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Trends in Peptide Therapeutics

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1.1 Introduction

The growing importance of peptide drugs within the pharmacopoeia has become evident over the past several decades. Among the factors that have contributed to this trend is the recognition that peptide ligands regulate a multitude of physiological pathways and are often suitable for therapeutic applications, in either their native or modified form. In addition, certain attributes that are unique to peptides, such as their high selectivity, potency, and lack of toxicity, have ultimately become appreciated. The alternative means of drugging peptide receptors through target-directed screening or rational design of orally available small molecules have, with few exceptions, proved unproductive. Mimicking the activity of a peptide agonist is highly challenging, particularly in the case of Class II G-protein-coupled receptor (GPCR) targets. Successful examples have typically involved receptor antagonists such as neurokinin, angiotensin, endothelin, and orexin. These lessons have increasingly led drug discovery scientists to consider peptides as legitimate drug candidates, rather than leads or proof-of-concept models for small-molecule programs. Peptide medicinal chemists have also had to confront and overcome shortcomings such as rapid metabolism, clearance, production costs, and limited alternative delivery options. In the present chapter, we highlight the role of peptides in therapeutic areas such as metabolic disease, where peptides have been well established, as well as in areas where their impact has been minor, but now rapidly expanding. We also emphasize examples where time-extension strategies and alternative delivery routes have helped establish and strengthen the position of peptide drugs in competitive markets. Finally, we explore two novel trends in peptide drug discovery, macrocyclic and cell-penetrating peptides, both of which may expand future opportunities for peptide therapeutics.

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1.2 Peptides in Metabolic Diseases

The global epidemic of type 2 diabetes and obesity continues unabated, impacting quality of life, life expectancy, and economic well-being. Health-care organizations have devoted enormous resources toward the treatment of metabolic diseases, often dramatically improving patient outcomes [1]. Perhaps more than in any other therapeutic area, peptides have had a unique and indispensable role in treating type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM) as well as obesity. This section will provide an overview of approved insulin, GLP-1 (glucagon-like peptide-1), and glucagon peptide drugs as well as those in late-stage clinical development.

1.2.1 Insulins

Insulin, which was discovered by Banting and Best in 1921, became commercially available only one year after its discovery (Figure 1.1) [2]. In spite of its miraculous potential, the short duration of action of early insulin preparations (four to six hours) required multiple daily injections and prompted the search for longer acting formulations. The first of these, insulin neutral protamine Hagedorn (NPH), developed in the 1940s, consisted of an insulin suspension complexed with protamine, a cationic protein isolated from fish sperm. The slow disassociation of the NPH complex delayed absorption from the injection site, prolonging insulin action to a range of 12 to 18 hours [3]. Insulin Lente, introduced in the 1950s, involved a neutral pH suspension of insulin formulated with excess zinc, which extended the duration of action to 24 hours and beyond [3]. Between the 1920s and the early 1980s, commercial insulin production relied on extraction of pancreatic glands from cows and pigs. The advent of biotechnology enabled the production of rDNA-derived human insulin in the early 1980s in sufficient quantity to satisfy the needs of the diabetic population, gradually

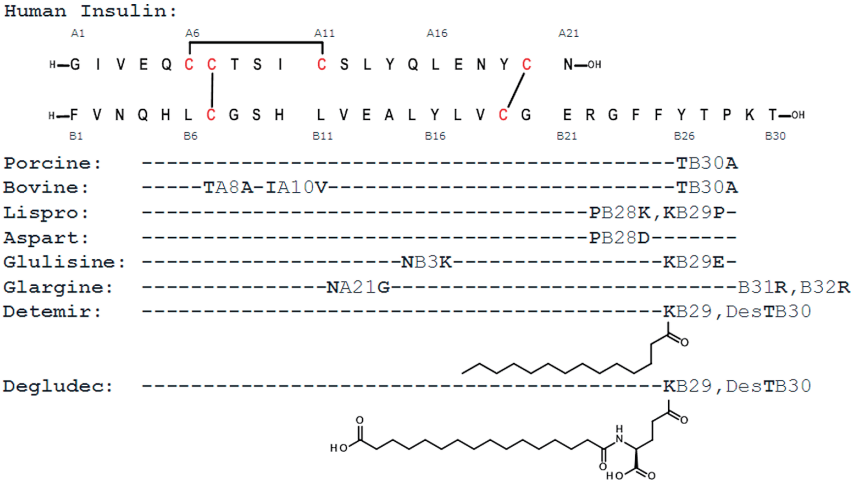


Figure 1.1 Sequences of human, porcine, bovine, and the commercially key insulin analogs.

displacing animal sourced insulins [4]. Recombinant DNA technology also furthered the development of short-acting analogs to manage prandial glucose excursion, as well as long-acting analogs to mimic basal insulin action [5, 6].

In the native state, the insulin hexamer complex consists of three non-covalent insulin dimers, which are in turn stabilized by two zinc ions coordinating the B10 His residues. Once injected, the diffusion of the zinc ion causes a sequential breakdown of the hexamer into insulin dimers, and further into monomers, the last being the rate-limiting step. Since physiological absorption occurs mainly via the monomeric form, research efforts have focused on weakening of the insulin dimer association. Examination of the X-ray structure indicated that the B-chain C-terminal regions of two insulin molecules form the dimer interface. Weakening of this interaction in order to accelerate dimer disassociation resulted in a more rapid onset of insulin action. Three rapid-acting insulin analogs were approved between 1996 and 2006: LysB28, ProB29, insulin lispro (Humalog®, Eli Lilly & Co.) [7]; AspB28, insulin aspart (Novolog®, Novo Nordisk A/S) [8]; and LysB3, GluB29, insulin glulisine (Apidra®, Sanofi S.A.) [9]. The onset of action for these analogs is within 5 to 15 minutes, with peak action at 30 to 90 minutes and duration of 4 to 6 hours [5]. An ultrafast, co-formulation of insulin aspart and niacinamide (Fiasp®, Novo Nordisk A/S) was approved in late 2017.

The long-acting, or "basal" insulins, use two independent strategies: isoelectric point shift and lipidation. Insulin glargine (Lantus®, Sanofi S.A.) is a human insulin analog with a mutation of AsnA21Gly, and an additional ArgB31 and ArgB32 residues. The presence of the two arginine residues shifts the isoelectric point of the hormone from 5.6 to 6.7, reducing its solubility at physiological pH. Aqueous solubility at pH 4.0 required the GlyA21 substitution to mitigate the acidic degradation of the native AsnA21. Upon injection, insulin glargine forms an insoluble depot that gradually dissipates, releasing the drug over a 20 to 24 hour period [10]. Insulin glargine undergoes extensive metabolism in the subcutaneous depot with only its metabolites released into systemic circulation. The earliest commercial application of the lipidation strategy was insulin detemir (Levemir®, Novo Nordisk A/S), a human desB30 insulin covalently modified with myristic acid at the LysB29 side chain. This modification induces hexamer and di-hexamer formation while also facilitating binding to serum albumin (98 % bound in plasma). The former phenomenon delays absorption from the injection site while the latter slows plasma clearance, contributing to the extended 12 to 24 hour duration of action as well as a less variable pharmacokinetic/pharmacodynamic (PK/PD) profile [11]. Insulin degludec (Tresiba®, Novo Nordisk A/S) represents the second generation of the lipidation strategy. LysB29 acylation with a γ-glutamate linked hexadecanedioic acid results in prolonged time action promoted by higher order multi-hexamer association at the subcutaneous injection site, and to a lesser degree, by higher affinity for albumin. Insulin degludec provides a longer duration of action (42 hours), which enables therapeutic accumulation using a once-daily dosing regimen, thus permitting administration during the day [12].

Alternative delivery of insulin has had a mixed record of success with two previously approved pulmonary products. Exubera® (Pfizer Inc.), an inhalable, spray-dried insulin powder administered using a reusable inhaler was approved

in 2006. It was withdrawn from the market after a brief time due to poor commercial performance attributed partly to the bulky device. Afrezza® (MannKind Corp.), a Technosphere® formulated human insulin delivered in a thumb-sized device, was approved in 2014. Peak plasma insulin levels with Afrezza® are achieved within 12 to 15 minutes after administration. Its commercial fate at this time remains uncertain [13].

A survey of clinical insulin programs indicates a high level of interest in various ultrafast bolus and ultra-long basal analogs, as well as orally administered insulins. The sponsoring companies include Adocia (ultrafast, BioChaperone formulation of insulin lispro, Phase 2; and premixed, Phase 1/2), AntriaBio (once weekly, Phase 1), Biocon/Mylan (insulin Tregopil, oral, Phase 2/3), Diasome (liver targeted, Phase 2), Eli Lilly (once-weekly, Phase 1; and ultrafast, Phase 3), Merck (glucose-sensitive, Phase 1), Novo Nordisk (once-weekly, Phase 2), Oramed (ORMD-0801, oral, Phase 2), and Sanofi S.A (ultrafast, Phase 3).

1.2.2 Glucagon-like Peptide-1

GLP-1 is a peptide hormone secreted from intestinal L-cells, which serve an essential role in glucose homeostasis (Figure 1.2). Activation of GLP-1 receptors on pancreatic β-cells stimulates glucose-dependent insulin secretion, and simultaneously suppresses glucagon levels under hyperglycemic conditions. Apart from helping maintain glucose homeostasis, GLP-1 also promotes satiety and delays gastric emptying, which together contribute to decreased food intake and lower body weight. This unique spectrum of biological and pharmacological properties has led to the development of a number of successful antidiabetic and anti-obesity medications [14]. Endogenous GLP-1 circulates in two equipotent bioactive isoforms, GLP-1 (7–36)-NH_2 and GLP-1 (7–37). Both have short half-lives of approximately two minutes because of dipeptidyl peptidase-4 (DDP-IV)-mediated cleavage of the N-terminal His^7-Ala^8 dipeptides. The short half-life diminishes the pharmacological effects and has promoted the search for longer acting, DPP-IV stabilized analogs [15]. The first approved GLP-1 analog, exenatide (Byetta®, Eli Lilly & Co. and Amylin Pharmaceuticals), was isolated from the saliva of the Gila monster lizard. Exenatide, the active pharmaceutical ingredient in Byetta, possesses similar *in vitro* potency as native GLP-1 with a half-life of

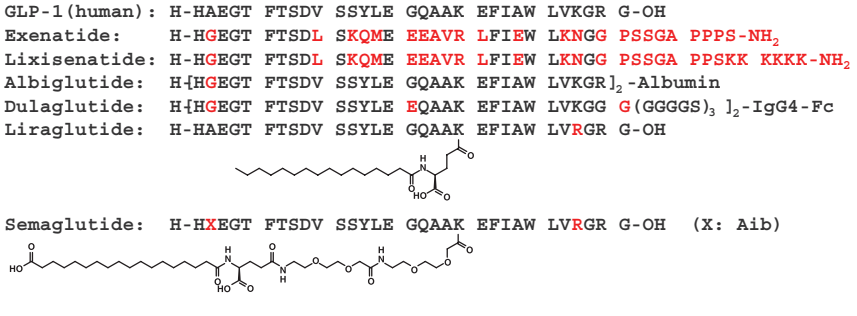


Figure 1.2 Structure of GLP-1 and marketed analogs.

two to four hours, attributed to resistance toward DPP-4 cleavage. It is approved for twice daily SC injection with a dose of 5 to 10 μg [16]. Lixisenatide, an analog of exenatide modified with six lysine residues at its C-terminus, is marketed as Adlyxin® in the United States and as Lyxumia® in the European Union by Sanofi S.A. [17]. Initial experience with GLP-1 agonists suggested that optimal patient compliance and outcomes might require once-daily, once-weekly, or even less frequent dosing intervals. Efforts to extend the Byetta dosing interval involved co-formulation with a poly(D,L-lactide-*co*-glycolide) (PLGA) polymer, which successfully prolonged the drug's half-life to five to six days in humans [15]. The new formulation was approved for once-weekly administration in 2012 (Bydureon®, AstraZeneca plc).

A validated strategy for extending peptide time action involves fusion to a macromolecular carrier as a means to mitigate proteolytic degradation, slow down renal clearance, and exploit FcRn trafficking to peripheral tissues. Human serum albumin (HSA) and the Fc portion of immunoglobulin G (IgG) as molecular fusions have both been utilized to extend GLP-1 duration of action to enable once-weekly dosing. Albiglutide (Tanzeum®, GlaxoSmithKline plc) is a recombinant HSA construct N-terminally extended with two tandem GLP-1 sequences. The DPP-IV cleavage of GLP-1 was minimized through the substitution of Gly for Ala at the second position. Albiglutide has a half-life of six to eight days in humans and is approved for once-weekly injection at a dose of 30 to 50 mg [18]. Dulaglutide (Trulicity®, Eli Lilly & Co.) is a human IgG4-Fc fusion protein N-terminally modified with a Gly4Ser flexible linker and a GLP-1 sequence. As in albiglutide, the DPP-IV degradation is suppressed by Gly substitution at the second position. The half-life of dulaglutide is approximately four days in humans, and the drug is approved for once-weekly injection at a dose of 0.75 to 1.5 mg [19].

The lipidation strategy utilized successfully to extend insulin pharmacokinetics has also been applied to GLP-1 peptides. It has resulted in two approved GLP-1 analogs, both from Novo Nordisk A/S. Liraglutide (Victoza®) utilizes a GLP-1 backbone with a palmitic acid attached to the Lys20 side chain via a γ-glutamate spacer. The native Lys at position 28, replaced with Arg, facilitates site-specific acylation at Lys20. Liraglutide's high degree of albumin binding not only slows kidney clearance but also effectively shields the native N-terminus from DPP-IV cleavage. Liraglutide has a half-life of approximately 13 hours in humans, and is approved for once-daily dosing of 1.2 to 1.8 mg [20]. A higher dose of liraglutide, approved for the obesity indication, is marketed as Saxenda®. Semaglutide (Ozempic®) is structurally similar to liraglutide, and uses an octadecanedioic acid in place of palmitic acid with two mini-PEG units as an additional spacer. Greater DPP-IV stability consistent with a once-weekly dosing requirement was achieved through substitution with 2-aminoisobutyric acid (Aib) at the second position. The combination of high-affinity albumin binding with greater DPP-IV stability prolongs the half-life of semaglutide in humans to six to seven days. It is approved for once-weekly injection at a dose of 0.5 to 1.0 mg [21].

The commercial success of the GLP-1 class has increased research and development investment in additional candidates. These include once-weekly efpeglenatide (Sanofi S.A., Phase 3), a subdermal osmotic mini-pump device, which delivers exendin-4 (Intarcia Therapeutics Inc, ITCA-650, food and drug

administration [FDA] complete response letter [CRL] received) and an oral tablet formulation of semaglutide (Novo Nordisk A/S, Phase 3).

1.2.3 Glucagon

Glucagon is the primary counter-regulatory hormone of insulin, which is secreted in response to hypoglycemic conditions [22, 23]. Its chief medicinal use is the emergency reversal of insulin-induced hypoglycemic shock, a relatively frequent event in the treatment of type-1 patients. Glucagon's poor biophysical and chemical stability makes liquid formulation challenging, thus requiring reconstitution of lyophilized glucagon powder in an acidic diluent immediately prior to use. This complicates not only its current emergency use but also hinders potential additional indications, which require a stable, soluble glucagon [22]. A recent survey revealed advanced glucagon programs at Adocia (BioChaperone formulation of human glucagon, Phase 1), Eli Lilly & Co. (Nasal glucagon, Phase 3; and novel soluble analog, Phase 1), Novo Nordisk A/S (novel, soluble analog, Phase 1), Xeris Pharmaceuticals (Xerisol, Phase 3, human glucagon in dimethyl sulfoxide [DMSO]), and Zealand Pharma (Dasiglucagon, Phase 3, novel, soluble analog).

1.2.4 Combination Therapies

Combination therapy, achieved through co-administration of two agents targeting independent pathways, produced additive or synergistic efficacy and a more tolerable side-effect profile [24]. Initiation of insulin therapy, either with a single agent or as part of a basal/bolus regimen, promotes weight gain and an increased incidence of hypoglycemia. In contrast, GLP-1 agonists provide not only improved glycemic control but also modest body weight loss. As expected, the combination of a basal insulin and a GLP-1 agonist consistently reduces HbA1c and body weight in most patients, while reducing the frequency of hypoglycemia [25]. Two combination products were approved by the FDA; insulin glargine plus lixisenatide (Soliqua®, Sanofi S.A.) and insulin degludec plus liraglutide (Xultophy® Novo Nordisk A/S).

Glucagon and glucose-dependent insulinotropic polypeptide (GIP) are two important metabolic hormones closely related to GLP-1. Glucagon has been shown to stimulate lipolysis, increase energy expenditure, and complement the weight-lowering effect of GLP-1 [22]. GIP is an incretin that functions as a "glucose-stat" stimulating insulin secretion under hyperglycemic conditions, while stimulating glucagon secretion in the hypoglycemic state [26]. Consequently, various combinations of GLP-1, GIP, and glucagon pharmacology have been explored in the form of unimolecular co-agonists [27]. A number of these programs reached the clinical development stage, including GLP-1/glucagon dual agonists from AstraZeneca plc (MEDI0382, Phase 2), Eli Lilly & Co. (Phase 1), Johnson & Johnson (JNJ-64565111, Phase 2), Novo Nordisk A/S (Phase 1), OPKO Health (POK88003, formerly TT401, Phase 2), Sanofi S.A. (SAR425899, Phase 2) and Zealand Pharma/Boehringer Ingelheim Gmbh (BI456906, Phase 1), GLP-1/GIP dual agonists from Eli Lilly & Co. (LY3298176, Phase 3) and Sanofi

S.A. (SAR438335, Phase 1), and a GLP-1/glucagon/GIP tri-agonist from Novo Nordisk A.S.(NN9423, Phase 1).

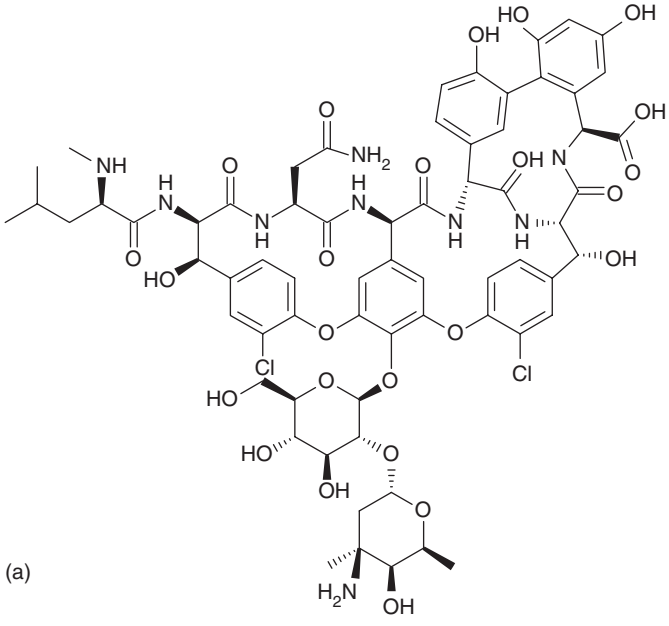
1.3 Peptide Antibiotics

While cyclic peptides such as gramicidin were among the earliest antibiotics discovered, their clinical application has been limited by their lack of oral availability, short half-life, and systemic toxicity. As a result, their use has been restricted to topical application in ophthalmology and dermatology. However, their inherent advantages, including their broad-spectrum activity, lesser susceptibility to microbial resistance, as well as the potential for broader indications, have combined to revive interest in this peptide category. The peptide antibiotics are structurally diverse and frequently highly complex natural products produced by either ribosomal or non-ribosomal biogenic pathways. Peptides such as the mammalian defensins, the insect-derived cecropins, and the amphibian antimicrobial peptides (AMPs), assembled by ribosomal synthesis, are occasionally posttranslationally modified. Peptides of microbial origin, assembled by non-ribosomal synthesis, incorporate a wide selection of non-native amino acids and are represented by the bacitracins, polymyxins, gramicidins, and vancomycin.

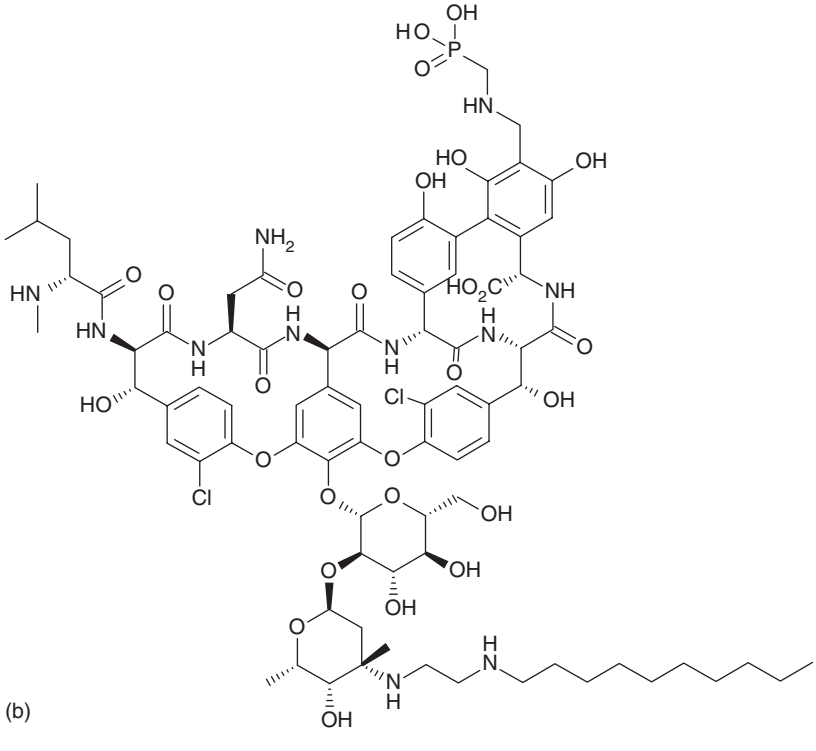
1.3.1 Non-ribosomally Synthesized

The isolation of tyrothricin (a mixture of gramicidins and tyrocidines) by R. Dubos in the late 1930s from *Bacillus brevis* provided the first clinically useful antibiotic for skin and throat infections [28]. Gramicidin S is mainly used for ophthalmic indications and treatment of surface wounds infected by gram-positive and gram-negative bacteria [29]. The polymyxins, discovered in the late 1940s, were used clinically for a number of years against gram-negative bacterial infections. As a result of reported systemic toxicity the polymyxins were all discontinued by the 1970s, but have recently re-emerged as a last resort treatment against resistant gram-negative bacteria. Recent reports have also described atypical synthetic polymyxin analogs with high antibiotic activity and minimal toxicity [30]. Bacitracin, first isolated in 1945 from *B. brevis* and used in combination with other antibiotics to treat skin and eye infections, is marketed as Neosporin®. A more recently developed lipidated cyclic depsipeptide, daptomycin (Cubicin®, Cubist Pharmaceuticals), was approved by the FDA in 2003 for complicated skin and skin-structure infections caused by *Staphylococcus aureus*. Daptomycin is also approved for systemic use in the treatment of bacteremia associated with right-sided endocarditis [31].

The glycopeptides (lipoglycopeptides) are an important class of antibiotics that has remained relevant 60 years after the discovery of vancomycin (Figure 1.3a), (Vancocin®, Eli Lilly & Co.). A group led by E.C. Kornfeldt isolated the molecule from soil samples collected in Borneo in 1953 [32]. Vancomycin moved quickly through development, with approval in 1958, and was used primarily against gram-positive strains. Its use declined with the introduction of newer antibiotics through the 1980s; however, increasing resistance to these



(a)



(b)

Figure 1.3 Structures of vancomycin (a) and telavancin (b).

newer agents led to vancomycin's re-introduction particularly for use against *S. aureus* [33]. Its complex structure was not elucidated until 1982 [34], which effectively limited exploration of its structure–activity relationships prior to that time. The unique efficacy of vancomycin stimulated extensive research into other glycopeptide antibiotics [32], leading to the discoveries of other naturally occurring analogs such as teicoplanin (Targocid®, Sanofi S.A.). A number of successful second generation semisynthetic glycopeptides based on vancomycin were subsequently introduced [35]. For example, telavancin (Vibativ®, Theravance Biopharma) introduced in 2009 demonstrates enhanced pharmacokinetic properties as well as efficacy against *S. aureus* strains (Figure 1.3b).

The echinocandins are semisynthetic lipidated, cyclic hexapeptides, which present an important option for combating invasive systemic fungal infections. All are structurally based on the parent echinocandin B natural product discovered in 1974 [36, 37]. The echinocandins exert their antifungal activity through a unique mechanism that involves potent inhibition of the (1 → 3)-β-D-glucan enzyme synthesis complex. Their main therapeutic indication is the treatment of *Candida* fungal infections, particularly those resistant to fluconazole and amphotericin B. They are also highly efficacious against a number of *Aspergillus* species [38]. Marketed echinocandins include caspofungin (Cancidas®, Merck & Co., Inc.), anidulafungin (Eraxis®, Pfizer Inc.), and micafungin (Mycamine, Astellas Pharma Inc.).

1.3.2 Ribosomally Synthesized

Lantibiotics (or lanthipeptides) are representative ribosomally synthesized peptides, which undergo posttranslational modification. The lantibiotics are characterized by thioether amino acid residues lanthionine and methyllanthionine [39]. The prototypical and oldest lantibiotic is nisin, originally isolated from *Lactococcus lactis* in the 1930s, which has been used for decades as a food preservative. More recently discovered analogs have attracted considerable interest for their multiple modes of action and high *in vitro* potency against a number of problematic organisms such as methicillin-resistant *S. aureus* and vancomycin resistant *Streptococcus pneumoniae* [40]. The clinical and commercial development of lantibiotics has been complicated by inefficient production and poor chemical and biophysical properties. Nevertheless, their unique therapeutic potential will likely help maintain interest in the lantibiotics class in the future.

Our understanding of the role and potential utility of peptide antibiotics was transformed in the 1980s by seminal discoveries of the cecropins, the magainins, and the defensins. The cecropins, isolated by Boman's group from the hemolymph of the cecropia moth, are 30 to 37 residue peptides that mediate cell-free immunity for the insect and are active against both gram-positive and gram-negative bacteria [41] with similar peptides identified in related insect species. Selsted et al. isolated homologous AMPs termed "defensins" from human and rabbit leukocytes with potent activity against bacterial and fungal organisms, suggesting that these peptides play a key role in mammalian host defense [42]. The defensins were subsequently also detected in mammalian respiratory secretions where they are thought to provide a first line of defense against microbial invasion [43].

Skin secretions from frogs and toads are a rich source of bioactive peptides. The serendipitous discovery of the magainins in the skin of the African clawed frog by Zasloff [44] was the first of many subsequently discovered ribosomally synthesized AMPs of amphibian origin. Other AMP families include dermaseptins, bombinins, brevinins, esculentins, and ranalexins with additional examples frequently reported. An updated database can found at http://aps.unmc.edu/AP. Many of these peptides have been shown to have potent, broad-spectrum activity against a variety of gram-positive and gram-negative bacterial as well as fungal pathogens. Despite their structural heterogeneity, most AMPs share a cationic, amphiphilic *a*-helical structure, which enables them to penetrate and disrupt bacterial cell membranes, their presumed mode of action [45, 46]. Their cationic nature, however, contributes to their hemolytic properties, which have thus far limited their use as systemic antibiotics. Despite this shortcoming, the use of AMPs in the clinical setting is being explored in wound healing [47] as well as skin and oral infections [47]. A number of AMPs have entered clinical trials: CLS001 (omniganan) in rosacea and acne vulgaris, AB-103 (reltecimod) in necrotizing soft tissue infections, SGX942 (dusquetide) and brilacidin each in oral mucositis [48]. A number of other candidates are either in early or preclinical stages of development.

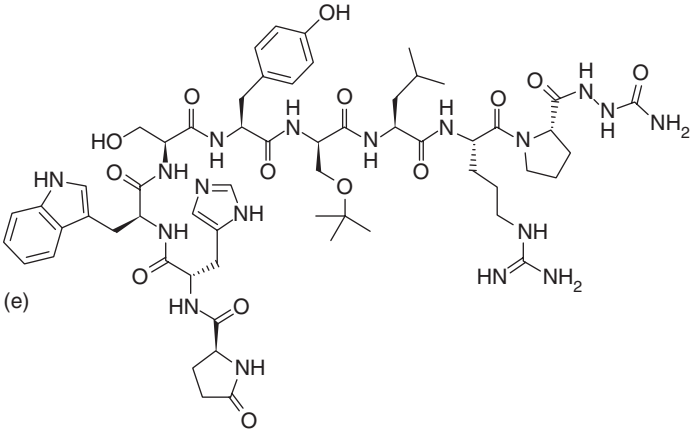
1.4 Peptides in Cancer

Until recently, peptides had only limited application in the treatment of cancer; specifically, they were used to induce hormonal deprivation as a means of slowing tumor growth and disease progression. Nevertheless, luteinizing hormone releasing hormone (LHRH) and somatostatin (SST) analogs remain the standard of care for numerous cancer indications, and currently provide a versatile platform for diagnostic and therapeutic innovation, which helps advance cancer treatment.

1.4.1 Luteinizing Hormone Releasing Hormone

The history of the LHRH peptide family was recently reviewed by Schally, whose group was principally involved in its discovery and subsequent introduction into reproductive medicine and oncology (see Figure 1.4) [49]. The demonstration that continuous administration of LHRH hormone (Figure 1.4a) downregulated LH and follicle-stimulating hormone (FSH) secretion was central to the clinical application of LHRH agonists to cancers of the prostate, breast, and endometrium [50]. The earliest approved examples of this class were short-acting *agonists* leuprolide (Lupron®, Abbott Laboratories), triptorelin (Trelstar®, Allergan, Inc.), buserelin (Suprecur®, Sanofi S.A.), and goserelin (Zoladex® AstraZeneca plc), which were all subsequently developed as depot formulations, providing continuous exposure for up to several months (Figure 1.4b–d respectively). Exploration of LHRH structure–activity relationships resulted in the discovery of LHRH *antagonists* such as degarelix (Figure 1.4e) (Firmagon®, Ferring Pharmaceuticals, Inc.) capable of inducing competitive LHRH receptor blockade

(a) pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2
(b) pGlu-His-Trp-Ser-Tyr-D-Leu-Leu-Arg-Pro-NHEt
(c) pGlu-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-Gly-NH_2
(d) pGlu-His-Trp-Ser-Tyr-D-Ser(tBu)-Leu-Arg-Pro-NHEt



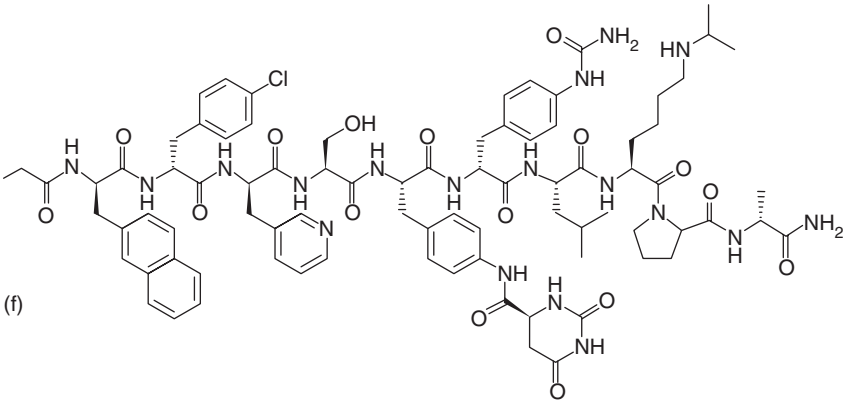


Figure 1.4 Structures of (a) LHRH, (b) leuprolide, (c) triptorelin, (d) buserelin, (e) goserelin, and (f) degarelix.

in the absence of an intrinsic agonist effect. In contrast to the earlier agonists, administration of LHRH antagonists produces immediate androgen deprivation, and importantly does not provoke the characteristic LH release or "flare" [51]. This was confirmed in a comparative study of the antagonist degarelix versus the agonist leuprolide, which demonstrated equally durable testosterone suppression for both agents. However, testosterone suppression occurred faster and with no reported "flare" in the degarelix arm [52].

1.4.2 Somatostatin

SST analogs represent the other major class of peptide therapeutics with application to cancer treatment. The initial observations noting that hypothalamic

extracts inhibited secretion of growth hormone (GH) from the pituitary led to the isolation and characterization of the 14 amino acid peptide, SST-14, by Vale and Guillemin [53, 54]. Early research demonstrated that in addition to the known inhibition of GH, somatostatin peptides are also potent suppressors of a number of other hormones, including insulin, TSH, and glucagon [55]. The potential application of SST ligands to cancer treatment was suggested by the finding that SST receptors are overexpressed in tumors originating within SST target tissues. This prompted Vale and coworkers, as well as investigators at the former Sandoz laboratories starting in the 1970s to find more potent and stable SST analogs [56]. The latter's efforts culminated in the development and approval of the potent SST agonist octreotide (Sandostatin® Novartis Pharmaceuticals Corporation) (Figure 1.5a). Octreotide demonstrated a three-fold higher potency in insulin inhibition as well as a nearly 20-fold higher potency in GH inhibition relative to the native hormone [57]. Following its launch in 1983, it proved a highly valuable treatment option for a number of conditions such as carcinoid syndrome, intestinal, pancreatic, and pituitary tumors [58]. Octreotide's ability to suppress pituitary secretion of GH also made it invaluable for the treatment of acromegaly. In addition to octreotide, a structurally similar SST analog lanreotide (Somatuline®, Ipsen S.A.S.) (Figure 1.5b) was approved in 2007 and is used to treat various gastro-enteropancreatic-neuroendocrine tumors (GEP-NETs). Both octreotide and lanreotide are available as extended time-action depot formulations: Sandostatin LAR Depot from Novartis and Somatuline Depot from Ipsen.

1.4.3 Peptide–Drug Conjugates

The clinical success of antibody–drug conjugates (ADCs) has validated Paul Ehrlich's century-old concept of a targeted "magic bullet" cancer therapy. The exquisite selectivity of peptide ligands has been successfully harnessed for the design of peptide–drug conjugates (PDCs), utilizing the same receptor targeting

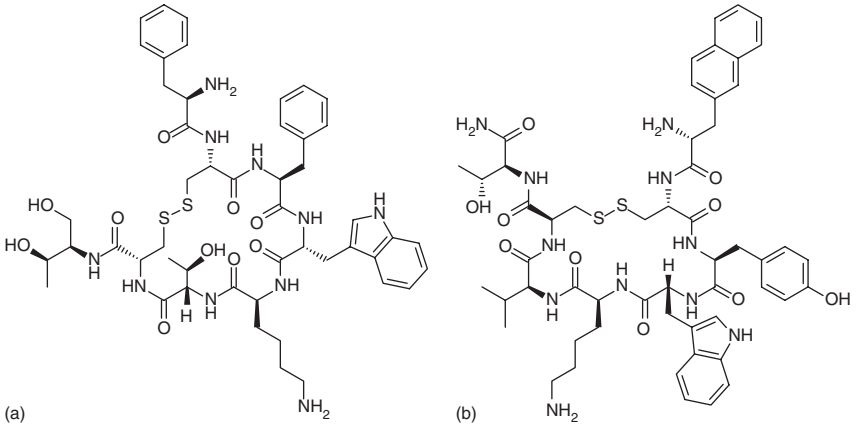


Figure 1.5 Structures of octreotide (a) and lanreotide (b).

strategy as the ADCs to deliver a cytotoxic payload directly to a tumor while minimizing systemic toxicity [59]. Substitution of an antibody with a peptide offers several advantages such as a reduction in immunogenicity, and a potential increase in cell and tissue penetration. From a technical standpoint, the installation of a linker and drug conjugation may be more straightforward in the case of a PDC. In addition, the peptide's lower molecular weight also permits higher drug loading relative to an ADC.

In the case of LHRH-based PDCs, several cytotoxic analogs have been evaluated clinically, including AEZS-152, a doxorubicin conjugate of [D-Lys6]-LHRH [60]. The octreotide platform also continues to be of great utility for both diagnostic and therapeutic applications [61]. The imaging agent 111 In-DPTA-octreotide (diethylene triamine pentaacetic acid) (OctreoScan® Mallinckrodt Pharmaceuticals) has been used extensively to localize somatostatin-expressing neuroendocrine tumors (NET) while 99mTc-depreotide (NeoTect® Diatide, Inc.) is used as a diagnostic for small cell lung cancer. The octreotide scaffold also serves as a targeting ligand in peptide radionuclide therapy. 177Lu octreotate (Lutathera® Advanced Accelerator Applications) was recently approved by the FDA for the treatment of somatostatin receptor-positive GEP-NETs [62].

Integrin motifs, such as ArgGlyAsp (RGD) and AsnGlyArg (NGR), are used to target tumor vasculature. NGR-hTNF is a fusion protein consisting of the CNGRCG tumor homing peptide and tumor necrosis factor (hTNF) cytokine [63]. NGR-hTNF is being investigated for the treatment of malignant pleural mesothelioma, either as a stand-alone therapy or in combination with standard chemotherapeutic regimens. Mipsagargin is a conjugate of a prostate-specific membrane antigen (PSMA) homing peptide fused with the cytotoxic sesquiterpene lactone thapsigargin, which is in clinical trials for hepatocellular carcinoma [64]. ANG 1005 is a conjugate formed with paclitaxel and angiopep-2, a peptide targeting the low density lipoprotein receptor-related protein 1 (LRP-1), being developed by Angiochem, Inc. for a glioblastoma indication [65].

1.4.4 Cancer Vaccines

Cancer vaccines have been previously tested in late-stage or metastatic settings and have shown only modest results [66]. Recently, however, this therapeutic approach has made enormous progress and for the first time gained substantial clinical validation [67]. The two basic peptide-based immunization strategies can be classified as utilizing either self-antigens or neo-antigens and are discussed briefly below. Successful vaccination with a self-antigen, typically directed at an overexpressed receptor, has been demonstrated in animal models and recently in the clinic. A breast cancer vaccine, Neuvax™, based on a human epidermal growth factor receptor-2 (HER-2) immuno-dominant epitope administered with granulocyte colony-stimulating factor (GMCSF) as an adjuvant has been evaluated extensively in a number of clinical trials designed to reduce breast cancer recurrence [68]. A meta-analysis has shown that Neuvax either as a single agent or in combination with Herceptin reduces risk of recurrence and prolongs both disease-free and overall survival [69]. Despite these promising results, the self-antigen strategy is based on a single epitope that may diminish the magni-

tude of the immune response [70]. The strategy is further limited by major histocompatibility complex (MHC) restriction to HLA-A2 and HLA-A3 positive patients [71].

An emerging vaccine paradigm involves amplification of tumor-specific T-cell response through immunization with multiple neo-antigens, unique sequences arising from cancer-specific somatic mutations. In contrast to self-antigens, neo-antigens bypass central thymic tolerance and are therefore more likely to produce a strong T-cell response [72]. The sequences of the neo-antigen peptides are predicted from algorithms utilizing genomic, proteomic, and predicted MHC binding data from an individual patient's tumor, synthesized and assembled into a personalized cancer vaccine. The strategy has been successful in small clinical trials, particularly in cases of tumors with a high mutagenic load. Of six patients diagnosed with advanced melanoma and treated with personalized neo-antigen vaccines, four were recurrence free 25 months post vaccination [73]. The two patients who experienced recurrence responded favorably to anti-programmed cell death protein-1 (anti-PD-1) therapy, an effect attributed to stimulation of their tumor-specific T cells. In another study, late-stage melanoma patients were treated with a poly-epitope vaccine constructed from two synthetic ribonucleic acids (RNAs) encoding linker connected antigens. Of the 13 patients in the study, eight remained recurrence free for 12 to 23 months. Of the five who relapsed, one achieved complete response to an anti-PD-1 antibody [74].

1.5 Peptides in Bone Diseases

As a molecular class, peptides are underrepresented in the treatment of bone disease. With the singular exception of calcitonin, used for the treatment of osteoporosis, most drugs approved for bone diseases have been orally administered – small molecules such as estrogen, the selective estrogen receptor modulator, raloxifene (Evista®, Eli Lilly & Co.), and the bisphosphonates. The competitive landscape changed dramatically with the introduction of teriparatide and later abaloparatide, peptides, which have demonstrated restoration of osteopenic bone and a reduction in fracture rates.

1.5.1 Calcitonin

A 32 amino acid peptide first described in the early 1960s, calcitonin (Figure 1.6a,b), is secreted by the parafollicular cells of the thyroid gland and is responsible for maintaining calcium homeostasis and regulation of bone turnover [75]. Because of its greater pharmacological potency, salmon calcitonin has been utilized in place of human calcitonin for most therapeutic applications. Initially approved in 1984 for the treatment of postmenopausal osteoporosis, calcitonin is currently also prescribed for Paget's disease and hypercalcemia. Its anti-resorptive activity at bone is mediated primarily through inhibition of osteoclast formation, although its precise mechanism is still not entirely clear. Clinical studies established that persistent parenteral administration of salmon calcitonin prevented postmenopausal bone loss and increased bone mineral

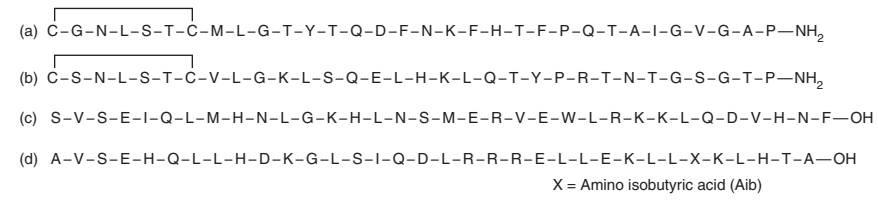


Figure 1.6 Structures of (a) human calcitonin, (b) salmon calcitonin, (c) teriparatide, and (d) abaloparatide.

density (BMD) [76]. Later studies with daily injectable salmon calcitonin also demonstrated an ability to reduce the risk of both vertebral [77] and hip fractures [78]. In addition to its potent *in vivo* activity, salmon calcitonin is reported to exhibit high bioavailability by nasal administration, estimated at 10 to 25 %. This offers an important option, which has facilitated greater patient acceptance and compliance. Clinical results with nasal calcitonin (Miacalcin®, Mylan), however, have demonstrated only a modest 1.7 % increase in BMD in osteoporotic women after one year [79] and also appeared inferior in a 12 month study versus alendronate [80]. The efficacy of nasal calcitonin in prevention of fractures is also less convincing, despite numerous clinical studies. A closer analysis of clinical trial data suggests that calcitonin pharmacology is, generally, more pronounced in patients with high bone turnover and established osteoporosis. The potential clinical utility of calcitonin is not limited to existing bone indications; it has also been investigated for its analgesic properties in the treatment of acute vertebral fracture pain [81] and osteoarthritis [82].

1.5.2 Parathyroid Hormone (PTH) (1–34) and (1–84)

Originally isolated by Collip in the 1920s [83], parathyroid hormone (PTH) was noted for its effects on calcium levels in the 1930s, but not fully investigated for its therapeutic potential until the 1970s. An early, small clinical study of 21 patients with osteoporosis confirmed that PTH (1–34) dosed daily at 100 μg for 6 to 24 months resulted in significant increases in new bone formation, particularly in trabecular bone [84]. This proved to be a seminal finding supporting therapeutic use of the hormone, since chronic overexposure to PTH as observed in hyperparathyroidism leads to osteoporosis. Subsequently, a number of clinical trials established the efficacy of PTH (1–34) in the management of severe osteoporosis in postmenopausal women, particularly those who had suffered a previous vertebral fracture. These studies revealed improvements in a number of clinical end points such as trabecular and cortical bone mass, mineral content, density, and fracture healing [85]. Additional studies also confirmed the importance of intermittent exposure to PTH (1–34) to achieve optimal anabolic activity, rather than continuous dosing with persistent plasma elevation of the hormone [86]. PTH (1–34) offers unprecedented therapeutic benefits relative to calcitonin, the bisphosphonates, and estrogen, which achieve their effect primarily through inhibition of osteoclast-mediated bone resorption. These clinical results demonstrated the seminal point that pharmacology differs from

physiology and pathology [87]. Through careful selection of clinical dosing and intermittent administration, the biological outcome derived from PTH is completely reversed from bone loss to bone growth. PTH (1–34) pharmacological effects are largely the result of its anabolic properties, which include bone-lining cell activation, osteoblast cell differentiation, and proliferation [88]. PTH (1–34) was approved in 2002 by the FDA for the treatment of osteoporosis in postmenopausal women and men with high risk of fracture as teriparatide (Forteo®, Eli Lilly & Co.) (Figure 1.6c). Clinical data collected prior to and post FDA approval support Forteo as the first truly regenerative medicine that stimulates active bone formation and restores osteopenic bone to near normal health. Despite some initial controversy, which pertained to the prospect of irreversible effects of bisphosphonates, teriparatide has been shown to work well with their concurrent administration, presumably because of the additive anti-resorptive effect of the oral agents [89]. The risks of teriparatide include increased blood calcium levels and osteosarcoma. The latter effect, which was specific to rodent studies, resulted in a restricted label with a clinical use limit of two years. The full-length PTH (1–84) protein was marketed for osteoporosis indication in the European Union as Preotact® starting in 2006, until its withdrawal for commercial reasons in 2014. Currently, it is approved for the treatment of chronic hypoparathyroidism in the European Union (as Natpar®, Shire Pharmaceuticals) and the United States (as Natpara®).

1.5.3 Parathyroid Hormone Related Protein

Parathyroid Related Protein (PTHrP) and PTH signal through a common receptor; however, there are notable differences in their respective modes of receptor interaction. A recent *in vitro* study examined the binding preference of PTH and PTHrP peptides to the R0 (G-protein-independent) and RG (G-protein-dependent) conformations of the PTHr1 [90]. The findings indicated that while both PTHrP and PTH peptides bind to the RG conformation of PTHr1, the PTHrP ligands bind the R0 conformation with much lower affinity. The high RG selectivity of PTHrP is characteristic of a transient signaling response and consistent with a pronounced anabolic effect relative to PTH. The recently approved 36 amino acid fragment of PTHrP, abaloparatide (Figure 1.6d) (Tymlos®, Radius Health, Inc.), exerts qualitatively similar pharmacological effects as teriparatide, with several notable therapeutically significant differences. Studies in human osteoblast cells indicated that abaloparatide exerts a lesser effect on the expression of bone-resorptive factors compared to teriparatide, supporting an overall greater net anabolic character than the latter [91]. Other clinical studies, notably a pivotal 18-month Phase 3 study (ACTIVE) in 2463 postmenopausal women using abaloparatide, teriparatide, and placebo revealed respective vertebral fracture rates of 0.6 %, 0.8 %, and 4.2 %, and non-vertebral fracture rates of 2.7 %, 3.3 %, and 4.7 %. In addition, the study noted lower hypercalcemia rates for abaloparatide than for teriparatide (3.4 % versus 6.4 %), consistent with the hypothesis that the former agent exerts a lesser bone-resorptive effect than the latter. While the overall results were supportive of greater efficacy of abaloparatide, the study noted slightly higher rates of adverse events for this agent [92].

1.5.4 Incretin Peptides

Recently, a number of peptides have been investigated for their bone remodeling/healing potential, and while these have not advanced into clinical trials, they nevertheless show promise. The growing interest in the extra-pancreatic actions of GLP-1 and GIP has revealed a strong link between incretin activity, bone strength, and fracture reduction [93]. A bone-densitometry study by Yamada [94] of GLP-1 receptor knockout mice and their littermate controls revealed cortical osteopenia and bone fragility in the receptor-deficient animals, due to increased osteoclast resorption. GLP-1 and exendin-4 also reversed osteopenia in hyperlipidic and hypercaloric rat models [95, 96]. Clinical data, however, appear mixed as a meta-analysis conducted by Su [97] found a significant decrease in bone fractures in patients treated with liraglutide, but an increase in patients treated with exenatide.

Preclinical evidence for a bone-related benefit seems more compelling in the case of GIP. GIP overexpressing mice exhibited increased bone formation and decreased bone resorption [98], while GIP receptor deficient mice exhibited bone weakening including decreased cortical thickness, increased resorption, and decreased bone mineralization [99]. An important link to human biology comes from association of functional GIP receptor polymorphism Glu354Gln and fracture risk conducted by Torekov et al. [100]. The Glu354Gln substitution attenuates GIP signaling, resulting in lower insulin secretion and higher glucose levels. Women with this allele in a 10 year period were found to have lower bone density and greater fracture risk.

1.5.5 Bone Morphogenic Protein-Derived Peptides

Bone morphogenic proteins (BMPs), members of the TGF-β (transforming growth factor) family, play an important role in bone formation and development [101, 102]. A number of peptide sequences derived from BMP proteins have shown potent osteogenic activity in animal models. Two peptides derived from BMP-7, bone-forming peptide-1 and 2, stimulated differentiation of bone marrow stem cells *in vitro* and *in vivo* [103, 104]. Work with BMP-9 identified a peptide that promoted the differentiation of pre-osteoblasts and deposition of calcium, conditions required for bone mineralization [105, 106].

1.6 Peptides in Gastrointestinal Diseases

The treatment of gastrointestinal (GI) disorders has been one therapeutic area where peptide drugs played virtually no role until very recently. The low pH and presence of proteolytic enzymes have made the GI track particularly unsuitable for peptide drugs. Nevertheless, in the last six years three new important peptide drugs have been launched, each addressing unmet needs in GI disease states.

1.6.1 Glucagon-like Peptide-2

A 33 amino acid peptide secreted by entero-endocrine L-cells, glucagon-like peptide-2 (GLP-2) exerts a number of effects on the GI tract. Most notably, it

increases intestinal nutrient absorption and the stimulation of intestinal growth mediated by the release of insulin-like growth factor-1, epidermal growth factor, and keratinocyte growth factor [107]. Much like GLP-1, GLP-2 has a short biological half-life of approximately seven minutes, due to DPP-IV inactivation. Teduglutide (Figure 1.7) is a DPP-IV-stabilized GLP-2 analog where the native Ala at the second position is replaced by Gly. The clinical development of teduglutide sponsored by NPS Pharmaceuticals focused on short bowel syndrome (SBS), a condition characterized by significant loss of bowel mass and function. Patients afflicted by SBS may lose sufficient absorptive function to the extent that they need to rely on parenteral support (PS) to maintain nutrient intake and electrolyte balance. A 21 day Phase II open label clinical study of teduglutide demonstrated a statistically significant increase in intestinal wet weight absorption in 15 of 16 patients [108]. The trial also noted favorable histological changes in most patients, specifically increases in villus height, crypt depth, and mitotic index. A subsequent Phase III study in SBS patients who had suffered intestinal failure met its primary end point, demonstrating a statistically significant reduction in PS requirements, as well as a secondary end point of allowing patients to gain additional days off PS, or entirely eliminating the need for it [109]. Teduglutide was approved by the FDA in 2012 for adults with SBS requiring PS support and marketed as Gattex® by NPS Pharmaceuticals. Zealand Pharma recently announced initiation of a Phase 3 trial of their GLP-2 analog glepaglutide in SBS.

1.6.2 Guanylate Cyclase-C Agonists

The natriuretic peptide hormones guanylin and uroguanylin (Figure 1.8a,b respectively) are produced by the entero-endocrine cells of the GI tract and sig-

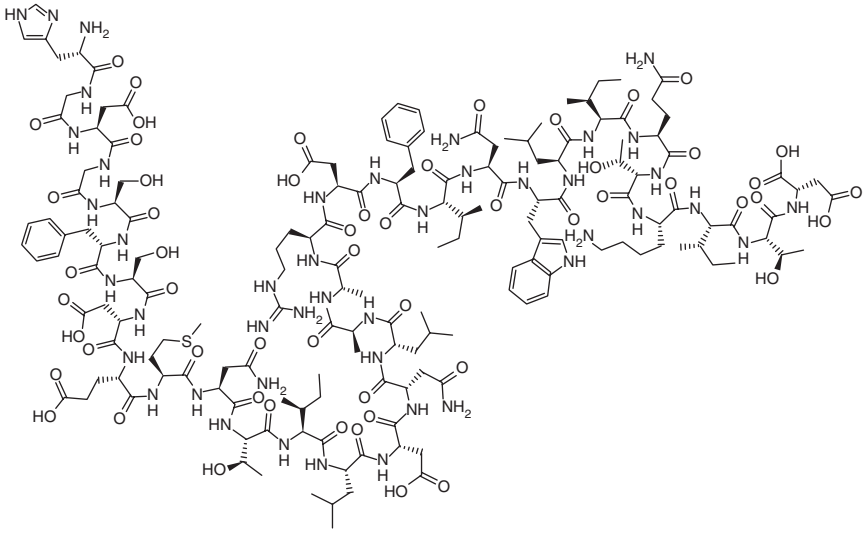


Figure 1.7 Structure of teduglutide.

nal through the guanylate cyclase (GC-C) receptors located on the intestinal enterocytes. Activation of the GC-C pathway is essential for maintaining fluid as well as chloride and bicarbonate ion homeostasis within the GI tract, which are supportive of healthy intestinal transit [110]. Linaclotide (Figure 1.8c), a peptide closely related to guanylin and uroguanylin, was developed by Ironwood Pharmaceuticals for symptoms related to constipation-predominant irritable bowel syndrome (IBS-C). Oral administration of linaclotide to healthy volunteers was shown to be safe and efficacious with a dose-dependent increase in stool frequency and weight [111]. Remarkably, the study also found no evidence of systemic absorption following oral dosing, suggesting that linaclotide works locally in the GI tract. In a subsequent placebo-controlled Phase II study in patients suffering from IBS-C, linaclotide improved bowel function in terms of frequency, severity of straining, stool consistency, and abdominal pain [112]. In a 26 week, double-blind, placebo-controlled Phase III trial in 804 patients, daily oral administration of 290 μg of linaclotide showed statistically significant improvements in the frequency of complete spontaneous bowel movements (CSBM) and a reduction of abdominal pain episodes [113]. Based on this as well as an additional Phase III clinical study linaclotide was approved by the FDA for adults suffering from IBS-C and chronic idiopathic constipation (CIC) in 2012 and marketed as Linzess® by Allergan and Ironwood Pharmaceuticals.

Plecanatide (Figure 1.8d) is a GC-C agonist peptide structurally related to uroguanylin, differing only in the substitution of Glu3 for the native Asp3. Plecanatide reversed symptoms of acute and chronic ulcerative colitis in several animal models [114], suggesting that uroguanylin and possibly guanylin deficiency may be a causative factor in GI inflammation. Synergy Pharmaceuticals undertook the development of plecanatide for various gastrointestinal disorders. A Phase I clinical study found plecanatide to be safe, and similarly to linaclotide, showed no evidence of systemic absorption [115]. A larger 12 week trial tested 3 and 6 mg doses of plecanatide or placebo in 1346 patients suffering from CIC. Both dose cohorts met the primary and secondary end points of significantly increasing CSBM and spontaneous bowel movements (SBM) [116]. The favorable results of

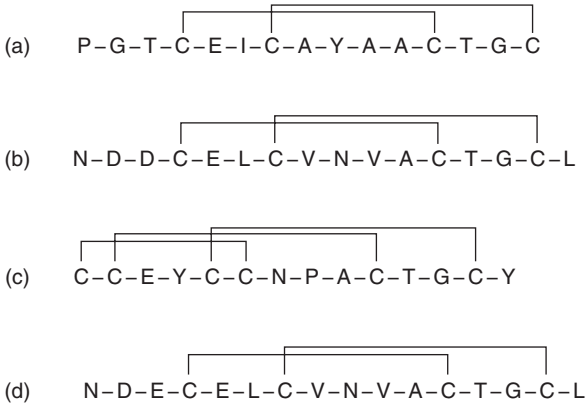


Figure 1.8 Structures of (a) guanylin, (b) uroguanylin, (c) linaclotide, and (d) plecanatide.

this trial, as well as additional Phase III trials in CIC and IBS-C patients, supported the FDA approval of plecanatide (Trulance®, Synergy Pharmaceuticals).

There are currently a number of peptide drugs in development for GI disorders and are summarized in a recent review [117]. These include larazotide, a peptide based on zonula occludent toxin, which is being advanced by Innovate Pharmaceuticals for celiac disease in patients who are symptomatic despite adhering to a gluten-free diet [118]. Relamorelin, a ghrelin agonist, is being investigated for several GI conditions including CIC and gastroparesis, the latter a currently an unmet medical condition [119].

1.7 Emerging Trends in Peptide Drug Discovery

1.7.1 Cell-Penetrating Peptides

The lack of membrane permeability, which effectively prevents access of various drugs to intracellular targets, is a common problem in drug discovery. Thirty years ago, novel peptides capable of transporting cargo across biological membranes were termed cell-penetrating peptides (CPPs). Members of this group range from 5 to 40 amino acids and have been successfully used to facilitate intracellular uptake of various cargoes including proteins, quantum dots, and siRNAs [120–122]. Recently, confirmation of the ability of CPPs to transport drug cargo safely into cells was demonstrated in preclinical and clinical studies. CPPs can be utilized in the form of a non-covalent complex or as a covalently bound drug conjugate. CPPs fall into three major categories and they include cationic, amphipathic, or hydrophobic. A representative of the cationic class, which was also the first CPP family member identified, is the trans-activating transcriptional (TAT) activator from human immunodeficiency virus 1 (HIV-1) (see Figure 1.9 for the sequences of various CPPs) [123, 124]. The minimal HIV-TAT derived sequences required for transduction were characterized several years later [125, 126]. Another important CPP is penetratin, a minimized 16 amino acid cationic sequence derived from Antennapedia, a homeoprotein of *Drosophila melanogaster* [127, 128]. Two examples of amphipathic CPPs containing both hydrophilic and hydrophobic amino acids, are transportan [129] and the model amphipathic peptide (MAP) [130]. Hydrophobic CPPs such as the Pep-7 peptide [131] are less common but are of particular interest as a result of their energy-independent mechanism. The list of CPPs is growing and their use is being explored for the treatment of infectious diseases [132], inflammation [133], and cancer [134] with several candidates in late-stage clinical development

HIV-TAT (48–60)	GRKKRRQRRRPPQ
Penetratin	RQIKIWFQNRRMKWKK
Transportan	GWTLNSAGYLLGKINLKALAALAKKIL
MAP	KLALKLALKALKAALKLA
Pep-7	SDLWEMMMVSLACQY

Figure 1.9 Structures of various CPPs.

[135]. The preferred CPP peptides used in preclinical and clinical trials today are generally based on the shorter TAT or penetratin sequences. Kai Pharmaceuticals (acquired by Amgen in 2012) had advanced at least two compounds containing TAT derivatives into clinical trials (KAI-9803 [136], KAI-1678 [137]). Sarepta Therapeutics, a company with multiple CPP-based clinical candidates, has developed eteplirsen, a phosphorodiamidate morpholino oligomer (PMO) conjugated to a proprietary arginine-rich CPP, to treat Duchenne muscular dystrophy, which received approval as Exondys 51™ [138]. Brimapitide, an arginine-rich sequence combined with a c-Jun-N-terminal kinase (JNK) inhibitor developed by Xigen, has received FDA fast track status for hearing loss treatment [139].

1.7.2 Macrocyclic Peptides

Natural product-derived macrocyclic peptides, such as vancomycin and cyclosporine, serve as an important historical precedent for peptide medicinal chemists. As a class, macrocyclic peptides possess properties that are atypical of those of conventional peptides, particularly protease stability, and in the rare case of cyclosporine, even oral availability [140]. A wide variety of chemistries can be used to construct macrocyclic peptides including disulfide, thioether, head-to-tail, and depsi-peptide bonds [141, 142]. In general, as the size of the macrocycle increases, the structure becomes more flexible and susceptible to proteolytic degradation. Additional constraint in the form of a second ring not only enhances proteolytic stability but can also increase affinity and selectivity, an approach pioneered by Bicycle Therapeutics. The company is advancing BT1718, a bicyclic macrocyclic peptide, for the treatment of advanced solid tumors. A second clinical candidate based on this technology, THR-149, a novel plasma kallikrein inhibitor for the treatment of diabetic macular edema, is being developed by Thrombogenics. PeptiDream has combined genetic code reprogramming to incorporate non-proteinogenic amino acids into vast libraries of macrocycles. The company's proprietary Peptide Discovery Platform System (PDPS) uses Flexizymes, artificial ribozymes capable of charging tRNAs with unnatural amino acids [143]. Aileron Therapeutics has applied the Grubbs olefin metathesis to construct "stapled peptides," which exhibit remarkable protease stability as well as unique ability to engage intracellular targets such as BCL-2 and MDMX and MDM2, by virtue of their covalently stabilized α-helical structure [144]. Aileron has used this platform to deliver their ALRN-6924 clinical candidate, an MDMX/MDM2 inhibitor currently in multiple Phase 1 and 2a clinical trials for the treatment of advanced solid tumors, peripheral T cell lymphoma (PTCL), acute myeloid leukemia, and advanced myelodysplastic syndrome [145]. Other companies focusing on the development of therapeutics based on cyclic peptides include Ra Pharma and Polyphor.

1.8 Summary

The peptide therapeutic class has experienced significant growth in recent years, as is evident from the approved and late-stage clinical programs highlighted in this

chapter. However, a comprehensive survey of earlier clinical and preclinical efforts suggests that this growth will accelerate in the future as peptides impact multiple therapeutic areas. A key reason for this trend is the inherent efficacy of peptide drugs in restoring normal physiological function to patients suffering from diabetes, osteoporosis, and other chronic diseases. This attribute has often enabled peptides to compete successfully against less efficacious oral agents. One example is the commercial success of injectable GLP-1 agonists when competing against orally administered DPP-4 inhibitors. Another is the superior performance of injectable PTH (1–34), relative to the oral bisphosphonates. The traditional advantages of oral agents over injectable drugs have recently narrowed through introduction of extended duration peptide analogs, which prolong the dosing interval from days to weeks or even longer. Additionally, a number of recently introduced peptide drugs such as the GC-C agonists can be delivered orally, and several more orally administered peptide candidates are in late stage clinical trials. Innovative concepts such as cell-penetrating peptides, stapled peptides, and peptide–drug conjugates promise to enable engagement of diverse, previously inaccessible targets. We anticipate that the above trends will expand the niche for peptide therapeutics in the pharmaceutical armamentarium of the future.

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List of Abbreviations

ADC	antibody–drug conjugates
Aib	2-aminoisobutyric acid
AMP	antimicrobial peptide
BMD	bone mineral density
BMP	bone morphogenic protein
CPP	cell-penetrating peptide
CRL	complete response letter
CSBM	complete spontaneous bowel movement
DDP-IV	dipeptidyl peptidase-4
CIC	chronic idiopathic constipation
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DTPA	diethylene triamine-pentaacetic acid
FDA	food and drug administration
FSH	follicle stimulation hormone
GC-C	guanylate cyclase C
GEP-NET	gastro-enteropancreatic neuroendocrine tumors
GH	growth hormone

GI	gastrointestinal
GIP	glucose-dependent insulinotropic polypeptide
GLP-1	glucagon-like peptide-1
GLP-2	glucagon-like peptide-2
GPCR	G-protein-coupled receptor
GMCSF	granulocyte colony-stimulating factor
HER-2	human epidermal growth factor receptor-2
HIV	human immunodeficiency virus
HSA	human serum albumin
hTNF	human tumor necrosis factor
IBS	irritable bowel syndrome
IgG	immunoglobulin G
JNK	c-Jun-N-terminal kinase
LHRH	luteinizing hormone releasing hormone
LRP-1	low-density lipoprotein receptor-related protein 1
MAP	model amphipathic peptide
MHC	major histocompatibility complex
NET	neuroendocrine tumor
NPH	neutral protamine Hagedorn
PD-1	programmed cell death protein-1
PDC	peptide-drug conjugate
PDPS	Peptide Discovery Platform System
PEG	polyethylene glycol
PK/PD	pharmacokinetic/pharmacodynamic
PLG	poly(D,L-lactide-*co*-glycolide)
PMO	phosphorodiamidate morpholino oligomer
PS	parenteral support
PSMA	prostate-specific membrane antigen
PTCL	peripheral T cell lymphoma
PTH	parathyroid hormone
PTHrP	parathyroid hormone related protein
RNA	ribonucleic acid
SBM	spontaneous bowel movement
SBS	short bowel syndrome
SST	somatostatin
TAT	trans-activating transcriptional activator
TGF	transforming growth factor

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