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Natural product-like chemical space: search for chemical dissectors of macromolecular interactions

Ayub Reayi and Prabhat Arya

Macromolecular interactions (i.e. protein–protein or DNA/RNA–protein interactions) play important cellular roles, including cellular communication and programmed cell death. Small-molecule chemical probes are crucial for dissecting these highly organized interactions, for mapping their function at the molecular level and developing new therapeutics. The lack of ideal chemical probes required to understand macromolecular interactions is the missing link in the next step of dissecting such interactions. Unfortunately, the classical combinatorial-chemistry community has not successfully provided the required probes (i.e. natural product inspired chemical probes that are rich in stereochemical and three-dimensional structural diversity) to achieve these goals. The emerging area of diversity-oriented synthesis (DOS) is beginning to provide natural product-like chemical probes that may be useful in this arena.

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Introduction

Interest in the dissection of macromolecular interactions (i.e. protein–protein or DNA/RNA–protein interactions) by small molecules is gaining momentum because the precise role of macromolecules at the molecular level is unclear. What is known is that highly organized interactions are central to cellular machinery [1••,2]. Both inside and outside the cell, proteins interact with each other and form complex networks [3•,4••]. Following the completion of the sequencing of the human genome, the post-genomic era has brought new challenges such as understanding the structure and function of complex macromolecular networks and, in turn, how this knowledge could transfer into developing new classes of therapeutics [5,6]. Because small molecules can be used as chemical modulators of macromolecular interactions [7,8], there is a growing demand for having an arsenal of available small molecules that can be used as chemical probes, provided that each small molecule has very high specificity in the context of multiple macromolecular interactions.

In this review, a few selected examples of bioactive natural products that act as highly specific modulators of macromolecular interactions are described. This is followed by a brief discussion on different combinatorial chemistry approaches with a few recent examples of a library generation of natural product-like compounds.

Natural products as chemical probes

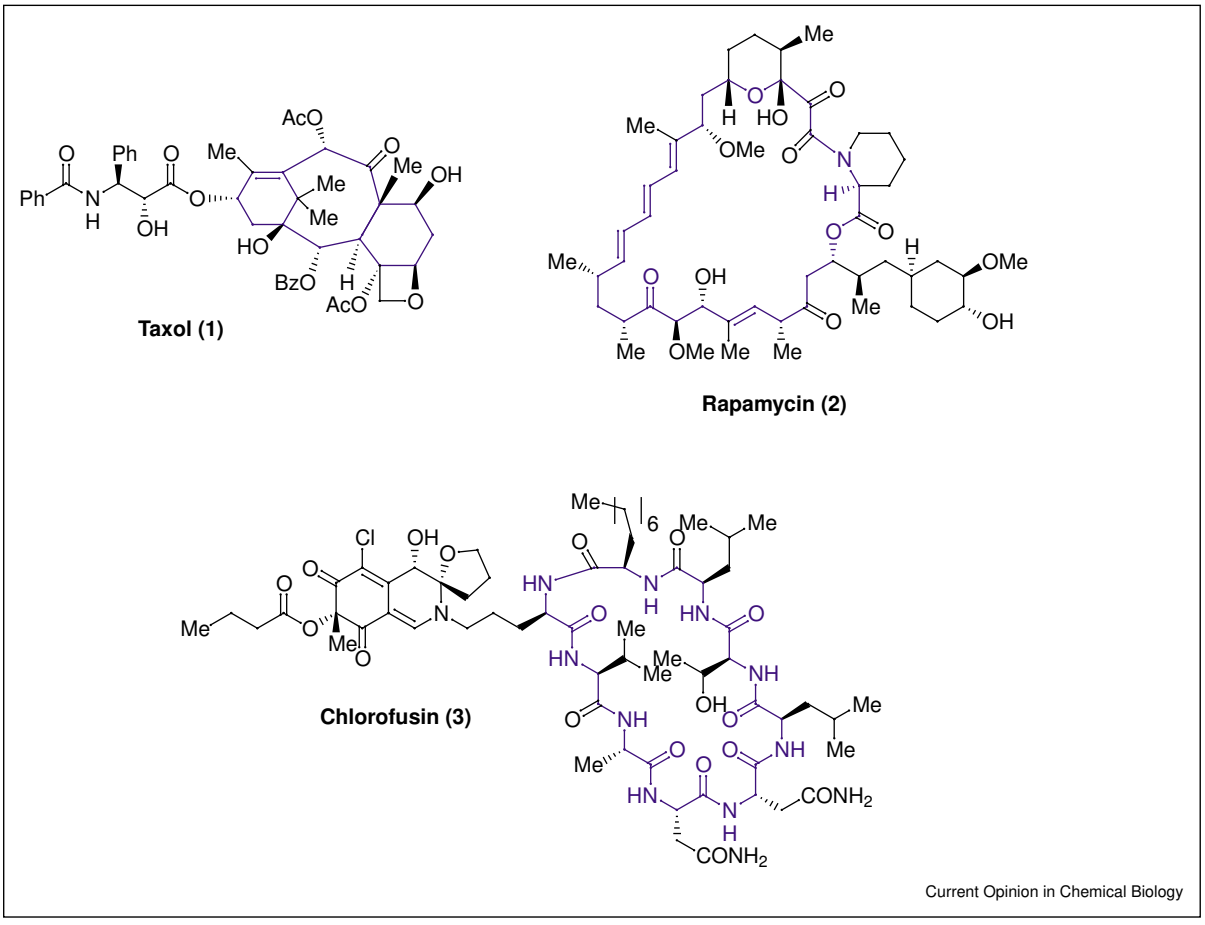
Because proteins are dynamic in nature and do not work in isolation, biological methods (e.g. mutagenesis) of understanding protein function at the molecular level are limited. One such example of these limitations applies to antibodies as therapeutics [9]. Although antibodies are highly specific for their molecular targets, they have been limited to the extracellular domain because of their poor cellular penetration. On the other hand, chemical methods for dissecting cellular processes have great potential [10]. Two compounds found in nature are known to interfere with protein–protein interactions. They are the natural products Taxol (**1**, Figure 1) and rapamycin (**2**).

Taxol **(1)** and its analogues are known to bind to the β-subunit of the tubulin heterodimer, thus stabilizing the heterodimer. These interactions enhance the polymerization of tubulin into microtubules and promote an arrest in the cell cycle, resulting in programmed cell death [11,12]. In recent years, several other natural products (i.e. epothilones [13], discodermolide [14–16], laulimalide [17] and eleutherobin [18], structures not shown) have been identified that also promote the stabilization of microtubules. As with Taxol, these natural products serve as interesting lead compounds in developing novel anti-tumour agents.

Rapamycin **(2)** interacts with a protein known as FKBP12, and this complex can associate with FRAP, a critical downstream signaling component of the PI3K/Akt pathway. Hence, rapamycin behaves as a chemical inducer of dimerization (CID). Rapamycin and its derivatives have also served as useful probes as modulators of protein–protein interactions and, therefore, as a tool in understanding pathways that respond to these interactions [19–22].

Using microorganisms, chlorofusin **(3)**, a novel fungal metabolite, was identified as an inhibitor of p53 binding to MDM2 during a screen of 5300 microbial extracts [23].

Figure 1



Taxol and rapamycin are bioactive natural products that are known to interfere with protein interactions. Chlorofusin is a fungal metabolite inhibitor of p53 and MDM2 interactions. The complete structures of bioactive natural products or the cyclic sub-structures (i.e. privileged scaffolds) are shown in blue.

The p53 tumour suppressor protein is a short-lived protein due to its rapid proteasomal degradation. Upon exposure of cells to various stress stimuli, levels of p53 rise as a consequence of reduced proteolytic activity. This results in induction of a programme of gene expression that leads to the arrest of the cell growth and/or apoptosis [24]. MDM2 is an important regulator of the stability of p53, as it directly interacts with p53 and promotes its ubiquitination and neddylation. Hence, a key function of MDM2 is to inhibit the growth-suppressing effects of p53. The co-crystal structure of p53 and MDM2 has shown that there is a hydrophobic cleft in MDM2 that acts as a 'hot spot' for the MDM2–p53 interaction, and that targeting this site by small-molecule intervention might be feasible [25•]. Tumors often express elevated levels of MDM2. Thus, it was envisioned that tumor cells with high levels of MDM2 could be treated with small molecules to interfere with p53–MDM2 interactions, and allow the cells to enter cell-cycle arrest or be eliminated by apoptosis. Although complex in nature, chlorofusin was identified as an antagonist of MDM2 and is a promising lead compound.

The examples discussed above clearly indicate that natural products can interfere with macromolecular interactions and present the need to develop new chemical approaches for creating architecturally complex compounds, leading to a wide variety of natural product-like compounds in high-throughput manner. Although classical combinatorial chemistry provides a rapid access to compounds, in most cases these compounds are poor in stereogenic functional groups and lack the three-dimensional architecture required for them to be effective chemical tools amenable to dissecting macromolecular interactions. Newer approaches to generate complex architectural compounds are therefore required.

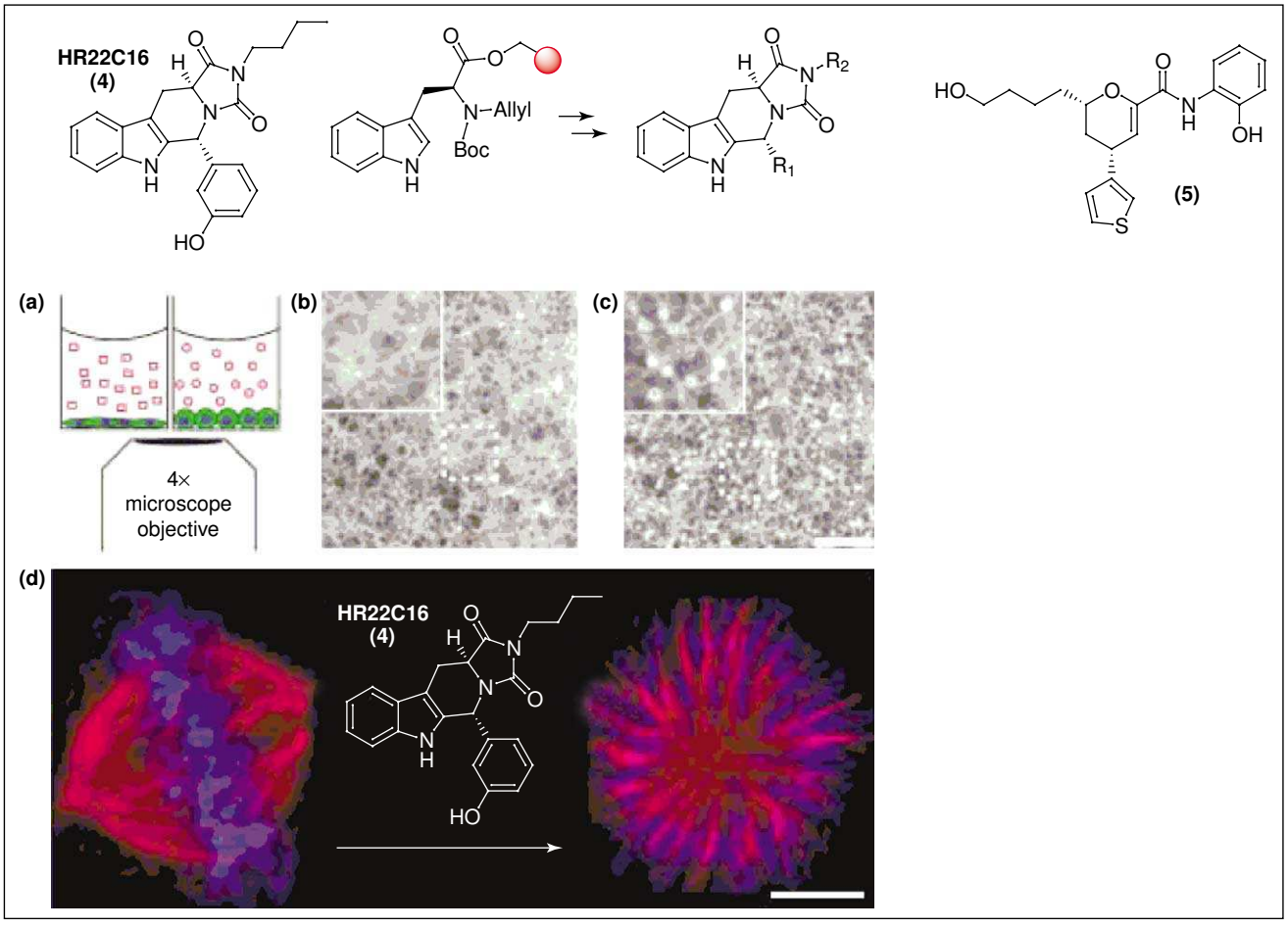
Diversity-oriented synthesis

Diversity-oriented synthesis (DOS) is aimed at addressing the present limitations of combinatorial chemistry by

providing rapid access to three-dimensional and stereochemically diverse, natural product-like compounds. Inspired by bioactive natural products, natural product-like compounds occupy the same chemical space that is currently available to natural products [26–28]. The presence of 3D architectures offers the advantage that it prevents the formation of artefacts during high-throughput screening due to non-specific binding. Having cyclic motifs may also be useful as this can make compounds more rigid, and, in turn, more acceptable to biological targets that have a requirement for stereochemical rigidity. For example, Kapoor and co-workers [29] have reported the discovery of HR22C16 (**4**, Figure 2), a small molecule identified by a high-throughput, microscopy-based forward chemical-genetic screen. There are several cyclic motifs present. HR22C16 was found to be a small-molecule inhibitor ($IC_{50} = 800 \pm 10$ nm) of cell division and targets Eg5, a protein required for cell division. The authors have also performed diastereoselective solid-phase synthesis of HR22C16 analogues using a DOS approach.

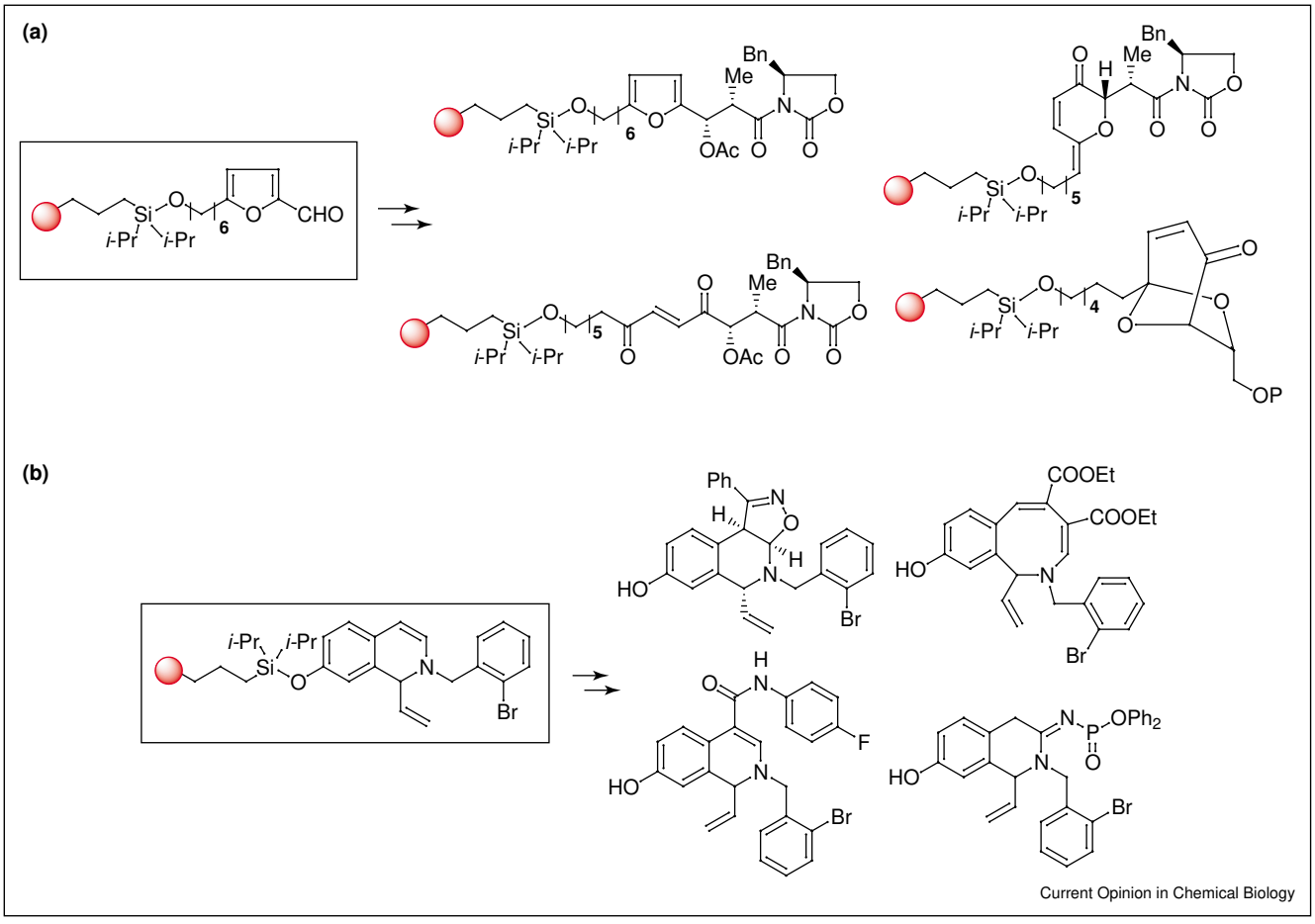
Using chiral Lewis acids, Schreiber and co-workers have demonstrated the importance of stereodefined functional diversity. The authors generated a library of different stereoisomers of 2H-pyrans using an enantiocontrolled methodology developed on solid phase. The authors tested their DOS-based library in search of small-molecule antagonists of transcription factors utilized in gene transcription [30•]. Transcription factors are known to be overactive in cancers, and disruption of such interaction with its target can be achieved by small molecules. From a library of dihydropyrancarboxamides, haptamide A (**5**) was found to have an IC_{50} value of 23.8 μM against Hap3p, a subunit of the yeast hap2/3/4 transcriptional factor complex. This study demonstrated that an *in vitro*, high-throughput screen of diverse small molecules can

Figure 2



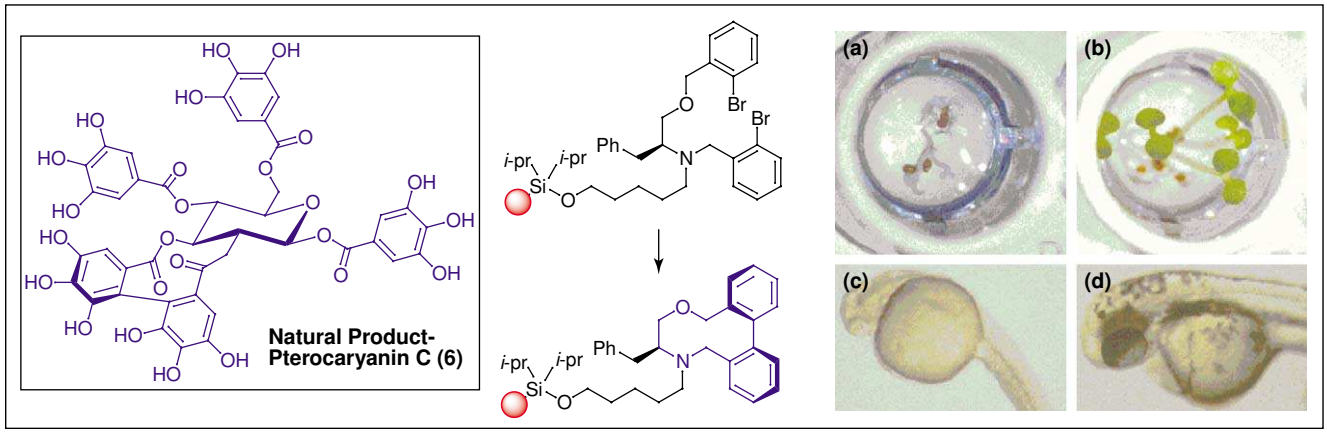
Kapoor and co-workers' solid-phase synthesis of HR22C16 analogues. **(a)** Schematic representation of the assay. Vertebrate cells (BS-C-1) in multi-well plates are stained for the actin cytoskeleton and imaged from below by using a 4X objective microscope. **(b)** The effect of a compound that causes no morphological change. **(c)** the effect of HR22C16. **(d)** Configuration of microtubules in a control cell (left) and a HR22C16 treated cell (right). At the top right is shown haptamide A **(5)**, a DOS-generated bioactive compound, reported by Schreiber and co-workers. It was found to target a transcription factor, Hap3p, a subunit of the yeast hap2/3/4 transcriptional factor complex. Parts of figure reproduced from [29] with permission. Copyright 2003, *Wiley-VCH*.

Figure 3



Approaches from Schreiber and co-workers using **(a)** a DOS approach to obtain skeletally diverse, natural-product-like compounds. and **(b)** an immobilized dihydroisoquinoline (reactive intermediate) to obtain skeletally different compounds.

Figure 4



Schreiber and co-workers' atropdiastereoselective, DOS approach to obtain a library of biaryl-containing medium ring-derived compounds. Plant and zebrafish development assays. **(a)** Seven days old *Arabidopsis* seedlings germinated on agar containing 1% DMSO and a small molecule library member (10 μm). **(b)** Seven day old control seedlings germinated on agar containing 1% DMSO. **(c)** Synchronized zebrafish embryos treated with a small-molecule library member (100 nM). Zebrafish are delayed developmentally; exhibiting lower than normal pigmentation, weak hearts, abnormal brains, and misshapen jaws. **(d)** Synchronized embryos treated with the same library member (5 μm). Zebrafish look indistinguishable to untreated controls. Parts of figure reproduced from [34•] with permission. Copyright 2002, the *American Chemical Society*.

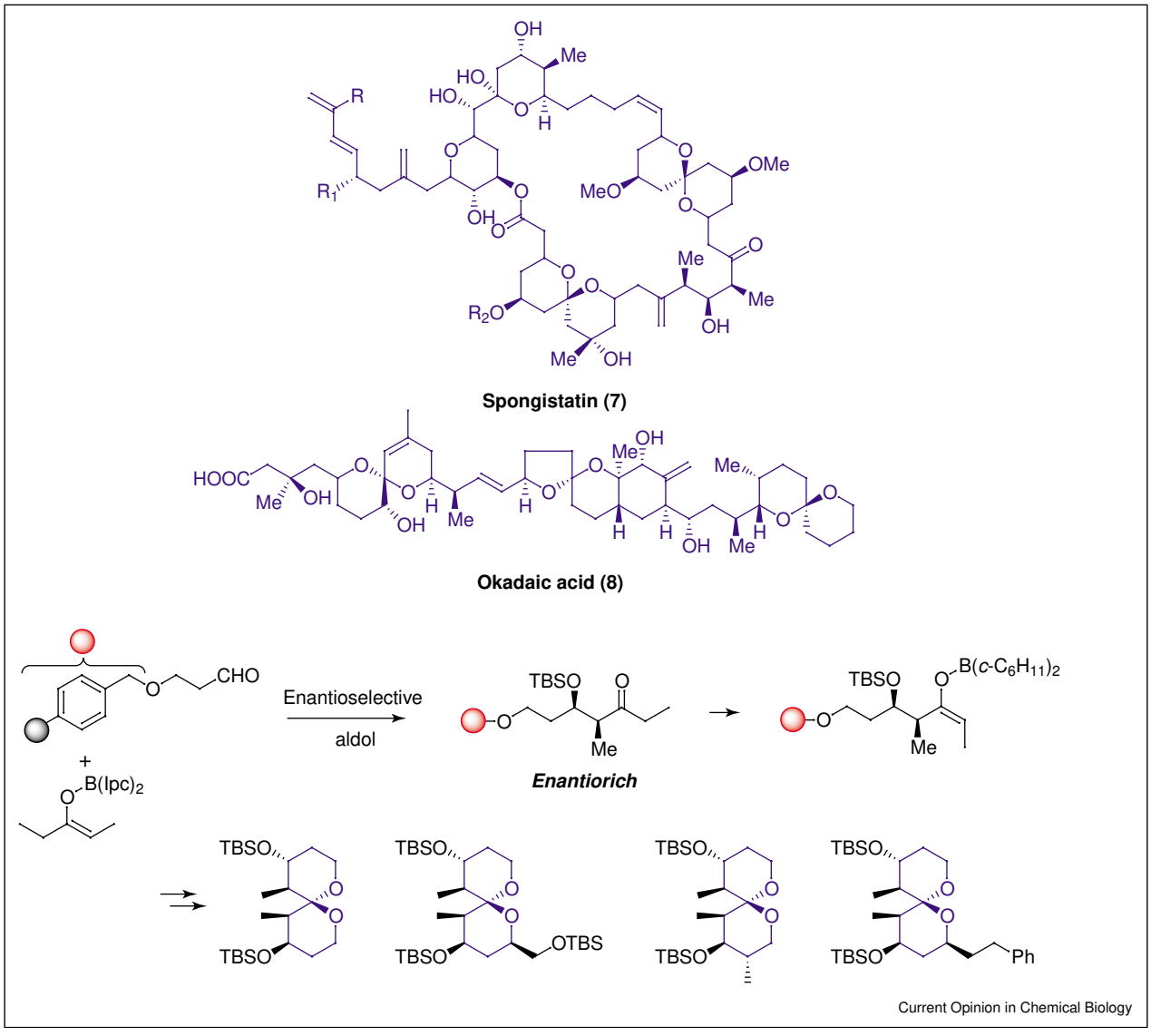
successfully target a subunit of a transcription factor and that 3D diversity is critical.

These results show that the presence of stereogenic functional groups and cyclic motifs in small molecules are crucial for dissecting macromolecular interactions. The potential of DOS-derived compounds can be endless; however, developing rapid access to skeletally diverse architectures is the real challenge in DOS.

DOS using simple starting materials

In the next generation of DOS pathways, there are two major aims. The first is to populate chemical space leading to complexity so that a common molecular skeleton is built using diverse building blocks. The second aim is to produce diverse molecular skeletons from simple building blocks. This second approach is utilized by Schreiber and co-workers such that a simple starting material, derived in few steps, is modified to generate a library of compounds in a divergent manner containing different skeletal shapes. The resultant molecules are populated with different groups and different shapes. The overall strategy is to perform combinatorial chemistry using DOS, leading to a diverse library. For example, Schreiber and co-workers have generated a collection of compounds representing many different molecular skeletons [31••,32]. Using the technique of split-and-pool synthesis, approximately 1260 compounds were synthesized, representing all possible combinations of building block, stereochemical and skeletal diversity elements (Figure 3a). Schreiber and co-workers have also described a new synthetic pathway using simple, readily available starting

Figure 5



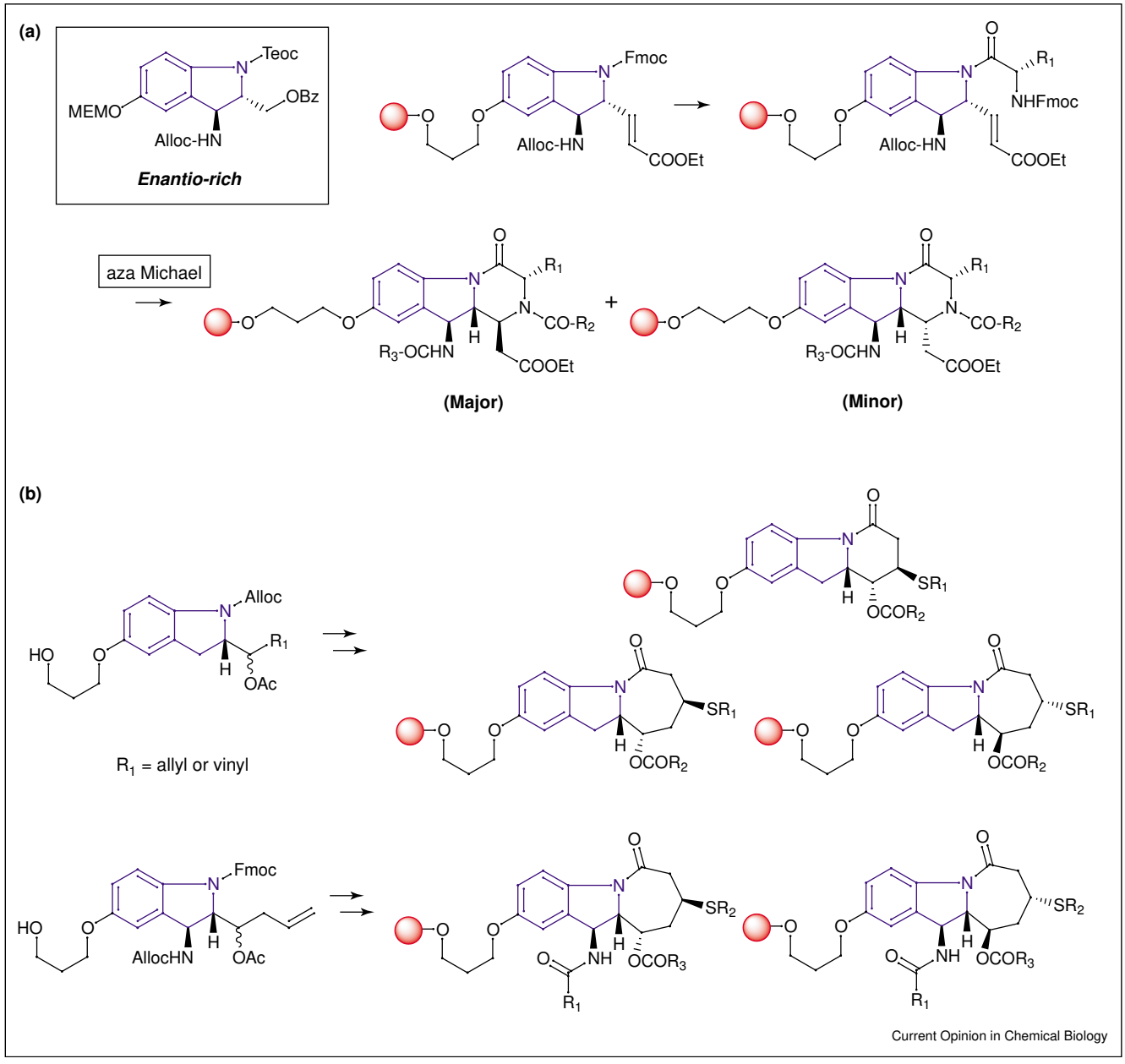
Waldmann and co-workers' solid-phase synthesis of a spiroketal library.

material to synthesize skeletally diverse alkaloid-like compounds [33]. Using DOS, dihydropyridines and dihydroisoquinolines intermediates were generated and subjected to further chemical treatment on solid support, producing skeletally diverse products. In three steps, 12 distinct skeletons were produced (Figure 3b).

DOS using a privileged scaffold

In this approach, a privileged scaffold (i.e. commonly found sub-structures in a wide variety of bioactive natural products) is used to perform DOS in a combinatorial manner. An example from Schreiber and co-workers involved the atropdiastereoselective synthesis of biaryl-containing medium rings using DOS [34•]. Pterocaryanin (**6**, Figure 4) is a natural product containing a biaryl-based medium sized ring. The library produced a maximum of 1412 theoretical members. The biology was also explored and protein binding assays in zebrafish and plant development studies produced unique developmental results, as shown in Figure 4. These developmental results will shed more light on the proteins involved at the molecular level.

Figure 6



Arya and co-workers' approach to obtain **(a)** a library of indoline-based, natural product-like, polycyclic compounds by a stereocontrolled aza Michael reaction, and **(b)** different natural product-like, polycyclic compounds by a ring-closing metathesis method on solid phase.

Waldmann and co-workers use the notion that a natural product (a validated structure) is a good starting point, and build a combinatorial chemistry programme around the privileged substructure present in the natural product. The authors recently described the solid-phase synthesis of 6,6-spiroketals, a natural product substructure [35•]. The 6,6-spiroketal is found in the natural products spongistatins and okadaic acid, and these natural products have been shown to possess biological activity. The authors reported the stereoselective solid-phase synthesis of 6,6-spiroketals (Figure 5), which proceeded in 12 linear steps. Waldmann's approach can be viewed as a structure–activity relationship on a natural-product scaffold using high-throughput combinatorial chemistry.

Arya and co-workers also utilize this approach, where a privileged scaffold (from a family of natural products) is used for DOS as the basis for populating the chemical space around the scaffold, leading to natural product-like compounds. For example, scaffolds such as the indole, indoline, aminoindoline and tetrahydroquinoline are found in natural products and are used for mapping the chemical space. This approach requires several steps of transformations. Using an enantio-rich aminoindoline scaffold, a library of 90 indoline alkaloid-like polycyclic derivatives was synthesized (Figure 6a) [36•]. This small-molecule library has yet to be screened for biological activity. Arya and co-workers have also used the indoline scaffold for the generation of both stereochemical and skeletal diversity, where six- and seven-membered ring-based polycyclic derivatives (Figure 6b) were synthesized (Reddy PT, Reayi A, Arya P, unpublished work).

Conclusions and future directions

In creating the ideal chemical probes, small molecules can be derived from different pathways depending on the source of the starting material and whether DOS (producing shape and skeletal diversity) is employed. In general, generation of a library of compounds by DOS is not directed towards a particular biological target. In this manner, unknown biological targets can be probed where the rational design of molecules is not possible. Because macromolecular interactions are complex, dynamic in nature and a challenge to understand, DOS is expected to provide the platform necessary to create complexity for the next generation of compounds. The overall aim is to generate 3D complex and diverse small molecules to explore their potential as highly specific binding agents and selective modulators of macromolecular interactions.

The field of organic chemistry is playing an increasing role in understanding structural and functional biology in a multidisciplinary approach. Because the events taking place inside a cell are extremely complex and difficult to investigate, our current knowledge of macromolecules involved in an organized and systemic manner is still lacking. To probe cellular interactions, experimental tools such as highly effective small molecules will be required and may be the only source for understanding macromolecular interactions, especially for poorly characterized or even unknown targets. The important criterion is to broaden natural product-like compounds with complexity while rendering the chemical space relevant to preserve biological significance.

Acknowledgements

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- •• of outstanding interest

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