Fragment-Based Drug Design

Fragments offer the prospect of a more efficient approach to drug discovery – resulting in the generation of high-quality leads with a better chance of success in clinical development.

By Dr Tim Mitchell and Dr Mike Cherry, Sareum Ltd

Dr Tim Mitchell is Founder and CEO of Sareum Ltd. Previously, he was Director of the Millennium Pharmaceuticals Structure-Based Discovery department and, prior to that, Director of Computational Chemistry at Cambridge Discovery Chemistry Ltd, and a team leader in the Computational and Structural Sciences department at SmithKline Beecham Pharmaceuticals. Dr Mitchell has a PhD in Computational Chemistry and a BSc in Chemistry.

Dr Mike Cherry is Head of Computational Chemistry at Sareum Ltd. Previously, he was a member of the Computational Chemistry team at Millennium Pharmaceuticals and Cambridge Discovery Chemistry. He started his industrial career as part of the Computational Design department at the Structural Materials Centre, DERA (UK Defence Evaluation and Research Agency). Dr Cherry has a BSc and PhD in Computational Chemistry.

Recent years have seen a tremendous increase in the technologies available for the discovery of new drugs. Functional genomics research has led to the identification of an unprecedented number of potential therapeutic protein targets; combinatorial chemistry has expanded the size of compound collections; and high-throughput screening (HTS) has enabled the screening of million-compound libraries.

Despite these advances, the number of NCEs entering development has remained more or less static, while pharmaceutical research productivity (in terms of NCEs developed per millions of research dollars invested) has continued to decline. Clearly, what is needed is a new approach for the generation of high-quality leads with a better rate of success in clinical development.

One of the most exciting new methods for lead generation is fragment-based discovery. Fragments are small, low molecular weight molecules that would usually form part of a drug compound. Once bound to the active site of a target protein, they can be developed into highly selective and potent drug candidates.

LIMITATIONS OF HTS

High-throughput screening (HTS) is the traditional approach for the discovery of most medicinal chemistry leads. Despite its many successes, the method has its drawbacks. It is a complex and expensive method, and – more importantly – it has proven to be relatively unproductive, with the attrition rate for compounds in preclinical research at an all-time high.

The HTS approach is also limited in terms of the number of complex fully-formed compounds that can practically be made and stored in a collection. Even very large libraries represent only a small fraction of the vast universe of potential compounds that could be made; for molecules containing up to 30 non-hydrogen atoms, this has been estimated at over 10^{60} (1).

The quality of the leads emanating from HTS has also fallen below expectations, with many requiring extensive optimisation and – even then – failing at the last hurdle. Furthermore, many of the compounds that have been developed using HTS have been for 'easy' targets, whereas it is the more difficult targets that are likely to be the more valuable.

Thus, after a decade of HTS, there is a need for a more efficient approach to lead discovery.

THE FRAGMENT-BASED APPROACH

In contrast to HTS, fragment-based lead discovery involves the identification of low molecular weight chemical fragments (also known as scaffolds or templates) from very much smaller compound libraries. These fragments are then combined or optimised to generate lead compounds.

The first stage of a fragment-based strategy is to generate a collection of fragments. Traditionally, researchers have turned to Lipinski's 'Rule of 5' for guidance on selecting compounds for screening that maximise the development chances for an oral drug candidate (2). These four 'rules' (all multiples of five) are common characteristics found in most drugs available today: not more than 5 hydrogen bond

donors (OH and NH groups), no more the 10 hydrogen bond acceptors (notably N and O), a molecular weight under 500 and CLogP under 5. For fragments, alternative selection criteria have been proposed (3, 3a) including a 'Rule of 3', namely: a molecular weight of less than 300, CLogP equal to 3, and not more than 3 hydrogen bond donors and three acceptors.

DETECTION OF FRAGMENTS

A small, less complex molecule is as a rule a weaker binder, which means that fragments are more difficult to detect using conventional HTS techniques. One approach is to screen compounds at high concentrations (250-1000μM compared with the 10-30μM concentrations typically used in HTS) (4). In addition to – or instead of – conventional bioassays, various biophysical screening methods are used including nuclear magnetic resonance (NMR), X-ray crystallography and mass spectrometry (MS). NMR and X-ray crystallography are particularly suitable as they can provide significant structural understanding of the ligand-protein binding event; this is critical in prioritising fragment hits and optimising them into leads.

NMR Screening

NMR has been shown to be the most productive of the fragment screening methods; furthermore, recent modifications have led to an improvement in the efficiency and throughput of the technique. Companies such as Abbott Laboratories, Novartis, Vertex Pharmaceuticals, Vernalis, Hoffmann-La Roche and Triad Therapeutics are reported to be using NMR for fragment-based discovery.

X-Ray Crystallography

This has the advantage that it provides a detailed profile of fragment-binding. Traditionally it has been regarded as too slow a method for screening, but with recent advances in the technology, solving crystal structures is becoming an increasingly high-throughput process. Abbott Laboratories, Astex Technology, Plexxikon Inc and Structural GenomiX are all reported to be using crystallography for fragment-screening.

FRAGMENTS TO LEADS

Fragments are generally less potent than hits obtained via HTS, so they are subjected to various processes in order to convert them into potential drug leads. A variety of strategies are available to do this: fragment evolution, fragment linking, fragment self-assembly and targeted libraries. In practice, there tends to be an overlap between these four methods; for example, fragment linking may also involve an element of fragment evolution.

Fragment Evolution

Initial fragments identified by direct binding techniques are built up into larger, more complex molecules that target additional interactions in the active site of the protein (5). This evolution leads to more tightly binding molecules which can be further developed including optimisation of their drug-like properties, for example, selectivity, oral activity and efficacy.

Fragment Linking

Two fragments are identified that bind in separate sites but are close enough together to be chemically linked – resulting in a larger, higher-affinity molecule.

Fragment Self-Assembly

This method exploits the ability of fragments to undergo self-assembly in the presence of a template, in this case the target protein. Separate fragments with complementary functional groups can thus be assembled in the presence of the target protein to form a larger, more potent molecule. In effect, the protein catalyses the synthesis of its own inhibitor by selecting fragments that can cross-link to each other when brought close together.

Targeted Libraries

Small, focused libraries using the fragment as the core template can efficiently map the features of the receptor allowing rapid generation of SAR. These libraries are also a means of accounting for issues such as induced fit that are otherwise difficult to predict.

ADVANTAGES OF THE APPROACH

The key to fragment-based drug discovery lies in the fact that fragments interact efficiently with the target protein and are selected to provide ideal starting points for further optimisation; the scientist then investigates, often with the help of X-ray crystallography, how the fragment and target interact, and designs and synthesises analogues containing the required desirable features. The approach has a number of advantages over the generation and screening of large compound libraries by HTS:

Scope for Development

The attrition of potential drugs in the clinic has been associated with unfavourable physio-chemical properties including molecular weight and LogP (6). For example, compounds over 500 daltons in weight have in general a much lower chance of being approved (7). The lead optimisation process typically adds to both molecular weight and LogP. A 250 dalton fragment, therefore, presents a far more attractive starting point for optimisation with greater scope for development compared with a higher molecular weight, more lipophilic HTS hit compound.

Ligand Efficiency

Hit fragments by their very nature should provide a better match to a target protein binding site. Being less complex, they are less likely to be hampered by other chemical moieties, enabling optimal binding with the target. Also, fragments have higher binding energies per unit molecular mass; this means that a high proportion of the atoms in a fragment hit are directly involved in the binding. By contrast, compounds detected via HTS – whilst more potent – tend to be larger and have redundant functionality making them less efficient binders.

Screening Efficiency

Screening of low molecular weight fragments results in a higher hit rate than HTS, and so fewer numbers need to be screened (anything from one hundred up a couple of thousand). The approach represents a very efficient way of screening chemical space and finding novel chemical structures; it also opens up the possibility of finding hits for 'intractable' targets – that is, those that have not been cracked by conventional bioassay-based screening.

THE FUTURE

Fragment-based lead discovery offers an alternative, complementary strategy to HTS; put simplistically, it emphasises efficiency and design, whereas with HTS, the emphasis is on affinity and numbers (5). Lead compounds emanating from fragment-based drug discovery have a better chance of being successful. Harren Jhoti of Astex Technology is quoted as saying that in a typical pharmaceutical company 70% of initial hits ultimately fail, whereas 80% of fragment hits prove useful (7).

For the future, the fragment-based approach can be seen as evolving along two parallel tracks (9): first the expansion and combination of fragments into libraries for functional screening; and second, the deconstruction of HTS hits into component fragments for individual optimisation. In particular, research will continue to focus on the discovery of fragments and their optimisation, and also the computational deconstruction of marketed drugs into fragments.

Over the next decade, it is hoped that the fragment approach will not only improve the productivity of pharmaceutical R&D, but also the quality of the lead compounds developed.

The authors can be contacted at
tim.mitchell@sareum.co.uk and mike.cherry@sareum.co.uk

References

1. **Bohacek RS, McMartin C and Guida WC (1996). The art and practice of structure-based drug design. *Med Res Rev*, 16, 3-50.**
2. **Lipinski CA, Lombardo F, Dominy DW and Feeney PJ (1997). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Delivery Rev*, 23, 3-25.**
3. **Congreve M, Carr R, Murray C and Jhoti HA (2003). A "rule of three" for fragment-based lead discovery. *Drug Discovery Today*, 8, 876-77.**

3a. **Baurin N, Aboul-ela F, Barril X, *et al*. (2004). Design and characterization of libraries of molecular fragments for use in NMR screening against protein targets. *J Chem Inf and Computer Science*, 44, 2157-2166.**

4. **Maly DJ, Choong IC, and Ellman JA (2000). Combinatorial target-guided ligand ligand assembly: Identification of potent subtype-selective c-Src inhibitors. *Proc Natl Acad Sci* USA, 97, 2419-2424.**
5. **Rees DC, Congreve M, Murray CW and Carr R (2004). Fragment-based lead discovery. *Nature Reviews, Drug Discovery*, 3, August 2004.**
6. **Wenlock MC, Austin RP, Barton P, *et al*. (2003). A comparison of physiochemical property profiles of development and marketed oral drugs. *J Med Chem*, 46, 7, 1250-56.**
7. **Branca MA (2004). Finding the perfect fit. *Bio-IT World*, April 2004.**
8. **Fezjo J, Lepre CA, Peng JW, *et al*. (1999). The SHAPES strategy: An NMR-based approach for lead generation in drug discovery. *Chem Biol*, 6, 755-769.**
9. **Erlanson DA, McDowell RS and O'Brien T (2004). Fragment-based drug discovery. *J Med Chem*, 47, 14, 3463-82.**