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 50 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, to both introduce chemical novelty and improve drug-like properties. Pharmacodynamic improvements are evolving to allow biasing ligands to activate specific downstream signalling pathways, in order to optimize efficacy and reduce side effects. In pharmacokinetics, modifications that increase plasma half-life have been revolutionary. Here, we discuss the current status of the peptide drugs targeting GPCRs, with a focus on evolving strategies to improve pharmacokinetic and pharmacodynamic properties.

G protein-coupled receptors (GPCRs) mediate a wide range of signalling processes and are targeted by one third of the 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 exist, including class C and the frizzled and adhesion classes, therapeutics have predominantly targeted class A and B GPCRs, so this Review is focused 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), where 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 the ligand to which they bind: calcitonin, corticotropin-releasing factor, glucagon, parathyroid hormone (which is generally considered to be a peptide, despite its 84-amino-acid length), 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. In the cases of 54 of these orphans, at least one publication has proposed an endogenous ligand, and some of these ligands are peptides[4]. The 'de-orphanization' 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 that these GPCRs may be functionally coupled in the regulation of feeding, and if substantiated, the 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: their 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.

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Cryo-electron microscopy (cryo-EM). Electron microscopy technique that allows near-atomic resolution of biomolecules such as G protein-coupled receptors (GPCRs) in samples cooled to low temperatures and embedded in an environment of vitreous water, avoiding the crystallization that is a prerequisite for X-ray crystallography.

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 by a lack of oral bioavailability due to a combination of low gastrointestinal stability and poor permeability. Therefore, strategies need to be developed that can address these aspects before most peptides can become effective medicines.

Peptide drugs occupy a structural space between biologics (antibodies and proteins) and small molecules. Whereas endogenous signalling peptides usually have 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 preclinical novel approaches (for example, 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. Complementary pharmacodynamic strategies are extending the repertoire of drugs that act at a given GPCR.

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

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) 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 of the more subtle aspects of conformational biased signalling, thereby enabling rational agonist or antagonist design to activate or block, respectively, a pathophysiological process.

In this Review, we discuss the clinically approved and preclinical GPCR-targeting peptide therapeutics (FIG. 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, the major receptor targets include μ and κ opioid receptors (abbreviated MOR and KOR, respectively) to relieve pain; oxytocin and vasopressin receptors for the induction of labour; and apelin and angiotensin receptors in cardiovascular disease (TABLES 1,2). More specialized targets include the somatostatin receptor, for acromegaly and Cushing's disease. Peptide therapies that target class B receptors are dominated by GLP-1 receptor agonists for the treatment of type 2 diabetes mellitus (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 desensitize the receptor in order 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 synthesized variants of endogenous peptides for class A receptors to enter clinical use, and they did so in the 1950s. These two peptides are amongst the ten peptides that are usually synthesized chemically or by recombinant technology but that have sequences identical to those of their endogenous peptide equivalents, and 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 to investigate the impact of those properties on drug development strategies.

The peptides that target class A GPCRs are mainly agonists that bind with both high affinity (median negative log of the dissociation constant (pK_i) = 8.4) and potency (median negative 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 min, reflecting their rapid degradation by peptidases and/or their 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 min. Consistent with their polar, hydrophilic chemical properties, they have low median volumes of distribution (~9.8 l, 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 is available to directly bind to and

Fig. 1 | **Overview of GPCR-targeted peptide drugs.** The numbers of peptide drugs that are approved, in phase III or in phase II clinical trials are shown for G protein-coupled receptors (GPCRs) from class A (blue), class B (red) and class C (green).

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-life.

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 min) and low volume of distribution (~9.9 l). Calcitonin (isolated from salmon in the 1970s) was one of the first native peptides to be used clinically and was used to treat Paget 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 to increase bone mass[15].

Paradoxically, at least some of the peptides in both classes are likely successful because their short plasma half-lives, which would usually be an undesirable trait, are exploited for clinical benefit. At one extreme, the peptide therapeutic with the shortest half-life is angiotensin II, first synthesized 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 5 min to adequately increase blood pressure following intravenous dosing[16]. The short half-life — less than 1 min — ensures that hypertension resulting from an overdose is very unlikely. Similarly, oxytocin, which was sequenced and synthesized in the 1950s[13,14], is used to induce labour and strengthen contractions immediately after intravenous administration, but the effects subside within an hour, reflecting a short half-life of a few minutes.

Modified peptides

Twenty-six synthetic peptides (20 agonists and 6 competitive antagonists) targeting 8 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 in order to maintain efficacy. In this regard, peptides targeting the gonadotropin-releasing hormone (GnRH) receptor in the pituitary are particularly intriguing for non-steroidal manipulation of the reproductive endocrine axis. For example, the agonist buserelin acts by an unusual pharmacological mechanism, as it desensitizes the GnRH receptor, reducing the amount of gonadotropin released from the pituitary gland, thereby inhibiting testosterone secretion in males and oestrogen secretion in females. 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, unwanted side effects are 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[17] (FIG. 2). 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.

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 i.v. 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 (for example, 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 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	GLP-1	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
	Postmenopausal osteoporosis	Teriparatide; 2002	5	PTH1	7.4[c]	ND
				PTH2	7.7-7.8[c]	ND

Endogenous peptides approved for clinical use were identified from the Guide to Pharmacology 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 pharmacodynamic parameters were curated from Guide to Pharmacology[3], DrugBank and the original citations. The peptides identified are in use in any geographical region, but largely they 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 have been reported. For peptides with affinities for more than one subtype within the family, all values are shown if 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 intramuscular or intranasal, 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, but it is included, as shorter sequences also activate target receptors. Teriparatide is a synthetic peptide comprising 1–34 of the N-terminal amino acids of human parathyroid hormone. GPCR, G protein-coupled receptor; i.v., intravenous; ND, no data are available from online resources; pEC_{50}, the negative logarithm of the base-10 molar concentration of an agonist that produces 50% of the maximal possible effect of that agonist; pK_i, the negative logarithm of the base-10 concentration of the competing ligand that would occupy 50% of receptors as determined in a competition binding assay. [a]pK_D, the negative logarithm to of the base-10 equilibrium dissociation constant for ligand receptor interactions. [b]Derived from studies of the rat receptor. [c]pIC_{50}, the negative logarithm of the base-10 concentration of a competing agonist or antagonist that inhibits the binding of a radioligand by 50% in a competition binding assay.

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

Family	Clinical indication	Therapeutic; approval date	Plasma half-life (hours following i.v. 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	10.2	8.0–9.4
Cholecystokinin	Diagnostic aid	Pentagastrin (ICI-50123; AY-6608; Peptavlon); 1974	0.16, <0.6[b]	CCK_2	9.05	ND
Gonadotrophin-releasing hormone	Endometriosis, pituitary desensitization 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]	$GnRH_1$	9.4–10.0, 9.5–10.4[c]	10.5
	Controlled ovarian stimulation	Cetrorelix[a] (SB-075; Cetrotide); 1999	5–62.8[b]	$GnRH_1$	9.3–10	8.7
	Controlled ovarian stimulation	Ganirelix[a] (Antagon; Orgalutran; Fyremadel; RS 26303); 2003	14.5, 13–16[b]	$GnRH_1$	ND	ND
	Advanced prostate cancer	Degarelix depot[a] (FE200486; Firmagon); 2008	996–1690, 696[b]	$GnRH_1$	8.8	ND
	Amenorrhea, hypogonadism	Gonadorelin (Abbott 41070; AY-24031; Hoe-471; RU-19847); 1986	0.16–0.67	$GnRH_1$	ND	ND
	Breast cancer, prostate cancer	Goserelin (Decapeptide I; ICI 118630; Zoladex); 1989	4.9	$GnRH_1$	8.8	ND
	Advanced prostate cancer	Histrelin (Supprelin LA; Vantas; ORF 17070; RWJ 17070); 2004	4[b]	$GnRH_1$	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	$GnRH_1$	8.5–9.1	ND
	Endometriosis, precocious puberty	Nafarelin (Synarel), 2005	3	$GnRH_1$	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	$GnRH_1$	8.5–8.8	ND
	Prostate cancer	Abarelix[a,d] (PPI 149; R 3827; Plenaxis); 2003	317 ± 77	$GnRH_1$	9.1–9.5	ND
Melanocortin	Erythropoietic protoporphyria	Afamelanotide (Scenesse); 2014	0.8–1.7	MC_1	10.0[e]	ND
				MC_3	8.9	ND
				MC_4	8.5–8.8	ND
				MC_5	9.0[e]	ND
	Sexual arousal disorder	Bremelanotide (PT-141; Rekynda); 2018	2	MC_1	ND	ND
				MC_3	ND	ND
				MC_4	8.0[e]	ND
				MC_5	ND	ND
Opioid	Pain following abdominal surgery	Difelikefalin (CR-845); 2019	2	κ	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	8.0[e]	ND
				SST_2	9.0[e]	ND
				SST_3	8.8[e]	ND
				SST_5	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	6.7[e]	ND
				SST_2	8.7–9.6	ND
				SST_3	7.2–8.0	ND
				SST_5	7.4–9.3	ND

Table 2 (cont.) | **Modified peptides targeting class A and class B GPCRs that are approved for clinical use**

Family	Clinical indication	Therapeutic; approval date	Plasma half-life (hours following i.v. administration)	Target receptor	Affinity (pK_i)	Potency (pEC_{50})
Class A (cont.)						
	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	8.7–9.9	ND
				SST_3	7.4–8.6	ND
				SST_5	7.2–9.5	ND
	Oesophageal variceal bleeding	Vapreotide (BMY 41606; CCRIS 6495; RC-160; Sanvar IR; Vapreotide acetate); 2009	0.5	SST_2	8.3–10.1	ND
				SST_3	7.4–7.9	ND
				SST_5	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; Tractocile; Antocin); 2000	1.7	OT	6.0–7.6	ND
	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	6.7–7.6	ND
				V_{1A}	7.0–7.7	ND
				V_{1B}	7.7–8.2	ND
				V_2	7.2–8.6	ND
	Haemostatic agent	Felypressin (PLV-2); 1969	ND	V_{1A}	ND	ND
	Hypotension, bleeding oesophageal varices	Terlipressin (Glypressin; Lucassin); 2006	0.4–1.1	V_{1A}	ND	ND
				V_{1B}	ND	ND
				V_2	ND	ND
	Postpartum haemorrhage	Carbetocin (Duratocin; Pabal; Lonacten; Depotocin; Comoton; Decomoton); 2016	1.4–1.7	OT	ND	ND
Class B						
Calcitonin	Paget disease	Calcitonin (salmon) (Fortical; Miacalcin); 1976	1–1.1[b]	AMY_1	8.7-9.7[e]	10.0
	Pain caused by osteoporosis	Elcatonin; 1981	ND	CT	ND	ND
	T1D, T2D	Pramlintide (AC-0137; AC-137; Symlin; Symlinpen); 2005	0.4–0.7, 0.8[b]	AMY_1	ND	9.4
				AMY_2	ND	8.6–8.9
				AMY_3	ND	9.1–9.3
				CT	ND	8.3
Glucagon	Dwarfism, HIV-associated weight loss	Sermorelin[g] (Sermorelin Acetate; Geref); 1990	0.17, 0.2[b]	GHRH	8.2	ND
	HIV-infected patients with lipodystrophy	Tesamorelin (TH9507; (3E)-hex-3-enoylsomatoliberin; Egrifta); 2010	0.43[b]	GHRH	10.2	ND
	T2D	Albiglutide protein, fused (GSK-716155; Eperzan; Tanzeum); 2014	96–168[b]	GLP-1	ND	7.7[h]
	T2D	Dulaglutide[i] (GLP-1Fc; LY2189265; Trulicity); 2014	120[b]	GLP-1	ND	ND
	T2D	Exenatide (Exendin-4, AC002993; AC 2993; AC2993A; Byetta; Bydureon); 2005	2.4[b]	GLP-1	8.7–9.0 9.2[e]	ND
	T2D	Liraglutide (NN-2211; Victoza; Saxenda); 2009	13[b]	GLP-1	8.3–10	10.2
	T2D	Lixisenatide (Adlyxin; AVE-0010; Lyxumia); 2013	3[b]	GLP-1	8.9	ND
	T2D	Semaglutide (NN-9535; Ozempic); 2017	168[b]	GLP-1	ND	11.2
	Short bowel syndrome	Teduglutide (ALX-0600; Gattex; Revestive; (Gly2)GLP-2); 2012	2.0	GLP-2	11.3[e,h]	10
Parathyroid hormone	Postmenopausal osteoporosis	Abaloparatide (BA058; Tymlos); 2017	1.7[b]	PTH1	9.7[e]	10.1

Table 2 (cont.) | **Modified peptides targeting class A and class B GPCRs that are approved for clinical use**

Family	Clinical indication	Therapeutic; approval date	Plasma half-life (hours following i.v. administration)	Target receptor	Affinity (pK_i)	Potency (pEC_{50})
Class C						
Calcium-sensing	Secondary hyper-parathyroidism	Etelcalcetide (Velcalcetide; KAI-4169; AMG-416; Parsabiv); 2016	84, 72–96[b]	CaS	ND	4.6

All molecules are agonists unless otherwise indicated. Compilation of the data was based on the databases and methods outlined in TABLE 1. TABLE 2 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 the formylpeptide receptor, has been omitted, as it is reported to be nonselective. The main clinical uses in DrugBank, RxList, the Electronic Medicines Compendium and ClinicalTrials.gov are listed under clinical indication. A range of affinities and potencies are given if ranges have been 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 thus 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 intramuscular or intranasal, which may alter these values. For example, the calcitonin (salmon) plasma half-lives are: intramuscular, 0.96 h; intranasal, 0.3–38 h. Information on volume of distribution and percent plasma binding can be found in Supplementary Table 3. GPCR, G protein-coupled receptor; i.v., intravenous; ND, no data available from online resources; pEC_{50}, the negative logarithm of the base-10 molar concentration of an agonist that produces 50% of the maximal possible effect of that agonist; pK_i, the negative logarithm of the base-10 concentration of the competing ligand that would occupy 50% of receptors as determined in a competition binding assay; T1D, type 1 diabetes; T2D, type 2 diabetes. [a]Antagonist. [b]Half-life following subcutaneous injection. [c]pK_D, the negative logarithm of the base-10 equilibrium dissociation constant for ligand receptor interactions. [d]Abarelix was discontinued in the United States in 2003, owing to allergic reactions, but clinical use has continued in Europe. [e]pIC_{50}, the negative logarithm of the base-10 concentration of competing agonist or antagonist that inhibits the binding of a radioligand by 50% in a competition binding assay. [f]Octreotide is a radiolabelled peptide that exploits radiation to kill tumours. [g]Withdrawn in the United States. [h]Derived from studies of the rat receptor. [i]Dulaglutide contains GLP-1 (amino acids 7–37) with substitutions of 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 disulfide bonds between Cys55-55 and Cys58-58.

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 the 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 l) following subcutaneous injection. The median plasma half-life is around 1 h (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 the pharmacodynamic effects and the 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 the 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 metabolized by proteolytic enzymes; therefore, for this peptide, reducing proteolytic cleavage is expected to have a greater effect on plasma exposure than would increasing plasma protein binding (TABLE 2).

Lessons from recent clinical approvals

During the last 3 years, 16 of the 195 FDA-approved new drugs have been peptides[25–27]. Abaloparatide, which was approved for postmenopausal 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 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, than to a second conformation (called R0), which results in comparatively prolonged binding and favours unwanted bone resorption[29]; R0 is bound by parathyroid hormone and analogues such as teriparatide.

The year 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 have a significantly increased half-life, in this case around 168 h. This increase was achieved by using a free fatty acid linker that allows the molecule to have 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 in order 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

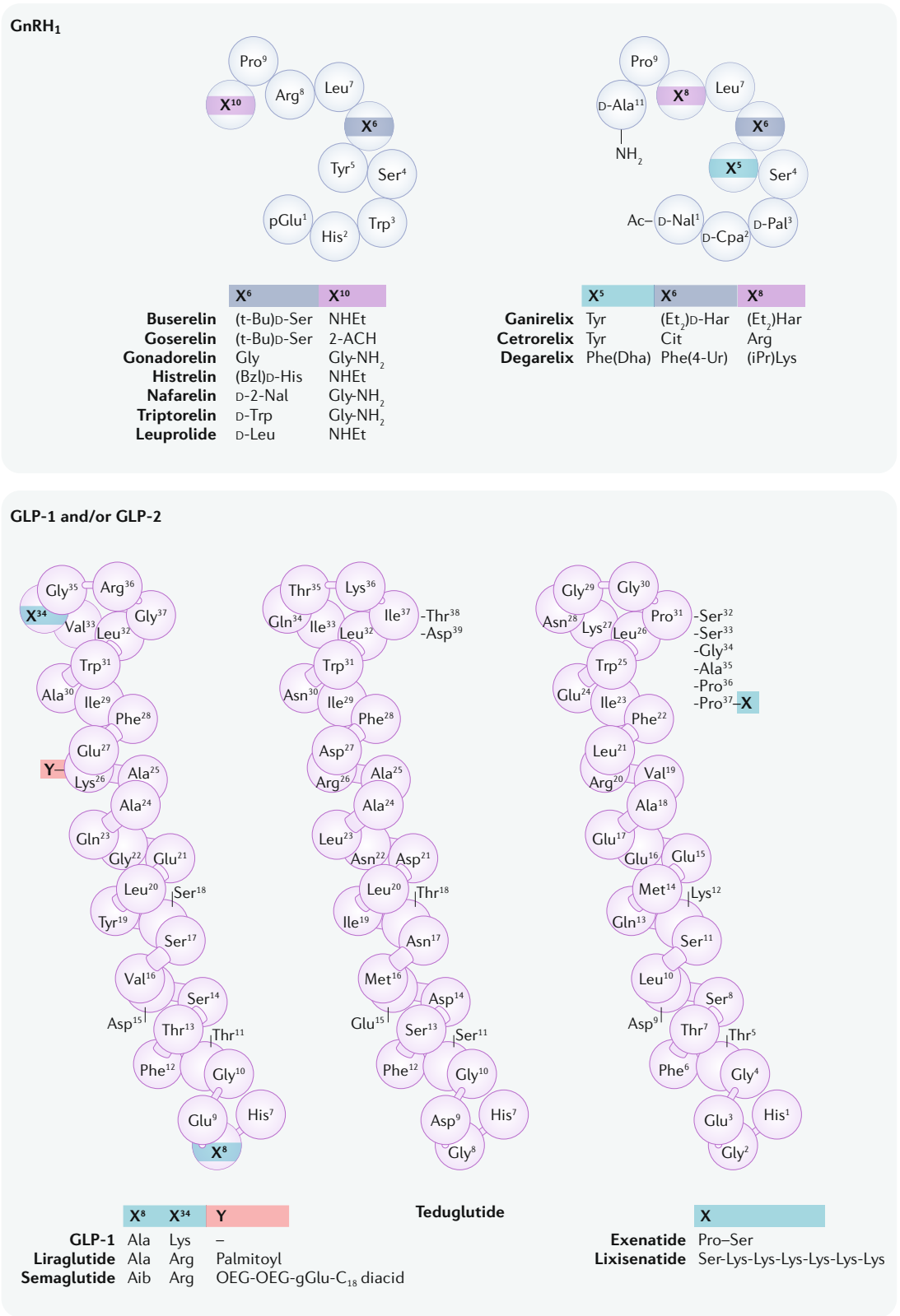


Fig. 2 | **2D and 3D diagrams of peptides that target receptors for $GnRH_1$ or GLP-1 and/or GLP-2.** Peptides are shown as beads, based on the 3D position of Cα atoms of the structural templates of these peptides bound to experimentally determined seven-transmembrane domain (7TM) structures of the associated 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 (FIG. 4), assuming a view in which transmembrane domain 1 (TM1) is on the left, TM5 is on the right, and the extracellular side is facing up. See Supplementary Fig. 2 for further structures of peptide drugs that target class A and B GPCRs.

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' 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, this treatment has few of the central nervous system (CNS) side effects, such as sedation, dysphoria, and hallucinations, that are associated with small-molecule analgesics[33]. This is consistent with the fact that little or no CNS permeability has been observed for difelikefalin, making this an example of a therapeutic for which lack of brain penetrance, as often occurs with peptides, is an advantage over a small molecule. The 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 III

Three of the peptides currently in phase III development are potentially first in their class (TABLE 3). BL-8040 is the first peptide antagonist for CXC-chemokine receptor 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 mobilization of haematopoietic stem cells to peripheral blood prior to collection for autologous cell transplantation[34]. The interaction between the chemokine CXCL12 (also known as SDF1) and its receptor, CXCR4, plays a key role in haematopoietic 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 cyclized (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 its 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 could potentially allow it to 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, in order to restore signalling in the MC_4 receptor pathway.

Phase II

In ongoing phase II trials, novel therapeutic strategies are exemplified by EP-100, which contains synthetic GnRH, consisting of 10 amino acids, attached to an 18-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 therefore are 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 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 hospitalization.

TABLE 3 also summarizes peptides that have been discontinued in phase II or phase III clinical trials. The reasons why trials have not progressed are often not disclosed in peer-reviewed articles and are mainly found on the relevant company websites. With this important caveat, the predominant reason cited for termination of a trial has been futility (the inability to achieve clinical objectives) rather than adverse side effects. In some cases the phase III trials were testing new peptides and clinical indications. For example, foot ulcers affect one in four patients with diabetes, and there is currently an unmet need for new treatments. Angiotensin II has been shown to promote wound healing[42]; however, aclerastide, an angiotensin II receptor agonist, failed to meet the primary efficacy end-point 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)

Table 3 | **Peptides in the pipeline**

Family	Receptor	Ligand; mechanism of action	Clinical indication	Year of study initiation	Synonyms
Ongoing phase III trials, class A GPCRs					
Chemokine	CXCR4	BL-8040; antagonist	Stem cell mobilization 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
Ongoing phase III trials, class B GPCRs					
Glucagon	GLP-2	Glepaglutide; agonist	Short bowel syndrome	2019	ZP1848
VIP and PACAP	$VPAC_1$	Aviptadil; agonist	Sarcoidosis	2019	Vasoactive intestinal peptide
Ongoing phase II 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 enkephalin; np2 enkephalin
Vasopressin and oxytocin	OT	Barusiban; antagonist	Infertility and IVF treatment	2019	None
Ongoing phase II 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 III 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 III trials, class B GPCRs					
Corticotropin-releasing factor	CRF_1	Corticorelin; agonist	Brain tumours and brain oedema	2012	Corticorelin acetate injection; human corticotropin-releasing factor (hCRF); Xerecept
Glucagon	GLP-1	Taspoglutide; agonist	T2D	2013	BIM-51077; ITM-077; R-1583
	Secretin	RG1068; agonist	Autism	2005	RG 1068; SecreFlo; synthetic human secretin
Discontinued during or after phase II trials, class A					
5-Hydroxytryptamine	$5\text{-}HT_{2A}$	Nemifitide; antagonist	Major depression	2010	None
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	NPY_2 and NPY_4	Obinepitide; agonist	Obesity	2007	TM30338; (34-L-glutamine) pancreatic hormone (human)
	NPY_4	TM30339; agonist	Obesity	2012	None

Table 3 (cont.) | **Peptides in the pipeline**

Family	Receptor	Ligand; mechanism of action	Clinical indication	Year of study initiation	Synonyms
Discontinued during or after phase II trials, class A (cont.)					
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 II 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]	T2D	2012	MAR-701; RG 7685; RO-6807952
		MAR709; agonist[c]	T2D	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	T2D	2010	E-1; E1-I.N.T.; E1; EGF analogue; G1; gastrin analogue; GLP-1-I.N.T.; TT 223
		Glymera; agonist	T2D	2016	PB1023
	GLP-2	Elsiglutide; agonist	Drug and/or toxin-induced diarrhoea	2017	GLP-2 analogue; ZP-1846
Parathyroid hormone	PTH1	Ostabolin-C; agonist	Hip fracture	2009	Cyclic PTH-(1–31); ZT 031

Data classified as in phase II or phase III 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. GPCR, G protein-coupled receptor; HSCT, haematopoietic stem cell transplantation; T2D, type 2 diabetes mellitus. [a]EP-100 comprises GnRH and CLIP-71 (an 18-amino-acid cationic α-helical lytic peptide). [b]Rusalatide comprises 23 amino acids from the receptor-binding domain of pro-thrombin. [c]MAR701 and MAR709 are PEGylated compounds.

phase IIB/III 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 h, excluding compounds with flip-flop kinetics, compared with ~5 min 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, occurring 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 the 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 synthesizing 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

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.

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

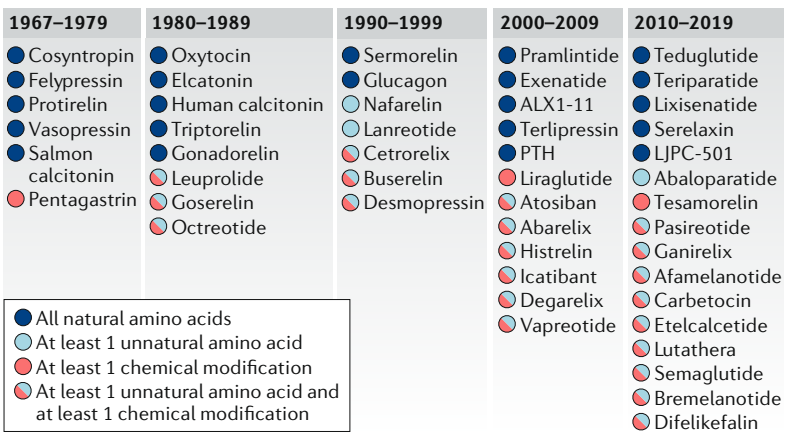
Many of the first peptide drugs were entirely composed of proteinogenic (naturally occurring) amino acids. These drugs had limited stability in the body, owing to rapid enzymatic degradation by peptidases and renal elimination. They required administration subcutaneously or intravenously because they had little or no oral bioavailablity[8]. Next-generation peptide drugs often incorporated unnatural amino acid 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 G protein-coupled receptors (GPCRs), 22 comprise all natural amino acids, 3 contain at least one unnatural amino acid, 3 have at least one chemical modification and 21 have both chemical modifications and unnatural amino acids (see the 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 11 peptides for GPCRs that were approved over the next 20 years (until 1987), only 1 (pentagastrin) contained an unnatural amino acid (β-alanine, butyloxycarbonyl-protected), and 1, 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 5 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, subtype selectivity, pharmacodynamics or pharmacokinetics[176]. The first of the gonadotropin-releasing hormone (GnRH) receptor-targeting peptides that have been approved for hormonal disorders (including breast, ovarian, endometrial or prostate carcinoma) was a natural peptide (gonadorelin), but subsequently approved drugs targeting the same receptor have incorporated unnatural residues that changed the route of administration and improved both potency and stability[177].

Octreotide was the first somatostatin receptor agonist, 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 good clinical efficacy[179]. Pasireotide (approved in 2005) is a heavily engineered cyclic peptide composed of six amino acids, five of which are unnatural. This enables both a reduction in the size of pasireotide versus octreotide and increased in vivo stability (half-life = 12 h versus 1.67 h)[180].

Glucagon-like peptide 1 (GLP-1) receptor agonists are important agents for the treatment of type 2 diabetes mellitus (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 (a GLP-1 dimer fused to human albumin) and dulaglutide (a GLP-1 analogue covalently linked to a human IgG4–Fc heavy chain by a small peptide linker), have also been developed.



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 T2D). The resulting drug, oral semaglutide[50], had increased transcellular permeability and bioavailability of ~4%[51]. The highest dose tested, 40 mg administered orally once daily, resulted in efficacy comparable to that of 1 mg of 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 tenfold. 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. 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 (FIG. 2; TABLE 4). To date, seven peptide GLP-1 receptor agonists have been approved for the treatment of T2D, with projected global sales in 2020 of at least US$10 billion (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 min) because of rapid enzymatic cleavage and enzymatic inactivation by DPP4, which precludes its use as an effective therapeutic treatment. Additional studies have 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

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 (h)	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	Free fatty acid linker binds albumin, which protects the peptide from dipeptyl peptidase 4 (DPP4) cleavage and reduces renal elimination
Exenatide QW	2011	53	Weekly	2.4	Reformulated version of exenatide encapsulated into microspheres 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 are from the Guide to Pharmacology[3], DrugBank, the Electronic Medicines Compendium and ClinicalTrials.gov (see Related links).

limits its 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 on 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.

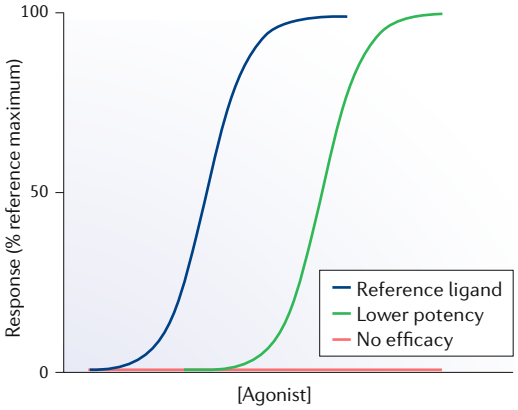
The other strategy focused on modifying the native GLP-1 peptide, leading to liraglutide, albiglutide, dulaglutide and, most recently, semaglutide (FIG. 2; TABLE 4). Because of the short half-life of native GLP-1, as a direct result of both cleavage by DPP4 and rapid renal elimination owing to its relatively 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 (Lys34Arg) 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, $GLP\text{-}1_{7–37}$. 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 Lys26, 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 two copies of GLP-1, either fused to 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 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 the 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

Box 2 | **Quantifying ligand bias**



The hypothesis that agonist-specific G protein-coupled receptor (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 that each elicits a particular pattern of downstream activation, is now well established[182]. Early evidence for biased agonists included the observation that the rank order of the 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, stabilize a particular conformation of the receptor that preferentially interacts with one or a subset of signalling pathways. Compounds might be compared in many assays, so as 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 biases from the assay, system and kinetics should be carefully avoided[185,186].

Biased agonists and antagonists. Compared to the reference ligand (blue in the figure), 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 if 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 internalization and desensitization), then it is probably not critical whether the biased compound has reduced or no potency in a 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 antagonize the effects of the endogenous peptide on this pathway. Other biased ligands might antagonize all pathways but show quantitatively greater functional affinities for one pathway than for another.

(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 for the design and optimization 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 likewise 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 (BOX 2). Bias is usually examined in the context of the two most thoroughly characterized GPCR signalling pathways, those initiated by the binding of either 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 might all be characterized as agonists, but each might 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 desensitization 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 internalized[64]. By contrast, the $GnRH_2$ receptor, which is found in other vertebrates, retains the C terminus, and stimulation induces phosphorylation of C-terminal residues, β-arrestin binding, receptor internalization and rapid receptor desensitization.

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 internalization will reduce desensitization that would limit 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 mechano-sensor in the absence of endogenous apelin[66], which is downregulated in disease. Replacing the lost apelin with a biased agonist might avoid activating the deleterious pathway (see The apelin receptor).

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 the 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 the following 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; 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 and was able to internalize the 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 that 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 antagonized AT_1 receptor-mediated G protein signalling and reduced mean arterial pressure, similar to the antihypertensive agent losartan. Crucially, its effect on cardiac contractility was opposite to that of losartan: 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 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 mmHg) reduction in blood pressure was reported, but no change was observed in volunteers with normal renin levels[78]. However, no benefits were observed over those of standard-of-care drugs through a 30-day follow-up in a phase IIb randomized, double-blind clinical trial[79]. The reasons for this lack of efficacy are unclear. Insufficient target engagement seems unlikely, as the 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 emphasizes the need for comprehensive analyses of the signalling pathways for biased peptide ligands, some of which are being evaluated for clinical use. Peptides have also been developed as tool compounds whose bias is reversed compared to that of TRV027, and which are presumed, therefore, to be deleterious, which should allow further insights into this signalling pathway.

The apelin receptor. The apelin system physiologically antagonizes 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), might play a role in the physiological regulation of the cardiovascular system. Dysregulation of the apelin system and loss of endogenous peptides have been 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 a reduced propensity to desensitize 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 of stretching; 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 into 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 internalization in cell-based assays[87,88]. These data suggest that putative endogenous apelin isoforms may demonstrate unique signalling profiles in vivo, but whether this might have physiological or pathophysiological consequences

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

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

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 an ~70-fold bias towards β-arrestin[89]. Modified peptides based on the apelin sequence have also demonstrated 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 internalization[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 internalization[91]. In terms of downstream signalling events, apelin-17 stimulates extracellular-signal-regulated kinase 1/2 (ERK1/2) phosphorylation in both a $G\alpha_i$- and a β-arrestin-dependent manner, whereas the action of Lys16Pro is 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 for β-arrestin-mediated signalling, but replacing Ser348 with Ala did not alter the cell surface expression of the receptor, binding of apelin ligands or activation of the $G\alpha_i$ or $G\alpha_q$ pathway[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 the data for linear peptides, the C-terminal amino acid is important for receptor binding, β-arrestin recruitment and receptor internalization. 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 the 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, as is exemplified by the macrocyclic peptide MM07 (REF.[96]). MM07 has induced vasodilatation and increased cardiac output in both rats and human volunteers, which may be desirable in the clinical setting, and importantly, the receptor was not desensitized 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 of similarly G protein-biased peptides, as we described above for GLP-1, may result in a peptide with improved plasma half-life to take forward to proof-of-principle clinical studies.

The ghrelin receptor. As we 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 the 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 its effects on food intake[99]. For another ghrelin-targeted peptide, ulimorelin (TABLE 3), the prokinetic effect on gut motility was sustained, unlike the tachyphylaxis seen with other ghrelin agonists. Perhaps ulimorelin does not activate β-arrestin signalling, which would limit receptor desensitization with continued use. Additionally, ulimorelin mimics the orexigenic and gut motility effects of ghrelin, but it 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 III trial in postoperative ileus was discontinued owing to a lack of efficacy over placebo.

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 extracellular loop 2 (ECL2) will form an extended α-helix, thereby constraining this part of the protein and resulting in the loss of constitutive activity[101]. Additionally, whereas ghrelin-stimulated $G\alpha_q$ 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 both full-length ($GLP\text{-}1_{1–36}$, $GLP\text{-}1_{1–37}$)

Non-proteinogenic
Not naturally encoded in any known organism.

and truncated (GLP-$1_{7–36}$, GLP-$1_{7–37}$; the mature isoforms) forms of 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-$1_{1–37}$ 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 the replacement of particular α-amino acids in the peptide backbone with β residues or with non-proteinogenic 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,

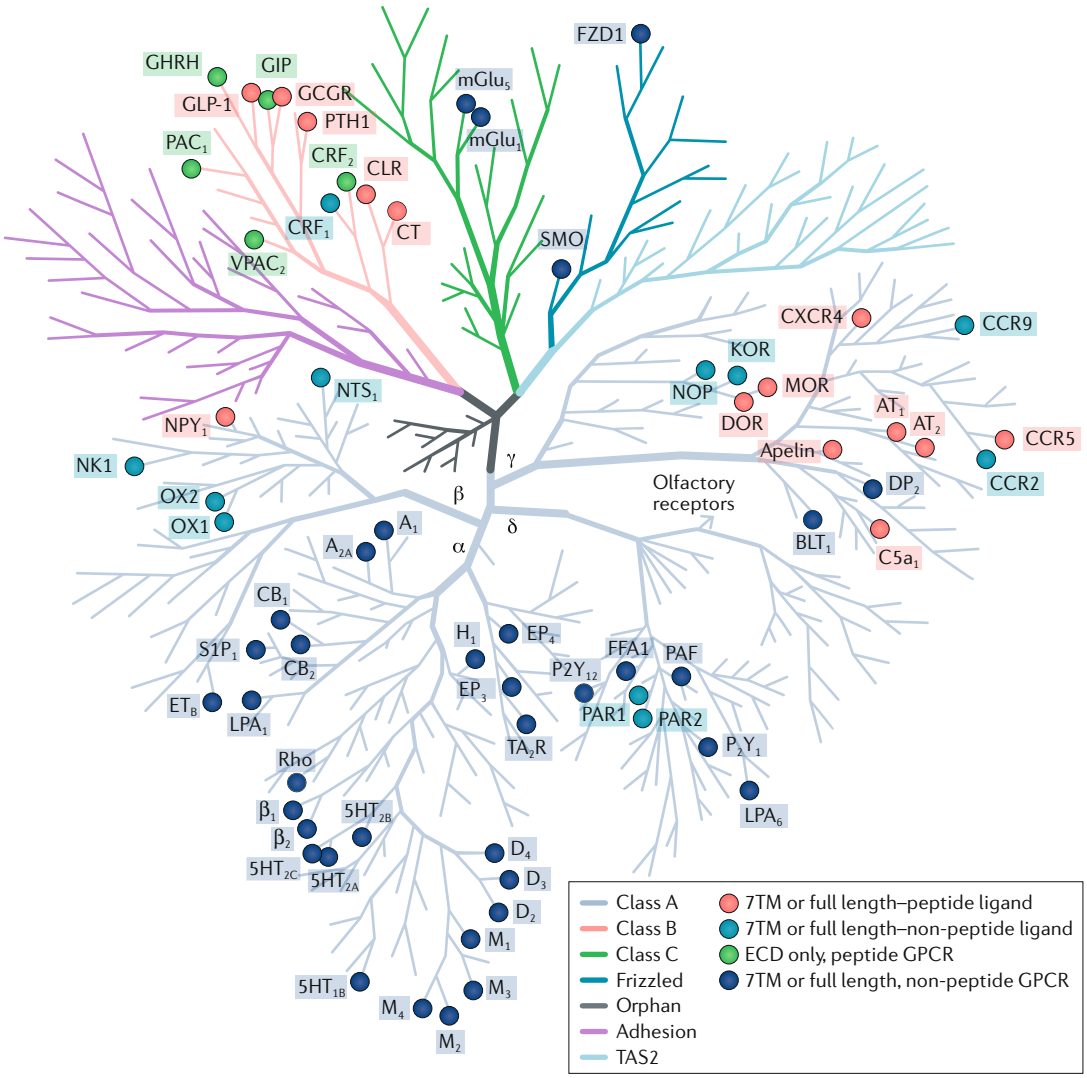
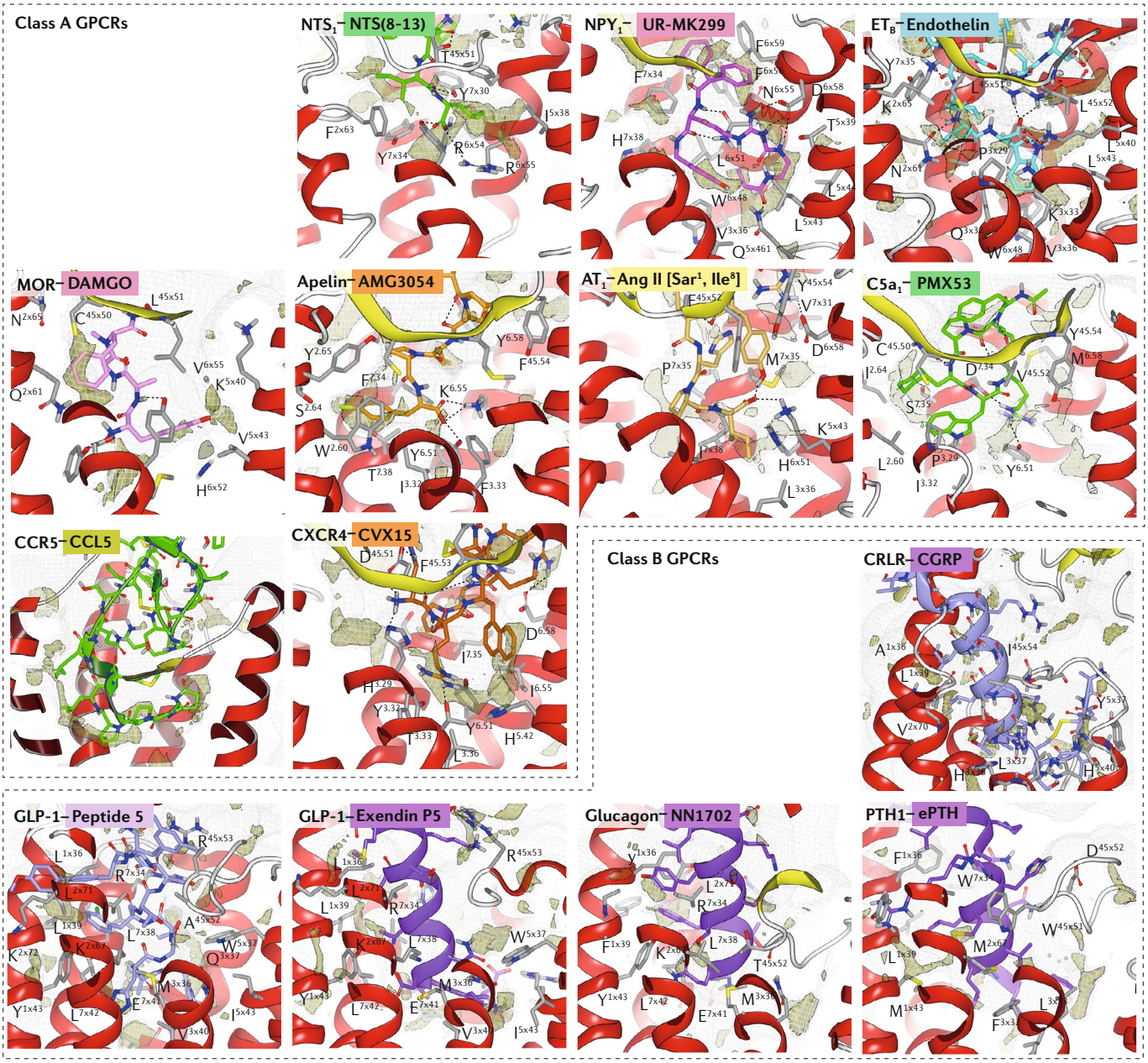


Fig. 3 | **Overview of structure determination for the GPCRs.** Peptide G protein-coupled receptors (GPCRs) for which seven-transmembrane domain (7TM) or full-length structures are available in complex with peptide ligands (red) or only in complex with non-peptide ligands (turquoise) are distinguished 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 endogenous ligands are not yet established. The Class A receptor family is shown classified into four groups: the 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; and the δ-group includes olfactory, purine and glycoprotein receptors.



Class A GPCRs

		1x31	1x35	1x39	2x60	2x61	2x63	2x64	3x29	3x32	3x33	3x36	4x61	4x65	45x50	45x51	45x52	45x53	45x54	5x39	5x40	5x33	5x34	5x461	6x48	6x51	6x52	6x54	6x55	6x58	7x30	7x31	7x34	7x35	7x38	7x39
NTS_1	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
NPY_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
MOR	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 GPCRs

		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-1	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-1	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
Glucagon	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
PTH1	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

Fig. 4 | **X-ray crystallography and cryo-electon microscopy structures of GPCRs.** The panels summarize the structural interactions for aligned binding-site residues of class A and class B peptide G protein-coupled receptors (GPCRs), forming polar H-bond or ionic interactions (red), or only lipophilic interactions (grey), with the peptide ligands shown in the individual binding-mode figure panels. Amongst the class A GPCRs, the binding sites of AMG3054-bound apelin receptor (Protein Data Bank identifier (PDB ID): 5VBL)[121], octapeptide partial agonist (Ang II [Sar^1, Ile^8]) bound AT_1 receptor (PDB: 6DO1)[122], PMX53-bound complement $C5a_1$ receptor (PDB: 6C1Q)[124], endothelin-bound ET_B receptor (PDB: 5GLH)[125], neurotensin-bound NTS_1 receptor (PDB: 3ZEV)[127], UR-MK299-bound neuropeptide NPY_1 receptor (PDB: 5ZBQ)[141], DAMGO-bound μ opioid receptor (MOR) (PDB: 6DDE)[128], and CCL5-bound CCR5 (PDB: 5UIW)[129] are shown. Amongst the class B GPCRs, the calcitonin gene-related peptide (CGRP) bound to the CGRP receptor (CRLR) and complexed with $Gα_s$ (PDB: 6E3Y)[134], NN1702-bound glucagon receptor (PDB: 5YQZ)[135], peptide 5-bound (PDB: 5NX2)[136] and exendin-P5-bound (PDB: 6B3J)[135] GLP-1 receptor, and ePTH-bound PTH1 receptor[139] are shown. All views are focused on the seven-transmembrane domain and are consistent with the orientations of the peptide diagrams shown in FIG. 2. The binding pocket surfaces (grey mesh) are contoured at 1 kcal mol^{-1} using the carbon sp^3 (C3) GRID probe[199], whereas lipophilic areas are defined using the C1 = (lipophilic) probe contoured at −2.8 to −3.0 kcal mol^{-1}, customized to the GPCR binding sites[200]. Generic GPCR residue numbers[201] are provided that are based on the Ballesteros–Weinstein class A GPCR (apelin receptor, AT_1, $C5a_1$, CCR5, ET_B, MOR, NPY_1)[202] and the Wootten[203] class B GPCR (CGRP, glucagon, GLP-1, PTH1) 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].

compared to 2 min 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 crystallize the understanding that differences in relative agonist potencies among 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 disease (TABLES 1,2). Interestingly, they exhibit distinct binding kinetics, affinity and functional efficacy in different G protein pathways, implying that they stabilize 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 that 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 internalized well and preferentially activates $Gα_s$ over $Gα_q$, relative to the $CT_{(a)}$ receptor[115]. Mutational data[116] mapped with molecular dynamic simulations have highlighted that ECL2 was important in conformational propagation linked to the $Gα_s$–cAMP pathway, which was distinct from the ligand-specific and pathway-specific effects propagated by ECL3. These observations highlighted differences in the mechanisms of ligand interaction and receptor activation of the CT receptor compared to another class B receptor, GLP-1 (REF.[116]).

Oxytocin receptors. Atosiban, described as an oxytocin receptor antagonist, is used clinically to prevent preterm labour, by blocking a $Gα_q$-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 $Gα_i$ (which is linked, via MAPK, to the inhibition of cell proliferation), in addition to antagonizing $Gα_q$ 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 the pathways downstream of different Gα proteins, it has little effect on β-arrestin signalling: receptor internalization 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 $Gα_{i3}$ over other $Gα_i$ 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 bind peptides or proteins with 65 solved unique receptor–ligand complexes[115,116,121–157]. So far, 22 of the experimentally determined GPCR structures (10%) (see the GPCR database in Related links) contain a peptide ligand, covering class A and B GPCRs (FIGS 3,4; 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 1,2,4 have been structurally modelled on the basis of homologous peptides and/or receptors. These peptides serve as potential templates for the structure-based optimization and design of novel GPCR peptide therapeutics.

Peptide ligands bind to GPCRs with numerous binding modes, reflecting the diverse chemical structures and properties of both the ligands and the receptor binding sites. Class A GPCR peptide ligands generally bind to ECL2 and a 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; 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 ligands' 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.

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

Biophysical, pharmacological, site-directed mutagenesis and biomolecular simulation studies, combined with comparative analysis of the different conformational states of recently released antagonist-bound and agonist-bound peptide G protein-coupled receptor (GPCR) structures (FIG. 3; Supplementary Table 1), have provided insights into the 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].

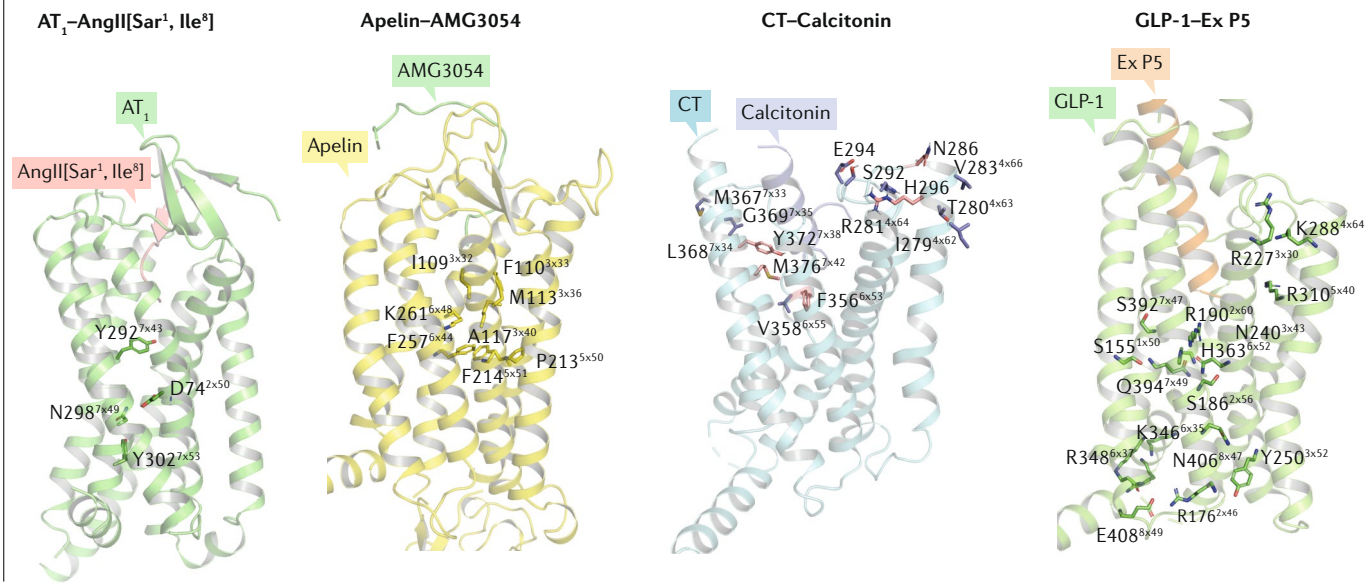
AT_1 receptor. Several residues in the AT_1 receptor are involved in signalling pathway bias[191], including $Y292^{7x43}$ (Ballesteros–Weinstein GPCR numbering; see FIG. 4 caption for an explanation) in the ligand binding site, $D74^{2x50}$ in the allosteric sodium binding site[192], and $N298^{7x49}$ and $Y302^{7x53}$ in the NPXXY tyrosine switch region[189,190]. Double electron–electron resonance (DEER) spectroscopic data[193] mapped on the peptide-bound, nanobody-stabilized active AT_1 structure[122] showed that $G\alpha_q$-biased peptides stabilize a receptor conformation in which the first transmembrane domain (TM1) is located far apart from both TM6 and intracellular loop 2 (ICL2); β-arrestin-biased ligands stabilize 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 the 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 $A117^{3x40}$, $P213^{5x50}$, $F214^{5x51}$, $F257^{6x44}$ and $K261^{6x48}$, which 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 (REF.[116]), including on $F356^{6x53}$, $V358^{6x55}$, $M367^{7x33}$, $L368^{7x34}$, $G369^{7x35}$, $Y372^{7x38}$ and $M376^{7x42}$.

GLP-1 receptor. Several residues in ECL1, ECL2 and ECL3, and in the tops of TM3 ($R227^{3x30}$), TM4 ($K288^{4x64}$) and TM5 ($R310^{5x40}$), which 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 the conformational differences between active and inactive GLP-1 receptor structures[133,136,137,149,198].

The residues involved in biased signalling in the AT_1 receptor and the apelin, CT and GLP-1 receptors in mutagenesis studies are highlighted and coloured on the corresponding receptor structures in the figure: aligned angiotensin II (AngII)[Sar^1, Ile^8], bound to the AT_1 receptor (Research Collaboratory for Structural Bioinformatics Protein Data Bank (PDB) ID: 6DO1)[122]; AMG3054, bound to the apelin receptor (PDB ID: 5VBL)[121]; calcitonin, bound to the CT receptor (PDB ID: 5UZ7; 6NIY)[116,133]; and exendin-P5 (Ex P5), bound to the GLP-1 receptor (PDB ID: 6B3J)[138].



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 3D structures of key regions for peptide recognition and selectivity by GPCRs. Methodological and technical improvements to cryo-electron microscopy are expanding the role of this technique, alongside X-ray crystallography, in solving GPCR structures. Emerging information on the 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, in which all endogenous ligands are peptides and there is the potential to discover new compounds based on exploiting the allosteric binding sites revealed by structural studies. Future studies will help unravel the importance of receptor dimerization and contribute to the rational, rather

Incretins
Metabolic hormones, induced upon eating, that decrease blood glucose by stimulating insulin release. The two known incretins are glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP).

than empirical, design of biased peptide ligands, as our knowledge expands of the key residues involved in the kinetics and dynamics of signalling by means such as β-arrestin.

Perspectives and conclusions

Nearly all of the 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-molecule therapeutics. This situation may, however, be set to change. The number of deduced structures of the 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 cryo-EM, 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 that have no effect on the activity of urotensin II-related peptide, the second endogenous agonist that binds the receptor[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 synthesize a better peptide drug? This Review has highlighted 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, to improve 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 being biased, in that it does not activate β-arrestin, thereby reducing internalization, 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, 68 missense variations that occurred within the coding regions of one-third of the GPCR drug targets; only 8 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 the 100,000 Genomes Project[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 the GLP-1 receptor along with either the glucagon[164] or glucose-dependent insulinotropic polypeptide (GIP)[165,166] receptor. Interestingly, a compound that binds to all three receptors is in phase I testing[166]. In a phase II 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. However, both are degraded by DPP4. Therefore, designing a peptide that contains both peptide sequences but that lacks DPP4 cleavage sites, in order 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) conformation 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 are lipidated to penetrate cells so they can access allosteric sites and stabilize GPCR conformations, 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 advances, and a range of strategies can 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 linking genetically engineered exendin-4 to a single-domain albumin-binding antibody (AlbudAb), which prolonged plasma half-life to 6–10 days while maintaining agonist activity (measured as 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 so as to release active peptide, thereby prolonging detectable activity in the plasma from 4 h 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 in order to transport compounds across the blood–brain barrier is being investigated experimentally. Finally, the number of potential new GPCR targets is expanding, as orphan GPCRs continue to be paired with peptide ligands[175].

It is not yet clear where the balance lies between the costs of developing clinical candidates 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 more than ten in the pipeline in phases II and III, which is mirrored by a rise in the estimated global value of the industry to US$25.4 billion.

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