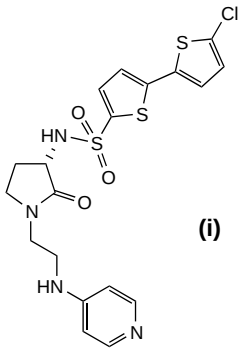
Combinatorial chemistry

Factor Xa inhibitors

The serine proteases of the coagulation cascade, thrombin (fIIa) and factor Xa (fXa), have been identified as logical targets for the development of antithrombotic agents. The inhibition of fXa, the central enzyme in the cascade, has emerged as an active area of research. Many of the small-molecule inhibitors of fXa discovered to date incorporate highly basic moieties, which impart poor physicochemical properties for oral administration. Replacing these highly basic groups with less basic ones is believed to lead to improved oral bioavailability and makes this an attractive strategy in the search for novel orally-available fXa inhibitors. A solid phase parallel approach was used to identify compounds that are capable of inhibiting fXa (Ref. 1). A library of 56 individual compounds was prepared by loading tetrafluorophenol derivatized solid phase resin with a range of sulfonyl chlorides. Displacement with bespoke-synthesized templates delivered products directly into solution. One of the most potent and selective compounds identified was (**i**), which possessed an anti-fXa-inhibition K_i of 15 nM. New structure–activity relationship (SAR) patterns have emerged for these small-molecule inhibitors, which contain less basic replacements of the benzamidines



(i)

and guanidines found in many previous fXa inhibitors. This work could, therefore, be useful as the basis for further exploration of SARs against this serine protease target.

Amino-acid–DNA contacts

Basic helix-loop-helix (bHLH) transcription factors are characterized by a conserved, parallel, four-helix bundle that recognizes a specific hexanucleotide DNA sequence in the major groove. The least characterized region of these proteins is the loop region, ranging in size from five to 23 amino acids, with the loop varying in amino acid content, especially between proteins of different subfamilies. Loop regions might play more than a structural role by contributing to DNA-binding affinity and/or specificity through phosphate–backbone or base-specific interactions. Protein–DNA recognition by the *Drosophila* bHLH transcription factor, Deadpan, was probed using combinatorial solid-phase peptide synthesis[2]. A depsipeptide unit (a peptide that contains an amide to ester substitution) was scanned through the loop region corresponding to the bHLH domain of the *Drosophila* transcription factor Deadpan. A series of bHLH peptide libraries that modulate amino acid content and length in the amino-terminal or carboxy-terminal region of the loop was screened with DNA and peptide affinity columns. From this work, a functional bHLH peptide with reduced loop length was found and Lys80 was unambiguously identified as the sole loop residue crucial for DNA binding. Therefore, this method provides a rapid alternative to standard recombinant techniques for the generation and assay of mutant proteins. The ability to replace key residues involved in protein–protein or protein–DNA recognition, especially with unnatural amino acids or other such small molecules, provides a powerful tool to probe energetic contributions to molecular recognition and, hence, new drug design.

Small-molecule enzyme inhibitors

In recent years, the rate of accumulation of genomic sequence information has rapidly accelerated, culminating in the sequencing of the human genome. This information has aided in the identification of genes whose products could serve as targets for pharmacological intervention. Although the accumulation of sequence information has been rapid, functional information for the corresponding gene products has lagged behind. For example, 40% of the open reading frames in *Escherichia coli* encode proteins of unknown function, even though this microbe has been scrutinized for decades. This lack of functional information makes it difficult to develop assays to exploit these genes as potential drug targets. Techniques and tools to facilitate the screening process, independent of functional assays, are needed to exploit available targets efficiently. A combinatorial approach was used to synthesize peptide libraries with a complexity of >5 x 10^8 members per library arising from either 7- or 12-residue peptides in a random 12-mer library, or random 11-residue peptides in which the central residue of each library was fixed with a different residue[3]. A broad range of enzymes was selected as targets for phage display and a series of peptides was isolated that bound specifically to each target. This methodology is useful in formatting assays for enzyme targets. The active peptides identified can be used in simple competitive binding assays to identify small-molecule inhibitors of enzyme function, targeting the same functional sites on enzymes to which effective therapeutic agents must be targeted. The binding assay can be used with a variety of detection systems and is readily adaptable to automation, making this platform suitable for high-throughput screening of compound libraries for drug discovery.

References

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Paul Edwards
Lead Discovery Technologies
Pfizer Global Research & Development
Sandwich, Kent, UK CT13 9NJ

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