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Prolyl carboxypeptidase and its inhibitors in metabolism

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Abstract

Proopiomelanocortin (POMC)-expressing neurons in the hypothalamus integrate a variety of central and peripheral metabolic inputs, and regulate energy homeostasis by controlling energy expenditure and food intake. To accomplish this, a precise balance of production and degradation of α -melanocyte-stimulating hormone (α -MSH), an anorexigenic neuropeptide and product of the *POMC* gene, in the hypothalamus, is crucial. Prolyl carboxypeptidase (PRCP) is a key enzyme that degrades α -MSH to an inactive form unable to inhibit food intake. Because it represents a new therapeutic target for the treatment of metabolic disorders, such as obesity and diabetes, efforts have been made to generate potent, brain-penetrant PRCP inhibitors. Here, we discuss the role of PRCP on energy metabolism and the development of PRCP inhibitors.

Keywords

hypothalamus; proopiomelanocortin; prolyl carboxypeptidase; enzymatic inhibitors; food intake

Hypothalamic circuitry regulating food intake

Hypothalamic neurons detect a variety of peripheral and central metabolic signals, and generate an adequate degree of molecular and behavioral actions to control energy balance through complex and interconnected signal cascades [1-4]. The hypothalamic POMC-expressing neurons have been shown to play a critical role in metabolic regulation [5-8]. The *POMC* gene is expressed in the central nervous system (CNS) as well as in the pituitary gland, the immune system, and the skin [9]. In the CNS, POMC is expressed in the hypothalamic arcuate nucleus (ARC; see Glossary) and in the nucleus tractus solitarius (NTS) of the brainstem [10]. The *POMC* gene encodes several peptides that are the products of a complex post-translational process including adrenocorticotrophin (ACTH), β -endorphin, α -, β -, and γ -melanocyte-stimulating hormones (MSH). To date, five melanocortin receptors (MCR) have been identified: MC1–5R. Of those, MC3R and MC4R have been shown to play an important role in the regulation of body weight [11-14]. In fact, deletion of either of these genes produces an obese phenotype in mice. Furthermore,

mutations in the *MC4R* gene in humans have been identified and were found to be the most common monogenetic form of human obesity described [15].

Although the ablation of these two receptors produces an obese phenotype in mice, several differences have been noted. First, MC4R-ablated mice show a hyperphagic phenotype whereas the MC3R mice are not hyperphagic [16,17]. In addition, the two receptors have a different expression pattern in the brain. Specifically in the hypothalamus, MC4R is expressed in several nuclei including the paraventricular nucleus (PVN), the dorsomedial hypothalamus (DMH), and the lateral hypothalamus (LH) [18,19]. MC4R is not expressed in the arcuate nucleus where MC3R is expressed in the POMC-containing neurons, suggesting that MC3R may play a key role in the feedback mechanism of these neurons [20,21].

ARC POMC neurons express several receptors to sense metabolic signals and, by producing and releasing α -MSH, they activate their downstream target neurons via MC4R and/or MC3R in several brain areas [22-24]. Thus, the precise production and degradation of α -MSH are critical steps in controlling the overall metabolic balance. Although the production and release of α -MSH has been largely studied [25,26], its degradation process was unknown for a long time. It was recently reported that PRCP, also known as lysosomal Pro-X carboxypeptidase, is a key enzyme responsible for the degradation and inactivation of α -MSH in the brain [27]. In support of its role in α -MSH degradation, *PRCP* gene-trap hypomorph (*PRCP^{gt/gt}*) mice, made using a gene-trap targeting vector containing the *lacZ* gene, show both decreased food intake [27] and increased energy expenditure [28], and are resistance to diet-induced obesity [29].

PRCP

PRCP is a highly conserved enzyme in eukaryotes with high amino acid homology between mouse, rat, and human [30]. PRCP is a serine protease with a unique protease structure that includes a novel helical structural domain, which caps the catalytic active site [31,32]. The enzyme cleaves the last amino acid at the C-terminal part of the target proteins if the substrate contains a proline as a penultimate amino acid [33]. Furthermore, substrate specificity studies have shown that PRCP has preferences for the amino acid sequences Xxx-Pro-Phe-OH and Xxx-Pro-Val-OH, where Xxx can be any amino acid [33].

PRCP was first identified over 40 years ago in swine kidney extract [34], and later cloned from a human kidney library. It is expressed in many other tissues including lung, liver, heart, and adipose as well as brain, suggesting possible multiple tissue- and/or cellular specific roles, with a variety of substrates. Within tissues, PRCP has been found in lysosomes and extracellularly either as a membrane-bound or as a soluble form [33,35].

PRCP has been shown to cleave angiotensin II and III, to produce angiotensins 1-7 and angiotensins 2-7, respectively [36,37]. Furthermore, the enzyme cleaves plasma prekallikrein to produce the active molecule kallikrein, and activates the vasodilator bradykinin [34]. Thus, PRCP is an important regulator of cardiovascular functions such as blood pressure and electrolyte balance. In support, recent data from the Schmaier group [38] showed that *PRCP^{gt/gt}* null mice are hypertensive, a finding consistent with human studies showing that a polymorphism E112D in PRCP is associated with hypertension and preeclampsia [39]. Interestingly, no difference in the levels of angiotensin and bradykinin were observed between *PRCP^{gt/gt}* null mice and their wild type (WT) controls [38,39], suggesting that the hypertension is not due to the alteration of these peptides. Along these lines, the use of melanocortin agonists as agents for controlling food intake has not been successful in human weight-loss trials because they resulted in increases in cardiovascular tone [40]. Indeed, MC4R-deficient humans exhibit markedly lower blood pressure, and

MC4R knockout (KO) mice do not develop hypertension [41]. Therefore, it is reasonable to hypothesize that the effect of PRCP depletion, which might result in more bioavailable α -MSH and increased activation of melanocortin receptors signaling, on blood pressure may be due to a central mechanism: in other words via the increased melanocortin-dependent sympathetic outflow. In addition, PRCP has also been suggested as a risk factor for inflammation [37,42] because upregulation of PRCP resulted in the development of inflammation in the endothelium. In support, α -MSH has long been known as a putative anti-inflammatory mediator through MCRs in the CNS, immune cells, and resident cells of peripheral tissues [43]. Thus, this suggests that PRCP, by modulating α -MSH, could also be a target for therapeutic treatment for inflammatory diseases, including obesity and leptin resistance.

In addition to its cardiovascular and proinflammatory functions [44], PRCP, as reported by a genome-wide association study (GWAS), is a risk factor for the metabolic syndrome because it was significantly associated with coronary heart disease in men [45]. This report supports recent data produced in rodents showing that central PRCP is an important regulator of energy metabolism because it is responsible for the degradation and thus inactivation of hypothalamic α -MSH [27]. In peripheral tissues such as in the kidney, PRCP has been shown to have an acidic pH optimum (5.0). However, PRCP retains significant enzymatic activity also at neutral pH [27]. Subsequently, it was shown that, at neutral pH, α -MSH is a better PRCP substrate than angiotensin II [27].

α -MSH is a 13 amino acid neuropeptide in which a proline in position 12 precedes a valine in position 13 [28]. By removing this valine residue, PRCP produces α -MSH₁₋₁₂ from α -MSH₁₋₁₃. The removal of the last amino acid is of physiological importance. This is demonstrated by the inability of α -MSH₁₋₁₂ to decrease food intake when administered centrally as well as by its inability to increase the action-potential frequency of hypothalamic MC4R-expressing neurons, compared to α -MSH₁₋₁₃ peptide [27]. Further support for the role of PRCP in the degradation of α -MSH₁₋₁₃ is provided by studies using the *PRCP^{gt/gt}* null mice. These mice have been shown to be sensitive to peripheral administration of α -MSH and to have higher α -MSH₁₋₁₃ levels in the hypothalamus compared to WT controls [27].

Although α -MSH is so far the only substrate involved in the regulation of food intake that has been identified as a PRCP substrate, we cannot exclude the possibility that more peptides with a proline as the penultimate amino acid and a function associated with body-weight regulation could be degraded by PRCP. However, data from human and rodent studies suggest that PRCP inhibition might provide a mechanism to increase central bioavailability of α -MSH, and thus reduce food intake.

Hypothalamic PRCP and metabolic regulation

Within the CNS, PRCP is distributed in several areas including the hippocampus, cerebral cortex, and hypothalamus [16]. Specifically, in the hypothalamus the highest levels of PRCP expression are detected in the DMH, ventromedial hypothalamus (VMH), and lateral hypothalamus (LH), whereas a lower expression level has been observed in the ARC and PVN. Within the LH, PRCP is produced in melanin-concentrating neurons (MCH) and hypocretin/orexin (Hcrt) neurons [16]. Given that numerous MC4R-expressing neurons are localized in the PVN, and both MCH and Hcrt neurons project into the PVN, where α -MSH is released from the terminals of POMC neurons (Figure 1), it is conceivable that PRCP may cleave α -MSH extracellularly at the synaptic level, thus increasing the antagonistic tone of the orexigenic neuropeptide agouti-related protein (AgRP), an endogenous inverse agonist of MC4R. However, in the DMH, where MC4R-expressing neurons are also present [18,19],

PRCP may cleave α -MSH after ligand-mediated receptor endocytosis (Figure 1) [46]. In the ARC, PRCP is not expressed in α -MSH-producing neurons [16]. However, ARC PRCP could be produced and released by the POMC neighboring NPY/AgRP neurons, and thus PRCP may act at the synaptic terminals in the PVN, as well as in the ARC, where it may interfere with the negative feedback loop via the action of α -MSH on MC3R [47,48]. Thus, if PRCP is responsible for α -MSH degradation, then depletion of PRCP will allow the accumulation of α -MSH and induce a prolonged effect of α -MSH in these regions of the hypothalamus. This in turn will affect downstream targets, such as the autonomic nervous system, and its functions. In support, studies on the *PRCP^{gt/gt}* mice indicate that these mice have an increased sympathetic outflow compared to WT controls [27-29]. For example, *PRCP^{gt/gt}* mice have increased energy expenditure, and possibly increased body temperature, as shown by higher levels of uncoupling protein 1 (UCP1) mRNA levels in the brown adipose tissue (BAT) [28]. The increased energy expenditure may be due in part to the increased thyroid hormone levels found in these animals. Furthermore, upregulation of thyrotropin-releasing hormone (TRH) gene expression in the PVN was also observed in *PRCP^{gt/gt}* mice, indicating that an impairment of the thyroid hormone feedback is possibly driven by the elevated α -MSH levels. In addition to increased energy expenditure and UCP1 mRNA levels in BAT, *PRCP^{gt/gt}* mice are protected against diet-induced obesity and from abnormalities in several metabolic parameters that are dysregulated in obesity. Supporting this notion, glucose tolerance, insulin sensitivity, and liver metabolism were significantly improved in these mice. Collectively, anatomical and functional data demonstrate that PRCP, by regulating α -MSH concentration in the hypothalamus, plays an important role in the modulation of whole-body energy homeostasis, and thus it might represent a potential therapeutic target for the treatment of obesity [27-29,49].

Several small PRCP inhibitors have been generated and tested *in vivo* and *in vitro* studies. Below we will discuss these in more detail.

Development of PRCP inhibitors

Because of its role in the regulation of energy metabolism, PRCP represents a potential new therapeutic target for the treatment of metabolic disorders such as obesity and type 2 diabetes. Since its discovery in 2009 as the enzyme responsible for the degradation of α -MSH, studies have been focused on the production of PRCP inhibitors as potential new drugs for obesity treatment. Furthermore, great attention has been paid to generating brain-penetrant PRCP inhibitors to target PRCP activity centrally.

Design and generation of small molecule inhibitors

Small-molecule PRCP inhibitors have been produced using chemical engineering and/or high-throughput screening mainly through modification of the founding lead chemical structures, such as a prolanyl compound [50,51]. These molecules have been tested for their efficacy in the inhibition of PRCP activity, their metabolic stability, oral bioavailability, plasma protein binding and clearance, tissue- and target-specific inhibition, and also for the minimalization of side effects [50,51].

Starting from a mechanism-based design for inhibiting serine proteases, a new class of reversible, potent and selective PRCP inhibitors was generated in which compound 80 was shown to be the most effective against PRCP (Table 1) [50]. Because the prolanyl compounds conjugated with heterocycles are generally a good source of serine protease inhibitors [52], several compounds have been generated by coupling of the benzimidazole pyrrolidinyl amides with carboxylic acids with and/or without brief transformational modification [50]. Among them, it was found that the inhibitory efficacy of compound 80 was highly specific to PRCP compared to other serine proteases families, showing a lower

plasma protein binding with its β -methyl group [50]. Furthermore, the effect of compound 8o on PRCP inhibition was tested both *in vitro* and *in vivo*. Specifically, when orally administered for 5 days to diet-induced obese mice at a dose of 100 mg/kg body weight, it was found that compound 8o induced a near-5% decrease in body weight. By contrast, the same treatment of PRCP knockout (PRCPKO) mice induced only a 1% reduction [50]. Food-intake analysis showed that the reduction in body weight was due to a significant decrease in food consumption in the WT mice compared to PRCPKO mice, and that the decreased body weight was mainly due to lower fat mass [50].

Other inhibitors were successively designed and synthesized in an attempt to simplify the structure, increase the polarity, and potentially decrease the serum shift. This was done by removing the α -aminoisobutyl amide and β -methyl groups from the founding structure, and by introducing a piperidinyl group. A compound named 9b was generated this way and its efficacy as a PRCP inhibitor was evaluated (Table 1) [51]. *In vitro* studies showed that compound 9b had an excellent PRCP inhibitory activity in mouse plasma. However, mouse pharmacokinetic profiles showed that compound 9b had high clearance, low oral bioavailability, and low oral exposure, indicating that compound 9b, similarly to compound 8o, is a substrate for P-glycoprotein *in vivo*, a promiscuous transport protein that limits drug pharmacokinetics, and thus its action on PRCP is mainly peripheral and not central [51]. Furthermore, when tested to evaluate whether peripheral PRCP inhibition by compound 9b could lead to a reduction in body weight, it was found that although food intake and body weight were reduced in treated mice, similar reductions were also observed in PRCPKO mice, indicating that the effects of this inhibitor on the development of obesity were not directly associated with PRCP function.

Later series of compounds

In a more recent effort to develop structurally diverse and potent PRCP inhibitors, a new class was discovered by high-throughput screening and subsequent structure–activity relationship (SAR) analysis [53]. This class possesses a tractable SAR for the optimization of biological and physical properties. *In vitro* results using plasma-shift assay, a measure of stability in plasma, indicated that some of these compounds have high levels of unbound compound in the plasma, and *ex vivo* target engagement assay suggested they are potent PRCP inhibitors [53,54]. However, *in vivo* studies suggested that this class of compound target mainly peripheral tissues because they exhibit low brain penetration.

More recently, several small compounds were generated as proline-based analogs using simple Z-Pro-Pro-NH-amide derivatives, where Z denotes ‘particular amino acids’, as the lead structure. Among them, a compound named UM8190 was generated through several chemical modifications, which included substituting the ethoxy-3-propyl repeating unit for the dodecane group, and deleting the proline A-ring carbamate from the *N*-carboxybenzyl group (Table 1) [55]. The efficacy of UM8190 as a PRCP inhibitor was tested using *in vitro* and *in vivo* studies. The inhibitory action of UM8190 was found to be highly specific for PRCP when compared to other serine proteases such as carboxypeptidases A, N, and M (CPA, CPN, and CPM, respectively) [55]. In addition, various metabolic parameters in kidney and liver, as well as its effect on food intake, were assessed after UM8190 administration. There were also no significant alterations in kidney and liver functions following UM8190 treatment [55]. However, intraperitoneal administration of UM8190 at 10 or 100 mg/kg successfully resulted in reduction of food intake in fasted animals, demonstrating the potential usage of UM8190 as an effective PRCP inhibitor, and more importantly for the treatment of metabolic disorders such as obesity [55]. Although these studies are encouraging, its oral bioavailability, plasma clearance, and cell permeability need to be further assessed. Moreover, the effect of UM8190 on the food intake of PRCP null

mice should also be tested to assess whether the observed effect on food regulation is directly associated with PRCP function.

Lessons learned

Aiming to overcome the limitations of previously developed small-molecule PRCP inhibitors, new efforts have been made to upgrade PRCP inhibitors both structurally and functionally. In this regard a major goal has been to enhance the inhibition efficacy of PRCP activity with low plasma-protein binding. In addition, tissue permeability, specifically the penetration of PRCP inhibitors into the CNS, has also been considered. As a result, several chemical compounds have been synthesized as novel candidates for brain-permeable PRCP inhibitors [56-59]. First, compounds were produced by replacing the dichlorobenzimidazole-substituted pyrrolidine with a series of substituted benzylamines. Among them, compound (S)-6e (Table 1) was found to be a good PRCP inhibitor in whole serum, and exhibited cell permeability with low affinity for P-glycoprotein, and thus was considered as a potent brain-penetrant inhibitor of PRCP [56]. However, when tested *in vivo*, because of its poor mouse pharmacokinetic profile, continuous subcutaneous dosing via infusion pump was required. When tested at low dose, 7 day infusion in diet-induced obesity (DIO) mice showed no statistically significant weight loss compared to vehicle treated mice. Similarly, food intake was no different from vehicle controls [56]. At a higher dose, the weight loss achieved by PRCPKO mice and their WT controls was similar, thus indicating that a non-mechanism-based weight loss was occurring in this study. Finally, significant skin-related adverse effects of compound (S)-6e were found, presumably due to the poor solubility of the compound [56].

Thus, in an attempt to improve oral pharmacokinetics and to enhance *in vivo* efficacy, benzodihydroisofurans were used as a core structure to synthesize potent PRCP inhibitors: among them, compound *ent*-3a (Table 1) was found to be brain-penetrant, and had the best pharmacokinetic profiles [57]. Compound *ent*-3a showed a good half-life, oral bioavailability, and low plasma clearance, with high efficiency for PRCP inhibition. Unfortunately, *ent*-3a failed to reduce body weight *in vivo*, even with almost complete PRCP inhibition in plasma and fairly high level of target engagement in CNS [57]. Next, by replacing the amide group from the basic founding structures with a heterocycle such as a pyridine ring, and sequentially modifying small chemical groups around them, several small molecules were produced including compound 32a (Table 1) [57]. *In vitro* studies have shown that compound 32a has a very good human PRCP-inhibitory activity and outstanding brain permeability [58]. However, no *in vivo* studies have been performed to assess its effect on body weight loss.

Similarly, in a most recent report, the proline residue, the centrally located amino acid at the founding structure of the compound, was replaced with other amino acids to obtain diverse classes of PRCP inhibitors. Inhibitory activity was achieved by an alanine-derived compound [59]. With several chemical modifications, such as coupling of α -isobutyrate and β -methyl groups from the alanine-derived compounds, compound 19 was obtained (Table 1) [59]. Compound 19 showed an upgraded potency as a PRCP inhibitor both in human and mice compared to the proline derivatives by *in vitro* tests, together with acceptable pharmacokinetic profiles. However, no further studies on its *in vivo* effects on body-weight regulation have been performed [59].

In summary, PRCP has been shown to play an important role in the regulation of energy metabolism, and thus it represents a potential new therapeutic target for the treatment of metabolic disorders such as obesity and type 2 diabetes [27-29,50,55]. As a consequence, many small-molecule PRCP inhibitors have been designed and tested both in *in vitro* and *in vivo* studies. As summarized in Table 1, compound 8o has been tested both in *in vitro* and *in*

vivo, with potentially promising effects, because this compound was shown to induce a greater decrease in body weight in WT compared to PRCP-null mice. However, its penetration to the CNS is modest, as shown by the study using *Pgp* KO mice. By contrast, when a compound such as *ent*-3a, with a fairly high level of target engagement in CNS, was tested it failed to reduce body weight *in vivo*. Thus, several observations can be made about these small-molecule PRCP inhibitors. First, their efficacy depends on many factors including the range of drug concentrations, pH, and the partition coefficient over which a drug is therapeutically beneficial. All of these factors and many others differ under *in vivo* compared to *in vitro* conditions. Second, PRCP is a member of the family of serine proteases, sharing certain chemical and functional properties with other members of this family. Thus, the specificity of these compounds as PRCP inhibitors may be limited as a result of crosstalk with other serine proteases *in vivo*. Finally, because PRCP may have several substrates in the CNS as well as in the periphery, and PRCP null mice show improved metabolic parameters that may be controlled by central and peripheral mechanisms, it is possible that the peripheral actions of PRCP play an important role in metabolic regulation. Thus, future studies using tissue- and cell-specific deletion of PRCP may shed light on the role of different organs and cell types in the control of energy metabolism by PRCP.

Concluding remarks

Hypothalamic melanocortin signaling has been shown to play a critical role in controlling molecular and behavioral aspect of metabolic homeostasis. Recently, it was shown that a serine protease, PRCP, plays a pivotal role in energy homeostasis by regulating central melanocortin signaling. Functional deletion of PRCP leads to an increased amount of α -MSH in the hypothalamus, with an overall improvement in energy metabolism. Thus, PRCP represents a potential new therapeutic target in the treatment of metabolic disorders such as obesity and type 2 diabetes.

In the past three years several structurally distinct series of compounds aiming to inhibit PRCP activity have been generated and tested to assess their inhibitory efficacy both *in vivo* and *in vitro*. Some of these compounds have been shown to act selectively in peripheral tissues, but with small effects on energy metabolism and potential negative side-effects. Efforts have been made to generate brain-penetrant compounds to target central PRCP activity. Although several such molecules have been generated, their *in vitro* inhibitory efficacy has been modest and limited *in vivo* studies have been reported.

Thus, additional optimization of this class, and/or the generation of a new class of brain-penetrant PRCP inhibitors, are needed before PRCP genetics and biochemistry can be translated into treatments for metabolic disorders.

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Glossary

Arcuate nucleus (ARC)	an aggregation of neurons located in the mediobasal hypothalamus, next to the third ventricle and the median eminence. The arcuate nucleus includes several important populations of neurons highly associated with metabolic homeostasis, such as proopiomelanocortin (POMC)-expressing neurons and neuropeptide Y (NPY)-expressing neurons
Bioavailability	the degree to which, or the rate at which, a medication or other substance is absorbed or becomes available at the targeted place in the body
Melanocortin receptors (MCRs)	there are five G protein-coupled MCRs with different affinities for the melanocyte-stimulating hormones. MC1R is expressed in skin cells and immune cells, and is important for the pigmentation of skin and hair as well as having anti-inflammatory activity. MC2R is the ACTH receptor for adrenal steroid secretion. MC3R and MC4R are expressed in the CNS and are important for metabolic regulation. MC5R is expressed broadly both in peripheral tissues and centrally, and has been suggested to play a role in exocrine secretion
Melanocyte-stimulating hormones (MSH)	a group of peptide hormones produced by post-translational modification of POMC in the pituitary and the hypothalamus. These include adrenocorticotrophic hormone (ACTH) and α -, β -, and γ -MSH. Of these, α -MSH is a well-known molecule controlling pigmentation, metabolism, cardiovascular function,

	and inflammation via a variety of melanocortin receptors in target tissues
P-glycoprotein	a promiscuous transport protein that limits drug uptake in the gut and brain, and regulates the distribution and bioavailability of drugs, including renal and hepatic drug clearance
Pharmacokinetics	the action of drugs in the body over a period of time, including the processes of absorption, distribution, localization in tissues, biotransformation, and excretion
Serum-shift assay	a method to evaluate the activity of drugs in the presence of serum protein <i>in vitro</i>
Structure-activity relationship (SAR)	the relationship between a chemical structure and its biological activity. SAR analysis permits the identification of the specific chemical groups that are responsible for a particular biological effect

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Figure 1.

Schematic illustration of hypothalamic circuits involved in metabolic regulation. Prolyl carboxypeptidase (PRCP) is anatomically positioned to determine α -melanocyte-stimulating hormone (α -MSH) signaling. PRCP is produced in lateral hypothalamic (LH) neurons, including the hypocretin (Hcrt)- and melanin-concentrating hormone (MCH)-expressing neurons, and are released in the paraventricular nucleus of the hypothalamus (PVN) to inactivate α -MSH at the synaptic levels, thus influencing body energy metabolism. A similar situation may occur in the arcuate nucleus (ARC) where PRCP may be expressed in neuropeptide Y/Agouti-related peptide (NPY/AgRP)-containing neurons affecting synaptic α -MSH levels in the PVN as well as in the ARC. In the dorsomedial nucleus of the hypothalamus (DMH) PRCP may cleave α -MSH intracellularly after ligand-mediated receptor endocytosis. Abbreviation: 3 V, third ventricle.

Table 1

Chemical structure and properties of representative PRCP inhibitors

Name/Structure	PRCP-Specific inhibition	Oral Bioavail ability	Clearance	Tissue specificity	Effect on Food intake	Organism tested		Ref
						<i>in vitro</i>	<i>in vivo</i>
Compound 80	Yes	Low	Low	Peripheral	Yes	Mice human	Mice	[50]
Compound 9b	Yes	Low	Moderate	Peripheral	Yes Non-mechanism Based	Mice Human	Mice	[51]
UM 8190	Yes	Not tested	Not tested	Not tested	Yes	Mice Human	Mice	[55]
(S)-6e	Yes	Moderate	High	Central, Peripheral	No	Mice Human	Mice	[56]
Ent-3a	Yes	Moderate	Moderate	Central, Peripheral	No	Mice Human Rat Dog	Mice	[57]

Name/Structure	PRCP-Specific inhibition	Oral Bioavail ability	Clearance	Tissue specificity	Effect on Food intake	Organism tested	Ref
						<i>in vitro</i>
Compound 19	Yes	Low	Moderate	Not tested	Not tested	Mice Human Mice	[59]
Compound 32a	Yes (human) No (mice)	Not tested	Not tested	Central, peripheral	Not tested	Mice human Mice	[58]