Combinatorial chemistry

NMR-based screening

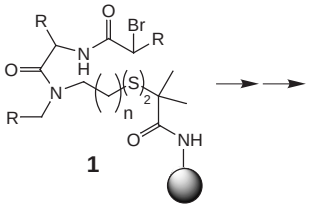
One of the challenges of combinatorial chemistry has been to devise methods that permit rapid screening of mixtures and subsequent identification of active compounds. Many screening techniques rely on the resynthesis of further compounds, or the decoding of tagging sequences to identify the active components. 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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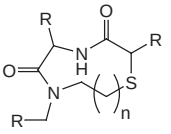

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The 172-member library was prepared on solid phase linked through a disulphide bond (**1**). Reduction of the disulphide using a phosphine and subsequent cyclization using basic tetramethylguanidinium resin gave the desired heterocycles, and these 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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Several broad-acting potent protease inhibitors have also been isolated from the sera of various animals, such as the opossum, the hedgehog, the hispid cotton rat and many snakes[6]. These inhibitors probably play a key role in the natural resistance of these species to snake venoms. Some are macroglobulins, while others are α1-globulin protease inhibitors. The potential use of these inhibitors in snakebite treatment has been suggested, but more studies on their chemical structure, mechanism of action and pharmacokinetic profile are required.

Synthetic metalloproteinase inhibitors

By contrast, the use of low molecular weight, synthetic metalloproteinase inhibitors is highly promising, as they are highly diffusible and could therefore effectively inhibit venom enzymes present in the tissues. For example, $CaNa_2EDTA$ inhibits metalloproteinases by chelating the zinc ion required for catalysis. Because it forms complexes with some heavy metals, it is successfully used in treating lead poisoning in humans. When incubated with the venom of *B. asper*, this EDTA salt inhibits the hemorrhagic and dermonecrotic effects of the venom[7]. Currently, the ability of $CaNa_2EDTA$ to inhibit tissue damage induced by BaP1 (a toxic metalloproteinase isolated from *B. asper* venom) is being tested by Gutiérrez and coworkers (University of Costa Rica) with encouraging results in various *in vivo* models. This suggests that administration of metalloproteinase inhibitors *in situ* rapidly after envenomation could become a useful approach in snakebite treatment.

The search for metalloproteinase inhibitors is an active field of research[8]. Patent activity in this field is growing and several inhibitors are being tested in clinical trials[9]. Most of these inhibitors are synthetic peptidomimetics that incorporate a zinc ligand in place of the scissile amide bond. The efforts being carried out in this field are mostly aimed at developing inhibitors of the matrix metalloproteinases (MMPs). This is a group of zinc-dependent enzymes that mediate extracellular matrix breakdown and are involved in such diverse pathologies as cancer, arthritis, and cardiovascular and neurodegenerative diseases[10].

Together with the ADAMs (membrane proteins with disintegrin and metalloproteinase domains), snake venom metalloproteinases form the reprolysin-family of zinc-metalloproteinases[11,12]. Their zinc-binding motif is very similar to that of MMPs, comprising three histidines. In addition, both reprolysins and MMPs share a methionine-containing turn, being classified as metzincins. Hence, snake venom metalloproteinases and MMPs have important structural similarities at their active site[13]. These similarities strongly suggest that the rapid developments in the search for new MMP inhibitors could find an application in the treatment of snakebite envenomations.

Moreover, the anti-snakebite activity of synthetic metalloproteinase inhibitors might be caused, not only by inhibiting venom metalloproteinases, but also by blocking the action of MMPs. It has been recently demonstrated in a mouse model that MMPs are activated after injection of a metalloproteinase isolated from *B. asper* venom[14], probably contributing to extracellular matrix degradation and tissue damage. In addition, venom metalloproteinases release active TNFα from its membrane-bound precursor[15]. Hence, metalloproteinase inhibitors could be highly beneficial in snakebite therapy by inhibiting venom metalloproteinase-induced tissue damage and activation of MMPs, as well as by interfering with the release of pro-inflammatory cytokines. These findings raise expectations in developing new treatments for the long-standing problem of local tissue damage after snakebite envenomations.

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