DATA-DIRECTED
DRUG DESIGN

In drug discovery, it has been fashionable to accentuate the rational, to salute the triumph of reason over serendipity, to reverence the molecular rather than the biological in molecular biology. Now there is a realization that, in molecular design, all the rationality of the computer-human combination is no substitute for data.

Until recently, there were no examples of strong drug candidates developed by predominantly rational processes. When the subject of clinical success arose, those in rational drug design would cough nervously and dust off examples such as the renin inhibitors developed by Abbott (Chicago, IL) or Merck’s (Rahway, NJ) angiotensin-converting enzyme (ACE) inhibitors—captopril, enalaprilat, and cilazapril. To regard the development of these compounds as wholly “rational” requires a pliable definition of terms. The structural information on the drug targets, renin and ACE, had been determined not experimentally through X-ray diffraction but by modeling based on homologous proteins—serine peptidase and thermolysin, respectively. It is now generally held that homology modeling does not provide a sufficiently stable base for drug design.

Within the last few months, however, a number of strong drug candidates have emerged which can lay better claim to rational foundations. Firstly, there were the HIV protease inhibitors developed by Abbott, followed more recently by Merck’s carbonic anhydrase inhibitors aimed at the substantial market in glaucoma treatments. Compounds from both projects are now in Phase I clinical trials. Joshua Boger, formerly with Merck and now President of Vertex (Cambridge, MA), believes that there are at least another half-dozen examples of compounds discovered by rational design which have yet to emerge because of the internal politics and code of secrecy within the pharmaceutical industry.

**DATA BASIS, NOT DATA BASES**

What the Abbott and Merck experiences have clearly demonstrated, says Boger, is that protein-based design is far from being a purely theoretical or computational activity. “Some people think that you can just determine the structure of the target protein by X-ray diffraction and then tell the crystallographers to go away. In fact you have to continue all the way through with the structural biology—it’s an experimental process.” The norm in the top-ranked pharmaceutical companies like Merck, Abbott, Glaxo (Greenford, U.K.), Hoechst (Frankfurt, Germany), Bayer (Wuppertal, Germany) and the Basel trio of Hoffmann-La Roche, Sandoz, and Ciba-Geigy is for interactive drug design teams—consisting of molecular biologists, computational chemists and modelers, medicinal chemists, and crystallographers—to be working on the design of agonists or antagonists of particular molecular targets. A similar pattern is also seen in the specialist drug design companies such as BioCryst (Birmingham, AL), CrysChem (Riverside, CA), Vertex, and Agouron Pharmaceuticals (La Jolla, CA). The U.K. “electronic biotechnology” start-up, Proteus Biotechnology (Marple) has a similar capability in the service it provides.

Crystal of the canine lymphoma antibody obtained by the Cryschem unit of Immunopharmaceutics.

**IMAGE UNAVAILABLE FOR COPYRIGHT REASONS**
The structure-based design and development process is, in fact, extremely iterative as Peter Johnson, president at Agouron, demonstrates for the company’s thymidylate synthase (TS) program. “At Agouron, we solve the structure of the target protein and then solve the structure of that protein with the inhibitor in place in order to check the extent that our modeling predictions are borne out in reality.” The same approach is used at Merck: the development of the carbonic anhydrase inhibitors, for instance, involved 10-15 repeats of the design/synthesis/structure cycle over a period of several years. But Agouron appears to have taken the approach to an extreme. “We’ve actually solved more than 100 complexes of TS with prototypic compounds of various stripes,” says Johnson. “These compounds have been not only inspired by—but also designed atom-by-atom—with reference to protein structure.” And that, he believes, is quite unlike the development history of any other class of compounds in the pharmaceutical industry.

Agouron expects its TS inhibitors to enter the clinical phase during 1991. They could be beaten there by the inhibitors of purine nucleoside phosphorylase (PNP) designed by BioCryst, the University of Alabama, Birmingham/Southern Research Institute start-up.

The clinical work (on applications in the AIDS market as potentiatiors of the antiviral drug dideoxynosine (ddI) and in the arthritis/inflammatory market) was undertaken by Geigy, which now owns all rights to the compounds. Steve Elick, who was the crystallographer on the PNP project, confirms the cyclical nature of the design process: “We made 60 or so compounds and determined the crystal structure [of protein-inhibitor complexes] for over half of them. If there is any one thing that is responsible for the success of the project, it’s the iteration in the process.”

Elick also points out that the emphasis on solving the structures of protein-inhibitor complexes has revealed many features that would probably not have been predicted by modeling alone. “We discovered that there were considerable conformational changes on binding. One always occurs: 15 amino acid residues of PNP form a flap, and when the enzyme binds substrate, the flap opens; when there is no substrate or inhibitor, the flap occupies most of the active site.” Other, more subtle changes, such as adjustments to optimize hydrogen-bonding, also occur.

The structure-based approach need not be as onerous as some people once believed. Elick says, because the frequent acquisition of data adds its own precision. “Modelers would argue that you need to have precise structures. But our PNP structure was solved at 2.8 Angstroms RMS—you don’t need to have super-high resolution for drug development...If you’re fortunate, you may be able to construct a model from data which only resolves at the theoretical limit of 3.5 Angstroms.”

**Design and Optimization**

At this point, with little clinical data available, it is difficult to assess whether an iterative design process can lead more rapidly to effective drugs. Peter Johnson puts forward two alternative criteria by which the “success” of structure-based design might be judged: “Firstly, to what extent can the approach yield new chemical entities which are both novel—in that they differ very substantially from any natural substrates or ligand of the target—and diverse—in that they differ from each other. Secondly, how rapidly can one generate such a lead and then optimize both its in vitro and pharmacological properties.”

The method can clearly generate significant numbers of credible new chemical entities. Around half of the 250 TS inhibitors synthesized by Agouron and the 60 PNP inhibitors produced by BioCryst went forward to crystallographic studies after showing significant activity in vitro. The extent to which the active compounds were diverse is not clear, although the strategies adopted by the companies—to design groups of molecules which interacted with different parts of the enzyme’s active site—would have led to diversity, at least initially. For the Abbott HIV protease inhibitor project, the early strategy was one of structure-based avoidance, according to team leader, John Erickson. “We tried to come up with a compound that looked less like a peptide than any of the transition state analogs that one would otherwise design for an aspartyl protease. We wanted to get away from peptides because, in general, they have terrible pharmacological properties. We found that [resultant compounds] were considerably more stable than their peptidic cousins and also had a clear advantage with respect to their first pass metabolism [usually 90-95 percent of an administered peptide is removed at a very early stage in the liver]. So the concept of trying to make something that is less of a peptide has worked—but the initial design element is not all that sophisticated.”

Where the structure-based design approach seems to come into its own is in the optimization of lead compounds. Ema Enna, executive vice president and senior scientist at Nova Pharmaceuticals (Baltimore, MD), explains that for improving the potency of compounds like the company’s bradykinin receptor antagonists for use against cold symptoms and a range of other inflammatory conditions, the computational chemistry/modeling approach is more efficient than “just making analogs and homologs” of leads. However, he believes it is difficult to quantify the sav-
ings that can be made by using structural knowledge to avoid chemical dead-ends. “Good medicinal chemists have a tremendous amount of insight and I wouldn’t want to offend any chemist by saying that, for instance, we’ve cut down on one tenth the number of compounds we need to look at. I don’t think you can establish a hard-and-fast number on that.”

That, nevertheless, is exactly what research teams at Abbott attempted to do within the company’s renin inhibitor development programme. What the company did, according to John Erickson, was to take two low-potency inhibitors in two separate projects and try to improve the activity of both compounds. The drug design team was involved in only one of the projects. Even when doubts about the validity of the “control” are taken into account, the results of this experiment are impressive: the production of neutralising antibodies against the virus and there have been similar findings in other laboratories with murine type III reovirus, poliovirus type II, rabies glycoprotein, Sendai virus, TMV, hepatitis B virus and HIV.

**Receptors Next**

“The next frontier for drug design,” is how BioCryst’s Steve Elick describes the prospects of extending rational drug design from enzyme targets to receptor targets. One of the problems in crossing that frontier is, of course, that there are no receptor structures available at a sufficient resolution—not even within industrial laboratories. A spectacular international collaboration involving researchers at the Medical Research Council Laboratory of Molecular Biology in Cambridge, U.K., the Fritz-Haber-Institut in Berlin and California’s Lawrence Berkeley Laboratory has led to the determination of the structure of bacteriorhodopsin by high-resolution electron cryomicroscopy. Bacteriorhodopsin is a membrane protein that naturally forms two-dimensional crystals and it is these that have been analyzed. The resolution of its structure is asymmetrical, 3.5 Angstroms in one direction but only 10 Angstroms in another. Nevertheless, this study has revealed many of the major features of the molecular interactions and has added to the data obtained by Robert Huber’s group on the photosynthetic reaction center.

The main significance of bacteriorhodopsin, however, is that it is a seven-helical transmembrane protein. Sequence data indicate that many of the pharmacologically important receptor targets—notably many of the receptors for biologically active peptides and the G-protein linked receptor family—which include the muscarinic and [beta]-adrenoreceptors—are also seven-helical proteins. The way is now starting to open, therefore, for modeling receptor structures and designing receptor agonists and antagonists.

As with enzymes, the use of modeled rather than experimentally determined receptor structures has its limitations. Although refined structures for receptors are probably 2-3 years away, a number of other interesting structures have recently been solved. One of them, the soluble CD4 antigen fragment, may provide new insights into the interaction of CD4 with HIV and with class II antigens. The structures of cyclophilin and macrophilin will soon be available, according to Sandoz’ Trevor Payne. However, the aldose reductase structure solved recently under contract by the French firm, Biostructure (Strasbourg), is likely to be kept under wraps for some time.

There are also some doubts about whether it will ever be possible to mimic or antagonize the activity of large ligands like growth factors or cytokines with low molecular mass compounds, even if experimental structural data are available. Trevor Payne of Sandoz believes “The chances of success for such an approach will be, in almost every case, very low, especially where the affinity between the endogenous ligand and its receptor is very high [sub-nanomolar]. His reasoning is essentially that the interactions of large ligands with their receptors have evolved to use a large number of relatively weak ligands over a large (600 Angstroms2) surface. Certainly, the number of amino acid residues involved in protein-protein interactions is high although that hasn’t prevented the design of enzyme inhibitors.

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