DDT Vol. 8, No. 20 October 2003

Structure-guided applications in drug discovery

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The *Chemistry-Driven Drug Design and Discovery* conference (San Francisco; 23–24 June 2003) (http://www.srinstitute.com/cs258) was the first in its series and was uniquely organized to integrate the applied chemistry and biology topics that currently drive drug discovery. Topics ranged from new screening techniques to structural biology to structure-guided medicinal chemistry applications. The main emphasis of the conference was on structure-based applications in drug discovery, focusing on hot areas, such as kinases and GPCRs.

Fragment-based screening and assembly

There is currently a need for more-frequent discovery of higher quality lead compounds to improve the productivity of R&D. Fragment-based strategies are, potentially, a powerful way to achieve this goal. Christopher Lepre (Vertex Pharmaceuticals; http://www.vpharm.com) described the 'SHAPES' strategy [1], which involves NMR screening of a small drug-like fragment-based library to identify initial weak hits (K_D values between low μM and 5 mM) that can be followed-up by a variety of complementary methods to produce high-quality leads.

Lepre outlined two successful applications of SHAPES screening for lead generation to the lipid binding protein, aP2, and the MAP kinase, JNK3, as pre-HTS and post-HTS examples, respectively. Screening only 100 compounds against aP2 by NMR methods resulted in 13 hits in the 0.3–800 μM range. X-ray structures of SHAPES hits bound to aP2, together with NMR follow-up screens, helped to generate a pharmacophore model and the development of several sub-μM leads for chemical optimization. In the JNK3 program, SHAPES NMR screening followed by a virtual screening strategy identified three scaffold classes with K_i values between 0.7 and 13 μM. These three classes of compounds showed distinct binding modes by x-ray crystallography; the resulting information providing an insight that could be useful across a wide variety of kinase targets.

Daniel Wyss (Schering-Plough Research Institute; http://www.schering-plough.com) described the application of 'structure-based NMR screening approaches' (SbN) [2] to a variety of drug discovery targets, for which HTS has failed to identify suitable leads. NMR methods were used to identify lead-like small-molecule hits from customized fragment libraries. These often initially weak hits (K_d values of μM–mM) were, after some optimization, turned into viable leads through focused chemical optimization, guided by SAR and 3D structural information. Wyss discussed novel tools that use the data generated in the NMR screen to rapidly provide an accurate structural representation of protein–ligand complexes under conditions that are not favorable in traditional structural work. The application of SbN to the discovery of sub-μM competitive inhibitors against the NS3 protease of the hepatitis C virus (HCV) was discussed, as was application to the discovery of lead inhibitors of the HCV helicase in the low μM range and leads in the 20–50 nM range against the tumor biology target, human MDM2. Wyss concluded by summarizing the application of SbN as a primary screening assay to seven drug discovery programmes, together with the chemistry approaches that were used to turn the NMR-detected weak hits into viable leads.

Johan Oslob (Sunesis Pharmaceuticals; http://www.sunesis.com) described the fragment assembly technique, 'tethering', which involves cysteine-captured ligands identified by MS from a small library of disulfide-containing molecules. Tethering enabled improved design of potent small-molecule antagonists of the IL-2/IL-2R interaction [3], the most potent analog having an IC_{50} value of 60 nM, weak cell-based assay activity (EC_{50} = 3 μM) and favorable PK profiles. Oslob demonstrated that a portion of the receptor-binding surface of IL-2 is highly adaptive but that tethering was still able to effectively probe ligand preferences and tolerances at this highly dynamic subsite.

Dean Artis (Plexxikon; http://www.plexxikon.com) described the company's 'Scaffold-Based Drug Discovery™' platform for lead generation using a highly integrated structure-guided approach that involves the screening of >20,000 selected protein family-targeted scaffold-like compounds by biochemical assays. These assays are tuned for the detection

  PII: S1359-6446(03)02853-8

of low-affinity compounds that can be followed-up by high-throughput co-crystallographic structure determination. This approach led to the discovery of 26 candidate kinase scaffolds, novel leads for kinase targets and nM leads for c-Abl kinase, which address the issue of 'Gleevec-resistant mutants'. Artis also discussed the rapid development of leads in the 100 nM range for the nuclear hormone receptor PPARγ (peroxisome proliferator-activated receptor γ), a drug target for the treatment of type II diabetes, by starting from x-ray structures of protein complexes with weak initial scaffolds in the 100 μM range that showed wide-ranging activity against this protein family.

Gene-family approaches to drug discovery

Similar gene-family drug discovery approaches, based on high-throughput x-ray crystallography, were described by David Webb (Syrrx; http://www.syrrx.com) and Janice Culpepper (Structural Genomix; http://www.stromix.com). Webb emphasized Syrrx's portfolio strategy which focuses on clinically validated targets whose structures are not known and the barrier to entry for others is high, important gene families such as kinases and proteases to build internal knowledge and expertise, and therapeutic areas with large unmet medical needs. He discussed the successful application to the rapid development of two chemotypes for the serine protease, DP-IV (dipeptidyl peptidase IV), with *in vitro* potencies of 4 and 25 nM, respectively. Syrrx's goal is to deliver two DP-4 clinical candidates within the next one or two years for the treatment of diabetes.

Peter Reiner (Active Pass Pharmaceuticals; http://www.activepass.com) described a gene-family approach to the discovery of ABC transporter-modulating compounds by using functional assays for the majority of human ABC transporters (31 out of 48), which enables the profiling of compounds for both potency and selectivity early in the drug discovery process. A subset of ABC transporters are implicated in ADME, therefore these assays also provide early indications of the bioavailability of potential drugs. Barry Bunin (Libraria; http://www.libraria.com) gave a live demonstration of the application of 'LUCIA™' (Libraria's Unique Chemically Intelligent Archive), which uses gene-family-wide SAR knowledge-bases and eScreens™ to help scientists rapidly scaffold-jump to novel chemotypes. Bunin presented case studies in which novel chemotypes against Abl kinase were discovered, having potencies approaching that of Gleevec™; a new chemotype with 400 nM inhibition against an undisclosed kinase target was identified, and Libraria's eScreen was able to pick out most of the top-scoring inhibitors from a set of 33 molecules with IC_{50} values ranging from 0.0007–2.5 μM against LCK kinase, using unrelated SAR training sets. Finally, Richard Eglen (DiscoveRx; http://www.discoverx.com) described the development of several technologies for use in cell-based assay development and HTS [4]. He demonstrated their applications to several drug discovery programmes, illustrating their broad applicability to compound screening of GPCRs and kinases.

Structure-guided drug discovery

Hing Sham (Abbott Laboratories; http://www.abbott.com) presented a great example of structure-guided drug discovery with the structure-based design of a potent second-generation HIV-1 protease inhibitor, lopinavir, that is effective against resistant HIV mutants emerging in response to therapy with the protease inhibitor ritonavir. The successful design of lopinavir was based on its X-ray crystal structure when bound to the active site of HIV-1 protease [5]. Moreover, by increasing the potency tenfold and reducing the serum binding, the trough concentration was increased enough to prevent future resistance – almost no resistance was observed in 470 patients after four years.

Conversely, James Rizzi (Array Biopharma; http://www.arraybiopharma.com/) focused on the challenges of using X-ray structures to guide medicinal chemistry efforts using the type II diabetes target, PTP-1B, as the case study. He stressed the significance of docking issues such as protein flexibility, structural water interactions, small-molecule initial geometry, and the scoring and ranking of docked molecules. Rizzi also described a novel scoring method, MASC (multiple active site correction), which addresses some of the shortcomings with current methods. Leslie Kuhn (Michigan State University; http://www.msu.edu) introduced a unified approach to protein–ligand flexibility and docking that enables the sampling of significant protein main-chain or ligand ring flexibility along with protein side-chain flexibility, thus, improving the realism of protein-ligand interaction modeling. Brian Shoichet (UCSF; http://www.ucsf.edu) noted that aggregate formation appears to explain the activity of many non-specific inhibitors and might account for the activity of many 'promiscuous' screening hits that appear to be a common problem among hits from both virtual and HTS screening [6].

Membrane proteins

Over half of all drugs on the market target cell membrane-embedded proteins, yet the study of their 3D structure remains limited by the inherent difficulty in growing 3D crystals suitable for X-ray diffraction and their poor solubility for solution NMR studies. John Bushweller (University of Virginia; http://www.virginia.edu) and James

Chou (Harvard Medical School; http://www.hms.harvard.edu) described solution NMR approaches to the determination of the 3D structures of integral membrane proteins. Bushweller illustrated the determination of the structure and dynamics of the transmembrane domain of OmpA (outer membrane protein A) in DPC (dodecylphosphocholine) micelles [7]. A key step in the study of membrane proteins by solution NMR is reconstituting the proteins to their native states in lipids and detergents that are more suitable for NMR spectroscopy. Chou demonstrated that the bicelle systems, in combination with the recently developed methods for acquiring residual dipolar couplings, offer an immediate solution for high-resolution structural studies of many smaller membrane proteins by NMR [8].

Concluding remarks

In general, the attendees considered this meeting very useful and application-driven. The backgrounds of the presenters were well balanced between pharma, biotech and academia and several successful structure-guided applications for the discovery of leads and drugs were demonstrated. 3D structural information can play an important role in the drug discovery process for many classes of drug targets and although novel approaches to the 3D structure determination of integral membrane proteins by solution NMR were also demonstrated, membrane proteins remain a big challenge for the structural biologist. It remains to be seen to what extent structural biology will be able to contribute to the drug discovery process of integral membrane proteins in the coming years.

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