

# The exploration of macrocycles for drug discovery — an underexploited structural class

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**Abstract** | Macrocyclic natural products have evolved to fulfil numerous biochemical functions, and their profound pharmacological properties have led to their development as drugs. A macrocycle provides diverse functionality and stereochemical complexity in a conformationally pre-organized ring structure. This can result in high affinity and selectivity for protein targets, while preserving sufficient bioavailability to reach intracellular locations. Despite these valuable characteristics, and the proven success of more than 100 marketed macrocycle drugs derived from natural products, this structural class has been poorly explored within drug discovery. This is in part due to concerns about synthetic intractability and non-drug-like properties. This Review describes the growing body of data in favour of macrocyclic therapeutics, and demonstrates that this class of compounds can be both fully drug-like in its properties and readily prepared owing to recent advances in synthetic medicinal chemistry.

## Natural product

A chemical compound or substance produced by a living organism, and thus found in nature.

## Rule of 5

A series of guidelines initially proposed by Lipinski. The molecular mass, lipophilicity and hydrogen-bonding groups collectively determine whether a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug.

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Many natural product small molecules have evolved to interact with proteins, and often have profound pharmacological activity, which has been harnessed in the development of a wide range of drugs. The activity arises either because a human protein has structural and functional similarities to a related non-human protein, or because many proteins of diverse function share similar small-molecule binding domains. Indeed, evidence suggests that the range of distinct protein family types is fairly restricted, and that the same domains are found in diverse proteins<sup>1</sup>.

One structural feature that is common in the larger natural products is the macrocycle: a ring architecture of 12 or more atoms. The macrocycle ring enables a molecule to achieve a degree of structural pre-organization, such that key functional groups can interact across extended binding sites in proteins without a major entropic loss on binding. Macrocycles can therefore be highly potent as well as selective. However, macrocycles are not rigid compounds. Instead, they provide a compromise between structural pre-organization and sufficient flexibility to mould to a target surface and maximize binding interactions. Furthermore, macrocycles are not just bigger versions of small molecules, but can be considered as the smallest examples of biomolecules that exhibit functional sub-domains. As demonstrated by examples in

this Review, there is much evidence that despite having molecular masses that are above 'rule of 5'-compliant small molecules<sup>2</sup>, macrocycles can demonstrate drug-like physicochemical and pharmacokinetic properties such as good solubility, lipophilicity, metabolic stability and bioavailability.

Despite the proven therapeutic potential of macrocyclic compounds, they have been under-explored and poorly exploited for the discovery of novel drug molecules. The reasons for this are many. There has been a growing reluctance in the pharmaceutical industry to investigate natural products because their structural complexity generates difficulties in analogue synthesis. Furthermore, preferential adoption of rule of 5-compliant compounds for screening has become widespread. However, as outlined in this article, several research groups are investigating the potential of synthetic macrocycles for drug discovery and have proved that such compounds can provide high target affinity and selectivity in structures that have acceptable drug-like properties. Several synthetic macrocycles, unrelated to natural products, are now under active preclinical and clinical development.

After reviewing the evolution and features of macrocyclic natural products that are relevant to their application as drugs, this Review will focus on the investigation of synthetic macrocycles. We have excluded macrocyclic

structures that act as therapeutic metal chelators as well as the synthetic receptors currently being explored in supramolecular chemistry. Synthetic methodology that is providing superior methods for the preparation of individual macrocycles, as well as libraries, will also be discussed.

### Evolution and variation in natural macrocycles

Many secondary metabolites produced by microbes and plants serve a variety of biological functions. These include roles as signalling molecules, in stimulating differentiation in plant growth (for example, formation of aerial myceliae, spores or conidia), or as a means to communicate between cells of the same species (for example, quorum sensing through acyl-homoserine lactones)<sup>3</sup>. In addition, such metabolites have frequently been identified as agents of chemical warfare between microbes competing for scarce resources in their soil or aqueous environmental niches. More than 100,000 natural-product secondary metabolites are known to exist, and of these, approximately 3% are macrocycles<sup>4</sup>. Despite the low percentage of macrocycles, this class of natural products includes a subset of powerful therapeutics that are used to modulate immune-system responses, to fight evolving infectious organisms and to treat cancer.

Natural selection has clearly contributed to the design and function of secondary metabolites, including macrocycles. The specific target–ligand interactions in which these molecules participate, coupled with the advantages they confer in binding to their targets, strongly support a history of selective optimization<sup>5–9</sup>. Clinical issues related to the development of microbial resistance to natural-product antibiotics are simply the latest step in this evolutionary process<sup>10</sup>. Recent insights additionally suggest that the re-use of a limited number of protein domains across wide stretches of phylogeny (from bacterial to mammalian) also increase the likelihood of finding natural products that have a biochemical function in organisms that differ from their natural host or competitor. Application of the principle that natural products have evolved general binding properties has led to the successful generation of natural-product-based combinatorial libraries that exhibit high hit rates against a number of pharmacologically important targets<sup>11,12</sup>. Combined with a growing capacity to generate synthetic macrocycles, this approach is now providing access to novel macrocyclic lead compounds for drug discovery.

Phenotypic variation provides the basic substrate on which natural selection can act. Horizontal gene transfer, gene duplication and mutation each contribute to the creation and maintenance of variation among families of secondary metabolites<sup>13–16</sup>. Comparison of structural conservation and variation among the macrocyclic natural products from distinct species can provide an insight into the range of functionality that a given macrocyclic scaffold can have. When coupled with data from screening those variants against a specific therapeutic target, the natural variation provides a ready-made structure–activity relationship guide. A set

of natural-product macrocycles containing the  $\alpha$ -keto homopropyl amide group, which share the ability to bind to the protein FKBP12 (also known as [FKBPA1](#)) and include the marketed drugs tacrolimus (1) and rapamycin (3), provide an instructive example (BOX 1).

### General features of macrocyclic drugs

Macrocycles represent a small subpopulation of the current drugs that are on the market or in late-stage development. TABLE 1 and TABLE 2 show the major classes of macrocyclic scaffolds that are repeatedly used as drugs. These examples (compounds 7–17) and the general features of macrocyclic drugs illustrate the following aspects that distinguish them from typical small-molecule drugs<sup>17</sup>.

**Marketed macrocyclic drugs are almost exclusively natural products.** Among the full list of marketed small-molecule drugs, approximately half are derived from synthetic sources and are not based on any naturally occurring compound or ligand (although many have been inspired by nature)<sup>4,18</sup>. By contrast, current macrocyclic drugs are almost exclusively derived from natural sources (primarily microorganisms) and are either identical to or closely derived from naturally occurring macrocycles<sup>17</sup>. For example, among the major classes of macrocyclic therapeutics, erythromycin (7, TABLE 1) was originally isolated from *Saccharopolyspora erythraea* (originally *Streptomyces erythraeus*); abamectin (avermectin, 11) from *Streptomyces avermitilis*; and the antituberculosis compound rifampin (rifampicin, 12) from *Amycolatopsis rifamycinica* (originally *Streptomyces mediterranei*). The immunosuppressant cyclic peptide cyclosporine A (see [Supplementary information S1](#) (box), part b) is produced by the fungus *Tolypocladium inflatum* Gams. Immunosuppressants produced by hybrid non-ribosomal peptide synthase–polyketide synthases include tacrolimus (1) from *Streptomyces chrysomallus* and *Streptomyces tsukubaensis*, and ascomycin and rapamycin (3), both from *Streptomyces hygroscopicus*. The cyclic glycopeptide antibiotic vancomycin (13) was originally isolated from *Streptomyces orientalis*. In addition, the potent antifungal compounds amphotericin B (9, from *Streptomyces nodosus*) and caspofungin (10, derived from fermentation broths of various *Aspergilli* species) are also macrocyclic compounds.

Modifications that have been made to the naturally isolated compounds — as in caspofungin (10), azithromycin (8) or temsirolimus (14) — tend to be at restricted positions, and although the topology of the compound might be modified, the original scaffold is readily discernable. Natural-product macrocycles often have high-affinity binding for their targets, which explains in part why they are used as therapeutics with minimal modification. For example, rapamycin has 0.2–0.4 nM affinity for FKBP12 (REF. 19). However, such macrocycles can also have undesirable medicinal attributes such as toxicity<sup>20</sup>, and modifications to overcome such features are typically challenging, owing to the large size and stereochemical complexity of these molecules.

#### Bioavailability

One of the principal pharmacokinetic properties of drugs, this is the proportion of an administered dose of drug that reaches the systemic circulation unchanged and is thus available to provide a pharmacological effect.

#### FKBP12

Also known as FKBPA1, FKBP12 is a human protein that binds the immunosuppressant molecule tacrolimus, which is used in treating patients after organ transplant and patients suffering from autoimmune disorders.

Box 1 | **Natural and synthetic variation converge in macrocycles binding to FKBP12**

Both natural and synthetic members of the  $\alpha$ -keto homopropyl amide family of macrocycles share the ability to bind to FKBP12 (also known as FKBP1). The structure in the dashed box summarizes the consensus of natural and synthetic variation: the conserved FKBP12-binding domain is shown as an explicit structure, while the variant portion of the natural and synthetic series is shown as a red ribbon.

The first such compound reported, tacrolimus (1), was identified as a product of *Streptomyces tsukubaensis*<sup>75</sup>, and a subsequent broad screening of 12,000 *Streptomyces* strains suggests that approximately 1% of this genus produces secondary metabolites that bind FKBP12 (REF. 42). In particular, among the positive strains, 65% produced the compound meridamycin, a close analogue of tacrolimus and rapamycin. The structurally related antascomicins (for example, compound 2), however, are produced by a strain of *Micromonospora*<sup>42</sup>, a species of *Actinomycete* that is phylogenetically distinct from *Streptomyces*<sup>76</sup>. The generation and maintenance of various macrocyclic  $\alpha$ -keto homopropyl amides that bind to FKBP12 is thus widely distributed among the *Actinomyces*, making it difficult to argue that any one structure provides significant selective advantage over the others.

The structural variation among the members of this metabolite family reveals the minimal sub-domain of the macrocycle that is essential for FKBP12 binding. The conserved region of the natural variants coincides well with the findings of synthetic structure–activity relationship studies (exemplified on the right half of the figure)<sup>40,41,77</sup>, as well as the crystal structure of bound FKBP12 and ternary complexes<sup>19,78</sup>. It thus appears that selective constraint preserves the key binding domain of these macrocycles, despite distribution into a variety of chemical, genetic and environmental contexts.

The series also exemplifies the general observation that macrocyclic compounds are among the smallest compounds known to exhibit functional sub-domains that are analogous to those in macromolecules: the variant region depicted here as a red ribbon in fact confers specific functionality to a bound FKBP12–macrocycle complex, depending on its particular structural characteristics. For example, calcineurin recognition (tacrolimus, 1) versus FKBP12–rapamycin binding recognition (rapamycin, 3) versus synthetic FKBP12–rapamycin binding variants (rapalogs<sup>41</sup>, 6). The subdivision of each macrocycle into functional sub-domains therefore allows latitude for divergent evolution in natural products, as well as synthetic chemical engineering for basic research or pharmaceutical optimization.

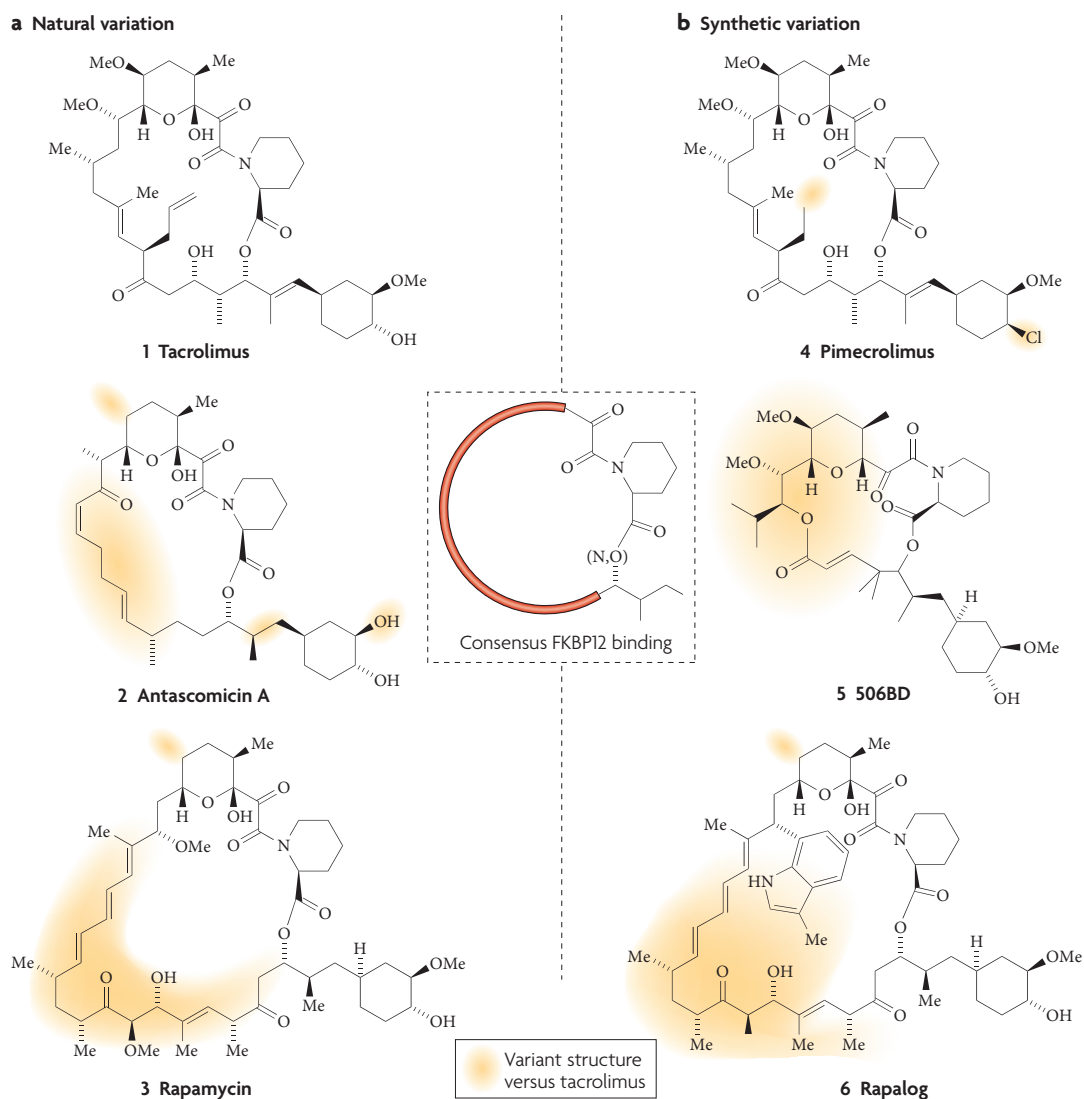
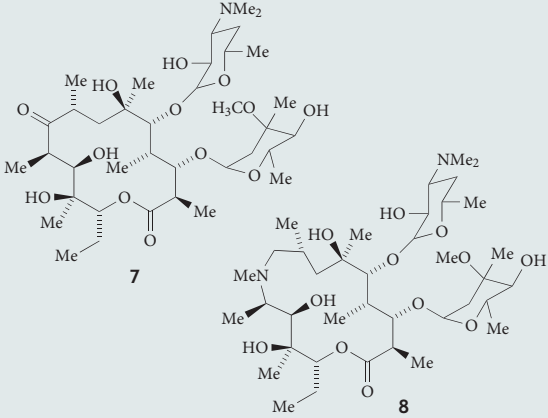
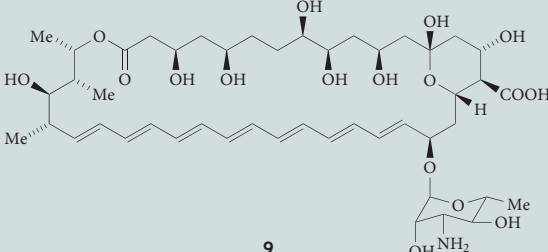
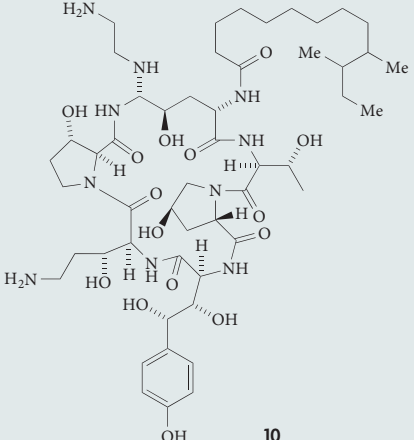
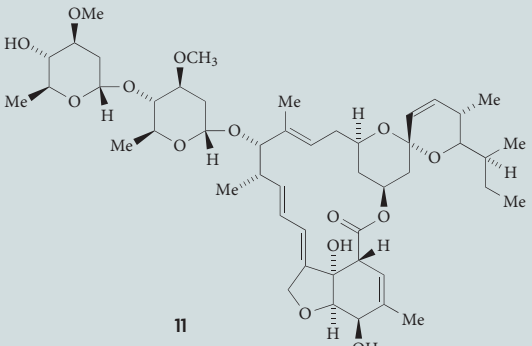
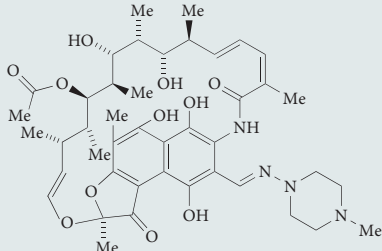
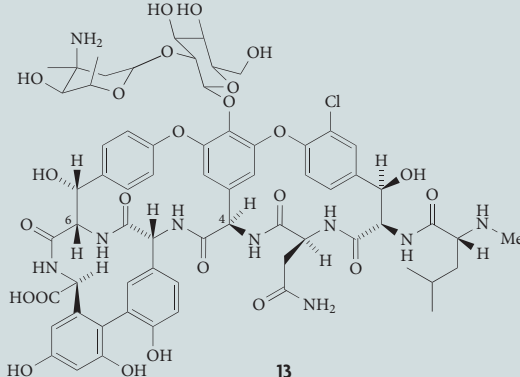
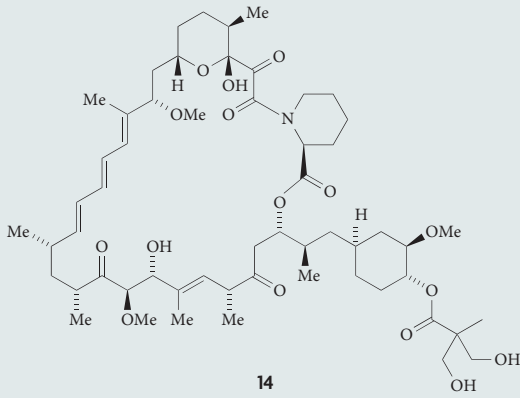
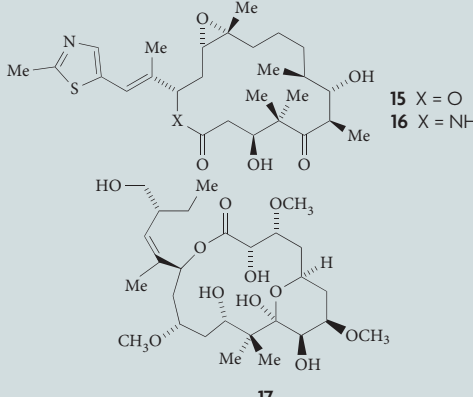


Table 1 | Families of macrocyclic drugs: representative members and their therapeutic applications\*

Family	Class description	Representative compounds	Application(s)
Macrolide antibiotics	12-, 14-, and 16-membered macrolactone rings, generally synthesized by modular polyketide synthases	Erythromycin (7), azithromycin (8), carbomycin, methymycin 	Antibiotics (target bacterial ribosome)
Polyene antifungals	Amphiphilic polyketide macrocycles, 38-membered rings	Amphotericin B (9) 	Systemic antifungals
Cyclic peptides	Broad class, produced by non-ribosomal peptide synthases; macrocyclic rings rich in amide bonds, although not exclusively peptidic	Caspofungin (10), cyclosporin, daptomycin, anidulofungin 	Anti-infectives, immunosuppression, cancer therapy
Avermectin-related	16-membered lactone, carrying three sites of unsaturation; variation in glycosylation state	Abamectin (11), milbemycin oxime, moxidectin 	Veterinary: antiparasitics, anthelmintic, insecticide

\*Continued in TABLE 2.

Table 2 | Families of macrocyclic drugs: representative members and their therapeutic applications\*

Family	Class description	Representative compounds	Application(s)
Rifampin-related	25-membered macrolactams containing ketal linkage to annular naphthohydroquinone; variation primarily in naphthyl moiety	Rifampacin (12), rifabutin  <b>12</b>	Antibiotic, mostly tuberculostatic
Vancomycin-related	Trimacrocyclic compounds, hybrid non-ribosomal peptide synthase–polyketide synthase products; variation in glycosylation pattern	Vancomycin (13)  <b>13</b>	Antibiotic: Gram-positive bacteria and methicillin-resistant <i>Staphylococcus aureus</i>
$\alpha$ -Keto homoprolyl amides	Hybrid amido and polyketide macrolactones of varying ring size	Temsirolimus (14), tacrolimus, rapamycin  <b>14</b>	Immunomodulators, cancer therapies
Tubulin binders	Modulates the interaction of $\alpha$ - and $\beta$ -tubulin	Epothilone B (15), ixabepilone (16), peloruside A (17)  <b>17</b>	Cancer chemotherapy

\*Continued from TABLE 1.

Box 2 | **Macromolecular and macrocyclic domains**

Examining structures of the biologically active species of macrocyclic drugs reveals a striking feature: these compounds frequently act as non-covalent assemblies of drug monomers, or as multimeric complexes of drug with endogenous biomolecules. Four out of the eight classes listed in TABLE 1 and TABLE 2 exhibit this feature (a molecular mechanism for the avermectin class is not yet known), making this a dominant mode of action among macrocyclic drugs (for example structures, see [Supplementary information S3](#) (box)).

Structurally, the allocation of a portion of the macrocycle to mediate the assembly, and another portion to interact with the target, requires the existence of separate 'domains'. Some macrocycle domains do form autonomously, as with rifampicin, and for these species, no multi-molecular assembly is needed. Used here, the term domain is thus intended to capture a concept that is distinct from identifying any simple covalent sub-region of a small molecule, that is, an arbitrary demarcation of a sub-portion of any structure for the sake of discussing function. Instead, these domains in macrocyclic drugs are analogous to macromolecular (especially protein) domains in that they are revealed through specific non-covalent as well as covalent interactions — a form of secondary or tertiary small molecule structure that can assemble (fold) and disassemble dynamically.

A second noteworthy point of analogy between protein domains and natural macrocycle domains is that the genetic encoding of macrocycle domains can often be transferred independently. Examples include the DNA encoding modular polyketide synthase elements, encoding glycosyl-epivancosamine portion of vancomycin, or the set of genes required for the  $\alpha$ -keto homopropyl amide pathway. This situation can enable divergent evolution, as seen in the creation of various macrocyclic functions from a relatively small repertoire of domain building blocks (BOX 1).

All macrocycles do not necessarily have domains in this defined sense; domains are not inherently a part of being cyclic. However, the forces of entropy make it difficult to achieve the formation of stable non-covalent structures in the absence of either sufficient size for the molecule to fold and exclude solvent (as in proteins), or a covalent cyclization, which provides sufficient constraint for non-covalent forces to stabilize the necessary functional structure in the presence of solvent or a binding partner. Macrocyclization is therefore a remarkably efficient way to introduce domains into small structures.

The preponderance of natural-product macrocycles among marketed drugs therefore reflects areas that synthetic medicinal chemistry have found challenging, as much as it demonstrates the rich pharmacopoeia provided from natural sources. In addition, the fact that the macrocycles that are currently on the market are so heavily represented by natural products correlates with two other general observations. First, macrocyclic drugs have been predominantly discovered and applied as anti-infectives, and second, the rate at which novel macrocyclic scaffolds have entered clinical trials has dropped in parallel with the general decline in the use of natural-product extracts as discovery tools<sup>18,21</sup>.

**Macrocycles can modulate challenging targets.** Macrocycles have demonstrated repeated success when addressing targets that have proved to be highly challenging for standard small-molecule drug discovery, especially in modulating macromolecular processes such as protein–protein interactions. For example, the macrocycle cyclosporine A (see [Supplementary information S1](#) (box), part b) has been extensively studied for its ability to enable the interaction of cyclophilin A with calcineurin. Rapamycin (**3**) similarly controls the mTOR pathway through its interaction with FKBP12 to create a hybrid macrocycle–protein surface that facilitates the binding of FRAP<sup>22</sup>. These examples have been extensively reported and reviewed<sup>23,24</sup>, and together suggest that macrocycles

function well to modulate macromolecular interactions of various types, often by creating new interaction surfaces for their targets that can induce a macrocycle-dependent gain-of-function for the complex.

Multiple classes of macrocyclic natural products are currently under investigation for their ability to modulate microtubule dynamics in mammalian cells, and thereby inhibit tumour growth<sup>25</sup>. Here again, these effects result from modulation of protein–protein interactions; in this case between the  $\alpha$  and  $\beta$  subunits of the tubulin heterodimer. Epothilone B (**15**), a macrocycle derived from myxobacteria, binds at the interface of the two tubulin subunits (a site that overlaps with the taxol binding site). Extensive re-configuration of the  $\alpha$ – $\beta$  interface stabilizes the dimer, and disturbs overall microtubule dynamics<sup>26</sup>. Ixabepilone (**16**), the lactam equivalent of epothilone B, has been approved for the treatment of metastatic breast cancer<sup>27</sup>. Dictyostatin is a second macrocycle known to bind at the taxol site to stabilize  $\alpha$ – $\beta$  tubulin dimers<sup>28</sup>. Moreover, an additional site has been identified that is recognized by laulimalide and peloruside A (**17**), macrocycles that have been found to be synergistic with taxol in stabilizing microtubules<sup>29</sup>. By contrast, binding at a different site by the complex natural-product macrocycle halichondrin B (and its analogue E7389) disrupts microtubule dynamics by destabilizing tubulin dimers<sup>30</sup>. While multiple examples of non-macrocyclic microtubule disrupters are also known, current knowledge indicates that nature has frequently identified macrocycles to modulate protein–protein interactions between microtubule subunits.

Natural-product antibiotics that target the ribosome and its structured RNA provide a further example of a biological effect that is mediated purely by a structural interaction with the target at a site where no reaction catalysis occurs. The macrolide antibiotic erythromycin (**7**) functions by binding to the inner surface of the ribosomal tunnel, to effectively create a new surface, and physically impede the exit of nascent peptides from the ribosome by narrowing the passage<sup>31</sup>. The modification of the ribosomal tunnel is conceptually similar to macrocycle-based modification of a protein–protein interaction surface. It is distinct from the enzymatic reaction inhibition mechanism used by smaller, non-macrocyclic natural-product drugs such as chloramphenicol, which target the peptidyl transferase reaction chemistry of the bacterial ribosome. The mechanism is conserved among various carbamycin macrolide derivatives as well as the spiramycin cyclic peptides, implying that this structural inhibition mechanism is readily accessible to various macrocyclic structures<sup>32</sup>.

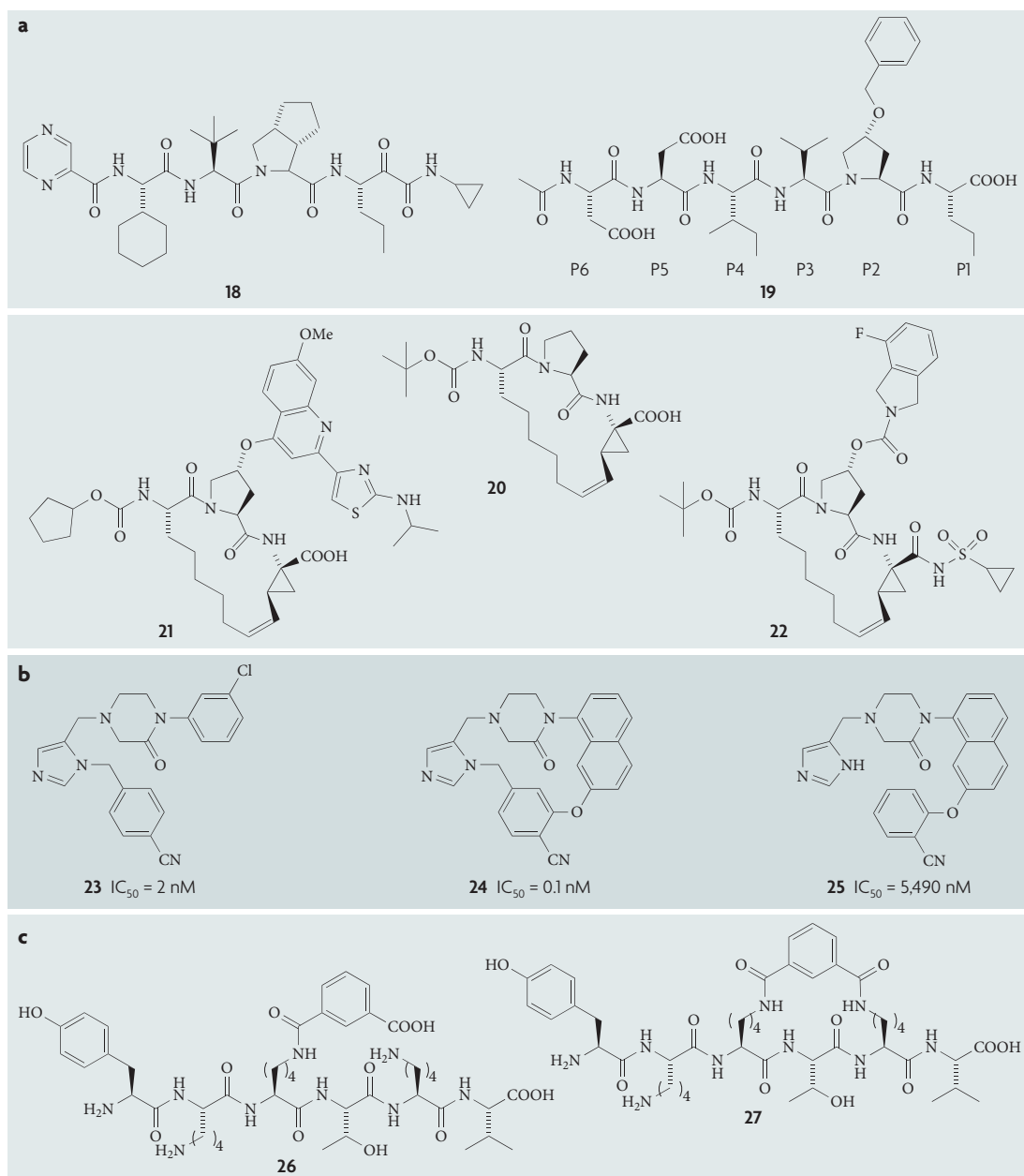
Macrocyclic compounds that function as classic enzyme inhibitors rarely occur. One example is the sponge-derived thrombin inhibitor cyclotheonamide A<sup>33</sup> (see [Supplementary information S1](#) (box)), which functions through a classic transition-state analogue mechanism to inhibit serine protease inhibitors such as thrombin<sup>34</sup> and human leukocyte elastase<sup>35</sup>. In this class, the macrocyclic architecture replicates the conformation of the protein substrate without the need for a protein scaffold to confer stabilization<sup>36</sup>.

**mTOR**

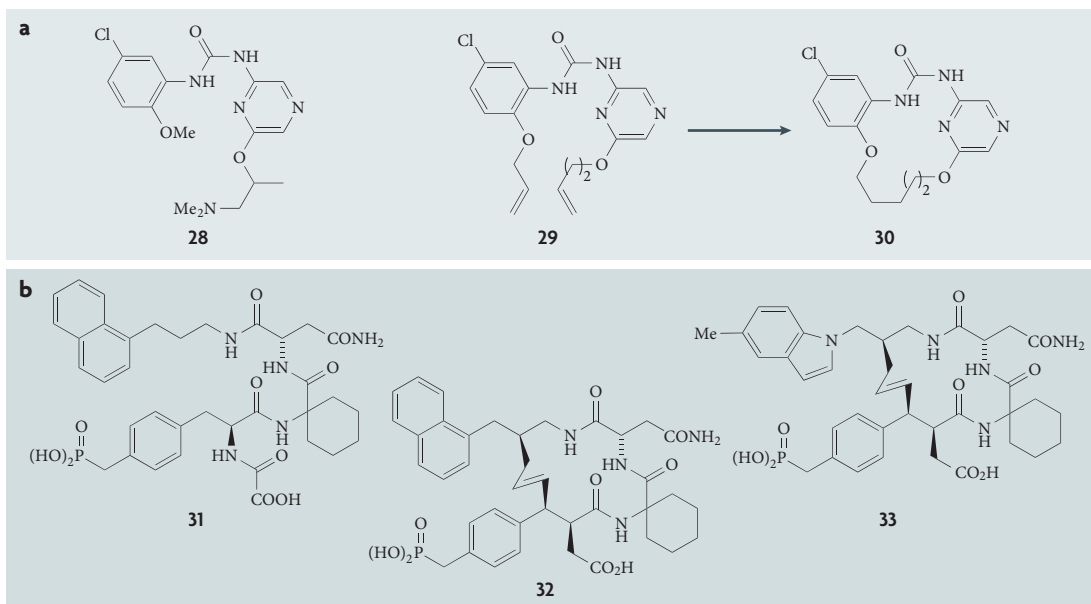
The mammalian target of rapamycin, which is analogous to the *Saccharomyces cerevisiae* proteins TOR1 and TOR2. The mTOR pathway is currently under investigation for its role in the control of the cell cycle and in human cancer.

**FRAP**

FKBP12-rapamycin-associated protein.



**Figure 1 | Improving target affinity through macrocyclization: part 1. a** | Potent hepatitis C virus (HCV) NS3 protease inhibitors. VX-950 (**18**) is a linear HCV NS3 protease inhibitor currently in clinical development. Dynamic nuclear magnetic resonance (NMR) methods revealed that a hexapeptide NS3 inhibitor related to VX-950 (**19**) binds in an extended conformation and undergoes rigidification on binding<sup>50</sup>. Close proximity of the solvent-exposed P1 and P3 side chains in the bound conformation suggested linking to convert **19** into a macrocyclic inhibitor such as **20** that preserved the geometry observed in the bound conformation. Modifying the proline and adding a new N-terminal carbamate substituent to **20** resulted in BILN 2061 (**21**), a potent NS3 protease inhibitor with a sub-nanomolar  $K_i$  value, which was advanced into clinical development<sup>52</sup>. A related structure, compound **22** (InterMune Inc.), containing an acylsulphonamide instead of the carboxylic acid is also in clinical development<sup>79</sup>. **b** | Enhancing the potency of farnesyltransferase inhibitors. A piperazinone farnesyltransferase inhibitor (**23**) was observed in dynamic structural NMR studies to be in a folded conformation, suggesting the opportunity for a cyclized analogue<sup>80</sup>. Synthesis of a macrocycle under high dilution conditions and chiral separation (as the molecule exhibited planar chirality), enabled identification of the active (+) enantiomer **24**. Compound **24** was 20-fold more potent than the open-chain inhibitor **23**, and was 55,000-fold more potent than the direct open chain analogue **25**. Even though both open-chain molecules can access an active conformation, and differences in the enthalpic contribution to binding should be small, these results suggest that the entropic disadvantage of forming a protein-inhibitor complex is significantly reduced by the process of macrocyclization. **c** | Macrocyclization of PDZ domain ligands. In a study of chain-bridged cyclic peptides with affinity for the third PDZ domain (PDZ3) of a mammalian neuronal protein, postsynaptic density protein 95 (PSD95), progressing from an open chain **26** to macrocyclic peptide **27**, improved  $K_d$  values by a factor of four, with the majority of this difference in Gibbs free energy a consequence of a favourable entropic change<sup>81</sup>.



**Figure 2 | Improving target affinity through macrocyclization: part 2. a** | Increasing CHK1 kinase affinity through macrocyclization. The observation from an X-ray co-crystal structure of CHK1 kinase with the inhibitor **28**, that the phenyl ring 2-position and the pyrazine 6' positions point towards the ribose binding site in the kinase, suggested that both could be connected<sup>82</sup>. Using Grubbs olefin metathesis to join two olefinic side chains gave a macrocyclic kinase inhibitor **30** with equivalent potency to the series lead, and 440-fold greater activity than the acyclic precursor **29**. **b** | Induction of conformational constraint through macrocyclization generates potent ligands for the growth factor receptor-bound protein 2 (GRB2). This target contains an Src homology 2 (SH2) domain and binds peptides in a bent rather than an extended conformation<sup>83</sup>. Macrocycle analogues (**32,33**) of the previously reported<sup>84</sup> tetrapeptide mimetic **31** exhibit vastly increased affinities. Ring-closing metathesis was used to generate a macrocycle analogue (**32**) in an attempt to stabilize the bent conformation, which increased affinity approximately 140-fold. Further modification of the aromatic functionality generated macrocycle **33** with a  $K_d$  value of 75 pM. Such significant affinity for the protein target translated into antiproliferative potency against cells that are mitogenically driven through GRB2-dependent signalling pathways.

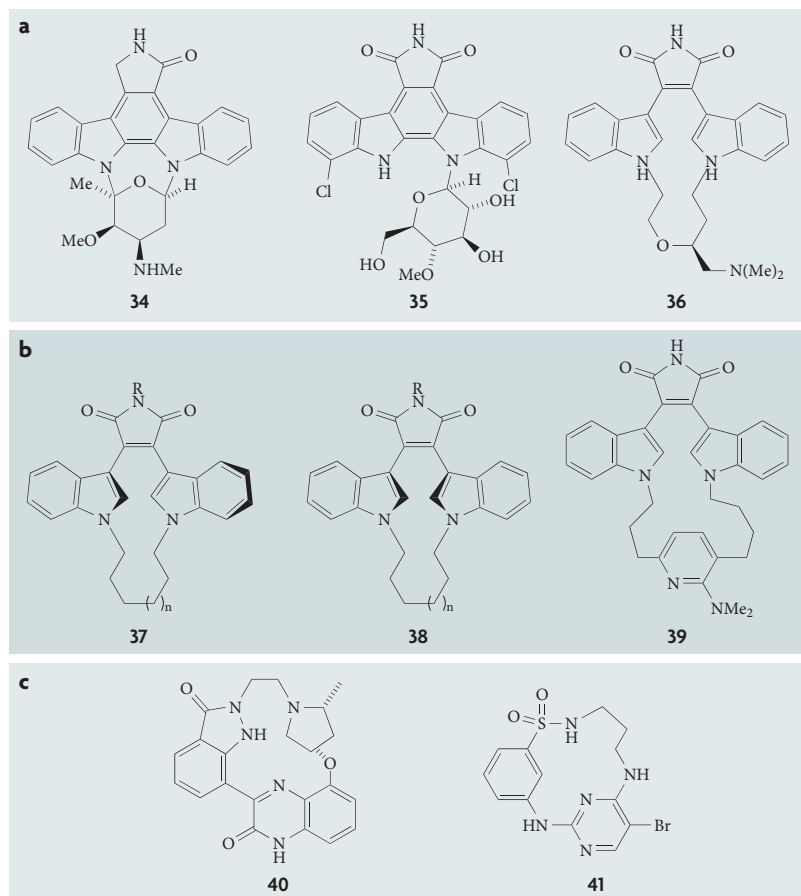
**Macrocyclic drugs have spatially organized functional domains.** The basic functional property of macromolecular domains, such as protein domains, is an ability to form (fold) and function independently. The macrocyclic drugs described in this section are too small to contain the multiple elements of secondary structure associated with protein domains. However, they have functionally independent sub-regions that enable these drugs to assemble non-covalently with each other, or to mediate the assembly of other macromolecules by adopting conformations stabilized by non-covalent interactions (BOX 2).

The localization of polar and non-polar functional groups along the circumference of macrocycles can serve to indicate the presence of functional macrocycle domains. Polar and apolar subregions are evident in many of the major classes of macrocyclic drugs. These include macrolide antibiotics; the immunosuppressants tacrolimus (**1**), ascomycin and related compounds; and the epothilones (**15**)<sup>4</sup>. A highly pronounced example of this feature is evident in the polyene antifungal compound amphotericin B (**9**)<sup>37</sup>, and related derivatives, in which it enables the amphotericin B monomers to assemble into a non-covalent octameric membrane pore.

Several macrocyclic drugs can be described as having a 'modulator' domain, a sub-region in which the details of molecular structure can be altered without affecting

target affinity, but which completes the macrocyclic architecture and positions functional groups for binding (for example the 'spacer' region of the bryostatins)<sup>38,39</sup>. More generally, these can serve as a domain that modulates physicochemical, pharmacokinetic and even biological properties. Again, such domains are often distinguished as runs of conserved polarity around the macrocycle ring.

Functional sub-domains that modulate biological function are evident and have been well studied in the various natural products and synthetic analogues related to tacrolimus (**1**) (see BOX 1 and earlier discussion). In this series of molecules, one domain provides FKBP12 binding activity (the  $\alpha$ -keto homopropyl amide and proximal moieties), while the other domain (generally the opposite side of the ring from the FKBP12 binding region) modulates the targeting of the bound complex. This latter domain is divergent among the various natural products that bind FKBP12, and even more divergence has been introduced in synthetic analogues (note that several of these analogues were produced before solid structural data were available that clearly defined the FKBP12 binding domain)<sup>40</sup>. For some variants, the geometry of the modulator domain confers specific biological activities, such as the ability to bridge FKBP12 alternatively with calcineurin (tacrolimus), mTOR/FRAP (rapamycin), or other engineered partners (for example, the rapalogs<sup>41</sup>).



**Figure 3 | Improving target selectivity through macrocyclization: part 1.**

**a** | Improving the selectivity of kinase inhibitors. Staurosporine (**34**) and rebecamycin (**35**) are potent but notoriously non-selective ATP-competitive kinase inhibitors that have indolocarbazole lactam rings that mimic the ATP adenine ring. However, disruption of the planarity of this indolocarbazole ring system by breaking the central aromatic ring, or the formation of other analogues containing a macrocyclic ring, fixes the conformation of the flexible mimic, giving novel and selective kinase inhibitors. In this way, the bisindolylmaleimide, ruboxistaurine (LY333531, **36**) selectively inhibits the  $\beta$  isoforms of protein kinase C (PKC)<sup>54</sup>. **b** | The conformation of bisindolylmaleimides is controlled by the size of the attached macrocycle ring, which subsequently affects affinity and selectivity for different kinases<sup>55</sup>. The AGC group protein kinases including PKC may be preferentially inhibited by bisindolylmaleimides that adopt a compressed, approximately C<sub>2</sub>-symmetric *anti* conformation (**37**), whereas glycogen synthase kinase 3 $\beta$  (GSK3 $\beta$ ) is best inhibited when the conformation is a distorted *syn* conformation (**38**). Compounds such as **39** have a *syn* conformation in the ATP binding pocket and are nanomolar inhibitors of GSK3 $\beta$ <sup>85</sup> exhibiting 500-fold selectivity over PKC- $\beta$ II. **c** | Selective cyclin-dependent kinase (CDK) macrocyclic inhibitors. The formation of a macrocycle to lock an arrangement of constituent rings into a coplanar arrangement that is deemed essential for activity was behind the design of the macrocyclic quinoxalin-2-one (**40**)<sup>86</sup>. The macrocycle had nanomolar affinities for CDK1, CDK2, CDK4 and CDK6 as well as GSK3 $\beta$ , although IC<sub>50</sub> values exceeded 1,000 nM for 62 off-target kinases. The preferred compound had optimized solubility and showed activity in a preclinical animal model. Similar compounds (for example, **41**) with two aromatic systems linked by a tether have been developed by Schering as inhibitors for CDK1 and CDK2 and vascular endothelial growth factor (VEGF) receptor 2 tyrosine kinase<sup>87</sup>.

The modulator domain of the antascomincins (**2**, BOX 1) completely eliminates secondary activities of their FKBP12 complexes, as opposed to immunosuppression or suppression of mTOR signalling, despite retaining high levels of FKBP12 affinity<sup>42</sup>. In the case of pimecrolimus (**4**),

synthetic modification to the modulator domain has led to improved pharmaceutical properties, while retaining potency<sup>20</sup>.

Cyclic peptides have demonstrated an ability to access alternative conformations that localize polar or apolar side-chain functionality as needed. In such cases, the functional domains of the macrocycle are not represented by covalent localization of ring polarity. Instead, the domains are revealed through a folding process that is analogous to protein folding. The best-studied example of this ability is cyclosporine A<sup>43</sup> (see Supplementary information S1 (box), part b).

Vancomycin (**13**) and related macrocycles also function through the activity of two separate functional domains. The most biologically active conformation of vancomycin is a non-covalent homodimer of the drug, and the two functional domains of the compound monomer independently mediate target binding and macrocycle dimerization. The target binding domain is comprised primarily of the heptapeptide backbone, which forms a carboxylate recognition pocket that binds tightly to acyl-D-Ala-D-Ala residues in peptidoglycan precursors<sup>44</sup>. The dimerization domain is comprised of the disaccharides attached to amino-acid residue 4, a highly modified D-phenylglycine, and, in some analogues, monosaccharides at residue 6, a modified D-tyrosine. Studies on a series of natural and synthetic analogues demonstrate that the two domains can be optimized independently. Synthetic, covalently linked dimeric species have been assayed<sup>45,46</sup>, whereas modifications that improve dimerization over four orders of magnitude have little impact on the affinity of those same monomers for acyl-D-Ala-D-Ala peptidoglycan elements<sup>47</sup>. Interestingly, despite the structural independence of the two domains in the monomer, it has been shown that dimerization is cooperative with acyl-D-Ala-D-Ala binding *in vivo*. Each independent function enhances the other through a feedback mechanism<sup>48</sup>, resulting in improved biological activity for vancomycin analogues showing increased dimerization.

The general conclusion of these examples is that macrocyclic drugs often function in a manner that is qualitatively distinct from small molecules. They can be productively considered as among the smallest examples of biomolecules that exhibit functional sub-domains. In addition, this multi-domain model is also evident in other natural non-drug macrocycles, such as dictyostatin<sup>28</sup>. It appears that evolution has repeatedly been driven towards the creation of domains in macrocyclic natural products. However, it is widely recognized that these molecules are challenging synthetic targets, and difficulties in making synthetic analogues of naturally occurring macrocycles has limited the wider application of this structural class for medicinal chemistry. Growth in the use of macrocycles for drug discovery has only occurred with the increasing focus on synthetic macrocycles that have some of the advantages of natural products but with greater accessibility. The range of success experienced with synthetic macrocycles is documented in the following sections.

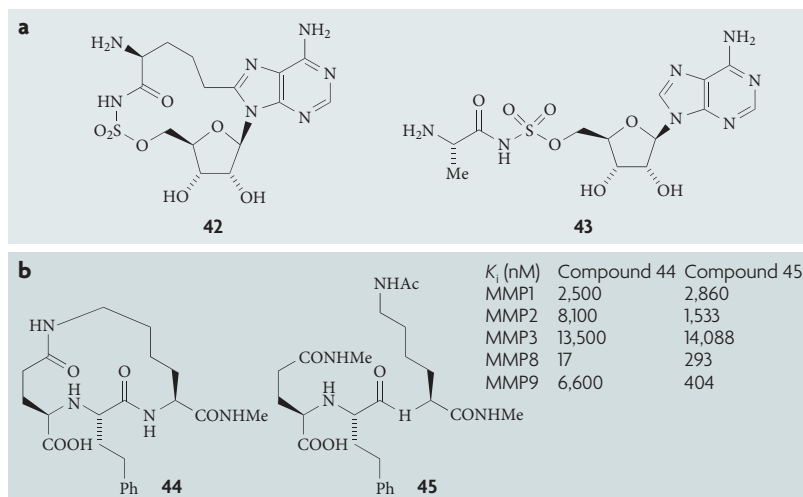


Figure 4 | **Improving target selectivity through macrocyclization: part 2.**

**a** | Inhibitors of amino-acid adenylation often also inhibit aminoacyl-tRNA synthetases. However, consideration of published ligand-bound crystal structures of the phenylalanine adenylation domain (PheA) and phenylalanyl-tRNA synthetase (PheRS) indicates a difference in bound substrate conformations. In the PheA structure, phenylalanine and AMP are held in a cisoid conformation, whereas in PheRS, a phenylalanine-AMP analogue is bound in a transoid conformation. This difference has been exploited in the design and synthesis of a macrocyclic inhibitor (**42**)<sup>88</sup> by the insertion of a two- or three-atom linker to generate a macrocycle that enforces the cisoid conformation. Compound **42** was shown to be a micromolar inhibitor in a cysteine adenylation assay, but unlike the linear aminoacyl-AMS analogue **43**, had no inhibitory activity against the corresponding aminoacyl-tRNA synthetases.

**b** | Selective matrix metalloproteinase (MMP) inhibitors. By using a macrocyclic ring to hold a unique backbone conformation, it has been possible to find selective inhibitors of MMP8 (REF. 89). An X-ray crystal structure of inhibitor-bound MMP3 demonstrated that the P1 and P2' side chains extend away from the active site into solvent, and thus could be connected into a macrocyclic structure to maintain the active backbone conformation. MMP8 modelling revealed that macrocycles could provide the same hydrogen-bonding interactions as the open-chain inhibitors. The 14-membered macrocycle **44**, was 17-fold more potent as an inhibitor of MMP8 compared with the corresponding open-chain inhibitor **45**, whereas activity against MMP1, MMP2, MMP3 and MMP9 decreased or was unchanged.

### Synthetic macrocycles in drug discovery

As with naturally occurring macrocycles, synthetic macrocycles represent a class of larger compounds that have been designed with a degree of conformational pre-organization that can bind to extended target binding sites with minimal entropic loss. The examples described below demonstrate that synthetic macrocycles can provide attractive ligands for disease-significant targets, and that such compounds can provide high levels of target affinity and selectivity, as well as presenting drug-like bioavailability and stability.

**Impact on target affinity.** The story of the discovery of macrocyclic hepatitis C virus (HCV) NS3 protease inhibitors typifies many of the key advantages of investigating macrocycles for drug discovery. Substrate recognition by this serine protease requires numerous interactions spanning residues P6 to P4' over a large surface area within the shallow, solvent-exposed substrate binding cleft. Finding a small rule of 5-compliant inhibitor structure with appreciable binding affinity has

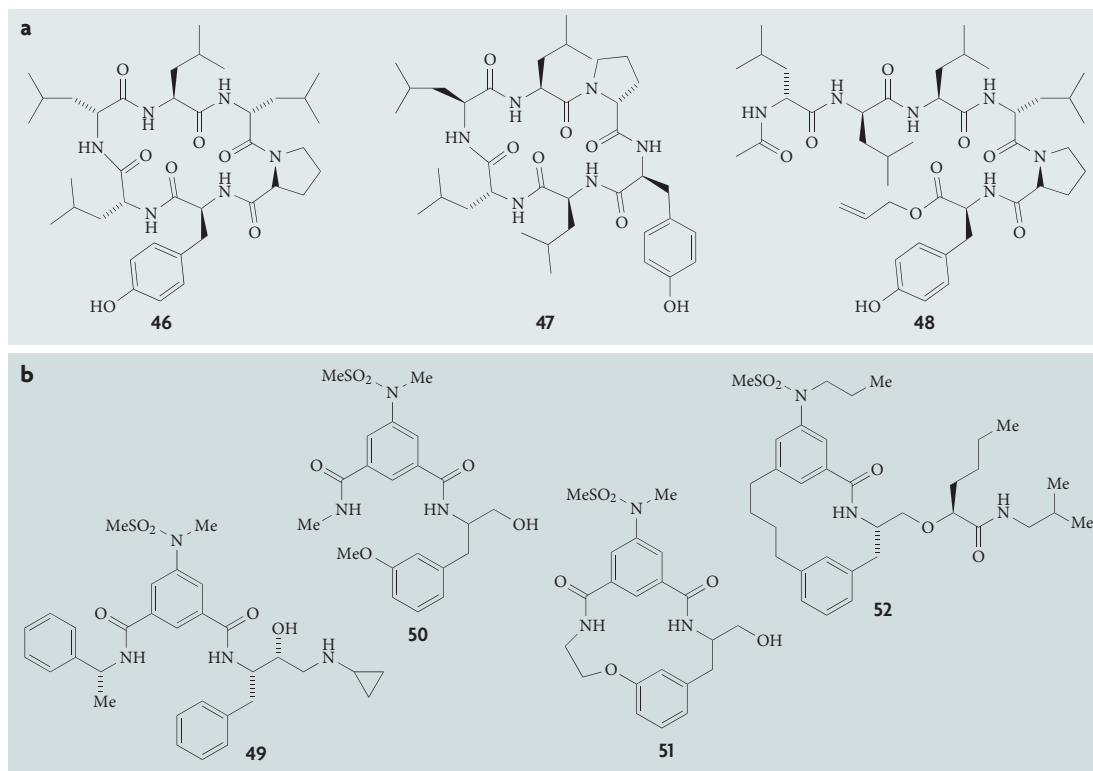
been a formidable drug discovery challenge, and the efforts of many groups have yielded modified peptidic structures containing active-site serine group chelation. Some of the linear inhibitors discovered have reasonable antiviral activity, but needed to contain a serine trap (for example, a ketoamide group) and be extensively fine-tuned for potency and bioavailability. The NS3 protease inhibitor VX-950 (**18**, FIG. 1) from Vertex is among the most advanced of this cohort and is currently in late-stage clinical development<sup>49</sup>.

Researchers at Boehringer Ingelheim demonstrated that the hexapeptide NS3 inhibitor **19** binds in an extended conformation and undergoes rigidification on binding<sup>50</sup>. Close proximity of solvent-exposed side chains in the bound conformation suggested linking to generate a macrocyclic inhibitor that preserved the bound conformation geometry. A simple macrocycle, compound **20**, demonstrated improved affinity over corresponding linear tetrapeptides. Further modifications resulted in BILN 2061 (**21**), a potent NS3 protease inhibitor<sup>51</sup>, which was advanced into clinical development before cardiac toxicity checked its progression<sup>52</sup>.

Additional examples highlighted in FIG. 1 and FIG. 2 suggest that the entropic loss on forming a protein-inhibitor complex is significantly reduced by using macrocyclic ligands, resulting in higher target affinity. As with all molecules with restricted conformations, macrocyclization is only beneficial if the correct spatial arrangement of key binding groups is maintained. In these examples, the macrocycles represent structures with conformations that demonstrate preferred functional group orientation; a necessity that has been ensured in many cases by evidence from direct experiments (for example, X-ray structural analysis) or by computational methods.

**Impact on selectivity.** Although improving potency often provides improved target selectivity, this is by no means automatic. However, macrocycles are able to provide enhanced selectivity as a consequence of fixing target-specific conformations. In a definitive study of selectivities conferred by macrocyclization, screening of libraries of 122 carbohydrate-derived open chain and the corresponding 122 macrocyclic analogues against 40 cell-based assays identified greater specificity of the macrocycle compounds, with only 2 out of the 19 active macrocycle compounds being active on more than one target<sup>53</sup>. By contrast, 20 of the 33 active open-chain analogues had activity in two or more assays.

When we consider individual drug discovery targets, macrocycles have repeatedly shown beneficial selectivity. The non-macrocycles, staurosporine (**34**, FIG. 3) and rebeccamycin (**35**) are non-selective kinase inhibitors that compete for the ATP binding site. Generation of macrocycles by the disruption of the indolocarbazole ring system, or the formation of macrocyclic analogues by other ring connections, can generate novel and selective kinase inhibitors. For example, ruboxistaurine (LY333531, **36**) selectively inhibits the  $\beta$  isoforms of protein kinase C (PKC) by a factor of 61-fold to 76-fold



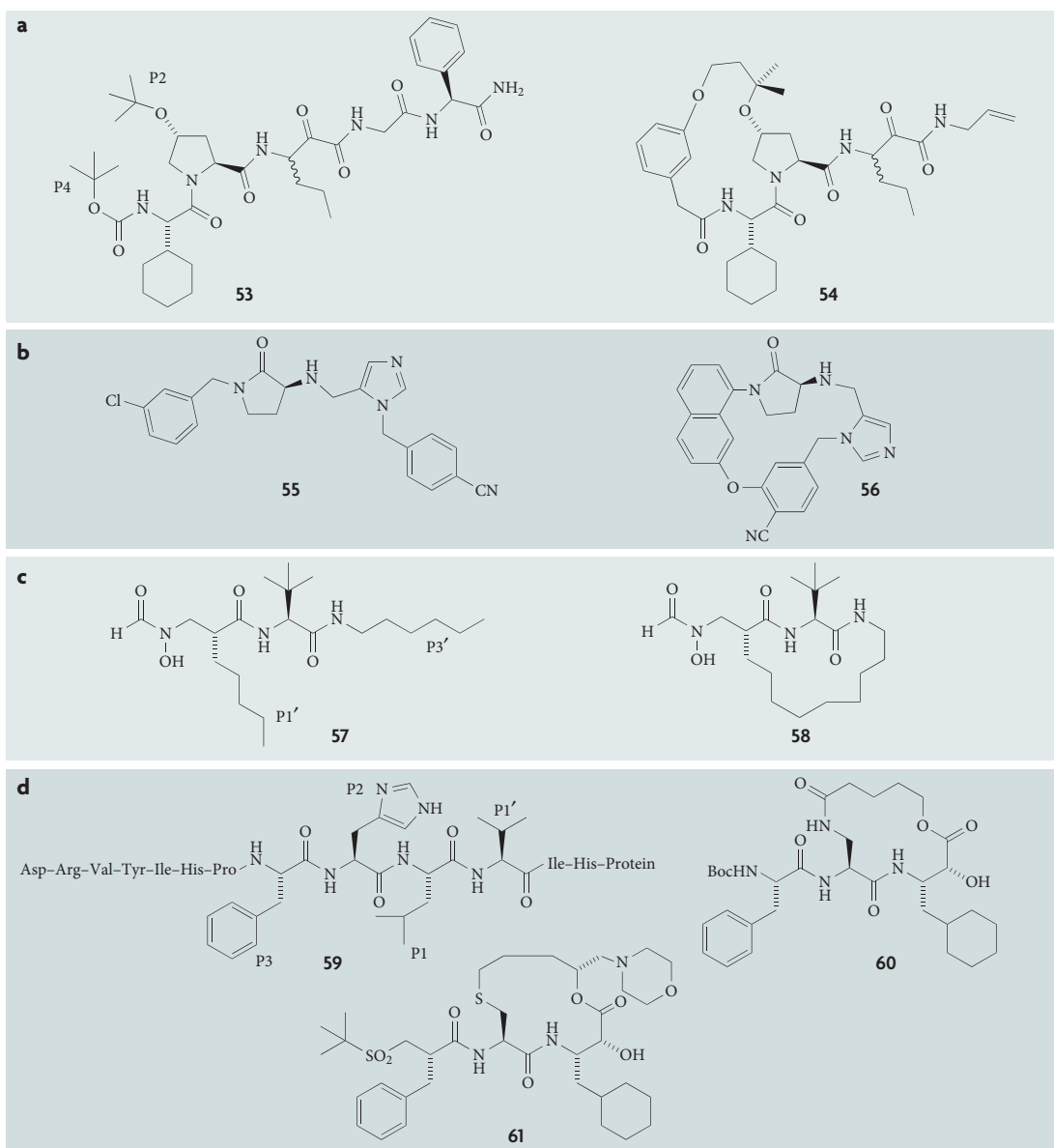
**Figure 5 | Improving physicochemical properties through macrocyclization. a** | The solubility of cyclic peptides. Nuclear magnetic resonance examination of solution conformations in  $\text{CDCl}_3$  revealed that the most permeable cyclic peptide diastereoisomer **46** could adopt a pseudosymmetric conformation with internally satisfied hydrogen bonds. By contrast, the least permeable diastereoisomer **47** exhibited an irregular, twisted conformation with some hydrogen-bonding groups oriented into the solvent<sup>56</sup>. A terminally protected linear analogue **48** of the most permeable cyclic peptide, exhibited permeability two log units lower than compound **46**, demonstrating the importance of the macrocyclic structure. **b** | Optimizing BACE inhibitors. 5-Substituted isophthalamides were reported as lead structures against BACE1 (also known as  $\beta$ -secretase), but utility was compromised by low cell-membrane permeability and recognition by P-glycoprotein (P-gp) transport mechanisms. When bound in the BACE1 catalytic site, the P1 and P3 side chains in the inhibitor **49** were found to be in close spatial proximity and might be linked in a macrocyclic analogue<sup>58</sup>. A simplified macrocyclic core structure **51** in a model system demonstrated at least a 30-fold increase in BACE1 affinity over the corresponding linear analogue **50**. Several rounds of structural optimization resulted in compound **52**, a macrocycle with an  $\text{IC}_{50}$  value of 4 nM, significantly improved permeability and reduced P-gp susceptibility.

over the  $\alpha$  isoform<sup>54</sup>. Conformation can be controlled by the macrocycle ring size, which subsequently affects kinase affinity and selectivity. A consideration of various inhibitors has demonstrated that adoption of a C2-symmetric *anti* conformation (compound **37**) or a distorted *syn* conformation (compound **38**) will influence kinase selectivity between PKC and glycogen synthase kinase 3 $\beta$  (GSK3 $\beta$ )<sup>55</sup>.

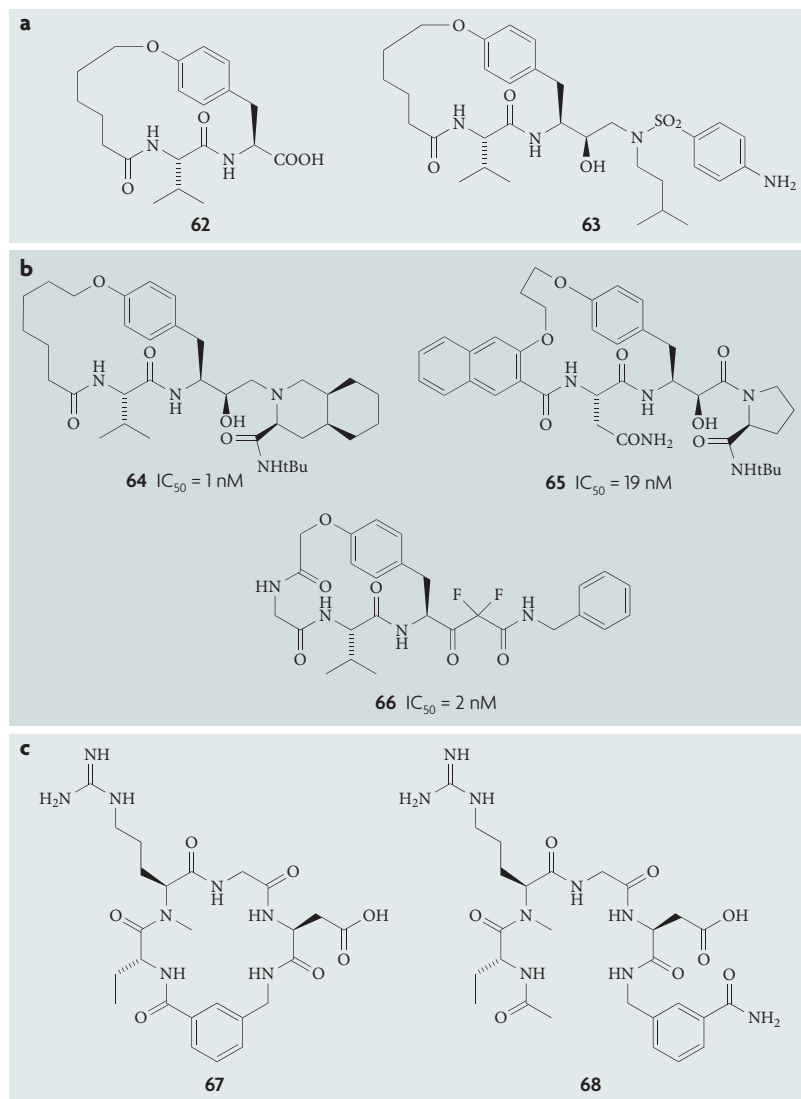
Further examples of macrocycle structures that gain selectivity by locking target-specific inhibitor conformations are provided in the design of compounds against non-ribosomal peptide synthetase amino-acid adenylation and in the development of selective matrix metalloproteinase (MMP) inhibitors (FIG. 4). In the latter case, the optimization of MMP inhibitors has frequently been compromised by poor selectivity between closely related isoenzymes, and the selectivity conferred by macrocycles provides a more important benefit than any increase in target affinity.

**Impact on physicochemical properties, membrane permeability and cellular activity.** Although peptides have long been recognized as a rich source of biologically active agents with numerous therapeutic applications, the use of peptide-like drugs is limited by metabolic instability and poor membrane permeability. As the problems arise from conformational flexibility and the exposure of polar functionalities in linear molecules, a possible solution is cyclization into macrocyclic structures to provide significant structure rigidity, reduce peptide characteristics and, consequently, significantly improve membrane permeability.

Investigation of macrocyclic peptides has demonstrated that passive membrane permeability is a feature of molecules that can readily take up conformations with internally satisfied hydrogen bonding<sup>56</sup>. A systematic survey of cyclic hexapeptides in the parallel artificial membrane permeability assay revealed that experimental permeability coefficients ( $P_e$ ) spanned



**Figure 6 | Improving pharmacokinetic properties through macrocyclization: part 1. a** | Improving the pharmacokinetics of hepatitis C virus (HCV) protease inhibitors. Macrocytic HCV protease inhibitors linking P2 and P4 were prepared from the linear pentapeptide  $\alpha$ -ketoamide **53**, giving significantly improved potency. Among the macrocycles evaluated, compound **54** demonstrated exceptional oral bioavailability of 97%<sup>60</sup>. **b** | Improving pharmacokinetics and reducing the potential cardiac side effects of farnesyltransferase (FTase) inhibitors. Macrocyclization connecting the two ends of the linear 3-aminopyrrolidinone FTase inhibitor **55** led to macrocycles such as compound **56**, which displayed excellent canine oral bioavailability ( $F = 68\%$ ), a much longer half-life and reduced affinity for the hERG potassium channel<sup>61</sup>. **c** | Improving the proteolytic stability of peptide deformylase (PDF) inhibitors. On the basis of observations that the P1' and P3' side chains of the acyclic PDF inhibitors (for example, compound **57**) were closely packed in the PDF-inhibitor complex, macrocytic PDF inhibitors were developed by linking the side chains<sup>90,91</sup>. Compared with their acyclic counterparts, the macrocytic inhibitors showed much-improved stability against proteolytic degradation. Cyclic inhibitor **58** was potent ( $K_i = 0.67$  nM) and stable in rat plasma, showing no detectable degradation after 5 hours, whereas the corresponding acyclic compound **57** showed time-dependent degradation. **d** | Metabolically stable renin inhibitors. Computational models<sup>92</sup> of the human renin active site suggested that the P2 and P1' side chains of angiotensinogen **59** could be linked to provide a novel renin inhibitor<sup>93</sup>. A number of 13- and 14-membered macrocytic compounds such as **60** ( $IC_{50} = 0.59$   $\mu$ M) were prepared with an ester linkage between the P2 and P1' positions, incorporating the (2*R*,3*S*)-3-amino-4-cyclohexyl-2-hydroxybutanoic acid as a transition-state isostere. Modification of the N terminus to mimic additional residues in the angiotensinogen structure led to increased potency. Inserting a serine in the P2 position linked to P1' through an ester led to increased potency but limited bioavailability due to ester hydrolysis and rapid hepatic extraction<sup>94</sup>. Replacement with a cysteine (**61**), led to compounds with improved activity, sufficient oral absorption and metabolic stability<sup>95</sup>.



**Figure 7 | Improving pharmacokinetic properties through macrocyclization: part 2. a** | Constrained HIV-1 protease inhibitors. Designing macrocyclic HIV-1 protease inhibitors incorporating lipophilic linkers mimicking the P1-P3 (or P1'-P3') Phe-Val-Ala tripeptide segments of the natural enzyme substrates has resulted in inhibitors such as compounds **62** and **63** with nanomolar inhibitory activity and enhanced proteolytic stability<sup>96,97</sup>. **b** | The same P1-P3 connection has also been used in the preparation of potent macrocyclic HIV protease inhibitors containing alternative hydroxyethylamine<sup>98</sup> (**64**), norstatine<sup>99</sup> (**65**) and difluoroketone<sup>100</sup> (**66**) transition-state mimicking groups. In the case of the norstatine-containing inhibitor, although the macrocycle was less active *in vitro* than acyclic analogues, it demonstrated superior cell penetration and/or metabolic stability. **c** | In a study towards developing high-affinity ligands for the platelet glycoprotein IIb/IIIa (GPIIb/IIIa), a series of macrocyclic compounds were prepared that contain the RGD motif with non-peptide rigid linkers<sup>63</sup>. The macrocyclic compound **67** was not only more potent than its corresponding linear version **68**, but also demonstrated superior metabolic stability, attributed to the successful rigidification of the RGD backbone.

nearly two log units between the most and least permeable (FIG. 5). The most permeable cyclic peptide diastereoisomers adopt conformations with internally satisfied hydrogen bonds, whereas cyclic peptides with lower permeability have conformations with some hydrogen-bonding groups oriented into the solvent.

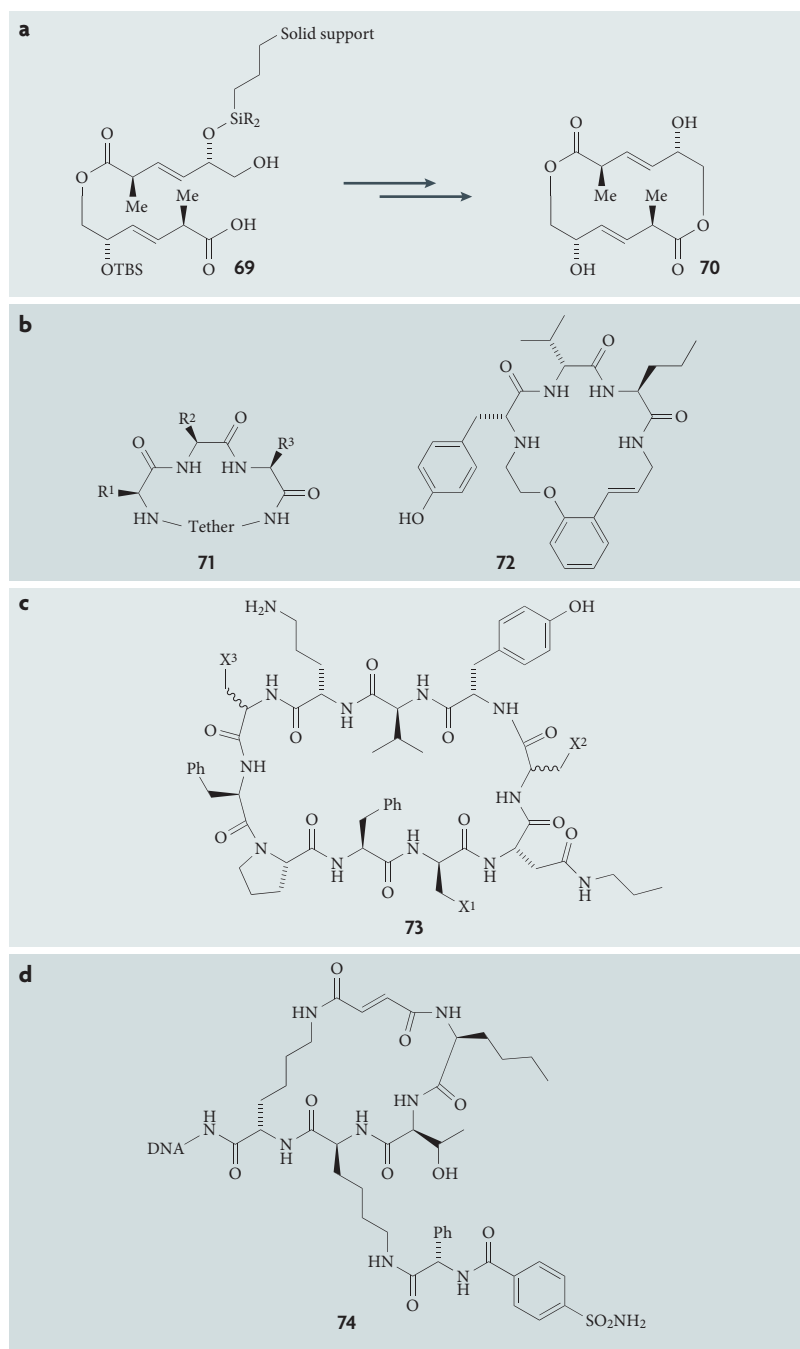
The studies resulted in an effective *in silico* model for predicting the relative permeabilities of macrocyclic peptides<sup>57</sup>.

The discovery of macrocyclic BACE1 ( $\beta$ -secretase) inhibitors for the treatment of Alzheimer's disease is a clear illustration of a design rationale applied to improve permeability (FIG. 5). As the utility of BACE1 inhibitors has been compromised by low cell-membrane permeability and recognition by P-glycoprotein (P-gp) transport mechanisms, macrocyclization has enhanced potency and improved pharmacokinetics by reducing the number of hydrogen-bonding groups. Structural optimization resulted in a macrocycle (compound **52**) with significantly improved permeability and reduced P-gp susceptibility. The macrocycle had reasonable CNS penetration and when administered intravenously to APP-YAC transgenic mice, a model of human Alzheimer's disease, amyloid precursor protein fragment levels of A $\beta$ 40 were reduced<sup>58</sup>.

**Impact on proteolytic and metabolic stabilities and overall pharmacokinetics.** There is a significant history of drug discovery based on naturally occurring peptides<sup>59</sup>, in which naturally occurring enzyme substrates have influenced inhibitor design. Flexible peptidic molecules have limited utility as drug candidates as a consequence of their inadequate proteolytic stabilities. Consequently, macrocyclization of linear molecules can significantly reduce accessible conformational space and has the potential to generate compounds with greater proteolytic and metabolic stability. Improved stability in combination with better membrane permeability of macrocycles, as outlined in the previous section, can translate into better *in vivo* pharmacokinetics.

As previously discussed, the HCV NS3 protease inhibitor, BILN2061 (**21**, FIG. 1), was developed by linking the P1 and P3 side chains of a linear peptide into a macrocyclic ring<sup>50,52</sup>. In addition to the dramatic improvement in potency over the linear peptide **19**, the macrocycle BILN2061 demonstrated superior metabolic stability and low clearance in various animal models. Despite its high molecular mass, BILN2061 showed good oral bioavailability in the dog. In the clinic, BILN2061 demonstrated a favourable oral pharmacokinetic profile, resulting in a twice-daily chronic dosing regimen before development of the compound was discontinued for reasons of cardiotoxicity. A similar macrocyclization approach has been taken in the search for other HCV protease inhibitors with good pharmacokinetic properties<sup>60</sup> (FIG. 6). Macrocyclic compounds have also been investigated to improve pharmacokinetics and reduce the potential cardiac side effects of farnesyltransferase inhibitors<sup>61</sup>. Examples in which the proteolytic stability of peptide deformylase and renin inhibitors have been improved by macrocyclization are given in FIG. 6.

Tripeptide  $\beta$ -strand conformations can be constrained by a macrocyclic scaffold (for example, compound **62**, FIG. 7) containing two *trans* amide bonds and a planar aromatic ring<sup>62</sup>. As many aspartyl proteases recognize this extended 'saw tooth' conformation in their substrates, holding the  $\beta$ -strand form in a conformationally



**Figure 8 | Examples of improved methods for synthesizing macrocycle libraries.** **a** | A library of 13- and 14-membered macrolactones (for example, compound **70**) has been prepared on solid-phase support by the cyclization of hydroxyacid precursors (for example, compound **69**)<sup>71</sup>. **b** | A library of at least 10,000 peptidomimetic macrocycles has been prepared by two complementary solid-phase parallel approaches<sup>72</sup>. The macrocycles **71** were tripeptides cyclized through a diverse tether region, and screened as antagonists to the human motilin receptor using a high-throughput fluorescence-based whole-cell assay. The initial high-throughput screen hit **72** had a  $K_i$  value of 137 nM and the introduction of unnatural amino acids containing basic side chains has resulted in single digit nanomolar antagonists<sup>101</sup>. **c** | Solid-phase synthesis has been used to generate a library of 1,716 tyrocidine A analogues **73** (REF. 102). Antibacterial assays have indicated active compounds that were identified by analysis of an encoding linear peptide still attached to the source bead. **d** | DNA-encoded synthesis has been used to make a library of macrocycles screened against carbonic anhydrase<sup>73</sup>. The DNA was subsequently amplified and decoded to reveal macrocycle **74** as having the highest target affinity.

pre-organized macrocycle may give rise to inhibitors that are both more potent and metabolically more stable than the open-chain equivalent. Such constrained templates can also potentially order their immediate enzyme environment and dampen any induced fit that may occur as a result of changing other substituents on the inhibitor, providing a more predictable picture of the structure–activity relationships. This approach has been applied to the design and synthesis of HIV-1 protease inhibitors, incorporating lipophilic linkers mimicking the P1–P3 (or P1′–P3′) Phe–Val–Ala tripeptide segments of the natural enzyme substrates. Macrocycles containing the RGD motif with superior metabolic stability have also been prepared in a study of high-affinity ligands for the platelet glycoprotein IIb/IIIa (GPIIb/IIIa)<sup>63</sup>.

### Macrocycle synthesis

Given the potential of synthetic macrocycles to address difficult drug discovery targets as outlined above, there is an increasing demand to find effective preparative approaches to these compounds. A widely held opinion based on naturally occurring macrocycle structures has been that they are difficult compounds to make. However, significantly less complex synthetic macrocycles can be readily prepared. Considerable numbers of macrocycles can be made using available building blocks and reliable synthetic transformations, and stereochemistry need not be a limiting factor. The only residual difficulty in macrocycle synthesis is finding conditions that allow good yields of cyclized materials from acyclic precursors. It is usually necessary to control the reaction to preferentially encourage intramolecular cyclization over intermolecular side reactions. For single compounds, this is usually achieved by resorting to the use of large reaction volumes and low reactant concentrations; conditions that were devised many years ago<sup>64</sup>.

There is an extensive and growing literature around macrocycle synthesis<sup>65</sup>, and a growing consensus on the use of preferred cyclization methods including ring-closing olefin metathesis<sup>66</sup>, multi-component reactions<sup>67</sup>, metal-templated chelation<sup>68</sup> or ring-closing-contraction sequences<sup>69</sup> such as the Staudinger ligation<sup>70</sup> (see [Supplementary information S2 \(box\)](#)).

Many of the synthetic macrocycles described above were designed based on an understanding of a specific ligand–target interaction, in many cases using structural evidence from X-ray crystallography. Methods are also required for generating libraries or parallel arrays of macrocycles that can address any target for which there is little or no prior structural knowledge, or for studies in which there may be structural information but a range of analogues are required. Synthesizing single macrocycles using high dilution is not a convenient method for making large numbers of macrocyclic products in parallel arrays. Approaches that do allow access to macrocycle libraries are being developed, and some groups have reported large libraries using techniques that isolate the individual acyclic precursors to prevent unwanted intermolecular reactions. The preferred methods used are synthesis on solid-phase or preparation on a DNA template (FIG. 8).

## Olefin metathesis

An organic chemical reaction catalysed by metals such as nickel, tungsten, ruthenium and molybdenum. It involves redistribution of double bonds resulting in new chemical compounds, or a new ring if the reaction occurs intramolecularly.

## Metal-templated chelation

The use of a metal ion to hold a linear precursor to a macrocycle in an energetically favourable conformation, resulting in higher yields or faster reaction rates.

## Staudinger ligation

A reaction between an azide and a phosphine in aqueous media that results in the production of an amide linkage.

## Wittig double-bond formation reaction

A reaction of a phosphorous ylid with an aldehyde to generate an alkene.

For example, a library of 13- and 14-membered macrolactones (for example, compound **70**) has been prepared on solid-phase support by the cyclization of hydroxyacid precursors (for example, compound **69**)<sup>71</sup>. The library of 36 stereochemically and regiochemically diverse products was used to demonstrate that chemical success was highly dependent on the protection strategy, solid support linker length and double-bond position.

A library of at least 10,000 peptidomimetic macrocycles (for example, compound **71**) has been prepared by two complementary solid-phase parallel approaches<sup>72</sup>. In the first approach, the acyclic precursor was attached to solid-phase through a thioester, and cyclization occurred through a macrolactamization cyclative release by amine attack and cleavage of the thioester. The second approach featured a ring-closing metathesis cyclative release, generating products with a double bond in the tether region. These macrocycles have been screened as antagonists to the human motilin receptor using a high-throughput whole-cell assay.

Furthermore, macrocycle libraries have been prepared on DNA templates, whereby the formation of a DNA duplex has driven the specific interaction of tethered building blocks and has also facilitated a ring-closing Wittig double-bond formation reaction<sup>73</sup>. This technology has been further developed to allow the preparation of libraries of tens of thousands of macrocycles<sup>74</sup>.

## Summary and conclusions

There is a growing body of evidence that macrocycles provide a viable and valuable area of structural space for drug discovery. Macrocycles are well preceded in nature, and many of these compounds have proved useful

in treating disease; the macrocycles providing a simple pre-organized scaffold that can optimally present functional binding domains. As natural products are often difficult to synthesize, several groups have used X-ray and nuclear magnetic resonance techniques to design both peptidic and non-peptidic synthetic macrocycles for a range of important disease targets, and several of these compounds are in preclinical or early clinical development. Macrocycles are most effective as ligands when the ring has a significant effect in restricting structural flexibility and provides a ground state cyclized conformation that approximates the optimal bound conformation. Such compounds will have greater potency, and in some cases provide greater selectivity between related targets. Macrocycles can have high levels of metabolic and proteolytic stability, as well as high levels of solubility and cell penetration that are required for acceptable pharmacokinetics. Reliable synthetic routes to macrocycles are now well explored and there are several classes of chemical transformations that are especially successful for forming rings. To allow the rapid screening of large numbers of unbiased compounds, library approaches to macrocycle synthesis and screening have been recently developed.

As more difficult drug discovery targets, such as those involving protein-protein interactions, are identified and the pursuit of conventional rule of 5-compliant compounds fails to deliver the drug-like lead molecules that medicine requires, there is a pressure to explore new structural space. Macrocycles provide a structural class with significant potential for drug discovery. Although they have been underexploited so far, it is anticipated that interest in, and success with, this class of compounds will rapidly grow in the coming years.

- Breinbauer, R. *et al.* From protein domains to drug candidate — natural products as guiding principles in the design and synthesis of compound libraries. *Angew. Chem. Int. Ed.* **41**, 2878–2890 (2002). **A detailed analysis of the make-up of proteins and a compelling argument for the evolution of protein functionality.**
- Lipinski, C. A., Lombardo, F., Dominy, B. W. & Feeney, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev.* **23**, 3–25 (1997).
- Demain, A. L. & Fang, A. The natural functions of secondary metabolites. *Adv. Biochem. Eng. Biotechnol.* **69**, 2–39 (2000).
- Wessjohann, L. A. *et al.* What can a chemist learn from nature's macrocycles? A brief, conceptual view. *Mol. Divers.* **9**, 171–186 (2005). **A perceptive overview of the range of natural product macrocycle properties.**
- Williams, D. H., Stone, M. J., Hauck, P. R. & Rahman, S. K. Why are secondary metabolites (natural products) biosynthesized? *J. Nat. Prod.* **52**, 1189–1208 (1989).
- Stone, M. J. & Williams, D. H. On the evolution of functional secondary metabolites (natural products). *Mol. Microbiol.* **6**, 29–34 (1992).
- Katz, E. & Demain, A. L. The peptide antibiotics of *Bacillus*: chemistry, biogenesis, and possible functions. *Bacteriol. Rev.* **41**, 449–474 (1977).
- Mulvenna, J. P. *et al.* Discovery of cyclotide-like protein sequences in graminaceous crop plants: ancestral precursors of circular proteins? *Plant Cell* **18**, 2134–2144 (2006).
- McArdle, B. M., Campitelli, M. R., Quinn, R. J. A common protein fold topology shared by flavonoid biosynthetic enzymes and therapeutic targets. *J. Nat. Prod.* **69**, 14–17 (2006).
- Blondeau, J. M. The evolution and role of macrolides in infectious diseases. *Expert Opin. Pharmacother.* **3**, 1131–1151 (2002).
- Nielsen, J. Combinatorial synthesis of natural product libraries. *Curr. Opin. Chem. Biol.* **6**, 297–305 (2002).
- Wessjohann, L. A. Synthesis of natural product-based compound libraries. *Curr. Opin. Chem. Biol.* **4**, 303–309 (2000).
- Jensen, P. R., Williams, P. G., Oh, D.-C., Zeigler, L. & Fenical, W. Species-specific secondary metabolite production in marine actinomycetes of the genus *Salinispora*. *Appl. Environ. Microbiol.* **73**, 1146–1152 (2007).
- Udway, D. W. *et al.* Genome sequencing reveals complex secondary metabolome in the marine actinomycete *Salinispora tropica*. *Proc. Natl Acad. Sci. USA* **104**, 10376–10381 (2007).
- Jenke-Kodama, H., Sandmann, A., Mueller, R. & Dittmann, E. Evolutionary implications of bacterial polyketide synthases. *Mol. Biol. Evol.* **22**, 2027–2039 (2005).
- Kim, T. K., Hewavitharana, A. K., Shaw, P. N. & Fuerst, J. A. Discovery of a new source of rifamycin antibiotics in marine sponge actinobacteria by phylogenetic prediction. *Appl. Environ. Microbiol.* **72**, 2118–2125 (2006).
- Merck. *The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals, Fourteenth Edition* (eds O'Neil, M. J., Heckelman, P. E., Koch, C. B. & Roman, K. J.) (Merck & Co., Inc., Whitehouse Station, NJ, USA, 2006).
- Newman, D. J., Cragg, G. M. & Snader, K. M. Natural products as sources of new drugs over the period 1981–2002. *J. Nat. Prod.* **66**, 1022–1037 (2003).
- Choi, J., Chen, J., Schreiber, S. L. & Clardy, J. Structure of the FKBP12–rapamycin complex interacting with the binding domain of human FRAP. *Science* **273**, 239–242 (1996).
- Nghiem, P., Pearson, G. & Langley, R. G. Tacrolimus and pimecrolimus: from clever prokaryotes to inhibiting calcineurin and treating atopic dermatitis. *J. Am. Acad. Dermatol.* **46**, 228–241 (2002).
- Butler, M. S. Natural products to drugs: natural product derived compounds in clinical trials. *Nat. Prod. Rep.* **22**, 162–195 (2005).
- Favre, S., Kroemer, G. & Raymond, E. Current development of mTOR inhibitors as anticancer agents. *Nature Rev. Drug Discov.* **5**, 671–688 (2006).
- Schreiber, S. L. Immunophilin-sensitive protein phosphatase action in cell signaling pathways. *Cell* **70**, 365–368 (1992).
- Schreiber, S. L. & Crabtree, G. R. The mechanism of action of cyclosporin A and FK506. *Immunol. Today* **13**, 136–142 (1992). **This review together with reference 23 summarize the early stages of research around FK506 and rapamycin, during which the requirement for FKBP12 complexation was discovered.**
- Feyen, F., Cachoux, F., Gertsch, J., Wartmann, M. & Altmann, K.-H. Epithilones as lead structures for the synthesis-based discovery of new chemotypes for microtubule stabilization. *Accounts Chem. Res.* **41**, 21–31 (2008).
- Nogales, E., Wolf, S. G., Khan, I. A., Luduena, R. F. & Downing, K. H. Structure of tubulin at 6.5 Å and location of the taxol-binding site. *Nature* **375**, 424–427 (1995).
- Mani, S. *et al.* Ixabepilone: antimetabolic drug: microtubule-stabilizing agent: epithilone. *Drug Future* **32**, 1033–1039 (2007).
- Madiraju, C. *et al.* Tubulin assembly, taxoid site binding, and cellular effects of the microtubule-stabilizing agent dictyostatin. *Biochemistry* **44**, 15053–15063 (2005).

29. Hamel, E. *et al.* Synergistic effects of peloruside A and laulimalide with taxoid site drugs, but not with each other, on tubulin assembly. *Mol. Pharmacol.* **70**, 1555–1564 (2006).
30. Dabyydeen, D. A. *et al.* Comparison of the activities of the truncated halichondrin B analog NSC 707389 (E7389) with those of the parent compound and a proposed binding site on tubulin. *Mol. Pharmacol.* **70**, 1866–1875 (2006).
31. Schlunzen, F. *et al.* Structural basis for the interaction of antibiotics with the peptidyl transferase center in eubacteria. *Nature* **413**, 814–821 (2001).
- Erythromycin and rifampicin share key mechanistic elements for their respective targets. However the rifampicin story has evolved. Compare this paper to that of Artsimovitch, I. *et al.* (Cell 122, 351–363; 2005), for an interesting perspective on the mechanisms used by these macrocyclic families.**
32. Tenson, T., Lovmar, M. & Ehrenberg, M. The mechanism of action of macrolides, lincosamides, and streptogramin B reveals nascent peptide exit path in the ribosome. *J. Mol. Biol.* **330**, 1005–1014 (2003).
33. Fusetani, N., Matsunaga, S., Matsumoto, H. & Takebayashi, Y. Bioactive marine metabolites. 33. Cyclotheonamides, potent thrombin inhibitors, from a marine sponge *Theonella* sp. *J. Am. Chem. Soc.* **112**, 7053–7054 (1990).
34. Maryanoff, B. E. Inhibitors of serine proteases as potential therapeutic agents: the road from thrombin to trypsin to cathepsin G. *J. Med. Chem.* **47**, 769–787 (2007).
35. Brauer, A. B., McBride, J. D., Kelly, G., Matthews, S. J. & Leatherbarrow, R. J. Resisting degradation by human elastase: commonality of design features shared by 'canonical' plant and bacterial macrocyclic protease inhibitor scaffolds. *Bioorg. Med. Chem.* **15**, 4618–4628 (2007).
36. Greco, M. N. & Maryanoff, B. E. Macrocyclic inhibitors of serine proteases. *Adv. Amino Acid Mimetics Peptidomimetics* **1**, 41–76 (1997).
37. Matsumori, N., Yamaji, N., Matsuoka, S., Oishi, T. & Murata, M. Amphoterin B covalent dimers forming sterol-dependent ion-permeable membrane channels. *J. Am. Chem. Soc.* **124**, 4180–4181 (2002).
38. Wender, P. A. *et al.* The design, computer modeling, solution structure, and biological evaluation of synthetic analogs of bryostatin 1. *Proc. Natl Acad. Sci. USA* **95**, 6624–6629 (1998).
39. Koehn, F. E. & Carter, G. T. The evolving role of natural products in drug discovery. *Nature Rev. Drug Discov.* **4**, 206–220 (2005).
40. Somers, P. K., Wandless, T. J. & Schreiber S. L. Synthesis and analysis of 506BD, a high affinity ligand for the immunophilin FKBP. *J. Am. Chem. Soc.* **113**, 8045–8056 (1991).
41. Bayle, J. H. *et al.* Rapamycin analogs with differential binding specificity permit orthogonal control of protein activity. *Chem. Biol.* **13**, 99–107 (2006). **Although not formally using the term 'domain', this work constitutes a very interesting practical exploration of the macrocycle domain concept in the context of one particular system: the rapamycin–FKBP12 interaction.**
42. Fehr, T. *et al.* Antascomincins A, B, C, D, E Novel FKBP12 binding compounds from a *Micromonospora* strain. *J. Antibiot. (Tokyo)* **49**, 230–233 (1996).
43. Navia, M. A. & Chaturvedi, P. R. Design principles for orally bioavailable drugs. *Drug Discov. Today* **1**, 179–189 (1996).
44. Loll, P. J., Bevivino, A. E., Korty, B. D. & Axelsen, P. H. Simultaneous recognition of a carboxylate-containing ligand and an intramolecular surrogate ligand in the crystal structure of an asymmetric vancomycin dimer. *J. Am. Chem. Soc.* **119**, 1516–1522 (1997).
45. Nicolaou, K. C. *et al.* Solid- and solution-phase synthesis of vancomycin and vancomycin analogues with activity against vancomycin-resistant bacteria. *Chem. Eur. J.* **7**, 3798–3823 (2001).
46. Nicolaou, K. C. *et al.* Synthesis and biological evaluation of vancomycin dimers with potent activity against vancomycin-resistant bacteria: target-accelerated combinatorial synthesis. *Chem. Eur. J.* **7**, 3824–3843 (2001).
47. Allen, N. E. & Nicas, T. I. Mechanism of action of oritavancin and related glycopeptide antibiotics. *FEMS Microb. Rev.* **26**, 511–532 (2003).
48. Williams, D. H. & Bardsley, B. The vancomycin group of antibiotics and the fight against resistant bacteria. *Angew. Chem. Int. Ed.* **38**, 1173–1193 (1999).
49. Perni, R. B. *et al.* Preclinical profile of VX-950, a potent, selective, and orally bioavailable inhibitor of hepatitis C virus NS3–4B serine protease. *Antimicrob. Agents Chemother.* **50**, 899–909 (2006).
50. Tsantrizos, Y. S. *et al.* Macrocyclic inhibitors of the NS3 protease as potential therapeutic agents of hepatitis C virus infection. *Angew. Chem. Int. Ed.* **42**, 1356–1360 (2003).
- The definitive description of the discovery of a macrocycle drug that targets HCV NS3 protease.**
51. Llinas-Brunet, M. *et al.* Structure–activity study on a novel series of macrocyclic inhibitors of the hepatitis C virus NS3 protease leading to the discovery of BILN 2061. *J. Med. Chem.* **47**, 1605–1608 (2004).
52. Lamarre, D. *et al.* An NS3 protease inhibitor with antiviral effects in humans infected with hepatitis C virus. *Nature* **426**, 186–189 (2003).
53. Kim, Y.-K. *et al.* Relationship of stereochemical and skeletal diversity of small molecules to cellular measurement space. *J. Am. Chem. Soc.* **126**, 14740–14745 (2004).
- An intriguing experiment to investigate the activity profiles of macrocycles and their corresponding open-chain analogues.**
54. Jirousek, M. R. *et al.* ([S]-13-[[[Dimethylamino)methyl]-10,11,14,15-tetrahydro-4,9:16,21-dimetheno-1*H*,13*H*-dibenzo[e],*k*]pyrrolo[3,4-*h*]]1,4,13]oxadiazacyclohexadecene-1,3(2*H*)-dione (LY333531) and related analogues: isozyme selective inhibitors of protein kinase Cβ. *J. Med. Chem.* **39**, 2664–2671 (1996).
55. Bartlett, S. *et al.* Comparison of the ATP binding sites of protein kinases using conformationally diverse bisindolylmaleimides. *J. Am. Chem. Soc.* **127**, 11699–11708 (2005).
56. Rezaei, T. *et al.* Testing the conformational hypothesis of passive membrane permeability using synthetic cyclic peptide diastereoisomers. *J. Am. Chem. Soc.* **128**, 2510–2511 (2006).
57. Rezaei, T. *et al.* Conformational flexibility, internal hydrogen bonding, and passive membrane permeability: successful *in silico* prediction of the relative permeabilities of cyclic peptides. *J. Am. Chem. Soc.* **128**, 14073–14080 (2006).
58. Stachel, S. J. *et al.* Macrocyclic inhibitors of β-secretase: functional activity in an animal model. *J. Med. Chem.* **49**, 6147–6150 (2006).
59. Wiley, R. A. & Rich, D. H. Peptidomimetics derived from natural products. *Med. Res. Rev.* **13**, 327–384 (1993).
60. Chen, K. X. *et al.* Novel potent hepatitis C virus NS3 serine protease inhibitors derived from proline-based macrocycles. *J. Med. Chem.* **49**, 995–1005 (2006).
61. Bell, I. M. *et al.* 3-Aminopyrrolidinones farnesyltransferase inhibitors: design of macrocyclic compounds with improved pharmacokinetics and excellent cell potency. *J. Med. Chem.* **45**, 2388–2409 (2002).
62. Reid, R. C., Kelso, M. J., Scanlon, M. J. & Fairlie, D. P. Conformationally constrained macrocycles that mimic tripeptide β-strands in water and aprotic solvents. *J. Am. Chem. Soc.* **124**, 5673–5683 (2002).
63. Jackson, S. *et al.* Template-constrained cyclic peptides: design of high-affinity ligands for GPIIb/IIIa. *J. Am. Chem. Soc.* **116**, 3220–3230 (1994).
64. Ruggli, P. A ring with triple bonds. *Justus Liebig's Ann. Chem.* **392**, 92–100 (1912).
65. Wessjohann, L. A. & Ruijter, E. Strategies for total and diversity-oriented synthesis of natural-product (like) macrocycles. *Top. Curr. Chem.* **243**, 137–184 (2005).
- A comprehensive review of synthetic approaches to macrocycles.**
66. Gradillas, A. & Perez-Castells, J. Macrocyclization by ring-closing metathesis in the total synthesis of natural products: reaction conditions and limitations. *Angew. Chem. Int. Ed.* **45**, 6086–6101 (2006).
- A thorough review of an increasingly important macrocyclization reaction.**
67. Wessjohann, L. A. & Ruijter, E. Macrocycles rapidly produced by multiple multicomponent reactions including bifunctional building blocks (MiBs). *Mol. Divers.* **9**, 159–169 (2005).
68. Gerbelev, N. V. & Arion, V. B. in *Complex Formation and Stereochemistry of Coordination Compounds* (ed. Buislaev, Y.) 133–204 (Nova Science Publishers, New York, NY, USA, 1996).
69. Meutermaans, W. D. F. *et al.* Difficult macrocyclizations: new strategies for synthesizing highly strained cyclic tetrapeptides. *Org. Lett.* **5**, 2711–2714 (2003).
70. David, O. *et al.* Intramolecular Staudinger ligation: a powerful ring-closure method to form medium-sized lactams. *Angew. Chem. Int. Ed.* **42**, 4373–4375 (2003).
71. Schmidt, D. R., Kwon, O. & Schreiber, S. L. Macrolactones in diversity-oriented synthesis: preparation of a pilot library and exploration of factors controlling macrocyclization. *J. Comb. Chem.* **6**, 286–292 (2004).
72. Marsault, E. *et al.* Discovery of a new class of macrocyclic antagonists to the human motilin receptor. *J. Med. Chem.* **49**, 7190–7197 (2006).
73. Gartner, Z. J. *et al.* DNA-templated organic synthesis and selection of a library of macrocycles. *Science* **305**, 1601–1605 (2004).
74. Driggers, E. M. *et al.* DNA-programmed assembly, characterization, and selection of small molecule libraries. 232nd ACS National Meeting, San Francisco, CA, United States Sept. 10–14, 2006. *ACS web site* [online], <<http://oasys2.confex.com/acs/232nm/techprogram/P982549.HTM>> (2006).
75. Kino, T. *et al.* FK-506, a novel immunosuppressant isolated from a *Streptomyces*. I. Fermentation, isolation, and physicochemical and biological characteristics. *J. Antibiot.* **40**, 1249–1255 (1987).
76. Kim, B.-J. *et al.* Phylogenetic analysis of the genera *Streptomyces* and *Kitasatospora* based on partial RNA polymerase β-subunit gene (*rpoB*) sequences. *Int. J. Syst. Evol. Microbiol.* **54**, 593–598 (2004).
77. Ikeda, Y., Schultz, L. W., Clardy, J. & Schreiber, S. L. Structural basis for peptidomimicry by a natural product. *J. Am. Chem. Soc.* **116**, 4143–4144 (1994).
78. Clardy, J. Structural studies on FK-506, cyclosporin A and their immunophilin complexes. *Perspect. Drug Discov. Design* **2**, 127–144 (1994).
79. Seiwert, S. *et al.* Preclinical characteristics of ITMN-191, an orally active inhibitor of the HCV NS3/4A protease nominated for preclinical development. Digestive Disease Week 2006, Los Angeles, USA, May 21–24 2006. *Array Biopharma web site* [online], <<http://www.arraybiopharma.com/documents/Publication/PubAttachment192.pdf>> (2006).
80. Dinsmore, C. J. *et al.* Conformational restriction of flexible ligands guided by the transferred noe experiment: potent macrocyclic inhibitors of farnesyltransferase. *J. Am. Chem. Soc.* **123**, 2107–2108 (2001).
81. Udagamasooriya, G., Saro, D. & Spaller, M. R. Bridged macrocycles as ligands for PDZ domain proteins. *Org. Lett.* **7**, 1203–1206 (2005).
82. Tao, Z.-F. *et al.* Structure-based design, synthesis, and biological evaluation of potent and selective macrocyclic checkpoint kinase 1 inhibitors. *J. Med. Chem.* **50**, 1514–1527 (2007).
83. Shi, Z.-D. *et al.* A novel macrocyclic tripeptide mimetic that exhibits low-picomolar Grb2 SH2 domain-binding affinity. *Biochem. Biophys. Res. Commun.* **310**, 378–383 (2003).
84. Wei, C.-Q., Li, B., Guo, R., Yang, D. & Burke, T. R. Development of a phosphatase-stable phosphotyrosyl mimetic suitably protected for the synthesis of high-affinity Grb2 SH2 domain-binding ligands. *Bioorg. Med. Chem. Lett.* **12**, 2781–2784 (2002).
85. Zhang, H.-C. *et al.* Novel bis(indolyl)maleimide pyridinophanes that are potent, selective inhibitors of glycogen synthase kinase-3. *Bioorg. Med. Chem. Lett.* **17**, 2863–2868 (2007).
86. Kawanishi, N. *et al.* Structure-based drug design of a highly potent CDK1,2,4,6 inhibitor with novel macrocyclic quinoxalin-2-one structure. *Bioorg. Med. Chem. Lett.* **16**, 5122–5126 (2006).
87. Lucking, U. *et al.* Macrocyclic aminopyrimidines as multitarget CDK and VEGF-R inhibitors with potent antiproliferative activities. *ChemMedChem* **2**, 63–77 (2007).
88. Cisar, J. S. *et al.* Exploiting ligand conformation in selective inhibition of non-ribosomal peptide synthetase amino acid adenylation with designed macrocyclic small molecules. *J. Am. Chem. Soc.* **129**, 7752–7753 (2007).
- Describes evidence that macrocycles can freeze molecular conformations to gain selectivity between related targets.**

89. Cherney, R. J. *et al.* Macrocyclic amino carboxylates as selective MMP-8 inhibitors. *J. Med. Chem.* **41**, 1749–1751 (1998).  
**A powerful demonstration of the ability of macrocycles to generate inhibitors that are selective for key enzymes within a closely related family of targets.**
90. Hu, X. *et al.* Structure-based design of a macrocyclic inhibitor for peptide deformylase. *J. Med. Chem.* **46**, 3771–3774 (2003).
91. Hu, X. *et al.* Macrocyclic inhibitors for peptide deformylase: a structure–activity relationship study of the ring size. *J. Med. Chem.* **47**, 4941–4949 (2004).
92. Williams, P. D. *et al.* Renin inhibitors containing conformationally restricted P<sub>1</sub>-P<sub>1</sub>' dipeptide mimetics. *J. Med. Chem.* **34**, 887–900 (1991).
93. Weber, A. E. *et al.* Design and synthesis of P<sub>2</sub>-P<sub>1</sub>'-linked macrocyclic human renin inhibitors. *J. Med. Chem.* **34**, 2692–2701 (1991).
94. Weber, A. E. *et al.* Highly potent, orally active diester macrocyclic human renin inhibitors. *J. Med. Chem.* **35**, 3755–3773 (1992).
95. Yang, L., Weber, A. E., Greenlee, W. J. & Patchett, A. A. Macrocyclic renin inhibitors: synthesis of a subnanomolar, orally active cysteine derived inhibitor. *Tetrahedron Lett.* **34**, 7035–7038 (1993).
96. Tyndall, J. D. A. *et al.* Synthesis, stability, antiviral activity, and protease-bound structures of substrate-mimicking constrained macrocyclic inhibitors of HIV-1 protease. *J. Med. Chem.* **43**, 3495–3504 (2000).
97. Reid, R. C. *et al.* Countering cooperative effects in protease inhibitors using constrained  $\beta$ -strand-mimicking templates in focused combinatorial libraries. *J. Med. Chem.* **47**, 1641–1651 (2004).
98. Smith, R. A. *et al.* Design, synthesis, and activity of conformationally-constrained macrocyclic peptide-based inhibitors of HIV protease. *Bioorg. Med. Chem. Lett.* **4**, 2217–2222 (1994).
99. Chen, J. J. *et al.* Synthesis and activity of conformationally-constrained macrocyclic norstatine-based inhibitors of HIV protease. *Bioorg. Med. Chem. Lett.* **6**, 435–438 (1996).
100. Podlogar, B. L. *et al.* Design, synthesis, and conformational analysis of a novel macrocyclic HIV-protease inhibitor. *J. Med. Chem.* **37**, 3684–3692 (1994).
101. Marsault, E. *et al.* Potent macrocyclic antagonists to the motilin receptor presenting novel unnatural amino acids. *Bioorg. Med. Chem. Lett.* **17**, 4187–4190 (2007).
102. Xiao, Q. & Pei, D. High-throughput synthesis and screening of cyclic peptide antibiotics. *J. Med. Chem.* **50**, 3132–3137 (2007).

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