The drug designers

BY NANCY MILLS

Drug development is a fragmented tale of hits, leads, reactions, protection and a molecule called Janet.

Drugs prescribed by medical professionals are big business, with worldwide spending on prescription drugs tipped to exceed US$1 trillion by 2015, according to the IMS Institute for Healthcare Informatics. Developing a new drug is an expensive long-term proposition involving laboratory research, approved animal trials, clinical studies and strict regulatory requirements that aim to protect the consumer.

'It takes about 20 years and over $1.5 billion to get a drug from the concept stage to the market,' says Dr Jerome Wielens, a senior researcher in structural biology at St Vincent's Institute of Medical Research in Melbourne.

Many potential drug candidates fail because of problems with side effects such as toxicity, or issues associated with oral delivery. Tablets are preferred by consumers, but that means a drug must negotiate the body's digestive system and metabolic processes without losing its potency. On its voyage through the human body, a typical drug molecule must also be absorbed into the bloodstream, travel to where it is needed, cross the cell membrane, deliver its intended impact, and then be quickly eliminated from the body.

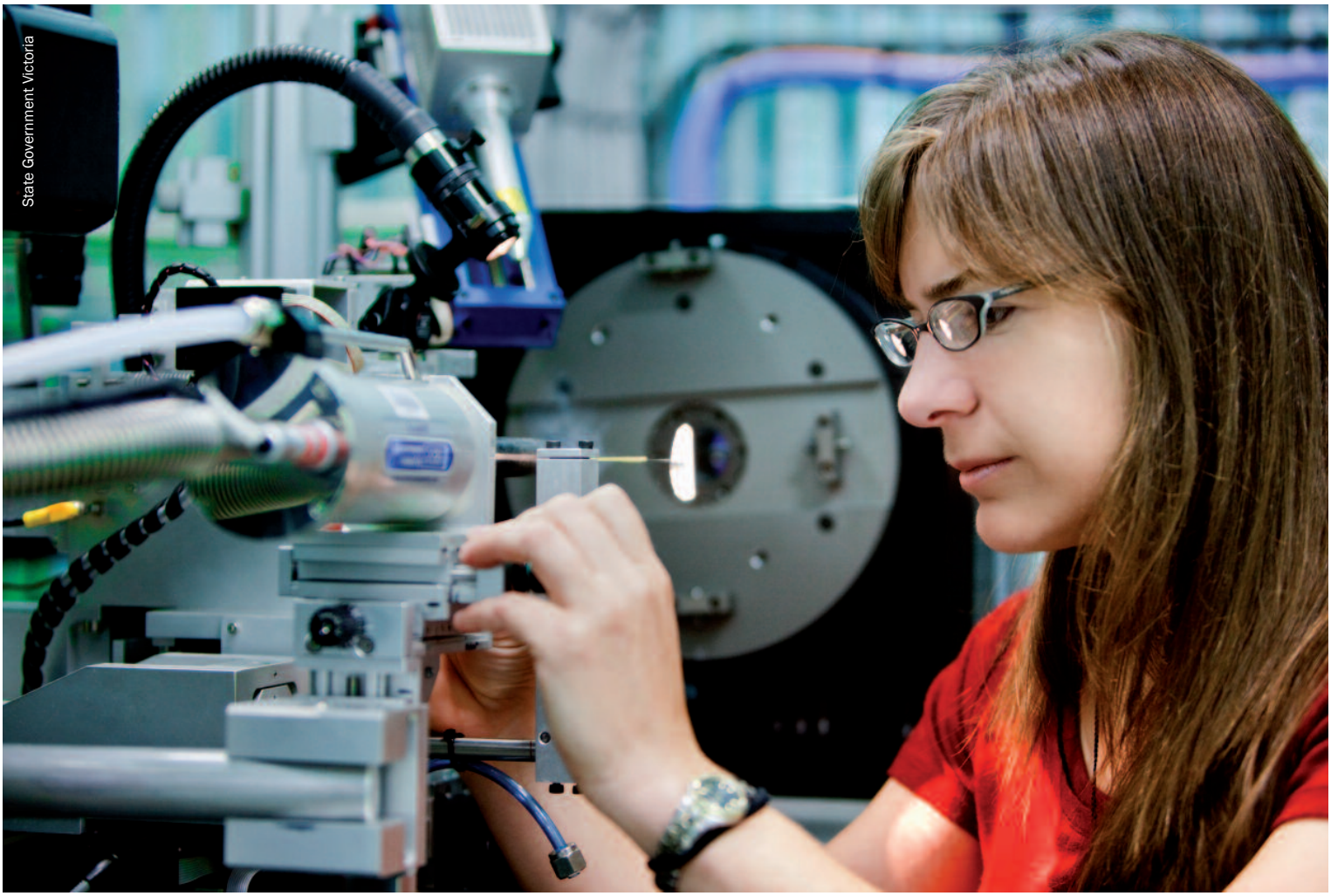
Drug development programs require expertise in areas such as chemistry, biology, crystallography, modelling and pharmacology. A drug development team will design, synthesise, assess and modify hundreds of compounds – or more – during the development of a single drug.

Selecting the right target

A team with a viral or bacterial disease in its sights begins by identifying a key protein or other biomolecule that is crucial to the organism's ability to survive, invade new cells or hosts, or replicate.

The challenge is then to find or synthesise a molecule that can block the action of the target protein so that the organism's survival is severely compromised and the human body's natural defence mechanisms can finish it off. This involves discovering one or more initial 'hits', compounds that bind well to a site on the target that will directly or indirectly modulate the target's activity. The hits are developed into 'lead' compounds with 'good potency in biological assays that reflect the targeted mechanism' (Murray C.W., Rees D.C. 2009, *Nature Chemistry* **1**, 187–92). The leads undergo more extensive assessment and further modification by medicinal chemists to optimise or introduce suitable drug-like properties.

Drug designers use several approaches. They might modify an



Dr Rachel Williamson (AS) checks the microcrystallography beamline, one of two dedicated X-ray crystallography beamlines at the facility.

existing drug or lead, work on a natural product known to be biologically active, use high-throughput screening methods to investigate as many as several million related compounds, or apply the principles of structure-based drug design or fragment-based drug discovery.

A fragmented approach

Fragment-based drug discovery is a relatively recent development. It is closely related to structure-based drug design, a method of designing drugs that uses the three-dimensional shape of a protein target to develop compounds that inhibit or otherwise modulate the activity of the protein to obtain a desired response.

A 'fragment' is a relatively simple, low-molecular-weight compound. Fragment-based drug discovery focuses on determining which types of functional groups have an affinity for sub-pockets within a binding site on the target protein. The technique relies on having high-resolution structural information for the protein and the protein–fragment complexes, which is where synchrotron X-ray crystallography comes in.

'Fragment screening enables you to identify molecules you'd otherwise never find,' says Professor Michael Parker, Deputy Director of St Vincent's Institute of Medical Research. 'But you'll need a synchrotron because robotic sample-handling capabilities, intense X-ray beams and high-resolution data are essential.'

At St Vincent's Institute, Jerome Wielens identifies hit compounds by screening a library of compounds, using NMR spectrometry to measure their binding affinity to the target protein.

'I use synchrotron radiation whenever we can crystallise the target

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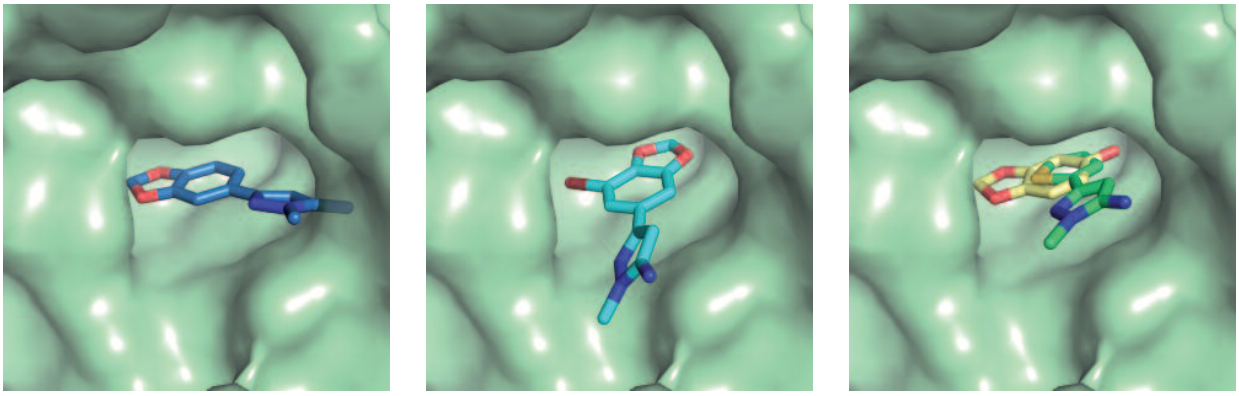
Jerome Wielens, SVI



Superimposed crystal structures of HIV-1 integrase in complex with two fragments (PDBID 3A01 and PDBID 3OVN) and the product of the merged fragments (PDBID 3A04). Integrase is represented as a Connolly surface and the ligands are shown in stick. Figure prepared using PyMOL.

protein bound to the hit compounds,' Jerome says. 'With synchrotron X-ray crystallography data, we can develop three-dimensional models to show in atomic detail how the hit compounds interact with the target protein.'

Each model is visualised on a computer where it can be rotated and modified to investigate different aspects of binding. Jerome uses his knowledge of chemistry and drug development to suggest changes to the compound that should improve its potency. He also identifies which regions of a hit compound can be used to engineer other properties into the drug, for example to improve its ability to pass through the digestive system unscathed.

Dr Martin Stoermer from the Institute for Molecular Bioscience (IMB) at the University of Queensland is developing drugs for infectious diseases, particularly flaviviruses such as dengue, West Nile, kunjin, Japanese encephalitis and yellow fever viruses that are spread by mosquitoes. Previously dismissed as 'tropical', these diseases are becoming more significant in Australia as global warming expands the range of their mosquito vectors.

As a medicinal chemist, Martin uses structure-based drug design as a critical tool in his research. He works closely with experienced collaborators such as IMB's Professor Jenny Martin, who regularly visits the Australian Synchrotron or uses the remote access capabilities of its high-throughput macromolecular and microcrystallography beamlines.

Dr Tom Peat from CSIRO Materials Science and Engineering in Melbourne is also a regular visitor to the Australian Synchrotron. He uses drug design methods to tackle a range of infectious diseases and cancers. Target molecules include HIV integrase (see Rhodes D.I. et al. 2011, *Antiviral Chem. Chemother.* **21**, 155–68), which plays a key role in incorporating viral DNA into the host cell's DNA, and hepatitis C polymerase, which is essential for viral replication.

Tom begins with structural information on the target (generally a protein) and any small molecules, substrates or products known to bind to the target. His group usually also has binding affinities obtained by surface plasmon resonance and either in vitro or cell-based assays.

'We might just start with the molecules that bind best and for which we have structural information,' Tom says. 'We use synchrotron X-ray crystallography at an early stage for fragment screening and then at every step going forward to improve our knowledge of structure-activity relationships (SARs) – and often until we have a candidate drug in preclinical development.'

Refining the hit list

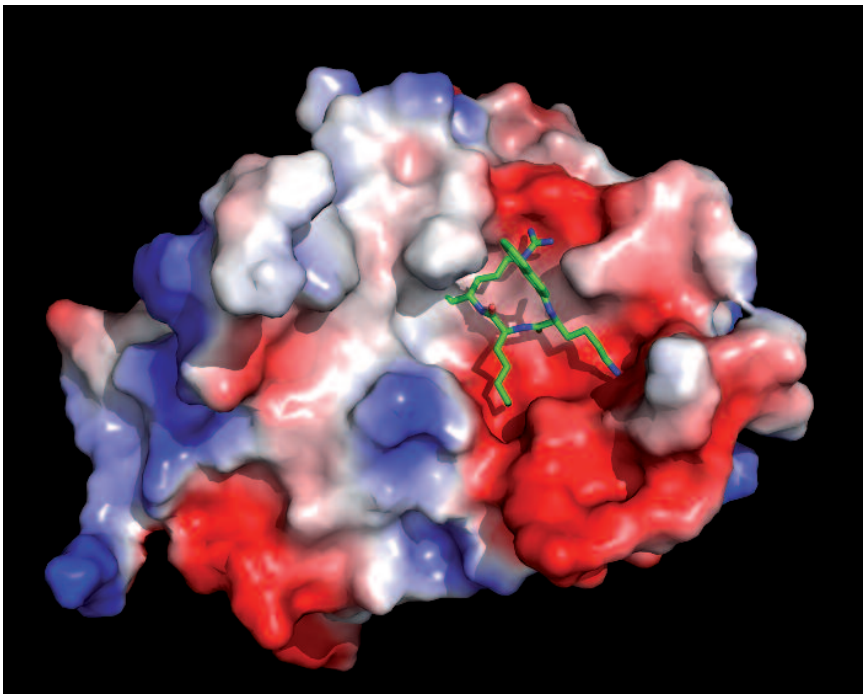
'Deciding which hit compounds to work with first can be difficult,' Jerome Wielens says. 'We have to consider potency, chemical tractability and availability of starting materials along with many other factors.'

In a recent project targeting the HIV-1 integrase protein (Wielens J. et al. 2011, *ChemMedChem* **6**, 258–61), Jerome and his colleagues had 16 hits to prioritise. Before they turned to chemistry, the team obtained crystal structures of each hit in complex with integrase. This enabled them to visualise each compound's binding environment, providing an excellent basis for further drug design work.

'In this case, we found by overlaying the crystal structures that several compounds had overlapping features as well as unique interactions with the protein. Two compounds stood out because each made good interactions with the binding site and an overlapping central ring meant that features from both compounds could be merged.'

Jerome and his collaborators synthesised the merged compound and determined its structure in complex with integrase. Their predictions were correct: the merged molecule made the same binding interactions as the two individual ones – and it was more tightly bound to integrase. The merged molecule

Martin Stoermer, IMB UQ



Shown here bound to its target protein, this molecule could lead to a potential drug for West Nile virus. Capable of causing severe neurological illness, the mosquito-borne West Nile virus could spread to Australia with global warming.

became the basis for further design work to improve its potency and develop it into a drug.

Enter the chemists

'Once a hit or 'active' is known to have the desired pharmacological activity, it's the chemist's job to optimise the compound's potency, physical properties and pharmacokinetic profile,' says Dr Michael Harding, a senior medicinal chemist at Biota.

For example, a chemist might decide to modify the active compound's shape and size to maximise binding affinity for the target protein. They could also increase or decrease side-chain lengths and ring sizes or add new groups to optimise or pick up new hydrogen-bonding or hydrophobic interactions. Adding polar groups such as amines and alcohols can also improve compound solubility.

Bioisosteric replacement is a commonly used technique that replaces a functional group with another of similar biological activity but enhanced physical or pharmacokinetic properties, such as substituting a tetrazole for a carboxylic acid. The tetrazole has a comparable pK_a (dissociation constant) but greater lipophilicity, resulting in improved absorption. Hydrogen is often replaced with fluorine, which is a similar size but will block positions prone to oxidation and consequently increase metabolic stability.

'The ideal synthetic procedure is general, robust, uses readily available reagents, proceeds under mild conditions and produces a high yield,' Michael says.

Commonly used reactions include alkylations, condensations (amide couplings, heterocycle formation), palladium-catalysed couplings (Suzuki, Buchwald–Hartwig, Sonogashira) and reductive aminations. Solid-phase reagents, automated flash chromatography and mass-directed HPLC systems have been developed to speed up the work-up of these reactions and the purification of the final products.

Michael says a useful strategy is to synthesise a library of compounds that will explore structure–activity relationships and guide further design. A medicinal chemist might aim to make around 20 milligrams of final compound for a biological assessment and some preliminary in vitro and in vivo pharmacokinetic profiling.

More chemistry, please

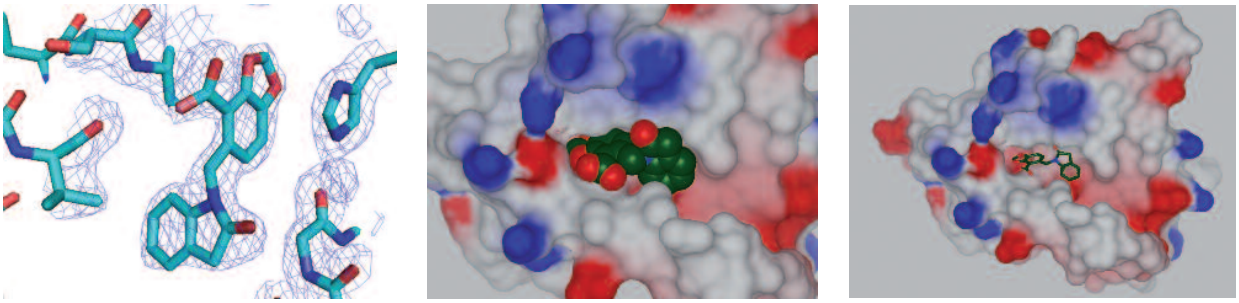
'Our throughput is most definitely not in the combichem ballpark,' Martin Stoermer says. 'We don't believe that the way to find a needle in a haystack is to make the haystack bigger!

'We would rather make small targeted libraries of up to 20 compounds in a structure–activity relationship series. Over the lifetime of a typical NHMRC grant of 3 years, on a particularly heavy med chem project we might make 100–200 carefully targeted compounds.'

In Martin's protease inhibitor research for the West Nile virus, the team started with the natural peptide sequences that the target enzyme processes. These natural peptide sequences had a lysine–arginine motif in a particular position, and a third basic residue (Lys–Lys–Arg) was added to optimise the substrates. The team then terminated the tripeptide sequence with an organic moiety at the nitrogen terminus and an aldehyde at the carbon terminus to create a low nanomolar inhibitor.

Martin says his 'bread and butter' reactions are similar to those outlined in a recent *Journal of Medical Chemistry* review by Roughley and Jordan (2011, **54**, 3451–79), including heteroatom substitutions, acylation, C–C bond formation, heterocycle formation, protection, deprotection,

Tom Peat, CSIRO



(*Z*)-5-((2-Oxoindolin-3-ylidene)methyl)benzo[*d*][1,3]dioxole-4-carboxylic acid, also known as 'Janet', is one of the lead compounds undergoing further investigation in a project that targets HIV integrase.

reduction, oxidation and addition or interconversion of functional groups.

Tom Peat and his colleagues use 'all the regular synthetic/medicinal chemistry tools' to modify compounds to get more specific binding and better inhibition. In the course of developing a potential new drug, they typically go through hundreds of molecules – such as the molecule shown above bound to its target HIV integrase protein – usually working with quantities ranging from tens to hundreds of milligrams.

Dr John Deadman from JDJ Bioservices, the chemist who worked on the molecule shown above, describes it as a 3-indanone linked to a 2-carboxybenzodioxolane.

'There isn't a common name as such, although sometimes we call it ''Janet'',' John says.

A fragment screen identified the benzodioxolane and carboxylic acid components and indicated that another aromatic ring could be incorporated. John chose the indanone. Starting with the benzodioxolane as the 5-aldehyde component, he used metallation, carboxylation, esterification, Knoevenagel condensation and deprotection reactions to convert it into the final molecule.

Technique, technique, technique

Synchrotron X-ray crystallography is an essential part of drug development when the target and potential drug molecules are available as crystals larger than a few microns across.

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When the molecules cannot be prepared as crystals of sufficient size, or cannot be crystallised at all, other synchrotron techniques such as powder diffraction, X-ray absorption spectroscopy and small angle X-ray scattering may be useful.

Powder diffraction can provide high-resolution diffraction data from polycrystalline materials, solve the structure of pharmaceuticals and evaluate the quantity of constituents in a tablet, for example. X-ray absorption spectroscopy yields useful data on chemical speciation, and small angle X-ray scattering provides information on the shapes of molecules in solution. Dr Ben Boyd from the Monash Institute of Pharmaceutical Sciences recently used small angle X-ray scattering to examine how conditions in the human digestive system affect the geometry of self-assembled lipid structures. Ben and his colleagues are using their synchrotron data to help identify structure–function relationships that could lead to more-effective drug products.

Think local, aim global

'Pharmaceutical companies around the world see structural biology, particularly crystallography, as a mandatory part of drug development,' says Michael Parker. 'The Australian Synchrotron has been absolutely essential for many of the drug development activities of Australian medical research institutes in collaboration with commercial companies such as Biota and with the CRC for Cancer Therapeutics.

'All major biotech and pharmaceutical companies actively developing drugs in Australia now use or aspire to use the Australian Synchrotron.'

Nancy Mills <nancy.mills@synchrotron.org.au> is the Australian Synchrotron's science writer. She thanks Jerome Wielens, Michael Parker, Martin Stoermer, Michael Harding, Tom Peat, John Deadman, Jenny Martin and the synchrotron crystallography team for their generous assistance with this article. The researchers acknowledge the support of various government grants, Avexa Limited and the Bio21 Collaborative Crystallisation Centre.