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Synthesis and Evaluation of Antifungal Properties of 2-((4-((6phenylimidazo[2,1-b][1,3,4]thiadiazole-2-yl)methoxy)phenoxy)methyl)-6-phenylimidazo[2,1-b][1,3,4] thiadiazole Derived from Hydroquinone

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Abstract

Thiadiazole, a heterocyclic core, has garnered significant interest from chemists seeking novel therapeutic agents. The synthetic route for the target compounds is depicted in Scheme 1. The compounds were synthesized with favorable yields through the condensation of hydroquinone with various organic reagents. They underwent physical characterization and were analyzed using spectroscopic methods, including infrared (FTIR) and NMR (1H, 13C) spectroscopy. The FT-IR spectrum of the product exhibited a ν(C=N) absorption band at 1580 cm^{-1}, indicative of the thiadiazole ring and confirming successful cyclization. The synthesized compounds were assessed in vitro for their antifungal activity against three plant pathogenic fungi—Helminthosporium tetramera, Aspergillus flavus, and Penicillium decombens—at two distinct concentrations, employing the paper-disc plate method as outlined by Thornberry, with standard potato dextrose agar medium. The prepared agar and petri dishes were sterilized via autoclaving at 121°C for 15 minutes. The agar plates were evenly inoculated using broth cultures of the test microorganisms. After solidification, wells with a diameter of 6 mm were created in the medium, and each well received 100 µl of the test compounds (1 mg of compound in 1 ml of DMSO). Dimethyl sulfoxide (DMSO) was used as the control solvent. The plates were incubated at 37 °C for 24 hours, after which the zones of inhibition were measured and recorded in millimetres. The synthesized compound demonstrated efficacy against all tested fungi at both concentrations. This research further investigates the biological properties of thiadiazoles, providing a succinct overview to aid researchers in expanding the utilization of these compounds in medicinal and coordination chemistry, and other relevant areas.

Key terms: thiadiazole, Helminthosporium tetramera, Aspergillus flavus, Penicillium decombens, Heterocyclic compounds, antifungal, Hydroquinone

Introduction:

Among the reported four isomeric thiadiazole structures, the results in the present review article

declared that most of inhibitory acitvities are attributed to the 1,3,4-thiadiazole one which is also involved in a number of antibiotic and antitumor drugs that are commercially available currently in the market (Figure 1).

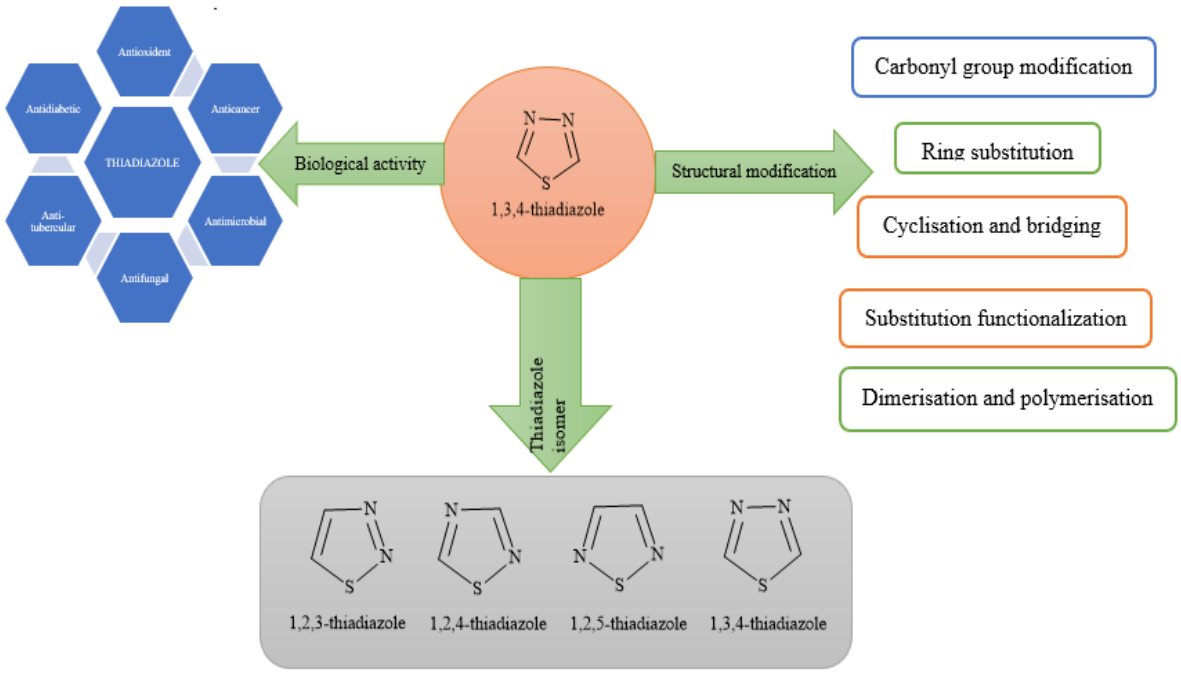

Figure 1: Graphical abstract representing structural modification, structural isomers and biological activities of thiadiazole.

Fungal infections represent a growing global health challenge, particularly among immunocompromised populations, and are associated with significant morbidity and mortality. Opportunistic fungal pathogens such as *Candida*, *Aspergillus*, and *Cryptococcus* species are responsible for a wide range of superficial and invasive infections. The growing number of these infections, combined with a lack of effective treatments, emphasizes the pressing requirement for better ways to fight fungal diseases. While several types of antifungal medications exist, such as azoles, polyenes, and echinocandins, their usefulness in practice is often reduced by the development of drug-resistant fungi, toxic side effects that limit dosage, poor targeting of fungi, and undesirable effects on how the body processes the drug. In addition, long-term antifungal treatment frequently results in side effects and interactions with other medications, further limiting their extended use. These limitations emphasize the need to constantly search for new antifungal structures that are more effective, safer, and less prone to resistance.

Heterocyclic compounds are essential in modern drug development because of their structural flexibility and ability to effectively interact with a wide range of biological targets. A significant percentage of drugs approved by the FDA contain heterocyclic elements, demonstrating their importance in improving molecular recognition, bioavailability, and metabolic stability. The inclusion of heteroatoms like nitrogen and sulfur in these structures often improves binding strength and adjusts key physicochemical properties related to biological activity.

Among heterocyclic systems, thiadiazole derivatives have gained significant attention as valuable structures in medicinal chemistry. Thiadiazole is a five-membered heterocyclic ring that consists of two nitrogen atoms and one sulfur atom. The presence of both nitrogen and sulfur atoms gives it unique electronic properties, enabling strong interactions with various biological targets like enzymes, receptors, and nucleic acidsOne of the major reasons for the extensive research on thiadiazole derivatives is their broad spectrum of biological activities. Thiadiazole-based compounds have been reported to exhibit antimicrobial, antifungal, anticancer, anti-inflammatory, antiviral, antitubercular, and antioxidant, activities[1-5]. The ability of a single heterocyclic nucleus to display such diverse pharmacological properties makes thiadiazole a privileged scaffold in drug discovery. Compared to other heterocyclic molecules, thiadiazole offers several advantages. The sulfur atom enhances lipophilicity, facilitating better membrane permeability, while the nitrogen atoms contribute to improved binding specificity through hydrogen bonding and coordination interactions. This synergistic effect often results in enhanced biological activity and binding affinity relative to many other heterocyclic systems

Another important advantage of thiadiazole is its metabolic stability. Many thiadiazole derivatives demonstrate strong resistance to metabolic breakdown, which enhances their pharmacokinetic characteristics and results in extended biological activity. In addition, several studies have reported high biological efficacy of thiadiazole derivatives with relatively low toxicity, making them suitable candidates for therapeutic development. Thiadiazole also provides excellent scope for structural modification. Various electron-donating and electron-withdrawing substituents can be easily introduced on the thiadiazole ring, enabling detailed structure–activity relationship (SAR) studies. Furthermore, the presence of nitrogen and sulfur atoms allows thiadiazole to form stable metal complexes, which often exhibit enhanced or

novel biological activities compared to the free ligands. This property further distinguishes thiadiazole from many other heterocyclic compounds.

In view of the increasing resistance to existing antifungal agents, the present work focuses on the development of organic thiadiazole derivatives and their in vitro antifungal evaluation using the zone of inhibition method.

Material and method

All chemicals, starting materials, and solvents utilized in this study were obtained from commercial suppliers and employed without any additional purification. The progress of the reactions was monitored using thin-layer chromatography (TLC). Fourier transform infrared (FTIR) spectra were recorded on a Perkin Elmer spectrometer within the range of 4000–400 cm^{-1}. Proton nuclear magnetic resonance (^1H NMR) spectra were measured using a 400 MHz spectrometer with d_6-DMSO as the solvent and tetramethylsilane (TMS) as the internal reference. The chemical shifts (δ) were expressed in parts per million (ppm) relative to TMS.

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The reaction mechanism is initiated by a nucleophilic attack in which the lone pair of electrons on the nitrogen atom of thiosemicarbazide attacks the sp^2 carbon of the carboxylic acid. This step forms an intermediate that subsequently undergoes dehydration. In the following stage, the lone pair on the sulfur atom attacks the carbonyl carbon, promoting cyclization of the molecule, after which the resulting intermediate again loses a molecule of water. Ultimately, electron rearrangement takes place, leading to the formation of the aromatic heterocyclic compound (Figure 2). The method reported by David Hoggarth in 1949 involved treating thiosemicarbazide derivatives with phosphoric acid to produce thiadiazoles [6-12].

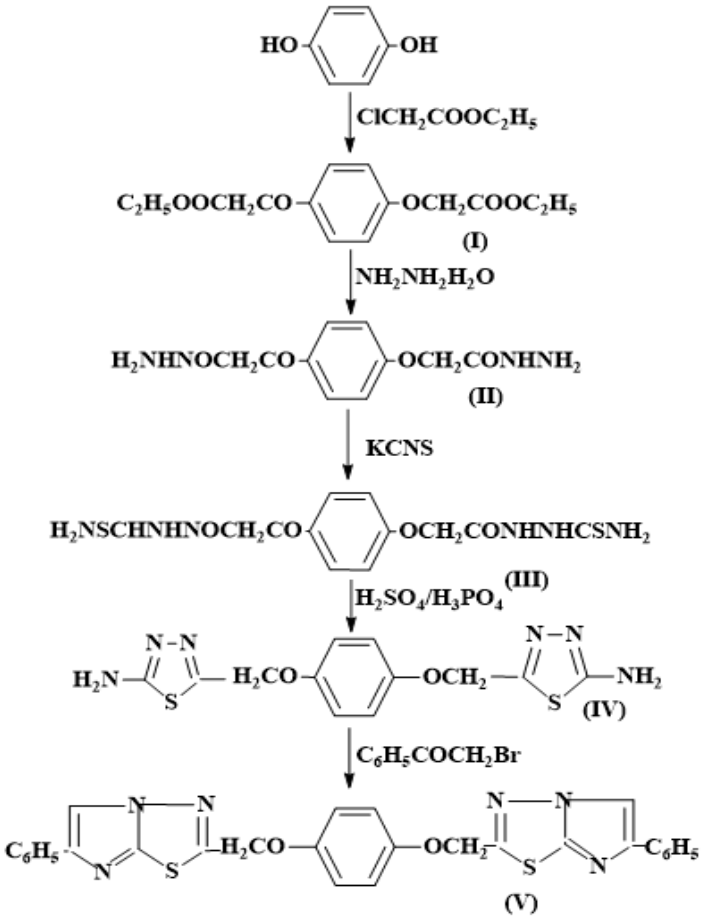

Figure 2: Synthesis of 2-((4-((6-phenylimidazo[2,1-b][1,3,4]thiadiazole-2-yl)methoxy)phenoxy)methyl)-6-phenylimidazo[2,1-b][1,3,4]thiadiazole.

This study details the synthesis of several hydroquinone derivatives, along with their characterization and antifungal activity.

Hydroquinone Diethyl Acetate (I):

Hydroquinone, chloroethyl acetate, and anhydrous potassium carbonate were mixed and heated. The mixture was occasionally shaken and then refluxed for 24 hours. After cooling, the reaction mixture was slowly added to ice-cold water. The precipitated residue was then collected by filtration, thoroughly washed with water, and recrystallized from ethanol, affording the product with a melting point of 72 °C and a yield of 92%.

Hydroquinone Diacetic Acid Hydrazide (II):

Hydroquinone diacetic acid hydrazide was prepared by refluxing hydroquinone with diethyl acetate in absolute alcohol until a homogeneous solution was obtained. Subsequently, hydrazine hydrate (98%) was added gradually in a dropwise manner, and the reaction mixture

was further refluxed for about 8 hours. The excess solvent was then eliminated through distillation under reduced pressure. The precipitated solid product was collected by filtration and purified through recrystallization from ethanol, producing the final compound with an 85% yield and a melting point of 225 °C.

Hydroquinone Diacetyl Bis(3-thiosemicarbazide) (III):

Hydroquinone diacetic acid hydrazide (1 mol), potassium thiocyanate (2 mol), concentrated hydrochloric acid (11.6 mL), and water (20 mL) were refluxed for 4 h. The reaction mixture was then poured into ice-cold water, leading to the formation of a precipitate, which was subsequently filtered, washed thoroughly, and dried. Recrystallization from glacial acetic acid yielded the product, with the molecular formula $C_{12}H_{16}N_6O_4S_2$ and a melting point greater than 250°C (64.42% yield). Elemental analysis confirmed the presence of sulfur (found: 17.12%, calculated: 17.20%) and nitrogen (found: 22.47%, calculated: 22.58%). The structure was further confirmed by IR and HNMR spectroscopy.

IR (KBr): Key absorptions were observed at 3210 cm-1 (N-H), 1680 cm-1 (-CO-NH-), 1190 cm-1 (C=S), and 820 cm-1 (1,4-disubstituted benzene).

HNMR (DMSO-d_6): Signals were observed at δ 6.75 ppm (singlet, 4H, Ar-H) and 4.5 ppm (singlet, 4H, 2 OCH2).

Bis-1,4-(5-amino-2-oxymethyl-1,3,4-thiadiazole) (IV):

Hydroquinone diacetyl bis(3-thiosemicarbazide) (0.01 mol) was gradually introduced into anhydrous phosphoric acid (20 mL) under continuous stirring over a period of 20 minutes. The flask was heated in an oil bath at 120 °C for 30 minutes, and the resulting slurry was poured into ice-cold water. The separated solid was filtered and recrystallized from ethanol, yielding the product with the molecular formula C12H12N6O2S2 and a melting point greater than 250°C (77.38% yield). Elemental analysis found S,19.13%; N, 28.86%, calculated S,28.86; N, 29%.

IR: At 3200 cm-1 (N-H), 1630 cm-1 (C=N, aromatic), 1580 cm-1 (C=C, aromatic), and 805 cm-1 (1,4-disubstituted benzene).

HNMR: Signals were observed at δ 6.75 ppm (singlet, 4H, Ar-H) and 4.45 ppm (singlet, 4H, 2 OCH2).

2-((4-((6-phenylimidazo[2,1-b][1,3,4]thiadiazole-2-yl)methoxy)phenoxy)methyl)-6-phenylimidazo[2,1-b][1,3,4]thiadiazole (V):

Bis-1,4-(5-amino-2-oxymethyl-1,3,4-thiadiazole) (0.005 mole) and phenacyl bromide (0.01 mole) were refluxed in a mixture of absolute alcohol and DMF (1:1) for 6 hours. The solvent was evaporated under reduced pressure, and the remaining residue was washed with ether followed by treatment with a sodium bicarbonate solution. After standing overnight, the resulting free base was filtered, washed, dried, and recrystallized from ethanol, yielding the product with the molecular formula C28H20N6O2S2 and a melting point of 200°C (46.76% yield). Elemental analysis found S,11.72%; N, 15.38%, calculated S,11.77; N, 15.45%.

IR: Key absorptions were observed at 1615 cm-1 (C=C, aromatic), 1580 cm-1 (C=N), and 810 cm-1 (1,4-disubstituted benzene).

Antifungal Activity:

The rising incidence of systemic fungal infections and the increasing prevalence of antimicrobial resistance, as highlighted by the World Health Organization, necessitate the development of new antifungal agents.

Results:

The synthesized compounds were evaluated in vitro for antifungal activity against Helminthosporium tetramera, Aspergillus flavus, and Penicillium decombens at two concentrations using the paper-disc plate method. Standard potato-dextrose-agar medium was used. Sterilized agar plates were inoculated with the test fungi. Wells (6 mm diameter) were created in the solidified medium and filled with 100 µL of the prepared compounds (1 mg/mL in DMSO). DMSO was used as a solvent control (Figure 3). The plates were incubated at 37°C for 24 hours, and the diameter of the resulting inhibition zones (mm) was measured (Table 1).

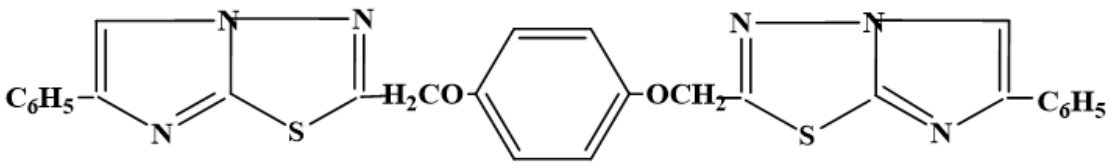

Figure 3: Structure of 2-((4-((6-phenylimidazo[2,1-b][1,3,4]thiadiazole-2-yl)methoxy)phenoxy)methyl)-6-phenylimidazo[2,1-b][1,3,4]thiadiazole.

	Diameter of zones of inhibition in mm for pathogenic fungi at concentration (%W/V)					
	Aspergillus flavus		Pennicillium decombens		Helmenthosporium tetramera	
	2	0.2	2	0.2	2	0.2
	16	–	–	–	16	–
Dithane Z-78	23	20	25	21	25	22
Thiram 75-W	30	26	27	22	28	24

Table 1: Antifungal activity of 2-((4-((6phenylimidazo[2,1-b][1,3,4]thiadiazole-2-yl)methoxy)phenoxy)methyl)-6-phenylimidazo[2,1-b][1,3,4]thiadiazole

It was also found that the synthesized compound was effective against all the test fungi. These compounds were found to be less effective in comparison to the reference fungicides dithane Z-78 and thiram 75-W.

Conclusion:

The aforementioned Scheme outlines the synthesis pathway for the target compounds. The cyclization of imine occurred via a reaction with H_2SO_4/H_3PO_4, resulting in the formation of thiadiazole. The FT-IR spectrum of compound (IV) displayed a ν(C=O) peak at 1700cm-1, attributed to the thiadiazole ring, serving as key evidence of successful cyclization. The synthesized compounds underwent in vitro screening for antifungal activity against three plant pathogenic fungi (Helminthosporium tetramera, Aspergillus flavus, and Penicillium decombens) at two concentrations using the paper-disc plate method. Results indicated the compounds' effectiveness against all tested fungi, albeit less so than the reference fungicides dithane Z-78 and thiram 75-W. This study investigated 1,3,4-thiadiazole derivative compounds with significant biological potency, systematically evaluating their effects through various in vitro assays. The findings highlight the 1,3,4-thiadiazole scaffold's critical role in enhancing biological activity. The exploration and creation of molecules with a 1,3,4-thiadiazole nucleus represent a compelling and promising avenue within medicinal chemistry. Pharmacophores incorporating this heterocyclic ring offer substantial potential for further structural modification and optimisation, leading to the development of innovative bioactive agents.

Future scope:

The findings of the present investigation provide a promising platform for the rational development of novel antifungal agents based on the imidazo[2,1-b][1,3,4]thiadiazole framework. Future research on this through substitution and functional group modification to enhance antifungal efficacy, selectivity, and physicochemical properties. Detailed structure–activity relationship (SAR) analyses will identify key molecular features governing biological activity. Evaluation against an expanded panel of fungal pathogens, including clinically relevant and drug-resistant strains, is warranted to establish the broader therapeutic potential of the synthesized compound.

Elucidation of the underlying mechanism of antifungal action using molecular, biochemical, and cell-based studies could provide valuable insight into its biological targets and pathways.

In vivo efficacy, safety assessment, and pharmacokinetic profiling represent essential next steps toward preclinical validation. Moreover, formulation strategies such as nano-carrier systems or advanced drug delivery approaches may be explored to improve solubility, stability, and bioavailability. Investigation of synergistic interactions with existing antifungal drugs could offer additional therapeutic advantages. Computational modeling and molecular docking studies may further support target identification and facilitate structure-guided drug design.

Overall, these future directions may contribute significantly to the development of effective antifungal therapeutics derived from this heterocyclic scaffold.

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