Combinatorial chemistry

Microautoradiographic screening

The screening of bead-supported libraries usually depends on a fluorescence or colour change in the bead containing an active structure. This allows that particular bead to be selected and the structure of the active ligand on the bead to be determined. However, the use of a dye on one of the components in the assay may give rise to misleading biological assay artefacts. The use instead of a radioisotope of carbon or hydrogen allows the biological screening to take place without altering the chemical properties of the labelled component. A ^{14}C-labelled macrobicyclic synthetic receptor molecule has been described in a recent paper [Nestler, H.P. *et al. Bioorg. Med. Chem. Lett.* (1996) 6, 1327–1330]. Individual beads carrying a peptide sequence with high affinity for this radioactive receptor could be identified by microautoradiographic screening.

2-Aminothiazoles library

Among all the excitement surrounding the solid-phase synthesis of large combinatorial libraries, it should not be forgotten that occasionally simple solution methods can be very successful in the preparation of drug discovery libraries. A modest set of 20 2-aminothiazoles have been prepared through the solution-phase Hantzsch reaction of thioureas with α-bromoketones [Watson, S.P. *et al. Bioorg. Med. Chem. Lett.* (1996) 6, 1409–1414] and characterized by NMR and high-resolution mass spectrometry. To demonstrate that these compounds have biological relevance, the anti-inflammatory agent fanetizole (**1**) was prepared as a standard within this library using the same methods. The authors have proceeded to use this simple approach for the preparation of 2,500 compounds using commercially available precursors.

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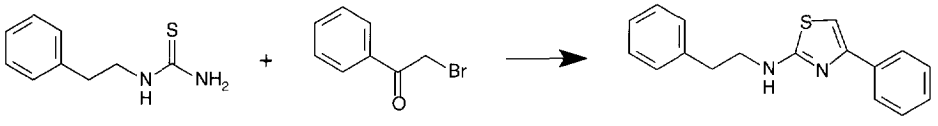
Emerging molecular targets

Another thrombin receptor?

It all seemed so well understood. Thrombin, a protease produced during tissue injury, was thought to have a single, well-defined G protein receptor expressed on numerous cells including platelets, leukocytes, fibroblasts and endothelial cells. After binding, thrombin cleaves its receptor, triggering intracellular signals that lead to the formation of a thrombus, an inflammatory reaction and the growth of new tissue. It made sense: acting through a single receptor, thrombin would trigger a coordinated response in different cells to stop bleeding, protect against infection and initiate tissue repair.

But now this neatly packaged scenario has been undermined by the unexpected findings of Andrew Connolly and coworkers at the University of California at San Francisco (San Francisco, CA, USA). They tested the single-receptor theory by using genetically altered mice in which the thrombin receptor was disrupted. Based on the prevailing theory, one would expect such mice to exhibit severe bleeding disorders; but this was not what Connolly and coworkers observed. Instead, they found that platelets from the genetically altered mice underwent a normal activation response to thrombin, and the mice had no apparent bleeding abnormalities. However, fibroblasts from the genetically altered mice could no longer respond to thrombin, and only about one-half of the homozygous progeny for the defective thrombin receptor survived to become normal adults [*Nature* (1996) 381, 516–519].

The surprised investigators concluded that at least one additional thrombin receptor exists, and that distinct thrombin receptors may regulate tissue-specific functions. This conclusion is of great importance from a drug discovery perspective; it suggests that compounds might be discovered that differentially regulate the multiple cellular effects of thrombin. The authors also conclude, based upon the progeny survival rates, that the known thrombin receptor has a role in development that is not yet understood. These findings should add additional impetus to the search for compounds that act through the thrombin receptor.



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L-Selectin protease and leukocyte-endothelial interactions

The adhesion molecule L-selectin sits on the surface of leukocytes and plays a prominent role in their interaction with vascular endothelium during the early stages of an inflammatory reaction. In the intact vascular bed, the initial interaction of the leukocyte with the endothelium is a transient attachment and rolling along the surface of the endothelial cell layer. In the presence of an inflammatory cytokine the leukocyte will eventually adhere to the endothelium, pass through the vascular bed and migrate to the site of inflammation.

L-selectin is also known to undergo proteolysis, which leads to shedding of the adhesion protein from the neutrophil surface. The protease responsible for the shedding is unusual, as its activity is not blocked by any of the common protease inhibitors. Now, Bruce Walcheck and colleagues at Boehringer Ingelheim Pharmaceuticals (Ridgefield, CT, USA), Khepri Pharmaceuticals (South San Francisco, CA, USA) and the University of Louisville (Louisville, KY, USA) have connected the shedding of L-selectin with the rolling reaction of the leukocyte [*Nature* (1996) 380, 720–723]. Using a novel hydroxamic acid-based peptidic protease inhibitor (KD-IX-73-4), they found that blocking the shedding of L-selectin from the surface of neutrophils led to a reduced neutrophil rolling activity under hydrodynamic flow and an increased neutrophil accumulation on a glass surface coated with MECA-79 antigen, an isolate from human tonsil, which is predominantly CD34. The protease inhibitor appeared to be selective for blocking the shedding of L-selectin; it had no effect on the upregulation of the Mac-1 adhesion protein or other measurable aspects of neutrophil activation. The authors conclude that the shedding of L-selectin during the early stages of the inflammatory reaction may be a critical determinant of the leukocyte rolling reaction and may play an important physiological role in leukocyte aggregation and accumulation at sites of inflammation.

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