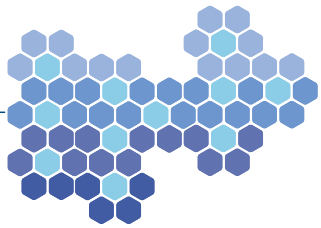

Circular Argument

By Nicholas K Terrett at Ensemble Therapeutics

Recent studies of the activities and properties of naturally occurring macrocycles seem to indicate that related synthetic versions could offer a solution to tough disease targets – an area that cannot be addressed with conventional small or biological molecule paradigms

Macrocycles – molecules with a core ring structure containing from 14 to over 30 atoms – have suddenly grabbed the attention of scientists engaged in drug discovery (1). They are not a new invention; macrocyclic natural products – such as erythromycin and cyclosporine – have been known for generations, and many are marketed as antibiotics, or for their anti-inflammatory or anti-cancer properties. However, two things have recently changed people's perception of their value: firstly, a realisation that they may offer solutions for challenging disease targets; and secondly, methods have been developed that permit rapid and efficient investigation of synthetic analogues. Together, these advances have fuelled a growing fascination with macrocycles as accessible and viable drug molecules (2).

The mainstay of the pharmaceutical industry for more than a century has been the small molecule. These compounds generally have molecular weights (MWs) below 500Da, are straightforward to manufacture, and are usually taken by mouth. Furthermore, during the discovery process, researchers are able to modify their structure to achieve the right balance of potency, selectivity, safety and physical properties, ensuring they get to the target tissue and stay there long enough to have a beneficial effect.

This approach has worked exceptionally well for numerous enzymes and receptors whose overexpression or misbalance has resulted in illness. However, as our understanding of disease mechanisms has grown in the last 20 years – in particular with new discoveries around the aetiology of inflammatory disease and cancer – we have discovered a wealth of new and important disease-relevant protein targets.

Keywords

Protein-protein interactions
Large-molecule biologics
Oral bioavailability
Synthetic variation
Hydrogen-bonding groups

However, many of these targets are components of protein-protein interactions (PPIs), whose evolution has generated large, sometimes flat and featureless protein surfaces that are difficult for small molecules to bind to. The key functional groups that lock the proteins together, the so-called 'hot spots', are widely dispersed on the protein surface – this can create problems because conventional small molecules lack the physical reach to enable them to successfully connect, in order to achieve an energetically favourable binding event. Small molecules have failed, and despite millions of compounds being screened empirically by dozens of companies against numerous PPI targets, effective hit discovery rates have remained meagre. It is this limitation that has stimulated the exceptional growth in biological drug research.

Biologic Concerns

Biological drugs – or biologics – represent a class of large molecule that is based on proteins naturally found in living systems. This includes recombinant proteins, antibodies, or more exotic species such as soluble forms of membrane-bound receptors. They have been developed as drugs because of extreme affinity and selectivity for their target proteins, and because in binding to those proteins, disease can be modified in beneficial ways for patients. One example is the monoclonal antibody, adalimumab – with the trade name HUMIRA® – an antagonist of tumour necrosis factor that is prescribed as therapy for rheumatoid arthritis and other inflammatory diseases. The drug is highly efficacious and has a half-life of between 10 and 20 days, meaning the injections required for administration can be widely spaced.

If they are so effective, why would anyone want to replace biologics? Firstly, the size of protein molecules means that these drugs cannot get into cells, so a significant number of compelling drug targets which occur only inside cells cannot be tackled with biologics. For the same reason, biologics essentially have no oral bioavailability, relying on a parenteral route of administration that might be inconvenient or even

distressing for patients. Finally, some biologics can produce dangerous or even unanticipated side-effects – for example, HUMIRA can suppress the natural immune response, resulting in infections that can be difficult to treat when the drug is eliminated slowly from the body.

Potential Solution

Many researchers and doctors have postulated the benefits of orally active drugs that could complement, rather than replace, injectable biologics, and in many cases would open up therapies that currently do not exist because of the limitations of biologics. What if we were able to find a class of molecule that could bind to the featureless protein surfaces targeted by biologics, but could possess properties that permit oral administration, and also address those compelling disease targets that lie within the cell?

Natural product macrocycles appear to provide a compelling precedent to a solution. Cyclosporine is marketed as an immunosuppressant drug, widely used in organ transplantation to prevent rejection, as well as for treating inflammatory diseases. It binds to cyclophilin and modifies the surface of this protein so that it is able to inhibit calcineurin – thereby suppressing the activity of inflammatory T cells. Cyclosporine is a large, cyclic peptide with an MW of over 1,200Da – such a size and structural complexity is required for binding to the cyclophilin surface. However, despite this large size, the drug is orally bioavailable.

Macrocycle Modification

How does cyclosporine achieve this drug-like character? It has been known for some time that the peptide bonds in the molecule are highly modified, with 7 of the 11 amides being methylated. These changes reduce the polarity inherent in peptides that usually mitigates against cell membrane permeability. In addition, the molecule has a remarkable flexibility that is used to great advantage. In some conformations, the molecule exists with the more polar hydrogen-bonding groups displayed externally, maximising solubility and making key interactions with cyclophilin.

But the macrocycle can also take-up another conformation, where the polar groups are hidden internally, generating intramolecular hydrogen bonds (3). This latter conformation is considerably more lipophilic, and also has a smaller cross-sectional area, thus readily passing through cell membranes. Indeed, surveying drug-like naturally occurring

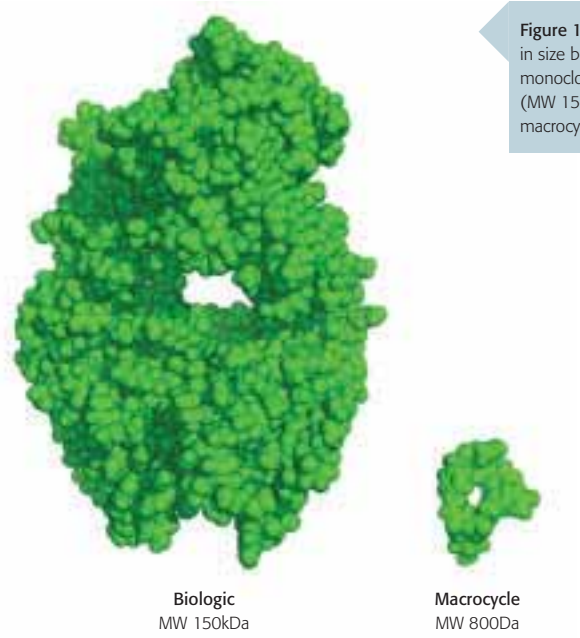

Figure 1: Comparison in size between a typical monoclonal antibody biologic (MW 150kDa) and a typical macrocycle (MW 800Da)

macrocycles shows that many have a similar capability for modifying their properties on demand.

Even with such examples, drug developers have, in the past, been reluctant to pursue macrocyclic drugs. The huge structural and stereochemical complexity frequently encountered with natural products makes them very challenging and economically unfeasible to synthesise on scale. Furthermore, even making modest adjustments to the structure to improve activity or the pharmacokinetic profile requires considerable synthetic work; modifications have been restricted to simple chemical changes in molecules where bulk material can be readily obtained from biological sources. For example, the successful development of the antibiotic macrocycle, azithromycin, was only feasible because the drug can be easily manufactured in just a few synthetic steps from erythromycin by the insertion of a methylated amine into the macrocycle backbone.

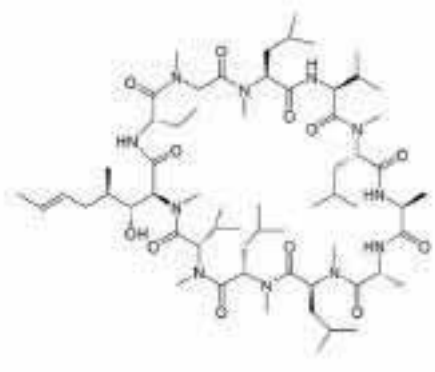

Figure 2: The structure of the natural macrocycle product, cyclosporine, showing the highly modified peptide backbone with seven methylated amide bonds

Using a chemical platform, it is possible to generate libraries of many millions of macrocyclic compounds. The technology – DNA-programmed chemistry – employs predefined synthetic DNA templates to permit controlled introduction of individual building blocks, ultimately resulting in collections of diverse macrocycles

Such natural product derivatives are in the minority, and the recent interest in this area is a consequence of new methods for macrocycle synthesis. In particular, generation of macrocycle libraries has been necessary to give scientists a starting point for drug discovery endeavours. It is especially important when seeking compounds that bind to PPIs to have large collections of macrocycles that can be screened empirically, as frequently there may be no useful structural information to inform lead compound design.

Using a chemical platform, it is possible to generate libraries of many millions of macrocyclic compounds. The technology – DNA-programmed chemistry – employs predefined synthetic DNA templates to permit controlled introduction of individual building blocks, ultimately resulting in collections of diverse macrocycles. The final library compounds are macrocycles with unique attached encoding DNA chains. Therefore, any compounds that bind to a disease target in an affinity-based selection assay can be immediately identified by DNA amplification and next-generation DNA sequencing.

New Modality

Other groups have been able to design macrocycles using structure-inspired rational design and synthetic macrocycles with activity; hepatitis C virus protease NS3/4a inhibitors and ghrelin agonists have been explored, and several are in clinical development. Many more macrocyclic drugs that work by targeting PPIs are in the pipeline, as well as some that address more conventional enzyme and receptor targets. For the vast majority of these programmes, the size of the macrocycle is a critical feature, as this allows reach across the protein surface to interact with widely dispersed hotspots. A recent review of how macrocycles interact with protein surfaces from an analysis of X-ray crystal structures revealed that many make edge-on interactions – cyclosporine is a good example of this – whereas others lie flat on the protein surface (4).

Macrocycles appear to make, on average, twice the contact area with their protein targets than generally seen with conventional small molecules. Yet, as we have seen, the macrocycles retain their conformational flexibility and a propensity for hiding polar groups that

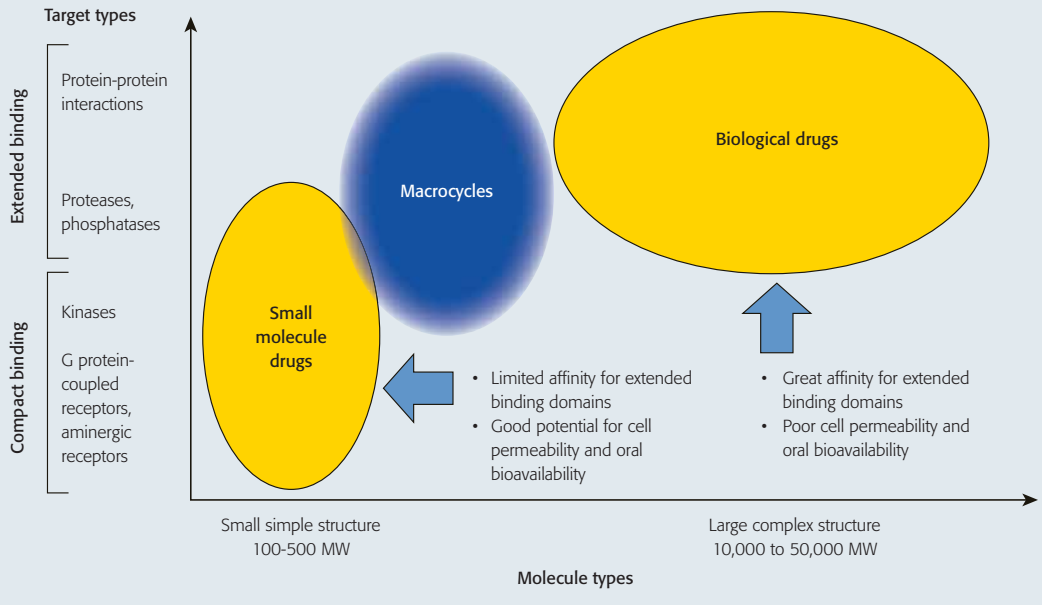

Figure 3: A plot of target complexity against drug complexity, showing how macrocycles fit between conventional small molecules and larger biologics

can facilitate cell membrane permeability. They also appear to suffer little from the proteolytic cleavage by metabolising enzymes generally seen for open-chain molecules, further contributing to their bioavailability. Analysing features of orally bioavailable macrocycles in clinical development is offering very clear pointers as to the preferred properties of this class of molecule (5).

In summary, there is growing interest in, and accumulating examples of, macrocycles providing new modality in drug design between the existing categories of conventional small molecules and larger biologics. While macrocycles appear to perform like biologics in binding with high affinity to challenging protein targets, they retain the pharmaceutical properties of small molecules. With this advantageous mix of properties, plus the development of new methods for synthetic access, macrocycles present an exciting direction for future drug discovery.

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