

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
22 June 2006 (22.06.2006)

PCT

(10) International Publication Number  
**WO 2006/063596 A2**

(51) International Patent Classification: Not classified

(21) International Application Number:  
PCT/DK2005/000796

(22) International Filing Date:  
15 December 2005 (15.12.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
PA 2004 01939 15 December 2004 (15.12.2004) DK

(71) Applicant (for all designated States except US):  
**ADITECH PHARMA AB** [SE/SE]; Medeon - Malmö  
Science Park, P.A. Hanssons väg 41, S-205 12 Malmö  
(SE).

(72) Inventor; and

(75) Inventor/Applicant (for US only): **NILSSON, Henrik**  
[DK/DK]; Cort Adelersgade 4, st. th., DK-1053 Copen-  
hagen K (DK).

(74) Agent: **HØIBERG A/S**; St. Kongensgade 59A, DK-1264  
Copenhagen K (DK).

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

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

(84) Designated States (unless otherwise indicated, for every  
kind of regional protection available): ARIPO (BW, GH,  
GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM,  
ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),  
European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI,  
FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT,  
RO, SE, SI, SK, 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))
- of inventorship (Rule 4.17(iv))

**Published:**

- without international search report and to be republished  
upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guid-  
ance Notes on Codes and Abbreviations" appearing at the begin-  
ning of each regular issue of the PCT Gazette.

(54) Title: A COMPOSITION COMPRISING PP FOR THE TREATMENT OF GASTROINTESTINAL DISORDERS

(57) Abstract: The present invention relates to PP or functional equivalents thereof for use in pharmaceutical compositions. The pharmaceutical compositions are in particular useful in the treatment of functional gastrointestinal disorders, such as irritable bowel disease and functional dyspepsia. The invention further relates to methods of treatment using said compositions. Further included is the combination of PP or functional equivalents thereof with a secondary active ingredient such as an anti-emetic drug.



WO 2006/063596 A2

**A composition comprising PP for the treatment of gastrointestinal disorders**

All patent and non-patent references cited in the application, or in the present application, are also hereby incorporated by reference in their entirety.

5

**Field of invention**

The present invention relates to a pharmaceutical composition comprising Pancreatic Polypeptide (in the following termed "PP") for use in the treatment of functional gastrointestinal disorders, such as Irritable Bowel Syndrome (IBS), Functional Dyspepsia (FD) and/or abdominal pain. Furthermore, the invention relates to the use of PP for the production of a pharmaceutical composition for the treatment of gastrointestinal disorders, such as IBS, FD and/or abdominal pain. The invention further relates to a method of treating such gastrointestinal disorders, which treatment comprises administration of said pharmaceutical composition, alone or in combination with a second active ingredient.

15

**Background of invention**

Gastrointestinal disorders are very common in the population. Some of these are very well characterised and thus suitable treatment regimes have been developed. It is more difficult to develop treatments for functional gastrointestinal disorder. This term refers to a disorder or disease where the primary abnormality is an altered physiological function (the way the body works) rather than an identifiable structural or biochemical cause. Thus, these types of gastrointestinal disorders have unknown aetiology, i.e. are lacking a clear biological explanation. Generally, this type of disorder can not be diagnosed in a traditional way; that is, as an inflammatory, infectious, or structural abnormality that can be seen by commonly used examination, x-ray, or laboratory test.

20

25

Gastrointestinal disorders with unknown aetiology include irritable bowel syndrome (IBS) also called irritable colon, functional dyspepsia (FD) or Non-ulcer dyspepsia. Therefore the diagnosis and development of suitable treatment for this subset of gastrointestinal disorders have so far not been sufficiently successful.

30

**Irritable Bowel Syndrome**

35

The predominant symptoms of IBS are abdominal pain, altered bowel habit, discomfort associated with disturbed defecation and bloating. Patients have an

**CONFIRMATION COPY**

increased mucus and nausea and feeling of constipation and distension. The criteria for irritable bowel syndrome are pain or discomfort for 12 weeks of the previous 12 months associated with two of the following; relief with defecation, looser or more frequent stools, harder or less frequent stools, according to Rome II (see below) reviewed by Talley, NJ and Spiller R (2002) and Talley, NJ (2003). The symptoms may be chronic and impair the quality of life for the patient.

#### ROME II SYMPTOM CRITERIA FOR IBS

At least 12 weeks or more, which need not be consecutive, in the preceding 12 months of abdominal discomfort or pain that has two out of three features:

- 1) Relieved with defecation; and/or
- 2) Onset associated with a change in frequency of stool; and/or
- 3) Onset associated with a change in form (appearance) of stool.

Other symptoms that are not essential but support the diagnosis of IBS:

- Abnormal stool frequency (greater than 3 bowel movements/day or less than 3 bowel movements/week);
- Abnormal stool form (lumpy/hard or loose/watery stool);
- Abnormal stool passage (straining, urgency, or feeling of incomplete evacuation);
- Passage of mucus;
- Bloating or feeling of abdominal distension.

It is estimated that 10-15 % of the population experience IBS symptoms.

The patients are grouped in three groups based on their predominant bowel habit (diarrhoea and/or constipation):

- IBS associated with abdominal pain, fecal urgency and diarrhoea
- IBS associated with abdominal discomfort, bloating and constipation
- IBS associated with alternating diarrhoea and constipation

#### Functional dyspepsia

The symptoms of functional dyspepsia (FD) are partially overlapping with the symptoms of IBS. Dyspepsia refers to persistent or recurrent epigastric pain or subjective upper abdominal discomfort that may be characterized by early satiety, postprandial fullness, or bloating. Dyspepsia is not restricted to meal-related symptoms. Patients are grouped in dysmotility-like FD and ulcer-like FD based on

the prevalent symptom centred in the upper abdomen (discomfort and pain respectively). Many patients experience symptoms after meal ingestion, including epigastric discomfort, fullness and pain. Further symptoms include inability to finish a normal-sized meal, bloating, belching, nausea and vomiting (Feinle-Bisset, C. et al. 2003). The underlying mechanism of functional dyspepsia is unclear. The role of delay gastric emptying is debated and currently not the favoured model.

#### Central nervous system, visceral hypersensitivity and stress

The brain-gut interaction plays a prominent role in the modulation of gut function in health and disease. The central nervous system (CNS) regulates the functions of the gastrointestinal tract, such as motility, secretion and blood flow. This occurs unperceived by the individual. In case of irregularities of the gastrointestinal an individual may experience short periods of pain. In the case of patients with gastrointestinal diseases the pain may be very strong and durable. There appears to be a connection with visceral hypersensitivity in the pathophysiology of functional gastrointestinal diseases including IBS and FD (Feinle-Bisset, C. et al. 2003). Furthermore, stress, anxiety or recall of aversive memories can enhance perception of painful events. Thus stress might have an inducing effect on hyperalgesia (Drossman DA, et al. 2002).

#### Psychological factors

Psychological and sociological factors appears relevant for the onset and severity of symptoms (Drossman DA, et al. 2002), it further appears that the most effective drugs for treatment of irritable bowel syndrome so far have been antidepressants (Talley, N.J. and Spiller R (2002)).

#### Treatments

Until now treatments of gastrointestinal disorders with unknown aetiology, such as irritable bowel syndrome and functional dyspepsia, have been based on empirical findings and aims for control of symptoms. The complexity of the symptoms indicates that a single cure will not be beneficial for all patients.

Symptoms relating to diarrhoea and constipation may be treated using laxatives and antidiarrhoeals but the success is modest. Furthermore, drugs such as loperamide may minimise diarrhoea but does not modulate the abdominal pain (review by

Talley, N.J. 2003), suggesting that the irregular bowel habit and the experienced abdominal pain is not directly linked.

As mentioned above, antidepressants have some effect on IBS. Mainly patients with diarrhoea-associated IBS may benefit from a treatment with tricyclic antidepressants, such as disipramine, nortryptiline and fluphenzine, but the trials have been of variably quality. It is further expected that selective serotonin reuptake inhibitors (SSRI) may be useful in IBS patients with constipation (review by Talley, N.J. 2003).

In addition, there are two pharmaceutical drugs, alosetron and tegaserod, registered for use in various subpopulations of IBS. Alosetron is registered for a subpopulation of women with severe diarrhoea-predominant IBS (US prescribing information), and tegaserod is indicated for short-term treatment of women with IBS whose primary bowel symptom is constipation (US prescribing information).

#### Gut hormones

Several gut hormones have been suggested to be related to symptoms of gastrointestinal disorders. VIP, CCK, and motilin relates to the motility of the upper gastrointestinal tract whereas polypeptide YY (PYY) and Neuropeptide Y (NPY) affect the absorption in the intestine. The latter (both PYY and NPY) have been suggested as treatment for diarrhoea (US 6,588, 708) by prolonging of the residence time. PYY and NPY constitute a family of hormones together with pancreatic polypeptide (PP), called the PP-fold family (Figure 1).

#### PP

PP as well as PYY and NPY exert their actions via the NPY receptors (Y1R, Y2R, Y4R and Y5R). These are all seven transmembrane domain receptors coupled to G<sub>i</sub> resulting in inhibition of adenylate cyclase. The PP, NPY and PYY peptides all consist of 36 amino acids with an amidated C-terminal. This COOH-terminal amidation appears to be necessary for activity (Stanley, S. et. al., 2004). In addition, all hormones of the PP-fold family share a common tertiary structure, the PP-fold (Figure 1). Two forms of PYY, PYY1-36 and PYY3-36, the latter being a truncated form of the former, have been found in circulation. PYY3-36 is produced by the cleavage of PYY1-36 by the enzyme dipeptidyl peptidase IV (DPP-IV). PYY1-36 binds to and activates Y1R, Y2R, Y4R as well as Y5R, whereas PYY3-36 is more

selective for the Y2 and Y5 receptors (Y2R and Y5R). PP, on the other hand, binds with the greatest affinity to Y4 receptors (Y4R), where its affinity is greater than that of PYY1-36. In addition, PP binds to Y5 receptors (Y5R) (Stanley, S. et. al., 2004).

- 5 The structure of PP varies from species to species, but there is a strong structural conservatism from an evolutionary point of view. The first form of PP to be isolated was the avian form (APP) in chicken in 1968 (Hazelwood, R. L. 1993).

10 PP is primarily expressed in the endocrine F-cells of the pancreas, especially in the duodenal portion of the pancreas. PP producing cells are also found elsewhere, such as in the exocrine pancreas and in the gastrointestinal tract (especially in the colon and rectum in humans), but only in small numbers. Plasma PP is regulated primarily by food intake, but there is also an intrinsic circadian rhythm. The postprandial PP plasma concentration is proportional to the caloric load, and the meal related PP release is biphasic. Furthermore, gastric distension, irrespective of  
15 caloric intake, also triggers PP release. PP release appears to be modulated by vagal tone. Adrenergic stimulation, e.g. by hypoglycaemia, increases PP release. In addition, various other pancreatic and gastrointestinal hormones affect PP release; ghrelin, motilin and secretin all stimulate PP release and somatostatin reduces  
20 plasma PP concentrations (Stanley, S. et. al., 2004).

PP inhibits exocrine pancreatic secretion of enzyme, bicarbonate, and water, and decreases gall bladder contractions, reducing bile secretion in mammals (Hazelwood, R. L. 1993). Gastric and small intestinal motilities are suppressed, but  
25 there are conflicting reports as to the effects of PP on gastric emptying in humans (Batterham, R. L. et al, 2003). Peripheral administration of PP as an intravenous infusion has been shown to reduce appetite and food intake in non-obese human subjects (Batterham, R. L. et al, 2003). PP is not able to cross the blood brain barrier (BBB), for which reason the peptide only can enter the central nervous  
30 system (CNS) in areas with a deficient BBB, such as the area postrema, where Y4R are highly expressed. It has therefore been speculated that the anorectic effects of PP are mediated via the Y4R (Stanley, S. et. al., 2004).

Pancreatic polypeptide circulates mainly as a dimer (>80%). It has a circulation half  
35 life of about 6-7 minutes in vivo, and is mainly cleared in its active form via the

kidneys (Hazelwood, R. L. 1993). There are varying reports on the plasma levels in humans, but the range appears to be about 15 to 100 pmol/L, with the lowest levels in the fasted state, and the highest levels within about 30 minutes after food intake (Batterham, R. L. et al, 2003).

References

Batterham, R.L.; Le Roux, C.W.; Cohen, M.A. et al. (2003) Pancreatic polypeptide reduces appetite and food intake in humans. J. Clin. Endocrinol. Metab. 88(8): 3989-3992.

5

Drossman, D.A.; Camilleri, M.; Mayer, E.A.; Whitehead WE (2002). AGA technical review on irritable bowel syndrome. Gastroenterology. Dec;123(6):2108-31.

Feinle-Bisset, C.; Vozzo, R.; Horowitz, M., and Talley, N.J.(2003). . American  
10 Journal of Gastroenterology, Jan.;99 (1):170-181

Hazelwood, R. L. The Pancreatic Polypeptide (PP-Fold) Family: Gastrointestinal, Vascular, and Feeding Behavioral Implications (1993), 202: 44-63.

15 Stanley, S.; Wynne, K. and Bloom, S. (2004). Gastrointestinal Satiety Signals III. Glucagon-like peptide 1, oxyntomodulin, peptide YY, and pancreatic polypeptide. Am. J. Physiol. Gastrointest. Liver Physiol. 286: G693-G697.

20 Talley, N.J. and Spiller R (2002). Irritable bowel syndrome: a little understood organic bowel disease. The Lancet, Vol 360, August 17, 555-564.

Talley, N.J.(2003). Evaluation of drug treatment in irritable bowel syndrome. J. Clin. Pharmacol, 56, 362-369.

**Summary of invention**

The invention relates to a composition comprising PP or a functional equivalent thereof or a pharmaceutically acceptable salt thereof, for the production of a medicament for the treatment or prophylaxis of a functional gastrointestinal disorder and/or the symptoms of such disorders.

Said medicament may be used for the treatment of irritable bowel syndrome wherein, the predominant bowel habit is constipation and/or the predominant bowel habit is alternating diarrhoea and constipation and/or the treatment is administered parenterally.

Said medicament may further be for the treatment of abdominal and visceral pain associated with functional gastrointestinal disorders such as irritable bowel syndrome and functional dyspepsia.

Said medicament may further be for the treatment of functional dyspepsia, for the relief of the symptom(s)

- a) sensation of fullness and/or
- b) inability to finish a normal sized meal and/or
- c) pain after food intake and/or
- d) nausea and/or
- e) vomiting and/or
- f) bloating and/or
- g) belching and/or
- h) regurgitation and/or
- i) epigastric pain and/or
- j) feeling of distension and/or
- k) excess flatus

and any combination of the above.

It is further contemplated that the composition according to the invention is pharmaceutically acceptable and especially may be adapted for parenteral, subcutaneous or intranasal administration. Likewise the composition may comprise a second active ingredient.

The invention further relates to a method of treatment comprising administration of said composition alone or in combination with a second pharmaceutical composition.

5

### Description of Drawings

Figure 1. Structure of the family of the PP-fold peptides

10

### Sequence listing

SEQ ID 1: Human PP (HPP).

### Definitions:

15

**AA:** See "Amino acid".

**Amino acid:** Entity comprising an amino terminal part (NH<sub>2</sub>) and a carboxy terminal part (COOH) separated by a central part comprising a carbon atom, or a chain of carbon atoms, comprising at least one side chain or functional group. NH<sub>2</sub> refers to the amino group present at the amino terminal end of an amino acid or peptide, and COOH refers to the carboxy group present at the carboxy terminal end of an amino acid or peptide. The generic term amino acid comprises both natural and non-natural amino acids. Natural amino acids of standard nomenclature as listed in J. Biol. Chem., 243:3552-59 (1969) and adopted in 37 C.F.R., section 1.822(b)(2) belong to the group of amino acids listed in Table 1 herein below. Non-natural amino acids are those not listed in Table 1. Examples of non-natural amino acids are those listed e.g. in 37 C.F.R. section 1.822(b) (4), all of which are incorporated herein by reference. Amino acid residues described herein can be in the "D" or "L" isomeric form.

20

25

30

| Symbols  |          | Amino acid    |
|----------|----------|---------------|
| 1-Letter | 3-Letter |               |
| Y        | Tyr      | tyrosine      |
| G        | Gly      | glycine       |
| F        | Phe      | phenylalanine |

35

|    |   |     |               |
|----|---|-----|---------------|
|    | M | Met | methionine    |
|    | A | Ala | alanine       |
|    | S | Ser | serine        |
|    | I | Ile | isoleucine    |
| 5  | L | Leu | leucine       |
|    | T | Thr | threonine     |
|    | V | Val | valine        |
|    | P | Pro | proline       |
|    | K | Lys | lysine        |
| 10 | H | His | histidine     |
|    | Q | Gln | glutamine     |
|    | E | Glu | glutamic acid |
|    | W | Trp | tryptophan    |
|    | R | Arg | arginine      |
| 15 | D | Asp | aspartic acid |
|    | N | Asn | asparagine    |
|    | C | Cys | cysteine      |

---

Table 1. Natural amino acids and their respective codes.

20

**Amino acid residue:** the term "amino acid residue" is meant to encompass amino acids, either standard amino acids, non-standard amino acids or pseudo-amino acids, which have been reacted with at least one other species, such as 2, for example 3, such as more than 3 other species. In particular amino acid residues may comprise an acyl bond in place of a free carboxyl group and/or an amine-bond and/or amide bond in place of a free amine group. Furthermore, reacted amino acid residues may comprise an ester or thioester bond in place of an amide bond.

25

**Antibody:** Are immunoglobulin molecules and active portions of immunoglobulin molecules. Antibodies are for example intact immunoglobulin molecules or fragments thereof retaining the immunologic activity.

30

**Antigen:** The molecule recognised by an antibody. Usually a peptide, polypeptide or a multimeric polypeptide. Antigens are preferably capable of eliciting an immune response.

35

**Chimera:** A molecule consisting of at least two parts not found together in nature. A chimeric peptide or protein is a peptide or protein constructed by the fusion of two

peptides or proteins. A chimeric DNA molecule is a DNA molecule that encodes a chimeric protein.

**Concentration equivalent:** A concentration equivalent is an equivalent dosage being defined as the dosage of a compound having the same response (as  
5 evaluated e.g. from a dosage-response curve) in vitro and/or in vivo as a known compound.

**Frequency:** The number of occurrences of a certain event within a certain period of time (e.g. the number of occurrences per day or per week).

**Individual:** A living animal or human. In preferred embodiments, the subject is a  
10 mammal, including humans and non-human mammals such as dogs, cats, pigs, cows, sheep, goats, horses, rats, and mice. In the most preferred embodiment, the subject is a human.

**Isolated:** is used to describe any of the various secretagogues, polypeptides and nucleotides disclosed herein, that have been identified and separated and/or  
15 recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would typically interfere with diagnostic or therapeutic uses for the polypeptide, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the polypeptide will be purified.

**Ligand:** A molecule, for example a peptide, capable of specific binding to one or  
20 more cognate receptors. An antigen is, for example, a ligand to its cognate antibodies.

**Medical disorder:** By the term "medical disorder" is meant any disease or syndrome having a detrimental effect on an individual's physical and/or mental  
25 health.

**MTD:** Maximum tolerated dose. The maximum tolerated dose of a substance in a given test period should not induce a) overt toxicity, such as appreciable death of cells or organ dysfunction; b) a material reduction in life span, except by cancer  
induction; c) 10% or greater retardation of body weight gain (in growing animals).

**Parenteral:** For the purpose of this document "parenteral" is defined as being outside the alimentary canal. Thus, the term "parenteral administration" of a compound encompasses e.g. subcutaneous, intramuscular, intravenous, intranasal, buccal,  
30 intradermal and transdermal administration, as well as inhalation of said compound.

**Peptide:** Plurality of covalently linked amino acid residues defining a sequence and  
35 linked by amide bonds. The term is used analogously with oligopeptide and poly-

peptide. The amino acids may be both natural amino acids and non-natural amino acids, including any combination thereof. The natural and/or non-natural amino acids may be linked by peptide bonds or by non-peptide bonds. The term peptide also embraces post-translational modifications introduced by chemical or enzyme-catalyzed reactions, as are known in the art.

**PP:** Pancreatic polypeptide, in particular, but not limited to human PP with the amino acid sequence shown in SEQ ID NO 1.

**PYY:** Peptide YY. Herein PYY represents both PYY1-36 and PYY3-36, the full length and a truncated version of PYY, respectively.

**Receptor:** A receptor is a molecule, such as a protein, glycoprotein and the like, that can specifically (non-randomly) bind to another molecule

**Recombinant DNA (rDNA) molecule:** A DNA molecule produced by operatively linking two DNA segments. Thus, a recombinant DNA molecule is a hybrid DNA molecule comprising at least two nucleotide sequences not normally found together in nature.

$T_{1/2}$ : The half-life is the time for the concentration of a compound to decrease 50 %.

#### Detailed description of the drawings

Figure 1. Structure of the family of the PP-fold peptides

NPY, PYY, and PP share a common hairpin-like three-dimensional structure called the PP-fold (Fuhlendorf, J. et al. (1990) J. Biol. Chem.). All three peptides are 36 amino acids long with an amidated carboxy-terminus. The general structure of the PP-fold peptides has been established using x-ray crystallography of avian PP and confirmed in several studies using nuclear magnetic resonance (Keire D. A. et al. (2000) Biochemistry). Amino acid residues 1–8 form a type II proline helix followed by a loop. Residues 15–32 form an  $\alpha$ -helix, and the four most carboxy-terminal residues are in a flexible loop conformation. NPY is one of the most evolutionary conserved peptides known. Despite a moderate degree of conservation in amino acid sequence between PP from different species as well as between PP and PYY and NPY, the general three-dimensional structure seems to be conserved in all PP-fold peptides.

#### Detailed description of the invention

##### Gastrointestinal disorders

The disorders described in the background section of the application are characterised by the lack of known aetiology, and are diagnosed based on patients' reports of symptoms. The symptoms of the patient may include, but are not limited to one or more of the following; constipation, diarrhoea, abdominal pain, visceral pain, abdominal discomfort, sensing of fullness, inability to finish a normal sized meal, bloating, vomiting, belching, nausea, epigastric pain and feeling of distension. Prevalent symptoms include abdominal discomfort and abdominal pain that may be accompanied by irregular stool frequency.

Individual in need

According to the invention, any individual who may draw benefit from the compositions of the present invention may be treated with said compositions. Thus an individual who would draw benefit of the treatment is considered an individual in need of treatment.

Preferably, said individual is suffering from a gastrointestinal disorder. An individual in need may suffer from a disorder such as any of the above mentioned. An individual in need may suffer from any of the symptoms of the above mentioned disorders, such as abdominal pain, visceral pain, abdominal discomfort, diarrhoea, constipation, nausea, vomiting, bloating, belching or feeling of distension.

An individual in need may be an individual in risk of acquiring any of the above mentioned disorders.

As described above the symptoms associated with such gastrointestinal disorders have been difficult to treat. Surprisingly, the present invention discloses successful use of PP and functional equivalents thereof for the treatment of gastrointestinal disorders.

Functional equivalents

Human PP is identified by SEQ ID NO: 1. Functional equivalents of PP include PP molecules originating from different species, such as mouse, rat, monkey, swine, bovine or other mammalian species. A functional equivalent may also be a homologue to PP.

Accordingly, in a first aspect the present invention relates to a composition comprising PP or a functional equivalent thereof for use in the prophylaxis or treatment of a functional gastrointestinal disorder and/or one or more symptoms of such disorder.

5

In a further aspect the invention relates to the use of PP or a functional equivalent thereof for the manufacture of a medicament for the treatment of a functional gastrointestinal disorder and/or one or more symptoms of such disorder.

10

The invention relates to a composition comprising PP or a functional equivalent thereof, for the preparation of a pharmaceutical composition for the treatment of a gastrointestinal disorder with unknown aetiology.

15

In an embodiment of the invention the composition is for the preparation of a pharmaceutical composition for the treatment a gastrointestinal disorder with unknown aetiology characterized by the symptoms

- a) constipation and/or
- b) diarrhoea and/or
- c) abdominal pain

20

In one embodiment the composition comprising PYY or a functional equivalent thereof is for the preparation of a pharmaceutical composition for the treatment of irritable bowel syndrome wherein

25

- a) the predominant bowel habit is constipation and/or
- b) the predominant bowel habit is alternating diarrhoea and constipation and/or
- c) the treatment is administered parenterally.

30

In another embodiment the composition comprising PP or a functional equivalent thereof is used for the treatment of irritable bowel syndrome.

In another embodiment the composition is used for the treatment of functional dyspepsia.

In yet a further embodiment the composition is used for the treatment of abdominal pain, visceral pain, such as pain in the left upper quadrant, pain in the right upper quadrant, pain in the left lower quadrant and/or pain in the right lower quadrant.

- 5 In another embodiment the composition is used for the treatment of pain associated with either irritable bowel syndrome (IBS) or functional dyspepsia (FD).

In an embodiment the composition is for the relief of the symptom(s)

- 10 a) sensation of fullness and/or  
b) inability to finish a normal sized meal and/or  
c) pain after food intake and/or  
d) nausea and/or  
e) vomiting and/or  
f) bloating and/or  
15 g) belching and/or  
h) regurgitation and/or  
i) epigastric pain and/or  
j) feeling of distension and/or  
k) excess flatus  
20 and any combinations hereof.

In another embodiment the composition is for the relief, treatment or prophylaxis of symptoms predominantly associated with the upper gastrointestinal (GI) tract or associated with the lower gastrointestinal tract.

25

In a preferred embodiment the composition comprises human PP. In a more preferred embodiment the composition comprises human PP as defined by SEQ ID NO.: 1.

30 Homologues

A homologue shall be construed as a molecule that shares some identity to the PP molecule. The homology may be expressed as the percentage of amino acid residues in the candidate sequence that are identical with the residue of a corresponding sequence of PP, after aligning the sequences and introducing gaps, if  
35 necessary to achieve the maximum percent identity for the entire sequence, and not

considering any conservative substitutions as part of the sequence identity. Neither N- or C-terminal extensions nor insertions shall be construed as reducing identity or homology. Methods and computer programs for the alignment are well known in the art. Sequence identity may be measured using sequence analysis software (e.g.,  
5 Sequence Analysis Software Package, Genetics Computer Group, University of Wisconsin Biotechnology Centre, 1710 University Ave., Madison, Wis. 53705). This software matches similar sequences by assigning degrees of homology to various substitutions, deletions, and other modifications.

10 A homologue of one or more of the sequences specified herein may vary in one or more amino acids as compared to the sequences defined, but is capable of performing the same function, i.e. a homologue may be envisaged as a "functional equivalent" of a predetermined sequence.

15 As described above a functional equivalent of any of the predetermined sequences herein may be defined as:

- i) homologues comprising an amino acid sequence capable of being recognised by an antibody, said antibody also recognising PP, and/or  
20
- ii) homologues comprising an amino acid sequence capable of binding selectively to a PP receptor (for example measured as described in example 1), and/or
- 25 iii) homologues having a substantially similar or higher binding affinity to PP receptors than PP and with a binding affinity preferably high than 100 nM.

Examples of homologues comprise one or more conservative amino acid substitutions including one or more conservative amino acid substitutions within the  
30 same group of predetermined amino acids, or a plurality of conservative amino acid substitutions, wherein each conservative substitution is generated by substitution within a different group of predetermined amino acids.

Homologues may thus comprise conservative substitutions independently of one  
35 another, wherein at least one glycine (Gly) of said homologue is substituted with an

amino acid selected from the group of amino acids consisting of Ala, Val, Leu, and Ile, and independently thereof, homologues, wherein at least one of said alanines (Ala) of said homologue thereof is substituted with an amino acid selected from the group of amino acids consisting of Gly, Val, Leu, and Ile, and independently thereof, homologues, wherein at least one valine (Val) of said homologue thereof is substituted with an amino acid selected from the group of amino acids consisting of Gly, Ala, Leu, and Ile, and independently thereof, homologues thereof, wherein at least one of said leucines (Leu) of said homologue thereof is substituted with an amino acid selected from the group of amino acids consisting of Gly, Ala, Val, and Ile, and independently thereof, homologues thereof, wherein at least one isoleucine (Ile) of said homologues thereof is substituted with an amino acid selected from the group of amino acids consisting of Gly, Ala, Val and Leu, and independently thereof, homologues thereof wherein at least one of said aspartic acids (Asp) of said homologue thereof is substituted with an amino acid selected from the group of amino acids consisting of Glu, Asn, and Gln, and independently thereof, homologues thereof, wherein at least one of said phenylalanines (Phe) of said homologues thereof is substituted with an amino acid selected from the group of amino acids consisting of Tyr, Trp, His, Pro, and preferably selected from the group of amino acids consisting of Tyr and Trp, and independently thereof, homologues thereof, wherein at least one of said tyrosines (Tyr) of said homologues thereof is substituted with an amino acid selected from the group of amino acids consisting of Phe, Trp, His, Pro, preferably an amino acid selected from the group of amino acids consisting of Phe and Trp, and independently thereof, homologues thereof, wherein at least one of said arginines (Arg) of said fragment is substituted with an amino acid selected from the group of amino acids consisting of Lys and His, and independently thereof, homologues thereof, wherein at least one lysine (Lys) of said homologues thereof is substituted with an amino acid selected from the group of amino acids consisting of Arg and His, and independently thereof, homologues thereof, wherein at least one of said asparagines (Asn) of said homologues thereof is substituted with an amino acid selected from the group of amino acids consisting of Asp, Glu, and Gln, and independently thereof, homologues thereof, wherein at least one glutamine (Gln) of said homologues thereof is substituted with an amino acid selected from the group of amino acids consisting of Asp, Glu, and Asn, and independently thereof, homologues thereof, wherein at least one proline (Pro) of said homologues thereof is substituted with an amino acid selected from the group of amino acids consisting

of Phe, Tyr, Trp, and His, and independently thereof, homologues thereof, wherein at least one of said cysteines (Cys) of said homologues thereof is substituted with an amino acid selected from the group of amino acids consisting of Asp, Glu, Lys, Arg, His, Asn, Gln, Ser, Thr, and Tyr.

5

Conservative substitutions may be introduced in any position of a preferred predetermined sequence. It may however also be desirable to introduce non-conservative substitutions, particularly, but not limited to, a non-conservative substitution in any one or more positions.

10

A non-conservative substitution leading to the formation of a functionally equivalent homologue of the sequences herein would for example i) differ substantially in polarity, for example a residue with a non-polar side chain (Ala, Leu, Pro, Trp, Val, Ile, Leu, Phe or Met) substituted for a residue with a polar side chain such as Gly, Ser, Thr, Cys, Tyr, Asn, or Gln or a charged amino acid such as Asp, Glu, Arg, or Lys, or substituting a charged or a polar residue for a non-polar one; and/or ii) differ substantially in its effect on polypeptide backbone orientation such as substitution of or for Pro or Gly by another residue; and/or iii) differ substantially in electric charge, for example substitution of a negatively charged residue such as Glu or Asp for a positively charged residue such as Lys, His or Arg (and vice versa); and/or iv) differ substantially in steric bulk, for example substitution of a bulky residue such as His, Trp, Phe or Tyr for one having a minor side chain, e.g. Ala, Gly or Ser (and vice versa).

15

20

25

30

Substitution of amino acids may in one embodiment be made based upon their hydrophobicity and hydrophilicity values and the relative similarity of the amino acid side-chain substitutions, including charge, size, and the like. Exemplary amino acid substitutions which take one of the foregoing characteristics into consideration are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

In a preferred embodiment the homologue has an amino acid sequence at least 60 % identical to SEQ ID NO 1.

35

More preferably the identity is at least 65 %, such as at least 70 % identical, such as at least 75 % identical, such as at least 80 % identical, such as at least 85 % identical, such as at least 90 % identical, such as at least 95 % identical, such as at least 98 % identical to SEQ ID NO 1.

5

In a preferred embodiment the functional equivalent comprise the amino acids corresponding to the 6 N- terminal amino acids of human PP as defined in SEQ ID NO.1 (Ala Pro Leu Glu Pro Val). The functional equivalent may comprise 8 N-terminal amino acids of human PP as defined in SEQ ID 1 (Ala Pro Leu Glu Pro Val Tyr Pro ), or such as 10 N-terminal amino acids of human PP as defined in SEQ ID 1 (Ala Pro Leu Glu Pro Val Tyr Pro Gly Asp) or such as 12 N-terminal amino acids of human PP as defined in SEQ ID 1 (Ala Pro Leu Glu Pro Val Tyr Pro Gly Asp Asn Ala), or such as 14 N-terminal amino acids of human PP as defined in SEQ ID 1 (Ala Pro Leu Glu Pro Val Tyr Pro Gly Asp Asn Ala Thr Pro), or such as 16 N-terminal amino acids of human PP as defined in SEQ ID 1 (Ala Pro Leu Glu Pro Val Tyr Pro Gly Asp Asn Ala Thr Pro Glu Gln), or such as 18 N-terminal amino acids of human PP as defined in SEQ ID 1 (Ala Pro Leu Glu Pro Val Tyr Pro Gly Asp Asn Ala Thr Pro Glu Gln Met Ala), or such as 20 N-terminal amino acids of human PP as defined in SEQ ID 1 (Ala Pro Leu Glu Pro Val Tyr Pro Gly Asp Asn Ala Thr Pro Glu Gln Met Ala Gln Tyr), or such as 22 N-terminal amino acids of human PP as defined in SEQ ID 1 (Ala Pro Leu Glu Pro Val Tyr Pro Gly Asp Asn Ala Thr Pro Glu Gln Met Ala Gln Tyr Ala Ala), or such as 24 N-terminal amino acids of human PP as defined in SEQ ID 1 (Ala Pro Leu Glu Pro Val Tyr Pro Gly Asp Asn Ala Thr Pro Glu Gln Met Ala Gln Tyr Ala Ala Asp Leu), or such as 26 N-terminal amino acids of human PP as defined in SEQ ID 1 (Ala Pro Leu Glu Pro Val Tyr Pro Gly Asp Asn Ala Thr Pro Glu Gln Met Ala Gln Tyr Ala Ala Asp Leu Arg Arg), or such as 28 N-terminal amino acids of human PP as defined in SEQ ID 1 (Ala Pro Leu Glu Pro Val Tyr Pro Gly Asp Asn Ala Thr Pro Glu Gln Met Ala Gln Tyr Ala Ala Asp Leu Arg Arg Tyr Ile), or such as 30 N-terminal amino acids of human PP as defined in SEQ ID 1 (Ala Pro Leu Glu Pro Val Tyr Pro Gly Asp Asn Ala Thr Pro Glu Gln Met Ala Gln Tyr Ala Ala Asp Leu Arg Arg Tyr Ile Asn Met), or such as 32 N-terminal amino acids of human PP as defined in SEQ ID 1 (Ala Pro Leu Glu Pro Val Tyr Pro Gly Asp Asn Ala Thr Pro Glu Gln Met Ala Gln Tyr Ala Ala Asp Leu Arg Arg Tyr Ile Asn Met Leu Thr), or such as 34N-terminal amino acids of human PP as defined in SEQ ID 1 (Ala Pro Leu Glu Pro Val

Tyr Pro Gly Asp Asn Ala Thr Pro Glu Gln Met Ala Gln Tyr Ala Ala Asp Leu Arg Arg Tyr Ile Asn Met Leu Thr Arg Pro).

In a preferred embodiment the functional equivalent comprise the amino acids  
5 corresponding to the 6 C- terminal amino acids of human PP as defined in SEQ ID NO 1 (Leu Thr Arg Pro Arg Tyr), or such as the 8 C-terminal amino acids of human PP as defined in SEQ ID NO 1 (Asn Met Leu Thr Arg Pro Arg Tyr), or such as the 10 C-terminal amino acids of human PP as defined in SEQ ID NO 1 (Tyr Ile Asn Met Leu Thr Arg Pro Arg Tyr), or such as the 12 C-terminal amino acids of human PP as defined in SEQ ID NO 1 (Arg Arg Tyr Ile Asn Met Leu Thr Arg Pro Arg Tyr), or such as the 14 C- terminal amino acids of human PP as defined in SEQ ID NO 1 (Asp Leu Arg Arg Tyr Ile Asn Met Leu Thr Arg Pro Arg Tyr), or such as the 16 C-terminal amino acids of human PP as defined in SEQ ID NO 1 (Ala Ala Asp Leu Arg Arg Tyr Ile Asn Met Leu Thr Arg Pro Arg Tyr), or such as the 18 C-terminal amino acids of human PP as defined in SEQ ID NO 1 (Gln Tyr Ala Ala Asp Leu Arg Arg Tyr Ile Asn Met Leu Thr Arg Pro Arg Tyr), or such as the 20 C- terminal amino acids of human PP as defined in SEQ ID NO 1 (Met Ala Gln Tyr Ala Ala Asp Leu Arg Arg Tyr Ile Asn Met Leu Thr Arg Pro Arg Tyr), or such as the 22 C-terminal amino acids of human PP as defined in SEQ ID NO 1 (Glu Gln Met Ala Gln Tyr Ala Ala Asp Leu Arg Arg Tyr Ile Asn Met Leu Thr Arg Pro Arg Tyr), or such as the 24 C-terminal amino acids of human PP as defined in SEQ ID NO 1 (Thr Pro Glu Gln Met Ala Gln Tyr Ala Ala Asp Leu Arg Arg Tyr Ile Asn Met Leu Thr Arg Pro Arg Tyr), or such as the 26 C-terminal amino acids of human PP as defined in SEQ ID NO 1 (Asn Ala Thr Pro Glu Gln Met Ala Gln Tyr Ala Ala Asp Leu Arg Arg Tyr Ile Asn Met Leu Thr Arg Pro Arg Tyr), or such as the 28 C-terminal amino acids of human PP as defined in SEQ ID NO 1 (Gly Asp Asn Ala Thr Pro Glu Gln Met Ala Gln Tyr Ala Ala Asp Leu Arg Arg Tyr Ile Asn Met Leu Thr Arg Pro Arg Tyr), or such as the 30 C-terminal amino acids of human PP as defined in SEQ ID NO 1 (Tyr Pro Gly Asp Asn Ala Thr Pro Glu Gln Met Ala Gln Tyr Ala Ala Asp Leu Arg Arg Tyr Ile Asn Met Leu Thr Arg Pro Arg Tyr), or such as the 32 C-terminal amino acids of human PP as defined in SEQ ID NO 1 (Pro Val Tyr Pro Gly Asp Asn Ala Thr Pro Glu Gln Met Ala Gln Tyr Ala Ala Asp Leu Arg Arg Tyr Ile Asn Met Leu Thr Arg Pro Arg Tyr), or such as the 34 C-terminal amino acids of human PP as defined in SEQ ID NO 1 (Leu Glu Pro Val Tyr Pro Gly Asp Asn Ala Thr Pro Glu Gln Met Ala Gln Tyr Ala Ala Asp Leu Arg Arg Tyr Ile Asn Met Leu Thr Arg Pro Arg Tyr).

In an embodiment the functional equivalent comprise any of the above sequences with conservative amino acid substitutions, such as one substitution, or such as two substitutions, or such as two substitutions, or such as more than two substitutions, or such as more than four substitutions.

Presently, in the most preferred embodiment the composition comprises human PP. In a more preferred embodiment the composition comprises human PP as defined by SEQ ID NO 1.

#### Covalent modifications

The functional equivalent may comprise any type of modifications. Nearly 200 structurally distinct covalent modifications have been identified thus far, ranging in size and complexity from conversion of amides to carboxylic acids, to the attachment of multiple complex oligosaccharides. Such modifications include phosphorylation, acetylation, ubiquination, lipidation (acetylation, prenylation, farnesylation, geranylation, palmitoylation, myristoylation), methylation, carboxylation, sulfonation and O- or N-glycosylations.

A subset of modifications is dependent on vitamin C as a cofactor. This includes proline and lysine hydroxylations and carboxy terminal amidation.

In an embodiment PP or the functional equivalent comprise a C-terminal amidation. In a preferred embodiment the C-terminal tyrosine residue of PP or a functional equivalent thereof is amidated.

#### Protecting group

The functional equivalent may according to the invention comprise protecting group at the N-terminus or the C-terminus or at both.

A protecting group covalently joined to the N-terminal amino group reduces the reactivity of the amino terminus under in vivo conditions. Amino protecting groups include - C1-10 alkyl, -C1-10 substituted alkyl, -C2-10 alkenyl, -C2-10 substituted alkenyl, aryl, -C1-6 alkyl aryl, -C(O)- (CH<sub>2</sub>) 1-6-COOH, -C(O)-C1-6 alkyl, -C(O)-aryl,

-C (O)-O-C1-6 alkyl, or-C (O)-O-aryl. Preferably, the amino terminus protecting group is acetyl, propyl, succinyl, benzyl, benzyloxycarbonyl or tbutyloxycarbonyl.

5 A protecting group covalently joined to the C-terminal carboxy group reduces the reactivity of the carboxy terminus under in vivo conditions. The carboxy terminus protecting group is preferably attached to the  $\alpha$ -carbonyl group of the last amino acid. Carboxy terminus protecting groups include amide, methylamide, and ethylamide.

#### 10 Conjugates

PP or the functional equivalent of PP may be conjugated to another entity, in order for example, to prolong its half-life. Conjugation can improve the delivery of targeted doses, prevent breakdown, and increase bioavailability in circulation. The other entity may be any substance that is capable of conferring improved properties to PP or a functional equivalent thereof, e.g. in terms of improved stability, half-life, etc.

The entity may be a polymer molecule. The polymer molecule may be any suitable polymer molecule, such as a natural or synthetic polymer, typically with a molecular weight in the range of about 1-100 kDa, such as about 3-20, kDa, e.g. 5-10 kDa.

20 The polymer is attached to a reactive group present on the PP polypeptide or functional equivalent thereof, e.g. an amine group or a thiol group.

Examples of suitable polymer molecules include polymer molecules selected from the group consisting of polyalkylene oxide (PAO), including polyalkylene glycol (PAG), such as linear or branched polyethylene glycol (PEG) and polypropylene glycol (PPG), poly-vinyl alcohol (PVA), poly-carboxylate, poly-(vinylpyrrolidone), polyethylene-co-maleic acid anhydride, polystyrene-co-maleic acid anhydride, dextran, including carboxymethyl-dextran. Preferably, the polymer molecule is a PEG molecule, in particular a monofunctional PEG, such as methoxypolyethylene glycol (mPEG). Suitable activated PEG molecules are available from Nektar Therapeutics Inc. (Huntsville Alabama, US) or from Valentis, Inc. (Burlingame, CA U.S.A.). Alternatively, the polymer molecules can be activated by conventional methods known in the art, e.g., as disclosed in WO 90/13540. Specific examples of activated PEG polymers include the following linear PEGs: NHS-PEG (e.g., SPA-PEG, SSPA-PEG, SBA-PEG, SS-PEG, SSA-PEG, SC-PEG, SG-PEG, and SCM-PEG), and NOR-PEG), BTC-PEG, EPOX-PEG, NCO-PEG, NPC-PEG, CDI-PEG,

ALD-PEG, TRES-PEG, VS-PEG, IODO-PEG, and MAL-PEG, and branched PEGs such as PEG2-NHS and those disclosed in US 5,932,462 and US 5,643,575, both of which are incorporated herein by reference.

- 5 The PEGylation (i.e. conjugation of PP or a functional equivalent thereof and the activated polymer molecule) is conducted in accordance with established procedures, e.g., as described in the following references (which also describe suitable methods for activation of polymer molecules): R. F. Taylor, (1991), "Protein immobilisation. Fundamental and applications", Marcel Dekker, N.Y.; S. S. Wong,  
10 (1992), "Chemistry of Protein Conjugation and Crosslinking", CRC Press, Boca Raton; G. T. Hermanson et al., (1993), "Immobilized Affinity Ligand Techniques", Academic Press, N.Y.).

- In yet another embodiment the PP polypeptide or functional equivalent thereof is  
15 conjugated to an oligosaccharide molecule, such as dextran, glycan, transferrin, etc. Such conjugation may be achieved in accordance with established technologies, e.g. those available from Neose Technologies, Inc. (Horsham, PA).

- In yet another embodiment, the PP or functional equivalent thereof is conjugated to  
20 an Fc region of an IgG molecule, typically in the form of a fusion protein, or to human serum albumin. Methods of such conjugation are known in the art.

- Other methods of preparing conjugates are well known in the art se for example Hermanson GT. *Bioconjugate Techniques*. New York: Academic Press; 1996,  
25 Aslam M, Dent AH. *Bioconjugation: protein coupling techniques for the biomedical sciences*. Houndsmills, England: Macmillan Publishers; 1999, and Wong SS. *Chemistry of protein conjugation and crosslinking*. Boca Raton, FL: CRC Press; 1991.

- 30 Most methods use amine-reactive reagents or thiol-reactive reagent. In the preparation of conjugates advantages may be achieved through the use of certain linkers. For example, linkers that contain a disulfide bond that is sterically "hindered" are often preferred, due to their greater stability in vivo, thus preventing release of

the toxin moiety prior to binding at the site of action. It is generally desired to have a conjugate that will remain intact under conditions found everywhere in the body except the intended site of action, at which point it is desirable that the conjugate have good "release" characteristics.

5

Different conjugates have been described, for example use of the A chain of ricin is described in US Pat. No. 4,340,535 incorporated herein by reference. Examples of peptide conjugates based on Ac-(RK)YY(RK)(WI)(RK)-NH<sub>2</sub> (where the brackets show allowable variations of amino acid residues) may be found in US patent application 2003040472.

10

In yet another embodiment the conjugated PP or functional equivalent thereof is provided as a fusion polypeptide. The fusion may be obtained by any suitable methods, for example, but not exclusively, by recombinant DNA technology. In a preferred embodiment the fusion of is made by recombinant DNA technology, such as a fusion of the nucleotide sequence encoding the PP or functional equivalent thereof and the nucleotide sequence encoding the other entity is made and the fusion is thereby encoded by a single nucleotide sequence. The fusion polypeptide may be expressed and purified as a single polypeptide molecule, using any suitable method, as described for the purification of a binding member. The fusion polypeptide may include insertion of a linker, such as a peptide of at least 2 AA, such as at least 5 AA, such as at least 8 AA, such as at least 15 AA, such as at least 20 AA. In one embodiment PP or a functional equivalent thereof is conjugated with another entity using a linker of at least 2AA.

15

20

25

#### Controlled release

The problem the invention solves is related to the reduction of symptoms of functional gastrointestinal disorders, such as IBS and functional dyspepsia. Often, the symptoms may be intermittent and of limited duration, i.e. come as "attacks" e.g. of pain. In such instances, it is important that the duration of action of the treatment in question, in this case PP or functional equivalents thereof, is adequate to treat the condition. PP has a relatively short T<sub>½</sub> of 6-7 minutes in vivo (Hazelwood, R. L. (1993)). Accordingly, compositions that enable a prolonged release of PP or a functional equivalent are within the scope of the present invention.

30

35

Such compositions are well-known to the skilled artisan and include e.g. modifications mentioned above, such as conjugates. Furthermore, the pharmaceutical compositions may be in the form of particles comprising a biodegradable polymer and/or a polysaccharide jellifying and/or bioadhesive polymer, an amphiphilic polymer, an agent modifying the interface properties of the particles and a pharmacologically active substance. These compositions exhibit certain biocompatibility features which allow a controlled release of the active substance (see e.g. U.S. Patent No. 5,700,486). Other controlled release systems are discussed in a review by Langer (Science 249:1527-1533, 1990). Moreover, there are pharmaceutical companies that based on a specific technology (such as mentioned above) can provide a specific composition with specific release characteristics of the active substance. Accordingly, a person skilled in the art will know how to obtain a suitable product once he has realized a specific need in respect of a particular drug substance. By way of example, MacroMed, Inc. (Sandy, UT, USA, <http://www.macromed.com/>) is one of such companies that offer technical solutions in order to obtain a controlled release pharmaceutical composition containing a specific active substance and having specific requirements with respect to the release of the active substance from the composition. MacroMed has developed a technology called ReGel® (see <http://www.macromed.com/> and US patent numbers 5,702,717; 6,004,573; 6,117,949 and 6,201,072) that is a drug delivery system that is composed of proprietary thermosensitive, biodegradable polymers/hydrogels which are solutions at administration temperature and become insoluble gels at the injection site. This system creates formulations which are easily administered through small-gauge needles or needle-free injectors. Because an insoluble gel depot is formed immediately upon injection, the formulation does not expel back through the needle track and remains at the site for a period of 4 weeks. The system is completely biocompatible and biodegradable and degrades into products which are well known to be easily metabolized and cleared. Drug release is controlled through a combination of diffusion from and degradation of the polymer. Alkermes® (Cambridge, Massachusetts, USA, <http://www.alkermes.com/>) is another such company that offers technical solutions for controlled release pharmaceutical compositions, including the ProLease® and Medisorb® injectable sustained-release technologies for both small and macromolecules (see [http://www.alkermes.com/technologies/prolease\\_medisorb.html](http://www.alkermes.com/technologies/prolease_medisorb.html)).

In one embodiment, the resulting controlled release composition gives rise to a release profile into plasma that has a prolonged effective plasma  $T_{1/2}$  compared to the natural half life of PP in plasma. In a preferred embodiment, the effective  $T_{1/2}$  is between 8 and 720 minutes, or such as between 8 and 600 minutes, or such as  
5 between 8 and 480 minutes, or such as between 8 and 360 minutes, or such as between 8 and 240 minutes, or such as between 8 and 180 minutes, or such as between 10 and 150 minutes, or such as between 20 and 120 minutes, or such as between 30 and 90 minutes, or such as between 45 and 75 minutes.

10 In another embodiment, the resulting controlled release composition gives rise to a release profile into plasma that has a prolonged effective plasma  $T_{1/2}$  compared to the natural half life of PP in plasma. In a further preferred embodiment, the effective  $T_{1/2}$  is between 1.2 and 120 times that of the natural half life of PP in plasma, or such as between 1.2 and 100 times that of the natural half life of PP in plasma, or such as  
15 between 1.2 and 80 times that of the natural half life of PP in plasma, or such as between 1.2 and 60 times that of the natural half life of PP in plasma, or such as between 1.2 and 40 times that of the natural half life of PP in plasma, or such as between 1.2 and 30 times that of the natural half life of PP in plasma, or such as between 1.5 and 25 times that of the natural half life of PP in plasma, or such as  
20 between 2 and 20 times that of the natural half life of PP in plasma, or such as between 3 and 15 times that of the natural half life of PP in plasma, or such as between 5 and 10 times that of the natural half life of PP in plasma.

The effective plasma  $T_{1/2}$  as mentioned above can be determined for said  
25 composition by administration of said composition to a human subject or a laboratory animal, depending on the species one wishes to determine the plasma  $T_{1/2}$  in. Typically, blood samples are drawn at baseline (time,  $T=0$ , i.e. immediately prior to administration of said compound), and subsequently with a certain frequency over a certain period of time. The frequency of blood sampling will in part be determined  
30 by the expected plasma  $T_{1/2}$ , and can be assessed by the skilled artisan. If one e.g. expects a plasma  $T_{1/2}$  of approximately 30 minutes, a frequency of blood sampling of once every five minutes for 60 to 90 minutes can be deemed satisfactory. Subsequently, plasma PP can be measured by radioimmunoassay, as described by e.g. Lamers et al (Lamers C B H W et al, Mechanisms of elevated serum pancreatic polypeptide concentrations in chronic renal failure. J Clin Endocrinol Metab 55:922-  
35

926, 1982) or Adrian et al (Adrian T E, Distribution and release of human pancreatic polypeptide. Gut 17:940-944, 1976).

#### Methods for production of PP

5 PP or a functional equivalent thereof can be produced using techniques well known in the art. For example, a polypeptide region of a PP can be chemically or biochemically synthesized and modified. Techniques for chemical synthesis of polypeptides are well known in the art such as solid phase peptide synthesis (see e. g., Vincent in Peptide and Protein Drug Delivery, New York, N. Y., Dekker, 1990).  
10 Examples of techniques for biochemical synthesis involving the introduction of a nucleic acid into a cell and expression of nucleic acids are provided in Ausubel, Current Protocols in Molecular Biology, John Wiley, 1987-1998, and Sambrook et al., in Molecular Cloning, A Laboratory Manual, 2 d Edition, Cold Spring Harbor Laboratory Press, 1989.

15 PP may according to the invention be synthesised by solid phase peptide synthesis (see Example 2).

A second example of how PP according to the invention is produced is described in  
20 brief below. The human PP of the present invention may be produced by the following process:

(a) constructing, by conventional techniques, an expression vector containing an operon with a DNA sequence encoding human PP or a functional equivalent  
25 thereof, thereby producing the vector of the invention;

(b) transfecting the expression vectors into a host cell by conventional techniques to produce the transfected host cell of the invention; and

30 (c) culturing the transfected cell by conventional techniques to produce the human PP of the invention.

The host cell may be cotransfected with a second vector for optimization of the production process. The two vectors may contain different selectable markers. The

coding sequences of PP may comprise cDNA or genomic DNA or both.

The host cell used to express the PP of the invention may be either a bacterial cell such as *Escherichia coli*, or a eukaryotic cell, such as *S. cerevisiae* or *P. pastoris*.

5 In particular a mammalian cell line may be used, such as Hela, CHO or any other suitable host cell known by the person skilled in the art.

The general methods by which the vectors of the invention may be constructed, transfection methods required to produce the host cell of the invention and culture  
10 methods required to produce the polypeptide of the invention from such host cells are all conventional techniques. Likewise, once produced, the polypeptide of the invention may be purified according to standard procedures as described below.

#### Purification of PP

15 After production, PP or a functional equivalent thereof is preferably purified. The method of purification used is dependent upon several factors, including the purity required, the source of PP or a functional equivalent, the intended use and the species in which PP or a functional equivalent was produced.

20 Any suitable conventional methods of purifying polypeptides including precipitation and column chromatography are well known to one of skill in the purification arts, including cross-flow filtration, ammonium sulphate precipitation, affinity column chromatography, gel electrophoresis and the like may be used.

#### Commercially available PP

25 PP, including human PP, is readily available from several commercial suppliers, such as PolyPeptide Laboratories Group (Wolfenbuttel, Germany and other locations, <http://www.polypeptide.com/>) and Bachem AG (Bubendorf, Switzerland, <http://www.bachem.com/>).

30

#### PP composition

According to the invention, a composition comprising PP or a functional equivalent thereof may preferably be produced by chemical or biochemical synthesized or recombinant methods, and may preferably be free of any contaminants present in  
35 blood such as infectious agents.

In a preferred embodiment the PP composition have a concentration of at least 10 nM such as at least 50 nM, such as at least 0.2  $\mu$ M, such as at least 0.5  $\mu$ M, such as at least 1  $\mu$ M, such as at least 2  $\mu$ M, such as at least 5  $\mu$ M, such as at least 10  $\mu$ M, such as at least 20  $\mu$ M, such as at least 50  $\mu$ M, such as at least 0.2 mM, such as at least 0.5  $\mu$ M, such as at least 1 mM, such as at least 2 mM, such as at least 5 mM of PP or a functional equivalent thereof.

The PP composition may be stored as a dry composition, for example lyophilized (freeze-dried) or spray dried to improve the stability of PP. Such compositions are reconstituted with liquid solutions prior to use. Generally, the protein concentration of the reconstituted formulation is about 2-40 times greater than the protein concentration in the mixture before lyophilization; thus this allows the production of a PP composition of a high concentration. When reconstituted with a diluent comprising a preservative (such as bacteriostatic water for injection, BWFI), the reconstituted formulation may be used as a multi-dose formulation. Such a formulation is useful, for example, where the patient requires frequent subcutaneous administrations.

In another embodiment the PP composition may be a liquid composition of high stability. The PP composition may be meant for mixing with a suitable diluent prior to use.

The PP composition may further comprise pharmaceutically acceptable salts, as well as pharmaceutically acceptable carriers and diluents.

#### Pharmaceutical compositions

Pharmaceutical compositions of the present invention may be prepared by conventional techniques, e.g. as described in Remington: The Science and Practice of Pharmacy 1995, edited by E. W. Martin, Mack Publishing Company, 19th edition, Easton, Pa. The compositions may appear in conventional forms, for example solutions or suspensions.

As used herein, the terms "pharmaceutically acceptable", "physiologically tolerable" and grammatical variations thereof, as they refer to compositions, carriers, diluents

and reagents, are used interchangeably and represent that the materials are capable of administration to or upon an individual without the production of undesirable physiological effects such as nausea, dizziness, gastric upset and the like.

5

According to the present invention, the pharmaceutical composition comprises PP or a functional equivalent thereof or a pharmaceutically acceptable salt of any of these and optionally one or more of a pharmaceutically acceptable carrier and/or a diluent. The pharmaceutical composition may further comprise vehicles, excipients and/or transport molecules.

10

The pharmaceutical composition may be produced prior to use by mixing a PP composition with an appropriate diluent.

15

The compositions of the present invention may preferably be administered to an individual in any way so as to achieve a beneficial effect, preferably for treatment of a gastrointestinal disorder.

20

An aspect of the invention relates to a method of treatment comprising administration of the pharmaceutical composition according to the invention.

25

The pharmaceutical composition according to the invention may be formulated for administered by any suitable route, such as peripherally, parenterally or orally. The pharmaceutical composition according to the invention is preferably formulated for parenteral administration, such as via a subcutaneous, intramuscular, intravenous, intranasal, inhalation, buccal, intradermal and transdermal administration route. Further including formulation for administration via rectal suppositories.

#### Second active ingredient

30

The patient suffering from a gastrointestinal disorder may benefit from additional treatments. This may e.g. involve loperamide, hyoscyamine sulphate, lactulose, YM-443, dexloxiglumide, bisacodyl, AZD 7371, talnetant (SB 223412), asimadoline, NT3, SLV305, AZD 3355, AZD 9343, pinaverine, Na picosulphate, calcium polycarpophil, trimebutine, dopamine D2 antagonists, such as itopride, motilin agonists, such as KC 11458 and GM-611, NK2 antagonists, such as saredutant,

35

NK3/NK2 receptor antagonists, such as SSR 241586, 5-hydroxytryptamine<sub>4</sub> (5-HT<sub>4</sub>) receptor partial agonists, such as tegaserod, 5-HT<sub>4</sub> receptor agonists, such as mosapride and prucalopride, antagonists of the serotonin 5-HT<sub>3</sub> receptor type, such as alosetron or cilansetron, dual 5-HT<sub>4</sub> agonist and 5-HT<sub>3</sub> –antagonists, such as renzapride and E-3620, GLP-1 or analogues/homologues thereof, such as exenatide, exenatide LAR, liraglutide, CJC-1131, ZP 10/AVE-0010 and GTP-010, DPP-IV inhibitors, such as LAF237, PYY(1-36) or analogues/homologues thereof, PYY(3-36) or analogues/homologues thereof, NPY(1-36) or analogues/homologues thereof, NPY(3-36) or analogues/homologues thereof and anti-depressants, such as selective serotonin reuptake inhibitors (SSRIs), serotonin noradrenalin reuptake inhibitors (SNRIs), norepinephrine serotonin reuptake inhibitors (NSRIs), selective noradrenalin reuptake inhibitors, tetracyclic antidepressants, non-selective monoamine reuptake inhibitors including tricyclic antidepressants (TCAs), selective reversible monoamine reuptake inhibitors and antidepressants with other mechanisms of action, e.g. mirtazapin. Examples of SSRIs are citalopram, escitalopram, fluoxetine, fluvoxamine, paroxetine and sertraline. An example of an SNRI is venlafaxine. An example of an NSRI is milnacipran. A further example of an anti-depressant is duloxetine.

In an embodiment of the invention, the composition comprises a second active ingredient in addition to PP or a functional equivalent thereof.

In another embodiment, an anti-emetic may be used as a second active ingredient. This is of particular interest in cases where the treatment with PP or a functional equivalent thereof gives rise to nausea and/or emesis.

#### Anti-emetic drugs

In the present context, an “anti-emetic” drug is one which counteracts (i.e. reduces or removes) nausea or emesis (vomiting). The experience of nausea and emesis may have many causes and the relief or reduction of the symptoms may be obtained by various mechanisms. The major groups of drugs useful for the treatment of nausea and emesis are; Neuroleptics/anti-psychotics, Antihistamines, Anticholinergic agents, Steroids (corticosteroids), 5HT<sub>3</sub>-receptor antagonists (serotonin receptor antagonist), NK1-receptor antagonists (Neurokinin 1 substance P receptor antagonists), Antidopaminergic agents/dopamine receptor antagonists,

Benzodiazapines, Cannabinoids. Here below is a non-exhaustive list of members of the different groups of compounds.

- 5           1. Neuroleptics/anti-psychotics
  - a. Dixyrazine
  - b. Haloperidol
  - c. Prochlorperazine (Compazine®)
- 10          2. Antihistamines
  - a. Piperazine derivatives
    - i. cyclizine
    - ii. meclizine
    - iii. cinnarizine
  - b. Promethazine
  - 15       c. Dimenhydrinate
  - d. Diphenhydramine
  - e. Hydroxyzine
  - f. Buclizine
  - g. Meclizine hydrochloride (Bonine, Antivert)
- 20          3. Anticholinergic agents (Inhibitors of the acetylcholine receptors.)
  - a. Scopolamine
  - b. Glycopyrron
  - c. Hyoscine
    - 25       i. Artane (Trihexy-5 trihexyphenidyl hydrochloride)
    - ii. Cogentin (benztropine mesylate)
    - iii. Akineton (biperiden hydrochloride)
    - iv. Disipal (Norflex orphenadrine citrate)
    - v. Kemadrin (procyclidine hydrochloride)
- 30          4. Steroids (corticosteroids)
  - a. Betamethasone
  - b. Dexamethasone
  - c. Methylprednisolone
  - 35       d. Prednisone®,
  - e. Trimethobenzamide (Tigan)
- 40          5. 5HT<sub>3</sub>-receptor antagonists (serotonin receptor antagonist)
  - a. Granisetron
  - b. Dolasetron
  - c. Ondansetron (hydrochloride)
  - d. Tropisetron
  - e. Ramosetron
  - f. Palonosetron
  - 45       g. Alosetron
  - h. Bemisetron
  - i. Zatisetron
  - j. Batanopirde
  - k. MDL-73147EF;
  - 50       l. Metoclopramide

- m. N-3389 (endo-3,9-dimethyl-3,9-diazabicyclo[3,3,1]non-7-yl-1 H-indazole-3-carboxamide dihydrochloride),
  - n. Y-25130 hydrochloride
  - o. MDL 72222
  - 5 p. Tropanyl-3,5-dimethylbenzoate
  - q. 3-(4-Allylpiperazin-1-yl)-2-quinoxalinecarbonitrile maleate
  - r. Zacopride hydrochloride
  - s. Mirtazepine (Antidepressant)
- 10 6. NK1-receptor antagonists (Neurokinin 1 substance P receptor antagonists)
    - a. Aprepitant
    - b. MPC-4505
    - c. GW597599
    - d. MPC-4505
    - 15 e. GR205171 (a selective tachykinin NK1 receptor antagonist)
    - f. L-759274
    - g. SR 140333
    - h. CP-96,345
    - i. BIIF 1149, NKP 608C, NKP 608A, CGP 60829, SR 140333  
 20 (Nolpitantium besilate/chloride), LY 303870 (Lanepitant), MDL-105172A, MDL-103896, MEN-11149, MEN-11467, DNK 333A, YM-49244, YM-44778, ZM-274773, MEN-10930, S-19752, Neuronorm, YM-35375, DA-5018, MK-869, L-754030, CJ-11974, L-758298, DNK-33A, 6b-I, CJ-11974.
    - 25 j. Benserazide and carbidopa
    - k. TAK-637 [(aR,9R)-7-[3,5-bis(trifluoromethyl)benzyl]-8,9,10,11-tetrahydro-9-methyl-5-(4-methylphenyl)-7H-[1,4]diazocino[2,1-g][1,7]naphthyridine-6,13-dione]
    - 30 l. PD 154075 [(2-benzofuran)-CH<sub>2</sub>OCO]-(R)-alpha-MeTrp-(S)-NHCH(CH<sub>3</sub>) Ph
    - m. FK888, chemical modification of the parent compound, (D-Pro4, D-Trp7, 9, 10, Phe11) SP4-11.
  - 35 7. Antidopaminergic agents/dopamine receptor antagonists
      - a. Domperidone
      - b. Prochlorperazine
      - c. Metoclopramide
      - d. Chlorpromazine (Thorazine)
      - e. Droperidol (Inapsine)
      - 40 f. Promethazine (Phenergan)
    8. Benzodiazapines(Valium® and others)
      9. Non-psychoactive cannabinoids,
          - 45 a. Cannabidiol (CBD)
          - b. Cannabidiol dimethylheptyl (CBD-DMH)
          - c. Tetra-hydro-cannabinol (THC)
          - d. Cannabinoid agonists such as WIN 55-212 (a CB1 and CB2 receptor agonist)
          - 50 e. Dronabinol (Marinol®)
        10. Further cannabinoids
            - a. Nabilone (Cesamet)

## 11. c-9280 (Merck)

5 A 5HT<sub>3</sub>-receptor antagonist is particularly preferred. The anti-emetic is used in an effective amount which is sufficient to either remove or reduce the nausea and/or emesis to an acceptable level.

Kit-in-part

10 The pharmaceutical composition may also be a kit-in-part further including loperamide, hyoscyamine sulphate, lactulose, YM-443, dexloxiglumide, bisacodyl, AZD 7371, talnetant (SB 223412), asimadoline, NT3, SLV305, AZD 3355, AZD 9343, pinaverine, Na picosulphate, calcium polycarpophil, trimebutine, dopamine D2 antagonists, such as itopride, motilin agonists, such as KC 11458 and GM-611, NK2 antagonists, such as saredutant, NK3/NK2 receptor antagonists, such as SSR 15 241586, 5-hydroxytryptamine<sub>4</sub> (5-HT<sub>4</sub>) receptor partial agonists, such as tegaserod, 5-HT<sub>4</sub> receptor agonists, such as mosapride and prucalopride, antagonists of the serotonin 5-HT<sub>3</sub> receptor type, such as alosetron or cilansetron, dual 5-HT<sub>4</sub> agonist and 5-HT<sub>3</sub> –antagonists, such as renzapride and E-3620, GLP-1 or analogues/homologues thereof, such as exenatide, exenatide LAR, liraglutide, CJC- 20 1131, ZP 10/AVE-0010 and GTP-010, DPP-IV inhibitors, such as LAF237, PYY(1-36) or analogues/homologues thereof, PYY(3-36) or analogues/homologues thereof, NPY(1-36) or analogues/homologues thereof, NPY(3-36) or analogues/homologues thereof or anti-depressants, such as selective serotonin reuptake inhibitors (SSRIs), serotonin noradrenalin reuptake inhibitors (SNRIs), norepinephrine serotonin 25 reuptake inhibitors (NSRIs), selective noradrenalin reuptake inhibitors, tetracyclic antidepressants, non-selective monoamine reuptake inhibitors including tricyclic antidepressants (TCAs), selective reversible monoamine reuptake inhibitors and antidepressants with other mechanisms of action, e.g. mirtazapin or duloxetine. Examples of SSRIs are citalopram, escitalopram, fluoxetine, fluvoxamine, 30 paroxetine and sertraline. An example of an SNRI is venlafaxine. The kit-in-part may be used for simultaneous, sequential or separate administration. An example of an NSRI is milnacipran.

In a preferred embodiment the pharmaceutical composition is a kit-in-part.

Pharmaceutically acceptable salts

Pharmaceutically acceptable salts of the present compounds, where they can be prepared, are also intended to be covered by this invention. These salts will be ones which are acceptable in their application to a pharmaceutical use. By that it is meant that the salt will retain the biological activity of the parent compound and the salt will not have untoward or deleterious effects in its application and use in treating diseases.

Pharmaceutically acceptable salts are prepared in a standard manner. If the parent compound is a base it is treated with an excess of an organic or inorganic acid in a suitable solvent. If the parent compound is an acid, it is treated with an inorganic or organic base in a suitable solvent.

The compounds of the invention may be administered in the form of an alkali metal or earth alkali metal salt thereof, concurrently, simultaneously, or together with a pharmaceutically acceptable carrier or diluent, especially and preferably in the form of a pharmaceutical composition thereof, whether by oral, rectal, or parenteral (including subcutaneous) route, in an effective amount.

The pharmaceutical composition of the present invention can include pharmaceutically acceptable salts of the compounds therein. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the polypeptide).

Such salts include pharmaceutically acceptable acid addition salts (formed with the free amino groups of the polypeptide), pharmaceutically acceptable metal salts, ammonium salts and alkylated ammonium salts. Acid addition salts include salts of inorganic acids as well as organic acids. Representative examples of suitable inorganic acids include hydrochloric, hydrobromic, hydriodic, phosphoric, metaphosphoric, sulphuric and nitric acids and the like. Representative examples of suitable organic acids include formic, acetic, trichloroacetic, trifluoroacetic, propionic, benzoic, cinnamic, citric, fumaric, glycolic, lactic, maleic, malic, malonic, mandelic, oxalic, picric, pyruvic, salicylic, succinic, methanesulfonic, ethanesulfonic, tartaric, ascorbic, pantoic, bismethylene salicylic, ethanedithionyl, gluconic, citraconic, aspartic, stearic, palmitic, ethylenediaminetetraacetic (EDTA), p-aminobenzoic,

glutamic, benzenesulfonic, arylsulphonic and p-toluenesulfonic acids and the like. Further examples of pharmaceutically acceptable inorganic or organic acid addition salts include the pharmaceutical acceptable salts listed in J. Pharm. Sci. 1977,66,2, which is incorporated herein by reference. Examples of metal salts include lithium,  
5 sodium, potassium and magnesium salts and the like.

According to the invention organic acid salts of organic acids such as for example acetic acid is preferred.

10 Examples of ammonium and alkylated ammonium salts include ammonium, methyl-ammonium, dimethylammonium, trimethylammonium, ethylammonium, hydroxy-ethylammonium, diethylammonium, butylammonium and tetramethylammonium salts and the like.

15 Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine and the like.

20 Also included within the scope of compounds or pharmaceutical acceptable acid addition salts thereof in the context of the present invention are any hydrates (hydrated forms) thereof.

The preparation of a pharmacological composition that contains active ingredients  
25 dissolved or dispersed therein is well understood in the art. Typically such compositions are prepared as sterile injectables either as liquid solutions or suspensions, aqueous or non-aqueous, however, solid forms suitable for solution, or suspensions, in liquid prior to use can also be prepared. The preparation can also be emulsified.

30

#### Pharmaceutically acceptable carriers and diluents

The active ingredient can be mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient and in amounts suitable for use in the therapeutic methods described herein. Suitable excipients are, for example,  
35 water, saline, dextrose, glycerol, ethanol or the like and combinations thereof. In

addition, if desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like which enhance the effectiveness of the active ingredient.

- 5      Liquid compositions can also contain liquid phases in addition to and to the exclusion of water. Exemplary of such additional liquid phases are glycerine, vegetable oils such as cottonseed oil, organic esters such as ethyl oleate, and water-oil emulsions.
- 10     Suitable pharmaceutical carriers include inert solid diluents or fillers, sterile aqueous solution and various organic solvents. Examples of solid carriers are lactose, terra alba, sucrose, cyclodextrin, talc, gelatine, agar, pectin, acacia, magnesium stearate, stearic acid or lower alkyl ethers of cellulose. Examples of liquid carriers are syrup, peanut oil, olive oil, phospholipids, fatty acids, fatty acid amines, polyoxyethylene or
- 15     water.

The pharmaceutical compositions formed by combining the compounds of the invention and the pharmaceutical acceptable carriers are then readily administered in a variety of dosage forms suitable for the disclosed routes of administration. The

20     compositions may conveniently be presented in unit dosage form or in multiple dosage form by methods known in the art of pharmacy.

In a still further aspect, the invention relates to a pharmaceutical composition comprising, as an active ingredient, a compound as defined above or a

25     pharmaceutical acceptable salt thereof together with a pharmaceutical acceptable carrier.

#### Stabilizers

The active compound of the invention may be unstable, thus the composition

30     preferably contain stabilizers, preservatives or conservatives to increase the stability of the compounds.

A pH-buffering agent may be used to stabilize the active compound of the composition. The buffering agent may be acetate, carbonate, bicarbonate,

phosphate, citrate, tris or hepes. In a preferred embodiment the buffering agent is acetate.

According to the invention the composition preferably has a pH between 2.0 and 9.0, or such as between 2.5 and 8.0, or such as 3.0 and 7.0, or such as between 3.5 and 6.0, or such as between 3.5 and 5.0 or such as between 4.0 and 5.5, or such as between 4.0 and 5.0, or such as between 4.0 and 4.5. Preferably the pH of the compositions is less than 6, preferably less than 5.5, preferably less than 5, preferably less than 4.8, preferably less than 4.6, preferably less than 4.4, preferably less than 4.2.

Tween 20, Tween 60, Tween 80, Span 80, cetostearyl alcohol, benzyl alcohol, myristyl alcohol, glyceryl mono-stearate, manitol, polysorbates and sodium lauryl sulphate are possible stabilizers.

In a preferred embodiment mannitol may be used as stabilizer.

For the preparation of a lyophilised composition ad lyoprotectant may be used to stabilize the active ingredient (Townsend and DeLuca, "Use of lyoprotectants in the freeze-drying of a model protein, ribonuclease A" Journal of Parenteral Science & Technology 42 (6): 190-199 (Nov.-Dec. 1988)).

The lyoprotectant may preferably be a sugar such as sucrose or trehalose such as sucrose, dextran, or hydroxypropyl-/142-cyclodextrin.

#### Transport molecules

Transport molecules act by having incorporated into or anchored to it the compound according to the invention. Any suitable transport molecules known to the skilled person may be used. Examples of transport molecules may be liposomes, micelles, and/or microspheres.

A variety of methods are available for preparing liposomes, as described in, e.g., Szoka et al., Ann. Rev. Biophys. Bioeng. 9:467 (1980), U.S. Pat. Nos. 4,235,871, 4,501,728 and 4,837,028, all of which are incorporated herein by reference.

Micelles are formed by surfactants (molecules that contain a hydrophobic portion and one or more ionic or otherwise strongly hydrophilic groups) in aqueous solution. As the concentration of a solid surfactant increases, its monolayers adsorbed at the air/water or glass/water interfaces become so tightly packed that further occupancy requires excessive compression of the surfactant molecules already in the two monolayers. Further increments in the amount of dissolved surfactant beyond that concentration cause amounts equivalent to the new molecules to aggregate into micelles. This process begins at a characteristic concentration called "critical micelle concentration".

The shape of micelles formed in dilute surfactant solutions is approximately spherical. The polar head groups of the surfactant molecules are arranged in an outer spherical shell whereas their hydrocarbon chains are oriented toward the centre, forming a spherical core for the micelle. The hydrocarbon chains are randomly coiled and entangled and the micellar interior has a nonpolar, liquid-like character. In the micelles of polyoxyethylated non-ionic detergents, the polyoxyethylene moieties are oriented outward and permeated by water. This arrangement is energetically favourable since the hydrophilic head groups are in contact with water and the hydrocarbon moieties are removed from the aqueous medium and partly shielded from contact with water by the polar head groups. The hydrocarbon tails of the surfactant molecules, located in the interior of the micelle, interact with one another by weak van der Waals forces.

The size of a micelle or its aggregation number is governed largely by geometric factors. The radius of the hydrocarbon core cannot exceed the length of the extended hydrocarbon chain of the surfactant molecule. Therefore, increasing the chain length or ascending homologous series increases the aggregation number of spherical micelles. If the surfactant concentration is increased beyond a few percent and if electrolytes are added (in the case of ionic surfactants) or the temperature is raised (in the case of non-ionic surfactants), the micelles increase in size. Under these conditions, the micelles are too large to remain spherical and become ellipsoidal, cylindrical or finally lamellar in shape.

Common surfactants well known to one of skill in the art can be used in the micelles of the present invention. Suitable surfactants include sodium laureate, sodium

oleate, sodium lauryl sulphate, octaoxyethylene glycol monododecyl ether, octoxynol 9 and PLURONIC F-127 (Wyandotte Chemicals Corp.). Preferred surfactants are non-ionic polyoxyethylene and polyoxypropylene detergents compatible with IV injection such as, TWEEN-80, PLURONIC F-68, n-octyl-beta-D-glucopyranoside, and the like. In addition, phospholipids, such as those described for use in the production of liposomes, may also be used for micelle formation.

#### Compositions for oral administration

The compounds of the present invention may be formulated in a wide variety of oral administration dosage forms. The pharmaceutical compositions and dosage forms may comprise the compounds of the invention or its pharmaceutically acceptable salt or a crystal form thereof as the active component. The pharmaceutically acceptable carriers can be either solid or liquid. Solid form preparations include powders, tablets, pills, capsules, cachets, suppositories, and dispersible granules. A solid carrier can be one or more substances which may also act as diluents, flavoring agents, solubilizers, lubricants, suspending agents, binders, preservatives, wetting agents, tablet disintegrating agents, or an encapsulating material.

Preferably, the 0.00002% to 2 % by weight of a compound or compounds of the invention, with the remainder consisting of suitable pharmaceutical excipients. For oral administration, such excipients include pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, gelatine, sucrose, magnesium carbonate, and the like.

In powders, the carrier is a finely divided solid which is a mixture with the finely divided active component. In tablets, the active component is mixed with the carrier having the necessary binding capacity in suitable proportions and compacted in the shape and size desired. The powders and tablets preferably containing from one to about seventy percent of the active compound. Suitable carriers are magnesium carbonate, magnesium stearate, talc, sugar, lactose, pectin, dextrin, starch, gelatine, tragacanth, methylcellulose, sodium carboxymethylcellulose, a low melting wax, cocoa butter, and the like. The term "preparation" is intended to include the composition of the active compound with encapsulating material as carrier providing a capsule in which the active component, with or without carriers, is surrounded by a carrier, which is in association with it. Similarly, cachets and lozenges are included.

Tablets, powders, capsules, pills, cachets, and lozenges can be as solid forms suitable for oral administration.

5 Drops according to the present invention may comprise sterile or non-sterile aqueous or oil solutions or suspensions, and may be prepared by dissolving the active ingredient in a suitable aqueous solution, optionally including a bactericidal and/or fungicidal agent and/or any other suitable preservative, and optionally including a surface active agent. The resulting solution may then be clarified by filtration, transferred to a suitable container which is then sealed and sterilized by  
10 autoclaving or maintaining at 98-100 °C for half an hour. Alternatively, the solution may be sterilized by filtration and transferred to the container aseptically. Examples of bactericidal and fungicidal agents suitable for inclusion in the drops are phenylmercuric nitrate or acetate (0.002%), benzalkonium chloride (0.01%) and chlorhexidine acetate (0.01%). Suitable solvents for the preparation of an oily  
15 solution include glycerol, diluted alcohol and propylene glycol.

Also included are solid form preparations which are intended to be converted, shortly before use, to liquid form preparations for oral administration. Such liquid forms include solutions, suspensions, and emulsions. These preparations may  
20 contain, in addition to the active component, colorants, flavours, stabilizers, buffers, artificial and natural sweeteners, dispersants, thickeners, solubilizing agents, and the like.

Other forms suitable for oral administration include liquid form preparations including  
25 emulsions, syrups, elixirs, aqueous solutions, aqueous suspensions, toothpaste, gel dentrifice, chewing gum, or solid form preparations which are intended to be converted shortly before use to liquid form preparations. Emulsions may be prepared in solutions in aqueous propylene glycol solutions or may contain emulsifying agents such as lecithin, sorbitan monooleate, or acacia. Aqueous  
30 solutions can be prepared by dissolving the active component in water and adding suitable colorants, flavours, stabilizing and thickening agents. Aqueous suspensions can be prepared by dispersing the finely divided active component in water with viscous material, such as natural or synthetic gums, resins, methylcellulose, sodium carboxymethylcellulose, and other well known suspending agents. Solid form  
35 preparations include solutions, suspensions, and emulsions, and may contain, in

addition to the active component, colorants, flavours, stabilizers, buffers, artificial and natural sweeteners, dispersants, thickeners, solubilizing agents, and the like.

5 It is well known to the skilled artisan that proteins and natural peptides are not readily amenable to absorption after oral administration, particularly due to degradation by acid and enzymes of the digestive tract. Furthermore, many macromolecules and polar compounds are poorly absorbed through certain membranes. Different technologies have been developed to try to overcome these and other problems associated with the oral administration of e.g. peptides. One  
10 example of such a technology is the eligen™ system (EMISPHERE® delivery agents) by Emisphere Technologies, Inc (Tarrytown, NY, USA, <http://www.emisphere.com/>). Under certain physiological conditions, drug molecules naturally exist in many different shapes, or "conformations". Some of these conformations can be transported across the cell membranes and some are too large or too charged to  
15 cross the membrane. EMISSPHERE® delivery agents enable molecules that are too large or too charged to cross cell membranes. Once the drug crosses the membrane, the delivery agent dissociates from the drug and the drug re-establishes its natural distribution of conformations, ensuring that the delivered drug is in its therapeutically active state. EMISSPHERE® delivery agents utilize the body's natural  
20 passive transcellular transport process, allowing macromolecules to cross membranes and yet remain therapeutically active. Thus, oral delivery using the eligen™ system occurs without chemical modification of the molecule or damage to the biological membrane.

25 Thus, in one embodiment of the invention, the composition according to the invention can be formulated using one or several of the EMISSPHERE® delivery agents (using the eligen™ system).

Another approach to the oral delivery of macromolecules including peptides is the  
30 approach taken by NOBEX Corporation (Research Triangle Park, NC, USA, <http://www.nobexcorp.com/>). The objective of the NOBEX approach is to provide multiple, new physical and chemical characteristics to drug molecules to enable oral delivery. By attaching low molecular weight polymers at specific sites, NOBEX creates a conjugated drug-polymer molecule. The conjugated drug-polymer is not  
35 degraded as readily by enzymes in the body compared with the drug in its native form. The conjugated drug is absorbed more efficiently across the gastrointestinal

wall than the drug in its native state due to its enhanced diffusion through both water and fatty portions of cells and tissues that make up barriers to absorption along the gastrointestinal pathway and into the bloodstream. Modifications to the molecule can be designed to favorably alter the drug's pharmacokinetics (the pattern of a drug's appearance in the bloodstream, the duration of its effects in the body, and its elimination from the body).

Thus, in another embodiment of the invention, the composition according to the invention is formulated using the NOBEX approach described above.

#### Compositions for parenteral administration

The compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, for example solutions in aqueous polyethylene glycol. Examples of oily or nonaqueous carriers, diluents, solvents or vehicles include propylene glycol, polyethylene glycol, vegetable oils (e.g., olive oil), and injectable organic esters (e.g., ethyl oleate), and may contain formulatory agents such as preserving, wetting, emulsifying or suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form, obtained by aseptic isolation of sterile solid or by lyophilisation from solution for constitution before use with a suitable vehicle, e.g., sterile, pyrogen-free water. Aqueous solutions should be suitably buffered if necessary, and the liquid diluents first rendered isotonic with sufficient saline or glucose. The aqueous solutions are particularly suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. The sterile aqueous media employed are all readily available by standard techniques known to those skilled in the art.

In a preferred embodiment of the invention, the composition comprising PP or a functional equivalent thereof or a salt thereof, is a lyophilised composition and the composition may further comprise a solvent. In another embodiment the composition is a solution of PP or a functional equivalent thereof according to the invention or a salt thereof. Preferably, the solvent may be any suitable solvents, such as described herein, and preferably the solvent is saline or a physiological buffer like phosphate buffer.

The pharmaceutical composition comprises PP or a functional equivalent thereof or a pharmaceutically acceptable salt thereof. Such compositions can be prepared in water or saline, and optionally mixed with a non-toxic surfactant. Compositions for intravenous or intra-arterial administration may include sterile aqueous solutions that may also contain buffers, liposomes, diluents and other suitable additives.

Oils useful in parenteral compositions include animal, vegetable, or synthetic oils. Specific examples of oils useful in such compositions include peanut, soybean, sesame, cottonseed, corn, olive, petrolatum, and mineral. Suitable fatty acids for use in parenteral compositions include oleic acid, stearic acid, and isostearic acid. Ethyl oleate and isopropyl myristate are examples of suitable fatty acid esters.

Suitable soaps for use in parenteral compositions include fatty alkali metal, ammonium, and triethanolamine salts, and suitable detergents include (a) cationic detergents such as, for example, dimethyl dialkyl ammonium halides, and alkyl pyridinium halides; (b) anionic detergents such as, for example, alkyl, aryl, and olefin sulfonates, alkyl, olefin, ether, and monoglyceride sulfates, and sulfosuccinates, (c) nonionic detergents such as, for example, fatty amine oxides, fatty acid alkanol-amides, and polyoxyethylenepolypropylene copolymers, (d) amphoteric detergents such as, for example, alkyl-beta-aminopropionates, and 2-alkyl-imidazoline quaternary ammonium salts, and (e) mixtures thereof.

The parenteral compositions typically will contain from about 0.5 to about 25% by weight of the active ingredient in solution. Preservatives and buffers may be used. In order to minimize or eliminate irritation at the site of injection, such compositions may contain one or more non-ionic surfactants having a hydrophile-lipophile balance (HLB) of from about 12 to about 17. The quantity of surfactant in such compositions will typically range from about 5 to about 15% by weight. Suitable surfactants include polyethylene sorbitan fatty acid esters, such as sorbitan monooleate and the high molecular weight adducts of ethylene oxide with a hydrophobic base, formed by the condensation of propylene oxide with propylene glycol. The parenteral compositions can be presented in unit-dose or multi-dose sealed containers, such as ampules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid excipient, for example, water, for injections, immediately prior to use. Extemporaneous injection solutions and

suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described.

5 The pharmaceutical dosage forms suitable for injection can include sterile aqueous solutions or dispersions comprising the active ingredient that are adapted for administration by encapsulation in liposomes. In all cases, the ultimate dosage form must be sterile, fluid and stable under the conditions of manufacture and storage.

10 Sterile injectable solutions are prepared by incorporating PP or a functional equivalent thereof or pharmaceutically acceptable salt thereof in the required amount in the appropriate solvent with several of the other ingredients enumerated above, as required, followed by filter sterilization.

#### Compositions for topical administration

15 The compounds of the invention can also be delivered topically. Regions for topical administration include the skin surface and also mucous membrane tissues of the rectum, nose, mouth, and throat. Compositions for topical administration via the skin and mucous membranes should not give rise to signs of irritation, such as swelling or redness.

20 The topical composition may include a pharmaceutically acceptable carrier adapted for topical administration. Thus, the composition may take the form of a suspension, solution, ointment, lotion, cream, foam, aerosol, spray, suppository, implant, inhalant, tablet, capsule, dry powder, syrup, balm or lozenge, for example. Methods for preparing such compositions are well known in the pharmaceutical industry.

30 The compounds of the present invention may be formulated for topical administration to the epidermis as ointments, creams or lotions, or as a transdermal patch. Ointments and creams may, for example, be formulated with an aqueous or oily base with the addition of suitable thickening and/or gelling agents. Lotions may be formulated with an aqueous or oily base and will in general also contain one or more emulsifying agents, stabilizing agents, dispersing agents, suspending agents, thickening agents, or colouring agents. Compositions suitable for topical administration in the mouth include lozenges comprising active agents in a flavoured

35 base, usually sucrose and acacia or tragacanth; pastilles comprising the active

ingredient in an inert base such as gelatine and glycerine or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

5 Creams, ointments or pastes according to the present invention are semi-solid compositions of the active ingredient for external application. They may be made by mixing the active ingredient in finely-divided or powdered form, alone or in solution or suspension in an aqueous or non-aqueous fluid, with the aid of suitable machinery, with a greasy or non-greasy base. The base may comprise hydrocarbons such as hard, soft or liquid paraffin, glycerol, beeswax, a metallic  
10 soap; a mucilage; an oil of natural origin such as almond, corn, arachis, castor or olive oil; wool fat or its derivatives or a fatty acid such as stearic or oleic acid together with an alcohol such as propylene glycol or a macrogel. The composition may incorporate any suitable surface active agent such as an anionic, cationic or non-ionic surfactant such as a sorbitan ester or a polyoxyethylene derivative thereof.  
15 Suspending agents such as natural gums, cellulose derivatives or inorganic materials such as siliceous silicas, and other ingredients such as lanolin, may also be included.

20 Lotions according to the present invention include those suitable for application to the skin or eye. An eye lotion may comprise a sterile aqueous solution optionally containing a bactericide and may be prepared by methods similar to those for the preparation of drops. Lotions or liniments for application to the skin may also include an agent to hasten drying and to cool the skin, such as an alcohol or acetone, and/or a moisturizer such as glycerol or an oil such as castor oil or arachis oil.

25 The pharmaceutical agent-chemical modifier complexes described herein can be administered transdermally. Transdermal administration typically involves the delivery of a pharmaceutical agent for percutaneous passage of the drug into the systemic circulation of the patient. The skin sites include anatomic regions for  
30 transdermally administering the drug and include the forearm, abdomen, chest, back, buttock, mastoidal area, and the like.

Transdermal delivery is accomplished by exposing a source of the complex to a patient's skin for an extended period of time. Transdermal patches have the added  
35 advantage of providing controlled delivery of a pharmaceutical agent-chemical

modifier complex to the body. See Transdermal Drug Delivery: Developmental Issues and Research Initiatives, Hadgraft and Guy (eds.), Marcel Dekker, Inc., (1989); Controlled Drug Delivery: Fundamentals and Applications, Robinson and Lee (eds.), Marcel Dekker Inc., (1987); and Transdermal Delivery of Drugs, Vols. 1-3, Kydonieus and Berner (eds.), CRC Press, (1987). Such dosage forms can be made by dissolving, dispersing, or otherwise incorporating the pharmaceutical agent-chemical modifier complex in a proper medium, such as an elastomeric matrix material. Absorption enhancers can also be used to increase the flux of the compound across the skin. The rate of such flux can be controlled by either providing a rate-controlling membrane or dispersing the compound in a polymer matrix or gel.

A variety of types of transdermal patches will find use in the methods described herein. For example, a simple adhesive patch can be prepared from a backing material and an acrylate adhesive. The pharmaceutical agent-chemical modifier complex and any enhancer are formulated into the adhesive casting solution and allowed to mix thoroughly. The solution is cast directly onto the backing material and the casting solvent is evaporated in an oven, leaving an adhesive film. The release liner can be attached to complete the system.

Alternatively, a polyurethane matrix patch can be employed to deliver the pharmaceutical agent-chemical modifier complex. The layers of this patch comprise a backing, a polyurethane drug/enhancer matrix, a membrane, an adhesive, and a release liner. The polyurethane matrix is prepared using a room temperature curing polyurethane prepolymer. Addition of water, alcohol, and complex to the prepolymer results in the formation of a tacky firm elastomer that can be directly cast onto the backing material.

A further embodiment of this invention will utilize a hydrogel matrix patch. Typically, the hydrogel matrix will comprise alcohol, water, drug, and several hydrophilic polymers. This hydrogel matrix can be incorporated into a transdermal patch between the backing and the adhesive layer.

The liquid reservoir patch will also find use in the methods described herein. This patch comprises an impermeable or semi permeable, heat sealable backing

material, a heat sealable membrane, an acrylate based pressure sensitive skin adhesive, and a siliconized release liner. The backing is heat sealed to the membrane to form a reservoir which can then be filled with a solution of the complex, enhancers, gelling agent, and other excipients.

5

Foam matrix patches are similar in design and components to the liquid reservoir system, except that the gelled pharmaceutical agent-chemical modifier solution is constrained in a thin foam layer, typically a polyurethane. This foam layer is situated between the backing and the membrane which have been heat sealed at the periphery of the patch.

10

For passive delivery systems, the rate of release is typically controlled by a membrane placed between the reservoir and the skin, by diffusion from a monolithic device, or by the skin itself serving as a rate-controlling barrier in the delivery system. See U.S. Pat. Nos. 4,816,258; 4,927,408; 4,904,475; 4,588,580, 4,788,062; and the like. The rate of drug delivery will be dependent, in part, upon the nature of the membrane. For example, the rate of drug delivery across membranes within the body is generally higher than across dermal barriers. The rate at which the complex is delivered from the device to the membrane is most advantageously controlled by the use of rate-limiting membranes which are placed between the reservoir and the skin. Assuming that the skin is sufficiently permeable to the complex (i.e., absorption through the skin is greater than the rate of passage through the membrane), the membrane will serve to control the dosage rate experienced by the patient.

15

20

25

Suitable permeable membrane materials may be selected based on the desired degree of permeability, the nature of the complex, and the mechanical considerations related to constructing the device. Exemplary permeable membrane materials include a wide variety of natural and synthetic polymers, such as polydimethylsiloxanes (silicone rubbers), ethylenevinylacetate copolymer (EVA), polyurethanes, polyurethane-polyether copolymers, polyethylenes, polyamides, polyvinylchlorides (PVC), polypropylenes, polycarbonates, polytetrafluoroethylenes (PTFE), cellulosic materials, e.g., cellulose triacetate and cellulose nitrate/acetate, and hydrogels, e.g., 2-hydroxyethylmethacrylate (HEMA).

30

Other items may be contained in the device, such as other conventional components of therapeutic products, depending upon the desired device characteristics. For example, the compositions according to this invention may also include one or more preservatives or bacteriostatic agents, e.g., methyl  
5 hydroxybenzoate, propyl hydroxybenzoate, chlorocresol, benzalkonium chlorides, and the like. These pharmaceutical compositions also can contain other active ingredients such as antimicrobial agents, particularly antibiotics, anesthetics, analgesics, and antipruritic agents.

#### 10 Compositions for administration as suppositories

The compounds of the present invention may be formulated for administration as suppositories. A low melting wax, such as a mixture of fatty acid glycerides or cocoa butter is first melted and the active component is dispersed homogeneously, for example, by stirring. The molten homogeneous mixture is then poured into  
15 convenient sized moulds, allowed to cool, and to solidify.

The active compound may be formulated into a suppository comprising, for example, about 0.5% to about 50% of a compound of the invention, disposed in a polyethylene glycol (PEG) carrier (e.g., PEG 1000 [96%] and PEG 4000 [4%]).

20

#### Compounds for nasal administration

The compounds of the present invention may be formulated for nasal administration. The solutions or suspensions are applied directly to the nasal cavity by conventional means, for example with a dropper, pipette or spray. The compositions may be  
25 provided in a single or multidose form. In the latter case of a dropper or pipette this may be achieved by the patient administering an appropriate, predetermined volume of the solution or suspension. In the case of a spray this may be achieved for example by means of a metering atomizing spray pump. A suitable formulation for nasal administration is described in EP 1 466 610.

30

#### Compounds for aerosol administration

The compounds of the present invention may be formulated for aerosol administration, particularly to the respiratory tract and including intranasal administration. The compound will generally have a small particle size for example

of the order of 5 microns or less. Such a particle size may be obtained by means known in the art, for example by micronization. The active ingredient is provided in a pressurized pack with a suitable propellant such as a chlorofluorocarbon (CFC) for example dichlorodifluoromethane, trichlorofluoromethane, or

5 dichlorotetrafluoroethane, carbon dioxide or other suitable gas. The aerosol may conveniently also contain a surfactant such as lecithin. The dose of drug may be controlled by a metered valve. Alternatively the active ingredients may be provided in a form of a dry powder, for example a powder mix of the compound in a suitable powder base such as lactose, starch, starch derivatives such as  
10 hydroxypropylmethyl cellulose and polyvinylpyrrolidone (PVP). The powder carrier will form a gel in the nasal cavity. The powder composition may be presented in unit dose form for example in capsules or cartridges of e.g., gelatine or blister packs from which the powder may be administered by means of an inhaler.

15 Compositions administered by aerosols may be prepared, for example, as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, employing fluorocarbons, and/or employing other solubilizing or dispersing agents.

## 20 Combinations

It is further envisaged that the composition of the present invention can be administered in combination with a second active ingredient. Said second active ingredient may be co-formulated with the other compound(s) according to the invention.

25

It is further envisaged that the active ingredient of the present invention can be administered in combination with a second active ingredient. By "in combination" is meant that said composition may be co-formulated with other compounds in the same composition, and/or that said second active ingredient and/or pharmaceutical  
30 composition(s) are administered before, during (including concurrently with) and/or after administration of the compositions of the present invention.

Thus an embodiment of the present invention relates to a composition comprising PP or a functional equivalent thereof and a second active ingredient.

35

Thus an embodiment of the present invention relates to a composition comprising PP or a functional equivalent thereof as defined herein and a second active ingredient. Said second active ingredient may be selected from the group of; loperamide, hyoscyamine sulphate, lactulose, YM-443, dexloxiglumide, bisacodyl, 5 AZD 7371, talnetant (SB 223412), asimadoline, NT3, SLV305, AZD 3355, AZD 9343, pinaverine, Na picosulphate, calcium polycarpophil, trimebutine, dopamine D2 antagonists, such as itopride, motilin agonists; such as KC 11458 and GM-611, NK2 antagonists, such as saredutant, NK3/NK2 receptor antagonists, such as SSR 241586, 5-hydroxytryptamine<sub>4</sub> (5-HT<sub>4</sub>) receptor partial agonists, such as tegaserod, 10 5-HT<sub>4</sub> receptor agonists, such as mosapride and prucalopride, antagonists of the serotonin 5-HT<sub>3</sub> receptor type, such as alosetron or cilansetron, dual 5-HT<sub>4</sub> agonist and 5-HT<sub>3</sub> –antagonists, such as renzapride and E-3620, GLP-1 or analogues/homologues thereof, such as exenatide, exenatide LAR, liraglutide, CJC-1131, ZP 10/AVE-0010 and GTP-010, DPP-IV inhibitors, such as LAF237, PYY(1-15 36) or analogues/homologues thereof, PYY(3-36) or analogues/homologues thereof, NPY(1-36) or analogues/homologues thereof, NPY(3-36) or analogues/homologues thereof and anti-hypertensives including (but not limited to) beta-adrenoceptor antagonists, alpha-adrenoceptor antagonists, calcium antagonists, angiotensin converting enzyme (ACE) inhibitors, angiotensin II receptor antagonists and 20 diuretics (including, but not limited to, thiazide diuretics and loop diuretics), insulin, DPP-IV inhibitors, corticosteroids as well as any other drug(s) that may increase body weight or body fat mass or that may be indicated for the treatment of hypertension, overweight, obesity, the metabolic syndrome (syndrome X) and/or diabetes mellitus and anti-depressant medications including (but not limited to), such 25 as selective serotonin reuptake inhibitors (SSRIs), serotonin noradrenalin reuptake inhibitors (SNRIs), norepinephrine serotonin reuptake inhibitors (NSRIs), selective noradrenalin reuptake inhibitors, tetracyclic antidepressants, non-selective monoamine reuptake inhibitors including tricyclic antidepressants (TCAs), selective reversible monoamine reuptake inhibitors and antidepressants with other 30 mechanisms of action, e.g. mirtazapine and mianserine, and classical as well as atypical antipsychotic drugs (neuroleptics). Examples of SSRIs are citalopram, escitalopram, fluoxetine, fluvoxamine, paroxetine and sertraline. An example of an SNRI is venlafaxine. An example of an NSRI is milnacipran. A further example of an anti-depressant is duloxetine.

Administration

Furthermore are provided in the scope of the present invention methods of treatment of an individual in need thereof, comprising administering to said individual an effective amount of one or more of the pharmaceutical compositions described herein. Said individual is preferably suffering, or at risk of, one or more of the health problems described earlier herein such as gastrointestinal disorders. By "treatment" is also meant prophylaxis and aftercare, and/or lessening of disease symptoms and/or possible disease prevention and/or cure. Said method of treatment may comprise improving the sense of well-being and the quality of life in an individual. Said method may involve one or more of the combination treatments as disclosed herein.

The pharmaceutical compositions of the invention may be used both prophylactically as well as for therapeutic administration. Thus, the pharmaceutical composition comprising PP or a functional equivalent thereof can be administered to patients in order to prevent the development of a gastrointestinal disorder, in order to minimise the severity of a disorder or to patients already suffering from a disorder. Furthermore the therapeutic method may prevent reoccurrence of disorders of the gastrointestinal.

The pharmaceutical composition may be prepared so it is suitable for one or more particular administration methods. The composition according to the invention may be administered parenterally, orally, nasally (inhalation or intranasal application), topically (to the skin or to the eye), rectally using suppositories or by intravaginal absorption.

The pharmaceutical composition comprising PP or a functional equivalent thereof may be administered to an individual in need thereof.

The composition comprising PP or a functional equivalent thereof can, according to the invention, be administered parenterally, e.g. by injection. Thus, PP or a functional equivalent thereof may, according to the invention, be administered parenterally, such as intravenously, intra-arterially, intraperitoneally, intramuscularly, subcutaneously, intranasally or transdermally. The composition may further be administered by inhalation, topical application to the eye, intranasal application,

intravaginal absorption, application to the skin, and rectal suppositories. Other drug-administration methods, which are effective to deliver the drug to a target site or to introduce the drug into the bloodstream, are also contemplated.

5 The pharmaceutical compositions containing PP or a functional equivalent thereof may be administered intravenously, for example by injection of a unit dose. The term "unit dose" when used in reference to a pharmaceutical composition of the present invention refers to physically discrete units suitable as unitary dosage for the sub-  
10 ject, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required diluent; i.e. carrier, or vehicle. Alternatively, the pharmaceutical composition may be prepared in a multiple dose form and, by use of the multi-dose delivery device, single doses can be administered when needed. As an alternative to standard injections the pharmaceutical composition may be administered by infusions, such infusions are  
15 preferably short.

The effect of PP or functional equivalents according to the invention is believed to be mediated via actions of PP outside the central nervous system.

20 The effect of PP or functional equivalents according to the invention is believed to be mediated via actions of PP outside the central nervous system, with the exception of the NPY neurons located in the hypothalamus. Thereby PP or functional equivalents do not affect NPY neurons located elsewhere in the central nervous system.

25 Accordingly, PP or functional equivalents capable of binding to the NPY Y2 receptor may circulate in the bloodstream to the receptors in the hypothalamus; however, these molecules should preferably not be capable of crossing the blood-brain barrier, and thereby not be able to enter into other parts of the central nervous system.

30 The composition according to the invention may be administered in combination with a second pharmaceutical composition. By "in combination" is meant that said composition may be co-formulated with other compounds in the same composition, and/or that said other compound(s) and/or pharmaceutical composition(s) are

administered before, during (including concurrently with) and/or after administration of the compositions of the present invention.

5 Thus in an embodiment the composition of the invention may be administered in combination with a second pharmaceutical composition. The compositions may be administered simultaneously, either as separate compositions or combined in a unit dosage form, or administered sequentially as two separate pharmaceutical compositions.

10 In one embodiment the pharmaceutical composition according to the invention is administered in combination with a second pharmaceutical composition selected from the group of pharmaceutical composition comprising ; loperamide, hyoscyamine sulphate, lactulose, YM-443, dexloxiglumide, bisacodyl, AZD 7371, talnetant (SB 223412), asimadoline, NT3, SLV305, AZD 3355, AZD 9343,  
15 pinaverine, Na picosulphate, calcium polycarpophil, trimebutine, dopamine D2 antagonists, such as itopride, motilin agonists, such as KC 11458 and GM-611, NK2 antagonists, such as saredutant, NK3/NK2 receptor antagonists, such as SSR 241586, 5-hydroxytryptamine<sub>4</sub> (5-HT<sub>4</sub>) receptor partial agonists, such as tegaserod, 5-HT<sub>4</sub> receptor agonists, such as mosapride and prucalopride, antagonists of the  
20 serotonin 5-HT<sub>3</sub> receptor type, such as alosetron or cilansetron, dual 5-HT<sub>4</sub> agonist and 5-HT<sub>3</sub> –antagonists, such as renzapride and E-3620, GLP-1 or analogues/homologues thereof, such as exenatide, exenatide LAR, liraglutide, CJC-1131, ZP 10/AVE-0010 and GTP-010, DPP-IV inhibitors, such as LAF237, PYY(1-36) or analogues/homologues thereof, PYY(3-36) or analogues/homologues thereof,  
25 NPY(1-36) or analogues/homologues thereof, NPY(3-36) or analogues/homologues thereof and anti-depressants, such as selective serotonin reuptake inhibitors (SSRIs), serotonin noradrenalin reuptake inhibitors (SNRIs), norepinephrine serotonin reuptake inhibitors (NSRIs), selective noradrenalin reuptake inhibitors, tetracyclic antidepressants, non-selective monoamine reuptake inhibitors including  
30 tricyclic antidepressants (TCAs), selective reversible monoamine reuptake inhibitors and antidepressants with other mechanisms of action, e.g. mirtazapin. Examples of SSRIs are citalopram, escitalopram, fluoxetine, fluvoxamine, paroxetine and sertraline. An example of an SNRI is venlafaxine. An example of an NSRI is milnacipran. A further example of an anti-depressant is duloxetine.

35

Method of treatment

The method of the invention for prophylaxis or treatment of a functional gastrointestinal disorder and/or one or more symptoms of such disorder aims at reducing or removing said symptom(s) or disorder.

5

In one embodiment the composition is administered after sensing of symptoms, such as 5 minutes after sensing of symptoms, such as 10 minutes after sensing of symptoms, such as 15 minutes after sensing of symptoms, such as up to 20 minutes after sensing of symptoms, such as up to 30 minutes after sensing of symptoms, such as up to 45 minutes after sensing of symptoms, such as up to 60 minutes after sensing of symptoms, such as up to 75 minutes after sensing of symptoms, such as up to 90 minutes after sensing of symptoms, such as up to 120 minutes after sensing of symptoms, such as up to 150 minutes after sensing of symptoms, such as up to 180 minutes after sensing of symptoms. In another embodiment the composition is administered prophylactically, i.e. in cases where the individual expects to obtain an attack of the functional gastrointestinal disorder and/or symptom in question.

15

Depending on the frequency and/or severity of attacks the composition may be administered acutely in connection with an attack or may be administered on a regular basis, e.g. once a day, or twice a day, or three times a day or every second day or twice a week or once a week.

20

The pharmaceutical composition may be administered prior to a meal, such as within 5 minutes of a meal, such as within 20 minutes of a meal, such as within 60 minutes of a meal. In another embodiment of the invention, the pharmaceutical composition according to the invention may be administered after the initiation of a meal, such as within 5 minutes after the initiation of a meal, such as within 20 minutes after the initiation of a meal, such as within 60 minutes after the initiation of a meal. In yet another embodiment of the invention, the pharmaceutical composition according to the invention may be administered at the completion of a meal. In yet a further embodiment of the invention, the pharmaceutical composition may be administered after the completion of a meal, such as within 5 minutes after the completion of a meal, such as within 20 minutes after the completion of a meal, such as within 60 minutes after the completion of a meal.

25

30

35

The pharmaceutical preparations described herein may also be arranged in unit dosage forms. In such a form, the preparation is subdivided into unit doses containing appropriate quantities of the active component. The unit dosage form can  
5 be a packaged preparation, the package containing discrete quantities of preparation, such as powders in compartments. In this embodiment the powders may be mixed with a solvent prior to or during use.

In one aspect of the present invention, a suitable dose of the compositions  
10 described herein is administered in pharmaceutically effective amounts to an individual in need of such treatment. Herein, "pharmaceutically effective amounts", is defined as an administration involving a total amount of each active component of the pharmaceutical composition or pharmaceutical composition or method that is  
15 sufficient to show a meaningful patient benefit. The term "unit dosage form" as used herein refers to physically discrete units suitable as unitary dosages for human and animal subjects, each unit containing a predetermined quantity of a compound, alone or in combination with other agents, calculated in an amount sufficient to produce the desired effect in association with a pharmaceutically acceptable diluent, carrier, or vehicle. The specifications for the unit dosage forms of the present  
20 invention depend on the particular compound or compounds employed and the effect to be achieved, as well as the pharmacodynamics associated with each compound in the host. The dose administered should be an "effective amount" or an amount necessary to achieve an "effective level" in the individual patient.

25 The pharmaceutical compositions may be administered by intravenous infusion. The duration of an infusion may be less than 120 minutes, such as less than 100 minutes, such as less than 80 minutes, such as less than 60 minutes, such as less than 40 minutes, such as less than 20 minutes, such as less than 10 minutes or such as less than 5 minutes.

30 The dosage requirements will vary with the particular drug composition employed, the route of administration and the particular subject being treated. Ideally, a patient to be treated by the present method will receive a pharmaceutically effective amount of the compound not exceeding the maximum tolerated dose (MTD), which is generally  
35 no higher than that required before drug resistance develops.

Suitable dosing regimens are preferably determined taking into account factors well known in the art including type of subject being dosed; age, weight, sex and medical condition of the subject; the route of administration; the renal and hepatic function of the subject; the desired effect; and the particular compound employed.

Optimal precision in achieving concentrations of drug within the range that yields efficacy without toxicity requires a regimen based on the kinetics of the drug's availability to target sites. This involves a consideration of the distribution, equilibrium, and elimination of a drug. For the present invention the dosage will vary depending on the compound employed and the mode of administration.

The dosage may be calculated based on the body weight of the subject but in certain situations the dosages may be calculated base on the fat free mass (FFM) of the subject. Thus the dosage may be in concentration equivalent to PP.

For the present invention the dosage will vary depending on the compound employed and the mode of administration. Dosage levels may vary between about 4 ng/kg body weight to 20 µg/kg body weight daily, preferably between about 10 ng/kg body weight to 1 µg/kg body weight, more preferably between 50 to 750 ng/kg body weight. Alternative dosages in relation to FFM may vary between about 5 ng/kg FFM to 25 µg/kg FFM daily, preferably between about 12.5 ng/kg FFM to 1.25 µg/kg FFM, more preferably between 62.5 to 875 ng/kg FFM. To obtain dosages in relation to FFM the dosage/kg bodyweight should be multiplied by the factor 1.25.

The dosage may be administered when needed, such as up to ten times daily, such as one to five times daily, such as two or three times daily, or preferably such as once a day, thus the daily dosage maybe up to 2-5 times the dosages mentioned above. Alternatively, the dosage may be administered less frequently than once daily as described herein above.

A preferred dosage of a composition employed according to the invention is in a concentration equivalent to PP or a functional equivalent there of from 4 ng to about 20 µg per kg bodyweight, or such as from 10 ng to 1 µg per kg bodyweight, more preferably from 20 to 100 ng per kg bodyweight. The preferred dosages may be in a

concentration equivalent to PP or a functional equivalent thereof from 1 pmol to 5 nmol per kg, such as from 5 pmol to 1 nmol per kg, or such as from 100 pmol to 900 pmol per kg, or such as 300 pmol to 600 pmol per kg bodyweight.

5 In one preferred embodiment of the present invention, the compositions of the present invention are administered in dosages of PP or a functional equivalent from about 400 ng to about 2 mg, more preferably from about 10  $\mu$ g to about 200  $\mu$ g, or from about 5  $\mu$ g to about 250  $\mu$ g, more preferably from about 20  $\mu$ g to about 200  $\mu$ g, more preferably from about 20  $\mu$ g to about 100  $\mu$ g. In a preferred embodiment the  
10 composition of the invention is administered in dosage of PP or a functional equivalent from 100 pmol to 500 nmol, or such from 500 pmol to 150 nmol, or such as from 1 nmol to 150 nmol, or such as from 5 to 100 nmol, or such as from 25 to 75 nmol of PP or a functional equivalent thereof.

15 The pharmaceutical preparations described herein may also be arranged in unit dosage forms. In such form, the preparation is subdivided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of preparation, such as powders in compartments. In this embodiment the powders may be mixed  
20 with a solvent prior to or during use.

In a preferred embodiment the pharmaceutical composition is administered in unit dosage form, from about 400 ng to about 2 mg of PP or a functional equivalent thereof, more preferably from about 10  $\mu$ g to about 200  $\mu$ g, or from about 5  $\mu$ g to  
25 about 250  $\mu$ g, more preferably from about 20  $\mu$ g to about 200  $\mu$ g, more preferably from about 20  $\mu$ g to about 100  $\mu$ g. The unit dosage form may comprise from 100 pmol to 500 nmol, or such from 500 pmol to 150 nmol, or such as from 1 nmol to 150 nmol, or such as from 5 to 100 nmol, or such as from 25 to 75 nmol of PP or a functional equivalent thereof.

30 When administered by infusion the dosage of PP or a functional equivalent thereof may be from 0.01 pmol/kg/minute to 500 pmol/kg/minute such as from 0.05 pmol/kg/minute to 100 pmol/kg/minute, or such as from 0.1 pmol/kg/minute to 50 pmol/kg/minute, or such as from 1 pmol/kg/min. to 25 pmol/kg/minute, or such as  
35 from 5 pmol/kg/min to 15 pmol/kg/min.

It is thereby included that the treatment may be administered during the night. Furthermore, since the "effective level" is used as the preferred endpoint for dosing, the actual dose and schedule can vary, depending on individual differences in pharmacokinetics, drug distribution, and metabolism. The "effective level" can be defined, for example, as the blood or tissue level desired in the patient that corresponds to a concentration of one or more compounds according to the invention.

The effective level may refer to an amount of the active ingredient of the composition according to the invention that is able to obtain certain blood or tissue levels of a desired compound in the patient. The effective level may be the amount of the active ingredient of the composition according to the invention that is able to diminish the symptoms in the patient.

The pharmaceutical composition may be administration during hospitalization. In addition it may be beneficial for the patient if the composition can be self-administered.

In one embodiment of the invention, the compositions of the present invention are self-administered. The pharmaceutical composition may be administered by use of an injection device, for example by the use of a system similar to insulin pens.

In one embodiment of the invention the composition is administered by use of a single or a multi-dose injection device.

The pharmaceutical composition may also be a kit-in-part further including loperamide, hyoscyamine sulphate, lactulose, YM-443, dexloxiglumide, bisacodyl, AZD 7371, talnetant (SB 223412), asimadoline, NT3, SLV305, AZD 3355, AZD 9343, pinaverine, Na picosulphate, calcium polycarpophil, trimebutine, dopamine D2 antagonists, such as itopride, motilin agonists, such as KC 11458 and GM-611, NK2 antagonists, such as saredutant, NK3/NK2 receptor antagonists, such as SSR 241586, 5-hydroxytryptamine<sub>4</sub> (5-HT<sub>4</sub>) receptor partial agonists, such as tegaserod, 5-HT<sub>4</sub> receptor agonists, such as mosapride and prucalopride, antagonists of the serotonin 5-HT<sub>3</sub> receptor type, such as alosetron or cilansetron, dual 5-HT<sub>4</sub> agonist

and 5-HT<sub>3</sub> –antagonists, such as renzapride and E-3620, GLP-1 or analogues/homologues thereof, such as exenatide, exenatide LAR, liraglutide, CJC-1131, ZP 10/AVE-0010 and GTP-010, DPP-IV inhibitors, such as LAF237, PYY(1-36) or analogues/homologues thereof, PYY(3-36) or analogues/homologues thereof, 5 NPY(1-36) or analogues/homologues thereof, NPY(3-36) or analogues/homologues thereof or anti-depressants, such as selective serotonin reuptake inhibitors (SSRIs), serotonin noradrenalin reuptake inhibitors (SNRIs), norepinephrine serotonin reuptake inhibitors (NSRIs), selective noradrenalin reuptake inhibitors, tetracyclic antidepressants, non-selective monoamine reuptake inhibitors including tricyclic 10 antidepressants (TCAs), selective reversible monoamine reuptake inhibitors and antidepressants with other mechanisms of action, e.g. mirtazapin or duloxetine. Examples of SSRIs are citalopram, escitalopram, fluoxetine, fluvoxamine, paroxetine and sertraline. An example of an SNRI is venlafaxine. The kit-in-part may be used for simultaneous, sequential or separate administration. An example of an 15 NSRI is milnacipran.

The preferred dosages may be in a concentration equivalent to PP or a functional equivalent thereof from 1 pmol/kg to 5 nmol/kg, such as from 5 pmol/kg to 1 nmol/kg, or such as from 20 pmol/kg to 500 pmol/kg alternatively such as from 40 to 20 160 pmol/kg or such as from 75 to 120 pmol/kg. PP is preferably administered in dosages of, such as from 30-350 pmol/kg, or 50-400 pmol/kg, such as 50-280 pmol/kg, or 80-320 pmol/kg, such as 80- 200 pmol/kg, or 150- 250 pmol/kg, such as about 120 pmol/kg or 200 pmol/kg. Dosages in relation to FFM may be calculated by multiplying the indicated dosages with 1.25.

25 In a second embodiment the dosage may be 5-50 pmol/kg, 5-40 pmol/kg, 5 to 30 pmol/kg, 10-40 pmol/kg, 10-30 pmol/kg such as 5 to 25 pmol/kg, such as 5 to 20 pmol/kg, and most preferably 10-20 pmol/kg, 15-30 pmol/kg or approximately 15 pmol/kg or 20 pmol/kg.

30 The dosages are preferably administrated once a day, or such as two times a day, or such as three times a day, or such as four times a day, or such as five times a day, or such as more than five times a day.

In one preferred embodiment of the present invention, the compositions are administered in dosages of PP or a functional equivalent from about 400 ng to about 2 mg, more preferably from about 10  $\mu$ g to about 200  $\mu$ g, or from about 5  $\mu$ g to about 250  $\mu$ g, more preferably from about 20  $\mu$ g to about 200  $\mu$ g, more preferably from about 20  $\mu$ g to about 100  $\mu$ g. Most preferably the dosage may be 1-20  $\mu$ g, 2-16  $\mu$ g, 4-16  $\mu$ g, 4-12  $\mu$ g, 4-8  $\mu$ g, 6-12  $\mu$ g, 6-10  $\mu$ g or approximately 8  $\mu$ g.

In a preferred embodiment the composition is administered in dosages of PP or a functional equivalent from 100 pmol to 500 nmol, or such from 500 pmol to 100 nmol, or such as from 1 nmol to 50 nmol, or such as from 2 to 25 nmol, or such as from 4 to 20 nmol. Alternatively the preferred dosage may be 0,25-5 nmol, 0,5-4 nmol, 1-4 nmol, 1-3 nmol, 1-2 nmol, 1,5-3 nmol or more preferably 1,5-2,5 nmol or most preferably approximately 2 nmol. In a further preferred embodiment a PP dosages includes such as from 5-40 nmol, such as 8-32 nmol, such as 15- 25 nmol, such as about 20 nmol, or a dosage of PP includes such as from 3-35 nmol, such as 5-28 nmol, such as 8-200 nmol, such as about 12 nmol.

In another embodiment, the PP or functional equivalent is administered subcutaneously in a dosage of 5-30 pmol/kg, such as 5-25 pmol/kg, such as 5-20 pmol/kg or such as 10-20 pmol/kg bodyweight, in order to achieve an effective level in the individual treated. The presently preferred dosage is 10-20 pmol/kg bodyweight of PP. In second preferred embodiment the dosages of PP is 150-250 or 80-150 pmol/kg.

The compositions are preferably administered after sensing of symptoms, such as 5 minutes after sensing of symptoms, such as 10 minutes after sensing of symptoms, such as 15 minutes after sensing of symptoms, such as up to 20 minutes after sensing of symptoms, such as up to 30 minutes after sensing of symptoms, such as up to 45 minutes after sensing of symptoms, such as up to 60 minutes after sensing of symptoms, such as up to 75 minutes after sensing of symptoms, such as up to 90 minutes after sensing of symptoms, such as up to 120 minutes after sensing of symptoms, such as up to 150 minutes after sensing of symptoms, such as up to 180 minutes after sensing of symptoms.

The PP composition of the present invention may be administered admixed with a pharmaceutically acceptable carrier or diluent.

In a preferred embodiment the pharmaceutical composition may be a kit-in-part.

5

### Examples

The following examples illustrate the invention without limiting it thereto.

#### 10 Example 1

##### Binding assay and functional assay

*Transfections and tissue culture:* COS-7 cells can be grown Dulbecco's Modified Eagle's Medium 1885 supplemented with 10% fetal calf serum, 2mM glutamine and 0.01 mg/ml gentamicin. The expression plasmids containing the cDNAs encoding the wild type or the mutated receptors can be transiently expressed after transfection according to the calcium phosphate precipitation method and assay can be performed 48 hour after transfection.

*Binding assay:* One day after transfection the cells will be transferred and seeded in multi-well plates for assay. The number of cells to be plated per well will be chosen so as to obtain 5 to 10% binding of the radioligand added. Two days after transfection the cells will be assayed in competition binding assays using <sup>125</sup>I-PP as a tracer. Radioligand will be bound in a buffer composed of 0.5 ml of 50 mM Hepes buffer, pH 7.4, supplemented with 1 mM CaCl<sub>2</sub>, 5 mM MgCl<sub>2</sub>, and 0.1% BSA, and displaced in a dose dependent manner by unlabelled ligands. The assay will be performed in duplicate for 3 hours at 4 °C, and stopped by washing twice in the buffer. Cell associated, receptor bound radioligand will be determined by the addition of lysis buffer (48% urea, 2% NP-40 in 3M acetic acid). The concentration of radioligand in the assay corresponds to a final concentration of approximately 20 pM.

##### *Functional assay.*

COS-7 can be cultured as described above and cotransfections can be performed. The activation of Phospholipase C by chimeric G-proteins (Conklin B) formed between both Gαq and Gαi the Y4 receptor can be measured through the inositol

35

phosphate (IP) turnover in the cell. The IP turnover may be recorded by use of following assay:

One day after transfection COS-7 cells are incubated for 24 hours with 5 :Ci of [<sup>3</sup>H]-*myo*-inositol (Amersham, PT6-271) in 1 ml medium supplemented with 10% fetal calf serum, 2 mM glutamine and 0.01 mg/ml gentamicin per well. Cells are washed twice in buffer, 20 mM HEPES, pH 7.4, supplemented with 140 mM NaCl, 5 mM KCl, 1 mM MgSO<sub>4</sub>, 1 mM CaCl<sub>2</sub>, 10 mM glucose, 0.05 % (w/v) bovine serum; and are incubated in 0.5 ml buffer supplemented with 10 mM LiCl at 37°C for 30 min. The indicated curves are furthermore incubated with adenosine deaminase ADA (200U/mg, Boeringer Mannheim, Germany) for 30 min in a concentration of 1U/ml . After stimulation with various concentrations of peptide for 45 min at 37 °C, cells will be extracted with 10 % ice-cold perchloric acid followed by incubation on ice for 30 min. The resulting supernatants are neutralized with KOH in HEPES buffer, and the generated [<sup>3</sup>H]-inositol phosphate is purified on Bio-Rad AG 1-X8 anion-exchange resin. Determinations will be made in duplicates.

## Example 2

### Synthetic production of PP and functional equivalents thereof

The polypeptide of the present invention may be produced by a conventional peptide synthesis method.

Amino acid derivatives and synthesis reagents can be obtained from commercial sources. Peptide chain extension is performed by mainly using Applied Biosystem 433A synthesizer produced by Perkin Elmer, and a protected peptide derivative-resin is constructed by the Boc or Fmoc method. The protected peptide resin obtained by the Boc method is deprotected with anhydrous hydrogen fluoride (HF) in the presence of p-cresol thereby releasing the peptide, which is then purified. The protected peptide resin obtained by the Fmoc method is deprotected with trifluoroacetic acid (TFA) or dilute with TFA containing various scavengers, and the released peptide is purified. Purification is performed in reversed phase HPLC on a C4 or C18 column. The purity of the purified product is confirmed by reverse phase HPLC, and its structure is confirmed by amino acid composition analysis and mass spectrometry.

### Example 3

#### Preparation of rats for electromyography.

5 Male Sprague-Dawley rats (e.g. B&K, Sollentuna, Sweden) weighing 300-350 g will be anaesthetized with pentobarbital ( $50 \text{ mg kg}^{-1}$  intraperitoneally; Apoteksbolaget, Umeå, Sweden) and, through a midline incision, three bipolar stainless steel electrodes (SS-5T, Clark Electromedical Instruments, Reading, UK) will be implanted into the muscular wall of the small intestine 5 (D), 15 (J1), and 25 (J2) cm  
10 distal to the pylorus. All animals will be supplied with a jugular vein catheter for administration of drugs. The electrodes and catheters will be tunnelled subcutaneously to exit at the back of the animal's neck. After surgery the animals will be housed singly and allowed to recover for at least 7 days before experiments is undertaken. During recovery the rats will be trained to accept experimental  
15 conditions. Experiments will be carried out in conscious animals after an 8-h fasting period in wire-bottomed cages with free access to water. During the experiments, the rats will be placed in Bollman cages. The electrodes will be connected to an EEG preamplifier (7P5B) operating a Grass Polygraph 7B (Grass Instruments, Quincy, MA). The time constant will be set at 0.015 s and the low and high cut-off  
20 frequencies will be set at 10 and 35 Hz, respectively.

#### *Design of electromyography studies.*

All experiments will start with a control recording of basal myoelectric activity with four activity fronts propagated over all three recording sites during a period of 60  
25 min.

Infusions will start immediately after the fourth activity front had passed the first electrode site using a microinjection pump (e.g. CMA 100, Carnegie Medicine, Stockholm, Sweden). In a first series of experiments, the natural ligand PP at doses of 0.5 to 400 pmol  $\text{kg}^{-1}\text{min}^{-1}$  will be administered intravenously for 60 min. A second  
30 series of experiments will be performed with administration of a NO synthase inhibitor  $\text{N}^{\omega}$ -nitro-L-arginine (L-NNA) at  $1 \text{ mg kg}^{-1}$  given as a bolus injection 45 min before infusion of PP at  $100 \text{ pmol kg}^{-1}\text{min}^{-1}$ . In a third series of experiments 3 mg

kg<sup>-1</sup> guanethidine will be administered on day one and the following day the effect of PP at 100 pmol kg<sup>-1</sup>min<sup>-1</sup> will be studied.

In a fourth series of experiments the effect of an infusion of PP at 100 pmol kg<sup>-1</sup>min<sup>-1</sup> will be studied in vagotomized and sham-vagotomized animals.

5

*Drugs:*

ratPP may be purchased from e.g. Neosystem (Strasbourg, France), L-NNA from e.g. Sigma-Aldrich, (St. Louis, Mo, USA) and guanethidine from e.g. Apoteksbolaget (Stockholm, Sweden).

10

*Data analysis.*

The main characteristic feature of myoelectric activity of the small intestine in the fasted state, the activity front, or phase III of MMC, is identified as a period of clearly distinguishable intense spiking activity with an amplitude at least twice that of the preceding baseline, propagating aborally through the whole recording segment and followed by a period of quiescence, phase I of MMC. The percent of the recording periods occupied by phase III activity fronts will be calculated for stimulatory periods (60 min). The effects of the peptides are expressed as per cent of time occupied by activity fronts. Dose-response curves will be generated by Graph-Pad Prism 4 (GraphPad, San Diego, Ca, USA). Data will be expressed as mean  $\pm$  SEM. Differences between individual data groups were determined using ANOVA, followed by Dunnet's test or students T-test, as appropriate, with statistical significance at  $p < 0.05$ .

15

20

25

Example 4

Effects of PP on pain sensation and the spontaneous motor activity in the rectum.

The study will be carried out as a dose escalating study. Each dose/group will be evaluated separately. The evaluation will be based on the primary and secondary endpoints, and tolerability.

30

The starting dose will be 10 pmol/kg, increasing to 30 pmol/kg, and 100 pmol/kg, and 300 pmol/kg, and 1 nmol/kg, and 3 nmol/kg, and 10 nmol/kg, and 100 nmol/kg, and 300 nmol/kg etc, until the dose is longer tolerated.

35

10 patients/dose group (men or women between 18 and 60 years of age) meeting the Rome II criteria will be included in the study.

*Conduct of the study*

- 5 All patients will come to the clinic after a 12-hour fasting period. Before the study patients will be given a tap water enema.

- 10 A probe (Barostat bag) will be inserted in the rectum and connected to an electronic barostat that is programmed to automatically perform distensions with fixed time lags and bag pressure increments.

The study will be performed as 2-way cross-over study with approx. 7 days between treatments.

- 15 A) S.c. administration of PP in 0.9% NaCl solution.  
B) S.c. administration of saline (0.9% NaCl solution).

- 20 After administration of PP or saline the distension according to a fixed protocol will begin. The patients will note the discomfort and pain using a visual analog scale (VAS). Patients will be given control over the protocol by their ability to deflate the bag instantaneously at any time of significant discomfort or pain.  
The threshold pressure and volume will be written down.

- 25 After a fixed period of time e.g. 20 or 30 minutes a saline meal with fixed energy content will be given p.o.

Hereafter a second distension similar to the first will begin.

- 30 In between to two distensions a fixed low pressure will be kept in the barostat bag. This will allow recording of the volume in the bag as a measure of the motor activity in the rectum.

Primary endpoint

Comparison of the pain threshold with or without food intake in patients with IBS and the effect of PP compared to placebo.

5

Second endpoint

Study of the physiological effects of PP on the spontaneous motor activity in the rectum compared to placebo.

10 The effect on symptoms will be evaluated by statistically comparing the result from group B with group A.

Statistical analysis will be performed by a non-parametric analysis of variance (Kruskal-Wallis test).

15

Example 5

Clinical protocol Gastrointestinal disorders

20 30 subjects that meet the proposed diagnostic criteria of IBS or functional dyspepsias are included in the study.

The study is performed in a double-blinded, placebo-controlled fashion. Subjects are divided into two groups (n=15 in each), groups A and B. The subjects are given diaries where they note their symptoms (bowel habits/pain/nausea etc) and eating information. This initial phase of the study is 4 weeks ("Run-in Phase"), after which the subjects start treatment with one of three regimens, as defined below. The subjects keep diaries where they note the timing, type and severity of experienced symptoms throughout the treatment phase. The treatment duration is 4 weeks ("Treatment Phase").

25

30

Dosing: The subjects of group A receive subcutaneous placebo injections (NaCl) three times daily (distributed evenly over the hours awake). The subjects of group B receive a dose of PP given s.c. according to body weight. The dose will be administered three times daily. The final dose to be given may be defined from the

35

study outlined in Example 4. The subjects' diaries are reviewed by the investigators and the severity of symptoms is determined in relation to food intake and timing.

- 5 The effect on symptoms is evaluated by comparing the results during the 4-week Treatment Phase with the results during the Run-in Phase for each subject, and then statistically comparing the result from group B with group A.

**Claims**

1. A composition comprising PP or a functional equivalent thereof or a pharmaceutical acceptable salt thereof, for the production of a medicament for the treatment or prophylaxis of functional gastrointestinal disorders and/or symptoms of such disorders.  
5
2. The composition according to claim 1, wherein the functional gastrointestinal disorder is irritable bowel syndrome (IBS) wherein,
  - a) the predominant bowel habit is constipation and/or
  - 10 b) the predominant bowel habit is alternating diarrhoea and constipation and/or
  - c) the treatment is administered parenterally.
3. The composition according to claim 1 for the treatment of abdominal pain.  
15
4. The composition according to claim 3 for the treatment of visceral pain.
5. The composition according to claim 3-4, wherein the functional gastrointestinal disorder is irritable bowel syndrome or functional dyspepsia.  
20
6. The composition according to claim 1, wherein the functional gastrointestinal disorder is functional dyspepsia.
7. The composition according to claim 6 for the relief of the symptom(s)
  - 25 (a) sensation of fullness and/or
  - (b) inability to finish a normal sized meal and/or
  - (c) pain after food intake and/or
  - (d) nausea and/or
  - (e) vomiting and/or
  - 30 (f) bloating and/or
  - (g) belching and/or
  - (h) regurgitation and/or
  - (i) epigastric pain and/or
  - (j) feeling of distension and/or
  - 35 (k) excess flatus

and any combination of the above.

- 5
8. The composition according to claim to any of the preceding claims, wherein the symptoms are predominantly upper GI symptoms.
9. The composition according to claim to any of the preceding claims, wherein the symptoms are predominantly lower GI symptoms.
- 10
10. The composition according to claim to any of the preceding claims, comprising human PP.
11. The composition according to any of the preceding claims, wherein the pH of the composition is between 2.0 and 9.0.
- 15
12. The composition according to any of the preceding claims, wherein the pharmaceutical composition is for parenteral administration.
13. The composition according to any of the preceding claims, wherein the pharmaceutical composition is for subcutaneous administration.
- 20
14. The composition according to any of the preceding claims, wherein the pharmaceutical composition is for intranasal administration.
15. The composition according to any of the preceding claims, wherein the composition comprises a second active ingredient.
- 25
16. The composition according to claim 15, wherein the second pharmaceutical composition is selected from the group of; anti-depressants, such as SSRIs (citalopram, escitalopram, fluoxetine, fluvoxamine, paroxetine and sertraline), SNRIs such as venlafaxine, NSRIs, tetracyclic antidepressants, non-selective monoamine reuptake inhibitors including tricyclic antidepressants (TCAs), selective reversible monoamine reuptake inhibitors and mirtazapin.
- 30
17. The composition according to claim 15, wherein the second pharmaceutical composition is an anti-emetic drug.
- 35

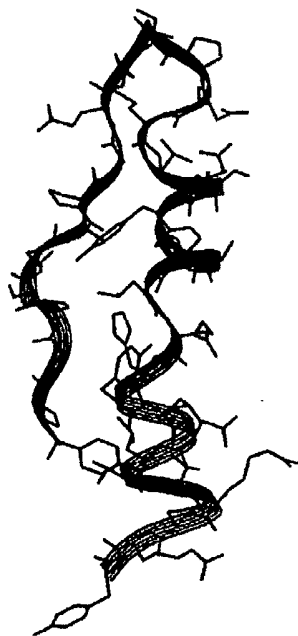
18. A controlled release pharmaceutical composition according to any of the preceding claims, wherein the effective plasma  $T_{1/2}$  is between 8 and 720 minutes, or such as between 8 and 600 minutes, or such as between 8 and 480 minutes, or such as between 8 and 360 minutes, or such as between 8 and 240 minutes, or such as between 8 and 180 minutes, or such as between 10 and 150 minutes, or such as between 20 and 120 minutes, or such as between 30 and 90 minutes, or such as between 45 and 75 minutes.
19. A controlled release pharmaceutical composition according to claims 1-10, wherein the effective plasma  $T_{1/2}$  is between 1.2 and 120 times that of the natural half life of PP in plasma, or such as between 1.2 and 100 times that of the natural half life of PP in plasma, or such as between 1.2 and 80 times that of the natural half life of PP in plasma, or such as between 1.2 and 60 times that of the natural half life of PP in plasma, or such as between 1.2 and 40 times that of the natural half life of PP in plasma, or such as between 1.2 and 30 times that of the natural half life of PP in plasma, or such as between 1.5 and 25 times that of the natural half life of PP in plasma, or such as between 2 and 20 times that of the natural half life of PP in plasma, or such as between 3 and 15 times that of the natural half life of PP in plasma, or such as between 5 and 10 times that of the natural half life of PP in plasma.
20. The composition according to any of the preceding claims for the treatment of irritable bowel syndrome or functional dyspepsia.
21. A method of treatment comprising administration of the pharmaceutical composition according to any of the preceding claims.
22. The method of treatment according to claim 21 wherein the pharmaceutical composition is administered parenterally.
23. The method of treatment according to any of claims 21-22 wherein the pharmaceutical composition is administered subcutaneously or intranasally.

24. The method of treatment according to any of the claims 21 to 23, comprising administration of the pharmaceutical composition according to claims 1 to 20 in combination with a second pharmaceutical composition.

- 5      25. The method of treatment according to claim 24 wherein the second pharmaceutical composition is selected from the group of; loperamide, hyoscyamine sulphate, lactulose, YM-443, dexloxiglumide, bisacodyl, AZD 7371, talnetant (SB 223412), asimadoline, NT3, SLV305, AZD 3355, AZD 9343, pinaverine, Na picosulphate, calcium polycarpophil, trimebutine, dopamine D2  
10      antagonists, such as itopride, motilin agonists, such as KC 11458 and GM-611, NK2 antagonists, such as saredutant, NK3/NK2 receptor antagonists, such as SSR 241586, 5-hydroxytryptamine<sub>4</sub> (5-HT<sub>4</sub>) receptor partial agonists, such as tegaserod, 5-HT<sub>4</sub> receptor agonists, such as mosapride and prucalopride, antagonists of the serotonin 5-HT<sub>3</sub> receptor type, such as alosetron or  
15      cilansetron, dual 5-HT<sub>4</sub> agonist and 5-HT<sub>3</sub> –antagonists, such as renzapride and E-3620, GLP-1 or analogues/homologues thereof, such as exenatide, exenatide LAR, liraglutide, CJC-1131, ZP 10/AVE-0010 and GTP-010, DPP-IV inhibitors, such as LAF237, PYY(1-36) or analogues/homologues thereof, PYY(3-36) or analogues/homologues thereof, NPY(1-36) or analogues/homologues thereof,  
20      NPY(3-36) or analogues/homologues thereof and anti-depressants, such as SSRIs (citalopram, escitalopram, fluoxetine, fluvoxamine, paroxetine and sertraline), SNRIs such as venlafaxine, NSRIs, tetracyclic antidepressants, non-selective monoamine reuptake inhibitors including tricyclic antidepressants (TCAs), selective reversible monoamine reuptake inhibitors, mirtazapin and  
25      duloxetine.

1/1

**Figure 1**



## SEQUENCE LISTING

&lt;110&gt; AdiTech Pharma AB

&lt;120&gt; A composition comprising PP for the treatment of gastrointestinal disorders

&lt;130&gt; P1010PC00

&lt;160&gt; 1

&lt;170&gt; PatentIn version 3.1

&lt;210&gt; 1

&lt;211&gt; 36

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 1

Ala Pro Leu Glu Pro Val Tyr Pro Gly Asp Asn Ala Thr Pro Glu Gln

1

5

10

15

Met Ala Gln Tyr Ala Ala Asp Leu Arg Arg Tyr Ile Asn Met Leu Thr

20

25

30

Arg Pro Arg Tyr

35