SUMMARY ~~OF THE INVENTION~~

[0024] The present disclosure includes~~invention is directed to~~ a composition of matter comprising an isolated polypeptide, which is a peripherally-restricted $Na_V1.7$ inhibitor.

[0024a] In one aspect, there is provided an isolated polypeptide comprising the amino acid sequence of the formula:

$X_{aa}^{1}X_{aa}^{2}\ Cys^{3}X_{aa}^{4}X_{aa}^{5}X_{aa}^{6}X_{aa}^{7}X_{aa}^{8}X_{aa}^{9}Cys^{10}X_{aa}^{11}X_{aa}^{12}X_{aa}^{13}X_{aa}^{14}Asp^{15}X_{aa}^{16}\ Cys^{17}$

$Cys^{18}X_{aa}^{19}X_{aa}^{20}X_{aa}^{21}X_{aa}^{22}X_{aa}^{23}\ Cys^{24}X_{aa}^{25}X_{aa}^{26}X_{aa}^{27}X_{aa}^{28}X_{aa}^{29}Trp^{30}$

$Cys^{31}Lys^{32}Tyr^{33}X_{aa}^{34}Phe^{35}X_{aa}^{36}X_{aa}^{37}X_{aa}^{38}$

or a pharmaceutically acceptable salt thereof,

wherein:

X_{aa}^{1} is absent and X_{aa}^{2} is any amino acid residue;

X_{aa}^{4} is an acidic, hydrophobic, basic, or neutral hydrophilic amino acid residue;

X_{aa}^{5} is a Gly, Ala, hydrophobic, or basic amino acid residue;

X_{aa}^{6} is a Gly, Ala, 2-Abu, 2-Aib, Ile, Leu, Val, Nle, or Nva;

X_{aa}^{7} is a Gly, Ala, aromatic, or hydrophobic amino acid residue;

X_{aa}^{8} is a basic, acidic, or neutral hydrophilic amino acid residue, or an Ala residue;

X_{aa}^{9} is a basic or neutral hydrophilic amino acid residue;

X_{aa}^{11} is any amino acid residue;

X_{aa}^{12} is a Pro, acidic, neutral, or hydrophobic amino acid residue;

X_{aa}^{13} is any amino acid residue;

X_{aa}^{14} is any amino acid residue;

X_{aa}^{16} is a basic, neutral hydrophilic, or acidic amino acid residue, or an Ala residue;

X_{aa}^{19} is any amino acid residue;

X_{aa}^{20} is a Pro, Gly, basic, or neutral hydrophilic residue;

X_{aa}^{21} is a basic, hydrophobic, or neutral hydrophilic amino acid residue;

X_{aa}^{22} is a hydrophobic or basic amino acid residue;

X_{aa}^{23} is a hydrophobic, basic, or neutral hydrophilic amino acid residue;

X_{aa}^{25} is a Ser, Ala, or a neutral hydrophilic amino acid residue;

X_{aa}^{26} is an Ala, acidic, basic, or neutral hydrophilic amino acid residue;

X_{aa}^{27} is an acidic, basic, neutral hydrophilic or hydrophobic residue;

X_{aa}^{28} is an aromatic or basic amino acid residue;

X_{aa}^{29} is an acidic, basic, or neutral hydrophilic amino acid residue;

X_{aa}^{34} is any amino acid residue;

each of X_{aa}^{36}, X_{aa}^{37}, and X_{aa}^{38} is independently absent or is independently a neutral, basic, or hydrophobic amino acid residue;

and wherein the isolated polypeptide selectively inhibits Nav1.7 sodium channels relative to Nav1.4 and Nav1.5 sodium channels.

[0024b] In one ~~embodiment~~example, the isolated polypeptide is a dual inhibitor of $Na_V1.7$ and $Na_V1.3$ having the amino acid sequence DCLGFMRKCIPDNDKCCRPNLVCSRTHKWCKYVF-NH_2 (SEQ ID NO:1), which is a peptide we have designated "GpTx-1", and which was isolated from the venom of the tarantula *Grammostola porteri*, as described herein. Other ~~embodiments~~examples of the present ~~invention~~disclosure comprise a peptide analog of GpTx-1. In other ~~embodiments~~examples, the present ~~invention~~disclosure is directed to a composition of matter comprising an isolated polypeptide comprising the amino acid sequence of the formula:

$X_{aa}^{1}X_{aa}^{2}X_{aa}^{3}X_{aa}^{4}X_{aa}^{5}X_{aa}^{6}X_{aa}^{7}X_{aa}^{8}X_{aa}^{9}X_{aa}^{10}X_{aa}^{11}X_{aa}^{12}X_{aa}^{13}X_{aa}^{14}Asp^{15}$
$X_{aa}^{16}X_{aa}^{17}X_{aa}^{18}X_{aa}^{19}X_{aa}^{20}X_{aa}^{21}X_{aa}^{22}X_{aa}^{23}X_{aa}^{24}X_{aa}^{25}X_{aa}^{26}X_{aa}^{27}X_{aa}^{28}X_{aa}^{29}$
$X_{aa}^{30}X_{aa}^{31}Lys^{32}X_{aa}^{33}X_{aa}^{34}X_{aa}^{35}X_{aa}^{36}X_{aa}^{37}X_{aa}^{38}$// SEQ ID NO:475

or a pharmaceutically acceptable salt thereof,

wherein:

$X_{aa}^{1}X_{aa}^{2}$ is absent; or X_{aa}^{1} is any amino acid residue and X_{aa}^{2} is any amino acid residue; or X_{aa}^{1} is absent and X_{aa}^{2} is any amino acid residue;

X_{aa}^{3} is Cys, if X_{aa}^{18} is Cys; or X_{aa}^{3} is SeCys, if X_{aa}^{18} is SeCys; or X_{aa}^{3} is an alkyl amino acid residue, if X_{aa}^{18} is an alkyl amino acid residue;

X_{aa}^{4} is an acidic, hydrophobic, basic, or neutral hydrophilic amino acid residue;

X_{aa}^{5} is a Gly, Ala, hydrophobic, or basic amino acid residue;

X_{aa}^{6} is a Gly, Ala, 2-Abu, Nle, Nva, or hydrophobic amino acid residue;

X_{aa}^{7} is a Gly, Ala, aromatic, or hydrophobic amino acid residue;

X_{aa}^{8} is a basic, acidic, or neutral hydrophilic amino acid residue, or an Ala residue;

X_{aa}^{9} is a basic or neutral hydrophilic amino acid residue;

X_{aa}^{10} is Cys if X_{aa}^{24} is Cys; or X_{aa}^{10} is SeCys if X_{aa}^{24} is SeCys;

X_{aa}^{11} is any amino acid residue;

X_{aa}^{12} is a Pro, acidic, neutral, or hydrophobic amino acid residue;

X_{aa}^{13} is any amino acid residue;

X_{aa}^{14} is any amino acid residue;

if X_{aa}^{17} and X_{aa}^{31} are both Cys residues, there is a disulfide bond between residue X_{aa}^{17} and residue X_{aa}^{31}; or if X_{aa}^{17} and X_{aa}^{31} are both SeCys residues, there is a diselenide bond between residue X_{aa}^{17} and residue X_{aa}^{31};

the amino-terminal residue is optionally acetylated, biotinylated, 4-pentynoylated, or PEGylated; and

the carboxy-terminal residue is optionally amidated.

In ~~particular embodiments~~some examples the composition of matter comprises an amino acid sequence selected from SEQ ID NOS: 3-30, 32-72, 74-134, 136-178, 180-211, 218-239, 241-305, 307-363, 366-386, 388-432, 434-474, 515-527, 532-588, 590, 591, 593-775, 777, 778, 780-788, 790-1049, and 1062-3086.

[0025] The present ~~invention~~disclosure includes a composition of matter, comprising an amino acid sequence selected from SEQ ID NOS: 3-134, 136-305, 307-386, 388-474, 515-527, 532-588, 590, 591, 593-1049, and 1062-3086.

[0026] The present ~~invention~~disclosure also encompasses a nucleic acid (e.g., DNA or RNA) encoding any of SEQ ID NOS: 3-134, 136-305, 307-386, 388-474, 515-527, 532-588, 590, 591, 593-1049, and 1062-3086 that does not include a non-canonical amino acid; an expression vector comprising the nucleic acid; and a recombinant host cell comprising the expression vector. In one aspect, there is provided the use of a prophylactically effective amount of the isolated polypeptide of the present invention in the manufacture of a medicament for preventing pain. In another aspect, there is provided the use of a therapeutically effective amount of the isolated polypeptide of the present invention in the manufacture of a medicament for treating pain. The compositions of the ~~invention~~disclosure provide an effective method of treating, or preventing, pain, for example acute, persistent, or chronic pain. For example, a dual inhibitor of Nav1.7 and Nav1.3 can be effective in some forms of chronic pain, particularly in cases where Nav1.7 inhibition alone is insufficient. Selectivity against off-target sodium channels, particularly those governing cardiac excitability (Nav1.5) and skeletal muscle excitability (NaV1.4), is cardinal for any systemically delivered therapeutic. This selectivity is a particularly high hurdle for a dual inhibitor. Compositions of the present ~~invention~~disclosure provide such selectivity against Nav1.5 and Nav1.4. For example, the GpTx-1 peptide (SEQ ID NO:1) is a weak inhibitor of Nav1.4. Moreover, the inhibition of Nav1.4 is strongly state dependent, such that GpTx-1 exerts negligible

effects on the physiological, non-inactivated states of Nav1.4. (This is a key distinction between GpTx-1 and tetrodotoxin, which has little selectivity against Nav1.4, and which inhibits equally the inactivated and non-inactivated states of Nav1.4).

[0027] In one aspect, there is provided a pharmaceutical composition, comprising the isolated polypeptide of the present invention, and a pharmaceutically acceptable carrier. T~~Consequently, t~~he present ~~invention~~disclosure ~~is~~ also includes~~directed to~~ a pharmaceutical composition or a medicament containing the ~~inventive~~ composition of matter as described herein, and a pharmaceutically acceptable carrier.

[0028] GpTx-1 and GpTx-1 peptide analogs are also useful as research tools. Heretofore, there have not been available specific biochemical probes for Nav1.7 or for Nav1.3, and particularly no probes for correctly folded, functional Nav1.7 in the plasma membrane of live cells. As GpTx-1 and its congeners inhibit Nav1.7 / Nav1.3, they clearly bind to the channel molecule with high potency and selectivity. Labeling with fluorescent or other tracer groups at the non-active sites of GpTx-1 as defined by NMR and by residue substitution can provide research tools suitable for, but not limited to, localizing sodium channels, sorting cells that express sodium channels, and screening or panning for peptides that bind to Nav1.7 or Nav1.3.

and wherein the isolated polypeptide selectively inhibits Nav1.7 sodium channels relative to Nav1.4 and Nav1.5 sodium channels.

~~Support: original claims 1, 4, 23, and 25; paragraphs [0026], [00222] and [00461]~~

2. The isolated polypeptide of Claim 1 wherein X_{aa}^4 is selected from Ala, Glu, Asp, Phe, Pro, Ile, Leu, Met, Val, Trp, Tyr, Arg, Lys, His, homolysine, ornithine, arginine, N-methyl-arginine, ω-aminoarginine, ω-methyl-arginine, 1-methyl-histidine, 3-methyl-histidine, homoarginine, N-methyl-lysine, N-ε-methyl lysine, Dab, cyclohexylglycine (Chg), cyclohexylalanine (Cha), glycine, norleucine, norvaline, 1-Nal, 2-Nal, 4-phenyl-phenylalanine (Bip), Gln, Asn, Ser, Thr, phosphoserine, phosphotyrosine, gamma-carboxyglutamic acid, and Cit residues.

3. The isolated polypeptide of Claim 1, wherein X_{aa}^5 is selected from Ala, Phe, Ile, Leu, Met, Val, Trp, Tyr, Arg, Lys, His, homolysine, ornithine, arginine, N-methyl-arginine, ω-aminoarginine, ω-methyl-arginine, 1-methyl-histidine, 3-methyl-histidine, homoarginine, N-methyl-lysine, N-ε-methyl lysine, Dab, glycine, norleucine, norvaline, 1-Nal, 2-Nal, 1'NMe-Trp, cyclohexylglycine (Chg), cyclohexylalanine (Cha), and 4-phenyl-phenylalanine (Bip) residues.

4. The isolated polypeptide of Claim 1, wherein X_{aa}^7 is selected from Gly, Ala, Phe, Ile, Leu, Met, Val, Trp, Tyr, norleucine, norvaline, Pro, 2-pyridinylalanine, 1-Nal, 2-Nal, 1'NMe-Trp, cyclohexylglycine (Chg), cyclohexylalanine (Cha), 2-chloro-phenylalanine, 4-chloro-phenylalanine, 3,4-dichlorophenylalanine, 4-trifluoromethyl-phenylalanine, and 4-phenyl-phenylalanine (Bip) residues.

5. The isolated polypeptide of Claim 1, wherein X_{aa}^8 is selected from Ala, Arg, Lys, His, homolysine, ornithine, N-methyl-arginine, ω-aminoarginine, ω-methyl-arginine, 1-methyl-histidine, 3-methyl-histidine, homoarginine, N-methyl-lysine, N-ε-methyl lysine, Dab, Ser, Thr, Asn, Gln, Cit, Asp, phosphoserine, phosphotyrosine, gamma-carboxyglutamic acid, and Glu residues.

6. The isolated polypeptide of Claim 1, wherein X_{aa}^9 is selected from Ala, Pro, Met, Arg, Lys, His, homolysine, ornithine, arginine, N-methyl-arginine, ω-aminoarginine, ω-methyl-arginine, 1-methyl-histidine, 3-methyl-histidine, homoarginine, N-methyl-lysine, N-ε-methyl lysine, Dab, Gln, Asn, Ser, Thr, and Cit residues.