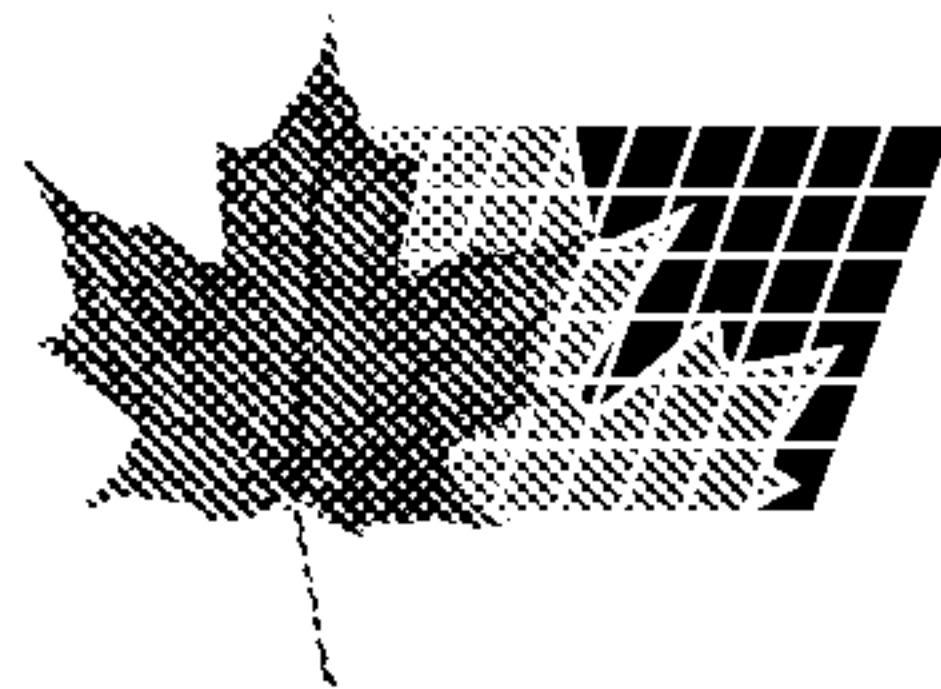




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(51) Int.Cl.⁷ A61K 31/185, A61P 15/18, A61P 31/04, A61P 7/02

(30) 1998/09/23 (09/159,814) US

(54) **PROCEDES ET COMPOSITIONS POUR TRAITER LES
MALADIES INDUITES PAR DES RECEPTEURS**

(54) **PHARMACEUTICAL COMPOSITIONS COMPRISING
DERIVATIVES OF SULPHUR ACIDS**

(57) Small molecule pharmaceuticals for treating sulfate receptor mediated diseases are disclosed.

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : A61K 31/185, A61P 7/02, 15/18, 31/04	A3	(11) International Publication Number: WO 00/16764 (43) International Publication Date: 30 March 2000 (30.03.00)
(21) International Application Number: PCT/US99/21797 (22) International Filing Date: 23 September 1999 (23.09.99) (30) Priority Data: 09/159,814 23 September 1998 (23.09.98) US (71) Applicant (for all designated States except US): PHYCOGEN, INC. [US/US]; Suite 440, 4 Moulton Street, Portland, ME 04101-5013 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): ALBERTE, Randall, S. [US/US]; 2551 Holly Manor Drive, Falls Church, VA 22043 (US). ZIMMERMAN, Richard, C. [US/US]; 392 Gibson Avenue, Pacific Grove, CA 93950 (US). (74) Agents: ARNOLD, Beth, E. et al.; Foley, Hoag & Eliot, LLP, One Post Office Square, Boston, MA 02109 (US).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> (88) Date of publication of the international search report: 23 November 2000 (23.11.00)
(54) Title: PHARMACEUTICAL COMPOSITIONS COMPRISING DERIVATIVES OF SULPHUR ACIDS (57) Abstract Small molecule pharmaceuticals for treating sulfate receptor mediated diseases are disclosed.		

Methods and Compositions for Treating Receptor Mediated Diseases

Background of the Invention

Cell surface receptors play a key role in the initiation of infection by pathogenic
5 viruses, bacteria and fungi. The host specificity of many pathogens is determined at the point
of initial contact and attachment to a eukaryotic plant or animal cell. For example, certain
fungal pathogens of plants release host-selective (or host-specific) toxins (HST) as a host
recognition factor during spore germination at the infection site on plants. Prior to penetration
of the pathogen into its host, the released toxin specifically binds to a receptor on the host
10 cells and initiates signaling mechanisms leading to pleiotropic effects on cells (Kohmoto and
Otani (1991) *Experientia* 47: 755-64). Of these, the crucial one negates the general and
inducible defense reactions of the cells. This is accomplished by a signal from the HST,
which is transduced through a path way at or near the step of plasma membrane modulation,
which is directly or indirectly triggered by the HST. The fungal spore is able to penetrate the
15 so- called 'narcotized cell' and completes the initial colonization of the host. The host
recognition process may take place without necessitating host cell death, even in the case of
perthophytic parasites. At the molecular level, HST-mediated recognition of the host by a
pathogen requires strict stereochemical precision like a lock and key between the host
receptor and the fungal pathogen. The criticality of pathogen/plant host receptor interactions
20 is further evident from genetic studies of plant disease resistance. For example, a major locus
of pathogen resistance in lettuce plants has been isolated and found to encode a family of
receptor-like genes (Meyers et al. (1998) *Plant Cell* 10: 1833-46).

The infection of animal cells by pathogenic bacteria is similarly dependent upon the
initial interaction between bacterium and host cell-surface protein. Enterotoxigenic
25 *Escherichia coli* (ETEC), which cause intestinal diarrhea in animals and humans, infect host
organism by initial attachment to mucosa surface receptors (Mouricout (1991) *Eur J*
Epidemiol 7: 588-604). Infection by ETEC further requires the action of specific bacterial
fimbrial adhesins which act as lectins in the process of bacterial recognition and attachment to
the host receptor. The ability of a large variety of pathogenic bacteria to adhere to and
30 colonize a host cell layer has been shown to involve the production of such hair-like fimbrial
adhesion or pili which project from the surface of the bacterial cell. Production of these pili is
controlled by a specific plasmid within the pathogenic bacterium and loss of this plasmid
renders the bacteria avirulent although they may continue to produce toxins (see Shaeffer

(1993) Urologe A 32: 7-15). Thus, adhesion of bacterial pili to host cell surface receptors plays an important role in the development of many infectious diseases, including enteric *E. coli* infections, urinary tract infections, and rheumatic fever.

Furthermore, numerous examples of receptor-mediated infection of human host cells by pathogenic viruses exist. The measles virus infects human host cells via the CD46 cell surface receptor (Gerlier et al. (1995) Trends Microbiol 3: 338-45); the poliovirus similarly binds to a specific human poliovirus receptor and this interaction is critical to the uncoating of the viral RNA and subsequent infectious events (Racaniello (1996) Proc Natl Acad Sci USA 93: 11378-81); and the role of the human CD4 cell surface receptor in binding the gp120 viral envelope glycoprotein of HIV and subsequent infection of T-cells is well established (see e.g. Bour et al. (1995) Microbiol Rev 59: 63-93).

Significantly, diseases which do not involve viral, bacterial or fungal pathogens may nonetheless similarly involve the participation of host cell surface receptors in the initiation or progression of the disease. An interesting case in point is the progression of transmissible spongiform encephalopathies in humans and animals where it appears that a host receptor may facilitate the cell to cell transmission of infectious prion proteins within an infected host (Martins (1999) Braz J Med Biol Res 32: 853-9). Another example is certain types of cancers. Accordingly, chemical compounds which are able to attach to cell surface receptors may be useful in treating and preventing a broad spectrum of infectious and noninfectious diseases and conditions.

One group of proteins known in nature to bind to a wide variety of cell surface moieties are the lectins. Lectins are a family of animal and plant cell proteins which bind with high affinity to specific plant and animal cell surface oligosaccharide groups. For example, concavilin A is a plant protein which binds to internal and nonreducing terminal α -mannosyl residues, while wheat-germ agglutinin is a plant protein which binds to terminal N-acetylglucosamine residues. Significantly, the binding of such plant lectins to the outer surface of a eukaryotic cell can have profound consequences on the biology of the bound cell. For example, binding of concavilin A stimulates mitosis and the lectin wheat phytohemagglutinin causes agglutination of mammalian red blood cells. Plant and microbial lectins have also been shown to affect numerous other biological processes (reviewed in Rudiger (1998) Acta Anat (Basel) 161: 130-52). For example, cereal root lectins specifically recognize N-acetyl-D-glucosamine extracellular polysaccharide components of the rhizobium bacteria *Azospirillum* and thereby mediate legume-Rhizobium symbiosis (Skvortsov and Ignatov (1998) FEMS Microbiol Lett 165: 223-9). Similarly, lectins are implicated in the

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recognition of the plant host by plant pathogenic fungi (Hardham and Mitchell (1998) Fungal Genet Biol 24: 252-84).

Numerous animal lectins have also been discovered and implicated in a broad range of biological functions. For example, the selectins, mediate specific cell-cell adhesion events, such as in the binding of leukocytes to activated endothelial cells which guides lymphocyte trafficking in inflammation (Kaltner and Stierstorfer (1998) Acta Anat (Basel) 161: 1-4). Certain other animal lectins are expressed in the vertebrate central and peripheral nervous system, where they appear to participate in ontogenetic processes, especially those involving cell adhesion and cell recognition mechanisms such as axonal growth and fasciculation, neuron migration, synaptogenesis, and myelination (Zanetta (1998) Acta Anat (Basel) 161: 180-95). Still other animal lectins may be involved in intracellular signaling events in the nucleus or at the cell surface by autocrine and paracrine mechanisms (*ibid*). The mannose-binding lectin (MBL) plays a key role in activation of the mammalian acute phase response by mediating the interaction of foreign microbial carbohydrate determinants to a serine protease (i.e. the MBL-associated serine protease or MASP) which triggers proteolytic activation of the complement component C3 and subsequent phagocytosis or cell lysis (Vasta et al. (1999) Acta Anat (Basel) 23: 401-20; Zhang et al. (1999) Immunopharmacology 42: 81-90). Animal lectins have also been implicated as mediators of the "glycocoding" of cell/ cell interactions during embryogenesis (reviewed in Mann and Waterman (1998) 161: 153-61).

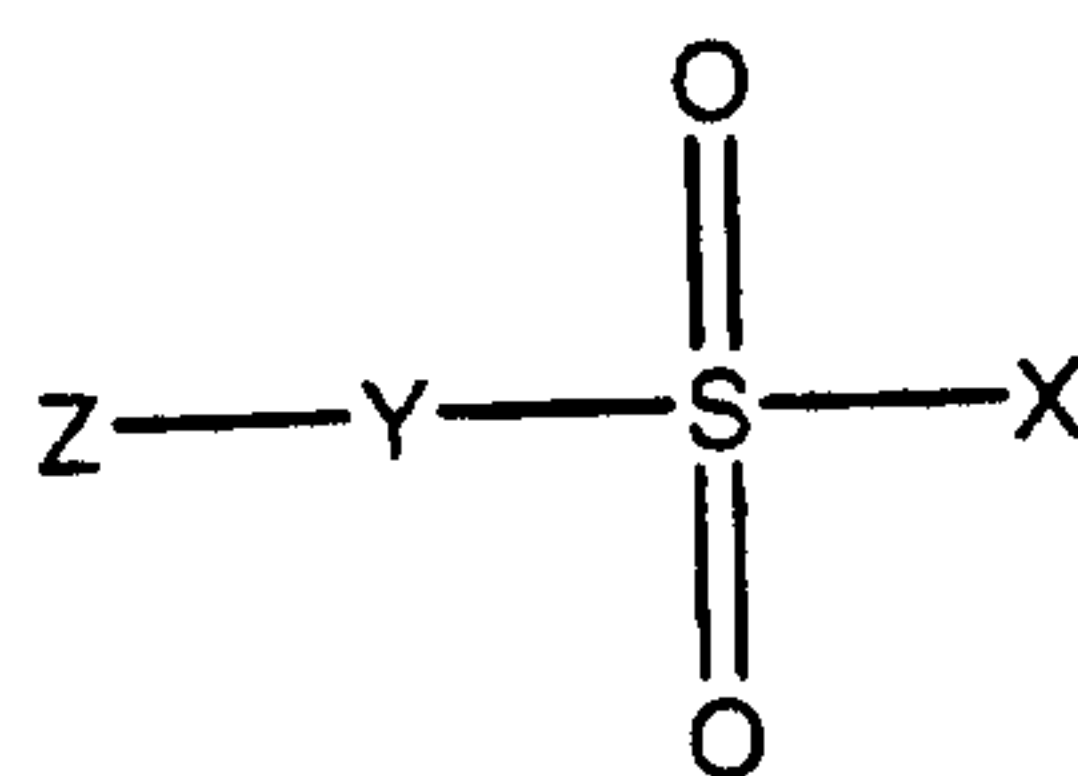
Although the lectins may be useful in targeting specific receptors and thereby blocking specific receptor-mediated disease processes, they have several disadvantages. In particular, their relative specificity requires that the chemical nature of the host cell target be known and, further, that a corresponding high-affinity receptor-binding lectin be available. Other disadvantages of the use of lectins to treat or prevent receptor mediated diseases include their relative high cost of production, and the difficulty of delivering them to an animal.

Compounds that can bind to receptors and thereby interfere with receptor mediated interactions would be useful pharmaceuticals.

Summary of the Invention

In one aspect, the invention features pharmaceutical compositions, comprising a pharmaceutically acceptable carrier and an effective amount of a compound having the general structure 1:

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1

wherein

X represents -OH, -O(aryl), -O(acyl), -O(sulfonyl), -CN, F, Cl, or Br;

Y represents O, S, Se, or NR;

5 Z represents optionally substituted alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, or $-(\text{CH}_2)_m\text{-R}_{80}$;

R represents independently for each occurrence hydrogen, alkyl, heteroalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, or $-(\text{CH}_2)_m\text{-R}_{80}$;

10 R_{80} represents independently for each occurrence aryl, cycloalkyl, cycloalkenyl, heterocyclyl, or polycyclyl; and m is an integer in the range 0 to 8 inclusive.

In another aspect the invention features methods for treating receptor mediated diseases utilizing the pharmaceutical compounds of the invention.

15 In addition, to being effective in treating or preventing a number of receptor mediated diseases, the compounds of the invention should prove relatively safe as they are derived from natural zosteric acid obtained from the marine eel grass (*Zostera marina*). In addition, many of the compounds are small molecules and therefore can be administered orally.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

20 Brief Description of the Drawings

Figure 1 is a diagrammatic representation of the results of marine algae attachment assays measuring the abundance of algal biofilm development on the inert coating RTV-11 compared to biofilm development on RTV-11 with octyl sulfate incorporated into the coating. Relative algal abundance represents the attachment of the marine algae to the tested surface.

25 Error bars indicate 1 standard error of the mean ($n = 3$) for each treatment. The ratio of the

optical densities measured at wavelengths 680 nm and 750 nm (A_{680}/A_{750}) at time 0 was used as a baseline reference for all samples.

Figure 2 is a diagrammatic representation of the results of bacterial attachment assays performed with the marine bacterium *Oceanosprillum*, cultured in the presence and absence of either zosteric acid or methyl sulfate.

Figure 3 is a diagrammatic representation of the results of bacterial attachment assays performed with the marine bacterium *Oceanosprillum*, cultured in the presence and absence of either zosteric acid or octyl sulfate.

Figure 4 is a diagrammatic representation of the results of bacterial attachment assays performed with the bacterium *Alteromonas atlantica*, performed in the presence and absence of either, zosteric acid, octyl sulfate, or methyl sulfate.

Figure 5 is a diagrammatic representation of the results of fungal attachment and growth assays using the fungus *Aureobasidium pullulans* (a shower fungus that stains grout) grown in the presence and absence of zosteric acid, where fungal abundance represents the attachment of *A. pullulans* to the exposed surface.

Figure 6 is a diagrammatic representation of the results of agglutination of the bacterium *Shewanella putrefaciens* induced by the presence of increased amounts of zosteric acid, measured by the percent transmission (%T) of the liquid cultures at wavelength 600 nm. Agglutination is indicated by the concentration-dependent increase in %T of cultures grown in the presence of zosteric acid. In this case, relatively high levels of %T exhibited by the zosteric acid-exposed cultures do not reflect differences in growth, as counts of viable colony forming units exhibited no difference in cell density at eight hours.

Figure 7 is a diagrammatic representation of data from prothrombin clotting time assays which displays the clotting time of erythrocytes in the presence of high molecular weight heparin compared to the clotting time of erythrocytes in the presence of zosteric acid.

Figure 8 is a diagrammatic representation of data measuring the effects of zosteric acid on the event of sea urchin egg fertilization. a) Dose dependent effect of zosteric acid on sea urchin fertilization. Percent fertilization represents a comparison of the number of eggs fertilized in the presence of the indicated concentration of zosteric acid, to the number of eggs fertilized under the same conditions, in the absence of zosteric acid. b) Relative effects of coumaric acid, heparin and zosteric acid at equal concentrations (1 mg/mL) on sea urchin egg fertilization.

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Figure 9 is a bar graph plots the fertilization of sea urchin eggs by various concentrations of 4 t-pentyl phenyl chlorosulfact (4-PPCS)

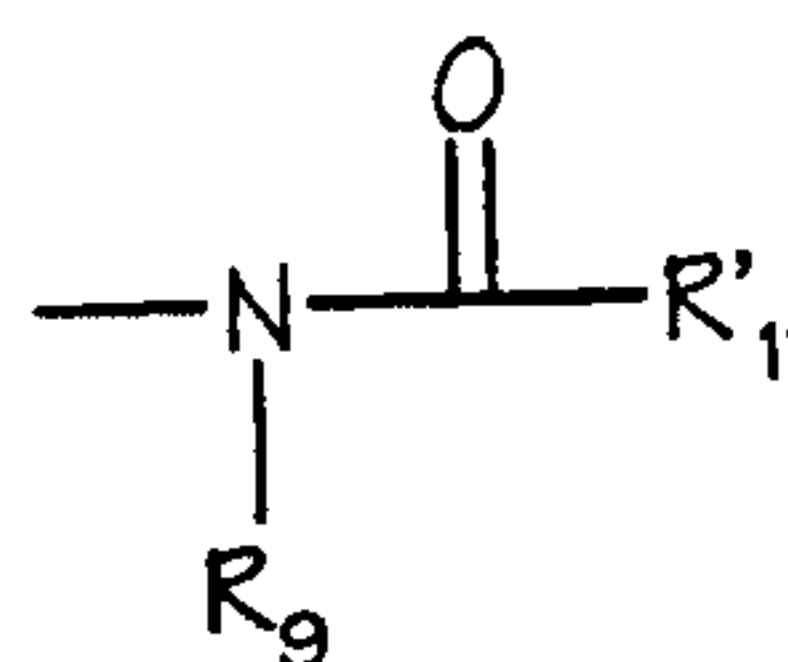
Detailed Description of the Invention

5

Definitions

For convenience, certain terms employed in the specification, examples, and appended claims are described below.

The term "acylamino" is art-recognized and refers to a moiety that can be represented
10 by the general formula:



wherein R_9 is as defined above, and R'_{11} represents a hydrogen, an alkyl, an alkenyl or $-(\text{CH}_2)_m\text{-R}_8$, where m and R_8 are as defined above.

The terms "alkenyl" and "alkynyl" refer to unsaturated aliphatic groups analogous in
15 length and possible substitution to the alkyls described above, but that comprise a double or triple bond, respectively.

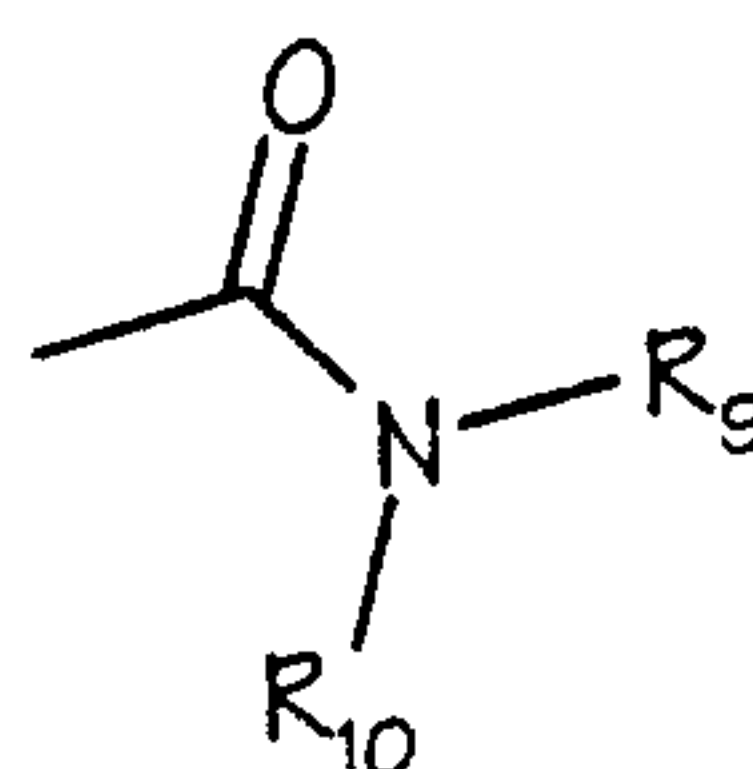
The terms "alkoxyl" or "alkoxy" as used herein refers to an alkyl group, as defined above, having an oxygen radical attached thereto. Representative alkoxyl groups include methoxy, ethoxy, propyloxy, tert-butoxy and the like. An "ether" is two hydrocarbons
20 covalently linked by an oxygen. Accordingly, the substituent of an alkyl that renders that alkyl an ether is or resembles an alkoxyl, such as can be represented by one of -O-alkyl, -O-alkenyl, -O-alkynyl, -O- $(\text{CH}_2)_m\text{-R}_8$, where m and R_8 are described above.

The term "alkyl" refers to the radical of saturated aliphatic groups, including straight-chain alkyl groups, branched-chain alkyl groups, cycloalkyl (alicyclic) groups, alkyl
25 substituted cycloalkyl groups, and cycloalkyl substituted alkyl groups. In preferred embodiments, a straight chain or branched chain alkyl has 30 or fewer carbon atoms in its backbone (e.g., $\text{C}_1\text{-C}_{30}$ for straight chain, $\text{C}_3\text{-C}_{30}$ for branched chain), and more preferably 20 or fewer. Likewise, preferred cycloalkyls have from 3-10 carbon atoms in their ring structure, and more preferably have 5, 6 or 7 carbons in the ring structure.

Moreover, the term "alkyl" (or "lower alkyl") as used throughout the specification and claims is intended to include both "unsubstituted alkyls" and "substituted alkyls", the latter of which refers to alkyl moieties having substituents replacing a hydrogen on one or more carbons of the hydrocarbon backbone. Such substituents can include, for example, a halogen, a hydroxyl, a carbonyl (such as a carboxyl, an ester, a formyl, or a ketone), a thiocarbonyl (such as a thioester, a thioacetate, or a thioformate), an alkoxyl, a phosphoryl, a phosphonate, a phosphinate, an amino, an amido, an amidine, an imine, a cyano, a nitro, an azido, a sulfhydryl, an alkylthio, a sulfate, a sulfonate, a sulfamoyl, a sulfonamido, a sulfonyl, a heterocyclyl, an aralkyl, or an aromatic or heteroaromatic moiety. It will be understood by those skilled in the art that the moieties substituted on the hydrocarbon chain can themselves be substituted, if appropriate. For instance, the substituents of a substituted alkyl may include substituted and unsubstituted forms of amino, azido, imino, amido, phosphoryl (including phosphonate and phosphinate), sulfonyl (including sulfate, sulfonamido, sulfamoyl and sulfonate), and silyl groups, as well as ethers, alkylthios, carbonyls (including ketones, aldehydes, carboxylates, and esters), $-CF_3$, $-CN$ and the like. Exemplary substituted alkyls are described below. Cycloalkyls can be further substituted with alkyls, alkenyls, alkoxys, alkylthios, aminoalkyls, carbonyl-substituted alkyls, $-CF_3$, $-CN$, and the like.

The term "alkylthio" refers to an alkyl group, as defined above, having a sulfur radical attached thereto. In preferred embodiments, the "alkylthio" moiety is represented by one of -S-alkyl, -S-alkenyl, -S-alkynyl, and $-S-(CH_2)_m-R_8$, wherein m and R_8 are defined above. Representative alkylthio groups include methylthio, ethylthio, and the like.

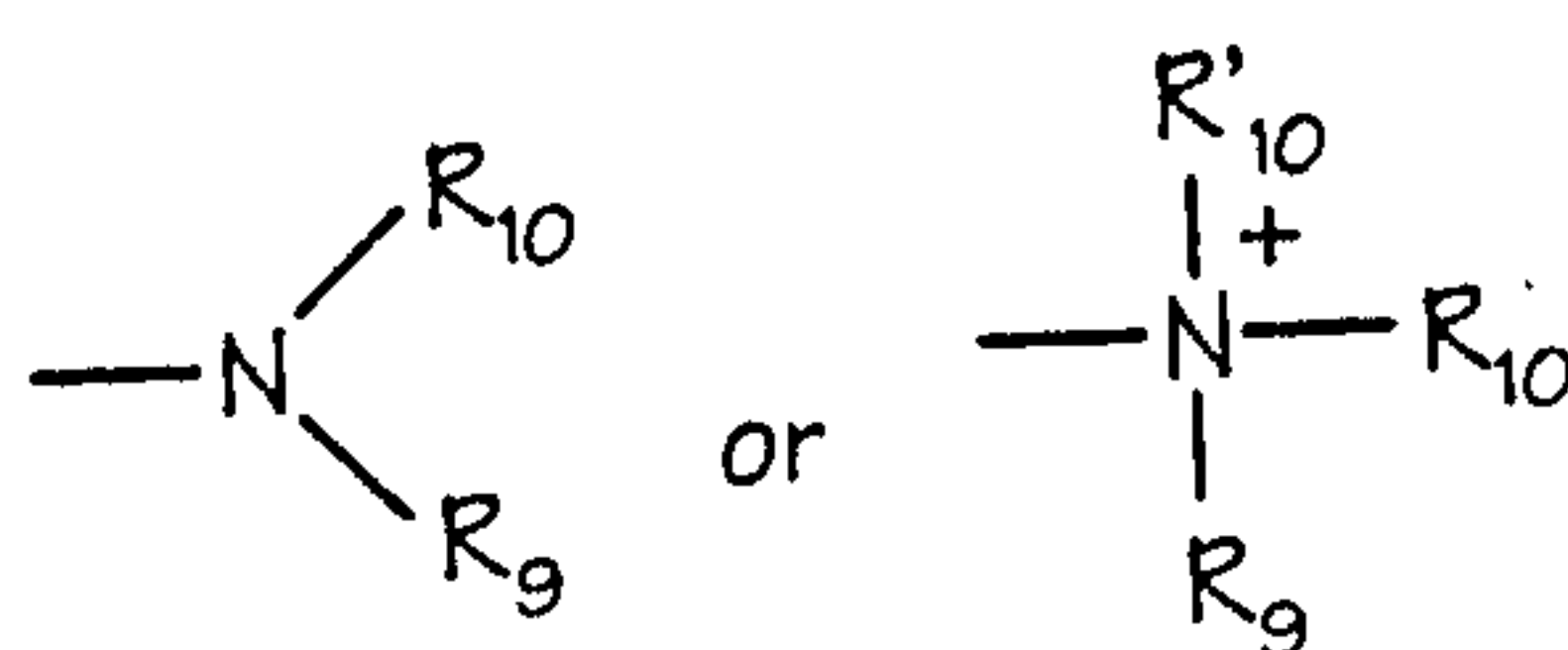
The term "amido" is art recognized as an amino-substituted carbonyl and includes a moiety that can be represented by the general formula:



wherein R_9 , R_{10} are as defined above. Preferred embodiments of the amide will not include imides which may be unstable.

The terms "amine" and "amino" are art recognized and refer to both unsubstituted and substituted amines, e.g., a moiety that can be represented by the general formula:

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wherein R_9 , R_{10} and R'_{10} each independently represent a hydrogen, an alkyl, an alkenyl, $-(CH_2)_m-R_8$, or R_9 and R_{10} taken together with the N atom to which they are attached complete a heterocycle having from 4 to 8 atoms in the ring structure; R_8 represents an aryl, a cycloalkyl, a cycloalkenyl, a heterocycle or a polycycle; and m is zero or an integer in the range of 1 to 8. In preferred embodiments, only one of R_9 or R_{10} can be a carbonyl, e.g., R_9 , R_{10} and the nitrogen together do not form an imide. In even more preferred embodiments, R_9 and R_{10} (and optionally R'_{10}) each independently represent a hydrogen, an alkyl, an alkenyl, or $-(CH_2)_m-R_8$. Thus, the term "alkylamine" as used herein means an amine group, as defined above, having a substituted or unsubstituted alkyl attached thereto, i.e., at least one of R_9 and R_{10} is an alkyl group.

An "aprotic solvent" means a non-nucleophilic solvent having a boiling point range above ambient temperature, preferably from about 25°C to about 190°C, more preferably from about 80°C to about 160°C, most preferably from about 80°C to 150°C, at atmospheric pressure. Examples of such solvents are acetonitrile, toluene, DMF, diglyme, THF or DMSO.

The term "aryl" as used herein includes 5-, 6- and 7-membered single-ring aromatic groups that may include from zero to four heteroatoms, for example, benzene, pyrrole, furan, thiophene, imidazole, oxazole, thiazole, triazole, pyrazole, pyridine, pyrazine, pyridazine and pyrimidine, and the like. Those aryl groups having heteroatoms in the ring structure may also be referred to as "aryl heterocycles" or "heteroaromatics". The aromatic ring can be substituted at one or more ring positions with such substituents as described above, for example, halogen, azide, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, amino, nitro, sulfhydryl, imino, amido, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, sulfonamido, ketone, aldehyde, ester, heterocyclyl, aromatic or heteroaromatic moieties, $-CF_3$, $-CN$, or the like. The term "aryl" also includes polycyclic ring systems having two or more rings in which two or more carbons are common to two adjoining rings (the rings are "fused") wherein at least one of the rings is aromatic, e.g., the other rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls and/or heterocyclyls.

The term "arylalkyl", as used herein, refers to an alkyl group substituted with an aryl group (e.g., an aromatic or heteroaromatic group).

"Biological activity" or "bioactivity" or "activity" or "biological function", which are used interchangeably, for the purposes herein means an effector or antigenic function that is directly or indirectly performed by a compound of the invention or a fragment thereof.

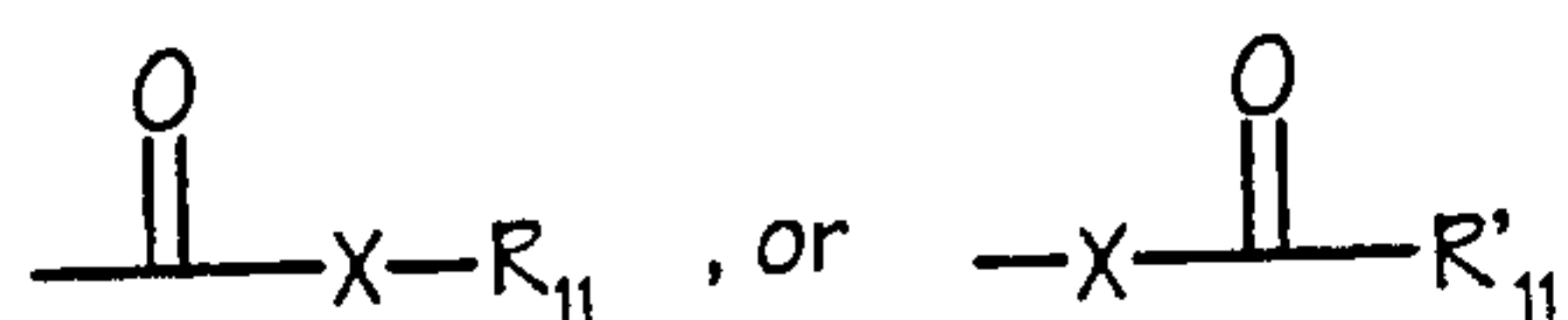
The term "bioavailable" is meant to refer to an appropriate location or orientation of a compound for performance of the compounds' bioactivity.

"Biofilm" refers to an accumulation of organisms on a surface. A mature biofilm can comprise a colony of microorganisms resident upon a surface surrounded by an exopolysaccharide.

"Biofilm resistant" or "antifouling" refers to inhibition or decrease in the amount of biofouling organisms that attach and/or grow.

The term "carbocycle", as used herein, refers to an aromatic or non-aromatic ring in which each atom of the ring is carbon.

The term "carbonyl" is art recognized and includes such moieties as can be represented by the general formula:



15

wherein X is a bond or represents an oxygen or a sulfur, and R_{11} represents a hydrogen, an alkyl, an alkenyl, $-(CH_2)_m-R_8$ or a pharmaceutically acceptable salt, R'_{11} represents a hydrogen, an alkyl, an alkenyl or $-(CH_2)_m-R_8$, where m and R_8 are as defined above. Where X is an oxygen and R_{11} or R'_{11} is not hydrogen, the formula represents an "ester". Where X is an oxygen, and R_{11} is as defined above, the moiety is referred to herein as a carboxyl group, and particularly when R_{11} is a hydrogen, the formula represents a "carboxylic acid". Where X is an oxygen, and R'_{11} is hydrogen, the formula represents a "formate". In general, where the oxygen atom of the above formula is replaced by sulfur, the formula represents a "thiolcarbonyl" group. Where X is a sulfur and R_{11} or R'_{11} is not hydrogen, the formula represents a "thiolester." Where X is a sulfur and R_{11} is hydrogen, the formula represents a "thiolcarboxylic acid." Where X is a sulfur and R'_{11} is hydrogen, the formula represents a "thiolformate." On the other hand, where X is a bond, and R_{11} is not hydrogen, the above formula represents a "ketone" group. Where X is a bond, and R_{11} is hydrogen, the above formula represents an "aldehyde" group.

The phrase "electron-withdrawing group" is recognized in the art, and denotes the tendency of a substituent to attract valence electrons from neighboring atoms, i.e., the substituent is electronegative with respect to neighboring atoms. A quantification of the level of electron-withdrawing capability is given by the Hammett sigma (σ) constant. This well known constant is described in many references, for instance, J. March, Advanced Organic Chemistry, McGraw Hill Book Company, New York, (1977 edition) pp. 251-259. The Hammett constant values are generally negative for electron donating groups (σ [P] = - 0.66 for NH_2) and positive for electron withdrawing groups (σ [P] = 0.78 for a nitro group), σ [P] indicating para substitution. Exemplary electron-withdrawing groups include nitro, ketone, aldehyde, sulfonyl, trifluoromethyl, -CN, chloride, and the like. Exemplary electron-donating groups include amino, methoxy, and the like.

The term "half-life" refers to the amount of time required for half of a compound to be eliminated or degraded by natural processes

The term "heteroatom" as used herein means an atom of any element other than carbon or hydrogen. Preferred heteroatoms are nitrogen, oxygen, sulfur and phosphorous.

The terms "heterocyclyl" or "heterocyclic group" refer to 3- to 10-membered ring structures, more preferably 3- to 7-membered rings, whose ring structures include one to four heteroatoms. Heterocycles can also be polycycles. Heterocyclyl groups include, for example, thiophene, thianthrene, furan, pyran, isobenzofuran, chromene, xanthene, phenoxathiin, pyrrole, imidazole, pyrazole, isothiazole, isoxazole, pyridine, pyrazine, pyrimidine, pyridazine, indolizine, isoindole, indole, indazole, purine, quinolizine, isoquinoline, quinoline, phthalazine, naphthyridine, quinoxaline, quinazoline, cinnoline, pteridine, carbazole, carboline, phenanthridine, acridine, perimidine, phenanthroline, phenazine, phenarsazine, phenothiazine, furazan, phenoxazine, pyrrolidine, oxolane, thiolane, oxazole, piperidine, piperazine, morpholine, lactones, lactams such as azetidinones and pyrrolidinones, sultams, sultones, and the like. The heterocyclic ring can be substituted at one or more positions with such substituents as described above, as for example, halogen, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, amino, nitro, sulfhydryl, imino, amido, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, ketone, aldehyde, ester, a heterocyclyl, an aromatic or heteroaromatic moiety, -CF₃, -CN, or the like.

Unless the number of carbons is otherwise specified, "lower alkyl" as used herein means an alkyl group, as defined above, but having from one to ten carbons, more preferably from one to six carbon atoms in its backbone structure. Likewise, "lower alkenyl" and "lower

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alkynyl" have similar chain lengths. Preferred alkyl groups are lower alkyls. In preferred embodiments, a substituent designated herein as alkyl is a lower alkyl.

The terms "infectious microorganisms" or "infectious agents" as used herein refers to disease causing or contributing bacteria (including gram-negative and gram-positive organisms, such as *Staphylococci* spp. (e.g. *Staphylococcus aureus*, *Staphylococcus epidermis*), *Enterococcus* sp. (*E. faecalis*), *Pseudomonas* sp. (*P. aeruginosa*), *Escherichia* sp. (*E. coli*), *Proteus* sp. (*P. mirabilis*)), fungi (including *Candida albicans*), viruses (including HIV, HCV, CMV, HBV) and protists (e.g. *Spirochaete* spp., *Treponema* spp.).

As used herein, the term "nitro" means -NO₂; the term "halogen" designates -F, -Cl, -Br or -I; the term "sulfhydryl" means -SH; the term "hydroxyl" means -OH; and the term "sulfonyl" means -SO₂-.

The terms *ortho*, *meta* and *para* apply to 1,2-, 1,3- and 1,4-disubstituted benzenes, respectively. For example, the names 1,2-dimethylbenzene and *ortho*-dimethylbenzene are synonymous.

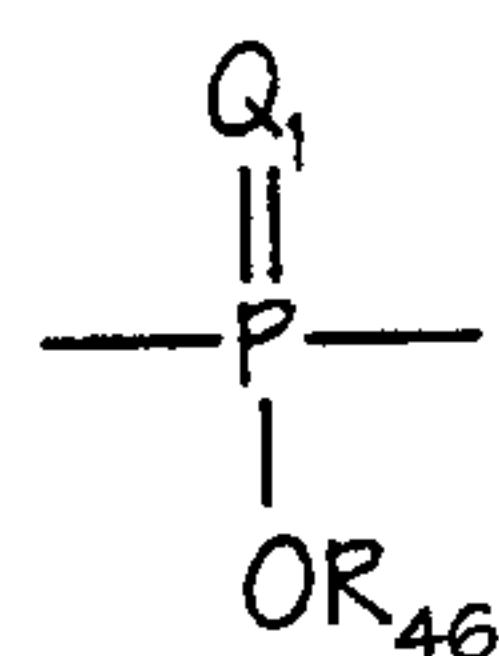
A "pharmaceutically effective amount" refers to an appropriate amount to obtain a therapeutic effect. Toxicity and therapeutic efficacy of compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining The Ld₅₀ (The Dose Lethal To 50% Of The Population) And The Ed₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD₅₀/ED₅₀. Compounds which exhibit large therapeutic indices are preferred. The effective amount may vary within a range depending upon the dosage form employed and the route of administration utilized. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ (i.e., the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture.

"Pharmaceutical effective carrier" refers to a physiologically acceptable carriers or excipient. Thus, the compounds and their physiologically acceptable salts and solvates may be formulated for administration by, for example, injection, inhalation or insufflation (either through the mouth or the nose) or oral, buccal, parenteral or rectal administration. For therapy, the compounds of the invention can be formulated for a variety of loads of administration, including systemic and topical or localized administration. Techniques and formulations generally may be found in Remington's Pharmaceutical Sciences, Meade

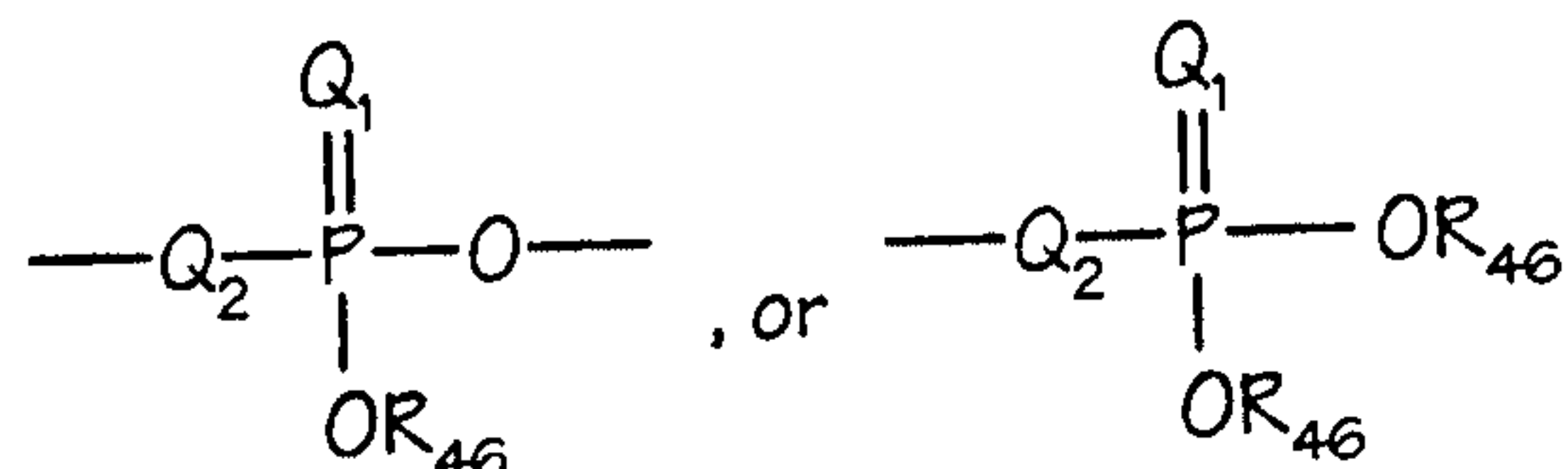
Publishing Co., Easton, PA. For systemic administration, injection is preferred, including intramuscular, intravenous, intraperitoneal, and subcutaneous. For injection, the compounds of the invention can be formulated in liquid solutions, preferably in physiologically compatible buffers such as Hank's solution or Ringer's solution. In addition, the compounds may be formulated in solid form and redissolved or suspended immediately prior to use. 5 Lyophilized forms are also included. For oral administration, the pharmaceutical compositions may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g., pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g., 10 magnesium stearate, talc or silica); disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulphate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may 15 be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., ationd oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring and sweetening agents as appropriate. Preparations for oral administration may be suitably formulated to give controlled release of the active compound. For buccal administration the compositions may take the form of tablets or lozenges formulated in conventional manner. For administration by inhalation, the compounds for use according to 25 the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebuliser, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of e.g., gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound 30 and a suitable powder base such as lactose or starch. The compounds may be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as

suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.. The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, e.g., containing conventional suppository bases such as cocoa butter or other glycerides. In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt. Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration bile salts and fusidic acid derivatives. in addition, detergents may be used to facilitate permeation. Transmucosal administration may be through nasal sprays or using suppositories. For topical administration, the oligomers of the invention are formulated into ointments, salves, gels, or creams as generally known in the art. A wash solution can be used locally to treat an injury or inflammation to accelerate healing.

A "phosphoryl" can in general be represented by the formula:



wherein Q_1 represented S or O, and R_{46} represents hydrogen, a lower alkyl or an aryl. When used to substitute, e.g., an alkyl, the phosphoryl group of the phosphorylalkyl can be represented by the general formula:



wherein Q₁ represented S or O, and each R₄₆ independently represents hydrogen, a lower alkyl or an aryl, Q₂ represents O, S or N. When Q₁ is an S, the phosphoryl moiety is a "phosphorothioate".

5 A "polar, aprotic solvent" means a polar solvent as defined above which has no available hydrogens to exchange with the compounds of this invention during reaction, for example DMF, acetonitrile, diglyme, DMSO, or THF.

A "polar solvent" means a solvent which has a dielectric constant (ϵ) of 2.9 or greater, such as DMF, THF, ethylene glycol dimethyl ether (DME), DMSO, acetone, acetonitrile, methanol, ethanol, isopropanol, n-propanol, t-butanol or 2-methoxyethyl ether. Preferred
10 solvents are DMF, DME, NMP, and acetonitrile.

The terms "polycyclyl" or "polycyclic group" refer to two or more rings (e.g., cycloalkyls, cycloalkenyls, cycloalkynyls, aryls and/or heterocyclyls) in which two or more carbons are common to two adjoining rings, e.g., the rings are "fused rings". Rings that are joined through non-adjacent atoms are termed "bridged" rings. Each of the rings of the
15 polycycle can be substituted with such substituents as described above, as for example, halogen, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, amino, nitro, sulfhydryl, imino, amido, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, ketone, aldehyde, ester, a heterocyclyl, an aromatic or heteroaromatic moiety, -CF₃, -CN, or the like.

The phrase "protecting group" as used herein means temporary modifications of a
20 potentially reactive functional group which protect it from undesired chemical transformations. Examples of such protecting groups include esters of carboxylic acids, silyl ethers of alcohols, and acetals and ketals of aldehydes and ketones, respectively. The field of protecting group chemistry has been reviewed (Greene, T.W.; Wuts, P.G.M. *Protective Groups in Organic Synthesis*, 2nd ed.; Wiley: New York, 1991).

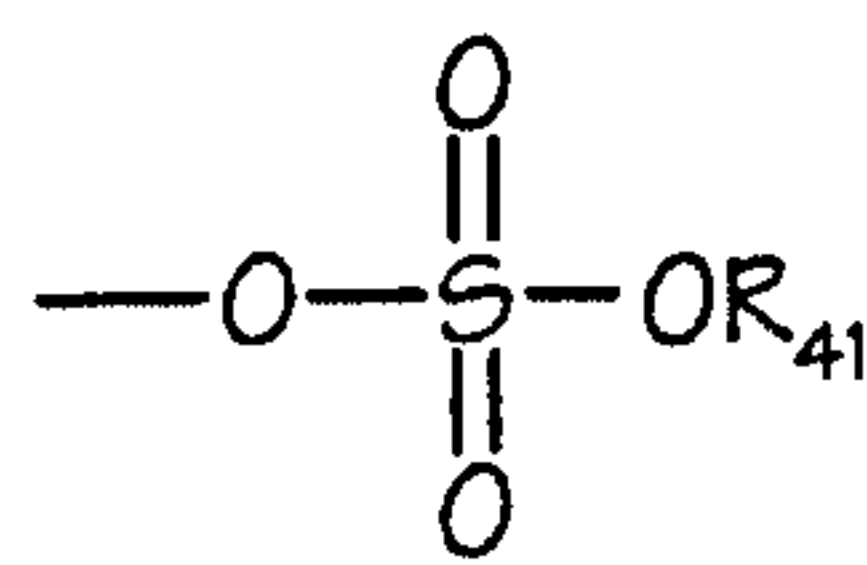
25 "Receptor mediated disease or condition" as used herein refers to any of a variety of diseases or conditions that involve receptor mediated pathways. Particularly preferred diseases or conditions are those involving sulfate receptor mediated pathways. Examples include: infection (by virus, bacteria, fungi, protists, prions), cancer, fertilization, and blood clotting associated diseases.

30 As used herein, the term "substituted" is contemplated to include all permissible substituents of organic compounds. In a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and

nonaromatic substituents of organic compounds. Illustrative substituents include, for example, those described hereinabove. The permissible substituents can be one or more and the same or different for appropriate organic compounds. For purposes of this invention, the heteroatoms such as nitrogen may have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valencies of the heteroatoms. This invention is not intended to be limited in any manner by the permissible substituents of organic compounds.

It will be understood that "substitution" or "substituted with" includes the implicit proviso that such substitution is in accordance with permitted valence of the substituted atom and the substituent, and that the substitution results in a stable compound, e.g., which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, etc.

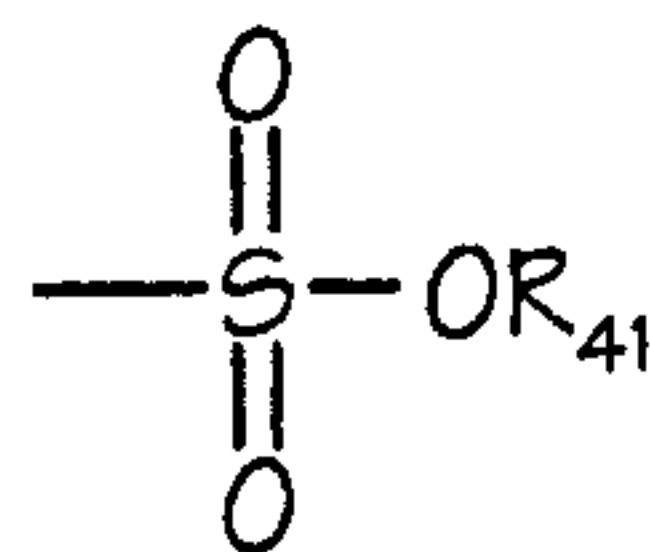
The term "sulfate" is art recognized and includes a moiety that can be represented by the general formula:



in which R_{41} is as defined above.

A "sulfate binding moiety" refers to a moiety that is capable of binding or otherwise associating with a sulfate or sulfonate group.

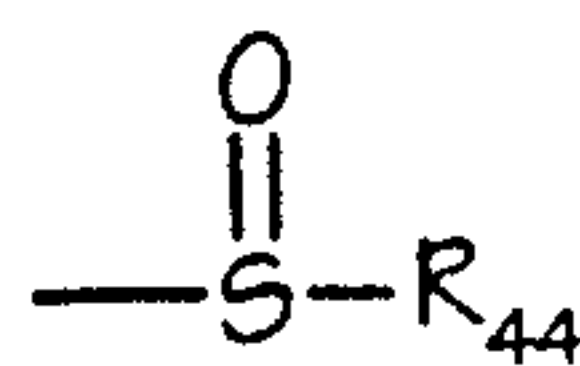
The term "sulfonate" is art recognized and includes a moiety that can be represented by the general formula:



20

in which R_{41} is an electron pair, hydrogen, alkyl, cycloalkyl, or aryl.

The terms "sulfoxido" or "sulfinyl", as used herein, refers to a moiety that can be represented by the general formula:



in which R_{44} is selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, aralkyl, or aryl.

Analogous substitutions can be made to alkenyl and alkynyl groups to produce, for example, aminoalkenyls, aminoalkynyls, amidoalkenyls, amidoalkynyls, iminoalkenyls, iminoalkynyls, thioalkenyls, thioalkynyls, carbonyl-substituted alkenyls or alkynyls.

“Sustained release” or “controlled release” refers to a relatively constant or prolonged
5 release of a compound of the invention from a surface. This can be accomplished through the use of diffusional systems, including reservoir devices in which a core of a compound of the invention is surrounded by a porous membrane or layer, and also matrix devices in which the compound is distributed throughout an inert matrix. Materials which may be used to form reservoirs or matrices include silicones, acrylates, methacrylates, vinyl compounds such as
10 polyvinyl chloride, olefins such as polyethylene or polypropylene, fluoropolymers such as polytetrafluorethylene, and polyesters such as terephthalates. The diffusional systems may be molded into a film or other layer material which is then placed in adherent contact with the structure intended for underwater use. Alternatively, the compounds of the invention may be mixed with a resin, e.g., polyvinyl chloride and then molded into a formed article, which
15 integrally incorporates the compound to form a structure having a porous matrix which allows diffusion of the compound, or a functional portion thereof, into the surrounding environment. Microencapsulation techniques can also be used to maintain a sustained focal release of a compound of the invention. Microencapsulation may also be used for providing improved stability. The encapsulated product can take the form of for example, spheres, aggregates of
20 core material embedded in a continuum of wall material, or capillary designs. The core material of a microcapsule containing a sulfate ester AF agent may be in the form of a liquid droplet, an emulsion, a suspension of solids, a solid particle, or a crystal. The skilled artisan will be aware of numerous materials suitable for use as microcapsule coating materials, including, but not limited to, organic polymers, hydrocolloids, lipids, fats, carbohydrates,
25 waxes, metals, and inorganic oxides. Silicone polymers are the most preferred microcapsule coating material for treatment of surfaces. Microencapsulation techniques are well known in the art and are described in the Encyclopedia of Polymer Science and Engineering, Vol. 9, pp. 724 et seq. (1989) hereby incorporated by reference.

The term “treating” as used herein is intended to encompass curing as well as
30 ameliorating at least one symptom of the condition or disease.

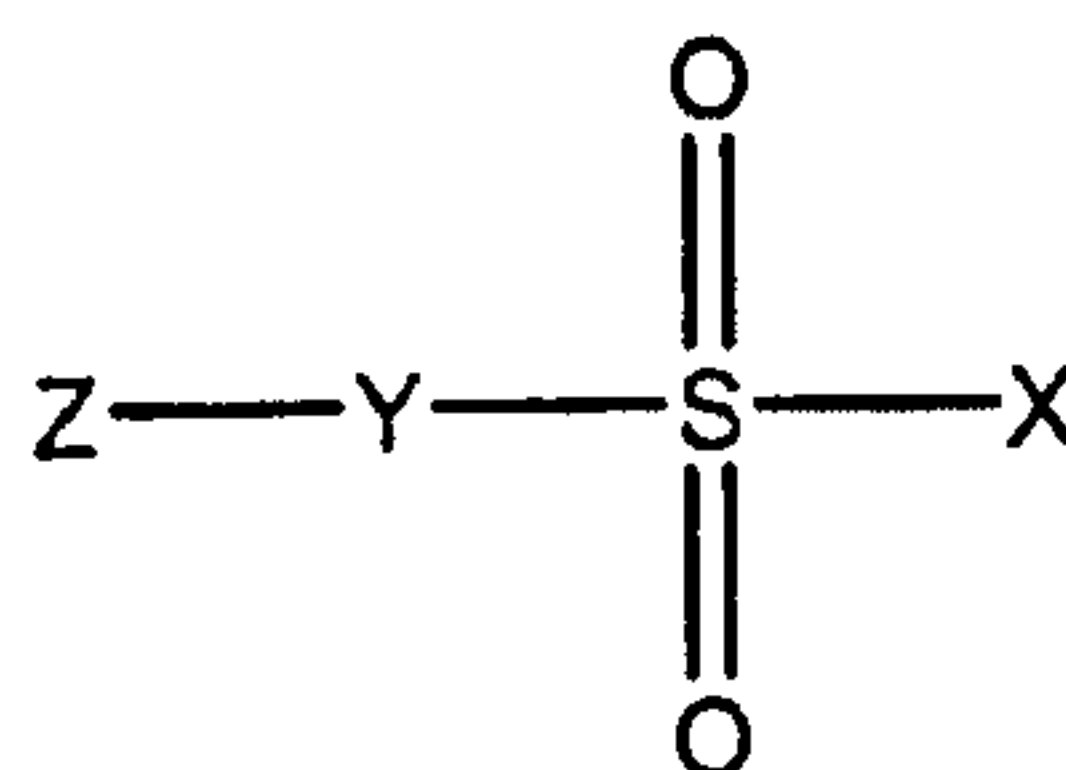
The abbreviations Me, Et, Ph, Tf, Nf, Ts, Ms, and dba represent methyl, ethyl, phenyl, trifluoromethanesulfonyl, nonafluorobutanesulfonyl, *p*-toluenesulfonyl, methanesulfonyl, and dibenzylideneacetone, respectively. A more comprehensive list of the abbreviations utilized by organic chemists of ordinary skill in the art appears in the first issue of each volume of the

Journal of Organic Chemistry; this list is typically presented in a table entitled Standard List of Abbreviations. The abbreviations contained in said list, and all abbreviations utilized by organic chemists of ordinary skill in the art are hereby incorporated by reference.

For purposes of this invention, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, Handbook of Chemistry and Physics, 67th Ed., 1986-87, inside cover. Also for purposes of this invention, the term "hydrocarbon" is contemplated to include all permissible compounds having at least one hydrogen and one carbon atom. In a broad aspect, the permissible hydrocarbons include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and nonaromatic organic compounds which can be substituted or unsubstituted.

Pharmaceutical Compositions of the Invention

In certain embodiments, the pharmaceutical compositions of the present invention comprise a pharmaceutical carrier and an effective amount of a compound represented by general structure 1:



1

wherein

X represents -OH, -O(aryl), -O(acyl), -O(sulfonyl), -CN, F, Cl, or Br;

Y represents O, S, Se, or NR;

Z represents optionally substituted alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, or $-(\text{CH}_2)_m\text{-R}_{80}$;

R represents independently for each occurrence hydrogen, alkyl, heteroalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, or $-(\text{CH}_2)_m\text{-R}_{80}$;

R_{80} represents independently for each occurrence aryl, cycloalkyl, cycloalkenyl, heterocyclyl, or polycyclyl; and

m is an integer in the range 0 to 8 inclusive. Other preferred compounds are salts of the compounds represented by the general structure 1.

Particularly stable compounds are represented by general structure 1 and the attendant definitions, wherein X represents -OH, F, Cl, or Br.

5 In other preferred embodiments, the compositions of the present invention comprise a compound represented by general structure 1 and the attendant definitions, wherein Y represents O.

In certain embodiments, the compositions of the present invention comprise a compound represented by general structure 1 and the attendant definitions, wherein Z
10 represents optionally substituted alkyl, aryl, or $-(CH_2)_m-R_{80}$.

In certain embodiments, the compositions of the present invention comprise a compound represented by general structure 1 and the attendant definitions, wherein Z represents optionally substituted alkylphenyl, heteroalkylphenyl, arylphenyl, or heteroarylphenyl.

15 In certain embodiments, the compositions of the present invention comprise a compound represented by general structure 1 and the attendant definitions, wherein Z represents 4-(2-methylpropyl)phenyl, 4-(1,1-dimethylethyl)phenyl, 4-(1,1-dimethylpropyl)phenyl, 4-pentylphenyl, 4-(1-methyl-1-phenylethyl)phenyl, or 4-(1-methylheptyl)phenyl.

20 In certain embodiments, the compositions of the present invention is a compound represented by general structure 1 and the attendant definitions, wherein R represents H or alkyl.

In certain embodiments, the compositions of the present invention comprise a compound represented by general structure 1 and the attendant definitions, wherein X
25 represents -OH, F, Cl, or Br; and Y represents O.

In certain embodiments, the compositions of the present invention comprise an a compound represented by general structure 1 and the attendant definitions, wherein X represents -OH or Cl; and Y represents O.

30 In certain embodiments, the compositions of the present invention comprise a compound represented by general structure 1 and the attendant definitions, wherein X represents -OH, F, Cl, or Br; and Z represents optionally substituted alkyl, aryl, or $-(CH_2)_m-R_{80}$.

In certain embodiments, the compositions of the present invention comprise an a compound represented by general structure 1 and the attendant definitions, wherein X represents -OH or Cl; and Z represents optionally substituted alkyl, aryl, or $-(CH_2)_m-R_{80}$.

5 In certain embodiments, the compositions of the present invention comprise an a compound represented by general structure 1 and the attendant definitions, wherein X represents -OH, F, Cl, or Br; and Z represents optionally substituted alkylphenyl, heteroalkylphenyl, arylphenyl, or heteroarylphenyl.

10 In certain embodiments, the compositions of the present invention comprise an a compound represented by general structure 1 and the attendant definitions, wherein X represents -OH or Cl; and Z represents optionally substituted alkylphenyl, heteroalkylphenyl, arylphenyl, or heteroarylphenyl.

15 In certain embodiments, the compositions of the present invention comprise an a compound represented by general structure 1 and the attendant definitions, wherein X represents -OH, F, Cl, or Br; and Z represents 4-(2-methylpropyl)phenyl, 4-(1,1-dimethylethyl)phenyl, 4-(1,1-dimethylpropyl)phenyl, 4-pentylphenyl, 4-(1-methyl-1-phenylethyl)phenyl, or 4-(1-methylheptyl)phenyl.

20 In certain embodiments, the compositions of the present invention comprise an a compound represented by general structure 1 and the attendant definitions, wherein X represents -OH or Cl; and Z represents 4-(2-methylpropyl)phenyl, 4-(1,1-dimethylethyl)phenyl, 4-(1,1-dimethylpropyl)phenyl, 4-pentylphenyl, 4-(1-methyl-1-phenylethyl)phenyl, or 4-(1-methylheptyl)phenyl.

In certain embodiments, the compositions of the present invention comprise an a compound represented by general structure 1 and the attendant definitions, wherein Y represents O; and Z represents optionally substituted alkyl, aryl, or $-(CH_2)_m-R_{80}$.

25 In certain embodiments, the compositions of the present invention comprise an a compound represented by general structure 1 and the attendant definitions, wherein Y represents O; and Z represents optionally substituted alkylphenyl, heteroalkylphenyl, arylphenyl, or heteroarylphenyl.

30 In certain embodiments, the compositions of the present invention comprise a compound represented by general structure 1 and the attendant definitions, wherein Y represents O; and Z represents 4-(2-methylpropyl)phenyl, 4-(1,1-dimethylethyl)phenyl, 4-

(1,1-dimethylpropyl)phenyl, 4-pentylphenyl, 4-(1-methyl-1-phenylethyl)phenyl, or 4-(1-methylheptyl)phenyl.

In certain embodiments, the compositions of the present invention comprise an a compound represented by general structure 1 and the attendant definitions, wherein X
5 represents -OH, F, Cl, or Br; Y represents O; and Z represents optionally substituted alkyl, aryl, or $-(CH_2)_m-R_{80}$.

In certain embodiments, the compositions of the present invention comprise an a compound represented by general structure 1 and the attendant definitions, wherein X
10 represents -OH or Cl; Y represents O; and Z represents optionally substituted alkyl, aryl, or $-(CH_2)_m-R_{80}$.

In certain embodiments, the compositions of the present invention comprise an a compound represented by general structure 1 and the attendant definitions, wherein X represents -OH, F, Cl, or Br; Y represents O; and Z represents optionally substituted alkylphenyl, heteroalkylphenyl, arylphenyl, or heteroarylphenyl.

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In certain embodiments, the compositions of the present invention comprise an a
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In certain embodiments, the compositions of the present invention comprise an a
25 compound represented by general structure 1 and the attendant definitions, wherein X represents -OH or Cl; Y represents O; and Z represents 4-(2-methylpropyl)phenyl, 4-(1,1-dimethylethyl)phenyl, 4-(1,1-dimethylpropyl)phenyl, 4-pentylphenyl, 4-(1-methyl-1-phenylethyl)phenyl, or 4-(1-methylheptyl)phenyl.

One of skill in the art will recognize that the composition of the invention can be
30 varied as required to optimize the overall chemical properties of the particular compound for specific uses, while retaining the AF activity. For example, the length of an alkyl chain can be extended or shortened to control the rate of dissolution of the compound from a structure

or a coating. Alternatively, additional functional groups can be added to the alkyl chain to further vary the chemical nature of the molecule.

Bioactivity of Compounds of the Invention

Compounds of the invention have been shown to provide one or more plant and
5 animal lectin-like activities. Lectins bind to cell surface proteoglycans, which function in the attachment of pathogens such as viruses and bacteria. Accordingly the lectin-like activities of compounds of the invention are useful in treating and preventing infections and other receptor-mediated diseases and conditions.

The ability of the compounds to bind to certain cell surface sites is useful for
10 agonizing or antagonizing certain cell surface interactions which are otherwise affected by animal or plant lectin proteins. The extracellular polysaccharides produced by fouling organisms are often highly sulfated, and these sulfate esters play an important role in polymerization (e.g. glue/gel formation) (Wetherbee et al., (1998) J. Phycol. 34: 9-15; Vreeland et al., (1998) J. Phycol. 34: 1-8).

15 As shown in the following examples, compounds of the invention inhibit these glue/gel interactions. The compounds of the invention therefore have numerous practical applications. Based on the exhibited broad based activity, the compounds of the invention obviate the need to determine the exact nature of the host receptor responsible for the disease process. Furthermore, the sulfated compounds of the invention provide a general class of
20 suitable lectin-like therapeutics which obviate the need to identify individual receptor-specific lectin species.

Furthermore, lectins are involved in a broad variety of processes and, accordingly, the lectin-like activities of zosteric acid and related sulfated compounds of the invention provide a correspondingly wide variety applications for the invention. For example, the involvement of
25 an animal lectins in the activation of host complement indicates that zosteric acid related compounds of the invention can be used to treat and prevent undesirable inflammatory diseases and conditions. Still further, zosteric acid and the related compounds of the invention have several unique properties useful in specific applications including an anti-clotting activity similar to that possessed by heparin.

30 Another important property of the compounds of the invention is their ability to affect the agglutination of bacterial and mammalian cells. The affects of the compounds on cell agglutination may involve the blocking of certain cell surface receptors and the activation of others - such as those involved in the attachment to extracellular surfaces and which thereby

mediate fouling. Thus, the compounds of the invention possess many of the activities of naturally-occurring proteins and glycoproteins which bind to sites on the surface of a cell and thereby affect cell/cell interactions. The cell hemagglutinin-like agglutination activities of compounds of the invention can be useful, for example, to promote wound healing.

5 As shown in the following examples, compounds of the invention have been found to possess certain anti-coagulation activities, such as possessed by the sulfated mucopolysaccharide heparin. Heparin is believed to inhibit the clotting cascade by binding to, and thereby activating, antithrombin III, a plasma protein that inactivates thrombin by forming an irreversible complex with it. This complex is similar to the acyl-enzyme complex
10 formed between trypsin and pancreatic trypsin inhibitor. Heparin is produced by mast cells near the walls of blood vessels and acts as an anticoagulant by increasing the rate of formation of the irreversible complex between thrombin and antithrombin III. Antithrombin III also inhibits other proteolytic components of the clotting cascade, such as Factors IX_a, X_a, and XI_a.

Although the activity for the low molecular weight zosteric acid molecule was
15 considerably less than that observed for heparin. Heparin was effective at preventing clot formation at concentrations well below 0.1 mg/ml, while zosteric acid was effective only at concentrations exceeding 10 mg/ml. The effectiveness of heparin-like anticoagulants is strongly linked to size, with high molecular weight molecules being more effective. Therefore certain of the higher molecular weight compounds of the invention should prove
20 more effective.

Heparin has been used extensively for prophylaxis and treatment of deep vein thrombosis. However, heparin has several limitations. For example, heparin, a relatively large molecule, has shown limited efficacy in inhibiting thrombin activity incorporated into a fibrin clot. In addition, heparin has a short intravenous half-life. Therefore, in certain applications,
25 the use of compounds of the invention can be advantageous in performing certain anti-clotting prophylaxis or therapies. The compounds of the invention can also be formulated as covalent derivatized for the treatment and prevention of clotting conditions. For example, a covalent antithrombin-heparin complex has proven to be more effective in reducing clot weight in vivo than thrombin and heparin combination therapy. Accordingly, the invention provides
30 methods and reagents for creating derivatized components of the fibrin clotting cascade, including antithrombin.

The compounds of the invention can potentially be orally delivered, whereas heparin must be delivered intravenously. In addition, the heparin-like property of the compounds of

the invention render them particularly suitable for incorporating into medical materials where an anti-coagulant effect is desired.

As shown in the following examples, compounds of the invention have also been found to inhibit fertilization, as a result, the compounds of the invention can be used as contraceptives. Contraceptives comprised of the instant claimed can be formulated as either routinely reapplied coatings, described above, foams, jellies and suppositories, or alternatively as semi-permanent coatings to be used on prophylactic articles of manufacture, (e.g., condoms or diaphragms). Alternatively, sulfate esters can be formulated as incorporations into such articles during manufacture.

The present invention is further illustrated by the following examples, which should not be construed as limiting in any way. The contents of all cited references including literature references, issued patents and published patent applications as cited throughout this patent application are hereby expressly incorporated by reference.

Examples

Example 1: Inhibition of Surface Attachment of Marine Bacteria by Alkyl Sulfates

Octyl sulfate is an alkyl sulfate surfactant with extensive industrial applications, and is manufactured by several large chemical companies. To investigate potential AF properties of the sulfate ester octyl sulfate, it was incorporated into an inert coating material that was then coated onto a surface to be exposed to conditions that support the formation of marine algal biofilms.

Materials and Methods

A 30%(w/v) solution of octyl sulfate in water (Stepan Chemical Co.) was evaporated to dryness under a stream of room temperature air, to recover pure octyl sulfate (Fig. 1). The dry octyl sulfate was incorporated into RTV-11 silicone polymer at a loading of 25% (wt/wt) (RTV-11 silicone, catalyst and primer obtained from General Electric). The mixture was applied to three glass slides previously primed with silicone primer, and allowed to cure to dryness. Three primed glass slides coated with pure RTV-11 served as agent-free controls. After complete drying, the absorption properties of each slide were measured using a Shimadzu UV-2101 spectrophotometer fitted with an integrating sphere. Slides were then placed in a tank of running raw seawater and allowed to incubate outdoors in natural sunlight

for 26 days. Water temperature was nominally 15 C. Spectrophotometric determination of biofilm accumulation was measured on each slide periodically. Relative algal biomass was calculated as the ratio of absorption at 680 nm, contributed by chlorophyll *a*, to that at 750 nm, a wavelength not absorbed by chlorophyll, to correct for differences in turbidity and scattering properties of the different slides.

Results

As shown in Figure 1, octyl sulfate incorporated into RTV-11 silicone, and then coated onto glass slides, significantly inhibited the formation of natural marine algal biofilms in natural seawater. After 26 days of incubation in running seawater, algal biofilm development on the octyl sulfate containing coatings was five fold less than that of controls lacking octyl sulfate, indicating that octyl sulfate possesses strong AF activity.

Studies were performed to evaluate the ability of the sulfate ester molecules octyl sulfate and methyl sulfate, to inhibit adhesion of the marine bacteriums *Oceanosprillum* and *Alteromonas atlantica* to glass surfaces.

Materials and Methods

Oceanosprillum adhesion test Each test consisted of a control set (with no sulfate esters) and sample sets containing the test molecules. The first test group consisted of a control sample set, a zosteric acid (5 mM) sample set, and an octyl sulfate (5 mM) sample set. The second test group consisted of a control sample set, a zosteric acid (5 mM) sample set, and a methyl sulfate (5 mM) sample set. Sample sets consisted of five 50 mL sterile centrifuge tubes, with each tube containing a glass microscope slide, 50 ml of artificial seawater (ASTM - American Society for testing and materials) with the dissolved sulfate ester, inoculated with an *Oceanosprillum* culture at 1×10^6 cells/mL. Sample sets were incubated at 23 C, with shaking so that the surface of the slides were horizontal. Over a 6-hour period, individual tubes were taken from the sample sets and tested for microbial adhesion.

Alteromonas atlantica adhesion tests. The tests consisted of a control sample set, a zosteric acid (5 mM) sample set, an octyl sulfate (5 mM) sample set, and a methyl sulfate (5 mM) set. A sample set consisted of six 60 mL sterile centrifuge tubes. Each tube contained a glass microscope slide and 50 mL of modified ASTM seawater (American Society for Testing

and Materials (1986) D1141-86, ASTM, Philadelphia, PA) with dissolved agent, inoculated with *Alteromonas atlantica* culture to an initial cell density of 1×10^6 cells/mL. The modified seawater consisted of normal ASTM seawater ingredients, however the carbon source glycerol, was only 1000th the normal strength, 0.1 L/L instead of 100 L/L, and was void of an amino acid source (casamino acids), in order to allow enough carbon for attachment, but not for significant cell growth.

Determination of bacterial adhesion. Samples were removed from the shaker and 1 mL of 50X acridine orange stain (0.5 g/L acridine powder in water) was added to the tube. The stain was allowed to react for 4 minutes. The slides were then removed and fitted with a long cover slip and immediately counted with an epifluorescent microscope fitted with a 100X (oil) objective lens on the under side of the slide. The size of the counting field was $10 \times 10 \mu\text{m}$. A total of 20 counts per slide were performed and averaged to yield the number of cells per μm^2 , which was in turn converted to cells per mm^2 . Error was assigned at 10% which is the standard accepted error for direct counting of bacterial cells.

Results

As shown in Figure 2, the presence of octyl sulfate or methyl sulfate in the medium significantly reduced bacterial adhesion to the glass slides when compared to controls in which no sulfate ester molecule was present. Methyl sulfate inhibited *Oceanosprillum* adhesion to an extent similar to the proven AF agent zosteric acid, with each compound promoting roughly a two fold reduction in bacterial attachment, relative to control. As shown in Figure 3, octyl sulfate inhibited *Oceanosprillum* adhesion to an even greater extent than zosteric acid.

25

As shown in Figure 4, the presence of dissolved zosteric acid, octyl sulfate, or methyl sulfate produced a significant reduction in the marine bacterium, *Alteromonas atlantica* adhesion relative the controls. The presence of methyl sulfate had the most dramatic effect upon adhesion, with adhesion remaining constant after 120 minutes at 150,000 cells/ mm^2 , while controls had greater than 700,000 cells/ mm^2 . Octyl sulfate also inhibited adhesion, demonstrating a slightly higher inhibitory activity than zosteric acid..

30

Example 2: Inhibition of Fungal Surface Attachment and Mycelial Development

To determine the effectiveness of sulfate esters at inhibiting fungal biofouling, the ability of zosteric acid to inhibit attachment of the fungus *Aureobasidium pullulans* to surfaces was examined.

5

Materials and Methods

Aureobasidium pullulans (ATCC 34261) was grown on potato-dextrose agar and harvested according to ASTM G-21-90 protocols (American Society for Testing and Materials (1986) D1141-86, ASTM, Philadelphia, PA). The resulting spore suspension was
10 used to inoculate liquid culture tubes containing 35 mL of growth medium (nutrient salts with 5 mM sucrose) and 15 mM zosteric acid. Zosteric acid-free medium was prepared as a control. A sterile microscope slide was added to each tube, the tubes were sealed and placed on a rotary shaker table at room temperature. One tube was harvested each day by removing the slide and counting the number of attached spores by direct microscopic counts, as
15 described above.

Results

Fungal spores were observed to grow in both the presence and absence of zosteric acid, as indicated by the clouding of all tubes after Day 1. However, as shown in Fig. 5, the
20 presence of zosteric acid prevented the attachment of the fungus to the glass slides. After 5 days incubation with *A. pullulans*, less than 20 germinated fungal colonies/mm² were observed on slides incubated in the additional presence of zosteric acid, compared to more than 600 germinated fungal colonies/mm² on control slides. Furthermore, fungal colonies in the media of zosteric acid free cultures were composed of multi-cellular (>20 cells) filaments,
25 indicative of mycelial growth. In contrast, colonies in the zosteric acid treated cultures were generally small and round, exhibiting no evidence of filamentous growth or mycelial development.

Example 3: Sulfate Esters Bind Cell Surfaces of Biofouling Organisms

30 To investigate the mechanism behind the AF activity of sulfate esters, polyclonal antibodies specific for the sulfate ester zosteric acid were generated (BAbCo, Berkeley, CA).

Preliminary testing of these antibodies for cross reactivity towards related compounds lacking the sulfate ester group (cinnamic acid, ferulic acid, coumaric acid) showed no cross reactivity, suggesting that the specific domain recognized by the antibodies probably includes the sulfate ester group. These antibodies were then used to investigate whether the sulfate ester AF agent
5 zosteric acid directly binds fouling organisms.

The marine bacterium *Shewanella putrefaciens* were grown in cultures containing zosteric acid and were subsequently examined for bound zosteric acid using immuno-gold staining with the antibody described above. Electron microscopic examination of immunoprobed *S. putrefaciens* detected zosteric acid molecules bound to the surface of the
10 bacteria. Furthermore, zosteric acid was observed to be present at high incidence at the sites of cell adhesion. In contrast to these agglutination sites, the majority of the cell surfaces as well as the continuous boundaries between daughter cells in dividing chains, showed no evidence of bound zosteric acid, as indicated by a lack of immuno-gold staining. These results indicate that sulfate esters bind to the surfaces of bacterial cells and suggest a possible
15 relationship between sulfate ester binding sites and the sites of bacterial agglutination.

Example 4: Zosteric Acid Promotes Bacterial Agglutination

To further investigate the role of sulfate esters in agglutination, the ability of sulfate esters to facilitate the agglutination of bacterial cells was investigated. Log-phase cultures
20 grown in the presence of zosteric acid were monitored spectrophotometrically (OD₆₀₀) for growth, and for agglutination in the presence of increasing amounts of zosteric acid.

Materials and Methods

Cell Surface Binding Assays. The marine bacterium *Shewanella putrefaciens* was
25 grown in marine broth in the presence of 16mM zosteric acid. Dense log phase cells were harvest after 5 hours growth, and preserved in 0.5 X Karnofsky's fixative (2% formaldehyde, 2.5% glutaraldehyde, 0.05 M sodium cacodylate, 0.25 M sucrose, pH 7.4) for 2 hours, and then transferred to a cacodylate buffer (0.05 M sodium cacodylate, pH 7.4) for storage. Cells were prepared for electron microscopic examination using immuno-gold staining techniques
30 (Harlow, E. and Laine, D., Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory, 359-421; Roth et al., *J. Histochem. Cytochem.* 26: 1074-1081 (1978)). The

primary antibody used in this study was an anti-zosteric acid polyclonal antibody (BAbCo, Richmond, CA).

Bacterial agglutination assays. Log-phase cultures of *Shewanella putrefaciens* were grown in complete seawater medium containing zosteric acid at a range of concentrations up to 20 mM. Cultures were counted for viable colony forming units at eight hours.

Results

Although zosteric acid concentrations up to 16 mM did not inhibit the growth of *S. putrefaciens* in liquid culture, the presence of zosteric acid caused significant agglutination of *S. putrefaciens* in a concentration dependent manner. The agglutination observed was visible to the naked eye, and was more quantitatively detected as a decrease in optical density absorbance in cultures containing zosteric acid (Fig. 6). Counts of viable colony forming units at eight hours revealed no difference in cell density among the different cultures, thus the observed differences in absorption resulted from differences in bacterial agglutination, not differences in growth (cell division) rates among the cultures. Thus, zosteric acid promoted cell agglutination, but did not affect cell growth.

Example 5: Zosteric Acid Binds Heparin-sensitive Sites

To investigate the ability of sulfate esters to mediate the interaction between biological surfaces involved in erythrocyte agglutination and blood clotting, erythrocyte agglutination assays and clot formation assays were performed using the sulfate ester zosteric acid.

Materials and Methods

Red Blood Cell agglutination assays. Washed equine erythrocytes suspended in 1 mg/mL sodium citrate were placed in microtiter plates designed with wells containing hemispherical bottoms. Negative controls (no zosteric acid) were diluted in isotonic saline solution. Zosteric acid treated cells were diluted with saline containing zosteric acid at eight concentrations ranging from 0.005 to 5.0 mg/mL. Positive controls were exposed to the same range of high molecular weight heparin sulfate concentrations.

Clotting assays. Clotting time assays were performed using commercial kits (Sigma Chemical Co.) for prothrombin clotting time. Serum was harvested from 30 mL of whole human blood obtained by venous puncture using centrifugation to remove blood cells. Zosteric acid and high MW heparin were added to separate aliquots of the serum, producing concentrations from 0 to 5.0 mg/mL. Clotting times were determined for each concentration in duplicate according to the protocols provided with the kit.

Results

In agglutination studies, equine erythrocytes were significantly agglutinated by zosteric acid at concentrations as low as 0.175 mg/mL. In contrast, the presence of high molecular weight heparin produced visible agglutination only at concentrations greater than 0.75 mg/mL. This result indicates that monomeric zosteric acid is eight times more reactive with cell surface glycoproteins and polysaccharides involved in cell agglutination, than high molecular weight heparin.

Zosteric acid was also effective at preventing clot formation, as measured by the prothrombin clotting time assays (Fig. 7), although this activity was considerably less than that observed for heparin. Heparin was effective at preventing clot formation at concentrations well below 0.1 mg/ml, while zosteric acid was effective only at concentrations exceeding 10 mg/ml. The effectiveness of heparin-like anticoagulants is strongly linked to size, with high molecular weight molecules being more effective. Thus, it is not surprising that the low molecular weight zosteric acid was considerably less effective than high molecular weight heparin in mediating clot formation. A derivative of zosteric acid or another sulfate ester that is higher in molecular weight may prove more effective. Nonetheless, these results indicate that zosteric acid interacts with cell surface glycoproteins and/or polysaccharides in a manner similar to that of heparin.

Example 6: Zosteric Acid Blocks Fertilization

The data above suggests that sulfate esters interact with sulfate ester-binding receptors in a variety of systems ranging from bacteria to mammalian erythrocytes. The fusion of sperm and egg cells in invertebrate and mammalian systems also appears to be mediated by organo-sulfate molecules such as the polysaccharides fucose sulfate and heparin. In light of

this, the following experiments were initiated to identify potential AF properties of sulfate esters in fertilization.

A simple sea urchin assay was used to detect and quantitate the ability of sulfate esters to block sperm-egg fusion. Sea urchin sperm was added to freshly collected eggs in the presence and absence of increasing amounts of the sulfate ester zosteric acid, and the eggs were subsequently scored for successful fertilization.

Materials and Methods

Fertilization assays. Healthy sea urchins were induced to spawn by injection with 0.5 M KCL solution. Freshly collected eggs were gently washed and resuspended in filtered sea water (FSW, pH 8.2) and aliquotted into separate tubes for fertilization assays. Zosteric acid was added to each tube from a concentrated stock dissolved in FSW (pH 8.2), along with additional FSW to ensure a constant volume in each tube. Equal amounts of sperm were added to each tube and percent fertilization was determined by direct microscopic counting. Eggs with elevated fertilization membranes were scored as fertilized. Assays were performed at sperm-limiting concentrations that allowed 95-99% fertilization in the absence of zosteric acid.

Sea urchin egg agglutination assays. Agglutination of unfertilized eggs by bindin was evaluated at the range of zosteric acid concentrations indicated in Table 1. Freshly spawned eggs were suspended in acidic seawater (pH 5) for 5 minutes to remove the outer jelly coat, and then washed 5 times in normal FSW (pH 8.2). Eggs were then transferred into plastic petri dished containing a range of zosteric acid concentrations and incubated for 15 minutes. Purified bindin (D. Epel, Stanford University) was added to the eggs at concentrations ranging from 1.2 to 12 $\mu\text{g/mL}$. The mixtures were gently agitated on a rotary shaker for 5 minutes and visually examined for agglutination. Bovine serum albumen (BSA) was used in separate assays to control for nonspecific agglutination of the dejellied eggs.

Dot blot assays. Serial dilutions of purified bindin, a covalently conjugated zosteric acid-BSA molecule, and an unconjugated mixture of free zosteric acid and BSA were pipetted onto a nitrocellulose membrane and allowed to air-dry. Standard immuno-blotting procedures were then employed to determine the reactivity between the blotted substrates and a

polyclonal anti-zosteric acid antibody. The membrane was blocked in blotto (1% nonfat dry milk in phosphate buffered saline (PBS)) for 1 hour prior to probing. Probing was done in blotto for 1 hour. Primary antibody was anti-zosteric acid antibody, used at a 1:1000 dilution. Secondary antibody was alkaline phosphatase conjugated goat anti-rabbit (Southern
5 Biotechnology Association, Inc.) and was used at a dilution of 1:1000. Rinses between probing were performed in triplicate in PBS-tween. The blot was developed in color reaction buffer (100 mM Tris, pH 9.5, 100 mM NaCl, 5 mM MgCl₂, 50 mg/mL Nitroblue Tetrazolium (Sigma), 50 ml/mL 5-bromo-4-chloro-3-indolyl phosphite (BCIP, Sigma)) for 20 minutes. Membranes were then transferred to stop buffer (10 mM Tris, pH 6.0, 5 mM EDTA) for 1
10 hour, and then transferred to freshwater, left overnight, and then dried.

Results

As shown in Fig. 8a, zosteric acid had a dose-dependent effect on sea urchin egg fertilization. Concentrations higher than 0.5 mg/mL (1.5 mM) completely blocked
15 fertilization. Re-exposure of unfertilized eggs from the highest zosteric acid treatment, to fresh sperm, after washing in sea water, resulted in the fertilization of all eggs. This result demonstrates that the zosteric acid inhibition is reversible. The presence of zosteric acid had no detected effect on sperm viability or motility. Sperm exposed to zosteric acid were observed to swim actively through the jelly layer surrounding the egg without adhering to the
20 egg surface or elevating the egg fertilization membrane, further supporting the conclusion that the antifouling effect of zosteric acid was mediated through inhibition of sperm-egg attachment.

The effectiveness of zosteric acid (1 mg/mL) at fertilization inhibition was compared to equal mass concentrations of coumaric acid (an unsulfated zosteric acid precursor) and high
25 MW heparin. The presence of coumaric acid had no effect on egg fertilization, while the presence of heparin reduced fertilization by approximately 50%. Zosteric acid was at least twice as effective as heparin at inhibiting fertilization, reducing fertilization to 21% at this concentration (Fig. 8b).

The ability of zosteric acid to compete for the binding of sulfate receptor sites on the
30 egg surface was investigated in egg agglutination assays. These experiments tested the ability of zosteric acid to interfere with the binding of the bindin molecule to unfertilized sea urchin eggs. Bindin added to unfertilized eggs causes them to agglutinate by cross linking sulfate receptors that are present on the surface of the eggs. Addition of zosteric acid inhibited this

agglutination in a dose dependent manner (Table 1), suggesting a competitive interaction of bindin and zosteric acid for the sulfate receptor sites on the egg surface.

[ZA] mg mL ⁻¹	Agglutination
3	No
1.5	No
0.75	Yes
0.30	Yes
0.15	Yes
0.075	Yes
0.03	Yes
0.015	Yes

5 **Table 1.** The effect of zosteric acid on the agglutination of sea urchin eggs by purified bindin.

Antibodies specific for zosteric acid (described above) exhibited strong cross reactivity with the bindin molecule in dot-blot assays, but not with other proteins, such as bovine serum albumin. This antibody cross reactivity indicates that zosteric acid and bindin share significant structural similarity at the site of antibody recognition, believed to be the sulfate moiety. Such structural similarities in the sulfate moieties between bindin and zosteric acid would explain why zosteric acid is an effective inhibitor of sea urchin fertilization.

Example 7: 4 t-Pentyl Phenyl Chlorosulfate (4-PPCS) Blocks Fertilization

The effect of 4-PPCS on inhibiting sea urchin fertilization was performed substantially as described for zosteric acid in Example 6. As can be seen in Figure 7, 4-PPCS was essentially 100% effective in blocking sea urchin fertilization in the range of 1-10mM, precisely the same range as zosteric acid was effective. As also can be seen in Figure 7, when more sperm were added to the medium, the effect of the PPCS inhibition could be washed out by exceeding the binding capacity of the 4-PPCS in solution. In contrast to most biocidal agents, contact with 4-PPCS resulted in no adverse impacts on sperm motility.

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Equivalents

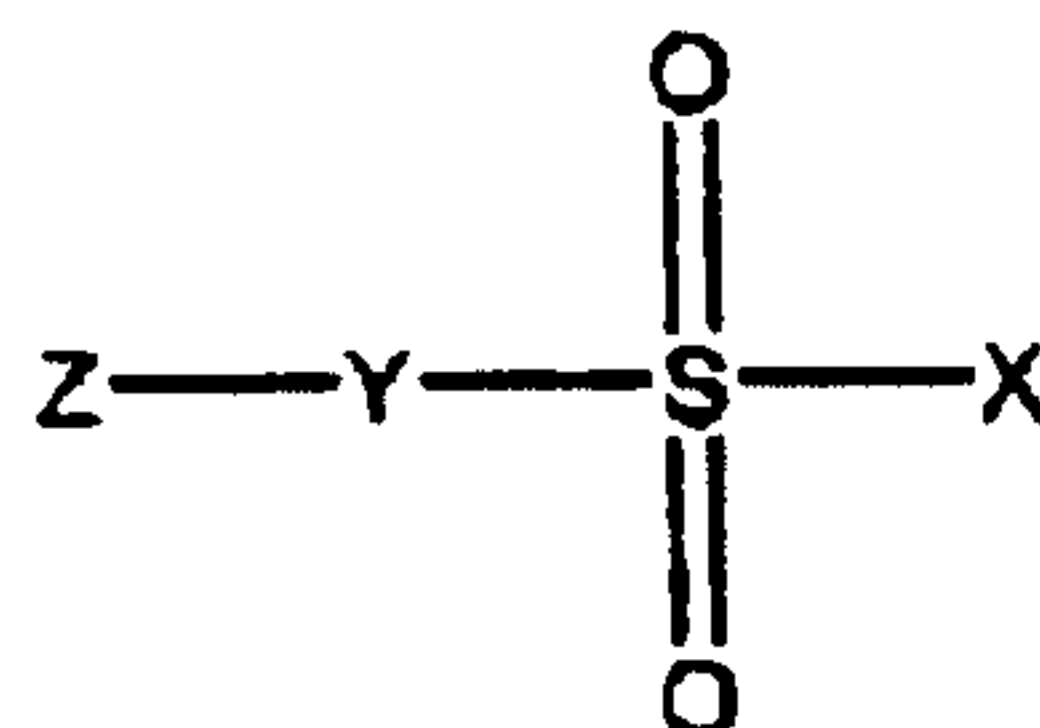
Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents of the specific embodiments of the invention
5 described herein. Such equivalents are intended to be encompassed by the following claims.

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CLAIMS

1. A pharmaceutical composition comprising a pharmaceutical carrier and an effective amount of a compound represented by general structure 1:



1

wherein

5 X represents -OH, -O(aryl), -O(acyl), -O(sulfonyl), -CN, F, Cl, or Br;

Y represents O, S, Se, or NR;

Z represents optionally substituted alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, or $-(\text{CH}_2)_m\text{-R}_{40}$;

10 R represents independently for each occurrence hydrogen, alkyl, heteroalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, or $-(\text{CH}_2)_m\text{-R}_{40}$;

R_{40} represents independently for each occurrence aryl, cycloalkyl, cycloalkenyl, heterocyclyl, or polycyclyl; and

m is an integer in the range 0 to 8 inclusive, or a salt thereof;

provided that when X is -OH, and Y is O, Z is not an alkyl group.

15

2. A pharmaceutical composition of claim 1, wherein X represents -OH, F, Cl, or Br.

3. A pharmaceutical composition of claim 1, wherein Y represents O.

20 4. A pharmaceutical composition of claim 1, wherein Z represents optionally substituted alkyl, aryl, or $-(\text{CH}_2)_m\text{-R}_{40}$.

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5. A pharmaceutical composition of claim 1, wherein Z represents optionally substituted alkylphenyl, heteroalkylphenyl, arylphenyl, or heteroarylphenyl.
6. A pharmaceutical composition of claim 1, wherein Z represents methyl, octyl, 4-(2-methylpropyl)phenyl, 4-(1,1-dimethylethyl)phenyl, 4-(1,1-dimethylpropyl)phenyl, 4-pentylphenyl, 4-(1-methyl-1-phenylethyl)phenyl, or 4-(1-methylheptyl)phenyl.
7. A pharmaceutical composition of claim 1, wherein R represents H or alkyl.
- 10 8. A pharmaceutical composition of claim 1, wherein X represents -OH, F, Cl, or Br; and Y represents O.
9. A pharmaceutical composition of claim 1, wherein X represents -OH or Cl; and Y represents O.
- 15 10. A pharmaceutical composition of claim 1, wherein X represents -OH, F, Cl, or Br; and Z represents optionally substituted alkyl, aryl, or $-(CH_2)_m-R_{80}$.
11. A pharmaceutical composition of claim 1, wherein X represents -OH or Cl; and Z represents optionally substituted alkyl, aryl, or $-(CH_2)_m-R_{80}$.
- 20 12. A pharmaceutical composition of claim 1, wherein X represents -OH, F, Cl, or Br; and Z represents optionally substituted alkylphenyl, heteroalkylphenyl, arylphenyl, or heteroarylphenyl.
- 25 13. A pharmaceutical composition of claim 1, wherein X represents -OH or Cl; and Z represents optionally substituted alkylphenyl, heteroalkylphenyl, arylphenyl, or heteroarylphenyl.

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14. A pharmaceutical composition of claim 1, wherein X represents -OH, F, Cl, or Br; and Z represents methyl, octyl, 4-(2-methylpropyl)phenyl, 4-(1,1-dimethylethyl)phenyl, 4-(1,1-dimethylpropyl)phenyl, 4-pentylphenyl, 4-(1-methyl-1-phenylethyl)phenyl, or 4-(1-methylheptyl)phenyl.

15. A pharmaceutical composition of claim 1, wherein X represents -OH or Cl; and Z represents methyl, octyl, 4-(2-methylpropyl)phenyl, 4-(1,1-dimethylethyl)phenyl, 4-(1,1-dimethylpropyl)phenyl, 4-pentylphenyl, 4-(1-methyl-1-phenylethyl)phenyl, or 4-(1-methylheptyl)phenyl.

16. A pharmaceutical composition of claim 1, wherein Y represents O; and Z represents optionally substituted alkyl, aryl, or $-(CH_2)_m-R_{40}$.

17. A pharmaceutical composition of claim 1, wherein Y represents O; and Z represents optionally substituted alkylphenyl, heteroalkylphenyl, arylphenyl, or heteroarylphenyl.

18. A pharmaceutical composition of claim 1, wherein Y represents O; and Z represents methyl, octyl, 4-(2-methylpropyl)phenyl, 4-(1,1-dimethylethyl)phenyl, 4-(1,1-dimethylpropyl)phenyl, 4-pentylphenyl, 4-(1-methyl-1-phenylethyl)phenyl, or 4-(1-methylheptyl)phenyl.

19. A pharmaceutical composition of claim 1, wherein X represents -OH, F, Cl, or Br; Y represents O; and Z represents optionally substituted alkyl, aryl, or $-(CH_2)_m-R_{40}$.

20. A pharmaceutical composition of claim 1, wherein X represents -OH or Cl; Y represents O; and Z represents optionally substituted alkyl, aryl, or $-(CH_2)_m-R_{40}$.

21. A pharmaceutical composition of claim 1, wherein X represents -OH, F, Cl, or Br; Y represents O; and Z represents optionally substituted alkylphenyl, heteroalkylphenyl, arylphenyl, or heteroarylphenyl.
- 5 22. A pharmaceutical composition of claim 1, wherein X represents -OH or Cl; Y represents O; and Z represents optionally substituted alkylphenyl, heteroalkylphenyl, arylphenyl, or heteroarylphenyl.
- 10 23. A pharmaceutical composition of claim 1, wherein X represents -OH, F, Cl, or Br; Y represents O; and Z represents methyl, octyl, 4-(2-methylpropyl)phenyl, 4-(1,1-dimethylethyl)phenyl, 4-(1,1-dimethylpropyl)phenyl, 4-pentylphenyl, 4-(1-methyl-1-phenylethyl)phenyl, or 4-(1-methylheptyl)phenyl.
- 15 24. A pharmaceutical composition of claim 1, wherein X represents -OH or Cl; Y represents O; and Z represents methyl, octyl, 4-(2-methylpropyl)phenyl, 4-(1,1-dimethylethyl)phenyl, 4-(1,1-dimethylpropyl)phenyl, 4-pentylphenyl, 4-(1-methyl-1-phenylethyl)phenyl, or 4-(1-methylheptyl)phenyl.

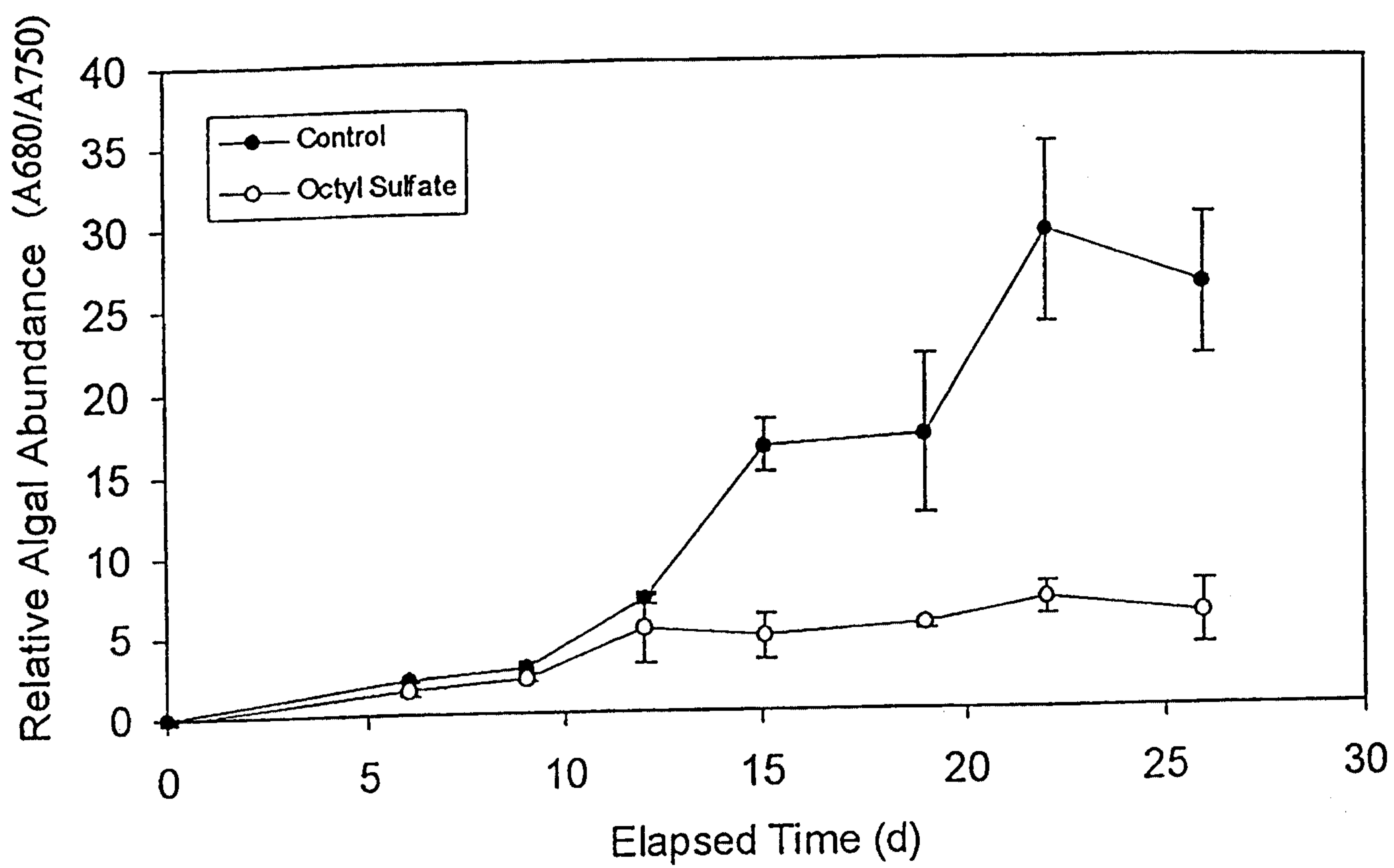


FIG. 1

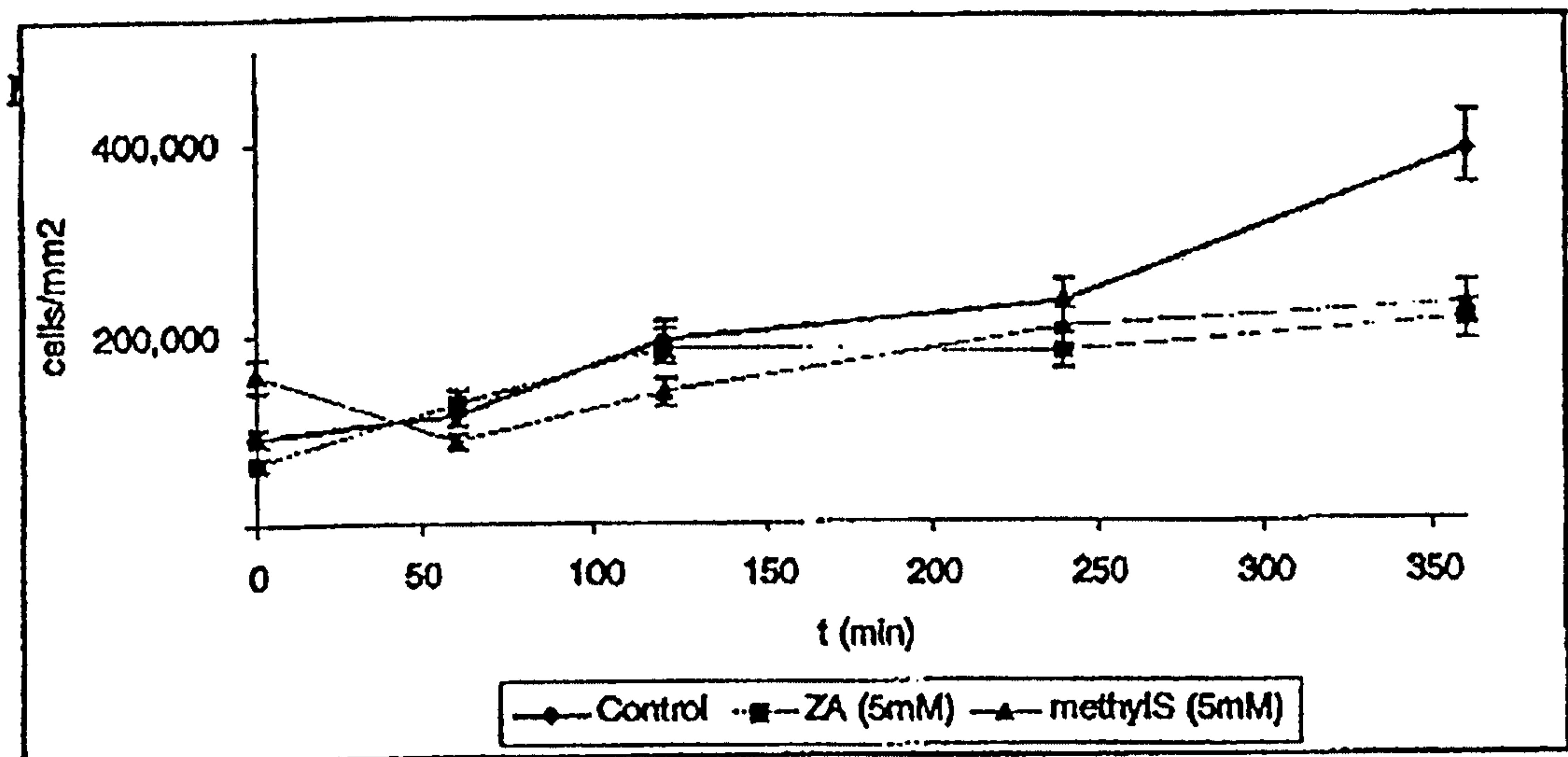


FIG. 2

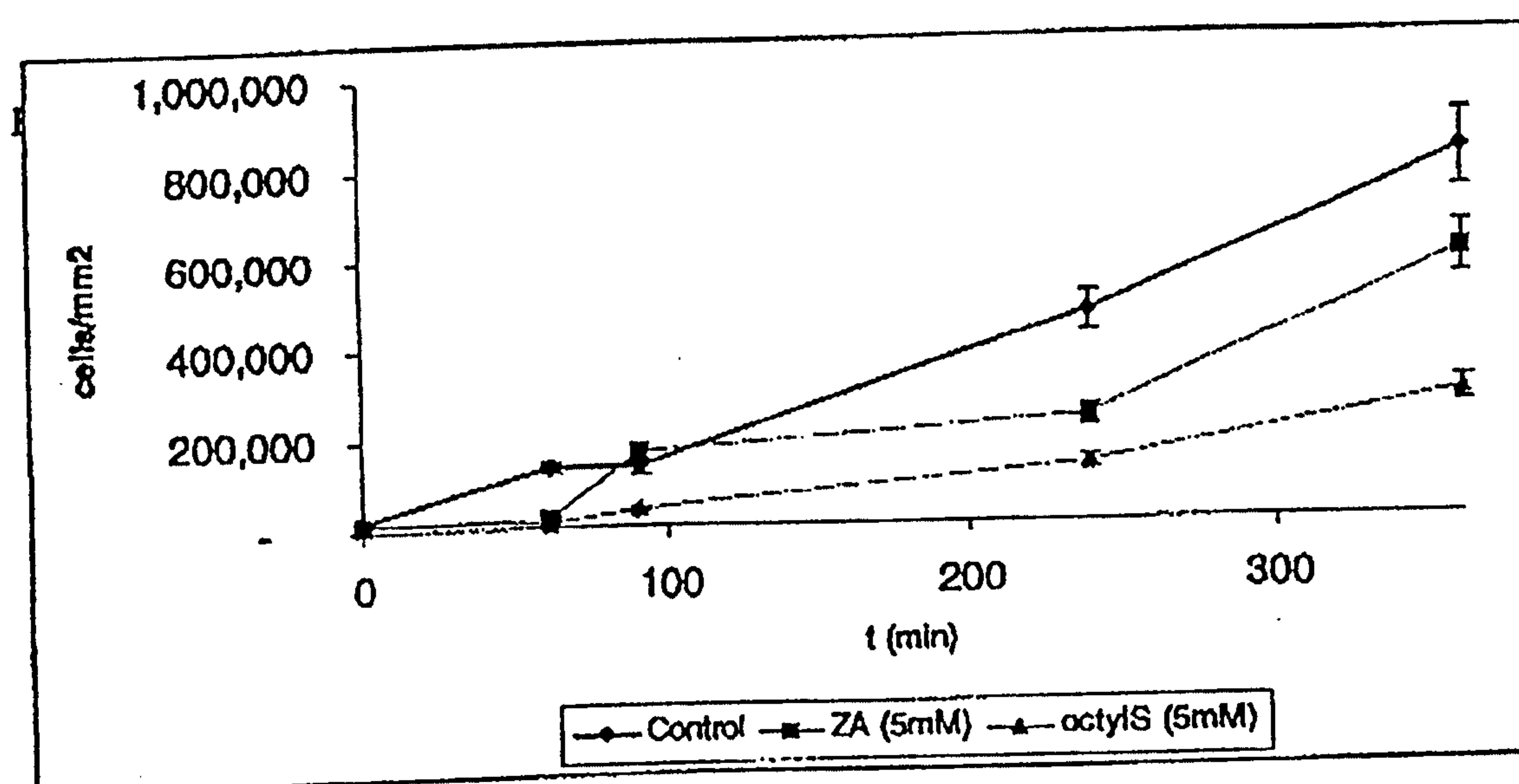


FIG. 3

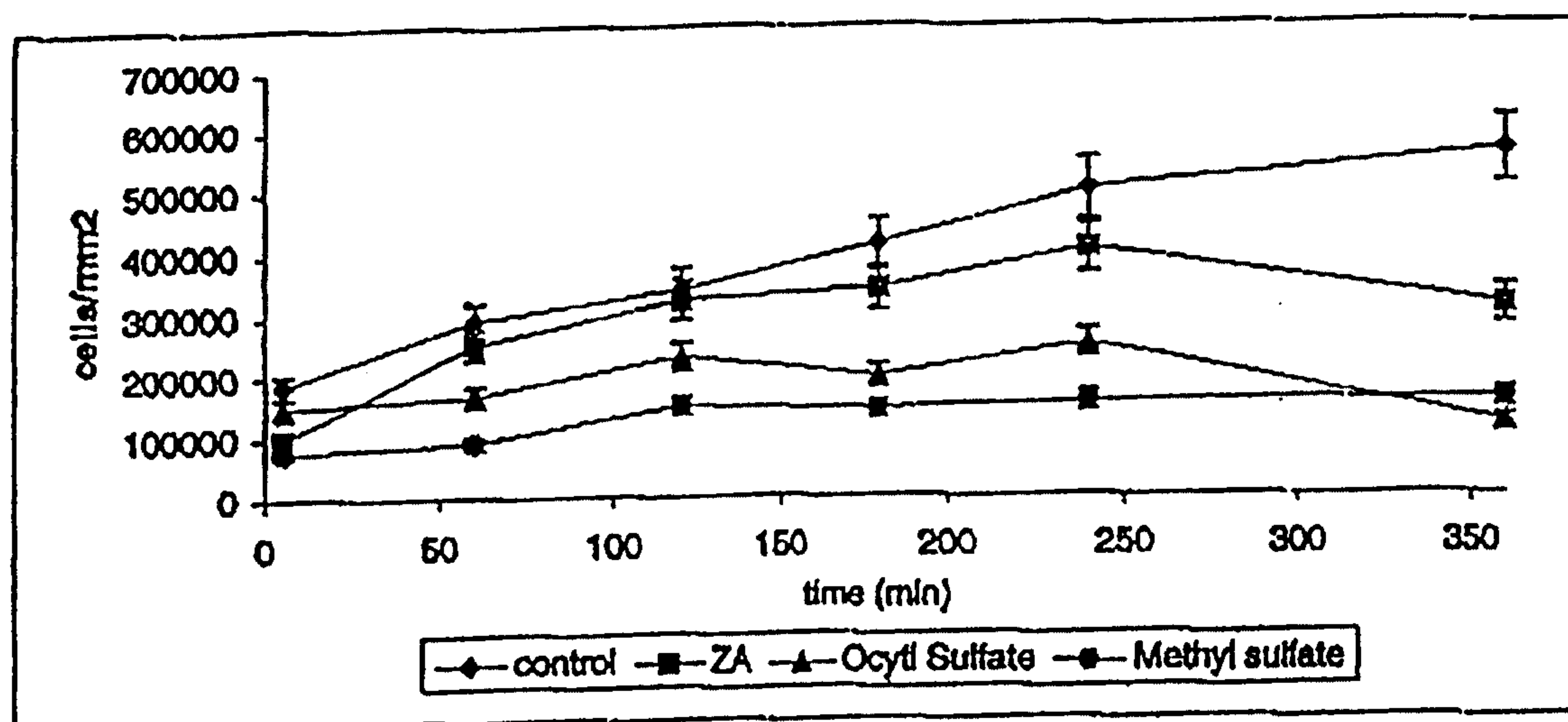


FIG. 4

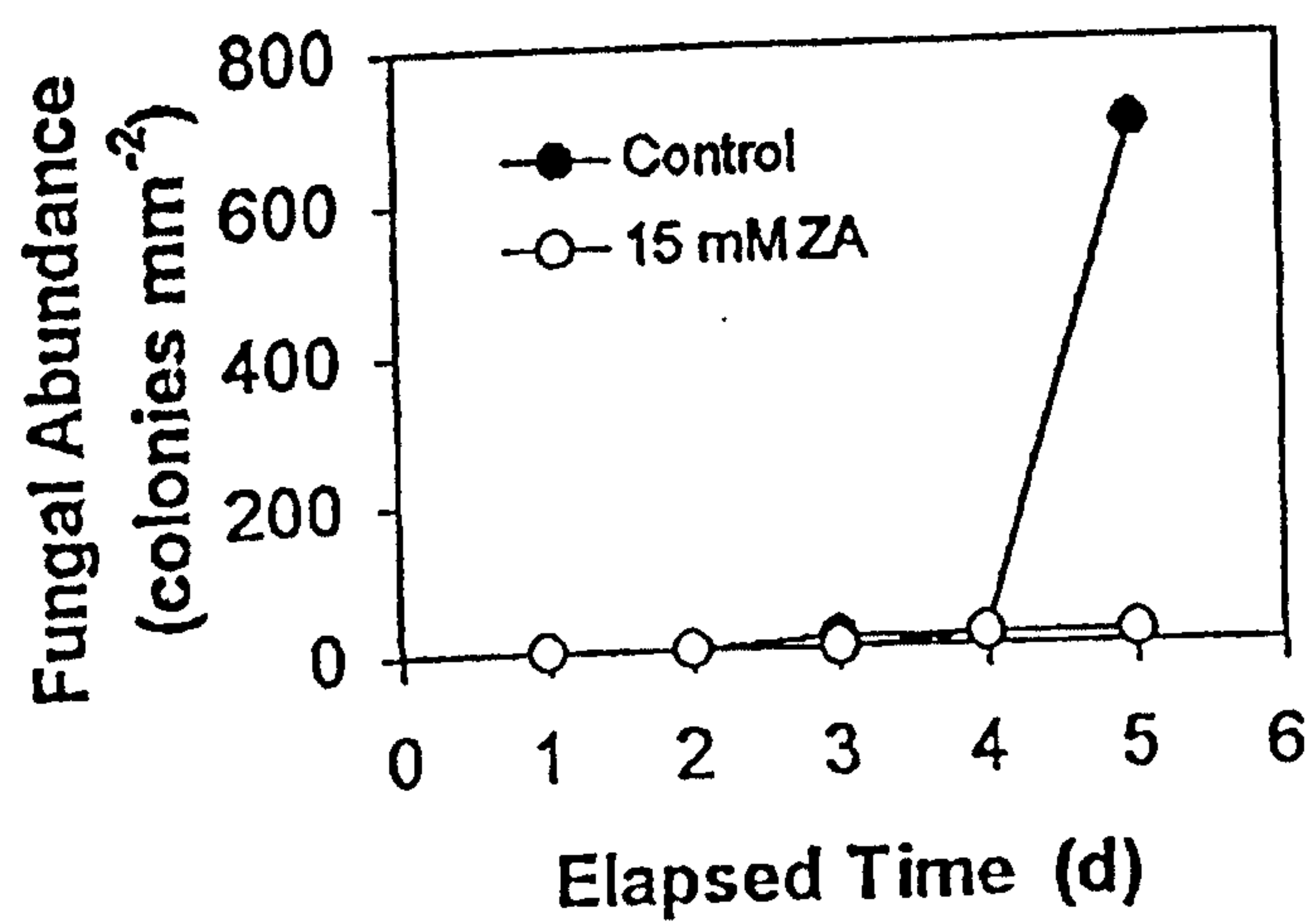


FIG. 5

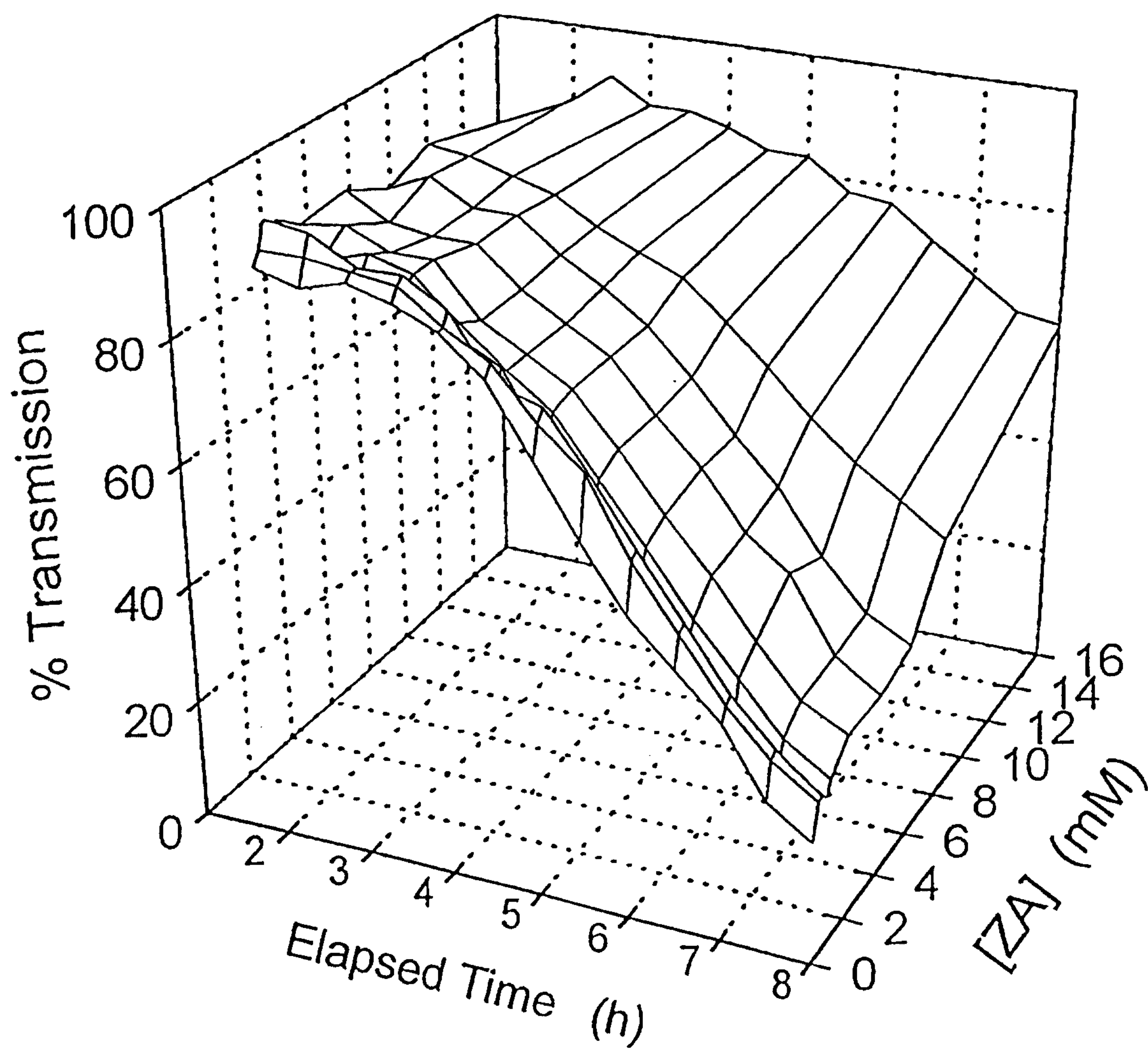


FIG. 6

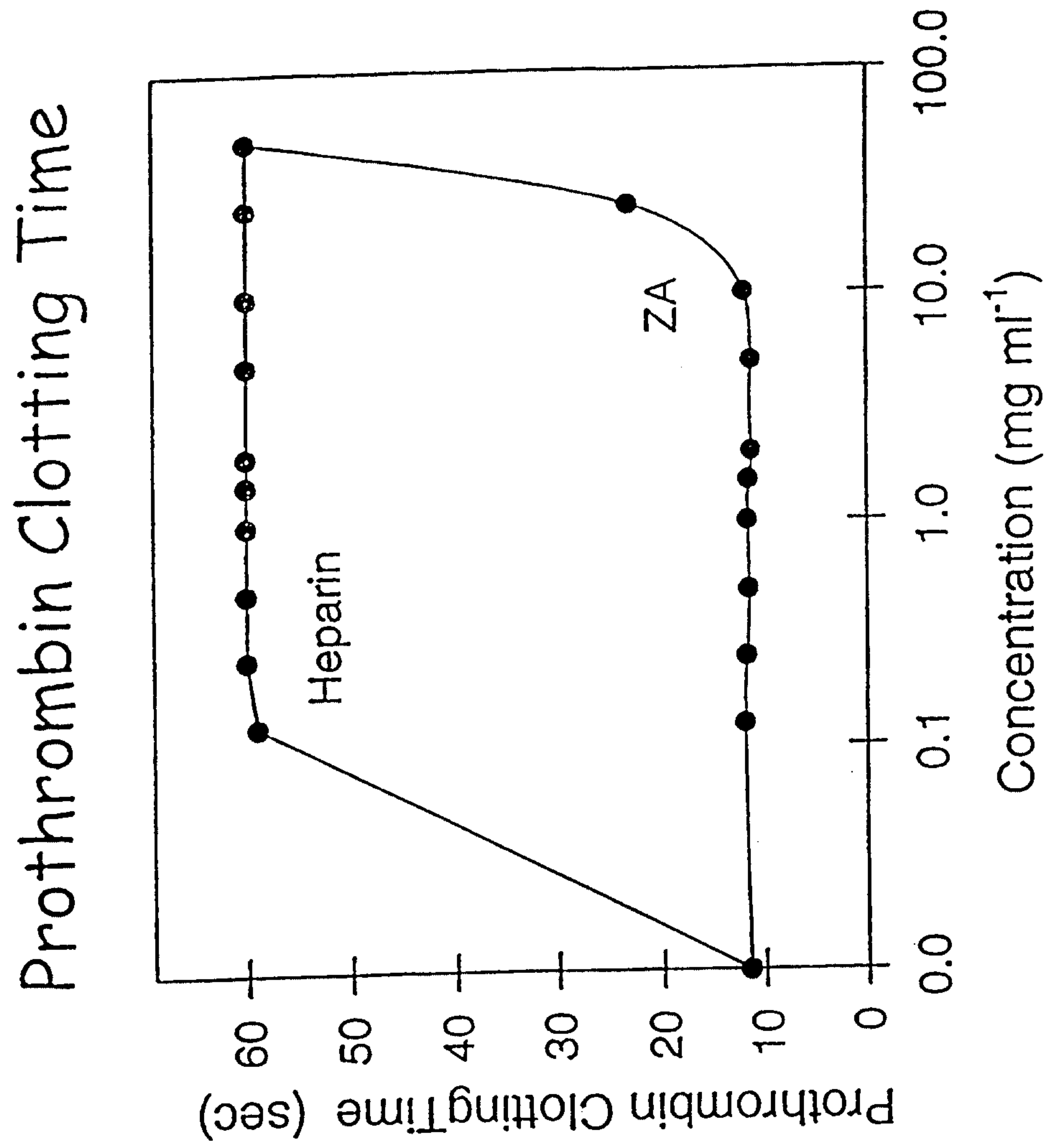


FIG. 7

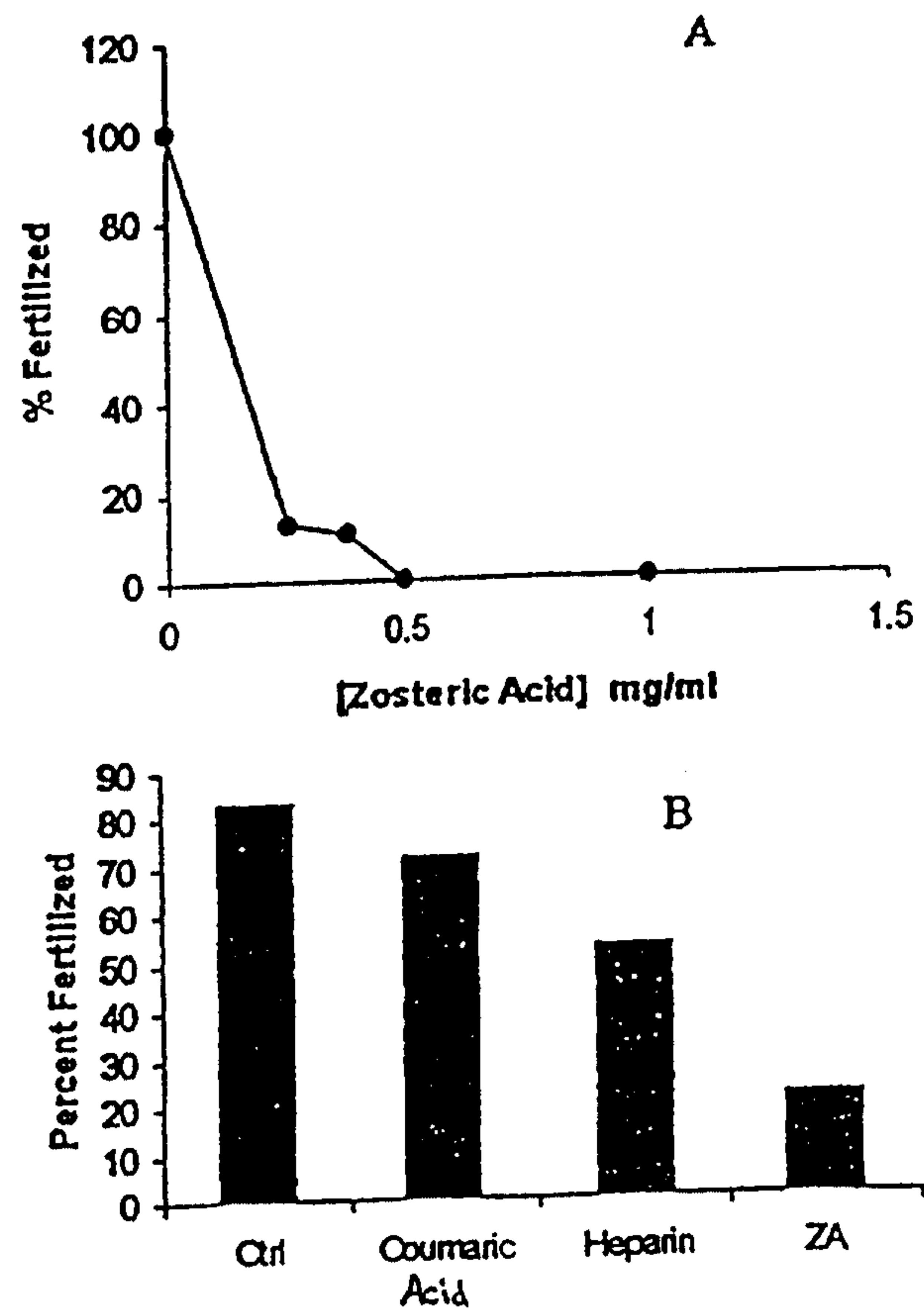


FIG. 8

