 97/44467A1, WO 99/01449A1, and WO 99/58523A1.

26. Phosphodiesterase Inhibitors

In another embodiment, the pharmacologically active compound is a phosphodiesterase inhibitor (e.g., CDP-840 (Pyridine, 4-[(2R)-2-[3-(cyclopentyloxy)-4-methoxyphenyl]-2-phenylethyl]- [CAS]), CH-3697, CT-2820,
30

- D-22888 (Imidazo[1,5-a]pyrido[3,2-e]pyrazin-6(5H)-one, 9-ethyl-2-methoxy-7-methyl-5-propyl-[CAS]), D-4418 (8-Methoxyquinoline-5-[N-(2,5-dichloropyridin-3-yl)]carboxamide), 1-(3-cyclopentyloxy-4-methoxyphenyl)-2-(2,6-dichloro-4-pyridyl) ethanone oxime, D-4396, ONO-6126, CDC-998, CDC-801, V-11294A
- 5 (3-[3-(Cyclopentyloxy)-4-methoxybenzyl]-6-(ethylamino)-8-isopropyl-3H-purine hydrochloride), S,S'-methylene-bis(2-(8-cyclopropyl-3-propyl-6-(4-pyridylmethylamino)-2-thio-3H-purine)) tetrahydrochloride, Rolipram (2-Pyrrolidinone, 4-[3-(cyclopentyloxy)-4-methoxyphenyl]- [CAS]), CP-293121, CP-353164 (5-(3-Cyclopentyloxy-4-methoxyphenyl)pyridine-2-carboxamide),
- 10 oxagrelate (6-Phthalazinecarboxylic acid, 3,4-dihydro-1-(hydroxymethyl)-5,7-dimethyl-4-oxo-, ethyl ester [CAS]), PD-168787, ibudilast (1-Propanone, 2-methyl-1-[2-(1-methylethyl)pyrazolo[1,5-a]pyridin-3-yl]- [CAS]), oxagrelate (6-Phthalazinecarboxylic acid, 3,4-dihydro-1-(hydroxymethyl)-5,7-dimethyl-4-oxo-, ethyl ester [CAS]), griseolic acid (Alpha-L-talo-Oct-4-enofuranuronic acid, 1-(6-amino-9H-purin-9-yl)-3,6-anhydro-6-C-carboxy-1,5-dideoxy- [CAS]), KW-4490,
- 15 KS-506, T-440, roflumilast (Benzamide, 3-(cyclopropylmethoxy)-N-(3,5-dichloro-4-pyridinyl)-4-(difluoromethoxy)- [CAS]), rolipram, milrinone, triflusinal (Benzoic acid, 2-(acetyloxy)-4-(trifluoromethyl)- [CAS]), anagrelide hydrochloride (Imidazo[2,1-b]quinazolin-2(3H)-one, 6,7-dichloro-1,5-dihydro-,
- 20 monohydrochloride [CAS]), cilostazol (2(1H)-Quinolinone, 6-[4-(1-cyclohexyl-1H-tetrazol-5-yl)butoxy]-3,4-dihydro-[CAS]), propentofylline (1H-Purine-2,6-dione, 3,7-dihydro-3-methyl-1-(5-oxohexyl)-7-propyl- [CAS]), sildenafil citrate (Piperazine, 1-((3-(4,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo(4,3-d)pyrimidin-5-yl)-4-ethoxyphenyl)sulfonyl)-4-methyl, 2-hydroxy-1,2,3-
- 25 propanetricarboxylate- (1:1) [CAS]), tadalafil (Pyrazino(1',2':1,6)pyrido(3,4-b)indole-1,4-dione, 6-(1,3-benzodioxol-5-yl)-2,3,6,7,12,12a-hexahydro-2-methyl-, (6R-trans) [CAS]), vardenafil (Piperazine, 1-(3-(1,4-dihydro-5-methyl(-4-oxo-7-propylimidazo(5,1-f)(1,2,4)-triazin-2-yl)-4-ethoxyphenyl)sulfonyl)-4-ethyl- [CAS]), milrinone ([3,4'-Bipyridine]-5-carbonitrile, 1,6-dihydro-2-methyl-6-oxo-
- 30 [CAS]), enoximone (2H-Imidazol-2-one, 1,3-dihydro-4-methyl-5-[4-(methylthio)benzoyl]- [CAS]), theophylline (1H-Purine-2,6-dione, 3,7-dihydro-

1,3-dimethyl- [CAS]), ibudilast (1-Propanone, 2-methyl-1-[2-(1-methylethyl)pyrazolo[1,5-a]pyridin-3-yl]- [CAS]), aminophylline (1H-Purine-2,6-dione, 3,7-dihydro-1,3-dimethyl-, compd. with 1,2-ethanediamine (2:1)- [CAS]), acebrophylline (7H-Purine-7-acetic acid, 1,2,3,6-tetrahydro-1,3-dimethyl-2,6-dioxo-, compd. with trans-4-[[2-amino-3,5-dibromophenyl)methyl]amino]cyclohexanol (1:1) [CAS]), plafibride (Propanamide, 2-(4-chlorophenoxy)-2-methyl-N-[[4-morpholinylmethyl]amino]carbonyl]- [CAS]), loprinone hydrochloride (3-Pyridinecarbonitrile, 1,2-dihydro-5-imidazo[1,2-a]pyridin-6-yl-6-methyl-2-oxo-, monohydrochloride- [CAS]), fosfosal (Benzoic acid, 2-(phosphonoxy)- [CAS]), amrinone ([3,4'-Bipyridin]-6(1H)-one, 5-amino- [CAS]) or an analogue or derivative thereof.

27. TGF beta Inhibitors

In another embodiment, the pharmacologically active compound is a TGF beta Inhibitor (e.g., mannose-6-phosphate, LF-984, tamoxifen (Ethanamine, 2-[4-(1,2-diphenyl-1-butenyl)phenoxy]-N,N-dimethyl-, (Z)- [CAS]), tranilast or an analogue or derivative thereof.

28. Thromboxane A2 Antagonists

In another embodiment, the pharmacologically active compound is a thromboxane A2 antagonist (e.g., CGS-22652 (3-Pyridineheptanoic acid, .gamma.-[4-[[4-(4-chlorophenyl)sulfonyl]amino]butyl]-, (.+.-)- [CAS]), ozagrel (2-Propenoic acid, 3-[4-(1H-imidazol-1-ylmethyl)phenyl]-, (E)- [CAS]), argatroban (2-Piperidinecarboxylic acid, 1-[5-[(aminoiminomethyl)amino]-1-oxo-2-[[1,2,3,4-tetrahydro-3-methyl-8-quinoliny]sulfonyl]amino]pentyl]-4-methyl-[CAS]), ramatroban (9H-Carbazole-9-propanoic acid, 3-[[4-fluorophenyl)sulfonyl]amino]-1,2,3,4-tetrahydro-, (R)- [CAS]), torasemide (3-Pyridinesulfonamide, N-[[1-methylethyl]amino]carbonyl]-4-[(3-methylphenyl)amino]- [CAS]), gamma linoleic acid ((Z,Z,Z)-6,9,12-Octadecatrienoic acid [CAS]), seratrodist (Benzeneheptanoic acid, zeta-(2,4,5-

trimethyl-3,6-dioxo-1,4-cyclohexadien-1-yl)-, (+/-)- [CAS]) or an analogue or derivative thereof.

29. TNFa Antagonists / TACE Inhibitors

In another embodiment, the pharmacologically active compound
 5 is a TNFa Antagonist / TACE Inhibitor (e.g., Celgene (CC10037, CC-11049, CC-10004, CC10083), E-5531 (2-Deoxy-6-O-[2-deoxy-3-O-[3(R)-[5(Z)-dodecenoyloxy]-decyl]-6-O-methyl-2-(3-oxotetradecanamido)-4-O-phosphono-β-D-glucopyranosyl]-3-O-[3(R)-hydroxydecyl]-2-(3-oxotetradecanamido)-Alpha-D-glucopyranose-1-O-phosphate), AZD-4717, glycoposphopeptical, UR-12715
 10 (Benzoic acid, 2-hydroxy-5-[[4-[3-[4-(2-methyl-1H-imidazol[4,5-c]pyridin-1-yl)methyl]-1-piperidiny]-3-oxo-1-phenyl-1-propenyl]phenyl]azo] (Z) [CAS]), PMS-601, AM-87, xyloadenosine (9H-Purin-6-amine, 9-β-D-xylofuranosyl-[CAS]), RDP-58, RDP-59, BB2275, benzydamine, E-3330 (Undecanoic acid, 2-[[4,5-dimethoxy-2-methyl-3,6-dioxo-1,4-cyclohexadien-1-yl)methylene]-, (E)-
 15 [CAS]), N-[D,L-2-(hydroxyaminocarbonyl)methyl-4-methylpentanoyl]-L-3-(2'-naphthyl)alanyl-L-alanine, 2-aminoethyl amide, CP-564959, MLN-608, SPC-839, ENMD-0997, Sch-23863 ((2-[10,11-Dihydro-5-ethoxy-5H-dibenzo [a,d]cyclohepten-S-yl]-N, N-dimethyl-ethanamine), SH-636, PKF-241-466, PKF-242-484, TNF-484A, cilomilast (Cis-4-cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]cyclohexane-1-carboxylic acid), GW-3333, GW-4459, BMS-561392, AM-87, cloricromene (Acetic acid, [[8-chloro-3-[2-(diethylamino)ethyl]-4-methyl-2-oxo-2H-1-benzopyran-7-yl]oxy]-, ethyl ester [CAS]), thalidomide (1H-Isoindole-1,3(2H)-dione, 2-(2,6-dioxo-3-piperidiny)- [CAS]), vesnarinone (Piperazine, 1-(3,4-dimethoxybenzoyl)-4-(1,2,3,4-tetrahydro-2-oxo-6-quinolinyl)-
 20 [CAS]), infliximab, lentinan, etanercept (1-235-Tumor necrosis factor receptor (human) fusion protein with 236-467-immunoglobulin G1 (human gamma1-chain Fc fragment) [CAS]), diacerein (2-Anthracenecarboxylic acid, 4,5-bis(acetyloxy)-9,10-dihydro-9,10-dioxo- [CAS]) or an analogue or derivative thereof.

30. Tyrosine Kinase Inhibitors

In another embodiment, the pharmacologically active compound is a tyrosine kinase inhibitor (e.g., SKI-606, ER-068224, SD-208, N-(6-Benzothiazolyl)-4-(2-(1-piperazinyl)pyrid-5-yl)-2-pyrimidineamine, celastrol

5 (24,25,26-Trinoroleana-1(10),3,5,7-tetraen-29-oic acid, 3-hydroxy-9,13-dimethyl-2-oxo-, (9 β ,13 α ,14 β ,20 α)- [CAS]), CP-127374 (Geldanamycin, 17-demethoxy-17-(2-propenylamino)- [CAS]), CP-564959, PD-171026, CGP-52411 (1H-Isoindole-1,3(2H)-dione, 4,5-bis(phenylamino)- [CAS]), CGP-53716 (Benzamide, N-[4-methyl-3-[[4-(3-pyridinyl)-2-

10 pyrimidinyl]amino]phenyl]- [CAS]), imatinib (4-((Methyl-1-piperazinyl)methyl)-N-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-phenyl]benzamide methanesulfonate), NVP-AAK980-NX, KF-250706 (13-Chloro,5(R),6(S)-epoxy-14,16-dihydroxy-11-(hydroylimino)-3(R)-methyl-3,4,5,6,11,12-hexahydro-1H-2-benzoxacyclotetradecin-1-one), 5-[3-[3-methoxy-4-[2-[(E)-2-phenylethenyl]-4-

15 oxazolylmethoxy]phenyl]propyl]-3-[2-[(E)-2-phenylethenyl]-4-oxazolylmethyl]-2,4-oxazolidinedione, genistein or an analogue or derivative thereof.

31. Vitronectin Inhibitors

In another embodiment, the pharmacologically active compound is a vitronectin inhibitor (e.g., O-[9,10-dimethoxy-1,2,3,4,5,6-hexahydro-4-

20 [(1,4,5,6-tetrahydro-2-pyrimidinyl)hydrazono]-8-benz(e)azulenyl]-N-[(phenylmethoxy)carbonyl]-DL-homoserine 2,3-dihydroxypropyl ester, (2S)-Benzoylcarbonylamino-3-[2-((4S)-(3-(4,5-dihydro-1H-imidazol-2-ylamino)-propyl)-2,5-dioxo-imidazolidin-1-yl)-acetylamino]-propionate, Sch-221153, S-836, SC-68448 (β -[[2-2-[[[3-[(aminoiminomethyl)amino]-

25 phenyl]carbonyl]amino]acetyl]amino]-3,5-dichlorobenzenepropanoic acid), SD-7784, S-247 or an analogue or derivative thereof.

32. Fibroblast Growth Factor Inhibitors

In another embodiment, the pharmacologically active compound is a fibroblast growth factor inhibitor (e.g., CT-052923 [(2H-benzo[d]1,3-

dioxalan-5-methyl)amino][4-(6,7-dimethoxyquinazolin-4-yl)piperazinyl]methane-1-thione) or an analogue or derivative thereof.

33. Protein Kinase Inhibitors

In another embodiment, the pharmacologically active compound
 5 is a protein kinase inhibitor (e.g., KP-0201448, NPC15437 (Hexanamide, 2,6-diamino-N-[[1-(1-oxotridecyl)-2-piperidinyl]methyl]- [CAS]), fasudil (1H-1,4-Diazepine, hexahydro-1-(5-isoquinolinylsulfonyl)- [CAS]), midostaurin (Benzamide, N-(2,3,10,11,12,13-hexahydro-10-methoxy-9-methyl-1-oxo-9,13-epoxy-1H,9H-diindolo[1,2,3-gh:3',2',1'-lm]pyrrolo[3,4-j][1,7]benzodiazonin-11-
 10 yl)-N-methyl-, (9Alpha,10Beta,11Beta,13Alpha)- [CAS]), fasudil (1H-1,4-Diazepine, hexahydro-1-(5-isoquinolinylsulfonyl)- [CAS]) or an analogue or derivative thereof.

34. PDGF Receptor Kinase Inhibitors

In another embodiment, the pharmacologically active compound
 15 is a PDGF receptor kinase inhibitor (e.g., RPR-127963E or an analogue or derivative thereof.

35. Endothelial Growth Factor Receptor Kinase Inhibitors

In another embodiment, the pharmacologically active compound
 is an endothelial growth factor receptor kinase inhibitor (e.g., CEP-7055, SU-
 20 0879 ((E)-3-(3,5-di-tert-Butyl-4-hydroxyphenyl)-2-(aminothiocarbonyl)acrylonitrile), BIBF-1000 or an analogue or derivative thereof.

36. Retinoic Acid Receptor Antagonists

In another embodiment, the pharmacologically active compound
 25 is a retinoic acid receptor antagonist (e.g., etarotene (Ro-15-1570) (Naphthalene, 6-[2-[4-(ethylsulfonyl)phenyl]-1-methylethenyl]-1,2,3,4-tetrahydro-1,1,4,4-tetramethyl-, (E)- [CAS]), (2E,4E)-3-Methyl-5-(2-((E)-2-(2,6,6-

trimethyl-1-cyclohexen-1-yl)ethenyl)-1-cyclohexen-1-yl)-2,4-pentadienoic acid, tocoretinate (Retinoic acid, 3,4-dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-2H-1-benzopyran-6-yl ester, [2R*(4R*,8R*)]-(±)- [CAS]), aliretinoin (Retinoic acid, cis-9, trans-13- [CAS]), bexarotene (Benzoic acid, 4-
 5 (1-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthalenyl)ethenyl)- [CAS]) or an analogue or derivative thereof.

37. Platelet Derived Growth Factor Receptor Kinase Inhibitors

In another embodiment, the pharmacologically active compound is a platelet derived growth factor receptor kinase inhibitor (e.g., leflunomide (4-
 10 Isoxazolecarboxamide, 5-methyl-N-[4-(trifluoromethyl)phenyl]- [CAS]) or an analogue or derivative thereof.

38. Fibrinogen Antagonists

In another embodiment, the pharmacologically active compound is a fibrinogen antagonist (e.g., picotamide (1,3-Benzenedicarboxamide, 4-
 15 methoxy-N,N'-bis(3-pyridinylmethyl)- [CAS]) or an analogue or derivative thereof.

39. Antimycotic Agents

In another embodiment, the pharmacologically active compound is an antimycotic agent (e.g., miconazole, sulconazole, parthenolide, rosconitine,
 20 nystatin, isoconazole, fluconazole, ketoconazole, imidazole, itraconazole, terpinafine, elonazole, bifonazole, clotrimazole, conazole, terconazole (Piperazine, 1-[4-[[2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-4-(1-methylethyl)-, cis- [CAS]), isoconazole (1-[2-(2,6-dichlorobenzyloxy)-2-(2,4-dichlorophenyl)ethyl]), griseofulvin
 25 (Spiro[benzofuran-2(3H),1'-[2]cyclohexane]-3,4'-dione, 7-chloro-2',4,6-trimethoxy-6'methyl-, (1'S-trans)- [CAS]), bifonazole (1H-Imidazole, 1-([1,1'-biphenyl]-4-ylphenylmethyl)- [CAS]), econazole nitrate (1-[2-[(4-chlorophenyl)methoxy]-2-(2,4-dichlorophenyl)ethyl]-1H-imidazole nitrate), croconazole (1H-Imidazole, 1-

- [1-[2-[(3-chlorophenyl)methoxy]phenyl]ethenyl]- [CAS]), sertaconazole (1H-Imidazole, 1-[2-[(7-chlorobenzo[b]thien-3-yl)methoxy]-2-(2,4-dichlorophenyl)ethyl]- [CAS]), omoconazole (1H-Imidazole, 1-[2-[2-(4-chlorophenoxy)ethoxy]-2-(2,4-dichlorophenyl)-1-methylethenyl]-, (Z)- [CAS]),
- 5 flutrimazole (1H-Imidazole, 1-[(2-fluorophenyl)(4-fluorophenyl)phenylmethyl]- [CAS]), fluconazole (1H-1,2,4-Triazole-1-ethanol, Alpha-(2,4-difluorophenyl)-Alpha-(1H-1,2,4-triazol-1-ylmethyl)- [CAS]), neticonazole (1H-Imidazole, 1-[2-(methylthio)-1-[2-(pentyloxy)phenyl]ethenyl]-, monohydrochloride, (E)- [CAS]),
- 10 butoconazole (1H-Imidazole, 1-[4-(4-chlorophenyl)-2-[(2,6-dichlorophenyl)thio]butyl]-, (+/-)-[CAS]), clotrimazole (1-[(2-chlorophenyl)diphenylmethyl]-1H-imidazole) or an analogue or derivative thereof.

40. Bisphosphonates

- In another embodiment, the pharmacologically active compound
- 15 is a bisphosphonate (e.g., clodronate, alendronate, pamidronate, zoledronate, etidronate) or an analogue or derivative thereof.

41. Phospholipase A1 Inhibitors

- In another embodiment, the pharmacologically active compound is a phospholipase A1 inhibitor (e.g., loteprednol etabonate (Androsta-1,4-
- 20 diene-17-carboxylic acid, 17-[(ethoxycarbonyl)oxy]-11-hydroxy-3-oxo-, chloromethyl ester, (11 β ,17 α)- [CAS] or an analogue or derivative thereof.

42. Histamine H1/H2/H3 Receptor Antagonists

- In another embodiment, the pharmacologically active compound is a histamine H1/H2/H3 receptor antagonist (e.g., ranitidine (1,1-
- 25 Ethenediamine, N-[2-[[[5-[(dimethylamino)methyl]-2-furanyl]methyl]thio]ethyl]-N'-methyl-2-nitro- [CAS]), niperotidine (N-[2-[[[5-[(dimethylamino)methyl]furfuryl]thio]ethyl]-2-nitro-N'-piperonyl-1,1-ethenediamine), famotidine (Propanimidamide, 3-[[[2-

[(aminoiminomethyl)amino]-4-thiazolyl)methyl]thio]-N-(aminosulfonyl)- [CAS]),
 roxitadine acetate HCl (Acetamide, 2-(acetyloxy)-N-[3-[3-(1-
 piperidinylmethyl)phenoxy]propyl]-, monohydrochloride [CAS]), lafutidine
 (Acetamide, 2-[(2-furanylmethyl)sulfinyl]-N-[4-[[4-(1-piperidinylmethyl)-2-
 5 pyridinyl]oxy]-2-butenyl]-, (Z)- [CAS]), nizatadine (1,1-Ethenediamine, N-[2-[[[2-
 [(dimethylamino)methyl]-4-thiazolyl)methyl]thio]ethyl]-N'-methyl-2-nitro- [CAS]),
 ebrotidine (Benzenesulfonamide, N-[[[2-[[[2-[(aminoiminomethyl)amino]-4-
 thiazolyl)methyl]thio]ethyl]amino]methylene]-4-bromo- [CAS]), rupatadine (5H-
 Benzo[5,6]cyclohepta[1,2-b]pyridine, 8-chloro-6,11-dihydro-11-[1-[(5-methyl-3-
 10 pyridinyl)methyl]-4-piperidinylidene]-, trihydrochloride- [CAS]), fexofenadine HCl
 (Benzeneacetic acid, 4-[1-hydroxy-4-[4(hydroxydiphenylmethyl)-1-
 piperidinyl]butyl]-Alpha,Alpha-dimethyl-, hydrochloride [CAS]) or an analogue or
 derivative thereof.

43. Macrolide Antibiotics

15 In another embodiment, the pharmacologically active compound
 is a macrolide antibiotic (e.g., dirithromycin (Erythromycin, 9-deoxo-11-
 deoxy-9,11-[imino[2-(2-methoxyethoxy)ethylidene]oxy]-, [9S(R)]- [CAS]),
 flurithromycin ethylsuccinate (Erythromycin, 8-fluoro-mono(ethyl butanedioate)
 (ester)- [CAS]), erythromycin stinoprate (Erythromycin, 2'-propanoate, compd.
 20 with N-acetyl-L-cysteine (1:1) [CAS]), clarithromycin (Erythromycin, 6-O-methyl-
 [CAS]), azithromycin (9-deoxo-9a-aza-9a-methyl-9a-homoerythromycin-A),
 telithromycin (3-De((2,6-dideoxy-3-C-methyl-3-O-methyl-Alpha-L-ribo-
 hexopyranosyl)oxy)-11,12-dideoxy-6-O-methyl-3-oxo-12,11-(oxycarbonyl((4-(4-
 (3-pyridinyl)-1H-imidazol-1-yl)butyl)imino))- [CAS]), roxithromycin
 25 (Erythromycin, 9-[O-[(2-methoxyethoxy)methyl]oxime] [CAS]), rokitamycin
 (Leucomycin V, 4B-butanolate 3B-propanoate [CAS]), RV-11 (erythromycin
 monopropionate mercaptosuccinate), midecamycin acetate (Leucomycin V,
 3B,9-diacetate 3,4B-dipropanoate [CAS]), midecamycin (Leucomycin V, 3,4B-
 dipropanoate [CAS]), josamycin (Leucomycin V, 3-acetate 4B-(3-
 30 methylbutanoate) [CAS]) or an analogue or derivative thereof.

44. GPIIb IIIa Receptor Antagonists

In another embodiment, the pharmacologically active compound is an GPIIb IIIa receptor antagonist (e.g., tirofiban hydrochloride (L-Tyrosine, N-(butylsulfonyl)-O-[4-(4-piperidiny)butyl]-, monohydrochloride- [CAS]),

- 5 eptifibatide (L-Cysteinamide, N6-(aminoiminomethyl)-N2-(3-mercapto-1-oxopropyl)-L-lysylglycyl-L-Alpha-aspartyl-L-tryptophyl-L-prolyl-, cyclic(1->6)-disulfide [CAS]) or an analogue or derivative thereof.

45. Endothelin Receptor Antagonists

- In another embodiment, the pharmacologically active compound
10 is an endothelin receptor antagonist (e.g., bosentan (Benzenesulfonamide, 4-(1,1-dimethylethyl)-N-[6-(2-hydroxyethoxy)-5-(2-methoxyphenoxy)[2,2'-bipyrimidin]-4-yl]- [CAS]) or an analogue or derivative thereof.

46. Peroxisome Proliferator-Activated Receptor Agonists

- In another embodiment, the pharmacologically active compound
15 is a peroxisome proliferators-activated receptor agonist (e.g., gemfibrozil (Pentanoic acid, 5-(2,5-dimethylphenoxy)-2,2-dimethyl- [CAS]), fenofibrate (Propanoic acid, 2-[4-(4-chlorobenzoyl)phenoxy]-2-methyl-, 1-methylethyl ester [CAS]), ciprofibrate (Propanoic acid, 2-[4-(2,2-dichlorocyclopropyl)phenoxy]-2-methyl- [CAS]), rosiglitazone maleate (2,4-Thiazolidinedione, 5-((4-(2-(methyl-
20 2-pyridinylamino)ethoxy)phenyl)methyl)-, (Z)-2-butenedioate (1:1) [CAS]), pioglitazone hydrochloride (2,4-Thiazolidinedione, 5-[[4-[2-(5-ethyl-2-pyridinyl)ethoxy]phenyl]methyl]-, monohydrochloride (+/-)- [CAS]), etofylline clofibrate (Propanoic acid, 2-(4-chlorophenoxy)-2-methyl-, 2-(1,2,3,6-tetrahydro-1,3-dimethyl-2,6-dioxo-7H-purin-7-yl)ethyl ester [CAS]), etofibrate (3-
25 Pyridinecarboxylic acid, 2-[2-(4-chlorophenoxy)-2-methyl-1-oxopropoxy]ethyl ester [CAS]), clinofibrate (Butanoic acid, 2,2'-[cyclohexylidenebis(4,1-phenyleneoxy)]bis[2-methyl-][CAS]), bezafibrate (Propanoic acid, 2-[4-[2-[(4-chlorobenzoyl)amino]ethyl]phenoxy]-2-methyl- [CAS]), binifibrate (3-

Pyridinecarboxylic acid, 2-[2-(4-chlorophenoxy)-2-methyl-1-oxopropoxy]-1,3-propanediyl ester [CAS]) or an analogue or derivative thereof.

47. Estrogen Receptor Agents

In another embodiment, the pharmacologically active compound
5 is an estrogen receptor agent (*e.g.*, estradiol, 17- β -estradiol) or an analogue or derivative thereof.

48. Somatostatin Analogues

In another embodiment, the pharmacologically active compound
is somatostatin or a somatostatin analogue (*e.g.*, angiopeptin, lanretide,
10 octreotide) or an analogue or derivative thereof.

49. JNK (Jun Kinase) Inhibitors

In another embodiment, the pharmacologically active compound
is a JNK Kinase inhibitor (*e.g.*, Celgene (SP600125, SPC105, SPC23105),
AS-602801 (Serono)) or an analogue or derivative thereof.

15 50. Melanocortin Analogues

In another embodiment, the pharmacologically active compound
is a melanocortin analogue (*e.g.*, HP228) or an analogue or derivative thereof.

51. RAF Kinase Inhibitors

In yet another embodiment, the pharmacologically active
20 compound is a raf kinase inhibitor (*e.g.*, BAY-43-9006 (N-(4-chloro-3-(trifluoromethyl)phenyl-N'-(4-(2-(N-methylcarbamoyl)-4-pyridyloxy)phenyl)urea)
or analogue or derivative thereof.

52. Lysylhydroxylase Inhibitors

In another embodiment, the pharmacologically active compound is a lysylhydroxylase inhibitor (e.g., minoxidil), or an analogue or derivative thereof.

5 53. IKK 1/2 inhibitors

In another embodiment, the pharmacologically active compound is an IKK 1/2 inhibitor (e.g., BMS-345541, SPC839) or an analogue or derivative thereof.

In addition to incorporation of a fibrosis-inhibiting agent into or
10 onto the formulation, another biologically active agent can be incorporated into or onto the formulation, for example an anti-inflammatory (e.g., dexamethazone or aspirin), antithrombotic agents (e.g., heparin, heparin complexes, hydrophobic heparin derivatives, aspirin or dipyridamole), and/or an antibiotic (e.g., amoxicillin, trimethoprim-sulfamethoxazole, azithromycin, clarithromycin,
15 amoxicillin-clavulanate, cefprozil, cefuroxime, cefpodoxime, or cefdinir).

In one aspect of the invention the pharmacologically active compound is capable of altering cellular and/or non-cellular processes involved in the development and/or maintenance of one or more processes involved in fibrosis or adhesions between tissues or between tissues and a medical device.
20 Fibrosis inducing compositions may be useful, for example, as tissue sealants, for effecting tissue adhesion, and for tissue augmentation and repair. Thus, pharmacological agents within the scope of this invention include but are not limited to those which increase one or a combination of processes such as cell division, cell secretion, cell migration, cell adhesion, extracellular matrix
25 production, cytokine (e.g., TNF alpha, IL-1, or IL-6), or other inflammatory activator (e.g., chemokines (e.g., MCP-1, IL-8)) production and/or release, angiogenesis, and/or free radical formation and/or release.

Suitable fibrosis-inducing agents may be readily determined based upon the *in vitro* and *in vivo* (animal) models such as those provided in
30 Examples 34-36.

Numerous therapeutic compounds have been identified that are of utility in the invention.

In one aspect, the fibrosis or adhesion-inducing agent is silk. Silk refers to a fibrous protein, and may be obtained from a number of sources, typically spiders and silkworms. Typical silks contain about 75% of actual fiber, referred to as fibroin, and about 25% sericin, which is a gummy protein that holds the filaments together. Silk filaments are generally very fine and long - as much as 300-900 meters long. There are several species of domesticated silkworm that are used in commercial silk production, however, *Bombyx mori* is the most common, and most silk comes from this source. Other suitable silkworms include *Philosamia cynthia ricini*, *Antheraea yamamai*, *Antheraea pernyi*, and *Antheraea mylitta*. Spider silk is relatively more difficult to obtain, however, recombinant techniques hold promise as a means to obtain spider silk at economical prices (see, e.g., U.S. Patent Nos. 6,268,169; 5,994,099; 5,989,894; and 5,728,810, which are exemplary only). Biotechnology has allowed researchers to develop other sources for silk production, including animals (e.g., goats) and vegetables (e.g., potatoes). Silk from any of these sources may be used in the present invention.

A commercially available silk protein is available from Croda, Inc., of Parsippany, N.J., and is sold under the trade names CROSILK LIQUID (silk amino acids), CROSILK 10,000 (hydrolyzed silk), CROSILK POWDER (powdered silk), and CROSILKQUAT (cocodiammonium hydroxypropyl silk amino acid). Another example of a commercially available silk protein is SERICIN, available from Pentapharm, LTD, a division of Kordia, BV, of the Netherlands. Further details of such silk protein mixtures can be found in U.S. Patent. No. 4,906,460, to Kim, et al., assigned to Sorencor. Silk useful in the present invention includes natural (raw) silk, hydrolyzed silk, and modified silk, i.e., silk that has undergone a chemical, mechanical, or vapor treatment, e.g., acid treatment or acylation (see, e.g., U.S. Patent No. 5,747,015).

Raw silk is typically twisted into a strand sufficiently strong for weaving or knitting. Four different types of silk thread may be produced by this

procedure: organzine, crepe, tram and thrown singles. Organzine is a thread made by giving the raw silk a preliminary twist in one direction and then twisting two of these threads together in the opposite direction. Crepe is similar to organzine but is twisted to a much greater extent. Twisting in only one direction
5 two or more raw silk threads makes tram. Thrown singles are individual raw silk threads that are twisted in only one direction. Any of these types of silk threads may be used in the present invention.

The silk used in the present invention may be in any suitable form that allows the silk to be joined with the medical implant, e.g., the silk may be in
10 thread or powder-based forms. Furthermore, the silk may have any molecular weight, where various molecular weights are typically obtained by the hydrolysis of natural silk, where the extent and harshness of the hydrolysis conditions determines the product molecular weight. For example, the silk may have an average (number or weight) molecular weight of 200 to 5,000. See,
15 e.g., JP-B-59-29199 (examined Japanese patent publication) for a description of conditions that may be used to hydrolyze silk.

A discussion of silk may be found in the following documents, which are exemplary only: Hinman, M.B., et al. "Synthetic spider silk: a modular fibre" *Trends in Biotechnology*, 2000, 18(9) 374-379; Vollrath, F. and
20 Knight, D.P. "Liquid crystalline spinning of spider silk" *Nature*, 2001, 410(6828) 541-548; and Hayashi, C.Y., et al. "Hypotheses that correlate the sequence, structure, and mechanical properties of spider silk proteins" *Int. J. Biol. Macromolecules*, 1999, 24(2-3), 265-270; and U.S. Patent No. 6,427,933.

Other representative examples of fibrosis and adhesion-inducing
25 agents include irritants (e.g., talc, talcum powder, copper, metallic beryllium (or its oxides), quartz dust, silica, crystalline silicates), polymers (e.g., polylysine, polyurethanes, poly(ethylene terephthalate), PTFE, poly(alkylcyanoactylates), and poly(ethylene-co-vinylacetate)); vinyl chloride and polymers of vinyl chloride; peptides with high lysine content; bleomycin and analogues and
30 derivatives thereof; growth factors and inflammatory cytokines involved in angiogenesis, fibroblast migration, fibroblast proliferation, ECM synthesis and

tissue remodeling, such as Epidermal Growth Factor (EGF) Family, Transforming Growth Factor- α (TGF- α), Transforming Growth Factor- β (TGF-9-1, TGF-9-2, TGF-9-3, Platelet-derived Growth Factor (PDGF), Fibroblast Growth Factor (acidic – aFGF; and basic - bFGF), Fibroblast stimulating factor-1, Activins, Vascular Endothelial Growth Factor (including VEGF-2, VEGF-3, VEGF-A, VEGF-B, VEGF-C, Placental Growth Factor - PlGF), Angiopoietins, Insulin-like Growth Factors (IGF), Hepatocyte Growth Factor (HGF), Connective Tissue Growth Factor (CTGF), Myeloid Colony-stimulating Factors (CSFs), Monocyte chemotactic protein, Granulocyte-Macrophage Colony-stimulating Factors (GM-CSF), Granulocyte Colony-stimulating Factor (G-CSF), Macrophage Colony-stimulating Factor (M-CSF), Erythropoietin, Interleukins (particularly IL-1, IL-8, IL-6), Tumor Necrosis Factor- α (TNF α), Nerve Growth Factor (NGF), Interferon- α , Interferon- β , histamine, endothelin-1, angiotensin II, growth hormone (GH), and synthetic peptides, analogues or derivatives of these factors are also suitable for release from specific implants and devices to be described later. Other examples include CTGF (connective tissue growth factor); inflammatory microcrystals (e.g., crystalline minerals such as crystalline silicates); Monocyte chemotactic protein, fibroblast stimulating factor 1, histamine, endothelin-1, angiotensin II, bovine collagen, bromocriptine, methylsergide, methotrexate, chitosan, N-carboxybutyl chitosan, carbon tetrachloride, Thioacetamide, Fibrosin, ethanol, naturally occurring or synthetic peptides containing the Arg-Gly-Asp (RGD) sequence, generally at one or both termini, described, e.g., in U.S. Patent No. 5,997,895, and tissue adhesives, such as cyanoacrylate and crosslinked poly(ethylene glycol) – methylated collagen compositions, such as CT3 (Cohesion Technologies, Palo Alto, CA). Other examples of fibrosis-inducing agents include bone morphogenic proteins (e.g., BMP-2, BMP-3, BMP-4, BMP-5, BMP-6 (Vgr-1), BMP-7 (OP-1), BMP-8, BMP-9, BMP-10, BMP-11, BMP-12, BMP-13, BMP-14, BMP-15, and BMP-16). Of these BMP's, BMP-2, BMP-3, BMP-4, BMP-5, BMP-6, and BMP-7 are of particular utility. Bone morphogenic proteins are described, for example, in U.S. Patent Nos. 4,877,864; 5,013,649; 5,661,007; 5,688,678; 6,177,406;

6,432,919; and 6,534,268 and Wozney, J.M., et al. (1988) Science: 242(4885); 1528-1534.

Other representative examples of fibrosis-inducing agents include components of extracellular matrix (e.g., fibronectin, fibrin, fibrinogen, collagen, including fibrillar and non-fibrillar collagen, adhesive glycoproteins, proteoglycans (e.g., heparin sulphate, chondroitin sulphate, dermatan sulphate), hyaluronan, Secreted Protein Acidic and Rich in Cysteine (SPARC), Thrombospondins, Tenacin, and Cell Adhesion Molecules (including integrins, vitronectin, fibronectin, laminin, hyaluronic acid, elastin, bitronectin), and proteins found in basement membranes, and fibrosin).

Within various embodiments of the invention, a composition which promotes fibrosis (and/or restenosis) also includes a compound which acts to stimulate cellular proliferation. Representative examples of agents that stimulate cellular proliferation include, e.g., dexamethasone, isotretinoin, 17- β -estradiol, diethylstilbestrol, cyclosporin A and all-trans retinoic acid (ATRA) and analogues and derivatives thereof. Other examples of agents that stimulate cellular proliferation include: Sphingosine 1-phosphate receptor agonist (e.g., FTY-720 (1,3-Propanediol, 2-amino-2-(2-(4-octylphenyl)ethyl)-,hydrochloride [CAS]; Immunostimulants, such as Imupredone (Methanone, [5-amino-2-(4-methyl-1-piperidiny)phenyl](4-chlorophenyl)- [CAS]), DiaPep227; and Nerve Growth Factor Agonist, such as, e.g., NG-012 (5H,9H,13H,21H,25H,-Dibenzo[k,u][1,5,9,15,19] pentaoxacyclotetracosin-5,9,13,21,25-pentone, 7,8,11,12,15,16,23,24,27,28-decahydro-2,4,18,20-tetrahydroxy-11-(hydroxymethyl)-7,15,23,27-tetramethyl- [CAS]), NG-121, SS-701 (2,2':6',2"-Terpyridine, 4'-(4-methylphenyl)-, trihydrochloride [CAS]), AMPAlex (Piperidine, 1-(6-quinoxaliny)carbonyl)- [CAS]), RGH-2716 (8-[4,4-bis(4-fluorophenyl)butyl]-3-(1,1-dimethylethyl)-4-methylene-1-oxa-3,8-diaza-spiro[4.5] decan-2-one [CAS]), TDN-345 (1-Oxa-3,8-diazaspiro[4.5]decan-2-one, 8-[4,4-bis(4-fluorophenyl)butyl]-3-(1,1-dimethylethyl)-4-methylene- [CAS]).

Within various embodiments of the invention, a stent graft is coated on one aspect with a composition which promotes fibrosis (and/or

restenosis), as well as being coated with a composition or compound which prevents thrombosis on another aspect of the device. Representative examples of agents that inhibit thrombosis include heparin, aspirin, dipyridamole, as well as analogues and derivatives thereof.

5 In another embodiment of the invention, the drug is a hydrophobic drug. The term "hydrophobic drug" refers to drugs that are insoluble or sparingly or poorly soluble in water. As used herein, such drugs will have a solubility below 10 mg/ml, usually below 1 mg/ml, sometimes below 0.01 mg/ml, and sometimes below 0.001 mg/ml. Exemplary hydrophobic drugs include
10 certain steroids, such as budesonide, testosterone, progesterone, estrogen, flunisolide, triamcinolone, beclomethasone, betamethasone; dexamethasone, fluticasone, methylprednisolone, prednisone, hydrocortisone, and the like; certain peptides, such as cyclosporin cyclic peptide, retinoids, such as all-cis retinoic acid, 13-trans retinoic acid, and other vitamin A and beta carotene
15 derivatives; vitamins D, E, and K and water insoluble precursors and derivatives thereof; prostaglandins and leukotrienes and their activators and inhibitors including prostacyclin (epoprostanol), and prostaglandins; tetrahydrocannabinol; lung surfactant lipids; lipid soluble antioxidants; hydrophobic antibiotics and chemotherapeutic drugs such as amphotericin B
20 and adriamycin and the like. In one aspect, the hydrophobic drug is selected from the following classes of compounds: chemotherapeutic, antibiotic, antimicrotubule, anti-inflammatory, and antiproliferative compounds. In a preferred aspect, the hydrophobic drug is selected from paclitaxel, hydrophobic paclitaxel derivatives and hydrophobic paclitaxel analogs. In another preferred
25 aspect, the hydrophobic drug is paclitaxel.

 The hydrophobic drug may be combined directly with Compound₁ and/or Compound₂. Alternatively, the hydrophobic drug may be combined with a secondary carrier, *e.g.*, a micelle, where the secondary carrier assists in solubilization and/or delivery of the drug. The drug/secondary carrier mixture is
30 then combined directly with Compound₁ and/or Compound₂, and/or added separately to the mixture of Compound₁ and Compound₂. The secondary

carrier is particularly useful in those instances where the drug is hydrophobic and does not readily dissolve in water. In one embodiment (e.g., in which the drug is hydrophobic), the drug is associated with a secondary carrier.

Optionally, this drug/carrier combination is present in an aqueous buffer

5 solution that is combined with Compound₁ and/or Compound₂ and/or the reaction product thereof. Suitable secondary carriers are described herein.

However, a preferred secondary carrier is described in PCT International Publication No. WO 02/072150 and U.S. Patent Application No. 10/251,659.

Optional Composition Constituents

10 In addition to the reactive compounds and the drug, the compositions of the present invention may also contain other compounds, which may be included in one or both of the components of the two-component compositions, or may be separately administered. In one embodiment, these compounds may become covalently incorporated into the matrix itself by

15 becoming crosslinked to one or both of the reactive compounds after they are mixed together. In another embodiment, (e.g., if the compound was unreactive with either of the reactive compounds), the compound may be administered in such a way that it becomes physically or ionically associated with the matrix-forming compounds after mixing, and thus becomes part of the matrix itself.

20 Additional compounds that may be added into the instant compositions include glycosaminoglycans and proteins. Suitable glycosaminoglycans include, inter alia, hyaluronic acid, chitin, chitosan, chondroitin sulfate A, B, or C, keratin sulfate, keratosulfate and heparin, and derivatives thereof. In another embodiment, proteins can be added for a variety

25 of purposes. For example, collagen may improve biocompatibility of the matrix, including the potential colonization by cells, promotion of wound healing, etc. Collagen and any amino group-containing proteins would also contribute to the structural integrity of the matrix by becoming crosslinked thereto along with the other matrix components. In particular, if PEG-succinimidyl esters are used,

30 the amide bonds formed with collagen will be more stable to hydrolytic

degradation than the bonds formed by the reaction of succinimidyl esters and sulfhydryls.

Suitable proteins include, inter alia, collagen, fibronectin, gelatin and albumin, as well as peptide fragments thereof. Particularly preferred is collagen, which may be in the form of afibrillar, microfibrillar or fibrillar collagen. Types I and III collagen isolated from bovine corium or human placenta, or prepared by recombinant DNA methods, are suitable. See PCT WO 90/05755 for a description of suitable collagens and collagen derivatives. It should be understood that when adding collagen to the composition, it is important to adjust the concentration of the other composition components to avoid precipitation.

Additional constituents which may be added to the composition include antibiotics, growth factors, hemostatic proteins (such as thrombin, fibrin, fibrinogen, blood factors, *etc.*), cells, genes, DNA, *etc.*

In one aspect, the compositions of the present invention include one or more preservatives or bacteriostatic agents, present in an effective amount to preserve the composition and/or inhibit bacterial growth in the composition, for example, bismuth tribromophenate, methyl hydroxybenzoate, bacitracin, ethyl hydroxybenzoate, propyl hydroxybenzoate, erythromycin, chlorocresol, benzalkonium chlorides, and the like. Examples of the preservative include paraoxybenzoic acid esters, chlorobutanol, benzylalcohol, phenethyl alcohol, dehydroacetic acid, sorbic acid, *etc.* In one aspect, the compositions of the present invention include one or more bactericidal (also known as bacteriacidal) agents.

In one aspect, the compositions of the present invention include one or more antioxidant, present in an effective amount. Examples of the antioxidant include sulfites and ascorbic acid.

In one aspect, the compositions of the present invention include one or more coloring agents, also referred to as dyestuffs, which will be present in an effective amount to impart observable coloration to the composition, *e.g.*, the gel. Examples of coloring agents include dyes suitable for food such as

those known as F. D. & C. dyes and natural coloring agents such as grape skin extract, beet red powder, beta carotene, annato, carmine, turmeric, paprika, and so forth.

Optional Composition Properties and Packaging

5 In one aspect, the compositions of the present invention are sterile. Many pharmaceuticals are manufactured to be sterile and this criterion is defined by the USP XXII <1211> where the term "USP" refers to U.S. Pharmacopeia (see www.usp.org, Rockville, MD). Sterilization in this embodiment may be accomplished by a number of means accepted in the
10 industry and listed in the USP XXII <1211>, including gas sterilization, ionizing radiation or, when appropriate, filtration. Sterilization may be maintained by what is termed aseptic processing, defined also in USP XXII <1211>. Acceptable gases used for gas sterilization include ethylene oxide. Acceptable radiation types used for ionizing radiation methods include gamma, for instance
15 from a cobalt 60 source and electron beam. A typical dose of gamma radiation is 2.5 MRad. Filtration may be accomplished using a filter with suitable pore size, for example 0.22 μm and of a suitable material, for instance Teflon.

 In another aspect, the compositions of the present invention are contained in a container that allows them to be used for their intended purpose,
20 *i.e.*, as a pharmaceutical composition. Properties of the container that are important are a volume of empty space to allow for the addition of a constitution medium, such as water or other aqueous medium, *e.g.*, saline, acceptable light transmission characteristics in order to prevent light energy from damaging the composition in the container (refer to USP XXII <661>), an acceptable limit of
25 extractables within the container material (refer to USP XXII), an acceptable barrier capacity for moisture (refer to USP XXII <671>) or oxygen. In the case of oxygen penetration, this may be controlled by including in the container, a positive pressure of an inert gas, such as high purity nitrogen, or a noble gas, such as argon.

Typical materials used to make containers for pharmaceuticals include USP Type I through III and Type NP glass (refer to USP XXII <661>), polyethylene, polytetrafluoroethylene (e.g., TEFLON from E. I. DuPont De Nemours and Company, Wilmington, DE), silicone, and gray-butyl rubber. For
5 parenterals, USP Types I to III glass and polyethylene are preferred.

Incorporation of biologically active agents into the compositions

Biologically active agents can be incorporated directly into the composition or they can be incorporated into a secondary carrier. Accordingly, a secondary carrier is another optional constituent of the compositions of the
10 present invention. For direct incorporation of the biologically active agent, the agent may or may not contain electrophilic or nucleophilic group or groups that can react with either the activated functional groups of the synthetic polymer of the composition. The biologically active agents can be incorporated into the composition before the components of the composition are brought together to
15 produce the crosslinked composition or after the components of the composition are brought together to form the crosslinked composition. The biologically active agent can be admixed with either of the starting components, admixed with both of the starting components, admixed with the admix of both starting components, admixed with either or both of the starting components at
20 the time of application or incorporated into the composition at a time after the starting components have been mixed or reacted with each other. A combination of these methods may also be used to incorporate the biologically active agent into the composition. The presence of the appropriate electrophilic or nucleophilic groups on the biologically active agent will allow the biologically
25 active agent to be incorporated into the final composition via chemical bonds. The absence of the appropriate electrophilic or nucleophilic groups on the biologically active agent will allow the biologically active agent to be incorporated into the final composition via physical entrapment, electrostatic interactions, hydrogen bonding, hydrophobic interactions, Van Der Waals
30 interactions or a combination of these interactive forces. A single biologically

active agent may be directly incorporated into the composition or a combination of biologically active agents may be incorporated into the composition using any of the possible approaches described above.

For the incorporation of the biologically active agent into the composition via the use of a secondary carrier, which is a preferred embodiment when the drug is hydrophobic, the biologically active agent can be incorporated into the secondary carrier by covalent linking to the secondary carrier, physical entrapment, adsorption, electrostatic interactions, hydrophobic interactions, partitioning effects, precipitation in the secondary carrier or a combination of these interactions. This biologically active agent/secondary carrier composition can then be incorporated directly into the composition (either with Compound₁ or with Compound₂ or with both Compound₁ and Compound₂) or they can be used as a separate component of the composition.

The secondary carriers that can be used to incorporate these biologically active agents may be in the form of particulates, microparticles, nanoparticles, nanocrystals, microspheres, nanospheres, liposomes, micelles, emulsions, microemulsions, dispersions, inclusion complexes, non-ionic surfactant vesicles (NISV), niosomes, proniosomes, cochleates, immunostimulating complexes (ISCOMs) and association complexes. In one embodiment, the microparticles, nanoparticles or microspheres can be prepared using polymers and copolymers that include one or more of the residue units from the following monomers: D-lactide, L-lactide, D,L-lactide, glycolide, ϵ -caprolactone, trimethylene carbonate, 1,4-dioxane-2-one, or 1,5-dioxepan-2-one. In another embodiment, the microparticles, nanoparticles, or microspheres can be prepared using block copolymers of the for A-B, A-B-A or B-A-B where A is a poly(alkylene oxide) (e.g., poly(ethylene glycol), poly(propylene glycol), copolymers of ethylene oxide and propylene oxide, or mono-alkyl ethers thereof) and B is a degradable polyester, for example polymers and copolymers comprising one or more of the residue units of the monomers D-lactide, L-lactide, D,L-lactide, glycolide, ϵ -caprolactone, trimethylene carbonate, 1,4-dioxane-2-one or 1,5-dioxepan-2-one). Micelles

can be prepared using small molecule surfactants (e.g., SDS) or polymeric compositions (e.g., PLURONIC F127 or PLURONIC F68 (both available from BASF Corporation, Mount Olive, NJ), block copolymers of the form A-B, A-B-A or B-A-B, where A is a poly(alkylene oxide) e.g., poly(ethylene glycol),
5 poly(propylene glycol), copolymers of ethylene oxide and propylene oxide, or mono-alkyl ethers thereof) and B is a degradable polyester, for example polymers and copolymers comprising one or more of the residue units of the monomers D-lactide, L-lactide, D,L-lactide, glycolide, ϵ -caprolactone, trimethylene carbonate, 1,4-dioxane-2-one or 1,5-dioxepan-2-one). Albumin,
10 alginate, gelatin, starch, collagen, chitosan, poly(anhydrides), poly(orthoesters), poly(phosphazines) can also be used to prepare these secondary carriers. Liposome compositions can include phosphatidyl choline, cholesterol, phosphatidyl ethanolamine as well as any of the commercially available lipids (for example, lipids available from Avanti Polar Lipids). Non-polymeric
15 compounds such as sucrose derivatives (e.g., sucrose acetate isobutyrate, sucrose oleate); sterols such as cholesterol, stigmasterol, β -sitosterol, and estradiol; cholesteryl esters such as cholesteryl stearate; C_{12} - C_{24} fatty acids such as lauric acid, myristic acid, palmitic acid, stearic acid, arachidic acid, behenic acid, and lignoceric acid; C_{18} - C_{36} mono-, di- and triacylglycerides such
20 as glyceryl monooleate, glyceryl monolinoleate, glyceryl monolaurate, glyceryl monodocosanoate, glyceryl monomyristate, glyceryl monodicenoate, glyceryl dipalmitate, glyceryl didocosanoate, glyceryl dimyristate, glyceryl didecenoate, glyceryl tridocosanoate, glyceryl trimyristate, glyceryl tridecenoate, glycerol tristearate and mixtures thereof; sucrose fatty acid esters such as sucrose
25 distearate and sucrose palmitate; sorbitan fatty acid esters such as sorbitan monostearate, sorbitan monopalmitate and sorbitan tristearate; C_{16} - C_{18} fatty alcohols such as cetyl alcohol, myristyl alcohol, stearyl alcohol, and cetostearyl alcohol; esters of fatty alcohols and fatty acids such as cetyl palmitate and cetearyl palmitate; anhydrides of fatty acids such as stearic anhydride;
30 phospholipids including phosphatidylcholine (lecithin), phosphatidylserine, phosphatidylethanolamine, phosphatidylinositol, and lysoderivatives thereof;

sphingosine and derivatives thereof; spingomyelins such as stearyl, palmitoyl, and tricosanyl spingomyelins; ceramides such as stearyl and palmitoyl ceramides; glycosphingolipids; lanolin and lanolin alcohols, calcium phosphate can also be used as part of the secondary carrier composition.

5 In one embodiment, one or more additives can be added to the drug component, the PEG components or the secondary carriers in order to modulate the pH or the composition and/or release of the drug from the composition. These additives can include neutral, positively or negatively charged lipids, fatty acids, amino-containing molecules or bile salts. Specific
10 examples of additives that can be used include histidine, spermidine, 1,2 dipalmitoyl-sn-glycero-3-phosphoethanolamine, 3-ethylphosphocholine chloride, palmitic acid or cholic acid.

 The biologically active agent/secondary carrier composition can be admixed with either of the starting components, admixed with both of the
15 starting components, admixed with the admix of both starting components, admixed with either or both of the starting components at the time of application or incorporated into the composition at a time after the starting components have been mixed or reacted with each other. A combination of these methods may also be used to incorporate the biologically active agent/secondary carrier
20 into the composition.

 The biologically active agent/secondary carrier composition can contain groups that may or may not be able to react with the electrophilic or nucleophilic groups of the starting components. In one embodiment, the secondary carrier does not contain electrophilic or nucleophilic groups that can
25 react with the starting polymer components, in which case the secondary carrier/biologically active agent is retained within the final composition through physical entrapment, hydrophobic, hydrogen bonding, Van der Waals interactions, electrostatic interactions or a combination of these interactive forces.

30 In another embodiment, the biologically active agent/secondary carrier composition may contain functional groups that can react with either the

electrophilic or nucleophilic groups of the starting components. Under these circumstances, the biologically active agent/secondary carrier composition is retained in the final composition via covalent bonds. Other interactions such as physical entrapment, hydrophobic, hydrogen bonding, Van der Waals

- 5 interactions, electrostatic interactions or a combination of these interactive forces may also contribute to the retention of the biologically active agent/secondary carrier in the final composition.

Compounds containing one or more of the following functional groups: -NH₂, -SH, -OH, -PH₂, -CO-NH-NH₂, -CO₂N(COCH₂), -CO₂H, -CHO, 10 -CHOCH₂, -N=C=O, -SO₂CH=CH₂, -N(COCH₂)₂, -S-S-(C₅H₄N), CH₂=CH-, CH₂=CH-COO-, CH₂=CH-CO-NH- etc. are compounds that can be incorporated into the secondary carriers thereby providing the secondary carriers with functional groups that are capable of reacting with the starting components of the crosslinked composition.

15 Examples of useful amino compounds that can be incorporated into the secondary carriers to provide functional groups on the secondary carrier include phosphatidyl ethanolamine lipids (for example, Avanti Polar Lipids, Inc. Catalogue # 850757, 850756, 850759, 850801, 850758, 850802, 850804, 850806, 850697, 850699, 850700, 850702, 850745, 850705, 850402, 20 850706, 830756C, 830756P, 850715, 850725, 85T725, 850755, 850795, 850800, 850797, 870125, 870122, 870140, 870142, 856705, 856715, 846725), alkyl amines, aryl amines, and cycloalkyl amines.

Examples of useful thiol compounds that can be incorporated into the secondary carriers to provide functional groups on the secondary carrier 25 includes 1,2-Dipalmitoyl-sn-Glycero-3-Phosphothioethanol (Sodium Salt) (Avanti Polar Lipids, Catalogue # 870160), alkyl thiols, and aryl thiols.

Other methods of incorporated a drug with Compound₁ and Compound₂ are illustrated in PCT International Publication No. WO 00/09087.

30 The cells or genes may be either allogeneic or xenogeneic in origin. For example, the compositions can be used to deliver cells or genes from other species which have been genetically modified. Because the

compositions of the invention are not easily degraded in vivo, cells and genes entrapped within the crosslinked polymer compositions will be isolated from the patient's own cells and, as such, will not provoke an immune response in the patient. In order to entrap the cells or genes within a crosslinked polymer

5 matrix, the first polymer and the cells or genes may be pre-mixed, then the second polymer is mixed into the first polymer/cell or gene mixture to form a crosslinked matrix, thereby entrapping the cells or genes within the matrix.

As discussed above for biologically active agents, when used to deliver cells or genes, the synthetic polymers preferably also contain

10 biodegradable groups to aid in controlled release of the cells or genes at the intended site of delivery.

Composition Formulation

The compositions of the present invention comprise two separate parts, or "components", which may be in liquid or solid form. In a preferred

15 embodiment, both components are liquids, such that each can be easily applied separately to the site of administration. Accordingly, one of the components may be in the form of a dry powder that becomes mixed with the second component, which is in liquid form, when each are sprayed separately onto the tissue, or by mixing at the tissue site. It is also possible to have both

20 components delivered to the site as powders, to be mixed with buffer at the site of administration.

In an alternative embodiment, both components can be mixed together in a single aqueous medium in which they are both unreactive, *i.e.*, such as in a low pH buffer. Thereafter, they can be sprayed onto the tissue site

25 along with a high pH buffer, after which they will rapidly react and form a gel.

The concentration of the reactive compounds in each of the composition components necessarily depends on a number of factors. For example, if the composition components are each 4-arm PEGs (*i.e.*, PEG-PEG compositions), a concentration of 20-25% by weight in each of the two

30 components before mixing results in a gel after mixing with an elastic modulus,

G', of approximately 10^5 - 10^6 dynes/cm², which is adequate for use as a surgical sealant. Using methylated collagen and 4-arm succinimidyl PEG, concentrations of 2-4% and 0.2-0.4%, respectively, result in gels with cohesive strengths that are comparable to PEG-PEG gels by about 10-15%. Using
5 albumin as one of the components, concentrations of 30% or more achieve a similar cohesive strength. The appropriate concentration of the compound, and other optional ingredients, in each component, and thus the relative concentration of the matrix components in the final gel matrix, can easily be optimized to achieve the desired gelation time and gel strength using routine
10 experimentation. Using the preferred four-arm PEGs described above, the synthetic polymer is generally present at a concentration of 2 to 50% (w/v), and more preferably 10-25%.

The liquid components of the compositions of the present invention are each separately prepared by adding the activated synthetic
15 polymer (in dry form or as a concentrated solution) to a liquid medium. Suitable liquid media include aqueous buffer solutions, such as monobasic sodium phosphate/dibasic sodium phosphate, sodium carbonate/sodium bicarbonate, glutamate or acetate, at a concentration of 0.5 to 300 mM. In general, the sulfhydryl-reactive PEG is prepared in water or a dilute buffer, with a pH of
20 between around 2 to 6. Buffers with pHs between about 8 to 10.5 for preparing the sulfhydryl-PEG component are useful to achieve fast gelation time of compositions containing mixtures of sulfhydryl-PEG/SG-PEG. These include carbonate, borate and AMPSO (3-[(1,1-dimethyl-2-hydroxyethyl)amino]2-hydroxy-propane-sulfonic acid). In contrast, using a combination of maleimidyl
25 PEG and sulfhydryl-PEG, a pH of around 5 to 9 is preferred for the liquid medium used to prepare the sulfhydryl PEG. A particularly preferred composition for hemostatic applications to actively bleeding tissue sites comprises a mixture of maleimidyl and succinimidyl PEG as the first component, and sulfhydryl PEG as the second component. Such compositions
30 produce gels with enhanced biodegradability and superior gel times when

compared to compositions having only maleimidyl PEG or succinimicyl PEG alone.

The pH of the aqueous buffer solution that is used for each of the two (or more) composition components should be adjusted using routine optimization to achieve a final pH that is conducive to rapid gelation, without causing instantaneous gelation which interferes with the delivery process. For example, both amino PEG and sulfhydryl PEG need a basic pH to enhance nucleophilicity. The effects of pH on gel time are discussed below in the Examples.

10 Use and Administration

The compositions of the present invention are generally delivered to the site of administration in such a way that the two (or more) individual reactive components of the composition come into contact with one another for the first time at the site of administration, or immediately preceding administration to the tissue. Thus, the compositions of the present invention are preferably delivered to the site of administration using an apparatus that allows the two components to be delivered separately. Such delivery systems usually involve two-compartment single exit or dual exit spray devices. Alternatively, the two reactive components can be delivered separately using any type of controllable extrusion system, or they can be delivered manually in the form of separate pastes, liquids or dry powders, and mixed together manually at the site of administration. Many devices that are adapted for delivery of two-component tissue sealants/hemostatic agents are well known in the art and can also be used in the practice of the present invention. In this regard, see, for example, U.S. Patent No. 6,328,229.

Yet another way of delivering the compositions of the present invention is to prepare the two reactive components (or the single reactive component in the case of sulfhydryl-containing components that are designed to form disulfide bonds) in inactive form as either a liquid or powder. Such compositions can then be activated after application to the tissue site, or

immediately beforehand, by applying an activator. In one embodiment, the activator is a buffer solution having a pH that will activate the composition once mixed therewith. See Example 12 for a description of a sulfhydryl-containing PEG composition that is maintained at a low pH until administration, then mixed
5 with a high pH buffer at the application site to initiate gelation.

The compositions of the present invention can be used in a variety of different pharmaceutical applications. In general, the compositions described herein can be adapted for use in any tissue engineering application where synthetic gel matrices are currently being utilized. For example, the
10 compositions of the present invention are useful as tissue sealants, in tissue augmentation, in tissue repair, as hemostatic agents, in preventing tissue adhesions, in providing surface modifications, and in drug/cell/gene delivery applications. One of skill in the art could easily determine the appropriate administration protocol to use with any composition having a known gel
15 strength and gelation time based on the principles described herein and well known scientific principles. A more detailed description of several specific applications is given below:

Tissue Sealants & Adhesives

In a preferred application, the compositions described herein can
20 be used for medical conditions that require a coating or sealing layer to prevent the leakage of gases, liquid or solids. The method entails applying both components to the damaged tissue or organ to seal 1) vascular and or other tissues or organs to stop or minimize the flow of blood; 2) thoracic tissue to stop or minimize the leakage of air; 3) gastrointestinal tract or pancreatic tissue to
25 stop or minimize the leakage of fecal or tissue contents; 4) bladder or ureters to stop or minimize the leakage of urine; 5) dura to stop or minimize the leakage of CSF; and 6) skin or serosal tissue to stop the leakage of serosal fluid.

These compositions may also be used to adhere tissues together such as small vessels, nerves or dermal tissue. The material can be used 1) by
30 applying it to the surface of one tissue and then a second tissue may be rapidly

pressed against the first tissue or 2) by bringing the tissues in close juxtaposition and then applying the material.

Surgical Adhesions

Another application is a method of reducing the formation of adhesions after a surgical procedure in a patient. The method entails applying the material onto the damaged tissue or organ either by spraying both components together or by applying previously admixed components. The components will react together to form a hydrogel on the tissue surface. The medical procedures include gynecological, abdominal, neurosurgical, cardiac, tendon and orthopedic indications.

General Procedure A

Sprague Dawley rats are prepared for surgery by anaesthetic induction with 5% halothane in an enclosed chamber. Animals are transferred to the surgical table, and anaesthesia maintained by nose cone on halothane throughout the procedure and Buprenorphen 0.035 mg/kg is injected intramuscularly. The abdomen is shaved, sterilized, draped and entered via a midline incision. The caecum is lifted from the abdomen and placed on sterile gauze dampened with saline. Dorsal and ventral aspects of the caecum are scraped a total of 45 times over the terminal 1.5 cm using a #10 scalpel blade, held at a 45° angle. Blade angle and pressure are controlled to produce punctuated bleeding, while avoiding severe tissue damage or tearing.

The left side of the abdominal cavity is retracted and everted to expose a section of the peritoneal wall nearest the natural resting caecal location. The exposed superficial layer of muscle (*transverses abdominis*) is excised over an area of 1.0 X 1.5 cm². Excision includes portions of the underlying internal oblique muscle, leaving behind some intact and some torn fibres from the second layer. Minor local bleeding is tamponaded until controlled.

A test formulation is deployed at the wounded areas, on the abraded sidewall, between the caecum and sidewall. The formulation is deployed using either a syringe spray system or an air-assisted syringe system. The abraded caecum is then positioned over the sidewall wound and sutured at
5 four points immediately beyond the dorsal corners of the wound edge. The large intestine is replaced in a natural orientation continuous with the caecum. The abdominal incision is closed in two layers with 4-0 silk sutures.

Healthy subjects are followed for one week, and then euthanized by lethal injection for *post mortem* examination to score. Severity of post-
10 surgical adhesions is scored by independently assessing the tenacity and extent of adhesions at the site of caecal-sidewall abrasion, at the edges of the abraded site, and by evaluating the extent of intestinal attachments to the exposed caecum. Adhesions are scored on a scale of 0-4 with increasing severity and tenacity. The extent of adhesion is scored as a percent of the
15 injured area that contained adhesions.

General Procedure B

Female New Zealand white rabbits weighing between 3-4 kg are used for surgeries. The animals are acclimated in the vivarium for a minimum of 5 days prior to study initiation and housed individually. Animals are
20 anesthetized by a single injection of ketamine hydrochloride (35 mg/kg) and xylazine hydrochloride (5 mg/kg). Once sedated, anesthesia is induced with halothane or isoflurane delivered through a mask until the animal is unconscious, when an endotracheal tube is inserted for delivery of halothane or isoflurane to sustain surgical anesthesia. The abdomen is shaved, swabbed
25 with antiseptic, and sterile-draped for surgery. A midline vertical incision 6-7 cm in length is made with a #10 scalpel blade. The uterine horns are brought through the incision and each horn is abraded 20 times in each direction with a #10 scalpel blade held at a 45° angle. A region of the uterine horn, approximately 2 cm in length is abraded along the circumference of the horn,
30 beginning 1 cm from the ovaric end. This injury results in generalized erythema

without areas of active bleeding. Each side of the abdominal cavity is retracted and everted to expose a section of the peritoneal wall nearest the natural resting location of the horn. The sidewall apposed to the abraded uterine horn is injured by removing a 2.0 X 0.5 cm² area of the peritoneum. The abraded
5 uterine horn is then positioned over the sidewall wound and sutured at four points of the wound edge. Following completion of the abrasion, before closure, animals are randomized into treatment and non-treatment groups. Treated animals have approximately 1 ml of the desired formulation applied to each horn at the site of attachment to the sidewall. Healthy subjects are
10 followed for one week, and then euthanized by lethal injection for *post mortem* examination to score the severity of inflammation and adhesions using established scoring systems. Post-surgical adhesions are scored by independently assessing the extent, severity and tenacity of adhesions of each horn to the peritoneal sidewall. Adhesions are scored on a scale of 0-4
15 depending involvement of the horn in adhesions and a scale of 0-3 with increasing severity and tenacity.

EXAMPLES

EXAMPLE 1

PREPARATION OF A TWO-COMPONENT TISSUE SEALANT COMPOSITION

20 a. First Component

Pentaerythritol poly(ethylene glycol)ether tetra-succinimidyl glutarate ("SG-PEG") (mol. wt. 10,000) is dissolved in 0.5 mM sodium phosphate pH 6.0 at a concentration of 20% w/v. (This solution is not stable in aqueous media due to the susceptibility of the active ester to hydrolysis and
25 should be used within one hour of preparation).

b. Second Component

Pentaerythritol poly(ethylene glycol)ether tetra-sulfhydryl (mol. wt. 10,000) is dissolved in 300 mM sodium phosphate/sodium carbonate buffer ("P/C buffer"), pH 9.6, at a concentration of 20% w/v. P/C buffer is prepared as

follows: 300 mM sodium monobasic phosphate is mixed with 300 mM sodium carbonate to achieve pH 9.6. The final molarity is approximately 117 mM phosphate and 183 mM carbonate. This solution is stable in aqueous media, but care should be taken to prevent the exposure of the solution to oxygen to prevent oxidation to disulfide. Although pH is preferred for certain compositions, a pH of 8 to 10.5 is generally believed to be suitable for use in the practice of the present invention.

EXAMPLE 2

SURGICAL SEALING OF ARTERIES

The right carotid artery of New Zealand white rabbits is exposed. The rabbits are treated with 200 U/kg of heparin and the vessel is clamped proximally and distally using atraumatic vascular clamps. A puncture hole is made in the carotid artery using a 27G needle. The control rabbits are treated with tamponade until hemostasis is achieved. For the treated rabbits, approximately 0.5 mL of each of the two components of the compositions prepared as described in Example 1 are delivered to the defect site using a two component sprayer (Duo Flow, Hemaedics, Malibu, Calif.). After the material is allowed to set for 30 sec, the clamps are removed and the time to hemostasis and the blood loss are measured. The arteries of the control rabbits also remain clamped for 30 sec for consistency. The results are shown in Table 1.

TABLE 1

<u>Blood Loss and Time to Hemostasis as a Function of Treatment</u>		
Treatment	Blood Loss (g)	Time to Hemostasis (sec)
Tamponade (n = 18)	5.7 ± 3.4	144 ± 34
Hydrogel (n = 17)	1.0 ± 2.5	31 ± 65

The above results illustrate that the composition significantly reduces the amount of blood loss and time to hemostasis from a punctured artery.

EXAMPLE 3

SURGICAL SEALING OF AN ePTFE GRAFT

The dogs are treated with heparin to achieve an activated clotting time of greater than 480 sec. The left iliac of the dogs is exposed and isolated using atraumatic vascular clamps placed distally and proximally. A 5 cm segment of the artery is excised and replaced with an ePTFE (polytetrafluoroethylene) graft of the same diameter. Prior to the completion of the anastomosis, the graft was de-aired using a 27G needle. Approximately 3.0 mL of each of the two components of the composition prepared according to Example 1 is delivered to the defect site using a two component sprayer (Cohesion Technologies, Inc., Palo Alto, Calif.). After the material is allowed to set for 30 sec, the clamps are removed and the time to hemostasis and the blood loss are measured. The procedure was repeated on the left iliac, with the exception of material application. The right iliac received only tamponade treatment. The results are shown in Table 2.

TABLE 2

<u>Blood Loss and Time to Hemostasis as a Function of Treatment</u>		
Treatment	Blood Loss (g)	Time to Hemostasis (sec)
Tamponade (n = 2)	244, 180	>15, >15
Hydrogel (n = 2)	18, 7	3.3, 2.3

The above results illustrate that this composition significantly reduces the amount of blood loss and time to hemostasis from an ePTFE anastomosis.

EXAMPLE 4

ENHANCED BIOCOMPATIBILITY OF THIOESTER-LINKED FORMULATIONS

Up to six subcutaneous pockets are made on the backs of New Zealand white rabbits. Approximately 1.0 mL of each of the components of the

composition described in Example 1 is delivered to the defect site using a two component sprayer (Cohesion Technologies, Inc., Palo Alto, Calif.) for liquid formulations or a spatula for formulations that are gelled ex-vivo. The grading key is shown in Table 3 and the results are shown in Table 4.

5

TABLE 3

<u>Grading Key for Biocompatibility Experiments</u>		
Score	Gross Observations	Histological Observations
-	All tissues appeared normal	all tissues appeared normal, no inflammation
+	mild foreign body response	mild inflammation
++	moderate foreign body response	moderate inflammation
+++	marked foreign body response	marked inflammation
++++	Severe foreign body response	severe inflammation

TABLE 4

<u>Results for Biocompatibility Experiments</u>			
Test	Description	Results	
		Gross Observations	Histological Observations
A	Surgical control	-	+
B	Fibrillar collagen	-	+
C	20% w/v tetra-SG PEG 10,000	++++	++++
	20% w/v tetra-amino PEG 10,000		
D	20% w/v tetra-SG PEG 10,000	++	++
	20% w/v tetra-sulfhydryl PEG 10,000		
E	20% w/v tetra-SG PEG 10,000	+	++
	20% w/v tetra-amino PEG 10,000; gelled ex-vivo; treated with mono-SG PEG 5000		
F	20% w/v tetra-SG PEG 10,000	++++	++++
	20% w/v di-sulfhydryl PEG 3,400; gelled ex-vivo; treated with di-amino PEG 3400		

Experiments A and B show a mild gross and histological response of fibrillar collagen (Cohesion Technologies, Palo Alto, CA) and the surgical control. Experiment C shows a severe response to hydrogels made with amino-PEG. The response consists of thick encapsulation of the hydrogel and abscess formation. By substitution of sulfhydryl-PEG for amino-PEG, as in Experiment D, the biocompatibility of the hydrogel is significantly improved.

Experiment E involves forming an amino hydrogel ex-vivo and incubating the hydrogel in a solution of mono-SG PEG, 5000 mol. wt. During the incubation period, the mono-SG PEG reacts with the free amines present on the hydrogel network, thus reducing the amount of free amines on the polymeric network.

- 5 This treatment enhances the biocompatibility of the hydrogel. Experiment F involves forming a sulfhydryl hydrogel ex-vivo and incubating the hydrogel in a solution of mono-SG PEG, 5000 mol. wt. During the incubation period, the di-amino PEG reacts with the free SG groups present on the hydrogel network, thus increasing the amount of free amines on the polymeric network. This
- 10 treatment decreases the biocompatibility of the hydrogel. Thus, these results show the enhanced biocompatibility of sulfhydryl formulations over amino formulations.

EXAMPLE 5

EFFECT OF BUFFER AND REACTIVE GROUP ON GEL TIMES

- 15 A desirable characteristic of the compositions described herein is their ability to rapidly achieve gelation. In this experiment, the effects of buffer strength and composition on gelation kinetics are studied. For all experiments, the tetra-functional SG PEG described in Example 1 is dissolved in 0.5 mM sodium phosphate, pH 6.0, and the tetra-sulfhydryl PEG described in Example
- 20 1, or the equivalent tetra-amino PEG is dissolved in the buffer listed in Table 5.

TABLE 5

Effect of Phosphate vs. Carbonate Buffer on Amino and Sulfhydryl Formulations			
Test	Formulation	Buffer	Gel Time (sec)
A	10% w/v tetra-SG PEG 10,000 + 10% w/v tetra-amino PEG 10,000	300 mM dibasic sodium phosphate pH 9	16
B	10% w/v tetra-SG PEG 10,000 + 10% w/v tetra-sulfhydryl PEG 10,000	300 mM dibasic sodium phosphate pH 9	55
C	10% w/v tetra-SG PEG 10,000 + 10% w/v tetra-amino PEG 10,000	300 mM sodium carbonate pH 9	14
D	10% w/v tetra-SG PEG 10,000 + 10% w/v tetra-sulfhydryl PEG 10,000	300 mM sodium carbonate pH 9	9
E	10% w/v tetra-SG PEG 10,000 + 10% w/v tetra-sulfhydryl PEG 10,000	P/C Buffer pH 9.6	3

Experiments A and B show the difference in gel times in amino formulations and sulfhydryl formulations in phosphate buffer. In this buffer, an increase in gelation rate is observed for sulfhydryl formulations compared to amino formulations. Experiments C and D show the difference in gelation times in amino formulations and sulfhydryl formulations in carbonate buffer. As shown, a decrease in gel time is observed for sulfhydryl formulations in carbonate buffer. In the preferred P/C Buffer, a gel time of 3 seconds is observed.

EXAMPLE 6

RHEOMETRIC MEASUREMENTS

The first component (tetra-functional Sulfhydryl-PEG, 10,000 mol. wt.) was prepared according to Example 1 and suspended in P/C Buffer. The second component (tetra-functional SG-PEG, 10,000 mol. wt.) was prepared according to Example 1 in 0.5 mM phosphate, pH 6.0. The two components (0.6 ml each) were loaded in a dual-syringe device with joiner and cannula. The cannula contained a mixing element. The solutions were mixed, and the resultant mixture was immediately delivered into a parallel plate cell of a Rheometrics Fluids Spectrometer 8500 (Rheometrics, Inc., Piscataway, NJ).

The upper platen had a diameter of 25 mm, and the gap between upper and lower parallel plates was 1.5 mm.

Gelation began immediately upon mixing of the formulation. The instrument was started, and G' and G'' (elastic and viscous moduli, respectively) were measured at 1% strain and 1 radian/sec. In less than a minute, G' was near 10^4 dynes/cm², which is characteristic of a soft rubbery material. G' began to plateau within 15 min, and continued to rise very gradually for more than an hour afterwards. G'' was in the order of 10^2 dynes/cm², and declined gradually. These results are consistent with a rapidly gelling material. G' and G'' for the unreacted starting materials was about 1-10 dynes/cm². These results are depicted in FIG. 4.

In this experiment, the rheometer cannot precisely quantitate G' and G'' below about 50 dynes/cm². In addition, the gelation occurred so rapidly that the mixture only filled 30 to 95% of the desired space-there was gelled fluid surrounding the plate, but not between the plates. Even with these limitations, a measurement of the elastic (G') and viscous modulus (G'') as a function of time can still be made, and the kinetics of gelation can be followed. As indicated in this experiment, a G' of greater than 10^2 dynes/cm² in less than one minute indicates rapid gelation.

20

EXAMPLE 7

EFFECTS OF BUFFERS ON GEL TIME USING SULFHYDRYL-PEG AND N-HYDROXY-SUCCINIMIDYL-PEG (NHS-PEG)

All tests were done with 50 ml of 20% (w/v) 4 arm, 10,000 mol. wt., tetrafunctional SG-PEG mixed with 50 ml of 20% (w/v) 4 arm, 10,000 mol. wt., tetra-functional sulfhydryl-PEG). Different buffers were used, and the times to gel were noted. The SG-PEG was dissolved in 0.5 mM phosphate, pH 6.0 for all tests. The sulfhydryl-PEG was dissolved in the buffers given below at a pH of 9.6 and times to gel are noted.

TABLE 6

<u>Effect Buffers on Gelation Time</u>		
Test	Buffer	Gel Time (Sec)
A	P/C Buffer	8
B	150 mM phosphate	35
C	58 mM phosphate 91 mM sodium chloride	138
D	58 mM phosphate 91 mM borate	<19
E	58 mM phosphate 91 mM AMPSO*	8

*(3[1,1-dimethyl-2-hydroxy-ethyl]amino]-2-hydroxypropane-sulfonic acid

- 5 As shown, buffers with pKs between 8 and 10.5 (borate, 8.1; carbonate, 10.3; AMPSO, 9.0), and mixtures thereof, are suitable

EXAMPLE 8

SULFHYDRYL-REACTIVE PEGs

The gelation characteristics of several different formulations are
10 described below:

8a: Gelation of Di Functional Maleimidyl-PEG, 3400 mol. wt.
(MAL-PEG) with Tetra-Sulfhydryl PEG, 10,000 mol. wt.

A 20% (w/v) solution of MAL-PEG in 0.5 mM sodium phosphate,
pH 6.0, was mixed rapidly with an equal volume of 20% (w/v) tetra-sulfhydryl
15 PEG in 150 mM sodium phosphate, pH 5.0. Gelation occurred in 15 sec. The
gel became a firm, rubbery solid in a minute or less.

8b: Gelation of Difunctional Iodoacetamide PEG, 3,400 mol. wt.
("IAM-PEG") with Tetra-Sulfhydryl PEG, 10,000 mol. wt.

IAM-PEG was dissolved at 20% (w/v) in 0.5 mM sodium
20 phosphate, pH 6.0, and mixed rapidly with a 20% (w/v) solution of tetra-
sulfhydryl PEG in P/C Buffer sodium phosphate-carbonate, pH 9.6. Gelation
occurred in less than 40 sec. A firm gel formed within 2 min.

8c: Gelation of Tetra-Sulfhydryl PEG, 10,000 mol. wt., with Dilute Hydrogen Peroxide

A 20% (w/v) solution of tetra-sulfhydryl PEG in P/C Buffer, was mixed with an equal volume of 0.1% (w/v) hydrogen peroxide. Gelation occurred in 15 sec. A firm gel formed in less than 2 min.

EXAMPLE 9

BLOOD COAGULATION ACTIVITY OF THROMBIN INCORPORATED INTO PEG COMPOSITIONS

This experiment demonstrates that hemostatic PEG gels containing active thrombin protein can be formed on tissue.

9a: Thrombin Incorporated into Tetra-Sulfhydryl PEG Gelled with Hydrogen Peroxide

20 mg of tetra-sulfhydryl PEG, 10,000 mol. wt., were dissolved in 80 µl of PC Buffer, and 11 µl of bovine thrombin at 8850 NH units/ml in 0.72 M sodium chloride (Thrombin topical, USP, Gentrac, Inc., Middleton, Wis.) were added. This solution of tetra-sulfhydryl PEG and thrombin was then mixed with 100 µl of 0.1% (w/v) hydrogen peroxide in water, by stirring rapidly in a 1.5 ml plastic tube. The mixture gelled in less than 40 sec, due to oxidation of the sulfhydryl groups to disulfide bonds. After 1.5 min, the gel was a firm, rubbery solid. On top of this gel was layered 200 µl of rabbit blood plasma. The plasma had been separated from citrated blood and contained approximately 11 mM citrate. Just prior to addition, this citrated blood plasma was re-calcified by addition of 8 µl of 0.5 M calcium chloride, to achieve a concentration of about 20 mM calcium. This re-calcified blood plasma was observed to form a fibrin clot 1.5 minutes after layering onto the PEG gel. The clotting reaction was taken as evidence for the presence of active thrombin in the PEG gel.

When control studies are performed, a second oxidized sulfhydryl-PEG gel without thrombin does not clot rabbit plasma until 20 minutes have elapsed. As a further control, re-calcified rabbit plasma is held in an identical plastic tube; and it clots spontaneously after 13 minutes. Therefore, the

sulfhydryl-PEG gel without thrombin clots blood no faster than control re-calcified plasma.

When the analogous experiment was attempted with tetra-sulfhydryl PEG and tetra-SG-PEG, plus thrombin, no enhanced clotting time of plasma was observed. Clotting of plasma was delayed beyond 25 minutes. This result is interpreted to indicate that SG-PEG inactivated thrombin, presumably by binding PEG to lysine side chains of thrombin and interfering with its enzymatic activity.

9b: Thrombin Incorporated into LAM-PEG/Sulfhydryl-PEG gel
20 mg of tetra-sulfhydryl PEG, 10,000 mol. wt. are dissolved in 80 μ l of PC Buffer along with 11 μ l of thrombin, as in 9a. above. 20 mg of LAM-PEG are dissolved in 80 μ l of 0.5 mM sodium phosphate, pH 6.0. The two solutions are rapidly mixed in a 1.5 ml plastic tube. The mixture has a gel time less than 30 sec and is a rubbery gel by 1.5 minutes. Re-calcified rabbit plasma (200 μ l) is layered on top of the gel, and a fibrin clot forms in this plasma in less than two minutes after layering onto the gel. A control reaction without thrombin forms a fibrin clot more than 18 minutes after layering onto the PEG gel. The rapid formation of a fibrin clot in the sample containing thrombin is taken as evidence for the presence of active thrombin in the PEG gel.

9c: Thrombin Incorporated into NEM-PEG/Sulfhydryl PEG gel
20 mg of tetra-sulfhydryl PEG, 10,000 mol wt., is dissolved in 80 μ l of 150 mM sodium phosphate, pH 5.0, along with 11 μ l of thrombin, as in 9a above. 20 mg of NEM-PEG are dissolved in 0.5 mM sodium phosphate, pH 6.0. The two solutions are rapidly mixed in a plastic tube. Gelation occurs in 15 sec. 15 ml of P/C Buffer, are layered onto the top of the PEG gel to adjust the pH to 7-9. Then, 200 μ l of re-calcified rabbit plasma are added. A fibrin clot formed in 1.5 min. after addition of the plasma. Control gels with no thrombin form a fibrin clot after 30 min. Again, the rapid formation of a fibrin clot in the PEG gel with thrombin is taken as evidence for the presence of active thrombin.

9d: Gelation of Layered Gels with Thrombin

In order to provide a gel formulation from SG-PEG and sulfhydryl-PEG to which thrombin can be added and remain active, a "gel layering" technique can be used. First, the tetra-sulfhydryl-PEG and tetra-Se-PEG gel at 20% solids, prepared according to Example 1 are sprayed onto sheets as
5 described in Example 2. The sheets are coarse fibered collagen hydrated by saline, which simulates a tissue surface. The total volume is approximately 0.5 ml. This formula gels in 18-15 sec. At 16 seconds, a second gel mixture of tetra-sulfhydryl PEG, di-maleimidyl PEG, both at 20% solids, and thrombin (700 NIH units/ml) of total gel mixture, total volume approx. 0.5 ml, are sprayed on
10 top of the first gel. This second gel layer gels at about 2 minutes. At 3 min after the first gel is sprayed, 0.4 ml of re-calcified rabbit blood plasma, prepared as described above are layered on top of the PEG gel. This plasma clots 1.5 minutes after it is layered onto the PEG gel. The formation of a fibrin clot at this early time, compared to a non-thrombin control, is taken as evidence for active
15 thrombin in the PEG gel.

EXAMPLE 10

GELATION USING POWDERED FORMULATIONS

10 mg of powdered tetra-SG PEG, 10,000 mol. wt., is spread on the surface of a piece of weighing paper. 10 mg of tetra-sulfhydryl PEG, 10,000
20 mol. wt., is dissolved in 80 μ l of P/C buffer. The sulfhydryl-PEG solution is loaded into a 1 cc syringe with a Haemedics (Malibu, Calif.) spray head and sprayed onto the SG-PEG on the weighing paper. The sprayed fluid is not stirred or mixed. It begins to gel in 27 seconds and forms a firm, rubbery layer by 2 min. This test shows that components in powdered form are also suitable
25 for use in the present invention.

EXAMPLE 11

COLLAGEN-CONTAINING COMPOSITIONS

Methylated collagen is prepared by the following process: bovine corium collagen is solubilized using pepsin and purified as described in U.S.

- 5 Pat. No. 4,233,360. This purified, solubilized collagen is precipitated by neutralization into 0.2M sodium phosphate, pH 7.2. The precipitate is isolated by centrifugation to a final concentration of 70 mg/ml. The material is dried for two days, and then pulverized. Dry methanol containing HCl (to 0.1 N) is added (40 ml) and stirred for four days. Collagen is separated from the acidic
- 10 methanol, vacuum dried and sterilized by irradiation. The final product is dissolved in water at a pH of 3-4.

- For delivery as a sealant, 10 mg of the methylated collagen, 100 mg of tetra-functional sulfhydryl-PEG, 10,000 mol. wt., and 100 mg of tetra-functional SG PEG, 10,000 mol. wt., are dissolved in water at pH 3-4 to a final
- 15 volume of 1 ml (first component). The second component is 1 ml of P/C Buffer. Each component is placed in a syringe and mixed and sprayed on the desired test site using a dual-syringe delivery system as described in Example 1. The applied mixture gels in less than 3 seconds.

- The adhesive and cohesive properties of the gel are examined in
- 20 a burst test. This test is conducted on a pressure gauge apparatus (PSI-Tronix, Model PG5000, Tulare, Calif.) connected by a pressure line to a circular sample plate with a 2 mm diameter central orifice. Sealant formulations are sprayed onto the plate to seal the orifice. To simulate bonding of the formulations to tissue, the sample plate has a circular sheet of coarse-fibered collagen
- 25 fastened to it, with a 2 mm hole pierced into it and displaced 2-3 mm from the sample plate orifice. Burst strength is measured as a function of the pressure it takes to force saline at a flow rate of 5 ml/min through the sealant gel.

The results are given below in Table 7.

TABLE 7

<u>Burst Strength Measurements of Collagen-Containing Compositions</u>	
<u>Material</u>	<u>Burst Strength, mm Hg</u>
Sulfhydryl-PEG/SG-PEG	100-180
Sulfhydryl-PEG/SG-PEG/ Methylated Collagen	122-205

Both formulations have gel times less than 3 seconds. As shown above, the addition of collagen to the formulation enhances burst strength.

5

EXAMPLE 12

SYNTHESIS OF "12-ARM" PEG COMPOUNDS

A 12-arm electrophilic PEG compound is formed from 1 mole of 4-arm sulfhydryl PEG, 10,000 mol. wt., and 4 moles of 4-arm SG-PEG, 10,000 mol. wt. The resulting compound is depicted in FIG. 5a. As shown, the compound core is pentaerythritol PEG ether tetra-sulfhydryl and the end functional group is succinimide. As long as the functional groups are reactive with one another to form chemical bonds, the sulfhydryl group, X, can be replaced with other nucleophilic groups, such as NH₂, *etc.*, and the succinimidyl group, Y, can be replaced with other electrophilic groups, such as maleimide, carbonyl imidazole, or isocyanate. This method is also used to prepare the 12-arm nucleophilic PEG compound depicted in FIG. 5b by reacting 4 moles of 4-arm sulfhydryl PEG with 1 mole of 4-arm SG-PEG. It should be understood that such reactions produce a heterogeneous population of activated PEG product, some having less than 12 arms, and some having more than 12 arms. As used herein, a "12-arm" PEG also refers to such heterogeneous reaction products that have an average of about 12 arms on each molecule.

12a: 12 arm Sulfhydryl PEG

Eight grams of pentaerythritol (polyethylene glycol)ether tetra sulfhydryl was dissolved in a mixture of 100 mL of methylene chloride and 100 mL of triethylamine. Two grams of pentaerythritol (polyethylene glycol)ether tetra succinimidyl glutarate in 40 mL of methylene chloride was slowly added

with stirring at room temperature under argon overnight. The solvent was removed and the product was isolated by recrystallization in ethanol and dried.

12b: 12 arm Succinimidyl PEG

Two grams of pentaerythritol (polyethylene glycol)ether tetra succinimidyl glutarate was dissolved in 50 mL of methylene chloride. 0.5 grams of pentaerythritol (polyethylene glycol)ether tetra amine in 10 mL of methylene chloride was slowly added with stirring at room temperature under argon overnight. The solvent was removed and the product was isolated by recrystallization in ethanol and dried.

When the two compounds were tested for burst strength as described in Example 12, they demonstrated a burst strength of greater than 150 mm Hg and a gel time of less than 2 seconds.

EXAMPLE 13

PREPARATION OF MICROSPHERES WITH AND WITHOUT PACLITAXEL

A) PVA solution preparation

1. In a 1000ml beaker, 1000ml of distilled water and 100g of PVA (Aldrich 13-23K, 98% hydrolyzed) are weighed. A two-inch stirrer bar is placed into the beaker. The suspension is heated up to 75-80°C during stirring. The PVA is dissolved completely (should form a clear solution).

2. The 10% PVA solution (w/v) is cooled down to room temperature and filtered through a syringe in-line filter. Stored at 2-8°C for use.

B) PLGA solution preparation with or without paclitaxel

1. Appropriate amount of paclitaxel and PLGA (for a total of 1.0g) are weighed and transferred into the 20ml scintillation vial.

2. 10mL of HPLC grade dichloromethane (DCM) is added into the vial to dissolve the PLGA with or without paclitaxel.

3. The polymer with or without paclitaxel is dissolved in DCM by placing the vial on an orbital shaker. The orbital shaker is set at 4.

Preparation of the microspheres with diameter less than 25mm

1. 100ml of 10% PVA solution is transferred into a 400ml beaker. The beaker is secured by a double side adhesive tape onto the fume-hood. A peddler with 3 blades is placed into the beaker with 0.5 cm above the bottom. The motor is turned on to 2.5 (Dyna-Mix from Fisher Scientific) at first.
- 5 The 10ml PLGA/paclitaxel solution is poured into the PVA solution during agitation. Gradually turn up the agitation rate to 5.0. The stirring is maintained for 2.5 to 3.0 hours.
2. The obtained microspheres are filtered through a set of sieves with 53mm (top) and 25mm (bottom) into a 100ml beaker. The
10 microspheres are washed using distilled water while filtering. The filtered microspheres are centrifuged (1000rpm, 10min.) and re-suspended/washed with 100ml distilled water three times to clean the PVA.
3. The washed microspheres are transferred into the freeze-dried beaker using a small amount of distilled water (20-30ml). The beaker is
15 then sealed and placed into a -20°C freezer over night.
4. The frozen microspheres are then freeze-dried using a freeze-drier for about 3 days. The dried microspheres are transferred into 20ml scintillation vial and stored at -20°C.

In a similar manner described above, other biologically active
20 agents, as described above, can be incorporated into a microsphere formulation.

EXAMPLE 14

MYCOPHENOLIC ACID INCORPORATION INTO MICROSPHERES

Mycophenolic acid was incorporated into microspheres in a
25 similar manner as described in Example 13.

EXAMPLE 15

INCORPORATION OF PACLITAXEL-LOADED MICROSPHERES – METHOD 1

Various amounts of the microspheres prepared in Example 13 are weighed out and mixed with the pentaerythritol poly(ethylene glycol)ether tetra-
5 succinimidyl glutarate. The formulation is then prepared in the same manner as that described in Example 1. Microspheres loaded with other agents, for example mycophenolic acid, are incorporated into the composition in a similar manner.

EXAMPLE 16

10 INCORPORATION OF PACLITAXEL-LOADED MICROSPHERES – METHOD 2

Various amounts of the microspheres prepared in Example 13 are weighed out and mixed with 0.5 mM sodium phosphate pH 6.0 buffer. The microsphere containing buffer is then used to prepare the formulation in the same manner as that described in example 1. Microspheres loaded with other
15 agents, for example mycophenolic acid, are incorporated into the composition in a similar manner.

EXAMPLE 17

PREPARATION OF CHLORPROMAZINE MICROSPHERES

Various amounts of chlorpromazine are dissolved in 1 mL 5%
20 PVA solution. This solution is then added to 10 mL dichloromethane (DCM) that is in a 25 mL beaker. The solution is homogenized (setting 5) for 2 minutes using a tissue homogenizer. The resultant solution is then poured into 50 mL 5% PVA solution. The solution is then homogenized (setting 5) for 2 minutes. The sample is then placed on the rotavap and the solvent is gradually removed
25 using a shallow increasing vacuum gradient. Once the majority of the DCM is removed, the sample is frozen and freeze dried.

EXAMPLE 18

EFFICACY OF DRUG LOADED FORMULATIONS – ADHESION PREVENTION

The compositions prepared in Examples 1, 15, 16 and 17 are tested in the rat cecal side wall model (see General Method A) and the rabbit
5 uterine horn model (see General Method B). The compositions as prepared in Examples 1, 15 and 16 were applied to the site of injury as a spray using an air assisted spray device (available from Cohesion Technologies or Micromedics) that mixed the 2 component solutions.

EXAMPLE 19

10 DIRECT INCORPORATION OF DRUGS INTO RAPID GELLING FORMULATION:

MYCOPHENOLIC ACID (MPA)-PREMIX

Reagents:

Syringe 1: A 1 mL syringe equipped with a BBraun luer-lock mixing connector (FDC1000/415080) containing PEG-SG4 (tetra functional poly(ethylene glycol)
15 succinimidyl glutarate) 50mg, PEG-SH4 (tetra functional poly(ethylene glycol) thiol 50mg and MPA (mycophenolic acid) 5 to 45 mg. The mycophenolic acid was less than 100 um in particles size. This was obtained by using a 100 um sieve.

Syringe 2: A 1 mL capped syringe with 0.25 mL 6.3mM HCl solution.

20 Syringe 3: A 1 mL capped syringe with 0.25 mL 0.12 M monobasic sodium phosphate and 0.2 M sodium carbonate (pH 9.7).

Applicator: Micromedics Y-shaped blending connector with a spray-tip (SA-3674), or similar.

25 Procedure:

Syringe 1 containing the solids and syringe 2 containing the acidic solution was mixed through the green mixing connector by repeatedly transferring from one syringe to the other by pushing the plungers back and forth. After complete mixing, all of the formulation was pushed into one of the
30 syringes which was attached to one inlet of the Y-shaped applicator equipped

with the spray tip. Syringe 3 containing the pH 9.7 solution was attached onto the other inlet of the Y-shaped applicator. A connector clip was attached to the plungers of the two syringes. The formulation was applied by quickly and evenly depressing the connected syringe plungers.

5 For mycophenolic acid amounts in the 50 to 100 mg range, a 1 mL capped syringe with 0.25 mL 0.24 M monobasic sodium phosphate adjusted to pH 10 with sodium carbonate was used as syringe 3.

EXAMPLE 20

DIRECT INCORPORATION OF DRUGS INTO RAPID GELLING FORMULATION: CELLCEPT- PREMIX

CELLCEPT (Syntex Laboratories, Inc., Palo Alto, CA) was incorporated into the composition in a similar manner to that described in Example 19. 5 mg CELLCEPT was added to the 2 PEG components in syringe 1. The composition was prepared and applied as described in Example 19.

EXAMPLE 21

DIRECT INCORPORATION OF DRUGS INTO RAPID GELLING FORMULATION:

CHLORPROMAZINE (CPZ)-PREMIX

In a similar manner to that described in Example 19,

20 Chlorpromazine was incorporated into the composition. Compositions containing between 5 and 20 mg Chlorpromazine were prepared in a similar manner as to that described in Example 19. No mycophenolic acid was included in these compositions.

EXAMPLE 22

25 DIRECT INCORPORATION OF DRUGS INTO RAPID GELLING FORMULATION:
MYCOPHENOLIC ACID – SEPARATE DRUG COMPONENT

Components :

Syringe 1: A 1 mL syringe equipped with a BBraun luer-lock mixing connector (FDC1000/415080) containing 50 mg PEG-SG4 (tetra functional poly(ethylene glycol) succinimidyl glutarate) and 50 mg PEG-SH4 (tetra functional poly(ethylene glycol) thiol).

- 5 Syringe 2: 1 mL syringe equipped with a BBraun luer-lock mixing connector (FDC1000/415080) containing between 5 and 45 mg MPA (mycophenolic acid) [sieved to a particle size less than 100 micron].

Syringe 3: A 1 mL capped syringe with 0.25 mL 6.3mM HCl solution.

- Syringe 4: A 1 mL capped syringe with 0.25 mL 0.12 M monobasic sodium
10 phosphate and 0.2 M sodium carbonate (pH 9.7).

Applicator: Micromedics Y-shaped blending connector with a spray-tip(SA-3674), or similar.

Procedure:

- Syringe 1 containing the solids was connected to syringe 3
15 containing the acidic solution through the green mixing connector. The contents were mixed by using the plungers to transfer the contents of one syringe into the other. This process was repeated at least 20 times. After complete mixing, all of the formulation was pushed into one of the syringes which was attached to one inlet of the Y-shaped applicator equipped with the spray tip. Syringe 4 and
20 2 (containing the drug) were similarly mixed and attached onto the other inlet of the Y-shaped applicator. A connector clip was attached to the plungers of the two syringes. The formulation was applied by quickly and evenly depressing the connected syringe plungers.

- For mycophenolic acid amounts in the 50 to 100 mg range, a 1
25 mL capped syringe with 0.25 mL 0.24 M monobasic sodium phosphate adjusted to pH 10 with sodium carbonate was used as syringe 4.

EXAMPLE 23

DIRECT INCORPORATION OF DRUGS INTO RAPID GELLING FORMULATION: CELLCEPT -
PREMIX

CELLCEPT was incorporated into the composition in a similar
5 manner to that described in Example 22. 5 mg CELLCEPT was contained in
syringe 2. The composition was prepared and applied as described in Example
22. Mycophenolic acid was included in these compositions.

EXAMPLE 23

MYCOPHENOLIC ACID-CONTAINING MICROSPHERES PREPARED BY SPRAY DRYING

10 Poly(L-lactic acid) (Mw 2000), was dissolved in methylene
chloride to result in a 0.2% solution. MPA was added in at different weight ratios
relative to the carrier polymer. These ranged from 10 to 50%. The resulting
solution was spray dried using a Buchi Research Spray Drier and the following
conditions: Inlet temperature 50 °C, outlet temperature < 39 °C, aspirator 100%,
15 flow rate 700 L/hr. The collected microspheres were further dried under
vacuum. MPA-containing microspheres were made in a similar manner to that
described above except that poly(caprolactone) (Mw 9,000), PLGA (Mw 54K),
PLURONIC-F127 or methoxy poly(ethylene glycol 5000)-block-poly (DL-lactide)
(65:35 or 60:40 PEG:PDLLA weight ratio) were used instead of the poly(L-
20 lactic acid).

EXAMPLE 23

CHLORPROMAZINE-CONTAINING MICROSPHERES PREPARED BY SPRAY DRYING

Methoxy poly(ethylene glycol 5000)-block-poly (DL-lactide) (65:35
PEG:PDLLA weight ratio) or PLURONIC-F127 was dissolved in methylene
25 chloride to result in a 0.2% solution. Chlorpromazine was added in 10% weight
ratio relative to the carrier polymer. The resulting solution was spray dried using
a Buchi Research Spray Drier and the following conditions: Inlet temperature 50

°C, outlet temperature < 39 °C, aspirator 100%, flow rate 700 L/hr. The collected microspheres were further dried under vacuum.

EXAMPLE 24

PACLITAXEL-CONTAINING MICROSPHERES PREPARED BY SPRAY DRYING

5 Methoxy poly(ethylene glycol 5000)-block-poly (DL-lactide) (65:35 or 60:40 PEG:PDLLA weight ratio) was dissolved in methylene chloride to result in a 0.2% solution. Paclitaxel was added in 10% weight ratio relative to the carrier polymer and the resulting solution was spray dried using a Buchi Research Spray Drier and the following conditions: Inlet temperature 50 °C,
10 outlet temperature < 39 °C, aspirator 100%, flow rate 700 L/hr. The collected microspheres were further dried under vacuum.

EXAMPLE 25

MYCOPHENOLIC ACID-CONTAINING MICROSPHERES (< 10 MICRONS) PREPARED BY EMULSION METHOD -

15 Into a 600mL beaker was added 100mL of freshly prepared 10% polyvinyl alcohol (PVA) solution and 10mL of pH 3 acetic acid solution saturated with MPA. This acidified PVA solution was stirred at 2000 rpm for 30 minutes. Meanwhile, solution containing between 80-400 mg MPA and 800 mg PLGA in 20mL dichloromethane were prepared. Each of these
20 dichloromethane solutions were individually added drop wise to a PVA solution while stirring at 2000 rpm with a Fisher Dyna-Mix. After addition was complete, the solution was allowed to stir for 45 minutes. The microsphere solution was transferred to falcon tubes, washed with a pH 3 acetic acid solution saturated with MPA, and centrifuged at 2600 rpm for 10 minutes. The aqueous layer was
25 decanted and the washing, centrifuging and decanting was repeated 3 times. The washed microspheres from each batch were freeze-dried.

EXAMPLE 26

MYCOPHENOLIC ACID-CONTAINING MICROSPHERES (50-130 MICRONS) PREPARED BY
EMULSION METHOD

Into a 600mL beaker was added 100mL of freshly prepared 1%
5 polyvinyl alcohol solution and 10mL of pH 3 acetic acid solution saturated with
MPA. This acidified PVA solution was stirred at 500 rpm for 30 minutes.
Meanwhile, a solution of 80-400 mg MPA and 800 mg PLGA in 20mL
dichloromethane was prepared. This dichloromethane solution was added drop
wise to the PVA solution while stirring at 500 rpm with a Fisher Dyna-Mix. After
10 addition was complete, the solution was allowed to stir for 45 minutes. The
microsphere solution was transferred to falcon tubes, washed with a pH 3
acetic acid solution saturated with MPA, and centrifuged at 2600 rpm for 10
minutes. The aqueous layer was decanted and the washing, centrifuging and
decanting was repeated 3 times. The combined, washed microspheres were
15 freeze-dried to remove any excess water. The product was sieved to isolate
microspheres of 53-125 μ m size.

EXAMPLE 27

INCORPORATION OF DRUG-LOADED CARRIERS INTO THE PEG COMPOSITIONS

Drug-loaded microspheres, 5 to 100 mg, were incorporated into
20 compositions as a mixture in a similar manner as to that described in Example
19 or as a separate component in a manner similar to that described in
Example 22.

EXAMPLE 28

INCORPORATION OF ADDITIVES INTO MPA-LOADED MICROSPHERES

25 Methoxy poly(ethylene glycol 5000)-block-poly (DL-lactide) (65:35
or 60:40 PEG:PDLLA weight ratio) was dissolved in the appropriate solvent
(see below) to result in a 0.2% solution. MPA was added in 10% weight ratio
relative to the carrier polymer. Different additives were then individually added

to the drug/polymer solution. The nature of the additive and the amounts used are described below:

	Additives:	Concentration:	Solvent:
5	Histidine	1-3 molar ratio to MPA	Methylene Chloride
	Spermidine	1-1/3 molar ratio to MPA	Methylene Chloride
	1,2 dipalmitoyl-sn-glycero- 3-phosphoethanolamine	1-15% (w/w) to carrier	Chloroform
10	1,2, dimyristoyl-sn-glycero- 3-ethylphosphocholine chloride	1-15% (w/w) to carrier)	Chloroform
	Palmitic Acid	1-15% (w/w) to carrier)	Methylene Chloride
	Cholic Acid	1-15% (w/w) to carrier)	Methylene Chloride

- 15 The resulting solution was spray dried using a Buchi Research Spray Drier and the following conditions: Inlet temperature 50°C, outlet temperature < 39°C, aspirator 100%, flow rate 700 L/hr. The collected microspheres were further dried under vacuum. The drug-loaded microspheres were used in direct combination with the PEG reagents, as described in
- 20 Example 19 or as a separate component as described in Example 22.

EXAMPLE 29

RAT SURGICAL ADHESIONS MODEL TO ASSESS FIBROSIS INHIBITING AGENTS

- Sprague Dawley rats are prepared for surgery by anaesthetic induction with 5% halothane in an enclosed chamber. Anaesthesia is
- 25 maintained by nose cone on halothane throughout the procedure and Buprenorphen 0.035 mg/kg is injected intramuscularly. The abdomen is shaved, sterilized, draped and entered via a midline incision. The caecum is lifted from the abdomen and placed on sterile gauze dampened with saline. Dorsal and ventral aspects of the caecum are scraped a total of 45 times over
- 30 the terminal 1.5 cm using a #10 scalpel blade, held at a 45° angle. Blade angle and pressure are controlled to produce punctuated bleeding, while avoiding severe tissue damage or tearing.

The left side of the abdominal cavity is retracted and everted to expose a section of the peritoneal wall nearest the natural resting caecal location. The exposed superficial layer of muscle (*transverses abdominis*) is excised over an area of 1.0 X 1.5 cm². Excision includes portions of the
5 underlying internal oblique muscle, leaving behind some intact and some torn fibres from the second layer. Minor local bleeding is tamponaded until controlled.

A test formulation is deployed at the wounded areas, on the abraded sidewall, between the caecum and sidewall. The formulation is
10 deployed using either a syringe spray system or an air-assisted syringe system. The abraded caecum is then positioned over the sidewall wound and sutured at four points immediately beyond the dorsal corners of the wound edge. The large intestine is replaced in a natural orientation continuous with the caecum. The abdominal incision is closed in two layers with 4-0 silk sutures.

15 Rats are followed for one week, and then euthanized by lethal injection for *post mortem* examination to score. Severity of post-surgical adhesions is scored by independently assessing the tenacity and extent of adhesions at the site of caecal-sidewall abrasion, at the edges of the abraded site, and by evaluating the extent of intestinal attachments to the exposed
20 caecum. Adhesions are scored on a scale of 0-4 with increasing severity and tenacity. The extent of adhesion is scored as a percent of the injured area that contained adhesions.

EXAMPLE 30

SCREENING ASSAY FOR ASSESSING THE EFFECT OF MITOXANTRONE 25 ON CELL PROLIFERATION

Fibroblasts at 70-90% confluency are trypsinized, replated at 600 cells/well in media in 96-well plates and allowed to attachment overnight. Mitoxantrone is prepared in DMSO at a concentration of 10⁻² M and diluted 10-fold to give a range of stock concentrations (10⁻⁸ M to 10⁻² M). Drug dilutions
30 are diluted 1/1000 in media and added to cells to give a total volume of 200

μL/well. Each drug concentration is tested in triplicate wells. Plates containing fibroblasts and mitoxantrone are incubated at 37°C for 72 hours (In vitro toxicol. (1990) 3: 219; Biotech. Histochem. (1993) 68: 29; Anal. Biochem. (1993) 213: 426).

5 To terminate the assay, the media is removed by gentle aspiration. A 1/400 dilution of CYQUANT 400X GR dye indicator (Molecular Probes; Eugene, OR) is added to 1X Cell Lysis buffer, and 200 μL of the mixture is added to the wells of the plate. Plates are incubated at room temperature, protected from light for 3-5 minutes. Fluorescence is read in a
10 fluorescence microplate reader at ~480 nm excitation wavelength and ~520 nm emission maxima. Inhibitory concentration of 50% (IC₅₀) is determined by taking the average of triplicate wells and comparing average relative fluorescence units to the DMSO control. An average of n=4 replicate experiments is used to determine IC₅₀ values. The results of the assay are
15 shown in FIG. 6 (IC₅₀=20 nM for proliferation of human fibroblasts).

EXAMPLE 31

SCREENING ASSAY FOR ASSESSING THE EFFECT OF MITOXANTRONE ON NITRIC OXIDE PRODUCTION BY MACROPHAGES

20 The murine macrophage cell line RAW 264.7 is trypsinized to remove cells from flasks and plated in individual wells of a 6-well plate. Approximately 2×10^6 cells are plated in 2 mL of media containing 5% heat-inactivated fetal bovine serum (FBS). RAW 264.7 cells are incubated at 37°C for 1.5 hours to allow adherence to plastic. Mitoxantrone is prepared in DMSO
25 at a concentration of 10^{-2} M and serially diluted 10-fold to give a range of stock concentrations (10^{-8} M to 10^{-2} M). Media is then removed and cells are incubated in 1 ng/mL of recombinant murine IFN γ and 5 ng/mL of LPS with or without mitoxantrone in fresh media containing 5% FBS. Mitoxantrone is added to cells by directly adding mitoxantrone DMSO stock solutions, prepared earlier,
30 at a 1/1000 dilution, to each well. Plates containing IFN γ , LPS plus or minus

mitoxantrone are incubated at 37°C for 24 hours (Chem. Ber. (1879) 12: 426; J. AOAC (1977) 60-594; Ann. Rev. Biochem. (1994) 63: 175).

At the end of the 24 hour period, supernatants are collected from the cells and assayed for the production of nitrites. Each sample is tested in triplicate by aliquoting 50 μ L of supernatant in a 96-well plate and adding 50 μ L of Greiss Reagent A (0.5 g sulfanilamide, 1.5 mL H_3PO_4 , 48.5 mL ddH_2O) and 50 μ L of Greiss Reagent B (0.05 g N-(1-Naphthyl)-ethylenediamine, 1.5 mL H_3PO_4 , 48.5 mL ddH_2O). Optical density is read immediately on microplate spectrophotometer at 562 nm absorbance. Absorbance over triplicate wells is averaged after subtracting background and concentration values are obtained from the nitrite standard curve (1 μ M to 2 mM). Inhibitory concentration of 50% (IC_{50}) is determined by comparing average nitrite concentration to the positive control (cell stimulated with IFN γ and LPS). An average of n=4 replicate experiments is used to determine IC_{50} values for mitoxantrone. The results of the assay are shown in FIG. 7 (Mitoxantrone IC_{50} =927 nM for Greiss assay in RAW 264.7 cells).

EXAMPLE 32

SCREENING ASSAY FOR ASSESSING THE EFFECT OF BAY 11-7082 ON TNF-ALPHA PRODUCTION BY MACROPHAGES

The human macrophage cell line, THP-1 is plated in a 12 well plate such that each well contains 1×10^6 cells in 2 mL of media containing 10% FCS. Opsonized zymosan is prepared by resuspending 20 mg of zymosan A in 2 mL of ddH_2O and homogenizing until a uniform suspension is obtained. Homogenized zymosan is pelleted at 250 g and resuspended in 4 mL of human serum for a final concentration of 5 mg/mL. and incubated in a 37°C water bath for 20 minutes to enable opsonization. Bay 11-7082 is prepared in DMSO at a concentration of 10^{-2} M and serially diluted 10-fold to give a range of stock concentrations (10^{-8} M to 10^{-2} M) (J. Immunol. (2000) 165: 411-418; J. Immunol. (2000) 164: 4804-4811; J. Immunol Meth. (2000) 235 (1-2): 33-40).

THP-1 cells are stimulated to produce TNF α by the addition of 1 mg/mL opsonized zymosan. Bay 11-7082 is added to THP-1 cells by directly adding DMSO stock solutions, prepared earlier, at a 1/1000 dilution, to each well. Each drug concentration is tested in triplicate wells. Plates are incubated
5 at 37°C for 24 hours.

After a 24 hour stimulation, supernatants are collected to quantify TNF α production. TNF α concentrations in the supernatants are determined by ELISA using recombinant human TNF α to obtain a standard curve. A 96-well MaxiSorb plate is coated with 100 μ L of anti-human TNF α Capture Antibody
10 diluted in Coating Buffer (0.1M Sodium carbonate pH 9.5) overnight at 4°C. The dilution of Capture Antibody used is lot-specific and is determined empirically. Capture antibody is then aspirated and the plate washed 3 times with Wash Buffer (PBS, 0.05% Tween-20). Plates are blocked for 1 hour at room temperature with 200 μ L/well of Assay Diluent (PBS, 10% FCS pH 7.0).
15 After blocking, plates are washed 3 times with Wash Buffer. Standards and sample dilutions are prepared as follows: (a) sample supernatants are diluted $1/8$ and $1/16$; (b) recombinant human TNF α is prepared at 500 pg/mL and serially diluted to yield as standard curve of 7.8 pg/mL to 500 pg/mL. Sample supernatants and standards are assayed in triplicate and are incubated at room
20 temperature for 2 hours after addition to the plate coated with Capture Antibody. The plates are washed 5 times and incubated with 100 μ L of Working Detector (biotinylated anti-human TNF α detection antibody + avidin-HRP) for 1 hour at room temperature. Following this incubation, the plates are washed 7 times and 100 μ L of Substrate Solution (Tetramethylbenzidine, H₂O₂)
25 is added to plates and incubated for 30 minutes at room temperature. Stop Solution (2 N H₂SO₄) is then added to the wells and a yellow colour reaction is read at 450 nm with λ correction at 570 nm. Mean absorbance is determined from triplicate data readings and the mean background is subtracted. TNF α concentration values are obtained from the standard curve. Inhibitory
30 concentration of 50% (IC₅₀) is determined by comparing average TNF α concentration to the positive control (THP-1 cells stimulated with opsonized

zymosan). An average of $n=4$ replicate experiments is used to determine IC_{50} values for Bay 11-7082. The results of the assay are shown in FIG. 8 (Bay 11-7082 $IC_{50}=810$ nM TNF α production by THP-1 cells).

EXAMPLE 33

5 RABBIT SURGICAL ADHESIONS MODEL TO ASSESS FIBROSIS INHIBITING AGENTS

The rabbit uterine horn model is used to assess the anti-fibrotic capacity of formulations *in vivo*. Mature New Zealand White (NZW) female rabbits are placed under general anesthetic. Using aseptic precautions, the abdomen is opened in two layers at the midline to expose the uterus. Both
10 uterine horns are lifted out of the abdominal cavity and assessed for size on the French Scale of catheters. Horns between #8 and #14 on the French Scale (2.5-4.5 mm diameter) are deemed suitable for this model. Both uterine horns and the opposing peritoneal wall are abraded with a #10 scalpel blade at a 45° angle over an area 2.5 cm in length and 0.4 cm in width until punctuate
15 bleeding is observed. Abraded surfaces are tamponaded until bleeding stops. The individual horns are then opposed to the peritoneal wall and secured by two sutures placed 2 mm beyond the edges of the abraded area. The formulation is applied and the abdomen is closed in three layers. After 14 days, animals are evaluated *post mortem* with the extent and severity of adhesions
20 being scored both quantitatively and qualitatively.

EXAMPLE 34

SCREENING PROCEDURE FOR ASSESSMENT OF PERIGRAFT REACTION

Large domestic rabbits are placed under general anesthetic. Using aseptic precautions, the infrarenal abdominal aorta is exposed and
25 clamped at its superior and inferior aspects. A longitudinal arterial wall arteriotomy is performed and a 2 millimeter diameter, 1 centimeter long segment of PTFE graft is inserted within the aorta and the proximal and distal aspect of the graft is sewn so that the entire aortic blood flow is through the

graft which is contained in the abdominal aorta in the manner of open surgical abdominal aortic repair in humans (except that no aneurysm is present in this model). The aortotomy is then surgically closed and the abdominal wound closed and the animal recovered.

- 5 The animals are randomized to receive standard PTFE grafts or grafts of which the middle 1 cm is coated alone circumferentially with nothing, or with an agent that induces a vessel wall reaction or adhesion between a stent graft and vessel wall alone or contained in a slow release, polymer.

- 10 The animals are sacrificed between 1 and 6 weeks post surgery, the aorta is removed en bloc and the area in relation to the graft is grossly examined for adhesive reaction. Any difference in morphology or histology of the vessel wall from portions of the artery which contain no graft, portion which contain graft without coating, and portion which contained graft with coating is noted.

15

EXAMPLE 35

ANIMAL ABDOMINAL AORTIC ANEURYSM MODEL

- Pigs or sheep are placed under general anesthetic. Using aseptic precautions the abdominal aorta is exposed. The animal is heparinized and the aorta is cross clamped below the renal arteries and above the bifurcation.
- 20 Collaterals are temporarily controlled with vessel loops or clips that are removed upon completion of the procedure. A longitudinal aortotomy is created in the arterial aspect of the aorta, and an elliptical shaped patch of rectus sheath from the same animal is sutured into the aortotomy to create an aneurysm. The aortic clamps from the lumbar arteries and collaterals are
- 25 removed and the abdomen closed. After 30 days, the animal is reanesthetized and the abdominal wall again opened. A cutdown is performed on the iliac artery and through this, a stent graft is positioned across the infrarenal abdominal aorta aneurysm extending from normal infrarenal abdominal aorta above to normal infrarenal abdominal aorta below the surgically created
- 30 aneurysm and the device is released in a conventional way.

Animals are randomized into groups of 5 receiving uncoated stent grafts, stent graft containing slow release polymer alone, and stent graft containing a biologically active or irritative substance as determined by the previously mentioned screening exam. After closure of the arteriotomy and of the abdominal wound, the animal is allowed to recover. At 6 weeks and 3 months post stent graft insertion, the animal is sacrificed and the aorta removed en bloc. The infrarenal abdominal aorta is examined for evidence of histologic reaction and perigraft leaking.

EXAMPLE 36

10 SCREENING PROCEDURE FOR ASSESSMENT OF PERIGRAFT REACTION

Large domestic rabbits are placed under general anesthetic. Using aseptic precautions, the infrarenal abdominal aorta is exposed and clamped at its superior and inferior aspects. A longitudinal arterial wall arteriotomy is performed and a 2 millimeter diameter, 1 centimeter long segment of PTFE graft is inserted within the aorta and the proximal and distal aspect of the graft is sewn so that the entire aortic blood flow is through the graft which is contained in the abdominal aorta in the manner of open surgical abdominal aortic repair in humans (except that no aneurysm is present in this model). The aortotomy is then surgically closed and the abdominal wound closed and the animal recovered.

The animals are randomized to receive standard PTFE grafts, silk stent grafts, or silk stent grafts coated with other agents as described above.

The animals are sacrificed between 1 and 6 weeks post surgery, the aorta is removed en bloc and the area in relation to the graft is grossly examined for adhesive reaction. Any difference in morphology or histology of the vessel wall from portions of the artery that contain no graft, portion which contain graft without coating, and portion which contained graft with coating is noted.

All of the above U.S. patents, U.S. patent application publications, U.S. patent applications, foreign patents, foreign patent applications and non-patent publications referred to in this specification and/or listed in the Application Data Sheet, are incorporated herein by reference, in their entirety.

- 5 From the foregoing it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

CLAIMS

We claim:

1. A biocompatible gel-forming drug-delivering composition for *in vivo* administration, comprising:
 - a drug;
 - a first component comprising at least one sulfhydryl group-containing compound in a liquid medium having an alkaline pH, wherein said sulfhydryl group-containing compound is given by the formula Compound₁ - (SH)_m, wherein $m \geq 2$; and
 - a second component comprising at least one sulfhydryl reactive group-containing compound in either a liquid medium having a neutral or acidic pH or in powder form, wherein said sulfhydryl reactive group-containing compound is given by the formula Compound₂ -Y_n, wherein Y is a sulfhydryl reactive group and wherein $n \geq 2$;wherein at least one of the first or second components is a polyalkylene oxide and wherein the sulfhydryl groups and the sulfhydryl reactive groups react with one another to form covalent bonds therebetween when said components are mixed together to form a gel in less than one minute.
2. The composition of claim 1, wherein m and n are each 4.
3. The composition of claim 1, wherein m and n are each 12.
4. The composition of claim 1, wherein the first component is a polyalkylene oxide.
5. The composition of claim 1, wherein the second component is a polyalkylene oxide.

6. The composition of claim 1, wherein the first and second components are polyalkylene oxides.

7. The composition of claim 6, wherein the polyalkylene oxides are polyethylene glycol.

8. The composition of claim 1, wherein only one of the first or second components is a polyalkylene oxide.

9. The composition of claim 8, wherein one of the components is a polyalkylene oxide and the other component is a functionally activated succinimidyl or maleimidyl compound which is not a polymer.

10. The composition of claim 1, wherein the covalent bonds are thioester linkages.

11. The composition of claim 1, wherein the covalent bonds are thioether linkages.

12. The composition of claim 1, wherein the covalent bonds are sulfhydryl linkages.

13. The composition of claim 1, wherein the drug is hydrophobic.

14. The composition of claim 1, wherein the drug is an angiogenesis inhibitor.

15. The composition of claim 1, wherein the drug is a 5-Lipoxygenase inhibitor or antagonist.

16. The composition of claim 1, wherein the drug is a chemokine receptor antagonist.
17. The composition of claim 1, wherein the drug is a cell cycle inhibitor or an analogue or derivative thereof.
18. The composition of claim 17, wherein the cell cycle inhibitor is a microtubule stabilizing agent.
19. The composition of claim 18, wherein the microtubule stabilizing agent is paclitaxel, docetaxel, or Peloruside A.
20. The composition of claim 17, wherein the cell cycle inhibitor is a taxane.
21. The composition of claim 18, wherein the taxane is paclitaxel or an analogue or derivative thereof.
22. The composition of claim 17, wherein the cell cycle inhibitor is an antimetabolite, an alkylating agent, or a vinca alkaloid.
23. The composition of claim 22, wherein the vinca alkaloid is vinblastine, vincristine, vincristine sulfate, vindesine, vinorelbine, or an analogue or derivative thereof.
24. The composition of claim 17, wherein the cell cycle inhibitor is camptothecin or an analogue or derivative thereof.
25. The composition of claim 17, wherein the cell cycle inhibitor is selected from the group consisting of mitoxantrone, etoposide, 5-fluorouracil,

doxorubicin, methotrexate, Mitomycin-C, CDK-2 inhibitors, and analogues and derivatives thereof.

26. The composition of claim 1, wherein the drug is a cyclin dependent protein kinase inhibitor or an analogue or derivative thereof.

27. The composition of claim 1, wherein the drug is an EGF (epidermal growth factor) kinase inhibitor or an analogue or derivative thereof.

28. The composition of claim 1, wherein the drug is an elastase inhibitor or an analogue or derivative thereof.

29. The composition of claim 1, wherein the drug is a factor Xa inhibitor or an analogue or derivative thereof.

30. The composition of claim 1, wherein the drug is a farnesyltransferase inhibitor or an analogue or derivative thereof.

31. The composition of claim 1, wherein the drug is a fibrinogen antagonist or an analogue or derivative thereof.

32. The composition of claim 1, wherein the drug is a guanylate cyclase stimulant or an analogue or derivative thereof.

33. The composition of claim 1, wherein the drug is a heat shock protein 90 antagonist or an analogue or derivative thereof.

34. The composition of claim 1, wherein the drug is an HMGCoA reductase inhibitor or an analogue or derivative thereof.

35. The composition of claim 1, wherein the drug is a hydroorotate dehydrogenase inhibitor or an analogue or derivative thereof.

36. The composition of claim 1, wherein the drug is an IKK2 inhibitor or an analogue or derivative thereof.

37. The composition of claim 1, wherein the drug is an IL-1, ICE, or IRAK antagonist or an analogue or derivative thereof.

38. The composition of claim 1, wherein the drug is an IL-4 agonist or an analogue or derivative thereof.

39. The composition of claim 1, wherein the drug is an immunomodulatory is rapamycin, tacrolimus, everolimus, biolimus, or an analogue or derivative thereof.

40. The composition of claim 1, wherein the drug is an inosine monophosphate dehydrogenase inhibitor or an analogue or derivative thereof.

41. The composition of claim 1, wherein the drug is a leukotrene inhibitor or an analogue or derivative thereof.

42. The composition of claim 1, wherein the drug is a MCP-1 antagonist or an analogue or derivative thereof.

43. The composition of claim 1, wherein the drug is a MMP inhibitor or an analogue or derivative thereof.

44. The composition of claim 1, wherein the drug is a NF kappa B inhibitor or an analogue or derivative thereof.

45. The composition of claim 1, wherein the drug is a NO antagonist or an analogue or derivative thereof.

46. The composition of claim 1, wherein the drug is a P38 MAP kinase inhibitor or an analogue or derivative thereof.

47. The composition of claim 1, wherein the drug is a phosphodiesterase inhibitor or an analogue or derivative thereof.

48. The composition of claim 1, wherein the drug is a TGF beta Inhibitor or an analogue or derivative thereof.

49. The composition of claim 1, wherein the drug is a thromboxane A2 antagonist or an analogue or derivative thereof.

50. The composition of claim 1, wherein the drug is a TNF α Antagonist, a TACE, or an analogue or derivative thereof.

51. The composition of claim 1, wherein the drug is a tyrosine kinase inhibitor or an analogue or derivative thereof.

52. The composition of claim 1, wherein the drug is a vitronectin inhibitor or an analogue or derivative thereof.

53. The composition of claim 1, wherein the drug is a fibroblast growth factor inhibitor or an analogue or derivative thereof.

54. The composition of claim 1, wherein the drug is a protein kinase inhibitor or an analogue or derivative thereof.

55. The composition of claim 1, wherein the drug is a PDGF receptor kinase inhibitor or an analogue or derivative thereof.

56. The composition of claim 1, wherein the drug is an endothelial growth factor receptor kinase inhibitor or an analogue or derivative thereof.

57. The composition of claim 1, wherein the drug is a retinoic acid receptor antagonist or an analogue or derivative thereof.

58. The composition of claim 1, wherein the drug is a platelet derived growth factor receptor kinase inhibitor or an analogue or derivative thereof.

59. The composition of claim 1, wherein the drug is a fibrinogen antagonist or an analogue or derivative thereof.

60. The composition of claim 1, wherein the drug is an antimycotic agent or an analogue or derivative thereof.

61. The composition of claim 1, wherein the drug is a bisphosphonate or an analogue or derivative thereof.

62. The composition of claim 1, wherein the drug is a phospholipase A1 inhibitor or an analogue or derivative thereof.

63. The composition of claim 1, wherein the drug is a histamine H1/H2/H3 receptor antagonist or an analogue or derivative thereof.

64. The composition of claim 1, wherein the drug is a macrolide antibiotic or an analogue or derivative thereof.

65. The composition of claim 1, wherein the drug is an GPIIb IIIa receptor antagonist or an analogue or derivative thereof.

66. The composition of claim 1, wherein the drug is an endothelin receptor antagonist or an analogue or derivative thereof.

67. The composition of claim 1, wherein the drug is a peroxisome proliferators-activated receptor agonist or an analogue or derivative thereof.

68. The composition of claim 1, wherein the drug is an estrogen receptor agent or an analogue or derivative thereof.

69. The composition of claim 1, wherein the drug is somatostatin or an analogue or derivative thereof.

70. The composition of claim 1, wherein the drug is a JNK Kinase inhibitor or an analogue or derivative thereof.

71. The composition of claim 1, wherein the drug is a melanocortin or an analogue or derivative thereof.

72. The composition of claim 1, wherein the drug is a raf kinase inhibitor or analogue or derivative thereof.

73. The composition of claim 1, wherein the drug is a lysylhydroxylase inhibitor or an analogue or derivative thereof.

74. The composition of claim 1, wherein the drug is an IKK 1/2 inhibitor or an analogue or derivative thereof.

75. The composition of claim 1, further comprising an anti-inflammatory agent, an antithrombotic agent, an antibiotic, or a combination thereof.

76. The composition of claim 1, wherein the drug further comprises a polymer.

77. The polymer of claim 76, wherein the polymer is a polymer or copolymer comprising one or more of the residue units of the monomers, lactic acid, glycolic acid, D-lactide, L-lactide, D,L-lactide, glycolide, ϵ -caprolactone, trimethylene carbonate, 1,4-dioxane-2-one or 1,5-dioxepan-2-one,

78. The polymer of claim 77, wherein the polymer is a block copolymers of the for A-B, A-B-A or B-A-B where A is a poly(alkylene oxide) and B is a degradable polyester.

79. The poly(alkylene oxide) in claim 78, wherein the poly(alkylene oxide) is poly(ethylene glycol), poly(propylene glycol), copolymers of ethylene oxide and propylene oxide or mono alkyl ethers thereof

80. The composition of claim 76, wherein the polymer is in the form of a microsphere.

81. The composition of claim 76, wherein the polymer is in the form of a nanosphere.

82. The composition of claim 76, wherein the polymer is in the form of a micelle.

83. The composition of claim 1, wherein the drug further comprises a non-polymeric carrier.

84. The composition of claim 1, wherein the drug is a hydrophobic drug in admixture with a secondary carrier to provide drug/carrier, the drug/carrier being in admixture with the first component to provide drug/carrier/first component, the drug/carrier/first component being suspended in an aqueous buffer solution.

85. The composition of claim 1, wherein the drug is hydrophilic.

86. The composition of claim 1, wherein the drug is a hydrophilic drug in admixture with a secondary carrier to provide drug/carrier, the drug/carrier being in admixture with the first component to provide drug/carrier/first component, the drug/carrier/first component being suspended in an aqueous buffer solution.

87. The composition of claim 1, wherein the first component is suspended in a buffer solution comprising a mixture of phosphate buffer and carbonate buffer.

88. The composition of claim 2, wherein the second component comprises a mixture of succinimidyl polyalkylene oxide and maleimidyl polyalkylene oxide.

89. A method for treating tissues, comprising the steps of:
administering to a tissue site a first component comprising at least one sulfhydryl group-containing compound in liquid medium having an alkaline pH, wherein said sulfhydryl group-containing compound is given by the formula Compound₁-(SH)_m, wherein $m \geq 2$; and

simultaneously or subsequently administering to the tissue site a second component comprising at least one sulfhydryl reactive group-containing compound either a liquid medium having a neutral or acidic pH or in powder form, wherein said sulfhydryl reactive group-containing compound is given by the formula Compound₂-Y_n, wherein Y is a sulfhydryl reactive group and wherein $n \geq 2$, and wherein at least one of the first or second components is a polyalkylene oxide; and

simultaneously or subsequently administering to the tissue site a drug; and

allowing the sulfhydryl groups and the sulfhydryl reactive groups to react with one another to form covalent bonds therebetween to form a gel in less than one minute.

90. A biocompatible gel-forming drug-delivering composition for *in vivo* administration with a gel time of less than one minute, comprising:
polyalkylene oxide-(SH)₄ and drug in a liquid medium having a pH of between 8 and 10.5; and
polyalkylene oxide-Y₄, wherein Y is succinimidyl, in a liquid medium having an acidic pH.

91. A biocompatible gel-forming drug-delivering composition for *in vivo* administration with a gel time of less than one minute, comprising:
polyalkylene oxide-(SH)₁₂ and drug in a liquid medium having an alkaline pH; and
polyalkylene oxide-Y₁₂ in a liquid medium having an acidic pH, wherein Y is a succinimidyl or maleimidyl group.

92. A biocompatible gel-forming composition for *in vivo* administration, comprising:

a sulfhydryl group-containing polyalkylene oxide in a liquid medium having an acidic pH, wherein said sulfhydryl group-containing polyalkylene oxide is given by the formula $\text{Core}-(\text{SH})_m$, wherein $m \geq 2$;

a buffer solution with an alkaline pH; and

drug in admixture with the polyalkylene oxide and/or the buffer solution;

wherein the sulfhydryl groups react with one another to form covalent bonds therebetween when said components are mixed together to form a gel in less than one minute.

93. A biocompatible gel-forming drug-delivering composition for *in vivo* administration, comprising:

at least one sulfhydryl group-containing compound in a liquid medium having an alkaline pH, wherein said sulfhydryl group-containing compound is given by the formula $\text{Compound}_1-(\text{SH})_m$, wherein $m \geq 2$;

at least one sulfhydryl reactive group-containing compound either a liquid medium having a neutral or acidic pH or in powder form, wherein said sulfhydryl reactive group-containing compound is given by the formula $\text{Compound}_2-\text{Y}_n$, wherein Y is a sulfhydryl reactive group and wherein $n \geq 2$;

at least one drug in admixture with either or both of the at least one sulfhydryl group-containing compound and the at least one sulfhydryl reactive group-containing compound; and

collagen;

wherein at least one of either the sulfhydryl group-containing compound or the sulfhydryl reactive group-containing compound is a polyalkylene oxide, and wherein the sulfhydryl groups and the sulfhydryl reactive groups are capable of reacting with one another to form covalent bonds therebetween.

94. The composition of claim 93, wherein m and n are each 4.

95. The composition of claim 93, wherein m and n are each 12.
96. The composition of claim 93 wherein the sulfhydryl group-containing compound is a polyalkylene oxide.
97. The composition of claim 93, wherein the sulfhydryl reactive group-containing compound is a polyalkylene oxide.
98. The composition of claim 93, wherein both the sulfhydryl group-containing compound and the sulfhydryl reactive group-containing compound are polyalkylene oxides.
99. The composition of claim 98, wherein both the sulfhydryl group-containing compound and the sulfhydryl reactive group-containing compound are polyalkylene oxides.
100. The composition of claim 93, wherein only one of the first or second components is a polyalkylene oxide.
101. The composition of claim 100, wherein one of the components is a polyalkylene oxide and the other component is a functionally activated succinimidyl or maleimidyl compound which is not a polymer.
102. The composition of claim 93, wherein the covalent bonds are thioester linkages.
103. The composition of claim 93, wherein the covalent bonds are thioether linkages.
104. The composition of claim 93, wherein the covalent bonds are sulfhydryl linkages.

105. The composition of claim 93, wherein the drug is a hydrophobic drug.

106. The composition of claim 93, wherein the drug is a hydrophobic drug in admixture with a secondary carrier to provide drug/carrier, the drug/carrier being in admixture with either or both of the at least one sulfhydryl group-containing compound and the at least one sulfhydryl reactive group-containing compound.

107. The composition of claim 93, wherein the sulfhydryl group-containing compound is suspended in a buffer solution comprising a mixture of phosphate buffer and carbonate buffer.

108. The composition of claim 93, wherein the sulfhydryl reactive group-containing compound comprises a mixture of succinimidyl polyalkylene oxide and maleimidyl polyalkylene oxide.

109. The composition of claim 93, wherein the collagen is methylated collagen.

110. A biocompatible gel-forming drug-delivering composition for *in vivo* administration, comprising:

(a) a first component in a liquid medium having an acidic pH comprising:

(i) at least one sulfhydryl group-containing compound given by the formula Compound₁-(SH)_m, wherein $m \geq 2$;

(ii) at least one sulfhydryl reactive group-containing compound given by the formula Compound₂-Y_n, wherein Y is a sulfhydryl reactive group and wherein $n \geq 2$; and

(iii) collagen; and

(b) a second component comprising a buffer having a pH of between 8 and 10.5;

wherein a drug is present in admixture with either or both of the first component or the second component; and

wherein at least one of either the sulfhydryl group containing compound or the sulfhydryl reactive group containing compound is a polyalkylene oxide.

111. The composition of claim 110 wherein the collagen is methylated collagen.

112. The composition of claim 110 wherein the second component is a buffer solution comprising a mixture of phosphate buffer and carbonate buffer.

113. A method for forming a drug delivery composition, comprising

a) selecting a first component, a second component and a drug, wherein

the first component comprises at least one sulfhydryl group-containing compound in a liquid medium having an alkaline pH, wherein said sulfhydryl group-containing compound is given by the formula Compound₁-(SH)_m, wherein $m \geq 2$; and

the second component comprises at least one sulfhydryl reactive group-containing compound in either a liquid medium having a neutral or acidic pH or in powder form, wherein said sulfhydryl reactive group-containing compound is given by the formula Compound₂-Y_n, wherein Y is a sulfhydryl reactive group and wherein $n \geq 2$;

at least one of the first or second components is a polyalkylene oxide;

the sulfhydryl groups and the sulfhydryl reactive groups react with one another to form covalent bonds therebetween when said components are mixed together to form a gel in less than one minute;

b) combining the first and second components in the presence of the drug, under conditions where the first component reacts with the second component.

114. A product produced by the method of claim 113.

115. A method for forming a drug delivery composition, comprising

a) forming an admixture of polyalkylene oxide-(SH)₄ and drug in a liquid medium having a pH of between 8 and 10.5; and

b) forming an admixture of polyalkylene oxide-Y₄, wherein Y is succinimidyl and liquid medium, the admixture having an acidic pH.

116. The method of claim 115 further comprising combining the admixtures of steps a) and b).

117. A product produced by the method of claim 116.

118. A method for forming a biocompatible gel-forming drug-delivering composition for *in vivo* administration with a gel time of less than one minute, comprising:

a) preparing an admixture of polyalkylene oxide-(SH)₁₂ and drug in a liquid medium having an alkaline pH; and

b) preparing polyalkylene oxide-Y₁₂ in a liquid medium having an acidic pH, wherein Y is a succinimidyl or maleimidyl group.

119. The method of claim 118 further comprising combining a) and b).

120. The product produced by the method of claim 119.

121. A method for forming a biocompatible gel-forming composition for *in vivo* administration, comprising:

a) preparing a sulfhydryl group-containing polyalkylene oxide in a liquid medium having an acidic pH, wherein said sulfhydryl group-containing polyalkylene oxide is given by the formula $\text{Core}-(\text{SH})_m$, wherein $m \geq 2$;

b) providing a buffer solution with an alkaline pH; and

c) adding drug to either or both of a) and b);

wherein the sulfhydryl groups react with one another to form covalent bonds therebetween when said components are mixed together to form a gel in less than one minute.

122. The method of claim 121 further comprising combining a) and b).

123. The product produced by the method of claim 122.

124. A method for forming a biocompatible gel-forming drug-delivering composition for *in vivo* administration, comprising:

a) providing an at least one sulfhydryl group-containing compound in a liquid medium having an alkaline pH, wherein said sulfhydryl group-containing compound is given by the formula $\text{Compound}_1-(\text{SH})_m$, wherein $m \geq 2$;

b) providing an at least one sulfhydryl reactive group-containing compound either in a liquid medium having a neutral or acidic pH or in powder form, wherein said sulfhydryl reactive group-containing compound is given by the formula $\text{Compound}_2-\text{Y}_n$, wherein Y is a sulfhydryl reactive group and wherein $n \geq 2$;

c) combining a drug with either or both of the at least one sulfhydryl group-containing compound and the at least one sulfhydryl reactive group-containing compound; and

d) providing collagen;

wherein at least one of either the sulfhydryl group-containing compound or the sulfhydryl reactive group-containing compound is a polyalkylene oxide; and

wherein the sulfhydryl groups and the sulfhydryl reactive groups are capable of reacting with one another to form covalent bonds therebetween.

125. A method for forming a biocompatible gel-forming drug-delivering composition for *in vivo* administration, comprising:

a) providing an at least one sulfhydryl group-containing compound in a liquid medium having an alkaline pH, wherein said sulfhydryl group-containing compound is given by the formula Compound₁-(SH)_m, wherein $m \geq 2$;

b) providing an at least one sulfhydryl reactive group-containing compound either in a liquid medium having a neutral or acidic pH or in powder form, wherein said sulfhydryl reactive group-containing compound is given by the formula Compound₂-Y_n, wherein Y is a sulfhydryl reactive group and wherein $n \geq 2$; and

c) providing collagen;

wherein at least one of either the sulfhydryl group-containing compound or the sulfhydryl reactive group-containing compound is a polyalkylene oxide; and

wherein the sulfhydryl groups and the sulfhydryl reactive groups are capable of reacting with one another to form covalent bonds therebetween.

126. The product produced by the method of claim 125.

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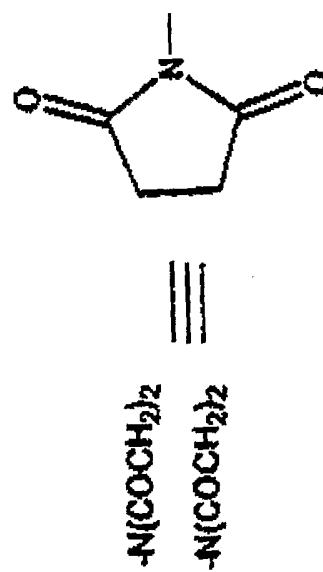
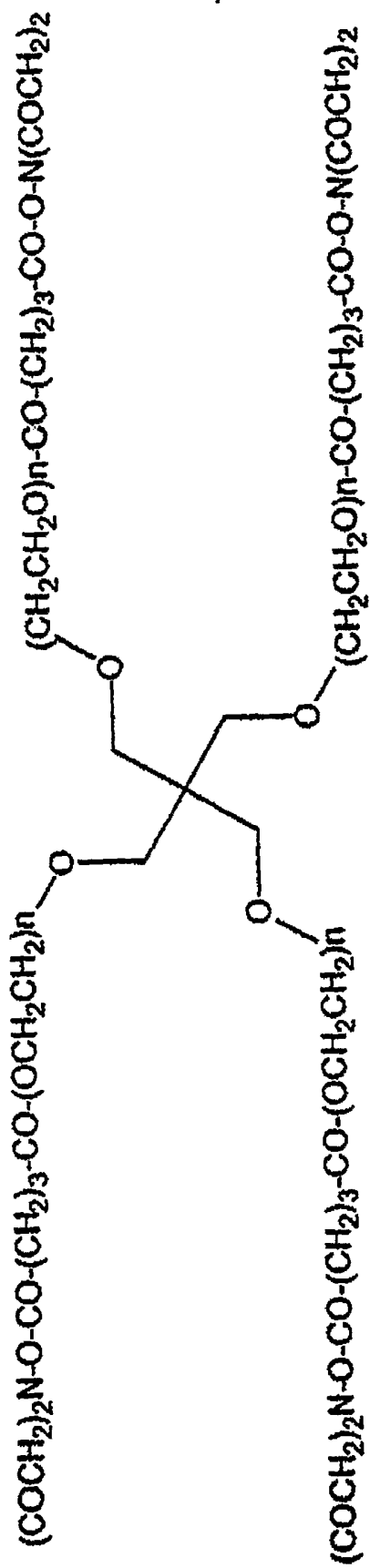


FIG. 1

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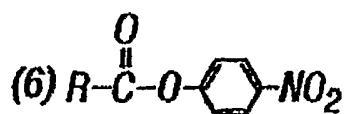
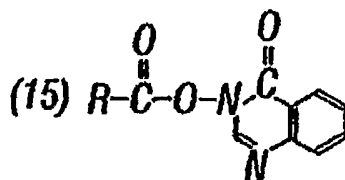
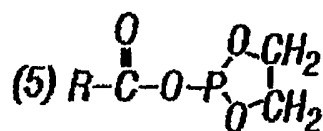
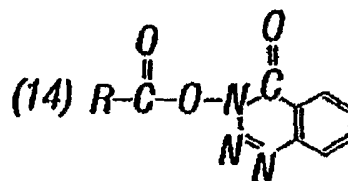
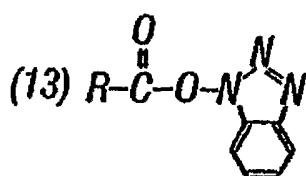
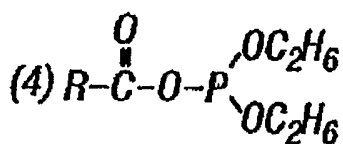
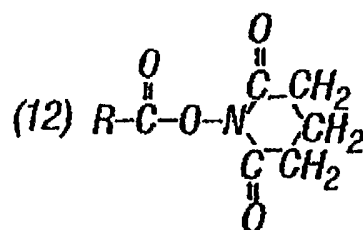
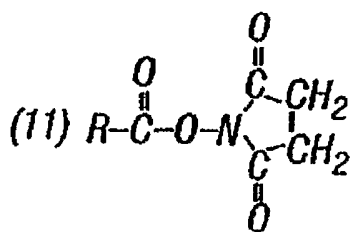
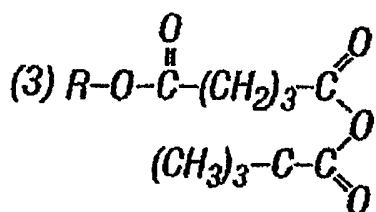
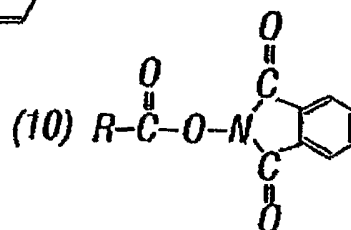
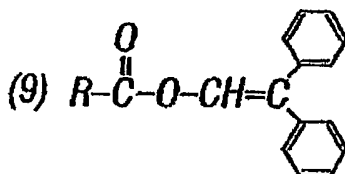
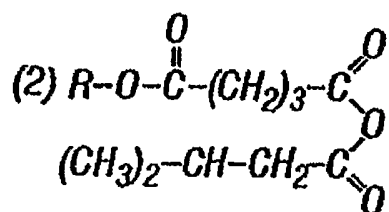
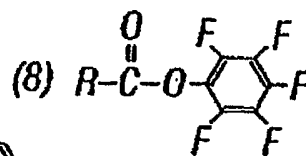
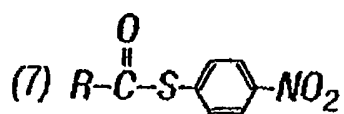
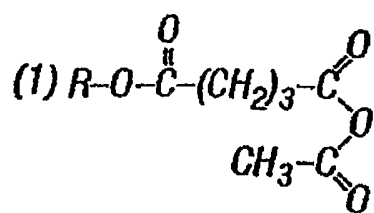


FIG. 2

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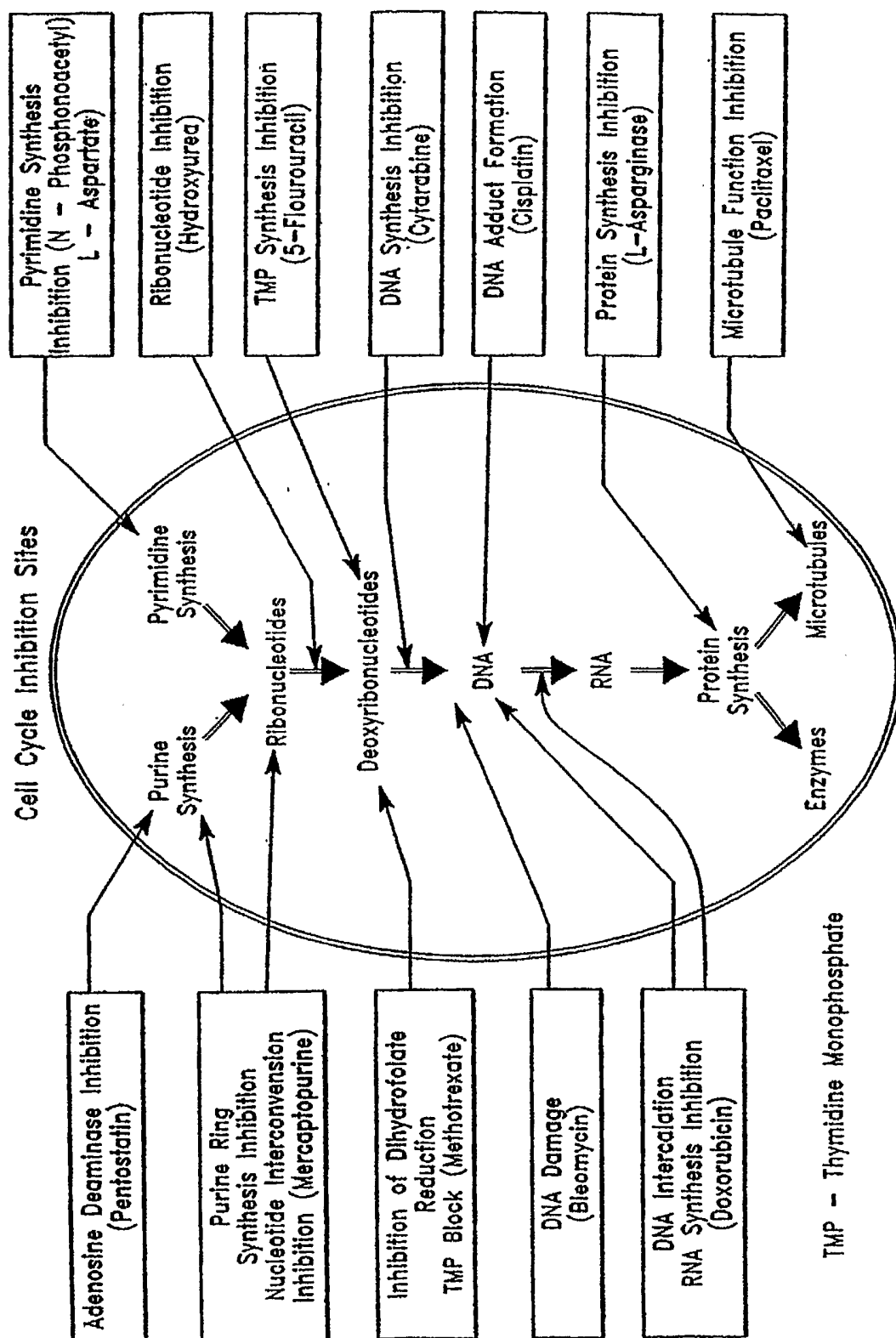


FIG. 3

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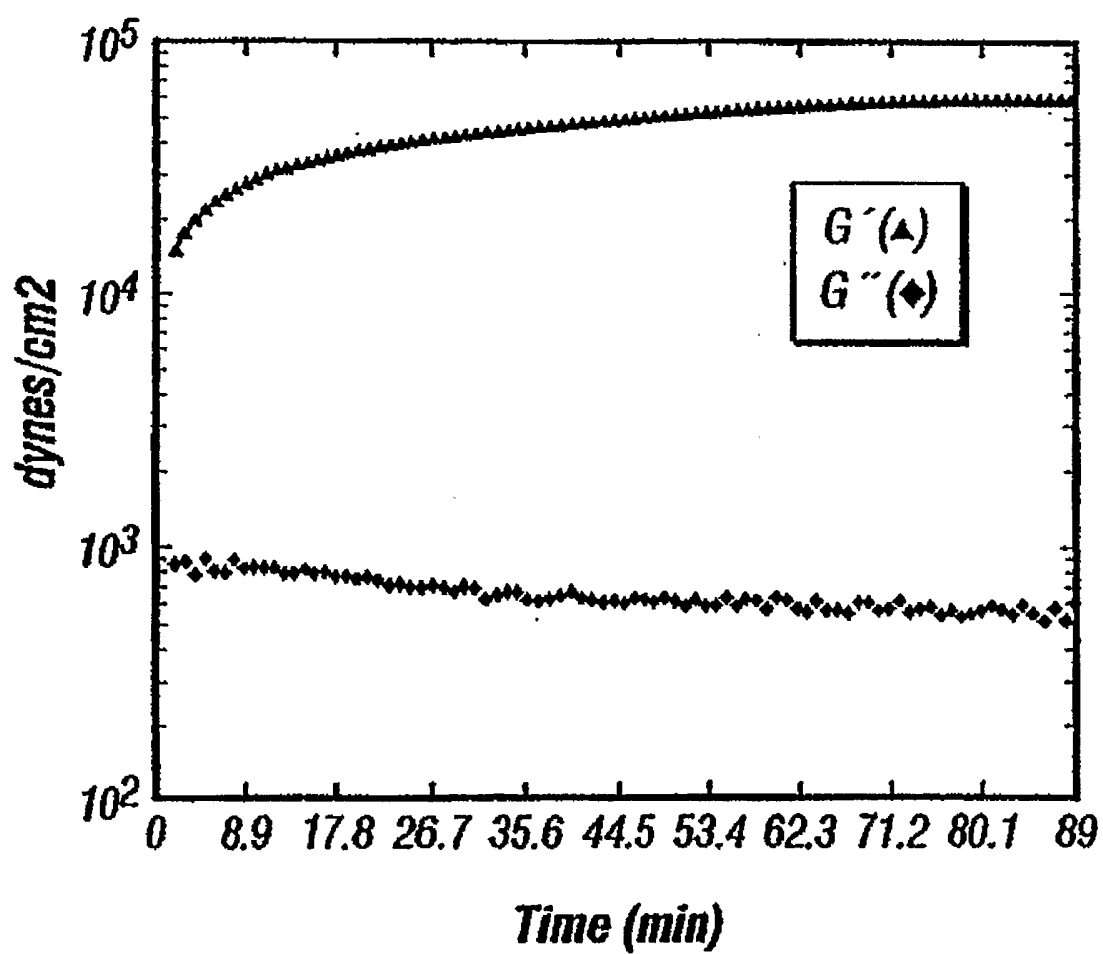


FIG. 4

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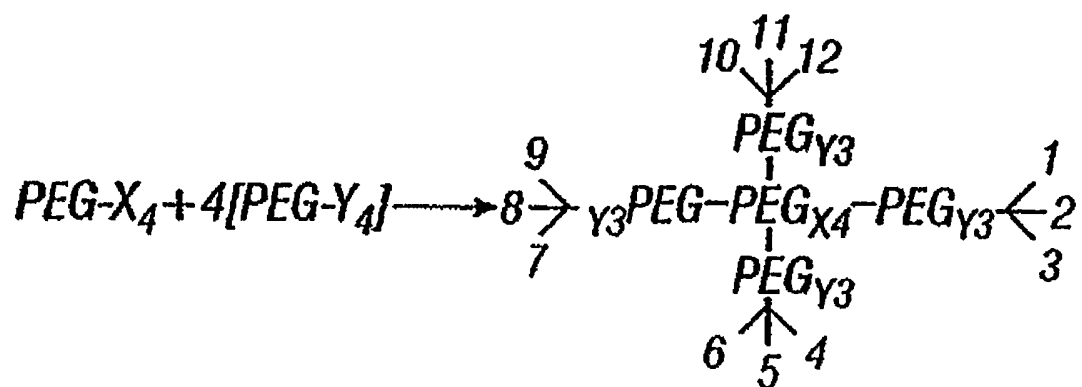


FIG. 5A

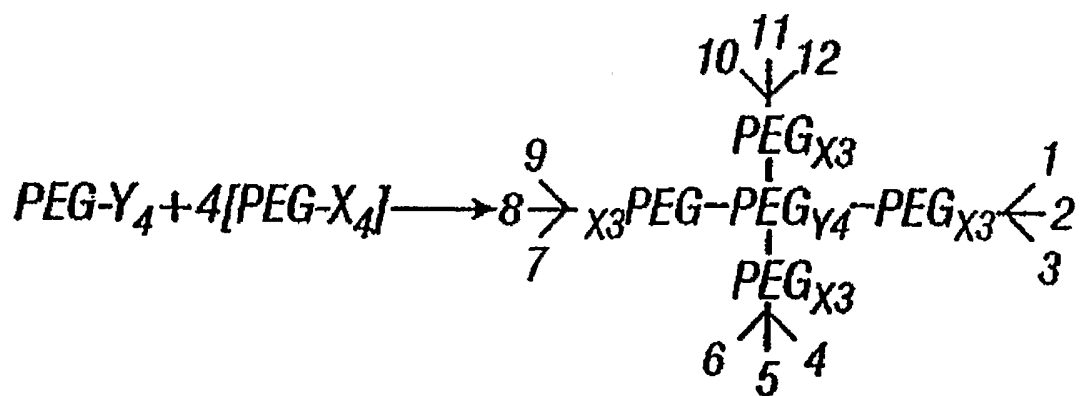


FIG. 5B

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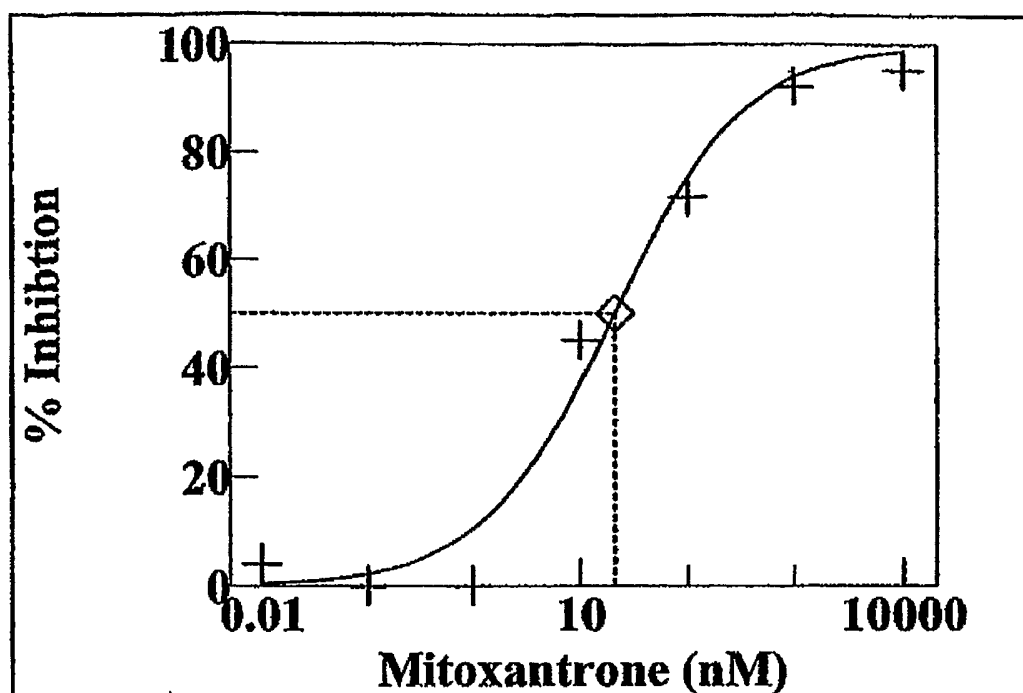


FIG. 6

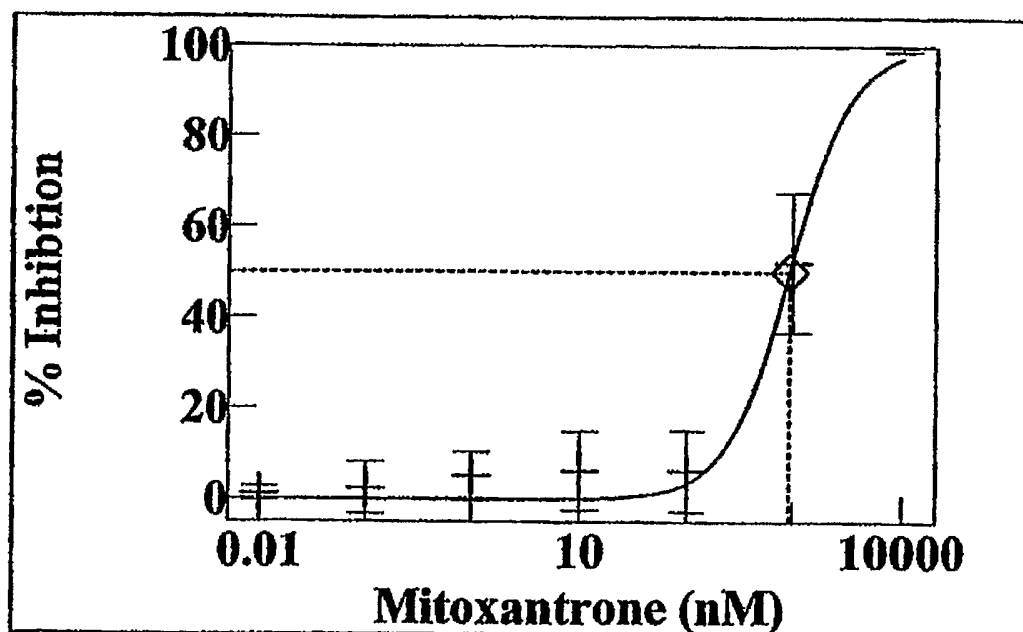
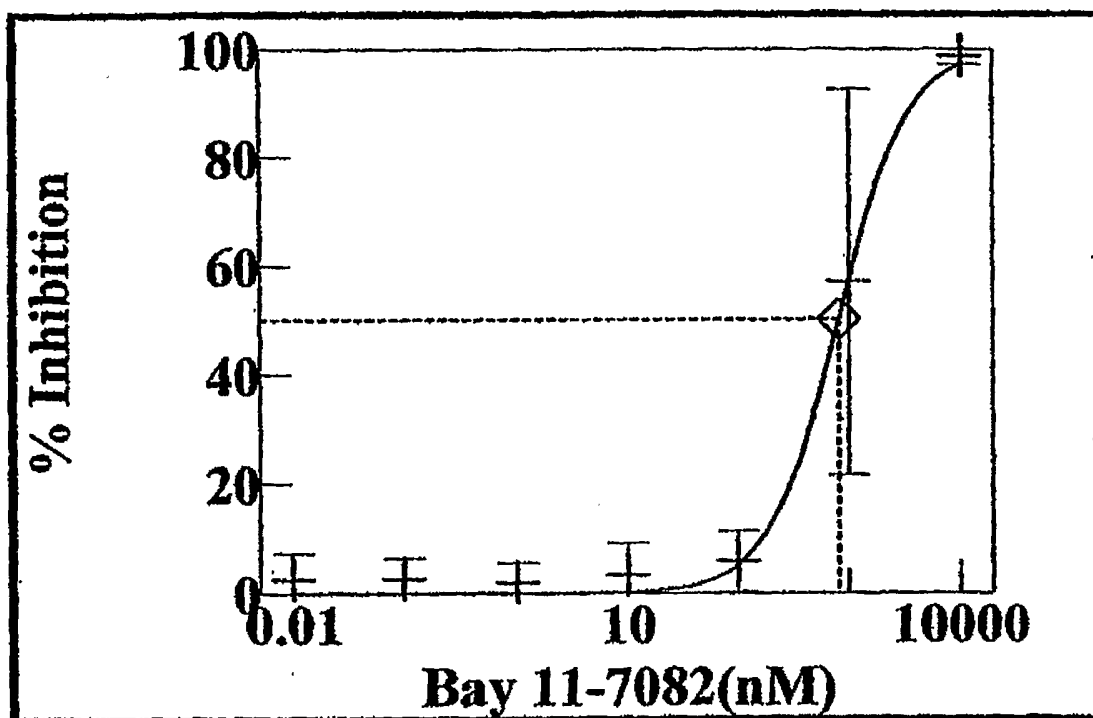


FIG. 7

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*FIG. 8*