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Current Organic Chemistry, 2016, *Volume* 1

The long and winding road to convert an antimicrobial compound in an antimicrobial drug. An overview from a medicinal chemistry point of view.

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Abstract: Fungi have emerged over the past decades as major causes of human infections, especially among immunocompromised hosts. Although it appears that we have a big armamentarium of antifungal drugs in clinical use, in fact only a modest number of drugs are available. Unfortunately, all antifungal drugs available for the treatment of fungal infections are not fully effective and possess a certain degree of toxicity or some drawbacks in pharmacokinetic aspects such as do not possess an adequate solubility. As complement, fungi have developed a rapid resistance against them because of their large-scale use. Considering such situation, it is clear an urgent need to develop new and more effective antifungal drugs. For such reason in recent years there has been an incredible increase in the number of studies looking for new compounds with antifungal effects; in particular new structures obtained from natural products. While there have been reported a large number of compounds with antifungal activities (some of them with novel structures), very few have managed to be used therapeutically. In this review article, we have identified and discussed the main reasons for the poor results that have been obtained so far in order to find new antifungal drugs. Also new strategies which could be the way to improve the search for new antifungal agents for therapeutic use are discussed here, remarking its scope and limitations.

Keywords: antimicrobial, natural products, antifungal, target-based screening, new drugs, new strategies.

THE NEED FOR NEW ANTIFUNGAL SYSTEMIC AGENTS FOR THERAPEUTIC USE

Until several years ago, fungal infections were relatively uncommon and systemic infections very unusual, particularly in cool and temperate climatic zones. Thus, a fungal infection usually meant athlete's foot or oral or vaginal thrush causing just discomfort, but not serious clinical situations. Unfortunately, since the 1970s the situation has changed notoriously. In fact, there has been a steady increase in the incidence of fungal infections which are more dangerous and life threatening. Probably the main factor favoring the spread of fungal diseases has been the widespread use of broad-spectrum antibiotics, which eliminate or decrease the non-pathogenic bacterial populations that normally compete with fungi. Further has been the increased number of individuals with reduced immune responses. This has led to an increased prevalence of opportunistic infections such as infections with fungi that rarely cause disease in healthy individuals.

The most common systemic fungal infection is candidiasis, but there are more serious conditions such as cryptococcal meningitis or endocarditis, invasive pulmonary aspergillosis, and rhinocerebral mucormycosis. Invasive pulmonary aspergillosis is now a leading cause of death in recipients of bone marrow transplants. In turn, the colonization of the lungs of patients with asthma or cystic fibrosis by aspergillus can lead to a similar condition, termed allergic bronchopulmonary aspergillosis.

While anyone can succumb to a fungal infection, some have more at risk than others. Older individuals, diabetics, pregnant women, and "burn wound victims", among others, are all more prone to fungal infections such as candidiasis [1-3]. Fungi also produce superficial fungal infections not only in immunocompromised hosts but also in healthy individuals. In addition, children living in the under-developed nations are potential victims of these fungal infections producing serious consequences for them [4,5].

The treatment for fungal infections can be topical or systemic. Topical antifungals are generally considered as first-line therapy for uncomplicated, superficial, relatively localized dermatomycoses due to their high efficacy and low potential for systemic adverse effects.

The oral route is usually the safest, the most economical, and the easiest for systemic antifungal drugs; however, the choice of an oral form involves a consideration of both drug and patient health situation.

The drugs for effective treatment of emerging fungal infections are in short supply, thereby contributing to a high

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mortality rate. The available drugs are essentially limited to four segments in the drug market: allylamines, azoles, polyene macrolides, and other treatments.

• Azole drugs, which inhibit the key enzyme that allows fungi to grow, are the largest segment in the antifungal group revenues of products sold, with leading products such as Vfend (Voriconazole), Noxafil (Posaconazole), and Diflucan (Fluconazole). The compound most recently entered to the market belongs to this family (Isavuconazonium Sulfate) (Fig. **1**).

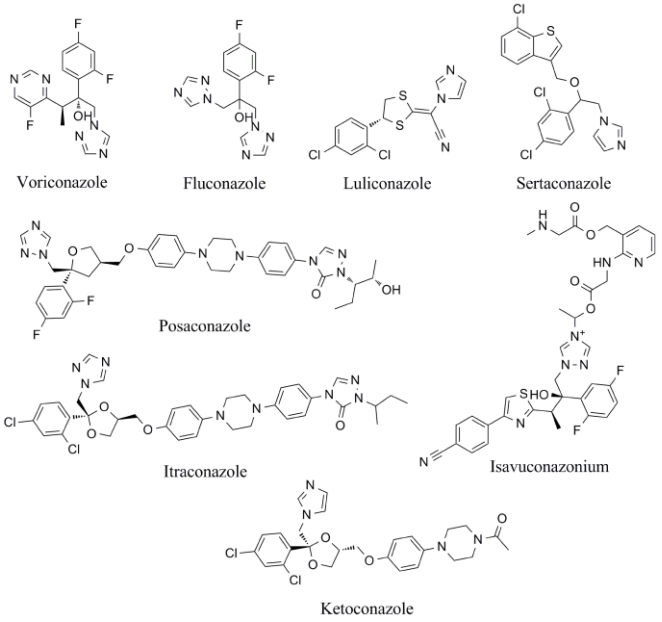

Fig. (1).

• Allylamines (Terbinafine and Naftifine, Fig. **2**), which prevent fungi cell walls from developing and thus block them from growing on human skin. This segment is largely driven by the terbinafine products, namely Lamisil and its generic counterparts. The market began to decline with the generic offering of terbinafine in the last years.

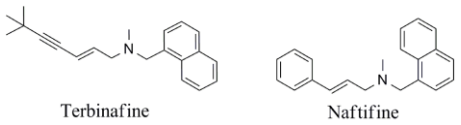

Fig. (2).

• Polyene macrolides (Fig. **3**) are powerful topical antifungals, but they constitute the smallest segment in the antifungal market. All products in this segment have generic counterparts, and there have not been new developments in this area in the past years

• Sales for other antifungal products (Fig. **4**) that do not fit into any of the three categories above have shown the highest growth.

All these agents suffer from a number of limitations that can render their use difficult; for example, dose-limiting nephrotoxicity associated with amphotericin B, rapid development of resistance with flucytosine and drug–drug interactions, fungistatic mode of action and resistance development with the azoles as well as the poor solubility of many of them.

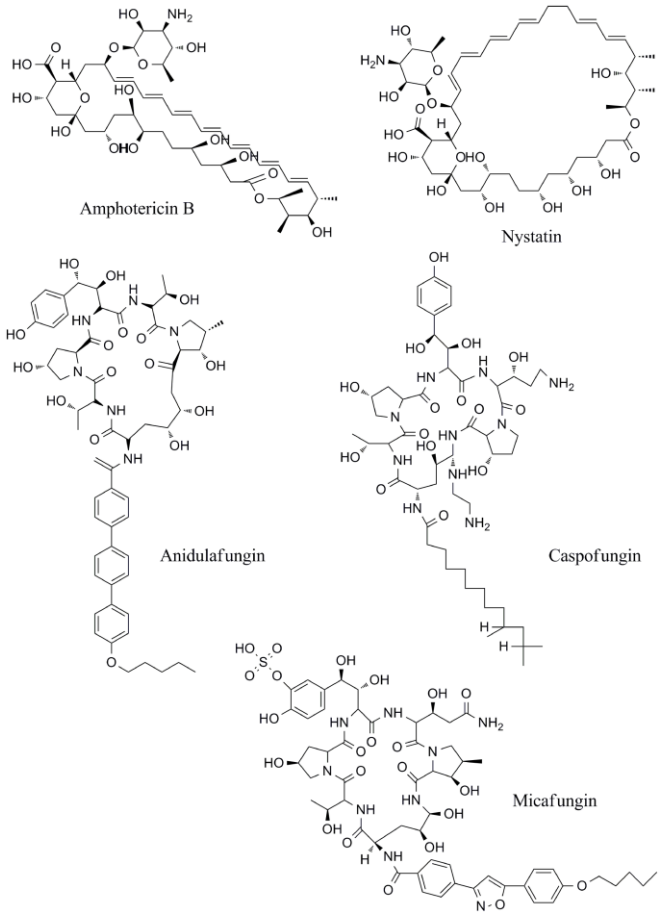

Fig. (3).

On the other hand, it is interesting to remark that generic competition is strong in the antifungal segment, with roughly 60% of total sales. The high generic product penetration creates a highly competitive environment and leads to added difficulty for new product introductions. Although we can expect to see a continued demand for new antifungal drugs, the low cost of generic products will probably generate an added pressure for the development of new products. Anyway, to remain competitive and secure a continued market share, current leaders will need to expand product lines and invest in new developments, specifically in niche areas such fungal infections for immunocompromised populations.

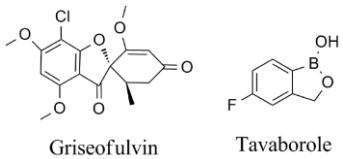

Fig. (4).

Another issue is that for the most part the systematic search for new antimicrobials has been abandoned by the major laboratories in the past decades, and thus, great part of the problem is being relegated to the efforts of academic research groups.

It is clear that there is an urgent need for new antifungals with a broad spectrum of action [6], and with fewer dose-limiting side effects [7,8]. Accordingly, it is particularly imperative to develop new agents for clinic use.

SOME USEFUL STATISTICS AND NUMERICAL DATA

Due to the need for new antifungal agents for clinical use a number of studies have been conducted to find new antifungal compounds from natural and synthetic sources. Our own research group has made numerous contributions to this topic, in which we have reported compounds obtained from natural products [9-14] and by synthesis [15-27]. The amazing amount and variety of compounds possessing antifungal effects reported in the past four decades can be also noted in several reviews [28-30].

Based on a survey conducted on the website of Scopus considering the production of scientific articles in the past 40 years (1975- 2015) with the keywords "antifungal, antimycotic and fungicidal", the results displayed a marked and consistent increase of information and general knowledge in this field. Thus, we found a total of 102,597 articles averaging about 470 annual reports for the first decade, while for the last decade was in approximately 5,500 articles with an annual increase in the same period of about 360 articles per year, denoting the strong interest in this field (black line of Fig. **5**).

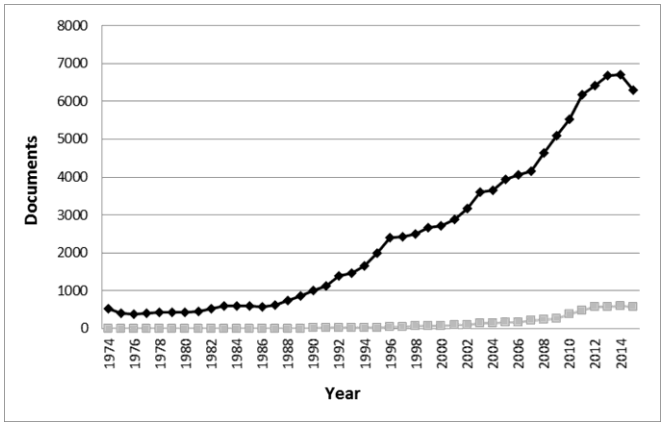

Fig. (5). Scientific articles reported in the period from 1975 to 2015. Black line (keywords used: antifungal, antimycotic and fungicidal); gray line (keywords: plant, extract, oil, specie or phytochemical). Source: unpublished personal analysis by the authors, 2015.

A more refined search on the sample obtain related to natural origin (plant, extract, oil, specie or phytochemical), also shows a steady increase with a total of 5,232 articles representing 5% of the previous total. Therefore, for the first decade, the average was below 3 reports per year, whereas for the last decade is about 400 with an increase of 55 papers per year (gray line in Fig. **5**). This result indicates that the exploration of antifungal activity is fully in force. Finally, if the search is restricted to articles reporting structures or new compounds (new structure, novel, synthesis, identification, in vitro evaluation or structure-activity), a more modest contribution is observed, reflecting the technical difficulties involved in this last stage, a total of 165 papers was representing 0.16% of the initial set with an approximate increase of three articles per year (Fig. **6**). It should be clarified that in the case of the 5,232 articles obtained with the second filter (gray line of Fig. **5**), all abstracts were read and discussed by us; while in the case of the 165 papers obtained by using the last filter (Fig. **6**), all the articles were fully analyzed and discussed in order to perform a properly classification of them.

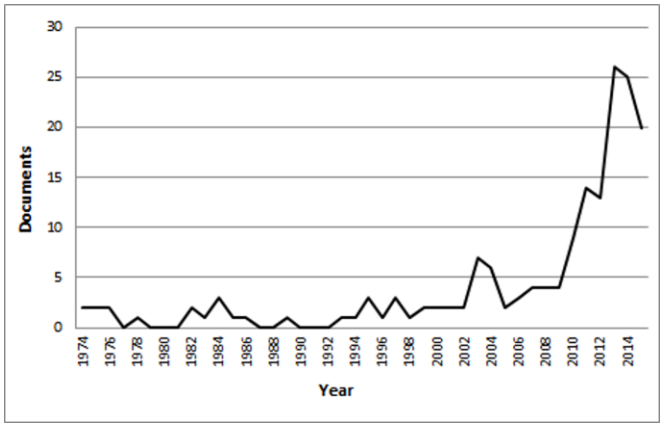

Fig. (6). Scientific articles reported in the period from 1975 to 2015 keywords used: new-structure, novel, synthesis, identification, in vitro evaluation or structure-activity. Source: unpublished personal analysis by the authors, 2015.

On the other hand, Table **1** displays the antifungal drugs approved by the FDA (Food and Drug Administration) in the last 15 years. This table shows that during this period, only 9 new molecules have entered to the market, of which 6 are for systemic or oral use and the rest are of topical use. Of these nine compounds, five are azoles (Fig. **2**), three are echinocandins (Fig. **3**), and the remaining one is tavaborole (Fig. **4**), a boron compound which does not fit in the other categories. From the above results, it is clear that in the antifungal area there has been a very scarce development of new drugs for therapeutic use.

THE LONG AND WINDING ROAD TO CONVERT AN ANTIMICROBIAL COMPOUND OBTAINED FROM NATURAL SOURCES INTO AN ANTIMICROBIAL DRUG. SOME ASPECTS EXPLAINING THE POOR RESULTS

From the data shown in the previous section it is clear that even though a large number of compounds with antifungal activity obtained from natural products is reported, very few can reach further development and almost none can be used therapeutically. The question that arises is if so many antifungal compounds have been discovered from natural sources, why is it so complicated to bring them to the market? [31]. There are many reasons explaining this limitation, probably the main ones are: that the discovered compounds are not potent enough or do not show the spectrum of action that makes them attractive for further research. Some compounds are not selective enough, also affecting mammalian cells, therefore they develop undesirable effects.

Table 1. Drugs approved by FDA during the last 15 years.

NDA Number	Proprietary Name	Established Name	Applicant	Appro-val Date
N021227	Cancidas	Caspofungin Acetate	Merck Research Labs	01-01
N021266	Vfend	Voriconazole	Pfizer	05-02
N021385	Ertaczo	Sertaconazole Nitrate	Mylan	12-03
N021506	Mycamine	Micafungin Sodium	Fujisawa	03-05
N021632	Eraxis	Anidulafungin	Vicuron	02-06
N022003	Noxafil	Posaconazole	Schering	09-06
NDA204153	Luzu	Luliconazole	Medicis Pharmaceutical Corp.	11-13
NDA204427	Kerydin	Tavaborole	Anacor Pharmaceuticals Inc.	06-14
NDA207500	Cresemba	Isavuconazo-nium Sulfate	Astellas Pharma US, Inc.	06-15

Many compounds possess promising in vitro but weak in vivo activities, probably due to poor absorption, inactivation by serum binding and high clearance rates among other reasons. Seen from a more general point of view there are two aspects which might justify these poor results. The first one is related to the characteristics and properties of the structures that are reported, but the second factor is more related to the type of methodology that has been used so far to the search for new antifungal agents.

According to Barrett [31], apart from a strong in vitro activity, the following aspects should be taken into account when we select a lead compound as antifungal. Is the compound structurally novel? Is the mechanism of action original or potentially useful? Is a clinical proof possible? Are chemical modifications of the structure possible? Unfortunately, in most of the studies analyzed in the previous section, these issues correctly mentioned by Barrett, have not been taken into account. Moreover, in most cases it has not been considered if these compounds meet the Lipinski's fifth rule [32] or the drug/non-drug concepts established by Veber [33] and Vieth [34]. Vieth et al. analyzed the different concepts for the drug/non-drug classification which has been reported so far (Lipinski,Veber and others) [34] by analyzing the properties of a group of drugs approved for oral use, certain general characteristics were found to classify between good and bad drug candidates. These properties are summarized in Table **2**. It is clear that these are very important properties that must meet these new compounds to really have good chances of becoming a leader structure. However, it is important to remark that, whereas these concepts are very important, there are exceptions. One example is the natural products macrocycles as drug candidates [35]. Terrett et al have highlighted the fact that the macrocyclic drug currently on the market (e.g. the antibiotics vancomycin and erythromycin, the immune suppressant scyclosporin A and FK506, the potent antifungal agent amphotericin B, and the anticancer agent epothilone B), are almost exclusively derived from microbial natural products. They remarked that natural products macrocycles are often ignored as drug candidates because they don't obey Lipinski's rules. These authors provide strong evidence that macrocycles as drug candidates have been underexplored so far, and they propose that the discovery of new molecules from this family might be of great interes in the future. In relation with this topic, a review by Zhang and Wilkinson displayed the applicability of the Lipinski's rule-of-five to natural products [36].

Regarding the limitations related with the methodology and strategies used in order to obtain new antifungal compounds from natural products, a particularly striking aspect is the lack of structural information of the potential molecular targets.

Table 2. **Properties select to classify the compounds as drug – non-drugs.** MW: Molecular Weight; CLogP: Computed logarithm of octanol-water partition coefficient; ONs: Quantity of nitrogens and oxygens; ONs: Quantity of OHs and NHs; NRING: Number of rings; ROT: Number of rotatable bonds; PSA: Polar surface area; NHal: Number of halogens.

Property	Lower limit	Upper limit
MW	200	500
CLogP	-0.8	5.2
ONs	2	9
OHNHs	0	3
NRING	1	4
ROT	1	10
PSA	22	134
NHal	0	2

Undoubtedly, azole compounds have experienced the most important development in recent years as compared to other antifungal drugs. The imidazole and triazole antifungal class has been the most successful in terms of numbers of different agents that have entered clinical use, but agents that are licensed for clinical use in invasive fungal disease are all triazoles: fluconazole, itraconazole, posaconazole and voriconazole. In turn, posaconazole and voriconazole represent specific advances in the understanding of structure–activity relationships for antifungal azoles, as evidenced by their progression in the past years [37-39]. A triazole based pharmacophore has replaced the earlier imidazole pharmacophore in systemically active azoles because the triazole group enhances the specificity for the fungal cytochrome p450 (erg11) target [40] (see Fig. **7**). In a fluconazole-based structural type, the extra methyl group at carbon atom number 3 enhances hydrophobic interactions at the active site and extends the antifungal spectrum [41].

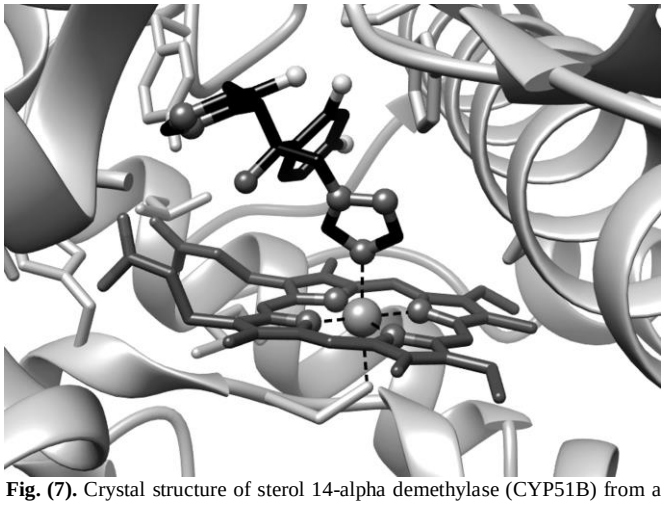

Fig. (7). Crystal structure of sterol 14-alpha demethylase (CYP51B) from a pathogenic filamentous fungus Aspergillusfumigatus in complex with voriconazole. This spatial view of the active site has been generated by using the Chimera software [45].

Replacement of the 1,3-dioxolane moiety in ketoconazole and itraconazole with a furan ring, as in posaconazole, alters and enhances activity, as extensively demonstrated in mouse models [42]. It is interesting to remark that from 2000 the structure of the fungal cytochrome p450 (erg11) (PDB code 1E9X), which is the principal molecular target of these compounds, is available [43]. Such crucial structural information, has allowed the design and development of novel azole compounds, some of which have been introduced into the market.

A completely different situation is that of amphotericin B (Amph B), whose structure was first reported in 1955 at the Squibb Institute and is still used today. This broad-spectrum polyene antibiotic became the early mainstay for the treatment of many types of invasive fungal infection [44]. However, Amph B can affect mammalian cells, and nephrotoxicity is a common sequel of clinical usage. The introduction of lipid complexed amphotericin B formulations considerably reduced the incidence of renal toxicity from amphotericin B.

Unlike the case of azoles, the mechanism of action of Amph B has not been fully elucidated and unfortunately, its molecular target becomes very difficult to establish structural changes that would improve the antifungal effect of this compound.

The current model proposed for the mechanism of action is based on the formation of 1:1 Amph B/sterol aggregates, which associates into a transmembrane barrel with a large-OH-lined aqueous pore down the middle [46,47] (Fig. **8**).

The result of interaction between Amph B and the sterols is a disturbance of the ergosterol function leading to an increased permeability, disruption of the proton gradient, and leakage of potassium ions. However, the complete mechanism of action of Amph B is not fully understood yet. Thus, new hypotheses are being analyzed, and many researchers studying the interaction with natural membranes or artificial bilayers are trying to prove such proposals. It should be noted that the conflicting membrane-permeability data suggest a multiplicity of Amph B channel structures as well as different modes of action [48].

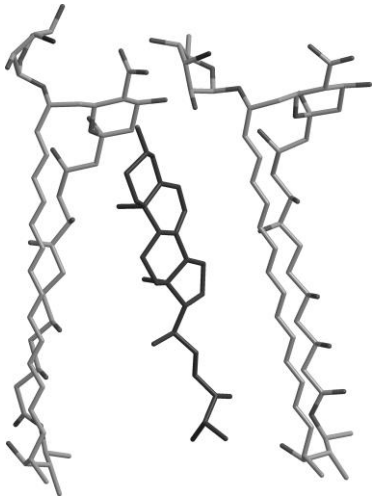

Fig. (8). Spatial view of the molecular interactions of Amph B with sterol aggregates. The figure shows the formation of a 1:2 amphotericin B/sterol aggregates. The complex was obtained from molecular calculations by using the program PATCHDOCK [49,50].

There are numerous new polyenes, modifications of existing polyenes and novel polyene formulations which have been reported, but we will mention here only two of them which deserve a main interest. A cochleate formulation of AmphB has shown efficacy in experimental models of candidiasis and aspergillosis [46-48,51]. This formulation entraps the Amph B molecules in a large, stable, spirally rolled lipid bilayer. The formulation promises oral bioavailability of Amph B, but to date the only pharmacokinetic study involves intravenous injection in mice [48]. The current development status is unclear. The other is a liposomal formulation of tetraenenystatin, which has undergone extensive preclinical and clinical trials [51]. However, the results of phase III clinical trials for treatment of fever in neutropenia and in cryptococcal meningitis have not been reported in detail or published in the peer-reviewed literature. The developmental status of liposomal nystatin is therefore unknown.

It should be noted that improvements in polyenes have been mainly in their pharmaceuticals forms that have allowed improve the pharmacokinetics properties, but there are not structural changes in the parent drug. The big disadvantage for the development of new antifungal polyenes compared to azoles is the lack of structural details of their molecular target which prevents a rational design of new structures.

There are many factors leading the development of new antifungal agents for clinical use into a very inefficient task. In our opinion the issues discussed above are the main ones. The question which arises is: how we can change the search for new antifungal agents to obtain more successful? In the next section some strategies that might improve future expectations are discussed.

NEW STRATEGIES FOR SEARCHING NEW ANTIFUNGAL AGENTS THAT CAN CHANGE THE CURRENT SITUATION

Approaches for identification of novel molecular targets; new trends in antifungal research.

As was previously discussed, the need to identify novel and specific targets for fungi is currently a major undertaking. It is interesting to remark that ideal molecular targets require two major features. First they need to be essential to cell viability, and second, they need to be unique to the fungal organism.

The sequencing of microbial genomes is revolutionizing the discovery of novel antifungal drugs, providing the tools for the rational identification of novel targets and compounds. In fact the genomic-based antifungal drug discovery begins when the complete DNA sequence of a fungal species has been determined. In this sense, important data were obtained from the complete sequentiation of the genome of *C. albicans* [52], *A. fumigatus* [53], *C. neoformans* [54] and *S. cerevisiae* [55]. Once such information is obtained, the sequences of fungal genes are compared with those of the human genome; thus, fungal genes with a low degree of homology to human genes are listed as potentially specific targets of antifungal interest [56]. However, it must be remarked that the list of genes of interest is reduced for several reasons that include the availability of a HTS (High-Throughput-Screening) assay targeting the selected genes [57]. After the design of the HTS assay, new natural sources or existing natural products must be screened with this assay. Thus, the high throughput genomic sequencing, combined with fragment assembly tools, has delivered a cornucopia of sequence information to assist in the search of new targets. Genomic information, combined with the ability to selectively delete or modify genes of interest, is proving to be useful in evaluating the selectivity of a target and its essentiality for growth. In addition, comparative genomics allows the identification of potential targets shared across fungal species. For instance, entire biochemical pathways can be reconstructed and compared in different pathogens. Two interesting review articles which explain the way in which genomic information can be translated into drug discovery are references [58] and [59].

Sequence comparisons may also provide some indication of potential mammalian toxicity if proteins of similar sequence exist in mammalian sequence databases. Numerous databases that contain both sequence and functionality information available over the Internet and easily downloaded onto local servers, are now available. In addition certain commercial databases are available for nonexclusive use by commercial subscribers.

Overall, the genomic revolution is expected might produce a profound impact on antifungal drug discovery, with the potential for the identification of new agents with novel mechanisms of action.

At present, several pharmaceutical companies are already using the information obtained from genomics and also from proteomics in order to find new targets for antifungal compounds [60]. Among them Genome Therapeutics [61] is a leader in the commercialization of genomics-based drug discovery. Another example is Elitra Pharmaceuticals [62], which shifted the traditional paradigm of screening targets singly, toward massive parallel screening. Although genomics open a big avenue for the discovery of antifungal drugs, it is important to remark that the rate of discovery of new antifungal agents has not perceptibly increased since the mid-1990s, when the first partial fungal genome sequences were available [56]. While the use of the genomic knowledge of fungi (in particular the target-based screening using genomic knowledge of fungi) is a promising strategy for the discovery of new molecular targets more specific, there is a need to improve and try to make these techniques more efficient in the near future.

What about peptides as antifungals?

More than 7,000 naturally occurring peptides have been identified, and these often have crucial roles in human physiology, including actions as hormones, neurotransmitters, growth factors, ion channel ligands, or anti-infectives [63]. At present there are over than 100 approved peptide-based therapeutics on the market, being the majority smaller than 20 amino acids [64]. In 2014-2015, US FDA approved 9 peptide drugs, being the most important: Myalept (Astra Zeneca) [65], Albiglutide (Tanzeum GSK) [66], Albiglutide (Tanzeum GSK) [67], Tresiba (Novo Nordisk) (for the treatment of type-1 & type-2 diabetes in adults) and Natpara (NPS Pharm.), for the hypocalcemia control in patients with hypoparathyroidism. This indicates the growing importance of peptides as potential drugs, in spite of their known weaknesses from a pharmacokinetic point of view. However, these peptides have not yet made a significant contribution as antifungals.

Our research group was the first to report the antifungal properties of penetratin, a well-known cell penetrating peptide [68] (Fig. **9**). Derivatives of penetratin have been also reported as antifungal peptides [69-71]. In addition, many peptides have been reported as potential antimicrobial agents [72-76]. Particularly interesting is the fact that our research group has recently reported a strong antimicrobial activity for small peptides [77]. In fact, these are the small-size peptides with the strongest antimicrobial activity

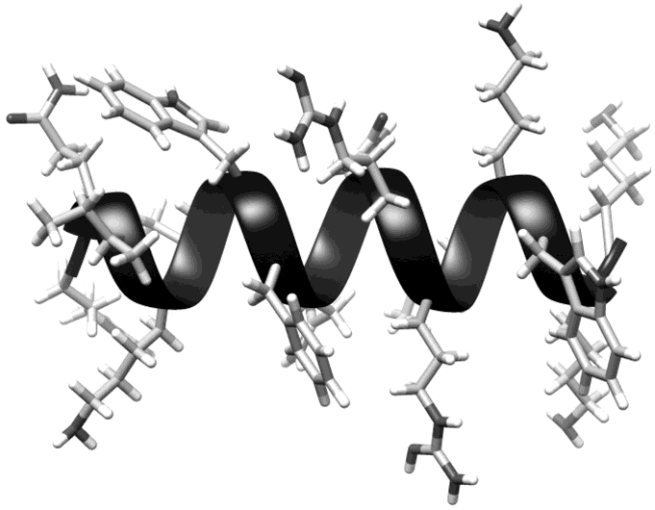

Fig. (9). Spatial view of the preferred helical conformation of penetratin near to the biological membrane. Structure obtained from molecular dynamics simulations performed in a mixed media 60:40 TFE/water.

reported to date. Numerous peptides with antimicrobial effects have been reporter [78]; however it has not been possible to achieve greater development for these compounds. Once again, probably the main difficulty is that their mechanism of action at the molecular level is unknown or at least it has not been yet clearly established. There are several mechanisms proposed to explain the effect of these peptides on the fungal membranes [79-82]. Our research group has made its own contribution to the understanding of the mechanism of action by extensive molecular dynamics simulations [83,84]. However, to date the mechanism of action at the molecular level of these peptides is not fully clear. Undoubtedly, the possibility of knowing the mechanism of action of these peptide might allow the development of new antifungal peptides more specific and effective and therefore all efforts in this regard are welcome.

The community approach strategy

In 2015 the Wellcome Trust and the University of Queensland founded the Community for Open Antimicrobial Drug Discovery (CO-ADD, http://www.co-add.org). CO-ADD is an open access facility, in that any chemist in the world can have their compounds tested for antimicrobial potency and cytotoxicity, free of charge and with no encumbrance on intellectual property. Thus, this program aims to tap into the potential of the millions of compounds distributed around the laboratories globally to be a source of new antibiotic or antifungal by offering free screening for antimicrobial properties. CO-ADD is a hybrid of existing successful models, such as GlaxoSmithKline´s Open Lab Foundation and the Health Molecular Libraries Program. An distinctive feature of CO-ADD is the specific focus on antimicrobial screening. The program has an extremely low barrier to participant entry. There are not limitations on geography or expert panels that review molecules for suitability to screen. The requirements are: the compound must be soluble in water or dimethyl sulfoxide, not radioactive, not pyrophoric and not an illicit drug,

The main motive of this program is that chemists around the world are isolating or synthesizing thousands of new organic compounds each year, but most of such compounds never leave their laboratories or at least they never will be tested as antimicrobials. This type of programs facilitates the testing and evaluation of many new compounds as potential antimicrobials. For more details about this program see reference [85].

Although these type of program has been received with enthusiasm by many individuals, laboratories, universities and research institutes, we cannot say that it has been used massively at least until now. However; it is clear that this type of programs and initiatives could play an important role in the near future and in theory could enhance the successful to obtain new lead-structures for the development of new antifungal drugs.

The key role of natural products for the discovery of new antifungal drugs in the genomic era

Newman and Cragg have reported a very interesting review in 2012 [86] in which it is reported that, from medicines approved by the FDA between 1981 and 2010, 34% of these drugs were natural products or direct derivatives of them. These compounds include statins, tubulin-binding anticancer drugs and immunosuppressants [87-90].

Natural products have provided the starting points for most of the major classes of antibiotics, including the β-lactams, aminoglycosides, macrolides, tetracyclines, rifamycins, glycopeptides, streptogramins and lipopeptides. Since the year 2000, 22 new antibiotics have been launched for treating infections in humans, but only five of these represented new compound classes [86]. In contrast to the antibacterial case, in the antifungal area, the contribution of natural products for antifungal compounds has been much more exiguous; only three agents from natural sources in the last 30 years, accounting for just over 10% of the approved drugs. This can be seen in the treatment regimens that still use agents such as amphotericin and griseofulvin, which are listed in the database launched in 1958. In fact in the case of antifungals, only one drug from natural sources was approved from 2006 to the date. This was the echinocandin derivative anidulafungin, approved for use in the U.S. in early 2006. It would appear that natural products have not yet made its full contribution to the development of new antifungal agents. Possibly the improvement of the current screening techniques might allow to obtain better results in the near future.

It is well-known that natural products exhibit a wide range of pharmacophores and a high degree of stereochemistry, and such properties are expected to contribute to the ability of such compounds to provide new hits. However, it is necessary to modify the traditional approach of bioassay-guided isolation of natural products to take advantage of technological advances. Like for example to accommodate the current understanding in medicinal chemistry, and to explore biologically relevant chemical space via cheminformatic approaches for the design of new libraries. A multidisciplinary approach to drug discovery is also necesary, involving the generation of truly novel molecular diversity from natural sources, combined with total or partial combinatorial chemistry and/or biosynthetic pathways, which without doubt will contribute to provide the best solution to the current crisis of productivity for the antifungal drug discovery and their later development.

When we perform a screening, there are many advantages that compounds from natural sources might have with respect to those obtained from synthesis. Among others, natural product samples can be enriched for drug-like properties by concentrating on fractions that contain compounds in the appropriate range of lipophilicity [91]. Besides the natural-product-likeness based on chemical structure, similarity based on physicochemical properties clearly enhance the results [92]. The closer a fraction library can approach the typical industry definition of a "lead molecule" with regard to physicochemical properties, the greater the likelihood of identifying a starting point that is attractive from a medicinal chemistry point of view. Natural products cover a different, wider and more drug-like chemical space than do synthetic derivatives [92,93].

Furthermore, it has been shown that 83% of core ring scaffolds that are present in natural products were absent from commercially available molecules and screening libraries [94]. It was concluded that including molecules with a natural-product-like scaffold into a screening library would increase hit rates [92]. A recent review by Harvey et al [95] about the re-emergence of natural products for drug discovery at the present is an excellent article on this topic.

As was manifested at the subtitle of this section, we believe that with the optimization of screening techniques that are available today, natural products once again could play a very important role for the research and development of new antifungal agents.

CONCLUDING REMARKS

Statistical data shown in the first part of this review have clearly shown that although a large number of structures with antifungal effects obtained from natural products has been reported, such results have unfortunately not allowed major developments for the development of new antifungal drugs for therapeutic use. It is important to remark that all information obtained from this large amount of research can be very useful in different aspects, for example from the standpoint of the chemistry of natural products and therefore it is not our intention to detract those efforts; however what we highlighted in the present review, is that if the main objective is to obtain a new antifungal drug, then there is no doubt that the type of studies and strategies that have been used until now, have been poorly successful. It should be noted that most of the current arsenal of antifungal drugs was originated from screening of natural products in the 1950s-1970s. However it is clear that "the low-hanging fruit" from this strategy appears to have been exhausted. Thus new strategies are necessary to obtain the required new antifungal compounds for the next generation.

There has been much written on the "superbug crisis" mainly due to the over-use of antibiotics in humans and animals and the exit of many companies from antibacterial drug research and development owing to the challenging market economics . The situation of the antifungal drugs is at least similar or even worse in some aspects and we can also speak of a "super-fungi crisis".

In this review we have discussed some of the main strategies currently used to obtain new molecular targets and new structures starting to enable the development of new antifungal compounds for therapeutic use. Some are relatively new and others are known strategies, which have been modified in order to make them more effective in the difficult goal of obtaining new antifungal compounds that might be converted in antifungal drugs. While all of these proposals can make their own contribution in this difficult task, it deserves a special mention the target-based screening using the genomic knowledge of fungi. Thus, in our opinion the advent of genomics in the investigation of pathogenic fungi is expected to reveal new targets that are unique to fungi and essential for their survival, hopefully might lead to the development of novel specific antifungal agents.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

The continuous financial support of the Universidad Nacional de San Luis-Argentina and the Consejo Nacional de Investigaciones Científias y Técnicas (CONICET) is gratefully acknowledged.

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