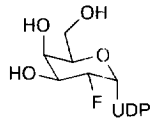


1

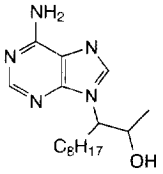
Varying the concentration of the inhibitors in the same MS experiment allowed the IC_{50} value to be determined as 119 μM.

Other experiments have demonstrated that mixtures of compounds can be simultaneously assessed against the enzyme using the ESMS method. Inactive mixtures appear as being totally inactive, while mixtures containing active components can be analysed by individual assessment of constituent compounds.

Adenosine deaminase inhibitor library

Solution-phase combinatorial libraries have been screened using a combination of pulsed ultrafiltration (PUF) and ESMS [Zhao, Y-Z. *et al. J. Med. Chem.* (1997) 40, 4006–4012]. PUF is an effective way of identifying ligands for a macromolecular target by pumping library compounds past a protein trapped by an ultrafiltration membrane in a flow-through cell. Compounds with high affinity for the protein target have their elution profile perturbed, and by coupling the cell to an ESMS the identity of the active ligand can be determined. Elution characteristics have also been used to calculate a range of classical binding parameters.

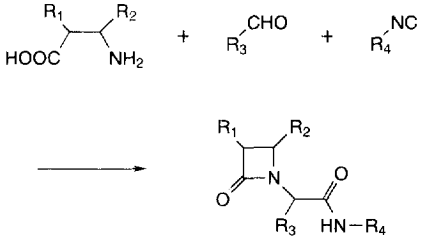
This approach has been used for the analysis of the binding of erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA) analogues to adenosine deaminase, an important enzyme in the inactivation of adenosine analogues used for the treatment of cancer and AIDS. From a mixture of eight analogues prepared without purification it was ascertained that three of the compounds bound to adenosine deaminase. Of these, one analogue (**2**) with an octyl side-chain had more than twofold greater affinity for the enzyme than EHNA itself.



2

β-Lactam library

Human leukocyte elastase (HLE) has degradative effects on lung elastin and has been implicated as a causative factor in several respiratory diseases including emphysema, bronchitis and cystic fibrosis. In the search for novel inhibitors of this enzyme, a library of β-lactams (**3**) has been prepared using a solution-phase combinatorial approach [Pitlik, J. and Townsend, C.A. *Bioorg. Med. Chem. Lett.* (1997) 7, 3129–3134]. The rationale for this library design is that the activated carbonyl of the β-lactam will acylate the key active-site serine residue resulting in enzyme inactivation.



3

A total of 126 compounds were prepared using the Ugi reaction, an approach that permits complex structures to be synthesized in one step. Three monomer sets consisting of seven β-amino acids, six isocyanides and three aldehydes were used to give library products as individual compounds analysed using electron ionization MS. Biological evaluation of the compounds against both HLE and chymotrypsin is ongoing.

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High-throughput screening

Accessible UHTS?

Screening-based drug discovery is a numbers game; according to current doctrine, the more compounds examined, the more a company is likely to succeed. The definition of high-throughput screening currently hovers between 100 and 1,000 data points per day, and increasingly there is discussion of ultra-high-throughput screening (UHTS), in which up to 100,000 data points would be generated per day. So far, this level of screening has not been achieved for most companies. However, Zymark (Hopkinton, MA, USA) is beta-testing its new Allegro™ system, which it claims will conduct automated assays at a rate of 1,000 microtitre plates per 24 h period. Assuming the use of 96-well plates and the ability to run the system around the clock, this new technology may allow more companies to contemplate the UHTS format.

According to Zymark, the Allegro™ is designed to be an open architecture system that will facilitate 96- or 386-well microtitre plates, and will allow the insertion of new instrumentation, such as nanoliter dispensing equipment, as it is developed. This flexibility should allow the system to keep up with the rapidly developing technology in areas such as liquid handling and new assay design. The Allegro™ is composed of a series of interlinked modular workstations and includes a transfer station, 8-channel dispensers, storage, source, and final destination carousels, plate washers, 96-well dispensers and plate readers. The system is claimed to be extremely flexible and fully compatible with all commonly used assay technologies including ELISA and SPA.

Zymark highlights the increased level of UHTS that can be achieved using the Allegro™ system when used with 386-well (or higher density) microplates. However, the most compelling reason for looking at the system for many companies may be the ability to enter the world of UHTS without completely redesigning their cherished 96-well plate assays.

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