

REVIEW ARTICLE

Melanocortin-4 receptor complexity in energy homeostasis, obesity and drug development strategies

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Abstract

The melanocortin-4 receptor (MC4R) has been critically investigated for the past two decades, and novel findings regarding MC4R signalling and its potential exploitation in weight loss therapy have lately been emphasized. An association between MC4R and obesity is well established, with disease-causing mutations affecting 1% to 6% of obese patients. More than 200 MC4R variants have been reported, although conflicting results as to their effects have been found in different cohorts. Most notably, some MC4R gain-of-function variants seem to rescue obesity and related complications via specific pathways such as beta-arrestin (β -arrestin) recruitment. Broadly speaking, however, dysfunctional MC4R dysregulates satiety and induces hyperphagia. The picture at the mechanistic level is complicated as, in addition to the canonical G stimulatory pathway, the β -arrestin signalling pathway and ions (particularly calcium) seem to interact with MC4R signalling to contribute to or alleviate obesity pathogenesis. Thus, the overall complexity of the MC4R signalling spectra has broadened considerably, indicating there is great potential for the development of new drugs to manage obesity and its related complications. Alpha-melanocyte-stimulating hormone is the major endogenous MC4R agonist, but structure-based ligand discovery studies have identified possible superior and selective agonists that can improve MC4R function. However, some of these agonists characterized in vitro and in vivo confer adverse effects in patients, as demonstrated in clinical trials. In this review, we provide a comprehensive insight into the genetics, function and regulation of MC4R and its contribution to obesity. We also outline new approaches in drug development and emerging drug candidates to treat obesity.

KEYWORDS

Ca²⁺, drug design, G_s, MC4R, obesity, β -arrestin

1 | INTRODUCTION

Obesity is characterized by excess fat mass, which affects physical health and increases the complexity of many associated diseases and

conditions, including type 2 diabetes, cardiovascular complications and cancer.¹ The associated healthcare costs are huge, and obesity is accompanied by significant morbidity and mortality. Obesity is defined in terms of body mass index (BMI), that is, weight (kg)/height (m²);

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people with a BMI of more than 30 kg/m² are considered obese, while those with a BMI of 35 to 39.9 30 kg/m² are severely obese.^{2–5} The intake of high-calorie food and a lack of sufficient physical activity leads to an increased positive energy balance and subsequent weight gain. In addition, studies have suggested there is a strong genetic influence on obesity, involving a complex neurochemical system that regulates appetite, energy expenditure and weight loss. There is an overall synergistic relationship between genes, the environment and lifestyle.⁶

The identification of genes involved in regulating body weight and maintaining energy homeostasis is crucial. Major genes associated with obesity include leptin, leptin receptor, proopiomelanocortin (*POMC*), proprotein convertase subtilin/kexin (*PCSK1*), adenylate cyclase 3, single-minded 1 (*SIM-1*), tyrosine kinase receptor tropomyosin-related kinase B (*TRKB*), brain-derived neurotrophic factor (*BDNF*), and melanocortin-4 receptor (*MC4R*).^{7,8} *PCSK1* and *POMC* are critical in children with obesity, while *MC4R* is the most significant gene for obesity overall and the most widely investigated so far.^{9–13} *MC4R* is localized at chromosome 18q22 and primarily expressed in the hypothalamus, encoding a 332-amino acid transmembrane protein.¹⁴ *MC4R* is a member of the heterotrimeric G-protein-coupled receptor (GPCR) family, and its primary functions are in regulating the intake of food, energy homeostasis and body weight.^{15,16}

The genetic basis of obesity was first discovered when disruption of the mouse *MC4R* was found to cause the accumulation of excess weight.¹⁷ Several reports of frameshift mutations in human *MC4R*s and their association with obesity were subsequently confirmed,^{18–22} with 17, 58 and more than 200 human *MC4R* mutations identified by the years 2000,^{23,24} 2006²⁵ and 2021,^{26–28} respectively. These mutations can cause partial or complete loss of function depending on the nature of the mutation and function of the mutated receptor.²⁵ Heterozygous *MC4R* variants alone are found in 1% to 6% of the obese population, and are particularly common in early-onset or childhood obesity, with variable penetrance and expressivity resulting in mild to severe manifestations of obesity and associated complications.^{7,16,29,30} Homozygous variants are reported less frequently but result in more severe manifestations of obesity.^{31,32} In recent studies, *MC4R* signalling has been implicated as a viable target for antiobesity treatment.^{10,13,33–36} For example, certain human *MC4R* variants are protective against obesity, and drugs targeting the receptor have shown efficacy for weight loss.

We discuss in the subsequent sections how *MC4R*, as a GPCR, relates to obesity. We begin with a brief introduction to GPCRs to promote a general understanding of the receptors. Then, *MC4R*-signalling pathways, including the broader, centrally regulated melanocortinergic system, the canonical G-stimulatory (G_s)-signalling pathway, and the newly proposed roles of the β -arrestin and calcium (Ca²⁺) pathways, are detailed. We also discuss the loss of function/gain of function (GoF) caused by mutations of the *MC4R* and how this affects or alleviates disease pathology. The crystal structure of *MC4R*, the importance of ion-binding sites, and the differences between *MC4R* and other GPCRs are discussed while focusing on the unique features of the receptor. Finally, we discuss in detail the development of *MC4R* agonist drugs and the current status of existing obesity drugs.

2 | MELANOCORTIN-4 RECEPTOR AS A GPCR

G-protein-coupled receptors are involved in most physiological functions in the body and in the manifestation of numerous diseases.³⁷ They represent the largest family of cell surface and transmembrane receptors, with more than 825 genes (~2% of the human genome) and corresponding gene products.^{38,39} The structure of GPCRs, which is largely conserved, comprises seven transmembrane glycoproteins spanning the plasma membrane,⁴⁰ with additional extracellular N-terminal and intracellular C-terminal domains. The overall structure of GPCRs enables them to receive extracellular stimuli (eg, ions or peptides), to communicate via the otherwise impermeable plasma membrane, and to transfer stimuli to the interior of cells to induce functional changes. These processes involve a series of protein interactions and changes in the expression levels of biochemical mediators to ultimately effect physiological or even behavioural changes. GPCRs are considered very clinically significant protein targets; of the 1500 drugs approved by the US Food and Drug Administration (FDA) by 2020, 460 targeted GPCRs.^{39,41} Among them, Class A GPCRs (rhodopsin-like receptors) are major drug targets (94%), followed by Class B (secretin family, 4%), Class C (metabotropic glutamate receptors, 2%), and Class F (frizzled and smoothed receptors, 2%).³⁹

The *MC4R* is a rhodopsin-like Class A GPCR, expressed in the paraventricular nucleus of the hypothalamus, and is a key component of the leptin-melanocortin pathway.¹⁷ *MC4R* is activated by *POMC*-derived polypeptides obtained by the posttranslational processing of *POMC* that yields alpha (α)-, beta (β)- and gamma (γ)-melanocyte-stimulating hormone (MSH) and adrenocorticotrophic hormone (ACTH). α -MSH is very effective in regulating eating behaviour and energy homeostasis, and in addition to its primary function in melanogenesis, it also activates all (*MC5R*, *MC4R*, *MC3R* and *MC1R*) but one (*MC2R*) melanocortin receptors. Of these, *MC4R* is the most crucial, as mutations in this receptor cause different forms of obesity in humans.^{15,42} The role of *MC3R* in energy homeostasis and in regulating satiety is also known and is under active investigation.^{6,43–45} It is a key component of the central melanocortin pathway.⁴⁶ *MC5R* is involved in fatty acid and lipid metabolism as well as exocrine secretion.⁶

3 | MELANOCORTIN-4 RECEPTOR SIGNALLING PATHWAYS

The next sections comprise an overview of the *MC4R*-signalling pathways, including the leptin-melanocortin pathway, followed by a more focused discussion of the specific details of G-protein-signalling pathways and, finally, a review of the latest proposed changes, particularly regarding the mechanisms of energy regulation via the β -arrestin and Ca²⁺-regulated pathways. The leptin-melanocortin pathway includes the overall, centrally (central nervous system) regulated pathway responses to anorectic/orectic signals. The canonical G_s pathway and the generation of cyclic adenosine monophosphate (cAMP) have been widely investigated for obesity-related/energy balance issues, and the

mechanisms have been fairly understood.^{16,47–49} However, the recently proposed involvement of β -arrestin in the regulation of MC4R, often referred to as β -arrestin bias, is a bias or shift toward recruiting β -arrestin as the mode of action,^{10,13} rather than canonical cAMP production via the G_s pathway. The role of Ca^{2+} , another new discovery,³⁵ is also detailed below.

3.1 | LEPTIN-MELANOCORTIN PATHWAY

Under a fed state, adipose tissues secrete the hormone leptin. Binding of leptin to the leptin receptor stimulates POMC neurons to secrete α -MSH, which binds to the MC4R, resulting in a satiety signal and hence reduced appetite and a signal to increase energy expenditure to achieve a reduced energy balance.^{50–52} MC4R, however, can be blocked by the inhibitor, agouti-related peptide (AgRP), expressed by neuropeptide Y (NPY) neurons in the arcuate nucleus.⁵ Lack of food induces the increased expression of NYP/AgRP, resulting in hunger signals. A balance between these two hormones is, therefore, critical in regulating food intake and energy metabolism. A few other molecules included in this pathway that are not yet fully elucidated include SIM-1, TRKB and BDNF. In brief, SIM-1 is a transcription factor that causes hyperphagia and a reduction of the periventricular nucleus, resulting in severe obesity.⁵³ Mutations in BDNF, which is involved in downstream MC4R signalling, and its receptor, TRKB, also contribute to hyperphagia and obesity.⁵⁴

3.2 | G-PROTEIN SIGNALLING PATHWAY

G-proteins are complex heterotrimeric guanine-binding proteins with G-alpha ($G\alpha$), G-beta ($G\beta$), and G-gamma ($G\gamma$) subunits. G-proteins are also classified into four distinct groups according to the $G\alpha$ subunits, $G\alpha_s$, $G\alpha_{i/o}$, $G\alpha_{q/11}$ and $G\alpha_{12/13}$.^{34,53} In G-protein-signalling pathways

(Figure 1), an exchange of guanosine triphosphate (GTP) for guanosine diphosphate (GDP) induces the dissociation of the α subunit from the $\beta\gamma$ dimer following communication with effectors.⁵⁵

Alpha melanocyte stimulating hormone activates MC4R and catalyses the exchange of GDP for GTP on the stimulatory G-protein ($G\alpha_s$), resulting in the activation of adenylyl cyclase (AC) and the generation of intracellular cAMP.⁵⁶ cAMP, which may also be triggered by $G\beta\gamma$ subunits to increase AC activity via certain isozymes,^{57,58} binds to protein kinase A (PKA) regulatory subunits, causing its dissociation and distribution to different cellular compartments. Activated PKA further affects numerous physiological processes by activating subsequent effector proteins (mostly via phosphorylation), including kinases, ion channels, and other signalling proteins/enzymes.^{13,49} cAMP-mediated transcriptional regulation is achieved via the binding of active PKA to the cAMP response element binding protein, causing PKA phosphorylation and the downstream transcription and translation of target genes and proteins. Coupling to $G\alpha_{i/o}$ deactivates or reduces AC activity, lowering cAMP levels.⁵⁶ $G\alpha_{q/11}$ and $G\alpha_{12/13}$ are mostly associated with functions other than obesity/energy homeostasis, such as the activation of phospholipase C.⁵⁹

In addition, MC4R also activates mitogen-activated protein kinases (MAPK) and extracellular signal-regulated kinases 1 and 2 (ERK1/2). Mo et al⁶⁰ reported various MC4R ligands, including AgRP and Ipsen 5i inverse agonists, at the G_s -cAMP signalling pathway, to regulate ERK activation in wild-type and six naturally occurring constitutively active mutant (CAM) MC4R. A significant increase in the phosphorylation of ERK was reported in some of them, suggesting that these MC4R inverse agonists could act as agonists in the MAPK pathway.⁶⁰ This study proposed the prevalence of multiple activation states of MC4R with ligand as well as mutant-specific conformations that could couple differentially to the MC4R, giving rise to distinct signalling pathways or its constitutive activity.⁶⁰ This suggests abundant potential for future investigations into new novel mechanisms.

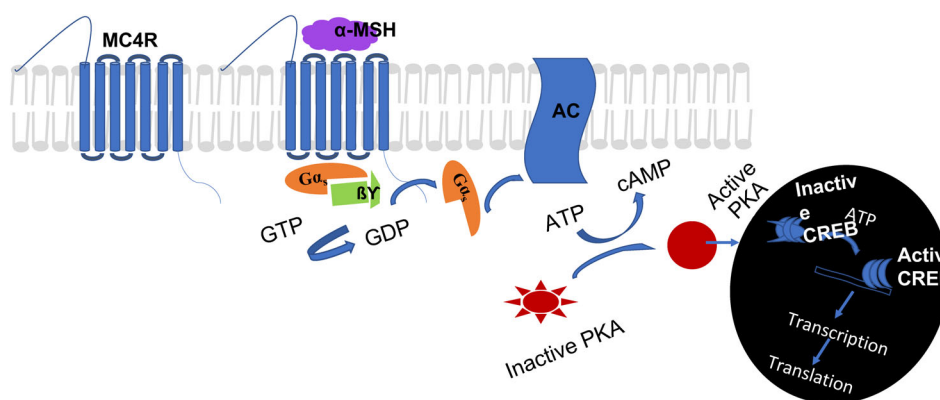


FIGURE 1 G-protein signalling pathway. Schematic showing the canonical stimulatory G-protein (G_s) pathway for melanocortin 4-receptor (MC4R) signalling and gene expression. Binding of α -melanocyte-stimulating hormone (MSH) to the MC4R causes the activation of the G-protein, with $\alpha\beta\gamma$ -subunits dissociating into α -/ $\beta\gamma$ -subunits. The dissociated $G\alpha_s$ causes the activation of adenylyl cyclase (AC), leading to the conversion of ATP to cyclic adenosine monophosphate (cAMP). cAMP activates inactive protein kinase A (PKA) which is translocated into the nucleus, activating the transcription factor cAMP response element binding protein (CREB) via phosphorylation of CREB, which regulates transcription. GTP, guanosine triphosphate

3.3 | β -ARRESTIN PATHWAY

The knowledge of constitutive MC4R activity is not new, as described above and also detailed in the GoF section. This constitutive activity leads to a GoF effect, which can alleviate obesity pathology. However, the underlying molecular mechanism has only recently been postulated.^{10,13} Mutations causing GoF may employ β -arrestin signalling, particularly those that are associated with a reduced risk of obesity-associated diseases such as type 2 diabetes and cardiac ailments. There is strong evidence that mutations, particularly Valine 103 Isoleucine (V103I) and Isoleucine 251 Leucine (I251L), help to effectively recruit β -arrestin.^{10,13} The binding of agonists, for example, α -MSH and [Nle⁴, DPhe⁷]- α -MSH (NDP-MSH; a synthetic analogue of α -MSH), to these mutant MC4Rs may induce changes in the conformation of the receptor, affecting ligand receptor-binding and either making the interaction strong enough to prevent internalization, resulting in longer retention of the MC4Rs on the plasma membrane, or allowing very rapid or much improved recycling so they are available in increased numbers on the plasma membrane compared with the wild type (Figure 2). This effect has yet to be investigated in detail, but research has been initiated by some groups^{10,13} and will be discussed later in this review.

3.4 | Ca^{2+} -REGULATED PATHWAYS

Ca^{2+} is recognized to be a cofactor for ligand-MC4R binding. The recent elucidation of the crystal structure of MC4R complexed with SHU9119, a potent cyclic peptide agonist, highlighted the role of Ca^{2+} -binding in the receptor's downstream signal regulation.^{35,61} Briefly, both the ligand agonist and Ca^{2+} ions complementarily activate MC4R, which induces closure of the inwardly rectifying

potassium channel (KIR7.1) to retain intracellular potassium levels (Figure 3). This leads to an overall anorexigenic effect with a negative energy balance and increased heart rate.⁶² However, the antagonist AgRP has inhibitory effects that open KIR7.1, causing K^+ to be pumped out of the cell. This promotes orexigenic effects by dysregulating energy homeostasis and ultimately creating a positive energy balance (Figure 3).

4 | LOSS-OF-FUNCTION MUTATIONS, DISEASE PATHOLOGY AND RESCUE

Evidence has shown that mutations in MC4R are largely associated with severe obesity. These mutations cause either partial or complete loss of function, depending on the nature and function of the mutation,^{16,27,48,63–65} and appear throughout the coding sequence.²⁵ A mutation-based classification scheme was proposed as early as 2003.^{25,66}

Most loss-of-function mutations are heterozygous, exhibiting a phenotype intermediate between wild-type and homozygous MC4R mutations.^{15,63} The extent of the functional defect is sometimes conflicting, owing to the complexity of gene-environment interactions and the varying expression of the dominant gene. Homozygous mutations, however, show pronounced effects on obesity, and patients carrying these mutations are characterized by high BMI, hyperphagia,⁶⁷ linear growth,³¹ increased bone mineral density,⁶⁸ and hyperinsulinaemia.³²

In the functional characterization of most MC4R loss-of-function mutants, their intracellular retention suggests they undergo impaired receptor trafficking to the plasma membrane. This reduces the number of MC4Rs available on the cell surface, ultimately diminishing cAMP generation and all effector responses and downstream

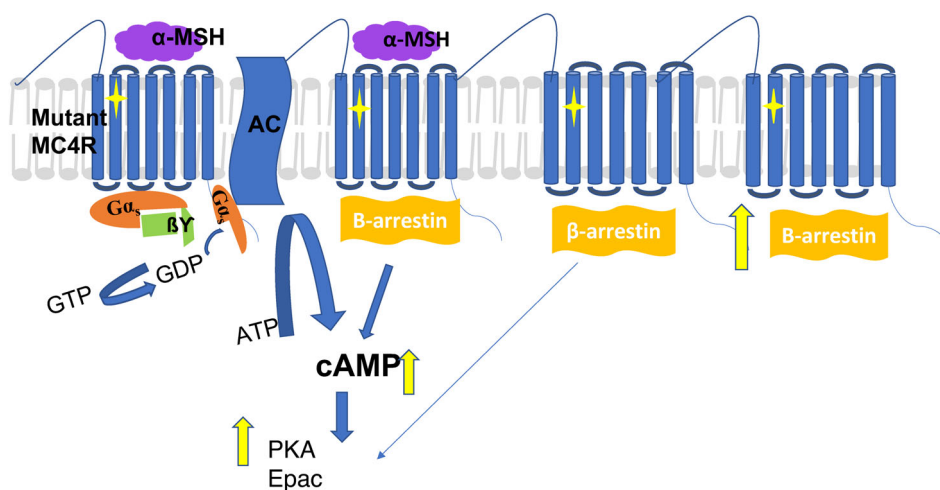


FIGURE 2 β -arrestin signalling pathway. Schematic elucidating the mechanism of gain-of-function induced by a mutation (yellow diamond) in the melanocortin 4-receptor (MC4R). The mutation causes an increase in the cell-surface expression of MC4R, possibly via reduced internalization or rapid recycling, causing increased cyclic adenosine monophosphate (cAMP) production, as well as increased production of protein kinase A (PKA) and cAMP-regulated guanine nucleotide exchange factors (Epac). AC, adenylyl cyclase; GDP, guanosine diphosphate; GTP, guanosine triphosphate; MSH, melanocyte-stimulating hormone;

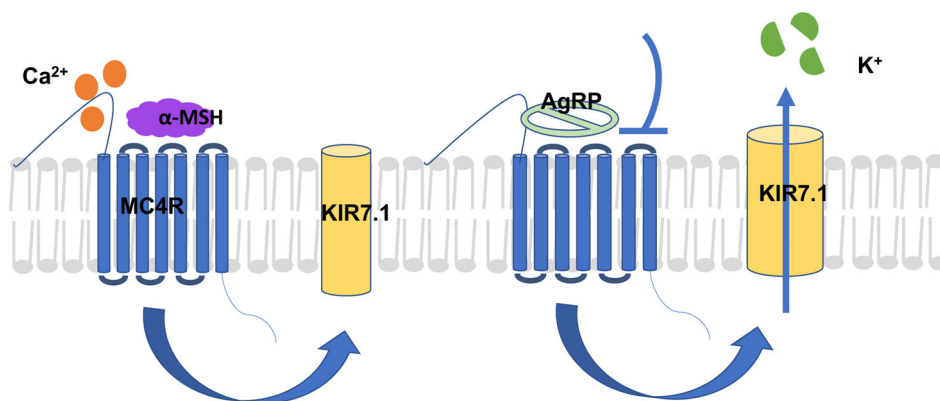


FIGURE 3 Ca²⁺ regulated pathway. The endogenous melanocortin 4-receptor (MC4R) pathway is regulated by agonist α -melanocyte-stimulating hormone (MSH) and antagonist agouti-related peptide (AgRP). Ca²⁺ ions are also important in the regulation of the MC4R pathway. α -MSH, along with Ca²⁺, induces satiety and reduces food intake. This is regulated partly by closure of the potassium inward rectifying channel KIR7.1. Conversely, AgRP regulates orexigenic signals, also via the opening of the KIR7.1, in addition to other possible mechanisms

signalling, which leads to enhanced manifestation of disease. Changes in amino acid residue(s) as a result of mutation may also weaken ligand-binding interactions, possibly because of changes in the protein conformation or reduced binding affinity, thus impairing or reducing downstream agonist-stimulated signalling.

The intracellular retention of MC4R, which is a major cause of its functional defectiveness, results when it remains in the endoplasmic reticulum as a misfolded/ubiquitinated protein. Therapeutic rescue mechanisms, such as using pharmacological or chemical chaperones, could aid proper folding and enhance receptor expression at the cell surface or prevent ubiquitination and degradation of the protein by proteasomes.⁶⁹ Granell et al^{70,71} first reported the rescuing potential of the chemical chaperone sodium 4-phenylbutyrate and the ubiquitin-activating enzyme inhibitor on MC4R in increasing the cell-surface expression of mutant MC4R associated with severe obesity. The clinical utility of chemical chaperones has, however, been challenging to elucidate. Various pharmacological chaperones have been developed as antagonists of MC4R.^{69,72} These return several misfolded MC4R mutants to the surface of the plasma membrane.⁶⁹ NBP (1-(1-(4-fluorophenyl)-2-(4-(4-[naphthalene-1-yl] butyl) piperazin-1-yl)ethyl)-4-methylpiperazine) showed potential to rescue many MC4R mutants but failed to restore their NDP-MSH binding responses, possibly due to the presence of a protracted binding, high-affinity antagonist of inhibition constant (Ki) 2.4 nM.⁷² ML00253764 (2-[2-[2-[5-Bromo-2-methoxyphenyl]ethyl]-3-fluorophenyl]-4,5-dihydro-2-1H-imidazole) and DCPMP (N-((2R)-3(2,4-dichlorophenyl)-1-(4-(2-((1-methoxypropan-2-ylamino)methyl)phenyl)piperazin-1-yl)-1-oxopropan-2-yl) propionamide) are efficient rescuers but have relatively low binding affinities, at Ki 0.17 μ M and 0.02 μ M, respectively, necessitating a high effective concentration (EC₅₀ 10 μ M).^{69,72} Encouragingly, Ipsen 5i and Ipsen 17 have wider rescue spectra and lower potency, facilitating rapid dissociation, thus they can rescue the mutant receptor at the plasma membrane at a low concentration yet still allow binding of endogenous ligands.^{73,74} THIQ (N-((3R)-1,2,3,4-Tetrahydroisoquinolinium-3-ylcarbonyl)-(1R)-1-(4-chlorobenzyl)-2-[4-

cyclohexyl-4-(1H-1,2,4-triazol-1-ylmethyl) piperidin-1-yl]-2-oxoethylamine) was reported to rescue seven of the 10 mutants investigated in neuronal cell lines but only three in human embryonic kidney 293 cells, suggesting it has effective chaperone activity in neuronal cells.⁷⁵ The structures and Ki, half maximum inhibitory concentration (IC₅₀) or effective concentration at a stable state inducing half of the maximum effect (EC₅₀) values of important chemical/pharmacological chaperones are shown in Table 1.

5 | GAIN-OF-FUNCTION MUTATIONS AND CONSTITUTIVE MC4R ACTIVITY

Interestingly, not all MC4R variants are associated with an increase in obesity pathology. A subset of variants has been reported to provide GoF, offering protection from obesity and associated complications.^{24,64,76} In vitro assays developed to determine ligand binding, cell-surface expression, and cAMP measurement as a function of G_s activation in wild-type and variant MC4Rs have been successfully developed over the past two decades. The mutations S127L,⁶⁴ P230L⁶⁴ and L250Q²⁴ augment the constitutive activity of MC4R in heterologous expression systems. A new addition to this knowledge has been the quantification of β -arrestin recruitment, which was proposed as the mechanism of action of GoF variants associated with a considerable decrease in the risk of obesity and associated disorders. Lotta et al¹³ screened UK Biobank data of 0.5 million people and characterized the variants to study their function as well as their association with BMI, type 2 diabetes and cardiometabolic diseases. Twelve of the 61 mutations identified in the UK population were nonsense/frameshift mutations, while 49 variants were functionally characterized and shown to be involved in the quantification of G_s-mediated cAMP production and the recruitment of β -arrestin to MC4R. Of the 49 variants, 11 exhibited GoF: T11S, T101N, F201L, G231S, R236C, V103I, I251L, I289L, I317V, L304F and Y332C.¹³ The first five exhibited bias toward cAMP production, the

TABLE 1 Chemical structures of potent melanocortin-4 receptor (MC4R) drug candidate examples with MC4R-specific $K_i/IC_{50}/EC_{50}$ values

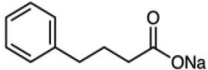
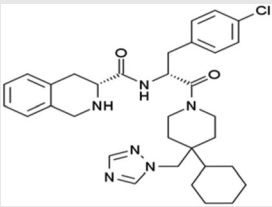
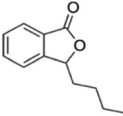
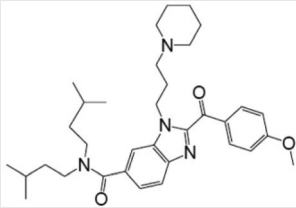
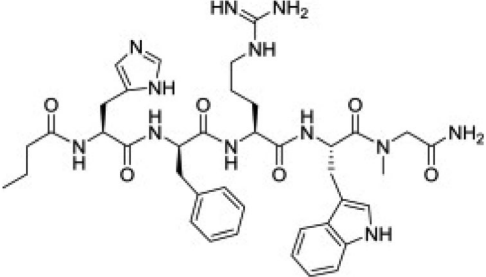
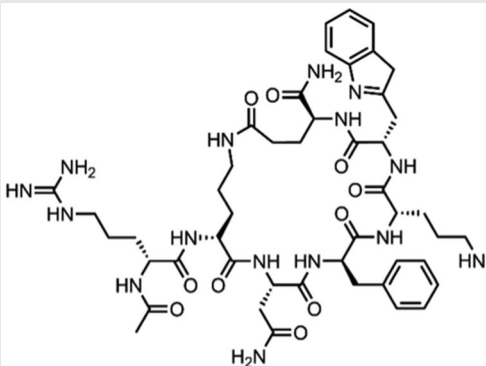
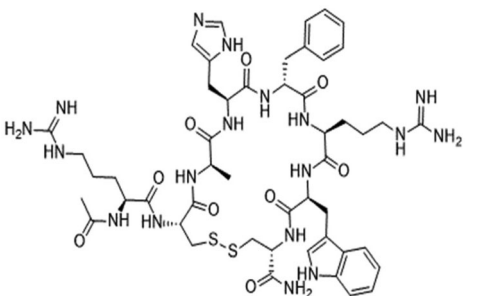
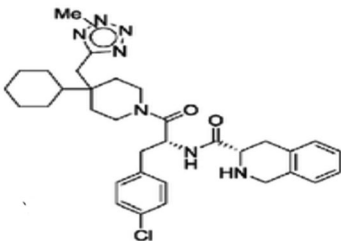
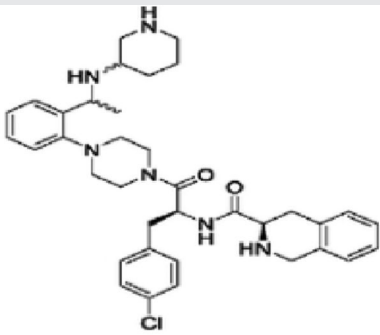
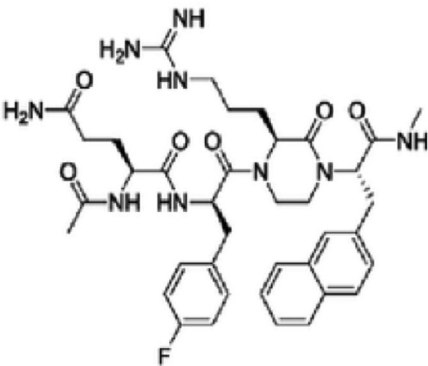
Name	Type	Structure	$K_i/IC_{50}/EC_{50}$ value for MC4R	Reference
Sodium 4-phenylbutyrate (4-PBA)	Chemical chaperone		–	70,71
THIQ	Pharmacological chaperone agonist		IC_{50} 1.2 nM	75
NPB	Pharmacological chaperone antagonist		K_i 2.4 nM	72
Ipsen 17	Pharmacological chaperone antagonist		K_i 0.96 nM	73
RO-273225 (Butyr-His-D-Phe-Arg-Trp-Sar-NH ₂)	Linear peptide		EC_{50} 1 ± 0.3	113
PL-8905	Cyclic peptide		High affinity	49
Setmelanotide	Cyclic peptide		EC_{50} 0.27 nM	36

TABLE 1 (Continued)

Name	Type	Structure	Ki/IC ₅₀ /EC ₅₀ value for MC4R	Reference
2Me-2H tetrazole derivative	Nonpeptide agonists		High affinity	101
Piperazine benzenes	Nonpeptide agonists		Ki 11 nM	103
1,3,4-trisubstituted-2-oxopiperazine	Nonpeptide agonists		Ki 5.7 nM	104

Abbreviations: EC₅₀, half maximum effective concentration; IC₅₀, half maximum inhibitory concentration; Ki, inhibition constant; MC4R, melanocortin-4 receptor; THIQ, N-[(3R)-1,2,3,4-Tetrahydroisoquinolinium-3-ylcarbonyl]-(1R)-1-(4-chlorobenzyl)-2-[4-cyclohexyl-4-(1H-1,2,4-triazol-1-ylmethyl) piperidin-1-yl]-2-oxoethylamine.

next four for β -arrestin recruitment, and the last two did not exhibit any bias for signalling. The frequency of reported heterozygous β -arrestin-biased GoF alleles in the UK Biobank was 6.1% and that of homozygous alleles was 0.1%. Compared with noncarriers, heterozygous carriers had an intermediate risk of obesity, type 2 diabetes, and coronary artery disease, while homozygous carriers had a 50% lower risk of these conditions.¹³ No change in protection from obesity was observed in carriers of the GoF variants with a preference toward cAMP production.¹³

A detailed examination of the molecular mechanisms may explain the observed effects. The GoF variants that showed bias for β -arrestin alone (V103I, I251L, I289L and I317V) exhibited enhanced signalling via the MAPK pathway,¹³ as confirmed by the over-expression of phosphorylated ERK 1/2, whereas no increase in the expression of this protein was observed in cAMP GoF variants. As expected, wild-type MC4Rs translocated from the membrane to the

cytoplasm upon agonist stimulation reduced the surface expression of MC4R, by 23%. The most frequently observed GoF variant, V103I, remained at the cell surface and showed no change in cell-surface expression,¹³ which could be because of impaired internalization or improved recycling.

6 | MELANOCORTIN-4 RECEPTOR CRYSTAL STRUCTURE FOCUSING ON THE ROLE OF Ca²⁺: A COMPARISON WITH OTHER GPCRS AND THEIR IONIC BINDING

An in-depth understanding of the function and pharmacological roles of ions and ion-binding sites in GPCRs has also become possible in the past decade, with advances in their biophysical, structural and functional characterization, marking the beginning of new avenues for

the discovery of potentially safer and more efficient drugs. Briefly, monovalent and divalent cations can act selectively and nonselectively at various sites of different GPCRs. Physiological concentrations of these ions can act as allosteric modulators in some cases.⁷⁷ Some GPCRs are selectively modulated by inorganic ions that facilitate the receptor's physiological function.^{78,79} Some unique or highly conserved sites for specific ions have also been reported; for example, Class A GPCRs have a Na⁺-binding site that functions as a near-universal allosteric modulator for GPCR structure and function, and the origin of this site can be traced as far back as prokaryotic rhodopsin channels.^{80,81} Fifteen residues in this highly conserved sodium pocket are conserved in 45 diverse receptors, with minor variants occurring in the majority of Class A GPCR members. Receptors lacking this Na⁺ pocket exhibit extremely compromised ligand-induced signaling.⁸² Also, structural changes that effect a shift in the position of the sodium pocket might result in changing the overall coordination and conservation pattern.⁸³ Crucial zinc (Zn²⁺)-binding sites have also been reported, for example, in the Class A GPCR, platelet-activating factor receptor.⁸⁴

With MC4R being an important drug target for obesity, gaining knowledge of its crystal structure is extremely beneficial. Yu et al³⁵ first reported the structure of human MC4R with the antagonist SHU9119 (a cyclic peptide) at 2.8 Å resolution. Analysis of the MC4R-SHU9119 complex revealed the classic seven-transmembrane helical structure in addition to details of the interactions involving the transmembrane and loop domains. Calcium was identified as a cofactor for ligand binding³⁵ and was complexed with amino acid residues of the receptor as well as the ligand (SHU9119). Intriguingly, extracellular calcium increased the affinity of endogenous α-MSH by 37 times and the potency of α-MSH more than 600-fold, while showing no selective effect on AgRP binding or the antagonist SUH9119.³⁵ The Ca²⁺-binding site in MC4R is distinct from the Na⁺- and Zn²⁺-binding sites in other GPCRs. Although MC4R is a member of Class A, it has been reported to differ from other Class A GPCRs in many respects, including its ion/ion-binding site, which confers its different functionality. MC4R shows more structural divergence as a GPCR, exhibiting a greater likeness to lipidic GPCRs than homologous peptidic GPCRs.³⁵ The structure of MC4R is in fact different from all other reported GPCRs. A comparison of the MC4R structure with other Class A GPCRs was carried out using the root-mean-square deviation of Ca atoms in the inactive state in the transmembrane regions. MC4R exceeded 2.2 Å,³⁵ which is closer to that of lysophosphatidic acid receptor 1 and is more than the 2.0 Å calculated for other GPCRs. The reason for the higher Ca value in MC4R could include the following: (a) the very short extracellular loop (ECL) 2; (b) the absence of the conserved disulphide bond that connects ECL2 to helix III in other Class A GPCRs⁸⁵; (c) the distinct outward position of the helix V; or (d) the presence of nonconserved residues, such as H^{5,5}, D^{3,25} and G^{2,58}.³⁵ It is interesting that Ca²⁺ binding only affects α-MSH and has no effect on AgRP binding. The relatively unexplored selectivity of the ionic cofactors should be investigated further with respect to transducer coupling and downstream signalling, to boost drug discovery prospects.⁸⁶

7 | ANTI-OBESITY DRUG DEVELOPMENT WITH MC4R AS A TARGET

Melanocortin-4 receptor, the key monogenic cause of obesity, is befitting as a strategic target for anti-obesity drugs. The ligands of this receptor, including ACTH and α-, β- and γ-MSH, are derived from the precursor POMC peptide.⁸⁷ ACTH [SYS MEHFRWGKPV GKKRRPVKVV PNGAEDESAE AFPLEF] is further processed to yield α-MSH [SYS MEHFRWGKPV].^{87,88} α-MSH adopts a β-turn conformation that presents histidine-phenylalanine-arginine-tryptophan (HFRW) for receptor binding, interacting with the ionic and aromatic amino acids in the upper second and third transmembrane domains.⁸⁹ MSHs lack selectivity in humans, and they have additional roles in pigmentation, hormone regulation and anti-inflammation, which limit their use in drug development. The hunt for a safe, potentially active, and highly specific drug continues.

The criteria for a good agonist include safety, selectivity, efficiency and bioavailability⁴⁹; the agonist must be harmless and incapable of causing any untoward effects in the body. The potency/efficiency relates to its capacity to induce the desired response at the minimum possible concentration. Its selectivity refers to its ability to activate a single desired pathway, while an agonist's bioavailability depends on its degree of solubility in body fluid and ease of assimilation in the body. Most synthetic MC4R agonists fall into one of three categories: linear peptides, cyclic peptides and nonpeptides. The structure and Ki/IC₅₀/EC₅₀ values of important peptides/nonpeptides are shown in Table 1.

7.1 | Linear peptides

Linear peptides are commonly between five and seven amino acid residues long, but may vary from four to 16 residues, and are held together by simple amide bonds. Most synthesis procedures are based on the substitution of amino acid residues in α-MSH [S¹Y²S³MEHFRWGKPV¹³],⁴⁹ particularly those in the core motif HFRW and/or two or three flanking sequences on either side. The core aim behind inducing and screening the various substitutions is to find novel potent ligand moieties with good selectivity and overall efficiency. A suitable agonist should have high potency (EC₅₀ < 10 nM), high selectivity (>50 times EC₅₀ for MC4R),⁴⁹ and better stability and safety compared with the unsubstituted parent peptide. The first step is the synthesis, which includes designing and inducing changes that might be useful, followed by a series of in vitro validation steps, including quantification of the functional activity of the proposed new peptide. For example, D-Phe (synthetic dextro isomer of Phenylalanine)/D-Phe analogues, the first substitutions reported, considerably increased the agonist's activity and ligand stability.⁹⁰ Haslach et al⁹¹ reported that the use of a D-Phe analogue with a halogen at the para position provided higher agonist activity and better ligand stability compared with that of D-Phe. Histidine has been substituted with Tyrosine, Atc (2-aminotetralin-2-carboxylic acid), Apc (1-amino-4-phenylcyclohexane-1-carboxylic acid), and other

residues to achieve more potent and selective peptides. Later, arginine was also proposed as a replacement for histidine.⁹¹ Tryptophan provides a better substitute than the Phe analogue, as it has an electron-withdrawing group at its para position that enhances ligand-receptor interactions. In addition to glutamine/glycine at the fifth position (first left of **H**), butyl and pentyl groups also act as effective ligands. Active ligands are formed by replacing glycine, first right of **W**, with acidic or neutral amino acids. However, in general, obtaining a potent/selective linear ligand for MC4R has been a challenge that has met with limited success.

Interestingly, the constitutive activity of MC4R is induced by its N-terminal domain [HLWNRSS] and its transmembrane domain, which undergo spontaneous conformational transformations to change inactive MC4R to active MC4R.⁹² The amino acid residues that take part in binding in case of constitutive activity differ from those involved in regular ligand binding, suggesting there is room for additional positive allosteric modulation and the potential to develop alternative therapeutic candidates.⁹³⁻⁹⁵

7.2 | Cyclic peptides

These are typically amino acids or amino acid analogues that are cyclized by disulphide bonds. The established core motif (**HFRW**) remains the same as in linear peptides; however, the potential substitutions and analogue designs differ to best fit the receptor, with the aim of conferring maximum efficiency and potency. Examples of the most effective substitutions include Phe to D-Phe and/or D-2-naphthylalanine and His to either polar or nonpolar moieties. Acidic amino acids (glutamate, aspartate, or D-alanine) are preferable at position 5, while alanine, lysine and cystine are optimum at position 10. In general, the replacement of Met at position 4 with lipophilic residues, such as norleucine or acidic residues, is favoured. Neutral or acidic compounds with short side chains at position 5 improve the potency of the ligand, while a change in chirality results in higher potency at position 7.

Multivalency can increase ligand-receptor affinity, and introducing bivalent agonists reportedly increases potency.⁹⁶ The melanocortin bivalent agonist CJL-1-87, with two repeats in the structure that are linked by an oligomer, shows approximately seven times the potency of the monovalent structure.⁹⁷ Fernandes et al⁹⁸ examined the effect of homo/hetero bivalency, combining a linear, truncated NDP-MSH with cyclic SHU9119 separated by a series of linkers of varying flexibility, for example, PEGO (19-amino-5-oxo-3-, 10,13,16-tetraoxa-6-azanodecan-1-oic acid) linkers. The heterobivalent ligand was five times more active against MC4R compared with the monovalent equivalents, indicating a cooperative effect upon binding, promoted by the flexible linker.⁹⁸

An example of an extremely efficient and safe cyclic peptide is setmelanotide. In addition to the tremendous (100-fold) increase in downstream signalling on MC4R activation, it also reduces the undesirable side effects. This is suggested to be the result of biased signalling of setmelanotide at the MC4R. Compared with other tested

drugs, setmelanotide is unique in that it is reported to activate nuclear factor of activated T cell (NFAT) signalling and restore the function of many MC4R variants.^{26,99} Setmelanotide is more effective in stimulating cAMP accrual in the presence of AgRP compared with α -MSH and LY2112688 (a first generation MC4R agonist). AgRP competes with α -MSH and LY2112688 in the MC4R binding pocket but fails to displace setmelanotide owing to the superior binding affinity of setmelanotide.^{26,99}

7.3 | Nonpeptide ligands

Substitutions within nonpeptide ligands are generally more effective than those in peptide ligands because the former have a compact, rigid structure.⁴⁹ Additionally, as nonpeptide ligands are resistant to proteolysis, they tend to be more stable compared with peptide ligands. Various nonpeptide agonists based on the β -turn motif were investigated by Haskell-Luevano et al¹⁰⁰ using cyclic lactam templates of the, then leading, structures melanotan-II and SHU9119. Sebhat et al¹⁰¹ used a piperidine core and introduced triazoles/tetrazoles to develop the first potent and selective nonpeptide MC4R agonist. Fotsch et al¹⁰² introduced tryptamine conjugated with cyclohexane 1,4-diamine or butyl guanidine to mimic tryptophan and arginine, respectively. The resulting agonist was potent yet lacked specificity for MC4R; however, introducing piperazine as the principal scaffold yielded the required selectivity.¹⁰³ Following this, Tian et al¹⁰⁴ synthesized various 1,3,4-trisubstituted 2-oxopiperazines and further capped the ligand with a tetrapeptide core. The designed dipeptide and tripeptide analogues showed excellent binding affinity (nanomole scale), potency and selectivity for MC4R compared with MC1R.¹⁰⁴

8 | DRUGS AT THE APPROVAL OR CLINICAL TRIAL STAGE

Structure-based ligand discovery has provided superior and selective agonists to promote MC4R function. Some of the drugs in clinical trials include LY2112688, melanotan-II, bremelanotide, PL-8905 and setmelanotide. Although well characterized in vitro and in vivo, undesirable side effects have been reported for many of these drugs in clinical trials. LY2112688 caused increased blood pressure.^{26,105} Melanotan-II, a super-potent cyclic MC4R agonist, caused penile erection in males and darkening of the skin.^{49,106} Bremelanotide was more closely linked to sexual dysfunction than weight reduction in both men and women and failed as an antiobesity drug in clinical trials.^{49,107} PL-8905 is in clinical trials after exhibiting minimal side effects, such as changes in blood pressure, in preclinical studies.^{49,108} Setmelanotide showed considerable promise with no observed side effects in phase III clinical trials³⁶ and has now been approved by the FDA.¹⁰⁸ Liraglutide, a glucagon-like peptide-1 receptor agonist (GLP-1RA), also causes weight loss by reducing appetite. It has been reported to be effective in many cases of monogenic obesity.¹⁰⁹ Liraglutide treatment is reported to increase bone mass in common

TABLE 2 List of antiobesity drugs

Drug	Target	Mechanism of action	Usage	Side-effects	Clinical status	Reference
Section I						
Setmelanotide	MC4R	Decreased food intake and increased energy expenditure via MC4R binding	LT	Reported safe	Approved	36,108
PL-8905	MC4R	-do-*	—	Reported safe	Clinical studies	49
LY2112688	MC4R	-do-	—	Increased systolic blood pressure	Failed in clinical studies	26,105
Melanotan-II	MC4R	-do-	—	Spontaneous penile erection; skin darkening	Failed for obesity	49,106
Bremelanotide	MC4R	-do-	—	Increase blood pressure and sexual activity	Failed for obesity	49,107
4-PBA	MC4R	Acts as chemical chaperone and helps rescue intracellular retention of variant MC4Rs	—	Lacks specificity	Preclinical	69–71
UBE-41	MC4R	-do-	—	Lacks specificity	Preclinical	70,71
THIQ	MC4R	Acts as pharmacological chaperone and helps rescuing intracellular retention of variant MC4Rs	—	Prolonged exposure decreases cell surface expression and signalling	Preclinical	75
NBP	MC4R	-do-	—	-do-	Preclinical	72
ML00253764	MC4R	-do-	—	High EC ₅₀	Preclinical	69,72
DCPMP	MC4R	-do-	—	High EC ₅₀	Preclinical	69,72
Ipsen 5i	MC4R	-do-	—	Reported efficient	Preclinical	75
Ipsen 17	MC4R	-do-	—	Reported efficient	Preclinical	73
Section II						
Orlistat	Pancreatic/stomach lipases	Decreases fat absorption	LT	Abdominal pain, diarrhea	Approved	114
Liraglutide	GLP-1R	Centrally (CNS) mediated	LT	Adverse GI effects	Approved	115,116
Semaglutide	GLP-1R	-do-	LT	-do-	Approved	117
Naltrexone-Bupropion	α -MSH/ β -endorphin	Possible modulation of melanocortin system	LT	Adverse GI effects; dizziness/insomnia	Approved	118
Lorcaserin	Serotonin/5HT receptor	Modulates melanocortin system	LT	Headache, weakness, bradycardia, cognitive impairment	Approved	119,120
Leptin	POMC/NPY neurons	Modulates the melanocortin system	LT	Exogenous administration largely ineffective	Approved as combinatorial therapy	121,122
Section III						
Amphetamine compounds	POMC/NPY neurons	High metabolic rate; stimulation of anorectic/inhibition of orectic signals	Short-term	Addictive in nature	Approved (less addictive analogues now available)	122–124
Methamphetamine desoxyephedrine	-do-	-do-	-do-	-do-	Approved	123,125
Deoxyphedrine	-do-	-do-	-do-	-do-	Approved	126
Amphetamine congeners (AC)	-do-	-do-	-do-	Additive in general	Approved	127
Diethylpropion (AC)	-do-	-do-	-do-	Limited drug efficiency	Approved	128
	-do-	-do-	-do-		Approved	129

TABLE 2 (Continued)

Drug	Target	Mechanism of action	Usage	Side-effects	Clinical status	Reference
Phendimetrazine (AC)				Insomnia, dry mouth, constipation		
Benzphetamine (AC)	-do-	-do-	-do-	Insomnia, dry mouth, mood swings	Approved	130
Phentermine	-do-	Increased energy consumption and anorexia	-do-	Insomnia, dry mouth, mood swings	Approved	131,132
Phentermine/topiramate (Qsymia)	Glutamate and GABA receptors	Weight loss and decrease in CNS neuronal activity via Ca ²⁺ channels	-do-	Insomnia, dry mouth, dizziness	Approved	108,120,133
Section IV						
MEDI0382	GLP-1R/GCGR	Bi-agonist targeting	—	—	Phase II	134
NNC0090-2746 (RG7697)	GLP-1R/GIPR	Bi-agonist targeting	—	—	Phase IIa	135
LY3298176	GLP-1R/GIPR	Bi-agonist targeting	—	—	Phase II complete	136
HM15211	GLP-1R/GCGR/GIPR	Tri-agonist targeting	—	—	Preclinical	137
NN9423/ NNC9204-1706	GLP-1R/GCGR/GIPR	Tri-agonist targeting	—	—	Phase I	138
Section V						
GLP-1 delivering Estrogen	Peptide mediated hormone delivery	Peripheral/central regulation by modulation of energy sensors	Long-term	Risk of breast cancer, heart ailments, stroke, dementia	Preclinical	139
17β-estradiol (E2)	-do-	-do-	Long-term	-do-	Preclinical	140
Glucagon/T3	-do-	Modulation of energy expenditure via BAT thermogenesis	—	—	Preclinical	141
GLP-1 delivering dexamethasone	-do-	Energy balance and weight loss via hypothalamic neurocircuits	—	—	Preclinical	142
Section VI						
Dinitrophenol	Mitochondrial uncoupling	High metabolic rate	—	Hyperthermia, tachycardia, nausea, vomiting	Withdrawn	143
Serotonergics	Serotonin/5HT	Serotonergic/Melanocortinergic system	—	Pulmonary hypertension; valvular heart disease	Withdrawn	128,131
Fenfluramine	-do-	-do-	—	-do-	Withdrawn	144,145
Dexfenfluramine	-do-	-do-	—	-do-	Withdrawn	144,145
Sibutramine	Serotonin/norepinephrine inhibitor	-do-	—	High BP, cardiac arrhythmia	Withdrawn	146
Rimonabant	Type I CB1R	Weight loss by modulating hemostatic and hedonic feeding circuits	—	Adverse psychiatric effects	Withdrawn	147

Section I: Antiobesity drugs targeting MC4R; Section II: General antiobesity drugs; Section III: Drugs approved for short-term use only because of potential side effects and addictive nature. Section IV: Bi- and tri-agonist drug targets (in developmental stage); Section V: Peptide-hormone based drugs (in developmental stage); Section VI: Drugs that have been withdrawn as a result of extreme side effects.

Note: *-do- Refers to repeat the exact words/content of the row above, in that specified column, to avoid writing the same information multiple times in the table.

Abbreviations: 4-PBA, sodium 4-phenylbutyrate; AC, adenylyl cyclase; ACTH, adrenocorticotropic hormone; BP, blood pressure; DCPMP, N-((2R)-3-(2,4-dichlorophenyl)-1-(4-(2-([1-methoxypropan-2-ylamino] methyl) phenyl) piperazin-1-yl)-1-oxopropan-2-yl) propionamide; ECL, extracellular loop; GABA, gamma-aminobutyric acid; GCGR, glucagon receptor; GI, gastrointestinal; GIPR, glucose-dependent insulinotropic polypeptide; GLP-1R, glucagon-like peptide-1 receptor; MC4R, melanocortin 4-receptor; NBP, 1-(1-(4-fluorophenyl)-2-(4-(4-[naphthalene-1-yl] butyl) piperazin-1-yl) ethyl)-4-methylpiperazine; NPY, neuropeptide Y; POMC, proopiomelanocortin; THIQ, N-[(3R)-1,2,3,4-Tetrahydroisoquinolinium-3-ylcarbonyl]-(1R)-1-(4-chlorobenzyl)-2-[4-cyclohexyl-4-(1H-1,2,4-triazol-1-ylmethyl) piperidin-1-yl]-2-oxoethylamine; UBE-41, ubiquitin activating enzyme inhibitor.

obesity, however, no change in bone metabolism was seen in obesity caused due to mutations in MC4R.¹¹⁰ A combination of liraglutide therapy and exercise improves maintenance of weight loss (as weight regain after a weight loss is a common problem) compared to either exercise or drug treatment alone.¹¹¹ Sun et al¹¹² have proposed a gut-intrinsic melanocortin signalling complex involving α -MSH release and MC4R activation on L cells secretion in humans. This could directly target mucosal MC4R to treat human metabolic disorders including obesity. A comprehensive summary of all drugs developed thus far as agents to treat obesity, including MC4R agonists, and their targets, mode of action, potential side effects, and status with regards to FDA approval, is shown in Table 2.

9 | CONCLUDING REMARKS

The dramatic increase in obesity, its associated disorders, and related mortality is alarming. Most drugs approved so far to treat obesity cause considerable side effects, especially to the nervous and gastrointestinal systems. Some have been withdrawn or are only prescribed for short-term use as a part of combination therapies. Overall, the development of effective drugs to treat obesity has been challenging. Encouragingly, however, our understanding of the genetics, molecular mechanisms, and structure of MC4R and other GPCRs, some of which are likely to contribute to the pathology of obesity, has increased tremendously. Setmelanotide, the latest FDA-approved drug for use against obesity, reportedly induced no side effects in clinical trials, which gives us hope for its sustained and efficient use in the future. The biased NFAT signalling of setmelanotide, in addition to its efficient ligand binding, is probably the reason behind its success as a drug candidate, emphasizing the importance of designing effective ligand substitutes and investigating novel molecular pathways. β -arrestin-biased signalling in the case of GoF variants, which provide protection against obesity and associated disorders, is another crucial example suggesting potential therapeutic approaches in the future would benefit from smart drug design and investigation of unconventional pathways as well as canonical ones. Thus, designing specific drugs that can selectively activate or block specific targets such as arrestin as well as improve ligand-receptor interactions may be a promising therapeutic direction. All these advancements that elucidate the finer details of obesity pathology will undoubtedly provide useful insights into how to effectively target specific receptors, leading to the design of safe and efficient drugs with which to treat obesity and other diseases.

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CONFLICT OF INTEREST

The author(s) declare(s) that they have no competing interests

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