High-throughput screening

1536-well plates at Pharmacopeia

While many companies debate the pros and cons of converting their screening operations from the 96-well format to 384- or 864-well plates, Pharmacopeia, Inc. (Princeton, NJ, USA) has taken the plunge to a 1536-well plate. According to Dr Jonathan Burnbaum, Group Leader for High-Throughput Screening and New Technologies, the new 1536-well plate accelerates the rate of drug discovery, because it significantly increases the rate of screening. At the same time it decreases the per assay cost of the screening operation because of the smaller size of each individual assay. Burnbaum described the new technology at the recent NMHCC conference on *Emerging Technologies for Drug Discovery* held 19–22 May, 1997 in Boston, MA, USA.

Sixteen for one

The 1536-well plate is designed to incorporate 96 distinct blocks of 16 wells. Each block is placed in a spacing interval of 9 mm; this same spacing interval contains only one well in the 96-well format. The wells of the new plates have a total volume of 2 μl, are 1.5 mm in diameter at the surface of the plate, and are spaced 2.25 mm from center to center. The plates are thinner than the normal 96-well plates, and the walls of the chambers are bevelled to help in the manufacturing process.

The 1536-well plates are used without a cover during assay setup and then covered with a glass plate during incubations to minimize problems due to evaporation. The sides of the plates contain eight control wells plus holes for both optical and pin alignment. The plates are available in clear, white or black and soon will be available in white or black with a clear bottom; Pharmacopeia is collaborating with Corning Costar to develop the plates.

To scale up from 96-well plates to the new 1536 plates incurs retooling costs in assay design, liquid handling and detection equipment. But according to Burnbaum, these costs are expected to be recovered quickly by the decreased use of expensive reagents. The goal at Pharmacopeia is to convert 90% of their assays to the new format by the second quarter of 1998. The major development necessitated by the new plates was new detection equipment. Fluorescent and luminescent assays are the way to go, according to Burnbaum, and Pharmacopeia uses a charged-coupled device (CCD)-camera-based detection system to read an entire plate of fluorescent assays simultaneously. For assays in which it is necessary to read each well individually, an automated microscopy-based detection system is being developed, which will be able to read an entire plate well-by-well in approximately 30 minutes.

Critical mass

It is necessary to have a critical mass of groups using the new 1536-well system in order to capitalize on the economy of scale. Pharmacopeia anticipates generating this critical mass by encouraging its seven major collaborators to convert a proportion of their assays to the high-density format.

Burnbaum welcomes questions and inquiries about the new 1536-well technology (tel: + 1 609 452 3712).

Emerging molecular targets

Targeting tyrosine kinases

Tyrosine kinases are interesting drug targets, but kinase inhibitors with sufficient specificity to be drugs are rare. Screening programs for kinase inhibitors usually turn up numerous compounds that act by blocking the ATP binding site. Such compounds frequently cross-react with many different kinases and other enzymes that utilize ATP, reflecting the conservative nature of the ATP-binding domain. But Dr Joseph Schlessinger and coworkers at New York University (New York, NY, USA) and SUGEN (Redwood City, CA, USA) argue that at least one class of compounds that bind to the ATP-binding domain of fibroblast growth factor receptor 1 (FGFR1) exhibit a sufficient degree of specificity to be taken seriously as drug candidates [*Science* (1997) 276, 955–960].

The general class of compounds investigated by Schlessinger's group have an oxindole core (indolinones). They were identified by traditional screening of synthetic libraries. Two tyrosine kinase inhibitors are reported, SU4984 and SU5402, which have IC_{50}s in the 10–40 μM range toward FGFR1 in the presence of 1 mM ATP. The authors state that this degree of inhibition in the presence of such a large excess of ATP indicates that the compounds must act by interacting with the catalytic domain of the kinase. One of the compounds, SU4984, also inhibited phosphorylation by the platelet-derived growth factor (PDGF) and the insulin receptor. However, the other compound, SU5402, inhibited PDGF only weakly and the insulin receptor not at all. Neither compound inhibited the kinase activity of epidermal growth factor.

An analysis of crystal structures of the kinase with the bound compounds shows that the compounds react with both the ATP-binding domain and the kinase domains. The oxindole portion of the compounds interacts with the adenine-binding site on FGFR1 and is thought to be responsible for the major affinity of the compound for the kinase. The chemical groups attached to the oxindole cores interact with the hinge region that connects the two kinase domains of FGFR1. It is apparently the latter interaction that confers the specificity on the SU5402 compound. By comparing the crystal structures of FGFR1 complexed to either of the two inhibitors, a rational explanation – based upon the structural interaction with the kinase of the chemical groups attached to the oxindole cores – was derived for why SU5402 is a specific inhibitor of FGFR1 while SU4984 is not. As a result of these studies, the authors conclude that it is now possible to use rational design approaches to produce highly specific tyrosine kinase inhibitors.

Robert W. Wallace
fax: +1 212 254 3322
e-mail: RobWallace@nasw.org

