STUDY OF ANTIFUNGAL COMPOUND

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ABSTRACT: Fungal infections have become problematic in recent years. There has been an emergence of new fungal pathogens, development of antifungal resistance, and increasing prevalence. In addition, toxicity, resistance, and lack of efficacy as a single agent limit the effectiveness of current antifungal drugs. So, new drugs targeting new pathways are really needed.

Key Words: antifungal compound, mode of action, new drug, clinical data, toxicity

1. INTRODUCTION:
Fungal infections which affect the keratinised tissue of the body like skin, hair and nail. It affect the stratum corneum of the epidermis. In worldwide Commonly called mycotic infection. Opportunistic fungal infections have become problematic in recent years. There has been an emergence of new fungal pathogens, development of antifungal resistance, and increasing prevalence. In addition, toxicity, resistance, and lack of efficacy as a single agent limit the effectiveness of current antifungal drugs. High rates of morbidity and mortality continue to be associated with infections that are caused by molds and yeast. The advancement in medicine and surgery has caused an alarming increase in immunocompromised patients that are susceptible to fungal infections. Many patients are at a higher risk of developing mycoses, such as those suffering from HIV infection, receiving organ transplantation and intensive cancer therapy. With such a wide range of risk factors, the prophylactic use of antifungal therapies is one of the reasons for frequent resistance to antifungal drugs. This section provides information on the major fungal pathogens, the current antifungal agents, the value of mechanism for action studies, and the novel PHL5-34A are introduced.

1.1 Major fungal pathogens
Candida species are the most common pathogens associated with fungal disease, and Aspergillus, Cryptococcus sp and Zygomycetes account for many fungal infections as well. These fungi are very common and can be acquired from host surroundings. After antifungal treatment, the mortality rate is still very high due to the patient’s immunodeficiency, late diagnosis, or fungal drug resistance. There are many invasive infections associated with Candida species. There are currently more than two hundred ascomycetous yeasts included in the genus Candida, and of these, only a few species of the genus are opportunistic pathogens of humans. Candida albicans are thought to be the most common cause of fungal infections in humans today. Identifying patients that are at a higher risk of developing Candida infections is an important
step in determining which patients should receive treatment. Some of the treatment options may include strategies that are presumptive (there are many risk factors that raise suspicion of infection) or prophylactic (preventative measure for high-risk factors). *Aspergillus fumigatus*, is an ascomycetous fungus found around the world and its spores are abundant in the environment due to their small size. *A. fumigatus* conidia are frequently present in food, tap water, at home, and in office rooms. A definitive diagnosis of invasive aspergillosis requires a positive culture from a sterile site, or it requires histologic or radiologic evidence in a patient at high risk with compatible clinical findings. Unfortunately, when the fungi are positively identified in the body, it could be too late for treatment. Techniques for an early diagnosis, preventative strategies, and new treatment methods are needed to reduce the mortality in invasive aspergillosis patients.

*Cryptococcus neoformans* is a saprophytic, basidiomycetous, dimorphic organism found worldwide. Its natural habitats are pigeon droppings and contaminated soil, and its small basidiospores can turn into yeast cells. The basidiospores or yeast cells may be inhaled by humans, then through the respiratory tract the pathogen can disseminate within the host causing pulmonary infections, and subsequently, due to the predilection of *C. neoformans* for the central nervous system, life threatening meningoencephalitis. It causes infections in both immunocompromised and immunocompetent patients. In addition, patients who have undergone organ transplantation and are receiving high-dose corticosteroids are at increased risk for development of cryptococcosis.

II. Anatomy of Skin:

**Epidermis**: The epidermis is stratified squamous epithelium. The thickness of the epidermis varies between 0.4 and 1.5 mm. More than 95% of epidermal cells is constituted by keratinocytes. Other cells in epidermis are Melanocytes, Langerhans cells, Merkel cells... Morphologically these are divided into
- Stratum basale
- Stratum spinosum
- Stratum granulosum
- Stratum lucidum
- Stratum corneum.

**Stratum Basale**: Only one cell thick, but may be two or three cells thick in glabrous skin and hyper proliferative epidermis. Basal cells - small and cuboidal (10–14 nm) with large, dark-staining nuclei and a dense cytoplasm. They contain ribosomes and tonofilaments, membrane-bound vacuoles which has melanosomes transferred from melanocytes by phagocytosis. The stratum basale is the primary site for mitotically active cells. The cells in basal layer are Stemcells, Transient amplifying cells, Post mitotic cells.

**Stratum Spinosum**: This layer contains 8 to 10 layers of cells. These cells are polyhedral with a round nucleus. The cells in the upper spinous layer are larger, more flattened and contain organelles called “lamellar granules”. Due to the presence of the spine-like appearance of the cell margins in histological sections (these spines correspond to the abundant desmosomes) this layer is called the spinous layer. Keratinocytes and provide a network for stability. Mechanical coupling between epidermal cells is provided by the desmosomes.
communication occurs at the gap junction.

**Stratum Granulosum**

Due to the presence of intracellular basophilic keratohyaline granules this name is given to this layer. Cells are 2 to 5 layer thick. Cell contains lamellated granules known as membrane coating granules.

**Stratum Corneum**

It is the outermost layer, has 20-25 layers corneocytes which are the largest cell of epidermis which contain soft keratin and are stabilized by intermolecular disulfide bond. Cells are flat with no nucleus.

**Stratum Lucidum**

Present over the palms and soles. This layer is electron lucent so is called by this name. This layer is present between the stratum granulosum and stratum corneum. These cells contain nucleus and are called as “transitional cells”.

**Blood vessels and lymphatics**: Skins have a rich vascular network. It includes arterioles, terminal arterioles, pre-capillary sphincters, arterial and venous capillaries, post-capillary venules and collecting venules. Cutaneous vessel network formed between the subcutaneous adipose tissue and the dermis arises from the deep plexus, the fascial network in the skin. From this various vessels branch out to reach the appendages and ascending arteriole arises to generate a sub papillary plexus from which capillary loops are formed which enters the papillary dermis. Epidermis is avascular. Vasculature has various roles in the skin: provide nutrients and oxygen, regulate body temperature. The blood flow is regulated where opening causes dissipation of more heat and constriction causes slowing of blood flow to the skin which in turn conserves energy. Endothelium constitutes the inner most component of the blood vessel. Arteriole contains a sub endothelial layer of elastic tissue in contrast to venules. Pericytes surround the endothelium of the capillaries, small arteries and venules.

**III. ANTIFUNGAL AGENTS CLASSES:**

There are currently five classes of antifungal agents that are used orally or intravenously for the treatment of fungal infections in humans. The classes are polyenes, pyrimidine analogues, allylamines, azoles.

**3.1. Mode of Action:**

**Azoles** are the second class to target the cell membrane of fungi. The major groups of azoles are the imidazoles and the triazoles. These two groups have five-membered organic rings containing either two or three nitrogen molecules. Cellular and mitochondrial membranes are both affected by azoles. The azoles inhibit cytochrome P450-dependent 14α-lanosteroldemethylation, which is a critical step in the synthesis of ergosterol, an important component of fungal membranes. The mode of administration is different in imidazoles and triazoles. Imidazoles are used by topical treatment while triazoles can be administered intravenously and orally. Since the azoles affect the P450 enzyme activity, their main toxicities are due to interactions with other compounds that...
induce or inhibit this system. Since the azoles are fungistatic drugs, their widespread use has resulted in the development of drug resistance.

**Flucytosine** is a *pyrimidine* analogue since 1972. Flucytosine is the only antimetabolite available for the treatment of fungal infections. Flucytosine is a fluorine analogue of cytosine that functions as an inhibitor of thymidylatesynthetase. It is only efficacious when administered in combination with amphotericin B. The major toxicity problems for flucytosine include bone marrow suppression, myocardial suppression, myocardial toxicity, and renal failure. The only *allylamine* in clinical use today is terbinafine. Terbinafinediscovered as a derivative of the topical antifungal naftifine, which was the original compound of the allylamine class. Terbinafine has limited activity for treatment of invasive fungal diseases and mainly effective against fungi such as dermatophytes. It can be combined with voriconazole for treatment of infections.

**Amphotericin B** is the *polyene* primarily in use therapeutically. Amphotericin B is a product of *Streptomyces nodosus*. Amphotericin B selectively and irreversibly binds fungal cell membrane sterols. The interaction of the antifungal with membrane sterols results in the formation of transmembrane pores, allowing for the leakage of ions and small molecules resulting in cellular damage or death. The major drawbacks for amphotericin B use are its significant side effects and that intravenous administration is required for treatment of invasive mycoses.

**The Novel antifungal compound:** The antifungal drugs that are being used today have several drawbacks including toxicity, resistance, lack of efficacy as a single agent, a limited spectrum of activity, high cost. In addition, their targets are mainly restricted to the cell membrane. Therefore, the need for new drugs targeting new pathways . Compounds that have a distinct mechanism of action (MOA) are at a higher demand because they can be used for combinatorial or chemical modifications. **PHL5-34A**: PHL5-34A is a synthetic compound based on the phloeodictine class of compounds found in marine sponges such as *Pellinaeusiphonia*. These compounds have a bicyclic tetrahyropyrrolopyrimidinium core with an aliphatic side chain at C-6

A novel analog of one of these compounds, PHL5-34A was synthesized chemically at the National Center.

![Mode of action of anti-fungal drug](https://www.jetir.org/figure.png)

**FIG. 3.1.1.Mode of action of anti-fungal drug**
IV. CLINICAL TEST\textsuperscript{12}:
Laboratory investigations play an important role
They are diagnosed by two important methods
i. Direct microscopy
ii. Culture

Direct Microscopy\textsuperscript{12,13}: Direct microscopic examination in 10\% KOH solution is considered one of the most important procedures in medical mycology. This procedure is done on an outpatient basis to establish evidence of fungal infection in skin, hair, and nail. We can obtain the results within 1-2 hours. It is an easier, reliable, and more useful procedure to diagnose fungal infections. The following steps:

Scraping\textsuperscript{13}
The presence of fungal infection of the skin, hair, nail can be established by doing a scraping. The scrapings are collected and examined under a microscope. First, the skin lesion is cleaned using alcohol. For easy sampling, little distilled water can be applied. By pulling the skin above the site with one hand and moving the scalpel edge across the lesion the sample is collected.

KOH mount\textsuperscript{13}
Skin scraping is collected on a black paper or directly on the slide using a scalpel from the edge of the lesion. 10-30\% KOH is usually used. After sample is collected on a glass slide from the advancing border of the lesion using a blunt scalpel, add KOH and place the cover slip. Now heat the slide gently and then examined for the presence of fungal elements. Special transport packs backed by black card when available can be used to transport the scrapings.

Microscopic examination\textsuperscript{12}
In order to view the presence of fungal spores and hyphae this procedure is done. In this procedure first we examine under low power magnification (x10) and then under high power magnification (x40) for better illumination so that the morphology of the fungus can be studied. Fungal spores vary from 2-10 mm in diameter. Sometimes the lines of juncture of normal epidermal cells dissolve into branching network and these are easily mistaken for a fungal structure. This is called 'mosaic fungus'. Cotton fibres and synthetic fibres can also mimic fungal hyphae. For microscopic examination 10\% to 40\% KOH is used. Counter stains such as Parker's blue black ink, Periodic acid Shiff or fluorescent stains such as Calcoflour can be used to visualise the fungal elements\textsuperscript{12}.

Modified Parker's ink and 1\% eosin method\textsuperscript{12}
Modified parker's stain is prepared by adding 1\% eosin to Parkers ink in 2:1 proportion. Mixture is applied over the affected area and allowed to dry. Then the cellophane tape is reapplied, gently pressed, removed and stuck over a glass slide and viewed. Pink appearance of background can be seen which is due to eosin and blue appearance of fungal elements can be seen which is due to ink\textsuperscript{12}. 


Calcofluor white \textsuperscript{12}
This is a colourless dye, a fluorochrome stain. When applied overscrapings from skin and mucous membrane it helps in rapid detection of the fungal elements. When viewed under ultraviolet light, fungal structures appear as brilliant apple-green or a ghostly blue-white colour. Though direct microscopy is an easy and reliable test, 5-10\% of false-negative results may occur because, inappropriate material or an insufficient quantity was obtained for analysis. Out dated or defective KOH used and adequate time was spent on examining the specimen.

USES OF KOH \textsuperscript{13}:
KOH is used in the diagnosis of, candida infection of the skin, nails, hair. Laboratories select a simple glucose/peptone agar, either with 4\% sugar, 1\% peptone and an acid pH (Sabouraud’s dextrose agar) or with 2\% sugar, 1\% peptone and a neutral pH (Emmon’s modification). In order to reduce contamination, antibacterial antibiotics such as gentamycin (0.0025\%) and/or chloramphenicol (0.005\%) are added. The addition of cycloheximide 0.04\% will inhibit the growth of non-dermatophyte moulds. For moulds, the temperature of incubation should be 26–28°C and cultures should be held for a maximum of 3–4 weeks. Microsporum- macroconidia are rough, thick walled, range from fusiform to obovate in shape with 1-12 or more septa. Trichophyton- thin walled, smooth, cylindrical, fusiform or clavate in shape with up to 12 transverse septa. Epidermophyton- macroconidium is clavate, broadened and rounded at its distal pole, thin walled and has up to 5 septa. In some species like M. audonii, T. verrucosum, T. simii, a third spore produced called chlamydospore. In T. schoeninii and T. violaceum there may be only one kind of spore \textsuperscript{13,14}.

IV. Drug – Drug interaction \textsuperscript{15}:
Polyene
Amphotericin B is not metabolized by hepatic CYP450 enzymes and has very few drug–drug interactions. The pertinent drug–drug interactions for amphotericin B are related to the nephrotoxicity and electrolyte similar renal side effects. One common example is the coadministration of amphotericin B with immunosuppressants, such as tacrolimus or cyclosporine \textsuperscript{15}, in transplant recipients. This combination is associated with increased risk of kidney injury and electrolyte disturbances.

Flucytosine
This is not a substrate or inhibitor of the CYP450 enzymes. There are very few drug–drug interactions. Because flucytosine is renally cleared, medications altering renal function may affect drug levels and the risk of toxicity \textsuperscript{15}.

Triazole
Drugs have the highest potential for serious drug–drug reactions. They are substrates and inhibitors of various hepatic CYP450 metabolic enzymes. The possible drug–drug interactions vary by individual drug because each has a variable affinity for the isoenzymes (CYP2C19, CYP3A4, CYP2C9) \textsuperscript{16}. As inhibitors of CYP450 enzymes, triazoles can impair metabolism of a coadministered drug, increasing the risk of toxicity. As substrates of the pathway, the concentrations of the triazoles can be substantially affected by concomitant use of medications that inhibit or induce the enzymes, as has been observed for itraconazole and voriconazole. lists
commonly used classes of medications that have the potential for serious interactions if administered with azoles. Closely examining a patient’s medication list is recommended before starting and stopping medications given the high potential for drug–drug interactions. Absorption of the itraconazole oral capsules and the posaconazole oral solution is optimized by gastric acidity, so proton pump inhibitors and histamine-2 blockers should be avoided. Drug–drug interactions may be encountered by the additive effect of additional QT prolonging agents. Isavuconazole is the only triazole that is not associated with QT prolongation.

V. Toxicity of Antifungal Compounds

![Fig. 5.1. Toxicity of antifungal compounds](image)

CNS = central nervous system; 5-FC = flucytosine; GI = gastrointestinal; IV = intravenous; QTc = corrected QT interval.

VI. FUTURE DIRECTIONS

The need to develop new therapy to treat fungal infections is being increasingly used in order to discover new antifungal compounds. The chemical characteristics and origin of the molecules have gained more importance in the screening phase. In combination with this strategy, genomics-era technologies promise to bring significant advances in the discovery of effective antifungal agents. Developing antifungal agents that explore unique factors in fungi is a challenge, since these organisms are eukaryotes such as mammals. Essential genes for fungal survival are very interesting targets and are already being useful for the design of new drugs. In addition, agents that can block genes related to drug resistance can also be an efficient alternative. Also, studies focused on several drugs are currently being conducted aiming at the potentiation of antifungal drugs, especially fluconazole.
VII. CONCLUSIONS
Further studies are needed to determine the chemical identity of the bioactive compounds responsible for antifungal activity. A MOA study was conducted on antifungal compound, the current antifungal drugs don’t inhibit cell cycle. Thus, this a potentially novel antifungal target for new drug discovery. Given the central role played by the cell cycle in cell division and growth, inhibiting the cell cycle will have a dramatic effect on cell viability. On the other hand, since the cell cycle is an important process in all eukaryotic cells, it is possible that PHL5-34A may cause an inhibitory effect on not only fungal cells but also on the human host cells.

VIII. REFERENCE


