

Various attempts to engineer toxin peptides for particular properties, including specificity or potency, have been described, e.g. in WO2006/002850, WO2006/042151, WO2008/088422, WO2006/116156, WO2010/105184 and WO2014/116937. International patent application PCT/EP2020/076187 also disclosed Kv1.3 blockers.

- 5 However, there remains a need for alternative Kv1.3 blockers. Blockers having improved specificity compared to known blockers may be particularly desirable, although improvements in other properties such as stability and potency may also be useful.

SUMMARY

- 10 The disclosure relates to ion channel blockers derived from a toxin peptide of the scorpion *Parabuthus transvaalicus*. The toxin peptide has the amino acid sequence QMDMRCSASVECKQKCLKAIGSIFGKCMNKKCKCYPR (SEQ ID NO: 1).

Amongst other desirable properties, this molecule and derivatives or variants thereof have been found to be extremely selective blockers for the Kv1.3 potassium ion channel over other voltage-gated potassium channels, and typically also have high potency at blocking the Kv1.3 channel.

- 15 Accordingly, in one aspect, the disclosure provides an ion channel blocker which has Kv1.3 inhibitor activity, or a pharmaceutically acceptable salt thereof, comprising a variant of the sequence QMDMRCSASVECKQKCLKAIGSIFGKCMNKKCKCYPR (SEQ ID NO: 1), wherein at least one amino acid in position 7, 8, 9, 10 or 11 of SEQ ID NO: 1 is substituted with an amino acid having a positively charged side chain and/or an amino acid having an aromatic side chain,
20 and wherein the variant does not comprise any of the following sequences:

NMDMRCKASVECKQKCLKAIGSIFGKCMNKKCKCYPR	SEQ ID NO: 2
NMDMRCSASRECKQKCLKAIGSIFGKCMNKKCKCYPR	SEQ ID NO: 3
N[Nle]D[Nle]RCRASVECKQKCLKAIGSIFGKC[Nle]NKKCKCYPR	SEQ ID NO: 4
N[Nle]D[Nle]RCSHSVECKQKCLKAIGSIFGKC[Nle]NKKCKCYPR	SEQ ID NO: 5
N[Nle]D[Nle]RCSASKECKQKCLKAIGSIFGKC[Nle]NKKCKCYPR	SEQ ID NO: 6
P[Nle]E[Nle]RCFASVECKQKCLAAIGSIFGKC[Nle]NKKCKCYPR	SEQ ID NO: 7
P[Nle]E[Nle]RCSYSVECKQKCLAAIGSIFGKC[Nle]NKKCKCYPR	SEQ ID NO: 8
P[Nle]E[Nle]RCSAFVECKQKCLAAIGSIFGKC[Nle]NKKCKCYPR	SEQ ID NO: 9

In one example the disclosure provides an ion channel blocker which has Kv1.3 inhibitor activity, or a pharmaceutically acceptable salt thereof, comprising a variant of the sequence QMDMRCSASVECKQKCLKAIGSIFGKCMNKKCKCYPR (SEQ ID NO: 1), wherein at least

one amino acid in position 7, 8, 9, 10 or 11 of SEQ ID NO: 1 is substituted with an amino acid having a positively charged side chain and/or an amino acid having an aromatic side chain.

5 In other aspects the disclosure provides a nucleic acid encoding an ion channel blocker of the disclosure, an expression vector comprising a nucleic acid of the disclosure, and a host cell comprising a nucleic acid of the disclosure or an expression vector of the disclosure and capable of expressing an ion channel blocker of the disclosure.

10 The disclosure also provides methods of synthesising the ion channel blocker or pharmaceutically acceptable salt of the disclosure, the method comprising synthesising the ion channel blocker by means of solid-phase or liquid-phase peptide synthesis methodology and recovering the peptide thus obtained; expressing the ion channel blocker from a nucleic acid construct that encodes the ion channel blocker and recovering the expression product; or expressing a precursor peptide from a nucleic acid construct that encodes the precursor peptide sequence, recovering the expression product, and modifying the precursor peptide to yield the ion channel blocker.

15 The disclosure further provides pharmaceutical compositions comprising the ion channel blocker or pharmaceutically acceptable salt of the disclosure.

20 The disclosure yet further provides medical use of the ion channel blocker, pharmaceutically acceptable salt or pharmaceutical composition of the disclosure and methods of treating disease using the ion channel blocker, pharmaceutically acceptable salt or pharmaceutical composition of the disclosure. In another aspect, the present disclosure provides a use of an ion channel blocker, a pharmaceutically acceptable salt or a pharmaceutical composition as disclosed herein in the manufacture of a medicament for the treatment of an inflammatory condition or disorder.

DETAILED DESCRIPTION

Ion channel blocker

25 The invention provides an ion channel blocker.

Ion channel blockers of the invention may be in the form of a pharmaceutically acceptable salt. All references to “an ion channel blocker” herein should be considered to encompass any pharmaceutically acceptable salt thereof, regardless of whether “pharmaceutically acceptable salt” is explicitly recited.

30 The term “ion channel blocker” is used to denote a compound having inhibitor (or blocking) activity against an ion channel, i.e. capable of inhibiting or eliminating ion flow through the respective ion

channel, typically by binding to the ion channel. Similarly, the terms “Kv1.3 inhibitor” and “Kv1.3 inhibitor component” refer to a peptide capable of inhibiting or eliminating ion flow through a Kv1.3 ion channel, typically by binding to the Kv1.3 channel. However, the terms “blocker” and “inhibitor” should not be taken to imply any particular mechanism of action, or any particular mode of interaction with the ion channel itself.

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The term Kv1.3 refers to potassium voltage-gated channel subfamily A member 3, also referred to as KCNA3, HPCN3, HGK5, HuKIII and HLK3. “Subfamily A” may also be referred

CLAIMS

1. An ion channel blocker which has Kv1.3 inhibitor activity, or a pharmaceutically acceptable salt thereof, comprising a variant of the sequence QMDMRCSASVECKQKCLKAIGSIFGKCMNKKCKCYPR (SEQ ID NO: 1),
- 5 wherein the variant differs from SEQ ID NO: 1 by up to ten substitutions, insertions or deletions in total,
- wherein at least one amino acid in position 7, 8, 9, 10 or 11 of SEQ ID NO: 1 is substituted with an amino acid having a positively charged side chain and/or an amino acid having an aromatic side chain, and wherein the variant does not comprise any of the following
- 10 sequences:
- | | |
|---|--------------|
| NMDMRCKASVECKQKCLKAIGSIFGKCMNKKCKCYPR | SEQ ID NO: 2 |
| NMDMRCSASRECKQKCLKAIGSIFGKCMNKKCKCYPR | SEQ ID NO: 3 |
| N[Nle]D[Nle]RCRASVECKQKCLKAIGSIFGKC[Nle]NKKCKCYPR | SEQ ID NO: 4 |
| N[Nle]D[Nle]RCSHSVECKQKCLKAIGSIFGKC[Nle]NKKCKCYPR | SEQ ID NO: 5 |
| N[Nle]D[Nle]RCSASKECKQKCLKAIGSIFGKC[Nle]NKKCKCYPR | SEQ ID NO: 6 |
| P[Nle]E[Nle]RCFASVECKQKCLAAIGSIFGKC[Nle]NKKCKCYPR | SEQ ID NO: 7 |
| P[Nle]E[Nle]RCSYSVECKQKCLAAIGSIFGKC[Nle]NKKCKCYPR | SEQ ID NO: 8 |
| P[Nle]E[Nle]RCSAFVECKQKCLAAIGSIFGKC[Nle]NKKCKCYPR | SEQ ID NO: 9 |
2. The ion channel blocker of any of the preceding claims wherein any substitutions or deletions in the variant of SEQ ID NO: 1 are at amino acid positions selected from positions 1-5, 7-11, 13-15, 17-23, 25, 28-31, 33 and 35-37 of SEQ ID NO: 1, preferably at amino acid
- 15 positions selected from positions 1-5, 7-11, 13, 15, 18, 28 and 30 of SEQ ID NO: 1.
3. The ion channel blocker of any of the preceding claims, wherein the ion channel blocker has an IC₅₀ for human Kv1.3 potassium channel of 50 nM or less, such as 20 nM or less, such as 10 nM or less, such as 5 nM or less, such as 2 nM or less, such as 1 nM or
- 20 less, such as 0.5 nM or less, such as 0.4 nM or less, such as 0.3 nM or less, such as 0.2 nM or less.
4. The ion channel blocker of any of the preceding claims wherein the amino acid having a positively charged side chain is selected from the group consisting
- 25 of H, K, hK, R, Orn, 2,3-diaminopropanoyl, 2,4-diaminobutanoyl, 2-amino-3-guanidinopropionyl, and 4-amino-phenylalanine (F(4-NH₂)), preferably from H, K, R, Orn, 2,3-diaminopropanoyl, 2-amino-3-guanidinopropionyl, and 2,4-diaminobutanoyl; and/or

the amino acid having an aromatic side chain is selected from the group consisting of F, W and Y, preferably Y.

5. The ion channel blocker of any of the preceding claims wherein

5 the amino acid in position 7 of SEQ ID NO: 1 is substituted with an amino acid having a positively charged side chain or an aromatic side chain, preferably H, K, R, Orn, 2,3-diaminopropanoyl, 2-amino-3-guanidinopropionyl, 2,4-diaminobutanoyl or Y;

the amino acid in position 8 of SEQ ID NO: 1 is substituted with an amino acid having a positively charged side chain or an aromatic side chain, or the amino acid in position 8 of the

10 variant is the same amino acid as position 8 in SEQ ID NO: 1, namely A;

the amino acid in position 9 of SEQ ID NO: 1 is substituted with an amino acid having a positively charged side chain or an aromatic side chain, preferably K, Orn or 2,3-diaminopropanoyl;

the amino acid in position 10 of SEQ ID NO: 1 is substituted with an amino acid having a

15 positively charged side chain or an aromatic side chain, preferably R; and/or

the amino acid in position 11 of SEQ ID NO: 1 is substituted with an amino acid having a positively charged side chain or an aromatic side chain, preferably R.

6. The ion channel blocker of any of the preceding claims wherein, in the variant of SEQ

20 ID NO: 1,

the amino acid at position 1 is N, P or Q, or is deleted,

the amino acid at position 2 is M or Nle, or is deleted,

the amino acid at position 3 is D or E, or is deleted,

the amino acid at position 4 is M or Nle, or is deleted,

25 the amino acid at position 5 is R or is deleted,

the amino acid at position 13 is A or K,

the amino acid at position 15 is K or S,

the amino acid at position 18 is A or K,

the amino acid at position 28 is M or Nle, and/or

30 the amino acid at position 30 is G or K.

7. The ion channel blocker of any of the preceding claims wherein, in the variant of SEQ ID NO: 1,

the amino acids at positions 1-5 consist of the amino acid sequence P[Nle]E[Nle]R,

35 the amino acid at position 13 is A or K,

the amino acid at position 15 is K or S,

the amino acid at position 18 is A,

the amino acid at position 28 is Nle, and
the amino acid at position 30 is G or K.

8. The ion channel blocker of any of the preceding claims wherein the variant of SEQ ID NO: 1 comprises or consists of one of the following sequences:

Sequence	SEQ ID NO
P[Nle]E[Nle]RC[2,4-Diaminobutanoyl]ASVECKQKCLAAIGSIFGKC[Nle]NKKCKCYPR	10
P[Nle]E[Nle]RC[Orn]ASVECKQKCLAAIGSIFGKC[Nle]NKKCKCYPR	11
P[Nle]E[Nle]RC[2-Amino-3-guanidinopropionyl]ASVECKQKCLAAIGSIFGKC[Nle]NKKCKCYPR	12
P[Nle]E[Nle]RCHASVECKQKCLAAIGSIFGKC[Nle]NKKCKCYPR	13
P[Nle]E[Nle]RCYASVECKQKCLAAIGSIFGKC[Nle]NKKCKCYPR	14
P[Nle]E[Nle]RCSA[Orn]VECKQKCLAAIGSIFGKC[Nle]NKKCKCYPR	15
P[Nle]E[Nle]RC[2,3-Diaminopropanoyl]ASVECKQKCLAAIGSIFGKC[Nle]NKKCKCYPR	16
P[Nle]E[Nle]RCSA[2,3-Diaminopropanoyl]VECKQKCLAAIGSIFGKC[Nle]NKKCKCYPR	17
P[Nle]E[Nle]RC[2,4-Diaminobutanoyl]ASVECAQSCLAAIGSIFGKC[Nle]NGKCKCYPR	18
P[Nle]E[Nle]RCRASVECAQSCLAAIGSIFGKC[Nle]NGKCKCYPR	19
P[Nle]E[Nle]RCSASRECAQSCLAAIGSIFGKC[Nle]NGKCKCYPR	20

9. The ion channel blocker of any of the preceding claims wherein the variant comprises or consists of one of the following compounds:

Cpd No.	Sequence	SEQ ID NO
1	H-P[Nle]E[Nle]RC(1)[2,4-Diaminobutanoyl]ASVEC(2)KQKC(3)LAAIGSIFGKC(1)[Nle]NKKC(2)KC(3)YPR-OH	10
2	H-P[Nle]E[Nle]RC(1)[Orn]ASVEC(2)KQKC(3)LAAIGSIFGKC(1)[Nle]NKKC(2)KC(3)YPR-OH	11
3	H-P[Nle]E[Nle]RC(1)[2-Amino-3-guanidinopropionyl]ASVEC(2)KQKC(3)LAAIGSIFGKC(1)[Nle]NKKC(2)KC(3)YPR-OH	12
4	H-P[Nle]E[Nle]RC(1)HASVEC(2)KQKC(3)LAAIGSIFGKC(1)[Nle]NKKC(2)KC(3)YPR-OH	13
5	H-P[Nle]E[Nle]RC(1)YASVEC(2)KQKC(3)LAAIGSIFGKC(1)[Nle]NKKC(2)KC(3)YPR-OH	14
6	H-P[Nle]E[Nle]RC(1)SA[Orn]VEC(2)KQKC(3)LAAIGSIFGKC(1)[Nle]NKKC(2)KC(3)YPR-OH	15
7	H-P[Nle]E[Nle]RC(1)[2,3-Diaminopropanoyl]ASVEC(2)KQKC(3)LAAIGSIFGKC(1)[Nle]NKKC(2)KC(3)YPR-OH	16

8	H-P[Nle]E[Nle]RC(1)SA[2,3-Diaminopropanoyl]VEC(2)KQKC(3)LAAIGSIFGKC(1)[Nle]NKKC(2)KC(3)YPR-OH	17
9	H-P[Nle]E[Nle]RC(1)[2,4-Diaminobutanoyl]ASVEC(2)AQSC(3)LAAIGSIFGKC(1)[Nle]NGKC(2)KC(3)YPR-OH	18
10	H-P[Nle]E[Nle]RC(1)[2,4-Diaminobutanoyl]ASVEC(2)AQSC(3)LAAIGSIFGKC(1)[Nle]NGKC(2)KC(3)YPR-[NH ₂]	18
11	H-P[Nle]E[Nle]RC(1)RASVEC(2)AQSC(3)LAAIGSIFGKC(1)[Nle]NGKC(2)KC(3)YPR-[NH ₂]	19
12	H-P[Nle]E[Nle]RC(1)SASREC(2)AQSC(3)LAAIGSIFGKC(1)[Nle]NGKC(2)KC(3)YPR-[NH ₂]	20

10. A nucleic acid encoding an ion channel blocker as defined in any of the preceding claims.
- 5 11. An expression vector comprising a nucleic acid according to claim 10.
12. A host cell comprising a nucleic acid according to claim 10 or an expression vector according to claim 11 and capable of expressing an ion channel blocker as defined in any of claims 1 to 9.
- 10 13. A method of synthesising an ion channel blocker according to any of claims 1 to 9, the method comprising:
- (a) synthesising the ion channel blocker by means of solid-phase or liquid-phase peptide synthesis methodology and recovering the peptide thus obtained;
- 15 (b) expressing the ion channel blocker from a nucleic acid construct that encodes the ion channel blocker and recovering the expression product; or
- (c) expressing a precursor peptide from a nucleic acid construct that encodes the precursor peptide sequence, recovering the expression product, and modifying the precursor peptide to yield the ion channel blocker.
- 20 14. A pharmaceutical composition comprising an ion channel blocker or pharmaceutically acceptable salt according to any one of claims 1 to 9.
15. Use of an ion channel blocker, a pharmaceutically acceptable salt or a
- 25 pharmaceutical composition according to any of the preceding claims in the manufacture of a medicament for the treatment of an inflammatory condition or disorder.