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— 发明人资格(细则 4.17(iv))

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— 包括说明书序列表部分(细则 5.2(a))。

(54) **Title**: NEW ANTIBIOTIC CONTAINING SIMULACRUM ANTIBODY, PREPARATION METHOD AND APPLICATION THEREOF

(54) **发明名称** : 一种含抗体模拟物的新型抗生素及其制备方法与应用

(57) **Abstract**: The invention provides a new antibiotic containing simulacrum antibody. The antibiotic consists of colicin E1, Ia, Ib, A, B, N, or aqueous channel-forming structural domain and the simulacrum antibody which is covalently linked to the carboxyl terminal of the peptide chain of said colicin or aqueous channel-forming structural domain. The simulacrum antibody is formed by the carboxyl terminal of the heavy chain variable (VH) complementarity determining region (CDR) 1 of immunoglobulin connected with the amino terminal of the VH framework region (FR) 2 where the carboxyl terminal is further connected with the amino terminal of the light chain variable (VL) CDR, and the immunoglobulin specifically recognizes the bacterial porin. The antibiotic is useful for preparing antibacterial agents such as anti-Neisseria meningitides, anti-vancomycin-resistant Enterococcus, anti-methicillin-resistant Staphylococcus aureus or anti-multiple drug resistant Pseudomonas aeruginosa.

(57) **摘要**:

本发明提供了一种含抗体模拟物的新型抗生素。该抗生素由大肠菌素 E1、Ia、Ib、A、B、N 或其水性孔道结构域及共价连接在所述大肠菌素或其水性孔道结构域的肽链羧基端的抗体模拟物构成。所述抗体模拟物由免疫球蛋白的 V_HCDR1 的羧基端连接 V_HFR2 的氨基端，V_HFR2 的羧基端再连接 V_LCDR 的氨基端构成；所述免疫球蛋白特异性识别细菌膜孔蛋白。所述抗生素可用于制备抗脑膜炎双球菌、抗耐万古霉素肠球菌、抗耐甲氧西林金葡菌或抗多重耐药绿脓杆菌等抗菌药物。

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Specification

A novel antibiotic comprising an antibody mimetic, preparation methods and uses thereof

FIELD OF THE INVENTION

The present invention belongs to the domain of biology and medicine, and especially relates to a novel antibiotic comprising an antibody mimetic, its preparation methods and uses thereof.

RELATED ART

Since Penicillin and other antibiotics were brought into use in 1944, Diplococcus meningitides, and other life-threatening pathogenic bacteria such as *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, and *Neisseria meningitidis* have acquired drug resistance. According to relative reports published by United States Disease Control Center (CDC) in recent years, these antibiotics would be likely to lose effectiveness completely in 10 or 20 years.

The present antibiotics kill pathogenic bacteria by restraining synthesis of cell wall, restraining or interfering the pathway of bacterial nucleic acid and protein metabolism and synthesis. However, these antibiosis methods are more likely to lead to drug resistance resulted by bacteria mutation. Therefore scientists are dedicated to develop novel antibiotics. One of the comparatively promising directions is to imitate the inter-killing mechanism among homogeneous heterologous strains so as to develop novel antibiotics. In Nature, a number of bacterial toxins can directly form ion channels on bacteria cytomembrane to kill bacteria. The typical specimen is a kind of bacterial toxin secreted by *E.col*, colicin. One of which is Colicin Ia, found in 1952 by Jacob. After efforts of generations, the transmembrane spatial structure of Colicin Ia when ion channels open as well as shut on the artificial lipide bimolecular film (Qiu et al, Major transmembrane movement associated with Colicin Ia channel gating. J. Gen. Physiology, 107: 313-328 (1996)) was disclosed in 1996. This established theoretical laid the basis for designing and preparing novel antibiotics on molecular level.

As mentioned above, colicin is a kind of ideal ion channel antibiotic model, but wild-type colicin can only act on homogeneous heterologous strains. We must change the targeting of the colicin so that they are capable of acting on other pathogenic bacteria. Porin, a kind of pore protein existing on the outer membrane of bacteria, mitochondria or chloroplast, allows bigger molecules to pass through. Porin also has a higher immunogenicity, and can induce high level expression of monoclonal antibody in host cells. It should be an ideal development direction for antibody research, if we can design an antibody mimetic with better recognizing ability to change the targeting of the colicin, by using the antibody specific for porins on outer membran of bacteria as the antetype of the antibody mimetic.

SUMMARY OF THE INVENTION

To overcome the above technical defects and make up a deficiency in the art, the present invention provides a novel antibiotic. Its antibacterial ability is a thousand-fold more powerful than regular antibiotics. Due to its unique action mechanism, drug resistance resulted in mutation can hardly be acquired by pathogenic bacteria. And the antibiotic will not hurt normal human cells when it kills pathogenic bacteria.

A novel antibiotic comprising an antibody mimetic and a colicin,

or comprising an antibody mimetic and a channel-forming domain of a colicin,

the antibody mimetic covalently bonded to the carboxyl end of the polypeptide of the colicin or the channel-forming domain of the colicin, wherein the colicin is selected from a group consisting of colicin E1, Ia, Ib, A, B, N; wherein said antibody mimetic being yielded by fusing two complementarity determining regions (CDRs), V_HCDR_1 and V_LCDR through a cognate framework region (V_HFR_2) of an immunoglobulin; wherein the immunoglobulin specifically recognizes bacterium porins.

Wherein the bacterium is *Neisseria meningitides*, the porin is PorA in the outer membrane of *Neisseria meningitidis* cells.

Wherein the immunoglobulin consist of two covalent bonded polypeptides with PUBMED Accession 2MPA_H and PUBMED Accession 2MPA_L.

Wherein the colicin is Ia.

A peptide molecule, comprising an antibody mimetic and a colicin,
or comprising an antibody mimetic and a channel-forming domain of a colicin,
the antibody mimetic covalently bonded to the carboxyl end of the polypeptide of the colicin or the channel-forming domain of the colicin, wherein the colicin is selected from a group consisting of colicin E1, Ia, Ib, A, B, N; wherein said antibody mimetic being yielded by fusing two complementarity determining regions (CDRs), V_HCDR_1 and V_LCDR3 through a cognate framework region (V_HFR_2) of an immunoglobulin; wherein the immunoglobulin specifically recognizes bacterium porins.

One of the foresaid peptide molecules, its amino acid sequence set forth in SEQ ID NO.6.

A nucleic acid molecule encoding any one of foresaid peptide molecules.

Wherein the nucleic acid molecule with nucleotide sequence set forth in SEQ ID NO.5.

A recombinant plasmids containing any one of foresaid nucleic acid molecules.

A preparation method of any one of foresaid novel antibiotics, one of foresaid recombinant plasmids is transfected into an expression system, and the novel antibiotic is separated and purified from expressed polypeptide.

Any one of foresaid novel antibiotics used for preparing antibacterial medicament.

Wherein the antibacterial medicament is used for killing *Neisseria meningitidis*, vancomycin-resistant *Enterococcus faecalis*, methicillin-resistant *Staphylococcus aureus* or multidrug-resistance *Pseudomonas aeruginosa*..

The novel antibiotics of this invention are based on the foundation of colicin's characteristic of

forming ion channels on the target pathogen membrane, which causes the pathogen to leak out its content and die. The targeting structure is an antibody mimetic with some domains of an immunoglobulin specifically recognizing porin protein on target pathogen. The antibody mimetic comprises two complementarity determining regions (CDRs), V_HCDR_1 and V_LCDR3, and a cognate framework region (V_HFR_2) of an immunoglobulin; the three regions covalently form a linear peptide molecule as V_HCDR1-V_HFR2-V_LCDR3 from amino end to carboxyl end. It is well known that the active regions of an immunoglobulin for recognition reaction are called complementary determining region that has only about several to a dozen of amino acids. The antibody mimetic in the novel antibiotic, compared with a nature antibody molecule or any present artificial reconstructed antibody such as seFv and Fab, has smaller molecular weight, nicer tissue penetration and simpler structure without most parts of frame structure and Fc fragment of a nature antibody. So it is be able to reduce immunoreaction in patients and easily guides colicin contained in the novel antibiotic to infected tissues and identifies the pathogenic bacteria. In clinical application, the novel antibiotic is directed to membrane of the target pathogenic bacteria by antibody mimetic contained, and the colicin contained forms ion channels on bacteria cytomembrane of the target bacteria and kills the target bacteria by leading to leak-out of its cytoplasm. The antibacterial ability of the novel antibiotic also applies to bacterial strains with drug resistance. The recognition sites are unique antigenic properties on bacterial surface, and not located on human cytomembrane, so the novel antibiotic is safe for human. Compared to other antibiotics that easily cause drug resistance, the antibiotic in this invention kills the pathogenic bacteria not by the porin but by the colicin acting on biomembrane of the pathogenic bacteria and forming ion channels to make the cell leaking out the cytoplasm to die. The antibody mimetic for targeting just needs to guide the colicin to the pathogenic bacteria. Bacteria's drug resistance is normally acquired by changing porin's structure and creating barrier for antibiotic's entry. The antibiotic in this invention only requires porin's antibody recognition sites for purpose of killing the pathogenic bacteria. The novel antibiotics identify the sites of porin on the membrane of bacteria, but the colicin of the novel antibiotic binds on other sites and forms ion channels to make the bacteria to leak out to die. The action sites are not the porin. So the pathogenic bacteria is not likely to acquire drug resistance to the novel antibiotic by mutating, evoluting, throwing away or changing the structure of porin which is necessary for survival. According to this inventive concept, the novel antibiotic has many variants due to the diversity of porin on bacterium surface and the diversity of the immunoglobulin recognizing the

porins.

Since the meningitis caused by *Neisseria meningitidis* severe threatens infants and children's health, the drug resistance of *Neisseria meningitidis* to common medicine is very serious. The dosage is higher and higher in order to inhibit the bacteria efficiently, which seriously endangers the health of patients. Therefore, on the basis of foresaid inventive concept of this invention, the inventor reconstructed and obtained an antibody mimetic of an antibody which was specific for the porin A of *Neisseria meningitidis*. The heavy chain peptide of the antibody had an accession number: 2MPA_H at PubMed Home. The light chain peptide had an accession number: 2MPA_L at PubMed Home. The antibody mimetic, of which the amino acid sequence set forth in Seq ID No.2, was connected on the C-terminus of the Colicin Ia's peptide to constitute a novel antibiotic PMC-AM1 with amino acid sequence set forth in Seq ID No.6. As survival curve of mice shown in Fig.7, the survival rate of the mice injected with fatal dose of *Neisseria meningitides* in PMC-AM1 group was 90% in 8 days. It showed that the antibacterial activity and protective effect in vivo of the novel antibiotics are superior to current normal antibiotics such as penicillin and gentamicin. Meanwhile a comparative test on bactericidal effect was set to compare the minimum inhibitory concentration (MIC value) of PMC-AM1, ceftazidime and ampicillin for *Neisseria meningitidis*. As shown in Fig.6A, the MIC value of PMC-AM1 was 0.11nMol, ceftazidime was 3.02nMol and ampicillin was 1.35nMol. This result indicated that the bactericidal ability of PMC-AM1 is significantly more powerful than antibiotics currently used for inhibiting *Neisseria meningitidis*.

In present invention, some experiments were set to test the bactericidal effect of the novel antibiotic PMC-AM1 on other pathogens with serious drug-resistance. The result showed the PMC-AM1 had extremely stronger antibacterial ability on multi-drug resistant *Pseudomonas aeruginosa*, vancomycin-resistant *Enterococcus faecalis*, methicillin-resistant *Staphylococcus aureus*, as shown in Fig.5, its antibacterial ability on multi-drug resistant *Pseudomonas aeruginosa*, was 127 to 3800 times stronger than that of ceftazidime, levofloxacin, gentamicin etc. And as shown in Fig. 6B and Fig. 6C, PMC-AM1 has obvious inhibition effect on vancomycin-resistant *Enterococcus faecalis*, methicillin-resistant *Staphylococcus aureus*.

The novel antibiotic of this invention can be used to prepare antibacterial drugs, especially for

Neisseria meningitidis, *Pseudomonas aeruginosa*, vancomycin-resistant *Enterococcus faecalis* or methicillin-resistant *Staphylococcus aureus* .

The nucleotide sequences encoding the peptides of the novel antibiotics can be cloned into the expression vector to construct recombinant plasmids, which express fusion protein in host cell. The novel antibiotic protein is isolated from expressed fusion protein.

According to degeneracy of nucleotide codons, the nucleotide sencoding antibiotic of the invention is adjustable. One of skill in the art would be able to adjust the nucleotide sequence according to the host cells' preference to the nucleotide codon. As long as the encoded polypeptide has no change, the nucleotide sequences are still in the scope of inventive concept of this invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig.1 shows the structure of recombinant plasmid comprising the gene of antibody mimetic and gene of Colicin Ia, referred to herein as pBHC-PorA1.

The peptide of the antibody mimetic bonded to the C-terminus of Colicin Ia, and the amino acid sequence of the antibody mimetic is set forth in Seq ID No.2.

Fig.2 shows the structure of the recombinant plasmid comprising the gene of antibody mimetic and gene of Colicin Ia, referred to herein as pBHC-PorA2.

The peptide of antibody mimetic is linked to the C-terminus of Ia's peptide, and the amino acid sequence of the antibody mimetic set forth in Seq ID No.4. In the antibody mimetic, a V_HCDR1 and a reversed V_LCDR3 are connected through a cognate framework region (V_HFR_2).

Fig.3 illustrates the construction of the novel antibiotic.

In which, T and R are signal recognition domains on the N-terminus of a Colicin Ia. The channel forming, a structure domain being capable of forming ion channels, is situated at C-terminus of the Colicin Ia. The AM is an antibody mimetic.

Fig.4 shows the result of experiment for inhibiting ability of the novel antibiotic PMC-AM1 to *Diplococcus intracellularis.*

In the curve, from left to right shows the control, 5μg/ml Ampicillin, 5μg/ml PMC-AM2, 5μg/ml PMC-AM1, 10μg /ml PMC-AM1.

The horizontal ordinate shows growth time of bacteria, the unit is hours; the longitudinal ordinate shows optical density of the bacteria medium at 600 nm, indicating the quantity of bacteria growth.

Fig.5 shows the minimum inhibitory concentration value (MIC) of the novel antibiotic tested by Agar dilution method.

The plates shows the MIC of the drugs to multi-drug resistant *Pseudomonas aeruginosa:* Con was blank control, (A) the MIC of ceftazidime was 16 μg / ml, (B) the MIC of levofloxacin was 8 μg / ml, (C) the MIC of gentamicin was greater than 512 μg / ml, (D) the MIC of PMC-AM1 was 8 μg / ml.

Fig.6 shows the comparison experiment of minimum inhibitory concentration of the novel antibiotic of this invention with the commonly used antibiotics to methicillin-resistant *Staphylococcus aureus* (ATCC BAA-42), vancomycin-resistant *Enterococcus faecalis* (ATCC 700802), multi-drug resistant *Pseudomonas aeruginosa* (isolated by West China Hospital, No.13578) and *Nesseria meningitidis* (No.29332 of bacteria Preservation Center in China).

In which, the longitudinal ordinate shows minimum inhibitory concentration (nMol);

A shows the result of *Nesseria meningitidi*: (1) PMC-AM1, MIC = 0.11 nMol, (2) ceftazidime, MIC = 3.02 nMol, (3) ampicillin, MIC = 1.35 nMol;

B shows the result of vancomycin-resistant *Enterococcus faecalis* (1) PMC-AM1, MIC = 0.23 nMol, (2) vancomycin, MIC = 21.54 nMol, (3) ampicillin, MIC = 10.78 nMol;

C shows the result for methicillin-resistant *Staphylococcus aureus*:(1) PMC-AM1, MIC = 0.06 nMol, (2) ampicillin, MIC = 21.55 nMol, (3) oxacillin, MIC =14.1 nMol;

D shows the result for multi-drug resistant *Pseudomonas aeruginosa*: (1) PMC-AM1, MIC=0.91nMol, (2) levofloxacin, MIC=43.2 nMol, (3) ceftazidime, MIC=29.3 nMol, (4) gentamicin, MIC>889.4 nMol.

Fig.7 shows survival curve of comparison of inhibition effective of the novel antibiotic, the wild-type colicin and the polypeptide anti-*Staphylococcal aureus* disclosed in China patent ZL 01128836.1 on methicillin-resistant *Staphylococcus aureus* (ATCC BAA-42), vancomycin-resistant *Enterococcus faecalis* (ATCC 700802), and multi-drug resistant *Pseudomonas aeruginosa* (isolated by West China Hospital, No.13578).

In which, longitudinal ordinate shows minimum inhibitory concentration(nMol);

A shows the result for vancomycin-resistant *Enterococcus faecalis*: (1) the polypeptide anti-*Staphylococcus aureus*, MIC = 0.91nMol, (2) the wild-type Colicin Ia, MIC =0.91 nMol, (3) PMC-AM1, MIC = 0.23 nMol;

B shows the result for methicillin-resistant *Staphylococcus aureus*: (1) the polypeptide anti-*Staphylococcus aureus*, MIC = 0.06nMol, (2) the wild-type Colicin Ia, MIC =0.23 nMol, (3) PMC-AM1, MIC = 0.06 nMol.

C shows the result for multidrug resistance *Pseudomonas aeruginosa* : (1) the polypeptide anti-*Staphylococcus aureus*, MIC = 0.91 nMol, (2) the wild-type Colicin Ia, MIC =0.91 nMol, (3) PMC-AM1,MIC=0.23 nMol.

Fig.8 shows survival curves, which were the result of in vivo experiment of the novel antibiotics to protect animals infected by *Nesseria meningitidis*.

In which, horizontal ordinate shows the survival time of mice, days; Longitudinal ordinate shows the number of animal survival. 1) PMC-AM1; 2) Gen was gentamicin; 3) PEN was penicillin; 4) Con. was blank control. The injection concentration of all test drugs is by 1.5mg/kg (drug's weight/ mouse's weight).

EMBODIMENTS

The invention is further illustrated by the following embodiments as well as the drawings.

Embodiment 1: Construction of plasmids expressing the novel antibiotics and preparation of the novel antibiotics

The original plasmid was pSELECT™-1 plasmid (8.3kb) with genes of Colicin Ia and immunity protein. By Double Strands Oligo nucleotide Point Mutation Technology (QuickChange™ Kit, Strategene corporation) the gene encoding the antibody mimetic set forth in SEQ ID NO. 1 or 3 were inserted to 626 amino acid position of Colicin Ia gene. Two recombinant plasmids, herein referred to as pBHC-PorA1 and pBHC-PorA2 shown in Fig.1 and Fig. 2, were constructed, which were used to prepare the novel antibiotics. The recombinant plasmids were respectively transfected into *E.coli* BL-21 engineering bacterium to express the novel antibiotics.

The mutation procedure was preceded according to the manual of Strategene Quick Change Site Directed Mutagenesis Kit (catalog #200518),

1. Point mutation reactant was prepared as follows:

5μl 10X buffer

2μl (10ng) original plasmid pSELECT^{TM}-1 with genes of Colicin Ia and immunity protein.

1.25μl (125ng) artificial 5'-3' oligo nucleotide primer (refer to below oligo nucleotide primer)

1.25μl (125ng) artificial 3'-5' oligo nucleotide primer (refer to below oligo nucleotide primer)

1μl dNTP

50μl de-ionized water

1μl Pfu

(The above drugs were all reagents in the medical kit, except plasmid, primer and de-ionized water.)

2. PCR amplification was proceeded with the amplification conditions as follow: denaturalize at 95 ℃ for 35 seconds, anneal at 53℃ for 70 seconds, extend at 68℃ for 17 minutes, totally 20 cycles.

3. 1μl endonuclease Dpn1 was incorporated to digest parent DNA chain(37℃, 1h); 1μl digestion product was placed on ice and incubated with 50μl XL1-Blue competent cells for 30 minutes, heat shock at 42℃ for 45 seconds, and then taken into ice for 2 minutes;

4. 0.5ml NZY culture medium was added, the bacteria solution (reactant of step 3, i.e. transformed cells from competent cells) was shaken at 220 rpm and 37℃ for 1 hour; then 50-100 μl reactant was taken out to plank on medium plate (LB culture medium with 1% agar and 50μg/ml ampicillin, at 37℃ over night);

5. The bacteria was picked out after cultivating 18 hours, the plasmid was abstracted and sequenced to ascertain it had successful mutation;

6. 100ng mutated plasmid was placed on ice and incubated with 40 μl of BL-21 competent cells for 5.minutes, heat shock at 42℃ for 30 seconds, and then placed on ice for 2 minutes. 160 μl SOC culture medium was added; bacteria was shaken at 220 rpm, 37℃ for 1 hour and taken out to plank on medium plate (LB culture medium with 1% agar, 50μg/ml ampicillin, at 37℃ cultivating one night); monoclone colonies are picked out for largely reproducing;

7. The bacteria was largely reproduced in 8-10L FB culture medium, at 250 rpm, 30℃ for 3-4 hours, and warmed to 42℃ at 250 rpm for 0.5hours and then cooled to 37℃at 250 rpm for 1.5hours. The thallus was centrifugated at 4℃, 6000g for 20 minutes, and then was suspended in 80-100 ml of 50mM boric acid buffer fluid (pH 9.0, with 2mM EDTA) at 4℃. After being added in 50μg PMSF the thallus was ultrasonicated at 4℃, 400W for 1 minute and repeated 4-5 times with 2-3 minutes interval for maintaining the temperature of the bacteria solution. The cracked thallus

was high-speed centrifugated at 4℃, 75,000g for 90 minutes. The 5,000,000 unit streptomycin sulfate was added into the supernatant to deposit DNA (stiring at 4℃ for 1 hour). After centrifugated at 10000g, 4℃ for 10 minutes, the supernatant was loaded in bag filter of 15,000 molecular weight and dialysed by 10L of 50 mM boric acid buffer fluid over night at 4℃; then centrifugated again at 10000g , 4℃ for 10 minutes. The supernatant was loaded on CM ion-exchange column. Then the CM ion-exchange column was washed thoroughly and the novel antibiotic was eluted by 0.3 M NaCl+50 mM boric acid buffer fluid. Corresponding to the above two kinds of recombinant plasmid, the novel antibiotics were named PMC-AM1 和 PMC-AM2 of which the amino acid sequences were set forth in Seq ID No.6 and Seq ID No.8 respectively.

The AM1 was a peptide chain comprising of the peptides of the first complementarity determining domain in variable region of the heavy chain, the peptide of the second frame region of the heavy chain and the peptide of the third complementarity determining domain in variable region of the light chain. Its amino acid sequence was set forth in Seq ID No.2. The AM2 was a peptide chain comprising of the peptide of the first complementarity determining domain in variable region of the heavy chain, the peptide of the second frame region of the heavy chain and the peptide of reversed third complementarity determining domain in variable region of the light chain. Its amino acid sequence was set forth in Seq ID No.4.

The PMC-AM2 was constructed as a control of the PMC-AM1 for testing the activity of the novel antibiotics of present invention when the domains composing the antibody mimetic were connected in different order.

The artificial oligo nucleotide sequences for preparing above two mutation plasmids respectively are as follow:

5'—3' (SEQ ID NO.9)

gcg aat aag ttc tgg ggt att *TCT TAT TGG CTG CAT TGG ATT AAA CAG* taa ata aaa tat aag aca ggc

3'—5' (SEQ ID NO.10)

gcc tgt ctt ata ttt tat tta *CTG TTT AAT CCA ATG CAG CCA ATA AGA* aat acc cca gaa ctt att cgc

5'—3' (SEQ ID NO.11)

tgg ctg cat tgg att aaa cag *AGA CCT GGT CAG GGA CTG TGG ATC GGA* taa ata aaa tat aag aca ggc

3'—5' (SEQ ID NO.12)

gcc tgt ctt ata ttt tat tta *TCC GAT CCA CAG TCC CTG ACC AGG TCT* ctg ttt aat cca atg cag cca

5'—3' (SEQ ID NO.13)

ggt cag gga ctg tgg atc gga *TCT CAG TCC ACG CAT GTG CCG AGA ACC* taa ata aaa tat aag aca ggc

3'—5' (SEQ ID NO.14)

gcc tgt ctt ata ttt tat tta *GGT TCT CGG CAC ATG CGT GGA CTG AGA* tcc gat cca cag tcc ctg acc

pBHC-PorA 2

5'—3' (SEQ ID NO.15)

gcg aat aag ttc tgg ggt att *TCT TAT TGG CTG CAT TGG ATT AAA CAG* taa ata aaa tat aag aca ggc

3'—5' (SEQ ID NO.16)

gcc tgt ctt ata ttt tat tta *CTG TTT AAT CCA ATG CAG CCA ATA AGA* aat acc cca gaa ctt att cgc

5'—3' (SEQ ID NO.17)

tgg ctg cat tgg att aaa cag *AGA CCT GGT CAG GGA CTG TGG ATC GGA* taa ata aaa tat aag aca ggc

3'—5' (SEQ ID NO.18)

gcc tgt ctt ata ttt tat tta *TCC GAT CCA CAG TCC CTG ACC AGG TCT* ctg ttt aat cca atg cag cca

5'—3' (SEQ ID NO.19)

ggt cag gga ctg tgg atc gga *ACC AGA CCG GTG CAT ACG TCC CAG TCT* taa ata aaa tat aag aca ggc

3'—5' (SEQ ID NO.20)

gcc tgt ctt ata ttt tat tta *AGA CTG GGA CGT ATG CAC CGG TCT GGT* tcc gat cca cag tcc ctg acc.

Embodiment 2: Inhibiting effect of the novel antibiotic on *Nesseria meningitidis*.

The bacteria was strain No. 29332 *Nesseria meningitidis* of Bacteria Preservation Center in China), two microliters (μl) of bacteria solution (10^5CFU/ml) was added in 10ml rabbit blood-chocolate medium (containing 50mg beef extract, 100mg tryptone, 50mg NaCl, 30 mg K_2HPO_4 and 0.5-0.8 ml off fiber rabbit blood). Five groups were prepared. The first group as control was added 0.3 M NaCl + 50 mM boric acid buffer fluid (i.e. blank preservative fluid for the novel antibiotic, by the same volume as the novel antibiotic solution in the experimental groups). Penicillin sodium was added in the second group by 5μg/ml. The novel antibiotic PMC-AM1 was added in the third group by 5μg/ml. The novel antibiotic PMC-AM2 was added in the forth group by 5μg/ml. The novel antibiotic PMC-AM1 was added in the fifth group by 10μg/ml.

Reactant liquid of the above five groups were respectively put into 100ml conical flask, and cultured at 37℃ by 200rpm. 100μl culture solution was sampled per hour and added onto 96-pore ELISA plate for measuring bacterial turbidity by spectrophotometer (A595nm) color comparison. The bacteria-growth curve was drawn up to compare the bacteriostatic efficacy of the novel antibiosis. The result, as shown in Fig.4, showed that *Nesseria meningitidis* could only be restrained by PMC-AM1.

Embodiment 3. Contrast experiment of the minimum inhibitory concentration of the novel antibiotic and normal antibiotics on multi-drug resistant *Pseudomonas aeruginosa.*

Testing the minimum inhibitory concentration (MIC) of the novel antibiotic by the agar dilution method. The bacteria were inoculated on the surface of agar plate containing different concentrations of drugs by multipoint inoculate instrument (Deneley A400). The bacteria concentration on per point was 10^5 CFU/ml. After incubated at 37℃ for 18-24 hours, the result can be observed. The least concentration of drugs in the plating medium without bacteria growth was Minimum Inhibitory Concentration (MIC) of the drug to the said bacteria.

Experimental strain was multi-drug resistant *Pseudomonas aeruginosa* which was a clinical isolated strain (isolated by West China Hospital, No.13578) using MH medium (per 100ml containing 500mg beef extract, 1.75g casein acid hydrolysate, 150mg soluble starch and 1.7g agar).

As the result showed in Fig.5, the MIC of the novel antibiotic (D) PMC-AM1 on multi-drug resistant *Pseudomonas aeruginosa* was 8 μg/ml, ceftazidime (A) was 16 μg/ml, levofloxacin (B) was 8 μg/ml, and gentamicin (C) was greater than 512 μg/ml. If in terms of molecular weight standard, the MIC of PMC-AM1 on multi-drug resistant *Pseudomonas aeruginosa* was 0.23 nMol, ceftazidime (A) was 29.3 nMol, levofloxacin was 43.2 nMol, and gentamicin(C) was greater than 890 nMol, i.e. the antibacterial effect of PMC-AM1 on multi-drug resistant *Pseudomonas aeruginosa* was stronger 127-3800 times than ceftazidime, levofloxacin and gentamicin.

Embodiment 4. Contrast experiment of the antibacterial activity in vitro between the novel antibiotic of this invention and normal antibiotics.

Testing the minimum inhibitory concentration (MIC) of the novel antibiotic by the agar dilution method. The bacteria were inoculated on the surface of agar plate containing different

concentrations of drugs by multipoint inoculate instrument (Deneley A400). The bacteria concentration per point was 10^5CFU/ml. After incubated at 37℃ for 18-24 hours, the result can be observed. The least concentration of drugs in the plating medium without bacteria growth was Minimum Inhibitory Concentration (MIC) of the drug to the said bacteria.

Experimental strains were multi-drug resistant *Pseudomonas aeruginosa* which is a clinical isolated strain (isolated by West China Hospital, No.13578) using MH medium (Per 100ml containing 500mg beef extract and 1.75g_casein acid hydrolyzate, 150mg soluble starch, 1.7g_gelose), methicillin-resistant *Staphylococcus aureus* (ATCC BAA-42) using BM medium (Per 100ml containing 1g tryptone, 0.5g yeast powder, 0.1g glucose, 100mg KH_2PO_4, 1g NaCL and 1g_agar), vancomycin-resistant *Enterococcus faecalis* (ATCC 700802) using the MH medium; *Nesseria meningitidis* (No.29332 of Bacteria Preservation Center in China) using the same medium used in the_embodiment 2 (in addition added Columbia blood agar base 3.9g).

The result shown in Fig.6, A showed the result of *Nesseria meningitidis*: (1) PMC-AM1, MIC = 0.11nMol, (2) ceftazidime, MIC=3.02 nMol, (3) ampicillin, MIC =1.35 nMol. B showed the result of vancomycin-resistant *Enterococcus faecalis*: (1) PMC-AM1, MIC=0.23 nMol, (2) vancomycin, MIC=21.54 nMol, (3) ampicillin, MIC=10.78 nMol. C showed the result for methicillin-resistant *Staphylococcus aureus:* (1) PMC-AM1, MIC=0.06nMol, (2) ampicillin MIC=21.55nMol, (3) oxacillin, MIC =14.1 nMol. D showed the result for multi-drug resistant *Pseudomonas aeruginosa*: (1) PMC-AM1, MIC=0.91nMol, (2) levofloxacin, MIC=43.2 nMol, (3) ceftazidime, MIC=29.3 nMol (4) gentamicin, MIC>889.4 nMol.

Embodiment 5. Contrast experiments of the antibacterial activity in vitro between the novel antibiotic of this invention, the polypeptide anti-*Staphylococcal aureus* and wild-type Colicin Ia.

Testing the minimum inhibitory concentration (MIC) of the novel antibiotic by the agar dilution method. The least concentration of drugs in the plating medium without bacteria growth was Minimum Inhibitory Concentration (MIC) of the drug to the said bacteria.

Experimental strains were multi-drug resistant *Pseudomonas aeruginosa* (isolated by West China Hospital, No.13578),methicillin-resistant *Staphylococcus aureus* (ATCC BAA-42), vancomycin-resistant *Enterococcus faecalis* (ATCC 700802), using MH medium; *Nesseria meningitidis* (No.29332 of bacteria Preservation Center in China).

The results shown in Fig.7, A showed the result for vancomycin-resistant *Enterococcus faecalis* : (1)the polypeptide anti-*Staphylococcal aureus*, MIC = 0.91nMol, (2) the wildtype Colicin Ia, MIC =0.91 nMol, (3) PMC-AM1,MIC = 0.23 nMol. B showed the result for methicillin-resistant *Staphylococcus aureus*: (1) the polypeptide anti-*Staphylococcal aureus*, MIC = 0.06nMol, (2) the wildtype Colicin Ia, MIC=0.23 nMol, (3) PMC-AM1, MIC=0.06nMol. C showed the result for multidrug resistance *Pseudomonas aeruginosa*:(1)the polypeptide anti-*Staphylococcal aureus*, MIC = 0.91nMol, (2) the wildtype Colicin Ia, MIC =0.91 nMol, (3) PMC-AM1, MIC = 0.23 nMol

Embodiment 6. In vivo protection experiments of the novel antibiotics for animals infected by *Nesseria meningitidis*.

Experimental materials

Drugs: PMC-AM1, gentamicin, ampicillin.

Experimental bacteria

Nesseria meningitides (No.29332 of Bacteria Preservation Center in China, i.e Center of Medical Devices of National Institute for the Control of Pharmaceutical & biological Products, SDA).

Experimental methods

As shown in Fig.7, 40 mice were divided into four experimental groups, 10 mice in each group. The mice were given an intraperitoneal injection of glucose solution of ferrous by 20mg/kg, and 1 hour later an intraperitoneal injection of 0.5 ml of bacteria culture containing 1 share of *Nesseria meningitidis*cultrue solution (its CFU was 2.36 x 10^9/ml) and 1.5 share of 5% dry yeast solution. One hour later after being given intraperitoneal injection of fatal dose of the bacteria culture, the mice in experimental group were given an intravenous injection of the drugs and the mice in control group were given an intravenous injection of normal saline, all drugs were injected by1.5mg/kg), observation every 2 hours for 8 days, the death of mice as the positive results.

In the Fig.7, 1.PMC-AM1 means the novel antibiotic obtained in this invention; 2. Gen means gentamicin; 3. PEN means penicillin; 4. Con. means control.

Results.

As the survive curve shown in Fig.7, after being given intraperitoneal injection of fatal dose of *Neisseria meningitides* solution, 1). Mice in the control group all died in two days, 2). Mice in penicillin group all died in two days, 3). Mice in gentamicin group had 50%survival rate in 8 days,

4).Mice in PMC-AM1 group had 90% survival rate in 8 days.

The result indicates the novel antibiotic PMC-AM1 of the present invention performed superior protection activity in vivo on mice infected by fatal dose of *Nesseria meningitidis* than common antibiotics.

Claims

1. A novel antibiotic comprising an antibody mimetic and a colicin,

or comprising an antibody mimetic and a channel-forming domain of a colicin,

the antibody mimetic covalently bonded to the carboxyl end of the polypeptide of the colicin or the channel-forming domain of the colicin, wherein the colicin is selected from a group consisting of colicin E1, Ia, Ib, A, B, N; wherein said antibody mimetic being yielded by fusing two complementarity determining regions (CDRs), V_HCDR_1 and V_LCDR through a cognate framework region (V_HFR_2) of an immunoglobulin; wherein the immunoglobulin specifically recognizes bacterium porins.

2. The novel antibiotic of claim 1, wherein the bacterium is *Neisseria meningitides*, the porin is PorA in the outer membrane of *Neisseria meningitidis* cells.

3. The novel antibiotic of claim 2, wherein the immunoglobulin consist of covalent bonded polypeptide with PUBMED Accession 2MPA_H and a polypeptide with PUBMED Accession 2MPA_L.

4. The novel antibiotic of claim 1 or claim 2 or claim 3, wherein the colicin is Ia.

5. A peptide molecule comprising an antibody mimetic and a colicin,

or comprising an antibody mimetic and a channel-forming domain of a colicin,

the antibody mimetic covalently bonded to the carboxyl end of the polypeptide of the colicin or the channel-forming domain of the colicin,, wherein the colicin is selected from the group consisting of colicin E1, Ia, Ib, A, B, N; wherein said antibody mimetic being yielded by fusing two complementarity determining regions (CDRs), V_HCDR_1 and V_LCDR3 through a cognate framework region (V_HFR_2) of an immunoglobulin; wherein the immunoglobulin specifically recognizes bacterium porins.,

6. One of the fusion peptide molecules of claim 5, its amino acid sequence sets forth in SEQ ID NO.6

7. A nucleic acid molecule encoding any fusion peptide molecule claimed in claim 5 or claim 6.

8.The nucleic acid molecule of claim 7, its nucleotide sequence sets forth in SEQ ID NO.5.

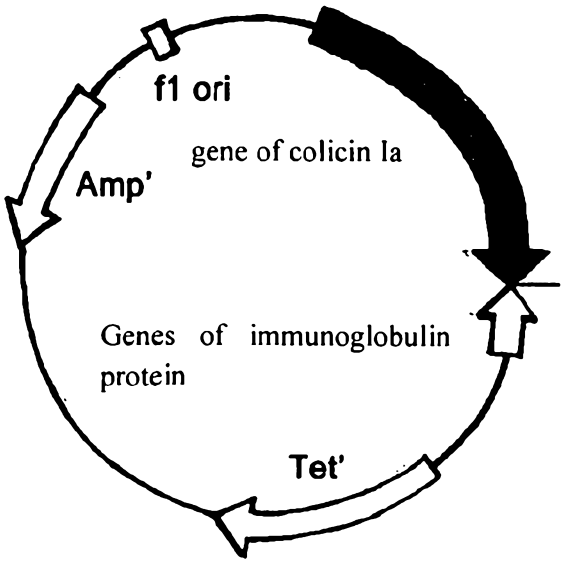
9. A recombinant plasmids containing any nucleic acid molecule of claim 7 or 8.

10. A preparation method of any one of the novel antibiotics claimed in claim1~4, one of the recombinant plasmids of claim 9 is transfected into an expression system, and the novel antibiotic is separated and purified from expressed polypeptide.

11. A use of any one novel antibiotics of claims 1~4 for preparing antibacterial medicament.

12. The use of claim 11, wherein the antibacterial medicament is used for killing *Neisseria meningitidis*, vancomycin-resistant *Enterococcus faecalis*, methicillin-resistant *Staphylococcus aureus* or multidrug-resistance *Pseudomonas aeruginosa.*

Fig.1



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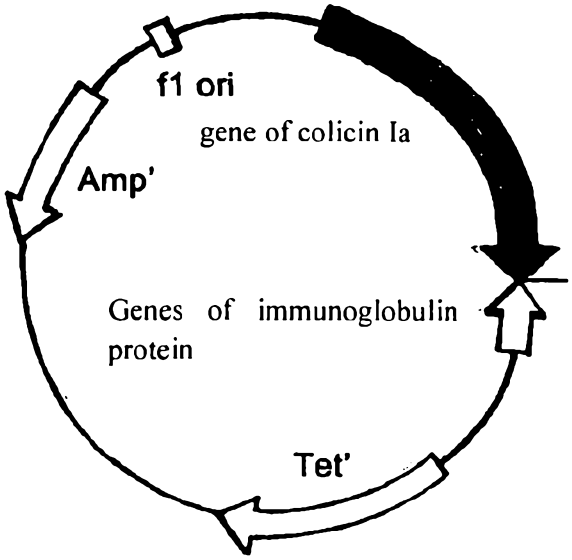

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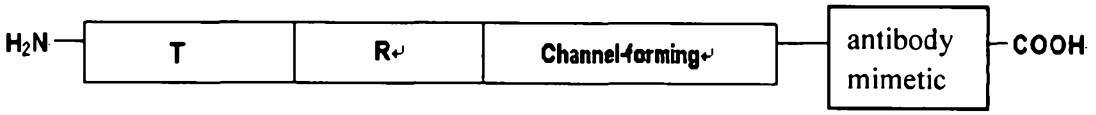
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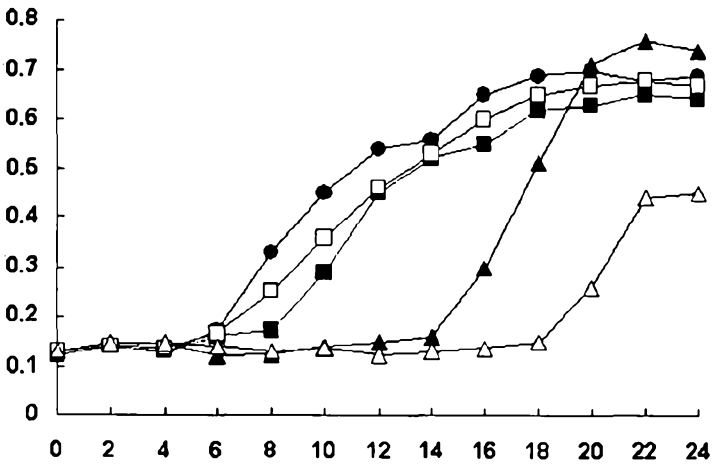
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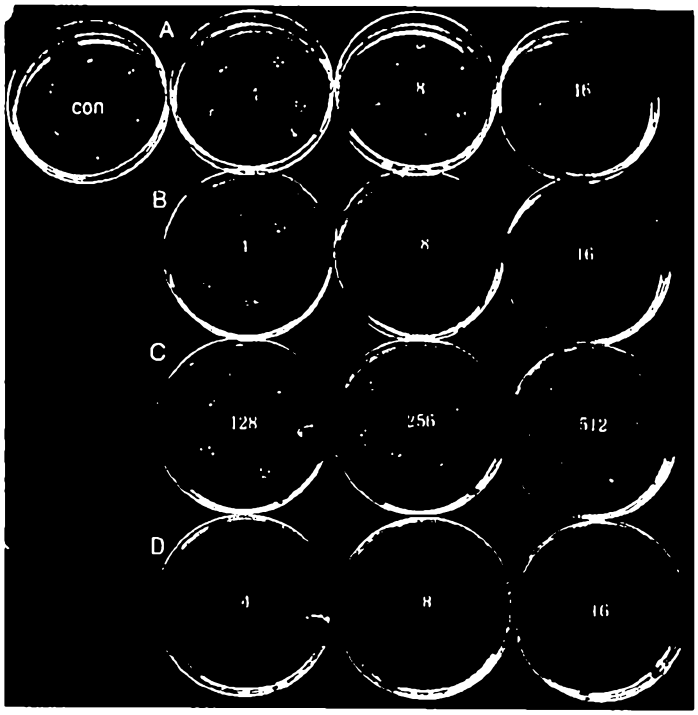
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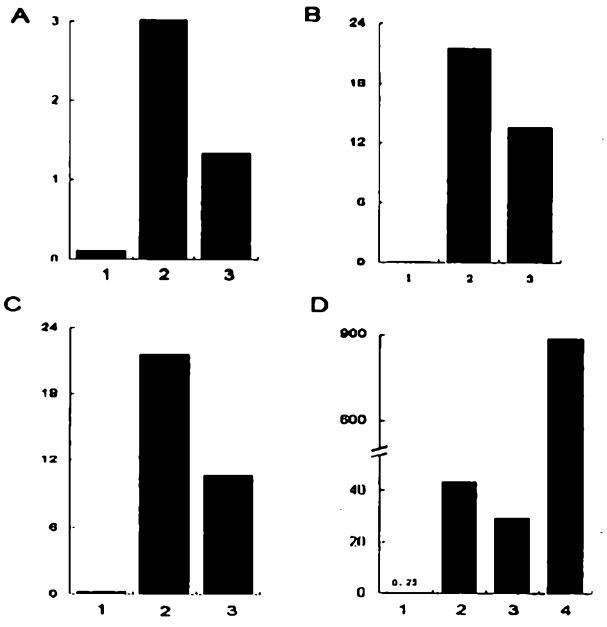
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Fig.7

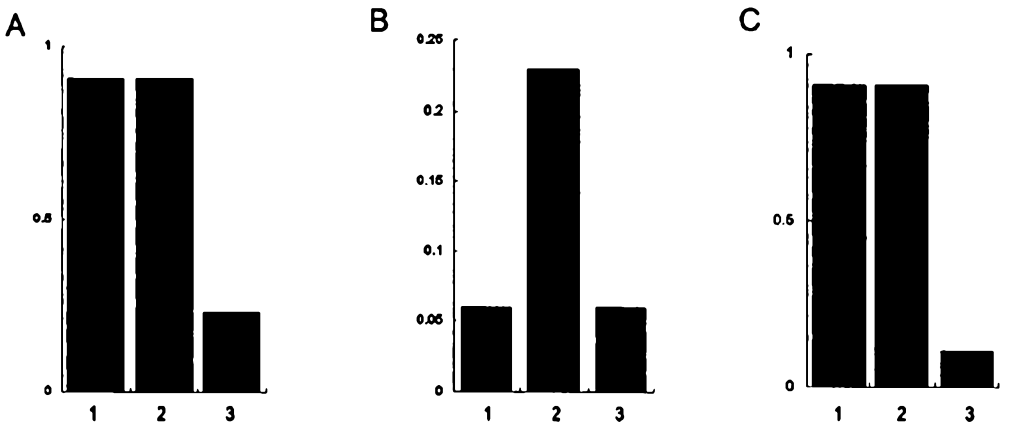
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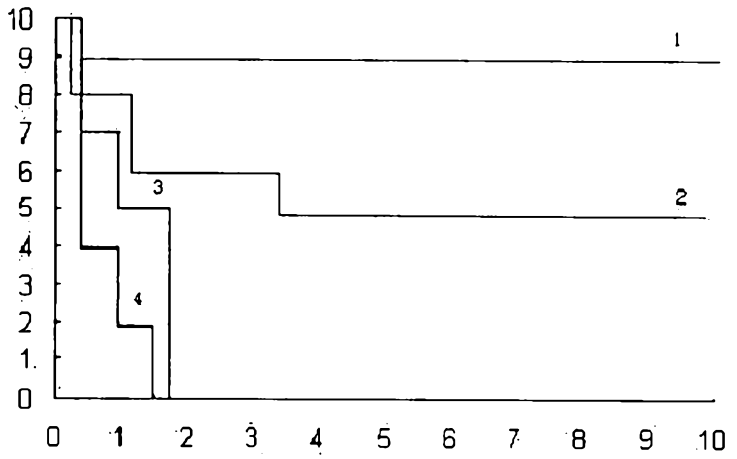
Fig.8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ctcagtgcca gaacaaatga gcaaaagcat gcaaatgacg ctcttaatgc cctgttgaag 1260

gaaaaagaga atatacgtaa ccagctttcc ggcatcaatc agaagatagc ggaagagaaa 1320

agaaaacagg atgaactgaa ggcaacgaaa gacgcaatta atttcacaac agagttcctg 1380

aaatcagttt cagaaaaata tggtgcaaaa gctgagcagt tagccagaga gatggccggg 1440

caggctaaag ggaagaaaat acgtaatgtt gaagaggcat taaaaacgta tgaaaagtac 1500

cgggctgaca ttaacaaaaa aattaatgca aaagatcgtg cagcgattgc cgcagccctt 1560

gagtctgtga agctgtctga tatatcgtct aatctgaaca gattcagtcg gggactggga 1620

tatgcaggaa aatttacaag tcttgctgac tggatcactg agtttggtaa ggctgtccgg 1680

acagagaact ggcgtcctct ttttgttaaa acagaaacca tcatagcagg caatgccgca 1740

acggctcttg tggcactggt cttcagtatt cttaccggaa gcgctttagg cattatcggg 1800

tatggtttac tgatggctgt caccggtgcg ctgattgatg aatcgcttgt ggaaaaagcg 1860

aataagttct ggggtatttc ttattggctg cattggatta aacagagacc tggtcaggga 1920

ctgtggatcg gaaccagacc ggtgcatacg tcccagtct 1959

<210> 8
<211> 651
<212> PRT
<213> The nucleotide sequence of antibiotic PMC-SA2

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Asp Ser Asp Gly His Glu Ile Met Ala Val Asp Ile Tyr Val Asn Pro
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Pro Arg Val Asp Val Phe His Gly Thr Pro Pro Ala Trp Ser Ser Phe
35 40 45

Gly Asn Lys Thr Ile Trp Gly Gly Asn Glu Trp Val Asp Asp Ser Pro

50 55 60

Thr Arg Ser Asp Ile Glu Lys Arg Asp Lys Glu Ile Thr Ala Tyr Lys
65 70 75 80

Asn Thr Leu Ser Ala Gln Gln Lys Glu Asn Glu Asn Lys Arg Thr Glu
85 90 95

Ala Gly Lys Arg Leu Ser Ala Ala Ile Ala Ala Arg Glu Lys Asp Glu
100 105 110

Asn Thr Leu Lys Thr Leu Arg Ala Gly Asn Ala Asp Ala Ala Asp Ile
115 120 125

Thr Arg Gln Glu Phe Arg Leu Leu Gln Ala Glu Leu Arg Glu Tyr Gly
130 135 140

Phe Arg Thr Glu Ile Ala Gly Tyr Asp Ala Leu Arg Leu His Thr Glu
145 150 155 160

Ser Arg Met Leu Phe Ala Asp Ala Asp Ser Leu Arg Ile Ser Pro Arg
165 170 175

Glu Ala Arg Ser Leu Ile Glu Gln Ala Glu Lys Arg Gln Lys Asp Ala
180 185 190

Gln Asn Ala Asp Lys Lys Ala Ala Asp Met Leu Ala Glu Tyr Glu Arg
195 200 205

Arg Lys Gly Ile Leu Asp Thr Arg Leu Ser Glu Leu Glu Lys Asn Gly
210 215 220

Gly Ala Ala Leu Ala Val Leu Asp Ala Gln Gln Ala Arg Leu Leu Gly
225 230 235 240

Gln Gln Thr Arg Asn Asp Arg Ala Ile Ser Glu Ala Arg Asn Lys Leu
245 250 255

Ser Ser Val Thr Glu Ser Leu Asn Thr Ala Arg Asn Ala Leu Thr Arg
260 265 270

Ala Glu Gln Gln Leu Thr Gln Gln Lys Asn Thr Pro Asp Gly Lys Thr
275 280 285

Ile Val Ser Pro Glu Lys Phe Pro Gly Arg Ser Ser Thr Asn His Ser
290 295 300

Ile Val Val Ser Gly Asp Pro Arg Phe Ala Gly Thr Ile Lys Ile Thr
305 310 315 320

Thr Ser Ala Val Ile Asp Asn Arg Ala Asn Leu Asn Tyr Leu Leu Ser

325 330 335

His Ser Gly Leu Asp Tyr Lys Arg Asn Ile Leu Asn Asp Arg Asn Pro
340 345 350

Val Val Thr Glu Asp Val Glu Gly Asp Lys Lys Ile Tyr Asn Ala Glu
355 360 365

Val Ala Glu Trp Asp Lys Leu Arg Gln Arg Leu Leu Asp Ala Arg Asn
370 375 380

Lys Ile Thr Ser Ala Glu Ser Ala Val Asn Ser Ala Arg Asn Asn Leu
385 390 395 400

Ser Ala Arg Thr Asn Glu Gln Lys His Ala Asn Asp Ala Leu Asn Ala
405 410 415

Leu Leu Lys Glu Lys Glu Asn Ile Arg Asn Gln Leu Ser Gly Ile Asn
420 425 430

Gln Lys Ile Ala Glu Glu Lys Arg Lys Gln Asp Glu Leu Lys Ala Thr
435 440 445

Lys Asp Ala Ile Asn Phe Thr Thr Glu Phe Leu Lys Ser Val Ser Glu
450 455 460

Lys Tyr Gly Ala Lys Ala Glu Gln Leu Ala Arg Glu Met Ala Gly Gln
465 470 475 480

Ala Lys Gly Lys Lys Ile Arg Asn Val Glu Glu Ala Leu Lys Thr Tyr
485 490 495

Glu Lys Tyr Arg Ala Asp Ile Asn Lys Lys Ile Asn Ala Lys Asp Arg
500 505 510

Ala Ala Ile Ala Ala Ala Leu Glu Ser Val Lys Leu Ser Asp Ile Ser
515 520 525

Ser Asn Leu Asn Arg Phe Ser Arg Gly Leu Gly Tyr Ala Gly Lys Phe
530 535 540

Thr Ser Leu Ala Asp Trp Ile Thr Glu Phe Gly Lys Ala Val Arg Thr
545 550 555 560

Glu Asn Trp Arg Pro Leu Phe Val Lys Thr Glu Thr Ile Ile Ala Gly
565 570 575

Asn Ala Ala Thr Ala Leu Val Ala Leu Val Phe Ser Ile Leu Thr Gly
580 585 590

Ser Ala Leu Gly Ile Ile Gly Tyr Gly Leu Leu Met Ala Val Thr Gly

595 600 605

Ala Leu Ile Asp Glu Ser Leu Val Glu Lys Ala Asn Lys Phe Trp Gly
610 615 620

Ile Ser Tyr Trp Leu His Trp Ile Lys Gln Arg Pro Gly Gln Gly Leu
625 630 635 640

Trp Ile Gly Thr Arg Pro Val His Thr Ser Gln
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<211> 69
<212> DNA
<213> Artificial sequence of constructing mutation plasmid pBHC-PorA1, 5'-3'

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aagacaggc 69

<210> 10
<211> 69
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<213> Artificial sequence of constructing mutation plasmid pBHC-PorA1, 3'-5'

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cttattcgc 69

<210> 11
<211> 69
<212> DNA
<213> Artificial sequence of constructing mutation plasmid pBHC-PorA1, 5'-3'

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aagacaggc 69

<210> 12
<211> 69
<212> DNA
<213> Artificial sequence of constructing mutation plasmid pBHC-PorA1, 3'-5'

<400> 12
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atgcagcca 69

<210> 13
<211> 69
<212> DNA
<213> Artificial sequence of constructing mutation plasmid pBHC-PorA1, 3'-5'

<400> 13
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aagacaggc 69

<210> 14
<211> 69
<212> DNA
<213> Artificial sequence of constructing mutation plasmid pBHC-PorA1, 3'-5'

<400> 14
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tccctgacc 69

<210> 15
<211> 69
<212> DNA
<213> Artificial sequence of constructing mutation plasmid pBHC-PorA2, 5'-3'

<400> 15
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aagacaggc 69

<210> 16
<211> 69
<212> DNA
<213> Artificial sequence of constructing mutation plasmid pBHC-PorA2, 3'-5'

<400> 16
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cttattcgc 69

<210> 17
<211> 69
<212> DNA
<213> Artificial sequence of constructing mutation plasmid pBHC-PorA2, 5'-3'

<400> 17
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aagacaggc 69

<210> 18
<211> 69
<212> DNA
<213> Artificial sequence of constructing mutation plasmid pBHC-PorA2, 3'-5'

<400> 18
gcctgtctta tattttattt atccgatcca cagtccctga ccaggtctct gtttaatcca 60

atgcagcca 69

<210> 19
<211> 69
<212> DNA
<213> Artificial sequence of constructing mutation plasmid pBHC-PorA2, 5'-3'

<400> 19
ggtcagggac tgtggatcgg aaccagaccg gtgcatacgt cccagtctta ataaaatat 60

aagacaggc 69

<210> 20
<211> 69
<212> DNA
<213> Artificial sequence of constructing mutation plasmid pBHC-PorA2, 3'-5'

<400> 20
gcctgtctta tattttattt aagactggga cgtatgcacc ggtctggttc cgatccacag 60

tccctgacc 69