Combinatorial chemistry

Anticancer agents from OBOC libraries

The one bead–one compound (OBOC) approach to combinatorial library synthesis is a highly effective method for the rapid production of large numbers of compounds. This procedure has been used in the synthesis of a library used in the high-volume cellular screening for cytotoxic anticancer agents [Salmon, S.E. *et al. Molecular Diversity* (1996) 2, 57–63]. The split-synthesis procedure was employed to prepare less than 100,000 compounds in three different libraries: tetrameric and trimeric peptide libraries (using both natural and unnatural amino acids) and a trimeric nonpeptidic library. These compounds were linked to the solid phase through two orthogonally cleavable linkers that could selectively release compounds at different pH.

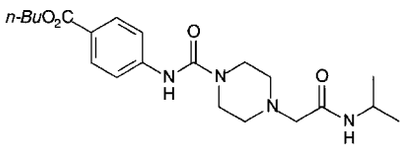
To perform the biological assay, the resin beads were mixed with tumour cells and these were plated in soft-agarose-containing tissue culture medium. At the neutral pH of the tissue culture medium, a 'reverse diketopiperazine' linker permitted release of one aliquot of compound from the beads. Within 48 hours, cytotoxic compounds could be detected by the clear ring of tumour cell lysis surrounding the active beads. The bead thus identified was isolated, the second aliquot of compound cleaved by base-catalysed cleavage of an ester linkage, and the structure determined by either Edman degradation for the peptides or LC-MS for the other structures.

Following screening of the three libraries, a compound with activity against P-388 leukaemia was identified from the tetrameric peptide library. A particular unnatural amino acid in the trimeric peptide library appeared to confer activity against MCF-7 breast cancer and several other solid tumour lines. The nonpeptide library yielded compounds active against 8226 human myeloma cells. No structures are revealed in the paper.

Human rhinovirus inhibitors

Combinatorial chemistry techniques have been used in a search for inhibitors of human rhinovirus, a primary cause of the common cold. Workers from Lilly (Indianapolis, IN, USA) have prepared a library of 4,000 ureas using the solution reaction of amines with an excess of isocyanates [Kaldor, S.W. *et al. Bioorg. Med. Chem. Lett.* (1996) 6, 3041–3044]. In particular, they have used functionalized resin beads as 'covalent scavengers' to purify the library products.

The 4,000 ureas were prepared as 400 ten-compound mixtures by reacting 1.25 equivalents of an isocyanate with a limiting amount of an equimolar mixture of ten amines. After completion of the reaction, the excess isocyanate was removed by the addition of aminomethylpolystyrene resin. Filtration to remove the resin and evaporation gave the product mixtures free of isocyanate. The library products were tested for activity against human rhinovirus-14 (HRV-14) in a whole-cell assay, and resynthesis of the constituent components of the most active mixture revealed novel low to submicromolar inhibitors (**1**, **2**) of human rhinovirus.



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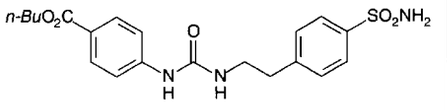

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Emerging molecular targets

Cell-permeable peptide import for use in target validation

A 'magic bullet' compound, capable of specifically blocking a cellular reaction, often serves as an enormously valuable reagent to prove the efficacy of an enzyme or protein as a new drug target. But, reagents with the specificity and potency needed to qualify as 'magic bullets' can be difficult to obtain, sometimes requiring as much chemistry and biology effort as the discovery of a drug candidate itself.

To get around this difficulty, drug targets for which an 'experiment of nature' exists are often selected. This 'experiment of nature' may be a particular enzyme or protein that exists, preferably in a population of human subjects, in a mutated form that mimics the action of the proposed drug. In such a case, the 'magic bullet' reagent is not needed to prove the efficacy of the target. The experimental phenotype exists naturally, and the effects of inactivating the particular protein or enzyme are apparent by observing the population with the mutation. In other cases, it is possible to use an animal model in which a particular gene has been 'knocked out' to determine if inactivating a potential drug target will have the effect predicted from theory. But there still remain many potential targets for which a 'magic bullet' reagent would be an invaluable tool.

Peptides as 'magic bullets'. Despite their well deserved reputation as unlikely drugs and challenging lead compounds for drug development, peptides sometimes serve as excellent 'magic bullet' reagents for proof-of-principle studies. Especially when designed on the basis of a naturally occurring sequence from a protein with multifunctional domains, peptides have proven to be of immense value for dissecting functional activity. Some protein kinases and phosphatases, for example, contain autoinhibitory domains as part of their primary structure. Such domains mimic the natural substrate of the enzyme and interact with the catalytic site to keep the kinase or phosphatase activity suppressed. But when an appropriate regulatory molecule – calcium ion, calmodulin or cAMP, for example – binds to the enzyme, its conformation is altered, so that the auto-inhibitory domain no longer blocks the active site, liberating the signaling activity of the kinase or phosphatase.

Peptides that mimic the autoinhibitory domains of such enzymes are often highly specific and potent kinase or phosphatase inhibitors. Because the peptides are not covalently connected to the other domains of the enzyme, their inhibitory activity is not released by the binding of the regulatory molecule. Such peptides can be extremely useful for determining the role a particular kinase or phosphatase plays in a complex metabolic mixture

and may offer valuable insight as to the efficacy of a possible drug targetting the particular kinase or phosphatase. Peptide inhibitors based on naturally occurring protein domains can also be used to perturb receptor–ligand interactions, the transport of specific proteins and enzymes to intracellular compartments or across cell membranes, and as inhibitors of other enzymes such as proteases.

The drawback of this approach, of course, is that the use of peptide inhibitors is often limited to biochemical studies and to protein targets on the exterior of the cell because of the difficulty of getting peptides to penetrate cell membranes. To get around this problem investigators have developed techniques to generate transient pores in cell membranes through which peptides may be slipped into the cell. Alternatively, microinjection techniques have been used to introduce peptides into intracellular compartments. Now Jacek Hawiger and colleagues at Vanderbilt University (Nashville, TN, USA) have used what in hindsight seems a very obvious, simple and convenient approach to moving peptides across cell membranes. They reasoned that if specific peptide leader sequences are required for peptide export, then the same leader sequences could be used to import a non-penetrable peptide into a cell. Their novel approach works and has been given the moniker cell-permeable peptide import (CPPI). Hawiger and coworkers have used CPPI to probe the transport of NF-κB into the nucleus and the structure and function of the cytoplasm tail of the β_3 integrin.

Nuclear translocation of NF-kB. Using the CPPI strategy, Hawiger and coworkers investigated the mechanism of translocation of the transcription factor NF-κB to the nucleus of NIH 3T3 cells, a critical step in its path to gene activation [*J. Biol. Chem.* (1995) 270, 14255–14258]. First, the researchers fused the hydrophobic region (h-region) from Kaposi fibroblast growth factor, a region known to attach to lipid bilayers, to the positively charged nuclear localization sequence (NLS) of NF-κB. Then they measured the ability of the peptide complex to penetrate the cell membrane of the NIH 3T3 cells and the functional consequences on the NF-κB activation pathway.

They found that the h-region facilitated a 20-fold increase in the amount of cargo peptide incorporated into the cells. The incorporation of the peptide was not receptor-dependent, not perturbed by inhibitors of endosomal or lysosomal function, and not dependent upon ATP, just as would be expected if the h-region was facilitating peptide incorporation through an increased rate of passive diffusion across the plasma membrane. Functionally, the cell permeable NLS peptide blocked the transport of NF-κB into the nucleus triggered by the proinflammatory compounds LPS and TNFα. Presumably, the cell-permeable NLS peptide competed with NF-κB for a limited number of binding sites on the nuclear membrane, resulting in the observed inhibition of NF-κB translocation.

It remains to be determined if the nuclear transport mechanisms of different transcription factors exhibit a sufficient degree of specificity to serve as drug targets, or if all transcription factors enter the nucleus through the same pathway. However, the strategy used in this study to produce a permeable peptide inhibitor of transcription factor translocation points a way for future detailed studies of this question.

β_3 integrin and cell signaling. Hawiger and coworkers have also used the CPPI technique to probe the structure and function of the 41-amino-acid cytoplasmic tail of the β_3 integrin, also known as glycoprotein IIIa [*Proc. Natl. Acad. Sci. U. S. A.* (1996) 93, 11819–11824]. It is well known that individuals with Glanzmann thrombasthenia – one of those convenient experiments of nature – have a point mutation in the cytoplasmic tail of the β_3 integrin and a loss of adhesive function in the extracellular domain of the protein. This, as well as additional data, suggests that a protein–protein interaction of the cytoplasmic tail of the β_3 integrin is essential for adhesion of the integrin to extracellular matrix proteins such as fibrinogen. The investigators set out to use the CPPI technique to map the specific region of the cytoplasmic tail of β_3 integrin that is essential for the adhesive properties of the extracellular domain.

Using different synthetic peptide analogs of the cytoplasmic tail coupled to the h-region from the β_3 integrin, the investigators found that one particular region of the cytoplasmic tail, corresponding to peptides 747 to 762, is essential for the protein's adhesive properties. When a synthetic peptide identical in sequence to this region was complexed to the h-region, it proved to be a highly effective inhibitor of adhesion. If the h-region was not included, a condition in which the peptide would not be expected to cross the cell membrane, it had no effect on cell adhesion. Presumably, once the peptide entered the cell it competed with the cytoplasmic tail of the β_3 integrin for binding to an essential intracellular component.

The results of this study are striking and certainly have implications for drug discovery. The adhesive properties of an extracellular protein known to be an important factor in immune reactions, atherosclerosis, thrombosis and tumor cell metastasis were blocked by an intracellular reagent. Further definition of the critical region of the cytoplasmic tail of the β_3 integrin may well provide an important new drug target. Moreover, the novel CPPI strategy for incorporation of peptides into intracellular compartments will undoubtedly prove highly useful in a wide variety of studies to identify or provide proof-of-principle studies for new intracellular drug targets.

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