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Innovations

Fragmentary Solutions
Astex Therapeutics Puts the Pieces Together

Stumbling across a promising drug candidate may not be mere statistical probability, but more a matter of putting the right fragments together. High throughput screening (HTS) which combines the advantages of combinatorial chemistry, advanced computing, and robotics, has been the mainstay of drug discovery over the past 15 years or so. Pushed by advances in analytical methods used to identify and quantify potential interactions, statistical data analysis, and automation, HTS is preferred over rational drug design for pharma and biotech alike.

HTS involves systematically testing libraries of drug candidates against a "target" protein for inhibition or activation. A basic knowledge of the system is all that is required; it is an unsentimental approach. HTS is essentially a brute force process of sorting through numbingly large amounts of data. "In certain cases it is the only way to do things," said Dean R. Artis, vice president, Lead Generation, Plexxikon, Inc. "It is the only technique that can be used for any target class. If you can develop an assay, you can drive an HTS approach. That is its strength and its weakness."

But despite impressive gains in screening capacity, the number of new drug submissions to the FDA has not increased accordingly. According to Joseph DiMasi, Ph.D., director of economic analysis at the Tufts Center for Drug Development, the average cost of discovery per drug has skyrocketed and, at last tally, averaged $802 million, with a nonclinical contribution of $336 million, including failures (based on year 2000 figures).

Don't Underestimate the Little Guys

In the same way that the tallest fellow in a bar is the one you notice first, HTS is biased toward compounds with strong interactions. Certain compounds that show only weak interactions (the shorter fellows) could be overlooked.

About three years ago, fragment-based drug discovery (FBDD) or fragment-based screening (FBS), an approach utilized at Abbott Laboratories in the mid-1990s, started gaining favor as a complement to combinatorial chemistry-driven screening efforts. After all, if everybody is screening similar compound libraries against fairly similar targets, the results might start looking the same as well. "The whole genesis for fragment-based drug discovery conceptually came about because [with it] you can explore chemical spaces of very weakly interacting compounds that have much greater potential diversity," said Artis.

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Companies like Cambridge, UK, based Astex Therapeutics, Ltd. (www.astex-therapeutics.com), and its competitors Vernalis, Sunesis, Sareum, and Plexxikon, rather than looking for a tight binding drug lead, examine the weak interactions of small fragments with a target and expand on or connect individual fragments to form a strongly bound new drug candidate. This approach requires knowledge of the three-dimensional structure of both the target and the target-fragment complex. This is usually determined by X-ray diffraction. Data on the interactions is often further verified by other tools such as NMR. However, researchers using this approach may be stymied by proteins or small molecule-protein complexes that resist crystallization, and, even with automated equipment, protein crystallization remains more of an art than a systematic technique.

Armed with preliminary structural information showing the basis for the fragment-protein interaction, promising fragments are expanded by chemists to fit the local protein environment in an iterative process, until a tight binding drug candidate is developed. It is a logical method, and the drug designer can easily follow Lipinski's Rule of Five, an empirical rule of thumb delineating properties that maximize an oral drug candidate's probability of surviving development [1], to narrow the universe of possibilities. According to Lipinski, a molecule has a greater chance of being orally active if it has no more than 5 hydrogen bond donors, not more than 10 hydrogen bond acceptors, a molecular weight under 500, and a LogP (a measure of the molecule's hydrophilicity) under 5. Advocates claim that FBDD is faster than high throughput screening at identifying promising lead candidates.

Structured around Structure

Dr. Harren Jhoti, chief scientific officer of Astex Therapeutics, developed his own corollary to Lipinski's rule. "In a nutshell, the idea here is, rather than screening drug sized molecules, which are between 300 and 500 molecular weight, in a kind of bioassay-based high throughput screen, one would look to screen libraries of fragments which are 100–200 molecular weight," said Jhoti. "Once you are able to visualize how these fragments are bound to your protein structure…it is really

very efficient and straightforward to build that fragment up by adding functional groups which improve the potency but also the selectivity of that compound for that target and ensuring that it stays within the appropriate space of drug-like features." Jhoti recalls that, with the exception of Abbott, big pharma companies were initially skeptical. "We may as well have been talking Klingon," Jhoti said. The fragment-based discovery methodology Astex dubbed "Pyramid" soon proved itself. "It is very chemistry efficient," said Jhoti. "That is something industry has always struggled with. The amount of attrition [of early stage leads] is huge."

Jhoti, formerly head of UK-based structural drug design and bioinformatics at Glaxo Wellcome, started Astex Therapeutics in 1999 with Professors Sir Tom Blundell and Chris Abell, both structural biologists at Cambridge University. Astex garnered $90 million in two rounds of financing from venture capitalists including Abingworth, Advent International, Alta Partners, Apax, GIMV, HypoVereinsbank, Oxford Bioscience Partners, Schering AG, and the University of Cambridge.

According to Jeremy Carmichael, Ph.D., Astex director of business development, the company's ratio of approximately one structural biologist to two medicinal chemists allows Astex to develop initial hits into leads much faster than other companies. Carmichael asserted that Astex can produce 200–300 protein-ligand structures a month, an order of magnitude greater than the throughput of most big pharma companies.

Astex Therapeutics has four cancer drug candidates in the pipeline. "We think we are the first biotech to put a compound into man which was based on a fragment hit," said Jhoti. "We went from first synthesis of AT7519 to first dosing in patients in 18 months. That is at least twice as fast as industry standards." Astex has two other product candidates, including AT9283, an aurora kinase inhibitor that impedes mitosis (cell division). AT9283 received FDA Investigational New Drug (IND) approval in April 2006 and is now in Phase I study in the U.S. AT9311, an oral cell cycle inhibitor, is in preclinical development with an IND/CTA planned for the second half of 2006. Additionally, AT13387, an Hsp90 inhibitor, just entered preclinical development.

Astex Therapeutics has drug discovery and development alliances with several large pharma companies potentially valued in excess of US $1 billion, including a US $520 million alliance signed with Novartis in 2005 to develop AT9311 and AT7519. AstraZeneca is also working with Astex Therapeutics to develop small molecule inhibitors of the anticancer target Protein Kinase B (also known as Akt), a $5 million agreement, following prior collaborations.

Puny Fragments Build Strong Drugs

Sunesis Pharmaceuticals (www.sunesis.com), a 130 person company located in South San Francisco, focuses on cell-cycle inhibitors for cancer. Sunesis calls its fragment-based discovery approach "tethering" and is focusing on weak binding fragments by looking at the binding event itself. Sunesis also uses another approach called extended tethering, where fragments are linked by a covalent bond to the target protein. "In one instance you fish off the bank and the next you fish off the pier," said Dr. James Wells, professor of pharmaceutical chemistry and cellular and molecular pharmacology at UCSF and Sunesis cofounder.

Sunesis has three compounds in the pipeline; SNS-595, in phase II clinical trials; SNS-032, in phase I clinical trials; and SNS-314 (preclinical.) The company also has five strategic collaborations with Biogen Idec, Johnson & Johnson PRD, and Merck. Founded in 1998, Sunesis raised $200 million from various investors and went public in 2005. "I think it [fragment based discovery] is complementary to high-throughput screening," said Robert McDowell, Ph.D, vice president, Discovery Chemistry.

On the other side of the Bay, Plexxikon (www.plexxikon.com) is a 65 person company located in Berkeley. Plexxikon starts with fragments between 150 and 350 Da in molecular weight, then screens them using biochemical assays, looking for very weak activity. Subsequent cocrystallographic structural analysis sorts the wheat from the chaff. Sometimes, to get additional interaction information, Plexxikon employs diverse techniques like surface plasmon-enhanced Raman spectroscopy and NMR to weed out the false positives from each technique. "You use an assay just to identify a measure of biochemical activity at high concentration," said Dean Artis, "We really let the crystals decide what is an interesting compound versus a confusing assay artifact. You get a very small, compact starting point."

Following this methodology, the company develops a "scaffold" that has binding affinity for multiple members of a protein family. Currently, Plexxikon uses several scaffolds to target each of three protein families (kinases, nuclear receptors, phosphodiesterases). Artis commented that Plexxikon has filed INDs for molecules generated using this approach. According to Artis, candidate compounds for preclinical testing can be generated in as little as 3–6 months. The company initiated collaborations with Genentech, Inc., in 2003 and Wyeth Pharmaceuticals in 2004.

"I came to Plexxikon to make drugs," Artis said. "This process gives you a very efficient path to do not just an exercise in technology, but to do drug design in a time frame that might actually help people sooner rather than later. Our first drug is in patients, and we've only been doing chemistry for four years."

What is the view of big pharma on this method? "We typically use it as the complementary method to HTS," said Dr. Andreas Marzinzik, group leader, Integrated Lead Discovery Program, Novartis Institute of Biomedical Research, "we would really want to focus on targets where HTS delivers no promising hits." Marzinzik cited, for example, non-ATP competitive inhibitors of kinases, which are hard to identify by high throughput screening. "The advantage is really that FBS technology is not prone to false positives." According to Marzinzik, Novartis assembled a working group to advance fragment-based discovery technology in 2005. Novartis has an internal project and also collaborates with outside companies for specific projects. "It is definitely a tool for us here at Novartis," Marzinzik said.

It remains an open question, after the iterative optimizing of weak

leads, if FBDD really shaves off that much time and effort from the discovery process. But it could pick up a few unprepossessing fragments that might grow up to be great drugs if they just got a bit of attention.

Selected Reading

1. Lipinski, C.A., Lombardo, F., Dominy, B.W., and Feeney, P.J. (1997). Adv. Drug Delivery Rev. *23*, 3–25.

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