

ECHINOCANDINS - NEW ANTIFUNGAL AGENTS

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ECHINOCANDINS - NEW ANTIFUNGAL AGENTS (Abstract): Over the past 10-15 years, the number of clinically available antifungal agents has increased substantially, due to rise in the number of invasive fungal infections, which are a real problem for specialists. Echinocandins are the new class of antifungal agents available for clinical use. This class comprises over 20 natural echinocandins and several semisynthetic ones. Natural echinocandins are not of clinical utility due to their toxicity and low water-solubility (which does not allow obtaining parenteral pharmaceutical forms), although they have good antifungal activity against *Candida* species. Consequently, semisynthetic echinocandins with minimal toxicity, good antifungal activity and high water-solubility were obtained. All echinocandins inhibit β -1,3-glucan-synthase, an essential component of the fungal cell wall. Echinocandins exhibit potent antifungal activity against key pathogenic fungi, including *Candida* species, *Aspergillus* species and *Pneumocystis carinii*. The available echinocandins lack in vitro activity against *Cryptococcus neoformans*. The semisynthetic echinocandins have great advantages, among which low toxicity, fast antifungal activity, favorable pharmacokinetics that allow once-daily administration. The echinocandins recently available for clinical use are: caspofungin, micafungin and anidulafungin. **Keywords:** MICAFUNGIN, ECHINOCANDINS, CASPOFUNGIN, PNEUMOCANDIN, ANIDULAFUNGIN

Antifungal agents were discovered quite late, although some species of pathogenic fungi were discovered in 1835 (*Beauveria bassiana*).

Despite intense research carried out in order to discover new antifungal agents, they appeared later than the antibacterial antibiotics on the pharmaceutical market. This happened because it was difficult to find good antifungal agents with low toxicity.

Antifungal agents can be categorized depending on how they are obtained as synthetic antifungal agents and natural

antifungal agents (tab. I) (1).

Nowadays, the natural antifungal agents most often used in therapy are: polyenic macrolides (nystatin, natamycin, amphotericin B), spiranic antibiotics (griseofulvin) and cyclic lipopeptide antibiotics (candins - the most popular being echinocandins).

Other natural compounds are in different stages of clinical testing:

- papulacandins – glycolipid antibiotics produced by *Papularia* species;

- pradimicins and benanomycins – antracyclic antifungal agents isolated from

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cultures of actinomycetes; gal agents isolated from cultures of acti-
 - nikkomycins and sardarins – antifun- nomycetes.

TABLE I
Natural antifungal agents

No	Antifungal agent	Producing miroorganism	Pharmaceutical products
1.	Amphotericin B	<i>Streptomyces nodosus</i>	Fungizone, Fungilin
2.	Azaserin	<i>Streptomyces</i> spp.	-
3.	Candicidin	<i>Streptomyces griseus</i>	Levorin, Vanobid
4.	Dermostatin	<i>Streptomyces viridogriseus</i>	Viridofulvin, Dermastatin
5.	Fungicromin	<i>Streptomyces cellulosa</i> <i>Streptomyces penticus</i> <i>Streptomyces roseoluteus</i> <i>Streptomyces griseus</i>	Cantricin
6.	Filipin	<i>Streptomyces filipenensis</i>	Filimarisin
7.	Griseofulvin	<i>Penicillium griseofulvum</i> <i>Penicillium janczewskii</i>	Fulvicin, Grisactin, Fulcin, Grifulvin
8.	Hachimycin	<i>Streptomyces hachijoensis</i>	Trichomicin, Trichonat
9.	Hamycin	<i>Streptomyces pimprina</i>	Primamycin
10.	Lucensomycin	<i>Streptomyces lucensis</i>	Etruscomycin,
11.	Natamycin	<i>Streptomyces natalensis</i> <i>Streptomyces chattanoogensis</i>	Pimaricin, Natacyn Pimafu- cin, Mycophyt
12.	Nystatin	<i>Streptomyces noursei</i> , <i>Streptomyces aureus</i>	Stamicin, Nistatin, Fungicidin, Mycostatin
13.	Partricin	<i>Streptomyces aureofaciens</i>	Ayfactin
14.	Pecilocin	<i>Paecilomyces varioti</i> Bainier var. <i>antibioticus</i>	Supral, Variotin
15.	Perimicin	<i>Streptomyces coelicolor</i> var. <i>aminophilus</i>	Aminomycin
16.	Pirrolnitrin	<i>Pseudomonas pyrrocinia</i>	Pyroace
17.	Siccanin	<i>Helminthosporium siccans</i> Drechsler	Tackle
18.	Trichomycin	<i>Streptomyces hachijoensis</i>	Trichomycin

Echinocandins were discovered in the 70's and 80's, being natural lipopeptidic antibiotics active against the resistant strains of *Candida* and *Aspergillus*. These are the most recently approved antifungal agents, after years of intense research (2, 3).

Candida species are the fourth leading cause of nosocomial infections in USA, mortality from candidiasis is reported to be 38% in immunocompromised persons, and complications could appear in over 15% of

patients (4, 5). Moreover, during the past decade, the incidence of invasive aspergillosis has increased, especially in immunocompromised, cancer, and transplant patients (6).

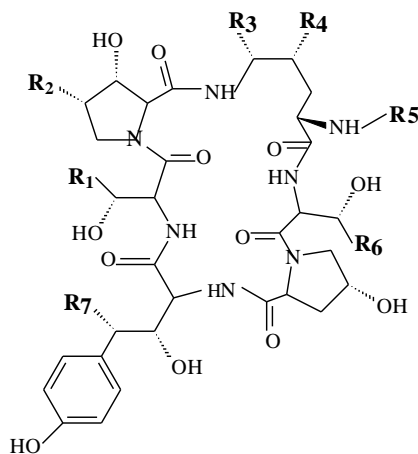
Amphotericin B or fluconazole are the first-line therapeutic options in the treatment of such fungal infections, but the utility of amphotericin B is limited due to its nephrotoxicity. Fluconazole is relatively safe, but there are already *Candida* species resistant to it.

Therefore, because invasive candidiasis and aspergillosis are severe nosocomial infections, there was an urgent need for new antifungal agents. The recent discovery and development of echinocandins is an alternative to amphotericin B and fluconazole as first-line treatment for candidiasis.

Echinocandins are large amphiphilic

cyclic hexapeptides molecules with the N-linked acyl fatty acid ("side chain") with a length of 14 to 18 carbon atoms (1).

Echinocandins are a class represented by over 20 isolated natural products, which are divided into several subclasses, and many semisynthetic analogues, derived from natural compounds (fig. 1).



No	Echinocandin	Fatty acid side chain (R5)
1.	Echinocandin B	Linoleic acid
2.	Echinocandin C	Linoleic acid
3.	Echinocandin D	Linoleic acid
4.	Aculeacin A γ	Palmitic acid
5.	Mulundocandin	12-Methylmyristic acid
6.	Sporiofungin A	10,12-Dimethylmyristic acid
7.	Pneumocandin A0	10,12-Dimethylmyristic acid
8.	Pneumocandin B0	10,12-Dimethylmyristic acid

Echinocandin	R1	R2	R3	R4	R6	R7
Echinocandin B	CH ₃	CH ₃	OH	OH	CH ₃	OH
Echinocandin C	CH ₃	CH ₃	OH	OH	CH ₃	H
Echinocandin D	CH ₃	CH ₃	H	H	CH ₃	H
Aculeacin A γ	CH ₃	CH ₃	OH	OH	CH ₃	OH
Mulundocandin	H	CH ₃	OH	OH	H	OH
Sporiofungin A	CH ₂ CONH ₂	CH ₃	OH	OH	H	OH
Pneumocandin A0	CH ₂ CONH ₂	CH ₃	OH	OH	CH ₃	OH
Pneumocandin B0	CH ₂ CONH ₂	H	OH	OH	CH ₃	OH

Fig. 1. Natural echinocandins

All natural echinocandins are active by inhibiting enzyme 1,3- β -D-glucan synthetase, being selective and noncompetitive inhibitors of the essential components of

the fungal cell wall biosynthesis, making it easy to lysate (1, 7). The enzyme is not present in the cell wall of higher animals, which explains the very few side effects

caused by echinocandins.

They have the advantage of low toxicity, fast antifungal activity and favorable pharmacokinetic that allows once-daily administration (having large molecules should only be given intravenously).

The spectrum of natural echinocandins is limited to *Candida* species (spp.), but some semisynthetic analogues have a wider spectrum that includes other endogenous fungi, such as: *Aspergillus* spp., *Pneumocystis carinii* etc. None of these compounds show activity against *Cryptococcus neoformans* strains.

Echinocandin B was first isolated and identified as the major component of the class (which includes echinocandin C and D). It was isolated from the fermentation broths of various fungi of the genus *Aspergillus nidulans* and *Aspergillus rugulosus* in 1974, at the same time by the researchers from Ciba-Geigy, Sandoz, and Eli-Lilly. Researchers from Toyo Jozo have isolated echinocandins from aculeacin class, and fermentations broths of *Aspergillus aculeatus*, those from Hoechst have isolated mulundocandin from *Aspergillus sydowi* and sporiofungin class was isolated from *Criptosporiopsis* spp. by Sandoz researchers. The pneumocandin class, with the main component pneumocandin B0, was isolated from the fermentations broths of some fungi of the genus *Zalerion arboricola* and *Glarea lozoyensis* by Merck researchers in 1985.

All these compounds are substances with low water solubility, being discovered quite recently. Of these, only the echinocandins and pneumocandins were studied more closely for the development of semisynthetic derivatives.

Natural echinocandins and natural pneumocandins have no therapeutic utility

due to their toxicity (they are hemolytic substances), although they have very good antifungal activity against *Candida* strains (8). They also have low water solubility, which makes them difficult for parenteral formulations. Thus, semisynthetic compounds, non-toxic, with high antifungal activity and enhanced water solubility were obtained. Today three semisynthetic echinocandins, caspofungin, micafungin and anidulafungin are licensed for clinical use (9).

Starting with the natural candins isolated from the culture medium of *Glarea lozoyensis* species a semisynthetic analogue of pneumocandin B0, called Caspofungin (fig. 2) was obtained and entered the U.S. market in 2001 by Merck (CANCIDAS - Merck, USA, vials 50 or 70 mg lyophilized powder). In Europe it was introduced in therapy in 2002 (CANCIDAS, Merck & Co. Inc., USA, MK-0991). The pharmaceutical product contains caspofungin acetate a water-soluble salt of caspofungin. (C52H88N10O15·2C2H4O2).

It has a good pharmacokinetics: 100% bioavailability, high plasma protein binding (97%) and a half-life of 9-11 h. It has a slow hepatic metabolism and a slow elimination from plasma with a clearance of 10-12 mL/minute. Excretion of drug is through hepatic (34%) and renal (41%) routes. A small amount of caspofungin is excreted unchanged in urine (1.4%).

Caspofungin acetate inhibits the synthesis of 1,3- β -D-glucan synthetase, an essential component of the cell wall of many filamentous fungi.

It is active against *Candida albicans*, *Candida tropicalis*, *Candida glabrata*, *Candida krusei*, *Candida lusitanae*, *Candida dubliniensis* and *Aspergillus* species, but is also active against *Pneumocystis jirovecii*

(7, 10, 11, 12). It is less active against *Candida parapsilosis* and *Candida guilliermondii*, with higher minimum inhibitory concentrations for these species. Caspofungin has limited activity against other fungi (e.g. *Trichosporon beigelii*, *Rhizopus*

arrhizus, *Fusarium* spp.) and its activity against other pathogens (e.g. protozoa) is undergoing clinical evaluation (13). The minimum inhibitory concentrations (MIC₅₀) of caspofungin against *Candida albicans* ranges from 0.01 µg/mL to 8 µg/mL (14).

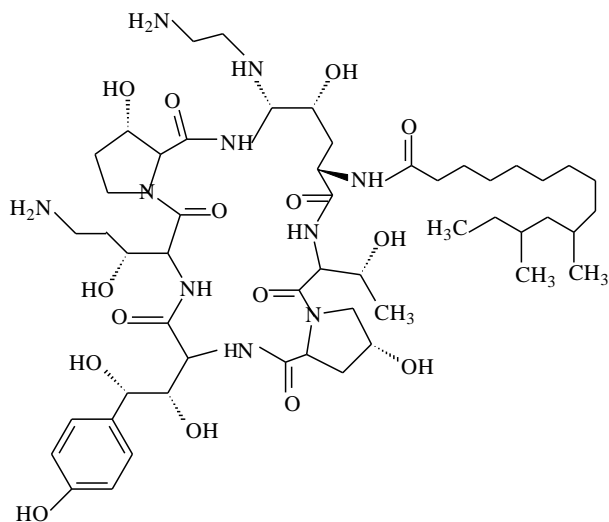


Fig. 2. Chemical structure of Caspofungin

It is administered only intravenously (i.v.), the solution is reconstituted by dissolving the powder exclusively in 0.9% NaCl solution. It is not compatible with dextrose, so diluents containing dextrose have to be avoided. Caspofungin is provided as a lyophilized powder and excipients include sucrose, mannitol, acetic acid, and sodium hydroxide. It can be stored (refrigerated) for up to 24 h after reconstitution (14).

Caspofungin (CANCIDAS) is administered intravenously as first-line treatment for *Candida* infections: intra-abdominal abscesses, peritonitis, pleural infections and in the treatment of esophageal candidiasis. It is indicated to treat adults, adolescents and children with invasive candidosis and invasive aspergillosis when the patient does not respond to or does not tolerate amphotericin B or itraconazole. (15, 16, 17,

18). It is used successfully in the treatment of invasive candidiasis in immunocompromised patients (19).

Another analog of pneumocandin B0 is Micafungin (fig. 3), a semisynthetic echinocandin obtained by conversion of the natural compounds isolated from fermentation broths of *Coleophoma empetri* F-11899.

Micafungin is manufactured in Japan and commercialized by Astellas Pharma under the trade name MYCAMINE (Micafungin sodium, 50 mg or 100 mg lyophilized powder per vial). The product has received U.S. market approval from Food and Drug Administration (FDA) on 16 March 2005. European Medicines Agency (EMA) has given approval for European market on 25 April 2008 (MYCAMINE, Astellas Pharma Inc., USA, FK-463).

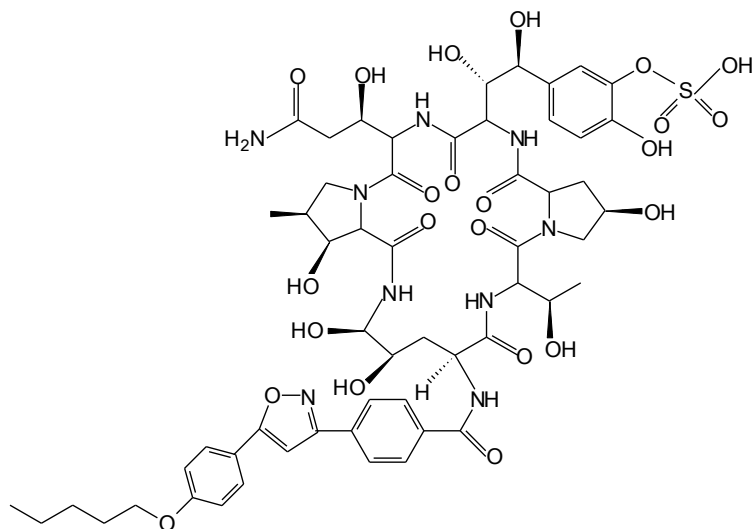


Fig. 3. Chemical structure of Micafungin

Micafungin is a semisynthetic echinocandin, presented as a white hygroscopic powder. The pharmaceutical product contains sodium micafungin a salt readily soluble in water (C₅₆H₇₀N₉NaO₂₃S).

It is active against strains of *Candida albicans*, *Candida glabrata*, *Candida krusei* and *Candida tropicalis*. It is less active against *Candida parapsilosis* and *Candida guilliermondii*, with higher minimum inhibitory concentrations for these species. The minimum inhibitory concentrations (MIC₅₀) of micafungin against *Candida albicans* ranges from 0.01 µg/mL to 0.5 µg/mL (14). Micafungin is characterized by linear pharmacokinetics. It is metabolized into three metabolites which are excreted slowly over many days, mainly in the bile. It is highly bound to plasma protein (99%), and the half-life is 10-17 hours. Micafungin is eliminated via digestive (40%) and renal (15%) routes.

Micafungin is administered intravenous-

ly, once daily, to treat *Candida* infections such as intra-abdominal abscesses and peritonitis (20, 21). Micafungin is approved for use in neonates, children, adults and adolescents in the treatment of invasive candidosis. It is also used, from January 2008, in the prophylaxis of candida infection in patients undergoing allogeneic hematopoietic stem cell transplantation or patients who are expected to have granulocytopenia (22). In addition, micafungin is used in the treatment of esophageal candidosis in adults.

It has few side effects, like other semisynthetic echinocandins being very well tolerated. However, patients who develop abnormal liver function tests during treatment with micafungin should be evaluated for the risk/benefit ratio.

The vials contain sodium micafungin and excipients which include lactose, citric acid and sodium hydroxide. The lyophilized powder is ready for reconstitution by dissolving with NaCl 0.9% or dextrose 5%.

Reconstituted solution is stable at room temperatures for 48 h, if protected from light.

An analogue of echinocandin B is Anidulafungin (fig. 4), marketed by Pfizer (U.K.).

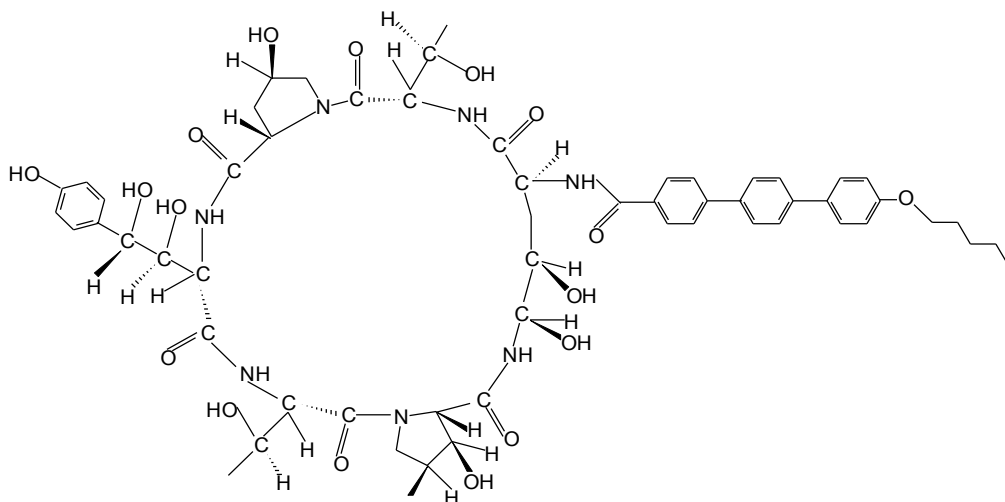


Fig. 4. Chemical structure of Anidulafungin

Pfizer received U.S. market approval from FDA for anidulafungin under the trade name ERAXIS (50 mg or 100 mg vials containing lyophilized powder) in February 2006. EMA has given approval for European market on November 2007 for anidulafungin under the trade name ECALTA (50 mg or 100 mg vial containing lyophilized powder, Pfizer Inc. UK).

It is a semisynthetic echinocandin obtained by converting natural echinocandin B isolated from *Aspergillus nidulans* species. It was initially researched by Eli Lilly, clinically developed by Vicuron company, and later marketed by Pfizer.

It is a white powder (C₅₈H₇₃N₇O₁₇), freely soluble in water, with an alkoxy-triphenyl side chain which intercalates with the phosphor-lipid bilayer of the cell membrane.

It is active against strains of *Candida* al-

bicans, *Candida glabrata*, *Candida krusei* and *Candida tropicalis*. It is less active against *Candida parapsilosis* and *Candida guilliermondii*, with higher minimum inhibitory concentrations for these species (23). The minimum inhibitory concentrations (MIC₅₀) of anidulafungin against *Candida albicans* ranges from 0.01 µg/mL to 8 µg/mL (14). On other fungi anidulafungin activity is limited (e.g. *Acremonium* spp., *Phialophora* spp., *Rhizopus* spp., *Fusarium* spp.).

Anidulafungin is slowly metabolized into an inactive open-ring peptide which is excreted in the bile. No liver metabolism has been observed. It is highly bound to plasma protein (99%), and the half-life is 0.5-1 hour. It is eliminated via digestive (30%) and renal (1%) routes.

Anidulafungin is administered intravenously to treat invasive candidosis, invasive aspergillosis in adults who are not

granulocytopenic, but not in children or adolescents below the age of 18 years (23). It is administered once-daily, the duration of treatment depending on patient response, usually at least 14 days. It has few side effects, like other semisynthetic echinocandins being very well tolerated. It can be used in patients with renal and hepatic impairment (14, 23, 24).

The vials contain anidulafungin and excipients as a lyophilized powder. The excipients include fructose, tartaric acid, mannitol, sodium hydroxide and hydrochloric acid. The lyophilized powder is ready for reconstitution with water for injections and subsequently diluted with NaCl 0.9% or glucose 5% for infusion. Reconstituted solution is stable at room temperatures for 24 h.

Echinocandins are the newest antifungal agents available for clinical use. The semisynthetic echinocandins have great advantages such as, low toxicity, great antifungal activity, high water solubility, and

favorable pharmacokinetics, which allow once-daily intravenous administration. They exhibit potent antifungal activity against key pathogenic fungi, including *Candida* species, *Aspergillus* species and *Pneumocystis* species. All clinical trials demonstrate their efficacy in the treatment of invasive candidosis and invasive aspergillosis in patients not responding to other antifungal agents. They can be used successfully in the treatment of invasive candidiasis in immunocompromised patients, or in patients with renal and hepatic impairment.

The echinocandins recently available for clinical use are: caspofungin, micafungin and anidulafungin.

Their safe use, route of administration, fewest interactions with other drugs and their spectrum of action make echinocandins a class of antifungal agents with a secure future, although today they are less accessible due to the cost.

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