

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
19 May 2011 (19.05.2011)

PCT

(10) International Publication Number
WO 2011/060352 A1

(51) International Patent Classification:
A01N 25/00 (2006.01)

(21) International Application Number:
PCT/US2010/056690

(22) International Filing Date:
15 November 2010 (15.11.2010)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/281,360 16 November 2009 (16.11.2009) US

(71) Applicant (for all designated States except US): **IPSEN PHARMA S.A.S.** [FR/FR]; 65, Quai Georges Gorse, F-92100 Boulogne-Billancourt (FR).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **DONG, Zheng Xin** [US/US]; 66 Fairview Street, Holliston, MA 01746 (US). **ZHANG, Jundong** [US/US]; 11 Sutcliffe Park, Newton, MA 02461 (US).

(74) Agents: **FEENEY, Alan, F.** et al.; Biomeasure, Incorporated, 27 Maple Street, Milford, MA 01757-3650 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM,

AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))

Published:

— with international search report (Art. 21(3))



WO 2011/060352 A1

(54) Title: PHARMACEUTICAL COMPOSITIONS OF MELANOCORTIN RECEPTOR LIGANDS

(57) Abstract: The present invention relates to improvements in compositions containing peptides that are ligands of one or more of the melanocortin receptors (MC-R), or pharmaceutically acceptable salts thereof, methods for preparing such compositions, and method of using such compositions to treat mammals. In particular, the present invention relates to a pharmaceutical composition comprising a pamoate salt of Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂ which is a ligand of the melanocortin receptor subtype 4 (MC4-R), and in which, after subcutaneous or intramuscular administration to a subject, the peptide forms a depot at physiological pH that is slowly dissolved and released into the body fluid and bloodstream. The present invention may further comprise an organic component such as dimethylacetamide (DMA) or polyethylene glycol (PEG) with an average molecular weight of lower than 1000.

PHARMACEUTICAL COMPOSITIONS OF MELANOCORTIN RECEPTOR LIGANDSBACKGROUND OF THE INVENTION

5 The present invention relates to improvements in compositions containing peptides that are ligands of one or more of the melanocortin receptors (MC-R), or pharmaceutically acceptable salts thereof, methods for preparing such compositions, and method of using such compositions to treat mammals. In particular, the present invention relates to a pharmaceutical composition comprising a pamoate salt of Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂ which is a ligand of the
10 melanocortin receptor subtype 4 (MC4-R), and in which, after subcutaneous or intramuscular administration to a subject, the peptide forms a depot at physiological pH that is slowly dissolved and released into the body fluid and bloodstream. The present invention may further comprise an organic component such as dimethylacetamide (DMA) or polyethylene glycol (PEG) with an average molecular weight of lower than 1000.

15 Melanocortins are a family of regulatory peptides which are formed by post-translational processing of pro-hormone pro-opiomelanocortin (POMC; 131 amino acids in length). POMC is processed into three classes of hormones; the melanocortins, adrenocorticotropin hormone, and various endorphins (e.g. lipotropin) (Cone, et al., Recent Prog. Horm. Res., 51:287-317, (1996); Cone et al., Ann. N.Y. Acad. Sci., 31:342-363, (1993)).

20 Melanocortins have been found in a wide variety of normal human tissues including the brain, adrenal, skin, testis, spleen, kidney, ovary, lung, thyroid, liver, colon, small intestine and pancreas (Tatro, J. B. et al., Endocrinol. 121:1900-1907 (1987); Mountjoy, K. G. et al., Science 257:1248-1251 (1992); Chhajlani, V. et al., FEBS Lett. 309:417-420 (1992); Gantz, I. et al. J. Biol. Chem. 268:8246-8250 (1993) and Gantz, I. et al., J. Biol. Chem. 268:15174-15179 (1993)).

25 Melanocortin peptides have been shown to exhibit a wide variety of physiological activities including the control of behavior and memory, affecting neurotrophic and antipyretic properties, as well as affecting the modulation of the immune system. Aside from their well known effects on adrenal cortical functions (adrenocorticotropin hormone or "ACTH") and on melanocytes (melanocyte stimulating hormone or "MSH"), melanocortins have also been shown to control the cardiovascular
30 system, analgesia, thermoregulation and the release of other neurohumoral agents including prolactin, luteinizing hormone and biogenic amines (De Wied, D. et al., Methods Achiev. Exp. Pathol. 15:167-199 (1991); De Wied, D. et al., Physiol. Rev. 62:977-1059 (1982); Guber, K.A. et al., Am. J. Physiol. 257:R681-R694 (1989); Walker J.M. et al., Science 210:1247-1249 (1980); Murphy, M. T. et al., Science 221:192-193 (1983); Ellerkmann, E. et al., Endocrinol. 130:133-138 (1992) and Versteeg, D.
35 H. G. et al., Life Sci. 38:835-840 (1986)).

It has also been shown that binding sites for melanocortins are distributed in many different tissue types including lachrymal and submandibular glands, pancreas, adipose, bladder, duodenum, spleen, brain and gonadal tissues as well as malignant melanoma tumors. Five melanocortin receptors have been characterized to date. These include melanocyte-specific receptor (MC1-R),
5 corticoadrenal-specific ACTH receptor (MC2-R), melanocortin-3 (MC3-R), melanocortin-4 (MC4-R) and melanocortin-5 receptor (MC5-R). All of the melanocortin receptors respond to the peptide hormone class of melanocyte stimulating hormones (MSH) (Cone, R. D. et al., *Ann. N.Y. Acad. Sci.*, 680:342-363 (1993); Cone, R. D. et al., *Recent Prog. Horm. Res.*, 51:287-318 (1996)).

MC1-R, known in the art as Melanocyte Stimulating Hormone Receptor (MSH-R),
10 Melanotropin Receptor or Melanocortin-1 Receptor, is a 315 amino acid transmembrane protein belonging to the family of G-Protein coupled receptors. MC1-R is a receptor for both MSH and ACTH. The activity of MC1-R is mediated by G-proteins which activate adenylate cyclase. MC1-R receptors are found in melanocytes and corticoadrenal tissue as well as various other tissues such as adrenal gland, leukocytes, lung, lymph node, ovary, testis, pituitary, placenta, spleen and uterus.
15 MC2-R, also called Adrenocorticotrophic Hormone Receptor (ACTH-R), is a 297 amino acid transmembrane protein found in melanocytes and the corticoadrenal tissue. MC2-R mediates the corticotrophic effect of ACTH. In humans, MC3-R is a 360 AA protein found in brain tissue; in mice and rats MC3-R is a 323 AA protein. MC4-R is a 332 amino acid transmembrane protein which is also expressed in brain as well as placental and gut tissues. MC5-R is a 325 amino acid
20 transmembrane protein expressed in the adrenals, stomach, lung and spleen and very low levels in the brain. MC5-R is also expressed in the three layers of adrenal cortex, predominantly in the aldosterone-producing zona glomerulosa cells.

The five known melanocortin receptors differ, however, in their functions. For example, MC1-R is a G-protein coupled receptor that regulates pigmentation in response to α -MSH, a potent
25 agonist of MC1-R. Agonism of the MC1-R receptor results in stimulation of the melanocytes which causes eumelanin and increases the risk for cancer of the skin. Agonism of MC1-R can also have neurological effects. Stimulation of MC2-R activity can result in carcinoma of adrenal tissue. Recent pharmacological confirmation has established that central MC4-R receptors are the prime mediators of the anorexic and orexigenic effects reported for melanocortin agonists and antagonists, respectively.
30 The effects of agonism of the MC3-R and MC5-R are not yet known.

There has been great interest in melanocortin (MC-R) receptors as targets for the design of novel therapeutics to treat disorders of body weight such as obesity and cachexia. Both genetic and pharmacological evidence points toward central MC4-R receptors as the principal target (Giraudou, S. Q. et al., *Brain Res.*, 809:302-306 (1998); Farooqi, I. S. et al., *NE J Med.*, 348:1085-1095 (2003);
35 MacNeil, D. J. et al., *Eu. J. Pharm.*, 44:141-157 (2002); MacNeil, D. J. et al., *Eu. J. Pharm.*, 450:93-109 (2002); Kask, A. et al., *NeuroReport*, 10:707-711 (1999)). The current progress with receptor-

selective agonists and antagonists evidences the therapeutic potential of melanocortin receptor activation, particularly MC4-R.

Agonist, antagonist or other ligand compounds activating one or more melanocortin receptor would be useful for treating a wide variety of indications in a subject in need thereof or at risk thereof including acute and chronic inflammatory diseases such as general inflammation (U.S. Patent No. 5 6,613,874; Catania, A. et al., *Pharm. Rev.*, 56:1-29 (2004)), inflammatory bowel disease (U.S. Patent No. 6,713,487; Catania, A. et al., *Pharm. Rev.*, 56:1-29 (2004)), brain inflammation (Catania, A. et al., *Pharm. Rev.*, 56:1-29 (2004)), sepsis (U.S. Patent No. 6,613,874; U.S. Patent No. 6,713,487; Catania, A. et al., *Pharm. Rev.*, 56:1-29 (2004)) and septic shock (U.S. Patent No. 6,613,874; Catania, A. et al., *Pharm. Rev.*, 56:1-29 (2004)); diseases with an autoimmune component such as rheumatoid 10 arthritis (U.S. Patent No. 6,713,487; Catania, A. et al., *Pharm. Rev.*, 56:1-29 (2004)), gouty arthritis (Catania, A. et al., *Pharm. Rev.*, 56:1-29 (2004)), Getting, S. J. et al., *Curr. Opin. Investig. Drugs*, 2:1064-1069 (2001)), and multiple sclerosis ((U.S. Patent No. 6,713,487); metabolic diseases and medical conditions accompanied by weight gain such as obesity (U.S. Patent No. 6,613,874; U.S. 15 Patent No. 6,600,015; Fehm, H. L. et al., *J. Clin. Endo. 2 Metab.*, 86:1144-1148 (2001); Hansen, M. J. et al., *Brain Res.*, 1039:137-145 (2005); Ye, Z. et al., *Peptides*, 26:2017-2025 (2005); Farooqi, I. S. et al., *NE J Med.*, 348:1085-1095 (2003); MacNeil, D. J. et al., *Eu. J. Pharm.*, 44:141-157 (2002); MacNeil, D. J. et al., *Eu. J. Pharm.*, 450:93-109 (2002); Kask, A. et al., *NeuroReport*, 10:707-711 (1999); Schwartz, M. W., *J. Clin. Invest.*, 108:963-964 (2001), Gura, T., *Science*, 287:1738-1740 20 (2000), Raffin-Sanson, M. L., *Eu. J. Endo.*, 144:207-208 (2001), Hamilton, B. S. et al., *Obesity Res.* 10:182-187 (2002)), feeding disorders (U.S. Patent No. 6,720,324; Fehm, H. L. et al., *J. Clin. Endo. 2 Metab.*, 86:1144-1148 (2001); Pontillo, J. et al., *Bioorganic 2 Med. Chem. Ltrs.*, 15:2541-2546 (2005)) and Prader-Willi Syndrome (GE, Y. et al., *Brain Research*, 957:42-45 (2002)); metabolic 25 diseases and medical conditions accompanied by weight loss such as anorexia (U.S. Patent No. 6,613,874; Wisse, B. R. et al., *Endo.*, 142:3292-3301 (2001)), bulimia (U.S. Patent No. 6,720,324), AIDS wasting (Marsilje, T. H. et al., *Bioorg. Med. Chem. Lett.*, 14:3721-3725 (2004); Markison, S. et al., *Endocrinology*, 146:2766-2773 (2005)), cachexia (U.S. Patent No. 6,613,874; Lechan, R. M. et al., *Endo.*, 142:3288-3291 (2001); Pontillo, J. et al., *Bioorganic 2 Med. Chem. Ltrs.*, 15:2541-2546 (2005)), cancer cachexia (U.S. Patent No. 6,639,123) and wasting in frail elderly (U.S. Patent No. 30 6,639,123); diabetes (U.S. Patent No. 6,713,487) and diabetological related conditions and complications of diabetes such as retinopathy (U.S. Patent No. 6,525,019); neoplastic proliferation (U.S. Patent No. 6,713,487) such as skin cancer (Sturm, R.A., *Melanoma Res.*, 12:405-416 (2002); Bastiens, M. T. et al., *Am. J. Hum. Genet.*, 68:884-894 (2001)), and prostate cancer (Luscombe, C. J. et al., *British J. Cancer*, 85:1504-1509 (2001)); reproductive or sexual medical conditions such as 35 endometriosis (U.S. Patent No. 6,713,487) and uterine bleeding in women (U.S. Patent No. 6,613,874), sexual dysfunction (U.S. Patent No. 6,720,324; Van der Ploeg, L. H. T. et al., *PNAS*,

99:11381-11386 (2002), Molinoff, P. B. et al., *Ann. N.Y. Acad. Sci.*, 994:96-102 (2003), Hopps, C. V. et al., *BJU International*, 92:534-538 (2003)), erectile dysfunction ((U.S. Patent No. 6,613,874; Diamond, L. E. et al., *Urology*, 65:755-759 (2005), Wessells, H. et al., *Int. J. Impotence Res.*, 12:S74-S79 (2000), Andersson, K-E. et al., *Int. J. Impotence Res.*, 14:S82-S92 (2002), Bertolini, A. et al.,
5 Sexual Behavior: Pharmacology and Biochemistry, Raven Press, NY, p 247-257 (1975); Wessells, H. et al., *Neuroscience*, 118:755-762 (2003), Wessells, H. et al., *Urology*, 56:641-646 (2000), Shadiack, A. M. et al., *Society for Neuroscience Abstract*, (2003); Wessells, H. et al., *J. Urology*, 160:389-393 (1998), Rosen, R. C. et al., *Int. J. Impotence Res.*, 16:135-142 (2004), Wessells, H. et al., *Peptides*, 26:1972-1977 (2005)) and decreased sexual response in females (U.S. Patent No. 6,713,487;
10 Fourcroy, J. L., *Drugs*, 63:1445-1457 (2003)); diseases or conditions resulting from treatment or insult to the organism such as organ transplant rejection (U.S. Patent No. 6,713,487; Catania, A. et al., *Pharm. Rev.*, 56:1-29 (2004)), ischemia and reperfusion injury (Mioni, C. et al., *Eu. J. Pharm.*, 477:227-234 (2003); Catania, A. et al., *Pharm. Rev.*, 56:1-29 (2004)), treatment of spinal cord injury and to accelerate wound healing (Sharma H. S. et al., *Acta. Neurochir. Suppl.*, 86:399-405 (2003);
15 Sharma H.S., *Ann. N.Y. Acad. Sci.* 1053: 407-421 (2005); U.S. Patent No. 6,525,019), as well as weight loss caused by chemotherapy, radiation therapy, temporary or permanent immobilization (Harris, R. B. et al., *Physiol. Behav.*, 73:599-608 (2001)) or dialysis; cardiovascular diseases or conditions such as hemorrhagic shock (Catania, A. et al., *Pharm. Rev.*, 56:1-29 (2004)), cardiogenic shock (U.S. Patent No. 6,613,874), hypovolemic shock (U.S. Patent No. 6,613,874), cardiovascular
20 disorders (U.S. Patent No. 6,613,874) and cardiac cachexia (Markison, S. et al., *Endocrinology*, 146:2766-2773 (2005); pulmonary diseases or conditions such as acute respiratory distress syndrome (U.S. Patent No. 6,350,430; Catania, A. et al., *Pharm. Rev.*, 56:1-29 (2004)), chronic obstructive pulmonary disease (U.S. Patent No. 6,713,487), asthma (U.S. Patent No. 6,713,487) and pulmonary fibrosis; to enhance immune tolerance (Luger, T. A. et al., *Pathobiology*, 67:318-321 (1999)) and to
25 combat assaults to the immune system such as those associated with certain allergies (U.S. Patent No. 6,713,487) or organ transplant rejection (U.S. Patent No. 6,713,487; Catania, A. et al., *Pharm. Rev.*, 56:1-29 (2004)); treatment of dermatological diseases and conditions such as psoriasis (U.S. Patent No. 6,713,487), skin pigmentation depletion (U.S. Patent No. 6,713,487; Ye, Z. et al., *Peptides*, 26:2017-2025 (2005)), acne (Hatta, N. et al., *J. Invest. Dermatol.*, 116:564-570 (2001); Bohm, M. et al., *J. Invest. Dermatol.*, 118:533-539 (2002)), keloid formation (U.S. Patent No. 6,525,019) and skin
30 cancer (Sturm, R.A., *Melanoma Res.*, 12:405-416 (2002); Bastiens, M. T. et al., *Am. J. Hum. Genet.*, 68:884-894 (2001)); behavioral, central nervous system or neuronal conditions and disorders such as anxiety (U.S. Patent No. 6,720,324; Pontillo, J. et al., *Bioorganic 2 Med. Chem. Ltrs.*, 15:2541-2546 (2005)), depression (Chaki, S. et al., *Peptides*, 26:1952-1964 (2005), Bednarek, M. A. et al., *Expert
35 Opinion Ther. Patents*, 14:327-336 (2004); U.S. Patent No. 6,720,324), memory and memory dysfunction (U.S. Patent No. 6,613,874; Voisey, J. et al., *Curr. Drug Targets*, 4:586-597 (2003)), modulating pain perception (U.S. Patent No. 6,613,874; Bertolini, A. et al., *J. Endocrinol. Invest.*,

4:241-251 (1981); Vrinten, D. et al., J. Neuroscience, 20:8131-8137 (2000)) and treating neuropathic pain (Pontillo, J. et al., Bioorganic 2 Med. Chem. Ltrs., 15:2541-2546 (2005)); conditions and diseases associated with alcohol consumption, alcohol abuse and/or alcoholism (WO 05/060985; Navarro, M. et al., Alcohol Clin. Exp. Res., 29:949-957 (2005)); and renal conditions or diseases such as the treatment of renal cachexia (Markison, S. et al., Endocrinology, 146:2766-2773 (2005)) or natriuresis (U.S. Patent No. 6,613,874).

Ligand compounds activating one or more melanocortin receptor would be useful for modulating a wide variety of normalizing or homeostatic activities in a subject in need thereof including thyroxin release (U.S. Patent No. 6,613,874), aldosterone synthesis and release (U.S. Patent No. 6,613,874), body temperature (U.S. Patent No. 6,613,874), blood pressure (U.S. Patent No. 6,613,874), heart rate (U.S. Patent No. 6,613,874), vascular tone (U.S. Patent No. 6,613,874), brain blood flow (U.S. Patent No. 6,613,874), blood glucose levels (U.S. Patent No. 6,613,874), bone metabolism, bone formation or development (Dumont, L. M. et al., Peptides, 26:1929-1935 (2005), ovarian weight (U.S. Patent No. 6,613,874), placental development (U.S. Patent No. 6,613,874), prolactin and FSH secretion (U.S. Patent No. 6,613,874), intrauterine fetal growth (U.S. Patent No. 6,613,874), parturition (U.S. Patent No. 6,613,874), spermatogenesis (U.S. Patent No. 6,613,874), sebum and pheromone secretion (U.S. Patent No. 6,613,874), neuroprotection (U.S. Patent No. 6,639,123) and nerve growth (U.S. Patent No. 6,613,874) as well as modulating motivation (U.S. Patent No. 6,613,874), learning (U.S. Patent No. 6,613,874) and other behaviors (U.S. Patent No. 6,613,874).

There exists a need for improved formulations of compositions containing peptides that are ligands of one or more of the melanocortin receptors, in particular ligands that selectively bind to MC4-R, that provide acceptable sustained release profile with improved pharmacokinetic parameters upon single subcutaneous injection. Ideally, such improved sustained release formulations comprise novel peptides that act as ligands for one or more of the melanocortin receptors as disclosed in the Applicant's own prior international publications WO2007/008704, WO2008/147556 and WO2008/156677. Moreover, among other therapeutic effects of the formulations of the present invention, are attenuated side effects and improved efficacy associated with flatter release profile. In addition, the present invention provides for a solution, which may or may not be a clear solution, devoid of excipients thus simplifying the manufacturing process.

SUMMARY OF THE INVENTION

The inventors of the present invention made a surprising discovery that a formulation of a pharmaceutical composition comprising a pamoate salt of a peptide that acts as a ligand of one or more of the melanocortin receptors exhibits an ideal solubility for slow release *in vivo*. Particularly preferred is the following peptide which is referred to hereinafter as "Example 1": Ac-Arg-

cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂, a ligand of the melanocortin receptor subtype 4, wherein after subcutaneous or intramuscular administration to a subject, the peptide forms a depot at physiological pH that is slowly dissolved and released into the body fluid and bloodstream, thereby resulting in attenuated side effects and improved efficacy.

5 The invention may be summarized in the following paragraphs below, as well as in the claims.

- 10 (1) In one aspect, the present invention is directed to a pharmaceutical composition of a solution, a gel or a semi-solid, or a suspension, comprising a peptide that acts as a ligand of one or more of the melanocortin receptors, or a pharmaceutically acceptable salt thereof, in which the peptide forms a depot after subcutaneous or intramuscular administration to a subject.
- 15 (2) The pharmaceutical composition according to paragraph 1, wherein said peptide in said solution forms a depot at an injection site after administration that is slowly dissolved and released into the body fluid and bloodstream, and wherein said solution is a purely aqueous solution, a purely organic solution, an aqueous solution having an organic component, an aqueous solution having an inorganic component, or an aqueous solution having both organic and inorganic components.
- (3) The pharmaceutical composition according to paragraph 1 or paragraph 2, wherein said peptide is Example 1, *i.e.*, Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂.
- 20 (4) The pharmaceutical composition according to any one of the preceding paragraphs, wherein said peptide is in a pamoate salt form.
- (5) The pharmaceutical composition according to any one of the preceding paragraphs, further comprising an organic component which increases the solubility of said peptide in an aqueous solution or decreases the viscosity of a gel or a semi-solid.
- 25 (6) The pharmaceutical composition according to paragraph 5, wherein said organic component is an organic polymer, an alcohol, DMSO, DMF, or DMA.
- (7) The pharmaceutical composition according to paragraph 6, wherein said organic polymer is PEG.
- (8) The pharmaceutical composition according to paragraph 7, wherein said PEG has an average molecular weight of from about 200 to about 10,000.
- 30 (9) The pharmaceutical composition according to paragraph 8, wherein said peptide is dissolved in a PEG200 or PEG400 aqueous solution, in which the volume-to-volume ratio of PEG to water is from about 1:99 to about 99:1.

- (10) The pharmaceutical composition according to paragraph 9, wherein said peptide is dissolved in a PEG200 or PEG400 aqueous solution, in which the volume-to-volume ratio of PEG to water is from about 1:9 to about 1:1.
- 5 (11) The pharmaceutical composition according to paragraph 6, wherein said alcohol is ethanol or isopropyl alcohol.
- (12) The pharmaceutical composition according to any one of the preceding paragraphs, wherein the weight-to-volume concentration of said peptide is between about 0.1 mg/mL and about 600 mg/mL.
- 10 (13) The pharmaceutical composition according to any one of the preceding paragraphs, wherein the pH of said composition is between about 3.0 and about 8.0.
- (14) The pharmaceutical composition according to paragraph 13, wherein said pamoate salt of Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂ is dissolved in a PEG400/aqueous solution, in which the volume-to-volume ratio of PEG400 to water is about 1:1, and in which the weight-to-volume concentration of the peptide is about 200 mg/mL.
- 15 (15) The pharmaceutical composition according to paragraph 13, wherein said pamoate salt of Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂ is dissolved in a PEG200/aqueous solution, in which the volume-to-volume ratio of PEG200 to water is about 1:1, and in which the weight-to-volume concentration of the peptide is about 200 mg/mL.
- 20 (16) The pharmaceutical composition according to paragraph 13, wherein said pamoate salt of Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂ is dissolved in a PEG400/PBS solution, in which the volume-to-volume ratio of PEG400 to PBS is about 1:1, and in which the weight-to-volume concentration of the peptide is about 300 mg/mL.
- 25 (17) The pharmaceutical composition according to paragraph 13, wherein said pamoate salt of Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂ is dissolved in a PEG400/saline solution, in which the volume-to-volume ratio of PEG400 to saline solution is about 1:1, and in which the weight-to-volume concentration of the peptide is about 300 mg/mL.
- (18) The pharmaceutical composition according to any one of the preceding paragraphs, further comprising a preservative.
- 30 (19) The pharmaceutical composition according to paragraph 18, wherein said preservative is selected from the group consisting of m-cresol, phenol, benzyl alcohol, and methyl paraben.
- (20) The pharmaceutical composition according to paragraph 19, wherein said preservative is present in a concentration from about 0.01 mg/mL to about 100 mg/mL.

- (21) The pharmaceutical composition according to any one of the preceding paragraphs, further comprising an isotonic agent.
- (22) The pharmaceutical composition according to paragraph 21, wherein said isotonic agent is present in a concentration from about 0.01 mg/mL to about 100 mg/mL.
- 5 (23) The pharmaceutical composition according to any one of the preceding paragraphs, further comprising a stabilizer.
- (24) The pharmaceutical composition according to paragraph 23, wherein said stabilizer is selected from the group consisting of imidazole, arginine and histidine.
- (25) The pharmaceutical composition according to any one of the preceding paragraphs, further comprising a surfactant.
- 10 (26) The pharmaceutical composition according to any one of the preceding paragraphs, further comprising a chelating agent.
- (27) The pharmaceutical composition according to any one of the preceding paragraphs, further comprising a buffer.
- 15 (28) The pharmaceutical composition according to paragraph 27, wherein said buffer is selected from the group consisting of Tris, ammonium acetate, sodium acetate, glycine, aspartic acid, and Bis-Tris.
- (29) The pharmaceutical composition according to any one of the preceding paragraphs, further comprising a divalent metal.
- 20 (30) The pharmaceutical composition according to paragraph 29, wherein said divalent metal is zinc.
- (31) The pharmaceutical composition according to any one of the preceding paragraphs, wherein said solution is a clear solution.

Although the preferred embodiment of the present invention is directed to Example 1, *i.e.*,
25 Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂, which is a ligand of MCR-R, the present invention is in no way limited to Example 1. The peptides of the present invention include, for example, all those peptides that act as ligands for one or more of the melanocortin receptors, as disclosed in the applicant's own prior international publication numbers published as
WO2007/008704, WO2008/147556 and WO2008/156677. These publications are herein
30 incorporated by reference to the same extent as if the disclosure of each independent publication was explicitly provided herein.

The following compounds from these publications may also be advantageously employed to constitute the pharmaceutical compositions of the present invention:

Example 2: Ac-D-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂;

Example 3: Ac-Tyr-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂;

5 Example 4: Ac-Tyr-D-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂; and

Example 5: hydantoin(Arg-Gly)-cyclo(Cys-Glu-His-D-Phe-Arg-Trp-Cys)-NH₂.

BRIEF DESCRIPTION OF THE DRAWINGS

10 FIG. 1A and FIG. 1B show full time course plots of the pharmacokinetic profiles (median values) obtained after a single subcutaneous administration to Sprague-Dawley rats dosed at 2.5 mg/kg body weight, of a formulation comprising 200 mg/mL (20% w/v) of pamoate salt of Example 1 dissolved in a 50% PEG400 and 50% water (v/v) solvent, on a normal scale and on a logarithmic scale, respectively.

15 FIG. 2A and FIG. 2B show full time course plots of the pharmacokinetic profiles (median values) obtained after a single subcutaneous administration to Sprague-Dawley rats dosed at 2.5 mg/kg body weight, of a formulation comprising 200 mg/mL (20% w/v) of pamoate salt of Example 1 dissolved in a 50% PEG200 and 50% water (v/v) solvent, on a normal scale and on a logarithmic scale, respectively.

20 FIG. 3A and FIG. 3B show full time course plots of the pharmacokinetic profiles (median values) obtained after a single subcutaneous administration to Sprague-Dawley rats dosed at 3.75 mg/kg body weight, of a formulation comprising 300 mg/mL (30% w/v) of pamoate salt of Example 1 dissolved in a 50% PEG400 and 50% PBS (v/v) solvent, on a normal scale and on a logarithmic scale, respectively.

25 FIG. 4A and FIG. 4B show full time course plots of the pharmacokinetic profiles (median values) obtained after a single subcutaneous administration to Sprague-Dawley rats dosed at 3.75 mg/kg body weight, of a formulation comprising 300 mg/mL (30% w/v) of pamoate salt of Example 1 dissolved in a 50% PEG400 and 50% saline (v/v) solvent, on a normal scale and on a logarithmic scale, respectively.

30 FIG. 5A and FIG. 5B show full time course plots of the pharmacokinetic profiles (median values) obtained after a single subcutaneous administration to Sprague-Dawley rats dosed at 0.5 mg/kg body weight of a formulation comprising acetate salt of Example 1 dissolved in a saline/2% heat inactivated mouse serum/5% DMA/2% tween-80 solvent, on a normal scale and on a logarithmic scale, respectively.

35

FIG. 6A and 6B show the same full time course plots of the pharmacokinetic profiles (median values) as shown in FIG. 1A through FIG. 5A on a normal scale as well as FIG 1B through 5B on a logarithmic scale as a side-by-side comparison of the different formulations used in the experiments as described herein.

DETAILED DESCRIPTION OF THE INVENTION

The nomenclature used to define the peptides herein is that typically used in the art wherein the amino group at the N-terminus appears to the left and the carboxyl group at the C-terminus appears to the right. Where the amino acid has isomeric forms, it is the L form of the amino acid that is represented unless otherwise explicitly indicated. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

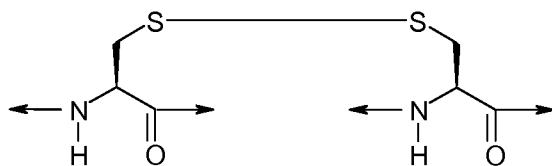
Abbreviations used herein are defined as follows:

10	Ac:	acetyl
	Ala or A:	alanine
	Arg or R:	arginine
	Cys or C:	cysteine
	Glu or E:	glutamic acid
15	Gly or G:	glycine
	His or H:	histidine
	Phe or F:	phenylalanine
	Trp or W:	tryptophan
	Tyr or Y:	tyrosine

20 Unless otherwise indicated, all abbreviations (*e.g.*, Ala) of amino acids in this disclosure stand for the structure of $-\text{NH}-\text{C}(\text{R})(\text{R}')-\text{CO}-$, wherein R and R' each is, independently, hydrogen or the side chain of an amino acid (*e.g.*, R = CH_3 and R' = H for Ala), or R and R' may be joined to form a ring system.

The designation “ NH_2 ” in, *e.g.*, Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)- NH_2 ,
25 indicates that the C-terminus of the peptide is amidated.

“-cyclo(Cys-Cys)-” denotes the structure:

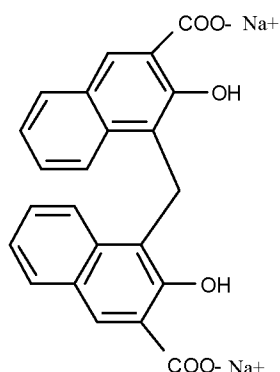


The term “about” as used herein, in associations with parameters and amounts, means that the parameter or amount is within $\pm 5\%$ of the stated parameter or amount.

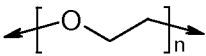
30 Certain other abbreviations used herein are defined as follows:

Boc:	<i>tert</i> -butyloxycarbonyl
BSA:	bovine serum albumin

- DCM: dichloromethane
 DIPEA: diisopropylethylamine
 DMF: dimethylformamide
 DTT: dithiothreitol
 5 Fmoc: 9-Fluorenylmethyloxycarbonyl
 HBTU: 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
 HOBt: 1-hydroxy-benzotriazole
 HPLC: high performance liquid chromatography
 IBMX: isobutylmethylxanthine
 10 Sodium pamoate: pamoic acid disodium salt having the structure of



- LC-MS: liquid chromatography mass spectrometry
 LOQ: limit of quantification
 MRM: multiple reaction monitoring
 15 NMP: N-methylpyrrolidone
 PBS: phosphate buffered saline

PEG: poly(ethylene glycol), which has the structure of  wherein n an integer between 1 and 2,000

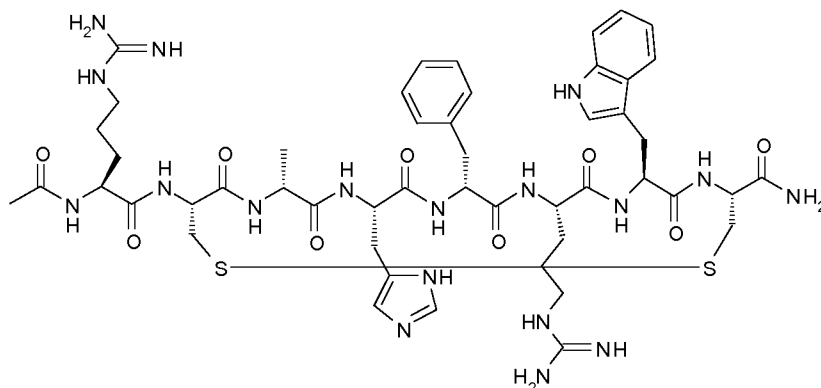
- PEG200: poly(ethylene glycol) with an average molecular weight of about 200 Da
 20 PEG400: poly(ethylene glycol) with an average molecular weight of about 400 Da
 TFA: trifluoroacetic acid
 TIS: triisopropylsilane
 Tris-HCl: tris(hydroxymethyl)aminomethane hydrochloride
 Trt: trityl

25

Synthesis

The peptides of this invention can be prepared by standard solid phase peptide synthesis. See, *e.g.*, Stewart, J.M., *et al.*, *Solid Phase Synthesis* (Pierce Chemical Co., 2d ed. 1984). The following examples describe synthetic methods for making a peptide of this invention, which methods are well-known to those skilled in the art. Other methods are also known to those skilled in the art. The examples are provided for the purpose of illustration and are not meant to limit the scope of the present invention in any manner.

- Synthesis of Example 1, i.e., Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂



The title peptide having the above structure was assembled using Fmoc chemistry on an Apex peptide synthesizer (Aapptec; Louisville, KY, USA). 220 mg of 0.91 mmol/g (0.20 mmoles) Rink Amide MBHA resin (Polymer Laboratories; Amherst, MA, USA) was placed in a reaction well and pre-swollen in 3.0 mL of DMF prior to synthesis. For cycle 1, the resin was treated with two 3-mL portions of 25% piperidine in DMF for 5 and 10 minutes respectively, followed by 4 washes of 3-mL DMF - each wash consisting of adding 3 mL of solvent, mixing for 1 minute, and emptying for 1 minute. Amino acids stocks were prepared in NMP as 0.45M solutions containing 0.45M HOBT. HBTU was prepared as a 0.45M solution in NMP and DIPEA was prepared as a 2.73M solution in NMP. To the resin, 2 mL of the first amino acid (0.9 mmoles, Fmoc-Cys(Trt)-OH) (Novabiochem; San Diego, CA, USA) was added along with 2 mL (0.9 mmoles) of HBTU and 1.5 mL (4.1 mmoles) of DIPEA. After one hour of constant mixing, the coupling reagents were drained from the resin and the coupling step was repeated. Following amino acid acylation, the resin was washed with two 3-mL aliquots of DMF for 1 minute. The process of assembling the peptide (deblock/wash/acylate/wash) was repeated for cycles 2-9 identical to that as described for cycle 1. The following amino acids were used: cycle 2) Fmoc-Trp(Boc)-OH (Genzyme; Cambridge, MA, USA); cycle 3) Fmoc-Arg(Pbf)-OH (Novabiochem); cycle 4) Fmoc-DPhe-OH (Genzyme); cycle 5) Fmoc-His(Trt)-OH (Novabiochem); cycle 6) Fmoc-D-Ala-OH (Genzyme); cycle 7) Fmoc-Cys(Trt)-OH, (Novabiochem); and cycle 8) Fmoc-Arg(Pbf)-OH (Genzyme). The N-terminal Fmoc was removed with 25% piperidine in DMF as

described above, followed by four 3-mL DMF washes for 1 minute. Acetylation of the N-terminus was performed by adding 0.5 mL of 3M DIPEA in NMP to the resin along with 1.45 mL of 0.45M acetic anhydride in NMP. The resin was mixed for 30 minutes and acetylation was repeated. The resin was washed with 3 mL of DMF for a total of 5 times followed with 5 washes with 5 mL of DCM each.

To cleave and deprotect the peptide, 5mL of the following reagent was added to the resin: 2% TIS/5% water/5% (w/v) DTT/88% TFA. The solution was allowed to mix for 3.5 hours. The filtrate was collected into 40 mL of cold anhydrous ethyl ether. The precipitate was pelleted for 10 minutes at 3500 rpm in a refrigerated centrifuge. The ether was decanted and the peptide was re-suspended in fresh ether. The ether workup was performed three times. Following the last ether wash, the peptide was allowed to air dry to remove residual ether.

The peptide was dissolved in 10% acetonitrile and analyzed by mass spectrometry and reverse-phase HPLC employing a 30x4.6cm C18 column (Vydac; Hesperia, CA, USA) with a gradient of 2-60% acetonitrile (0.1% TFA) over 30 minutes. This analysis identified a product with ~53% purity. Mass analysis employing electrospray ionization identified a main product containing a mass of 1118.4 corresponding to the desired linear product. The crude product (~100 mg) was diluted to a concentration of 2mg/mL in 5% acetic acid. To this solution, 0.5M iodine/methanol was added dropwise with vigorous stirring until a pale yellow color was achieved. The solution was vigorously stirred for another 10 minutes. Excess iodine was then quenched by adding 1.0M sodium thiosulfate under continuous mixing until the mixture was rendered colorless. The peptide was re-examined by mass spectrometry analysis and HPLC. Mass spectrometry analysis identified a main species with a mass of 1116.4 which indicated successful oxidation to form the cyclic peptide. The peptide solution was purified on a preparative HPLC equipped with a C18 column using a similar elution gradient. The purified product was re-analyzed by HPLC for purity (>95%) and mass spectrometry (1116.9 which is in agreement with the expected mass of 1117.3) and subsequently lyophilized. Following lyophilization, 28 mg of purified product was obtained representing a 24% yield.

The other exemplified peptides were synthesized substantially according to the procedure described for the above-described synthetic process. Physical data for select exemplified peptides are given in Table 1.

TABLE 1

Example Number	Mol. Wt. (calculated)	Mol. Wt. (ES-MS)	Purity (HPLC)
1	1117.3	1116.9	95.1%
2	1117.3	1116.8	99.2%
3	1280.5	1280.6	98.0%
5	1216.37	1216.20	99.9%

- Preparation of Pamoate Salt of Example 1

The acetate salt of Example 1 (200 mg, 0.18 mmole) was dissolved in 10 mL of water. Sodium pamoate (155 mg, 0.36 mmole) was dissolved in 10 mL of water. The two solutions were combined and mixed well. The precipitates were collected by centrifugation at 3000 rpm for 20 minutes, washed for three times with water, and dried by lyophilization.

In Vitro Studies

Compounds of the present invention can be and were tested for activity as ligands of one or more of the melanocortin receptors according to the following procedures. One skilled in the art would know that procedures similar to those described herein may be used to assay the binding activities of the compounds of the invention to melanocortin receptor molecules.

- Radioligand Binding Assays

Cellular membranes used for the *in vitro* receptor binding assays were obtained from transgenic CHO-K1 cells stably expressing hMC-R receptor subtypes 1, 3, 4 or 5. The CHO-K1 cells expressing the desired hMC-R receptor type were sonicated (Branson®, CT, USA; setting 7, approximately 30 sec) in ice-cold 50 mM Tris-HCl at pH 7.4 and then centrifuged at 39,000 g for 10 minutes at a temperature of approximately 4°C. The pellets were resuspended in the same buffer and centrifuged at 50,000 g for 10 minutes at a temperature of approximately 4°C. The washed pellets containing the cellular membranes were stored at approximately -80°C.

Competitive inhibition of [¹²⁵I](Tyr²)-(Nle⁴-D-Phe⁷) α -MSH ([¹²⁵I]-NDP- α -MSH; Amersham Biosciences®, Piscataway, NJ, USA) binding was carried out in polypropylene 96 well plates. Cell membranes (1-10 μ g protein/well), prepared as described above, were incubated in 50 mM Tris-HCl at pH 7.4 containing 0.2% BSA, 5 mM MgCl₂, 1 mM CaCl₂ and 0.1 mg/mL bacitracin, with increasing concentrations of the test compound and 0.1-0.3 nM [¹²⁵I]-NDP- α -MSH for approximately 90-120 minutes at approximately 37°C. Bound [¹²⁵I]-NDP- α -MSH ligand was separated from free [¹²⁵I]-NDP- α -MSH by filtration through GF/C glass fiber filter plates (Unifilter®, Meriden, CT, USA) presoaked with 0.1 % (w/v) polyethylenimine (PEI), using a Packard Filtermate® harvester (Millipore, Danvers, MA, USA). Filters were washed three times with 50 mM Tris-HCl at pH 7.4 at a temperature of approximately 0-4°C and then assayed for radioactivity using a Packard Topcount® scintillation counter (GMI, Inc., Ramsey, MN, USA). Binding data were analyzed by computer-assisted non-linear regression analysis (XL fit; IDBS, Burlington, MA, USA).

A selection of the preferred embodiments was tested using the above-discussed assay and the binding constants (K_i in nM) are reported in Table 2.

TABLE 2

Example Number	Ki (nM) hMC1-R	Ki (nM) hMC3-R	Ki (nM) hMC4-R	Ki (nM) hMC5-R
1	3.87	10.1	2.09	430
2	4.01	12.1	1.76	352
3	8.53	21.2	3.72	714
5	8.59	94.1	2.44	7760

- *cyclic AMP Bioassay*

Intracellular cyclic AMP (cAMP) levels were determined by an electrochemiluminescence (ECL) assay (Meso Scale Discovery, Gaithersburg, MD, USA; referred to hereinafter as “MSD”).

5 CHO-K1 cells stably expressing the hMC receptor subtypes were suspended in RMPI 1640[®] assay buffer (RMPI 1640 buffer contains 0.5mM IBMX, and 0.2% protein cocktail (MSD blocker A)). Transgenic CHO-K1 cells stably expressing hMC receptor subtypes 1, 3, 4 or 5 were dispensed at a density of approximately 7,000 cells/well in 384-well Multi-Array plates (MSD) containing integrated

10 carbon electrodes and coated with anti-cAMP antibody. Increasing concentrations of the test compounds were added and the cells were incubated for approximately 40 minutes at approximately 37°C. Following this incubation, lysis buffer (HEPES-buffered saline solution with MgCl₂ and Triton X-100[®] at pH 7.3) containing 0.2% protein cocktail and 2.5 nM TAG[™] ruthenium-labeled cAMP (MSD) was added and the cells were incubated for approximately 90 minutes at room temperature.

15 At the end of the second incubation period, read buffer (Tris-buffered solution containing an ECL co-reactant and Triton X-100 at pH 7.8) was added and the cAMP levels in the cell lysates were immediately determined by ECL detection with a Sector Imager 6000 reader[®] (MSD). Data were analyzed using a computer-assisted non-linear regression analysis (XL fit; IDBS) and reported as either an EC₅₀ value or a Kb value.

20 EC₅₀ represents the concentration of an agonist compound needed to obtain 50% of the maximum reaction response, *e.g.*, 50% of the maximum level of cAMP as determined using the assay described above. The Kb value reflects the potency of an antagonist and is determined by Schild analysis. In brief, concentration-response curves of an agonist are carried out in the presence of increasing concentrations of an antagonist. The Kb value is the concentration of antagonist which

25 would produce a 2-fold shift in the concentration-response curve for an agonist. It is calculated by extrapolating the line on a Schild plot to zero on the y-axis.

A selection of compounds was tested using the above-discussed assays and the results are reported in Table 3.

TABLE 3

Example Number	EC ₅₀ hMC1-R	EC ₅₀ hMC3-R	EC ₅₀ hMC4-R	EC ₅₀ hMC5-R
1	5.79	5.25	0.313	1630
2	6.17	5.6	0.397	1020
3	6.42	2.39	0.194	1540
5	8.83	7.86	0.0979	4010

Solubility Studies

- Preparation of Formulation of Pamoate Salt of Example 1

5 Pamoate salt of Example 1 (50 mg) was weighted into a microcentrifuge tube, and 125 μ L of PEG400 and 125 μ L of water were added thereafter. The mixture was sonicated to facilitate dissolution. A clear solution may be, and was, obtained.

The solubility of acetate salt of Example 1 was determined by weighing a certain amount of the peptide and dissolving it in a proper volume of water, saline, or PBS, and the results are shown in

10 Table 4A.

TABLE 4A

Solvent	Concentration (w/v)	pH	ZnCl ₂	Solubility
Water	10%	7.0	no	soluble
Water	10%	7.0	1:1 molar	soluble
Water	20%	7.0	no	soluble
Water	20%	7.0	1:1 molar	soluble
Water	50%	7.0	no	soluble
Water	50%	7.0	1:1 molar	soluble
Water	70%	7.0	no	soluble
Water	70%	7.0	1:1 molar	soluble
Water	90%	7.0	no	soluble
Water	90%	7.0	1:1 molar	soluble
Water	50%	8.0	no	soluble
Saline	50%	7.0	no	soluble
Saline	50%	7.0	1:1 molar	soluble
Saline	50%	8.0	no	soluble
PBS	50%	7.0	no	soluble
PBS	50%	7.0	1:1 molar	soluble
PBS	50%	8.0	no	soluble

The solubility of pamoate salt of Example 1 was determined by mixing the peptide in water or PBS, followed by HPLC determination of the concentration in the supernatant, and the results are shown in Table 4B.

TABLE 4B

Solubility of	Water, pH 7.0	PBS, pH 7.4
Pamoate salt of Example 1	0.06 mg/mL	0.21 mg/mL
Pamoate salt of Example 1 with Zn	0.05 mg/mL	0.12 mg/mL

5

- Pharmacokinetic Studies of Formulations of Fatty Acid Salts of Example 1 in

10

The following “Formulations 1-6” of Example 1 were prepared:

- (1) “Formulation 1”: Pamoate salt of Example 1 dissolved in a 50% PEG400 and 50% water (v/v) solution, at a concentration of 200 mg/mL (20% w/v).
- (2) “Formulation 2”: Pamoate salt of Example 1 dissolved in a 50% PEG200 and 50% water (v/v) solution, at a concentration of 200 mg/mL (20% w/v).
- (3) “Formulation 3”: Pamoate salt of Example 1 dissolved in a 50% PEG400 and 50% PBS (v/v) solution, at a concentration of 300 mg/mL (30% w/v).
- (4) “Formulation 4”: Pamoate salt of Example 1 dissolved in a 50% PEG400 and 50% saline (v/v) solution, at a concentration of 300 mg/mL (30% w/v).
- (5) “Formulation 5”: Acetate salt of Example 1 dissolved in a saline/2% heat inactivated mouse serum/5% DMA/2% tween-80 solution.

20

- Dosing

For Formulations 1 and 2, Sprague-Dawley rats were dosed via subcutaneous injection at a fixed amount of either 5 μ L/rat or 1.0 mg/rat, or a variable amount of 2.5 mg/kg body weight.

25

For Formulations 3 and 4, Sprague-Dawley rats were dosed via subcutaneous injection at a fixed amount of either 5 μ L/rat or 1.5 mg/rat, or a variable amount of 3.75 mg/kg body weight.

For Formulation 5, Sprague-Dawley rats were dosed via subcutaneous injection at a variable amount of 0.5 mg/kg body weight.

- Sample Preparation

30

For Formulations 1 and 2, 100 μ L of plasma was acidified with 5 μ L of formic acid and precipitated with 300 μ L of acetonitrile. The supernatant was collected by centrifugation and dried by

speed-vac. The dried pellet was dissolved in 100 μ L of water which was then centrifuged. 50 μ L of the preparation was injected for LC-MS/MS analysis.

For Formulations 3 and 4, 200 μ L of plasma was acidified with 10 μ L of formic acid and precipitated with 600 μ L of acetonitrile. The supernatant was collected by centrifugation and dried by speed-vac. The dried pellet was dissolved in 150 μ L of water which was then centrifuged. 50 μ L of the preparation was injected for LC-MS/MS analysis.

For Formulation 5, 200 μ L of plasma was acidified with 10 μ L of formic acid and precipitated with 600 μ L of acetonitrile. The supernatant was collected by centrifugation and dried by speed-vac. The dried pellet was dissolved in 150 μ L of 30% acetonitrile which was then centrifuged. 50 μ L of the preparation was injected for LC-MS/MS analysis.

- LC-MS/MS Analysis

For Formulations 1 and 2, LC-MS/MS analysis was performed with an API4000 mass spectrometer system equipped with a Turbo Ionspray probe. The MRM mode of molecular ion detection with an ion pair of 559.5 and 110.1 was used. HPLC separation was performed with a Luna C8(2) 2x30 mm 3 μ column run from 0% B to 80% B in 10 minutes at a flow rate of 0.3 mL/minute. Buffer A is 1% formic acid in water and buffer B is 1% formic acid in acetonitrile. LOQ was 5 ng/mL.

For Formulations 3 and 4, LC-MS/MS analysis was performed with an API4000 mass spectrometer system equipped with a Turbo Ionspray probe. The MRM mode of molecular ion detection with an ion pair of 559.5 and 110.1 was used. HPLC separation was performed with a Luna C8(2) 2x30 mm 3 μ column run from 0% B to 80% B in 10 minutes at a flow rate of 0.3 mL/minute. Buffer A is 1% formic acid in water and buffer B is 1% formic acid in acetonitrile. LOQ was 2 ng/mL.

For Formulation 5, LC-MS/MS analysis was performed with an API4000 mass spectrometer system equipped with a Turbo Ionspray probe. The MRM mode of molecular ion detection with an ion pair of 559.5 and 110.1 was used. HPLC separation was performed with a Luna C8(2) 2x30 mm 3 μ column run from 0% B to 90% B in 10 minutes at a flow rate of 0.3 mL/minute. Buffer A is 1% formic acid in water and buffer B is 1% formic acid in acetonitrile. LOQ was 1 ng/mL.

- Results and Summary

The plasma concentrations of Example 1, dosed with the Formulations 1-5, were calculated with its standard calibration plot and the results are shown in Table 5.

TABLE 5

Time	Plasma concentration (ng/mL) of Example 1, dosed with Formulation 1	Plasma concentration (ng/mL) of Example 1, dosed with Formulation 2	Plasma concentration (ng/mL) of Example 1, dosed with Formulation 3	Plasma concentration (ng/mL) of Example 1, dosed with Formulation 4	Plasma concentration (ng/mL) of Example 1, dosed with Formulation 5
5 minutes	55.35	84.40	44.1	56.0	83.8
10 minutes	57.05	153.50	69.5	84.0	200.7
15 minutes	83.65	187.50	94.7	109.0	245.0
30 minutes	94.80	212.00	165.0	150.7	298.3
1 hour	123.00	237.00	N/A	193.0	292.0
2 hours	127.00	304.50	559.7	269.0	160.7
3 hours	N/A	N/A	N/A	N/A	72.4
4 hours	182.50	420.00	768.3	644.5	38.4
5 hours	N/A	N/A	N/A	N/A	16.6
8 hours	135.00	235.50	478.7	495.0	N/A
12 hours	71.30	86.00	239.0	167.5	N/A
16 hours	42.85	42.25	142.5	181.0	N/A
20 hours	24.30	31.60	112.8	68.0	N/A
24 hours	16.50	27.85	86.9	75.9	N/A

5 Full time course plots of the pharmacokinetic profiles of Formulation 1 are shown on a normal scale in FIG. 1A, and on a logarithmic scale in FIG. 1B.

Full time course plots of the pharmacokinetic profiles of Formulation 2 are shown on a normal scale in FIG. 2A, and on a logarithmic scale in FIG. 2B.

Full time course plots of the pharmacokinetic profiles of Formulation 3 are shown on a normal scale in FIG. 3A, and on a logarithmic scale in FIG. 3B.

10 Full time course plots of the pharmacokinetic profiles of Formulation 4 are shown on a normal scale in FIG. 4A, and on a logarithmic scale in FIG. 4B.

Full time course plots of the pharmacokinetic profiles of Formulation 5 are shown on a normal scale in FIG. 5A, and on a logarithmic scale in FIG. 5B.

15 FIG. 6A and 6B show the same full time course plots of the pharmacokinetic profiles (median values) as shown in FIG. 1A through FIG. 5A on a normal scale, as well as FIG 1B through 5B on a logarithmic scale, as a side-by-side comparison of the different formulations used in the experiments as described herein.

Some pharmacokinetic parameters of Example 1, dosed with the Formulations 1-5, are shown in Table 6.

TABLE 6

5

	Example 1 dosed with Formulation 1	Example 1 dosed with Formulation 2	Example 1 dosed with Formulation 3	Example 1 dosed with Formulation 4	Example 1 dosed with Formulation 5
Dose (mg/kg)	2.5	2.5	3.75	3.75	0.5
T _{max} (hours)	4	4	4	4	1
C _{max} (ng/mL)	182	420	768	644	290
AUC (ng-hr/mL)	2141	3873	7519	6366	697
CL (ml/hour)	934	516	465	549	716
T _{1/2} (hours)	5.3	5.3	6.6	6.1	0.94

The results indicate that the formulations of Example 1 according to the present invention as described herein provide for acceptable sustained release formulations with improved pharmacokinetic parameters and flatter release profiles which may result in attenuated side effects and improved efficacy. For instance, Formulation 1 is shown to have an approximately 24-hour release profile after a single subcutaneous injection, with significantly low C_{max} and long T_{max}. Moreover, all of the pamoate salt formulations of Example 1, *i.e.*, Formulations 1-4, are shown to have significantly increased T_{1/2} compared to the acetate salt formulation of Example 1, *i.e.*, Formulation 5, as shown in Table 6.

10

15

Additional embodiments of the present invention will be apparent from the foregoing disclosure and are intended to be encompassed by the invention as described fully herein and defined in the following claims.

CLAIMS

What is claimed is:

1. A pharmaceutical composition of a solution, a gel or a semi-solid, or a suspension,
5 comprising a peptide that acts as a ligand of one or more of the melanocortin receptors, or a
pharmaceutically acceptable salt thereof, in which the peptide forms a depot after subcutaneous or
intramuscular administration to a subject.
2. The pharmaceutical composition according to claim 1, wherein said peptide in said
10 solution forms a depot at an injection site after administration that is slowly dissolved and released
into the body fluid and bloodstream, and wherein said solution is a purely aqueous solution, a purely
organic solution, an aqueous solution having an organic component, an aqueous solution having an
inorganic component, or an aqueous solution having both organic and inorganic components.
- 15 3. The pharmaceutical composition according to claim 1 or claim 2, wherein said
peptide is:
Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂;
Ac-D-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂;
Ac-Tyr-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂;
20 Ac-Tyr-D-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂; or
hydantoin(Arg-Gly)-cyclo(Cys-Glu-His-D-Phe-Arg-Trp-Cys)-NH₂;
or a pharmaceutically acceptable salt thereof.
4. The pharmaceutical composition according to claim 3, wherein said peptide is Ac-
25 Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂.
5. The pharmaceutical composition according to any one of the preceding claims,
wherein said pharmaceutically acceptable salt is a pamoate salt.
- 30 6. The pharmaceutical composition according to claim 5, further comprising an organic
component.
7. The pharmaceutical composition according to claim 6, wherein said organic
component is an organic polymer, an alcohol, DMSO, DMF, or DMA.
- 35 8. The pharmaceutical composition according to claim 7, wherein said organic polymer
is PEG.

9. The pharmaceutical composition according to claim 8, wherein said PEG has an average molecular weight of from about 200 to about 10,000.

10. The pharmaceutical composition according to claim 9, wherein said peptide is
5 dissolved in a PEG200 or PEG400 aqueous solution, in which the volume-to-volume ratio of PEG to water is from about 1:99 to about 99:1.

11. The pharmaceutical composition according to claim 10, wherein said peptide is
10 dissolved in a PEG200 or PEG400 aqueous solution in which the volume-to-volume ratio of PEG to water is from about 1:9 to about 1:1.

12. The pharmaceutical composition according to claim 7, wherein said alcohol is ethanol or isopropyl alcohol.

13. The pharmaceutical composition according to any one of the preceding claims,
15 wherein the weight-to-volume concentration of said peptide is between about 0.1 mg/mL and about 600 mg/mL.

14. The pharmaceutical composition according to any one of the preceding claims,
20 wherein the pH of said composition is between about 3.0 and about 8.0.

15. The pharmaceutical composition according to claim 14, wherein said pamoate salt of Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂ is dissolved in a PEG400/aqueous solution, in which the volume-to-volume ratio of PEG400 to water is about 1:1 and the weight-to-volume
25 concentration of the peptide is about 200 mg/mL.

16. The pharmaceutical composition according to claim 14, wherein said pamoate salt of Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂ is dissolved in a PEG200/aqueous solution, in which the volume-to-volume ratio of PEG200 to water is about 1:1 and the weight-to-volume
30 concentration of the peptide is about 200 mg/mL.

17. The pharmaceutical composition according to claim 14, wherein said pamoate salt of Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂ is dissolved in a PEG400/PBS solution, in which the volume-to-volume ratio of PEG400 to PBS is about 1:1 and the weight-to-volume
35 concentration of the peptide is about 300 mg/mL.

18. The pharmaceutical composition according to claim 14, wherein said pamoate salt of Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂ is dissolved in a PEG400/saline solution, in which the volume-to-volume ratio of PEG400 to saline solution is about 1:1 and the weight-to-volume
40 concentration of the peptide is about 300 mg/mL.

19. The pharmaceutical composition according to any one of the preceding claims further comprising a preservative.

5 20. The pharmaceutical composition according to claim 19, wherein said preservative is selected from the group consisting of m-cresol, phenol, benzyl alcohol, and methyl paraben.

21. The pharmaceutical composition according to claim 20, wherein said preservative is present in a concentration from about 0.01 mg/mL to about 100 mg/mL.

10

22. The pharmaceutical composition according to any one of the preceding claims further comprising an isotonic agent.

15

23. The pharmaceutical composition according to claim 22, wherein said isotonic agent is present in a concentration from about 0.01 mg/mL to about 100 mg/mL.

24. The pharmaceutical composition according to any one of the preceding claims further comprising a stabilizer.

20

25. The pharmaceutical composition according to claim 24, wherein said stabilizer is selected from the group consisting of imidazole, arginine and histidine.

26. The pharmaceutical composition according to any one of the preceding claims further comprising a surfactant.

25

27. The pharmaceutical composition according to any one of the preceding claims further comprising a chelating agent.

30

28. The pharmaceutical composition according to any one of the preceding claims further comprising a buffer.

29. The pharmaceutical composition according claim 28, wherein said buffer is selected from the group consisting of Tris, ammonium acetate, sodium acetate, glycine, aspartic acid, and Bis-Tris.

35

30. The pharmaceutical composition according to any one of the preceding claims further comprising a divalent metal.

40

31. The pharmaceutical composition according to claim 30, wherein said divalent metal is zinc.

32. The pharmaceutical composition according to any one of the preceding claims, wherein said solution is a clear solution.

FIG. 1A

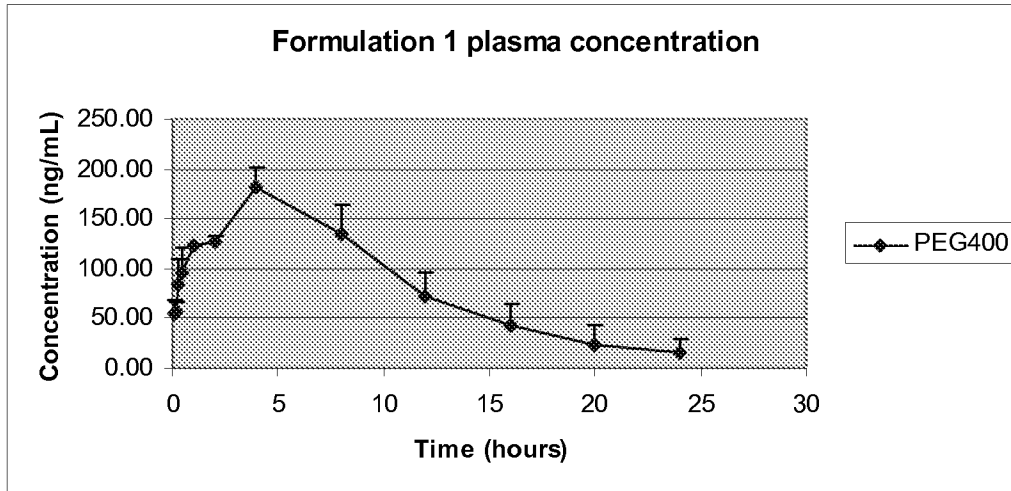


FIG. 1B

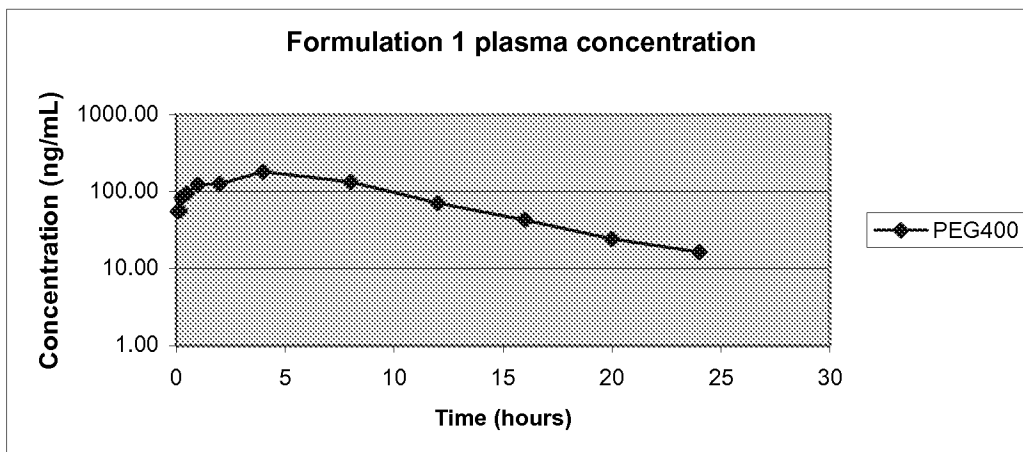


FIG. 2A

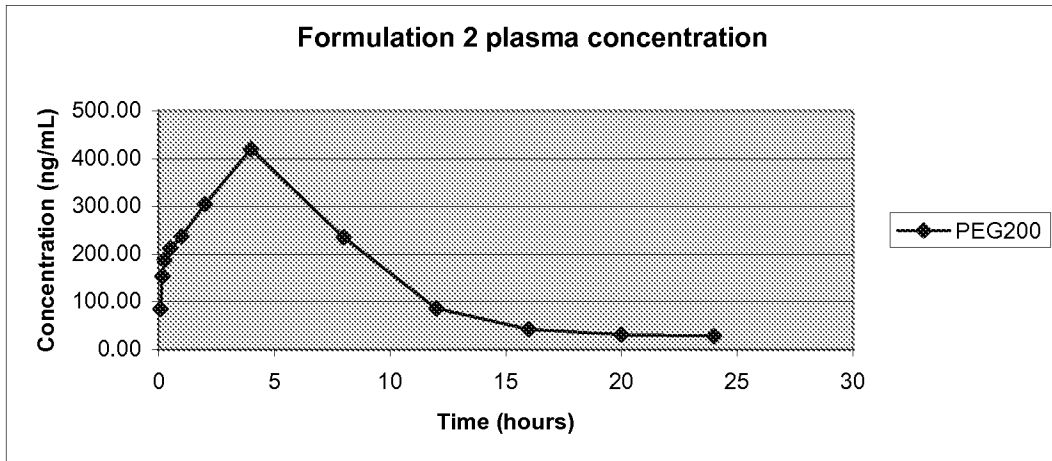


FIG. 2B

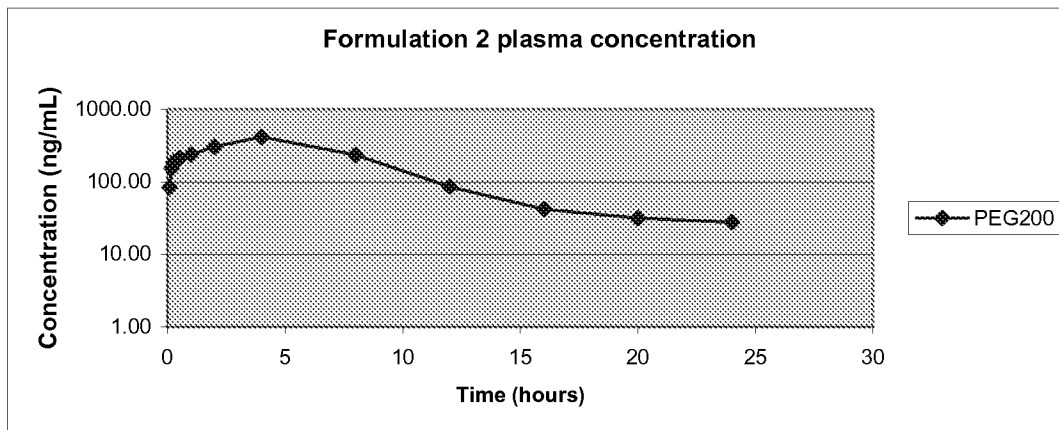


FIG. 3A

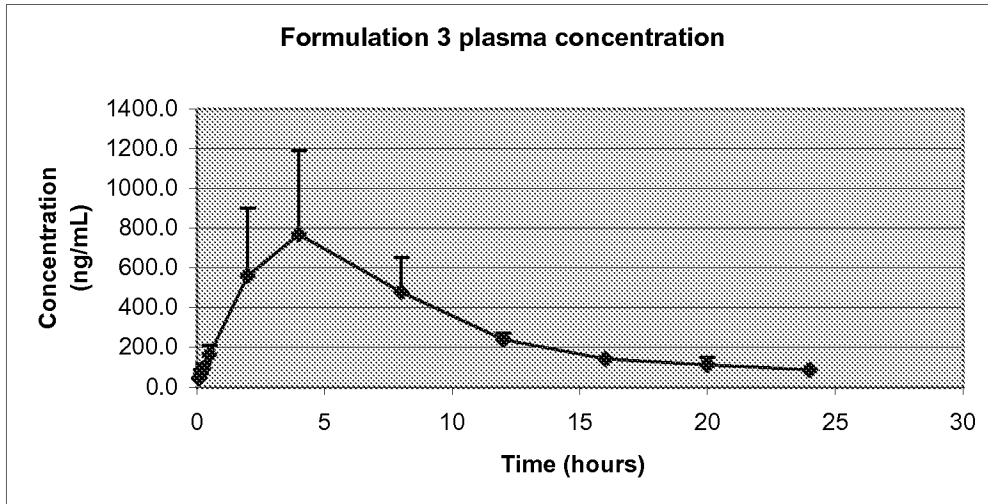


FIG. 3B

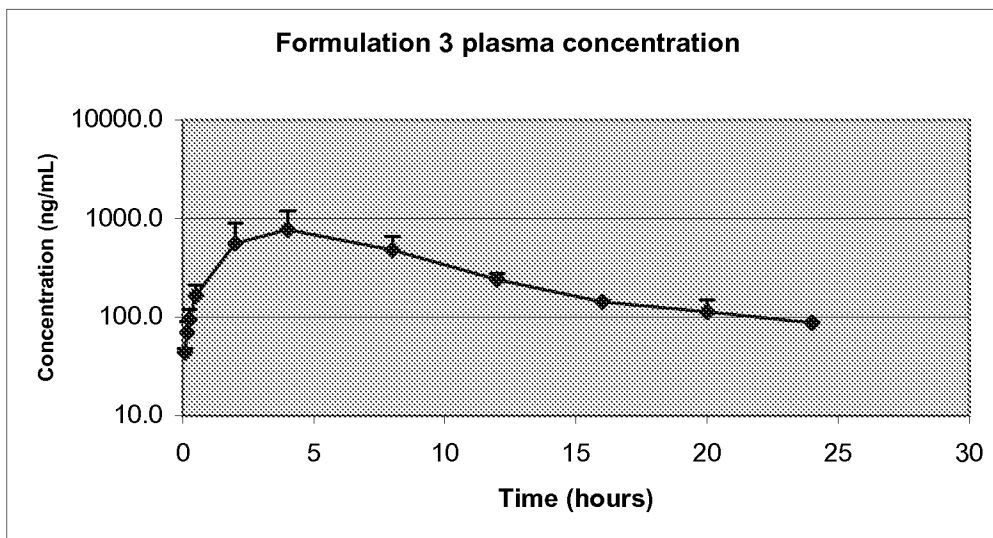


FIG. 4A

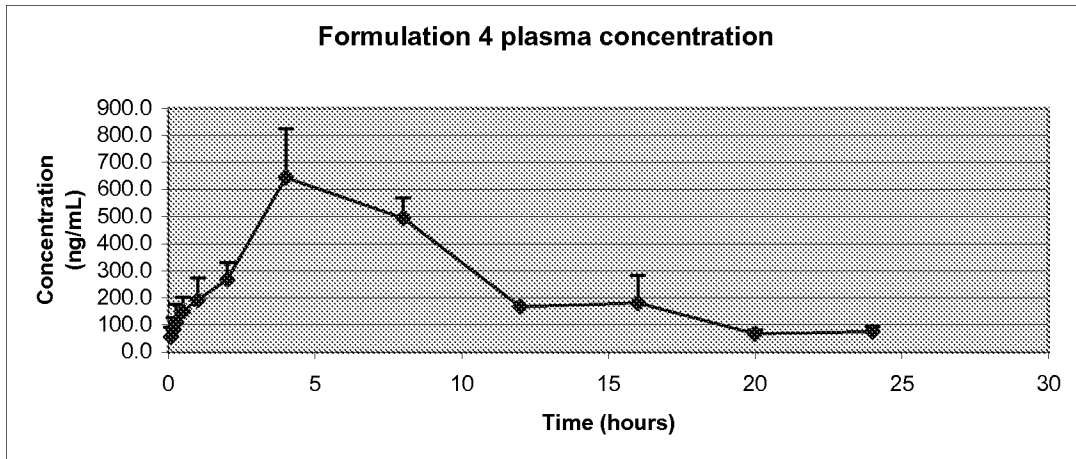


FIG. 4B

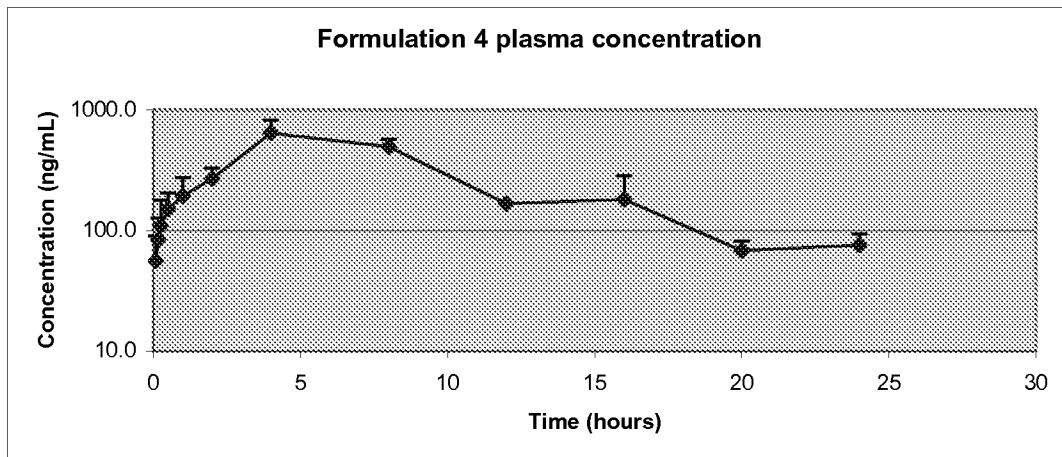


FIG. 5A

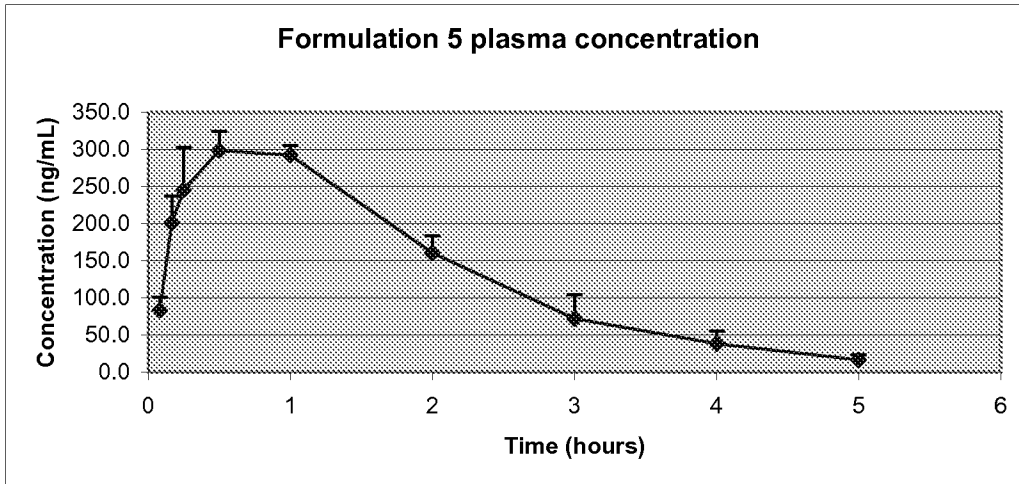


FIG. 5B

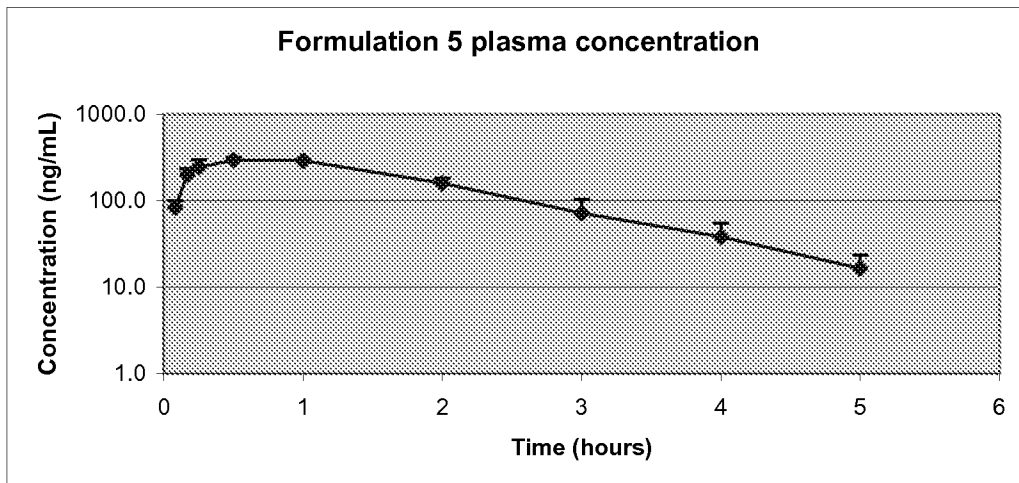


FIG. 6A

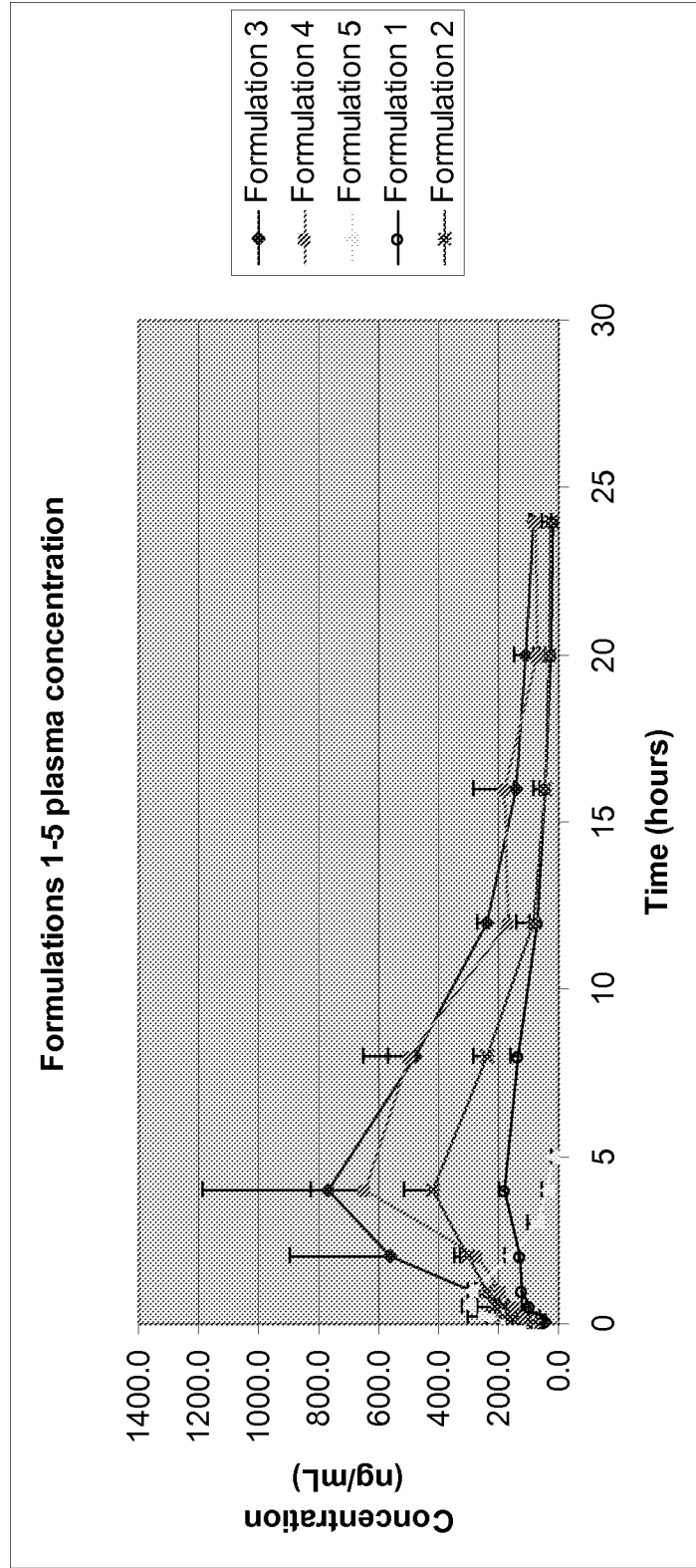
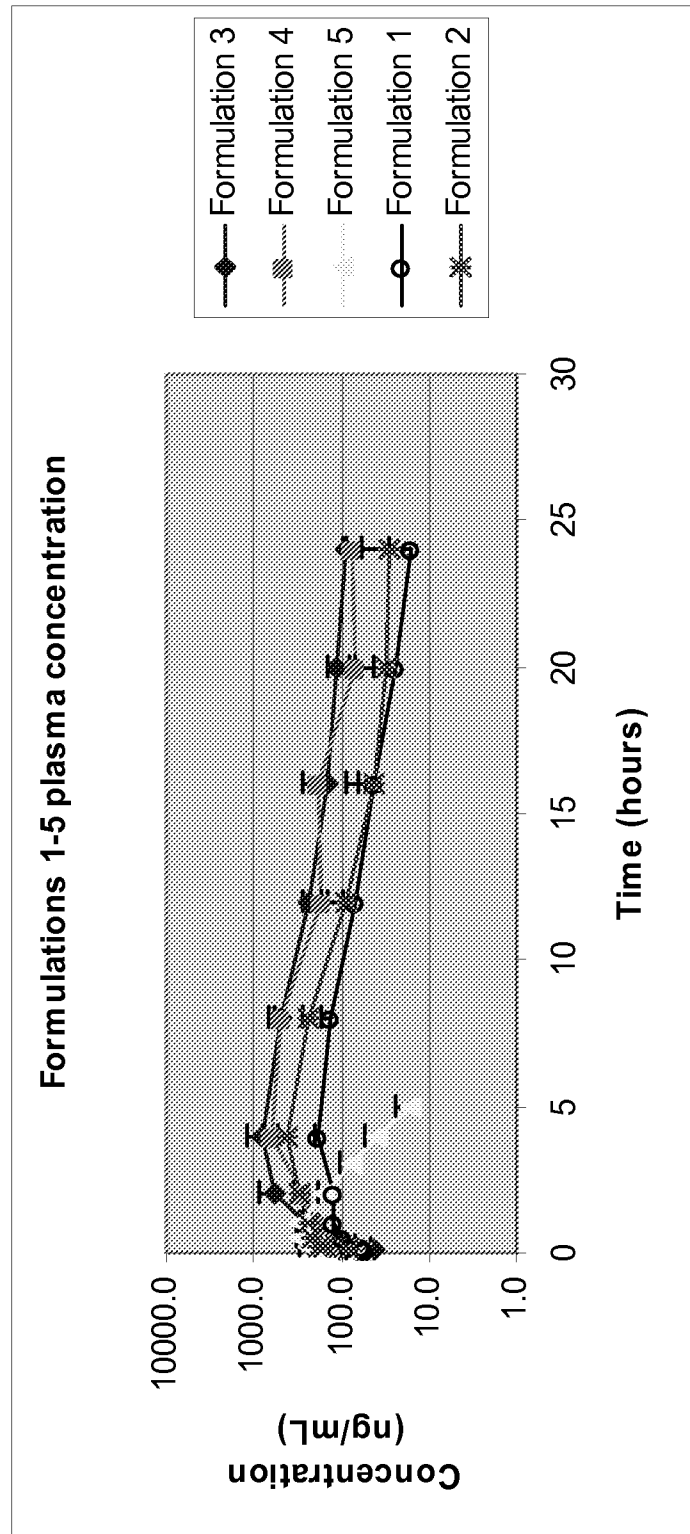


FIG. 6B



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 10/56690

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A01N 25/00 (2010.01)

USPC - 514/773

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

USPC 514/773

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC: 514/773,2.9;424/456,478

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PubWEST (PGPB, USPT, EPAB, JPAB)

organic ,dimethyl acetamide, polyethylene glycol, aqueous , solution, a gel or a semi-solid, a suspension, peptide, ligand, melanocortin receptors, depot, subcutaneous, intramuscular, injection, inject, administer, administration, subject, patient, animal, mammal, human,

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2007/008704 A2 (DONG et.al.) 18 January 2007 (18.01.2007) page 7, para 2-3; page 21, para 2; page 70, para 3; page 71, para 5-6; page 72, para 2, 6	1-4

 Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

29 December 2010 (29.12.2010)

Date of mailing of the international search report

20 JAN 2011

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-3201

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 10/56690

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. Claims Nos.: 5-32
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.