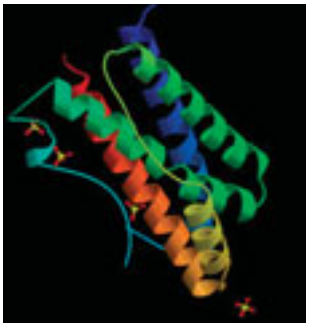


Interleukin-6.

Prognostic biomarker

IL-6 is a 30-kDa glycoprotein produced by several cell types, including B and T cells, macrophages, and fibroblasts, and it performs various functions in the healthy and diseased body. Among other things, IL-6 is involved in the proliferation and differentiation of a variety of cell types. It is this function that has researchers investigating the possible role of IL-6 in tumor progression and the molecule's potential to act as a prognostic biomarker for cancers that have metastasized.

Although IL-6 is expressed in a number of carcinoma cell lines and IL-6 receptors have been found in many cancer cells, it is uncertain what role IL-6 plays in mediating tumor growth. Recently, Luc Dirix and colleagues at the Oncology Centre St.-Augustinus and the University of Antwerp (Wilrijk, Belgium) looked for a correlation between IL-6 and tumor progression in untreated metastatic breast cancer (*Int. J. Cancer* **2003,** *103,* 642-646).

The researchers collected blood samples from 96 unselected breast cancer patients at the moment of diagnosis of metastasis and determined the serum levels of IL-6 using an enzyme-linked immunosorbent assay, which had a detection limit of 0.7 pg/mL. In 10% of patients, IL-6 was undetectable, and of the remaining patients, 51% had IL-6 levels above the median of 6.6 ± 2.1 pg/mL. The researcher found that patients with multiple metastatic sites had higher IL-6 levels (8.15 ± 1.7 pg/mL) than patients with only one metastatic site (3.06 ± 6.6 pg/mL). Also, patients with IL-6 levels above the median had a median survival time of 277 days, whereas those with levels below the median had a survival time of at least 722 days.

Interestingly, IL-6 levels showed no correlation with clinicopathological factors such as age, menopausal status, or tumor grade, indicating independent prognostic value for the biomarker.

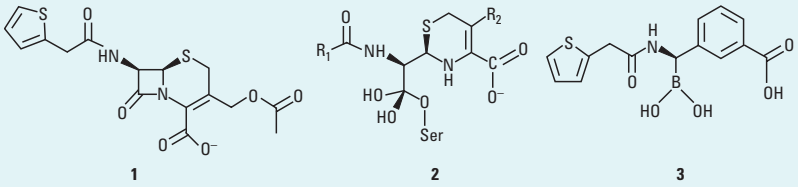
—RANDALL C. WILLIS

ILLUSTRATION: PROTEIN DATA BANK

Boronic β-lactamase busters?

For each class of antibiotics, there is a different set of rapidly evolving mechanisms of bacterial resistance, each posing a serious threat to public health and an escalating challenge to medicinal chemists. For β-lactam antibiotics, including penicillin and cephalosporins, the principal rivals are the β-lactamase enzymes, the genes for which are spreading widely among bacteria. New generations of β-lactams with enzyme inhibitory power have been introduced to combat these enzymes, but their similarity to the original drugs has allowed resistance to develop further. Scientists led by Brian Shoichet from the University of California, San Francisco, and Fabio Prati from Università degli Studi di Modena e Reggio Emilia (Modena, Italy), however, have taken a different course (*J. Am. Chem. Soc.* **2003,** *125,* 685–695).

The multi-institute team synthesized structural analogues of cephalosporin β-lactamase-bound intermediates, replacing the β-lactam motif with a boronic acid (see figure), a moiety the Shoichet group had recently shown to be a strong inhibitor of the broad-spectrum β-lactamase AmpC. By synthesizing a range of compounds with these parameters, the researchers found molecules that inhibit AmpC, even at very low concentrations, with K_i values as low as 1 nM as measured in enzyme-binding assays. The two most effective inhibitors were shown in bacterial cell cultures to reverse the resistance of several clinical pathogens to the third-generation cephalosporin ceftazidime. Furthermore, in-depth X-ray crystallography pointed to clear structural explanations for the variation in inhibitory activity among the synthesized compounds. "The crystal structures will be a jumping-off point for the next design cycle," says Shoichet, "and we expect to be able to improve affinity still further."



Evolving inhibitors. The cephalosporin antibiotic cephalothin (**1**), an intermediate (**2**) of a cephalosporin in a serine β-lactamase, and a glycylboronic acid AmpC inhibitor (**3**).

The results, however, raise some serious questions, according to Shoichet. To reach the affinity levels that they did, his team had to add more and more functional groups reminiscent of those on the original β-lactam substrates. "They are still inhibitors because they are boronic acids," he says, "but we worry that we have entered a sort of Faustian bargain with resistance evolution. By decorating the inhibitors with substrate groups, it is possible that they will be inactive against resistance mutants that have already appeared against β-lactams."

The solution to this puzzle will be vital to determining whether the family of inhibitors can be of actual therapeutic use as antiresistance agents. This may take a while, says Shoichet, "but new inhibitors of some sort are really needed."

—DAVID FILMORE

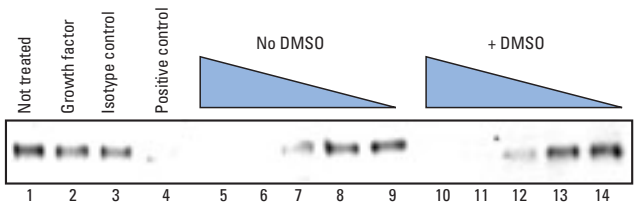
mAb fuel

Wai Lam Ling and colleagues at the Schering-Plough Research Institute (Union, NJ) recently demonstrated the efficacy of a simple stimulatory agent to boost production of hybridoma cell-derived monoclonal antibodies, which are in great demand in pharmaceutical research for their potential applications as diagnostic reagents and clinical therapeutics. Low antibody titers have often hampered the progress of such endeavors, but the Schering team has shown that dimethyl sulfoxide (DMSO), added in the right amounts and with the right timing, can significantly increase production from hydridoma c19 cells with the full retention of mAb bioactivity (*Biotechnol. Prog.* **2002,** ASAP).

The researchers found that at concentrations above 0.3% DMSO, c19 cell density and viability were reduced, but at lower concentrations, these parameters were comparable to those of untreated cultures, even five days after cell seeding. Thus, the group investigated the effect that treatment of the c19 cultures with 0–0.3% DMSO had on mAb production—first in 125-mL Erlenmeyer flasks and then in 10-L bioreactors. In both cases, they found that there was a pronounced effect and that the optimal time to add the DMSO was after three days, the time of maximum cell density. Adding 0.2% DMSO at the three-day mark increased mAb production twofold seven days after seeding on the small and the large scale.

Parallel assays of mAbs derived from DMSO-treated and untreated cells clearly showed that bioactivity—specifically, the inhibition of autophosphorylation of the targeted receptor—was unchanged. In addition, heavy- and light-chain compositions determined by SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) and glycosylation patterns measured by fluorophore-assisted carbohydrate electrophoresis were similar, indicating that structural integrity was maintained with DMSO addition.

—DAVID FILMORE



DMSO treatment did not alter the in vitro activity of the mAb on DU146 prostate cancer cells. Equal amounts of mAb in lanes 5 and 10; 6 and 11; 7 and 12; 8 and 13; 9 and 14.

Crystallize to deliver

Dry-powder inhalers offer several advantages over the oral ingestion of drugs—not only for pulmonary targets but for systemic delivery as well. Inhaled drugs are not degraded in the gastrointestinal tract, and they are exposed to the surface of the lung, which is well supplied with blood. However, only about 10% of the inhaled drug typically reaches the alveoli. This lack of performance can stem from limitations in both the inhaler and the powder formulation. For optimal drug delivery, the micronized drug powder should have a tight particle-size distribution and a low cohesive force that prevents agglomeration.

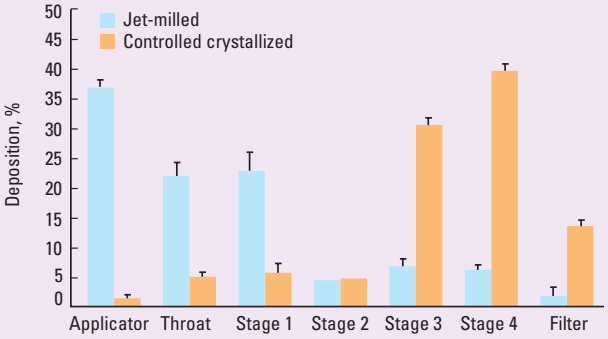
The traditional method of drug powder micronization is jet-milling, but this approach provides only a limited opportunity for control of parameters such as size, shape, morphology, and surface properties of the milled particles, and its high energy input can promote chemical degradation. The resulting powders are generally amorphous, which negatively affects their flow properties, and have broad size distributions. Thus, an alternative method is sought to prepare microsized drug particles. To that end, Bernd Müller and colleagues at Christian Albrecht University Kiel (Germany) recently explored a controlled crystallization process. They demonstrated this strategy with steroids and an anti-inflammatory drug by using a solvent change process (*J. Pharm. Sci.* **2003,** *92,* 35–44).

The researchers poured an aqueous solution of hydroxypropyl methylcellulose (HPMC) into organic solutions of the various drugs with constant mixing. Upon exposure to water, the drugs form a disperse solution of crystals, and the HPMC binds to the surface of the newly formed crystals, keeping them from growing or agglomerating. The dispersion is then spray-dried to generate microsized particles.

Scanning electron microscopy indicated that the resulting powders were homogeneous and had narrow particle-size distributions. Similarly, X-ray diffraction studies clearly showed crystallinity. The researchers also noted that compounds with high lipophilicity (as defined by their log P values) were better able to stabilize the microsized particles. The authors used a multistage liquid impinger to determine the aerodynamic properties of the powders and found that the controlled crystallized drugs travel significantly farther than their jet-milled counterparts (see figure) and, thus, have an increased respirable fraction. Finally, the researchers examined the dissolution properties of the drugs and found that the hydrophilic properties of the HPMC caused the drugs to dissolve faster, increasing drug effectiveness.

The authors point out that this strategy could lower the effective doses of inhaled drugs and cut down on side effects.

—RANDALL C. WILLIS



Drug race. Deposition characteristics of jet-milled and crystallized ECU-R2, an anti-inflammatory drug, as measured by a liquid impinger. Higher stage numbers indicate greater distance.

Computer dating for GPCRs

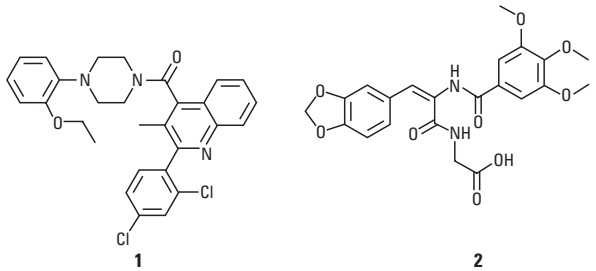
It's hard to go looking for ligands in all the wrong places, especially when you are a pharmaceutical company trying to find promising lead candidates. Instead of relying on a total hit-and-miss cruising of combinatorial libraries, researchers are attempting to find shortcuts to match up potential drug targets with compatible ligands.

GPCRs (G protein-coupled receptors) represent one of the most important classes of drug targets, acting as receptors for 50% of currently available pharmaceuticals. Ideally, if a particular set of GPCR-binding prerequisites for molecules could be found, a significant amount of "blind-dating" could be avoided along with all the ancillary costs involved. Konstantin Balakin from Chemical Diversity Labs, Inc. (San Diego), reported a method of producing GPCR-targeted libraries using a property-based design approach (*J. Chem. Inf. Comput. Sci.* **2002,** *42,* 1332–1342). This is a tailored advancement over the concept of determining whether a compound is simply druglike or non-druglike, following parameters such as Lipinski's rule of five.

The scientists developed an algorithm that used simple, automated procedures for designing combinatorial libraries that would show preferential GPCR-binding activity. A neural network approach was taken to correlate structure with binding. Parameters were developed by screening a structural database of 5376 known GPCR ligands compared with 7506 compounds without GPCR affinity that could act as a "negative training set". Eight structural descriptors were analyzed for each compound and then correlated to GPCR (+) or GPCR (–) binding. These included molecular weight, number of H-bond donors and acceptors, number of bonds that could be rotated, number of aromatic bonds, and various solubility factors. The resulting model produced a global description of GPCR-binding-like qualities that could separate (+) and (–) with more than 90% accuracy.

Compared with other therapeutic agents, the idealized GPCR binding ligand showed less flexibility, lower polarity (fewer H-bond donors and acceptors), and distinctly more hydrophobicity. The researchers successfully translated their model to designing a combinatorial library that showed a high percentage of these GPCR-ligandlike traits.

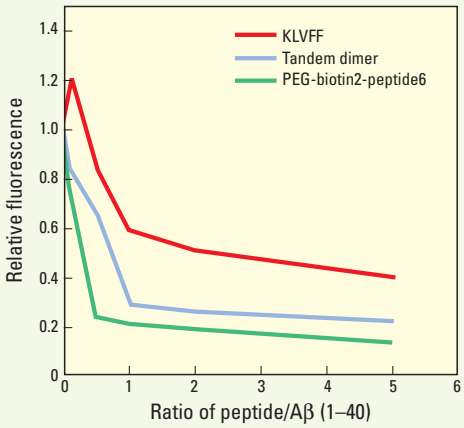
—MARK S. LESNEY



Hot and not. Compound **1** was among the highest-scoring test compounds selected from a GPCR-targeted library, whereas **2** had one of the lowest scores.

β-Amyloid binders

The neuropathology of Alzheimer's disease (AD) is largely caused by the formation of extracellular lesions comprising deposits or fibrils of the β-amyloid peptide (Aβ). Fibril formation is thought to occur in two stages: nucleation and extension. In the nucleation phase, Aβ monomers associate to create disordered aggregates. These aggregates then rapidly accrete more monomers to form the structured and neurotoxic antiparallel β-sheet fibrils. Many researchers are looking for ways to inhibit the formation of the Aβ peptide, hoping that this will prevent fibril formation. There is evidence, however, that Aβ might serve some biological function. Thus, rather than inhibit Aβ formation, other researchers are trying to inhibit aggregation.



Peptide power. The inhibition of ordered fibril formation leads to decreased fluorescence intensity.

Previous studies have shown that within the Aβ peptide is a minimal stretch of five residues—$K^{16}LVFF^{20}$—that support peptide aggregation. Stanley Stein from Rutgers University (Piscataway, NJ) and colleagues at various research centers recently explored the use of this small peptide to disrupt the aggregation of Aβ and its formation into fibrils (*Bioconjugate Chem.* **2003,** *14,* 86–92). In addition, the researchers tested the retro-inverso peptide ffvlk, comprised of D-amino acids, which is resistant to proteolytic degradation. They also added an extra lysine residue to the retro-inverso peptide (ffvlkk), thinking that this might increase the peptide's avidity to the fibrils.

Both retro-inverso peptides had slightly higher affinity for the fibrils than did KLVFF. A tandem dimer of ffvlk, however, exhibited a 100-fold higher affinity, as did a conjugate of KLVFF-PEG-KLVFF. Conjugating six retro-inverso peptides to polyethylene glycol (PEG) increased the affinity to fibrils a further 100-fold (10^4-fold total). Although none of the peptides was able to disrupt fibrils that had already formed, they did appear to prevent fibril formation by binding to and precipitating the Aβ monomer.

The researchers suggest that this last finding offers the possibility of a future therapeutic role for the conjugate. Soluble Aβ peptide is in equilibrium between the central nervous system and peripheral bloodstream, and earlier work using anti-Aβ antibodies in a mouse model of AD showed that it was possible to generate a sink effect, pulling Aβ across the blood–brain barrier (BBB) and thereby preventing fibril formation. According to the authors, "The antibody results suggest that even if the multivalent conjugates do not cross the BBB, they may prove useful for diagnosis and treatment of AD."

—RANDALL C. WILLIS

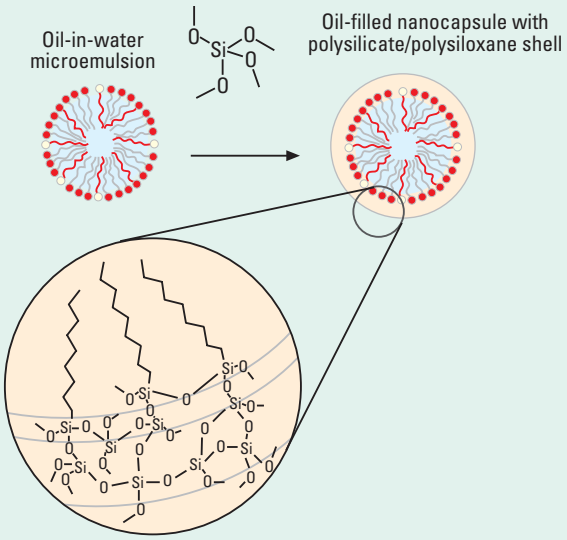
Nano-antidotes

Drug developers are typically more concerned with getting their products into a patient's bloodstream than extracting them out again. However, at present, antidotes are not available to treat many harmful, even life-threatening, drug reactions. Randolph Duran and colleagues at the University of Florida (Gainesville) and Hamdard University (New Delhi) are working to remedy this situation with their studies of silica-encased oil droplets that remove lipophilic drug compounds from saline solution (*Chem. Mater.* **2002,** *14,* 4919–4925). Drug removal differs from drug delivery in that encapsulated drugs are often designed for slow release, whereas drug removal must often be accomplished rapidly. Removal agents must reduce the available drug concentration to below the toxicity threshold, and they must be biocompatible.

Duran's group uses thermodynamically stable oil-in-water microemulsions containing oil droplets less than 250 nm in diameter, which can pass through even the smallest human capillaries (typically about 5000 nm across). Encasing the droplets in silica shells stabilizes them against coalescence and rupture.

Duran and co-workers used normal saline solution to simulate human plasma and hexadecane as the oil phase. Buffers kept the pH at 7.4 (physiological pH) throughout the process. Adding two surfactants, a nonionic detergent and an organic silane, produced self-assembled droplets of hexadecane surrounded by a surfactant layer. Tetramethoxysiloxane was added to form the silica shell.

The silica-encased droplets were added to a solution of bupivacaine hydrochloride (a lipophilic local anesthetic) in saline solution. At pH 7.4, the ratio of ionic to electrically neutral bupivacaine is about 5:1. The neutral form is soluble in the oil core of the droplet, and as it is progressively removed from the aqueous phase, the equilibrium shifts to produce more of the neutral form. HPLC analysis indicated that more than 99% of the bupivacaine was removed from a saline solution with an initial concentration of 200 µM. Nanocapsules having 0.1% w/v oil had a measured uptake capacity of 1900 µM. Uptake studies in serum are ongoing.



Nanocapsule containment. A stable shell forms through the condensation of a tetramethoxysiloxane around the microemulsion droplet. The inset illustrates the network forming the shell. (Adapted from *Chem. Mater.* **2002,** *14,* 4919–4925.)

—NANCY K. McGUIRE

Paring probes

High-density oligonucleotide microarrays have, without question, become routine and essential tools for genomic analysis. One threshold that has not yet been reached with oligonucleotide chips, however, is a report of a single array representing the entire human genome. This will require researchers to maximize the number of genes assayable on a single slide, while maintaining high sensitivity and specificity. Todd Golub and colleagues at the Whitehead Institute (Cambridge, MA) and the Dana-Farber Cancer Institute (Boston) recently demonstrated a simple strategy for doing just that (*Genome Biol.* **2003,** *3,* 0073.1–0073.4).

Generally, oligonucleotide arrays contain multiple numbers of distinct probes per gene to assure high sensitivity and specificity. For example, the GeneChips used in Golub's study contained 20 probe pairs—that is, 20 pairs of perfect match (PM) and single mismatch (MM) oligonucleotide sequences—for each of 7129 genes. A typical measurement would consolidate the hybridization difference data from these 20 pairs into a single expression level, or "average difference", for each gene. In this study, the researchers used a training set of 141 human tumor samples to determine the individual probe pairs with difference values (PM minus MM binding) that best correlated with the average difference values for each gene.

The researchers evaluated the capabilities of these optimized probe pairs—eliminating 95% of the original GeneChip probes—against an independent, nonoverlapping test set of 176 human tumor samples. They found that 79.3% of the hybridization values were within twofold of their respective average difference value compared with 57.8 % for randomly selected oligonucleotide probe pairs. More important, using only the values from the optimized probe pairs produced nearly identical results to full array average difference analysis in the subclassifications of acute myeloid leukemia versus acute lymphoblastic leukemia (ALL), T-cell ALL versus B-cell ALL, diffuse large B-cell lymphoma survival prediction, and medulloblastoma brain tumor survival prediction, all of which have genomic signatures that have been previously described.

The researchers see this systematic optimization as a promising strategy for future chip design, particularly for high-throughput screening applications, in which small, genomewide arrays will be of most use.

—DAVID FILMORE

Key terms: drug delivery (p 12), genomics (p 16), informatics (p 15), medicinal chemistry (p 11), modeling (p 15), process (p 12), screening (pp 11, 15), technique (p 16)