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Review on Basic Chemistry, Mode of Action and Clinical Uses of Clotrimazole.

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ABSTRACT

Clotrimazole, one of the early imidazole antifungal drugs, was synthesized in 1969 by Bayer AG, with its synthesis reported in 1972. It exhibits strong in vitro activity against dermatophytes, pathogenic yeasts, fungi, and some gram-positive bacteria, making it effective in treating various infections such as dermatophytic and superficial fungal infections, including tinea versicolor, oral thrush, and vaginal candidiasis. [1] Its proven effectiveness led to its use as a control drug in clinical trials for newer azole derivatives. Clotrimazole's broad spectrum and potent activity have been confirmed in multiple studies, and it acts quickly with a short contact time, disturbing fungal cell membranes. [2]

❖ **SYNONYMS**

- Bay b 5097
- Canesten
- Clotrimazole
- FB b 5097
- Kanesten
- Klotrimazole
- Lotrimin

INTRODUCTION

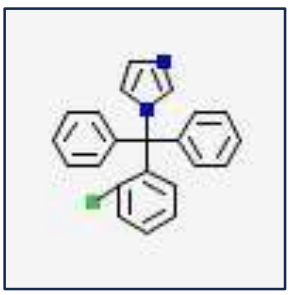
Clotrimazole is a versatile medication known for its effectiveness against various fungal infections. It belongs to the imidazole class and acts as a broad-spectrum antifungal agent. Initially discovered for its antifungal properties in the late 1960s, clotrimazole falls into the category of azole antifungals. [3]

This drug is available in different forms, including creams, pessaries, and troche formulations. Its minimal side effects and straightforward metabolic profile have made it widely accepted for treating fungal infections such as vaginal yeast infections and athlete's foot. [4]

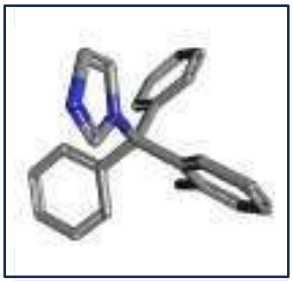
Beyond its antifungal applications, clotrimazole has attracted interest in treating other conditions like sickle cell disease, malaria, and certain cancers. Overall, its broad-ranging efficacy and low risk of side effects have contributed to its widespread use for managing various mycotic outbreaks. [5]

STRUCTURE OF CLOTRIMAZOLE

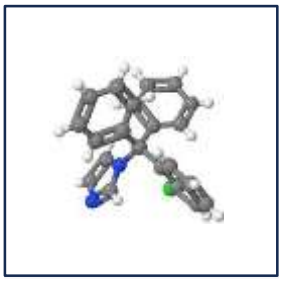
2D



3D



Crystal



PHYSICAL PROPERTIES

- Molecular Formula - C22H17ClN2
- Molecular Weight - 344.8 g/mol
- Physical Description
  - Solid
- Color / Form
  - Crystals
- Odor
  - Odourless
- Melting Point
  - 148
- Solubility
  - Soluble in Acetone, Chloroform, and Ethyl Acetate.
  - Slightly Soluble in Water.
  - Slightly Soluble in Benzene and Toluene.

MECHANISM OF ACTION OF CLOTRIMAZOLE

Clotrimazole works by disrupting the protective barrier within the cell membrane of fungi. It achieves this by inhibiting the biosynthesis of ergosterol, a crucial component of fungal cell membranes. When the synthesis of ergosterol is hindered, the cellular membrane loses its ability to form a complete and functional structure. Ergosterol is essential for the growth of fungal cells, and its inhibition prevents the formation of a healthy cellular membrane. [6]

The primary mechanism of clotrimazole's antifungal properties involves the inhibition of lanosterol 14-demethylase (also known as CYP51), leading to a decrease in ergosterol production. However, clotrimazole also exhibits other pharmacological effects. These include the inhibition of sarcoplasmic reticulum Ca2+ -ATPase, reduction of intracellular calcium levels, and blocking of calcium-dependent potassium channels and voltage-dependent calcium channels. These additional effects contribute to the overall effectiveness of clotrimazole in combating fungal infections. [7]

PHARMACOKINETICS

Absorption:

Clotrimazole has poor oral bioavailability, with less than 3% absorbed from mucosal surfaces and less than 0.5% via the skin.

Topical administration of the drug also results in limited absorption, and systemic administration is generally avoided.

The drug can be administered orally or transmucosally through lozenges (troches), topically, or intravaginally.

Time to Peak, Serum:

A mean peak serum level of clotrimazole, equivalent to only 0.03 µg/mL, is reached 1 to 2 days after application.

Salivary levels appear within 3 hours after a half-hour of dissolution time for oral and topical routes, while high vaginal levels take 8-24 hours for vaginal cream. [8]

Distribution:

Clotrimazole is minimally distributed locally when applied to the skin surface.

Topically applied clotrimazole has very low absorption into blood serum and tissues, limiting its systemic effects.

Metabolism:

Most absorbed drug undergoes metabolism in the liver on the first pass, resulting in inactivated compounds. [9]

Excretion:

Clotrimazole is mostly excreted via feces and urine as metabolites, and a small amount is excreted via bile.

It is unclear whether clotrimazole is excreted in human milk. [10]

Administration:

Clotrimazole is not intended for systemic administration; it is administered through oral/transmucosal lozenges, topically, or intravaginally.

Only small amounts are absorbed, metabolized in the liver, and excreted via bile. [11]

Dosage Forms:

Oral lozenges: 10 mg

Topical cream: 1% (available in different sizes) Topical lotion: 1% (30 mL)

Topical solution: 1% (10 mL, 30 mL)

Vaginal tablets: 100 mg, 200 mg, 500 mg

Combination pack: Vaginal tablets 500 mg/topical cream 1% 7 g Vaginal cream: 1% (available in different sizes), 2% (25g) Vaginal tablets: 100 mg, 200 mg, 500 mg [12]

SIDE EFFECTS, INTERACTIONS AND CONTRAINDICATION

Safety and Side Effects of Clotrimazole:

Topical Forms:

Over-the-counter topical forms of clotrimazole are generally considered safe with minimal serious side effects.

Limited case reports suggest rare instances of contact allergic dermatitis with clotrimazole creams, not related to allergies to vehicles or excipients but to the active ingredient itself. [13]

Intravaginal Use:

Intravaginal clotrimazole, when applied via a pessary, may damage latex contraceptives (condoms), requiring additional contraceptive measures during administration. [14]

Oral Lozenges:

Oral lozenges used for oral candidiasis may cause side effects such as nausea, vomiting, unpleasant mouth sensations, pruritus, and elevated liver enzymes.

Clotrimazole lozenges are not available in the European Union but are widely used in the USA and other countries. [15]

Oral Tablets/Capsules:

Clotrimazole tablets or capsules designed for swallowing are no longer used due to associated gastrointestinal disturbances, dysuria, and mental depression.

Other imidazole antifungal drugs have replaced clotrimazole in oral capsule formulations. [16]

Drug Interactions:

Clotrimazole's limited water solubility and gastrointestinal toxicity have led to its replacement in oral capsule formulations.

As clotrimazole is not systemically absorbed, it has minimal drug interactions.

Safe for use with alcohol, does not affect driving ability, and no evidence suggests a risk to the developing fetus during pregnancy. [17]

Special Populations:

Pessaries are not recommended for use in children or infants, although the drug itself poses no special risk to this subpopulation.

Clotrimazole is considered safe for use in the elderly population and breastfeeding mothers. [18]

BRIEF SUMMARY OF ANTIMICROBIAL ACTIVITY

Mechanism of Action of Azole-Type Antimycotic Drugs, Including Clotrimazole:

1. Interference with Ergosterol Biosynthesis:

All azole-type antimycotic drugs, including clotrimazole, disrupt the biosynthesis of ergosterol, a key component of the fungal cytoplasmic membrane. [19]

2. Inhibition of Cytochrome P450 (CYP450)-Dependent Demethylation:

Azoles, such as clotrimazole, inhibit the microsomal cytochrome P450 (CYP450)-dependent process of 14-a-lanosterol demethylation.

This step is crucial in the biosynthesis of ergosterol by fungi. [20]

3. Depletion of Ergosterol and Formation of Aberrant Sterols:

Inhibition of demethylation results in the depletion of ergosterol.

Ergosterol is replaced by aberrant sterol species, specifically 14-a-methylsterol. This alteration disrupts normal membrane permeability and fluidity. [21]

4. Downstream Effects on Fungal Cells:

Reduced activity of membrane-bound enzymes, including those involved in cell wall synthesis. Increased cell wall leakiness and leakage of cell contents. [22]

5. Inhibition of Fungal Growth:

Ergosterol stimulates the growth of fungal cells in a hormone-like manner.

Depletion of ergosterol leads to a dose- and time-dependent inhibition of fungal growth. [23]

6. Fungistatic vs. Fungicidal Effects:

Clotrimazole is generally considered fungistatic, but at higher concentrations, it exhibits fungicidal effects.

Rapid onset of membrane disruption and other effects contributes to the inhibition of fungal growth. [24]

7. Selectivity for Fungal Cells:

Azole drugs show greater affinity for fungal cytochrome P450 enzymes compared to mammalian counterparts.

Specifically target the cytochrome P450-Erg11p or Cyp51p enzyme in fungi. [25]

8. Binding to Active Site of Cyp51p:

Azole compounds bind to the iron atom of the iron protoporphyrin moiety in the active site of Cyp51p.

Binding occurs via a nitrogen atom (N-3 or N-4) in the imidazole (N-3) or triazole (N-4) ring of the azole drug. [26]

9. Exploiting Differences for Drug Design:

Differences in the conformation of the active site among fungal species and mammalian P450 enzymes provide opportunities for designing drugs with improved efficacy against specific fungal species. [27]

Clotrimazole Resistance:

Issue: Clotrimazole resistance is a concern, especially in immunocompromised patient populations.

Linked to Overexpression of Genes:

Overexpression of efflux pump genes, including Candida drug resistance1, Candida drug resistance2, and multidrug resistance1 genes, has been linked to resistance. [28]

In Candida glabrata, the antiporter protein CgTpo3 contributes to clotrimazole resistance. Changes in Drug Target:

Mutations or overexpression of the ERG11 gene, responsible for lanosterol 14-a-demethylase (the

drug target), may lead to resistance.

Alternative Treatments:

In cases of resistance, non-azole treatments or second-generation drugs might be suitable as alternative pharmacotherapies. [29]

Diverse Pharmacological Actions of Clotrimazole:

Antimycotic Properties:

CYP450 inhibition primarily contributes to clotrimazole's antimycotic properties.

Additional Pharmacological Actions:

Inhibition of Sarcoplasmic Reticulum Ca2+-ATPase:

Impacts cellular calcium regulation.

Depletion of Intracellular Calcium Stores:

Influences intracellular calcium levels.

Blockade of Calcium-Dependent Potassium Channels and Voltage-Dependent Calcium Channels: Affects cellular signaling and ion channels. [30]

Biological Effects Independent of Antimycotic Action:

Cell Proliferation Inhibition:

Inhibits proliferation of various normal and cancer cell lines in vitro.

Adhesion Molecule Expression Inhibition:

Inhibits the expression of adhesion molecules by TNF-a.

Neuroprotective Effects:

Exhibits neuroprotective effects.

Modification of Cytotoxicity:

Modifies the cytotoxicity of certain metal cations. [31] Therapeutic Applications Beyond Antifungal Properties:

Sickle Cell Disease

Considered in the therapeutic application of sickle cell disease, as its metabolite, ICA 17043, is believed to affect erythrocyte dehydration.

Antimalarial Activity:

Exhibits antimalarial activity in vitro, potentially by inhibiting hemoperoxidase and causing oxidative stress in the parasite.

THERAPEUTIC CLASS AND PHARMACEUTICAL USE

Clotrimazole Overview:

Classification: Clotrimazole belongs to the azole class of synthetic antimycotic agents. Discovery: Discovered in the 1960s.

Azole Class:

Azoles form the largest class of antimycotic drugs in clinical use.

Two main subclasses based on chemical structure: imidazoles and triazoles. Clotrimazole falls into the imidazole subclass.

Medical Applications:

Topical Treatment: Clotrimazole, along with econazole and miconazole, is the drug of choice for the topical treatment of various fungal infections:

Tinea pedis (athlete's foot) Tinea cruris

Tinea corporis

Caused by: Isolates of Trichophyton rubrum, Trichophyton mentagrophytes, Epidermophyton floccosum, Microsporum canis, and Candida albicans.

Other Uses: Widely used in the topical treatment of vulvovaginal and oropharyngeal candidiasis. [32]

SIMILAR DRUGS

Antimycotic Azole Drugs Related to Clotrimazole:

Imidazole Drugs:

Miconazole Econazole Ketoconazole

Triazole Drugs:

Fluconazole Itraconazole

Oral Absorption Exceptions:

Ketoconazole and Itraconazole:

Notably, ketoconazole and itraconazole are exceptions, as they can be absorbed orally to some extent. [33]

Clinical Applications:

Miconazole and Econazole:

Used similarly to fluconazole in treating topical fungal infections.

Miconazole is also administered intravenously for systemic infections in patients unable to tolerate amphotericin.

Itraconazole:

Active against Aspergillus.

Antifungal Activity and Cytochrome P450 Interaction:

Antifungal activity for each azole drug is heavily influenced by the interaction with cytochrome P450.

Rational drug design approaches have led to the development of a new generation of azole drugs, incorporating triazole structures.

Second-Generation Azole Drugs:

Voriconazole and Ravuconazole:

Structurally based on fluconazole.

Developed to address limitations of first-generation azole compounds. Extended antimycotic spectrums.

Posaconazole:

Closely related to itraconazole.

Advancements and Goals:

Second-generation triazole compounds aim to overcome limitations of existing azoles, including the emergence of drug-resistant fungal strains. [34]

These advancements are crucial in addressing the evolving challenges in antifungal therapy. Pediatric Medicine Considerations:

Use of second-generation drugs is reviewed in the context of paediatric medicine, considering their efficacy and safety in treating fungal infections in children. (Refer to Valerio et al., 2013)

PHARMACEUTICAL DOSAGE FORMS AND ADMINISTRATION

Clotrimazole is a medication available in the European Union and the USA for treating fungal infections. In the EU, it comes in cream and pessary forms under various names, while in the USA, you can find lotions, powders, lozenges, topical solutions, and vaginal inserts/tablets. Some clotrimazole preparations may also include steroids like hydrocortisone or betamethasone. Generally, you can get standalone clotrimazole without a prescription, but combined formulations might need one. [35] For fungal skin issues, it's often in 1% cream, lotion, spray, or solution. Vulvovaginal candidiasis is treated with daily doses of 100 mg, 200 mg, or 500 mg pessaries, or by applying 1%, 2%, or 10% creams. Oropharyngeal candidiasis involves using 10 mg

clotrimazole lozenges, sucked slowly until dissolved, five times daily for 14 days. In prophylactic prevention for immunosuppressed individuals, the dose is reduced to 10 mg, three times daily. Quality standards, according to USP, ensure clotrimazole is 98.0-102.0% pure. The British and European Pharmacopoeias specify 98.5-100.5% clotrimazole purity concerning the dried substance. [36]

CONCERNS SORROUNDING ENVIRONMENTAL TOXICITY

Growing concerns revolve around the presence of chemicals in our environment, where insufficient data exist about their potential harm. While pharmaceuticals undergo rigorous human toxicity assessments before release, their environmental impact is often less scrutinized. Some chemicals, notably those persistent and prone to bioaccumulation, pose risks to aquatic wildlife exposed to wastewater discharges. Clotrimazole, targeting cytochrome P450 and having a long environmental half-life, falls into this category. In 2002, it made the OSPAR priority action list for marine protection. Despite concerns, a thorough assessment in 2005 deemed clotrimazole not a significant environmental risk. However, recent studies hint at potential effects on marine microalgae even at low concentrations. [37]

HUMAN TOXICITY EXCERPTS

In some cases, applying clotrimazole on the skin might lead to mild adverse effects, including stinging, redness, swelling, blistering, peeling, itching, and hives. When used vaginally, approximately 1.6% of users may experience a mild burning sensation, and rarely, lower abdominal cramps, increased urinary frequency, or skin rash. There have been occasional reports of penile or urethral irritation in sexual partners. When taken orally, clotrimazole can cause mild gastrointestinal irritation, with an incidence of about 5% in patients using troches (lozenges). Troche users may also experience nausea and vomiting (around 5% incidence), and 15% have shown reversible elevation of liver enzyme levels. It's important to monitor hepatic function in patients with pre-existing liver conditions. Clotrimazole's safety and efficacy in children under 3 years old have not been established, so the use of troches in this age group is not recommended. In a study involving rheumatoid arthritis patients, those receiving clotrimazole showed improvements in grip strength, joint count, and pain assessment, but also experienced more gastrointestinal complaints, leading to some patients discontinuing the therapy. [38]

NON-HUMAN TOXICITY EXCERPTS

Clotrimazole is a medication that can prevent the growth or kill various fungi, including yeast and dermatophytes. It also shows activity against certain gram-positive bacteria. In laboratory conditions, clotrimazole concentrations of 1 ug/ml or less can inhibit most strains of fungi like Trichophyton rubrum, T. mentagrophytes, Epidermophyton floccosum, and Microsporum canis. At a concentration of 3 ug/ml or less, it can inhibit other susceptible organisms, including Malassezia furfur, Aspergillus fumigatus, Candida albicans, some strains of Staphylococcus aureus, Streptococcus pyogenes, Proteus vulgaris, and Salmonella. Clotrimazole also demonstrates activity against Sporothrix, Cryptococcus, Cephalosporium, and Fusarium. Higher concentrations (100 ug/ml) are needed to inhibit Trichomonas vaginalis. [39]

In animal studies with rats and mice, clotrimazole has been found to be harmful to the developing embryos when given in oral doses 100 times the usual human dose, likely due to maternal toxicity. However, it did not cause birth defects in mice, rabbits, or rats when given in oral doses up to 200, 180, or 100 times the human dose, respectively. Reproduction studies in mice and rats using oral clotrimazole at higher doses resulted in some adverse effects on mating, number of viable offspring, and survival to weaning. These effects were observed at doses significantly higher than the usual human dose. [40]

METABOLISM / METABOLITES

The study investigated the impact of clotrimazole, an antifungal imidazole compound, on the metabolism of benzo[a]pyrene in cultured keratinocytes derived from BALB/c mouse epidermis. The addition of varying concentrations of clotrimazole to the cultured keratinocytes resulted in a dose-dependent inhibition of the activities of cytochrome P450-dependent monooxygenases, specifically aryl hydrocarbon hydroxylase and 7-ethoxycoumarin O-deethylase. [41]

The primary metabolites of benzo[a]pyrene identified in the cultured cells included trans-7,8- dihydro-7,8-dihydroxybenzo[a]pyrene, 9-hydroxybenzo[a]pyrene, and 3-hydroxybenzo[a]pyrene, with smaller amounts of other metabolites. In both intracellular and extracellular environments, water-soluble metabolites consisted mainly of glucuronide conjugates of 3- hydroxybenzo[a]pyrene, 9-hydroxybenzo[a]pyrene, and benzo[a]pyrene-3,6-dione, along with sulfate conjugates. [42]

Clotrimazole demonstrated a dose-dependent inhibition of the formation of both organic solvent- soluble and water-soluble conjugates. Moreover, clotrimazole inhibited the in vitro metabolism of benzo[a]pyrene by microsomes prepared from both control and benz(a)anthracene-induced cultured keratinocytes. The inhibitory effect was more pronounced in benz(a)anthracene-induced keratinocytes, particularly concerning the formation of diols and quinones. [43]

Furthermore, clotrimazole substantially reduced the enzyme-mediated covalent binding of benzo[a]pyrene to mouse keratinocyte DNA and protein in a dose-dependent manner. This suggests that clotrimazole, commonly used for managing superficial dermatophyte infections of the skin, serves as a potent inhibitor of cytochrome P450-dependent transformation of polycyclic aromatic hydrocarbons in cultured murine keratinocytes. This system provides a convenient approach for studying inhibitors of carcinogen metabolism in the epidermis. [44]

CONCLUSION AND FUTURE DIRECTIONS

In recent years, invasive fungal infections have become more common, especially affecting immunocompromised individuals. The rise in these infections is partly due to the success in treating conditions like HIV, cancer, and organ transplants, leading to a larger immunocompromised population. Clotrimazole, once rarely associated with resistance, is now more common in specific patient groups with candidiasis. While these trends may not significantly impact clotrimazole's general use, they drive the development of new drugs with improved efficacy. Newer drugs like posaconazole may replace clotrimazole in treating high-risk patient populations.

Despite potential challenges, ongoing research explores clotrimazole's further pharmaceutical development. There are prospects for its use in new applications and the creation of novel formulations. Clotrimazole's structure is utilized in designing affordable antimalarial drugs and investigating metal–clotrimazole complexes for potential anticancer agents. Researchers are also exploring clotrimazole's role in treating sickle cell disease. New formulations, such as buccal bio adhesive films and thermosensitive vaginal gels, aim to enhance clotrimazole's effectiveness. Slow-release formulations, like liposomes and nano-capsules, may offer advantages in vaginal applications. Clotrimazole remains widely used and ongoing efforts focus on expanding its clinical applications, utilizing its unique chemistry in drug design, and optimizing formulations for enhanced drug delivery.

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