Amit Basak · Ranadhir Chakraborty
Santi M. Mandal *Editors*

Recent Trends in Antifungal Agents and Antifungal Therapy

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Preface

In the history of discoveries of fungal pathogens, the nineteenth century has witnessed two important events. The causal organism of a silkworm disease, *muscardine*, a fungus named later as *Beauveria bassiana*, was revealed by Agostino Bassi in 1835. Six years later, in 1841, the causal agent of the human scalp disease, favus, being a fungus was discovered by David Gruby. The Gruby's unique and innovative method for the isolation of fungus from the infected scalp and on potato slices, repeated infection of the healthy tissues by the isolated fungus (parallel to the Koch's postulate) was left ignored in the pages of science history due to reasons not related to science. The fact remains that even after the seminal researches by Bassi and Gruby, the knowledge of the fungal diseases remained much less than that of bacterial diseases. Compared to bacterial diseases (among which some of them were epidemic) of human beings, diseases caused by fungi were not epidemic in nature and often are occasional but consequences of some mycoses that can be severe to lethal.

Nevertheless, fungal infections are difficult to treat because fungi are eukaryotes with similarity in biochemical composition and phylogenetic nearness to animals. Hence, treatment of an internal infection caused by a fungus is often very complicated as finding a drug that would specifically kill the fungus and not the animal is very difficult. Most fungi are killed by the immune system, and if the host immune system is overpowered by the fungus, the result is most likely death. Abnormalities in the function of neutrophils and neutropenia help the spread of infections caused by *Candida*, *Aspergillus*, and *Mucoraceae* strains, while altered T-lymphocyte mononuclear phagocyte function will allow dissemination of *C. neoformans*, *Histoplasma*, and *Coccidioides*. Treatment and diagnosis of fungal infections in the immunocompromised host are very tricky and difficult, and in obtaining enough tissue for histology and culture, it is most often required to perform invasive procedures. Moreover, fungal infections have taken a new spectrum due to the increased incidence of multidrug-resistant fungal pathogens. The freedom of choice for drugs to treat fungal infections is also narrow because of lesser probability of discovering drugs that would bypass affecting human cells and target fungal cells producing fewer side effects in patients.

The book is edited in such a way that it will serve as an important resource material for not only the students and researchers but also the physicians and infectious disease scientists. It consists of a series of chapters that dealt in details with the development of antifungal compounds; the prospect of finding newer antifungal drugs including natural, synthetic, and designed; the panorama of combinational therapy including immunotherapy, and the susceptibility testing of dermatophytes. Medical relevance is emphasized throughout the text. On a more immediate level, the editors are grateful to all contributing authors for their intelligence, enthusiasm, and cooperation and for their expert and exhaustive scientific review.

Kharagpur, West Bengal, India — Amit Basak
Darjeeling, West Bengal, India — Ranadhir Chakraborty
Midnapore, West Bengal, India — Santi M. Mandal

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About the Editors

Amit Basak, currently Professor of Chemistry and Chairman, School of Bioscience, IIT Kharagpur, obtained his Ph.D. (natural product chemistry) from Calcutta University and D. Phil. (penicillin biosynthesis) from University of Oxford. He then worked on clavulanic acid biosynthesis as a postdoctoral fellow at the Johns Hopkins University. His research interests involve understanding the mechanism of diradical generating reactions and their applications, development of enzyme inhibitors as antimicrobial agents and molecular capture chemistry. He has received several prestigious awards and fellowships for his research contribution.

Ranadhir Chakraborty was born in Darjeeling. He obtained his Ph.D. from Calcutta University. He worked on "Repetitive DNA sequences in Acidithiobacillus ferrooxidans and their role in regulation of sulfur metabolism" under the supervision of Dr. Pradosh Roy, in the Department of Microbiology, Bose Institute. He is at present serving the Department of Biotechnology, University of North Bengal, in the capacity of Professor and Head. He maintains a perfect blend of classical and modern microbiology in his ongoing journey of Science. He probes some basic scientific problems including antimicrobial resistance with cutting edge technology of every passing time period.

Santi M. Mandal obtained his Ph.D. in the field of Molecular Microbiology and continuing research with major focus in Antimicrobial Chemotherapy. He visited UTMB-USA and NUS-Singapore for his postdoctoral training. At present, he is working as an Assistant Professor of Microbiology at Vidyasagar University, India. He has published more than 90 research papers in reputed journals and conferred upon several prestigious awards for his research contribution.

Fungi Fights Fungi: Tip-off in Antifungal Chemotherapy

1

Santi M. Mandal, Anupam Roy, Debarati Paul, Suresh Korpole, Shanker Lal Shrivastava, Ranadhir Chakraborty, and Amit Basak

Abstract

Fungal infections have taken a new spectrum due to the increased incidence of multi-drug resistant fungal pathogens. Freedom of choice for drugs to treat fungal infections is also narrow because of lesser probability of discovering drugs that would bypass affecting human cells and target fungal cells producing fewer side effects in patients. An approach has gained prominence in research is to look for bioactive antifungal compounds from natural sources and discover new classes of antifungals to control the recent emergence of fungal infections. Most of antifungal drugs are originated from fungi. A conservative estimate of total number of fungal species on this planet would exceed 10^6 if taken into account the ones yet to be discovered from diverse habitats ranging from forest land to marine ecosystem. While attempting to summarize the status of reported fungi-derived antifungal compounds discovered since ancient times, the subset of such compounds were found to be anticancer too. Antifungal compounds with the promise of inducing challenge to rediscover the new effective molecules from drug prototype are also discussed.

Anupam Roy and Santi M. Mandal are equally contributed in literature survey.

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1.1 Introduction

Diversity in species characterized by unique and unusual biochemical pathways facilitates fungi to offer several bioactive molecules (Keller and Turner 2005). These compounds generally come from fungal cellular components in the form of secondary metabolites (Magdalena et al. 2013). Fungal bioactive compounds suitably combats several diseases in plants and animals. Biological activities such as antibacterial, antifungal, antitumor, anti-cholesterol, cytotoxic, mutagenic, carcinogenic, teratogenic, immunosuppressive, enzyme inhibitory effect, etc. make 'fungal origin' as a potential area of research in natural product discovery. Rapid increase in fungal infections contributing to higher mortality rates has become a major concern. Resistance to currently available antifungal drugs necessitates the discovery of new classes of antifungals from both natural and synthetic approach. In agriculture, infection or contamination from fungi in pre- or post-harvest is a major problem leading to economic loss. Fungal pathogen e.g. *Aspergillus* and *Fusarium* spp. not only relates to economical loss but also creates health problem producing mycotoxins. A details top to bottom outline of fungal derived antifungal compounds with their modifications or synthetic analogues may be helpful to understand the structure-activity relationship, which leads to new compound development in antifungal chemotherapy.

1.2 Fungi-Derived Antifungal Agents

1.2.1 Griseofulvin

Griseofulvin, a metabolic product of *Penicillium griseofulvum*, was first isolated by Oxford et al. in 1939 (1939). In 1946, Brain et al. reported that *Penicillium janczewski* was found to produce a substance capable of shrinking and stunting of fungal hyphae (Brian and Curtis 1946). The physical, chemical and biological identity of the isolated compound was established by several researchers (Brian 1949; Grove and McGowan 1947; Brian and Curtis 1949). Subsequent studied had established that *P. platulum* and *P. raistrickii* also produces Griseofulvin (Brian and Curtis 1949, 1955). Thereafter, the production of griseofulvin from various fungi has been thoroughly studied (Brian 1949; Araujo et al. 1990; Petit et al. 2004; Oxford et al. 1939; Wright 1955; Brian and Curtis 1955; Clarke and Mckenzie 1967). The molecule offers in vitro fungistatic action against dermatophytes, such as Microsporum, Epidermophyton and Trichophyton, whereas activity was restricted to yeast, actinomyces and Nocardia. The minimum inhibitory concentrations (MIC) were observed as low as 5–20 μg/ml and bind to microtubules comprising the spindles and inhibit mitotic cell division (Huber and Gottlieb 1968). Initially the structure of griseofulvin was reported as 7-chloro-4,6 dimethoxy-3-cumarone-2-spiro-1′,2′-methoxy- 6′-methyl-2′-cyclohexen-4′-one (Fig. 1.1). The structures proposed by several groups are inconsistent (Oxford and Raistrick 1939; Grove et al. 1951, 1952). Recently approaches for strain improvement by mutation are studied to enhance griseofulvin production (Aytoun and Mcwilliam 1957; Songgang and Yunshen 1983; Kommmunarskaya 1969, 1970).

1.2.2 Strobilurins

Strobilurins (methyl (E)-3-methoxy-2-(5-phenylpenta-2,4-dienyl) acrylate) are another class of fungal metabolites reported by Anke et al. (1977). *Strobilurus tenacellus*, a basidiomycetes fungus produce strobilurins A and B, showed high activity against yeasts and filamentous fungi but inactive against bacteria (Anke et al. 1977). Strobilurins inhibits the mitochondrial respiration in fungi and binds at the Qo-centre on cytochrome b which blocks the electron transfer between cytochrome b and cytochrome c1 (Balba 2007; Bartlett et al. 2002). Therefore, it is called as Qo inhibitors (QoI), or Quinone outside inhibitors. Anke and his

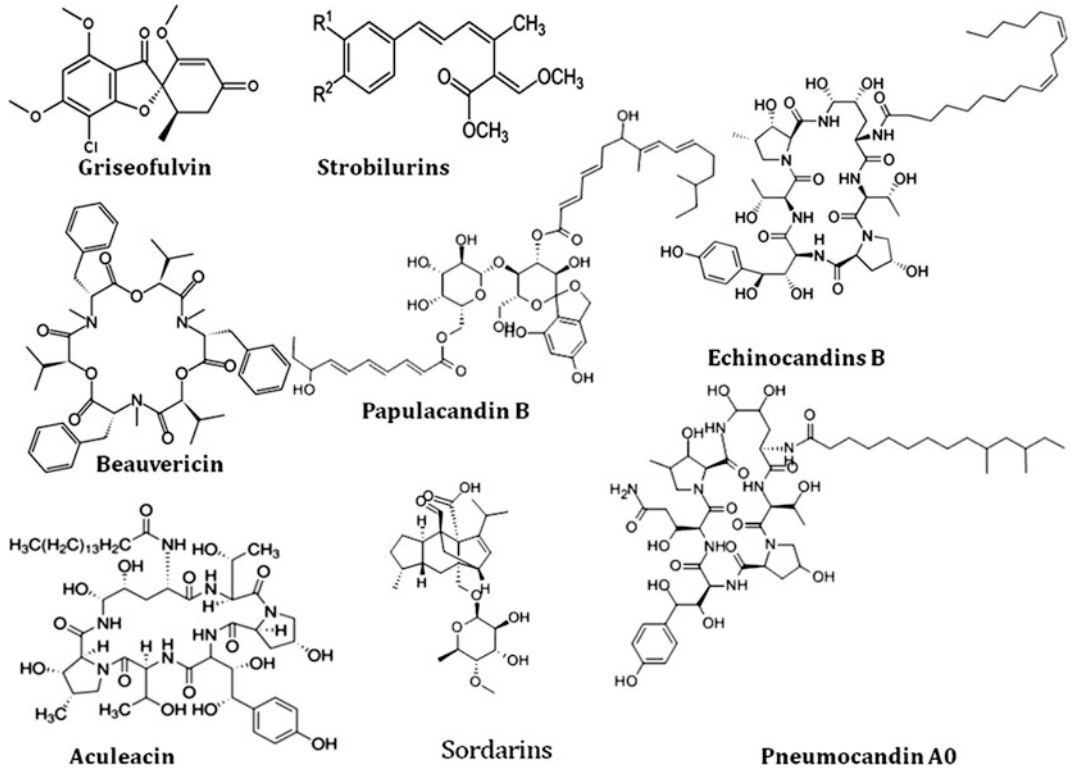


Fig. 1.1 The representative chemical structure of some fungi-derived antifungal agent

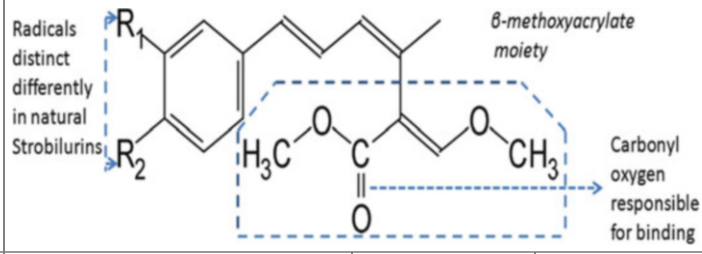
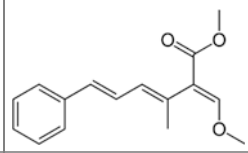
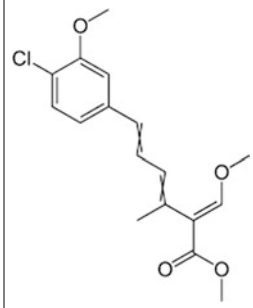
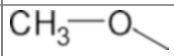
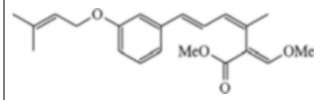
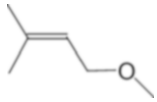
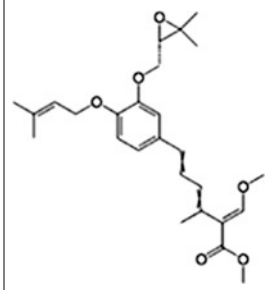
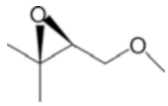
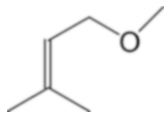
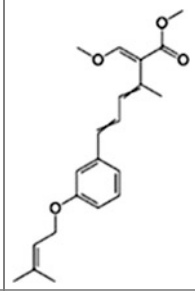
coworker first attempt to resolve the structure and variable structure of strobilurins are listed in Table 1.1. The structure of strobilurin may vary in only in the aromatic ring substitutions at 3 and 4 positions. Strobilurin in natural form break down easily under light (Dolores et al. 2010).

1.3 Echinocandins, Pneumocandin and Papulacandin

In 1970s, two structurally important antifungals were screened. The first one belonged to the lipopeptide class termed as echinocandins and second one was glycopeptides class as papulacandin, affecting cell wall components are the prime target of fungal inhibition. Fungi-derived echinocandins and pneumocandin are the antifungal compounds having inhibitory effect on the synthesis of glucan by noncompetitive inhibition of the enzyme 1,3-β glucan synthase (Morris and Villmann 2006a, b). Besides that, these molecules are recognized as potent backbones or as a basic molecular structure for synthesis and developing analogues.

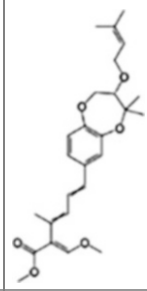
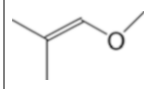
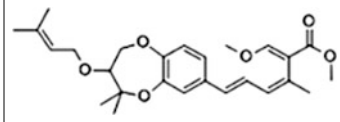
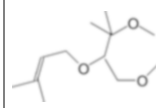
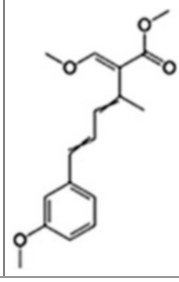
Echinocandins are cyclic antifungal hexapeptides core N-acylated with different aliphatic carboxylic acids. The first report of echinocandin discovery was in early 1974. Researchers of Ciba-Geigy, Sandoz and Eli Lilli isolated echinocandins B from the fermentation broth of *Aspergillus nidulans* var. *echinolatus*, *Aspergillus nidulansvar roseus* and *Aspergillus rugulosus* in random screening of the available strain collections (Benz et al. 1974; Keller-Juslén et al. 1976; Nyfeler and Keller 1974; Geiser et al. 2007). Afterword, a series of fungi now has come into existence having ability of synthesizing natural echinocandin (Nyfeler and Keller 1974; Geiser et al. 2007; Traber et al. 1979) (Table 1.2). The presence of different substituents in the hexapeptide ring or a distinct fatty acid chain makes echinocandins different

Table 1.1 Natural strobilurins with their respective structure

Natural isolated Strobilurin	Structure	Substitution in R1 and R2	Name of the fungus	Reference
General structure				
Strobilurin A		H− in both R1 and R2	*Strobilurus tenacellus*	Anke et al. (1977), Balba (2007), and Schramm et al. (1978)
Strobilurin B		 in R1 and Cl− in R2	*Strobilurus tenacellus*	Anke et al. (1977), Balba (2007), and Schramm et al. (1978)
Strobilurin C		 in R1 and Cl− in R2	*Xerula* sp. (agaricales)	Balba (2007) and Anke et al. (1983)
Strobilurin D		 in R1 and  in R2	*Cyphellopsis anomala*	Balba (2007) and Weber et al. (1990b)
Strobilurin E			*Crepidotus fulvotomentosus.*	Weber et al. (1990a)

(continued)

Table 1.1 (continued)

Natural isolated Strobilurin	Structure	Substitution in R1 and R2	Name of the fungus	Reference
Strobilurin F		−OH in R1 and  in R2	*Cyphellopsis anomala* and *Bolinea lutea*. **I**.	Balba (2007), Weber et al. (1990b), Fredenhagen et al. (1990a, b)
Strobilurin G		 in R2	*Bolinea lutea*. **I**.	Balba (2007), Weber et al. (1990a), and Fredenhagen et al. (1990a, b)
Strobilurin H		−OH in R1 and −H in R2	*Bolinea lutea*. **I**.	Balba (2007), and Fredenhagen et al. (1990a b)

from each other (Fig. 1.2). Several unusual amino acids like dihydroxyornithine, 4-hydroxyproline, dihydrooxy homotyrosine and 3-hydroxy-4-methylproline, as well as two threonine component of hexapeptide nucleus are reported (Kurtz and Rex 2001).

In 1977, a new antifungal antibiotic, *Aculeacin* is isolated from the mycelial cake of *Aspergillus aculeatus* M-4214 (Mizuno et al. 1977b). Subsequently, another six new antibiotics were isolated as the minor components related to aculeacin A from the same culture named as aculeacins B, C, D, E, F and G. The structure of Aculeacin is similar to echinocandin B but differs in the acyl moiety. Their acyl moiety is either the myristoyl (aculacin Aα–Dα) or palmytoyl (aculacin Aγ–Dγ) group. Physicochemical properties aculeacins B, C, D, E, F and G were analogous to those of aculeacin A and they all showed significant activity against fungi (Satoi et al. 1977).

Pneumocandin is another fungi-mediated antifungal compound. Pneumocandin has a sulfate moiety in the molecule and is differentiated from echinocandins by their structural difference (Fig. 1.2). The first member of pneumocandin class was pneumocandin B0 and was isolated from *Glarea lozoyensis* in 1985 at CIBE, a subsidiary of Merck located in Madrid, Spain. Subsequently, pneumocandin Ao was also reported from same culture by the same research group (Schwartz et al. 1989, 1992). Pneumocandin Ao is less haemolytic than other member of naturally occurring echinocandins (Boeck et al. 1989), whereas pneumocandin Bo appears to be the most potent glucan synthase inhibitor compared to other pneumocandin and in vitro and in vivo. Pneumocandin Bo differs from pneumocandin Ao only by the absence of a methyl on one of