Advances in therapeutic peptides targeting G protein-coupled receptors

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Abstract

Dysregulation of peptide-activated pathways causes a range of diseases, fostering the discovery and clinical development of peptide drugs. Many endogenous peptides activate G protein-coupled receptors (GPCRs) — nearly fifty GPCR peptide drugs have been approved to date, most of them for metabolic disease or oncology, and more than 10 potentially first-in-class peptide therapeutics are in the pipeline. The majority of existing peptide therapeutics are agonists, which reflects the currently dominant strategy of modifying the endogenous peptide sequence of ligands for peptide-binding GPCRs. Increasingly, novel strategies are being employed to develop both agonists and antagonists, and both to introduce chemical novelty and improve drug-like properties. Pharmacodynamic improvements are evolving to bias ligands to activate specific downstream signalling pathways in order to optimise efficacy and reduce side effects. In pharmacokinetics, modifications that increase plasma-half life have been revolutionary. Here, we discuss the current status of peptide drugs targeting GPCRs, with a focus on evolving strategies to improve pharmacokinetic and pharmacodynamic properties.

Introduction

G protein-coupled receptors (GPCRs) mediate a wide range of signalling processes and are targeted by one third of drugs in clinical use[1]. Although most GPCR-targeting therapeutics are small molecules[2], the endogenous ligands for many GPCRs are peptides (comprising 50 or fewer amino acids), which suggests that this class of molecule could be therapeutically useful.

GPCRs are divided into families based on structural similarities. The largest group is the Class A (rhodopsin-like) family, followed by the Class B (secretin) family. Although other families, such as the Class C, frizzled, and adhesion classes exist, therapeutics predominantly target Class A and B GPCRs, so this Review is focussed on these two groups. *The International Union of Basic and Clinical Pharmacology* (IUPHAR) *Guide to Pharmacology*[3] currently lists 197 Class A receptors with known ligands (excluding olfactory, vision, taste and vomeronasal sensory receptors); 64 (32%) of these bind to endogenous peptides[3]. In GPCR Class B, there are 20 receptors activated by 15 endogenous peptides. These GPCRs are grouped in the following families based on which ligand they bind: calcitonin, corticotropin-releasing factor, glucagon, parathyroid hormone (which is generally considered to be a peptide, despite its 84-amino acid length), or vasoactive intestinal peptide (VIP) or pituitary adenylate cyclase-activating peptide (PACAP).
A further 87 'orphan' receptors from different families — for which the endogenous ligand is not yet known — have been identified in the human genome. For 54 of these orphans, at least one publication has proposed an endogenous ligand, some of which are peptides[4]. 'De-orphanisation' of these receptors is ongoing. For example, G protein-coupled receptor 171 (GPR171) and GPR83 were recently found to interact with the neuropeptides PEN and LEN respectively, which are abundant in mouse brain (and highly conserved in humans). Initial studies suggest they may be functionally coupled in the regulation of feeding, and if substantiated, these receptors could be new potential drug targets[5,6].

Endogenous peptides that bind to GPCRs on cell surfaces spatiotemporally span paracrine and autocrine signalling, from long-acting hormones to locally released mediators of cellular functions and neurotransmitters. Peptides are one of the largest and most ancient classes of intercellular chemical messengers[7]. The pioneering development, using these naturally occurring peptides as therapeutics, was the use of insulin in the 1920s. Insulin principally targets a tyrosine kinase receptor[8] and the development of this therapeutic exploited the remarkable pharmacodynamic properties of peptides: high affinity, selectivity and potency. In line with the observed effects of insulin, most other peptide therapies are well tolerated with few off-target effects.

Naturally occurring peptides, however, do not typically make good therapeutics. The development of peptides as drugs has been limited by poor pharmacokinetics (short half-life, rapid degradation and high levels of clearance) and a lack of oral bioavailability due to a combination of low gastrointestinal stability and poor permeability. Therefore, strategies need to be developed to address these aspects before most peptides can be effective medicines.

Peptide drugs occupy a structural space between biologics (antibodies and proteins) and small molecules. Whereas endogenous signalling peptides have usually 50 or fewer

residues, the FDA defines peptide drugs[9] as having a maximum of 40 residues (a few exceptions have been noted), not limited to the 20 genetically encoded amino acids. These therapeutics are undergoing a renaissance. Emerging novel strategies include half-life extension platforms, stapling and resistance to proteolysis, all of which significantly improve pharmacokinetics and oral bioavailability. These strategies are perhaps best exemplified by the development of glucagon-like peptide 1 (GLP-1) receptor agonists that have increased resistance to proteolytic degradation and reduced renal clearance. Several successfully marketed products and a multitude of pre-clinical novel approaches (e.g., stapling, cyclization, and glycosylation) have come from these efforts. This success has also fostered the development of multifunctional peptides that combine agonism for two or more GPCRs in the same peptide, based on relatively high sequence homology and similar binding sites. Complimentary pharmacodynamic strategies are extending the repertoire of drugs that act at a given GPCR.

Peptide ligands that can selectively activate downstream signalling pathways and are described as biased towards, for example, G proteins or β-arrestins (the two main pathways downstream of GPCRs), are also emerging. These pathways may be linked to distinct physiological or pathophysiological responses — beneficial or detrimental — so biased ligands can be designed to have the optimum therapeutic activity but with reduced side effects or receptor internalisation.

The application of structure-based design has significantly altered all aspects of small molecule drug discovery, including drugs targeting GPCRs[10, 11]. Peptides are also amenable to structure-based design strategies. X-ray crystallography or cryo-electron microscopy (cryo-EM) [G] structures of the binding domains of over 27 peptide-binding or protein-binding GPCRs have been reported — amongst these 65 unique receptor–ligand complexes, 22 contain a peptide ligand. The rapidly expanding repertoire of receptor structures will substantially advance understanding of the basic peptide–receptor structure activity relationship (SAR) and more subtle aspects of conformational biased signalling, thereby enabling rational agonist or antagonist design to activate or block a pathophysiological process.

In this Review, we discuss the clinically approved and preclinical GPCR-targeting peptide therapeutics (Figure 1) and outline the challenges in the field. We also highlight key strategies to improve pharmacokinetics (mainly via increases in plasma half-life) and pharmacodynamics (via increased potency), as well as ligand bias.

Approved peptide therapeutics

The majority of GPCR-targeting peptide drugs that are either approved for clinical use or in development function as agonists, and are used to replace or enhance low levels of

endogenous peptides. In Class A, major receptor targets include μ and κ opioid receptors (abbreviated to MOR and KOR, respectively) to relieve pain; oxytocin and vasopressin receptors for induction of labour; and apelin and angiotensin receptors in cardiovascular disease (Table 1, Table 2). More specialised targets include the somatostatin receptor for acromegaly and Cushing's disease. Peptide therapies targeting Class B receptors are dominated by GLP-1 receptor agonists for the treatment of type 2 diabetes (T2D), along with synthetic or modified versions of peptide hormones.

Few antagonists have made it to the clinic. Most of those that have made this leap target Class A GPCRs. Antagonists that block the action of gonadotrophin releasing hormone, such as Degarelix and Abarelix, or agonists that desensitise the receptor to have the same effect, such as Buserelin, are used for the treatment of cancer.

Synthetic endogenous peptide analogues

Oxytocin[12, 13] and vasopressin were the first chemically synthesised variants of endogenous peptides for Class A receptors to enter clinical use, and did so in the 1950s. These two peptides are amongst the ten peptides that are usually synthesised chemically or by recombinant technology, but that have an identical sequence to their endogenous peptide equivalents, that have been approved for clinical use in one or more countries (Table 1). Evaluation of those ten peptides provides an opportunity to examine the properties of native peptides that may limit or enable their widespread therapeutic application as well as the impact of those properties on drug development strategies.

Peptides targeting Class A GPCRs are mainly agonists that bind with both high affinity (median –log of the dissociation constant (pK_i) = 8.4) and potency (median –log of the half maximal effective concentration (pEC_{50}) = 8.5), compared with the average clinically used drug[14]. However, they typically demonstrate very short plasma half-lives following intravenous administration, with a median value of ~5.3 minutes, reflecting their rapid degradation by peptidases and/or high rates of excretion, particularly by the kidney. Theoretically, peptide levels will have therefore declined to <1% of the original dose within 6 half-lives, which is about 32 minutes. Consistent with their polar, hydrophobic chemical properties, they have low median volumes of distribution (~9.8 litres, which is close to the expected total volume of interstitial fluid), indicating that the protein is restricted to the fluid compartment with negligible distribution into tissues. Finally, endogenous peptides typically have low protein plasma binding, which ensures that the majority of the circulating peptide is in the unbound state and available to directly bind to and activate its cognate receptor. However, unbound peptide is also highly susceptible to renal elimination and cleavage by serum or tissue proteases; both of these processes decrease plasma half-lives.

The endogenous ligands for all Class B GPCRs are peptides. For peptides that interact with Class B GPCRs, the median pharmacodynamic and pharmacokinetic values are similar to those for peptides that target Class A receptors: high potency (pEC_{50} = 9.9) combined with short plasma half-life (15 minutes) and low volume of distribution (~9.9 litres). Calcitonin (isolated from salmon in the 1970s) was one of the first native peptides to be used clinically, and was used to treat Paget's disease in patients who were unresponsive to first-line treatments. Human calcitonin is used in patients who develop antibodies to the salmon calcitonin. The goal of treatment is to inhibit bone resorption by osteoclasts and therefore increase bone mass[15].

Paradoxically, at least some of the peptides in both classes are likely successful because their short plasma half-lives, which is usually an undesirable trait, are exploited for clinical benefit. At one extreme, the peptide therapeutic with the shortest half-life is angiotensin II, first synthesised in 1947 but only approved in 2017 (as Giapreza). This synthetic analogue of angiotensin II is used for the treatment of critically ill patients with septic shock, in whom an abnormal distribution of blood to the smallest blood vessels results in inadequate systemic blood supply, which can be fatal. The drug binds to vascular smooth muscle AT_1 receptors and takes a median time of five minutes to adequately increase blood pressure following intravenous dosing[16]. The short half-life — less than one minute — ensures that hypertension resulting from an overdose is very unlikely. Similarly, oxytocin, which was sequenced and synthesised in the 1950s[13,14], is used to induce labour and strengthen contractions immediately after intravenous administration, but then subsides within an hour, reflecting its short half-life of a few minutes.

Modified peptides

Twenty six synthetic peptides (20 agonists and six competitive antagonists) targeting eight Class A receptor families have been approved for clinical use (Table 2). The few antagonists that have been developed (such as Icatibant and Cetrorelix) usually have sub-nanomolar affinity, which is higher than the affinity of the endogenous peptide. This may be an important requirement for a clinically successful peptide antagonist, given the high potency and affinity of the endogenous peptide agonists, suggesting that effective antagonists require high levels of receptor occupancy to maintain efficacy. In this regard, peptides targeting the gonadotrophin-releasing hormone (GnRH) receptor in the pituitary are particularly intriguing in the non-steroidal manipulation of the reproductive endocrine axis. For example, the agonist Buserelin acts by an unusual pharmacological mechanism as it desensitises the GnRH receptor, reducing the amount of gonadotropin released from the pituitary gland, thereby inhibiting testosterone secretion in males and oestrogen secretion in females. Therefore, this agonist peptide effectively switches off the GnRH receptor by

removing receptors from the cell surface and reducing further stimulation. Whereas transient administration of Buserelin — for example, in the setting of in-vitro fertilization — suppresses the premature surge of luteinizing hormone, there are unwanted side effects caused by the initial agonist activity of this peptide, such as hyperstimulation of the ovaries, which has led to the development of GnRH receptor antagonists such a Ganirelix (Figure 2)[17]. To date, no peptide inverse agonists or allosteric modulators have been reported in clinical use; most of the current drugs are based on endogenous peptides and therefore bind to the orthosteric site.

In Class B, all 13 existing therapeutics are agonists that target one of three receptor families (principally the GLP-1 receptor in the glucagon family), and, like Class A-targeting synthetic peptides, have a high median affinity and potency ($pK_i = 8.9$; $pEC_{50} = 9.7$) and a comparatively low volume of distribution (~16 litres) following subcutaneous injection. The median plasma half-life is around one hour (but this value excludes peptides modified specifically to have very long half-lives, measured in days), which has been achieved by a combination of selective amino acid substitutions at known sites of enzymatic cleavage and conjugation to ligands that bind serum proteins, such as albumin, which protects the peptides from enzymatic cleavage and reduces renal clearance[18-22].

Plasma protein binding of the remaining synthetic peptides in Table 2 is, in many cases, also high despite a lack of specific modifications that link the peptides to plasma proteins. According to the free drug hypothesis, only unbound drug is available to bind to and act at physiological sites of action. Therefore, plasma protein binding can influence both pharmacodynamic effects and pharmacokinetic properties of peptides. Liu et al.[23] have argued that, because many small molecule drugs (~30%) have high plasma binding, this is not necessarily an undesirable trait. Smith et al.[24] have maintained that increasing plasma binding, which increases metabolic stability and reduces clearance by organs such as the kidney, will lead to better drugs. The effects of plasma binding on the elimination (and therefore half-life) of peptides can be complex. For peptides excreted in their intact form by renal glomerular filtration (such as Cetrorelix, 86% of which is plasma-bound), increasing plasma binding would be expected to decrease the rate of elimination, because only the free peptide is filtered. Conversely, Buserelin (15% of which is plasma-bound) is metabolised by proteolytic enzymes; therefore, reducing proteolytic cleavage is expected to have a greater effect than increasing plasma protein binding on plasma exposure (Table 2).

Lessons from recent clinical approvals

During the last three years, 16 of the 195 FDA-approved new drugs were peptides[25,26,27]. Abaloparatide, which was approved for post-menopausal osteoporosis in 2017, was the first analogue of human parathyroid hormone-related protein to be developed. Although this

molecule is distinct from Teriparatide (a parathyroid hormone analogue), both of them target the PTH1 receptor for the same clinical condition and have similar side effects; however, Abaloparatide induces a greater increase in bone density[28]. Abaloparatide binds to the receptor with an affinity that is two orders of magnitude higher than that of Teriparatide. In addition, Abaloparatide binds with higher selectivity to a G protein-dependent receptor conformation (called RG), which results in transient responses and favours bone-formation versus a second confirmation (called R0), which is comparatively prolonged and favours unwanted bone-resorption[29]; R0 is bound by parathyroid hormone and analogues such as Teriparatide.

2017 also saw the approval of Semaglutide[30], the fifth GLP-1 receptor agonist to be approved for T2D. Of note, Semaglutide is one of several drugs that has a significantly increased half-life, around 168 hr. This increase was achieved by using a free fatty acid linker that allows the molecule to form a non-covalent reversible interaction with albumin, which reduces renal excretion. A different protein-linking strategy was used in the design of Albiglutide and Dulaglutide. These two GLP-1 receptor agonists become covalently linked (irreversibly) to large proteins; albumin and an Fc fragment of human IgG4, respectively. Importantly, an α-aminobutyric acid was engineered into Semaglutide at position 8 to reduce metabolism by dipeptyl peptidase 4 (DPP4), a cell-surface protein that cleaves numerous circulating peptides. The GLP-1 sequence in position 8 of Albiglutide has also been modified by substituting glycine for alanine adjacent to the DPP4 hydrolysis site to reduce metabolism. Modifications to GLP-1 receptor agonists have led to substantial increases in plasma half-lives, reducing the frequency of dosing from twice daily to weekly, which should improve patient compliance.

Although it does not target a GPCR, Plecanatide, an engineered peptide agonist of guanylate cyclase C, is notable in that it is administered orally and acts directly in the gastrointestinal tract, demonstrating that local targeting approaches are feasible for intestinally restricted targets. GPCRs are abundant in the gut, so this could provide a novel approach for future therapeutic strategies.

Other peptide therapeutics have been approved for novel indications or uses. In 2018, the second melanocortin agonist, Bremelanotide, was approved, but the approval was for a new clinical indication, sexual arousal disorder[31]. Lutathera (lutetium Lu 177 dotatate) was also approved in 2018 for the treatment of neuroendocrine tumours and is the most recent example of a peptide receptor radionuclide therapy. In this compound, the peptide DOTATATE, a somatostatin receptor agonist, is labelled with lutetium-177. After binding to somatostatin receptors, which are found at a high density on the surface of tumours, the radiation emitted by the peptide causes tumour cell death but, owing to the limited particle range, it causes little toxicity to adjacent healthy tissue[32].

In 2019, Difelikefalin was approved as a 'first-in-class' κ-opioid receptor (KOR) agonist that has a high degree of selectivity for KOR over other opioid receptors and is used in the treatment of pain after abdominal surgery. Importantly, there are few of the CNS side effects such as sedation, dysphoria, and hallucinations that are associated with small molecule analgesics[33]. This is consistent with the little or absent CNS permeability observed for Difelikefalin and is an example of a therapeutic for which lack of brain penetrance, as often occurs with peptides, is an advantage over a small molecule. Development of compounds selective for peripheral KORs is crucial for managing pain in the context of the emerging opiate crisis.

Peptides in the pipeline

Phase 3

Three of the peptides currently in Phase 3 development are potentially first-in-class (Table 3). BL-8040 is the first peptide antagonist for C-X-C chemokine receptor type 4 (CXCR4) and, if it progresses to clinic, BL-8040 would compete with an established small molecule antagonist (Plerixafor) for the same clinical indication – the mobilisation of hematopoietic stem cells to peripheral blood prior to collection for autologous cell transplantation[34]. The interaction between the chemokine CXCL12 (also known as stromal cell-derived factor 1, SDF1) and its receptor, CXCR4, plays a key role in hematopoietic stem cell mobilization.

Livoletide is a first-in-class analogue of unacylated ghrelin (UAG) and is being evaluated for the treatment of Prader–Willi syndrome, a rare genetic disease, to reduce hyperphagia (excessive eating) and obesity[35,36]. Ghrelin, the 'hunger hormone', stimulates appetite, increases food intake and promotes fat storage, all of which are severely upregulated in patients with Prader–Willi syndrome. Interestingly, Livoletide is a fully cyclised (cyclo(-Ser-Pro-Glu-His-Gln-Arg-Val-Gln) variant, which protects this peptide from metabolism (at least in vitro in human blood) and extends plasma half-life[35,36]. However, the exact mechanism of action is still unclear. A number of studies suggest that when UAG is co-administered with ghrelin it acts as a functional antagonist or acts via another receptor[37]. Other studies suggest that UAG is a full agonist[38], so the data from late-stage clinical trials could be informative.

Setmelanotide[39] is proposed as a possible first-in-class MC_4 receptor agonist, as it has a modest ~20-fold selectivity over the MC_3 receptor, which would potentially avoid cardiovascular side effects. The MC_4 receptor is a key regulator in the hypothalamic control of food intake. The synthetic peptide is in development for the treatment of individuals with genetic variants in the pro-opiomelanocortin and leptin receptors, which cause severe early onset obesity and hyperphagia, to restore signalling in the MC_4 receptor pathway.

Phase 2

In ongoing Phase 2 trials, novel therapeutic strategies are exemplified by EP-100, which contains synthetic GnRH, which is 10 amino acids, attached to an eighteen amino acid cationic α-helical lytic peptide (CLIP-71) without a linker[40]. This lytic domain interacts with the negatively charged membrane to induce cell death. Cancerous cells overexpress $GnRH_1$ receptors compared with normal tissue and are therefore thought to bind the GnRH peptide, so binding of EP-100 to these receptors would target the lytic CLIP-71 domain preferentially to cancer cells. One potential advantage of this strategy is that the lytic peptide does not need to be released from GnRH by cleavage of a linker, which avoids possible toxicity.

Among Class B receptor ligands, the naturally occurring peptide stresscopin (urocortin 3) targets the corticotropin-releasing factor CRF_2 receptor and has shown short-term, dose-dependent efficacy in improving cardiac index and systemic vascular resistance[41]. Stresscopin (as RT-400) is in clinical trials for acute decompensated heart failure, a major cause of hospitalisation.

Table 3 also summarises peptides that have been discontinued in Phase 2 or 3 clinical trials. The reasons why trials have not progressed are often not disclosed in peer reviewed papers and are mainly found on the relevant company websites. With this important caveat, the predominant reason cited for termination of a trial was futility (the inability to achieve a clinical objectives) rather than adverse side effects. In some cases, the Phase 3 trials were testing new peptides and clinical indications. For example, foot ulcers affects one in four diabetic patients and there is currently an unmet need for new treatments. Angiotensin II has been shown to promote would healing[42]; however, Aclerastide, an angiotensin II receptor agonist, failed to meet the primary efficacy endpoint of confirmed complete wound closure of the target ulcer within 12 weeks of the start of treatment. For others, peptides lacked efficacy against established targets. Terlipressin has been used in the treatment of hypotension and septic shock (Table 2) since 2006; surprisingly, in 2018, a large (868-patient) phase 2B/3 clinical trial of Selepressin, which targets the same receptors, in sepsis[43] was terminated early for futility[44].

Strategies to improve peptide design

Potency, half-life and administration

What pharmacokinetic and pharmacodynamic properties contribute to an efficacious peptide drug? Currently there is no consensus, but a trend is emerging amongst recently approved drugs for high sustained target potency combined with increased plasma half-life and reduced enzymatic metabolism and renal elimination. Synthetically modified peptides targeting Class A GPCRs have maintained a high affinity (median $pK_{i\,=}$ 8.8), similar to that observed for native peptides, but have a substantially increased median plasma half-life (3

hours, excluding compounds with flip-flop kinetics [G], compared with ~5 minutes for native peptide equivalents). Flip-flop kinetics have also been used to increase plasma half-life. For example, Degarelix (Table 2) is a synthetic derivative of GnRH that blocks binding of the endogenous peptide to receptors in the pituitary gland, and is used to treat prostate cancer. Following subcutaneous administration, Degarelix forms a depot at the site of injection, from which the drug is slowly released into circulation to produce a plasma half-life from 42–70 days. Clearance is unaffected, and occurs mainly via hydrolysis in the hepatobiliary system and excretion of the unchanged drug by the kidney[45, 46]. Similarly, Lanreotide, a somatostatin agonist that acts mainly by binding to the SST_2 and SST_5 receptors, and is used to inhibit growth hormone release to treat acromegaly, also forms a drug depot at the site of injection, giving a plasma half-life of 22 days[47], and also contains unnatural amino acids (Box 1).

The majority of peptides that target GPCRs are administered by injection, although other routes (for example, intranasal administration is used for Desmopressin) are increasingly being exploited. Charged and hydrophilic molecules such as peptides are typically not orally bioavailable. After several decades of synthesising modified peptides, the inherent disadvantages of low membrane permeability, which limits oral bioavailability and tissue distribution, including to the CNS, are still applicable to most peptide drugs. However, there are a number of encouraging examples of the application of permeation enhancer strategies[48] and detailed SARs are being explored, especially for cyclic and conformationally constrained peptides[49]. The most advanced, and exciting, of these approaches has been pioneered by Emisphere using a pharmaceutically inactive small-molecule enhancer N-[8-(2-hydroxybenzoyl)amino]caprylate (SNAC) co-formulated with Semaglutide (a GLP-1 receptor agonist already approved as an injectable for the treatment of T2DM). The resulting drug, oral Semaglutide[50], had increased transcellular permeability and bioavailability of ~4%[51]. The highest dose tested, 40 mg administered once daily orally, resulted in comparable efficacy to 1mg Semaglutide injected once weekly[52]. Oral Semaglutide was approved by the FDA in 2019. For drugs with high hydrophilicity and poor membrane permeability, such as peptides, absorption enhancers can promote membrane permeability and improve oral bioavailability.

Experimental approaches to enhance brain permeability include linking neurotensin to a brain-penetrant peptide, angiopep-2, thereby increasing transport across the blood–brain barrier via receptor-mediated transcytosis by about ten-fold. This was sufficient to reverse pain behaviours in animal models of neuropathic and bone cancer pain[53].

Desmopressin is one of the few examples of a peptide that can be administered orally. Cyclization contributes to its resistance to metabolism, and its hydrophobic nature enhances cellular absorption across the gut. Bioavailability is very low by this route (0.08–0.16%)[54] but this level is sufficient to achieve a plasma concentration that is clinically

effective in T2D. The success of desmopressin has proven the potential of engineering peptides to have oral activity. As a result of the range of strategies outlined above, a main area of growth for peptide drugs is in targeting peripheral peptide receptors, particularly those linked to metabolic diseases (Table 2).

Strategies for GLP-1 receptor agonists

The history of the development of GLP-1 receptor agonists exemplifies the broad range of approaches used to address the pharmacokinetic challenges in peptide development (Figure 2, Table 4). To date, seven peptide GLP-1 receptor agonists are approved for the treatment of T2D with projected global sales in 2020 of at least US$10bn (see EvaluatePharma in Related links).

GLP-1 has multiple effects that are beneficial in the treatment of T2D. Despite this, the natural peptide has a very short plasma half-life (~2 minutes) because of rapid enzymatic cleavage and enzymatic inactivation by DPP4, which precludes its use as an effective therapeutic treatment. Additional studies confirmed high plasma clearance following the subcutaneous route of administration[55, 56].

Exenatide (Table 4) was the first GLP-1 receptor agonist approved for clinical use. In 1992, Eng et al.[57] identified exendin-4, a new peptide hormone from the saliva of the Gila monster (*Heloderma suspectum*). Exendin-4 had many of the same pharmacological properties as GLP-1 — it increased insulin secretion and reduced plasma glucose levels. However, unlike GLP-1, exendin-4 is resistant to cleavage and inactivation by DPP4. Exendin-4, as Exenatide, received approval for the treatment of T2D in 2005 as an adjunctive therapy and in 2009 as a monotherapy[58]. Although Exenatide has been widely used for the treatment of T2D, its short plasma half-life requires frequent (twice daily) subcutaneous injection, which limits efficacy, results in poor patient compliance and increases the risks of additional side effects, such as infection at the sites of injection.

Following the success of Exenatide, multiple strategies were employed to increase plasma stability and half-life, reduce renal elimination and improve oral bioavailability for GLP-1 receptor agonists. These strategies can be broadly divided into two approaches. One is based around extending the plasma half-life of Exenatide, leading to Exenatide once weekly (QW) and Lixisenatide. Exenatide QW[59] is a reformulation of Exenatide into microspheres consisting of a biodegradable polymer, poly-(D,L-lactide-co-glycolide), to extend the dosing interval to weekly administration. In Lixisenatide[60], modification of the Exenatide sequence, including addition of a C-terminal lysine tail, conferred resistance to DPP4 cleavage and increased plasma half-life.

The other strategy focused on modifying the native GLP-1 peptide, leading to Liraglutide, Albiglutide, Dulaglutide and, most recently, Semaglutide (Figure 2, Table 4).

Because of the short half-life of native GLP-1, as a direct result of both cleavage by DPP4 and rapid renal elimination owing to its relatively small size, a combination of strategic solutions has been employed. Liraglutide[61] was approved in 2009 and is a human GLP-1 receptor agonist based on the native GLP-1 peptide with one amino acid substitution (Lys24Arg) and a C16 palmitic acid side chain attached via a glutamyl spacer at position 26. These modifications increase its binding to serum albumin, which significantly reduces renal elimination and DPP4 cleavage. The active modified GLP-1 is released from albumin at a slow, constant rate, resulting in slower degradation and reduced renal elimination compared, for example, to that of the mature endogenous form of GLP-1, GLP-$1_{7\text{-}37}$. Semaglutide[62], which was approved in 2017, incorporates the unnatural amino acid α-aminoisobutyric acid (Box 1) at position 8 to reduce DPP4 cleavage and contains an alternative C18 fatty diacid at Arg26, which provides strong albumin binding. Albiglutide[22] and Dulaglutide[63] are variants of peptide fusion proteins. Both are protected from DPP4 cleavage because of substitutions at position 8 and both contain 2 copies of GLP-1, fused to either human serum albumin (Albiglutide) or covalently linked to a human IgG4-Fc heavy chain by a small peptide linker (Dulaglutide), to increase the half-life of the molecule owing to recycling by the neonatal Fc receptor (FcRn) and/or increased molecular weight.

All of these GLP-1 receptor agonists require subcutaneous administration. However, several promising oral delivery approaches are in various stages of development, including the recently approved oral Semaglutide, which is co-formulated with SNAC as described in the section on potency, half-life and administration.

Ligand bias

Traditional drug–receptor theory posits that drugs have two properties: affinity and intrinsic efficacy. Affinity is the quantifiable measure of how tightly a drug binds to its target and is constant for each drug–receptor pair, supporting medicinal-chemistry-led SAR investigations and the application of concepts such as drug selectivity for target over non-target receptors. However, affinity says nothing about the functional consequences of a drug–receptor interaction. This is defined by the term 'intrinsic efficacy', which describes the effect a drug has on receptor activity. Using this original definition, drugs are either agonists (with a combination of affinity and intrinsic efficacy) or antagonists (with affinity but no intrinsic efficacy), an approach that has underpinned drug development for the past few decades. However, there is increasing evidence that simple concepts of agonism and antagonism only scratch the surface of the drug–receptor signalling landscape. We now know that receptors adopt a range of conformational states, thus giving rise to important new pharmacological concepts such as constitutive activity and biased signalling (or functional selectivity). Because of their relatively large size, peptides often interact at multiple key positions within

both the extracellular and transmembrane domains of GPCRs. These contemporary pharmacological concepts may have major implications in the design and optimisation of new peptide drugs, especially when used in combination with the structural biology techniques described later in this article.

A receptor may be able to engage with a spectrum of downstream signalling pathways, but a ligand with affinity for that receptor may affect only a subset of these pathways, and may be an agonist of some and an antagonist of others. This observation underpins the concept of ligand bias and is the definition of a biased peptide ligand used in this review. This principle has been used as a criterion to identify biased peptides from the literature (see Box 2). Bias is usually examined in the context of the two most thoroughly characterised GPCR signalling pathways, those initiated by the binding of β-arrestin or G proteins to the GPCR complex. Importantly, however, biased signalling can refer to any signalling pathway measured, for example those involving different subtypes of G proteins, and can be considered to be specific to both the context and the pathway. As such, multiple drugs acting at a single receptor may all be characterised as agonists, but each may have a different functional selectivity profile for the cellular pathways regulated by that receptor.

How important is bias in a particular pathway? Physiologically, this is exemplified with the $GnRH_1$ receptor, which is unusual amongst the peptide-binding GPCRs because it lacks the C-terminal intracellular domain. Prolonged agonist stimulation of GPCRs usually results in phosphorylation of residues in the intracellular C-terminus, which then interact with β-arrestin[64]. This interaction induces receptor endocytosis, which terminates receptor signalling and results in desensitisation of the receptor to the peptide. The $GnRH_1$ receptor does not undergo agonist-induced phosphorylation or couple to β-arrestin and is therefore slowly internalised[64]. In contrast, the $GnRH_2$ receptor, which is found in other vertebrates, retains the C-terminus and stimulation induces phosphorylation of C-terminus residues, β-arrestin binding, receptor internalisation and rapid receptor desensitisation.

How important is biased agonism in drug discovery? This dichotomy outlined for the GnRH receptors suggests that biasing compounds away from β-arrestin recruitment and receptor internalisation will reduce desensitisation, which limits drug efficacy. Bias may have additional clinically important consequences. For example, β-arrestin-mediated respiratory depression is an adverse consequence of treatment with MOR agonists, which could potentially be reduced with biased agonists[65]. Stretch induced-myopathy in the heart occurs as a consequence of the apelin receptor acting as a mechanosensor in the absence of endogenous apelin[66] that is down regulated in disease. Replacing the lost apelin with a biased agonist may avoid activating the deleterious pathway (see apelin receptor section below).

Biased signalling could herald a new, more specific pharmacological strategy for GPCR agonists, some examples of which are described in the following sections. However, the vast majority of existing examples of biased signalling have been defined using relatively simple in vitro cellular outputs. Predicting clinical benefit will require an understanding of the relevant cellular mechanisms that contribute to disease and identification of biased ligands from appropriate in vitro cell signalling assays. Many receptors, including those activated by GnRH[67], opioids[68,69], chemokines[70], neuropeptide S[71], proteinases[72] or parathyroid hormone[73], also exhibit bias but are not discussed in these sections.

Angiotensin II and AT_1 receptor. Biased peptide agonists that target the angiotensin (AT_1) receptor are the most extensively studied of the peripheral GPCR targets. This peptide–receptor pair is important in regulating blood pressure and the AT_1 receptor is targeted by the ‘sartan’ class of small molecule antagonists, which are used as antihypertensive agents.

Pioneering studies revealed a synthetic angiotensin II analogue, SII, that bound with high affinity, was able to internalise AT_1 receptor (presumably by β-arrestin recruitment, but this was not measured) and activate the β-arrestin effector mitogen-activated protein kinase (MAPK), but did not induce the G protein-mediated production of inositol triphosphate (IP_3)[74]. More potent compounds that stimulated β-arrestin-mediated signalling but competitively antagonized G protein coupling were subsequently developed, including TRV027 (Sar-Arg-Val-Tyr-Ile-His-Pro-D-Ala-OH; Table 3). In rats, TRV027 antagonised AT_1 receptor-mediated G protein signalling and reduced mean arterial pressure, similar to the antihypertensive agent losartan. Crucially, it had the opposite effect to losartan on cardiac contractility: TRV027 induced the β-arrestin-mediated activation of kinase pathways and increased endothelial nitric-oxide synthase phosphorylation[75], with a resulting increase in cardiac contractility. This pharmacological profile of an antihypertensive action combined with an increase in cardiac output was demonstrated to be beneficial in a dog model of heart failure in which TRV027, when co-administered with the commonly used loop diuretic furosemide, was shown to preserve furosemide-mediated natriuresis and diuresis[76].

This molecule was then evaluated in patients with acute heart failure with the objective of reducing afterload [G] while increasing cardiac performance and maintaining stroke volume[77]. In individuals with elevated plasma renin levels (indicative of acute heart failure), a short, reversible and modest (5 mm Hg) reduction in blood pressure was reported, but no change was observed in volunteers with normal renin levels[78]. However, no benefits were observed on top of standard of care drugs through a 30-day follow-up in a Phase 2B randomised, double-blind clinical trial[79]. The reasons for this lack of efficacy are unclear. Insufficient target engagement seems unlikely, as peak plasma concentration (C_{max}) at the highest dose was ~580 nM which, combined with low plasma binding and high affinity (16

nM), would be expected to result in significant receptor occupancy[78]. Signalling pathways may be subtly altered in conditions such as heart failure, which could affect drug efficacy. Angiotensin II, SII and TRV027 have distinct downstream phosphorylation events and gene expression profiles[80], which emphasises the need for comprehensive analyses of signalling pathways for biased peptide ligands, some of which are being evaluated for clinical use. Peptides have also been developed as tool compounds where the bias is reversed compared to TRV027 and presumed, therefore, to be deleterious that should allow further insights into this signalling pathway.

The apelin receptor. The apelin system physiologically antagonises angiotensin-II signalling. Although it is not currently targeted by any approved drug, this Class A GPCR and its ligands, apelin and Elabela (also called apelin receptor early endogenous ligand or Toddler), may have a role in the physiological regulation of the cardiovascular system. Dysregulation of the apelin system and loss of endogenous peptides are proposed to contribute to a number of conditions, such as pulmonary arterial hypertension[81–83] and heart failure[84,85], indicating potential for more precise targeting of apelin signalling pathways using biased ligands. Specifically, a G protein biased agonist, if used to replace the missing ligand, would show reduced propensity to desensitise the apelin receptor with repeated use. Interestingly, mice lacking the apelin receptor were protected from cardiac hypertrophy and heart failure associated with chronic pressure overload, whereas mice lacking apelin itself were not[66]. In the heart, apelin normally stimulates $G_{\alpha i}$ -mediated protective responses. However, the cardiac apelin receptor, in the absence of apelin, acts, via β-arrestin pathways, as a mechano-sensor to stretch; cardiomyocytes from apelin receptor knockout mice have a reduced hypertrophic stretch response[66]. Therefore, apelin receptor ligands that are G protein-biased or that preferentially block β-arrestin signalling may be beneficial in patients with heart failure.

Preproapelin is a 77 amino acid peptide that is predicted to be cleaved to biologically active peptides including apelin-36 (corresponding to amino acids 42-77), apelin-17, apelin-13 (corresponding to amino acids 65-77) and a pyroglutamate modified form, [Pyr[1]]apelin-13. In human cardiovascular tissues *in vitro*, apelin-13, [Pyr[1]]apelin-13 (which was identified as the predominant isoform) and apelin-36 were found to be equipotent as vasodilators and inotropes [86]; however, apelin-13 and apelin-36 elicited different patterns of receptor internalisation in cell based assays [87,88]. These data suggest that putative endogenous apelin isoforms may demonstrate unique signalling profiles *in vivo.* Whether this might have physiological or pathophysiological consequences is not yet known. However, specific pathway bias has been described *in vitro*: for example, in cAMP and β-arrestin assays,

compared to [Pyr[1]]apelin-13, the N-terminally extended apelin-17 demonstrated ~70 fold bias towards β-arrestin[89]. Modified peptides based on the apelin sequence also demonstrate pathway bias. Compared to apelin-17, a truncated apelin-17 that lacks the C-terminal phenylalanine (Lys16Pro), or versions of apelin-17 and [Pyr[1]]apelin-13 in which the C-terminal phenylalanine is replaced by an alanine (Lys17Ala and pGlu13Ala, respectively), retained similar binding affinity and potency in inhibiting cAMP but did not induce receptor internalisation[90]. Indeed, interactions between the C-terminal phenylalanine and residues in an aromatic pocket (Phe255 and Trp259 in the rat apelin receptor) are required for apelin-mediated internalisation[91]. In terms of downstream signalling events, apelin-17 stimulates extra-cellular signal-regulated kinase 1/2 (ERK1/2) phosphorylation in both a Gαi- and β-arrestin-dependent manner, whereas the action of Lys16Pro was G protein-dependent but β-arrestin-independent[92]. The highly conserved Ser348 in the C-terminus of the apelin receptor is critical for interaction with G protein-coupled receptor kinase (GRK), and β-arrestin-mediated signalling, but replacing Ser348 with Ala did not alter cell surface expression of the receptor, binding of apelin ligands or activation of $G_{\alpha i}$ or G_q pathways[93].

The SAR of biased signalling at the apelin receptor has been assessed with a novel series of cyclic peptides based on the apelin-13 structure[94]. The main conclusion was that, consistent with data for linear peptides, the C-terminal amino acid is important for receptor binding, β-arrestin recruitment and receptor internalisation. In addition, the [Pyr[1]]apelin-13 sequence incorporates an N-terminal RPRL motif that is absolutely necessary for receptor binding. Earlier SAR studies demonstrated that His7 and Met11 substitutions did not affect binding or function of the ligand[95]. From these SAR studies it is apparent that modified peptides can be designed that may show G protein or β-arrestin signalling bias and this is exemplified by the macrocyclic peptide MM07[96]. MM07 induced vasodilatation and increased cardiac output in rats and in human volunteers, which may be desirable in the clinical setting, and, importantly, the receptor was not desensitised on repeated application. This peptide has subsequently been shown to have efficacy in a rat model of pulmonary arterial hypertension[97]. Further modification of MM07 or similarly G protein biased peptides, as described above for GLP-1, may result in a peptide with improved plasma half-life to take forward to proof of principle clinical studies.

As mentioned above, ghrelin is a gut hormone with a role in hunger signalling which has made the ghrelin receptor a potential target for anti-obesity drugs. However, the physiological actions of ghrelin are diverse, including effects on gastric motility, growth hormone release, reward behaviour and mood. Therefore, drugs that mimic or block ghrelin may have a number of therapeutic uses but the impetus for developing such agents is

hampered by the potential for undesirable on-target side effects. The discovery of compounds that can distinguish between ghrelin responses now suggests that it may be possible to develop biased ligands that, for example, selectively reduce body weight. Modification of the ghrelin inverse agonist, [D-Arg^{1},D-Phe^{5},D-Trp^{7,9},Leu^{11}]substance P, generated compounds containing an N-terminal D-Trp-Phe-D-Trp (wFw) motif with differing C-terminal peptide-mimetic spacers[98]. One of these, wFw-isonipecotic acid, was the first ghrelin receptor biased agonist as, unlike known ghrelin mimetics, it did not signal through the serum-response element (SRE) pathways (presumably downstream of $G\alpha_{12/13}$) but did activate the $G\alpha_q$ and ERK1/2 pathways. A likely explanation was revealed by mutagenesis and modelling studies: wFw-isonipecotic acid does not interact with receptor residues that are important for the binding and function of ghrelin or other ghrelin mimetics[98]. wFw-isonipecotic acid did not stimulate feeding in rats, which may reflect the relative importance of SRE pathways for this function. If confirmed, these data imply that biased ghrelin ligands could be designed that distinguish its effects on growth hormone release from effects on food intake[99]. For another ghrelin-targeted peptide, Ulimorelin (Table 3), the prokinetic effect on gut motility was sustained, unlike the tachyphylaxis [G] seen with other ghrelin agonists. Perhaps Ulimorelin does not activate β-arrestin signalling, which would limit receptor desensitisation with continued use. Additionally, Ulimorelin mimics the orexigenic and gut motility effects of ghrelin, but is not a growth hormone secretagogue. Whether this unique pharmacology is explained by pathway bias is unclear. Unfortunately, despite Ulimorelin's apparent advantages over other ghrelin mimetics, a Phase 3 trial in postoperative ileus was discontinued owing to lack of efficacy over.

The ghrelin receptor exhibits high constitutive activity, independent of the cellular environment[100], that is abolished in a naturally occurring human mutant receptor (Ala204Glu)[101] that is associated with short stature. This mutation increases the probability that the C-terminal section of extra-cellular loop (ECL) 2 will form an extended α-helix, thereby constraining this part of the protein and resulting in loss of constitutive activity[101]. Additionally, whereas ghrelin-stimulated G_q and $G\alpha_{12/13}$ signalling were essentially unaffected by the mutation, β-arrestin responses were substantially reduced, indicating that ECL2 is also important for determining ligand bias. A role for constitutive receptor activity in fasting-induced hyperphagia in mice[102] has been proposed. Biased signalling at the ghrelin receptor can also be achieved with inverse agonists[103], which could inform future drug discovery efforts.

GLP-1 receptors. Class B receptors are also tractable to ligand bias. Insulin secretion and regulation of blood glucose in response to GLP-1 may result from receptor engagement with a number of different G proteins and several signalling pathways. Pathway preference may be determined by the agonist and/or the cell type. Endogenous peptides derived from the proglucagon peptide include full-length (GLP-$1_{1\text{-}36}$, GLP-$1_{1\text{-}37}$) and truncated (GLP-$1_{7\text{-}36}$, GLP-$1_{7\text{–}37}$; the mature isoforms) GLP-1, each of which can each also exist in an amidated form, as well as oxyntomodulin. The effects of the endogenous peptide agonists, as well as the clinically used peptide mimetic Exenatide, have been compared in physiologically relevant assays that measure cAMP, Ca^{2+} mobilization, and ERK1/2 phosphorylation. Using GLP-$1_{1\text{-}37}$ amide as the reference ligand, the shorter GLP-1 isoforms and Exenatide had a similar pathway activation profile whereas the longer GLP-1 isoforms and oxyntomodulin exhibited some pathway bias[104]. Subsequently, from a peptide library screen an N-terminally modified version of Exenatide, Exendin-P5, was identified. P5 was shown to be G protein-biased, lacking β-arrestin activity and was more effective than Exenatide in a chronic mouse model of T2D[105] suggesting that G protein-biased ligands may be advantageous in this condition.

A novel strategy to generate biased versions of GLP-1 involves replacement of particular α-amino acids in the peptide backbone with β residues, or with non-proteinogenic [G] α-amino acids[106,107]. The resulting α–β peptides are resistant to degradation by endogenous peptidases. For example, α-aminoisobutyric acid, a strong helix inducer that occurs rarely in nature, protects the N-terminus from degradation by DPP4 and neprilysin. Thioamidation limits degradation and thioamidated GLP-1 analogues have an in vitro half-life of many hours compared to two minutes for the native peptide[108]. Thioamides do not have appreciable β-arrestin agonist activity and are therefore also G protein biased. An alternative, but challenging, strategy may be to develop allosteric modulators that affect both endogenous peptide binding kinetics and signalling bias[109].

Calcitonin receptors. Experiments using the calcitonin (CT) receptor were some of the first to crystallise the understanding that differences in relative agonist potencies in different tissues did not necessarily mean that these tissues expressed different receptor subtypes[110]. Both human and salmon calcitonin have FDA approval for treatment of Paget's disease (Table 1 and Table 2). Interestingly, they exhibit distinct binding kinetics, affinity and functional efficacy in different G protein pathways, implying that they stabilise different active conformations of the CT receptor[111,112]. The response to activation of the human receptor is complicated by the existence of two major splice variants that exhibit tissue specific expression patterns and couple to different signalling pathways[113]. The predominant human

receptor isoform, $CT_{(a)}$ receptor, lacks a 16 amino acid insert in the first ECL that is present in the less abundant $CT_{(b)}$ receptor isoform[114]. Both variants bind calcitonin peptides with comparable affinity, however, the $CT_{(b)}$ receptor is not internalised well and preferentially activates G_S over G_q relative to $CT_{(a)}$ receptor[115]. Mutational data[116] mapped with molecular dynamic simulations highlighted that ECL2 was important in conformational propagation linked to the $G_{\alpha s}$–cAMP pathway, which was distinct to the ligand-specific and pathway-specific effects propagated by ECL3. These observations highlighted differences in the mechanism of ligand interaction and receptor activation of the CT receptor compared to another class B receptor, GLP-1[116].

Oxytocin receptors. Atosiban, described as an oxytocin receptor antagonist, is used clinically to prevent preterm labour by blocking the G_q-linked increase in intracellular Ca^{2+} that normally promotes uterine contractility. However, Atosiban is more correctly identified as a biased ligand as it promotes coupling of the oxytocin receptor to $G\alpha_i$ (which is linked, via MAPK, to inhibition of cell proliferation) in addition to antagonising $G\alpha_q$ signalling[117]. This is of interest because oxytocin receptors are overexpressed in several cancers and therefore Atosiban could be repurposed as a chemotherapy. Interestingly, whilst Atosiban can activate or inhibit pathways downstream of different $G\alpha$ proteins, it has little effect on β-arrestin signalling: receptor internalisation was markedly attenuated following exposure to Atosiban, whereas these receptors are rapidly and profoundly lost in response to oxytocin[117]. Furthermore, oxytocin-triggered IP_3 accumulation was competitively blocked by pre-exposure to Atosiban[117]. It has subsequently been confirmed that Atosiban does not recruit β-arrestin to the oxytocin receptor and shows selectivity for $G\alpha_{i3}$ over other G_i isoforms[118].

Insights from structural studies

Experimental X-ray crystallography or cryo-EM structures have been reported for the 7-transmembrane domains of 62 GPCRs, covering 212 distinct GPCR–ligand complexes and 200 unique ligands[119,120]. Of these GPCRs with solved structures, 27 of them bind peptides or proteins, with 65 solved unique receptor–ligand complexes[115,116,121-157]. Twenty two of the experimentally determined GPCR structures (10%) so far (see the GPCR database in Related links), contain a peptide ligand, covering Class A and B GPCRs (Figures 3 and 4, Supplementary Table 1). The peptide-bound structures of these receptors provide structural templates and detailed insights into the structural determinants of ligand binding and functional activity (Box 3, Supplementary Text and Supplementary Table 1). About half of the clinically relevant peptides reported in Tables 1, 2 and 4 have been structurally modelled based on homologous peptides and/or receptors. These peptides serve as potential

templates for the structure-based optimisation and design of novel GPCR peptide therapeutics.

Peptide ligands bind to GPCRs with numerous different binding modes, reflecting the diverse chemical structures and properties of both ligands and receptor binding sites. Class A GPCR peptide ligands generally bind to ECL2, and polar or ionic interaction site at the top of ECL3 (the portion of the protein between transmembrane region 6 (TM6) and TM7) and bind differentially located and shaped lipophilic regions deeper in the receptor pocket. Most Class B GPCR ligands are helical, and the helical ligands have a lipophilic interaction with a site in the region between TM1, TM2 and TM7 that is less accessible in Class A GPCRs, and some of the Class B ligand specificity is determined by polar interaction networks that can form with different relative orientations of the extracellular N-terminal domains (ECDs) and the transmembrane domains[149].

Class B GPCRs contain an ECD of 120–160 residues and a transmembrane domain of 310–420 residues. In addition to the transmembrane-domain-only and full-length structures of class B GPCRs that have been described, several structures of isolated class B GPCR ECDs have been solved[149] (Supplementary Table 1). These ECD–peptide complexes have conserved hydrophobic interactions between conserved lipophilic residues in the C-terminal part of the ligand and hydrophobic interaction sites in the ECD of the corresponding receptor.

Considerable progress has been made in elucidating the three dimensional structures of key regions for peptide recognition and selectivity by GPCRs. Methodological and technical improvements to cryo-electron microscopy are expanding the role this technique occupies alongside X-ray crystallography in solving GPCR structures. Emerging information on different activation states and structural features responsible for activation or inhibition are being exploited to guide drug discovery. This is particularly important for Class B, where all endogenous ligands are peptides and there is potential to discovery new compounds based on exploiting allosteric binding sites revealed by structural studies. Future studies will help to unravel the importance of receptor dimerization and the rational, rather than empirical, design of biased peptide ligands, as our knowledge expands of key residues involved in the kinetics and dynamics of signalling processes such as β-arrestin.

Perspectives and conclusions

Nearly all peptide drugs approved for clinical use to date function as full agonists. This probably reflects the predominant strategy for the discovery of clinical candidates, which is based on structural modifications to naturally occurring peptide sequences, rather than high-throughput screens against target receptors, which are often used to identify small molecules leads. This may, however, be set to change. The number of deduced structures of

GPCRs that bind peptides is rapidly increasing using crystallography (Supplementary Table 1), which will enable the rational structure-guided design of peptides. This approach will expand further because of cryoEM, from which, crucially, structures can be determined from GPCRs bound to an agonist in an active state, as has been successfully done for Class B receptors[133,137,140]. Indeed, peptide allosteric modulators have been proposed for the urotensin II receptor that block urotensin II-mediated contraction of aortic rings, but have no effect on the activity of the second endogenous agonist that binds the receptor, urotensin II-related peptide[158]. Structure-guided design may therefore enable peptides drugs to selectively distinguish between and modulate the action of two endogenous peptides that act at the same receptor, one of which causes a detrimental pathophysiological action, perhaps owing to differences in spatial or temporal signalling. Strategies such as screening phage display peptides have also been effective in discovering novel peptide ligands; for example, antagonists of the Class B VIP_2 receptor that have nanomolar affinity were identified using this approach[159].

How do we use this information to synthesise a better peptide drug? This Review highlights one major trend over the last two decades: the successful exploitation of unnatural amino acids and chemical modifications to manipulate physicochemical properties, principally to improve pharmacokinetics but also, to a lesser extent, pharmacodynamics. Another, earlier stage trend stems from the discovery of peptides that can be biased towards G protein-dependent or G protein-independent β-arrestin-mediated pathways.

Biased ligands have enormous potential to selectively activate pathways that produce beneficial clinical effects while reducing signalling via pathways that may cause unwanted on-target side effects. Biased peptide agonists have been identified for a number of receptor families and clinical proof of concept studies are emerging[94]. As proof of principle, but subsequent to clinical approval, the OT receptor antagonist Atosiban was identified as biased as it does not activate β-arrestin, thereby reducing internalisation, which is an important aspect of its mechanism of action[118].

Particularly compelling evidence for the need for biased agonists has emerged from studies of the MC_4 receptor. Remarkably, gain of function mutations identified in humans that were associated with reduced body mass index and protection from T2D and coronary artery disease were biased towards the β-arrestin pathway. This suggests that β-arrestin biased MC_4 agonists that act at the native receptor may be a new strategy for the treatment of obesity[160]. Individuals from the 1000 Genomes Project had, on average, sixty eight missense variations that occurred within the coding regions of one-third of the GPCR drug targets; only eight of these variants had previously known clinical associations with altered drug response[161]. In data from ~68,000 individuals in the Exome Aggregation Consortium (EXAC), variants were found in the drug binding sites of 108 GPCRs[162]. Interestingly,

variants of MOR and CCK_A, which binds cholecystokinin, were shown experimentally to have an altered drug response. These results suggest that mining databases such as that from 100,000 Genomes,[163] in which disease phenotypes are linked with whole genome sequencing and patients can be recalled, will yield additional variants that experimentally lead to a loss or gain of function that can be used to identify new GPCR targets for novel treatments.

Dual agonist peptides that activate two different GPCRs are also emerging. The focus to date has been on combinations that target GLP-1 receptor with either the glucagon[164] or GIP receptors[165,166]. Interestingly, a compound that binds to all three receptors is in Phase 1[166]. In a Phase 2 trial, a molecule that contained both GLP-1 and GIP sequences significantly improved glycaemic control and reduced body weight in patients with T2D[167]. The rationale was that the two peptides account for most of the effects of incretins [G]. However both are degraded by DPP4. Therefore, designing a peptide that contains both peptide sequences but lacks DPP4 cleavage sites to enhance plasma half-life could mimic the beneficial effects of incretins. Clearly a single molecule is an attractive strategy for synergy and patient compliance, but empirical determination of the optimum relative balance between the potency of the two agonistic effects of this single molecule is challenging. Stapled peptides that constrain α-helices to lock a peptide in a particular (often active) confirmation are being explored, for example, as modified orexin[168] and oxyntomodulin[169] ligands. Similarly, pepducins, which are derived from short sequences of intracellular loops of GPCRs and lipidated to penetrate cells so they can access allosteric sites and stabilise GPCR confirmations are being developed[170] and some are being tested in experimental medicine studies in the clinic[171].

In pharmacokinetics, modifications to dramatically increase plasma-half life from a few minutes to days have been the most revolutionary, and a range of strategies effectively reduce metabolism and/or renal excretion. Sustained release formulations are another key development. Theoretically, these innovations can be applied to virtually any peptide.

Promising experimental or early stage trials include genetically engineered exendin-4 linked to a single domain albumin binding antibody (AlbudAb), which prolonged plasma half-life to 6-10 days while maintaining agonist activity (measured as the expected reduced postprandial glucose and insulin levels, and delayed gastric emptying)[172]. An alternative strategy has been employed to chemically link an apelin peptide analogue to a single domain antibody[173]. The advantage of using a chemical link is that non-genetically encoded amino acids can be introduced, so this agonist has high affinity for the apelin receptor but is also resistant to peptidase-mediated degradation. Genetically engineered peptide-AlbudAb conjugates, for example, cannot be made resistant to peptidases in the same way.

Nanotechnology strategies have been applied to induce reversible peptide self-assembly to prolong the bioactivity of a peptide in vivo. As proof of concept, Ouberai et al.[174] demonstrated that oxyntomodulin self-assembled into a stable nanofibril formulation, which subsequently dissociated in vivo to release active peptide, thereby prolonging detectable activity in the plasma from 4 hr to 5 days. Oral (albeit with low bioavailability) and nasal delivery are being used in the clinic to avoid daily injections and these strategies will be increasingly explored. The challenge of engineering peptides that cross the blood–brain barrier remains, and many GPCRs with peptide ligands reside in the brain. Linking GPCR-targeting peptides to a brain-penetrant peptide to transport compounds across the blood–brain barrier is being investigated experimentally. Finally, there is an expanding number of potential new GPCR targets as orphan GPCRs continue to be paired with peptide ligands[175].

It is not yet clear where the balance lies in the cost of developing a clinical candidate based on a peptide versus a small molecule. However, the development of new GPCR peptide drugs continues on an upward trajectory, with seven approved by the FDA in 2017-2019 and over ten in the pipeline in Phase 2 and 3, which is mirrored by the rise in the estimated global value of US$25.4 billion.

Glossary terms

Cryo-electron microscopy

Electron microscopy technique that allows near atomic resolution of biomolecules such as GPCRs in samples cooled to low temperatures and embedded in an environment of vitreous water, avoiding the need for crystallization required for X-ray crystallography.

Flip-flop kinetics

A property of compounds that are administered by subcutaneous injection and slowly absorbed, resulting in fairly continuous release of the compound into the blood.

Afterload

The pressure against which the heart must work to eject blood.

Tachyphylaxis

The rapidly diminishing response to repeated doses of a therapeutic agent.

Non-proteinogenic

Not naturally encoded in any known organism.

Incretins

Metabolic hormones, induced upon eating, that decrease blood glucose by stimulating insulin release. The two known incretins are GLP-1 and GIP

Related links

Clinicaltrials.gov
https://clinicaltrials.gov

DrugBank
https://www.drugbank.ca

Electronic Medicines Compendium
https://www.medicines.org.uk/emc

EvaluatePharma
https://www.evaluate.com/ (2019).

Globaldata
https://www.globaldata.com/store/search/pharmaceuticals

GPCR database
https://gpcrdb.org

RCSB Protein Data Bank
https://www.rcsb.org

RxList
https://www.rxlist.com

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Competing interests

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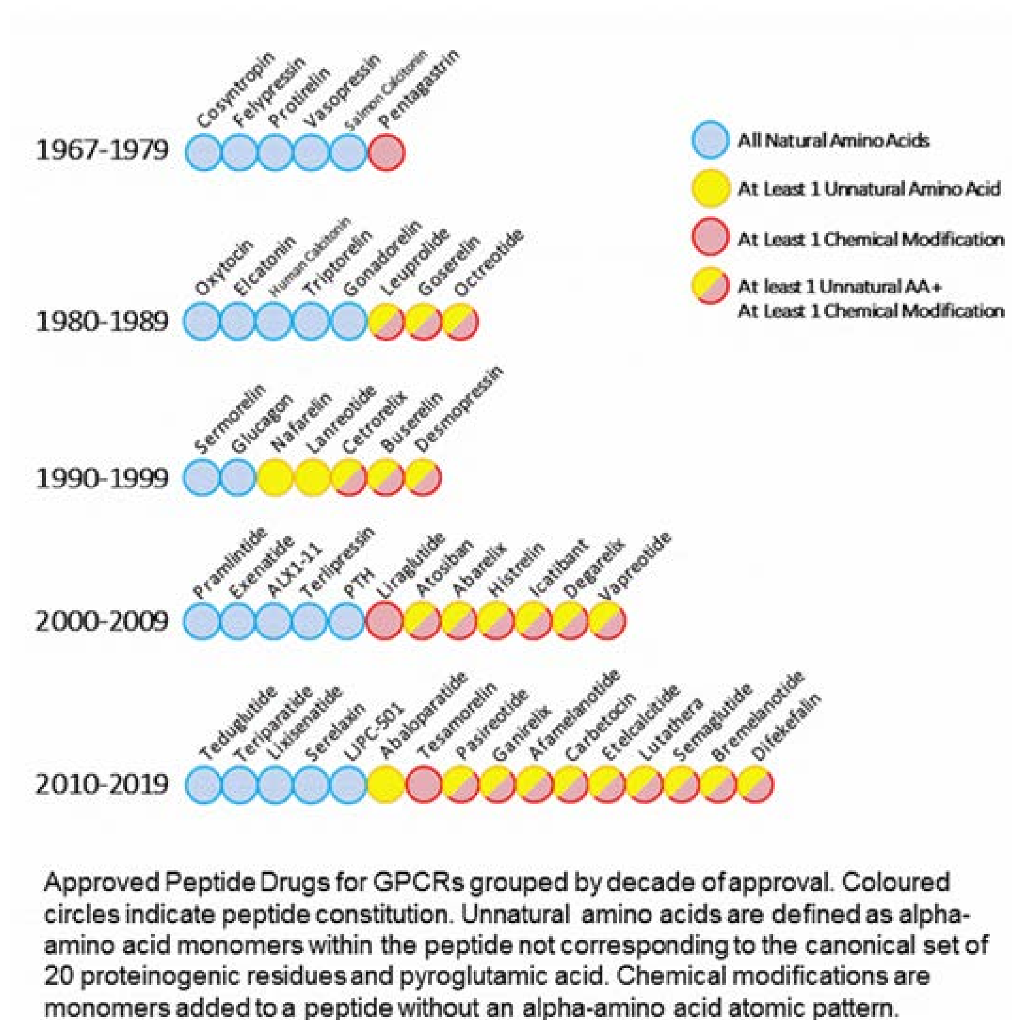
Box 1 | **Unnatural amino acids and chemical modifications**



Approved Peptide Drugs for GPCRs grouped by decade of approval. Coloured circles indicate peptide constitution. Unnatural amino acids are defined as alpha-amino acid monomers within the peptide not corresponding to the canonical set of 20 proteinogenic residues and pyroglutamic acid. Chemical modifications are monomers added to a peptide without an alpha-amino acid atomic pattern.

Many of the first peptide drugs were entirely comprised of proteinogenic (naturally occurring) amino acids. These drugs had limited stability in the body owing to rapid enzymatic degradation by peptidases and renal elimination. They required administration subcutaneously or intravenously because they had little or no oral bioavailablity[8]. Next-generation peptide drugs often incorporated unnatural amino acids residues such as D-amino acids, N-methyl amino acids or residues with unnatural side chains. Chemical modifications — monomeric groups other than amino acids that are added to peptides during synthesis — also became more relevant. These modifications include N-terminal modifications, C-terminal caps, fatty acids and polyethylene glycol (PEG) groups. Of the 49 approved peptide drugs for GPCRs, 22 comprise all natural amino acids, three contain at least one unnatural amino acid, three have at least one chemical modification and 21 have both chemical modifications and unnatural amino acids (see Figure).

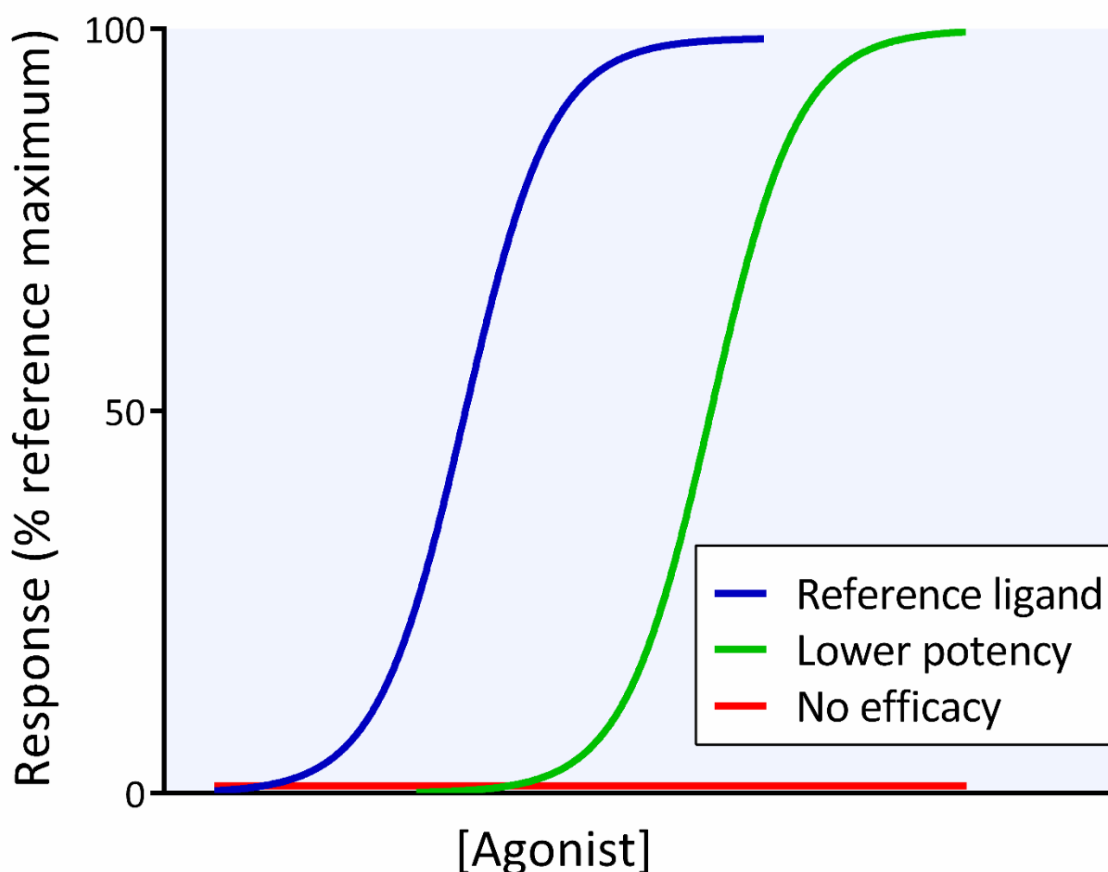
Cosyntropin (an adrenocorticotropic hormone analogue) was the first peptide targeting a GPCR to be approved for therapeutic use, in 1967, and consisted of only natural amino acids (including pyroglutamic acid, which is produced in the human body). Amongst the next eleven peptides for GPCRs that were approved over the next 20 years (until 1987), only one (Pentagastrin) contained an unnatural amino acid (β-alanine, butyloxycarbonyl-protected), and one, Leuprolide, had a C-terminal N-ethyl cap and an unnatural D-Leu residue. Since then, the ratio of approved peptide drugs that contain an unnatural residue or chemical modification to all-natural amino acid peptides has continued to rise. From 2010–2019, 16 new peptide drugs targeting GPCRs were approved, only five of which comprised exclusively natural amino acids.

The addition of unnatural residues or chemical modifications is generally used to alter properties such as efficacy, potency, sub-type selectivity, pharmacodynamics or pharmacokinetics[176]. The first of the gonadotrophin-releasing hormone (GnRH) receptor-targeting peptides that was approved for hormonal disorders (such as breast, ovarian, endometrial or prostate carcinoma) was a natural peptide (Gonadorelin), but subsequently approved drugs targeting the same receptor incorporated unnatural residues that changed the route of administration, and improved potency and stability[177].

Octreotide was the first somatostatin receptor agonist and was approved in 1987 for acromegaly and gastroentero-pancreatic tumours. Octreotide has an unnatural D-Trp residue and a modified Thr residue, which enable the eight-residue Octreotide to function as an effective mimetic of the endogenous peptide, somatostatin (which has 14 residues)[178]. Further research resulted in the approval of Lanreotide (in 1994), which incorporates an unnatural D-2-Naphthylalanine residue, and has superior clinical efficacy[179]. Pasireotide (approved in 2005) is a heavily engineered cyclic peptide comprised of six amino acids, five of which are unnatural. This enables both a reduction in size of Pasireotide versus Octreotide, and increased *in vivo* stability (half- life = 12 hours versus 1.67 hours)[180].

Glucagon-like peptide 1 (GLP-1) receptor agonists are important agents for the treatment of type 2 diabetes (T2D) and other metabolic disorders. The first approved GLP-1 receptor agonist for T2D was Exenatide, which was approved in 2005, and since then six more agents have been brought to market for the same indication[181]. How the structure of these peptides affects their pharmacology and effectiveness as drugs is discussed in detail in the text. Peptides linked to larger molecules, including Albiglutide (GLP-1 dimer fused to human albumin) and Dulaglutide (GLP-1 analogue covalently linked to a human IgG4-Fc heavy chain by a small peptide linker) have also been developed.

Box 2 **Quantifying ligand bias**

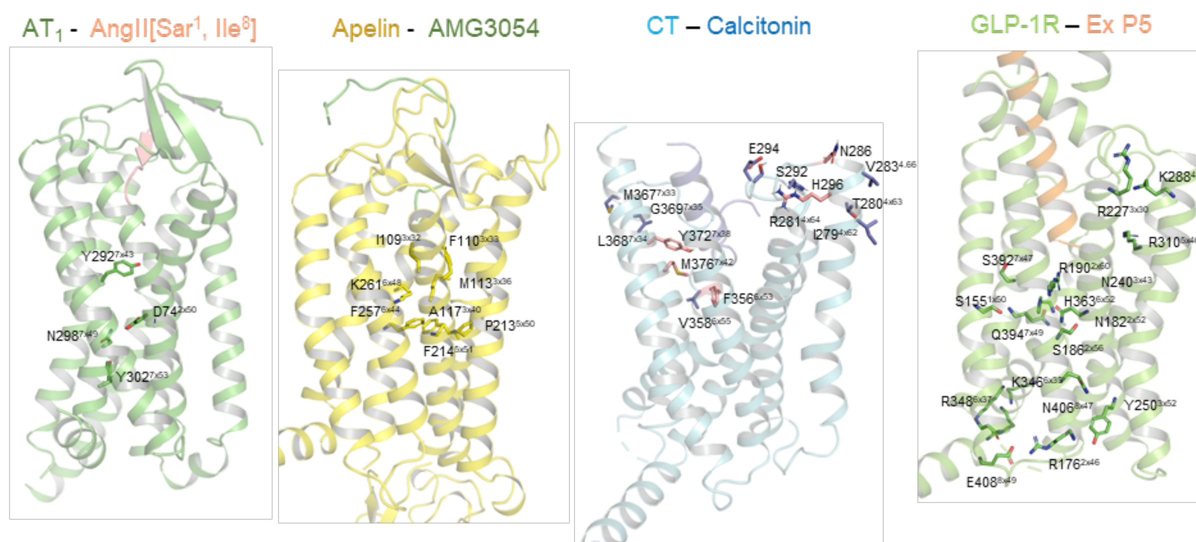


The hypothesis that agonist-specific GPCR active states result in agonist-specific activation of signalling pathways was proposed almost two decades ago[110] in response to data that did not fit the accepted two-state receptor models. The concept that compounds with affinity for the same receptor can induce ligand-specific GPCR conformations, and each elicit a particular pattern of downstream activation, is now well established[182]. Early evidence for biased agonists includes the observation that the rank order of relative potencies and/or maximal responses for ligands could be different for different signalling pathways regulated by a unique receptor[110]. In the extreme, compounds might activate one pathway and inhibit another pathway, both of which are activated by the same receptor[182, 183]. This simple measure of bias has been superseded by more sophisticated analyses that compare test compounds to a reference ligand and allow for assay differences in receptor density and coupling efficiency. One such analysis[184] uses a reference ligand, which can be any compound, although the endogenous agonist for the target receptor may be most appropriate (as used in this Review), and is used to distinguish system bias from ligand bias. Biased ligands, which may include the reference compound, stabilise a particular conformation of the receptor that preferentially interacts with one or a subset of signalling pathways. Compounds might be compared in many assays to determine an exhaustive

signalling profile but, in practice, clinically relevant signalling pathways are examined to define lead compounds with the desired pharmacological signature. Confounding bias from the assay, system and kinetics should be carefully avoided[185, 186].

Biased agonists and antagonists. Compared to the reference ligand (blue) a biased compound may have similar potency in pathway A but demonstrate lower potency (green), or no agonist activity (red) in pathway B. Whether a biased agonist with normal activity in pathway A but no effect on pathway B behaves as an antagonist of the endogenous agonist is not usually reported, but could be relevant. If the biased agonist is being used to replace a missing endogenous peptide and there is benefit in activating some downstream signalling pathways but not others (for example, activating G protein-dependent pathways but not β-arrestin recruitment, which should limit receptor internalisation and desensitisation) then it is probably not critical whether the biased compound has reduced or no potency in this second pathway. However, in conditions in which there is overactivation of a receptor system, a biased ligand with no effect on pathway B would specifically antagonise the effects of the endogenous peptide on this pathway. Other biased ligands may antagonise all pathways but show quantitatively greater functional affinities for one pathway compared to another.

Box 3 | **Structural determinants of receptor ligand bias**



Biophysical, pharmacological, site-directed mutagenesis and biomolecular simulation studies, combined with comparative analysis of different conformational states of recently released antagonist-bound and agonist-bound peptide GPCR structures (Supplementary Table 1, Figure 3), have provided insights into structural interaction networks that determine biased signalling for several peptide-binding GPCRs, including those in class A (such as the receptors for angiotensin (AT_1 and AT_2)[122] or apelin[121]) and class B (such as the receptors for calcitonin[116] or glucagon-like peptide 1 (GLP-1)[187, 188]). These structural determinants of biased signalling connect ligand binding sites, transmission switches in the core of the receptor, and intracellular interaction networks linked to G-protein and β-arrestin coupling binding sites[189, 190].

AT_1 receptor. Several residues in the AT_1 receptor are involved in signalling pathway bias[191], including $Y292^{7.43}$ (Ballesteros Weinstein GPCR numbering) in the ligand binding site, $D74^{2x50}$ in the allosteric sodium binding site[192], and $N298^{7.49}$ and $Y302^{7.53}$ in the NPXXY tyrosine switch region[189,190] . Double electron-electron resonance (DEER) spectroscopic data[193] mapped on the peptide-bound, nanobody-stabilised active AT_1 structure[122] showed that: G_q-biased peptides stabilize a receptor conformation in which the first transmembrane domain (TM1) is located far apart from TM6 and intracellular loop 2 (ICL2); β-arrestin-biased ligands stabilise a conformation in which TM1 and ICL2 are in closer proximity and TM1 is far apart from TM6; and inverse agonists stabilize a receptor population in which TM1 and TM6 are relatively close to each other and ICL2 is far apart from TM7, consistent with conformational differences between active[122] and inactive [194,195] AT_1 receptor structures.

Apelin receptor. A cluster of residues (I109^{3x32}, F110^{3x33}, M113^{3x36}) that forms an interaction network with Phe13 of apelin plays an important role in biased signalling[196]. This cluster is connected to the transmission switch region between A117^{3x40}, P213^{5x50}, F214$^{5.51}$, F257$^{6.44}$ and K261^{6X48} that are part of a hydrophobic hindering mechanism that locks class A GPCRs in the inactive state[189,190].

Calcitonin (CT) receptor. Residues in the top of TM4 and extracellular loop 2 (ECL2, including I279^{4x62}, T280^{4x63}, R281^{4x64}, V283^{4x64}, N286^{45x46}, S292^{45x53}, E294^{45x55} and H296^{45x57}) are important in conformational propagation linked to the $G_{\alpha s}$–cAMP pathway. This network is specific to the ligand and the pathway. Effects are propagated by the top of TM6/TM7/EL3, and the top of TM7[116], including F356$^{6.53}$, V358$^{6.55}$, M367^{7x33}, L368^{7x34}, G369^{7x35}, Y372^{7x38}, M376^{7x42}.

GLP-1 receptor. Several residues in ECL1, ECL2 and ECL3, and in the top of TM3 (R227^{3x30}), TM4 (K288^{4x64}) and TM5 (R310$^{5.40}$) that line the orthosteric hormone binding site play ligand-specific roles in biased signalling [187, 197]. Several additional regions connecting the peptide ligand binding site and the intracellular G-protein and β-arrestin binding regions have been identified in the GLP-1 receptor[188], consistent with conformational differences between active and inactive GLP-1 receptor structures[133,136,137,149,198].

Residues involved in biased signalling in AT_1 receptor, and apelin, CT and GLP-1 receptors in mutagenesis studies are highlighted and coloured red on the corresponding receptor structures in the figure: aligned Ang II [Sar^1, Ile^8] bound to AT_1 receptor (Research Collaboratory for Structural Bioinformatics (RCSB) protein data bank (PDB) ID: 6DO1)[122]; AMG3054 bound to apelin receptor (PDB ID: 5VBL)[121]; calcitonin bound to CT receptor (PDB ID: 5UZ7; 6NIY) [116,133]; and Exendin-P5 bound to GLP-1 receptor (PDB ID: 6B3J)[138].

Figure legends:

Figure 1 | Overview of GPCR-targeted peptide drugs. The number of peptide drugs that are approved, in Phase 3 or in Phase 2 clinical trials are shown for receptors from Class A (blue), Class B (red), or Class C (green).

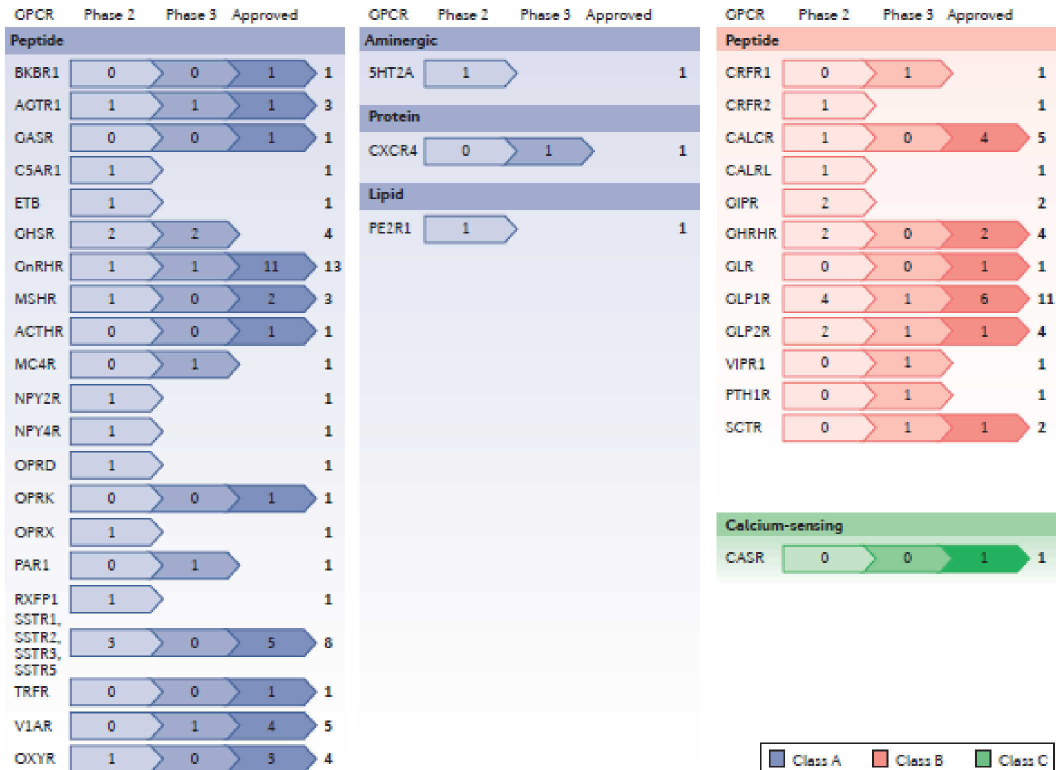


Figure 2 | **2D and 3D diagrams of peptides targeting receptors for GnRH$_1$ or GLP-1 and/or GLP-2.** Peptides are shown as beads based on the three-dimensional position of Cα atoms of the structural templates of these peptides bound to experimentally determined 7-transmembrane domain (7TM) structures of associated G protein-coupled receptors (GPCRs) (Supplementary Table 1). The orientations of the 3D peptide bead strings are consistent with their binding mode in the GPCR binding site (Figure 4), assuming a view in which transmembrane domain 1 (TM1) is on the left, TM5 is on the right, and the extra-cellular side is facing up. See Supplementary Figure 2 for further structures of peptide drugs targeting Class A and B GPCRs.

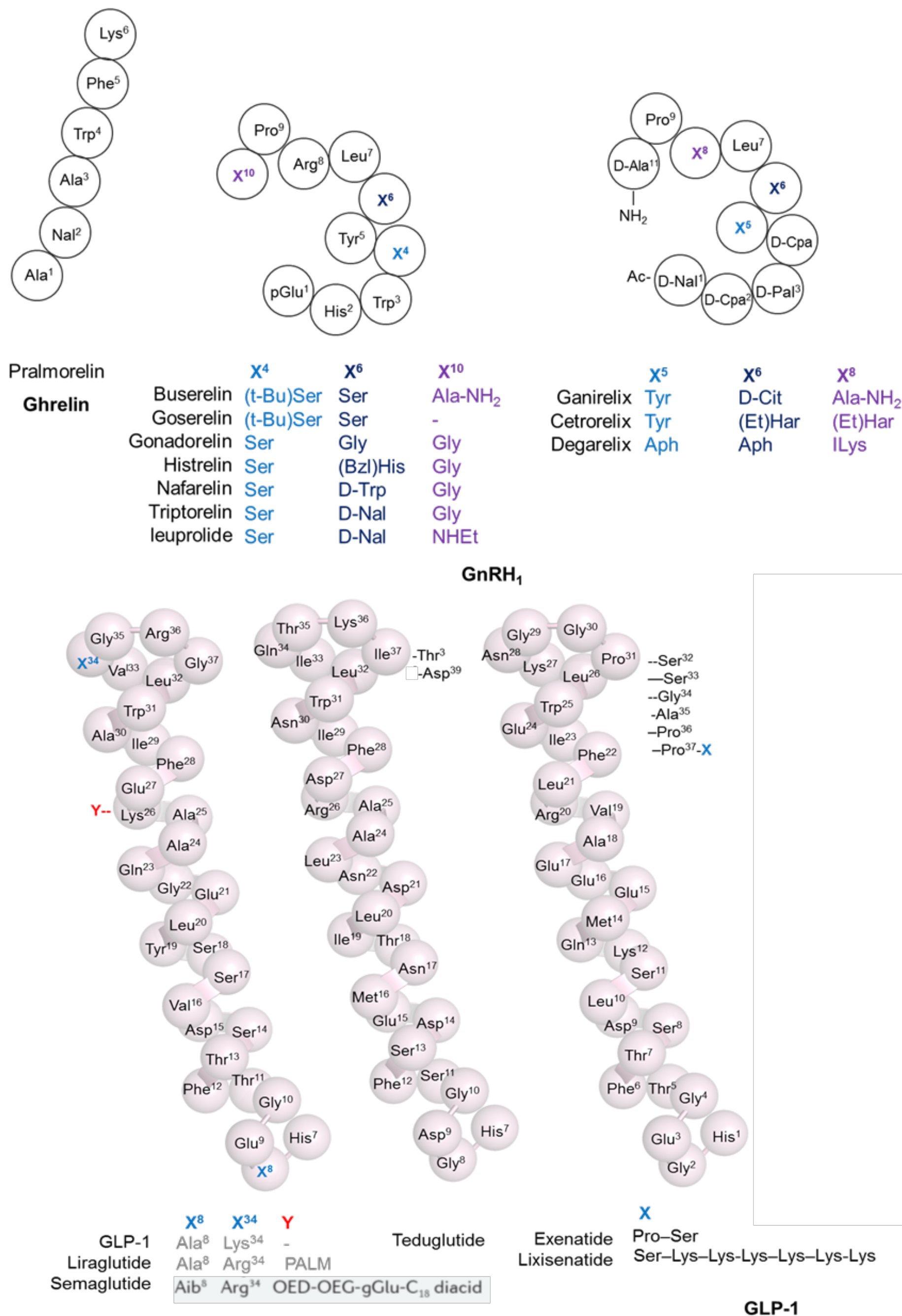


Figure 3 │ Overview of structure determination for the GPCRs. Peptide GPCRs for which 7-transmembrane domain (7TM) or full-length structures are available in complex with peptide ligands (red) or only in complex with non-peptide ligands (turquoise) are distinguished from peptide GPCRs for which only extracellular domain (ECD) structures are available (green) and non-peptide GPCRs for which 7TM or full-length structures are available (blue). For details, see Supplementary table 1. Related GPCR families — adhesion, glutamate, frizzled and taste (TAS2) are shown for reference. Receptors are classified as orphans when the endogenous ligand(s) is not yet established. The rhodopsin family is shown classified into four groups: α-group includes amine, peptide and prostaglandin receptors; the β-group includes only receptors that bind peptides; the γ-group contains chemokine receptors, some receptors that bind peptides such as somatostatins, galanin, and opioids and receptors that bind other types of ligands; the δ-group includes olfactory, purine and glycoprotein receptors.

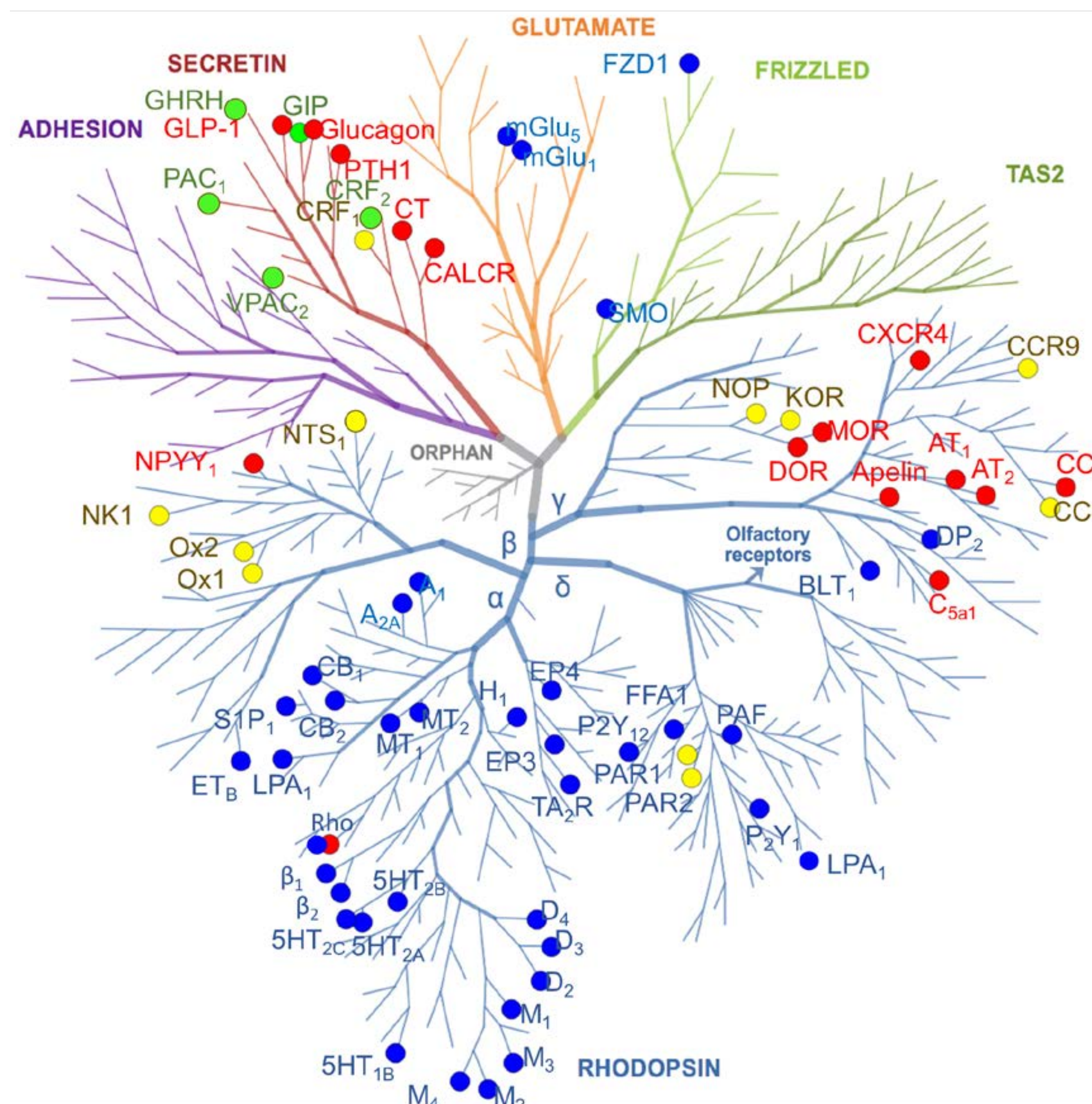
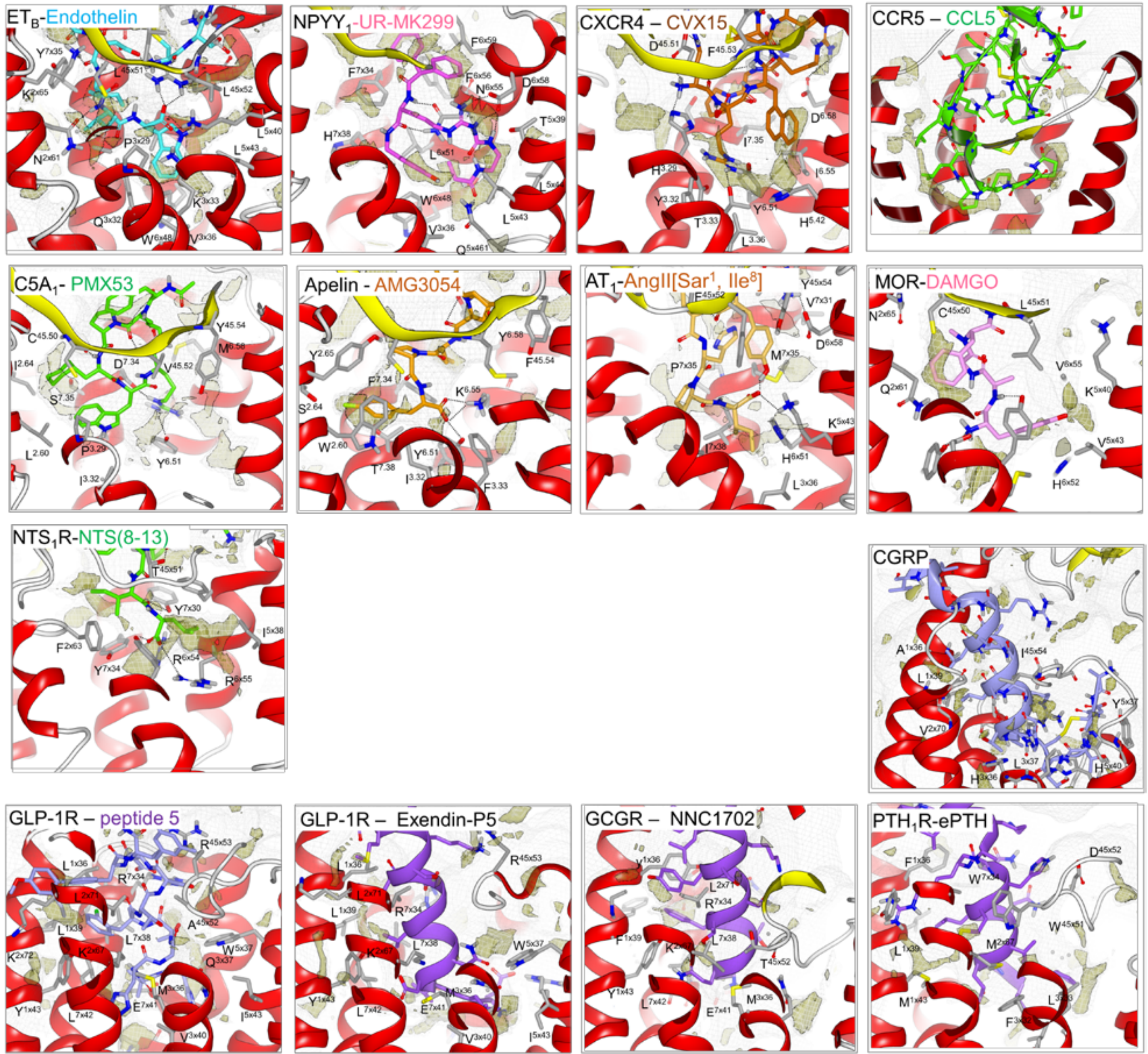


Figure 4 │ X-ray crystallography and cryo-electon microscopy structures of GPCRs. The panels summarise structural interactions for aligned binding site residues of class A and class B peptide GPCRs forming polar H-bond or ionic interactions (red), or only lipophilic interactions (grey) with peptide ligands shown in the individual binding mode figure panels. For the Class A GPCRs, the binding sites of AMG3054-bound apelin receptor (protein data bank identifier (PDB ID): 5VBL)[121], octapeptide Ang II [Sar^1, Ile^8] partial agonist-bound angiotensin AT_1 (PDB: 6DO1)[122], Complement $C5a_1$ bound to PMX53 (PDB: 6C1Q)[124], Endothelin-bound ET_B (PDB: 5GLH)[125], neurotensin-bound NTS_1 (PDB: 3ZEV) [125], UR-MK299-bound neuropeptide NPY1 (PDB: 5ZBQ)[141], DAMGO-bound MOR (PDB: 6DDE)[128], and CCL5-bound CCR5 (PDB: 5UIW)[129] are shown. For class B GPCRs, calcitonin gene-related peptide (CGRP) receptor bound to G_s [Au: Edit ok?] (PDB: 6E3Y)[134], NNC-1702-bound glucagon receptor (GCGR) (PDB: 5YQZ)[135], peptide 5-bound (PDB: 5NX2)[136] and Exendin-P5-bound (PDB: 6B3J)[135] GLP-1 receptor, and ePTH-bound parathyroid receptor (PTH1) [139] are shown. Views are focused on the 7-transmembrane domain and are consistent with the orientations of peptide diagrams shown in Figure 2. The binding pocket surfaces (grey mesh) are contoured at 1 kcal/mol using the C3 (carbon sp3) GRID probe[199], whereas lipophilic areas are defined using the C1= (lipophilic) probe contoured at -2.8 to -3.0 kcal/mol, customized to GPCR binding sites[200]. Generic GPCR residue numbers[201] are provided based on Ballesteros–Weinstein Class A GPCR (apelin receptor, AT_1, $C5a_1$, CCR5, ET_B, MOR, NPY1R)[202] and Wootten[203] Class B GPCR (CGRP, Glucagon, GLP-1, $PTHR_1$) numbering schemes. According to these schemes, the first number (1-7) denotes the transmembrane helix, and the following number indicates the residue position relative to the most conserved amino-acid in the helix (which is assigned the number 50), considering numbering offset due to helical bulges or constrictions[201].



	class A GPCRdb	1x31	1x35	1x39	2x60	2x61	2x63	2x64	3x29	3x32	3x33	3x36	4x61	4x65	45x50	45x51	45x52	45x53	45x54	5x39	5x40	5x43	5x44	5x461	6x48	6x51	6x52	6x54	6x55	6x58	7x30	7x31	7x34	7x35	7x38	7x39
NTS_1R	NTS(8-13)	S	V	Y	E	L	F	I	Y	R	D	T	M	M	C	T	P	T	I	V	I	N	T	S	W	Y	H	R	R	F	Y	H	Y	M	N	A
$NPYY_1$	UR-MK299	M	L	Y	T	F	Y	T	P	Q	C	I	F	Q	C	F	D	Q	F	Y	T	L	L	Q	W	L	T	F	N	F	H	N	F	L	H	L
ET_B	Endothelin	F	N	S	N	V	K	L	P	Q	K	V	E	F	C	L	L	H	P	K	D	L	F	Y	W	L	H	S	R	K	L	L	D	Y	I	N
Mu	DAMGO	T	I	Y	Q	S	N	Y	I	D	Y	M	M	T	C	T	L	T	F	L	K	V	F	A	W	I	H	Y	V	K	Q	T	W	H	I	A
Apelin	AMG3054	S	I	Y	W	A	Y	T	S	I	F	M	V	R	C	Y	M	D	Y	L	G	S	T	G	W	Y	H	V	K	Y	L	M	F	P	T	C
AT_1	Ang II[Sar[1],Ile[8]]	L	I	Y	W	A	Y	T	S	V	S	L	A	R	C	A	F	H	Y	L	G	K	N	G	W	H	Q	F	T	D	V	D	M	P	I	L
$C5a_1$	PMX53	P	A	F	L	F	I	V	P	I	L	M	S	R	C	G	V	D	Y	V	A	R	L	G	W	Y	Q	T	G	M	L	K	D	S	V	S
CCR5	CCL5	A	I	Y	W	A	Y	A	T	Y	F	F	N	T	C	S	S	H	F	Q	T	I	V	G	W	Y	N	V	L	N	L	D	M	Q	E	T
CXCR4	CVX15	N	L	Y	W	A	D	A	H	Y	T	L	D	A	C	D	R	F	Y	F	Q	H	I	G	W	Y	Y	G	I	D	V	H	I	S	E	A

	class B GPCRdb	1x36	1x39	1x43	2x67	2x68	2x71	2x72	3x33	3x36	3x37	3x40	4x60	4x64	45x50	45x51	45x52	45x53	45x54	5x36	5x37	5x39	5x40	5x43	6x53	6x56	6x57	6x59	6x60	EL3	7x34	7x35	7x38	7x39	7x41	7x42
CRLR	CGRP	A	L	T	H	L	V	A	Q	H	L	M	H	R	C	W	I	S	S	L	Y	I	H	I	F	I	P				E	E	D	Y	M	H
$GLP\text{-}_1R$	peptide 5	L	L	Y	K	D	L	K	F	M	Q	V	W	K	C	W	A	R	N	Y	W	I	R	I	E	F	A	V	M		R	F	L	F	E	L
$GLP\text{-}_1R$	Exendin P5	L	L	Y	K	D	L	K	F	M	Q	V	W	K	C	W	T	R	N	Y	W	I	R	I	E	F	A	V	M		R	F	L	F	E	L
GCGR	NN1702	Y	F	Y	I	D	L	R	A	M	Q	I	W	K	C	W	T	S	N	F	W	L	R	V	E	F	A	V	T		R	S	L	F	D	L
PTH_1R	ePTH	F	L	I	M	D	L	Y	V	F	L	L	W	R	C	W	D	L	S	N	K	I	Q	I	Y	F	M	T	P		W	Q	M	H	E	M

Table 1| **Endogenous peptides targeting Class A and B GPCRs that are approved for clinical use**

Family	Clinical indication	Therapeutic; approval date	Plasma half-life (minutes, following iv administration)	Target receptor	Affinity (pK_i)	Potency (pEC_{50})
Class A						
Angiotensin	Septic shock	Angiotensin II (Giapreza; LJPC501); 2017	< 1	AT_1	8.8	9.0–9.3
				AT_2	10.2[a]	ND
Melanocortin	Diagnosis, adrenal insufficiency	Cosyntropin (Tetracosactide); 1967	15	MC_2	ND	ND
Thyrotropin-releasing hormone	Testing response of anterior pituitary gland for thyroid disorders (eg secondary hypothyroidism or acromegaly)	TRH (Thyroliberin; Protirelin); 1976	5.3	TRH_1	7.4[b]	8.5
				TRH_2	7.4[b]	ND
Vasopressin and oxytocin	Induction of labour	Oxytocin (Otx; Pitocin; Syntocinon); 1980	1–6	OT	8.2–9.6	7.8–10.4
				V_{1A}	6.9–8.3	8
				V_{1B}	5.7–7.0	6.6–7.6
				V_2	5.4–6.8	8.1
		Vasopressin (ADH; antidiuretic hormone; arginine vasopressin; argipressin); 2014	10–20	OT	7.3–9.3	ND
				V_{1A}	8.5–9.3	9–9.6
				V_{1B}	9.9	8.3–8.7
				V_2	7.9–9.1	10.3
Class B						
Calcitonin	Paget's Disease	Calcitonin (Thyrocalcitonin; LS-173874); 1981	10–38	AMY_1	ND	8.9–11.3
				AMY_2	ND	11.4
				AMY_3	ND	8.0–10.6

				CT	9	9.0–11.2
Glucagon	Severe hypoglycaemia	Glucagon; 1998	8–18	GLP1R	6.9–7.0	ND
				Glucagon	ND	9.0
	Diagnostic	Secretin; 2004	45	Secretin	ND	9.7
Parathyroid hormone	Hypocalcaemia, parathyroid deficiency	PTH (Natpara; ALX1-11; rhPTH(1-84)); 2006	90	PTH1	ND	ND
				PTH2	ND	ND
	Post-menopausal osteoporosis	Teriparatide; 2002	5	PTH1	7.4[c]	ND
				PTH2	7.7-7.8[c]	ND

[a] pK_D (negative logarithm to base 10 of the equilibrium dissociation constant for ligand receptor interactions); [b] Derived from studies of the rat receptor; [c] pIC_{50} (negative logarithm to base 10 of the concentration of competing agonist or antagonist that inhibits the binding of a radioligand by 50% in a competition binding assay). Endogenous peptides approved for clinical use were identified from the GuidetoPharmacology database[3], which has the most extensive classification. The list was compared with the DrugBank database, with further information from RxList, Global Data or relevant company websites (See Related links). Pharmacokinetic and pharmacodynamics parameters were curated from GuidetoPharmacology[3], DrugBank and the original citations. The peptides identified are in use in any geographical region, but largely reflect those used widely in major pharmaceutical markets such as North America, Asia and Europe, and our analysis may not have captured peptides licensed in just one country. A range of affinities and potencies are given if ranges were reported. For peptides with affinities for more than one subtype within the family, all values are shown where available. Affinity and potency data are derived from human receptors, except where indicated. Plasma half-life calculations are based on intravenous administration, but clinical administration may be via other routes, such as intra-muscular or intra-nasal, that may alter these values. Information on volume of distribution and percent plasma binding can be found in Supplementary Table 2. Human parathyroid hormone (1-84), manufactured as a recombinant form with the full 1-84 amino acids was approved for clinical use as Natpara, (ALX1-11, rhPTH1-84) but it is included as shorter sequences are also effective in activating target receptors. Teriparatide is a synthetic peptide comprising 1-34 of the N-terminal amino acids of human parathyroid hormone. iv, intravenous; ND, no data available from online resources; pEC_{50}, the negative logarithm to base 10 of the molar concentration of an agonist that produces 50% of the maximal possible effect of that agonist; pK_i, negative logarithm to base 10 of the concentration of the competing ligand that would occupy 50% of receptors as determined in a competition binding assay.

Table 2 | **Modified peptides targeting Class A and B GPCRs that are approved for clinical use**

Family	Clinical indication	Therapeutic; approval date	Plasma half-life (hours, following iv administration)	Target receptor	Affinity (pK_i)	Potency (pEC_{50})
Class A						
Bradykinin	Hereditary angioedema	Icatibant[a] (D-Arg-[Hyp[3],Thi[5],D-Tic[7],Oic[8]]BK; HOE140; Firazyr); 2008	1–2 1.4[b]	B_2 receptor	10.2	8.0–9.4
Cholecystokinin	Diagnostic aid	Pentagastrin (ICI-50123; AY-6608; Peptavlon); 1974	0.16 <0.6[b]	CCK_2 receptor	9.05	ND
Gonadotrophin-releasing hormone	Endometriosis, pituitary desensitisation prior to ovulation induction, advanced prostate cancer	Buserelin (HOE 766; HOE 766A; ICI 123215; Suprefact; Receptal; Etilamide; Metrelef); 1999	0.08–1.3 1.33[b]	$GnRH_1$ receptor	9.4–10.0 9.5–10.4[c]	10.5
	Controlled ovarian stimulation	Cetrorelix[a] (SB-075; Cetrotide); 1999	5–62.8[b]	$GnRH_1$ receptor	9.3–10	8.7
	Controlled ovarian stimulation	Ganirelix[a] (Antagon; Orgalutran; Fyremadel; RS 26303); 2003	14.5 13–16 [b]	$GnRH_1$ receptor	ND	ND
	Advanced prostate cancer	Degarelix depot[a] (FE200486; Firmagon); 2008	996–1690 696 [b]	$GnRH_1$ receptor	8.8	ND
	Amenorrhea, hypogonadism	Gonadorelin (Abbott 41070; AY-24031; Hoe-471; RU-19847); 1986	0.16–0.67	$GnRH_1$ receptor	ND	ND
	Breast cancer, prostate Cancer	Goserelin (Decapeptide I; ICI 118630; Zoladex); 1989	4.9	$GnRH_1$ receptor	8.8	ND
	Advanced prostate cancer	Histrelin (Supprelin LA; Vantas; ORF 17070; RWJ 17070); 2004	4 [b]	$GnRH_1$ receptor	8.7–9.7 9.0–10.4[c]	ND
	Advanced prostate cancer	Leuprolide (Leuprorelin; Lupron; Viadu; ABBOTT-43818; TAP-144; Eligard; Carcinil; Prostap; Lutrate); 1985	3	$GnRH_1$ receptor	8.5–9.1	ND

	Endometriosis, precocious puberty	Nafarelin (Synarel), 2005	3	GnRH$_1$ receptor	10	ND
	Prostate cancer, endometriosis, precocious puberty	Triptorelin (Triptodur; Trelstar; Pamorelin; CL-118532; Decapeptyl; Diphereline; Gonapeptyl; Variopeptyl); 1986	3 phases: 0.1, 0.75, 3	GnRH$_1$ receptor	8.5–8.8	ND
	Prostate cancer	Abarelix[a, d] (PPI 149; R 3827; Plenaxis); 2003	317±77	GnRH$_1$ receptor	9.1–9.5	ND
Melanocortin	Erythropoietic protoporphyria	Afamelanotide (Scenesse); 2014	0.8–1.7	MC$_1$ receptor	10.0[e]	ND
				MC$_3$ receptor	8.9	ND
				MC$_4$ receptor	8.5–8.8	ND
				MC$_5$ receptor	9.0[e]	ND
	Sexual arousal disorder	Bremelanotide (PT-141; Rekynda); 2018	2	MC$_1$ receptor	ND	ND
				MC$_3$ receptor	ND	ND
				MC$_4$ receptor	8.0[e]	ND
				MC$_5$ receptor	ND	ND
Opioid	Pain following abdominal surgery	Difelikefalin (CR-845); 2019	2	κ receptor	ND	9.8
Relaxin	Heart failure	Serelaxin (Reasanz; RLX-030; recombinant human relaxin 2); 2016	8–9	RXFP1	10[e]	9.2
Somatostatin	Acromegaly, Cushing's Disease	Pasireotide (SOM 230; Signifor), 2012	12	SST$_1$ receptor	8.0[e]	ND
				SST$_2$ receptor	9.0[e]	ND
				SST$_3$ receptor	8.8[e]	ND
				SST$_5$ receptor	9.8[e]	ND
	Acromegaly, gastroentero-pancreatic tumours	Lanreotide depot (DC 13-116; Somatuline; Ipstyl; BIM 23014; Lanreotide acetate); 1994	1.14 528[b]	SST$_1$ receptor	6.7[e]	ND
				SST$_2$ receptor	8.7–9.6	ND
				SST$_3$ receptor	7.2–8.0	ND
				SST$_5$ receptor	7.4–9.3	ND
	Acromegaly, gastroentero-pancreatic tumours	Octreotide[f] (DRG-0115; Longastatin; SMS 201 995; Sandostatin; Atrige; Sandostatin LAR; Lutathera); 1987	1.67 4.2[b]	SST$_2$ receptor	8.7–9.9	ND
				SST$_3$ receptor	7.4–8.6	ND
				SST$_5$ receptor	7.2–9.5	ND
	Oesophageal variceal bleeding	Vapreotide (BMY 41606; CCRIS 6495; RC-160; Sanvar IR; Vapreotide acetate); 2009	0.5	SST$_2$ receptor	8.3–10.1	ND
				SST$_3$ receptor	7.4–7.9	ND
				SST$_5$ receptor	7.3–9.2	ND
Vasopressin and oxytocin	Delaying imminent pre-term birth	Atosiban[a] (ORF22164; RWJ 22164; d[D-Tyr(Et)2, Thr^4,Orn^8] vasotocin; d [D-Tyr(Et)^2 Thr^4]OVT;	1.7	OT receptor	6.0–7.6	ND

		Tractocile; Antocin); 2000				
	Cranial diabetes insipidus (post hypophysectomy or for polyuria or polydipsia post head trauma), haemophilia, von Willebrand's Disease	Desmopressin (1-deamino; D-AVP; [deamino-Cys1,D-Arg^{8}]vasopressin; dDAVP; Adiuretin; Concentraid; Minirin; Stimate; Noctiva); 1999	0.9–2.6	OT receptor	6.7–7.6	ND
				V_{1A} receptor	7.0–7.7	ND
				V_{1B} receptor	7.7–8.2	ND
				V_2 receptor	7.2–8.6	ND
	Haemostatic agent	Felypressin (PLV-2); 1969	ND	V_{1A} receptor	ND	ND
	Hypotension, bleeding oesophageal varices	Terlipressin (Glypressin; Lucassin); 2006	0.4–1.1	V_{1A} receptor	ND	ND
				V_{1B} receptor	ND	ND
				V_2 receptor	ND	ND
	Postpartum haemorrhage	Carbetocin (Duratocin; Pabal; Lonacten; Depotocin; Comoton; Decomoton); 2016	1.4–1.7	OT receptor	ND	ND
Class B						
Calcitonin	Paget's Disease	Calcitonin (salmon) (Fortical; Miacalcin); 1976	1–1.1 [b]	AMY_1 receptor	8.7-9.7 [e]	10.0
	Pain caused by osteoporosis	Elcatonin; 1981	ND	CT receptor	ND	ND
	T1DM, T2DM	Pramlintide (AC-0137; AC-137; Symlin; Symlinpen); 2005	0.4–0.7 0.8 [b]	AMY_1 receptor	ND	9.4
				AMY_2 receptor	ND	8.6–8.9
				AMY_3 receptor	ND	9.1–9.3
				CT receptor	ND	8.3
Glucagon	Dwarfism, HIV-associated weight loss	Sermorelin [g] (Sermorelin acetate; Geref); 1990	0.17 0.2 [b]	GHRH receptor	8.2	ND
	HIV-infected patients with lipodystrophy	Tesamorelin (TH9507; (3E)-hex-3-enoylsomatoliberin; Egrifta); 2010	0.43 [b]	GHRH receptor	10.2	ND
	T2DM	Albiglutide protein, fused (GSK-716155; Eperzan; Tanzeum); 2014	96–168 [b]	GLP-1 receptor	ND	7.7 [h]

	T2DM	Dulaglutide[i] (GLP-1Fc; LY2189265; Trulicity); 2014	120 [b]	GLP-1 receptor	ND	ND
	T2DM	Exenatide (Exendin-4, AC002993; AC 2993; AC2993A; Byetta; Bydureon); 2005	2.4 [b]	GLP-1 receptor	8.7–9.0 9.2[e]	ND
	T2DM	Liraglutide (NN-2211; Victoza; Saxenda); 2009	13 [b]	GLP-1 receptor	8.3–10	10.2
	T2DM	Lixisenatide (Adlyxin; AVE-0010; Lyxumia); 2013	3 [b]	GLP-1 receptor	8.9	ND
	T2DM	Semaglutide spacer (NN-9535; Ozempic); 2017	168 [b]	GLP-1 receptor	ND	11.2
	Short bowel syndrome	Teduglutide (ALX-0600; Gattex; Revestive; (Gly2)GLP-2); 2012	2.0	GLP-2 receptor	11.3 [e, h]	10
Parathyroid Hormone	Post-menopausal osteoporosis	Abaloparatide (BA058; Tymlos); 2017	1.7 [b]	PTH1 receptor	9.7 [e]	10.1
Class C						
Calcium-Sensing	Secondary hyper-parathyroidism	Etelcalcetide (Velcalcetide; KAI-4169; AMG-416; Parsabiv); 2016	84 72–96 [b]	CaS	ND	4.6

[a] Antagonist; [b] half-life following subcutaneous injection; [c] pK_D (negative logarithm to base 10 of the equilibrium dissociation constant for ligand receptor interactions); [d] Abarelix was discontinued in United States in 2003, owing to allergic reactions, but clinical use has continued in Europe; [e] pIC_{50} (negative logarithm to base 10 of the concentration of competing agonist or antagonist that inhibits the binding of a radioligand by 50% in a competition binding assay); [f] Lutathera is a radiolabelled peptide that exploits radiation to kill tumours; [g] withdrawn in United States; [h] Derived from studies of the rat receptor; [i] Dulaglutide contains GLP-1 (amino acids 7-37) with substitutions Ala8Gly, Gly22Glu, Arg36Gly, a 16 amino acid linker sequence and a 228 amino acid synthetic human Fc fragment (immunoglobulin G4) - two identical peptide chains form a dimer, linked by inter-monomer disulphide bonds between Cys55-55 and Cys58-58. All molecules are agonists unless otherwise indicated. Compilation of the data used the databases and methods as outlined in Table 1. The table reflects the consensus that synthetic peptides act primarily on a single receptor or family of receptors (such as the somatostatin family). Cyclosporin, which is reported to be an antagonist of formylpeptide receptor, has been omitted as it is reported to be nonselective. The main clinical uses in DrugBank, RxList, Electronic Medicines Compendium and Clinical Trials.Gov are listed under clinical indication. A range of affinities and potencies are given if ranges were reported. For peptides binding to somatostatin receptors, the relative importance of the affinities for the different subtypes in the therapeutic action is unclear and all values have been included. Affinity and potency data are derived from human receptors, except where indicated. Plasma half-life calculations are based on intravenous or subcutaneous administration, but clinical administration may be via other routes, such as intra-muscular or intra-nasal, that may alter these values. For example, calcitonin (salmon) plasma half-lives are: intra-muscular, 0.96 hr, intra-nasal 0.3-38 hr. Information on volume of distribution and percent plasma binding can be found in Supplementary Table 3. iv, intravenous; ND,

no data available from online resources; pEC50, the negative logarithm to base 10 of the molar concentration of an agonist that produces 50% of the maximal possible effect of that agonist; pKi, negative logarithm to base 10 of the concentration of the competing ligand that would occupy 50% of receptors as determined in a competition binding assay.

Table 3 | **Peptides in the pipeline**

Family	Receptor	Ligand; mechanism of action	Clinical indication	Year of study initiation	Synonyms
Ongoing Phase 3 trials, Class A GPCRs					
Chemokine	CXCR4	BL-8040; antagonist	Stem cell mobilisation for HSCT for multiple myeloma	2019	None
Ghrelin	Ghrelin	Livoletide; mechanism of action unclear	Prader–Willi Syndrome, hyperphagia	2019	AZP-531; unacylated Ghrelin
Melanocortin	MC_4	Setmelanotide; agonist	Obesity	2019	RM-493; BIM-22493; IRC-022493
VIP and PACAP	$VPAC_1$	Aviptadil; agonist	Sarcoidosis	2019	Vasoactive intestinal peptide
Ongoing Phase 3 trials, Class B GPCRs					
Glucagon	GLP-2	Glepaglutide; agonist	Short bowel syndrome	2019	ZP1848
Ongoing Phase 2 trials, Class A GPCRs					
Gonadotrophin-releasing hormone	$GnRH_1$	EP-100; agonist[a]	Ovarian cancer	2017	None
Opioid	δ	Enkephalin; agonist	Intractable cancer pain	2016	IRT-101; MENK; methionine encephalin; np2 enkephalin
Vasopressin and oxytocin	OT	Barusiban; antagonist	Infertility and IVF treatment	2019	None
Ongoing Phase 2 trials, Class B GPCRs					
Corticotropin-releasing factor	CRF_2	RT-400; agonist	Acute decompensated heart failure	2017	JNJ-39588146; JNJ-9588146; RT 400; Stresscopin acetate; Stresscopin program; Urocortin III; Urocortin-3
Glucagon	GLP-1	Exendin (9-39); antagonist	Congenital hyperinsulinism, postbariatric hypoglycaemia	2018	None
	GLP-2	Apraglutide; agonist	Short bowel syndrome	2018	FE203799
Discontinued during or after Phase 3 trials, Class A GPCRs					
Angiotensin	AT_1	Aclerastide; agonist	Diabetic foot ulcers	2015	DSC-127; NorLeu3-A(1-7); USB-005
Ghrelin	Ghrelin	Ulimorelin; agonist	Gastrointestinal dysmotility	2012	TZP-101

Gonadotrophin-releasing hormone	GnRH$_1$	Ozarelix; antagonist	Benign prostatic hyperplasia	2010	D-63153; Ozarelix acetate; SPI-153
Proteinase-activated	PAR1	Rusalatide; agonist[b]	Radius fracture	2008	Chrysalin; TP-508; TRAP-508
Vasopressin and Oxytocin	V_{1A}	Selepressin; agonist	Septic shock	2018	None
Discontinued during or after Phase 3 trials, Class B GPCRs					
Corticotropin-releasing factor	CRF$_1$	Corticorelin; agonist	Brain tumours and brain oedema	2012	Corticorelin acetate injection; hCRF; human corticotropin-releasing factor; Xerecept
Glucagon	GLP-1	Taspoglutide; agonist	T2DM	2013	BIM-51077; ITM-077; R-1583
	Secretin	RG1068; agonist	Autism	2005	RG 1068; SecreFlo; synthetic human secretin
Discontinued during or after Phase 2 trials, Class A					
5-Hydroxytryptamine	5-HT$_{2A}$	Nemifitide; antagonist	Major depression	2010	NO
Angiotensin	AT$_1$	TRV027; biased ligand	Heart failure	2016	TRV120027
Endothelin	ET$_B$	SPI-1620; agonist	Non small cell lung cancer, biliary tract cancer	2016	PMZ-1620; IRL-1620; SPI-1620
Ghrelin	Ghrelin	GTP-200; agonist	Cancer cachexia or anorexia	2007	Ghrelin; GTP 200
		TZP-102; agonist	Diabetic gastroparesis	2012	None
		Pralmorelin; agonist	Severe hypoglycaemia	2010	Growth hormone-releasing peptide-2; GHRP-2; KP 102
Melanocortin	MC$_1$	Modimelanotide; agonist	Acute kidney injury	2014	AP-214; ABT-719; ZP-1480
Neuropeptide Y	Y$_2$ and Y$_4$	Obinepitide; agonist	Obesity	2007	TM30338; (34-L-glutamine) pancreatic hormone (human)
	Y$_4$	TM30339; agonist	Obesity	2012	None
Opioid	NOP	Ser100; agonist	Isolated systolic hypertension	2014	None

Somatostatin	SST_1	Veldoreotide; agonist	Acromegaly	2007	COR-005; DG3173
	SST_5	BIM23A760; agonist	Acromegaly, carcinoid syndrome	2010	BIM 23A-760; TBR 760
Discontinued during or after Phase 2 trials, Class B					
Calcitonin	CGRP	CGRP; agonist	Myocardial infarction, asthma	2007	None
	CT	Davalintide; agonist	Obesity	2010	AC2307
Glucagon	GHRH	AKL-0707; agonist	Malnutrition associated with chronic kidney disease	2007	AKL 0707; GHRH analogue
		DAC:GRF; agonist	Obesity	2006	CJC-1295
	GIP	MAR701; agonist[c]	T2DM	2012	MAR-701; RG 7685; RO-6807952
		MAR709; agonist[c]	T2DM	2015	GLP-1/GIP dual agonist; MAR-709; NN-9709; NNC-00902746; RG 7697; RO-6811135
	GLP-1	GTP-010; agonist	Irritable bowel syndrome	2009	GTP 010; LY-307161; ROSE 010; ROSE-010GTP010
		TT-223; agonist	T2DM	2010	E-1; E1-I.N.T.; E1; EGF analog; G1; gastrin analog; GLP1-I.N.T.; TT 223
		Glymera; agonist	T2DM	2016	PB1023
	GLP-2	Elsiglutide; agonist	Drug and/or toxin-induced diarrhoea	2017	GLP-2 analog; ZP-1846
Parathyroid hormone	PTH1	Ostabolin-C; agonist	Hip fracture	2009	Cyclic PTH-(1-31); ZT 031

[a] EP-100 comprises GnRH and CLIP-71 (an 18 amino acid cationic α-helical lytic peptide). [b] Rusalatide comprises 23 amino acids of the receptor binding domain of pro-thrombin. [c] MAR701 and MAR709 are PEGylated compounds. Data classified as in Phase 2 or 3 as of 2019 were retrieved primarily from Global Data, ClinicalTrials.gov or relevant company websites (see Related links). Studies can be in any geographical region. Trials were considered discontinued if no activity had been recorded in three years prior to 2019 or if the drug had disappeared from the company pipeline.

Table 4 | **Evolution of GLP-1 peptide agonists to increase plasma half-life and reduce dosing interval**

Agonist	**FDA approval date**	**Sequence similarity to native GLP-1**	**Clinical dosing**	**Plasma half-life** (hr)	**Summary of modifications**
Exenatide	2005	53%	Twice daily	2.4	39-amino-acid synthetic peptide analogue Exendin-4, a toxin from Gila monster saliva
Liraglutide	2009	97%	Daily	13	The free fatty acid linker binds albumin, which protects the peptide from DPP4 cleavage and reduces renal elimination
Exenatide QW	2011	53%	Weekly	2.4	Reformulated version of exenatide encapsulated into micropsheres for slow plasma release
Lixisenatide	2013	48%	Daily	3	Contains a modified version of exendin-4 to extend plasma half life
Albiglutide	2014	95%	Weekly	96-168	GLP-1 peptide–albumin fusion protein
Dulaglutide	2014	90%	Weekly	120	GLP-1 peptide–Fc fusion protein (peptibody)
Semaglutide	2017	94%	Weekly	168	GLP-1 peptide modified at positions 8 and 34 with a free fatty acid linker at Lys26
Semaglutide, oral	2019	94%	Daily	168	Co-formulation with the absorption enhancer sodium N-(8-[2-hydroxylbenzoyl] amino) caprylate.

Data from GuidetoPharmacology[3], DrugBank, Electronic Medicines Compendium and Clinicaltrials.gov (see Related links).

Supplementary Text

Insights from structural studies

Currently experimental X-ray crystallography or cryo-electron microscopy (cryoEM) structures have been reported for the seven-transmembrane domain (7TM) domains of 62 GPCRs, covering 212 distinct GPCR-ligand complexes and 200 unique ligands. Structures of 27 peptide or protein binding GPCRs have been reported, covering 65 unique receptor-ligand complexes. Twenty two of the experimentally determined GPCR structures (10%) contain a peptide ligand, covering Class A GPCRs apelin, angiotensin AT_1 and AT_2, complement $C5a_1$, endothelin ET_B, neurotensin NTS_1,opioid receptors δ (DOR) and MOR, chemokine CCR5, CXCR4 and US28 receptors and Class B GPCRs calcitonin CT, CGRP, glucagon, GLP-1, and parathyroid PTH1 receptors (Supplementary Table 1, Figures 2 and 4). The Supplementary Table 1 and Supplementary Figure 1 show how the peptide bound structures of AT_1, AT_2, ET_B, CT, DOR, MOR, CXCR4, CT, CGRP, glucagon, GLP-1 and PTH1 receptors provide structural templates and detailed insights into the structural determinants of ligand binding and functional activity (Box 3). This is shown for four identical and 21 homologous therapeutically relevant peptides, covering four of the 10 clinically relevant endogenous peptide ligands (Table 1), 15 of the 40 synthetic peptide drugs (Table 2) and six of the 11 peptide ligands that are currently in development (Table 3). The structures of several of the previously mentioned receptors, as well as neuropeptide $NPYY_1$, KOR, NOP, CT and corticotropin releasing factor CRF_1 receptors provide templates to model the three-dimensional binding modes of an additional 32 clinically relevant peptides to these and/or closely related receptors (Supplementary Table 1). The accumulated portion of GPCR-peptide complexes that can be structurally modelled based on homologous peptides and/or receptors covers about half of all clinically relevant peptides reported in Tables 1, 2 and 3. The following section summarises the binding modes of these GPCR-peptide complexes (Supplementary Table 1, Supplementary Figure 1, Figures 2 and 4) providing insights into the structural determinants of binding and functional activity (Box 3) of several of these clinically relevant peptides and representing potential templates for the structure-based optimisation and design of novel GPCR peptide therapeutics.

Figures 2, 4 and Supplementary Figure 1 show how peptide ligands adopt diverse binding modes across GPCRs, reflecting the diverse chemical structures and properties of both ligands and receptor binding sites. Class A GPCR peptide ligands generally target ECL2 and a polar/ionic interaction site at the top of TM6/7/ECL3 and bind differentially located and shaped lipophilic regions deeper in the 7TM receptor pocket. Helical Class B GPCR ligands share an apolar interaction site with the region between TM1/2/7 that is less accessible in Class A GPCRs, but form differential polar interaction networks associated with

different relative orientations of the extracellular N-terminal domain (ECD) and the 7TM domain[1].

The octapeptide Ang II [Sar^1, Ile^8] partial agonist bound AT_1 and AT_2 crystal structures[122,123] show an extensive polar interaction network between C_{ter}-K^{5x43}, Tyr^4-F/M^{45x52}, His^6/Pro^7-$R^{4.63x64}$, Arg^2-D^{6x58}/D^{7x31}, whereas Ile^1 and Pro^7 target lipophilic hotspots between W^{2x60}, V/L^{3x32} and $I^{7.39x38}$ (Supplementary Table 1, Figures 2-3). The conformation of Ang II [Sar^1, Ile^8] is stabilised by aromatic stacking between Tyr^4 and His^6 and an intramolecular H-bond between Tyr^4 and the C-terminus. Comparative analysis of different conformational states of antagonist and agonist bound AT_1 and AT_2 structures (Supplementary Table 1), combined with biophysical and pharmacological studies provide insights into determinants of biased angiotensin receptor signalling (Box 3). The homologous clinically relevant natural peptide angiotensin-II, Aclerastide (discontinued Phase 3) and TRV027 (discontinued Phase 2) (Table 1, Table 3, Figure 2) likely adopt similar binding modes in AT_1.

The apelin receptor structure[121](Figure 4) shows how the 17-amino acid apelin mimetic peptide agonist AMG3054 adopts binding mode targeting the major pocket in the 7TM domain and the top of ECL 1 and ECL 2 (Supplementary Table 1, Figures 2 and 3, Supplementary Figure 1) and provides a structural template to identify determinants of biased apelin signalling (Box 3). The C-terminal carboxylic acid group of the apelin mimetic interacts with the cationic $R168^{ECL2}$ and $K268^{6x55}$ residues. The C-terminal chlorinated phenyl residue is stacked between $Y35^{1x39}$, $W85^{2x60}$, $Y88^{2x63}$, $Y299^{7.43x42}$ and forms a halogen bond with the backbone carbonyl of $W85^{2x60}$. Nle^{15} binds another lipophilic region between $Y271^{6.58}$ and $F290^{7.34}$. The identified structural determinants are consistent with apelin mutation studies[121], showing the importance of for example of $Y35^{1x39}$, $W85^{2x60}$, and $R168^{ECL2}$, suggesting that features of the AMG3504 binding mode are transferrable to apelin analogues.

The endothelin ET_B receptor structure[125] shows how the C-terminus of the 21-residue endothelin-1 (ET-1) peptide targets lipophilic pockets in the major pocket of ET_B, whereas the extra-cellular vestibule of the receptor folds around the α-helix and N-terminus of the peptide ligand (Supplementary Table 1, Figures 2 and 4, Supplementary Figure 1). The C-terminal carboxylic acid group of ET-1 forms a tight ionic H-bond interaction network with the cationic $K182^{3x33}$, $K273^{5.39x38}$, and $R343^{6x55}$ residues, whereas the N-terminal Cys^1 and Ser^2 residues form polar interactions with the backbone of $L257^{45x52}$ in EL2. The C-terminal Trp^{21} and Ile^{20} residues of ET-1 bind a lipophilic pockets between TM5/6 ($L277^{5.42x43}$, $W336^{6x48}$, $I339^{6x51}$) and TM2/3/EL1 ($W167^{23x50}$, $I181^{3x32}$ and $Y148^{3x33}$.), respectively. The conformation of ET is stabilised by disulfide bridges between the Cys^1 and Cys^{15} and between Cys^3 and Cys^{11}. The homologous shorter 14-mer SPI-1620 (discontinued Phase 2, Table 3, Figure 2)

is expected to adopt a similar binding mode in ET_A as the C-terminal region of ET-1 in the ET_B structure, consistent with mutation studies[125].

The pentapeptide agonist DAMGO adopts a binding mode in the MOR cryoEM structure[128] in which the N-terminal Tyr^1 forms ionic interactions with $D147^{3x32}$ and targets a small lipophilic region between $I296^{6x51}$, $H278^{6x52}$ and $V281^{6x55}$ (Supplementary Table 1, Figures 2-3). The $MePhe^4$ residue of DAMGO targets a second lipophilic hotspot between $W133^{23x50}$, $P143^{3.28}$ and $I144^{3x29}$. The Tyr^1 and Phe^4 residues of the dual DOR/MOR pentapeptide agonist Met-enkephalin (Tyr^1-Gly^2-Gly^3-Phe^4-Met^5, Ph2) Table 3, Figure 2) are expected to adopt a similar DOR/MOR binding mode as the Tyr^1 and $MePhe^4$ residues of DAMGO.

The CXCR4 chemokine receptor structure (Supplementary Table 1, Figure 4)[130] shows how positively charged arginine residues of the cyclic 16-mer peptide antagonist CVX15 form ionic/H-bond interaction networks with CXCR4 (Arg^1-$D187^{45.51}$, Arg^2-$T117^{3.33}$/$D171^{4.61x60}$, Arg^{11}-$D272^{6x58}$/$D277^{7.31x30}$), while the naphthalene moiety of Aln^3 binds a lipophilic pocket formed by $Y190^{45x54}$/$F199^{5.39x38}$/$H203^{5.42x43}$/$I259^{6x55}$. The beta-strand conformation of CVX15 is further stabilised by a disulfide bridges between the Cys^4 and Cys^{13}. The homologous 15-mer BL-8040 (Phase 3, Table 3, Figure 2) is expected to adopt a similar binding mode in CXCR4, targeting an additional lipophilic pocket between $W94^{2x60}$, $V112^{3x28}$, $H113^{3x29}$ with its additional N-terminal 4-fluorinated benzyl residue, potentially forming additional intra/intermolecular ionic interactions with its C-terminal diaminopropoanoic acid residue. The binding mode of CVX15/BL-8084 to CXCR4 is distinct from the binding mode of chemokine ligands which bind the receptor N-terminus with their conserved disulfide stabilised C-terminal region and target different lipophilic regions in the TM pocket[2] as observed in CCL5 bound CCR5[129] (Figure 3), vMIP-II bound CXCR4, and CX3CL1 bound US28[132, 3] 8 new crystal structures.

The PMX53 bound $C5a_1$ crystal structure[124] shows how the C-terminal Arg^6 residue of the discontinued (Phase 1A/2B) hexamer peptide antagonist PMX53 forms polar interactions with Y^{6x51} and $D^{7.35x34}$, while the apolar Phe^1 and $dCha^4$/Trp_5 residues target lipophilic regions between $R264^{EL2}$/$C274^{45x50}$/$V276^{45x52}$ and between $L278^{2x60}$/$I182^{2x64}$/$P199^{3x29}$/$C274^{45x50}$/$C369^{7.36x35}$, respectively (Figure 2, Figure 3).

The peptidomimetic antagonist UR-MK299 forms H-bond interactions with $Q219^{5x461}$, $N283^{6x55}$ and $D286^{6.58}$ and targets lipophilic regions between in the neuropeptide $NPYY_1$ crystal structure[141](Figure 3), providing a structural template (Supplementary Table 1) for modelling the binding mode of the N-terminal Arg-Try residues of Obinepitide and TM30339 (Table 3, discontinued Phase 2) in homologous $NPYY_2$/$NPYY_4$.

Class B GPCRs comprise an ECD of 120–160 residues and a 7TM of 310–420 residues. In addition to the 7TM and full length (7TM and ECD) structures of class B GPCRs

described below several structures of isolated class B GPCR ECDs have been solved[149](Supplementary Table 1), including calcitonin analogue bound CT receptor[144], GIP bound GIP receptor[4] and GLP-1, Ex[9-39] and semaglutide bound GLP-1 receptor[25,5,6]. These ECD-peptide complexes show conserved hydrophobic interactions between conserved apolar residues in the C-terminal part of the ligand (such as GLP-1: Phe^{28}, Ile^{29}, Leu^{32}) and similar hydrophobic interaction sites with the ECD of the corresponding receptor (for example, GLP-1 receptor: L32, T35, V36, and $W39^{ECD}$ in α_1; $Y69^{ECD}$ in β turn 1 connecting β1–β2; Y88, L89, and P90 in β turn 2 connecting β3–β4)[149].

The full-length calcitonin-like receptor (CLR) cryoEM structure shows how the C-terminal region of the agonist CGRP binds the ECD, while the N-terminal helical region binds the 7TM domain of CLR[133]. The N-terminal Phe^{36} targets a hydrophobic pocket between $G71^{ECD}$/$W72^{ECD}$ in CLR and W84/P85 of RAMP1. The CLR/CGRP/RAMP1 complex is further stabilised by amongst others polar interaction networks between N-terminal amide group of CGRP and $T122^{ECD}$ and between Lys^{35} (CGRP), $R119^{ECD}$ (CLR), and W84 (RAMP2), consistent with the function of RAMP1 as allosteric modulator of CLR. Val^{8} ($F349^{6x53}$/$M369^{7x41}$/$H370^{7x42}$/$M373^{7x45}$) and Leu^{12}/Leu^{16} ($V135^{1X35}$/$A138^{1x36}$/ $L139^{1x37}$/$L141^{1x39}$/$F142^{1x40}$/$L195^{2x68}$/$a199^{2x72}$/$H370^{7x42}$) in the C-terminal helix of CGRP target lipophilic regions in CLR, consistent with SAR and mutation studies showing the role of several of these residue in CGRP-peptide binding[133] (Figure 3). The CGRP bound CLR structure provides a structural basis for modelling the binding mode of several homologous clinically relevant peptides to CLR and/or CR receptors and RAMP bound $AMY_{1/2/3}$ complexes, including adrenomedullin, calcitonin, Davalintide, Elcatonin, and Pramlintide (Tables 1, 2, Supplementary Table 1 and Figure 2).

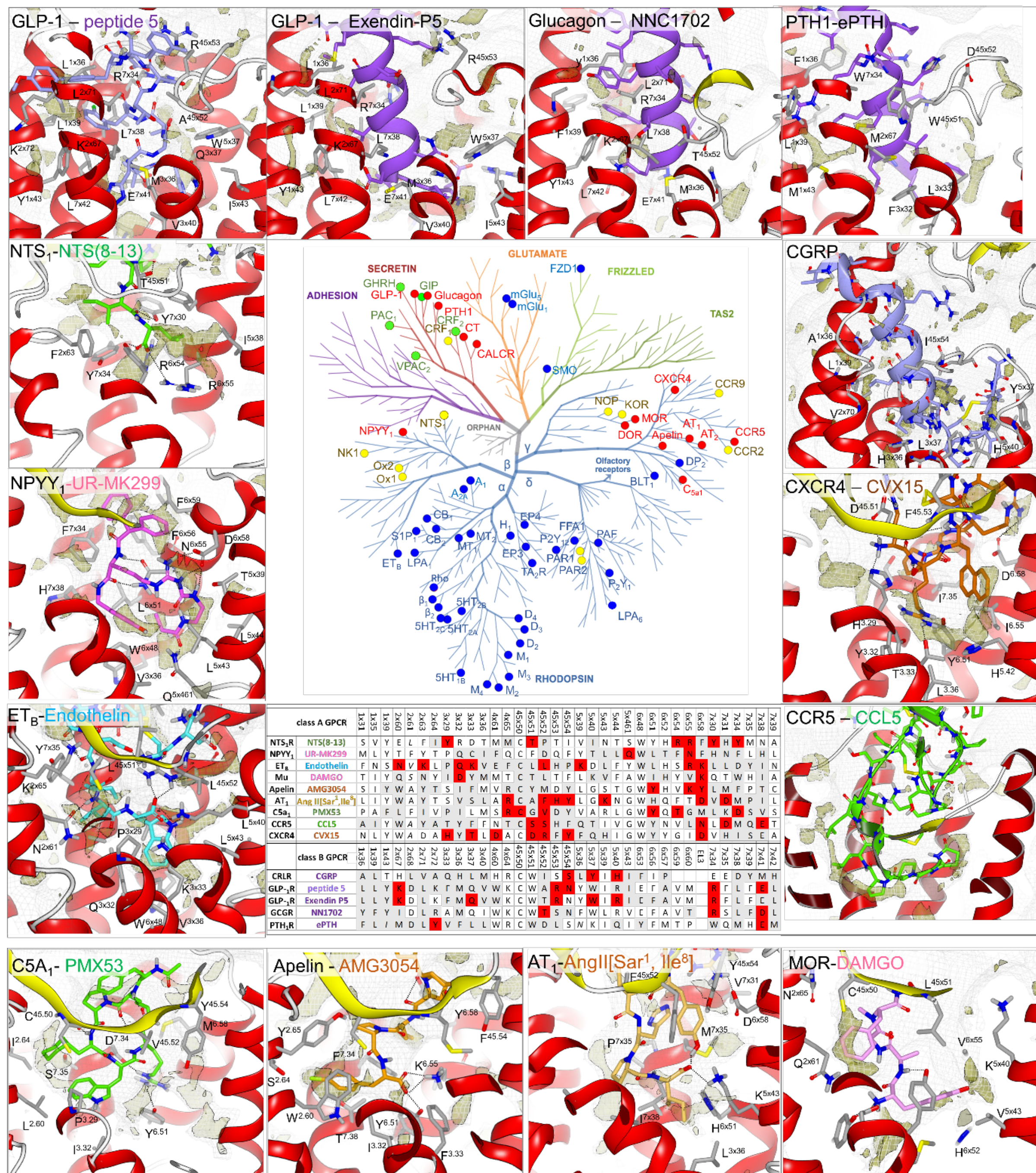
The agonist 31-mer GLP-1, biased agonist 36-mer Ex-P5, and truncated agonist 15-mer peptide 5 adopt a similar overall binding conformation and orientation within the 7TM domain of full-length GLP-1 receptor X-ray crystallography and cryoEM structures, but show different structural interaction patterns[133,136,137,149](Figure 2 and 4, Supplementary Figure 1). Val3 and Val7 of Ex-P5 and the fluorinated Phe12 homolog X_1 and the gem-dimethyl of peptide 5 target the same lipophilic pocket defined ($L141^{1x36}$, $L144^{1x39}$, $Y145^{1x40}$, $Y148^{1x43}$, $L384^{7x38}$, $E387^{7x41}$, $L388^{7x42}$). In the lower resolution GLP-1 bound cryoEM structure the homologous Phe12 and Val16 are located at a similar position, but TM1 seems to be rotated, with for example, $L144^{1x39}$ and $Y148^{1x43}$ towards the membrane. The substituted biphenyl residue X_2 of peptide 5 extends between TM1 ($L142^{1x37}$/$Y145^{1x40}$) and TM2 ($L201^{2x72}$/$K202^{2x73}$), whereas the L-Norvalin derivative X_3 of peptide 5 further extends between the ECD (L32), TM2 ($M204^{2x75}$), ECL2 ($C296^{45x50}$), targeting additional lipophilic regions to compensate for the limited interaction with the ECD and explaining the similar

potency of peptide 5 compared to GLP-1. Ex-P5 and peptide 5 share polar interactions with R299^{45x53} and R380$^{7.35b}$, and form ligand specific polar interactions with Y152^{1x47}/R190^{2x60} (peptide 5/c-tetrazole-Ala), R310$^{5.44}$ (Ex-P5/Glu^{1}), and E387$^{7.42b}$ (peptide 5/Cap1) that are not observed in the GLP-1-bound GLP-1 receptor structure. The binding modes of Ex-P5 and peptide 5 are consistent with GLP-1 receptor peptide ligand SAR and mutation studies and provide a template for the elucidation of determinants of biased signalling (Box 3) and for modelling structural interactions of the clinically relevant peptides glucagon, GLP-1, Albiglutide, Dulaglutide, Exendin-4, Liraglutide. Lixisenatid, Semaglutide, GTP-010, Taspoglutide, TT-223 with the GLP-1 receptor (Tables 1, 2, 4 Supplementary Table 1, Figure 2). The GLP-1 receptor-peptide ligand complexes furthermore can serve as basis for the construction of GLP-2 (Apraglutide, Elsiglutide, Glepaglutide,Teduglutide) and GIP (MAR701, MAR709) in complex with homologous peptide ligands, guided by SAR and mutagenesis studies[149] (Tables 1, 2, 4; Supplementary Table 1; Figure 2).

The Ser2/Phe6 and Val10 residues of the partial agonist NNC1702 target the same lipophilic pockets defined by TM1 and TM7 (Y138^{1x36}, F141^{1x39}, Y148^{1x43}, L382^{7x38}, D385^{7x41}, L386^{7x42}) and TM1/TM2 (Y138^{1x36}, L198^{2x72}) in full-length glucagon receptor[135] as the Ala8/Phe12 and Val16 homologs of GLP-1/Ex-P5/peptide 5 in the GLP-1 receptor structures described above. This glucagon receptor-peptide ligand binding mode is consistent with glucagon receptor ligand SAR and receptor mutation studies[149].

Also in the full-length PTH1 crystal structure[139] and cryoEM structures[140], ePTH (Aib3, Leu7) and PTH analogue (Ala3, Leu7), target the lipophilic pockets between TM1/2 (F184^{1x36}, L187^{1x39}, C191^{1x43}, M441^{7x38}, E444^{7x41}, M445^{7x42}), in line with PTH1 SAR and mutation studies[149]. The peptide bound PTH1 structures provide a template to model the interactions of PTH1/PTH2 receptors with PTH, PTHrP, Abaloparatide, Teriparatide, Ostabolin-C as well as the construction of homology models of GHRH with AKL-0707, DAC:GRs, Sermorelin, Tesamorelin (Tables 1, 2 and 4; Supplementary Table 1; Figure 2 and 4; Supplementary Figure 1)

Supplementary Figure 1



Supplementary Figure 1 **│ X-ray crystallography and cryo-electron microscopy structures of GPCRs.** In the central GPCR phylogenetic tree peptide GPCRs for which 7TM/full-length structures are available in complex with peptide ligands (red) or only in complex with non-peptide ligands (yellow) are distinguished from peptide GPCRs for which only ECD structures are available (green) and non-peptide GPCRs for which 7TM/full-length structures are available (blue), consistent with Table 5 (Supplementary). Related GPCR families, Adhesion (purple), Glutamate (orange), Frizzled (light green) and Taste (dark

green) are shown for reference. Receptors are classified as orphans when the endogenous ligand(s) is not yet established. The bottom panel summarises structural interactions for aligned binding site residues of class A and class B peptide GPCRs forming polar H-bond or ionic interactions (red), or only lipophilic interactions (grey) with peptide ligands shown in the individual binding mode figure panels.

Binding sites of AMG3054 bound Apelin (PDB ID: 5VBL)[121,], octapeptide Ang II [Sar[1], Ile[8]] partial agonist bound angiotensin AT_1 (PDB: 6DO1)[122] Complement $C5a_1$ (PDB: 6C1Q)[124], Endothelin ET_B (PDB: 5GLH)[125], neurotensin NTS_1R (PDB: 3ZEV) [126], UR-MK299 bound neuropeptide $NPYY_1$ (PDB: 5ZBQ)[141], MOR (PDB: 6DDE)[128], chemokine CCR5 (PDB: 5UIW)[129] receptors, and class B GPCRs calcitonin Calcitonin gene-related peptide bound CGRP (PDB: 6E3Y)[134], NNC-1702 bound glucagon receptor (PDB: 5YQZ)[135], peptide 5 (PDB: 5NX2)[136] and Exendin-P5 (PDB: 6B3J)[135] bound GLP-1 receptor, and ePTH bound parathyroid PTH1 [139] receptors. Views are focused on the 7TM domain and consistent with the orientations of peptide diagrams shown in Figure 2. The binding pocket surfaces (grey mesh) are contoured at 1 kcal/mol using the C3 (carbon sp3) GRID probe[203], whereas lipophilic areas are defined using the C1= (lipophilic) probe contoured at -2.8 to -3.0 kcal/mol, customized to GPCR binding sites[204]. Generic GPCR residue numbers[205] are provided based on Ballesteros-Weinstein Class A GPCR (Apelin, AT_1, $C5a_1$, CCR5, ET_B, MOR, $NPYY_1$)[206] and Wootten[207] Class B GPCR (CGRP, Glucagon, GLP-1, $PTHR_1$) numbering schemes. According to these schemes, the first (1-7) denotes the transmembrane helix (TM), and the following number indicates the residue position relative to the most conserved amino-acid in the helix (which is assigned the number 50), considering numbering offset due to helical bulges or constrictions[205].

Supplementary Figure 2

Supplementary Figure 2 | **2D and 3D diagrams of clinically relevant GPCR peptides (Table 1) and synthetic GPCR peptide drugs (Table 2).** Peptides are shown as beads based on the three-dimensional position of Cα atoms of structural templates of peptides bound to experimentally determined 7TM structures of associated GPCRs (Supplementary Table 5). The orientations of the 3D peptide bead strings are consistent with their binding mode in the GPCR binding site (Figure 3), assuming a view in which TM1 is on the left, TM5 is on the right, and the extra-cellular side is up. Peptides for which no 7TM GPCR structure bound 3D template is available, or depicted as 2D diagrams reflecting the putative structure based on (secondary) structure information of the free peptide, disulphide bridge constraints and binding mode information (showing an orientation consistent with the 3D diagrams).

Supplementary Table 1| **Experimentally determined structures of peptide binding GPCRs**

Family	Target Receptor	Ligand	Ligand mode of action	Similar peptide drug in Development	PDB
Class A					
Serotonin	$5HT_{2A}$	Risperidone	ANT	None	6A93
		Zotepine	ANT		6A94
Angiotensin	AT_1	Olmersartan	iAGO	None	4ZUD[195]
		ZD7155	ANT		4YAY[196]
		Ang II [Sar^1,^{8}Ile]	AGO		6DO1[122]
Angiotensin	AT_2	8EM	ANT	None	5UNH[121]
		8ES	ANT		5UNF[137]; 5UNG
		Ang II [Sar^1,^{8}Ile]*	AGO	Angiotensin II Aclerastide (AT_1), TRV120027 (AT_1)	5XJM[199]
Apelin	Apelin	AMG305	AGO	Apelin	5VBL[121]
Complement Peptide	$C5a_1$	NDT 9513727; PMX53	ANT alloANT		6C1Q[124]
		Avacopan; PMX53	ANT alloANT		6C1R[124]
		NDT 9513727	alloANT		5O9H
Endothelin	ET_B	Endothelin-1*	AGO	Endothelin 1, SPI-1620	5GLH; 5GLI[125]
		Bosentan	ANT	None	5XPR
		K-8794	ANT		5X93
Neuropeptide Y	Y_1	BMS-193885	ANT	None	5ZBH
		UR-MK299	ANT		5ZBQ
Neurotensin	NTS_1	$NT_{(8-13)}$*	AGO	Neurotensin	4XEE; 4XES; 3ZEV[126]; 4BUO; 4BV0; 4BWB; 4GRV; 5T04
opioid	δ	DIPP-NH_2*	ANT	None	4RWA; 4RWD[127]
		Naltrindole	ANT		4N6H; 4EJ4
	κ	MP1104	AGO	None	6B73[142]
		JDTic	ANT		4DJH
	μ	BU72	AGO	None	5C1M
		DAMGO*	AGO		6DDE; 6DDF [128]
		Morphinan	ANT		4DKL
	NOP	C-24	ANT	None	4EA3[143]
		C-35	ANT		5DHG
		SB-612111	ANT		5DHH

Orexin	OX_1	Suvorexant	ANT	None	4ZJ8
		SB-674042	iAGO		4ZJC
	OX_2	EMPA	ANT		5WQC; 5WS3
		Suvorexant	ANT		4S0V
Proteinase-Activated	PAR1	Vorapaxar	ANT	None	3VW7
	PAR2	AZ3451	ANT	None	5NDZ
		AZ8838	ANT		5NDD
		Fab*	ANT		5NJ6
Tachykinin	NK_1	Aprepitant	ANT	None	6HLO
		CP-99,994	ANT		6HLL
		L760735	ANT		6E59
		Netupitant	ANT		6HLP
Chemokine	CCR2	BMS-681; CCR2-RA-[R]	ANT; NAM	None	5T1A
		MK-0812	ANT		6GPS, 6GPX
	CCR5	Maraviroc	ANT	None	4MBS
		5P7-CCL5*	AGO	CCL3, CCL5	5UIW[129]
	CCR9	Vercirnon	ANT	None	5LWE
	CXCR4	IT1t	ANT	None	3ODU; 3OE6; 3OE8; 3OE9[130]
		CVX15*	ANT	BL-8040	3OE0[130]
		vMIP-II*	ANT	CXCL12	4RWS [131]
	US28	CX3CL1*	AGO	None	4XT1[*]; 4XT3[132]
		CX3CL1.35*	AGO		5WB2[151]
		Nb7[$]	AGO		5WB1[151]
	Class B				
Calcitonin	AM_1	Adrenomedullin*	AGO	Adrenomedullin	3AQF (ECD), 4RWF (ECD)
		CGRP analog*	AGO	CGRP	4RWG (ECD)
	CT	Calcitonin*	AGO	Calcitonin, Elcatonin	5UZ7
					5II0 (ECD)[144] 5UZ7[a]; 6NIY
	CGRP	CGRP*	AGO	CGRP	6E3Y[133]
		Apo		None	3N7P (ECD)
		Telcagepant; Olcegepant	ANT	None	3N7R (ECD)
		9968071l; Olcegepan	ANT		3N7S (ECD)
Corticotropin-releasing factor	CRF_1	CRF*	AGO	CRF, Corticorelin	3EHU[148], 2L27
		CP-376395	NAM	None	4K5Y[*145]; 4Z9G
	CRF_2	Astressin*	AGO	Astressin, Urocortin-1, Urocortin-2, RT-400, Stresscopin	3N93[146]
		Urocortin-1*	AGO		1U34[147]
		Urocortin-2*	AGO		3N96[146]

Glucagon	Glucagon	NNC1702*	AGO	Glucagon, GLP-1	5YQZ[135]
		ZP3780*	AGO		
		NNC0640 mAb1[$]	ANT		5XEZ; 5XF1[202]
		mAb1[$]	ANT		4ERS (ECD)
		NNC0640[a]	NAM		4L6R
		MK-0893	NAM		5EE7
	GLP-1	Exendin-P5*	AGO	Glucagon, GLP-1 Albiglutide, Dulaglutide, Exendin-4, Liraglutide. Lixisenatid, Semaglutide GTP-010, Taspoglutide, TT-223	6B3J[137]
		GLP-1[a]*	AGO		5VAI[133]
		Peptide 5*	ANT		5NX2[136]
		GLP-1*	AGO		3IOL (ECD)[154]
		Semaglutide*	AGO		4ZGM (ECD)[25]
		$Ex_{[9-39]}$*	ANT		3C59 (ECD)[153]
		Exendin-4 analog*	AGO		5OTT (ECD)
		Exendin-4 analog*	AGO		5OTU (ECD)
		Exendin-4 analog*	AGO		5OTV (ECD)
		Exendin-4 analog*	AGO		5OTW (ECD)
		Fab 3F52[$]	ANT	None	5E94 (ECD)
		NNC0640	NAM	None	5VEX
		PF-06372222	NAM		5VEW
	GIP	GIP*	AGO	GIP, MAR701, MAR709	2QKH (ECD)[152]
	GIP	Gipg013 Fab[$]	ANT	None	4HJ0 (ECD)
	GHRH	Apo		None	2XDG (ECD)
Parathyroid Hormone	PTH1	ePTH*	AGO	PTH, PTHrP Abaloparatide, Teriparatide, Ostabolin-C	6FJ3[139]
		PTH analog*	AGO		6NBF; 6NBH; 6NBI[140]
		PTH*	AGO		3C4M (ECD)
		PTHrP*	AGO		3H3G (ECD)
VIP and PACAP	PAC_1	$PACAP_{6-38}$*	ANT	None	2JOD, 1GEA (ECD)
		Apo			3N94 (ECD)
	$VPAC_2$	Apo			2X57 (ECD)

[a)] Ligand included in crystallisation studies, but ligand coordinates not determined or low resolution (<4Å). [b)] The following ligand types are defined: Full agonist (AGO), partial agonist (pAGO) inverse agonist (iAGO), antagonist (ANT) and negative allosteric modulators (NAM). [c)] Peptide ligands are indicated with "P", antibodies with ('Ab'), other ligands are small molecules. [d)] Endogeneous peptides and endogeneous/synthetic peptide drugs/in development indicated in Tables 1-3 that share sequence/structural similarity to the peptide ligand in the experimentally determined (X-ray crystallography, cryoEM, NMR) structure. [e)] Assessment of structural receptor-peptide ligand models including endogeneous and/or clinically relevant peptide ligands (Table 1-3) that can be constructed by combining the specified structural templates (with indicated additional templates indicated between brackets). [f)] Structures of truncated Extracellular Domain are indicated with 'ECD' between brackets, other structures contain transmembrane domain. Protein Data Bank[200, 201] (PDB) ID codes of experimentally determined structures (crystallography, cryo-EM) of complexes of peptide ligands bound to the full-length transmembrane domain (TM-peptide) or extracellular domain (ECD-peptide) of the receptor, as well as non-peptide ligands bound to the receptor transmembrane domain (TM). Structures of free peptide ligands (NMR, crystallography) are only reported for receptors for which no peptide ligand structures are available.

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Supplementary Table 2| **Endogenous peptides targeting Class A and B GPCRs that are approved for clinical use**

Family	Target Receptor	Ligand/ Approval Date	Clinical Indication	Affinity pK$_i$ *pK$_d$ **pIC$_{50}$	Potency pEC$_{50}$	Plasma half-life iv (min)	Volume of distribution (L)	Plasma binding (%)	Synonym Ligand peptide
Class A									
Angiotensin	AT$_1$ AT$_2$	Angiotensin II 2017	Septic shock	8.8 *10.2	9.0–9.3 -	< 1	-	-	Giapreza LJPC501
Melanocortin	MC$_2$	Cosyntropin 1967	Diagnosis, adrenal insufficiency	-	-	15	-	-	Tetracosactide
Thyrotropin-Releasing Hormone	TRH$_1$ TRH$_2$	TRH 1976	Testing Response of Anterior Pituitary, GI and in Secondary Hypothyroidism, Acromegaly.	†7.4 †7.4	8.5 -	5.3	15.7	-	Thyroliberin Protirelin
Vasopressin and Oxytocin	OT V$_{1A}$ V$_{1B}$ V$_2$	Oxytocin 1980	Induction of Labour	8.2–9.6 6.9–8.3 5.7–7.0 5.4–6.8	7.8-10.4 8 6.6-7.6 8.1	1-6	12.2	Negligible	Otx Pitocin Syntocinon
	OT V$_{1A}$ V$_{1B}$ V$_2$	Vasopressin 2014		7.3–9.3 8.5-9.3 9.9 7.9-9.1	- 9-9.6 8.3-8.7 10.3	10-20	9.8	Negligible	ADH Antidiuretic Hormone Arginine Vasopressin Argipressin
Class B									
Calcitonin	AMY$_1$ AMY$_2$ AMY$_3$ CT	Calcitonin 1981	Paget's Disease	- - - 9	8.9–11.3 11.4 8.0–10.6 9.0–11.2	10-38	4.9	-	Thyrocalcitonin LS-173874
Glucagon	GLP-1 Glucagon	Glucagon 1998	Severe Hypoglycaemia	6.9-7.0 -	- 9.0	8-18	17.5	-	
	Secretin	Secretin 2004	Diagnostic	-	9.7	45	2.7	-	
Parathyroid Hormone	PTH1 PTH2	PTH 2006	Hypocalcaemia, Parathyroid Deficiency	-	-	90	5.4	Negligible	Natpara, ALX1-11, rhPTH(1-84), **Note 1**
	PTH1 PTH2	Teriparatide 2002	Post-Menopausal Osteoporosis	**7.4 **7.7-7.8	- -	5	8.4	-	**Note 1**

Identification of endogenous peptides approved for clinical use was identified from the GuidetoPharmacology database. This has the most extensive classification with other groups being antibodies, natural products, metabolites or synthetic organics. The list was compared with DrugBank with further information from RxList Global Data or relevant company websites.

Pharmacokinetic and pharmacodynamics parameters were curated from GuidetoPharmacology, DrugBank as well as original citations. The peptides identified in use in any geographical region, will largely reflect those used widely in major pharmaceutical markets such as North America, Asia and Europe but may not capture peptides licensed in just one country.

Class, Target receptor and Ligand. Unlike small molecules that may target multiple receptors**,** the table reflects the consensus that peptides act primarily on a single receptor or family of receptors (such as the somatostatin family).

Affinity and potency: Where a range of affinities and potencies were reported, these are shown. Where peptides have affinities for more than one subtype within the family, all values are shown where available. For example, the relative importance of the affinities for the five different subtypes of somatostatin receptors in the therapeutic action is unclear and all values have been included. Data are derived from human receptors except where indicated from rat[†].

pK_i : negative logarithm to base 10 of the concentration of the competing ligand that would occupy 50% of receptors, determined in a competition binding assay.

pK_D : negative logarithm to base 10 of the equilibrium dissociation constant for ligand receptor interactions.

pIC_{50} : negative logarithm to base 10 of the concentration of competing agonist or antagonist that inhibits the binding of a radioligand by 50% in a competition binding assay.

pEC_{50} : the negative logarithm to base 10 of the molar concentration of an agonist that produces 50% of the maximal possible effect of that agonist.

Plasma half-life (min) and route indicates calculations based on intravenous administration. Clinical administration may also be via other routes such as intra-muscular or intra-nasal that may alter these values.

Volumes of distribution (L) have been recalculated where these were reported as litres/kg based on 70 kg body weight.

Synonyms are given for clinical names of the peptide based on the information in databases indicated.

Note 1. Human parathyroid hormone (1-84), manufactured as a recombinant form with the full 1-84 amino acids was approved for clinical use as Natpara, (ALX1-11, rhPTH1-84) but it is included as shorter sequences are also effective in activating target receptors. Teriparatide is a synthetic peptide comprising 1-34 of the N-terminal amino acids of human parathyroid hormone.

Supplementary Table 3 | **Modified peptides targeting Class A and B GPCRs that are approved for clinical use**											
Family	**Target Receptor**	**Ligand Mode of Action Approval Date**	**Clinical Indication**	**Affinity pK_i *pK_D **pIC_{50}**	**Potency pEC_{50}**	**Plasma Half-life (hr), iv *sc**	**Volume of Distribution (L) iv *sc**	**Plasma Binding (%)**	**Clearance (L/hr)**	**Route of Elimination**	**Synonyms**
Class A											
Bradykinin	B_2	Icatibant Antagonist 2008	Hereditary Angioedema	10.2	8.0–9.4	1-2 *1.4	29	44	14.7±4.0	Proteolytic Enzymes	D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]BK; HOE140;Firazyr
Cholecystokinin	CCK_2	Pentagastrin Agonist 1974	Diagnostic Aid	9.05	-	0.16 *<0.6	-	-	-	-	ICI-50123; AY-6608;Peptavlon
Gonadotrophin-Releasing Hormone	$GnRH_1$	Buserelin Agonist 1999	Endometriosis, Pituitary Desensitisation Prior to Ovulation Induction, Advanced Prostate Cancer	9.4-10.0 *9.5-10.4	10.5	0.08-1.3 *1.33	-	15	-	Proteolytic Enzymes	HOE 766; HOE 766A; ICI 123215 Suprefact; Receptal; Etilamide; Metrelef
		Cetrorelix Antagonist 1999	Controlled Ovarian Stimulation	9.3-10	8.7	*5-62.8	81	86	5.4	Renal	SB-075; Cetrotide
		Ganirelix Antagonist 2003	Controlled Ovarian Stimulation	-	-	14.5 *13-16	43.7	82	2.4	Renal, Biliary	Antagon; Orgalutran; Fyremadel; RS 26303
		Degarelix Depot Antagonist 2008	Advanced Prostate Cancer	8.8	-	996-1690 *696	40.9	90	-	Faecal (70-80%), Renal (20-30%)	FE200486; Firmagon
		Gonadorelin Agonist 1986	Amenorrhea, Hypogonadism.	-	-	0.16-0.67	-	-	-	Metabolism by Hydrolysis	Abbott 41070; AY-24031; Hoe-471; RU-19847
		Goserelin Agonist 1989	Breast Cancer, Prostate Cancer	8.8	-	4.9	44.1 *13.7	27	7.3±2.5	Renal, Biliary	Decapeptide I; ICI 118630; Zoladex
		Histrelin Agonist 2004	Advanced Prostate Cancer	8.7-9.7 *9.0-10.4	-	*4	58	30	10.4		Supprelin LA; Vantas; ORF 17070; RWJ 17070
		Leuprolide Agonist 1985	Advanced Prostate Cancer	8.5-9.1	-	3	27	43-49	8.3	Renal	Leuprorelin; Lupron; Viadu

											ABBOTT-43818; TAP-144; Eligard; Carcinil; Prostap Lutrate
		Nafarelin Agonist 2005		10	-	3	-	80	-		Synarel
		Triptorelin Agonist 1986	Prostate Cancer, Endometriosis, Precocious Puberty	8.5-8.8	-	3 phases 0.1, 0.75, 3	30-33	0	12.7	Renal, Biliary	Triptodur; Trelstar; Pamorelin; CL-118532; Decapeptyl; Diphereline; Gonapeptyl; Variopeptyl
		Abarelix Antagonist 2003 **Note 1**	Prostate cancer	9.1-9.5	-	317±77	-	96-99	-	-	PPI 149; R 3827; Plenaxis
Melanocortin	MC_1 MC_3 MC_4 MC_5	Afamelanotide Agonist 2014	Erythropoietic Protoporphyria	**10.0 8.9 8.5–8.8 **9.0	-	0.8-1.7	-	-	-	-	Scenesse
	MC_1 MC_3 MC_4 MC_5	Bremelanotide Agonist 2018	Sexual Arousal Disorder	- - **8.0 -	-	2	-	-	-	-	PT-141; Rekynda
Opioid	κ	Difelikefalin Agonist 2019	Post Abdominal Surgery Pain	-	9.8	2	-	-	-	Unchanged in Bile and Urine	CR-845
Relaxin	RXFP1	Serelaxin Agonist 2016	Heart failure	**10	9.2	8-9	0.3-1.0	-	0.12-0.14	Proteolytic Enzymes	Reasanz; RLX-030 Recombinant Human Relaxin 2
Somatostatin	SST_1 SST_2 SST_3 SST_5	Pasireotide Agonist 2012	Agromegaly, Cushing's Disease	**8.0 **9.0 **8.8 **9.8	-	12	>100.	88	-	Mainly Hepatic, Renal	SOM 230; Signifor
	SST_1 SST_2 SST_3 SST_5	Lanreotide (DEPOT) Agonist 1994	Agromegaly, Gastroentero-Pancreatic Tumours	**6.7 8.7-9.6 7.2-8.0 7.4-9.3	-	1.14 *528	16	-	23.7	Billiary, Renal (<5%)	DC 13-116; Somatuline; Ipstyl; BIM 23014; Lanreotide acetate

	SST_2 SST_3 SST_5	Octreotide Agonist 1987	Agromegaly, Gastroentero-Pancreatic Tumours	8.7-9.9 7.4-8.6 7.2-9.5	-	1.67 *4.2	13.6	65	7.6	Mainly Hepatic, Renal	DRG-0115; Longastatin; SMS 201 995; Sandostatin; Atrige; Sandostatin LAR, Lutathera **Note 2**
	SST_2 SST_3 SST_5	Vapreotide Agonist 2009	Oesophageal Variceal Bleeding	8.3-10.1 7.4-7.9 7.3-9.2	-	0.5	-	-	-	Mainly Hepatic, Renal	BMY 41606; CCRIS 6495; RC-160; Sanvar IR; Vapreotide acetate
Vasopressin and Oxytocin	OT	Atosiban Antagonist 2000	Delaying Imminent Pre-Term Birth	6.0-7.6	-	1.7	18 l	46-48	41.8	Proteolytic Enzymes	ORF22164; RWJ 22164 d[D-Tyr(Et)2, Thr^4,Orn^8] vasotocin d [D-Tyr(Et)^2 Thr^4]OVT; Tractocile; Antocin
	OT V_{1A} V_{1B} V_2	Desmopressin Agonist 1999	Vasopressin-Sensitive Cranial Diabetes Insipidus, Post Hypophysectomy Polyuria/Polydipsia.	6.7-7.6 7.0-7.7 7.7-8.2 7.2-8.6	-	0.9-2.6	33	17-50	-	Renal	1-deamino,D-AVP [deamino-Cys1,D-Arg^8]vasopressin dDAVP; Adiuretin; Concentraid; Minirin; Stimate; Noctiva
	V_{1A}	Felypressin Agonist 1969	Haemostatic Agent	-	-	-	-	-	-	-	PLV-2
	V_{1A} V_{1B} V_2	Terlipressin Agonist 2006	Hypotension, Bleeding Oesophageal Varices	-	-	0.4-1.1	35	30	37.8	-	Glypressin; Lucassin
	OT	Carbetocin Agonist 2016	Postpartum Haemorrhage	-	-	1.4-1.7	-	-	-	Partly Proteolytic Enzymes, Negilible Renal	Duratocin Pabal Lonacten; Depotocin; Comoton Decomoton
Class B											
Calcitonin	AMY_1	Calcitonin (Salmon) Agonist 1976	Paget's Disease	**8.7-9.7	10.0	*1-1.1	16	30-40	-	Renal	Fortical; Miacalcin
	CT	Elcatonin Agonist 1981	Pain Caused by Osteoporosis	-	-	-	-	-	-	-	

	AMY_1 AMY_2 AMY_3 CT	Pramlintide Agonist 2005	T1DM, T2DM	-	9.4 8.6-8.9 9.1-9.3 8.3	0.4-0.7 *0.8	56	60	60	Renal	AC-0137; AC-137; Symlin; Symlinpen
Glucagon	GHRH	Sermorelin Agonist 1990 **Note 3**	Dwarfism, HIV-Associated Weight Loss	8.2	-	0.17 *0.2	24-26	-	144-168	-	Sermorelin Acetate; Geref
		Tesamorelin Agonist 2010	HIV-Infected Patients with Lipodystrophy.	10.2	-	*0.43	*658	-	-	-	TH9507; (3E)-hex-3- enoylsomatoliberin; Egrifta
	GLP-1	Albiglutide Protein Fused Agonist 2014	HIV-Infected Patients with Lipodystrophy.	-	†7.7	*96-168	*11	-	0.07	Human Serum Albumin, Catabolized Primarily in Vascular Endothelium.	GSK-716155; Eperzan; Tanzeum
		Dulaglutide Agonist 2014 **Note 4**	T2DM	-	-	*120	*17-19	-	0.11	-	GLP-1Fc; LY2189265; Trulicity
		Exenatide Agonist 2005	T2DM	8.7-9.0 **9.2	-	*2.4	*28.3	-	9.1	Renal	Exendin-4, AC002993; AC 2993; AC2993A; Byetta; Bydureon;
		Liraglutide Agonist 2009	T2DM	8.3-10	10.2	*13	4.9 *11-17	>98	1.2	Proteolytic Enzymes	NN-2211; Victoza; Saxenda
		Lixisenatide Agonist 2013	T2DM	8.9	-	*3	*100	55	35	Proteolytic Enzymes	Adlyxin; AVE-0010; Lyxumia
		Semaglutide Spacer Agonist 2017	T2DM	-	11.2	*168	*9.4	99	0.04	Renal	NN-9535; Ozempic
	GLP-2	Teduglutide Agonist 2012	Short Bowel Syndrome	**†11.3	10	2.0	7.2 *26	-	8.6	Renal	ALX-0600; Gattex; Revestive; (Gly2)GLP-2;
Parathyroid Hormone	PTH1	Abaloparatide Agonist 2017	Post-Menopausal Osteoporosis	**9.7	10.1	*1.7	50	70 (in vitro)	-	Renal	BA058; Tymlos

Class C											
Calcium-Sensing	CaS	Etelcalcetide Agonist 2016	Secondary Hyper-Parathyroidism	-	4.6	84 *72-96	796	47	7.7	Renal	Velcalcetide KAI-4169 AMG-416 Parsabiv

Class, Target Receptor and Ligand. Compilation of the data used the same databases and methods as outline in Table 1. The table reflects the consensus that synthetic peptides act primarily on a single receptor or family of receptors (such as the somatostatin family). Cyclosporin reported to be an antagonist of formylpeptide FPR1 receptor has been omitted as it is reported to interact with multiple targets and is not selective for a particular target.

Clinical indication. These represent the main clinical uses in DrugBank[210], RxList[211], Electronic Medicines Compendium[213] and Clinical Trials.Gov[214].

Affinity and potency: Where a range of affinities and potencies were reported, these are shown. For peptides binding to somatostatin receptors, the relative importance of the affinities for the different subtypes in the therapeutic action is unclear and all values have been included. Data are derived from human receptors except where indicated (†) where values at the rat receptor are reported.

Plasma half-life (hr): time taken for the concentration of a drug in plasma to decline to half its original level.

Route: indicates calculations based on intravenous and sub-cutaneous administration. Clinical administration for a small number of peptides may also be via other routes such as intra-muscular or intra-nasal and can alter these values. For example, calcitonin (salmon) plasma half-lives are: intra-muscular, 0.96 hr, intra-nasal 0.3-38 hr.

Volumes of distribution (L): Where values were reported as L/kg these have been recalculated based on 70 kg body weight.

Plasma binding (%): The percentage of peptide bound to blood proteins including human serum albumin, lipoprotein, glycoprotein, and α, β, and γ globulins.

Clearance (L/hr): Where values were reported as L/hr/kg, these have been recalculated based on 70 kg body weight**.**

Route of Elimination: The main routes of metabolism and/or elimination are reported**.**

Synonyms are given for clinical names of the synthetic peptide based on the information in databases indicated.

NA: Not available

Note 1. Abarelix was discontinued in United States in 2003, owing to allergic reactions but clinical use has continued in Europe.

Note 2. Lutathera is a radiolabelled peptide that exploits radiation to kill tumours.

Note 3. Withdrawn in United States.

Note 4. Dulaglutide contains GLP-1(3- 37) with substitutions Ala8Gly, Gly22Glu, Arg36Gly, 16 amino acids linker sequence and 228 amino acid synthetic human Fc fragment (immunoglobulin G4). Two identical peptide chains form a dimer, linked by inter-monomer disulphide bonds between Cys55-55 and Cys58-58.