Clinical Medicine Insights: Dermatology

ORIGINAL RESEARCH

Evaluation of the Biological Activity of a New Hydrocortisone Derivative against Some Pathogenic Skin Fungi

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Abstract: The resistance of different types of fungi against ordinary antifungal drugs has been widely reported. The continuous search for a new compound with effective antifungal properties is therefore important and continuous. The present descriptive study aims to estimate the effectiveness of a new compound (HCX), prepared from the reaction of hydrocortisone with cefotaxime, as an in vitro antifungal drug. Five pathogenic fungi samples (*Mentagrophytes canis, Mentagrophytes gypseum, Trichophyton mentagrophyte, Trichophyton rubrum, and Trichophyton verrucosum*) were collected in this study. The antifungal activity of the HCX was determined via agar dilution method using three different concentrations of HCX (1, 2, and 3 µg/mL, respectively). The inhibition efficacy of each HCX concentration on the fungus growth was expressed as the mean inhibition percentage and the mean inhibition concentration.
The results indicate the effective inhibition of growth of the fungi, especially at higher concentrations of the HCX compound. At 3 µg/mL of HCX concentration, 100% inhibition occurred, except in the case of *M. canis,* which was at 77.8% at the relatively lower concentration of 1%. The new compound, formed from the reaction between cefotaxime and hydrocortisone (HCX), has the ability to inhibit the growth of five types of dermatophytes in low doses, comparable to well-known antifungal drugs.

Keywords: antifungal, cefotaxime, hydrocortisone derivative, trychophytone, therapy

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Introduction

Dermatophytes are fungi that cause infections (known as tinea) of the hair, nails, and skin due to their ability to metabolize keratin. Superficial mycoses are among the most prevalent infectious diseases in countries worldwide,[1,2] including Iraq.[3,4] Tinea pedis (athlete's foot) and onychomycosis (infection of the toenails) are caused by the dermatophyte fungi, *Trichophyton*s, which are highly prevalent in adults.[5] This fungus represents a serious problem when associated with other diseases.[6] *Trichophyton rubrum* (*T. rubrum*) is the most frequently isolated dermatophyte, accounting for 80% to 90% of the infectious strains,[7] followed by *Trichophyton mentagrophytes* (*T. mentagrophytes*). These fungi are connected with the increase in the incidence of tinea pedis. *Trichophyton* infections increased in recent years[8] and azole compounds (Econazole, Micanazole, Clotrimazole, Ketanozole) are widely used as antifungal drugs.[9] However, the ineffective treatment of different types of tinea with polyenes, such as nystatin, commonly occurred.[10] Limited treatment options and diverse modes of transmission complicate the clinician's ability to address this disease adequately.[11] The development of more effective and less toxic antifungal agents is required for the treatment of dermatophytosis. Hydrocortisone, a steroid that has anti-inflammatory action, restores hemodynamic stability and differentially modulates the immunological response to stress via anti-inflammation instead of immunosuppression.[12] Furthermore, low-dose hydrocortisone therapy is useful in the treatment of septic shock.[13] Cefotaxime (Claforan) is a third-generation cephalosporin antibiotic that has broad spectrum activity against Gram positive and Gram negative bacteria for infections of different body organs.[14] The antibiotic inhibits bacterial cell wall synthesis and causes cell membrane lyses due to the ongoing activity of cell wall autolytic enzymes while cell wall assembly is arrested.

Most antifungal agents inhibiting fungal growth by inhibiting the formation of sterol precursors, such as ergosterol, which is necessary for the cytoplasmic membrane function.[15]

In the present study, a new compound (HCX) was synthesized from the reaction between cefotaxime, a bacterial cell wall inhibitor, and hydrocortisone. This compound was examined for its effectiveness as an antifungal drug against different fungi: *M. canis*, *M. gypseum, T. mentagrophyte, T. rubrum, and T. verrucosum.* This study hypothesizes that the HCX may exhibit biological activity from its parent compounds, such as the bacterial cell wall inhibitor from cefotaxime, and that the steroid nature from hydrocortisone may interfere with sterol synthesis in fungi cell walls, an important target in the action of ordinary antifungal drugs.

Experimental

Preparation of the compound

Our group previously prepared and diagnosed a new compound,[16] which we will be briefly discussed. A total of 2.384 g of hydrocortisone (Yicheng Chem. Co., China) and 3.00 g of Cefotaxime (Hainan Pharchem Inc., China) were dissolved in 50 mL of 70% ethanol acidified with a few drops of acetic acid. The mixture then refluxed for 2 h and then cooled and filtered to exclude any undissolved particles. After the evaporation of alcohol solvent at room temperature, the solid precipitate was recrystallized in aliquots of ethanol. The new compound was diagnosed and identified via different techniques: CHN, IR, NMR, and UV. The results indicated the formation of a new Schiff's base (HCX) compound and the suggested structural formula is shown in Figure 1. The IUPAC name of the suggested formula is (7*R*)-3-(acetoxymethyl)-7-((*E*)-2-((*Z*)-1-((8*S*,9*S*,10*R*,11*R*,13*S*,14*S*,17*R*)-11,17-dihydroxy-10-13-dimethyl-3-oxo-2,3,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)-2-hydroxyethylideneamino) thiazol-4-yl)-2-(methoxyimino)acetamido)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid. The chemical formula of HCX is $C_{37}H_{45}N_5O_{11}S_2$ with a molecular weight of 799.91 g/moL. The purity of the new compound was obtained through several recrystallization steps, until the melting point of the HCX became constant and confirmed by the IR spectra.

Biological Methods

Specimens collection

The pathogenic fungi samples (*M. canis*, *M. gypseum, T. mentagrophyte, T. rubrum,* and *T. verrucosum)* were obtained from scraping skin, hair, nails, and between fingers from clinically diagnosed infected patients at Al-Dewaniya Teaching Hospital, Al-Qadesiya, Iraq. Oral consent was taken

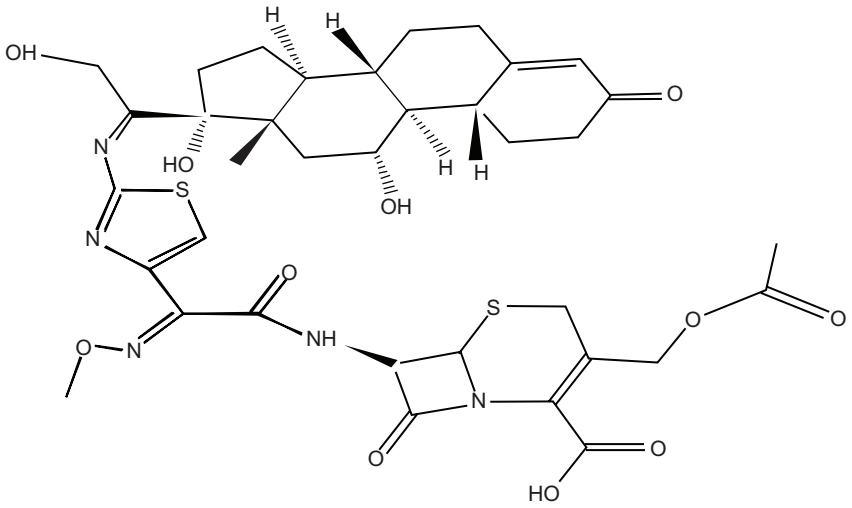

Figure 1. Structural formula of the HCX compound.

from patients for the participation of their samples in the study. Diagnosis of the species was further confirmed by direct microscopic examination of the samples after incubation with a drop of 10% KOH solution in addition to the culture examination of the fungal samples. The identification process was carried out based on the phenotypic characteristics of the colony, such as shape, color, and diameter of the colonies. Furthermore, microscopic qualities such as shape, size and color conidia were adopted, in addition to previously mentioned biochemical tests.[17,18] Each species was then separately isolated and cultured.

Antifungal activity

The antifungal activity of the HCX was determined via the agar dilution method.[19] Cycloheximide (SDA) dissolved and cooled at 50 °C was added to prevent the growth of most yeast cells. The DMSO stock solution of HCX (500 ppm) was prepared and used to prepare HCX serial dilutions. These diluted HCX solutions were mixed with Sabouraud dextrose agar–with chloramphenicol to prevent bacterial growth—to obtain final concentrations of HCX in volume units of agar (µg/mL): 0, 1, 2, and 3 µg/mL. These concentrations were chosen after extensive initial experiments determining the optimal concentrations that produce suitable antifungal activity. Each experiment was repeated three times. After solidification of the media, a fungi colony disk 6 mL in diameter, previously cultured for 7–10 days in SDA media, was placed in the center of the disk. The Petri dishes were incubated at 25 °C to 28 °C for 14 days. The mean inhibition percentages (MIP) were calculated from the equation MIP = ((R1 – R2)/R1)*100%, where R1 is the diameter of fungus colonies that grow in the absence of HCX compound in the agar and R2 in the presence of HCX in the agar.

Parallel experiments were done using four well-known antifungal drugs (Clotrimazole, Flouconazole, Itraconazole, and Ketoconazole) instead of HCX for comparison of antifungal activity of HCX.

Statistical Analysis

The results were analyzed statistically and expressed as mean ± standard deviation. The level of significance was determined by employing the pooled (*t*) test. When the *P*-value was less than 0.05, the difference between the groups was considered statistically significant. All the statistical analysis processes were carried out using Microsoft Excel® 2007.

Results and Discussion

The mean inhibition concentrations (MIP) of the different concentrations of HCX compound (1, 2, and 3 µg/mL) against five types of pathogenic fungi are shown in Table 1.

The results showed effective inhibition of growth of the fungi, especially at higher concentrations of the HCX compound. At 3 µg/mL of HCX concentration, the inhibition was at 100%, except for *M. canis,* which exhibited an inhibition of 77.8% at the relatively lower (1%) concentration. Figure 2 shows an example of the activity of HCX on *T. rubrum* growth; Image B

Table 1. Mean inhibition percentages (MIP) of the different concentrations of HCX compound (1, 2, and 3 μg/mL) against different fungi.

Fungus	Mean inhibition percentages %				
	1 μg/mL	2 μg/mL	3 μg/mL	4 μg/mL	5 μg/mL
M. canis	16.7	23.4	77.8	100	100
M. gypseum	74.3	73.1	100	100	100
T. mentagrophytes	0	35.5	100	100	100
T. rubrum	83.1	100	100	100	100
T. verrucosum	4.4	35.6	100	100	100

exhibits an inhibition of 100% in comparison with Image A (free of HCX).

In the comparison of HCX antifungal activity with the minimum inhibitory concentration of well-known antifungal drugs (Table 2), HCX exhibited good activity comparable with that of flouconazole and Ketoconazole; the HCX compound exhibited higher activity than flouconazole.

A reference method for the antifungal susceptibility testing of dermatophytes is not available. In the recent years, several studies on in vitro susceptibility of dermatophytes to antifungal drugs have been conducted, with results showing considerable variation.[20,21] This variability is likely due to important methodological differences between laboratories. The comparison of HCX antifungal activity, expressed as a concentration of HCX (μg/mL) that produced 100% inhibition in comparison with four antifungal drugs, showed that HCX exhibits higher activity than flouconazole and is comparable with Ketoconazole. These antifungal activities of HCX are comparable with the activity of different other antifungals used previously.[22] The in vitro activity of HCX compound should be monitored in vivo in addition to the present in vitro results.

A new antifungal compound was researched and created due to various reports of increased fungal infections in addition to resistance to antifungal drugs. The emergence of resistance to drugs has led to a growing demand for discovering new effective antifungal drugs.[23,24] Furthermore, dermatophytes that cause lesions in nails do not respond well to ordinary treatment,[25] leading to the need of new treatment discovery.

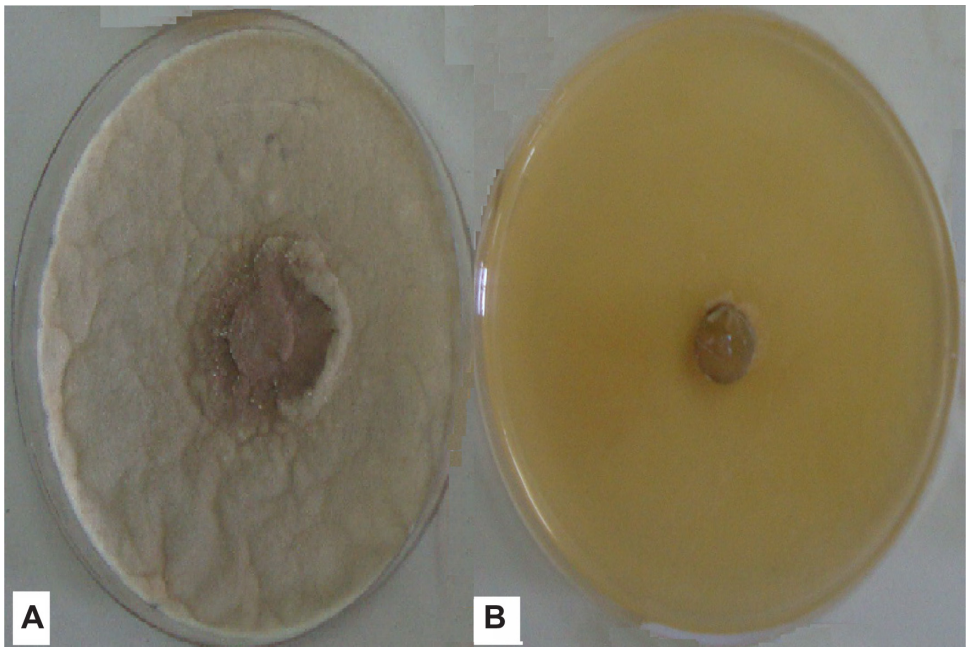

Figure 2. The growth zone of *T. rubrum* in (**A**) Agar free of HCX (**B**) Agar mixed with 3 μg/mL of HCX.

Table 2. Concentration of HCX (μg/mL) that produces 100% inhibition in comparison with four antifungal drugs against the studied fungi.

Fungus	Mean inhibition percentages %				
	1 μg/mL	2 μg/mL	3 μg/mL	4 μg/mL	5 μg/mL
M. canis	16.7	23.4	77.8	100	100
M. gypseum	74.3	73.1	100	100	100
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Successful treatment depends on the ability of a given antimycotic to eradicate the fungal isolate.[26] To predict this ability, in vitro susceptibility testing becomes helpful, allowing clinicians to select the correct treatment for their patients. Antifungal agents affect fungal cells through different mechanisms. Some antifungals act by inhibiting mitosis and nucleic acid synthesis of fungal cells,[27] or lysis of cell membrane;[28] however, the majority of the drugs act by binding to ergosterol in the cell membrane, inhibiting ergosterol synthesis.[15] The effect of the HCX compound may also interfere with sterol synthesis as it also contains a steroid nucleus (cyclopentanophenanthroline). The steroidal property of HCX compound may act as an inhibitor for certain enzymes, as a substrate analogue, or by changing the properties of the fungal cell membrane when HCX is incorporated with these cells. The multi-conjugated double bonds that resemble to the structure of polyene antifungal drugs, including nystatin and amphotericin, comprise another important structural feature of the HCX molecule. These polyenes increase cell membrane permeability by binding to membrane sterols.[29]

Furthermore, the chemistry of the HCX molecule plays a principal role in its antifungal activity due to chemical, electronic, and spatial configurations. The molecule contains the carboxylic acid group that may partially dissociate and decrease the pH of the aqueous medium, which in turn affects the growth of fungi in the media. In earlier QSAR studies on carboxylate ions, researchers noticed that antifungal activity was dependent on the lipophilicity of the compounds.[30]

Research has shown that the common imidazole and triazole antifungal drugs inhibit different types of P450 enzymes in sterol biosynthesis. The basic nitrogen of the azole ring forms a tight bond with the heme iron of the fungal P450-preventing substrate and oxygen binding. The thiazole ring bound to the nitrogen atom in the HCX molecule may have an identical function; however, this requires more studies to be confirmed. The presence of several hydroxyl groups attached to large hydrophobic structures, as shown in the chemical structure of the HCX molecule, is another important feature. Earlier QSAR studies on monohydroxy alcohols revealed that antifungal activity is mainly dependent on the hydrophobicity of the alkyl moiety.[31,32] Furthermore, there is a dramatic role of the environment within the molecule on the antifungal activity of organic compounds with hydroxyl groups.[33]

Hydrocortisone has the ability to inhibit thymidine incorporation into lymphocytes and inhibit DNA synthesis in a dose-dependent manner. This property of hydrocortisone may be still conserved in the HCX compound, and may also play a role in the inhibition of DNA synthesis in fungal cells and inhibit fungal growth.[34] Moreover, a physical combination of hydrocortisone with known antifungal drugs in different experiment types resulted in improved antifungal activity over the antifungal drug alone.[35,36] This property may reinforce the previous suggestions for the special activity of the new compound in the present study. The possible effect of DMSO on the growth of the studied fungi, as recorded in few studies including that of Randhwa (2006),[37] was eliminated using DMSO in all experiments, including the blank Petri dish with no added drugs.

We conclude that the new compound, formed from the reaction between cefotaxime and hydrocortisone (HCX), has the ability to inhibit growth of five types of dermatophytes in low doses, comparable to well-known antifungal drugs. A pharmacological mechanism for the antifungal action of the HCX is recommended to be studied with more advanced pharmacological tools.

Author Contributions

Conceived and designed the experiments: HKAH. Analysed the data: HKAH.Wrote the first draft of the manuscript: HKAH.Contributed to the writing of the manuscript: HKAH.All authors agree with manuscript results and conclusions. All authors developed the structure and arguments for the paper. All authors made critical revisions and approved final version. All authors reviewed and approved of the final manuscript. HKAH and MAKH were responsible for synthesis of (HCX). HKAH and AHM were responsible for Biological activity. All authors reviewed and approved of the final manuscript.

Disclosures and Ethics

As a requirement of publication author(s) have provided to the publisher signed confirmation of compliance with legal and ethical obligations including but not limited to the following: authorship and contributorship, conflicts of interest, privacy and confidentiality and (where applicable) protection of human and animal research subjects. The authors have read and confirmed their agreement with the ICMJE authorship and conflict of interest criteria. The authors have also confirmed that this article is unique and not under consideration or published in any other publication, and that they have permission from rights holders to reproduce any copyrighted material. Any disclosures are made in this section. The external blind peer reviewers report no conflicts of interest.

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