**Zeitschrift:** Mycologia Helvetica
**Herausgeber:** Swiss Mycological Society
**Band:** 7 (1995)
**Heft:** 2

**Artikel:** Mycology in the pharmaceutical industry
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**DOI:** https://doi.org/10.5169/seals-1036384

Fungi, Spores and Environment
Compte-rendu du colloque de Neuchâtel le 26 novembre 1994

# Mycology in the Pharmaceutical Industry

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## Introduction

The main objective of pre-clinical research in the pharmaceutical industry is to identify compounds that are suitable for development as drugs. Mycology research is not different: the study of fungi is directed to finding ways of destroying those which cause disease in man. In some cases fungi pathogenic to animals or plants are the target. The search for these "New Chemical Entities" involves the newest techniques of biochemistry and molecular biology as well as the traditional methods of microbiology. The chemical laboratories become involved as soon as a "lead structure" has been identified – a molecule which has some antifungal activity and can be chemically modified to optimise its properties. If this optimisation leads to a compound with suitable characteristics it then starts the long and expensive process of clinical trials which may lead to its approval for use in the treatment of human mycoses.

## Lead generation – random screening or rational drug design

There are two approaches to getting a drug discovery programme running by finding that essential first lead structure. Many companies run high-flux screening programmes which search for active metabolites secreted by micro-organisms into culture broths. This approach has been aided by advances in automation and robotics, and the scope of testing can be widened to include plant extracts and collections of compounds synthesised for other projects.

Once a suitable lead structure has been found in a screening programme, one of the first tasks is to determine its mode of action, by what mechanism it inhibits the growth of the fungal cell. The so-called "rational approach" turns this around. A suitable target enzyme in the biochemical pathways of the cell is chosen, ideally one which is essential for the cell, which is absent in man, and about which there is a considerable amount of information. This ideal is seldom found, so compromises have to be made, but the goal is to know enough about the enzyme and its mechanism to be able to design molecules that inhibit its action. This involves techniques such as the study of enzyme kinetics, sequencing, genetic mutation, X-ray crystallography and computer modelling.

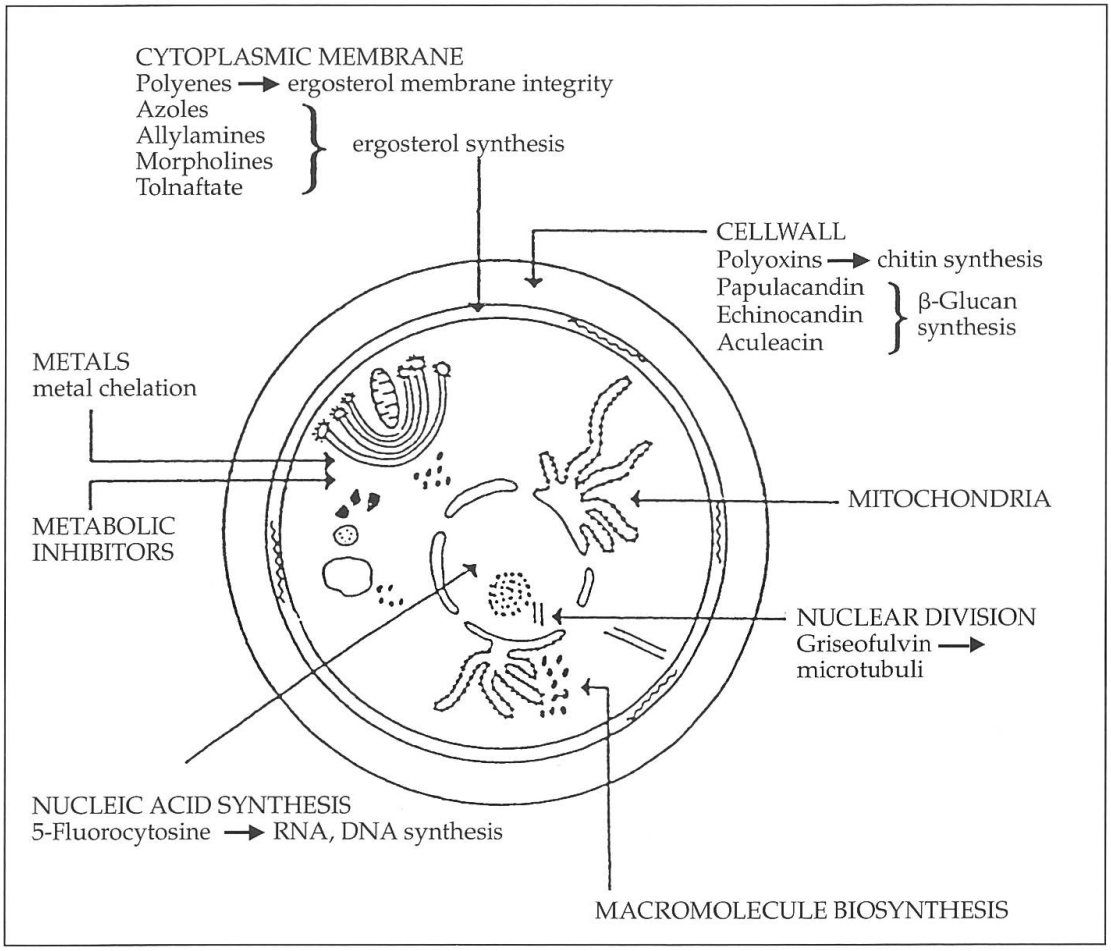


*Figure 1. Potential targets for antifungal activity*

These two approaches can be to some extent combined in that a suitable assay of enzyme activity can be used for random screening, resulting in a lead structure that is an enzyme inhibitor which may or may not have antifungal activity. It is often a major task to modify such compounds in such a way that they can penetrate into the cell to reach their enzyme target.

## Antifungal targets

There are many biochemical processes that would theoretically be good targets for antifungal drugs, but very few have been used in practice. This is illustrated in Figure 1. The most obvious, easy to reach and selective target is the cell wall, but in contrast to the field of antibacterials no antifungal on the market attacks the fungal cell wall or synthesis of its components, despite much work in this area. Apart from 5-fluorocytosine and griseofulvin, all serious

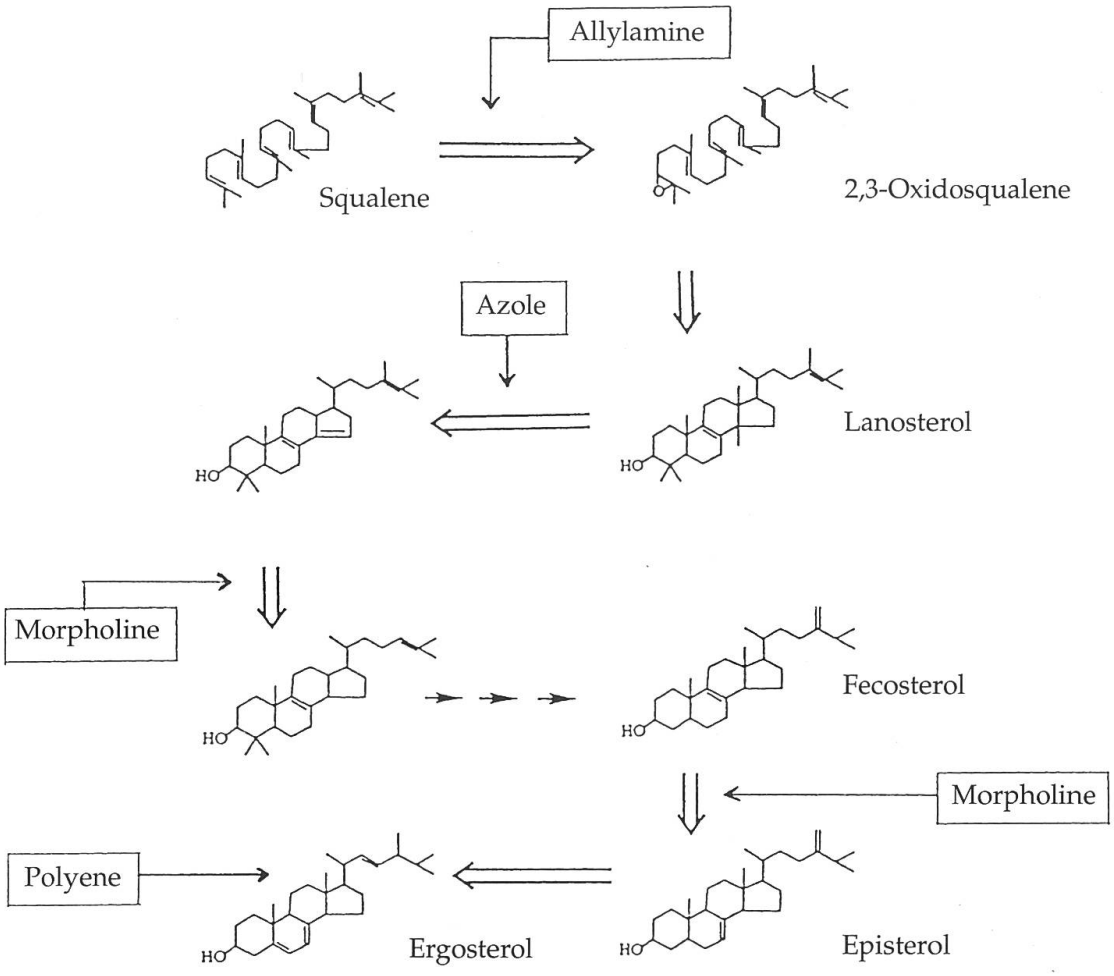


*Figure 2. Drugs acting on fungal ergosterol synthesis*

drugs against human mycoses affect in some way or another the cytoplasmic membrane. The polyenes, such as amphotericin B, directly disrupt the integrity of the membrane, while the majority inhibit the synthesis of ergosterol, a major membrane component, a lack of which alters its mechanical properties and disturbs the function of important membrane-bound enzymes. Figure 2 shows the targets of the major classes of Sterol Biosynthesis Inhibitor (SBI). The allylamines naftifine and terbinafine inhibit the epoxidation of squalene, a very early step in the pathway, while amorolfine, a morpholine derivative, attacks two distinct enzymes and thus benefits from an in-built synergism. The most important drugs for systemic infections are undoubtably the azoles fluconazole, itraconazole and ketoconazole, which inhibit the demethylation of lanosterol.

We decided to investigate another sterol biosynthesis enzyme, the 2,3-epoxysqualene-lanosterol cyclase, which catalyses the transformation of the linear squalene epoxide molecule into the polycyclic sterol structure of lanosterol, as an antifungal target.

## From lead structure to clinical candidate

A comprehensive test system is used to evaluate the hundreds of derivatives of the lead structure that are synthesised in an attempt to find a molecule which fulfils the stringent criteria of a new drug. The first step is to measure enzyme inhibition, which in the case of SBIs is readily achieved by adding radiolabelled acetate to cells growing in the presence of the inhibitor. The radiolabel is incorporated into the various intermediates of the biosynthetic pathway, which can be extracted and separated on thin layer chromatography plates, and the extent of inhibition estimated by the degree of accumulation of a particular intermediate. By using radiolabelled 2,3-epoxysqualene in cell-free extracts and measuring the concentration of inhibitor required to inhibit the cyclase activity, more details of the enzyme inhibiting capabilities can be gained. This is the primary information which guides the synthetic effort.

Whether enzyme inhibition results in antifungal activity is studied using standard *in vitro* methodology, measuring by agar or broth dilution techniques the Minimum Inhibitory Concentration (MIC), the amount of potential drug required for complete inhibition of fungal growth. We routinely tested our compounds against *Candida albicans,* the opportunistic pathogen that most often attacks the weakened or immune-depressed host; against *Aspergillus fumigatus,* a mold which causes infections that are probably the most difficult to treat; against *Trichophyton mentagrophytes,* a representative dermatophyte; and against *Histoplasma capsulatum* as an example of a dimorphic fungus and primary pathogen (one able to cause disease in healthy individuals).

Substances are then tested in models of fungal infections in mice. Systemic candidosis and histoplasmosis were found to be the simplest, least labour intensive, most reproducible and clinically relevant murine models, and these were used to routinely test for *in vivo* activity. After a further selection process, promising compounds are subjected to a far broader screening, *in vitro* against most species of fungi pathogenic to man, and against as many as practicable *in vivo.* Included are mouse or rat models of cryptococcosis and aspergillosis, trychophytosis and other models of superficial infection, candidosis with non-albicans species, and chronic models which evaluate the organ load of infecting fungus.

The cyclase project gradually produced compounds that were very active against a broad spectrum of fungi *in vitro,* and examples are shown in Table 1

| | *C. albicans* | *C. spp.* | Crypto. | Dermat. | Dimorph. | *Asp.* |
|---|---|---|---|---|---|---|
| Ro 44-7319 | 1.49 | 1.33 | 5 | 1.25 | 1.75 | 5 |
| Ro 46-6523 | 1.68 | 0.71 | 1.25 | 0.15 | 0.23 | 0.73 |
| Ro 46-7349 | 0.3 | 0.29 | 5.2 | 2.25 | 1.1 | 54.7 |
| Itraconazole | 1.25 | 0.07 | 0.15 | 5 | 1.25 | 1.5 |
| Terbinafine | 100 | 0.15 | 2.5 | <0.0007 | 1.5 | 1.2 |

*Table 1. Inhibitors of the 2,3-oxidosqualene-lanosterol cyclase. In vitro activity: MIC in μg/ml*

where their MICs are compared with those of itraconazole (Sporanox) and terbinafine (Lamisil), two antifungals on the market. Unfortunately, this high activity was not transferable to an *in vivo* situation. Due to rapid metabolism and extremely high protein binding these compounds were not active in animal models, a problem which further derivatisation could not overcome. Antifungal activities have now been transferred to the Roche research centre in Kamakura, Japan, where the process has started over again with new enzyme targets.

## The clinical candidate

A newcomer to the pharmaceutical industry might think that once a substance is found that has sufficient *in vivo* activity the clinical trials can begin. However, this is not the case. A well orchestrated process involving a diversity of activities swings into action, and only the best compounds survive. Most immediate are the toxicology studies, as it is an absolute requirement that extended dosage in at least two animal species precedes the first application to man. Pharmacokinetics is no less important. Does the drug reach the source of infection? Is it rendered inactive by metabolism or binding to plasma proteins? Does it stay in the body for long enough to be useful, and not too long to be a problem? A drug that must be given six times a day is unlikely to be a commercial success, but if it is retained in the body for weeks there is an increased likelihood of toxicity. Suitable galenical formulations have to be developed for i.v. and oral applications in humans, and a synthetic process capable of producing large quantities of the drug safely and at reasonable cost has to be found. In short, the clinicians have to be convinced that the drug will work, the authorities must be convinced that it works and is safe, and the marketing department have to believe they can sell it.

## Clinical development

At this stage a drug goes out of the hands of the pre-clinical scientist, but it does not disappear from his sight as it goes through the most lengthy and costly part of development, clinical trials. The first steps are relatively straightforward. Healthy volunteers are the first humans to take the drug, and they are carefully monitored to ensure that there are no adverse effects that were not picked up by animal studies. Then the drug is tested, usually in mild infections, to make sure that it really does work in man – there have been cases where a drug which was very active in animal models did not work at all in humans. The most difficult part is the programme of comparative trials comparing the drug to those already on the market, where large numbers of patients and convincing results are required. It is no longer possible for a com-

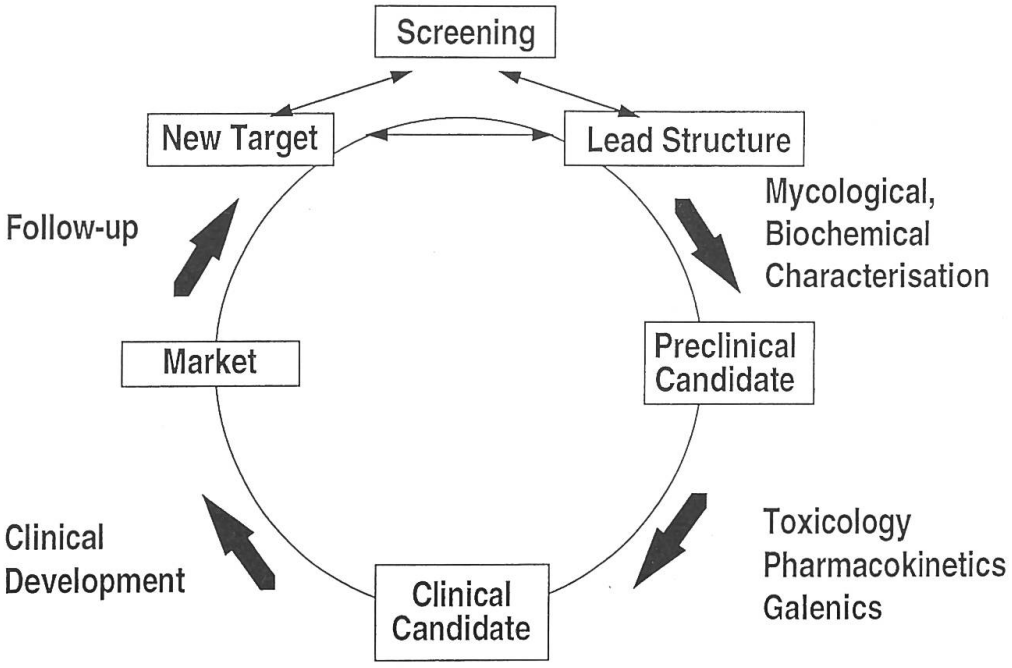


*Figure 3. The never-ending circle*

pany to introduce a drug to the market just because it wants to. A significant benefit in terms of activity, safety or cost-saving must be clearly demonstrated before a drug can be registered.

## The never ending circle (Figure 3)

Long before the drug reaches the market, the search has begun for the next. There is a constant race to keep ahead. Ahead of advances in medical science such as cancer chemotherapy, organ transplantation, intensive care medicine, major surgery, chronic medication, increased survival of the elderly and the prematurely born, all of which produce an ever increasing population with a suppresed immune defence who are highly susceptible to a vast range of fungal pathogens. To keep ahead of fungi – species that were never thought pathogenic are now infecting risk patients. And, of course, to keep ahead of the competition.

The area of antifungal chemotherapy is one of the fastest growing areas of medical research, and most processes of cell metabolism and their regulation are being investigated for their potential as antifungal targets. This will lead not only to more and better drugs against mycoses, but also to an increased knowledge of the biochemistry, molecular biology and genetics of the fungi. It is a fascinating and rewarding area of research, and will remain so for a long time to come.