Superficial fungal infections and their pharmacotherapy

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Abstract

In the current scenario immunocompromised patients are increasing day by day due to tuberculosis, cancer, AIDS and various new pandemic diseases, these patients are very prone to fungal infection also. Dermatophyte infections (Tinea) caused by group of organisms belongs to three genera namely *Epidermatophyton*, *Trichophyton* and *Microsporum* belonging to the family arthrodermataceae. Invasive fungal infections are caused by human fungal pathogens *Candida albicans*, *Aspergillus fumigatus* & *Cryptococcus neoformans.* Pathogenic fungal strain develops due to resistance towards available pharmacotherapy so new molecular targets are required to find and explore new molecular targets. New curative therapy is also required in current scenario to explore the natural & synthetic libraries for the development of new drugs. The Discovery of a new molecular target & its validation requires a new therapeutic approach the most interesting targets explored and covered under this review are inhibitors of cell wall biosynthetic pathways, inhibitors of DNA and protein synthesis, inhibitors of nucleic acid and protein biosynthesis and other cellular functions. Further this article also covers the latest drugs registered for clinical trials and their therapeutical indications thus providing a comprehensive insight into the available pharmacotherapy and their limitations, recently approved drugs for dermatophytosis, along with the available molecular targets for antifungal drugs.

Keywords: - Superficial infections, dermatophytes, Fungal pathogens, Pharmacotherapy, Immunocompromised infections.

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1. Introduction

The fungal infections are increasing day by day, so is the demand for improvements in antifungal therapy, approximate 2.5 percent of people worldwide suffer from serious infections, and approximately 1.47 million people have affected, annually by dermatophyte infections as there is a constant increase in the number of immunosuppressed patients. Frequent prescription of immunosuppressant drugs, substantial use of chemotherapeutic agents and corticosteroid therapy, and medical implants are also responsible for invasive fungal infections. Dermatophyte infections (tinea) belong to the three genera, *Trichophyton*, *Microsporum*, and *Epidermophyton* (White TC, *et al.*,2014) all belong to the family *Arthrodermataceae* Superficial fungal infections have a high affinity for keratinized tissues present in nails, skin, and hair & slowly they make a network-like structure and cause dermatophytosis (Gnat S *et al*.,2020).The dermatophytes show resistance to available pharmacotherapy so the requirement for recognition of new antifungal molecular targets and new curative therapy is needed in the current scenario. The recognition of new molecular targets encourages us to explore the discovery of new antifungal agents. It is also necessary to investigate the natural and synthetic products that are anticipated to bring new advancements in the development of new strategies to overcome the resistance developed by fungal strains. Dermatophytes as well as invasive fungi develop resistance to available treatment of fungal infections. (Khurana A *et al*.,2019). The discovery of a new molecular target & its validation requires a new therapeutic approach among which the most interesting target are fungal cell wall biosynthetic pathways, DNA and protein synthesis, topoisomerases nucleases enzymes, elongation factors, and transduction pathways various elongation factors (Ostrosky-Zeichner L *et al.*,2010).

1.1. Reluctance/Resistance development in dermatophytes

Resistance of fungi is described as the unresponsiveness towards the available antifungals. Antifungal resistance has been divided into three categories: intrinsic or internal resistance, acquired or secondary resistance, and sometimes resistance shown by a fungus strain that exhibits the genotypic changes referred to as "clinical resistance" (Khurana A *et al*.,2019) . .Intrinsic or internal resistance develops before exposure to antifungal therapy (Cowen *et al.*,2015). Clinical resistance is seen in patients with a low immune system (e.g., AIDS, neutropenia), long-term use of corticosteroid therapy, use of catheters, and sometime suboptimum drug concentrations in the blood also contributes to clinical resistance. Drugs frequently fail to completely stop the biological reaction because the intended enzyme is overexpressed (Scorzoni L *et al.* 2017) so the drug target is changed, making it impossible for the medication to attach to the target figure -1 shows the possible causes of resistance shown

in figure-1 .

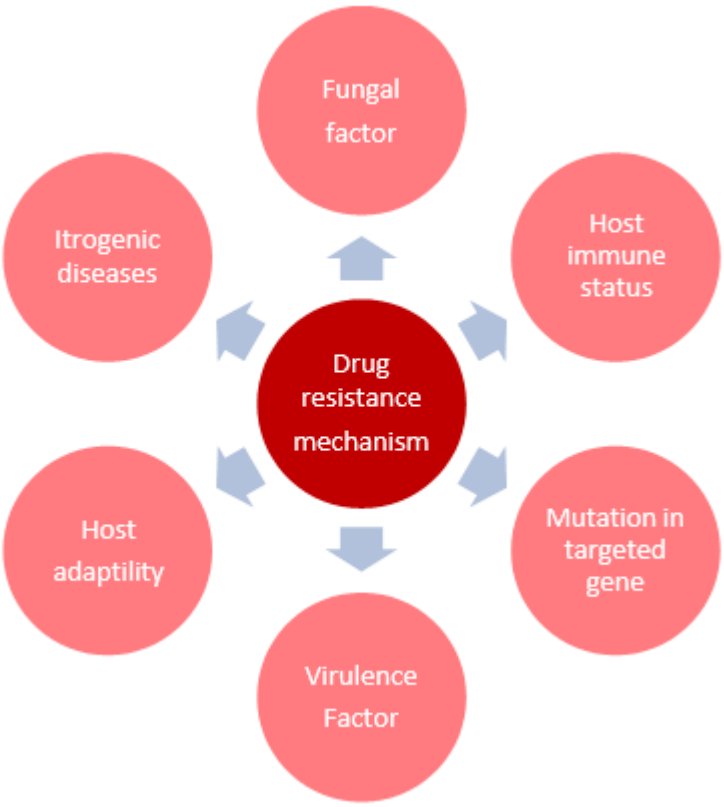


Figure 1: **Possible causes of resistance in fungi**

1.2. Risk factors of fungal infection and their systemic infections

The initiation of antifungal resistance is a complex phenomenon on multiple hosts and microbial factors (White TC, *et al.*,2014) . The host's immune response is also the main factor for the development of resistance. There are some risk factors -

- Diabetes mellitus
- Immunocompromises diseases (HIV, Cancer,)
- Poor circulation of blood in the body (peripheral arterial disease)

- Maceration of skin (Athlete's foot)
- Low CD4 count
- Long use of antibiotics and anticancer drugs
- Acute leukemia
- Severe neutropenia

Symptoms

- Rashes
- Scaling
- Itching
- Thickening of the nail (hyperkeratosis) onycholysis, and discoloration of nails
- Small white specks with crumbling nails on the nail plate's surface.
- Lymphadenopathy
- Distorted nail shape

2. Available pharmacotherapy

For the development of antifungal resistance for currently available therapy, we need to develop new pharmacotherapy before it spreads and acquires antifungal drug resistance. To overcome fungal resistance developing new medicines with improved antifungal activity and better pharmacokinetic profiles is needed in the development of better restorative approaches for the treatment of fungal infections. The available medications, including synthetic medications like allylamines, azoles, and fluorinated pyrimidines as well as naturally occurring antibiotics like polyenes and echinocandins, not only cause adverse effects but also cause fungal pathogens to develop drug resistance. (Ostrosky-Zeichner L *et al.*,2010) . To overcome these problems, there is a requirement for the discovery of new antifungal agents with new therapeutics value. Figure 2 shows the pathway for ergosterol biosynthesis and the steps where the currently used drugs inhibit the pathway and show their antifungal effects. Table 1 enlists the chemical class of topical & systemic antifungals along with their generic, trade name and their available dosage forms.

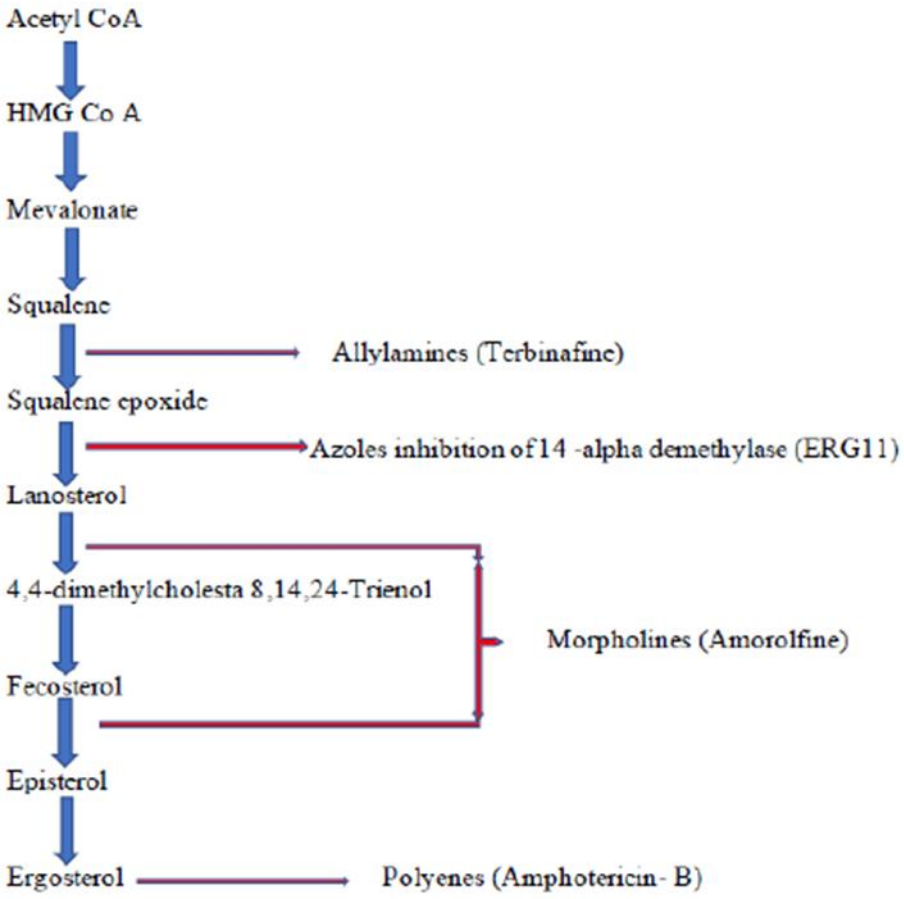


Figure 2: Biosynthetic pathway of ergosterol

TOPICAL ANTIFUNGALS				
Chemical Class		**Generic Name**	**Trade Name**	**Dosage Form**
Azoles	Imidazole	Ketoconazole Clotrimazole	Nizoral Crux	2 % cream, shampoo 1 % cream
	Triazole	Tetraconazole	Terazol	0.4 – 0.8 % cream
Allylamines		Butenafine Naftifine Terbinafine Tolnaftate	Lotrimin ultra, Mentax Naftin Lamisil, Derma Gel Tinactin, Foot Cream	1 % cream 1 % cream 1 % cream 1% cream, 1% cream, solution, powder spray liquid
Polyenes		Nystatin Natamycin	Myostatin, Nistat Alcon	100,000 U/g cream, ointment, powder 5% ophthalmic suspension
Miscellaneous		Ciclopirox	Loprox	0.77 % cream, gel, suspension

SYSTEMIC ANTIFUNGALS				
Chemical Class		**Generic Name**	**Trade Name**	**Dosage Form**
Azoles	Imidazole	Ketoconazole	Nizoral	200 mg tablets
	Triazole	Fluconazole Voriconazole Itraconazole	Diflucan Vfend Sporanox	50, 100,150, and 200 mg tablets 50 and 200mg tablets, 200 mg powder for injection 100 mg capsules, 10 mg/ml injection
Allylamines		Terbinafine	Lamisil	250 mg tablets
Polyenes		Amphotericin B	Amphocin Abelcet Fungizone	50 mg powder for injection 100 mg/20 ml suspension 50 mg powder for injection
Echinocandins		Caspofungin Micafungin	Cancidas Mycamine	50 mg 70 mg powder for injection 50 mg powder for injection
Miscellaneous		Flucytosine Griseofulvin	Ancobon Fulvicin, Grifulvin	250 and500 mg capsules 250 and 500mg microsize tablets and 125 to 330 mg ultra-micro size tablets

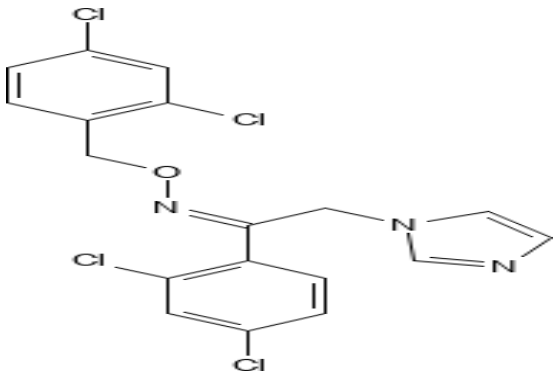
(Sahni K, *et al.*, 2018) , (Sheehan DJ *et al.*, 1999)

Table 1: Topical & Systemic antifungals

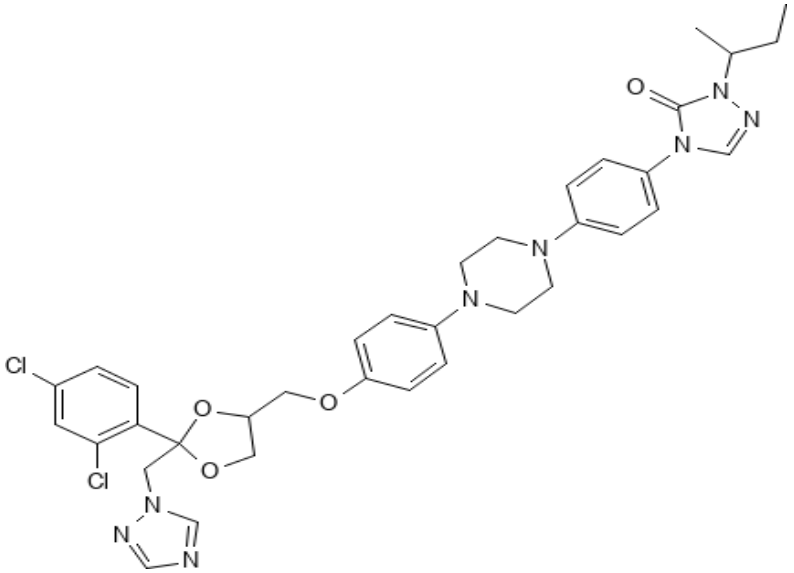
2.1. Currently used chemical classes of drugs

Azoles: Imidazole and Triazoles –

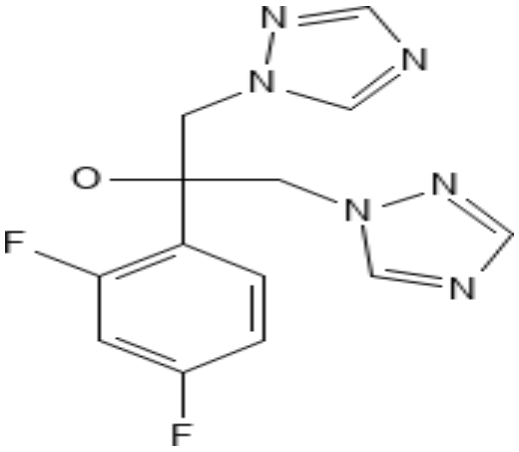
All azoles including imidazole and triazole were also known as ergosterol biosynthesis inhibitors. Azoles interfere with ergosterol biosynthesis by inhibiting the enzyme 14- alpha demethylase that is used during the biosynthesis of ergosterol and stop the conversion of lanosterol into ergosterol so, that toxic methylated sterols are accumulated, affecting the cell organelles' functions and homeostasis of the cell. (Nucci M, Perfect JR. 2008) . The most prevalent class of antimycotics now in use, azoles antifungal medications are used topically to treat systemic fungal infections as well as superficial dermatophytes infections. (Arnold TM *et al.*,2010) . Around twenty-five drugs are commonly available in the market under azoles category like ketoconazole, fluconazole, voriconazole, and Itraconazole. Figure 3 depicts the site of action of azoles.



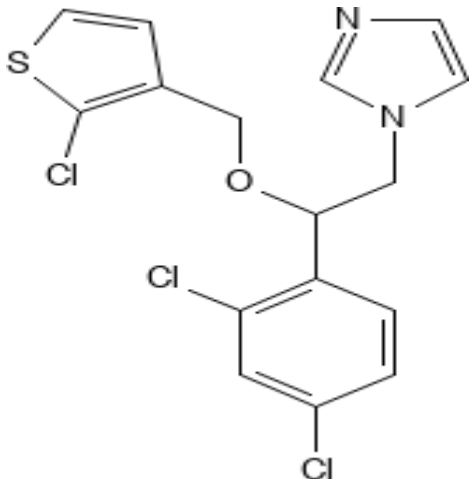
Oxiconazole



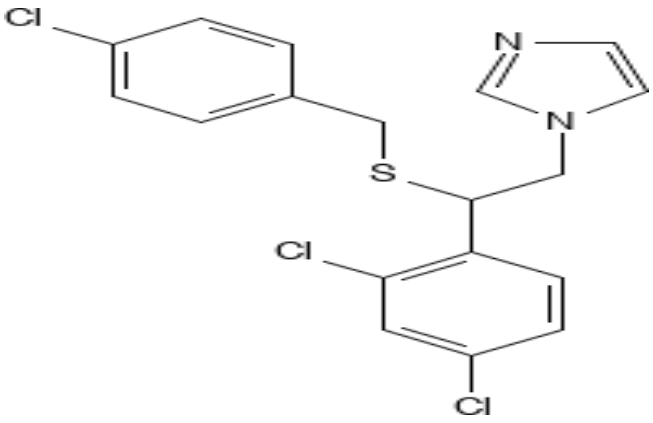
Itraconazole



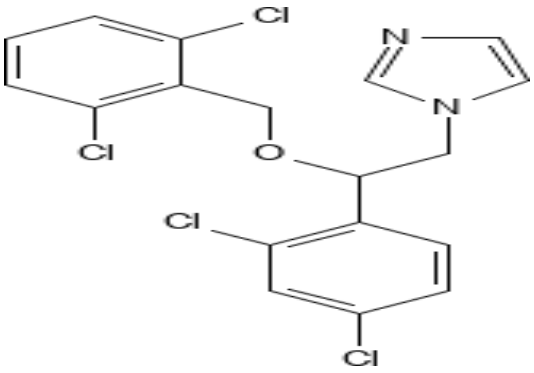
Fluconazole



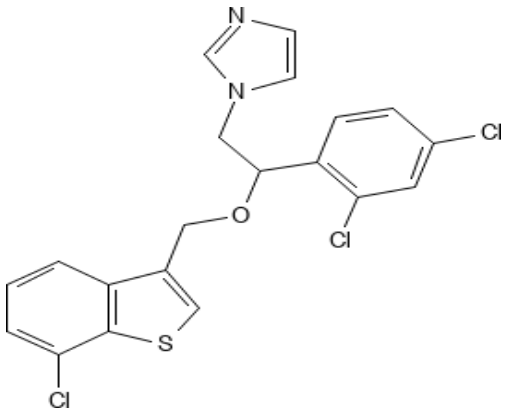
Tioconazole



Sulconazole

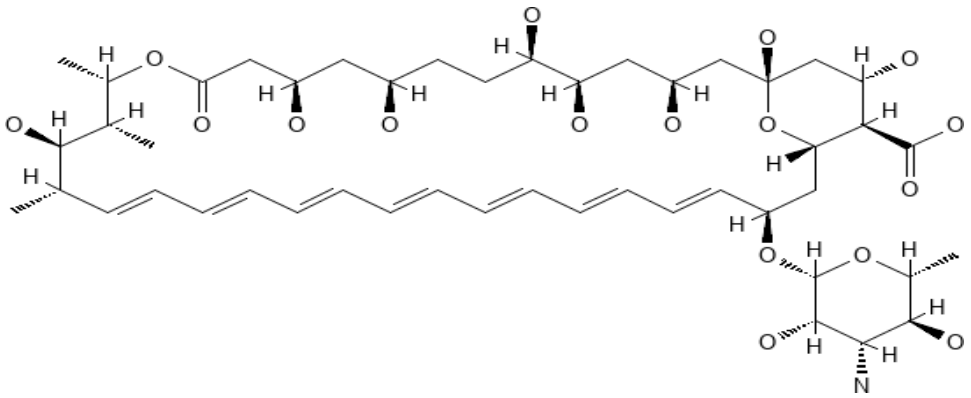


Isoconazole

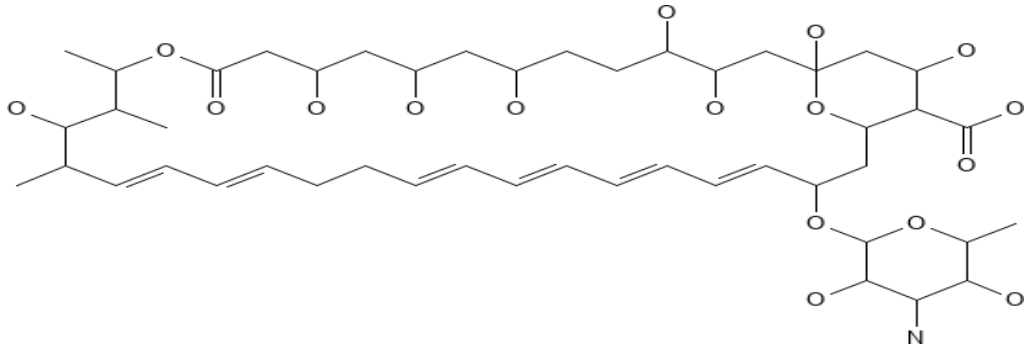


Sertaconazole

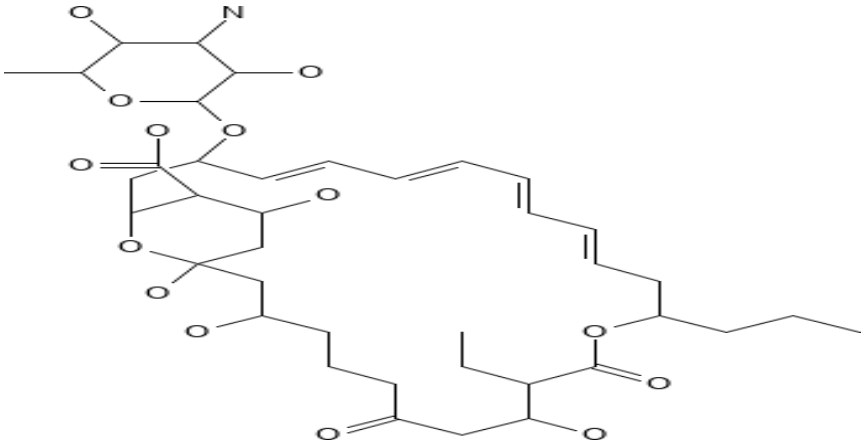
Polyenes: Polyene antibiotics bind with ergosterol of the cell membrane and then cell contents like ions and small molecules are leaked out from the cell membrane resulting in the cellular death thus showing a fungicidal effect. Amphotericin B is a polyenes antibiotic made up of a 38-membered lactone ring and an amino sugar moiety that are covalently bonded. It binds the sterols in fungal cell membranes specifically and permanently. The amphotericin B choice in the treatment of invasive fungal infections. The main negative effects of amphotericin B are caused by its interaction with membrane sterols. Significant side effects from long-term use include anemia, arrhythmia, hypotension, respiratory distress, type IV renal tubular acidosis, fever, chills, and chills. (Zotchev SB.2003)



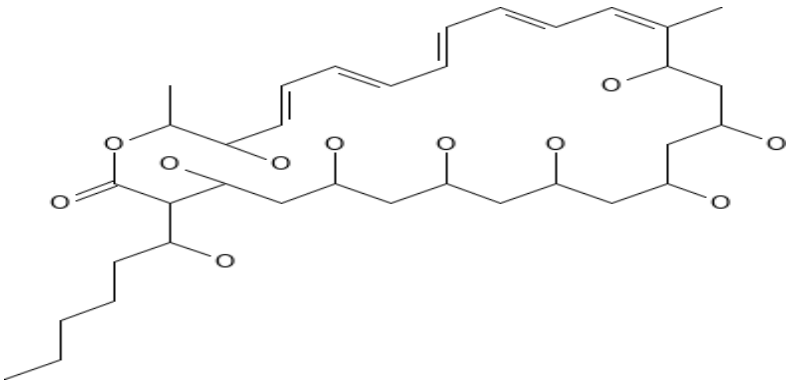
Amphotericin B



Nystatin

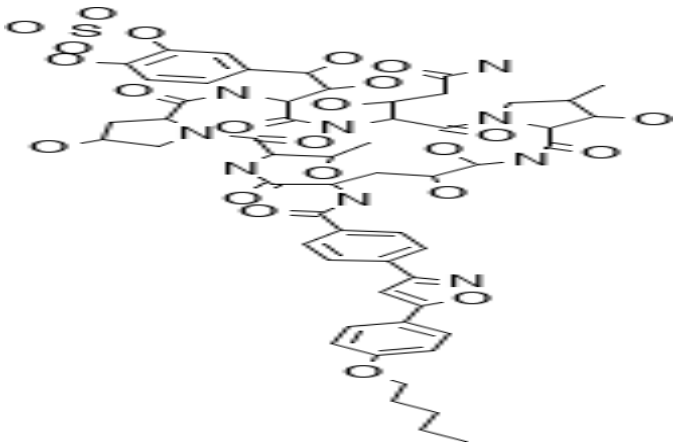


Rimocidin

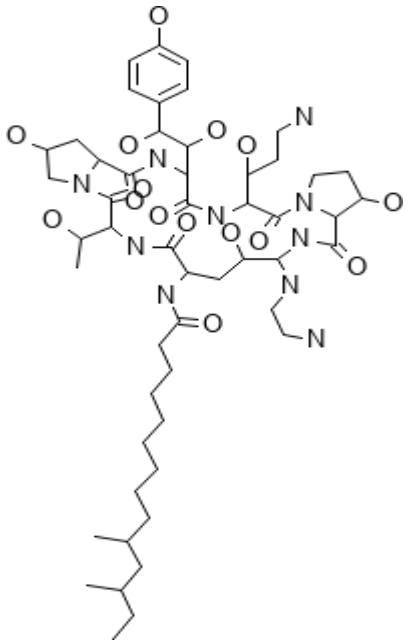


Filipin-

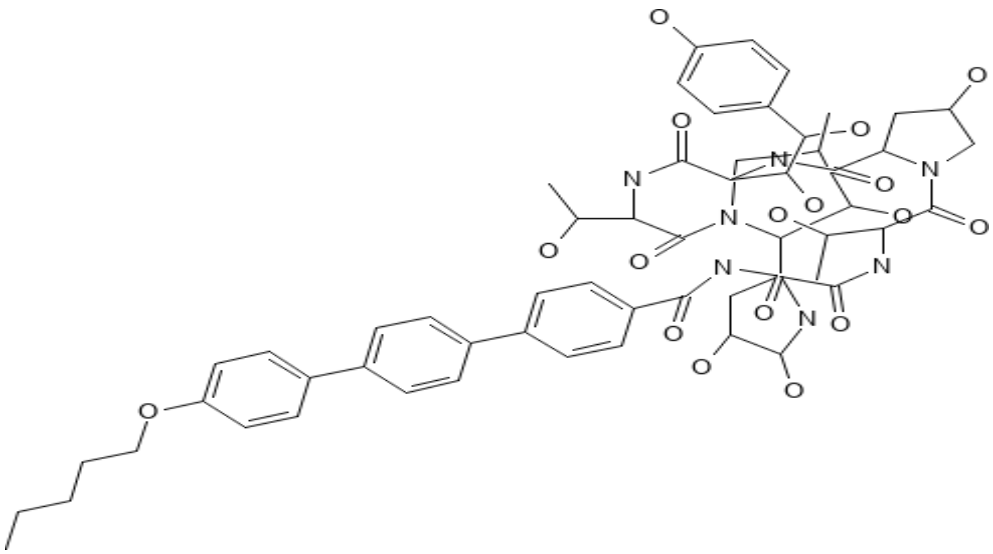
Echinocandins: Echinocandins are a class of antifungal drugs whose molecular targets are fungal cell walls. They are lipoprotein in nature that noncompetitively inhibits (1,3) – beta- D-glucan synthase enzyme. The glucan synthase enzyme is the major component of fungal cell walls (Grover ND *et al.*,2010) , therefore by inhibiting its synthesis systemic infections can be restricted. Examples are Caspofungin, Micafungin and Anidulafungin.



Micafungin

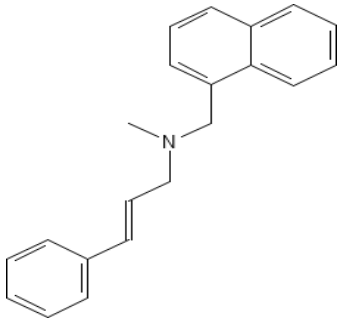


Caspofungin

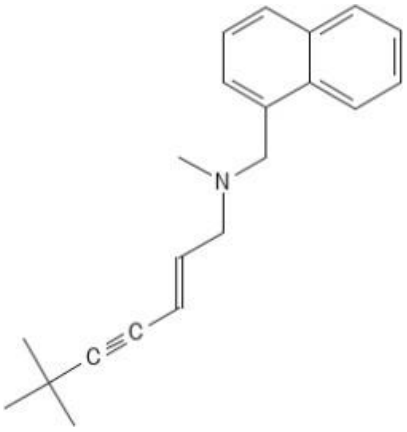


Anidulafungin

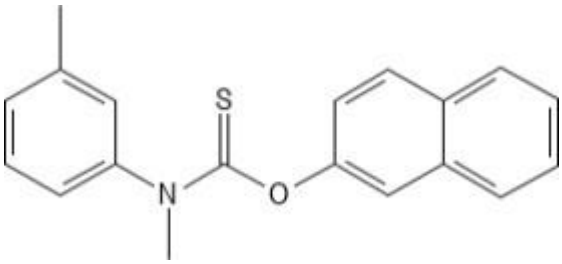
Allylamines and Thiocarbamates: The two most effective inhibitors of the fungal cell membrane are thiocarbamate and allylamines. The allylamines have a limited spectrum of activity and are basically effective against superficial fungal infections and are used in the treatment of fungal infections of nails & skin. Allylamines have also come in the category of ergosterol biosynthesis inhibitors in the biosynthetic pathway of ergosterol synthesis starting from squalene when squalene is converted to squalene epoxide by the enzyme squalene epoxidase (Birnbaum JE *et al.*,1990) so that squalene accumulation and ergosterol deficiency gives a fungicidal and fungistatic actions. Allylamines includes Terbinafine, Butenafine, Naftifine, and Tolnaftate.



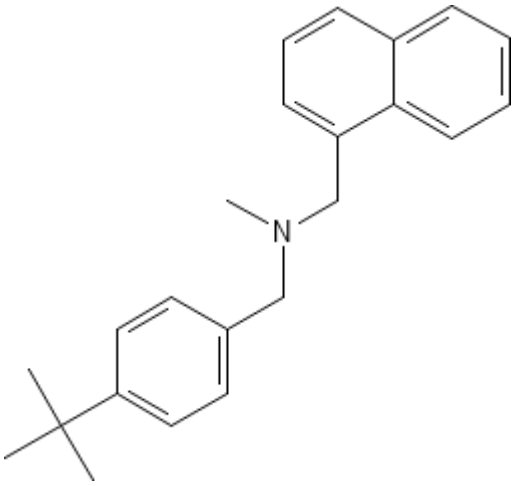
Naftifine



Terbinafine

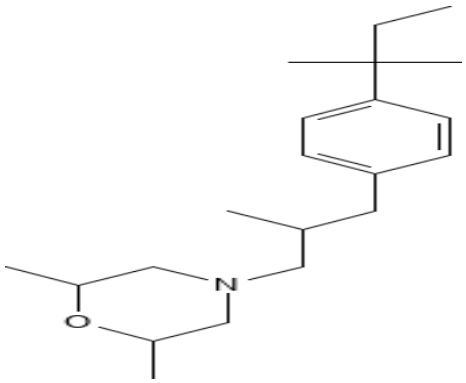


Tolnaftate



Butenafine

Morpholines: Morpholines were found in the 1970s and are used to treat nail infections topically. They are also employed as fungicides in agriculture. They interfere with the biosynthesis of ergosterol by blocking the activity of the enzymes 14-reductase and 4,8-isomerase. (Nucci M, Perfect JR *et al.*,2008). Amorolfine is a morphinone and has fungicidal and antifungal properties.



Amorolfine

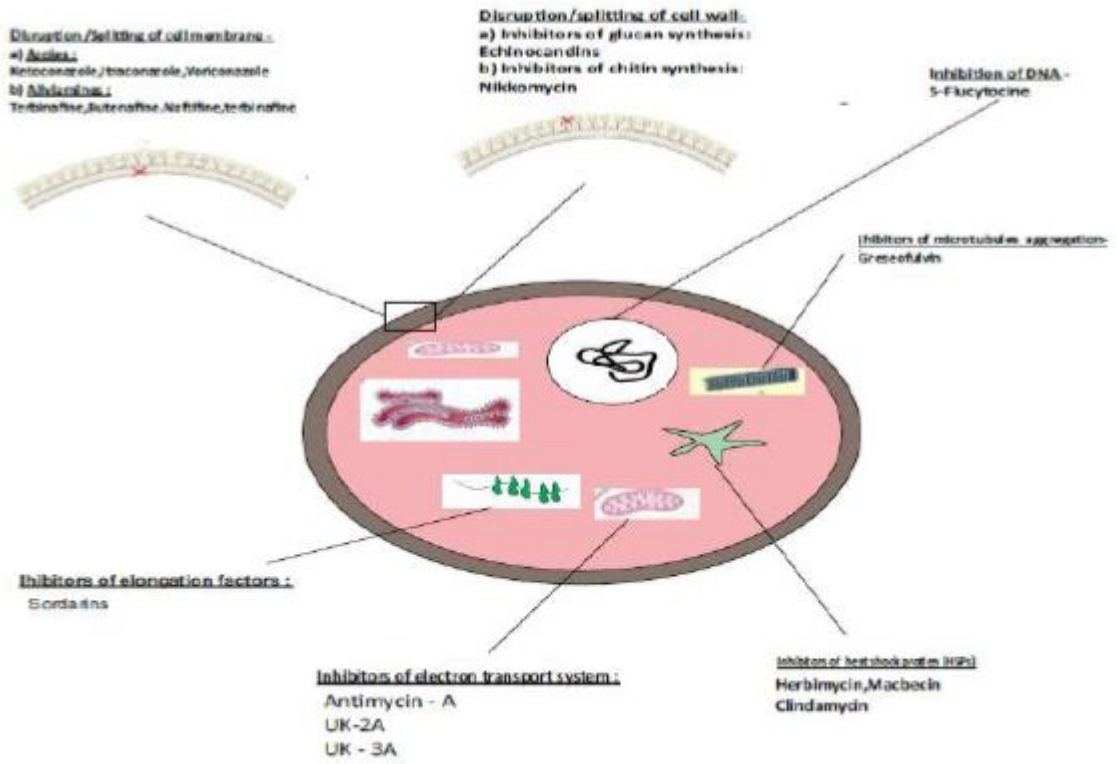


Figure 3: Site of mechanism of action of available antifungal drugs

2.2 Recently approved drugs for dermatophytosis:

Newer investigational drugs ME1111 causes inhibition of succinate dehydrogenase enzyme, this enzyme interferes with the electron transport system of mitochondria and shows the fungicidal effect. (Sangamwar AT *et al.*, 2008). In the current scenario epidemic diseases increase the recurrent chronic dermatophytosis due to the development of resistance by fungal strains increasing the demands for antifungal agents and these formulations. Many drugs under the investigational stage of clinical trials like UK-109-496., Pfizer., SCH-56592 Schering., BMS-207147/ER-30346 Bristol, Myers Squib/Eisai; TAK-187., Takeda; UR-9825, Uriach and UR-9751., T-8581 (Toyama)., SYN −2835.2869,29 No.03,2921., Phar/Taiho., SSPharma & Yoshitomi. Table 2 enlists the antifungal agents which are currently under clinical trials (https://clinicaltrials.gov/)

S.No.	Antifungal agent	Status	Conditions	Clinical trials.gov Identifier No.	Drugs under clinical trial
1.	Ibrexafungerp (SCY-078)	Recruiting 15 Nov2017 to December	Candidiasis, Invasive Candidemia	NCT03363841	Phase 3
2.	P-3058(Terbinafine hydrochloride 10%)	Withdrawn May 2017-Dec2019	Onychomycosis of Toenail	NCT03094468	Phase 3
3.	Griseofulvin 500 mg Griseofulvin 250 mg	Completed in 13 Jan 2020 to 13 February	Antifungal agents	NCT04318535	Phase I
4.	Loceryl nail Lacquer Bifonazole Cream	Completed Jan 2016 to Sep 2016	Foot dermatoses	NCT02705664	Phase 4
5.	Drug: BB2603-1 Drug:BB2603-3 Drug:BB2603-10	Active not recruiting. 6 Dec2019 to Sep2022 (Lastverified)	Distal subungual onychomycosis Fungal infections	NTCT04188574	Phase 2
6.	Drug: CGB-400 Topical gel	Recruiting 8 Feb 2022 to 15 Sep 2022	Fungal infection Onychomycosis by *Tinea unguium*	NCT05202366	Phase 3
7.	Drug: P-3058	Withdrawn August 2014 to September 2017	Fungal infection of Nail	NCT03094468	Phase 3
8.	Drug: Lozanoc 50 mg Sporanox 100mg	Completed July 2015 - October 2015	Fungal infection of skin	NCT02493738	Phase I

(Van Daele R *et al*., 2019)

Table 2: Antifungal agents under Clinical Trials.

2.3 Identification of suitable drug targets for therapeutic intervention

Table-3 summarizes the presently available targets of dermatophytes understanding of the molecular targets will help in the development and designing of more promising drugs which has a larger spectrum of activity and show less resistance to currently available pharmacotherapy (K Mazu T *et al.*,2016).

2.3.1 Available fungal target:

S. No	Name of fungal targets	Drugs	Mechanism of action
1.	**FUNGAL CELL – MEMBRANE**		
	Glucan biosynthesis	Caspofungin Micafungin	Inhibition of beta- 1,3 glucan synthase enzyme
	Chitin biosynthesis	Polyoxins Nikkomycins	Inhibition of chitinase enzyme
	Mannoprotein biosynthesis	Benacomicin Pradimicins	It binds to the polysaccharide's units of the cell surface
2.	**CELL – MEMBRANE BIOSYNTHESIS**		
	Sphingolipid biosynthesis	Lipoxamicin Myriocin Pramanicin	Inhibition of sphingosine-acetyltransferase (ceramide synthase)
	Ergosterol biosynthesis	Fluconazole Voriconazole Posaconazole Ketoconazole	Inhibition of lanosterol 14 -alpha demethylase enzymes required for the conversion of lanosterol into ergosterol
		Polyene antibiotic- Amphotericin –B	It forms a complex with fungal sterol ergosterol and affected the permeability of

		Nystatin Natamycin (Pimaricin)	cell membrane causing the leakage of cytoplasmic contents finally cell death
	Inhibitors of proton ATPases	Naftifine Terbinafine Tolnaftate Foliomycin	Inhibition of squalene epoxidase enzyme, this enzyme is required to be responsible for the cyclization of squalene into lanosterol so that ergosterol is depleted and squalene accumulated that affecting cell membrane structure & functions. Inhibition of proton ATPases that affect intracellular pH of the cell
	Other antifungal agents	Ciclopirox	The mechanism of action shows chelation of polyvalent metal cations like Fe3+ and Al3+ interfere with the biosynthesis of ergosterol
3.	**INHIBITORS OF NUCLEIC ACID AND PROTEIN BIOSYNTHESIS**		
	Interfere with normal processes	Flucytosine or 5 - Fluro cytosine	It causes miscoding during the translation process of protein synthesis & also causes inhibition of thymidylate synthase and affects the DNA replication
	Inhibition of elongation factors	Sordarins	It affects the polypeptide chain elongation factor (EF-3) and interferes with the protein synthesis translation process
4.	**INHIBITORS OF OTHER CELLULAR FUNCTIONS**		
	Inhibitors of microtubule aggregation	Griseofulvin	Inhibits microtubule aggregation interacting along with the β-tubulin protein of spindle fibers
	Inhibitors of signal transduction Pathways	cyclosporine Tacrolimus, Rapamycin,	Inhibition of signal transduction cascades

	Inhibitors of calcineurin-dependent signaling	Wortmannin	Calcineurin affects the homeostasis of the cell under stress conditions
	Inhibitors of heat shock proteins	Herbimycin Macbecin Clindamycin	Inhibition of HSP-90 depending on the signaling
	Inhibitors of electron transport	Antimycin-A UK-2A UK-3A	Inhibition of mitochondrial electron transport system

Table 3: Currently used drugs with their molecular targets and mechanism of action.

2.3.2 Genomics of molecular targets

The genome of fungi includes all the genetic information encoded by chromosomal genes and extrachromosomal components, each of these can contribute significantly to the phenotype of fungi. The DNA sequencing of the complete genome of a living organism is a helpful resource for research to understand the physiology of an organism and the identification of newtargets (Isaacson RE 2002), genomics is a key tool for identifying new therapeutic targets. After the determination of the complete gene sequencing of pathogens then computerized approaches are used to identify potential targets. The genomic approach is used for the quantification and recognition of molecular targets in antimicrobial agents. Recently, the full database of Saccharomyces known as Saccharomyces Genome Database (SGD) showed that yeast contains 6,604 open reading frames, these gene sequences are predicted to encode the protein.

3. Summary & Conclusion

The growing immunocompromised diseases cause recurrent chronic dermatophytosis and also the development of fungal resistance that provides a route to search for newer antifungal agents through optimization techniques, specificity studies of targets and drugs aim to make limitations like decreased effectiveness of drugs that are less toxic to the patients. The chemical modifications of drugs have some limitations like decreased effectiveness of drugs resistance development. Because of these reasons there is a constant need to identify novel drugs for fungal

infections. The ideal targets require two major characteristics, specificity & fungal organism cell viability. The genomic databases can be used as sources of knowledge to find prospective target genes. It is anticipated that the genetic analysis employed to study pathogenic fungus will give way to the hunt for new targets that can be exploited to create new antifungal medications.

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Author contribution conceptualization

Ms. Sarita did the literature survey drafted the manuscript; Dr. Neha Mathur supervised and reviewed the literature and corrected the drafted manuscript. Dr. Pooja Chawla contributed to correcting the final manuscript.

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