ORALLY DELIVERABLE NON-NATURALLY OCCURRING MELANOCORTIN ANALOGS AND USES THEREOF FOR TREATING SUBSTANCE USE DISORDERS

CROSS-REFERENCE TO RELATED APPLICATION(S)

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 63/554,944, filed February 16, 2024; U.S. Provisional Patent Application No. 63/557,409, filed February 23, 2024; U.S. Provisional Patent Application No. 63/572,896, filed April 1, 2024; U.S. Provisional Patent Application No. 63/573,433, filed April 2, 2024; U.S. Provisional Patent Application No. 63/632,496, filed April 10, 2024; U.S. Provisional Patent Application No. 63/637,285, filed April 22, 2024; U.S. Provisional Patent Application No. 63/640,864, filed April 30, 2024; U.S. Provisional Patent Application No. 63/647,545, filed May 14, 2024; U.S. Provisional Patent Application No. 63/650,368, filed May 21, 2024; U.S. Provisional Patent Application No. 63/654,876, filed May 31, 2024; U.S. Provisional Patent Application No. 63/656,579, field June 5, 2024; U.S. Provisional Patent Application No. 63/663,689, filed June 24, 2024; U.S. Provisional Patent Application No. 63/675,219, filed July 24, 2024; U.S. Provisional Patent Application No. 63/681,055, filed August 8, 2024; and of U.S. Provisional Patent Application No. 63/692,605, filed September 9, 2024, all of which are incorporated herein by reference in their entirety.

INCORPORATION BY REFERENCE OF SEQUENCE LISTING

[0002] This application contains an ST.26 compliant Sequence Listing, which is submitted concurrently in xml format and hereby incorporated by reference in its entirety. The .xml copy, created on February 13, 2025, is titled "146316_8023_WO02_SL.xml" and is 1,048,420 bytes in size.

BACKGROUND

[0003] Melanocortin receptors (e.g., melanocortin 3 receptor, melanocortin 4 receptor) are major molecular targets for pain management. The vast majority of clinically prescribed pain medications are melanocortin receptor ligands (i.e., opioid drugs) that are structurally similar to or derived from morphine or other opiates. While these medications serve as effective painkillers, usage can result in several unwanted

adverse side effects, including increased tolerance, addiction, overdose, respiratory distress, and constipation.

[0004] Opioid and other substance addictions, for example, alcohol and nicotine addictions, contribute to millions of premature deaths each year. Although current therapies aimed at treating addiction can be effective, many substance users are unable to overcome their addiction, despite having a desire to quit.

[0005] Even after recent advances in pain medication development, there remains considerable need for new medications to manage pain with improved therapeutic profiles (e.g., robust therapeutic activity and reduced unwanted adverse side effects) compared to conventional opioid drugs. There is also a growing demand for new therapies to treat opioid use disorders (i.e., opioid addictions) and other addictions (e.g., alcohol use disorder, and nicotine addiction).

SUMMARY

[0006] The present technology comprises non-naturally occurring melanocortin analogs and associated used. In some embodiments, the present technology comprises non-naturally occurring melanocortin analog comprising a sequence according to Formula (IC):

$$R^1\text{-}R^2\text{-}R^3\text{-}R^4\text{-}R^5\text{-}R^6\text{-}R^7\text{-}Y^1\text{-}Y^2 \text{ (IC)},$$

wherein:

R^1 is Nle;

R^2 is Asp or Glu;

R^3 is Pro;

R^4 is p(F)dPhe or dPhe;

R^5 is Arg;

R^6 is Trp;

R^7 is Orn;

Y^1 is dVal;

Y^2 is dPro; and

the non-naturally occurring melanocortin analog is cyclized through a lactam bridge between Asp or Glu at R^2 and Orn at R^7,

provided that:

when R^4 is dPhe, then R^2 is Glu

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] FIG 1. illustrates 24-hour plasma concentrations of non-naturally occurring melanocortin analogs of the present technology following oral administration of Compound Ac-Nle-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 19; M1) to Cynomolgus Monkeys at 30.0 mg/kg.

[0008] FIGS. 2A-2C illustrate 24-hour plasma concentrations of non-naturally occurring melanocortin analogs of the present technology following oral (PO) administration of Compound D (Ac-Nle-c[Glu-Pro-p(F)dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2; SEQ ID NO: 218; O7) to Cynomolgus Monkeys at 3 mg/kg, 10.0 mg/kg, 30.0 mg/kg, or 60 mg/kg.

[0009] FIGS. 3A-3D illustrate 24-hour plasma and cerebrospinal fluid (CSF) concentrations of non-naturally occurring melanocortin analogs of the present technology following subcutaneous (SC) administration of Compound D (FIGS. 3A-3D) to Rats at 1.0 mg/kg, 3.0 mg/kg, and 10.0 mg/kg.

[0010] FIGS. 4A-4D illustrate plasma and CSF concentrations of non-naturally occurring melanocortin analogs following IP administration of Compound D to Rats at 1.0 mg/kg, 3.0 mg/kg, and 10.0 mg/kg.

[0011] FIGS. 5A-5C illustrate 24-hour plasma and CSF concentrations of non-naturally occurring melanocortin analogs of the present technology following PO administration of Compound D to Rats at 10.0 mg/kg and 30.0 mg/kg.

[0012] FIGS. 6A-6D show the plasma and CSF concentration of Compound F (Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2; SEQ ID NO: 31; A3) following SC administration to Rats at 1.0 mg/kg, 3.0 mg/kg, and 10.0 mg/kg.

[0013] FIGS. 7A-7C show the plasma and CSF concentration of Compound F of FIGS. 6A-6D following IP administration to Rats at 1.0 mg/kg, 3.0 mg/kg, and 10.0 mg/kg.

[0014] FIGS. 8 shows the plasma and CSF concentration of Compound F of FIGS. 6A-6D following IV bolus administration to Rats at 1.0 mg/kg.

[0015] FIG. 9 shows the mean plasma concentration of Compound F of FIGS. 6A-6D over time following SC, IP, or IV bolus administration in rats at different dosages.

[0016] FIGS. 10A and 10B show preliminary weight loss and daily food intake results in rats administered 3 mg/kg of A3 (Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2; SEQ ID NO: 31), O7 (Ac-Nle-c[Glu-Pro-p(F)dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2; SEQ ID NO: 218) and O11 (Ac-Nle-c[Glu-Pro-dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2; SEQ ID NO: 216).

[0017] FIGS. 11A-11D show food intake (g) at baseline in monkeys having diet-induced obesity (n=6) from 0.5 to 72 hours after saline administration at day 1 (D1). FIG. 11A shows net weight of food intake among individual monkeys. FIG. 11B shows net weight of food intake for each group average. FIG. 11C shows cumulative food intake among individual monkeys. FIG. 11D shows cumulative food intake for each group average.

[0018] FIGS. 12A-12H show food intake (g) in the same monkeys as FIGS. 11A-11D administered 1 mg/kg or 3 mg/kg of O7 (Ac-Nle-c[Glu-Pro-p(F)dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2; SEQ ID NO: 218) or O11 (Ac-Nle-c[Glu-Pro-dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2; SEQ ID NO: 216) on day 8 (D8). FIG. 12A shows net weight of food intake among individual monkeys at day 8 (D8). FIG. 12B shows net weight of food intake for each group average of monkeys administered the same compound at D8. FIG. 12C shows cumulative food intake among individual monkeys of FIG. 12A at D8. FIG. 12D shows cumulative food intake for each group average of monkeys of FIG.12C administered the same compound at D8. FIG. 12E shows net weight of food intake among individual monkeys at day 12 (D12). FIG. 12F shows net weight of food intake for each group average of monkeys administered the same compound at D12. FIG. 12G shows cumulative food intake among individual monkeys at D12. FIG. 12H shows cumulative food intake for each group average of monkeys administered the same compound at D12.

[0019] FIGS. 13A-13D show food intake patterns in the monkeys administered saline of FIGS. 11A-11D, relative compared to the same monkeys administered 1mg/kg or 3 mg/kg of O7 (Ac-Nle-c[Glu-Pro-p(F)dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2; SEQ ID

NO: 218) or O11 (Ac-Nle-c[Glu-Pro-dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2; SEQ ID NO: 216) (FIGS. 13C and 13D) in FIGS. 12A-12H.

[0020] FIGS. 14A-14C show changes in daily caloric intake (FIG. 14A), cumulative caloric intake (FIG. 14B), percent change in caloric consumption from baseline through day 6 (FIG. 14C) for diet-induced obese monkeys orally administered 10 mg/kg of O7 (Ac-Nle-c[Glu-Pro-p(F)dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2; SEQ ID NO: 218). (PO: oral administration; QD: once daily).

[0021] FIGS. 15A-15J show changes in cumulative caloric intake, percent change in caloric consumption from baseline, food intake of a normal diet, food intake of a high fat diet, food intake in calories, and food preference for diet-induced obese monkeys orally administered O7 (Ac-Nle-c[Glu-Pro-p(F)dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2; SEQ ID NO: 218) or O11 (Ac-Nle-c[Glu-Pro-dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2; SEQ ID NO: 216). (PO: oral administration; QD: once daily; BID: twice daily).

[0022] FIG. 15K shows normalized cumulative food consumption in rats subcutaneously administered MC4R selective agonists (A07D (Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2; SEQ ID NO: 31), O7 (Ac-Nle-c[Glu-Pro-p(F)dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2; SEQ ID NO: 218) and O11 (Ac-Nle-c[Glu-Pro-dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2; SEQ ID NO: 216)) (n=5 per group) or a same dose of MC3R/MC4R co-agonists (O10 (Ac-Nle-c[Glu-His-p(F)dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2; SEQ ID NO: 326)) (n=5) compared to saline controls (n=4). Rats were administered 0.5 mg/kg A07D, O7, O10, or O11 for days 1-7 and 1 mg/kg for days 8-17.

[0023] FIGS. 16A-16G show changes in body weight for diet included obese monkeys orally administered O7 (Ac-Nle-c[Glu-Pro-p(F)dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2; SEQ ID NO: 218). FIGS. 16A and 16B show daily body weight (FIG. 16A) and percent change in body weight from baseline (FIGS. 16B) for the diet-induced obese monkeys of FIGS. 14A-14C through day 8. FIGS. 16C shows percent change in body weight as a percentage of initial weight for the monkeys of FIGS. 16A and 16B through day 15. FIG. 16D shows body weight change as a percentage of initial weight in diet induced obese monkeys orally administered O7 or O10 at increasing dosages. FIG. 16E shows body weight change in the monkeys of FIG. 16D. FIG. 16F shows body weight changes as a percentage of initial in monkeys orally administered a non-naturally

occurring melanocortin analog of the present technology (PO) under different dosing regimens. FIG. 16G shows body weight percent change from initial weight in monkeys orally administered O7 at different doses (PO: oral administration; SC: subcutaneous administration; QD: once daily; BID: twice daily; BIW: twice weekly).

[0024] FIG. 16K shows changes in body weight as a percentage of day 1 in rats administered saline, a melanocortin 4 receptor (MC4R) selective agonist (A07D (Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2; SEQ ID NO: 31), O7 (Ac-Nle-c[Glu-Pro-p(F)dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2; SEQ ID NO: 218) and O11 (Ac-Nle-c[Glu-Pro-dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2; SEQ ID NO: 216)), or a same dose of melanocortin 3 receptor (MC3R)/MC4R coagonist ((O10)(Ac-Nle-c[Glu-His-p(F)dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2; SEQ ID NO: 326)).

[0025] FIG. 17 shows plasma concentration of O7 after oral (PO) administration to cynomolgus monkeys at 10 mg/kg or 30 mg/kg.

[0026] FIG. 18 shows plasma concentration of C29 (Ac-dLys-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dLys-dVal-dPro-NH_2; SEQ ID NO: 160) after oral administration to cynomolgus monkeys at 10 mg/kg or 30 mg/kg.

[0027] FIGS. 19A-19D show blood glucose levels following administration of A07D to Sprague-Dawley rats at 1 mg/kg or 3 mg/kg. OGTT: Oral Glucose Tolerance Test.

[0028] FIGS. 20A-20G show measurements of diastolic blood pressure (DBP), systolic blood pressure (SBP), heart rate, and heart rate corrected QT interval (QTc) in rats administered setmelanotide at 0.5 mg/kg, 1.0 mg/kg, 3.0 mg/kg, or 6.0 mg/kg, relative to control rats administered saline (FIGS. 20A-20D) and cardiac data for A07D ("A3"; Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2; SEQ ID NO: 31) up to 180 minutes after subcutaneous injection (FIGS. 20E-20G).

[0029] FIGS. 21A-21D show the plasma and cerebrospinal fluid (CSF) concentration of A07D ("A3"; Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2; SEQ ID NO: 31) in rats over time following subcutaneous administration.

[0030] FIG. 22 shows plasma concentration of A07D (Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2; SEQ ID NO: 31) administered at 1mg/kg intravenously, 10 mg/kg orally, or 30 mg/kg orally to fasted male cynomolgus monkeys.

[0031] FIGS. 23A and 23B show results of a morphine conditioned placed preference (CPP) study in rats administered vehicle and saline, vehicle and morphine, or A07D (Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2; SEQ ID NO: 31) and morphine. FIG 23A illustrates the experimental timeline. FIG 23B illustrates CPP scores in each group before and after each test.

DETAILED DESCRIPTION

[0032] The present technology comprises methods of treating, preventing, reducing, or otherwise ameliorating one or more symptoms or conditions associated with pain, addiction, addictive agent use, or withdrawal from an addictive substance using a non-naturally occurring melanocortin analog. In some embodiments, the non-naturally occurring melanocortin analog is administered orally. In some embodiments, the non-naturally occurring melanocortin analog is a melanocortin receptor agonist and/or an melanocortin receptor agonist. In some embodiments, the method comprises preventing, stabilizing, or reducing one or more of a dose used or consumed of an addictive substance, a dependency on an addictive substance, an amount/frequency of an addictive substance, use (e.g., dose or dosage) of an opioid reversal agent, relapse associated with use of an addictive substance, use (e.g., frequency/amount) of a conventional medication used to treat an addiction, an incidence of or need for hospitalization associated with use of an addictive substance, or an incidence in or a need for rehabilitation from an addiction. In some embodiments, the methods promote or increase cessation of an addictive substance.

[0033] The following description is merely exemplary in nature and is not intended to limit the present technology, its applications, or its uses. It should be understood that throughout the drawings, corresponding reference numerals indicate like or corresponding parts and features. The description of specific examples indicated in various embodiments of the present technology are intended for purposes of illustration only and are not intended to limit the scope of the present technology disclosed herein. Moreover, recitation of multiple embodiments having stated features is not intended to exclude other embodiments having additional features or other embodiments incorporating different combinations of the stated features.

[0034] The following description is merely exemplary in nature and is not intended to limit the present technology, its applications, or its uses. It should be understood that

throughout the drawings, corresponding reference numerals indicate like or corresponding parts and features. The description of specific examples indicated in various embodiments of the present technology are intended for purposes of illustration only and are not intended to limit the scope of the present technology disclosed herein. Moreover, recitation of multiple embodiments having stated features is not intended to exclude other embodiments having additional features or other embodiments incorporating different combinations of the stated features.

[0035] While the embodiments are described in sufficient detail to enable those skilled in the art to practice the present technology, it should be understood that other embodiments may be realized, and that logical changes may be made without departing from the spirit and scope of the present technology. Thus, the detailed description herein is presented for purposes of illustration only and not of limitation. For example, steps recited in any method or process, may be executed in any order and are not limited to the order presented. Moreover, any of the steps thereof may be outsourced to or performed by one or more third parties.

Definitions

[0036] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which the present technology belongs. For the purposes of the present technology, the following terms are defined below.

[0037] The articles “a” and “an” are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element. Likewise, any reference to singular includes plural embodiments, and any reference to more than one component may include a singular embodiment.

[0038] The term “about” means a quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length that varies by acceptable levels in the art. Typically, such variation may be as much 10% above and below a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length and such variation may be influenced by standard applicable measurement practices. When the term “about” is used in conjunction with a numerical range, it

modifies that range by extending the boundaries above and below the numerical values set forth.

[0039] The terms "administering" or "administer" include delivery of therapies (e.g., non-naturally occurring melanocortin analogs (also referred to herein as peptides)) of the present technology to a subject either by local or systemic administration.

[0040] The terms "active ingredient" and "active compound" refer to a biologically active substance, whether naturally or non-naturally occurring, that is the main component of the pharmaceutical composition which elicits the intended effect of an administered therapeutic. This may be any component that drives the pharmacological activity or direct effect in the diagnosis, cure, mitigation, treatment, or prevention of the conditions associated with the present technology, such as but not limited to, substance use disorders.

[0041] As used herein, a "composition" or a "pharmaceutical composition" refers to a mixture of the active ingredient with other chemical components, such as pharmaceutically acceptable carriers and/or excipients.

[0042] As used herein, a "pharmaceutically acceptable carrier" of the pharmaceutical composition refers to a carrier or diluent that does not cause significant irritation to an organism, does not abrogate the biological activity and properties of the administered active ingredient, and/or does not interact in a deleterious manner with the other components of the composition in which it is contained. The term "carrier" encompasses any excipient, binder, diluent, filler, salt, buffer, solubilizer, lipid, stabilizer, or other material well known in the art for use in pharmaceutical formulations. The choice of a carrier for use in a composition will depend upon the intended route of administration for the composition. The preparation of pharmaceutically acceptable carriers and formulations containing these materials is described in, e.g., Remington's Pharmaceutical Sciences, 21st Edition, ed. University of the Sciences in Philadelphia, Lippincott, Williams & Wilkins, Philadelphia Pa., 2005, which is incorporated herein by reference in its entirety). Some examples of physiologically acceptable carriers include antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other

carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN® (ICI, Inc.; Bridgewater, N.J.), polyethylene glycol (PEG), and PLURONICS™ (BASF; Florham Park, N.J.). An "excipient" of the first or the pharmaceutical composition refers to an inert substance added to a composition to further facilitate administration of a compound. Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils, and polyethylene glycols.

[0043] The terms "treat", "treatment", and "treating" may also refer to the reduction or inhibition of the progression and/or duration of a disease, the reduction or amelioration of the severity of the disease, and/or the amelioration of one or more symptoms thereof resulting from the administration of one or more therapies.

[0044] As used herein, the terms "effective amount" or "therapeutically effective amount," refer to that amount of the active ingredient being administered which will relieve to some extent one or more of the symptoms of the disease or disorder being treated.

[0045] The term "after administration" refers to any duration of time after the non-naturally occurring melanocortin analog or pharmaceutical composition thereof has been administered to a subject. Likewise, the term "prior to" refers to any duration of time before the non-naturally occurring melanocortin analog or pharmaceutical composition thereof has been administered to a subject. Unless otherwise specified, durations of time encompassed by "after administration" or "prior to administration" may include seconds, minutes, hours, days, weeks, months, and years.

[0046] The terms "subject" and "patient" refer to anyone being evaluated for disease, disorder, or condition or being administered a therapeutic or pharmaceutical composition. This includes people without diagnosed or confirmed disease or condition. This also includes people with diagnosed or confirmed disease or condition, such as an alcohol use disorder or an opioid use disorder.

[0047] The term "control subject," as used herein, refers to any subject used as a basis for comparison to the subject (e.g., test subject). A control subject includes, but is not limited to, any subject who has not been administered the therapeutic or

pharmaceutical composition (e.g., the non-naturally occurring melanocortin analog, a therapeutically effective amount of the non-naturally occurring melanocortin analog or a pharmaceutical composition thereof) or administered a placebo.

[0048] "Melanocortin analogs," "non-naturally occurring melanocortin analogs," "melanocortin peptides," "melanocortin receptor peptides," or "melanocortins," are used interchangeably and refer to melanocortin-receptor ligands, which are macromolecules containing at least one melanocortin pharmacophore. Melanocortin analogs are typically peptides that bind melanocortin receptors under physiological conditions. Melanocortin analogs include naturally occurring non-naturally occurring melanocortin analogs (i.e., "synthetic peptides" or "synthetic analogs") and truncated and/or modified versions of melanocortin full-length protein or peptides. For example, the full-length pro-opiomelanocortin protein (POMC), prior to proteolytic cleavage of "sub-peptides," consists of 241 amino acids. Tissue-specific proteolytic cleavage of POMC yields peptides ranging in size from 13 amino acids to 76 amino acids. See Bicknell and Lawry, Encyclopedia of Stress, vol. 3, 257-265, Academic Press (2000). Synthesized, non-naturally occurring melanocortin analogs having increased melanocortin receptor activity as discussed herein are approximately 7-12 amino acids in size. Melanocortin analogs exhibit binding functionality with melanocortin receptors. In addition to peptides, the non-naturally occurring melanocortin analogs include small molecule analogs of melanocortin or portions thereof comprised of organic compounds, inorganic compounds, or combinations of peptide and small molecule—i.e., peptide mimetics, or various combinations thereof. "Non-naturally occurring melanocortin analogs" may be structurally similar and/or functionally similar to biological melanocortin proteins in their ability to bind melanocortin receptors. Further, the melanocortin analogs generally contain the pharmacophore: His-Phe-Arg-Trp (SEQ ID NO: 1) or a modified version thereof, or a structural or functional peptide mimetic thereof.

[0049] A "pharmacophore" is the minimum set of amino acid residues necessary to achieve a physiological effect; or a small molecule that is (with respect to a receptor) a structural mimic of the amino acid residues required for binding to and activation of a receptor. His-Phe-Arg-Trp (SEQ ID NO: 1) and their analogs are the pharmacophore of melanocortin for the regulated physiological effect. Therefore, non-naturally occurring melanocortin pharmacophore analogs may be small peptides or organic molecules

designed to mimic the appearance or function (including activation or deactivation of receptor activity) of the melanocortin pharmacophore core sequence peptide.

[0050] A melanocortin receptor "agonist" is a naturally occurring substance or manufactured drug substance or composition that may interact with a melanocortin receptor and initiate a pharmacological response characteristic of the melanocortin receptor.

[0051] A melanocortin receptor "antagonist" is a naturally occurring substance or manufactured drug substance or composition that opposes the melanocortin receptor-associated responses normally induced by a melanocortin receptor agonist agent.

[0052] "Potentiated therapeutic activity" refers to an increase in melanocortin activity in a non-naturally occurring melanocortin analog that has undergone derivatization at the N- and/or C-terminus. Such derivatizations do not necessarily involve the pharmacophore, but do imply a relative increase in in vivo biological half-life.

[0053] The terms "bind," "binding," "complex," and "complexing," refer to all types of physical and chemical binding, reactions, complexing, attraction, chelating and the like.

[0054] The "peptides" of the present technology may be (a) naturally-occurring, (b) produced by chemical synthesis, (c) produced by recombinant DNA technology, (d) produced by biochemical or enzymatic fragmentation of larger molecules, (e) produced by methods resulting from a combination of methods (a) through (d) listed above, or (f) produced by any other means for producing peptides.

[0055] The term "peptide" as used herein includes any structure comprised of two or more amino acids, including chemical modifications and derivatives of amino acids. The amino acids forming all or a part of a peptide may be naturally occurring amino acids, stereoisomers and modifications of such amino acids, non-protein amino acids, post-translationally modified amino acids, enzymatically modified amino acids, constructs or structures designed to mimic amino acids, and the like, so that the term "peptide" includes pseudopeptides and peptidomimetics, including structures which have a non-peptidic backbone. The term "peptide" also includes dimers or multimers of peptides. A "manufactured" peptide includes a peptide produced by chemical synthesis,

recombinant DNA technology, biochemical, or enzymatic fragmentation of larger molecules, combinations of the foregoing or, in general, made by any other method. The term "peptide" includes peptides containing a variable number of amino acid residues, optionally with non-amino acid residue groups at the N- and C-termini, such groups including acyl, acetyl, alkenyl, alkyl, N-alkyl, amine, or amide groups, among others.

[0056] By employing chemical synthesis, a useful means of production, it is possible to introduce various amino acids which do not naturally occur along the chain, modify the N- or C-terminus, and the like, thereby providing for improved stability and formulation, resistance to protease degradation, and the like.

[0057] "Amino acids" are molecules containing an amine group, a carboxylic acid group, and a side-chain that is specific to each amino acid. The key elements of an amino acid are carbon, hydrogen, oxygen, and nitrogen and have the generic formula H_2N—CHR—COOH, wherein R represents a side chain group. The various α-amino acids differ in the side-chain moiety that is attached to the α-carbon. The "amino acids" of the present technology include the known naturally occurring protein amino acids, which are referred to by both their common three letter abbreviation and single letter abbreviation. See generally *Synthetic Peptides: A User's Guide*, G. A. Grant, editor, W.H. Freeman & Co., New York (1992), the teachings of which are incorporated herein by reference, including the text and table set forth at pages 11 through 24. As set forth above, the term "amino acid" also includes stereoisomers and modifications of naturally occurring protein amino acids, non-protein amino acids, post-translationally modified amino acids, enzymatically synthesized amino acids, derivatized amino acids, constructs or structures designed to mimic amino acids, and the like. Modified and unusual amino acids are described generally in *Synthetic Peptides: A User's Guide*, supra; Hruby et al., *Biochem. J.* 268:249-262 (1990); and Toniolo, *Int. J. Peptide Protein Res.* 35:287-300 (1990); the teachings of all of which are incorporated herein by reference.

[0058] The phrase "amino acid side chain moiety" used herein, including as used in the specification and claims, includes any side chain of any amino acid, as the term "amino acid" is defined herein. This thus includes the side chain moiety present in naturally occurring amino acids. It further includes side chain moieties in modified

naturally occurring amino acids, such as glycosylated amino acids. It further includes side chain moieties in stereoisomers and modifications of naturally occurring protein amino acids, non-protein amino acids, post-translationally modified amino acids, enzymatically synthesized amino acids, derivatized amino acids, constructs, or structures designed to mimic amino acids, and the like. For example, the side chain moiety of any amino acid of the present technology is included within the definition. A "derivative" of an amino acid side chain moiety is included within the definition of an amino acid side chain moiety.

[0059] The "derivative" of an amino acid side chain moiety includes any modification to or variation in any amino acid side chain moieties, including a modification of naturally occurring amino acid side chain moieties. By way of example, derivatives of amino acid side chain moieties include straight chain or branched, cyclic or noncyclic, substituted or unsubstituted, saturated or unsaturated, alkyl, aryl or aralkyl moieties.

[0060] In the peptides of the present technology, conventional amino acid residues have their conventional meaning as given in Chapter 2400, of the *Manual of Patent Examining Procedure,* 8th Ed. Thus, "Ala" is alanine; "Arg" is arginine; "Asn" is asparagine; "Asp" is aspartic acid; "Cys" is cysteine; "Gln" is glutamine; "Glu" is glutamic acid; "His" is histidine; "Ile" is isoleucine; "Leu" is leucine; "Lys" is lysine; "Met" is methionine; "Phe" is phenylalanine; "Pro" is proline; "Ser" is serine; "Thr" is threonine; "Trp" is tryptophan; "Tyr" is tryosine; and "Val" is valine. Unless otherwise indicated, all amino acids abbreviations represent either isomer, i.e., the L-isomer, the D-isomer, or combinations thereof may be used. Thus, for example, "L-Phe" is L-phenylalanine; "D-Phe" is D-phenylalanine; "D-/L-Phe" is D-phenylalanine, L-phenylalanine, or combinations thereof; "Phe" is also D-phenylalanine, L-phenylalanine, or combinations thereof, and so on.

[0061] An alpha (α)-amino acid has the generic formula $H_2N—C_\alpha HR—COOH$, where R is a side chain moiety and the amino group is attached to the carbon atom immediately adjacent to the carboxylate group (i.e., the α-carbon). Other types of amino acids exist when the amino group is attached to a different carbon atom. For example, beta (β)-amino acids, the carbon atom to which the amino group is attached is separated from the carboxylate group by one carbon atom, C_β.

[0062] When β-amino acids are incorporated into peptides, two main types of β-peptides exist: those with the side chain residue, R, on the carbon next to the amine are called β^3 peptides and those with the side chain residue on the carbon next to the carbonyl group are called β^2 amino acids.

[0063] Gamma (γ)-amino acids are amino acids where the carbon atom to which the amino group attaches is separated from the carboxylate moiety by two carbon atoms.

[0064] For additional modified and unusual amino acids, see §2422 of the MPEP, particularly Table 4 at 2400-24. Additionally, "Ac" indicates N-acetyl and "cyclo" refers to a cyclic structure, which is also shown as "c." "NH_2" indicates an amine group, typically added on the C-terminus of a polypeptide. Accordingly, as used herein, an —NH_2 moiety on the C-terminus of a peptide indicates an amide, i.e., —CO—NH_2.

[0065] Additional abbreviations are used as herein follows: Nle is norleucine, Nal(2') is 2′-naphthylalanine, Nal(1') is 1′-naphthylalanine, Tle is tert-leucine, Orn is ornithine, Bip is biphenylalanine; *cis*Pro(guan) is *cis*-4-guanidyl-proline, *trans*Pro(guan) is *trans*-4-guanidyl-proline, Hyp is hydroxyproline; Pen is penicillamine; Tic is 1,2,3,4-tetrahydroisoquinoline-3-carboxylic Acid; Aba is 4-amino-1,2,4,5-tetra-hydro-2-benzazepin-3-one; Oic is octohydroindole-2-carboxylic acid; Atc is 2-aminotetraline-2-carboxylic acid, APC is 1-amino-4-phenylcyclohexane-carboxylic acid, APPC is 4-aminophenylpiperidine-4-carboxylic acid, Ata is 7-amino-7,8-dihydro-4H-[1,2,3]triazolo-[1,5-a][1,4]diazepin-6(5H)-one, Aia is 4-amino-1,4,5,6-tetrahydroazepino[4,3-*b*]indol-3(*2H*)-one, Che is 1-amino-1-cyclohexanecarboxylic acid, Ioc is indoline-2-carboxylic acid, Cpe is 1-amino-1-cyclopentane carboxylic, and p(Cl)dPhe is para-chloro-phenylalanine (F – fluoro, Br – bromo).

[0066] The term "acyl" includes a group RCO—, where R is an organic group. An example is the acetyl group CH_3CO—, referred to herein as "Ac."

[0067] A peptide or aliphatic moiety is "acylated" when an alkyl or substituted alkyl group as defined above is bonded through one or more carbonyl {—(C=O)—} groups. A peptide is most usually acylated at the N-terminus.

[0068] An "amine" includes compounds that contain an amine group (—NH_2).

[0069] An "amide" includes compounds that have a trivalent nitrogen attached to a carbonyl group (i.e., —CO—NH_2), such as for example methylamide, ethylamide, propylamide, and the like. A peptide is most usually amidated at the C-terminus by the addition of an amine (—NH_2) moiety to the C-terminal carboxyl group.

[0070] Amino acids, including stereoisomers and modifications of naturally occurring amino acids, protein amino acids, non-protein amino acids, post-translationally modified amino acids, enzymatically synthesized amino acids, derivatized amino acids, constructs, or structures designed to mimic amino acids (peptide mimetics), and the like, including all of the foregoing, are sometimes referred to herein as "residues."

[0071] "Substantial degradation" refers to the degradation of the N-terminal extension, the C-terminal extension, both N- and C-terminal degradation or degradation to other regions of the non-naturally occurring melanocortin analog by physiological enzymes and other factors, in such a manner or to a degree that side effects appear. According to one embodiment, a non-naturally occurring melanocortin analog having a C-terminal extension that resists substantial degradation is one where no more than 50% of the administered peptide causes side effects and/or displays a low half-life. In some embodiments, no more than 25% of the administered peptide causes side effects and/or displays a low half-life. More preferably, in some embodiments, less than 10% of the administered peptide causes side effects and/or displays a low half-life, as compared to a non-naturally occurring melanocortin analog that lacks a C-terminal extension.

[0072] The disclosure of all publications, patents, and published patent applications listed herein are hereby incorporated by reference in their entireties, including but not limited to U.S. Patent Nos. 8,541,545 and 9,534,018.

Non-naturally Occurring Melanocortin Analogs

[0073] The non-naturally occurring melanocortin analogs of the present technology may comprise a non-naturally occurring melanocortin analog or a pharmaceutically acceptable salt, solvate, or stereoisomer thereof. Non-naturally occurring melanocortin analogs of the present technology may be selective for the melanocortin 4 receptor (MC4R) and/or melanocortin 3 receptor (MC3R) over other melanocortin receptors, i.e., the melanocortin 1 receptor (MC1R), the melanocortin 2

receptor (MC2R), and the melanocortin 5 receptor (MC5R). Some of the non-naturally occurring melanocortin analogs may bind only the MC3R or the MC4R. Alternatively, some of the non-naturally occurring melanocortin analogs may bind the MC3R with greater affinity than the MC4R, whereas other melanocortin analogs may bind the MC4R with greater affinity than the MC3R. Certain melanocortin analogs may bind the MC3R with the same or generally similar affinity as the MC4R.

[0074] The non-naturally occurring melanocortin analogs of the present technology may be full agonists or full antagonists for one or more melanocortin receptors. A full agonist may comprise a non-naturally occurring melanocortin analog having a maximum effect (E_{max}) agonist value of greater than or equal to 85%. Similarly, a full antagonist may comprise a non-naturally occurring melanocortin analog having an E_{max} antagonist value of greater than or equal to 85%.

[0075] The non-naturally occurring melanocortin analogs of the present technology may be partial agonists or partial antagonists. A partial agonist may comprise a non-naturally occurring melanocortin analog having a maximum effect E_{max} agonist value of less than 85%. Similarly, a partial antagonist may comprise a non-naturally occurring melanocortin analog having an E_{max} antagonist value of less than 85%.

[0076] If a non-naturally occurring melanocortin analog's E_{max} agonist value is greater than it's E_{max} antagonist value, then the non-naturally occurring melanocortin analog may be classified as an agonist (e.g., a full agonist or a partial agonist).

[0077] If a non-naturally occurring melanocortin analog's E_{max} antagonist value is greater than it's E_{max} agonist value, then the non-naturally occurring melanocortin analog may be classified as an antagonist (e.g., a full antagonist or a partial antagonist).

[0078] The non-naturally occurring melanocortin analogs of the present technology may be one or more of (i) a full MC4R agonist and a full MC3R antagonist; (ii) a full MC4R agonist and a partial MC3R antagonist; and (iii) a full MC4R agonist having no MC3R activity.

[0079] The non-naturally occurring melanocortin analogs of the present technology may avoid cardiac activation typically seen in conventional melanocortin peptide and small molecule agonists. For example, a subject may maintain a stable

heart rate, systolic blood pressure, and/or diastolic blood pressure following administration of a melanocortin analog of the present technology.

[0080] The non-naturally occurring melanocortin analogs in accordance with the present technology may have certain structural features that impart specific properties on the analogs, such as, for example, degradation resistance, enhanced epithelial, gastrointestinal, and/or blood brain barrier transport, and binding affinity for the melanocortin 4 receptor and/or melanocortin 3 receptor. Accordingly, in some embodiments, the non-naturally occurring melanocortin analogs have one or more beta hairpin (β-hairpin) and/or beta turn (β-turn) structures. The presence of amino acids that are structurally rigid, such as, for example, Aia, Aba, Ata, Hyp, dHyp, Pro, dPro, *trans*Pro(guan), and *cis*Pro(guan), may lead to formation of β-hairpin and/or β-turn structures in the non-naturally occurring melanocortin analog. Additionally, disulfide bridges (e.g., cyclization via disulfide bond) may induce and/or stabilize beta-turn structures of the non-naturally occurring melanocortin analogs. In general, cyclization may stabilize beta-turns, and D-amino acids may induce and/or stabilize beta-turns. Further, in some embodiments, melanocortin analogs include D-valine-D-proline (dVal-dPro) chain as their C-terminus, which may provide enhanced transport and resistance to degradation.

[0081] The presence of certain structural features may impart the non-naturally occurring melanocortin analogs of the present technology with specific binding properties. For example, inclusion of p(F)dPhe or dPhe at the R^4 position may result in enhanced binding and activation of the melanocortin 4 receptor. Accordingly, melanocortin analogs having p(F)dPhe or dPhe at R^4 may be full agonists on MC4R. Further, inclusion of Pro at the R^3 position may result in partial agonism of the melanocortin 3 receptor, whereas inclusion of His at the R^3 position may result in full agonism of the melanocortin 3 receptor.

[0082] In some embodiments, the non-naturally occurring melanocortin analog comprises a sequence of Formula (I):

$$X^1\text{-}X^2\text{-}X^3\text{-}R^1\text{-}R^2\text{-}R^3\text{-}R^4\text{-}R^5\text{-}R^6\text{-}R^7\text{-}R^8\text{-}Y^1\text{-}Y^2\text{-}Y^3\text{-}Y^4\text{-}Y^5\text{-}Y^6\text{-}Y^7 \quad (I),$$

wherein:

X^1 is absent or selected from the group consisting of norleucine (Nle), arginine (Arg), D-arginine (dArg), alanine (Ala), lysine (Lys), D-lysine (dLys), histidine (His), and D-histidine (dHis);

X^2 is absent, phenylalanine (Phe), or Nle;

X^3 is absent, Phe, or Nle;

R^1 is selected from the group consisting of Nle, D-norleucine (dNle), Ala, D-alanine (dAla), Arg, dArg, Lys, dLys, His, dHis, ornithine (Orn), D-ornithine (dOrn), D-leucine (dLeu), D-tyrosine (dTyr), Phe, D-phenylalanine (dPhe), tryptophan (Trp), D-tryptophan (dTrp), aspartic acid (Asp), cysteine (Cys), and D-cysteine (dCys);

R^2 is selected from the group consisting of Asp, proline (Pro), D-aspartic acid (dAsp), Cys, dCys, D-penicillamine (dPen), Ala, dAla, Trp, Phe, dPhe, His, dHis, Tyr, glutamic acid (Glu), and dTyr;

R^3 is absent or selected from the group consisting of His, Ala, Pro, hydroxyproline (Hyp), leucine (Leu), D-glutamine (dGln), Phe, dPhe, Trp, dTrp, Tyr, dTyr, 4-amino-1,2,4,5-tetrahydro-2-benzazepin-3-one (Aba), 7-amino-7,8-dihydro-4H-[1,2,3]triazolo-[1,5-a][1,4]diazepin-6(5H)-one (Ata), 4-amino-1,4,5,6-tetrahydroazepino[4,3-*b*]indol-3(*2H*)-one (Aia), 2-aminotetraline-2-carboxylic acid (Atc), 1-amino-4-phenylcyclohexane-carboxylic acid (APC), 4-aminophenylpiperidine-4-carboxylic acid (APPC), tetrahydro-isoquinoline-3-carboxylic acid (Tic), biphenylalanine (Bip), octohydroindole-2-carboxylic acid (Oic), 1-amino-1-cyclohexanecarboxylic acid (Che), indoline-2-carboxylic acid (Ioc), and 1-amino-1-cyclopentane carboxylic (Cpe);

R^4 is selected from the group consisting of Phe, dPhe, para-chloro-D-phenylalanine (p(Cl)dPhe), para-bromo-D-phenylalanine (p(Br)dPhe), and para-fluoro-D-phenylalanine (p(F)dPhe);

R^5 is selected from the group consisting of Arg, His, *cis*-4-guanidyl-proline (*cisPro*(guan)), and *trans*-4-guanidyl-proline (*trans*Pro(guan));

R^6 is selected from the group consisting of Trp, dTrp, Phe, Aia, Aba, Ata, 2'-D-naphthylalanine (dNal(2')), 2'-naphthylalanine (Nal(2')), and 1'-naphthylalanine (Nal(1'));

R^7 is selected from the group consisting of Gly, Pro, Lys, dLys, Cys, dCys, penicillamine (Pen), dPen, and Orn;

R^8 is absent or Lys;

Y^1 is absent or selected from the group consisting of valine (Val), D-valine (dVal), β^2-valine (β^2-Val), β^3-valine (β^3-Val), Pro, D-proline (dPro), β-proline (β-Pro), Hyp, D-hydroxyproline (dHyp), dLeu, D-tert-leucine (dTle), Trp, Arg, dArg, Lys, dLys, Gly, Ala, dAla, Asp, dAsp, asparagine (Asn), D-asparagine (dAsn), and D-threonine (dThr);

Y^2 is absent or selected from the group consisting of Val, dVal, β^2-Val, β^3-Val, Pro, dPro, dTle, β-Pro, Hyp, dHyp, dTle, Arg, dArg, Gly, Ala, dAla, Asp, and dAsn;

Y^3 is absent or selected from the group consisting of Val, dVal, Pro, dPro, dThr, and dLys;

Y^4 is absent or selected from the group consisting of dVal, dPro, and dAsp;

Y^5 is absent or dVal;

Y^6 is absent or dVal;

Y^7 is absent or dPro; and

the non-naturally occurring melanocortin analog is cyclized through a moiety selected from the group consisting of:

a disulfide bond between R^1 and R^7 when R^1 and R^7 are each independently Cys or dCys;

a disulfide bond between R^2 and R^7, when R^2 and R^7 are each independently selected from Cys, dCys, Pen, and dPen;

a lactam bridge between R^1 or R^2 and R^7 or R^8 when R^1 or R^2 is Asp and R^7 or R^8 is Lys; and

a lactam bridge between R^2 and R^7 when R^2 is Asp, dAsp, or Glu and R^7 is dLys or Orn,

provided that:

when R^4 is p(Br)dPhe, then R^2 is dCys;

when R^4 is p(Cl)dPhe, then (i) R^3 is selected from Ata, Aia, and Aba; or (ii) R^3 is His, R^5 is Arg; R^6 is Trp; R^7 is Lys or dCys, and R^1 is not Ala, wherein when X^1 is present, then Y^1 is dTle and when X^1 is absent and Y^1-Y^2 is dVal-dPro or dTle-dPro, then either

R^1 is not Nle or the non-naturally occurring melanocortin analog is cyclized through a disulfide bond between R^2 and R^7;

when R^4 is p(F)dPhe, then (i) R^3 is selected from Pro, dGln, and dTyr, R^6 is Trp, and R^2 is not Cys; or (ii) R^3 is His and R^6 is Nal(2') or the non-naturally occurring melanocortin analog is cyclized through a disulfide bond between R^2 and R^7;

when R^4 is dPhe and R^1 is Asp, then either (i) R^2 is selected from Trp, Phe, His, Tyr, dPhe, dHis, and dTyr, R^3 is selected from Pro, Ala, and Leu, R^5 is Arg, and R^6 is selected from Trp, Nal(1'), dNal(2'), and Nal(2'); or (ii) R^2 is selected from Ala, dAla, and Phe, and R^3 is His, wherein when R^2 is dAla, then Y^1-Y^7 are absent and when R^2 is Ala, then R^1 is not Nle;

when R^4 is dPhe, and R^2 is Asp, Glu, or dPen, then R^3 is not His or absent; and

when R^4 is dPhe, R^2 is Asp, and R^3 is Pro, then R^5 is not dNal(2') or Nal(2') and Y^3-Y^7 are absent if any of X^1-X^3 are present.

[0083] In some embodiments, the non-naturally occurring melanocortin analog comprises a sequence of Formula (I), wherein:
X^1 is absent or selected from the group consisting of norleucine (Nle), arginine (Arg), D-arginine (dArg), alanine (Ala), lysine (Lys), D-lysine (dLys), histidine (His), and D-histidine (dHis);

X^2 is absent, phenylalanine (Phe), or Nle;

X^3 is absent, Phe, or Nle;

R^1 is selected from the group consisting of Nle, D-norleucine (dNle), Ala, D-alanine (dAla), Arg, dArg, Lys, dLys, His, dHis, ornithine (Orn), D-ornithine (dOrn), D-leucine (dLeu), D-tyrosine (dTyr), Phe, D-phenylalanine (dPhe), tryptophan (Trp), D-tryptophan (dTrp), aspartic acid (Asp), cysteine (Cys), and D-cysteine (dCys);

R^2 is selected from the group consisting of Asp, proline (Pro), D-aspartic acid (dAsp), Cys, dCys, D-penicillamine (dPen), Ala, dAla, Trp, Phe, dPhe, His, dHis, Tyr, glutamic acid (Glu), and dTyr;

R^3 is absent or selected from the group consisting of His, Ala, Pro, hydroxyproline (Hyp), leucine (Leu), D-glutamine (dGln), Phe, dPhe, Trp, dTrp, Tyr, dTyr, 4-amino-1,2,4,5-tetrahydro-2-benzazepin-3-one (Aba), 7-amino-7,8-dihydro-4H-[1,2,3]triazolo-

[1,5-a][1,4]diazepin-6(5H)-one (Ata), 4-amino-1,4,5,6-tetrahydroazepino[4,3-*b*]indol-3(*2H*)-one (Aia), 2-aminotetraline-2-carboxylic acid (Atc), 1-amino-4-phenylcyclohexane-carboxylic acid (APC), 4-aminophenylpiperidine-4-carboxylic acid (APPC), tetrahydro-isoquinoline-3-carboxylic acid (Tic), biphenylalanine (Bip), octohydroindole-2-carboxylic acid (Oic), 1-amino-1-cyclohexanecarboxylic acid (Che), indoline-2-carboxylic acid (Ioc), and 1-amino-1-cyclopentane carboxylic (Cpe);

R^4 is selected from the group consisting of Phe, dPhe, para-chloro-D-phenylalanine (p(Cl)dPhe), para-bromo-D-phenylalanine (p(Br)dPhe), and para-fluoro-D-phenylalanine (p(F)dPhe);

R^5 is selected from the group consisting of Arg, His, *cis*-4-guanidyl-proline (*cisPro*(guan)), and *trans*-4-guanidyl-proline (*trans*Pro(guan));

R^6 is selected from the group consisting of Trp, dTrp, Phe, Aia, Aba, Ata, 2'-D-naphthylalanine (dNal(2')), 2'-naphthylalanine (Nal(2')), and 1'-naphthylalanine (Nal(1'));

R^7 is selected from the group consisting of Gly, Pro, Lys, dLys, Cys, dCys, penicillamine (Pen), dPen, and Orn;

R^8 is absent or Lys;

Y^1 is absent or selected from the group consisting of valine (Val), D-valine (dVal), β^2-valine (β^2-Val), β^3-valine (β^3-Val), Pro, D-proline (dPro), β-proline (β-Pro), Hyp, D-hydroxyproline (dHyp), dLeu, D-tert-leucine (dTle), Trp, Arg, dArg, Lys, dLys, Gly, Ala, dAla, Asp, dAsp, asparagine (Asn), D-asparagine (dAsn), and D-threonine (dThr);

Y^2 is absent or selected from the group consisting of Val, dVal, β^2-Val, β^3-Val, Pro, dPro, dTle, β-Pro, Hyp, dHyp, dTle, Arg, dArg, Gly, Ala, dAla, Asp, and dAsn;

Y^3 is absent or selected from the group consisting of Val, dVal, Pro, dPro, dThr, and dLys;

Y^4 is absent or selected from the group consisting of dVal, dPro, and dAsp;

Y^5 is absent or dVal;

Y^6 is absent or dVal;

Y^7 is absent or dPro; and

the non-naturally occurring melanocortin analog is cyclized through a moiety selected from the group consisting of:

a disulfide bond between R^1 and R^7 when R^1 and R^7 are each independently Cys or dCys;

a disulfide bond between R^2 and R^7, when R^2 and R^7 are each independently selected from Cys, dCys, Pen, and dPen;

a lactam bridge between R^1 or R^2 and R^7 or R^8 when R^1 or R^2 is Asp and R^7 or R^8 is Lys; and

a lactam bridge between R^2 and R^7 when R^2 is Asp, dAsp, or Glu and R^7 is dLys or Orn,

provided that the non-naturally occurring melanocortin analog does not comprise a sequence selected from the group consisting of:

Ac-Nle-c[Cys-His-p(Br)dPhe-Arg-Trp-Cys]-dVal-dPro-NH_2 (SEQ ID NO: 232);

Ac-Ala-c[Asp-His-p(Br)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 233);

Ac-Nle-Nle-c[Asp-His-p(Br)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 234);

Ac-Nle-c[Asp-His-p(Br)dPhe-His-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 235);

Ac-Nle-c[Asp-His-p(Br)dPhe-Arg-dNal(2')-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 236);

Ac-Nle-c[Asp-His-p(Br)dPhe-Arg-Nal(2')-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 237);

Ac-Nle-c[Asp-His-p(Br)dPhe-Arg-Trp-Lys]-dTle-dTle-dVal-NH_2 (SEQ ID NO: 238);

Ac-Nle-c[Asp-His-p(Br)dPhe-Arg-Trp-Lys]-dPro-dVal-dPro-NH_2 (SEQ ID NO: 239);

Ac-Nle-c[Asp-His-p(Br)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 240);

Ac-Nle-Nle-c[Asp-His-p(Br)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 241);

Ac-Ala-c[Asp-His-p(Br)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 242);

Ac-Nle-c[Asp-dPhe-p(Br)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 243);

Ac-Nle-c[Asp-dGln-p(Br)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 244);

Ac-Nle-c[Asp-Trp-p(Br)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 245);

Ac-Nle-c[Asp-dTrp-p(Br)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 246);

Ac-Nle-c[Asp-Tyr-p(Br)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 247);

Ac-Nle-c[Asp-dTyr-p(Br)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 248);

Ac-Nle-c[Asp-Pro-p(Br)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 249);

Ac-Nle-c[Asp-His-p(Br)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 250);

Ac-dArg-c[Asp-His-p(Br)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 251);

Ac-Arg-c[Asp-His-p(Br)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 252);

Ac-Lys-c[Asp-His-p(Br)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 253);

Ac-dLys-c[Asp-His-p(Br)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 254);

Ac-His-c[Asp-His-p(Br)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 255);

Ac-dHis-c[Asp-His-p(Br)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 256);

Ac-Nle-c[Asp-His-p(Br)dPhe-Arg-Trp-Lys]-dVal-dVal-dVal-dPro-NH_2 (SEQ ID NO: 257);

Ac-Nle-Nle-c[Asp-His-p(Br)dPhe-Arg-Trp-Lys]-dPro-dVal-dPro-NH_2 (SEQ ID NO: 258);

Ac-dArg-c[Asp-His-p(Br)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 259);

Ac-Arg-c[Asp-His-p(Br)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 260);

Ac-Lys-c[Asp-His-p(Br)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 261);

Ac-dLys-c[Asp-His-p(Br)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 262);

Ac-His-c[Asp-His-p(Br)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 263);

Ac-dHis-c[Asp-His-p(Br)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 264);

Ac-Nle-c[Asp-His-p(Br)dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2 (SEQ ID NO: 265);

Ac-Nle-c[Glu-His-p(Br)dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2 (SEQ ID NO: 266);

Ac-Nle-c[Asp-Bip-p(Br)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 267);

Ac-Nle-c[Asp-dHis-p(Br)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 268);

Ac-Nle-c[Asp-Phe-p(Br)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 269);

Ac-Nle-c[Asp-dGln-p(Cl)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 270);

Ac-Nle-c[Asp-dTrp-p(Cl)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 271);

Ac-Nle-c[Asp-dPhe-p(Cl)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 272);

Ac-Nle-c[Asp-dTyr-p(Cl)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 273);

Ac-Nle-c[Asp-Trp-p(Cl)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 274);

Ac-Nle-c[Asp-Tyr-p(Cl)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 275);

Ac-Nle-c[Asp-Pro-p(Cl)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 276);

Ac-Nle-c[Asp-Pro-p(Cl)dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2 (SEQ ID NO: 277);

Ac-Nle-c[Glu-Pro-p(Cl)dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2 (SEQ ID NO: 278);

Ac-Nle-c[Asp-His-p(Cl)dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2 (SEQ ID NO: 279);

Ac-Nle-c[Glu-His-p(Cl)dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2 (SEQ ID NO: 280);

Ac-Nle-c[dCys-His-p(Cl)dPhe-Arg-Trp-Cys]-dVal-dPro-NH_2 (SEQ ID NO: 281);

Ac-Nle-c[Asp-His-p(Cl)dPhe-Arg-dNal(2')-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 282);

Ac-Nle-c[Asp-His-p(Cl)dPhe-Arg-Nal(2')-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 283);

Ac-Nle-c[Cys-His-p(Cl)dPhe-Arg-Trp-Cys]-dVal-dPro-NH_2 (SEQ ID NO: 284);

Ac-Ala-c[Asp-His-p(Cl)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 285);

Ac-Nle-c[Asp-His-p(Cl)dPhe-His-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 286);

Ac-Ala-c[Asp-His-p(Cl)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 287);

Ac-Nle-Nle-c[Asp-His-p(Cl)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 288);

Ac-Nle-Nle-c[Asp-His-p(Cl)dPhe-Arg-Trp-Lys]-dPro-dVal-dPro-NH_2 (SEQ ID NO: 289);

Ac-Nle-c[Asp-His-p(Cl)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 290);

Ac-Nle-c[Asp-His-p(Cl)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 291);

Ac-Nle-c[Asp-Phe-p(Cl)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 292);

Ac-Nle-c[Asp-Bip-p(Cl)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 293);

Ac-Nle-c[Asp-dHis-p(Cl)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 294);

Ac-Nle-c[Asp-dTrp-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 295);

Ac-Nle-c[Asp-dPhe-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 296);

Ac-Ala-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 297);

Ac-dArg-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 298);

Ac-Nle-c[Asp-His-p(F)dPhe-Arg-dNal(2')-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 299);

Ac-Nle-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dTle-dTle-dVal-NH_2 (SEQ ID NO: 300);

Ac-Ala-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 301);

Ac-dArg-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 302);

Ac-Nle-c[Asp-Pro-p(F)dPhe-Arg-Nal(2')-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 303);

Ac-Nle-c[Asp-Trp-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 304);

Ac-Nle-c[Asp-Tyr-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 305);

Ac-Nle-c[Cys-Pro-p(F)dPhe-Arg-Trp-Cys]-dVal-dPro-NH_2 (SEQ ID NO: 306);

Ac-Nle-c[Asp-Pro-p(F)dPhe-Arg-dNal(2')-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 307);

Ac-Arg-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 308);

Ac-Lys-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 309);

Ac-dLys-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 310);

Ac-His-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 311);

Ac-dHis-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 312);

Ac-Nle-Nle-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 313);

Ac-Nle-c[Asp-His-p(F)dPhe-His-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 314);

Ac-Nle-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dVal-dVal-dVal-dPro-NH_2 (SEQ ID NO: 315);

Ac-Nle-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dPro-dVal-dPro-NH_2 (SEQ ID NO: 316);

Ac-Nle-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 317);

Ac-Nle-Nle-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 318);

Ac-Nle-Nle-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dPro-dVal-dPro-NH_2 (SEQ ID NO: 319);

Ac-Arg-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 320);

Ac-Lys-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 321);

Ac-dLys-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 322);

Ac-His-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 323);

Ac-dHis-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 324);

Ac-Nle-c[Asp-His-p(F)dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2 (SEQ ID NO: 325);

Ac-Nle-c[Glu-His-p(F)dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2 (SEQ ID NO: 326);

Ac-Nle-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 327);

Ac-Nle-c[Asp-Bip-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 328);

Ac-Nle-c[Asp-dHis-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 329);

Ac-Nle-c[Asp-Phe-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 330);

Ac-Nle-c[Asp-Pro-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 331);

Ac-Nle-c[Asp-Pro-His-dPhe-His-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 332);

Ac-Nle-c[Asp-Trp-Pro-dPhe-His-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 333);

Ac-Nle-c[Asp-Trp-Pro-dPhe-Arg-Phe-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 334);

Ac-dArg-c[Asp-dAla-His-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 335);

Ac-Nle-c[Asp-Ala-His-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 336);

Ac-Nle-c[Asp-Pro-His-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 337);

Ac-Nle-c[Asp-dAla-His-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 338);

Ac-Arg-c[Asp-dAla-His-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 339);

Ac-Nle-c[Asp-dHis-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 340);

Ac-Arg-c[Asp-dAla-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 341);

Ac-dArg-c[Asp-dAla-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 342);

Ac-Nle-c[Asp-His-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 343);

Ac-Nle-c[Asp-His-dPhe-Arg-Trp-Pro-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 344);

Ac-Nle-c[Asp-His-dPhe-Arg-Trp-Lys]-dPro-dVal-NH_2 (SEQ ID NO: 345);

Ac-Nle-c[Glu-His-dPhe-His-Trp-Orn]-dVal-dPro-NH_2 (SEQ ID NO: 346);

Ac-Nle-c[dPen-dPhe-Arg-Trp-dPen]-dVal-dPro-NH_2 (SEQ ID NO: 347);

Ac-Nle-c[Asp-His-dPhe-transPro(guan)-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 348);

Ac-Nle-c[Asp-His-dPhe-cisPro(guan)-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 349);

Ac-Nle-c[Asp-Pro-dPhe-Arg-dNal(2')-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 350);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Nal(2')-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 351); and

Ac-Nle-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dPro-dVal-dPro-NH_2 (SEQ ID NO: 352).

[0084] In some embodiments, the non-naturally occurring melanocortin analog comprises a sequence of Formula (I), wherein R^4 is dPhe or p(F)dPhe. Accordingly, in some embodiments, the sequence of Formula (I) is a sequence of Formula (IA):

X^1-X^2-X^3-R^1-R^2-R^3-R^4-R^5-R^6-R^7-R^8-Y^1-Y^2-Y^3-Y^4-Y^5-Y^6-Y^7 (IA),

wherein:

X^1 is absent or selected from the group consisting of Nle, Arg, dArg, Ala, Lys, dLys, His, and dHis;

X^2 is absent, Phe, or Nle;

X^3 is absent, Phe, or Nle;

R^1 is selected from the group consisting of Nle, dNle, Ala, dAla, Arg, dArg, Lys, dLys, His, dHis, Orn, dOrn, dLeu, dTyr, Phe, dPhe, Trp, dTrp, Asp, Cys, and dCys;

R^2 is selected from the group consisting of Asp, dAsp, Glu, Cys, dCys, dPen, Ala, dAla, Trp, Phe, dPhe, His, dHis, Tyr, and dTyr;

R^3 is absent or selected from the group consisting of His, Ala, Pro, Hyp, Leu, dGln, Phe, dPhe, Trp, dTrp, Tyr, dTyr, Aba, Ata, Aia, Atc, APC, APPC, Tic, Bip, Oic, Che, Ioc, and Cpe;

R^4 is dPhe or p(F)dPhe;

R^5 is selected from the group consisting of Arg, His, *cisPro*(guan), and *trans*Pro(guan);

R^6 is selected from the group consisting of Trp, dTrp, Phe, Aia, Aba, Ata, dNal(2'), Nal(2'), and Nal(1');

R^7 is selected from the group consisting of Gly, Pro, Lys, dLys, Cys, dCys, Pen, dPen, and Orn;

R^8 is absent or Lys;

Y^1 is absent or selected from the group consisting of Val, dVal, β^2-Val, β^3-Val, Pro, dPro, β-Pro, Hyp, dHyp, dLeu, dTle, Trp, Arg, dArg, Lys, dLys, Gly, Ala, dAla, Asp, dAsp, Asn, dAsn, and dThr;

Y^2 is absent or selected from the group consisting of Val, dVal, β^2-Val, β^3-Val, Pro, dPro, dTle, β-Pro, Hyp, dHyp, dTle, Arg, dArg, Gly, Ala, dAla, Asp, and dAsn;

Y^3 is absent or selected from the group consisting of Val, dVal, Pro, dPro, dThr, and dLys;

Y^4 is absent or selected from the group consisting of dVal, dPro, and dAsp;

Y^5 is absent or dVal;

Y^6 is absent or dVal;

Y^7 is absent or dPro; and

the non-naturally occurring melanocortin analog is cyclized through a moiety selected from the group consisting of:

a disulfide bond between R^1 and R^7 when R^1 and R^7 are each independently Cys or dCys;

a disulfide bond between R^2 and R^7, when R^2 and R^7 are each independently selected from Cys, dCys, Pen, and dPen;

a lactam bridge between R^1 or R^2 and R^7 or R^8 when R^1 or R^2 is Asp and R^7 or R^8 is Lys; and

a lactam bridge between R^2 and R^7 when R^2 is Asp, dAsp, or Glu and R^7 is dLys or Orn.

[0085] In some embodiments, the non-naturally occurring melanocortin analog comprises a sequence of Formula (IA):

X^1-X^2-X^3-R^1-R^2-R^3-R^4-R^5-R^6-R^7-R^8-Y^1-Y^2-Y^3-Y^4-Y^5-Y^6-Y^7 (IA),

wherein:

X^1 is absent or selected from the group consisting of Nle, Arg, dArg, Ala, Lys, dLys, His, and dHis;

X^2 is absent, Phe, or Nle;

X^3 is absent, Phe, or Nle;

R^1 is selected from the group consisting of Nle, dNle, Ala, dAla, Arg, dArg, Lys, dLys, His, dHis, Orn, dOrn, dLeu, dTyr, Phe, dPhe, Trp, dTrp, Asp, Cys, and dCys;

R^2 is selected from the group consisting of Asp, dAsp, Glu, Cys, dCys, dPen, Ala, dAla, Trp, Phe, dPhe, His, dHis, Tyr, and dTyr;

R^3 is absent or selected from the group consisting of His, Ala, Pro, Hyp, Leu, dGln, Phe, dPhe, Trp, dTrp, Tyr, dTyr, Aba, Ata, Aia, Atc, APC, APPC, Tic, Bip, Oic, Che, Ioc, and Cpe;

R^4 is dPhe or p(F)dPhe;

R^5 is selected from the group consisting of Arg, His, *cisPro*(guan), and *trans*Pro(guan);

R^6 is selected from the group consisting of Trp, dTrp, Phe, Aia, Aba, Ata, dNal(2'), Nal(2'), and Nal(1');

R^7 is selected from the group consisting of Gly, Pro, Lys, dLys, Cys, dCys, Pen, dPen, and Orn;

R^8 is absent or Lys;

Y^1 is absent or selected from the group consisting of Val, dVal, β^2-Val, β^3-Val, Pro, dPro, β-Pro, Hyp, dHyp, dLeu, dTle, Trp, Arg, dArg, Lys, dLys, Gly, Ala, dAla, Asp, dAsp, Asn, dAsn, and dThr;

Y^2 is absent or selected from the group consisting of Val, dVal, β^2-Val, β^3-Val, Pro, dPro, dTle, β-Pro, Hyp, dHyp, dTle, Arg, dArg, Gly, Ala, dAla, Asp, and dAsn;

Y^3 is absent or selected from the group consisting of Val, dVal, Pro, dPro, dThr, and dLys;

Y^4 is absent or selected from the group consisting of dVal, dPro, and dAsp;

Y^5 is absent or dVal;

Y^6 is absent or dVal;

Y^7 is absent or dPro; and

the non-naturally occurring melanocortin analog is cyclized through a moiety selected from the group consisting of:

a disulfide bond between R^1 and R^7 when R^1 and R^7 are each independently Cys or dCys;

a disulfide bond between R^2 and R^7, when R^2 and R^7 are each independently selected from Cys, dCys, Pen, and dPen;

a lactam bridge between R^1 or R^2 and R^7 or R^8 when R^1 or R^2 is Asp and R^7 or R^8 is Lys; and

a lactam bridge between R^2 and R^7 when R^2 is Asp, dAsp, or Glu and R^7 is dLys or Orn,

provided that:

when R^4 is p(F)dPhe, then (i) R^3 is selected from Pro, dGln, and dTyr, R^6 is Trp, and R^2 is not Cys; or (ii) R^3 is His and R^6 is Nal(2') or the non-naturally occurring melanocortin analog is cyclized through a disulfide bond between R^2 and R^7;

when R^4 is dPhe and R^1 is Asp, then either (i) R^2 is selected from Trp, Phe, His, Tyr, dPhe, dHis, and dTyr, R^3 is selected from Pro, Ala, and Leu, R^5 is Arg, and R^6 is selected from Trp, Nal(1'), dNal(2'), and Nal(2'); or (ii) R^2 is selected from Ala, dAla, and Phe, and R^3 is His, wherein when R^2 is dAla, then Y^1-Y^7 are absent and when R^2 is Ala, then R^1 is not Nle;

when R^4 is dPhe, and R^2 is Asp, Glu, or dPen, then R^3 is not His or absent; and

when R^4 is dPhe, R^2 is Asp, and R^3 is Pro, then R^5 is not dNal(2') or Nal(2') and Y^3-Y^7 are absent if any of X^1-X^3 are present.

[0086] In some embodiments, the non-naturally occurring melanocortin analog comprises a sequence of Formula (IA):

$$X^1\text{-}X^2\text{-}X^3\text{-}R^1\text{-}R^2\text{-}R^3\text{-}R^4\text{-}R^5\text{-}R^6\text{-}R^7\text{-}R^8\text{-}Y^1\text{-}Y^2\text{-}Y^3\text{-}Y^4\text{-}Y^5\text{-}Y^6\text{-}Y^7 \text{ (IA)},$$

wherein:

X^1 is absent or selected from the group consisting of Nle, Arg, dArg, Ala, Lys, dLys, His, and dHis;

X^2 is absent, Phe, or Nle;

X^3 is absent, Phe, or Nle;

R^1 is selected from the group consisting of Nle, dNle, Ala, dAla, Arg, dArg, Lys, dLys, His, dHis, Orn, dOrn, dLeu, dTyr, Phe, dPhe, Trp, dTrp, Asp, Cys, and dCys;

R^2 is selected from the group consisting of Asp, dAsp, Glu, Cys, dCys, dPen, Ala, dAla, Trp, Phe, dPhe, His, dHis, Tyr, and dTyr;

R^3 is absent or selected from the group consisting of His, Ala, Pro, Hyp, Leu, dGln, Phe, dPhe, Trp, dTrp, Tyr, dTyr, Aba, Ata, Aia, Atc, APC, APPC, Tic, Bip, Oic, Che, Ioc, and Cpe;

R^4 is dPhe or p(F)dPhe;

R^5 is selected from the group consisting of Arg, His, *cisPro*(guan), and *trans*Pro(guan);

R^6 is selected from the group consisting of Trp, dTrp, Phe, Aia, Aba, Ata, dNal(2'), Nal(2'), and Nal(1');

R^7 is selected from the group consisting of Gly, Pro, Lys, dLys, Cys, dCys, Pen, dPen, and Orn;

R^8 is absent or Lys;

Y^1 is absent or selected from the group consisting of Val, dVal, β^2-Val, β^3-Val, Pro, dPro, β-Pro, Hyp, dHyp, dLeu, dTle, Trp, Arg, dArg, Lys, dLys, Gly, Ala, dAla, Asp, dAsp, Asn, dAsn, and dThr;

Y^2 is absent or selected from the group consisting of Val, dVal, β^2-Val, β^3-Val, Pro, dPro, dTle, β-Pro, Hyp, dHyp, dTle, Arg, dArg, Gly, Ala, dAla, Asp, and dAsn;

Y^3 is absent or selected from the group consisting of Val, dVal, Pro, dPro, dThr, and dLys;

Y^4 is absent or selected from the group consisting of dVal, dPro, and dAsp;

Y^5 is absent or dVal;

Y^6 is absent or dVal;

Y^7 is absent or dPro; and

the non-naturally occurring melanocortin analog is cyclized through a moiety selected from the group consisting of:

a disulfide bond between R^1 and R^7 when R^1 and R^7 are each independently Cys or dCys;

a disulfide bond between R^2 and R^7, when R^2 and R^7 are each independently selected from Cys, dCys, Pen, and dPen;

a lactam bridge between R^1 or R^2 and R^7 or R^8 when R^1 or R^2 is Asp and R^7 or R^8 is Lys; and

a lactam bridge between R^2 and R^7 when R^2 is Asp, dAsp, or Glu and R^7 is dLys or Orn,

provided that the non-naturally occurring melanocortin analog does not comprise a sequence selected from the group consisting of:

Ac-Nle-c[Asp-dTrp-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 295);

Ac-Nle-c[Asp-dPhe-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 296);

Ac-Ala-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 297);

Ac-dArg-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 298);

Ac-Nle-c[Asp-His-p(F)dPhe-Arg-dNal(2')-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 299);

Ac-Nle-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dTle-dTle-dVal-NH_2 (SEQ ID NO: 300);

Ac-Ala-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 301);

Ac-dArg-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 302);

Ac-Nle-c[Asp-Pro-p(F)dPhe-Arg-Nal(2')-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 303);

Ac-Nle-c[Asp-Trp-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 304);

Ac-Nle-c[Asp-Tyr-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 305);

Ac-Nle-c[Cys-Pro-p(F)dPhe-Arg-Trp-Cys]-dVal-dPro-NH_2 (SEQ ID NO: 306);

Ac-Nle-c[Asp-Pro-p(F)dPhe-Arg-dNal(2')-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 307);

Ac-Arg-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 308);

Ac-Lys-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 309);

Ac-dLys-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 310);

Ac-His-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 311);

Ac-dHis-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 312);

Ac-Nle-Nle-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 313);

Ac-Nle-c[Asp-His-p(F)dPhe-His-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 314);

Ac-Nle-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dVal-dVal-dVal-dPro-NH_2 (SEQ ID NO: 315);

Ac-Nle-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dPro-dVal-dPro-NH_2 (SEQ ID NO: 316);

Ac-Nle-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 317);

Ac-Nle-Nle-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 318);

Ac-Nle-Nle-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dPro-dVal-dPro-NH_2 (SEQ ID NO: 319);

Ac-Arg-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 320);

Ac-Lys-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 321);

Ac-dLys-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 322);

Ac-His-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 323);

Ac-dHis-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 324);

Ac-Nle-c[Asp-His-p(F)dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2 (SEQ ID NO: 325);

Ac-Nle-c[Glu-His-p(F)dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2 (SEQ ID NO: 326);

Ac-Nle-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 327);

Ac-Nle-c[Asp-Bip-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 328);

Ac-Nle-c[Asp-dHis-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 329);

Ac-Nle-c[Asp-Phe-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 330);

Ac-Nle-c[Asp-Pro-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 331);

Ac-Nle-c[Asp-Pro-His-dPhe-His-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 332);

Ac-Nle-c[Asp-Trp-Pro-dPhe-His-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 333);

Ac-Nle-c[Asp-Trp-Pro-dPhe-Arg-Phe-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 334);

Ac-dArg-c[Asp-dAla-His-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 335);

Ac-Nle-c[Asp-Ala-His-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 336);

Ac-Nle-c[Asp-Pro-His-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 337);

Ac-Nle-c[Asp-dAla-His-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 338);

Ac-Arg-c[Asp-dAla-His-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 339);

Ac-Nle-c[Asp-dHis-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 340);

Ac-Arg-c[Asp-dAla-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 341);

Ac-dArg-c[Asp-dAla-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 342);

Ac-Nle-c[Asp-His-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 343);

Ac-Nle-c[Asp-His-dPhe-Arg-Trp-Pro-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 344);

Ac-Nle-c[Asp-His-dPhe-Arg-Trp-Lys]-dPro-dVal-NH_2 (SEQ ID NO: 345);

Ac-Nle-c[Glu-His-dPhe-His-Trp-Orn]-dVal-dPro-NH_2 (SEQ ID NO: 346);

Ac-Nle-c[dPen-dPhe-Arg-Trp-dPen]-dVal-dPro-NH_2 (SEQ ID NO: 347);

Ac-Nle-c[Asp-His-dPhe-transPro(guan)-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 348);

Ac-Nle-c[Asp-His-dPhe-cisPro(guan)-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 349);

Ac-Nle-c[Asp-Pro-dPhe-Arg-dNal(2')-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 350);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Nal(2')-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 351); and

Ac-Nle-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dPro-dVal-dPro-NH_2 (SEQ ID NO: 352).

[0087] In some embodiments, the non-naturally occurring melanocortin analog comprises a sequence of Formula (I) or (IA), wherein R^4 is dPhe. In further embodiments, the sequence of Formula (I) or (IA) is cyclized between R^2 and R^7 or R^8. Accordingly, in some embodiments, the sequence of Formula (I) or (IA) is a sequence of Formula (IA(i)):

$$X^1\text{-}X^2\text{-}X^3\text{-}R^1\text{-}R^2\text{-}R^3\text{-}R^4\text{-}R^5\text{-}R^6\text{-}R^7\text{-}R^8\text{-}Y^1\text{-}Y^2\text{-}Y^3\text{-}Y^4\text{-}Y^5\text{-}Y^6\text{-}Y^7 \text{ (IA(i))},$$

wherein:

X^1 is absent or Nle;

X^2 is absent or Nle;

X^3 is absent or Nle;

R^1 is selected from the group consisting of Nle, dNle, Ala, dAla, Arg, dArg, Lys, dLys, His, dHis, Orn, dOrn, dLeu, dTyr, Phe, dPhe, Trp, and dTrp;

R^2 is selected from the group consisting of Asp, dAsp, Glu, Cys, dCys, and dPen;

R^3 is absent or selected from the group consisting of Ala, Pro, Hyp, Leu, dGln, Phe, dPhe, Trp, dTrp, Tyr, dTyr, Aba, Ata, Aia, Atc, APC, APPC, Tic, Bip, Oic, Che, Ioc, and Cpe;

R^4 is dPhe;

R^5 is selected from the group consisting of Arg, His, *cisPro*(guan), and *trans*Pro(guan);

R^6 is selected from the group consisting of Trp, dTrp, Phe, Aia, Aba, Ata, and Nal(1');

R^7 is selected from the group consisting of Gly, Pro, Lys, dLys, Cys, dCys, Pen, dPen, and Orn;

R^8 is absent or Lys;

Y^1 is absent or selected from the group consisting of Val, dVal, β^2-Val, β^3-Val, Pro, dPro, β-Pro, Hyp, dHyp, dLeu, dTle, Trp, Arg, dArg, Lys, dLys, Gly, Ala, dAla, Asp, dAsp, Asn, dAsn, and dThr;

Y^2 is absent or selected from the group consisting of Val, dVal, β^2-Val, β^3-Val, Pro, dPro, dTle, β-Pro, Hyp, dHyp, dTle, Arg, dArg, Gly, Ala, dAla, Asp, and dAsn;

Y^3 is absent or selected from the group consisting of Val, dVal, Pro, dPro, dThr, and dLys;

Y^4 is absent or selected from the group consisting of dVal, dPro, and dAsp;

Y^5 is absent or dVal;

Y^6 is absent or dVal;

Y^7 is absent or dPro; and

the non-naturally occurring melanocortin analog is cyclized through a moiety selected from the group consisting of:

a disulfide bond between R^2 and R^7, when R^2 and R^7 are each independently selected from Cys, dCys, Pen, and dPen;

a lactam bridge between R^2 and R^7 or R^8 when R^2 is Asp and R^7 or R^8 is Lys; and

a lactam bridge between R^2 and R^7 when R^2 is Asp, dAsp, or Glu and R^7 is dLys or Orn.

[0088] In some embodiments, the non-naturally occurring melanocortin analog comprises a sequence of Formula (IA(i)):

X^1-X^2-X^3-R^1-R^2-R^3-R^4-R^5-R^6-R^7-R^8-Y^1-Y^2-Y^3-Y^4-Y^5-Y^6-Y^7 (IA(i)),

wherein:

X^1 is absent or Nle;

X^2 is absent or Nle;

X^3 is absent or Nle;

R^1 is selected from the group consisting of Nle, dNle, Ala, dAla, Arg, dArg, Lys, dLys, His, dHis, Orn, dOrn, dLeu, dTyr, Phe, dPhe, Trp, and dTrp;

R^2 is selected from the group consisting of Asp, dAsp, Glu, Cys, dCys, and dPen;

R^3 is absent or selected from the group consisting of Ala, Pro, Hyp, Leu, dGln, Phe, dPhe, Trp, dTrp, Tyr, dTyr, Aba, Ata, Aia, Atc, APC, APPC, Tic, Bip, Oic, Che, Ioc, and Cpe;

R^4 is dPhe;

R^5 is selected from the group consisting of Arg, His, *cisPro*(guan), and *trans*Pro(guan);

R^6 is selected from the group consisting of Trp, dTrp, Phe, Aia, Aba, Ata, and Nal(1');

R^7 is selected from the group consisting of Gly, Pro, Lys, dLys, Cys, dCys, Pen, dPen, and Orn;

R^8 is absent or Lys;

Y^1 is absent or selected from the group consisting of Val, dVal, β^2-Val, β^3-Val, Pro, dPro, β-Pro, Hyp, dHyp, dLeu, dTle, Trp, Arg, dArg, Lys, dLys, Gly, Ala, dAla, Asp, dAsp, Asn, dAsn, and dThr;

Y^2 is absent or selected from the group consisting of Val, dVal, β^2-Val, β^3-Val, Pro, dPro, dTle, β-Pro, Hyp, dHyp, dTle, Arg, dArg, Gly, Ala, dAla, Asp, and dAsn;

Y^3 is absent or selected from the group consisting of Val, dVal, Pro, dPro, dThr, and dLys;

Y^4 is absent or selected from the group consisting of dVal, dPro, and dAsp;

Y^5 is absent or dVal;

Y^6 is absent or dVal;

Y^7 is absent or dPro; and

the non-naturally occurring melanocortin analog is cyclized through a moiety selected from the group consisting of:

a disulfide bond between R^2 and R^7, when R^2 and R^7 are each independently selected from Cys, dCys, Pen, and dPen;

a lactam bridge between R^2 and R^7 or R^8 when R^2 is Asp and R^7 or R^8 is Lys; and

a lactam bridge between R^2 and R^7 when R^2 is Asp, dAsp, or Glu and R^7 is dLys or Orn,

provided that:

when R^2 is Asp, Glu, or dPen, then R^3 is not absent; and

when R^2 is Asp and R^3 is Pro, then Y^3-Y^7 are absent if any of X^1-X^3 are present.

[0089] In some embodiments, the non-naturally occurring melanocortin analog comprises a sequence of Formula (IA(i)), wherein:

X^1 is absent or Nle;

X^2 is absent or Nle;

X^3 is absent or Nle;

R^1 is selected from the group consisting of Nle, dNle, Ala, dAla, Arg, dArg, Lys, dLys, His, dHis, Orn, dOrn, dLeu, dTyr, Phe, dPhe, Trp, and dTrp;

R^2 is selected from the group consisting of Asp, dAsp, Glu, Cys, dCys, and dPen;

R^3 is absent or selected from the group consisting of Ala, Pro, Hyp, Leu, dGln, Phe, dPhe, Trp, dTrp, Tyr, dTyr, Aba, Ata, Aia, Atc, APC, APPC, Tic, Bip, Oic, Che, Ioc, and Cpe;

R^4 is dPhe;

R^5 is selected from the group consisting of Arg, His, *cisPro*(guan), and *trans*Pro(guan);

R^6 is selected from the group consisting of Trp, dTrp, Phe, Aia, Aba, Ata, and Nal(1');

R^7 is selected from the group consisting of Gly, Pro, Lys, dLys, Cys, dCys, Pen, dPen, and Orn;

R^8 is absent or Lys;

Y^1 is absent or selected from the group consisting of Val, dVal, β^2-Val, β^3-Val, Pro, dPro, β-Pro, Hyp, dHyp, dLeu, dTle, Trp, Arg, dArg, Lys, dLys, Gly, Ala, dAla, Asp, dAsp, Asn, dAsn, and dThr;

Y^2 is absent or selected from the group consisting of Val, dVal, β^2-Val, β^3-Val, Pro, dPro, dTle, β-Pro, Hyp, dHyp, dTle, Arg, dArg, Gly, Ala, dAla, Asp, and dAsn;

Y^3 is absent or selected from the group consisting of Val, dVal, Pro, dPro, dThr, and dLys;

Y^4 is absent or selected from the group consisting of dVal, dPro, and dAsp;

Y^5 is absent or dVal;

Y^6 is absent or dVal;

Y^7 is absent or dPro; and

the non-naturally occurring melanocortin analog is cyclized through a moiety selected from the group consisting of:

a disulfide bond between R^2 and R^7, when R^2 and R^7 are each independently selected from Cys, dCys, Pen, and dPen;

a lactam bridge between R^2 and R^7 or R^8 when R^2 is Asp and R^7 or R^8 is Lys; and

a lactam bridge between R^2 and R^7 when R^2 is Asp, dAsp, or Glu and R^7 is dLys or Orn,

provided that the non-naturally occurring melanocortin analog does not comprise a sequence of:

Ac-Nle-c[dPen-dPhe-Arg-Trp-dPen]-dVal-dPro-NH_2 (SEQ ID NO: 347); or

Ac-Nle-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dPro-dVal-dPro-NH_2 (SEQ ID NO: 352).

[0090] Alternatively, in some embodiments, when the non-naturally occurring melanocortin analog comprises a sequence of Formula (I) or (IA), wherein R^4 is dPhe, then the sequence of Formula (I) or (IA) is cyclized between R^1 and R^7. Accordingly, in some embodiments, the sequence of Formula (I) or (IA) is a sequence of Formula (IA(ii)):

$$X^1\text{-}X^2\text{-}X^3\text{-}R^1\text{-}R^2\text{-}R^3\text{-}R^4\text{-}R^5\text{-}R^6\text{-}R^7\text{-}Y^1\text{-}Y^2\text{-}Y^3\text{-}Y^4 \text{ (IA(ii))},$$

wherein:

X^1 is absent or selected from the group consisting of Nle, Arg, dArg, Ala, Lys, dLys, His, and dHis;

X^2 is absent, Phe, or Nle;

X^3 is absent or Phe;

R^1 is selected from the group consisting of Asp, Cys, and dCys;

R^2 is selected from the group consisting of Ala, dAla, Trp, Phe, dPhe, His, dHis, Tyr, and dTyr;

R^3 is absent or selected from the group consisting of His, Pro, Ala, and Leu;

R^4 is dPhe;

R^5 is Arg;

R^6 is selected from the group consisting of Trp, dNal(2'), Nal(2'), and Nal(1');

R^7 is selected from the group consisting of Lys, Cys, and dCys;

Y^1 is absent or selected from the group consisting of dVal, dLeu, dTle, dArg, and dLys;

Y^2 is absent or selected from the group consisting of dVal, dPro, and dHyp;

Y^3 is absent, dVal, or dPro;

Y^4 is absent or dPro; and

the non-naturally occurring melanocortin analog is cyclized through a moiety selected from the group consisting of:

a disulfide bond between R^1 and R^7 when R^1 and R^7 are each independently Cys or dCys; and

a lactam bridge between R^1 and R^7 when R^1 is Asp and R^7 is Lys.

[0091] In some embodiments, the non-naturally occurring melanocortin analog comprises a sequence of Formula (IA(ii)):

$$X^1\text{-}X^2\text{-}X^3\text{-}R^1\text{-}R^2\text{-}R^3\text{-}R^4\text{-}R^5\text{-}R^6\text{-}R^7\text{-}Y^1\text{-}Y^2\text{-}Y^3\text{-}Y^4 \text{ (IA(ii))},$$

wherein:

X^1 is absent or selected from the group consisting of Nle, Arg, dArg, Ala, Lys, dLys, His, and dHis;

X^2 is absent, Phe, or Nle;

X^3 is absent or Phe;

R^1 is selected from the group consisting of Asp, Cys, and dCys;

R^2 is selected from the group consisting of Ala, dAla, Trp, Phe, dPhe, His, dHis, Tyr, and dTyr;

R^3 is absent or selected from the group consisting of His, Pro, Ala, and Leu;

R^4 is dPhe;

R^5 is Arg;

R^6 is selected from the group consisting of Trp, dNal(2'), Nal(2'), and Nal(1');

R^7 is selected from the group consisting of Lys, Cys, and dCys;

Y^1 is absent or selected from the group consisting of dVal, dLeu, dTle, dArg, and dLys;

Y^2 is absent or selected from the group consisting of dVal, dPro, and dHyp;

Y^3 is absent, dVal, or dPro;

Y^4 is absent or dPro; and

the non-naturally occurring melanocortin analog is cyclized through a moiety selected from the group consisting of:

a disulfide bond between R^1 and R^7 when R^1 and R^7 are each independently Cys or dCys; and

a lactam bridge between R^1 and R^7 when R^1 is Asp and R^7 is Lys,

provided that:

when R^1 is Asp, then either (i) R^2 is selected from Trp, Phe, His, Tyr, dPhe, dHis, and dTyr, R^3 is selected from Pro, Ala, and Leu, R^5 is Arg, and R^6 is selected from Trp, Nal(1'), dNal(2'), and Nal(2'); or (ii) R^2 is selected from Ala, dAla, and Phe, and R^3 is His, wherein when R^2 is dAla, then Y^1-Y^7 are absent and when R^2 is Ala, then R^1 is not Nle.

[0092] In some embodiments, the non-naturally occurring melanocortin analog comprises a sequence of Formula (IA(ii)), wherein:

X^1 is absent or selected from the group consisting of Nle, Arg, dArg, Ala, Lys, dLys, His, and dHis;

X^2 is absent, Phe, or Nle;

X^3 is absent or Phe;

R^1 is selected from the group consisting of Asp, Cys, and dCys;

R^2 is selected from the group consisting of Ala, dAla, Trp, Phe, dPhe, His, dHis, Tyr, and dTyr;

R^3 is selected from the group consisting of His, Pro, Ala, and Leu;

R^4 is dPhe;

R^5 is Arg;

R^6 is selected from the group consisting of Trp, dNal(2'), Nal(2'), and Nal(1');

R^7 is selected from the group consisting of Lys, Cys, and dCys;

Y^1 is absent or selected from the group consisting of dVal, dLeu, dTle, dArg, and dLys;

Y^2 is absent or selected from the group consisting of dVal, dPro, and dHyp;

Y^3 is absent, dVal, or dPro;

Y^4 is absent or dPro; and

the non-naturally occurring melanocortin analog is cyclized through a moiety selected from the group consisting of:

a disulfide bond between R^1 and R^7 when R^1 and R^7 are each independently Cys or dCys; and

a lactam bridge between R^1 and R^7 when R^1 is Asp and R^7 is Lys,

provided that the non-naturally occurring melanocortin analog does not comprise a sequence selected from the group consisting of:

Ac-Nle-c[Asp-Trp-Pro-dPhe-His-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 333);

Ac-Nle-c[Asp-Trp-Pro-dPhe-Arg-Phe-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 334);

Ac-dArg-c[Asp-dAla-His-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 335); and

Ac-Nle-c[Asp-Ala-His-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 336).

[0093] In some embodiments, when the non-naturally occurring melanocortin analog comprises a sequence of Formula (I) or (IA), wherein R^4 is p(F)dPhe. Accordingly, in some embodiments, the sequence of Formula (I) or (IA) is a sequence of Formula (IA(iii)):

$$X^1\text{-}R^1\text{-}R^2\text{-}R^3\text{-}R^4\text{-}R^5\text{-}R^6\text{-}R^7\text{-}Y^1\text{-}Y^2\text{-}Y^3\text{-}Y^4 \text{ (IA(iii))},$$

wherein:

X^1 is absent or Nle;

R^1 is selected from the group consisting of Nle, Ala, Arg, dArg, Lys, dLys, His, and dHis;

R^2 is selected from the group consisting of Asp, Glu, Cys, and dCys;

R^3 is selected from the group consisting of His, Pro, dGln, and dTyr;

R^4 is p(F)dPhe;

R^5 is Arg or His;

R^6 is Trp or Nal(2');

R^7 is selected from the group consisting of Lys, Cys, dCys, and Orn;

Y^1 is selected from the group consisting of dVal, dPro, and dTle;

Y^2 is selected from the group consisting of dVal, dPro, and dTle;

Y^3 is absent, dVal, or dPro;

Y^4 is absent or dPro; and

the non-naturally occurring melanocortin analog is cyclized through a moiety selected from the group consisting of:

a disulfide bond between R^2 and R^7, when R^2 and R^7 are each independently Cys or dCys;

a lactam bridge between R^2 and R^7 when R^2 is Asp and R^7 is Lys; and

a lactam bridge between R^2 and R^7 when R^2 is Asp, or Glu and R^7 is Orn.

[0094] In some embodiments, the non-naturally occurring melanocortin analog comprises a sequence of Formula (IA(iii)):

$$X^1\text{-}R^1\text{-}R^2\text{-}R^3\text{-}R^4\text{-}R^5\text{-}R^6\text{-}R^7\text{-}Y^1\text{-}Y^2\text{-}Y^3\text{-}Y^4 \text{ (IA(iii))},$$

wherein:

X^1 is absent or Nle;

R^1 is selected from the group consisting of Nle, Ala, Arg, dArg, Lys, dLys, His, and dHis;

R^2 is selected from the group consisting of Asp, Glu, Cys, and dCys;

R^3 is selected from the group consisting of His, Pro, dGln, and dTyr;

R^4 is p(F)dPhe;

R^5 is Arg or His;

R^6 is Trp or Nal(2');

R^7 is selected from the group consisting of Lys, Cys, dCys, and Orn;

Y^1 is selected from the group consisting of dVal, dPro, and dTle;

Y^2 is selected from the group consisting of dVal, dPro, and dTle;

Y^3 is absent, dVal, or dPro;

Y^4 is absent or dPro; and

the non-naturally occurring melanocortin analog is cyclized through a moiety selected from the group consisting of:

a disulfide bond between R^2 and R^7, when R^2 and R^7 are each independently Cys or dCys;

a lactam bridge between R^2 and R^7 when R^2 is Asp and R^7 is Lys; and

a lactam bridge between R^2 and R^7 when R^2 is Asp, or Glu and R^7 is Orn,

provided that:

when R^3 is selected from Pro, dGln, and dTyr, R^6 is Trp, then R^2 is not Cys; and

when R^3 is His, then R^6 is Nal(2') or the non-naturally occurring melanocortin analog is cyclized through a disulfide bond between R^2 and R^7.

[0095] In some embodiments, the non-naturally occurring melanocortin analog comprises a sequence of Formula (IA(iii)), wherein:

X^1 is absent or Nle;

R^1 is selected from the group consisting of Nle, Ala, Arg, dArg, Lys, dLys, His, and dHis;

R^2 is selected from the group consisting of Asp, Glu, Cys, and dCys;

R^3 is selected from the group consisting of His, Pro, dGln, and dTyr;

R^4 is p(F)dPhe;

R^5 is Arg or His;

R^6 is Trp or Nal(2');

R^7 is selected from the group consisting of Lys, Cys, dCys, and Orn;

Y^1 is selected from the group consisting of dVal, dPro, and dTle;

Y^2 is selected from the group consisting of dVal, dPro, and dTle;

Y^3 is absent, dVal, or dPro;

Y^4 is absent or dPro; and

the non-naturally occurring melanocortin analog is cyclized through a moiety selected from the group consisting of:

a disulfide bond between R^2 and R^7, when R^2 and R^7 are each independently Cys or dCys;

a lactam bridge between R^2 and R^7 when R^2 is Asp and R^7 is Lys; and

a lactam bridge between R^2 and R^7 when R^2 is Asp, or Glu and R^7 is Orn,

provided that the non-naturally occurring melanocortin analog does not comprise a sequence selected from the group consisting of:

Ac-Ala-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 297);

Ac-dArg-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 298);

Ac-Nle-c[Asp-His-p(F)dPhe-Arg-dNal(2')-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 299);

Ac-Nle-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dTle-dTle-dVal-NH_2 (SEQ ID NO: 300);

Ac-Ala-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 301);

Ac-dArg-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 302);

Ac-Nle-c[Asp-Pro-p(F)dPhe-Arg-Nal(2')-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 303);

Ac-Nle-c[Cys-Pro-p(F)dPhe-Arg-Trp-Cys]-dVal-dPro-NH_2 (SEQ ID NO: 306);

Ac-Nle-c[Asp-Pro-p(F)dPhe-Arg-dNal(2')-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 307);

Ac-Arg-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 308);

Ac-Lys-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 309);

Ac-dLys-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 310);

Ac-His-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 311);

Ac-dHis-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 312);

Ac-Nle-Nle-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 313);

Ac-Nle-c[Asp-His-p(F)dPhe-His-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 314);

Ac-Nle-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dVal-dVal-dVal-dPro-NH_2 (SEQ ID NO: 315);

Ac-Nle-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dPro-dVal-dPro-NH_2 (SEQ ID NO: 316);

Ac-Nle-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 317);

Ac-Nle-Nle-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 318);

Ac-Nle-Nle-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dPro-dVal-dPro-NH_2 (SEQ ID NO: 319);

Ac-Arg-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 320);

Ac-Lys-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 321);

Ac-dLys-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 322);

Ac-His-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 323);

Ac-dHis-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 324);

Ac-Nle-c[Asp-His-p(F)dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2 (SEQ ID NO: 325);

Ac-Nle-c[Glu-His-p(F)dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2 (SEQ ID NO: 326); and

Ac-Nle-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 327).

[0096] In some embodiments, when the non-naturally occurring melanocortin analog comprises a sequence of Formula (I), wherein R^4 is p(Cl)dPhe or p(Br)dPhe. When R^4 is p(Cl)dPhe or p(Br)dPhe in the sequence of Formula (I), then R^8 is absent. Accordingly, in some embodiments, the sequence of Formula (I) is a sequence of Formula (IB):

$$X^1\text{-}R^1\text{-}R^2\text{-}R^3\text{-}R^4\text{-}R^5\text{-}R^6\text{-}R^7\text{-}Y^1\text{-}Y^2\text{-}Y^3\text{-}Y^4 \text{ (IB)},$$

wherein:

X^1 is absent or Nle;

R^1 is selected from the group consisting of Nle, Arg, dArg, Lys, dLys, His, and dHis;

R^2 is Asp or dCys;

R^3 is selected from the group consisting of His, Aba, Aia, and Ata;

R^4 is p(Cl)dPhe or p(Br)dPhe;

R^5 is Arg;

R^6 is Trp;

R^7 is selected from the group consisting of Lys, Cys, and dCys;

Y^1 is selected from the group consisting of dVal, dPro, and dTle;

Y^2 is selected from the group consisting of dVal, dPro, and dTle;

Y^3 is absent, dVal, or dPro;

Y^4 is absent or dPro; and

the non-naturally occurring melanocortin analog is cyclized through a moiety selected from the group consisting of:

a disulfide bond between R^2 and R^7, when R^2 and R^7 are each independently Cys or dCys; and

a lactam bridge between R^2 and R^7 when R^2 is Asp and R^7 is Lys.

[0097] In some embodiments, the non-naturally occurring melanocortin analog comprises a sequence of Formula (IB):

$$X^1\text{-}R^1\text{-}R^2\text{-}R^3\text{-}R^4\text{-}R^5\text{-}R^6\text{-}R^7\text{-}Y^1\text{-}Y^2\text{-}Y^3\text{-}Y^4 \text{ (IB)},$$

wherein:

X^1 is absent or Nle;

R^1 is selected from the group consisting of Nle, Arg, dArg, Lys, dLys, His, and dHis;

R^2 is Asp or dCys;

R^3 is selected from the group consisting of His, Aba, Aia, and Ata;

R^4 is p(Cl)dPhe or p(Br)dPhe;

R^5 is Arg;

R^6 is Trp;

R^7 is selected from the group consisting of Lys, Cys, and dCys;

Y^1 is selected from the group consisting of dVal, dPro, and dTle;

Y^2 is selected from the group consisting of dVal, dPro, and dTle;

Y^3 is absent, dVal, or dPro;

Y^4 is absent or dPro; and

the non-naturally occurring melanocortin analog is cyclized through a moiety selected from the group consisting of:

a disulfide bond between R^2 and R^7, when R^2 and R^7 are each independently Cys or dCys; and

a lactam bridge between R^2 and R^7 when R^2 is Asp and R^7 is Lys,

provided that:

when R^4 is p(Br)dPhe, then R^2 is dCys; and

when R^4 is p(Cl)dPhe, then (i) R^3 is selected from Ata, Aia, and Aba; or (ii) R^3 is His, R^5 is Arg; R^6 is Trp; R^7 is Lys or dCys, and R^1 is not Ala, wherein when X^1 is present, then Y^1 is dTle and when X^1 is absent and Y^1-Y^2 is dVal-dPro or dTle-dPro, then either R^1 is not Nle or the non-naturally occurring melanocortin analog is cyclized through a disulfide bond between R^2 and R^7.

[0098] In some embodiments, the non-naturally occurring melanocortin analog comprises a sequence of Formula (IB), wherein:

X^1 is absent or Nle;

R^1 is selected from the group consisting of Nle, Arg, dArg, Lys, dLys, His, and dHis;

R^2 is Asp or dCys;

R^3 is selected from the group consisting of His, Aba, Aia, and Ata;

R^4 is p(Cl)dPhe or p(Br)dPhe;

R^5 is Arg;

R^6 is Trp;

R^7 is selected from the group consisting of Lys, Cys, and dCys;

Y^1 is selected from the group consisting of dVal, dPro, and dTle;

Y^2 is selected from the group consisting of dVal, dPro, and dTle;

Y^3 is absent, dVal, or dPro;

Y^4 is absent or dPro; and

the non-naturally occurring melanocortin analog is cyclized through a moiety selected from the group consisting of:

a disulfide bond between R^2 and R^7, when R^2 and R^7 are each independently Cys or dCys; and

a lactam bridge between R^2 and R^7 when R^2 is Asp and R^7 is Lys,

provided that the non-naturally occurring melanocortin analog does not comprise a sequence selected from the group consisting of:

Ac-Nle-Nle-c[Asp-His-p(Br)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 234);

Ac-Nle-c[Asp-His-p(Br)dPhe-Arg-Trp-Lys]-dTle-dTle-dVal-NH_2 (SEQ ID NO: 238);

Ac-Nle-c[Asp-His-p(Br)dPhe-Arg-Trp-Lys]-dPro-dVal-dPro-NH_2 (SEQ ID NO: 239);

Ac-Nle-c[Asp-His-p(Br)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 240);

Ac-Nle-Nle-c[Asp-His-p(Br)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 241);

Ac-Nle-c[Asp-His-p(Br)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 250);

Ac-dArg-c[Asp-His-p(Br)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 251);

Ac-Arg-c[Asp-His-p(Br)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 252);

Ac-Lys-c[Asp-His-p(Br)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 253);

Ac-dLys-c[Asp-His-p(Br)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 254);

Ac-His-c[Asp-His-p(Br)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 255);

Ac-dHis-c[Asp-His-p(Br)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 256);

Ac-Nle-c[Asp-His-p(Br)dPhe-Arg-Trp-Lys]-dVal-dVal-dVal-dPro-NH_2 (SEQ ID NO: 257);

Ac-Nle-Nle-c[Asp-His-p(Br)dPhe-Arg-Trp-Lys]-dPro-dVal-dPro-NH_2 (SEQ ID NO: 258);

Ac-dArg-c[Asp-His-p(Br)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 259);

Ac-Arg-c[Asp-His-p(Br)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 260);

Ac-Lys-c[Asp-His-p(Br)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 261);

Ac-dLys-c[Asp-His-p(Br)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 262);

Ac-His-c[Asp-His-p(Br)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 263);

Ac-dHis-c[Asp-His-p(Br)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 264);

Ac-Nle-c[Asp-His-p(Cl)dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2 (SEQ ID NO: 279);

Ac-Nle-c[Glu-His-p(Cl)dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2 (SEQ ID NO: 280);

Ac-Nle-c[dCys-His-p(Cl)dPhe-Arg-Trp-Cys]-dVal-dPro-NH_2 (SEQ ID NO: 281);

Ac-Nle-Nle-c[Asp-His-p(Cl)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 288);

Ac-Nle-Nle-c[Asp-His-p(Cl)dPhe-Arg-Trp-Lys]-dPro-dVal-dPro-NH_2 (SEQ ID NO: 289);

Ac-Nle-c[Asp-His-p(Cl)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 290); and

Ac-Nle-c[Asp-His-p(Cl)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 291).

[0099] In some embodiments, when the non-naturally occurring melanocortin analog comprises a sequence of Formula (I) or (IA), wherein R^4 is dPhe or p(F)dPhe. In further embodiments, the sequence of Formula (I) or (IA) is cyclized through a lactam bond between Asp or Glu at R^2 and Orn at R^7. Accordingly, in some embodiments, the sequence of Formula (I) is a sequence of Formula (IC):

$$R^1\text{-}R^2\text{-}R^3\text{-}R^4\text{-}R^5\text{-}R^6\text{-}R^7\text{-}Y^1\text{-}Y^2 \text{ (IC)},$$

wherein:

R^1 is Nle;

R^2 is Asp or Glu;

R^3 is Pro;

R^4 is p(F)dPhe or dPhe;

R^5 is Arg;

R^6 is Trp;

R^7 is Orn;

Y^1 is dVal;

Y^2 is dPro; and

the non-naturally occurring melanocortin analog is cyclized through a lactam bridge between Asp or Glu at R^2 and Orn at R^7.

[0100] In some embodiments, the non-naturally occurring melanocortin analog comprises a sequence of Formula (IC):

R^1-R^2-R^3-R^4-R^5-R^6-R^7-Y^1-Y^2 (IC),

wherein:

R^1 is Nle;

R^2 is Asp or Glu;

R^3 is Pro;

R^4 is p(F)dPhe or dPhe;

R^5 is Arg;

R^6 is Trp;

R^7 is Orn;

Y^1 is dVal;

Y^2 is dPro; and

the non-naturally occurring melanocortin analog is cyclized through a lactam bridge between Asp or Glu at R^2 and Orn at R^7,

provided that:

when R^4 is dPhe, then R^2 is Glu.

[0101] In some embodiments, the non-naturally occurring melanocortin analog comprises a sequence of Formula (IC), wherein:

R^1 is Nle;

R^2 is Asp or Glu;

R^3 is Pro;

R^4 is p(F)dPhe or dPhe;

R^5 is Arg;

R^6 is Trp;

R^7 is Orn;

Y^1 is dVal;

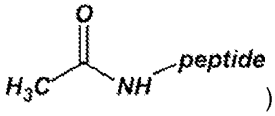
Y^2 is dPro; and

the non-naturally occurring melanocortin analog is cyclized through a lactam bridge between Asp or Glu at R^2 and Orn at R^7,

provided that the non-naturally occurring melanocortin analog does not comprise a sequence of: Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2 (SEQ ID NO: 23).

[0102] In some embodiments, the non-naturally occurring melanocortin analog has one or more beta hairpin (β-hairpin) and/or beta turn (β-turn) structures. In some embodiments, the presence of Pro, dPro, Hyp, dHyp, *trans*Pro(guan), and/or *cis*Pro(guan), provides the β-hairpin and/or β-turn structures of the non-naturally occurring melanocortin analog. In some embodiments, the disulfide bond of the sequence according to Formula (I), if present, provides the β-hairpin and/or β-turn structures of the non-naturally occurring melanocortin analog.

[0103] As will be appreciated by the skilled artisan, non-naturally occurring melanocortin analogs comprising a sequence of any one of Formulae (I)-(IC), have an N-terminus and a C-terminus. The melanocortin analogs of the present technology are written beginning with the N-terminus at the left-most amino acid residue and ending with the C-terminus at the right most residue. Accordingly, the N-terminus of a non-naturally melanocortin analog comprising a sequence of any one of Formulae (I)-(IC) may be at any of X^1, X^2, X^3, and R^1. Analogously, the C-terminus of a non-naturally occurring melanocortin analog comprising a sequence of any one of Formulae (I)-(IC) may be at any of R^7, R^8, Y^1, Y^2, Y^3, Y^4, Y^5, Y^6, and Y^7.

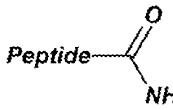
[0104] In some embodiments, the N-terminus of the non-naturally occurring melanocortin analog is modified by an acyl group. In some embodiments, the acyl group is acetyl group (e.g., 

).

[0105] In some embodiments, the N-terminus of the non-naturally occurring melanocortin analog is not modified.

[0106] As discussed above, $Y^1Y^2Y^3Y^4Y^5Y^6Y^7$ represents a C-terminus of the non-naturally occurring melanocortin analog. In some embodiments, Y^1-Y^7 are absent. In some embodiments, Y^1 is present and Y^2-Y^7 are absent. In some embodiments, Y^1 and

Y^2 are present and Y^3-Y^7 are absent. In some embodiments, Y^1-Y^3 are present and Y^4-Y^7 are absent. In some embodiments, Y^1-Y^4 are present and Y^5-Y^7 are absent. In some embodiments, Y^1-Y^5 are present and Y^6-Y^7 are absent. In some embodiments, Y^1-Y^6 are present and Y^7 is absent. In some embodiments, Y^1-Y^7 are present.

[0107] In some embodiments, the C-terminus of the non-naturally occurring melanocortin analog is modified by an amide group (e.g., 

). In the sequence of any one of Formulae (I)-(ID), a non-naturally occurring melanocortin analog with a C-terminus modified by an amide may be represented by a terminal -NH_2.

[0108] In some embodiments, the C-terminus of the non-naturally occurring melanocortin analog is not modified. In the sequence of any one of Formulae (I)-(ID), a non-naturally occurring melanocortin analog with an unmodified C-terminus may be represented by -OH.

[0109] Non-naturally occurring melanocortin analogs comprising a sequence of any one of Formulae (I)-(IC) are cyclized. For example, the non-naturally occurring melanocortin analog may be cyclized through a moiety selected from the group consisting of: a disulfide bond between R^1 and R^7 when R^1 and R^7 are each independently Cys or dCys; a disulfide bond between R^2 and R^7, when R^2 and R^7 are each independently selected from Cys, dCys, Pen, and dPen; a lactam bridge between R^1 or R^2 and R^7 or R^8 when R^1 or R^2 is Asp and R^7 or R^8 is Lys; and a lactam bridge between R^2 and R^7 when R^2 is Asp, dAsp, or Glu and R^7 is dLys or Orn.

[0110] In some embodiments of the sequence of Formula (I), R^4 is Phe or dPhe. In further embodiments, R^1 is Nle, R^3 is Pro or His, R^5 is Arg, R^6 is Trp, Y^1 is dVal and Y^2 is dPro. In some embodiments, the sequence of Formulae (I) is:

Ac-Nle-c[Asp-Pro-Phe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 2);

Ac-Nle-c[Asp-Pro-His-Phe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 3); and

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 31),

wherein c represents cyclization through R^1 or R^2 and R^7 via a lactam bond.

[0111] In some embodiments of the sequence of any one of Formulae (I)-(IC), R^4 is dPhe. In further embodiments, sequence of any one of Formulae (I)-(IC) is cyclized through a lactam bond between Asp R^2 and Lys at R^7.

[0112] In some embodiments, X^1-X^3 are absent. Alternatively, in some embodiments, one or more of , X^1-X^3 is present. In some embodiments, X^1 is present and X^2-X^3 are absent. In some embodiments, X^1-X^2 are present and X^3 is absent. In some embodiments, X^1-X^3 are present. In further embodiments, R^1 is Nle, R^3 is Pro, R^5 is Arg, R^6 is Trp, Y^1 is dVal or dTle and Y^2 is dPro. In some embodiments, the sequence of any one of Formulae (I)-(IC) is selected from the group consisting of:

Ac-Nle-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH2 (SEQ ID NO: 53);

Ac-Nle-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dTle-dPro-NH2 (SEQ ID NO: 40);

Ac-Nle-Nle-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 188); and

Ac-Nle-Nle-Nle-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 189),

wherein c represents cyclization through R^2 and R^7 via a lactam bond.

[0113] In some embodiments, R^1 is Nle. Alternatively, in some embodiments, R^1 is an amino acid other than Nle. In further embodiments, R^1 is selected from dLeu, dAla, Ala, dPhe, Phe, Trp, dTrp, dArg, Arg, dLys, His, dHis, Orn, and dOrn. In still further embodiments, R^3 is Pro, R^5 is Arg, and R^6 is Trp. In some embodiments, Y^1 is dVal and Y^2 is dPro. In some embodiments, the sequence of any one of Formulae (I)-(IC) is selected from the group consisting of:

Ac-dLeu-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH2 (SEQ ID NO: 66);

Ac-dAla-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH2 (SEQ ID NO: 94);

Phe-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH2 (SEQ ID NO: 144);

His-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH2 (SEQ ID NO: 145);

Trp-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH2 (SEQ ID NO: 147);

dPhe-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH2 (SEQ ID NO: 148);

dHis-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH2 (SEQ ID NO: 149);

dTrp-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH2 (SEQ ID NO: 150);

Ac-Arg-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH2 (SEQ ID NO: 151);

Ac-dLys-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH2 (SEQ ID NO: 152);

Ac-Lys-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH2 (SEQ ID NO: 153);

Ac-His-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH2 (SEQ ID NO: 154);

Ac-dHis-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH2 (SEQ ID NO: 155);

Ac-Ala-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH2 (SEQ ID NO: 156);

Ac-Orn-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH2 (SEQ ID NO: 158);

Ac-dOrn-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH2 (SEQ ID NO: 159);

Ac-dArg-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 187);

Ac-dPhe-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 193); and

Ac-dTyr-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 195),

wherein c represents cyclization through R^2 and R^7 via a lactam bond.

[0114] In other embodiments, Y^1 is dTle and Y^2 is dPro. In some embodiments, the sequence of any one of Formulae (I)-(IC) is selected from the group consisting of:
Ac-Ala-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 167);

Ac-dArg-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 168);

Ac-Arg-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 169);

Ac-Lys-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 170);

Ac-dLys-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 171);

Ac-His-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 172); and

Ac-dHis-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 173),

wherein c represents cyclization through R^2 and R^7 via a lactam bond.

[0115] In still other embodiments, Y^1 is selected from dArg, dLys, dVal, and dTle, Y^2 is selected from dPro, dVal, and dHyp, Y^3 is absent, dVal, or dPro, and Y^4 is absent or dPro. In some embodiments, the sequence of any one of Formulae (I)-(IC) is selected from the group consisting of:
Ac-Arg-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dArg-dVal-dPro-NH_2 (SEQ ID NO: 39);

Ac-dLys-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dLys-dVal-dPro-NH_2 (SEQ ID NO: 160);

Ac-Lys-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dArg-dPro-NH_2 (SEQ ID NO: 161);

Ac-His-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dLys-dPro-NH_2 (SEQ ID NO: 162);

Ac-dHis-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dHyp-NH_2 (SEQ ID NO: 163);

Ac-Orn-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 164); and

Ac-dOrn-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dVal-dVal-dPro-NH_2 (SEQ ID NO: 165),

wherein c represents cyclization through R^2 and R^7 via a lactam bond.

[0116] In some embodiments, R^3 is Pro. Alternatively, in some embodiments, R^3 is an amino acid other than Pro. In further embodiments, R^3 is selected from Aba, Aia, Ata, APC, APPC, Ioc, Tyr, Trp, Atc, Ala, Leu, Hyp, Phe, dPhe, dGln, dTyr, dTrp, Bip, Tic, Cpe, Che, and Oic. In still further embodiments, R^1 is Nle, R^5 is Arg, R^6 is Trp, Y^1 is dVal and Y^2 is dPro. In some embodiments, the sequence of any one of Formulae (I)-(IC) is selected from the group consisting of:

Ac-Nle-c[Asp-Aba-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 4);

Ac-Nle-c[Asp-Aia-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 5);

Ac-Nle-c[Asp-Ata-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 6);

Ac-Nle-c[Asp-APC-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 10);

Ac-Nle-c[Asp-APPC-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 11);

Ac-Nle-c[Asp-Ioc-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 18);

Ac-Nle-c[Asp-Trp-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 42);

Ac-Nle-c[Asp-Tyr-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 43);

Ac-Nle-c[Asp-Atc-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 63);

Ac-Nle-c[Asp-Ala-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 88);

Ac-Nle-c[Asp-Hyp-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 142);

Ac-Nle-c[Asp-dPhe-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 174);

Ac-Nle-c[Asp-dGln-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 175);

Ac-Nle-c[Asp-dTrp-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 176);

Ac-Nle-c[Asp-dTyr-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 177);

Ac-Nle-c[Asp-Cpe-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 190);

Ac-Nle-c[Asp-Che-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 191);

Ac-Nle-c[Asp-Oic-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 192);

Ac-Nle-c[Asp-Leu-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 220);

Ac-Nle-c[Asp-Tic-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 221);

Ac-Nle-c[Asp-Phe-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 222); and

Ac-Nle-c[Asp-Bip-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 223),

wherein c represents cyclization through R^2 and R^7 via a lactam bond.

[0117] In some embodiments, R^5 is Arg. Alternatively, in some embodiments, R^5 is an amino acid other than Arg. In further embodiments, R^5 is selected from *trans*Pro(guan), *cis*Pro(guan), and His. In still further embodiments, when R^1 is Nle, R^3 is Pro, R^6 is Trp, R^7 is Lys, Y^1 is dVal, and Y^2 is dPro. In some embodiments, the sequence of any one of Formulae (I)-(IC) is selected from the group consisting of:

Ac-Nle-c[Asp-Pro-dPhe-transPro(guan)-Trp-Lys]-dVal-dPro-NH2 (SEQ ID NO: 12);

Ac-Nle-c[Asp-Pro-dPhe-cisPro(guan)-Trp-Lys]-dVal-dPro-NH2 (SEQ ID NO: 13); and

Ac-Nle-c[Asp-Pro-dPhe-His-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 92),

wherein c represents cyclization through R^2 and R^7 via a lactam bond.

[0118] In some embodiments, R^6 is Trp. Alternatively, in some embodiments, R^6 is an amino acid other than Trp. In further embodiments, R^6 is selected from Aia, Aba, Ata, dTrp, Nal(1'), and Phe. In still further embodiments, when R^1 is Nle, R^3 is Pro, R^5 is Arg, R^7 is Lys, Y^1 is dVal, and Y^2 is dPro. In some embodiments, the sequence of any one of Formulae (I)-(IC) is selected from the group consisting of:

Ac-Nle-c[Asp-Pro-dPhe-Arg-Aia-Lys]-dVal-dPro-NH2 (SEQ ID NO: 14);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Aba-Lys]-dVal-dPro-NH2 (SEQ ID NO: 15);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Ata-Lys]-dVal-dPro-NH2 (SEQ ID NO: 16);
Ac-Nle-c[Asp-Pro-dPhe-Arg-dTrp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 65);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Nal(1')-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 95); and

Ac-Nle-c[Asp-Pro-dPhe-Arg-Phe-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 96),

wherein c represents cyclization through R^2 and R^7 via a lactam bond.

[0119] In some embodiments, Y^1 is dVal, Y^2 is dPro, and the C-terminus is modified by NH_2. Alternatively, in some embodiments, Y^1 is an amino acid other than dVal, Y^2 is an amino acid other than dPro, and/or the C-terminus is not modified. In further embodiments Y^1 is selected from dThr, dPro, Val, β-Val, Hyp, dHyp, Pro, β-Pro, Ala, dAla, Gly, Asp, Arg, Asn, dAsp, dArg, dAsn, Lys, dLys, and dTle and Y^2 is selected from dThr, dVal, Val, β-Val, Hyp, dHyp, Pro, β-Pro, Ala, dAla, Gly, Asp, Arg, Asn, dAsp, dArg, dAsn, and dTle. In still further embodiments, R^1 is Nle, R^3 is Pro, R^5 is Arg, and R^6 is Trp. In some embodiments, the sequence of any one of Formulae (I)-(IC) is selected from the group consisting of:

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-Hyp-NH2 (SEQ ID NO: 36);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dHyp-NH2 (SEQ ID NO: 37);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-β2Val-βPro-NH2 (SEQ ID NO: 38);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-Arg-NH2 (SEQ ID NO: 41);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dArg-NH2 (SEQ ID NO: 44);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-Lys-Pro-Val-NH2 (SEQ ID NO: 45);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dLys-dVal-dPro-NH2 (SEQ ID NO: 46);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-Arg-Pro-Val-NH2 (SEQ ID NO: 47);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-Arg-Val-Pro-NH2 (SEQ ID NO: 48);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dArg-dVal-dPro-NH2 (SEQ ID NO: 49);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dTle-dPro-NH2 (SEQ ID NO: 50);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dTle-NH2 (SEQ ID NO: 51);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dVal-dVal-dPro-NH2 (SEQ ID NO: 52);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-Trp-NH2 (SEQ ID NO: 54);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dAsn-NH_2 (SEQ ID NO: 57);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-Asn-dPro-NH_2 (SEQ ID NO: 58);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-Ala-Ala-NH_2 (SEQ ID NO: 59);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-Gly-Gly-NH_2 (SEQ ID NO: 60);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dAsn-dPro-NH_2 (SEQ ID NO: 61);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-Val-Pro-NH_2 (SEQ ID NO: 104);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-Val-NH_2 (SEQ ID NO: 129);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dThr-dPro-dThr-NH_2 (SEQ ID NO: 130);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dThr-dPro-dThr-OH (SEQ ID NO: 131);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dPro-dVal-NH_2 (SEQ ID NO: 132);

Ac-Nle-c[dAsp-Pro-dPhe-Arg-Trp-dLys]-dPro-dVal-NH_2 (SEQ ID NO: 133);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-βPro-β2Val-NH_2 (SEQ ID NO: 134);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-βPro-β3Val-NH_2 (SEQ ID NO: 135);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-OH (SEQ ID NO: 136);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-β3Val-βPro-NH_2 (SEQ ID NO: 137);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-Pro-NH_2 (SEQ ID NO: 138);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-βPro-βPro-NH_2 (SEQ ID NO: 139);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-Arg-NH_2 (SEQ ID NO: 146);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dArg-NH_2 (SEQ ID NO: 157);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dArg-dPro-NH_2 (SEQ ID NO: 166);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dLys-dPro-NH_2 (SEQ ID NO: 178);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-Lys-Val-Pro-NH_2 (SEQ ID NO: 179);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-Lys-dVal-dPro-NH_2 (SEQ ID NO: 180);

Ac-dNle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 181);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dTle-dTle-dPro-NH_2 (SEQ ID NO: 182);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dTle-dTle-dVal-NH_2 (SEQ ID NO: 183);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dVal-dPro-NH_2 (SEQ ID NO: 184);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dPro-dVal-dPro-NH_2 (SEQ ID NO: 185);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-dVal-dPro-NH_2 (SEQ ID NO: 186);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-Lys-dPro-dVal-NH_2 (SEQ ID NO: 197);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dAla-dAla-NH_2 (SEQ ID NO: 198);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-Asp-NH_2 (SEQ ID NO: 199);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dHyp-NH_2 (SEQ ID NO: 200);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-Asp-NH_2 (SEQ ID NO: 201);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-Hyp-NH_2 (SEQ ID NO: 203);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-Asp-dPro-NH_2 (SEQ ID NO: 204);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-Asn-NH_2 (SEQ ID NO: 205);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dAsp-NH_2 (SEQ ID NO: 206);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dAsn-NH_2 (SEQ ID NO: 207);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-Lys-dPro-NH_2 (SEQ ID NO: 208);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dPro-dVal-OH (SEQ ID NO: 209);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dVal-dVal-dVal-dVal-dVal-dPro-NH_2 (SEQ ID NO: 17),

wherein c represents cyclization through R^2 and R^7 via a lactam bond.

[0120] In some embodiments, R^4 is dPhe and the sequence of any one of Formulae (I)-(IC) is cyclized through a lactam bond between Glu or Asp at R^2 and Orn at R^7. In some embodiments, the sequence of any one of Formulae (I)-(IC) is:
Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2 (SEQ ID NO: 23); or

Ac-Nle-c[Glu-Pro-dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2 (SEQ ID NO: 216),

wherein c represents cyclization through R^2 and R^7 via a disulfide bond.

[0121] In some embodiments, R^4 is dPhe and the sequence of any one of Formulae (I)-(IC) is cyclized through a disulfide bond between R^2 and R^7, where R^2 and R^7 are each independently selected from Cys, dCys, and dPen. In further embodiments, R^1 is Nle, R^3 is Pro or absent, R^5 is Arg, R^6 is Trp, Y^1 is dVal and Y^2 is dPro. In some embodiments, the sequence of any one of Formulae (I)-(IC) is selected from the group consisting of:
Ac-Nle-c[dCys-Pro-dPhe-Arg-Trp-Cys]-dVal-dPro-NH_2 (SEQ ID NO: 62);

Ac-Nle-c[dPen-Pro-dPhe-Arg-Trp-dPen]-dVal-dPro-NH_2 (SEQ ID NO: 67);

Ac-Nle-c[dCys-Pro-dPhe-Arg-Trp-dCys]-dVal-dPro-NH_2 (SEQ ID NO: 74);

Ac-Nle-c[Cys-Pro-dPhe-Arg-Trp-Cys]-dVal-dPro-NH_2 (SEQ ID NO: 202); and

Ac-Nle-c[Cys-dPhe-Arg-Trp-Pen]-dVal-dPro-NH_2 (SEQ ID NO: 210),

wherein c represents cyclization through R^2 and R^7 via a disulfide bond.

[0122] In some embodiments, R^4 is dPhe and the sequence of any one of Formulae (I)-(IC) is cyclized through a lactam or disulfide bond between R^1 and R^7. In some embodiments, R^2 is Trp and/or R^3 is Pro. In further embodiments, X^1 is selected from Nle, Ala, Lys, dLys, Arg, His, and dHis; R^2 is selected from Trp, Phe, His, Tyr, dPhe, dHis, and dTyr; R^3 is selected from Pro, Ala, and Leu, R^5 is Arg, and R^6 is selected from Trp, dNal(2'), Nal(2'), and Nal(1'). In some embodiments, the sequence of any one of Formulae (I)-(IC) is selected from the group consisting of:

Ac-Nle-c[Asp-Trp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 64);

Ac-Nle-c[Cys-Trp-Pro-dPhe-Arg-Trp-Cys]-dVal-dPro-NH_2 (SEQ ID NO: 99);

Ac-Nle-c[dCys-Trp-Pro-dPhe-Arg-Trp-Cys]-dVal-dPro-NH_2 (SEQ ID NO: 100);

Ac-Nle-c[dCys-Trp-Pro-dPhe-Arg-Trp-dCys]-dVal-dPro-NH_2 (SEQ ID NO: 101);

Ac-Ala-c[Asp-Trp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 102);

Ac-Lys-c[Asp-Trp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 103);

Ac-dLys-c[Asp-Trp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 105);

Ac-Arg-c[Asp-Trp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 106);

Ac-dArg-c[Asp-Trp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 107);

Ac-dHis-c[Asp-Trp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 108);

Ac-His-c[Asp-Trp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 109);

Ac-Nle-Nle-c[Asp-Trp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 110);

Ac-Nle-c[Asp-Trp-Ala-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 111);

Ac-Nle-c[Asp-Trp-Leu-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 112);

Ac-Nle-c[Asp-Trp-Pro-dPhe-Arg-Nal(1')-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 113);

Ac-Nle-c[Asp-Trp-Pro-dPhe-Arg-dNal(2')-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 114);

Ac-Nle-c[Asp-Trp-Pro-dPhe-Arg-Nal(2')-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 115);

Ac-Nle-c[Asp-Trp-Pro-dPhe-Arg-Trp-Lys]-dArg-dVal-dPro-NH_2 (SEQ ID NO: 116);

Ac-Nle-c[Asp-Trp-Pro-dPhe-Arg-Trp-Lys]-dLys-dVal-dPro-NH_2 (SEQ ID NO: 117);

Ac-Nle-c[Asp-Trp-Pro-dPhe-Arg-Trp-Lys]-dArg-dPro-NH_2 (SEQ ID NO: 118);

Ac-Nle-c[Asp-Trp-Pro-dPhe-Arg-Trp-Lys]-dLys-dPro-NH_2 (SEQ ID NO: 119);

Ac-Nle-c[Asp-Trp-Pro-dPhe-Arg-Trp-Lys]-dVal-dHyp-NH_2 (SEQ ID NO: 120);

Ac-Nle-c[Asp-Trp-Pro-dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 121);

Ac-Nle-c[Asp-Trp-Pro-dPhe-Arg-Trp-Lys]-dVal-dVal-dVal-dPro-NH_2 (SEQ ID NO: 122);

Ac-Nle-c[Asp-Phe-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 123);

Ac-Nle-c[Asp-His-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 124);

Ac-Nle-c[Asp-Tyr-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 125);

Ac-Nle-c[Asp-dPhe-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 126);

Ac-Nle-c[Asp-dHis-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 127); and

Ac-Nle-c[Asp-dTyr-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 128),

wherein c represents cyclization through R^1 and R^7 via a lactam bond.

[0123] In other embodiments, R^2 is Ala or dAla and R^3 is His. In further embodiments, X^1 is Arg or dArg; R^5 is Arg, and R^6 is Trp, Y^1 is absent or dVal, and Y^2 is absent or dPro. In some embodiments, the sequence of any one of Formulae (I)-(IC) is selected from the group consisting of:

Ac-Arg-c[Asp-Ala-His-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 194);

Ac-dArg-c[Asp-Ala-His-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 196); and

Ac-Arg-c[Asp-dAla-His-dPhe-Arg-Trp-Lys]-NH_2 (SEQ ID NO: 230),

wherein c represents cyclization through R^1 and R^7 via a lactam bond.

[0124] In still other embodiments, R^2 is Phe and R^3 is His. In further embodiments, X^1 is Nle or Phe; R^5 is Arg, and R^6 is Trp, Y^1 is selected from dVal, dLeu, and dTle, and Y^2 is dPro. In some embodiments, the sequence of any one of Formulae (I)-(IC) is selected from the group consisting of:

Ac-Nle-Phe-Phe-c[Asp-Phe-His-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 140);

Ac-Nle-c[Asp-Phe-His-dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 141);

Ac-Nle-c[Asp-Phe-His-dPhe-Arg-Trp-Lys]-dLeu-dPro-NH_2 (SEQ ID NO: 224); and

Ac-Nle-c[Asp-Phe-His-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 225),

wherein c represents cyclization through R^1 and R^7 via a lactam bond.

[0125] In some embodiments, R^4 is dPhe and the sequence of any one of Formulae (I)-(IC) is cyclized through a lactam or disulfide bond between R^2 and R^8. In some embodiments, R^7 is Pro or Gly. In further embodiments, R^1 is Nle R^3 is Pro or Hyp, R^5 is Arg, and R^6 is Trp. In some embodiments, the sequence of any one of Formulae (I)-(IC) is selected from the group consisting of:
Ac-Nle-c[Asp-Hyp-dPhe-Arg-Trp-Pro-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 143);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Gly-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 55);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Gly-Lys]-dPro-dPro-dLys-dAsp-NH_2 (SEQ ID NO: 56);

Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Gly-Lys]-dPro-dPro-Lys-Asp-NH_2 (SEQ ID NO: 219);

wherein c represents cyclization through R^2 and R^8 via a lactam bond.

[0126] In some embodiments of the sequence of any one of Formulae (I)-(IC), R^4 is p(F)dPhe. In further embodiments, sequence of any one of Formulae (I)-(IC) is cyclized through a lactam bond between Asp R^2 and Lys at R^7.

[0127] In some embodiments, X^1-X^3 are absent. Alternatively, in some embodiments, one or more of , X^1 is present and X^2-X^3 are absent. In further embodiments, R^1 is Nle, R^3 is Pro, R^5 is Arg, R^6 is Trp, Y^1 is dVal or dTle and Y^2 is dPro. In some embodiments, the sequence of any one of Formulae (I)-(IC) is selected from the group consisting of:
Ac-Nle-Nle-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 20);

Ac-Nle-Nle-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 76); and

Ac-Nle-Nle-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dPro-dVal-dPro-NH_2 (SEQ ID NO: 77),

wherein c represents cyclization through R^2 and R^7 via a lactam bond.

[0128] In some embodiments, R^1 is Nle. Alternatively, in some embodiments, R^1 is an amino acid other than Nle. In further embodiments, R^1 is selected from Ala, Arg, dArg, dLys, His, and dHis. In still further embodiments, R^3 is Pro, R^5 is Arg, and R^6 is

Trp. In some embodiments, Y^1 is dVal and Y^2 is dPro. In some embodiments, the sequence of any one of Formulae (I)-(IC) is selected from the group consisting of:
Ac-Ala-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 213);

Ac-dArg-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 214);

Ac-Arg-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 215);

Ac-Lys-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 68);

Ac-dLys-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 69);

Ac-His-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 70); and

Ac-dHis-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 71),

wherein c represents cyclization through R^2 and R^7 via a lactam bond.

[0129] In other embodiments, Y^1 is dTle and Y^2 is dPro. In some embodiments, the sequence of any one of Formulae (I)-(IC) is selected from the group consisting of:
Ac-Ala-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH2 (SEQ ID NO: 78);

Ac-dArg-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH2 (SEQ ID NO: 79);

Ac-Arg-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH2 (SEQ ID NO: 80);

Ac-Lys-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH2 (SEQ ID NO: 81);

Ac-dLys-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH2 (SEQ ID NO: 82);

Ac-His-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH2 (SEQ ID NO: 83); and

Ac-dHis-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH2 (SEQ ID NO: 84),

wherein c represents cyclization through R^2 and R^7 via a lactam bond.

[0130] In some embodiments, R^3 is Pro. Alternatively, in some embodiments, R^3 is an amino acid other than Pro. In further embodiments, R^3 is selected from His, dGln and dTyr. In still further embodiments, R^1 is Nle, R^5 is Arg, R^6 is Trp or dNal(2'), Y^1 is dVal and Y^2 is dPro. In some embodiments, the sequence of any one of Formulae (I)-(IC) is selected from the group consisting of:
Ac-Nle-c[Asp-dGln-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 97);

Ac-Nle-c[Asp-dTyr-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 98); and

Ac-Nle-c[Asp-His-p(F)dPhe-Arg-Nal(2')-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 226),

wherein c represents cyclization through R^2 and R^7 via a lactam bond.

[0131] In some embodiments, R^5 is Arg. Alternatively, in some embodiments, R^5 is an amino acid other than Arg. In further embodiments, R^5 is His. In some embodiments, the sequence of any one of Formulae (I)-(IC) is: Ac-Nle-c[Asp-Pro-p(F)dPhe-His-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 21), wherein c represents cyclization through R^2 and R^7 via a lactam bond.

[0132] In some embodiments, R^1 is Nle, R^3 is Pro, R^5 is Arg, and R^6 is Trp. In further embodiments, Y^1 is selected from dVal, dPro and dTle, Y^2 is selected from dVal, dPro, and dTle, Y^3 is absent, dVal, or dPro, and Y^4 is absent or dPro. In some embodiments, the sequence of any one of Formulae (I)-(IC) is selected from the group consisting of:

Ac-Nle-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 19);

Ac-Nle-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 22);

Ac-Nle-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dTle-dTle-dVal-NH_2 (SEQ ID NO: 72);

Ac-Nle-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dVal-dVal-dVal-dPro-NH_2 (SEQ ID NO: 73); and

Ac-Nle-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dPro-dVal-dPro-NH_2 (SEQ ID NO: 75),

wherein c represents cyclization through R^2 and R^7 via a lactam bond.

[0133] In some embodiments, R^4 is p(F)dPhe and the sequence of any one of Formulae (I)-(IC) is cyclized through a lactam bond between Glu or Asp at R^2 and Orn at R^7. In some embodiments, the sequence of any one of Formulae (I)-(IC) is:
Ac-Nle-c[Asp-Pro-p(F)dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2 (SEQ ID NO: 217); or

Ac-Nle-c[Glu-Pro-p(F)dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2 (SEQ ID NO: 218),

wherein c represents cyclization through R^2 and R^7 via a disulfide bond.

[0134] In some embodiments, R^4 is p(F)dPhe and the sequence of any one of Formulae (I)-(IC) is cyclized through a disulfide bond between R^2 and R^7, where R^2 and R^7 are each independently Cys or dCys. In further embodiments, R^1 is Nle, R^3 is Pro or His, R^5 is Arg, R^6 is Trp, Y^1 is dVal and Y^2 is dPro. In some embodiments, the sequence of any one of Formulae (I)-(IC) is selected from the group consisting of:
Ac-Nle-c[dCys-Pro-p(F)dPhe-Arg-Trp-Cys]-dVal-dPro-NH_2 (SEQ ID NO: 211);

Ac-Nle-c[dCys-Pro-p(F)dPhe-Arg-Trp-dCys]-dVal-dPro-NH_2 (SEQ ID NO: 212);

Ac-Nle-c[dCys-His-p(F)dPhe-Arg-Trp-dCys]-dVal-dPro-NH_2 (SEQ ID NO: 93);

Ac-Nle-c[Cys-His-p(F)dPhe-Arg-Trp-Cys]-dVal-dPro-NH_2 (SEQ ID NO: 227); and

Ac-Nle-c[dCys-His-p(F)dPhe-Arg-Trp-Cys]-dVal-dPro-NH_2 (SEQ ID NO: 231),

wherein c represents cyclization through R^2 and R^7 via a disulfide bond.

[0135] In some embodiments of the sequence of any one of Formulae (I)-(IC), R^4 is p(Cl)dPhe or p(Br)dPhe. In further embodiments, sequence of any one of Formulae (I)-(IC) is cyclized through a lactam bond between Asp R^2 and Lys at R^7.

[0136] In some embodiments, R^1 is Nle. Alternatively, in some embodiments, R^1 is an amino acid other than Nle. In further embodiments, R^1 is selected from Arg, dArg, dLys, His, and dHis. In still further embodiments, R^3 is His, R^4 is p(Cl)dPhe, R^5 is Arg, and R^6 is Trp. In some embodiments, Y^1 is dVal and Y^2 is dPro. In some embodiments, the sequence of any one of Formulae (I)-(IC) is selected from the group consisting of:
Ac-Arg-c[Asp-His-p(Cl)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 24);

Ac-Lys-c[Asp-His-p(Cl)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 25);

Ac-His-c[Asp-His-p(Cl)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 26);

Ac-dHis-c[Asp-His-p(Cl)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 27);

Ac-dArg-c[Asp-His-p(Cl)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 86); and

Ac-dLys-c[Asp-His-p(Cl)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 87),

wherein c represents cyclization through R^2 and R^7 via a lactam bond.

[0137] In other embodiments, Y^1 is dTle and Y^2 is dPro. In some embodiments, the sequence of any one of Formulae (I)-(IC) is selected from the group consisting of:
Ac-dArg-c[Asp-His-p(Cl)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 33);

Ac-Arg-c[Asp-His-p(Cl)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 34);

Ac-dHis-c[Asp-His-p(Cl)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 35);

Ac-Lys-c[Asp-His-p(Cl)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 228);

Ac-His-c[Asp-His-p(Cl)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 229); and

Ac-dLys-c[Asp-His-p(Cl)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 89),

wherein c represents cyclization through R^2 and R^7 via a lactam bond.

[0138] In some embodiments, R^3 is Pro. Alternatively, in some embodiments, R^3 is an amino acid other than Pro. In further embodiments, R^3 is selected from Aia, Aba, and Ata. In still further embodiments, R^1 is Nle, R^5 is Arg, R^6 is Trp, Y^1 is dVal and Y^2 is dPro. In some embodiments, the sequence of any one of Formulae (I)-(IC) is selected from the group consisting of:

Ac-Nle-c[Asp-Aba-p(Cl)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 7);

Ac-Nle-c[Asp-Aia-p(Cl)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 8); an

Ac-Nle-c[Asp-Ata-p(Cl)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 9),

wherein c represents cyclization through R^2 and R^7 via a lactam bond.

[0139] In some embodiments, Y^1 is dVal and Y^2 is dPro. Alternatively, in some embodiments, Y^1 is an amino acid other than dVal and/or Y^2 is an amino acid other than dPro. In further embodiments, R^1 is Nle, R^5 is Arg, and R^6 is Trp. In some embodiments, the sequence of any one of Formulae (I)-(IC) is selected from the group consisting of:

Ac-Nle-c[Asp-His-p(Cl)dPhe-Arg-Trp-Lys]-dTle-dTle-dVal-NH_2 (SEQ ID NO: 28);

Ac-Nle-c[Asp-His-p(Cl)dPhe-Arg-Trp-Lys]-dVal-dVal-dVal-dPro-NH_2 (SEQ ID NO: 29);

Ac-Nle-c[Asp-His-p(Cl)dPhe-Arg-Trp-Lys]-dPro-dVal-dPro-NH_2 (SEQ ID NO: 30); and

Ac-Nle-Nle-c[Asp-His-p(Cl)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2 (SEQ ID NO: 32),

wherein c represents cyclization through R^2 and R^7 via a lactam bond.

[0140] In some embodiments, R^4 is p(Cl)dPhe or p(Br)dPhe and the sequence of any one of Formulae (I)-(IC) is cyclized through a disulfide bond between R^2 and R^7, where R^2 and R^7 are each independently Cys or dCys. In further embodiments, R^1 is Nle, R^3 is Pro or His, R^5 is Arg, R^6 is Trp, Y^1 is dVal and Y^2 is dPro. In some embodiments, the sequence of any one of Formulae (I)-(IC) is selected from the group consisting of:

Ac-Nle-c[dCys-His-p(Cl)dPhe-Arg-Trp-dCys]-dVal-dPro-NH_2 (SEQ ID NO: 85);

Ac-Nle-c[dCys-His-p(Br)dPhe-Arg-Trp-Cys]-dVal-dPro-NH_2 (SEQ ID NO: 90); and

Ac-Nle-c[dCys-His-p(Br)dPhe-Arg-Trp-dCys]-dVal-dPro-NH_2 (SEQ ID NO: 91),

wherein c represents cyclization through R^2 and R^7 via a disulfide bond.

[0141] In some embodiments, the non-naturally occurring melanocortin analog comprises a sequence of Formula (I). In some embodiments, the non-naturally occurring melanocortin analog comprises any one of the sequences of SEQ ID NOs: 2-231.

[0142] In some embodiments, the non-naturally occurring melanocortin analog comprises a sequence of Formula (IA). In some embodiments, the non-naturally occurring melanocortin analog comprises any one of the sequences of SEQ ID NOs: 4-6, 10-23, 31, 36-84, 88, 92-227, 230, and 231.

[0143] In some embodiments, the non-naturally occurring melanocortin analog comprises a sequence of Formula (IA(i)). In some embodiments, the non-naturally occurring melanocortin analog comprises any one of the sequences of SEQ ID NOs: 4-6, 10-18, 23, 31, 36-63, 65-67, 74, 88, 92, 94-96, 104, 129-139, 142-193, 195, 197-210, 216, and 219-223.

[0144] In some embodiments, the non-naturally occurring melanocortin analog comprises a sequence of Formula (IA(ii)). In some embodiments, the non-naturally occurring melanocortin analog comprises any one of the sequences of SEQ ID NOs: 64, 99-103, 105-128, 140, 141, 194, 196, 145, 147, and 230.

[0145] In some embodiments, the non-naturally occurring melanocortin analog comprises a sequence of Formula (IA(iii)). In some embodiments, the non-naturally occurring melanocortin analog comprises any one of the sequences of SEQ ID NOs: 19-22, 68-73, 75-84, 93, 97, 98, 211-215, 217, 218, 226, 227, and 231.

[0146] In some embodiments, the non-naturally occurring melanocortin analog comprises a sequence of Formula (IB). In some embodiments, the non-naturally occurring melanocortin analog comprises any one of the sequences of SEQ ID NOs: 7-9, 24-30, 32-35, 85-87, 89-91, 228, and 229.

[0147] In some embodiments, the non-naturally occurring melanocortin analog comprises a sequence of Formula (IC). In some embodiments, the non-naturally occurring melanocortin analog comprises any one of the sequences of SEQ ID NOs: 216-218.

Non-Naturally Occurring Melanocortin Analog Synthesis

[0148] The non-naturally occurring melanocortin analogs of the present technology may be readily synthesized by any known conventional procedure for the formation of a peptide linkage between amino acids. Such conventional procedures include, for example, any solution phase procedure permitting a condensation between the free alpha amino group of an amino acid or residue thereof having the carboxyl group or other reactive groups protected and the free primary carboxyl group of another amino acid or residue thereof having the amino group or other reactive groups protected. In an exemplary procedure, the peptides of the present technology may be synthesized by solid-phase synthesis and purified according to methods known in the art. Any of a number of well-known procedures utilizing a variety of resins and reagents may be used to prepare the peptides of the present technology.

[0149] The process for synthesizing the peptides may be carried out by a procedure whereby each amino acid in the desired sequence is added one at a time in succession to another amino acid or residue thereof or by a procedure whereby peptide fragments with the desired amino acid sequence are first synthesized conventionally and then condensed to provide the desired peptide. The resulting peptide is then cyclized to yield a cyclic peptide.

[0150] Solid phase peptide synthesis methods are well known and practiced in the art. In such methods, the synthesis of peptides may be carried out by sequentially incorporating the desired amino acid residues one at a time into the growing peptide chain according to the general principles of solid phase methods. These methods are disclosed in numerous references, including Merrifield, Angew Chem. 24:799-810 (1985) and Barany et al., The Peptides, Analysis, Synthesis and Biology, Vol. 2, Gross E. and Meienhofer J., Eds. Academic Press 1-284 (1980).

[0151] In chemical syntheses of peptides, reactive side chain groups of the various amino acid residues are protected with suitable protecting groups, which prevent a chemical reaction from occurring at that site until the protecting group is removed. Also common is the protection of the alpha amino group of an amino acid residue or fragment while that entity reacts at the carboxyl group, followed by the selective removal of the alpha amino protecting group to allow a subsequent reaction to take place at that site.

Specific protecting for solid phase synthesis methods and solution phase synthesis methods groups are known to those having ordinary skill in the art.

[0152] Alpha amino groups may be protected by a suitable protecting group, including a urethane-type protecting group, such as benzyloxycarbonyl (Z) and substituted benzyloxycarbonyl, such as p-chlorobenzyloxycarbonyl, p-nitrobenzyloxycarbonyl, p-bromobenzyloxycarbonyl, p-biphenyl-isopropoxycarbonyl, 9-fluorenylmethoxycarbonyl (Fmoc) and p-methoxybenzyloxycarbonyl (Moz); aliphatic urethane-type protecting groups, such as t-butyloxycarbonyl (Boc), diisopropylmethoxycarbonyl, isopropoxycarbonyl, and allyloxycarbonyl. Fmoc is useful for alpha amino protection.

[0153] Guanidino groups may be protected by a suitable protecting group, such as nitro, p-toluenesulfonyl (Tosyl), Z, pentamethylchromanesulfonyl (Pmc), adamantyloxycarbonyl, pentamethyldihydrobenzofuran-5-sulfonyl (Pbf) and Boc. Pmc is a useful protecting group for Arg.

[0154] Solid phase synthesis is commenced from the C-terminal end of the peptide by coupling a protected alpha amino acid to a suitable resin. Such starting material is prepared by attaching an alpha amino-protected amino acid by an ester linkage to a p-benzyloxybenzyl alcohol (Wang) resin or a 2-chlorotrityl chloride resin, by an amide bond between an Fmoc-Linker, such as p-[(R,S)-α-[1-(9H-fluor-en-9-yl)-methoxyformamido]-2,4-dimethyloxybenzyl]-phenoxyacetic acid (Rink linker) to a benzhydrylamine (BHA) resin, or by other means well known in the art. Fmoc-Linker-BHA resin supports are commercially available and generally used when feasible. The resins are carried through repetitive cycles as necessary to add amino acids sequentially. The alpha amino Fmoc protecting groups are removed under basic conditions. Piperidine, piperazine, diethylamine, or morpholine (20-40% v/v) in N,N-dimethylformamide (DMF) may be used for this purpose.

[0155] Following removal of the alpha amino protecting group, the subsequent protected amino acids are coupled stepwise in the desired order to obtain an intermediate, protected peptide-resin. The activating reagents used for coupling of the amino acids in the solid phase synthesis of the peptides are well known in the art. After the peptide is synthesized, if desired, the orthogonally protected side chain protecting

groups may be removed using methods well known in the art for further derivatization of the peptide.

[0156] Reactive groups in a peptide may be selectively modified, either during solid phase synthesis or after removal from the resin. For example, peptides may be modified to obtain N-terminus modifications, such as acetylation, while on resin, or may be removed from the resin by use of a cleaving reagent and then modified. Methods for N-terminus modification, such as acetylation, and for C-terminus modification, such as amidation, are known in the art. Similarly, methods for modifying side chains of amino acids are well known to those skilled in the art of peptide synthesis. The choice of modifications made to reactive groups present on the peptide will be determined, in part, by the characteristics that are desired in the peptide.

[0157] The peptide may be cyclized prior to cleavage from the peptide resin. For cyclization through reactive side chain moieties, the desired side chains are deprotected, and the peptide suspended in a suitable solvent and a cyclic coupling agent added. Suitable solvents include, for example DMF, dichloromethane (DCM) or 1-methyl-2-pyrrolidone (NMP). Suitable cyclic coupling reagents include, for example, 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU), 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), benzotriazole-1-yl-oxy-tris(dimethylamino)phosphoniumhexafluorophosphate (BOP), benzotriazole-1-yl-oxy-tris(pyrrolidino)phosphoniumhexafluorophosphate (PyBOP), 2-(7-aza-1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TATU), 2-(2-oxo-1 (2H)-pyridyl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TPTU) or N,N′-dicyclohexylcarbodiimide/1-hydroxybenzotriazole (DCCI/HOBt). Coupling is convention initiated by use of a suitable base, such as N,N-diispropylethylamine (DIPEA), sym-collidine or N-methylmorpholine (NMM).

[0158] Following cleavage of peptides from the solid phase following their synthesis, the peptide may be purified by any number of methods, such as reverse phase high performance liquid chromatography (RP-HPLC), using a suitable column, such as a C18 column. Other methods of separation or purification, such as methods based on the size or charge of the peptide, may also be employed. Once purified, the peptide may be characterized by any number of methods, such as high-performance liquid chromatograph (HPLC), amino acid analysis, mass spectrometry, and the like.

Salt Forms of Non-Naturally Occurring Melanocortin Analogs

[0159] The non-naturally occurring melanocortin analog peptides of the present technology may be in the form of any salt. The term "pharmaceutically acceptable salts" refers to salts prepared from non-toxic bases or acids including inorganic or organic bases and inorganic or organic acids. Salts derived from inorganic bases include aluminum, ammonium, calcium, copper, ferric, ferrous, lithium, magnesium, manganic salts, manganous, potassium, sodium, zinc, and the like. Exemplary salts are the ammonium, calcium, lithium, magnesium, potassium, and sodium salts. Salts derived from organic non-toxic bases include salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines, and basic ion exchange resins, such as arginine, betaine, caffeine, choline, N,N′-dibenzylethylenediamine, diethylamine, 2-diethylaminoethanol, 2-dimethylaminoethanol, ethanolamine, ethylenediamine, N-ethyl-morpholine, N-ethylpiperidine, glucamine, glucosamine, histidine, hydrabamine, isopropylamine, lysine, methylglucamine, morpholine, piperazine, piperidine, polyamine resins, procaine, purines, theobromine, triethylamine, trimethylamine, tripropylamine, tromethamine, and the like.

[0160] When the non-naturally occurring melanocortin analogs of the present technology are basic, acid addition salts may be prepared from non-toxic acids, including inorganic and organic acids. Such acids include acetic, benzenesulfonic, benzoic, camphorsulfonic, carboxylic, citric, ethanesulfonic, formic, fumaric, gluconic, glutamic, hydrobromic, hydrochloric, isethionic, lactic, maleic, malic, mandelic, methanesulfonic, malonic, mucic, nitric, pamoic, pantothenic, phosphoric, propionic, succinic, sulfuric, tartaric, p-toluenesulfonic acid, trifluoroacetic acid, and the like. Acid addition salts of the peptides of the present technology are prepared in a suitable solvent from the peptide and an excess of an acid, such as hydrochloric, hydrobromic, sulfuric, phosphoric, acetic, trifluoroacetic, citric, tartaric, maleic, succinic or methanesulfonic acid. The acetate salt form is especially useful. Where the peptides of the present technology include an acidic moiety, suitable salts may include alkali metal salts, such as sodium or potassium salts, or alkaline earth metal salts, such as calcium or magnesium salts.

Conjugates

[0161] The present technology further includes conjugates comprising a non-naturally occurring melanocortin analog. In some embodiments, the non-naturally melanocortin analog is conjugated to a pharmaceutical agent. Non-limiting examples of suitable pharmaceutical agents include peptides, monoclonal antibodies, and small molecules. In yet other embodiments, the non-naturally occurring melanocortin analog is conjugated to a small molecule MCR agonist.

[0162] Conjugates of the present technology further comprise a linker connecting the non-naturally occurring melanocortin analog to the pharmaceutical agent. In some embodiments, the linker is rigid. In some embodiments, the linker is flexible. In some embodiments, the linker is a cleavable linker. In some embodiments, the linker comprises a glycine-serine (Gly/Ser) linker, a proline-threonine-glycine linker, an alanine linker, a lysine linker, a threonine linker, a valine-glycine-serine-threonine linker, an elastin-like peptide linker, a hexahistidine linker, a polyethylene glycol linker, a fatty acid linker, or a hydrocarbon linker.

[0163] In some embodiments, the linker is a peptide linker. The peptide linkers of the present technology may vary from 2 to 31 amino acids of any primary sequence in length and do not impose any constraints on the conformation or interactions of the linked partners. In some embodiments, the linkers vary from about 2-30, 2-29, 2-28, 2-27, 2-26, 2-25, 2-24, 2-23, 2-22, 2-21, 2-20, 2-19, 2-18, 2-17, 2-16, 2-15, 2- 14, 2-13, 2-12, 2-11, 2-10, 2-9, 2-8, 2-7, 2-6, 2-5, 2-4, 2-3, 3-31, 3-30, 3-29, 3-28, 3-27, 3-26, 3-25, 3-24, 3-23, 3-22, 3-21, 3-20, 3-19, 3-18, 3-17, 3-16, 3-15, 3-14, 3-13, 3-12, 3-11, 3-10, 3-9, 3-8, 3-7, 3-6, 3-5, 3-4, 4-31, 4-30, 4-29, 4-28, 4-27, 4-26, 4-25, 4-24, 4-23, 4-22, 4-21, 4- 20, 4-19, 4-18, 4-17, 4-16, 4-15, 4-14, 4-13, 4-12, 4-11, 4-10, 4-9, 4-8, 4-7, 4-6, 4-5, 5-31, 5- 30, 5-29, 5-28, 5-27, 5-26, 5-25, 5-24, 5-23, 5-22, 5-21, 5-20, 5-19, 5-18, 5-17, 5-16, 5-15, 5- 14, 5-13, 5-12, 5-11, 5-10, 5-9, 5-8, 5-7, 5-6, 6-31, 6-30, 6-29, 6-28, 6-27, 6-26, 6-25, 6-24, 6-23, 6-22, 6-21, 6-20, 6-19, 6-18, 6-17, 6-16, 6-15, 6-14, 6-13, 6-12, 6-11, 6-10, 6-9, 6-8, 6- 7, 7-31, 7-30, 7-29, 7-28, 7-27, 7-26, 7-25, 7-24, 7-23, 7-22, 7-21, 7-20, 7-19, 7-18, 7-17, 7- 16, 7-15, 7-14, 7-13, 7-12, 7-11, 7-10, 7-9, 7-8, 8-31, 8-30, 8-29, 8-28, 8-27, 8-26, 8-25, 8-24, 8-23, 8-22, 8-21, 8-20, 8-19, 8-18, 8-17, 8-16, 8-15, 8-14, 8-13, 8-12, 8-11, 8-10, 8-9, 9-31, 9-30, 9-29, 9-28, 9-27, 9-26, 9-25, 9-24, 9-23, 9-22, 9-21, 9-20, 9-19, 9-18, 9-17, 9-16, 9-15, 9-14, 9-13, 9-12, 9-11, 9-

10, 10-31, 10-30, 10-29, 10-28, 10-27, 10-26, 10-25, 10-24, 10-23, 10-22, 10-21, 10-20, 10-19, 10-18, 10-17, 10-16, 10-15, 10-14, 10-13, 10-12, 10-11, 11-31, 11-30, 11-29, 11-28, 11-27, 11-26, 11-25, 11-24, 11-23, 11-22, 11-21, 11-20, 11-19, 11-18, 11-17, 11-16, 11-15, 11-14, 11-13, 11-12, 12-31, 12-30, 12-29, 12-28, 12-27, 12-26, 12-25,12-24, 12-23, 12-22, 12-21, 12-20, 12-19, 12-18, 12-17, 12-16, 12-15, 12-14, 12-13, 13-31, 13-30, 13-29, 13-28, 13-27, 13-26, 13-25, 13-24, 13-23, 13-22, 13-21, 13-20, 13-19, 13-18, 13-17, 13-16, 13-15, 13-14, 14-31, 14-30, 14-29, 14-28, 14-27, 14-26, 14-25, 14-24, 14-23, 14-22, 14-21, 14-20, 14-19, 14-18, 14-17, 14-16, 14-15, 15-31, 15-30, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 15-16, 16-31, 16-30, 16-29, 16-28, 16-27, 16-26, 16-25, 16-24, 16-23, 16-22, 16-21, 16-20, 16-19, 16-18, 16-17, 17-31, 17-30, 17-29, 17-28, 17-27, 17-26, 17-25, 17-24, 17-23, 17-22, 17-21, 17-20, 17-19, 17-18, 18-31, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 18-19, 19-31, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-31, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 20-21, 21-31, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, 21-22, 22-31, 22-30, 22-29, 22-28, 22-27, 22-26, 22-25, 22-24, 22-23, 23-31, 23-30, 23-29, 23-28, 23-27, 23-26, 23-25, 23-24, 24-31, 24-30, 24-29, 24-28, 24-27, 24-26, 24-25, 25-31, 25-30, 25-29, 25-28, 25-27, 25-26, 26-31, 26-30, 26-29, 26-28, 26-27, 27-31, 27-30, 27-29, 27-28, 28-31, 28-30, 28-29, 29-31, 29-30, or 30-31 amino acids of any primary sequence in length. The peptide linkers may be designed as appropriate for an intended use.

[0164] The peptide linkers may comprise one or more of a Gly-rich linker (e.g., a flexible linker connecting various domains in a single protein without interfering with the function of each domain; a linker forming stable covalently linked dimers; a linker to connect two independent domains that create a ligand-binding site or recognition sequence), a Serine linker (e.g., a coiled structure linker); a coiled structure linker comprising a Gln, Arg, Glu, Ser, and/or Pro amino acids; a rigid space linker comprising one or more of a Pro, Arg, Phe, Thr, Glu, and/or Gln residues; a linker comprising a flexible Gly-rich regions that may may generate loops connecting domains; or a linker comprising a Thr, Ser, Gly, and/or Ala residue.

[0165] In some embodiments, the linker comprises an amino acid sequence about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of any one of Linker A, Linker

B, Linker C, Linker D, Linker E, Linker F, Linker G, Linker H, Linker I, Linker J, Linker K, Linker L, Linker M, Linker N, Linker O, Linker P, Linker Q, Linker R, or Linker S in Table 0.

[0166] In some embodiments, the linker comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of any one of Linker A, Linker B, Linker C, Linker D, Linker E, Linker F, Linker G, Linker H, Linker I, Linker J, Linker K, Linker L, Linker M, Linker N, Linker O, Linker P, Linker Q, Linker R, or Linker S in Table 0.

In some embodiments, the linker comprises an amino acid sequence at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of any one of Linker A, Linker B, Linker C, Linker D, Linker E, Linker F, Linker G, Linker H, Linker I, Linker J, Linker K, Linker L, Linker M, Linker N, Linker O, Linker P, Linker Q, Linker R, or Linker S in Table 0.

Table 0: Peptide Linker Amino Acid Sequences

Name	Amino Acid	SEQ ID NO
Linker A	$(GGS)_{1-5}$	369
Linker B	GGGGSLVPRGSGGGGS	370
Linker C	GSGSGS	371
Linker D	GS_8	372
Linker E	GGSGGHMGSGG	373
Linker F	GGSGGSGGSGG	374
Linker G	GGSGG	375
Linker H	GGSGGGGG	376

Name	Amino Acid	SEQ ID NO
Linker I	GSGSGSGS	377
Linker J	GSGSGSGSGSGSGSGSGSGSGSGSGSGSGSG	378
Linker K	GGGSEGGGSEGGGSEGGG	379
Linker L	AAGAATAA	380
Linker M	GGGGG	381
Linker N	GGSSG	382
Linker O	GSGGGTGGGSG	383
Linker P	GT	384
Linker Q	GSGSGSGSGGSG	385
Linker R	GSGGSGSGGSGGSG	386
Linker S	SGGGGSRGGGSGGGG**SGGGGSGGGGSGGGG**	387

Pharmaceutical Compositions

[0167] In some embodiments, the non-naturally occurring melanocortin analogs of the present technology are present in a pharmaceutical composition.

[0168] In some embodiments, the non-naturally occurring melanocortin analog is present in the pharmaceutical composition in a concentration of 0.1 mg/mL to 500 mg/mL, relative to a total volume of the composition. For example, the non-naturally occurring melanocortin analog is present in the pharmaceutical composition in a concentration of 0.1 mg/mL to 500 mg/mL, 0.5 mg/mL to 250 mg/mL, 1 mg/mL to 100 mg/mL, 2.5 mg/mL to 50 mg/mL, or 5 mg/mL to 25 mg/mL, relative to a total volume of the composition. In some embodiments, the non-naturally occurring melanocortin analog is present in the pharmaceutical composition in a concentration of about 50 mg/mL, relative to a total volume of the pharmaceutical composition.

[0169] In some embodiments, the non-naturally occurring melanocortin analog comprises any one of SEQ ID NOs: 31, 216, and 218, and the non-naturally occurring melanocortin analog is present in the pharmaceutical composition in a concentration of 5 mg/mL to 100 mg/mL, relative to a total volume of the pharmaceutical composition. For example, the non-naturally occurring melanocortin analog comprising any one of SEQ ID NOs: 31, 216, and 218is present in the pharmaceutical composition in a concentration of 5 mg/mL to 100 mg/mL, 10 mg/mL to 75 mg/mL, 15 mg/mL to 50 mg/mL, 20 mg/mL to 40 mg/mL, or 25 mg/mL to 30 mg/mL, relative to a total volume of the pharmaceutical composition. In some embodiments, the non-naturally occurring melanocortin analog comprising any one of SEQ ID NOs: 31, 216, and 218 is present in the pharmaceutical composition in a concentration of about 50 mg/mL, relative to a total volume of the pharmaceutical composition.

[0170] In some embodiments, the composition comprises the non-naturally occurring melanocortin analog of formula (I) at a concentration at about 0.001 nmol, 0.005 nmol, 0.01 nmol, 0.02 nmol, 0.05 nmol, 0.1 nmol, 0.25 nmol, 0.5 nmol, 1 nmol, 2.5 nmol, 5 nmol, 10 nmol, 20 nmol, 25 nmol, 50 nmol, 100 nmol, 250 nmol, 500 nmol, or 1000 nmol, or more, depending on the specific peptide selected, the desired response, the route of administration, the formulation and other factors known to those of skill in the art. In some embodiments, the non-naturally occurring melanocortin analog comprises any one of SEQ ID NOs: 31, 216, and 218.

[0171] The non-naturally occurring melanocortin analogs may be formulated with one or more pharmaceutically acceptable carriers and/or excipients. The carriers and/or excipients of the present technology facilitate delivery of the non-naturally occurring melanocortin analog to a subject. Other pharmaceutically acceptable carriers and/or excipients may be included in the pharmaceutical composition to enhance dispersion, solubility, and/or stability of the non-naturally occurring melanocortin analog, and/or to reduce adverse injection site reactions.

[0172] In some embodiments, the pharmaceutical composition comprises 0.1 to 99.9999 wt.%, 1 to 99.999 wt.%, 5 to 99.99 wt.%, 10 to 99.9 wt.%, 15 to 99 wt.%, 20 to 90 wt.%, 30 to 85 wt.%, 40 to 80 wt.%, 50 to 75 wt.%, or 60 to 70 wt.% of the pharmaceutically acceptable carrier and/or excipient relative to a total weight of the pharmaceutical composition.

[0173] In some embodiments, the pharmaceutically acceptable carrier and/or excipient of the pharmaceutical composition is selected from the group consisting of water, a buffer, an inorganic salt, a fatty acid, a vegetable oil, a synthetic fatty ester, a surfactant, and a polymer.

[0174] In some embodiments, the pharmaceutically acceptable carrier and/or excipient of the pharmaceutical composition is water. In some embodiments, the pharmaceutically acceptable carrier and/or excipient of the pharmaceutical composition is a buffer.

[0175] In some embodiments, the pharmaceutical composition contains about 0.1 to about 99.9999 wt.%, about 1 to about 99.999 wt.%, about 5 to about 99.99 wt.%, about 10 to about 99.9 wt.%, about 15 to about 99 wt.%, about 20 to about 90 wt.%, about 30 to about 85 wt.%, about 40 to about 80 wt.%, about 50 to about 75 wt.%, or about 60 to about 70 wt.% of one or more pharmaceutically acceptable carriers and/or excipients relative to a total weight of the pharmaceutical composition.

[0176] In some embodiments, the pharmaceutical composition contains at least 0.1 to at least 99.9999 wt.%, at least 1 to at least 99.999 wt.%, at least 5 to at least 99.99 wt.%, at least 10 to at least 99.9 wt.%, at least 15 to at least 99 wt.%, at least 20 to at least 90 wt.%, at least 30 to at least 85 wt.%, at least 40 to at least 80 wt.%, at least 50 to at least 75 wt.%, or at least 60 to at least 70 wt.% of one or more pharmaceutically acceptable carriers and/or excipients relative to a total weight of the pharmaceutical composition.

[0177] In some embodiments, the pharmaceutical composition contains at least about 0.1 to at least about 99.9999 wt.%, at least about 1 to at least about 99.999 wt.%, at least about 5 to at least about 99.99 wt.%, at least about 10 to at least about 99.9 wt.%, at least about 15 to at least about 99 wt.%, at least about 20 to at least about 90 wt.%, at least about 30 to at least about 85 wt.%, at least about 40 to at least about 80 wt.%, at least about 50 to at least about 75 wt.%, or at least about 60 to at least about 70 wt.% of one or more pharmaceutically acceptable carriers and/or excipients relative to a total weight of the pharmaceutical composition.

[0178] Any pharmaceutically acceptable carriers and/or excipients known in the art may be included in the pharmaceutical composition. Non-limiting examples of pharmaceutically acceptable carriers and/or excipients include buffers, binders,

excipients, stabilizers, lubricants, oils, adjuvants, preservatives, lipids, and antioxidants. The pharmaceutical composition may comprise any combination of the one or more pharmaceutically acceptable carriers and/or excipients previously described in relation to the first and pharmaceutical compositions. In some embodiments, the one or more pharmaceutically acceptable carriers and/or excipients comprise water.

[0179] The carriers and/or excipients of the composition may generally include one or more of the following components: (i) one or more antioxidants, (ii) one or more preservatives, (iii) one or more buffers, (iv) one or more tonicity adjustors, (v) one or more surfactants, (vi) flavor, (vii) propellants, and/or (viii) a vehicle or solvent. In some embodiments, all components are compatible with the non-naturally occurring melanocortin analog (i.e., do not react or cause the non-naturally occurring melanocortin analog to react) and are homogeneously dispersed or dissolved uniformly in the composition.

[0180] In some embodiments, the one or more pharmaceutically acceptable carriers and/or excipients are isotonic. In some embodiments, the carrier and/or excipient is isotonic to nasal fluids.

[0181] In some embodiments, the pharmaceutical composition further comprises a pharmaceutical salt. Any pharmaceutical salt known in the art may be included in the pharmaceutical composition. For examples, to achieve a desirable tonicity, the pharmaceutical composition may include a salt selected from the group consisting of sodium chloride, sodium succinate, sodium sulfate, potassium chloride, magnesium chloride, magnesium sulfate, and calcium chloride.

[0182] In some embodiments, the salt is present in the pharmaceutical composition in a concentration of about 0.1 mg/mL to about 50 mg/mL, about 1 mg/mL to about 25 mg/mL, or about 5 mg/mL to about 10 mg/mL, relative to a total volume of the composition.

[0183] In some embodiments, the salt is present in the pharmaceutical composition in a concentration of at least 0.1 mg/mL to at least 50 mg/mL, at least 1 mg/mL to at least 25 mg/mL, or at least 5 mg/mL to at least 10 mg/mL, relative to a total volume of the composition.

[0184] In some embodiments, the salt is present in the pharmaceutical composition in a concentration of at least about 0.1 mg/mL to at least about 50 mg/mL, at least about 1 mg/mL to at least about 25 mg/mL, or at least about 5 mg/mL to at least about 10 mg/mL, relative to a total volume of the composition.

[0185] Pharmaceutically acceptable carriers and/or excipients that may be included in the pharmaceutical composition generally include a pH buffered aqueous solution comprising one or more of the following components: (a) sodium acetate, (b) Tris, and (c) water. In some embodiments, all components are compatible with the non-naturally occurring melanocortin analog (i.e., do not react or cause the non-naturally occurring melanocortin analog to react) and are homogeneously dispersed or dissolved uniformly in the composition.

[0186] In the pH buffered solution of the pharmaceutical composition, the water may act as a diluent and include, without limitation, water for injection (WFI), sterile water, bacteriostatic water for injection (BWFI), distilled water, bidistilled water, deionized water, deionized distilled water, and reverse osmosis water. In some embodiments, the water present in the pH buffered aqueous solution is water for injection.

[0187] In some embodiments, the pharmaceutical composition includes water in an amount of about 1 wt% to about 90 wt%, about 10 wt% to about 75 wt%, or about 25 wt% to about 50 wt%, relative to a total weight of the composition.

[0188] In some embodiments, the pharmaceutical composition includes water in an amount of at least 1 wt% to at least 90 wt%, at least 10 wt% to at least 75 wt%, or at least 25 wt% to at least 50 wt%, relative to a total weight of the composition.

[0189] In some embodiments, the pharmaceutical composition includes water in an amount of at least about 1 wt% to at least about 90 wt%, at least about 10 wt% to at least about 75 wt%, or at least about 25 wt% to at least about 50 wt%, relative to a total weight of the composition.

[0190] In some embodiments, sodium acetate is present in the pharmaceutical composition in a concentration of 0.5 mg/mL to 50 mg/mL, relative to a total volume of the composition. For example, sodium acetate may be present in the pharmaceutical composition in a concentration of 0.5 mg/mL to 50 mg/mL, 1 mg/mL to 40 mg/mL, 2

mg/mL to 30 mg/mL, 4 mg/mL to 20 mg/mL, 5 mg/mL to 15 mg/mL, 6 mg/mL to 12 mg/mL, or 8 mg/mL to 10 mg/mL, relative to a total volume of the composition.

[0191] In some embodiments, sodium acetate is present in the pharmaceutical composition in a concentration of about 6 mg/mL to about 8 mg/mL, relative to a total volume of the composition. For example, sodium acetate may be present in the pharmaceutical composition in a concentration of 6 mg/mL, 6.5 mg/mL, 7 mg/mL, 7.1 mg/mL, 7.5 mg/mL, or 8 mg/mL, relative to a total volume of the composition.

[0192] In some embodiments, sodium acetate is present in the pharmaceutical composition in a molar concentration of 5 mM to 700 mM, relative to a total volume of the composition. For example, sodium acetate may be present in the pharmaceutical composition in a molar concentration of 5 mM to 700 mM, 10 mM to 600 mM, 20 mM to 500 mM, 30 mM to 400 mM, 40 mM to 300 mM, 50 mM to 200 mM, 60 mM to 100 mM, or 70 mM to 80 mM, relative to a total volume of the composition.

[0193] In some embodiments, sodium acetate is present in the pharmaceutical composition in a molar concentration of about 80 mM to about 100 mM, relative to a total volume of the composition. For example, sodium acetate may be present in the pharmaceutical composition in a molar concentration of 80 mM, 85 mM, 87 mM, 90 mM, 95 mM, or 100 mM, relative to a total volume of the composition.

[0194] The term “Tris” refers to tris(hydroxymethyl)aminomethane, which is also known as Tris buffer, Tris base, TRIS, tromethamine, tromethamine buffer, Trizma®, Trisamine, Trometamol, Tromethane, Trisaminol, or THAM. In some embodiments, Tris is present in the pharmaceutical composition in a concentration of 0.5 mg/mL to 50 mg/mL, relative to a total volume of the composition. For example, Tris may be present in the pharmaceutical composition in a concentration of 0.5 mg/mL to 50 mg/mL, 1 mg/mL to 40 mg/mL, 2 mg/mL to 30 mg/mL, 4 mg/mL to 20 mg/mL, 5 mg/mL to 15 mg/mL, 6 mg/mL to 12 mg/mL, or 8 mg/mL to 10 mg/mL, relative to a total volume of the composition.

[0195] In some embodiments, Tris is present in the pharmaceutical composition in a concentration of about 6 mg/mL to about 8 mg/mL, relative to a total volume of the composition. For example, Tris may be present in the pharmaceutical composition in a concentration of 6 mg/mL, 6.5 mg/mL, 7 mg/mL, 7.3 mg/mL, 7.6 mg/mL, or 8 mg/mL, relative to a total volume of the composition.

[0196] In some embodiments, Tris is present in the pharmaceutical composition in a molar concentration of 2 mM to 500 mM, relative to a total volume of the composition. For example, Tris may be present in the pharmaceutical composition in a molar concentration of 2 mM to 500 mM, 5 mM to 400 mM, 10 mM to 300 mM, 20 mM to 200 mM, 30 mM to 150 mM, 40 mM to 100 mM, 50 mM to 80 mM, or 60 mM to 70 mM, relative to a total volume of the composition.

[0197] In some embodiments, Tris is present in the pharmaceutical composition in a molar concentration of about 50 mM to about 70 mM, relative to a total volume of the composition. For example, Tris may be present in the pharmaceutical composition in a molar concentration of 50 mM, 55 mM, 60 mM, 65 mM, or 70 mM, relative to a total volume of the composition.

[0198] In some embodiments, the pH buffered aqueous solution provides the pharmaceutical composition with a pH equivalent or close to the physiological pH levels. This may reduce adverse injection site reactions and also provide the non-naturally occurring melanocortin analog with enhanced stability and resistance to aggregation and degradation.

[0199] In some embodiments, a weight ratio of sodium acetate to Tris is about 1:4 to about 4:1, about 2:7 to about 7:2, about 1:3 to about 3:1, about 2:5 to about 5:2, about 1:2 to about 2:1, about 2:3 to about 3:2, or about 1:1. In some embodiments, the weight ratio of sodium acetate to Tris is about 1:1.

[0200] In some embodiments, a weight ratio of sodium acetate to Tris is at least 1:4 to at least 4:1, at least 2:7 to at least 7:2, at least 1:3 to at least 3:1, at least 2:5 to at least 5:2, at least 1:2 to at least 2:1, at least 2:3 to at least 3:2, or at least 1:1. In some embodiments, the weight ratio of sodium acetate to Tris is at least 1:1.

[0201] In some embodiments, a weight ratio of sodium acetate to Tris is at least about 1:4 to at least about 4:1, at least about 2:7 to at least about 7:2, at least about 1:3 to at least about 3:1, at least about 2:5 to at least about 5:2, at least about 1:2 to at least about 2:1, at least about 2:3 to at least about 3:2, or at least about 1:1. In some embodiments, the weight ratio of sodium acetate to Tris is at least about 1:1.

[0202] In some embodiments, a weight ratio of the non-naturally occurring melanocortin analog to sodium acetate is about 1:1 to about 20:1, about 3:2 to about

15:1, about 2:1 to about 12:1, about 3:1 to about 10:1, about 4:1 to about 9:1, about 5:1 to about 8:1, or about 6:1 to about 7:1. In some embodiments, the weight ratio of the non-naturally occurring melanocortin analog to sodium acetate is about 7:1.

[0203] In some embodiments, a weight ratio of the non-naturally occurring melanocortin analog to sodium acetate is at least 1:1 to at least 20:1, at least 3:2 to at least 15:1, at least 2:1 to at least 12:1, at least 3:1 to at least 10:1, at least 4:1 to at least 9:1, at least 5:1 to at least 8:1, or at least 6:1 to at least 7:1. In some embodiments, the weight ratio of the non-naturally occurring melanocortin analog to sodium acetate is at least 7:1.

[0204] In some embodiments, a weight ratio of the non-naturally occurring melanocortin analog to sodium acetate is at least about 1:1 to at least about 20:1, at least about 3:2 to at least about 15:1, at least about 2:1 to at least about 12:1, at least about 3:1 to at least about 10:1, at least about 4:1 to at least about 9:1, at least about 5:1 to at least about 8:1, or at least about 6:1 to at least about 7:1. In some embodiments, the weight ratio of the non-naturally occurring melanocortin analog to sodium acetate is at least about 7:1.

[0205] In some embodiments, a weight ratio of the non-naturally occurring melanocortin analog to Tris is about 1:1 to about 20:1, about 3:2 to about 15:1, about 2:1 to about 12:1, about 3:1 to about 10:1, about 4:1 to about 9:1, about 5:1 to about 8:1, or about 6:1 to about 7:1. In some embodiments, the weight ratio of the non-naturally occurring melanocortin analog to Tris is about 7:1.

[0206] In some embodiments, a weight ratio of the non-naturally occurring melanocortin analog to Tris is at least 1:1 to at least 20:1, at least 3:2 to at least 15:1, at least 2:1 to at least 12:1, at least 3:1 to at least 10:1, at least 4:1 to at least 9:1, at least 5:1 to at least 8:1, or at least 6:1 to at least 7:1. In some embodiments, the weight ratio of the non-naturally occurring melanocortin analog to Tris is at least 7:1.

[0207] In some embodiments, a weight ratio of the non-naturally occurring melanocortin analog to Tris is at least about 1:1 to at least about 20:1, at least about 3:2 to at least about 15:1, at least about 2:1 to at least about 12:1, at least about 3:1 to at least about 10:1, at least about 4:1 to at least about 9:1, at least about 5:1 to at least about 8:1, or at least about 6:1 to at least about 7:1. In some embodiments, the weight ratio of the non-naturally occurring melanocortin analog to Tris is at least about 7:1.

[0208] In addition to sodium acetate and Tris, the pharmaceutical composition may include other buffering agents. Non-limiting examples of additional buffering agents include saline, phosphate, phosphoric acid, citrate, succinate, gluconate, histidine, acetic acid, ascorbate, tartartic acid, maleic acid, glycine, lactate, lactic acid, ascorbic acid, imidazole, bicarbonate, carbonic acid, succinic acid, sodium benzoate, benzoic acid, gluconate, edetate, malate, imidazole, and mixtures thereof. In some embodiments, the pharmaceutical composition comprises acetic acid as an additional buffering agent.

[0209] The pharmaceutical composition may further comprise one or more chelating agents. Suitable chelating agents include, but are not limited to edetate disodium dihydrate, calcium disodium edetate, sodium edetate, calcium versetamide sodium, calteridol, and diethylenetriaminepentaacetic acid. In some embodiments, the pharmaceutical composition further comprises edetate disodium dihydrate.

[0210] The pharmaceutical composition may further comprise a preservative agent. Exemplary preservative agents include, but are not limited to, ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite, ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, phenol, m-cresol, benzyl alcohol, alpha-tocopherol, citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, benzalkonium chloride, phenoxyethanol, and methyl paraben. In some embodiments, when the pharmaceutical composition comprises a preservative agent, the preservative agent is phenol, benzyl alcohol, or a combination thereof.

[0211] If present in the pharmaceutical composition, the concentration of the preservative agent may range from 0.001 mg/mL to 50 mg/mL, 0.01 mg/mL to 25 mg/mL, 0.1 mg/mL to 10 mg/mL, or 1 mg/mL to 5 mg/mL, relative to a total volume of the composition.

[0212] The pharmaceutical composition may further comprise an emulsifier. Non-limiting examples of emulsifiers that may be included in the pharmaceutical composition include sodium carboxymethylcellulose, cetyl alcohol, glycerol monostearate, methylcellulose, and stearic acid. In some embodiments, when the pharmaceutical composition comprises an emulsifier, the emulsifier is sodium carboxymethylcellulose.

[0213] The pharmaceutical composition may further comprise a lipid. Lipids may enhance solubility and/or improve permeability of the non-naturally occurring melanocortin analog. In some embodiments, the lipid is a phospholipid. Non-limiting examples of phospholipids that may be included in the pharmaceutical composition include egg phosphatidylcholine, hydrogenated soybean phoshphaditylcholine, glycerophosphocholine, lecithin, and N-(carbonyl-methoxypolyethylene glycol 2000)-1,2-distearoyl-glycero-3-phosphoethanolamine sodium salt. In some embodiments, when the pharmaceutical composition comprises a lipid, the lipid is N-(carbonyl-methoxypolyethylene glycol 2000)-1,2-distearoyl-glycero-3-phosphoethanolamine sodium salt

[0214] The pharmaceutical composition may further comprise a bulking agent. Inclusion of a bulking agent may increase the stability of the pharmaceutical composition. Non-limiting examples of bulking agents that may be included in the pharmaceutical composition include sucrose, lactose, trehalose, mannitol, sorbitol, glucose, raffinose, glycine, histidine, and polyvinyl pyrrolidone. In some embodiments, when the pharmaceutical composition comprises a bulking agent, the bulking agent is mannitol.

[0215] In some embodiments, the pharmaceutical composition is in the form of an aqueous solution or a suspension. In some embodiments, the pharmaceutical composition is in the form of an emulsion. In some embodiments, the pharmaceutical composition is in the form of an aqueous solution. In some embodiments, the pharmaceutical composition is in the form of an aqueous solution which is clear, colorless, and/or free of visible foreign matter.

[0216] In some embodiments, the pharmaceutical composition has a pH ranging from about 6.5 to about 8.5. In some embodiments, the pharmaceutical composition has a pH of about 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1, 8.2, 8.3, 8.4, or 8.5.

[0217] In some embodiments, the pharmaceutical composition has a pH ranging from at least 6.5 to at least 8.5. In some embodiments, the pharmaceutical composition has a pH of at least 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1, 8.2, 8.3, 8.4, or 8.5.

[0218] In some embodiments, the pharmaceutical composition has a pH ranging from at least about 6.5 to at least about 8.5. In some embodiments, the pharmaceutical composition has a pH of at least about 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1, 8.2, 8.3, 8.4, or 8.5.

[0219] In some embodiments, the pharmaceutical composition is basic and has a pH of about 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1, 8.2, 8.3, 8.4, or 8.5. In some embodiments, the pharmaceutical composition has a pH ranging from about 7.3 to about 7.4. In some embodiments, the pharmaceutical composition has a pH of 7.3 or 7.4.

[0220] In some embodiments, the pharmaceutical composition is basic and has a pH of at least 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1, 8.2, 8.3, 8.4, or 8.5. In some embodiments, the pharmaceutical composition has a pH ranging from at least 7.3 to at least 7.4. In some embodiments, the pharmaceutical composition has a pH of 7.3 or 7.4.

[0221] In some embodiments, the pharmaceutical composition is basic and has a pH of at least about 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1, 8.2, 8.3, 8.4, or 8.5. In some embodiments, the pharmaceutical composition has a pH ranging from at least about 7.3 to at least about 7.4. In some embodiments, the pharmaceutical composition has a pH of 7.3 or 7.4.

[0222] In some embodiments, the pharmaceutical composition has an osmolality ranging from 250 mOsm/kg to 350 mOsm/kg. For example, the pharmaceutical composition may have an osmolality ranging from 250 mOsm/kg to 360 mOsm/kg, 260 mOsm/kg to 340 mOsm/kg, 270 mOsm/kg to 330 mOsm/kg, 280 mOsm/kg to 320 mOsm/kg, 290 mOsm/kg to 310 mOsm/kg, or about 300 mOsm/kg. In some embodiments, the pharmaceutical composition has an osmolarity of about 250 mOsm/kg, about 260 mOsm/kg, about 270 mOsm/kg, about 280 mOsm/kg, about 290 mOsm/kg, about 300 mOsm/kg, about 310 mOsm/kg, about 320 mOsm/kg, about 330 mOsm/kg, about 340 mOsm/kg, about 350 mOsm/kg, or about 360 mOsm/kg. In some embodiments, the pharmaceutical composition has an osmolality ranging from about 275 mOsm/kg to about 330 mOsm/kg. In some embodiments, the pharmaceutical composition has an osmolality of about 279 mOsm/kg, about 314 mOsm/kg, or about 329 mOsm/kg.

[0223] In some embodiments, the pharmaceutical composition has an osmolality ranging from 250 mOsm/kg to 350 mOsm/kg. For example, the pharmaceutical composition may have an osmolality ranging from 250 mOsm/kg to 360 mOsm/kg, 260 mOsm/kg to 340 mOsm/kg, 270 mOsm/kg to 330 mOsm/kg, 280 mOsm/kg to 320 mOsm/kg, 290 mOsm/kg to 310 mOsm/kg, or at least 300 mOsm/kg. In some embodiments, the pharmaceutical composition has an osmolarity of at least 250 mOsm/kg, at least 260 mOsm/kg, at least 270 mOsm/kg, at least 280 mOsm/kg, at least 290 mOsm/kg, at least 300 mOsm/kg, at least 310 mOsm/kg, at least 320 mOsm/kg, at least 330 mOsm/kg, at least 340 mOsm/kg, at least 350 mOsm/kg, or at least 360 mOsm/kg. In some embodiments, the pharmaceutical composition has an osmolality ranging from at least 275 mOsm/kg to at least 330 mOsm/kg. In some embodiments, the pharmaceutical composition has an osmolality of at least 279 mOsm/kg, at least 314 mOsm/kg, or at least 329 mOsm/kg.

[0224] In some embodiments, the pharmaceutical composition has an osmolality ranging from 250 mOsm/kg to 350 mOsm/kg. For example, the pharmaceutical composition may have an osmolality ranging from 250 mOsm/kg to 360 mOsm/kg, 260 mOsm/kg to 340 mOsm/kg, 270 mOsm/kg to 330 mOsm/kg, 280 mOsm/kg to 320 mOsm/kg, 290 mOsm/kg to 310 mOsm/kg, or at least about 300 mOsm/kg. In some embodiments, the pharmaceutical composition has an osmolarity of at least about 250 mOsm/kg, at least about 260 mOsm/kg, at least about 270 mOsm/kg, at least about 280 mOsm/kg, at least about 290 mOsm/kg, at least about 300 mOsm/kg, at least about 310 mOsm/kg, at least about 320 mOsm/kg, at least about 330 mOsm/kg, at least about 340 mOsm/kg, at least about 350 mOsm/kg, or at least about 360 mOsm/kg. In some embodiments, the pharmaceutical composition has an osmolality ranging from at least about 275 mOsm/kg to at least about 330 mOsm/kg. In some embodiments, the pharmaceutical composition has an osmolality of at least about 279 mOsm/kg, at least about 314 mOsm/kg, or at least about 329 mOsm/kg.

[0225] In some embodiments, the pharmaceutical composition has a viscosity ranging from about 0.5 cP to about 5 cP. For example, the pharmaceutical composition may have a viscosity ranging from about 0.5 cP to about 5 cP, about 0.75 cP to about 4.5 cP, about 1.0 cP to about 4 cP, about 1.2 cP to about 3.5 cP, about 1.3 cP to about 3 cP, about 1.4 cP to about 2.5 cP, about 1.5 cP to about 2 cP, or about 1.6 cP to about 1.8 cP. In some embodiments, the pharmaceutical composition has a viscosity of about

0.5 cP, 0.6 cP, 0.7 cP, 0.8 cP, 0.9 cP, 1.0 cP, 1.1 cP, 1.2 cP, 1.3 cP, 1.4 cP, 1.5 cP, 1.6 cP, 1.7 cP, 1.8 cP, 1.9 cP, or 2.0 cP. In some embodiments, the pharmaceutical composition has a viscosity of about 1.4 cP or about 1.6 cP.

[0226] In some embodiments, the pharmaceutical composition has a viscosity ranging from at least 0.5 cP to at least 5 cP. For example, the pharmaceutical composition may have a viscosity ranging from at least 0.5 cP to at least 5 cP, at least 0.75 cP to at least 4.5 cP, at least 1.0 cP to at least 4 cP, at least 1.2 cP to at least 3.5 cP, at least 1.3 cP to at least 3 cP, at least 1.4 cP to at least 2.5 cP, at least 1.5 cP to at least 2 cP, or at least 1.6 cP to at least 1.8 cP. In some embodiments, the pharmaceutical composition has a viscosity of at least 0.5 cP, 0.6 cP, 0.7 cP, 0.8 cP, 0.9 cP, 1.0 cP, 1.1 cP, 1.2 cP, 1.3 cP, 1.4 cP, 1.5 cP, 1.6 cP, 1.7 cP, 1.8 cP, 1.9 cP, or 2.0 cP. In some embodiments, the pharmaceutical composition has a viscosity of at least 1.4 cP or at least 1.6 cP.

[0227] In some embodiments, the pharmaceutical composition has a viscosity ranging from at least about 0.5 cP to at least about 5 cP. For example, the pharmaceutical composition may have a viscosity ranging from at least about 0.5 cP to at least about 5 cP, at least about 0.75 cP to at least about 4.5 cP, at least about 1.0 cP to at least about 4 cP, at least about 1.2 cP to at least about 3.5 cP, at least about 1.3 cP to at least about 3 cP, at least about 1.4 cP to at least about 2.5 cP, at least about 1.5 cP to at least about 2 cP, or at least about 1.6 cP to at least about 1.8 cP. In some embodiments, the pharmaceutical composition has a viscosity of at least about 0.5 cP, 0.6 cP, 0.7 cP, 0.8 cP, 0.9 cP, 1.0 cP, 1.1 cP, 1.2 cP, 1.3 cP, 1.4 cP, 1.5 cP, 1.6 cP, 1.7 cP, 1.8 cP, 1.9 cP, or 2.0 cP. In some embodiments, the pharmaceutical composition has a viscosity of at least about 1.4 cP or at least about 1.6 cP.

[0228] In some embodiments, the pharmaceutical composition disclosed is formulated for parenteral administration, such as, for example, in the form of aqueous or non-aqueous isotonic sterile injection solutions or suspensions. The term “parenteral,” as used herein, includes subcutaneous, intravenous, intraperitoneal, intramuscular, and intralesional, or infusion techniques.

[0229] When the pharmaceutical composition is formulated for parenteral administration (e.g., subcutaneous administration), the active ingredient(s) (e.g., the non-naturally occurring melanocortin analog) may be dissolved or suspended in the

aforementioned carrier and/or excipient. Additional aqueous or non-aqueous carriers that may facilitate dissolution of the active ingredient include, but are not limited to, ethanol, benzyl alcohol, DMSO, polyethylene glycol, propylene glycol, corn oil, cottonseed oil, peanut oil, sesame oil, and/or various buffers.

[0230] In some embodiments, the pharmaceutical composition is formulated for parenteral administration (e.g., subcutaneous administration) and comprises a non-naturally occurring melanocortin analog in a concentration of about 0.001 nmol, 0.005 nmol, 0.01 nmol, 0.02 nmol, 0.05 nmol, 0.1 nmol, 0.25 nmol, 0.5 nmol, 1 nmol, 2.5 nmol, 5 nmol, 10 nmol, 20 nmol, 25 nmol, 50 nmol, 100 nmol, 250 nmol, 500 nmol, or 1000 nmol, or even more, depending on the specific peptide selected, the desired therapeutic response, the route of administration, the formulation and other factors known to those of skill in the art.

[0231] In some embodiments, the pharmaceutical composition is formulated for parenteral administration (e.g., subcutaneous administration) and comprises sodium acetate in a concentration of about 5 mM, 10 mM, 15 mM, 20 mM, 25 mM, 30 mM, 35 mM, 40 mM, 45 mM, 50 mM, 55 mM, 60 mM, 65 mM, 70 mM, 75 mM, 76 mM, 77 mM, 78 mM, 79 mM, 80 mM, 81 mM, 82 mM, 83 mM, 84 mM, 85 mM, 86 mM, 87 mM, 88 mM, 89 mM, 90 mM, 91 mM, 92 mM, 93 mM, 94 mM, 95 mM, 96 mM, 97 mM, 98 mM, 99 mM, 100 mM, 105 mM, 110 mM, 120 mM, 130 mM, 140 mM, 150 mM, 160 mM, 170 mM, 180 mM, 190 mM, or 200 mM.

[0232] In some embodiments, the pharmaceutical composition is formulated for parenteral administration (e.g., subcutaneous administration) and comprises Tris in a concentration of about 5 mM, 10 mM, 15 mM, 20 mM, 25 mM, 30 mM, 3 5mM, 40 mM, 45 mM, 50 mM, 55 mM, 60 mM, 65 mM, 70 mM, 75 mM, 80 mM, 85 mM, 90 mM, 95 mM, 100 mM, 105 mM, 110 mM, or 120 mM.

[0233] In some embodiments, the pharmaceutical composition comprises one or more antioxidants. For example, the pharmaceutical composition may comprise ascorbic acid, cysteine, sodium metabisulfite, propyl gallate, butylated hydroxytoluene, and/or butylated hydroxyanisole.

[0234] In some embodiments, the pharmaceutical composition comprises a surfactant, such as a sorbitan ester.

[0235] In some embodiments, the pharmaceutical composition comprises a flavoring or scent, such as an aromatic oil.

[0236] In some embodiments, the non-naturally occurring melanocortin analog is solubilized or suspended in a solvent or vehicle. The solvent or vehicle may be purified water, ethyl alcohol, and/or propylene glycol. In some embodiments, the pharmaceutical composition comprises between 0.03 wt% and 1 wt% melanocortin analog solubilized or suspended in a solvent or vehicle. For example, the pharmaceutical composition may comprise the non-naturally occurring melanocortin analog in an amount of about 0.03 wt%, about 0.05 wt%, about 0.1 wt%, about 0.15 wt%, about 0.2 wt%, about 0.25 wt%, about 0.3 wt%, about 0.35 wt%, about 0.4 wt%, about 0.45 wt%, about 0.5 wt%, about 0.55 wt%, about 0.6 wt%, about 0.65 wt%, about 0.7 wt%, about 0.75 wt%, about 0.8 wt%, about 0.85 wt%, about 0.9 wt%, about 0.95 wt%, or about 1 wt%.

[0237] In some embodiments, the pharmaceutical composition may comprise the non-naturally occurring melanocortin analog in an amount of at least 0.03 wt%, at least 0.05 wt%, at least 0.1 wt%, at least 0.15 wt%, at least 0.2 wt%, at least 0.25 wt%, at least 0.3 wt%, at least 0.35 wt%, at least 0.4 wt%, at least 0.45 wt%, at least 0.5 wt%, at least 0.55 wt%, at least 0.6 wt%, at least 0.65 wt%, at least 0.7 wt%, at least 0.75 wt%, at least 0.8 wt%, at least 0.85 wt%, at least 0.9 wt%, at least 0.95 wt%, or at least 1 wt%.

[0238] In some embodiments, the pharmaceutical composition may comprise the non-naturally occurring melanocortin analog in an amount of at least about 0.03 wt%, at least about 0.05 wt%, at least about 0.1 wt%, at least about 0.15 wt%, at least about 0.2 wt%, at least about 0.25 wt%, at least about 0.3 wt%, at least about 0.35 wt%, at least about 0.4 wt%, at least about 0.45 wt%, at least about 0.5 wt%, at least about 0.55 wt%, at least about 0.6 wt%, at least about 0.65 wt%, at least about 0.7 wt%, at least about 0.75 wt%, at least about 0.8 wt%, at least about 0.85 wt%, at least about 0.9 wt%, at least about 0.95 wt%, or at least about 1 wt%.

[0239] The non-naturally occurring melanocortin analogs of the present technology may be formulated for administration using any means known in the art, including orally, rectally, vaginally, ocularly, intranasally, topically, parenterally, or by injection. If administered by injection, the peptide injection may be intravenous (IV), subcutaneous (SC), intramuscular (IM), intraperitoneal (IP), intracerebroventricular

(ICV), or other means known in the art. The non-naturally occurring melanocortin analog of the combination therapy may be formulated by any means known in the art, including but not limited to formulation as tablets, capsules, caplets, suspensions, powders, lyophilized preparations, suppositories, pessaries, ocular drops, skin patches, orally soluble formulations, enteric formulations, solutions sprays, aerosols and the like, and may be mixed and formulated with buffers, binders, excipients, stabilizers, lubricants, oils, adjuvants, anti-oxidants and other agents known in the art. In general, any route of administration by which the peptides are introduced across an epidermal layer of cells may be employed. Administration includes topical delivery. Administration includes delivery across the blood brain barrier. Administration includes delivery through mucous membranes, buccal administration, ophthalmic administration, oral administration, dermal administration, inhalation administration, nasal administration, urethral administration, vaginal administration, rectal administration, and the like.

[0240] In some embodiments, the pharmaceutical composition formulated for intranasal administration comprises a non-naturally occurring melanocortin analog at a concentration at about 0.001 nmol, 0.005 nmol, 0.01 nmol, 0.02 nmol, 0.05 nmol, 0.1 nmol, 0.25 nmol, 0.5 nmol, 1 nmol, 2.5 nmol, 5 nmol, 10 nmol, 20 nmol, 25 nmol, 50 nmol, 100 nmol, 250 nmol, 500 nmol, or 1000 nmol, or more, depending on the specific peptide selected, the desired therapeutic response, the route of administration, the formulation and other factors known to those of skill in the art.

[0241] In some embodiments, the pharmaceutical composition is formulated for oral administration. For example, the pharmaceutical composition may be in the form of a tablet, capsule, lozenge, pill, sachet, or any other orally deliverable form know in the art.

[0242] The composition may be formulated to be delivered by nose drop, spray device, or topical solution. In some embodiments, the pharmaceutical composition may be formulated as an aerosol, atomizer, inhalation, insufflation, metered-dose inhaler, or nebulizer. In some embodiments, the pharmaceutical composition includes a propellant, such as hydrofluoroalkane.

[0243] In some embodiments, the pharmaceutical composition may be configured to be administered using a spray device or nasal inhaler. The spray device or nasal inhaler may be configured to deliver 1ug to 100ug per spray. In some embodiments, the

spray device or nasal inhaler may be configured to deliver 1ug to 100ug, 5ug to 90ug, 10ug to 80ug, 15ug to 70ug, 20ug to 60ug, 25ug, to 50ug, or 30ug to 40ug per spray.

Dosing

[0244] In some embodiments, the non-naturally occurring melanocortin analog or a pharmaceutical composition thereof (e.g., the pharmaceutical composition) is administered hourly once a day, or twice a day. In some embodiments, the non-naturally occurring melanocortin analog is administered at least once daily in an amount ranging from 0.001 mg/kg to 25 mg/kg. In some embodiments, the non-naturally occurring melanocortin analog is present in the pharmaceutical composition in a concentration of 5 mg/mL to 100 mg/mL, relative to a total volume of the pharmaceutical composition.

Methods

[0245] The non-naturally occurring melanocortin analogs of the present technology may interact with one or more ligand binding sites of a melanocortin receptor, thereby modulating melanocortin receptor activity. This may modulate melanocortin receptor signaling pathways, including, but not limited to, reward and reinforcement-type pathways, thereby reducing pain, cravings, and/or withdrawal symptoms. As such, the non-naturally occurring melanocortin analog of the present technology may be useful pain management, opioid and other substance addictions (e.g., alcohol or nicotine addiction), or reducing a symptom thereof.

[0246] In some embodiments, the non-naturally occurring melanocortin analogs selectively bind at least a portion of an melanocortin receptor. The portion may comprise one or more amino acids of an melanocortin receptor. In some embodiments, the non-naturally occurring melanocortin analogs selectively bind two or more amino acids of an melanocortin receptor. The melanocortin receptor may be a MC3R or a MC4R.

[0247] The non-naturally occurring melanocortin analogs may comprise one or more motifs that confer binding to a melanocortin receptor or at least a portion thereof. The motif may be selected from the group consisting of Pro-p(F)dPhe; Trp-Pro-p(F)dPhe; dAla-Pro-p(F)dPhe; dAla-Pro-dPhe; Pro-dPhe; Ioc-dPhe; dAla-Pro-dPhe; Trp-Pro-dPhe; Pro-His-dPhe; His-p(Cl)dPhe; Aba-p(Cl)dPhe; Aia-p(Cl)dPhe; Ata-p(Cl)dPhe; Oic-p(F)dPhe; Oic-dPhe; dPhe at position R^4; and p(F)dPhe at position R^4.

[0248] The non-naturally occurring melanocortin analogs of the present technology may have a dissociation constant (K_d) value for an melanocortin receptor or one or more amino acids thereof that is less than that of a conventional melanocortin analog.

Subjects

[0249] In some embodiments, the subject has used or is using an addictive substance. In some embodiments, the subject has used or is using a medication to treat an addiction. In some embodiments, the frequency or amount of the addictive substance and/or rescue medication is reduced during or after administration of the non-naturally occurring melanocortin analog. In some embodiments, the frequency or amount of the addictive substance and/or rescue medication is reduced as the dose or dosage of the non-naturally occurring melanocortin analog increases. In some embodiments, the frequency or amount of the addictive substance and/or rescue medication comprises a downward titration during or after administration of a non-naturally occurring melanocortin analog.

[0250] In some embodiments, the subject is a mammal, including but not limited to a human, a non-human primate such as a chimpanzee, a domestic livestock or a farm animal such as a cow, a bison, sheep, a pig, a goat, a horse, a chicken, and a rooster, a domestic pet animal such as a dog, a cat, a rat, a mouse, and a rabbit, and a laboratory subject such as a rodent, including a rat, a mouse, and a guinea pig. In some embodiments, the subject is a human. In some embodiments, the subject is an animal such as a rat or a dog.

Controls

[0251] The controls of the present technology may comprise the subject at baseline. In some embodiments, the controls of the methods of the present technology comprise a subject that is not administered a non-naturally occurring melanocortin analog of the present technology or a subject subjected to a method lacking one or more steps of the methods of the present technology.

Pharmacokinetics and Pharmacodynamics

[0252] The non-naturally occurring melanocortin analogs of the present technology exhibit pharmacokinetic (pK) and/or pharmacodynamic (pD) parameters.

Such pK and/or pD may be expressed or otherwise determined relative to a control, which, in some instances, may be a non-naturally occurring melanocortin analog lacking one or more features of the non-naturally occurring melanocortin analogs of the present technology.

[0253] In some embodiments, the pK and/or pD of the non-naturally occurring melanocortin analogs may be assessed using concentration and/or temporal measurements (e.g., T_{final}, C_{max}, T ½ (h)), AUC, or $T_{max.}$ In some embodiments, the non-naturally occurring melanocortin analogs have reduced clearance and/or metabolism, increased uptake, absorption, and/or stability, relative to a control.

Clearance

[0254] "Clearance" may refer to the elimination, absorption, and/or metabolism of the non-naturally occurring melanocortin analogs in the subject's plasma. Clearance may be assessed as volume of plasma cleared of the non-naturally occurring melanocortin analogs over time (e.g., mL/min, L/hr, or L/day) and/or may be normalized to body weight of the subject (e.g., mL/min/kg). Reduced clearance may also be represented by an increase in half-life or volume of distribution (V_d). In some embodiments, measuring clearance comprises measuring a terminal elimination rate constant (λ_z) or an inter-compartmental clearance (Q).

[0255] In some embodiments, the reduction in clearance comprises a measurement about 10 minutes, 20 minutes, 30 minutes, 40 minutes, 50 minutes, 1 hour, 1.5 hours, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 12 hours, 1 day, 2 days, 3 days, 4 days, 5 day, 6 days, or 1 week after administration of the non-naturally occurring melanocortin analogs.

[0256] In some embodiments, the reduction in clearance comprises a measurement during administration of the non-naturally occurring melanocortin analogs. In some embodiments, the reduction in clearance comprises a measurement at the completion of administration of the non-naturally occurring melanocortin analogs.

Concentration

[0257] In some embodiments, the non-naturally occurring melanocortin analogs of the present technology comprise an increased tissue, plasma, and/or serum concentration relative to a control. "Concentration" may comprise a measurement

reflecting one or more of the absolute amounts of the non-naturally occurring melanocortin analogs, the absorption of the non-naturally occurring melanocortin analogs, the metabolism of non-naturally occurring melanocortin analogs, or the elimination of non-naturally occurring melanocortin analogs.

[0258] The increased tissue, plasma, and/or serum concentration may be an increase in concentration of the non-naturally occurring melanocortin analogs at a given time point relative to a control administered at the same dose and measured at the same time point. The concentration may be measured at an intermediate time point or a final time point and may be measured as a mean residence time (MRT), an average concentration (C_{avg}), a trough concentration (C_{trough}), or a concentration at the end of administration (e.g., infusion) time (C_T).

[0259] In some embodiments, the increased concentration is reflected by an increase in peak plasma concentration (C_{max}). An increase in C_{max} may suggest increased absorption, reduced metabolism, or slower elimination of the non-naturally occurring melanocortin analogs, relative to a control. In some embodiments, C_{max} comprises a dose normalized C_{max} (DNC_{max}).

[0260] In some embodiments, the increased concentration is reflected by an increase in minimum plasma concentration (C_{min}). An increase in C_{min} may suggest increased absorption, reduced metabolism, or slower elimination of the non-naturally occurring melanocortin analogs, relative to a control. In some embodiments, C_{min} comprises a dose normalized C_{min} (DNC_{min}).

[0261] In some embodiments, the increased concentration is reflected by a reduction in time to reach C_{max} (T_{max}). A reduced T_{max} may suggest increased absorption, reduced metabolism, or slower elimination of the non-naturally occurring melanocortin analogs, relative to a control.

[0262] In some embodiments, the increased concentration is reflected by a final measurable concentration (T_{final}). An increased T_{final} may suggest increased absorption, reduced metabolism, or slower elimination of the non-naturally occurring melanocortin analogs, relative to a control.

[0263] In some embodiments, the increased concentration is reflected by an increase in area under the curve (AUC). An increase in AUC may signify increased

exposure to the non-naturally occurring melanocortin analogs and/or may suggest increased absorption, reduced metabolism, or slower elimination of the non-naturally occurring melanocortin analogs, relative to a control. The AUC measurement may comprise an Area Under the Curve for Concentration of Drug in Non-Compartmental Analysis (DNAUC).

[0264] In some embodiments, the increase in tissue, plasma, and/or serum concentration comprises a measurement about 10 minutes, 20 minutes, 30 minutes, 40 minutes, 50 minutes, 1 hour, 1.5 hours, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 12 hours, 1 day, 2 days, 3 days, 4 days, 5 day, 6 days, or 1 week after administration of the non-naturally occurring melanocortin analogs.

[0265] In some embodiments, the increase in tissue, plasma, and/or serum concentration comprises a measurement at least 10 minutes, 20 minutes, 30 minutes, 40 minutes, 50 minutes, 1 hour, 1.5 hours, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 12 hours, 1 day, 2 days, 3 days, 4 days, 5 day, 6 days, or 1 week after administration of the non-naturally occurring melanocortin analogs.

[0266] In some embodiments, the increase in tissue, plasma, and/or serum concentration comprises a measurement at least about 10 minutes, 20 minutes, 30 minutes, 40 minutes, 50 minutes, 1 hour, 1.5 hours, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 12 hours, 1 day, 2 days, 3 days, 4 days, 5 day, 6 days, or 1 week after administration of the non-naturally occurring melanocortin analogs.

[0267] In some embodiments, the increase in tissue, plasma, and/or serum concentration comprises a measurement during administration of the non-naturally occurring melanocortin analogs.

[0268] In some embodiments, the increase in tissue, plasma, and/or serum concentration comprises a measurement at the completion of administration of the non-naturally occurring melanocortin analogs.

[0269] In some embodiments, the concentration of the non-naturally occurring melanocortin analog in the plasma or a tissue of the subject is at least about 5 ng/mL to at least about 2000 ng/mL about 24 hours after administration of the non-naturally occurring melanocortin analog.

[0270] In some embodiments, the non-naturally occurring melanocortin analog is administered to the subject until the concentration of the non-naturally occurring melanocortin analog in the plasma or a tissue of the subject is at least about 5 ng/mL to at least about 2000 ng/mL after administration of the non-naturally occurring melanocortin analog. In some embodiments, the concentration is at least about 5 ng/mL after administration of the non-naturally occurring melanocortin analog. In some embodiments, the concentration is at least about 10 ng/mL after administration of the non-naturally occurring melanocortin analog. In some embodiments, the concentration is at least about 50 ng/mL after administration of the non-naturally occurring melanocortin analog. In some embodiments, the concentration is at least about 100 ng/mL after administration of the non-naturally occurring melanocortin analog. In some embodiments, the concentration is at least about 200 ng/mL after administration of the non-naturally occurring melanocortin analog. In some embodiments, the concentration is at least about 300 ng/mL after administration of the non-naturally occurring melanocortin analog. In some embodiments, the concentration is at least about 400 ng/mL after administration of the non-naturally occurring melanocortin analog. In some embodiments, the concentration is at least about 500 ng/mL after administration of the non-naturally occurring melanocortin analog. In some embodiments, the wherein concentration is at least about 750 ng/mL after administration of the non-naturally occurring melanocortin analog. In some embodiments, the concentration is at least about 1000 ng/mL after administration of the non-naturally occurring melanocortin analog. In some embodiments, the is at least about 1500 ng/mL after administration of the non-naturally occurring melanocortin analog. In some embodiments, the concentration is at least about 2000 ng/mL after administration of the non-naturally occurring melanocortin analog. In some embodiments, the concentration is a C_{max}.

Distribution

[0271] In some embodiments, the non-naturally occurring melanocortin analogs of the present technology comprise an increased distribution relative to a control. The increased distribution may be an increase in distribution of the non-naturally occurring melanocortin analogs at a given time point relative to a control administered at the same dose and measured at the same time point. The distribution may be measured at an intermediate time point or a final time point.

[0272] In some embodiments, the measurement of distribution comprises measuring a volume of distribution at the terminal phase (V_d or $V_{d\beta}$), a central volume of distribution (V), a peripheral volume of distribution (V2), an apparent volume of distribution (V_z), or a measurement of distribution comprises measuring a volume of distribution at steady state (V_{ss}). A high or increased V_d, $V_{d\beta}$, V_z, and/or V_{ss} may suggest large distribution beyond the tissue, plasma, and/or serum compartment, relative to the control.

[0273] In some embodiments, the increase in distribution comprises a measurement about 10 minutes, 20 minutes, 30 minutes, 40 minutes, 50 minutes, 1 hour, 1.5 hours, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 12 hours, 1 day, 2 days, 3 days, 4 days, 5 day, 6 days, or 1 week after administration of the non-naturally occurring melanocortin analogs.

[0274] In some embodiments, the increase in distribution comprises a measurement at least 10 minutes, 20 minutes, 30 minutes, 40 minutes, 50 minutes, 1 hour, 1.5 hours, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 12 hours, 1 day, 2 days, 3 days, 4 days, 5 day, 6 days, or 1 week after administration of the non-naturally occurring melanocortin analogs.

[0275] In some embodiments, the increase in distribution comprises a measurement at least about 10 minutes, 20 minutes, 30 minutes, 40 minutes, 50 minutes, 1 hour, 1.5 hours, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 12 hours, 1 day, 2 days, 3 days, 4 days, 5 day, 6 days, or 1 week after administration of the non-naturally occurring melanocortin analogs.

[0276] In some embodiments, the increase in distribution comprises a measurement during administration of the non-naturally occurring melanocortin analogs.

[0277] In some embodiments, the increase in distribution comprises a measurement at the completion of administration of the non-naturally occurring melanocortin analogs.

Additional pD and pK Embodiments

[0278] In some embodiments, the non-naturally occurring melanocortin analog of the present technology exhibits one or more of the following:

(a) an increase in half-life relative to a control;

(b) a reduction in clearance relative to a control;

(c) an increase in tissue concentration relative to a control;

(d) an increase in plasma concentration relative to a control;

(e) an increase in serum concentration relative to a control;

(f) an increase in distribution relative to a control;

(g) an increase in an AUC measurement relative to a control;

(h) an increase in a DNAUC measurement relative to a control;

(i) an increase in T_{final} relative to a control;

(j) an increase in C_{max} relative to a control;

(k) an increase in C_{min} relative to a control;

(l) an increase in DNC_{min} relative to a control;

(m) an increase in MRT relative to a control;

(n) an increase in C_{avg} relative to a control;

(o) an increase in C_{trough} relative to a control;

(p) an increase in C_T relative to a control;

(q) an increase in V_d relative to a control;

(r) a reduction in T_{max} relative to a control; or

(s) a reduction in Q relative to a control.

[0279] In some embodiments, the non-naturally occurring melanocortin analog of the present technology exhibits one or more of the following, relative to a control:

(a) an increase in half-life by at least about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, 500%, 600%, 700%, 800%, 900%, or 1000% relative to a control;

(b) a reduction in clearance by at least about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% relative to a control;

(c) an increase in tissue concentration by at least about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, 500%, 600%, 700%, 800%, 900%, or 1000% relative to a control;

(d) an increase in plasma concentration by at least about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, 500%, 600%, 700%, 800%, 900%, or 1000% relative to a control;

(e) an increase in serum concentration by at least about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, 500%, 600%, 700%, 800%, 900%, or 1000% relative to a control;

(f) an increase in distribution by at least about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, 500%, 600%, 700%, 800%, 900%, or 1000% relative to a control;

(g) an increase in an AUC by at least about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, 500%, 600%, 700%, 800%, 900%, or 1000% measurement relative to a control;

(h) an increase in a DNAUC measurement by at least about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, 500%, 600%, 700%, 800%, 900%, or 1000% relative to a control;

(i) an increase in T_{final} by at least about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, 500%, 600%, 700%, 800%, 900%, or 1000% relative to a control;

(j) an increase in C_{max} by at least about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, 500%, 600%, 700%, 800%, 900%, or 1000% relative to a control;

(k) an increase in C_{min} by at least about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, 500%, 600%, 700%, 800%, 900%, or 1000% relative to a control;

(l) an increase in DNC_{min} by at least about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, 500%, 600%, 700%, 800%, 900%, or 1000% relative to a control;

(m) an increase in MRT by at least about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, 500%, 600%, 700%, 800%, 900%, or 1000% relative to a control;

(n) an increase in C_{avg} by at least about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, 500%, 600%, 700%, 800%, 900%, or 1000% relative to a control;

(o) an increase in C_{trough} by at least about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, 500%, 600%, 700%, 800%, 900%, or 1000% relative to a control;

(p) an increase in C_T by at least about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, 500%, 600%, 700%, 800%, 900%, or 1000% relative to a control;

(q) an increase in V_d by at least about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, 500%, 600%, 700%, 800%, 900%, or 1000% relative to a control;

(r) a reduction in T_{max} by at least about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% relative to a control; or

(s) a reduction in Q by at least about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% relative to a control.

EXAMPLES

[0280] The following examples are intended to illustrate various embodiments of the present technology. As such, the specific embodiments discussed are not to be construed as limitations on the scope of the present technology. It will be apparent to one skilled in the art that various equivalents, changes, and modifications may be made without departing from the scope of present technology, and it is understood that such equivalent embodiments, are to be included herein. Further, all references cited herein are hereby incorporated by reference in their entirety, as if fully set forth herein.

Example 1: Peptide Synthesis-Generic

[0281] The non-naturally occurring melanocortin analogs of the present technology were synthesized by conventional procedures (e.g., solution-phase procedure, solid-phase synthesis) for the formation of a peptide linkage between amino acids. The solution-phase procedure involved a condensation between the free alpha amino group of an amino acid or derivative thereof having the carboxyl group or other reactive groups protected and the free primary carboxyl group of another amino acid or derivative thereof having the amino group or other reactive groups protected. The solid-phase synthesis utilized a variety of resins and reagents and may involve additional purification steps.

[0282] The process for synthesizing the non-naturally occurring melanocortin analogs was generally performed by a procedure as follows. Each amino acid in the desired sequence of the non-naturally occurring melanocortin analogs was added one at a time in succession to another amino acid or derivative thereof or by a procedure whereby peptide fragments with the desired amino acid sequence were first synthesized conventionally and then condensed to provide the desired peptide. In most cases, the resulting peptide was then cyclized to yield a cyclic peptide.

[0283] Solid-phase peptide synthesis was carried out by sequentially incorporating the desired amino acid residues one at a time into the growing peptide chain coupled to a solid-phase support according to the general principles of solid phase methods (see Merrifield, Angew Chem. 24:799-810 (1985) and Barany et al., The Peptides, Analysis, Synthesis and Biology, Vol. 2, Gross E. and Meienhofer J., Eds. Academic Press 1-284 (1980)). An exemplary solid-phase synthesis of non-naturally occurring melanocortin analogs is provided below.

[0284] Initially, the C-terminal amino acid residue of the non-naturally occurring melanocortin analog was coupled to a solid-phase support, e.g., a solid-phase resin. Coupling of the C-terminal amino acid residue and the solid-phase support may be carried out according to any method know in the art. Depending on the coupling method, the alpha-amine of the C-terminal amino acid residue may or may not be protected with an amine protecting group, as described below. Likewise, the carboxyl group of the amino acid residue may or may not be activated prior to coupling to the solid-phase support in order to increase its electrophilicity. Some methods of coupling rely on the

formation of an ester bond between the carboxyl group of the amino acid and a reactive handle on the solid-phase resin. For example, an amino acid residue may be coupled to a p-benzyloxybenzyl alcohol resin (Wang) or a 2-chlorotrityl chloride resin via an ester linkage. Some methods of coupling rely on the formation of an amide bond between the carboxyl group of the amino acid and a reactive handle on the solid-phase resin For example, an amino acid residue may be coupled to a benzhydrylamine (BHA) resin through an Fmoc-linker such as, for example, p-[(R,S)-α-[1-(9H-fluor-en-9-yl)-methoxyformamido]-2,4-dimethyloxybenzyl]-phenoxyacetic acid (Rink linker) via an amide linkage.

[0285] The non-naturally occurring melanocortin analog was then synthesized by sequential amino acid addition or combination of peptide fragments. Subsequently, the peptide was cleaved from the solid-phase support and purified by methods known in the art, such as, for example, reverse phase high performance liquid chromatography (RP-HPLC) using a suitable column, such as a C18 column. Additionally, or alternatively, other methods of separation or purification were employed, including, but not limited to, methods based on the size or charge of the peptide. Once purified, the peptide was characterized by methods such as high-performance liquid chromatograph (HPLC), amino acid analysis, mass spectrometry, and the like.

Example 2: Peptide Synthesis-Protecting Groups

[0286] During synthesis of the non-naturally occurring melanocortin analogs, reactive side chain groups of the various amino acid residues were protected with suitable protecting groups, which prevented undesirable chemical reaction from occurring at that site until the protecting group was removed.

[0287] Additionally, protection of the alpha amino group of an amino acid residue or fragment was performed while that entity reacting with the carboxyl group, followed by the selective removal of the alpha amino protecting group to allow a subsequent reaction to take place at that site. Specific protecting groups for solid phase synthesis methods and solution phase synthesis methods are known to those having ordinary skill in the art. Alpha amino groups were protected by a suitable protecting group, including a urethane-type protecting group, such as benzyloxycarbonyl (Z) and substituted benzyloxycarbonyl, such as p-chlorobenzyloxycarbonyl, p-nitrobenzyloxycarbonyl, p-bromobenzyloxycarbonyl, p-biphenyl-isopropoxycarbonyl, 9-fluorenylmethoxycarbonyl

(Fmoc) and p-methoxybenzyloxycarbonyl (Moz); aliphatic urethane-type protecting groups, such as t-butyloxycarbonyl (Boc), diisopropylmethoxycarbonyl, isopropoxycarbonyl, and allyloxycarbonyl. Fmoc was also used for alpha amino protection. Guanidino groups, if present, were protected by a suitable protecting group, such as nitro, p-toluenesulfonyl (Tos), Z, pentamethylchromanesulfonyl (Pmc), adamantyloxycarbonyl, pentamethyldihydrobenzofuran-5-sulfonyl (Pbf) and Boc. Pmc was used as a protecting group for Arg.

[0288] Alpha aminoprotecting groups may be removed under basic conditions, such as, for example, using a solution of piperidine, piperazine, diethylamine, or morpholine (20-40% v/v) in N,N-dimethylformamide (DMF). In synthesis methods in which alpha amino protecting groups were used, protecting groups were removed after synthesis of the peptide and before or after cleavage of the solid-phase support.

Example 3: Peptide Synthesis-Additional Modifications

[0289] If necessary, the peptides were further modified to obtain N-terminus modifications, such as acetylation, while on resin, or were removed from the resin by use of a cleaving reagent and then modified. Likewise, C-terminus modification (e.g., amidation), was performed if needed.

[0290] Additionally, the cyclized peptide structures were obtained prior to cleavage from the peptide resin. For cyclization through reactive side chain moieties, the desired side chains were deprotected, and the peptide suspended in a suitable solvent and a cyclic coupling agent added. Suitable solvents, for example DMF, dichloromethane (DCM) or 1-methyl-2-pyrrolidone (NMP), were used for the cyclization. Suitable cyclic coupling reagents (e.g., 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU), 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), benzotriazole-1-yl-oxy-tris(dimethylamino)phosphoniumhexafluorophosphate (BOP), benzotriazole-1-yl-oxy-tris(pyrrolidino)phosphoniumhexafluorophosphate (PyBOP), 2-(7-aza-1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TATU), 2-(2-oxo-1 (2H)-pyridyl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TPTU), N,N′-dicyclohexylcarbodiimide/1-hydroxybenzotriazole (DCCI/HOBt)) were also used for the cyclization. Coupling was initiated by a suitable base, such as N,N-diispropylethylamine (DIPEA), sym-collidine or N-methylmorpholine (NMM).

Example 4: Biological Data

[0291] The agonist and antagonist activity of exemplary non-naturally occurring melanocortin analogs at the melanocortin receptors (e.g., MC1R, MC3R, MC4R, and MC5R) were measured via cAMP accumulation assay, according to the following procedure. Experimental design and execution were conducted by Epics Therapeutics S.A. EuroscreenFast (Bruxelles, Belgium).

Compound Handing

[0292] Compounds were delivered as powder (1 mg) or 10 mM solutions (100 µl) in 100% DMSO. Powders were solubilized in 100% DMSO at a concentration of 10 mM (master solution) in a solvent volume defined. Serial dilutions were performed from master solution in 100% DMSO to obtain intermediate concentrations 200-, 300- or 400-fold higher than the concentrations to be tested, depending on the assay. Each sample was diluted 100-fold in the assay buffer and dispensed in a test plate. Amounts, solvents, and dilutions were estimated based on standard small-molecule drugs. Cell lines used for functional assays are shown in Table 1.

Table 1. Cell lines

Receptor	Cell Line	Reference agonist	Reference antagonist
MC1R	CHO-K1	NDP-α-MSH	N/A
MC3R	CHO-K1	NDP-α-MSH	SHU9119
MC4R	CHO-K1	NDP-α-MSH	SHU9119
MC5R	CHO-K1	NDP-α-MSH	N/A

Compound Testing

[0293] Compounds were tested for (i) agonist and/or antagonist activity at the human MC3 (FAST-0232C) and MC4 (FAST-0233C) receptors, (ii) agonist activity at the human MC1 (FAST-0230C) receptor, and/or (iii) agonist activity at the human MC5 (FAST-0233C) receptor at the following nanomolar concentrations, in duplicate: 0.0001, 0.001, 0.01, 0.03, 0.1, 0.3, 1, 10, 100, and 1,000.

Testing Protocol

[0294] Cyclic AMP (cAMP) Homogenous Time-Resolved Fluorescence (HTRF) assay for Gs coupled receptor:

[0295] CHO-K1 cells expressing recombinant human receptor grown prior to the test in media without antibiotic were detached by gentle flushing with PBS-EDTA (5 mM EDTA), recovered by centrifugation and resuspended in assay buffer (KRH: 5 mM KCl, 1.25 mM MgSO4, 124 mM NaCl, 25 mM HEPES, 13.3 mM Glucose, 1.25 mM KH2PO4, 1.45 mM CaCl2, 0.5 g/l BSA, supplemented with 1mM IBMX or 25µM Rolipram).

[0296] Dose response curves were performed in parallel with the reference compounds.

[0297] For agonist test (384well): 5 µl of cells were mixed with 5 µl of the test compound at increasing concentrations and then incubated 30 min at room temperature. After addition of the lysis buffer containing cAMP-d2 and anti-cAMP cryptate detection reagents, plates were incubated 1-hour at room temperature, and fluorescence ratios were measured according to the manufacturer specification, with the HTRF kit.

[0298] For antagonist test (384well): 5 µl of cells were mixed in the wells of an assay plate with 5 µl of a mix of test compound at increasing concentrations and reference agonist for a final concentration corresponding to the historical EC80. The plates were then incubated 30 min at room temperature. After addition of the lysis buffer containing cAMP-d2 and anti-cAMP cryptate detection reagents, plates were incubated 1-hour at room temperature, and fluorescence ratios were measured according to the manufacturer specification, with the HTRF kit.

Quality Control for Compound Testing

[0299] On each day of experimentation and prior to the testing of compounds, reference compounds were tested at several concentrations in duplicate (n=2) to obtain a dose-response curve and an estimated EC50 and/or IC50 values.

[0300] Reference values thus obtained for the test were compared to historical values obtained from the same receptor and used to validate the experimental session.

[0301] A session was considered as valid only if the reference value was found to be within a 0.5 logs interval from the historical value.

[0302] For replicate determinations, the maximum variability tolerated in the test was of +/-20% around the average of the replicates.

Non-naturally Occurring Melanocortin Analog Grouping

[0303] Group A included non-naturally occurring melanocortin analogs A1 to A3, all of which are cyclic peptides comprising the motif Pro-dPhe-Arg-Trp (SEQ ID NO: 353) or Pro-Phe-Arg-Trp (SEQ ID NO: 358) and cyclized through a lactam bond between Asp and Lys. Group A non-naturally occurring melanocortin analogs are provided in Table 2.

Table 2. Group A non-naturally occurring melanocortin analogs

Melanocortin analog	SEQ ID NO	Sequence
A1	2	Ac-Nle-c[Asp-Pro-Phe-Arg-Trp-Lys]-dVal-dPro-NH_2
A2	3	Ac-Nle-c[Asp-Pro-His-Phe-Arg-Trp-Lys]-dVal-dPro-NH_2
A3	31	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2

[0304] Group B included non-naturally occurring melanocortin analogs B1 to B4, all of which are cyclic peptides comprising the motif Pro-dPhe-Arg-Trp (SEQ ID NO: 353) and cyclized through a lactam bond between Asp and Lys. Group B melanocortin analogs include an N-terminal extension. Group B non-naturally occurring melanocortin analogs are provided in Table 3.

Table 3. Group B non-naturally occurring melanocortin analogs

Melanocortin analog	SEQ ID NO	Sequence
A1	53	Ac-Nle-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
A2	40	Ac-Nle-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2
A3	188	Ac-Nle-Nle-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
A4	189	Ac-Nle-Nle-Nle-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2

[0305] Group C included non-naturally occurring melanocortin analogs C1 to C34, all of which are cyclic peptides comprising the motif Pro-dPhe-Arg-Trp (SEQ ID NO: 353) and cyclized through a lactam bond between Asp and Lys. Group C melanocortin analogs include an N-terminal amino acid other than Nle. Group C non-naturally occurring melanocortin analogs are provided in Table 4.

Table 4. Group C non-naturally occurring melanocortin analogs

Melanocortin analog	SEQ ID NO	Sequence
C1	66	Ac-dLeu-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
C2	94	Ac-dAla-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
C3	144	Phe-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
C4	145	His-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
C6	147	Trp-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
C7	148	dPhe-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
C8	149	dHis-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
C9	150	dTrp-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
C10	151	Ac-Arg-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
C11	152	Ac-dLys-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
C12	153	Ac-Lys-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
C13	154	Ac-His-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
C14	155	Ac-dHis-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
C15	156	Ac-Ala-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
C16	158	Ac-Orn-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
C17	159	Ac-dOrn-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
C18	187	Ac-dArg-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
C19	193	Ac-dPhe-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
C20	195	Ac-dTyr-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
C21	167	Ac-Ala-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2
C22	168	Ac-dArg-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2
C23	169	Ac-Arg-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2
C24	170	Ac-Lys-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2
C25	171	Ac-dLys-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2
C26	172	Ac-His-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2
C27	173	Ac-dHis-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2
C28	39	Ac-Arg-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dArg-dVal-dPro-NH_2
C29	160	Ac-dLys-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dLys-dVal-dPro-NH_2
C30	161	Ac-Lys-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dArg-dPro-NH_2
C31	162	Ac-His-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dLys-dPro-NH_2

C32	163	Ac-dHis-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dHyp-NH_2
C33	164	Ac-Orn-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2
C34	165	Ac-dOrn-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dVal-dVal-dPro-NH_2

[0306] Group D included non-naturally occurring melanocortin analogs D1 to D23, all of which are cyclic peptides comprising a derivative of the motif Pro-dPhe-Arg-Trp (SEQ ID NO: 353) and cyclized through a lactam bond between Asp and Lys. Derivatives of the motif of SEQ ID NO: 353 included in Group D melanocortin analogs include substitution of Pro for another amino acid. Group D non-naturally occurring melanocortin analogs are provided in Table 5.

Table 5. Group D non-naturally occurring melanocortin analogs

Melanocortin analog	SEQ ID NO	Sequence
D1	4	Ac-Nle-c[Asp-Aba-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
D2	5	Ac-Nle-c[Asp-Aia-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
D3	6	Ac-Nle-c[Asp-Ata-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
D4	10	Ac-Nle-c[Asp-APC-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
D6	11	Ac-Nle-c[Asp-APPC-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
D7	18	Ac-Nle-c[Asp-Ioc-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
D8	42	Ac-Nle-c[Asp-Trp-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
D9	43	Ac-Nle-c[Asp-Tyr-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
D10	63	Ac-Nle-c[Asp-Atc-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
D11	88	Ac-Nle-c[Asp-Ala-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
D12	142	Ac-Nle-c[Asp-Hyp-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
D13	174	Ac-Nle-c[Asp-dPhe-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
D14	175	Ac-Nle-c[Asp-dGln-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
D15	176	Ac-Nle-c[Asp-dTrp-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
D16	177	Ac-Nle-c[Asp-dTyr-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
D17	190	Ac-Nle-c[Asp-Cpe-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
D18	191	Ac-Nle-c[Asp-Che-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
D19	192	Ac-Nle-c[Asp-Oic-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
D20	220	Ac-Nle-c[Asp-Leu-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2

D21	221	Ac-Nle-c[Asp-Tic-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
D22	222	Ac-Nle-c[Asp-Phe-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
D23	223	Ac-Nle-c[Asp-Bip-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2

[0307] Group E included non-naturally occurring melanocortin analogs E1 to E9, all of which are cyclic peptides comprising a derivative of the motif Pro-dPhe-Arg-Trp (SEQ ID NO: 353) and cyclized through a lactam bond between Asp and Lys. Derivatives of the motif of SEQ ID NO: 353 included in Group E melanocortin analogs include substitution of Arg for another amino acid or substitution of Trp for another amino acid. Group E non-naturally occurring melanocortin analogs are provided in Table 6.

Table 6. Group E non-naturally occurring melanocortin analogs

Melanocortin analog	SEQ ID NO	Sequence
E1	12	Ac-Nle-c[Asp-Pro-dPhe-transPro(guan)-Trp-Lys]-dVal-dPro-NH_2
E2	13	Ac-Nle-c[Asp-Pro-dPhe-cisPro(guan)-Trp-Lys]-dVal-dPro-NH_2
E3	14	Ac-Nle-c[Asp-Pro-dPhe-Arg-Aia-Lys]-dVal-dPro-NH_2
E4	15	Ac-Nle-c[Asp-Pro-dPhe-Arg-Aba-Lys]-dVal-dPro-NH_2
E5	16	Ac-Nle-c[Asp-Pro-dPhe-Arg-Ata-Lys]-dVal-dPro-NH_2
E6	92	Ac-Nle-c[Asp-Pro-dPhe-His-Trp-Lys]-dVal-dPro-NH_2
E7	65	Ac-Nle-c[Asp-Pro-dPhe-Arg-dTrp-Lys]-dVal-dPro-NH_2
E8	95	Ac-Nle-c[Asp-Pro-dPhe-Arg-Nal(1')-Lys]-dVal-dPro-NH_2
E9	96	Ac-Nle-c[Asp-Pro-dPhe-Arg-Phe-Lys]-dVal-dPro-NH_2

[0308] Group F included non-naturally occurring melanocortin analogs F1 to F57, all of which are cyclic peptides comprising the motif Pro-dPhe-Arg-Trp (SEQ ID NO: 353) and cyclized through a lactam bond between Asp and Lys. Group F melanocortin analogs include a C-terminal extension other than dVal-dPro. Group F non-naturally occurring melanocortin analogs are provided in Table 7.

Table 7. Group F non-naturally occurring melanocortin analogs

Melanocortin analog	SEQ ID NO	Sequence
F1	17	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dVal-dVal-dVal-dVal-dVal-dPro-NH_2

F2	36	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-Hyp-NH_2
F3	37	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dHyp-NH_2
F4	38	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-β2Val-βPro-NH_2
F5	39	Ac-Arg-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dArg-dVal-dPro-NH_2
F6	41	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-Arg-NH_2
F7	44	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dArg-NH_2
F8	45	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-Lys-Pro-Val-NH_2
F9	46	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dLys-dVal-dPro-NH_2
F10	47	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-Arg-Pro-Val-NH_2
F11	48	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-Arg-Val-Pro-NH_2
F12	49	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dArg-dVal-dPro-NH_2
F13	50	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2
F14	51	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dTle-NH_2
F15	52	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dVal-dVal-dPro-NH_2
F16	54	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-Trp-NH_2
F17	57	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dAsn-NH_2
F18	58	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-Asn-dPro-NH_2
F19	59	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-Ala-Ala-NH_2
F20	60	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-Gly-Gly-NH_2
F21	61	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dAsn-dPro-NH_2
F22	104	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-Val-Pro-NH_2
F23	129	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-Val-NH_2
F24	130	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dThr-dPro-dThr-NH_2
F25	131	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dThr-dPro-dThr-OH
F26	132	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dPro-dVal-NH_2
F27	133	Ac-Nle-c[dAsp-Pro-dPhe-Arg-Trp-dLys]-dPro-dVal-NH_2
F28	134	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-βPro-β2Val-NH_2
F29	135	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-βPro-β3Val-NH_2
F30	136	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-OH
F31	137	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-β3Val-βPro-NH_2
F32	138	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-Pro-NH_2
F33	139	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-βPro-βPro-NH_2

F34	146	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-Arg-NH_2
F35	157	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dArg-NH_2
F36	166	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dArg-dPro-NH_2
F37	178	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dLys-dPro-NH_2
F38	179	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-Lys-Val-Pro-NH_2
F39	180	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-Lys-dVal-dPro-NH_2
F40	181	Ac-dNle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
F41	182	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dTle-dTle-dPro-NH_2
F42	183	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dTle-dTle-dVal-NH_2
F43	184	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dVal-dPro-NH_2
F44	185	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dPro-dVal-dPro-NH_2
F45	186	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-dVal-dPro-NH_2
F46	197	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-Lys-dPro-dVal-NH_2
F47	198	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dAla-dAla-NH_2
F48	199	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-Asp-NH_2
F49	200	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dHyp-NH_2
F50	201	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-Asp-NH_2
F51	203	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-Hyp-NH_2
F52	204	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-Asp-dPro-NH_2
F53	205	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-Asn-NH_2
F54	206	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dAsp-NH_2
F55	207	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dAsn-NH_2
F56	208	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-Lys-dPro-NH_2
F57	209	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dPro-dVal-OH

[0309] Group G included non-naturally occurring melanocortin analogs G1 to G4, all of which are cyclic peptides comprising a derivative of the motif Pro-dPhe-Arg-Trp (SEQ ID NO: 353) and cyclized through a lactam bond between Asp and Lys. Group G melanocortin analog include a Pro or Gly after the Trp residue. Group G non-naturally occurring melanocortin analogs are provided in Table 8.

Table 8. Group G non-naturally occurring melanocortin analogs

Melanocortin analog	SEQ ID NO	Sequence
G1	55	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Gly-Lys]-dVal-dPro-NH_2
G2	56	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Gly-Lys]-dPro-dPro-dLys-dAsp-NH_2
G3	143	Ac-Nle-c[Asp-Hyp-dPhe-Arg-Trp-Pro-Lys]-dVal-dPro-NH_2
G4	219	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Gly-Lys]-dPro-dPro-Lys-Asp-NH_2

[0310] Group H included non-naturally occurring melanocortin analogs H1 to H5, all of which are cyclic peptides comprising a derivative of the motif Pro-dPhe-Arg-Trp (SEQ ID NO: 353) and cyclized through a disulfide bond. Group H non-naturally occurring melanocortin analogs are provided in Table 9.

Table 9. Group H non-naturally occurring melanocortin analogs

Melanocortin analog	SEQ ID NO	Sequence
H1	62	Ac-Nle-c[dCys-Pro-dPhe-Arg-Trp-Cys]-dVal-dPro-NH_2
H2	67	Ac-Nle-c[dPen-Pro-dPhe-Arg-Trp-dPen]-dVal-dPro-NH_2
H3	74	Ac-Nle-c[dCys-Pro-dPhe-Arg-Trp-dCys]-dVal-dPro-NH_2
H4	202	Ac-Nle-c[Cys-Pro-dPhe-Arg-Trp-Cys]-dVal-dPro-NH_2
H5	210	Ac-Nle-c[Cys-dPhe-Arg-Trp-Pen]-dVal-dPro-NH_2

[0311] Group I included non-naturally occurring melanocortin analogs I1 to I31, all of which are cyclic peptides comprising a derivative of the motif Trp-Pro-dPhe-Arg-Trp (SEQ ID NO: 354) or a derivative thereof. Derivatives of the motif of SEQ ID NO: 354 may include substitution of Trp for another amino acid or substitution of Pro for another amino acid. Group I non-naturally occurring melanocortin analogs are provided in Table 10.

Table 10. Group D non-naturally occurring melanocortin analogs

Melanocortin analog	SEQ ID NO	Sequence
I1	64	Ac-Nle-c[Asp-Trp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
I2	99	Ac-Nle-c[Cys-Trp-Pro-dPhe-Arg-Trp-Cys]-dVal-dPro-NH_2
I3	100	Ac-Nle-c[dCys-Trp-Pro-dPhe-Arg-Trp-Cys]-dVal-dPro-NH_2

I4	101	Ac-Nle-c[dCys-Trp-Pro-dPhe-Arg-Trp-dCys]-dVal-dPro-NH_2
I6	102	Ac-Ala-c[Asp-Trp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
I7	103	Ac-Lys-c[Asp-Trp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
I8	105	Ac-dLys-c[Asp-Trp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
I9	106	Ac-Arg-c[Asp-Trp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
I10	107	Ac-dArg-c[Asp-Trp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
I11	108	Ac-dHis-c[Asp-Trp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
I12	109	Ac-His-c[Asp-Trp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
I13	110	Ac-Nle-Nle-c[Asp-Trp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
I14	111	Ac-Nle-c[Asp-Trp-Ala-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
I15	112	Ac-Nle-c[Asp-Trp-Leu-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
I16	113	Ac-Nle-c[Asp-Trp-Pro-dPhe-Arg-Nal(1')-Lys]-dVal-dPro-NH_2
I17	114	Ac-Nle-c[Asp-Trp-Pro-dPhe-Arg-dNal(2')-Lys]-dVal-dPro-NH_2
I18	115	Ac-Nle-c[Asp-Trp-Pro-dPhe-Arg-Nal(2')-Lys]-dVal-dPro-NH_2
I19	116	Ac-Nle-c[Asp-Trp-Pro-dPhe-Arg-Trp-Lys]-dArg-dVal-dPro-NH_2
I20	117	Ac-Nle-c[Asp-Trp-Pro-dPhe-Arg-Trp-Lys]-dLys-dVal-dPro-NH_2
I21	118	Ac-Nle-c[Asp-Trp-Pro-dPhe-Arg-Trp-Lys]-dArg-dPro-NH_2
I22	119	Ac-Nle-c[Asp-Trp-Pro-dPhe-Arg-Trp-Lys]-dLys-dPro-NH_2
I23	120	Ac-Nle-c[Asp-Trp-Pro-dPhe-Arg-Trp-Lys]-dVal-dHyp-NH_2
I24	121	Ac-Nle-c[Asp-Trp-Pro-dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2
I25	122	Ac-Nle-c[Asp-Trp-Pro-dPhe-Arg-Trp-Lys]-dVal-dVal-dVal-dPro-NH_2
I26	123	Ac-Nle-c[Asp-Phe-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
I27	124	Ac-Nle-c[Asp-His-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
I28	125	Ac-Nle-c[Asp-Tyr-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
I29	126	Ac-Nle-c[Asp-dPhe-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
I30	127	Ac-Nle-c[Asp-dHis-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
I31	128	Ac-Nle-c[Asp-dTyr-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2

[0312] Group J included non-naturally occurring melanocortin analogs J1 to J3, all of which are cyclic peptides comprising a derivative of the motif Ala-His-dPhe-Arg-Trp (SEQ ID NO: 355) or a derivative thereof. Derivatives of the motif of SEQ ID NO: 355

may include substitution of Ala for another amino acid, for example dAla. Group J non-naturally occurring melanocortin analogs are provided in Table 11.

Table 11. Group J non-naturally occurring melanocortin analogs

Melanocortin analog	SEQ ID NO	Sequence
J1	194	Ac-Arg-c[Asp-Ala-His-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
J2	196	Ac-dArg-c[Asp-Ala-His-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
J3	230	Ac-Arg-c[Asp-dAla-His-dPhe-Arg-Trp-Lys]-NH_2

[0313] Group K included non-naturally occurring melanocortin analogs K1 to K4, all of which are cyclic peptides comprising a derivative of the motif Phe-His-dPhe-Arg-Trp (SEQ ID NO: 356). Group K non-naturally occurring melanocortin analogs are provided in Table 12.

Table 12. Group K non-naturally occurring melanocortin analogs

Melanocortin analog	SEQ ID NO	Sequence
K1	140	Ac-Nle-Phe-Phe-c[Asp-Phe-His-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
K2	141	Ac-Nle-c[Asp-Phe-His-dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2
K3	224	Ac-Nle-c[Asp-Phe-His-dPhe-Arg-Trp-Lys]-dLeu-dPro-NH_2
K4	225	Ac-Nle-c[Asp-Phe-His-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2

[0314] Group L included non-naturally occurring melanocortin analogs L1 to L22, all of which are cyclic peptides comprising a derivative of the motif His-p(Cl)dPhe-Arg-Trp (SEQ ID NO: 357), or a derivative thereof. Derivatives of the motif of SEQ ID NO: 357 may include substitutions of His for another amino acid, for example, Aba, Aia, and Ata, or substitutions of p(Cl)dPhe for another amino acid, for example, p(Br)dPhe. Group L non-naturally occurring melanocortin analogs are provided in Table 13.

Table 13. Group L non-naturally occurring melanocortin analogs

Melanocortin analog	SEQ ID NO	Sequence
L1	7	Ac-Nle-c[Asp-Aba-p(Cl)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
L2	8	Ac-Nle-c[Asp-Aia-p(Cl)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
L3	9	Ac-Nle-c[Asp-Ata-p(Cl)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2

L4	24	Ac-Arg-c[Asp-His-p(Cl)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
L5	25	Ac-Lys-c[Asp-His-p(Cl)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
L6	26	Ac-His-c[Asp-His-p(Cl)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
L7	27	Ac-dHis-c[Asp-His-p(Cl)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
L8	28	Ac-Nle-c[Asp-His-p(Cl)dPhe-Arg-Trp-Lys]-dTle-dTle-dVal-NH_2
L9	29	Ac-Nle-c[Asp-His-p(Cl)dPhe-Arg-Trp-Lys]-dVal-dVal-dVal-dPro-NH_2
L10	30	Ac-Nle-c[Asp-His-p(Cl)dPhe-Arg-Trp-Lys]-dPro-dVal-dPro-NH_2
L11	32	Ac-Nle-Nle-c[Asp-His-p(Cl)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2
L12	33	Ac-dArg-c[Asp-His-p(Cl)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2
L13	34	Ac-Arg-c[Asp-His-p(Cl)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2
L14	35	Ac-dHis-c[Asp-His-p(Cl)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2
L15	85	Ac-Nle-c[dCys-His-p(Cl)dPhe-Arg-Trp-dCys]-dVal-dPro-NH_2
L16	86	Ac-dArg-c[Asp-His-p(Cl)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
L17	87	Ac-dLys-c[Asp-His-p(Cl)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
L18	89	Ac-dLys-c[Asp-His-p(Cl)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2
L19	90	Ac-Nle-c[dCys-His-p(Br)dPhe-Arg-Trp-Cys]-dVal-dPro-NH_2
L20	91	Ac-Nle-c[dCys-His-p(Br)dPhe-Arg-Trp-dCys]-dVal-dPro-NH_2
L21	228	Ac-Lys-c[Asp-His-p(Cl)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2
L22	229	Ac-His-c[Asp-His-p(Cl)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2

[0315] Group M included non-naturally occurring melanocortin analogs M1 to M31, all of which are cyclic peptides comprising a derivative of the motif Pro-p(F)dPhe-Arg-Trp (SEQ ID NO: 359), or a derivative thereof. Derivatives of the motif of SEQ ID NO: 359 may include substitutions of Pro for another amino acid, for example, His, dGln, and dTyr, or substitutions of Trp for another amino acid, for example, dNal(2'). Group M non-naturally occurring melanocortin analogs are provided in Table 14.

Table 14. Group M non-naturally occurring melanocortin analogs

Melanocortin analog	SEQ ID NO	Sequence
M1	19	Ac-Nle-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
M2	20	Ac-Nle-Nle-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2

M3	21	Ac-Nle-c[Asp-Pro-p(F)dPhe-His-Trp-Lys]-dVal-dPro-NH_2
M4	22	Ac-Nle-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2
M5	68	Ac-Lys-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
M6	69	Ac-dLys-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
M7	70	Ac-His-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
M8	71	Ac-dHis-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
M9	72	Ac-Nle-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dTle-dTle-dVal-NH_2
M10	73	Ac-Nle-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dVal-dVal-dVal-dPro-NH_2
M11	75	Ac-Nle-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dPro-dVal-dPro-NH_2
M12	76	Ac-Nle-Nle-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2
M13	77	Ac-Nle-Nle-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dPro-dVal-dPro-NH_2
M14	78	Ac-Ala-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2
M15	79	Ac-dArg-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2
M16	80	Ac-Arg-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2
M17	81	Ac-Lys-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2
M18	82	Ac-dLys-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2
M19	83	Ac-His-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2
M20	84	Ac-dHis-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH_2
M21	93	Ac-Nle-c[dCys-His-p(F)dPhe-Arg-Trp-dCys]-dVal-dPro-NH_2
M22	97	Ac-Nle-c[Asp-dGln-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
M23	98	Ac-Nle-c[Asp-dTyr-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
M24	211	Ac-Nle-c[dCys-Pro-p(F)dPhe-Arg-Trp-Cys]-dVal-dPro-NH_2
M25	212	Ac-Nle-c[dCys-Pro-p(F)dPhe-Arg-Trp-dCys]-dVal-dPro-NH_2
M26	213	Ac-Ala-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
M27	214	Ac-dArg-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
M28	215	Ac-Arg-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2
M29	226	Ac-Nle-c[Asp-His-p(F)dPhe-Arg-Nal(2')-Lys]-dVal-dPro-NH_2
M30	227	Ac-Nle-c[Cys-His-p(F)dPhe-Arg-Trp-Cys]-dVal-dPro-NH_2
M31	231	Ac-Nle-c[dCys-His-p(F)dPhe-Arg-Trp-Cys]-dVal-dPro-NH_2

[0316] Group O included non-naturally occurring melanocortin analogs O1, O2, O7, O11, all of which are cyclic peptides comprising the motif Pro-dPhe-Arg-Trp (SEQ ID NO: 353) or Pro-p(F)dPhe-Arg-Trp (SEQ ID NO: 359), and are cyclized through a lactam bond between Asp or Glu and Orn. Group O non-naturally occurring melanocortin analogs are provided in Table 15.

Table 15. Group O non-naturally occurring melanocortin analogs

Melanocortin analog	SEQ ID NO	Sequence
O1	23	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2
O2	217	Ac-Nle-c[Asp-Pro-p(F)dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2
O7	218	Ac-Nle-c[Glu-Pro-p(F)dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2
O11	216	Ac-Nle-c[Glu-Pro-dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2

Agonist activity of melanocortin analogs on melanocortin 1 and 5 receptors

[0317] Administration of some non-naturally occurring melanocortin analogs activated melanocortin 1 receptor (MC1R) and/or melanocortin 5 receptor (MC5R) activity, as measured by cAMP levels (Table 16).

Table 16. Dose-response results of melanocortin analogs and control against the melanocortin 1 receptor (MC1R) and melanocortin 5 receptor (MC5R)

SEQ ID NO	Compound ID	MC1R		MC5R	
		EC50 (nM))	Max Activation (Emax)	EC50 (nM))	Max Activation (Emax)
	NDP-a-MSH	0.039	101.06	0.12	101.44
31	A3	0.03	102.62	0.08	102.61
66	C1	0.02	102.84	0.57	98.53
94	C2	6.75	105.70	7.50	103.05
156	C15	1.66	101.79	2.74	102.12
181	F40	0.02	101.39	0.08	101.90
187	C18	1.88	100.29	2.69	103.33
193	C19	0.58	101.44	1.58	102.54
195	C20	8.77	101.34	51.43	100.24

144	C3	0.11	100.37	0.24	105.08
145	C4	3.25	98.64	11.81	104.41
147	C6	0.19	103.43	0.63	104.13
148	C7	0.28	102.54	1.77	103.35
149	C8	4.93	104.87	11.43	103.61
150	C9	0.24	103.25	1.61	103.27
151	C10	1.31	102.01	0.69	104.40
152	C11	4.67	104.03	4.54	102.85
153	C12	2.56	104.39	1.57	103.33
154	C13	6.87	102.37	6.97	104.63
155	C14	11.47	100.84	22.23	103.69
158	C16	4.78	102.51	4.02	103.38
159	C17	5.53	102.95	7.68	103.90
202	H4	0.17	101.44	0.10	102.07
62	H1	0.02	100.65	0.01	102.58
67	H2	18.04	102.68	78.67	99.36
74	H3	0.01	102.99	0.01	101.67
23	O1	0.04	102.33	0.20	102.38
216	O11	0.03	102.66	0.03	103.23
210	H5	192.92	100.00	616.59	100.00
219	G4	0.01	102.11	0.00	103.30
56	G2	0.01	102.71	0.01	105.20
88	D11	0.01	101.74	0.01	103.65
220	D20	0.05	102.90	0.61	101.56
190	D17	0.07	100.60	0.36	101.34
191	D18	1.25	100.27	138.39	112.09
192	D19	0.21	101.61	59.80	100.26
18	D7	0.74	96.39	1.19	100.41
221	D21	0.01	103.06	0.02	104.44
142	D12	0.03	100.86	0.28	100.84
222	D22	0.02	101.89	0.05	104.20
174	D13	3.09	97.55	14.95	99.27

223	D23	1.23	104.40	0.22	104.54
175	D14	22.29	101.68	101.95	106.50
42	D8	0.07	101.02	0.07	102.56
176	D15	21.66	99.44	57.67	104.43
43	D9	0.01	102.05	0.03	101.45
177	D16	12.10	101.41	98.25	117.39
194	J1	0.15	102.19	432.03	100.00
196	J2	0.22	100.80	181.61	98.22
99	I2	0.49	107.77	7.59	104.79
100	I3	1.89	106.79	25.37	103.05
101	I4	38.25	100.23	174.89	97.25
102	I6	12.99	106.71	45.52	103.83
103	I7	3.01	105.24	34.35	96.96
105	I8	6.95	106.61	53.13	97.06
106	I9	6.44	104.37	54.26	97.19
107	I10	18.84	102.22	448.00	102.49
108	I11	7.33	104.83	102.30	100.00
109	I12	5.29	105.71	104.72	100.87
110	I13	3.77	108.98	4.55	101.78
111	I14	12.92	102.68	195.24	100.00
112	I15	7.76	103.52	40.93	100.20
113	I16	261.34	116.55	1683.41	100.00
114	I17	7.00	99.16	57.31	95.79
115	I18	11.88	97.12	267.75	100.00
116	I19	2.42	102.93	82.73	100.46
117	I20	3.31	100.39	89.44	100.92
118	I21	2.08	102.23	27.61	101.25
119	I22	2.31	102.04	45.73	103.66
120	I23	2.75	102.03	43.46	97.63
121	I24	8.31	100.84	89.00	98.47
122	I25	20.77	103.20	128.18	96.70
123	I26	33.74	100.69	155.75	108.18

124	I27	375.94	98.62	133.53	97.82
125	I28	28.49	103.09	112.65	93.67
126	I29	31.25	101.18	214.61	92.30
127	I30	29.79	101.53	245.07	100.00
128	I31	70.55	99.37	473.61	100.00
143	G3	9.20	103.43	59.94	99.02
140	K1	0.18	101.72	43.59	96.63
224	K3	0.02	101.80	8.41	94.67
141	K2	0.02	102.85	15.82	99.77
225	K4	0.03	103.31	21.17	95.88
92	E6	7.09	102.64	1.16	100.06
95	E8	0.23	102.10	4.50	101.15
96	E9	0.81	103.92	7.72	102.29
211	M24	0.02	102.24	0.01	102.56
212	M25	0.01	101.40	0.01	103.56
213	M26	2.57	102.60	0.87	104.42
214	M27	1.42	103.74	0.76	106.55
215	M28	1.30	100.89	0.21	103.00
68	M5	1.99	101.09	0.58	104.66
69	M6	3.54	102.03	1.57	105.10
70	M7	5.17	99.39	1.39	106.28
71	M8	9.12	108.52	3.86	106.88
20	M2	0.02	100.36	0.01	105.13
21	M3	1.63	100.37	0.06	102.60
72	M9	0.49	100.42	0.35	102.49
73	M10	0.01	100.40	0.01	103.78
75	M11	0.10	101.31	0.27	100.96
22	M4	0.01	102.11	0.01	103.06
76	M12	0.01	101.76	0.01	103.31
77	M13	0.51	101.11	0.35	101.99
78	M14	1.10	102.96	0.57	102.59
79	M15	0.45	98.69	0.30	101.03

80	M16	0.67	102.62	0.15	103.78
81	M17	0.89	103.71	0.24	104.77
82	M18	2.38	102.82	0.93	104.96
83	M19	2.01	101.33	0.92	103.80
84	M20	3.44	102.73	2.82	104.14
227	M30	0.00	102.51	0.06	101.66
231	M31	0.14	101.37	1.82	103.21
93	M21	4.67	102.91	31.64	101.43
226	M29	0.01	101.98	0.08	98.77
97	M22	33.22	102.68	140.72	100.00
98	M23	52.92	99.79	191.20	88.87
85	L15	16.02	99.35	11.87	103.59
86	L16	0.01	101.17	0.10	102.35
24	L4	0.01	100.46	0.09	100.92
25	L5	0.01	99.29	0.16	102.51
87	L17	0.02	101.04	0.55	101.75
26	L6	0.02	100.53	0.21	101.97
27	L7	0.03	99.65	0.83	100.86
28	L8	0.01	100.79	0.08	103.93
29	L9	0.01	101.48	0.01	102.79
30	L10	0.00	102.02	0.02	103.42
32	L11	0.01	101.98	0.02	102.59
33	L12	0.01	102.27	0.18	103.84
34	L13	0.01	101.88	0.08	102.90
228	L21	0.01	102.66	0.19	102.49
89	L18	0.03	102.53	0.54	104.16
229	L22	0.02	102.65	0.36	103.05
35	L14	0.02	102.99	0.71	102.84
90	L19	0.10	100.98	0.12	100.74
91	L20	5.49	102.34	7.05	102.33
217	O2	0.02	101.30	0.01	102.83
218	O7	0.02	101.68	0.01	103.33

104	F22	0.01	101.56	0.02	100.77
36	F2	0.01	103.32	0.02	103.36
37	F3	0.03	100.32	0.02	101.72
129	F23	0.02	101.85	0.05	103.15
138	F32	0.21	102.22	0.09	103.09
146	F34	0.01	101.90	0.02	102.23
157	F35	0.01	102.00	0.02	102.58
166	F36	0.01	100.58	0.01	103.14
41	F6	0.00	101.30	0.01	102.71
44	F7	0.00	99.14	0.00	101.77
45	F8	0.00	103.89	0.01	103.95
178	F37	0.01	101.14	0.02	103.59
179	F38	0.01	101.68	0.02	102.08
180	F39	0.01	101.41	0.01	102.09
46	F9	0.00	104.98	0.02	102.83
47	F10	0.00	100.24	0.00	102.98
48	F11	0.00	104.81	0.00	104.64
49	F12	0.00	104.01	0.01	103.56
50	F13	0.00	105.00	0.02	102.89
182	F41	0.01	104.63	0.03	102.10
183	F42	0.18	101.47	0.53	103.92
51	F14	0.03	102.57	0.10	103.54
184	F43	0.02	101.52	0.07	102.03
185	F44	0.11	101.63	0.25	103.21
52	F15	0.02	102.56	0.09	105.07
186	F45	0.02	102.13	0.04	101.26
54	F16	0.01	102.79	0.01	104.27
197	F46	0.01	101.21	0.03	100.56
198	F47	0.05	101.27	0.06	102.47
199	F48	0.19	101.72	0.57	102.52
200	F49	0.20	101.68	0.11	101.46
57	F17	0.02	99.49	0.04	101.35

201	F50	0.09	100.11	0.18	101.59
58	F18	0.03	102.27	0.10	103.58
203	F51	0.57	102.73	0.29	102.14
59	F19	0.02	102.73	0.03	103.56
60	F20	0.02	103.30	0.04	104.04
204	F52	0.16	103.63	0.44	103.48
61	F21	0.02	101.17	0.03	103.18
205	F53	0.02	102.56	0.03	102.32
206	F54	0.30	102.38	0.26	101.84
207	F55	0.03	101.65	0.03	101.39
208	F56	0.06	101.66	0.47	102.62
209	F57	0.61	100.61	9.35	97.69
130	F24	0.06	101.31	0.23	101.00
131	F25	0.11	101.57	0.52	102.47
132	F26	0.23	102.09	0.93	100.69
133	F27	9.59	101.13	73.51	101.01
134	F28	0.71	101.00	0.56	100.86
135	F29	0.55	100.49	0.53	101.84
136	F30	0.07	101.38	0.45	101.42
38	F4	0.02	101.95	0.13	102.11
137	F31	0.06	100.71	0.28	101.29
139	F33	0.54	101.82	0.56	102.19
53	A1	0.02	101.31	0.03	100.71
188	A3	0.04	99.81	0.07	101.97
189	A4	0.21	99.28	0.27	102.77
230	J3	0.15	101.67	2607.20	100.00
39	C28	0.42	103.32	0.36	104.49
160	C29	1.52	102.32	4.64	106.94
161	C30	0.49	105.33	0.29	106.44
162	C31	2.31	103.88	1.90	104.00
163	C32	5.59	103.83	10.83	100.26
164	C33	3.56	102.53	3.14	103.35

165	C34	2.78	102.61	11.45	106.28
40	A2	0.02	104.14	0.04	104.55
167	C21	2.53	102.24	3.80	101.63
168	C22	1.82	102.37	3.55	103.87
169	C23	1.08	102.81	1.01	104.67
170	C24	2.49	102.55	2.11	103.82
171	C25	4.54	104.26	6.12	104.94
172	C26	9.98	101.60	8.97	101.26
173	C27	4.98	98.90	14.58	102.18
65	E7	0.75	102.34	1.60	99.32
63	D10	13.48	99.07	7.55	104.69
19	M1	0.03	99.13	0.04	101.84
64	I1	4.25	100.95	33.33	99.53

Agonist activity of melanocortin analogs on melanocortin 3 and 4 receptors

[0318] Administration of some non-naturally occurring melanocortin analogs activated melanocortin 3 receptor (MC3R) activity and/or melanocortin 4 receptor (MC4R) activity, as measured by cAMP levels (Table 17).

Table 17. Dose-response results of melanocortin analogs and control against the melanocortin 3 receptor (MC3R) and melanocortin 4 receptor (MC4R)

SEQ ID NO	Compound ID	MC3R		MC4R	
		EC50 (nM))	Max Activation (Emax)	EC50 (nM))	Max Activation (Emax)
	NDP-a-MSH	0.17	106.05	0.07	103.19
31	A3	172.84	32.44	0.61	93.64
66	C1	— n.c. —		3.91	97.00
94	C2	— n.c. —		18.32	94.68
156	C15	— n.c. —		8.74	87.32
181	F40	— n.c. —		2.56	95.73
187	C18	— n.c. —		5.70	96.49
193	C19	— n.c. —		19.46	96.37

195	C20	— n.c. —		24.77	97.54
144	C3	16.63	37.79	0.06	104.36
145	C4	— n.c. —		0.82	106.76
147	C6	7.49	29.97	0.09	102.19
148	C7	— n.c. —		0.51	104.38
149	C8	42.74	28.39	1.23	101.39
150	C9	— n.c. —		0.53	102.59
151	C10	1.86	21.79	0.15	102.38
152	C11	— n.c. —		0.59	103.37
153	C12	26.00	40.68	0.82	104.73
154	C13	— n.c. —		1.91	102.89
155	C14	— n.c. —		0.91	99.31
158	C16	96.87	33.38	0.63	105.62
159	C17	11.92	20.65	0.74	103.53
202	H4	— n.c. —		2.78	86.97
62	H1	1.05	42.70	0.01	102.78
67	H2	— n.c. —		110.62	95.13
74	H3	— n.c. —		0.11	99.71
23	O1	5.20	23.80	1.71	91.87
216	O11	881.487	23	0.670	93
210	H5	— n.c. —		519.38	100.00
219	G4	0.21	61.73	0.03	97.55
56	G2	0.67	70.46	0.02	103.16
88	D11	0.43	47.58	0.03	102.99
220	D20	— n.c. —		2.93	94.23
190	D17	9.60	71.26	3.04	86.02
191	D18	— n.c. —		416.34	100.00
192	D19	— n.c. —		304.07	100.00
18	D7	— n.c. —		333.53	100.00
221	D21	0.45	73.31	0.02	102.80
142	D12	— n.c. —		1.35	95.82
222	D22	4.09	61.18	0.12	97.46

174	D13	— n.c. —		24.33	121.06
223	D23	4.40	62.29	0.15	92.85
175	D14	— n.c. —		14.70	103.29
42	D8	4.50	48.85	0.24	91.71
176	D15	— n.c. —		54.42	91.73
43	D9	1.08	47.46	0.10	91.80
177	D16	— n.c. —		20.73	95.26
194	J1	— n.c. —		7.75	100.02
196	J2	— n.c. —		10.03	100.75
99	I2	— n.c. —		3.41	99.98
100	I3	— n.c. —		26.80	99.01
101	I4	— n.c. —		589.92	100.00
102	I6	— n.c. —		1.61	100.03
103	I7	— n.c. —		44.78	96.39
105	I8	— n.c. —		33.95	100.01
106	I9	— n.c. —		12.98	98.15
107	I10	— n.c. —		39.16	98.06
108	I11	— n.c. —		28.00	102.28
109	I12	— n.c. —		26.27	99.71
110	I13	— n.c. —		54.94	101.59
111	I14	— n.c. —		16.10	94.34
112	I15	— n.c. —		39.71	85.73
113	I16	— n.c. —		461.80	96.18
114	I17	— n.c. —		125.57	87.87
115	I18	— n.c. —		42.06	98.58
116	I19	— n.c. —		4.63	100.71
117	I20	— n.c. —		4.13	99.32
118	I15	— n.c. —		1.91	99.42
119	I22	— n.c. —		2.82	100.91
120	I23	— n.c. —		9.59	98.38
121	I24	— n.c. —		60.58	100.79
122	I25	— n.c. —		45.98	99.76

123	I26	— n.c. —		326.11	87.61
124	I27	— n.c. —		475.33	100.00
125	I28	— n.c. —		123.60	97.41
126	I29	— n.c. —		169.49	100.00
127	I30	— n.c. —		488.54	100.00
128	I31	— n.c. —		163.45	100.00
143	G3	— n.c. —		853.10	100.00
140	K1	20.90	66.90	0.87	104.37
224	K3	3.31	67.45	0.03	103.26
141	K2	4.18	69.29	0.24	103.06
225	K4	4.42	62.02	0.27	100.60
92	E6	— n.c. —		19.69	92.69
95	E8	— n.c. —		7.39	86.22
96	E9	— n.c. —		133.74	89.11
211	M24	— n.c. —		0.05	100.96
212	M25	— n.c. —		0.17	99.46
213	M26	3.39	22.11	0.10	98.22
214	M27	— n.c. —		0.13	99.98
215	M28	— n.c. —		0.10	103.20
68	M5	7.26	26.63	0.19	101.79
69	M6	21.88	23.10	0.12	101.62
70	M7	— n.c. —		0.17	102.32
71	M8	9.25	21.62	0.40	102.39
20	M2	0.78	41.37	0.10	99.05
21	M3	2.34	41.54	0.19	96.76
72	M9	— n.c. —		7.26	88.81
73	M10	2.02	22.14	0.53	95.74
75	M11	— n.c. —		0.90	93.00
22	M4	3.76	30.97	0.12	97.24
76	M12	0.40	38.57	0.08	98.23
77	M13	— n.c. —		1.17	95.47
78	M14	8.23	22.17	0.14	96.36

79	M15	— n.c. —		0.07	102.74
80	M16	— n.c. —		0.10	102.49
81	M17	63.31	31.98	0.18	104.46
82	M18	— n.c. —		0.15	101.76
83	M19	— n.c. —		0.43	102.66
84	M20	— n.c. —		0.22	103.19
227	M30	1.72	63.73	0.09	99.98
231	M31	344.26	100.00	1.69	100.23
93	M21	— n.c. —		134.09	89.34
226	M29	1.66	48.65	0.28	96.77
97	M22	— n.c. —		85.13	99.23
98	M23	— n.c. —		984.11	100.00
85	L15	— n.c. —		192.88	100.00
86	L16	— n.c. —		0.21	92.87
24	L4	0.21	34.74	0.17	91.87
25	L5	0.71	42.05	0.28	93.53
87	L17	— n.c. —		0.42	95.20
26	L6	3.73	41.53	0.25	97.28
27	L7	10.69	32.44	0.44	97.19
28	L8	0.58	42.87	0.99	85.36
29	L9	0.24	42.08	0.29	87.89
30	L10	0.23	57.56	0.12	89.99
32	L11	1.30	52.48	0.35	86.04
33	L12	2.07	31.98	0.02	99.25
34	L13	0.17	59.05	0.02	98.79
228	L21	0.18	69.45	0.01	99.43
89	L18	0.13	24.79	0.03	99.42
229	L22	7.87	63.93	0.02	101.79
35	L14	1.90	52.37	0.02	102.43
90	L19	— n.c. —		4.78	90.30
91	L20	— n.c. —		272.76	100.00
217	O2	0.11	48.42	0.04	96.69

218	O7	130.896	25	0.351	91
104	F22	— n.c. —		0.87	93.70
36	F2	0.21	32.39	0.09	96.76
37	F3	8.85	57.37	0.09	98.50
129	F23	— n.c. —		1.78	93.61
138	F32	— n.c. —		1.06	95.09
146	F34	— n.c. —		0.69	93.12
157	F35	— n.c. —		0.27	96.29
166	F36	— n.c. —		0.29	91.59
41	F6	2.41	23.33	0.31	93.95
44	F7	4.36	22.74	0.30	95.22
45	F8	3.34	21.86	0.29	94.58
178	F37	— n.c. —		0.29	91.69
179	F38	— n.c. —		0.38	91.57
180	F39	— n.c. —		0.97	91.88
46	F9	7.57	51.84	0.03	100.20
47	F10	3.14	46.33	0.02	101.37
48	F11	3.14	33.14	0.03	99.75
49	F12	1.15	47.06	0.03	102.71
50	F13	4.71	35.17	0.08	98.38
182	F41	23.57	36.84	0.23	100.20
183	F42	4.86	28.27	0.42	100.45
51	F14	1.35	43.14	0.05	100.47
184	F43	— n.c. —		0.66	96.62
185	F44	— n.c. —		0.93	101.29
52	F15	3.65	45.43	0.06	101.01
186	F45	— n.c. —		1.20	90.48
54	F16	0.85	45.60	0.02	100.93
197	F46	— n.c. —		0.55	94.26
198	F47	— n.c. —		0.41	95.61
199	F48	— n.c. —		7.13	88.67
200	F49	— n.c. —		0.81	94.70

57	F17	8.99	22.06	2.73	93.06
201	F50	— n.c. —		5.07	91.77
58	F18	1.35	21.10	0.80	94.30
203	F51	— n.c. —		1.05	95.26
59	F19	4.20	21.20	0.76	92.22
60	F20	2.82	30.43	0.21	96.81
204	F52	— n.c. —		3.61	94.92
61	F21	2.63	20.84	0.36	94.81
205	F53	— n.c. —		0.52	91.80
206	F54	— n.c. —		3.19	93.90
207	F55	— n.c. —		0.72	94.19
208	F56	— n.c. —		1.35	94.18
209	F57	— n.c. —		8.66	93.55
130	F24	— n.c. —		1.49	90.06
131	F25	— n.c. —		7.08	85.34
132	F26	— n.c. —		1.89	95.42
133	F27	— n.c. —		37.89	100.84
134	F28	— n.c. —		1.92	95.63
135	F29	— n.c. —		1.25	94.65
136	F30	— n.c. —		7.92	86.80
38	F4	5.12	23.46	0.74	94.20
137	F31	— n.c. —		2.00	93.74
139	F33	— n.c. —		2.04	97.01
53	A1	3.39	20.71	1.11	92.35
188	A3	— n.c. —		2.15	92.02
189	A4	— n.c. —		9.63	93.74
230	J3	40.85	95.01	0.73	99.37
39	F5	21.28	31.71	0.01	104.66
160	C29	610.89	25.90	0.27	101.93
161	C30	10.88	45.59	0.09	102.44
162	C31	— n.c. —		0.09	99.76
163	C32	— n.c. —		31.74	99.40

164	C33	— n.c. —		3.20	102.76
165	C34	— n.c. —		0.27	102.96
40	A2	6.30	36.37	0.27	97.54
167	C21	— n.c. —		0.42	94.40
168	C22	— n.c. —		0.36	97.33
169	C23	— n.c. —		0.37	100.93
170	C24	24.13	31.62	0.74	100.93
171	C25	— n.c. —		0.84	101.48
172	C26	— n.c. —		1.96	101.47
173	C27	— n.c. —		1.57	100.85
65	E7	— n.c. —		31.25	85.85
63	D10	— n.c. —		29.60	96.67
19	M1	0.89	27.74	0.26	92.13
64	I1	— n.c. —		7.38	98.82

n.c. = not calculated

Antagonist activity of melanocortin analogs on melanocortin 3 or 4 receptor

[0319] Administration of some non-naturally occurring melanocortin analogs inhibited melanocortin 3 receptor (MC3R) and/or melanocortin 4 receptor (MC4R) activity, as measured by cAMP levels (Table 18).

Table 18. Dose-response results of melanocortin analogs and control against the melanocortin 3 receptor (MC3R) and melanocortin 4 receptor (MC4R)

SEQ ID NO	Compound ID	MC3R		MC4R	
		EC50 (nM))	Max Activation (Emax)	EC50 (nM))	Max Activation (Emax)
	SHU9119	2.34	97.24	2.56	98.58
31	A3	19.75	72.59	6549.22	0.86
66	C1	17.50	79.59	— n.c. —	
94	C2	572.62	100.00	— n.c. —	
156	C15	309.36	81.42	— n.c. —	
181	F40	49.90	82.70	— n.c. —	

187	C18	217.69	83.76	— n.c. —	
193	C19	97.56	95.98	— n.c. —	
195	C20	374.81	100.92	— n.c. —	
144	C3	76.22	98.38	— n.c. —	
145	C4	319.98	100.00	— n.c. —	
147	C6	40.28	95.51	— n.c. —	
148	C7	94.54	103.47	— n.c. —	
149	C8	415.54	100.00	— n.c. —	
150	C9	80.26	103.58	— n.c. —	
151	C10	51.05	88.38	— n.c. —	
152	C11	393.75	100.00	— n.c. —	
153	C12	424.34	99.76	— n.c. —	
154	C13	654.90	100.00	— n.c. —	
155	C14	885.21	100.00	— n.c. —	
158	C16	946.89	100.00	— n.c. —	
159	C17	643.22	100.00	— n.c. —	
202	H4	124.99	84.39	— n.c. —	
62	H1	43.72	71.22	— n.c. —	
67	H2	— n.c. —		— n.c. —	
74	H3	198.95	100.00	— n.c. —	
23	O1	35.74	66.65	— n.c. —	
216	O11	5.981	76	— n.c. —	
210	H5	— n.c. —		— n.c. —	
219	G4	3.27	62.01	— n.c. —	
56	G2	9.33	48.53	— n.c. —	
88	D11	21.69	71.10	— n.c. —	
220	D20	54.30	76.00	— n.c. —	
190	D17	7.15	20.34	— n.c. —	
191	D18	— n.c. —		— n.c. —	
192	D19	— n.c. —		— n.c. —	
18	D7	180.86	97.17	— n.c. —	
221	D21	12.43	36.47	— n.c. —	

142	D12	52.21	69.24	— n.c. —	
222	D22	118.54	55.89	— n.c. —	
174	D13	— n.c. —		— n.c. —	
223	D23	49.24	58.38	— n.c. —	
175	D14	— n.c. —		— n.c. —	
42	D8	48.42	61.46	— n.c. —	
176	D15	— n.c. —		— n.c. —	
43	D9	35.86	66.47	— n.c. —	
177	D16	— n.c. —		— n.c. —	
194	J1	— n.c. —		— n.c. —	
196	J2	— n.c. —		— n.c. —	
99	I2	339.19	100.00	— n.c. —	
100	I3	920.15	100.00	— n.c. —	
101	I4	— n.c. —		— n.c. —	
102	I6	— n.c. —		— n.c. —	
103	I7	715.98	100.00	— n.c. —	
105	I8	— n.c. —		— n.c. —	
106	I9	699.91	100.00	— n.c. —	
107	I10	— n.c. —		— n.c. —	
108	I11	— n.c. —		— n.c. —	
109	I12	— n.c. —		— n.c. —	
110	I13	— n.c. —		— n.c. —	
111	I14	— n.c. —		— n.c. —	
112	I15	741.56	89.60	— n.c. —	
113	I16	— n.c. —		— n.c. —	
114	I17	726.01	100.00	— n.c. —	
115	I18	— n.c. —		— n.c. —	
116	I19	— n.c. —		— n.c. —	
117	I20	— n.c. —		— n.c. —	
118	I21	740.95	100.00	— n.c. —	
119	I22	— n.c. —		— n.c. —	
120	I23	— n.c. —		— n.c. —	

121	I24	— n.c. —		— n.c. —	
122	I25	— n.c. —		— n.c. —	
123	I26	— n.c. —		— n.c. —	
124	I27	— n.c. —		— n.c. —	
125	I28	— n.c. —		— n.c. —	
126	I29	— n.c. —		— n.c. —	
127	I30	— n.c. —		— n.c. —	
128	I31	— n.c. —		— n.c. —	
143	G3	— n.c. —		— n.c. —	
140	K1	969.05	100.00	— n.c. —	
224	K3	79.55	62.22	— n.c. —	
141	K2	788.30	100.00	— n.c. —	
225	K4	63.31	42.51	— n.c. —	
92	E6	659.23	100.00	— n.c. —	
95	E8	294.96	100.00	— n.c. —	
96	E9	254.32	100.00	— n.c. —	
211	M24	9.49	63.87	— n.c. —	
212	M25	56.71	73.38	— n.c. —	
213	M26	63.41	91.45	— n.c. —	
214	M27	27.50	93.24	— n.c. —	
215	M28	34.48	104.31	— n.c. —	
68	M5	165.88	88.68	— n.c. —	
69	M6	118.17	92.99	— n.c. —	
70	M7	233.94	105.85	— n.c. —	
71	M8	153.54	93.16	— n.c. —	
20	M2	15.10	81.16	— n.c. —	
21	M3	82.66	66.65	— n.c. —	
72	M9	110.73	80.80	— n.c. —	
73	M10	10.95	86.81	— n.c. —	
75	M11	563.18	110.16	— n.c. —	
22	M4	14.53	84.02	— n.c. —	
76	M12	37.54	90.33	— n.c. —	

77	M13	532.29	100.00	— n.c. —	
78	M14	97.74	95.08	— n.c. —	
79	M15	124.42	111.31	— n.c. —	
80	M16	57.75	93.42	— n.c. —	
81	M17	210.23	85.29	— n.c. —	
82	M18	320.59	102.66	— n.c. —	
83	M19	296.08	110.19	— n.c. —	
84	M20	364.88	112.56	— n.c. —	
227	M30	7.81	29.23	— n.c. —	
231	M31	— n.c. —		— n.c. —	
93	M21	— n.c. —		— n.c. —	
226	M29	2.60	36.45	— n.c. —	
97	M22	— n.c. —		— n.c. —	
98	M23	— n.c. —		— n.c. —	
85	L15	— n.c. —		— n.c. —	
86	L16	4.88	87.66	— n.c. —	
24	L4	1.58	61.19	— n.c. —	
25	L5	2.85	48.99	— n.c. —	
87	L17	13.18	77.70	— n.c. —	
26	L6	9.18	57.04	— n.c. —	
27	L7	25.31	69.15	— n.c. —	
28	L8	14.98	63.55	— n.c. —	
29	L9	1.21	56.49	— n.c. —	
30	L10	4.80	61.69	— n.c. —	
32	L11	9.76	77.05	13.01	25.98
33	L12	9.90	77.76	— n.c. —	
34	L13	2.31	65.28	— n.c. —	
228	L21	3.59	54.84	— n.c. —	
89	L18	30.90	89.18	— n.c. —	
229	L22	13.81	58.67	— n.c. —	
35	L14	23.87	62.98	— n.c. —	
90	L19	110.01	87.53	— n.c. —	

91	L20	— n.c. —		— n.c. —	
217	O2	9.60	63.66	— n.c. —	
218	O7	2.581	76	— n.c. —	
104	F22	141.73	100.00	— n.c. —	
36	F2	44.81	84.83	— n.c. —	
37	F3	46.51	72.89	— n.c. —	
129	F23	213.43	100.00	— n.c. —	
138	F32	876.72	100.00	— n.c. —	
146	F34	42.67	83.72	— n.c. —	
157	F35	31.59	79.24	— n.c. —	
166	F36	11.29	68.56	— n.c. —	
41	F6	9.10	70.59	— n.c. —	
44	F7	17.29	74.16	— n.c. —	
45	F8	3.81	75.84	— n.c. —	
178	F37	17.60	84.38	— n.c. —	
179	F38	11.00	84.98	— n.c. —	
180	F39	12.75	77.21	— n.c. —	
46	F9	45.77	73.07	— n.c. —	
47	F10	7.99	75.03	— n.c. —	
48	F11	20.77	81.03	— n.c. —	
49	F12	55.62	83.59	— n.c. —	
50	F13	48.28	82.75	— n.c. —	
182	F41	100.60	85.83	— n.c. —	
183	F42	455.16	100.00	— n.c. —	
51	F14	85.30	77.24	— n.c. —	
184	F43	26.62	79.66	— n.c. —	
185	F44	— n.c. —		— n.c. —	
52	F15	103.98	81.82	— n.c. —	
186	F45	14.35	78.39	— n.c. —	
54	F16	18.89	62.05	— n.c. —	
197	F46	17.97	74.84	— n.c. —	
198	F47	37.75	73.58	— n.c. —	

199	F48	145.75	100.00	— n.c. —	
200	F49	119.87	74.83	— n.c. —	
57	F17	29.71	67.41	— n.c. —	
201	F50	74.46	70.45	— n.c. —	
58	F18	26.34	71.47	— n.c. —	
203	F51	169.13	67.13	— n.c. —	
59	F19	14.74	77.00	— n.c. —	
60	F20	16.55	67.68	— n.c. —	
204	F52	105.45	71.83	— n.c. —	
61	F21	35.71	75.57	— n.c. —	
205	F53	15.53	71.70	— n.c. —	
206	F54	115.32	80.11	— n.c. —	
207	F55	31.71	73.19	— n.c. —	
208	F56	28.92	75.21	— n.c. —	
209	F57	847.61	100.00	— n.c. —	
130	F24	102.81	100.00	— n.c. —	
131	F25	381.33	100.00	— n.c. —	
132	F26	994.99	100.00	— n.c. —	
133	F27	— n.c. —		— n.c. —	
134	F28	— n.c. —		— n.c. —	
135	F29	623.30	100.00	— n.c. —	
136	F30	254.18	100.00	— n.c. —	
38	F4	26.27	80.25	— n.c. —	
137	F31	95.80	86.26	— n.c. —	
139	F33	964.23	100.00	— n.c. —	
53	A1	17.55	81.85	— n.c. —	
188	A3	42.62	88.49	— n.c. —	
189	A4	96.42	79.92	— n.c. —	
230	J3	— n.c. —		— n.c. —	
39	F5	54.74	79.91	— n.c. —	
160	C29	3055.93	100.00	— n.c. —	
161	C30	605.48	100.00	— n.c. —	

162	C31	686.80	100.00	— n.c. —	
163	C32	560.69	100.00	— n.c. —	
164	C33	— n.c. —		— n.c. —	
165	C34	— n.c. —		— n.c. —	
40	A2	86.13	72.08	— n.c. —	
167	C21	141.38	66.80	— n.c. —	
168	C22	122.01	72.86	— n.c. —	
169	C23	224.95	101.22	— n.c. —	
170	C24	845.19	100.00	— n.c. —	
171	C25	838.02	100.00	— n.c. —	
172	C26	— n.c. —		— n.c. —	
173	C27	— n.c. —		— n.c. —	
65	E7	163.42	79.43	— n.c. —	
63	D10	— n.c. —		— n.c. —	
19	M1	5.18	56.65	— n.c. —	
64	I1	— n.c. —		— n.c. —	

n.c. = not calculated

Comparison of agonist and antagonist activity of O7 and O11 to similar melanocortin analogs

[0320] To determine the impact that certain features of the melanocortin analogs of the present technology had on agonist activity at the different melanocortin receptors, the agonist and antagonist activity of O7 and O11 was compared to the agonist and antagonist activity of similar sequences. Specifically, O7 and O11 were compared to sequences with different lactam cyclization types, different residues at the R^4 and R^3 positions, and different C-terminal residues. The agonist and antagonist activity of O7 and comparative sequences is provided in Table 19 and Table 20 and the agonist and antagonist activity of O11 and comparative sequences is provided in Table 21 and 22.

Table 19. Agonist activity of O7 and melanocortin analogs with point mutations relative to O7

Feature	Sequence	SEQ ID NO	MC3R		MC4R	
			EC50 (nM)	Max Activation	EC50 (nM)	Max Activation
O7	Ac-Nle-[Glu-Pro-p(F)dPhe-Arg-Trp-Orn]-dVal-dPro-NH2	218	130.896	25	0.351	91
Linkage	Ac-Nle-c[Orn-Pro-p(F)dPhe-Arg-Trp-Glu]-dVal-dPro-NH2	360	0.214	29	0.138	90
	Ac-Nle-c[Lys-Pro-p(F)dPhe-Arg-Trp-Asp]-dVal-dPro-NH2	361	0.242	41	0.027	100
	Ac-Nle-c[Asp-Pro-p(F)dPhe-Arg-Trp-Orn]-dVal-dPro-NH2	217	0.110	48	0.041	97
	Ac-Nle-c[Glu-Pro-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH2	362	1.139	49	0.060	98
R^4	Ac-Nle-c[Glu-Pro-p(Cl)dPhe-Arg-Trp-Orn]-dVal-dPro-NH2	278	n.c.	-	0.298	51
	Ac-Nle-c[Asp-Pro-p(Cl)dPhe-Arg-Trp-Lys]-dVal-dPro-NH2	276	n.c.	-	39.427	24
	Ac-Nle-c[Asp-Pro-p(Br)dPhe-Arg-Trp-Lys]-dVal-dPro-NH2	249	n.c.	-	n.c.	-
	Ac-Nle-c[Asp-Pro-p(I)dPhe-Arg-Trp-Lys]-dVal-dPro-NH2	364	n.c.	-	n.c.	-
R^3	Ac-Nle-c[Glu-His-p(F)dPhe-Arg-Trp-Orn]-dVal-dPro-NH2	326	0.058	97	0.032	101
	Ac-Nle-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH2	327	0.106	94	0.047	102
C-terminus	Ac-Nle-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH2	22	3.763	31	0.119	97

Table 20. Antagonist activity of O7 and melanocortin analogs with point mutations relative to O7

Feature	Sequence	SEQ ID NO	MC3R		MC4R	
			EC50 (nM)	Max Activation	EC50 (nM)	Max Activation
O7	Ac-Nle-[Glu-Pro-p(F)dPhe-Arg-Trp-Orn]-dVal-dPro-NH2	218	2.581	76	n.c.	-

Linkage	Ac-Nle-c[Orn-Pro-p(F)dPhe-Arg-Trp-Glu]-dVal-dPro-NH2	360	4.561	79	n.c.	-
	Ac-Nle-c[Lys-Pro-p(F)dPhe-Arg-Trp-Asp]-dVal-dPro-NH2	361	3.534	62	n.c.	-
	Ac-Nle-c[Asp-Pro-p(F)dPhe-Arg-Trp-Orn]-dVal-dPro-NH2	217	9.603	64	n.c.	-
	Ac-Nle-c[Glu-Pro-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH2	362	10.257	63	n.c.	-
R^4	Ac-Nle-c[Glu-Pro-p(Cl)dPhe-Arg-Trp-Orn]-dVal-dPro-NH2	278	1.244	92	2.686	50
	Ac-Nle-c[Asp-Pro-p(Cl)dPhe-Arg-Trp-Lys]-dVal-dPro-NH2	276	1.980	90	4.591	61
	Ac-Nle-c[Asp-Pro-p(Br)dPhe-Arg-Trp-Lys]-dVal-dPro-NH2	249	0.499	97	1.662	91
	Ac-Nle-c[Asp-Pro-p(I)dPhe-Arg-Trp-Lys]-dVal-dPro-NH2	364	1.697	103	3.274	101
R^3	Ac-Nle-c[Glu-His-p(F)dPhe-Arg-Trp-Orn]-dVal-dPro-NH2	326	n.c.	4	n.c.	-
	Ac-Nle-c[Asp-His-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH2	327	n.c.	-	n.c.	-
C-terminus	Ac-Nle-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dTle-dPro-NH2	22	14.528	84	n.c.	-

Table 21. Agonist activity of O11 and melanocortin analogs with point mutations relative to O11

Feature	Sequence	SEQ ID NO	MC3R		MC4R	
			EC50 (nM)	Max Activation	EC50 (nM)	Max Activation
O11	Ac-Nle-c[Glu-Pro-dPhe-Arg-Trp-Orn]-dVal-dPro-NH2	216	881.487	23	0.670	93
Linkage	Ac-Nle-c[Orn-Pro-dPhe-Arg-Trp-Glu]-dVal-dPro-NH2	365	n.c.	-	0.409	89
	Ac-Nle-c[Lys-Pro-dPhe-Arg-Trp-Asp]-dVal-dPro-NH2	366	0.583	47	0.063	101
	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Orn]-dVal-dPro-NH2	23	5.204	24	1.714	92

	Ac-Nle-c[Glu-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH2	367	3.941	42	0.235	98
	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH2	31	n.c.	-	2.963	89
	Ac-Nle-c[Glu-Pro-p(Cl)dPhe-Arg-Trp-Orn]-dVal-dPro-NH2	278	n.c.	-	0.298	51
	Ac-Nle-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH2	19	0.890	28	0.256	92
	Ac-Nle-c[Asp-Pro-p(Cl)dPhe-Arg-Trp-Lys]-dVal-dPro-NH2	276	n.c.	-	39.427	24
	Ac-Nle-c[Asp-Pro-p(Br)dPhe-Arg-Trp-Lys]-dVal-dPro-NH2	249	n.c.	-	n.c.	-
	Ac-Nle-c[Asp-Pro-p(I)dPhe-Arg-Trp-Lys]-dVal-dPro-NH2	364	n.c.	-	n.c.	-
R^3	Ac-Nle-c[Asp-His-dPhe-Arg-Trp-Lys]-dVal-dPro-NH2	343	0.728	92	0.675	101
	Ac-Nle-c[Glu-His-dPhe-Arg-Trp-Orn]-dVal-dPro-NH2	363	0.041	100	0.024	102
C-terminus	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dTle-dPro-NH2	50	4.706	35	0.081	98

Table 22. Antagonist activity of O11 and melanocortin analogs with point mutations relative to O11

Feature	Sequence	SEQ ID NO	MC3R		MC4R	
			EC50 (nM)	Max Activation	EC50 (nM)	Max Activation
O11	Ac-Nle-[Glu-Pro-dPhe-Arg-Trp-Orn]-dVal-dPro-NH2	216	5.981	76	n.c.	
Linkage	Ac-Nle-c[Orn-Pro-dPhe-Arg-Trp-Glu]-dVal-dPro-NH2	365	7.584	77	n.c.	
	Ac-Nle-c[Lys-Pro-dPhe-Arg-Trp-Asp]-dVal-dPro-NH2	366	4.365	56	n.c.	
	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Orn]-dVal-dPro-NH2	23	35.741	67	n.c.	
	Ac-Nle-c[Glu-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH2	367	21.377	62	n.c.	
	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH2	31	21.116	81	n.c.	

R^4	Ac-Nle-c[Glu-Pro-p(Cl)dPhe-Arg-Trp-Orn]-dVal-dPro-NH2	278	2.581	76	n.c.	
	Ac-Nle-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH2	19	1.244	92	2.686	50
	Ac-Nle-c[Asp-Pro-p(Cl)dPhe-Arg-Trp-Lys]-dVal-dPro-NH2	276	5.181	57	n.c.	
	Ac-Nle-c[Asp-Pro-p(Br)dPhe-Arg-Trp-Lys]-dVal-dPro-NH2	249	1.980	90	4.591	61
	Ac-Nle-c[Asp-Pro-p(I)dPhe-Arg-Trp-Lys]-dVal-dPro-NH2	364	0.499	97	1.662	91
	Ac-Nle-c[Asp-His-dPhe-Arg-Trp-Lys]-dVal-dPro-NH2	343	1.697	103	3.274	101
R^3	Ac-Nle-c[Glu-His-dPhe-Arg-Trp-Orn]-dVal-dPro-NH2	363	n.c.		n.c.	
	Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dTle-dPro-NH2	50	n.c.		n.c.	
C-terminus	Ac-Nle-c[Orn-Pro-dPhe-Arg-Trp-Glu]-dVal-dPro-NH2	216	48.280	83	n.c.	

[0321] As shown in Tables 19-22, all else equal, the Glu/Orn cyclization of O7 and O11 clearly impacts the agonism on MC3R, however, the cyclization does not appear to have the same impact on agonism of MC4R and antagonism of MC3R and MC4R. Even more stark is the difference in agonism/antagonism of MC3R and MC4R observed when p(F)dPhe or dPhe is substituted for a larger residue, such as p(Br)dPhe or p(I)dPhe. For example, SEQ ID NOs: 216 and 218, which have smaller p(F)dPhe and dPhe at the R^4 position, are full agonists on MC4R, whereas SEQ ID NOs: 249 and 364, which have larger p(Br)dPhe and p(I)dPhe at the R^4 position, are full antagonists on MC4R. Additionally, a substantial change in agonist activity on the MC3 receptor was observed when His was substituted for Pro at position R^3 in the melanocortin analogs. Specifically, SEQ ID NOs: 216 and 218, which have Pro at R^3, exhibited moderate binding and partial agonism on MC3R, whereas SEQ ID NOs: 326 and 363, which have His at R^3, exhibited strong binding and full agonism on MC3R.

Residual binding of melanocortin analogs on melanocortin 1 receptor

[0322] The residual binding of some of the non-naturally occurring melanocortin analogs was measured on the melanocortin 1 receptor (MC1R) by RLB assay (Table 23).

Table 23. Dose-response binding results of melanocortin analogs and control against the melanocortin 1 receptor (MC1R)

Synthetic Peptide	**Max Residual Binding %**	**EC_{50} (nM)***	**Hill Coefficient**	**Top (%)**
NDP-a-MSH	99.06	0.85	-1.23	-2.35
A3	120.40	2.22	-0.88	-2.79
M1	100.88	2.85	-1.22	0.87
O7	108.44	0.83	-0.85	0.66
O11	95.76	1.05	-0.86	0.54

Residual binding of melanocortin analogs on melanocortin 3 receptor

[0323] The residual binding of some of the non-naturally occurring melanocortin analogs was measured on the melanocortin 3 receptor (MC3R) by RLB assay (Table 24).

Table 24. Dose-response binding results of melanocortin analogs and control against the melanocortin 3 receptor (MC3R)

Synthetic Peptide	**Max Residual Binding %**	**EC_{50} (nM)***	**Hill Coefficient**	**Top (%)**
NDP-a-MSH	100.61	1.34	-0.87	-1.08
A3	107.27	10.5	-0.95	-2.61
M1	97.71	3.90	-0.96	-1.68
O7	104.76	1.66	-1.02	2.43
O11	117.97	3.88	-0.92	1.63

Residual binding of melanocortin analogs on melanocortin 4 receptor

[0324] The residual binding of some of the non-naturally occurring melanocortin analogs was measured on the melanocortin 4 receptor (MC4R) by RLB assay (Table 25).

Table 25. Dose-response binding results of melanocortin analogs and control against the melanocortin 4 receptor (MC4R)

Synthetic Peptide	**Max Residual Binding %**	**EC_{50} (nM)***	**Hill Coefficient**	**Top (%)**
NDP-a-MSH	95.89	1.07	-1.37	1.61
A3	107.75	2.21	-0.86	-0.97
M1	106.82	0.88	-1.19	4.52
O7	92.91	0.81	-1.16	2.51
O11	9950	1.50	-0.91	0.97

Residual binding of melanocortin analogs on melanocortin 5 receptor

[0325] The residual binding of some of the non-naturally occurring melanocortin analogs was measured on the melanocortin 5 receptor (MC5R) by RLB assay (Table 26).

Table 26. Dose-response binding results of melanocortin analogs and control against the melanocortin 5 receptor (MC5R)

Synthetic Peptide	**Max Residual Binding %**	**EC_{50} (nM)***	**Hill Coefficient**	**Top (%)**
NDP-a-MSH	103.88	2.07	-1.36	2.39
A3	108.55	14.9	-0.96	0.15
M1	107.08	5.09	-1.13	-0.38
O7	101.15	2.27	-1.21	0.24
O11	101.97	3.56	-1.03	0.72

Agonist activity on GPR54

[0326] The agonist activity of some of the non-naturally occurring melanocortin analogs was measured on GPR54 by Aequorin assay. Results from this assay are provided in Table 27.

Table 27. Dose-response results of melanocortin analogs and control against GPR54

Synthetic Peptide	**Max Activation %**	**EC_{50} (nM)***	**Hill Coefficient**	**Top (%)**
Kisspeptin	105.10	6.43	1.60	99.83
LY2112688 trifluoroacetate	1.75	>2000	-	-

A3	1.32	>2000	-	-
O7	0.32	>2000	-	-
O11	0.55	>2000	-	-

Antagonist activity on GPR54

[0327] The antagonist activity of some of the non-naturally occurring melanocortin analogs was measured on GPR54 by Aequorin assay. Results from this assay are provided in Table 28.

Table 28. Dose-response results of melanocortin analogs and control against GPR54

Synthetic Peptide	**Max Inhibition %**	**IC_{50} (nM)***	**Hill Coefficient**	**Top (%)**
LY2112688 trifluoroacetate	6.38	>1000	-	-
A3	18.74	>1000	-	-
O7	9.78	>1000	-	-
O11	10.68	>1000	-	-

Agonist activity on QRFP

[0328] The agonist activity of some of the non-naturally occurring melanocortin analogs was measured on QRFP by Aequorin assay. Results from this assay are provided in Table 29.

Table 29. Dose-response results of melanocortin analogs and control against QRFP

Synthetic Peptide	**Max Activation %**	**EC_{50} (nM)***	**Hill Coefficient**	**Top (%)**
P518	105.91	1.44	1.99	100.33
LY2112688 trifluoroacetate	0.80	>2000	-	-
A3	0.46	>2000	-	-
O7	2.86	>2000	-	-
O11	1.57	>2000	-	-

Antagonist activity on QRFP

[0329] The antagonist activity of some of the non-naturally occurring melanocortin analogs was measured on QRFP by Aequorin assay. Results from this assay are provided in Table 30.

Table 30. Dose-response results of melanocortin analogs and control against QRFP

Synthetic Peptide	**Max Inhibition %**	**IC_{50} (nM)***	**Hill Coefficient**	**Top (%)**
LY2112688 trifluoroacetate	25.70	>1000	-	-
A3	14.66	>1000	-	-
O7	18.92	>1000	-	-
O11	17.97	>1000	-	-

Agonist activity on PrRP

[0330] The agonist activity of some of the non-naturally occurring melanocortin analogs was measured on PrRP by Aequorin assay. Results from this assay are provided in Table 31.

Table 31. Dose-response results of melanocortin analogs and control against PrRP

Synthetic Peptide	**Max Activation %**	**EC_{50} (nM)***	**Hill Coefficient**	**Top (%)**
PrRP 31	112.19	0.33	2.11	104.34
LY2112688 trifluoroacetate	2.33	>2000	-	-
A3	1.15	>2000	-	-
O7	0.49	>2000	-	-
O11	0.79	>2000	-	-

Antagonist activity on PrRP

[0331] The antagonist activity of some of the non-naturally occurring melanocortin analogs was measured on PrRP by Aequorin assay. Results from this assay are provided in Table 32.

Table 32. Dose-response results of melanocortin analogs and control against PrRP

Synthetic Peptide	**Max Inhibition %**	**IC_{50} (nM)***	**Hill Coefficient**	**Top (%)**

LY2112688 trifluoroacetate	17.52	>1000	-	-
A3	10.26	>1000	-	-
O7	20.21	>1000	-	-
O11	5.71	>1000	-	-

Agonist activity on NPFF2S

[0332] The agonist activity of some of the non-naturally occurring melanocortin analogs was measured on NPFF2S by Aequorin assay. Results from this assay are provided in Table 33.

Table 33. Dose-response results of melanocortin analogs and control against NPFF2S

Synthetic Peptide	**Max Activation %**	**EC_{50} (nM)***	**Hill Coefficient**	**Top (%)**
hNPFF	101.15	0.033	1.45	95.41
LY2112688 trifluoroacetate	0.23	>2000	-	-
A3	1.15	>2000	-	-
O7	0.28	>2000	-	-
O11	0.20	>2000	-	-

Antagonist activity on NPFF2S

[0333] The antagonist activity of some of the non-naturally occurring melanocortin analogs was measured on NPFF2S by Aequorin assay. Results from this assay are provided in Table 34.

Table 34. Dose-response results of melanocortin analogs and control against NPFF2S

Synthetic Peptide	**Max Inhibition %**	**IC_{50} (nM)***	**Hill Coefficient**	**Top (%)**
LY2112688 trifluoroacetate	17.01	>1000	-	-
A3	6.95	>1000	-	-
O7	11.68	>1000	-	-
O11	27.09	>1000	-	-

Agonist activity on NPY1

[0334] The agonist activity of some of the non-naturally occurring melanocortin analogs was measured on NPY1 by cAMP assay. Results from this assay are provided in Table 35.

Table 35. Dose-response results of melanocortin analogs and control against NPY1

Synthetic Peptide	**Max Activation %**	**EC_{50} (nM)***	**Hill Coefficient**	**Top (%)**
NPY	99.68	9.42	1.26	101.45
A3	3.30	>1000	-	-
O7	2.67	>1000	-	-
O11	0.84	>1000	-	-

Antagonist activity on NPY1

[0335] The antagonist activity of some of the non-naturally occurring melanocortin analogs was measured on NPY1 by cAMP assay. Results from this assay are provided in Table 36.

Table 36. Dose-response results of melanocortin analogs and control against NPY1

Synthetic Peptide	**Max Inhibition %**	**IC_{50} (nM)***	**Hill Coefficient**	**Top (%)**
BVD10	98.1	22.9	0.86	100.72
A3	17.00	>1000	-	-
O7	37.45	>1000	-	-
O11	29.52	>1000	-	-

Agonist activity on NPFF1

[0336] The agonist activity of some of the non-naturally occurring melanocortin analogs was measured on NPFF1 by cAMP assay. Results from this assay are provided in Table 37.

Table 37. Dose-response results of melanocortin analogs and control against NPFF1

Synthetic Peptide	**Max Activation %**	**EC_{50} (nM)***	**Hill Coefficient**	**Top (%)**
hNPFF	91.83	108	0.81	91.52
A3	10.27	>1000	-	-
O7	9.18	>1000	-	-

O11	11.26	>1000	-	-

Antagonist activity on NPFF1

[0337] The antagonist activity of some of the non-naturally occurring melanocortin analogs was measured on NPFF1 by cAMP assay. Results from this assay are provided in Table 38.

Table 38. Dose-response results of melanocortin analogs and control against NPFF1

Synthetic Peptide	**Max Inhibition %**	**IC_{50} (nM)***	**Hill Coefficient**	**Top (%)**
A3	27.75	>1000	-	-
O7	6.90	>1000	-	-
O11	21.95	>1000	-	-

Example 5: Pharmacokinetic Assessment of Non-Naturally Occurring Melanocortin Analogs Following Oral Administration to Rats and Cynomolgus Monkeys

Study Objective

[0338] The objective of this study is to determine the pharmacokinetics of non-naturally occurring melanocortin analogs of the present technology following oral gavage administration in male rats and single oral administration to non-naïve male cynomolgus monkeys. In rats, the test article will be monitored in plasma for up to 24 hours post each dose. Study design and sample collection will be conducted as outlined in Table 39 and Table 40.

Table 39. Study Design

Animal	**N**	**Compound**	**Compound Formula**	**Target Dose Level (mg/kg)**	**Target Dose Volume (mL/kg)**	**Target Dose Concen-tration (mg/mL)**	**Vehicle**	**Dose Route**
Cynomolgus Monkey	3	B	M1	30	5	6	5%DMSO/95% saline	Oral
Cynomolgus Monkey	3	D	O7	10	5	2	physiological saline	PO
Cynomolgus Monkey	3	D	O7	30	5	6	physiological saline	PO

Rat	3	B	M1	15	10	1.5	5%DMSO/95% saline	PO
Rat	3	B	M1	30	10	3	5%DMSO/95% saline	PO
Rat	3	B	M1	60	10	6	5%DMSO/95% saline	PO
Rat	3	D	O7	1	1	1	physiological saline	SC
Rat	3	D	O7	3	1	3	physiological saline	SC
Rat	3	D	O7	10	1	10	physiological saline	SC
Rat	3	D	O7	1	1	1	physiological saline	IP
Rat	3	D	O7	3	1	3	physiological saline	IP
Rat	3	D	O7	10	1	10	physiological saline	IP
Rat	3	D	O7	10	10	1	physiological saline	PO
Rat	3	D	O7	30	10	3	physiological saline	PO
Rat	3	D	O7	60	10	6	physiological saline	PO
Rat	3	F	A3	1	1	1	physiological saline	SC
Rat	3	F	A3	3	1	3	physiological saline	SC
Rat	3	F	A3	10	1	10	physiological saline	SC
Rat	3	F	A3	1	1	1	physiological saline	IP
Rat	3	F	A3	3	1	3	physiological saline	IP
Rat	3	F	A3	10	1	10	physiological saline	IP
Rat	6	F	A3	1	1	1	physiological saline	IV bolus

Table 40. Sample Collections

Group	Dosage	Sampling time points (hr)								
	(mg/kg)	0.083	0.25	0.5	1	2	4	6	8	24
Cynomolgus Monkey	10	P	P	P	P	P	P	P	P	P
Cynomolgus Monkey	30	P	P	P	P	P	P	P	P	P
Rat	1	P/C*	P/C*	P/C*	P	P	P	P	P	P
Rat	3	P	P	P	P	P	P	P	P	P
Rat	10	P	P	P	P	P	P	P	P	P/C*
Rat	15	P	P	P	P	P	P	P	P	P
Rat	30	P	P	P	P	P	P	P	P	P
Rat	60	P	P	P	P	P	P	P	P	P

E: Anticoagulant: Potassium (K_2) EDTA;

P: plasma

C: cerebrospinal fluid

*Sampled only for certain compounds

[0339] Dose Solution Analysis Samples: After each dose preparation, remove approximately 0.5 mL aliquots from the formulations, transfer the aliquots into amber HPLC vials and stored at -60°C or lower until assayed in duplicate for dose validation.

[0340] Disposition of Remaining Test Article Formulations: Remaining formulations will be stored at -60°C or lower.

[0341] Disposition of Remaining Test Article (dry powder or solid): Remaining test article will be stored at room temperature desiccated, and protected from light until shipment or discard.

[0342] Animals will be fasted overnight through 4 hours post dosing.

Vehicle and Formulation Preparation:

[0343] Appropriate amount of test article will be accurately weighed and mixed with appropriate volume of vehicle to get a clear solution or suspension.

[0344] Formulation samples will be removed from each of the formulation solutions, transferred into 1.5 mL of polypropylene microcentrifuge tubes and run dose validation by LC/UV or LC-MS/MS.

[0345] Compounds to be assessed are outlined in Table 41.

Table 41. Compounds

Compound ID	Animal Group	MW (g/mol)	FW (g/mol)	Exact Mass	Purity (%)	CF	Storage Condition
B (M1)	Rats	1198.41	1257.45	N/A	98	N/A	-20°C
D (O7)	Rats	1198.41	1258.46	N/A	98.7	N/A	N/A
F (A3)	Rats	1180.42	1239.68	N/A	95	N/A	N/A
B (M1)	Cynomolgus Monkeys	1198.41	1258.46	N/A	95	N/A	-20°C
D (O7)	Cynomolgus Monkeys	1198.41	1258.46	N/A	98.7	N/A	N/A

CF: FW/MW/purity

Animal Specifications: Cynomolgus Monkeys

[0346] Cynomolgus Monkey specifications are outlined in Table 42.

Table 42. Cynomolgus Monkey Specifications

Species	Cynomolgus Monkeys
Justification for Species Selection	This is an acceptable species to support PK studies for compounds intended to use in humans.
History of Dosing	Non-naïve animals
Body Weight Range	≥ 2 kg
Age	≥ 1.5 years old
Sex	Male
Method of Identification	Unique skin tattoo on chest
Number of Animals for Acclimation	4 males
Number of Animals for Dosing	3 males
Justification for number of Animals	The number of animals in each group is the minimum number of animals necessary for assessment of interanimal variability.
Selection of Animals	Animals will have undergone a physical examination for general health by a staff veterinarian. 3 males confirmed as being healthy, will be assigned to study.
Acclimation Period	Selected animals will be acclimated at the testing facility prior to the study.

[0347] Environmental Conditions: Environment controls will be set to maintain a temperature range of 20-26°C, a relative humidity range of 40 to 70%, and a 12-hour light/12-hour dark cycle. The light/dark cycle may be interrupted for study-related activities.

[0348] Housing: Animals will be group-housed (up to four animals/sex/cage) in polysulfone cages with certified aspen shaving bedding or corncob bedding during acclimation and study period. While animals may be individually housed after surgery or when there is special requirement in protocol, as well as, for behavioral or health reasons or due to cage mate death.

[0349] Diet and Feeding: Animals were offered certified rodent breeding and growth diet ad libitum every day, unless fasted for study procedures. Each lot of the diet is analyzed for nutrients, chemical contaminant and microorganisms, the results are reviewed and evaluated by veterinarians before provided to animals.

[0350] Drinking Water: Autoclaved RO (reverses osmosis) water will be available to all animals, ad libum.

[0351] Feed and Water Analyses: Autoclaved RO water will be provided ad libitum via water bottle. Water samples are periodically analyzed by a certified laboratory for specified microorganisms and environment contaminants. The diet is routinely analyzed by the manufacturer for specified microorganisms, nutritional components and environmental contaminants.

[0352] Environmental Enrichment: Enrichment toys will be provided.

[0353] Dose Administration: The dose volume will be determined by the animals' body weight collected on the morning of dosing day.

Animal Specifications: Rats

[0354] Rat specifications are outlined in Table 43.

Table 43. Rat Specifications

Species	Rat
Strain	SD
Source	Approved Vendor.
Gender	Male
Body weight	＞200g
Age	7-10 W
Total number of animals	9 male rats
Justification	The SD rat is a standard species used in pharmacokinetic evaluations and is acceptable to regulatory agencies. The number of animals used in this study is, in the judgment of the investigator involved, the

	minimum necessary to properly perform this plasma pharmacokinetic evaluation.

Observations and Examinations

[0355] Clinical Observations: All animals will be observed at dosing and each scheduled collection. All abnormalities will be recorded.

[0356] Body Weight: All animals will be weighed on the dosing day prior to dosing to determine the dose volume to be administered.

Sample Collection and Processing

[0357] Blood Sample Collection and Process: At least 0.1 mL blood will be collected at each time point. All blood samples will be collected via jugular vein. All blood samples will be transferred into low binding EP tube with anticoagulant (0.5 M Potassium (K2) EDTA will be pre-added as a ratio of 50:1 for blood: anticoagulant), 0.05% Triton X-100 (e.g., 100uL Blood+2uL 2.5% Triton X-100) will be used for desorption the blood samples will be placed on wet ice.

[0358] Blood samples will be centrifuged within 1hr of collection at 3,200 g 4°C for 10 minutes. Following centrifugation, plasma samples will be transferred into their respective pre-labeled low binding EP tube and immediately frozen over dry ice. The plasma samples will be stored lower than -60°C until bioanalysis.

[0359] LC-MS/MS method development:

[0360] A LC-MS/MS method for the quantitative determination of test compound in biological matrix will be developed.

[0361] N in 1 cassette LC-MS/MS method may be developed for samples coming from different studies as long as these studies belong to the same sponsor.

[0362] Cassette administration assay could be performed if the mass difference (ΔMass) among different analytes is ≥4 Da. In this case, interference evaluation is not necessary.

[0363] If ΔMass among different analytes is less than 4 Da, there is a potential risk that interference would occur during LC-MS/MS analysis. If such kind of cassette assay

is still requested by client, interference among analytes will not be evaluated but the LC separation of those analytes by using a generic method will be attempted.

[0364] Sample analysis:

[0365] A calibration curve with at least 6 non-zero calibration standards will be applied for each batch including LLOQ.

[0366] If sample number within a batch is ≤ 12, at least one set of standard curve separated with two parts through begin and end of the sequence should be included in the run and QCs are not required. The recommended injection order is C8, C6, C4, C2, study samples, C7, C5, C3, C1.

[0367] If sample number within a batch is > 12, one standard curve and two sets of QCs with low, middle and high concentrations will be applied for bioanalysis. Meanwhile, QCs number should be more than 5% of study sample number.

[0368] Samples, coming from one client with the same type of matrix in different studies, are allowed to be quantified in one analysis run by using the developed N in 1 cassette LC-MS/MS method.

[0369] Acceptance criteria:

[0370] (1) Linearity: At least 75% of the calibration standards should fall within ±20% of their nominal values for biofluid and within ±25% of their nominal values for tissue and feces samples. If the endpoints, such as LLOQ and ULOQ, on the calibration curve are eliminated, the calibration curve will be truncated. The truncated calibration curve should consist of at least 75% of the initial STDs.

[0371] (2) Accuracy: At least 67% of QCs should fall within ±20% of their nominal values for biofluid and within ±25% of their nominal values for tissue and feces samples. At least half of QCs at each concentration should be passed.

[0372] (3) Specificity: The mass response of analyte in the double blank and blank should be ≤ 50% LLOQ.

[0373] (4) Sensitivity: The LLOQ will be tried to target ≤ 3 ng/mL. Any adjustment of LLOQ will be informed to client in advance.

[0374] (5) Carryover: The mean calculated carryover peak area in the double blanks or blanks immediately after the highest standard injection should be less than

that of LLOQ. If the carryover couldn't meet the criteria, the impact of the carryover on unknown samples should be re-evaluated according to the below procedure:

[0375] Carryover should be re-evaluated based on absolute carryover. Absolute carryover is calculated by carryover contribution multiplies carryover impact, where the carryover contribution is calculated by the area ratio of the double blank or blank with the highest carryover (Area max of carryover blank) to the ULOQ with the minimum calculated value (Area min of ULOQ), and the carryover impact is calculated by the area ratio of one injection (Area of one injection) to the following injection (Area of the following injection). The absolute carryover should be below the acceptable accuracy of the studies (e.g., 20% or 25%).

[0376] Carryover contribution = $Area_{max}$ of carryover blank / $Area_{min}$ of ULOQ Carryover impact = Area of one injection / Area of the following injection Absolute carryover = Carryover contribution * Carryover impact

Data Analysis

[0377] Plasma concentration versus time data for Compounds A-C in Rats and Cynomolgus Monkeys will be plotted in graph and analyzed by non-compartmental approaches. Related PK parameters will be calculated according to dosing route, e.g., Cl, V_{dss} and C0 for intravenous administration, C_{max}, T_{max} or %F for extravascular administration, and T½, $AUC_{(0-t)}$, $AUC_{(0-inf)}$, $MRT_{(0-t)}$, $MRT_{(0-inf)}$ for all routes. Preliminary plasma results after 24 hours for 60 mg/kg oral administration in Rats and 30mg/kg of Compounds A-C in Cynomolgus Monkeys are shown in Table 44 and FIG. 1.

Table 44. Plasma Pharmacokinetic Results of Compounds A-C after 24 hours

Com-pound Name	Formula	60 mg/kg PO plasma Rat Data 24 h					30 mg/kg PO plasma Cynomolgus Monkeys data 24 h				
		Cmax (ng/mL)	Tmax (h)	AUC (ng.h/mL)	Tfinal	T1/2 (h)	Cmax (ng/mL)	Tmax (h)	AUC (ng.h/mL)	Tfinal	T1/2 (h)
B (M1)	Ac-Nle-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH2	32.1	1.67	102	6 h - 8h	1.19	522	1.67	4,170	24	6.13

[0378] Plasma concentration versus time data for Compound D (O7) (FIGS. 2A-2C) in Cynomolgus Monkeys was plotted in graphs and analyzed by non-compartmental

approaches. Related PK parameters were calculated according to dosing route, e.g., Cl, V_{dss} and C0 for intravenous administration, C_{max}, T_{max} or %F for extravascular administration, and T½, $AUC_{(0-t)}$, $AUC_{(0-inf)}$, $MRT_{(0-t)}$, $MRT_{(0-inf)}$ for all routes. Preliminary plasma results after 24 hours of 3 mg/kg, 10 mg/kg, 30 mg/kg, or 60 mg/kg PO administration of Compound D (Ac-Nle-c[Glu-Pro-p(F)dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2; O7) in Cynomolgus Monkeys are shown in Table 45.

Table 45. Plasma Pharmacokinetic Results of Compound (O7) in Cynomolgus Monkeys after 24 hours

Comp. Name	Dose (mg/kg)	Cmax (ng/mL)	Tmax (h)	Tfinal	T1/2 (h)	AUC_{0-last} (ng.h/mL)	AUC_{0-inf} (ng.h/mL)	MRT_{0-last} (h)	MRT_{0-inf} (h)	AUC_{EX} (%)	$AUMC_{EX}$ (%)
D	10	129	1.67	8h	1.75	387	410	2.80	3.23	5.65	17.7
D	30	193	1.50	24h	6.44	798	831	4.72	5.87	5.01	22.8

[0379] Preliminary plasma and cerebrospinal fluid (CSF) results after 24 hours of administration of Compound D (Ac-Nle-c[Glu-Pro-p(F)dPhe-Arg-Trp-Orn]-dVal-dPro-NH2; O7) and Compound F (Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH2) in Rats are shown in Table 46 and Table 47. Graphs of plasma concentration and CSF concentration in Rats are shown in FIGS 3A-3D (SC administration), FIGS. 4A-4D (IP administration), and FIGS. 5A-5C (PO administration).

[0380] Plasma and CSF concentrations over 24 hours after administration of Compound D (O7) or setmelanotide were also assessed (FIGS. 4H-4K). It was predicted

Table 46. Plasma Pharmacokinetic Results of Compound D (O7) in Rats after 24 hours

Comp Name	Dose (mg/kg)	Admin Route	Cmax (ng/mL)	Tmax (h)	Tfinal	T1/2 (h)	AUC_{0-last} (ng.h/mL)	AUC_{0-inf} (ng.h/mL)	MRT_{0-last} (h)	MRT_{0-inf} (h)	AUC_{EX} (%)	$AUMC_{EX}$ (%)
D	1	SC	297	1.67	6h	1.03	1066	1077	2.37	2.45	1.02	3.97
D	3	SC	1011	2.00	10h-24h	1.93	3877	3881	3.11	3.13	0.096	0.753
D	10	SC	1384	2.00	24h	2.17	10475	10488	5.42	5.45	0.123	0.612
D	1	IP	792	0.47	4h-10h	1.02	1120	1156	1.24	1.53	2.87	15.1
D	3	IP	3639	0.833	6h-10h	1.40	4810	4813	1.17	1.17	0.0566	0.569
D	10	IP	6067	1.00	24h	15.6	13182	13259	1.53	1.80	0.58	15.0
D	10	PO	9.51	0.250	4h	1.23	20.6	23.1	1.52	1.99	11.1	32.3
D	30	PO	22.9	0.583	4h-6h	1.35	49.5	53.2	1.73	2.12	7.50	24.4

D	60	PO	94.0	0.083	8h	1.56	111	115	2.22	2.52	3.69	15.0

Table 47. CSF Pharmacokinetic Results of Compound D (O7) in Rats after 24 hours

Comp. Name	Dose (mg/kg)	Admin Route	Cmax (ng/mL)	Tmax (h)	Tfinal	T1/2 (h)	$AUC_{0\text{-last}}$ (ng.h/mL)	$AUC_{0\text{-inf}}$ (ng.h/mL)	$MRT_{0\text{-last}}$ (h)	$MRT_{0\text{-inf}}$ (h)	AUC_{EX} (%)	$AUMC_{EX}$ (%)
D	10	SC	32.6	8.00	10h	ND	236	ND	5.55	ND	ND	ND
D	10	IP	119	2.00	10h	2.52	489	527	3.38	4.12	23.9	0.0371
D	60	OP	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

ND = Not determined

[0381] Dose proportionality of Compound D (O7) following a single administration via SC, IP, and PO in Rats is shown in Tables 48-50.

Table 48. Dose Proportionality of Compounds D and E in Rats after a single SC administration

Compound Name	Compare Doses (mg/kg)			Dose Ratio	Cmax Values	Cmax Ratio	$AUC_{0\text{-last}}$ Values	$AUC_{0\text{-last}}$ Ratio
D	3.00	Over	1.00	3.00	1011/297	3.40	3877/1066	3.64
D	10.0	Over	1.00	3.33	1384/1011	1.37	10475/3877	2.70
D	10.0	Over	3.00	10.0	1384/297	4.66	10475/1066	9.83

Table 49. Dose Proportionality of Compound D in Rats after a single IP administration

Compound Name	Compare Doses (mg/kg)			Dose Ratio	Cmax Values	Cmax Ratio	$AUC_{0\text{-last}}$ Values	$AUC_{0\text{-last}}$ Ratio
D	3.00	Over	1.00	3.00	3639/792	4.59	4810/1120	4.29
D	10.0	Over	1.00	3.33	6067/792	7.66	13182/1120	11.8
D	10.0	Over	3.00	10.0	6067/3639	1.67	13182/4810	2.74

Table 50. Dose Proportionality of Compound D in Rats after a single PO administration

Compound Name	Compare Doses (mg/kg)			Dose Ratio	Cmax Values	Cmax Ratio	$AUC_{0\text{-last}}$ Values	$AUC_{0\text{-last}}$ Ratio
D	30.0	Over	10.0	3.00	22.9/7.94	2.88	49.5/19.5	2.54
D	60.0	Over	10.0	6.00	94.0/7.94	11.8	111/19.5	5.69
D	60.0	Over	30.0	2.00	94.0/22.9	4.10	111/49.5	2.24

[0382] Preliminary plasma and cerebrospinal fluid (CSF) results after 24 hours of SC and IP administration of Compound F (Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2) in Rats are shown in Table 51 and Table 52. Graphs of plasma concentration

and CSF concentration in Rats are shown in FIGS 6A-6D (SC administration) and 7A-7C (IP administration).

Table 51. Concentration of Compound F in plasma and CSF of Rats over time

Comp. Name	**Admin Route**	**Dose (mg/kg)**	**Plasma Concentration (ng/mL) over Time**								**CSF Conc. (ng/mL) over Time**		**Plasma/CSF ratio**	
			0.25h	**0.5 h**	**1 h**	**2 h**	4 h	**6 h**	**8 h**	**24 h**	**2 h**	**4 h**	**2h**	**4 h**
F	SC	1	228	253	319	249	122	17.1	1.47	ND	-	-	-	-
F	SC	3	303	369	570	620	431	267	93.9	ND	-	-	-	-
F	SC	10	274	364	747	1169	1139	844	575	7.23	8.60	15.9	0.00749	0.0141
F	IP	1	714	686	629	215	7.31	ND	ND	ND	-	-	-	-
F	IP	3	1667	1773	1564	548	30.9	3.02	ND	ND	-	-	-	-
F	IP	10	4629	5295	5320	2856	198	42.6	7.45	1.74	-	-	-	-

ND = not determined

Table 52. Plasma Pharmacokinetic Results of Compound F in Rats after 24 hours

Comp. Name	Dose (mg/kg)	Admin Route	Cmax (ng/mL)	Tmax (h)	Tfinal	T1/2 (h)	$AUC_{0\text{-}last}$ (ng.h/mL)	$AUC_{0\text{-}inf}$ (ng.h/mL)	$MRT_{0\text{-}last}$ (h)	$MRT_{0\text{-}inf}$ (h)	AUC_{EX} (%)	$AUMC_{EX}$ (%)	Bioavailability (%)
F	1	SC	319	1.00	8h-24h	2.58	1066	1106	3.02	3.87	3.02	14.0	95.1
F	3	SC	647	1.67	8h	1.85	2986	3305	3.23	3.94	8.97	22.3	94.7
F	10	SC	1324	2.67	24h	2.50	9002	9032	5.65	5.72	0.319	1.50	77.7
F	1	IP	743	0.333	4h-6h	0.495	1102	1106	1.09	1.10	0.356	1.57	95.1
F	3	IP	1778	0.833	4h-6h	0.473	2802	2805	1.13	1.13	0.104	0.537	80.4
F	10	IP	5591	0.75	8h-24h	2.12	10641	10647	1.42	1.43	0.0554	0.954	91.5

[0383] Dose proportionality of Compound F in male rats following a single SC or IP administration in Rats is shown in Table 53 and Table 54 and FIG 8.

Table 53. Dose Proportionality of Compound F in Rats after a single SC administration

Compound Name	Compare Doses (mg/kg)			Dose Ratio	Cmax Values	Cmax Ratio	$AUC_{0\text{-}last}$ Values	$AUC_{0\text{-}last}$ Ratio
F	3.00	Over	1.00	3.00	647/319	2.03	2986/1066	2.80
F	10.0	Over	1.00	3.33	1324/647	2.05	9002/298	3.01
F	10.0	Over	3.00	10.0	13824/319	4.15	9002/1066	8.44

Table 54. Dose Proportionality of Compound F in Rats after a single IP administration

Compound Name	Compare Doses (mg/kg)			Dose Ratio	Cmax Values	Cmax Ratio	$AUC_{0\text{-}last}$ Values	$AUC_{0\text{-}last}$ Ratio
F	3.00	Over	1.00	3.00	1778/743	2.39	2802/1102	2.54
F	10.0	Over	1.00	3.33	5591/1778	3.14	10641/2802	3.80

F	10.0	Over	3.00	10.0	5591/743	7.52	10641/1102	9.66

[0384] Preliminary plasma and cerebrospinal fluid (CSF) results after 1.5 hours of IV bolus administration of 1.0 mg/kg of Compound F in Rats are shown in Table 55 and Table 56.

Table 55. Concentration of Compound F in plasma and CSF of Rats over time

Comp. Name	**Plasma Concentration (ng/mL) over Time (h)**				**CSF Concentration (ng/mL) over Time (h)**				**Plasma/CSF ratio**			
	0.083	**0.25**	**0.5**	**1.5**	**0.083**	**0.25**	**0.5**	**1.5**	**0.083h**	**0.25h**	**0.5h**	**1.5h**
F	2373	1273	759	108	6.56	7.03	21.0	6.72	0.00278	0.0056	0.0274	0.026

Table 56. Plasma and CSF Pharmacokinetic Results of Compound F in Rats after IV bolus administration

	C_0 (ng/mL)	Cmax (ng/mL)	Tmax (h)	Tfinal	T1/2 (h)	Vdss (L/kg)	Cl (mL/min/kg)	$AUC_{0\text{-last}}$ (ng.h/mL)	$AUC_{0\text{-inf}}$ (ng.h/mL)	$MRT_{0\text{-last}}$ (h)	$MRT_{0\text{-inf}}$ (h)	AUC_{EX} (%)	$AUMC_{EX}$ (%)
Plasma	3234	-	-	1.50	0.352	0.399	14.3	1108	1163	0.387	0.464	4.72	20.5
CSF	-	21.0	0.5	1.50	ND	-	-	17.4	ND	ND	ND	ND	ND

ND = Not determined

[0385] FIG. 9 illustrates the average plasma concentration of Compound F over time in rats following SC, IP, and IV bolus administration.

Plasma Concentration

[0386] Plasma pharmacokinetic measurements using 30 mg/kg were assessed in Cynomolgus monkey plasma up to 24 hours. Results are detailed in Table 57.

Table 57: Pharmacokinetic Data

Compound Name	30 mg/kg PO Cynomolgus Monkey	
	Cmax (ng/mL)	AUC (ng.h/mL)
B (M1)	522 ± 277	4170 ± 2003
D (O7)	193	798

[0387] The antagonist activity of exemplary non-naturally occurring melanocortin analogs at specific ion channels was measured using the Qube electrophysiological platform. The non-naturally occurring melanocortin analogs and specific ion channel targets are provided in Table 58.

Table 58. Ion channel targets and melanocortin analogs

Melanocortin Analog	Ion channel targets	MW + Saly	Condition
A3	Voltage-Gated Sodium: HEK-Nav1.5 (Peak), HEK-Nav1.5 (Late, Antagonist) Voltage-Gated Potassium: HEK-Kv4.3/KChIP2, CHO-hERG, CHO-KCNQ1/minK Voltage-Gated Calcium: HEK-Cav1.2 Inward-Rectifying Voltage-Gated Potassium: HEK-Kir2.1	1179.70	Dry powder
O2	Voltage-Gated Potassium: CHO-hERG	1183.63	Dry powder
O7	Voltage-Gated Potassium: CHO-hERG	1197.65	Dry powder

Methods

[0388] The non-naturally occurring melanocortin analogs identified above were tested for antagonist activity at various ion channels at concentrations ranging from 0.1 mM to 30 mM. In each experiment and if applicable, the respective reference compounds were tested concurrently with the test compounds, and the data were compared with known historical values.

hNav1.5 Sodium Channel Assay – Qube APC

[0389] Onset and steady state block of peak Nav1.5 current is measured using a pulse pattern, repeated every 5 sec, consisting of a hyperpolarizing pulse to -120mV for a 200ms duration, depolarization to -15mV amplitude for a 40ms duration, followed by step to 40mV for 200ms and finally a 100ms ramp (1.2 V/s) to a holding potential of -80mV. Peak current is measured during the step to -15mV.

[0390] The parameters measured were difference between the peak inward current on stepping to -15mV (i.e., peak of the current) and the leak current. All data were filtered for seal quality, seal drop, and current amplitude. The peak current amplitude was calculated before and after compound addition and the amount of block was assessed by dividing the Test compound current amplitude by the Control current amplitude. Control is the mean Nav1.5 current amplitude collected 10 seconds at the end of the vehicle control; Test Compound is the mean Nav1.5 current amplitude collected 10 seconds at the end of test concentration application for each concentration.

hKv4.3/hKChIP2 Potassium Channel Assay – Qube APC

[0391] After whole cell configuration is achieved, the cells are held at -80mV. Onset and steady state block of hKv4.3 current is measured using a pulse pattern from -80mV to 40mV amplitude for a 110ms duration, and finally a 100ms ramp (1.2 V/s) to -80mV. This paradigm is delivered once every 5s to monitor the current amplitude.

[0392] The parameters measured were the maximum outward current evoked on stepping to 40mV from holding potential of -80mV. All data were filtered for seal quality, seal drop, and current amplitude. The peak current amplitude was calculated before and after compound addition and the amount of block was assessed by dividing the Test compound current amplitude by the Control current amplitude. Control data is the mean Kv4.3/KChIP2 current amplitude collected 10 seconds at the end of the vehicle control period; Test compound data is the mean Kv4.3/KChIP2 current amplitude collected 10 seconds at the end of test concentration application for each concentration.

hCav1.2 (L-type) CiPA Calcium Channel Assay – Qube APC

[0393] Onset and steady state block of peak hCav1.2 current is measured using a pulse pattern, repeated every 15 sec. Cells were held at -80mV for a 50ms before stepping to -90mV for 100ms to measure leak current and then stepped back to -80mV for 50ms, depolarization to 0mV amplitude for a 40ms duration, followed by step to 40mV for 200ms and finally a 100ms ramp (1.2 V/s) to a holding potential of -80 mV. Peak current is measured during the step to 0mV. Each concentration is applied for 5 minutes.

[0394] The calcium current amplitude is calculated by measuring the difference between the peak inward current on stepping to 0mV or the peak inward current at the ramp (i.e. peak of the current) and the leak current. The calcium current is assessed in vehicle control conditions and at the end of each five (5) minute compound application.

hNav1.5 Late Current Sodium Channel Assay – Qube APC

[0395] Onset and steady state block of Late Nav1.5 current is measured using a pulse pattern, repeated every 5 sec, consisting of a hyperpolarizing pulse to -120mV for a 200ms duration, depolarization to -15mV amplitude for a 40ms duration, followed by step to 40mV for 200ms and finally a 100ms ramp (1.2 V/s) to a holding potential of -80mV. Late current is measured as charge current elicited during the ramp with 50nM ATXII.

[0396] The parameters measured were the ramp current charge (AUC) evoked on ramping back to -80mV from 40mV test pulse in the presence of 50nM ATXII. All data were filtered for seal quality, seal drop, and current. The peak and ramp current amplitude was calculated before and after compound addition and the amount of current was assessed by dividing the Test compound current by the Control current. Control data is the mean hNav1.5 late current collected 15 seconds at the end of 50nM ATXII application (50nM ATXII control); Test compound data is the mean ramp hNav1.5 current collected 15 seconds at the end of test concentration application for each concentration.

hERG Potassium Channel Assay - Qube APC

[0397] After whole cell configuration is achieved, the cells are held at -80mV. Cells are held at this voltage for 50ms to measure the leak current, which is subtracted from the tail current on-line. The cells are depolarized to +40mV for 500ms and then to -80 mV over a 100ms ramp to elicit the hERG tail current. This paradigm is delivered once every 8s to monitor the current amplitude. All compounds were tested in the presence of 0.1% Pluronic F-68 Non-Ionic Surfactant and at approximately room temperature.

[0398] The parameters measured were the maximum tail current evoked ramping back to -80mV from the test pulse of 40mV. All data were filtered for seal quality, seal drop, and current amplitude. The peak current amplitude was calculated before and after compound addition and the amount of block was assessed by dividing the Test compound current amplitude by the Control current amplitude. Control data is the mean hERG current amplitude collected for three pulses (24 seconds) at the end of the vehicle control; Test compound data is the mean hERG current amplitude collected for three pulses (24 seconds) at the end of test concentration application for each concentration.

hKCNQ1/hminK Potassium Channel Assay – Qube APC

[0399] After whole cell configuration is achieved, the cells are held at -80mV. KCNQ1/minK currents are evoked by a 1000ms pulse from -80mV to 60mV followed by a ramp from 60mV to -80mV over 115ms with the outward peak currents measured upon depolarization of the cell membrane. This paradigm is delivered once every 15s to monitor the current amplitude.

[0400] The parameters measured were the maximum outward current evoked on stepping to +60mV from a holding potential of -80mV. All data were filtered for seal quality, seal drop, and current amplitude. The peak current amplitude was calculated before and after compound addition and the amount of block was assessed by dividing the Test compound current amplitude by the Control current amplitude. Control data is the mean hKCNQ1/hminK current amplitude collected 30 seconds at the end of vehicle control period; Test compound data is the mean hKCNQ1/hminK current amplitude collected 30 seconds at the end of test concentration application for each concentration.

hKir2.1 Potassium Channel Assay – Qube APC

[0401] After whole cell configuration is achieved, the cells are held at -30mV. Kir2.1 currents are evoked by a single 500ms pulse to -120mV before returning to the holding potential of -30mV. This paradigm is delivered once every 20s to monitor the current amplitude.

[0402] The parameters measured were the maximum inward current elicited on stepping to -120mV for 500ms from a holding potential of -30 mV. All data were filtered for seal quality, seal drop, and current amplitude. The peak current amplitude was calculated before and after compound addition. Residual non-Kir2.1 current was eliminated via normalization to residual current after application of 100uM Barium Chloride. The amount of Test compound effect was then assessed by dividing the Test compound current amplitude by the Control current amplitude. Control data is the mean Kir2.1 current amplitude collected 40 seconds at the end of the vehicle control; Test compound data is the mean Kir2.1 current amplitude collected 30 seconds at the end of test concentration application for each concentration.

Results

[0403] Where presented, IC_{50} values were determined by a non-linear, least squares regression analysis. Reference standards were run as an integral part of each assay to ensure the validity of the results obtained. Results from the ion channel assessment of non-naturally occurring melanocortin analogs are provided in Table 59.

Table 59. Dose-response results of melanocortin analogs and reference compounds against ion channels.

Compound	**Ion channel target**	**Max Inhibtion %**	**Estimated IC_{50} (nM)***
Tetracaine	Nav1.5	95.17	2.67
Flecainide	Kv4.3/ChIP2	96.97	7.60
Nifedipine	Cav1.2	88.16	0.127
Ranolazine	Nav1.5 Late Current	92.16	37.1
Verapamil_01	hERG	105.19	0.217
Verapamil_02	hERG	85.05	0.426
Chromanol 293B	KNQ1/mink	75.00	32.2
Barium Chloride	Kir2.1	103.10	9.12
A3	Nav1.5	2.32	N/C
A3	Kv4.3/ChIP2	14.90	N/C
A3	Cav1.2	18.72	N/C
A3	Nav1.5 Late Current	13.80	N/C
A3	hERG	14.58	N/C
A3	KNQ1/mink	1.01	N/C
A3	Kir2.1	2.17	N/C
O2	hERG	7.61	N/C
O7	hERG	14.76	N/C

*N/C: Indicates observed mean maximal inhibition was <25%

Example 7: Assessment of physical biological properties of non-naturally occurring melanocortin analogs

[0404] Physical biological properties including solubility, in vitro absorption and in vitro metabolism of nine exemplary non-naturally occurring melanocortin analogs were assessed according to the following procedures. In each experiment and if applicable, the respective reference compound was tested concurrently with the test compounds, and the data were compared with known values. Stock solutions of the tested compounds were prepared at a concentration of 0.01M in DMSO.

Solution Properties

[0405] Solution properties of non-naturally occurring melanocortin analogs and reference compounds in various biological media were assessed according to the conditions provided in Table 60.

Table 60. Assay conditions for in vitro absorption assessment

Assay	**Technique**	**Incubation**	**Detection Method**
Aqueous solubility (simulated intestinal fluid)	shake-flask	24h, RT	HPLC-UV/Vis
Aqueous solubility (PBS, pH 7.4)	shake-flask	24h, RT	HPLC-UV/Vis
Aqueous solubility (simulated gatric fluid)	shake-flask	24h, RT	HPLC-UV/Vis
Protein binding (plasma, human)	Equilibrium dialysis	4h, 37°C	HPLC-MS/MS
Partition coefficient	shake-flask	60 min, RT	HPLC-UV/Vis
Half-life (simulated gastric fluid)		0, 15, 30, 45 and 60 min, 37°C	HPLC-MS/MS
Half-life (simulated intestinal fluid)	Caco-2 cell line	0, 1, 2, 3, and 4h, 37°C	HPLC-MS/MS

Aqueous Solubility

[0406] Aqueous solubility (μM) was determined by comparing the peak area of the principal peak in a calibration standard (200 μM) containing organic solvent (methanol/water, 60/40, v/v) with the peak area of the corresponding peak in a buffer sample. In addition, chromatographic purity (%) was defined as the peak area of the principal peak relative to the total integrated peak area in the HPLC chromatogram of the calibration standard. A chromatogram of the calibration standard of each test compound, along with a UV/VIS spectrum with labeled absorbance maxima, was generated.

[0407] A chromatogram of the test compound (200 μM) along with a UV/VIS spectrum with labeled absorbance maxima, was generated.

Protein Binding

[0408] The peak areas of the test compound in the buffer and test samples were used to calculate percent binding and recovery according to the following formulas:

Protein binding(%) = $((Area_p-Area_b)/Area_p) \times 100$

Recovery(%) = $((Area_p-Area_b)/Area_c) \times 100$

where: $Area_p$ = peak area of analyte in protein matrix; $Area_b$ = peak area of analyte in buffer; and $Area_c$ = peak area of analyte in control sample.

Partition Coefficient

[0409] The total amount of compound was determined as the peak area of the principal peak in a calibration standard (100 µM) containing organic solvent (methanol/water, 60/40, v/v). The amount of compound in buffer was determined as the combined, volume-corrected, and weighted areas of the corresponding peaks in the aqueous phases of three organic-aqueous samples of different composition. An automated weighting system was used to ensure the preferred use of raw data from those samples with well quantifiable peak signals. The amount of compound in organic was calculated by subtraction. Subsequently, Log D was calculated as the Log10 of the amount of compound in the organic phase divided by the amount of compound in the aqueous phase.

Half-Life Determination

[0410] At the end of the incubation at each of the time points, an equal volume of an organic mixture (acetonitrile/methanol, 50/50, v/v) was added to the incubation mixture. Samples were analyzed by HPLC-MS/MS and corresponding peak areas were recorded for each analyte. The ratio of precursor compound remaining after each time point relative to the amount present at time 0, expressed as percent, is reported as chemical stability. The half-life (T1/2) was estimated from the slope of the initial linear range of the logarithmic curve of compound remaining (%) versus time, assuming first order kinetics.

[0411] Results of the solubility assessment detailed above are provided in Tables 61-64.

Table 61. Protein binding of melanocortin analogs in plasma

Compound	Test Concentration (M)	Average % Protein Bound	Average % Recovery
Acebutolol	1.0E-5	15.17	112.61
Quinidine_01	1.0E-5	54.99	105.22
Quinidine_02	1.0E-5	66.10	114.10
Sertraline_01	1.0E-5	97.52	64.76
Sertraline_02	1.0E-5	98.25	97.34
Warfarin_01	1.0E-5	98.98	91.90
Warfarin_02	1.0E-5	98.58	107.98
A3	1.0E-5	58.35	94.47
M1	1.0E-5	51.07	108.08
O7	1.0E-5	52.72	104.88
O11	1.0E-5	48.76	115.09

Table 62. Half-life of melanocortin analogs in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF)

Compound	Test Concentration (M)	Average Half-life (min)	
		SGF	SIF
Erythromycin	1.0E-6	42.9	-
Propantheline	1.0E-6	>120	113.6
Chlorambucil	1.0E-6	-	20.0
A3	1.0E-6	>120	115.4
M1	1.0E-6	>120	69.3
O7	1.0E-6	>120	101.7
O11	1.0E-6	>120	>120

Table 63. Aqueous solubility of melanocortin analogs in plasma (PBS), simulated gastric fluid (SGF), and simulated intestinal fluid (SIF)

Compound	Test Concentration (M)	Average Solubility (mM)		
		PBS*	SGF	SIF
Diethylstilbestrol	2.0E-4	5.21	-	-
Ketoconazole	2.0E-4	133.32	-	-
Metoprolol	2.0E-4	190.93	-	-
Phenytoin	2.0E-4	89.72	-	-
Rifampicin	2.0E-4	174.01	-	-
Simvastatin	2.0E-4	36.04	-	-
Tamoxifen	2.0E-4	4.12	-	-
A3	2.0E-4	0.05	142.19	>200
M1	2.0E-4	>200	>200	129.54
O7	2.0E-4	198.75	196.88	133.12
O11	2.0E-4	190.73	185.73	110.16

*pH = 7.4

Table 64. Partition coefficient of melanocortin analogs and reference compounds

Compound	Test Concentration (M)	Partition Coefficient*
Haloperidol	1.0E-4	2.53
Ketoconazole	1.0E-4	3.35
Metoprolol	1.0E-4	-0.32
Phenytoin	1.0E-4	2.20
Rifampicin	1.0E-4	1.08
Simvastatin	1.0E-4	>4.01
A3	1.0E-4	0.98
M1	1.0E-4	0.36
O7	1.0E-4	-0.07
O11	1.0E-4	-0.34

*log D, n-octanol/PBS, pH 7.4

In Vitro Absorption

[0412] *In vitro* absorption of non-naturally occurring melanocortin analogs was determined using permeability assays. Assay conditions are provided in Table 65.

Table 65. Assay conditions for in vitro absorption assessment

Assay	Source	pH	Incubation	Detection Method
A-B permeability	Caco-2 cell line	6.5/7.4	0 and 60 min, 37°C	HPLC-MS/MS
B-A permeability	Caco-2 cell line	6.5/7.4	0 and 60 min, 37°C	HPLC-MS/MS

Permeability

[0413] The apparent permeability coefficient (Papp) of the test compound was calculated as follows: $P_{app}(cm/s) = (V_R*C_{R,end}/Dt) \times (1/A*(C_{D,mid}-C_{R,mid})$

where V_R is the volume of the receiver chamber; $C_{R,end}$ is the concentration of the test compound in the receiver chamber at the end time point; Δt is the incubation time; A is the surface area of the cell monolayer; $C_{D,mid}$ is the calculated mid-point concentration of the test compound in the donor side, which is the mean value of the donor concentration at time 0 minute and the donor concentration at the end time point; and $C_{R,mid}$ is the mid-point concentration of the test compound in the receiver side, which is one half of the receiver concentration at the end time point. Concentrations of the test compound were expressed as peak areas of the test compound.

Recovery of the Test Compound from the Permeability Assay

[0414] The recovery of the test compound was calculated as follows:

[0415] $Recovery(\%) = ((V_D*C_{D,end}+V_R*C_{R,end})/V_D*C_{D0}) \times 100$

[0416] where V_D and V_R are the volumes of the donor and receiver chambers, respectively; $C_{D,end}$ is the concentration of the test compound in the donor sample at the end time point; $C_{R,end}$ is the concentration of the test compound in the receiver sample at the end time point; and C_{D0} is the concentration of the test compound in the donor sample at time zero. Concentrations of the test compound are expressed as peak areas of the test compound.

Fluorescein assessment for Permeability assays

[0417] Fluorescein was used as the cell monolayer integrity marker. Fluorescein permeability assessment (in the A-B direction at pH 7.4 on both sides) was performed after the permeability assay for the test compound. The cell monolayer that had a

fluorescein permeability of less than 1.5 x 10^{-6} cm/s for Caco-2 and MDR1-MDCKII cells and 2.5 x 10^{-6} cm/s for MDCKII cells was considered intact, and the permeability result of the test compound from intact cell monolayer is reported.

[0418] Results of the *in vitro* absorption assessments described above are provided in Table 66.

Table 66. *In vitro* absorption of melanocortin analogs and reference compounds

Compound	**Test Concentration (M)**	**A-B permeability**		**B-A permeability**	
		Permeability (10^{-8} cm/s)	**Recovery (%)**	**Permeability (10^{-8} cm/s)**	**Recovery (%)**
Colchicine	1.0E-5	0.2	83	3.4	89
Labetalol	1.0E-5	6.1	100	26.0	93
Propranolol	1.0E-5	26.4	90	28.9	91
Ranitidine	1.0E-5	0.2	96	1.1	99
A3	1.0E-5	<0.014	88	<0.023	74
M1	1.0E-5	<0.007	89	<0.021	69
O7	1.0E-5	<0.006	86	<0.015	79
O11	1.0E-5	<0.013	90	<0.02	75

In Vitro Metabolism

[0419] *In vitro* metabolism of non-naturally occurring melanocortin analogs was determined using the assay conditions provided in Table 67.

Table 67. Assay conditions for in vitro metabolism assessment

Assay	**Source**	**Incubation**	**Detection Method**
Intrinsic clearance	Human liver microsomes (0.1 mg/mL)	0, 15, 30, 45, and 60 min, 37°C	HPLC-MS/MS

[0420] Metabolic stability, expressed as percent of the parent compound remaining, was calculated by comparing the peak area of the compound at the time point relative to that at time-0. The half-life ($T_{1/2}$) was estimated from the slope of the initial linear range of the logarithmic curve of compound remaining (%) vs. time,

assuming the first-order kinetics. The apparent intrinsic clearance (CL_{int}, in µL/min/pmol, µL/min/mg or µL/min/Mcell) was calculated according to the following formula:

CL_{int}= 0.693/($T_{1/2}$*(mg protein/µL or million cells/µL or pmol CYP isoyme/µL)).

[0421] Results from the intrinsic clearance assay are provided in Table 68.

Table 68. *In vitro* metabolism of melanocortin analogs and reference compounds

Compound	Test Concentration (M)	Half-life	Clint (uL/mi/mg)
Imipramine	1.0E-7	>120	<57.8
Propranolol	1.0E-7	>120	<57.8
Terfenadine	1.0E-7	13.2	524.9
Verapamil	1.0E-7	33.8	205.1
A3	1.0E-7	>120	<57.8
M1	1.0E-7	>120	<57.8
O7	1.0E-7	>120	<57.8
O11	1.0E-7	>120	<57.8

Example 8: CYP Inhibition of Non-Naturally Occurring Melanocortin Analogs

[0422] The inhibitory potential of exemplary non-naturally occurring melanocortin analogs was tested on seven human Cytochrome P450 (CYP) enzymes: CYP1A, CYP2B6, CYP2C19, CYPC8, CYP2C9, CYP2D6, and CYP3A. Exemplary non-naturally occurring melanocortin analog, O7, and comparator non-naturally occurring melanocortin analog Ac-Nle-c[Asp-Pro-dNal(2')-Arg-Trp-Lys]-dVal-dPro-NH2 (SEQ ID NO: 368) were tested at concentrations ranging from 0.1 mM to 100 mM for inhibition of the seven CYPs. The CYP inhibition assays were performed using human liver microsomes (HLM) and human recombinant CYP isozymes in 96-well plate format. Direct inhibition (e.g., zero-min incubation) and time-dependent (e.g., 30 min preincubation) inhibition assays were performed. The results of the CYP inhibition assays of O7 at 10 mM are shown in Table 69 and the results of the comparator CYP inhibition assays including IC_{50} values at each of the CYP enzymes are shown in Table 70.

Table 69. CYP Inhibition Studies of O7

Assay	Substrate	NADPH+/-	O7 µm

CYP1A inhibition	phenacetin		15%
CYP2B6 inhibition	bupropion		4%
CYP2C19 inhibition	omeprazole		9%
CYP2C8 inhibition	amodiaquine		23%
CYP2C9 inhibition	diclofenac		-4%
CYP2D6 inhibition	dextromethorphan		18%
CYP3A inhibition	midazolam		21%
CYP3A inhibition	testosterone		21%
Time-dependent CYP1A inhibition	phenacetin	- NADPH	1%
Time-dependent CYP1A inhibition	phenacetin	+ NADPH	17%
Time-dependent CYP2B6 inhibition	bupropion	- NADPH	-9%
Time-dependent CYP2B6 inhibition	bupropion	+ NADPH	0%
Time-dependent CYP2C19 inhibition	omeprazole	- NADPH	-1%
Time-dependent CYP2C19 inhibition	omeprazole	+ NADPH	0%
Time-dependent CYP2C8 inhibition	Amodiaquine	- NADPH	-1%
Time-dependent CYP2C8 inhibition	Amodiaquine	+ NADPH	4%
Time-dependent CYP2C9 inhibition	diclofenac	- NADPH	0%
Time-dependent CYP2C9 inhibition	diclofenac	+ NADPH	-1%
Time-dependent CYP2D6 inhibition	dextromethorphan	- NADPH	-6%
Time-dependent CYP2D6 inhibition	dextromethorphan	+ NADPH	0%
Time-dependent CYP3A inhibition	midazolam	- NADPH	2%
Time-dependent CYP3A inhibition	midazolam	+NADPH	3%

Table 70. CYP Inhibition of B07

CYP	Marker Substrate (Conc.)	Isoform-Catalyzed Reaction	TCMCB07 IC50 Values (µm)		IC50 Shift Fold[b]
			No Pre-Incubation	30-min Pre-Incubation (IC 50,30min)	
1A2	Phenacetin (50 µM)	O-Deethylation	>100	>100	NA
2B6	Bupropion (50 µM)	Hydroxilation	>100	>100	NA
2C8	Amodiaquine (2 µM)	N-Deethylation	>100	>100	NA
2C9	Diclofenac (5 µM)	4' -Hydroxylation	>100	>100	NA
2C19	S-Mephenytoin (20 µM)	4' -Hydroxylation	>100	>100	NA
2D6	Bufuralol (5 µM)	1' -Hydroxylation	68.3	96.4	0.7
3A	Midazolam (2 µM)	1' -Hydroxylation	>100	>100	NA
3A	Testosterone (50 µM)	6ß' Hydroxylation	>100	>100	NA

Example 9: Assessing Weight and Food Intake in an Animal Model

[0423] Weight and food intake were assessed in Sprague-Dawley rats after administration of 3 mg/kg (3mg/kg) A3, O7, and O11 for three or four days are shown in FIGS. 10A (weight) and 10B (daily food intake).

Example 10: Body Weight, Complete Blood Count, Clinical Chemistry, and Food Intake in an Animal Model

Objective

[0424] The objective of this study was to evaluate the pharmacodynamic (PD) characteristics and effects on food intake, body composition, and clinical chemistry following administration of exemplary non-naturally occurring melanocortin analogs of the present technology. Non-naturally occurring melanocortin analogs were administered to Cynomolgus monkeys via two subcutaneous (s.c.) injections at different doses.

Primate Specifications

[0425] Primate specifications are outlined in Table 71.

Table 71: Primate Specifications

Species:	Cynomolgus monkey
Sex and Number:	Male, 6
Age:	10~25 years old
Body Weight:	>8 kg
Dosing History:	Non-naïve

Animal Husbandry

[0426] Animals were supplied with unlimited Certified Monkey Diet for 72 hours after dosing on days 1, 8, and 11. Otherwise, the animals were supplied with Certified Monkey Diet twice a day. Animals received fruit daily as nutritional enrichment.

[0427] Animals were supplied with fresh reverse-osmosis (RO) water using an automated watering system.

Study Design

[0428] Non-naturally occurring melanocortin analogs were each administered to a group of Cynomolgus monkeys (n=2 per group) via s.c. injections as outlined in Table

72. The start of treatment is designed as Day 1. The end time of dosing was recorded as 0h.

Table 72. Group & Dosing Information

Group	Test article	N	1st Dose Level (mg/kg)	2nd Dose Level (mg/kg)	Vehicle	Dose volume (mL/kg)	Dose Frequency	Route of Administration (ROA)
1, 2, & 3	Saline	6	N/A	N/A	N/A	0.5	Day 1	s.c.
1	O7	2	1	3	Saline	0.5	Day 8 (Day 11)	s.c.
3	O11	2	1	3	Saline	0.5	Day 8 (Day 11)	s.c.

Examination and Observations

[0429] General physical examinations were completed once in the acclimation period. Cage-side observations for general health and appearance, such as nausea and vomiting, were done every hour for 6 hours after dosing on Days 8 and 11. Otherwise, cage-side clinical observations were done once daily during the pre-study and after dosing period.

[0430] Acclimatization Period (Day 1-7): Each animal was examined at least once during the acclimation period to determine suitability for the study. Body weight, clinical observations, and food intake observations were recorded daily. Measurement of initial body composition using Dual-Energy X-Ray Absorptiometry (DEXA) was recorded at least once during this time. Standard blood markers (e.g., glucose, insulin, HDL-c, LDL-c, triglycerides, total cholesterol, alanine aminotransferase, aspartate aminotransferase) were measured once.

[0431] Treatment Phase (Days 8-49): Body weight was recorded twice a week, clinical observation and food intake were recorded once a day. Standard hematology and clinical chemistry readouts as above were conducted before dosing and at the conclusion of the study on day 49. DEXA measurements of body composition were conducted biweekly.

Food intake measurement

[0432] Food intake was observed at about 0.5 hours, 1 hour, 1.5 hours, 2 hours, 3 hours, 4 hours, 24 hours, 48 hours, and 72 hours after administration of saline or non-naturally occurring melanocortin analogs at 1 mg/kg and 3 mg/kg (Day 8 and Day 11) dosing. On Days 4-7, monkeys were acclimatized to a normal diet of two meals per day.

On Day 8, the lower dose of the non-naturally occurring melanocortin analogs were administered, and observations continued through Day 10. On Day 11, the higher dose of the non-naturally occurring melanocortin analogs was administered, and observations continued through Day 13, the conclusion of the study.

Blood collection

[0433] Blood collection according to Table 73 was conducted for the following: clinical chemistry (Serum), hematology (whole blood). Whole blood was collected at each time point with syringe via the cephalic or saphenous veins into labeled polypropylene tubes.

Table 73. Blood Sampling

Animal Group	**Time Point**	**Test Type**	**Blood Sample Type**	**Minimum Volume of Blood Samples**
All Groups	Day -1, Day 8 (2h), Day 11 (2h).	CBC	Whole Blood	~1 mL
	Day -1, Day 8 (2h), Day 11 (2h).	Clinical Chemistry	Serum	~0.5 mL

Sample Measurement and Analysis

[0434] Clinical chemistry and hematology assessments were conducted, assessing the parameters outlined in Tables 74 and 75.

Table 74: Clinical Chemistry

Parameter	**Abbreviation**	**Unit**
Albumin	ALB	g/L
Globulin (calculated)	GLB	g/L
Albumin/Globulin Ratio	A/G	-
Alkaline Phosphatase	ALP	U/L
Alanine Aminotransferase	ALT	U/L
Aspartate Aminotransferase	AST	U/L
Total Bilirubin	TBIL	μmol/L
Direct Bilirubin	DBIL	μmol/L
Total Cholesterol	CHO	mmol/L
Glucose	GLU	mmol/L
Creatinine Kinase	CK	U/L
Creatinine	CREA	μmol/L

Urea Nitrogen	UREA	mmol/L
Uric Acid	UA	µmol/L
Gamma-glutamyl transferase	GGT	U /L
Total Protein	TP	g/L
Triglycerides	TG	mmol/L
Lactate Dehydrogenase	LDH	U/L
C-reactive Protein	CRP	mg/L

Table 75: Hematology

Parameter	**Abbreviation**	**Unit**
Red blood cell count	RBC	10^{12}/L
Hemoglobin	HGB	g/L
Hematocrit	HCT	%
Mean corpuscular volume	MCV	fL
Mean corpuscular hemoglobin	MCH	pg
Mean corpuscular hemoglobin concentration	MCHC	g/L
Red cell distribution width	RDW	%
Platelet count	PLT	10^9 /L
Mean platelet volume	MPV	fL
Total white blood cell count	WBC	10^9/L
Routine CBC Differential		
Neutrophils	#NEUT	10^9/L
Lymphocytes	#LYM	
Monocytes	#MONO	
Eosinophils	#EOS	
Basophils	#BASO	
Neutrophils	%NEUT	%
Lymphocytes	%LYM	
Monocytes	%MONO	
Eosinophils	%EOS	
Basophils	%BASO	

Body Weight

[0435] Body weight was assessed in male Cynomolgus monkeys having diet-induced obesity of age 14-19 years old (n=6). Monkeys were subcutaneously injected with O7 or O11 at 1 mg/kg and 3 mg/kg. Monkeys were fasted overnight before dosing. Body weight was measured on days 1, 8, and 15 and percent change in body weight was calculated. Results are shown in Tables 76 and 77, respectively.

Table 76: Body Weight (kg)

Group	Animal ID	Day 1	Day 8	Day 15
Group 1 O7, 1mg/kg, 3mg/kg, SC, two dose, n=2	1	8.49	8.45	8.89
	2	10.48	10.30	10.80
	Mean	9.49	9.38	9.85
	SD	1.41	1.31	1.35
Group 3 O11, 1mg/kg, 3mg/kg, SC, two doses, n=2	5	8.53	8.57	8.98
	6	9.41	9.50	9.84
	Mean	8.97	9.04	9.41
	SD	0.62	0.66	0.61

Table 77: Body Weight (% Change)

Group	Animal No.	Day 1	Day 8	Day 15
Group 1 O7, 1mg/kg, 3mg/kg, SC, two dose, n=2	1	0.0	-0.5	4.7
	2	0.0	-1.7	3.1
	Mean	0.0	-1.1	3.9
	SD	0.0	0.9	1.2
Group 3 O11, 1mg/kg, 3mg/kg, SC, two doses, n=2	5	0.0	0.5	5.3
	6	0.0	1.0	4.6
	Mean	0.0	0.7	4.9
	SD	0.0	0.3	0.5

Complete Blood Count

[0436] Complete blood count (CBC) was assessed on days 1, 8, and 12. Results are detailed in Tables 78-83.

Table 78: Complete Blood Count Day 1 (Part 1)

CBC	Measurement	Total white blood cell count	Neutro-phils	Lympho-cytes	Mono-cytes	Eosino-phils	Baso-phils	Neutro-phils	Lym-pho-cytes	Mono-cytes	Eosino-phils	Baso-phils
		WBC	%NEUT	%LYMP	%MONO	%EO	%BASO	#NEUT	#LYMP	#MONO	#EO	#BASO
Group (Day 1)		10^9/L	%	%	%	%	%	10^9/L	10^9/L	10^9/L	10^9/L	10^9/L
Group 1 O7, 1mg/kg, 3mg/kg, SC, two doses, n=2	Mean	11.68	58.15	35	4.9	1.2	0.7	7	3.6	1	0.2	0.08
	SD	4.79	19.59	21	0.7	0.4	0.1	5	0.7	0	0.1	0.04
Group 3 O11, 1mg/kg, 3mg/kg, SC, two doses, n=2	Mean	14.01	50.00	43	5.1	1.3	0.5	7	6.0	1	0.2	0.08
	SD	1.24	3.68	2	1.5	0.0	0.1	1	0.2	0	0.0	0.02
	SD	1.00	1.41	1.98	9.55	3.677	9.192	0.212	6.364	0.424		

Table 79: Complete Blood Count Day 1 (Part 2)

CBC	Measurement	Red blood cell count	Hemo-globin	Hema-tocrit	Mean corpus-cular volume	Mean corpus-cular hemo-globin	Mean corpus-cular hemo-globin concen-tration	Red cell distribu-tion width	Platelet count	Mean platelet volume
		RBC	HGB	HCT	MCV	MCH	MCHC	RDW-CV	PLT	MPV
Group (Day 1)		10^{12}/L	g/L	%	fL	pg	g/L	%	10^9/L	fL
Group 1 O7, 1mg/kg, 3mg/kg, SC, two doses, n=2	Mean	5.57	150.00	42.40	76.20	27.20	356.00	12.45	564.00	10.50
	SD	0.45	19.80	2.12	2.40	5.66	63.64	0.07	219.20	0.71
Group 3 O11, 1mg/kg, 3mg/kg, SC, two doses, n=2	Mean	6.26	152.00	48.90	78.85	24.500	310.500	11.550	489.500	9.700
	SD	1.00	1.41	1.98	9.55	3.677	9.192	0.212	6.364	0.424

Table 80: Complete Blood Count Day 8 (Part 1)

CBC	Measurements	Total white blood cell count	Neutro-phils	Lympho-cytes	Mono-cytes	Eosino-phils	Baso-phils	Neutro-phils	Lym-pho-cytes	Mono-cytes	Eosino-phils	Baso-phils
		WBC	%NEUT	%LYMP	%MONO	%EO	%BASO	#NEUT	#LYMP	#MONO	#EO	#BASO
Group (Day 8)		10^9/L	%	%	%	%	%	10^9/L	10^9/L	10^9/L	10^9/L	10^9/L
Group 1 O7, 1mg/kg, 3mg/kg, SC, two doses, n=2	Mean	12.48	61.85	30	5.9	1.1	0.8	8	3.7	1	0.2	0.09
	SD	3.66	6.43	7	0.0	0.6	0.1	3	0.2	0	0.1	0.03
Group 3 O11, 1mg/kg, 3mg/kg, SC, two doses, n=2	Mean	12.14	55.25	39	4.8	0.8	0.5	7	4.7	1	0.1	0.06
	SD	0.69	1.63	0	1.0	0.0	0.3	0	0.3	0	0.0	0.04

Table 81: Complete Blood Count Day 8 (Part 2)

CBC	Measurements	Red blood cell count	Hemo-globin	Hema-tocrit	Mean corpus-cular volume	Mean corpus-cular hemo-globin	Mean corpus-cular hemo-globin concen-tration	Red cell distribu-tion width	Platelet count	Mean platelet volume
		RBC	HGB	HCT	MCV	MCH	MCHC	RDW-CV	PLT	MPV
Group (Day 8)		10^{12}/L	g/L	%	fL	pg	g/L	%	10^9/L	fL
Group 1 O7, 1mg/kg, 3mg/kg, SC, two doses, n=2	Mean	5.57	136.50	42.00	75.45	24.55	325.50	12.30	636.50	10.55
	SD	0.37	3.54	1.27	2.76	2.19	17.68	0.14	415.07	0.78
Group 3 O11, 1mg/kg, 3mg/kg, SC, two doses, n=2	Mean	5.98	145.50	46.70	78.65	24.500	311.500	11.550	457.500	9.650
	SD	0.75	0.71	0.42	9.12	3.253	4.950	0.212	34.648	0.212

Table 82: Complete Blood Count Day 12 (Part 1)

CBC	Measurements	Total white blood cell count	Neutro-phils	Lympho-cytes	Mono-cytes	Eosino-phils	Baso-phils	Neutro-phils	Lym-pho-cytes	Mono-cytes	Eosino-phils	Baso-phils
		WBC	%NEUT	%LYMP	%MONO	%EO	%BASO	#NEUT	#LYMP	#MONO	#EO	#BASO
Group (Day 8)		10^9/L	%	%	%	%	%	10^9/L	10^9/L	10^9/L	10^9/L	10^9/L
Group 1 O7, 1mg/kg, 3mg/kg, SC, two doses, n=2	Mean	13.04	53.90	40	5.4	1.3	0.0	7	4.9	1	0.2	0.00
	SD	2.23	20.93	20	1.6	0.4	0.0	4	1.7	0	0.1	0.00
Group 3 O11, 1mg/kg, 3mg/kg, SC, two doses, n=2	Mean	11.29	52.35	40	6.6	1.3	0.0	6	4.5	1	0.1	0.00
	SD	1.11	1.91	1	1.6	0.6	0.0	0	0.5	0	0.0	0.00

Table 83: Complete Blood Count Day 12 (Part 2)

CBC	Measurements	Red blood cell count	Hemo-globin	Hema-tocrit	Mean corpus-cular volume	Mean corpus-cular hemo-globin	Mean corpus-cular hemo-globin concen-tration	Red cell distribu-tion width	Platelet count	Mean platelet volume
		RBC	HGB	HCT	MCV	MCH	MCHC	RDW-CV	PLT	MPV
Group (Day 8)		10^{12}/L	g/L	%	fL	pg	g/L	%	10^9/L	fL
Group 1 O7, 1mg/kg, 3mg/kg, SC, two doses, n=2	Mean	5.77	127.50	41.10	71.30	22.15	310.00	12.95	619.50	11.40
	SD	0.01	6.36	2.26	3.82	1.06	1.41	0.07	396.69	2.12
Group 3 O11, 1mg/kg, 3mg/kg, SC, two doses, n=2	Mean	6.46	144.50	47.20	73.75	22.650	306.500	12.200	470.500	10.600
	SD	0.94	0.71	1.41	8.56	3.182	7.778	0.000	24.749	0.849

Clinical Chemistry

[0437] Clinical chemistry, including triglycerides, creatine kinase, lactate dehydrogenase, alanine amino transferase, aspartate amino transferase, bilirubin, alkaline phosphatase, total protein, albumin, globulin, glucose, urea nitrogen, creatine, urea acid, and C-reactive protein was assessed in monkey serum on days 1, 8 (2h), and 11 (2h). Results of each measurement are outlined in Tables 84-89.

Table 84: Clinical Chemistry Day 1 (Part 1)

Clinical Chemistry	Measurement	Direct bili-rubin	(Total) Cho-lesterol	Trigly-cerides	Creati-nine Kinase	Lactate Dehy-droge-nase	Alanine Amino-trans-ferase	Aspar-tate Amino-trans-ferase	Total Bilirubin	Alkaline Phos-phatase
		DBIL	CHO	TG	CK	LDH	ALT	AST	BIL	ALP
Group (Day 1)		umol/L	mmol/L	mmol/L	U/L	U/L	U/L	U/L	umol/L	U/L
Group 1 O7, 1mg/kg, 3mg/kg, SC, two doses, n=2	Mean	0.00	19.80	13.15	289.00	310.0	115.5	83.50	0.5	188.0
	SD	0.00	23.33	11.67	91.92	41.0	71.4	79.90	0.7	49.5
Group 3 O11, 1mg/kg, 3mg/kg, SC, two doses, n=2	Mean	0.50	13.45	5.05	301.00	319.5	81.5	47.00	2.0	203.0
	SD	0.71	14.21	3.46	124.45	145.0	38.9	32.53	0.0	53.7

Table 85: Clinical Chemistry Day 1 (Part 2)

Clinical Chemistry	Measurement	Total Protein	Albu-min	Globu-lin (calcu-lated)	Albu-min/ Globu-lin Ratio	Gamma-glutamyl trans-ferase	Glu-cose	Urea Nitro-gen	Creati-nine	Urea acid	C-reac-tive Protein
		TP	ALB	G	A/G	GGT	GLU	BUN	Cr	UA	CRP
Group (Day 1)		g/L	g/L	g/L	%	u/L	mmol/L	mmol/L	umol/L	umol/L	mg/L
Group 1 O7, 1mg/kg, 3mg/kg, SC, two doses, n=2	Mean	99.5	43.75	55.75	0.8	170.3	11.94	5.50	79.0	2	5.47
	SD	4.9	2.76	7.71	0.2	102.8	3.49	2.12	12.7	2	0.71
Group 3 O11, 1mg/kg, 3mg/kg, SC, two doses, n=2	Mean	74.9	39.60	35.30	1.1	101.4	9.83	5.50	73.0	3	2.85
	SD	0.7	4.38	3.68	0.2	20.7	1.09	2.12	14.1	1	2.60

Table 86: Clinical Chemistry Day 8 (Part 1)

Clinical Chemistry	Measurement	Direct bili-rubin	(Total) Cho-lesterol	Trigly-cerides	Creati-nine Kinase	Lactate Dehy-droge-nase	Alanine Amino-trans-ferase	Aspar-tate Amino-trans-ferase	Total Bilirubin	Alkaline Phos-phatase
		DBIL	CHO	TG	CK	LDH	ALT	AST	BIL	ALP
Group (Day 8)		umol/L	mmol/L	mmol/L	U/L	U/L	U/L	U/L	umol/L	U/L
Group 1 O7, 1mg/kg, 3mg/kg, SC, two doses, n=2	Mean	0.00	20.55	12.30	245.00	291.0	145.0	15.50	1.5	171.0
	SD	0.00	23.69	10.04	55.15	114.6	91.9	12.02	0.7	69.3
Group 3 O11, 1mg/kg, 3mg/kg, SC, two doses, n=2	Mean	0.50	13.15	3.25	253.50	276.5	85.5	33.50	2.5	157.0
	SD	0.71	11.95	0.64	200.11	94.0	36.1	9.19	0.7	22.6

Table 87: Clinical Chemistry Day 8 (Part 2)

Clinical Chemistry	Measurement	Total Protein	Albu-min	Globu-lin (calcu-lated)	Albu-min/ Globu-lin Ratio	Gamma-glutamyl trans-ferase	Glu-cose	Urea Nitro-gen	Creati-nine	Urea acid	C-reac-tive Protein
		TP	ALB	G	A/G	GGT	GLU	BUN	Cr	UA	CRP
Group (Day 8)		g/L	g/L	g/L	%	u/L	mmol/L	mmol/L	umol/L	umol/L	mg/L
Group 1 O7, 1mg/kg, 3mg/kg, SC, two doses, n=2	Mean	91.0	41.90	49.05	0.9	111.5	10.32	5.50	83.0	3	4.22
	SD	12.8	1.98	10.82	0.2	14.8	2.94	2.12	9.9	1	1.99
Group 3 O11, 1mg/kg, 3mg/kg, SC, two doses, n=2	Mean	78.5	39.35	39.15	1.0	97.0	8.58	5.50	74.0	2	2.47
	SD	4.5	4.60	9.12	0.4	22.6	0.48	0.71	15.6	1	2.07

Table 88: Clinical Chemistry Day 12 (Part 1)

Clinical Chemistry	Measurement	Direct bili-rubin	(Total) Cho-lesterol	Trigly-cerides	Creati-nine Kinase	Lactate Dehy-droge-nase	Alanine Amino-trans-ferase	Aspar-tate Amino-trans-ferase	Total Bilirubin	Alkaline Phos-phatase
		DBIL	CHO	TG	CK	LDH	ALT	AST	BIL	ALP
Group (Day 8)		umol/L	mmol/L	mmol/L	U/L	U/L	U/L	U/L	umol/L	U/L
Group 1 O7, 1mg/kg, 3mg/kg, SC, two doses, n=2	Mean	0.00	19.45	8.05	373.50	363.0	125.5	31.00	2.5	130.5
	SD	0.00	21.00	2.47	181.73	155.6	74.2	4.24	0.7	27.6
Group 3 O11, 1mg/kg, 3mg/kg, SC, two doses, n=2	Mean	0.50	11.80	3.20	260.00	309.5	117.5	36.50	4.0	143.5
	SD	0.71	11.17	1.84	172.53	54.4	19.1	0.71	1.4	2.1

Table 89: Clinical Chemistry Day 12 (Part 2)

Clinical Chemistry	Measurement	Total Protein	Albu-min	Globu-lin (calcu-lated)	Albu-min/ Globu-lin Ratio	Gamma-glutamyl trans-ferase	Glu-cose	Urea Nitro-gen	Creati-nine	Urea acid	C-reac-tive Protein
		TP	ALB	G	A/G	GGT	GLU	BUN	Cr	UA	CRP
Group (Day 8)		g/L	g/L	g/L	%	u/L	mmol/L	mmol/L	umol/L	umol/L	mg/L
Group 1 O7, 1mg/kg, 3mg/kg, SC, two doses, n=2	Mean	87.3	37.90	49.35	0.8	93.5	9.42	6.50	77.5	1	3.07
	SD	11.0	5.52	5.44	0.0	7.4	0.43	3.54	7.8	1	0.66
Group 3 O11, 1mg/kg, 3mg/kg, SC, two doses, n=2	Mean	80.3	39.00	41.30	1.0	99.5	6.10	5.50	72.0	2	2.47
	SD	0.8	5.23	4.38	0.2	21.9	2.64	0.71	14.1	0	1.65

Food Intake

[0438] Food intake (g) was assessed in monkeys on days 2, 3, 4, 9, 10, 11, and 13-15. Results are detailed in Table 90.

Table 90: Food Intake

Group	Animal ID	Day 2	Day 3	Day 4	Day 9	Day 10	Day 11	Day 13	Day 14	Day 15
Group 1 O7,1mg/kg, 3mg/kg, SC, two doses, n=2	1	166.52	246.12	234.87	152.86	237.29	284.19	149.92	258.51	358.11
	2	109.94	175.37	215.02	162.10	167.67	199.36	97.50	168.68	222.84
	Mean	138.23	210.75	224.95	157.48	202.48	241.78	123.71	213.60	290.48
	SD	40.01	50.03	14.04	6.53	49.23	59.98	37.07	63.52	95.65
Group 3 O11, 1mg/kg, 3mg/kg, SC, two doses, n=2	5	143.70	203.06	219.30	142.35	214.93	199.62	123.36	200.82	234.52
	6	74.27	170.21	148.89	56.48	139.89	163.28	56.50	118.36	162.10
	Mean	108.99	186.64	184.10	99.42	177.41	181.45	89.93	159.59	198.31
	SD	49.09	23.23	49.79	60.72	53.06	25.70	47.28	58.31	51.21

[0439] Food intake was also assessed on days 1, 8, and 12 at 0.5-, 1-, 1.5-, 2-, 3-, 4-, 24-, 48-, and 72-hours post-dose. Results are detailed in Tables 91 and 92.

Table 91: Food intake Days 1 and 8

Group		Day 1									Day 8								
		0.5 h	1 h	1.5 h	2 h	3 h	4 h	24 h	48 h	72 h	0.5 h	1 h	1.5 h	2 h	3 h	4 h	24 h	48 h	72 h
Group 1 O7 1mg/kg, 3mg/kg, SC, two doses, n=2	Mean	70.62	36.88	2.84	0.00	33.25	8.25	138.23	210.75	224.95	102.82	25.60	0.00	25.11	6.77	0.00	157.48	202.48	241.78
	SD	43.29	19.06	4.02	0.00	23.29	11.67	40.01	50.03	14.04	61.21	4.85	0.00	35.51	9.57	0.00	6.53	49.23	59.98
Group 3 O11, 1mg/kg, 3mg/kg, SC, two doses, n=2	Mean	55.26	5.71	0.00	0.00	45.86	2.05	108.99	186.64	184.10	48.12	20.72	8.60	39.07	0.00	0.00	99.42	177.41	181.45
	SD	12.67	8.07	0.00	0.00	7.01	2.90	49.09	23.23	49.79	27.03	29.30	12.16	16.14	0.00	0.00	60.72	53.06	25.70

Table 92: Food Intake Day 12

Group		Day 12								
		0.5 h	1 h	1.5 h	2 h	3 h	4 h	24 h	48 h	72 h
Group 1 O7 1mg/kg, 3mg/kg, SC, two doses, n=2	Mean	65.58	50.03	22.21	5.69	7.70	9.33	123.71	213.60	290.48
	SD	38.29	0.06	28.26	8.05	10.89	13.19	37.07	63.52	95.65
Group 3 O11, 1mg/kg, 3mg/kg, SC, two doses, n=2	Mean	57.82	49.26	6.04	9.87	12.27	0.00	89.93	159.59	198.31
	SD	7.32	1.02	8.54	13.96	17.35	0.00	47.28	58.31	51.21

Example 11: Acute Food Intake

[0440] Food intake was assessed in 6 diet-induced obese Cynomolgus monkeys (n=6). Monkeys were first administered saline on day 1 (D1) and food intake was measured (FIGS. 11A-11D). On day 8 and day 12, monkeys were administered 1 mg/kg O7 (Group 1; n=2), or O11 (Group 3; n=2) (FIGS. 12A-12H). Monkeys were fasted overnight prior to administration. Food was administered 2 hours after each dose and intake was measured at 0.5-, 1-, 1.5-, 2-, 3-, 4-, 24-, 48-, and 72-hours after dosing. Group 2 monkeys demonstrated a decrease in food intake relative to saline dosing (FIGS. 13A-13D).

Example 12: Caloric Intake, Body Weight, and Body Composition

Food Intake and Body Composition in Monkeys

[0441] Changes in caloric intake and body weight were assessed in diet-induced obese Cynomolgus monkeys (n=6). Monkeys were orally administered 10 mg/kg O7 (MC4R selective agonist). One day 15, dosing changed to 15/mg/kg administered twice daily, and dosing was maintained until at least day 28 and monkeys were assessed until at least day 35. Control monkeys were administered saline. Daily caloric intake (FIGS. 14A), cumulative caloric intake (FIGS. 14B and 15A), percent change in caloric consumption, relative to baseline (FIGS. 14C, 15B), and caloric intake per body weight were measured in addition to food intake (see also FIGS. 15A-15J; Tables 93-111). Cumulative caloric intake and percent change in caloric intake, relative to baseline, were each reduced in the monkeys administered O7, relative to those administered saline. Changes in body composition and body weight were also assessed (Tables 113-115; FIGS. 16A-16G).

Table 93: Animal Pre-screening and Day 1 Food Intake

Food intake (g)										
Group	**Measurement**	**animal pre-screening**			**animal pre-screening**			**Day 1**		
		AM	**PM**	**Fruit**	**AM**	**PM**	**Fruit**	**AM**	**PM**	**Fruit**
Group 1 saline, p.o., QD, n=8	Mean	28.72	129.69	--	9.66	137.21	--	--	101.98	134.85
	SD	20.42	23.14	--	18.57	24.27	--	--	46.60	33.20

Food intake (g)										
Group	Measurement	animal pre-screening			animal pre-screening			Day 1		
		AM	PM	Fruit	AM	PM	Fruit	AM	PM	Fruit
Group 3 O7, p.o., n=8 10mg/kg (Day 1-8), QD 20mg/kg (Day 9-14), QD 15mg/kg (Started on Day 15), BID	Mean	37.50	142.88	--	19.82	143.68	--	--	123.59	126.65
	SD	50.62	9.70	--	21.86	16.05	--	--	48.41	46.08

Table 94: Days 2-4 Food Intake

Food intake (g)										
Group	Measurement	Day 2			Day 3			Day 4		
		AM	PM	Fruit	AM	PM	Fruit	AM	PM	Fruit
Group 1 saline, p.o., QD, n=8	Mean	1.81	137.23	133.89	3.51	132.73	133.32	14.41	122.99	144.17
	SD	2.15	17.31	44.67	5.30	28.92	46.34	16.15	36.95	11.40
Group 3 O7, p.o., n=8 10mg/kg (Day 1-8), QD 20mg/kg (Day 9-14), QD 15mg/kg (Started on Day 15), BID	Mean	6.89	88.00	113.70	7.57	105.59	120.80	23.43	111.29	135.31
	SD	7.93	36.53	49.36	12.26	30.27	44.77	31.69	27.69	26.67

Table 95: Days 5-7 Food Intake

Food intake (g)										
Group	Measurement	Day 5			Day 6			Day 7		
		AM	PM	Fruit	AM	PM	Fruit	AM	PM	Fruit
Group 1 saline, p.o., QD, n=8	Mean	25.21	102.17	149.29	19.46	108.73	150.05	10.91	95.27	148.06
	SD	26.74	43.46	4.08	25.03	41.71	0.97	16.18	49.78	6.52
Group 3 O7, p.o., n=8 10mg/kg (Day 1-8), QD 20mg/kg (Day 9-14), QD 15mg/kg (Started on Day 15), BID	Mean	14.41	111.62	151.36	14.83	120.38	133.92	8.00	101.08	135.91
	SD	17.93	32.27	3.94	19.57	29.22	39.74	10.71	42.92	27.34

Table 96: Days 8-10 Food Intake

Food intake (g)										
Group	Measurement	Day 8			Day 9			Day 10		
		AM	PM	Fruit	AM	PM	Fruit	AM	PM	Fruit
Group 1 saline, p.o., QD, n=8	Mean	27.26	128.54	148.97	15.15	125.90	149.79	18.03	117.30	150.51
	SD	32.10	22.81	3.49	21.49	25.72	1.99	19.40	41.84	2.50
Group 3 O7, p.o., n=8 10mg/kg (Day 1-8), QD 20mg/kg (Day 9-14), QD 15mg/kg (Started on Day 15), BID	Mean	28.74	106.34	120.35	8.11	132.51	150.78	13.54	125.25	143.26
	SD	17.39	31.69	46.38	10.89	16.50	2.72	19.14	24.52	22.03

Table 97: Days 11-13 Food Intake

Food intake (g)										
Group	Measurement	Day 11			Day 12			Day 13		
		AM	PM	Fruit	AM	PM	Fruit	AM	PM	Fruit
Group 1 saline, p.o., QD, n=8	Mean	20.47	116.25	150.87	24.48	127.92	150.63	27.38	119.84	151.49
	SD	26.24	40.09	1.98	26.57	33.06	1.66	26.31	34.66	1.69
Group 3 O7, p.o., n=8 10mg/kg (Day 1-8), QD 20mg/kg (Day 9-14), QD 15mg/kg (Started on Day 15), BID	Mean	22.09	126.58	147.00	17.49	132.44	150.56	21.04	128.96	142.00
	SD	16.81	22.78	7.73	14.36	18.08	1.96	28.86	18.35	25.57

Table 98: Day 14 Food Intake

Food intake (g)				
Group	Measurement	Day 14		
		AM	PM	Fruit
Group 1 saline, p.o., QD, n=8	Mean	19.44	111.72	150.31
	SD	26.36	35.18	1.48
Group 3 O7, p.o., n=8 10mg/kg (Day 1-8), QD 20mg/kg (Day 9-14), QD 15mg/kg (Started on Day 15), BID	Mean	17.60	113.79	127.35

Food intake (g)				
Group	Measurement	Day 14		
		AM	PM	Fruit
	SD	9.07	38.89	31.65

Table 99: Days 15 and 16 Food Intake

Group	Animal measurement	Day 15			Day 16		
		AM	PM	Fruit	AM	PM	Fruit
Group 1 saline, p.o., QD, n=8	Mean	38.5925	113.9	149.4325	25.0475	114.545	150.6875
	SD	31.80052	52.34367	3.785973	31.71758	27.82156	1.56245
Group 3 O7, p.o., n=8 10mg/kg (Day 1-8), QD 20mg/kg (Day 9-14), QD 15mg/kg (Started on Day 15), BID	Mean	24.9225	118.36	135.0813	14.92375	107.4925	128.7475
	SD	17.2302	28.41367	23.24318	7.975183	30.82759	32.694

Table 100: Days 17 and 18 Food Intake

Group	Animal measurement	Day 17			Day 18		
		AM	PM	Fruit	AM	PM	Fruit
Group 1 saline, p.o., QD, n=8	Mean	21.95375	115.435	137.9788	46.33	94.53875	151.525
	SD	26.0538	49.55535	39.41042	23.94622	34.91529	3.11345
Group 3 O7, p.o., n=8 10mg/kg (Day 1-8), QD 20mg/kg (Day 9-14), QD 15mg/kg (Started on Day 15), BID	Mean	26.09	100.43	126.2113	32.9525	114.09	97.38875
	SD	16.33548	23.0996	43.83899	23.52256	18.05141	66.04801

Table 101: Days 19 and 20 Food Intake

Group	Animal measurement	Day 19			Day 20		
		AM	PM	Fruit	AM	PM	Fruit
Group 1 saline, p.o., QD, n=8	Mean	33.3325	115.7438	146.6225	16.05625	133.62	122.9888
	SD	32.53471	40.65942	10.73163	23.02026	26.20351	54.93729
Group 3 O7, p.o., n=8 10mg/kg (Day 1-8), QD 20mg/kg (Day 9-14), QD 15mg/kg (Started on Day 15), BID	Mean	26.685	96.25	128.36	12.84	95.26125	113.4925
	SD	15.79153	34.65562	45.9885	10.77898	27.15248	57.53523

Table 102: Days 21 and 22 Food Intake

Group	Animal measurement	Day 21			Day 22		
		AM	PM	Fruit	AM	PM	Fruit (Apple)
Group 1 saline, p.o., QD, n=8	Mean	28.42875	107.7988	149.7738	14.545	122.4825	149.9025
	SD	28.2244	31.03882	1.900263	19.27357	30.43795	1.79632
Group 3 O7, p.o., n=8 10mg/kg (Day 1-8), QD 20mg/kg (Day 9-14), QD 15mg/kg (Started on Day 15), BID	Mean	32.8825	103.7088	144.2675	17.18	113.7175	126.9188
	SD	24.11543	36.88398	19.42615	20.22404	24.62287	41.96281

Table 103: Days 24 and 25 Food Intake

Group	Measurement	Day 24			Day 25		
		AM	PM	Fruit	AM	PM	Fruit
Group 1 saline, p.o., QD, n=8	Mean	15.1875	125.255	150.7588	18.29875	122.3863	139.4363
	SD	16.80568	27.85392	1.14588	20.85324	43.51322	29.77701
Group 3 O7, p.o., n=8 10mg/kg (Day 1-8), QD 20mg/kg (Day 9-14), QD 15mg/kg (Started on Day 15), BID	Mean	18.6825	127.1013	105.1063	10.93625	104.0063	122.5688
	SD	17.23201	25.16601	56.42719	14.95893	26.4806	41.49931

Table 104: Days 26 and 27 Food Intake

Group	Measurement	Day 26			Day 27		
		AM	PM	Fruit	AM	PM	Fruit
Group 1 saline, p.o., QD, n=8	Mean	12.57	121.77	149.07	22.03375	98.49875	145.58
	SD	24.40542	34.99332	4.822779	22.90044	46.41777	12.71348
Group 3 O7, p.o., n=8 10mg/kg (Day 1-8), QD 20mg/kg (Day 9-14), QD 15mg/kg (Started on Day 15), BID	Mean	19.55125	119.0588	117.25	23.6375	126.07	126.8338
	SD	18.66904	22.21352	50.64362	17.21742	34.07497	40.74194

Table 105: Days 28 and 29 Food Intake

Group	Measurement	Day 28			Day 29		
		AM	PM	Fruit	AM	PM	Fruit
Group 1 saline, p.o., QD, n=8	Mean	21.51125	118.5263	150.7088	N/A	56.8875	68.36875
	SD	23.64851	34.31948	3.056176	N/A	46.25776	60.77436
	Mean	15.2875	103.43	131.76	14.945	81.82	117.8388

Group 3 O7, p.o., n=8 10mg/kg (Day 1-8), QD 20mg/kg (Day 9-14), QD 15mg/kg (Started on Day 15), BID	SD	18.42535	36.34806	37.07991	19.00773	44.25872	46.24757

Table 106: Day 30 Food Intake

Group	Measurement	Day 30		
		AM	PM	Fruit
Group 1 saline, p.o., QD, n=8	Mean	11.67375	104.2775	144.1688
	SD	11.46482	50.89431	10.84557
Group 3 O7, p.o., n=8 10mg/kg (Day 1-8), QD 20mg/kg (Day 9-14), QD 15mg/kg (Started on Day 15), BID	Mean	19.62625	113.3375	134.1988
	SD	19.08369	27.37875	23.29181

Table 107: Food Intake (Calorie) Prescreening and Days 1-4

Food intake (calorie)							
Group	**Measurement**	**animal pre-screening**	**animal pre-screening**	**Day 1**	**Day 2**	**Day 3**	**Day 4**
Group 1 saline, p.o., QD, n=8	Mean	659.49	625.15	509.65	667.41	653.67	655.49
	SD	132.80	139.20	194.71	74.58	131.91	192.58
Group 3 O7, p.o., n=8 10mg/kg (Day 1-8), QD 20mg/kg (Day 9-14), QD 15mg/kg (Started on Day 15), BID	Mean	747.06	688.64	598.53	462.52	544.41	632.00
	SD	190.97	104.27	229.48	171.21	156.80	134.76

Table 108: Days 5-10 Food Intake (Calorie)

Food intake (calorie)							
Group	**Measurement**	**Day 5**	**Day 6**	**Day 7**	**Day 8**	**Day 9**	**Day 10**
Group 1 saline, p.o., QD, n=8	Mean	606.22	614.76	525.76	726.88	634.60	607.80
	SD	251.70	194.12	236.27	146.38	136.17	212.02

Food intake (calorie)							
Group	Measurement	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Group 3 O7, p.o., n=8 10mg/kg (Day 1-8), QD 20mg/kg (Day 9-14), QD 15mg/kg (Started on Day 15), BID	Mean	610.24	640.35	534.32	621.52	638.70	624.46
	SD	151.87	167.12	200.58	173.88	79.96	156.75

Table 109: Days 11-14 Food Intake (Calorie)

Food intake (calorie)					
Group	Measurement	Day 11	Day 12	Day 13	Day 14
Group 1 saline, p.o., QD, n=8	Mean	611.92	676.21	651.73	627.71
	SD	192.20	198.49	207.14	200.32
Group 3 O7, p.o., n=8 10mg/kg (Day 1-8), QD 20mg/kg (Day 9-14), QD 15mg/kg (Started on Day 15), BID	Mean	661.09	671.20	666.36	618.23
	SD	126.98	117.15	158.99	174.49

Table 110: Days 15-22 Food Intake (Calorie)

Group	Measure-ment	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22
Group 1 saline, p.o., QD, n=8	Mean	703.6877	659.7127	646.1119	648.41	691.763	696.0532	603.0544	656.7564
	SD	286.849	149.1018	237.1083	181.2112	199.8555	160.5247	178.8588	169.8756
Group 3 O7, p.o., n=8 10mg/kg (Day 1-8), QD 20mg/kg (Day 9-14), QD 15mg/kg (Started on Day 15), BID	Mean	667.6026	582.4745	589.7982	657.7038	574.9822	514.5321	599.583	616.2502
	SD	172.8821	145.7327	135.4153	155.6394	187.2511	144.5283	238.6645	163.3052

Table 111: Days 24-30 Food Intake (Calorie)

Group	Measurement	Day 24	Day 25	Day 26	Day 27	Day 28	Day 29	Day 30
Group 1 saline, p.o.,	Mean	671.39985	669.8996857	639.0352714	611.6815571	635.8829571	303.0223429	560.8711571
	SD	142.3724953	248.2354969	179.5501003	237.7878076	176.5516399	224.4573731	255.6706537

QD, n=8								
Group 3 O7, p.o., n=8 10mg/kg (Day 1-8), QD 20mg/kg (Day 9-14), QD 15mg/kg (Started on Day 15), BID	Mean	888.7684	803.1586	839.8799	923.7256	786.0597	830.0146	911.7182
	SD	621.0722	457.6885	526.0957	633.5051	368.6682	376.9992	516.0133

Table 112: Body Composition at Baseline (Part 1)

Group	Measurement	Body weight	Body height	BMI	Whole body bone mineral content	Trunk bone mineral content	Bone density	Trunk fat mass
		kg	m	kg/m2	g	g	g/cm^2	g
Group 1 saline, p.o., QD, n=8	Mean	11.3925	0.46875	52.39938907	395.65	135.4875	0.83825	2489
	SD	2.05281514	0.03044315 5	11.14473132	23.2690475	14.9020073	0.04560310 1	1268.159972
Group 3 O7, p.o., n=8 10mg/kg (Day 1-8), QD 20mg/kg (Day 9-14), QD 15mg/kg (Started on Day 15), BID	Mean	11.385	0.45875	53.92335511	405.5125	139.8625	0.8525	2511.25
	SD	2.35897677 6	0.02356601 7	8.64225431 8	45.3776357	15.4321498 8	0.04200680 2	1645.133842

Table 113: Body Composition at Baseline (Part 2)

Group	Measurement	Trunk fat mass	Trunk muscle mass	Trunk muscle mass	Fat mass	Fat mass	Muscle mass	Muscle mass
		%	g	%	g	%	g	%
Group 1 saline, p.o., QD, n=8	Mean	38.7625	3605.375	59.83061715	3287	30.75	6970.25	66.5853178
	SD	11.65405356	424.2802098	11.17204892	1583.54557	9.653570768	668.0149164	8.876878753
Group 3 O7, p.o., n=8 10mg/kg (Day 1-8), QD 20mg/kg (Day 9-14), QD 15mg/kg (Started on Day 15), BID	Mean	38.5875	3590.625	59.93177536	3317.75	30.7625	6856.5	66.44445658
	SD	10.81327722	369.9768091	10.39278614	2102.126729	10.69057495	586.1135921	9.957953076

Table 114: Body Weight (kg) (Day 1-22)

		Day 1	Day 8	Day 15	Day 22	Day 29
Group 1 saline, p.o., QD, n=8	Mean	11.44	11.36	11.39	11.46	11.45
	SD	1.99	2.05	2.04	2.00	2.01
Group 3 O7, p.o., n=8 10mg/kg (Day 1-8), QD 20mg/kg (Day 9-14), QD 15mg/kg (Started on Day 15), BID	Mean	11.29	11.21	11.33	11.27	11.22
	SD	2.08	2.02	1.92	1.96	1.94

Table 115: Body Weight (Percent Change)

Group	Animal Measurement	Day 1	Day 8	Day 15	Day 22	Day 29
Group 1 saline, p.o., QD, n=8	Mean	0.0	-0.8	-0.5	0.2	0.1
	SD	0.0	1.2	1.3	1.7	2.0

Group	Animal Measurement	Day 1	Day 8	Day 15	Day 22	Day 29
Group 3 O7, p.o., n=8 10mg/kg (Day 1-8), QD 20mg/kg (Day 9-14), QD 15mg/kg (Started on Day 15), BID	Mean	0.0	-0.6	0.6	0.1	-0.4
	SD	0.0	2.2	3.2	3.8	4.1

[0442] Blood chemistry, clinical chemistry, gastric impact, and nausea levels were also assessed (Tables 116-127). Cage side observations showed no signs of gastric distress (assessed by stool changes) or nausea (assessed by food refusal).

Table 116: Clinical Chemistry Day 1 (Part A)

Clinical Chemistry		Glucose	Alanine Aminotransferase	Aspartate Aminotransferase	Alkaline Phosphatase	Gamma-glutamyl transferase	Total Bilirubin	Total Protein	Triglycerides
		GLU	ALT	AST	ALP	GGT	TBIL	TP	TG
Day 1		mmol/L	U/L	U/L	U/L	u/L	umol/L	g/L	mmol/L
Group 1 saline, p.o., QD, n=8	Mean	3.9287 5	32.125	44	164.0375	90.5375	2.25	79.1125	1.7125
	SD	0.6745 25	8.576338	5.451081	65.57861	30.35664	0.886405	6.001771	1.418689
Group 3 O7, p.o., n=8 10mg/kg (Day 1-8), QD 20mg/kg (Day 9-14), QD 15mg/kg (Started on Day 15), BID	Mean	3.885	50.625	55.75	165.6625	84.875	2.375	78.225	0.625
	SD	0.7733 78	44.30717	27.47856	84.43068	23.5047	0.744024	6.845802	0.271241

Table 117: Clinical Chemistry Day 1 (Part B)

Clinical Chemistry		(Total) Cholesterol	Albumin	High density lipoprotein cholesterol	Low density lipoprotein cholesterol	Lactate Dehydrogenase	Creatinine	Urea Nitrogen	Creatinine Kinase
		CHO	ALB	HDL-C	LDL-C	LDH	CREA	UREA	CK
Day 1		mmol/L	g/L	mmol/L	mmol/L	U/L	umol/L	mmol/L	U/L
Group 1 saline, p.o., QD, n=8	Mean	8	38.225	1.4875	4.65	693.875	102.75	5.125	238.75
	SD	3.824358	2.860944	0.313676	3.061279	231.6318	15.69122	0.991031	86.59553
Group 3 O7, p.o., n=8 10mg/kg (Day 1-8), QD 20mg/kg (Day 9-14), QD 15mg/kg (Started on Day 15), BID	Mean	7.5	38.0125	1.725	3.975	604.125	114.5	5.75	530.25
	SD	3.274141	4.363301	0.286606	2.574462	170.6956	28.29942	1.669046	837.5634

Table 118: Clinical Chemistry Day 15 (Part A)

Clinical Chemistry	Measurement	Glucose	Alanine Aminotransferase	Aspartate Aminotransferase	Alkaline Phosphatase	Gamma-glutamyl transferase	Total Bilirubin	Total Protein	Triglycerides
		GLU	ALT	AST	ALP	GGT	TBIL	TP	TG
Day 15		mmol/L	U/L	U/L	U/L	u/L	umol/L	g/L	mmol/L

Group 1 saline, p.o., QD, n=8	Mean	8.813 75	28	25.5	142.5625	84.6125	1.625	76.75	1.75
	SD	4.7206 54	9.242758	4.6291	47.90019	30.27009	0.517549	5.35883 8	1.75987
Group 3 O7, p.o., n=8 10mg/kg (Day 1-8), QD 20mg/kg (Day 9-14), QD 15mg/kg (Started on Day 15), BID	Mean	6.157 5	40.625	27.25	241.1375	78.1125	1.5	76.6125	1.0625
	SD	1.9235 22	23.36626	5.175492	115.0788	23.88642	0.755929	5.95877 2	0.66319

Table 119: Clinical Chemistry Day 15 (Part B)

Clinical Chemistry	Measurement	(Total) Cholesterol	Albumin	High density lipoprotein cholesterol	Low density lipoprotein cholesterol	Lactate Dehydrogenase	Creatinine	Urea Nitrogen	Creatinine Kinase
		CHO	ALB	HDL-C	LDL-C	LDH	CREA	UREA	CK
Day 15		mmol/L	g/L	mmol/L	mmol/L	U/L	umol/L	mmol/L	U/L
Group 1 saline, p.o., QD, n=8	Mean	8.8	38.5	1.7	5.4125	266.625	95.875	3.625	129.5
	SD	5.218648	3.685493	0.40708	4.058655	59.62247	15.16987	1.505941	28.15462
Group 3 O7, p.o., n=8 10mg/	Mean	7.6375	36.5625	1.7375	4.5625	309.25	98.625	3	147.0625
	SD	3.35897	3.599	0.35431	2.481899	122.4427	20.47952	0.92582	81.1532

kg (Day 1-8), QD 20mg/kg (Day 9-14), QD 15mg/kg (Started on Day 15), BID			181						

Table 120: Clinical Chemistry Day 29 (Part A)

Clinical Chemistry	Measurement	Glucose	Alanine Aminotransferase	Aspartate Aminotransferase	Alkaline Phosphatase	Gamma-glutamyl transferase	Total Bilirubin	Total Protein	Triglycerides
		GLU	ALT	AST	ALP	GGT	TBIL	TP	TG
Day 29		mmol/L	U/L	U/L	U/L	u/L	umol/L	g/L	mmol/L
Group 1 saline, p.o., QD, n=8	Mean	8.48125	25.125	28.5	160	88.7125	1.125	73.65	2.375
	SD	4.454434	10.52124	5.682052	42.4937	34.42484	0.64087	4.809811	1.891038 9
Group 3 O7, p.o., n=8 10mg/kg (Day 1-8), QD 20mg/kg (Day 9-14), QD 15mg/kg (Started	Mean	5.6375	35	26.625	311	81.7	1.375	75.025	0.8625
	SD	2.754932	18.58571	4.405759	120.4563	27.97029	0.744024	7.185054	0.531675

d on Day 15), BID									

Table 121: Clinical Chemistry Day 29 (Part B)

Clinical Chemistry	Measurement	(Total) Cholesterol	Albumin	High density lipoprotein cholesterol	Low density lipoprotein cholesterol	Lactate Dehydrogenase	Creatinine	Urea Nitrogen	Creatinine Kinase
		CHO	ALB	HDL-C	LDL-C	LDH	CREA	UREA	CK
Day 29		mmol/L	g/L	mmol/L	mmol/L	U/L	umol/L	mmol/L	U/L
Group 1 saline, p.o., QD, n=8	Mean	9.6375	35.425	1.7	5.9875	399.125	93.625	6	120.825
	SD	5.233119	3.162165	0.414039	4.046317	123.6891	13.75227	2	44.37901
Group 3 O7, p.o., n=8 10mg/kg (Day 1-8), QD 20mg/kg (Day 9-14), QD 15mg/kg (Started on Day 15), BID	Mean	8.05	33.55	1.6625	4.775	293.25	99.375	4.25	121.15
	SD	3.04584	3.185234	0.329231	2.195287	57.20327	22.01907	0.886405	45.52343

Table 122: Complete Blood Count Day 1 (Part A)

CBC	Measurement	Total white blood cell count	Red blood cell count	Hemoglobin	Hematocrit	Mean corpuscular	Mean corpuscular hem	Mean corpuscular hem	Red cell distribution width	Platelet count	Mean platelet volume

						volume	oglobin	oglobin concentration			
		WBC	RBC	HGB	HCT	MCV	MCH	MCHC	RDW	PLT	MPV
Day 1		109/L	1012/L	g/L	%	fL	pg	g/L	%	109/L	fL
Group 1 saline, p.o., QD, n=8	Mean	23.52	5.96125	137	46.275	77.4375	22.9375	296.125	13.6125	430.625	11.75
	SD	5.864472	0.604375	18.53953	6.136716	4.133465	1.622113	7.079901	0.865922	36.52763	1.409154
Group 3 O7, p.o., n=8 10mg/kg (Day 1-8), QD 20mg/kg (Day 9-14), QD 15mg/kg (Started on Day 15), BID	Mean	18.5675	5.98	131.625	44.3625	74.225	22.025	296.875	13.8625	443.625	11.175
	SD	3.61778	0.443976	11.2496	3.645325	3.686171	1.268013	7.453427	0.755811	78.89403	0.906721

Table 123: Complete Blood Count Day 1 (Part B)

CBC	Measurement	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
		#NEUT	#LYMP	#MONO	#EOS	#BASO	%NEUT	%LYMP	%MONO	%EOS	%BASO
Day 1		109/L	109/L	109/L	109/L	109/L	%	%	%	%	%
Group	Mean	17.47125	4.53	1.42625	0.0925	0	74.025	19.475	6.1	0.4	0

1 saline, p.o., QD, n=8	SD	4.98689	1.737354	0.5439	0.070051	0	6.980534	6.10708	1.705453	0.282843	0
Group 3 O7, p.o., n=8 10 mg/kg (Day 1-8), QD 20 mg/kg (Day 9-14), QD 15 mg/kg (Started on Day 15), BID	Mean	14.32375	3.3425	0.83375	0.0675	0	76.35	18.6125	4.6125	0.425	0
	SD	3.835291	1.001581	0.327891	0.113484	0	7.580426	6.901229	1.899953	0.772288	0

Table 124: Complete Blood Count Day 15 (Part A)

CBC	Measurement	Total white blood cell count	Red blood cell count	Hemoglobin	Hematocrit	Mean corpuscular volume	Mean corpuscular hemoglobin	Mean corpuscular hemoglobin concentration	Red cell distribution width	Platelet count	Mean platelet volume
		WBC	RBC	HGB	HCT	MCV	MCH	MCHC	RDW	PLT	MPV

Day 15		109/L	1012/L	g/L	%	fL	pg	g/L	%	109/L	fL
Group 1 saline, p.o., QD, n=8	Mean	15.565	6.0275	138.375	47.15	78.125	23	293.5	13.8	423.75	11.2125
	SD	2.62518	0.530815	15.59247	5.160288	3.732268	1.600893	8.194075	0.884792	50.56467	1.409597
Group 3 O7, p.o., n=8 10mg/kg (Day 1-8), QD 20mg/kg (Day 9-14), QD 15mg/kg (Started on Day 15), BID	Mean	15.16	5.805	127.5	43.375	74.8125	21.9875	294.125	14.125	516	9.525
	SD	4.559254	0.577359	11.91638	4.209428	3.527216	1.244918	5.642631	0.820714	164.711	0.582482

Table 125: Complete Blood Count Day 15 (Part B)

CBC	Measurement	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
		#NEUT	#LYMP	#MONO	#EOS	#BASO	%NEUT	%LYMP	%MONO	%EOS	%BASO
Day 15		109/L	109/L	109/L	109/L	109/L	%	%	%	%	%
Group 1 saline, p.o., QD, n=8	Mean	6.4775	7.53875	1.24875	0.29125	0.00875	41.7	48.475	8	1.775	0.05
	SD	2.365271	2.116604	0.381143	0.208562	0.00991	13.48947	11.73551	1.797617	1.072714	0.053452
Group 3	Mean	7.06875	6.62625	1.1675	0.28875	0.00875	45.65	45.0125	7.3875	1.8875	0.0625

O7, p.o., n=8 10mg/kg (Day 1-8), QD 20mg/kg (Day 9-14), QD 15mg/kg (Started on Day 15), BID	SD	4.026539	2.742246	0.61981	0.30498	0.008345	14.87932	15.44635	1.727457	1.648755	0.051755

Table 126: Complete Blood Count Day 29 (Part A)

		WBC	RBC	HGB	HCT	MCV	MCH	MCHC	RDW	PLT	MPV
Day 29		109/L	1012/L	g/L	%	fL	pg	g/L	%	109/L	fL
Group 1 saline, p.o., QD, n=8	Mean	15.8825	6.1775	143.25	48.225	78.0125	23.1875	297.25	13.65	418.5	11.9125
	SD	3.156778	0.523416	14.99286	5.10091	3.985845	1.535706	7.025464	0.81416	45.64772	1.453506
Group 3 O7, p.o., n=8 10mg/kg (Day 1-8), QD 20mg/kg (Day 9-14), QD 15mg/kg (Started on Day 15), BID	Mean	13.3	5.7975	127.125	43.1875	74.65	21.9625	294.25	13.925	476.5	10.5625
	SD	4.643998	0.588066	12.28748	4.083219	3.50795	1.203492	5.175492	0.77817	107.2767	0.641288

Table 127: Complete Blood Count Day 29 (Part B)

CBC	Measurement	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
		#NEUT	#LYMP	#MONO	#EOS	#BASO	%NEUT	%LYMP	%MONO	%EOS	%BASO
Day 29		109/L	109/L	109/L	109/L	109/L	%	%	%	%	%
Group 1 saline, p.o., QD, n=8	Mean	5.888 75	8.415	1.218 75	0.355	0.005	37.96 25	52.38 75	7.55	2.075	0.025
	SD	1.712 729	2.739 859	0.417 182	0.269 603	0.009 258	12.21 942	11.01 498	1.481 312	1.309 035	0.046 291
Group 3 O7, p.o., n=8 10mg/kg (Day 1-8), QD 20mg/kg (Day 9-14), QD 15mg/kg (Started on Day 15), BID	Mean	6.691 25	5.373 75	0.978 75	0.25	0.006 25	49.12 5	41.87 5	7.275	1.675	0.05
	SD	3.343 712	1.916 857	0.435 97	0.331 318	0.007 44	12.48 379	12.87 642	1.383 319	1.748 264	0.053 452

Food Intake and Body Composition in Rats

[0443] Normalized cumulative food consumption (FIG. 15K) and percent change in body weight (FIG. 16K) were assessed in rats administered MC4R selective agonists (A07D, O7, O11) (n=5) or MC3R/MC4R co-agonists (O10) compared to saline controls (n=4). For each treatment group, rats were subcutaneously administered 0.5 mg/kg of A07D, O7, O10, or O11 for days 1-7 and 1 mg/kg for days 8-17. O11 and O7 did not appear to influence food consumption as a percent of food consumption in saline-treated rats, relative to O10 (FIG. 15K). At 17 days, O10 treated rats gained only about 0.64% of their body weight compared to saline, which gained about 8.98% body weight (**) (FIG. 16J). O10-treated rats gained less weight than O7-treated rats (****), A07D-treated rats (*), and O11-treated rats (p=.066). O10, an MC3R/MC4R coagonist, also exhibited a stronger influence on caloric consumption, as measures by cumulative food consumption) than the MC4R selective agonists. However, both the MC4R selective

agonists and the MC3R/MC4R coagonists were each more effective in reducing body weight relative to saline controls.

[0444] Rats were also assessed for blood glucose levels following subcutaneous administration of 1 mg/kg or 3 mg/kg A07D using an oral glucose tolerance test (OGTT). Blood glucose was monitored at day 0 (FIG. 19A), day 8 (FIG. 19B), and day 19 (FIG. 19C). Blood glucose was significantly reduced by day 8 in rats administered A07D at both 1 mg/kg and 3 mg/kg compared to saline controls (FIG. 19D).

Example 13: Assessing Bioavailability

A07D

[0445] Various pharmacokinetic parameters of A07D were assessed in the plasma of male cynomolgus monkeys fasted prior to dosing A07D at 1 mg/kg intravenously, 10 mg/kg orally, or 30 mg/kg orally. Results are shown in Table 128 and FIG. 22.

Table 128: A07D Results

IV bolus1 (1 mg/kg)							
Time (h)	C1001	C1002	C1003	Mean		SD	CV (%)
--	Plasma Concentration (ng/mL)						
0.083	9594	5458	5566	6873	±	2357	34.3
0.25	6334	3905	3783	4674	±	1439	30.8
0.5	5668	3474	3125	4089	±	1379	33.7
1	4917	2312	2499	3243	±	1453	44.8
2	3191	1673	1687	2184	±	872	40.0
4	1869	868	830	1189	±	589	49.6
6	1249	602	465	772	±	419	54.2
8	871	324	238	478	±	343	71.9
24	82.4	17.3	12.6	37.4	±	39.0	104
PK Parameters	C1001	C1002	C1003	Mean		SD	CV (%)
Rsq_adj	0.999	0.995	0.989	--	±	--	--
No. points used for $T_{1/2}$	3.00	4.00	3.00	ND	±	--	--
C_0 (ng/mL)	11793	6446	6744	8328	±	3005	36.1
$T_{1/2}$ (h)	4.63	3.57	3.57	3.92	±	0.612	15.6
Vd_{ss} (L/kg)	0.201	0.328	0.313	0.281	±	0.0694	24.7
Cl(mL/min/kg)	0.633	1.37	1.48	1.16	±	0.461	39.7
T_{last} (h)	24.0	24.0	24.0	24.0	±	--	--
$AUC_{0\text{-}last}$ (ng.h/mL)	25795	12071	11189	16352	±	8190	50.1
$AUC_{0\text{-}inf}$ (ng.h/mL)	26346	12160	11254	16587	±	8464	51.0
$MRT_{0\text{-}last}$ (h)	4.76	3.81	3.37	3.98	±	0.710	17.8
$MRT_{0\text{-}inf}$ (h)	5.31	3.99	3.52	4.27	±	0.928	21.7
AUC_{Extra} (%)	2.09	0.733	0.577	1.13	±	0.832	73.4

$AUMC_{Extra}$ (%)	12.1	5.35	4.78	7.41	±	4.07	54.9

PO2 (10 mg/kg)							
Time (h)	C2001	C2002	C2003	Mean		SD	CV (%)
--	Plasma Concentration (ng/mL)						
0.083	2.85	3.83	8.58	5.09	±	3.06	60.3
0.25	20.2	101	23.2	48.1	±	45.8	95.2
0.5	67.0	418	74.9	187	±	200	107
1	85.1	450	111	215	±	204	94.5
2	174	408	79.6	221	±	169	76.7
4	136	240	59.8	145	±	90.5	62.3
6	90.9	171	45.3	102	±	63.6	62.1
8	53.7	71.5	23.2	49.5	±	24.4	49.4
24	8.75	7.42	2.45	6.21	±	3.32	53.5
PK Parameters	C2001	C2002	C2003	Mean		SD	CV (%)
Rsq_adj	0.974	0.963	0.987	--	±	--	--
No. points used for $T_{1/2}$	3.00	5.00	5.00	ND	±	--	--
C_{max} (ng/mL)	174	450	111	245	±	180	73.6
T_{max} (h)	2.00	1.00	1.00	1.33	±	0.577	43.3
$T_{1/2}$ (h)	5.61	3.93	4.37	4.64	±	0.871	18.8
T_{last} (h)	24.0	24.0	24.0	24.0	±	--	--
$AUC_{0\text{-}last}$ (ng.h/mL)	1251	2441	613	1435	±	928	64.7
$AUC_{0\text{-}inf}$ (ng.h/mL)	1321	2483	628	1477	±	937	63.4
$MRT_{0\ last}$ (h)	6.94	5.01	5.74	5.90	±	0.974	16.5
$MRT_{0\text{-}inf}$ (h)	8.29	5.43	6.34	6.69	±	1.46	21.9
AUC_{Extra} (%)	5.36	1.69	2.46	3.17	±	1.94	61.0
$AUMC_{Extra}$ (%)	20.7	9.24	11.7	13.9	±	6.03	43.5
Bioavailability (%)[a]	-	-	-	0.890	--	--	--

PO3 (30 mg/kg)							
Time (h)	C3001	C3002	C3003	Mean		SD	CV (%)
--	Plasma Concentration (ng/mL)						
0.083	17.0	10.4	33.8	20.4	±	12.1	59.1
0.25	108	24.0	97.6	76.5	±	45.8	59.8
0.5	431	37.3	171	213	±	200	93.9
1	504	122	236	287	±	196	68.3
2	502	427	326	418	±	88.3	21.1
4	332	320	244	299	±	47.7	16.0
6	220	190	180	197	±	20.8	10.6
8	120	124	163	136	±	23.8	17.5
24	57.7	19.1	39.6	38.8	±	19.3	49.8
PK Parameters	C3001	C3002	C3003	Mean		SD	CV (%)
Rsq_adj	0.785	0.989	0.996	--	±	--	--
No. points used for $T_{1/2}$	5.00	3.00	3.00	ND	±	--	--
C_{max} (ng/mL)	504	427	326	419	±	89.3	21.3
T_{max} (h)	1.00	2.00	2.00	1.67	±	0.577	34.6

$T_{1/2}$ (h)	7.80	5.61	8.08	7.16	±	1.35	18.9
T_{last} (h)	24.0	24.0	24.0	24.0	±	--	--
$AUC_{0\text{-}last}$ (ng.h/mL)	3873	2772	3154	3266	±	559	17.1
$AUC_{0\text{-}inf}$ (ng.h/mL)	4522	2927	3615	3688	±	800	21.7
$MRT_{0\ last}$ (h)	7.44	7.05	8.39	7.63	±	0.689	9.04
$MRT_{0\text{-}inf}$ (h)	11.4	8.37	11.9	10.6	±	1.91	18.1
AUC_{Extra} (%)	14.4	5.28	12.8	10.8	±	4.87	45.0
$AUMC_{Extra}$ (%)	44.3	20.2	38.4	34.3	±	12.6	36.6
Bioavailability (%)[a]	-	-	-	0.741	--	--	--

[0446] ND = Not determined (Parameters not determined due to inadequately defined terminal elimination phase).

[0447] BQL = Below the lower limit of quantitation (LLOQ).

[0448] If the adjusted rsq (linear regression coefficient of the concentration value on the terminal phase) is less than 0.9, $T_{1/2}$ might not be accurately estimated.

[0449] If the % AUC_{Extra} > 20%, $AUC_{0\text{-}inf}$, Cl, $MRT_{0\text{-}inf}$ and Vd_{ss} might not be accurately estimated.

[0450] If the % $AUMC_{Extra}$ > 20%, $MRT_{0\text{-}inf}$ and Vd_{ss} might not be accurately estimated.

[0451] The adjusted linear regression coefficient of the concentration value on the terminal phase is less than 0.9, $T_{1/2}$ might not be accurately estimated.

[0452] a: Bioavailability (%) was calculated using $AUC_{0\text{-}inf}$ (if all $AUC_{Extra} \leq 20\%$) or $AUC_{0\text{-}last}$ (if one or more AUC_{Extra} > 20%) with Nominal Dose.

O7 & C29

[0453] Plasma concentration of O7 and C29 were next assessed up to 24 hours following oral administration to cynomolgus monkeys at 10 mg/kg or 30 mg/kg, results of which are shown in FIGS. 17 and 18.

Example 14: Cardiac Effects

O7 & O11

[0454] Changes in cardiac effects were assessed in rats subcutaneously administered O7 or O11 at either 0.5 mg/kg, 1 mg/kg, or 3 mg/kg. Control rats were administered saline. Animal specifications are outlined in Table 129.

Table 129: Animal Specifications

Species Level Age	Sprague-Dawley (SD) Rats SPF 6-7 weeks
Sex	Male
Animal Number (N)	30

Animal Housing

[0455] 6-7 week old male SD rats were obtained and housed in a controlled environment (target conditions: temperature 20 to 24°C, relative humidity 30 to 70%). Temperature and relative humidity was monitored daily. An electronic time-controlled lighting system was used to provide a 12-hour light/12-hour dark cycle. Rats were housed in plastic cages. Enrichment toys were provided. During the housing and study period, the rats were fed with normal chow and fresh water, *ad libitum.*

Animal Acclimation

[0456] After arrival, the animals acclimated to environment for at least 5 days.

Study Design

[0457] Before the experiment, the weight of each animal was measured and rats were be separated into different groups based on weight, with 3 rats per group (Table 130).

Table 130: Study Groups

Group	Treatment	Dosage (mg/kg)	Route	Volume (mL/kg)	Concentration (mg/ml)	Monitoring time	Number of rats	Frequency
1	Control (Saline)	-	SC	5ml/kg	-	3h	3	Single dose
2	O7	0.5mg/kg			0.1	3h	3	
3	O7	1mg/kg			0.2	3h	3	
4	O7	3mg/kg			0.6	3h	3	
8	O11	0.5mg/kg			0.1	3h	3	
9	O11	1mg/kg			0.2	3h	3	
10	O11	3mg/kg			0.6	3h	3	

Blood Pressure and 2-Lead ECG Monitoring

[0458] After acclimatization, animals were anesthetized with urethane as well as subjected to femoral vein for drug injection and carotid common artery to measure blood pressure. Meanwhile, electrocardiogram (ECG) was detected by subcutaneous puncture needle electrodes. The body temperature of the rats was maintained at about 37°C during assessments and experimentation. The blood pressure (BP) and ECG was recorded for 15 minutes before administration and for 3 hours after administration continuously for each rat.

Readouts

[0459] Systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) were recorded with blood pressure measurements.

[0460] QRS, ST, QT, P wave, T wave, and HR were assessed with ECG measurements.

[0461] Recording duration occurred from -15 min (pre-dose) to 180 min (after dosing).

Blood Collection

[0462] After recording, animals were euthanized with CO_2 immediately and blood was collected for *in vitro* analysis, including plasma corticosterone by ELISA Kit.

Data Processing and Analysis

[0463] All values were expressed as mean ± SEM. The significances of the differences among groups and within groups was evaluated by one-way ANOVA followed by Dunnett's test using Graph Pad statistic software. A p value of less than 0.05 was considered statistically significant. Results for the BP analysis are shown for all groups in Tables 131-133. ECG results are shown in Tables 134-138.

Table 131: Blood Pressure Analysis (Part 1)

Group	Animal Measurement	SBP(mmHg)								
		0min	5min	15min	30min	60min	90min	120min	150min	180min
G1: Control	Mean	108	112	112	113	104	106	108	107	107
	SEM	5	2	3	3	9	4	9	8	5
G2: O7 0.5mg/kg	Mean	101	107	102	101	106	107	104	105	105
	SEM	1	3	2	3	3	1	3	1	1
	Mean	103	105	104	105	103	108	108	103	107

G3: O7 1mg/kg	SEM	3	2	2	0	2	3	2	2	1
G4: O7 3mg/kg	Mean	106	108	107	110	109	110	111	111	110
	SEM	1	0	2	1	3	1	2	2	2
G8: O11 0.5mg/kg	Mean	109	108	111	110	108	105	110	112	111
	SEM	1	1	2	1	2	5	5	2	2
G9: O11 1mg/kg	Mean	109	110	109	112	113	111	111	110	113
	SEM	0	1	2	2	4	2	3	3	3
G10: O11 3mg/kg	Mean	108	105	105	109	108	110	112	110	112
	SEM	1	5	4	1	1	1	4	1	2

Table 132: Blood Pressure Analysis (Part 2)

Group	**Animal Measurement**	**DBP(mmHg)**								
		0min	**5min**	**15min**	**30min**	**60min**	**90min**	**120min**	**150min**	**180min**
G1: Control	Mean	77	83	81	81	72	73	78	75	74
	SEM	6	4	3	4	9	4	9	8	6
G2: O7 0.5mg/kg	Mean	66	74	67	65	71	73	71	71	72
	SEM	3	5	3	5	2	2	3	4	1
G3: O7 1mg/kg	Mean	71	74	72	73	69	76	75	70	75
	SEM	4	4	3	1	3	4	2	3	1
G4: O7 3mg/kg	Mean	75	75	75	78	77	77	79	79	78
	SEM	2	1	2	1	3	2	3	2	3
	SEM	5	6	4	7	5	6	1	7	7
G8: O11 0.5mg/kg	Mean	79	79	81	81	77	76	80	81	79
	SEM	3	2	3	3	3	5	6	1	2
G9: O11 1mg/kg	Mean	79	78	78	80	82	80	82	80	83
	SEM	0	2	2	1	5	2	3	2	4
G10: O11 3mg/kg	Mean	78	74	73	77	75	78	81	78	80
	SEM	4	5	2	5	4	5	5	2	2

Table 133: Blood Pressure Analysis (Part 3)

Group	**Animal Measurement**	**HR(BPM)**								
		0min	**5min**	**15min**	**30min**	**60min**	**90min**	**120min**	**150min**	**180min**
G1: Control	Mean	370	385	367	374	377	354	352	358	353
	SEM	27	20	23	1	26	31	12	4	27
G2: O7 0.5mg/kg	Mean	344	383	355	335	371	392	369	378	373
	SEM	20	19	19	19	51	23	23	25	16
G3: O7 1mg/kg	Mean	385	379	367	367	348	377	369	344	353
	SEM	37	29	23	22	20	9	23	22	27
G4: O7 3mg/kg	Mean	315	346	325	337	328	345	350	349	348
	SEM	21	21	22	8	26	16	9	17	23
	SEM	8	15	11	15	20	16	16	16	14

G8: O11 0.5mg/kg	Mean	415	403	419	422	413	381	426	421	420
	SEM	64	51	42	40	39	33	7	18	23
G9: O11 1mg/kg	Mean	363	355	362	391	382	386	387	390	389
	SEM	12	8	1	9	26	23	7	12	19
G10: O11 3mg/kg	Mean	381	376	377	391	372	385	399	397	414
	SEM	27	15	5	16	35	29	23	23	21

Table 134: ECG Results (Part 1)

Group	**Animal Measurement**	**Heart Rate (BPM)**								
		0min	**5min**	**15min**	**30min**	**60min**	**90min**	**120min**	**150min**	**180min**
G1: Control	Mean	369	385	367	374	377	358	352	358	353
	SEM	28	20	23	1	26	35	12	5	27
G2: O7 0.5mg/kg	Mean	344	382	355	335	371	392	369	377	372
	SEM	20	19	19	19	51	23	23	26	16
G3: O7 1mg/kg	Mean	385	379	367	367	348	377	369	343	352
	SEM	37	29	23	22	20	9	23	22	27
G4: O7 3mg/kg	Mean	314	346	325	337	328	345	349	349	348
	SEM	21	20	22	8	26	16	9	16	23
	SEM	8	15	11	15	20	16	16	16	13
G8: O11 0.5mg/kg	Mean	414	403	419	421	413	380	425	420	420
	SEM	64	51	42	40	39	33	8	18	23
G9: O11 1mg/kg	Mean	363	355	362	391	382	386	387	390	389
	SEM	12	8	1	9	26	23	7	12	19
G10: O11 3mg/kg	Mean	381	376	377	392	372	385	399	397	412
	SEM	27	15	5	16	36	29	23	24	20

Table 135: ECG Results (Part 2)

Group	**Animal Measurement**	**PR Interval (s)**								
		0min	**5min**	**15min**	**30min**	**60min**	**90min**	**120min**	**150min**	**180min**
G1: Control	Mean	0.0494	0.0496	0.0501	0.0502	0.0505	0.0508	0.0543	0.0531	0.0533
	SEM	0.0026	0.0023	0.0021	0.0007	0.0009	0.0027	0.0009	0.0008	0.0020
G2: O7 0.5mg/kg	Mean	0.0503	0.0486	0.0495	0.0510	0.0507	0.0490	0.0489	0.0486	0.0491
	SEM	0.0013	0.0009	0.0013	0.0007	0.0012	0.0013	0.0016	0.0018	0.0016
G3: O7 1mg/kg	Mean	0.0510	0.0516	0.0522	0.0553	0.0563	0.0549	0.0548	0.0562	0.0561
	SEM	0.0012	0.0008	0.0012	0.0013	0.0010	0.0005	0.0013	0.0008	0.0014
G4: O7 3mg/kg	Mean	0.0557	0.0549	0.0550	0.0575	0.0581	0.0581	0.0575	0.0570	0.0570
	SEM	0.0015	0.0011	0.0015	0.0007	0.0018	0.0014	0.0007	0.0006	0.0006
	SEM	0.0031	0.0028	0.0016	0.0023	0.0019	0.0019	0.0035	0.0018	0.0016

G8: O11 0.5mg/kg	Mean	0.0482	0.0480	0.0485	0.0485	0.0487	0.0484	0.0486	0.0477	0.0485
	SEM	0.0040	0.0037	0.0031	0.0037	0.0036	0.0034	0.0010	0.0017	0.0023
G9: O11 1mg/kg	Mean	0.0523	0.0521	0.0522	0.0503	0.0501	0.0492	0.0500	0.0489	0.0478
	SEM	0.0019	0.0019	0.0013	0.0010	0.0008	0.0001	0.0007	0.0012	0.0015
G10: O11 3mg/kg	Mean	0.0528	0.0535	0.0539	0.0521	0.0532	0.0519	0.0500	0.0499	0.0488
	SEM	0.0009	0.0027	0.0030	0.0016	0.0008	0.0005	0.0007	0.0007	0.0011

Table 136: ECG Results (Part 3)

Group	**Animal Measurement**	**QRS Interval (s)**								
		0min	**5min**	**15min**	**30min**	**60min**	**90min**	**120min**	**150min**	**180min**
G1: Control	Mean	0.0153	0.0157	0.0158	0.0167	0.0166	0.0167	0.0170	0.0167	0.0165
	SEM	0.0020	0.0018	0.0018	0.0020	0.0020	0.0019	0.0021	0.0019	0.0020
G2: O7 0.5mg/kg	Mean	0.0168	0.0166	0.0167	0.0170	0.0170	0.0164	0.0164	0.0163	0.0167
	SEM	0.0011	0.0010	0.0009	0.0008	0.0007	0.0010	0.0012	0.0013	0.0011
G3: O7 1mg/kg	Mean	0.0164	0.0165	0.0167	0.0168	0.0171	0.0173	0.0171	0.0170	0.0170
	SEM	0.0008	0.0010	0.0009	0.0009	0.0010	0.0011	0.0010	0.0008	0.0009
G4: O7 3mg/kg	Mean	0.0137	0.0143	0.0140	0.0141	0.0142	0.0143	0.0144	0.0141	0.0144
	SEM	0.0012	0.0009	0.0011	0.0011	0.0010	0.0010	0.0011	0.0012	0.0011
	SEM	0.0012	0.0008	0.0010	0.0005	0.0010	0.0006	0.0007	0.0011	0.0007
G8: O11 0.5mg/kg	Mean	0.0147	0.0149	0.0148	0.0148	0.0150	0.0156	0.0151	0.0155	0.0149
	SEM	0.0010	0.0011	0.0010	0.0008	0.0011	0.0006	0.0004	0.0011	0.0004
G9: O11 1mg/kg	Mean	0.0170	0.0170	0.0173	0.0173	0.0168	0.0169	0.0169	0.0168	0.0168
	SEM	0.0006	0.0005	0.0008	0.0011	0.0009	0.0005	0.0009	0.0009	0.0010
G10: O11 3mg/kg	Mean	0.0179	0.0178	0.0184	0.0177	0.0179	0.0177	0.0177	0.0180	0.0182
	SEM	0.0010	0.0008	0.0004	0.0010	0.0004	0.0008	0.0006	0.0002	0.0003

Table 137: ECG Results (Part 4)

Group	**Animal Measurement**	**QTc (s)**								
		0min	**5min**	**15min**	**30min**	**60min**	**90min**	**120min**	**150min**	**180min**
G1: Control	Mean	0.1498	0.1534	0.1489	0.1531	0.1563	0.1528	0.1612	0.1637	0.1618
	SEM	0.0015	0.0029	0.0044	0.0018	0.0050	0.0064	0.0136	0.0112	0.0047

G2: O7 0.5mg/kg	Mean	0.1366	0.1469	0.1421	0.1361	0.1567	0.1622	0.1549	0.1549	0.1552
	SEM	0.0037	0.0029	0.0030	0.0034	0.0152	0.0082	0.0104	0.0094	0.0096
G3: O7 1mg/kg	Mean	0.1474	0.1505	0.1492	0.1513	0.1475	0.1687	0.1680	0.1560	0.1663
	SEM	0.0080	0.0094	0.0107	0.0056	0.0119	0.0074	0.0099	0.0152	0.0105
G4: O7 3mg/kg	Mean	0.1058	0.1192	0.1110	0.1157	0.1170	0.1260	0.1232	0.1253	0.1287
	SEM	0.0318	0.0319	0.0333	0.0347	0.0380	0.0379	0.0379	0.0397	0.0409
	SEM	0.0040	0.0061	0.0030	0.0078	0.0064	0.0058	0.0049	0.0064	0.0072
G8: O11 0.5mg/kg	Mean	0.1555	0.1571	0.1623	0.1649	0.1669	0.1621	0.1745	0.1792	0.1811
	SEM	0.0193	0.0168	0.0164	0.0183	0.0172	0.0140	0.0133	0.0156	0.0183
G9: O11 1mg/kg	Mean	0.1393	0.1392	0.1408	0.1512	0.1532	0.1576	0.1575	0.1589	0.1651
	SEM	0.0068	0.0068	0.0052	0.0043	0.0077	0.0049	0.0081	0.0068	0.0094
G10: O11 3mg/kg	Mean	0.1596	0.1640	0.1619	0.1678	0.1596	0.1685	0.1736	0.1667	0.1823
	SEM	0.0092	0.0036	0.0064	0.0112	0.0111	0.0105	0.0048	0.0048	0.0050

Table 138: ECG Results (Part 5)

Group	**Animal Measurement**	**ST Height**								
		0min	**5min**	**15min**	**30min**	**60min**	**90min**	**120min**	**150min**	**180min**
G1: Control	Mean	0.2026	0.1896	0.2176	0.3175	0.3091	0.2614	0.3198	0.2771	0.2747
	SEM	0.1758	0.1558	0.1512	0.1324	0.1234	0.1749	0.1303	0.1402	0.1268
G2: O7 0.5mg/kg	Mean	0.8593	0.8364	0.8514	0.8680	0.8567	0.8527	0.8372	0.7977	0.7969
	SEM	0.1244	0.1025	0.1163	0.1195	0.1043	0.0463	0.0742	0.0837	0.0846
G3: O7 1mg/kg	Mean	0.2289	0.1916	0.2046	0.1947	0.1331	0.2699	0.3537	0.4510	0.4644
	SEM	0.1652	0.2281	0.2551	0.2591	0.3194	0.1860	0.1114	0.1596	0.1545
G4: O7 3mg/kg	Mean	0.2615	0.0707	0.1596	0.0463	0.0998	0.0319	0.0914	0.0480	0.0119
	SEM	0.2106	0.3526	0.2888	0.4242	0.4138	0.4416	0.3763	0.3743	0.3979
	SEM	0.1434	0.1294	0.1456	0.1638	0.1484	0.1576	0.2083	0.1746	0.1939
G8: O11 0.5mg/kg	Mean	0.5898	0.6187	0.5868	0.6072	0.6318	0.6663	0.5882	0.5971	0.5867
	SEM	0.1780	0.1722	0.1625	0.1696	0.1733	0.1770	0.1553	0.1596	0.1663
G9: O11 1mg/kg	Mean	0.2356	0.2427	0.2324	0.1934	0.2260	0.2101	0.2149	0.2287	0.2471
	SEM	0.1217	0.1903	0.1964	0.2240	0.2453	0.2298	0.2476	0.2475	0.2334

G10: O11 3mg/kg	Mean	0.3048	0.3728	0.3354	0.3920	0.4311	0.4135	0.3946	0.4352	0.4193
	SEM	0.1178	0.1478	0.1085	0.1467	0.1726	0.1752	0.1830	0.2104	0.2045

[0464] No significant changes in systolic blood pressure (SBP), diastolic blood pressure (DBP), nor heart rate (HR) were observed in rats administered O7 or O11 at any dose, relative to controls. Comparably, setmelanotide-treated animals showed at some doses, there were changes in QTc, suggesting altered cardiac repolarization. (FIGS. 20A-20D).

A07D (A3; SEQ ID NO: 31)

[0465] The effect of the melanocortin analogs on cardiac activation in rats was tested using A3 according to the following procedure. Baseline measurements of heart rate, systolic blood pressure, and diastolic blood pressure of rats were taken 15 minutes prior to administration of A3. The rats were then administered saline, 3 mg/kg A3, or 6 mg/kg A3 via subcutaneous injection and the heart rate, systolic blood pressure, and diastolic blood pressure were measure at the following time points post injection: 5 minutes, 15 minutes, 30 minutes, 60 minutes, 120 minutes, and 180 minutes.

[0466] FIGS. 20E-20G show the cardiac activation data from 15 minutes before subcutaneous injection of A3 to 180 minutes after injection of A3. As shown in FIG. 20E, the heart rate of rats administered 3 mg/kg A3 (G1) decreased directly after injection, increased steadily from 30 minutes to 120 minutes, and then decreased again at 180 minutes to just slightly above the starting heart rate (i.e., at -15 min). Rats administered 6 mg/kg A3 (G2) maintained a steady heart rate until 120 minutes after injection when an increase in heart rate may be seen, however, the final heart rate of G2 rats was similar to that of G1 and control (G3) rats.

[0467] As may be seen in FIG. 20F the systolic blood pressure (SBP) of the rats in each group was similar for all time points, with a slight increase overall after injection for all three groups. The diastolic blood pressure (DBP) of rats administered 3 mg/kg A3 and 6 mg/kg A3 was higher than the diastolic blood pressure of the control rats at all time points post injection, however, no group of rats had a diastolic blood pressure above 100 at any time point (FIG. 20G).

[0468] This data suggests that melanocortin analogs in accordance with the present technology such as A3 avoid cardiac activation, which has been seen in earlier peptide and small molecule agonists of the melanocortin receptors.

Example 15: Pharmacokinetic Assessment of Non-Naturally Occurring Melanocortin Analogs Following Subcutaneous Administration to Rats

Study Objective

[0469] The objective of this study is to determine the pharmacokinetics of non-naturally occurring melanocortin analogs of the present technology following subcutaneous administration in male rats. In rats, the test article will be monitored in plasma for up to 24 hours post each dose. Study design and sample collection will be conducted as outlined in Table 139 and 140.

Table 139. Study Design

Animal	**N**	**Compound**	**Target Dose Level (mg/kg)**	**Target Dose Volume (mL/kg)**	**Target Dose Concentration (mg/mL)**	**Vehicle**	**Dose Route**
Rat	3	A07D	1	1	1	physiological saline	SC
Rat	3	A07D	3	1	3	physiological saline	SC
Rat	3	A07D	10	1	10	physiological saline	SC

Table 140. Sample Collections

Group	Dosage	Sampling time points (hr.)								
	(mg/kg)	0.083	0.25	0.5	1	2	4	6	8	24
Rat	1	P	P	P	P	P	P	P	P	P
Rat	3	P	P	P	P	P	P	P	P	P
Rat	10	P	P	P	P	P/C	P/C	P	P	P

E: Anticoagulant: Potassium (K_2) EDTA;

P: plasma

C: cerebrospinal fluid (CSF)

[0470] Dose Solution Analysis Samples: After each dose preparation, remove approximately 0.5 mL aliquots from the formulations, transfer the aliquots into amber HPLC vials and stored at -60°C or lower until assayed in duplicate for dose validation.

[0471] Disposition of Remaining Test Article Formulations: Remaining formulations will be stored at -60°C or lower.

[0472] Disposition of Remaining Test Article (dry powder or solid): Remaining test article will be stored at room temperature desiccated, and protected from light until shipment or discard.

[0473] Animals will be fasted overnight through 4 hours post dosing.

Vehicle and Formulation Preparation:

[0474] Appropriate amount of test article will be accurately weighed and mixed with appropriate volume of vehicle to get a clear solution or suspension.

[0475] Formulation samples will be removed from each of the formulation solutions, transferred into 1.5 mL of polypropylene microcentrifuge tubes and run dose validation by LC/UV or LC-MS/MS.

Animal Specifications: Rats

[0476] Rat specifications are outlined in Table 141.

Table 141. Rat Specifications

Species	Rat
Strain	SD
Source	Approved Vendor.
Gender	Male
Body weight	＞200g
Age	7-10 W
Total number of animals	9 male rats
Justification	The SD rat is a standard species used in pharmacokinetic evaluations and is acceptable to regulatory agencies.

	The number of animals used in this study is, in the judgment of the investigator involved, the minimum necessary to properly perform this plasma pharmacokinetic evaluation.

Observations and Examinations

[0477] Clinical Observations: All animals will be observed at dosing and each scheduled collection. All abnormalities will be recorded.

[0478] Body Weight: All animals will be weighed on the dosing day prior to dosing to determine the dose volume to be administered.

Sample Collection and Processing

[0479] Blood Sample Collection and Process: At least 0.1 mL blood will be collected at each time point. All blood samples will be collected via jugular vein. All blood samples will be transferred into low binding EP tube with anticoagulant (0.5 M Potassium (K2) EDTA will be pre-added as a ratio of 50:1 for blood: anticoagulant), 0.05% Triton X-100 (e.g., 100uL Blood+2uL 2.5% Triton X-100) will be used for desorption the blood samples will be placed on wet ice.

[0480] Blood samples will be centrifuged within 1hr of collection at 3,200 g 4°C for 10 minutes. Following centrifugation, plasma samples will be transferred into their respective pre-labeled low binding EP tube and immediately frozen over dry ice. The plasma samples will be stored lower than -60°C until bioanalysis.

[0481] LC-MS/MS method development:

[0482] A LC-MS/MS method for the quantitative determination of test compound in biological matrix will be developed.

[0483] N in 1 cassette LC-MS/MS method can be developed for samples coming from different studies as long as these studies belong to the same sponsor.

[0484] Cassette administration assay could be performed if the mass difference (ΔMass) among different analytes is ≥4 Da. In this case, interference evaluation is not necessary.

[0485] If ΔMass among different analytes is less than 4 Da, there is a potential risk that interference would occur during LC-MS/MS analysis. If such kind of cassette assay is still requested by client, interference among analytes will not be evaluated but the LC separation of those analytes by using a generic method will be attempted.

[0486] Sample analysis:

[0487] A calibration curve with at least 6 non-zero calibration standards will be applied for each batch including LLOQ.

[0488] If sample number within a batch is ≤ 12, at least one set of standard curves separated with two parts through begin and end of the sequence should be included in the run and QCs are not required. The recommended injection order is C8, C6, C4, C2, study samples, C7, C5, C3, C1.

[0489] If sample number within a batch is > 12, one standard curve and two sets of QCs with low, middle and high concentrations will be applied for bioanalysis. Meanwhile, QCs number should be more than 5% of study sample number.

[0490] Samples, coming from one client with the same type of matrix in different studies, are allowed to be quantified in one analysis run by using the developed N in 1 cassette LC-MS/MS method.

[0491] Acceptance criteria:

[0492] (1) Linearity: At least 75% of the calibration standards should fall within ±20% of their nominal values for biofluid and within ±25% of their nominal values for tissue and feces samples. If the endpoints, such as LLOQ and ULOQ, on the calibration curve are eliminated, the calibration curve will be truncated. The truncated calibration curve should consist of at least 75% of the initial STDs.

[0493] (2) Accuracy: At least 67% of QCs should fall within ±20% of their nominal values for biofluid and within ±25% of their nominal values for tissue and feces samples. At least half of QCs at each concentration should be passed.

[0494] (3) Specificity: The mass response of analyte in the double blank and blank should be ≤ 50% LLOQ.

[0495] (4) Sensitivity: The LLOQ will be tried to target ≤ 3 ng/mL. Any adjustment of LLOQ will be informed to client in advance.

[0496] (5) Carryover: The mean calculated carryover peak area in the double blanks or blanks immediately after the highest standard injection should be less than that of LLOQ. If the carryover couldn't meet the criteria, the impact of the carryover on unknown samples should be re-evaluated according to the below procedure:

[0497] Carryover should be re-evaluated based on absolute carryover. Absolute carryover is calculated by carryover contribution multiplies carryover impact, where the carryover contribution is calculated by the area ratio of the double blank or blank with the highest carryover (Area max of carryover blank) to the ULOQ with the minimum calculated value (Area min of ULOQ), and the carryover impact is calculated by the area ratio of one injection (Area of one injection) to the following injection (Area of the following injection). The absolute carryover should be below the acceptable accuracy of the studies (e.g., 20% or 25%).

[0498] Carryover contribution = $Area_{max}$ of carryover blank / $Area_{min}$ of ULOQ Carryover impact = Area of one injection / Area of the following injection Absolute carryover = Carryover contribution * Carryover impact

Data Analysis

[0499] Preliminary plasma and cerebrospinal fluid (CSF) results after 24 hours of subcutaneous (SC) administration of A07D in Rats are shown in Table 142 and Table 143. Graphs of plasma concentration and CSF concentration in Rats are shown in FIGS 21A-21D.

Table 142. Concentration of A07D in plasma and CSF of Rats over time

Comp. Name	**Dose (mg/kg)**	**Plasma Concentration (ng/mL) over Time**								**CSF Concentration (ng/mL) over Time**		**Plasma/CSF ratio**	
		0.25h	**0.5 h**	**1 h**	**2 h**	4 h	**6 h**	**8 h**	**24 h**	**2 h**	**4 h**	**2h**	**4 h**
A07D	1	228	253	319	249	122	17.1	1.47	ND	-	-	-	-
A07D	3	303	369	570	620	431	267	93.9	ND	-	-	-	-
A07D	10	274	364	747	1169	1139	844	575	7.23	8.60	15.9	0.00749	0.0141

ND = not determined

Table 143. Plasma Pharmacokinetic Results of A07D in Rats after 24 hours

Comp Name	Dose (mg/kg)	Cmax (ng/mL)	Tmax (h)	Tfinal	T1/2 (h)	$AUC_{0\text{-last}}$ (ng.h/mL)	$AUC_{0\text{-inf}}$ (ng.h/mL)	$MRT_{0\text{-last}}$ (h)	$MRT_{0\text{-inf}}$ (h)	AUC_{EX} (%)	$AUMC_{EX}$ (%)	Bioavailability (%)
A07D	1	319	1.00	8h-24h	2.58	1066	1106	3.02	3.87	3.02	14.0	95.1
A07D	3	647	1.67	8h	1.85	2986	3305	3.23	3.94	8.97	22.3	94.7
A07D	10	1324	2.67	24h	2.50	9002	9032	5.65	5.72	0.319	1.50	77.7

[0500] Dose proportionality of A07D in male rats following a single subcutaneous administration in Rats is shown in Table 144.

Table 144. Dose Proportionality of A07D in Rats after a single SC administration

Compound Name	Compare Doses (mg/kg)			Dose Ratio	Cmax Values	Cmax Ratio	$AUC_{0\text{-last}}$ Values	$AUC_{0\text{-last}}$ Ratio
D	3.00	Over	1.00	3.00	647/319	2.03	2986/1066	2.80
D	10.0	Over	1.00	3.33	1324/647	2.05	9002/298	3.01
D	10.0	Over	3.00	10.0	13824/319	4.15	9002/1066	8.44

Example 17: Effects of A07D in the Expression of Morphine Conditioned Place Preference (CPP)

[0501] The non-naturally occurring melanocortin analogs of the present technology are share structural similarities with A07D, which has the sequence: Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2 (SEQ ID NO: 31). For example, the melanocortin analogs of the present technology include one or more of the following features: Pro at R^3, dPhe at R^4; lactam cyclization; and dVal-dPro-NH_2 C-terminus. Without intending to be limiting, these features, and in particular, the motif Pro-dPhe, and derivatives thereof, such as, for example, Pro-p(F)dPhe, impart the melanocortin analogs with partial agonism on the MC3R and full agonism on the MC4R. Accordingly, effects of A07D on morphine preference was assessed in rats using a CPP assay (FIG. 23A). Rats were habituated in animal facility for 7 days and were handled for 1-2 minutes per day for 3 days before pre-test.

[0502] For pre-testing, rats were allowed to freely explore both sides of the box for 15 min to rule out a preference for either side (rats with a CPP score ≥+150 or ≤ -150

were excluded from the study). After pre-test, group assignment was conducted within each group according to the pre-test results such that morphine was paired with the less-preferred compartment.

[0503] Following the pretest phase, rats underwent place conditioning for 8 days, with alternating treatment-compartment pairings. For a conditioning session, saline (2 mL/kg, intraperitoneal injection (i.p)) or morphine (10 mg/kg, i.p.) was administered, and rats were confined to the paired compartment for 45 minutes.

[0504] For post-testing, one day later, rats were randomly placed into one compartment and have access to both compartments during the 15-minute test period, and the time spend in each compartment was recorded. Animals that with a CPP score ≥ 100 in the morphine group were included for the further tests.

[0505] In the Expression test 1, A07D at 3 mg/kg and vehicle were administered subcutaneously (s.c) once 2 hours before the CPP expression test. The CPP score was defined as the time (in seconds) spent in the morphine-paired compartment minus the time spent in the saline-paired compartment during the CPP test. Groups included group 1 (vehicle + saline administration), group 2 (vehicle + morphine administration), and group 3 (A07D + morphine administration) (N = 10 rats per group).

[0506] For the Expression test 2, 24 hours later, A07D at 3 mg/kg was administered (s.c) once 1 hour before the CPP expression test. The CPP score was defined as the time (in seconds) spent in the morphine-paired compartment minus the time spent in the saline-paired compartment during the CPP test.

[0507] Data were analyzed by the two-way ANOVA followed by Bonferroni's pos hoc analysis.

Results

[0508] There was no difference in the pre-test for all groups (FIG. 23B). In the post-test, morphine-conditioned groups showed significant increased CPP score compared with saline-conditioned group ($p<0.0001$). There was no difference between vehicle + morphine group and A07D + morphine group in the post-test ($p>0.9999$). In the Expression test 1, A07D at the dose of 3 mg/kg almost significantly reduced the morphine CPP score when administered 2 hours prior to the test ($p = 0.08$). In the Expression test 2, A07D at the dose of 3 mg/kg significantly reduced the morphine CPP

score when administered 1 hour prior to the test (p = 0.01). Results are further detailed in Tables 145 and 146.

[0509] A07D significantly decreased the expression of morphine CPP in rats, indicating a potential effect of A07D in attenuating morphine addiction-related behaviors.

Table 145: Summary Statistics for CPP Scores (N=10 per group)

Group	Measurement	Pre-test	Post-test	Test1	Test2
Vehicle+saline	Mean	-69.420	-70.610	-79.000	-57.060
	SEM	15.840	43.013	54.855	74.686
Vehicle+morphine	Mean	-55.550	246.370	199.250	199.600
	SEM	14.304	28.173	29.830	34.138
A07D+morphine	Mean	-64.620	200.980	84.280	47.080
	SEM	14.650	20.689	26.341	34.188

Table 146: Individual CPP Scores

Treatment Group	Animal No.	Drug-paired Side	Pre-test	Post-test	Test 1	Test 2
Vehicle+saline	1	A	-25.5	-302.3	-329.2	-318.2
	2	A	-120.9	-90.9	-59.5	194.7
	3	A	-20.3	-53.5	274.4	217.1
	4	B	-115.9	-102.5	-143.1	204.5
	5	B	-49.6	-5.7	-391.3	124.1
	6	B	-35.0	-179.7	-69.3	-31.1
	7	A	-17.5	-89	-33.6	259.9
	8	B	-147.4	222.6	-237.3	4.7
	9	A	-43.6	-66.6	-64.4	193.9
	10	A	-118.5	-95.5	-54.3	43.1
Vehicle+morphine	1	A	-36.4	-102.5	-175.3	132.2
	2	B	-83.0	-5.7	107.9	58.8
	3	A	360.4	-179.7	293.3	--
	4	B	89.0	274.0	--	--
	5	B	-113.5	-222.6	112.6	--
	6	B	258.5	-277.6	317.8	--
	7	B	-35.1	-278.8	219.2	--
	8	A	66.6	142.8	--	--
	9	B	-138.5	95.5	48.5	--
	10	A	-135.2	87.6	145.5	--

Treatment Group	Animal No.	Drug-paired Side	Pre-test	Post-test	Test 1	Test 2
A07D+morphine	1	B	-97.7	174.6	276.6	--
	2	B	-97.4	159.1	329.3	--
	3	A	177.9	164.1	279.7	--
	4	B	-21.1	302.5	126.2	--
	5	A	350.0	274.2	--	--
	6	B	-125.0	170.5	332.7	--
	7	B	-54.2	57.5	106.5	--
	8	B	29.3	143.2	--	--
	9	B	-25.0	233.0	149.0	--
	10	B	120.4	32.1	--	--
Saline	1	A	-25.5	-302.3	-329.2	-318.2
	2	A	-120.9	-90.9	-59.5	194.7
	3	A	-20.3	-53.5	274.4	217.1
	4	B	-115.9	-102.5	-143.1	-391.3
	5	B	-49.6	-5.7	-69.3	204.5
	6	B	-35	-179.7	-33.6	-237.3
	7	A	-17.5	89	-64.4	-54.3
	8	B	-147.4	-222.6	-277.6	-278.8
	9	A	-43.6	66.6	-175.3	124.1
	10	A	-118.5	95.5	87.6	-31.1
Vehicle-Morphine	1	A	-36.4	174.6	107.9	259.9
	2	B	-72.8	159.1	293.3	4.7
	3	A	-6.5	312.1	274	193.9
	4	B	-83	360.4	112.6	317.8
	5	A	-25	276.2	219.2	142.8
	6	B	-42.3	106.2	48.5	145.5
	7	B	-113.5	258.5	276.6	329.3
	8	B	-35.1	164.1	279.7	126.2
	9	A	-2.4	302.5	274.2	332.7
	10	B	-138.5	350	106.5	143.2
A07D-Morphine	1	A	-135.2	170.5	57.5	29.3
	2	B	-97.7	233	149	32.1
	3	B	-97.4	177.9	-72.8	-104.5
	4	B	-21.1	120.4	142.6	58.3
	5	A	-23.2	289.4	37.4	280.4
	6	B	-8	327.4	98.2	-92.7
	7	B	-125	156.7	203.7	33.8
	8	B	-54.2	-157.4	-15.6	43.1

Treatment Group	Animal No.	Drug-paired Side	Pre-test	Post-test	Test 1	Test 2
	9	B	-59.4	160.5	127.7	132.2
	10	B	-25	216.6	115.1	58.8

Example 18: Assessing absorption, distribution, metabolism, and excretion of non-naturally occurring melanocortin analogs

[0510] Non-naturally occurring melanocortin analogs of the present technology, A07D, O7, O11,and M1 (Ac-Nle-c[Asp-Pro-p(F)dPhe-Arg-Trp-Lys]-dVal-dPro-NH_2), were assessed for solution properties, in vitro absorption, and in vitro metabolism for absorption, distribution, metabolism, and excretion (ADME) properties. Various ADME outcomes are outlined in Table 147 ([Test]: Test Compound Concentration; Comp.: Compound; Rep: replicate; recov.: recovery; T1/2: half-life (minutes); Avg.: mean; Perm.: permeability).

Aqueous Solubility

[0511] Aqueous solubility (µM) was determined by comparing the peak area of the principal peak in a calibration standard (200 µM) containing organic solvent (methanol/water, 60/40, v/v) with the peak area of the corresponding peak in a buffer sample. Additionally, chromatographic purity (%) was defined as the peak area of the principal peak relative to the total integrated peak area in the HPLC chromatogram of the calibration standard. A chromatogram of the calibration standard of each test compound, along with a UV/VIS spectrum with labeled absorbance maxima, was generated.

HPLC-UV Screen

[0512] A chromatogram of the test compound (200 µM) along with a UV/VIS spectrum with labeled absorbance maxima, was generated.

Protein Binding

[0513] The peak areas of the test compound in the buffer and test samples were used to calculate percent binding and recovery according to the following formulas:

Protein binding (%)= ($Area_p$–$Area_b Area_p$)∗100

Recovery (%)=(($Area_p$+$Area_b$)/$Area_c$)∗100

where:

$Area_p$ = Peak area of analyte in protein matrix

$Area_b$ = Peak area of analyte in buffer

$Area_c$ = Peak area of analyte in control sample

Partition Coefficient

[0514] The total amount of compound was determined as the peak area of the principal peak in a calibration standard (100 µM) containing organic solvent (methanol/water, 60/40, v/v). The amount of compound in buffer was determined as the combined, volume-corrected, and weighted areas of the corresponding peaks in the aqueous phases of three organic-aqueous samples of different composition. An automated weighting system was used to ensure the preferred use of raw data from those samples with well quantifiable peak signals. The amount of compound in organic was calculated by subtraction. Subsequently, LogD was calculated as the Log10 of the amount of compound in the organic phase divided by the amount of compound in the aqueous phase.

Half-Life Determination

[0515] At the end of the incubation at each of the time points, an equal volume of an organic mixture (acetonitrile/methanol, 50/50, v/v) was added to the incubation mixture. Samples were analyzed by HPLC-MS/MS and corresponding peak areas were recorded for each analyte. The ratio of precursor compound remaining after each time point relative to the amount present at time 0, expressed as percent, is reported as chemical stability. The half-life ($T_{1/2}$) was estimated from the slope of the initial linear range of the logarithmic curve of compound remaining (%) versus time, assuming first-order kinetics.

<u>ADME-Tox: In Vitro Absorption</u>

Permeability

[0516] The apparent permeability coefficient (Papp) of the test compound was calculated as follows:

$$P_{app}(cm/s)=(V_R \cdot C_{R,end}/\Delta t) \cdot (1/A^*(C_{D,mid}-C_{R,mid}))$$

where:

V_R is the volume of the receiver chamber.

$C_{R,end}$ is the concentration of the test compound in the receiver chamber at the end time point.

Δt is the incubation time.

A is the surface area of the cell monolayer.

$C_{D,mid}$ is the calculated mid-point concentration of the test compound in the donor side, which is the mean value of the donor concentration at time 0 minute and the donor concentration at the end time point.

$C_{R,mid}$ is the mid-point concentration of the test compound in the receiver side, which is one half of the receiver concentration at the end time point. Concentrations of the test compound were expressed as peak areas of the test

Recovery of the Test Compound from the Permeability Assay

[0517] The recovery of the test compound was calculated as follows:

$$\text{Recovery } (\%)=((V_D \cdot C_{D,end}+V_R \cdot C_{R,end})/(V_D \cdot C_{D0}))*100$$

where: V_D and V_R are the volumes of the donor and receiver chambers, respectively.

$C_{D,end}$ is the concentration of the test compound in the donor sample at the end time point.

$C_{R,end}$ is the concentration of the test compound in the receiver sample at the end time point.

C_{D0} is the concentration of the test compound in the donor sample at time zero. Concentrations of the test compound are expressed as peak areas of the test compound.

Fluorescein Assessment for Permeability Assays

[0518] Fluorescein was used as the cell monolayer integrity marker. Fluorescein permeability assessment (in the A-B direction at pH 7.4 on both sides) was performed after the permeability assay for the test compound. The cell monolayer that had a fluorescein permeability of less than 1.5×10^{-6} cm/s for Caco-2 and MDR1-MDCKII cells and 2.5×10^{-6} cm/s for MDCKII cells was considered intact, and the permeability result of the test compound from intact cell monolayer is reported.

ADME-Tox: In Vitro Metabolism

Intrinsic Clearance (microsomes, S9, cryopreserved hepatocytes, recombinant CYP, recombinant UGT)

[0519] Metabolic stability, expressed as percent of the parent compound remaining, was calculated by comparing the peak area of the compound at the time point relative to that at time-0. The half-life ($T_{1/2}$) was estimated from the slope of the initial linear range of the logarithmic curve of compound remaining (%) vs. time, assuming first-order kinetics. The apparent intrinsic clearance (CL_{int}, in µL/min/pmol, µL/min/mg or µL/min/Mcell) was calculated according to the following formula:

$CL_{int} = 0.693/(T_{1/2}*(\text{mg protein/µL or million cells/µL or pmol CYP isoyme/µL}))$

Table 147: ADME Assessment of Non-Naturally Occurring Analogs and Reference Compounds

Comp.	[Test]	% Protein Bound (1st Rep)	% Protein Bound (2nd Rep)	% Protein Bound (mean)	% recov. (1st rep)	% recov. (2nd Rep)	% recov. (Avg)
A07D	1.0E-05	56.30	60.40	58.35	96.27	92.66	94.47
O11	1.0E-05	48.33	49.20	48.76	109.77	120.41	115.09
O7	1.0E-05	53.73	51.70	52.72	106.26	103.51	104.88
M1	1.0E-05	50.27	51.88	51.07	106.45	109.72	108.08
Acebutolol	1.0E-05 M	16.16	14.18	15.17	116.82	108.4	112.61
Quinidine	1.0E-05 M	45.09	64.89	54.99	107.43	103	105.22
Quinidine	1.0E-05 M	63.03	69.17	66.1	115.06	113.14	114.1
Sertraline	1.0E-05 M	97.2	97.84	97.52	58.41	71.11	64.76
Sertraline	1.0E-05 M	98.01	98.5	98.25	96.63	98.05	97.34
Warfarin	1.0E-05 M	98.94	99.01	98.98	93.13	90.66	91.9
Warfarin	1.0E-05 M	98.11	99.05	98.58	112.92	103.05	107.98

Half Life: simulated gastric fluid								
Comp.	[Test]	Incubation (minutes)	% Comp. Remaining (1st Rep.)	% Comp. Remaining (2nd Rep.)	% Comp.Remaining (Avg)	T1/2 (first Rep.)	T1/2 (2nd Rep.)	T1/2 (Avg rep.)
A07D	1.0E-06	0	100.00	100.00	100.00	533.4	460.8	>120
A07D	1.0E-06	30	90.28	95.70	92.99			
A07D	1.0E-06	60	81.52	92.27	86.90			
A07D	1.0E-06	90	85.55	87.09	86.32			
A07D	1.0E-06	120	84.53	{94.3}	84.53			
O11	1.0E-06	0	100.00	100.00	100.00	1314.8	1090.9	>120
O11	1.0E-06	30	89.17	100.25	94.71			
O11	1.0E-06	60	94.86	99.13	97.00			
O11	1.0E-06	90	{81.9}	95.41	95.41			
O11	1.0E-06	120	91.04	93.19	92.11			
O7	0	100.00	100.00	100.00	343.3	455.0	>120	0
O7	30	93.28	81.00	87.14				
O7	60	86.21	87.07	86.64				
O7	90	84.64	{93.9}	84.64				
O7	120	77.55	79.25	78.40				
M1	1.0E-06	0	100.00	100.00	100.00	514.6	559.1	>120
M1	1.0E-06	30	90.52	{85.6}				
M1	60	95.57	85.20	90.38				

M1	90	92.70	88.04	90.37				
M1	120	80.74	85.71	83.22				
Erythromycin	1.0E-06 M	--	--	--	--	42.4	43.4	42.9
Propantheline	1.0E-06 M	--	--	--	--	433.7	515.3	>120
Half-life: Simulated intestinal fluid								
Comp.	[Test]	Incubation (minutes)	% Comp. Remaining (1st Rep.)	% Comp. Remaining (2nd Rep.)	% Comp. Remaining (Avg)	T1/2 (first Rep.)	T1/2 (2nd Rep.)	T1/2 (Avg rep.)
A076	1.0E-06	0	100.00	100.00	100.00	110.6	120.2	115.4
A076	1.0E-06	30	85.58	81.63	83.60			
A076	1.0E-06	60	68.53	67.20	67.86			
A076	1.0E-06	90	56.15	56.28	56.22			
A076	1.0E-06	120	48.21	50.72	49.47			
O11	1.0E-06	0	100.00	100.00	100.00	156.8	162.7	>120
O11	1.0E-06	30	89.89	90.71	90.30			
O11	1.0E-06	60	81.27	81.00	81.14			
O11	1.0E-06	90	68.77	66.27	67.52			
O11	1.0E-06	120	58.92	61.74	60.33			
O7	1.0E-06	0	100.00	100.00	100.00	100.6	102.8	101.7
O7	1.0E-06	30	81.56	83.82	82.69			

O7	1.0E-06	60	65.61	68.68	67.14			
O7	1.0E-06	90	56.42	53.02	54.72			
O7	1.0E-06	120	42.77	45.75	44.26			
M1	1.0E-06	0	100.00	100.00	100.00	67.0	71.7	69.3
M1	1.0E-06	30	83.71	81.53	82.62			
M1	1.0E-06	60	57.23	60.90	59.07			
M1	1.0E-06	90	44.96	46.68	45.82			
M1	1.0E-06	120	28.87	31.01	29.94			
Chloram bucil	1.0E-06 M	--	--	--	--	19.9	20	20
Propant heline	1.0E-06 M	--	--	--	--	123. 9	103.3	113.6

Aqueous solubility: simulated intestinal fluid				
Comp.	[Test]	Solubility (µM, 1st Rep)	Solubility (µM, 2nd Rep)	Solubility (µM, mean)
A07D	2.0E-04 M	275.72	281.44	>200
O11	2.0E-04 M	109.07	111.25	110.16
O7	2.0E-04 M	130.68	135.56	133.12
M1	2.0E-04 M	116.81	142.26	129.54
Aqueous solubility: PBS, pH 7.4				
A07D	2.0E-04 M	0.08	0.02	0.05
O11	2.0E-04 M	193.62	187.83	190.73
O7	2.0E-04 M	198.61	198.89	198.75

M1	2.0E-04 M	204.15	214.25	>200	
Aqueous solubility: simulated gastric fluid					
A07D	2.0E-04 M	125.87	158.51	142.19	
O11	2.0E-04 M	179.50	191.97	185.73	
O7	2.0E-04 M	199.16	194.59	196.88	
M1	2.0E-04 M	207.86	204.77	>200	
Diethylstilbesterol	2.0E-04 M	5.13	5.3	5.21	
Ketoconazole	2.0E-04 M	140.23	126.41	133.32	
Metoprolol	2.0E-04 M	195.25	186.61	190.93	
Phenytoin	2.0E-04 M	87.35	92.08	89.72	
Rifampicin	2.0E-04 M	172.46	175.57	174.01	
Simvastatin	2.0E-04 M	36.93	35.16	36.04	
Tamoxifen	2.0E-04 M	1.38	6.86	4.12	
Partition coefficient (log D, n-octanol/PBS, pH 7.4)					
Comp.	[Test]	LogD			
A07D	1.0E-04 M	0.98			
O11	1.0E-04 M	-0.34			
O7	1.0E-04 M	-0.07			
M1	1.0E-04 M	0.36			
Haloperidol	1.0E-04 M	2.53			

Ketoconazole	1.0E-04 M	3.35						
Metoprolol	1.0E-04 M	-0.32						
Phenytoin	1.0E-04 M	2.2						
Rifampicin	1.0E-04 M	1.08						
Simvastatin	1.0E-04 M	>4.01						
in vitro absorption: A-B permeability (Caco-2, pH 6.5/7.4)								
Comp.	[Test]	Perm. (10-6 cm/s, 1st Rep.)	Perm. (10-6 cm/s, 2nd Rep.)	Perm. (10-6 cm/s, Avg.)	Flags	% recov. (%, 1st Rep.)	% recov. (%, 2nd Rep.)	% recov. (%, Avg.)
A07D	1.0E-05	0.01	0.02	<0.014	BLQ	86	91	88
O11	1.0E-05	0.02	0.01	<0.013	BLQ	89	91	90
O7	1.0E-05	0.01	0.00	<0.006	BLQ	82	90	86
M1	1.0E-05	0.01	0.01	<0.007	BLQ	87	90	89
Colchicine	1.0E-05 M	0.18	0.13	0.2		80	86	83
Labetalol	1.0E-05 M	6.02	6.15	6.1		98	101	100
Propranolol	1.0E-05 M	25.54	27.19	26.4		89	91	90
Ranitidine	1.0E-05 M	0.21	0.18	0.2		91	100	96
in vitro absorption: B-A permeability (Caco-2, pH 6.5/7.4)								
Comp.	[Test]	Perm. (10-6 cm/s, 1st Rep.	Perm. (10-6 cm/s, 2nd Rep.)	Perm. (10-6 cm/s, Avg.)	Flags	% recov. (%,	% recov. (%, 2nd rep.)	% recov. (%, Avg.)

						1st rep.)		
A07D	1.0E-05	0.02	0.03	<0.023	BLQ	71	78	74
O11	1.0E-05	0.02	0.02	<0.02	BLQ	65	85	75
O7	1.0E-05	0.02	0.02	<0.015	BLQ	78	80	79
M1	1.0E-05	0.02	0.02	<0.021	BLQ	66	72	69
Colchicine	1.0E-05 M	3.37	3.46	3.4		87	91	89
Labetalol	1.0E-05 M	27.48	24.62	26		93	92	93
Propranolol	1.0E-05 M	29.61	28.21	28.9		90	92	91
Ranitidine	1.0E-05 M	1.08	1.03	1.1		104	94	99

Intrinsic clearance (liver microsomes - human)									
Comp.	[Test]	Incubation (minutes)	% Comp. Remaining (1st Rep.)	% Comp. Remaining (2nd Rep.)	% Comp. Remaining (Avg)	T1/2 (minutes, 1st Rep)	T1/2 (minutes, 2nd Rep)	T1/2 (Avg)	Clint (µl/min/mg)
A07D	1.0E-07 M	0	100.00	100.00	100.00	368.4	1251.4	>120	<57.8
A07D	1.0E-07 M	15	95.20	97.55	96.38				
A07D	1.0E-07 M	30	97.56	94.27	95.91				
A07D	1.0E-07 M	45	95.33	95.99	95.66				
A07D	1.0E-07 M	60	86.78	96.71	91.74				

O11	1.0E-07	0	100.00	100.00	100.00	672.0	275.5	>120	<57.8
O11	1.0E-07	15	96.82	{90.7}	96.82				
O11	1.0E-07	30	96.10	92.82	94.46				
O11	1.0E-07	45	92.45	93.00	92.72				
O11	1.0E-07	60	94.72	84.51	89.62				
O7	1.0E-07	0	100.00	100.00	100.00	1677.2	338.2	>120	<57.8
O7	1.0E-07	15	100.37	105.70	103.04				
O7	1.0E-07	30	104.06	102.19	103.13				
O7	1.0E-07	45	108.43	95.46	101.95				
O7	1.0E-07	60	93.28	90.24	91.76				
M1	1.0E-07	0	100.00	100.00	100.00	659.0	>120	>120	<57.8
M1	1.0E-07	15	104.15	105.92	105.03				
M1	1.0E-07	30	104.90	108.28	106.59				
M1	1.0E-07	45	102.12	103.56	102.84				
M1	1.0E-07	60	93.32	101.69	97.51				
imipramine	1.0E-07 M	180.1	184.3	>120	<57.8				
Propranolol	1.0E-07 M	217.0	394.0	>120	<57.8				
Terfenadine	1.0E-07 M	13.3	13.1	13.2					
Verapamil	1.0E-07 M	34.2	33.4	33.8					

Additional Embodiments

[0520] Various embodiments of the present technology are set forth below in paragraphs [0521] to [0535]:

[0521] Embodiment 1. A non-naturally occurring melanocortin analog comprising a sequence according to Formula (IC):

$$R^1\text{-}R^2\text{-}R^3\text{-}R^4\text{-}R^5\text{-}R^6\text{-}R^7\text{-}Y^1\text{-}Y^2 \text{ (IC)},$$

wherein:

R^1 is Nle;

R^2 is Asp or Glu;

R^3 is Pro;

R^4 is p(F)dPhe or dPhe;

R^5 is Arg;

R^6 is Trp;

R^7 is Orn;

Y^1 is dVal;

Y^2 is dPro; and

the non-naturally occurring melanocortin analog is cyclized through a lactam bridge between Asp or Glu at R^2 and Orn at R^7,

provided that:

when R^4 is dPhe, then R^2 is Glu.

[0522] Embodiment 2. The non-naturally occurring melanocortin analog of embodiment 2, comprising a sequence according to Formula (IC), wherein:

R^1 is Nle;

R^2 is Asp or Glu;

R^3 is Pro;

R^4 is p(F)dPhe or dPhe;

R^5 is Arg;

R^6 is Trp;

R^7 is Orn;

Y^1 is dVal;

Y^2 is dPro; and

the non-naturally occurring melanocortin analog is cyclized through a lactam bridge between Asp or Glu at R^2 and Orn at R^7,

provided that the non-naturally occurring melanocortin analog does not comprise a sequence of: Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2 (SEQ ID NO: 23).

[0523] Embodiment 3. The non-naturally occurring melanocortin analog of embodiment 1 or 2, wherein the sequence of Formula (IC) is selected from the group consisting of:

Ac-Nle-c[Glu-Pro-dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2 (SEQ ID NO: 216);
Ac-Nle-c[Asp-Pro-p(F)dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2 (SEQ ID NO: 217); and

Ac-Nle-c[Glu-Pro-p(F)dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2 (SEQ ID NO: 218),

wherein c represents cyclization through R^2 and R^7 via a lactam bond.

[0524] Embodiment 4. The non-naturally occurring melanocortin analog of any one of embodiments 1-3, wherein the sequence of Formula (IC) is cyclized through a lactam bond between Glu at R^2 and Orn at R^7.

[0525] Embodiment 5. The non-naturally occurring melanocortin analog of embodiment 4, wherein the sequence of Formula (IC) is:

Ac-Nle-c[Glu-Pro-dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2 (SEQ ID NO: 216); or
Ac-Nle-c[Glu-Pro-p(F)dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2 (SEQ ID NO: 218),

wherein c represents cyclization through R^2 and R^7 via a lactam bond.

[0526] Embodiment 6. The non-naturally occurring melanocortin analog of any one of embodiments 1-3, wherein R^4 is p(F)dPhe.

[0527] Embodiment 7. The non-naturally occurring melanocortin analog of embodiment 6, wherein the sequence of Formula (IC) is:
Ac-Nle-c[Asp-Pro-p(F)dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2 (SEQ ID NO: 217);

Ac-Nle-c[Glu-Pro-p(F)dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2 (SEQ ID NO: 218),

wherein c represents cyclization through R^2 and R^7 via a lactam bond.

[0528] Embodiment 8. The non-naturally occurring melanocortin analog of embodiment 7, wherein the sequence of Formula (IC) is: Ac-Nle-c[Glu-Pro-p(F)dPhe-

Arg-Trp-Orn]-dVal-dPro-NH_2 (SEQ ID NO: 218), wherein c represents cyclization through R^2 and R^7 via a lactam bond.

[0529] Embodiment 9. The non-naturally occurring melanocortin analog of any one of embodiments 1-3, wherein R^4 is dPhe.

[0530] Embodiment 10. The non-naturally occurring melanocortin analog of embodiment 4, wherein the sequence of Formula (IC) is: Ac-Nle-c[Glu-Pro-dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2 (SEQ ID NO: 216), wherein c represents cyclization through R^2 and R^7 via a lactam bond.

[0531] Embodiment 11. The non-naturally occurring melanocortin analog of any one of embodiments 1-10, wherein the non-naturally occurring melanocortin analog binds at least a portion of a melanocortin receptor.

[0532] Embodiment 12. The non-naturally occurring melanocortin analog of embodiment 11, wherein the non-naturally occurring melanocortin analog binds one or more amino acids of the melanocortin receptor.

[0533] Embodiment 13. The non-naturally occurring melanocortin analog of embodiment 12, wherein the non-naturally occurring melanocortin analog binds two or more amino acids of the melanocortin receptor.

[0534] Embodiment 14. The non-naturally occurring melanocortin analog of any one of embodiments 11-13, wherein the non-naturally occurring melanocortin analog comprises a dissociation constant (Kd) value for the melanocortin receptor or the one or more amino acids thereof that is less than that of a conventional melanocortin analog

[0535] Embodiment 15. The non-naturally occurring melanocortin analog of any one of embodiments 11-14, wherein the melanocortin receptor is a melanocortin 3 receptor or a melanocortin 4 receptor.

[0536] From the foregoing, it will be appreciated that specific embodiments of the present technology have been described herein for purposes of illustration, but that various modifications may be made without deviating from the scope of the present technology. Accordingly, the present technology is not limited except as by the appended claims.

CLAIMS

I/We claim:

1. A non-naturally occurring melanocortin analog comprising a sequence according to Formula (IC):

R^1-R^2-R^3-R^4-R^5-R^6-R^7-Y^1-Y^2 (IC),

wherein:

R^1 is Nle;

R^2 is Asp or Glu;

R^3 is Pro;

R^4 is p(F)dPhe or dPhe;

R^5 is Arg;

R^6 is Trp;

R^7 is Orn;

Y^1 is dVal;

Y^2 is dPro; and

the non-naturally occurring melanocortin analog is cyclized through a lactam bridge between Asp or Glu at R^2 and Orn at R^7,

provided that:

when R^4 is dPhe, then R^2 is Glu.

2. The non-naturally occurring melanocortin analog of claim 2, comprising a sequence according to Formula (IC), wherein:

R^1 is Nle;

R^2 is Asp or Glu;

R^3 is Pro;

R^4 is p(F)dPhe or dPhe;

R^5 is Arg;

R^6 is Trp;

R^7 is Orn;

Y^1 is dVal;

Y^2 is dPro; and

the non-naturally occurring melanocortin analog is cyclized through a lactam bridge between Asp or Glu at R^2 and Orn at R^7,

provided that the non-naturally occurring melanocortin analog does not comprise a sequence of: Ac-Nle-c[Asp-Pro-dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2 (SEQ ID NO: 23).

3. The non-naturally occurring melanocortin analog of claim 1 or 2, wherein the sequence of Formula (IC) is selected from the group consisting of:

Ac-Nle-c[Glu-Pro-dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2 (SEQ ID NO: 216);
Ac-Nle-c[Asp-Pro-p(F)dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2 (SEQ ID NO: 217); and

Ac-Nle-c[Glu-Pro-p(F)dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2 (SEQ ID NO: 218),

wherein c represents cyclization through R^2 and R^7 via a lactam bond.

4. The non-naturally occurring melanocortin analog of any one of claims 1-3, wherein the sequence of Formula (IC) is cyclized through a lactam bond between Glu at R^2 and Orn at R^7.

5. The non-naturally occurring melanocortin analog of claim 4, wherein the sequence of Formula (IC) is:

Ac-Nle-c[Glu-Pro-dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2 (SEQ ID NO: 216); or
Ac-Nle-c[Glu-Pro-p(F)dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2 (SEQ ID NO: 218),

wherein c represents cyclization through R^2 and R^7 via a lactam bond.

6. The non-naturally occurring melanocortin analog of any one of claims 1-3, wherein R^4 is p(F)dPhe.

7. The non-naturally occurring melanocortin analog of claim 6, wherein the sequence of Formula (IC) is:
Ac-Nle-c[Asp-Pro-p(F)dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2 (SEQ ID NO: 217);

Ac-Nle-c[Glu-Pro-p(F)dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2 (SEQ ID NO: 218),

wherein c represents cyclization through R^2 and R^7 via a lactam bond.

8. The non-naturally occurring melanocortin analog of claim 7, wherein the sequence of Formula (IC) is: Ac-Nle-c[Glu-Pro-p(F)dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2 (SEQ ID NO: 218), wherein c represents cyclization through R^2 and R^7 via a lactam bond.

9. The non-naturally occurring melanocortin analog of any one of claims 1-3, wherein R^4 is dPhe.

10. The non-naturally occurring melanocortin analog of claim 4, wherein the sequence of Formula (IC) is: Ac-Nle-c[Glu-Pro-dPhe-Arg-Trp-Orn]-dVal-dPro-NH_2 (SEQ ID NO: 216), wherein c represents cyclization through R^2 and R^7 via a lactam bond.

11. The non-naturally occurring melanocortin analog of any one of claims 1-10, wherein the non-naturally occurring melanocortin analog binds at least a portion of a melanocortin receptor.

12. The non-naturally occurring melanocortin analog of claim 11, wherein the non-naturally occurring melanocortin analog binds one or more amino acids of the melanocortin receptor.

13. The non-naturally occurring melanocortin analog of claim 12, wherein the non-naturally occurring melanocortin analog binds two or more amino acids of the melanocortin receptor.

14. The non-naturally occurring melanocortin analog of any one of claims 11-13, wherein the non-naturally occurring melanocortin analog comprises a dissociation

constant (K_d) value for the melanocortin receptor or the one or more amino acids thereof that is less than that of a conventional melanocortin analog.

15. The non-naturally occurring melanocortin analog of any one of claims 11-14, wherein the melanocortin receptor is a melanocortin 3 receptor or a melanocortin 4 receptor.