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(54) **BROAD-SPECTRUM ANTIVIRAL DRUG FOR ENTEROVIRUS, AND APPLICATION**

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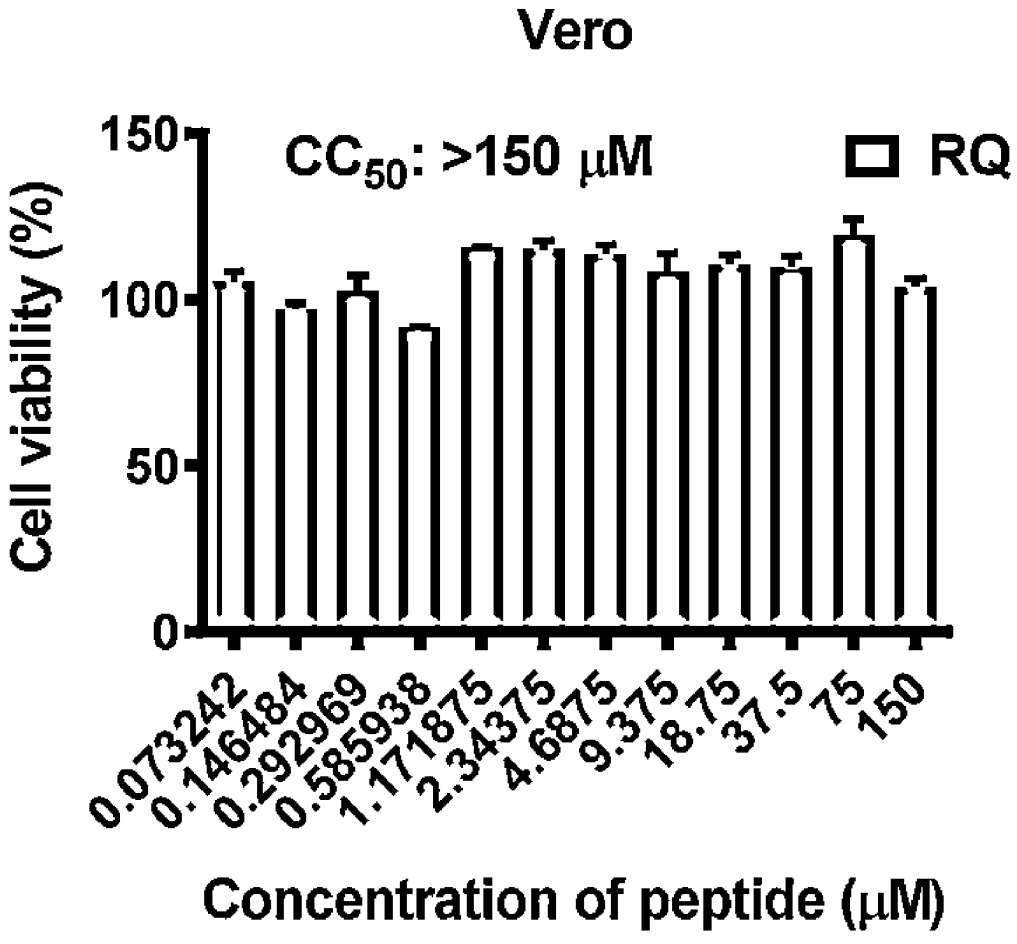
(52) **U.S. Cl.**

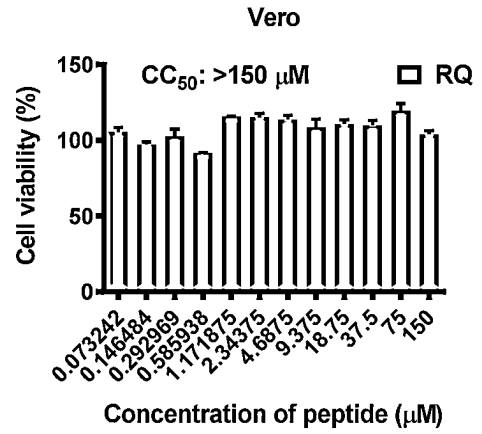
CPC ***C07K 7/08*** (2013.01); ***A61K 38/10*** (2013.01); ***A61P 31/14*** (2018.01); ***C07K 19/00*** (2013.01)

(57) **ABSTRACT**

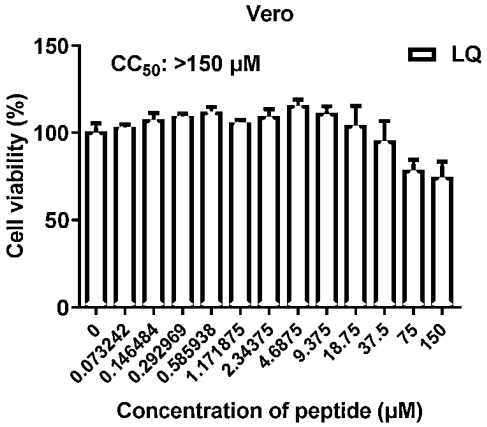
One of the core sequences of a polypeptide inhibitor provided by the present invention is as shown in SEQ ID NO.1, and the sequence of a polypeptide comprising a cell-penetrating peptide is as shown in SEQ ID NO.2. The polypeptide provided by the present invention uses enterovirus 2C protein multimerization as a target. Compared with other inhibitors targeting an enterovirus 2C protein, the present invention has high inhibition efficiency, good safety, and provides a new policy for enterovirus prevention and control.

Specification includes a Sequence Listing.

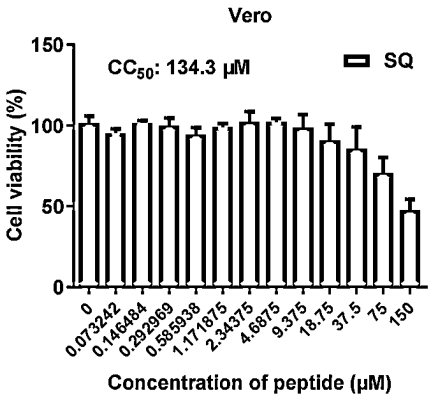



Vero
CC50: >150 μM
RQ
Cell viability (%)
150
100
50
0
0.073242
0.146484
0.292969
0.585938
1.171875
2.34375
4.6875
9.375
18.75
37.5
75
150
Concentration of peptide (μM)

A


Vero
CC50: >150 μM
LQ
Cell viability (%)
150
100
50
0
0
0.073242
0.146484
0.292969
0.585938
1.171875
2.34375
4.6875
9.375
18.75
37.5
75
150
Concentration of peptide (μM)

B


Vero
CC50: 134.3 μM
SQ
Cell viability (%)
150
100
50
0
0
0.073242
0.146484
0.292969
0.585938
1.171875
2.34375
4.6875
9.375
18.75
37.5
75
150
Concentration of peptide (μM)

C

FIG. 1A-C

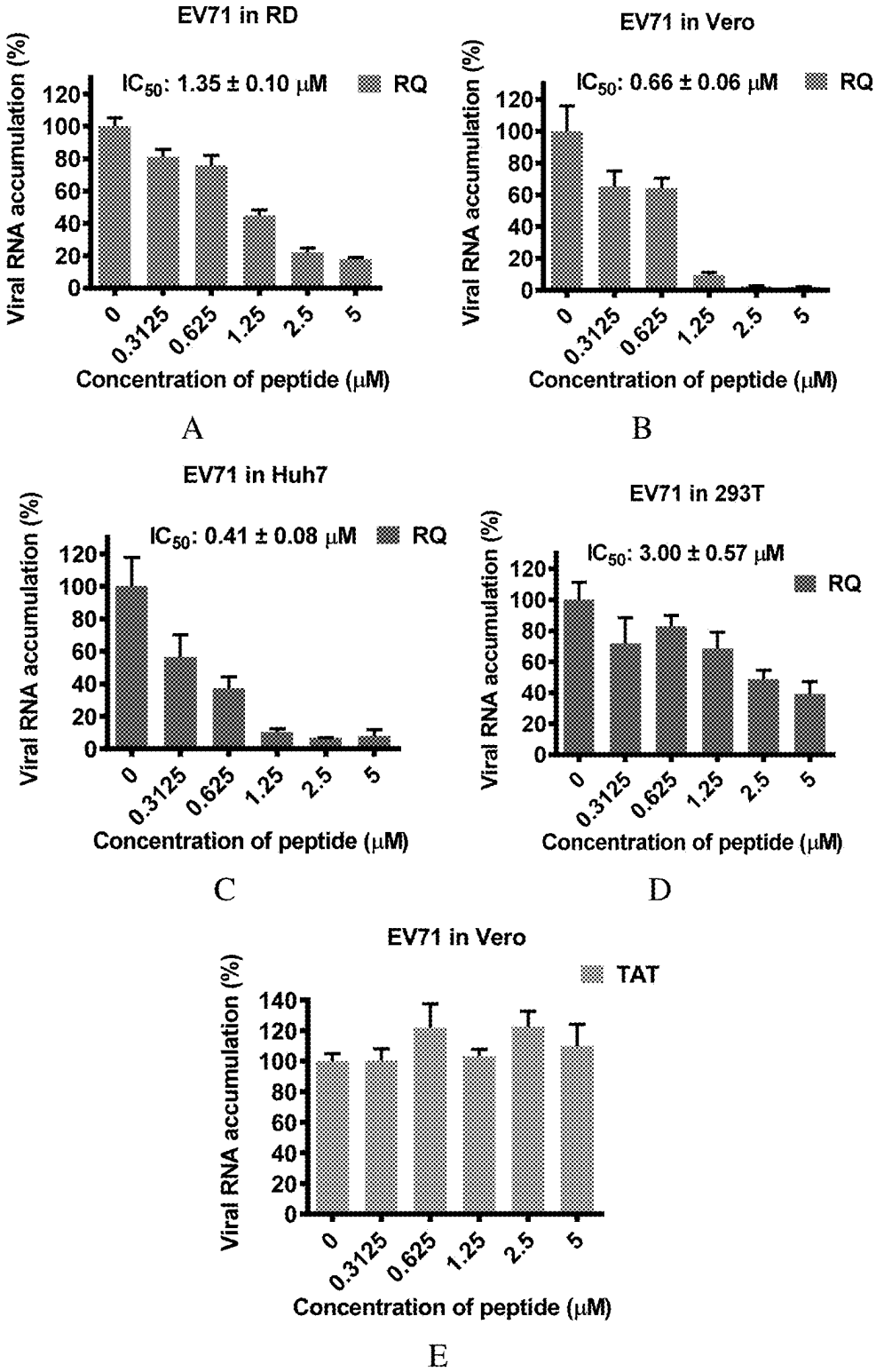
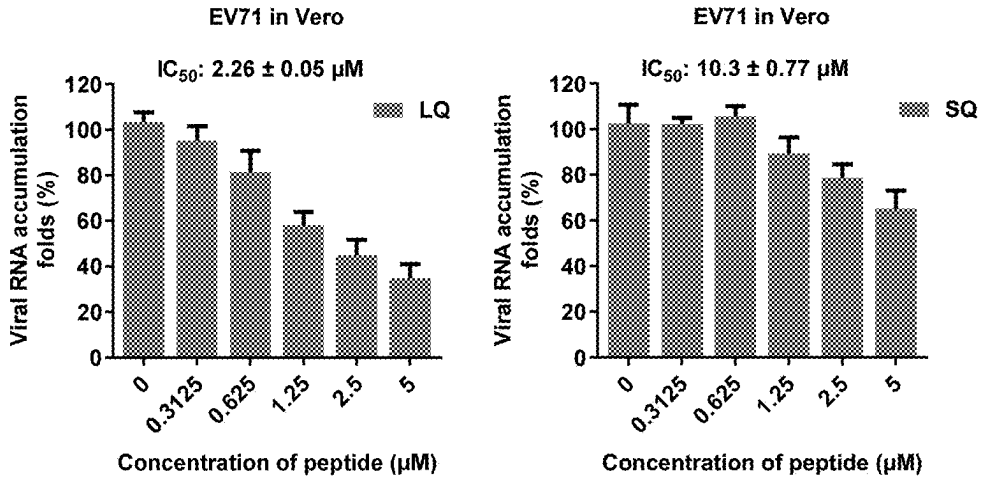

EV71 in RD
IC50: 1.35 ± 0.10 μM
RQ
Viral RNA accumulation (%)
120
100
80
60
40
20
0
0
0.3125
0.625
1.25
2.5
5
Concentration of peptide (μM)
A
EV71 in Vero
IC50: 0.66 ± 0.06 μM
RQ
Viral RNA accumulation (%)
Concentration of peptide (μM)
B
EV71 in Huh7
IC50: 0.41 ± 0.08 μM
RQ
Viral RNA accumulation (%)
Concentration of peptide (μM)
C
EV71 in 293T
IC50: 3.00 ± 0.57 μM
RQ
Viral RNA accumulation (%)
Concentration of peptide (μM)
D
EV71 in Vero
TAT
Viral RNA accumulation (%)
140
120
100
80
60
40
20
0
Concentration of peptide (μM)
E

FIG. 2A-E



A B

FIG. 3A-B

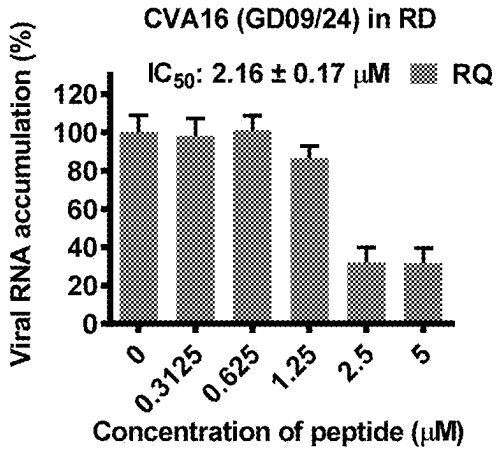


FIG. 4

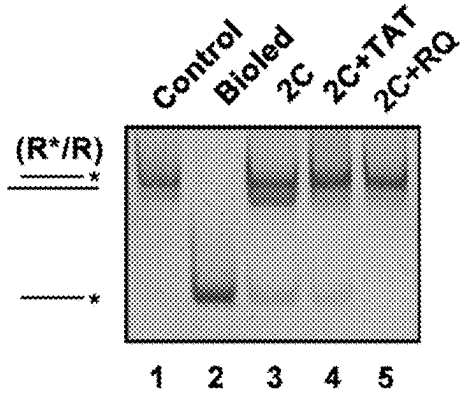
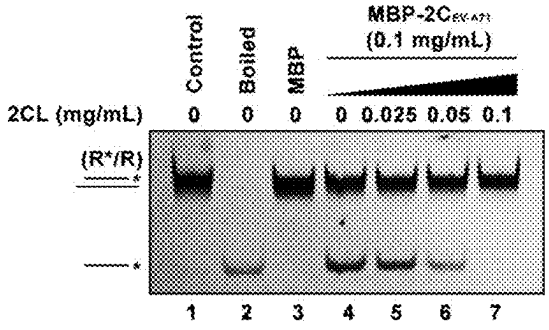
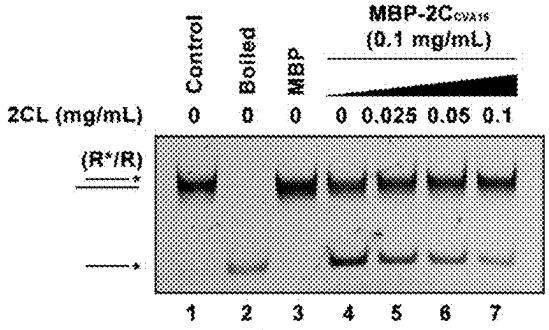


FIG. 5



A



B

FIG. 6A-B

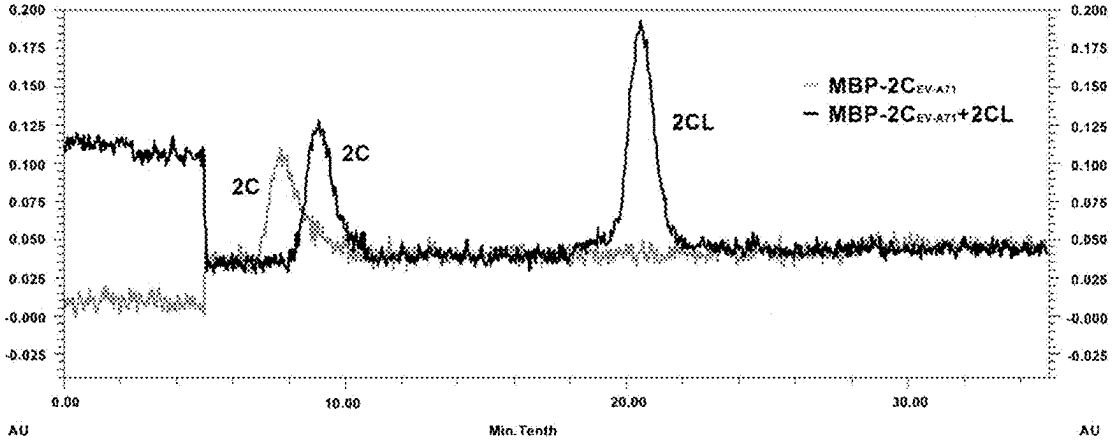


FIG. 7

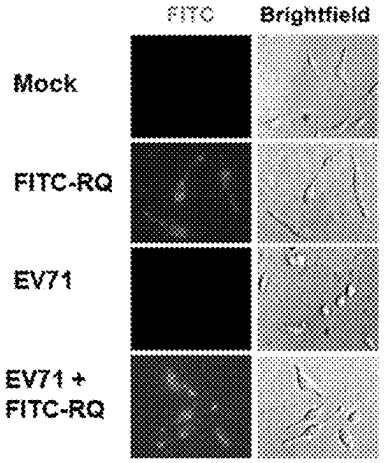
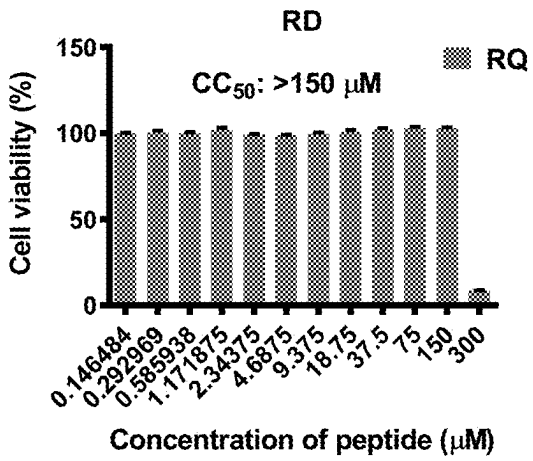
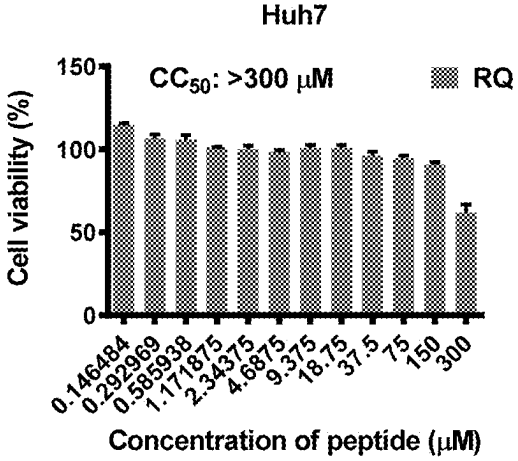


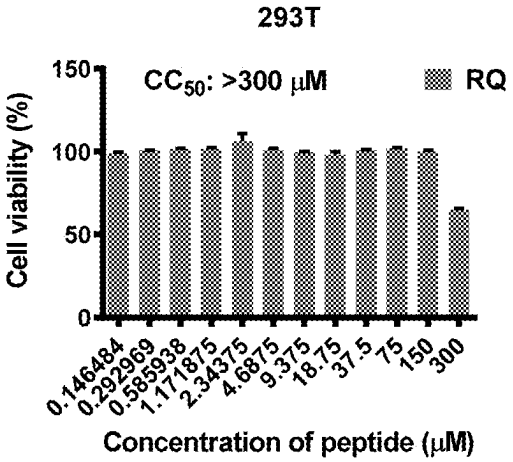
FIG. 8



A



B



C

FIG. 9A-C

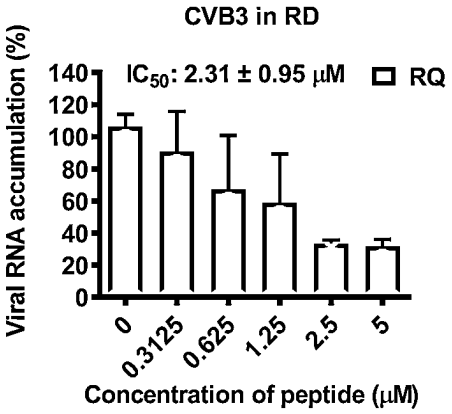

CVB3 in RD
IC50: 2.31 ± 0.95 μM
RQ
Viral RNA accumulation (%)
140
120
100
80
60
40
20
0
0
0.3125
0.625
1.25
2.5
5
Concentration of peptide (μM)

FIG. 10

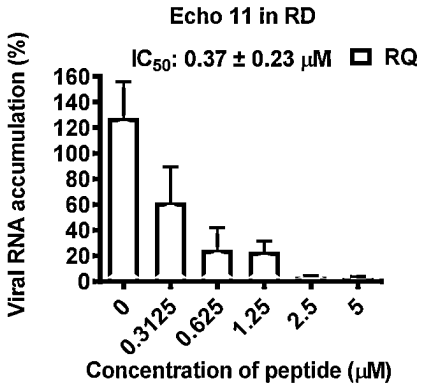
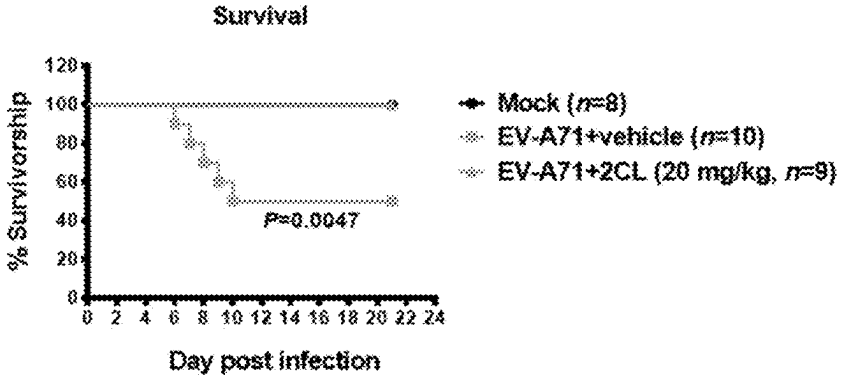
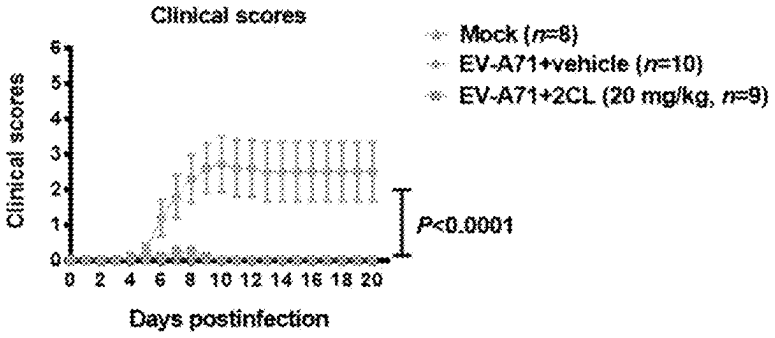

Echo 11 in RD
IC50: 0.37 ± 0.23 μM
RQ
Viral RNA accumulation (%)
160
140
120
100
80
60
40
20
0
0
0.3125
0.625
1.25
2.5
5
Concentration of peptide (μM)

FIG. 11


Survival
% Survivorship
120
100
80
60
40
20
0
0 2 4 6 8 10 12 14 16 18 20 22 24
P=0.0047
Day post infection
Mock (n=8)
EV-A71+vehicle (n=10)
EV-A71+2CL (20 mg/kg, n=9)

A


Clinical scores
Clinical scores
6
5
4
3
2
1
0
0 2 4 6 8 10 12 14 16 18 20
P<0.0001
Days postinfection
Mock (n=8)
EV-A71+vehicle (n=10)
EV-A71+2CL (20 mg/kg, n=9)

B

FIG. 12A-B

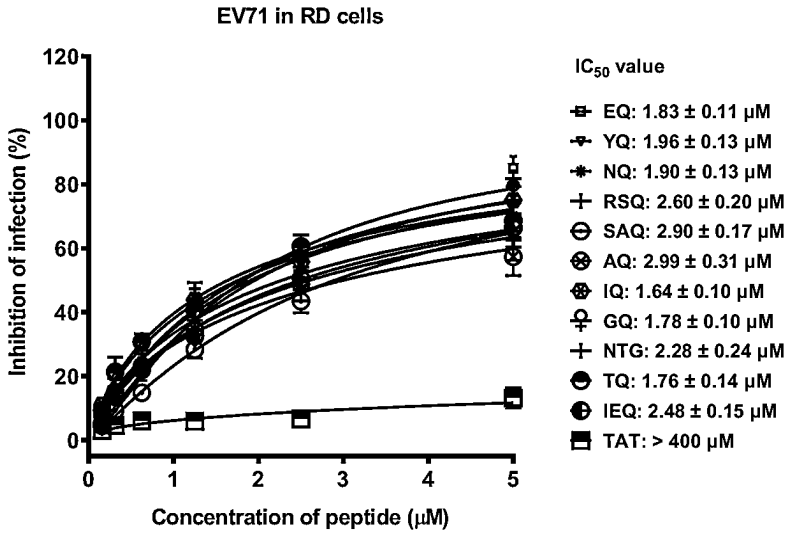


FIG. 13

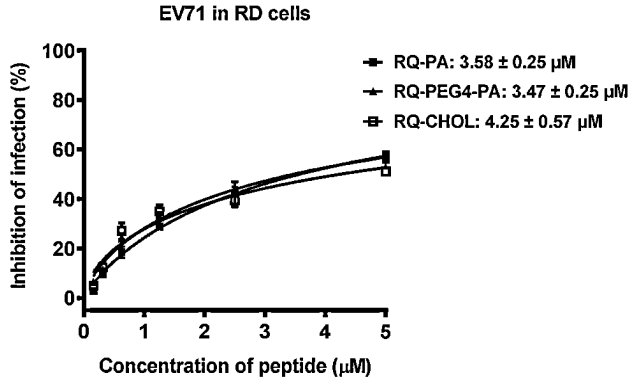


FIG. 14

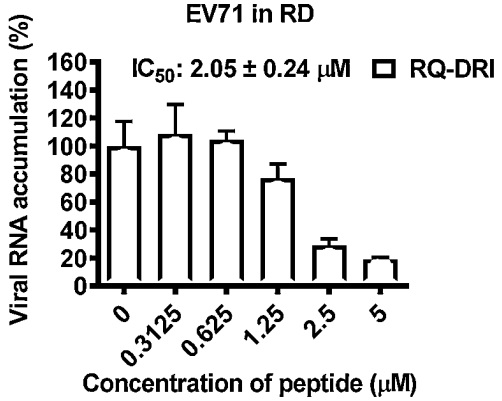


FIG. 15

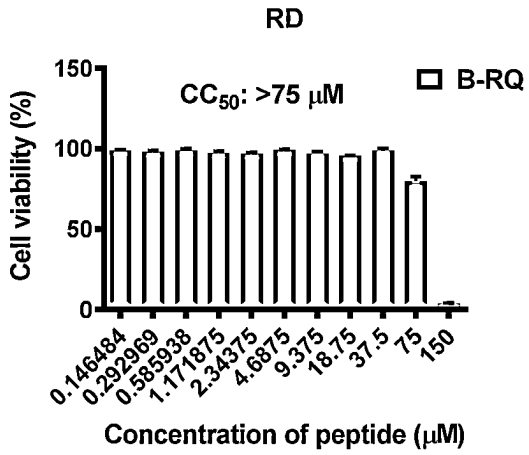
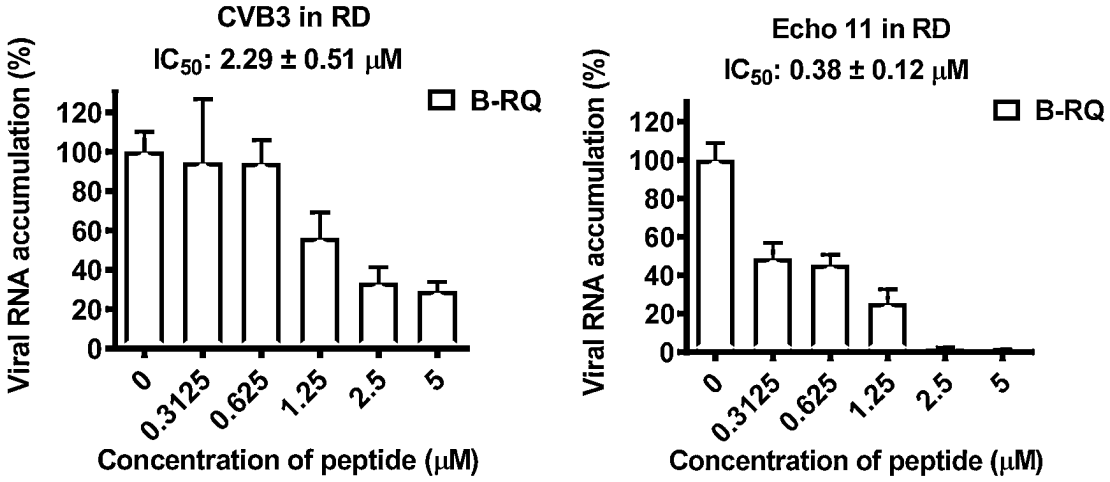


FIG. 16



A B

FIG. 17A-B

BROAD-SPECTRUM ANTIVIRAL DRUG FOR ENTEROVIRUS, AND APPLICATION

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a national phase application under 35 U.S.C. § 371 of International Application No. PCT/CN2021/081723, filed Mar. 19, 2021, which claims the priority of Chinese Patent Application No. 202010735992.8, filed Jul. 28, 2020, each of which are hereby incorporated by reference in their entirety.

REFERENCE TO A SEQUENCE LISTING

[0002] Pursuant to 37 C.F.R. 1.821 (c), a sequence listing is submitted herewith as an ASCII compliant text file named "UNITP0094US_Corrected_Sequence_Listing", created on Sep. 1, 2023, and having a size of ~10 kilobytes. The content of the aforementioned file is hereby incorporated by reference in its entirety.

FIELD

[0003] The present invention relates to the technical field of biomedicine, and in particular to a broad-spectrum antiviral drug for enterovirus and an application thereof.

BACKGROUND

[0004] Enterovirus, as a positive-sense single-stranded RNA virus, belongs to the Enterovirus genus of the Picornaviridae family, and mainly includes human enterovirus (EV), coxsackie A virus (CVA), coxsackie B virus (CVB), echovirus, rhinovirus, poliovirus, etc. Enterovirus infections are widely distributed all over the world, and exhibit complex and diverse clinical manifestations, ranging from mild low-grade fever, fatigue and respiratory diseases, to herpetic angina, hand-foot-mouth disease, severe aseptic meningitis, myocarditis, encephalitis, poliomyelitis and the like. At present, there are no specific drugs for effectively treating or preventing against enterovirus infections.

[0005] Herpetic angina is mainly caused by coxsackie A virus type 2 (CVA2), CVA4, CVA6, CVA9, CVA16, CVA22, coxsackie B virus type 1 (CVB1), CVB2, CVB3, CVB4 or CVB5. Herpetic angina often presents with acute fever of mostly low or moderate degree and occasionally as high as 40° C. or above, and even causes convulsion. The course of the fever is about 2-4 days. Older children may complain of sore throat which can affect swallowing. Infants and young children present with salivation, refusal to eat, and restlessness, sometimes accompanied by headache, stomachache or myalgia. About 25% of children under 5 years old may be accompanied by vomiting. Typical symptoms appear in the pharynx, and manifest as hyperemia in the pharynx, and several (as few as 1-2, or as many as 10) small (1-2 mm in diameter) grey-white herpes surrounded by redness in the oral mucosa within 2 days of onset. After 2-3 days, the redness intensifies and expands, and the herpes break out to form yellow ulcers. Such mucosal herpes commonly appear in the tonsil anterior pillar, and can also appear in the soft palate, uvula and tonsils, but do not involve the gums and buccal mucosa. The course of the disease is generally 4-6 days, and occasionally extends to 2 weeks.

[0006] Hand-foot-mouth disease is mainly caused by enterovirus 71 (EV71), CVA6, CVA8, CVA10, CVA16, CVB3 and CVB5, and has common clinical manifestations of acute fever, mouth pain, anorexia, and scattered herpes or ulcers in the oral mucosa, which mostly appear in the tongue, buccal mucosa, and hard palate, and can also appear in the soft palate, gums, tonsils, and pharynx. Maculopapular rashes appear on hands, feet, buttocks, arms, and legs, and then turn into herpes, which may be surrounded by inflammatory redness and have little liquid inside. There are more rashes on hands and feet, both on the dorsum and the vola from a few to dozens. After the rashes subside, no trace and no pigmentation are left. Some children with hand-foot-mouth disease present with herpetic angina as the first symptom, and then red rashes on palms, soles, and buttocks. When the disease develops rapidly, a small number of children can develop from hand-foot-and-mouth disease to severe aseptic meningitis and encephalitis, manifested as fever, headache, nausea, vomiting, and then meningeal irritation, as well as great fluctuation in body temperature, with low-grade fever in the most case and sometimes with fever up to 40° C. or above, often bimodal fever in the course of the disease. Other symptoms include such as sore throat, muscle aches, skin rash, photophobia, diarrhea, swollen lymph nodes, and sometimes mild paralysis.

[0007] Myocarditis is mainly caused by CVB1-61 and Echovirus. The clinical manifestations of patients with viral myocarditis depend on the extent and location of the lesion. Mild cases can be asymptomatic, while severe cases can present with heart failure, cardiogenic shock and sudden death. Patients often have a history of infection in upper respiratory tract or intestines 1-3 weeks before the onset, manifested as symptoms such as fever, body aches, sore throat, fatigue, nausea, vomiting, and diarrhea, followed by palpitations, chest tightness, chest pain or precordial dull pain, dizziness, dyspnea, edema, and even Adams-Stokes syndrome. An extremely small number of patients develop heart failure or cardiogenic shock.

[0008] Enterovirus is a positive-sense single-stranded RNA virus with a genome of about 7.5 kb containing a large ORF that encodes a polyprotein. The polyprotein is further hydrolyzed into 4 structural proteins (VP1-VP4) and 7 non-structural proteins (2A-2C and 3A-3D). Protein 2C is a very conservative non-structural protein in enterovirus (including EV71, CVA, CVB, echovirus, etc.), and exists in the form of homopolymer. Enterovirus protein 2C has an activity of RNA helicase, and is a classical superfamily 3 (SF3) helicase. A large number of studies on EV71 and PV have proved that the helicase activity of 2C is necessary for the replication and proliferation of the virus, and the multimerization of protein 2C is crucial for its helicase function. Therefore, the present invention designs a polypeptide drug targeting the multimerization domain of 2C to inhibit its multimerization, so as to inhibit the helicase function, finally achieving the purpose of inhibiting virus replication.

[0009] The polypeptide provided by the present invention has high-efficiency and broad-spectrum antiviral activity, which provides a new strategy for the prevention and control of enteroviruses such as EV71, CVA16, CVA4, CVA6, CVA10, CVB3, CVB5 and Echo 11, and also provides a new theoretical basis for accelerating the development of polypeptide and small molecule drugs against human enteroviruses.

SUMMARY

[0010] In view of this, an object of the present invention is to provide use of a preparation inhibiting multimerization

of enterovirus protein 2C as a target in the manufacture of a medicament for preventing and/or treating a viral disease;

[0011] Another object of the present invention is to provide a broad-spectrum anti-enterovirus polypeptide inhibitor. The inhibitor has a core sequence shown in SEQ ID NO.21. Specifically, the inhibitor has a sequence shown in SEQ ID NO.1 or SEQ ID NO.24, and the sequence containing a cell-penetrating peptide are respectively shown in SEQ ID NO.2 and SEQ ID NO.20.

[0012] Another object of the present invention is to provide use of the above polypeptide inhibitor in the manufacture of an enterovirus inhibitor.

[0013] In order to realize the above objects of the present invention, the present invention provides the following technical solution:

[0014] A broad-spectrum anti-enterovirus polypeptide inhibitor is provided, which has a sequence of:

I.
(X1)E(X2)(X3)(X4)R(X5)(X6)(X7)(X8)(X9)(X10)(X11)
EALFQ

[0015] wherein:

[0016] X1 is selected from the group consisting of arginine (R), asparagine (N) and lysine (K);

[0017] X2 is selected from the group consisting of tyrosine (Y) and arginine (R);

[0018] X3 is selected from the group consisting of serine(S), asparagine (N) and arginine (R);

[0019] X4 is selected from the group consisting of asparagine (N), arginine (R), threonine (T) and histidine (H);

[0020] X5 is selected from the group consisting of serine(S), asparagine (N) and histidine (H);

[0021] X6 is selected from the group consisting of alanine (A), asparagine (N) and serine(S);

[0022] X7 is selected from the group consisting of isoleucine (I), threonine (T) and valine (V);

[0023] X8 is selected from the group consisting of glycine (G) and glutamine (Q);

[0024] X9 is selected from the group cFIGURESonsisting of asparagine (N), aspartic acid (D) and alanine (A);

[0025] X10 is selected from the group consisting of threonine (T), cysteine (C) and lysine (K);

[0026] X11 is selected from the group consisting of isoleucine (I) and leucine (L);

[0027] and the sequence is shown in SEQ ID NO.21;

[0028] II. a sequence with deletion, addition or substitution of at least one amino acid compared to the sequence in I;

[0029] III. a sequence that has at least 50% homology with the amino acid sequence in I or II and inhibits enterovirus activity; and

[0030] IV. a complementary sequence to the sequence in I or II or III.

[0031] The "amino acid" in the present invention includes natural amino acids or unnatural amino acids. Amino acids commonly known to those skilled in the art are all within the protection scope of the present invention.

[0032] The above sequence is preferably: REYN (X4) R (X5) (X6) (X7) (X8) (X9) (X10) (X11) EALFQ, as shown in SEQ ID NO.22; further preferably: REYN (X4) R (X5) (X6) (X7) G (X9) T (X11) EALFQ, as shown in SEQ ID NO.23;

[0033] In a specific embodiment of the present invention, the sequence is as shown in SEQ ID NO.1 or SEQ ID NO.24, both of which can be added with a cell-penetrating peptide. A sequence with addition of a cell-penetrating peptide to the sequence shown in SEQ ID NO.1 is shown in SEQ ID NO.2. A sequence with addition of a cell-penetrating peptide to the sequence shown in SEQ ID NO.24 is shown in SEQ ID NO.20;

[0034] The protection content of the present invention also includes a polypeptide sequence for inhibiting enterovirus containing the sequence shown in SEQ ID NO.1 or SEQ ID NO.24, and an inhibitor with inhibitory activity on enterovirus obtained by replacing different cell-penetrating sequences, performing polypeptide modification, or designing and modifying unnatural amino acids on the basis of polypeptide RQ (SEQ ID NO.2) or B-RQ (SEQ ID NO.20).

[0035] In the present invention, the polypeptide with the sequence shown in any one of SEQ ID NOs.21-23 is a core polypeptide of the present invention, and a polypeptide with addition/deletion of amino acids at the N-terminal, or a polypeptide with modification at the C-terminal, or a D configuration of the polypeptide can be used in the manufacture of an enterovirus inhibitor, or in the manufacture of a medicament for treating or preventing enterovirus infections.

[0036] Preferably, the modifications to the above core polypeptide include:

[0037] addition of 1-5 amino acids to the N-terminal, deletion of 1-13 amino acids from the N-terminal or modification to the C-terminal, or a D configuration of the polypeptide, all of which have the same inhibitory activity as the core polypeptide. Specifically, for addition of amino acids, amino acids such as S, E, L and I can be added to the N-terminal, for example, the addition of LI dipeptide and SELI tetrapeptide; for amino acids deletion, 1-13 (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or 13) amino acids can be sequentially deleted from the N-terminal; for modification to the C-terminal, amino acids A (BA) and K, PEG4 (tetrapolyethylene glycol), C16 (palmitic acid), Chol (cholesterol) and the like can be modified to the C-terminal. In specific modifications of the present invention, AK dipeptide is usually added at the C-terminal with or without PEG4, C16, Chol and the like (A\K can be added between PEG4, C16 and Chol), for example, AK-C16, AK-PEG4-K-C16 and AK-Chol. In a specific embodiment of the present invention, the sequence shown in SEQ ID NO.1 is taken as an example to perform the above modifications and changes, which can also be performed on the basis of the sequence shown in SEQ ID NO.24.

[0038] The inhibitor of the present invention is obtained by adding a cell-penetrating peptide to the polypeptide with inhibitory activity. The cell-penetrating peptide is added according to conventional methods by, for example, linking a cell-penetrating peptide with the polypeptide with inhibitory activity of the present invention via a linking peptide as, for example, a cell-penetrating peptide+a linking peptide+active polypeptide provided by the present invention. The polypeptide with modification to the C-terminal does not need to be added with a cell-penetrating peptide.

[0039] The above sequences obtained by conventional methods in the art are all within the protection scope of the present invention. The conventional methods include but are

not limited to artificial synthesis, prokaryotic or eukaryotic expression of recombinant proteins comprising the above proteins.

[0040] Use of a broad-spectrum anti-enterovirus polypeptide inhibitor, includes use of a polypeptide containing the sequence shown in SEQ ID NO.1, or a polypeptide with addition/deletion of amino acids at the N-terminal or a polypeptide with modification at the C-terminal, or a D configuration of the polypeptide as mentioned above in the manufacture of an enterovirus inhibitor or in the manufacture of a medicament for treating or preventing enterovirus infection.

[0041] In the above use, preferably, the enterovirus includes but is not limited to: the Enterovirus genus of the Picornaviridae family, including human enterovirus (EV), coxsackie A virus (CVA), coxsackie B virus (CVB), echovirus, rhinovirus, poliovirus, etc.

[0042] In the above use, a disease caused by the enterovirus infection comprises hand-foot-mouth disease, myocarditis, herpetic angina, aseptic meningitis, encephalitis, viral cold, etc.

[0043] Compared with the prior art, the present invention has the following advantages:

[0044] The polypeptides and derivatives thereof involved in the present invention can inhibit multimerization of enterovirus 2C, thereby inhibiting its helicase function, and they are a novel type of medicament for treating enterovirus against a new target, showing great significance for antiviral drug resistance.

[0045] The polypeptide RQ screened out by the present invention has high-efficiency antiviral activity, which provides a new strategy for the prevention and control of enterovirus, and also provides a new theoretical basis for accelerating the development of polypeptide and small molecule drugs against human enteroviruses. Moreover, the clear antiviral mechanism of the RQ series polypeptides can ensure the safety of their uses and the clarity of approach optimization, which is convenient for further development in the future.

BRIEF DESCRIPTION OF DRAWINGS

[0046] FIG. **1**A-C shows the results of the determined cytotoxicity of polypeptides SQ, LQ and RQ;

[0047] FIG. **2**A-E shows the results of the determined efficiency of polypeptide RQ in inhibiting EV71 in RD, Vero, huh7, and 293T cells;

[0048] FIG. **3**A-B shows the results of the determined efficiencies of polypeptides LQ and SQ in inhibiting EV71 in Vero cells

[0049] FIG. **4** shows the results of the determined efficiency of polypeptide RQ in inhibiting CVA16 in RD cells;

[0050] FIG. **5** shows the results of the inhibition of 2C helicase activity of EV71 by polypeptide RQ;

[0051] FIG. **6**A-B shows the results of the inhibition of 2C helicase activity of EV71 and CVA16 by polypeptide RQ;

[0052] FIG. **7** shows the results of the inhibition of the multimerization of EV71 2C protein by polypeptide RQ;

[0053] FIG. **8** shows the results of the detected cell-penetrating efficiency of polypeptide RQ;

[0054] FIG. **9**A-C shows the results of the determined toxicity of polypeptide RQ in various cells;

[0055] FIG. **10** shows the results of the determined efficiency of polypeptide RQ in inhibiting CVB3 in RD cells;

[0056] FIG. **11** shows the results of the determined efficiency of polypeptide RQ in inhibiting Echo 11 in RD cells;

[0057] FIG. **12**A-B shows the results of the detected antiviral activity of polypeptide RQ against EV71 in mice;

[0058] FIG. **13** shows the results of the detected antiviral activity of the variants of polypeptide RQ;

[0059] FIG. **14** shows the results of the detected antiviral activity of the modifiers of polypeptide RQ;

[0060] FIG. **15** shows the results of the determined efficiency of polypeptide RQ-DRI in inhibiting EV71 in RD cells;

[0061] FIG. **16** shows the results of the detected toxicity of polypeptide B-RQ in RD cells;

[0062] FIG. **17**A-B shows the results of the determined efficiency of polypeptide B-RQ in inhibiting CVB3 and Echo 11 in RD cells.

DETAILED DESCRIPTION

[0063] The present invention discloses a broad-spectrum antiviral drug for enterovirus and an application thereof (a broad-spectrum anti-enterovirus polypeptide inhibitor targeting enterovirus protein 2C and an application thereof). Those skilled in the art can refer to the content of this application and appropriately improve the process parameters for realization. In particular, it should be noted that all similar replacements and modifications are apparent to those skilled in the art, and they are all considered to be included in the present invention. The polypeptide inhibitor, antiviral drug and application of the present invention have been described through preferred embodiments. Those skilled can apparently make modifications or appropriate changes and combinations to the polypeptide inhibitor, antiviral drug and application described herein without departing from the content, spirit and scope of the present invention, to realize and apply the technology of the present invention.

[0064] The present invention takes EV71 virus as an example to verify the inhibitory effect of the polypeptide provided by the present invention. Actually, the present invention designs an inhibitor specifically for enterovirus protein 2C as the target, and the inhibitor of the present invention has effect on any virus with enterovirus protein 2C, such as coxsackie A virus (CVA), coxsackie B virus (CVB), echovirus, rhinovirus and poliovirus. In view of space constraints, no more details.

[0065] One of the sequences of the inhibitory proteins designed for enterovirus protein 2C in the present invention is REYNNRSAIGNTIEALFQ as shown in SEQ ID NO.1, which is a core sequence. In order to make it work in vivo, a cell-penetrating peptide is linked to the core protein, and the polypeptide with a cell-penetrating peptide linked has a sequence of YGRKKRRQRRRGSGREYNNRSAIGNTIEALFQ as shown in SEQ ID NO.2, named as polypeptide RQ.

[0066] The applicant also designed another two inhibitory polypeptides containing a cell-penetrating peptide for enterovirus protein 2C, which have sequences of:

[0067] YGRKKRRQRRRGSGLIREYNNRSAIGNTIEALFQ, SEQ ID NO.3, named as polypeptide LQ; and

[0068] YGRKKRRQRRRGSGSELIREYNNRSAIGNTIEALFQ, SEQ ID NO.4, named as polypeptide SQ.

[0069] EV71 2C protein has the ability of multimerization, which is crucial for correct helicase function by 2C. Based on the structural composition and sequence features of the 2C multimerization domain, the applicant designed a series

of polypeptide sequences for REYNNRSAIGNTIEALFQ (SEQ ID No.1), one of the core sequences necessary for protein 2C multimerization. After screening, the applicant found that the polypeptide RQ has a strong virus-inhibiting ability. It has been proved by experiments that RQ can efficiently enter cells, and can inhibit the correct multimerization of protein 2C and thus the helicase function of protein 2C in vitro. The applicant also conducted structural modifications on the basis of RQ to construct a series of variants, and found that these variants also have good anti-virus ability.

[0070] The polypeptides involved in the present invention are shown in Table 1:

TABLE 1

Peptide	Number	Sequence	Tested	Antiviral(s)	IC_{50}/EC_{50}	CC_{50}
Core sequence	SEQ ID No. 1	REYNNRSAIGNTIEALFQ	/	/	/	/
SQ	SEQ ID No. 4	YGRKKRRQRRRGSGSELIREYNNRSAIGNTIEALFQ	In vitro	EV71	10.3 μM	134.3 μM
LQ	SEQ ID No. 3	YGRKKRRQRRRGSGLIREYNNRSAIGNTIEALFQ	In vitro	EV71	2.26 μM	>150 μM
RQ	SEQ ID No. 2	YGRKKRRQRRRGSGREYNNRSAIGNTIEALFQ	In vivo	EV71/CVA16/CVA4/CVA6/CVA10/CVB3/Echo 11	0.41 μM/0.37	>300 μM
EQ	SEQ ID No. 5	YGRKKRRQRRRGSGEYNNRSAIGNTIEALFQ	In vitro	EV71	1.83 μM	
YC	SEQ ID No. 6	YGRKKRRQRRRGSGYNNRSAIGNTIEALFQ	In vitro	EV71	1.96 μM	/
NQ	SEQ ID No. 7	YGRKKRRQRRRGSGNNRSAIGNTIEALFQ	In vitro	EV71	1.90 μM	/
RSQ	SEQ ID No. 8	YGRKKRRQRRRGSGRSAIGNTIEALFQ	In vitro	EV71	2.60 μM	/
SAQ	SEQ ID No. 9	YGRKKRRQRRRGSGSAIGNTIEALFQ	In vitro	EV71	2.90 μM	/
AQ	SEQ ID No. 10	YGRKKRRQRRRGSGAIGNTIEALFQ	In vitro	EV71	2.99 μM	/
IQ	SEQ ID No. 11	YGRKKRRQRRRGSGIGNTIEALFQ	In vitro	EV71	1.64 μM	/
GQ	SEQ ID No. 12	YGRKKRRQRRRGSGGNTIEALFQ	In vitro	EV71	1.78 μM	/
NTQ	SEQ ID No. 13	YGRKKRRQRRRGSGNTIEALFQ	In vitro	EV71	2.28 μM	/
TQ	SEQ ID No. 14	YGRKKRRQRRRGSGTIEALFQ	In vitro	EV71	1.76 μM	/
IEQ	SEQ ID No. 15	YGRKKRRQRRRGSGIEALFQ	In vitro	EV71	2.48 μM	/
RQ-PA	SEQ ID No. 16	REYNNRSAIGNTIEALFQ-βAK-C16	In vitro	EV71	3.58 μM	/
RQ-PEG4-PA	SEQ ID No. 17	REYNNRSAIGNTIEALFQ-βAK-PEG4-K-C16	In vitro	EV71	3.47 μM	/
RQ-CHOL	SEQ ID No. 18	REYNNRSAIGNTIEALFQ-βAK-Chol	In vitro	EV71	4.25 μM	/
RQ-DRI	SEQ ID No. 19	AC-qflaeitngiasrnnyergsgrrrqrrkkrgy-NH_2 (D-configuration amino acid)	In vitro	EV71	2.05 μM	/

TABLE 1-continued

Peptide	Number	Sequence	Tested	Antiviral(s)	IC_{50}/EC_{50}	CC_{50}
B-RQ	SEQ ID No. 20	YGRKKRRQRRRGSGREYNHR HSVGATLEALFQ	In vitro	CVB3/ Echo 11	0.38 μM	>75 μM
Core sequence	SEQ ID No. 21	(X1)E(X2)(X3)(X4)R(X5)(X6) (X7)(X8)(X9)(X10)(X11)EALFQ				
	SEQ ID No. 22	REYN(X4)R(X5)(X6)(X7)(X8) (X9)(X10)(X11)EALFQ				
	SEQ ID No. 23	REYN(X4)R(X5)(X6)(X7)G(X9) T(X11)EALFQ				
	SEQ ID No. 24	REYNHRHSVGATLEALFQ				

[0071] In the polypeptide sequence of the present invention, YGRKKRRQRRR (TAT) is the cell-penetrating peptide, GSG is the linking peptide, and the amino acid sequence of each polypeptide after removal of the cell-penetrating peptide and linking peptide is the sequence or partial sequence of the core polypeptide. The polypeptides having sequences shown in SEQ ID Nos.3 and 4 are the polypeptides of a cell-penetrating peptide+a linking peptide+the core polypeptide having the sequence shown in SEQ ID No.1 with addition of LI and SELI to the N-terminal. The polypeptides having sequences shown in SEQ ID Nos.5-15 are the polypeptides of a cell-penetrating peptide+a linking peptide+the core polypeptide having the sequence shown in SEQ ID No.1 with sequential deletion of 1-12 amino acids from the N-terminal. The polypeptides having sequences shown in SEQ ID Nos.16-18 are the polypeptides of the core polypeptide having the sequence shown in SEQ ID No.1 with modification of amino acids A (BA) and K, PEG4 (tetrapolyethylene glycol), C16 (palmitic acid) and Chol (cholesterol) to the C-terminal. The polypeptide having a sequence shown in SEQ ID No.19 is the D configuration of the core polypeptide of the sequence shown in SEQ ID No.1. The polypeptide having a sequence shown in SEQ ID No.20 is the polypeptides of a cell-penetrating peptide+a linking peptide+the core polypeptide having the sequence shown in SEQ ID No.24;

[0072] Negative controls were set for the polypeptides in each example of the present invention, which prove that the core sequences of the polypeptides provided by the present invention have corresponding antiviral efficacy.

[0073] The raw materials and reagents used in the polypeptide and application thereof provided by the present invention are all commercially available.

[0074] The present invention is further described in conjunction with examples below.

Example 1: Toxicity of Polypeptides RQ, LQ and SQ in Vero Cells

1. Experimental Materials

[0075] Vero E6 cells; DMEM medium (Thermo) and serum (Gibco) were purchased from Invitrogen Co., Ltd; CCK-8 reagent (MCE) was purchased from Promoter Company.

[0076] Polypeptide SQ was synthesized by Nanjing GenScript Co., Ltd, with a sequence shown in SEQ ID NO.4. Polypeptide LQ was synthesized by Nanjing GenScript Co., Ltd, with a sequence shown in SEQ ID NO.3. Peptide RQ was synthesized by Nanjing GenScript Co., Ltd, with a sequence shown in SEQ ID NO.2.

2. Experimental Process

[0077] In the anti-virus process, the polypeptide needs to not only inhibit the virus, but also ensure no toxicity to cells. Therefore, this standard was testified by cytotoxicity assay, and the cells without any treatment were used as a control group.

[0078] The steps are as follows:

[0079] (1) Vero cells were plated in a 96-well plate at 100 μL per well.

[0080] (2) When the cells grew to a confluence of 70%-80%, the DMEM medium containing 10% serum was replaced with a DMEM medium containing 2% serum, and a certain concentration gradient of polypeptide RQ or LQ or SQ was added, so that the final concentrations of the polypeptide in the wells were 0.073242 μM, 0.146484 μM, 0.292969 μM, 0.585938 μM, 1.171875 μM, 2.34375 μM, 4.6875 μM, 9.375 μM, 18.75 μM, 37.5 μM, 75 μM and 150 μM, respectively.

[0081] (3) The samples were collected 24 h after the addition of the polypeptide, and then 10 μL of live cell detection reagent CCK-8 was added to each well and mixed well.

[0082] (4) The plate was placed at 37° C. for 2 h.

[0083] (5) The absorbance value at OD450 was detected by a microplate reader.

[0084] The results are shown in FIG. **1** and Tables 2-4. The CC50 of each polypeptide was calculated taking the cell viability of untreated cells as 100%. RQ had a CC50 of >150 μM (FIG. **1**A), LQ had a CC50 of >150 μM (FIG. **1**B), and SQ had a CC50 of 134.3 μM (FIG. **1**C);

TABLE 2

Concentration of RQ polypeptide (μM)	Cell viability (%)		
0.073242	100.6749	110.2362	105.9618
0.146484	93.81327	99.10011	98.98763
0.292969	92.68841	108.2115	106.7492
0.585938	92.23847	91.56355	90.77615
1.171875	116.0855	115.2981	115.5231
2.34375	115.8605	111.0236	119.1226
4.6875	112.7109	109.7863	118.7852
9.375	117.5478	109.3363	99.10011
18.75	111.1361	115.4106	105.7368
37.5	105.6243	116.0855	108.5489
75	110.0112	127.5591	120.5849
150	108.2115	103.5996	100.1125

TABLE 3

Concentration of LQ polypeptide (μM)	Cell viability (%)		
0.073242	100	105.88408	97.01806
0.146484	102.8559	104.7744	101.9913
0.292969	111.8018	106.5265	104.7619
0.585938	110.1638	110.5563	108.0087
1.171875	111.3398	115.1555	110.4762
2.34375	104.8299	106.3075	107.1861
4.6875	110.8858	104.8489	112.5108
9.375	114.0277	119.5795	113.5931
18.75	108.6518	115.6373	110.3247
37.5	117.0937	99.1853	97.1429
75	108.2318	87.6084	90.8658
150	72.1798	83.0968	80.9091

TABLE 4

Concentration of SQ polypeptide (μM)	Cell viability (%)		
0.073242	104.2439	103.4468	96.06877
0.146484	98.04083	94.606	92.7401
0.292969	101.0204	103.1624	100.4524
0.585938	94.40455	103.9959	100.6225
1.171875	90.14603	94.06821	98.81252
2.34375	96.60283	101.1325	99.03158
4.6875	105.1932	106.7734	95.3706
9.375	99.4719	102.9859	103.7701
18.75	105.7187	100.481	90.24951
37.5	100.7644	90.65433	80.59708
75	90.35876	95.75345	70.30992
150	70.01401	80.35016	60.9948

Example 2: Determination of Efficiency of Polypeptide RQ in Inhibiting EV71 in RD, Vero, Huh7, And 293T Cells

1. Experimental Materials

[0085] RD cells, Vero E6 cells, huh7 cells, and 293T cells; DMEM medium (Thermo) and serum (Gibco) were purchased from Invitrogen Co., Ltd; Total RNA extraction kit (Omega) and One step qRT-PCR kit (Takara) were purchased from U-MeBiotech Co., Ltd. The water used in the processes of RNA extraction and qRT-PCR was DEPC water, and the entire experiment was performed in an RNase-free environment.

[0086] Polypeptide RQ was synthesized by Nanjing GenScript Co., Ltd, with a sequence shown in SEQ ID NO.2. The cell-penetrating peptide YGRKKRRQRRR (TAT) was used as a control and synthesized by Nanjing GenScript.

2. Experimental Process

[0087] (1) Different cells were plated in a 24-well plate.

[0088] (2) When the cells grew to a confluence of 70%-80%, the DMEM medium containing 10% serum was replaced with a DMEM medium containing 2% serum (the DMEM medium containing 2% serum was added in each well at an amount of 0.5 mL), and 5 μL of EV71 virus of 1×10^6 PFU/mL was added to each well.

[0089] (3) After 1 h, different polypeptides (RQ or control TAT) with final concentrations of 0.3125 μM, 0.625 μM, 1.25 μM, 2.5 μM, and 5 μM were added respectively. The group without polypeptide added was used as a control.

[0090] (4) The samples were collected 24 h after the infection of EV71 virus, and RNA was extracted with the total RNA extraction kit.

[0091] (5) The supernatant was discarded, then 350 μL of TRK lysate was added to each well, and the plate was put on a shaker for 5 min.

[0092] (6) 350 μL of 70% ethanol (DEPC) was added to each well, and then the plate was put on a shaker for 5 min.

[0093] (7) The solution in each well was transferred to a RNA extraction column for 1 min of centrifugation at 12000 g.

[0094] (8) The solution obtained in the recovery tube was reloaded on the column again for 1 min of centrifugation at 12000 g.

[0095] (9) RNA washing buffer 1 was added in the column for 30 s of centrifugation at 12000 g.

[0096] (10) RNA washing buffer 2 was added in the column for 1 min of centrifugation at 12000 g.

[0097] (11) Step (10) was repeated.

[0098] (12) The column alone was centrifuged at 12000 g for 2 min to completely remove the residual RNA washing buffer.

[0099] (13) 50 μL of DEPC water was added to the column for 2 min of centrifugation at 12000 g.

[0100] (14) 2 μL of RNA sample was subjected to a fluorescence quantitative experiment using the one step qRT-PCR kit.

[0101] The results are shown in FIG. **2** and Tables 5-9. The results of the determined anti-EV71 effect of polypeptide RQ in different cells show an IC50 of 1.35 μM in RD cells (FIG. **2**A), an IC50 of 0.66 μM in Vero cells (FIG. **2**B), an IC50 of 0.41 μM in huh7 cells (FIG. **2**C), and an IC50 of 3 μM in 293T cells (FIG. **2**D). The cell-penetrating peptide control TAT had no anti-EV71 effect in Vero cells (FIG. **2**E);

TABLE 5

Concentration of RQ (μM)	Percentage of viral RNA in RD cells (%)								
0	98.74564	96.68729	104.5671	82.17592	130.0352	87.78892	84.09811	100.7668	115.1351
0.3125	75.34828	80.42976	84.75739	79.07512	85.1167	65.50396	105.6539	93.32565	61.22043
0.625	73.54402	85.43979	113.5023	86.56448	56.64673	58.60146	62.52759	60.61082	84.55632
1.25	30.05595	42.79864	38.42442	49.01438	63.21512	36.02987	48.88676	46.65231	50.16218
2.5	14.68721	22.11395	20.34741	13.63269	8.986402	25.41335	28.37401	31.44852	32.99647
5	ND	15.74388	16.39792	19.14505	16.02361	15.46365	21.74359	23.04845	14.24234

ND, not detected

TABLE 6

Concentration of RQ (μM)	Percentage of viral RNA in Vero cells (%)					
0	82.32617	153.297	64.37678	ND	117.1999	82.80011
0.3125	87.78578	47.76442	71.67834	86.67063	70.51145	25.67804
0.625	46.90411	82.11225	73.54103	51.71642	65.58883	ND
1.25	6.624615	16.23832	12.20634	9.196032	5.526034	7.367869
2.5	2.980446	0.826799	1.137232	3.105048	2.98824	2.97166
5	2.389376	0.957253	2.002879	1.797167	1.955004	2.113301

ND, not detected

TABLE 7

Concentration of RQ (μM)	Percentage of viral RNA in Huh7 cells (%)		
0	135.2764	80.15702	84.56655
0.3125	52.90425	81.62619	35.41682
0.625	26.90658	51.32333	32.9747
1.25	11.6287	6.87045	12.90771
2.5	7.068214	6.953349	6.49349
5	0.715564	7.820489	14.67491

TABLE 8

Concentration of RQ (μM)	Percentage of viral RNA in 293T cells (%)					
0	140.6661	89.65254	69.68134	86.48279	127.9453	85.57189
0.3125	66.50167	26.53837	41.68405	142.3522	86.73876	67.75856
0.625	90.63467	82.72855	62.9566	112.2779	72.71821	76.02054
1.25	46.14077	61.77934	42.09579	110.8795	85.43184	64.39499
2.5	50.03924	21.79971	51.62416	51.28562	65.19374	51.55135
5	23.20045	ND	24.63166	65.17771	46.72863	36.78709

ND, not detected

TABLE 9

Concentration of TAT (μM)	Percentage of viral RNA in Vero cells (%)		
0	91.39502	100.2266	108.3784
0.3125	115.6133	93.74802	91.92313
0.625	121.4818	94.86778	149.0928
1.25	111.6527	97.24053	100.8214
2.5	125.8799	103.6078	137.9907
5	136.8661	104.0616	88.68295

Example 3: Determination of Efficiency of Polypeptides LQ and SQ in Inhibiting EV71 in Vero Cells

1. Experimental Materials

[0102] Vero E6 cells; DMEM medium (Thermo) and serum (Gibco) were purchased from Invitrogen Co., Ltd; Total RNA extraction kit (Omega) and One step qRT-PCR kit (Takara) were purchased from U-MeBiotech Co., Ltd. The water used in the processes of RNA extraction and qRT-PCR was DEPC water, and the entire experiment was performed in an RNase-free environment.

[0103] Polypeptide LQ was synthesized by Nanjing GenScript Co., Ltd, with a sequence shown in SEQ ID NO.3. Polypeptide SQ was synthesized by Nanjing GenScript Co., Ltd, with a sequence shown in SEQ ID NO.4

2. Experimental Process

[0104] (1) Vero E6 cells were plated in a 24-well plate.

[0105] (2) When the cells grew to a confluence of 70%-80%, the DMEM medium containing 10% serum was replaced with a DMEM medium containing 2% serum (the DMEM medium containing 2% serum was added in each well at an amount of 0.5 mL), and 5 μL of EV71 virus of 1×10^6 PFU/mL was added to each well.

[0106] (3) After 1 h, different polypeptides (LQ or SQ) with final concentrations of 0.3125 μM, 0.625 μM, 1.25 μM, 2.5 μM, and 5 μM were added respectively. The group without polypeptide added was used as a control.

[0107] (4) The samples were collected 24 h after the infection of EV71 virus, and RNA was extracted with the total RNA extraction kit.

[0108] (5) The supernatant was discarded, then 350 μL of TRK lysate was added to each well, and the plate was put on a shaker for 5 min.

[0109] (6) 350 μL of 70% ethanol (DEPC) was added to each well, and then the plate was put on a shaker for 5 min.

[0110] (7) The solution in each well was transferred to a RNA extraction column for 1 min of centrifugation at 12000 g.

[0111] (8) The solution obtained in the recovery tube was reloaded on the column again for 1 min of centrifugation at 12000 g.

[0112] (9) RNA washing buffer 1 was added in the column for 30 s of centrifugation at 12000 g.

[0113] (10) RNA washing buffer 2 was added in the column for 1 min of centrifugation at 12000 g.

[0114] (11) Step (10) was repeated.

[0115] (12) The column alone was centrifuged at 12000 g for 2 min to completely remove the residual RNA washing buffer.

[0116] (13) 50 μL of DEPC water was added to the column for 2 min of centrifugation at 12000 g.

[0117] (14) 2 μL of RNA sample was subjected to a fluorescence quantitative experiment using the one step qRT-PCR kit.

[0118] The results are shown in FIG. **3** and Tables 10-11, which indicate that LQ polypeptide had an IC50 of 2.26 μM in Vero cells (FIG. **3**A), and SQ polypeptide had an IC50 of 10.3 μM in Vero cells (FIG. **3**B).

[0119] The above results show that the inhibitory proteins designed for protein 2C had significantly different inhibition efficiency against the virus, where the polypeptide RQ had an IC50 of 0.66 μM in Vero cells, showing significantly higher inhibition efficiency than those of polypeptide LQ and polypeptide SQ;

TABLE 10

Concentration of LQ (μM)	Percentage of viral RNA in Vero cells (%)		
0	101.0805	100.8891	108.1782
0.3125	92.0208	91.1729	102.4602
0.625	90.7271	81.3501	71.8857
1.25	60.5336	51.4135	62.3497
2.5	52.6153	41.584	40.6992
5	41.9179	31.799	30.9085

TABLE 11

Concentration of SQ (μM)	Percentage of viral RNA in Vero cells (%)		
0	94.938	111.0771	101.7717
0.3125	101.0203	100.3849	105.3364
0.625	110.1337	105.2279	100.9028
1.25	90.2836	95.7988	81.9498
2.5	81.2941	82.75	71.6192
5	60.1186	61.1934	74.2715

Example 4: Determination of Efficiency of Polypeptide RQ in Inhibiting CVA16 in RD Cells

1. Experimental Materials

[0120] RD cells; DMEM medium (Thermo) and serum (Gibco) were purchased from Invitrogen Co., Ltd; Total RNA extraction kit (Omega) and One step qRT-PCR kit (Takara) were purchased from U-MeBiotech Co., Ltd. The water used in the processes of RNA extraction and qRT-PCR was DEPC water, and the entire experiment was performed in an RNase-free environment.

[0121] Polypeptide RQ was synthesized by Nanjing GenScript Co., Ltd with a sequence shown in SEQ ID NO.2.

2. Experimental Process

[0122] (1) RD cells were plated in a 24-well plate.

[0123] (2) When the cells grew to a confluence of 70%-80%, the DMEM medium containing 10% serum was replaced with a DMEM medium containing 2% serum (the DMEM medium containing 2% serum was added in each well at an amount of 0.5 mL), and 5 μL of CVA16 virus of 1×10^6 PFU/mL was added to each well.

[0124] (3) After 1 h, different polypeptides with final concentrations of 0.3125 μM, 0.625 μM, 1.25 μM, 2.5 μM, and 5 μM were added respectively. The group without polypeptide added was used as a control.

[0125] (4) The samples were collected 24 h after the infection of CVA16 virus, and RNA was extracted with the total RNA extraction kit.

[0126] (5) The supernatant was discarded, then 350 μL of TRK lysate was added to each well, and the plate was put on a shaker for 5 min.

[0127] (6) 350 μL of 70% ethanol (DEPC) was added to each well, and then the plate was put on a shaker for 5 min.

[0128] (7) The solution in each well was transferred to a RNA extraction column for 1 min of centrifugation at 12000 g.

[0129] (8) The solution obtained in the recovery tube was reloaded on the column again for 1 min of centrifugation at 12000 g.

[0130] (9) RNA washing buffer 1 was added in the column for 30 s of centrifugation at 12000 g.

[0131] (10) RNA washing buffer 2 was added in the column for 1 min of centrifugation at 12000 g.

[0132] (11) Step (10) was repeated.

[0133] (12) The column alone was centrifuged at 12000 g for 2 min to completely remove the residual RNA washing buffer.

[0134] (13) 50 μL of DEPC water was added to the column for 2 min of centrifugation at 12000 g.

[0135] (14) 2 μL of RNA sample was subjected to a fluorescence quantitative experiment using the one step qRT-PCR kit.

[0136] The results of the determined anti-CVA16 effect of polypeptide RQ in RD cells are shown in Table 12 and FIG. **4**, which indicate an IC50 of 2.16 μM.

TABLE 12

Concentration of RQ (μM)	Percentage of viral RNA in RD cells (%)								
0	78.38927	97.34762	124.2631	53.65574	123.7802	122.564	93.49458	77.16421	129.3412
0.3125	100.0216	148.3965	77.29282	125.3469	101.8237	69.03192	64.02673	86.24284	110.1741
0.625	115.4959	79.1848	91.90599	99.67949	99.63403	80.72403	145.8942	96.4369	ND
1.25	83.98713	74.20358	80.83334	111.5916	81.89601	63.92798	62.81053	100.5664	117.5637
2.5	68.28515	40.30227	61.91291	27.90528	46.74052	21.57059	4.815019	2.49757	13.56626
5	38.35756	89.90899	32.58479	25.69166	15.84122	34.43394	18.04668	7.616083	21.36344

ND, not detected

Example 5: Inhibition of EV71 2C Helicase Activity by RQ

1. Experimental Materials

[0137] Baculovirus for fusion expression of MBP-EV71 2C protein; *Spodoptera frugiperda* cells (Sf9) were obtained from China Center for Type Culture Collection (CCTCC), culture medium (SF-HM) was purchased from Beijin Compamy, maltose binding protein (MBP) filler was purchased from NEB, Amicon Mltra-30KDa (ultrafiltration tube) was purchased from Millipore; binding buffer (pH 7.4): 20 mM

Tris-HCl (pH 7.4), 0.5 M EDTA, 200 mM NaCl, 10 mM B-mercaptoethanol, anhydrous ethanol of 5% by volume, and glycerol of 10% by volume. Elution buffer: 10 mM maltose solution. 50 mM HEPES solution at pH 7.5.

[0138] HEX fluorescently labeled RNA single strand at a length of 42 nt, and a RNA single strand at a length of 54 nt complementary to the HEX labeled RNA strand.

[0139] Polypeptide RQ was synthesized by Nanjing GenScript Co., Ltd with a sequence shown in SEQ ID NO.2.

2. Experimental Process

[0140] 2.1 In vitro expression and purification of EV71 2C protein

[0141] (1) 1 mL of baculovirus expressing MBP-EV71 2C protein was added to each of 6 flasks (T75) of Sf9 cells with a density of 80-90%. The flasks were placed at 27.5° C. for 3 days of infection. When the cells presented with obvious symptoms of virus infection (the cells became larger and rounder, and a large number of them were suspended), Sf9 cells were blown off with the original medium, and then centrifuged at 1000 g for 5 min. The supernatant was discarded, and the cells were resuspended with 15 mL of the binding buffer for purifying MBP fusion protein.

[0142] (2) Sf9 cells were broken by ultrasonication (250 W, 15-20 min) to be transparent, then aliquoted into 1.5 mL centrifuge tubes, and centrifuged at 12000 g and 4° C. for 15 min. Then the supernatant was transferred into a 15 mL centrifuge tube and placed on ice.

[0143] (3) A chromatography column was added with 2-3 mL of Amylose Resin, washed with 30 mL of ddH2O, and then added with 30 mL of the binding buffer to equilibrate the filler. It should be noted that air bubbles were not allowed to exist in the filler during the washing process.

[0144] (4) The supernatant containing the target protein was slowly added into the equilibrated column, and a constant flow pump was set at a flow rate of 50 or 60, so that the flow rate of the protein sample was 7-8 s/drop. 15 mL of supernatant was loaded 3 times.

[0145] (5) After binding, the filler was washed with 100 mL of the binding buffer at a flow rate of the constant flow pump of 130 to wash off impurities.

[0146] (6) After washing, the filler was eluted with 10 mM maltose eluent at a flow rate of the constant flow pump of 10. The collected eluate (containing the target protein) was added into a 30 KD ultrafiltration tube and centrifuged at 7200 g and 4° C. for ultrafiltration to concentrate the target protein (about 200-300 μL, with a concentration of about 1 mg/mL).

[0147] (7) After the ultrafiltration was completed, the buffer system of the target protein was replaced with 50 mM HEPES at pH 7.5 (by ultrafiltration with HEPES-KOH for 3-4 times).

[0148] (8) 2 μL of the purified protein sample was subjected to SDS-PAGE electrophoresis, and the remaining protein was stored at −80° C. for later use.

[0149] EV71 2C protein labeled with MBP was successfully purified.

[0150] 2.2 Inhibition of helicase activity of EV71 2C protein by RQ in vitro

[0151] (1) A strand labeled with HEX at a concentration of 0.2 pmol/μL was added with a complementary strand RNA at the same concentration to prepare a HEX-labeled double-stranded dsRNA substrate by annealing.

[0152] (2) Annealing process: the reaction system was kept at 75° C. for 3 min, cooled to 25° C. at a rate of 1° C. per minute, and then kept at 25° C. for 2 min.

[0153] (3) The target protein and the double-stranded substrate were prepared according to the standard unwinding experiment reaction system. 5 μg of polypeptide RQ and control TAT were added respectively, and single and double strands controls were set. The single strand sample was boiled at 75° C. for 3 min and then placed on ice for 2 min.

[0154] (4) The prepared system was mixed well and then placed at 37° C. for 50 min of reaction.

[0155] (5) A mixture obtained after the reaction was subjected to electrophoresis.

[0156] (6) Finally, Typhoon 9500 was used for direct scan to obtain a HEX signal.

[0157] During electrophoresis, the single strand ran faster than the double strand. Therefore, if the MBP-2C protein had helicase activity, it can unwind the double-stranded dsRNA substrate to release a single-stranded RNA, and then the lane would show two bands, upper and lower. The single-stranded RNA (lane 2) prepared by boiling at 75° C. was used as a positive control. The reaction without protein added (lane 1) was used as a negative control. As shown in lane 3 of FIG. **5**, EV71 2C had helicase activity and can unwind the double-stranded dsRNA substrate; whereas the addition of RQ inhibited the helicase activity of 2C (lane 5), and the control TAT did not affect the helicase activity of 2C (lane 4). The above results indicate that RQ can indeed inhibit the helicase function of EV71 2C.

Example 6: Inhibition of 2C helicase activity of EV71 and CVA16 by RQ

1. Experimental Materials

[0158] Purified MBP-EV71 2C protein; baculovirus for fusion expression of MBP-CVA16 2C protein; *Spodoptera frugiperda* cells (Sf9) was obtained from China Center for Type Culture Collection (CCTCC), culture medium (SF-HM) was purchased from Beijin Company, maltose-binding protein (MBP) filler was purchased from NEB, Amicon Mltra-30KDa (ultrafiltration tube) was purchased from Millipore; binding buffer (pH 7.4): 20 mM Tris-HCl (pH 7.4), 0.5 M EDTA, 200 mM NaCl, 10 mM B-mercaptoethanol, anhydrous ethanol of 5% by volume, and glycerol of 10% by volume. Elution buffer: 10 mM maltose solution. 50 mM HEPES solution at pH 7.5.

[0159] HEX fluorescently labeled RNA single strand at a length of 42 nt, and a RNA single strand at a length of 54 nt complementary to the HEX labeled RNA strand.

[0160] Polypeptide RQ was synthesized by Nanjing GenScript Co., Ltd with a sequence shown in SEQ ID NO.2.

2. Experimental Process

2.1 In Vitro Expression and Purification of CVA16 2C Protein

[0161] (1) 1 mL of baculovirus expressing MBP-CVA16 2C protein was added to each of 6 flasks (T75) of Sf9 cells with a density of 80-90%. The flasks were placed at 27.5° C. for 3 days of infection. When the cells presented with obvious symptoms of virus infection (the cells became larger and rounder, and a large number of them were suspended), Sf9 cells were blown off with the original medium, and then centrifuged at 1000 g for 5 min. The

supernatant was discarded, and the cells were resuspended with 15 mL of the binding buffer for purifying MBP fusion protein.

[0162] (2) Sf9 cells were broken by ultrasonication (250 W, 15-20 min) to be transparent, then aliquoted into 1.5 mL centrifuge tubes, and centrifuged at 12000 g and 4° C. for 15 min. Then the supernatant was transferred into a 15 mL centrifuge tube and placed on ice.

[0163] (3) A chromatography column was added with 2-3 mL of Amylose Resin, washed with 30 mL of ddH2O, and then added with 30 mL of the binding buffer to equilibrate the filler. It should be noted that air bubbles were not allowed to exist in the filler during the washing process.

[0164] (4) The supernatant containing the target protein was slowly added into the equilibrated column, and a constant flow pump was set at a flow rate of 50 or 60, so that the flow rate of the protein sample was 7-8 s/drop. 15 mL of supernatant was loaded 3 times.

[0165] (5) After binding, the filler was washed with 100 mL of the binding buffer at a flow rate of the constant flow pump of 130 to wash off impurities.

[0166] (6) After washing, the filler was eluted with 10 mM maltose eluent at a flow rate of the constant flow pump of 10. The collected eluate (containing the target protein) was added into a 30 KD ultrafiltration tube and centrifuged at 7200 g and 4° C. for ultrafiltration to concentrate the target protein (about 200-300 μL, with a concentration of about 1 mg/mL).

[0167] (7) After the ultrafiltration was completed, the buffer system of the target protein was replaced with 50 mM HEPES at pH 7.5 (by ultrafiltration with HEPES-KOH for 3-4 times).

[0168] (8) 2 μL of the purified protein sample was subjected to SDS-PAGE electrophoresis, and the remaining protein was stored at −80° C. for later use.

[0169] EV71 2C protein labeled with MBP was successfully purified.

2.2 Inhibition of Helicase Activity of EV71 and CVA16 2C Proteins by RQ In Vitro

[0170] (1) A strand labeled with HEX at a concentration of 0.2 pmol/μL was added with a complementary strand RNA at the same concentration to prepare a HEX-labeled double-stranded dsRNA substrate by annealing.

[0171] (2) Annealing process: the reaction system was kept at 75° C. for 3 min, cooled to 25° C. at a rate of 1° C. per minute, and then kept at 25° C. for 2 min.

[0172] (3) The target protein and the double-stranded substrate were prepared according to the standard unwinding experiment reaction system. 5 μg of polypeptide RQ and control TAT were added respectively, and single and double strands controls were set. The single strand sample was boiled at 75° C. for 3 min and then placed on ice for 2 min.

[0173] (4) The prepared system was mixed well and then placed at 37° C. for 50 min of reaction.

[0174] (5) A mixture obtained after the reaction was subjected to electrophoresis.

[0175] (6) Finally, Typhoon 9500 was used for direct scan to obtain a HEX signal.

[0176] As shown in FIG. **6**A, RQ inhibited the helicase activity of EV71 2C in a dose-dependent manner; and as shown in FIG. **6**B, RQ inhibited the helicase activity of CVA16 2C in a dose-dependent manner.

Example 7: Inhibition of Multimerization of EV71 2C Protein by RQ

1. Experimental Materials

[0177] Purified MBP-EV71 2C protein; Superdex 200 Increase 10/300 GL chromatography column was purchased from GE Healthcare Co., Ltd; Amicon Ultra centrifugal filters were purchased from Merck Co., Ltd; BioLogic DuoFlow system was purchased from Bio-Rad Co., Ltd; 50 mM HEPES-KOH (pH 8.5).

[0178] Polypeptide RQ was synthesized by Nanjing GenScript Co., Ltd with a sequence shown in SEQ ID NO.2.

2. Experimental Process

[0179] (1) The purified MBP-EV71 2C was concentrated to 1 mg/mL with Amicon Ultra centrifugal filters.

[0180] (2) The concentrated protein 2C was mixed with 20 μM polypeptide RQ for 1 h of incubation on ice, and the incubation of 2C with ddH2O of the same volume was set as a control.

[0181] (3) The above samples were equilibrated with 50 mM HEPES-KOH (pH 8.5), and then loaded onto a Superdex 200 Increase 10/300 GL chromatography column. The flow rate was controlled to be 1 mL/min by the BioLogic DuoFlow system.

[0182] (4) The duration of the protein passing through the chromatography column was recorded by ultraviolet (UV) signal, and changes in protein molecular weight were analyzed.

[0183] As shown in FIG. **7**, in a case that protein 2C was only co-incubated with ddH_2O, 2C formed into a polymer, which was eluted from the system rapidly (light-colored line) with a peak elution time of 8 min (light-colored peak on the left); in a case that RQ was co-incubated with 2C (dark-colored line), the peak elution time of 2C polymer changed obviously (dark-colored peak on the left), and the dark-colored peak on the right represents free polypeptide RQ. The above results indicate that RQ in co-incubation with 2C inhibited the formation of 2C polymer.

Example 8: Determination of Cell-Penetrating Efficiency of Polypeptide RQ

1. Materials

[0184] MEM medium (Thermo) and serum (Gibco) were purchased from Invitrogen Co., Ltd, immunofluorescence dish (NEST) was purchased from Promoter Company, PBS, DAPI and paraformaldehyde were purchased from Diyue Chuangxin Company.

[0185] Polypeptide RQ was synthesized by Nanjing GenScript Co., Ltd with a sequence shown in SEQ ID NO.2.

2. Experimental Process

[0186] The experiment was performed with two groups. In order to avoid the impact of adding EV71 virus on the polypeptide entering cells, in one group of the experiment, EV71 virus was added before the addition of polypeptide RQ, while in the other group, no virus was added before the addition of the polypeptide, and each group was set with a negative control.

[0187] An immunofluorescence experiment was conducted according to the following steps:

[0188] (1) 1 mL of RD cells was added in an immunofluorescence special dish, and cells were collected when grew to a confluence of 30%.

[0189] (2) The culture medium was discarded, and the residual culture medium was washed off by washing with 1 mL of PBS of 0.01 mol/L and pH 7.4 three times, 5 min each time.

[0190] (3) 4% paraformaldehyde solution was prepared by dissolving 4 g of paraformaldehyde in 100 mL of PBS. 1 mL of the prepared 4% paraformaldehyde was added to each dish for 5 min of reaction to fix the cells.

[0191] (4) The 4% paraformaldehyde was discarded, and then the residual paraformaldehyde was washed off by washing with 1 mL of PBS of 0.01 mol/L and pH 7.4 three times, 5 min each time.

[0192] (5) 1 mg/mL DAPI solution was diluted to 100 ng/mL with PBS, and then added to the dish for 15 min of reaction.

[0193] (6) The reaction solution was discarded, and then the residual reaction solution was washed off by washing with 1 mL of PBS of 0.01 mol/L and pH 7.4 three times, 5 min each time.

[0194] (7) The dish was placed under a fluorescence microscope for observation.

[0195] The fluorescently labeled (FITC) polypeptide was detected for its cell-penetrating efficiency in RD cells. Two groups of experiments were set up. The first group was an untreated control group, which was added with FITC-RQ. The second group was an EV71-infected group, which was added with FITC-RQ after EV71 infection. The two groups of experiments were performed simultaneously, with a virus MOI of 0.1 and a concentration of the polypeptide added of 1 μM. The samples were collected 12 h after the addition of the polypeptide, and the cells were fixed and subjected to an immunofluorescence experiment. The results show that the polypeptide can enter the cells with or without infection, showing a good cell-penetrating ability.

[0196] As shown in FIG. **8**, the polypeptide can be observed entering the cells with or without virus added, proving that polypeptide RQ had a good cell-penetrating ability.

Example 9: Toxicity Assay of Polypeptide RQ in Various Cells

1. Experimental Materials

[0197] RD cells, Huh7 cells, and 293T cells; DMEM medium (Thermo) and serum (Gibco) were purchased from Invitrogen Co., Ltd; CCK-8 reagent (MCE) was purchased from Promoter Company.

[0198] Polypeptide RQ was synthesized by Nanjing GenScript Co., Ltd with a sequence shown in SEQ ID NO.2.

2. Experimental Process

[0199] (1) Different cells were plated in a 96-well plate at 100 μL per well.

[0200] (2) When the cells grew to a confluence of 70%-80%, the DMEM medium containing 10% serum was replaced with a DMEM medium containing 2% serum, and a certain concentration gradient of polypeptide RQ was added, so that the final concentrations of the polypeptide in the wells were 0.073242 μM, 0.146484 μM, 0.292969 μM, 0.585938 μM, 1.171875 μM, 2.34375 μM, 4.6875 μM, 9.375 μM, 18.75 μM, 37.5 μM, 75 μM, 150 μM, and 300 μM, respectively.

[0201] (3) The samples were collected 24 h after the addition of the polypeptide, and then 10 μL of live cell detection reagent CCK-8 was added to each well and mixed well.

[0202] (4) The plate was placed at 37° C. for 2 h.

[0203] (5) The absorbance value at OD450 was detected by a microplate reader.

[0204] The results are shown in FIG. **9** and Tables 13-15. The CC50 of each RQ in different cells was calculated taking the cell viability of untreated cells as 100%. CC50 was >150 μM in RD cells (FIG. **9**A), CC50 was >300 μM in Huh cells (FIG. **9**B), and CC50 was >300 μM in 293T cells (FIG. **1**C).

TABLE 13

Concentration of RQ polypeptide (μM)	Viability of RD cell (%)		
0.073242	99.7786	100.3321	99.8893
0.146484	99.22509	102.3247	99.94465
0.292969	100.7749	100.2214	99.16974
0.585938	98.94834	103.5978	102.7122
1.171875	99.61255	99.00369	99.22509
2.34375	98.78229	99.22509	98.50554
4.6875	98.33948	99.44649	100.8856
9.375	98.39483	100.7196	102.6568
18.75	100.6089	103.4317	102.4354
37.5	101.4945	103.9852	103.5424
75	102.1033	103.5978	102.9889
150	8.726937	8.782288	8.00738

TABLE 14

Concentration of RQ polypeptide (μM)	Viability of Huh7 cell (%)		
0.073242	113.2507	113.9801	116.7518
0.146484	110.6978	106.6132	101.8721
0.292969	110.9895	104.4979	102.018
0.585938	101.2157	100.7051	101.5074
1.171875	104.3521	97.20399	99.02747
2.34375	98.73572	100.1945	96.03696
4.6875	98.00632	103.5497	101.2886
9.375	103.4768	101.4345	97.71456
18.75	100.851	93.41114	94.28641
37.5	95.0158	97.05811	92.02529
75	90.93119	93.26526	88.08655
150	65.84002	67.73645	51.47095

TABLE 15

Concentration of RQ polypeptide (μM)	Viability of Huh7 cell (%)		
0.073242	98.39022	100.4267	97.86656
0.146484	100.6594	100.8922	100.9503
0.292969	100.7758	102.4631	100.8922
0.585938	100.4849	102.8123	101.6486
1.171875	99.67029	115.4383	103.5687
2.34375	99.08844	100.3685	102.8704
4.6875	98.56478	99.96121	100.0776
9.375	99.67029	99.96121	94.02638

TABLE 15-continued

Concentration of RQ polypeptide (μM)	Viability of Huh7 cell (%)		
18.75	100.2521	99.90303	101.8231
37.5	101.8231	101.1249	102.9286
75	101.5322	98.97207	99.49573
150	66.56323	63.82855	64.4104

Example 10: Determination of Efficiency of Polypeptide RQ in Inhibiting CVB3 in RD Cells

1. Experimental Materials

[0205] RD cells; DMEM medium (Thermo) and serum (Gibco) were purchased from Invitrogen Co., Ltd; Total RNA extraction kit (Omega) and One step qRT-PCR kit (Takara) were purchased from U-MeBiotech Co., Ltd. The water used in the processes of RNA extraction and qRT-PCR was DEPC water, and the entire experiment was performed in an RNase-free environment.

[0206] Polypeptide RQ was synthesized by Nanjing GenScript Co., Ltd with a sequence shown in SEQ ID NO.2.

2. Experimental Process

[0207] (1) RD cells were plated in a 24-well plate.

[0208] (2) When the cells grew to a confluence of 70%-80%, the DMEM medium containing 10% serum was replaced with a DMEM medium containing 2% serum (the DMEM medium containing 2% serum was added in each well at an amount of 0.5 mL), and 5 μL of CVB3 virus of 1×10^6 PFU/mL was added to each well.

[0209] (3) After 1 h, the polypeptides with final concentrations of 0.3125 μM, 0.625 μM, 1.25 μM, 2.5 μM, and 5 μM were added respectively. The group without polypeptide added was used as a control.

[0210] (4) The samples were collected 24 h after the infection of CVB3 virus, and RNA was extracted with the total RNA extraction kit.

[0211] (5) The supernatant was discarded, then 350 μL of TRK lysate was added to each well, and the plate was put on a shaker for 5 min.

[0212] (6) 350 μL of 70% ethanol (DEPC) was added to each well, and then the plate was put on a shaker for 5 min.

[0213] (7) The solution in each well was transferred to a RNA extraction column for 1 min of centrifugation at 12000 g.

[0214] (8) The solution obtained in the recovery tube was reloaded on the column again for 1 min of centrifugation at 12000 g.

[0215] (9) RNA washing buffer 1 was added in the column for 30 s of centrifugation at 12000 g.

[0216] (10) RNA washing buffer 2 was added in the column for 1 min of centrifugation at 12000 g.

[0217] (11) Step (10) was repeated.

[0218] (12) The column alone was centrifuged at 12000 g for 2 min to completely remove the residual RNA washing buffer.

[0219] (13) 50 μL of DEPC water was added to the column for 2 min of centrifugation at 12000 g.

[0220] (14) 2 μL of RNA sample was subjected to a fluorescence quantitative experiment using the one step qRT-PCR kit.

[0221] The determination results of the anti-CVB3 effect of polypeptide RQ in RD cells are shown in Table 16 and FIG. **10**, which indicate an IC50 of 2.31 μM.

TABLE 16

Concentration of SQ (μM)	Percentage of viral RNA in Vero cells (%)		
0	106.8374	119.2124	93.16261
0.3125	62.25856	140.3936	70.09917
0.625	30.54633	134.359	36.93837
1.25	119.4583	30.84876	26.36559
2.5	35.87058	29.1883	35.07889
5	31.34734	39.17466	25.33601

Example 11: Determination of Efficiency of Polypeptide RQ in Inhibiting Echo 11 in RD Cells

1. Experimental Materials

[0222] RD cells; DMEM medium (Thermo) and serum (Gibco) were purchased from Invitrogen Co., Ltd; Total RNA extraction kit (Omega) and One step qRT-PCR kit (Takara) were purchased from U-MeBiotech Co., Ltd. The water used in the processes of RNA extraction and qRT-PCR was DEPC water, and the entire experiment was performed in an RNase-free environment.

[0223] Polypeptide RQ was synthesized by Nanjing GenScript Co., Ltd with a sequence shown in SEQ ID NO.2.

2. Experimental Process

[0224] (1) RD cells were plated in a 24-well plate.

[0225] (2) When the cells grew to a confluence of 70%-80%, the DMEM medium containing 10% serum was replaced with a DMEM medium containing 2% serum (the DMEM medium containing 2% serum was added in each well at an amount of 0.5 mL), and 5 μL of Echo 11 virus of 1×10^6 PFU/mL was added to each well.

[0226] (3) After 1 h, the polypeptides with final concentrations of 0.3125 μM, 0.625 μM, 1.25 μM, 2.5 μM, and 5 μM were added respectively. The group without polypeptide added was used as a control.

[0227] (4) The samples were collected 24 h after the infection of Echo 11 virus, and RNA was extracted with the total RNA extraction kit.

[0228] (5) The supernatant was discarded, then 350 μL of TRK lysate was added to each well, and the plate was put on a shaker for 5 min.

[0229] (6) 350 μL of 70% ethanol (DEPC) was added to each well, and then the plate was put on a shaker for 5 min.

[0230] (7) The solution in each well was transferred to a RNA extraction column for 1 min of centrifugation at 12000 g.

[0231] (8) The solution obtained in the recovery tube was reloaded on the column again for 1 min of centrifugation at 12000 g.

[0232] (9) RNA washing buffer 1 was added in the column for 30 s of centrifugation at 12000 g.

[0233] (10) RNA washing buffer 2 was added in the column for 1 min of centrifugation at 12000 g.

[0234] (11) Step (10) was repeated.

[0235] (12) The column alone was centrifuged at 12000 g for 2 min to completely remove the residual RNA washing buffer.

[0236] (13) 50 μL of DEPC water was added to the column for 2 min of centrifugation at 12000 g.

[0237] (14) 2 μL of RNA sample was subjected to a fluorescence quantitative experiment using the one step qRT-PCR kit.

[0238] The determination results of the anti-Echo 11 effect of polypeptide RQ in RD cells are shown in Table 17 and FIG. **11**, which indicate an IC50 of 0.37 μM.

TABLE 17

Concentration of SQ (μM)	Percentage of viral RNA in Vero cells (%)		
0	144.9111	165.5089	72.39416
0.3125	117.2316	36.60896	30.65337
0.625	59.40357	6.90746	7.106392
1.25	33.44803	29.3462	7.071973
2.5	1.051358	5.505496	3.538126
5	4.488181	1.195551	2.309541

Example 12: Detection of Antiviral Activity of Polypeptide RQ on EV71 in Mice

1. Experimental Materials

[0239] Newborn 1-day-old ICR suckling mice. Polypeptide RQ was synthesized by Nanjing GenScript Co., Ltd with a sequence shown in SEQ ID NO.2.

2. Experimental Process

[0240] (1) Twenty-seven 1-day-old ICR suckling mice were randomly divided into 3 groups; one group of 10 suckling mice was challenged with virus and injected with the same amount of PBS (vehicle) as a positive control, one group of 9 suckling mice was challenged with virus and then injected with RQ, and one group of 8 suckling mice was not challenged with virus and not administered with drugs as a negative control. These 19 suckling mice were challenged with EV71 at a dose of 107 PFU by intraperitoneal injection.

[0241] (2) Simultaneously with the challenge, one group was intraperitoneally injected with polypeptide RQ at 20 mg/kg as a treatment group, and the other group was injected with the same amount of PBS as a control group.

[0242] (3) The polypeptide and PBS were injected every 12 h until the 7th day after the challenge.

[0243] (4) The clinical symptoms and death of suckling mice were observed until the 21st day.

[0244] (5) The clinical symptoms were evaluated by a clinical scoring system: 0 point: healthy, 1 point: exhibiting slow and hunchbacked movements, 2 points: weak in one limb, 3 points: one limb paralyzed, 4 points: two limbs paralyzed, and 5 points: died.

[0245] The results are shown in FIG. **12**A, which indicate that all the suckling mice in the negative control group (Mock) survived, 5 suckling mice in the group with challenge but no drug administration died on the 10th day, showing a mortality rate of 50%, while all the suckling mice in the group with RQ administration survived. As shown in FIG. **12**B, the group with challenge but no drug administration had a significantly higher clinical score than that of the group with drug administration after challenge. The above results indicate that RQ can effectively treat suckling mice infected with a lethal dose of EV71 and prevent them from dying.

Example 13: Detection of Antiviral Activity of Polypeptide RQ Variants

1. Experimental Materials

[0246] Polypeptides EQ (shown in SEQ ID NO.5), YQ (shown in SEQ ID NO.6), NQ (shown in SEQ ID NO.7), RSQ (shown in SEQ ID NO.8), SAQ (shown in SEQ ID NO.9), AQ (shown in SEQ ID NO.10), IQ (shown in SEQ ID NO.11), GQ (shown in SEQ ID NO.12), NTQ (shown in SEQ ID NO.13), TQ (shown in SEQ ID NO.14), and IEQ (shown in SEQ ID NO.15). The sequences were all commercially synthesized. CCK-8 reagent (MCE) was purchased from Promoter Company.

2. Experimental Process

[0247] (1) RD cells were plated in a 96-well plate at 100 μL per well.

[0248] (2) When the cells grew to a confluence of 70%-80%, the DMEM medium containing 10% serum was replaced with a DMEM medium containing 2% serum.

[0249] (3) The polypeptide drug was gradiently diluted with the DMEM containing 2% FBS into concentrations of 0.15625 μM, 0.3125 μM, 0.625 μM, 1.25 μM, 2.5 μM, and 5 μM, and then added to a new 96-well plate at 100 μL per well, with 3 replicate wells for each concentration.

[0250] (4) The diluted virus was added to the above wells at 100 μL per well. The wells with no drug and no virus added and the wells with no drug but virus added were set as controls respectively, with a final concentration of virus of 0.1 MOI.

[0251] (4) The mixture was transferred into the 96-well plate plated with cells for another 24 h of culture, and the inhibitory activity of the polypeptide on the virus was determined by a CCK8 kit.

[0252] (5) The inhibition rates of different concentrations of the polypeptides on virus infection were calculated according to a calculation formula of: inhibition rate of polypeptide=(well with drug-well with virus)×100%/(well with no drug-well with virus).

[0253] As shown in Tables 18-21 and FIG. **13**, the determination of the inhibitory activity of the polypeptides on the virus by a CCK8 method indicates that EQ had an IC50 of 1.83 μM, YQ had an IC50 of 1.96 μM, NQ had an IC50 of 1.90 μM, RSQ had an IC50 of 2.60 μM, SAQ had an IC50 of 2.90 μM, AQ had an IC50 of 2.99 μM, IQ had an IC50 of 1.64 μM, GQ had an IC50 of 1.78 μM, NTG had an IC50 of 2.28 μM, TQ had an IC50 of 1.76 μM, IEQ had an IC50 of 2.48 μM, and control TAT had no antiviral activity.

TABLE 18

Concentration of polypeptide (μM)	Inhibition rate on EV71 virus (%)								
	EQ			YQ			NQ		
0.15625	6.88487	4.67462	10.9434	5.8298	7.32961	11.0089	4.27801	5.36773	8.33654
0.3125	10.2126	12.8194	16.4536	15.102	10.7124	13.3881	11.5056	17.809	19.7228
0.625	21.5745	20.64	25.9399	23.6157	20.9642	29.1606	16.7886	21.2091	24.9365
1.25	31.5814	30.7778	32.9503	30.9365	28.1124	32.0401	41.6538	40.8779	50.5607
2.5	62.8263	54.1779	55.7124	62.0331	53.8159	52.1987	51.933	60.1502	57.7744
5	86.8541	90.3885	78.0023	80.0886	71.2711	87.578	60.6122	74.6708	75.3331

TABLE 19

Concentration of polypeptide (μM)	Inhibition rate on EV71 virus (%)								
	RSQ			SAQ			AQ		
0.15625	1.47093	6.58837	2.82634	3.54678	2.72622	6.81941	9.88833	7.50558	5.9715
0.3125	11.34	21.2746	16.4228	8.96419	7.90219	11.0435	15.9607	11.702	12.6022
0.625	18.633	25.8298	26.1918	14.4055	11.5364	18.402	26.3573	23.7158	19.5264
1.25	40.5468	36.4575	35.2676	27.4779	24.0778	33.4848	33.3847	31.7366	38.7678
2.5	53.4848	40.7778	47.2122	47.0158	47.2468	36.2572	58.9642	46.161	41.3092
5	58.6022	68.9642	62.4952	75.1367	61.8367	63.2191	51.0435	51.8675	69.1952

TABLE 20

Concentration of polypeptide (μM)	Inhibition rate on EV71 virus (%)								
	IQ			GQ			NTQ		
0.15625	12.0331	13.5849	7.36773	7.21948	10.3851	5.4678	2.6541502	4.74383	5.74339
0.3125	18.7401	22.4297	22.9573	14.8883	12.0678	14.91259	14.830508	10.2542	13.3898
0.625	26.0539	31.1744	34.8606	28.8333	30.5125	26.48479	20.841686	30.339	24.339
1.25	41.6022	42.578	47.4848	43.1263	40.6161	31.07124	39.174089	47.6271	50.8202
2.5	55.7952	50.9781	60.5503	51.8367	58.9472	57.2507	45.423729	51.9492	59.661
5	73.2884	68.7332	83.2884	71.5094	77.9438	84.7439	68.271186	52.5424	61.6949

TABLE 21

Concentration of polypeptide (μM)	Inhibition rate on EV71 virus (%)								
	TQ			IEQ			TAT		
0.15625	3.31377	5.35593	7.23007	11.724	8.44024	7.62688	1.38042	5.23131	2.29427
0.3125	12.3437	24.3588	27.3869	18.8643	15.5487	10.6223	2.46255	5.24217	6.28153
0.625	33.3736	28.4011	30.3085	20.7161	25.7224	19.5166	8.37385	2.33994	7.23439
1.25	35.3209	40.3898	46.2471	33.6936	35.484	28.4286	8.36531	3.23706	6.20318
2.5	59.3508	67.3559	55.2235	45.6995	48.4526	57.4377	7.37254	4.24089	8.21274
5	71.3029	65.3559	69.2301	69.4739	61.3527	68.272	12.2608	8.20447	19.1522

Example 14: Detection of Antiviral Activity of Polypeptide RQ Modifier

1. Experimental Materials

[0254] Polypeptides RQ-PA (shown in SEQ ID NO.16), RQ-PEG4-PA (shown in SEQ ID NO.17) and RQ-CHOL (shown in SEQ ID NO.18). The sequences were all commercially synthesized. CCK-8 reagent (MCE) was purchased from Promoter Company.

2. Experimental Process

[0255] (1) RD cells were plated in a 96-well plate at 100 μL per well.

[0256] (2) When the cells grew to a confluence of 70%-80%, the DMEM medium containing 10% serum was replaced with a DMEM medium containing 2% serum.

[0257] (3) The polypeptide drug was gradiently diluted with the DMEM containing 2% FBS into concentrations of 0.15625 μM, 0.3125 μM, 0.625 μM, 1.25 μM, 2.5 μM, and 5 μM, and then added to a new 96-well plate at 100 μL per well, with 3 replicate wells for each concentration. (4) The diluted virus was added to the above wells at 100 μL per well. The wells with no drug and no virus added and the wells with no drug but virus added were set as controls respectively, with a final concentration of virus of 0.1 MOI.

[0258] (4) The mixture was transferred into the 96-well plate plated with cells for another 24 h of culture, and the inhibitory activity of the polypeptide on the virus was determined by a CCK8 kit.

[0259] (5) The inhibition rates of different concentrations of the polypeptides on virus infection were calculated according to a calculation formula of: inhibition rate of polypeptide=(well with drug-well with virus)×100%/(well with no drug-well with virus).

[0260] As shown in Table 22 and FIG. **14**, the determination of the inhibitory activity of the polypeptides on the virus by a CCK8 method indicates that RQ-PA had an IC50 of 3.58 μM, RQ-PEG4-PA had an IC50 of 3.47 μM, and RQ-CHOL had an IC50 of 4.25 p.M.

TABLE 22

Concentration of polypeptide (μM)	Inhibition rate on EV71 virus (%)								
	RQ-PA			RQ-PEG4-PA			RQ-CHOL		
0.15625	1.01454	2.28411	5.27651	7.27824	4.14494	8.12615	5.14141	6.97113	3.01327
0.3125	7.00885	10.3563	13.2967	11.2937	10.1514	13.1404	10.1389	15.972	11.0215
0.625	14.0139	21.402	20.3253	25.328	21.1587	27.1395	21.154	32.986	27.0328
1.25	26.9703	29.3073	30.2687	30.2434	35.1393	34.127	30.1246	38.9729	35.9495
2.5	35.0322	41.4947	50.486	44.43	40.1798	45.1788	34.1616	42.9746	41.0404
5	53.0512	57.3238	59.3155	62.2879	53.136	52.1554	49.1465	51.9169	52.0145

Example 15: Determination of Efficiency of RQ-DRI in Inhibiting EV71 in RD Cells

1. Experimental Materials

[0261] RD cells; DMEM medium (Thermo) and serum (Gibco) were purchased from Invitrogen Co., Ltd; Total RNA extraction kit (Omega) and One step qRT-PCR kit (Takara) were purchased from U-MeBiotech Co., Ltd. The water used in the processes of RNA extraction and qRT-PCR was DEPC water, and the entire experiment was performed in an RNase-free environment.

[0262] Polypeptide RQ-DRI was synthesized by Nanjing GenScript Co., Ltd with a sequence shown in SEQ ID NO.19.

2. Experimental Process

[0263] (1) Different cells were plated in a 24-well plate.

[0264] (2) When the cells grew to a confluence of 70%-80%, the DMEM medium containing 10% serum was replaced with a DMEM medium containing 2% serum (the DMEM medium containing 2% serum was added in each well at an amount of 0.5 mL), and 5 μL of EV71 virus of 1×10^6 PFU/mL was added to each well.

[0265] (3) After 1 h, different polypeptides (RQ or control TAT) with final concentrations of 0.3125 μM, 0.625 μM, 1.25 μM, 2.5 μM and 5 μM were added respectively. The group without polypeptide added was used as a control.

[0266] (4) The samples were collected 24 h after the infection of EV71 virus, and RNA was extracted with the total RNA extraction kit.

[0267] (5) The supernatant was discarded, then 350 μL of TRK lysate was added to each well, and the plate was put on a shaker for 5 min.

[0268] (6) 350 μL of 70% ethanol (DEPC) was added to each well, and then the plate was put on a shaker for 5 min.

[0269] (7) The solution in each well was transferred to a RNA extraction column for 1 min of centrifugation at 12000 g.

[0270] (8) The solution obtained in the recovery tube was reloaded on the column again for 1 min of centrifugation at 12000 g.

[0271] (9) RNA washing buffer 1 was added in the column for 30 s of centrifugation at 12000 g.

[0272] (10) RNA washing buffer 2 was added in the column for 1 min of centrifugation at 12000 g.

[0273] (11) Step (10) was repeated.

[0274] (12) The column alone was centrifuged at 12000 g for 2 min to completely remove the residual RNA washing buffer.

[0275] (13) 50 μL of DEPC water was added to the column for 2 min of centrifugation at 12000 g.

[0276] (14) 2 μL of RNA sample was subjected to a fluorescence quantitative experiment using the one step qRT-PCR kit.

[0277] The results are shown in FIG. **15** and Table 23, which indicate that the polypeptide RQ-DRI had an IC50 of 2.05 M in RD cells.

TABLE 23

Concentration of RQ-DRI (μM)	Percentage of viral RNA in RD cells (%)		
0	68.13518	128.4527	103.4121
0.3125	90.20158	150.7832	85.03728
0.625	113.2834	92.13482	108.0728
1.25	93.12259	79.48209	58.28617
2.5	24.39655	38.77366	23.45334
5	21.2764	19.04182	17.18671

Example 16: Toxicity Assay of B-RQ in RD Cells

1. Experimental Materials

[0278] CCK-8 reagent (MCE) was purchased from Promoter Company. Polypeptide B-RQ was synthesized by Nanjing GenScript Co., Ltd with a sequence shown in SEQ ID NO.20.

2. Experimental Process

[0279] (1) RD cells were plated in a 96-well plate at 100 μL per well.

[0280] (2) When the cells grew to a confluence of 70%-80%, the DMEM medium containing 10% serum was replaced with a DMEM medium containing 2% serum, and B-RQ was added so that the final concentrations in the wells were 0.46 μM, 2.34 μM, 4.68 μM, 9.37 μM, 18.75 μM, 37.5 μM, 75 μM, and 150 μM.

[0281] (3) The samples were collected 24 h after the addition of the polypeptide, and then 10 μL of live cell detection reagent CCK-8 was added to each well and mixed well.

[0282] (4) The plate was placed at 37° C. for 2 h.

[0283] (5) The absorbance value at OD450 was detected by a microplate reader.

[0284] The results are shown in FIG. **16** and Table 24, which indicate that B-RQ had a CC50 of >75 μM taking the cell viability of untreated cells as 100%,.

TABLE 24

Concentration of B-RQ polypeptide (μM)	Cell viability (%)		
0.46875	99.01384	99.46899	98.27423
2.34375	99.12763	97.93287	97.19325
4.6875	98.67248	100.7775	96.96567
9.375	99.29831	96.62431	96.16916
18.75	97.81908	95.54333	97.36393
37.5	99.35521	99.63967	99.24142
75	95.99848	95.65712	99.29831
150	95.54333	95.94159	95.31576

Example 17: Determination of Efficiency of B-RQ in Inhibiting CVB3 and Echo 11 in RD Cells

1. Experimental Materials

[0285] RD cells; DMEM medium (Thermo) and serum (Gibco) were purchased from Invitrogen Co., Ltd; Total RNA extraction kit (Omega) and One step qRT-PCR kit (Takara) were purchased from U-MeBiotech Co., Ltd. The water used in the processes of RNA extraction and qRT-PCR was DEPC water, and the entire experiment was performed in an RNase-free environment.

[0286] Polypeptide B-RQ was synthesized by Nanjing GenScript Co., Ltd with a sequence shown in SEQ ID NO.20.

2. Experimental Process

[0287] (1) Different cells were plated in a 24-well plate.

[0288] (2) When the cells grew to a confluence of 70%-80%, the DMEM medium containing 10% serum was replaced with a DMEM medium containing 2% serum (the DMEM medium containing 2% serum was added in each well at an amount of 0.5 mL), and 5 μL of EV71 virus of 1×10^6 PFU/mL was added to each well.

[0289] (3) After 1 h, the polypeptides with final concentrations of 0.3125 μM, 0.625 μM, 1.25 μM, 2.5 μM and 5 μM were added respectively. The group without polypeptide added was used as a control.

[0290] (4) The samples were collected 24 h after the infection of EV71 virus, and RNA was extracted with the total RNA extraction kit.

[0291] (5) The supernatant was discarded, then 350 μL of TRK lysate was added to each well, and the plate was put on a shaker for 5 min.

[0292] (6) 350 μL of 70% ethanol (DEPC) was added to each well, and then the plate was put on a shaker for 5 min.

[0293] (7) The solution in each well was transferred to a RNA extraction column for 1 min of centrifugation at 12000 g.

[0294] (8) The solution obtained in the recovery tube was reloaded on the column again for 1 min of centrifugation at 12000 g.

[0295] (9) RNA washing buffer 1 was added in the column for 30 s of centrifugation at 12000 g.

[0296] (10) RNA washing buffer 2 was added in the column for 1 min of centrifugation at 12000 g.

[0297] (11) Step (10) was repeated.

[0298] (12) The column alone was centrifuged at 12000 g for 2 min to completely remove the residual RNA washing buffer.

[0299] (13) 50 μL of DEPC water was added to the column for 2 min of centrifugation at 12000 g.

[0300] (14) 2 μL of RNA sample was subjected to a fluorescence quantitative experiment using the one step qRT-PCR kit.

[0301] The results are shown in FIG. **17** and Tables 25-26, which indicate that the polypeptide B-RQ had an IC50 of 2.29 μM for inhibiting CVB3 in RD cells (FIG. **17**A), and an IC50 of 0.38 μM for inhibiting Echo 11 in RD cells (FIG. **17**B).

TABLE 25

Concentration of B-RQ (μM)	Percentage of CVB3 viral RNA (%)		
0	110.1778	109.7637	80.05847
0.3125	ND	126.7007	62.25837
0.625	116.425	88.76191	77.23717
1.25	80.22625	52.01695	36.74467
2.5	26.8204	49.13786	24.49975
5	22.86381	37.99408	26.97489

ND, not detected

TABLE 26

Concentration of B-RQ (μM)	Percentage of Echo 11 viral RNA (%)					
0	90.48197	75.34791	134.1701	105.4064	82.09798	112.4956
0.3125	78.63341	46.42134	35.11162	43.24912	24.63388	64.67318
0.625	50.9076	51.05015	20.07373	51.16597	51.93671	48.38548
1.25	47.75375	35.28912	14.8342	3.086194	12.76211	39.50013
2.5	0.685338	0.532339	0.784078	4.1758	1.177338	3.252157
5	0.616862	1.284713	0.869355	1.744232	0.571171	1.849904

[0302] The above are only preferred embodiments of the present invention. It should be noted that for those of ordinary skill in the art, multiple improvements and modifications can be made without departing from the principle of the present invention, and these improvements and modifications should be considered as the protection scope of the present invention.

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Ala Leu Phe Gln
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1 5 10 15

Phe Gln Xaa

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<223> OTHER INFORMATION: Xaa 19 =beta-AK-Chol

<400> SEQUENCE: 18

-continued

Arg Glu Tyr Asn Asn Arg Ser Ala Ile Gly Asn Thr Ile Glu Ala Leu
1 5 10 15

Phe Gln Xaa

<210> SEQ ID NO 19
<211> LENGTH: 32
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Polypeptide RQ-DRI

<400> SEQUENCE: 19

Gln Phe Leu Ala Glu Ile Thr Asn Gly Ile Ala Ser Arg Asn Asn Tyr
1 5 10 15

Glu Arg Gly Ser Gly Arg Arg Arg Gln Arg Arg Lys Lys Arg Gly Tyr
20 25 30

<210> SEQ ID NO 20
<211> LENGTH: 32
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Polypeptide B-RQ

<400> SEQUENCE: 20

Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg Gly Ser Gly Arg Glu
1 5 10 15

Tyr Asn His Arg His Ser Val Gly Ala Thr Leu Glu Ala Leu Phe Gln
20 25 30

<210> SEQ ID NO 21
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Core Sequence
<220> FEATURE:
<221> NAME/KEY: UNSURE
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Xaa (1) = arginine (R) or asparagine (N) or lysine (K)
<220> FEATURE:
<221> NAME/KEY: UNSURE
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: Xaa (3) = tyrosine (Y) or arginine (R)
<220> FEATURE:
<221> NAME/KEY: UNSURE
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: Xaa (4) = serine (S) or asparagine (N) or arginine (R)
<220> FEATURE:
<221> NAME/KEY: UNSURE
<222> LOCATION: (5)..(5)
<223> OTHER INFORMATION: Xaa (5) = asparagine (N) or arginine (R) or threonine (T) or histidine (H)
<220> FEATURE:
<221> NAME/KEY: UNSURE
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa (7) = serine (S) or asparagine (N) or histidine (H)
<220> FEATURE:
<221> NAME/KEY: UNSURE
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Xaa (8) = alanine (A) or asparagine (N) or serine (S)
<220> FEATURE:
<221> NAME/KEY: UNSURE
<222> LOCATION: (9)..(9)

-continued

<223> OTHER INFORMATION: Xaa (9) = isoleucine (I) or threonine (T) or valine (V)
<220> FEATURE:
<221> NAME/KEY: UNSURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa (10) = glycine (G) or glutamine (Q)
<220> FEATURE:
<221> NAME/KEY: UNSURE
<222> LOCATION: (11)..(11)
<223> OTHER INFORMATION: Xaa (11) = asparagine (N) or aspartic acid (D) or alanine (A)
<220> FEATURE:
<221> NAME/KEY: UNSURE
<222> LOCATION: (12)..(12)
<223> OTHER INFORMATION: Xaa (12) = threonine (T) or cysteine (C) or lysine (K)
<220> FEATURE:
<221> NAME/KEY: UNSURE
<222> LOCATION: (13)..(13)
<223> OTHER INFORMATION: Xaa (13) = isoleucine (I) or leucine (L)

<400> SEQUENCE: 21

Xaa Glu Xaa Xaa Xaa Arg Xaa Xaa Xaa Xaa Xaa Xaa Xaa Glu Ala Leu
1 5 10 15

Phe Gln

<210> SEQ ID NO 22
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Core Sequence
<220> FEATURE:
<221> NAME/KEY: UNSURE
<222> LOCATION: (5)..(5)
<223> OTHER INFORMATION: Xaa (5) = asparagine (N) or arginine (R) or threonine (T) or histidine (H)
<220> FEATURE:
<221> NAME/KEY: UNSURE
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa (7) = serine (S) or asparagine (N) or histidine (H)
<220> FEATURE:
<221> NAME/KEY: UNSURE
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Xaa (8) = alanine (A) or asparagine (N) or serine (S)
<220> FEATURE:
<221> NAME/KEY: UNSURE
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: Xaa (9) = isoleucine (I) or threonine (T) or valine (V)
<220> FEATURE:
<221> NAME/KEY: UNSURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa (10) = glycine (G) or glutamine (Q)
<220> FEATURE:
<221> NAME/KEY: UNSURE
<222> LOCATION: (11)..(11)
<223> OTHER INFORMATION: Xaa (11) = asparagine (N) or aspartic acid (D) or alanine (A)
<220> FEATURE:
<221> NAME/KEY: UNSURE
<222> LOCATION: (12)..(12)
<223> OTHER INFORMATION: Xaa (12) = threonine (T) or cysteine (C) or lysine (K)
<220> FEATURE:
<221> NAME/KEY: UNSURE
<222> LOCATION: (13)..(13)
<223> OTHER INFORMATION: Xaa (13) = isoleucine (I) or leucine (L)

<400> SEQUENCE: 22

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Arg Glu Tyr Asn Xaa Arg Xaa Xaa Xaa Xaa Xaa Xaa Xaa Glu Ala Leu
1 5 10 15

Phe Gln

<210> SEQ ID NO 23
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Core Sequence
<220> FEATURE:
<221> NAME/KEY: UNSURE
<222> LOCATION: (5)..(5)
<223> OTHER INFORMATION: Xaa (5) = asparagine (N) or arginine (R) or threonine (T) or histidine (H)
<220> FEATURE:
<221> NAME/KEY: UNSURE
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa (7) = serine (S) or asparagine (N) or histidine (H)
<220> FEATURE:
<221> NAME/KEY: UNSURE
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Xaa (8) = alanine (A) or asparagine (N) or serine (S)
<220> FEATURE:
<221> NAME/KEY: UNSURE
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: Xaa (9) = isoleucine (I) or threonine (T) or valine (V)
<220> FEATURE:
<221> NAME/KEY: UNSURE
<222> LOCATION: (11)..(11)
<223> OTHER INFORMATION: Xaa (11) = asparagine (N) or aspartic acid (D) or alanine (A)
<220> FEATURE:
<221> NAME/KEY: UNSURE
<222> LOCATION: (13)..(13)
<223> OTHER INFORMATION: Xaa (13) = isoleucine (I) or leucine (L)

<400> SEQUENCE: 23

Arg Glu Tyr Asn Xaa Arg Xaa Xaa Xaa Gly Xaa Thr Xaa Glu Ala Leu
1 5 10 15

Phe Gln

<210> SEQ ID NO 24
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Core Sequence

<400> SEQUENCE: 24

Arg Glu Tyr Asn His Arg His Ser Val Gly Ala Thr Leu Glu Ala Leu
1 5 10 15

Phe Gln

1. A polypeptide used as a broad-spectrum anti-enterovirus inhibitor, having a sequence selected from the group consisting of:

I.
(X1)E(X2)(X3)(X4)R(X5)(X6)(X7)(X8)(X9)(X10)(X11)
EALFQ

wherein:

X1 is selected from the group consisting of arginine (R), asparagine (N) and lysine (K);

X2 is selected from the group consisting of tyrosine (Y) and arginine (R);

X3 is selected from the group consisting of serine(S), asparagine (N) and arginine (R);

X4 is selected from the group consisting of asparagine (N), arginine (R), threonine (T) and histidine (H);

X5 is selected from the group consisting of serine(S), asparagine (N) and histidine (H);

X6 is selected from the group consisting of alanine (A), asparagine (N) and serine(S);

X7 is selected from the group consisting of isoleucine (I), threonine (T) and valine (V);

X8 is selected from the group consisting of glycine (G) and glutamine (Q);

X9 is selected from the group consisting of asparagine (N), aspartic acid (D) and alanine (A);

X10 is selected from the group consisting of threonine (T), cysteine (C) and lysine (K);

X11 is selected from the group consisting of isoleucine (I) and leucine (L);

II. a sequence with deletion, addition or substitution of at least one amino acid compared to the sequence in I;

III. a sequence that has at least 50% homology with the amino acid sequence in I or II and inhibits enterovirus activity; and

IV. a complementary sequence to the sequence in I or II or III.

2. The polypeptide according to claim 1, having a sequence shown in SEQ ID NO.1 or SEQ ID NO.24, or a D-configuration polypeptide thereof.

3. The polypeptide according to claim 1, comprising a sequence shown in SEQ ID NO.1 or SEQ ID NO.24.

4. The polypeptide according to claim 2, wherein the polypeptide has a sequence shown in SEQ ID NO.2 or SEQ ID NO.20.

5. The polypeptide according to claim 1, having a sequence with addition of 1-5 amino acids to the N-terminal of the polypeptide with the sequence shown in SEQ ID NO.21, with deletion of 1-13 amino acids from the N-terminal of the polypeptide with the sequence shown in SEQ ID NO.21, or with modification to the C-terminal of the polypeptide with the sequence shown in SEQ ID NO.21, or a D configuration polypeptide thereof.

6. The polypeptide according to claim 5, further comprising a cell-penetrating peptide.

7. The polypeptide according to claim 6, having a sequence shown in any one of SEQ ID NOs.3-20.

8. A method for inhibiting an enterovirus, comprising administering the polypeptide according to claim 1 to a subject in need thereof.

9. A method for treating or preventing enterovirus infection, comprising administering the polypeptide according to claim 1 to a subject in need thereof.

10. The method according to claim 8, wherein the enterovirus is selected from the group consisting of human enterovirus (EV), coxsackie A virus (CVA), coxsackie B virus (CVB), echovirus, rhinovirus and poliovirus.

11. The method according to claim 9, wherein the enterovirus infection causes a disease selected from the group consisting of hand-foot-mouth disease, myocarditis, herpetic angina, aseptic meningitis, encephalitis, and viral cold.

12. A method for preventing and/or treating a viral disease, comprising administering a preparation inhibiting multimerization of enterovirus protein 2C as a target to a subject in need thereof.

13. The method according to claim 9, wherein the enterovirus is selected from the group consisting of human enterovirus (EV), coxsackie A virus (CVA), coxsackie B virus (CVB), echovirus, rhinovirus and poliovirus.

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