Combi-extensions

Covalent tethering, developed by scientists at Sunesis Pharmaceuticals, Inc. (San Francisco, *www.sunesis.com*), is a dynamic combinatorial chemistry method in which the target molecule is used as a template upon which to assemble its own inhibitor. Proteins with unpaired cysteine residues at or near the active site are probed with a library of disulfide-containing small molecules. When a molecule with even low affinity for the target binds near the active site, its disulfide reacts with the cysteine to form a complex that can be isolated and identified.

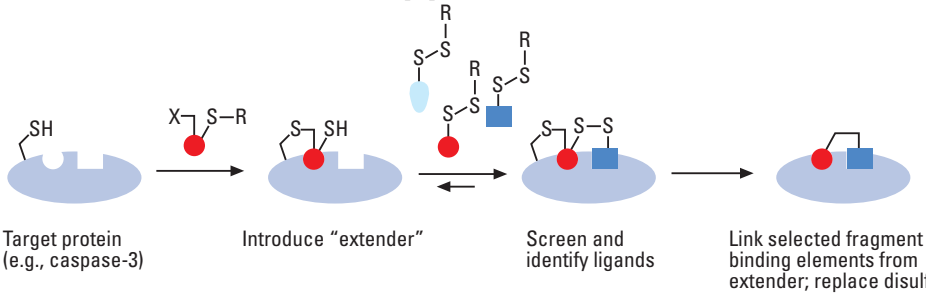
Recently, Daniel Erlanson and colleagues from Sunesis improved on this method by creating the extended tethering strategy (*Nat. Biotechnol.* **2003,** *21,* 308–314). In this approach, the target cysteine residue is modified with a small molecule with affinity for the protein and a protected thiol group. Disulfide-containing small molecules with affinity for the target then react with the tether to form a higher-affinity complex.

The researchers used their method to identify inhibitors of caspase-3, an enzyme involved in programmed cell death that has an active site containing cysteine. They reacted the enzyme with a tether composed of an arylacyloxymethyl ketone that interacts irreversibly with the cysteine, an aspartyl group that fits into part of the active site, and a masked thiol in the form of a thioester group.

After probing this complex with a combinatorial library, the researchers identified two molecules that bound the complex—a salicylic acid sulfonamide and a thiophene sulfone. These complexes served as models for reversible binding compounds that were shown to inhibit in vitro caspase-3 activity at the micromolar range. Further structural refinements led to the development of potent inhibitors of in vivo apoptosis.

"By developing family-specific extenders," says Erlanson, "we can rapidly develop inhibitors against a wide range of targets within a given family."

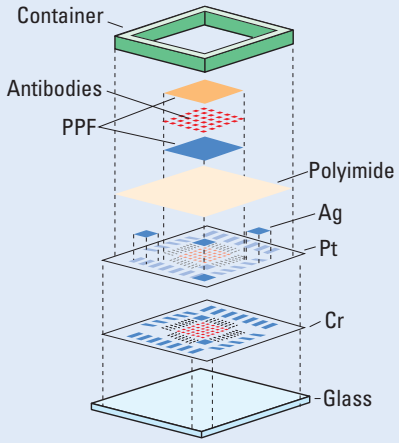
—RANDALL C. WILLIS



The extended tethering strategy. (Adapted with permission from *Nat. Biotechnol.* **2003,** *21,* 308–314. Copyright Nature Publishing Group 2003.)

Protein chip

Protein counterparts to the technologies developed for parallel DNA detection could provide a substantial leap forward in diagnosing disease. Unfortunately, the optical detection technologies used for high-density arrays with tens to hundreds of thousands of sites are less efficient for the smaller arrays (fewer than 100 sites) that would be used for clinical diagnostics. Electrochemical detection offers a solution by virtue of its low detection limits, fast response times, and ability to contain the entire system (including signal-processing elements) in a compact space.



Chip sandwich. Layer-by-layer construction of the electrochemical protein chip. (Adapted with permission from *Anal. Chem.* **2003,** *75,* 1116–1122.)

Isao Karube from the University of Tokyo and co-workers at the same and other Japanese schools have developed an electrochemical protein chip that capitalizes on immobilized capture antibodies contained in a plasma-polymerized hexamethyldisiloxane film (*Anal. Chem.* **2003,** *75,* 1116–1122). Using their device, the researchers demonstrated the detection of α-1-fetoprotein (AFP, a marker for liver disease) and β_2-microglobulin (β_2MG, a marker for kidney disease and certain cancers of the white blood cells) with high specificity and negligible cross-talk between neighboring sites.

The chip was constructed on a 17 mm × 17 mm glass substrate that supported an array of 36 platinum working electrodes (see figure). The leads were insulated with a polyimide film, over which was deposited a layer of rabbit antihuman AFP IgG and rabbit antihuman β_2MG IgG antibodies sandwiched between two layers of plasma-polymerized film (PPF).

The researchers tested the system by adding solutions of AFP or β_2MG antigens, followed by mouse antihuman AFP IgG or mouse antihuman β_2MG IgG secondary antibodies, and then goat antimouse IgG tertiary antibodies labeled with glucose oxidase. When the capture antibodies bound the target proteins, the resulting enzymatic reaction of glucose oxidase produced hydrogen peroxide, which was oxidized, producing a distinct increase in the electrical current.

—NANCY K. McGUIRE

Eggs, not butter

Increased vegetable fat and dietary fiber consumed during adolescence might lead to a reduced risk of breast cancer, according to a recent case-control study by researchers at Harvard Medical School and School of Public Health (Boston). The study also revealed that greater egg intake in these years could reduce risk, while butter consumption increases it.

Several lines of evidence have led researchers to investigate a link between teenage diet and breast cancer, including differences in incidence among populations where diet arises as a significant variable and indications that adolescent breast tissue is more vulnerable to carcinogenic exposure.

Looking for more specifics, Lindsay Frazier and Harvard colleagues analyzed questionnaire data from 843 women diagnosed with breast cancer against controls (*Breast Cancer Res.* **2003,** *5,* R59–R64). The subjects were participants in the Nurses' Health Study, which began in 1976 with about 122,000 women between the ages of 30 and 55 years and, in 1986, included a food-frequency questionnaire that asked about diet between the ages of 12 and 18 years (diagnosis for all study cases was between 1976 and 1986).

The analysis probed adolescent consumption of 24 different foods, ranging from milk and orange juice to broccoli, beef, and French fries. Most of the foods—all except eggs and butter—were not significantly related to risk of breast cancer, negatively or positively, as determined by relative risks calculated through multiple logistic regression adjusted for various factors such as age at diagnosis, menopausal status, and family history.

The researchers also determined adolescent nutrient intake (14 nutrients, including protein, animal fat, vitamin A, and vitamin D) of the survey respondents by performing a simple ratio calculation based on the specified food consumption and the nutrient content of the food. Again, consumption of most nutrients did not correlate with breast cancer risk or prevention; however, vegetable fat and dietary fiber did show some statistically significant links suggestive of the latter.

"Although the news media has put more emphasis on eggs, I think that the vegetable fat and fiber findings are more interesting," says Frazier. "Vegetable fat is a primary vehicle of fat-soluble vitamins (A, D, E), which all have anticarcinogenic effects, and fiber binds free estrogen and thereby may decrease risk." More precise research into the dietary effects will be necessary.

For now, the authors warn that the study's findings do not serve as conclusive evidence and only warrant more complete assessments of the diet–cancer link.

—DAVID FILMORE

PHOTO: PHOTODISC

FDA looks forward

An initiative launched by the FDA intends to make innovative medical technologies available sooner and reduce the cost of developing safe and effective products. The proposal recommends three areas in which to achieve these goals.

One is to encourage early communication to improve the quality of New Drug Applications and avoid, whenever possible, multiple cycles of FDA review. This will reduce delays and product development costs. Another component of the proposal is to improve the effectiveness of the review process by adopting a quality-systems approach to medical product reviews that includes improved training of FDA review staff, more defined review templates to be followed, and augmented quality control measures. Finally, the plan seeks to facilitate new product development by providing clearer, up-to-date guidance for particular diseases and emerging technologies.

The initiative, which involves all four of the FDA's medical product review centers (drugs, biologics, devices, and veterinary medicine) is outlined in the report *Improving Innovation in Medical Technology: Beyond 2002.*

As the report points out, 2002 had a varied outcome. For example, there was an increase in the total number of products that were approved, including new treatments for hepatitis B and C and various cancers, but the year brought fewer marketing applications and longer total approval times in some significant product areas, including new molecular entities for drugs and for Class III medical devices.

FDA Commissioner Mark B. McClellan said in a press release, "FDA approved a variety of important new medical products last year, and FDA largely met its user fee review goals. However, we also noted a decline in product applications from manufacturers in some key areas, which contributed to an increase in average and median review times."

Many of the FDA initiatives will be developed in collaboration with other government agencies and expert groups, particularly the NIH. The FDA is confident that it can expedite bringing potentially significant up-and-coming technologies to the market by reducing regulatory doubt and increasing the certainty of product development. To do this, the FDA plans to make the regulatory pathways clearer in three rising areas of technology: cell and gene therapy, pharmacogenomics, and novel drug delivery systems.

—FELICIA M. WILLIS

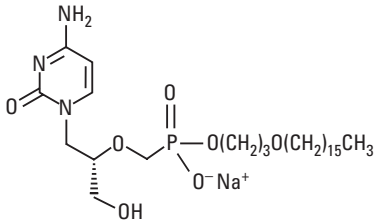
Pox pill penetration

Last year, at the 15th International Conference on Antiviral Research, Karl Hostetler and colleagues from the San Diego VA Medical Center and University of California at San Diego reported on a promising oral treatment for the smallpox virus. Now, in a more recent study, they have established a metabolic basis for the activity of this drug, 1-O-hexadecyloxypropyl-cidofovir (HDP-CDV), which has also been shown to be effective in mice against a range of other viruses, such as vaccinia, cytomegalovirus, and herpes simplex (*Mol. Pharmacol.* **2003,** *63,* 678–681).

HDP-CDV is a derivative of cidofovir (CDV, marketed as Vistide), an FDA-approved treatment for cytomegaloviral eye infections. Although CDV has activity against this and other double-stranded DNA-based viruses (including those mentioned above), the drug must be given at high doses intravenously—which is not favorable, for example, for a quick response to a smallpox bioterrorism outbreak—and has dose-limiting renal side effects. HDP-CDV, on the other hand, has demonstrated oral bioavailability and more than 100-fold greater antiviral activity than CDV in rodents.

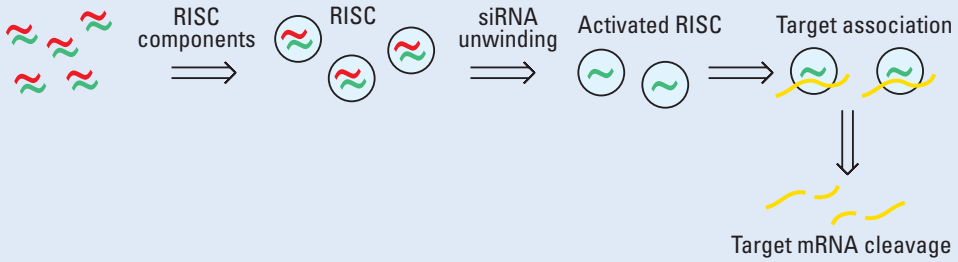
To further understand the therapeutic, the San Diego team studied the in vitro cellular uptake and anabolic metabolism of CDV and its derivative. They found that the uptake of HDP-CDV over 24 h is 11- to 23-fold greater than that of CDV, indicating that increased cell penetration is likely an important factor in the antiviral improvements. Furthermore, the levels of the active antiviral metabolite CDV diphosphate (CDV-DP) that are formed 24 and 48 h after cellular exposure to HDP-CDV are 102-fold more than the levels resulting from CDV exposure. With the use of ^{14}C labeling, the researchers also assessed the half-lives of the various metabolites. Notably, CDV-DP had a 10-day half-life after HDP-CDV treatment, compared with a 2.7-day half-life after CDV exposure.

The researchers suggest that the lipid packaging of the derivative molecule avoids the slow cellular uptake process (fluid endocytosis) undergone by CDV through a rapid association with cellular membrane phospholipids, allowing a more effective metabolic pathway to antiviral activity. According to Hostetler, Chimerix, Inc., a company he founded in 2002, is now developing HDP-CDV for the prevention and treatment of smallpox.



HDP-CDV.

—DAVID FILMORE



Interfering mechanism. The RNAi pathway involves assemblage of the siRNAs in endoribonulease-containing complexes known as RNA-induced silencing complexes (RISCs), unwinding to form activated RISCs, and cleavage of complementary HCV RNA.

Interfering with HCV

Chronic hepatitis C virus (HCV) can lead to the development of cirrhosis of the liver and hepatocellular carcinoma. Current treatment combines two antiviral medications: interferon (IFN) and ribavirin—however, most patients are not cured using this dosing regime. Recently, Francis V. Chisari and colleagues at The Scripps Research Institute (La Jolla, CA) demonstrated the use of an IFN-free antiviral strategy called RNA interference (RNAi) as an alternative (*Proc. Natl. Acad. Sci. U.S.A.* **2003,** *100,* 2014–2018).

This novel antiviral mechanism induces double-stranded RNA (dsRNA) degradation. Because dsRNA acts as an intermediate in the replication of some animal viruses and can function independently of IFN-induced pathways, RNAi targeting of HCV is a natural application. In the Scripps study, RNAi was used to inhibit ongoing HCV RNA replication and protein expression in derived human hepatoma (Huh-7) cells that replicate the HCV genome.

To test the effectiveness of RNAi in inhibiting HCV replication, specific HCV and human cells were transfected with targeted sequences of short interfering RNAs (siRNAs) and screened by reverse transcription (RT)-PCR. The siRNAs with the greatest specific inhibition were further transfected, and total RNA was harvested for isolation and immunoanalysis. Selected harvested cells were then treated with IFN, and total RNA was harvested and analyzed by RT-PCR. The researchers performed Western blot analysis to determine the level of protein expression, Northern blot analysis to determine the kinetics of RNAi, and RT-PCR to quantitate the level of HCV RNA.

Within 2 days of siRNA transfection, HCV RNA replication was inhibited 12.5-fold, and this inhibition held for at least 6 days, demonstrating that RNAi can significantly suppress viral and cellular transcripts. Protein expression, detected by immunofluorescence analysis, was decreased in cells, suggesting that some or all cells were transfected or that RNAi may have spread. Although the results indicate that HCV is susceptible to inhibition by RNAi, and that RNAi demonstrates a potential role in the treatment of HCV infection, the investigators believe further studies are needed to fully analyze the ability of RNAi to inhibit replication of a full-length HCV replicon.

—KIMBERLY S. CLEAVES

Thiamine to tumor

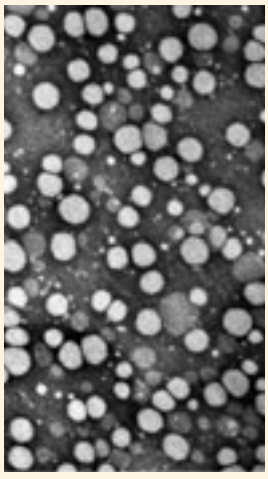
The fact that all eukaryotic cells have specific uptake mechanisms for vitamins has attracted interest in using these compounds as targeting ligands for cell-specific drug delivery. In particular, attention has been directed toward ligand attachment to nanoparticle drug formulations. Researchers from the University of Kentucky (Lexington) recently investigated the cell-specific ligand potential of thiamine (vitamin B_1), previously untested for this purpose, for gadolinium (Gd) nanoparticle delivery to breast cancer cells (*Bioconjugate Chem.* **2003,** *14,* 404–411).

The lanthanide series metal Gd has been proposed as an agent for neutron capture therapy, in which it would act as a selective neutron beam absorber at tumor cells to bring about localized cytotoxic radiation. The crucial factor, of course, is the preferentiality toward the cancer over healthy cells, and this is where the thiamine could be significant.

Scientists transfected a breast cancer cell line with the thiamine transporter genes *THTR1* and *THTR2* as experimental targets for their 100-nm thiamine-coated Gd nanoparticles—formed by oil-in-water microemulsions—and transfected other cells from the same line with an empty expression vector.

Nanoparticle–cell association was significantly greater with the *THTR1* and *THTR2* cells than with the controls. For example, with 180 µg/mL of nanoparticles, the concentration associated with the transporter-transfected cells was 1.9- to 2.9-fold greater than with the controls. Furthermore, the researchers compared the *THTR* cell association of thiamine-coated nanoparticles with that of uncoated nanoparticles and found, at a temperature of 37 °C, 2.5- to 5-fold greater association in the former. At 4 °C, however, at which a reduced activity of the thiamine transporters is expected, thiamine-coated nanoparticle cell linkage fell off dramatically.

Overall, say the researchers, the study demonstrated the high specificity of thiamine-bound nanoparticle association to cells that express thiamine transporters and its dependence on the extent that transporters are expressed. Thus, the next step toward a tumor-targeted therapy is determining the degree of thiamine transporter expressed by various human tumors compared with normal tissue. Studies cited by the authors that support preferential binding conditions provide some cause for optimism. For instance, increased thiamine use in tumor cells—because of the importance of B_1 in the biosynthesis of many cell constituents—and depleted thiamine stores in the surrounding tissues, which might be a result of this augmented use of the vitamin, have been observed.



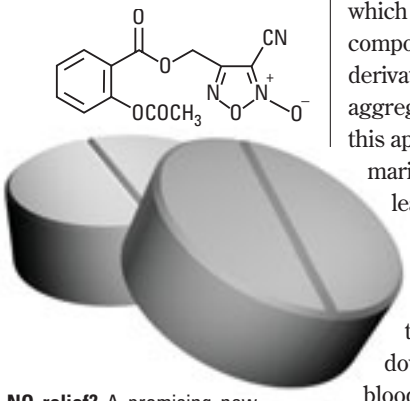
Thiamine-coated nanoparticles. (Adapted with permission from *Bioconjugate Chem.* **2003,** *14,* 404–411.)

—DAVID FILMORE

Aspirin toxicity—NO way

Nonsteroidal anti-inflammatory drugs (NSAIDs) act by inhibiting cyclooxygenase (COX) enzymes, but they have a significant long-term side effect—gastrotoxicity. Not only can this prove debilitating to patients and force cessation of treatment, but it can also be fatal, as in the case of the 16,500 arthritis patients who died from these effects in the United States in 1997.

For this reason, the pursuit of NSAID alternatives has been increasing. COX-2 inhibitors are one such alternative that led to several blockbusters, including celecoxib (Celebrex) and rofecoxib (Vioxx). Because nitric oxide (NO) protects gastric mucosa through a number of physiological mechanisms, there is also a strong focus on NO-releasing NSAIDs. Researchers Clara Cena and colleagues from the Università degli Studi di Torino and the Università degli di Parma (both in Italy) and the University of Edinburgh (Scotland) recently reported on their efforts to develop NO-donor esters of aspirin—the prototype of NSAIDs—that maintain the benefits of the original drug while eliminating side effects (*J. Med. Chem.* **2003,** *46,* 747–754).



NO relief? A promising new NO-donating NSAID.

They synthesized furoxanyloxypropyl and furoxanylmethyl esters of aspirin and related derivatives such as furazans, cyanos, and a nitrooxy derivative, and evaluated NO release.

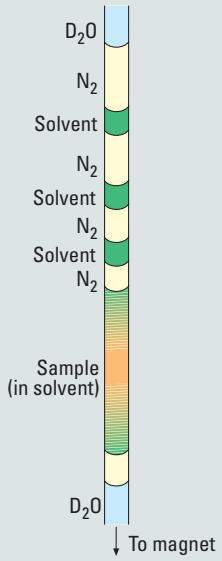
The scientists determined anti-inflammatory activity by injecting the seaweed extract carrageenan into the hind paw of a rat. This increases paw volume significantly within an hour. Simultaneous intragastric administration of aspirin reduces the inflammation significantly. Because aspirin in the dose used produces significant gastric lesions in rats, as measured by mucosal lesions and hemorrhage, it was easy to compare these deleterious effects with those of the derivative compounds.

Not only did several of the furoxan derivatives show equivalent or better anti-inflammatory effects than aspirin—one showed 100-fold greater inhibition—but these ester derivatives showed no ulcerogenic properties at equimolar concentrations. Contrary to theoretical expectations, NO-releasing ability was not correlated to the protection, although this was not completely unexpected, because gastrotoxicity from aspirin has been reported to depend on the carboxyl group, which was altered in these compounds. In addition, the derivatives inhibited platelet aggregation, as did aspirin, but this appeared to be based primarily on their ability to release NO. HPLC analysis of the compounds incubated in human serum showed no evidence of the derivatives breaking down into aspirin in the blood.

—MARK S. LESNEY

Going with the flow

For many years, pharmaceutical researchers have relied on the combined techniques of liquid chromatography, nuclear magnetic resonance (NMR) spectroscopy, and mass spectrometry to rapidly characterize and screen the myriad compounds that they generate. The need for automation led to the development of autosamplers that exchange the numerous glass NMR tubes required for a single experiment, but this method of sample exchange comes with its own problems. Not the least of these challenges are sample loss caused by tube breakage and the introduction of foreign matter from the environment into the spinner bearings. To address these shortcomings, Gregory Leo and colleagues at Johnson & Johnson Pharmaceutical Research and Development (Spring House, PA) recently created a flow injection system that eliminates NMR tubes and minimizes sample-handling errors (*Anal. Chem.* **2003,** ASAP).

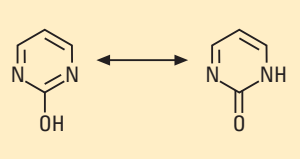


Plugging along. Using a series of solvent, D_2O, and nitrogen gas plugs, researchers have developed a flow injection system for high-throughput NMR analysis.

The researchers designed their flow injection system so that a compound sample (dissolved in deuterated solvent) is automatically drawn from a sample vial. The sample is pushed through an NMR flow probe by a series of nitrogen gas, deuterated solvent, and deuterium oxide (D_2O) plugs (see figure) into the magnet, and it is finally recovered in a second vial. The scientists discovered that the NMR spectra suffered from severe line broadening if the sample chamber was not completely filled, but that they could eliminate this problem by simply increasing the sample size. Doing so, they achieved spectra that were effectively identical to those derived using standard methods. Furthermore, by adjusting the volumes and solution contents of the wash steps, the researchers eliminated carryover.

The scientists then compared the costs associated with their flow injection and the autosampler systems. A quick calculation indicated that although flow injection requires more solvent, it is less expensive than the other method because it does not require NMR tubes. This difference translates into possible savings of thousands of dollars per month. Thus, the researchers are confident that "as the vendors continue to develop and improve the technology, [flow injection] may soon be the common mode of operation in open-access industrial environments."

—RANDALL C. WILLIS



Proton shuffle. In lactim–lactam tautomerism, proton migration produces a gain or loss of aromaticity.

Chemical matchmaker

Why pay for the same thing twice? It often happens that several of the drug candidate compounds in commercially marketed proprietary libraries are actually tautomeric forms of the same compounds. The large registry databases, including CAS and Beilstein, have computational methods and well-defined rules for identifying tautomers, but proprietary compounds do not appear in these registries.

Sergey Trepalin and colleagues at Chemical Diversity Labs, Inc. (San Diego), have devised a program that runs on a personal computer and can distinguish among several types of tautomeric compounds as well as compounds containing ionic and semipolar bonds (*J. Chem. Inf. Comput. Sci.* **2003,** ASAP). Once the compound list is reduced to a set of canonical structures (basic forms defined by a set of rules), the library database can be searched in real time. Various forms of the same compound can be identified, saving time and money in the early stages of drug discovery.

The structure search algorithm is an integrated utility of the ChemSoft chemical database management environment (ChemSoft, Inc., San Jose, CA), and it runs on a Windows platform. At present, the software is set up to identify tautomers that differ by the migration of a proton. The most favorable tautomeric form, however, which depends on a complex combination of physical variables, is not identified.

Tautomers can be identified in both aromatic and aliphatic systems as long as the proton does not migrate more than four atoms away and there are no transformations of the molecular scaffold. The software works well for hydrogen migrations in acyclic compounds (1,3-type), five-member aromatic systems, and heteroaromatic systems in which the compound loses aromaticity upon proton migration (see figure). The ionic and semipolar bond algorithm is useful for identifying related compounds, such as amines and carboxylic acids, that are commonly isolated as salts and for matching charged and charge-neutral representations of functionalities such as nitro groups. A database of canonical structures can be compiled from a library of 100,000 structural entries in about 25 min using a 1.8-GHz processor, and searching takes only a few seconds. The system was tested on 15 tautomeric forms of guanine; if any one of the structures was queried, all others were retrieved effectively.

—NANCY K. McGUIRE

KEY WORDS: automation (p 19), cell biology (pp 15, 16), clinical (p 12), combinatorial chemistry (p 11), drug delivery (p 16), high throughput (p 11), informatics (p 19), medicinal chemistry (p 16), proteomics (p 11), regulations (p 12), screening (p 16), technique (pp 11, 15, 19)