

3.08 Natural Products Drug Discovery

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3.08.1 Introduction

Over the past two decades natural products drug discovery has been increasingly de-emphasized by pharmaceutical companies. Although heralded on the verge of a comeback several times,^{1,2} attention for natural products has so far failed to materialize in big pharmaceutical companies (pharma), remaining relegated, with a few exceptions, to small biotech companies. Many review articles have analyzed this issue, pointing out the past success of natural products in drug discovery and their big and still largely untapped potential to provide new drugs for a host of unmet medical needs.^{3–19} In this chapter, we will attempt to analyze from a pharma perspective why natural products have fallen out of favor in drug discovery despite their intrinsic utility for biomedical research. After an introduction on the current state of drug discovery, the reasons for the inbuilt utility of natural products for biomedical research will be highlighted, attempting next to explain why, despite so many advantages, it is so difficult for mainstream drug discovery to interface with natural products research. Finally, several strategies to improve natural products drug discovery and make it more efficient and attractive from a pharma side will be discussed.

3.08.2 The Current Pharmaceutical Scenario

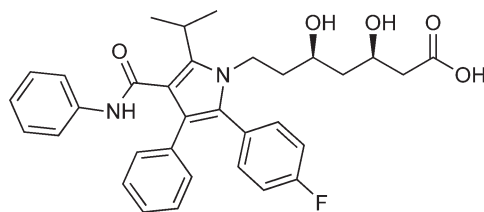
A byproduct of the late-19th-century chemical business, pharmaceutical research thrived for more than a century by finding chemical combinations to treat diseases. But, after contributing substantially both to human health and drug-industry profits, it has failed to produce significant innovations in recent years.

A. Johson, *Wall St. J.* 12 December 2007

Despite the introduction of a wealth of ingenious and innovative strategies to help and/or direct drug discovery (combinatorial chemistry, diversity-oriented synthesis, fragment-based drug discovery, chemical biology, *in silico* screening),²⁰ the number of new chemical entities (NCEs) reaching the pharmaceutical market has suffered a downward trend over most of the past decade. To explain this dismal performance, a host of arguments have been proposed, from the shortage of low-hanging drug fruits to the suitability of the much-hyped modern techniques to pick the higher-hanging fruits identified by genomics in the drug orchard.²⁰ Thus, aminergic G-protein coupled receptors (CPCRs) are relatively easy, highly druggable targets since their endogenous ligands are small molecules (serotonin, dopamine, adrenaline, histamine), while interfering with protein–protein interactions requires larger and more lipophilic agents and this inflation of physical properties promotes binding to unwanted targets and raises problems regarding absorption, distribution, metabolism, and excretion (ADME).²⁰

Depending on the therapeutic area, the capital needed to bring a drug to the market has now skyrocketed to \$0.8–1.7 billion, with cost splitting (breakdown) being approximately 10% for discovery, 15% for preclinical, 15% for manufacturing and process, 55% for clinical trials, and 5% for postmarketing.²¹ Since the attrition rate of clinical development is currently estimated at an appalling 93–96%,²² a market potential of at least \$300 million is considered the lowest limit for a big pharmaceutical company to be interested in a product.²³ This combined with the lengthening of the R&D time, currently at around 12 years, is responsible for the ‘blockbuster-itis’ that is plaguing the pharmaceutical industry.

Faced with a looming and massive patent cliff in the first half of the next decade and with an arthritic drug pipeline, drug companies have been increasingly relying for innovation on biological big molecules (monoclonal antibodies, vaccines, and nucleic acids) and techniques (stem cells). Developments in this area have been remarkably fast. Thus, the first paper describing RNA interference (RNAi) gene silencing in mammal cells was published in 2001,²⁴ but six therapeutic programs based on this concept had already moved to clinical trials in 2007,²⁴ and also cutting-edge genetic tools like small inhibitory RNAs (siRNA) made the leap to drug candidates in record time.²⁴ Compared to small molecules, biologics enjoy a longer exclusivity, since clinical trials of bioequivalence are generally needed for generic versions (biosimilars),²⁵ but have higher costs of development and manufacturing and are therefore more expensive. A recent Swedish survey estimated that the annual cost of four biological antirheumatic drugs (etanercept, infliximab, adalimumab, and anakinara) is 50–70-fold higher than the average annual drug cost per person (€10 800–14 400 per year versus €170 per year),²⁶ and the financial burden of the biological anticancer agents is even higher. When generalized, these costs will become unsustainable for any national health insurance, since the total health costs in Western countries has already reached very high levels (16% of the gross domestic product (GDP) in the United States in 2005).²⁷ Despite these limitations, the pharmaceutical industry has embraced a ‘biopharma’ approach to drug discovery, diversifying its traditional focus on small molecules with a growing commitment on biologics. Thus, the term ‘new molecular entities’ is rapidly replacing the term NCEs in new drugs inventories. Unsurprisingly, drug companies have also started to shed their chemical workforce at an unprecedented rate. The laying off of Robert Sliskovic, the discoverer of atorvastatin (Lipitor, **1**), made headline news and was considered emblematic of the pharmaceutical industry’s declining fortunes.²⁸ Atorvastatin is the best-selling pharmaceutical product ever, having generated over \$80 billion sales to Pfizer since its introduction into the clinic, and the laying off of Sliskovic was commented in a front page article by *The Wall Street Journal*.²⁸ The list of major drug companies that announced programs of cost reduction and biotech refocusing in 2007 includes, apart from Pfizer, Bristol–Myers Squibb (BMS), Novartis, and Astra–Zeneca, with Merck and Lilly already adopting this model a few years earlier.²⁹



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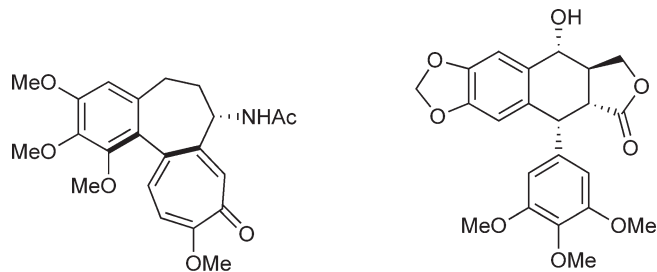
With a mean time of approximately 12 years from drug discovery to launch, the current problems of pharmaceutical research have their roots in choices dating to the 1990s and any strategy pursued today will have only marginal influence on the near-future late-stage development pipelines. The current downsizing of natural products drug discovery should therefore be seen as a part of a general trend of pharma research to focus on biological drugs at the expenses of small molecules. Confronted with an acute productivity crisis, mainstream pharmaceutical research will undoubtedly explore alternative strategies of discovery, with 'old' assets like natural products possibly coming of age again. Newer screening paradigms, shortened discovery times, and integration into a multifaceted drug discovery scenario will, however, be necessary to foster the long-awaited renaissance of natural products drug discovery.

3.08.3 Why Natural Products Are Intrinsically Useful for Drug Discovery

When you have no idea where to begin in a drug discovery program, Nature is a good starting point.

L. H. Caporale, *Chem. Eng. News* 13 October 2003, p 89

A large number of biological processes involve the interaction of a small molecule (ligand) with a macromolecular target (receptor). Preeminent examples are the interaction of neurotransmitters with their protein targets, of intercalating agents with specific DNA or RNA polynucleotidic sequences, and of macromolecular carriers with the ligands they transport across biological membranes. In general, the interaction of a small molecule with a macromolecular target represents an opportunity to perturb a biological system, to study its function, and to assess its druggability, that is, its amenability to pharmaceutical exploitation.³⁰ In molecular terms, perturbing is equal to knowing and natural products hold a special position as molecular perturbators, since their role to reveal interesting biology, to provide the tools necessary for its study, and to generate molecular clues (hits and leads) for its ultimate therapeutic exploitation is at the very base of modern medicine. Tubulin, one of the most important anticancer targets, is a remarkable example of this process.³¹ Thus, tubulin was discovered because of the availability of colchicine (**2**), a specific ligand obtained from the medicinal plant *Colchicum autumnale* L. The biological profile of tubulin was next furthered using a variety of natural products, including podophyllotoxin (**3**), and the manipulation of tubulin was eventually translated chemically with the development of *Vinca* alkaloids, taxanes, and epothilones into effective anticancer drugs. Natural products were also instrumental for the identification of hsp90 as an anticancer target and to study its function.³² Several natural products drugs aimed at the inhibition of this chaperone are currently under clinical development, making it possible that, thanks to natural products, hsp90 biology will also soon be translated into hsp90 drugs³³ (for more details, see Chapter 3.06).



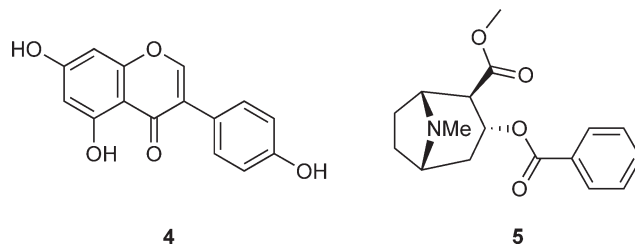
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Natural products have a personality: they come with attractive names, awe-inspiring structures and dazzling biological properties, and there are clear molecular, evolutionary, and structural bases for their relevance in drug discovery. Because of their three-dimensional structural complexity and inbuilt affinity for biological surfaces, natural products are in fact privileged structures for drug discovery from both a chemical and a biological standpoint and qualify nature not only as the ultimate synthetic chemist but also as the ultimate pharmacist.

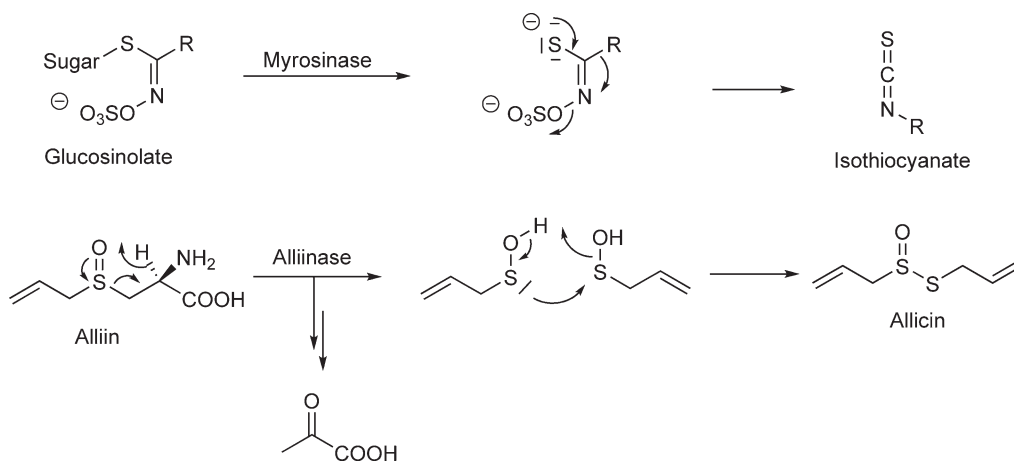
3.08.3.1 Molecular Bases for the Biomedical Relevance of Natural Products

Macromolecular biological targets (proteins, nucleic acids) 'see' small molecules as three-dimensional surfaces bearing specific binding elements (charges, polarities, and hydrogen-bonding donor and acceptor elements). Surreptitious complementarity between biogenetic enzymes and animal protein domains is the general molecular basis for the preeminence of natural products in biomedical research and drug discovery.³⁴ Most natural products are the results of enzymatic reactions and their shape must therefore be complementary to that of the biological protein surface that fitted their ultimate precursor(s). A biosynthetic origin therefore involves the imprinted capacity to recognize protein surfaces. All organisms, whether bacteria, plants, or animals, are the results of permutations and combinations of the same four basic nucleotides and 22 amino acids. The number of unmodified, or 'naked' proteins coded by the human genome is between 80 000 and 100 000 but the total number of different proteins produced by human cells is magnified by posttranslational modifications like glycosylation, acetylation, methylation, hydroxylation, and phosphorylation and is probably around 1 million.³⁵ Assuming a 10% of druggability,³⁶ the number of drug targets is therefore enormous, around 100 000, but only 324 of them have so far been identified for all the approved therapeutic drugs.³⁷ Despite the large number and structural variety of these protein targets, the number of peptide domains, which is the way secondary structures like α -helices and β -sheets can arrange themselves in space independently from the rest of the structure, is fairly limited. It has been estimated that the widely diverse function of proteins derives from the combination of only 600–8000 domains. The number of distinct protein families is therefore fairly limited³⁸ since a similar structural domain can be used by many proteins in more or less modified forms generated by divergent evolution, as shown, for instance, by leukotriene A4 hydrolase and thermolysin. These two enzymes catalyze different reactions (vinylogous opening of an epoxide ring and peptide hydrolysis) but share a very similar fold and profile of inhibitors.³⁸ Therefore, natural products are characterized by an intrinsic, biogenetically imprinted shape complementary to biological surfaces and this topological property can be translated into a reversible interaction with a druggable target totally unrelated to their original biosynthetic enzymes. In other words, since protein domains are conserved, biological surfaces homologues to those present in biogenetic enzymes might exist in animals, even though they fundamentally lack the enzyme machinery to produce secondary metabolites. For instance, the flavonoid biogenetic enzymes chalcone isomerase, chalcone synthase, and anthocyanidin synthase share a flavonoid recognition site with mammalian protein kinases and it is therefore unsurprising that the flavonoid genistein (**4**) was one of the first inhibitors of tyrosine kinases to be discovered.³⁹ The biosynthetic enzymes/drug targets correlation makes it possible that small-molecule ligands might exist for all the druggable human targets and neuroactive plant alkaloids represent a striking example of this assumption. Cocaine (**5**) inhibits the reuptake of mammalian biogenic amine neurotransmitters (dopamine, serotonin, noradrenaline, adrenaline), a system that has no counterpart in plants, which, in turn, also contain a host of compounds that target the cholinergic, adrenergic, dopaminergic, and gamma-aminobutyric acid (GABA)ergic systems of animals, all structures that do not fundamentally exist, even in cognate form, in plants.³⁴

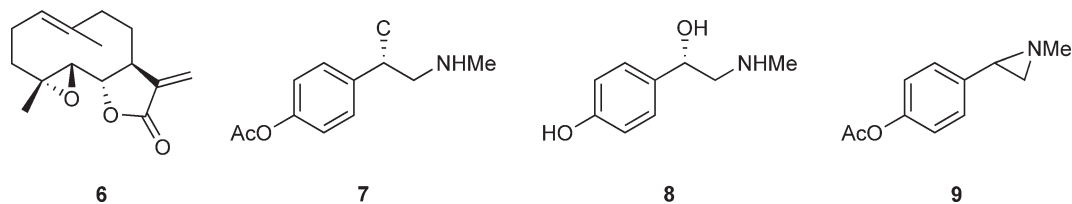


Some natural products are not the direct result of an enzyme reaction but rather of a spontaneous reaction cascade triggered by an enzyme reaction. In this so-called bomb-like strategy, a stable precursor is enzymatically transformed into an unstable species (chemical bomb) that undergoes an intramolecular rearrangement to a more stable but still reactive compound, capable of interacting with a variety of targets rather than with a single target. Examples from plant secondary metabolites are isothiocyanates from cruciferous plants and thiosulfonates from garlic, formed from glucosinolates and sulfoxidic amino acids by the action of specific enzymes (myrosinase and alliinase, respectively) compartmentalized in different cells or cellular stores with respect to their substrates.⁴⁰ Hydrolysis of the thioglucosidic bond of glucosinolates generates the unstable sulfate of an *N*-hydroxythioimidate, which then undergoes Lossen rearrangement to a reactive isothiocyanate,⁴⁰ while alliinase triggers a β -elimination reaction that splits alliin into pyruvic acid and a sulfinic acid, which then spontaneously dimerizes to allicin, a reactive thiosulfonate ester (Scheme 1).⁴¹ Compounds like isothiocyanates and thiosulfonates target nucleophilic sulfhydryl sites of a host of proteins via a polar trapping mechanism, while the microbial enediyne antibiotics cleave DNA with a radical mechanism, mediated by the formation of a 1,4-diyne (*p*-benzine) via a Bergman reaction.⁴²

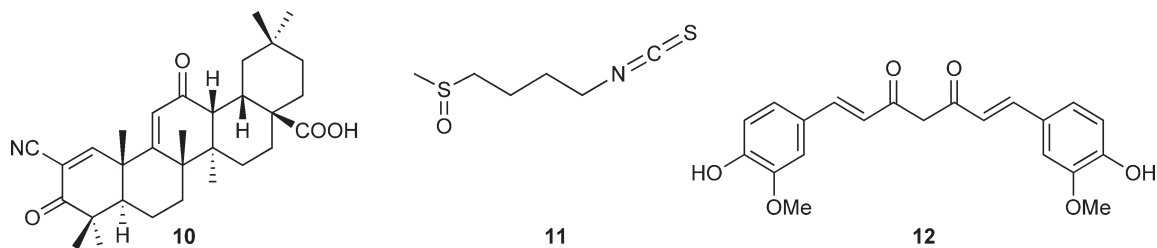
Reactive secondary metabolites have generally been perceived as nonselective and indiscriminate in their activity and therefore of little relevance for drug discovery. Lists of chemically or biologically reactive groups that should be avoided in drug discovery (aldehydes, exomethylene enones, epoxides, furans, etc.) can be found in any book on drug discovery. Nevertheless, reactive functional groups do occur in natural products and are, surprisingly, often associated with a specific activity. Thus, the exomethylene- γ -lactone parthenolide (6), an NF- κ B inhibitor and the hallmark constituent of feverfew (*Tanacetum parthenium* L.) has been considered for clinical development for a variety of conditions, including headache, but has also raised considerable interest as a selective cytotoxic agent for stem cells,⁴³ while the even more reactive nitrogen mustard CpdA (Compound A, 7) is a selective glucocorticoid receptor modifier, capable of dissecting the transactivation and the transrepression properties of these hormones.⁴⁴ CpdA is as effective as dexamethasone in counteracting acute inflammation *in vivo* but lacks the hyperglycemic side effects of glucocorticoids and, despite its reactive nature, is considered of 'great potential for therapeutic use'.⁴⁵ CpdA is a stable (*sic*) analogue of the unstable active principle of the Namibian shrub *Salsola tuberculatififormis* Botsch., a plant used in the bushman folklore as a contraceptive, and implicated in a syndrome of prolonged gestation and fetal postmaturity in sheep.⁴⁴ The active principle of this plant is a very unstable compound that quickly decomposes to synephrine (8) in acidic water and that has been suggested to be a precursor of the phenolic aziridine (9). CpdA was prepared from synephrine by treatment with acetyl chloride and its biological profile was comparable to that of the elusive active principle of the plant.⁴⁴ Although phenolic aziridines are extremely unstable *in vitro*, the active principle of *S. tuberculatififormis* and its derived aziridine are apparently stabilized *in vivo* by binding to plasma proteins.^{44,46}



Scheme 1 Enzymatic 'detonation' of glucosinolate and alliin chemical bombs.



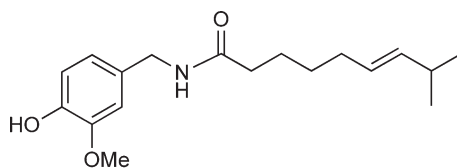
There is also growing evidence that reactive Michael acceptors can interact with biomolecules in a reversible fashion, not remaining bound to their ‘proximate’ targets but bouncing within a multitude of thiol-containing proteins and eventually ‘landing’ on key regulatory proteins having thiol group especially sensitive to electrophiles. This mechanism is the basis of the activity of CDDO (**10**), a semisynthetic homotriterpenoid nitrile considered one of the most promising anticancer agents in current clinical development.^{47,48} Compounds like CDDO do not fit into the conventional models of drug action based on a single high-affinity receptor–ligand interaction, since their activity involves transient molecular interactions with multiple targets that share the critical and reactive cysteine residues.⁴⁹ If Michael acceptors that react reversibly with thiol groups have different affinity for different target proteins, then low concentrations of these compounds would preferentially interact with the most sensitive targets. A host of networks, in this case those responsible to the redox state of a cell, are therefore affected by their activity. This mechanism might well apply to a series of multifunctional chemopreventive agents like sulforafane (**11**), curcumin (**12**), and the organosulfur compounds from garlic,⁴⁹ all compounds capable of targeting multiple proteins through Michael adduct mechanisms.⁵⁰ Compounds like CDDO undoubtedly stretch the concept of ligand–receptor interaction well beyond the classic lock-and-key metaphor and it should be remarked that several tyrosine kinase inhibitors also interact in a combinatorial way with a host of their enzyme substrates, modifying the whole ‘kinomic’ profile of a cell rather than a single effector target and thus modulating the activity of several signaling networks.⁵¹



3.08.3.2 Evolutionary Bases for the Biomedical Relevance of Natural Products

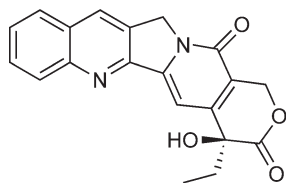
The term ‘secondary’ in secondary metabolites does not imply a ‘less important’ status but simply addresses a different functional level, just like the ‘secondary’ structure of a protein is equally important as the ‘primary’ or the ‘tertiary’ structure. Indeed, natural products have been detected in fossils^{52,53} and presumably appeared very early in the evolution of life, possibly already in the RNA world, since many antibiotics bind specific RNA sequences.^{52,53} Evolutionarily, the synthesis of natural products is a conserved and highly successful trait. Since it is not uncommon that scores of specific enzymes are involved in the biosynthesis of a single natural product, the production of secondary metabolites involves an enormous investment in terms of protein-coding DNA. Given this investment, natural products must have beneficial functions for their producers, all based on the interaction with macromolecular targets and their functional perturbation in terms of promotion or inhibition. The activity of natural products has been shaped to overcome environmental stress and provide defense against natural enemies and an adverse environment and was optimized by evolution during millions of years of environmental high throughput screening (HTS). In nature, the struggle for survival is waged at the molecular level and natural products, by reflecting eons of wisdom and refinement, have an intrinsic evolutionary and ecological meaning. They represent working examples of biological ‘intelligent design’ and it is therefore regrettable that the futile cat-and-mouse contest between science and creationism has not been waged at a

molecular level, spurring research into the elusive meaning of secondary metabolites. The ecological role of secondary metabolites is extremely difficult to investigate. They are like words of a language that we can read but do not understand, just like Etruscan. An important exception is capsaicin (**13**), probably the only secondary metabolite whose natural function has been elucidated in detail.⁵⁴ After survival and reproduction, dispersal of seeds is third on the list of priorities for a plant. To this purpose, peppers produce fleshy and colored fruits to attract consumers and colonize new areas. By using nonpungent peppers consumed by both mammals and birds, it was found that fruit ingestion by mammals inhibits seed germination, while, conversely, consumption by birds does not damage seed germinability, rather promotes it. Birds swallow the fruits and promote the dispersion of seeds while mammals chew the fruits with their teeth and physically damage them. Hence, mammals behave as seed predators and birds as seed dispersers, acting as living ‘vessels’ to carry chillies to new turfs. Capsaicin targets the vanilloid receptor TRPV1, a molecular thermometer whose activation is perceived as pungent pain by mammals but not by birds, which have a mutated form of TRPV1, insensitive to capsaicin.⁵⁵ Owing to the selective sensitivity of the mammalian version of TRPV1 to capsaicin, this compound functions therefore as a specific inhibitor of seed predation.

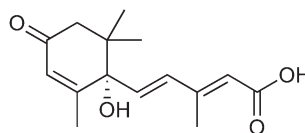


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There are strict relationships between sensitivity to natural products and genomics, with the possibility of adaptive coevolution between the production of secondary metabolites and their target. For instance, it has been demonstrated that camptothecin (CPT, **14**)-producing plants like *Camptotheca acuminata*, *Ophiorrhiza pumila*, and *Ophiorrhiza liukiensis* have Topo1's with point mutations that confer resistance to CPT.⁵⁶ Remarkably, one of these substitutions (Asn722Ser, human Topo1 numbering) is identical to that found in CPT-resistant human cancer cells while a phylogenetic analysis of Topo1's in CPT-producing and CPT-nonproducing plants suggests that mutations in Topo1 occurred before the CPT-producing enzymatic machinery appeared.⁵⁶ In other words, CPT was ‘planned’ taking advantage of a previous biochemical enzymatic unicity. Since the evolutionary history of most natural products is unknown, we lack information on the molecular milieu and the temporal range in which their production evolved. In this context, a theory alternative to surreptitious complementary has been developed to explain why animals have receptors for compounds produced by plants and, conversely, plants produce compounds for receptors they lack. According to the vestigial receptor hypothesis,³⁴ receptor–ligand pairs evolved in primitive organisms that predated the divergence of animals and plants. Since then, organisms that then evolved into plants lost the need of receptors and maintained that of ligands, while those that evolved into animals retained receptors but lost the need for their ligands. In this context, it is instructive to ponder that many compounds involved in cell signaling in plant cells have receptors also in animals, such as abscisic acid (**15**),⁵⁷ salicylates,⁵⁸ and genistein (**4**).⁵⁹ While being necessarily vague and simplistic, evolutionary observations and hypotheses on the function of natural products can nevertheless have interesting implications for drug discovery, as shown by the adaptation strategies developed by plants and microorganisms to overcome self-poisoning from their own metabolic products.

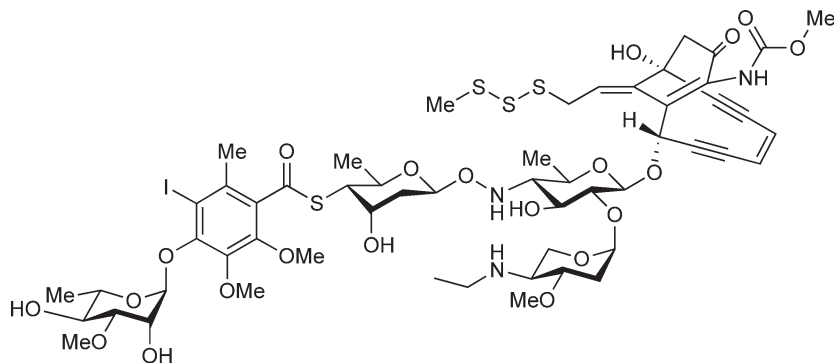


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As already discussed with CPT, organisms accumulating inhibitors of basic biological processes like mitotic poisons (taxanes, *Vinca* alkaloids, colchicine) and antibiotics, must also have evolved strategies to avoid self-poisoning. These involve, apart from compartmentalization, the expression of export pumps, of antibiotic-modifying enzymes, and of target-protecting mechanisms that can mimic the molecular bases of drug resistance in the clinical setting.⁶⁰ Thus, antibiotics are produced to create, by disrupting basic biological processes, a local protective environment inhospitable to invading organisms. Clearly, antibiotic-producing microorganisms must have evolved self-resistance mechanisms prior to the production of antibiotics and, indeed, horizontal gene transfer from nonpathogenic bacteria, the major source of antibiotic resistance, has been ultimately traced to the antibiotic-producing organisms themselves.^{60,61} In other words, acquired antibiotic resistance among dangerous bacterial pathogens, an increasing medical problem, relies on mechanisms primed at the stage of antibiotic production. Just like with CPT-producing plants, also in antibiotic-producing *Actinomycetes*, protection must have evolved before the production of active metabolites. A possible mechanism has been dubbed 'feed-forward' and involves inactive precursors that act as signal to prepare the organism for the later buildup of toxic levels of antibiotics.⁶¹ More sophisticated mechanisms of self-resistance to 'endogenous' secondary metabolites have also been discovered and they might afford interesting mechanistic clues to the development of clinical resistance to them. The enediyne anticancer antibiotic calicheamicin (**16**) is an interesting case. This agent is a real 'chemical nuclease' that fragments DNA via a cycloaromatization-induced radical mechanism.⁴² Because of its extraordinary toxicity, in the femtomolar range for some organisms, calicheamicin is used in the clinics only as a monoclonal antibody conjugate (Mylotarg) and the mechanism of self-resistance to this toxin has long remained a mystery. Enediyne-binding proteins (chromoproteins) stabilizing these highly unstable compounds and possibly aiding in self-protection have been detected in some ene-dyne-producing microorganisms but not in *Micromonospora calicheamicensis*, the source of calicheamicins. Recently, a gene (*calC*) conferring *in vivo* resistance to calicheamicin has been characterized in the genome of *M. calicheamicensis*.⁶² The encoded CalC protein turned out to be a stoichiometric self-sacrificing agent against calicheamicin-induced double strand DNA scission, capable of quenching activated calicheamicin through a direct and specific hydrogen abstraction that mimics the action of the antibiotic on DNA. The production of CalC, along with a tightly sequestered biosynthesis and exportation system, makes it possible to escape self-toxicity from this agent and similar self-sacrificing strategies might underlie the development of resistance to enediyne also in nonproducing organisms, including cancer cells.



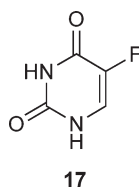
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The examples we have described highlight how closely related genomics, metabolomics, and ecology are and how information from this fertile interface can have far-reaching clinical implications. Indeed, the evolutionary study of natural products can afford interesting clues for drug discovery in terms of both mechanism of activity and resistance to it.

3.08.3.3 Structural Bases for the Biomedical Relevance of Natural Products

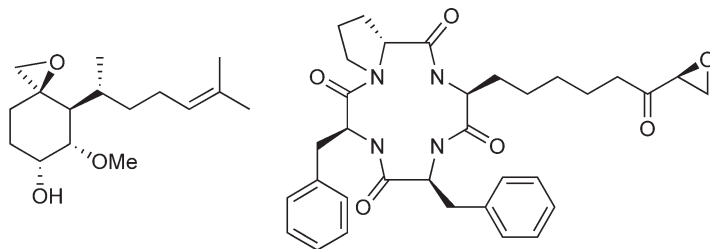
The chemical complexity and diversity of natural products supercedes anything pharmaceutical companies or chemists can design, making it possible to explore areas of the biological space that are difficult, or downright

impossible, to access with compounds obtained by random synthesis campaigns. Thus, a statistical investigation of the structural complementarity of natural and synthetic compounds showed that 40% of the chemical scaffolds of natural products are absent in synthetic compounds.⁶³ Organic synthesis has evolved rapidly and nowadays, given enough students and funds, most natural products are within the reach of synthetic preparative chemistry. However, simple statistic considerations make it very unlikely that the constitutional and configurational structural subtleties of natural products might evolve *ex novo* from a random synthetic program. The chemical space is in fact huge but fundamentally void in biological terms. Most small biomolecules are made only by four elements (carbon, hydrogen, nitrogen, and oxygen) and have molecular weight (MW) <500 Da, while biological peptides are based on only 20 building blocks and have an average of 300 amino acids. Even with these structural constraints, the number of possible structural choices (permutations in the statistic lingo) is appalling. Thus, the number of small molecules having MW <500 Da and based on the four most common biological elements (C, H, N, and O) is 10^{60} , while the number of 300 amino acid-long linear sequences based on the 20 proteogenic amino acids is 10^{360} .⁶³ Life has therefore been extremely selective in molecular terms, since less than 18 000 natural products are known and, as we have seen, the human genome contains sequences of less than 100 000 proteins. Most synthetic chemical compounds are therefore biological chuff and, without opportune clues, it is extremely difficult to enter the highly selective biological space of natural products with compounds derived from a random synthetic campaign. An apparent exception is the anticancer drug 5-fluorouracil (5FU, **17**), which was synthesized over 40 years before being actually described as a natural product.⁶⁴ However, its synthesis was inspired by the RNA base uracil and was not the result of a program of random synthesis.⁶⁵ Considerations of this type, better than the much-hyped similarity between the drug space and the natural products space, support the view that natural products are special tickets in the drug discovery lottery. Since natural products have been the major source of chemical diversity for drug discovery, the drug space was filled mostly by research programs based on endogenous small molecules (neurotransmitters, hormones) or natural products and therefore this convergence might, in principle, be viewed as the mere result of past strategies of drug discoveries.



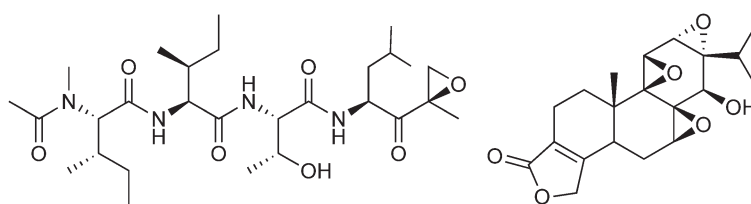
Synthetic libraries are straightforward to assemble but the relatively limited number of synthetic reactions and building blocks amenable to combinatorial strategies means that the resulting compounds often lack the structural complexity and diversity required to efficiently explore the biological space. For instance, rigid molecules occur rarely in combinatorial libraries since they are more difficult to assemble and the higher number of rotatable bonds means that conformational constraint, often an affinity booster, is missing from these libraries.⁶³ Indeed, combinatorial libraries are mainly based on flat structures. These, while very useful to explore ATP mimics and kinase ligands, are much less suitable to discover agents capable of interfering with complex processes like protein–protein interactions. Finally, synthetic libraries are often biased by the requirements of previous focused drug discovery programs and/or by the compliance to certain predefined criteria like the Lipinsky rule of 5 (RO5)⁶³ and the absence of reactive functional groups like epoxides, furans, and β -unsubstituted enones. Many bioactive natural products violate RO5 and feature ‘undesirable’ reactive groups. Thus, the alkylation of an epoxide moiety is essential for the bioactivity of important drug leads, such as the antiangiogenic methionine aminopeptidase2 (MetAP-2) inhibitor fumagillin (**18**),⁶⁶ the histone deacetylase (HDAC) inhibitor trapoxin (**19**),⁶⁷ and the exquisitely selective proteasome inhibitor epoxomicin (**20**),⁶⁸ while the anti-inflammatory agent triptolide (**21**), a putative transient receptor potential canonical (TRPC) ligand,⁶⁹ features a lineup of three adjacent epoxide groups. Other reactive, more exotic, and still biologically critical functional groups can occur in natural products drug leads, as exemplified by the hydroxamic HDAC inhibitor trichostatin A (**22**),⁷⁰ the β -lactone proteasome inhibitor lactacystin (**23**),⁷¹ and the diazo derivative kinamycin (**24**).⁷² Given a suitable molecular framework, reactive functional groups can indeed be implanted in drug leads

but this operation is forbidden under current ‘rational’ drug discovery rules, which, by doing so, precludes the exploration of a significant portion of the biological space. Finally, structural complexity aids specificity and potency in biological interactions, limiting target promiscuity,⁷³ while diversity is important to broaden the chemical space ‘interrogated’ during the biological evaluation.



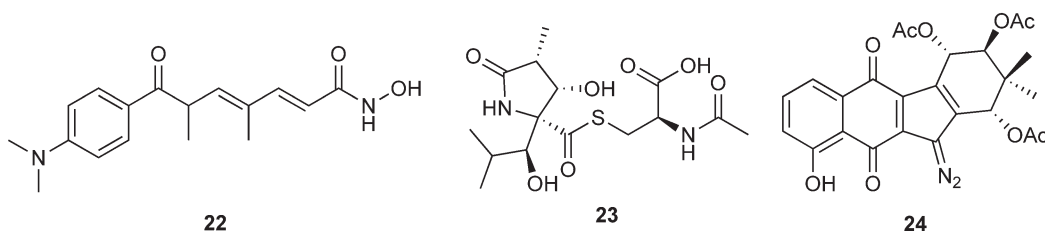
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A host of molecular, evolutionary, and structural reasons therefore underlie the idea that natural products, as evolutionarily selected ligands to structurally conserved but genetically mobile protein domains, represent the most validated starting point to explore the druggable section of the chemical space. This view, while amply shared within the drug discovery environment, is nevertheless difficult to explicitly translate into numbers since structural diversity is hard to assess objectively with measurable parameters.⁷⁴ Even using simple structural elements, natural products are clearly differentiable from combinatorial chemistry products in terms of MW (414 versus 393), number of stereogenic centers (6.2 versus 0.4), and cycles (4.1 versus 3.2) and they incorporate fewer nitrogen, halogen, and sulfur atoms but are more rich in terms of oxygen and are sterically more complex, with more rings and bridgehead carbon atoms.⁷⁴ Based on these observations, a topological analysis of combinatorial libraries and natural products showed that combinatorial compounds densely populate a small area of the chemical space while natural products are more largely distributed in terms of occupancy of chemical space and are more diverse.⁷⁴

3.08.4 Possible Reasons for the Current Downsizing of Natural Products Drug Discovery

The pharmaceutical industry has a conception of the format through which future discoveries will be made, and natural products are not on their radar. The mavens (sic) of the pharmaceutical industry seem to think that a discovery made outside that format can't be worth much. Some of these guys would have turned down the

Gettysburg Address because it was handwritten by an aging single author rather than turned out by some pricey word-manufacturing institute that hit upon it by chance.

S. Danishefsky, *Chem. Eng. News* 13 October 2003, p 107

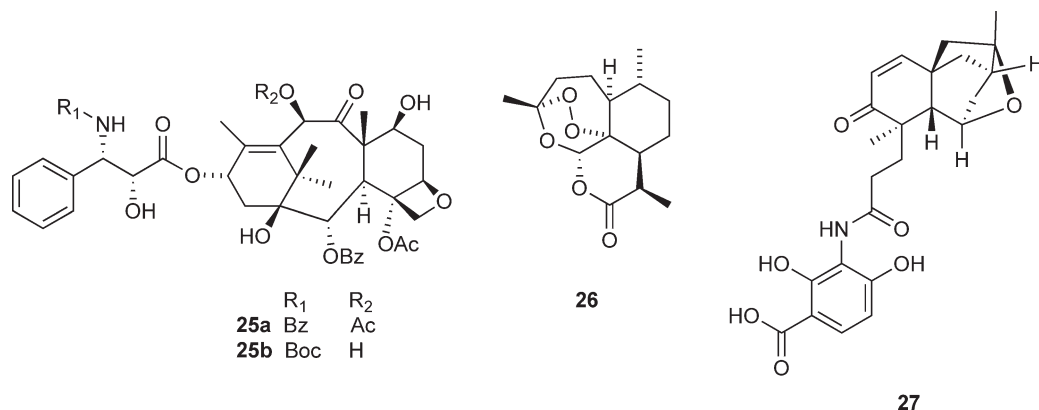
The exquisite biological specificity of natural products has laid the foundations of modern medicine. Natural products save millions of lives every year and generate annual commercial sales of billions of dollars to their developers and discoverers. Undoubtedly, natural products represent the most successful and validated strategy of drug discovery and it has been calculated that 60% of the current drugs are natural products, derivatives of natural products, or synthetic analogues of natural products.⁷⁵ Furthermore, high-speed approaches of fractionation and structure elucidation based on hyphenated techniques⁷⁶ have considerably expedited the construction of natural products libraries, alleviating the burden of dereplication.⁷⁷ Interestingly, some of these techniques, such as automated high-performance liquid chromatography (HPLC) separation with fast gradient elution, were originally developed for the production of combinatorial libraries.^{78,79} Overall, the processing time of crude extracts has been substantially reduced through pretreatment, automated separation, and computer-assisted structure elucidation. In particular, iterative automated fractionation makes it possible to detect minor compounds once below the threshold of chemical and biological revelation and therefore inaccessible.^{78,79} The structural complexity and the diversity of secondary metabolites are also ideal to fill the gap between the growing number of drug targets disclosed by genomics and the dismally low number of specific ligands available for them.

Nature is undoubtedly the largest and most diverse combinatorial library available but unlocking it is far from trivial since it requires multidisciplinary expertise, is more time consuming and costly than most current drug discovery approaches, and poses problems unfamiliar to corporate culture. It is therefore hardly surprising that, over the past two decades, drug discovery has gradually, and probably myopically, prematurely dismissed research into natural products as an old-fashioned delivering tool, investing instead in nonvalidated surrogates of biodiversity like combinatorial chemistry. The profitability of natural diversity to provide templates for drug discovery has been questioned and bioprospecting has lost out to high-throughput drug discovery, a process that relies on combinatorial chemistry and computational drug discovery. Thus, in 2008, Merck cut its natural products program entirely, despite a long and successful history in this area (lovastatin, caspofungin acetate) and, within the major pharmaceutical companies, only Novartis and Wyeth retain natural products research divisions with activities that go beyond the semisynthesis of antibiotics and steroids. Remarkably, less than a decade ago, it was still possible to claim that “most major pharmaceutical companies maintain important efforts in natural products’ research.”⁸⁰ The demise of natural products in drug discovery is even more surprising when one considers that, within the 15 small-molecule drugs approved by Food and Drug Administration (FDA) in 2007, six were natural products or derivatives of natural products.⁸¹ This ratio is somewhat skewed by the inclusion, within the count of NCEs, of three ‘old’ drugs (azithromycin, topotecan, and temsirolimus) for which new indications were approved but is nevertheless remarkable. Clearly, there must be solid reasons for the pharma industry to abandon the beaten track of natural products and adventure into remote areas of the chemical space to discover new drugs.

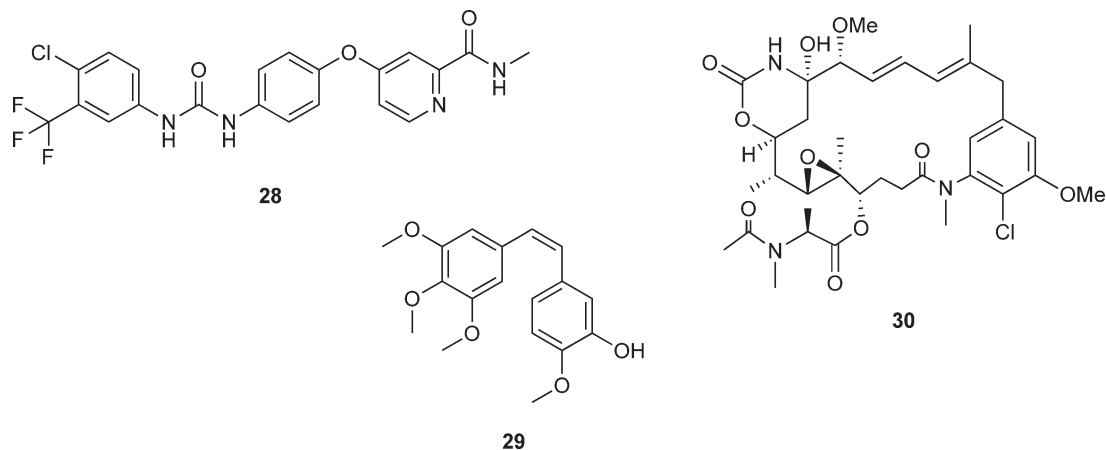
On the other hand, natural products isolation is also becoming a rare area of interest in the US and European universities. Natural products have long been pursued in academia only because of their unique chemical structure but, over the past decades, the focus has shifted on the discovery of their properties and the academic demise of natural products isolation is therefore unjustified. The academic woes of Georg Pettit, a hero of natural products drug discovery, exemplify this trend.⁸² Just like taxonomy, natural products are also becoming extinct in academia, serving mostly as models for total synthesis. The shift in focus from isolation to synthesis is ironical at an age when more and more emphasis is placed on applied research since synthesis is often pursued essentially as a training ground for Ph.D. students and lacks practical application.

The transition from natural products to synthetic drugs was preceded by that from pharmacognosy to medicinal chemistry. Indeed, by the 1940s, heroic plant drugs like opium, cinchona, ipecacuhana, and mayapple had already been replaced by their isolated active constituents (morphine, quinine, emetine, and podophyllo-toxin). Since the activity of most medicinal plants, even as popular as valerian and chamomile, could not be traced to a single constituent amenable to pharmaceutical development, their gradual downsizing to the health food realm was inexorable, while, in the wake of the wartime efforts to produce penicillin, large fermentation programs of drug discovery were launched, filling up the dwindling pipeline of plant natural products drugs.

Despite several decades of success, in the past two decades microbial natural products research has also been de-emphasized in pharmaceutical companies and considered as overexploited and poised to fail to significantly enrich the yield of pharmaceuticals. Natural products are no longer considered a focal point for hit discovery, making up for only a tiny percentage of the compound archives of large pharmaceutical companies, typically comprising up to 5 million compounds. Alternative opportunities to discover novel low MW leads have taken over and drug discovery is nowadays associated with dazzling new concepts and technologies, such as functional genomics, combinatorial synthesis, structure-based ligand design, and ultrahigh-throughput screening (UHTS, >100 000 compounds per week).²⁰ Conversely, and despite noteworthy methodological evolution, the strategy to develop new natural products drugs has instead remained the same, involving primary screening of crude extracts, bioassay-guided fractionation, dereplication of active compounds, and isolation and structure elucidation of new bioactive constituents. Furthermore, despite the current success of natural products drugs, there is no recent record of plant-derived drug discovered by big pharmaceutical companies and modern successful plant drugs like paclitaxel (**25a**),^{83,84} CPT (**14**),⁸⁴ and artemisinin (**26**)⁸⁵ originated from publicly funded research. After the success of rapamycin, chaliceamicin, tacrolimus, and epothilones, the pipeline of fermentation products also seems to have become arthritic and doubts linger if platensimycin (**27**), a lipid biosynthesis inhibitor and the most notable antimicrobial agent discovered in the past few years,⁸⁶ will ever be developed by Merck.



Conversely, several unsuccessful case histories of natural product-based drug discovery projects have been reported. Thus, in the early 1990s, Merck paid \$1.14 million to InBio, a Costa Rican conservation group, to screen rainforest species (plants, insects, and microorganisms) for novel chemicals of interest for drug discovery.⁸⁷ Nothing useful apparently came out of this project, which was terminated in 1999. In those years, Shaman Pharmaceuticals, a company founded in 1989 to develop modern drugs from traditional medicines, went as far as late-stage clinical trials for an antiviral plant extract but then went bankrupt,⁸⁸ while the 1970s–1990s had witnessed the Herculean efforts of National Cancer Institute (NCI) to discover new natural products anticancer drugs. The program was terminated after the screening of 114 000 extracts originating from 35 000 plant samples representing 12 000–13 000 species had apparently failed to produce a single natural product-based anticancer drug.⁸⁹ For the sake of comparison, we can mention that the discovery of the kinase inhibitor sorafenib (**28**) by Bayer involved the screening of a combinatorial library of 200 000 compounds, followed by the parallel synthesis of 1000 further analogues and was accomplished in only 4 years.⁹⁰ However, since sorafenib is the first, and so far the only, drug emerging from the screening of combinatorial libraries, it is not clear how general for modern drug discovery its genesis is and if its success can be considered as a real validation of combinatorial chemistry as a delivering tool.⁹¹ Furthermore, the clinical potential of many leads discovered during the NCI campaign was realized only after its termination. Thus, CPT derivatives and paclitaxel were introduced into the clinic almost three decades after their discovery, while a host of compounds from the original NCI program are being developed only now, such as combretastatin A4 (**29**)⁹² and maytansine (**30**).⁹³



Of great relevance to the current demise of natural products is also the growing trend toward a more rational approach to drug discovery, where ligands are designed *ex novo* or assembled by fragments and where emphasis is apparently placed on design rather than on discovery. Natural products research is also intrinsically multidisciplinary and requires the combined efforts of natural products chemists, pharmacologists, and medicinal chemists. These expertises are difficult to coordinate and focus and require a highly trained team, difficult to assemble in a pharmaceutical scenario characterized by a high personnel mobility.

For these reasons, there is no doubt that, compared to synthetic libraries, natural products and extract libraries pose a host of problems, which, albeit all singularly soluble, nevertheless conjure up to provide an overall unattractive scenario for big pharma. The future of natural products in drug discovery and their promotion to full potential will therefore critically depend on how well and quickly these issues will be solved, making the process less time and resource intensive.

3.08.4.1 Intellectual Property Issues

Natural products cannot be patented as a structure but semisynthetic analogues can, as well as their uses, isolation/manufacture processes, and specific drug formulations, although intellectual protection is obviously weaker.⁹⁴ Furthermore, the technological asymmetry between biodiversity- and technology-rich countries has raised considerable and still unresolved proprietary issues on natural products drugs.⁹⁵ According to the UN Convention on Biological Diversity (CBD), countries have sovereign rights over the biological resources within their boundaries and should establish conditions for the preservation and sustainable use of their biodiversity.⁹⁶ Source countries should be involved in projects related to their biodiversity and should share any commercial benefit resulting from its use. These claims are objectively difficult to translate in terms of pharmaceutical intellectual property (IP), whose reinforcement is, nevertheless, essential to contribute to the local development of resources and to make prospecting an engine for biodiversity conservation.⁹⁷ As a result of this ambiguity, many countries have placed barriers on the exporting of biological materials, even for noncommercial researches. CBD is not retrospective and therefore the examples of earlier natural products discovery that failed to produce commercial rewards to the source country lack legal meaning. Although the political debate on biopiracy is colored with examples from developing countries (rapamycin from Easter Island, teprotide, on which the angiotensin converting enzyme (ACE) inhibitors were molded, and tubocurarine from Brazil),⁹⁸ there are far many examples from developed countries, such as cephalosporin, rifampicin, daunomycin, and mycophenolic acid from Italy, cyclosporin A from Norway, or paclitaxel from United States, just to mention a few examples.⁹⁹

3.08.4.2 Access to Natural Chemical Diversity

Gaining access to biodiversity from natural habitats is legally complicated, especially for broadscale corporate campaigns involving the collection of hundreds, or even thousands, of species. Developing societies that possess

important biodiversity and developed societies endowed with advanced technologies should interact on the basis of a principle of equity but, as we have seen, it is difficult to specify how.^{97,98} In principle, countries with rich biological resources should be able to charge companies for bioprospecting for either drugs or genetic information that could lead to new drugs but legally binding formulas to control this 'trade' are difficult to conceive and to implement. In 2002, countries signatory of the Rio Biodiversity Convention agreed on a set of rules (Bonn Guidelines on Access and Benefit Sharing) that specify how each country should frame licenses to allow companies to access natural resources but this arrangement was fiercely opposed by many environmental and economic organizations. Thus, Jeremy Rifkin, who heads the Foundation on Economic Trends claimed that "nobody has the right to enter into exclusive deals over the products of millions of years of evolution."¹⁰⁰ Political sensitivity regarding access to biodiversity from developing countries is undoubtedly one of the reasons underlying the phasing out of natural products from big pharma.

Given the current downsizing of basic natural products research in drug companies, it is likely that many, if not most, of the natural products drugs of the future will originate from publicly funded research, via government organizations and academic institutions, or from venture capital small biotech companies. Taxol (paclitaxel, **25a**) was the first anticancer drug to reach a billion-dollar yearly sale and the NCI–BMS deal on the pharmaceutical development of this compound was undoubtedly highly profitable from a corporate viewpoint.¹⁰¹ It was also vociferously criticized, in particular for the hijacking of the taxol name from BMS. On the other hand, the deal made rapid access to this drug possible and its price sank after it acquired a generic state. Aventis (then still Rhône–Poulenc Rhorer) developed a semisynthetic analogue of the natural product (docetaxel, **25b**) that enjoyed a longer protection status and topped the list of best-selling anticancer drugs for several years.¹⁰¹ Compared to natural products, synthetic compounds undoubtedly enjoy the advantage of clarity in terms of IP but the development of taxane anticancer drugs provides an important example of how the intrinsically difficult IP protection issues associated with natural products can be solved in a pharmaceutical way. Furthermore, NIH has sponsored several bioprospecting projects of International Cooperative Biodiversity Groups (ICBG) that combine academic and industrial groups and that might serve as models for programs that combine drug discovery, conservation of environmental and genetic resources, and the establishing of sustainable economic activities.¹⁰²

3.08.4.3 The Biodiversity Crisis

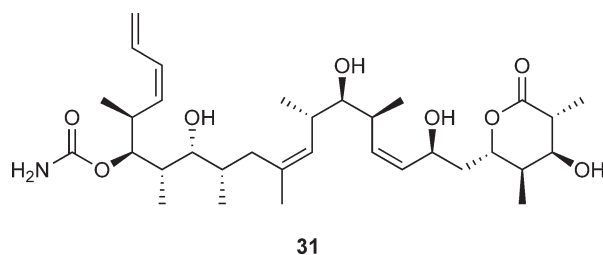
The prospect of turning natural resources to practical use has fuelled human activity for thousands of years and biodiversity is one of the most valuable but least appreciated natural resources. Some of the most important wonder drugs came from organisms not usually associated with healing, such as poisonous plants and animals, and every species has the potential to teach us something new. The calculated yearly loss of 20 000 living species therefore means that thousands of undiscovered and unique chemicals with potential utility will be lost forever, along with the genetic information necessary for their assembly.¹⁰³ The current inventory of biodiversity is very incomplete and will undoubtedly be enlarged as exotic regions and habitats are studied, with marine ecosystems being the largest unexplored habitat of life. We are monumentally uncertain as to how many living species there are on earth and even the tenet that most of the higher terrestrial plants have been discovered has been hotly debated. It has been estimated that there are at least 250 000 species of higher plants, 30 million insect species, 1.5 million species of fungi, and a similar number of algal and prokaryote species.¹⁰⁴ The uncertainty on these number is big since it has been reckoned that over 50 000 plants are still waiting to be discovered.¹⁰⁴ Whatever the case, only a fraction of the plants known have been investigated chemically or for a specific bioactivity and many of them were investigated decades ago with relatively crude techniques. Considerable disagreement also exists as to where plant biodiversity is most concentrated, although a certain consensus exists that the richest regions in terms of flowering plants are the Amazon basin, Southeast Asia, and the Mediterranean region.¹⁰⁵ Owing to difficulties in cultivation, only a tiny percentage of bacteria and fungi are known (12 and 5%, respectively) and most insects and nematodes living are still undiscovered.¹⁰⁵

Translating biological diversity into chemical diversity has long been the aim of phytochemistry and represents the first step toward a rational utilization of bioresources. Modern developments in separation techniques and spectroscopy have expedited the 'molecular cataloguing' aspect of phytochemistry, just like technological advances and genomics have simplified the work of taxonomists. Nevertheless, and paradoxically,

both taxonomy and phytochemistry are facing a lack of academic prestige and resources that is crippling the cataloguing of biological and chemical diversity just at the time when it has become most urgent. Taxonomy and phytochemistry are enabling sciences. They do not generate new ideas or test hypotheses but make it possible to open new areas of research and translational sciences, with clearly achievable and relevant goals, which are favored by the current funding system. Thus, a project on the health benefits of a certain diet will surely have more chances of being funded than one on the phytochemistry of the plants on which the program is based.

3.08.4.4 Supply Issues

Biodiversity can be lost by natural catastrophes (fires, eruptions, diseases) or by human activity and is basically less reliable than oil-derived feedstocks to secure continuous access to a product. Indeed, resupplying of an active extract is a major drawback in natural products drug discovery and the lack of a backup sample can cause substantial delay, especially for nonfermentable biomasses like plants and marine organisms while many nonmicrobial natural products cannot be produced in bulk by isolation or can be produced only after considerable efforts, as exemplified by the development of paclitaxel (**25a**) as an anticancer agent.¹⁰¹ Furthermore, many nonmicrobial natural products cannot be produced in bulk by isolation. In general, the reliability of marine feedstock as a bulk source of natural products has often been questioned, being much less than that of plants and microorganisms, and the belief that marine-derived natural products may furnish better opportunities to synthetic chemists than to medicinal chemists is still rife. The incredible ordeal represented by the 60 g synthesis of discodermolide (**31**), a marine biological analogue of paclitaxel (**25a**), gives credit to this idea. Discodermolide was prepared for preclinical studies by a total synthesis that required the combined efforts of more than 43 Novartis chemists to produce 60 g of product in an overall yield of 0.65%.¹⁰⁶ While a commercial chemical synthesis of discodermolide is still elusive, there is strong evidence that many marine secondary metabolites are actually of microbial origin and that the marine source simply represent a macroscopic host for the microbial colony actually producing the compound of interest.¹⁰⁷ Thus, it has been firmly established that tetrodotoxin, possibly the most famous marine natural product, is actually a microbial compound and that the puffer fish only accumulates it, using it as a hormone.¹⁰⁸ On the positive side, several techniques have been developed to gain access to natural products or natural products-like compounds, in nonnatural ways, with plant tissue cultures and combinatorial genetics being the most investigated 'rain forest' surrogates in terms of availability of natural products.¹⁰⁷



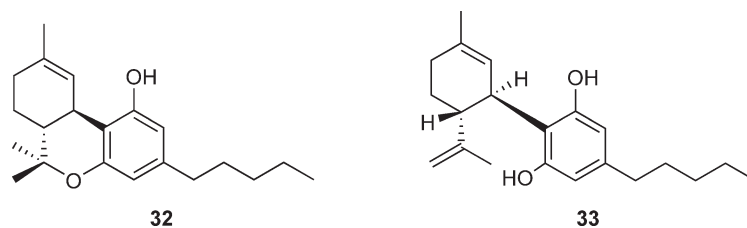
3.08.4.5 Methodological Issues

The *de novo* construction of libraries of pure natural products is prohibitively costly compared to synthetic libraries but a very big library of pure natural products (>10 000 compounds, >85% purity) has been assembled by the German biotech company AnalytiCon Discovery and made commercially available, unifying, in terms of the internal logistic of pharmaceutical companies, the screening of natural products and that of synthetic compounds.¹⁰⁹ However, libraries of crude extracts rather than pure compounds are typically screened in natural products-based drug discovery campaigns. Screening extracts in both biochemical and cell-based assays is operatively similar to screening libraries of synthetic compounds but the readouts are plagued by factors that occur more rarely in synthetic libraries and there is therefore great interest in the production of 'assay-friendly' libraries of extracts.

3.08.4.5.1 Entourage effects

The isolation of morphine from opium in 1805¹¹⁰ was the first demonstration that the activity of a medicinal plant could be attributed to a single chemical constituent, initiating natural products chemistry and the search for similar ‘quintessential’ principles in other medicinal plants. This approach was successful only for highly active or poisonous medicinal plants (heroic drugs) while the activity of the majority of medicinal plants could not be traced to a single constituent (magic bullet).

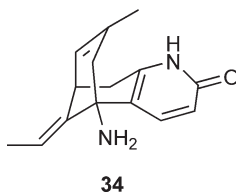
There is now growing awareness that the activity of most medicinal plants is the result of the synergistic action of several constituents (magic shotgun).¹¹¹ These concepts were deftly exploited to develop Sativex, a combination of two strains of *Cannabis* characterized by a high contents of tetrahydrocannabinol (THC; **32**) and cannabidiol (CBD; **33**), used to relieve the symptoms of multiple sclerosis and which is also under clinical development for the treatment of cancer pain.¹¹² CBD, long considered pharmacological ballast, shows anti-inflammatory activity and modulates the psychotropic effects of THC via its CB1 reverse agonism and by interfering with the hepatic 11-hydroxylation of THC, which increases the brain penetration of this psychotropic compound.¹¹³ The ‘entourage effect’ has been a deterrent for the mainstream and reductionist pharmaceutical exploitation of medicinal plants. In other words, extracts of natural origin are complex systems and we do not know how much we can simplify (fractionate) them and still have them functioning. Chronic degenerative diseases like cancer and Alzheimer’s disease are multifactorial and mixtures of compounds, or compounds with a pleiotropic mechanism of activity, are in principle more useful to treat these diseases than a single compound. Indeed, cancer and HIV are treated with cocktails of drugs and not with a single agent, while synergistic combination drugs like Augmentin, an association of a β -lactam antibiotic and a lactamase inhibitor, have been developed. Nevertheless, synergies are better deduced than planned and entourage effects are unmanageable in mainstream, magic bullet style, drug discovery campaigns.



3.08.4.5.2 False positives/negatives and reproducibility

False positives can originate from various causes, such as nonspecific hydrophobic binding, poor solubility, the tendency to form aggregates, or the presence of denaturing agents (tannins), pigments, fluorescent compounds, nonselective and widespread ligands like linoleic acid, or functional groups that react in a nonspecific way with protein targets (aldehydes, epoxides, and Michael acceptors).¹¹⁴ All these issues are more severe in extracts than in synthetic libraries, where hydrophobicity, solubility, and presence of reactive functional groups and color can be minimized at the planning stage. Conversely, extracts are generally characterized by a total lack of information on their molecular composition and, in this sense, they are black boxes. False negatives might originate from a too low concentration of an active compound in an extract, its chemical instability, the interferences with the assay readout, and/or the presence of compounds with opposite activity. Again, these issues are nonexistent or rare in synthetic libraries. Extracts are intrinsically ‘dirtier’ than synthetic libraries but can be cleaned by prefractionation, an operation that minimizes most of the false positive issues and increases the concentration of constituents, therefore improving the detectability of trace constituents. Several methods to remove tannins, protein-precipitating agents, and reactive chemicals from plant extracts have been developed.^{115,116} False negatives might also originate from the presence of compounds with opposite bioactivity and some potent natural products could probably never have been discovered using modern HTS campaigns. Thus, fiber cannabis contains THC, a cannabinoid agonist, but also CBD, a cannabinoid reverse agonist that is much more abundant than THC.¹¹³ Another case is *Lycopodium* extract, which, despite containing the very powerful nicotinic agent huperzin A (**34**), also contains anticholinergic compounds with, overall, little, if any, cholinergic activity.¹¹⁷ Clearly, the interrogation of a novel target with a high-throughput campaign based on natural products extracts might well fail to produce any useful results, since few bioassays are robust enough to

withstand the screening of complex mixtures and previous prefractionation is therefore necessary. This operation of molecular simplification limits the possibility of false positive and negative but it is undoubtedly labor intensive, time consuming, and costly. Finally, reproducibility of activity and/or composition is often an issue, being observed in approximately 40% of plant extracts as a result of differences in geography and time of plant collection, or of the presence of microbial elicitors of the production of secondary metabolites.¹¹⁸

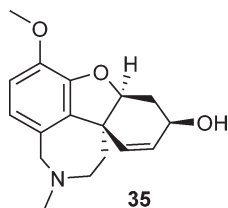


3.08.4.6 Dereplication

Natural product-based hit discovery campaigns suffer from a complete lack of information on the composition of the compounds to screen and assays are *per se* incapable of distinguishing between known and novel compounds. Dereplication, the identification of known compounds responsible for the activity of an extract before bioassay-guided fractionation,¹¹⁹ is therefore important before screening, at least in campaigns aimed at the identification of structurally novel ligands. It is therefore possible, at least in principle, that obvious ligands are ‘rediscovered’ in any nondereplicated phytochemical screening. For instance, GABA is widespread within plants and its presence interferes with assays of GABAergic activity, masking the presence of both GABA inhibitors (false negative readout) and GABA mimetics (false positive readout).¹²⁰ To minimize this problem, the NCI has developed a dereplication strategy based on HPLC fractionation with diode array detection, collection of fractions into 96-well microtiter plates, and preparation of daughter plates for either biological testing or mass spectrometry–electrospray ionization (MS–ESI) detection.¹²¹

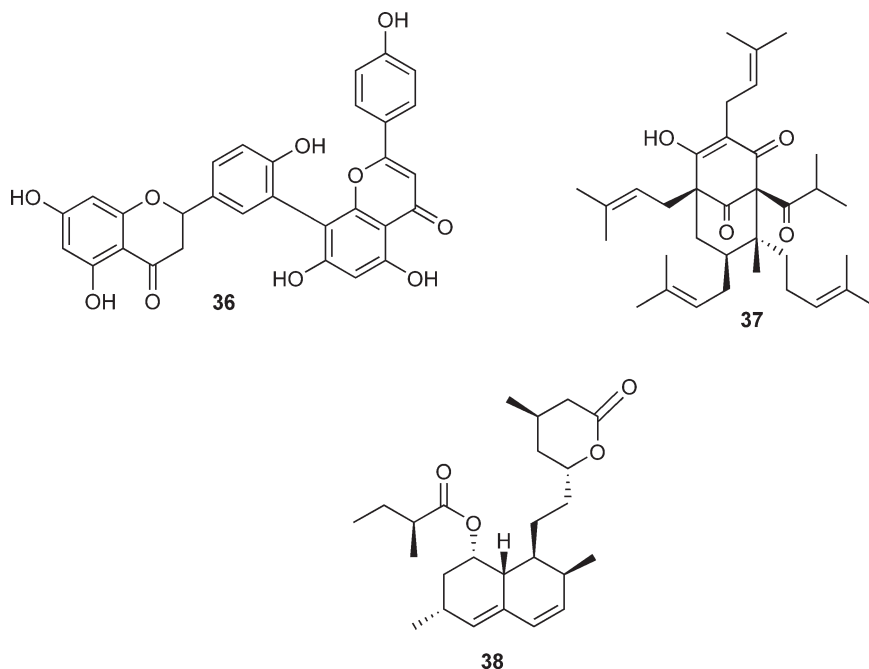
3.08.4.7 Advent of Combinatorial Chemistry and Progress in Synthetic Chemistry

The rapid identification of protein, DNA, and RNA pharmaceutical targets has driven the need for easily prepared, chemically diverse, and target-specific small-molecule ligands.¹²² HTS and combinatorial chemistry have emerged to meet this need. HTS, whose flow rate far exceeded the capacity of standard proprietary libraries, predates combinatorial chemistry and spurred its development. The design and synthesis of combinatorial libraries have focused mainly on functional group variation within members of the library, with, at least at the beginning, little, if any, stereochemical or skeletal diversification.¹²³ Considerable advances have been achieved in the past years in terms of purity and structural diversity of combinatorial libraries, which, however, remain dismally inferior to natural products in terms of diversity. Since it is nowadays accepted that biological relevance and chemical diversity are more important than the library size, several groups have been involved in the development of natural products-like libraries based on the combinatorial elaboration of scaffolds inspired by natural products.¹²³ Current pharmaceutical research needs increasingly larger number of compounds spanning as many molecular architectures as possible and phytochemical techniques minimizing manipulation and purification steps must be developed. Clearly, no magic techniques of high-throughput isolation exist and, despite all the impressive progress in isolation and structure elucidation techniques, natural products libraries will never be competitive in terms of availability and rapidity of assembly with synthetic libraries. At the same time, progress in synthetic chemistry and the spiraling of drug prices have made it possible to produce by total synthesis drugs that rival the complexity and polyfunctionalization of natural products. The anti-HIV drug enfuvirtide (Fuzeon) is a remarkable example. This 26 amino acid peptide is not produced by Roche recombinantly in engineered cells but by total synthesis, with an investment that led to a worldwide overall cost lowering of all peptide synthesis reagents, starting materials, and equipment.¹²⁴ Complex natural products like huperzine A (34) and galanthamine (35) are nowadays competitively produced by synthesis rather than by isolation,¹²⁵ and the enormous progress of the past years in synthetic methodologies and efficiency have undoubtedly made synthesis a rival of isolation for both the discovery of new drug hits and the production of bioactive natural products.



3.08.4.8 Poor Relevance to Noncytotoxic Targets

Since natural products are essentially chemical weapons, natural product-derived drugs are preeminent in the field of oncology and anti-infective therapy,¹²⁶ while chances to identify natural products leads in screening for other activities (cardiovascular, neurological, and metabolic) is undoubtedly weaker, since the source organism and human proteins did not coevolve. These low hit rates should, however, be compared to those of purely synthetic libraries and there is no shortage of examples of recent discoveries of new natural products leads and new natural product-related targets in hot areas of research like diabetes, metabolic diseases, and Alzheimer disease. A recent example is the identification of the dimeric flavone isoginkgetin (**36**) as a mechanistically new promoter of adiponectin secretion, an important antidiabetic target.¹²⁷ Adiponectin is a hormone secreted by adipocytes that increases insulin sensitivity and whose plasma level are low in diabetic and obese people. Screening of a library of drug-like synthetic compounds and natural products identified isoginkgetin, a constituent of ginkgo leaves, as a powerful inducer of adiponectin secretion, acting in a fundamentally distinct way compared to thiazolidinediones, and involving not peroxisome proliferator-activated receptor- γ (PPAR- γ) but rather AMP-activated protein kinase (AMPK).¹²⁷ Regarding the natural product-inspired discovery of new targets, a recent example is the identification of TRPC6 as the antidepressant target of the phloroglucinol hyperforin (**37**).¹²⁸ This constituent of St. John's Worth inhibits the neuronal reuptake of serotonin, dopamine, and norepinephrine, behaving as a functional biological analogue of synthetic antidepressants. However, hyperforin acts with a basically different mechanism, inducing sodium and calcium entry mediated by specific binding to TRPC6, a nonselective ion channel. Since neurotransmitter reuptake requires an efficient sodium gradient, its impairment translates into a decreased amine reuptake. The therapeutic areas of infectious diseases and oncology have undoubtedly benefited most from natural products but natural products have been successfully developed to treat human diseases in almost all therapeutic areas and it should be remarked that statins, the commercially most successful drugs ever, were molded on the microbial product lovastatin (**38**) (for more details on Natural Products of Therapeutic, see Chapter 2.19). In 2006 alone, the sales of statins were over 20 billion dollars.¹²⁹



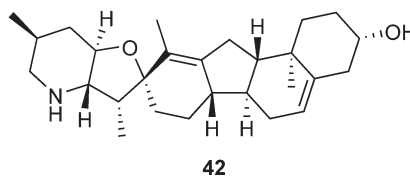
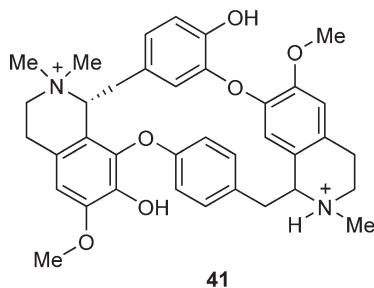
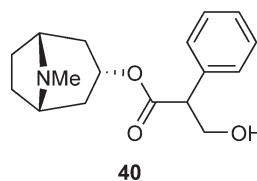
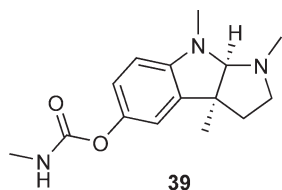
3.08.5 Strategies in Natural Products Drug Discovery

How would penicillin have fared had the initial discovery occurred in 2007, in the absence of a clearly defined molecular target against which were screened a mind-numbing collection of low-pedigree samples, often of questionably purity?

S. Danishefsky, *Chem. Eng. News* 13 October 2003, p 103

3.08.5.1 Ethnopharmacology

Traditional medicinal practices predate modern medicine by thousands of years. All indigenous populations have derived a pharmacopoeia unique to their environment and an enormous amount of information on the medicinal properties of plants, fungi, and animals exists in ethnic cultures.¹³⁰ By analyzing extensive databases of bioactivity such as the NCI list of 'active plants', it was calculated that plants with a traditional use in medicine were 2–5 times more likely to generate 'active (cytotoxic) extracts' compared to plants without an ethnopharmacological record.¹³¹ Of special interest are poisonous organisms (plants, animals, mushrooms, and microorganisms), since their 'bad' properties can be potentially translated into successful therapeutic drugs.¹³² Physostigmine (39), atropine (40), and tubocurarine (41) and botulinum toxin are important examples from the past and cyclopamine¹³³ (42) and conotoxins¹³⁴ from current research on poisonous organisms.



The use of medicinal plants in traditional medicine represents in principle a sort of preexisting clinical testing and a shortcut to biologically active compounds but the translation of ethnobotanical knowledge into commercialized products is far from simple.¹³⁵ For one thing, many traditional medicines are not based on the Hippocratic principles of disease. Thus, traditional Chinese medicine (TCM) takes a holistic approach to treatment, emphasizing the balance and harmony of the human body. Central to its practice are concepts like *yin* and *yang*, primal and opposite forces, and the spiritual energy known as *qi*, whose block causes illness. These concepts cannot be translated into molecular terms and it is therefore hardly surprising that TCM has so far contributed so little to mainstream drug discovery.¹³⁶ Furthermore, while issues like claim validation and standardization can be addressed by current pharmaceutical expertise, others like sustainability of the source and ownership of the intellectual knowledge are unusual, or downright alien, to mainstream pharmaceutical corporate culture, as is the use of mixtures of compounds like extracts, or even of mixtures of extracts. These problems are no doubt exacerbated by the current pharmaceutical legislation, which is well suited to cope with monomolecular drugs or mixtures of active pharmaceutical ingredients (APIs) but is at a loss with complex active matrixes like extracts. For this reason, special channels have been devised in the US and European Union

pharma legislation to accommodate drug derived from 'evidence-based' ethnobotanical medicinal discovery.¹³⁷ Extracts, a fundamentally rudimentary form of drug even in purified and standardized form for current pharmaceutical standards, represent an important area of drug discovery and the recent FDA approval of Veregen (polyphenon A), a standardized polyphenolic extract from green tea, for the management of genital papilloma warts represent an important example on a basically new type of natural products drug, which was approved without any evidence of mechanism of activity and on the basis of highly positive clinical results only.¹³⁸ Traditional knowledge is disappearing faster than biodiversity and many 'islands' of traditional knowledge remain to be investigated and will undoubtedly get lost forever with the current pace of globalization. The study of folk pharmacopoeias and ethnomedicine is the basis of the discovery of several important drugs and biological leads, as exemplified by digoxin, tubocurarine, ephedrine, atropine, and quinine.¹³⁹ Not only plant-derived compounds but microbial products also owe their origin to ethnopharmacology, as cogently shown by cephalosporins, whose discovery was related to the study of the so-called 'Cagliari paradox', namely the very low incidence of cholera in this Sardinian town despite the lack of a public sewage system and the habit of the inhabitants to take a bath in the polluted waters of the Su Siccu beach, later found by Brotzu to be sterile because of the presence of the antibiotic-producing mold *Cephalosporium acremonium*.¹⁴⁰ After their isolation by Brotzu in Cagliari, the development of cephalosporins as antibacterial agents was eventually carried out in England and their introduction into the clinic brought rich dividends to the National Research Development Corporation, a body set up in 1949 to exploit discoveries made by British universities and government laboratories.¹⁴¹ The clinical translation of the original discovery by Brotzu required considerable efforts from both academy and industry but in the highly politicized context of bioprospecting, can also be perceived as a blatant case of exploitation of both tangible (genetic resources) and intangible (knowledge) indigenous resources.

While ethnopharmacology is undoubtedly an asset for natural products plant discovery, this approach has some obvious limitations, even under a Hippocratic medicinal context, since many diseases are ill defined in terms of symptoms. Thus, most cancers show little if any symptoms until the late stages of the disease and they are not specific. It is therefore difficult to translate ethnopharmacological information into clinical clues for cancer, despite a monumental attempt by Hartwell.¹⁴² Even for diseases well defined in terms of symptoms, such as fever and malaria, traditional use might have missed important plants. A striking case is artemisinin (26). This antimalarial drug was discovered in a Chinese medicinal plant (*Artemisia annua* L.) that was substantially overlooked in terms of antimalarial use in the TCM.¹³⁶ Indeed, the Jesuit penetration in China in the seventeenth century was spurred by the healing of the Chinese emperor by *Cinchona*, the miracle antimalarial plant traded by Jesuits. Pure artemisinin is not orally available, although it was reported that a certain absorption takes place from crude extracts containing flavonoids,¹⁴³ and *A. annua*, even with all the limitations implicit in the translation of folklore indications into modern medicine, was not sufficiently emphasized as an antimalarial agent in TCM.¹³⁶

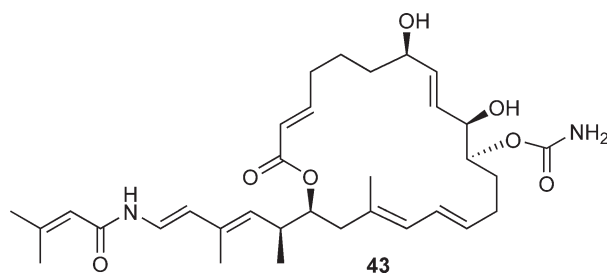
3.08.5.2 Ecology

The preservation of biodiversity goes beyond the simple cataloguing of living species but also involves the study of their physiology and the preservation of their relationships. Biodiversity is therefore strictly related to the conservation of a specific environment as a whole and it would be limiting to associate it to botanical herbaria, fungal collections, or aquaria. The study of the ecology of a species can afford interesting clues for drug discovery, as exemplified by exenatide (Byetta), a drug derived from a lizard venom and the first incretin mimetic introduced into the clinic.¹⁴⁴ The Gila monster (*Heloderma suspectum*), a poisonous desert reptile from the American Southwest and Northern Mexico, can withstand long periods of fasting, eating only 3 or 4 times a year. The physiological bases for this remarkable feeding behavior was traced to the presence of a salivary hormone (exendin-4) that slow down the digestion and the absorption of food.¹⁴⁵ Exendin-4, a 39 amino acid peptide, shows an approximately 50% analogy with glucagon-like peptide-1 (GLP-1), a hormone that increases the production of insulin when blood sugar levels are high. Exendin-4 is more potent than GLP-1 to enhance glucose-dependent insulin synthesis from pancreatic beta cells, to decrease glucagon production, and to slow down gastric emptying. Furthermore, exendin-4 has longer duration of action than GLP-1, with a half-life of

over 2 h versus less than 1 min for the human hormone, being resistant to enzymatic inactivation by dipeptidyl peptidase-IV (DPP-IV). A synthetic form of exendin-4 (exenatide, Byetta) was approved by FDA in April 2005 for the control of type II diabetes in patients whose blood glucose cannot be controlled with oral diabetic agents (metformin, sulfonylureas, or thiazolidinediones) alone.¹⁴⁴ The wild population of Gila monster is declining rapidly due to habitat loss and illegal hunting for the pet trade. The project *Heloderma* has been established to save the Gila monster and related species from extinction and Eli Lilly, the company that commercializes Byetta, is making a charitable contribution to this project. Byetta is an interesting example of drug coming from a threatened species and whose clinical exploitation is actually helping its preservation. The limitation of the ecological approach to natural products drug discovery is that most targets of high-throughput screens are not easily translated into observable phenomena that can provide prospecting clues. Thus, while the observation of a fruit that does not rot can suggest the presence of antibacterial compounds, most drug targets cannot benefit from this type of observation.

3.08.5.3 Unconventional Natural Products Sources

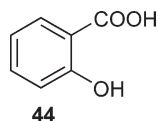
Plants and microorganisms, especially *Actinomycetes*, are the most validated sources of natural products drugs, especially in consideration of the facility of their cultivation or fermentation. Even so, only a fraction of the known plants and microbial species have been investigated for their pharmaceutical potential and other biodiversity sources are still largely or completely unexplored and untapped.¹⁴⁶ In general, the taxonomic and geographical diversity of bioprospecting has constantly increased and now encompasses cyanobacteria, endophytic fungi, sponges, mollusks, seaweeds, insects, and amphibians. Particularly impressive is the bewildering variety of structurally unique natural products isolated from marine organisms, often with no counterpart in terrestrial organisms.¹⁴⁷ However, the difficulties of collection and scale-up of marine natural products are formidable, also because the identity of the actual biological producer is often unknown and its propagation in a commercial setting unpractical. Thus, it seems well established that, especially in sponges, the production of secondary metabolites is due to coexisting microorganisms, especially cyanobacteria, and not due to the their host.¹⁴⁸ The identification and fermentation of these marine microorganisms could represent a revolutionary twist in marine natural products chemistry, paving the way for the clinical exploitation of an area of the chemical space distinct from that of terrestrial natural products that has lagged far behind in terms of pharmaceutical exploitation essentially because of the lack of a sustainable supply.¹⁴⁹ Some environmental niches are still completely pristine in terms of bioprospecting, with Antarctica being a preminent example. Despite its harshness, this habitat supports a thriving community of invertebrates and algae that produce very interesting products, such as the polyketide palmerolide A (**43**) from the tunicate *Synoicum adareanum*.¹⁵⁰ Palmerolide A, so named from the Palmer Station on the Antarctic Peninsula in whose vicinity its animal source was collected, is a potent antimelanoma agent and a one-digit nanomolar inhibitor of V-ATPase, a vacuolar proton-translocating enzyme that acidifies organelles of both constitutive and regulated secretory pathways.^{150–152} Extremophile microorganisms from a variety of inhospitable terrestrial and marine sources, such as acidic hot springs (acidophiles), alkaline lakes (halophiles), deep-sea vents (baro- and thermophiles), polar waters, and alpine lakes (psychrophiles) hold great promise. It is not unreasonable that, just like enzymes from extremophiles supported the discovery of PCR, also interesting drug leads might come from the study of their secondary metabolites.¹⁵³



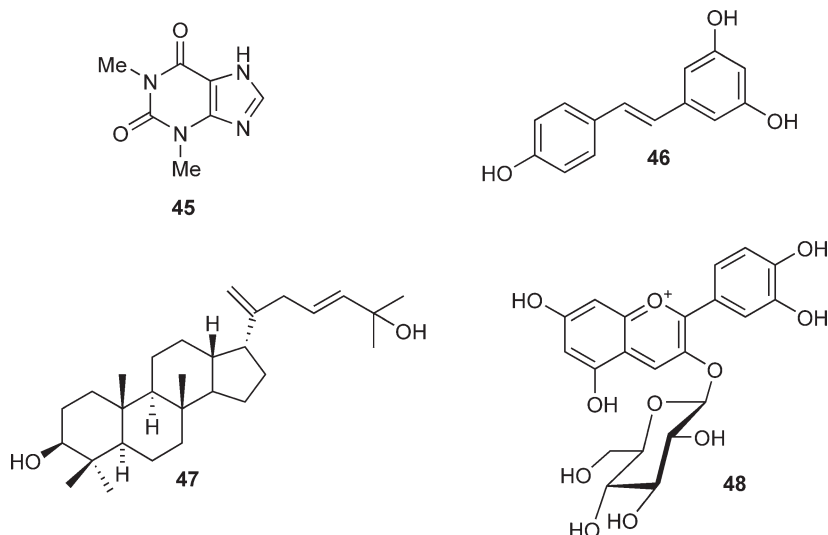
Apart from these exotic sources, it should also be pointed out that only a fraction of soil microorganisms can be cultured and have therefore been investigated for the production of secondary metabolites.¹⁵⁴ To get around this issue, genetic material coding for secondary metabolites can be obtained directly from the soil and expressed in a host organism. The secondary metabolites obtained so far from environmental DNA are rather similar to those produced by fermentable microorganisms,¹⁵⁵ but there have been only few studies of this type and more systematic investigations might lead to uncharted areas of the biological chemical space. Overall, there is no shortage of areas of the world and habitats where new and unusual chemodiversity can be discovered and the major limitation of these studies is that, since we know so little on the ecology of unconventional environments, there are no clues to select in biorational ways the organisms to study.

3.08.5.4 Edible Plants

Humans are daily exposed to a multitude of secondary metabolites contained in edible plants and spices. These compounds have accompanied us during evolution, playing a role in the shaping of our genome and making us not what we eat but rather what our ancestors have eaten.¹⁵⁶ Dietary secondary metabolites are not considered as nutrients but appear to play a role, still undefined in molecular terms, in the maintenance of health, and there is therefore great interest in their identification and in the characterization of their biological profile. Dietary compounds are the basis of the development of highly successful drugs, such as lovastatin (38) and salicylic acid (44), the archetypal statin and nonsteroid anti-inflammatory drugs, respectively. Lovastatin occurs in the red yeast of rice (*Monascus ruber*), an ingredient of Eastern cuisine used to give a red color to the Pekinese duck,¹⁵⁷ while salicylic acid is ubiquitous in plants.¹⁵⁸ Remarkably, the isolation of lovastatin from the dietary mold *M. ruber* was reported by Endo 1 year before Merck described its obtaining from *Aspergillus terreus*.¹⁵⁹ Other important dietary drug candidates are curcumin (12) from turmeric¹⁶⁰ and capsaicin from hot pepper (13),⁵⁴ while traces of pharmaceutical benzodiazepins (including diazepam) occur in common edible plants like potatoes and cherries.¹⁶¹

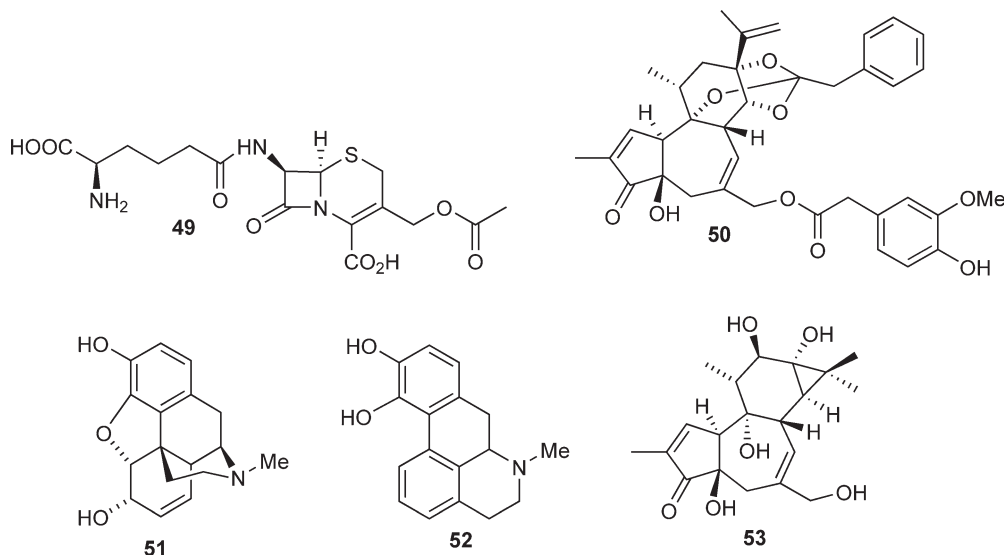


Dietary observations have afforded many clues to drug discovery. The antiasthmatic properties of theophylline (45), a caffeine metabolite and a minor constituent of tea, were discovered because of the improvement of breathing problems of asthmatic patients who consumed strong black coffee,¹⁶² and resveratrol (46) came under the limelight because of the alleged protective effect of red wine in the fat-rich French diet (French paradox).¹⁶³ Resveratrol, a pleiotropic agent that has raised considerable interest as a sirtuin ligand, was recently granted orphan drug status for the treatment of encephalomyopathy, a rare disease.¹⁶⁴ Also, negative dietary correlations can afford clues to drug discovery. Thus, the potent immunosuppressant dammarane triterpenoid (47) was discovered because of epidemiological correlations between the incidence of cancer and the consumption of palmyrah flour (*Borassus flabellifer*), a staple food of Sri Lankan Tamils.¹⁶⁵ The major limitation of the many dietary clues is that the beneficial or detrimental effects of health resist a reductionistic analysis, being the results of a combination of principles and their bacterial and hepatic metabolites. Anthocyanosides are remarkable examples. They are the most abundant dietary flavonoids and show a remarkable pattern of activity *in vitro* but are also chemical chameleons, varying in structure, polarity, and overall charge according to the pH of the medium and suffering from an outmost complex enteric and hepatic metabolism as well as entourage effect in their activity.¹⁶⁶ Anthocyanosides such as cyanidin glucoside (48) have recently raised great interest as antiobesity agents, due to their inhibiting properties on the differentiation of adipocytes and their lack of toxicity.¹⁶⁷

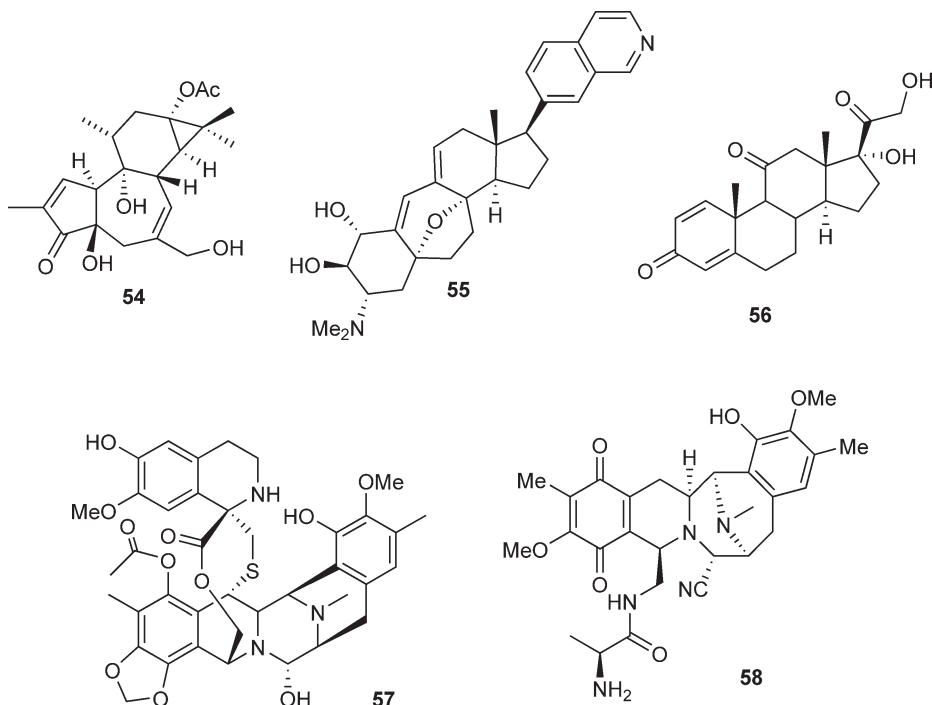


3.08.5.5 Derivatization, Diverted Total Synthesis, Diversity-Oriented Synthesis, and Semisynthesis

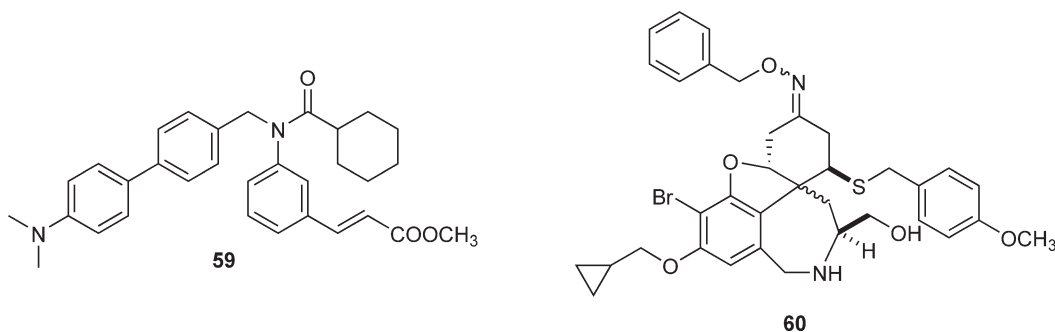
Because of toxicity, modest activity, poor solubility and stability, or overall unsatisfactory ADMET (absorption, distribution, metabolism, elimination, toxicology) profile, many natural products are of limited clinical use as such. Cephalosporin C (49), CPT (14), and curcumin (12) exemplify this situation in terms of suboptimal potency, toxicity, and oral bioavailability, respectively. However, natural products can be ‘domesticated’ by suitable chemical derivatization. In some cases, chemical modification can revert activity (iodination of the ultrapotent vanilloid resiniferatoxin (RTX; 50),¹⁶⁸ *N*-methyl for *N*-allyl swap in morphine (51)¹⁶⁹) or redirect it to unnatural targets, as observed for morphine (51, an opioid agonist) and its acidic rearrangement product apomorphine (52, a dopamine ligand).¹⁶⁹ However, natural products are often too complex for straightforward chemical derivatization and the exuberance of functional groups means that their reactivity is often unpredictable, with the need to develop *ad hoc* solution of specific and tailored applicability. For instance, the secondary hydroxyl of phorbol (53), a key element of its pharmacophore, is less reactive than the adjacent tertiary hydroxyl, which can be esterified chemoselectively even in the presence of the primary allylic hydroxyl.¹⁷⁰ Patterns of reactivity like this are difficult to predict and require a careful preliminary study, with a consequent slowing down of the drug discovery campaign. Furthermore, in complex natural products, the reactivity of functional groups can be quenched by an unfavorable steric environment, as exemplified by the endocyclic double bond of paclitaxel (25a), which is resistant to hydrogenation,¹⁷¹ or the C-9 tertiary hydroxyl of phorbol (53), which is characterized by total chemical inertness.¹⁷² The manipulation of these cryptic functional groups might be of enormous biological relevance and could provide a solution to long-standing biological issues, such as the mode of binding of phorbol esters to PKC. Finally, there are limitations in the extent of the structure–activity relationships that can be studied using the functionalization pattern of a natural product. This is especially marked for apolar moieties that lack functional groups or that only bear functional groups redundant for activity. To address these issues, the concept of diverted total synthesis has been proposed by Wilson and Danishefsky.¹⁷³ The most straightforward way to assemble a complex target is by using a convergent synthesis, where smaller modules are combined sequentially en route to the target. The reactivity pattern of these small fragments is generally predictable and by feeding these modified fragments into the pipeline of the synthetic scheme, a full exploration of the structure–activity relationships can be achieved. Major applications of this strategy were described in the field of anticancer compounds, using epothilones and radicicol as leads.¹⁷³



Over the previous years, there has also been an increased interest for the semisynthesis of complex natural products, with notable achievements by Wender *et al.* (prostratin (**54**) from phorbol (**53**))¹⁷⁴ and Baran and coworkers (cortistatin (**55**) from prednisone (**56**)).¹⁷⁵ By elaborating easily available compounds, semisynthesis can provide a scalable access to complex structures difficult to source. It requires great ingenuity since synthetic creativity is constrained by the connectivity and configuration of the starting material. The industrial production of paclitaxel (**25a**)¹⁷¹ and of ecteinascidin-743 (**57**) are examples of important industrial applications of semisynthesis to the production of natural products drugs. The marine anticancer compound ecteinascidin-743 (Yondelis), used for the treatment of soft-tissue sarcoma, was originally isolated from the marine tunicate *Ecteinascidia turbinata*. Wild harvest of this organism could not have supported its clinical development, which relied on aquaculture to afford the small amounts required at that stage. A total synthesis was reported by Corey *et al.*¹⁷⁶ but the supply problem was eventually solved by semisynthesis from a related microbial compound, cyanosafrafin B (**58**), from *Pseudomonas fluorescens*.¹⁷⁷

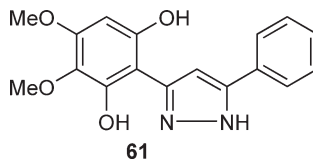


Many natural products are easily available in multigram amounts by isolation and boast a rich decoration of reactive functional groups as well as complex skeleton amenable to rearrangement. This chemical exuberance could be coupled to efficient technology platforms like combinatorial chemistry or diversity-oriented synthesis^{178,179} to expand the pool of natural products and generate new modulators of biological activity.¹⁸⁰ Many attempts have also been made to combine the quality of natural products and the speed and efficiency of modern synthetic technologies by using natural products motifs as scaffolds to build combinatorial libraries. The efficiency of this process is exemplified by the discovery of fexaramine (**59**), an inhibitor of farnesoid X-receptor,¹⁸¹ and of secramine (**60**), an inhibitor of protein trafficking by the Golgi apparatus.¹⁸² These molecular probes emerged from synthetic combinatorial libraries built on the 2,2-dimethylbenzopyran motif¹⁸³ and on the tetracyclic core of galanthamine.¹⁸²



3.08.5.6 Extract Engineering

Crude extracts often contain a series of related compounds that share a common functionality that can make up for a large proportion of the extract. The crude extracts can be directly treated with a reagent specific for this functionality, generating a modified 'secondary' extract containing semisynthetic compounds that can be screened for a useful activity. In this way, the exploitable molecular diversity from a given biological source can be substantially increased. This principle was proposed by Furlan, who investigated the antifungal activity of a series of natural extracts containing flavones. Noticing the paucity of N–N motifs in natural products compared to their abundance in drugs, the extract was treated with hydrazine, affording an engineered extract where the flavone constituents had been converted to their corresponding pyrazoles by remodeling of the central C ring. Remarkably, while the natural extract lacked antifungal activity, the engineered one showed interesting activity against human fungal pathogens, traced by bioassay-directed fractionation, to the flavone-derived pyrazole (**61**).¹⁸⁴ This ingenious strategy should be further investigated for its generality and holds undoubtedly great potential, although not many extracts are amenable to simple engineering.

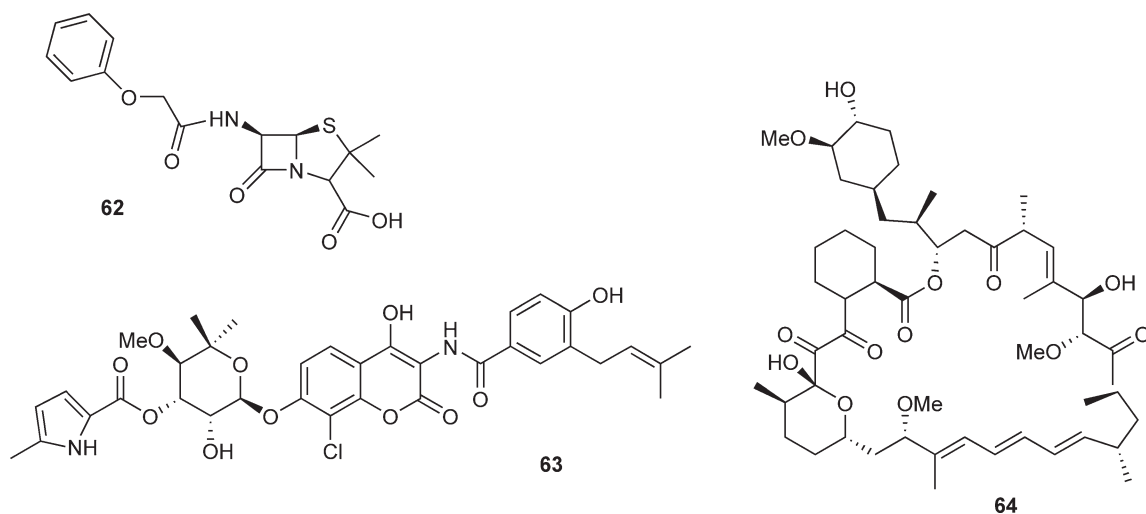


3.08.5.7 Engineered Biosynthesis (Mutasynthesis, Combinatorial, and Transgenic Biosynthesis)

The living organisms are just a tiny fraction of those that have inhabited the earth and that went extinct during evolution. The extraordinary metabolic richness and unicity of living fossils like the ginkgo tree points to a chemically exuberant past that we will never be able to recapture. Millions of transient natural products were evolutionarily deselected along the pathway that eventually led to the natural products of today. Thus, hydrophobic hopanoid pentacyclic triterpenoids arose early in evolution (*Archaeobacteria*) as integral stabilizers

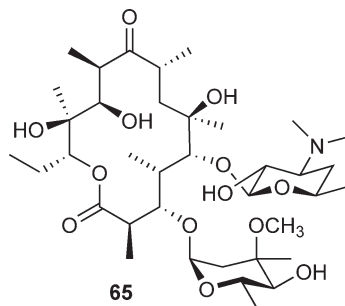
of hydrophobic membranes, followed by phytoesters in plants, and eventually cholesterol in animal cells.¹⁸⁵ The very fact that squalene and squalene oxide can be cyclized in almost 100 different folding patterns to afford cyclosqualenoids gives a glimpse of the approach followed by nature to optimize natural products and generate today's chemodiversity, and of the intrinsic potential of biosynthetic pathways to generate a bewildering array of different structures.¹⁸⁵ Plants and microorganisms have biogenetic pathways that are expressed only under certain conditions and there is an enormous hidden chemical diversity apparent only at the genome level. We might ignore the reasons as to why most folding of squalene and squalene oxide were either never considered by nature or evolutionarily deselected but, thanks to molecular genetics, we are now in the position to randomly mutate key biogenetic enzymes, generating natural (since enzyme-derived) products in an unnatural way (molecular biology) and somehow mimicking evolution (for more details, see Chapter 2.20).

While biosynthetic engineering is still in its infancy, modification of a biosynthetic way by the addition of suitable building blocks has been pursued since the early studies on β -lactam antibiotics, as testified by the industrial production of penicillin V (phenoxymethylpenicillin, **62**) by the addition of phenoxyacetic acid to fermentation of *Penicillium chrysogenum*, a process established already in the 1950s.¹⁸⁵ Since the capacity to produce the natural compounds is retained, precursor-directed biosynthesis leads to a mixture of natural and unnatural compounds, resulting from competition between the natural building block and its unnatural analogue. To overcome this limitation, mutasynthesis, which is the use of microorganisms where the production of a specific building block is deficient because of an induced genetic mutation, has been developed. By blocking the biosynthesis of a specific precursor, the production of a complex compound becomes dependent on the supplementation with that specific precursor, which acts as a sort of metabolic 'vitamin'. The loose substrate specificity of many biosynthetic enzymes makes it possible to replace the natural precursor with modified versions of it. Mutasynthesis is especially suitable for modification of compounds having a modular structure. Thus, the aminocoumarin hsp90 inhibitor antibiotic chlorobiocin (**63**) consists of three elements, an aminocoumarin core, an acylated novobiiose moiety, and a 3-prenyl-4-hydroxybenzoyl group (dimethylallyl-hydroxybenzoic acid, DMAHB). The introduction of the prenyl group is achieved by the dimethylallyl transferase CloQ and, by using molecular engineering, a strain of *Streptomyces roseochromogenes* was constructed where the *cloQ* gene was inactivated. Supplementation with analogues of DMAHB led to their incorporation into the biogenetic pathway and to the generation of chlorobiocin analogues.¹⁸⁶ A similar strategy but based on the shikimate-derived 4,5-dihydroxycyclohex-1-enecarboxylic acid was employed to generate analogues of the immunosuppressant polyketide rapamycin (**64**).¹⁸⁷

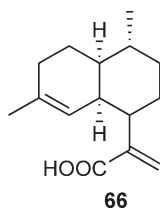


In combinatorial biosynthesis, genes from different but related biosynthetic pathways are combined to produce new compounds and this strategy has been particularly successful with polyketides.¹⁸⁸ These modular compounds represent the single most successful class of natural products drugs, with a lineup of compounds that encompasses first-in-the-class agents like lovastatin, erythromycin, tetracycline, doxorubicin,

amphotericin B, tacrolimus, and avermectin. Polyketides are built from a linear chain of carbon atoms generated by sequential reactions governed by polyketide synthases (PKSs), basically enzymic complexes that act like an assembly line tethering a starter unit and growing it. At the end of the process, the chain is untethered and cyclized by non-PKS enzymes (see Chapters 1.02–1.07). Additional enzymatic reactions introduce further decorations, such as sugars and methyl groups, while some PKSs also have ketone-modifying properties. Since genes in a polyketide pathway are always clustered together in contiguous DNA sequences, their isolation is easy, unlike other biogenetic pathways whose genes are dispersed in different chromosomal locations and must be isolated one at a time. Thus, a study of the biosynthesis of the polyketide antibiotic erythromycin (**65**) has resulted in the identification of some 28 domains. Repositioning the sequence of the corresponding genes enabled then to produce new ‘unnatural’ natural products.¹⁸⁹ A similar combinatorial approach was applied to the production of epothilones and to nonribosomal peptides.¹⁹⁰



Natural products can, in principle, be also obtained from a direct biotechnological route, where all the genes involved in its biosynthesis are expressed in a fermentable host. The transgenic production of the antimalarial sesquiterpene lactone artemisinin (**26**) is currently investigated as a cheap alternative to isolation from *A. annua* L. or to total synthesis.¹⁹¹ A biochemical and chemical precursor of artemisinin (artemisinic acid, **66**) has been produced in acceptable yield from the fermentation of an engineered strain of the yeast *Saccharomyces cerevisiae* where the production of farnesyl diphosphate was diverted from the triterpenoid sink to the sesquiterpene pool. The amorphaadiene synthase gene and a cytochrome P-450 monooxygenase from *A. annua* were then expressed in this engineered yeast, overall resulting in the conversion of farnesyl diphosphate into artemisinic acid.¹⁹¹



There are clearly several strategies to ‘take the nature out of natural products’ and produce them in a nonnatural way. Remarkably, these strategies have relevance not only for the mass production of a natural products drug but also for providing access to natural products-related chemodiversity.

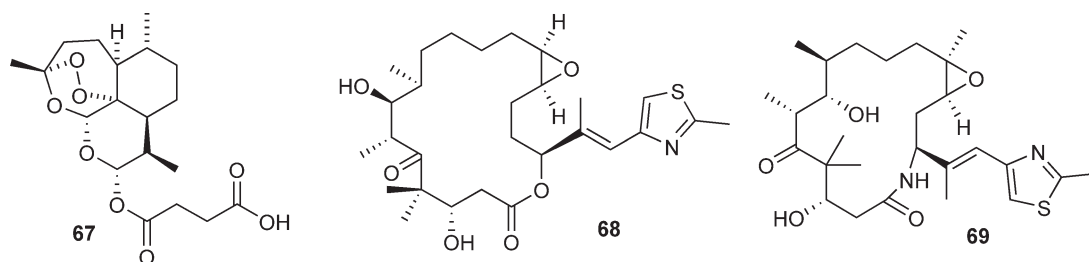
3.08.6 Conclusions

The point is not that natural products will solve all problems. It is that a lot of problems are not being solved because natural products are not being examined.

S. J. Gould, *Chem. Eng. News* 13 October 2003, p 103

There is no doubt that natural products represent the best and most validated source to start a drug discovery campaign to a new druggable target but natural products can be difficult to access efficiently and effectively, unsuitable for further development due to poor ADMET properties, and plagued by IP issues. In the current

scenario of drug discovery, the dwindling use of natural products as pharmaceutical leads seems related to the intrinsically slower and more resource-intensive nature of natural products research compared to combinatorial chemistry and rational (*ab initio*) drug design. To remain competitive in drug discovery, natural products research should sharpen its tools by proper methodological evolution, interfacing with the current strategies of drug discovery, and overall, moving to higher throughput. In general, natural product-based drug discovery activities should be integrated with complementary technologies, such as combinatorial chemistry and rational drug discovery, and not be pursued alone in an independent fashion. They should also take advantage of techniques complementary to bioprospecting, such as derivatization of existing and easily available natural products, diverted total synthesis, and the high-throughput *de novo* construction of natural product-like scaffolds. Natural products have a function in the environment and nature is the functional filter that is lacking in combinatorial chemistry. A small collection of ‘smart’ compounds like those present in a plant extract or a fermentation broth will always be more valuable than a collection of randomly assembled synthetic compounds but the access to these ‘intelligent’ collections should be made technically easier and legally transparent, while the pharmacokinetic and proprietary profile of natural products could be improved by tailor-made chemical modification. The transition from paclitaxel (**25a**) to docetaxel (**25b**), from artemisinin (**26**) to artesunate (**67**),¹⁹² or from epothilone B (**68**) to ixabepilone (**69**),¹⁹³ just to mention only recent examples, cogently demonstrates the success of this approach.



Given a promising natural product lead, there seems to be no difficulty in convincing big pharma to invest in its chemical derivatization and development. What is getting increasingly difficult is, paradoxically, to convince corporate decision makers that interesting natural products ligands, hits, leads, and even readymade drugs can originate from the study of biodiversity and of natural products libraries. It seems therefore logical to end up with a quotation from Samuel Danishefsky, possibly the most outspoken paladin for natural products in drug discovery, who, “at the risk of sounding Neanderthal,” urged drug companies to “get back to the screening of natural products” and “critically examine the prevailing supposition that synthesizing zillions of compounds at a time is necessarily going to cut the costs of drug discovery or fill pharma pipelines with new drugs anytime soon.”¹⁹⁴

References

1. A. M. Rouhi, *Chem. Eng. News* 13 October 2003, 77–91.
2. I. Paterson; E. A. Anderson, *Science* **2005**, *310*, 451–453.
3. W. R. Strohl, *Drug Discov. Today* **2000**, *5*, 39–41.
4. G. Cragg; D. Newman, *Chem. Br.* **2001**, 22–26.
5. J. Meinwald; T. Eisner, *Helv. Chim. Acta* **2003**, *86*, 3633–3637.
6. D. J. Newman; G. M. Cragg; K. M. Snader, *J. Nat. Prod.* **2003**, *66*, 1022–1037.
7. A. M. Rouhi, *Chem. Eng. News* 13 October 2003, 93–103, 104–106.
8. D. J. Newman; G. M. Cragg; D. G. I. Kingston, *Natural Products as Pharmaceuticals and Sources for Lead Structures*. In *The Practice of Medicinal Chemistry*; 2nd ed.; C. G. Wermuth, Ed.; Academic Press: London; 2003; pp 91–110.
9. M. S. Butler, *J. Nat. Prod.* **2004**, *67*, 2141–2153.
10. J. Clardy; C. Walsh, *Nature* **2004**, *432*, 829–837.
11. F. E. Koehn; G. T. Carter, *Nat. Rev. Drug Discov.* **2005**, *4*, 206–220.
12. M. S. Butler, *Nat. Prod. Rep.* **2005**, *22*, 162–195.
13. D. D. Baker; M. Chu; U. Oza; V. Rajgarhia, *Nat. Prod. Rep.* **2007**, *24*, 1225–1244.
14. J. D. McChesney; S. K. Venkataraman; J. T. Henri, *Phytochemistry* **2007**, *68*, 2015–2022.
15. A. Saklani; S. K. Kuttly, *Drug Discov. Today* **2008**, *13*, 161–171.

16. M. Pucheault, *Org. Biomol. Chem.* **2008**, *6*, 424–432.
17. M. S. Butler, *Nat. Prod. Rep.* **2008**, *25*, 475–516.
18. O. Potterat; M. Hamburger, *Progr. Drug Res.* **2008**, *65*, 47–118.
19. D. J. Newman, *J. Med. Chem.* **2008**, *51*, 2589–2599.
20. P. J. Hajduk; J. R. Huth; S. W. Fesik, *J. Med. Chem.* **2005**, *48*, 2518–2525.
21. C. P. Adams; V. V. Brantner, *Drug Dev.* **2006**, *25*, 23–24.
22. I. Kola; J. Landis, *Nat. Rev. Drug Discov.* **2004**, *3*, 711–716.
23. L. Smith, *The Pink Sheet* 10 December 2008, 13.
24. N. Blow, *Nature* **2007**, *450*, 1117–1120.
25. C. Dombrowski, *The Pink Sheet* **2008**, *70* (23), 27–28.
26. P. Geborek; E. Nitelius; S. Noltorp; H. Petri; L. Jacobsson; L. Larson; T. Saxne; I. Leden, *Ann. Rheum. Dis.* **2005**, *64*, 1805–1807.
27. A. Catlin; C. Cowan; S. Heffler; B. Washington, *Health Aff. (Millwood)* **2007**, *26*, 142–153.
28. A. Johson, *The Wall Street Journal* 12 December 2007.
29. J. Merrill, *The Pink Sheet* 10 December 2008, p 5.
30. P. Imming; C. Sinning; A. Meyer, *Nat. Rev. Drug Discov.* **2006**, *5*, 821–832.
31. K. H. Altmann; J. Gertsch, *Nat. Prod. Rep.* **2007**, *24*, 327–352.
32. Y. Uehara, *Curr. Cancer Drug Targets* **2003**, *3*, 325–330.
33. P. Workman, *Curr. Cancer Drug Targets* **2003**, *3*, 297–300.
34. J. M. McPartland; G. W. Guy, The Evolution of Cannabis and Coevolution with the Cannabinoid Receptor – A Hypothesis. In *The Medicinal Uses of Cannabis and Canabinoids*; G. W. Guy, B. A. Whittle, P. J. Robson, Eds.; Pharmaceutical Press: London, 2004; pp 71–101.
35. E. Jung; A.-L. Veuthney; E. Gasteiger; A. Bairoch, *Proteomics* **2001**, *1*, 262–268.
36. A. L. Hopkins; C. R. Groom, *Nat. Rev. Drug Discov.* **2002**, *1*, 727–730.
37. J. P. Overington; B. Al-Lazikani; A. L. Hopkins, *Nat. Rev. Drug Discov.* **2006**, *5*, 993–996.
38. R. Breinbauer; I. R. Vetter; H. Waldmann, *Angew. Chem. Int. Ed.* **2002**, *41*, 2878–2890.
39. B. M. McArdle; M. R. Campitelli; R. J. Quinn, *J. Nat. Prod.* **2006**, *69*, 14–17.
40. B. A. Halkier; J. Gershenzon, *Ann. Rev. Plant Biol.* **2006**, *57*, 303–333.
41. E. Block, *Angew Chem Int. Ed.* **1992**, *104*, 1158–1203.
42. K. C. Nicolaou; W. M. Dai, *Ang. Chem. Int. Ed.* **1991**, *30*, 1387–1416.
43. M. L. Guzman; R. M. Rossi; L. Karnischky; X. Li; D. R. Peterson; D. S. Howard; C. T. Jordan, *Blood* **2005**, *105*, 4163–4169.
44. A. Louw; P. Swart; S. S. de Kock; K. J. van der Merwe, *Biochem. Pharmacol.* **1997**, *53*, 189–197.
45. A. Louw; P. Swart, *Endocrinology* **1999**, *140*, 2044–2053.
46. P. Dewint; V. Gossye; K. De Bosscher; W. Vanden Berghe; K. Van Beneden; D. Derocher; S. Van Calenbergh; U. Mueller-Ladner; B. Vander Cruyssen; G. Verbruggen; G. Haegeman; D. Elewaut, *J. Immunol.* **2008**, *180*, 2608–2615.
47. R. D. Couch; R. G. Browning; T. Honda; G. W. Gribble; D. L. Wright; M. B. Sporn; A. C. Anderson, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2215–2219.
48. K. T. Liby; M. M. Yore; M. B. Sporn, *Nat. Rev. Cancer* **2007**, *7*, 357–369.
49. J. C. Espin; M. T. Garcia-Conesa; F. A. Tomás-Barberán, *Phytochemistry* **2007**, *68*, 2986–3008.
50. J. W. Fahey; T. W. Kensler, *Chem. Res. Toxicol.* **2007**, *20*, 572–576.
51. D. M. Goldstein; N. S. Gray; P. P. Zarrinkar, *Nat. Rev. Drug Discov.* **2008**, *7*, 391–395.
52. J. Jossang; H. Bel-Kassoui; A. Jossang; M. Seuleman; A. Nel, *J. Org. Chem.* **2008**, *73*, 412–417.
53. C. Spickler; M. N. Brunelle; L. Brakier-Gingras, *J. Mol. Biol.* **1997**, *273*, 586–599.
54. G. Appendino, Capsaicin and Capsaicinoids. In *Modern Alkaloids: Structure, Isolation, Synthesis and Biology*; E. Fattorusso, O. Tagliatela-Scafati, Eds.; Wiley: Weinheim, 2007; pp 75–112.
55. G. Appendino; A. Minassi; A. Pagani; A. Ech-Chahad, *Curr. Pharm. Design* **2008**, *14*, 2–17.
56. S. Sirikantaramas; M. Yanazaki; K. Saito, *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 6782–6786.
57. S. Bruzzone; I. Moreschi; C. Usai; L. Guida; G. Damonte; A. Salis; S. Scarfi; E. Millo; A. De Flora; E. Zocchi, *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 5759–5764.
58. V. A. Halim; A. Vess; D. Scheel; S. Rosahl, *Plant Biol. (Stuttg.)* **2006**, *8*, 307–313.
59. A. A. Begum; S. Leibovitch; P. Migner; F. Zhang, *J. Exp. Bot.* **2001**, *52*, 1537–1543.
60. E. Cundliffe, *Annu. Rev. Microbiol.* **1989**, *43*, 207–233.
61. D. A. Hopwood, *Mol. Microbiol.* **2007**, *63*, 937–940.
62. S. Singh; M. H. Hager; C. Zhang; B. R. Griffith; M. S. Lee; K. Hallenga; J. L. Markley; J. S. Thorson, *ACS Chem. Biol.* **2006**, *1*, 451–460.
63. T. Henkel; R. M. Brunne; H. Mueller; F. Reichel, *Angew. Chem. Int. Ed. Engl.* **1999**, *38*, 643–647.
64. X.-H. Xo; G.-M. Yao; Y.-M. Li; J.-H. Lu; C. J. Lin; X. Wang; C.-H. Kong, *J. Nat. Prod.* **1993**, *66*, 285–288.
65. W. Sneader, *Drug Prototypes and their Exploitation*; Wiley: Chichester, 1996; p 446.
66. B. Lefkove; B. Govindarajan; J. L. Arbiser, *Expert Rev. Anti Infect. Ther.* **2007**, *5*, 373–379.
67. M. Yoshida; S. Horinouchi; T. Beppu, *Bioassays* **1995**, *17*, 423–430.
68. M. Groll; R. Huber, *Biochim. Biophys. Acta* **2004**, *1695*, 33–44.
69. X. Tao; F. Fan; V. Hoffmann; C. Y. Gao; N. S. Longo; P. Zerfas; P. E. Lpsky, *Arthritis Rheum.* **2008**, *58*, 1774–1783.
70. P. Jones; C. Steinkueler, *Curr. Pharm. Des.* **2008**, *14*, 545–561.
71. S. Omura; K. Matsuzaki; T. Fujimoto; K. Kosuge; T. Furuya; S. Fujita; A. Nakagawa, *J. Antibiot. (Tokyo)* **1991**, *44*, 117–118.
72. D. P. Arya, *Top. Heterocycl. Chem.* **2006**, *2*, 129–152.
73. C. Lipinski; A. Hopkins, *Nature* **2004**, *432*, 855–861.
74. M. Feher; J. M. Schmidt, *J. Chem. Inf. Comput. Sci.* **2003**, *43*, 218–227.
75. G. M. Cragg; D. J. Newman; K. M. Snader, *J. Nat. Prod.* **1997**, *60*, 52–60.
76. K. P. Mishra; L. Ganjum; M. Sairam; P. K. Banerjee; R. C. Sawhney, *Biomed. Pharmacother.* **2008**, *62*, 94–98.

77. G. R. Elridge; H. C. Vervoort; C. M. Lee; P. A. Cremin; C. T. Williams; S. M. Hart; M. G. Goering; M. O'Neil; M. Johnson; L. Zeng, *Anal. Chem.* **2002**, *74*, 3963–3971.
78. H. N. Woller, *Mol. Diversity* **1999**, *4*, 47–52.
79. M. Hamburger, *Chimia* **2006**, *60*, 14–18.
80. R. N. Young, *Pure Appl. Chem.* **1999**, *71*, 1655–1661.
81. A. I. Graul; E. Cruces; L. Revel; N. Serradell; E. Rosa, *Drug News Perspect.* **2008**, *21*, 44–58.
82. E. K. Wilson, *Chem. Eng. News*, 13 February 2006, pp 86–87.
83. G. M. Cragg, *Med. Res. Rev.* **1998**, *18*, 315–331.
84. M. E. Wall; M. C. Wani, *Cancer Res.* **1995**, *55*, 753–760.
85. E. Hsu, *Br. J. Clin. Pharmacol.* **2006**, *61*, 666–670.
86. J. Wang; S. M. Soisson; K. Young; W. Shoop; S. Kodali; A. Galgoci; R. Painter; G. Parthasarathy; Y. S. Tang; R. H. S. Cummings; K. Dorso; M. Motyl; H. Jayasuriya; J. Ondeyka; K. Herath; C. Zhang; L. Hernandez; J. Allocco; A. Basilio; J. R. Tormo; O. Genilloud; F. Vicente; F. Pelaez; L. Colwell; S. H. Lee; B. Michael; T. Felcetto; C. Gill; L. L. Silver; J. D. Hermes; K. Bartizal; J. Barrett; D. Schmatz; J. W. Becker; D. Cully; S. B. Singh, *Nature* **2006**, *441*, 358–361.
87. M. D. Coughlin, Jr.; M. Columb, *J. Transn. Law* **1993**, *31*, 337–375.
88. Challenges in Negotiating and Implementing ABS Agreements. http://www.iisd.org/pdf/2006/abs_session2_augustine.ppt
89. G. M. Cragg; M. R. Boyd; J. H. Cardellina; M. R. Grever; S. A. Schepartz; K. M. Snader; M. Suffness, *Human Medicinal Agents from Plants*; ACS Symposium Series 534; American Chemical Society: Washington, 1993.
90. T. B. Lowinger; B. Riedl; J. Dumas; R. A. Smith, *Curr. Pharm. Des.* **2002**, *8*, 2269–2278.
91. D. Pytel; S. Sliwinski; T. Poplawski; D. Ferriola; I. Maisterek, *Anticancer Agents Med. Chem.*, **2009**, *9*, 66–76.
92. G. C. Tron; T. Pirali; G. Sorba; F. Pagliai; S. Busacca; A. A. Genazzani, *J. Med. Chem.* **2006**, *49*, 3033–3044.
93. W. C. Widdison; S. D. Wilhelm; E. E. Cavanagh; K. R. Whiteman; B. A. Leece; Y. Kovtun; V. S. Goldmacher; H. Xie; R. M. Steeves; R. J. Lutz; R. Zhao; L. Wang; W. A. Blättler; R. V. Chari, *J. Med. Chem.* **2006**, *49*, 4392–4408.
94. M. R. Boyd, *J. Ethnopharmacol.* **1996**, *51*, 17–25.
95. G. A. Cordell, *Phytochemistry* **2000**, *55*, 463–480.
96. G. A. Cordell, *Phytochemistry* **1995**, *40*, 1585–1612.
97. T. D. Mays; K. D. Mazan, *J. Ethnopharmacol.* **1996**, *51*, 93–102.
98. M. A. Gollin, *Nat. Biotechnol.* **1999**, *17*, 921–922.
99. W. Snader, *Drug Prototypes and Their Exploitation*; Wiley: Chichester, 1996.
100. F. Pearce, *New Scientist* 18 April 2002, p 14.
101. J. Goodman; V. Walsh, *The Story of Taxol. Nature and Politics in the Pursuit of an Anti-Cancer Drug*; Cambridge University Press: Cambridge, 2001.
102. A. M. Rouhi, *Chem. Eng. News* 7 April 1997, 14–29.
103. U. Schueklenk; A. Kleinsmidt, *Dev. World Bioeth.* **2006**, *6*, 112–134.
104. S. L. Pimm; G. J. Russellm; J. L. Gittleman; T. M. Brooks, *Science* **1995**, *269*, 347–350.
105. F. Pearce, *New Scientist* 29 June 2006, p 11.
106. S. J. Mickel, *Curr. Opin. Drug Discov. Devel.* **2004**, *7*, 869–881.
107. M. Saleem; M. S. Ali; S. Hussain; A. Jabbar; M. Ashraf; Y. S. Lee, *Nat. Prod. Rep.* **2007**, *24*, 1142–1152.
108. J. W. Daly, *J. Nat. Prod.* **2004**, *67*, 1211–1215.
109. Analyticon Discovery. <http://www.ac-discovery.com/english/go.html>
110. F. W. Sertuerner, *Trommsdorff's J. Pharm.* **1805**, *13*, 234.
111. R. Mechoulam; S. Ben-Shabat, *Nat. Prod. Rep.* **1999**, *16*, 131–143.
112. M. P. Barnes, *Expert Opin. Pharmacother.* **2006**, *7*, 607–615.
113. R. G. Pertwee, *Br. J. Pharmacol.* **2008**, *153*, 199–215.
114. R. Verpoorte, *Drug Discov. Today* **1998**, *3*, 232–238.
115. M. Zhu; J. D. Phillipson; P. M. Greengrass; N. E. Bowery; Y. Cai, *Phytochemistry* **1999**, *44*, 441–447.
116. G. M. Rishton, *Drug Discov. Today* **1997**, *2*, 382–384.
117. X. Ma; C. Tan; D. Zhu; D. R. Gang; P. Xiao, *J. Ethnopharmacol.* **2007**, *113*, 15–34.
118. G. A. Cordell, *Phytochemistry* **2000**, *55*, 463–480.
119. N. CanMiddlesworth; R. J. P. Cannell, *Natural Products Isolation*. In *Methods in Biotechnology*; R. J. P. Cannell, Ed.; Humana Press: Totowa, NJ, 1998; Vol. 4, pp 279–327.
120. H. J. Kim; I. Baburin; S. Khom; S. Hering; M. Hamburger, *Planta Med.* **2008**, *74*, 521–526.
121. P. L. Smith; K. N. Maooney; R. G. Pothen; J. Clardy; D. E. Clapham, *J. Biol. Chem.* **2006**, *281*, 29897–29904.
122. S. L. Schreiber, *Science* **2000**, *287*, 1964–1969.
123. L. A. Marcaurelle; C. W. Johannes, *Prog. Drug Res.* **2008**, *66*, 189–216.
124. B. L. Bray, *Nat. Rev. Drug Discov.* **2003**, *2*, 587–593.
125. J. Marco-Contelles; M. do Carmo Carreiras; C. Rodríguez; M. Villaroya; A. G. Garcia, *Chem. Rev.* **2006**, *106*, 116–133.
126. M. S. Butler; D. J. Newman, *Prog. Drug Res.* **2008**, *65*, 3–44.
127. G. Liu; M. Grifman; J. Macdonald; P. Moller; F. Wong-Stall; Q. X. Li, *J. Endocrinol.* **2007**, *194*, 569–578.
128. K. Leuner; V. Kazanski; M. Müller; K. Essin; B. Henke; M. Gollasch; C. Harteneck; W. E. Muller, *FASEB J.* **2007**, *21*, 4101–4111.
129. P. Evers, *SCRIP* 21 May 2008, pp 31–34.
130. M. J. Plotkin, *Medicine Quest: In Search of Nature's Healing Secrets*; Viking: New York, 2000.
131. R. W. Spjut; A. R. E. Perdue, *Cancer Treat. Rep.* **1976**, *60*, 979–985.
132. Y. Gaillard; G. Pepin, *J. Chromatogr. B* **1999**, *733*, 181–229.
133. E. E. Bar; D. Stearns, *Expert Opin. Investig. Drugs* **2008**, *17*, 185–195.
134. B. M. Olivera; R. W. Teichert, *Mol. Interv.* **2007**, *7*, 251–260.
135. M. Heinrich; S. Gibbons, *J. Pharm. Pharmacol.* **2001**, *53*, 425–432.
136. W. F. Li; J. C. Jiang; J. Chen, *Arch. Med. Res.* **2008**, *39*, 246–251.
137. B. M. Schmidt; D. M. Ribnicky; P. E. Lipsky; I. Raskin, *Nat. Chem. Biol.* **2007**, *3*, 360–366.

138. G. Gross, *Hautarzt* **2008**, *59*, 31–35.
139. M. Heinrich; P. Bremner, *Curr. Drug Targets* **2006**, *7*, 239–245.
140. G. Bo, *Clin. Microbiol. Infect.* **2000**, *6* (Suppl. 3), 6–9.
141. W. Sneader, *Drug Prototypes and their Exploitation*; Wiley: Chichester, 1996; pp 489–491.
142. J. L. Hartwell, *Lloydia* **1967**, *30*, 379–426 (and the other seven articles in the series).
143. B. C. Elford; M. F. Roberts; J. D. Phillipson; R. J. Wilson, *Trans. R. Soc. Trop. Med. Hyg.* **1987**, *81*, 434–436.
144. C. Triplitt; E. J. Chiquette, *Am. Pharm. Assoc.* **2003**, *46*, 44–52.
145. J. Eng; W. A. Kleinman; L. Singh; G. Singh; J. P. Raufman, *J. Biol. Chem.* **1992**, *15*, 7402–7405.
146. A. Harvey, *Drug Disc. Today* **2000**, *7*, 294–300.
147. T. E. Adrian, *Curr. Pharm. Des.* **2007**, *13*, 3417–3426.
148. T. L. Simmons; R. C. Coates; B. R. Clark; N. Eugene; D. Gonzalez; E. Esquenazi; P. C. M. Dorrestein; W. H. Gerwick, *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 4587–4594.
149. M. Gordaliza, *Clin. Transl. Oncol.* **2007**, *9*, 767–776.
150. T. Divabalange; C. D. Amsler; J. B. McClintock; B. J. Baker, *J. Am. Chem. Soc.* **2006**, *128*, 5630–5631.
151. X. Jiang; B. Liu; S. Lebreton; J. K. Brabander, *J. Am. Chem. Soc.* **2007**, *129*, 6386–6387.
152. K. C. Nicolaou; R. Guduru; Y. P. Sun; B. Banerji; D. Y. Chen, *Angew. Chem. Int. Ed. Engl.* **2007**, *46*, 5896–5900.
153. C. Chiraldi; M. De Rosa, *Trends Biotechnol.* **2002**, *20*, 515–521.
154. C. A. Roessner; A. L. Scott, *Annu. Rev. Microbiol.* **1996**, *50*, 467–490.
155. S. F. Brady; C. J. Chao; J. Clardy, *Appl. Environ. Microbiol.* **2004**, *70*, 6865–6870.
156. G. Appendino; O. Tagliatela-Scafati, Drug-Like Compounds from Food Plants and Spices. In *Dietary Supplements of Plant Origin*; M. Maffei, Ed.; Taylor & Francis: London, 2003; pp 43–74.
157. A. Endo, *J. Antibiot.* **1979**, *32*, 852–854.
158. I. Raskin, *Plant Physiol.* **1992**, *99*, 799–803.
159. A. W. Alberts; J. Chen; G. Kuron; V. Hunt, *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 3957–3961.
160. A. Goel; A. B. Kunnumakara; B. B. Aggarwal, *Biochem. Pharmacol.* **2008**, *75*, 787–809.
161. J. H. Medina; C. Pena; M. Piva; C. Wolfman; M. L. de Stein; C. Wasowski; C. Da Cunha; I. Izquierdo; A. C. Paladini, *Mol. Neurobiol.* **1992**, *6*, 377–386.
162. H. Salter, *Asthma. Its Pathology and Treatment*; Churchill: London, 1860; p 181.
163. J. C. Espin; M. T. Garcia-Conesa; F. A. Tomás-Barberán, *Phytochemistry* **2007**, *68*, 2986–3008.
164. *SCRIP* 3362, 16 May 2008, p 19.
165. L. Révész; P. Hiestand; L. La Vecchia; R. Naef; H. U. Naegeli; L. Overer; H. J. Roth, *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1521–1526.
166. F. Galvano; L. la Fauci; P. Vitaglione; V. Fogliano; L. Vanella; C. Felgines, *Ann. Ist. Super. Sanità* **2007**, *43*, 382–393.
167. T. Tsuda, *J. Agric. Food Chem.* **2008**, *56*, 642–646.
168. P. Wahlm; C. Foged; S. Tullin; C. Thomsen, *Mol. Pharmacol.* **2001**, *59*, 9–15.
169. W. Sneader, *Drug Prototypes and their Exploitation*; Wiley: Chichester, 1996; pp 85–89.
170. N. Márquez; M. A. Calzado; G. Sánchez-Duffhues; M. Pérez; A. Minassi; A. Pagani; G. Appendino; L. Diaz; M. A. Muñoz-Fernández; E. Muñoz, *Biochem. Pharmacol.* **2008**, *75*, 1370–1380.
171. D. G. I. Kingston; P. G. Jagtap; H. Yuan; L. Samala, *Fortschr. Chem. Org. Naturst.* **2002**, *84*, 53–225.
172. G. Appendino; A. Bertolino; A. Minassi; R. Annunziata; A. Szallasi; L. De Petrocellis; V. Di Marzo, *Eur. J. Org. Chem.* **2004**, 3413–3421.
173. R. M. Wilson; S. J. Danishefsky, *J. Org. Chem.* **2006**, *71*, 8329–8351.
174. P. A. Wender; J. M. Kee; J. M. Warrington, *Science* **2008**, *320*, 649–652.
175. R. A. Shenvi; C. A. Guerrero; C. C. Li; P. S. Baran, *J. Am. Chem. Soc.* **2008**, *130*, 7241–7243.
176. E. J. Corey; D. Y. Gin; R. S. Kania, *J. Am. Chem. Soc.* **1996**, *118*, 9202–9203.
177. C. Cuevas; M. Pérez; M. J. Martín; J. L. Chicharro; C. Fernandez-Rivas; M. Flores; A. Francesch; P. Gallego; M. Zarzuelo; F. de la Calle; J. García; C. Polanco; I. Rodríguez; I. Manzanares, *Org. Lett.* **2000**, *2*, 2545–2548.
178. R. J. Spandl; A. Bender; D. R. Spring, *Org. Biomol. Chem.* **2008**, *6*, 1149–1158.
179. L. A. Marcaurelle; C. W. Johannes, *Prog. Drug Res.* **2008**, *66*, 189–216.
180. G. Appendino; G. C. Tron; T. Jarevang; O. Sterner, *Org. Lett.* **2001**, *3*, 1609–1612.
181. M. Downes; M. A. Verdecia; A. J. Roecker; R. Hughes; J. B. Hogenesch; H. R. Kast-Woelbern; M. E. Bowman; J. L. Ferrer; A. M. Anisfeld; P. A. Edwards; J. M. Rosenfeld; J. G. Alvarez; J. P. Noel; K. C. Nicolaou; R. M. Evans, *Mol. Cell.* **2003**, *11*, 1079–1092.
182. H. E. Pelish; N. J. Westwood; Y. Feng; T. Kirchhausen; M. D. Shair, *J. Am. Chem. Soc.* **2001**, *123*, 6740–6741.
183. K. C. Nicolaou; J. A. Pfefferkorn; A. J. Roecker; G. Q. Cao; S. Barluenga, *J. Am. Chem. Soc.* **2000**, *122*, 9939–9953.
184. S. N. López; I. A. Ramallo; M. Gonzalez Sierra; S. A. Zacchino; R. L. E. Furlan, *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 441–444.
185. J. Kennedy, *Nat. Prod. Rep.* **2008**, *25*, 25–34.
186. U. Galm; S. Heller; S. Shapiro; M. Page; M. S. M. Li; L. Heide, *Antimicrob. Agents Chemother.* **2004**, *48*, 1307–1312.
187. K. J. Weissman, *Trends Biotechnol.* **2007**, *25*, 139–142.
188. J. K. Borchardt, *Mod. Drug Discov. July/August 1999*, 22–29.
189. Y. Volcegursky; Z. Hu; R. McDaniel, *Mol. Microbiol.* **2000**, *37*, 752–762.
190. D. E. Cane; C. T. Walsh; C. Khosla, *Science* **1998**, *282*, 63–68.
191. D. K. Ro; E. M. paradise; M. Ouellet; K. J. Fischer; K. L. Newnam; J. M. Ndungu; K. A. Ho; R. A. Eachus; T. S. Ham; J. Kirby; M. C. Chang; S. T. Withers; Y. Shiba; R. Sarpong; J. D. Keasling, *Nature* **2006**, *440*, 940–943.
192. G. Li; X. Guo; R. Jin; Z. Wang; H. Jian; Z. Li, *J. Tradit. Chin. Med.* **1982**, *2*, 125–130.
193. F. Y. Lee; R. Barzilleri; C. R. Fairchild; S. H. Kim; B. H. Long; C. Reventos-Suarez; G. D. Vite; W. C. Rose; R. A. Kramer, *Clin. Cancer Res.* **2001**, *7*, 1429–1437.
194. Quoted in S. Borman, *Chem. Eng. News* 14 January 2002, 23–24.

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Gabriele Fontana was born in Magenta, Italy, in 1967. After he graduated from the University of Milano in 1992 and obtained his Ph.D. in Chemistry in 1996, he was research assistant at the University of Newcastle Upon Tyne (UK) till 1998 under the guidance of Professor Roger J. Griffin. He then moved to Glaxo-Wellcome, Italy, as medicinal chemistry scientist under the direction of Dr. Romano di Fabio. In September 2000 he joined Indena SpA, Milan, Italy, where he became head of medicinal chemistry in 2008.



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