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Thymol as an Interesting Building Block for Promising Fungicides against *Fusarium solani*

Mariana Alves Eloy, Rayssa Ribeiro, Leandra Martins Meireles, Thiago Antonio de Sousa Cutrim, Carla Santana Francisco, Clara Lirian Javarini, Warley de Souza Borges, Adilson Vidal Costa, Vagner Tebaldi de Queiroz, Rodrigo Scherer, Valdemar Lacerda, Jr.,*,# and Pedro Alves Bezerra Morais*,#

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ABSTRACT: The semisynthesis of 15 new thymol derivatives was achieved through Williamson synthesis and copper-catalyzed azide−alkyne cycloaddition (CuAAC) approaches. The reaction of CuAAC using the "*Click Chemistry*" strategy, in the presence of an alkynyl thymol derivative and commercial or prepared azides, provided nine thymol derivatives under microwave irradiation. This procedure reduces reaction time and cost. All molecular entities were elucidated by ^{1}H and ^{13}C NMR, IR, and HRMS data. These derivatives were evaluated *in vitro* for their fungicidal activity against *Fusarium solani* sp. Among the nine triazolic thymol derivatives obtained, seven of them were found to have moderated antifungal activity. In contrast, naphthoquinone/thymol hybrid ether **2b** displayed activity comparable with that of the commercial fungicide thiabendazole. The structure−activity relationship for the most active compound **2b** was discussed, and the mode of action was predicted by a possible binding to the fungic ergosterol and interference of osmotic balance of K^{+} into the extracellular medium.

KEYWORDS: *thymol derivatives, triazole, Fusarium solani, black pepper root rot, agrochemicals*

INTRODUCTION

Agricultural inputs, such as fungicides, have been applied and grown significantly in organic farming for the management and control of fungal diseases, especially as a "green revolution" technology.[1] Plant fungal pathogens have relevant impacts on a wide range of crops since several phytopathogenic fungi release prejudicial mycotoxins to animal and human health.[2−4] Thus, controlling fungal diseases is essential to keep higher agricultural productivity and minimize monetary losses.[1] Considering the need to develop new fungicides with novel molecular frameworks and mechanisms of action, the agrochemical industry has successfully produced and filled this profitable market. However, continuous resistance to commercially available agrochemicals is an intrinsic event related to the application of fungicides. Moreover, more rigorous environmental and toxicological regulations have been broadly introduced worldwide. Thus, research and development toward a novel approach for safer and more effective fungicides in comparison to available agrochemicals remain important.[1]

Terpenoids reveal a broad and potent class of natural products with a remarkable role in the enzyme systems of plants and highlight an important biological activity against several pests.[5] Furthermore, semisynthesis using these natural products as scaffolds has been reported to improve biological activities.[6−9] Thymol is a naturally occurring phenolic monoterpenoid, well-known biocide, and major compound of the oils of thyme (*Thymus vulgaris* L.) and oregano (*Origanum vulgare* L.). Derivatives of this natural product are easily observed in Asteraceae plants, particularly in Senecioneae, Eupatorieae, Inuleae and Helenieae tribes.[10−13] Several works have highlighted its biological activities like antimutagenic,[14,15] anthelmintic,[16−19] antiseptic;[20] gastro protective activity against several strains of *Helicobacter pylori*.[21,22] In addition, thymol has interesting antitumor properties and is an effective fungicide, particularly against fluconazole-resistant strains, and it has inhibitory activity against plants' pathogenic fungi.[12,23] The mode of thymol action against fungi is due to its ability to modify the hyphal structure and hyphal agglomeration, promoting reduction in hyphal diameters and lysis of hyphal wall. Moreover, antifungal activity is related to hydrophobicity and hence the capacity to pass through the fungal cell membrane, further affecting pH homeostasis and disturbing the cell structures.[24,25]

During the past decades, 1,2,4-triazolic compounds have demonstrated large peculiar biological activities such as pesticidal and antifungal. Successful representatives of this large class of fungicides include tebuconazole, tebuconazole, flutriafol, hexaconazole, and cyproconazole.[26−29] The Bayer company initially launched triadimefon (Bayleton) in 1973, and after that, several other triazoles were developed. The longevity of these heterocyclic fungicides is due to the high-

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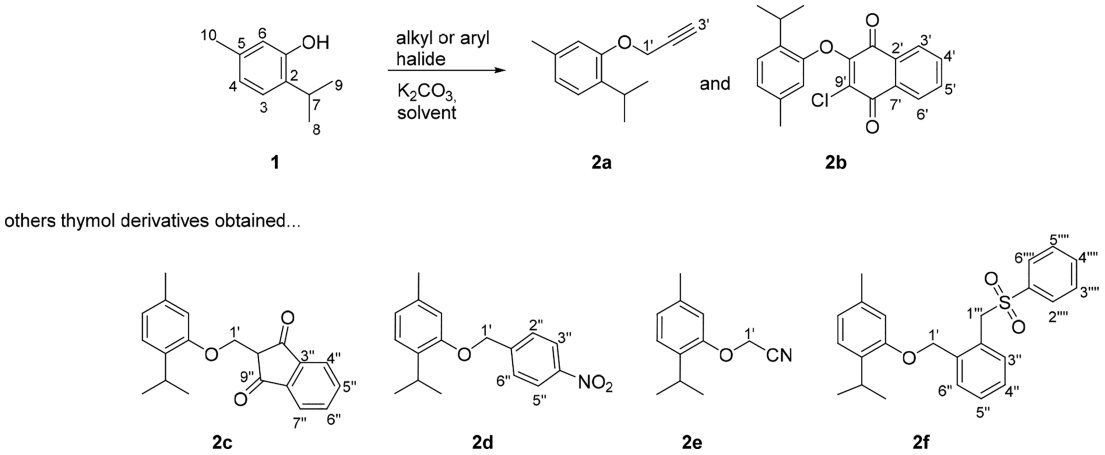
Scheme 1. Synthesis of Thymol-*O*-alkylated Derivatives



efficiency, broad-spectrum, and slowly developing resistance, resulting in a decreased sensitivity to their mode of action as demethylation inhibitors. The isomeric 1,2,3-1*H*-triazole heterocyclic is a well well-established and elegant pharmacophore in medicinal chemistry which has been introduced as a linker into a large library of molecules with important biological properties. Novel and improved lead compounds have continuously been designed by molecular hybridization through coupling with 1,2,3-1*H*-triazole.[30] Reports highlight 1,2,3-triazole as anticancer, antifungal, antibacterial, antituberculosis, and antiviral agents. Indeed, there are already drugs in which the 1,2,3-triazole ring is present, for example, cefatrizine (antibiotic), tazobactum (antibacterial), and carboxyamidotriazol (anticancer).[31−34]

Black pepper (*Piper nigrum* L.) is a high-value-added product for the agroindustry worldwide. Vietnam stands out as the largest producer and exporter, followed by India, Brazil, and Indonesia. Numerous diseases that are caused by viruses, fungi, algae, and nematodes affect the black pepper crop.[35] However, fungal diseases present the greater impact in black pepper crop losses, specifically the root rot (*Nectria hematococca* Berk & Br. f. sp. *piperis* Albuq., anamorph *Fusarium solani* Mart. (Sacc.) f. sp. *Piperis* Albuq.), which is responsible for reducing the useful life of a crop from 12 years to 5 or 6 years.[36] In terms of dollars, this means perhaps $10 million dollars a year.

Fusarium solani (Mart.) Sacc. f. sp. *piperis* Albuq. naturally inhabits soils and survives both in the plant and in the soil's organic matter, as a saprophyte. This fungus belongs to the phylum Ascomycota, class Ascomycetes, order Hypocreales, and family Hypocreaceae. The control usually applied for *F. solani* f. sp. *piperis* is focused on prevention, such as the use of seedlings of a high phytosanitary standard, field monitoring, identification, and eradication of symptomatic plants.[36,37] Nevertheless, because of the absence of useful agrochemicals or commercial cultivars resistant to the disease, a further search for novel and safer molecular entities is necessary to lead to an improvement of potency and duration of action and decreasing their toxic effects.

In connection with the aforementioned terms, the present investigation was concerned with performing the preparation of 15 thymol derivatives comprising triazole pharmacophore and/or substituted aromatic rings, both promising biologically active to achieve potential fungicidal agents against *Fusarium solani* sp. The newly prepared compounds structural assignments were elucidated on the basis of ^{1}H and ^{13}C NMR spectra, IR, and mass spectra. Compounds **2a−f** were synthesized by Williamson synthesis, while **3d−3l** were prepared under microwave irradiation by the CuAAC reaction between of *O*-propargyl thymol derivative **2a** and commercials or prepared azides. All tested compounds, with the exception of **3e** and **3k**, showed a moderate MIC (minimum inhibitory concentration) value of 57 μg mL^{-1}, in comparison with thiabendazole, although more active than tebuconazole (absence of activity). Hybrid compound **2b** displayed *in vitro* fungitoxicity comparable to that of the commercial fungicide thiabendazole against *Fusarium solani* sp.

As supported by references, it is important to emphasize that the use of thymol as scaffold for antifungal activity against Fusarium species is quite promising.[38−40] In relation to the phenolic hydroxyl and its importance for biological properties of thymol, in this study, we investigated the substitution of this portion by a triazol nucleus with well-established biological properties and also known in medicinal chemistry for its application as a bioisosteric ring of acid groups. Therefore, the expectations, by coupling thymol and triazole rings, are to improve the potency of this natural product against *Fusarium solani* and maintain the physicochemical and electronic properties of this scaffold.

EXPERIMENTAL SECTION

Analytical grade solvents with purity higher than 99.5% were purchased from Synth (São Paulo, Brazil). Thymol, dimethylformamide (DMF), acetone, azides, bromides, potassium carbonate, sodium ascorbate, copper sulfate, propargyl bromide solution (80 wt % in toluene, containing 0.3% of magnesium as a stabilizer), and deuterated solvents were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Microwave reactions were conducted using a CEM Discover Synthesis Unit (CEM Corp., Matthews, U.S.A.). The instrument consists of a continuous focused microwave power delivery system with operator-selectable power output from 0 to 300 W. Reactions were performed in glass vessels (capacity 10 mL) sealed with a septum. Column chromatography (CC) was carried out using stationary phase silica gel 60 (70−230 mesh, Merck (Darmstadt, Germany)) and silica gel 60 ACC (6−35 μm, Chromagel-SDS), respectively, and mixtures of hexane and ethyl acetate were used as eluent. For thin-layer chromatography (TLC), silica gel F254 was used as a stationary phase, and plates were 20 cm × 20 cm × 0.20 mm. NMR (nuclear magnetic resonance) spectra were recovered on a Varian 400 MHz instrument (Palo Alto, U.S.A.) using deuterated chloroform ($CDCl_3$) or deuterated dimethyl sulfoxide (DMSO-d_6) as solvent and tetramethylsilane (TMS) as

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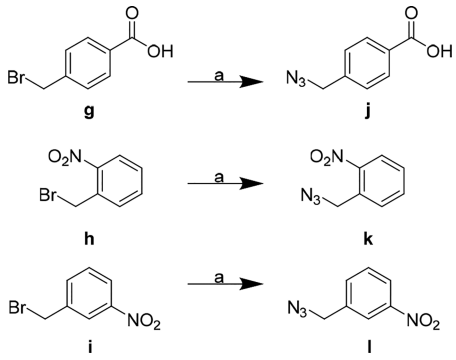
the internal standard. The chemical shift (δ) is in ppm, and J values are in Hertz (Hz). High-resolution mass spectra were obtained with a Bruker Daltonics micrOTOF QII/ESI-TOF (Billerica, U.S.A.) by direct injection of the compound dissolved in methanol. This provided an unambiguous molecular formula assignment for singly charged molecular ions, such as $[M + H]^+$ or $[M - H]^-$ and $[M + Na]^+$, and also DBE (double-bound equivalents) values. The infrared (IR) spectrum was recorded on a Bruker Tensor 27 FT-IR Spectrometer (Bremen, Germany) scanning from 4000 to 500 cm^{-1}.

General Procedure for the Synthesis of Thymol Ethers. A reaction solution of thymol (**1**) (0.05 g; 3.3 mmol) and potassium carbonate (K_2CO_3) (5 mmol) in acetone (4 mL) was stirred at 0 °C for 20 min. Hereafter, alkyl or aryl halides, such as propargyl bromide (33 mmol) and 2,3-dichloro-1,4-naphthoquinone (5 mmol), were added to the reaction mixture and stirred at room temperature for 4 h, leading to compounds **2a** and **2b**, respectively. After the solvent evaporated, the residues were purified by column chromatography using silica gel 60 (70−230) mesh as stationary phase and hexane:ethyl acetate or hexane:ether as eluent (Scheme 1).[41,42]

Synthesis of the Azide Compounds. In a 100 mL round-bottom flask containing commercial bromide (**g**, **h**, and **i**) (1 equiv), in 10 mL of acetone at 0 °C, NaN_3 (1.5 equiv) dissolved in water was slowly added. The reaction mixture was stirred at room temperature for 3 h. Then, acetone was evaporated under vacuum, and the residue was extracted with an ethyl acetate/water partition. After the organic solvent was subjected to rotary evaporation under pressure, the compounds **j**, **k**, and **l** were obtained (Scheme 2).[43]

Scheme 2. (a) Sodium Azide, Acetone/Water, 0°C, r.t



General Procedure for the Synthesis of Triazolic Thymol Derivatives 3d−3l. Microwave-assisted click reaction in the presence of Cu(I)/Na ascorbate, to generate the copper(I) catalyst *in situ* was performed, and in a solution DMF (2 mL), in a microwave reactor vessel (10 mL), was added **2a** (0.05 g; 0.27 mmol), commercial azide (1.08 mmol), copper(II) sulfate 0.1 M (81 μL; 0.0081 mmol), and sodium ascorbate (5.2 mg; 0.027 mmol). The reaction mixture was placed in a microwave reactor, 150 W, heated at 70 °C for 10 min, and then cooled to room temperature. After evaporation of the solvent, the residual solid containing the triazolic thymol derivatives were purified by silica gel chromatographic column [ethyl acetate−hexane 7:3 (v/v)].[44−46]

Susceptibility Testing. The susceptibility test was performed for the new compounds **2a−f** and **3d−l** according to the CLSI (Clinical and Laboratory Standards Institute) reference protocol M38-A2 (CLSI 2008) for *Fusarium solani* ATCC 40099.[47] The concentrations ranged from 115 μg mL^{-1} to 0.1. The commercial pesticides thiabendazole and tebuconazole were included in the test. The inoculum was prepared by the spectrophotometric method (530 nm and OD 0.15 to 0.17) to obtain a final concentration between 0.4×10^4 CFU/mL and 5×10^4 CFU/mL. The lowest concentration that resulted in complete inhibition of the microorganism growth compared to the control (microorganism without the drug) was considered MIC (minimum inhibitory concentration). The MIC were determined after 48 h of incubation at 35 °C by visual reading with a reading mirror aid. The results were validated with the strain *A. fumigatus* ATCC 40014. The experiments were performed in duplicate.

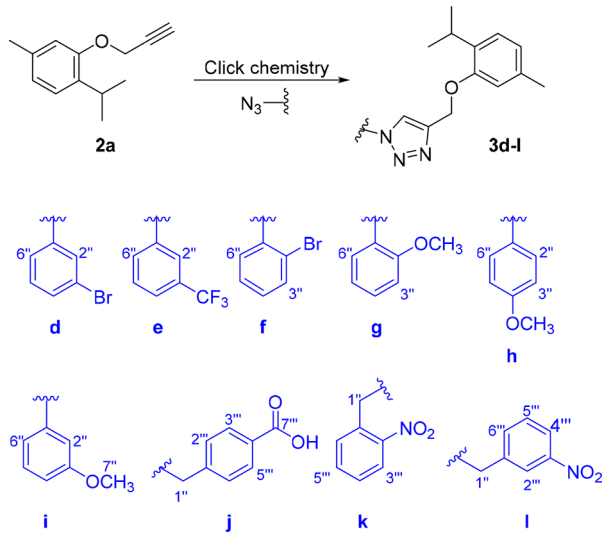
Minimum Fungicidal Concentration. The minimum fungicidal concentration was performed according to Espinel-Ingroff et al. 2002.[48] Briefly, the minimum fungicidal concentration (MFC) was determined by transferring 20 μL from each well-considered MIC that demonstrated complete inhibition (100% or a clear optical well) and 2× MIC (discrete fungal growth) in potato dextrose agate plates. The contents of the wells were not agitated before the application of the specified volumes. The plates were incubated at 35 °C until growth was observed in the growth control subculture (growth of the microorganism in the absence of drug) before 48 h. An MFC was the lowest concentration of drugs that did not grow or had fewer than three colonies (approximately 99 to 99.5% of death activity).

Cytotoxicity Assay. The cytotoxicity assay was performed for **2b**, since it was the compound with the best antifungal activity. A colorimetric MTT assay was proposed by Mosmann.[49] Briefly, L929 fibroblast cells, H1c1c7 hepatocyte cells, and Hacat keratinocyte cells were added to the 96-well microplate at a final concentration of 7×10^5 cells/mL, followed by incubation within 18 h. After the incubation period, the cells were exposed to different concentrations of naphthoquinone thymol (1 μg mL^{-1}, 10 μg mL^{-1} and 100 μg mL^{-1}) and incubated for a further 24 h. Then 0.1 mL of MTT (1 mg mL^{-1}) was added to the microplate and incubated for a further 2 h. Next, 0.1 mL of DMSO was added to dissolve the formazan crystals. The purple formazan absorbance, proportional to the number of viable cells, was measured at 595 nm using a microplate reader (Molecular Devices, Spectra Max 190, Sunnyvale, CA). The experiments were performed in triplicate.

RESULTS AND DISCUSSION

Chemistry. The synthetic route designed to achieve thymol derivatives **2a−f** and **3d−l** is outlined in Schemes 1 and 3. The

Scheme 3. Azide Compounds d−l, Sodium Ascorbate, Copper(II) Sulfate, Dimethylformamide, MW 70 °C, 10 min



synthesis began with the Williamson method applied to commercially available thymol (**1**) and alkyl/aryl halides in basic medium (K_2CO_3), in acetone at room temperature, to give ethers derivatives **2a−f**. This classic method occurs initially with deprotonation of phenolic hydroxyl in **1**, followed by the nucleophilic attack to halides. These steps proceeded in good yield and can be conducted on a multigram scale mainly for **2a−c** compounds (Table 1). The pivotal intermediate 6-*O*-

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Table 1. Spectral Data of Compounds 2a−2f

compd	mol formula	yield (%)	Rf (hexane/ether)	M^+ (m/z)	^{1}H NMR (400 MHz, $CDCl_3$ or DMSO-d_6) (δ ppm)	^{13}C NMR (100 MHz, $CDCl_3$ or DMSO-d_6) (δ ppm)
2a	$C_{13}H_{16}O$	70.0	0.81 (9:1)	HRESIMS [M + Na]$^+$ Found: 211.1090. Calc. for $C_{13}H_{16}NaO^+$: 211.1093.	1.20 (6H, d, CH_3-8 and CH_3-9); 2.32 (3H, s, CH_3-10); 2.48 (1H, t, $J_{3',1'}$ 2.44, H-3′) 3.26−3.34 (1H, m, H-7); 4.69 (2H, d, $J_{1',3'}$ 2.44, CH_2−1′); 6.76 (1H, s, H-6); 6.79 (1H, d, $J_{4,3}$ 7.82, H-4); 7.11 (1H, d. $J_{3,4}$ 7.82, H-3).	21.3 (C-10); 22.9 (C-8 and C-9); 26.5 (C-7); 56.1 (C-1′); 75.0 (C-3′); 79.1 (C-2′); 113.1 (C-6); 122.2 (C-4); 126.1 (C-3); 134.8 (C-2); 136.3 (C-5); 154.8 (C-1).
2b	$C_{20}H_{17}ClO_3$	84.1	0.56 (10:1)	HRESIMS [2 M + Na]$^+$ Found: 703.1629. Calc. for $C_{40}H_{34}Cl_2NaO_6^+$: 703.1625.	1.24 (6H, d, CH_3-8 and CH_3-9); 2.11 (3H, s, CH_3-10);3.28−3.33 (1H, m, H-7); 6.80 (1H, s, H-6); 6.85 (1H, d, $J_{4,3}$ 7.82, H-4); 7.19 (1H, d, $J_{3,4}$ 7.82. H-3); 7.87 (2H, m. $J_{4',5'}$ 8.60, H-4′ and H-5′); 7.95 (1H, dd, $J_{3',6'}$ 8.60, H-3′); 8.09 (1H. dd. $J_{6',4'}$ 8.60, H-6′).	20.8 (C-10); 23.0 (C-8. C-9); 27.0 (C-7); 115.7 (C-6); 124.7 (C-4); 126.8 (C-3); 126.9 (C-3′); 127.0 (C-6′); 131.1 (C-2′); 131.9 (C-7′); 133.9 (C-2); 134.8 and 135.0 (C-4′ and C-5′); 136.8 (C-5); 154.1 (C-1); 178.3 (C-1′); 178.5 (C-8′).
2c	$C_{19}H_{19}NO_3$	76.5	0.33 (7:3)	HRESIMS [M + Na]$^+$ Found: 332.1245. Calc. for $C_{19}H_{19}NNaO_3^+$: 332.1263	1.14 (6H, d, CH_3-8 and CH_3-9); 2.33 (3H, s, CH_3-10); 3.22−3.29 (1H, m, H-7); 5.64 (2H, s, CH_2−1′); 6.81 (1H, d, $J_{4,3}$ 7.82, H-4); 6.99 (1H, s, H-6); 7.09 (1H, d, $J_{3,4}$ 7.82, H-3); 7.73−7.76 (2H, m, $J_{5'',7''}$ 8.21, H-5″and H-6″); 7.89−7.91 (2H, m, $J_{4'',6''}$ 8.21, H-4″ and H-7″).	21.2 (C-10); 23.0 (C-8. C-9); 26.4 (C-7); 65.7 (C-1′); 115.0 (C-6); 123.1 (C-4); 123.8 (C-4″ and C-7″); 126.2 (C-3); 131.8 (C-3″ and C-8″); 134.5 (C-2); 135.5 (C-5″ and C-6″); 136.4 (C-5); 153.3 (C-1); 167.8 (2″ and 9″).
2d	$C_{17}H_{19}NO_3$	52.4	0.34 (7:3)	HRESIMS [M + Na]$^+$ Found: 308.1260 Calc. for $C_{17}H_{19}NNaO_3^+$: 308.1263	1.25 (6H, d, CH_3-8 and CH_3-9); 2.31 (3H, s, CH_3-10); 3.34−3.40 (1H, m, H-7); 5.17 (2H, s, CH_2−1′); 6.69 (1H, s, H-6); 6.80 (1H, d, $J_{4,3}$ 7.78, H-4); 7.15 (1H, d, $J_{3,4}$ 7.78, H-3); 7.62 (2H, d, $J_{2'',3''}$ 8.69, H-2″ and H-6″); 8.26 (2H, d, $J_{3'',6''}$ 8.69, H-3″ and H-5″).	21.3 (C-10); 22.8 (C-8 and C-9); 26.7 (C-7); 68.7 (C-1′); 112.6 (C-6); 122.1 (C-4); 123.9 (C-3″. C-5″); 126.2 (C-3); 127.3 (C-2″. C-6″); 134.3 (C-2); 136.51 (C-5); 145.1 (C-1″); 147.5 (C-4″); 155.2 (C-1).
2e	$C_{12}H_{15}NO$	58.7	0.73 (9:1)	HRESIMS [M + Na]$^+$ Found: 212.1045. Calc. for $C_{12}H_{15}NaO^+$: 212.1046.	1.12 (6H, d, CH_3-8 and CH_3-9); 2.26 (3H, s, CH_3-10); 3.11−3.18 (1H, m, H-7); 5.13 (2H, s, CH_2−1′); 6.82 (1H, d, $J_{4,3}$ 7.82, H-4); 6.89 (1H, s, H-6); 7.11 (1H, d, $J_{3,4}$ 7.82, H-3).	21.3 (C-10); 23.1 (C-8. C-9); 26.4 (C-7); 54.2 (C-1′); 113.6 (C-6); 117.3 (C-2′); 123.5 (C-4); 126.6 (C-3); 134.3 (C-2); 136.6 (C-5); 153.9 (C-1).
2f	$C_{24}H_{26}O_3S$	43.0	0.19 (7:3)	HRESIMS [M + Na]$^+$ Found: 417.1494 Calc. for $C_{24}H_{26}NaO_3S^+$: 417.1500	1.14 (6H, d, CH_3-8 and CH_3-9); 2.34 (3H, s, CH_3-10); 3.18−3.24 (1H, m, H-7); 4.52 (2H, s, CH_2−1‴); 4.93 (2H, s, CH_2−1′); 6.69 (1H, s, H-6); 6.79 (1H, d, $J_{4,3}$ 7.78, H-4); 7.05 (1H, d, $J_{6'',5''}$ 8.24, H-6″); 7.10 (1H, d, $J_{3,4}$ 7.78, H-3); 7.24 (1H, m, $J_{5'',3''}$ 8.24, H-5″); 7.37 (1H, t, $J_{4''',3'''}$ 7.47, H-4‴′); 7.47 (3H, m, $J_{3'',4''}$ 8.24, $J_{4'',6''}$ 8.24, $J_{3''',5'''}$ 7.47, H-3″, H-4″ and H-3‴′); 7.63 (1H, m, $J_{5''',6'''}$ 7.47, H-5‴′); 7.68 (2H, m, $J_{2''',4'''}$ 7.47, $J_{6''',3'''}$ 7.47, H-2‴′ and H-6‴′).	21.4 (C-10); 22.9 (C-8. C-9); 26.4 (C-7); 59.6 (C-1′); 68.2 (C-1‴); 112.8 (C-6); 121.8 (C-4); 126.03 (C-3); 126.56 (C-2″); 128.2 (C-4″); 128.6 (C-3‴′); 129.6 (C-5″); 132.6 (C-5‴′); 133.9 (C-2); 136.5 (C-5); 137.6 (C-1″); 138.8 (C-1‴′); 155.5 (C-1).

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Table 2. Spectral Data of Compounds 3d–l

compd	mol formula	yield (%)	Rf (hexane/ether)	M^+ (m/z)	^{1}H NMR (400 MHz, $CDCl_3$ or DMSO-d_6) (δ ppm)	^{13}C NMR (100 MHz, $CDCl_3$ or DMSO-d_6) (δ ppm)
3d	$C_{19}H_{20}BrN_3O$	34.3	0.10 (9:1)	HRESIMS [M + Na]$^+$ Found: 408.0693 Calc. for $C_{19}H_{20}BrN_3NaO^+$: 408.0687	1.05 (6H, d, CH_3-8 and CH_3-9); 2.24 (3H, s, CH_3-10); 3.10−3.18 (1H, m, H-7); 5.16 (2H, s, CH_2−1′); 6.70 (1H, d, $J_{4,3}$ 7.82, H-4); 6.95 (1H, s, H-6); 7.02 (1H, d, $J_{3,4}$ 7.82, H-3); 7.52 (1H, t, $J_{5'',4''}$ 8.21, H-5″); 7.66 (1H, dd, $J_{4'',6''}$ 8.21, H-4″); 7.92 (1H, dd, $J_{6'',5''}$ 8.21, H-6″); 8.13 (1H, t, H-2″); 8.91 (1H, s, H-triazole).	21.4 (C-10); 23.1 (C-8 and C-9); 26.3 (C-7); 61.7 (C-1′); 113.6 (C-6); 119.6 (C-4″); 122.1 (C-4); 122.9 (C-2″); 123.1 (C-3′); 123.1 (C-1″);126.1 (C-3); 132.0 (C-6″); 132.3 (C-5″); 133.9 (C-2); 136.4 (C-5); 138.1 (C-3″); 144.9 (C-2′); 155.4 (C-1).
3e	$C_{20}H_{20}F_3N_3O$	63.0	0.20 (9:1)	HRESIMS [M + Na]$^+$ Found: 398.1461 Calc. for $C_{20}H_{20}F_3N_3NaO^+$: 398.1456	1.06 (6H, d, CH_3-8 and CH_3-9); 2.24 (3H, s, CH_3-10); 3.13−3.20 (1H, m, H-7); 5.19 (2H, s, CH_2−1′); 6.70 (1H, d, $J_{4,3}$ 7.82, H-4); 6.96 (1H, s, H-6); 7.10 (1H, d, $J_{3,4}$ 7.82, H-3); 7.83−7.77 (2H, m, $J_{5'',6''}$ 8.21, H-5″ and H-6″); 8.27−8.20 (2H, m, $J_{4'',5''}$ 8.21, H-2″ and H-4″); 9.05 (1H, s, H-triazole).	21.3 (C-10); 23.1 (C-8, C-9); 26.3 (C-7); 61.8 (C-1′); 113.6 (C-6); 117.2 (C-2″); 122.0 (C-4); 123.3 (C-3′); 124.4 (C-4″); 125.7 (C-6″); 126.1 (C-3); 131.7 (C-5″); 133.9 (C-2); 136.4 (C-5); 137.4 (C-3″); 145.0 (C-2′); 155.4 (C-1).
3f	$C_{19}H_{20}BrN_3O$	92.0	0.47 (7:3)	HRESIMS [M + H]$^+$ Found: 386. 0873 Calc. for $C_{19}H_{21}BrN_3O^+$: 386.0868.	1.08 (6H, d, CH_3-8 and CH_3-9); 2.25 (3H, s, CH_3-10); 3.13−3.20 (1H, m, H-7); 5.21 (2H, s, CH_2−1′); 6.71 (1H, d, $J_{4,3}$ 7.82, H-4); 6.97 (1H, s, H-6); 7.03 (1H, d, $J_{3,4}$ 7.82, H-3); 7.52 (1H, td, $J_{6'',5''}$ 7.82, H-6″); 7.59 (1H, td, $J_{5'',4''}$ 7.82, H-5″); 7.63 (1H, dd, $J_{4'',6''}$ 7.82, H-4″); 7.88 (1H, dd, $J_{3'',5''}$ 7.82, H-3″); 8.58 (1H, s, H-triazole).	21.4 (C-10); 23.1 (C-8, C-9); 26.5 (C-7); 61.9 (C-1′); 113.8 (C-6); 119.2 (C-1″); 122.0 (C-4); 126.1 (C-3); 126.8 (C-3′); 129.1 (C-4″); 129.4 (C-5″); 132.4 (C-6″); 134.0 (C-2); 134.1 (C-3″); 136.4 (C-2″); 136.5 (C-5); 143.7 (C-2′); 155.5 (C-1).
3g	$C_{20}H_{23}N_3O_2$	39.8	0.36 (7:3)	HRESIMS [M + H]$^+$ Found: 338.1861 Calc. for $C_{20}H_{24}N_3O_2^+$: 338.1869.	1.08 (6H, d, CH_3-8 and CH_3-9); 2.26 (3H, s, CH_3-10); 3.12−3.19 (1H, m, H-7); 3.82 (3H, s, O−CH_3); 5.19 (2H, s, CH_2−1′); 6.71 (1H, d, $J_{4,3}$ 7.82, H-4); 6.98 (1H, s, H-6); 7.03 (1H, d, $J_{3,4}$ 7.82, H-3); 7.12 (1H, t, $J_{5'',6''}$ 8.60, H-5″); 7.29 (1H, d, $J_{3'',5''}$ 8.60, H-3″); 7.50 (1H, m, $J_{4'',6''}$ 8.60, H-4″); 7.62 (1H, dl, $J_{6'',3''}$ 8.60, H-6″); 8.51 (1H, s, H-triazole).	21.4 (C-10); 23.1 (C-8, C-9); 26.5 (C-7); 56.5 (O−CH_3); 61.8 (C-1′); 113.5 (C-3″); 113.7 (C-6); 121.3 (C-5″); 121.9 (C-4); 126.1 (C-6″); 126.1 (C-3); 126.6 (C-3′); 131.1 (C-4″); 133.9 (C-2); 136.3 (C-5); 143.4 (C-2′); 151.6 (C-2″); 155.5 (C-1).
3h	$C_{20}H_{23}N_3O_2$	36.2	0.27 (7:3)	HRESIMS [M + H]$^+$ Found: 338.1863 Calc. for $C_{20}H_{24}N_3O_2^+$: 338.1869.	0.94 (6H, d, CH_3-8 and CH_3-9); 2.20 (3H, s, CH_3-10); 2.78−2.86 (1H, m, H-7); 3.79 (3H, s, O−CH_3); 5.17 (2H, s, CH_2−1′); 6.70 (1H, d, $J_{4,3}$ 7.82, H-4); 6.77 (1H, s, H-6); 6.99 (1H, d, $J_{3,4}$ 7.82, H-3); 7.09 (2H, dd, $J_{2'',3''}$ 7.43, H-2″ and H-6″); 7.53 (2H, dd, $J_{5'',6''}$ 7.43, H-3″ and H-5″); 7.98 (1H, s, H-triazole).	21.3 (C-10); 23.1 (C-8, C-9); 25.9 (C-7); 56.0 (O−CH_3); 58.8 (C-1′); 113.3 (C-6); 115.1 (C-2″ and C-6″); 122.3 (C-4); 126.1 (C-3); 126.4 (C-3″ and C-5″); 129.3 (C-1″); 133.9 (C-2′); 134.3 (C-2); 134.9 (C-3′); 136.3 (C-5); 154.7 (C-1); 160.4 (C-4″).
3i	$C_{20}H_{23}N_3O_2$	39.3	0.20 (9:1)	HRESIMS [M + H]$^+$ Found: 338.1861 Calc. for $C_{20}H_{24}N_3O_2^+$: 338.1869.	1.06 (6H, d, CH_3-8 and CH_3-9); 2.24 (3H, s, CH_3-10); 3.10−3.17 (1H, m, H-7); 3.82 (3H, s, O−CH_3); 5.16 (2H, s, CH_2−1′); 6.71 (1H, d, $J_{4,3}$ 7.82, H-4); 6.96 (1H, s, H-6); 7.01−7.06 (2H, m, $J_{6'',5''}$ 7.43, $J_{3,4}$ 7.82, H-6″ and H-3); 7.43 (1H, d, $J_{2'',4''}$ 7.43, H-2″); 7.45 (1H, t, $J_{5'',6''}$ 7.43, H-5″); 7.47 (1H, d, $J_{4'',5''}$ 7.43, H-4″); 8.87 (1H, s, H-triazole).	21.4 (C-10); 23.1 (C-8 and C-9); 26.4 (C-7); 56.0 (O−CH_3); 61.7 (C-1′); 106.2 (C-2″); 112.6 (C-4″); 113.6 (C-6); 114.9 (C-6″); 122.0 (C-4); 123.1 (C-3′); 126.1 (C-3); 131.4 (C-5″); 133.9 (C-2); 136.4 (C-5); 138.0 (C-1″); 144.6 (C-2′); 155.4 (C-1); 160.6 (C-3″).
3j	$C_{21}H_{23}N_3O_3$	4.9	0.14 (7:3)	HRESIMS [M + H]$^+$ Found: 366.1817 Calc. for $C_{21}H_{24}N_3O_3^+$: 366.1818.	1.05 (6H, d, CH_3-8 and CH_3-9); 2.23 (3H, s, CH_3-10); 3.06−3.12 (1H, m, H-7); 5.10 (2H, s, CH_2−1′); 5.68 (2H, s, CH_2−1″); 6.69 (1H, d, $J_{4,3}$ 7.82, H-4); 6.90 (1H, s, H-6); 7.01 (1H, d, $J_{3,4}$ 7.82, H-3); 7.32 (2H, d, $J_{2''',5'''}$ 8.60, H-2‴ and H-6‴); 7.89 (2H, d, $J_{3''',6'''}$ 8.60, H-3‴ and H-5‴); 8.25 (1H, s, H-triazole).	21.4 (C-10); 23.1 (C-8 and C-9); 26.4 (C-7); 52.8 (C-1″); 62.0 (C-1′); 113.7 (C-6); 121.9 (C-4); 125.0 (C-3′); 126.1 (C-3); 128.2 (C-2‴ and C-6‴); 130.1 (C-3‴ and C-5‴); 133.9 (C-2); 136.3 (C-5); 140.9 (C-1‴); 143.9 (C-2′); 155.4 (C-1); 167.5 (C-OOH).
3k	$C_{20}H_{22}N_4O_3$	83.7	0.20 (7:3)	HRESIMS [M + H]$^+$ Found: 367.1693 Calc. for $C_{20}H_{23}N_4O_3^+$: 367.1770.	1.06 (6H, d, CH_3-8 and CH_3-9); 2.23 (3H, s, CH_3-10); 3.08−3.15 (1H, m, H-7); 5.14 (2H, s, CH_2−1′); 5.96 (2H, s, CH_2−1″); 6.69 (1H, d, $J_{4,3}$ 7.82, H-4); 6.91 (1H, s, H-6); 6.99 (1H, d, $J_{6''',3'''}$ 8.21, H-6‴); 7.01 (1H, d, $J_{3,4}$ 7.82, H-3); 7.60 (1H, t, $J_{5''',4'''}$ 8.21, H-5‴); 7.69 (1H, t, $J_{4''',3'''}$ 8.21, H-4‴); 8.11 (1H, d, $J_{3''',5'''}$ 8.21, H-3‴); 8.22 (1H, s, H-triazole).	21.4 (C-10); 23.0 (C-8 and C-9); 26.5 (C-7); 50.4 (C-1″); 62.0 (C-1′); 113.7 (C-6); 121.9 (C-4); 125.5 (C-3′); 125.5 (C-3‴); 126.1 (C-3); 130.0 (C-5‴); 130.3 (C-6‴); 131.4 (C-1‴); 133.8 (C-2); 134.7 (C-4‴); 136.3 (C-5); 144.0 (C-2′); 148.0 (C-2‴); 155.4 (C-1).
3l	$C_{20}H_{22}N_4O_3$	42.4	0.13 (9:1)	HRESIMS [M + H]$^+$ Found: 367.1764 Calc. for $C_{20}H_{23}N_4O_3^+$: 367.1770.	1.02 (6H, d, CH_3-8 and CH_3-9); 2.21 (3H, s, CH_3-10); 3.04−3.11 (1H, m, H-7); 5.09 (2H, s, CH_2−1′); 5.75 (1H, s, CH_2−1″); 6.68 (1H, d, $J_{4,3}$ 7.82, H-4); 6.80 (1H, s, H-6); 6.99 (1H, d, $J_{3,4}$ 7.82, H-3); 7.64 (1H, t, $J_{5''',2'''}$ 8.60, H-5‴); 7.73 (1H, d, $J_{6''',5'''}$ 8.60, H-6‴); 8.15 (2H, m, $J_{2''',5'''}$ 8.60; $J_{4''',2'''}$ 8.60, H-4‴ and H-2‴); 8.29 (1H, s, H-triazole).	21.3 (C-10); 22.3 (C-8 and C-9); 26.4 (C-7); 52.2 (C-1″); 61.9 (C-1′); 113.7 (C-6); 122.0 (C-4); 123.1 (C-2‴); 123.5 (C-4‴); 125.1 (C-3); 130.8 (C-5‴); 133.9 (C-2); 135.1 (C-6‴); 136.3 (C-5); 138.5 (C-1‴); 144.1 (C-2′), 148.3 (C-3‴); 155.4 (C-1).

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propargyl thymol **2a** was obtained as the precursor of triazole derivatives **3d**−**l**, and strategically speaking, a noteworthy perspective must be given to compound **2b** because it is easily obtained, in one step, with the molecular hybridization approach. This useful concept in medicinal chemistry employs a combination of pharmacophoric portions of bioactive compounds with improved affinity and efficacy.

The greatest synthetic success encountered during our efforts was fabricating nine triazole heterocyclic compounds' thymol derivatives. In brief, the facile one-pot CuAAC methodology was used to prepare **3d**−**l**, through Huisgen's 1,3-dipolar cycloaddition between alkyne and azide, which can be performed in water with an organic cosolvent. The microwave-assisted technique had become a landmark and substantial contribution to mantain the environment by decreasing the waste as well, as it gives a useful internal heat transfer that shortens the reaction time and increases the rate of reaction and yield.[50−53]

O-Propargyl thymol (**2a**) was then used to optimize the 1,3-dipolar cycloaddition with 4 molar equiv of azides' derivatives, under microwave conditions to shorten reaction times, in the presence of $CuSO_4$/sodium ascorbate in DMF to yield the triazole derivatives. The solids obtained were washed with water/ethyl acetate, and the organic phase was dried in a rotary evaporator. The crude products were purified by column chromatography using hexane:ethyl acetate or hexane:ether as eluent to afford the desired compounds (Table 2).

During the procedure to prepare the triazol derivatives, the following conditions were tested: changing temperature, 70 or 80 °C; ratio of azide reagents, 3 and 4 molar equiv; and time, 10, 15, and 20 min. The complete consumption of compound **2a** was observed only using the ratio of 4 molar equiv, under microwave conditions, which made the purification easier and faster. However, using this ratio, some yields were not satisfactory.

Spectral data of the 15 successful derivatives prepared were confirmed from ^{1}H NMR, ^{13}C NMR, FTIR, and HRMS. The ^{1}H NMR spectrum exhibited four sets of aromatic hydrogen signals characteristic of the expected moiety of **1**. In addition, isopropyl and methyl hydrogens of the natural product also appeared at δ 1.12−1.25 (CH_3-8 and CH_3-9) as a doublet and 2.11−2.34 (CH_3-10) as a singlet in **2a**−**f**, while δ 0.94−1.08 (CH_3-8 and CH_3-9) and 2.20−2.26 (CH_3-10) appeared in **3d**−**l**. Furthermore, the methylenic group (CH_2−1″), in whole derivatives except **2b**, is shown in the range δ 4.69−5.64 as a singlet (doublet for **2a**), and the substituent signals are in agreement with the nature of an aromatic moiety. The hydrogen of the triazole ring appears in the range δ 7.98−9.05 as a singlet and plays a crucial role in identifying of thymol triazolic derivatives **3d**−**l**.

Infrared spectroscopy confirmed the presence of characteristic absorption bands for the derivatives prepared. The IR spectra showed the following for compounds **2**: two bands related to the ether group linked to C═C bond at 1246 and 1029 cm^{-1} (**2a**), 1237 and 1073 cm^{-1} (**2b**), 1257 and 1052 cm^{-1} (**2d**), 1239 and 1048 cm^{-1} (**2e**), and 1254 and 1034 cm^{-1} (**2f**). An absorption band at 2119 cm^{-1} verified compound **2a** and corresponded to the triple bond C−C, while 720 cm^{-1} was detected to the C−Cl bond for compound **2b**. Two vibration bands were observed at 1502 and 1330 cm^{-1} and related to the nitro group (compound **2d**), and two bands at 1315 and 1150 cm^{-1} corresponded to the sulfonyl group (compound **2f**). In the IR spectra for compounds **3**, the characteristic absorption bands for azide scaffold were revealed at 2102 cm^{-1} (**3d**−**f** and **3k**) and 2106 cm^{-1} (**3h** and **3i**), and for compound **3l**, the corresponding band appeared at 2108 cm^{-1}.

Mass spectrometry analysis validated the structure of the examined derivatives (Tables 1 and 2).

Fungicidal Activity. All the target compounds were screened for their inhibitory activity against *Fusarium solani* ATCC 40099. Fusarium species show reduced sensitivity to azole compounds because of an intrinsic resistance as observed in previous works.[48,54] The low sensitivity is reflected in susceptibility assays characterized by high minimum inhibitory concentration values or absence of activity observed for the compound tebuconazole (Table 3), a triazole pesticide commonly used in crops. The new triazolic compounds **3d**−**l** described here in this work are present in structural groups such as halogenated, carboxylic acid, and nitro with broad biological properties. The Fusarium strain showed moderate sensitivity to the new triazole compounds, characterized by the MIC 57 μg/mL. The literature reports that triazole compounds with alkyl chains and/or aromatic substituents containing halogenated radical have important antimicrobial activities, and therefore, they have been widely used in medicinal chemistry research to produce molecules with specificity to the target enzyme, potency, and a broad spectrum of action.[55] The compound thiabendazole is a benzimidazole that acts as an inhibitor of nuclear division by binding to the tubulin protein. Its mechanism of action different from triazole compounds explains the lower MIC value observed for the Fusairum strain in the present work.[56]

In this study, most azole compounds tested showed a moderate MIC, 57 μg mL^{-1}, or low sensitivity, in comparison with thiabendazole, 7 μg mL^{-1}, although they are more active than tebuconazole (absence of activity), which leads to an important perspective of molecular hybridization by coupling

Table 3. Antifungal Activity for New Compounds and Commercial Pesticides against *Fusarium solani* ATCC 40099[a]

Fusarium solani ATCC 40099	
entry	MIC (μg mL^{-1})
2a	-[b]
2b	14
2c	-
2d	-
2e	-
2f	-
3d	57
3e	-
3f	57
3g	57
3h	57
3i	57
3j	57
3k	-
3l	57
thiabendazole	7
tebuconazole	-

[a]Results were validated with strain *A. fumigatus* ATCC 40014 and showed MIC values of 115 μg mL^{-1} for all compounds tested. [b]-absence of activity.

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triazole and natural products' scaffolds toward to an antifungal synergism. This concept can be corroborated with a simple structure activity relationship based on the MIC result for compounds **2d** and **3l** because, although both present the nitro group, only the triazole derivative showed an inhibition.

Strengthening the concept of synergism from the coupling between natural products scaffold, the MIC result observed for the most active compound **2b**, thymol/naphthoquinone hybrid, 14 μg mL^{-1}, deeply illustrates the importance of the mentioned since presented as an inhibitor quite similar to thiabendazole (Table 3). This promising compound has great potential as an agrochemical because of the facile and low-cost synthesis even with similar potency to control. In addition azole compounds have fungistatic activity for most filamentous fungi, as evidenced by the MFC test in this study. The microorganism returned to growth after not being in contact with the azole drug. However, fungicidal activity was observed for compound **2b** and thiabendazole (Table 4), and the fungicidal action makes the use of these compounds more attractive. They reduce the possibility of resistance development over time.

Table 4. Fungicidal Activity for Hybrid Compound 2b and Commercial Pesticide Thiabendazole against *Fusarium solani*[a]

Fusarium solani ATCC 40099	
entry	MFC (μg mL^{-1})
2b	14
thiabendazole	7

[a]Results were validated with strain *A. fumigatus* ATCC 40014 and showed MIC values of 115 μg mL^{-1} for all compounds tested.

Since agricultural fungicides are dispersed in the environment and promote acute effects on human organisms because of long-term exposure,[57] the MTT bioassay was performed to evaluate the cytotoxic effect of compound **2b** against human cells by determining the IC_{50} value, as expressed in Table 5.

Table 5. Expresses the Cytotoxic Concentration Capable of Killing Fibroblast (L929), Hepatocyte (H1c1c7), and Keratinocyte (Hacat) Cells by 50% (IC_{50})

compound	cell viability (μg mL^{-1})		
	IC_{50}		
	L929	H1c1c7	Hacat
2b	4.0 ± 0.3	7.2 ± 3.4	19.0 ± 6.0

Compound **2b** revealed a cytotoxic effect for hepatocytes (H1c1c7, IC50 7.2 ± 3.4), fibroblasts (L929, IC50 4.0 ± 0.3), and keratinocytes (Hacat, 19.0 ± 6.0). This result is comparable to the obtained by Portis et al. (2015), who evaluated the cytotoxic profile of Tebuconazole, which can be harmful to the health of individuals who handle this product.[58] However, the MTT assay performed in this study is not conclusive to assess the cytotoxic profile of compound **2b**.

Further insight into the mode of action of the hybrid compound **2b** can be predicted from thymol and naphthoquinone antifungal mode of action as an isolated nucleus. The literature describes that thymol antifungal properties are not related to the biosynthetic pathways of the cell wall. In contrast, the MIC value of thymol against *C. albicans* increased 8 times in the presence of exogenous ergosterol concluding that thymol appears to bind to the ergosterol in the membrane, increasing ion permeability and occurring cell death.[59] Concerning the naphthoquinone nucleus, a recent study revealed that it did not interfere with the fungal cell wall synthesis in the presence of sorbitol as osmotic support because the synthetic naphthoquinones MIC value did not change, and the addition of free exogenous ergosterol showed that the MIC of the naphthoquinone derivatives against *C. albicans* ATCC 36232 did not change in the presence of different concentrations of exogenous ergosterol because it is unable to induce changes in the fungal membrane. In contrast, a potassium efflux test was conducted to evaluate whether synthetic naphthoquinones interfere with the osmotic balance of *C. albicans* (ATCC 36232), which revealed that the efflux of K^+ into the extracellular medium increased with the naphthoquinones treatment.[60]

Through our synthetic efforts, we investigated the antifungal activity of semisynthetic thymol derivatives against *Fusarium solani*. We have shown that the proposed synthetic methods, Williamson and CuAAC, were quite plausible. It was discovered that most triazolic derivatives revealed moderated MIC against *Fusarium solani* sp. compared with the control thiabendazole and in contrast with the low sensitivity broadly encountered for azoles, such as tebuconazole assayed herein. The further discovery showed **2b** as a promising antifungal agent against *Fusarium solani* with MIC similar to the positive control. We have also provided a possible mode of action supporting the interesting antifungal activity of the hydrid ether **2b**. Therefore, our results indicate that the thmyol is a potential building block for further chemical investigations toward new agrochemicals applied to the root rot fungal disease occasioned by *Fusarium solani*.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.0c07439.

^{1}H NMR spectra of **2a−f** and **3d−l**; ^{13}C NMR spectra of **2a−f** and **3d−l**; COSY, HMBc, and HSQC spectra **2a−f** and **3d−l**; infrared (FTIR) spectra of **2a−f** and **3d−l**; high-resolution mass spectrometry (HRMS) spectra of **2a−f** and **3d−l** (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

Valdemar Lacerda, Jr. − *Programa de Pós-Graduação em Química, Universidade Federal do Espírito Santo, 29075910 Vitória, Espirito Santo, Brazil*; Email: valdemar.lacerda@ufes.br

Pedro Alves Bezerra Morais − *Programa de Pós-Graduação em Agroquímica, Universidade Federal do Espírito Santo, 29500000 Alegre, Espirito Santo, Brazil*; orcid.org/0000-0001-5501-7350; Email: pedro.morais@ufes.br

Authors

Mariana Alves Eloy − *Programa de Pós-Graduação em Agroquímica, Universidade Federal do Espírito Santo, 29500000 Alegre, Espirito Santo, Brazil*

Rayssa Ribeiro − *Programa de Pós-Graduação em Agroquímica, Universidade Federal do Espírito Santo, 29500000 Alegre, Espirito Santo, Brazil*

Leandra Martins Meireles − *Programa de Pós-Graduação em Ciências Farmacêuticas, Universidade Vila Velha, 29103-900 Vila Velha, Espirito Santo, Brazil*
Thiago Antonio de Sousa Cutrim − *Programa de Pós-Graduação em Ciências Farmacêuticas, Universidade Vila Velha, 29103-900 Vila Velha, Espirito Santo, Brazil*
Carla Santana Francisco − *Programa de Pós-Graduação em Química, Universidade Federal do Espírito Santo, 29075910 Vitória, Espirito Santo, Brazil*
Clara Lirian Javarini − *Programa de Pós-Graduação em Química, Universidade Federal do Espírito Santo, 29075910 Vitória, Espirito Santo, Brazil*
Warley de Souza Borges − *Programa de Pós-Graduação em Química, Universidade Federal do Espírito Santo, 29075910 Vitória, Espirito Santo, Brazil*
Adilson Vidal Costa − *Programa de Pós-Graduação em Agroquímica, Universidade Federal do Espírito Santo, 29500000 Alegre, Espirito Santo, Brazil*
Vagner Tebaldi de Queiroz − *Programa de Pós-Graduação em Agroquímica, Universidade Federal do Espírito Santo, 29500000 Alegre, Espirito Santo, Brazil*
Rodrigo Scherer − *Programa de Pós-Graduação em Ciências Farmacêuticas, Universidade Vila Velha, 29103-900 Vila Velha, Espirito Santo, Brazil*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jafc.0c07439

Author Contributions

[#]V.L. and P.A.B.M. contributed equally to this work.

Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Singh, P. K. Synthesis and fungicidal activity of novel 3-(substituted/unsubstituted phenylselenonyl)-1-ribosyl/deoxyribosyl-1 H-1, 2, 4-triazole. *J. Agric. Food Chem.* **2012**, *60* (23), 5813−5818.
(2) Sui, G.; Zhang, W.; Zhou, K.; Li, Y.; Zhang, B.; Xu, D.; Zou, Y.; Zhou, W. Trialkylamine derivatives containing a triazole moiety as promising ergosterol biosynthesis inhibitor: design, synthesis, and antifungal activity. *Chem. Pharm. Bull.* **2017**, *65* (1), 82−89.
(3) Bräse, S.; Encinas, A.; Keck, J.; Nising, C. F. Chemistry and biology of mycotoxins and related fungal metabolites. *Chem. Rev.* **2009**, *109* (9), 3903−3990.
(4) Hou, Z.; Zhu, L.-F.; Yu, X.-c.; Sun, M.-Q.; Miao, F.; Zhou, L. Design, Synthesis, and Structure−Activity Relationship of New 2-Aryl-3, 4-dihydro-β-carbolin-2-ium Salts as Antifungal Agents. *J. Agric. Food Chem.* **2016**, *64* (14), 2847−2854.
(5) Breitmaier, E. *Terpenes: flavors, fragrances, pharmaca, pheromones*; John Wiley & Sons: 2006.
(6) Lazarević, J. S.; Kolarević, A.; Đorđević, A.; Stojanović, G.; Šmelcerović, A.; Ciuffreda, P.; Santaniello, E. Synthesis, antimicrobial activity and in silico studies on thymol esters. *Acta Chim. Slov.* **2017**, *64* (3), 603−612.
(7) Hedin, P. A. *Naturally Occurring Pest Bioregulators*; ACS Publications: 1991.
(8) Baker, D. R.; Fenyes, J. G.; Basarab, G. S. *Synthesis and Chemistry of Agrochemicals IV*; ACS Publications: 1995.
(9) Mathela, C. S.; Singh, K. K.; Gupta, V. K. Synthesis and in vitro antibacterial activity of thymol and carvacrol derivatives. *Acta Polym. Pharm.* **2010**, *67* (4), 375−380.
(10) Stojakowska, A.; Malarz, J.; Kisiel, W. Thymol derivatives from a root culture of Inula helenium. *Z. Naturforsch., C: J. Biosci.* **2004**, *59* (7−8), 606−608.
(11) Wajs-Bonikowska, A.; Malarz, J.; Stojakowska, A. Composition of Essential Oils from Roots and Aerial Parts of Carpesium divaricatum, a Traditional Herbal Medicine and Wild Edible Plant from South-East Asia, Grown in Poland. *Molecules* **2019**, *24* (23), 4418.
(12) Zhao, J.; Li, Y.; Liu, Q.; Gao, K. Antimicrobial activities of some thymol derivatives from the roots of Inula hupehensis. *Food Chem.* **2010**, *120* (2), 512−516.
(13) Paolini, J.; Muselli, A.; Bernardini, A. F.; Bighelli, A.; Casanova, J.; Costa, J. Thymol derivatives from essential oil of Doronicum corsicum L. *Flavour Fragrance J.* **2007**, *22* (6), 479−487.
(14) Mezzoug, N.; Elhadri, A.; Dallouh, A.; Amkiss, S.; Skali, N.S.; Abrini, J.; Zhiri, A.; Baudoux, D.; Diallo, B.; El Jaziri, M.; Idaomar, M. Investigation of the mutagenic and antimutagenic effects of Origanum compactum essential oil and some of its constituents. *Mutat. Res., Genet. Toxicol. Environ. Mutagen.* **2007**, *629* (2), 100−110.
(15) Carmona-Aparicio, L.; Cárdenas-Rodríguez, N.; Huerta-Gertrudis, B.; Rodríguez-Chávez, J. L.; Coballase-Urrutia, E., Hetherotheca inuloides (Mexican Arnica) a Potent Antioxidant Effect as Neuro and Hepato-Protective. In *Medicinal Plants*, Vol. *227* Pereira, D. A. M., Ed.; Nova Science Publishers, **2013**.
(16) Wilson, C. O.; Gisvold, O.; Block, J. H.; Beale, J. M. *Wilson and Gisvold's textbook of organic medicinal and pharmaceutical chemistry/ edited by*; Block, J. H., Beale, J. M., Jr., Eds.; Lippincott Williams & Wilkins, Philadelphia: 2004.
(17) Guo, N.; Liu, J.; Wu, X.; Bi, X.; Meng, R.; Wang, X.; Xiang, H.; Deng, X.; Yu, L. Antifungal activity of thymol against clinical isolates of fluconazole-sensitive and-resistant Candida albicans. *J. Med. Microbiol.* **2009**, *58* (8), 1074−1079.
(18) Shin, S.-C.; Park, I.-K.; Choi, I.-H. Nematicidal activity of onion (Allium cepa) oil and its components against the pine wood nematode (Bursaphelenchus xylophilus). *Nematology* **2007**, *9* (2), 231−235.
(19) Kong, J.-O.; Lee, S.-M.; Moon, Y.-S.; Lee, S.-G.; Ahn, Y.-J. Nematicidal activity of plant essential oils against Bursaphelenchus xylophilus (Nematoda: Aphelenchoididae). *J. Asia-Pac. Entomol.* **2006**, *9* (2), 173−178.
(20) Filoche, S.; Soma, K.; Sissons, C. Antimicrobial effects of essential oils in combination with chlorhexidine digluconate. *Oral Microbiol. Immunol.* **2005**, *20* (4), 221−225.
(21) El-Miligy, M. M.; Hazzaa, A. A.; El-Zemity, S. R.; Al-Kubeisi, A. K. Synthesis of Thymol Derivatives as Potential Non-Irritant Antimicrobial and Insecticidal Agents. *Curr. Bioact. Compd.* **2019**, *15* (1), 125−137.
(22) Zaidi, S. F. H.; Yamada, K.; Kadowaki, M.; Usmanghani, K.; Sugiyama, T. Bactericidal activity of medicinal plants, employed for the treatment of gastrointestinal ailments, against Helicobacter pylori. *J. Ethnopharmacol.* **2009**, *121* (2), 286−291.
(23) Tawata, S.; Taira, S.; Kobamoto, N.; Ishihara, M.; Toyama, S. Synthesis and fungicidal activity of new thiophosphorylated monoterpenoids and related compounds. *J. Pestic. Sci. (Tokyo, Jpn.)* **1996**, *21*, 141.

(24) Numpaque, M. A.; Oviedo, L. A.; Gil, J. H.; García, C. M.; Durango, D. L. Thymol and carvacrol: biotransformation and antifungal activity against the plant pathogenic fungi Colletotrichum acutatum and Botryodiplodia theobromae. *Tropical plant pathology* **2011**, *36* (1), 3−13.
(25) Šegvić Klarić, M.; Kosalec, I.; Mastelić, J.; Pieckova, E.; Pepeljnak, S. Antifungal activity of thyme (Thymus vulgaris L.) essential oil and thymol against moulds from damp dwellings. *Lett. Appl. Microbiol.* **2007**, *44* (1), 36−42.
(26) Arnoldi, A.; Dallavalle, S.; Merlini, L.; Musso, L.; Farina, G.; Moretti, M.; Jayasinghe, L. Synthesis and antifungal activity of a series of N-substituted [2-(2, 4-dichlorophenyl)-3-(1, 2, 4-triazol-1-yl)] propylamines. *J. Agric. Food Chem.* **2007**, *55* (20), 8187−8192.
(27) Mares, D.; Romagnoli, C.; Andreotti, E.; Manfrini, M.; Vicentini, C. B. Synthesis and antifungal action of new tricyclazole analogues. *J. Agric. Food Chem.* **2004**, *52* (7), 2003−2009.
(28) Arnoldi, A.; Carzaniga, R.; Morini, G.; Merlini, L.; Farina, G. Synthesis, fungicidal activity, and QSAR of a series of 2-dichlorophenyl-3-triazolylpropyl ethers. *J. Agric. Food Chem.* **2000**, *48* (6), 2547−2555.
(29) Ferreira, E.; Alfenas, A.; Maffia, L.; Mafia, R.; Mounteer, A. Effectiveness of systemic fungicides in the control of Quambalaria eucalypti and their effects on production of eucalypt mini-cuttings for rooting. *Crop Prot.* **2008**, *27* (2), 161−170.
(30) Cardoso, M. F.C.; Rodrigues, P. C.; Oliveira, M. E. I.M.; Gama, I. L.; da Silva, I. M.C.B.; Santos, I. O.; Rocha, D. R.; Pinho, R. T.; Ferreira, V. F.; de Souza, M. C. B.V.; da Silva, F. d. C.; Silva-Jr, F. P. Synthesis and evaluation of the cytotoxic activity of 1, 2-furanonaphthoquinones tethered to 1, 2, 3−1H-triazoles in myeloid and lymphoid leukemia cell lines. *Eur. J. Med. Chem.* **2014**, *84*, 708−717.
(31) Pokhodylo, N.; Shyyka, O.; Matiychuk, V. Synthesis and anticancer activity evaluation of new 1, 2, 3-triazole-4-carboxamide derivatives. *Med. Chem. Res.* **2014**, *23* (5), 2426−2438.
(32) Kharb, R.; Shahar Yar, M.; C. Sharma, P. New insights into chemistry and anti-infective potential of triazole scaffold. *Curr. Med. Chem.* **2011**, *18* (21), 3265−3297.
(33) Melo, J. O. F.; Donnici, C. L.; Augusti, R.; Ferreira, V. F.; Souza, M. C. B. V. d.; Ferreira, M. L. G.; Cunha, A. C. Heterociclos 1, 2, 3-triazólicos: histórico, métodos de preparação, aplicações e atividades farmacológicas. *Quim. Nova* **2006**, *29* (3), 569−579.
(34) Bozorov, K.; Zhao, J.; Aisa, H. A. 1, 2, 3-Triazole-containing hybrids as leads in medicinal chemistry: A recent overview. *Bioorg. Med. Chem.* **2019**, *27* (16), 3511−3531.
(35) Rocha, F. d. S.; Ferreira, G. H. S.; Silva, T. C. S. R.; Amaral, F. L.; Muniz, M. d. F. S.; Pereira, E. A. Caracterização de Fusarium solani f. sp. piperis, produção de fitotoxina e incidência da fusariose no norte de Minas Gerais. *Summa Phytopathologica* **2016**, *42* (1), 67−72.
(36) Tremacoldi, C. Principais doenças fúngicas da pimenteira-do-reino no Estado do Pará e recomendações de controle. *Embrapa Amazônia Oriental. Documentos* **2010**, *24*, 8.
(37) Oliveira, B. S.; Neto, A. P. D.; da Silva, M. B. Pimenta-do-reino: importância da defesa fitossanitária para a sustentabilidade da atividade na região norte do Espírito Santo. *Revista Brasileira de Agropecuária Sustentável* **2011**, *1*, 88−92.
(38) Kong, J.; Xie, Y.; Yu, H.; Guo, Y.; Cheng, Y.; Qian, H.; Yao, W. Synergistic antifungal mechanism of thymol and salicylic acid on Fusarium solani. *LWT* **2021**, *140*, 110787.
(39) Gill, T.; Li, J.; Saenger, M.; Scofield, S. Thymol-based submicron emulsions exhibit antifungal activity against Fusarium graminearum and inhibit fusarium head blight in wheat. *J. Appl. Microbiol.* **2016**, *121* (4), 1103−1116.
(40) Oukhouia, M.; Hamdani, H.; Jabeur, I.; Sennouni, C.; Remmal, A. In-vitro study of anti-Fusarium effect of thymol, carvacrol, eugenol and menthol. *Journal of Plant Pathology & Microbiology* **2017**, *8* (10), 1000423.
(41) Thanh, N. D.; Hai, D. S.; Bich, V. T.N.; Hien, P. T.T.; Duyen, N. T.K.; Mai, N. T.; Dung, T. T.; Van, H. T.K.; Toan, V. N.; Toan, D. N.; Dang, L. H. Using Sodium Hydride and Potassium Carbonate as Bases in Synthesis of Substituted 2-Amino-4-aryl-7-propargyloxy-4H-chromene-3-carbonitriles. *Curr. Org. Synth.* **2019**, *16* (3), 423−430.
(42) Suresh Babu, K.; Hari Babu, T.; Srinivas, P.V.; Hara Kishore, K.; Murthy, U.S.N.; Rao, J. M. Synthesis and biological evaluation of novel C (7) modified chrysin analogues as antibacterial agents. *Bioorg. Med. Chem. Lett.* **2006**, *16* (1), 221−224.
(43) Sa, M. M.; Ramos, M. D.; Fernandes, L. Fast and efficient preparation of Baylis−Hillman-derived (E)-allylic azides and related compounds in aqueous medium. *Tetrahedron* **2006**, *62* (50), 11652−11656.
(44) Amblard, F.; Cho, J. H.; Schinazi, R. F. Cu (I)-catalyzed Huisgen azide− alkyne 1, 3-dipolar cycloaddition reaction in nucleoside, nucleotide, and oligonucleotide chemistry. *Chem. Rev.* **2009**, *109* (9), 4207−4220.
(45) Aragão-Leoneti, V.; Campo, V. L.; Gomes, A. S.; Field, R. A.; Carvalho, I. Application of copper (I)-catalysed azide/alkyne cycloaddition (CuAAC)'click chemistry'in carbohydrate drug and neoglycopolymer synthesis. *Tetrahedron* **2010**, *66* (49), 9475−9492.
(46) Trujillo, M.; Hull-Crew, C.; Outlaw, A.; Stewart, K.; Taylor, L.; George, L.; Duensing, A.; Tracey, B.; Schoffstall, A. Green Methodologies for Copper (I)-Catalyzed Azide-Alkyne Cycloadditions: A Comparative Study. *Molecules* **2019**, *24* (5), 973.
(47) CLSI. Clinical and Laboratory Standards Institute (CLSI). *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi; Approved Standard-Second Edition*. CLSI document M38-A2; CLSI, 2008; Vol. *28* (16), p 13.
(48) Espinel-Ingroff, A.; Fothergill, A.; Peter, J.; Rinaldi, M.; Walsh, T. Testing conditions for determination of minimum fungicidal concentrations of new and established antifungal agents for Aspergillus spp.: NCCLS collaborative study. *Journal of clinical microbiology* **2002**, *40* (9), 3204−3208.
(49) Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **1983**, *65* (1−2), 55−63.
(50) Ashok, D.; Reddy, M. R.; Dharavath, R.; Ramakrishna, K.; Nagaraju, N.; Sarasija, M. Microwave-assisted synthesis of some new 1, 2, 3-triazole derivatives and their antimicrobial activity. *J. Chem. Sci.* **2020**, *132* (1), 1−9.
(51) Aarjane, M.; Slassi, S.; Tazi, B.; Maouloua, M.; Amine, A. Novel series of acridone-1, 2, 3-triazole derivatives: microwave-assisted synthesis, DFT study and antibacterial activities. *J. Chem. Sci.* **2019**, *131* (8), 85.
(52) Schumacher, R. F.; Von Laer, P. B.; Betin, E. S.; Cargnelutti, R.; Perin, G.; Alves, D. Synthesis of 3-(1H-1, 2, 3-Triazol-1-yl)-2-(arylselanyl) pyridines by Copper-Catalyzed 1, 3-Dipolar Cycloaddition of 2-(Arylselanyl)-3-azido-pyridines with Terminal Alkynes. *J. Braz. Chem. Soc.* **2015**, *26* (11), 2298−2306.
(53) Bouillon, C.; Meyer, A.; Vidal, S.; Jochum, A.; Chevolot, Y.; Cloarec, J.-P.; Praly, J.-P.; Vasseur, J.-J.; Morvan, F. Microwave assisted "click" chemistry for the synthesis of multiple labeled-carbohydrate oligonucleotides on solid support. *J. Org. Chem.* **2006**, *71* (12), 4700−4702.
(54) Taj-Aldeen, S. J.; Salah, H.; Al-Hatmi, A. M.; Hamed, M.; Theelen, B.; van Diepeningen, A. D.; Boekhout, T.; Lass-Flörl, C. In vitro resistance of clinical Fusarium species to amphotericin B and voriconazole using the EUCAST antifungal susceptibility method. *Diagn. Microbiol. Infect. Dis.* **2016**, *85* (4), 438−443.
(55) Shalini, K.; Kumar, N.; Drabu, S.; Sharma, P. K. Advances in synthetic approach to and antifungal activity of triazoles. *Beilstein J. Org. Chem.* **2011**, *7* (1), 668−677.
(56) Zhou, Y.; Xu, J.; Zhu, Y.; Duan, Y.; Zhou, M. Mechanism of action of the benzimidazole fungicide on Fusarium graminearum: interfering with polymerization of monomeric tubulin but not polymerized microtubule. *Phytopathology* **2016**, *106* (8), 807−813.
(57) Sabarwal, A.; Kumar, K.; Singh, R. P. Hazardous effects of chemical pesticides on human health−Cancer and other associated disorders. *Environ. Toxicol. Pharmacol.* **2018**, *63*, 103−114.

https://doi.org/10.1021/acs.jafc.0c07439
J. Agric. Food Chem. 2021, 69, 6958−6967

(58) Portis, I. G.; et al. BIOENSAIOS EM TOXICOGENÉTICA DO FUNGICIDA TEBUCONAZOLE. *Enciclopédia Biosfera* **2015**, *11* (21), 2080−2093.
(59) de Castro, R. D.; de Souza, T. M. P. A.; Bezerra, L. M. D.; Ferreira, G. L. S.; de Brito Costa, E. M. M.; Cavalcanti, A. L. Antifungal activity and mode of action of thymol and its synergism with nystatin against Candida species involved with infections in the oral cavity: an in vitro study. *BMC Complementary Altern. Med.* **2015**, *15* (1), 1−7.
(60) Carriço, M. d. P. S. B.; do Carmo Cardoso, M. F.; Da Silva, F. D. C.; Ferreira, V. F.; Lima, E. S.; Souza, J. V. B. Antifungal activity of synthetic naphthoquinones against dermatophytes and opportunistic fungi: preliminary mechanism-of-action tests. *Ann. Clin. Microbiol. Antimicrob.* **2014**, *13* (1), 26.

https://doi.org/10.1021/acs.jafc.0c07439
J. Agric. Food Chem. 2021, 69, 6958−6967