Synthesis and Activity Evaluation of New Benzofuran-1,3,4-Oxadiazole Hybrids Against Wood-Degrading Fungi

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A series of novel benzofuran-1,3,4-oxadiazole hybrids were synthesized and evaluated as antifungal agents. The synthetic pathway was started from salicylaldehyde, which afforded 5-(substituted benzylthio)-1,3,4-oxadiazole derivatives in moderate to good yields. The compounds were investigated for their antifungal potential against white-rot, *Trametes versicolor* and brown-rot, *Poria placenta* and *Coniophora puteana* fungi at different concentrations (500, 1000 ppm). The obtaining results demonstrated that most of the compounds at 500 ppm concentration did not exhibit acceptable antifungal effects but they had better antifungal activity at 1000 ppm concentration. Compounds **5a**, **5c**, and **5i** showed inhibition percentages of 14.6%, 23.0%, and 14.7%, against the growth of *P. placenta* and *C. puteana*, respectively. Among the compounds, the 2-(benzofuran-2-yl)-5-((2,6-difluorobenzyl)thio)-1,3,4-oxadiazole (**5h**) hybrid was the most active one.

Keywords: Oxadiazole; Synthesis; Antifungal activity; Wood-degrading fungi

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INTRODUCTION

Azoles, a class of five-membered heterocyclic compounds containing a nitrogenatom, are commonly found in molecules with interesting bioactivities. The excellent therapeutic activities of azole derivatives represent a vast number of synthetic compounds with a high therapeutic index and low toxicity to the library of bioactive agents (Karyotakis and Anaissie 1994). The beneficial features of azoles allow their use in the drug market, especially as antifungal agents, including fluconazole, itraconazole, posaconazole, ketoconazole, miconazole, and tioconazole. 1,3,4-Oxadiazole is an important member of the azole family (Khalilullah *et al*. 2012). It is incorporated in different bioactive compounds with a variety of biological functions including antifungal, potassium channel opener, antidepressant, analgesic, muscle relaxant, anticancer, antibacterial, and anticonvulsant (Maslat *et al*. 2002; Wang *et al*. 2006; Khanum *et al*. 2009; Ishii *et al*. 2011; Xu *et al*. 2011; Xu *et al*. 2012; Khalilullah *et al*. 2012; Subhashinia *et al*. 2017).

The –N=C-O structural unit in this heterocyclic core is responsible for most of the observed bioactivities (Yurttaş *et al*. 2017). Having been identified as an active scaffold in medicinal chemistry, many compounds incorporating the oxadiazole motif have been designed and synthesized to achieve more effective medicines. The 5-phenyl-2-furans are privileged building blocks for medicinal chemists, as anti-fungal agents (Owens 1959; Burch *et al*. 1980; Kupchik *et al*. 1982; Chen *et al*. 1991; Wei *et al*. 1992; Ye *et al*. 2004; Xue *et al*. 2004; Ke *et al*. 2005; Xue *et al*. 2008; Vedantham *et al*. 2008; Kort *et al*. 2008; Cui *et al*. 2012).

Brown rot fungi, *i.e*., *Coniophora puteana* and *Gloeophyllum trabeum*, produce two types of cellobiohydrolases (cellobiosedehydrogenase and endoglucanase) (Schmidhalter and Canevascini 1993; Hyde and Wood 1997) that depolymerize and metabolize the holocellulose (cellulose and hemicelluloses) fraction of wood (Green *et al*. 1991), leading to a rapid loss in wood strength in early stages of decay (Koenigs 1974; Illman *et al*. 1988; Blanchette *et al*. 1990; Eriksson *et al*. 1990; Daniel 1994). In artificial media, laccase production is observed in the brown rot fungi *G. trabeum* and *Postia placenta* (*Poria placenta*) (D'Souza *et al*. 1996). Lignin loss or metabolism of wood middle lamella by brown rot fungi occurs in later stages of degradation (Kim *et al*. 1991). Laccases penetrate the cell wall *via* the production of bore holes, passing through both cell wall and middle lamella regions, and consequently removes lignin (Jin *et al*. 1990). However, a white-rot fungus is capable of degrading all components of the wood cell wall during decay. In white-rot fungi cultures with high levels of phenol oxidase activity by phenol-oxidizing enzymes such as LiP, MnP, or laccase, there is a significant degradation of wood with preferential degradation of the lignin (Tanaka *et al*. 1985, 1986, 1999a). Phenol oxidase-activity in cultures of white-rot fungi is not necessarily correlated with the rate of wood degradation. Hydroxyl radicals (·OH) in combination with phenol oxidase may play a role in lignin degradation by white-rot fungi. *Trametes versicolor* and *Phanerochaete crysosporium* produce hydroxyl radicals in wood-degrading cultures in the redox reaction between electron donors and O_2 catalyzed by the low-molecular-weight glycopeptide (Tanaka *et al*. 1999b, 2000).

Molecular hybridization strategies in modern drug discovery may require the synthesis of bioactive heterocycles (Mahdavi *et al*. 2017; Pouramiri *et al*. 2017; Moghimi *et al*. 2018; Ayati *et al*. 2018a,b; Moradi *et al*. 2018). The use of conventional wood preservatives has been limited due to their health and environmental toxicity profile. Accordingly, to develop more effective and less toxic compounds, in this work new rigid forms of 5-phenyl-2-furans were designed and synthesized. Benzofuran was combined with 5-(substituted benzylthio)-1,3,4-oxadiazole. The compounds were evaluated for *in vitro* activity against white and brown-rot fungi including *T. versicolor*, *P. placenta*, and *C. puteana*.

EXPERIMENTAL

Materials

All chemical compounds were purchased from Merck (Darmstadt, Germany), Sigma-Aldrich (Darmstadt, Germany), and Acros Chemical (Schwerte, Germany) and used without further purification.

The progress of the reaction was monitored by thin layer chromatography (TLC) (silica gel 250 micron, F254 plates, Merck). Melting points were measured on a Kofler

hot stage apparatus (Munich, Germany). ^{1}H NMR spectra were recorded using Bruker 500 MHz instrument (Zurich, Switzerland). The chemical shifts (δ) and coupling constants (J) were presented in parts per million (ppm) and Hertz (Hz), respectively. Elemental analyses were performed with CHN-Rapid Heraeus Elemental Analyzer (Darmstadt, Germany). The results of the elemental analyses (C, H, N) were within ± 0.5% of the calculated values.

Synthesis of ethyl benzofuran-2-carboxylate **2**

A mixture of 2-hydroxybenzaldehyde **1** (0.05 mol), ethyl bromoacetate (0.05 mol), anhydrous potassium carbonate (0.075 mol) in dry dimethylformamide (DMF) (70 mL) was heated at 92 to 94 °C for 4 h. After this time, the mixture was poured into ice-water (100 mL). The resulting precipitate was filtered and washed with water (Abedinifar *et al*. 2018).

Synthesis of benzofuran-2-carbohydrazide **3**

Compound **2** (0.1 mol) was dissolved in ethanol (100 mL), and hydrazine hydrate (0.5 mol, 25 mL) was added to the solution. The mixture was stirred at room temperature for 12 h. The reaction was monitored by TLC and after completion, the solid was filtered, washed, and dried (Parekh *et al*. 2011).

Synthesis of 5-(benzofuran-2-yl)-1,3,4-oxadiazole-2-thiol **4**

In a 250 mL round bottom flask, benzofuran-2-carbohydrazide **3** (0.01 mol) was dissolved in absolute ethanol (10 mL). Carbon disulfide (0.03 mol) and the aqueous solution of potassium hydroxide (0.02 mol, 5 mL) were added to the solution. The reaction mixture was heated at reflux temperature for 6 h. After this time, the mixture was diluted with distilled water (50 mL) and acidified with hydrochloric acid to reach pH 1 to 2. The precipitate was filtered, washed with ethanol (96%), and dried under vacuum. The crude solid was recrystallized from ethanol to give pure product **4** (Saitoh *et al*. 2009).

General procedure for the synthesis of compounds **5a-i**

Benzofuran-1,3,4-oxadiazole-2-thiol **4** (1 mmol) was dissolved in the KOH solution (1.5 mmol in 0.1 mL H_2O). After 5 min, the corresponding benzyl halide (1 mmol) and absolute EtOH were added dropwise to the mixture with vigorous stirring. The reaction progress was monitored by TLC. After completion of the reaction, the mixture was poured into ice water, washed with ether, and dried under air. The crude product was recrystallized from EtOH to yield product **5** in good yield.

2-(Benzofuran-2-yl)-5-(benzylthio)-1,3,4-oxadiazole (**5a**)

Yield: 0.21 g (70%).- M. p. 129-131 °C.- ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ = 7.80 (d, J = 8.5 Hz, 1H, benzofuran), 7.79 (s, 1H, benzofuran), 7.76 (d, J = 8.5 Hz, 1H, benzofuran), 7.50-7.55 (m, 3H, phenyl), 7.38 (t, J = 7.3 Hz, 1H, benzofuran), 7.35-7.38 (m, 2H, phenyl), 7.29 (t, J = 7.3 Hz, 1H, benzofuran), 4.61 (s, 2H, CH_2) ppm.- Anal. Calcd (%). for $C_{17}H_{12}N_2O_2S$: C 66.22, H 3.92, N 9.08; found: C 66.45, H 3.77, N 9.26. ESI-MS *m/z*: 309.1 $[M+H]^+$.

2-(Benzofuran-2-yl)-5-((3-methoxybenzyl)thio)-1,3,4-oxadiazole (**5b**)

Yield: 0.25 g (75%).- M. p. 103-105 °C.- ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ = 7.82 (d, J = 8.4 Hz, 1H, benzofuran), 7.81 (s, 1H, benzofuran), 7.77 (d, J = 8.4

Hz, 1H, benzofuran), 7.51 (t, *J* = 7.5 Hz, 1H, benzofuran), 7.39 (t, *J* = 7.5 Hz, 1H, benzofuran), 7.31 (s, 1H, phenyl), 7.28 (d, *J* = 7.4 Hz, 1H, phenyl), 7.23 (t, *J* = 7.4 Hz, 1H, phenyl), 7.11 (d, *J* = 7.4 Hz, 1H, phenyl), 4.57 (s, 2H, CH_2), 3.71 (s, 3H, OCH_3) ppm.- Anal. Calcd (%). for $C_{18}H_{14}N_2O_3S$: C 63.89, H 4.17, N 8.28; found: C 63.67, H 4.32, N 8.45. ESI-MS *m/z*: 339.2 $[M+H]^+$.

2-(Benzofuran-2-yl)-5-((3-methylbenzyl)thio)-1,3,4-oxadiazole (**5c**)

Yield: 0.21 g (68%).- M. p. 106 °C.- ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ = 7.82 (d, J = 8.4 Hz, 1H, benzofuran), 7.81 (s, 1H, benzofuran), 7.78 (d, J = 8.4 Hz, 1H, benzofuran), 7.52 (t, J = 7.6 Hz, 1H, benzofuran), 7.40 (t, J = 7.6 Hz, 1H, benzofuran), 7.31 (s, 1H, phenyl), 7.29 (d, J = 7.5 Hz, 1H, phenyl), 7.24 (t, J = 7.5 Hz, 1H, phenyl), 7.11 (d, J = 7.5 Hz, 1H, phenyl), 4.57 (s, 2H, CH_2), 2.23 (s, 3H, CH_3) ppm.- Anal. Calcd (%). for $C_{18}H_{14}N_2O_2S$: C 67.06, H 4.38, N 8.69; found: C 67.29, H 4.52, N 8.91.

2-(Benzofuran-2-yl)-5-((2-fluorobenzyl)thio)-1,3,4-oxadiazole (**5d**)

Yield: 0.23 g (71%).- M. p. 126 °C.- ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ = 7.82 (d, J = 8.4 Hz, 1H, benzofuran), 7.80 (s, 1H, benzofuran), 7.77 (d, J = 8.4 Hz, 1H, benzofuran), 7.59 (t, J = 7.6 Hz, 1H, benzofuran), 7.52 (t, J = 7.6 Hz, 1H, benzofuran), 7.38-7.43 (m, 2H, phenyl), 7.19-7.22 (m, 2H, phenyl), 4.63 (s, 2H, CH_2) ppm.- Anal. Calcd (%). for $C_{17}H_{11}FN_2O_2S$: C 62.57, H 3.40, N 8.58; found: C 62.32, H 3.26, N 8.77. ESI-MS *m/z*: 327.0 $[M+H]^+$.

2-(Benzofuran-2-yl)-5-((3-chlorobenzyl)thio)-1,3,4-oxadiazole (**5e**)

Yield: 0.23 g (68%).- M. p. 119-121 °C.- ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ = 7.82 (d, J = 8.3 Hz, 1H, benzofuran), 7.80 (s, 1H, benzofuran), 7.77 (d, J = 8.3 Hz, 1H, benzofuran), 7.61 (s, 1H, phenyl), 7.47-7.50 (m, 2H, benzofuran), 7.38-7.40 (m, 3H, phenyl), 4.61 (s, 2H, CH_2) ppm.- Anal. Calcd (%). for $C_{17}H_{11}ClN_2O_2S$: C 59.56, H 3.23, N 8.17; found: C 59.22, H 3.41, N 8.35.

2-(Benzofuran-2-yl)-5-((4-chlorobenzyl)thio)-1,3,4-oxadiazole (**5f**)

Yield: 0.24 g (72%).- M. p. 139-141 ºC.- ^{1}H NMR (500 MHz, DMSO-d_6, 25 ºC, TMS): δ = 7.82 (d, J = 8.3 Hz, 1H, benzofuran), 7.81 (s, 1H, benzofuran), 7.78 (d, J = 8.3 Hz, 1H, benzofuran), 7.51-7.54 (m, 3H), 7.42-7.45 (m, 3H), 4.63 (s, 2H, CH_2) ppm.- Anal. Calcd (%). for $C_{17}H_{11}ClN_2O_2S$: C 59.56, H 3.23, N 8.17; found: C 59.31, H 2.97, N 8.38. ESI-MS *m/z*: 343.1 $[M+H]^+$.

2-(Benzofuran-2-yl)-5-((4-bromobenzyl)thio)-1,3,4-oxadiazole (**5g**)

Yield: 0.28 g (74%).- M. p. 155-157 °C.- ^{1}H NMR(500 MHz, DMSO-d_6, 25 °C, TMS): δ = 7.82 (d, J = 8.4 Hz, 1H, benzofuran), 7.80 (s, 1H, benzofuran), 7.77 (d, J = 8.4 Hz, 1H, benzofuran), 7.55 (d, J = 8.2 Hz, 2H, phenyl), 7.51 (t, J = 7.6 Hz, 1H, benzofuran), 7.46 (d, J = 8.2 Hz, 2H, phenyl), 7.39 (t, J = 7.6 Hz, 1H, benzofuran), 4.58 (s, 2H, CH_2) ppm.- Anal. Calcd (%). for $C_{17}H_{11}BrN_2O_2S$: C 52.73, H 2.86, N 7.23; found: C 52.58, H 2.65, N 7.46.

2-(Benzofuran-2-yl)-5-((2,6-difluorobenzyl)thio)-1,3,4-oxadiazole (**5h**)

Yield: 0.29 g (86%).- M. p. 106-108 °C.- ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ = 7.83 (d, J = 7.8 Hz, 1H, benzofuran), 7.79 (s, 1H, benzofuran), 7.77 (d, J = 7.8

Hz, 1H, benzofuran), 7.52 (t, J = 7.9 Hz, 1H, benzofuran), 7.45 (t, J = 7.9 Hz, 1H, benzofuran), 7.39 (t, J = 6.9 Hz, 1H, phenyl), 7.16 (t, J = 7.6 Hz, 2H, benzofuran), 4.60 (s, 2H, CH_2) ppm.- Anal. Calcd (%). for $C_{17}H_{10}F_2N_2O_2S$: C 59.30, H 2.93, N 8.14; found: C 59.51, H 2.75, N 8.41. ESI-MS *m/z*: 345.4 $[M+H]^+$.

2-(Benzofuran-2-yl)-5-((4-nitrobenzyl)thio)-1,3,4-oxadiazole (**5i**)

Yield: 0.22 g (67%).- M. p. >190 °C; .- ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ = 8.23 (d, 2H, benzofuran), 7.79 (m, 5H), 7.52 (t, J = 7.5 Hz, 1H, benzofuran), 7.39 (t, J = 7.5 Hz, 1H, benzofuran), 4.74 (s, 2H, CH_2) ppm.- Anal. Calcd (%). for $C_{17}H_{11}N_3O_4S$: C 57.78, H 3.14, N 11.89; found: C 57.95, H 3.00, N 11.59.

Antifungal Activity Assay

To evaluate the toxicity of target compounds, the work solution with 1000 ppm concentration was prepared with dissolving 12 mg of dry powder of synthetic fungicide into 12 mL of methanol. The solution was passed through a 0.45 μm Microsolve filter and poured into a glass vial. The media were sterilized in an autoclave at 120 °C. Approximately 25 mL of the media was poured into Petri plates, and 20 μL of solution was added at different concentrations (500 and 1000 ppm on four antibiogram discs as replicates for every concentration) to media containing malt extract agar (MEA) (48 g/L), which was poured into the one Petri plate.

The chemical synthetic fungicide ketoconazole (at 30 and 60 ppm concentrations) was used as the positive control to due to its inhibitory effects against the radial growth of 12 wood-degrading fungi such as *Schizophyllum commune* and *Pycnoporus sanguineus*. Methanol was used as the negative control to confirm that there was no inhibitory effect (Teoh *et al*. 2015).

The plates were cooled in a sterile hood and inoculated with 0.50 cm plugs of *Trametes versicolor*, *Poria placenta*, and *Coniophora puteana* fungi mycelia into the center of the Petri plate. Inoculated plates were incubated at 23 °C and 75% relative humidity without light. Four replicate antibiogram discs were used per treatment. The fungus was also grown on non-compound MEA (*i.e.*, with methanol) as a negative control. The fungal growth was monitored daily by measuring the percentage of area that was covered by fungus in the plates.

The percentage of fungal growth was plotted against the compound concentrations, and the toxic level was determined by the compound concentration at which the fungal growth was one day remaining to completely inhibited in similar to reported methods (Hosseinihashemi *et al*. 2016a,b).

The fungal growth (colony diameter) was measured and percentage inhibition was calculated according to the formula.

$$\text{Percentage inhibition} = [(C\text{-}T)/C] \times 100 \quad (1)$$

where *C* is the colony diameter (mm) of the negative control and *T* is the colony diameter (mm) of the test plate.

RESULTS AND DISCUSSION

Chemistry

The synthetic route toward the title compounds **5a-i** is outlined in Fig. 1. In the

first step, the cyclocondensation reaction between salicylaldehyde and ethyl bromoacetate was carried out by using DMF as the solvent at 92 to 94 °C (Abedinifar *et al*. 2018). Ethyl benzofuran-2-carboxylate **2** underwent a nucleophilic substitution reaction with hydrazine monohydrate at ambient temperature to produce compound **3** (Parekh *et al*. 2011). Treatment of acid hydrazide **3** and carbon disulfide in EtOH yielded the cyclized product 5-(benzofuran-2-yl)-1,3,4-oxadiazole-2-thiol **4** (Saitoh *et al*. 2009). The reaction of compound **4** and benzyl halide with KOH as a base led to *S*-benzylated targets in moderate to good yields (Fig. 2).

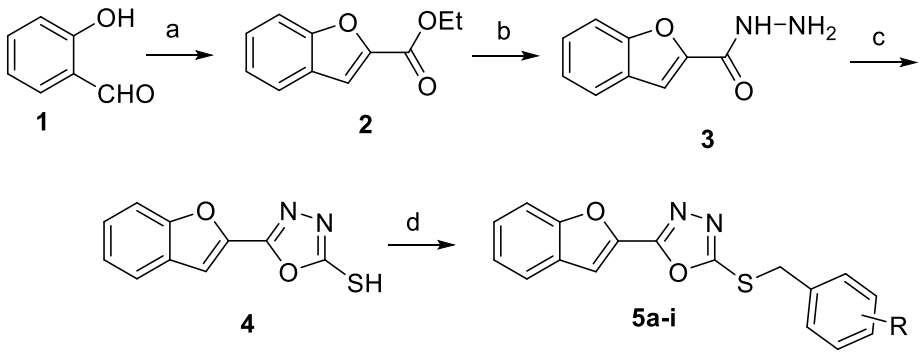

Fig. 1. Synthesis of compounds **5a-i**. Reagents and conditions: a) Bromoethylacetate, DMF, K_2CO_3, 92-94 ˚C, 4h; b) EtOH, hydrazine hydrate, r.t., 12h; c) CS_2, EtOH, KOH, reflux, 12h; d) EtOH, KOH, benzylhalides, r.t., 2h

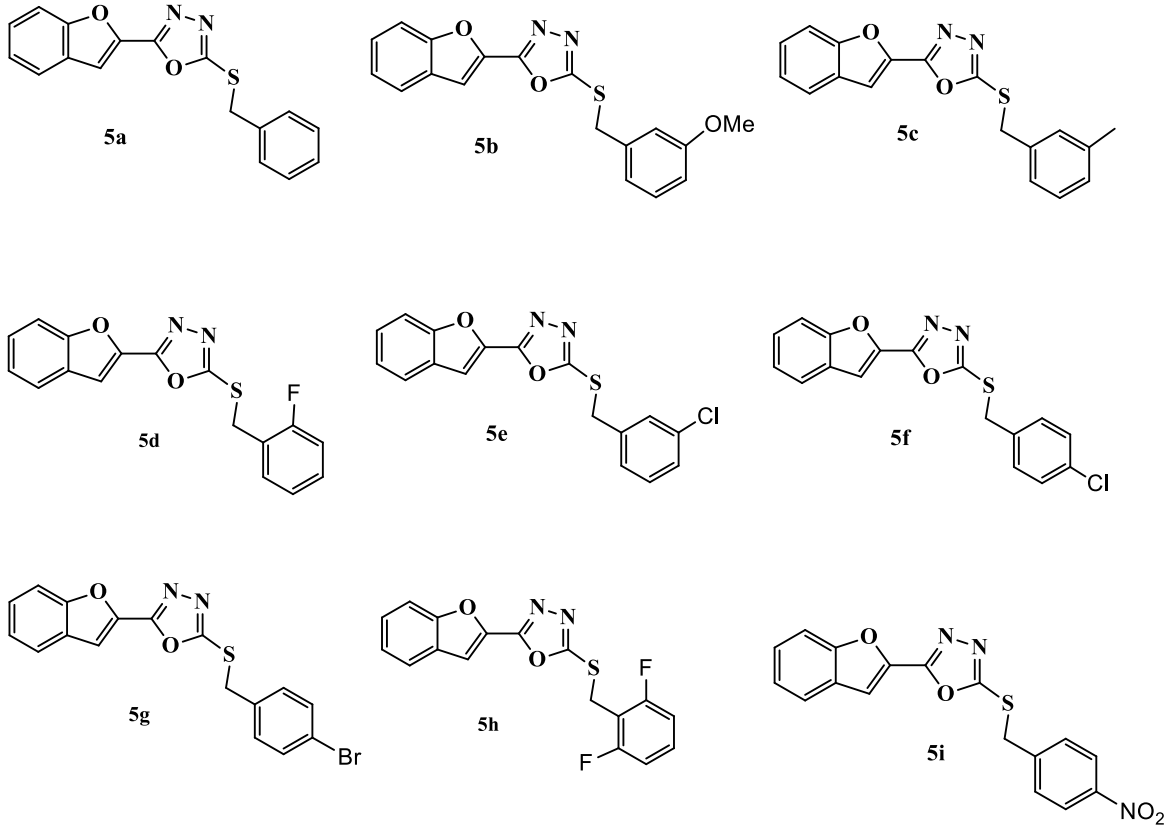

Fig. 2. Final compounds **5a-i**

Antifungal Activity of Target Compounds Against Fungi

The wood protection efficiency of the compounds **5a-i** was assessed by determining the percentage of mycelial growth inhibition against three wood-degrading fungi including *Trametes versicolor*, *Poria placenta*, and *Coniophora puteana* fungi at 500 and 1000 ppm. Ketoconazole and methanol were used as a positive and negative control, respectively.

According to the corresponding results (Table 1 and Fig. 3), most of the target compounds did not exhibit acceptable antifungal activity at 500 ppm. Compounds **5g** showed better antifungal activity at 500 ppm against *T. versicolor* with percentage inhibition of 7.01% in comparison to the positive control (6.37%). In addition, compounds **5d** and **5h** showed inhibition of 7.60%, and 6.38%, respectively, against *C. puteana* which were more effective than ketoconazole (-26.87%). At 1000 ppm, better antifungal activity was observed for most of the synthetic compounds. Particularly, compounds **5a**, **5c**, **5g**, **5h**, and **5i** exhibited inhibition of 14.61%, 23.04%, 12.36%, 17.95%, and 18.64%, respectively, against the growth of *P. placenta*, *T. versicolor*, and *C. puteana* fungi. Propiconazole (PCZ) as a representative synthetic fungicide from triazoles family and commonly preservative in wood protection has shown good antifungal property (Buschhaus and Valcke 1995), where exhibited inhibition of 49.33% against the growth of *T. versicolor* fungus at 450 ppm (Hosseinihashemi *et al*. 2016a).

Table 1. Mean ± Std. Values of Percentage of Mycelial Growth Inhibition of Three Wood-Degrading Fungi to Nine Synthetic Fungicide Solutions and Ketoconazole per 25 cm^3 of Malt Extract Agar (MEA) in One Day Prior to Complete Inhibition

Compound	*T. versicolor*		*P. placenta*		*C. puteana*	
5a	3.05 ± 2.34[a]	-10.90 ± 4.38[b]	3.18 ± 2.08[a]	14.61 ± 1.83[b]	-25.94 ± 1.12[a]	-5.95 ± 2.38[b]
5b	4.88 ± 1.99	1.92 ± 5.69	-1.91 ± 2.08	6.74 ± 2.90	4.76 ± 10.10	7.91 ± 6.49
5c	-1.83 ± 1.22	-12.82 ± 2.09	-5.10 ± 2.44	23.04 ± 1.13	-25.75 ± 1.29	-4.76 ± 4.76
5d	1.83 ± 1.22	5.13 ± 2.09	-5.74 ± 1.46	13.48 ± 3.89	7.60 ± 5.27	-26.95 ± 1.42
5e	0.61 ± 1.22	10.90 ± 2.46	-2.55 ± 1.28	10.11 ± 1.83	-29.85 ± 1.36	1.13 ± 3.38
5f	6.10 ± 3.15	1.92 ± 2.46	3.85 ± 3.20	-1.12 ± 0.00	-28.36 ± 1.22	2.26 ± 2.84
5g	2.44 ± 0.00	8.97 ± 2.57	7.01 ± 3.29	12.36 ± 3.18	-26.87 ± 2.65	0.00 ± 2.16
5h	6.71 ± 2.34	17.95 ± 2.09	-3.19 ± 1.47	7.87 ± 3.18	6.38 ± 4.01	18.64 ± 2.61
5i	0.00 ± 1.99	10.90 ± 2.46	2.55 ± 3.82	7.87 ± 0.00	-5.36 ± 3.57	14.69 ± 2.16
Ketoconazole (C(+))	14.03 ± 2.23	12.82 ± 2.09	6.37 ± 4.35	25.28 ± 2.83	-26.87 ± 6.22	2.26 ± 7.91
[a] Mean ± std. amounts at 500 ppm; [b] Mean ± std. amounts at 1000 ppm						

The introduction of a strong electron-donating group (methoxy group) resulted in better activity against *T. versicolor* and *C. puteana* in both concentrations. The presence of a methyl group provided similar activity to ketoconazole against *P. placenta* at 1000 ppm. No logical pattern was observed in the electron-withdrawing group. At 500 ppm,

changing the position of -Cl substitution from *meta* to *para* position led to more potent compounds, but this effect was not observed in 1000 ppm, which was related to the low solubility of the compound at this concentration. In compound **5g**, the presence of bromine, a less electronegative atom, resulted in better inhibition growth result against *P. placenta.*

The synthetic 1,3,4-oxadiazole derivatives showed moderate control over the growth of fungi rather than their positive control, ketoconazole, at 30 and 60 ppm concentrations. Among all synthetic compounds, 2-(benzofuran-2-yl)-5-((2,6-difluorobenzyl)thio)-1,3,4-oxadiazole (**5h)** was the most active antifungal agent against mycelium growth of all fungi.

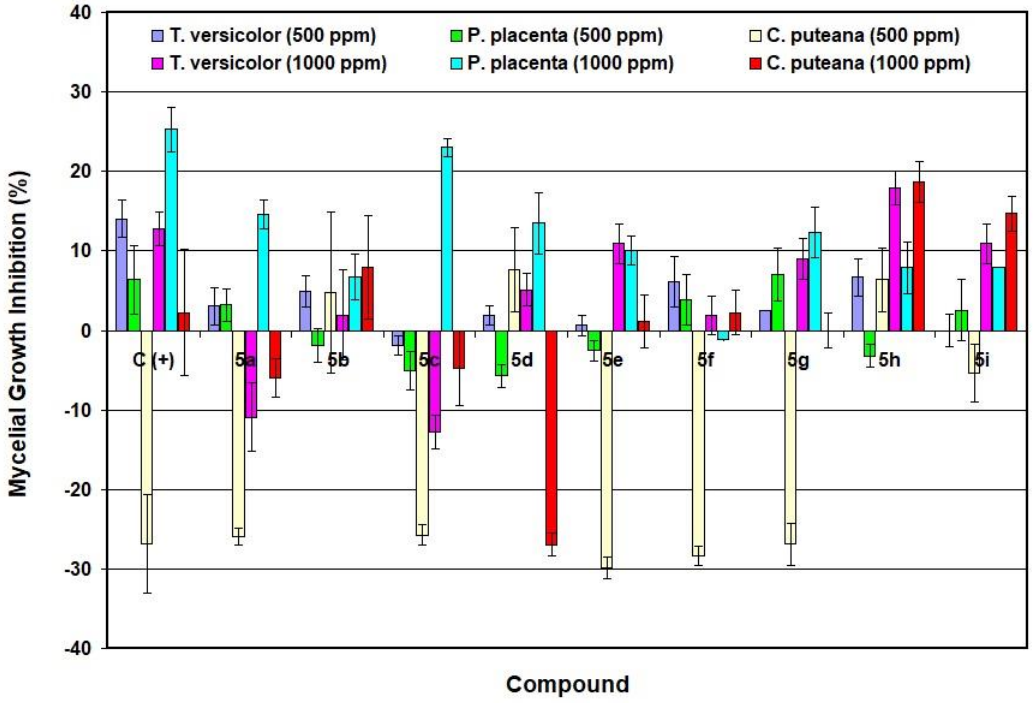

Fig. 3. Percentage of mycelial growth inhibition of synthetic fungicides

The results suggested that the substitution position on the benzyl ring affected the antifungal activity of all the synthesized compounds at 500 and 1000 ppm against the wood-degrading fungi. Furthermore, at higher concentrations of the test compound, there was more probable control. Due to the low activity of these compounds, further modification and investigation should be carried out to increase their wood preservation property.

CONCLUSIONS

The purpose of this study was the synthesis and evaluation of new benzofuran-1,3,4-oxadiazole hybrids as antifungal agents. The wood protection efficiency of compounds **5a-i** was evaluated against white- and brown-rot fungi *T. versicolor*, *P. placenta*, and *C. puteana* in different concentrations (500, 1000 ppm) using *in vitro* disk diffusion. The synthesized compounds with oxadiazole linkage at 1000 ppm concentration may help for further development of new wood preservatives against the

three wood rotting fungi. From the above results, **5c** showed the highest antifungal activity against *P. placenta* at 1000 ppm with mycelial growth inhibition of 23.04%, followed by **5h** at 1000 ppm (mycelial fungal growth with 18.64%).

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