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(54) Title: HEPCIDIN AND MINI-HEPCIDIN ANALOGUES AND USES THEROF

(57) Abstract:

HEPCIDIN AND MINI-HEPCIDIN ANALOGUES AND USES THEROF

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 62/018,382, filed on June 27, 2014, which is incorporated by reference herein in its entirety.

FIELD OF THE INVENTION

[0002] The present invention relates, inter alia, to certain hepcidin peptide analogues, including both peptide monomers and peptide dimers, and conjugates and derivatives thereof, as well as compositions comprising the peptide analogues, and to the use of the peptide analogues in the treatment and/or prevention of a variety of diseases, conditions or disorders, including treatment and/or prevention of iron overload diseases such as hereditary hemochromatosis, iron-loading anemias, and other conditions and disorders described herein.

BACKGROUND

[0003] Hepcidin (also referred to as LEAP-1), a peptide hormone produced by the liver, is a regulator of iron homeostasis in humans and other mammals. Hepcidin acts by binding to its receptor, the iron export channel ferroportin, causing its internalization and degradation. Human hepcidin is a 25-amino acid peptide (Hep25). See Krause et al. (2000) FEBS Lett 480:147-150, and Park et al. (2001) J Biol Chem 276:7806-7810. The structure of the bioactive 25-amino acid form of hepcidin is a simple hairpin with 8 cysteines that form 4 disulfide bonds as described by Jordan et al. J Biol Chem 284:24155-67. The N terminal region is required for iron-regulatory function, and deletion of 5 N-terminal amino acid residues results in a loss of iron-regulatory function. See Nemeth et al. (2006) Blood 107:328-33.

[0004] Abnormal hepcidin activity is associated with iron overload diseases, including hereditary hemochromatosis (HH) and iron-loading anemias. Hereditary hemochromatosis is a genetic iron overload disease that is mainly caused by hepcidin deficiency or in some cases by hepcidin resistance. This allows excessive absorption of iron from the diet and development of iron overload. Clinical manifestations of HH may include liver disease (e.g., hepatic cirrhosis and hepatocellular carcinoma), diabetes, and heart failure. Currently, the only treatment for HH is regular phlebotomy, which is very burdensome for the patients. Iron-loading anemias are hereditary anemias with ineffective erythropoiesis such as β-thalassemia, which are accompanied by severe iron overload. Complications from iron overload are the main cause of morbidity and mortality for these patients. Hepcidin

deficiency is the main cause of iron overload in non-transfused patients, and contributes to iron overload in transfused patients. The current treatment for iron overload in these patients is iron chelation which is very burdensome, sometimes ineffective, and accompanied by frequent side effects.

[0005] Hepcidin has a number of limitations which restrict its use as a drug, including a difficult synthesis process due in part to aggregation and precipitation of the protein during folding, which in turn leads to high cost of goods. What are needed in the art are compounds having hepcidin activity and also possessing other beneficial physical properties such as improved solubility, stability, and/or potency , so that hepcidin-like biologics might be produced affordably, and used to treat hepcidin-related diseases and disorders such as, e.g., those described herein.

[0006] The present invention addresses such needs, providing novel peptide analogues, including both peptide monomer analogues and peptide dimer analogues, having hepcidin activity and also having other beneficial properties making the peptides of the present invention suitable alternatives to hepcidin.

BRIEF SUMMARY OF THE INVENTION

[0007] The present invention generally relates to peptide analogues, including both monomer and dimers, exhibiting hepcidin activity and methods of using the same.

[0008] In some embodiments, the invention provides peptides, which may be isolated and/or purified, comprising, consisting essentially of, or consisting of, the following structural formula I:

R^1-X-Y-R^2 (I) (SEQ ID NO:1)

[0009] or a pharmaceutically acceptable salt or solvate thereof,

[0010] wherein R^1 is hydrogen, a C1-C6 alkyl, a C6-C12 aryl, a C6-C12 aryl C1-C6 alkyl, or a C1-C20 alkanoyl, and including PEGylated versions alone or as spacers of any of the foregoing;

[0011] R^2 is OH or NH_2;

[0012] X is a peptide sequence having the formula Ia:

X1-X2-X3-X4-X5-X6-X7-X8-X9-X10 (Ia) (SEQ ID NO:2)

[0013] wherein

X1 is Asp, Ser, Glu, Ida, pGlu, bhAsp, D-Asp or absent;

X2 is Thr, Ser, Lys, Glu, Pro, Ala or absent;

X3 is His, Ala, or Glu;

X4 is Phe, Ile or Dpa;

X5 is Pro, bhPro, Val, Glu, Sarc or Gly;

X6 is Cys or (D)-Cys;

X7 is absent or any amino acid except Ile, Cys or (D)-Cys;

X8 is absent or any amino acid except Cys or (D)-Cys;

X9 is Phe, Ala, Ile, Thr, Tyr, Lys, Arg, bhPhe, D-Phe or absent; and

X10 is Lys, Phe or absent; and

[0014] Y is absent or present;

[0015] provided that if Y is present, Y is a peptide having the formula Im:

Y1-Y2-Y3-Y4-Y5-Y6-Y7-Y8-Y9-Y10-Y11-Y12 (Im) (SEQ ID NO:3)

[0016] wherein

Y1 is Gly, PEG3, Sarc, Lys, Glu, Ala, Phe, Pro, Glu, Lys, D-Pro, Val, Ser or absent;

Y2 is Pro, Ala, Cys, Gly or absent;

Y3 is Arg, Lys, Pro, Gly, His, Ala, Trp or absent;

Y4 is Ser, Arg, Gly, Trp, Ala, His, Glu, Tyr or absent;

Y5 is Lys, Met, Ser, Arg, Ala or absent;

Y6 is Gly, Sarc, Glu, Lys, Arg, Ser, Lys, Ile, Ala, Pro, Val or absent;

Y7 is Trp, Lys, Gly, Ala, Ile, Val or absent;

Y8 is Val, Trp, His, Thr, Gly, Cys, Met, Tyr, Ala, Glu, Lys, Asp, Arg or absent;

Y9 is Val, Asp, Asn, Cys, Tyr or absent;

Y10 is Cys, Met, Lys, Arg, Tyr or absent;

Y11 is Arg, Met, Cys, Lys or absent; and

Y12 is Arg, Lys, Ala or absent.

[0017] In one alternative embodiment, the present invention provides a hepcidin analogue peptide of formula Ia, wherein X5 is Pro, bhPro, Val, Glu, Sarc, Gly, or any N-methylated amino acid.

[0018] In one embodiment, the invention provides peptides, which may be isolated and/or purified, comprising, consisting essentially of, or consisting of formula I, wherein X is a peptide sequence having the formula Ib:

X1-X2-X3-X4-X5-X6-X7-X8-X9-X10 (Ib) SEQ ID NO:18

[0019] wherein

X1 is Asp, Glu, Ida, pGlu, bhAsp, D-Asp or absent;

X2 is Thr, Ser, Lys, Glu, Pro, Ala or absent;

X3 is His, Ala, or Glu;

X4 is Phe, Ile or Dpa;

X5 is Pro, bhPro, Sarc or Gly;

X6 is Cys;

X7 is absent or any amino acid except Ile, Cys or (D)-Cys;

X8 is absent or any amino acid except Cys or (D)-Cys;

X9 is Phe, Ile, Tyr, bhPhe or D-Phe or absent; and

X10 is Lys, Phe or absent; and

[0020] wherein Y is absent or present, provided that if Y is present, Y is a peptide having the formula In:

Y1-Y2-Y3-Y4-Y5-Y6-Y7-Y8-Y9-Y10-Y11-Y12 (In) SEQ ID NO:19

[0021] wherein

Y1 is Gly, PEG3, Sarc, Lys, Glu, Ala, Phe, Pro, Glu, Lys, D-Pro, Val, Ser or absent;

Y2 is Pro, Ala, Gly or absent;

Y3 is Arg, Lys, Pro, Gly, His, Ala, or absent;

Y4 is Ser, Arg, Glu or absent;

Y5 is Lys, Ser, Met, Arg, Ala or absent;

Y6 is Gly, Sarc, Glu, Leu, Phe, His or absent;

Y7 is Trp, N-Methyl Trp, Lys, Thr, His, Gly, Ala, Ile, Val or absent;

Y8 is Val, Trp, Ala, Asn, Glu or absent;

Y9 is Val, Ala, Asn, Asp, Cys or absent;

Y10 is Cys, (D)Cys, Glu or absent;

Y11 is Tyr, Met or absent; and

Y12 is Trp or absent.

[0022] In related embodiments, the invention provides peptides, which may be isolated and/or purified, comprising, consisting essentially of, or consisting of, the following structural formula II:

R^1-X-Y-R^2 (II) (SEQ ID NO:4)

[0023] or a pharmaceutically acceptable salt or solvate thereof,

[0024] wherein R^1 is hydrogen, a C1-C6 alkyl, a C6-C12 aryl, a C6-C12 aryl C1-C6 alkyl, or a C1-C20 alkanoyl, and including PEGylated versions alone or as spacers of any of the foregoing;

[0025] R^2 is OH or NH_2;

[0026] X is a peptide sequence having the formula IIa:

X1-X2-X3-X4-X5-X6-X7-X8-X9-X10 (IIa) (SEQ ID NO:5)

[0027] wherein

X1 is Asp, Glu or Ida;

X2 is Thr, Ser or absent;

X3 is His;

X4 is Phe or Dpa;

X5 is Pro, bhPro, Sarc or Gly;

X6 is Cys or D-Cys;

X7 is Arg, Glu, Phe, Gln, Leu, Val, Lys, Ile, Ala, Ser, Dapa or absent;

X8 is Ile, Arg, Lys, Arg, Ala, Gln, Phe, Glu, Asp, Tyr, Ser, Leu, Val, D-Ile, D-Lys, D-Arg, Dapa or absent;

X9 is Phe, Tyr, bhPhe, D-Phe or absent; and

X10 is Lys, Phe or absent; and

[0028] wherein Y is absent or present, provided that if Y is present, Y is a peptide having the formula IIm:

Y1-Y2-Y3-Y4-Y5-Y6-Y7-Y8-Y9-Y10-Y11-Y12 (IIm) (SEQ ID NO:6)

[0029] wherein

Y1 is Gly, Sarc, Lys, Glu or absent;

Y2 is Pro, Ala, Gly or absent;

Y3 is Arg, Lys, Pro, Gly, His, Ala or absent;

Y4 is Ser, Arg, Glu or absent;

Y5 is Lys, Ser, Met, Arg, Ala or absent;

Y6 is Gly, Sarc, Glu, Leu, Phe, His or absent;

Y7 is Trp, N-MethylTrp, Lys, Thr, His, Gly, Ala, Ile, Val or absent;

Y8 is Val, Trp, Ala, Asn, Glu or absent;

Y9 is Cys;

Y10 is Met or absent;

Y11 is Tyr, Met, or absent; and

Y12 is Trp or absent.

[0030] In certain embodiments, X6 in formula IIa is Cys.

[0031] In certain alternative embodiments, X7 in formula IIa is Arg, Glu, Phe, Gln, Leu, Val, Lys, Ala, Ser, Dapa or absent.

[0032] In certain embodiments, Y10 is absent.

[0033] In certain embodiments, Y11 is absent.

[0034] In certain embodiments, Y12 is absent.

[0035] In other related embodiments, the invention provides peptide homo- or heterodimers, which may be isolated and/or purified, comprising two hepcidin analogues, each hepcidin analogue comprising, consisting essentially of, or consisting of the structure of Formula I, the structure of Formula II, the structure of Formula III, the structure of Formula IV, the structure of Formula V, the structure of Formula VI, the structure of Formula VII, the structure of Formula VIII, the Structure of Formula IX, the structure of Formula X, or a sequence or structure shown in any one of Tables 2-4, 6-10, 12, 14, or 15, provided that when the dimer comprises a hepcidin analogue having the structure of Formula III, Formula IV, Formula V, or Formula VI, the two hepcidin analogues are linked via a lysine linker.

[0036] In certain embodiments, a hepcidin analogue dimer of the present invention is dimerized by more than one means. In particular embodiments, a hepcidin analogue dimer of the present invention is dimerized by at least one intermolecular disulfide bridge and at least one linker moiety (e.g., an IDA linker, such as an IDA-Palm). In particular embodiments, a hepcidin analogue dimer of the present invention is dimerized by at least one intermolecular disulfide bridge and at least one linker moiety (e.g., an IDA linker, such as an IDA-Palm), wherein the linker moiety is attached to a lysine residue in each of the peptide monomers.

[0037] In certain embodiments, one or both hepcidin analogue has the Formula III:

R^1-X-Y-R^2 (III) (SEQ ID NO:7)

[0038] or a pharmaceutically acceptable salt or solvate thereof, wherein

[0039] R^1 is hydrogen, a C1-C6 alkyl, a C6-C12 aryl, a C6-C12 aryl C1-C6 alkyl, or a C1-C20 alkanoyl, and including PEGylated versions thereof, alone or as spacers of any of the foregoing;

[0040] R^2 is -NH_2 or -OH;

[0041] X is a peptide sequence having the formula (IIIa)

X1-X2-X3-X4-X5-X6-X7-X8-X9-X10 (IIIa) (SEQ ID NO:8)

[0042] wherein

X1 is Asp, Glu, Ala, Gly, Thr, Ida, pGlu, bhAsp, D-Asp, Tyr, Leu or absent;

X2 is Thr, Ala, Aib, D-Thr, Arg or absent;

X3 is His, Lys, Ala, or D-His;

X4 is Phe, Ala, Dpa or bhPhe;

X5 is Pro, Glu, Ser, Gly, Arg, Lys, Val, Ala, D-Pro, bhPro, Sarc, Abu or absent;

X6 is Ile, Cys, Arg, Leu, Lys, His, Glu, D-Ile, D-Arg, D-Cys, Val, Ser or Ala;

X7 is Cys, Ile, Ala, Leu, Val, Ser, Phe, Dapa, D-Ile or D-Cys;

X8 is Ile, Lys, Arg, Ala, Gln, Phe, Glu, Asp, Tyr, Ser, Leu, Val, D-Ile, D-Lys, D-Arg, or Dapa;

X9 is Phe, Ala, Ile, Tyr, Lys, Arg, bhPhe or D-Phe; and

X10 is Lys, Phe or absent; and

[0043] Y is absent or present, and when present, Y is a peptide having the formula (IIIm)

Y1-Y2-Y3-Y4-Y5-Y6-Y7-Y8-Y9-Y10-Y11-Y12-Y13-Y14-Y15 (IIIm) (SEQ ID NO:9)

[0044] wherein

Y1 is Gly, Cys, Ala, Phe, Pro, Glu, Lys, D-Pro, Val, Ser or absent;

Y2 is Pro, Ala, Cys, Gly or absent;

Y3 is Arg, Lys, Pro, Gly, His, Ala, Trp or absent;

Y4 is Ser, Arg, Gly, Trp, Ala, His, Tyr or absent;

Y5 is Lys, Met, Arg, Ala or absent;

Y6 is Gly, Ser, Lys, Ile, Arg, Ala, Pro, Val or absent;

Y7 is Trp, Lys, Gly, Ala, Ile, Val or absent;

Y8 is Val, Thr, Gly, Cys, Met, Tyr, Ala, Glu, Lys, Asp, Arg or absent;

Y9 is Cys, Tyr or absent;

Y10 is Met, Lys, Arg, Tyr or absent;

Y11 is Arg, Met, Cys, Lys or absent;

Y12 is Arg, Lys, Ala or absent;

Y13 is Arg, Cys, Lys, Val or absent;

Y14 is Arg, Lys, Pro, Cys, Thr or absent; and

Y15 is Thr, Arg or absent;

[0045] wherein if Y is absent from the peptide of formula (III), X7 is Ile; and

[0046] wherein said compound of formula (III) is optionally PEGylated on R^1, X, or Y.

[0047] In certain embodiments, one or both hepcidin analogue has the structure of Formula (IV):

R^1-X-Y-R^2 (IV) (SEQ ID NO:10)

[0048] or a pharmaceutically acceptable salt or solvate thereof,

[0049] wherein R^1 is hydrogen, a C1-C6 alkyl, a C6-C12 aryl, a C6-C12 aryl C1-C6 alkyl, or a C1-C20 alkanoyl, and including PEGylated versions alone or as spacers of any of the foregoing;

[0050] R^2 is $-NH_2$ or -OH;

[0051] X is a peptide sequence having the formula (IVa)

X1-X2-X3-X4-X5-X6-X7-X8-X9-X10 (IVa) (SEQ ID NO:11)

[0052] wherein

X1 is Asp, Glu, Ala, Gly, Thr, Ida, pGlu, bhAsp, D-Asp, Tyr, Leu or absent;

X2 is Thr, Ala, Aib, D-Thr, Arg or absent;

X3 is His, Lys, Ala, or D-His;

X4 is Phe, Ala, Dpa, bhPhe or D-Phe;

X5 is Pro, Glu, Ser, Gly, Arg, Lys, Val, Ala, D-Pro, bhPro, Sarc, Abu or absent;

X6 is Ile, Cys, Arg, Leu, Lys, His, Glu, D-Ile, D-Arg , D-Cys, Val, Ser or Ala;

X7 is Cys, Ile, Ala, Leu, Val, Ser, Phe, Dapa, D-Ile or D-Cys;

X8 is Ile, Lys, Arg, Ala, Gln, Phe, Glu, Asp, Tyr, Ser, Leu, Val, D-Ile, D-Lys, D-Arg or Dapa;

X9 is Phe, Ala, Ile, Tyr, Lys, Arg, bhPhe or D-Phe; and

X10 is Lys, Phe or absent;

[0053] wherein Y is present or absent, and provided that if Y is absent, X7 is Ile; and

[0054] Y is a peptide having the formula (IVm):

Y1-Y2-Y3-Y4-Y5-Y6-Y7-Y8-Y9-Y10-Y11-Y12-Y13-Y14-Y15 (IVm) (SEQ ID NO:12)

[0055] wherein

Y1 is Gly, Cys, Ala, Phe, Pro, Glu, Lys, D-Pro, Val, Ser or absent;

Y2 is Pro, Ala, Cys, Gly or absent;

Y3 is Arg, Lys, Pro, Gly, His, Ala, Trp or absent;

Y4 is Ser, Arg, Gly, Trp, Ala, His, Tyr or absent;

Y5 is Lys, Met, Arg, Ala or absent;

Y6 is Gly, Ser, Lys, Ile, Arg, Ala, Pro, Val or absent;

Y7 is Trp, Lys, Gly, Ala, Ile, Val or absent;

Y8 is Val, Thr, Gly, Cys, Met, Tyr, Ala, Glu, Lys, Asp, Arg or absent;

Y9 is Cys, Tyr or absent;

Y10 is Met, Lys, Arg, Tyr or absent;

Y11 is Arg, Met, Cys, Lys or absent;

Y12 is Arg, Lys, Ala or absent;

Y13 is Arg, Cys, Lys, Val or absent;

Y14 is Arg, Lys, Pro, Cys, Thr or absent; and

Y15 is Thr, Arg or absent;

[0056] wherein said compound of formula (IV) is optionally PEGylated on R^1, X, or Y; and

[0057] wherein when said compound of formula (IV) comprises two or more cysteine residues, at least two of said cysteine residues being linked via a disulfide bond.

[0058] In certain embodiments, one or both hepcidin analogue has the structure of Formula V:

$$R^1\text{-}X\text{-}Y\text{-}R^2 \quad (V) \quad \text{(SEQ ID NO:13)}$$

[0059] or a pharmaceutically acceptable salt or solvate thereof, wherein

[0060] wherein R^1 is hydrogen, a C1-C6 alkyl, a C6-C12 aryl, a C6-C12 aryl C1-C6 alkyl, or a C1-C20 alkanoyl, and including PEGylated versions alone or as spacers of any of the foregoing;

[0061] R^2 is $-NH_2$ or -OH;

[0062] X is a peptide sequence having the formula (Va):

X1-X2-X3-X4-X5-X6-X7-X8-X9-X10 (Va) (SEQ ID NO:14)

[0063] wherein

X1 is Asp, Glu, Ala, Gly, Thr, Ida, pGlu, bhAsp, D-Asp, Tyr, Leu or absent;

X2 is Thr, Ala, Aib, D-Thr, Arg or absent;

X3 is His, Lys, Ala, D-His or Lys;

X4 is Phe, Ala, Dpa, bhPhe or D-Phe;

X5 is Pro, Glu, Ser, Gly, Arg, Lys, Val, Ala, D-Pro, bhPro, Sarc, Abu or absent;

X6 is Ile, Cys, Arg, Leu, Lys, His, Glu, D-Ile, D-Arg, D-Cys, Val, Ser or Ala;

X7 is Cys, Ile, Ala, Leu, Val, Ser, Phe, Dapa, D-Ile or D-Cys;

X8 is Ile, Lys, Arg, Ala, Gln, Phe, Glu, Asp, Tyr, Ser, Leu, Val, D-Ile, D-Lys, D-Arg, or Dapa;

X9 is Phe, Ala, Ile, Tyr, Lys, Arg, bhPhe or D-Phe; and

X10 is Lys, Phe or absent;

[0064] wherein Y is present or absent, and provided that if Y is absent, X7 is Ile;

[0065] wherein said compound of formula V is optionally PEGylated on R^1, X, or Y; and

[0066] wherein when said compound of formula V comprises two or more cysteine residues, at least two of said cysteine residues being linked via a disulfide bond.

[0067] In certain embodiments, one or both hepcidin analogue has the structure of formula VI:

R^1-X-Y-R^2 (VI) (SEQ ID NO:15)

[0068] or a pharmaceutically acceptable salt or solvate thereof, wherein

[0069] wherein R^1 is hydrogen, a C1-C6 alkyl, a C6-C12 aryl, a C6-C12 aryl C1-C6 alkyl, or a C1-C20 alkanoyl, and including PEGylated versions alone or as spacers of any of the foregoing;

[0070] R^2 is $-NH_2$ or -OH;

[0071] X is a peptide sequence having the formula (VIa):

X1-X2-X3-X4-X5-X6-X7-X8-X9-X10 (VIa) (SEQ ID NO:16)

[0072] wherein

X1 is Asp, Glu, Ida or absent;

X2 is Thr, Ser, Pro, Ala or absent;

X3 is His, Ala, or Glu;

X4 is Phe or Dpa;

X5 is Pro, bhPro, Sarc or Gly;

X6 is Cys, (D)-Cys, Arg, Glu, Phe, Gln, Leu, Val, Lys, Ala, Ser, Dapa or absent;

X7 is Cys, (D)-Cys, Arg, Glu, Phe, Gln, Leu, Val, Lys, Ala, Ser, Dapa or absent;

X8 is Ile, Arg, Lys, Ala, Gln, Phe, Glu, Asp, Tyr, Ser, Leu, Val, D-Ile, D-Lys, D-Arg, Dapa or absent;

X9 is Phe, Ala, Ile, Thr, Tyr, Lys, Arg, bhPhe, D-Phe or absent; and

X10 is Lys, Phe or absent;

[0073] Y is absent or present, provided that if Y is present, Y is a peptide having the formula (VIm)

Y1-Y2-Y3 (VIm) (SEQ ID NO:17)

[0074] wherein

Y1 is Ile, Arg, Lys, Ala, Gln, Phe, Glu, Asp, Tyr, Ser, Leu, Val, D-Ile, D-Lys, D-Arg, Dapa or absent;

Y2 is Phe, Ala, Ile, Thr, Tyr, Lys, Arg, bhPhe or D-Phe or absent; and

Y3 is Lys, Phe or absent.

[0075] In one embodiment, the present invention provides peptide homo- or heterodimers, which may be isolated and/or purified, comprising two hepcidin analogues, each hepcidin analogue comprising, consisting essentially of, or consisting of the structure of Formula I or the structure of Formula II, wherein the two hepcidin analogues are linked via an Ida linker (e.g., an IDA-Palm linker), wherein the Ida linker is attached to a lysine (e.g., via a lysine sidechain) in each of the two hepcidin analogues. In one such embodiment, the dimer is a homodimer, and in another embodiment, the dimer is a heterodimer.

[0076] In other embodiments, the present invention includes polynucleotide comprising a sequence encoding a hepcidin analogue described herein.

[0077] In further embodiments, the present invention includes a vector comprising a polynucleotide comprising a sequence encoding a hepcidin analogue described herein.

[0078] In additional embodiments, the present invention includes a pharmaceutical composition comprising a peptide or hepcidin analogue described herein, and a pharmaceutically acceptable carrier, excipient or vehicle.

[0079] In related embodiments, the present invention includes method of binding a ferroportin or inducing ferroportin internalization and degradation, comprising contacting the ferroportin with at least one peptide or hepcidin analogue described herein.

[0080] In further related embodiments, the present invention includes a method for treating a disease of iron metabolism in a subject comprising providing to the subject an effective amount of at least one peptide or hepcidin analogue described herein.

[0081] In another embodiment, the present invention includes a device comprising a peptide or hepcidin analogue described herein, for delivery of the hepcidin analogue, dimer or composition to a subject.

[0082] In another related embodiment, the present invention includes a kit comprising at least one peptide or hepcidin analogue described herein, packaged with a reagent, a device, or an instructional material, or a combination thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0083] **Figure 1** shows an in vivo dose response of illustrative hepcidin analogues at two concentrations, 300 nmol/kg and 1000 nmol/kg (subcutaneous or "s.c."; 2 h), in C-57 (mouse) presented as serum iron levels (n=4). The sequences of the hepcidin analogue monomer peptides used in this experiment are shown in Table 14.

DETAILED DESCRIPTION OF THE INVENTION

[0084] The present invention relates generally to hepcidin analogue peptides and methods of making and using the same. In certain embodiments, the hepcidin analogues exhibit one or more hepcidin activity. In certain embodiments, the present invention relates to hepcidin peptide analogues comprising one or more peptide subunit that forms a cyclized structures through an intramolecular bond, e.g., an intramolecular disulfide bond. In particular embodiments, the cyclized structure has increased potency and selectivity as compared to non-cyclized hepcidin peptides and analogies thereof.

Definitions and Nomenclature

[0085] Unless otherwise defined herein, scientific and technical terms used in this application shall have the meanings that are commonly understood by those of ordinary skill in the art. Generally, nomenclature used in connection with, and techniques of, chemistry, molecular biology, cell and cancer biology, immunology, microbiology, pharmacology, and protein and nucleic acid chemistry, described herein, are those well-known and commonly used in the art.

[0086] As used herein, the following terms have the meanings ascribed to them unless specified otherwise.

[0087] Throughout this specification, the word "comprise" or variations such as "comprises" or "comprising" will be understood to imply the inclusion of a stated integer (or components) or group of integers (or components), but not the exclusion of any other integer (or components) or group of integers (or components).

[0088] The singular forms "a," "an," and "the" include the plurals unless the context clearly dictates otherwise.

[0089] The term "including" is used to mean "including but not limited to." "Including" and "including but not limited to" are used interchangeably.

[0090] The terms "patient," "subject," and "individual" may be used interchangeably and refer to either a human or a non-human animal. These terms include mammals such as humans, primates, livestock animals (e.g., bovines, porcines), companion animals (e.g., canines, felines) and rodents (e.g., mice and rats). The term "mammal" refers to any mammalian species such as a human, mouse, rat, dog, cat, hamster, guinea pig, rabbit, livestock, and the like.

[0091] The term "peptide," as used herein, refers broadly to a sequence of two or more amino acids joined together by peptide bonds. It should be understood that this term does not connote a specific length of a polymer of amino acids, nor is it intended to imply or distinguish whether the polypeptide is produced using recombinant techniques, chemical or enzymatic synthesis, or is naturally occurring.

[0092] The term "peptide analogue," as used herein, refers broadly to peptide monomers and peptide dimers comprising one or more structural features and/or functional activities in common with hepcidin, or a functional region thereof. In certain embodiments, a peptide analogue includes peptides sharing substantial amino acid sequence identity with hepcidin, e.g., peptides that comprise one or more amino acid insertions, deletions, or substitutions as compared to a wild-type hepcidin, e.g., human hepcidin, amino acid sequence. In certain embodiments, a peptide analogue comprises one or more additional modification, such as, e.g., conjugation to another compound. Encompassed by the term "peptide analogue" is any peptide monomer or peptide dimer of the present invention. In certain instances, a "peptide analog" may also or alternatively be referred to herein as a "hepcidin analogue," "hepcidin peptide analogue," or a "hepcidin analogue peptide."

[0093] The recitations "sequence identity", "percent identity", "percent homology", or, for example, comprising a "sequence 50% identical to," as used herein, refer to the extent that sequences are identical on a nucleotide-by-nucleotide basis or an amino acid-by-amino acid basis over a window of comparison. Thus, a "percentage of sequence identity" may be calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (e.g., A, T, C, G, I) or the identical amino acid residue (e.g., Ala, Pro, Ser, Thr, Gly, Val, Leu, Ile, Phe, Tyr, Trp, Lys, Arg, His, Asp, Glu, Asn, Gln, Cys and Met) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (i.e., the window size), and multiplying the result by 100 to yield the percentage of sequence identity.

[0094] Calculations of sequence similarity or sequence identity between sequences (the terms are used interchangeably herein) can be performed as follows. To determine the percent identity of two amino acid sequences, or of two nucleic acid sequences, the sequences can be aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In certain embodiments, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, 60%, and even more preferably at least 70%, 80%, 90%, 100% of the length of the reference sequence. The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position.

[0095] The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

[0096] The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In some embodiments, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch, (1970, J. Mol. Biol. 48: 444-453) algorithm which has been incorporated into the GAP program in the GCG software package, using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package, using an NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. Another exemplary set of parameters includes a Blossum 62 scoring matrix with a gap penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5. The percent identity between two amino acid or nucleotide sequences can also be determined using the algorithm of E. Meyers and W. Miller (1989, Cabios, 4: 11-17) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

[0097] The peptide sequences described herein can be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related

sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, et al., (1990, J. Mol. Biol, 215: 403-10). BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al. (Nucleic Acids Res. 25:3389-3402, 1997). When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used.

[0098] The term "conservative substitution" as used herein denotes that one or more amino acids are replaced by another, biologically similar residue. Examples include substitution of amino acid residues with similar characteristics, e.g., small amino acids, acidic amino acids, polar amino acids, basic amino acids, hydrophobic amino acids and aromatic amino acids. See, for example, the table below. In some embodiments of the invention, one or more Met residues are substituted with norleucine (Nle) which is a bioisostere for Met, but which, as opposed to Met, is not readily oxidized. Another example of a conservative substitution with a residue normally not found in endogenous, mammalian peptides and proteins is the conservative substitution of Arg or Lys with, for example, ornithine, canavanine, aminoethylcysteine or another basic amino acid. In some embodiments, one or more cysteines of a peptide analogue of the invention may be substituted with another residue, such as a serine. For further information concerning phenotypically silent substitutions in peptides and proteins, see, for example, Bowie et. al. Science 247, 1306-1310, 1990. In the scheme below, conservative substitutions of amino acids are grouped by physicochemical properties. I: neutral, hydrophilic, II: acids and amides, III: basic, IV: hydrophobic, V: aromatic, bulky amino acids.

I	**II**	**III**	**IV**	**V**
A	N	H	M	F
S	D	R	L	Y
T	E	K	I	W
P	Q		V	
G			C	

[0099] In the scheme below, conservative substitutions of amino acids are grouped by physicochemical properties. VI: neutral or hydrophobic, VII: acidic, VIII: basic, IX: polar, X: aromatic.

VI	VII	VIII	IX	X
A	E	H	M	F
L	D	R	S	Y
I		K	T	W
P			C	
G			N	
V			Q	

[00100] The term "amino acid" or "any amino acid" as used here refers to any and all amino acids, including naturally occurring amino acids (e.g., a-amino acids), unnatural amino acids, modified amino acids, and non-natural amino acids. It includes both D- and L-amino acids. Natural amino acids include those found in nature, such as, e.g., the 23 amino acids that combine into peptide chains to form the building-blocks of a vast array of proteins. These are primarily L stereoisomers, although a few D-amino acids occur in bacterial envelopes and some antibiotics. The 20 "standard," natural amino acids are listed in the above tables. The "non-standard," natural amino acids are pyrrolysine (found in methanogenic organisms and other eukaryotes), selenocysteine (present in many noneukaryotes as well as most eukaryotes), and N-formylmethionine (encoded by the start codon AUG in bacteria, mitochondria and chloroplasts). "Unnatural" or "non-natural" amino acids are non-proteinogenic amino acids (i.e., those not naturally encoded or found in the genetic code) that either occur naturally or are chemically synthesized. Over 140 natural amino acids are known and thousands of more combinations are possible. Examples of "unnatural" amino acids include β-amino acids (β^3 and β^2), homo-amino acids, proline and pyruvic acid derivatives, 3-substituted alanine derivatives, glycine derivatives, ring-substituted phenylalanine and tyrosine derivatives, linear core amino acids, diamino acids, D-amino acids, and N-methyl amino acids. Unnatural or non-natural amino acids also include modified amino acids. "Modified" amino acids include amino acids (e.g., natural amino acids) that have been chemically modified to include a group, groups, or chemical moiety not naturally present on the amino acid.

[00101] As is clear to the skilled artisan, the peptide sequences disclosed herein are shown proceeding from left to right, with the left end of the sequence being the N-terminus of the peptide and the right end of the sequence being the C-terminus of the peptide. Among sequences disclosed herein are sequences incorporating a "Hy-" moiety at the amino terminus (N-terminus) of the sequence, and either an "-OH" moiety or an "-NH_2" moiety at the carboxy terminus (C-terminus) of the sequence. In such cases, and unless otherwise indicated, a "Hy-" moiety at the N-terminus of the sequence in question indicates a hydrogen atom, corresponding to the presence of a free primary or secondary amino group at the N-terminus, while an "-OH" or an "–NH_2" moiety at the C-terminus of the sequence indicates a hydroxy group or an amino group, corresponding to the presence of an amido ($CONH_2$) group at the C-terminus, respectively. In each sequence of the invention, a C-terminal "–OH" moiety may be substituted for a C-terminal "–NH_2" moiety, and vice-versa. It is further understood that the moiety at the amino terminus or carboxy terminus may be a bond, e.g., a covalent bond, particularly in situations where the amino terminus or carboxy terminus is bound to a linker or to another chemical moiety, e.g., a PEG moiety.

[00102] The term "NH_2," as used herein, refers to the free amino group present at the amino terminus of a polypeptide. The term "OH," as used herein, refers to the free carboxy group present at the carboxy terminus of a peptide. Further, the term "Ac," as used herein, refers to Acetyl protection through acylation of the C- or N-terminus of a polypeptide.

[00103] The term "carboxy," as used herein, refers to –CO_2H.

[00104] For the most part, the names of naturally occurring and non-naturally occurring aminoacyl residues used herein follow the naming conventions suggested by the IUPAC Commission on the Nomenclature of Organic Chemistry and the IUPAC-IUB Commission on Biochemical Nomenclature as set out in "Nomenclature of α-Amino Acids (Recommendations, 1974)" Biochemistry, 14(2), (1975). To the extent that the names and abbreviations of amino acids and aminoacyl residues employed in this specification and appended claims differ from those suggestions, they will be made clear to the reader. Some abbreviations useful in describing the invention are defined below in the following Table 1.

Table 1. Abbreviations of Non-Natural Amino Acids and Chemical Moieties

Abbreviation	Definition
DIG	Diglycolic acid

Dapa	Diaminopropionic acid
Daba	Diaminobutyric acid
Pen	Penicillamine
Sarc	Sarcosine
Cit	Citroline
Cav	Cavanine
NMe-Arg	N-Methyl-Arginine
NMe-Trp	N-Methyl-Tryptophan
NMe-Phe	N-Methyl-Phenylalanine
Ac-	Acetyl
2-Nal	2-Napthylalanine
1-Nal	1-Napthylalanine
Bip	Biphenylalanine
βAla	beta-Alanine
Aib	2-aminoisobutyric acid
Azt	*azetidine*-2-carboxylic acid
Tic	(3S)-1,2,3,4-Tetrahydroisoquinoline-hydroxy-3-carboxylic acid
Phe(OMe)	Tyrosine (4-Methyl)
N-MeLys	N-Methyl-Lysine
N-MeLys(Ac)	N-e-Acetyl-D-lysine
Dpa	β,β diphenylalanine
NH_2	Free Amine
$CONH_2$	Amide
COOH	Acid
Phe(4-F)	4-Fluoro-Phenylalanine

PEG3	$NH_2CH_2CH_2(OCH_2CH_2)_3CH_2CH_2CO_2H$
m-PEG3	$CH_3OCH_2CH_2(OCH_2CH_2)_2CH_2CH_2CO_2H$
m-PEG4	$CH_3OCH_2CH_2(OCH_2CH_2)_3CH_2CH_2CO_2H$
m-PEG8	$CH_3OCH_2CH_2(OCH_2CH_2)_7CH_2CH_2CO_2H$
PEG11	O-(2-aminoethyl)-O′-(2-carboxyethyl)-undecaethyleneglycol $NH_2CH_2CH_2(OCH_2CH_2)_{11}CH_2CH_2CO_2H$
PEG13	Bifunctional PEG linker with 13 PolyEthylene Glycol units
PEG25	Bifunctional PEG linker with 25 PolyEthylene Glycol units
PEG1K	Bifunctional PEG linker with PolyEthylene Glycol Mol wt of 1000Da
PEG2K	Bifunctional PEG linker with PolyEthylene Glycol Mol wt of 2000Da
PEG3.4K	Bifunctional PEG linker with PolyEthylene Glycol Mol wt of 3400Da
PEG5K	Bifunctional PEG linker with PolyEthylene Glycol Mol wt of 5000Da
IDA or Ida	Iminodiacetic acid
IDA-Palm	(Palmityl)-Iminodiacetic acid
hPhe	homoPhenylalanine
Ahx	Aminohexanoic acid
DIG-OH	Glycolic monoacid
Triazine	Amino propyl Triazine di-acid
Boc-Triazine	Boc-Triazine di-acid
Trifluorobutyric acid	4,4,4-Trifluorobutyric acid
2-Methylltrifluorobutyric acid	2-methyl-4,4,4-Butyric acid
Trifluorpentanoic acid	5,5,5-Trifluoropentanoic acid
1,4- Phenylenediacetic acid	*para*-Phenylenediacetic acid
1,3 - Phenylenediacetic acid	*meta*-Phenylenediacetic acid
DTT	Dithiothreotol
Nle	Norleucine

βhTrp or bhTrp	β-homoTryptophane
βhPhe or bhPhe	β-homophenylalanine
Phe(4-CF_3)	4-TrifluoromethylPhenylalanine
βGlu or bGlu	β-Glutamic acid
βhGlu or bhGlu	β-homoglutamic acid
2-2-Indane	2-Aminoindane-2-carboxylic acid
1-1-Indane	1-Aminoindane-1-carboxylic acid
hCha	homocyclohexylalanine
Cyclobutyl	Cyclobutylalanine
hLeu	Homoleucine
Gla	γ-Carboxy-glutamic acid
Aep	3-(2-aminoethoxy)propanoic acid
Aea	(2-aminoethoxy)acetic acid
IsoGlu-octanoic acid	octanoyl-γ-Glu
K-octanoic acid	octanoyl-ε-Lys
Dapa(Palm)	Hexadecanoyl-β-Diaminopropionic acid
IsoGlu-Palm	hexadecanoyl-γ-Glu
C-StBu	S-tert-butylthio-cysteine
C-tBu	S-tert-butyl-cysteine
Dapa(AcBr)	NY-(bromoacetyl)-2,3- diaminopropionic acid
Tle	*tert*-Leucine
Phg	phenylglycine
Oic	octahydroindole-2-carboxylic acid
Chg	α-cyclohexylglycine
GP-(Hyp)	Gly-Pro-HydroxyPro

Inp	isonipecotic acid
Amc	4-(aminomethyl)cyclohexane carboxylic acid
Betaine	(CH3)3NCH2CH2CO2H

[00105] Throughout the present specification, unless naturally occurring amino acids are referred to by their full name (e.g. alanine, arginine, etc.), they are designated by their conventional three-letter or single-letter abbreviations (e.g. Ala or A for alanine, Arg or R for arginine, etc.). In the case of less common or non-naturally occurring amino acids, unless they are referred to by their full name (e.g. sarcosine, ornithine, etc.), frequently employed three- or four-character codes are employed for residues thereof, including, Sar or Sarc (sarcosine, i.e. N-methylglycine), Aib (α-aminoisobutyric acid), Daba (2,4-diaminobutanoic acid), Dapa (2,3-diaminopropanoic acid), γ-Glu (γ-glutamic acid), pGlu (pyroglutamic acid), Gaba (γ-aminobutanoic acid), β-Pro (pyrrolidine-3-carboxylic acid), 8Ado (8-amino-3,6-dioxaoctanoic acid), Abu (4-aminobutyric acid), bhPro (β-homo-proline), bhPhe (β-homo-L-phenylalanine), bhAsp (β-homo-aspartic acid]), Dpa (β,β diphenylalanine), Ida (Iminodiacetic acid), hCys (homocysteine), bhDpa (β-homo-β,β -diphenylalanine).

[00106] Furthermore, R^1 can in all sequences be substituted with isovaleric acids or equivalent. In some embodiments, wherein a peptide of the present invention is conjugated to an acidic compound such as, e.g., isovaleric acid, isobutyric acid, valeric acid, and the like, the presence of such a conjugation is referenced in the acid form. So, for example, but not to be limited in any way, instead of indicating a conjugation of isovaleric acid to a peptide by referencing isovaleroyl, in some embodiments, the present application may reference such a conjugation as isovaleric acid.

[00107] The term "L-amino acid," as used herein, refers to the "L" isomeric form of a peptide, and conversely the term "D-amino acid" refers to the "D" isomeric form of a peptide. In certain embodiments, the amino acid residues described herein are in the "L" isomeric form, however, residues in the "D" isomeric form can be substituted for any L-amino acid residue, as long as the desired functional is retained by the peptide.

[00108] Unless otherwise indicated, reference is made to the L-isomeric forms of the natural and unnatural amino acids in question possessing a chiral center. Where appropriate, the D-isomeric form of an amino acid is indicated in the conventional manner by the prefix "D"

before the conventional three-letter code (e.g. Dasp, (D)Asp or D-Asp; Dphe, (D)Phe or D-Phe).

[00109] The term "DRP," as used herein, refers to disulfide rich peptides.

[00110] The term "dimer," as used herein, refers broadly to a peptide comprising two or more monomer subunits. Certain dimers comprise two DRPs. Dimers of the present invention include homodimers and heterodimers. A monomer subunit of a dimer may be linked at its C- or N-terminus, or it may be linked via internal amino acid residues. Each monomer subunit of a dimer may be linked through the same site, or each may be linked through a different site (e.g., C-terminus, N-terminus, or internal site).

[00102] As used herein, in the context of certain disclosed peptide sequences (such as those depicted in Tables 2-4, 6-15), parentheticals, e.g., (__), represent side chain conjugations and brackets, e.g., [__], represent unnatural amino acid substitutions. Generally, where a linker is shown at the N-terminus of a peptide sequence, it indicates that the peptide is dimerized with another peptide, wherein the linker is attached to the N-terminus of the two peptides. Generally, where a linker is shown at the C-terminus of a peptide sequence, it indicates that the peptide is dimerized with another peptide, wherein the linker is attached to the C-terminus of the two peptides.

[00103] The term "isostere replacement" or "isostere substitution" are used interchangeably herein to refer to any amino acid or other analog moiety having chemical and/or structural properties similar to a specified amino acid. In certain embodiments, an isostere replacement is a conservative substitution with a natural or unnatural amino acid.

[00104] The term "cyclized," as used herein, refers to a reaction in which one part of a polypeptide molecule becomes linked to another part of the polypeptide molecule to form a closed ring, such as by forming a disulfide bridge or other similar bond.

[00105] The term "subunit," as used herein, refers to one of a pair of polypeptide monomers that are joined to form a dimer peptide composition.

[00106] The term "linker moiety," as used herein, refers broadly to a chemical structure that is capable of linking or joining together two peptide monomer subunits to form a dimer.

[00107] The term "solvate" in the context of the present invention refers to a complex of defined stoichiometry formed between a solute (e.g., a hepcidin analogue or pharmaceutically

acceptable salt thereof according to the invention) and a solvent. The solvent in this connection may, for example, be water, ethanol or another pharmaceutically acceptable, typically small-molecular organic species, such as, but not limited to, acetic acid or lactic acid. When the solvent in question is water, such a solvate is normally referred to as a hydrate.

[00108] As used herein, a "disease of iron metabolism" includes diseases where aberrant iron metabolism directly causes the disease, or where iron blood levels are dysregulated causing disease, or where iron dysregulation is a consequence of another disease, or where diseases can be treated by modulating iron levels, and the like. More specifically, a disease of iron metabolism according to this disclosure includes iron overload diseases, iron deficiency disorders, disorders of iron biodistribution, other disorders of iron metabolism and other disorders potentially related to iron metabolism, etc. Diseases of iron metabolism include hemochromatosis, HFE mutation hemochromatosis, ferroportin mutation hemochromatosis, transferrin receptor 2 mutation hemochromatosis, hemojuvelin mutation hemochromatosis, hepcidin mutation hemochromatosis, juvenile hemochromatosis, neonatal hemochromatosis, hepcidin deficiency, transfusional iron overload, thalassemia, thalassemia intermedia, alpha thalassemia, sideroblastic anemia, porphyria, porphyria cutanea tarda, African iron overload, hyperferritinemia, ceruloplasmin deficiency, atransferrinemia, congenital dyserythropoietic anemia, anemia of chronic disease, anemia of inflammation, anemia of infection, hypochromic microcytic anemia, iron- deficiency anemia, iron-refractory iron deficiency anemia, anemia of chronic kidney disease, erythropoietin resistance, iron deficiency of obesity, other anemias, benign or malignant tumors that overproduce hepcidin or induce its overproduction, conditions with hepcidin excess, Friedreich ataxia, gracile syndrome, Hallervorden-Spatz disease, Wilson's disease, pulmonary hemosiderosis, hepatocellular carcinoma, cancer, hepatitis, cirrhosis of liver, pica, chronic renal failure, insulin resistance, diabetes, atherosclerosis, neurodegenerative disorders, multiple sclerosis, Parkinson's disease, Huntington's disease, and Alzheimer's disease.

[00109] In some embodiments, the disease and disorders are related to iron overload diseases such as iron hemochromatosis, HFE mutation hemochromatosis, ferroportin mutation hemochromatosis, transferrin receptor 2 mutation hemochromatosis, hemojuvelin mutation hemochromatosis, hepcidin mutation hemochromatosis, juvenile hemochromatosis, neonatal hemochromatosis, hepcidin deficiency, transfusional iron overload, thalassemia, thalassemia intermedia, alpha thalassemia.

[00110] In some embodiments, the hepcidin analogues of the invention are used to treat diseases and disorders that are not typically identified as being iron related. For example, hepcidin is highly expressed in the murine pancreas suggesting that diabetes (Type I or Type II), insulin resistance, glucose intolerance and other disorders may be ameliorated by treating underlying iron metabolism disorders. See Ilyin, G. et al. (2003) FEBS Lett. 542 22-26, which is herein incorporated by reference. As such, peptides of the invention may be used to treat these diseases and conditions. Those skilled in the art are readily able to determine whether a given disease can be treated with a peptide according to the present invention using methods known in the art, including the assays of WO 2004092405, which is herein incorporated by reference, and assays which monitor hepcidin, hemojuvelin, or iron levels and expression, which are known in the art such as those described in U.S. Patent No. 7,534,764, which is herein incorporated by reference.

[00111] In certain embodiments of the present invention, the diseases of iron metabolism are iron overload diseases, which include hereditary hemochromatosis, iron-loading anemias, alcoholic liver diseases and chronic hepatitis C.

[00112] The term "pharmaceutically acceptable salt," as used herein, represents salts or zwitterionic forms of the peptides or compounds of the present invention which are water or oil-soluble or dispersible, which are suitable for treatment of diseases without undue toxicity, irritation, and allergic response; which are commensurate with a reasonable benefit/risk ratio, and which are effective for their intended use. The salts can be prepared during the final isolation and purification of the compounds or separately by reacting an amino group with a suitable acid. Representative acid addition salts include acetate, adipate, alginate, citrate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, camphorate, camphorsulfonate, digluconate, glycerophosphate, hemisulfate, heptanoate, hexanoate, formate, fumarate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethansulfonate (isethionate), lactate, maleate, mesitylenesulfonate, methanesulfonate, naphthylenesulfonate, nicotinate, 2-naphthalenesulfonate, oxalate, pamoate, pectinate, persulfate, 3-phenylproprionate, picrate, pivalate, propionate, succinate, tartrate, trichloroacetate, trifluoroacetate, phosphate, glutamate, bicarbonate, para-toluenesulfonate, and undecanoate. Also, amino groups in the compounds of the present invention can be quaternized with methyl, ethyl, propyl, and butyl chlorides, bromides, and iodides; dimethyl, diethyl, dibutyl, and diamyl sulfates; decyl, lauryl, myristyl, and steryl chlorides, bromides, and iodides; and benzyl and phenethyl bromides. Examples of acids which can be employed to form therapeutically acceptable addition salts include inorganic acids such as hydrochloric, hydrobromic, sulfuric, and

phosphoric, and organic acids such as oxalic, maleic, succinic, and citric. A pharmaceutically acceptable salt may suitably be a salt chosen, e.g., among acid addition salts and basic salts. Examples of acid addition salts include chloride salts, citrate salts and acetate salts. Examples of basic salts include salts where the cation is selected among alkali metal cations, such as sodium or potassium ions, alkaline earth metal cations, such as calcium or magnesium ions, as well as substituted ammonium ions, such as ions of the type N(R1)(R2)(R3)(R4)+, where R1, R2, R3 and R4 independently will typically designate hydrogen, optionally substituted C1-6-alkyl or optionally substituted C2-6-alkenyl. Examples of relevant C1-6-alkyl groups include methyl, ethyl, 1-propyl and 2-propyl groups. Examples of C2-6-alkenyl groups of possible relevance include ethenyl, 1-propenyl and 2-propenyl. Other examples of pharmaceutically acceptable salts are described in "Remington's Pharmaceutical Sciences", 17th edition, Alfonso R. Gennaro (Ed.), Mark Publishing Company, Easton, PA, USA, 1985 (and more recent editions thereof), in the "Encyclopaedia of Pharmaceutical Technology", 3rd edition, James Swarbrick (Ed.), Informa Healthcare USA (Inc.), NY, USA, 2007, and in J. Pharm. Sci. 66: 2 (1977). Also, for a review on suitable salts, see *Handbook of Pharmaceutical Salts: Properties, Selection, and Use* by Stahl and Wermuth (Wiley-VCH, 2002). Other suitable base salts are formed from bases which form non-toxic salts. Representative examples include the aluminum, arginine, benzathine, calcium, choline, diethylamine, diolamine, glycine, lysine, magnesium, meglumine, olamine, potassium, sodium, tromethamine, and zinc salts. Hemisalts of acids and bases may also be formed, e.g., hemisulphate and hemicalcium salts.

[00113] The term "N(alpha)Methylation", as used herein, describes the methylation of the alpha amine of an amino acid, also generally termed as an N-methylation.

[00114] The term "sym methylation" or "Arg-Me-sym", as used herein, describes the symmetrical methylation of the two nitrogens of the guanidine group of arginine. Further, the term "asym methylation" or "Arg-Me-asym" describes the methylation of a single nitrogen of the guanidine group of arginine.

[00115] The term "acylating organic compounds", as used herein refers to various compounds with carboxylic acid functionality that are used to acylate the N-terminus of an amino acid subunit prior to forming a C-terminal dimer. Non-limiting examples of acylating organic compounds include cyclopropylacetic acid, 4-Fluorobenzoic acid, 4-fluorophenylacetic acid, 3-Phenylpropionic acid, Succinic acid, Glutaric acid, Cyclopentane carboxylic acid, 3,3,3-trifluoropropeonic acid, 3-Fluoromethylbutyric acid, Tetrahedro-2H-Pyran-4-carboxylic acid.

[00116] The term "alkyl" includes a straight chain or branched, noncyclic or cyclic, saturated aliphatic hydrocarbon containing from 1 to 24 carbon atoms. Representative saturated straight chain alkyls include, but are not limited to, methyl, ethyl, *n*-propyl, *n*-butyl, *n*-pentyl, *n*-hexyl, and the like, while saturated branched alkyls include, without limitation, isopropyl, *sec*-butyl, isobutyl, *tert*-butyl, isopentyl, and the like. Representative saturated cyclic alkyls include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and the like, while unsaturated cyclic alkyls include, without limitation, cyclopentenyl, cyclohexenyl, and the like.

[00117] As used herein, a "therapeutically effective amount" of the peptide agonists of the invention is meant to describe a sufficient amount of the peptide agonist to treat an hepcidin-related disease, including but not limited to any of the diseases and disorders described herein (for example, a disease of iron metabolism). In particular embodiments, the therapeutically effective amount will achieve a desired benefit/risk ratio applicable to any medical treatment.

Peptide Analogues of Hepcidin

[00118] The present invention provides peptide analogues of hepcidin, which may be monomers or dimers (collectively "hepcidin analogues").

[00119] In some embodiments, a hepcidin analogue of the present invention binds ferroportin, e.g., human ferroportin. In certain embodiments, hepcidin analogues of the present invention specifically bind human ferroportin. As used herein, "specifically binds" refers to a specific binding agent's preferential interaction with a given ligand over other agents in a sample. For example, a specific binding agent that specifically binds a given ligand, binds the given ligand, under suitable conditions, in an amount or a degree that is observable over that of any nonspecific interaction with other components in the sample. Suitable conditions are those that allow interaction between a given specific binding agent and a given ligand. These conditions include pH, temperature, concentration, solvent, time of incubation, and the like, and may differ among given specific binding agent and ligand pairs, but may be readily determined by those skilled in the art. In some embodiments, a hepcidin analogue of the present invention binds ferroportin with greater specificity than a hepcidin reference compound (e.g., any one of the hepcidin reference compounds provided herein). In some embodiments, a hepcidin analogue of the present invention exhibits ferroportin specificity that is at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 700%, 1000%, or 10,000% higher than a hepcidin reference compound (e.g., any one of the hepcidin reference compounds provided herein. In some embodiments, a

hepcidin analogue of the present invention exhibits ferroportin specificity that is at least about 5 fold, or at least about 10, 20, 50, or 100 fold higher than a hepcidin reference compound (e.g., any one of the hepcidin reference compounds provided herein.

[00120] In certain embodiments, a hepcidin analogue of the present invention exhibits a hepcidin activity. In some embodiments, the activity is an *in vitro* or an *in vivo* activity, e.g., an *in vivo* or an *in vitro* activity described herein. In some embodiments, a hepcidin analogue of the present invention exhibits at least about 50%, 60%, 70%, 80%, 90%, 95%, 97%, 98%, 99%, or greater than 99% of the activity exhibited by a hepcidin reference compound (e.g., any one of the hepcidin reference compounds provided herein.

[00121] In some embodiments, a hepcidin analogue of the present invention exhibits at least about 50%, 60%, 70%, 80%, 90%, 95%, 97%, 98%, 99%, or greater than 99% of the ferroportin binding ability that is exhibited by a reference hepcidin. In some embodiments, a hepcidin analogue of the present invention has a lower IC_{50} (i.e., higher binding affinity) for binding to ferroportin, (e.g., human ferroportin) compared to a reference hepcidin. In some embodiments, a hepcidin analogue the present invention has an IC_{50} in a ferroportin competitive binding assay which is at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 700%, or 1000% lower than a reference hepcidin.

[00122] In certain embodiments, a hepcidin analogue of the present invention exhibits increased hepcidin activity as compared to a hepcidin reference peptide. In some embodiments, the activity is an *in vitro* or an *in vivo* activity, e.g., an *in vivo* or an *in vitro* activity described herein. In certain embodiments, the hepcidin analogue of the present invention exhibits 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, or 200-fold greater hepcidin activity than a reference hepcidin. In certain embodiments, the hepcidin analogue of the present invention exhibits at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 97%, 98%, 99% or greater than 99%, 100%, 200% 300%, 400%, 500%, 700%, or 1000% greater activity than a reference hepcidin.

[00123] In some embodiments, a peptide analogue of the present invention exhibits at least about 50%, 60%, 70%, 80%, 90%, 95%, 97%, 98%, 99%, or greater than 99%, 100%, 200% 300%, 400%, 500%, 700%, or 1000% greater *in vitro* activity for inducing the degradation of human ferroportin protein as that of a reference hepcidin, wherein the activity is measured according to a method described herein.

[00124] In some embodiments, a peptide or a peptide dimer of the present invention exhibits at least about 50%, 60%, 70%, 80%, 90%, 95%, 97%, 98%, 99%, or greater than 99%, 100%, 200% 300%, 400%, 500%, 700%, or 1000% greater *in vivo* activity for inducing the reduction of free plasma iron in an individual as does a reference hepcidin, wherein the activity is measured according to a method described herein.

[00125] In some embodiments, the activity is an *in vitro* or an *in vivo* activity, e.g., an *in vivo* or an *in vitro* activity described herein. In certain embodiments, a hepcidin analogue of the present invention exhibits 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, or 200-fold greater or at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500% , 700%, or 1000% greater activity than a reference hepcidin, wherein the activity is an *in vitro* activity for inducing the degradation of ferroportin, e.g., as measured according to the Examples herein; or wherein the activity is an *in vivo* activity for reducing free plasma iron, e.g., as measured according to the Examples herein.

[00126] In some embodiments, the hepcidin analogues of the present invention mimic the hepcidin activity of Hep25, the bioactive human 25-amino acid form, are herein referred to as "mini-hepcidins". As used herein, in certain embodiments, a compound (e.g., a hepcidin analogue) having a "hepcidin activity" means that the compound has the ability to lower plasma iron concentrations in subjects (e.g. mice or humans), when administered thereto (e.g. parenterally injected or orally administered), in a dose-dependent and time-dependent manner. See e.g. as demonstrated in Rivera et al. (2005), Blood 106:2196-9. In some embodiments, the peptides of the present invention lower the plasma iron concentration in a subject by at least about 1.2, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, or 10-fold, or at least about 5%, 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or about 99%.

[00127] In some embodiments, the hepcidin analogues of the present invention have *in vitro* activity as assayed by the ability to cause the internalization and degradation of ferroportin in a ferroportin-expressing cell line as taught in Nemeth et al. (2006) Blood 107:328-33. In some embodiments, *in vitro* activity is measured by the dose-dependent loss of fluorescence of cells engineered to display ferroportin fused to green fluorescent protein as in Nemeth et al. (2006) Blood 107:328-33. Aliquots of cells are incubated for 24 hours with graded concentrations of a reference preparation of Hep25 or a mini-hepcidin. As provided herein, the EC_{50} values are provided as the concentration of a given compound (e.g. a hepcidin analogue peptide or peptide dimer of the present invention) that elicits 50% of the maximal

loss of fluorescence generated by a reference compound. The EC_{50} of the Hep25 preparations in this assay range from 5 to 15 nM and in certain embodiments, preferred hepcidin analogues of the present invention have EC_{50} values in *in vitro* activity assays of about 1,000 nM or less. In certain embodiments, a hepcidin analogue of the present invention has an EC_{50} in an *in vitro* activity assay (e.g., as described in Nemeth et al. (2006) Blood 107:328-33 or the Example herein) of less than about any one of 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 200 or 500 nM. In some embodiments, a hepcidin analogue or biotherapeutic composition (e.g., any one of the pharmaceutical compositions described herein) has an EC_{50} value of about 1nM or less.

[00128] Other methods known in the art for calculating the hepcidin activity and *in vitro* activity of the hepcidin analogues according to the present invention may be used. For example, in certain embodiments, the *in vitro* activity of the hepcidin analogues or the reference peptides is measured by their ability to internalize cellular ferroportin, which is determined by immunohistochemistry or flow cytometry using antibodies which recognizes extracellular epitopes of ferroportin. Alternatively, in certain embodiments, the *in vitro* activity of the hepcidin analogues or the reference peptides is measured by their dose-dependent ability to inhibit the efflux of iron from ferroportin-expressing cells that are preloaded with radioisotopes or stable isotopes of iron, as in Nemeth et al. (2006) Blood 107:328-33.

[00129] In some embodiments, the hepcidin analogues of the present invention exhibit increased stability (*e.g.,* as measured by half-life, rate of protein degradation) as compared to a reference hepcidin. In certain embodiments, the stability of a hepcidin analogue of the present invention is increased at least about 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, or 200-fold greater or at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, or 500% greater than a reference hepcidin. In some embodiments, the stability is a stability that is described herein. In some embodiments, the stability is a plasma stability, e.g., as optionally measured according to the method described herein.

[00130] In particular embodiments, a hepcidin analogue of the present invention exhibits a longer half-life than a reference hepcidin. In particular embodiments, a hepcidin analogue of the present invention has a half-life under a given set of conditions (e.g., temperature, pH) of at least about 5 minutes, at least about 10 minutes, at least about 20 minutes, at least about 30

minutes, at least about 45 minutes, at least about 1 hour, at least about 2 hour, at least about 3 hours, at least about 4 hours, at least about 5 hours, at least about 6 hours, at least about 12 hours, at least about 18 hours, at least about 1 day, at least about 2 days, at least about 4 days, at least about 7 days, at least about 10 days, at least about two weeks, at least about three weeks, at least about 1 month, at least about 2 months, at least about 3 months, or more, or any intervening half-life or range in between, about 5 minutes, about 10 minutes, about 20 minutes, about 30 minutes, about 45 minutes, about 1 hour, about 2 hour, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 12 hours, about 18 hours, about 1 day, about 2 days, about 4 days, about 7 days, about 10 days, about two weeks, about three weeks, about 1 month, about 2 months, about 3 months, or more, or any intervening half-life or range in between. In some embodiments, the half-life of a hepcidin analogue of the present invention is extended due to its conjugation to one or more lipophilic substituent, e.g., any of the lipophilic substituents disclosed herein. In some embodiments, the half-life of a hepcidin analogue of the present invention is extended due to its conjugation to one or more polymeric moieties, e.g., any of the polymeric moieties disclosed herein. In certain embodiments, a hepcidin analogue of the present invention has a half-life as describe above under the given set of conditions wherein the temperature is about 25 °C, about 4 °C, or about 37 °C, and the pH is a physiological pH, or a pH about 7.4.

[00131] In some embodiments, the half-life is measured *in vitro* using any suitable method known in the art, e.g., in some embodiments, the stability of a hepcidin analogue of the present invention is determined by incubating the hepcidin analogue with pre-warmed human serum (Sigma) at 37 ° C. Samples are taken at various time points, typically up to 24 hours, and the stability of the sample is analyzed by separating the hepcidin analogue from the serum proteins and then analyzing for the presence of the hepcidin analogue of interest using LC-MS.

[00132] In some embodiments, the stability of the hepcidin analogue is measured *in vivo* using any suitable method known in the art, e.g., in some embodiments, the stability of a hepcidin analogue is determined in vivo by administering the peptide or peptide dimer to a subject such as a human or any mammal (e.g., mouse) and then samples are taken from the subject via blood draw at various time points, typically up to 24 hours. Samples are then analyzed as described above in regard to the *in vitro* method of measuring half-life. In some embodiments, in vivo stability of a hepcidin analogue of the present invention is determined via the method disclosed in the Examples herein.

[00133] In some embodiments, the present invention provides a hepcidin analogue as described herein, wherein the hepcidin analogue exhibits improved solubility or improved aggregation characteristics as compared to a reference hepcidin. Solubility may be determined via any suitable method known in the art. In some embodiments, suitable methods known in the art for determining solubility include incubating peptides (e.g., a hepcidin analogue of the present invention) in various buffers (Acetate pH4.0, Acetate pH5.0, Phos/Citrate pH5.0, Phos Citrate pH6.0, Phos pH 6.0, Phos pH 7.0, Phos pH7.5, Strong PBS pH 7.5, Tris pH7.5, Tris pH 8.0, Glycine pH 9.0, Water, Acetic acid (pH 5.0 and other known in the art) and testing for aggregation or solubility using standard techniques. These include, but are not limited to, visual precipitation, dynamic light scattering, Circular Dichroism and fluorescent dyes to measure surface hydrophobicity, and detect aggregation or fibrillation, for example. In some embodiments, improved solubility means the peptide (e.g., the hepcidin analogue of the present invention) is more soluble in a given liquid than is a reference hepcidin.

[00134] In certain embodiments, the present invention provides a hepcidin analogue as described herein, wherein the hepcidin analogue exhibits a solubility that is increased at least about 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, or 200-fold greater or at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, or 500% greater than a reference hepcidin in a particular solution or buffer, e.g., in water or in a buffer known in the art or disclosed herein.

[00135] In certain embodiments, the present invention provides a hepcidin analogue as described herein, wherein the hepcidin analogue exhibits decreased aggregation, wherein the aggregation of the peptide in a solution is at least about 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, or 200-fold less or at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, or 500% less than a reference hepcidin in a particular solution or buffer, e.g., in water or in a buffer known in the art or disclosed herein.

[00136] In some embodiments, the present invention provides a hepcidin analogue, as described herein, wherein the hepcidin analogue exhibits less degradation (i.e., more degradation stability), e.g., greater than or about 10% less, greater than or about 20% less, greater than or about 30% less, greater than or about 40 less, or greater than or about 50% less than a reference hepcidin. In some embodiments, degradation stability is determined via

any suitable method known in the art. In some embodiments, suitable methods known in the art for determining degradation stability include the method described in Hawe et al J Pharm Sci, VOL. 101, NO. 3, 2012, p 895-913, incorporated herein in its entirety. Such methods are in some embodiments used to select potent sequences with enhanced shelf lives.

[00137] In some embodiments, the hepcidin analogue of the present invention is synthetically manufactured. In other embodiments, the hepcidin analogue of the present invention is recombinantly manufactured.

[00138] The various hepcidin analogue monomer and dimer peptides of the invention may be constructed solely of natural amino acids. Alternatively, these hepcidin analogues may include unnatural or non-natural amino acids including, but not limited to, modified amino acids. In certain embodiments, modified amino acids include natural amino acids that have been chemically modified to include a group, groups, or chemical moiety not naturally present on the amino acid. The hepcidin analogues of the invention may additionally include D-amino acids. Still further, the hepcidin analogue peptide monomers and dimers of the invention may include amino acid analogs. In particular embodiments, a peptide analogue of the present invention comprises any of those described herein, wherein one or more natural amino acid residues of the peptide analogue is substituted with an unnatural or non-natural amino acid, or a D-amino acid.

[00139] In certain embodiments, the hepcidin analogues of the present invention include one or more modified or unnatural amino acids. For example, in certain embodiments, a hepcidin analogue includes one or more of Daba, Dapa, Pen, Sar, Cit, Cav, HLeu, 2-Nal, 1-Nal, d-1-Nal, d-2-Nal, Bip, Phe(4-OMe), Tyr(4-OMe), βhTrp, βhPhe, Phe(4-CF_3), 2-2-Indane, 1-1-Indane, Cyclobutyl, βhPhe, hLeu, Gla, Phe(4-NH_2), hPhe, 1-Nal, Nle, 3-3-diPhe, cyclobutyl-Ala, Cha, Bip, β-Glu, Phe(4-Guan), homo amino acids, D-amino acids, and various N-methylated amino acids. One having skill in the art will appreciate that other modified or unnatural amino acids, and various other substitutions of natural amino acids with modified or unnatural amino acids, may be made to achieve similar desired results, and that such substitutions are within the teaching and spirit of the present invention.

[00140] The present invention includes any of the hepcidin analogues described herein, e.g., in a free or a salt form.

[00141] The hepcidin analogues of the present invention include any of the peptide monomers or dimers described herein linked to a linker moiety, including any of the specific linker moieties described herein.

[00142] The hepcidin analogues of the present invention include peptides, e.g., monomers or dimers, comprising a peptide monomer subunit having at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% amino acid sequence identity to a hepcidin analogue peptide sequence described herein (e.g., any one of the peptides disclosed in Tables 1-4 or 6-15).

[00143] In certain embodiments, a peptide analogue of the present invention, or a monomer subunit of a dimer peptide analogue of the present invention, comprises or consists of 7 to 35 amino acid residues, 8 to 35 amino acid residues, 9 to 35 amino acid residues, 10 to 35 amino acid residues, 7 to 25 amino acid residues, 8 to 25 amino acid residues, 9 to 25 amino acid residues, 10 to 25 amino acid residues, 7 to 18 amino acid residues, 8 to 18 amino acid residues, 9 to 18 amino acid residues, or 10 to 18 amino acid residues, and, optionally, one or more additional non-amino acid moieties, such as a conjugated chemical moiety, e.g., a PEG or linker moiety. In particular embodiments, a monomer subunit of a hepcidin analogue comprises or consists of 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, or 35 amino acid residues. In particular embodiments, a monomer subunit of a hepcidin analogue of the present invention comprises or consists of 10 to 18 amino acid residues and, optionally, one or more additional non-amino acid moieties, such as a conjugated chemical moiety, e.g., a PEG or linker moiety. In various embodiments, the monomer subunit comprises or consists of 7 to 35 amino acid residues, 9 to 18 amino acid residues, or 10 to 18 amino acid residues. In particular embodiments of any of the various Formulas described herein, X comprises or consists of 7 to 35 amino acid residues, 8 to 35 amino acid residues, 9 to 35 amino acid residues, 10 to 35 amino acid residues, 7 to 25 amino acid residues, 8 to 25 amino acid residues, 9 to 25 amino acid residues, 10 to 25 amino acid residues, 7 to 18 amino acid residues, 8 to 18 amino acid residues, 9 to 18 amino acid residues, or 10 to 18 amino acid residues.

Peptide Monomer Hepcidin Analogues

[00144] In certain embodiments, hepcidin analogues of the present invention comprise a single peptide subunit. In certain embodiments, these hepcidin analogues form cyclized structures through intramolecular disulfide or other bonds. In one embodiment, the present invention

provides a cyclized form of any one of the hepcidin analogues listed in Tables 2-4, or 12-15, provided that the analogue has two or more Cys residues.

[00145] In certain embodiments, the present invention includes a peptide analogue, wherein the peptide analogue has the structure of Formula I:

R^1-X-Y-R^2 (I) (SEQ ID NO:1)

[00146] or a pharmaceutically acceptable salt or solvate thereof,

[00147] wherein R^1 is hydrogen, a C1-C6 alkyl, a C6-C12 aryl, a C6-C12 aryl C1-C6 alkyl, or a C1-C20 alkanoyl, and including PEGylated versions alone or as spacers of any of the foregoing;

[00148] R^2 is OH or NH_2; and

[00149] X is a peptide sequence having the formula Ia:

X1-X2-X3-X4-X5-X6-X7-X8-X9-X10 (Ia) (SEQ ID NO:2)

[00150] wherein

X1 is Asp, Ser, Glu, Ida, pGlu, bhAsp, D-Asp or absent;

X2 is Thr, Ser, Lys, Glu, Pro, Ala or absent;

X3 is His, Ala, or Glu;

X4 is Phe, Ile or Dpa;

X5 is Pro, bhPro, Val, Glu, Sarc or Gly;

X6 is Cys or (D)-Cys;

X7 is absent or any amino acid except Ile, Cys or (D)-Cys;

X8 is absent or any amino acid except Cys or (D)-Cys;

X9 is Phe, Ala, Ile, Thr, Tyr, Lys, Arg, bhPhe, D-Phe or absent; and

X10 is Lys, Phe or absent;

Y is absent or present; and

[00151] provided that if Y is present, Y is a peptide having the formula Im:

Y1-Y2-Y3-Y4-Y5-Y6-Y7-Y8-Y9-Y10-Y11-Y12 (Im) (SEQ ID NO:3)

[00152] wherein

Y1 is Gly, PEG3, Sarc, Lys, Glu, Ala, Phe, Pro, Glu, Lys, D-Pro, Val, Ser or absent;

Y2 is Pro, Ala, Cys, Gly or absent;

Y3 is Arg, Lys, Pro, Gly, His, Ala, Trp or absent;

Y4 is Ser, Arg, Gly, Trp, Ala, His, Glu, Tyr or absent;

Y5 is Lys, Met, Ser, Arg, Ala or absent;

Y6 is Gly, Sarc, Glu, Lys, Arg, Ser, Lys, Ile, Ala, Pro, Val or absent;

Y7 is Trp, Lys, Gly, Ala, Ile, Val or absent;

Y8 is Val, Trp, His, Thr, Gly, Cys, Met, Tyr, Ala, Glu, Lys, Asp, Arg or absent;

Y9 is Val, Asp, Asn, Cys, Tyr or absent;

Y10 is Cys, Met, Lys, Arg, Tyr or absent;

Y11 is Arg, Met, Cys, Lys or absent; and

Y12 is Arg, Lys, Ala or absent.

[00153] In certain alternative embodiments, X7 is absent or any amino acid except Cys, or (D)-Cys.

[00154] In certain embodiments, X7 is Arg, Glu, Phe, Gln, Leu, Val, Lys, Ala, Ser, Dapa or absent.

[00155] In certain embodiments, X8 is Ile, Arg, Lys, Ala, Gln, Phe, Glu, Asp, Tyr, Ser, Leu, Val, D-Ile, D-Lys, D-Arg, Dapa or absent.

[00156] In certain embodiments of any of the peptide analogues having any of the various Formulae set forth herein, R^1 is selected from methyl, acetyl, formyl, benzoyl, trifluoroacetyl, isovaleryl, isobutyryl, octanyl, and conjugated amides of lauric acid, hexadecanoic acid, and γ-Glu-hexadecanoic acid.

[00157] In certain embodiments of any of the Formulae set forth herein, wherein the amino acid residue immediately carboxy to X6 is not Ile. In particular embodiments, wherein X6 is Cys or (D)-Cys, the amino acid residue immediately carboxy to X6 is not Ile. For example, in certain embodiments, wherein X7 is absent and X8 is present, X8 is not Ile, or wherein X7 and X8 are absent, X9 is not Ile.

[00158] In certain embodiments of any of the Formulae set forth herein, X either or both does not comprise or does not consist of an amino acid sequence set forth in US Patent No. 8,435,941.

[00159] In certain embodiments of the peptide analogue of Formula I,

[00160] X is a peptide sequence having the formula Ib:

X1-X2-X3-X4-X5-X6-X7-X8-X9-X10 (Ib) (SEQ ID NO:18)

[00161] wherein

X1 is Asp, Glu, Ida, pGlu, bhAsp, D-Asp or absent;

X2 is Thr, Ser, Lys, Glu, Pro, Ala or absent;

X3 is His, Ala, Glu or Ala;

X4 is Phe, Ile or Dpa;

X5 is Pro, bhPro, Sarc or Gly;

X6 is Cys;

X7 is absent or any amino acid except Ile, Cys or (D)-Cys;

X8 is absent or any amino acid except Cys or (D)-Cys;

X9 is Phe, Ile, Tyr, bhPhe or D-Phe or absent; and

X10 is Lys, Phe or absent;

[00162] wherein Y is absent or present, provided that if Y is present, Y is a peptide having the formula In:

Y1-Y2-Y3-Y4-Y5-Y6-Y7-Y8-Y9-Y10-Y11-Y12 (In) (SEQ ID NO:19)

[00163] wherein

Y1 is Gly, PEG3, Sarc, Lys, Glu, Ala, Phe, Pro, Glu, Lys, D-Pro, Val, Ser or absent;

Y2 is Pro, Ala, Gly or absent;

Y3 is Arg, Lys, Pro, Gly, His, Ala, or absent;

Y4 is Ser, Arg, Glu or absent;

Y5 is Lys, Ser, Met, Arg, Ala or absent;

Y6 is Gly, Sarc, Glu, Leu, Phe, His or absent;

Y7 is Trp, NMe-Trp, Lys, Thr, His, Gly, Ala, Ile, Val or absent;

Y8 is Val, Trp, Ala, Asn, Glu or absent;

Y9 is Val, Ala, Asn, Asp, Cys or absent;

Y10 is Cys, (D)Cys, Glu or absent;

Y11 is Tyr, Met or absent; and

Y12 is Trp or absent.

[00164] In certain embodiments, X7 is Arg, Glu, Phe, Gln, Leu, Val, Lys, Ala, Ser, Dapa or absent.

[00165] In certain embodiments, X7 is Arg, Glu, Phe, Gln, Leu, Ile, Val, Lys, Ala, Ser, Dapa or absent.

[00166] In certain alternative embodiments, X7 is absent or any amino acid except Cys, or (D)-Cys.

[00167] In certain embodiments, X8 is Ile, Arg, Lys, Ala, Gln, Phe, Glu, Asp, Tyr, Ser, Leu, Val, D-Ile, D-Lys, D-Arg, Dapa or absent.

[00168] In some embodiments, the peptides of formula (I) comprise at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, or at least 12 amino acid residues in Y.

[00169] In some embodiments, Y1 to Y3 are present and Y4 to Y12 are absent.

[00170] In some embodiments, Y1 to Y11 are present and Y12 is absent.

[00171] In some embodiments, Y1 to Y10 are present and Y11 to Y12 are absent.

[00172] Illustrative embodiments of peptide analogues of Formula I are provide in Table 2. In particular embodiments, a peptide analogue of the present invention comprises or consists of an amino acid sequence set forth in Table 2, or has a structure shown in Table 2. Table 2 also provides the EC_{50} values of illustrative peptide analogues as determined via the ferroportin internalization/degradation assay described in the accompanying Examples.

Table 2. Illustrative Peptide Monomer Hepcidin Analogues

SEQ ID No.	Sequence	EC_{50} (nM)
440	Hy-DTHFPCAIF-NH_2	>1000
441	Hy-DTHFPCRRF-NH_2	> 10 μM
442	[IDA]-TH-[Dpa]-[bhPro]CRR-[bhPhe]-NH_2	206
443	Hy- DTHFPCEIF-NH_2	>1000
444	Hy-DTHFPCFIF-NH_2	1191.8
445	Hy- DTHFPCQIF-NH_2	>1000
446	Hy-DTHFPCRIF-NH_2	>1000
447	Hy- [pGlu]-THFPCRKF-NH_2	>1000
448	Hy- DTHFPCLIF-NH_2	> 10 μM
449	Hy-DTHFPCVIF-NH_2	81% at 10 uM
450	Hy-DTHFPCEIF-NH_2	19% at 10 uM
451	Hy-DTHFPCRIF-NH_2	31% at 10 uM
452	Hy- DTHFPCKIF-NH_2	9% at 10 uM
453	Hy- DTHFPCLF-NH_2	39% at 1 uM
454	Hy- DTHFPCEF-NH_2	17% at 10 uM
455	Hy-DTHFPCRF-NH_2	31% at 10 uM
456	Hy-DTHFPRRFGPRSKGWVC-NH_2	>1000
457	[IDA]-THF-[bhPro]-CRR-[bhPhe]GPRSKGWVC-NH_2	>1000
458	Hy- DTHFPCIFGPRSKGWVC-NH_2	>1000
459	Hy-DTHFPCRIFGPRSRGWVCK-NH_2	>1000
460	Isovaleric acid-DTHFPCLIFGPRSKGWVCK-NH_2	19.2
461	Isovaleric acid-DTHFPCVIFGPRSKGWVCK-NH_2	41
462	Isovaleric acid-DTHFPCSIFGPRSKGWVCK-NH_2	78

463	Isovaleric acid-DTHFPCQIFGPRSKGWVCK-NH_2	157
464	Isovaleric acid-DTHFPCKIFGPRSKGWVCK-NH_2	86
465	Isovaleric acid-DTHFPC-[Dapa]-IFGPRSKGWDCK-NH_2	65
466	Isovaleric acid-DTHFPC-[Dapa]-IFGPRSKGWECK-NH_2	151
467	Isovaleric acid-DTHFPCKIFGPRSKGWECK-NH_2	163
468	Isovaleric acid-DTHFPCRRFGPRSKGWVCK-NH_2	>1000
469	Isovaleric acid-DTHFPCTIFGPRSKGWVCK-NH_2	Not Tested

[00173] In certain embodiments, the present invention includes a peptide analogue, wherein the peptide analogue has the structure of Formula II:

R^1-X-Y-R^2 (II) (SEQ ID NO:4)

[00174] or a pharmaceutically acceptable salt or solvate thereof,

[00175] wherein R^1 is hydrogen, a C1-C6 alkyl, a C6-C12 aryl, a C6-C12 aryl C1-C6 alkyl, or a C1-C20 alkanoyl, and including PEGylated versions alone or as spacers of any of the foregoing;

[00176] R^2 is OH or NH_2; and

[00177] X is a peptide sequence having the formula IIa:

X1-X2-X3-X4-X5-X6-X7-X8-X9-X10 (IIa) (SEQ ID NO:5)

[00178] wherein

X1 is Asp, Glu or Ida;

X2 is Thr, Ser or absent;

X3 is His;

X4 is Phe or Dpa;

X5 is Pro, bhPro, Sarc or Gly;

X6 is Cys or (D)-Cys;

X7 is Arg, Glu, Phe, Gln, Leu, Val, Lys, Ile, Ala, Ser, Dapa or absent;

X8 is Ile, Arg, Lys, Arg, Ala, Gln, Phe, Glu, Asp, Tyr, Ser, Leu, Val, D-Ile, D-Lys, D-Arg, Dapa or absent;

X9 is Phe, Tyr, bhPhe, D-Phe or absent; and

X10 is Lys, Phe or absent; and

[00179] wherein Y is absent or present, provided that if Y is present, Y is a peptide having the formula IIm:

Y1-Y2-Y3-Y4-Y5-Y6-Y7-Y8-Y9-Y10-Y11-Y12 (IIm) (SEQ ID NO:6)

wherein

Y1 is Gly, Sarc, Lys, Glu or absent;

Y2 is Pro, Ala, Gly or absent;

Y3 is Arg, Lys, Pro, Gly, His, Ala or absent;

Y4 is Ser, Arg, Glu or absent;

Y5 is Lys, Ser, Met, Arg, Ala or absent;

Y6 is Gly, Sarc, Glu, Leu, Phe, His or absent;

Y7 is Trp, NMe-Trp, Lys, Thr, His, Gly, Ala, Ile, Val or absent;

Y8 is Val, Trp, Ala, Asn, Glu or absent;

Y9 is Cys;

Y10 is Met or absent;

Y11 is Tyr, Met or absent; and

Y12 is Trp or absent.

[00180] In certain embodiments, X6 is Cys.

[00181] In some embodiments, X7 is Arg, Glu, Phe, Gln, Leu, Val, Lys, Ala, Ser, Dapa or absent.

[00182] In certain embodiments, Y10 is absent.

[00183] In certain embodiments, Y11 is Tyr.

[00184] In certain embodiments, Y11 is absent.

[00185] In certain embodiments, Y12 is absent.

[00186] In certain embodiments, Y11 and Y12 or Y10, Y11 and Y12 are absent.

[00187] In certain embodiments of any of the peptide analogues having any of the Formulae set forth herein, R^1 is selected from methyl, acetyl, formyl, benzoyl, trifluoroacetyl, isovaleryl, isobutyryl, octanyl, and the conjugated amides of lauric acid, hexadecanoic acid, and γ-Glu-hexadecanoic acid.

[00188] In certain embodiments of any of the Formulae set forth herein, X either or both does not comprise or does not consist of an amino acid sequence set forth in US Patent No. 8,435,941.

[00189] In some embodiments, the peptides of formula (II) comprise at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, or at least 12 amino acid residues in Y.

[00190] In some embodiments, Y1 to Y3 are present and Y4 to Y12 are absent.

[00191] In some embodiments, Y1 to Y11 are present and Y12 is absent.

[00192] In some embodiments, Y1 to Y10 are present and Y11 to Y12 are absent.

[00193] Illustrative embodiments of peptide analogues of Formula II are provide in Table 3. In particular embodiments, a peptide analogue of the present invention comprises or consists of an amino acid sequence set forth in Table 3, or has a structure shown in Table 3. Table 3 also provides the EC_{50} values of illustrative peptide analogues as determined via the ferroportin internalization/degradation assay described herein.

Table 3. Illustrative Peptide Monomer Hepcidin Analogues

SEQ ID No.	**Sequence**	**Ferroportin internalization assay EC_{50} (nM)**
470	Hy- DTHFPIAICI-NH_2	Not Active
471	Hy- DTHFPIICI-NH_2	Not Active
472	Hy- DTHICIAIF-NH_2	Not Active
473	Hy- DTHCPIAIF-NH_2	Not Active
474	Hy- ATHFPCIIF-NH_2	>1000
475	Hy- ADHFPCIIF-NH_2	>1000
476	Hy- DTHFPCIIFKC-NH_2	6398.0

477	Hy- DTHFPCIIFAC-NH_2	>1000
478	Hy- DTHFPCIIFAA-NH_2	59% at 1 uM
479	Hy- DEHFPCIIF-NH_2	34% at 10 uM
480	Hy- DPHFPCIIF-NH_2	64% at 10 uM
481	Hy- DTHKPCIIF-NH_2	45 % at 10 uM
482	Hy- DTHVPCIIF-NH_2	34% at 10 uM
483	Hy- DTHFVCIIF-NH_2	50% at 10 uM
484	Hy- DTHFPCIIY-NH_2	75% at 10 uM
485	Hy- DTHFPCIIT-NH_2	23% at 1 uM
486	Hy- DTHFPCILY-NH_2	85% at 1 uM
487	Hy- DTHFPCIEY-NH_2	8% at 1 uM
488	Isovaleric acid-DTHFPCIIFGPRSKG-[N-MeTrp]-VC-NH_2	32
489	Isovaleric acid-DTHFPCIIF-[Sarc]-PRSKG-[N-MeTrp]-VC-NH_2	10
490	Isovaleric acid-DTHFPCIIF-[Sarc]-PHSKG-[N-MeTrp]-VC-NH_2	9
491	Isovaleric acid-DTHFPCIIFEPRSKHWVCK-NH_2	15
492	Isovaleric acid-DTHFPCIIFEPRSKEWVCK-NH_2	19

493	Isovaleric acid-DTHFPCIIFEPRSKLWVCK-NH_2	7
494	Isovaleric acid-DTHFPCIIFEPRSKFWVCK-NH_2	10
495	Isovaleric acid-DTHFPCIKFEPHSK-[Sarc]-CK-NH_2	28
496	Isovaleric acid-DTHFPCIKFKPHSKEWVCE-NH_2	46
497	Isovaleric acid-DTHFPCIKFEPRSKEWVCK-NH_2	20
498	Isovaleric acid-DTHFPCIKFEPRSKLWVCK-NH_2	9
499	Isovaleric acid-DTHFPCIKFEPRSKEWVCK-OH	46
500	Isovaleric acid-DTHFPCIKFEPRS-K(isoGlu-octanoic acid)-ECK-NH_2	48
501	Hy-DTHFPCIIFGPRSKGWAVCYW-NH_2	197
502	Hy-DTHFPICIFGPHRSKGWVCM-NH_2	149
503	Hy-DTHFPCIIFGPRSKGWVAC-NH_2	281
504	Hy-DTHFP-[(D)Cys]-IIFGPRSKGWVA-[(D)Cys]-NH_2	Not active
505	Hy-DTHFPCIIFGPRSKGWVACY-NH_2	Not active
506	Hy-DTHFPCIIFGPRSRGHVCK-NH_2	>1000
507	Hy-DTHFPCIIFGPRSKGWNCK-NH_2	>1000
508	Hy-DTHFPCINFGPRSKGWVCK-NH_2	>1000
509	Hy-DTHFPCIDFGPRSKGWVCK-NH_2	>1000
510	Isovaleric acid-DTHFECIIFGPRSKGWVCK-NH_2	>1000
511	Hy-DTHFPCIIFGGPRSRGWVCK-NH_2	520
512	Hy-DTHFPCIIFGGPRSKGWNCK-NH_2	404
513	Hy-DTHFPCIIFGGPRSKGWDCK-NH_2	679
514	Isovaleric acid-DTHFPCIFEPRSKGTCK-NH_2	57
515	Isovaleric acid-DTHFPCIIF-[PEG3]-C-NH_2	157
516	Isovaleric acid-DTHAPCIKF-[Sarc]-PRSKGWECK-NH_2	Not active
517	Isovaleric acid-DTHAPCIKFEPRSK-[Sarc]-WECK-NH_2	Not active
518	Isovaleric acid-DTHAPCIKFEPRSKEWECK-NH_2	Not active
519	Isovaleric acid-STHAPCIKFEPRSKGWECK-NH_2	Not active
520	Isovaleric acid-SKHAPCIKFEPRSKGWECK-NH_2	Not active
521	Isovaleric acid-DTHFPCIKFEPHSKEWVCK-NH_2	80
522	Isovaleric acid-DTAFPCIKFEPRSKEC-NH_2	Not active
523	Isovaleric acid-DTHFGCIKFEPRSKEWVCK-NH_2	>1000
524	Isovaleric acid-DTEFPCIKFEPRSKEWVCK-NH_2	>1000
525	Isovaleric acid-DTHFPCIKFEPRS-K(octanoic acid)-EWVCK-NH_2	62
526	Isovaleric acid-ETHFPCIKFEPRSKEWVCK-NH_2	181

Peptide Dimer Hepcidin Analogues

[00194] In certain embodiments, the present invention includes dimers of the monomer hepcidin analogues described herein, including dimers comprising any of the monomer peptides sequences or structures set forth in Tables 2-4, and certain dimers of sequences or structures set forth in Tables 6-10, 12, 14, and 15. In particular embodiments, the invention includes dimers of any of the monomer peptide sequences or structure set forth in Table 11 or 13. These dimers fall within the scope of the general term "hepcidin analogues" as used herein. The term "dimers," as in peptide dimers, refers to compounds in which two peptide monomer subunits are linked. A peptide dimer of the present invention may comprise two identical monomer subunits, resulting in a homodimer, or two non-identical monomer subunits, resulting in a heterodimer. A cysteine dimer comprises two peptide monomer subunits linked through a disulfide bond between a cysteine residue in one monomer subunit and a cysteine residue in the other monomer subunit.

[00195] In particular embodiments, a peptide dimer hepcidin analogue comprises one or more, e.g., two, peptide monomer subunits shown in Table 4 or described in US Patent No. 8,435,941, which is herein incorporated by reference in its entirety.

Table 4. Illustrative peptide monomer subunits

SEQ ID NO	Sequence
376	DTHFPICIFC
377	FPIC
378	HFPIC
379	HFPICI
380	HFPICIF
381	DTHFPIC
381	DTHFPICI
382	DTHFPICIF
383	DTHFPIAIFC
384	DTHAPICIF
385	DTHAPI-[C-StBu]-IF
386	DTHAPI-[C-tBu]-IF
387	DTHFPIAIF
388	DTHFPISIF
389	DTHFPI-([D)-Cys]-IF
390	DTHFPI-[homoCys]-IF
391	DTHFPI-[Pen]-IF
392	DTHFPI-[(D)-Pen]-IF
393	DTHFPI-[Dapa(AcBr)]-IF
394	CDTHFPICIF
395	DTHFPICIF-$NHCH_2CH_2S$

396	CHFPICIF
397	HFPICIF-$NHCH_2CH_2S$
398	D-[Tle]-H-[Phg]-[Oic]-[Chg]-C-[Chg]-F
399	D-[Tle]-HP-[Oic]-[Chg]-C-[Chg]-F
400	[(D)Phe]-[(D)Ile]-[(D)Cys]-[(D)Ile]-[(D)pro]-[(D)Phe]-[(D)His]-[(D)Thr]-[(D)Asp]
401	[(D)Phe]-[(D)Ile]-[(D)Cys]-[(D)Ile]-[(D)Pro]-[(D)Phe]-[(D)His]
402	Chenodeoxycholate-(PEG11)-[(D)Phe]-[(D)Ile]-[(D)Cys]-[(D)Ile]-[(D)Pro]-[(D)Phe]-[(D)His]-[(D)Thr]-[(D)Asp]
403	Ursodeoxycholate-(PEG11)-[(D)Phe]-[(D)Ile]-[(D)Cys]-[(D)Ile]-[(D)Pro]-[(D)Phe]-[(D)His]-[(D)Thr]-[(D)Asp]
404	F-[(D)Ile]-[(D)Cys]-[(D)Ile]-[(D)Pro]-[(D)Phe]-[(D)His]-[(D)Thr]-[(D)Asp]-(Peg11)-GYIPEAPRDGQAYVRKDGEWVLLSTFL
405	F-[(D)Ile]-[(D)Cys]-[(D)Ile]-[(D)pro]-[(D)Phe]-[(D)His]-[(D)Thr]-[(D)Asp]-$[GP\text{-}(Hyp)]_{10}$
406	Palmitoyl-(PEG11)-[(D)Phe]-[(D)Ile]-[(D)Cys]-[(D)Ile]-[(D)Pro]-[(D)Phe]-[(D)His]-[(D)Thr]-[(D)Asp]
407	2(Palmitoyl)-[Dapa]-(Peg11)-[(D)Phe]-[(D)Ile]-[(D)Cys]-[(D)Ile]-[(D)Pro]-[(D)Phe]-[(D)His]-[(D)Thr]-[(D)Asp]
408	DTH-[bhPhe]-PIICIF
409	DTH-[Dpa]-PICI.
410	DTH-[Bip]-PICIF
411	DTH[1-Nal]-PICIF
412	DTH-[bhDpa]-PICIF
413	DTHFP-ICI-bhPhe
414	DTHFPICI-[Dpa]
415	DTHFPICI-[Bip]
416	DTHFPICI-[1-Nal]
417	DTHFPICI-[bhDpa]
418	DTH-[Dpa]-PICI-[Dpa]
419	D-[Dpa]-PICIF
420	D-[Dpa]-PICI-[Dpa]
421	DTH-[Dpa]-P-[(D)Arg]-CR-[Dpa]
422	DTH-[Dpa]-P-[(D)Arg]-C-[(D)Arg]-[Dpa]
423	DTH-[Dpa]-[Oic]-ICIF
424	DTH-[Dpa]-[Oic]-ICI-[Dpa]
425	DTH-[Dpa]-PCCC-[Dpa]
426	DTHFPICIF-[(D)Pro]-PK
427	DTHFPICIF-[(D)Pro]-PR
428	DTHFPICIF-[bhPro]-PK
429	DTHFPICIF-[bhPro]-PR
430	DTHFPICIF-[(D)Pro]-[bhPro]-K
431	DTHFPICIF-[(D)-Pro]-[bhPro]-R
432	DTHFPICI-[bhPhe]-[(D)Pro]-PK
433	DTHFPICI-[bhPhe]-[(D)Pro]-PR
434	DTHFPICI-[bhPhe]-[(D)Pro]-[bhPro]-K
435	DTHFPICI-[bhPhe]-[(D)Pro]-[bhPro]-R
436	C-[Inp]-[(D)Dpa]-[Amc]-R-[Amc]-[Inp]-[Dpa]-Cysteamide
437	CP-[(D)Dpa]-[Amc]-R-[Amc]-[Inp]-[Dpa]-Cysteamide
438	C-[(D)Pro]-[(D)Dpa]-[Amc]-R-[Amc]-[Inp]-[Dpa]-Cysteamide
439	CG-[(D)Dpa]-[Amc]-R-[Amc]-[Inp]-[Dpa]-Cysteamide

[00196] In some embodiments, the hepcidin analogues of the present invention are active in a dimer conformation, in particular when free cysteine residues are present in the peptide. In certain embodiments, this occurs either as a synthesized dimer or, in particular, when a free cysteine monomer peptide is present and under oxidizing conditions, dimerizes. In some embodiments, the dimer is a homodimer. In other embodiments, the dimer is a heterodimer.

[00197] In certain embodiments, a hepcidin analogue dimer of the present invention is a peptide dimer comprising two hepcidin analogue peptide monomers of the invention.

[00198] In various embodiments, the amino acid sequences listed in Tables 2-4 and Tables 6-15 are shown using one letter codes for amino acids. Wherein only the hepcidin analogue monomer peptide sequence is shown, it is understood that, in certain embodiments, these hepcidin analogue monomer peptides, i.e., monomer subunits, are dimerized to form peptide dimer hepcidin analogues, in accordance with the present teachings. Thus, in one embodiment, the present invention provides a dimer of a peptide monomer shown in any one of Tables 2-4, 6-10, 12, 14, or 15.

[00199] The monomer subunits may be dimerized by a disulfide bridge between two cysteine residues, one in each peptide monomer subunit, or they may be dimerized by another suitable linker moiety, as defined herein. Some of the monomer subunits are shown having C- and N-termini that both comprise free amine. Thus, to produce a peptide dimer inhibitor, the monomer subunit may be modified to eliminate either the C- or N-terminal free amine, thereby permitting dimerization at the remaining free amine. Further, in some instances, a terminal end of one or more monomer subunits is acylated with an acylating organic compound selected from the group consisting of 2-me-Trifluorobutyl, Trifluoropentyl, Acetyl, Octonyl, Butyl, Pentyl, Hexyl, Palmityl, Trifluoromethyl butyric, cyclopentane carboxylic, cyclopropylacetic, 4-fluorobenzoic, 4-fluorophenyl acetic, 3-Phenylpropionic, tetrahedro-2H-pyran-4carboxylic, succinic acid, and glutaric acid. In some instances, monomer subunits comprise both a free carboxy terminal and a free amino terminal, whereby a user may selectively modify the subunit to achieve dimerization at a desired terminus. One having skill in the art will, therefore, appreciate that the monomer subunits of the instant invention may be selectively modified to achieve a single, specific amine for a desired dimerization.

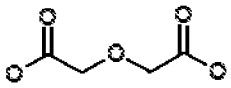
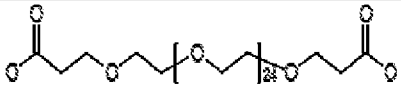
[00200] It is further understood that the C-terminal residues of the monomer subunits disclosed herein are amides, unless otherwise indicated. Further, it is understood that, in certain embodiments, dimerization at the C-terminus is facilitated by using a suitable amino

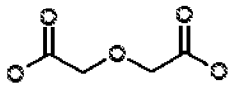
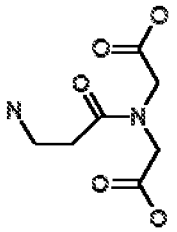
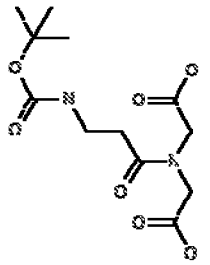
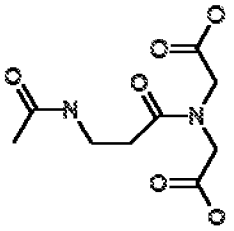
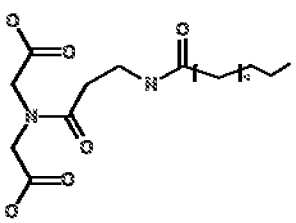
acid with a side chain having amine functionality, as is generally understood in the art. Regarding the N-terminal residues, it is generally understood that dimerization may be achieved through the free amine of the terminal residue, or may be achieved by using a suitable amino acid side chain having a free amine, as is generally understood in the art.

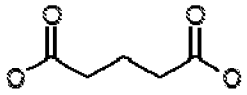
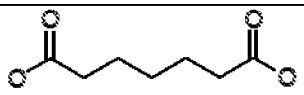
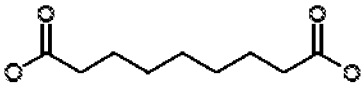
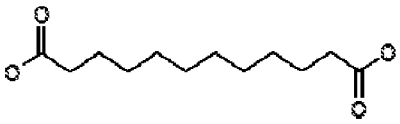
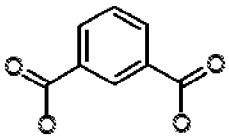
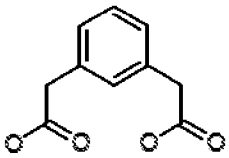
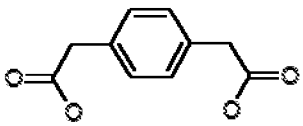
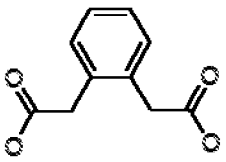
[00201] Moreover, it is understood that the side chains of one or more internal residue comprised in the hepcidin analogue peptide monomers of the present invention can be utilized for the purpose of dimerization. In such embodiments, the side chain is in some embodiments a suitable natural amino acid (e.g., Lys), or alternatively it is an unnatural amino acid comprising a side chain suitable for conjugation, e.g., to a suitable linker moiety, as defined herein.

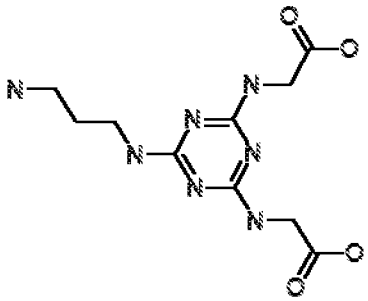
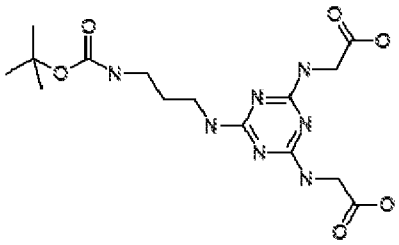
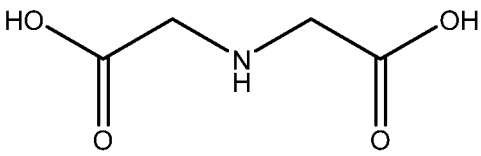
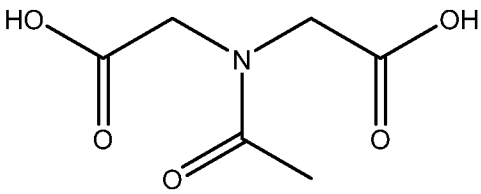
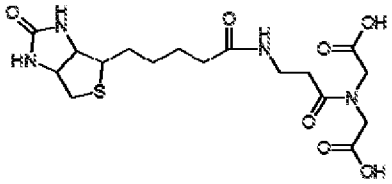
[00202] The linker moieties connecting monomer subunits may include any structure, length, and/or size that is compatible with the teachings herein. In at least one embodiment, a linker moiety is selected from the non-limiting group consisting of: cysteine, lysine, DIG, PEG4, PEG4-biotin, PEG13, PEG25, PEG1K, PEG2K, PEG3.4K, PEG4K, PEG5K, IDA, IDA-Palm, ADA, Boc-IDA, Glutaric acid, Isophthalic acid, 1,3-phenylenediacetic acid, 1,4-phenylenediacetic acid, 1,2-phenylenediacetic acid, Triazine, Boc-Triazine, IDA-biotin, PEG4-Biotin, AADA, suitable aliphatics, aromatics, heteroaromatics, and polyethylene glycol based linkers having a molecular weight from approximately 400Da to approximately 40,000Da. Non-limiting examples of suitable linker moieties are provided in Table 5.

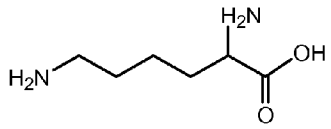
Table 5. Illustrative Linker Moieties

Abbreviation	Description	Structure
DIG	DIGlycolic acid	
PEG4	Bifunctional PEG linker with 4 PolyEthylene Glycol units	
PEG13	Bifunctional PEG linker with 13 PolyEthylene Glycol units	
PEG25	Bifunctional PEG linker with 25 PolyEthylene Glycol units	

PEG1K	Bifunctional PEG linker with PolyEthylene Glycol Mol wt of 1000Da	
PEG2K	Bifunctional PEG linker with PolyEthylene Glycol Mol wt of 2000Da	
PEG3.4K	Bifunctional PEG linker with PolyEthylene Glycol Mol wt of 3400Da	
PEG5K	Bifunctional PEG linker with PolyEthylene Glycol Mol wt of 5000Da	
DIG	Diglycolic acid	
β-Ala-IDA	β-Ala-Iminodiacetic acid	
Boc- β-Ala-IDA	Boc-β-Ala-Iminodiacetic acid	
Ac- β-Ala-IDA	Ac-β-Ala-Iminodiacetic acid	
Palm-β-Ala-IDA-	Palmityl-β-Ala-Iminodiacetic acid	

GTA	Glutaric acid	
PMA	Pemilic acid	
AZA	Azelaic acid	
DDA	Dodecanedioic acid	
IPA	Isopthalic acid	
1,3-PDA	1,3- Phenylenediacetic acid	
1,4-PDA	*1,4*- Phenylenediacetic acid	
1,2-PDA	1,2 - Phenylenediacetic acid	

Triazine	Amino propyl Triazine di-acid	
Boc-Triazine	Boc-Triazine di-acid	
IDA	Iminodiacetic acid	
AIDA	n-Acetyl imino acetic acid	
Biotin-β-ala-IDA-	N-Biotin-β -Ala-Iminodiacetic acid	

Lys	Lysine	

[00203] One having skill in the art will appreciate that the C- and N-terminal and internal linker moieties disclosed herein are non-limiting examples of suitable linker moieties, and that the present invention may include any suitable linker moiety. Thus, some embodiments of the present invention comprise a homo- or heterodimer hepcidin analogue comprised of two monomer subunits selected from the peptides shown herein, e.g., in Tables 2-4 and 11-15 or comprising or consisting of a sequence presented herein, e.g., in Tables 2-4 and 11-15, wherein the C- or N-termini of the respective monomer subunits are linked by any suitable linker moiety to provide a hepcidin analogue dimer peptide having hepcidin activity. In some embodiments the present invention comprises a homo- or heterodimer hepcidin analogue comprised of two monomer subunits described herein, e.g., selected from the peptides shown in Tables 2-4 and 11-15 or comprising or consisting of a sequence presented in Tables 2-4 or 10-15, wherein the respective monomer subunits are linked internally by any suitable linker moiety conjugated to the side chain of one or more internal amino acids to provide a hepcidin analogue dimer peptide having hepcidin activity.

[00204] In particular embodiments, a hepcidin analogue of the present invention comprises two or more polypeptide sequences of the monomer hepcidin analogues described herein.

[00205] In one embodiment, a peptide dimer hepcidin analogue of the present invention comprises two peptide monomer subunits connected via one or more linker moieties or intermolecular linkages (e.g., a cysteine disulfide bridge), wherein each peptide monomer subunit is a compound of Formula I, wherein X is hepcidin analogue of the present invention comprises two peptide monomer subunits connected via one or more linker moieties or intermolecular linkages (e.g., a cysteine disulfide bridge), or wherein each peptide monomer subunit is a compound of Formula II, e.g., wherein X is IIa and Y is IIm. In certain embodiments, a peptide dimer hepcidin analogue of the present invention comprises two peptide monomer subunits connected via one or more linker moieties or intermolecular linkages (e.g., a cysteine disulfide bridge), wherein each peptide monomer subunit is a

compound of Formula I, wherein X is Ia and Y is Im, or wherein X is Ib and Y is In, or a compound of Formula II, wherein X is IIa and Y is IIm. In certain embodiments, the peptide dimer is a homodimer, and in other embodiments, the peptide dimer is a heterodimer.

[00206] In certain embodiments, a peptide dimer inhibitor has the structure of Formula VII:

$(R^1\text{-}X\text{-}Y\text{-}R^2)_2\text{-}L$ (VII) SEQ ID NO:20

[00207] or a pharmaceutically acceptable salt or solvate thereof,

[00208] wherein each R^1 is independently selected from a bond (e.g., a covalent bond), hydrogen, a C1-C6 alkyl, a C6-C12 aryl, a C6-C12 aryl C1-C6 alkyl, a C1-C20 alkanoyl, and including PEGylated versions alone or as spacers of any of the foregoing;

[00209] each R^2 is independently absent, a bond (e.g., a covalent bond), or selected from OH or NH_2;

[00210] L is a linker moiety; and

[00211] wherein each X and Y combination is independently selected from those present in any of the Formulae described herein, such as Formulas I, II, III, IV, V, or VI. In certain embodiments, each X and Y combination is independently selected from the group consisting of:

Ia and Im;

Ib and In;

IIa and IIm;

IIIa-IIId and IIIm-IIIs;

IVa-IVd and IVm-Ivs;

Va-Vd and Vm-Vn; and

VIa and VIm.

In one embodiment of the peptide dimer of Formula VII,

each X is an independently selected peptide sequence having the formula VIIa:

X1-X2-X3-X4-X5-X6-X7-X8-X9-X10 (VIIa) SEQ ID NO: 21

wherein

X1 is Asp, Glu, Ida, Lys or absent;

X2 is Thr, Ser, Lys or absent;

X3 is His, Ala or Lys;

X4 is Phe, Dpa or Lys,

X5 is Pro, bhPro, Gly or Lys;

X6 is Cys,

X7 is Arg, Glu, Phe, Gln, Leu, Val, Lys, Ala, Ser, Dapa, Thr or absent;

X8 is Ile, Arg, Lys, Glu, Asn, Asp, Ala, Gln, Phe, Glu, Tyr, Ser, Leu, Val, D-Ile, D-Lys, D-Arg, or Dapa or absent;

X9 is Phe, Tyr, bhPhe, Lys or absent; and

X10 is Lys, Phe or absent; and

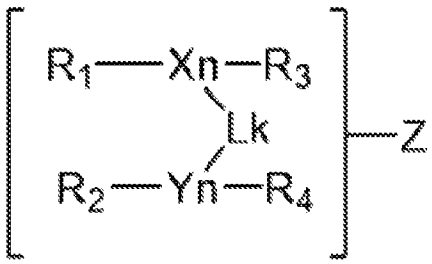
[00212] each Y is absent.

[00213] In certain alternative embodiments of Formula VII, X7 is Arg, Glu, Phe, Gln, Leu, Val, Ile, Lys, Ala, Ser, Dapa, Thr or absent.

[00214] In certain embodiments of Formula VII, the linker is Lys or Phe. In particular embodiments, the linker is Lys.

[00215] In certain embodiments of Formula VII, the two X peptides are linked via a disulfide bond.

[00216] In some embodiments, the invention provides peptides, which may be isolated and/or purified, comprising, consisting essentially of, or consisting of the following structural formula VIII:



(VIII)

[00217] or a pharmaceutically acceptable salt or solvate thereof,

wherein

[00218] R_1 and R_2 are each independently selected from a bond, a hydrogen, a C1-C6 alkyl, a C6-C12 aryl, a C6-C12 aryl C1-C6 alkyl, and a C1-C20 alkanoyl, and including PEGylated versions (e.g. PEG3 to PEG11), alone or as spacers of any of the foregoing;

[00219] R_3 and R_4 are each independently selected from a bond, -NH2 and -OH;

[00220] Xn and Yn are each independently selected peptide sequences having the formula VIIIa

X1-X2-X3-X4-X5-X6-X7-X8-X9-X10 (VIIIa) SEQ ID NO: 22

wherein

X1 is Asp, Glu, Ida, Lys or absent;

X2 is Thr, Ser, Lys or absent;

X3 is His, Ala, Lys;

X4 is Phe, Dpa or Lys;

X5 is Pro, bhPro, Gly or Lys;

X6 is Cys;

X7 is Arg, Glu, Phe, Gln, Leu, Val, Lys, Ala, Ser, Dapa, Thr or absent;

X8 is Ile, Arg, Lys, Glu, Asn, Asp, Ala, Gln, Phe, Glu, Tyr, Ser, Leu, Val, D-Ile, D-Lys, D-Arg, or Dapa or absent;

X9 is Phe, Tyr, bhPhe, Lys or absent; and

X10 is Lys, Phe or absent;

[00221] Lk is a linker or absent;

[00222] Xn and Yn are optionally linked by a disulfide bond; and

[00223] wherein Z is absent or it is a conjugate as described herein, (e.g., a conjugate to enhance drug like characteristics of the hepcidin analogue, such as extending in vivo half-life solubility, etc.), wherein if Z is present, it is optionally linked to the Xn peptide (e.g., at its N-terminus, C-terminus, or internally via a side chain, e.g., a lysine side chain), the Yn peptide

(e.g., at its N-terminus, C-terminus, or internally via a side chain, e.g., a lysine side chain), or to an Lk linker.

[00224] In certain embodiments, Z is a palmyltyl moiety, a PEG moiety, or a lipidic moiety.

[00225] In certain embodiments, Lk links the two monomer subunits via an amino acid residue in Xn and/or an amino acid residue in Yn.

[00226] In certain alternative embodiments, R1, R2, R3, and R4 are selected from a bond, -NH2 and –OH, hydrogen, a C1-C6 alkyl, a C6-C12 aryl, a C6-C12 aryl C1-C6 alkyl, and a C1-C20 alkanoyl, and including PEGylated versions (e.g. PEG3 to PEG11), alone or as spacers of any of the foregoing.

[00227] In certain embodiments, Lk links the two monomer subunits via R_3 and/or R_4.

[00228] In certain embodiments, Lk links the monomer subunits via R_1 and/or R_2.

[00229] In certain embodiments, Lk links the monomer subunits via any one of R_1, Xn or R_3 and any one of R_2, Yn and R_4.

[00230] In certain embodiments of Formula VIII, the linker is Lys or Phe. In particular embodiments, the linker is Lys.

[00231] In certain embodiments of Formula VIII, the two X peptides are linked via a disulfide bond.

[00232] In some embodiments, the present invention provides a hepcidin analogue monomer, or a homodimer or heterodimer thereof, comprising a peptide that comprises, consists of, or consists essentially of a sequence DTX_1FPC, wherein X_1 is any amino acid. In one embodiment, the present invention provides a peptide that comprises, consists of, or consists essentially of a sequence $DTX_1FPCX_2X_3F$, wherein X_1 is any amino acid, X_2 is any amino acid, and X_3 is any amino acid or it is absent. In one such embodiment, X_2 is any amino acid except for Cys. In one embodiment, X_1, X_2, and/or X_3 is an unnatural amino acid. In some embodiments, a dimer comprising such a hepcidin analogue monomer comprises a linker (e.g., a lysine linker). In some embodiments, such a dimer comprises a first hepcidin analogue monomer and a second monomer (which monomers are optionally identical in sequence), and the dimer further comprises at least one intermolecular disulfide bridge linking a Cys in the first monomer (e.g., the Cys shown in either one of the above formulae) to a Cys in the second monomer.

[00233] In some embodiments, the present invention provides a hepcidin analogue monomer, or a homodimer or heterodimer thereof, comprising a peptide that comprises, consists of, or consists essentially of a sequence $X_1X_2X_3FX_4CY_1X_5F$, wherein any of X_1, X_2, and X_3 are absent or any amino acid, X_4 and X_5 are any amino acid, and Y_1 is any amino acid except for D-Cys, D-Ser, D-Ala, Cys(S-tBut), homoC, Pen, (D)Pen, Dap(AcBr), Inp, or D-His. In one such embodiment, Y_1 is any lipidic amino acid. In particular embodiments, Y_1 is selected from Val, Ile, and Leu. In one embodiment, Y_1 is Ile. In one embodiment, X_5 is Lys. In one embodiment, any of X_1, X_2, X_3, X_4, X_5, and/or Y_1 is an unnatural amino acid. In some embodiments, a dimer comprising such a hepcidin analogue monomer comprises a linker (e.g., a lysine linker). In some embodiments, such a dimer comprises a first hepcidin analogue monomer and a second monomer (which monomers are optionally identical in sequence), and the dimer further comprises at least one intermolecular disulfide bridge linking a Cys in the first monomer (e.g., the Cys shown in either one of the above formulae) to a Cys in the second monomer.

[00234] In some embodiments, the present invention provides a hepcidin analogue monomer, or a homodimer or heterodimer thereof, comprising a peptide that comprises, consists of, or consists essentially of a sequence $DTX_1FX_2CY_1X_3F$, wherein X_1 is any amino acid, Y_1 is any amino acid except for D-Cys, D-Ser, D-Ala, Cys(S-tBut), homoC, Pen, (D)Pen, Dap(AcBr), Inp, or D-His, and X_2 is any amino acid or it is absent. In one such embodiment, Y_1 is any amino acid except for Cys. In one such embodiment, Y_1 is any lipidic amino acid. In particular embodiments, Y_1 is selected from Val, Ile, and Leu. In one embodiment, Y_1 is Ile. In one embodiment, X_1, X_2 (if not absent), and/or Y_1 is an unnatural amino acid. In some embodiments, a dimer comprising such a hepcidin analogue monomer comprises a linker (e.g., a lysine linker). In some embodiments, such a dimer comprises a first hepcidin analogue monomer and a second monomer (which monomers are optionally identical in sequence), and the dimer further comprises at least one intermolecular disulfide bridge linking a Cys in the first monomer (e.g., the Cys shown in either one of the above formulae) to a Cys in the second monomer.

[00235] In some embodiments, the present invention provides a hepcidin analogue homodimer or heterodimer comprising a hepcidin analogue monomer peptide that comprises, consists of, or consists essentially of a sequence DTX_1FPX_2C, wherein X_1 is any amino acid. In one embodiment, the present invention provides a hepcidin analogue homodimer or heterodimer comprising a hepcidin analogue monomer peptide that comprises, consists of, or consists essentially of a sequence $DTX_1FPX_2CX_3F$, wherein X_1 is any amino acid, X_2 is any amino

acid, and X_3 is any amino acid or it is absent. In one such embodiment, X_2 is any amino acid except for Cys. In one embodiment, X_1, X_2, and/or X_3 is an unnatural amino acid. In some embodiments, a dimer comprising such a hepcidin analogue monomer comprises a linker (e.g., a lysine linker). In some embodiments, such a dimer comprises a first hepcidin analogue monomer and a second monomer (which monomers are optionally identical in sequence), and the dimer further comprises at least one intermolecular disulfide bridge linking a Cys in the first monomer (e.g., the Cys shown in either one of the above formulae) to a Cys in the second monomer.

[00236] In some embodiments, the present invention provides a hepcidin analogue monomer, or a homodimer or heterodimer thereof, comprising a peptide that comprises, consists of, or consists essentially of a sequence $X_1X_1X_1FX_2X_2CY_1F$ wherein X_1 is absent or it is any amino acid, X_2 is any amino acid, and Y_1 is any amino acid. In one such embodiment, Y is any natural amino acid. In particular embodiments, Y_1 is selected from Arg, Val, Ile, and Leu. In one embodiment, Y_1 is Ile. In one embodiment, X_1, X_2, and/or Y_1 is an unnatural amino acid. In some embodiments, a dimer comprising such a hepcidin analogue monomer comprises a linker (e.g., a lysine linker). In some embodiments, such a dimer comprises a first hepcidin analogue monomer and a second monomer (which monomers are optionally identical in sequence), and the dimer further comprises at least one intermolecular disulfide bridge linking a Cys in the first monomer (e.g., the Cys shown in either one of the above formulae) to a Cys in the second monomer.

[00237] In some embodiments, the present invention provides a hepcidin analogue monomer, or a homodimer or heterodimer thereof, comprising a peptide that comprises, consists of, or consists essentially of a sequence $DTX_1FX_2X_3CY_1F$, wherein X_1 is any amino acid, X_2 is any amino acid or it is absent, X_3 is any amino acid, and Y_1 is any amino acid. In one such embodiment, Y_1 is any lipidic amino acid. In particular embodiments, Y_1 is selected from Val, Ile, and Leu. In one embodiment, Y_1 is Ile. In one embodiment, X_1, X_2 (if not absent), and/or Y_1 is an unnatural amino acid. In some embodiments, a dimer comprising such a hepcidin analogue monomer comprises a linker (e.g., a lysine linker). In some embodiments, such a dimer comprises a first hepcidin analogue monomer and a second monomer (which monomers are optionally identical in sequence), and the dimer further comprises at least one intermolecular disulfide bridge linking a Cys in the first monomer (e.g., the Cys shown in either one of the above formulae) to a Cys in the second monomer.

[00238] In some embodiments, the present invention provides a homodimer or heterodimer of one or more hepcidin analogue monomer that comprises, consists of, or consists essentially of a sequence $X_1X_1X_1FX_2X_2CX_3F$ wherein X_1 is absent or it is any amino acid, X_2 is any amino acid, and X_3 is any amino acid. In one such embodiment, X_3 is any natural amino acid. In particular embodiments, X_3 is selected from Arg, Val, Ile, and Leu. In one embodiment, X_3 is Ile. In one embodiment, X_1, X_2, and/or X_3 is an unnatural amino acid. In some embodiments, a dimer comprising such a hepcidin analogue monomer comprises a linker (e.g., a lysine linker). In some embodiments, such a dimer comprises a first hepcidin analogue monomer and a second monomer (which monomers are optionally identical in sequence), and the dimer further comprises at least one intermolecular disulfide bridge linking a Cys in the first monomer (e.g., the Cys shown in either one of the above formulae) to a Cys in the second monomer.

In some embodiments, the present invention provides a homodimer or heterodimer of one or more hepcidin analogue monomer that comprises, consists of, or consists essentially of a sequence $DTX_1FX_2X_3CX_4F$, wherein X_1 is any amino acid, X_2 is any amino acid or it is absent, X_3 is any amino acid, and X_4 is any amino acid. In one such embodiment, X_4 is any amino acid except for Cys. In one such embodiment, X_4 is any lipidic amino acid. In particular embodiments, X_4 is selected from Val, Ile, and Leu. In one embodiment, X_4 is Ile. In one embodiment, X_1, X_2 (if not absent), and/or X_4 is an unnatural amino acid. In one embodiment, Cys is linked through a disulphide forming a dimer. In some embodiments, a dimer comprising such a hepcidin analogue monomer comprises a linker (e.g., a lysine linker). In some embodiments, such a dimer comprises a first hepcidin analogue monomer and a second monomer (which monomers are optionally identical in sequence), and the dimer further comprises at least one intermolecular disulfide bridge linking a Cys in the first monomer (e.g., the Cys shown in either one of the above formulae) to a Cys in the second monomer.

[00239] In certain embodiments, a peptide dimer (e.g., a hepcidin analogue or inhibitor) of the present invention comprises two peptide monomer subunits connected via one or more linker moieties or intermolecular linkages (e.g., a cysteine disulfide bridge), wherein each peptide monomer subunit comprises a sequence shown in any of Tables 2-4 or Tables 11-15. In certain embodiments, the peptide dimer is a homodimer, and in other embodiments, the peptide dimer is a heterodimer. In some embodiments, a linker moiety or intermolecular linkage that dimerizes two monomers is bound to any of the N-terminus, the C-terminus, or an internal amino acid (e.g., a lysine sidechain) of one or more of the monomer peptides.

[00240] In certain embodiments, a peptide dimer (e.g., a hepcidin analogue or inhibitor) of the present invention comprises two peptide monomer subunits connected via one or more linker moieties or intermolecular linkages (e.g., a cysteine disulfide bridge), wherein each peptide monomer subunit is: a compound of Formula I, wherein X is Ia and Y is Im, or wherein X is Ib and Y is In; a compound of Formula II, wherein X is IIa and Y is IIm; or a compound having a sequence shown in any of Tables 2-4, 10, 12, 14, and 15. In certain embodiments, the peptide dimer is a homodimer, and in other embodiments, the peptide dimer is a heterodimer. In particular embodiments, the peptide dimer is a peptide dimer as shown in any one of Tables 6-10, and 15.

[00241] In certain embodiments, at least two cysteine residues of the hepcidin analogue peptide dimers are linked by a disulfide bridge.

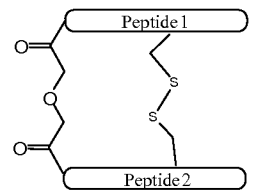
[00242] In particular embodiments of the hepcidin analogue peptide dimer of the present invention, the linker moiety (L) is any of the linkers shown in Table 5. In certain embodiments, the linker is a lysine linker, a diethylene glycol linker, an iminodiacetic acid (IDA) linker, a β-Ala-iminodiaceticacid (β-Ala-IDA) linker, or a PEG linker.

[00243] In certain embodiments of any of the hepcidin analogue peptide dimers, the N-terminus of each peptide monomer subunit is connected by a linker moiety.

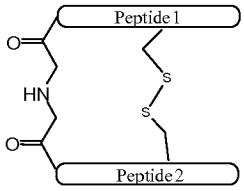
[00244] In certain embodiments of any of the hepcidin analogue peptide dimers, the C-terminus of each peptide monomer subunit is connected by a linker moiety.

[00245] In certain embodiments, the side chains of one or more internal amino acid residues (e.g., Lys residues) comprised in each peptide monomer subunit of a hepcidin analogue peptide dimer are connected by a linker moiety.

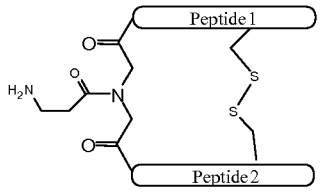
[00246] In certain embodiments of any of the hepcidin analogue peptide dimers, the C-terminus, the N terminus, or an internal amino acid (e.g., a lysine sidechain) of each peptide monomer subunit is connected by a linker moiety and at least two cysteine residues of the hepcidin analogue peptide dimers are linked by a disulfide bridge. In some embodiments, a peptide dimer has a general structure shown below. Non-limiting schematic examples of such hepcidin analogues are shown in the following illustration:



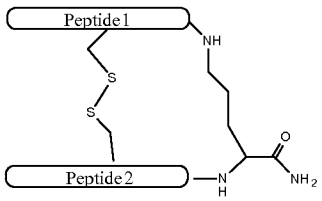
DIG (Diethyleneglycol)



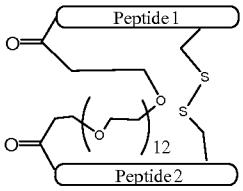
IDA (Iminodiaceticacidl)



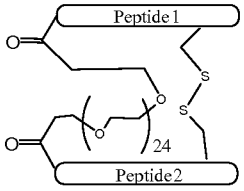
β-Ala-IDA



Lys



PEG13



PEG25

[00247] Illustrative examples of peptide dimer hepcidin analogues of the present invention are provided in Tables 6-8 with in vitro activity data in the ferroportin internalization/degradation assay described in the accompanying Examples.

Table 6. Illustrative Peptide Dimer Hepcidin Analogues

SEQ ID NO	**Sequence**	**Potency EC_{50} (nM)**
527	$([pGlu]\text{-}THFPCRKF\text{-}NH_2)_2$	31% at 10 uM
528	$(Hy\text{-}DTHFPCLF\text{-}NH_2)_2$	297

Table 7. Illustrative Peptide Dimer Hepcidin Analogues

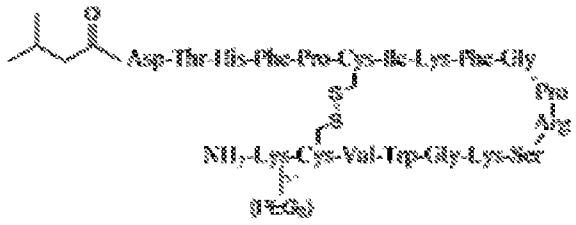
SEQ ID NO	**Sequence**	**Potency EC_{50} (nM)**
529	$(isovaleric\ acid\ \text{-}DTHFPICIFK(Palm)\text{-}NH_2)_2$	580
530	$(isovaleric\ acid\text{-}DTHFPCIK(Palm)\text{-}F\text{-}NH_2)_2$	294
531	$(isovaleric\ acid\text{-}DTHFPCIKFAA\text{-}NH_2)_2$	47

Table 8. Illustrative Peptide Dimer Hepcidin Analogues

SEQ ID NO	**Sequence**	**Potency EC_{50} (nM)**
532	$([(D)Phe]\text{-}[(D)Ile]\text{-}[(D)Cys]\text{-}[(D)Ile]\text{-}[(D)Pro]\text{-}[(D)Phe]\text{-}[(D)His]\text{-}[(D)Thr]\text{-}[(D)Asp])_2$	Not active at 10 uM

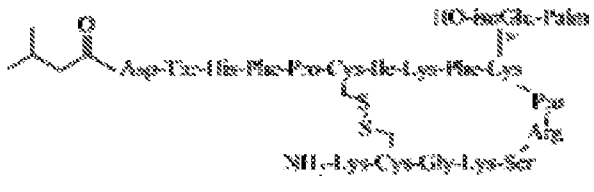
533	$(Hy\text{-}DTHFPICIF\text{-}NH_2)_2$	146
534	$(Ida\text{-}TH\text{-}Dpa\text{-}bhPro\text{-}RCR\text{-}bhPhe\text{-}PEG3\text{-}Palm)_2$	31

[00248] In one embodiment, a peptide monomers of the present invention has the following structure:



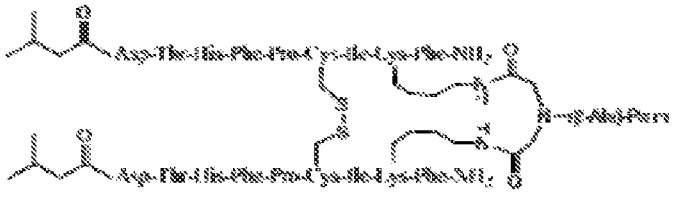
SEQ ID NO:535

[00249] In one embodiment, a peptide monomers of the present invention has the following structure:



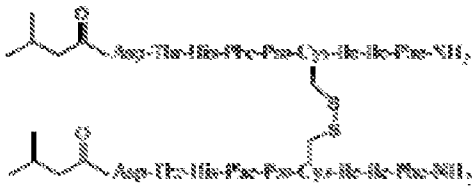
SEQ ID NO:536

[00250] In one embodiment, a peptide dimer of the present invention has the following structure:



SEQ ID NO:537

[00251] In one embodiment, the peptide dimer of the present invention has the following structure:



SEQ ID NO:538

[00252] In certain embodiments, a peptide dimer inhibitor has the structure of Formula X:

$(R^1\text{-}X\text{-}R^2)_2\text{-}L$ (X) SEQ ID NO:23

[00253] or a pharmaceutically acceptable salt or solvate thereof,

[00254] wherein each R^1 is independently absent, a bond (e.g., a covalent bond), or selected from hydrogen, a C1-C6 alkyl, a C6-C12 aryl, a C6-C12 aryl C1-C6 alkyl, a C1-C20 alkanoyl, and including PEGylated versions alone or as spacers of any of the foregoing;

[00255] each R^2 is independently absent, a bond (e.g., a covalent bond), or selected from OH or NH_2;

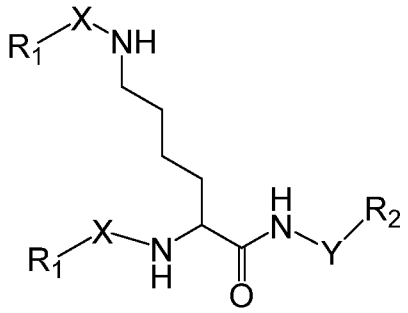
[00256] L is a linker moiety; and

[00257] each X is an independently selected peptide monomer subunit comprising or consisting of 7 to 35 amino acid residues, 8 to 35 amino acid residues, 9 to 35 amino acid residues, 10 to 35 amino acid residues, 7 to 25 amino acid residues, 8 to 25 amino acid residues, 9 to 25 amino acid residues, 10 to 25 amino acid residues, 7 to 18 amino acid residues, 8 to 18 amino acid residues, 9 to 18 amino acid residues, or 10 to 18 amino acid residues amino acids in length, each comprising or consisting of the sequence of Formula I or Formula II, or set forth in Tables 2-4, Tables 12-14, or a monomer sequence set forth in Table 15..

Lysine Dimer Hepcidin Analogues

[00258] In certain embodiments, a peptide dimer hepcidin analogue of the present invention comprises two peptide monomer subunits linked via a lysine linker.

[00259] In some embodiments, a peptide dimer hepcidin analogue of the present invention has a structure of Formula IX:



Formula IX
(SEQ ID NO:24)

[00260] or a pharmaceutically acceptable salt of solvate thereof,

[00261] wherein each X is an independently selected peptide sequence having the formula IXa:

X1-X2-X3-X4-X5-X6-X7-X8-X9-X10 (IXa) SEQ ID NO:25

[00262] wherein

X1 is Asp, Glu, Ida or absent;

X2 is Thr, Ser, Pro, Ala or absent;

X3 is His, Ala, Glu or Ala;

X4 is Phe or Dpa;

X5 is Pro, bhPro, Sarc or Gly;

X6 is Cys, (D)-Cys, Arg, Glu, Phe, Gln, Leu, Val, Lys, Ala, Ser, Dapa or absent;

X7 is Cys, (D)-Cys, Arg, Glu, Phe, Gln, Leu, Val, Lys, Ala, Ser, Dapa or absent;

X8 is Ile, Arg, Lys, Ala, Gln, Phe, Glu, Asp, Tyr, Ser, Leu, Val, D-Ile, D-Lys, D-Arg, Dapa or absent;

X9 is Phe, Ala, Ile, Thr, Tyr, Lys, Arg, bhPhe, D-Phe or absent; and

X10 is Lys, Phe or absent;

[00263] wherein each R^1 is independently absent, a bond (e.g., a covalent bond), or selected from hydrogen, a C1-C6 alkyl, a C6-C12 aryl, a C6-C12 aryl C1-C6 alkyl, a C1-C20 alkanoyl, and including PEGylated versions alone or as spacers of any of the foregoing;

[00264] each R^2 is independently absent, a bond (e.g., a covalent bond), or selected from OH or NH_2;

[00265] Y is absent or present, and provided that if Y is present, Y is a peptide having the formula IXm:

Y1-Y2-Y3 (IXm) SEQ ID NO:26

[00266] wherein

Y1 is Ile, Arg, Lys, Ala, Gln, Phe, Glu, Asp, Tyr, Ser, Leu, Val, D-Ile, D-Lys, D-Arg, Dapa or absent;

Y2 is Phe, Ala, Ile, Thr, Tyr, Lys, Arg, bhPhe, D-Phe or absent; and

Y3 is Lys, Phe or absent.

[00267] In certain embodiments, one or more of Y1, Y2 and Y3 is present.

[00268] In certain embodiments, Y is conjugated to one or more chemical substituents, including but not limited to any of those described herein.

[00269] In some embodiments, one or both X is cyclized via a disulfide bond.

[00270] In some embodiments, the two X peptides are linked via a disulfide bond.

[00271] In certain embodiments, a lysine linked peptide dimer hepcidin analogue of the present has a structure set forth in Table 9.

Table 9. Illustrative Lysine-linked Dimer Hepcidin Analogues

SEQ ID NO	Sequence	EC_{50} (nM) (n>3)
539	(isobutyric acid-DTHFPCIKF)$_2$[Lys]K(iso-Glu-Palm)-NH_2	24
540	(isovaleric acid-DTHFPCIKF)$_2$[Lys]K(iso-Glu-Palm)-NH_2	14
541	(cyclohexanoic acid-DTHFPCIKF)$_2$[Lys]K(iso-Glu-Palm)-NH_2	17

542	(Isovaleric acid-DTHFPCIRF)$_2$[Lys]-K(iso-Glu-Palm)-NH_2	4
543	(Isovaleric acid-DTHFPCIKF)$_2$[Lys]-NH_2	30
544	(Isovaleric acid-DTHFPCIKF)$_2$[Lys]-Lys(Palm)-NH_2	17

[00272] In certain embodiments, each of the peptide monomer subunits of a lysine-linked peptide dimer hepcidin analogue of the present invention comprises or consists of a structure of Formula III:

R^1-X-Y-R^2 (III) SEQ ID NO:7

[00273] or a pharmaceutically acceptable salt or solvate thereof, wherein

[00274] R^1 is hydrogen, a C1-C6 alkyl, a C6-C12 aryl, a C6-C12 aryl C1-C6 alkyl, or a C1-C20 alkanoyl, and including PEGylated versions thereof, alone or as spacers of any of the foregoing;

[00275] R^2 is -NH_2 or -OH;

[00276] X is a peptide sequence having the formula (IIIa)

X1-X2-X3-X4-X5-X6-X7-X8-X9-X10 (IIIa) SEQ ID NO:8

[00277] wherein

X1 is Asp, Glu, Ala, Gly, Thr, Ida, pGlu, bhAsp, D-Asp, Tyr, Leu or absent;

X2 is Thr, Ala, Aib, D-Thr, Arg or absent;

X3 is His, Lys, Ala, or D-His;

X4 is Phe, Ala, Dpa or bhPhe;

X5 is Pro, Glu, Ser, Gly, Arg, Lys, Val, Ala, D-Pro, bhPro, Sarc, Abu or absent;

X6 is Ile, Cys, Arg, Leu, Lys, His, Glu, D-Ile, D-Arg, D-Cys, Val, Ser or Ala;

X7 is Cys, Ile, Ala, Leu, Val, Ser, Phe, Dapa, D-Ile or D-Cys;

X8 is Ile, Lys, Arg, Ala, Gln, Phe, Glu, Asp, Tyr, Ser, Leu, Val, D-Ile, D-Lys, D-Arg, or Dapa;

X9 is Phe, Ala, Ile, Tyr, Lys, Arg, bhPhe or D-Phe; and

X10 is Lys, Phe or absent;

Y is absent or present, and when present, Y is a peptide having the formula (IIIm)

Y1-Y2-Y3-Y4-Y5-Y6-Y7-Y8-Y9-Y10-Y11-Y12-Y13-Y14-Y15 (IIIm) SEQ ID NO:9

[00278] wherein

Y1 is Gly, Cys, Ala, Phe, Pro, Glu, Lys, D-Pro, Val, Ser or absent;

Y2 is Pro, Ala, Cys, Gly or absent;

Y3 is Arg, Lys, Pro, Gly, His, Ala, Trp or absent;

Y4 is Ser, Arg, Gly, Trp, Ala, His, Tyr or absent;

Y5 is Lys, Met, Arg, Ala or absent;

Y6 is Gly, Ser, Lys, Ile, Arg, Ala, Pro, Val or absent;

Y7 is Trp, Lys, Gly, Ala, Ile, Val or absent;

Y8 is Val, Thr, Gly, Cys, Met, Tyr, Ala, Glu, Lys, Asp, Arg or absent;

Y9 is Cys, Tyr or absent;

Y10 is Met, Lys, Arg, Tyr or absent;

Y11 is Arg, Met, Cys, Lys or absent;

Y12 is Arg, Lys, Ala or absent;

Y13 is Arg, Cys, Lys, Val or absent;

Y14 is Arg, Lys, Pro, Cys, Thr or absent; and

Y15 is Thr, Arg or absent;

[00279] wherein if Y is absent from the peptide of formula (III), X7 is Ile; and

[00280] wherein said compound of formula (III) is optionally PEGylated on R^1, X, or Y.

[00281] In certain embodiments, R^1 is selected from methyl, acetyl, formyl, benzoyl, trifluoroacetyl, isovaleryl, isobutyryl, octanyl, and the conjugated amides of lauric acid, hexadecanoic acid, and γ-Glu-hexadecanoic acid.

[00282] In certain embodiments, X does not comprise and/or does not consist of an amino acid sequence set forth in US Patent No. 8,435,941.

[00283] In some embodiments, the compound or peptide of formula (III) comprises two or more cysteine residues, wherein at least two of said cysteine residues are linked via a disulfide bond.

[00284] In some embodiments, X is a peptide sequence according to formula (IIIa), described herein, wherein

X1 is Asp, Ala, Ida, pGlu, bhAsp, Leu, D-Asp or absent;

X2 is Thr, Ala, or D-Thr;

X3 is His, Lys, or D-His;

X4 is Phe, Ala, or Dpa;

X5 is Pro, Gly, Arg, Lys, Ala, D-Pro or bhPro;

X6 is Ile, Cys, Arg, Lys, D-Ile or D-Cys;

X7 is Cys, Ile, Leu, Val, Phe, D-Ile or D-Cys;

X8 is Ile, Arg, Phe, Gln, Lys, Glu, Val, Leu or D-Ile;

X9 is Phe or bhPhe; and

X10 is Lys, Phe or absent.

In some embodiments, X is a peptide sequence having the formula (IIIb)

X1-Thr-His-X4-X5-X6-X7-X8-Phe-X10 (IIIb) SEQ ID NO:27

[00285] wherein

X1 is Asp, Ida, pGlu, bhAsp or absent;

X4 is Phe or Dpa;

X5 is Pro or bhPro;

X6 is Ile, Cys or Arg;

X7 is Cys, Ile, Leu or Val;

X8 is Ile, Lys, Glu, Phe, Gln or Arg; and

X10 is Lys, Phe or absent;

[00286] In some embodiments, X is a peptide sequence according to formula (IIIb), as described herein, wherein

X1 is Asp, Glu, Ida, pGlu, bhAsp or absent;

X4 is Phe or Dpa;

X5 is Pro or bhPro;

X6 is Ile, Cys or Arg;

X7 is Cys, Ile, Leu or Val;

X8 is Ile, Lys, Glu, Phe, Gln or Arg; and

X10 is Lys or absent.

[00287] In some embodiments, X is a peptide sequence having the formula (IIIc)

X1-Thr-His-X4-X5-Cys-Ile-X8-Phe-X10 (IIIc) SEQ ID NO:571

[00288] wherein

X1 is Asp, Glu, Ida, pGlu, bhAsp or absent;

X4 is: Phe or Dpa;

X5 is Pro or bhPro;

X8 is Ile Lys, Glu, Phe, Gln or Arg; and

X10 is Lys or absent.

[00289] In some embodiments, X is a peptide sequence having the formula (IIId)

X1-Thr-His-Phe-X5-Cys-Ile-X8-Phe-X10 (IIId) SEQ ID NO:572

[00290] wherein

X1 is Asp, Glu, or Ida;

X4 is: Phe;

X5 is Pro or bhPro;

X8 is Ile, Lys or Phe; and

X10 is absent.

[00291] In some embodiments, Y is a peptide sequence having the formula IIIn

Y1-Y2-Y3-Y4-Y5-Y6-Y7-Y8-Cys-Y10 (IIIn) SEQ ID NO:573

[00292] wherein

Y1 is Gly, Ala, Lys, Pro or D-Pro;

Y2 is Pro, Ala or Gly;

Y3 is Arg, Ala, Lys or Trp;

Y4 is Ser, Gly or Ala;

Y5 is Lys, Met, Arg or Ala;

Y6 is Gly, Arg or Ala;

Y7 is Trp, Ala or absent;

Y8 is Val, Thr, Lys, Ala, Glu or absent; and

Y10 is Met, Lys or absent.

[00293] In some embodiments, Y is a peptide sequence according to formula (IIIn), as described herein,

[00294] wherein

Y1 is Gly, Ala, Lys, Pro or D-Pro;

Y2 is Pro, Ala or Gly;

Y3 is Arg, Ala, Lys or Trp;

Y4 is Ser, Gly or Ala;

Y5 is Lys, Met, Arg or Ala;

Y6 is Gly, Arg or Ala;

Y7 is Trp or Ala;

Y8 is Val, Thr, Ala, or Glu; and

Y10 is Met, Lys or absent.

[00295] In some embodiments, Y is a peptide sequence having the formula (IIIo)

Y1-Y2-Y3-Ser-Lys-Gly-Trp-Y8-Cys-Y10 (IIIo) SEQ ID NO:574

[00296] wherein

Y1 is Gly, Pro or D-Pro;

Y2 is Pro or Gly;

Y3 is Arg or Lys;

Y8 is Val or Thr; and

Y10 is Met, Lys or absent.

[00297] In some embodiments, Y is a peptide sequence having the formula (IIIp)

Y1-Cys-Y3-Y4-Arg-Y6-Y7-Y8-Cys-Y10-Y11-Y12-Y13-Y14-Y15 (IIIp) SEQ ID NO:575

[00298] wherein

Y1 is Val, Ala or absent;

Y3 is Gly, Pro or absent;

Y4 is His, Trp or Tyr;

Y6 is Ser, Gly or Pro;

Y7 is Ile, Gly or Lys;

Y8 is Gly, Met or absent;

Y10 is Tyr or Cys;

Y11 is Arg, Lys, Met or Ala;

Y12is Arg or Ala;

Y13 is Cys or Val or absent;

Y14 is Cys, Lys, Pro, Arg, Thr or absent; and

Y15 is Arg, Thr or absent.

[00299] In some embodiments, Y is a peptide sequence having the formula (IIIq)

Val-Cys-Y3-His-Arg-Y6-Y7-Y8-Cys-Tyr-Arg-Y12-Y13-Y14-Y15 (IIIq) SEQ ID NO

[00300] wherein

Y3 is Gly or absent;

Y6 is Ser or Pro;

Y7 is Ile or Lys;

Y8 is Gly or absent;

Y12 is Arg or Ala;

Y13 is Cys, Val or absent;

Y14 is Cys, Arg, Thr or absent; and

Y15 is Arg or absent.

[00301] In some embodiments, Y is a peptide sequence having the formula (IIIr)

Y1-Pro-Y3-Ser-Y5-Y6-Y7-Y8-Cys-Y10 (IIIr) SEQ ID NO:576

[00302] wherein

Y1 is Gly, Glu, Val, or Lys;

Y3 is Arg or Lys;

Y5 is Arg or Lys;

Y6 is Gly, Ser, Lys, Ile or Arg;

Y7 is Trp or absent;

Y8 is Val, Thr, Asp, Glu or absent; and

Y10 is Lys or absent.

[00303] In some embodiments, Y is a peptide sequence having the formula (IIIs)

Y1-Pro-Y3-Ser-Y5-Y6-Y7-Y8-Cys-Y10 (IIIs) SEQ ID NO:577

[00304] wherein

Y1 is Glu or Lys;

Y3 is Arg or Lys;

Y5 is Arg or Lys;

Y6 is Gly, Ser, Lys, Ile or Arg;

Y7 is Trp or absent;

Y8 is Val or absent; and

Y10 is Lys or absent.

[00305] In some embodiments, the peptide of formula (III) comprises at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve, at least thirteen, at least fourteen or at least fifteen Y residues in Y.

[00306] In some embodiments, Y1 to Y3 are present and Y4 to Y15 are absent.

[00307] In some embodiments, Y1 to Y11 are present and Y12 to Y15 are absent.

[00308] In some embodiments, Y1 to Y10 are present and Y11 to Y15 are absent.

[00309] In some embodiments, Y8 and Y15 are absent.

[00310] In some embodiments, Y3 and Y15 are absent.

[00311] In some embodiments, Y3, Y14 and Y15 are absent.

[00312] In some embodiment Y5 is absent.

[00313] In some embodiments Y1, Y5, Y7, Y12, Y13, Y14 and Y15 are absent.

[00314] In some embodiments Y1, Y5, and Y7 are absent. In some embodiments, Y8 is absent. In some embodiments, Y3 is absent. In some embodiments Y1, Y5, Y7, and Y11-Y15 are absent. In some embodiments, Y8 and Y11-Y15 are absent. In some embodiments, Y3 and Y11-Y15 are absent.

[00315] In certain embodiments, a peptide dimer hepcidin analogue of the present invention comprises two peptide monomer subunits linked via a lysine linker, comprising, consisting essentially of, or consisting of, the following structural formula:

R^1-X-Y-R^2 (IV) SEQ ID NO:10

[00316] or a pharmaceutically acceptable salt or solvate thereof, wherein

wherein R^1 is hydrogen, a C1-C6 alkyl, a C6-C12 aryl, a C6-C12 aryl C1-C6 alkyl, or a C1-C20 alkanoyl, and including PEGylated versions alone or as spacers of any of the foregoing;

[00317] R^2 is $-NH_2$ or -OH;

[00318] X is a peptide sequence having the formula (IVa)

X1-X2-X3-X4-X5-X6-X7-X8-X9-X10 (IVa) SEQ ID NO: 11

[00319] wherein

X1 is Asp, Glu, Ala, Gly, Thr, Ida, pGlu, bhAsp, D-Asp, Tyr, Leu or absent;

X2 is Thr, Ala, Aib, D-Thr, Arg or absent;

X3 is His, Lys, Ala, or D-His;

X4 is Phe, Ala, Dpa, bhPhe or D-Phe;

X5 is Pro, Glu, Ser, Gly, Arg, Lys, Val, Ala, D-Pro, bhPro, Sarc, Abu or absent;

X6 is Ile, Cys, Arg, Leu, Lys, His, Glu, D-Ile, D-Arg , D-Cys, Val, Ser or Ala;

X7 is Cys, Ile, Ala, Leu, Val, Ser, Phe, Dapa, D-Ile or D-Cys;

X8 is Ile, Lys, Arg, Ala, Gln, Phe, Glu, Asp, Tyr, Ser, Leu, Val, D-Ile, D-Lys, D-Arg or Dapa;

X9 is Phe, Ala, Ile, Tyr, Lys, Arg, bhPhe or D-Phe; and

X10 is Lys, Phe or absent;

[00320] and provided that if Y' is absent, X7 is Ile; and

[00321] Y is absent or is a peptide having the formula (IVm):

Y1-Y2-Y3-Y4-Y5-Y6-Y7-Y8-Y9-Y10-Y11-Y12-Y13-Y14-Y15 (IVm) SEQ ID NO:12

[00322] wherein

Y1 is Gly, Cys, Ala, Phe, Pro, Glu, Lys, D-Pro, Val, Ser or absent;

Y2 is Pro, Ala, Cys, Gly or absent;

Y3 is Arg, Lys, Pro, Gly, His, Ala, Trp or absent;

Y4 is Ser, Arg, Gly, Trp, Ala, His, Tyr or absent;

Y5 is Lys, Met, Arg, Ala or absent;

Y6 is Gly, Ser, Lys, Ile, Arg, Ala, Pro, Val or absent;

Y7 is Trp, Lys, Gly, Ala, Ile, Val or absent;

Y8 is Val, Thr, Gly, Cys, Met, Tyr, Ala, Glu, Lys, Asp, Arg or absent;

Y9 is Cys, Tyr or absent;

Y10 is Met, Lys, Arg, Tyr or absent;

Y11 is Arg, Met, Cys, Lys or absent;

Y12 is Arg, Lys, Ala or absent;

Y13 is Arg, Cys, Lys, Val or absent;

Y14 is Arg, Lys, Pro, Cys, Thr or absent; and

Y15 is Thr, Arg or absent;

[00323] wherein said compound of formula (IV) is optionally PEGylated on R^1, X, or Y; and

[00324] wherein when said compound of formula (IV) comprises two or more cysteine residues, at least two of said cysteine residues being linked via a disulfide bond.

[00325] In certain embodiments, R^1 is selected from methyl, acetyl, formyl, benzoyl, trifluoroacetyl, isovaleryl, isobutyryl, octanyl, and the conjugated amides of lauric acid, hexadecanoic acid, and γ-Glu-hexadecanoic acid.

[00326] In some embodiments, $R^{1'}$ is hydrogen, isovaleric acid, isobutyric acid or acetyl.

[00327] In certain embodiments, X either or both does not comprise or does not consist of an amino acid sequence set forth in US Patent No. 8,435,941.

[00328] In some embodiments of the peptide compound of formula (IV), X is a peptide sequence according to formula (IVa), wherein

X1 is Asp, Ala, Ida, pGlu, bhAsp, Leu, D-Asp or absent;

X2 is Thr, Ala, or D-Thr;

X3 is His, Lys, D-His or Lys;

X4 is Phe, Ala, Dpa or D-Phe;

X5 is Pro, Gly, Arg, Lys, Ala, D-Pro or bhPro;

X6 is Ile, Cys, Arg, Lys, D-Ile or D-Cys;

X7 is Cys, Ile, Leu, Val, Phe, D-Ile or D-Cys;

X8 is Ile, Arg, Phe, Gln, Lys, Glu, Val, Leu or D-Ile;

X9 is Phe or bhPhe; and

X10 is Lys, Phe or absent.

[00329] In some embodiments of the peptide compound of formula IV, X is a peptide sequence having the formula (IVb)

X1-Thr-His-X4-X5-X6-X7-X8-Phe-X10 (IVb) SEQ ID NO:578

[00330] wherein

X1 is Asp, Ida, pGlu, bhAsp or absent;

X4 is Phe or Dpa;

X5 is Pro or bhPro;

X6 is Ile, Cys or Arg;

X7 is Cys, Ile, Leu or Val;

X8 is Ile Lys, Glu, Phe, Gln or Arg; and

X10 is Lys or absent.

[00331] In some embodiments of the peptide compound of formula IV, X is a peptide sequence having the formula (IVc)

X1-Thr-His-X4-X5-Cys-Ile-X8-Phe-X10 (IVc) SEQ ID NO:579

[00332] wherein

X1 is Asp, Ida, pGlu, bhAsp or absent;

X4 is: Phe or Dpa;

X5 is Pro or bhPro;

X8 is Ile Lys, Glu, Phe, Gln or Arg; and

X10 is Lys or absent;

[00333] In some embodiments of the peptide compound of formula IV, X is a peptide sequence having the formula (IVd)

X1-Thr-His-Phe-X5-Cys-Ile-X8-Phe-X10 (IVd) SEQ ID NO:580

[00334] wherein

X1 is Asp, Glu, or Ida;

X4 is: Phe;

X5 is Pro or bhPro;

X8 is Ile, Lys, or Phe; and

X10 is absent;

[00335] In some embodiments of the peptide compound of formula IV, Y is a peptide sequence having the formula (IVn)

Y1-Y2-Y3-Y4-Y5-Y6-Y7-Y8-Cys-Y10 (IVn) SEQ ID NO:581

[00336] wherein

Y1 is Gly, Ala, Lys, Pro or D-Pro;

Y2 is Pro, Ala or Gly;

Y3 is Arg, Ala, Lys or Trp;

Y4 is Ser, Gly or Ala;

Y5 is Lys, Met, Arg or Ala;

Y6 is Gly, Arg or Ala;

Y7 is Trp or Ala;

Y8 is Val, Thr, Ala or Glu; and

Y10 is Met, Lys or absent.

[00337] In some embodiments of the peptide compound of formula IV, Y is a peptide sequence having the formula (IVo)

Y1-Y2-Y3-Ser-Lys-Gly-Trp-Y8-Cys-Y10 (IVo) SEQ ID NO:582

[00338] wherein

Y1 is Gly, Pro or D-Pro;

Y2 is Pro or Gly;

Y3 is Arg or Lys;

Y8 is Val or Thr; and

Y10 is Met, Lys or absent.

[00339] In some embodiments of the peptide compound of formula IV, Y is a peptide sequence having the formula (IVp)

Y1-Cys-Y3-Y4-Arg-Y6-Y7-Y8-Cys-Y10-Y11-Y12-Y13-Y14-Y15 (IVp) SEQ ID NO:583

[00340] wherein

Y1 is Val or Ala or absent;

Y3 is Gly, Pro or absent;

Y4 is His, Trp or Tyr;

Y6 is Ser, Gly or Pro;

Y7 is Ile, Gly or Lys;

Y8 is Gly, Met or absent;

Y10 is Tyr or Cys;

Y11 is Arg, Lys, Met or Ala;

Y12is Arg or Ala;

Y13 is Cys or Val or absent;

Y14 is Cys, Lys, Pro, Arg, Thr or absent; and

Y15 is Arg, Thr or absent.

[00341] In some embodiments of the peptide compound of formula IV, Y is a peptide sequence having the formula (IVq)

Val-Cys-Y3-His-Arg-Y6-Y7-Y8-Cys-Tyr-Arg-Y12-Y13-Y14-Y15 (IVq) SEQ ID NO

[00342] wherein

Y3 is Gly or absent;

Y6 is Ser or Pro;

Y7 is Ile or Lys;

Y8 is Gly or absent;

Y12 is Arg or Ala;

Y13 is Cys, Val or absent;

Y14 is Cys, Arg, Thr or absent; and

Y15 is Arg or absent.

[00343] In some embodiments of the peptide compound of formula IV, Y is a peptide sequence having the formula (IVr)

Y1-Pro-Y3-Ser-Y5-Y6-Y7-Y8-Cys-Y10 (IVr) SEQ ID NO

[00344] wherein

Y1 is Gly, Glu, Val, or Lys;

Y3 is Arg or Lys;

Y5 is Arg or Lys;

Y6 is Gly, Ser, Lys, Ile or Arg;

Y7 is Trp or absent;

[00345] Y8 is Val, Thr, Asp, Glu or absent; and

[00346] Y10 is Lys or absent.

[00347] In some embodiments of the peptide compound of formula IV, Y is a peptide sequence having the formula (IVs)

Y1-Pro-Y3-Ser-Y5-Y6-Y7-Y8-Cys-Y10 (IVs) SEQ ID NO

[00348] wherein

Y1 is Glu or Lys;

Y3 is Arg or Lys;

Y5 is Arg or Lys;

Y6 is Gly, Ser, Lys, Ile or Arg;

Y7 is Trp or absent;

Y8 is Val or absent; and

Y10 is Lys or absent.

[00349] In some embodiments, the peptide of formula IV comprises at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve, at least thirteen, at least fourteen or at least fifteen Y residues in Y.

[00350] In some embodiments, Y1 to Y3 are present and Y4 to Y15 are absent.

[00351] In some embodiments, Y1 to Y11 are present and Y12 to Y15 are absent.

[00352] In some embodiments, Y1 to Y10 are present and Y11 to Y15 are absent.

[00353] In some embodiments, Y8 and Y15 are absent.

[00354] In some embodiments, Y3 and Y15 are absent

[00355] In some embodiments, Y3, Y14 and Y15 are absent.

[00356] In some embodiment Y5 is absent.

[00357] In some embodiments Y1, Y5, Y7, Y12, Y13, Y14 and Y15 are absent.

[00358] In certain embodiments, a peptide dimer hepcidin analogue of the present invention comprises two peptide monomer subunits linked via a lysine linker, comprising, consisting essentially of, or consisting of, the following structural formula:

R^1-X-Y-R^2 (V) SEQ ID NO:13

[00359] or a pharmaceutically acceptable salt or solvate thereof, wherein

[00360] R^1 is hydrogen, a C1-C6 alkyl, a C6-C12 aryl, a C6-C12 aryl C1-C6 alkyl, or a C1-C20 alkanoyl, and including PEGylated versions alone or as spacers of any of the foregoing;

[00361] R^2 is $-NH_2$ or -OH;

[00362] X is a peptide sequence having the formula (Va)

X1-X2-X3-X4-X5-X6-X7-X8-X9-X10 (Va) SEQ ID NO:14

[00363] wherein

X1 is Asp, Glu, Ala, Gly, Thr, Ida, pGlu, bhAsp, D-Asp, Tyr, Leu or absent;

X2 is Thr, Ala, Aib, D-Thr, Arg or absent;

X3 is His, Lys, Ala, D-His or Lys;

X4 is Phe, Ala, Dpa, bhPhe or D-Phe;

X5 is Pro, Glu, Ser, Gly, Arg, Lys, Val, Ala, D-Pro, bhPro, Sarc, Abu or absent;

X6 is Ile, Cys, Arg, Leu, Lys, His, Glu, D-Ile, D-Arg, D-Cys, Val, Ser or Ala;

X7 is Cys, Ile, Ala, Leu, Val, Ser, Phe, Dapa, D-Ile or D-Cys;

X8 is Ile, Lys, Arg, Ala, Gln, Phe, Glu, Asp, Tyr, Ser, Leu, Val, D-Ile, D-Lys, D-Arg, or Dapa;

X9 is Phe, Ala, Ile, Tyr, Lys, Arg, bhPhe or D-Phe; and

X10 is Lys, Phe or absent;

wherein Y is present or absent, and provided that if Y is absent, X7 is Ile;

[00364] wherein said compound of formula V is optionally PEGylated on R^1, X, or Y; and

[00365] wherein when said compound of formula V comprises two or more cysteine residues, at least two of said cysteine residues being linked via a disulfide bond.

[00366] In certain embodiments, R^1 is selected from methyl, acetyl, formyl, benzoyl, trifluoroacetyl, isovaleryl, isobutyryl, octanyl, and the conjugated amides of lauric acid, hexadecanoic acid, and γ-Glu-hexadecanoic acid.

[00367] In some embodiments, $R^{1'}$ is hydrogen, isovaleric acid, isobutyric acid or acetyl.

[00368] In certain embodiments, X either or both does not comprise or does not consist of an amino acid sequence set forth in US Patent No. 8,435,941.

[00369] In some embodiments of the compound of formula (V), X is a peptide sequence according to formula (Va), wherein

X1 is Asp, Ala, Ida, pGlu, bhAsp, Leu, D-Asp or absent;

X2 is Thr, Ala, or D-Thr;

X3 is His, Lys, or D-His;

X4 is Phe, Ala, or Dpa;

X5 is Pro, Gly, Arg, Lys, Ala, D-Pro or bhPro;

X6 is Ile, Cys, Arg, Lys, D-Ile or D-Cys;

X7 is Cys, Ile, Leu, Val, Phe, D-Ile or D-Cys;

X8 is Ile, Arg, Phe, Gln, Lys, Glu, Val, Leu or D-Ile;

X9 is Phe or bhPhe; and

X10 is Lys or absent.

[00370] In some embodiments of the compound of formula (V), X is a peptide sequence having the formula (Vb)

X1-Thr-His-X4-X5-X6-X7-X8-Phe-X10 (Vb) SEQ ID NO:584

[00371] wherein

X1 is Asp, Ida, pGlu, bhAsp or absent;

X4 is Phe or Dpa;

X5 is Pro or bhPro;

X6 is Ile, Cys or Arg;

X7 is Cys, Ile, Leu or Val;

X8 is Ile, Lys, Glu, Phe, Gln or Arg; and

X10 is Lys, Phe or absent.

[00372] In some embodiments of the compound of formula (V), X is a peptide sequence having the formula (Ic'')

X1-Thr-His-X4-X5-Cys-Ile-X8-Phe-X10 (Vc) SEQ ID NO:585

[00373] wherein

X1 is Asp, Ida, pGlu, bhAsp or absent;

X4 is Phe or Dpa;

X5 is Pro or bhPro;

X8 is Ile, Lys, Glu, Phe, Gln or Arg; and

X10 is Lys or absent.

[00374] In some embodiments of the compound of formula (V), X is a peptide sequence having the formula (Vd)

X1-Thr-His-Phe-X5-Cys-Ile-X8-Phe-X10 (Vd) SEQ ID NO:586

[00375] wherein

X1 is Asp, Glu or Ida;

X4 is Phe;

X5 is Pro or bhPro;

X8 is Ile, Lys, or Phe; and

X10 is absent.

[00376] In embodiments of the compound of formula (V) where Y is present, Y is a peptide having the formula (Vm)

Y1-Y2-Y3-Y4-Y5-Y6-Y7-Y8-Cys-Y10 (Vm) SEQ ID NO:587

[00377] wherein

Y1 is Gly, Ala, Lys, Pro or D-Pro;

Y2 is Pro, Ala or Gly;

Y3 is Arg, Ala, Lys or Trp;

Y4 is Ser, Gly or Ala;

Y5 is Lys, Met, Arg or Ala;

Y6 is Gly, Arg or Ala;

Y7 is Trp, Ala or absent;

Y8 is Val, Thr, Lys, Ala, Glu or absent; and

Y10 is Met, Lys or absent.

[00378] In some embodiments of the compound of formula (V), Y is a peptide sequence according to formula (Vm), wherein

Y1 is Gly, Glu, Val, or Lys

Y2 is Pro

Y3 is Arg or Lys;

Y4 is Ser

Y5 is Arg or Lys;

Y6 is Gly, Ser, Lys, Ile or Arg

Y7 is Trp or absent

Y8 is Val, Thr, Asp, Glu or absent; and

Y10 is Lys or absent.

[00379] In some embodiments of the compound of formula (V), Y is a peptide sequence according to formula (Vm), wherein

Y1 is Glu or Lys

Y2 is Pro

Y3 is Arg or Lys;

Y4 is Ser

Y5 is Arg or Lys;

Y6 is Gly, Ser, Lys, Ile or Arg;

Y7 is Trp or absent;

Y8 is Val or absent; and

Y10 is Lys or absent

[00380] In some embodiments of the compound of formula (V), Y is a peptide sequence according to formula (Vm), wherein

Y1 is Gly, Pro or D-Pro;

Y2 is Pro or Gly;

Y3 is Arg or Lys;

Y4 is Ser;

Y5 is Lys;

Y6 is Gly;

Y7 is Trp;

Y8 is Val or Thr; and

Y10 is Met, Lys or absent.

[00381] In some embodiments of the compound of formula (V), Y is a peptide sequence having the formula (Vn):

Y1-Y2-Y3-Ser-Lys-Gly-Trp-Y8-Cys-Y10 (Vn) SEQ ID NO:588

[00382] wherein

Y1 is Gly, Pro or D-Pro;

Y2 is Pro or Gly;

Y3 is Arg or Lys;

Y8 is Val or Thr; and

Y10 is Met, Lys or absent.

[00383] In some embodiments the peptide of formula (V) comprises at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, or at least ten amino acid residues of Y. In some embodiments, Y1 to Y3 are present and Y4 to Y10 are absent. In some embodiments, Y5 is absent. In some embodiments Y1, Y5, and Y7 are absent. In some embodiments, Y8 is absent. In some embodiments, Y3 is absent.

[00384] In certain embodiments, a peptide dimer hepcidin analogue of the present invention comprises two peptide monomer subunits linked via a lysine linker, comprising, consisting essentially of, or consisting of, the following structural formula VI:

R^1-X-Y-R^2 (VI) SEQ ID NO:15

[00385] or a pharmaceutically acceptable salt or solvate thereof, wherein

wherein R^1 is hydrogen, a C1-C6 alkyl, a C6-C12 aryl, a C6-C12 aryl C1-C6 alkyl, or a C1-C20 alkanoyl, and including PEGylated versions alone or as spacers of any of the foregoing;

[00386] R^2 is $-NH_2$ or -OH;

[00387] X is a peptide sequence having the formula (VIa):

X1-X2-X3-X4-X5-X6-X7-X8-X9-X10 (VIa) SEQ ID NO:16

[00388] wherein

X1 is Asp, Glu, Ida or absent;

X2 is Thr, Ser, Pro, Ala or absent;

X3 is His, Ala, or Glu;

X4 is Phe or Dpa;

X5 is Pro, bhPro, Sarc or Gly;

X6 is Cys, (D)-Cys, Arg, Glu, Phe, Gln, Leu, Val, Lys, Ala, Ser, Dapa or absent;

X7 is Cys, (D)-Cys, Arg, Glu, Phe, Gln, Leu, Val, Lys, Ala, Ser, Dapa or absent;

X8 is Ile, Arg, Lys, Ala, Gln, Phe, Glu, Asp, Tyr, Ser, Leu, Val, D-Ile, D-Lys, D-Arg, Dapa or absent;

[00389] X9 is Phe, Ala, Ile, Thr, Tyr, Lys, Arg, bhPhe, D-Phe or absent; and

[00390] X10 is Lys, Phe or absent;

[00391] Y is absent or present, provided that if Y is present, Y is a peptide having the formula (VIm)

Y1-Y2-Y3 (VIm) SEQ ID NO:17

[00392] wherein

Y1 is Ile, Arg, Lys, Ala, Gln, Phe, Glu, Asp, Tyr, Ser, Leu, Val, D-Ile, D-Lys, D-Arg, Dapa or absent;

Y2 is Phe, Ala, Ile, Thr, Tyr, Lys, Arg, bhPhe or D-Phe or absent; and

[00393] Y3 is Lys, Phe or absent;

[00394] and wherein said compound of formula VI is optionally PEGylated on R^1, X, or Y.

[00395] As used herein, the term "having" means "comprising," "consisting of" or "consisting essentially of" and encompasses each of these various embodiments in each instance.

[00396] In certain embodiments, a peptide analogue of formula VI comprises two or more cysteine residues, at least two of said cysteine residues being linked via a disulfide bond.

[00397] In certain embodiments, R^1 is selected from methyl, acetyl, formyl, benzoyl, trifluoroacetyl, isovaleryl, isobutyryl, octanyl, and the conjugated amides of lauric acid, hexadecanoic acid, and γ-Glu-hexadecanoic acid.

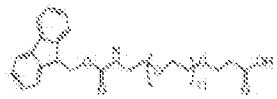
[00398] In some embodiments, $R^{1'}$ is hydrogen, isovaleric acid, isobutyric acid or acetyl.

[00399] In certain embodiments, X either or both does not comprise or does not consist of an amino acid sequence set forth in US Patent No. 8,435,941.

[00400] In certain embodiments, a dimer hepcidin analogue of the present invention, e.g., a lysine dimer hepcidin analogue of the present invention, comprises one or two peptide monomers having an amino acid sequence shown as any one of compound numbers 1-361 in Table 12 with ferroportin internalization/degradation assay EC_{50} values.

[00401] For certain compounds comprising an N-terminal PEG11 moiety, the following was used in their synthesis:

Fmoc-amino PEG propionic acid



[00402] In certain embodiments, a lysine dimer peptide analogue of the present invention has a structure or comprises a peptide sequence shown in Table 10 with ferroportin internalization/degradation assay EC50 values.

Table 10. Illustrative Lysine dimer peptide analogues

SEQ ID NO	Sequence	EC_{50} (nM) (n>3)
539	(isobutyric acid-DTHFPCIKF)$_2$[Lys]K(iso-Glu-Palm)-NH_2	24
540	(isovaleric acid-DTHFPCIKF)$_2$[Lys]K(iso-Glu-Palm)-NH_2	14
541	(cyclohexanoic acid-DTHFPCIKF)$_2$[Lys]K(iso-Glu-Palm)-NH_2	17

542	(Isovaleric acid-DTHFPCIRF)$_2$[Lys]-K(iso-Glu-Palm)-NH$_2$	4
543	(Isovaleric acid-DTHFPCIKF)$_2$[Lys]-NH$_2$	30
544	(Isovaleric acid-DTHFPCIKF)$_2$[Lys]-Lys(Palm)-NH$_2$	16
570	(Isovaleric acid-DTHFPCIKF)2[Lys]-Lys[(isoGlu(octanoic acid)]-NH2	17

Peptide Analogue Conjugates and Analogues

[00403] In certain embodiments, hepcidin analogues of the present invention, including both monomers and dimers, comprise one or more conjugated chemical substituents, such as lipophilic substituents and polymeric moieties. Without wishing to be bound by any particular theory, it is believed that the lipophilic substituent binds to albumin in the bloodstream, thereby shielding the hepcidin analogue from enzymatic degradation, and thus enhancing its half-life. In addition, it is believed that polymeric moieties enhance half-life and reduce clearance in the bloodstream, and in some cases enhance permeability through the epithelium and retention in the lamina propria. Moreover, it is also surmised that these substituents in some cases may enhance permeability through the epithelium and retention in the lamina propria. The skilled person will be well aware of suitable techniques for preparing the compounds employed in the context of the invention. For examples of non-limiting suitable chemistry, see, e.g., WO98/08871, WO00/55184, WO00/55119, Madsen et al (J. Med. Chem. 2007, 50, 6126-32), and Knudsen et al. 2000 (J. Med Chem. 43, 1664-1669).

[00404] In one embodiment, the side chains of one or more amino acid residues (e.g., Lys residues) in a hepcidin analogue of the invention is further conjugated (e.g., covalently attached) to a lipophilic substituent. The lipophilic substituent may be covalently bonded to an atom in the amino acid side chain, or alternatively may be conjugated to the amino acid side chain via one or more spacers. The spacer, when present, may provide spacing between the hepcidin analogue and the lipophilic substituent.

[00405] In certain embodiments, the lipophilic substituent comprises a hydrocarbon chain having from 4 to 30 C atoms, for example at least 8 or 12 C atoms, and preferably 24 C atoms or fewer, or 20 C atoms or fewer. The hydrocarbon chain may be linear or branched and may be saturated or unsaturated. In certain embodiments, the hydrocarbon chain is substituted with a moiety which forms part of the attachment to the amino acid side chain or the spacer, for example an acyl group, a sulfonyl group, an N atom, an O atom or an S atom. In some embodiments, the hydrocarbon chain is substituted with an acyl group, and accordingly the

hydrocarbon chain may form part of an alkanoyl group, for example palmitoyl, caproyl, lauroyl, myristoyl or stearoyl.

[00406] A lipophilic substituent may be conjugated to any amino acid side chain in a hepcidin analogue of the invention. In certain embodiment, the amino acid side chain includes a carboxy, hydroxyl, thiol, amide or amine group, for forming an ester, a sulphonyl ester, a thioester, an amide or a sulphonamide with the spacer or lipophilic substituent. For example, the lipophilic substituent may be conjugated to Asn, Asp, Glu, Gln, His, Lys, Arg, Ser, Thr, Tyr, Trp, Cys or Dbu, Dpr or Orn. In certain embodiments, the lipophilic substituent is conjugated to Lys. An amino acid shown as Lys in any of the formula provided herein may be replaced by, e.g., Dbu, Dpr or Orn where a lipophilic substituent is added.

[00407] In further embodiments of the present invention, alternatively or additionally, the side-chains of one or more amino acid residues in a hepcidin analogue of the invention may be conjugated to a polymeric moiety, for example, in order to increase solubility and/or half-life *in vivo* (e.g. in plasma) and/or bioavailability. Such modifications are also known to reduce clearance (e.g. renal clearance) of therapeutic proteins and peptides.

[00408] As used herein, "Polyethylene glycol" or "PEG" is a polyether compound of general formula H-(O-CH2-CH2)n-OH. PEGs are also known as polyethylene oxides (PEOs) or polyoxyethylenes (POEs), depending on their molecular weight PEO, PEE, or POG, as used herein, refers to an oligomer or polymer of ethylene oxide. The three names are chemically synonymous, but PEG has tended to refer to oligomers and polymers with a molecular mass below 20,000 g/mol, PEO to polymers with a molecular mass above 20,000 g/mol, and POE to a polymer of any molecular mass. PEG and PEO are liquids or low-melting solids, depending on their molecular weights. Throughout this disclosure, the 3 names are used indistinguishably. PEGs are prepared by polymerization of ethylene oxide and are commercially available over a wide range of molecular weights from 300 g/mol to 10,000,000 g/mol. While PEG and PEO with different molecular weights find use in different applications, and have different physical properties (e.g. viscosity) due to chain length effects, their chemical properties are nearly identical. The polymeric moiety is preferably water-soluble (amphiphilic or hydrophilic), non-toxic, and pharmaceutically inert. Suitable polymeric moieties include polyethylene glycols (PEG), homo- or co-polymers of PEG, a monomethyl-substituted polymer of PEG (mPEG), or polyoxyethylene glycerol (POG). See, for example, Int. J. Hematology 68:1 (1998); Bioconjugate Chem. 6:150 (1995); and Crit. Rev. Therap. Drug Carrier Sys. 9:249 (1992). Also encompassed are PEGs that are prepared

for purpose of half-life extension, for example, mono-activated, alkoxy-terminated polyalkylene oxides (POA's) such as mono-methoxy-terminated polyethyelene glycols (mPEG's); bis activated polyethylene oxides (glycols) or other PEG derivatives are also contemplated. Suitable polymers will vary substantially by weights ranging from about 200 to about 40,000 are usually selected for the purposes of the present invention. In certain embodiments, PEGs having molecular weights from 200 to 2,000 or from 200 to 500 are used. Different forms of PEG may also be used, depending on the initiator used for the polymerization process, e.g., a common initiator is a monofunctional methyl ether PEG, or methoxypoly(ethylene glycol), abbreviated mPEG. Other suitable initiators are known in the art and are suitable for use in the present invention.

[00409] Lower-molecular-weight PEGs are also available as pure oligomers, referred to as monodisperse, uniform, or discrete. These are used in certain embodiments of the present invention.

[00410] PEGs are also available with different geometries: branched PEGs have three to ten PEG chains emanating from a central core group; star PEGs have 10 to 100 PEG chains emanating from a central core group; and comb PEGs have multiple PEG chains normally grafted onto a polymer backbone. PEGs can also be linear. The numbers that are often included in the names of PEGs indicate their average molecular weights (e.g. a PEG with n = 9 would have an average molecular weight of approximately 400 daltons, and would be labeled PEG 400.

[00411] As used herein, "PEGylation" is the act of coupling (e.g., covalently) a PEG structure to the hepcidin analogue of the invention, which is in certain embodiments referred to as a "PEGylated hepcidin analogue". In certain embodiments, the PEG of the PEGylated side chain is a PEG with a molecular weight from about 200 to about 40,000. In some embodiments, a spacer of a peptide of formula I, formula I', or formula I'' is PEGylated. In certain embodiments, the PEG of a PEGylated spacer is PEG3, PEG4, PEG5, PEG6, PEG7, PEG8, PEG9, PEG10, or PEG11. In certain embodiments, the PEG of a PEGylated spacer is PEG3 or PEG8.

[00412] In some embodiments, the present invention includes a hepcidin analogue peptide (or a dimer thereof) conjugated with a PEG that is attached covalently, e.g., through and amide, a thiol, via click chemistry, or via any other suitable means known in the art. In particular embodiments PEG is attached through an amide bond and, as such, certain PEG derivatives used will be appropriately functionalized. For example, in certain embodiments, PEG11,

which is O-(2-aminoethyl)-O′-(2-carboxyethyl)-undecaethyleneglycol, has both an amine and carboxylic acid for attachment to a peptide of the present invention. In certain embodiments, PEG25 contains a diacid and 25 glycol moieties.

[00413] Other suitable polymeric moieties include poly-amino acids such as poly-lysine, poly-aspartic acid and poly-glutamic acid (see for example Gombotz, et al. (1995), Bioconjugate Chem., vol. 6: 332-351; Hudecz, et al. (1992), Bioconjugate Chem., vol. 3, 49-57 and Tsukada, et al. (1984), J. Natl. Cancer Inst., vol. 73, : 721-729. The polymeric moiety may be straight-chain or branched. In some embodiments, it has a molecular weight of 500-40,000 Da, for example 500-10,000 Da, 1000-5000 Da, 10,000-20,000 Da, or 20,000-40,000 Da.

[00414] In some embodiments, a hepcidin analogue of the invention may comprise two or more such polymeric moieties, in which case the total molecular weight of all such moieties will generally fall within the ranges provided above.

[00415] In some embodiments, the polymeric moiety may be coupled (by covalent linkage) to an amino, carboxyl or thiol group of an amino acid side chain. Certain examples are the thiol group of Cys residues and the epsilon amino group of Lys residues, and the carboxyl groups of Asp and Glu residues may also be involved.

[00416] The skilled worker will be well aware of suitable techniques which can be used to perform the coupling reaction. For example, a PEG moiety bearing a methoxy group can be coupled to a Cys thiol group by a maleimido linkage using reagents commercially available from Nektar Therapeutics AL. See also WO 2008/101017, and the references cited above, for details of suitable chemistry. A maleimide-functionalised PEG may also be conjugated to the side-chain sulfhydryl group of a Cys residue.

[00417] As used herein, disulfide bond oxidation can occur within a single step or is a two-step process. As used herein, for a single oxidation step, the trityl protecting group is often employed during assembly, allowing deprotection during cleavage, followed by solution oxidation. When a second disulfide bond is required, one has the option of native or selective oxidation. For selective oxidation requiring orthogonal protecting groups, Acm and Trityl is used as the protecting groups for cysteine. Cleavage results in the removal of one protecting pair of cysteine allowing oxidation of this pair. The second oxidative deprotection step of the cysteine protected Acm group is then performed. For native oxidation, the trityl protecting group is used for all cysteines, allowing for natural folding of the peptide.

[00418] A skilled worker will be well aware of suitable techniques which can be used to perform the oxidation step.

Illustrative Hepcidin Analogue Peptide Monomers and Hepcidin Analogue Peptide Dimers

[00419] Illustrative hepcidin analogues and hepcidin analogue peptide dimers of the present invention are shown in Tables 2-4, 6-10, 12, 14, and 15. These tables provides the amino acid sequence of selected monomer hepcidin analogues and hepcidin analogue peptide dimers, and in some cases indicate the linker moiety present in the hepcidin analogue peptide dimers. According to the protocols discussed herein, a number of the hepcidin analogues monomer peptides and hepcidin analogue peptide dimers shown were synthesized. The IC50 values for selected monomer hepcidin analogues and hepcidin analogue peptide dimers for inducing the internalization/degradation of human ferroportin protein *in vitro* are provided.

[00420] The present invention thus provides various hepcidin analogues which bind or associate with ferroportin (e.g., human ferroportin), inducing internalization of the transporter.

[00421] In some embodiments, the present invention provides a dimer of any one of the peptide monomers disclosed herein. In one embodiment, the present invention provides a hepcidin analogue dimer that is a homodimer of any one of the monomer peptide sequences disclosed herein. In one embodiment, the present invention provides a hepcidin analogue dimer that is a heterodimer of any two different monomer peptide sequences disclosed herein. In one embodiment, the present invention provides a hepcidin analogue dimer that is a heterodimer of any one monomer peptide sequence disclosed herein and any other peptide sequence known in the art to have hepcidin activity including a wildtype hepcidin peptide or a hepcidin analogue. In various embodiments, the present invention provides hepcidin homodimers and heterodimers that are dimerized by a disulfide linkage. In various embodiments, the present invention provides hepcidin homodimers and heterodimers that are dimerized via a linker, e.g., any one or more of the linkers disclosed herein or known in the art. In still further embodiments, the present invention provides hepcidin homodimers and heterodimers that are dimerized by one or more disulfide linkages and one or more linker, e.g., any one or more of the linkers disclosed herein or known in the art.

[00422] The hepcidin analogues of the present invention may be synthesized by many techniques that are known to those skilled in the art. In certain embodiments, monomer subunits are synthesized, purified, and dimerized using the techniques described in the accompanying Examples.

[00423] In related embodiments, the present invention includes polynucleotides that encode a polypeptide having a sequence set forth in any one of Formula I-IX, or as shown in any of Tables 2-4, 6-10, 12, 14, or 15.

[00424] In addition, the present invention includes vectors, e.g., expression vectors, comprising a polynucleotide of the present invention.

[00425] In certain embodiments, the present invention provides a hepcidin analogue monomer, or a homodimer or heterodimer comprising such a monomer, according to any one of the formulae disclosed herein, wherein the monomer comprises a Cys in position 6 or 7 and wherein the amino acid directly C-terminal to such a Cys is any natural or unnatural amino acid except for Ile.

Methods of Treatment

[00426] In some embodiments, the present invention provides methods for treating a subject afflicted with a disease or disorder associated with dysregulated hepcidin signaling, wherein the method comprises administering to the subject a hepcidin analogue of the present invention. In some embodiments, the hepcidin analogue that is administered to the subject is present in a composition (e.g., a pharmaceutical composition). In one embodiment, a method is provided for treating a subject afflicted with a disease or disorder characterized by increased activity or expression of ferroportin, wherein the method comprises administering to the individual a hepcidin analogue or composition of the present invention in an amount sufficient to (partially or fully) bind to and agonize ferroportin in the subject. In one embodiment, a method is provided for treating a subject afflicted with a disease or disorder characterized by dysregulated iron metabolism, wherein the method comprises administering to the subject a hepcidin analogue or composition of the present invention.

[00427] In some embodiments, methods of the present invention comprise providing a hepcidin analogue or a composition of the present invention to a subject in need thereof. In particular embodiments, the subject in need thereof has been diagnosed with or has been determined to be at risk of developing a disease or disorder characterized by dysregulated iron levels (e.g., diseases or disorders of iron metabolism; diseases or disorders related to iron overload; and diseases or disorders related to abnormal hepcidin activity or expression). In particular embodiments, the subject is a mammal (e.g., a human).

[00428] In certain embodiments, the disease or disorder is a disease of iron metabolism, such as, e.g., an iron overload disease, iron deficiency disorder, disorder of iron biodistribution, or another disorder of iron metabolism and other disorder potentially related to iron metabolism, etc. In particular embodiments, the disease of iron metabolism is hemochromatosis, HFE mutation hemochromatosis, ferroportin mutation hemochromatosis, transferrin receptor 2 mutation hemochromatosis, hemojuvelin mutation hemochromatosis, hepcidin mutation hemochromatosis, juvenile hemochromatosis, neonatal hemochromatosis, hepcidin deficiency, transfusional iron overload, thalassemia, thalassemia intermedia, alpha thalassemia, beta thalassemia, sideroblastic anemia, porphyria, porphyria cutanea tarda, African iron overload, hyperferritinemia, ceruloplasmin deficiency, atransferrinemia, congenital dyserythropoietic anemia, anemia of chronic disease, anemia of inflammation, anemia of infection, hypochromic microcytic anemia, iron- deficiency anemia, iron-refractory iron deficiency anemia, anemia of chronic kidney disease, transfusion-dependent anemia, hemolytic anemia, erythropoietin resistance, iron deficiency of obesity, other anemias, benign or malignant tumors that overproduce hepcidin or induce its overproduction, conditions with hepcidin excess, Friedreich ataxia, gracile syndrome, Hallervorden-Spatz disease, Wilson's disease, pulmonary hemosiderosis, hepatocellular carcinoma, cancer (e.g., liver cancer), hepatitis, cirrhosis of liver, pica, chronic renal failure, insulin resistance, diabetes, atherosclerosis, neurodegenerative disorders, dementia, multiple sclerosis, Parkinson's disease, Huntington's disease, or Alzheimer's disease.

[00429] In certain embodiments, the disease or disorder is related to iron overload diseases such as iron hemochromatosis, HFE mutation hemochromatosis, ferroportin mutation hemochromatosis, transferrin receptor 2 mutation hemochromatosis, hemojuvelin mutation hemochromatosis, hepcidin mutation hemochromatosis, juvenile hemochromatosis, neonatal hemochromatosis, hepcidin deficiency, transfusional iron overload, thalassemia, thalassemia intermedia, alpha thalassemia.

[00430] In certain embodiments, the disease or disorder is one that is not typically identified as being iron related. For example, hepcidin is highly expressed in the murine pancreas suggesting that diabetes (Type I or Type II), insulin resistance, glucose intolerance and other disorders may be ameliorated by treating underlying iron metabolism disorders. See Ilyin, G. et al. (2003) FEBS Lett. 542 22-26, which is herein incorporated by reference. As such, peptides of the invention may be used to treat these diseases and conditions. Those skilled in the art are readily able to determine whether a given disease can be treated with a peptide according to the present invention using methods known in the art, including the assays of

WO 2004092405, which is herein incorporated by reference, and assays which monitor hepcidin, hemojuvelin, or iron levels and expression, which are known in the art such as those described in U.S. Patent No. 7,534,764, which is herein incorporated by reference.

[00431] In certain embodiments, the disease or disorder is postmenopausal osteoporosis.

[00432] In certain embodiments of the present invention, the diseases of iron metabolism are iron overload diseases, which include hereditary hemochromatosis, iron-loading anemias, alcoholic liver diseases, heart disease and/or failure, cardiomyopathy, and chronic hepatitis C.

[00433] In particular embodiments, any of these diseases, disorders, or indications are caused by or associated with a deficiency of hepcidin or iron overload.

[00434] In some embodiments, methods of the present invention comprise providing a hepcidin analogue of the present invention (i.e., a first therapeutic agent) to a subject in need thereof in combination with a second therapeutic agent. In certain embodiments, the second therapeutic agent is provided to the subject before and/or simultaneously with and/or after the pharmaceutical composition is administered to the subject. In particular embodiments, the second therapeutic agent is iron chelator. In certain embodiments, the second therapeutic agent is selected from the iron chelators Deferoxamine and Deferasirox (Exjade ™). In another embodiment, the method comprises administering to the subject a third therapeutic agent.

[00435] The present invention provides compositions (for example pharmaceutical compositions) comprising one or more hepcidin analogues of the present invention.

[00436] In certain embodiments, the compositions comprise two or more hepcidin analogues disclosed herein. In certain embodiments, the combination is selected from one of the following: (i) any two or more of the hepcidin analogue peptide monomers, such as, e.g., any one of those disclosed in Tables 2-4, 6-10, 12, 14, or 15, or dimers of any monomers shown therein; (ii) any two or more of the hepcidin analogue peptide dimers disclosed in Tables 2-4 or 6-10, 12, 14, or 15; (iii) any one or more of the hepcidin analogue peptide monomers disclosed herein, and any one or more of the hepcidin analogue peptide dimers disclosed herein.

[00437] In certain embodiments, the present invention includes pharmaceutical compositions comprising one or more hepcidin analogues of the present invention and a pharmaceutically acceptable carrier, diluent or excipient. A pharmaceutically acceptable carrier, diluent or

excipient refers to a non-toxic solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents such as sugars, sodium chloride, and the like.

[00438] The term "pharmaceutically acceptable carrier" includes any of the standard pharmaceutical carriers. Pharmaceutically acceptable carriers for therapeutic use are well known in the pharmaceutical art and are described, for example, in "Remington's Pharmaceutical Sciences", 17th edition, Alfonso R. Gennaro (Ed.), Mark Publishing Company, Easton, PA, USA, 1985. For example, sterile saline and phosphate-buffered saline at slightly acidic or physiological pH may be used. Suitable pH-buffering agents may, e.g., be phosphate, citrate, acetate, tris(hydroxymethyl)aminomethane (TRIS), N-tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid (TAPS), ammonium bicarbonate, diethanolamine, histidine, arginine, lysine or acetate (e.g. as sodium acetate), or mixtures thereof. The term further encompasses any carrier agents listed in the US Pharmacopeia for use in animals, including humans.

[00439] It is to be understood that the inclusion of a hepcidin analogue of the invention (i.e., one or more hepcidin analogue peptide monomers of the invention or one or more hepcidin analogue peptide dimers of the present invention) in a pharmaceutical composition also encompasses inclusion of a pharmaceutically acceptable salt or solvate of a hepcidin analogue of the invention. In particular embodiments, the pharmaceutical compositions further comprise one or more pharmaceutically acceptable carrier, excipient, or vehicle.

[00440] In certain embodiments, the invention provides a pharmaceutical composition comprising a hepcidin analogue, or a pharmaceutically acceptable salt or solvate thereof, for treating a variety of conditions, diseases, or disorders as disclosed herein or elsewhere (see, e.g., Methods of Treatment, herein). In particular embodiments, the invention provides a pharmaceutical composition comprising a hepcidin analogue peptide monomer, or a pharmaceutically acceptable salt or solvate thereof, for treating a variety of conditions, diseases, or disorders as disclosed herein elsewhere (see, e.g., Methods of Treatment, herein). In particular embodiments, the invention provides a pharmaceutical composition comprising a hepcidin analogue peptide dimer, or a pharmaceutically acceptable salt or solvate thereof, for treating a variety of conditions, diseases, or disorders as disclosed herein elsewhere (see, e.g., Methods of Treatment, herein).

[00441] The hepcidin analogues of the present invention may be formulated as pharmaceutical compositions which are suited for administration with or without storage, and which typically comprise a therapeutically effective amount of at least one hepcidin analogue of the invention, together with a pharmaceutically acceptable carrier, excipient or vehicle.

[00442] In some embodiments, the hepcidin analogue pharmaceutical compositions of the invention are in unit dosage form. In such forms, the composition is divided into unit doses containing appropriate quantities of the active component or components. The unit dosage form may be presented as a packaged preparation, the package containing discrete quantities of the preparation, for example, packaged tablets, capsules or powders in vials or ampoules. The unit dosage form may also be, e.g., a capsule, cachet or tablet in itself, or it may be an appropriate number of any of these packaged forms. A unit dosage form may also be provided in single-dose injectable form, for example in the form of a pen device containing a liquid-phase (typically aqueous) composition. Compositions may be formulated for any suitable route and means of administration, e.g., any one of the routes and means of administration disclosed herein.

[00443] In particular embodiments, the hepcidin analogue, or the pharmaceutical composition comprising a hepcidin analogue, is suspended in a sustained-release matrix. A sustained-release matrix, as used herein, is a matrix made of materials, usually polymers, which are degradable by enzymatic or acid-base hydrolysis or by dissolution. Once inserted into the body, the matrix is acted upon by enzymes and body fluids. A sustained-release matrix desirably is chosen from biocompatible materials such as liposomes, polylactides (polylactic acid), polyglycolide (polymer of glycolic acid), polylactide co-glycolide (copolymers of lactic acid and glycolic acid) polyanhydrides, poly(ortho)esters, polypeptides, hyaluronic acid, collagen, chondroitin sulfate, carboxylic acids, fatty acids, phospholipids, polysaccharides, nucleic acids, polyamino acids, amino acids such as phenylalanine, tyrosine, isoleucine, polynucleotides, polyvinyl propylene, polyvinylpyrrolidone and silicone. One embodiment of a biodegradable matrix is a matrix of one of either polylactide, polyglycolide, or polylactide co-glycolide (co-polymers of lactic acid and glycolic acid).

[00444] In certain embodiments, the compositions are administered enterally or parenterally. In particular embodiments, the compositions are administered orally, intracisternally, intravaginally, intraperitoneally, intrarectally, topically (as by powders, ointments, drops, suppository, or transdermal patch, including delivery intravitreally, intranasally, and via inhalation) or buccally. The term "parenteral" as used herein refers to modes of

administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous, intradermal and intraarticular injection and infusion. Accordingly, in certain embodiments, the compositions are formulated for delivery by any of these routes of administration.

[00445] In certain embodiments, pharmaceutical compositions for parenteral injection comprise pharmaceutically acceptable sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders, for reconstitution into sterile injectable solutions or dispersions just prior to use. Examples of suitable aqueous and nonaqueous carriers, diluents, solvents or vehicles include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), carboxymethylcellulose and suitable mixtures thereof, beta-cyclodextrin, vegetable oils (such as olive oil), and injectable organic esters such as ethyl oleate. Proper fluidity may be maintained, for example, by the use of coating materials such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants. These compositions may also contain adjuvants such as preservative, wetting agents, emulsifying agents, and dispersing agents. Prolonged absorption of an injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption, such as aluminum monostearate and gelatin.

[00446] Injectable depot forms include those made by forming microencapsule matrices of the hepcidin analogue in one or more biodegradable polymers such as polylactide-polyglycolide, poly(orthoesters), poly(anhydrides), and (poly)glycols, such as PEG. Depending upon the ratio of peptide to polymer and the nature of the particular polymer employed, the rate of release of the hepcidin analogue can be controlled. Depot injectable formulations are also prepared by entrapping the hepcidin analogue in liposomes or microemulsions compatible with body tissues.

[00447] The injectable formulations may be sterilized, for example, by filtration through a bacterial-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium just prior to use.

[00448] Topical administration includes administration to the skin or mucosa, including surfaces of the lung and eye. Compositions for topical lung administration, including those for inhalation and intranasal, may involve solutions and suspensions in aqueous and non-aqueous formulations and can be prepared as a dry powder which may be pressurized or non-pressurized. In non-pressurized powder compositions, the active ingredient may be finely

divided form may be used in admixture with a larger-sized pharmaceutically acceptable inert carrier comprising particles having a size, for example, of up to 100 micrometers in diameter. Suitable inert carriers include sugars such as lactose.

[00449] Alternatively, the composition may be pressurized and contain a compressed gas, such as nitrogen or a liquefied gas propellant. The liquefied propellant medium and indeed the total composition may be such that the active ingredient does not dissolve therein to any substantial extent. The pressurized composition may also contain a surface active agent, such as a liquid or solid non-ionic surface active agent or may be a solid anionic surface active agent. It is preferred to use the solid anionic surface active agent in the form of a sodium salt.

[00450] A further form of topical administration is to the eye. A hepcidin analogue of the invention may be delivered in a pharmaceutically acceptable ophthalmic vehicle, such that the hepcidin analogue is maintained in contact with the ocular surface for a sufficient time period to allow the hepcidin analogue to penetrate the corneal and internal regions of the eye, as for example the anterior chamber, posterior chamber, vitreous body, aqueous humor, vitreous humor, cornea, iris/ciliary, lens, choroid/retina and sclera. The pharmaceutically acceptable ophthalmic vehicle may, for example, be an ointment, vegetable oil or an encapsulating material. Alternatively, the hepcidin analogues of the invention may be injected directly into the vitreous and aqueous humour.

[00451] Compositions for rectal or vaginal administration include suppositories which may be prepared by mixing the hepcidin analogues of this invention with suitable non-irritating excipients or carriers such as cocoa butter, polyethylene glycol or a suppository wax, which are solid at room temperature but liquid at body temperature and, therefore, melt in the rectum or vaginal cavity and release the active compound.

[00452] Hepcidin analogues of the present invention may also be administered in liposomes or other lipid-based carriers. As is known in the art, liposomes are generally derived from phospholipids or other lipid substances. Liposomes are formed by mono- or multi-lamellar hydrated liquid crystals that are dispersed in an aqueous medium. Any non-toxic, physiologically acceptable and metabolizable lipid capable of forming liposomes can be used. The present compositions in liposome form can contain, in addition to a hepcidin analogue of the present invention, stabilizers, preservatives, excipients, and the like. In certain embodiments, the lipids comprise phospholipids, including the phosphatidyl cholines (lecithins) and serines, both natural and synthetic. Methods to form liposomes are known in the art.

[00453] Pharmaceutical compositions to be used in the invention suitable for parenteral administration may comprise sterile aqueous solutions and/or suspensions of the peptide inhibitors made isotonic with the blood of the recipient, generally using sodium chloride, glycerin, glucose, mannitol, sorbitol, and the like.

[00454] In some aspects, the invention provides a pharmaceutical composition for oral delivery. Compositions and hepcidin analogues of the instant invention may be prepared for oral administration according to any of the methods, techniques, and/or delivery vehicles described herein. Further, one having skill in the art will appreciate that the hepcidin analogues of the instant invention may be modified or integrated into a system or delivery vehicle that is not disclosed herein, yet is well known in the art and compatible for use in oral delivery of peptides.

[00455] In certain embodiments, formulations for oral administration may comprise adjuvants (e.g. resorcinols and/or nonionic surfactants such as polyoxyethylene oleyl ether and n-hexadecylpolyethylene ether) to artificially increase the permeability of the intestinal walls, and/or enzymatic inhibitors (e.g. pancreatic trypsin inhibitors, diisopropylfluorophosphate (DFF) or trasylol) to inhibit enzymatic degradation. In certain embodiments, the hepcidin analogue of a solid-type dosage form for oral administration can be mixed with at least one additive, such as sucrose, lactose, cellulose, mannitol, trehalose, raffinose, maltitol, dextran, starches, agar, alginates, chitins, chitosans, pectins, gum tragacanth, gum arabic, gelatin, collagen, casein, albumin, synthetic or semisynthetic polymer, or glyceride. These dosage forms can also contain other type(s) of additives, e.g., inactive diluting agent, lubricant such as magnesium stearate, paraben, preserving agent such as sorbic acid, ascorbic acid, alpha-tocopherol, antioxidants such as cysteine, disintegrators, binders, thickeners, buffering agents, pH adjusting agents, sweetening agents, flavoring agents or perfuming agents.

[00456] In particular embodiments, oral dosage forms or unit doses compatible for use with the hepcidin analogues of the present invention may include a mixture of hepcidin analogue and nondrug components or excipients, as well as other non-reusable materials that may be considered either as an ingredient or packaging. Oral compositions may include at least one of a liquid, a solid, and a semi-solid dosage forms. In some embodiments, an oral dosage form is provided comprising an effective amount of hepcidin analogue, wherein the dosage form comprises at least one of a pill, a tablet, a capsule, a gel, a paste, a drink, a syrup, ointment, and suppository. In some instances, an oral dosage form is provided that is

designed and configured to achieve delayed release of the hepcidin analogue in the subject's small intestine and/or colon.

[00457] In one embodiment, an oral pharmaceutical composition comprising a hepcidin analogue of the present invention comprises an enteric coating that is designed to delay release of the hepcidin analogue in the small intestine. In at least some embodiments, a pharmaceutical composition is provided which comprises a hepcidin analogue of the present invention and a protease inhibitor, such as aprotinin, in a delayed release pharmaceutical formulation. In some instances, pharmaceutical compositions of the instant invention comprise an enteric coat that is soluble in gastric juice at a pH of about 5.0 or higher. In at least one embodiment, a pharmaceutical composition is provided comprising an enteric coating comprising a polymer having dissociable carboxylic groups, such as derivatives of cellulose, including hydroxypropylmethyl cellulose phthalate, cellulose acetate phthalate and cellulose acetate trimellitate and similar derivatives of cellulose and other carbohydrate polymers.

[00458] In one embodiment, a pharmaceutical composition comprising a hepcidin analogue of the present invention is provided in an enteric coating, the enteric coating being designed to protect and release the pharmaceutical composition in a controlled manner within the subject's lower gastrointestinal system, and to avoid systemic side effects. In addition to enteric coatings, the hepcidin analogues of the instant invention may be encapsulated, coated, engaged or otherwise associated within any compatible oral drug delivery system or component. For example, in some embodiments a hepcidin analogue of the present invention is provided in a lipid carrier system comprising at least one of polymeric hydrogels, nanoparticles, microspheres, micelles, and other lipid systems.

[00459] To overcome peptide degradation in the small intestine, some embodiments of the present invention comprise a hydrogel polymer carrier system in which a hepcidin analogue of the present invention is contained, whereby the hydrogel polymer protects the hepcidin analogue from proteolysis in the small intestine and/or colon. The hepcidin analogues of the present invention may further be formulated for compatible use with a carrier system that is designed to increase the dissolution kinetics and enhance intestinal absorption of the peptide. These methods include the use of liposomes, micelles and nanoparticles to increase GI tract permeation of peptides.

[00460] Various bioresponsive systems may also be combined with one or more hepcidin analogue of the present invention to provide a pharmaceutical agent for oral delivery. In

some embodiments, a hepcidin analogue of the instant invention is used in combination with a bioresponsive system, such as hydrogels and mucoadhesive polymers with hydrogen bonding groups (e.g., PEG, poly(methacrylic) acid [PMAA], cellulose, Eudragit®, chitosan and alginate) to provide a therapeutic agent for oral administration. Other embodiments include a method for optimizing or prolonging drug residence time for a hepcidin analogue disclosed herein, wherein the surface of the hepcidin analogue surface is modified to comprise mucoadhesive properties through hydrogen bonds, polymers with linked mucins or/and hydrophobic interactions. These modified peptide molecules may demonstrate increase drug residence time within the subject, in accordance with a desired feature of the invention. Moreover, targeted mucoadhesive systems may specifically bind to receptors at the enterocytes and M-cell surfaces, thereby further increasing the uptake of particles containing the hepcidin analogue.

[00461] Other embodiments comprise a method for oral delivery of a hepcidin analogue of the present invention, wherein the hepcidin analogue is provided to a subject in combination with permeation enhancers that promote the transport of the peptides across the intestinal mucosa by increasing paracellular or transcellular permeation. For example, in one embodiment, a permeation enhancer is combined with a hepcidin analogue, wherein the permeation enhancer comprises at least one of a long-chain fatty acid, a bile salt, an amphiphilic surfactant, and a chelating agent. In one embodiment, a permeation enhancer comprising sodium N-[hydroxybenzoyl)amino] caprylate is used to form a weak noncovalent association with the hepcidin analogue of the instant invention, wherein the permeation enhancer favors membrane transport and further dissociation once reaching the blood circulation. In another embodiment, a hepcidin analogue of the present invention is conjugated to oligoarginine, thereby increasing cellular penetration of the peptide into various cell types. Further, in at least one embodiment a noncovalent bond is provided between a peptide inhibitor of the present invention and a permeation enhancer selected from the group consisting of a cyclodextrin (CD) and a dendrimers, wherein the permeation enhancer reduces peptide aggregation and increasing stability and solubility for the hepcidin analogue molecule.

[00462] Other embodiments of the invention provide a method for treating a subject with a hepcidin analogue of the present invention having an increased half-life. In one aspect, the present invention provides a hepcidin analogue having a half-life of at least several hours to one day *in vitro* or *in vivo* (e.g., when administered to a human subject) sufficient for daily (q.d.) or twice daily (b.i.d.) dosing of a therapeutically effective amount. In another embodiment, the hepcidin analogue has a half-life of three days or longer sufficient for

weekly (q.w.) dosing of a therapeutically effective amount. Further, in another embodiment, the hepcidin analogue has a half-life of eight days or longer sufficient for bi-weekly (b.i.w.) or monthly dosing of a therapeutically effective amount. In another embodiment, the hepcidin analogue is derivatized or modified such that is has a longer half-life as compared to the underivatized or unmodified hepcidin analogue. In another embodiment, the hepcidin analogue contains one or more chemical modifications to increase serum half-life.

[00463] When used in at least one of the treatments or delivery systems described herein, a hepcidin analogue of the present invention may be employed in pure form or, where such forms exist, in pharmaceutically acceptable salt form.

Dosages

[00464] The total daily usage of the hepcidin analogues and compositions of the present invention can be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular subject will depend upon a variety of factors including: a) the disorder being treated and the severity of the disorder; b) activity of the specific compound employed; c) the specific composition employed, the age, body weight, general health, sex and diet of the patient; d) the time of administration, route of administration, and rate of excretion of the specific hepcidin analogue employed; e) the duration of the treatment; f) drugs used in combination or coincidental with the specific hepcidin analogue employed, and like factors well known in the medical arts.

In particular embodiments, the total daily dose of the hepcidin analogues of the invention to be administered to a human or other mammal host in single or divided doses may be in amounts, for example, from 0.0001 to 300 mg/kg body weight daily or 1 to 300 mg/kg body weight daily. In certain embodiments, a dosage of a hepcidin analogue of the present invention is in the range from about 0.0001 to about 100 mg/kg body weight per day, such as from about 0.0005 to about 50 mg/kg body weight per day, such as from about 0.001 to about 10 mg/kg body weight per day, e.g. from about 0.01 to about 1 mg/kg body weight per day, administered in one or more doses, such as from one to three doses.

[00465] In various embodiments, a hepcidin analogue of the invention may be administered continuously (e.g. by intravenous administration or another continuous drug administration method), or may be administered to a subject at intervals, typically at regular time intervals, depending on the desired dosage and the pharmaceutical composition selected by the skilled

practitioner for the particular subject. Regular administration dosing intervals include, e.g., once daily, twice daily, once every two, three, four, five or six days, once or twice weekly, once or twice monthly, and the like.

[00466] Such regular hepcidin analogue administration regimens of the invention may, in certain circumstances such as, e.g., during chronic long-term administration, be advantageously interrupted for a period of time so that the medicated subject reduces the level of or stops taking the medication, often referred to as taking a "drug holiday." Drug holidays are useful for, e.g., maintaining or regaining sensitivity to a drug especially during long-term chronic treatment, or to reduce unwanted side-effects of long-term chronic treatment of the subject with the drug. The timing of a drug holiday depends on the timing of the regular dosing regimen and the purpose for taking the drug holiday (e.g., to regain drug sensitivity and/or to reduce unwanted side effects of continuous, long- term administration). In some embodiments, the drug holiday may be a reduction in the dosage of the drug (e.g. to below the therapeutically effective amount for a certain interval of time). In other embodiments, administration of the drug is stopped for a certain interval of time before administration is started again using the same or a different dosing regimen (e.g. at a lower or higher dose and/or frequency of administration). A drug holiday of the invention may thus be selected from a wide range of time-periods and dosage regimens. An exemplary drug holiday is two or more days, one or more weeks, or one or more months, up to about 24 months of drug holiday. So, for example, a regular daily dosing regimen with a peptide, a peptide analogue, or a dimer of the invention may, for example, be interrupted by a drug holiday of a week, or two weeks, or four weeks, after which time the preceding, regular dosage regimen (e.g. a daily or a weekly dosing regimen) is resumed. A variety of other drug holiday regimens are envisioned to be useful for administering the hepcidin analogues of the invention.

[00467] Thus, the hepcidin analogues may be delivered via an administration regime which comprises two or more administration phases separated by respective drug holiday phases.

[00468] During each administration phase, the hepcidin analogue is administered to the recipient subject in a therapeutically effective amount according to a pre-determined administration pattern. The administration pattern may comprise continuous administration of the drug to the recipient subject over the duration of the administration phase. Alternatively, the administration pattern may comprise administration of a plurality of doses

of the hepcidin analogue to the recipient subject, wherein said doses are spaced by dosing intervals.

[00469] A dosing pattern may comprise at least two doses per administration phase, at least five doses per administration phase, at least 10 doses per administration phase, at least 20 doses per administration phase, at least 30 doses per administration phase, or more.

[00470] Said dosing intervals may be regular dosing intervals, which may be as set out above, including once daily, twice daily, once every two, three, four, five or six days, once or twice weekly, once or twice monthly, or a regular and even less frequent dosing interval, depending on the particular dosage formulation, bioavailability, and pharmacokinetic profile of the hepcidin analogue of the present invention.

[00471] An administration phase may have a duration of at least two days, at least a week, at least 2 weeks, at least 4 weeks, at least a month, at least 2 months, at least 3 months, at least 6 months, or more.

[00472] Where an administration pattern comprises a plurality of doses, the duration of the following drug holiday phase is longer than the dosing interval used in that administration pattern. Where the dosing interval is irregular, the duration of the drug holiday phase may be greater than the mean interval between doses over the course of the administration phase. Alternatively the duration of the drug holiday may be longer than the longest interval between consecutive doses during the administration phase.

[00473] The duration of the drug holiday phase may be at least twice that of the relevant dosing interval (or mean thereof), at least 3 times, at least 4 times, at least 5 times, at least 10 times, or at least 20 times that of the relevant dosing interval or mean thereof.

[00474] Within these constraints, a drug holiday phase may have a duration of at least two days, at least a week, at least 2 weeks, at least 4 weeks, at least a month, at least 2 months, at least 3 months, at least 6 months, or more, depending on the administration pattern during the previous administration phase.

[00475] An administration regime comprises at least 2 administration phases. Consecutive administration phases are separated by respective drug holiday phases. Thus the administration regime may comprise at least 3, at least 4, at least 5, at least 10, at least 15, at least 20, at least 25, or at least 30 administration phases, or more, each separated by respective drug holiday phases.

[00476] Consecutive administration phases may utilise the same administration pattern, although this may not always be desirable or necessary. However, if other drugs or active agents are administered in combination with a hepcidin analogue of the invention, then typically the same combination of drugs or active agents is given in consecutive administration phases. In certain embodiments, the recipient subject is human.

[00477] In some embodiments, the present invention provides compositions and medicaments comprising at least one hepcidin analogue as disclosed herein. In some embodiments, the present invention provides a method of manufacturing medicaments comprising at least one hepcidin analogue as disclosed herein for the treatment of diseases of iron metabolism, such as iron overload diseases. In some embodiments, the present invention provides a method of manufacturing medicaments comprising at least one hepcidin analogue as disclosed herein for the treatment of diabetes (Type I or Type II), insulin resistance, or glucose intolerance. Also provided are methods of treating a disease of iron metabolism in a subject, such as a mammalian subject, and preferably a human subject, comprising administering at least one hepcidin analogue, or composition as disclosed herein to the subject. In some embodiments, the hepcidin analogue or the composition is administered in a therapeutically effective amount. Also provided are methods of treating diabetes (Type I or Type II), insulin resistance, or glucose intolerance in a subject, such as a mammalian subject, and preferably a human subject, comprising administering at least one hepcidin analogue or composition as disclosed herein to the subject. In some embodiments, the hepcidin analogue or composition is administered in a therapeutically effective amount.

[00478] In some embodiments, the invention provides a process for manufacturing a hepcidin analogue or a hepcidin analogue composition (e.g., a pharmaceutical composition), as disclosed herein.

[00479] In some embodiments, the invention provides a device comprising at least one hepcidin analogue of the present invention, or pharmaceutically acceptable salt or solvate thereof for delivery of the hepcidin analogue to a subject.

[00480] In some embodiments, the present invention provides methods of binding a ferroportin or inducing ferroportin internalization and degradation which comprises contacting the ferroportin with at least one hepcidin analogue, or hepcidin analogue composition as disclosed herein.

[00481] In some embodiments, the present invention provides kits comprising at least one hepcidin analogue, or hepcidin analogue composition (e.g., pharmaceutical composition) as disclosed herein packaged together with a reagent, a device, instructional material, or a combination thereof.

[00482] In some embodiments, the present invention provides a method of administering a hepcidin analogue or hepcidin analogue composition (e.g., pharmaceutical composition) of the present invention to a subject *via* implant or osmotic pump, by cartridge or micro pump, or by other means appreciated by the skilled artisan, as well-known in the art.

[00483] In some embodiments, the present invention provides complexes which comprise at least one hepcidin analogue as disclosed herein bound to a ferroportin, preferably a human ferroportin, or an antibody, such as an antibody which specifically binds a hepcidin analogue as disclosed herein, Hep25, or a combination thereof.

[00484] In some embodiments, the hepcidin analogue of the present invention has a measurement (e.g., an EC50) of less than 500 nM within the Fpn internalization assay. As a skilled person will realize, the function of the hepcidin analogue is dependent on the tertiary structure of the hepcidin analogue and the binding surface presented. It is therefore possible to make minor changes to the sequence encoding the hepcidin analogue that do not affect the fold or are not on the binding surface and maintain function. In other embodiments, the present invention provides a hepcidin analogue having 85% or higher (e.g., 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5%) identity or homology to an amino acid sequence of any hepcidin analogue described herein that exhibits an activity (e.g., hepcidin activity), or lessens a symptom of a disease or indication for which hepcidin is involved.

[00485] In other embodiments, the present invention provides a hepcidin analogue having 85% or higher (e.g., 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5%) identity or homology to an amino acid sequence of any hepcidin analogue presented herein, e.g., in any one of Tables 2-4 or Tables 6-10, 12, 14, or 15, or a peptide according to any one of the formulae described herein, e.g., formulae I, II, III, IV, V, and VI.

[00486] In some embodiments, a hepcidin analogue of the present invention may comprise functional fragments or variants thereof that have at most 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid substitutions compared to one or more of the specific peptide analogue sequences recited herein.

[00487] In addition to the methods described in the Examples herein, the hepcidin analogue peptides and the peptide dimers of the present invention may be produced using methods known in the art including chemical synthesis, biosynthesis or in vitro synthesis using recombinant DNA methods, and solid phase synthesis. See e.g. Kelly & Winkler (1990) Genetic Engineering Principles and Methods, vol. 12, J. K. Setlow ed., Plenum Press, NY, pp. 1-19; Merrifield (1964) J Amer Chem Soc 85:2149; Houghten (1985) PNAS USA 82:5131-5135; and Stewart & Young (1984) Solid Phase Peptide Synthesis, 2ed. Pierce, Rockford, IL, which are herein incorporated by reference. The hepcidin analogues of the present invention may be purified using protein purification techniques known in the art such as reverse phase high-performance liquid chromatography (HPLC), ion-exchange or immunoaffinity chromatography, filtration or size exclusion, or electrophoresis. See Olsnes, S. and A. Pihl (1973) Biochem. 12(16):3121-3126; and Scopes (1982) Protein Purification, Springer- Verlag, NY, which are herein incorporated by reference. Alternatively, the hepcidin analogues of the present invention may be made by recombinant DNA techniques known in the art. Thus, polynucleotides that encode the polypeptides of the present invention are contemplated herein. In certain preferred embodiments, the polynucleotides are isolated. As used herein "isolated polynucleotides" refers to polynucleotides that are in an environment different from that in which the polynucleotide naturally occurs.

EXAMPLES

The following examples demonstrate certain specific embodiments of the present invention. The following examples were carried out using standard techniques that are well known and routine to those of skill in the art, except where otherwise described in detail. It is to be understood that these examples are for illustrative purposes only and do not purport to be wholly definitive as to conditions or scope of the invention. As such, they should not be construed in any way as limiting the scope of the present invention.

ABBREVIATIONS:

DCM: dichloromethane

DMF: N,N-dimethylformamide

NMP: N-methylpyrolidone

HBTU: O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate

HATU: 2-(7-aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate

DCC: Dicyclohexylcarbodiimide

NHS: N-hydoxysuccinimide

DIPEA: diisopropylethylamine

EtOH: ethanol

Et2O: diethyl ether

Hy: hydrogen

TFA: trifluoroacetic acid

TIS: triisopropylsilane

ACN: acetonitrile

HPLC: high performance liquid chromatography

ESI-MS: electron spray ionization mass spectrometry

PBS: phosphate-buffered saline

Boc: t-butoxycarbonyl

Fmoc: Fluorenylmethyloxycarbonyl

Acm: acetamidomethyl

IVA: Isovaleric acid (or Isovaleryl)

K( ): In the peptide sequences provided herein, wherein a compound or chemical group is presented in parentheses directly after a Lysine residue, it is to be understood that the compound or chemical group in the parentheses is a side chain conjugated to the Lysine residue. So, e.g., but not to be limited in any way, K-[(PEG8)]- indicates that a PEG8 moiety is conjugated to a side chain of this Lysine. For a few non-limiting examples of such a conjugated Lysines, please see, e.g., compounds 54 and 90.

Palm: Indicates conjugation of a palmitic acid (palmitoyl).

As used herein "C( )" refers to a cysteine residue involved in a particular disulfide bridge. For example, in Hepcidin, there are four disulfide bridges: the first between the two C(1) residues; the second between the two C(2) residues; the third between the two C(3) residues; and the fourth between the two C(4) residues. Accordingly, in some embodiments, the sequence for Hepcidin is written as follows:

Hy-DTHFPIC(1)IFC(2)C(3)GC(2)C(4)HRSKC(3)GMC(4)C(1)KT-OH (SEQ ID NO:335); and the sequence for other peptides may also optionally be written in the same manner.

EXAMPLE 1

SYNTHESIS OF PEPTIDE ANALOGUES

[00488] Unless otherwise specified, reagents and solvents employed in the following were available commercially in standard laboratory reagent or analytical grade, and were used without further purification.

Procedure for solid-phase synthesis of peptides

[00489] Peptide analogues of the invention were chemically synthesized using optimized 9-fluorenylmethoxy carbonyl (Fmoc) solid phase peptide synthesis protocols. For C-terminal amides, rink-amide resin was used, although wang and trityl resins were also used to produce C-terminal acids. The side chain protecting groups were as follows: Glu, Thr and Tyr: O-tButyl; Trp and Lys: t-Boc (t-butyloxycarbonyl); Arg: N-gamma-2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl; His, Gln, Asn, Cys: Trityl. For selective disulfide bridge formation, Acm (acetamidomethyl) was also used as a Cys protecting group. For coupling, a four to ten-fold excess of a solution containing Fmoc amino acid, HBTU and DIPEA (1:1:1.1) in DMF was added to swelled resin [HBTU: O-(Benzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium hexafluorophosphate; DIPEA: diisopropylethylamine; DMF: dimethylformamide]. HATU (O-(7-azabenzotriazol-1-yl)-1,1,3,3,-tetramethyluronium hexafluorophosphate) was used instead of HBTU to improve coupling efficiency in difficult regions. Fmoc protecting group removal was achieved by treatment with a DMF, piperidine (2:1) solution.

Procedure for cleavage of peptides off resin

[00490] Side chain deprotection and cleavage of the peptide analogues of the invention (e.g., Compound No. 2) was achieved by stirring dry resin in a solution containing trifluoroacetic acid, water, ethanedithiol and tri-isopropylsilane (90:5:2.5:2.5) for 2 to 4 hours. Following TFA removal, peptide was precipitated using ice-cold diethyl ether. The solution was centrifuged and the ether was decanted, followed by a second diethyl ether wash. The peptide was dissolved in an acetonitrile, water solution (1:1) containing 0.1% TFA (trifluoroacetic acid) and the resulting solution was filtered. The linear peptide quality was assessed using electrospray ionisation mass spectrometry (ESI-MS).

Procedure for purification of peptides

[00491] Purification of the peptides of the invention (e.g., Compound No. 2) was achieved using reverse-phase high performance liquid chromatography (RP-HPLC). Analysis was performed using a C18 column (3μm, 50 x 2mm) with a flow rate of 1 mL/min. Purification of the linear peptides was achieved using preparative RP-HPLC with a C18 column (5μm, 250 x 21.2 mm) with a flow rate of 20 mL/min. Separation was achieved using linear gradients of buffer B in A (Buffer A: Aqueous 0.05% TFA; Buffer B: 0.043% TFA, 90% acetonitrile in water).

Procedure for oxidation of peptides

[00492] Method A (Single disulfide oxidation). Oxidation of the unprotected peptides of the invention was achieved by adding drop-wise iodine in MeOH (1 mg per 1 mL) to the peptide in a solution (ACN: H_2O, 7: 3, 0.5% TFA). After stirring for 2 min, ascorbic acid portion wise was added until the solution was clear and the sample was immediately loaded onto the HPLC for purification.

[00493] Method B (Selective oxidation of two disulfides). When more than one disulfide was present, selective oxidation was often performed. Oxidation of the free cysteines was achieved at pH 7.6 NH_4CO_3 solution at 1mg /10 mL of peptide. After 24 h stirring and prior to purification the solution was acidified to pH 3 with TFA followed by lyophilization. The resulting single oxidized peptides (with ACM protected cysteines) were then oxidized / selective deprotection using iodine solution. The peptide (1 mg per 2 mL) was dissolved in MeOH/H_20, 80:20 iodine dissolved in the reaction solvent was added to the reaction (final concentration: 5 mg/mL) at room temperature. The solution was stirred for 7 minutes before ascorbic acid was added portion wise until the solution is clear. The solution was then loaded directly onto the HPLC.

[00494] Method C (Native oxidation). When more than one disulfide was present and when not performing selective oxidations, native oxidation was performed. Native oxidation was achieved with 100 mM NH4CO3 (pH7.4) solution in the presence of oxidized and reduced glutathione (peptide/GSH/GSSG, 1:100:10 molar ratio) of (peptide: GSSG: GSH, 1:10, 100). After 24 h stirring and prior to RP-HPLC purification the solution was acidified to pH 3 with TFA followed by lyophilization.

[00495] Procedure of cysteine oxidation to produce dimers. Oxidation of the unprotected peptides of the invention was achieved by adding drop-wise iodine in MeOH (1 mg per 1 mL) to the peptide in a solution (ACN: H2O, 7: 3, 0.5% TFA). After stirring for 2 min, ascorbic acid portion wise was added until the solution was clear and the sample was immediately loaded onto the HPLC for purification.

Procedure for dimerization.

[00496] Glyoxylic acid (DIG), IDA, or Fmoc-β-Ala-IDA was pre-activated as the N-hydoxysuccinimide ester by treating 1 equivalent (abbreviated "eq") of the acid with 2.2 eq of both N-hydoxysuccinimide (NHS) and dicyclohexyl carbodiimide (DCC) in NMP (N-methyl pyrolidone) at a 0.1 M final concentration. For the PEG13 and PEG25 linkers, these chemical entities were purchased pre-formed as the activated succinimide ester. The activated ester ~ 0.4 eq was added slowly to the peptide in NMP (1mg/mL) portionwise. The solution was left stirring for 10 min before 2-3 additional aliquots of the linker ~0.05 eq were slowly added. The solution was left stirring for a further 3 h before the solvent was removed under *vaccuo* and the residue was purified by reverse phase HPLC. An additional step of stirring the peptide in 20% piperidine in DMF (2 x 10 min) before an additional reverse phase HPLC purification was performed.

[00497] One of skill in the art will appreciate that standard methods of peptide synthesis may be used to generate the compounds of the invention.

Linker activation and dimerization

[00498] Peptide monomer subunits were linked to form hepcidin analogue peptide dimers as described below.

[00499] Small Scale DIG Linker Activation Procedure: 5mL of NMP was added to a glass vial containing IDA diacid (304.2 mg, 1 mmol), N-hydroxysuccinimide (NHS, 253.2 mg, 2.2 eq. 2.2mmol) and a stirring bar. The mixture was stirred at room temperature to completely dissolve the solid starting materials. N, N'-Dicyclohexylcarbodiimide (DCC, 453.9mg, 2.2 eq., 2.2 mmol) was then added to the mixture. Precipitation appeared within 10 min and the reaction mixture was further stirred at room temperature overnight. The reaction mixture was then filtered to remove the precipitated dicyclohexylurea (DCU). The activated linker was kept in a closed vial prior to use for dimerization. The nominal concentration of the activated linker was approximately 0.20 M.

[00500] For dimerization using PEG linkers, there was no pre-activation step involved. Commercially available pre-activated bi-functional PEG linkers were used.

[00501] Dimerization Procedure: 2mL of anhydrous DMF was added to a vial containing peptide monomer (0.1 mmol). The pH of the peptide was the adjusted to 8~9 with DIEA. Activated linker (IDA or PEG13, PEG 25) (0.48eq relative to monomer, 0.048 mmol) was then added to the monomer solution. The reaction mixture was stirred at room temperature for one hour. Completion of the dimerization reaction was monitored using analytical HPLC. The time for completion of dimerization reaction varied depending upon the linker. After completion of reaction, the peptide was precipitated in cold ether and centrifuged. The supernatant ether layer was discarded. The precipitation step was repeated twice. The crude dimer was then purified using reverse phase HPLC (Luna C18 support, 10u, 100A, Mobile phase A: water containing 0.1% TFA, mobile phase B: Acetonitrile (ACN) containing 0.1% TFA, gradient of 15%B and change to 45%B over 60min, flow rate 15ml/min). Fractions containing pure product were then freeze-dried on a lyophilizer.

EXAMPLE 2

ACTIVITY OF PEPTIDE ANALOGUES

[00502] Peptide analogues were tested *in vitro* for induction of internalization of the human ferroportin protein. Following internalization, the peptides are degraded. The assay measures a decrease in fluorescence of the receptor.

[00503] The cDNA encoding the human ferroportin (SLC40A1) was cloned from a cDNA clone from Origene (NM_014585). The DNA encoding the ferroportin was amplified by PCR using primers also encoding terminal restriction sites for subcloning, but without the termination codon. The ferroportin receptor was subcloned into a mammalian GFP expression vector containing a neomycin (G418) resistance marker in such that the reading frame of the ferroportin was fused in frame with the GFP protein. The fidelity of the DNA encoding the protein was confirmed by DNA sequencing. HEK293 cells were used for transfection of the ferroportin-GFP receptor expression plasmid. The cells were grown according to standard protocol in growth medium and transfected with the plasmids using Lipofectamine (manufacturer's protocol, Invitrogen). The cells stably expressing ferroportin-GFP were selected using G418 in the growth medium (in that only cells that have taken up and incorporated the cDNA expression plasmid survive) and sorted several times on a

Cytomation MoFlo ™ cell sorter to obtain the GFP-positive cells (488nm/530 nm). The cells were propagated and frozen in aliquots.

[00504] To determine activity of the hepcidin analogues (compounds) on the human ferroportin, the cells were incubated in 96 well plates in standard media, without phenol red. Compound was added to desired final concentration for at least 18 hours in the incubator. Following incubation, the remaining GFP-fluorescence was determined either by whole cell GFP fluorescence (Envision plate reader, 485 / 535 filter pair), or by Beckman Coulter Quanta ™ flow cytometer (express as Geometric mean of fluorescence intensity at 485nm/525nm). Compound was added to desired final concentration for at least 18 hours but no more than 24 hours in the incubator.

[00505] Reference compounds included native Hepcidin, Mini-Hepcidin, and R1-Mini-Hepcidin, which is an analog of mini-hepcidin. The "RI" in RI-Mini-Hepcidin refers to Retro Inverse. A retro inverse peptide is a peptide with a reversed sequence in all D amino acids. An example is that Hy-Glu-Thr-His-NH2 becomes Hy-DHis-DThr-DGlu-NH2. The EC_{50} of these reference compounds for ferroportin degradation was determined according to the activity assay described above. These peptides served as control standards for many of the subsequence studies.

Table 11. Reference compounds

Name	**Sequence**	**Potency EC50 (nM)**
Hepcidin	Hy-DTHFPIC(1)IFC(2)C(3)GC(2)C(4)HRSKC(3)GMC(4)C(1)KT-OH (SEQ ID NO: 335)	34
Mini-Hepcidin 1-9	Hy-DTHFPICIF-NH_2 (SEQ ID NO: 545)	712
RI-Mini Hepcidin	Hy-DPhe-DIle-DCys-DIle-DPro-DPhe-DHis-DThr-DAsp-NH_2 (SEQ ID NO: 546)	> 10 μM

The EC_{50} values determined for various peptide analogues of the present invention are provided below and in other tables herein.

Table 12. Activity of Illustrative Peptide Analogues

CMPD No.	SEQ ID No.	Sequence	Potency EC_{50} (nM)
1	28	Hy-DTHFPCIIF-NH_2	133
2	29	Isovaleric acid-DTHFPICIFGPRSKGWVC-NH_2	5
3	30	Isovaleric acid-DTHFPCIIFGPRSRGWVCK-NH_2	15
4	31	Isovaleric acid-DTHFPCIIFGPRSKGWVC-NH_2	19
5	32	[Ida]-TH-[Dpa]-[bhPro]-ICIFGPRSKGWVCM-NH_2	17
6	33	Isovaleric acid-DTHFPCIFFGPRSKGWVCK-NH_2	23
7	34	Isovaleric acid-DTHFPCIIFGPRSKGWTCK-NH_2	24
8	35	[Ida]-TH-[Dpa]-[bhPro]-CIIFGPRSRGWVCK-NH_2	29
9	36	Isovaleric acid-DTHFPCIKFGPRSKGWVCK-NH_2	32
10	37	Isovaleric acid-DTHFPCIQFGPRSKGWVCK-NH_2	35
11	38	Isovaleric acid-DTHFPCIIFGPRSKGWVCK-NH_2	9
12	39	Hy-DTHFPIC_1IFVC_2GHRSIC_2YRRC_1R-NH_2	77
13	40	Isobutyric acid-DTHFPIC_1IFVC_2HRSKGC_2YRRC_1R-NH_2	63
14	41	Hy-DTHFPIC_1IFVC_2HRSKGC_2YRAC_1-NH_2	69
15	42	Isovaleric acid-DTHFPCIEFGPRSKGWVCK-NH_2	79
16	43	Hy-DTHFPICIFGPRAKGWVCM-NH_2	88
17	44	Isobutyric acid-DTHFPIC_1IFVC_2HRSKGC_2YRRC_1R-NH_2	93
18	45	Hy-DTHFPICIFGPRSKGWVCM-NH_2	125
19	46	Hy-DTHFPIC_1IFVC_2HRSKGC_2YRRC_1R-NH_2	140
20	47	Hy-DTHFPICIFGPRSRGWVCK-NH_2	101
21	48	Hy-DTHFPCIIFGPRSKGWVCM-NH_2	46

22	49	Hy-DTHFPICIFAPRSKGWVCM-NH_2	9430
23	50	Hy-DTHFPICIFGPRSKGWVCM-OH	131
24	51	Hy-DTHFPCIQF-NH_2	138
25	52	Hy-DTHFPIC_1IFVC_2GHRSKGC_2YRRC_1R-NH_2	144
26	53	Hy-DTHFAICIFGPRSKGWVCM-NH_2	147
27	54	Hy-DTHFPICIFGPHRSKGWVCM-NH_2	149
28	55	Hy-DTHFPICIFGPRAKGWVCM-NH_2	88
29	56	Hy-DTHFPACIFGPRSKGWVCM-NH_2	157
30	57	Hy-DTHFPC_1IIFVC_2HRPKGC_2YRRVC_1R-NH_2	173
31	58	Hy-DTHFPICIFGPRSKAWVCM-NH_2	175
32	59	Hy-DTHFPIC_1IFVC_2GHRGKGC_2YRRC_1R-NH_2	182
33	60	Hy-ATHFPICIFGPRSKGWVCM-NH_2	184
34	61	Hy-DTHFPICIFGPASKGWVCM-NH_2	206
35	62	Hy-DTHFPIC_1IFVC_2HRSKGC_2YARC_1-NH_2	214
36	63	Ac-DTHFPICIFGPRSKGWVCM-NH_2	239
37	64	Hy-DTHFPICIFGPRSAGWVCM-NH_2	239
38	65	Hy-DTHAPICIFGPRSKGWVCM-NH_2	254
39	66	Hy-DTHFPIC_1IFVC_2HRSKGC_2YRRC_1-NH_2	256
40	67	pGlu-THFPIC_1IFVC_2HRSKGC_2YRRC_1R-NH_2	260
41	68	Ac-DTHFPICIFKPRSKGWVCM-NH_2	262
42	69	Hy-DTHFPIC_1IFVC_2GHRSKGC_2YMRC_1KT-NH_2	265
43	70	Hy-DAHFPICIFGPRSKGWVCM-NH_2	265
44	71	Hy-DTHFPIC_1IFVC_2YRGIC_2YRRC_1R-NH_2	269

45	72	Ac-DTHFPICIFGPRSKGWVCM-NH_2	272
46	73	Hy-[bhAsp]-THFPICIFGPRSKGWVC-NH_2	274
47	74	Hy-DTHFPICIFGPRSKGWACM-NH_2	313
48	75	[Ida]-TH-[Dpa]-[bhPro]-RCR-[bhPhe]-GPRSKGWVCM-NH_2	331
49	76	Hy-DTHFPCIRF-NH_2	334
50	77	Isovaleric acid-THFPCIIFGPRSKGWVCM-NH_2	345
51	78	Hy-DTHFPCIAF-NH_2	382
52	79	Hy-DAHFPCIIF-NH_2	388
53	80	Hy-DTHFPIC_1IFVC_2HRPKGC_2YRRC_1P-NH2	393
54	81	Ac-DTHFPICIFKPRS-K(m-PEG8)-GWVCM-NH_2	479
55	82	Hy-DTHFPCIIFK-NH_2	419
56	83	Hy-DTHFPCIFF-NH_2	441
57	84	Hy-DTHFPICIFGPRSK-K(m-PEG8)-WVCM-NH_2	462
58	85	Ac-DTHFPICIFGPRSKKWVCM-NH_2	472
59	86	Hy-DTHFPIC_1IFC_2PWGM$C_2$$C_1$K-NH2	495
60	87	Hy-DTAFPICIFGPRSKGWVCM-NH_2	498
65	88	Hy-DTHFPIC_1IFVC_2YRGIC_1YMRC_2KT-NH_2	763
66	89	Hy-DTHFPICIFGPRSKGAVCM-NH_2	520
67	90	Hy-DTHFPICIAGPRSKGWVCM-NH_2	2466
68	91	Hy-DTHFPICAFGPRSKGWVCM-NH_2	>10 μM
69	92	Hy-DTHFPIAIFGPRSKGWVAM-NH_2	>10 μM
70	93	Hy-DTHFPCRRFGPRSKGWVC-NH_2	> 10 μM
71	94	[Ida]-THF-[bhPro]-CRR-[bhPhe]-GPRSKGWVC-NH_2	N/A

73	96	Hy-DTHFPC$_1$IIFVC$_2$HRSKGC$_2$YWAVC$_1$-NH$_2$	2640
74	97	Hy-DTHFP-(D)Cys$_1$-IIFVC$_2$HRSKGC$_2$YWAV-(D)Cys$_1$-F-NH$_2$	356
75	98	Hy-DTHFPC$_1$IIFVC$_2$HRSKGC$_2$YWAVC$_1$FW-NH$_2$	>10 μM
76	99	Ac-DTHFPICIF-K-[(m-PEG8)]--PRSKGWVCM-NH$_2$	610
78	101	Hy-DTH-[Dpa]-PCIIFGPRSRGWVCK-NH$_2$	> 1 μM
79	102	Hy-DTHF-[bhPro]-CIIFGPRSRGWVCK-NH $_2$	> 1 μM
80	103	Hy-DTHFPCIIFGPRSRGWRCK-NH$_2$	> 1 μM
81	104	Hy-DTHFPCIRFGPRSRGWVCK-NH$_2$	> 1 μM
82	105	Hy-DTHFPCIRFGPRSRGWRCK-NH$_2$	> 1 μM
83	106	Hy-DTHFPCIIFGPRSRGWVCK-NH$_2$	> 1 μM
84	107	Hy-DTHFPCIIFGPRSRGVCK-NH$_2$	> 1 μM
85	108	Hy-DTHFPCIYFGPRSKGWVCK-NH$_2$	705
86	109	Hy-DTHFPCIIFGPRSKGWVCK-NH$_2$	> 1 μM
87	110	Hy-DTHFPCIIFGPRARGWVCK-NH$_2$	> 1 μM
88	111	Octanoic acid-DTHFPCIIFGPRSRGWVCK-NH$_2$	> 1 μM
89	112	Palm-PEG11-DTHFPCIIFGPRSRGWVCK-NH$_2$	> 1 μM
90	113	Ac-DTHFPICIF-K(2K PEG)-PRSKGWVCK-NH$_2$	107
91	114	Hy-DTHFPCIIFGPRSKGWKCK-NH$_2$	Not Tested
92	115	Hy-DTHFPCIKFGPRSKGWKCK-NH$_2$	Not Tested
93	116	Isovaleric acid-DTHFPCLIFGPRSKGWVCK-NH$_2$	19
94	117	Isovaleric acid-DTHFPCVIFGPRSKGWVCK-NH$_2$	41
95	118	Isovaleric acid-DTHFPCSIFGPRSKGWVCK-NH$_2$	78
96	119	Isovaleric acid-DTHFPCQIFGPRSKGWVCK-NH$_2$	157

97	120	Hy-THFPCIIFGPRSKGWVCK-NH_2	>10 μM
98	121	Isovaleric acid-THFPCIIFGPRSKGWVCK-NH_2	>10 μM
99	122	Hy-HFPCIIFGPRSKGWVCK-NH_2	>10 μM
100	123	Isovaleric acid-HFPCIIFGPRSKGWVCK-NH_2	>10 μM
101	124	Hy-DTHFPCISFGPRSKGWVCK-NH_2	> 1 μM
102	125	Hy-DTHFPCIKFGPRSKGWVCK-NH_2	> 1 μM
103	126	Hy-EDTHFPCIIFGPRSKGWVCK-NH_2	> 1 μM
105	128	Isovaleric acid-DTHFPCIIFEPRSKGWVCK-NH_2	10
106	129	Isovaleric acid-DTHFPCIIFSPRSKGWVCK-NH_2	44
107	130	Isovaleric acid-DTHFSCIIFGPRSKGWVCK-NH_2	50
108	131	Octanoic acid-PEG11-DTHFPCIIFGPRSRGWVCK-NH_2	> 1 μM
109	132	Isobutyric acid-PEG11-DTHFPCIIFGPRSRGWVCK-NH_2	> 1 μM
110	133	[Ida]-THFPCIIFGPRSRGWVCK-NH_2	> 300 nM
111	134	Isovaleric acid-DTHFPCIIFGPKSKGWVCK-NH_2	12
112	135	Isovaleric acid-DTHFPCIKFGPKSKGWVCK-NH_2	15
113	136	Isovaleric acid-DTHFPCIIFGPRSKGWCK-NH_2	15
114	137	Isovaleric acid-DTHFPCIIFGPRSKGVC-NH_2	18
115	138	Isovaleric acid-DTHFPCIIFGPRSKGCK-NH_2	21
117	140	Isovaleric acid-DTHFPC-[Dapa]-IFGPRSKGWDCK-NH_2	65
118	141	Isovaleric acid-DTHFPCI-[Dapa]-FGPRSKGWDCK-NH_2	17
119	142	Isovaleric acid-DTHFPC-[Dapa]-IFGPRSKGWECK-NH_2	151
120	143	Isovaleric acid-DTHFPCI-[Dapa]-FGPRSKGWECK-NH_2	15
121	144	Isovaleric acid-DTHFPCIKFGPRSKGWECK-NH_2	14

122	145	Isovaleric acid-DTHFGCIIFGPRSKGWVCK-NH_2	57
123	146	Hy-DTHFGCIIFGPRSKGWVCK-NH_2	>10 μM
124	147	Isovaleric acid-DTHFRCIIFGPRSKGWVCK-NH_2	106
125	148	Hy-DTHFRCIIFGPRSKGWVCK-NH_2	>10 μM
126	149	Isovaleric acid-DTHF-[Sarc]-CIIFGPRSKGWVCK-NH_2	31
127	150	Hy-DTHF-[Sarc]-CIIFGPRSKGWVCK-NH_2	>10 μM
128	151	Isovaleric acid-DTHF-[β-Ala]-CIIFGPRSKGWVCK-NH_2	264
129	152	Hy-DTHF-[β-Ala]-CIIFGPRSKGWVCK-NH_2	>10 μM
130	153	Isovaleric acid-DTHFKCIIFGPRSKGWVCK-NH_2	150
131	154	Hy-DTHFKCIIFGPRSKGWVCK-NH_2	>10 μM
132	155	Hy-THFPCIIFGPRSKGWVCM-NH_2	>1 μM
133	156	Hy-HFPCIIFGPRSKGWVCM-NH_2	>1 μM
134	157	Isovaleric acid-HFPCIIFGPRSKGWVCM-NH_2	>1 μM
135	158	Hy-DTHFPCISFGPRSKGWVCM-NH_2	545
136	159	Hy-DTHFPCIKFGPRSKGWVCM-NH_2	669
137	160	Hy-EDTHFPCIIFGPRSKGWVCM-NH_2	873
139	162	Hy-DTHFPCIIFEPRSKGWVCM-NH_2	N/A
140	163	Isovaleric acid-DTHFKCIEFGPRSKGWVCK-NH_2	>1 μM
141	164	Isovaleric acid-DTHFPCIIFGPRSKGWACK-NH_2	11
142	165	Isovaleric acid-DTHFPCIIFEPRSKGWVCK-NH_2	9
143	166	Isovaleric acid-DTHFPCIIFGPRSKGWVCKKKK-NH_2	24
144	167	Isovaleric acid-DTHFPCIIFEPRSKGWVCKKKK-NH_2	15
145	168	Isovaleric acid-DTHFPCIIFGPRSKGWVCKK-NH_2	9

146	169	Isovaleric acid-DTAFPCIIFGPRSKGWVCK-NH_2	24
147	170	Isovaleric acid-DTKFPCIIFGPRSKGWVCK-NH_2	20
148	171	Isovaleric acid-DTHFPC_1IIFVC_2HRPKGC_2YRRVC_1R-NH_2	2.2
149	172	Isovaleric acid-DTHFPCI-K(m-PEG8)-FGPRSKGWVCK-NH_2	9
150	173	Isovaleric acid-DTHFPCIKF-K(m-PEG8)-PRSKGWVCK-NH_2	7
151	174	Isovaleric acid-DTHFPCIKFGP-K(m-PEG8)-SKGWVCK-NH_2	13
152	175	Isovaleric acid-DTHFPCIKFGPRS-K(m-PEG8)-GWVCK-NH_2	16
153	176	Isovaleric acid-DTHFPCIKFGPRSKGWVC-K(m-PEG8)-NH_2	18
154	177	Isovaleric acid-DTHFPCIKFGPRSKGWTCK-NH_2	18
155	178	Isovaleric acid-DTHFPCIEFGPRSKGWTCK-NH_2	38
156	179	Isovaleric acid-DTHFPICIFGPRS-K(Betaine)-GWVC-NH_2	Not Tested
157	180	Isovaleric acid-DTHFPCIKFGPRS-K(Betaine)-GWVCK-NH_2	18
158	181	Isovaleric acid-DTHFPCI-K(Betaine)-FGPRSKGWVCK-NH_2	16
159	182	Isovaleric acid-DTHFPCIKFGPRSKGWVC-K(Betaine)-NH_2	17
160	183	Ac-DTHFPCIKFGPRSKGWVCK-NH_2	464
161	184	Isovaleric acid-PEG3-DTHFPCIKFGPRSKGWVCK-NH_2	666
162	185	Isobutyric acid-DTHFPCIKFGPRSKGWVCK-NH_2	41
163	186	Valeric acid-DTHFPCIKFGPRSKGWVCK-NH_2	64
164	187	Hy-VDTHFPCIKFGPRSKGWVCK-NH_2	146
165	188	Hy-LDTHFPCIKFGPRSKGWVCK-NH_2	107
166	189	Hexanoic acid-DTHFPCIKFGPRSKGWVCK-NH_2	36
167	190	5-Methylpentanoic acid-DTHFPCIKFGPRSKGWVCK-NH_2	99
168	191	Cyclohexanoic acid-DTHFPCIKFGPRSKGWVCK-NH_2	30

169	192	Heptanoic acid-DTHFPCIKFGPRSKGWVCK-NH_2	91
170	193	Octanoic acid-DTHFPCIKFGPRSKGWVCK-NH_2	183
171	194	Isovaleric acid-DTHFPCIIFGPRSKGWKCK-NH_2	48
172	195	Isovaleric acid-DTHFPCIIFGPRSKGWECK-NH_2	15
173	196	Isovaleric acid-DTHFPCRRFGPRSKGWVCK-NH_2	Not Tested
176	199	Isovaleric acid-DTHFPICIFGPRS-K(m-PEG8)-GWVC-NH_2	6
177	200	Isovaleric acid-DTHFPICIFGPRS-K-[(m-PEG4)]-GWVC-NH_2	6
178	201	Isovaleric acid-DTHFPCIIFGPRSRGWVC-K(m-PEG8)-NH_2	3
179	202	Isovaleric acid-DTHFPCIIFGPRSRGWVC-K-[(m-PEG4)]--NH_2	4
180	203	Isovaleric acid-DTHFPCIIFGPRSRGWVC-K(PEG2)-NH_2	9
181	204	Isovaleric acid-DTHFPCIKFEPRSKGWVCK-NH2	15
182	205	Isovaleric acid-DTHFPCIKFEPRSKGWTCK-NH_2	13
183	206	Isovaleric acid-DTHFPCIKFEPRSKGWCK-NH_2	17
184	207	Isovaleric acid-DTHFPCIKFEPRSKGCK-NH_2	23
185	208	Isovaleric acid-DTHFPCIFEPRSKGCK-NH_2	54
186	209	Isovaleric acid-DTHFPCIFEPRSKGWCK-NH_2	12
187	210	Isovaleric acid-DTHFPCIKFGPRSKCK-NH_2	21
188	211	Isovaleric acid-DTHFPCIKFGPRSCK-NH_2	30
189	212	Isovaleric acid-DTHFPCIKFGPRCK-NH_2	36
190	213	Isovaleric acid-DTHFPCIKFGPCK-NH_2	55
191	214	Isovaleric acid-DTHFPCIKFGCK-NH_2	97
192	215	Isovaleric acid-DTHFPCIKFCK-NH_2	48
193	216	Isovaleric acid-DTHFPCIKFC-NH_2	80

194	217	Isovaleric acid-DTHFPCI-K(Palm)-FGPRSKGWVCK-NH_2	4
195	218	Isovaleric acid-DTHFPCIKF-K(Palm)-PRSKGWVCK-NH_2	9
196	219	Isovaleric acid-DTHFPCIKFGP-K(Palm)-SKGWVCK-NH_2	2
197	220	Isovaleric acid-DTHFPCIKFGPRS-K(Palm)-GWVCK-NH_2	1
198	221	Isovaleric acid-DTHFPCIKFGPRSKGWVC-K(Palm)-NH_2	7
199	222	Isovaleric acid-DTHFPCI-K(PEG3-Palm)-FGPRSKGWVCK-NH_2	7
200	223	Isovaleric acid-DTHFPCIKF-K(PEG3-Palm)-PRSKGWVCK-NH_2	6
201	224	Isovaleric acid-DTHFPCIKFGP-K(PEG3-Palm)-SKGWVCK-NH_2	4
202	225	Isovaleric acid-DTHFPCIKFGPRS-K(PEG3-Palm)-GWVCK-NH_2	3
203	226	Isovaleric acid-DTHFPCIKFGPRSKGWVC-K(PEG3-Palm)-NH_2	4
204	227	Hy-DTHFPCI-K(IVA)-FGPRSKGWVCK-NH_2	>300 nM
205	228	Hy-DTHFPCIKF-K(IVA)-PRSKGWVCK-NH_2	>300 nM
206	229	Hy-DTHFPCIKFGP-K(IVA)-SKGWVCK-NH_2	624
207	230	Hy-DTHFPCIKFGPRS-K(IVA)-GWVCK-NH_2	318
208	231	Hy-DTHFPCIKFGPRSKGWVC-K(IVA)-NH_2	109
209	232	Hy-DTHFPCI-K(PEG3-IVA)-FGPRSKGWVCK-NH_2	342
210	233	Hy-DTHFPCIKF-K(PEG3-IVA)-PRSKGWVCK-NH_2	457
211	234	Hy-DTHFPCIKFGP-K(PEG3-IVA)-SKGWVCK-NH_2	>300 nM
212	235	Hy-DTHFPCIKFGPRS-K(PEG3-IVA)-GWVCK-NH_2	>300 nM
213	236	Hy-DTHFPCIKFGPRSKGWVC-K(PEG3-IVA)-NH_2	233
214	237	Isovaleric acid-DTHFPCIKFEPRSKKWVCK-NH_2	15
215	238	Hy-DTHFPCIKFGPRSKGWVCK-NH_2	>1 μM
216	239	Palm-DTHFPCIKFGPRSKGWVCK-NH_2	>1 μM

217	240	Palm-PEG3-DTHFPCIKFGPRSKGWVCK-NH_2	>1 μM
218	241	Isovaleric acid-DTHFPCI-K(isoglu-Palm)-FEPRSKGCK-NH_2	10
219	242	Isovaleric acid-DTHFPCIKF-K(isoglu-Palm)-PRSKGCK-NH_2	9
220	243	Isovaleric acid-DTHFPCIKFEP-K(isoglu-Palm)-SKGCK-NH_2	5
221	244	Isovaleric acid-DTHFPCIKFEPRS-K(isoglu-Palm)-GCK-NH_2	4
222	245	Isovaleric acid-DTHFPCIKFEPRSK-K(isoglu-Palm)-CK-NH_2	4
223	246	Isovaleric acid-DTHFPCIKFEPRSKGC-K(isoglu-Palm)-NH_2	5
224	247	Isovaleric acid-DTHFPCIKFEPRSKGCK-K(isoglu-Palm)-NH_2	4
225	248	Isovaleric acid-DTHFPCI-K(dapa-Palm)-FEPRSKGCK-NH_2	17
226	249	Isovaleric acid-DTHFPCIKF-K(dapa-Palm)-PRSKGCK-NH_2	14
227	250	Isovaleric acid-DTHFPCIKFEP-K(dapa-Palm)-SKGCK-NH_2	10
228	251	Isovaleric acid-DTHFPCIKFEPRS-K(dapa-Palm)-GCK-NH_2	7
229	252	Isovaleric acid-DTHFPCIKFEPRSK-K(dapa-Palm)-CK-NH_2	13
230	253	Isovaleric acid-DTHFPCIKFEPRSKGC-K(dapa-Palm)-K-NH_2	10
231	254	Isovaleric acid-DTHFPCIKFEPRSKGCK-K(dapa-Palm)-NH2	11
232	255	Isovaleric acid-DTHFPCIKFGPRSKGWVCK-NH_2	Not Tested
233	256	Isovaleric acid-AAHFPCIKFGPRSKGWVCK-NH_2	320
234	257	Isovaleric acid-ATHFPCIKFGPRSKGWVCK-NH_2	60
235	258	Isovaleric acid-DAHFPCIKFGPRSKGWVCK-NH_2	203
236	259	Isovaleric acid-DTHAPCIKFGPRSKGWVCK-NH_2	>500 nM
237	260	Isovaleric acid-DTHFPCIKAGPRSKGWVCK-NH_2	50
238	261	Isovaleric acid-DTHFPCIKFEPRSKGWVCK-OH	47
239	262	Isovaleric acid-DTHFPCIKFEPRSKGWECK-OH	101

240	263	Isovaleric acid-DTHFPCIIFEPRSKGWEC-OH	139
241	264	Isovaleric acid-DTHFPCIKFK(isoGlu-Palm)-PRSKGWECK-NH_2	6
242	265	Isovaleric acid-DTHFPCIKFEPK(isoGlu-Palm)-SKGWECK-NH_2	8
243	266	Isovaleric acid-DTHAPCIKFEPRSKGWECK-NH_2	>10 μM
244	267	Ida-THFPCIKFEPRSK-K(isoGlu-Palm)CK-NH_2	25
245	268	Isovaleric acid-DTHFPCI-K(isoGlu-Palm)-FEPRSKGWEC-OH	131
246	269	4,4-5,5-6,6,6-Heptafluorohexanoic acid-DTHFPCIKFGPRSKGWVCK-NH_2	480
247	270	Isovaleric acid-DTHFPCIKF-K(mysteric acid)-PRSKGWVC-NH_2	7
248	271	Isovaleric acid-DTHFPCIKF-K(lauric acid)-PRSKGWVC-NH_2	10
249	272	Isovaleric acid-DTHFPCIKF-K(decanoic acid)-PRSKGWVC-NH_2	22
250	273	Isovaleric acid-DTHFPCIKF-K(octanoic acid)-PRSKGWVC-NH_2	30
251	274	Isovaleric acid-DTHFPCIKF-K(hexanoic acid)-PRSKGWVC-NH_2	21
252	275	Isovaleric acid-DTHFPCIKF-K(butyric acid)-PRSKGWVC-NH_2	37
253	276	Isovaleric acid-DTHFPCIKF-K(Ac)-PRSKGWVC-NH_2	29
254	277	Ida-THFPCIKFEPRSKGWVC-K(mysteric acid)-NH_2	20
255	278	[Ida]-THFPCIKFEPRSKGWVC-K(lauric acid)-NH_2	52
256	279	[Ida]-THFPCIKFEPRSKGWVC-K(decanoic acid)-NH_2	116
257	280	[Ida]-THFPCIKFEPRSKGWVC-K(octanoic acid)-NH_2	129
258	281	[Ida]-THFPCIKFEPRSKGWVC-K(hexanoic acid)-NH_2	191
259	282	[Ida]-THFPCIKFEPRSKGWVC-K(butyric acid)-NH_2	355
260	283	[Ida]-THFPCIKFEPRSKGWVC-K(Ac)-NH_2	502
261	284	Isovaleric acid-HFPCIKFEPRSKGWVC-K(octanoic acid)-NH_2	>300 nM
262	285	Isovaleric acid-HFPCIKFEPRSKGWVC-K(lauric acid)-NH_2	77

263	286	Isovaleric acid-DTHFPCIKFEPHSKGCK-NH2	62
264	287	Isovaleric acid-DTHFPCIHFEPHSKGC-NH_2	118
265	288	Isovaleric acid-DTHFPCIKFEPHS-K(Albu)-GCK-NH_2	6
266	289	Isovaleric acid-DTHFPCIKFEPREKEC-NH_2	183
267	290	Isovaleric acid-DTAFPCIKFEPRSKEC-NH_2	>1 μM
268	291	Isovaleric acid-DTHFPCIKFECK-NH_2	107
269	292	Hy-DTHFPIAIFAAGICI-NH_2	>10 μM
270	293	Hy-DTHFPIAIFAAICI-NH_2	>10 μM
271	294	Hy-DTHFPIAIFAICI-NH_2	>10 μM
272	295	Hy-DTHFPIAIFICI-NH_2	>10 μM
273	296	Hy-DTHFPIAIICI-NH_2	>10 μM
274	297	Hy-DTHFPIAICI-NH_2	>10 μM
275	298	Hy-DTHFPIICI-NH_2	>10 μM
276	299	Hy-DTHICIAIF-NH_2	>10 μM
277	300	Hy-DTHCPIAIF-NH_2	>10 μM
278	301	Hy-DTHFPCIIA-NH_2	>1 μM
279	302	Hy-DTHFPCAIF-NH_2	>1 μM
280	303	Hy-DTHFACIIF-NH_2	>1 μM
281	304	Hy-DTHF-(D)-Ala-CIIF-NH_2	>10 μM
282	305	Hy-DTHAPCIIF-NH_2	>10 μM
283	306	Hy-DTAFPCIIF-NH_2	739 nM
284	307	Hy-ATHFPCIIF-NH2	>1 μM
285	308	[Ida]-THF-[bhPro]-CIIF-NH_2	>1 μM

286	310	Hy-DTHFPCIEF-NH_2	>1 μM
287	298	Hy-DTHFPCIEF-NH_2	>1 μM
288	311	Isovaleric acid-DTHFPCIIF-NH_2	16 nM
289	312	Isovaleric acid-DTHFPAIIF-NH2	Inactive
290	313	Isovaleric acid-DTHFPSIIF-NH2	Inactive
291	314	Isovaleric acid-DTHFPCIKF-NH_2	7 nM
293	316	Hy-DTHFPCIF-NH_2	52% at 1 μM
297	320	Hy-DTHFPCIKFF-NH_2	64% at 1 μM
298	321	Hy-YTHFPCIIF-NH_2	Not Tested
299	322	Hy-LTHFPCIIF-NH_2	64% at 1 μM
300	323	Hy-ETHFPCIIF-NH_2	77% at 1 μM
301	324	Hy-DRHFPCIIF-NH_2	Not Tested
302	325	Hy-DTKFPCIIF-NH_2	60% at 1 μM
303	326	Hy-DTHFECIIF-NH_2	Not Tested
304	327	Hy-DTHFPCIIK-NH_2	55% at 1 μM
305	328	Hy-DTHFPCIIR-NH_2	62% at 1μM
306	329	Hy-DTHFPCIEF-NH_2	Not Tested
307	330	Hy-DTHFPCIVF-NH_2	75% at 1 μM
308	331	Hy-DTHFPCILF-NH_2	89% at 1 μM
309	332	Hy-DTHFPCILK-NH_2	55% at 1 μM
310	333	Hy-DTHFPCIEK-NH_2	0% at 1 μM
355	369	Isovaleric acid-DTHFPCIKFEPRSKECK-NH_2	48
356	370	Isovaleric acid-DTHFPCIKFEPHSKECK-NH_2	181

357	371	Isovaleric acid-DTHFPCIKKEPHSKECK-NH_2	>1μM
358	372	Isovaleric acid-DTHFPCIKF-K(isoglu-Palm)-PHSKECK-NH_2	6
359	373	Isovaleric acid-DTHFPCIKFEPRECK-NH_2	64
360	374	Isovaleric acid-DTHFPCIKFEPHECK-NH_2	138
361	375	Isovaleric acid-DTHFPCIKFEPRCK-NH_2	29

	376	DTHFPICIFC	
	377	FPIC	
	378	HFPIC	
	379	HFPICI	
	380	HFPICIF	
	381	DTHFPIC	
	381	DTHFPICI	
	382	DTHFPICIF	
	383	DTHFPIAIFC	
	384	DTHAPICIF	
	385	DTHAPI-[C-StBu]-IF	
	386	DTHAPI-[C-tBu]-IF	
	387	DTHFPIAIF	
	388	DTHFPISIF	
	389	DTHFPI-([D)-Cys]-IF	
	390	DTHFPI-[homoCys]-IF	
	391	DTHFPI-[Pen]-IF	
	392	DTHFPI-[(D)-Pen]-IF	
	393	DTHFPI-[Dapa(AcBr)]-IF	
	394	CDTHFPICIF	
	395	DTHFPICIF-NHCH2CH2S	
	396	CHFPICIF	
	397	HFPICIF-NHCH2CH2S	

	398	D-[Tle]-H-[Phg]-[Oic]-[Chg]-C-[Chg]-F	
	399	D-[Tle]-HP-[Oic]-[Chg]-C-[Chg]-F	
	400	[(D)Phe]-[(D)Ile]-[(D)Cys]-[(D)Ile]-[(D)pro]-[(D)Phe]-[(D)His]-[(D)Thr]-[(D)Asp]	
	401	[(D)Phe]-[(D)Ile]-[(D)Cys]-[(D)Ile]-[(D)Pro]-[(D)Phe]-[(D)His]	
	402	Chenodeoxycholate-(Peg11)-[(D)Phe]-[(D)Ile]-[(D)Cys]-[(D)Ile]-[(D)Pro]-[(D)Phe]-[(D)His]-[(D)Thr]-[(D)Asp]	
	403	Ursodeoxycholate-(Peg11)-[(D)Phe]-[(D)Ile]-[(D)Cys]-[(D)Ile]-[(D)Pro]-[(D)Phe]-[(D)His]-[(D)Thr]-[(D)Asp]	
	404	F-[(D)Ile]-[(D)Cys]-[(D)Ile]-[(D)Pro]-[(D)Phe]-[(D)His]-[(D)Thr]-[(D)Asp]-(Peg11)-GYIPEAPRDGQAYVRKDGEWVLLSTFL	
	405	F-[(D)Ile]-[(D)Cys]-[(D)Ile]-[(D)pro]-[(D)Phe]-[(D)His]-[(D)Thr]-[(D)Asp]-$([GP-(Hyp)]_{10}$	
	406	Palmitoyl-(Peg11)-[(D)Phe]-[(D)Ile]-[(D)Cys]-[(D)Ile]-[(D)Pro]-[(D)Phe]-[(D)His]-[(D)Thr]-[(D)Asp]	
	407	2(Palmitoyl)-[Dapa]-(Peg11)-[(D)Phe]-[(D)Ile]-[(D)Cys]-[(D)Ile]-[(D)Pro]-[(D)Phe]-[(D)His]-[(D)Thr]-[(D)Asp]	
	408	DTH-[bhPhe]-PIICIF	
	409	DTH-[Dpa]-PICI.	
	410	DTH-[Bip]-PICIF	
	411	DTH[1-Nal]-PICIF	
	412	DTH-[bhDpa]-PICIF	
	413	DTHFP-ICI-bhPhe	
	414	DTHFPICI-[Dpa]	
	415	DTHFPICI-[Bip]	
	416	DTHFPICI-[1-Nal]	
	417	DTHFPICI-[bhDpa]	
	418	DTH-[Dpa]-PICI-[Dpa]	
	419	D-[Dpa]-PICIF	
	420	D-[Dpa]-PICI-[Dpa]	

	421	DTH-[Dpa]-P-[(D)Arg]-CR-[Dpa]	
	422	DTH-[Dpa]-P-[(D)Arg]-C-[(D)Arg]-[Dpa]	
	423	DTH-[Dpa]-[Oic]-ICIF	
	424	DTH-[Dpa]-[Oic]-ICI-[Dpa]	
	425	DTH-[Dpa]-PCCC-[Dpa]	
	426	DTHFPICIF-[(D)Pro]-PK	
	427	DTHFPICIF-[(D)Pro]-PR	
	428	DTHFPICIF-[bhPro]-PK	
	429	DTHFPICIF-[bhPro]-PR	
	430	DTHFPICIF-[(D)Pro]-[bhPro]-K	
	431	DTHFPICIF-[(D)-Pro]-[bhPro]-R	
	432	DTHFPICI-[bhPhe]-[(D)Pro]-PK	
	433	DTHFPICI-[bhPhe]-[(D)Pro]-PR	
	434	DTHFPICI-[bhPhe]-[(D)Pro]-[bhPro]-K	
	435	DTHFPICI-[bhPhe]-[(D)Pro]-[bhPro]-R	
	436	C-[Inp]-[(D)Dpa]-[Amc]-R-[Amc]-[Inp]-[Dpa]-Cysteamide	
	437	CP-[(D)Dpa]-[Amc]-R-[Amc]-[Inp]-[Dpa]-Cysteamide	
	438	C-[(D)Pro]-[(D)Dpa]-[Amc]-R-[Amc]-[Inp]-[Dpa]-Cysteamide	
	439	CG-[(D)Dpa]-[Amc]-R-[Amc]-[Inp]-[Dpa]-Cysteamide	
	440	Hy-DTHFPCAIF-NH_2	>1000
	441	Hy-DTHFPCRRF-NH_2	Not active
	442	[IDA]-TH-[Dpa]-[bhPro]CRR-[bhPhe]-NH_2	206
	443	Hy- DTHFPCEIF-NH_2	>1000

	444	Hy-DTHFPCFIF-NH_2	1191.8
	445	Hy- DTHFPCQIF-NH_2	>1000
	446	Hy-DTHFPCRIF-NH_2	>1000
	447	Hy- [pGlu]-THFPCRKF-NH_2	>1000
	448	Hy- DTHFPCLIF-NH_2	> 10 μM
	449	Hy-DTHFPCVIF-NH_2	81% at 10 uM
	450	Hy-DTHFPCEIF-NH_2	19% at 10 uM
	451	Hy-DTHFPCRIF-NH_2	31% at 10 uM
	452	Hy- DTHFPCKIF-NH_2	9% at 10 uM
	453	Hy- DTHFPCLF-NH_2	39% at 1 uM
	454	Hy- DTHFPCEF-NH_2	17% at 10 uM
	455	Hy-DTHFPCRF-NH_2	31% at 10 uM
	456	Hy-DTHFPRRFGPRSKGWVC-NH_2	>1000
	457	[IDA]-THF-[bhPro]-CRR-[bhPhe]GPRSKGWVC-NH_2	>1000
	458	Hy- DTHFPCIFGPRSKGWVC-NH_2	>1000
	459	Hy-DTHFPCRIFGPRSRGWVCK-NH_2	>1000
	460	Isovaleric acid-DTHFPCLIFGPRSKGWVCK-NH_2	19.2
	461	Isovaleric acid-DTHFPCVIFGPRSKGWVCK-NH_2	41
	462	Isovaleric acid-DTHFPCSIFGPRSKGWVCK-NH_2	78
	463	Isovaleric acid-DTHFPCQIFGPRSKGWVCK-NH_2	157
	464	Isovaleric acid-DTHFPCKIFGPRSKGWVCK-NH_2	86
	465	Isovaleric acid-DTHFPC-[Dapa]-IFGPRSKGWDCK-NH_2	65
	466	Isovaleric acid-DTHFPC-[Dapa]-IFGPRSKGWECK-NH_2	151

	467	Isovaleric acid-DTHFPCKIFGPRSKGWECK-NH_2	163
	468	Isovaleric acid-DTHFPCRRFGPRSKGWVCK-NH_2	>1000
	469	Isovaleric acid-DTHFPCTIFGPRSKGWVCK-NH_2	Not Tested
	470	Hy- DTHFPIAICI-NH_2	>10 μM
	471	Hy- DTHFPIICI-NH_2	>10 μM
	472	Hy- DTHICIAIF-NH_2	>10 μM
	473	Hy- DTHCPIAIF-NH_2	>10 μM
	474	Hy- ATHFPCIIF-NH_2	>1000
	475	Hy- ADHFPCIIF-NH_2	>1000
	476	Hy- DTHFPCIIFKC-NH_2	6398.0
	477	Hy- DTHFPCIIFAC-NH_2	>1000
	478	Hy- DTHFPCIIFAA-NH_2	59% at 1 uM
	479	Hy- DEHFPCIIF-NH_2	34% at 10 uM
	480	Hy- DPHFPCIIF-NH_2	64% at 10 uM
	481	Hy- DTHKPCIIF-NH_2	45 % at 10 uM
	482	Hy- DTHVPCIIF-NH_2	34% at 10 uM
	483	Hy- DTHFVCIIF-NH_2	50% at 10 uM
	484	Hy- DTHFPCIIY-NH_2	75% at 10 uM
	485	Hy- DTHFPCIIT-NH_2	23% at 1 uM
	486	Hy- DTHFPCILY-NH_2	85% at 1 uM

	487	Hy- DTHFPCIEY-NH_2	8% at 1 uM
	488	Isovaleric acid-DTHFPCIIFGPRSKG-[N-MeTrp]-VC-NH_2	32
	489	Isovaleric acid-DTHFPCIIF-[Sarc]-PRSKG-[N-MeTrp]-VC-NH_2	10
	490	Isovaleric acid-DTHFPCIIF-[Sarc]-PHSKG-[N-MeTrp]-VC-NH_2	9
	491	Isovaleric acid-DTHFPCIIFEPRSKHWVCK-NH_2	15
	492	Isovaleric acid-DTHFPCIIFEPRSKEWVCK-NH_2	19
	493	Isovaleric acid-DTHFPCIIFEPRSKLWVCK-NH_2	7
	494	Isovaleric acid-DTHFPCIIFEPRSKFWVCK-NH_2	10
	495	Isovaleric acid-DTHFPCIKFEPHSK-[Sarc]-CK-NH_2	28
	496	Isovaleric acid-DTHFPCIKFKPHSKEWVCE-NH_2	46
	497	Isovaleric acid-DTHFPCIKFEPRSKEWVCK-NH_2	20
	498	Isovaleric acid-DTHFPCIKFEPRSKLWVCK-NH_2	9
	499	Isovaleric acid-DTHFPCIKFEPRSKEWVCK-OH	46
	500	Isovaleric acid-DTHFPCIKFEPRS-K(isoGlu-octanoic acid)-ECK-NH_2	48
	501	Hy-DTHFPCIIFGPRSKGWAVCYW-NH_2	197
	502	Hy-DTHFPICIFGPHRSKGWVCM-NH_2	149
	503	Hy-DTHFPCIIFGPRSKGWVAC-NH_2	281
	504	Hy-DTHFP-[(D)Cys]-IIFGPRSKGWVA-[(D)Cys]-NH_2	>10 μM
	505	Hy-DTHFPCIIFGPRSKGWVACY-NH_2	>10 μM
	506	Hy-DTHFPCIIFGPRSRGHVCK-NH_2	>1000
	507	Hy-DTHFPCIIFGPRSKGWNCK-NH_2	>1000
	508	Hy-DTHFPCINFGPRSKGWVCK-NH_2	>1000

	509	Hy-DTHFPCIDFGPRSKGWVCK-NH_2	>1000
	510	Isovaleric acid-DTHFECIIFGPRSKGWVCK-NH_2	>1000
	511	Hy-DTHFPCIIFGGPRSRGWVCK-NH_2	520
	512	Hy-DTHFPCIIFGGPRSKGWNCK-NH_2	404
	513	Hy-DTHFPCIIFGGPRSKGWDCK-NH_2	679
	514	Isovaleric acid-DTHFPCIFEPRSKGTCK-NH_2	57
	515	Isovaleric acid-DTHFPCIIF-[PEG3]-C-NH_2	157
	516	Isovaleric acid-DTHAPCIKF-[Sarc]-PRSKGWECK-NH_2	>10 μM
	517	Isovaleric acid-DTHAPCIKFEPRSK-[Sarc]-WECK-NH_2	>10 μM
	518	Isovaleric acid-DTHAPCIKFEPRSKEWECK-NH_2	>10 μM
	519	Isovaleric acid-STHAPCIKFEPRSKGWECK-NH_2	>10 μM
	520	Isovaleric acid-SKHAPCIKFEPRSKGWECK-NH_2	>10 μM
	521	Isovaleric acid-DTHFPCIKFEPHSKEWVCK-NH_2	80
	522	Isovaleric acid-DTAFPCIKFEPRSKEC-NH_2	>10 μM
	523	Isovaleric acid-DTHFGCIKFEPRSKEWVCK-NH_2	>1000
	524	Isovaleric acid-DTEFPCIKFEPRSKEWVCK-NH_2	>1000
	525	Isovaleric acid-DTHFPCIKFEPRS-K(octanoic acid)-EWVCK-NH_2	62
	526	Isovaleric acid-ETHFPCIKFEPRSKEWVCK-NH_2	181

[00506] To determine whether a given peptide modifies the internalization and degradation of endogenous ferroportin, the protein levels and cellular distribution of ferroportin in hepatocytes and macrophages treated with the peptide may be assayed using Western blotting, immunohistochemistry and ferroportin antibodies known in the art.

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EXAMPLE 3

SERUM STABILITY ASSAY

[00507] Serum stability experiments were undertaken to complement the *in vivo* results and assist in the design of potent, stable Ferroportin agonists. Key peptides (10 μM) were incubated with pre-warmed human serum (Sigma), fresh rat serum or plasma at 37 degrees. Samples were taken at various time points up to 24 hours. The samples were separated from serum proteins and analysed for the presence of the peptide of interest using LC-MS. The amount of intact peptide in each sample was calculated using the analyte peak area in relation to the zero time point. Percent remaining at each timepoint is calculated based on the peak area response ratio of test to compound to internal standard. Time 0 is set to 100%, and all later timepoints are calculated relative to time 0. Half-lives are calculated by fitting to a first-order exponential decay equation using Graphpad. The full list of *ex vivo* stability human and rat is shown in Table 15.

Table 15. Examples of analogues possessing Serum /Plasma Half life

SEQ ID NO	Sequence	Rat serum t½ (h)	Rat plasma t½ (h)	Human serum t½ (h)
547	Hy-DTHFPICIFCCGCCHRSKCGMCCKT-OH (Hepcidin)			2.76 (variable)
545	Hy-DTHFPICIF-NH_2	-	-	0.1
548	Palm-PEG11-ficipfhtd-NH_2	-	-	0.06
28	Hy-DTHFPCIIF-NH_2	-	-	0.18
549	DTHFPICIFGPRSKGWVCM-NH_2	0.18	-	2.32
533	(Hy-DTHFPICIF-NH_2)$_2$	-	-	0.67
93	Hy-DTHFPCRRFGPRSKGWVC-NH_2	-	-	0.46
550	Ida-THF-[bhPro]-CRR-[bhPhe]-GPRSKGWVC-NH_2	-	-	1.14
551	Hy- [bhAsp]-THFPICIFGPRSKGWVC-NH_2	-	-	2.1
552	Hy-[bhAsp]-TH-[NMePhe]-PICIFGPRSKGWVC-NH_2	0.16	4	1.93
29	Isovaleric acid-DTHFPICIFGPRSKGWVC-NH_2	0.15	4	1.99
68	Ac-DTHFPICIFKPRSKGWVCM-NH_2	0.31	5.9	-
553	Ac-DTHFPICIF-K(m-PEG8)-PRSKGWVCM-NH_2	1.81	19.5	40
554	Ac-DTHFPICIFGPRS-K(m-PEG8)-GWVCM-NH_2	1.82	6	40
555	Ida-TH-Dpa-bhPro-CIIFGPRSRGWVCK-NH_2	-	-	0.51
556	Hy-IPFIDTCFHGPRSRGWVCK-NH_2	-	-	0.18
30	Isovaleric acid-DTHFPCIIFGPRSRGWVCK-NH_2	0.08	0.43	0.51

63	Ac-DTHFPICIFGPRSKGWVCM-NH_2	0.38	6	-
128	Isovaleric acid-DTHFPCIIFEPRSKGWVCK-NH_2	0.68	-	2.22
31	Isovaleric acid-DTHFPCIIFGPRSKGWVC-NH_2	0.13	4	0.94
557	Isovaleric acid-DTHFPCIIFGPRSKGVCK-NH_2	0.27	-	1.17
138	Isovaleric acid-DTHFPCIIFGPRSKGCK-NH_2	0.19	-	1.33
558	Isovaleric acid-DTHFPCIFGPRSKGWCK-NH_2	0.21	-	0.99
144	Isovaleric acid-DTHFPCIKFGPRSKGWECK-NH_2	0.38	-	1.19
38	Isovaleric acid-DTHFPCIIFGPRSKGWVCK-NH_2	0.14	-	-
36	Isovaleric acid-DTHFPCIKFGPRSKGWVCK-NH_2	0.14	-	0.57
37	Isovaleric acid-DTHFPCIQFGPRSKGWVCK-NH_2	0.12	-	0.61
42	Isovaleric acid-DTHFPCIEFGPRSKGWVCK-NH_2	0.15	-	0.74
172	Isovaleric acid-DTHFPCI-K(m-PEG8)-FGPRSKGWVCK-NH_2	0.32	-	1.13
173	Isovaleric acid-DTHFPCIKF-K(m-PEG8)-PRSKGWVCK-NH_2	0.42	-	1.35
175	Isovaleric acid-DTHFPCIKFGPRS-K(m-PEG8)-GWVCK-NH_2	1.16	-	11.09
176	Isovaleric acid-DTHFPCIKFGPRSKGWVC-K(m-PEG8)-NH_2	0.41	-	3.36
181	Isovaleric acid-DTHFPCI-K(Betaine)-FGPRSKGWVCK-NH_2	0.14	-	1.22
559	(Isovaleric acid-DTHFPCIIF-NH_2)$_2$	18	-	>24
560	Isovaleric acid-DTHFPICIFGPRS-K(m-PEG8)-GWVC-NH_2	1.62	-	15
561	Isovaleric acid-DTHFPICIFGPRS-K(m-PEG4)-GWVC-NH_2	1.1	-	12
562	(Isovaleric acid-DTHFPCIIFGPRSRGWVCK)$_2$-DIG-NH_2	0.59	-	9
563	Isovaleric acid-DTHFPICIFGPRSKG-[NMeTrp]-VC-NH_2	0.07	-	0.4
564	Isovaleric acid-DTHFPICIF-[Sar]-PRSKG-[NMeTrp]-VC-NH_2	0.24	-	1.36
565	Isovaleric acid-DTHFPICIF-[Sar]-PHSKG-[NMeTrp]-VC-NH_2	11.3	-	>24
207	Isovaleric acid-DTHFPCIKFEPRSKGCK-NH_2	2.12	-	8.06
218	Isovaleric acid-DTHFPCIKF-K(Palm)-PRSKGWVCK-NH_2	24	-	>24
220	Isovaleric acid-DTHFPCIKFGPRS-K(Palm)-GWVCK-NH_2	>24	-	>>24
223	Isovaleric acid-DTHFPCIKF-K(PEG3-Palm)-PRSKGWVCK-NH_2	3.95	-	22.2
228	DTHFPCIKF-K(IVA)-PRSKGWVCK-NH_2	0.19	-	0.31
233	DTHFPCIKF-K(PEG3-IVA)-PRSKGWVCK-NH_2	0.35	-	0.58
491	Isovaleric acid-DTHFPCIIFEPRSKHWVCK-NH_2	1.29	-	4.71

492	Isovaleric acid-DTHFPCIIFEPRSKEWVCK-NH_2	7.7	-	>24
493	Isovaleric acid-DTHFPCIIFEPRSKLWVCK-NH_2	3.7	-	>24
566	Isovaleric acid-DTHFPCIIFEPRSKKWVCK-NH_2	0.89	-	5.06
494	Isovaleric acid-DTHFPCIIFEPRSKFWVCK-NH_2	2.69	-	20
567	Isovaleric acid –DTHFPCIIF-PEG3-C-NH_2	>24	-	>>24
568	DIG-(DTHFPCIIF-NH_2)$_2$	>24	-	>>24
242	Isovaleric acid-DTHFPCIKF-K(Isoglu-Palm)-PRSKGCK-NH_2	16	-	>>24
569	Isovaleric acid-DTHFPCIKFK(dapa-Palm)PRSKGCK-NH_2	14	-	24

EXAMPLE 4

REDUCTION OF FREE PLASMA IRON IN RATS

[00508] To investigate whether the peptide analogues are effective in decreasing free Fe^{2+} in serum, Retro Inverse mini Hepcidin is used as a reference peptide. Although RI mini-Hep has a very low potency *in vitro*, it is highly active *in viv*o as reported by Presza et al. J Clin Invest. 2011.

[00509] At Day 1, the animals are monitored for free Fe^{2+} in serum. In order to reach a homogenous serum level, Fe^{2+} is analyzed and a homogenous cohort of 7 or 8 animals is randomized to each treatment group. At Day 2, an acute experiment is performed where the animals are subjected to intraperitoneal (i.p.) dosing of test compound and subsequent tail vein blood samples. Prior to dosing, the animals are put under a heating lamp for 3-5 minutes. Blood samples are drawn from the tail vein from all animals in order to determine serum iron levels prior to vehicle or compound dosing. Animals are dosed i.p. with 1 ml of test substance in vehicle or just vehicle and blood samples of 250 µl are drawn from each animal at t=0, 60, 120, 240, 360 min and 24 hours in the study of the reference compound. The dose response study performed with Retro Inverse (RI) mini-Hepcidin (Reference compound), and the efficacy study performed with test compounds are performed as separate experiments.

[00510] Analysis of Fe^{2+} from Day 0 and 1 is done at a later time point not later than 10 days after. The chemicals and equipment used are shown below in Table 13.

Table 13. Chemicals and equipment used

Drug Name	Cmpd. No.	SEQ ID No.	MW (g/mol)	Peptide Content Calculated %	Peptide Content Determined %	Purity %	Solvent
Isovaleric acid-DTHFPICIFGPRSKGWVC-NH_2	2	29	2144.52	86.2	86.2	90	Na-Acetate buffer
RI-Hepcidin1-9		546	1091.3	82.7	82.7	94.2	Strong PBS

[00511] Initially, all compounds, including peptides analogues, are solubilized in acidic H_2O in pH=2.5 and to a concentration of 3 mg/ml API. Compounds are thereafter either dissolved in Na-Acetate buffer (50 mM Acetic Acid, 125 mM NaCl, pH 5.0) or strong PBS, (25 mM sodium phosphate, 125 mM NaCl, pH 7.4).

[00512] Male Sprague-Dawley rats weighing 200-250 g are used in the study. They are housed in groups for n=2 in a light-, temperature- and humidity-controlled room (12-hour light: 12-hour dark cycle, lights on/off at 0600/1800 hour; 23 degrees Celsius; 50% relative humidity). Humane endpoints are applied, according to OECD's 'Guidelines for Endpoints in Animal Study Proposals." The animals are monitored daily. In case of significantly affected condition (based on signs such as weight loss > 30% (obese animals); abnormal posture; rough hair coat; exudate around eyes and/or nose; skin lesions; abnormal breathing; difficulty with ambulation; abnormal food or water intake; or self-mutilation), or other conditions causing significant pain or distress, the animals are euthanized immediately.

[00513] Iron content in plasma/serum is measured for iron content using a colorimetric assay on the Cobas c 111 according to instructions from the manufacturer of the assay (assay: IRON2**:** ACN 661).

[00514] The data obtained from the cobas Iron2 analysis is presented as mean values +/- SEM.

[00515] Dosing of peptide analogues of the present invention is expected to result in a decrease in serum iron level that is comparable to that observed after injection of the positive control Retro Inverse mini Hepcidin (RI-Mini-Hepcidin).

EXAMPLE 5

IN VIVO VALIDATION OF PEPTIDE ANALOGUES

[00516] Peptide analogues of the present invention were tested for in vivo activity, as described in the previous Example, with the following changes. Instead of rats, mice (C57-BL6) were tested. Peptides or vehicle controls were administered to the mice (n=8/group) with the compounds of the present invention dosed at 3000 nmol / kg, and a hepcidin control administered via subcutaneous injection at 1000 nmol/kg. Peptides tested are shown in Table 14 with internalization/degradation assay potency values.

Table 14. Potency of illustrative hepcidin analogues

Compound number	**SEQ ID NO:**	**Sequence**	***Potency* EC50 (nM)**
Hepcidin	335	DTHFPICIFCCGCCHRSKCGMCCKT-OH	34
Cmpd1	207	Isovaleric acid-DTHFPCIKFEPRSKG_ _CK-NH2	23
Cmpd2	36	Isovaleric acid - DTHFPCIKFGPRSKGWVCK-NH_2	35
Cmpd3	76	Isovaleric acid – DTHFPCIKFGPRSKGWVCK-[(m-PEG8)]--NH_2	17
Cmpd4	199	Isovaleric acid – DTHFPICIFGPRSK-[(m-PEG8)]-GWVC-NH_2	6.4
Cmpd5	492	Isovaleric acid-DTHFPCIIFEPRSKEWVCK-NH_2	19
Cmpd6	490	Isovaleric acid-DTHFPCIIF-[Sarc]-PHSKG-[N-MeTrp]-VC-NH_2	9

[00517] The primary goal of this experiment was to validate, in a mouse model, the activity of peptide analogues of the present invention. Serum iron levels were assessed as in the previous Example two hours after peptide or vehicle administration. A significant reduction in serum iron was observed in compound-treated animals as compared to the vehicle control. Furthermore, the max-dose responses of compounds of the present invention are expected to be similar to the max-dose response achieved with Hepcidin.

[00518] A similar experiment was performed with lower doses to assess the dose response of these compounds for inducing serum iron reduction. Methods were as described above in this Example, except for the following parameters: n = 4 mice / group, however n = 8 for the

vehicle, as two groups are pooled. Mice were administered test compounds at two separate dosages (300 nmol/kg or 1000 nmol/kg), *via* subcutaneous injection. Serum iron levels were assessed as in the previous Example two hours after peptide or vehicle injection. These peptides induced similar iron reductions as native hepcidin *in vivo*. The results of this experiment are shown in Figure 1, which provides an in vivo dose response of illustrative hepcidin analogues at two concentrations, 300 nmol/kg and 1000 nmol/kg (subcutaneous or "s.c."; 2 h), in C-57 (mouse) presented as serum iron levels (n=4).

[00519] Other peptides are tested similarly, either in rats as described in the previous Example, or in mice as described above in the present Example. The route of peptide administration is *via* subcutaneous injection, unless otherwise indicated as being *via* intraperitoneal injection

[00520] The peptides are also tested for other pharmacokinetic/ pharmacodynamic (PK/PD) parameters using methods commonly known by the skilled artisan. These parameters include determinations regarding stability (hours stable in plasma from the indicated human or rat subject), half-life in mice, and *in vitro* activity (EC_{50}). The PK/PD properties of peptide analogues of the present invention are compared with hepcidin to determine their PK/PD effects in C57BL6 mice. The peptide analogues are expected to produce a decrease in serum iron, which may be transient or sustained.

[00521] All of the above U.S. patents, U.S. patent application publications, U.S. patent applications, foreign patents, foreign patent applications and non-patent publications referred to in this specification and/or listed in the Application Data Sheet, are incorporated herein by reference, in their entirety.

[00522] From the foregoing it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention.

CLAIMS

What is claimed is:

1. A hepcidin analogue having the structure of Formula I:

R^1-X-Y-R^2 (I) SEQ ID NO:1

or a pharmaceutically acceptable salt or solvate thereof,

wherein R^1 is hydrogen, a C1-C6 alkyl, a C6-C12 aryl, a C6-C12 aryl C1-C6 alkyl, or a C1-C20 alkanoyl, and including PEGylated versions alone or as spacers of any of the foregoing;

R^2 is OH or NH_2;

X is a peptide sequence having the formula Ia:

X1-X2-X3-X4-X5-X6-X7-X8-X9-X10 (Ia) SEQ ID NO:2

wherein

X1 is Asp, Ser, Glu, Ida, pGlu, bhAsp, D-Asp or absent;

X2 is Thr, Ser, Lys, Glu, Pro, Ala or absent;

X3 is His, Ala, or Glu;

X4 is Phe, Ile or Dpa;

X5 is Pro, bhPro, Val, Glu, Sarc or Gly;

X6 is Cys or (D)-Cys;

X7 is absent or any amino acid except Ile, Cys or (D)-Cys;

X8 is absent or any amino acid except Cys or (D)-Cys;

X9 is Phe, Ala, Ile, Thr, Tyr, Lys, Arg, bhPhe, D-Phe or absent; and

X10 is Lys, Phe or absent; and

Y is absent or present;

provided that if Y is present, Y is a peptide having the formula Im:

Y1-Y2-Y3-Y4-Y5-Y6-Y7-Y8-Y9-Y10-Y11-Y12 (Im) SEQ ID NO:3

wherein

Y1 is Gly, PEG3, Sarc, Lys, Glu, Ala, Phe, Pro, Glu, Lys, D-Pro, Val, Ser or absent;

Y2 is Pro, Ala, Cys, Gly or absent;

Y3 is Arg, Lys, Pro, Gly, His, Ala, Trp or absent;

Y4 is Ser, Arg, Gly, Trp, Ala, His, Glu, Tyr or absent;

Y5 is Lys, Met, Ser, Arg, Ala or absent;

Y6 is Gly, Sarc, Glu, Lys, Arg, Ser, Lys, Ile, Ala, Pro, Val or absent;

Y7 is Trp, Lys, Gly, Ala Ile, Val or absent;

Y8 is Val, Trp, His, Thr, Gly, Cys, Met, Tyr, Ala, Glu, Lys, Asp, Arg or absent;

Y9 is Val, Asp, Asn, Cys, Tyr or absent;

Y10 is Cys, Met, Lys, Arg, Tyr or absent;

Y11 is Arg, Met, Cys, Lys or absent; and

Y12 is Arg, Lys, Ala or absent.

2. The hepcidin analogue of claim 1, wherein X is a peptide sequence having the formula Ib:

X1-X2-X3-X4-X5-X6-X7-X8-X9-X10 (Ib) SEQ ID NO:18

wherein

X1 is Asp, Glu, Ida, pGlu, bhAsp, D-Asp or absent;

X2 is Thr, Ser, Lys, Glu, Pro, Ala or absent;

X3 is His, Ala, or Glu;

X4 is Phe, Ile or Dpa;

X5 is Pro, bhPro, Sarc or Gly;

X6 is Cys;

X7 is absent or any amino acid except Ile, Cys or (D)-Cys;

X8 is absent or any amino acid except Cys or (D)-Cys;

X9 is Phe, Ile, Tyr, bhPhe or D-Phe or absent; and

X10 is Lys, Phe or absent; and

wherein Y is absent or present, provided that if Y is present, Y is a peptide having the formula In:

Y1-Y2-Y3-Y4-Y5-Y6-Y7-Y8-Y9-Y10-Y11-Y12 (In) SEQ ID NO:19

wherein

Y1 is Gly, PEG3, Sarc, Lys, Glu, Ala, Phe, Pro, Glu, Lys, D-Pro, Val, Ser or absent;

Y2 is Pro, Ala, Gly or absent;

Y3 is Arg, Lys, Pro, Gly, His, Ala, or absent;

Y4 is Ser, Arg, Glu or absent;

Y5 is Lys, Ser, Met, Arg, Ala or absent;

Y6 is Gly, Sarc, Glu, Leu, Phe, His or absent;

Y7 is Trp, N-Methyl Trp, Lys, Thr, His, Gly, Ala, Ile, Val or absent;

Y8 is Val, Trp, Ala, Asn, Glu or absent;

Y9 is Val, Ala, Asn, Asp, Cys or absent;

Y10 is Cys, (D)Cys, Glu or absent;

Y11 is Tyr, Met or absent; and

Y12 is Trp or absent.

3. The hepcidin analogue of claim 1, wherein the hepcidin analogue comprises an amino acid sequence or a structure shown in Table 2.

4. A hepcidin analogue having the structure of Formula II:

R^1-X-Y-R^2 (II) SEQ ID NO:4

or a pharmaceutically acceptable salt or solvate thereof,

wherein R^1 is hydrogen, a C1-C6 alkyl, a C6-C12 aryl, a C6-C12 aryl C1-C6 alkyl, or a C1-C20 alkanoyl, and including PEGylated versions alone or as spacers of any of the foregoing;

R^2 is OH or NH_2;

X is a peptide sequence having the formula IIa:

X1-X2-X3-X4-X5-X6-X7-X8-X9-X10 (IIa) SEQ ID NO:5 SEQ ID NO:5

wherein

X1 is Asp, Glu or Ida;

X2 is Thr, Ser or absent;

X3 is His;

X4 is Phe or Dpa;

X5 is Pro, bhPro, Sarc or Gly;

X6 is Cys or (D)-Cys;

X7 is Arg, Glu, Phe, Gln, Leu, Val, Lys, Ile, Ala, Ser, Dapa or absent;

X8 is Ile, Arg, Lys, Arg, Ala, Gln, Phe, Glu, Asp, Tyr, Ser, Leu, Val, D-Ile, D-Lys, D-Arg, Dapa or absent;

X9 is Phe, Tyr, bhPhe, D-Phe or absent; and

X10 is Lys, Phe or absent; and

wherein Y is absent or present, provided that if Y is present, Y is a peptide having the formula IIm:

Y1-Y2-Y3-Y4-Y5-Y6-Y7-Y8-Y9-Y10-Y11-Y12 (IIm) SEQ ID NO:6

wherein

Y1 is Gly, Sarc, Lys, Glu or absent;

Y2 is Pro, Ala, Gly or absent;

Y3 is Arg, Lys, Pro, Gly, His, Ala or absent;

Y4 is Ser, Arg, Glu or absent;

Y5 is Lys, Ser, Met, Arg, Ala or absent;

Y6 is Gly, Sarc, Glu, Leu, Phe, His or absent;

Y7 is Trp, NMe-Trp, Lys, Thr, His, Gly, Ala Ile, Val or absent;

Y8 is Val, Trp, Ala, Asn, Glu or absent;

Y9 is Cys;

Y10 is absent;

Y11 is absent; and

Y12 is absent.

5. The hepcidin analogue of claim 4, wherein the hepcidin analogue comprises an amino acid sequence or a structure shown in Table 3.

6. A dimer comprising two hepcidin analogues, each hepcidin analogue having the structure of Formula I, the structure of Formula II, the structure of Formula III, the structure of Formula IV, the structure of Formula V, the structure of Formula VI, or a sequence or structure shown in any one of Tables 2-4 and 6-8, or 10-12, provided that when the dimer comprises a hepcidin analogue having the structure of Formula III, Formula IV, Formula V, or Formula VI, the two hepcidin analogues are linked via a lysine linker.

7. The dimer of claim 6, wherein one or both hepcidin analogue has the structure of Formula I.

8. The dimer of claim 6, wherein one or both hepcidin analogue has the structure of Formula II.

9. The dimer of claim 6, wherein one or both hepcidin analogue has the Formula III:

R^1-X-Y-R^2 (III) SEQ ID NO:7

or a pharmaceutically acceptable salt or solvate thereof, wherein

R^1 is hydrogen, a C1-C6 alkyl, a C6-C12 aryl, a C6-C12 aryl C1-C6 alkyl, or a C1-C20 alkanoyl, and including PEGylated versions thereof, alone or as spacers of any of the foregoing;

R^2 is -NH_2 or -OH;

X is a peptide sequence having the formula (IIIa)

X1-X2-X3-X4-X5-X6-X7-X8-X9-X10 (IIIa) SEQ ID NO:8

wherein

X1 is Asp, Glu, Ala, Gly, Thr, Ida, pGlu, bhAsp, D-Asp, Tyr, Leu or absent;

X2 is Thr, Ala, Aib, D-Thr, Arg or absent;

X3 is His, Lys, Ala, or D-His;

X4 is Phe, Ala, Dpa or bhPhe;

X5 is Pro, Glu, Ser, Gly, Arg, Lys, Val, Ala, D-Pro, bhPro, Sarc, Abu or absent;

X6 is Ile, Cys, Arg, Leu, Lys, His, Glu, D-Ile, D-Arg, D-Cys, Val, Ser or Ala;

X7 is Cys, Ile, Ala, Leu, Val, Ser, Phe, Dapa, D-Ile or D-Cys;

X8 is Ile, Lys, Arg, Ala, Gln, Phe, Glu, Asp, Tyr, Ser, Leu, Val, D-Ile, D-Lys, D-Arg, or Dapa;

X9 is Phe, Ala, Ile, Tyr, Lys, Arg, bhPhe or D-Phe; and

X10 is Lys, Phe or absent; and

Y is absent or present, and when present, Y is a peptide having the formula (IIIm)

Y1-Y2-Y3-Y4-Y5-Y6-Y7-Y8-Y9-Y10-Y11-Y12-Y13-Y14-Y15 (IIIm) SEQ ID NO:9

wherein

Y1 is Gly, Cys, Ala, Phe, Pro, Glu, Lys, D-Pro, Val, Ser or absent;

Y2 is Pro, Ala, Cys, Gly or absent;

Y3 is Arg, Lys, Pro, Gly, His, Ala, Trp or absent;

Y4 is Ser, Arg, Gly, Trp, Ala, His, Tyr or absent;

Y5 is Lys, Met, Arg, Ala or absent;

Y6 is Gly, Ser, Lys, Ile, Arg, Ala, Pro, Val or absent;

Y7 is Trp, Lys, Gly, Ala, Ile, Val or absent;

Y8 is Val, Thr, Gly, Cys, Met, Tyr, Ala, Glu, Lys, Asp, Arg or absent;

Y9 is Cys, Tyr or absent;

Y10 is Met, Lys, Arg, Tyr or absent;

Y11 is Arg, Met, Cys, Lys or absent;

Y12 is Arg, Lys, Ala or absent;

Y13 is Arg, Cys, Lys, Val or absent;

Y14 is Arg, Lys, Pro, Cys, Thr or absent; and

Y15 is Thr, Arg or absent;

wherein if Y is absent from the peptide of formula (III), X7 is Ile; and

wherein said compound of formula (III) is optionally PEGylated on R^1, X, or Y.

10. The dimer of claim 6, wherein one or both hepcidin analogue has the structure of Formula (IV):

R^1-X-Y-R^2 (IV) SEQ ID NO:10

or a pharmaceutically acceptable salt or solvate thereof,

wherein R^1 is hydrogen, a C1-C6 alkyl, a C6-C12 aryl, a C6-C12 aryl C1-C6 alkyl, or a C1-C20 alkanoyl, and including PEGylated versions alone or as spacers of any of the foregoing;

R^2 is $-NH_2$ or -OH;

X is a peptide sequence having the formula (IVa)

X1-X2-X3-X4-X5-X6-X7-X8-X9-X10 (IVa) SEQ ID NO:11

wherein

X1 is Asp, Glu, Ala, Gly, Thr, Ida, pGlu, bhAsp, D-Asp, Tyr, Leu or absent;

X2 is Thr, Ala, Aib, D-Thr, Arg or absent;

X3 is His, Lys, Ala, or D-His;

X4 is Phe, Ala, Dpa, bhPhe or D-Phe;

X5 is Pro, Glu, Ser, Gly, Arg, Lys, Val, Ala, D-Pro, bhPro, Sarc, Abu or absent;

X6 is Ile, Cys, Arg, Leu, Lys, His, Glu, D-Ile, D-Arg , D-Cys, Val, Ser or Ala;

X7 is Cys, Ile, Ala, Leu, Val, Ser, Phe, Dapa, D-Ile or D-Cys;

X8 is Ile, Lys, Arg, Ala, Gln, Phe, Glu, Asp, Tyr, Ser, Leu, Val, D-Ile, D-Lys, D-Arg or Dapa;

X9 is Phe, Ala, Ile, Tyr, Lys, Arg, bhPhe or D-Phe; and

X10 is Lys, Phe or absent;

wherein Y is present or absent, and provided that if Y is absent, X7 is Ile; and

Y is a peptide having the formula (IVm):

Y1-Y2-Y3-Y4-Y5-Y6-Y7-Y8-Y9-Y10-Y11-Y12-Y13-Y14-Y15 (IVm) SEQ ID NO:12

wherein

Y1 is Gly, Cys, Ala, Phe, Pro, Glu, Lys, D-Pro, Val, Ser or absent;

Y2 is Pro, Ala, Cys, Gly or absent;

Y3 is Arg, Lys, Pro, Gly, His, Ala, Trp or absent;

Y4 is Ser, Arg, Gly, Trp, Ala, His, Tyr or absent;

Y5 is Lys, Met, Arg, Ala or absent;

Y6 is Gly, Ser, Lys, Ile, Arg, Ala, Pro, Val or absent;

Y7 is Trp, Lys, Gly, Ala, Ile, Val or absent;

Y8 is Val, Thr, Gly, Cys, Met, Tyr, Ala, Glu, Lys, Asp, Arg or absent;

Y9 is Cys, Tyr or absent;

Y10 is Met, Lys, Arg, Tyr or absent;

Y11 is Arg, Met, Cys, Lys or absent;

Y12 is Arg, Lys, Ala or absent;

Y13 is Arg, Cys, Lys, Val or absent;

Y14 is Arg, Lys, Pro, Cys, Thr or absent; and

Y15 is Thr, Arg or absent;

wherein said compound of formula (IV) is optionally PEGylated on R^1, X, or Y; and

wherein when said compound of formula (IV) comprises two or more cysteine residues, at least two of said cysteine residues being linked via a disulfide bond.

11. The dimer of claim 6, wherein one or both hepcidin analogue has the structure of Formula V:

R^1-X-Y-R^2 (V) SEQ ID NO:13

or a pharmaceutically acceptable salt or solvate thereof, wherein

wherein R^1 is hydrogen, a C1-C6 alkyl, a C6-C12 aryl, a C6-C12 aryl C1-C6 alkyl, or a C1-C20 alkanoyl, and including PEGylated versions alone or as spacers of any of the foregoing;

R^2 is $-NH_2$ or -OH;

X is a peptide sequence having the formula (Va):

X1-X2-X3-X4-X5-X6-X7-X8-X9-X10 (Va) SEQ ID NO:14

wherein

X1 is Asp, Glu, Ala, Gly, Thr, Ida, pGlu, bhAsp, D-Asp, Tyr, Leu or absent;

X2 is Thr, Ala, Aib, D-Thr, Arg or absent;

X3 is His, Lys, Ala, D-His or Lys;

X4 is Phe, Ala, Dpa, bhPhe or D-Phe;

X5 is Pro, Glu, Ser, Gly, Arg, Lys, Val, Ala, D-Pro, bhPro, Sarc, Abu or absent;

X6 is Ile, Cys, Arg, Leu, Lys, His, Glu, D-Ile, D-Arg, D-Cys, Val, Ser or Ala;

X7 is Cys, Ile, Ala, Leu, Val, Ser, Phe, Dapa, D-Ile or D-Cys;

X8 is Ile, Lys, Arg, Ala, Gln, Phe, Glu, Asp, Tyr, Ser, Leu, Val, D-Ile, D-Lys, D-Arg, or Dapa;

X9 is Phe, Ala, Ile, Tyr, Lys, Arg, bhPhe or D-Phe; and

X10 is Lys, Phe or absent;

wherein Y is present or absent, and provided that if Y is absent, X7 is Ile;

wherein said compound of formula V is optionally PEGylated on R^1, X, or Y; and

wherein when said compound of formula V comprises two or more cysteine residues, at least two of said cysteine residues being linked via a disulfide bond.

12. The dimer of claim 6, wherein one or both hepcidin analogue has the structure of formula VI:

R^1-X-Y-R^2 (VI) SEQ ID NO:15

or a pharmaceutically acceptable salt or solvate thereof, wherein

wherein R^1 is hydrogen, a C1-C6 alkyl, a C6-C12 aryl, a C6-C12 aryl C1-C6 alkyl, or a C1-C20 alkanoyl, and including PEGylated versions alone or as spacers of any of the foregoing;

R^2 is $-NH_2$ or -OH;

X is a peptide sequence having the formula (VIa):

X1-X2-X3-X4-X5-X6-X7-X8-X9-X10 (VIa) SEQ ID NO:16

wherein

X1 is Asp, Glu, Ida or absent;

X2 is Thr, Ser, Pro, Ala or absent;

X3 is His, Ala, or Glu;

X4 is Phe or Dpa;

X5 is Pro, bhPro, Sarc or Gly;

X6 is Cys, (D)-Cys, Arg, Glu, Phe, Gln, Leu, Val, Lys, Ala, Ser, Dapa or absent;

X7 is Cys, (D)-Cys, Arg, Glu, Phe, Gln, Leu, Val, Lys, Ala, Ser, Dapa or absent;

X8 is Ile, Arg, Lys, Ala, Gln, Phe, Glu, Asp, Tyr, Ser, Leu, Val, D-Ile, D-Lys, D-Arg, Dapa or absent;

X9 is Phe, Ala, Ile, Thr, Tyr, Lys, Arg, bhPhe, D-Phe or absent; and

X10 is Lys, Phe or absent;

Y is absent or present, provided that if Y is present, Y is a peptide having the formula (VIm)

Y1-Y2-Y3 (VIm) SEQ ID NO:17

wherein

Y1 is Ile, Arg, Lys, Ala, Gln, Phe, Glu, Asp, Tyr, Ser, Leu, Val, D-Ile, D-Lys, D-Arg, Dapa or absent;

Y2 is Phe, Ala, Ile, Thr, Tyr, Lys, Arg, bhPhe or D-Phe or absent; and

Y3 is Lys, Phe or absent.

13. The dimer of claim 6, wherein one or both hepcidin analogue has a sequence or structure shown in in Table 4.

14. The dimer of claim 6, having a sequence or structure shown in any one of Tables 6, 7 and 8.

15. The dimer of claim 6, comprising a sequence or structure shown in any one of compounds 1-361 in Table 12.

16. The dimer of claim 6, comprising a sequence or structure shown in Table 10 or Table 12.

17. The dimer of any one of claims 6-16, wherein the dimer is a homodimer.

18. The dimer of any one of claims 6-16, wherein the dimer is a heterodimer.

19. The hepcidin analogue of any one of claims 1-5 or the dimer of any one of claims 6-16, wherein two cysteine residues of one or more hepcidin analogue are linked by an intramolecular disulfide bridge.

20. The dimer of any one of claims 6-16, wherein the two hepcidin analogues are linked by a linker moiety selected from diethylene glycol linker, an iminodiacetic acid (IDA) linker, a β-Ala-iminodiaceticacid (β-Ala-IDA) linker, or a PEG linker, and wherein the dimer does not include a peptide analogue having a structure of Formula III, IV, V, or VI, or a sequence shown in any of compounds 1-361 in Table 12 or a sequence shown in Table 10.

21. A polynucleotide comprising a sequence encoding the hepcidin analogue of any one of claims 1-5 or a hepcidin analogue of the dimer of any one of claims 6-20.

22. A vector comprising the polynucleotide of claim 21.

23. A pharmaceutical composition comprising the hepcidin analogue of any one of claims 1-5 or the dimer of any one of claims 6-20, and a pharmaceutically acceptable carrier, excipient or vehicle.

24. A method of binding a ferroportin or inducing ferroportin internalization and degradation, comprising contacting the ferroportin with at least one hepcidin analogue of any one of claims 1-5, dimer of any one of claims 6-20, or composition of claim 23.

25. A method for treating a disease of iron metabolism in a subject comprising providing to the subject an effective amount of at least one hepcidin analogue of any one of claims 1-5, dimer of any one of claims 6-20, or the composition of claim 23.

26. The method of claim 25, wherein the pharmaceutical composition is provided

to the subject by an oral, intravenous, peritoneal, intradermal, subcutaneous, intramuscular, intrathecal, inhalation, vaporization, nebulization, sublingual, buccal, parenteral, rectal, vaginal, or topical route of administration.

27. The method of claim 25, wherein the disease of iron metabolism is an iron overload disease.

28. A device comprising the hepcidin analogue of any one of claims 1-5, the dimer of any one of claims 6-20, or the composition of claim 23, for delivery of the hepcidin analogue, dimer or composition to a subject.

29. A kit comprising at least one hepcidin analogue of any one of claims 1-5, dimer of any one of claims 6-20, or composition of claim 23, packaged with a reagent, a device, or an instructional material, or a combination thereof.

30. The hepcidin analogue of claim 4, wherein X6 is Cys.

31. The hepcidin analogue of claim 4, wherein X7 is Arg, Glu, Phe, Gln, Leu, Val, Lys, Ala, Ser, Dapa or absent.

32. The dimer of claim 6, comprising a monomer peptide having a sequence or structure shown in Table 14 or 15.

Fig. 1

In vivo dose response of a select series of hepcidin anlogues at two concentrations (s.c.; 2 h)
C-57 (Mouse) Serum Iron levels (*n = 4*)