HHS Public Access

Author manuscript

Curr Med Chem. Author manuscript; available in PMC 2024 June 28.

Published in final edited form as:

Curr Med Chem. 2021 ; 28(13): 2565–2576. doi:10.2174/0929867327666200615152804.

Ghrelin Based Therapy of Metabolic Diseases

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Abstract

Background: Ghrelin, a unique 28 amino acid peptide hormone secreted by the gastric X/A like cells, is an endogenous ligand of the growth hormone secretagogue receptor (GHSR). Ghrelin-GHSR signaling has been found to exert various physiological functions, including stimulation of appetite, regulation of body weight, lipid and glucose metabolism, and increase of gut motility and secretion. This system is thus critical for energy homeostasis.

Objective: The objective of this review is to highlight the strategies of ghrelin-GHSR based intervention for therapy of obesity and its related metabolic diseases.

Results: Therapeutic strategies of metabolic disorders targeting the ghrelin-GHSR pathway involve neutralization of circulating ghrelin by antibodies and RNA spiegelmers, antagonism of ghrelin receptor by its antagonists and inverse agonists, inhibition of ghrelin O-acyltransferase (GOAT), as well as potential pharmacological approach to decrease ghrelin synthesis and secretion.

Conclusion: Various compounds targeting the ghrelin-GHSR system have shown promising efficacy for the intervention of obesity and relevant metabolic disorders in animals and *in vitro*. Further clinical trials to validate their efficacy in human beings are urgently needed.

Keywords

Ghrelin; growth hormone secretagogue receptor (GHSR); therapeutic strategies; obesity; body weight; food intake

1. INTRODUCTION

Obesity, a major risk factor of non-communicable diseases, reduces the expected life span by 5 to 20 years, depending on the severity of the condition and its associated comorbidities [1-4]. Current therapy of obesity focuses mainly on weight management, with limited effect.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

Defining a novel strategy that could provide a lasting effect of weight reduction is urgently needed.

Ghrelin, an acylated peptide hormone, was first discovered in the gastric X/A like endocrine cells through a reverse pharmacological approach in 1999 [5]. It is the endogenous ligand of growth hormone secretagogue receptor (GHSR), a seven-transmembraneG protein-coupled receptor, which is now named as ghrelin receptor (GRLN-R) [6]. This receptor has demonstrated high expression in the pituitary gland, pancreatic islets, heart, thyroid gland and a variety of brain regions [7-12]. X/A like cells are the second abundant type of endocrine cells in the stomach, found in the gastric fundus, accounting for 20-30% of the oxyntic endocrine cells. These cells synthesize and release ghrelin [13]. Ghrelin is also expressed in alpha cells of the embryonic pancreas [14], as well as in pituitary [15, 16], hypothalamus [17, 18], kidney [19], liver [9], spleen, heart [20], lung [21], adipose tissue, skin [9] and testis [9, 22-24] in lower amounts.

Ghrelin gene composing five exons and four introns have been demonstrated to encode prepro-ghrelin of 117-amino acid. Prepro-ghrelin is cleaved to proghrelin and a signal peptide of 23-amino acid subsequently [5]. In golgi apparatus, pro-ghrelin is cleaved by prohormone convertases 1/3 (pc1/3) to produce mature ghrelin containing 28 amino acids [25]. Ghrelin binds to GHSR to exert its biological action. The acylation of ghrelin by ghrelin O-acyl-transferase (GOAT) to attach octanoate to serine-3 of ghrelin is necessary for ghrelin to bind to GHSR [26, 27]. The acylated ghrelin could not be detected in the circulation of GOAT deficiency mice, indicating that GOAT is the only enzyme capable of octanoylate ghrelin *in vivo* [28, 29]. However, not all ghrelin is octanoylated. Actually, only a fraction (less than 10%) of the ghrelin in circulation is acylated [30]. The unmodified des-n-octanoyl form of ghrelin (desacyl ghrelin) does not activate GHSR [23]. The acylated ghrelin increases food intake, gastric emptying, lipogenesis, blood glucose and insulin resistance, as well as reduces thermogenesis and insulin secretion [31]. Conversely, desacyl ghrelin decreases food intake, gastric emptying, lipogenesis, blood glucose and insulin resistance, while stimulates thermogenesis and insulin secretion via a yet-to-be-defined receptor [32].

Subcutaneous or intraventricular injection of ghrelin increases body weight by stimulating food intake [33-35] and inhibiting fat utilization in rodents [31, 36]. Subsequent studies have showed that ghrelin increases lipid deposition by concurrently increasing mRNA expression of enzymes responsible for fat storage and decreasing carnitine palmitoyltransferase-1a levels to reduce β-oxidation and lipolysis [37-39]. In the hypothalamic feeding center, GHSR1a is localized in neurons expressing neuropeptide Y (NPY) and agoutirelated peptide (AGRP), the orexigenic neuropeptides [40]. In humans, the anteprandial rise and postprandial fall of ghrelin indicate that ghrelin is a signal of hanger that promotes meal initiation [41]. In addition to stimulation of food intake and weight gain, the ghrelin-GHSR axis has also been demonstrated to regulate glucose and lipid metabolism *via* mTOR signal pathway [42-45], indicating a critical role in energy homeostasis. Thus, a strategy targeting the ghrelin-GHSR system may hold promise for the therapy of obesity and its related metabolic diseases. The present review summarizes the recent advances in ghrelin-GHSR based intervention of energy imbalance.

2. NEUTRALIZATION OF GHRELIN

2.1. Antibodies of Ghrelin

Traditionally, vaccination has been used to prevent infectious diseases. This notion has recently been redefined to allow vaccination as a pharmacological application to regulate body weight. Antibodies directed against acylated ghrelin were produced by active vaccination of rats through the injection of ghrelin immunoconjugates. The ghrelin antibody produced has been demonstrated to decrease significantly feeding efficiency, and weight gain [46]. A similar effect has been shown in pigs actively immunized against ghrelin, which reduces 10% of body weight and 15% of food intake relative to the control pigs at the end of the experiment [47]. Recently, another vaccine has been developed to lose weight targeting the ghrelin-GHSR system. The vaccine was developed in an advanced materialnanogel to allow intranasal administration. This approach reduces body weight in high fat diet-induced obese mice and ob/ob mice, thereby improving glucose tolerance and insulin sensitivity [48]. Unfortunately, ghrelin vaccine shows limited effect in the human phase IIIa clinical trial. Despite that strong antibody response to ghrelin reduced hunger, weight loss was not significant in 111 obese subjects [49]. Therefore, the clinical application of vaccination against ghrelin in the treatment of obesity remains to be proven [50].

2.2. RNA Spiegelmers

RNA spiegelmers (German *Spiegel* = mirror) are a unique brand of reagents to inhibit ghrelin action [51, 52]. Spiegelmers are oligonucleotides that are synthesized with unnatural L-enantiomers of ribose in the sugar-phosphate framework for stability. They arc designed to bind to acylated ghrelin, thereby blocking ghrelin receptor activation *in vitro* [53]. RNA spiegelmer has been shown to block the effect of ectogenic ghrelin on the activity of neurons in the hypothalamic arcuate nucleus [54]. Administration of ghrelin RNA spiegelmer into diet-induced obese mice results in weight loss and reduction of food intake and fat deposition relative to control mice during the first week of treatment. Spiegelmer NOX-B11-2 blocks the activation of GHSR1a *in vitro* and effectually accelerates weight loss in diet-induced obese mice [55]. However, the weight loss relapses during the second week of treatment. Another ghrelin RNA spiegelmer, NOX-B11-3, effectually blocks the stimulation of arcuate nucleus neurons induced by ghrelin in rats [54]. Further studies are required to explicate the therapeutic clinical effects of ghrelin RNA spiegelmers.

2.3. GHSR-Fc Fusion Protein

GHSR-Fc is a recombinant protein containing the ligand-binding domains of the GHSR1a fused with a human IgG constant region (Fc). The GHSR-Fc is secretable because its structure does not contain a sequence motif corresponding to the transmembrane domain of the receptor. *In vivo* expression of GHSR/Fc reduces levels of acylated ghrelin but not unacylated ghrelin. Thus, this fusion structure is able to deplete circulating acylated-ghrelin. Intramuscular injection of GHSR-Fc decreases weight gain in high fat diet-induced obese mice accompanied by reduction of peritoneal fat accumulation, alteration of gene expression in adipocyte and improvement of glucose tolerance [56]. GHSRl-Fc fusion protein may provide an alternative potential for the treatment of obesity.

3. PHARMACOLOGICAL TARGET OF GHRELIN RECEPTOR

3.1. Ghrelin Receptor Antagonists

Ghrelin is known to exercise its orexigenic and metabolic function through GHSR [57, 58]. Mice with GHSR deficiency show reduced fat deposition and healthier lipid structure, along with increasing energy expenditure and basal metabolic rate [59]. Thus, suppression of ghrelin-GHSR signaling is an attractive strategy for therapy of obesity, type 2 diabetes, and metabolic syndrome. Recently, various GHSR antagonists have been reported as new approaches for weight reduction (Table 1).

[D-Lys3]-growth hormone-releasing peptide 6 ([D-Lys3]-GHRP-6) was discovered in 1992 during the study of met-enkephalin-derivatives of growth hormone-releasing peptide [60]. Its special amino acid sequence His-D-Trp-A1a-Trp-D-Phe-Lys-NH2 inhibits GHRP combining with the pituitary gland and membranes of hypothalamus [60]. In a recent study, [D-Lys3]-GHRP-6 has been shown to behave as a partial antagonist with a strong bias toward GHSR-1a-β-arrestin signaling [61]. Administration of [D-Lys3]-GHRP-6 reduces growth hormone secretion and weight gain in mice [62-64], as well as attenuates alcohol-induced hepatic steatosis by regulating lipid metabolism [65]. However, [D-Lys3]-GHRP-6 unexpectedly increases food intake and reduces plasma insulin levels, leading to increased blood glucose and insulin resistance [62]. This result casts a cloud on the use of [D-Lys3]-GHRP-6 as a new treatment option for weight control, mainly on account of its side effects relevant to glycemic control.

Another growth hormone secretagogue analogs, met-enkephalin-derived GHSR antagonists, with His-D2NaI-DLys-Trp-DPhe-LysNH2 amino acid sequence which carry 1, 2, 4-triazole structure, have also been studied in energy metabolism. For example, a subcutaneous injection of compound 21b (JMV2810) inhibits hexareline-stimulated food intake [66]. Similarly, JMV2844 (sc), JMV2959 (icv, sc, ip), JMV3002 (icv) and JMV3021 (sc) also suppress food intake in rats [66-69]. The effects of these antagonists on body weight control in humans remain unknown.

BIM-28163, a ghrelin-derived ligand, was identified as a GHSR antagonist blocking ghrelin-induccd GH secretion. However, BIM-28163 acts unexpectedly as a GHSR agonist to induce body weight gain in male SD rats [70].

The effect of small molecule GHSR antagonists, such as piperidine-substituted derivatives of quinazolinone seems promising [71]. YIL-781, a piperidine-substituted quinazolinone derivative, acts as an effective GHSR1a antagonist to improve insulin release and weight loss and to reduce food intake in obese mice [72]. Compound D (CpdD), another small molecule GHSR antagonist, leads to a transient reduction in food intake. Similarly, compound B (CpdB) imposes continuous weight loss on account of reduced fat mass, which is associated with improved insulin sensitivity and resistance to hepatic steatosis in diet-induced obese mice [73].

GHSR1a antagonists also include several derivatives of carbohydrazide [74]. However, GSK1614343, one of these compounds, increases weight gain and food intake in both rats and dogs [75]. Thus, their metabolic benefit requires further investigation.

Although short-term use of ghrelin receptor antagonists has shown promising effects for weight control, its long-term efficacy is limited.

3.2. Inverse Ghrelin Receptor Agonists

Inverse GHSR1a agonists may provide an alternative strategy for the intervention of obesity because of the constitutive ghrelin receptor activity, which could be reduced by its inverse agonists [76-78]. In the hypothalamus, prolonged fasting results in an increase of GHSR1a expression, leading to higher appetite and lower energy expenditure. Thus, reducing the constitutive activity of GHSR1a through inverse agonists improves the sensitivity to anorectic signals such as leptin, PYY or insulin and eliminates the hunger between meals and the craving for snacks [79]. [D-Arg-1, D-Phe-5, D-Trp-7, 9 and Leu-11]-substance P was identified as the first inverse agonist for GHSR1a [63]. This substance P derivate reduces food intake in lean, diet-induced obese or ob/ob mice [63].

Liver enriched antimicrobial peptide 2 (LEAP-2) is a potent antagonist of the GHSR. LEAP-2 blocks the major effect of ghrelin on food intake, and maintenance of glucose levels in a steady state during fasting in mice [80]. The n-terminus sequence of LEAP-2 acts as an inverse agonist of the GHSR. Subcutaneous administration of the n-terminus region of LEAP-2 inhibits the food intake induced by ghrelin [81]. In a recent study, Mani *et al.* found that LEAP-2 regulates body mass and food intake by changing the LEAP-2/acyl-ghrelin ratio in humans and mice [82]. Therefore, decreasing LEAP-2 degradation or increasing LEAP-2 expression may be another promising strategy to antagonize ghrelin's effect.

A series of 2-alkylamino nicotinamide analogs has been defined as the inverse ghrelin receptor agonist with oral bioactivity. Among these, compound 33 (Table 2) decreases weight gain in obese rats [83]. As a GHSR inverse agonist, the core structure of compound 33 is a diazabicyclo ring at the 5-position of the pyridine ring. Oral administration of compound 33 displays a moderate concentration in brain, suggesting its brain permeability [84].

An acylurea series of ghrelin receptor agonists have been identified by high throughput screening and affinity optimizing through structural modifications. Two chemical compounds; the non-CNS penetrant inverse agonist 22 (AZ-GHS-22) and the CNS penetrant inverse agonist 38 (AZ-GHS-38), have been developed through modification of specific substructure and optimization of physical and chemical properties to convert partial agonist activity to inverse agonist activity [85]. The effects of these compounds *in vivo* remain unknown.

Novel synthetic inverse ghrelin receptor agonists GHSR-IA and GHSR-IA2 have also been developed. Both compounds are able to reduce food intake in mice. Oral GHSR-IA2 decreases glucose levels, blood triglyceride levels and body weight, with more efficacy [86].

Another small-molecule, PF-5190457, has been identified as a potent and selective GHSR inverse agonist with high oral bioavailability and potential of clinical application [87]. PF-5190457 is from a spiroazetidino-piperidine series. Through the evaluation of the pharmacokinetics, PF-5190457 appears to be a promising molecule. It increases insulin secretion in a glucose-dependent manner in human entire pancreas and dispersed islets. Initial clinical trial in healthy people shows that PF-5190457 is quickly absorbed orally, leading to attenuated secretion of GH, impaired motility and evacuation of stomach, and reduced postprandial glucose [88]. Recently, PF-5190457 has finished preclinical safety experiments and phase 1b of human laboratory study. The study demonstrates that the inverse ghrelin receptor agonist PF-5190457 is safe and tolerable [89].

Overall, several GHSR inverse agonists have been shown to be efficient for weight control in animals (Table 2). Additional researches are necessary to evaluate the long-term use of these compounds in the treatment of obesity and related metabolic diseases.

4. INHIBITION OF GOAT

Octanoylated pentapeptides were identified as the first inhibitor of ghrelin O-acyltransferase (GOAT) [29]. Another specific GOAT inhibitor, GO-CoA-Tat, a bisubstrate analog that antagonizes GOAT, was developed in a subsequent study [90]. In wild-type mice, intraperitoneal injection of GO-CoA-Tat reduces body weight and improves glucose tolerance. This reagent does not improve metabolic conditions in ghrelindeficient mice [90], Circulating acyl ghrelin levels and weight gain are reduced by GO-CoA-Tat in diet-induced obese mice [91]. Among mice treated with GO-CoA-Tat, body weight, triglyceride (TG), total cholesterol (TC) and glucose are reduced. Indicators of liver injury, such as glutamate pyruvate transaminase (GPT) and glutamic oxalacetic transaminase (GOT) are also significantly decreased [92]. Thus, GO-CoA-Tat may hold promise for the intervention of metabolic diseases. However, it is worth noting that this GOAT inhibitor has shown limited efficacy in human beings.

Compound B ((4-chloro-6-[[2-methyl-6-(tntluoromethyl) pyridin-3-yl]methoxy}-1-benzothiophen-3-yl) acetic acid), another GOAT inhibitor discovered by high-throughput screening, decreases gastric acylghrelin of mice after oral administration [93]. These novel discoveries suggest that GOAT is a therapeutic target, and the effects of GOAT inhibition by compound B on body weight warrant further investigation.

5. POTENTIAL PHARMACOLOGICAL TARGET TO REDUCE GHRELIN SECRETION

The discovery that ghrelin activates its receptor GHSR to regulate food intake stimulates the development of over 20 drugs targeting the ghrelin system as interventions for obesity. While ghrelin-GHSR antagonists initially held promise, long term efficacy has been minimal. In a recent study [94, 95], we have demonstrated that long term administration of the ghrelin receptor antagonist, D-Lys-3-GHRP6, leads to significant increases in ghrelin production. An increase in ghrelin production with long term use of receptor antagonists may explain the clinical inefficacy of ghrelin receptor antagonists. Shifting focus from

ghrelin receptor antagonism to direct blockade of ghrelin production provides a novel strategy for the treatment of obesity and related diseases. For this goal, we have identified the mechanistic target of rapamycin (mTOR) as a critical molecule linking the production of ghrelin with organism energy levels within gastric X/A like cells [95]. Activation of mTOR signaling in these endocrine cells by deletion of tuberous sclerosis complex 1 (TSC1), an upstream inhibitor of mTOR complex, suppresses the synthesis and secretion of ghrelin, leading to the subsequent amelioration of liver steatosis in high fat diet-induced obese mice [42]. Further, these transgenic mice develop pancreatic fibrosis, which reduces the secretion of insulin [96]. Whether mTOR in gastric X/A like cells may serve as a novel target for the intervention of obesity and metabolic diseases requires further investigation.

Bitter taste receptor (T2R) has been demonstrated to stimulate ghrelin secretion to regulate food intake and gastric emptying [97]. Plasma ghrelin levels increase in an α-gustducin dependent manner in mice by intragastric administration of a mixture of T2R agonists [97]. A recent study has found that the sympathetic drive, controlling ghrelin release in the fundus, is impaired in obese mice. This attenuates the sensitivity of the X/A like cells to bitter and sweet signals in the gastrointestinal tract leading to the reduction of ghrelin level [98]. All these findings suggest that manipulation of endogenous ghrelin by T2R antagonists may provide a potential approach to treat obesity and its related metabolic diseases.

6. SUMMARY AND FUTURE DIRECTIONS

Ghrelin and GFISR play important roles in the modulation of energy homeostasis. A great effort has been made to develop novel strategies targeting the ghrelin-GHSR system and to evaluate the potential clinical application in the intervention of obesity and metabolic diseases. These strategies include neutralization of ghrelin by antibodies, RNA spiegelmers and GFISR-Fc, antagonism of ghrelin receptor by its an-tagonists and inverse agonists, inhibition of ghrelin O-acyltransferase (GOAT) enzyme, as well as potential pharmacological target to decrease ghrelin secretion (Fig. 1). While short-term use of ghrelin or GFISR antagonism holds promise in animal experiments, a long-term application has shown limited efficacy. The mechanism underlying their minimal long-term effect may be attributed to the compensatory increment in the newly synthesized ghrelin associated with these treatments. Defining novel pathways to alter the production of acyl-ghrelin from gastric X/A like cells would shift therapeutic focus to novel gastric targets and ultimately provide a therapeutic benefit for people with metabolic diseases.

It is also worth noting that most of the tested compounds have been shown to be effective only in animals and *in vitro.* So far, only the ghrelin vaccine and PF-5190457 have been tested in humans. Clinical trials of other ghrelin-GHSR based interventions are urgently needed to prove their effectiveness in human beings, especially on reduction of appetite and body gain.

CONCLUSION

The long-term efficacy in weight reduction and therapy of metabolic dysfunctions by blocking the action of ghrelin has been limited, likely because of the negative feedback

up-regulation in ghrelin production. Blockade of ghrelin production by direct targeting the gastric X/A like cells may provide an alternative strategy.

FUNDING

This study has been supported by the National Key R&D Program of China (2017YFC0908900); National Natural Science Foundation of China (81730020, 81930015 and 81700516); and National Institutes of Health (R01 DK112755 and R01-DK-110273-01A1).

LIST OF ABBREVIATIONS

GHSR	Growth Hormone Secretagogue Receptor
GOAT	Ghrelin O-Acyltransferase
GRLN-R	Ghrelin Receptor
NPY	Neuropeptide Y
AGRP	Agouti Related Peptide
[D-LYS3]-GHRP-6	[D-Lys3]-Growth Hormone-Releasing Peptide 6
LEAP-2	Liver Enriched Antimicrobial Peptide 2
AZ-GHS-22	Non-CNS Penetrant Inverse Agonist 22
AZ-GHS-38	CNS Penetrant Inverse Agonist 38
TG	Triglyceride
TC	Total Cholesterol
GPT	Glutamate Pyruvate Transaminase
GOT	Glutamic Oxalacetic Transaminase
mTOR	Mechanistic Target of Rapamycin
TSC1	Tuberous Sclerosis Complex 1
T2R	Bitter Taste Receptor

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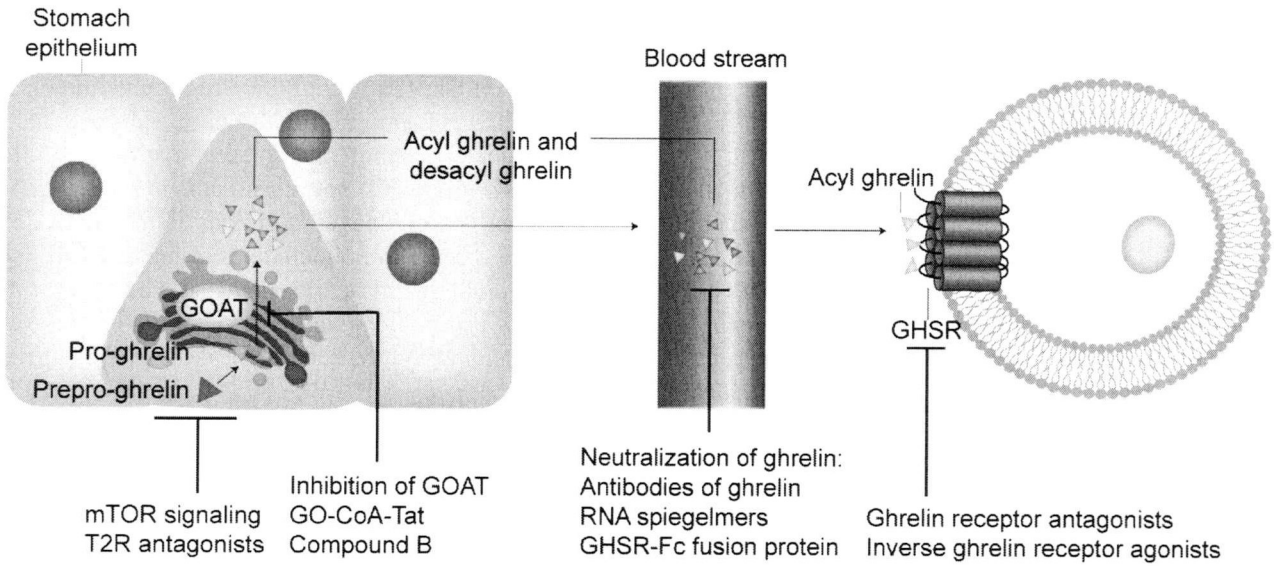

Fig. (1).

Summary of ghrelin-based strategies for intervention of metabolic dysfunction. Intervention of ghrelin synthesis, processing, release and activation of its receptor GHSR1a has been developed for the therapy of obesity and its related metabolic dysfunctions.

Table 1.

Ghrelin receptor antagonists.

Chemical types	Compounds	Functions
Met-enkephalin-derived GH-releasing peptides	[D-Lys3]-growth hormone–releasing peptide 6 (GHRP-6)	1. Increases cumulative food intake in non obese diabetic MKR mice [65]. 2. Reduces GH secretion, weight gain and plasma insulin [65]. 3. Attenuates alcohol-induced hepatic steatosis [69].
Met-enkephalin-derived GRLN-R antagonists	JMV2810 JMV2844 JMV2959 JMV3002 JMV3021	Suppresses food intake [70-73].
Ghrelin-derived ligand	BIM-28163	Induces body weight gain [74].
Small molecule GHSR antagonists-piperidine-substituted quinazolinone derivatives	YIL-781	Improves glucose-stimulated insulin secretion and reduce food intake and weight loss [76].
	Compound D (CpdD)	Reduces food intake [77].
	Compound B (CpdB)	Reduces body weight, white adipose tissue, hepatic steatosis and improves insulin sensitivity [77].
Carbohydrazide derivatives	GSK 1614343	Increases weight gain and food intake [79].

Table 2.

Inverse ghrelin receptor agonists.

Chemical types	Compounds	Functions
Substance P derivate	[D-Argl, D-Phe5, D-Trp7,9, Leul 1]-substance P	Reduces food intake [66]
Endogenous full antagonist of the GHSR	Antimicrobial peptide 2 (LEAP-2)	Reduces food intake and body mass [84-86]
2-alkylamino nicotinamide analogs	Compound 33	Decreases weight gain [87]
Acylurea series of ghrelin modulators	Non-CNS penetrant inverse agonist 22 (AZ-GHS-22) and the CNS penetrant inverse agonist 38 (AZ-GHS-38)	Unknown [89]
Synthetic ghrelin receptor inverse agonists	GHSR-IA GHSR-1A2	Reduces food intake, glucose levels, blood triglyceride levels and body weight [90]
Spiro-azetidino-piperidine	PF-5190457	Increases glucose-stimulated insulin secretion, inhibits ghrelin-induced secretion of GH, delays gastric emptying, and reduces postprandial glucose [92]