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Translational advances of melanocortin drugs: Integrating biology, chemistry and genetics

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ABSTRACT

Melanocortin receptors have emerged as important targets with a very unusual versatility, as their widespread distribution on multiple tissues (e.g. skin, adrenal glands, brain, immune cells, exocrine glands) together with the variety of physiological processes they control (pigmentation, cortisol release, satiety mechanism, inflammation, secretions), place this family of receptors as genuine therapeutic targets for many disorders. This review focuses in the journey of the development of melanocortin receptors as therapeutic targets from the discovery of their existence in the early 1990 s to the approval of the first few drugs of this class. Two major areas of development characterise the current state of melanocortin drug development: their role in obesity, recently culminated with the approval of setmelanotide, and their potential for the treatment of chronic inflammatory and autoimmune diseases like rheumatoid arthritis, multiple sclerosis or fibrosis. The pro-resolving nature of these drugs offers the advantage of acting by mimicking the way our body naturally resolves inflammation, expecting fewer side effects and a more balanced (i.e. non-immunosuppressive) response from them. Here we also review the approaches followed for the design and development of novel compounds, the importance of the GPCR nature of these receptors in the process of drug development, therapeutic value, current challenges and successes, and the potential for the implementation of precision medicine approaches through the incorporation of genetics advances.

1. Introduction

The journey of melanocortin receptors (MCRs) started in 1992 when Mountjoy et al. reported for the first time the characterisation of two receptors that mediated the biological actions of the hormones alpha-melanocyte stimulating hormone (αMSH) and adrenocorticotropin hormone (ACTH) [1]. These receptors corresponded to the now named as MC_1 and MC_2 receptors, while other three MCRs ($MC_{3,4,5}$) were soon discovered within the next three years [2–4]. This seminal work prompted the study of these receptors as potential therapeutic targets, as the discovery that they were G-protein coupled receptors (GPCRs) was immediately envisaged as an opportunity, as will be discussed later.

ACTH was first isolated in 1942 [5] and its role in modulating the immune system was quickly identified although attributed, at that time, solely to the induction of cortisol release upon stimulation of MC_2 expressed in the adrenal glands. Sixty years passed before Getting et al. discovered that ACTH preserved its anti-inflammatory activity in a model of arthritis using rats that had their adrenal glands removed, and to identify MC_3 receptor as a likely mediator of this action [6]. This work hit another milestone in the MC timeline (Fig. 1) as it unfolded the possibility of designing melanocortin drugs to treat inflammatory diseases, devoid of adrenal effects, by designing new molecules selective for MC_3 and possibly MC_1 and MC_4. A crucial third moment was marked by the demonstration that MC compounds can also enhance neutrophil phagocytosis [7] and accelerate the resolution of inflammation [8], granting these drugs an upgrade from anti-inflammatory to pro-resolving agents.

Hence, melanocortin drugs are part of the *Resolution Pharmacology* movement [9,10] which postulates that therapeutic strategies focused on the active promotion of our body's endogenous pro-resolving

Abbreviations: ACTH, adrenocorticotropic hormone; GPCR, G-protein coupled receptor; MC, melanocortin; MCR, melanocortin receptor; MSH, melanocyte stimulating hormone.

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mechanisms may lead to better and safer drugs. MC compounds are under investigation as pro-resolving drugs as well as for many other indications due to their widespread expression and diversity of biological actions that they control. In this review, we summarise the different approaches to MC drug development, advantages and challenges of targeting this system, therapeutic uses with ongoing investigations, and the current clinical success of this class of drugs.

2. The druggability of the melanocortin pathway

2.1. Melanocortin pathway components

Like most GPCRs, the essential components of the MC pathway include ligands, receptors, accessory proteins, signalling effectors and regulators (Fig. 2), and targeting several of these components may have therapeutic opportunities. There are four endogenous ligands with agonistic activity, all derived from a common larger precursor protein called pro-opiomelanocortin protein, POMC, from which the shorter peptides ACTH, α-,β-, and γMSH derive through the cleavage and processing by the enzymes prohormone convertases PCSK1 and PCSK2, peptidyl α-amidating monooxygenase (PAM), carboxypeptidase E (CPE) and N-acetyltransferase (NAT) [10]. Antagonistic activity at melanocotin receptors (MCRs) is mediated by the proteins agouti signalling protein (ASIP) and agouti related neuropeptide (AGRP), making the MC system unique for being the only GPCR family for which natural antagonists have been identified. ASIP strongly prevents cyclic adenosine monophosphate (cAMP) accumulation by αMSH on $MC_{1,2,4}$ and mildly on $MC_{3,5}$ [11], while AGRP presents antagonistic activity for the production of cAMP at MC_3, MC_4 and very mildly at MC_5 [12]. To add complexity, pharmacologically speaking these two proteins are not precisely antagonists (see Fig. 3 for Pharmacology terms). The fact that both ASIP and AGRP proteins are able to reduce the ligand-independent constitutive activity of different MCRs indicates that they are *inverse agonists* [13–15]. Moreover, although they prevent the activation of cAMP by αMSH (i.e. antagonistic activity), the fact that they can activate alternative signalling pathways like G(i/o) or β-arrestin, renders them as *biased agonists* [15–17]. Most drug discovery efforts are focused on mimicking the actions of the agonists at $MC_{1,3,4,5}$ and the strictly antagonistic activity at MC_2 or MC_5. The biased profile of these endogenous molecules is largely unexplored because we still ignore the functional and potential therapeutic value of those alternative signalling pathways triggered by the endogenous antagonists.

Melanocortin receptors activity is also modulated by a number of accessory proteins like MRAP, crucial for MC_2 function in adrenal glands and where deficiency causes familial glucocorticoid deficiency. MRAP2 is also determinant for MC_4 activity and its loss of function leads to obesity, similarly to MC_4 deficiency [18]. Although no specific approaches have been reported yet, it has been suggested that targeting these accessory proteins may bring therapeutic usefulness [19]. Additional accessory proteins have been described to interact and affect the function of the mouse MC_1. Attractin (ATRN) and mahogunin ring finger 1 (MGRN1) appear to be important for the actions of ASIP, as mice mutants for these proteins present with black coat, suggesting the inability of ASIP to antagonise MC_1 in the absence of these accessory proteins [20]. How these proteins affect the human MC_1 and what therapeutic potential their modulation may have are largely unknown.

As a GPCR system, the melanocortin pathway may also be susceptible to modulation by targeting the effectors and regulatory components. G-proteins could potentially be targeted to cause, for example, biased responses by blocking a subset of the signals induced by a given GPCR. This strategy may however lead to very broad actions as G-proteins are not specific for their receptors [21]. We could speculate that molecules that block the *interaction* between the desired receptor and the G-protein may achieve higher specificity without affecting signalling via other GPCRs, although strategies of this kind have not been reported yet. G-protein coupled receptor kinases, GRKs, are enzymes that cause the phosphorylation of GPCRs, terminating the signalling cascade and favouring the binding of arrestin proteins, which subsequently lead to receptor internalisation and desensitisation [22]. GRK and arrestin inhibitors could also be developed, for example, to enhance the activity of a receptor by preventing its desensitisation [23,24]. Another level of GPCR regulation potentially susceptible of intervention is the formation of homo- or heterodimers, which can be disrupted using stapled transmembrane peptides [25]. Dimerisation has been described for melanocortin receptors [26,27], although their functional relevance remains unclear. Interestingly, dimerisation may also be targeted using bivalent ligands [28]. Finally, a novel recent strategy that could also be applied to target GPCR is known as targeted protein degradation (TPD), which uses molecules that can re-direct a specific protein for degradation by artificially recruiting an E3 ligase, promoting its ubiquitylation [29]. This strategy has been successfully applied to induce selective degradation of a GPCR receptor, the α1A-adrenergic receptor, with potential

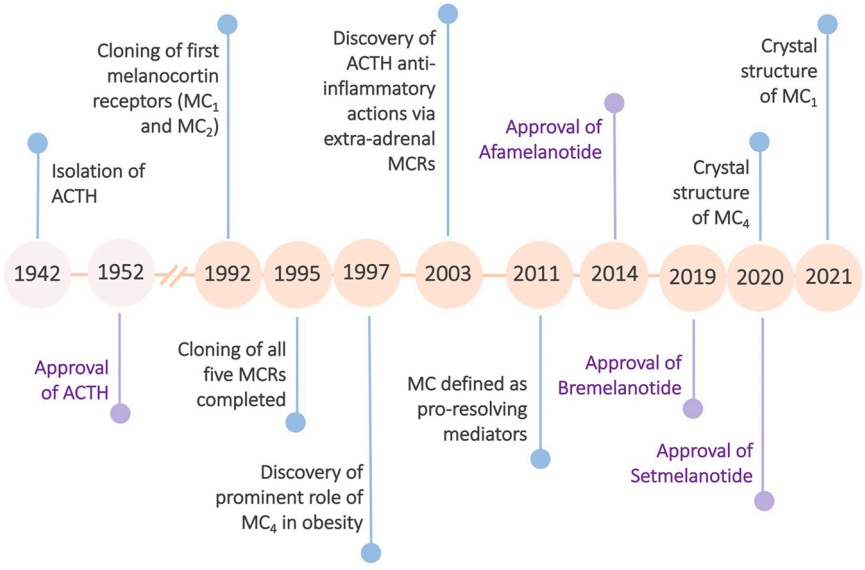

Fig. 1. Melanocortin discoveries. This figure highlights some of the most important discoveries related to the melanocortin system with relevance to their development as drugs and their clinical translation into approved products.

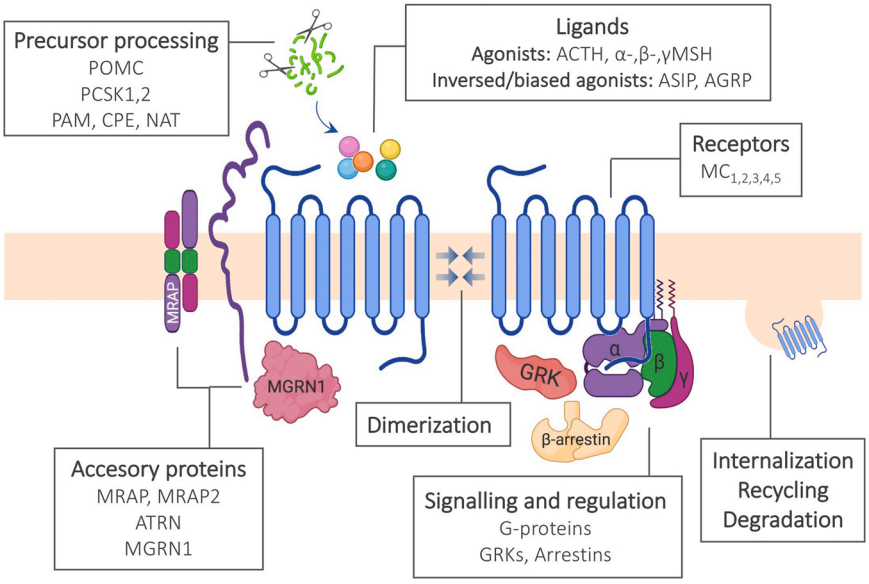

Fig. 2. Components of the melanocortin pathway. The essential components of the MC pathway include: (i) the agonists adrenocorticotropic hormone (ACTH) and the peptides α-, β- and γ-melanocyte stimulating hormones (MSH), which are produced from the cleavage of the pro-opiomelanocortin protein (POMC) by the prohormone convertases PCSK1 and PCSK2, and further modification by peptidyl α-amidating monooxygenase (PAM), carboxypeptidase E (CPE) and N-acetyltransferase (NAT); (ii) the antagonists - or inverse/biased agonists agouti signalling protein (ASIP) and agouti related neuropeptide (AGRP); (iii) five melanocortin receptors ($MC_{1,2,3,4,5}$); (iv) two melanocortin 2 receptor accessory proteins (MRAP, MRAP2), attractin, (ATRN) and mahogunin ring finger 1 (MGRF1); (v) common to other GPCRs, the several G-proteins, GPCR kinases (GRKs) and arrestin proteins are involved in melanocortin functioning and regulation.

	Agonist – A compound that can bind and activate a receptor to produce a biological response, for example, an increase of cyclic-AMP or phosphorylation of ERK1/2. Receptor sites where agonists bind to are known as orthosteric sites.
no response	**Antagonist** – A compound that binds but does not activate the receptor, i.e. it does not induce a biological response. Antagonists typically prevent agonists from exerting their actions by occupying the receptor and are known as competitive antagonists.
	Inverse agonist – A compound that binds to the receptor and induces the opposite biological response of an agonist, for example, a decrease on cyclic-AMP. Inverse agonists also reduce the basal or constitutive activity of receptors.
	Biased agonist – A compound that binds to the receptor but activates only a subset of biological responses, compared with the endogenous agonist. Biased agonism can lead to functional selectivity when those subsets of responses lead to different final biological outcomes.
	Allosteric agonist – A compound that binds to a different site of the receptor compared to typical agonists (i.e. allosteric site), and causes its full or biased activation. Compounds that binding to allosteric sites prevent the activation of the receptor by an agonist are known as non-competitive antagonists.
no response	**Allosteric modulator** – A compound that, binding to allosteric sites, and only in the presence of an orthosteric ligand, enhances (positive allosteric modulator, PAM) or decreases (negative allosteric modulator, NAM) the response of that orhtosteric ligand.

Fig. 3. Pharmacology terms and definitions. GPCRs mediate a vast array of cellular responses to multiple stimuli like hormones, chemokines or neurotransmitters, and can be of very different nature including proteins, lipids, peptides, nucleotides or even light. Once understood as a linear cascade of events (i.e. ligand→ receptor→ signalling pathway→ biological effect), GPCR activation is now recognised as a highly complex network of events in which different binding sites, multiple possible conformations, availability of secondary effectors or pathway dynamics (i.e. early and late secondary signalling), all determine the final biological outcome. The most common terms with interest for drug discovery are defined in this figure.

implications for the treatment of prostate cancer [30].

In summary, modulating accessory proteins, GPCR effectors and regulators and interfering with dimerisation or degradation may represent additional opportunities for intervening with MC system that are still unexplored. Meanwhile, current avenues for the development of new therapies targeting the MC system focus on the direct targeting of the five MCRs.

2.2. Melanocortin receptors: why five?

Tetrapod species, including mammals, have five melanocortin receptors. Zebrafish have six, while pufferfish have only four [31]. GPCRs are a family of families: in addition to five MCRs, the human genome contains four adenosine receptors, one bile acid receptor and thirteen serotonin receptors, among ~800 GPCRs classified in almost 70 different families [32]. All these receptors share a common architecture consisting of seven transmembrane (TM) helical domains, connected by three extracellular (EC) and three intracellular (IC) loops, with an extracellular N-terminus and an intracellular C-terminus. Functionally, the TM regions are responsible for ligand binding and signal transduction through conformational changes and the IC loops interact with G-proteins and other downstream signalling effectors [33]. How the human genome ended up with around 800 highly similar genes is still a mystery but the fact that GPCRs are present in the genomes of all living organisms including vertebrates, invertebrates, plants, fungi and even bacteria, i.e. prokaryotic organisms, suggests a very early evolutionary

origin of GPCRs and possibly the existence of a common GPCR ancestor. Mendoza et al. showed that the last eukaryotic common ancestor, typically referred as LECA, which dates back to 1.6–2.5 billion years ago, already had most of the genes involved in GPCR signalling [34] and that the evolutionary conservation of this signalling transduction machinery and receptor diversification may have been crucial in the transition to multicellularity.

Focusing on melanocortin receptors, Baron et al. [35] proposed the 2 R hypothesis to explain the existence of five vertebrate MCRs. The 2 R hypothesis suggests that the genomes of early vertebrates underwent two rounds of whole genome duplications around 500–600 million years ago [36,37] which allowed vertebrates to achieve their complexity by providing a substrate for increasing genome diversity. It is speculated that an ancestral *MCR* gene was duplicated during the first round (1 R) of genome duplication, leading to *MCRa* and *MCRb*, which are the receptors found in the lamprey, an ancient jawless fish considered a living fossil as it has barely evolved since it first arose during the Palaeozoic era, before the first dinosaurs. Then, these ancestral melanocortin receptors where further duplicated during the second duplication event, 2 R, leading to *MC1R* and *MC2R/5R* (emerging from *MCRa*) and *MC4R* and *MC3R* (derived from *MCRb*). The fact that *MC2R* and *MC5R* are found in the same chromosome led the authors to suggest that these two receptors may be descendants of a common ancestor and emerged from a local duplication event, resulting in the five MCRs as we know them today [31,35]. Similar investigations had proposed models for the origin of the MC peptides, the antagonists and the accessory proteins [31,38].

The appearance of new genes then lacks purpose or directedness and are more the result of chance. The relevant aspect then is not why new genes or variations emerge, but why they are conserved during evolution. Duplicated genes can acquire new functions (neo-functionalization), specialise (subfunctionalization), or degenerate into a pseudogene or be lost from the genome (non-functionalization) [39]. What forces drove the functional and molecular diversification of MCRs is not well described. It has been suggested that MCRs in early vertebrates had a preference for ACTH, while sensitivity for the shorter peptides α-, β-, and γ-MSH appeared later in the evolutionary journey as the receptor subtypes acquired more specialised functions [40]. The distinctive actions exerted by each MCR subtype therefore may derive in part by the acquisition of different affinities for the different endogenous agonists, suggesting a mechanism for specialisation. However, this explanation is very reductionist. Although the affinities vary, all endogenous melanocortin peptides can bind and activate all MCRs (with the exception of MC_2), and the particular peptide which is available in each tissue, may largely determine the physiological activity. Moreover, the preference for each receptor to be expressed in different tissues and hence different cellular contexts (e.g. MC_2 in adrenal glands, MC_4 in the brain, or MC_1 in melanocytes) may also be largely responsible for the specific activity of each receptor.

These aspects prompt us to reflect on what exactly promotes the activity of the melanocortin system within an organism and how multiple factors contribute to that. What drives the activity? The ligand? The receptor? Or the signalling machinery specific to each cell type or tissue? Possibly a combination of all. If MC_4 in the brain was swapped with MC_1, would then MC_1 regulate appetite? Or if we replace MC_1 in melanocytes with MC_4, would then MC_4 drive the production of melanin? Fibroblasts express MC_1 but they do not produce melanin upon activation with a selective MC_1 ligand [41], possibly because the pigmentary pathway is epigenetically silenced in this type of cells. Does the functional specialisation then derive from the receptor sequence or from the specific tissue that drives its preferential expression? Interestingly, the same ligand could behave as an agonist or antagonist depending upon which receptor it acts, like the synthetic peptide SHU9119, which has agonistic and antagonistic activity at MC_1 and MC_4, respectively. Recent work by Israeli H et al. showed that SHU9119 surprisingly behaves as an agonist at MC_4 when a particular point mutation is introduced at amino acid L133 [42], prompting us to think on where does the activity reside, on the ligand or the receptor. A better understanding of molecular determinants of receptor activity will greatly aid in the development of the five MCRs as therapeutic targets.

2.3. Advantages and challenges for drug discovery

Belonging to the GPCR family confers MCRs multiple advantages for drug development. It has been estimated that more than 130 GPCRs are targeted by ~700 existing medicines approved in the United States and the European Union. This means that ~35% of all approved drugs act by modulating the activity of a GPCR, thereby constituting the largest family of proteins currently targeted by approved therapeutics [43]. The attractiveness for drug discovery derives primarily because they are involved in a vast diversity of physiological functions and diseases but also, importantly, because they are easy to screen for at a high throughput scale. While the final biological function differs for different GPCRs, the secondary messengers and other aspects of GPCR regulation are essentially the same for most of them. Thus, any assay can be used to screen for almost any GPCR. For example, a cAMP assay can be used to study any GPCR that signals through the Gαs of Gαi, while measuring Ca^{2+} can detect activation of any GPCR that couples with Gαq. β-arrestin assays can be used to study any GPCR, as well as receptor internalisation and dimerisation events [44,45]. MCRs couple to Gαs, being cAMP the canonical pathway although many other mediators are also activated, like ERK1/2-phosphorylation and intracellular Ca^{2+}. The use of compound libraries is therefore common in the screening of GPCRs, allowing scientists to test thousands of compounds in a relatively short time [46]. Their high promiscuity also permits the screening for multiple types of ligands including peptides, small molecules or lipids and the recent elucidation of the crystal structures of MC_4 [42,47] and MC_1 [48] will likely prompt the rational design of new melanocortin molecules and the use of *in-silico* drug discovery strategies [49].

Further to this, GPCRs offer additional angles for intervention by exploiting the complexity of their signalling and regulation. The same receptor can elicit different signalling responses by the use of biased agonists (Fig. 3), which are molecules that only activate a subset of the pathways activated by that receptor, typically compared to the activity of the endogenous agonist [50]. For example, the melanocortin small molecule AP1189 can trigger ERK1/2 phosphorylation without activating the canonical pathway cAMP [8]. The therapeutic implications for this specific bias are substantial, as phosphorylation of ERK mediates the anti-inflammatory actions while cAMP is responsible for the unwanted pigmentary effects. This division of functions derived from ligand bias is known as functional selectivity. The innovation that this compound achieves is that although the drug is not selective for a 'receptor', the selectivity for the 'function' provides the solution. Allosteric modulation also offers hopes for achieving receptor selectivity because allosteric regions are typically less conserved. We identified that the drug fenoprofen acted as an allosteric modulator at MC_3 [46]. This activity confers the molecule with pro-resolving properties that other similar anti-inflammatory drugs like aspirin or ibuprofen lack, although in this case, there was no improvement on selectivity.

Availability of genetic information is also an advantage and it has been reported that targets for which this type of information is available are more likely to succeed in clinical trials [51–53]. Recent efforts in understanding loss and gain of function variants at MC_4 [54,55] might have favoured the successful development of setmelanotide, the first anti-obesity drug targeting the MC system.

From a physiological perspective, the focus on endogenous resolution mechanisms offers the rationale that these drugs will exhibit fewer side effects as they mimic the way our body naturally resolves inflammation [9,10]. Besides resolution, the wide distribution of these receptors and their involvement in multiple organ systems grants their exploitation for many other conditions, as will be discussed in Section 4.1.

Regarding challenges, the lack of selectivity has traditionally been

seen as a problem to MC drug discovery, which is caused by the high sequence homology between the five receptors [10], rendering it difficult to activate one without acting on the others. Interestingly, this concern has not been an obstacle for the approval of the first few MC drugs currently on the market, as none of them are fully selective for any MCRs (Table 1). Although generally mild and well tolerated, these drugs present with side effects mostly associated with the interaction with another MCR. Selectivity therefore would improve the profile of existing drugs. Little is known about how to achieve selectivity, but the recent deciphering of the long-awaited crystal structure of MC_4 [42,47] and MC_1 [48] may pave the way to accomplish that. Indeed, the MC_4 structure has already guided the rational design of new peptides and the identification of key residues determining MC_4/MC_3 selectivity [56].

A screening based on a single signalling cascade may recognise receptor binding with lack of signalling as proof of antagonism (Fig. 3). A biased agonist may however have the ability to activate another pathway and mistakenly be categorised as an antagonist. It is likely that many screenings performed in the past may have been hampered by this misinterpretation. Hence, the approach to MC drug discovery should include multiplexing of several signalling pathways [44], or at least those known to be involved in therapeutic/side effects, instead of solely focusing on cAMP, which is the strategy that has prevailed in the MC field until now. However, to overcome this challenge, first we need more comprehensive and systematic studies of the repertoire of signalling pathways that each receptor is able to activate as well as what is their biological role.

The peptidic nature of endogenous melanocortins renders them unsuitable for oral administration, making the development of small molecules an attractive alternative. In addition, the half-life of αMSH in circulation is less than fifteen minutes [57], hence unsuitable to be used directly as a drug. Strategies have been focusing on the introduction of modifications to the amino acid sequence of αMSH and the other MC peptides in order to enhance the stability. Next, we discuss strategies that have been followed in order to develop and improve the properties of novel MC drugs.

3. Evolution of strategies to develop melanocortin drugs: past and current ambitions

3.1. Aiming for stability and selectivity

The initial efforts in developing melanocortin drugs were mainly, and expectedly, based on the introduction of modifications into the amino acid sequences of endogenous melanocortin ligands, with the aims of improving the stability as well as achieving selectivity for specific MCRs subtypes. Those early studies helped to identify the core sequence HFRW that is required to activate all MCRs [58], and led to the design of a large number of peptides that, although many of them did not reach clinical development, have been invaluable as research tools. Common approaches included the incorporation of non-proteinogenic amino acids like norleucine or D-amino acids to increase stability, as these are more resistant to proteolytic cleavage. This approach led to the development of the peptides [Nle^4,D-Phe^7]-αMSH (NDP, also called melanotan I or afamelanotide [59]), and DTrp^8-γMSH [60], two of the most commonly used tools in melanocortin research. Cyclic peptides were also designed, leading to highly selective pan-agonists like melanotan II [61], which was designed using NDP as a scaffold. Then, using melanotan II as scaffold, it was found that the single substitution of D-Phe with the amino acid D-Nal(2') converted this peptide, known as SHU9119, into an antagonist to MC_3 and MC_4, while retaining agonistic activity at MC_1 and MC_5 [62].

To overcome the limitations of peptide-based drugs, strategies to develop small molecules soon followed, either by using peptides or amino acids as starting scaffolds, or by performing compound library screenings [46,63]. The compound BMS-470539 was developed to mimic the central HFRW pharmacophore of MC peptides, an approach that resulted in selectivity to MC_1 receptor [64]. MC_4 selectivity was achieved with a compound known as THIQ (tetrahydroisoquinolone) [65], which together with BMS-470539, has been fundamental to understanding the roles of MC_4 and MC_1, respectively. Additional compounds and strategies to produce MC ligands were recently reviewed by Ericson et al. [66].

In summary, these strategies have provided novel insights into the physiological role of the different MCRs, new clues on how to achieve receptor selectivity, how to improve stability, how to modulate agonism vs antagonism, and the potential side effects that targeting the MC system may produce.

3.2. Exploring GPCR versatility

The deeper understanding of GPCR biology and the incorporation of this knowledge into melanocortin drug development has resulted in the design of innovative molecules. Although GPCR signalling was previously understood as a linear cascade of events (i.e. ligand → receptor → signalling pathway → biological effect), we now know that GPCR activity is highly complex and the final outcome depends on the combination of multiple aspects including dimerisation, multiple signalling pathways with different dynamics, existence of signalling bias, allosteric modulation, desensitisation, receptor co-expression, and more. Some of these aspects have actually been exploited to produce molecules with improved therapeutic profiles. For example, allosteric modulation can be exploited to obtain molecules with higher receptor selectivity, as allosteric sites tend to be more variable than orthosteric ones (see Fig. 3). Another advantage of allosteric modulation is the possibility of achieving more directed effects, as the action of an allosteric modulator depends on the presence of the endogenous ligand in the target tissues. Then, we found, for example, that the presence of endogenous melanocortin peptides in synovial fluids of arthritic joints could be exploited with the use of an allosteric modulator like fenoprofen [46]. Furthermore, Roger Cone also proposed an interesting application of melanocortins targeting MC_4 for the treatment of obesity [67,68]. Based on the fact that positive allosteric modulators (PAM) act by enhancing the activity of the endogenous ligand, a PAM directed at MC_4 could be used to treat obesity in patients carrying a loss-of-function mutation only in one copy of the gene (i.e. haploinsufficiency), with the expectation that a PAM may enhance and compensate for the loss activity at MC_4 caused by the mutant allele.

Ligand bias occurs when a compound shows preferential activation

Table 1
Melanocortin drugs currently approved for the use in humans.

Product	MC molecule	Activity	Indications	Source
Acthar® Gel	ACTH (pan agonist)	$MC_{3,4}$	Infantile spasm, rheumatic disorders, multiple sclerosis, etc	Mallinckrodt Pharmaceuticals
Cortrosyn™	$ACTH_{1-24}$(pan agonist)	MC_2	Adrenal insufficiency diagnosis	Amphastar Pharmaceuticals
Synacthen®Depot	$ACTH_{1-24}$(pan agonist)	MC_2	Adrenal insufficiency diagnosis	Atnahs Pharma UK Ltd
		MC_3	Gout, colitis, arthritis, etc	
Scenesse®	Afamelanotide(pan agonist)	MC_1	Erythropoietic protoporphyria	Clinuvel Pharmaceuticals
Vyleesi®	Bremelanotide (pan agonist)	MC_4	Hypoactive sexual desire disorder	Palatin Technologies
Imcivree®	Setmelanotide (pan agonist)	MC_4	Obesity	Rhythm Pharmaceuticals

Last updated on 1st Nov 2021. See available structures in Table 3.

of a particular signalling pathway, with respect to the activity of the endogenous ligand (Fig. 3). The different biological outcomes that can derive form the activation of a receptor may depend on different signalling pathways. For example, although activation of MC_1 on melanocytes results in cAMP accumulation and phosphorylation of ERK1/2, it is known that the production of eumelanin is entirely dependent on cAMP [69]. On the other hand, the discovery of the bias agonist AP1189, which shows preference for ERK1/2 phosphorylation over cAMP, has helped to reveal that the anti-inflammatory actions of melanocortins may depend more on phospho-ERK1/2 than on cAMP [8]. The advantages are self-explanatory: ligands may then be designed to preferentially activate the therapeutically relevant pathway while evading activation of the pathways that may lead to undesired effects. The full exploitation of signalling bias is still in its infancy though, as it may be driven by multiple reasons other than the specific ligand, such as the specific cell type and context (e.g. depending on which G proteins are expressed), or even by the presence of single nucleotide polymorphisms [54,70].

3.3. Pharmacogenetics: aiming for precision medicine

As discussed above, the focus for MC drug development has shifted from exploring chemical modifications in ligands to exploiting the complex biology of the receptors, i.e. from improving the properties of the actual molecules to refining the biological actions to seek for. We envisage that the next trend and stage in melanocortin drug development will focus on patients and their specific needs, in order to deliver personalised treatments and to increase the chances of success. Genetic variants are very frequently found in MCR genes. The most well-known are those occurring in the *MC1R* gene [71], which determine skin and hair pigmentation and risk of developing skin cancer, and variants in the *MC4R* gene, which strongly impact body weight regulation [54,55]. Genetic variants can affect drug efficacy through multiple mechanisms. First, they can cause loss-of-function by altering surface expression, maximal response or by affecting the engagement with G-proteins [72–74]. Genetic variants may also lead to biased signalling. The variant D294H on *MC1R* retains the ability to induce cAMP accumulation upon activation with NDP-αMSH but results in a loss of ERK1/2 phosphorylation [70,75]. Interestingly, certain amino acid variations can also cause radical changes in the pharmacology of a ligand, like for example, the substitution of leucine at position 133 of MC_4 with a methionine, which converts the MC_4 antagonist SHU9119 into an agonist [42].

Thus, a deep characterisation of the impact of genetic variants on MCRs is needed in order to improve the process of drug development, given the high frequency of these variants in the population. Patients carrying certain variants may not respond to the treatment while at the same time may still suffer from the unwanted off-target effects. In addition, the incorporation of this knowledge into the early phases of clinical development may allow the identification of subsets of patients that are most likely to respond and consequently increase the chances of success during clinical trials [76].

4. Melanocortin drugs: from potential to reality

4.1. Therapeutic value of melanocortin drugs

Three major areas have dominated MC research and drug discovery efforts in recent years: inflammatory diseases, obesity and cancer. A better understanding of the complex role of MCs in disease mechanisms, as well as of GPCR biology, culminated with the recent elucidation of the crystal structures of MC_4 [42,47] and MC_1 [48], suggesting that the development and approval of additional MC drugs for new indications may be certain. The approval of the first few MC drugs also confers a 'safety net' for the development of new compounds, as they have already demonstrated that they are generally safe and well tolerated, which is commonly the most important bottleneck for the approval of first-in-class drugs.

The widespread distribution of MCRs in many organ systems and their remarkable involvement in the control of multiple and varied physiological functions form the basis for their high therapeutic value. The receptor MC_1 is highly expressed in melanocytes and is responsible for the formation of the photoprotective dark melanin, known as eumelanin. Ultraviolet (UV) radiation induces the release of the ligand αMSH in the skin which activates MC_1, leading to the upregulation of the enzymes responsible for converting red/yellow pheomelanin into dark eumelanin [77]. This mechanism can be therapeutically harnessed to induce photoprotection and increase tolerance to light to treat photodermatoses like erythropoietic protoporphyria, to induce re-pigmentation in the auto-immune condition vitiligo and for the prevention of melanoma. However, the protection exerted by MC_1 activation extends beyond the production of photoprotective melanin by inducing the repair of UV-induced DNA damage [78]. This activity is being investigated for the treatment of xeroderma pigmentosum, a devastating rare genetic condition caused by mutations in the components of the nucleotide excision repair mechanism, in which patients, referred to as "moon children", present with extreme sensitivity to sun exposure, causing sunburns and skin cancer. Recently, we also discovered a mechanism of senescence induction in fibroblasts treated with the MC_1 selective compound BMS470539, an action with potential therapeutic value in fibroblasts-mediated conditions [41].

MC_2 differs significantly from the other four MCRs. Limited by its confined expression in the adrenal glands and by being solely activated by ACTH, its role consists of promoting transcription of steroidogenic genes leading to the production of cortisol. The potential therapeutic value of targeting MC_2 may reside in the use of antagonists for the treatment of conditions caused by excess of ACTH, like congenital adrenal hyperplasia and Cushing's disease caused by ACTH overexpressing pituitary adenoma [79–81].

MC_3 is expressed in the central nervous system and plays a role in the regulation of energy homoeostasis. It has been reported that loss of function mutations in humans increases the risk of obesity [82]. Its role however, is not as clear as the one for MC_4. *Mc3r*$^{-/-}$ mice are obese but they do not suffer from the hyperphagia and the early onset-obesity characteristic of *Mc4r*$^{-/-}$ mice, while behavioural phenotypes, like responses to hypocaloric conditioning or binge-feeding, are compromised in *Mc3r*$^{-/-}$ but not in *Mc4r*$^{-/-}$ mice [83,84]. Thus, the use of MC_4 agonism for the treatment of obesity is based in the regulation of appetite, an action that will not be achieved by activating MC_3. On the other hand, the use of MC_4 antagonists may be effective in inducing appetite in patients with anorexia as well as cachexia [85]. However, the antagonism at MC_3 may also be beneficial for the regulation of anorexia although through a complex mechanism, distinct from that afforded by MC_4, involving the interaction with hypothalamic AGRP neurons [86]. More recently, a potential use of MC_3 agonists in patients with delayed puberty has also been suggested [87].

MC receptors, particularly MC_1 and MC_3, are also expressed peripherally in immune cells [88] and are extensively investigated for the anti-inflammatory actions exerted by agonists at these receptor. MC_4 activation has also been related to ethanol consumption and hence could be used to treat alcohol use disorders [83]. On the other hand, the expression of MC_4 in central nociceptive regions suggests the possible use of MC_4 antagonists for the treatment of neuropathic pain [89].

The last receptor, MC_5 is the least studied of the five members of this family. The potential value as a therapeutic target is based mainly on its expression in exocrine glands and its role in the regulation of secretions from sebaceous glands in the skin, making its antagonism a potential treatment for acne vulgaris (see Section 4.3). On the other hand, agonism at MC_5 may help reduce inflammatory damage in the retina associated with uveitis [90].

Besides these disparate actions, what all MCRs share in common is their role in inflammation, albeit through different mechanisms. While MC_2 activation leads to anti-inflammatory actions through the

production of cortisol (steroid-dependent mechanism), the other four receptors have shown, to different extents, to have anti-inflammatory properties via two different steroid-independent mechanisms: the vagus nerve-mediated cholinergic-anti-inflammatory pathway [91], and direct effects on immune cells and other cell types like endothelial cells and fibroblasts [10]. The current efforts in the development of MC anti-inflammatory drugs are based on this last mechanism, which affords reduction in immune infiltration, a decrease in the release of pro-inflammatory mediators and activation of pro-resolving pathways. These actions provide the basis for investigating the potential use of these drugs, in addition to the already approved inflammatory conditions (see below), for kidney diseases like nephrotic glomerulopathy [92], ischaemia reperfusion injury [93,94], colitis [95], periodontal disease [96,97], non-alcoholic fatty liver disease [98], myocardial infarct [99,100], pulmonary inflammation [101], Alzheimer's disease [102], sarcoidosis [103], and many other conditions with an inflammatory component as disease driver.

4.2. Approved melanocortin drugs

Acthar® Gel, the first melanocortin drug used for therapeutic use in humans, was approved in the early 1950 s [104], about forty years before MC receptors had even been discovered [1]. This scenario is not unusual, as currently it is estimated that between 20% and 30% of approved drugs have no known target or clear mode of action [105,106]. Acthar® Gel, also known as repository corticotropin injection, is manufactured from porcine pituitary extracts and hence contains other pituitary peptides in addition to the melanocortin ACTH, which remains the main mediator of its efficacy, but details on those other peptides and possible actions are not available. Currently, it is approved for nineteen different indications which include infantile spasms (also known as West syndrome), acute exacerbations of multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, serum sickness, sarcoidosis or nephrotic syndrome, among others.

Another form of ACTH is also approved and consists of the first twenty-four amino acids of the peptide. Commercialised as Cortrosyn™ and Synacthen® Depot, $ACTH_{1-24}$ is commonly used as a diagnostic tool for the detection of adrenal insufficiency, as plasma cortisol levels roughly double within one hour from the intramuscular administration of 1 mg of this peptide. Synacthen® Depot is also approved for the treatment of inflammatory conditions like rheumatoid arthritis, osteoarthritis, inflammatory bowel disease and more commonly for gout, as this condition requires short term treatments [107,108]. However, as could be anticipated, adrenal effects derived from MC_2 activation currently limit the long-term use of ACTH-based drug, required for chronic conditions. Innovative ACTH forms devoid of MC_2-derived adrenal affects may then be ideal to bypass these limiting side effects.

Although, as mentioned earlier, knowing the mode of action of a drug is not a requirement for its approval, this knowledge can provide a more rational and informed route for the development of successful approaches. Our significantly deeper current understanding of melanocortin physiopathology and pharmacology is what favoured the recent success of setmelanotide, branded as Imcivree® [109,110]. Thanks to the elucidation of the complex neural mechanisms that control appetite, MC_4 activation was proposed as an anti-obesity drug for patients with deficiency in the appetite regulators proopiomelanocortin (*POMC*), proprotein convertase subtilisin/kexin type 1 (*PCSK1*), or leptin receptor (*LEPR*) genes [109]. The deep understanding of satiation regulation by MC_4 gained over many years of research with a recognition of how genetic variants affect this regulation, provided a new basis for precision medicine. Thus, patients with obesity which is not related to deficiency in those genes are not treated with this drug as it is not expected to display any efficacy. Although the anti-obesity actions of setmelanotide are mediated by MC_4, this molecule is a pan-agonist and effects derived from the activation of the other MCRs are common. For example, ~50% of patients experience darkening of the skin due to activation of MC_1 in melanocytes. However, the unwanted sexual reactions caused by setmelanotide in obesity patients represent indeed the therapeutic action of another approved MC_4 drug, bremelanotide, commercialised under the name of Vyleesi® [111]. Importantly, patients non-eligible for setmelanotide treatment, while not benefiting from the anti-obesity effect, will still suffer from its side effects, evidencing the importance of a precision medicine approach that guarantees the provision of the right drug for the right patient. Furthermore, an incomplete elucidation of the regulatory mechanisms exerted by MC_4 and role of genetic variants would have likely resulted in the failure of setmelanotide clinical trials.

As discussed in previous sections, the wide range of actions and potential therapeutic indications highlights the fact that a biological effect considered unwanted in one case, can indeed be the therapeutic action in another situation. The 'unwanted' skin darkening of setmelanotide and bremelanotide is actually a therapeutic opportunity for the treatment of devastating conditions like erythropoietic protoporphyria (EPP). The active ingredient of the drug Scenesse®, known as afamelanotide, is based on the endogenous αMSH peptide, with the structure $[Nle^4,D\text{-}Phe^7]$-αMSH. This pan-agonist thirteen-amino acid peptide is extensively used as a research tool, and it is commonly referred to in literature as NDP or melanotan-I. This drug, by inducing the production of eumelanin in the skin, increases tolerance to sunlight and artificial light thus preventing the phototoxic reactions characteristic of EPP patients. Moreover, the mode of action may extend beyond the physical barrier protection conferred by eumelanin, as MC_1 activation also regulates DNA damage repair mechanisms [112,113], suggesting new potential indications for skin cancer prevention and other conditions associated with defective DNA repair mechanisms.

In this section we have summarised the current existing melanocortin medicines and their approved indications. Next, we will discuss the current efforts under clinical investigation.

4.3. In the pipeline

The expectations for novel successful MC drugs are high. Currently, multiple clinical trials are being conducted for already approved MC drugs to test the efficacy on novel indications (Table 2, and Table 3 for structures). The peptide $ACTH_{1-24}$ (also known by the names cosyntropin, tetracosactide or MNK-1411) is under investigation for the treatment of Duchenne muscular dystrophy. The study, a randomised, double-blind, placebo-controlled trial, will address the effect of $ACTH_{1-24}$ in motor function on male patients between 4 and 8 years old. While not addressing the cause of the disease, the efficacy of $ACTH_{1-24}$ is expected to be driven by reducing inflammation and muscle damage (trial # EUCTR2017-004139-35-BE). The other currently available form of ACTH, Acthar® Gel, is being tested for skin conditions (keratitis, psoriatic arthritis), renal diseases (glomerunonephritis, nephrotic syndrome), eye related conditions (acute optic neuritis, vitreoretinopathy) and other inflammatory diseases like sarcoidosis. Although more research is needed to fully understand the mechanism of action, Acthar® Gel has been included in the 2021 European Respiratory Society guidelines for the treatment of sarcoidosis when other therapies fail or are contra-indicated [114]. Afamelanotide (approved for the treatment of EPP, marketed as Scenesse®) is under investigation to expand its panel of skin-related indications, including vitiligo, solar urticaria, xeroderma pigmentosum and acne vulgaris. The potential efficacy of MC drugs in the treatment of acne vulgaris is, however, puzzling. MC_5 deficiency in mice causes severe disruption of sebaceous lipid production in the skin of mice [115], as well as in human sebocytes [116]. Consequently, the use of *antagonism* rather than agonism at MC_5 was proposed as a potential strategy [117], and efficacy has been shown for the compound JNJ10229570 in clinical trials (trial # NCT01326780). On the other hand, the beneficial effect of *agonism* with afamelanotide might then be related to its anti-inflammatory properties [118], although it is not clear if the expected increase in sebum production may

Table 2
Non-exhaustive list of registered clinical trials investigating drugs targeting the melanocortin system. Source: ClinicalTrials.gov and WHO International Clinical Trials Registry Platform. Search was performed on September 2021. *Asterisks indicate results are available at ClinicalTrials.gov. See available structures in Table 3.

Intervention	Drug type	Indications	Trial ID	Phase/Status
ACTH	MCR agonist	Duchenne Muscular Dystrophy	EUCTR2017-004139-35-BE	2/Authorised
		Refractory Gout	NCT04808856	Na/Recruiting
Acthar® Gel	Pituitary extract	Keratitis	NCT04169061	4/Completed*
		Psoriatic arthritis	NCT03419650	4/Completed
		Glomerulopathy	NCT04080076	4/Recruiting
		Sarcoidosis	NCT02523092	4/Not recruiting
		Rheumatoid arthritis	NCT02030028	Na/Recruiting
		Acute optic neuritis	NCT01838174	4/Active
		Nephrotic syndrome	NCT02132195	3/Completed*
		Multiple sclerosis	NCT02290444	3/Completed*
		Vitreoretinopathy	NCT03727776	1/Recruiting
Afamelanotide	MCR agonist	Arterial ischemic stroke	NCT04962503	2/Recruiting
		Acne vulgaris	NCT04943159	2/Completed
		Solar urticaria	NCT00859534	2/Completed
		Vitiligo	NCT04525157	2/Completed*
		Xeroderma pigmentosum	EUCTR2019-000597-34-DE	2/Recruiting
AP1189	Biased MCR agonist	Idiopathic Membranous Nephropathy	NCT04456816	2/Recruiting
		Rheumatoid Arthritis	NCT04004429	2/Recruiting
		COVID-19	RBR-59447vn	2/Completed
JNJ10229570	$MC_{1,5}$ antagonist	Acne vulgaris	NCT01326780	2/Completed*
MT-7117	MC_1 agonist	Scleroderma	NCT04440592	2/Recruiting
		Erythropoietic Protoporphyria	NCT05005975	3/Recruiting
PF-07258669	MC_4 antagonist	Anorexia	NCT04628793	1/Completed
PL-8177	MC_1 agonist	Non-infectious uveitis	NCT04105452	2/Not recruiting
PL-9643	$MC_{1,5}$ agonist	Dry eye disease	NCT04268069	2/Completed
Setmelanotide	MCR agonist	Bardet-Biedl Syndrome	NCT04966741	3/Not recruiting
		Hypothalamic obesity	NCT04725240	2/Recruiting
		Prader-Willi Syndrome	NCT02311673	2/Recruiting
TTP435	AGRP inhibitor	Obesity	NCT00779519	2/Completed

confound or clash with this anti-inflammatory activity. Theoretically, therefore, agonism at MC_1 combined with antagonism at MC_5 might yield the best outcome. Deciphering which of these activities is more relevant to determine clinical efficacy for patients with acne vulgaris will help to improve future approaches.

In addition to repurposing strategies for already approved drugs, new molecular entities are under investigation like the small molecule MT-7117, for the treatment of EPP and scleroderma. Also known as dersimelagon, this compound is reported to act selectively via MC_1 and can be administered orally [121]. Although the structure is available (PubChem CID 134817217), no further information nor data have been reported in the literature regarding the actions or mode of action of this compound. Agonism at MC_4 drives the anti-obesity actions of setmelanotide, by activating the satiety mechanism in the brain. Hence, antagonism at MC_4 (compound PF-07258669) is now being tested for the contrary, to induce appetite in patients with anorexia. Other examples of promising MC drugs include two selective peptides (PL-8177, PL-9643) for the treatment of various ocular diseases [119], as well as a new strategy to target obesity, with the use of an AGRP inhibitor. This molecule, TTP435, is the only ongoing strategy that is not based on mimicking the actions of endogenous ligands, but instead on inhibiting the natural antagonist AGRP. Excessive AGRP causes the same effects on hyperphagia and obesity caused by deficiency on MC_4 [120]. Hence, blockade of AGRP may represent a novel therapeutic alternative, although results on this trial have not been published yet.

5. Conclusions

Intensive research efforts on melanocortin receptors as therapeutic targets started as soon as these receptors were discovered in the early 1990 s. Their GPCR nature and involvement in multiple organ systems and physiological processes (e.g. skin, metabolism, adrenal function, obesity, inflammation, etc) was soon identified as an opportunity for the treatment of multiple clinical situations. Early efforts focused on understanding the specific pathways and actions activated by these receptors and endogenous ligands and on the development of multiple compounds that served as invaluable tools for advancing our understanding on this complex family of receptors.

Importantly, the approval of several MC drugs has also revealed that the initial concerns on possible side effects have been overcome and that the use of MC drugs are safe and well tolerated. For example, for the early unfounded claims that MC_1 activation could lead to melanoma, scientific evidence now shows that, not only this is not the case, but that the activation of this receptor indeed activates DNA repair mechanisms that could be exploited for the prevention of skin cancer. However, the development of more selective compounds that activate only one receptor of the family at the time are still desired to further improve these drugs, an objective we can now envisage as feasible in the near future thanks to the recent elucidation of the crystal structure of the receptors MC_1 and MC_4, which will likely be completed soon with further structures for the other MCRs.

The integration of multiple chemistry approaches for the development of MC ligands, with the complex biological roles and versatility of these GPCRs, as well as with the incorporation of genetics knowledge may soon deliver additional MC drugs to the clinics for the treatment of new conditions, like chronic inflammatory diseases. In the context of resolution of inflammation, MC drugs may offer solutions for high-need patients suffering from rheumatoid arthritis, fibrosis, inflammatory bowel disease, periodontitis or multiple sclerosis, among many other conditions for which MC drugs are under pre-clinical and clinical investigation. Given the growing number of innovative molecules and clinical trials currently ongoing, we envisage that the now 'unforgotten' therapy, may soon deliver the first *Resolution Pharmacology* drug to help to improve the quality of life of patients suffering from chronic inflammatory conditions.

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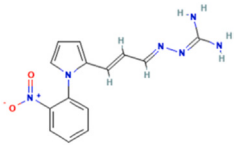
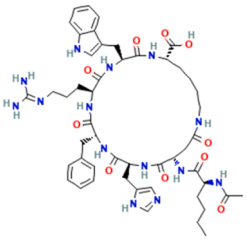
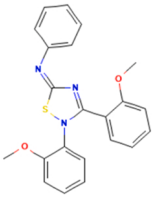
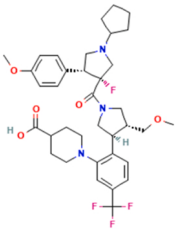
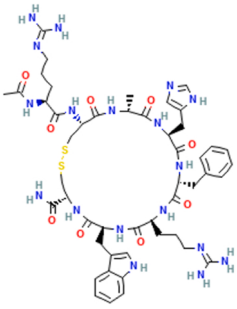
Table 3
Amino acid sequence and chemical structures. Available/disclosed sequences and structures for compounds indicated in Tables 1 and 2.

MC molecule	Peptide sequence / Structure	IUPAC Name
ACTH	SYSMEHFRWGKPVGKKRRPVKVYPNGAEDESAEAFPLEF	
$ACTH_{1-24}$	SYSMEHFRWGKPVGKKRRPVKVYP	
Afamelanotide	SYSXEHFRWGKPV	
AP1189		2-[(*E*)-[(*E*)−3-[1-(2-nitrophenyl)pyrrol-2-yl]prop-2-enylidene]amino]guanidine
Bremelanotide		(3 *S*,6 *S*,9 *R*,12 *S*,15 *S*,23 *S*)−15-[[(2 *S*)−2-acetamidohexanoyl]amino]−9-benzyl-6-[3-(diaminomethylideneamino)propyl]−12-(1*H*-imidazol-5-ylmethyl)−3-(1*H*-indol-3-ylmethyl)−2,5,8,11,14,17-hexaoxo-1,4,7,10,13,18-hexazacyclotricosane-23-carboxylic acid
JNJ10229570		2,3-bis(2-methoxyphenyl)-*N*-phenyl-1,2,4-thiadiazol-5-imine
MT-7117		1-[2-[(3 *S*,4 *R*)−1-[(3 *R*,4 *R*)−1-cyclopentyl-3-fluoro-4-(4-methoxyphenyl)pyrrolidine-3-carbonyl]−4-(methoxymethyl) pyrrolidin-3-yl]−5-(trifluoromethyl)phenyl]piperidine-4-carboxylic acid
Setmelanotide		(4 *R*,7 *S*,10 *S*,13 *R*,16 *S*,19 *R*,22 *R*)−22-[[(2 *S*)−2-acetamido-5-(diaminomethylideneamino)pentanoyl]amino]−13-benzyl-10-[3-(diaminomethylideneamino)propyl]−16-(1*H*-imidazol-5-ylmethyl)−7-(1*H*-indol-3-ylmethyl)−19-methyl-6,9,12,15,18,21-hexaoxo-1,2-dithia-5,8,11,14,17,20-hexazacyclotricosane-4-carboxamide

Source: PubChem.

Authors contribution

TMM Collected information and wrote manuscript. TB collected information and revised manuscript. TENJ collected information and revised manuscript.

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Biorender.com was used in the preparation of Fig. 2.

Declaration of interest

TMM has received research funding and conducted consultancy work for SynAct Pharma AB and TXP Pharma AG. TENJ is shareholder in SynAct Pharma AB, TXP Pharma AG, and Resother Pharma ApS, and owns intellectual property on AP1189. TB holds managerial positions in SynAct Pharma AB and in TXP pharma AG, and is shareholder in SynAct Pharma AB, TXP Pharma AG, and Resother Pharma ApS. SynAct Pharma owns intellectual property rights on AP1189.

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