# Antibiotics II

(antibiotics with antifungal activity)

# Antifungal compounds

- Fungi and pathologic fungi infections
- Drugs for treatment fungal infections
- Classification of antifungal drugs

#### Aspergillus fumigatus





https://de.wikipedia.org/wiki/Cryptococcus\_neoformans

Candida auris



https://de.wikipedia.or g/wiki/Candida\_auris

https://www.studocu.com/en-au/document/university-of-technology-sydney/pharmacology-2/lecture-notes/antifungal-drugs-2018-hermily-gave-this-lecture-payattention-to-the-mechanism-of-action-and-side/3100365/view University of technology Sydney

http://old.vscht.cz/obsah/fakulty/fpbt/ostatni/miniatlas/images/plisne/kolonie/P enicillium%20chrysogenum%20CCF%202878%20CYA%2010-25.jpg

# Funghi

aspergillus-

fumigatus-fungus





https://www.researchgate.net/figure/Culture s-of-Candida-albicans-colonies-based-ondifferent-concentrations-ofthymoquinone fig1 318336650



- > 200,000 known fungi species, estimated total size of the Fungi Kingdom 1 000 000 including yeasts, molds, mushrooms, smuts, the pathogens Aspergillus fumigatus and Candida albicans, and the source of penicillin, Penicillium chrysogenum.
- approximately 400 fungi cause animal disease, less cause significant human diseases.
- immunosuppressed patients e.g., AIDS and patients with compromised immune systems are susceptible to mycoses.

# Pathogenic fungi – clinical importance

- yeasts (e.g. Cryptococcus neoformans)
- yeast-like fungi, fungi that produce a mycelium resembling structure (e.g. *Candida albicans*)
- filamentous fungi, they have true mycelium (e.g. Aspergillus fumigatus)
- "dimorphic" fungi are depending on nutritional constraints, can grow as yeasts or filamentous fungi (e.g. *Histoplasma capsulatum*)



http://thunderhouse4yuri.blogspot.com/2010/09/cryptococcusneoformans.html



https://peerj.com/articles/7870/

https://alchetron.com/ Aspergillus-fumigatus



# Fungal infections

• Systemic mycoses – systemic fungal infections

a) <u>opportunistic infections</u> - <u>candidiasis</u>, aspergillosis, cryptococcosis - are present mainly in immunocompromised hosts (such as cancer, transplant, AIDS patients), rarely seen in case a "healthy" host.

b) <u>non-opportunistic infections</u> - occur in any host (uncommon)

 Superficial mycoses – mostly a) candida, or, b) filamentous fungi (such as epidermophyton, trichophyton and microsporum). Candida infections often occur in mucous membranes and moist skins. Contrary filamentous fungi are generally oriented towards the skin, hair and nails.

> https://www.creative-biolabs.com/drugdiscovery/therapeutics/trichophytonmentagrophytes.htm



https://de.wikipedia.org/wiki/Candidose

#### Trichophyton Mentagrophytes



#### Candida albicans

- Candidiasis infections can be ranging from superficial (such as oral thrush and vaginitis) to systemic (life-threatening candidosis).
- Sometimes CA "Embarrassing infection"



https://www.leibniz-hki.de/en/newsdetails/369.html



Candida albicans infection in female ... sciencephoto.com



https://www.123rf.com/photo\_1318492 53\_a-close-up-of-a-foot-with-the-toewith-the-toe-with-candida-albicans-.html

## Ringworm and Tinea

• Tinea unguium

Infection of finger and toe nails Often associated with *T. pedis* 

• Tinea cruris

Starts in groin area ("Jock itch") Causes by *Trychophytum rubrum* 

• Tinea capitis - Ringworm of scalp and hair

#### Tinea unguium



https://www.sciencephoto.com/me dia/262850/view/toenails-showingtinea-unguium-fungal-infections

Tinea capitis



https://www.blueberrytherape utics.com/ringworm-of-thescalp-tinea-capitis/

# Superficial Mycoses



https://umvie. com/toutsavoir-sur-lacandidose/

- most common infections
- In the U.S., 50% of women experience vulvovaginal candidiasis/candidosis by the age of 25, with the majority of these infections being caused by *Candida albicans*.
- Risk factors corticosteroid therapy, broad-spectrum antibiotic, diabetes, and immunosuppression. Tight clothing, oral contraceptive use have also been associated with candidosis, and it is fairly common during pregnancy.

#### Treatment



- Fungal infections are difficult to treat particularly in the immunocompromised patients. Problem: fungi resistence to conventional antimicrobial agents. Limited number of drugs for the treatment of systemic fungal diseases.
- Amphotericin B, azoles (fluconazole, itraconazole, ketoconazole, and voriconazole) – drugs of choice used in systemic infections.
- Amphotericin and azoles have selective toxicity to fungi inhibition of ergosterol synthesis (a sterol unique to fungal cell membranes, mammals have cholesterol in the cell membranes).

#### Fungal cell wall composition.

- chitin (brown) located close to the cell membrane. Chitin is synthesized by transferring *N*-acetylglucosamine residues from uridine diphosphate-*N*-acetylglucosamine (UDPGlcNAc; brown hexagon) to a growing fiber that is shuttled through the cell membrane by the transmembrane chitin synthase (light blue).

ß-1,3-and ß-1,6-glucan (green) adjacent to the chitin fibers b-1,3glucan is synthesized by a b-1,3glucan synthase (yellow) that uses uridine diphosphate-*N*-glucose (UDPGlc; green hexagon) as a donor to transfer glucose to the extruded b-1,3-glucan fiber.

mannoproteins (red) – polymer galactose and mannose (galactomannan)

# Fungal cell membrane



Fungal Genetics and Biology, DOI:10.1016/j.fgb.2015.12.004

#### Antifungal drugs (in the clinics)



• Antifungal drugs pipeline



Nature reviews, An insight into the antifungal pipeline: selected new molecules and beyond doi:10.1038/nrd3074



Robbins N, Wright GD, Cowen LE. 2016. Antifungal drugs: the current armamentarium and development of new agents. Microbiol Spectrum 4(5):FUNK-0002-2016. doi:10.1128 /microbiolspec.FUNK-0002-2016.



Robbins N, Wright GD, Cowen LE. 2016. Antifungal drugs: the current armamentarium and development of new agents. Microbiol Spectrum 4(5):FUNK-0002-2016. doi:10.1128 /microbiolspec.FUNK-0002-2016.

#### Azoles

- The azoles (clotrimazole, econazole, fenticonazole, ketoconazole, miconazole, tioconazole, sulconazole, itraconazole, voriconazole and fluconazole) are a group of synthetic fungistatic agents with a broad spectrum of activity and represent an alternative to amphotericin B for most systemic fungal infections.
- azoles have lower toxicity and can be administered orally.
- Azoles inhibit fungal cytochrome P450 3A enzyme, lanosterol 14α-demethylase, which is responsible for converting lanosterol to ergosterol, the main sterol in the fungal cell membrane. This leads to depletion of ergosterol, alteration of membrane fluidity, resulting in inhibition of replication.

#### Ketoconazole

S. Emami 246 et al. / European Journal of Medicinal Chemistry 135 (2017) 241e259



- The first azole that could be given orally to treat systemic fungal infections.
- Use: Is an alternative to amphotericin for systemic mycoses, much less toxic and less effective
- Unwanted effects: The main hazard is liver toxicity which can be fatal. Adverse interactions with other drugs: cyclosporine, terfenadine and astemizole all interfere with drug-metabolising enzymes.

Azoles – mode of action is inhibition of lanosterol  $14\alpha$ -demethylase



Fig. 5. Mechanism of lanosterol 14a-demethylation by CYP51-dependent 14a-demethylase.

#### Fluconazole



- reaches high concentrations in the cerebrospinal fluid and ocular fluids.
- becomes the drugs of first choice for most types of fungal meningitis (such as candidosis and both cryptococcal and coccidioidal). Fungicidal concentrations are also achieved in vaginal tissue, saliva, skin and nails.
- Side effects: can cause nausea, vomiting or rash. There are significant drug interactions between fluconazole and warfarin, cyclosporine, phenytoin, lovastatin, oral hypoglycemics, and protease inhibitors.



- Is an alternative to amphotericin B for several systemic mycoses. The drug is safer than amphotericin and has the added advantage of oral dosing.
- A drug of choice for blastomycosis, histoplasmosis, paracoccidioidomycosis, an alternative to amphotericin for aspergillosis, candidasis, and coccidioimycosis, may also be used for superficial mycoses.

# **Amphotericin B**

- macrocycle with powerful antifungal activity isolated from cultures of *Streptomyces*.
- Currently: 4 formulations of amphotericin B commercially available: conventional amphotericin B (C-AMB), liposomal amphotericin B (L-AMB), amphotericin B lipid complex (ABLC), and amphotericin B colloidal dispersion (ABCD). All are IV infusion formulations.



# Mechanism of action

- Target: fungal cell membranes, the most important mechanism is probably its ability to form large pores in the membrane causing gross disturbances in ion balance including the loss of intracellular K<sup>+</sup>
- Amphotericin B binds to ergosterol, antifungal activity of amphotericin B depends on ergosterol binding, ergosterol have to be in the membrane of sensitive fungi.
- Bacteria are NOT affected as bacterial membrane lack ergosterol



https://drfungus.org/knowledge-base/antifungal-pharmacology/



Burke group, https://doi.org/10.10 73/pnas.1117280109

## Therapeutic uses



- A drug of choice, for most systemic mycoses. Active against most fungi and yeasts. Gold standard in the treatment disseminated infections caused by *Aspergillus* and *Candida*.
- useful synergistic combinations with second drug such as 5-fluorocytosine.
- should be used only against infections that are progressive and potentially fatal
- treatment could be prolonged from 6-8 weeks or 3-4 m

 Adverse effects: Highly toxic and dangerous drug- "Amphoterrible". Infusion reactions: fever, chills, nausea, headache, nephrotoxicity — renal impairment occurs in all patients, dose dependent.



- polyene macrolide antibiotic similar in structure to amphotericin and with the same mechanism of action (less toxic compared to amphotericin).
- Use is mainly limited to Candida infections of the skin, mucous membranes and the gastrointestinal tract. Cryptococcus is also sensitive to nystatin.

# Echinocandins - Caspofungin

- Echinocandins are ones the newest class of antifungal drugs.
- In contrast to amphoteicin and azoles, which disrupt the fungal cell membrane, the echinocandins disrupt the fungal cell wall.
- The echinocandins inhibit the synthesis of 1,3-β-glucan, a glucose polymer that is necessary for maintaining the structure of fungal cell walls. In the absence of this polymer, fungal cells lose integrity and lysis quickly follows.
- Caspofungin is active in vitro against a wide variety of fungi, and it has proved effective in the treatment of candidiasis and forms of invasive aspergillosis that are refractory to amphotericin.
- It is given intravenously, once daily, and its plasma half-life in humans is 9-10 hours.

#### Mechanism of action



**Figure 3** Cartoon illustrating the binding of echinocandins on the glucan synthase enzyme complex. Echinocandins non-competitively inhibit the activity of glucan synthase and disrupts the  $\beta$ -(1,3)-p-glucan synthesis leading to fungal cell lysis. [Color figure can be viewed at wileyonlinelibrary.com]

© 2017 Royal Pharmaceutical Society, Journal of Pharmacy and Pharmacology, 69 (2017), pp. 1635–1660

1639



Pneumocandin B0 – a naturaly occurring lipophilic peptide isolated from fungus *Glarea lozoyensis*. Caspofungin - Developed by Merck.



Genomics-driven discovery of the pneumocandin biosynthetic gene cluster in the fungus Glarea lozoyensis, <u>BMC Genomics</u> 14(1):339 DOI:10.1186/1471-2164-14-339

Nat. Prod. Rep., 2014, 31, 15–34

## Chemistry of caspofungin (Cancidas)





Cmpd #	<i>C. albicans</i> GS (IC <sub>50</sub> , nM)	C. albicans MFC ( $\mu g m L^{-1}$ )	C. parapsilosis MFC ( $\mu g m L^{-1}$ )	A. fumigatus MEC ( $\mu g m L^{-1}$ )	TOKA ED <sub>99.9</sub> , (mg kg <sup>-1</sup> , % MB cure)	$\begin{array}{l} \text{ASP} \left( \text{ED}_{50} \right. \\ \text{mg kg}^{-1} \right) \end{array}$
2a	70	0.25	4	1	6 (20%)	>20
3	10	0.125	1	0.015	0.75 (80%)	0.06
4	10	0.125	0.125	2	0.375 (70%)	>20
5	1	<0.06	0.125	0.015	0.09 (80%)	0.03

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Table 6 Co	Comparative activities of semisynthetic pneumocandins 5, 6 and amphotericin B (AmB)								
Cmpd #	<i>C. albicans</i> GS (IC <sub>50</sub> , nM)	C. albicans MFC ( $\mu g m L^{-1}$ )	A. fumigatus MEC ( $\mu g m L^{-1}$ )	TOKA (ED <sub>99</sub> , mg kg <sup><math>-1</math></sup> )	$\begin{array}{l} \text{ASP} \left( \text{ED}_{50}, \right. \\ \text{mg} \ \text{kg}^{-1} \right) \end{array}$	Acute tox (mg $kg^{-1}$ )			
5	1	<0.06	0.015	0.045	0.03	30			
6	0.60	0.125	0.008	0.027	0.05	50			
Amb	NA	0.25	1	0.013	0.06	4			

#### Caspofungin fluorescent probe



Medical Mycology January 2013, 51, 103–107.



*C. albicans* was labeled for 1 h with CSF-BODIPY at 0.12 mg/ml.

#### Fluorocytosine – pyrimidine analogue



- Is a synthetic orally active antifungal agent that is effective against a limited range (mainly yeasts) of systemic fungal infections.
- Mechanism: Flucytosine is a pro-drug and converted to the antimetabolite 5fluorouracil in fungal but not human cells. 5-Fluorouracil inhibits thymidylate synthetase and thus DNA synthesis→disruption of fungal DNA and RNA synthesis.
- It is a narrow spectrum antifungals and usually combined with amphotericin for severe systemic infections such as candidiasis and cryptococcal meningitis.

## Flucytosine drawbacks



- Development of resistance during therapy is common and continues a serious clinical problem
- Relatively harmless. Bone marrow suppression (anaemia, neutropenia, thrombocytopenia), GI disturbances, and alopecia have occurred, but these are usually mild (but may be more significant in AIDS patients) and are easily reversed when therapy ceases.
- Drug interactions: advantages and problems

## Terbinafine



- From the group of allylamines, sold under brand name Lamisil. Used to treat pityriasis versicolor fungal nail infection (ringworm, jock itch, athlete's foot).
- Inhibits squalene epoxidase (biosynthesis of ergosterol). Treated fungi accumulate squalene while becoming deficient in ergosterol.



- Is a narrow-spectrum antifungal agent isolated from cultures of Penicillium griseofulvum.
- It causes a fungistatic action by interacting with fungal microtubules and interfering with mitosis.
- Used to treat dermatophyte infections of skin or nails when local treatment is ineffective, but treatment needs to be very prolonged. It has largely been superseded by other drugs. is given orally.

# Griseofulvin chemistry

(water soluble prodrugs)



International Journal of Medicinal Chemistry, https://doi.org/10.1155/2017/7386125

## Other antifungals Sordarins



 Sordarins: Sordarins are a class of semi-synthetic natural products that were shown to have antifungal activity in the early 1970s (developed preclinically in the 1990s by Glaxo-wellcome and merck). The compounds were found to inhibit a novel target for antifungal agents: elongation factor 2 in protein biosynthesis.

# Nikkomycin Z $HO_{V} \xrightarrow{CH_3 O} \xrightarrow{COOH}_{HO} \xrightarrow{COO}_{HO} \xrightarrow{CO$

 Nikkomycin Z: nikkomycin Z was first identified by Bayer pharmaceutical Company in the 1970s. However, its development has only recently been renewed. The mode of action of nikkomycin Z is to competitively inhibit chitin synthases and thereby interfere with fungal cell wall construction. As mammalian hosts do not possess this target, nikkomycin Z is potentially pathogen selective.

#### MK-3118



• Enfumafungin is a structurally distinct natural product class that is similar to the echinocandins in targeting glucan synthase. The current development candidate MK-3118 is an orally active, semisynthetic derivative of enfumafungin with potent in vitro activity against glucan synthase and potent in vivo activity against Candida and Aspergillus species.

## Parnafungin A



 Parnafungins inhibit poly(A) polymerase and display potent broad-spectrum activity against clinically relevant Candida species and therapeutic efficacy in murine models of candidiasis.

#### BHBM



 A screen of 49,120 small molecules to identify inhibitors of fungal sphingolipid GlcCer synthesis identified two compounds: [N'-(3-bromo-4hydroxybenzylidene)-2-methylbenzohydrazide (BHBM). BHBM wsa efficacious against *C. neoformans* in vitro, in an intranasal infection model, and in an invasive infection model of the central nervous system. Paradoxically, although BHBM did not show potent antifungal activity against *C. albicans* in vitro, it was efficacious in a model of invasive candidiasis.

#### 17-AAG



 17-AAG transforms fluconazole from ineffective to highly efficacious without host toxicity in the context of this localized infection and drug delivery. In the context of systemic fungal infections, toxicity associated with inhibition of host Hsp90 precludes the use of current Hsp90 inhibitors as antifungals and demands the development of fungal-selective Hsp90 inhibitors or agents that target fungal-specific regulators of Hsp90 function.

#### Fellutamide A



• inhibitors targeting the fungal 26S proteasome.

#### **Overview**

M. K Kathiravan et al. / Bioorg. Med. Chem. 20 (2012) 5678-5698



Figure 1. Targets for antifungal therapy.