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ASSESSMENT OF ANTIFUNGAL ACTIVITY OF DICHLORODIAZADIENES AND HYDROZO-BASED COMPOUND DERIVATIVES

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Abstract

In this study, four novel compounds were synthesized and evaluated for their antifungal activity against three clinically significant *Candida* strains, including *Candida albicans* BDU MI-44, *Candida guilliermondii* BDU-217 and *Candida tropicalis* BDU LK-30. All compounds were tested at a uniform concentration of 0.3%, and their antifungal efficacy was assessed through the agar well diffusion method. The inhibition zones ranged from 11.0 mm to 24.1 mm, indicating varying degrees of antifungal activity. Among the tested strains, *C. tropicalis* BDU LK-30 was the most susceptible to all four compounds. The compound methyl (Z)-2-(4-(tert-butyl) phenyl)-2-(2-(p-tolyl) hydrazineylidene) acetate (Compound II) demonstrated the highest antifungal potency, producing an inhibition zone of 24.1 mm against *C. tropicalis*, and consistently outperformed the other compounds across all tested strains. Comparative analysis revealed that hydrazineylidene acetate moieties significantly enhance antifungal activity. Overall, the results highlight the potential of these synthesized compounds, particularly compound II as promising antifungal agents, with selective and enhanced activity against *Candida* species.

Keywords*: Antifungal activity; novel compounds; pathogenic fungi; Agar well diffusion method*

1. Introduction

Fungal infections continue to pose a significant threat to public health worldwide, especially among immunocompromised individuals. Among the opportunistic fungal pathogens, species of the genus *Candida*, notably *Candida albicans, Candida guilliermondii*, and *Candida tropicalis* are recognized as leading causes of both superficial and systemic mycoses [13, 19, 25]. *Candida albicans* is a common human commensal yeast responsible for mucosal infections affecting the oral cavity, vaginal tract, esophagus, and nails, as well as severe systemic candidiasis impacting vital organs such as the bloodstream, central nervous system, liver, and kidneys. Despite the availability of antifungal therapies, systemic candidiasis continues to carry a high mortality rate [15, 17]. While *Candida albicans* is the most extensively studied, other species like *Candida guilliermondii*, an emerging pathogen with inherent resistance to azoles and echinocandins [16, 18, 21] and *Candida tropicalis*, noted for its high virulence, biofilm formation, and multi-drug resistance, represent growing clinical challenges [1, 7-9].

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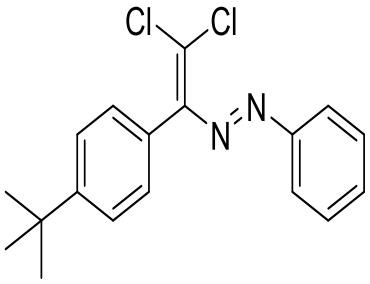
The escalating incidence of antifungal resistance among these pathogens has significantly compromised the efficacy of conventional antifungal classes such as azoles, polyenes, and echinocandins, underscoring the urgent need for novel antifungal agents with unique mechanisms of action [6, 14, 20]. In this context, nitrogen-containing heterocyclic compounds have garnered considerable attention due to their diverse bioactivities. Dichlorodiazadienes, a class of heterocycles characterized by their versatile chemical framework, have demonstrated promising antimicrobial, anticancer, and anti-inflammatory effects; however, their potential as antifungal agents remains underexplored [22]. Similarly, hydrozo compound derivatives, which contain distinctive nitrogen-nitrogen (-N=N-) or nitrogen-oxygen (-N-O-) linkages, have shown encouraging biological activities in preliminary studies, suggesting possible mechanisms of action involving fungal enzyme inhibition or disruption of membrane integrity [23, 24].

This study aims to systematically assess the antifungal efficacy of selected dichlorodiazadiene and hydrozo compound derivatives against *Candida albicans, Candida guilliermondii*, and *Candida tropicalis*. The outcomes of this research have the potential to contribute significantly to the development of novel heterocyclic antifungal agents, offering new therapeutic avenues to combat resistant *Candida* infections and address the global shortage of effective antifungal drugs [10, 12].

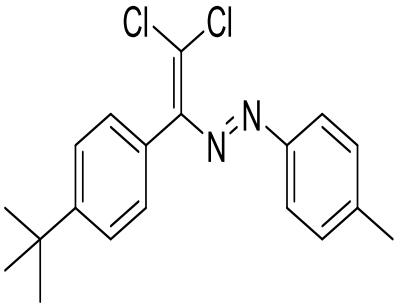
2. Material and methods

2.1. The selected compounds

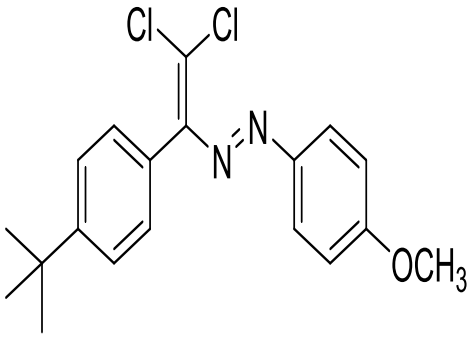
Four synthesized compounds were employed in this study. Their chemical structures are shown in the Figure. These compounds were obtained from the Department of Organic Chemistry at Baku State University, Azerbaijan [22].

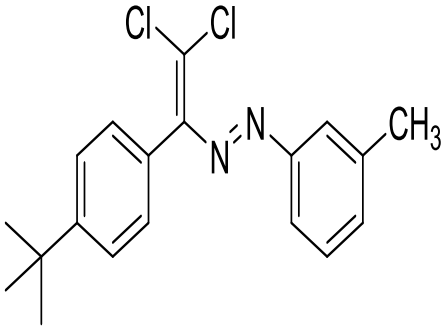


Compound I



Compound II





Compound III Compound IV

Fig. 1. Structure of (E)-1-(1-(4-(tert-butyl) phenyl)-2, 2-dichlorovinyl)-2-phenyldiazene (compound I), methyl (Z)-2-(-4-(tert-butyl) phenyl)-2-(2-(p-tolyl)hydrazineylidene) acetate (compound II), (E)-1-(1-(4-(tert-butyl) phenyl)-2, 2-dichlorovinyl)-2-(4-methoxy-phenyl)diazene (compound III) and (E)-1-(1-(4-(tert-butyl) phenyl)-2, 2-dichlorovinyl)-2-(m-tolyl) diazene (compound IV)

2.2. The fungal strains

All four synthesized compounds were tested against three yeast of the genus *Candida*: *Candida albicans* BDU MI-44, *Candida guilliermondii* BDU-217 and *Candida tropicalis* BDU LK-30. These microbial strains were obtained from the Research Laboratory of Microbiology and Virology at Baku State University in Azerbaijan [3, 11].

2.3. Agar well diffusion method

The antifungal activity of the synthesized compounds was determined using the agar well diffusion method at a 0.3% concentration. Due to its inert nature, dimethyl sulfoxide (DMSO) was chosen as the solvent to dissolve the compounds. To prepare a 0.3% solution, 0.003 g of each solid compound or 3 µL of each liquid compound was dissolved in 1 mL of DMSO. All fungal strains were grow on sabouraud dextrose agar (Liofilchem, Italy) with composition g/L (Glucose 40.0; enzymatic digestion of animal tissues 5.0; chloramphenicol 0.5; enzymatic digestion of casein 5.0; agar 15.0; pH 5.6±0.2 at 25 °C) [138]. Briefly, 150 µL of a 24-hour fresh broth culture (0.5 McFarland standard) of each fungus was aseptically spread over the surface of agar plates. Four wells, each 8 mm in diameter, were aseptically punched into the agar using sterile tips and labeled 1, 2, 3, and 4. Then, 100 µL of the respective test solutions were added to each well. The plates were incubated at 28 °C for 24 hours. After incubation, the diameters of the inhibition zones were measured using a graduated ruler. All experiments were performed in quadruplicate [2, 4, 5].

3. Results and discussion

The antifungal activity results of all four synthesized compounds were evaluated at a uniform concentration of 0.3%, and the results are summarized in the Table. The diameters of inhibition zones ranged from 11.0 mm to 24.1 mm, indicating a varied spectrum of antifungal efficacy among the tested compounds.

Table. Antifungal activity results of compounds against *candida* species

Fungal strains	***Diameter of inhibition zone (mm), M ± m***			
	I	**II**	**III**	**IV**
***Candida albicans* BDU MI-44**	19.0 ± 0.5	20.3 ± 0.6	17.0 ± 0.6	14.3 ± 0.5

***Candida guilliermondii* BDU-217**	11.0 ± 0.2	16.4 ± 0.3	11.2 ± 0.2	12.2 ± 0.2
***Candida tropicalis* BDU LK-30**	19.0 ± 0.5	24.1 ± 0.7	22.0 ± 0.6	22.0 ± 0.6

Compound I-(E)-1-(1-(4-(tert-butyl) phenyl)-2,2-dichlorovinyl)-2-phenyldiazene exhibited moderate to strong antifungal activity. It was particularly effective against *Candida albicans* BDU MI-44 and *Candida tropicalis* BDU LK-30, producing identical inhibition zones of 19.0 mm for both strains. This activity was 1.7 times greater than its effect on *Candida guilliermondii* BDU-217, which displayed a significantly smaller inhibition zone, suggesting a relatively lower susceptibility of this strain to compound I. This indicates that Compound I possesses selective antifungal properties with a preference for *C. albicans* and *C. tropicalis.*

Compound II-methyl (Z)-2-(4-(tert-butyl) phenyl)-2-(2-(p-tolyl) hydrazine ylidene) acetate demonstrated the most potent antifungal activity among the four compounds. The inhibition zone reached a maximum of 24.1 mm against *C. tropicalis* BDU LK-30, highlighting a strong fungicidal effect. The same compound was 1.2 times and 1.5 times more effective against *C. tropicalis* compared to *C. albicans* and *C. guilliermondii*, respectively. This suggests that Compound II exhibited a broad-spectrum antifungal profile with superior activity, especially against *C. tropicalis*, which appeared to be the most susceptible strain. Additionally, when comparing compound II with the other compounds against *C. albicans*, it showed increased activity being 1.1, 1.2, and 1.4 times more effective than compounds I, III, and IV, respectively. A similar trend was observed for *C. guilliermondii*, with compound II outperforming compounds IV, I, and III by factors of 1.3, 1.5, and 1.5, respectively.

Compound III-(E)-1-(1-(4-(tert-butyl) phenyl)-2,2-dichlorovinyl)-2-(4-methoxyphenyl) diazene showed its highest activity against *C. tropicalis*, yielding an inhibition zone of 22.0 mm. It was 1.3 and 2.0 times more effective on this strain than on *C. albicans* and *C. guilliermondii*, respectively, again underscoring the enhanced susceptibility of *C. tropicalis* to this class of compounds. However, in comparison to compound II, its overall antifungal activity was somewhat lower.

Compound IV-(E)-1-(1-(4-(tert-butyl) phenyl)-2,2-dichlorovinyl)-2-(m-tolyl) diazene also displayed relatively strong antifungal activity, particularly against *C. tropicalis* BDU LK-30, with a maximum inhibition zone of 22.0 mm. Its activity against *C. albicans* and *C. guilliermondii* was 1.5 and 1.8 times lower, respectively, suggesting that its antifungal efficacy, like the other compounds, was strain-dependent. When directly compared to compound II, it was 1.1 times less effective against *C. tropicalis*.

When comparing the performance of all tested compounds on each fungal strain. Against *C. albicans* BDU MI-44, Compound II was 1.1, 1.2, and 1.4 times more effective than Compounds I, III, and IV, respectively. For *C. guilliermondii* BDU-217, compound II showed enhanced activity, being 1.3, 1.5, and 1.5 times more effective than compounds IV, I, and III, respectively. Regarding *C. tropicalis* BDU LK-30, compound II again outperformed the others, with activities 1.1, 1.1, and 1.3 times higher than Compounds IV, III, and I, respectively.

4. Conclusion

Across all experimental observations, *Candida tropicalis* BDU LK-30 was identified as the most susceptible fungal strain, showing consistently larger zones of inhibition for all four compounds. Conversely, *Candida guilliermondii* BDU-217 emerged as the most resistant strain, particularly to compounds I and III. Among the tested compounds, methyl (Z)-2-(4-(tert-butyl) phenyl)-2-(2-(p-tolyl) hydrazineylidene)acetate proved to be the most broad-spectrum and potent antifungal agent, demonstrating superior activity against all three *Candida* species, with a notably strong effect on *C. tropicalis*. These results suggest that structural modifications, particularly involving hydrazineylidene acetate functionalities, significantly influence antifungal potency and strain selectivity.

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