Combinatorial chemistry

NMR-based screening

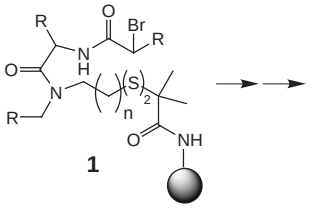
One of the challenges of combinatorial chemistry has been to devise methods that permit rapid screening of mixtures and subsequent identification of active compounds. Many screening techniques rely on the resynthesis of further compounds, or the decoding of tagging sequences to identify the active components. More recently, NMR-based screening techniques have been described that rely on observing changes in ^{15}N–^{1}H-amide chemical shifts that occur when a ligand binds to a labelled protein. In the latest modification of this technique, Hajduk, P.J. and coworkers have used cryogenic NMR-probe technology to obtain two-dimensional ^{15}N–^{1}H correlation spectra in less than ten minutes using only 50 μM protein samples [*J. Med. Chem.* (1999) 42, 2315–2317].

Screening at these concentrations allows mixtures of up to 100 compounds to be rapidly assessed resulting in screening of >100,000 compounds per day. This technique has been used to examine the binding of mixtures to stromelysin. A known inhibitor of stromelysin, 3-[4-(4-cyanophenyl)phenoxy]propanohydroxamic acid, was included and large unambiguous chemical-shift changes were observed.

Although limited to relatively small proteins (<40 kDa) that can be ^{15}N-labelled, there are advantages to NMR-based screening in that no background signals from the ligands are observed. Furthermore, the specific binding-site on the protein can be determined from the particular amide-bond chemical-shift changes.

Somatostatin-receptor ligands

The significance of endogenous peptidic mediators in biological systems, and the generic instability of exogenously administered peptide therapeutics have driven the design of physiologically stable peptidomimetics. One successful strategy for the design of peptidomimetics has been to place the key amino acid side-chains on a rigid template that mimics the biologically active peptide conformation. The design and synthesis of combinatorial libraries of scaffolds based on the β-turn structure has received the most attention. Souers, A.J. and coworkers have now applied this strategy to the preparation of a library of medium-ring heterocyclic β-turn mimetics (**2**) targeted against a panel of five cloned human somatostatin receptors ($hSST_1$– $hSST_5$) [*J. Am. Chem. Soc.* (1999) 121, 1817–1825].



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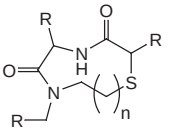

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The 172-member library was prepared on solid phase linked through a disulphide bond (**1**). Reduction of the disulphide using a phosphine and subsequent cyclization using basic tetramethylguanidinium resin gave the desired heterocycles, and these were then screened against the somatostatin receptors. Integral to the design of the library was the ability to vary the side chains and their relative stereochemistry. One compound from this library was found to have 87 nM affinity for the SST_5 receptor.

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Metalloproteinase inhibitors in snakebite envenomations

Pit viper envenomations are characterized by prominent local tissue damage, such as necrosis, hemorrhage and inflammation. These effects are relatively difficult to neutralize with antivenoms because of their rapid onset and development[1]. When treatment is delayed, as often occurs in tropical regions of the world, patients are at risk of developing permanent sequelae such as tissue loss or dysfunction. Horse- or sheep-derived antivenoms continue to be the mainstay in the treatment of snakebite envenomations, as they effectively neutralize systemically acting venom toxins, and partially decrease the extent of venom-induced local tissue damage. However, there is a need to develop ancillary treatments to inhibit locally acting toxins that could be used in addition to immunotherapy.

Metalloproteinases are widely distributed in crotaline and viperine snake venoms[2]. They play a significant role in local tissue damage by inducing hemorrhage, oedema, myonecrosis, dermonecrosis and inflammation. Inhibitors of venom metalloproteinases, which could be injected directly at the site of venom injection, could offer a means of addressing this problem.

Natural metalloproteinase inhibitors

Borkow, G. and coworkers[3] have screened several natural and synthetic substances for their ability to inhibit local hemorrhagic activity induced by the venom of *Bothrops asper*, the medically most important snake in Central America. Besides antibodies, the most effective compounds were proteinase inhibitors isolated from the sera of the snakes *Natrix tessellata* and *B. asper*[4,5], and calcium sodium ethylene diamine tetraacetate ($CaNa_2EDTA$) that chelates the zinc required for metalloproteinase activity.

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