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Review

MC4R Antagonists: A Promising New Hope for the Treatment of Cachexia and Wasting Diseases

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Abstract

Melanocortin-4 receptor (MC4R) is a key receptor in the hypothalamus regulating appetite and energy metabolism. Research has indicated that MC4R agonists have demonstrated potential for weight loss; its antagonists may stimulate appetite and weight gain, showing potential therapeutic applications in patients with anorexia or cachexia or those requiring weight restoration. However, the signaling mechanism, clinical applications, and safety profile of MC4R antagonists still require in-depth investigation. The purpose of this review is to comprehensively summarize the research progress of MC4R antagonists, analyze their mechanism of action and clinical applications, evaluate their safety profile, and explore future research directions. Future studies should focus on developing safer and more effective MC4R antagonists and exploring the therapeutic potential of MC4R in other diseases.

Keywords: MC4R antagonist; appetite; cachexia; wasting diseases; mechanism of action

1. Introduction

Weight disorders include obesity, anorexia, cachexia, etc., which cause abnormal changes in body weight and appetite. Obese patients experience continuous accumulation of body fat due to energy intake exceeding expenditure, which may be associated with excessive appetite and dysregulation of energy -metabolism, resulting in sustained weight gain [1–3]. Anorexia patients experience a significant decline in appetite due to psychological or physiological factors, consuming far less than their body's requirements, leading to rapid weight loss [4]. Cachexia, often secondary to severe chronic diseases such as cancer or AIDS, is characterized not only by reduced appetite but also by substantial loss of muscle and fat tissue, causing pronounced weight decline [5,6]. These diseases exert significant impacts on patients' physical and mental health, making it of paramount importance to thoroughly investigate their pathogenesis and identify effective intervention measures.

The melanocortin receptors belongs to a subfamily of the G protein-coupled receptor (GPCR) superfamily with therapeutic potential, comprising MC1R, MC2R, MC3R, MC4R, and MC5R [7]. Melanocortin receptors (MC1R-MC5R) are located on various cell types and distributed across different body systems. MC1R is expressed in skin melanocytes and plays a crucial role in determining skin and hair pigmentation; it is also expressed in leukocytes, potentially mediating anti-inflammatory properties. MC2R, which refers to the adrenocorticotropic hormone (ACTH) receptor, mediating the effect of ACTH on steroid secretion. MC3R is distributed in multiple regions of the central nervous system and peripheral tissues, participating in the regulation of energy homeostasis. MC4R is primarily expressed in the central nervous system and plays a critical regulatory role in the processes of food intake and energy metabolism. MC5R is found in peripheral tissues and primarily contributes to exocrine functions [7,8].

Melanocortin-4 receptor (MC4R) is widely distributed in the central nervous system (such as the hypothalamic arcuate nucleus and paraventricular nucleus) and peripheral tissues of mammals, and is primarily involved in regulating energy balance, appetite, and body weight homeostasis. Studies have shown that abnormalities in the MC4R gene are a major cause of severe obesity [9,10]. Gene mutations often manifest as complete or partial loss of function, impairing receptor signal transduction and subsequently leading to metabolic abnormalities such as obesity, elevated leptin levels, hyperinsulinemia, and insulin resistance [11]. The statement indicates that the functional level of MC4R plays a pivotal role in the body's energy metabolism and weight regulation. Under normal functional conditions, activation of MC4R suppresses appetite and increases energy expenditure (e.g., raising basal metabolic rate and thermogenic capacity). By modulating the distribution and metabolism of adipose tissue, it reduces fat accumulation. MC4R agonists have shown highly effective in appetite

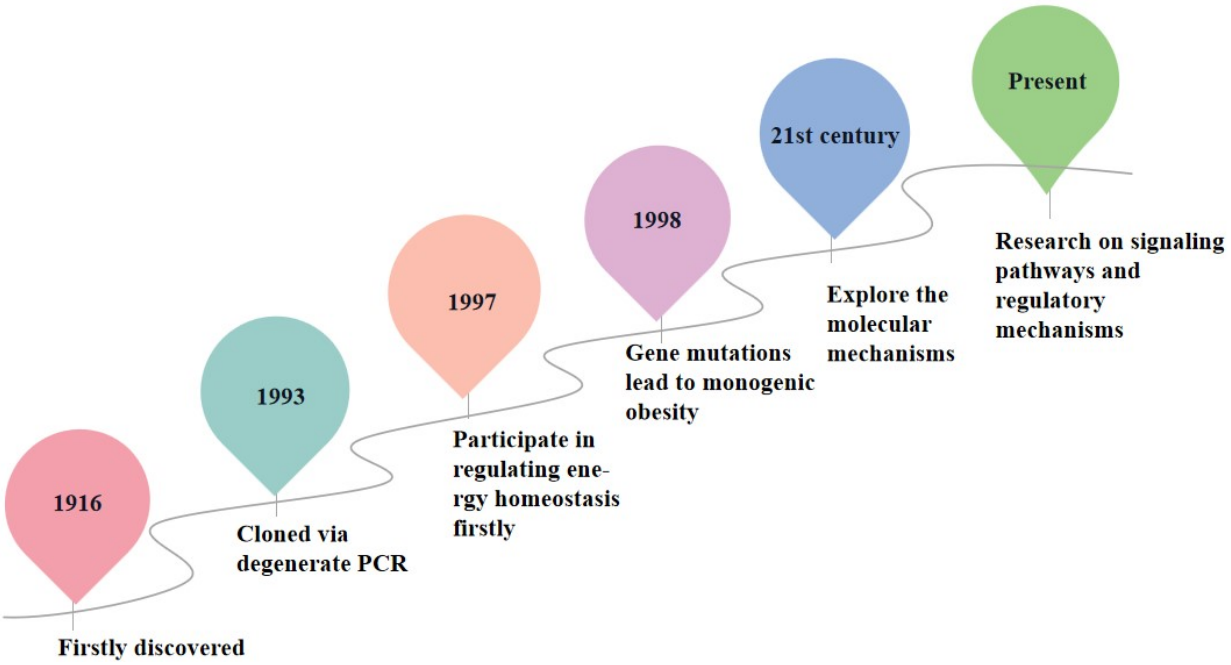

Fig. 1. The discovery process of MC4R. MC4R, Melanocortin-4 receptor.

suppression and weight loss, but long-term use may trigger compensatory metabolic adaptations and cannot meet the needs of catabolic conditions such as anorexia nervosa or cachexia.

Recent studies have shown that, beyond its classic role in energy metabolism, MC4R also plays a crucial neuroprotective role, primarily through anti-inflammatory mechanisms [12]. Activation of MC4R can inhibit signaling pathways such as NF-κB, thereby down-regulating pro-inflammatory cytokines like IL-6 and reducing neuronal injury. Key evidence comes from two disease models: in ischemic stroke models, MC4R agonists reduce infarct size and improve neurological function [12], and in Alzheimer's disease (AD) models, MC4R activation suppresses microglial activation and delays cognitive decline [13], with these effects being dependent on MC4R activation. Together, these findings highlight the therapeutic potential of the MC4R signaling pathway in neurodegenerative diseases.

Given that MC4R plays a crucial role in both metabolic diseases and neurological disorders, precise regulation of its function is of paramount importance. MC4R antagonists dynamically regulate energy balance through reversible inhibition of MC4R, and are applicable for the treatment of anorexia, cachexia, anxiety, depression, and metabolic disorders [14,15]. The development of MC4R antagonists has gained attention due to their higher selectivity and specificity, as well as their potential therapeutic value in treating cachectic diseases. This article primarily outlines the current research and development status of MC4R antagonists, aiming to provide insights for subsequent studies.

2. Structure and Function of MC4R

Melanocortins are a class of hormones and proteins with diverse biological functions, first discovered in 1916 [16]. They exert their effects by interacting with G protein-coupled melanocortin receptors, activating multiple intracellular signaling pathways. In humans, melanocortins primarily function through regulating energy balance, immune responses, and pigmentation. In 1993, MC4R was cloned using degenerate PCR, but its function remained unknown [17]. Subsequent studies have shown that MC4R may be involved in regulating energy homeostasis. In 1997, a series of groundbreaking studies on mice validated this hypothesis. In 1998, human genetic studies discovered that MC4R gene mutations could cause monogenic obesity [17], revealing the critical role of MC4R in human weight regulation. In the early 2000s, researchers began to explore the molecular mechanism of MC4R. For example, in 2001, studies demonstrated that MC4R gene mutations lead to receptor internalization and functional inactivation [18]. Additionally, studies in 2003 revealed the role of MC4R in energy homeostasis and its binding properties with α-melanocyte-stimulating hormone (α-MSH) [19,20]. Since the 2000s, researchers have been continuously conducting in-depth studies on the signaling pathways and regulatory mechanisms of MC4R. These investigations have not only enhanced the understanding of MC4R's functions but also provided potential molecular targets for the treatment of obesity and related diseases (as shown in Fig. 1).

The MC4R protein, composed of 332 amino acids, is encoded by a gene located at chromosome 18q21.3, which contains only one exon [21]. MC4R is composed of an extracellular N-terminus, seven transmembrane segments,

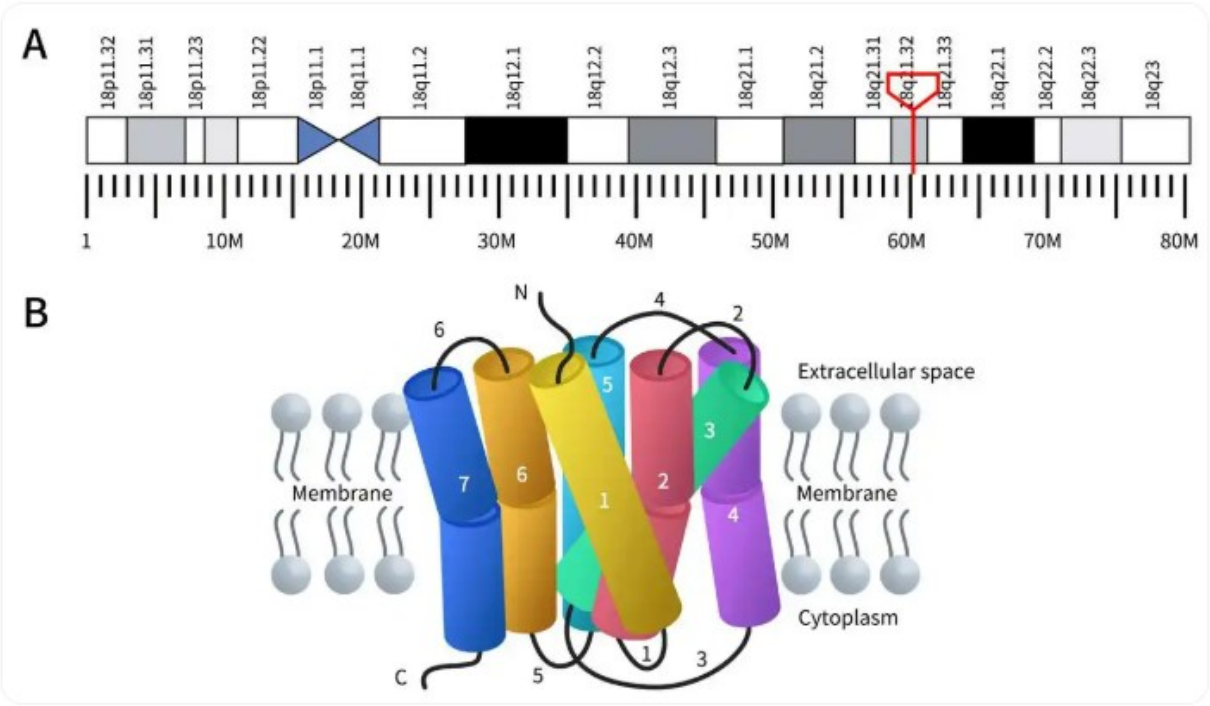

Fig. 2. Gene location and protein structure of MC4R. (A) The genomic location of MC4R. The red box indicates the location of the gene encoding the MC4R protein. (B) Its protein structure. (The genomic location data derived from WIKIPWDIA.)

three intracellular loops, three extracellular loops, and a cytoplasmic tail [22–24], as shown in Fig. 2. This structural configuration enables it to regulate signal transduction by binding to G proteins. In 2020, Yu *et al.* [25] resolved the crystal structure of MC4R bound to the antagonist **SHU9119** [26], this structure revealed the three-dimensional conformation of MC4R and its binding mode with **SHU9119**. The study demonstrated that MC4R is a seven-transmembrane helical protein (TM1–TM7) containing a binding pocket for **SHU9119** and a divalent calcium ion (Ca^{2+}) cofactor [26].

MC4R is a key receptor regulating energy balance and appetite. Its active sites are primarily located in the transmembrane domains (especially TM3 and TM6), which interact with various agonists and antagonists to trigger or inhibit signal transduction, thereby influencing energy metabolism and appetite regulation. For example, the endogenous ligand α-MSH activates MC4R by binding to specific amino acid residues (e.g., $D122^{3.25}$, $D126^{3.29}$, and $E100^{3.29}$), while the natural antagonist **AgRP** inhibits receptor activity through interactions with these residues [27]. **SHU9119** as an MC4R antagonist, with its binding pocket located in the extracellular loop 2 (EL2) region of the receptor, form a complex binding pattern with the transmembrane helices of TM3 and TM6, as shown in Fig. 3 (Ref. [25]).

SHU9119 prevents the transition of $L133^{3.36}$ from the inactive state to the active state through steric hindrance. $L133^{3.36}$ is located in the third transmembrane region (TM6) of MC4R, which acts as a key regulator for the activation of Class A GPCRs. It interacts with $W258^{6.48}$ to influence the displacement of TM6. In the inactive state, $L133^{3.36}$ maintains a CH-π contact with $W258^{6.48}$, while in the active state, the orientation of $L133^{3.36}$ shifts, allowing $W258^{6.48}$ to move freely. This facilitates conformational rearrangement of TM6, thereby promoting G protein coupling [28], as shown in Fig. 4 (Ref. [28]). The naphthyl group of **SHU9119** replaces methionine at position $M133^{8.24}$ in $L133^{3.36}$, thereby stabilizing the inactive state of MC4R [28,29]. Additionally, the binding of **SHU9119** to EL2 also involves hydrogen bonding and hydrophobic interactions, such as hydrogen bonds formed between TM2-H4 and the aromatic backbone of EL2, as well as interactions with the hydrophobic regions of EL2 [28].

In addition, under certain circumstances, Ca^{2+} ions also participate in the ligand binding process. When MC4R binds to the agonist setmelanotide, the carbonyl oxygen atom forms a coordination bond with Ca^{2+}, thereby enhancing binding stability [28,29]. This binding pattern suggests that Ca^{2+} plays a pivotal role in the ligand-binding process, possibly by stabilizing the receptor conformation or facilitating interactions between the ligand and receptor. The structure of the **SHU9119**-MC4R complex (DB ID: 6W25) reveals the binding mode of the antagonist **SHU9119** and further demonstrates the critical role of Ca^{2+} ions within the binding pocket [28]. In this complex, **SHU9119** binds to amino acid residues F184, Y268, and F284 through hydrophobic interactions, while the carbonyl

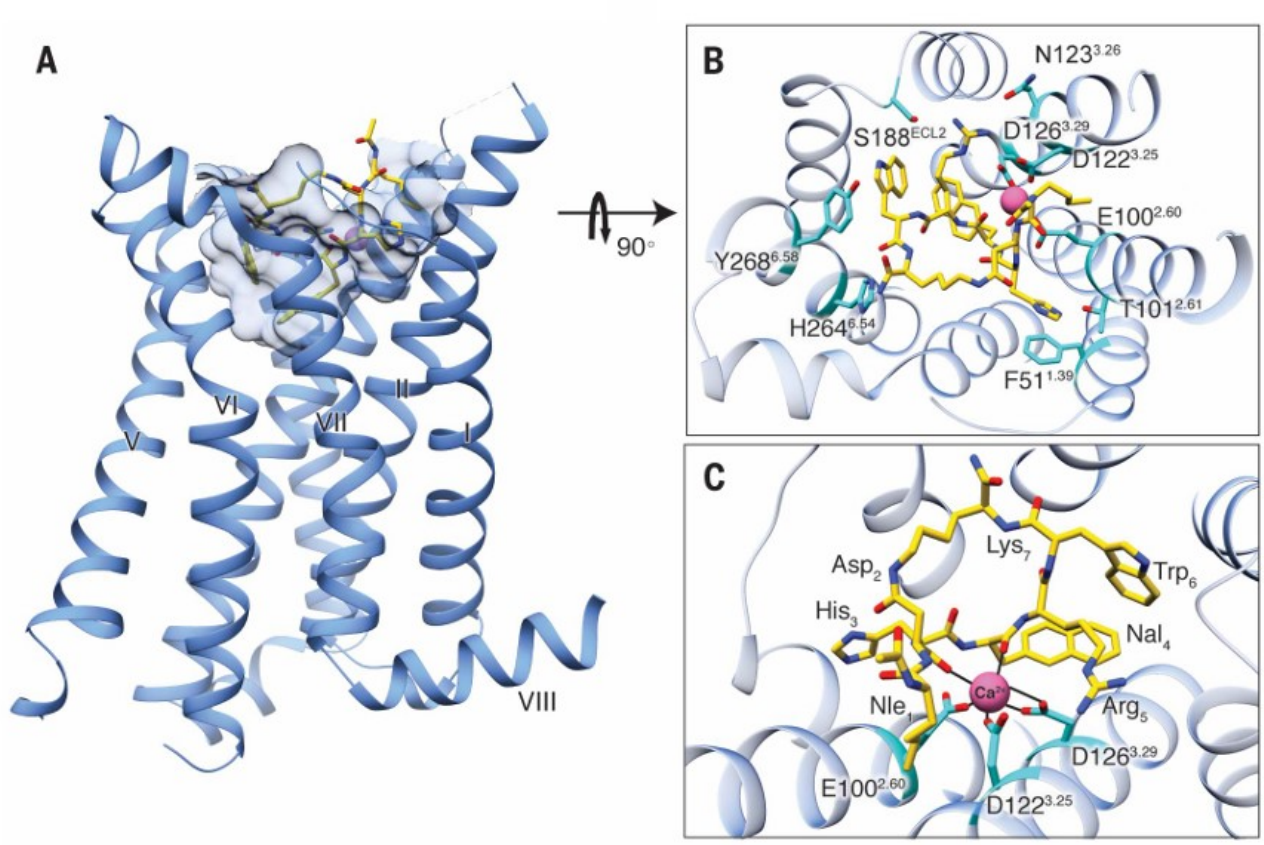

Fig. 3. Structure of MC4R bound with SHU9119 [25]. (A) Side view of the crystal structure of the MC4R-**SHU9119** complex. (B) Structure viewed from the extracellular side, illustrating the interaction network among MC4R, **SHU9119**, and metal ions. (C) Expanded view of the metal ion-binding site in the protein.

oxygen atom forms hydrogen bonds with Ca^{2+}, thus enhancing the binding affinity between the ligand and the receptor [28].

The Ca^{2+}-mediated fine-tuning mechanism ensures that MC4R can sensitively respond to fluctuations in upstream signals, thereby establishing its structural basis as a central hub for energy balance. The most representative regulatory pathway is the leptin-α-MSH (melanocyte-stimulating hormone) axis: leptin, secreted by adipose tissue, acts on leptin receptors (LepR) in the arcuate nucleus (ARC) of the hypothalamus, activating α-MSH-producing neurons. These neurons then release α-MSH, which binds to MC4R, generating signals that suppress appetite and increase energy expenditure [30,31]. This pathway is the core circuit for regulating body-weight balance, and any functional defect at any step can lead to an obese phenotype. Although the leptin-α-MSH pathway occupies a central position in long-term energy-balance regulation, the body's fine-tuned control of energy homeostasis cannot be achieved by a single pathway alone. MC4R integrates heterogeneous hormonal signals from various pathways (see Table 1, Ref. [30,31]), ultimately achieving dynamic regulation of energy intake and expenditure.

3. MC4R Signaling Mechanism

The primary signaling pathways involved in MC4R include the G protein, β-arrestin, Ca^{2+} regulatory, and leptin–melanocortin pathways, all of which are linked to obesity and energy metabolism.

3.1 G Protein Signaling Pathway

The complexity of the MC4R signaling pathway is not only reflected in its diverse physiological functions and regulatory mechanisms but also in its close association with G protein-coupled receptors. When delving into the complexity of the MC4R signaling pathway, we must focus on one of its core mechanisms, the G protein-coupled signaling pathway. MC4R activates the cAMP-PKA signaling pathway through direct coupling with G proteins, which constitutes its primary signal transduction mechanism (see Table 2, Ref. [32–34]). This mechanism plays a critical role in regulating appetite suppression and energy balance within the hypothalamus [35]. Specifically, after MC4R binds to the G protein, the α subunit (Gαs) is activated, stimulating adenylate cyclase (AC) to catalyze the synthesis of cAMP [36]. As a second messenger, cAMP subsequently activates protein kinase A (PKA), thereby regulating the function of

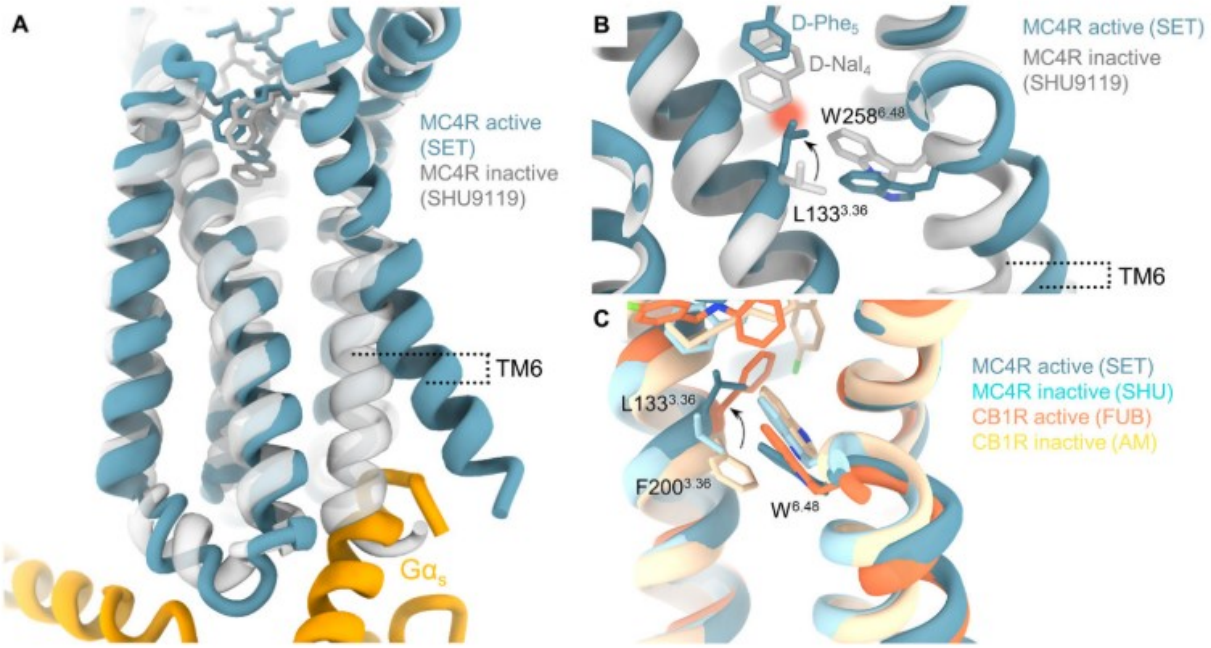

Fig. 4. MC4R activation switch [28]. (A) Superimposition of the MC4R active complex with the antagonist-bound receptor. (B) The D-Nal_4 fragment in **SHU9119** stabilizes the inactive switch conformation. (C) Superimposition of the MC4R activation switch (active—light blue, inactive—sky blue) with the classical "toggle switch" of CB1R (active—pink, inactive—orange conformation).

downstream target proteins [35], as shown in Fig. 5A (Ref. [34,37]). For example, endogenous agonists such as α-MSH generate satiety signals through this pathway to suppress appetite [32]. Beyond this, MC4R can also couple with other G protein subtypes (e.g., $G\alpha_{q/11}$ and $G\alpha_{i/o}$), activating phospholipase C (PLC) or inhibiting cAMP production. Certain MC4R mutations (e.g., H158R) bias signaling toward the $G_{q/11}$ pathway, leading to the release of IP3 and DAG, thereby regulating intracellular calcium dynamics [33].

3.2 Non-G Protein-Dependent Signaling Pathways

In addition to the classical G protein signaling pathway, the MC4R signaling pathway also involves non-G protein-dependent pathways, adding flexibility and diversity to its signal transduction and further enriching its complex signaling network.

β-arrestin pathway plays a critical role in the regulation of MC4R, particularly in cases of loss-of-function (LoF) or gain-of-function (GoF) caused by MC4R mutations. Mutations causing gain-of-function (GoF) may activate β-arrestin signaling, particularly those associated with reduced risks of obesity-related diseases. Research indicates that MC4R mutations (e.g., V103I and I251L) facilitate the recruitment of β-arrestin. When these mutate MC4Rs bind agonists (e.g., α-MSH or its analogs), they induce conformational changes in the receptor, altering ligand-receptor interactions. This mechanism may enhance ligand-receptor binding stability, thereby either preventing MC4R internalization into the cell or accelerating its recycling, ultimately prolonging MC4R retention on the plasma membrane [34]. This mutation may increase the cell surface expression of MC4R by reducing internalization or promoting rapid recycling, leading to elevated production of cyclic adenosine monophosphate (cAMP), as well as increased generation of PKA and cAMP-regulated guanine nucleotide exchange factor (Epac) [34] (as shown in Fig. 5B).

Studies show that after MC4R is activated by ligands, G protein-coupled receptor kinases (GRKs) phosphorylate its C-terminal domain and recruit β-arrestin proteins to form signaling complex. This complex directly activates the ERK1/2 pathway in the mitogen-activated protein kinase (MAPK) signaling cascade independent of G proteins [32]. Activated ERK1/2 pathway plays a crucial role in regulate energy homeostasis, which phosphorylates multiple substrates to influence cell proliferation, differentiation, and metabolism. In obesity caused by MC4R mutations, aberrant activation of the ERK1/2 pathway may lead to energy balance dysregulation [31,38]. For example, MC4R carrying V103I or I251L gain-of-function mutations exhibit significant β-arrestin-biased signaling, with ERK phosphorylation levels increased 3-5-fold compared to wild-type, while cAMP signaling remains unaffected [32]. This signaling differentiation provides a novel therapeutic approach for obesity: β-arrestin pathway activation is significantly associated with lower BMI and reduced cardioascular risk,

Table 1. Other key signaling pathways and their roles in obesity/energy metabolism [30,31].

Pathway	Main downstream molecules	Effect on obesity/energy metabolism
G-protein	G protein alpha s ($G\alpha s$) $\rightarrow$ Adenylate Cyclase (AC) $\rightarrow$ Cyclic Adenosine Monophosphate (cAMP) $\rightarrow$ Protein Kinase A (PKA)	PKA phosphorylates Melanocortin-4 receptor (MC4R), strengthening its anorectic (appetite-suppressing) effect.
β-Arrestin	β-Arrestin-2 $\rightarrow$ Mitogen-Activated Protein Kinase (MAPK)/Extracellular signal-regulated kinase (ERK)	Mediates MC4R's G-protein-independent signaling, contributing to long-term regulation of energy balance.
Ca^{2+} pathway	Phospholipase C beta ($PLC\beta$) $\rightarrow$ Inositol 1,4,5-trisphosphate (IP_3)/Diacylglycerol (DAG) $\rightarrow$ Ca^{2+} release	Alters neuronal excitability, thereby modulating feeding impulses.
PI3K/Akt	Phosphoinositide 3-kinase (PI3K) $\rightarrow$ Protein Kinase B (Akt) $\rightarrow$ Mechanistic target of rapamycin (mTOR)	Interacts with leptin signaling and regulates hypothalamic energy sensing.

Table 2. Differences in mechanisms and signaling pathways between G protein-coupled and non-G protein-dependent signaling [32–34].

Features	G protein-coupled signaling	Non-G protein-dependent signaling
Core mediators	G protein subtypes such as $G\alpha$/$G\alpha i$/o	β-arrestin, GRK (G protein-coupled receptor kinase)
Signal initiation conditions	After ligand binding, it induces conformational changes in the receptor and activates G proteins	After the receptor is phosphorylated by GRK, it recruits β-arrestin to form a signaling complex
Main pathways	$G\alpha s$-cAMP-PKA (classical pathway); $G\alpha_{q/11}$-PLC-IP3/DAG (branch pathway)	β-arrestin-MAPK pathway; receptor endocytosis and formation of scaffold signaling complexes
Signal initiation speed	Fast (in seconds), such as rapid accumulation of cAMP	Slow (in minutes to hours), such as ERK phosphorylation regulating gene transcription
Signal duration	Transient (depending on cAMP degradation)	Persistent (continuous activation of endosomal signals)
Main effects	Acute appetite suppression and metabolic regulation	Regulation of gene expression and receptor recycling
Case associations	Inactivating mutations in MC4R lead to obesity	Signal imbalance is associated with insulin resistance

without inducing the blood pressure elevation side effects commonly observed in the classical Gs pathway.

MC4R also activates the Ca^{2+} signaling pathway, demonstrating its functional complexity [35]. Ca^{2+} can cooperate with α-MSH to activate MC4R, thereby inducing the closure of the inward-rectifying potassium channel (KIR7.1) to maintain intracellular potassium levels. This leads to an overall anorexigenic effect, manifested as enhanced satiety and reduced food intake. Conversely, **AgRP** can open KIR7.1 channels, leading to the efflux of K^+ ions from cells and thereby promoting appetite [34], as shown in Fig. 5C.

Leptin–Melanocortin Pathway is a key system for regulating energy balance and appetite, functioning through specific neurons and receptors in the hypothalamus. Under satiety conditions, adipose tissue secretes leptin, which activates the leptin receptor (LEPR) in the arcuate nucleus. After leptin binds to its receptor, it stimulates pro-opiomelanocortin (POMC) neurons to secrete α-MSH, which then activates MC4R in the paraventricular nucleus (PVN), generating satiety signals that suppress appetite while promoting energy expenditure. Additionally, MC4R inhibits the secretion of agouti-related protein (**AgRP**), an inverse agonist of MC4R, which is expressed by neuropeptide Y (NPY)/**AgRP** neurons in the ARC. In a state of fasting, the expression of NPY/**AgRP** increases, generating hunger signals [37], as shown in Fig. 5D.

As a member of the GPCR family, MC4R not only transmits signals through the classical $G\alpha s$/cAMP pathway, but also activates non-canonical downstream effector networks such as β-arrestin and MAPK, even exhibiting ligand-dependent signaling bias. These multidimensional and dynamic signal transduction characteristics enable MC4R to precisely coordinate feeding behavior, energy metabolism, and neuroendocrine regulation. However, such complexity poses challenges in pharmacological interventions, as traditional antagonists may induce off-target effects by broadly blocking all downstream pathways, for instance, in obesity treatment, appetite suppression may interfere with cardiovascular or reproductive system functions. Developing functional antagonists that can selectively modulate specific downstream pathways has become a central focus of future precision drug design.

Under the theoretical framework of "pathway-selective inhibition", the biased signaling of MC4R plays a critical role. Biased signaling refers to the phenomenon where specific ligands or receptor variants preferentially activate one of several downstream signaling pathways of the receptor [39]. Human genetic studies have confirmed that gain-of-function mutants, such as V103I and I251L, exhibit a characteristic β-arrestin bias: compared to the wild-type, these mutants show a significant increase in β-arrestin recruitment, while activation of the Gs-cAMP pathway is relatively diminished [40].

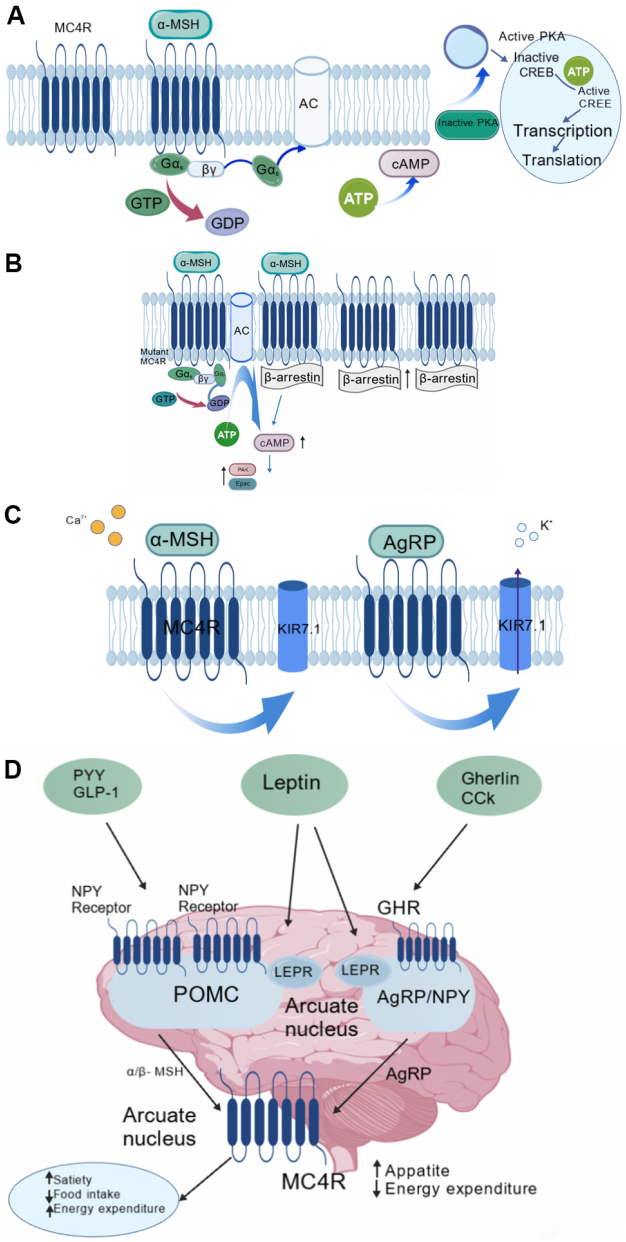

Fig. 5. Signaling pathways of MC4R [34,37]. (A) G protein-coupled signaling pathway. (B) β-arrestin pathway. (C) Ca^{2+} signaling pathway. (D) Leptin–melanocortin pathway. Created with BioGDP.com.

Molecular mechanism studies have shown that enhanced β-arrestin recruitment is accompanied by sustained phosphorylation of the MAPK/ERK1/2 pathway. In mutant cells, a higher and more persistent p-ERK signal is observed. This signaling network also attenuates the antagonistic effect of **AGRP** (Agouti-related peptide) on MC4R, making the mutants approximately two times less sensitive to **AGRP** inhibition, thereby enhancing satiety signaling at the level of hypothalamic POMC neurons [32]. Animal studies further support these findings: mice carrying the V103I or I251L mutation show a 5–8% reduction in body weight and a 10–15% decrease in fat tissue weight under standard housing conditions. Indirect calorimetry measurements reveal a 12% increase in their basal metabolic rate (VO_2), and they maintain a lower food intake even when subjected to high-calorie dietary interventions. Glucose tolerance (IP-GTT) and insulin tolerance (ITT) tests show that the blood glucose curve of the mutant mice is more stable, with enhanced insulin sensitivity [40].

Additionally, studies have reported that certain agonists preferentially activate the Gq/11 pathway rather than the Gs pathway. These ligands significantly reduce food intake by enhancing meal-related satiety, while avoiding full activation of the Gs-cAMP pathway, which leads to a significant reduction in cardiovascular side effects (e.g., blood pressure elevation) in preclinical models [41,42]. This suggests that activation of the β-arrestin pathway is sufficient to regulate energy homeostasis, and "decoupling" it from the Gs/cAMP pathway may be key to mitigating adverse effects.

In conclusion, from the molecular to the animal level, the V103I and I251L mutants provide comprehensive evidence that biased signaling mediated by the β-arrestin-ERK axis of MC4R regulates energy metabolism and confers obesity resistance. This lays a solid theoretical and experimental foundation for the development of drugs based on pathway-selective inhibition.

To achieve this precise design goal, future research methods must also be innovated: for example, single-cell analysis can be used to resolve the dynamics of MC4R signaling pathways across different cell types in spatial and temporal dimensions [43]; additionally, label-free assays and other non-invasive techniques can capture whole-cell responses to ligand stimulation, identifying unique biased signaling fingerprints [44]. These advancements will enable rational design of highly selective antagonists that target specific signaling branches, effectively controlling body weight while maximizing therapeutic safety.

4. Progress in MC4R Antagonists

MC4R plays a critical role in regulating appetite and metabolism. Its dysfunction is associated with diseases such as obesity, cachexia, and anorexia, making it a crucial target for drug development. This article primarily analyzes the research status of MC4R antagonists from two stages: preclinical and clinical.

4.1 Pharmacological Classification of MC4R Antagonists: Neutral Antagonists and Inverse Agonists

Before discussing specific MC4R antagonists, it is essential to clarify an important pharmacological subdivision: the distinction between neutral antagonists and inverse agonists. Although both can block the action of endogenous agonists (e.g., α-MSH), they have opposite effects on the receptor's basal activity, which directly determines their potential therapeutic application scenarios.

Table 3. Comparative pharmacological properties of MC4R ligands.

Compound	Type	Mode of action	Key activity data (IC_{50}/Ki)
AGRP (86-132) [47]	Endogenous peptide	Inverse agonist (reduces basal cAMP)	$IC_{50} \approx 10$ nM
SHU 9119 [51]	Synthetic peptide	Neutral antagonist (exhibits inverse-agonist activity in specific neurons and cellular contexts)	$IC_{50} \approx 30$ nM (inverse agonism)
MCL0020 [48]	Small molecule	Inverse agonist (suppresses basal signaling)	$IC_{50} \approx 11.6$ nM
ML00253764 [48]	Small molecule	Inverse agonist (suppresses basal signaling)	/
PF 07258669 [47]	Small molecule	Neutral antagonist (does not affect basal activity)	$IC_{50} \approx 13$ nM
TCMCB-07 [49]	Peptide	Neutral antagonist	$IC_{50} \approx 10$ nM (*in vitro*)
GSC000580 [50]	Small molecule	Neutral antagonist	IC_{50} <1 nM; selectivity ≈ 500–1000-fold
Ipsen 5i [16]	Small molecule	Inverse agonist (suppresses basal signaling)	Ki ≈ 2 nM

GPCRs generally exhibit a certain level of constitutive activity (i.e., basal activity), and MC4R is no exception [45]. Inverse agonists can actively suppress this basal activity, lowering receptor signaling below baseline levels; in contrast, neutral antagonists merely block the binding of exogenous agonists (e.g., α-MSH or **Setmelanotide**) without altering the receptor's basal activity [46]. This mechanistic difference leads to distinct physiological effects and therapeutic potentials: inverse agonists act by further reducing the basal activity of MC4R. Their endogenous counterpart, **AgRP**, can inhibit the MC4R Gs/cAMP signaling pathway and serves as a key "hunger signal" that promotes appetite [32]. In pathological conditions such as cachexia, inverse agonists can markedly increase food intake and body weight, demonstrating therapeutic value; neutral antagonists, on the other hand, restore appetite by "releasing" the inhibition imposed by exogenous agonists (e.g., α-MSH or **Setmelanotide**). Such ligands are suitable for scenarios that simply require blocking excessive agonist signaling, such as cancer-related anorexia or unintended weight loss in the elderly. For example, the small-molecule oral antagonist **PF-07258669** (which has entered Phase I clinical trials) significantly increased body weight in cachexia animal models in preclinical studies, without affecting the receptor's constitutive activity and with good safety [47].

In summary, clearly distinguishing inverse agonists from neutral antagonists provides key insights for developing MC4R-targeted drugs that address different pathophysiological mechanisms. In recent years, numerous MC4R ligands with well-defined pharmacological properties have been reported, and their diversity is summarized in Table 3 (Ref. [16,47–51]).

4.2 Preclinical MC4R Antagonists

4.2.1 Non-Selective Antagonists

The primary objective of early studies was to validate the feasibility of MC4R as a therapeutic target rather than pursuing subtype selectivity, therefore, researchers have prioritized the development of compounds with antagonistic activity, while placing relatively lower requirements on selectivity. During this phase, researchers developed a series of non-selective MC4R antagonists (e.g., **SHU9119**).

SHU9119, as an antagonist in early studies, exhibits inhibitory effects on both melanocortin receptors (MC3R and MC4R), thereby leading to off-target effects. Research has shown that **SHU9119** was initially designed as a non-selective antagonist capable of acting on both MC3R and MC4R, demonstrating significant pharmacological effects in obesity models and acute inflammation models in mice. The primary mechanism of action is primarily through competitive binding to MC3R and MC4R, blocking the activation of endogenous ligands such as α-MSH, thereby inhibiting the activity of these receptors [52]. Experimental results in animal models demonstrated that **SHU9119** increased food intake only when administered via intracerebroventricular (ICV) injection, while peripheral administration showed no effect on food consumption [53]. This suggests that **SHU9119** may not be able to effectively cross the blood–brain barrier, or it cannot activate the signal pathways related to appetite regulation in peripheral tissues, further confirming the importance of MC4R in the mechanism of action of **SHU9119**. With the progression of research, scientist have discovered that **SHU9119** exhibits high antagonistic activity against both MC3R and MC4R, but shows lower selectivity for MC4R [54]. Furthermore, research has shown that **SHU9119** not only exhibits antagonistic effects on MC4R but also acts as an agonist for MC1R [28]. This cross-reactivity may lead to non-specific side effects such as metabolic disturbances, mood disorders, and increased appetite [55]. Its structure is shown in Fig. 6 (Ref. [51]).

Early compounds demonstrated significant antagonistic activity against MC4R, but exhibited cross-reactivity with other melanocortin receptors (e.g., MC1R, MC3R). Through studies on early-stage compounds, researchers not only validated the feasibility of MC4R as a drug target, but also provided critical structural foundations and mechanistic insights for subsequent MC4R-targeted drug design.

4.2.2 Highly Selective MC4R Antagonists

Although non-selective antagonists have successfully validated the therapeutic potential of MC4R, their cross-reactive inhibitory activity against subtypes such as MC1R and MC3R leads to off-target effects (e.g., metabolic dis-

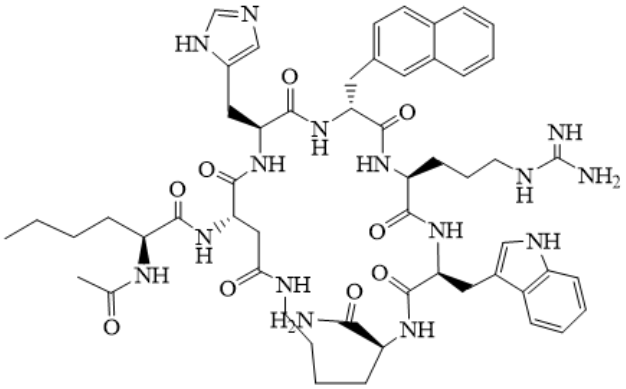

Fig. 6. The structure of SHU9119 [51].

orders, anxiety-like behaviors), severely limiting clinical translation. The root of this issue lies in the high sequence homology of the melanocortin receptor family (MC1R-MC5R) [56,57], particularly in the transmembrane regions of MC3R and MC4R, which exhibit particularly high homology, making it challenging for traditional phenotypic screening-based compounds to achieve selective binding. The research focus has shifted to the development of highly selective MC4R antagonists based on this, aiming to circumvent interference from MC3R and other subtypes through rational design, while optimizing pharmacokinetic properties (such as brain permeability and metabolic stability) to meet the long-term therapeutic requirements for chronic disease treatment.

Supported by structural biology, Structure-Based Drug Design (SBDD) has become a critical strategy for developing highly selective MC4R antagonists. By leveraging the crystallographic data of MC4R, researchers can precisely identify and optimize the interactions between small molecules and the receptor. Specifically, these strategies include molecular docking, virtual screening, and structural optimization strategies, which are used to design and optimize antagonists with high selectivity and good pharmacokinetic properties. The application of these methods not only accelerated the discovery of novel antagonists but also provided crucial insights into understanding the structure-function relationship of MC4R.

HS014 (Fig. 7A), as a synthetic cyclic MSH (melanocyte-stimulating hormone) analog, is a selective MC4R antagonist. It suppresses MC4R, thereby reducing the appetite-suppressing effect induced by α-MSH, leading to increased food intake. Research indicates that **HS014** significantly increases food intake in rats and leads to body weight gain, suggesting that its antagonistic effect on MC4R interferes with normal satiety regulation [58,59]. In free-feeding experiments, **HS014** significantly increased food intake in a dose-dependent manner. For example, After ICV injection of **HS014** at doses ranging from 0.33 to 3.3 nmol, food intake increased within the first hour, and the cumulative intake reached its peak (100%) after

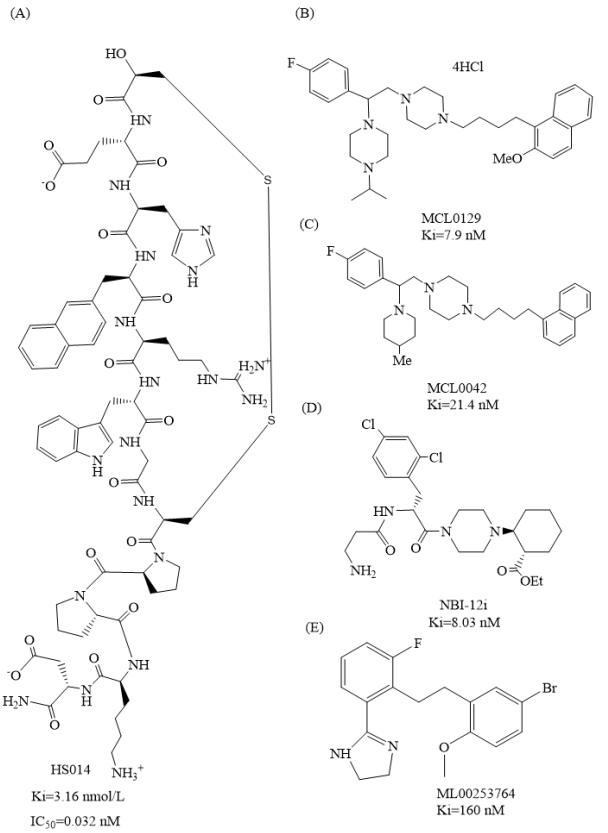

Fig. 7. Structures of highly selective MC4R antagonists. Panels (A–E) show the chemical structures of the five compounds **HS014**, **MCL0129**, **MCL0042**, **NBI-12i**, and **ML00253764**, along with their corresponding affinity values.

four hours [58]. Additionally, **HS014** can induce significant weight gain and fat deposition. In a long-term experiment, rats receiving continuous infusion of **HS014** via a permeable micro-pump showed a 20% increase in body weight compared with the control group, and their blood glucose levels were significantly elevated, suggesting that this compound may exacerbate obesity by promoting disordered lipid metabolism [59], long-term use of **HS014** can lead to significant obesity and metabolic disorders.

HT1-0 is a linear peptide extracted from conotoxin, it exhibits the property of competitive antagonism on G protein-coupled receptors. Specifically, it acts as an MC4R antagonist with relatively lower binding affinity, but requires the combination of hydrophobic groups and negatively charged residues to bind to MC4R. This demonstrates that **HT1-0** is a unique high-affinity ligand, representing the first MC4R antagonist derived from snake venom peptides, which provides a new direction for the development of novel MC4R antagonists [60].

Highly selective MC4R antagonists can also be achieved through non-peptidic compounds. Studies have identified **MCL0129** and **MCL0042** as potent and selective MC4R antagonists, which function through MC4R signaling pathways and exhibit unique pharmacological prop-

erties. In animal models, they demonstrate anxiolytic and antidepressant effects.

MCL0129 (Fig. 7B) is a novel MC4R antagonist developed by Taisho Pharmaceutical Co., Ltd. (Tokyo, Japan), currently at the preclinical stage as its highest development phase. **MCL0129** exerts its effects by inhibiting the binding of α-MSH to the MC4R receptor, demonstrating high selectivity for MC4R (Ki = 7.9 nM) while showing negligible affinity for MC1R and MC3R [60]. **MCL0129** demonstrated significant efficacy in various rodent models of anxiety and depression. For example, in the swimming stress-induced model, mice were subjected to 10 min of swimming in water, followed by a light-dark exploration test. The result demonstrated that swimming stress significantly reduced the time mice spent in the bright area of the light-dark exploration test; Additionally, rats were subjected to 2 min of swimming, followed by the elevated plus maze test. The result indicated that swim stress significantly reduced the time rats spent in the open arms of the maze. These two experimental results demonstrate that **MCL0129** can reverse stress-induced anxiety-like behavior [61]. In the marble burying test in mice, **MCL0129** significantly reduced marble burying behavior. Other known anxiolytic drugs (such as diazepam) also significantly reduced this behavior [60], this further demonstrates the anxiolytic and antidepressant effects of **MCL0129**. However, due to the lack of data from human clinical trials, its specific effects and side effects in humans still require further research.

MCL0042 (Fig. 7C) is a novel compound developed by Taisho Pharmaceutical Co., Ltd. Currently, the drug's highest development stage is preclinical, intended for the treatment of anxiety and depression. **MCL0042** is a non-peptide MC4R antagonist that exerts its effects by antagonizing the MC4 receptor and inhibiting the serum transporter [61]. **MCL0042** demonstrates significant efficacy in animal models of anxiety and depression. For example, in swimming stress-induced animal models, **MCL0042** administration reduces the time spent with arms extended by swimming-stressed rats in the elevated plus maze task [62], demonstrating its anxiolytic potential; in the olfactory bulbectomy (OBX)-induced rat model, OBX rats exhibited a significant increase in locomotor activity. Administration of **MCL0042** significantly attenuated the locomotor activity in OBX rats [62], suggesting that **MCL0042** possesses antidepressant-like potential.

NBI-12i (Fig. 7D) is a small-molecule MC4R antagonist characterized by high affinity and selectivity, it exhibits nanomolar affinity for the MC4R receptor (Ki = 8.03 nM) with 30 to 200-fold selectivity over other melanocortin receptor subtypes [63], and can penetrate the central nervous system following peripheral administration, by blocking MC4R, it significantly ameliorated the cachexia state in uremic mice, including increased food intake, body weight, and body composition, while reducing basal metabolic rate. Its mechanism of action may involve the regulation of appetite signals in the central nervous system and energy metabolism in brown adipose tissue. This study provides an important theoretical basis for developing novel anti-cachectic drugs targeting MC4R [64].

ML00253764 (Fig. 7E) (developed by Millennium Pharmaceuticals, Inc., Cambridge, MA, USA) is an MC4R antagonist that, besides its potential applications in obesity research, has increasingly attracted attention for its role in oncology. This compound exhibits low affinity for MC4R (Ki = 160 nM) and displays inverse agonist activity in the Gs-cAMP pathway, selectively inhibiting both wild-type and various constitutively active human MC4R forms [16]. Notably, its pharmacological effects show marked species specificity: in humans and mouse models it primarily acts as an antagonist/inverse agonist, whereas in fish MC4R (e.g., grass carp and zebrafish) it functions as an agonist, an effect that can be competitively blocked by other ligands [48,64]. In tumor biology, functional expression of MC4R has been confirmed in several malignancies. For instance, in glioblastoma (GBM), targeted inhibition of MC4R blocks the ERK1/2 and Akt signaling pathways, producing anti-proliferative and pro-apoptotic effects; moreover, **ML00253764** combined with temozolomide shows remarkable synergistic efficacy in preclinical studies [65]. Similarly, in melanoma models, MC4R antagonism suppresses ERK1/2 phosphorylation and down-regulates BCL-XL, thereby inhibiting tumor growth. The combination of **ML00253764** with the BRAF inhibitor vemurafenib demonstrates strong synergistic activity both *in vitro* and *in vivo*, with preclinical safety assessments revealing no significant toxicity and indicating a favorable safety profile [66]. In summary, **ML00253764** is a pharmacologically complex MC4R modulator that not only serves as a crucial tool for elucidating the diverse functions of the melanocortin system but also holds significant translational value in cancer therapy, especially within combination treatment strategies.

Studies have shown that highly selective MC4R antagonists demonstrate potential therapeutic value in treating obesity, depression, and anxiety disorders. Although highly selective MC4R antagonists hold great theoretical potential, they still face several challenges in practical applications. The current comprehensive evaluation of various MC4R subtypes remains insufficient, and the biological activity and selectivity of some compounds still require further validation. Furthermore, how to balance the selectivity and efficacy of antagonists to avoid adverse reactions remains an important research direction [67]. In summary, significant progress has been made in the design and development of highly selective MC4R antagonists, but further research is still required to overcome existing challenges and fully realize their therapeutic potential in disease treatment.

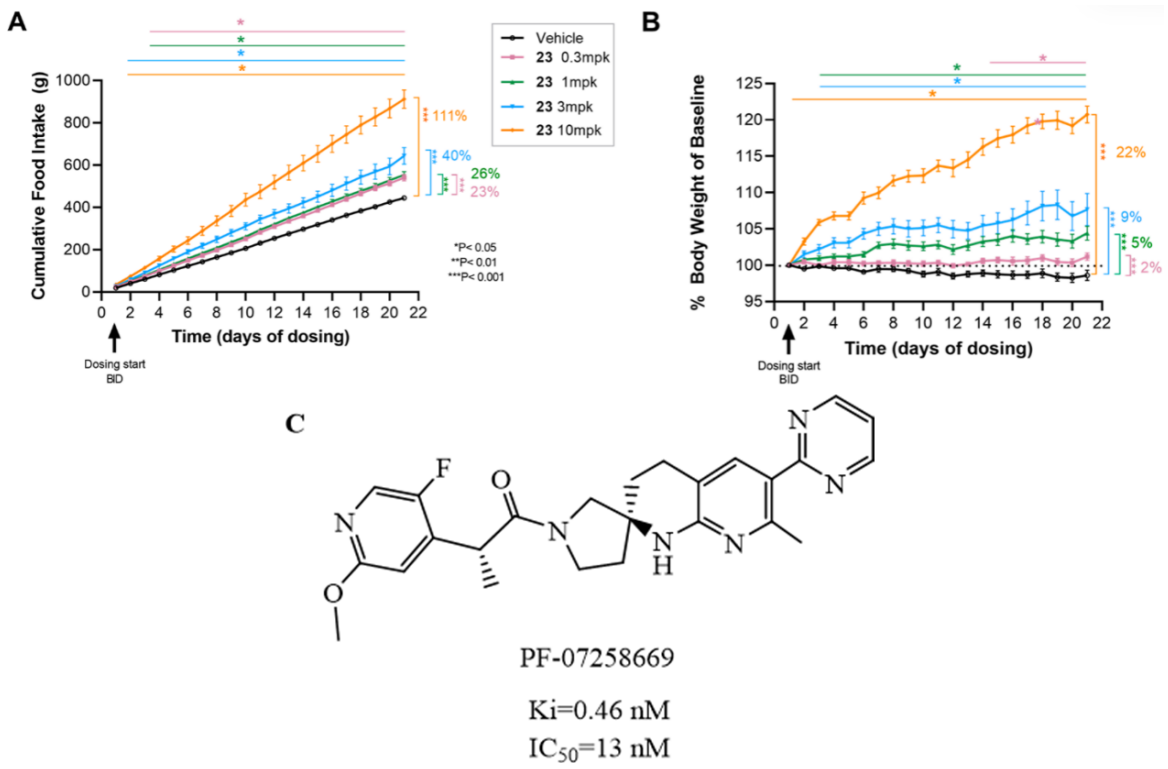

Fig. 8. Effects of different doses of PF-07258669 on food intake and body weight in rats and the structure of PF-07258669 [47]. (A) Cumulative food intake since the baseline of self-pretreatment. (*$p < 0.05$, ***$p < 0.001$ vs Vehicle) (B) Percentage change in body weight since the baseline of self-pretreatment. (*$p < 0.05$, ***$p < 0.001$ vs Vehicle) (C) The chemical structure and affinity values of **PF-07258669**. ("Reproduced with permission from Ref. [47]. © 2023 American Chemical Society.")

4.3 Clinical-Stage MC4R Antagonists

In research on MC4R antagonists, certain compounds have garnered notable attention due to their unique pharmacological effects and promising clinical application prospects. These compounds not only demonstrate significant efficacy in regulating appetite and energy metabolism, but also hold potential value in the treatment of various diseases. The following section will focus on elaborating the pharmacological effects and clinical development progress of two representative MC4R antagonists in clinical stages (e.g., **PF-07258669** and **TCMCB07**).

PF-07258669 (Fig. 8C) (Ref. [47]) is an innovatively designed MC4R antagonist. Developed by Pfizer as a small-molecule drug, it aims to treat appetite loss-related disorders by targeting the MC4R signaling pathway. Currently, the drug's highest development stage is Phase I clinical trial, intended for the treatment of nutritional deficiency. The design of **PF-07258669** employs a spirocyclic structure, which not only optimizes the pharmacological efficacy of the MC4 receptor antagonist but also improves ADME (absorption, distribution, metabolism, and excretion) properties, while avoiding the endogenous hormone-activating activity (hERG) observed in earlier series. For example, by optimizing the spirocyclic structure, the antagonist **PF-07258669** was ultimately obtained, which exhibits potent MC4R antagonist activity (IC_{50} = 13 nM, K_i = 0.46 nM) and excellent metabolic stability; and exhibits a lower MDR efflux ratio (MDR efflux ratio of 4.7), which is beneficial for brain penetration; it does not produce hERG-active metabolites, significantly reducing the associated safety risks [47]. Additionally, **PF-07258669** demonstrated potent efficacy in animal models, particularly exhibiting significant appetite stimulation and weight gain effects in aged rat models. For example, after orally administering **PF-07258669** at different doses of 0.3, 1, 3, and 10 mg/kg twice daily to rats for 20 days, compared to the control group, the cumulative food intake in each dose group increased by 23%, 26%, 40%, and 111%, respectively, the body weight increased by 2%, 5%, 9%, and 22% respectively [47], as shown in Fig. 8A,B (Ref. [47]). From a clinical development perspective, **PF-07258669** is currently undergoing Phase 1 clinical trials, aimed at evaluating its safety, pharmacokinetics, and pharmacodynamics risks in healthy adult subjects [47]; this indicates that the safety and effectiveness of the drug in humans are being further validated, and it may potentially become a candidate drug for treating cachexia-related diseases in the future.

Bile acid transport peptides are an emerging class of peptide therapeutics that have attracted attention because they can be actively transported by multispecific bile acid transporters and have the potential to cross the blood–brain barrier. However, the generally rapid hepatic clear-

ance of these peptides severely limits their druggability. **TCMCB07** (Endevica Bio Inc., Northbrook, IL, USA) represents a breakthrough case in this context. As a member of the bile acid peptide family, it incorporates a clever modification of the C-terminal amino-acid sequence that slows hepatic clearance [68]. This key modification not only addresses the core bottleneck of this peptide class but also provides a platform approach that can be applied to other similar peptides, opening a new pathway for developing drug-like peptides with desirable pharmacokinetic properties.

TCMCB07 (Fig. 9, Ref. [69]) is a cyclic nonapeptide MC4R antagonist developed by Endevica Bio Inc. that is currently in Phase II clinical trials for the treatment of cachexia. Its mechanism of action involves inhibition of central MC4R signaling, which stimulates appetite and improves energy balance. Additionally, it has been shown to reduce the expression of inflammatory genes in the hypothalamus [70]. Evidence in inflammatory models: For example, in a lipopolysaccharide (LPS)-induced systemic inflammation model, LPS triggers a severe cytokine storm that leads to multi-organ damage [71]. Studies have demonstrated that, regardless of administration route—intraperitoneal, subcutaneous, or oral—**TCMCB07** significantly improves 24-hour food intake and body-weight changes in LPS-treated rats, confirming its efficacy under acute inflammatory conditions. Effects in cachexia disease models: In a cancer-associated cachexia model, subcutaneous injection of **TCMCB07** markedly suppresses the expression of several hypothalamic inflammatory genes (such as *Il1b*, *Il1r1*, *Il6*, and *Selp*), consistent with its anti-inflammatory action. In a chronic kidney disease (CKD) cachexia model, although typical inflammatory gene expression changes are not pronounced, **TCMCB07** treatment significantly attenuates the up-regulation of the Agrp gene, which may represent a key mechanism by which it improves appetite through a different pathway [70]. These preclinical studies, which demonstrate improvements in feeding, body weight, and the regulation of neuroinflammation and appetite signaling, collectively reveal the therapeutic potential of **TCMCB07** and provide a theoretical basis for further investigation in human patients.

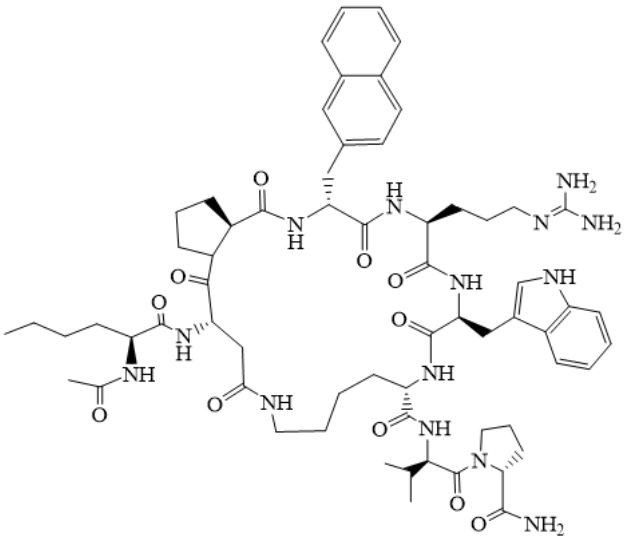

Fig. 9. Structure of TCMCB07 [69].

5. Challenges and Future Directions

Research on MC4R antagonists has achieved significant progress in structural elucidation, selectivity optimization, and preclinical evaluation, revealing the pivotal role of MC4R in energy metabolism and appetite regulation, while providing a structural foundation for the design of novel antagonists. However, there are still challenges such as enhancing subtype-specific selectivity, optimizing pharmacokinetic properties (including blood–brain barrier permeability and metabolic stability), and achieving personalized treatment strategies. Future research should focus on optimizing the subtype selectivity of antagonists through structural biology and computational chemistry, exploring novel strategies to enhance pharmacokinetic performance, and developing precision treatment regimens integrated with individualized medicine to promote breakthroughs in clinical applications.

5.1 Strategies for Enhancing MC4R Subtype Selectivity

Melanocortin receptors are key regulators of energy balance, and the tissue distribution and functional differences among their multiple subtypes pose challenges for the development of subtype-selective antagonists. Current research primarily focuses on the development of subtype-specific ligands, rational design based on cryo-EM structures, and the development of allosteric modulators and dual-target antagonists.

Subtype-specific ligands achieve specific binding to target subtypes through screening and optimization, thereby reducing side effects and enhancing therapeutic efficacy. For example, certain ligands can achieve higher selectivity by binding to non-conserved regions [72]. However, due to the high structural homology of MC4R with other subtypes, traditional drug design approaches often rely on low-resolution models and empirical screening methods, making it particularly challenging to accurately identify and target the unique structural features of MC4R. The advancements in cryo-electron microscopy (cryo-EM) technology have provided revolutionary tools for the structural elucidation of MC4R. Its high-resolution three-dimensional structure has revealed key binding pockets and allosteric sites, enabling researchers to perform rational design at the atomic level [73,74]. Based on these structural insights, researchers have initiated the exploration of developing allosteric modulators and dual-target antagonists to further enhance the selectivity and pharmacological efficacy of MC4R antagonists. Additionally, combining allosteric modulators with traditional antagonists can further enhance the drug's selectivity and therapeutic efficacy [74].

These strategies have been successfully applied to the drug development of other GPCRs, providing new opportunities for the innovative design of MC4R antagonists [75–77].

5.2 Key Issues in Translational Medicine

Despite progress in basic research and drug design, the clinical translation of MC4R antagonists still faces challenges, including optimization of blood–brain barrier penetration, personalized therapy, and biomarker research.

MC4R is highly expressed in the central nervous system, and developing drugs capable of penetrating the blood–brain barrier is crucial. Traditional small molecules and peptide compounds have limitations in penetrating the blood–brain barrier, resulting in insufficient drug concentration in the brain and compromising therapeutic efficacy [78]. By optimizing the chemical structure of drug molecules (e.g., introducing lipophilic groups), their ability to cross the blood–brain barrier can be enhanced. Furthermore, due to individual variations in MC4R subtype expression, it is particularly important to develop individualized treatment regimens based on genotype and receptor expression levels. Biomarker research aids in predicting therapeutic outcomes and adverse reactions, thereby promoting precision medicine advancements.

In summary, research on MC4R antagonists should focus on enhancing subtype selectivity, optimizing pharmacokinetic properties, and addressing key challenges in translational medicine to overcome the limitations of existing drugs and provide novel therapeutic solutions for the treatment of obesity, cachexia, metabolic disorders, and related diseases.

6. Discussion

MC4R, as a key receptor in the melanocortin system, plays a crucial role in regulating energy balance, body weight control, and appetite. MC4R antagonists can improve appetite decline and weight loss in patients with anorexia or cachexia by blocking its activation. In early studies, non-selective MC4R antagonists such as **SHU9119** were widely used to investigate the physiological roles of MC4R, including its effects on feeding behavior and energy homeostasis, which validated the feasibility of targeting MC4R. However, due to the activity of **SHU9119** on other melanocortin receptors, off-target effects have limited its clinical application. To overcome the limitations of non-selective antagonists, researchers have developed selective MC4R antagonists. For example, compounds such as **HS014**, **MCL0129**, and **MCL0042** have been demonstrated to be selective for MC4R and exhibit potential therapeutic value in treating obesity, depression, and anxiety disorders. Recently, studies have found that **PF-07258669** and **TCMCB07** demonstrate broad therapeutic potential in the treatment of cachexia and related diseases.

However, Cachexia is characterized not only by loss of appetite and weight loss, but also by significant muscle wasting and emotional disorders such as anxiety and depression. Recent studies have shown that MC3R plays an important role in the development of cachexia. Unlike MC4R, which primarily regulates appetite, MC3R agonists can both stimulate feeding and alleviate negative emotions such as anxiety during treatment; conversely, MC3R antagonists suppress feeding [79]. This suggests that MC3R may be a novel therapeutic target. Therefore, an ideal cachexia treatment strategy should act on both MC4R and MC3R: using an MC4R antagonist to increase appetite while modulating MC3R signaling to improve anxiety, depression, and other symptoms.

Although MC4R antagonists exhibit potential in treating conditions such as appetite regulation, they still face challenges including insufficient selectivity, poor blood–brain barrier penetration, low metabolic stability, unclear dosing and administration regimens, and a lack of effective biomarkers to guide personalized treatment. Future research should focus on developing safer, more efficient, and personalized MC4R antagonists while exploring individualized treatment strategies to achieve more precise disease intervention.

7. Conclusions

This review systematically dissects the central role of MC4R in the regulation of energy balance and argues for the therapeutic potential of MC4R antagonists in alleviating disease-related anorexia, such as that seen in cachexia. At the same time, we recognize that the pathophysiology of cachexia is far more complex than merely reduced appetite; it also involves independent pathways mediated by MC3R that drive muscle wasting. To overcome the current challenges of MC4R-targeted therapy—such as selectivity and blood–brain barrier penetration—and to achieve comprehensive and effective management of cachexia, the MC4R field needs to focus on structure-based drug design, develop biomarker-guided personalized strategies, and actively explore combinatorial treatments targeting both MC4R and MC3R. Through these efforts, a novel, integrated therapeutic solution for cachexia may become attainable.

Author Contributions

JS was responsible for the overall conceptualization of the review, literature search and drafting of the initial manuscript. JX was responsible for the overall conception and design of this review and conducted multiple rounds of critical revisions of the key content. CH contributed to the review's design and the preparation of figures and tables. LH was primarily responsible for the creation of the figures and diagrams, including the key structural and mechanistic illustrations in this review. YC, as the corresponding author, oversaw the overall conceptualization of the review, the final manuscript approval, responses to reviewer comments, and communication with the editorial office. All authors have read and approved the final manuscript, ensuring

the authenticity and integrity of the research content. All authors contributed to editorial changes in the manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

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Conflict of Interest

Despite Ling Huang being employed by Grand Medical Nutrition Science (Wuhan) Co., LTD., the judgments in data interpretation and writing were not influenced by this relationship. The remaining authors declare no financial conflicts of interest that could have influenced the results of this study.

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