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Natural Products as Enzyme Inhibitors

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1.1 Why Are Natural Products Good Enzyme Inhibitors?

Natural products are widely distributed and their unique properties have been explored for centuries by our earliest ancestors to treat diseases and injuries. Throughout evolution, the potential of natural products as modulators of biological functions has been increasingly realized [1].

Over the past decades, there has been a decrease in the use of natural products by pharmaceutical companies as a starting point for drug discovery, essentially due to the belief that natural products were somehow incompatible with drug discovery approaches that were based on high-throughput screening directed towards molecular targets [2]. Furthermore, there was also the assumption that combinatorial chemistry techniques would be able to generate all the chemical diversity needed for successful lead discovery. However, the results of many large combinatorial screening collections have proved to be quite discouraging and it has already been recognized that diversity within biologically relevant 'chemical space' is more important than library size. To a certain point, libraries of synthetic molecules have been designed to mimic the chemical properties of the natural compounds [3].

Despite the deficiency of investment in natural products as main leads in drug discovery over the past decades, 34% of the medicines approved by the US Food and Drug Administration (FDA) between 1981 and 2010 were actually natural products or directly derived from them [4].

The great potential of molecules of natural origin in drug discovery arises from their remarkable chemical and structural diversity. About 40% of the chemical scaffolds found in natural products are indeed absent in today's medicinal chemistry synthetic libraries. For this reason, the use of nature-inspired molecules is a good complement to synthetically produced molecules [5, 6].

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One of the most relevant reasons for the success of natural products as a source of bioactive molecules arises from their 'drug-likeness', which frequently surpasses that of synthetic compounds. Considering their biosynthetic processes in living organisms, it is not surprising that natural molecules display greater similarity and binding potential with biological structures, thus increasing the probability of an effective interaction with different biological targets [6].

One of the most outstanding features of natural products is their three-dimensional conformation, which is attributed to the complex and unique structure that is mostly beyond the synthetic capacity of medicinal chemistry. Natural products are often described for their 'privileged' scaffold, allowing them to work as ligands for a diverse array of enzymes and receptors. This term, first mentioned by Evans in the late 1980s, was originally used to address the benzodiazepines scaffold, privileged by their ability to bind not only to their receptors at the central and peripheral nervous system, but also to cholecystokinin receptors. In this way, and according to Evans's definition, a privileged structure displays affinity to several receptors/proteins [7, 8]. In 2010, Matthew *et al.*, presented an exhaustive review by providing a comprehensive list of privileged scaffolds found in both synthetic drugs and natural ones. Spiket-p, integramycin and routiennocin, despite having the same scaffold (6,6-spiroacetal), display different bioactivities and are found in different species, which demonstrates the evolution-driven predisposition for repetition, once a suitable solution to a particular biochemical problem has been found (Figure 1.1). This can also explain the non-random patterning of macromolecular structures in living systems. Consequently, 6,6-spiroacetal is a 'privileged' scaffold found in a number of natural products displaying the ability to bind to different targets thereby exerting different pharmacological effects [9].

Considering the enormous variety of compounds occupying the 'chemical space', it can easily be assumed that natural products cover distinct regions when compared with synthetic ones, having wider and more drug-like properties. Rosén *et al.* demonstrated throughout computational screenings that natural products cover parts of the chemical space that lack representation by medicinal chemistry compounds and, by doing so, these compounds may be useful for novel leads [10].

For obvious reasons, the difference between natural products and other sources of molecules, with relevance to their ability to display biological properties, has a chemical basis [11, 12]. In general, the composition of natural molecules is distinct from that of synthetic ones, as they display fewer nitrogen, sulfur or halogen atoms, being richer in oxygen and containing more hydrogen bond donors. The sterical complexity of natural products also plays a role in this equation, these molecules presenting a larger number of rings and, overall, more chiral centres.

But why? Why do natural products present such chemical traits? The obvious answer is that they are not randomly synthesized, instead resulting from biosynthetic processes that are highly targeted. For this reason, these molecules are meant to interact with molecular targets that are, themselves, three-dimensional and chiral. In addition, the enzymes involved in the biosynthesis are usually chiral in the way they usually yield a single isomer, a trait not always found in

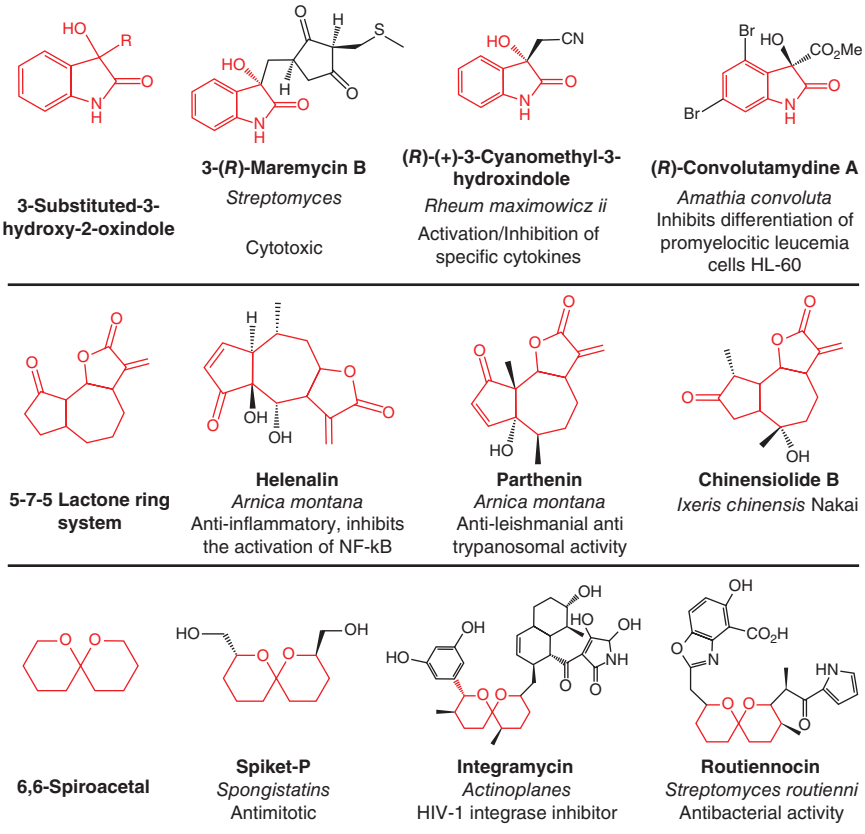

Figure 1.1 Examples of 'privileged' scaffolds found in natural products. (Adapted from [9].)

medicinal chemistry, where racemic mixtures are frequently produced [12]. Enzymes involved in their natural biosynthesis, as well as the molecular targets the natural product is meant to interact with, are inherently three-dimensional and chiral as human enzymes are. Thus, there is actually a link that can explain why natural products can display good results as enzyme inhibitors [12].

The fact that the majority of natural products exhibits such characteristics, shows that they result from an evolutionary drive that selects molecules displaying a certain arrangement of atoms [12, 13]. In addition, the probability of finding a bioactive molecule is much higher in natural products when compared with a randomly synthesized molecule [8]. This is not surprising if we consider that it arises from nature's own high-throughput screening: not only are molecules prone to display the well-defined three-dimensional structure described above, but they are also produced to target well-conserved biological targets in a certain mechanism of action, meaning that they are synthesized to display some kind of activity towards a biological target [9, 14].

Another striking difference between natural products and randomly synthesized molecules rests in the underlying synthesis strategy in both cases. Unlike combinatorial chemistry, which can make use of tens of thousands of different

scaffolds as building blocks, no such mechanism is available in nature. In fact, for biosynthesis, nature has a limited number of building blocks available from a small number of biosynthetic pathways and, for this reason, any chemical novelty can only be achieved by branching out intermediates and creating a multitude of biosynthetic routes, ultimately generating different chemical entities. A different strategy is adopted in combinatorial chemistry, in which it is frequent to follow the same sequence of reactions using different starting molecules.

Nature's chemistry is, in its essence, oxophilic and, hence, it has the enzymatic tools performing site-selective C—H activation, in order to introduce oxygen atoms and discriminate between functional groups with different degrees of oxidation [15, 16]. Natural products have coexisted over time with several species and environments, thus undergoing the same iterative cycle of improvements and evolution with ever changing biotic systems.

Several natural compounds or their derivatives act as enzyme inhibitors in different therapeutic areas, such as in the treatment of cancer, diabetes, hypertension and infectious diseases [17]. Teprotide, isolated from *Bothrops jararaca* venom, is a classic example. This compound displayed long-lasting *in vivo* activity against angiotensin-converting enzyme and was chosen as a lead compound for the development of angiotensin carboxypeptidase inhibitors [18]. Galanthamine, an anticholinesterase drug isolated from the snowdrop plant *Galanthus nivalis* L., has been prescribed broadly for the treatment of Alzheimer's [19]. Vialinin A, a *p*-terphenyl compound obtained from the edible Chinese mushroom *Thelephora vialis* plant, strongly inhibits tumour necrosis factor-α production and release [20].

Given the complex structural variety of these products, it is reasonable to question the likelihood of these compounds actually presenting viability as orally active drugs. In 1997, Lipinski provided a set of four parameters common to 90% of more than 2000 drugs and candidate drugs at, or beyond, phase II clinical trials. These parameters state that in order for a candidate drug to be drug-like it should have less than five donor groups of hydrogen, less than five hydrogen acceptor groups, a molecular weight lower than 500 Da and log *P* lower than 5. However, it is irrefutable that many biologically active natural compounds do not fulfil all of these requirements. In this way, 12 of the 24 natural drugs approved between 1970 and 2006 do not agree with Lipinski's rule [11, 21]. Actually, in 2006 only 51% of all FDA-approved small molecule drugs were used orally and comply with this rule [22].

Nonetheless, it is important to take into account that Lipinski's rules leave out some aspects that are imperative to discuss. For example, natural products frequently benefit from transport mechanisms, which results in characteristics, such as molecular weight, to be less important regarding intestinal absorption [11].

1.2 Drawbacks of Natural Products

Despite the general potential of natural products in drug discovery, there are still some drawbacks that can hinder pharmaceutical development. Technologies and methodologies that allow natural products to be used effectively and efficiently in drug discovery are not yet fully matured to the point of sustaining the

demand of pharmaceutical companies, thus leading to a loss of interest in their development over the past decades [23].

One of the most relevant issues is related to the difficulty in scaling-up. Indeed, given the fact that most bioactive natural products are secondary metabolites, they are frequently found in small amounts in a given extract/species and cannot meet the pharmaceutical market demand, which can reach a scale of hundreds to thousands of kilograms per year [24]. However, as we will discuss subsequently, this situation can be addressed by relying on biotechnological approaches, such as fermentation, provided their cost does not limit the economic sustainability, which is pivotal to guarantee the progression to preclinical development. In this regard, it should be highlighted that the recent advances in drug discovery from non-conventional sources, such as marine organisms or extremophiles, may frequently result in a problem of tractability, as the bioactive molecules may be produced by symbiotic organisms [23, 25, 26].

Another aspect regarding drug discovery is the lack of high-quality libraries of natural products, due to the difficulty in their construction and maintenance. Nonetheless, when screening natural product libraries, rediscovery of known compounds is also a leading problem because of the lack of robust dereplication methodologies for both natural product sourcing and compounds in the natural product libraries [23].

Another constraint is the lack of novelty. As expected, most of the times when a sample is under study within a context of activity-guided isolation, the probability of finding an already known molecule is quite high. In certain cases, when novel molecules are obtained, structure elucidation can be challenging for some classes of natural compounds with high chemical complexity [12]. When a new lead compound is found, molecular modifications of the lead structure may be a challenge mainly due to the complexity of these compounds that, as referred above, display many functional groups that need to be protected for analogue synthesis [23].

Time is yet another variable that sometimes is against drug discovery from natural products. Despite the development and increasingly robust analytical techniques and instrumentation, the process from hit identification to chemical elucidation is very time-consuming, not always compatible with the objectives of industry players [23, 24].

Finally, intellectual property can also be a relevant issue that hinders further development of drug development from natural products. With the increasing awareness regarding the economic potential of natural products, many countries already have regulations in place to prevent or hinder sampling of natural products without prior authorization. Researchers and companies are claiming intellectual property rights over herbal medicine resources and the fast growth of patent applications for these products shows precisely this trend [27].

1.3 The Future of Natural Products Drug Discovery

1.3.1 New Sources and New Production Methods

Over the past years, the development of new technologies and methods strongly contributed to the beginning of a new era for natural products, considered by

many as a 'New Golden Age'. Developments in metagenomics and microbial genomics, as well as innovations in microbial culture, have opened new doors towards the understanding and manipulation of biosynthetic pathways, thus contributing to the rational discovery of natural products. Furthermore, advancements in bioassays and high-throughput screening technology also has a pronounced impact in this field [24, 28].

In order to cover the large biodiversity of biological systems, the interest in different sources of natural products has also spread. Currently, there is high concern on the research of species from different ecosystems, such as deep oceans (marine organisms) and extreme environments [29].

Marine environment, which covers 70% of the earth, represents a natural habitat for a notable number of species. In fact, considering their biodiversity, marine organisms, such as sponges, tunicates, fishes, soft corals, nudibranchs, sea hares, opisthobranch molluscs, echinoderms, bryozoans, prawns, shells, sea slugs, and microorganisms are valuable resources for drug discovery [30].

Secondary metabolites from these organisms have been studied for the past 30 years, first and foremost by academics chemists, who first began to isolate and elucidate novel compounds from these organisms in the 1970s. Since then, increasing attention has been given to the molecules found in those species [31].

When compared to terrestrial natural products, marine ones have shown higher chemical novelty (71% of the molecular scaffolds in the Dictionary of Marine Natural Products were exclusively found in marine organisms) and higher incidence of significant bioactivity (1% of marine samples tested for anti-tumour activity in a cytotoxicity screen performed by National Cancer Institute showed potential as anti-tumour, against 0.1% of terrestrial samples) [32, 33].

Furthermore, the unique structure of some metabolites has led to the discovery of novel mechanisms of action. Ziconotide, for example, has an analgesic effect by reversibly blocking N-type voltage-sensitive calcium channels, which inhibits the activity of pain-sensing primary nociceptors [34]. This analgesic mechanism was not reported before this drug was discovered and is now a subject pertinent for the development of other analgesic drugs [35]. The potential of marine organisms in drug discovery led to the development of new classes of therapeutic agents, some of them already used in clinics and others in clinical trials mainly for their anti-microbial, anti-tumour, anti-inflammatory and analgesic activity (Table 1.1).

Currently, seven marine drugs (ziconotide, vidarabine, cytarabine, trabectedin, brentuximab vedotin, eribulin mesylate and omega-3-acid ethyl esters), have already been approved by the FDA. Ziconotide, a peptide originally from a tropical marine cone snail, was the first drug approved (in 2004) for the treatment of chronic pain in spinal cord injury. Trabectedin, an alkaloid of tetrahydroisoquinoline class, was the first anti-cancer marine drug approved in the Europe Union for the treatment of soft-tissue sarcoma [36].

At the microbial level, there is spreading interest for marine actinomycetes, due to their diversity and proven ability to produce novel bioactive compounds with particular structure diversity and unique biological activities [37]. For example, salinoporamide A, a γ-lactam-β-lactone from *Salinispora tropica*, is a potent 20S proteasome inhibitor that is on phase I clinical trials for the treatment of

Table 1.1 Current clinical status of marine drugs.

Compound name	Marine organism	Chemical class	Disease area
Approved			
Cytarabine, ara-C	Sponge	Nucleoside	Cancer, leukaemia
Brentuximab vedotin	Mollusc/ cyanobacterium	ADC (MMAE)	Cancer, lymphoma
Vidarabine, ara-A	Sponge	Nucleoside	Anti-viral
Omega-3-acid ethyl esters	Fish	Omega-3 fatty acid	Hypertriglyceridemia
Ziconotide	Cone snail	Peptide	Pain
Eribulin mesylate	Sponge	Macrolide	Breast cancer
Trabectedin	Tunicate	Alkaloid	Cancer
Phase III			
Plitidepsin	Tunicate	Depsipeptide	Cancer
Tetrodotoxin	Pufferfish	Guanidinium alkaloid	Chronic pain
Soblidotin	Bacterium	Peptide	Cancer
Phase II			
Plinabulin	Fungus	Diketopiperazine	Cancer
Glembatumumab vedotin	Mollusc/ cyanobacterium	ADC (MMAE)	Breast cancer, melanoma
Elisidepsin	Mollusc	Depsipeptide	Cancer
PM1004	Nudibranch	Alkaloid	Cancer
TAsidotin, synthadotin	Bacterium	Peptide	Cancer
Pseudopterosins	Soft coral	Diterpene glycoside	Wound healing
Phase I			
Bryostatin 1	Bryozoa	Polyketide	Cancer
Pinatuzumab vedotin	Mollusc/ cyanobacterium	ADC (MMAE)	N-HL, CLL
Hemiasterlin	Sponge	Tripeptide	Cancer
HuMax®-TF-ADC	Mollusc/ cyanobacterium	ADC (MMAE)	Cancer

ADC, antibody drug conjugate; MMAE, monomethyl aurisatin E; N-HL, non-Hodgkin lymphoma; CLL, chronic lymphocytic leukaemia.
Source: Malve 2006 [36]. Reproduced with permission of Wolters Kluwer Medknow Publications.

multiple myeloma [38]. The difficulty to isolate and cultivate rare actinomycetes still remains an obstacle to their use as a source of natural products. However, the continued development of improved cultivation methods and molecular technologies promises to ensure overcoming these limitations [37, 39].

On the other hand, organisms that live in extreme conditions and found in the marine environment have also been widely explored. Palmerolide A is a potent

cytotoxic macrolide isolated from *Synoicum adareanum*, a circumpolar tunicate from Antarctica displaying a potent anti-cancer activity against melanoma cell lines [40].

Other marine organisms receiving increasing attention are cyanobacteria, photosynthetic bacteria recognized as sources of a variety of bioactive metabolites. A survey of natural products with FDA approval and of those in clinical trials indicated that 20% of these small molecules are likely to have cyanobacteria as predicted biosynthetic sources [41].

Supply difficulty, as previously mentioned, is one of the major problems encountered in natural products drug discovery. Nonetheless, outstanding improvements in chemistry, fermentation and biotechnology have provided solutions regarding this issue [29]. Advances in total chemical synthesis have greatly contributed to overcome this issue, allowing industrial scale production of these compounds for structure–activity relationship (SAR) studies, hit to lead optimization and further industrial production [42]. Largazole, a histone deacetylase inhibitor isolated from cyanobacterium of the genus *Symploc*, has been chemically synthesized for the preparation of analogues and SAR studies, which revealed that the group is the pharmacophore responsible for anti-proliferative activity [43]. Further examples of compounds first isolated from natural products and those now obtained by synthetic pathways are amphotericin B (an antibiotic isolated from *Streptomyces nodosus*), calicheamicin γ1 (an anti-cancer agent from *Micromonospora echinospora* ssp. *calichensi*), efrotomycin (an antibiotic from *Nocardia lactamdurans*), rapamycin (an immunosuppressant agent *Streptomyces hygroscopicus*) and taxol (an anti-cancer agent from *Taxus brevifolia*) [44].

Another important tool to improve supply problems is the fermentation of a starting natural product, or of a major intermediate. Yet, not all natural products or starting materials can be produced by fermentation routes [29, 42]. However, fermentation is still an important tool for the industrial production of many drugs: trabectedin, for example, is produced by initial fermentation of the starting material cyanosafracin B from *Pseudomonas fluorescens*, followed by semisynthetic steps to reach the final drug [45]. Mixed fermentation is also validated as an effective approach to access the metabolic potential of cultivatable microbes and can increase yields of both previously described and undetected metabolites [46]. For example, emericellamides A and B were not detected by normal LC–MS analysis in the pure culture of *Emericella* sp., but a greater enrichment was observed upon inoculating the bacterial culture with the marine actinomycete *Salinispora arenicola* [47].

Furthermore, advances in biotechnology and metabolic engineering have had a strong impact on the supply field, allowing for better knowledge of the metabolic pathways leading to the production of these secondary metabolites. In this way, knowing the biosynthetic route by which these metabolites are produced *in vivo* enables the increase of their production rate *in vitro*, by manipulating biosynthetic pathways and redirecting the metabolic flows towards the desired product. This can be done using different strategies, such as increasing the precursor supply, overexpressing or increasing the efficiency of bottleneck enzymes, altering the regulation of gene expression, reducing flux towards unwanted by-products or competing pathways and reconstituting entire pathways in a heterologous

host [48]. For example, genetic engineering of daptomycin biosynthetic pathway of *Streptomyces roseosporus* provided sufficient amounts of compound for the purposes of *in vitro* screening, structure elucidation and further drug development, by exchanges of the non-ribosomal peptide synthase subunit and inactivation of the tailoring enzyme (glutamic acid 3-methyltransferase) [49, 50].

Likewise, metagenomics approaches have been used to assess a broader range of synthetic capabilities of bacteria, leading to the discovery of novel compounds. This strategy involves sampling the entire bacterial DNA from an environmental sample and cloning the DNA in host organisms, such as *Escherichia coli*, which are then cultured and tested for the expression of bioactive metabolites [51]. For example, bryostatins are a family of protein kinase C modulators that display potent bioactivity in the central nervous system. These compounds are found in *Candidatus Endobugula sertula*, an uncultivated marine bacterial symbiont of the marine bryozoan *Bugula neritina*. After the identification of bryostatin biosynthetic genes, the cultivation of this bacterial symbiont and heterologous expression of these genes was the strategy adopted for the development of bryostatins and derived compounds [52].

These biotechnological tools play an important role in the field of drug discovery, allowing the production of new biologically active molecules that are similar to the parent compound, while offering different chemical and sometimes biological properties [29]. Weissman *et al*. engineered bacterial multi-enzyme polyketide synthases by combinatorial biosynthesis and have produced more than 200 new polyketides as novel drug candidates [53].

Advances in purification and characterization processes made the screening of natural product more compatible with the ordinary timescale of high-throughput screening campaigns [54]. In the past years, separation technologies, such as high-performance liquid chromatography (HPLC), counter-current chromatography (CCC), supercritical fluid chromatography (SFC) and capillary electrophoresis (CE) experienced significant improvements of resolving power and efficiency [29]. Furthermore, the emerging of new hyphenated spectroscopy technologies such as HPLC-MS, HPLC-NMR and HPLC-NMR-MS allows rapid compound identification and accelerates dereplication processes [55].

1.3.2 New Strategies for Delivery

Despite the bioactivity displayed by many natural compounds in *in vitro* assays, their transposition for *in vivo* use is often disappointing, essentially due to their limited absorption rate. In fact, most of these drugs have poor solubility, limited dissolution rate and instability in extreme pH, which compromise their bioavailability [56]. It is also important to notice that, many natural compounds do not meet the structural requirements of Lipinski's rule and some of them may not display oral bioavailability, although, as previously stated, this is not a necessary requirement. However, technological advances, essentially in the nanotechnology field, allowed the control of drugs pharmacokinetics and pharmacodynamics, through the development of drug delivery systems. In addition to the improvement of bioavailability, these new strategies can be helpful in the management of harmful side effects by directing the drug to their specific target [57].

Microencapsulation is an approach used in a drug delivery system. Small particles, such as nanoparticles are packaged within an encapsulating matrix. Nanoparticles are broadly defined as particles of size less than 100 nm; however, some authors subdivide them into two distinct groups according to their dimensions: microparticles, microspheres or microcapsules (1–800 μm range), and nanoparticles, nanospheres or nanocapsules (below 1 μm) [58]. The most common types of nanoparticles used for drug delivery are polymer nanoparticles (nanocapsules and nanospheres), solid lipid nanoparticles, crystal nanoparticles, liposomes, micelles and dendrimers [58]. Polymeric nanoparticles have been used to enhance the bioavailability of molecules such as luteolin, epigallocatechin gallate and tea polyphenols, using polymers such as polyethylene glycol and polyvinyl alcohol [59, 60].

Liposomes are nanoparticles composed of phospholipid bilayers similar to cell membranes, having a hydrophilic tail that can work as a vehicle for more polar drugs and a hydrophobic tail that can deliver lipophilic drugs. The type of delivery system should be selected based on the physicochemical properties of the drug of interest. The liposomal aqueous compartment, formed by the hydrophilic head groups of the phospholipids, can contain one or more hydrophilic drugs. However, lipophilic drugs are better suited for delivery within a micelle, in which the lipophilic tail of the phospholipid forms the drug-containing compartment [61]. Bergamot essential oil has anti-cancer properties, but lacks the acquired lipophilic properties for solubilization. The encapsulation of this essential oil in liposomes improved the solubility of the drug and led to increased cell death *in vitro* [62].

Solid lipid nanoparticles contain a solid hydrophobic core surrounded by phospholipids, and are a good choice for hydrophobic drug delivery, being more stable than liposomes and less toxic than polymeric nanoparticles [63]. Curcumin, a diarylheptanoid obtained from turmeric, has caused wide enthusiasm as a lead compound against several conditions, including cancer, inflammation, microbial infection and angiogenesis. In this case, poor bioavailability was a noteworthy restraint to the therapeutic efficacy of curcumin in clinical trials. However, it has been demonstrated that orally administered liposome-encapsulated curcumin could dramatically increase the bioavailability of this compound (up to 155 times at 1 mg/kg) [64–66].

The solubility of the compounds can also be enhanced by solid dispersion, a strategy that uses a hydrophilic inert carrier matrix at the molecular level that can reduce the aggregation size of the compound and increase its dispersibility [67]. Apigenin, a plant-derived flavone, has low lipid and water solubility. It was demonstrated that its bioavailability could be largely improved by using solid dispersions of the compound with novel carbon nanopowder: drug release profiles showed that apigenin dissolution from the system improved by 275% [68].

Another approach used to increase the water solubility of natural drugs and enhance their bioavailability and stability, is the use of cyclodextrins. Cyclodextrins are a family of cyclic oligosaccharides with a hydrophilic outer surface and a lipophilic central cavity that work as a vehicle for both lipophilic and hydrophilic drugs [69]. The supramolecular systems that result from encapsulation depend on the molecular combination of both host and visitor particles [70]. The

physiological stability and insolubility problems of camptothecin, an anti-cancer agent can be solved through a loaded amphiphilic cyclodextrins that may support its delivery [71].

Directing the drug to a specific target of a particular tissue/organ can also enhance drug bioavailability. In addition, targeting strategies can also reduce side effects once the drug is released in a localized area of the body, narrowing toxicity to other organs. Targeting strategies can fall under two general classifications. The first is passive targeting, which does not involve chemical/biological interaction, but rather depends on physical transport of the particles according to their natural properties (size, shape and surface charge). The second sort is active targeting, in which a ligand that is selectively recognized by receptors on the surface of the cells is attached to the surface of a nanoparticle. Typical active targeting is generally accomplished by functionalizing the nanoparticle with a protein, peptide or antibody [72].

Passive targeting is a successful and cheaper choice that is frequently used in cancer treatment. Due to their flawed vasculature, many tumours have enhanced permeability, a feature that is relevant for the development of nanoparticles intended to distinguish between tumour and healthy tissue. One example that reflects this delivery system is the encapsulated gambogic acid and vitamin E-containing telodendrimers for colon cancer treatment [73].

Antibody–drug conjugates are typical examples of active targeting and have been effectively used in therapeutics for haematological diseases and cancer, due to their ability to convey the cytotoxic compound to a particular growth cell without influencing normal cells. An antibody–drug conjugate has three noteworthy constituents: a monoclonal antibody, a synthetic linker and a powerful cytotoxic payload [74]. There are a lot of antibody–drug conjugates already in use, but only a few of them are derivatives from natural products. Brentuximab vedotin is a chimeric antibody derived from dolastatin 10, an antitubulin agent found in *Symploca* sp., currently approved for the treatment of Hodgkin's lymphoma and anaplastic large cell lymphoma [41]. Trastuzumab-DM1 is an antibody drug approved for metastatic breast cancer, derived from maytansine, a potent antimitotic compound isolated from the shrub *Maytenus ovatus* [75]. Calicheamicin from *Micromonospora calichensis* is one of the most potent DNA cleaving agents and a derivative of calicheamicin-γ-1, *N*-acetyl-γ-calicheamicin, is now being used to conjugate to a monoclonal antibody. Anti-CD22-calicheamicin conjugate is actually in phase 3 clinical trials for relapsed or refractory CD22-positive acute lymphoblastic leukaemia [76].

Conjugation of nanoparticles with folic acid is also an interesting approach to direct drug for cancer cells that have folic acid receptors overexpressed. In this context, quercetin nanoparticles conjugated with folic acid have already demonstrated their selectivity to cancer cells in a folate-dependent manner in some experiments [77].

Targeting can likewise be accomplished by using external forces. The use of magnetic fields to coordinate a conveyance framework has been widely considered. A novel magnetic drug delivery nanosystem was developed for oncocalyxone A, an anti-tumour compound isolated from *Auxemma oncocalyx*. Iron oxide nanoparticles coated in oleic acid and oncocalyxone A were

incorporated into the hydrophobic cores of copolymer micelles, thus allowing the targeting of the system by a magnetic field to the tumour [78].

The strategies discussed above are often solutions for most of the problems regarding lack of bioavailability of natural products as drug leads. Deeper research on these approaches is being pursued enthusiastically in many laboratories. Novel drug delivery systems not only increase the therapeutic value by reducing toxicity and increasing the bioavailability, but also overcome non-compliance to the therapeutic by reducing repeated drug administrations.

1.3.3 New Targets?/Drug Repurposing

Considering the above presented, natural products have undoubtedly been a helpful source for the improvement and development of new drugs for quite some time. However, their macromolecular targets are still unknown to a great extent, thus hampering the objective rational drug design and optimization methodologies [79]. The arrival of the sequenced human genome and new molecular biological approaches prompted the ascension of rational molecular target-based screening as another view in drug discovery [80]. In this context, computational target prediction strategies using chemical descriptors have been exhaustively applied for drug discovery, in order to elucidate the mechanisms-of-action of natural compounds and enlighten researchers on their new potential therapeutic applications [81]. In fact, there has been a cross-over regarding the use of many natural compounds in such a way that agents that were initially isolated and purified for potential therapeutic applicability are now being studied for complete different targets. In this way, the concept of drug redirection, previously applied in medicinal chemistry, has been extended to natural products in the drug discovery field [82].

One classic example of this cross-over is the microbial product rapamycin. Originally isolated as an anti-fungal agent produced by the bacterium *Streptomyces hygroscopicus*, it has been approved as an immunosuppressive drug and is also being tested (as a derivative) as an anti-neoplastic agent, by the specific inhibition of the mTOR protein kinase [83, 84]. Other drugs, such as canagliflozin (a SGLT-2 inhibitor) and darunavir (a HIV protease inhibitor), have also been taken into account for the possibility of displaying cross-over activity as renin inhibitors, besides their classical approved targets, which makes them reasonable options for drug repurposing [85].

AMP-activated protein kinase, activated by metformin (the main drug used for the treatment of type 2 diabetes) is also a target for many natural compounds. It has been discussed that the non-steroid anti-inflammatory drug salsalate (a salicylate prodrug) may also interfere with AMPK, thus improving metabolic parameters in subjects with insulin resistance. In this way, a possible repurposing of this drug could be effective in the treatment of metabolic disorders and also in cancer, where AMPK activation is frequently sought [86].

Archazolid A, a macrolide from the myxobacterium *Archangium gephyra*, has also been pointed for other relevant macromolecular targets, such as 5-lipoxygenase and farnesoid X receptor, besides its main target and it is now undergoing further studies for repurposing approaches [79].

This strategy of repurposing approved drugs is a promising approach that could enhance the potential of natural products. Besides the financial advantages, repurposing has also safety rewards. Existing drugs that are either approved or have been shown to be safe in clinical trials can leverage their inherently reduced development risk into potentially new indications.

1.4 Conclusion

Natural products have traditionally played a crucial part in drug discovery and were the premise for the development of general early drugs. In the course of the past years progresses in methodologies, such as X-ray crystallography and NMR, as well as alternative drug discovery, mainly rational drug design and combinatorial chemistry, have set considerable weight on natural products research, leading to a decay of interest of most pharmaceutical organizations, which have ended or significantly scaled down their operations based on these compounds [17]. Natural products often feature biologically relevant molecular scaffolds and pharmacophore patterns that have evolved as preferred ligand–protein binding motifs. It is not a coincidence that Nature has already selected natural products as lead structures of biological significance in the almost infinite universe of chemical space and, therefore, their chemical diversity and biochemical specificity must be explored. It is still also important to notice that natural compounds can suffer further enhancements regarding target efficacy and selectivity, in order to accomplish ideal pharmacokinetic and pharmacodynamic properties [87].

Between 2008 and 2013 a total of 25 natural molecules, as well as natural products derived drugs, were approved for marketing and several of new natural compounds are in different stages of clinical trials, which demonstrate their value [17]. Recently, and essentially due to advances in biotechnology and genomic approaches, there has been a renewed interest in natural product research in different therapeutic areas. The fundamental breakthroughs in purification and structure elucidation technologies have brought down the obstacles intrinsic to the screening of these molecules. The conjunction of these advances with advances in genomics, metabolic engineering and chemical synthesis now offer an alternative to explore the notable chemical diversity of natural products in the pursuit for new drugs. Those recent approaches are expected to bring again natural products to the front line of the drug development process. Furthermore, new sources of natural products, such as marine species, have been widely explored and their contribution might be the largest asset towards finding novel structures with novel modes of action that can cover biologically relevant chemical space.

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