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1,3,4-Oxadiazole derivatives as potent antifungal agents: Synthesis, biological evaluation and an *in silico* study

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ABSTRACT

*Ten 1,3,4-oxadiazole derivatives were prepared and evaluated for their anti-fungal activities. The results showed that compounds **4a**, **7a**, and **7f** displayed activity against F. oxysporum. Molecular docking study indicated that compounds **4a**, **7a**, and **7f** exhibited affinity towards F. oxysporum's β-tubulin by showing low binding energies as well as interactions with the key amino acids in the binding sites of the receptor.*

1. INTRODUCTION

Phytopathogenic fungi and oomycetes have drawn attention because of their significant impact on agricultural production activities. (Sanzani et al., 2014). They can attack and harm a variety of important crops, including wheat, rice, pepper, rapeseed, potatoes, soybeans, blueberries, cucumbers, and corn (Xuan et al., 2006). The invasion of pathogens causes host plants to lose their natural metabolic balance, which hinders plant growth and causes catastrophic symptoms like etiolation, necrosis, and malformation (Zhang et al., 2014). This consequence has a significant impact on agricultural product productivity and quality, and it causes enormous yearly economic losses (MacLean et al., 2018). Currently, the application of fungicide agents remains an effective and economic strategy in crop protection (Gisi et al., 2008).

The 1,3,4-oxadiazole structural silhouette has been widely used to create biopharmaceutical substrates with a variety of biological effects and acceptable resistance profiles. The 1,3,4-oxadiazole's toxophoric moiety (N-C-O), accentuating its significance as a potential biodynamic molecule (Somani et al., 2011) has been extensively researched and reported for biological activities such as antibacterial (Bordei Telehoiu et al., 2020), antifungal (Song et al., 2021), and antiviral activities (Gan et al., 2017). The classical 1,3,4-oxadiazole derivatives, including oxadiazone and metoxadiazone, have been developed as the corresponding herbicides and insecticides (Yamamoto et al., 1993).

Based on the above findings and also to develop new potential antifungal agents, ten derivatives of 1,3,4-oxadiazoles and 1,3,4-oxadiazole/benzimidazole conjugates were prepared and evaluated for their antifungal activity. Molecular docking was conducted to clarify their activity.

2. MATERIALS AND METHOD

2.1. Materials

General procedures for the synthesis of the tested compounds can be found in the literature (Quy et al., 2022).

2.2. Antifungal bioassay

The antifungal efficacy of the tested compounds was evaluated under laboratory conditions (i*n vitro*) using the poisoned food technique. Antifungal activity was tested on Petri dishes with *Potato Dextrose Agar* (PDA) medium. The isolated and purified fungal strains were perforated with a punch tool with a diameter of 4 mm. Once performed, the canopy diameter was then placed on the PDA medium mixed with the test specimen. The tested sample was dissolved with Tween 0.05% and 2% methanol or 2% dimethyl sulfoxide (DMSO) at the test concentrations. The sample was then mixed into molten PDA at 50°C, sterilized and cooled. Each different concentration will be repeated 3 times on PDA medium. Fungal strains were cultured at 25°C after being placed on petri dish agar. Fungal growth was tracked over a span of 1 to 3 days. Fungal colonies were observed and their diameters were measured. The diameter of mycelial growth was measured and recorded when fungal colonies nearly reached the edge of the negative control dishes. The percentage inhibition of mycelial growth (%) for the tested samples was calculated by using the formula % inhibition = $100 \times [(A - B)/(A - 4)]$ where A is the diameter of the mycelial growth of fungus in the negative control dishes (mm), B is the diameter of mycelial growth of fungus in treated dishes (mm), and 4 is the diameter of PDA plug of fungal inoculum (mm).

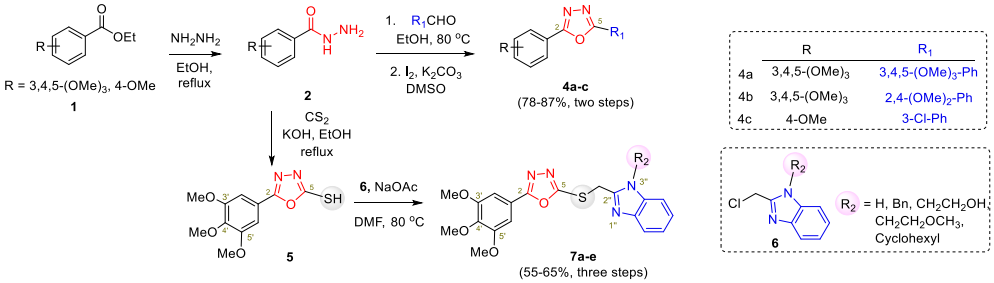
2.3. Molecular docking studies

The 3D structure of *F. oxysporum* β-tubulin has been reported (ID: 5ZXH) (Li et al., 2019; Borrego-Muoz et al., 2022). The ligands were optimized in Gauview, and the docking was done using LeadIT software. The center was selected at the co-crystallization ligand site and the spatial region was set at 7.5 Å. Other parameters were inserted by default. Images were processed with Discovery Studio. The re-docking process was performed, and the space was selected when RMSD < 2 Å.

3. RESULTS AND DISCUSSION

3.1. Synthesis of 1,3,4-oxadiazole derivatives

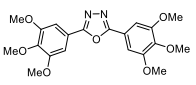
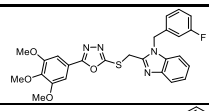
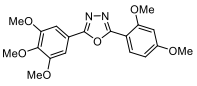
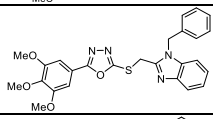
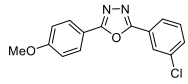
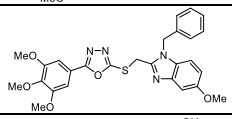
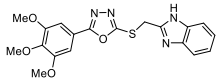
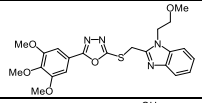
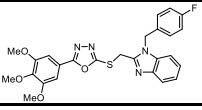
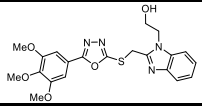
The synthetic pathway towards 1,3,4-oxadiazole derivatives (**4a-c**) is illustrated in Scheme 1 (Quy et al., 2022). In the first step of the synthetic sequence, the ester moiety of the commercially available substituted ethyl benzoates **1** was aminolyzed using hydrated hydrazine solution in ethanol to produce the corresponding benzohydrazides **2**. These intermediates were then reacted with various aromatic aldehydes (**3a-c**) in the presence of iodine as the oxidant and K_2CO_3 as the base to produce three different 1,3,4-oxadiazole derivatives (**4a-c**) in very good total yields (83-87%). Reliable spectroscopic data including IR, HR-MS and NMR firmly confirmed the structures of the synthesized compounds (Quy et al., 2022).



Scheme 1. Synthesis of 1,3,4-oxadiazole derivatives

The hybrid 1,3,4-oxadiazole/benzimidazole derivatives (**7a-f**) were synthesized by condensing benzohydrazide **2** with CS_2 using KOH as the base to accomplish the heterocycle 1,3,4-oxadiazole **5** with the thiol moiety attached at the C-5 position of the oxadiazole ring (Quy et al., 2022). The benzimidazole pharmacophore was then introduced into the molecule *via* a thiol ether linkage based on the nucleophilic substitution reaction with 2-(chloromethyl)benzimidazoles **6.** The reaction proceeded smoothly under mild conditions using sodium acetate as the base and DMSO as the solvent to afford the desired hybrid derivatives 1,3,4-oxadiazole/benzimidazole (**7a-f**) in rather good total yields (54-65%). Structural characterization was accomplished using the reliable spectroscopic data including IR, HR-MS and NMR (Quy et al., 2022).

Table 1. Synthesis of 1,3,4-oxadiazole compounds

Compd.	Structure	Total yield (%)	Compd.	Structure	Total yield (%)
4a		87	**7b**		60
4b		87	**7c**		56
4c		83	**7d**		62
7a		65	**7e**		62
7b		54	**7f**		55

3.2. Antifungal activity of 1,3,4-oxadiazole compounds against phytopathogenic fungi

The inhibition activity of the synthesized 1,3,4-oxadiazoles against phytopathogenic fungi was studied. *Fusarium oxysporum*, a typical fungus that is often present in the agro-ecosystem, was chosen for screening the anti-fungicide activity based on mycelial growth inhibitory bioassay. In a preliminary test, all tested compounds inhibited *F. oxysporum*, in which compounds **4a**, **7a**, and **7f** exerted clear inhibition after 2 days of treatment at the concentration ranging from 12.5 to 100 µg/mL and somewhat remained after 3 days (Figure 1). Interestingly, all the compounds inhibited this fungal strain in a dose-dependent manner (Figure 1 and Figure 2).

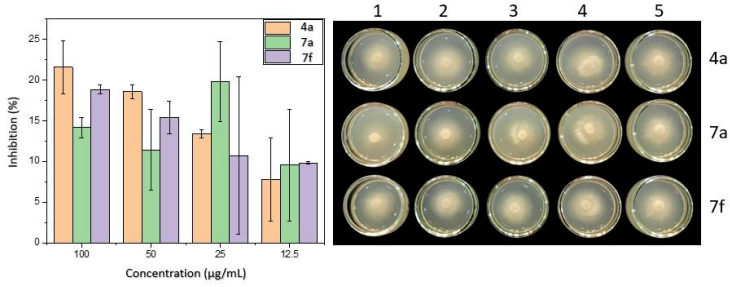


Figure 1. Inhibition of 4a, 7a, and 7f against Fusarium oxysporum after 2 days of treatment

Lane 1 = 100 µg/mL; 2 = 50 µg/mL; 3 = 25 µg/mL; 4 = 12.5 µg/mL; 5 = Negative control

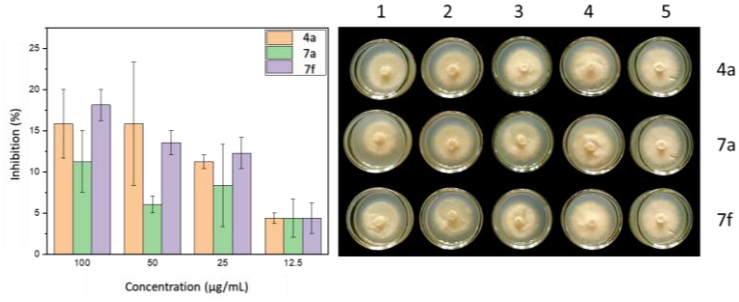


Figure 2. Inhibition of 4a, 7a, and 7f against *Fusarium oxysporum* after 3 days of treatment

Lane 1 = 100 µg/mL; 2 = 50 µg/mL; 3 = 25 µg/mL; 4 = 12.5 µg/mL; 5 = Negative control

When the concentration of 100 µg/mL was applied, all the tested compounds displayed moderate inhibition activity against *F. oxysporum*. Among them, compounds **4a**, **7a** and **7f** showed the inhibition activity after 3 days, with the control efficacies of 16.0%, 11.0%, and 18.0%, respectively. These initial results have suggested the potential uses of 1,3,4-oxadiazole containing compounds treating fungal diseases in plants. Further investigation of the inhibition activities of these compounds against *F. oxysporum* at higher concentrations has been continued in our lab in order to calculate their IC_{50} and IC_{90}. In addition, the mode of action of these compounds against *F. oxysporum* has also been conducted for designing more potent 1,3,4-oxadiazole-based antifungal agents in the future.

3.3. Docking results

Tubulins are the well-known target of clinically relevant fungicide. *β*-Tubulin polymerization inhibitors help induce mitotic arrest by binding to this enzyme. Carbendazim is a benzimidazole fungicide that inhibits microtubule formation by binding to free *β*-tubulin monomers (Zhang et al., 2020). Based on the structural similarity compared to carbendazim, the synthesized 1,3,4-oxadiazoles were expected to display inhibition activity toward *β*-tubulin. Therefore, the three active compounds in the *in vitro* test (**4a, 7a,** and **7f**) were docked into the *F. oxysporum β*-tubulin. Because the *β*-tubulin model of *F. oxysporum* has not been published in the literature yet, a structural model of *F. oxysporum β*-tubulin was employed (Borrego-Muoz et al., 2022). The docking results showed that compounds **7a** and **7f** containing the benzimidazole ring showed lower bonding energy than compound **4a** (-17.88, -14.83, -4.40 kJ/mol, respectively). This suggested that compound **4a** would probably target other receptors than *β*-tubulin. Whereas, the control compound MT189, which had the IC_{50} value of 3.41 µM toward this receptor in the *in vitro* assays (Li et al., 2019), showed the bonding energy of -21.88 kJ/mol. In addition, the benzimidazole ring exhibited stacking interactions with some key amino acids such as Cys239, Leu253, Ala248, and Ala314 and the 1,3,4-oxadiazole ring showed common features when interacting with the amino acid Leu246, Lys350 and Thr179. These key amino acids have been proven to be crucial in determining the activity of the ligand toward the examined *β*-tubulin target (Li et al., 2019; Zhang et al, 2020; Borrego-Muñoz et al., 2022). This result is very promising for the development of *β*-tubulin inhibition compounds based on the 1,3,4-oxadiazole/benzimidazole structure.

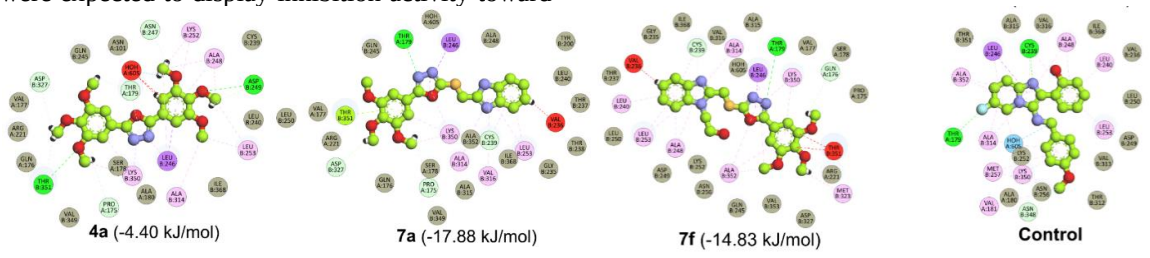


Figure 3. The structures of the docking poses of three ligand–*F. oxysporum β*-tubulin complexes

Relevant interactions are depicted by dashed line (H-bond: green; alkyl or Pi-alkyl: violet; carbon hydrogen: cyan; unfavorable bump: red)

4. CONCLUSION

Ten derivatives of 1,3,4-oxadiazoles **4a-c,** and **7a-f** have been synthesized and tested for anti-fungal activities. The compounds **4a, 7a** and **7f** inhibited the growth of *F. oxysporum* in the *in vitro* test. Molecular docking study revealed the affinity of compounds **4a, 7a,** and **7f** towards *F. oxysporum β*-tubulin, the well-known target of clinically relevant fungicide. These results show the potential of 1,3,4-oxadiazole heterocycle for the design and development of fungicides.

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