Design and Synthesis of New Benzofuran-1,2,3-Triazole Hybrid Preservatives and the Evaluation of Their Antifungal Potential Against White and Brown-Rot Fungi

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A series of novel benzofuran-1,2,3-triazole hybrids were synthesized and investigated as fungicidal preservatives. The compounds were evaluated for their antifungal potential against white-rot (*Trametes versicolor*), dry brown-rot (*Poria placenta*), and wet brown-rot (*Coniophora puteana* and *Gloeophyllum trabeum*) fungi, at different concentrations (500 ppm and 1000 ppm). The tests of the final products (**8a**, **8b**, **8c**, **8d**, **8e**, **8f**, and **8g**) demonstrated that compound N-((1-(4-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)benzofuran-2-carboxamide (**8f**) at a concentration of 500 ppm was the most active against wet brown-rot *C. puteana* (23.86% inhibition) and *G. trabeum* (47.16% inhibition) fungi. However, testing demonstrated that compounds **8a**, **8b**, **8c**, **8d**, and **8g** at a concentration of 500 ppm did not exhibit acceptable antifungal effects against white-rot *T. versicolor* and dry brown-rot *P. placenta* fungi.

Keywords: Triazoles; Synthesis; Antifungal activity; White and brown-rot fungi

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INTRODUCTION

Carbamates, creosotes, isothiazolones, quaternary ammonium compounds, sulfamides, and triazoles are the main classes of metal-free organic fungicides used for wood protection (Reinprecht 2010). Many of these preservatives are effective against a specific class of microorganism; however, synergistic effects have been reported for a group of triazole fungicides, which help overcome this selectivity (Bauschhaus and Valcke 1995; Christen *et al.* 2014). Furthermore, triazoles have an added advantage of exhibiting high antifungal activity in both organic solvent- and water-based formulations (Bruns *et al.* 2005).

Triazole fungicides have great importance in agriculture and medicine and are commonly used in two categories: (1) triazole antifungal drugs, *e.g.*, fluconazole, voriconazole, isavuconazole, itraconazole, *etc*., and (2) triazole plant protection fungicides, *e.g.*, tebuconazole, triadimefon, triadimenol, paclobutrazol, and flutriafol. The two triazole ring isomers, 1,2,3-triazole and 1,2,4-triazole, are found in both plant protection fungicides and the others antifungal drugs, respectively (Carisse 2010).

Propiconazole, tebuconazole, and azaconazole are well known synthetic fungicides that are commonly used in commercial formulations for wood preservation

against all types of wood-rotting fungi (Wüstenhöfer *et al.* 1993). However, 1,2,4-triazoles have additional benefits of having a low toxicity to animals and being environmentally stable (Bakhsous *et al.* 2006). Propiconazole, tebuconazole, and chlorothalonile have shown inhibitory activity of 49.3%, 40.4%, and 7.1%, respectively, against the growth of *T. versicolor* fungus at 450 ppm (Hosseinihashemi *et al.* 2016a, Nazari and Hosseinihashemi 2017a; Nazari and Hosseinihashemi 2017b).

Shi *et al.* (2014) studied the synthesis and *in vitro* activity of 1-((benzofuran-2-yl)methyl)-1H triazole derivatives against a panel of five different human tumor cell lines and showed that benzotriazole, *i.e.*, a 1,2,3-triazole ring with a substitution of the triazolyl-3-position with a naphthylacyl, 4-bromophenacyl, or 4-methylbenzyl group, could be crucial for promoting cytotoxic activity. Likewise, some of the studied compounds were found most potent with selectively against HL-60, SMMC-7721, and MCF-7 cell lines, respective to the above substitutions, and in particular, compound 20 was selective against the HL-60 and A549 cell lines with IC_{50} values of 0.62 μM and 1.60 μM.

A series of benzofuran-triazole hybrids were designed and synthesized by Liang *et al.* (2016), and the *in vitro* antifungal activity of the final compounds were evaluated using the microdilution broth method against five strains of pathogenic fungi. This study indicated that the target compounds exhibited moderate to satisfactory activity.

Some representative 1,2,3-triazole derivatives with antifungal activity are shown in Fig. 1 (Liang *et al.* 2016).

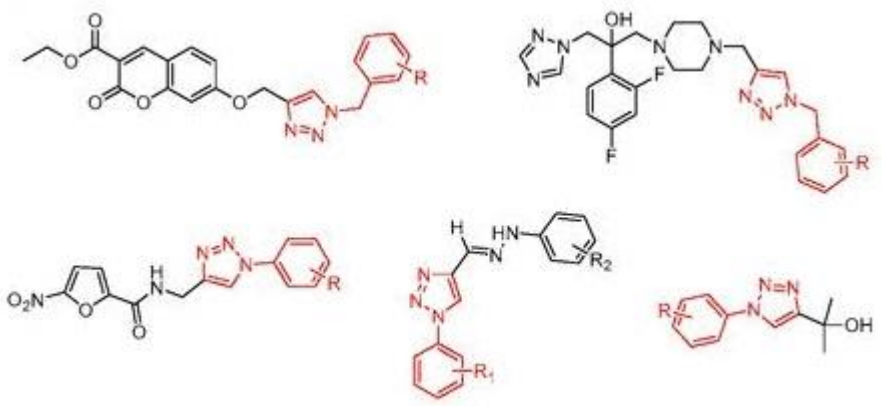

Fig. 1. Representative 1,2,3-triazole derivatives with antifungal activity

Recently, the 1,2,3-triazole scaffold became popular prominent fragment with the emergence of click chemistry. The 1,2,3-triazole-containing compounds have been reported to possess a variety of biological activities (Giffin *et al.* 2008; Weide *et al.* 2010; Xu *et al.* 2013; Kumar *et al.* 2014; Mohammadi-Khanaposhtani *et al.* 2015), especially as antifungal agents (Lima-Neto *et al.* 2012; Dai *et al.* 2015; Kamal *et al.* 2015). Furthermore, hybridizations of the 1,2,3-triazole moiety with other antifungal agents were reported (Giffin *et al.* 2008).

Reports by Hosseinihashemi *et al.* (2020), in regards to the effects of new benzofuran-1,3,4-oxadiazole hybrids on the inhibition of mycelial growth in wood-degrading fungi, showed 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 at a concentration of 1000 ppm showed

inhibition percentages of 14.6%, 23.0%, and 14.7%, respectively, against the growth of *P. placenta* and *C. puteana*. Among the tested compounds, 2-(benzofuran-2-yl)-5-((2,6-difluorobenzyl)thio)-1,3,4-oxadiazole (5h) hybrid was the most active one.

It is unclear whether such fungi (*P. placenta* and *C. puteana*) have a generic ability to degrade pesticides, and whether similar degradative abilities are ubiquitous among white-rot fungi (Bending *et al*. 2002).

Triazole fungicides, *e.g.*, propiconazole and tebuconazole, inhibit the C14 demethylation step in the fungal ergosterol biosynthesis as demethylation inhibitors (DMIs) and thereby interfere with the basic metabolism of the fungal cell walls and contents (Copping *et al.* 1984).

Encouraged by the above results, the authors attempted to design and synthesize a series of new benzofuran-1,2,3-triazole hybrids to evaluate the *in vitro* antifungal activity against wood-deterioration fungi.

EXPERIMENTAL

Preparation of Synthesized Materials

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

Synthesis of ethyl benzofuran-2-carboxylates (**2**)

A mixture of 2-hydroxybenzaldehydes (**1**) (0.05 mol), ethyl bromoacetate (0.05 mol), anhydrous potassium carbonate (0.075 mol), and dried dimethylformamide (DMF) (70 mL) was heated at 90 °C for 4 h. This solution was decanted into ice water and the precipitate was filtered off and washed with cold water to afford ethyl benzofuran-2-carboxylate as cream powder (yield: 75%; M. p. 32 °C).

Synthesis of benzofuran-2-carboxylic acids (**3**)

Ethyl-1-benzofuran-2-carboxylate (**2**) (1 mmol) containing ethanol was added to water (at a 2 to 1 ratio, 30 mL) and potassium hydroxide (2 mmol); this mixture was refluxed for 2 h. The reaction was monitored *via* thin layer chromatography (TLC). After the reaction was completed, the reaction mixture was decanted into ice water and extracted with ethyl acetate. The ethyl acetate layer was separated, dried, and evaporated under vacuum to yield benzofuran-2-carboxylic acid (**3**) as a white crystal (yield: 85%; M. p. 190 °C).

Synthesis of N-(prop-2-ynyl)benzofuran-2-carboxamides (**5**)

A mixture of benzofuran-2-carboxylic acid (**3**) (1.5 mmol), HOBt (1.7 mmol), and EDC.HCl (1.7 mmol) in dried acetonitrile (10 mL) was stirred for 30 min at ambient temperature. Propargylamine (1.8 mmol) was added and the mixture was stirred for 24 h to 48 h. After the reaction was completed (checked *via* TLC), the crude product was extracted with chloroform, a citric acid solution (10%), and sodium hydrogen carbonate (10%). The organic layer was separated, dried, and evaporated under a vacuum to yield N-(prop-2-ynyl)benzofuran-2-carboxamides as a cream powder (**5**) (yield: 78%, M. p. 160.1-164 °C.

General procedure for the synthesis of product **8a-g**

Triethylamine (1 mmol) and H_2O/t-BuOH (1:1) (5 mL) were added to a mixture of benzyl bromide (**6**) (1.3 mmol) and sodium azide (1 mmol) and stirred for 1 h at room temperature. After that, *N*-(prop-2-yn-1-yl)benzofuran-2-carboxamide (**5**) (1 mmol), sodium ascorbate, and $CuSO_4$.$5H_2O$ (7 mol%) were added to the reaction mixture and stirred at room temperature for 24 h to 48 h. Then, the reaction mixture was diluted with cold water and poured into crushed ice and precipitated products **8a**, **8b**, **8c**, **8d**, **8e**, and **8g** were filtered off, washed with water, and purified *via* recrystallization in ethanol.

The proposed procedure for the synthesis of designed compounds **8a** through **8g** is depicted in Fig. 2.

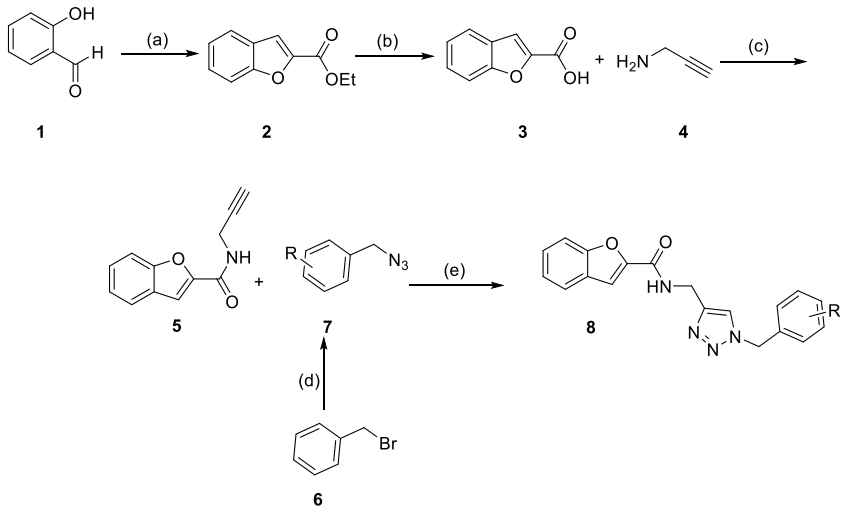

Fig. 2. Synthesis of benzofuran-1,2,3-triazole hybrids: (a) Ethyl bromoacetate, K_2CO_3, Dry DMF, 90 °C, 4 h to 6 h; (b) KOH, Ethanol: H_2O (2 to 1 ratio), reflux; (c) HOBT, EDC.HCl, dry CH_3CN, room temperature (r.t.), 24 h to 48 h; (d) NaN_3, NEt_3, H_2O/t-BuOH (1 to 1 ratio), 1 h, r.t.; and (e) CuSO4 .$5H_2O$, Sodium ascorbate, r.t., 24 h to 48 h

N-((1-(2-methylbenzyl)-1H-1,2,3-triazol-4-yl)methyl)benzofuran-2-carboxamide (**8a**)

White powder; 0.298 g, Yield: (86%); M. p. 147-150 ºC. IR (KBr) (ν_{max}/cm^{-1}): 3421 (NH), 1659 (CO). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.30 (3H, s, Me) , 4.75 (2H, bs), 5.51 (2H, s), 7.15 (1H, bs) , 7.21 (2H, d, J = 6 Hz), 7.26 to 7.28 (2H, m), 7.39 (2H, t, J = 6 Hz), 7.44 to 7.47 (2H, m), 7.64 (1H, d, J = 6 Hz). ^{13}C NMR (125 MHz, DMSO-d_6): δ 18.60, 34.34, 50.80, 109.44, 109.57, 111.73, 122.71, 123.05, 123.19, 123.71, 126.17, 126.85, 128.25, 128.69, 130.35, 136.27, 144.79, 148.88, 154.17, and 158.05. Combustion elemental analysis (Anal.) calculated (Calcd) for $C_{20}H_{18}N_4O_2$: C, 69.35; H, 5.24; N, 16.17. Found: C, 69.48; H, 5.51; N, 16.04.

N-((1-(2-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)benzofuran-2-carboxamide (**8b**)

White powder; 0.319 g, Yield: (87%); M. p. 149-152 ºC. IR (KBr) (ν_{max}/cm^{-1}): 3417 (NH), 1656 (CO). ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.79 (2H, bs) , 5.67 (2H s) , 7.20 (1H, bs), 7.26 to 7.33 (3H, m) , 7.40 (1H, s), 7.43 (1H, t, J = 7.5 Hz), 7.47 (1H, d, J = 5.8 Hz), 7.49 (1H, s), 7.80 (1H, bs, NH-). ^{13}C NMR (125 MHz, DMSO-d_6): δ 34.69, 51.43, 110.54, 111.78, 122.62, 123.64, 126.93, 127.43, 127.55, 129.92, 130.26, 130.36, 132.21, 144.63, 148.32, 154.72, and 158.84. EI-MS, *m/z*: 366.2 (Calcd for $C_{19}H_{15}ClN_4O_2$:

366.81). Anal. Calcd for $C_{19}H_{15}ClN_4O_2$: C, 62.21; H, 4.12; N, 15.27. Found: C, 62.13; H, 4.02; N, 15.46.

N-((1-(3-methylbenzyl)-1H-1,2,3-triazol-4-yl)methyl)benzofuran-2-carboxamide (**8c**)

White powder; 0.283 g, Yield: 82%; M. p. 174.5-176.3 ºC. IR (KBr) (ν_{max}/cm^{-1}): 3420 (NH), 1655 (CO). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.32 (3H, s, Me), 4.75 (2H, bs), 5.46 (2H, s), 7.07 to 7.09 (2H, m), 7.15 (1H, d, *J* = 8 Hz), 7.23-7.28 (2H, m), 7.38 (1H, t, *J* = 7.5 Hz), 7,44 (1H, d, *J* = 6 Hz), 7.45 (1H, s), 7.58 (1H, bs, NH-), 7.63 (1H, d, *J* = 7.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 21.28, 34.80, 54.32, 110.50, 111.82, 122.63, 123.67, 125.23, 126.93, 127.49, 128.88, 129.00, 120.55, 134.36, 138.96, 148.47, 154.80, and 158.92. EI-MS, *m/z*: 346.3 (Calcd for $C_{20}H_{18}N_4O_2$: 346.3). Anal. Calcd for $C_{20}H_{18}N_4O_2$: C, 69.35; H, 5.24; N, 16.17. Found: C, 69.53; H, 5.11; N, 16.28.

N-((1-(3-methoxybenzyl)-1H-1,2,3-triazol-4-yl)methyl)benzofuran-2-carboxamide (**8d**)

White powder; 0.309 g, Yield: 86%; M. p. 127-128 ºC. IR (KBr) (ν_{max}/cm^{-1}): 3462 (NH), 1657 (CO). ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.73 (3H, s, -OMe), 4.71 (2H, d, *J* = 5.3 Hz), 5.47 (2H, s), 6.78 (1H, s), 6.82 to 6.85 (2H, m), 7.24 (1H, t, *J* = 7.7 Hz), 7.26 (1H, s), 7.36 (1H, t, *J* = 7.7 Hz), 7.41 (1H, d, *J* = 6.6 Hz), 7.42 (1H, s), 7.53 (1H, bs, NH), 7.59 to 7.62 (2H, d, *J* = 8 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 34.68, 54.27, 55.24, 110.57, 111.79, 113.74, 114.19, 120.29, 122.62, 123.64, 126.90, 126.94, 127.40, 130.18, 135.75, 148.28, 154.70, 158.85, and 160.02. EI-MS, *m/z*: 362.3 (Calcd for $C_{20}H_{18}N_4O_3$: 362.3). Anal. Calcd for $C_{20}H_{18}N_4O_3$: C, 66.29; H, 5.01; N, 15.46. Found: C, 66.37; H, 5.10; N, 15.39.

N-((1-(4-methoxybenzyl)-1H-1,2,3-triazol-4-yl)methyl)benzofuran-2-carboxamide (**8e**)

White powder; 0.297 g, Yield: 81%; M. p. 179-181 ºC. IR (KBr) (ν_{max}/cm^{-1}): 3457 (NH), 1656 (CO). ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.80 (3H, s, -OMe), 4.74 (2H, bs), 5.44 (2H, s), 6.88 (2H, d, *J* = 8.5 Hz), 7.23 (2H, d, *J* = *8.5* Hz), 7.26 to 7.28 (2H, m), 7.41 (1H, t, *J* = 7.5 Hz), 7.45 (1H, s), 7.46 (1H, d, *J* = 7.5 Hz), 7.60 (1H, bs, -NH), 7.65 (1H, d, *J* = 7.5Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 34.55, 54.43, 55.30, 110.65, 111.81, 114.47, 122.64, 123.67, 126.31, 126.97, 127.44, 129.79, 133.10, 137.11, 139.10, 146.10, 148.33, 154.76, 158.82, and 160.02. Anal. Calcd for $C_{20}H_{18}N_4O_3$: C, 66.29; H, 5.01; N, 15.46. Found: C, 66.18; H, 5.16; N, 15.25.

N-((1-(4-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)benzofuran-2-carboxamide (**8f**)

Pale yellow powder; 0.301 g, Yield: 86%; M. p. 173-175 ºC. ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.70 (2H, bs), 5.48 (2H, s), 7.06 (2H, t, *J* = 8 Hz), 7.26 to 7.30 (4H, m), 7.41 (1H, m), 7.46 to 7.49 (2H, m), 7.66 (1H, m). ^{13}C NMR (125 MHz, $CDCl_3$): δ 34.39, 54.43, 110.72, 111.80, 116.02, 116.19, 122.67, 123.71, and 127.01, 127.44, 130.08. Anal. Calcd for $C_{19}H_{15}FN_4O_2$: C, 65.14; H, 4.32; N, 15.99. Found: C, 65.23; H, 4.21; N, 16.08.

N-((1-(4-bromobenzyl)-1H-1,2,3-triazol-4-yl)methyl)benzofuran-2-carboxamide (**8g**)

White powder; 0.370 g, Yield: 90%; M. p. 197-199 ºC. IR (KBr) (ν_{max}/cm^{-1}): 3411 (NH), 1655 (CO). ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.76 (2H, bs), 5.47 (2H, s), 7.15 (2H, d, *J* = 8 Hz), 7.26 (1H, s), 7.29 (1H, t, *J* = 7.5 Hz), 7.42 (2H, m), 7.42 (1H, t, *J* = 7.5 Hz), 7.46 to 7.51 (4H, m), 7.66 (1H, d, *J* = 7.7 Hz). EI-MS, m/z: 410.2 (Calcd for $C_{19}H_{15}BrN_4O_2$: 411.2). Anal. Calcd for $C_{19}H_{15}BrN_4O_2$: C, 55.49; H, 3.68; N, 13.62. Fund: C, 55.38; H, 3.79; N, 13.53.

Antifungal Activity Evaluation

To evaluate the antifungal properties of various compounds, 12 mg of each of the synthetic fungicides (dry powder) was dissolved into 12 mL of methanol (MeOH) (Merck, Darmstadt, Germany), used as a solvent. The solution was passed by syringe from a 0.45 μm Microsolve filter and poured into a sterile glass vial. Approximately 25 mL of the sterilized medium was placed in an autoclave for 20 min at 120 °C and 1.2 atm, and then it was poured into Petri plates. Then 25 μL of the solution was added *via* micro-sampler at various concentrations (500 ppm and 1000 ppm on four antibiogram discs) to medium containing malt extract agar (MEA) for microbiology (Merck KgaA 64271 Darmstadt, Germany), pH value: 5.6 (48 g/L, H_2O, 22 °C) after autoclaving and were each poured into one of the Petri plates.

Ketoconazole, as the representative synthetic fungicide (at 500 ppm and 1000 ppm concentrations), was used as the positive control due to its inhibitory effects against radial growth of three wood-degrading fungi, *i.e.*, *T. versicolor*, *P. placenta*, and *C. puteana*. Methanol was selected as the negative control to confirm that there was no inhibitory effect (Teoh *et al.* 2015; Hosseinihashemi *et al.* 2020).

The plates were cooled in a sterile hood and inoculated with 0.50 cm plugs of *Trametes versicolor*, *Poria placenta*, *Coniophora puteana*, and *Gloeophyllum trabeum* fungi mycelia, which were introduced into the center of the Petri plate. The inoculated plates were incubated at 23 °C and 75% relative humidity without light. Four replicate antibiogram discs and one plate were used per treatment. The fungi were also grown on plates with non-compound MEA (*i.e.*, with methanol) as a negative control. The percentage of fungal mycelial growth was plotted against the compound concentration, and the toxicity level was determined by the compound concentration. In other words, the fungal growth was monitored daily by measuring the percentage of area that was covered by fungus in the test plates until one day prior to complete coverage of Petri plate, similar to reported methods (Hosseinihashemi *et al.* 2016a, 2016b; Hosseinihashemi *et al.* 2020).

The colony fungal growth (shown by radius) was measured, and the percentage inhibition was calculated according to Eq 1.,

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

where $C=[\frac{(C_1+C_2+C_3+C_4)}{4}]$ is the average colony radius (mm) of the negative control and $T=[\frac{(T_1+T_2+T_3+T_4)}{4}]$ is the average colony radius (mm) of the test plate.

Chemistry Methods and Procedures

The progress of the reaction was monitored *via* thin layer chromatography (silica gel 250 micron, F254 plates, Merck, Darmstadt, Germany). Melting points were determined using an Electrothermal 9100 apparatus. The IR spectra were recorded with a Shimadzu IR-460 spectrometer. The ^{1}H and ^{13}C NMR spectra were measured with a Bruker DRX-500 AVANCE (at 500.1 MHz and 125.8 MHz) instrument. The mass spectra were recorded with an Agilent Technology (HP) mass spectrometer, which operated at an ionization potential of 20 eV. The elemental analysis was performed with an ElementarAnalysen system GmbH VarioEL (CHN mode).

Statistical analysis

Percentage inhibition was calculated and an analysis of variance for the different treatments was conducted using Duncan's test.

RESULTS AND DISCUSSION

Chemistry

Figure 2 describes the condensation reaction between salicylaldehyde (1) and ethyl bromoacetate in the presence of K_2CO_3, which afforded ethyl benzofuran-2-carboxylates (**2**). The subsequent hydrolysis in aqueous ethanol/KOH yielded benzofuran-2-carboxylic acid (**3**). The propargylation of the latter compounds with propargyl amine (**4**) in the presence of hydroxybenzotriazole (HOBt) and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC.HCl) as coupling agents resulted in compound (**5**). The *N*-(prop-2-ynyl)benzofuran-2-carboxamide (**5**) compounds were used for the click reaction. For this purpose, various benzyl bromide derivatives (**6**) and sodium azide, reacted in the presence of Et_3N in H_2O/t-BuOH at room temperature. Then, *N*-(prop-2-ynyl)benzofuran-2-carboxamide (**5**), $CuSO_4$.$5H_2O$, and sodium ascorbate were added to the freshly prepared azide derivatives (**7**) and the reaction was continued at room temperature for 8 h to 12 h to give the corresponding products **8a** through **8g** with sufficient yields, *i.e.*, 80% to 90% (as shown in Fig. 3).

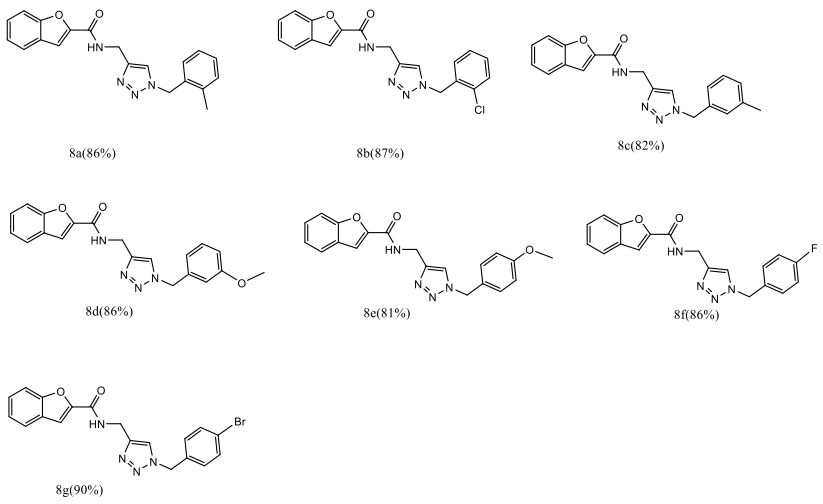

Fig. 3. Final compounds: **8a**, **8b**, **8c**, **8d**, **8e**, **8f**, and **8g**

Activity Evaluation of Antifungal Compounds Against Fungi

The *in vitro* antifungal activity of the target compounds was measured *via* the malt extract agar disk diffusion method with ketoconazole as the control fungicide or drug. The results are summarized in Table 1 and Fig. 4. The potential of synthetic compounds **8a**, **8b**, **8c**, **8d**, **8e**, **8f**, and **8g** as wood preservatives were evaluated by determining the percentage of mycelial growth inhibition against the four tested wood-degrading fungi, *Trametes versicolor*, *Poria placenta*, *Coniophora puteana*, and *Gloeophyllum trabeum*, at 500 ppm and 1000 ppm. Methanol and ketoconazole were used as a negative and positive control, respectively.

Table 1. Mean ± (Std.) Values of the % Mycelial Growth Inhibition of Four Tested Wood-Degrading Fungi by the Seven Novel Synthetic Fungicide Preservatives and Ketoconazole at Two Concentrations per 25 cm^3 of Malt Extract Agar (in One Day Prior to Complete Coverage by Fungus in the Negative Control Plates)

Compound	*T. versicolor*		*P. placenta*		*C. puteana*		*G. trabeum*	
8a	-3.57a (4.12)	2.38b (2.75)	0.00a (2.29)	10.18b (1.39)	1.14a (4.35)	8.53b (9.16)	1.71a (5.37)	7.39b (2.17)
8b	0.00 (5.14)	5.95 (3.07)	-3.59 (1.20)	12.57 (2.40)	3.98 (2.18)	7.39 (2.17)	9.66 (2.86)	3.98 (2.18)
8c	1.19 (1.37)	10.71 (3.08)	-0.60 (1.96)	4.79 (2.29)	10.23 (6.02)	-1.72 (1.32)	15.34 (2.17)	4.55 (1.86)
8d	2.38 (1.94)	4.76 (0.00)	-1.80 (2.30)	10.18 (3.09)	1.71 (7.96)	-1.15 (3.43)	3.41 (1.32)	28.22 (6.85)
8e	2.98 (1.19)	2.98 (2.99)	-4.79 (2.29)	2.99 (4.59)	-1.14 (1.31)	11.93 (6.78)	1.71 (2.86)	13.07 (5.68)
8f	1.79 (1.19)	-0.60 (2.99)	-4.19 (1.39)	0.00 (1.20)	23.87 (3.94)	6.82 (4.91)	47.16 (3.41)	15.91 (0.00)
8g	-4.17 (3.57)	2.38 (5.50)	0.00 (1.20)	0.60 (1.39)	5.12 (9.16)	11.36 (6.43)	3.98 (2.18)	11.93 (3.88)
Descriptions	A: Mean ± (std.) values at 500 ppm; b: Mean ± (std.) values at 1000 ppm							
C(+) 500 and 1000 (ppm)	16.67c (3.89)	23.81d (2.75)	2.40c (1.20)	6.59d (3.39)	12.50c (7.06)	17.62d (7.51)	64.78c (2.27)	65.34d (5.68)
C (+) 30 and 60 (ppm)	14.03 (2.23)	12.82 (2.09)	6.37 (4.35)	25.28 (2.83)	-26.87 (6.22)	2.26 (7.91)	14.63 (5.97)	29.27 (1.99)
Descriptions	C: Mean ± (std.) values at 30 ppm and 500 ppm d: Mean ± (std.) values at 60 ppm and 1000 ppm							

8a: *N-((1-(2-methylbenzyl)-1H-1,2,3-triazol-4-yl)methyl)benzofuran-2-carboxamide*; **8b**: *N-((1-(2-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)benzofuran-2-carboxamide*; **8c**: *N-((1-(3-methylbenzyl)-1H-1,2,3-triazol-4-yl)methyl)benzofuran-2-carboxamide*; **8d**: *N-((1-(3-methoxybenzyl)-1H-1,2,3-triazol-4-yl)methyl)benzofuran-2-carboxamide*; **8e**: *N-((1-(4-methoxybenzyl)-1H-1,2,3-triazol-4-yl)methyl)benzofuran-2-carboxamide*; **8f**: *N-((1-(4-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)benzofuran-2-carboxamide*; **8g**: *N-((1-(4-bromobenzyl)-1H-1,2,3-triazol-4-yl)methyl)benzofuran-2-carboxamide*; C (+): Ketoconazole

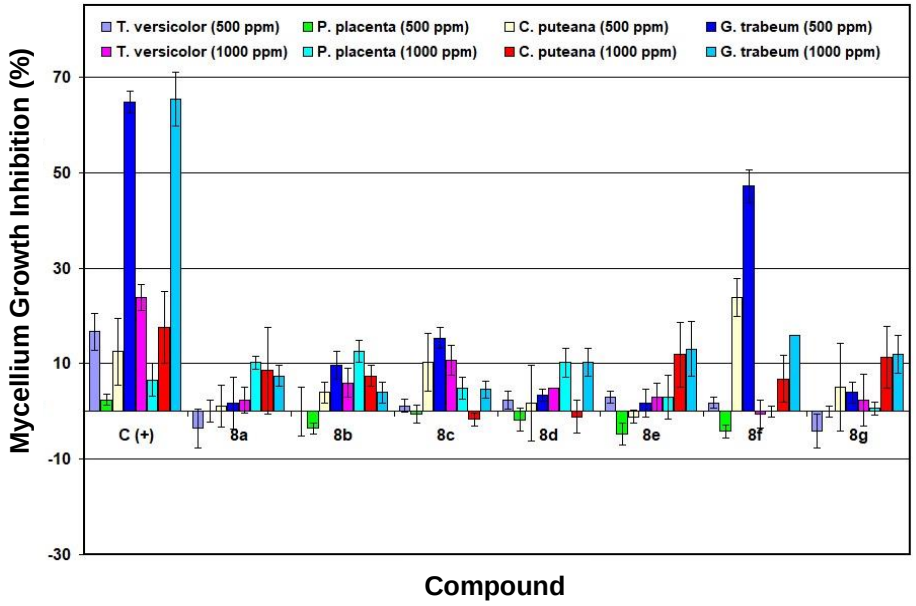

Fig. 4. Percentage of mycelial growth inhibited by synthetic fungicides

Statistically, there was a significant difference between the compounds and their concentrations on the percentage of mycelial growth inhibition of wood-degrading fungi (Table 2).

Table 2. Univariate Test Results for Percentage of Mycelial Growth Inhibition of the Four Tested Wood-Degrading Fungi by the Seven Novel Synthetic Fungicide Preservatives and Ketoconazole at Two Concentrations per 25 cm^3 of Malt Extract Agar (in One Day Prior to Complete Coverage by Fungus in the Negative Control Plates)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	45951.662	63	729.391	20.170	0.000
Intercept	16982.814	1	16982.814	469.627	0.000
Fungi (F)	10251.709	3	3417.236	94.497	0.000
Concentrations (Con)	645.382	1	645.382	17.847	0.000
Compounds (Com)	13360.626	7	1908.661	52.780	0.000
F * Con	581.503	3	193.834	5.360	0.001
F * Com	15120.924	21	720.044	19.911	0.000
Con * Com	2639.349	7	377.050	10.427	0.000
F * Con * Com	3352.168	21	159.627	4.414	0.000
Error	6943.166	192	36.162		
Total	69877.642	256			
Corrected Total	52894.828	255			

According to the results, some of the target compounds exhibited acceptable antifungal activity at 500 ppm and 1000 ppm. Compound **8c** showed better antifungal activity at 1000 ppm against *T. versicolor* (with a percentage inhibition of 10.71%) in comparison to the other compounds with the exception of the positive control at all concentrations. In addition, compounds **8b**, **8a**, and **8d** showed inhibition rates of 12.57%, 10.18%, and 10.18%, respectively, against *P. placenta*, which were more effective than the ketoconazole at 30 ppm, 500 ppm, and 1000 ppm. However, ketoconazole showed better antifungal activity at 60 ppm against *P. placenta* with a inhibition rate of 25.28%. At 1000 ppm, better antifungal activity against *T. versicolor* and *P. placenta* was observed for most of the synthetic compounds. Particularly, compounds **8c**, **8f**, **8e**, and **8g** exhibited inhibition rates of 10.23%, 17.61%, 11.93%, and 11.36%, respectively, against the growth of *C. puteana* fungus. The greatest antifungal activities were for compound **8c** at 500 ppm and compound **8f** at 500 ppm and 1000 ppm showing inhibition of 15.34%, 47.16%, and 15.91%, respectively, against *G. trabeum*. These two compounds were more effective than ketoconazol at 30 ppm and 60 ppm. However, ketoconazole showed better antifungal activity at 500 ppm and 1000 ppm against *G. trabeum* fungus with an inhibition rate of 64.78% and 65.34%, respectively.

The synthetic 1,2,3-triazole derivatives showed moderate control over the growth of fungi rather than their positive control for ketoconazole, at 30 ppm, 60 ppm, 500 ppm, and 1000 ppm concentrations. Among all the novel synthetic compounds, N-((1-(4-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)benzofuran-2-carboxamide (**8f**) was the most active antifungal agent against the mycelium growth of *G. trabeum* fungus.

The trends of the percentage inhibition of white-rot (*T. versicolor*) growth after 6 d (one day prior to complete coverage by fungus in the negative control plates) were shown in Fig. 5. As shown in Fig. 5, the compounds with greater and lower than 10%

inhibition are indicated by solid and dashed lines, respectively. Among the synthesized compounds that inhibited the growth of *T. versicolor*, N-((1-(4-methoxybenzyl)-1H-1,2,3-triazol-4-yl)methyl)benzofuran-2-carboxamide (**8c**) had the greatest performance, with an inhibition rate of 10.71% at a 1000 ppm concentration.

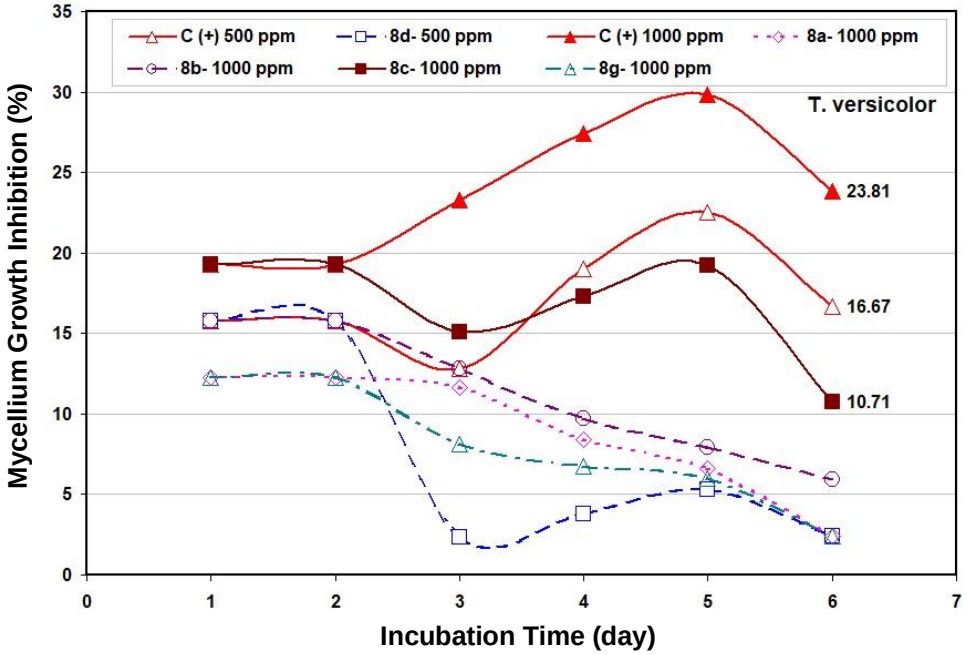

Fig. 5. Trends of the percentage inhibition of *T. versicolor* growth by different synthetic fungicides solutions with 500 ppm and 1000 ppm concentrations in 6 d (the 7th d was the final time)

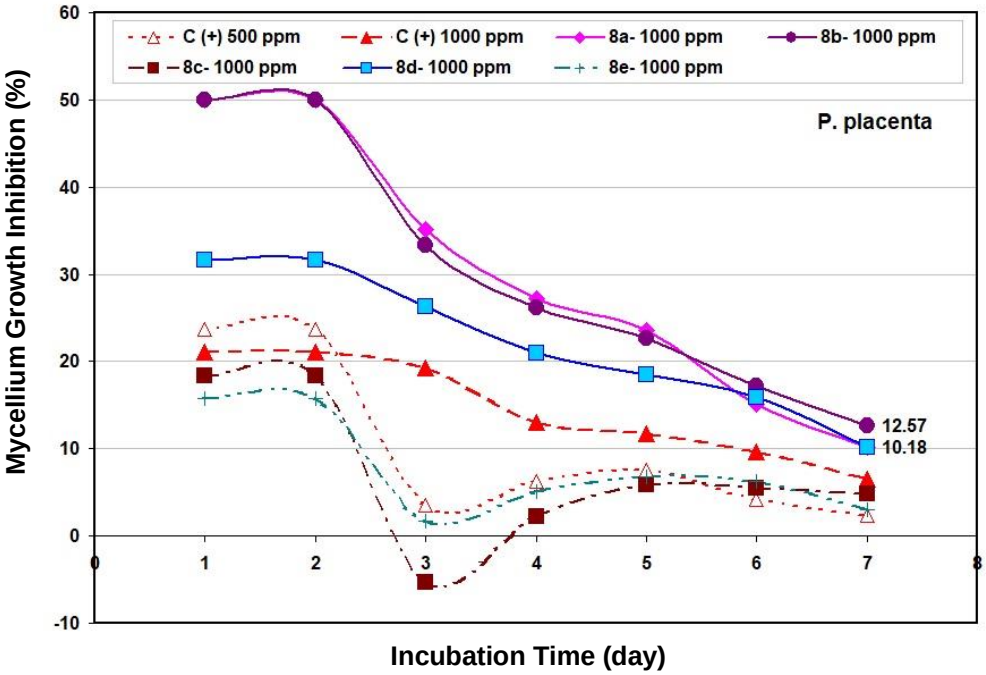

Fig. 6. Trends of the percentage inhibition of *P. placenta* growth by different synthetic fungicides solutions with 500 ppm and 1000 ppm concentrations in 7 d (the 8th d was the final time)

Figure 6 illustrates the trends of percentage inhibition of dry brown-rot (*P. placenta*) growth after 7 d (one day prior to complete coverage by fungus in the negative control plates). In Fig. 6, the compounds above 0% to 10% of inhibition and above 10% of inhibition, were indicated by dashed and solid lines, respectively.

The most effective synthesized compounds that inhibited the growth of *P. placenta* were N-((1-(2-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)benzofuran-2-carboxamide (**8b**), with the position of 2-Cl substitution, N-((1-(2-methylbenzyl)-1H-1,2,3-triazol-4-yl)methyl)benzofuran-2-carboxamide (**8a**), with the position of 2-CH_3 substitution, and N-((1-(3-methoxybenzyl)-1H-1,2,3-triazol-4-yl)methyl)benzofuran-2-carboxamide (**8d**), with the position of 3-OCH_3 substitution, at a concentration of 1000 ppm (12.57%, 10.18%, and 10.18%, respectively).

The trends of percentage inhibition of wet brown-rot (*C. puteana*) growth after 18 d (one day prior to complete coverage by fungus in the negative control plates) are shown in Fig. 7. As shown in Fig. 7, the compounds that had greater than 10% inhibition are indicated by solid lines. Among the novel synthesized compounds, N-((1-(4-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)benzofuran-2-carboxamide (**8f**) at a concentration of 500 ppm could inhibited the growth of *C. puteana* by 23.86%, which more effective than ketoconazole at the four various concentrations.

Figure 8 shows the trends of percentage inhibition of wet brown-rot (*G. trabeum*) growth after 11 d (one day prior to complete coverage by fungus in the negative control plates). As shown in Fig. 8, the compounds that had greater than 10% inhibition were indicated by solid lines. Three synthesized compounds showed high growth percentage inhibition amounts (15.34%, 15.91%, and 47.16%), with the compound with the highest inhibition value was N-((1-(4-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)benzofuran-2-carboxamide (**8f**), at 500 ppm concentration.

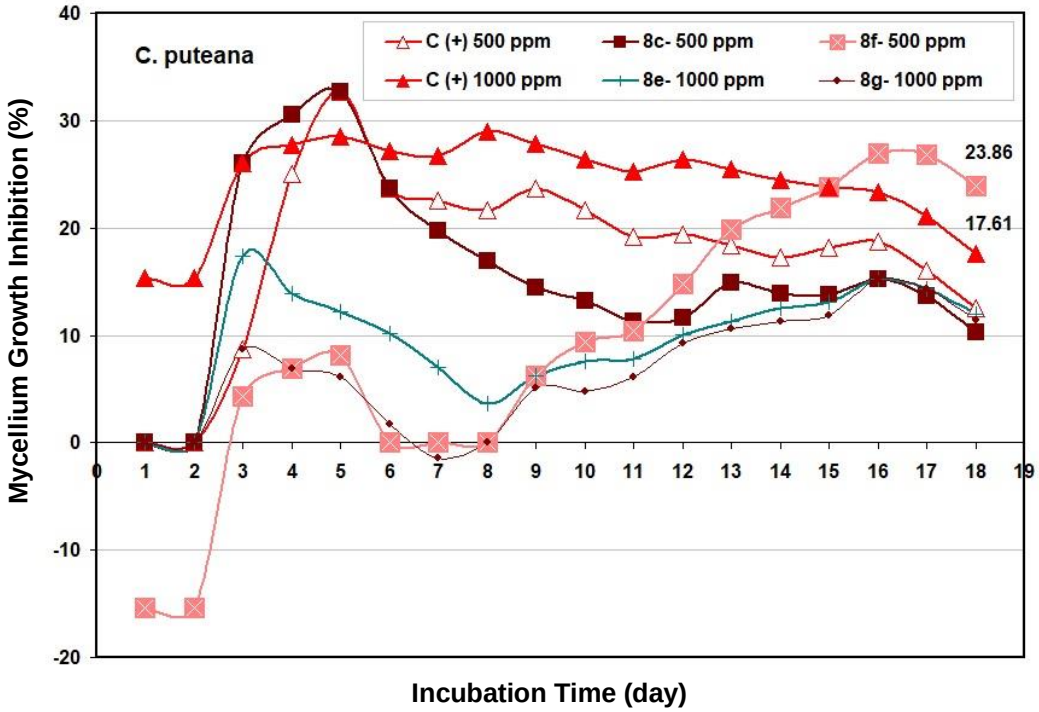

Fig. 7. Trends of the percentage inhibition of *C. puteana* growth by different synthetic fungicides solutions with 500 ppm and 1000 ppm concentrations in 18 d (the 19th d was the final time)

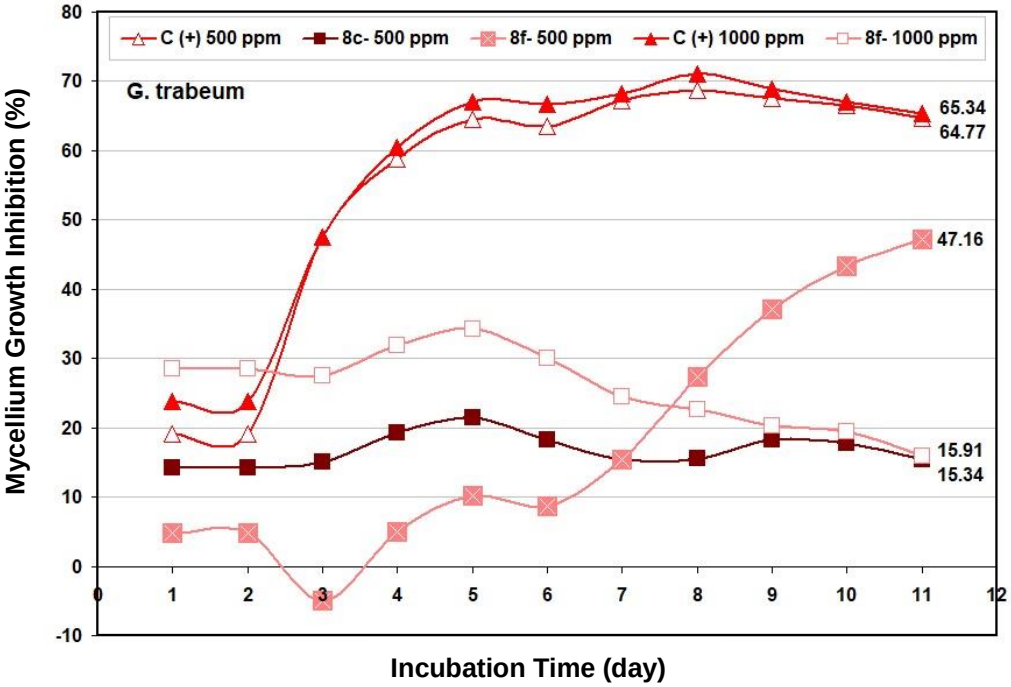

Fig. 8. Trends of the percentage inhibition of *G. trabeum* growth by different synthetic fungicides solutions with 500 ppm and 1000 ppm concentrations in 11 d (the 12th d was the final time)

As shown in Fig. 8 and Fig. 9b, the growth of *G. trabeum* stopped after 6 d when tested with compound **8f**, which at the final day of growth (12th d) reached a percentage of inhibition of 48.33%.

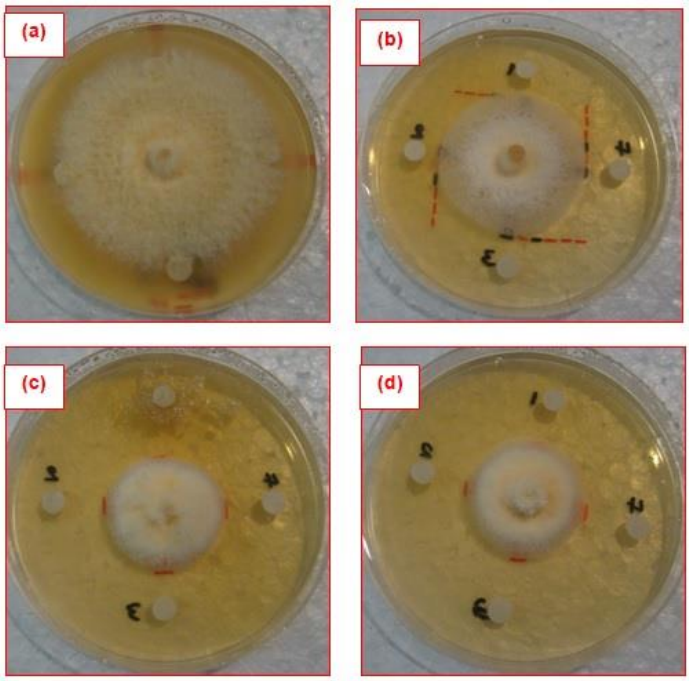

Fig. 9. Mycelial growth areas of the *G. trabeum* fungus; a: Negative control (44 mm); b: **8f** at 500 ppm (23.25 mm); c: C (+) at 1000 ppm (15.25 mm); d: C (+) at 500 ppm (15.50 mm)

Researchers have described both the biotransformation and biodegradation of some triazoles, such as tebuconazole and propiconazole, by the surrounding microbial species (fungi or bacteria) in liquid media (Obanda 2008; Obanda *et al.* 2008; Obanda and Shupe 2009), soil (Potter *et al.* 2005; White *et al.* 2010), and wood matrixes (Woo *et al.* 2010). Cleavage of the triazole ring is one of the major pathways of tebuconazole degradation by bacteria, mold, and soft and brown-rot fungi (Obanda 2008; Obanda and Shupe 2009). The hydroxyl group can be further acetylated or oxidized to form an ester or carboxyl group. Acetylation causes the deactivation of tebuconazole by decreasing its hydrophilicity (Kukowski 2018).

The presence of a methyl group provided similar activity to ketoconazole against *T. versicolor* and *P. placenta* at 1000 ppm (Hosseinihashemi *et al.* 2020). A strong electron-donating group (methoxy group) with a *meta* position resulted in better activity against *P. placenta* than a *para* position at 1000 ppm. When the authors changed the position of the -F substitution (strong electron-donating group) in the new benzofuran-1,2,3-triazole hybride synthetic fungicide from the previous benzofuran-1,3,4-oxadiazole hybrid synthetic fungicide 5h (*meta* to *para* position) led to more a potent compound **8f** at 500 ppm against *C. puteana* and specially *G. trabeum* (Hosseinihashemi *et al.* 2020). However, this effect was not observed at 1000 ppm, which could be related to the low solubility of the compound at this concentration in the MEA medium. In compound **8g**, the presence of bromine in the *para* position, thus making a less electronegative atom, resulted in lower inhibition of the growth of *T. versicolor* and *C. puteana* fungi at 1000 ppm.

CONCLUSIONS

The synthesis and evaluation of new benzofuran-1,2,3-triazole hybrids as antifungal agents were investigated. Seven compounds (**8a** to **8g**), for use as wood preservatives, were evaluated against white- and brown-rot fungi (*T. versicolor, P. placenta*, *C. puteana*, and *G. trabeum*) at different concentrations (500 ppm and 1000 ppm) using *in vitro* disk diffusion.

1. The synthesized compounds with triazole linkage at 500 ppm concentration may advance further development of new wood preservatives against the two wood brown-rotting fungi.
2. Compound **8f** showed the highest antifungal activity against *C. puteana* and *G. trabeum* at 500 ppm with a mycelial growth inhibition rate of 23.86% and 47.16%, respectively.
3. Among the novel synthesized compounds, the complete inhibition of mycelial growth of *G. trabeum* fungus has been occurred in 6^{th} d of incubation time only by **8f** at a concentration of 500 ppm.

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