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Study of antifungal activity of (Z)-2, 2', 4, 4', 5, 5'-Hexahydroxy stilbene

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Abstract

Stilbenes constitute a prominent class of plant-derived secondary metabolites belonging to the polyphenolic family and are widely distributed in dietary sources such as grapes, peanuts, and related foods. These compounds occur in two geometric isomeric forms, namely trans-stilbene (E-form) and cis-stilbene (Z-form), both of which contribute significantly to biological activity. Naturally occurring stilbene derivatives, including resveratrol, pterostilbene, and combretastatin A-4, have been extensively studied for their diverse pharmacological properties, such as antimicrobial, anticancer, anti-inflammatory, and neuroprotective effects, along with their roles in preventing cardiovascular diseases, Alzheimer's disease, and diabetes. Among these, cis-stilbenes exhibit unique structural and physicochemical characteristics that render them valuable scaffolds in photochemistry, materials science, and medicinal chemistry. Recent studies have highlighted the enhanced antimicrobial potential of stilbenoid compounds. In the present study, a novel cis-stilbene derivative, (Z)-2,2′,4,4′,5,5′-hexahydroxy stilbene, was successfully synthesized for the first time via a single-step transformation from its precursor, (Z)-2,2′,4,4′,5,5′-hexamethoxy stilbene. The synthesized compound was subjected to spectral characterization and evaluated for its fungicidal activity against selected fungal pathogens, demonstrating its potential as a promising antifungal agent.

Key words: Hydroxy stilbene, Antifungal, Disc diffusion method.

1. Introduction

Stilbenes are phenylpropanoid compounds characterized by a 1,2-diphenylethylene backbone and constitute a relatively small yet biologically significant subclass of phenylpropanoids. They are widely distributed in plant species and are known for their diverse and vital biological activities [1]. Most stilbene derivatives, collectively referred to as stilbenoids, are naturally occurring compounds that function as phytoalexins, playing a crucial role in plant defense mechanisms. Beyond their protective role in plants, stilbenoids have demonstrated the ability to disrupt microbial biofilms and attenuate virulence factors, highlighting their growing importance as antimicrobial agents. Consequently, these compounds have attracted considerable interest in medical research as potential alternatives or adjuncts to conventional antimicrobial therapies, particularly in the context of increasing drug resistance. In agriculture, stilbenoids offer a sustainable and environmentally friendly alternative to synthetic pesticides due to their efficacy and reduced ecological impact [2].

Among naturally occurring stilbenoids, trans-resveratrol, found in grapes, berries, and peanuts, is one of the most extensively studied compounds. It exhibits notable antifungal activity, particularly against Botrytis cinerea, a fungal pathogen responsible for gray mold in fruits. This activity is primarily attributed to its ability to disrupt fungal cell membranes and inhibit key metabolic enzymes [3]. Structurally, stilbenes exist as two diastereoisomeric forms, namely E- (trans) and Z- (cis) isomers, which can interconvert through E/Z isomerization. Although the trans isomer is generally more stable and widely investigated, recent studies have increasingly focused on cis-stilbene derivatives due to their unique geometry, where both phenyl rings reside on the same side of the double bond. This configuration significantly influences their chemical behavior, stability, and biological properties, thereby positioning cis-stilbenes as valuable scaffolds in photochemistry, materials science, and medicinal chemistry [4]. Given these promising attributes, considerable efforts have been directed toward the synthesis of stilbene derivatives with enhanced biological activity. Synthetic strategies allow precise structural modification, including the introduction of hydroxyl groups, which are known to significantly improve antifungal efficacy [5]. Hydroxylation enhances interactions with fungal cell membranes, leading to increased permeability and cellular damage. Additionally, hydroxylated stilbenoids can inhibit fungal enzymes and induce oxidative stress through the generation of reactive oxygen species (ROS), thereby contributing to fungal cell death [6].

In view of the biological significance of hydroxylated stilbenes, the present study focuses on the development of a new hydroxylated stilbene derivative of E-geometry synthesized in a single step from the corresponding methoxy stilbene. The synthesized compound was structurally characterized using FT-IR, ^{1}H and ^{13}C NMR, mass spectrometry, and X-ray crystallographic analysis [7]. Furthermore, its biological potential was investigated through density functional theory (DFT) studies followed by molecular docking to examine interactions with a fungal protein, providing insights into its binding affinity, specificity, and possible antifungal mechanism of action.

2. Materials and methods

2.1. General methods

All chemicals and solvents were of LR and analytical grade, respectively. TLC was performed on silica gel 60 F254 plates (Merck). Analytical HPLC was carried out using an Atlantis T3 column with acetonitrile–water (50:50) as the mobile phase and UV detection at 290 nm. Column chromatography was performed on silica gel using ethyl acetate–hexane as the eluent.

2.2. Preparation of (Z) - 2, 2', 4, 4', 5, 5'- Hexahydroxy stilbene

The synthetic scheme carried out as described in Figure 1, to a solution of TEA (triethyl amine) in chlorobenezene under nitrogen atmosphere at 0-5°C was added anhydrous $AlCl_3$ in small portions over 30 min. The obtained reaction mixture was then maintained at ambient temperature for 30 minutes with continuous stirring. Slowly raise the temperature to 60° C and maintained at same temperature for another 1hour. Added (*E*) - 2, 2', 4, 4', 5, 5'- Hexamethoxy stilbene [8] in chlorobenezene at 60-65° C in 30 minutes. The reaction mixture was cooled to room temperature and quenched into ice water.

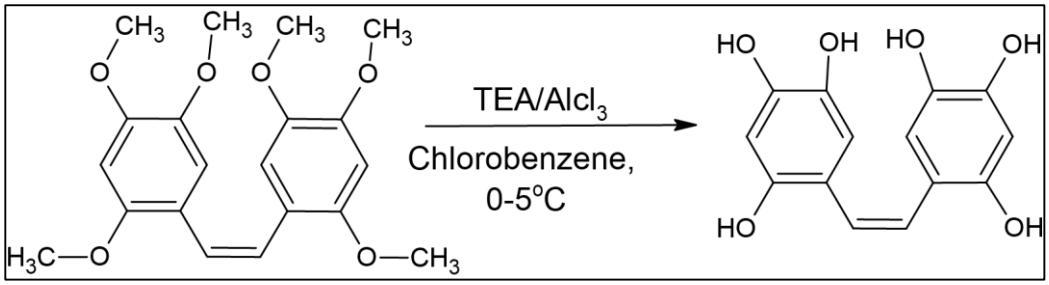

Figure 1: Synthesis of (Z)-2, 2', 4, 4', 5, 5'-Hexahydroxy stilbene

3. Results and Discussion

3.1 Biological evaluation of 2, 2', 4, 4', 5, 5'-hexahydroxy stilbene

Screening for antifungal target of selected ligand: The three-dimensional (3D) crystal structure of *Candida albicans* (PDB ID: 4HOE) was obtained from the **Protein Data Bank (PDB)** (https://www.rcsb.org/structure/4HOE). The structure was prepared using the Protein Preparation Wizard in the Schrödinger Suite (Maestro v.2023). The PDB ID: 4HOE structure was imported and refined by assigning bond orders, optimizing the hydrogen-bonding network, and adding missing hydrogen atoms. Unnecessary water molecules beyond 5 Å from the binding pocket were deleted, while the metal ion was retained for maintaining the enzyme's structural integrity. The structure was minimized using the Optimized Potentials for Liquid Simulations (OPLS4) force field, ensuring proper geometry and energy stabilization. The active site was identified based on the heme-binding region, where the native ligand and essential catalytic residues were preserved for docking studies.

3.1.1 Antifungal activity

The antifungal potential of synthesized compounds was assessed using *Candida albicans*, a human pathogenic fungus. The microorganism samples were obtained from a recognized Microbial Type Culture Collection and Gene Bank (MTCC). Antifungal activity was evaluated using the agar dilution method within a concentration range of 50 to 200 µg / mL and the minimal inhibitory concentration (MIC) of each compound was determined,[8] 5 grams of Itraconazole served as a reference antifungal agent.

The MIC values were established for yeast isolates that exhibited sensitivity in the disc diffusion assay. The inoculum was prepared from 12-hour broth cultures of *Candida albicans*. The test compounds were initially dissolved in ethanol and diluted to a maximum concentration of 200 µg /mL; Serial two-fold dilutions were performed to achieve concentrations ranging from 50, 100, 150, and 200 µg /mL; The MIC values were determined using the disc diffusion method on a Potato Dextrose Agar (PDA) plate named P1.

3.1.2 Antifungal activity by disc diffusion method

The disc diffusion assay of 2, 2', 4, 4', 5, 5'-Hexahydroxy stilbene against *Candida albicans* shows the approaching effectiveness at the concentration ranging from 200µg/ml - 1000 µg/ml with zone of inhibition 0.8mm - 18.2mm, respectively. Itraconazole (10 µg/ml) is used as a standard with a zone of inhibition 16.1mm, as shown in Figure 2 & Table 1.

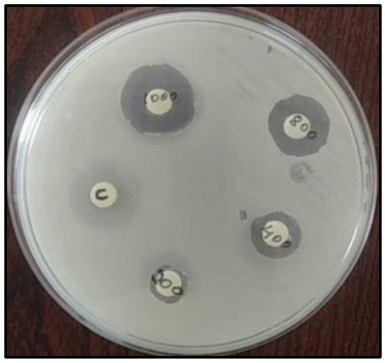

Figure 2: The inhibition of *Candida albicans* treated with 2, 2', 4, 4', 5, 5'-Hexahydroxystilbene at varying concentrations

Table 1: Minimum inhibition concentration values

Compound	**Concentration (**µg/ml)	***Candida albicans*** **(**mm)
2, 2', 4, 4', 5, 5'-Hexahydroxystilbene	200	0.8
	400	2.4
	800	15.1
	1000	18.2
Itraconazole	10	16.1

A comparison study[9] on benzo [4,5] imidazole [1,2-d] [1,2,4] triazine derivatives shown significant antifungal activity. Primarily due to their inhibition of ergosterol biosynthesis, which disrupts fungal cell membrane integrity. Similarly, our compound, 2, 2', 4, 4', 5, 5'-Hexahydroxy stilbene, exhibits strong antifungal properties, but its mechanism of action is distinct. The presence of multiple hydroxyl groups in Hexahydroxy stilbene plays a critical role in generating reactive oxygen species (ROS). These ROS induce oxidative stress and trigger apoptosis in fungal cells, contributing to the compound's antifungal efficacy [10].

In addition to ROS generation, the hydroxyl groups in the targeted compound interact with the fungal membrane causing destabilization of the lipid bilayers. This leads to ion leakage and membrane disruption, which are key factors in its antifungal activity [11]. Such dual-action mechanisms involving both oxidative stress and membrane disruption are essential to the compound's effectiveness against fungal pathogens.

Moreover, the structure of 2,2',4,4',5,5'-Hexahydroxy stilbene shows similarities to other antifungal compounds, such as the benzo[4,5]imidazo[1,2-d][1,2,4]triazine derivatives [12]. Both classes of compounds share the ability to disrupt fungal membranes and inhibit critical biosynthetic pathways. However, the hexahydroxylation of stilbene enhances its hydrophilicity, improving the compound's ability to diffuse into fungal cells more efficiently. This increased solubility contributes to its heightened antifungal activity and bioavailability.

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4. Conclusion

The current work underscores the importance of synthetic stilbene derivatives as a promising class of antifungal agents. By combining advanced synthesis and characterization techniques with computational approaches such as molecular docking and biological activity, we aim to contribute to the on-going search for effective and sustainable solutions to combat fungal pathogens. The FTIR analysis confirms the structural features of 2, 2', 4, 4', 5, 5'-Hexahydroxy stilbene, which align with its antifungal activity. The XRD analysis confirms the successful synthesis of highly crystalline 2, 2', 4, 4', 5, 5'-Hexahydroxy stilbene. This research represents a significant step towards addressing the challenges posed by fungal infections, particularly in the face of rising resistance to conventional antifungal therapies.

In conclusion, the antifungal potential of 2, 2', 4, 4', 5, 5'-Hexahydroxy stilbene is attributed to its polyphenolic structure, which facilitates ROS generation and membrane destabilization. Its ability to disrupt fungal cell membranes and induce oxidative stress makes it a valuable candidate for further research into effective antifungal therapies. Further experimental validation, including in-vitro and in-vivo studies, is warranted to confirm these findings and to explore the therapeutic potential of hydroxy stilbene in antifungal applications.

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