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The historical delivery of antibiotics from microbial natural products—Can history repeat?

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ABSTRACT

Microbial natural products are the origin of most of the antibiotics on the market today. However, research in antibiotics and natural products has declined significantly during the last decade as a consequence of diverse factors, among which the lack of interest of industry in the field and the strong competition from collections of synthetic compounds as source of drug leads. As a consequence, there is an alarming scarcity of new antibiotic classes in the pipelines of the pharmaceutical industry. Still, microbial natural products remain the most promising source of novel antibiotics, although new approaches are required to improve the efficiency of the discovery process. The impact of microbial biodiversity, the influence of growth conditions on the production of secondary metabolites, the choice of the best approach at the screening step and the challenges faced during the isolation and identification of the active compounds are examined in this review as the critical factors contributing to success in the effort of antibiotic discovery from microbial natural products.

1. Introduction

It is a well known fact that natural products isolated from microorganisms have been the source of most of the antibiotics currently on the market. The discovery of penicillin and its use in the clinic in the 1940s was soon followed by the discovery of a huge number of antibiotics from microbes, in particular from members of the actinomycetes and fungi. Many antibiotics discovered until the early 1970s reached the market, and their chemical scaffolds were later used as leads to generate new generations of clinically useful antibiotics by chemical modification (Table 1). Actually, antibiotics and natural products are intimately linked terms. The word antibiotic was originally coined for those natural compounds with antimicrobial properties [1,2] versus antibacterials, to designate synthetic compounds with similar activity.

Although the word antibiotic no longer refers only to natural compounds, it is true that most of the marketed antibiotics, even in the recent past, are based on natural chemotypes. Thus, 70 out of the 90 antibiotics marketed in the years 1982–2002 originated in natural products [3]. Most of the remaining products belonged to the fluoroquinolones class, and it could even be argued that this category has its roots in nature as well, since they originate from nalidixic acid, a compound discovered during the attempts to synthesize the natural antimalarial agent chloroquin. What becomes evident from the analysis of the last 20 years is the paucity of new scaffolds that reached the market. Only the streptogramins dalfopristin/quinupristin (Synercid®), linezolid (Zyvox®), and more recently the lipopeptide daptomycin (Cubicin®) can be considered as new antibiotic classes [2,4,5]. Remarkably, only linezolid can be considered a purely synthetic drug, the other two being natural products. The latest antibiotic approved by the FDA, tigecylcine, a glycilcycline, is a derivative of tetracycline, also an old class of natural antibiotics [6]. The rest of products launched during the last 20 years belonged to

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Table 1 – Examples of marketed antibiotics originated in microbial natural products

Original metabolite	Commercial products[a]	Producing organism
Penicillins	Penicillin G, V, Ampicillin, Methicillin, Amoxicillin, Carbenicillin	*Penicillium* spp., *Aspergillus* spp.
Cephalosporins	MEFOXIN (Cefoxitin), CECLOR (Cefaclor), CLAFORAN (Cefotaxime), ROCEPHIN (Ceftriaxone), CEFTIN (Cefuroxime)	*Acremonium* spp., *Emericellopsis* spp., *Amycolatopsis lactamdurans*, *Streptomyces clavuligerus*
Thienamycin	PRIMAXIN (Imipenem), INVANZ (Ertapenem)	*Streptomyces cattleya*
Erythromycin	ERYTHROCIN, ZITHROMAX (Azithromycin), BIAXIN (Clarithromycin), KETEK (Telithromycin)	*Saccharopolyspora erythraea*
Vancomycin	VANCOCIN	*Streptomyces orientalis*
Fosfomycin	MONURIL	*Streptomyces fradiae*
Mupirocin (pseudomonic acid)	BACTROBAN	*Pseudomonas fluorescens*
Fusidic acid	FUSIDIN LEO[b]	*Fusidium griseum*
Streptogramins	SYNERCID (Dalfopristin/quinupristin)	*Streptomyces pristinaespiralis*
Daptomycin	CUBICIN	*Streptomyces roseosporus*

[a] Trade names are in capitals. Non-capitalized names between parentheses refer to marketed semisynthetic derivatives from the original natural compound. Only some representatives are indicated.
[b] In Canada.

older classes, mostly β-lactams and macrolides, though these new generations represented significant advantages over the original products in spectrum, potency or pharmacokinetic properties [5]. Other compounds cited as under development in recent literature belong to the classes of antibiotics mentioned above or to others equally old (glycopeptides, rifamycins), the few exceptions being those of the diaminopyrimidine AR-100 (iclaprim), an inhibitor of dihydrofolate reductase; the glycolipodepsipeptide ramoplanin, an inhibitor of peptidoglycan transglycosylation [7], and the peptide deformylase inhibitor LBM-415, progressing into phase II/III [2,5,8].

The poor state of the pipeline speaks about the general failure of the involved stakeholders (big pharma, small biotech, academic groups and governmental agencies) in delivering new antibiotics. However, it is still critically important to find new antibiotic classes. The reasons have been analyzed in detail in a number of reviews [2,9,10] and will not be discussed here. It may be enough to mention the increasing incidence of resistant pathogens, which ultimately appeals to the sense of social responsibility of pharmaceutical industry: if we do not invest heavily in discovering and developing new antibiotic classes, we might well end up in a situation akin to the pre-antibiotic era.

Being the emergence of resistance in bacteria the key reason why antibiotics become obsolete, an alternative strategy to the design of new antibiotics could be the combination of known antibiotics with compounds able to revert the bacteria resistant phenotype to a sensitive one. This idea has been successfully exploited in the combinations of β-lactam antibiotics with β-lactamase inhibitors, three of which are on the market (clavulanic acid, sulbactam and tazobactam). The combination of clavulanic acid with amoxicillin in particular (Augmentin®) has become one of the most successful antibiotics ever on the market. Another related approach is the development of inhibitors of bacterial efflux pumps, drug transporters that are responsible for the resistance to a number of antibiotic classes (fluoroquinolones, tetracyclines, macrolides and others) in both gram positive as well gram-negative bacteria [11,12]. A number of efflux pump inhibitors have been described in recent literature, some of which have shown efficacy in animal models [12,13]. A good example is the compound MC-207,110 and related inhibitors of the RND transporters, which contribute to fluoroquinolone resistance in *Pseudomonas aeruginosa* [11,14]. However, none of the compounds reported to date have reached clinical trials yet. The difficulties in developing these agents arise from diverse factors, such as the challenging task of finding or designing inhibitors affecting multiple pumps, since bacterial resistance to an antibiotic is often mediated by more than one transporter, and the difficulty to eliminate adverse effects [12,13,15].

2. The decline of natural products and antibiotics research

Examining the evolution of the pharmaceutical industry in the last decade, two trends in the focus of the in-house research efforts can be identified, one being the abandonment or downsizing of antibiotics research, and the other one the near-demise of natural products research. Antibiotics research was abandoned mainly due to the perception of solved medical need (no real need of new agents) and of a poor return of investment [2,10,16,17]. At the same time, natural products were abandoned due to several factors, including the lack of success stories in the late 1980s and early 1990s (exceptions such as the echinocandin class of antifungal agents notwithstanding); its association with antibiotics; and the introduction of two technologies that changed the paradigm of drug discovery in the industry: combinatorial chemistry, as a new way of generating the chemical diversity that was formerly expected from nature, and high throughput screening (HTS) [18]. In addition, the structural complexity of many natural products has often been perceived as an obstacle, since it may impose serious challenges to chemical synthesis and derivatization during the lead optimization process [19].

The introduction of the HTS paradigm in the drug discovery process brought two negative consequences for natural products research. One, the perception that the

Table 2 – Examples of HTS approaches to antibiotic discovery reported in recent literature

Pathway	Target protein	Function	Reference	Main outcome
Peptidoglycan synthesis	Multiple	Whole pathway	[74]	MurA inhibitors found with very weak antibacterial activity (*S. aureus* MIC 16 μg/ml). Whole cell assay
	MurA	UPD-N-acetylglucosamine enolpyruvil-transferase	[75]	Enzyme inhibitors with weak antibacterial activity (*S. aureus* MIC 4 μg/ml)
			[76]	Enzyme inhibitors found without reported antibacterial activity
	MurC	UDP-N-acetylmuramyl-L-ala ligase	[77]	Enzyme inhibitors found without reported antibacterial activity
	MurG	Nucleoside diphospho-glycosyltransferase	[78]	Enzyme inhibitors found without reported antibacterial activity
	MraY	Transferase[a]	[79]	Description of methodology, no hits reported
	PBP1b	Transglycosylase/transpeptidase	[80]	Description of methodology, no hits reported
Protein synthesis	Phe-RS	Phenylalanyl-tRNA synthetase	[81]	Enzyme inhibitors found with in vitro and in vivo antibacterial activity antagonized by phenylalanine
	Pdf1	Peptide deformylase	[82]	Screening of focused libraries identified a lead with in vitro and modest in vivo antibacterial activity
	Multiple	Transcription–translation	[83]	Cell-free transcription–translation assay in *S. aureus* Description of methodology, no hits reported
	Multiple	Transcription–translation	[84]	Cell-free transcription–translation assay in *S. pneumoniae*. Hits found with weak antibacterial activity, slightly improved by medicinal chemistry
	Multiple[b]	Ribosome assembly	[85]	Description of methodology. Piloted with a focused library of aminoglycosides derivatives
Fatty acid synthesis	FabI	Enoyl-ACP-reductase	[26]	Enzyme inhibitors found in primary screening without antibacterial activity. Medicinal chemistry produced compounds with potent activity against *S. aureus* and in vivo activity in a rat model, though limited spectrum (substrate of efflux pumps)
	Multiple[c]	Most of the type II fatty acid synthesis pathway	[25]	Enzyme inhibitors with weak antibacterial activity identified from a natural products library
Others	FtsZ	Tubulin-like protein involved in septum formation	[86]	Description of methodology, no hits reported
	spsB	Signal peptidase I	[87]	Enzyme inhibitors with weak antibacterial activity identified from a natural products library

The screens are cell-free assays and the inhibitors reported are from synthetic origin, unless indicated otherwise.
[a] UDP-*N*-acetylmuramyl-pentapeptide: undecaprenyl-phophate phospho-*N*-acetylmuramyl-pentapeptide transferase.
[b] Ribosomal protein S15 and 16S rRNA.
[c] FabD, FabF/B, FabG, FabA/Z and FabI.

complex extracts generated from microbial broths were incompatible with the modern detection techniques developed for HTS. Second, the perception that the time required to go from the detection of an extract hitting in the screen to the active compound was far too long to compete effectively with the screening of massive collections of synthetic compounds. Regarding the first obstacle, it is true that strongly colored extracts tend to interfere with some assay detection methods, but this has not been a serious problem with natural product libraries in screening campaigns using technologies such as FRET, fluorescent polarization and others [18,20]. The second caveat, the time required from hit (extract) to lead (compound), is harder to refute, though a number of new technologies have been added to the toolkit of natural products chemistry groups, helping to reduce this time significantly. It is generally accepted however that this is still the major bottleneck affecting natural product lead discovery. However, the promise of structural novelty available only by exploring the world of secondary metabolism may well justify the investment of time and resources [18].

The HTS paradigm was also applied to the field of antibiotics discovery. In combination with the burst of new potential targets unveiled by the rapid progress of microbial genomics, it promised a renewed source of leads for antibiotic development [21–24]. However, it is now clear that this approach has not been as successful as initially expected [2,10,16]. A number of HTS assays potentially useful to find inhibitors of essential bacterial enzymes or processes have been described (Table 2). To date, HTS campaigns of collections of synthetic compounds against bacterial enzymes in cell-free assays have mostly failed in delivering compounds useful as clinical candidates. It is relatively easy to find submicromolar inhibitors of bacterial targets from synthetic scaffolds, but in most of the cases these compounds lack antibacterial activity or they are just too weak to make them real drug leads, most likely because they are unable to reach their targets due to poor penetration and/or active efflux [25]. Lack of permeability is usually very difficult to correct by medicinal chemistry, although some examples of success exist [26]. An obvious factor is that most of the synthetic compounds in the collections available for screening have been designed for other purposes rather than for antibiotic activity.

In summary, the failure of the paradigm based on HTS of synthetic compounds using target-based in vitro screens provides a window of opportunity for natural products as the source for the next generation of antibiotics.

3. Key factors for success in natural products research

One could question why it has apparently become so difficult to discover new antibiotics from natural products. It is generally accepted that the number of antibiotics in nature is vast [27]. Data generated in our lab show that the percentage of actinomycete and fungal strains producing antimicrobial activities in standard agar diffusion assays ranges between 30 and 80%, depending on the ecological or taxonomic groups [28,29]. However, though the number of antibiotics in nature may be really huge, most of them are already known or useless (not specific for bacteria, toxic, too weak, lacking the desired pharmacokinetic properties, etc.). This hyperabundance of antibiotics in nature implies that the challenges faced by antibiotic discovery programs based on natural products are going to be very different from the ones typically faced by other therapeutic areas when screening synthetic libraries. The only field in which some similarities could be found is in the search for natural antitumor agents, since cytotoxic metabolites are equally abundant in nature.

Success in discovering new antibiotics from microbial natural products (Fig. 1) requires having a given microorganism grown in conditions appropriate to induce the production of the desired metabolite, which is then extracted and tested in a screen able to detect this as a hit. This compound has to be isolated from the original mixture and identified. Every strategy put in place along the process should focus on how to address the issues surrounding those steps, how to maximize their efficiency and how to solve the potential bottlenecks that will appear, as in any other process.

One of the perceived liabilities of this field is that it requires a substantial amount of manual work. Automation has been regarded as necessary to improve the efficiency of the early steps in the drug discovery process, but it can not be used with equal success and efficiency throughout the process of natural products lead discovery, the screening step being clearly more amenable to automation than the generation of natural products libraries.

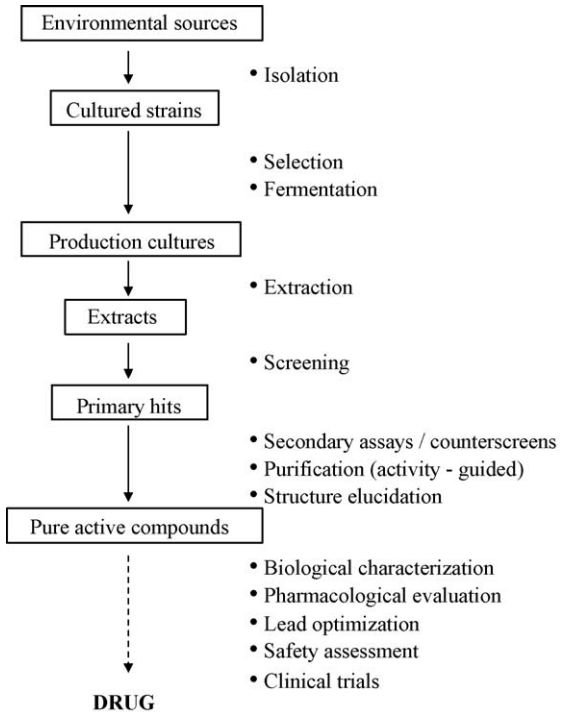

Fig. 1 – The process of antibiotic discovery from microbial natural products.

4. Natural products and biodiversity

One of the basic questions to address in any discovery effort of new natural antibiotics is which groups of organisms should be selected to improve the probability of success.

Actinomycetes have traditionally been the most prolific group in antibiotic production, and have been the origin of a good number of marketed antibiotics (Table 1). Fungi are another rich source of antibiotics [30], though only a few examples have reached the market (Table 1). These two microbial groups have been the focus of most of the efforts by industrial and academic laboratories for the last 60 years. It could be argued that since they have already been so extensively studied, the chances of finding anything new are too low to be worth the effort. However, the evidence is that only a minor fraction of all the species or genetically distinct strains of actinomycetes and fungi existing in nature have been grown in culture [27,31]. Mathematical models suggest that the number of antibiotics still to be discovered from actinomycetes could well be above 10^5 [27]. New species and even major taxa of fungi and actinomycetes are being discovered every day, opening windows of opportunity and proving that our knowledge of these microorganisms is far from exhaustive. As an example, new major marine actinomycete taxa have recently been described and shown to produce biological activities, including antibiotics [32–34]. Marine microbes are particularly attractive because they have not been as extensively exploited as their terrestrial counterparts, and because of the high potency required for bioactive compounds to be effective in the marine environment, due to the diluting effect of seawater [35]. Significant progress has been made recently in the high throughput cultivation of marine microorganisms, in some cases recovering organisms unattainable by traditional methods [35,36]. Furthermore, there is compelling evidence that compounds formerly attributed to marine invertebrates are actually synthesized by bacterial simbionts [37].

Overall, it is clear that expanding the diversity of actinomycetes and fungi is feasible and can be achieved by exploring little explored ecological niches and developing new ways of growing previously uncultivable strains [38].

Other microbial groups well known to produce bioactive secondary metabolites include the cyanobacteria and the myxobacteria [38]. Although these seem to offer more promise in areas such as oncology [39–41], a recent report shows that myxobacteria are able to produce activities against gram-negative bacteria [42]. However, extensive exploitation of these groups necessitates improved methods for isolation and cultivation, to allow generating the high number of isolates required for industrial purposes with less effort than the time consuming techniques currently available [39,42]. Finally, plants and other well-proven sources of bioactive compounds, such as marine invertebrates, seem to have a higher potential as source of leads in oncology and other therapeutic areas.

Maximizing biodiversity within the groups selected as our focus, even if not ensuring success, is one of the cornerstones of any rationale strategy designed to find new antibiotics, but it remains a critical challenge. It is relatively easy to isolate hundreds of strains of fungi, actinomycetes and other bacteria in short order by using indirect methods [29,43]. Moreover, semi-automated platforms for microbial isolation have been described, although not universally accepted [43]. However, deciding which strains are worth being tested in the screening process is a much more difficult task. A number of strategies have been described to facilitate the ''dereplication'' of genetically identical strains, using tools ranging from morphology to chemotaxonomic markers and diverse molecular approaches [29]. A critical point here is that even a minimum level of taxonomic analysis will strongly impact the capacity of the group to generate isolates for screening. Furthermore, many antibiotics are produced by multiple different species. This makes biodiversity an imperfect surrogate of chemical diversity, which is actually what should be maximized. However, the principle that maximizing diversity increases the chances of success remains as one of the few still valid axioms in industrial natural products screening programs.

5. Non-conventional sources for natural products discovery

An idea that was heralded in the 1990s as the potential solution to the lack of productivity in natural products research was the use of environmental DNA (or DNA from non-cultivable organisms) as a source for genes involved in secondary metabolite biosynthesis [44,45]. This approach was rooted in the notion that only a very small fraction of microorganisms in any environmental sample can be cultured by standard techniques [46], thus leaving a vast genetic pool unexplored by these conventional methods. Actually, the number of bacterial species found per gram of soil could be as high as 10^6 [47].

In essence, the approach was based on the isolation of DNA from soil or other environments, followed by the generation of ''metagenomic'' libraries, using large DNA fragments cloned in *E. coli* or *Streptomyces* spp. [44,45]. These libraries were subsequently screened for bioactive metabolites. Although proof of concept was obtained that this approach can indeed deliver the desired output [48,49], the truth is that it has so far failed to bring in the expected leads useful for antibiotic development. Perhaps the main challenge is how to translate those early proof-of-concept experiments into a technology suitable for drug discovery at the industrial scale. Some of the companies that were working in this field have abandoned this strategy or re-focused their efforts. Thus, considering the small effort placed into these approaches at present, it seems unlikely that the next useful antibiotic will come from this strategy.

A different group of natural products that has been explored as a potential source of antimicrobial therapies are the so-called antimicrobial peptides. The term refers to peptides of variable length (typically below 25–30 kDa), synthesized in ribosomes and widespread in nature, from microorganisms to higher eukaryotes. In animals, these peptides (defensins, cathelicinds, protegrins, magainins and many others) play a critical role in the innate immune response against pathogens. These peptides are composed mainly of cationic and hydrophobic aminoacids, organized as an amphipatic structure. This structure is believed to confer to antimicrobial peptides their capacity to disrupt cell mem-

branes, and their net positive charge is the reason why they are selective against bacterial cell membranes. The structural characteristics and mode of action of antimicrobial peptides have been extensively reviewed recently [50,51].

Antimicrobial peptides have been the subject of some interest as leads to generate therapeutic products. Many of them show potent and broad spectrum in vitro activity against bacteria resistant to conventional antibiotics and, due to their particular mode of action, the development of resistance may be limited. A number of drug candidates from several companies have been reported in clinical and preclinical studies (reviewed in [52]), some of which may reach the market in the near future. In most of the cases, these compounds are being developed for topical application in indications such as acne infections or infected foot ulcers in diabetic patients. Their use for systemic infections is much less obvious, given the inherent issues of instability, potential toxicity and the cost of large-scale production of these peptides [50,51]. Several approaches to circumvent the problem of lack of stability are under development, from the concomitant use of protease inhibitors to the modification of the structure to peptoids, which are not easily cleaved by proteases, or to cyclicized sequences of D-aminoacids [52,53]. Overall, the impact of antimicrobial peptides in the field of conventional antibiotic therapy is unclear at present, although there are several companies that are exploring this area [52].

6. Fermentations and extract generation

Another argument supporting the potential of fungi and actinomycetes as valuable sources for antibiotic discovery relies on the effect of growth conditions on the production of microbial secondary metabolites. Thus, a single strain, grown under different conditions may produce substantially different compounds. This has been known since the early days of the development of the industrial production of penicillin [1]. This old concept has received even further support after the publication of the genomes of two *Streptomyces* species, *S. coelicolor* [54] and *S. avermitilis* [55]. In both cases the genomic analysis revealed the presence of more than 20 gene clusters encoding for the synthesis of polyketides or non-ribosomal peptides, many more than the number of compounds actually isolated from these strains. Similar results have been found in fungi [56], thus suggesting we are still far from having a good understanding about how to fully exploit the metabolic potential of microbes under laboratory conditions.

The main issue here is how to translate this concept to the discovery process when there is no previous knowledge on which metabolites are expected. A common strategy in industrial screening programs consists of growing each strain under multiple growth conditions. However, using a high number may set limits to the number of strains screened, another parameter that is critical to maximize. As a compromise, usually only a small number of conditions (three to five) are applied to each strain [57,58]. In addition, without previous knowledge about the required conditions for a given microorganism to produce the desired antibiotic, assigning many growth conditions at random may result in an unnecessary redundancy of metabolites screened and in the proliferation of extracts without relevant levels of secondary metabolites. These problems may be exacerbated if a library of extracts is built to be tested in diverse screens. A number of strategies have been described to address these issues [59], but this remains one of the unsolved problems in natural products research.

In recent times, new methods to grow microbes are being reported that could help to exploit the metabolic potential of selected strains. For instance, a method has been described to grow bacterial strains in microtiter plates that allows parallel handling of strains, making it easy to increase the number of growth conditions assigned to each strain [60]. This system has been also piloted on *Streptomyces* with success [61].

The extraction process is the next critical step. As mentioned above, one of the reasons why natural products lost its popularity in industry stems from the perception that extracts are prone to produce artifacts and interferences in the sensitive assays used in HTS. It is also commonplace to hear that interesting compounds can be overlooked due to the presence of other molecules, or are simply undetected because of low titers [18,62]. One way to overcome these issues is by including a fractionation step after the extraction [18,62]. The obvious disadvantage of this system is the proliferation of testable samples, at the expense of including more different strains or extracts in the library. An extension of this approach is the generation of libraries containing pure natural compounds [63], a strategy that may seem initially attractive but is not free of hurdles, mainly the difficulties in isolating significant amounts of low titer compounds and the cost required to prepare those libraries. Cost–benefit considerations are essential to address whether it is really efficient to invest major resources before the screening process.

7. Screens and targets

The lack of success of the HTS approaches already mentioned suggests that the chances may be higher if looking upfront for molecules able to kill bacteria, rather than just enzyme inhibitors in a cell-free assay. Indeed, the old antibiotics were discovered by measuring the ability of microbial extracts to inhibit bacterial growth. However, this paradigm was finally exhausted as it started to deliver again and again the same old antibiotics.

As already mentioned, the increasing knowledge of genomics, bioinformatics and microbial physiology, as well as our better understanding of the mode of action of empirically discovered old antibiotics, has resulted in the identification of a number of targets (Table 2) with suitable properties for antibiotic discovery: essentiality, conservation in the desired range of microbial pathogens and lack of (or different enough) human homologs [5,23]. The challenge is how to combine this knowledge with assay technologies able to detect molecules that not only interfere with those targets, but are actually killing the bacteria by doing so.

A possible answer may rely on the use of bacterial strains genetically modified to either overexpress or underexpress targeted essential genes, rendering them resistant or hypersensitive to antibiotics acting on those targets when compared with a wild type strain. The progress of molecular micro-

biology accumulated in the last years allows the generation of such strains by a number of approaches, e.g. by replacing the native promoter by a regulated promoter, or by means of antisense technology [23,64,65]. These strains can be used in liquid or agar diffusion assays in parallel with unmodified strains, to facilitate the detection of antibiotics acting specifically on the desired target. Furthermore, robotic systems able to fully integrate the screening process using microbiological agar diffusion assays have been reported [66].

An alternative strategy would be the use of empiric screening looking for activity against a target microorganism, much in the way it was done in the early days of antibiotics discovery, followed by the examination of the mode of action using for instance a multicopy suppression screen as described recently [67]. Only the extracts acting on targets known to be acceptable would be prioritized for follow-up studies.

8. From hit to lead

The next step after the screening process is the purification and identification of the compound(s) responsible for the biological activity detected in the extract. At this point it is critical to have an efficient system to identify uninteresting or already known antibiotics as early as possible, to be able to focus the resources on the important ones. These ''dereplication'' issues are of paramount importance, since many known antibiotics are produced by different species and they will be found repeatedly in antimicrobial screens. In the last years, this process of dereplication has made enormous progress by the use of databases containing LC–MS [18,68] or LC–NMR data [69].

This step has been one of the points blamed as a disadvantage of natural products when compared to the collections of defined synthetic compounds [63]. To be able to compete successfully, this process should be as rapid and efficient as possible. This can be hampered by the fact that screens often present a high hit rate, making it necessary to prioritize the extracts selected in some way. The use of secondary assays or counterscreens that help to eliminate the uninteresting biological activities is essential at this point [20]. However, the use of secondary assays at this early stage may yield misleading results, since the extract may contain several active compounds, and they may be masking each other in the secondary assay. The most immediate solution is to subject all the selected extracts from primary screening to some type of fractionation, but this is costly and time-consuming.

The introduction of parallel chromatographic systems and other separation technologies, as well as the dramatic progress in the spectroscopic techniques used for structure elucidation, are obviously an enormous improvement in this part of the process [18]. However, the complexity of the process, which involves not only bioassay-guided isolation chemistry, but also re-supply of extract in volume to obtain sufficient pure material, is such that this is still recognized as the rate limiting step of natural products lead discovery. It is clear that this is one of the key areas to improve if natural products research groups are aspiring to again play an important role in the pharmaceutical research business.

9. Natural products as leads for drug development

As already mentioned, one of the arguments often heard against natural products refers to their structural complexity, which may pose challenges for their use as leads for medicinal chemistry or in their synthesis, if necessary for scale up. However, it is important to remember that structural complexity in natural products is diverse, going from the very simple to the highly complex. More importantly, structural complexity has not been an obstacle in developing some natural products as useful drugs, not only antibiotics (vancomycin and daptomycin would be good examples), but also in other areas (for instance taxol, an anticancer agent which has become a top selling drug). The diversity of natural compounds is higher than what is found in combinatorial libraries and they have properties that often make them advantageous as leads, such as their comparatively higher rigidity, a property that has been associated to a reduced entropic cost of binding to macromolecules and improved oral bioavailability [70]. It is also important to remember that the synthesis of natural compounds, precisely due to the unparalleled structural diversity that can be found in nature, has historically been a driver for tremendous progress in discovery and research, ultimately leading to critical advances in drug development [71].

In any case, the field of natural product synthesis has experienced significant advances in recent times [18,70–72]. As time passes on, more is known about polyketide and nonribosomal biosynthetic pathways, the two biosynthetic classes to which most useful antibiotics belong. The increasing body of knowledge in this field, including research tools, genes and enzymes, may be added to the usual toolkit of medicinal chemists to facilitate the manipulation of natural product structures to generate new compounds with better properties [73].

10. Conclusions

Microbial natural products still appear as the most promising source of the future antibiotics that society is expecting. The arguments supporting this idea are the unparalleled structural diversity that can be found in nature, the fact that natural antibiotics have apparently been shaped by evolution to make them effective in killing microorganisms, and the suggestions that the field still unexplored is huge, in terms of microbial diversity, potential to trigger the expression of silent pathways by manipulating the cultivation conditions, and number of molecular targets still to be exploited for antibiotic therapy. The process of antibiotic discovery from natural products is complex and difficult, but significant progress has been made during the last years that has improved our understanding of the key factors required for success. Any new efforts in this area will benefit from this understanding, as well as from the new technologies that are contributing to improve the efficiency of the process. The resources required may look significant, but the reward for companies willing to take the risk is worthwhile: the solution to one of the most serious health threats that we may be facing in the years to come.

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