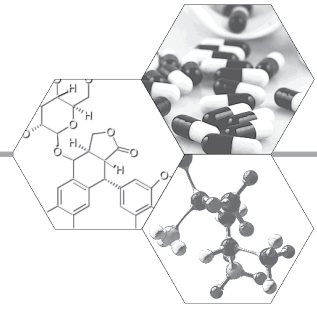

Design and development of peptides and peptide mimetics as antagonists for therapeutic intervention

The concept of peptides as therapeutic agents has been historically disregarded by the pharmaceutical industry on account of their susceptibility to degradation, their size and consequent limitations in methods of delivery. Recently, however, there has been a surge of interest in peptides and their mimetics as potential antagonists for therapeutic intervention. This is in part due to the increased half-life and oral availability that has been achieved for a number of peptide-based systems, the introduction and acceptance of alternative delivery methods, and the prevalence of proteomics to identify countless protein–protein interaction targets. The use of peptides and molecules that mimic their function therefore has great potential to effectively target a range of proteins that are pathogenically implicated in numerous diseases.

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An enormous area that has been avoided in favor of small-molecule approaches is the use of short peptides (e.g., from two up to 50 residues in length) and their mimetics to inhibit **protein–protein interactions** (PPIs) implicated in disease pathways. There is increasing pressure on research and development and yet fewer drugs are being brought to market. This has led to a surge in the search for peptide-based antagonists, or 'biodrugs'. Their use as therapeutics is particularly exciting in this context since most PPIs bring large surface areas into close contact to form an interaction that lacks a clearly defined binding pocket suiting traditional small molecules. In addition, their high specificity and low toxicity offer a viable alternative to the small molecule. The consequent advent of proteomics and interactomics to define such disease pathways has resulted in this becoming an immense area in the design and identification of interfering peptide-based antagonists, with knowledge of PPI pathways and targets that do not suit small-molecule intervention increasing rapidly. Indeed, in recent years, while the number of new chemical entities attributable to small molecules discovered each year has remained unchanged, those attributable to peptides and proteins has seen a steady increase [1].

Pros & cons of peptides as therapeutic agents

There are numerous potential benefits as well as some drawbacks to the use of peptides. Many of these drawbacks (e.g., poor tissue penetration, serum resistance, oral bioavailability and quick elimination) were discovered during investigation of the pharmacological properties of peptide antagonists, and have led pharmaceutical companies instead to invest in small-molecule approaches. However, more recently it has been shown that many drawbacks (e.g., protease resistance, solubility and logP) can be dealt with by modification of the peptide, while others (e.g., the size of the molecule) are more difficult to circumvent. Some of the advantages and drawbacks in peptide-based discovery, methods available to circumvent these issues and methods for developing peptide-based antagonists in general are discussed in this article.

■ Potential benefits of peptide based drugs

The largest benefit of peptides over small molecules is their innate ability to block PPIs where binding pockets amenable to small molecule inhibition can be more difficult to be found. A second advantage is that small peptides are unlikely to invoke an immune response since they fall below the immunogenic threshold. A third advantage is that peptides can display high levels of chemical and biological diversity, allowing a more specific interaction to be formed than with small-molecule approaches, giving rise to a greater efficacy for their desired target. The physiological dexterity of peptides opens up a broad range of pharmacological effects for peptide chemistry, and companies can hope to generate many structures of importance. Although

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Key Term

Protein–protein interaction: The interaction of two similar (homodimeric) or dissimilar (heterodimeric) protein chains to bring about a biological function.

peptide chemistry may not be as broad as small-molecule chemistry, this is compensated for by the fact that problems can be readily addressed and that the molecules being mimicked are particularly important or interesting [2]. A fourth advantage, owing to the biological nature of peptides, is that they often have fewer toxicology issues arising from xenobiotic metabolism. Indeed, they are often potent and display fewer toxicity issues than small-molecule compounds as a result of high specificity. The ability to modify the rate of turnover and susceptibility to protease action is also likely to influence the level of toxicity and immunogenicity.

■ Potential drawbacks of peptide-based drugs

Potential drawbacks of peptides that have historically meant they have not been pursued as therapeutics includes low oral availability; a delivery method that is a convenient and necessary for many drugs. However, low oral availability has meant that peptides often need to be injected to be effective. However, in recent years there has been a wider acceptance of such alternative methods of delivery, with lack of oral availability becoming less of a discouragement for further investigation. There are also obvious stability issues and transporting peptides across membranes can be problematic. Additionally, there can be issues with solubility and peptides can potentially be cleared from the body rather rapidly (often in minutes). Their increased size means that they can be difficult to synthesize recombinantly (since they are often too small for this approach) and are yet expensive to synthesize chemically owing to the resins, protected amino acids, and coupling agents that must be used, and the large amount of peptide required for them to be effective. In addition, the larger the molecule, the larger the complexity and therefore the cost involved in its synthesis, with a 5000-Da peptide costing in excess of 10-times the cost of a 500-Da peptide to produce owing to the increased amount of starting materials required [3,4]. Another peptide drawback that is not observed with small molecules, which have been more extensively investigated, is the knowledge that peptide research is only recently beginning to mature to the extent that investigators can readily develop synthetic analogs of natural peptides with the desired pharmacological properties. However this is changing with the advent of semi-rational library based-design combined with directed evolution approaches (see discussion later). Finally, there are very few examples of unmodified peptides that have made good drug candidates, with one huge problem for therapeutic peptides being proteolytic instability. A large range of proteases recognize structural features common to a vast range of peptides. However, methods are now arising by which peptides are selected first and later structurally modified to improve these pharmacokinetic properties. Indeed, innovative synthetic strategy approaches are maturing. This combined with routine peptide synthesis and a lower Fmoc amino acid price is helping to accelerate the path to peptide-based drug development.

Peptide discovery

■ Rational design

Non-library techniques are often based on a known or predicted structure to derive a sequence predicted to interfere with its target. For example, in the design of β-sheet breaker peptides to abolish the formation of amyloid implicated in neurological diseases such as Alzheimer's, CJD and Parkinson's [5,6]. Examples include the use by Soto *et al.* of a short amyloidogenic region of $A\beta_{1\text{-}42}$, whereby a proline residue was inserted into it to form a kink in the natural β-sheet peptide [7]. Murphy *et al.* created a peptide based on the same amyloidogenic sequence in which a charged region was added and found to increase amyloid formation while lower toxicity [8]. In addition, modification of the peptide with nonpeptidic constraints such as replacing the backbone N-C groups with alternative atoms of similar geometry, *N*-methylation of the peptides [9,10], or attaching bulky non-native groups to the N- or C-terminus of the peptide [11] are all methods that have been exploited to improve the druggability of lead molecules.

Combinatorial methods for deriving peptide antagonists

There are a range of systems that have been used to generate and screen peptide libraries to identify tight-binders as the starting point for investigation. The advantage of such systems is that one can use a limited amount of information both structurally and chemically in the design of the antagonist (e.g., sequence-based knowledge of the region targeting the binding partner) and introduce randomizations into the sequence followed by sequence enrichment techniques to select peptides with improved binding properties. Importantly, all provide a means of linking the sequence of interest to the DNA that

encoded it. Once undertaken, the approach can be followed by iterations of randomization and selection to refine the sequence that is selected. Some of the approaches used in both semi-rational as well as fully randomized library-based design approaches are highlighted.

Protein-fragment complementation assays

Protein-fragment complementation assays (PCAs) are based on split protein systems, which then report on the formation of a target–antagonist complex either by generating a measurable color change (e.g., by using a split GFP) in a process known as bimolecular fluorescence complementation (BiFC) [12,13], or by cell survival using a split essential enzyme (e.g., ubiquitin or DHFR) [14]. This system involves transformation or transfection of cell lines using libraries to screen and select peptide antagonists that are capable of binding to their target and recombining the split enzyme under selective conditions. The assay is limited by the number of colonies that can be generated to represent the library but is carried out entirely *in vivo* (albeit in the context of bacterial or mammalian cell lines), therefore ensuring that selected peptides are not susceptible to protease action in the selected cell line, and are soluble and nontoxic [15,16]. For cell survival assays, growth competitions can be used to identify sequences that confer the tightest binders [17]. The attractiveness of this technique stems from the yeast two-hybrid assay [18], but the selection is more robust, giving rise to almost no false positives. More recently this technology has been modified to impose a greater degree of competitive and negative design on the system; by expressing potentially competing proteins in the system, nonspecific binders, or those unable to compete with competitor–target interactors are removed from the bacterial pool, generating peptides that bind their target with both high affinity and specificity. This technique is termed a 'competitive and negative design initiative' (CANDI) and has been used by the author and co-workers in the Jun-Fos system to create a peptide that has been derived to bind to cFos without binding to cJun. This CANDI–PCA technique generated a more specific antagonist relative to conventional PCA; by using cJun directly in the assay as a competitor the energy gap between the nondesired antagonist–cJun competing complex and the desired antagonist–cFos complex was maximized [19,20].

■ Phage display

Phage display is a widely used *in vitro* technique in which the target protein/peptide is immobilized and the library is expressed as a phage coat protein fusion [21]. The phage-containing solution is then washed such that if there is a genotype–phenotype linkage the phage can be sequenced to reveal the sequence of the tight-binding peptide [22–24]. Increasing stringency can be used to purify those peptides that bind with highest affinity to the immobilized target. Phage display is used extensively in the generation of antibodies in which the variable regions can be randomized at length. In addition, multivalent display on the surface can lead to avidity effects and the possibility to detect low-affinity binders. An additional and elegant method known as mirror-image phage display has been to convert the target into D-amino acid-containing peptides, such that the protein 3 fusion antagonist is selected as an L-peptide against the D-peptide epitope. The antagonist can then be directly translated into the proteolytically stable D-peptide form and provided that the target is extended in the form of a straightforward α-helical or β-sheet structure, it should bind with equally high affinity to the original L-amino acid target peptide [25,26] (see discussion on retro-inverso strategies later). Although less well used, examples of yeast display [27] and bacterial display [28] also exist but are not discussed here.

■ Ribosome display

Ribosome display is an *in vitro* technique that utilizes mRNA-based libraries that lack a stop codon to prevent halting of translation of the peptide during synthesis [29]. The technique uses low temperatures and cations (e.g., magnesium) to help stabilize the ribosome–mRNA complex. Thus, by placing additional residues at the C-terminus of the peptide, one is able to generate a peptide antagonist that is capable of binding its target while being linked to the mRNA that encoded its production. The mRNA can then be converted to a cDNA, sequenced [30,31], and the resulting sequence readily mutated using error prone PCR and rescreened and selected in an iterative process. An advantage of this technique is that efficiency is not limited by the number of cells that can be transformed. However, a disadvantage is that the technique is carried out *in vitro* and can therefore be expensive and difficult to setup. In addition, the noncovalent nature of the peptide–mRNA linkage means

that problems can arise from background binding. A 20,000-KDa ribosome in close proximity to antagonist and target can also lead to false positive interactions between the target and the ribosome.

■ mRNA display

Using mRNA display translated proteins and peptides become physically attached to their mRNA via a puromycin linkage [32,33]. Again the target is immobilized and panning rounds are performed to identify peptides that can bind to the target. Reverse transcriptase converts the mRNA to a cDNA, which can be readily sequenced. It allows large library sizes of up to 10^{14}–10^{15} to be sampled as again it is not limited by transformation efficiency or artefacts arising from the coupling of the peptide to the mRNA via a large ribosome complex. Once again, error-prone PCR can be performed quickly without having to retransform the cell line, and reselection can then occur to optimize binding affinity. Like CIS-display and ribosome display, it is an *in vitro* technique and thus removes selection pressures such as protein expression and protease resistance, but unlike ribosome display, the coupling of mRNA to peptide is more straightforward to carry out and there are fewer issues arising from the conjugation to a ribosome complex.

■ CIS-display

CIS display is an *in vitro* library selection system that exploits the ability of a DNA replication initiator protein (RepA) to bind exclusively to the template DNA from which it is expressed, a property called CIS-activity [34,35]. Thus, by fusing the library of interest to RepA, tight binders are linked to the mRNA encoding the fusion protein, and the sequence can be determined. Like phage display technology, this allows very large libraries to be generated and screened and high affinity binders can be identified. Again, library generation is *in vitro*, it is not restricted by bacterial transformation, thus the library size can be considerably increased. CIS-display is a proprietary technology belonging to Isogenica Ltd (Chesterford Research Park, UK).

■ Peptide aptamers & combinatorial chemical library approaches

Although not strictly the subject of this article, it is worthy of mention that there is also considerable promise for peptide aptamers and combinatorial chemical libraries. Aptamers provide a protein scaffold by which desired peptides can be 'presented' to their target in a proteolytically and structurally stable frame that circumvents conformational flexibility problems that can arise with free peptides [36]. Examples include both IgG-based monoclonal antibodies derived by both active and passive administration as well as small single chain antibodies [37]. However, the downside of this approach is that peptides are less likely to be able to penetrate further into tissues, or to cross membranes and the blood–brain barrier. In addition, there has been much attention focused on combinatorial chemical libraries that can serve to identify non-natural amino acid entities that can later be incorporated and even combined by introduction into peptide scaffolds while retaining their desired binding properties.

Peptide optimization methods

Numerous methods are being imposed to create a more intelligent approach to peptide antagonist development, namely the derivation of peptide analogues to expand the drug-like traits of the molecule (**Figure 1**). This involves engineering the desired chemical and pharmacological properties using the raw peptide, either rationally derived, semi-rationally derived, or derived using complete randomization as the starting point. While nature optimizes for physiological properties, protein engineers and chemists are optimizing for pharmacological properties, so it stands to reason that there is considerable scope within the amino acid repertoire to engineer these properties.

■ Peptide production

Once sequences are identified, peptides can be produced recombinantly using bacteria or yeast, or extracted from transgenic animals. Alternatively peptides may be produced in cell-free transcription/translation-based systems. This can be used to modify existing sequences, since they can utilize redundancy in the genetic code to permit the incorporation of non-native amino acids into the growing chain. Finally, chemical synthesis using Boc or more commonly Fmoc chemistry, allows for many more modifications to the chain that are not available by other means – for example, incorporation of unnatural amino acids, pseudo-peptide bonds, cyclic peptides, as well as the possibility to *N*-terminally acetylate and C-terminally amidate for increased stability (see later discussion). Finally, chemically produced peptides can be easily separated from their side products to yield a pure and homogenous

sample. This has made chemical synthesis the most preferred method for peptide synthesis, and additionally has implications for intellectual property as more unique molecules are able to be patented [4]. Indeed, many companies have sprung up to provide custom peptide-synthesis services. Much has been made from the small-molecule viewpoint that peptides must be modified to render them more 'Lipinski'-like [38,39], and there are numerous methods that have been developed to modify natural peptide sequences and to improve their pharmacological profile. However, Lipinski-like molecules are no longer considered essential for drug design, and the advent of non-Lipinski-like molecules increasingly suggests that larger molecules are not considered an immediate barrier to pharmacological success.

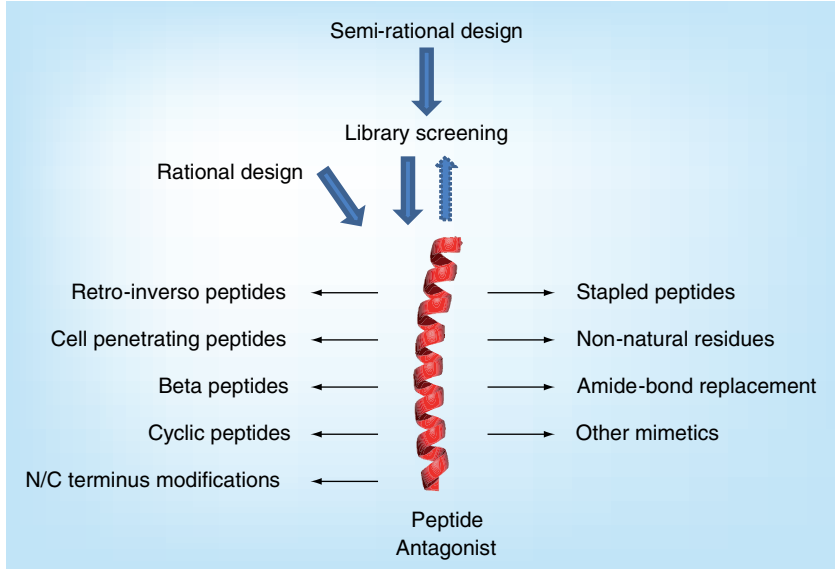

Figure 1. Using rational and semi-rational design followed by modification to derive molecules that retain desired drug-like attributes while circumventing some of the problems that can arise from unmodified peptides. In rational design, known binding regions are directly tested. Semi-rational design takes regions known to be important and uses them as the basis for a library scaffold in which improved binders can be identified. A range of modification techniques discussed in this article can then be applied to improve the pharmacological profile of a peptide.

■ Replacing peptide bonds with nonpeptidic constraints

There are a number of strategies in existence to replacement the N-C bond of peptides with alternatives with improved pharmacological properties. The overarching theme in all of these techniques is to design structures that are capable of effectively mimicking the overall topology of secondary structure on which they are based. There is a range of amide surrogates that mimic the natural peptide bond including peptoids [40] (whereby amino acid sidechains are attached to the amide group, rather than the α-carbon), oligoureas, peptidosulfonamide, and depsipeptides (in which amide bonds are replaced by ester bonds, thus removing a potential hydrogen bond) [41]. These serve to create amide bond surrogates with defined 3D structures similar to those of natural peptides, yet with significant differences in polarity, hydrogen bonding capability, and acid–base character. These strategies serve to lock the peptide into a biologically relevant conformation, stabilizing it both structurally and chemically, and helping to improve half life and oral availability. In addition, there is the possibility to replace side chains with non-natural groups (e.g., butylglycine, pyroglutamic acid norleucine and hydroxy proline), *N*-methylation of the N-H peptide group [9,10] and introduction of phosphate groups to create phosphonopeptides. Additonal stabilization strategies include acetylation and amidation of the termini, which can assist with endopeptidases, use of D-peptides in the peptide chain (see later discussion), peptide aldehydes, PEGylation [42] (e.g., Pegasys, a PEGylated IFN-α-2a, can be used to treat for hepatitis C) and β-peptides (in which the amide group is attached to the β-carbon, rather than the α-carbon). In addition, for conventional helical peptides, helix-capping motifs are often introduced. These serve to counter the fact that the first four N-H donors and final four C=O acceptors in a helix are not satisfied, which can cause helical fraying at the helix termini. Therefore, key residues at the termini can counter this effect by providing alterative bonds while addressing the helix dipole [43,44]. An elegant method for stabilizing further the N-terminal of an α-helical peptide is the introduction of a hydrogen bond surrogate to introduce a carbon–carbon bond at the N-terminus between i–i+4 terminal residues in such a way that it falls into the expected distances and angles required of a fully hydrogen-bonded helix [45,46]. The process works by forcing the helix termini into a helical conformation, thus overcoming the intrinsic nucleation barrier normally required to initiate helix formation. This has been used effectively in the stabilization of a peptide inhibitor to inhibit HIV-1 fusion [47].

■ Cyclic peptides & stapled peptides

These strategies represent an attractive methodology for constraining the peptide into a chemically and structurally stable, biologically active

Key Terms

Stapled peptide: The use of non-peptidic constraints such as hydrocarbon staples to lock peptides into a biologically active conformation.

Retro-inverso peptides: Peptides in which L-amino acids have been substituted for D-amino acids and the sequence reversed in order to mimic the function of the parent peptide while reducing proteolytic susceptibility.

Cell-penetrating peptides: Small positively charged/hydrophobic sequences that can be coupled to molecular cargo to facilitate its intracellular internalisation.

Peptide mimetic: A small peptide-like molecule designed to mimic a natural peptide while bringing desirable drug-like features such as stability and biological activity to the molecule.

conformation without the need for a large aptameric scaffold. It also has the additional advantage of reducing the susceptibility of the peptide to protease action. Peptides and mimeitcs can either be circularized between the N and C termini, or a covalent bond can be introduced between intervening parts of the molecule to create a peptide staple. Examples of such structures are known to occur naturally and again render the peptide resistant to protease action, leading such structures to be the focus of investigation for drug development. For example, by connecting *i*–*i*+4 or *i*–*i*+7 residues within a very small α-helical region of a protein, helicity and biological activity can be maintained [48–51]. This allows some of the advantages of proteins (e.g., specificity) to be combined with those of small-molecule inhibitors (e.g., cost, stability, delivery and uptake), and is also of use when context-dependant helicity can be problematic. **Stapled peptides** are particularly exciting in the context of downsizing antagonistic helices to generate very short yet highly stable α helical peptides that are resistant to protease action and denaturant [50]. It has even been speculated that 'stapled peptides' can inhibit targets that were previously considered undruggable owing to their large protein interface targets, for example inhibition of the Notch signaling pathway [52], or short peptides capable of targeting the GAG polyprotein of HIV [53]. Encouragingly, there are increasing numbers of 'stapled peptides' that impose helicity on very short (5–10-mer) sequences. Some of these peptides are small and charged and able to traverse membranes in isolation, while the staple has the additional benefit of inhibiting protease action [49]. Cyclic peptides, in which the N- and C- termini form a peptide bond with each other also show great promise, and methodologies have been developed to use this template as the basis for combinatorial design [54].

■ D-peptides & retro-inverso strategies

Approaches have also been used to maintain biological activity and specificity, while circumventing issues arising from proteases. As stated previously, this has been made use of in a technique known as mirror-image phage display where it has been used to generate a range of D-peptide antagonists [55–57]. Indeed, replacing the entire peptide with D-amino acids, either by a combinatorial library-based approach, or by rational means, can render the peptide immune to proteolytic attack, meaning that the peptide is entirely stable in human plasma and tissue. This process requires that, in order to mimic the natural peptide, all peptide bonds must also be inverted to generate **retro-inverso peptides**. Thus, a structural mimic of the parent peptide is maintained along with biological activity [58]. This approach has been used effectively for a variety of small extended α-helical- or β-sheet-containing peptide inhibitors [59,60]

■ Cell-penetrating peptides

There has been much discussion in the literature regarding the advent of **cell-penetrating peptides** (CPPs), or protein-transduction domains (PTDs). These are typically short (10–30 amino acids) and rich in the basic amino acids arginine and lysine and possibly interspersed with hydrophobic residues. The idea is that peptides or proteins, particularly those that are too large or hydrophilic to cross by natural diffusion, can either contain or be conjugated to such sequences and pulled across biological membranes as cargo [61]. It is thought that these sequences interact with both hydrophobic protective barriers and acidic surface lipids, pulling the peptides across the membranes due to the negative transmembrane potentials. These residues thus help to facilitate their proximity and, ultimately, transport across biological membranes. This is particularly relevant to peptides that must traverse the blood–brain barrier, a natural membrane that surrounds the brain and an impediment to the design of neurological drugs. CPP examples include a 16-mer region known as penetratin taken from drosophila antenaepedia [62] and a 13-mer corresponding to residues 48–60 of the HIV transactivator of transcription domain (Tat), which has been particularly favored for larger protein cargo [63,64]. It is thought that such peptides share a common theme: they can form amphipathic helices able to interact with hydrophobic and hydrophilic environments. Interestingly, retro-inverso forms of such CPPs have been shown to be just as effective as parent peptides, while having the additional benefit of protease resistance [65]. There is additionally the possibility of creating s–s linkages so that peptide or even oligonucleotide cargo [66,67] can be released from the PTD upon entry into the reducing environment of the cell. There is therefore great promise over the next years for the delivery of peptide drugs by oral means, both in terms of resistance to proteases, ability to traverse membranes and oral availability [68,69].

Existing examples of peptides & their mimetics as drugs

There are currently in excess of 60 marketed peptides worldwide (see elsewhere [4] for a list), approximately 270 peptides in clinical-phase testing, and approximately 400 in advanced preclinical phases. While naturally occurring peptides such as insulin, vancomycin, oxytocin and cyclosporine have been around for some time, recently synthetically produced peptides have been brought on to market. Examples include Fuzeon® (enfuvirtide) and Integrilin® (eptifibatide). Fuzeon is a 36-amino acid acetylated and amidated (to prevent terminal peptidase action) peptide, containing only natural amino acids, which is designed to bind to the six-helix bundle that composes gp41, thus preventing the fusion of the HIV with CD4+ cells. Oral availability issues mean that it must be injected regularly and subcutaneously and is used in instances where the patient displays multiple drug-resistant strains of the virus. Fuzeon is particularly interesting as it has marked a turning point; it has caused the pharmaceutical industry to take notice and rethink its approach to peptide-based drugs and has helped peptide synthesis to become a routine and more cost–effective approach than was considered previously possible. Integrilin is a cyclic heptapeptide derived from rattlesnake venom and is used as an antiplatelet drug to prevent ischemic events. A ligand of the CCR5 receptor called RANTES is a 68-mer with *N*-terminal modifications and non-natural amino acids substitutions in positions 1–3 to increase potency and protease resistance [70]. Another example includes Cubicin (daptomycin), a lipopeptide antibiotic used to treat infection with Gram-positive organisms. Antimicrobial peptides are particularly exciting in this respect, since they offer the potential for modification in generating a first-line immune defense by protecting against bacterial infection [71].

■ Venom-derived peptides

Peptides derived from venom are very bioactive and are the subject of much interest. Venoms often contain structurally diverse and complex peptides that have the advantage of being stable and displaying great specificity for their target. Stability is often achieved via disulphide bridges and a range of post-translational modifications, which are introduced by additional enzymes (e.g., amidation of the C-terminus, sulfation (Tyr), bromination (Trp), glycosylation (Thr), N-C-cyclization and isomerization to D-amino acids) [72]. Thus, they are very useful as research tools and hold promise as potential therapeutics. They have been shown to act on receptors and gated channels to exert their effects. One such example is ziconotide, which is derived from a toxin of the cone snail and has been shown to be effective against chronic pain by blocking calcium-dependent pain signal transmission [73]. Another, chlorotoxin is a 36-amino acid peptide extracted from the venom of the deathstalker scorpion. It is capable of blocking chloride channels and binds exclusively to glioma cells, opening the possibility for treatment and diagnosis of particular cancers. Indeed, synthetic variants have been derived that are capable of binding to malignant brain-tumor cells without affecting healthy tissue [74].

Future perspective

It is clear that there is growing promise in the use of peptides as drugs. Rising evidence suggests that many of the traditional barriers to druggable agents can be circumvented by a range of techniques, many of which have been discussed in this article. This field is moving at an increasing rate; with fewer small-molecule compounds reaching the market each year scientists have turned their attention to creating **peptide mimetics** and restraints of known binding regions using high-resolution structural information in their scaffolds for design. These molecules are likely to give rise to future therapeutics. An additional promising approach for delivery and stabilisation of biodrugs is via their encapsulation into nanoparticles (e.g., lipids, synthetic polymers, dendrimers and fullerenes) to shield the drug until it is required, at which point the nanoparticle can be degraded [75,76]. Some nanoparticles are also able to function in the delivery of the drug to the area of interest. Research into methods of delivery and release is likely to become an intense area of research over the coming years. Lastly, Fmoc chemistry has now become routine, with more efficient coupling agents meaning that greater length peptides can be synthesized. Price is a big issue, especially when lesser used non-natural amino acids are incorporated. Increasing numbers of individual amino acid mimetics are becoming widely available and this is likely to improve further as the technology becomes more widely adopted.

Executive summary

- Peptides as therapeutics contravene some of the strict limitations that have traditionally been imposed by the pharmaceutical industry. A major caveat has been that peptides that do not conform to Lipinski-like 'rules of 5' are unlikely to be successful as potential starting molecules for effective drug candidates. Others have since applied more stringent 'rules of 4' and even 3. Peptides antagonists are therefore often simply too large to apply these rules, with even the smallest residue, glycine, having a molecular weight of 75 Da.
- However, with fewer small-molecule drugs reaching the market and the explosion in proteomic and interactomic information, attention is turning to the vast and relatively untapped area of protein–protein interaction (PPI) inhibition, and particularly non-Lipinski-like peptides and their mimetics. This area suits peptides and their mimetics well since PPIs lack well-defined binding pockets amenable to traditional small-molecule inhibition. Indeed, numerous examples now exist of peptide-based drugs, which has encouraged research into this area.
- Examples are emerging of peptides that can traffic either themselves, or larger peptide cargo into the nucleus and efficiently compete with cellular transcription factors, either via positive charge [64,77], via modification of the peptide, or possibly even via the addition of covalent staples to the peptide [78].
- Modification of the peptide can bring chemical and structural stability to the molecule and resistance to the action of proteases, leading to increased oral availability and decreased turnover.
- Peptide-based inhibitors can be produced by rational or semi-rational design, with a range of novel approaches used in screening and selecting antagonists from peptide libraries.
- Because the level of molecular complexity in peptides and their mimetics is great and the fact that they are less well investigated relative to small molecule research, there remains much to be learnt about designing for therapeutic application.
- Various strategies are being employed to implement increases in stability, specificity and druggability of peptides, using smallest known binding elements as starting points in design.

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