

eIF4G impairs the assembly of the eIF4F complex on the 5' cap structure and hinders cap-dependent translation.

[0006] There is therefore a need to identify eIF4E binding agents possessing a distinct mechanism of action from that described above to circumvent emerging mechanisms of resistance that have been reported for compounds that target signalling pathways upstream of eIF4E involved in regulating protein synthesis. There is also a need to develop eIF4E binding agents for use in anti-cancer therapy.

SUMMARY

[0007] In one example, there is provided a peptide comprising the amino acid sequence CEX₁GX₂X₃X₄X₅C (SEQ ID NO: 1), wherein X₁ is an amino acid selected from the group consisting of threonine (T), methionine (M) or leucine (L); wherein X₂ is an amino acid selected from the group consisting of a hydrophobic amino acid, an aromatic amino acid, a modified hydrophobic amino acid and a modified aromatic amino acid; wherein X₃ is an amino acid selected from the group consisting of a hydrophobic amino acid, an aromatic amino acid, a modified hydrophobic amino acid and a modified aromatic amino acid; wherein X₄ is any amino acid; wherein X₅ is any amino acid; wherein the two cysteine residues are joined by a disulphide bond; and wherein the peptide binds to elongation initiation factor 4E (eIF4E).

[0007a] In one aspect, there is provided a peptide comprising the amino acid sequence CEX₁GX₂X₃X₄X₅C (SEQ ID NO: 1), wherein X₁ is an amino acid selected from the group consisting of threonine (T), methionine (M) or leucine (L); wherein X₂ is an amino acid selected from the group consisting of a hydrophobic amino acid, an aromatic amino acid, a modified hydrophobic amino acid and a modified aromatic amino acid; wherein X₃ is an amino acid selected from the group consisting of a hydrophobic amino acid, an aromatic amino acid, a modified hydrophobic amino acid and a modified aromatic amino acid; wherein X₄ is any amino acid; wherein X₅ is any amino acid; wherein the two cysteine residues are joined by a disulphide bond; wherein the peptide binds to elongation initiation factor 4E (eIF4E); and wherein X₂ and X₃ are independently selected from the group consisting of phenylalanine (F), tyrosine (Y) and a modified phenylalanine.

[0008] In another aspect, there is provided a peptide as described herein for use as a medicament.

[0009] In another example, there is provided a peptide as described herein for use in treating a condition associated with dysregulated cap-dependent translation.

[0010] In another aspect, there is provided a pharmaceutical composition comprising the peptide as described herein and a pharmaceutically acceptable carrier.

5 [0011] In another aspect, there is provided a use of the peptide as described herein, or the pharmaceutical composition as described herein in the manufacture of a medicament for treating a condition associated with dysregulated cap-dependent translation.

[0012] In another example, there is provided a method of treating a condition associated with dysregulated cap-dependent translation in a subject in need thereof, comprising the
10 administering the peptide as described herein, or the pharmaceutical composition as described herein to the subject.

the formation of a β -hairpin with the correct geometry in the binding pocket. Substitution of Q8 with A did not affect binding. However, replacement of D9 with A causes a two-fold decrease in binding.

[00140] **Table 3:** Targeted mutagenesis on position E3 and M4 of EE-02

Name	N-term	1	2	3	4	5	6	7	8	9	10	11	C-term	mean KD (nM)	SD of KD	SEQ ID NO.
EE-2	amine	A	C	E	M	G	F	F	Q	D	C	G	amide	207	57	2
EE-19	amine	A	C	D	M	G	F	F	Q	D	C	G	amide	26000		20
EE-20	amine	A	C	E	I	G	F	F	Q	D	C	G	amide	1600		21
EE-21	amine	A	C	E	L	G	F	F	Q	D	C	G	amide	2500		22
EE-22	amine	A	C	E	Nle	G	F	F	Q	D	C	G	amide	520		23
EE-23	amine	A	C	E	Cba	G	F	F	Q	D	C	G	amide	35000		24

[00141] E3 could not be replaced by D3 highlighting the important of correct orientation of the carboxyl group to interact with R112 of eIF4E. M4 was replaced with shorter and smaller aliphatic groups such as I and L that resulted in loss of binding. M4 was also replaced with the non-natural aliphatic amino acids norleucine and cyclobutylalanine, which are similar in size to methionine. However, the K_d s determined for these molecules still decreased against eIF4E, highlighting the importance of the sulphur atom interaction with S92.

[00142] **Table 4:** Targeted mutagenesis of residue G5

Name	N-term	1	2	3	4	5	6	7	8	9	10	11	C-term	mean KD (nM)	SD of KD	SEQ ID NO.
EE-2	amine	A	C	E	M	G	F	F	Q	D	C	G	amide	207	57	2
EE-27	amine	A	C	E	M	bA	F	F	Q	D	C	G	amide	>50000		25
EE-28	amine	A	C	E	M	Sar	F	F	Q	D	C	G	amide	4200		26
EE-29	amine	A	C	E	M	P	F	F	Q	D	C	G	amide	>50000		27

[00143] G5 was replaced with different amino acids (beta-alanine (bA), Sarcosine (Sar), P) to explore different stereo-chemical restraints around this position. All resulted in dramatic decreases in macrocyclic binding to eIF4E, further highlighting the role of G5 in allowing the beta hairpin to form and to orientate the key interacting residues into their most optimal positions to bind eIF4E.

[00144] **Table 5:** Targeted mutagenesis of positions F6 and F7 in EE-02

Name	N-term	1	2	3	4	5	6	7	8	9	10	11	C-term	mean KD (nM)	SD of KD	SEQ ID NO.
EE-2	amine	A	C	E	M	G	F	F	Q	D	C	G	amide	207	57	2

EE-30	amine	A	C	E	M	G	Y	F	Q	D	C	G	amide	85	31	28
EE-31	amine	A	C	E	M	G	W	F	Q	D	C	G	amide	5900		29
EE-33	amine	A	C	E	M	G	L	F	Q	D	C	G	amide	>50000		30
EE-34	amine	A	C	E	M	G	F	L	Q	D	C	G	amide	>50000		31
EE-35	amine	A	C	E	M	G	F	Y	Q	D	C	G	amide	137	6	32
EE-36	amine	A	C	E	M	G	F	W	Q	D	C	G	amide	2600		33
EE-37	amine	A	C	E	M	G	Y	Y	Q	D	C	G	amide	350		34
EE-38	amine	A	C	E	M	G	L	L	Q	D	C	G	amide	>50000		35

[00145] Binding to eIF4E can be incrementally improved by exploring ring substituents on F6 and F7. Replacement of F6 with Y6 or F7 with Y7 produced two molecules that bound more tightly than EE-02 (EE-30 and EE-35, respectively). These results that these positions

5 can be explored further to generate better binding analogues.

[00146] **Table 6:** Target mutagenesis of position Q8 in EE-02

Name	N-term	1	2	3	4	5	6	7	8	9	10	11	C-term	mean KD (nM)	SD of KD	Fold change	SEQ ID NO.
EE-2	amine	A	C	E	M	G	F	F	Q	D	C	G	amide	207	57	1	2
EE-2F	amine	A	C	E	M	G	F	F	A	D	C	G	amide	167	38	0.8	4
EE-39	amine	A	C	E	M	G	F	F	K	D	C	G	amide	109	12	0.5	5
EE-40	amine	A	C	E	M	G	F	F	G	D	C	G	amide	400		1.9	36
EE-41	amine	A	C	E	M	G	F	F	L	D	C	G	amide	190		0.9	6

[00147] Position 8 of EE-02 shows no strict preference for a particular residues. This correlates with the fact in forms no interactions with eIF4E in the crystal structure. However,

10 the slight decrease in binding seen with the G mutation suggest that rigidification strategies at this position could improve binding via a mechanism of entropy reduction.

[00148] **Table 7:** Truncation studies on EE-02

Name	N-term	1	2	3	4	5	6	7	8	9	10	11	C-term	mean KD (nM)	SD of KD	Fold change	SEQ ID NO.
EE-2	amine	A	C	E	M	G	F	F	Q	D	C	G	amide	207	57	1	2
EE-2FAM	5FAM	A	C	E	M	G	F	F	Q	D	C	G	amide	2100		10.1	2
EE-2G	amine	A	C	E	M	G	F	F	Q	A	C	G	amide	530	0	2.6	19
EE-2H	Ac		C	E	M	G	F	F	Q	D	C		amide	1700		8.2	38
EE-11	Ac	A	C	E	M	G	F	F	Q	D	C	G	amide	1000		4.9	2
EE-12	amine		C	E	M	G	F	F	Q	D	C	G	amide	77	6	0.4	3
EE-44	amine		C	E	M	G	F	F	A	D	C		amide	79	9	0.4	7

[00149] These studies were designed to find the smallest macrocyclic by molecular

15 weight (MW) could bind eIF4E. Additionally N-terminal capping of the macrocyclics were

Claims

1. A peptide comprising the amino acid sequence CEX₁GX₂X₃X₄X₅C (SEQ ID NO: 1),
wherein X₁ is an amino acid selected from the group consisting of threonine (T),
methionine (M) or leucine (L);
wherein X₂ is an amino acid selected from the group consisting of a hydrophobic
amino acid, an aromatic amino acid, a modified hydrophobic amino acid and a
modified aromatic amino acid ;
wherein X₃ is an amino acid selected from the group consisting of a hydrophobic
amino acid, an aromatic amino acid, a modified hydrophobic amino acid and a
modified aromatic amino acid;
wherein X₄ is any amino acid;
wherein X₅ is any amino acid;
wherein the two cysteine residues are joined by a disulphide bond;
wherein the peptide binds to elongation initiation factor 4E (eIF4E); and
wherein X₂ and X₃ are independently selected from the group consisting of
phenylalanine (F), tyrosine (Y) and a modified phenylalanine.
2. The peptide according to claim 1, wherein the peptide binds to eIF4E at the mRNA
5' cap-binding site.
3. The peptide according to claims 1 or 2, wherein binding of the peptide to eIF4E
inhibits eIF4E activity.
4. The peptide according to any one of claims 1 to 3, wherein the peptide binds to eIF4E
in an open conformation.
5. The peptide according to any one of claims 1 to 4, wherein X₁ is methionine (M).
6. The peptide according to any one of claims 1 to 5, wherein X₄ and X₅ are
independently glutamine (Q), aspartic acid (D), alanine (A), lysine (K), glycine
(G) or leucine (L).

7. The peptide according to any one of claims 1 to 6, wherein X₅ is D-aspartic acid or L-aspartic acid.
8. The peptide according to any one of claims 1 to 7, wherein the peptide comprises the amino acid sequence selected from the group consisting of ACEMGFFQDCG (SEQ ID NO: 2), CEMGFFQDCG (SEQ ID NO: 3), ACEMGFFADCG (SEQ ID NO: 4), ACEMGFFKDCG (SEQ ID NO: 5), ACEMGFFLDCG (SEQ ID NO: 6), CEMGFFADC (SEQ ID NO: 7), ACEMGYFQDCG (SEQ ID NO: 28), and ACEMGFYQDCG (SEQ ID NO: 32).
9. The peptide according to any one of claims 1 to 8, wherein the peptide consists of the amino acid sequence CEMGFFADC (SEQ ID NO: 7).
10. The peptide according to any one of claims 1 to 9, wherein the peptide is conjugated to one or more additional peptides.
11. The peptide according to claim 10, wherein the one or more additional peptides is a cell penetrating peptide.
12. The peptide according to any one of claims 1 to 11 for use as a medicament.
13. A pharmaceutical composition comprising the peptide according to any one of claims 1 to 11 and a pharmaceutically acceptable carrier.
14. Use of the peptide according to any one of claims 1 to 11, or the pharmaceutical composition according to claim 13 in the manufacture of a medicament for treating a condition associated with dysregulated cap-dependent translation.
15. The use of claim 14, wherein the condition is cancer, an infectious disease, autism or fragile X syndrome; wherein the infectious disease is a viral infection.