Natural products: chemical instruments to apprehend biological symphony

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Received 23rd August 2007, Accepted 29th October 2007
First published as an Advance Article on the web 19th November 2007
DOI: 10.1039/b713022h

As a striking variety of biological activities are elicited by natural products, these chemicals have been used for decades to study biological phenomena. Understanding how these products interfere with normal cell functions at a molecular level led to a wide range of discoveries including new signaling pathways and proteins. Moreover, as natural products often act as chemical inhibitors, such studies often allow the identification of their binding partners as relevant targets for drug design. This article aims to emphasize how natural products or engineered analogs can be used as chemical tools to apprehend some biological problems from the point of view of a chemical biologist.

1 Introduction

While much has been written about the biodiversity of our planet, the chemical diversity produced by wildlife on Earth nevertheless remains impressive. Hence, natural products have long been attractive tools for cell biologists. These molecules are often synthesized by organisms in response to stress and can exhibit a plethora of biological activities. As a consequence, clinical, pharmacological and chemical studies of natural products, mostly derived from plants, were conducted as early as the beginning of the 19th century, and molecules such as aspirin, digitoxin, morphine, quinine, streptomycin, chloramphenicol, erythromycin and vancomycin, which have been described decades or centuries ago, continue to be used as drugs.[1]

Natural products are often isolated based on a potent biological activity without the knowledge of how that biological activity is elicited at a molecular level. However, in the last decades studies of their activity have elucidated the cellular mechanisms of many such products (Fig. 1). This review does not aim to provide an exhaustive list of pathways in which natural products interfere; it rather intends to assess how natural products can be used as molecular probes to the discover important biological processes, validate target proteins for drug treatment, and be subsequently modified to address specific problems.

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Mathieu Pucheault was born in Villeneuve St Georges (France) in 1979. After graduating form the Ecole Normale Supérieure (Paris, France), he joined the group of Prof. J.-P. Genet, working in the field of organometallic catalysis where he got his PhD degree in 2004. During a post doctoral stay in Dr C. M. Crews' group at Yale University (New Haven, USA), he studied the degradation of proteins in cells induced by small molecules, supported with a HFSP Cross Disciplinary Fellowship. Since 2006, he is a permanent researcher (CNRS) in Rennes (France), where his research interests cover various areas including sustainable chemistry (use of onium salts as scaffold for synthesis), boron chemistry and chemical biology (chemical tools for identifying protein interactions).

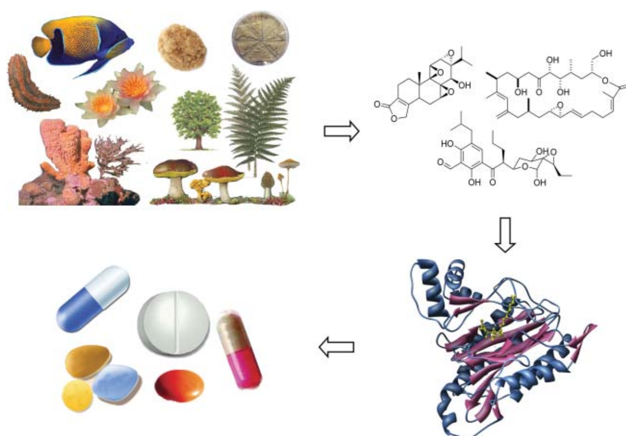

Fig. 1 Chemical genomics approach to a biological problem leading to drug development.

Structurally defined, natural products can be used directly to interfere with biological functions. Upon binding to their natural target(s), they perturb normal cascades of events in cell leading to (an) abnormal macroscopic readout(s).[2] This perturbation method has proved to be useful in understanding protein function in the past, and it will undoubtedly continue to play an even larger role in the post-genomic area. Indeed, now that the sequence of the human genome is available, understanding the protein function within complex intracellular networks represents one of the major challenges facing cell biologists today.

The use of small molecules to explore cell biology offers several advantages over traditional genetic approaches. First, biologically active natural products can serve as reversible "conditional alleles", and therefore be used to mimic lethal mutations or inhibit protein function at specific points during the cell cycle

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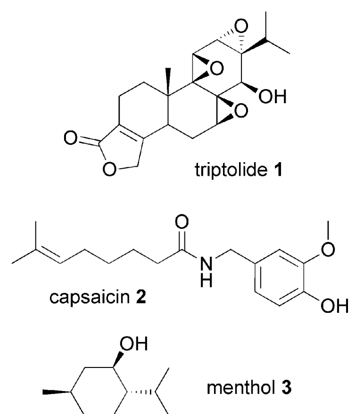
or developmental process. Thus, this 'chemical genetic' approach provides new tools to investigate protein function and signaling events within a short temporal window and therefore addresses problems that would be difficult to solve *via* more traditional genetic methods.[3,4]

2 Natural products as molecular biology tools

The first question regarding the interest of a potent natural product is related to its *in vivo* relevant target. In many cases, the global macroscopic influence of a given molecule can be explained by protein function interference, whether it is elicited at a molecular level by an enzyme inhibition/overactivation or by protein–protein interaction disruptions. If for many natural products standard tests can give a good apprehension of the identity of the protein involved, some cases appeared trickier and allowed for the discovery of entirely new families of protein (*i.e.* ion channel modulators, see section 2.1). As a result of new protein identification, studies of the mode of action of natural products can lead to draw out a complete signaling pathway (*i.e.* TOR signaling pathway, section 2.2). Along with finding new pathways, some biological phenomena can even be unraveled, such as angiogenesis where the use of small molecules for disrupting the process turned out to be critical (section 2.3)

2.1 Identification and characterization of unknown proteins

The example of ion channel modulators. Using small molecules and trying to understand their activity at a molecular level has often allowed new proteins or new functions for known proteins to be discovered; such was the case for triptolide **1** (Scheme 1). Triptolide **1** is a diterpene triepoxide that was originally isolated from the traditional Chinese medicinal vine *Trypterygium wilfordii.*[5] Its anti-inflammatory effects have been known for several centuries and have recently been attributed to the inhibition of NF-κB transactivation.[6] However, unlike other NF-κB pathway inhibitors previously described triptolide **1** blocks this signaling cascade after DNA binding, demonstrating a different mode of action. More recently, studies have shown strong calcium dependence for the antiproliferative activity of triptolide and revealed multiple possible modes of action *in vivo.*[7]



Scheme 1 Ion-channel modulators.

The TRPC calcium channel family member, polycystin 2, was found to be associated with a [^{3}H]-labeled triptolide binding activity and might be a triptolide binding protein. Polycystin 2 function is required for mechanosensation-induced cell cycle arrest during kidney formation. Interestingly, triptolide **1** was shown to induce a Ca^{2+} flux *via* a polycystin-2-dependent mechanism[5] and also to block cyst formation in a murine model of polycystic kidney disease. Natural products also played a crucial role in the identification and characterization of thermo-sensitive ion channels.[8] For example, the mechanism through which capsaicin **2**, the pungent ingredient of hot chili peppers, elicits a hot sensation remained unknown for many years[9] until its receptor was cloned and named transient receptor potential vanilloid 1 (TRPV1).[10] Additionally, this receptor was shown to be a heat-activated ion channel involved in pain sensation, opening a new field of research on temperature-dependent ion channels.[11] Similarly, menthol **3** was found to activate the cold-sensitive receptor TRPM8 in a comparable manner as its natural stimulus.[12] TRPM8 was later shown to be permeable to ions (Na^+, K^+, Ca^{2+} or Ba^{2+}) under both natural and provoked stimuli.[13]

2.2 Identification of signaling pathways

Immunosuppressive properties of rapamycin unravel TOR signaling pathway. It was recently discovered that the molecular mechanisms through which cyclosporine A **4** (CsA), FK506 **5**, rapamycin **6** and sanglifehrin A **7** (Scheme 2) suppress the immune response is by inhibiting conserved signaling pathways.[14] Elucidating their modes of action has provided great insights into the understanding of the mechanisms leading to T-cell activation.[15] These natural products have been shown to associate with their intracellular receptors, immunophilins, and form complexes which subsequently bind their target protein, cyclophilin for CsA **4** and calcineurin for FK506 **5** in a complex with its binding protein FKBP.[16] Inhibition of this calcium-dependent phosphatase prevents dephosphorylation of transcription factor NFAT, which is required for its translocation into the nucleus. The normal NFAT function in regulating cytokines gene expression among other genes is thereby inhibited, leading to a general immunosuppressive activity.[17]

Studies on the mode of action of rapamycin **6** have revealed a new signaling pathway which is central to cell growth control (Fig. 2(a)).[18] Despite structural similarities with FK506 **5** (Scheme 2), rapamycin **6** was shown to bind to mTOR (mammalian Target Of Rapamycin), a member of the phosphatidylinositol kinase-related kinase (PIKK) family. This large (~280 kDa) protein subsequently appeared critical for regulation of two major events regarding cell growth: when and where cells grow.

When conditions are favorable, TOR initiates anabolic processes and disables catabolic processes. However, only TOR2 controls the spatial aspects of yeast cell growth which, intriguingly, appears to be insensitive to rapamycin. In mammals, mTOR regulates cell growth through the PI3K pathway responding to exterior stimuli such as growth factors, nutrients, energy or stress. For example, amino acid starvation induces dephosphorylation of mTOR1 effectors; AMP-activated protein kinase inhibits mTORC1-dependent phosphorylation depending on the AMP/ATP ratio in cytosol; protein synthesis is downregulated under hypoxic conditions *via* inhibition of TOR signaling pathway at different levels. Downstream events of TOR are critical for cell survival and growth including regulation of translation (through

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Scheme 2 Natural products with immunosuppressive properties.

Fig. 2 Rapamycin: (a) simplified scheme of mTOR signaling pathway, (b) activation of gene expression induced by a natural product, (c) conditional protein splicing, (d) analog design using "bumped" ligand-"hole" protein strategy.

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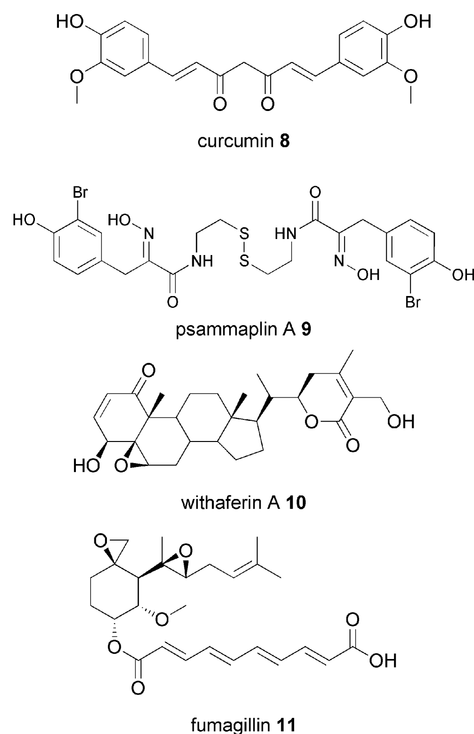
the control of phosphorylation of S6K1 and 4E-BP), ribosome biogenesis (by regulating RNA Polymerase I gene transcription), macroautophagy (*via* the inhibition of ATG1), transcription (through transcription factors UBF and TIF1A), metabolism (such as glucose homeostasis and amino acids synthesis) and actin organization. All these essential functions in cells have been clarified thanks to the use of rapamycin **6**. This molecule additionally turned out to be a critical tool for studying molecular biology as it induces the formation of a ternary FRB–rapamycin–FKBP complex (Fig. 2).

2.3 Identification of biological processes

Hijacking angiogenesis. Angiogenesis or the formation of blood vessels is essential for various processes such as wound healing, tumor growth and metastasis.[19] In response to numerous regulators such as vascular endothelial growth factor (VEGF), endothelial cells in preexisting blood vessels degrade their basement membrane, migrate, proliferate and ultimately form new blood vessels. Inhibiting any of these steps has proved to be effective against angiogenesis. The molecular binding partners of natural products known to have anti-angiogenic activities are thus attractive targets for drug development (Scheme 3).



Scheme 3 Natural products with anti-angiogenic activities.

For example, curcumin **8**, a component of the food flavor turmeric (*Curcuma longa*), inhibits proliferation of various tumor cell lines; and its target has recently been identified.[20] Curcumin **8** binds to CD13/Aminopeptidase N (APN) with a low micromolar affinity and irreversibly inhibits its activity. APN was subsequently validated as a target for chemotherapeutic treatment: curcumin was shown to specifically inhibit APN-positive tumor cell invasion and growth factor induced angiogenesis; it has no significant effect on APN-negative tumors. APN inhibition was thought to also lead to the downregulation of Hypoxia-Inducing Factor-1 (HIF-1) in hypoxia induced angiogenesis.[21] APN is also a target for another anti-angiogenic agent, marine natural product psammaplin A **9**, which was shown to inhibit APN with an IC_{50} of 18 μM.[22]

Withaferin A **10**, a compound which was originally isolated from the medicinal plant *Withania somnifera*, is known for its anti-inflammatory and its cardioactive effects, as well as for its effects on the central nervous system. Its antitumor properties were discovered back in 1970,[23] but more recently a new mode of action has been elucidated. Indeed, at a 500-fold lower dose than for its antitumor activity (IC_{50} = 12 nM), it exerts a strong anti-angiogenic effect *in vivo*, confirming a possible second mechanism of action for this natural product.[24] Withaferin A **10** is thought to inhibit NF-κB in HUVEC cells by interfering with the ubiquitin proteasome pathway and therefore preventing ubiquitination of IκBα. However its true target protein is still unclear despite efforts to synthesize affinity probes.[25]

One of the most potent antiangiogenic compounds, fumagillin **11**, was isolated from an *A. fumigatus fresenius* colony contaminating an endothelial cell culture in the Folkman laboratory.[26] The efforts of medicinal chemists subsequently led to the identification of an analog (TNP-470) with increased potency, which is now used in antitumor clinical trials. The molecular target of fumagillin **11** was identified in 1997 using a biotinylated fumagillin **11** affinity reagent: the intracellular metalloprotease Methionine AminoPeptidase 2 (MetAP-2) is covalently inhibited by fumagillin.[27] A bond between fumagillin **11** and the catalytic site of MetAP2 is generated by the nucleophilic addition of HIS231 to one of the two epoxides present on the natural product (Fig. 3). The loss of angiogenesis in MetAP-2 knockout mice confirms MetAP-2 to be an important anti-angiogenic target.[28] Moreover, a recent study has shown that fumagillin **11**/TNP-470 are potent inhibitors of the non-canonical Wnt signaling pathway.[29]

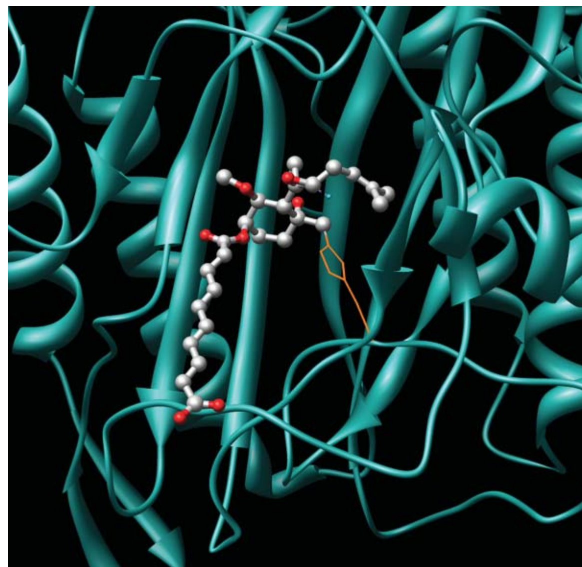


Fig. 3 Fumagillin covalently bound to MetAP-2 HIS 231[30] (PDB 1BOA).

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3 Natural products validate strategy and targets for drug design

Making a connection between molecular targets and activities is essential for medicinal chemistry and ultimately relies on understanding how the natural product activity is elicited at the molecular level. For example, antiproliferative compounds that have a unique mode of action can give alternative strategies for treatments (section 3.1) such as inhibiting transcription (HDAC inhibitors) or translation (protein synthesis inhibitors). Alternatively, when the natural product target is clearly identified as a protein or a family of proteins, one can use complementary tools such as molecular modeling and traditional medicinal chemistry for achieving a better drug design. For example, conceiving proteasome inhibitors is now one of the most promising strategies for developing antiproliferative drugs thanks to the use of several natural products which have been shown to inhibit proteolysis upon binding to the proteasome (section 3.2.).

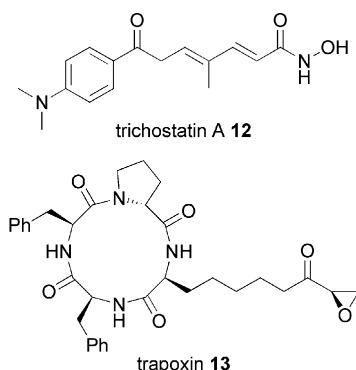
3.1 Treatment strategy validation

Tackling cancer at the transcription level with HDAC inhibitors. As mentioned previously, the understanding of how natural products affect normal biological processes at the molecular level has often guided new strategies for drug design. Strikingly, studies on the regulation of gene transcription led to the development of an entire new family of anticancer molecules, inhibitors of Histone DeACetylatylases (HDACs, Scheme 4). Indeed, access of transcription factors and RNA polymerase to specific chromosomal *loci* is regulated by chromatin structure which is itself controlled *via* the modification of histones, protein components of chromatin. Much of our knowledge of how histone modification controls chromatin structure has come through the use of natural products that inhibit HDACs.[31] Trichostatin A **12** (TSA) was initially isolated from *Streptomyces hygroscopicus* in 1976[32] on the basis of its antiproliferative activity. Namely, exposing ovarian cancer cells to TSA **12** induced a change in cell morphology, differentiation and proliferation.[33] This effect has been shown to be associated with changes in p21$^{CIP/WAF}$, retinoblastoma protein (Rb) and Id proteins induced by the specific inhibition of HDACs by TSA **12** with an IC_{50} of 3.4 nM.[34] A more detailed analysis of the pathway showed the overexpression of the CDK inhibitor p21 $^{CIP/WAF}$ leads to a decrease in Rb phosphorylation. TSA **12** was also shown to suppress growth in pancreatic adenocarcinoma cells by stopping the cell cycle in G_2 phase and inducing apoptotic cell death.[35] As is the case in ovarian cancer cells, levels of p21 $^{CIP/WAF}$ were shown to be strongly increased in pancreatic adenocarcinoma cells[36,37] confirming that HDAC inhibition is a potential therapeutic strategy for cancer treatment.

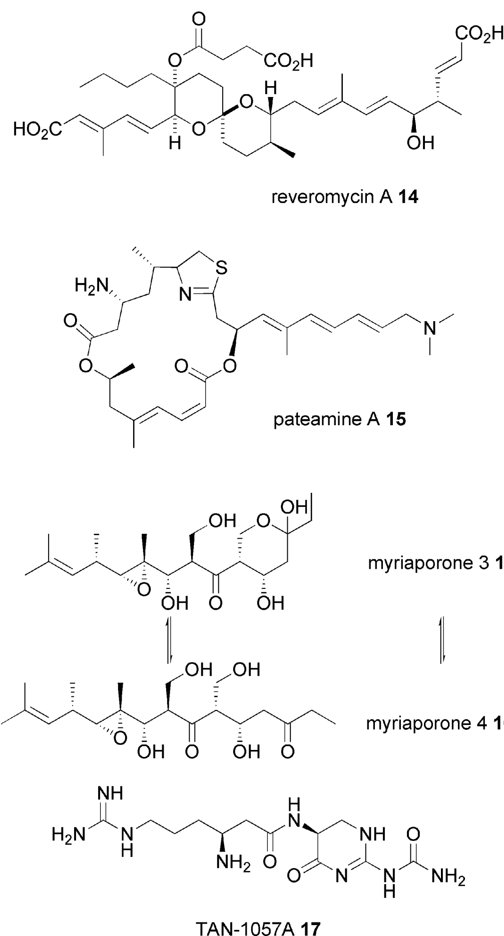
Similarly, trapoxin **13** (TPX) is a cyclic tetrapeptide that was reported to induce morphological reversion in v-*sis*-transformed NIH3T3 cells. It was through TPX's effect that the first HDAC was identified. The activity of TPX **13** is similar to that of TSA **12** except that it is irreversible.[38] HDAC inhibition was subsequently confirmed to be relevant to the mode of action of TPX **13** and the epoxide moiety of TPX **13** was found to be critical for enzyme inhibition.[38]

Traptoxin **13** and trichostatin A **12** have proven useful in the exploration of HDAC function in gene regulation as well as differentiation processes.[39] However, despite their low affinity and the appealing strategy for anticancer therapy, their intrinsic instability has prevented them so far from be used directly as drugs although synthetic analogs are currently developed for therapeutic applications.[31,40]

Protein synthesis inhibition. Another similar strategy for inhibiting protein function simply relies on preventing their synthesis. This tactic has been developed thanks to natural products such as reveromycin **14**, pateamine A **15** or myriaporone **16** (Scheme 5). For example, reveromycin A **14** has diverse biological activities that include morphological reversion of *src*^{ts}-NRK cells



Scheme 4 HDACs and protein synthesis inhibitors.



Scheme 5 Protein synthesis inhibitors.

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from spherical transformed cells to flat shaped cells without cytotoxicity (EC_{50} = 1.58 μg mL^{-1}),[41] antifungal activity and antiproliferative activity against human tumor cell lines (IC_{50} = 1.3 μg mL^{-1}).[42,43] These biological activities have been shown to be mediated by reveromycin *via* inhibition of isoleucyl-tRNA synthetase (IC_{50} of 1.3 ng mL^{-1}) resulting in loss of protein synthesis.[44] Similarly, TAN-1057A **17**, another natural product, particularly effective against Gram positive organisms including *Staphyllococcus aureus*, is believed to target the 50S ribosomeal article and thus blocking translation with an inhibitory effect that is comparable to the classic antibiotics erythromycin and clarithromycin (IC_{50} = 4.5 μg mL^{-1}).[45,46]

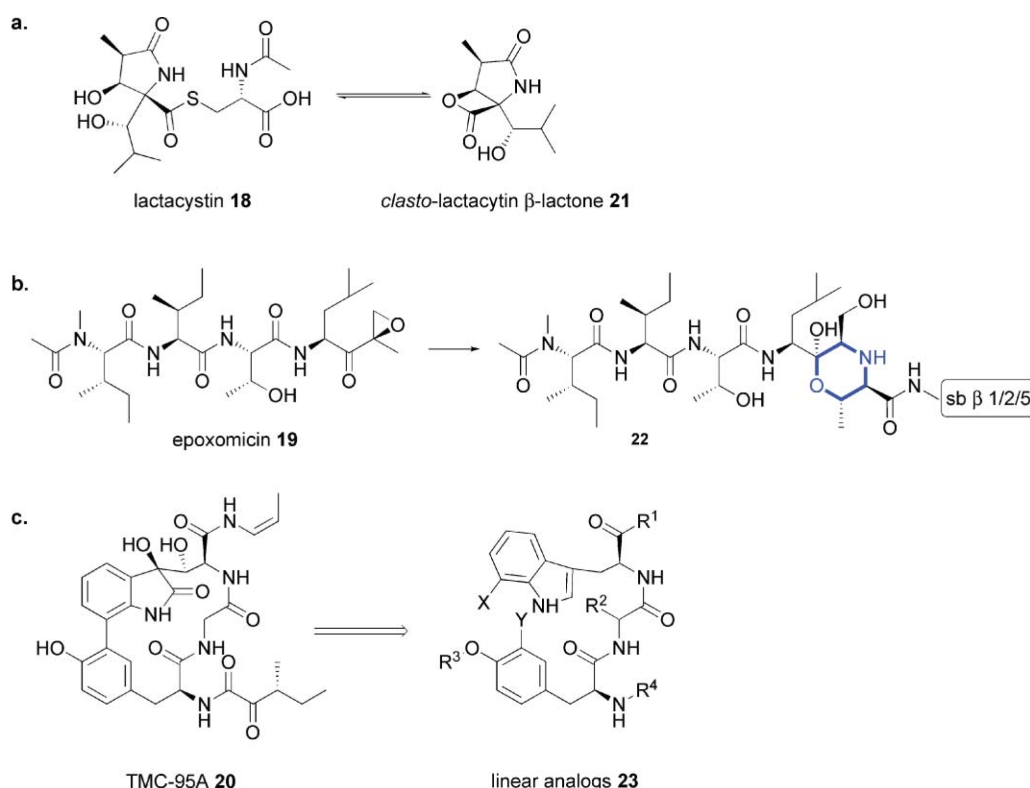
Recently, two marine natural products have been shown to inhibit eukaryotic protein synthesis in unique ways. Pateamine A **15** is an antiproliferative and antitumor marine natural product[47,48] that targets elF4A, a part of the elF4F complex responsible for recognition of mRNA at the 5′ cap. By binding to elF4A, pateamine A **15** causes the stalling of initiation complexes on mRNA *in vitro* and induces stress granule formation *in vivo*.[49] These results suggest pateamine A **15** should be useful in the study of eukaryotic translation initiation. Another marine natural product, myriaporone 3/4 **16**, isolated from the Mediterranean false coral *Myriapora truncata*, also has been potent antiproliferative activity.[50] It has recently been shown that in mammalian cells, myriaporone **16** exhibits low nanomolar reversible and rapid inhibition of protein synthesis and cell proliferation independent of p21 activity by blocking the cell cycle in the S phase. Given the antiproliferative activities of myriaporone **16** and pateamine A **15**, these natural products could serve as the starting points for the development of novel antitumor drugs.

3.2 Target validation of drug treatments

Proteasome inhibitors. Among other pathways, intracellular protein turnover is a regulated by the ubiquitin-dependent proteasome pathway. In this pathway, proteins targeted for degradation are first labeled with chains of ubiquitin, a highly conserved 76 amino acids protein, before being recognized and degraded by the 26 S proteasome. The 26 S proteasome plays a key role in important cellular processes such as apoptosis, cell differentiation, NF-κB activation, tumor suppression and cell division. This protein complex is composed of two 19 S regulatory particles which cap the central 20 S proteolytic core. Given the central role of the proteasome in cell cycle regulation, proteasome inhibition has attracted much interest recently as an anti-proliferative chemotherapeutic strategy[51–53] and natural products such as lactacystin **18**, epoxomicin **19** or TMC-95A **20** (Scheme 6)[54] have been critical in this line of research.

Lactacystin **18**, a secondary metabolite isolated from a *Streptomyces* strain, was first identified based on its ability to inhibit cell cycle progression and induce differentiation in a murine neuroblastoma cell line. Lactacystin **18** was subsequently shown to covalently bind to the N-terminal threonine of the 20 S proteasome[55] *via* a *clasto*-lactacystin β-lactone intermediate **21** (Scheme 5(a)), which irreversibly modifies all catalytic β subunits.[56]

The full therapeutic potential of proteasome inhibition was recognized when the antitumor natural product epoxomicin **19** was identified as a proteasome inhibitor. It was first isolated from an *Actinomycetes* strain based on its ability to inhibit cell division in M16 murine melanoma tumors. Epoxomicin **19** has been shown to inhibit proinflammatory signaling as well as plant cell wall



Scheme 6 Proteasome inhibitors: intermediates and analogs.

synthesis.[57] Interestingly, the unique proteasome specificity of epoxomicin's epoxyketone pharmacophore was elucidated upon determination of the structure of epoxomicin bound to the 20 S proteasome.[58,59] The co-crystal structure revealed that an unexpected morpholino ring derivative **22** (Scheme 4(b)) forms upon condensation of both the nucleophilic side chain's hydroxyl groups and the aminoterminus of Thr1.[60] Unlike most peptide-aldehyde proteasome inhibitors, epoxomicin **19** is selective and does not inhibit non-proteasomal proteases such as trypsin, chymotrypsin, cathepsin or papain. *In vitro* and *in vivo* anti-inflammatory activities have been attributed to interference with the NF-κB signaling pathway. By preventing the ubiquitin-dependent degradation of IκBα, epoxomicin **19** blocks NF-κB translocation into the nucleus and therefore inhibits pro-inflammatory cytokine-mediated gene transcription.[61]

TMC-95A **20**, a cyclic modified peptide isolated from *Apiospora Montagnei*, has also been shown to have antitumor and anti-inflammatory activities. Indeed, this natural product inhibits the 20 S proteasome activity albeit without covalently binding to the β subunits. A crystal structure of TMC-95A **20** in the 20 S core particle[62] has provided a better understanding of the binding mode of this natural product and provided insights for design of new proteasome inhibitors (Scheme 4(c)).[63–65]

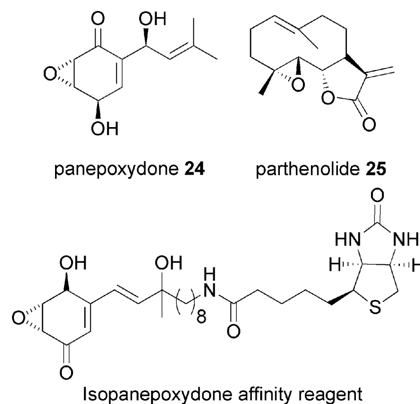
4 Where natural products are modified and improved to continue their journey

The main advantage of natural products is their low molecular weight and ease of use. But after the initial discovery of their target, natural products often appear too limited for further analysis or unsuitable for drug treatment. This is where chemistry allows the design of tailor-made probes to address specific issues. A great deal of effort has been made in the last decade to synthesize chemical derivatives of natural products derivatives, and use them as probes in cell biology.

A bi-functional molecule can be generated where a light reactive (typically fluorescent) is juxtaposed to a natural compound. Fluorescent probes (Table 1) provide useful preliminary information relative to the cellular location of the natural product. It is usually one of the first steps to understand the biological function of a small molecule. Furthermore, using two-photon excitation fluorescence microscopy[66] precise 4D images of tissues can be obtained. This basic strategy has been tremendously developed including by using photoaffinity probes.[67]

A now commonly used tactic for identifying target of small molecules is to synthesize an affinity based probe (Table 1). This heterodimeric molecule binds on one end to a given protein (typically streptavidin when biotin is used) while the other one is recruiting its target. Starting from this simple concept, a wide range of applications can be envisioned from simple analysis to protein purification using polymer-immobilized avidin chromatography column. If these approaches are often limited to *in vitro* applications due to biotin poor membrane permeability, alternative strategies can be developed especially when the natural product is covalently bound to its target. In this case the biotin moiety can be attached to the assembly in a second stage (after cell lysis). Finally, the biotin/(strep)avidin pair is undoubtedly the most widely used system although other affinity reagents can target other proteins and be used for specific problems.[68]

As an example of this technique, synthesizing an affinity reagent derived from the natural product panepoxydone **24** (Scheme 7) unraveled the mechanism through which this fungal metabolite displayed anti-inflammatory activity. Indeed panepoxydone **24** has been shown to interfere with the NF-κB signaling pathway by inducing higher molecular weight species of IκBα.[69–71] However, other natural products have also played an important role in the exploration of the NF-κB signaling pathway; in this mechanism IKK is phosphorylated leading to the ubiquitin dependent proteolysis of IκB and subsequent NF-κB translocation in the nucleus. Inhibition of NF-κB transcription activity results in the downregulation of genes involved in inflammation. Parthenolide **25**, an anti-inflammatory sesquiterpene lactone isolated from the medicinal herb Feverfew (*Tanacetum parthenium*) has been



Scheme 7 Inhibitors of NF-κB signaling pathway and affinity reagents.

Table 1 Natural product derivatized heterobifunctional molecules for chemical biology applications

	Fluorescent probe	Affinity based probe	PROTAC
Typical probe[a]	Nat P — Fluo	Nat P — Biotin	Nat P — E3LBM
Main applications	Imaging, analysis	Purification, analysis	Chemical genetics
(+)	*In vivo* application, multicolor labeling, 4-D imaging	Structural diversity, wide applicability, cheap	*In vivo* application, chemical analog of RNAi, catalytic, virtually applicable to all proteins
(−)	Expensive, no direct proof of target	Mainly restricted to *in vitro* application	Sensitive to proteolysis, high molecular weight

[a] Fluo: Fluorescent moiety; E3LBM: E3 Ligase Binding Molecule.

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shown to covalently bind to IKKβ *via* cysteine 179 and inhibit its kinase activity.[72] IκBα is therefore stabilized and prevents NF-κB translocation into the nucleus. The epoxyquinone A monomer is structurally similar to panepoxydone and it has the same mechanism of inhibition of NF-κB activity. However, it has been shown that the epoxyquinone A monomer blocks two molecular targets, IκB kinase IKKβ and NF-κB subunit p65.[73] Moreover, in IκBα deficient cells, the epoxyquinone A monomer has been shown to induce apoptosis by directly inhibiting the DNA binding activity of transcription factor Rel, an NF-κB subunit.[74]

Finally, a unique approach using natural product derivatives has recently been described by Craig Crews' group. The PROTAC (for PROteolysis TArgeting Chimera, Table 1) strategy relies on artificially generating a special proximity between the target of a natural product and an E3 ubiquitin ligase as part of a bigger complex of proteins (with E2 conjugating enzyme and ubiquitin). Upon formation of this complex, ubiquitin is transferred to the protein target, which is subsequently recruited for degradation by the proteasome.[75] After an initial *in vitro* proof of principle,[76,77] the same group managed to induce the selective degradation of FKBP12 in cells using PROTAC with a heterodimeric molecule which binds to VHL through a peptidic moiety bearing a hydroxylatable proline at one end, and FKBP12 *via* the so-called "bumped ligand" at the other end.[78] This strategy provides a nice tool to selectively remove a protein from the entire proteome without otherwise affecting the cell. Moreover, degradation of the target protein can be regulated in time. This strategy also allows the cellular concentration of the target protein to be regulated. It does not require any genetic modification and can target structural proteins as well as enzymes.

5 Conclusions

Clearly, chemistry tools can be quite helpful to unravel the complexity of biological processes. This article aimed to employ a few specific examples to underline how rational design and synthesis of natural products analogs or probes led to critical progress in understanding biology. For instance, a wide range of chemical dimerizers and three hybrid systems has now been developed allowing the specific activation/inactivation of biological processes without requiring invasive methods.[79] Rapamycin **6** was widely used as a chemical scaffold around which FRB and FKBP associate to give a ternary complex.[80] This trick allowed great insight into activation of gene transcription (Fig. 2(b)),[81] receptor signaling,[82] induction of protein splicing (Fig. 2(c))[83,84] and recently recruiting protein for degradation similarly to the PROTAC strategy.[85] Additionally, by designing mutant FKBP and rapamycin-like molecules, the affinity and specificity of the interactions can be increased by creating a lock in the binding pocket of FKBP corresponding to a key on the chemical analog (Fig. 2(d)).[86,87] As illustrated here, natural products and their mode of action studies have proven to be instrumental in the exploration of many areas in cell biology.

The journey of natural products doesn't end with biological activity. Indeed, biologically active natural products provide a means by which intracellular functions can be chemically perturbed, and, as such, combined with more classical molecular biology techniques to investigate cellular processes. In addition, current efforts in the design and generation of natural product-like libraries of compounds acknowledge the inherent advantages of using natural products as the basis for drug development.[88] Further studies on theses products mode of action often lead to striking discoveries and open widely new fields of research in stimulating generations of chemical biologists.

Importantly, the rational design of the molecular instruments resulting from natural products studies is critical and despite a lot of synthesis efforts, a poor design will inevitably lead to failure. Nonetheless as shiny as they could appear, chemical tools, natural products, probes, affinity reagents are not the ultimate answer to all biological problems either. Luckily for scientific creativity, we are still far from having in our hand a method that could tell us the relevant procedure for studying any given system. If chemistry and biology have been playing together for millennia, the arsenal of techniques available in the biologists' toolbox has recently beneficiated of interdisciplinary connections. Whether serving as cell biology probes, the basis for therapeutic development or as inspiration in the design of novel compound libraries, natural products and their biological activities will undoubtedly continue to surprise and aid researchers for many years to come. It's probably fair to say that chemistry radically changed our way of understanding and tackling biology questions as much as molecular biology modified apprehension of medicinal issues in the second part of the last century.

Acknowledgements

I would like to thank earnestly Craig M. Crews for his strong support and countless fruitful discussions. I am also grateful of the Human Frontier Science Program Organization for supporting my research with a cross-disciplinary fellowship.

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