PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : A61K	A2	(11) International Publication Number: **WO 00/40204**
		(43) International Publication Date: 13 July 2000 (13.07.00)

(21) International Application Number: PCT/US00/00480

(22) International Filing Date: 7 January 2000 (07.01.00)

(30) Priority Data:
09/226,666 8 January 1999 (08.01.99) US

(71) Applicant: PERIODONTIX, INC. [US/US]; 313 Pleasant Street, Watertown, MA 02472 (US).

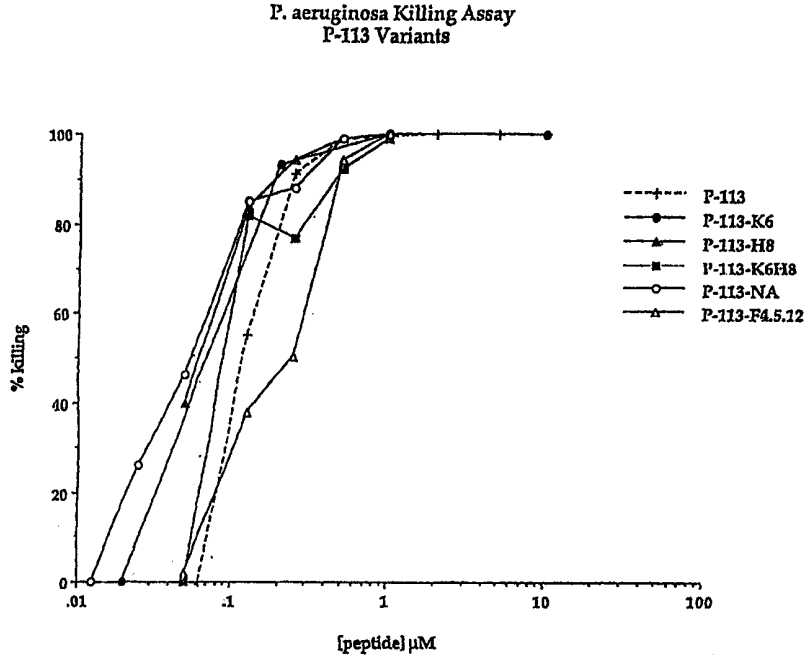
(72) Inventors: SPACCIAPOLI, Peter; 9 High Road, Newbury, MA 01951 (US). ROTHSTEIN, David, M.; 107 Cedar Street, Lexington, MA 02173 (US). FRIDEN, Phillip, M.; 32 Washington Street, Bedford, MA 01730 (US).

(74) Agent: CLARK, Paul, T.; Clark & Elbing LLP, 176 Federal Street, Boston, MA 02110–2214 (US).

(81) Designated States: AU, CA, NZ, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: METHODS FOR TREATING CYSTIC FIBROSIS



(57) Abstract

Methods for treating cystic fibrosis in a mammal that include administering to the mammal an effective amount of a histatin, a histatin fragment, or a histatin–related peptide are disclosed.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

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

METHODS FOR TREATING CYSTIC FIBROSIS

Background of the Invention

The invention relates to methods for treating cystic fibrosis.

Cystic fibrosis is a hereditary disease that affects a number of organs, particularly the lungs and pancreas. The exocrine glands of a cystic fibrosis patient secrete abnormally thick mucous, which blocks the patient's bronchi. As a result, many cystic fibrosis patients have chronic bronchitis; they are also susceptible to pneumonia and other pulmonary infections. In particular, cystic fibrosis patients are susceptible to *Pseudomonas* infections.

As there is currently no cure for cystic fibrosis, treatments for this disabling disease focus on alleviating the symptoms of the disease. Unfortunately, the infections of many cystic fibrosis patients do not respond to the antibiotics traditionally used to treat pulmonary infections.

Summary of the Invention

In one aspect, the invention features a method for treating cystic fibrosis in a mammal, such as a human; the method includes administering to the mammal an effective amount of a histatin or a histatin fragment. In preferred methods, the histatin fragment has between 8 and 20 amino acids, inclusive, and more preferably has between 8 and 12 amino acids, inclusive.

For example, the histatin fragment may have the amino acid sequence Ala-Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His. Preferably, at least one of the amino acids in this sequence is a D-amino acid. In one preferred method, the fragment has the amino acid sequence D-Ala-D-Lys-D-Arg-D-His-D-His-D-Gly-D-Tyr-D-Lys-D-Arg-D-Lys-D-Phe-D-His.

In another preferred method, the histatin is histatin 5 (shown in Fig.

2); preferably, at least one amino acid of the histatin 5 is a D-amino acid. In yet another preferred method, the histatin or histatin fragment has at least one modification selected from the group consisting of an acyl addition at the N-terminus; a carbamyl addition at the N-terminus; and an amide addition at the C-terminus.

In a second aspect, the invention features a method for treating cystic fibrosis in a mammal, such as a human; the method includes administering to the mammal an effective amount of a histatin-related peptide having between 8 and 20 amino acids, inclusive, where the peptide has the amino acid sequence: R1-R2-R3-R4-R5-R6-R7-R8-R9-R10-R11-R12-R13-R14-R15-R16-R17-R18-R19-R20-R21-R22-R23, where R1 is Asp or is absent; R2 is Ser or is absent; R3 is His or is absent; R4 is Ala, His, Leu, or is absent; R5 is Lys, Gln, Arg, Orn, or another basic amino acid; R6 is Arg, Gln, Lys, or another basic amino acid; R7 is His, Phe, Tyr, Leu, or another hydrophobic amino acid; R8 is His, Phe, Tyr, Leu, or another hydrophobic amino acid; R9 is Gly, Lys, Arg, Ser, or a basic amino acid; R10 is Tyr; R11 is Lys, His, Phe, Leu, or another hydrophobic amino acid; R12 is Arg, Gln, Lys, or another basic amino acid; R13 is Lys, Gln, Arg, Orn, another basic amino acid, or is absent; R14 is Phe or is absent; R15 is His, Phe, Tyr, Leu, another hydrophobic amino acid, or is absent; R16 is Glu or is absent; R17 is Lys or is absent; R18 is His or is absent; R19 is His or is absent; R20 is Ser or is absent; R21 is His or is absent; R22 is Arg or is absent; and R23 is Gly or is absent.

In preferred methods, at least one of R7, R8, R11, or R15 is Phe. For example, R7 is Phe; R8 is Phe; R11 is Phe; and/or R15 is Phe. In one preferred method, all of R7, R8, and R15 are Phe. In other preferred methods, R9 is Lys and/or R11 is His. In other preferred methods, at least one of R7, R8, or R15 is Tyr. Preferably, all of R7, R8, and R15 are Tyr.

In another preferred method, at least one of R5 and R13 is Gln;

preferably, both R5 and R15 are Gln. In a different preferred method, at least one of R5 and R13 is Orn; preferably, both R5 and R13 are Orn.

In some preferred methods, all of R1, R2, and R3 are absent. In other preferred methods, both R22 and R23 are absent, all of R20, R21, R22, and R23 are absent, or all of R18, R19, R20, R21, R22, and R23 are absent. Examples of preferred peptides include peptides which have the amino acid sequences: Ala-Lys-Arg-Phe-Phe-Gly-Tyr-Lys-Arg-Lys-Phe-Phe (P-113-F4.5.12); Ala-Lys-Arg-His-His-Lys-Tyr-Lys-Arg-Lys-Phe-His (P-113-K6); Ala-Lys-Arg-His-His-Gly-Tyr-His-Arg-Lys-Phe-His (P-113-H8); Ala-Lys-Arg-His-His-Lys-Tyr-His-Arg-Lys-Phe-His (P-113-K6H8); Ala-Lys-Arg-Tyr-Tyr-Gly-Tyr-Lys-Arg-Lys-Phe-Tyr-NH_2 (P-113-Y4.5.12); Ala-Gln-Arg-His-His-Gly-Tyr-Lys-Arg-Gln-Phe-His-NH_2 (P-113-Q2.10); or Ala-Orn-Arg-Tyr-Tyr-Gly-Tyr-Lys-Arg-Orn-Phe-Tyr-NH_2 (P-113-K2.10O-H4.5.12Y).

Preferably, the peptide has at least one modification selected from the group consisting of an acyl addition at the N-terminus; a carbamyl addition at the N-terminus; and an amide addition at the C-terminus.

By "hydrophobic amino acid" is meant an amino acid selected from the group consisting of Ala, Val, Leu, Ile, Phe, Trp, Met, and Thr.

By "basic amino acid" is meant an amino acid selected from the group consisting of Lys, Arg, Orn, Gln, and Asn.

The invention provides effective methods for treating cystic fibrosis. According to the invention, histatins, histatin fragments, and histatin-related peptides can be used to combat *Pseudomonas* infections and other pulmonary infections in cystic fibrosis patients. Histatin derivatives are effective in treating these infections, even in cases where the infections are resistant to traditional antibiotics.

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

Brief Description of the Figures

Fig. 1 is the amino acid sequence of histatin 3.

Fig. 2 is the amino acid sequence of histatin 5.

Figs. 3, 4, 5, and 6 are graphs showing the percentages of cells in *P. aeruginosa* assays killed by peptides of the invention.

Detailed Description

The invention features peptides containing 8 to 20 amino acids; these peptides include defined portions of the amino acid sequence of the naturally occurring protein histatin 3 (SEQ ID NO: 1), which is shown in Fig. 1. In addition, the peptides of the invention include defined portions of the amino acid sequence of histatin 3, with amino acid substitutions at particular positions of the peptides. These peptides are referred to herein as "histatin-related peptides."

Histatins (also referred to in the literature as histidine-rich proteins or HRPs) are salivary proteins that are synthesized in the parotid and submandibular-sublingual secretory glands of humans and Old World monkeys and are believed to be part of the extraimmunologic defense system of the oral cavity. The family of naturally occurring human histatins is a group of twelve low molecular weight peptides.

The peptides of the present invention can thus be obtained from naturally occurring sources of histatin; alternatively, they can be obtained by recombinant DNA techniques as expression products from cellular sources. The peptides can also be chemically synthesized.

For example, cloned DNA encoding the histatins or histatin-related peptides may be obtained as described by L. M. Sabatini et al., *Biochem. Biophys. Res. Comm.* 160: 495-502 (1989) and J. C. Vanderspek et al., *Arch. Oral Biol.* 35(2): 137-43 (1990). cDNA encoding the histatin-related peptides

can be cloned by recombinant DNA techniques, for instance, by using degenerate oligonucleotides based on the amino acid sequence of histatin-related peptides as primers for polymerase chain reaction amplification.

Alternatively, oligonucleotides encoding histatins or histatin-related peptides can be synthesized chemically using commercially available equipment. They can then be made double-stranded and cloned into vectors for amplification. The histatin-related peptides can be produced in a variety of expression vector/host systems, which are available commercially or can be reproduced according to recombinant DNA and cell culture techniques. The vector/host expression systems can be prokaryotic or eukaryotic, and can include bacterial, yeast, insect, mammalian, and viral expression systems. The construction of expression vectors encoding histatin-related peptides, transfer of the vectors into various host cells, and production of peptides from transformed host cells can be accomplished using genetic engineering techniques, as described in manuals such as J. Sambrook et al., *Molecular Cloning* (2d ed. 1989) and *Current Protocols in Molecular Biology*, (F.M. Ausubel et al., eds.).

The histatin-related peptides encoded by expression vectors may be modified by post-translational processing in a particular expression vector/host cell system. In addition, these peptides can be altered by minor chemical modifications, such as by adding small substituents or by modifying one or more of the covalent bonds within or between the amino acid residues. The substituent groups can be bulky and may include one or more natural or modified amino acids. Useful modifications include the addition of a substituent to either the amino terminus, the carboxyl terminus, or to both termini of the peptide. Particularly useful modifications include acylation or carbamylation of the amino terminus of the peptide, or amidation of the carboxyl terminus of the peptide. These alterations do not significantly

diminish the antifungal or antibacterial activities of the peptides and appear to stabilize the peptide in its active form and to aid in the prevention of enzymatic degradation of these peptides.

The peptides can also be made by standard solid phase synthetic methods.

The peptides described herein can be used in preventive treatment as well. The compositions may contain combinations of histatin-related peptides, in order to obtain maximum activity against all developmental forms of fungi or bacteria that cause pulmonary infections. The ionic strength, presence of various mono- and divalent ions, and pH of the compositions may be adjusted to obtain maximum activity of the histatin-related peptides, as described in T. Xu et al., *Infect. Immun.* 59(8): 2549-54 (1991).

Carriers appropriate for administration of pharmaceutical agents to the respiratory system are known and described, for instance, in Pollock et al., U.S. Patent No. 4,725,576. Compositions for treatment of cystic fibrosis can be administered by various routes; for example, they may be administered by inhalation.

There now follow particular examples that describe the preparation of histatin-related peptides and the antibacterial activity of various histatin-related peptides against strains of *P. aeruginosa*. These examples are provided for the purpose of illustrating the invention, and should not be construed as limiting.

Example 1: Isolation of Histatin-related Peptides

The isolation and amino acid sequence determination of human histatins are performed as described in F. G. Oppenheim et al., *J. Biol. Chem.* 263(16): 7472-7477 (1988). Human parotid secretion from healthy adults is stimulated using sour lemon candies, collected with Curby cups in ice-chilled

graduated cylinders, pooled, dialyzed and lyophilized. The total protein in the human parotid secretion is subjected to fractionation on Bio-Gel P-2 (Bio-Rad Laboratories, Richmond, CA) developed in 0.05 M ammonium formate buffer, pH 4.0. The protein fractionation enriched with histatins is further purified using reverse-phase high-performance liquid chromatography on a C_{18} column. Purified histatins are evaporated to dryness, dissolved in deionized water, quantified by amino acid analysis, lyophilized, and stored at -20°C until use.

Example 2: Chemical Synthesis of Histatin-related Peptides

Histatin-related peptides are synthesized by the solid phase method of B. Merrifield, *Science* 232:341-47 (1986). Peptides are synthesized on a MilliGen/Bioresearch Sam-Two Peptide Synthesizer using Fmoc L-amino acid kits (Millipore, Bedford, MA), and purified on a TSK ODS-i20T C_{18} column (5μm, 4.6 x 250 nm) using RP-HPLC (Pharmacia-LKB). The purified peptides are quantified by amino acid analysis on a Beckman System 6300 amino acid analyzer.

Example 3: Antibacterial activity of a histatin-related peptide derivative P-113 against various strains of *P. aeruginosa*

Pseudomonas clinical isolates were gathered from cystic fibrosis patients, ranging in age from 14 to 51, from different parts of the country. These isolates were exposed to a variety of antibiotics that are commonly used to treat pulmonary infections, including ceftazidime, imipenem, amikacin, piperacillin, aztreonam, tobramycin, ciprofloxacin, gentamicin, ticarcillin, and timentin. All of the isolates were resistant to the majority of these antibiotics, and some were resistant to all of these antibiotics. Five of these isolates were resistant to all antibiotics tested, including high doses of tobramycin and gentamicin. The susceptibility of these isolates to these antibiotics is

summarized in Table 1.

Table 1. Background information of cystic fibrosis clinical isolates

Strain ID	Age	Organism	City	State	Susceptibility
1-71 AM	18	*B. cepacia*	Toledo	OH	RR
1-76 AM	51	*P. aeruginosa*	Cleveland	OH	R
1-77 AM	27	*P. aeruginosa*	Dallas	TX	R
1-78 AM	20	*P. aeruginosa*	Birmingham	AL	R
1-82 AM	14	*P. aeruginosa*	St. Louis	MO	R
2-17 AM	10	*P. aeruginosa*	Los Angeses	CA	RR
2-24 AM	15	*P. aeruginosa*	Atlanta	GA	R
2-25 AM	21	*P. aeruginosa*	Durham	NC	R
2-87 AM	21	*P. aeruginosa*	Falls Church	VA	R
2-90 AM	26	*P. aeruginosa*	Cincinnati	OH	R
2-98-AL	33	*P. aeruginosa*	Cleveland	OH	RR
3-16 AL	23	*P. aeruginosa*	Providence	RI	R
3-20 AL	23	*P. aeruginosa*	Fort Worth	TX	R
3-36 AM	26	*P. aeruginosa*	Tulsa	OK	R
3-47 AM	24	*P. aeruginosa*	Peoria	IL	R
3-54 AM	18	*B. cepacia*	Lewisburg	PA	RR
3-66 AM	23	*P. aeruginosa*	Oklahoma City	OK	RR
4-34 AL	19	*P. aeruginosa*	Chattanooga	TN	R
4-36 AL	22	*P. aeruginosa*	Oklahoma City	OK	RR
4-68 AL	17	*P. aeruginosa*	Pittsburgh	PA	r_1
4-87AL	23	*B. pickettii*	Atlanta	GA	r_2
4-97 AL	17	*P. aeruginosa*	San Francisco	CA	R
5-39 AM	21	*P. aeruginosa*	Phoenix	AZ	r_3

Antibiotics tested against CF isolates: ceftazidime, imipenem, amikacin, piperacillin, aztreonam, tobramycin, ciprofloxacin, gentamicin, ticarcillin, and timentin.

RR = Resistant to all antibiotics, including high doses of tobramycin and gentamicin
R= Resistant to all antibiotics tested
r_1 = Resistant to all antibiotics tested except ticarcillin, timentin
r_2 = Resistant to all antibiotics tested except piperacillin, ticarcillin, timentin
r_3 = Resistant to all antibiotics tested except tobramycin, amikacin

These *Pseudomonas* clinical isolates, as well as a variety of *Pseudomonas* ATCC strains, were tested for their susceptibility to the histatin derivative having the sequence Ala-Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-NH_2 (P-113). P-113 was used at a concentration of 100 μg/mL to 0.75 μg/mL. *P. aeruginosa* ATCC 27853 was used as a control in each assay. The ATCC strains were tested using Direct Antibacterial Assay SOP 98-020; the clinical isolates were tested using either the Direct Antibacterial Assay (D) or Alamar Blue Antimicrobial/Antifungal Assay SOP 96-018 (A).

The Direct Assay is an MIC test in microtiter plate format in which cells resuspended in LM broth are exposed to two-fold dilutions of P-113, and the cell density is determined directly after an overnight incubation. Cells are inoculated onto a blood agar plate and incubated for 18 hours at 37°C, and then stored at room temperature for use later in the day. Cells are resuspended and diluted based on the optical density to a final concentration of 5×10^5 per ml in LM broth containing two-fold dilutions of antibiotics. After 16-18 hours at 37°C, the optical density is measured with a Molecular Devices Thermonix plate reader. A reading of ~0.3 over background is observed for cells grown in the absence of drug. Readings $\leq$ 0.01 correlated with no visible growth. At least two independent tests were conducted with each strain, and if matching MICs were not obtained, at least one additional test was conducted to determine the MIC.

The composition of LM broth is as follows: 5% Cation-Adjusted Mueller Hinton Broth Supplemented with 2.2 mM Sodium Phosphate, 0.1 mM Magnesium Sulfate, 1.0 mM Sodium Citrate, all to final concentrations. In addition, 0.4 mg $ZnCl_2$, 2.0 mg $FeCl_3*10H_2O$, all per liter of medium. Also Glucose, Amino Acid Mix, and Vitamin Mix all from Life Technologies RPMI-1640 Select-Amine Kit, supplemented as instructed by the vendor (Accumed International, Inc., Westlake, OH.

The Alamar Blue biological assay was used to compare the effects of

various antimicrobial and antifungal agents on *Candida albicans* with that of a reference standard. The effect of the antimicrobial agent on the growth of *C. albicans* was monitored using Alamar Blue, a growth indicator dye that is based on the detection of metabolic activity. The dye incorporates an oxidation-reduction (redox) indicator that changes color in response to chemical reduction of growth medium resulting from cell growth. The redox indicator exhibits a colorimetric change in the appropriate oxidation-reduction range relating to cellular metabolic activity and produces a clear, stable and distinct change which can be quantitated using a spectrophotometric microtiter plate reader. A dose response curve was generated using both the test article and the reference standard (positive control). Zero % inhibition was defined as the result obtained when buffer without peptide is added to the cells (negative control). The 50% inhibition points (IC_{50}) were used to calculate the activity of the test article, which was expressed as percent activity relative to the reference standard. The IC_{50} for the reference standard fell within the range of 0.60 to 2.24 μg/mL under the conditions of this assay.

The results are shown in Table 2.

Table 2. P-113 susceptibility of ATCC strains and clinical cystic fibrosis isolates

Strain ID[1]	MIC (μg/mL)	Assay **
ATCC 19142	3.1	D
ATCC 19143	3.1	D
ATCC 27853	6.2	D
ATCC 43393	6.25	D
ATCC 51674	1.56	D
332*	3.1	D
8050-2*	3.12	D
5681-1*	6.2	D
1-76 AM	3.1	D

1-77 AM	25	A
1-78 AM	1.6	A
1-82 AM	1.56	D
2-17 AM	3.12	D
2-24 AM	1.6	A
2-25 AM	1.56	D
2-87 AM	1.56	D
2-90 AM	1.56	D
2-98-AL	>100	D
3-16 AL	1.6	A
3-20 AL	3.12	D
3-36 AM	3.12	D
3-47 AM	1.6	A
3-66 AM	3.12	D
4-34AL	3.1	A
4-36 AM	1.6	A
4-68 AL	4.1	A
4-97 AL	3.12	D
5-39 AM	6.25	D
2192*	6.25	D

[1] Strains from ATCC are designated. Most of the other strains are clinical CF isolates obtained from Dr. Lisa Saiman at Columbia University. Four strains (indicated with an asterisk) were obtained from Dr. Gerry Pier at Harvard University

As shown in Table 2, the peptide P-113 was effective against nearly all of the *Pseudomonas* strains tested.

Example 4: Antibacterial activity of a first group of histatin-related peptides against *P. aeruginosa*

Histatin derivatives having the following amino acid sequences were tested for activity against *P. aeruginosa*:

Ala-Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-NH_2 (P-113); Ala-Lys-

Arg-His-His-Lys-Tyr-Lys-Arg-Lys-Phe-His-NH_2 (P-113-K6); Ala-Lys-Arg-His-His-Gly-Tyr-His-Arg-Lys-Phe-His-NH_2 (P-113-H8); Ala-Lys-Arg-His-His-Lys-Tyr-His-Arg-Lys-Phe-His-NH_2 (P-113-K6H8); Ac-Ala-Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-NH_2 (P-113-NA); and Ala-Lys-Arg-Phe-Phe-Gly-Tyr-Lys-Arg-Lys-Phe-Phe-NH_2 (P-113-F4.5.12).

The standard procedure used for testing activity was incubation of the bacterial strain with the peptide for one hour at 37°C, followed by plating at two dilutions.

The results are shown in Fig. 3. As shown there, the peptides all showed antimicrobial activity against *P. aeruginosa*. All of the peptides were capable of killing at least 80% of the bacteria at peptide concentrations of about 0.5 μM and higher.

Example 5: Antibacterial activity of a second group of histatin-related peptides against *P. aeruginosa*

Peptides having the following amino acid sequences (including P-113, as in Example 4) were tested for activity against *P. aeruginosa* under the conditions described above:

Ala-Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-NH_2 (P-113); Ala-Lys-Arg-Tyr-Tyr-Gly-Tyr-Lys-Arg-Lys-Phe-Tyr-NH_2 (P-113-Y4.5.12); Ala-Lys-Arg-Leu-Leu-Gly-Tyr-Lys-Arg-Lys-Phe-Leu-NH_2 (P-113-L4.5.12); Ala-Gln-Arg-His-His-Gly-Tyr-Lys-Arg-Gln-Phe-His-NH_2 (P-113-Q2.10); Ala-Lys-Gln-His-His-Gly-Tyr-Lys-Gln-Lys-Phe-His-NH_2 (P-113-Q3.9); and Ala-Gln-Gln-His-His-Gly-Tyr-Lys-Gln-Gln-Phe-His-NH_2 (P-113-Q2.3.9.10).

The results are shown in Fig. 4. As shown there, P-113, P-113 Y4.5.12, and P-113 Q2.10 were very active against *P. aeruginosa*; P-113 Q3.9 and P-113 L4.5.12 were also active. P-113 Q2.3.9.10 was inactive against *P. aeruginosa*, even at high peptide concentrations.

Example 6: Antibacterial activity of a third group of histatin-related peptides against *P. aeruginosa*

Peptides having the following amino acid sequences were tested for antibacterial activity against *P. aeruginosa*:

Ala-Lys-Arg-Leu-Leu-Ser-Tyr-Lys-Arg-Lys-Phe-Leu-NH_2 (P-113-G6S-H4.5.12L); Ala-Orn-Arg-Tyr-Tyr-Gly-Tyr-Lys-Arg-Orn-Phe-Tyr-NH_2 (P-113-K2.10O-H4.5.12Y); Ala-Lys-Lys-His-His-Gly-Tyr-Lys-Lys-Lys-Phe-His-NH_2 (P113-R3.9K); His-Lys-Lys-His-His-Lys-Tyr-His-Lys-Lys-Phe-His-NH_2 (P-113 H-amphipathic); Leu-Lys-Lys-Phe-Phe-Gly-Tyr-Leu-Lys-Lys-Phe-Phe-NH_2 (P-113 amphipathic); and Ala-Gln-Gln-His-His-Gly-Tyr-Lys-Gln-Gln-Phe-His-NH_2 (P-113-KR2.3.9.10Q).

The results for two runs of the same assay are shown in Figs. 5 and 6. As shown there, all of the peptides tested in this series had activity against *P. aeruginosa*. All of the peptides killed at least 80% of the cells at peptide concentrations of about 2 μM.

Example 6: Antibacterial Activity of histatin-5 and P-113 against *P. aeruginosa*

P-113 and His-5 were tested against *P. aeruginosa* under the conditions described in the preceding examples. The dilution values were as follows: expected-2000 cfu/plate (in broth) from dilution A. A= 1/2.5 x dilution; plate 50 µl. B = 1/2.5 x dilution; plate 25 µl.

The results are shown in Table 3. Both peptides were effective in killing these bacteria, with P-113 exhibiting greater efficacy than His-5.

Table 3: P-113 and His 5: Killing of *P. aeruginosa*

	cfu/plate				% killing
Control 1	A	168	179	165	
	B	76	85	97	
Control 2	A	234	217	248	
	B	124	152	130	
His-5 200μM	A	0	0		100
	B	0	0		100
P-113 200 μM	A	0	0		100
	B	0	0		100
His-5 50μM	A	0	1		99
	B	0	2		98
P-113 50μM	A	0	0		100
	B	0	1		99
His-5 5μM	A	143	159		24
	B	73	68		36
P-113 5μM	A	1	4		99
	B	0	4		98
His-5 0.5μM	A	150	164		19
	B	90	96		16
P-113 5μM	A	133	170		24
	B	88	85		22

All publications and patents mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent was specifically and individually indicated to be incorporated by reference.

Other Embodiments

From the foregoing description, it will be apparent that variations and

modifications may be made to the invention described herein to adopt it to various usages and conditions. Such embodiments are also within the scope of the following claims.

What is claimed is:

Claims

1. A method for treating cystic fibrosis in a mammal comprising administering to said mammal an effective amount of a histatin or a histatin fragment.

2. The method of claim 1, wherein said histatin fragment has between 8 and 20 amino acids, inclusive.

3. The method of claim 2, wherein said histatin fragment has between 8 and 12 amino acids, inclusive.

4. The method of claim 1, wherein said histatin fragment has the amino acid sequence Ala-Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His.

5. The method of claim 4, wherein at least one of the amino acids in said sequence is a D-amino acid.

6. The method of claim 5, wherein said histatin fragment has the amino acid sequence D-Ala-D-Lys-D-Arg-D-His-D-His-D-Gly-D-Tyr-D-Lys-D-Arg-D-Lys-D-Phe-D-His.

7. The method of claim 1, wherein said histatin is histatin 5.

8. The method of claim 7, wherein at least one amino acid of said histatin 5 is a D-amino acid.

9. The method of claim 1, wherein said histatin, or said histatin fragment, has at least one modification selected from the group consisting of

(a) an acyl addition at the N-terminus;

(a) a carbamyl addition at the N-terminus; and

(b) an amide addition at the C-terminus.

10. The method of claim 1, wherein said mammal is a human.

11. The method of claim 1, wherein sais histatin, or said histatin fragment, is administered by inhalation.

12. A method for treating cystic fibrosis in a mammal comprising administering to said mammal an effective amount of a peptide having between 8 and 20 amino acids, inclusive, wherein said peptide has the amino acid sequence:

R1-R2-R3-R4-R5-R6-R7-R8-R9-R10-R11-R12-R13-R14-R15-R16-R17-R18-R19-R20-R21-R22-R23,

wherein

R1 is Asp or is absent;
R2 is Ser or is absent;
R3 is His or is absent;
R4 is Ala, His, Leu, or is absent;
R5 is Lys, Gln, Arg, Orn, or another basic amino acid;
R6 is Arg, Gln, Lys, or another basic amino acid;
R7 is His, Phe, Tyr, Leu, or another hydrophobic amino acid;
R8 is His, Phe, Tyr, Leu, or another hydrophobic amino acid;
R9 is Gly, Lys, Arg, Ser, or a basic amino acid;
R10 is Tyr;
R11 is Lys, His, Phe, Leu, or another hydrophobic amino acid;
R12 is Arg, Gln, Lys, or another basic amino acid;
R13 is Lys, Gln, Arg, Orn, another basic amino acid, or is absent;
R14 is Phe or is absent;
R15 is His, Phe, Tyr, Leu, another hydrophobic amino acid, or is absent;
R16 is Glu or is absent;
R17 is Lys or is absent;
R18 is His or is absent;
R19 is His or is absent;
R20 is Ser or is absent;
R21 is His or is absent;

R22 is Arg or is absent; and
R23 is Gly or is absent.

13. The method of claim 12, wherein at least one of R7, R8, R11, or R15 is Phe.

14. The method of claim 13, wherein R7 is Phe.

15. The method of claim 13, wherein R8 is Phe.

16. The method of claim 13, wherein R11 is Phe.

17. The method of claim 13, wherein R15 is Phe.

18. The method of claim 13, wherein all of R7, R8, and R15 are Phe.

19. The method of claim 12, wherein R9 is Lys.

20. The method of claim 12, wherein R11 is His.

21. The method of claim 12, wherein R9 is Lys and R11 is His.

22. The method of claim 12, wherein at least one of R7, R8, or R15 is Tyr.

23. The method of claim 21, wherein all of R7, R8, and R15 are Tyr.

24. The method of claim 12, wherein at least one of R5 and R13 is Gln.

25. The method of claim 23, wherein both R5 and R13 are Gln.

26. The method of claim 12, wherein at least one of R5 and R13 is Orn.

27. The method of claim 25, wherein both R5 and R13 are Orn.

28. The method of claim 12, wherein all of R1, R2, and R3 are absent.

29. The method of claim 12, wherein both R22 and R23 are absent.

30. The method of claim 12, wherein all of R20, R21, R22, and R23 are absent.

31. The method of claim 12, wherein all of R18, R19, R20, R21, R22, and R23 are absent.

32. The method of claim 12, wherein said peptide has the amino acid sequence Ala-Lys-Arg-Phe-Phe-Gly-Tyr-Lys-Arg-Lys-Phe-Phe.

33. The method of claim 12, wherein said peptide has the amino acid sequence Ala-Lys-Arg-His-His-Lys-Tyr-Lys-Arg-Lys-Phe-His.

34. The method of claim 12, wherein said peptide has the amino acid sequence Ala-Lys-Arg-His-His-Gly-Tyr-His-Arg-Lys-Phe-His.

35. The method of claim 12, wherein said peptide has the amino acid sequence Ala-Lys-Arg-His-His-Lys-Tyr-His-Arg-Lys-Phe-His.

36. The method of claim 12, wherein said peptide has the amino acid sequence Ala-Lys-Arg-Tyr-Tyr-Gly-Tyr-Lys-Arg-Lys-Phe-Tyr-NH_2.

37. The method of claim 12, wherein said peptide has the amino acid sequence Ala-Gln-Arg-His-His-Gly-Tyr-Lys-Arg-Gln-Phe-His-NH_2.

38. The method of claim 12, wherein said peptide has the amino acid sequence Ala-Orn-Arg-Tyr-Tyr-Gly-Tyr-Lys-Arg-Orn-Phe-Tyr-NH_2.

39. The method of claim 12, wherein said peptide has at least one modification selected from the group consisting of

(a) an acyl addition at the N-terminus;

(b) a carbamyl addition at the N-terminus; and

(c) an amide addition at the C-terminus.

40. The method of claim 12, wherein said mammal is a human.

41. The method of claim 12, wherein said peptide is administered by inhalation.

Histatin 3: Asp-Ser-His-Ala-Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His
1 5 10 15

-His-Ser-His-Arg-Gly-Tyr-Arg-Ser-Asn-Tyr-Leu-Tyr-Asp-Asn

20 25 30

Fig. 1

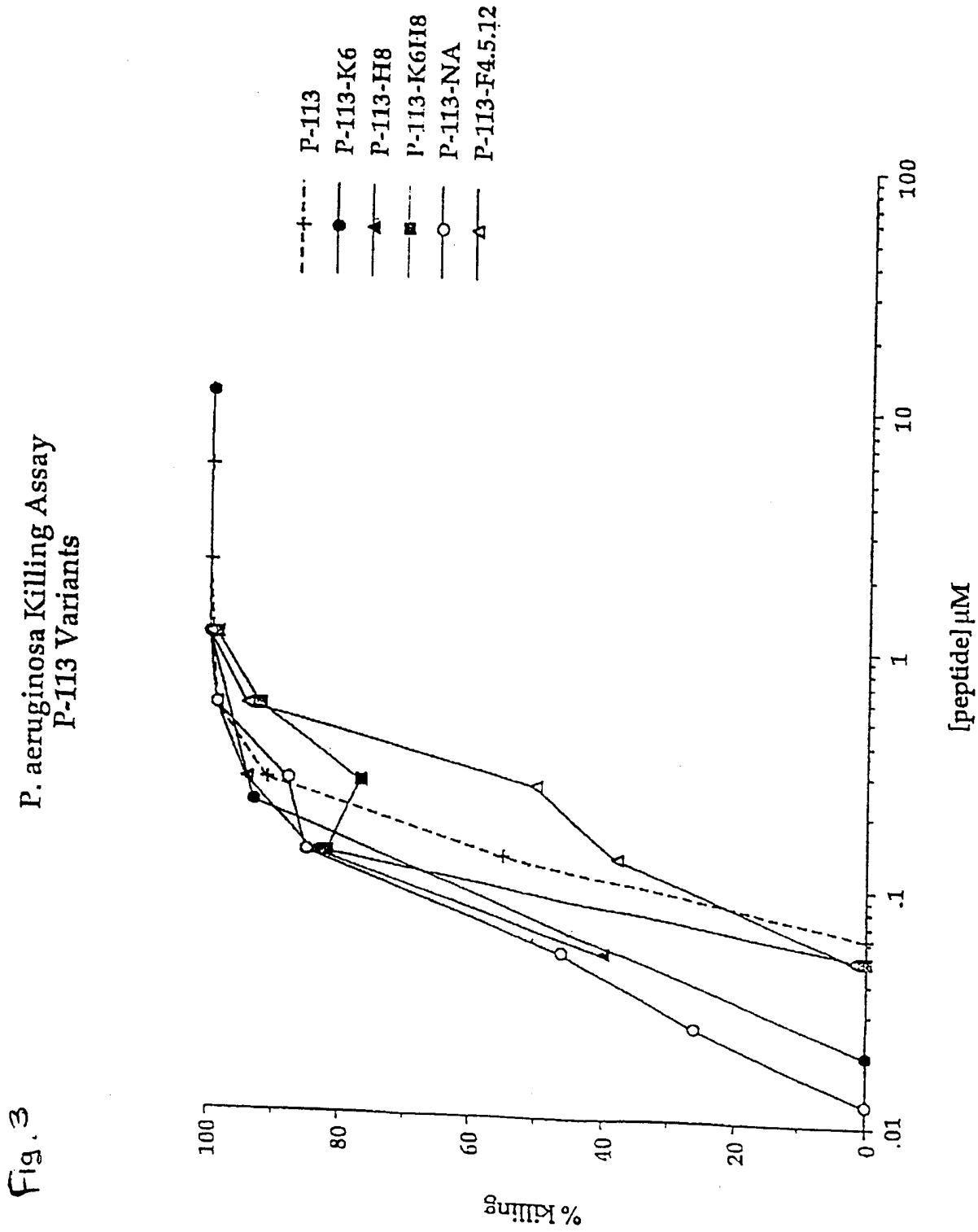
Histatin 5: Asp-Ser-His-Ala-Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His
1 5 10 15

-His-Ser-His-Arg-Gly-Tyr

20

Fig. 2

Fig. 3


P. aeruginosa Killing Assay
P-113 Variants
P-113
P-113-K6
P-113-H8
P-113-K6H8
P-113-NA
P-113-F4.5.12
% killing
0
20
40
60
80
100
.01
.1
1
10
100
[peptide] µM

Second Set of P-113 Variants
Effect on P. aeruginosa

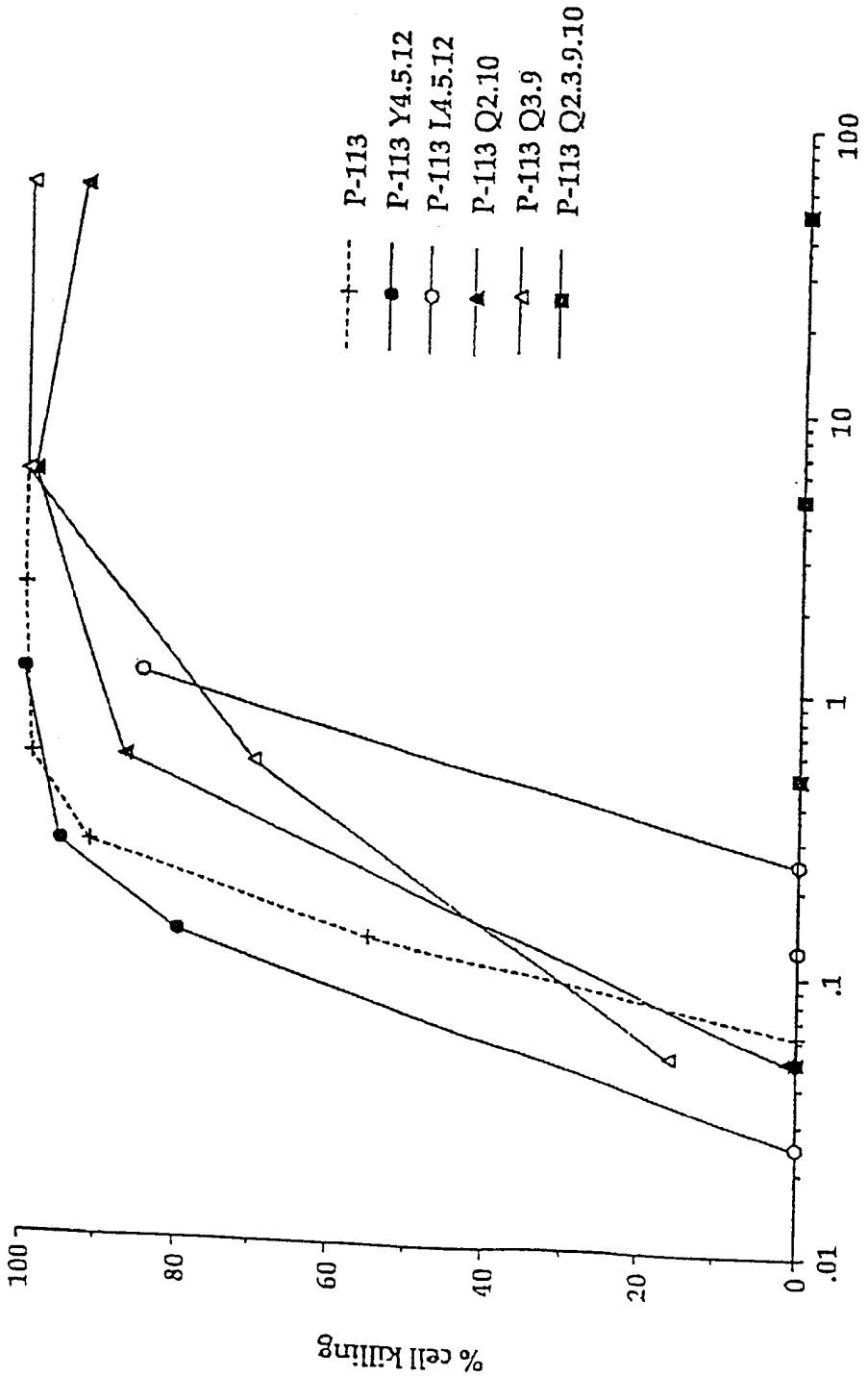


Fig. 4

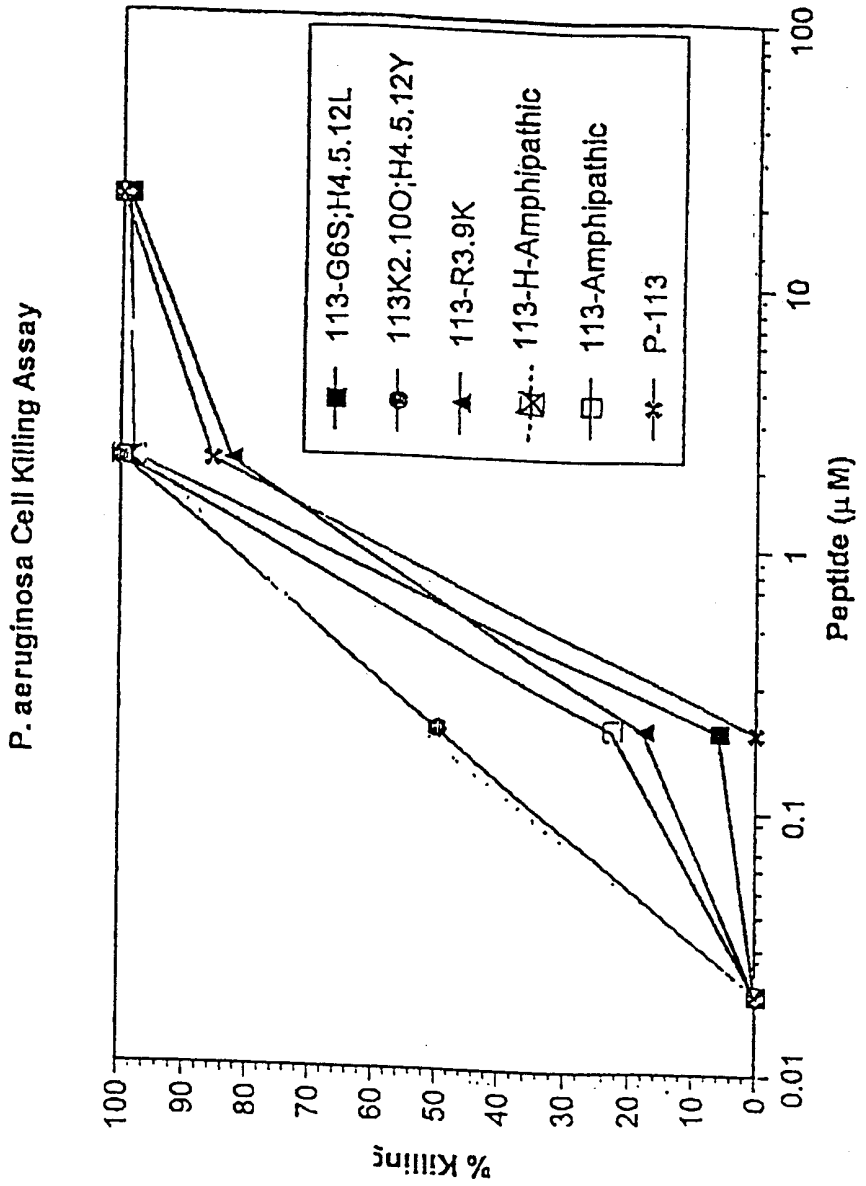

P. aeruginosa Cell Killing Assay
% Killing
Peptide (μM)
0.01
0.1
1
10
100
0
10
20
30
40
50
60
70
80
90
100
113-G6S;H4.5.12L
113K2.10O;H4.5.12Y
113-R3.9K
113-H-Amphipathic
113-Amphipathic
P-113

Fig. 5

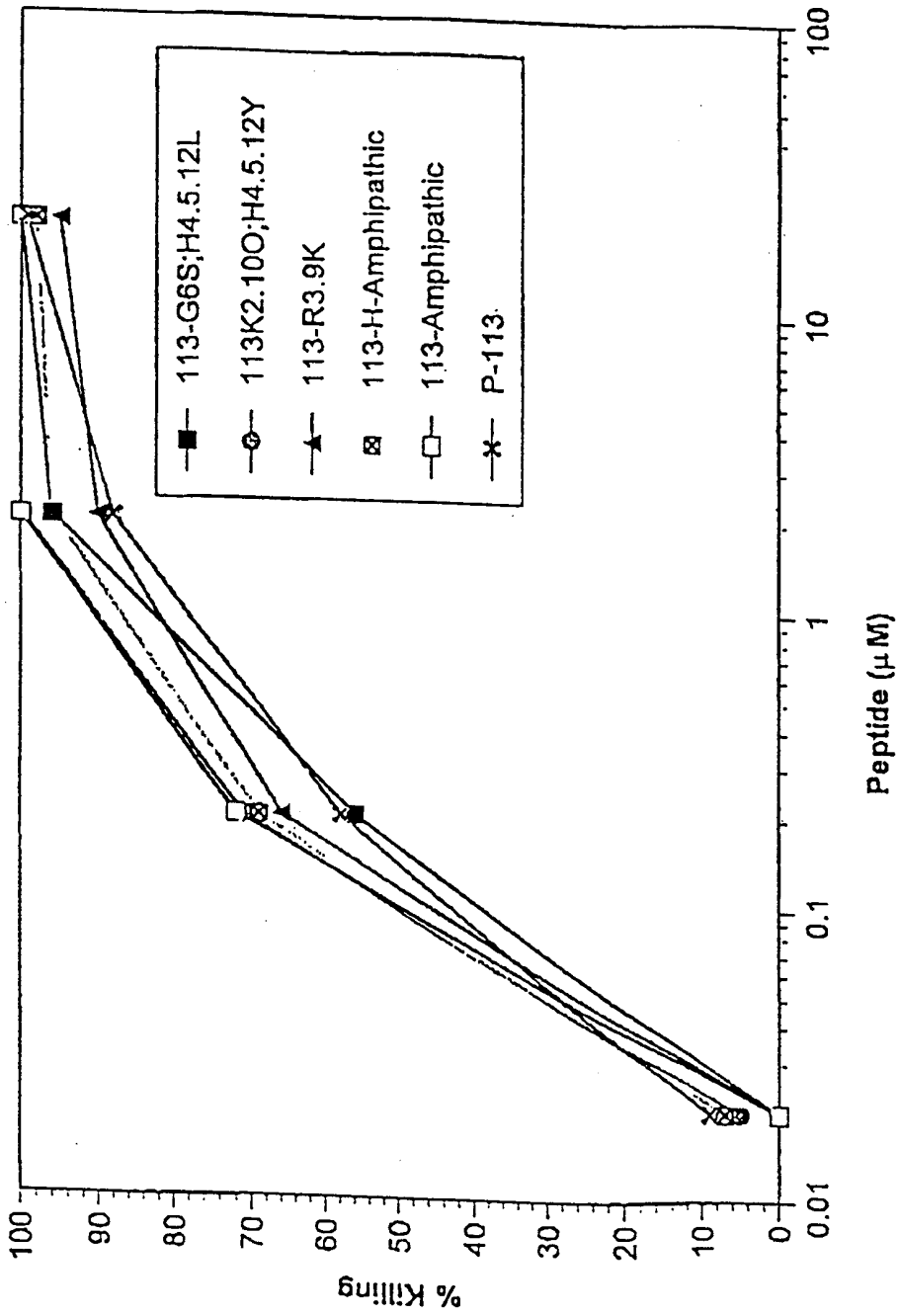

P. aeruginosa Cell Killing Assay #2
% Killing
Peptide (μM)
0
10
20
30
40
50
60
70
80
90
100
0.01
0.1
1
10
100
113-G6S;H4.5.12L
113K2.10O;H4.5.12Y
113-R3.9K
113-H-Amphipathic
113-Amphipathic
P-113

Fig. 6