Small-molecule mimetics

Dr V. Hruby (University of Arizona, Tucson, AZ, USA) opened the *Fourth Annual Development of Small Molecular Mimetic Drugs* meeting in Washington DC in May with a good introduction to the topic of peptidomimetic design. This was the predominant theme of the two-day meeting, which was organized by Cambridge Healthtech Institute (CHI). Presentations covered both experimental and theoretical methods for drug discovery.

Dr Hruby explained the strengths and weaknesses of peptides and nonpeptides as therapeutic agents, and discussed processes for the optimization of peptides and/or peptidomimetics. As an example, he showed how Leu-enkephalin was converted to [D-Pen^2, D-Pen^5]-enkephalin, which is selective for δ receptors. He also pointed out the value of non-standard amino acids (e.g. β-methyl phenylalanine); such non-standard residues often have unusual Ramachandran plots and may be used to force a particular conformation within a peptide or peptidomimetic.

ADME/PK

Dr J. Gibbons (Chiron, Emeryville, CA, USA) provided an excellent overview of absorption, distribution, metabolism and elimination (ADME) and pharmacokinetics (PK) from an industrial perspective. A series of assays are used to screen libraries of small molecules for desirable properties, such as favorable oral absorption, gastrointestinal stability, resistance to hepatic clearance and oral bioavailability. A single property, such as 'oral bioavailability', is influenced by many factors (e.g. transport, metabolism in the gut and hepatic clearance), so it is desirable to have a variety of assays that allow for the examination of each process independently. Microsomal assays, for example, include homogenized livers to examine xenobiotic metabolism. An alternative approach is to test a compound against biotransforming enzymes, such as members of the cytochrome P-450 family.

Using advanced MS techniques, the sensitivity of measurement of oral bioavailability can be greatly improved (e.g. from recovery of compounds in urine). Furthermore, simultaneous measurements of oral bioavailability for many compounds can be made. This is essential for handling chemical mixtures derived from combinatorial chemistry.

Finally, Dr Gibbons pointed out that no single 'QSAR parameter' is predictive of ADME/PK properties; rather, many different parameters, such as log*P*, pK_a, solubility and hydrodynamic volume, may play a role, and modern pharmaceutical ADME/PK research requires that assays for all these properties be available.

Dr Ronald Borchardt (University of Kansas, Lawrence, KS, USA) described the development and use of the Caco-2 assay for intestinal adsorption. Caco-2 cells provide a model of intestinal mucosa. The Caco-2 assay therfore allows an estimate of the diffusion (either passive or paracellular) of drugs across the intestinal wall. By examining a series of drug molecules, it is possible to evaluate how changes to the chemical structure and physicochemical properties of a drug can affect adsorption. For example, adsorption can be greatly increased by cyclization or a reduction of the number of hydrogen bonding groups.

Thrombospondin-1

Dr D. Roberts (NCI, Bethesda, MD, USA) discussed the extracellular matrix protein thrombospondin-1 (TSP), which inhibits endothelial cell growth and motility, and inhibits tumor growth when overexpressed in human breast cancer cells. TSP has many biological activities, including platelet aggregation, modulation of fibrin clot formation, inhibition of neutral proteases and activation of transforming growth factor-β (TGF-β). It promotes cell adhesion of both normal and cancerous cells, but also inhibits cell adhesion of endothelial cells. TSP can either promote or inhibit cell motility, as well as both stimulate and inhibit growth of various cell types. The antitumor properties of TSP can be replicated from much smaller peptides derived from a single region – the Type I repeats. Peptides containing the WSXW motif inhibit the action of basic fibroblast growth factor. Peptides containing the KRFK motif activate latent TGF-β. Stable retro-inverso analogs containing both of these peptide regions (18 amino acids in total) were prepared and also show significant antitumor activity at 6 mg/kg intravenous dosing in mouse xenograft models for breast carcinoma.

Dr M. Pearson (Molecumetics, Bellevue, WA, USA) described the design of orally active thrombin inhibitors. A variety of secondary structure templates have been developed to mimic the more commonly found conformational motifs in peptides (sheet, turn, helix). Using these templates in conjunction with combinatorial chemistry and crystallographic studies, potent inhibitors of thrombin, trypsin and other serine proteases have been developed. The best compounds are subnanomolar inhibitors that are orally bioavailable in animal models and possess modest (10-fold) selectivity for thrombin versus trypsin.

Dr M. Murcko (Vertex Pharmaceuticals, Cambridge, MA, USA) discussed the importance of considering strain energy in drug design. In order to bind to its receptor, a drug molecule generally must alter its conformation; that is, the bound conformation is higher in energy than the global minimum energy conformation found in solution. The greater the strain energy in the bound state, the less potent the compound. Structural information as well as molecular mechanics and *ab initio* calculations may be used to estimate

this strain energy, and to design compounds that will minimize it, leading to more potent drugs.

Hammerhead program

Dr A. Jain (Arris Pharmaceuticals, South San Francisco, CA, USA) described a very exciting new computer program, Hammerhead, which is a fully automated, flexible docking system. Tens of thousands of compounds can be docked per week. Its scoring function has been carefully tuned using the geometries of many known receptor–ligand crystal structures. Hammerhead is able to reproduce the bound conformation of these ligands. The application of Hammerhead in the design of novel inhibitors of streptavidin was discussed.

Dr W. Hoekstra (R.W. Johnson Research Institute, Raritan, NJ, USA) described the design of fibrinogen receptor antagonists. A series of nonpeptide turn mimetics were designed to mimic the solution NMR structure of a portion of the sequence of γ-fibrinogen that is known to interact with platelet receptor glycoprotein IIb/IIIa. The synthetic exploration was done using solid-phase combinatorial chemistry, and potent oral inhibitors of platelet aggregation in the dog model were produced.

Dr W. Miller (SmithKline Beecham, King of Prussia, PA, USA) discussed the use of NMR structural information in the design of receptor antagonists – in this case, antagonists of the vitronectin receptor ($\alpha_v\beta_3$). Such antagonists may be useful in the treatment of osteoporosis, restenosis and angiogenesis. The vitronectin receptor is in the integrin superfamily, and potent antagonists do possess an RGD mimic, but the spatial relationship between the basic and acidic moieties is shorter than in fibrinogen antagonists. In model peptide vitronectin receptor antagonists, the glycine was in a turn conformation rather than an extended one. This information led the design team towards a somewhat different-looking set of scaffolds and substitution patterns, and compounds effective in both an osteoclast adhesion assay and a bone resorption assay were discovered.

Dr V. Goodfellow (Cortech, Denver, CO, USA) described a series of bradykinin (B_2) antagonists. These compounds, derived from 1,4-piperazine scaffolds, were prepared on the solid phase. The library was designed based on a pharmacophore hypothesis derived from structure–activity data for many other known bradykinin antagonists. The compounds prepared were designed to be as diverse as possible within the constraints of matching the pharmacophore model, as well as possessing the properties usually thought to be desirable in drug molecules, such as good solubility, lack of secondary amide bonds and low M_r. Novel nonpeptide B_2 receptor antagonists were discovered.

Protein farnesyl transferase inhibitors

Dr N. Kohl (Merck Research Laboratories, Rahway, NJ, USA) described the data supporting the hypothesis that protein farnesyl transferase (PFTase) inhibitors may be safe and effective antitumor agents. PFTase transfers farnesyl pyrophosphate to Ras p21, which then is able to anchor to the cell membrane, where it is active. A mimetic of the Ras C-A-A-Ser/Met tetrapeptide, L 739750 is a potent and selective inhibitor of human PFTase *in vitro*. An ester prodrug of this compound inhibited Ras processing in cell culture and blocked the anchorage-independent growth of human tumor epithelial cells and *ras*-transformed rodent fibroblasts. A wide variety of tumor cell lines, including breast, colon and pancreas cell lines, were sensitive to this inhibitor. These included both tumor types containing a mutant Ras and those without. These inhibitors change the morphology of *ras*-transformed cells, but not *raf*-transformed. Daily administration of the ester prodrug dramatically inhibited the growth of H-Ras-dependent tumor xenografts in nude mice. However, microscopic tumors remain, even after many weeks' treatment, and when the PFTase inhibitor is withdrawn, tumor growth resumes. No systemic toxicity was found upon necropsy of the treated mice.

Dr D. Matthews (Arris Pharmaceuticals, South San Francisco, CA, USA) discussed strategies for developing small-molecule mimetic drugs for cell surface receptors, such as erythropoietin and human growth hormone. Activation of these receptors requires binding of the growth factor to the extracellular portion of the receptor, followed by dimerization of the receptor. This dimerization must occur such that a signal is transduced through the cell membrane into the cytosol; this signal in turn leads to downstream activation of various pathways that are currently only partially understood. Thus, growth factor mimetic drugs are agonists that must mimic much larger protein molecules in order to be active. Mutational analysis of the receptors, as well as the growth factors themselves, provides information regarding the receptor–hormone interactions at the atomic level; this in turn can be used as a basis for rational design of mimetics – a very challenging but important problem.

TRAP screening approach

Dr L. Kauvar (Terrapin Technologies, South San Francisco, CA, USA) discussed the 'TRAP' approach to screening. A set of 30,000 compounds is screened against a panel of diverse reference proteins. The data are analyzed and a set of 18 proteins is chosen to be statistically uncorrelated in their ligand binding properties. The result of this analysis is a 'fingerprint' for each enzyme that describes the types of compounds that bind to it. The fingerprints are used to calculate a 'surrogate function', which explains the binding profile to new targets. This surrogate function can then be used to predict how well compounds from the database will bind to the new target. He reported that 25 therapeutic targets have been tested, and in 20 of these, hits of 10 μM or better have been seen. For 12 targets, the leads were judged to be chemically useful, and two of these are being pursued in-house. It was even claimed that the approach works when the original data is derived from whole-cell assays rather than *in vitro* binding.

In conclusion, as often happens at such meetings, some presenters were peddling not-quite-ready-for-prime-time technologies, but most of the meeting focused on discussion of serious science.

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