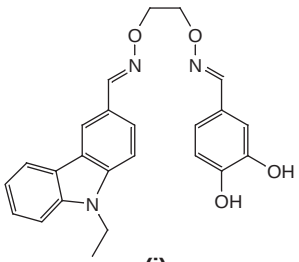
Combinatorial chemistry

Potent subtype-selective c-Src inhibitors

A new method for the rapid identification of ligands to biological targets has been reported and used for the discovery of novel ligands for the non-receptor tyrosine kinase, c-Src (Ref. 1). The approach proceeds by first identifying several monomeric compounds that might have activity against the biological target of interest. Screening these compounds reveals the set of monomers with highest affinity. A limited combinatorial library can be constructed by joining all pairs of this set through a flexible linker and testing these compounds against the biological target.

A set of 305 aldehydes derivatized as their *O*-methyl oximes were prepared and screened against the tyrosine kinase, c-Src. From this set, 37 were selected as having the highest affinity (>70% inhibition at 500 μM). These aldehydes were then linked in pairs through five different *O,O′*-diaminoalkanediol linkers to give a combinatorial set. Screening the library of linked binding elements using a microtitre-based ELISA screening format revealed several potent ligands including the most active compound (**i**) which had an IC_{50} value of 64 nM.



(i)

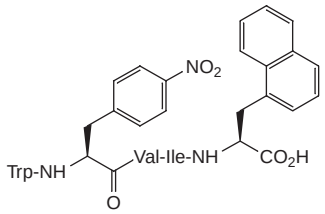
This approach has been demonstrated to be a novel and effective method for the identification of new ligands. In this case, the library generated a potent ligand of c-Src that is also selective over other tyrosine kinases.

1 Maly, D.J. *et al.* (2000) Combinatorial target-guided ligand assembly: Identification of potent subtype-selective c-Src inhibitors. *Proc. Natl. Acad. Sci. U. S. A.* 97, 2419–2424

Human serotonin *N*-acetyltransferase

Arylalkylamine *N*-acetyltransferase (AANAT) is the enzyme responsible for the conversion of serotonin into *N*-acetylserotonin by reaction with acetyl-CoA. As this enzyme plays a key role in the biochemical pathway leading to melatonin, it has a crucial function in regulating the vertebrate circadian rhythm. Combinatorial chemistry techniques have been used to identify peptidic substrates and inhibitors of AANAT (Ref. 2).

Using the mix-and-split procedure, libraries of tri-, tetra- and pentapeptides were prepared on solid-phase supports and screened against human cloned AANAT expressed in bacteria. One of the best inhibitors discovered was the pentapeptide (**ii**), which had an IC_{50} of 4.3 μM for AANAT. Three-dimensional models of the inhibitor–enzyme complex suggest that the pentapeptide partly occupies the binding site of acetyl CoA, explaining the observed competitive inhibition.



(ii)

Tetrapeptide inhibitors derived from (**ii**) might provide useful leads in the development of novel non-peptide AANAT inhibitors.

2 Ferry, G. *et al.* (2000) Substrate specificity and inhibition studies of human serotonin *N*-acetyltransferase. *J. Biol. Chem.* 275, 8794–8805

Inhibitors of bacterial signal transduction kinase

The emergence of bacterial resistance to antibiotics is causing significant concern and has stimulated the search for novel classes of antibacterial agents. Indeed, numerous bacterial genes encode for proteins that might serve as novel targets that bypass the existing mechanisms of antibiotic resistance. Combinatorial chemistry has been used in the search for inhibitors of regulatory proteins such as the two-component histidine kinases[3].

Libraries of N-acetylated, C-amidated hexapeptides were constructed from D-amino acids to avoid the intrinsic *in vivo* instability of natural peptide sequences. The combinatorial libraries were prepared as mixtures using both, mix-and-split and the positional scanning methods. Screening the mixtures against CheA, the kinase involved in chemotaxis in *Escherichia coli*, and following routine deconvolution strategies, several inhibitors were revealed. Amongst these, the most potent were Ac-wfvirr-NH_2 and Ac-wfvilr-NH_2 (single D-amino acid code). Minimum inhibitory concentrations against various bacterial strains were evaluated and were generally in the 8–64 μM range. The study demonstrated that both combinatorial approaches yielded similar results with respect to key residues in the inhibitor structures.

3 Roychoudhury, S. *et al.* (2000) Use of combinatorial library screening to identify inhibitors of a bacterial two-component signal transduction kinase. *Mol. Diversity* 4, 173–182

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