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New Synthesis and Biological Evaluation of Benzothiazole Derivates as Antifungal Agents

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Supporting Information

ABSTRACT: In search of new antifungal agrochemicals that could replace commercially available, aryl-2-mercaptobenzothiazoles were synthesized. They were prepared by two methodologies, using both photostimulated reaction and microwave assisted reaction. These reactions took place without the use of metallic catalyst by a one-pot procedure with excellent yields (70−98%). Synthesized compounds were evaluated for fungal growth inhibition against *Botrytis cinerea*. Most of the compounds have an excellent antifungal activity, and three of these showed a superior inhibitory effect to commercial fungicide Triadimefon. IC_{50} values observed for 2-(phenylthio)benzothiazole, 2-(2-chlorophenylthio)benzothiazole, and 2-(3-chlorophenyl thio)-benzothiazole were 0.75, 0.69, and 0.65 $\mu g\ mL^{-1}$, respectively.

KEYWORDS: *synthesis, antifungal activities, Botrytis cinerea, microwave irradiation*

INTRODUCTION

Compounds with benzothiazole ring such as 1-(1,3-benzothiazol-2-yl)-3-isopropylurea (Bentaluron), 4-chloro-3-methyl-1,3-benzothiazol-2(3*H*)-one (Chlorobenthiazone), or 2-(thiocyanomethylthio)benzothiazole (TCMTB) are widely used as fungicides (Figure 1). 2-(Arylthio)benzothiazoles are an important class of bioactive and industrially important organic compounds.[1] In agriculture, benzothiazoles are used for control and prevention of phytopathogenic fungi found in soil, which affect crops. Usually, to control fungus such as *Botrytis cinerea*[2] in grapevine, 1-(4-chlorofenoxy)-3,3-dimethyl-1-(1*H*-1,2,4-triazol-1-yl)butan-2-one (Triadimefon) is used (Figure 1).[2a] It is very important because grapes infections caused by *B. cinerea* origin great losses for the wine industry.[3]

Recently, Keri et al. showed that the structural benzothiazole ring plays an important role in medicinal chemistry.[4] These compounds possess potential application as anticancer,[5] antimicrobial,[6] anticonvulsant,[7] antiviral,[8] antitubercular,[9] antimalarial,[10] antihelmintic,[11] analgesic,[12] antiinflammatory,[13] antidiabetic,[14] antifungal,[15] antibacterial, antioxidant,[16] etc., and soon have been successfully developed, marketed, and extensively used in the clinic in preventing and treating various types of diseases with low toxicity, high bioavailability, and good biocompatibility and curative effects.

It is well-known that aryl halides can afford substitution products with different aryl thiolates at good yields by the unimolecular radical nucleophilic substitution ($S_{RN}1$) mechanism.[17] Aryldihalides and phenyl thiolate afforded monosubstitution with retention of halide, monosubstitution with loss of halide, or disubstitution products, depending on relative reactivity of halides.[18]

2-Mercaptobenzothiazole-2-benzylsulfanyl derivatives were synthesized in 94−98% yields by reaction between 2-mercaptobenzothiazole and benzyl bromides in refluxing acetone in the presence of K_2CO_3. Synthesized compounds were found to be either weakly active or inactive against *Escherichia coli*, *Candida albicans*, etc.[19] Moreover, 2-arylbenzothiazole scaffold has provided the inspiration for the discovery of a number of new antitumor agents.[20] Direct thiolation of substituted benzothiazoles with thiophenol was performed using *N*-heterocyclic carbene copper(I) complexes in moderate-to-good yields.[21] Another approach to prepare 2-thio-substituted benzothiazoles starting from benzothiazoles, aryl iodides, and sulfur by copper complex has been reported.[22] It is important to mention that activity against *B. cinerea* was not tested for these products.

It is clear that compounds that have a benzothiazole ring are important for biological activity.[1] It is thus necessary to develop simple and eco-friendly synthetic methods. The main goal of this study was to carry out a new synthetic method to obtain benzothiazole derivates and prove its activity against *B. cinerea*. To obtain different derivates containing benzothiazole core, we performed the arylation of 2-mercaptobenzothiazole using two methodologies. In the first way, reactions were realized under irradiation using DMSO as solvent. In the second way, reactions were microwave assisted and water was the solvent choice (Figure 2).

MATERIALS AND METHODS

Chemicals. 2-mercaptobenzothiazole, dihalobenzenes, iodobenzene, *t*-BuOK, K_2CO_3, and KOH were commercially available and used as received (Aldrich). Liquid ammonia was distilled under nitrogen and metallic Na. It was used immediately after the distillation.

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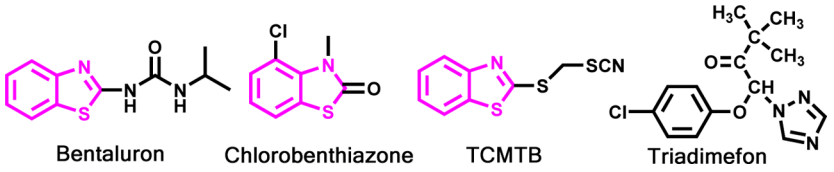

Figure 1. Examples of fungicides structure.

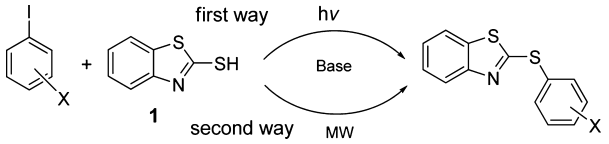

Figure 2. Methodologies employed for the arylation of 2-mercaptobenzotiazole.

DMSO (Carlo Erba) was also distilled under nitrogen and stored in molecular sieve 4 Å (Aldrich). Potato glucose agar (PGA) and Triadimefon were commercially available and used as received. Distilled water was sterilized before their use.

Photostimulated Reactions of Dihalobenzenes with Benzothiazole-2-thiolate Ions in DMSO. The following procedure is representative of these reactions. 2-Mercaptobenzothiazole (1.1 mmol) and *t*-BuOK (1.1 mmol) were added to 8 mL of degassed DMSO. Then, after 10 min when no more solid was present, benzothiazole-2-thiolate ions were ready for use (characterized by a yellow solution). Substrate (1.0 mmol) was added to the solution and the reaction mixture irradiated for 180 min. The reaction was then quenched by adding an excess of water. The solution was then extracted with dichloromethane and water (25 mL). The organic extract was analyzed by GC-MS, and the products were isolated by column chromatography.

A similar procedure was carried out for reactions with substrate excess, adding 1.0 mmol of 2-mercaptobenzothiazole and *t*-BuOK and 5.0 mmol of substrate. These reaction mixtures were irradiated for 30 min.

Microwave Assisted Reactions of Dihalobenzenes with Benzothiazole-2-thiolate Ions in Microwave Oven. 2-Mercaptobenzothiazole (0.5 mmol) and KOH (0.5 mmol) was added to 2 mL of water. Then, after 2 min when no more solid was present, the benzothiazole-2-thiolate ions were ready for use (characterized by a yellow solution). Substrate (0.6 mmol) was added to the solution and the reaction mixture irradiated for 2−7 min. The solid was extracted with dichloromethane. The organic extract was analyzed by GC-MS.

Data for 2-(Phenylthio)benzothiazole (2). The product was isolated as yellow oil after column chromatography using dichloromethane:petroleum ether (70:30). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.27−7.57 (m, 5H), 7.62−7.82 (m, 3H), 7.90 (dd, J = 12.55 Hz, J = 8.17 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 121.1, 121.5, 122.2, 124.7, 124.9, 126.5, 130.3, 130.8, 135.7, 135.9, 153.3. HRMS $[MH]^+$ exact mass calculated for $C_{13}H_{10}S_2N$, 244.0249; found, 244.0317.

Data for 1,4-Bis(2-Mercaptobenzothiazol)benzene (3). The nucleophile excess was extracted from the reaction mixture by washing with water. The product was isolated as a white solid after filtrating and washing the precipitate with ethyl acetate; mp: 197.5−199.3 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.39 (td, J = 8.14 Hz, J = 1.15 Hz, 2H), 7.49 (td, J = 8.22 Hz, J = 1.27 Hz, 2H), 7.89 (ddd, J = 8.17 Hz; J = 1.00 Hz, J = 0.54 Hz, 2H), 7.92 (s, 4H), 8.00 (ddd, J = 8.05 Hz, J = 1.17 Hz, J = 0.55 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) ppm: 121.7, 121.9, 125.0, 126.6, 132.1, 135.1, 135.5, 153.2. HRMS $[MH]^+$ exact mass calculated for $C_{20}H_{13}S_4N_2$, 408.9962; found, 408.9986.

Data for 2-(4-Bromophenylthio)benzothiazole[23] (4). The product was isolated as white solid after column chromatography using dichloromethane:petroleum ether (70:30); mp 55.6−56.8 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.29 (ddd, J = 7.97 Hz, J = 7.37 Hz, J = 1.21 Hz, 1H), 7.42 (ddd, J = 8.13 Hz, J = 7.28 Hz, J = 1.25 Hz, 1H), 7.56−7.64 (m, J = 3.00 Hz; J = 1.14 Hz, J = 0.52 Hz, 4H), 7.69 (ddd, J = 8.28 Hz, J = 0.53 Hz, 1H), 7.89 (dd, J = 8.28 Hz, J = 0.53 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 120.9, 122.1, 124.6, 125.2, 126.3, 129.1, 133.1, 135.6, 136.5, 139.1, 153.81, 168.0. HRMS $[MH]^+$ exact mass calculated for $C_{13}H_9S_2NBr$, 321.9354; found, 321.9356.

Data for 2-(4-Iodophenylthio)benzothiazole (5). The product was isolated as yellow oil after column chromatography using dichloromethane:petroleum ether (70:30). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.28−7.32 (m, 1H), 7.40−7.45 (m. 3H), 7.56−7.78 (m, 2H), 7.80 (d, J = 8.12 Hz, 1H) 7.89 (dd, J = 8.12 Hz, J = 3.03 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 121.2, 122.5, 124.9, 126,7, 130.2, 130.3, 130.6, 135.7, 136.0, 136.9, 139.4, 154.1. HRMS $[MH]^+$ exact mass calculated for $C_{13}H_9S_2NI$, 369.9221; found, 369.9239.

Data for 2-(2-Chlorophenylthio)benzothiazole (6). The product was isolated as brown solid after column chromatography using dichloromethane:petroleum ether (70:30). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.28−7.34 (td, 1H, J = 1.17 Hz, J = 8.69 Hz), 7.34−7.39 (dd, 1 H, overlapping, J = 1.23 Hz, J = 8.38 Hz), 7.39−7.46 (tt, 2H, overlapping, J = 1.23 Hz, J = 8.01 Hz), 7.66−7.72 (dt, 1H, J = 1.48 Hz, J = 7.54 Hz), 7.73−7.78 (dt, 1H, J = 8.26 Hz), 7.78−7.82 (dt, 1H, J = 1.97 Hz), 7.89−7.95 (dt, 1H, J=8.14 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) (ppm): 120.93, 122.27, 124.63, 126.26, 128.65, 129.73, 131.71, 131.87, 134.19, 135.87, 136.92, 153.87, 166.97. HRMS $[MH]^+$ exact mass calculated for $C_{13}H_8S_2NCl$, 277.9865; found, 277.9860.

Data for 2-(3-Chlorophenylthio)benzothiazole[24] (7). The product was isolated as colorless oil after column chromatography using dichloromethane:petroleum ether (70:30). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.28−7.34 (J = 1.17 Hz, J = 8.69 Hz, td, 1H), 7.37−7.44 (t, 1H, overlapping, J = 7.77 Hz), 7.37−7.44 (dd, 1H, overlapping, J = 1.23 Hz, J = 8.38 Hz), 7.44−7.50 (tt, 1H, overlapping, J = 1.23 Hz, J = 8.01 Hz), 7.58−7.64 (dt, 1H, J = 1.48 Hz, J = 7.54 Hz), 7.68−7.72 (d, 1H, J = 8.26 Hz), 7.72−7.74 (t, 1H, J = 1.97 Hz), 7.83−8.03 (d, 1H, J = 8.14 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 120.9, 122.3, 124.7, 126.3, 130.4, 130.9, 131.9, 132.9, 134.5, 135.4, 135.7, 153.7. HRMS $[MH]^+$ exact mass calculated for $C_{13}H_9S_2NCl$, 277.9865; found, 277.9860.

Data for 2-(4-Fluorophenilthio)benzothiazole (8). The product was isolated as brownish solid after column chromatography using dichloromethane:petroleum ether (70:30); mp 54.3−55.5 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.11−7.21 (m, 2H), 7.22−7.31 (m, 1H), 7.40 (ddd, J = 8.65 Hz, J = 7.30 Hz, J = 1.20 Hz, 1H), 7.65 (d, J = 8.17 Hz, 1H), 7.68−7.73 (m, 2H), 7.86 (d, J = 8.17 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 117.5, 117.7, 121.1, 122.3, 124.7, 126.8, 135.9, 138.1, 138.2, 154.2, 163.3, 165.8, 169.8. HRMS $[MH]^+$ exact mass calculated for $C_{13}H_9S_2NF$, 262.0155; found, 262.0169.

Bioassays of Fungicidal Activities. Five bunches of botrytized grape clusters (*Vitis vinifera* cv. Chardonnay) were randomly hand-harvested in a vineyard in the district Barrancas, Maipú, province Mendoza (Argentina) in 2010. Solutions of compounds **2**−**8** were prepared with ethanol and were sterilized by filtration with 0.2 μm GHP HPLC filter membrane. Those solutions were each incorporated into polystyrene Petri dishes which contained potato glucose agar (PGA) and the required amount of water to reach the desired concentration of each compound. Fungal growth in the presence of each compound was evaluated at four concentrations (12.5, 25, 50, and 100 μg mL^{-1}) in PGA. Then the culture media were allowed to solidify. The final concentration of ethanol was 1.2% of the final volume of media for all treatments and control. For this instance, ethanol, water, PGA, and HPLC filters, membranes, and syringes were previously sterilized by autoclave for 15 min at 120 °C. For each analysis, three replicates were performed. Radial growth was measured after incubation at 28 °C for 8 days in the dark. For all assays, the fungi were cultured on PGA/water (positive control) and PGA/water/

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ethanol (ethanol control). More details are included in the Supporting Information.

RESULTS AND DISCUSSION

Synthesis under Light Irradiation. The photostimulated reaction of benzothiazole-2-thiolate anion with iodobenzene afforded 60% yields of monosubstitution product (**2**) within 180 min (Table 1, entry 1, photostimulated reactions). Photostimulated reaction between 2 mmol of 2-mercaptobenzothiazole and 1 mmol of *p*-diiodobenzene afforded mainly 65% yields of disubstitution product (**3**) within 180 min (Table 1, entry 2).

To decrease the formation of disubstitution product and to produce monosubstitution product with retention of halide, reactions were performed with different benzenedihalides using a relation substrate:nucleophile (1:1). However, the best yields were obtained using a relation substrate:nucleophile (5:1). Thus, benzothiazole-2-thiolate ions reacted with *p*-bromoiodobenzene at a relation substrate:nucleophile (1:1) and (5:1), affording 37% and 98% yields of monosubstitution product with retention of bromine (**4**), respectively (Table 1, entry 3). This reaction did not occur in the dark and was inhibited by *p*-dinitrobenzene (*p*-DNB), a well-known inhibitor of $S_{RN}1$ reactions.[17] These reactions and more details about the optimization for *p*-bromoiodobenzene with **1** are included in the Supporting Information.

Reaction of benzothiazole-2-thiolate anion with *p*-diiodobenzene in a relation (1:1) afforded disubstitution product within 60 min, but by reducing the concentration of benzothiazole-2-thiolate anion and reaction time (30 min), 25% yields of monosubstitution product with retention of iodine (**5**) was obtained. The reaction performed in a relation substrate:nucleophile (5:1) afforded 93% yields of **5** within 30 min (Table 1, entry 4).

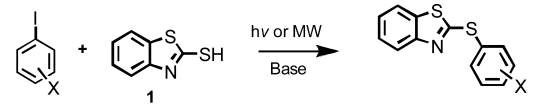
Photostimulated reaction of *o*-chloroiodobenzene afforded 44% and 98% yields of monosubstitution product with retention of chloride (**6**) within 30 min (Table 1, entry 5) at a relation substrate:nucleophile (1:1) and (5:1), respectively.

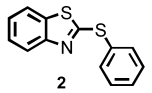
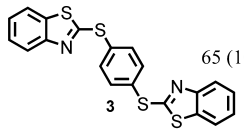
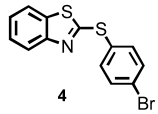
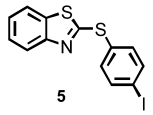
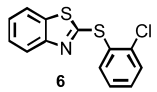
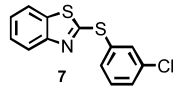
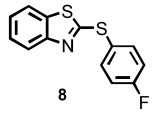
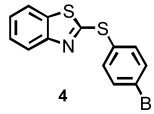
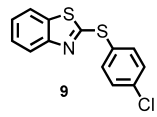
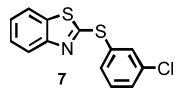
Furthermore, benzothiazole-2-thiolate ions reacted with *m*-chloroiodobenzene and *p*-fluoroiodobenzene, rendering 58% (**7**) and 63% (**8**) yields, respectively, under irradiation during 180 min with a relation substrate:nucleophile (1:1) (Table 1, entries 6 and 7). It has been observed that *o*-chloroiodobenzene was less reactive than *m*-chloroiodobenzene, probably due to hindering.

By contrast, *p*-dibromobenzene, *p*-chlorobromobenzene, or *m*-clorobromobenzene did not react with benzothiazole-2-thiolate ions under these reaction conditions (Table 1, entries 8−10). It is clear that those substrates with reduction potential greater than −1.60 V[25] are able to initiate the reaction while those with lower potential such as *p*-dibromobenzene, *m*-chlorobromobenzene, and *p*-chlorobromobenzene remain unreacted (see reduction potential in the Supporting Information).

Synthesis under Microwave Radiation. As an alternative to reaction initiation and in search of an environmentally benign process, microwave irradiation was employed.[26] Microwave irradiation in polar solvents allows rapid heating to high temperatures enabling short reaction times and often improved yields.[27] The solvent polarity affects the rate of a reaction carried out in a microwave oven because polar molecules absorb microwave causes heating of the substance through frictional effects. It would follow that the rates at which a solvent absorbs microwave radiation depends on its loss factor. The larger the loss factor the greater the coupling with microwaves. Thus, solvents such as water, methanol, DMF,

Table 1. Reactions of Arylhalides with 1: Comparison between Light and Microwave Irradiation



Entry	X	Product	Isolated Product (%) (Substrate recovered) hν/DMSO[a]	MW/H_2O[i]	IC_{50} μg mL^{-1}
1	H	 2	60 (34)	78 (20)	0.75
2	*p*-I[b]	 3	65 (10)	-	1.03
3	*p*-Br	 4	37 (50)[c] 98 (nq)[d]	88 (nq)	1.93
4	*p*-I	 5	25 (68)[e] 93 (nq)[d]	72 (nq)[j]	0.97
5	*o*-Cl	 6	44 (50) 98 (nq)[d]	70 (nq)	0.69
6	*m*-Cl	 7	58 (30)	81 (nq)	0.65
7	*p*-F	 8	63 (34)	18 (nq)	1.17
8	*f*	 4	- (95)	- (96)	-
9	*g*	 9	- (98)	- (97)	-
10	*h*	 7	- (98)	- (95)	-

[a] *tert*-BuOK and **1** concentrations were 13.7 × 10^{-2} M, substrate concentration was 12.5 × 10^{-2} M. [b] Base and **1** concentrations were 27.5 × 10^{-2} M, 18% of **5**. [c] 4% of **2** and 2% of **3**. [d] Substrate concentration was 62.5 × 10^{-2} M, reaction time was 30 min. nq: not quantified. [e] Base and **1** concentrations were 7.5 × 10^{-2} M, reaction time was 45 min, 4% of **2**. [f] *p*-Dibromobenzene. [g] *p*-Chlorobromobenzene. [h] *m*-Chlorobromobenzene. [i] KOH and **1** concentrations were 25 × 10^{-2} M, substrate concentration was 30 × 10^{-2} M, TBABr, reaction time was 7 min. [j] Reaction time was 2 min.

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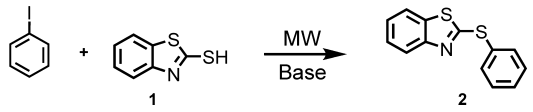
DMSO, etc., are all heated rapidly when irradiated with microwaves.[28]

Arylation of commercial fungicides were also studied in a previous report, allowing the formation of new compounds with potential fungicide activity under microwave irradiation.[29] The synthesis of polyfluorinated 2-benzylthiobenzothiazole derivatives under microwave radiation has been also reported.[30] Recently, Peñéñory et al. reported that microwave irradiation promotes a quick aromatic nucleophilic substitution by a thermally induced electron transfer process to form new C−C bonds by the coupling of aryl radicals and enolate nucleophiles.[31]

The reaction of 0.5 mmol of the benzothiazole-2-thiolate anion with 0.6 mmol of iodobenzene in DMSO was made at low and high temperatures (60, 90, 120 °C), short times (5−60 min), and different microwave radiation modes, but the outcome was nucleophile decomposition in all conditions assessed, which did not allow achievement of the desired product (Table 2, entries 1 and 2).

Table 2. Optimization of 2[a] under Microwave Irradiation



1 2

entry	conditions (time, min)[c]	product yields[b] (substrate recovered)
1	DMSO, 60 °C (5−60)[d]	decomposition
2	DMSO, 60−120 °C (5−60)	decomposition
3	DMF, 120 °C (20)[e,f]	**2**, − (97)
4	DMF, 120 °C (20)[g]	**2**, 5 (94)
5	DMF, 120 °C (20)[h]	**2**, 30 (65)
6	DMF, 120 °C (20)[i,j]	**2**, 70 (24)
7	H_2O/polyethylenglycol, 150 °C	**2**, − (95)
8	H_2O, 90 °C (20)[d,e,g,h,i,k]	**2**, − (96)
9	H_2O, 120 °C (20)[a,k]	**2**, 78 (20)
10	H_2O, 120 °C (10)[a,k]	**2**, 78 (20)
11	H_2O, 120 °C (7)[a,k]	**2**, 75 (16)

[a]Base and **1** concentrations were 25 × 10^{-2} M. [b]Yields of isolated products. [c]Dynamic mode and 150 W power was applied. [d]Pulsed mode. [e]With K_2CO_3 as base. [f]With *t*-BuOK as base. [g]With 1 equiv of KOH as base. [h]With 2 equiv of KOH. [i]With 3 equiv of KOH. [j]200 W power was applied. [k]With tetra-*n*-butylammonium bromide (TBABr).

Reaction of 2-mercaptobenzothiazole with iodobenzene in DMF at 120 °C using both K_2CO_3 and *t*-BuOK as base did not occur (Table 2, entry 3). Nevertheless, using KOH as base, the coupling reaction was observed (Table 2, entry 4). Moreover, reaction yields were increased using excess of KOH, proving 30 and 70% yields of (**2**) (Table 2, entries 5 and 6). Thus, this reaction was also carried out using a power of 200 W, and similar results were observed. Under N_2, no changes were observed.

To find an environmental friendly procedure, the reaction was performed in *t*-butanol, isopropyl alcohol, polyethylene glycol, or acetonitrile, which did not allow achieving the desired product. The reaction was also carried out using a solvent mixture which did not allow achievement of the desired product (Table 2, entry 7).

When the reaction was made using water as solvent at 90 °C with different bases and other conditions, no product was detected (Table 2, entry 8), while at 120 °C, the coupling reaction product was observed (Table 2, entries 9−11). Reactions were efficient using solvents with loss factor between 0.1 and 0.2. The best product yields were obtained using water as solvent at 120 °C under microwave irradiation for 7 min. So, subsequent reactions were performed using this last condition.

Thus, the reaction of **1** with *p*-bromoiodobenzene, *p*-diiodobenzene, *o*-chloroiodobenzene, *m*-chloroiodobenzene, and *p*-fluoriodobenzene, rendered 88% (**4**), 72% (**5**), 70% (**6**), 81% (**7**), and 18% (**8**) yields, respectively (Table 1, entries 3−7). Similar to photoestimulation, *p*-dibromobenzene, *p*-chlorobromobenzene, and *m*-clorobromobenzene did not react with **1** under these reaction conditions (Table 1, entries 8−10).

No disubstitution product was obtained from 2 mmol of benzothiazole-2-thiolate anion and 1 mmol of *p*-diiodobenzene using microwave irradiation because the nucleophile got decomposed (Table 1, entry 2). It is very important to notice that this reaction was efficient under photostimulation.

The use of microwave decreased reaction times considerably in comparison to photostimulation. The synthetic procedure reported here is simple and reproducible, and the products are obtained in high yields using water as solvent. It is worthy of mention that this novel process shows environmental and economic advantages over previously reported protocols.

Bioassays of Fungicidal Activity against *Botrytis cinerea*. *B. cinerea* is responsible for the gray mold disease on more than 200 host plants. This necrotrophic ascomycete displays the capacity to kill host cells through the production of toxins, reactive oxygen species, and the induction of a plant-produced oxidative burst.[32] It is well-known that Triadimefon inhibits *B. cinerea*'s growth as well. Triadimefon is widely used to control other fungal diseases. For this reason, it was used as assay control.[33]

Toxicity Test of Solvents on B. cinerea. Solvents used to dissolve tested compounds needed to be nontoxic against *B. cinerea*. Results showed that a solution of ethanol 1.2% final in potato glucose agar (PGA) culture media did not exhibit significant suppression on fungus growth (Figure 3, control with ethanol).

B. cinerea Growth Inhibition Assay. In vitro fungal growth inhibition assay modified from Huang et al. was performed.[33] Fungal growth in the presence of compounds **2**−**8** was evaluated at four concentrations (12.5, 25, 50, and 100 μg mL^{-1}) in PGA.[33] Results in Figure 3 show that all compounds assayed exhibited strong antifungal activity against *B. cinerea* at four concentrations.

Each treatment was performed in triplicate, and the effective concentration 50 (IC_{50}), defined as the concentration required to inhibit 50% of the fungal growth, was used to describe the inhibitory activity of the compounds. IC_{50} of the target compounds along with the standard drug for comparison are summarized in Table 1.

By analyzing the graphs at 12.5 μg mL^{-1}, it can be seen that compounds **2**, **3**, **4**, **6**, and **7** inhibit fungal growth better than Triadimefon. Apparently, the presence of a halogen, either Br, I, or F, in the aromatic ring does not cause large effects in terms of activity against *B. cinerea*. This can be seen if compounds **4**, **5**, and **8** are compared at 25 μg mL^{-1}.

Compounds **2**, **6**, and **7** are those that caused greater inhibition in fungus growth at different concentrations than Triadimefon (IC_{50} values: 0.75, 0.69, 0.65 vs 1.05 μg mL^{-1}). Furthermore, it is easy to see that compounds **6** and **7** showed three times more inhibition than Triadimefon at 25 μg mL^{-1}, presenting the highest antifungal activity.

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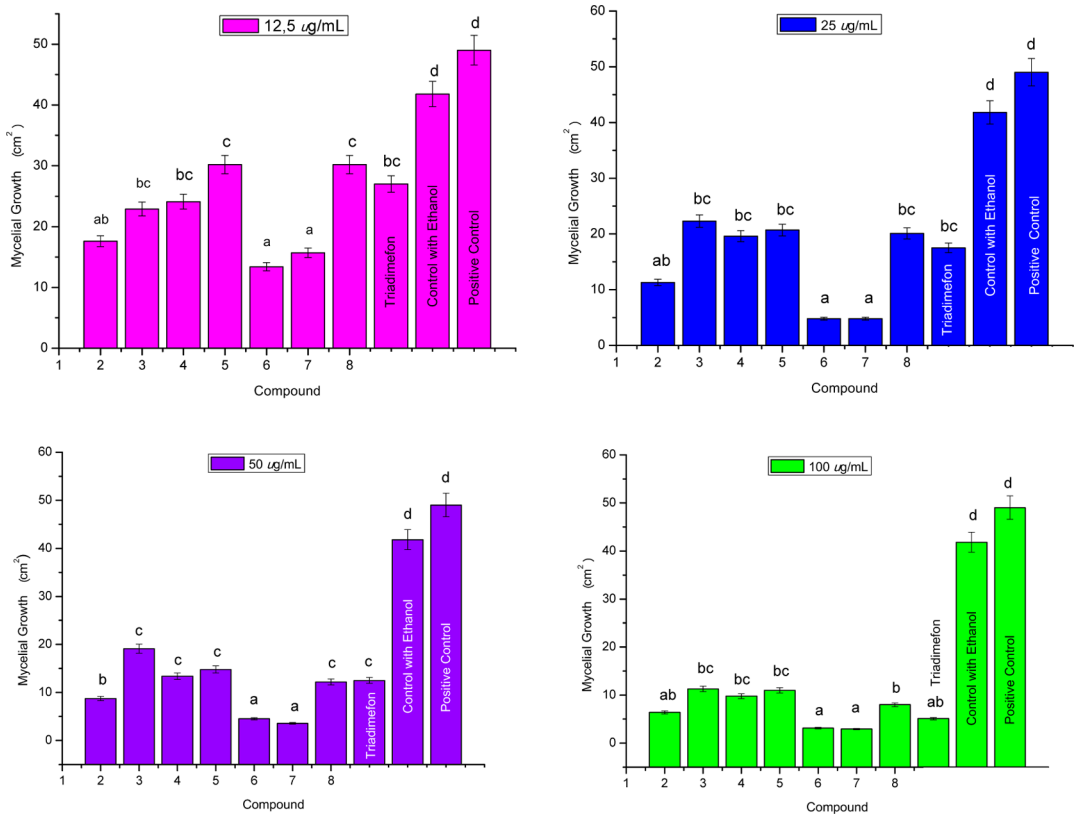

Figure 3. *Botrytis cinerea* growth inhibition assay of aryl-2-thiobenzothiazoles family at different concentrations. Radial growth areas were calculated using the formula for the area of a circle ($A = \pi r^2$) (cm^2). Different letters indicate significant differences ($p \leq 0.05$).

In conclusion, two alternative routes for the arylation of 2-mercaptobenzothiazole have been evaluated. The first pathway involved a photostimulated nucleophilic substitution, while the second consisted in a microwave assisted reaction. Both methodologies were shown to be complementary. Reactivity of the family of dihalobenzene explained by their reduction potentials was reported.

The synthetic procedures reported here are one-pot, reliable, simple, and highly reproducible processes. These are also energy efficient as they involves very short reaction times and in high reaction yields. The methodology using water and microwave irradiation is economical and eco-friendly. Moreover, thermal initiated $S_{RN}1$ reactions of dihalobenzene using controlled microwave heating to promote the reaction were reported.

All compounds assayed have an excellent growth inhibition for *B. cinerea* in the conditions employed. Furthermore, compounds **2**, **6**, and **7** inhibit fungal growth better than Triadimefon. Thus, these compounds could be used as alternative to Triadimefon for fungi control in plants. Further toxicity, persistence, and structure−activity relationship studies for those compounds are underway.

■ ASSOCIATED CONTENT

Supporting Information

General information, optimization of *p*-bromoiodobenzene with **1**, reduction potential, more details about bioassays of fungicidal activities, isolation and spectroscopy data for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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■ ABBREVIATIONS USED

GC-MS, Gas chromatography−mass spectroscopy detector; IC_{50}, half-maximal inhibitory concentration; DMSO, dimethyl sulfoxide; PGA, potato glucose agar; DMF, dimethylformamide; TBABr, tetra-*n*-butylammonium bromide

■ REFERENCES

(1) Azam, M. A. B.; Suresh, B. Biological Activities of 2-Mercaptobenzothiazole Derivatives: A Review. *Sci. Pharm.* **2012**, *80*, 789−823.

(2) (a) Roberts, T. R.; Hutson, D. H. *Metabolic Pathways of Agrochemicals, Part 2: Insecticides and Fungicides*; RSC, Cambridge, 1999. (b) Ten Have, A.; Dekkers, E.; Kay, J.; Phylip, L. H.; Van Kan, J. A. L. An aspartic proteinase gene family in the filamentous fungus *Botrytis cinerea* contains members with novel features. *Microbiology* **2004**, *150*, 2475−2489. (c) Hong, Y. S.; Martinez, A.; Liger-Belair, G.; Jeandet, P.; Nuzillard, J.-M.; Cilindre, C. *J. Exp. Bot.* **2012**, *63*, 5773−

DOI: 10.1021/acs.jafc.5b00150
J. Agric. Food Chem. 2015, 63, 3681−3686

5785. (d) Cilindre, C.; Jégou, S.; Hovasse, A.; Schaeffer, C.; Castro, A. J.; Clément, C.; Van Dorsselae, A.; Jeandet, P.; Marchal, R. Proteomic Approach To Identify Champagne Wine Proteins as Modified by *Botrytis cinerea* Infection. *J. Proteome Res.* **2008**, *7*, 1199−1208.

(3) Steel, C. C.; Blackman, J. W.; Schmidtke, L. M. Grapevine bunch rots: Impacts on wine composition, quality, and potential procedures for the removal of wine faults. *J. Agric. Food Chem.* **2013**, *61*, 5189−5206.

(4) Keri, R. S.; Patil, M. R.; Patil, S. A.; Budagumpi, S. A comprehensive review in current developments of benzothiazole-based molecules in medicinal chemistry. *Eur. J. Med. Chem.* **2015**, *89*, 207−251.

(5) Huang, S. T.; Hsei, I. J.; Chen, C. Synthesis and anticancer evaluation of bis(-benzimidazoles), bis(benzoxazoles), and benzothiazoles. *Bioorg. Med. Chem.* **2006**, *14*, 6106−6119.

(6) Singh, M.; Singh, S. K.; Gangwar, M.; Nath, G.; Singh, S. K. Design, synthesis and mode of action of some benzothiazole derivatives bearing an amide moiety as antibacterial agents. *RSC Adv.* **2014**, *4*, 19013−19023.

(7) Siddiqui, N.; Pandeya, S. N.; Khau, S. A.; Stables, J.; Rana, A.; Alam, M.; MdArshad, F.; Bhat, M. A. Synthesis and anticonvulsant activity of sulfonamide derivatives hydrophobic domain. *Bioorg. Med. Chem.* **2007**, *17*, 255−259.

(8) Akhtar, T.; Hameed, S.; Al-Masoudi, N.; Loddo, R.; Colla, P. In vitro antitumor and antiviral activities of new benzothiazole and 1,3,4-oxadiazole-2-thione derivatives. *Acta Pharm.* **2008**, *58*, 135−149.

(9) Palmer, F. J.; Trigg, R. B.; Warrington, J. V. Benzothiazolines as antituberculous agent. *J. Med. Chem.* **1971**, *14*, 248−251.

(10) Burger, A.; Sawhey, S. N. Antimalarials. III. Benzothiazole amino alcohols. *J. Med. Chem.* **1968**, *11*, 270−273.

(11) Suresh, C. H.; Rao, J. V.; Jayaveera, K. N.; Subudhi, S. K. Synthesis and anthelmintic activity of 3-(2-hydrozinobenzothiazole)-substituted indole-2-one. *Int. J. Pharm.* **2013**, *2*, 257−261.

(12) Siddiqui, N.; Alam, M.; Siddiqui, A. A. Synthesis and analgesic activity of some 2-[{4-(alkylthioureido)phenyl}sulphonamido]-6-substituted benzothiazoles. *Asian J. Chem.* **2004**, *16*, 1005−1008.

(13) Gurupadayya, B. M.; Gopal, M.; Padmashali, B.; Vaidya, V. P. Synthesis and bological activities of fuorobenzothiazoles. *Ind. J. Heterocy Chem.* **2005**, *15*, 169−172.

(14) Pattan, S. R.; Suresh, Ch.; Pujar, V. D.; Reddy, V. V. K.; Rasal, V. P.; Koti, B. C. Synthesis and antidiabetic activity of 2-amino[5″(4-sulphonylbenzylidene-2,4-thiazolidenedinone]-7-chloro-6-flourobenzothiazole. *Indian J. Chem.* **2005**, *44B*, 2404−2408.

(15) Singh, S. P.; Segal, S. Study of fungicidal activities of some benzothiazoles. *Indian J. Chem.* **1988**, *27B*, 941−943.

(16) Rao, A. J.; Rao, P. V.; Rao, V. K.; Mohan, C.; Raju, C. N.; Suresh Reddy, C. Microwave assisted one-pot synthesis of novel a-aminophosphonates and their biological activity. *Bull. Korean Chem. Soc.* **2010**, *31*, 1863−1868.

(17) (a) Bardagí, J. I., Vaillard, V. A., Rossi, R. A. In *Encyclopedia of Radicals in Chemistry, Biology & Materials*; Chatgilialoglu, C., Studer, A., Eds.; John Wiley & Sons Ltd.: Chichester, UK, 2012; pp 333−364. (b) Rossi, R. A.; Pierini, A. B.; Peñéñory, A. B. Nucleophilic Substitution Reactions by Electron Transfer. *Chem. Rev.* **2003**, *103*, 71−167. (c) Rossi, R. A.; Pierini, A. B.; Santiago, A. N. Aromatic Substitution by $S_{RN}1$ Reaction. In *Organic Reactions*; Paquette, L. A., Bittman, R., Eds.; Wiley & Sons: New York, 1999, *54*, pp 1−271.

(18) Uranga, J. G.; Montañez, J. P.; Santiago, A. N. Theoretical and experimental study on the reactivity of methyl dichlorobenzoates with sulfur-centered nucleophiles by electron transfer reactions. *Tetrahedron* **2012**, *68*, 584−589.

(19) Ramnathan, V. K.; Kotha, V. S. R. S. K.; Kotarkonda, R. G. Facile synthesis of 2-(substitutedbenzylsulfanyl)-benzothiazoles and their antimicrobial activity screening. *J. Heterocycl. Chem.* **2005**, *42*, 153−156.

(20) Aiello, S.; Wells, G.; Stone, E. L.; Kadri, H.; Bazzi, R.; Bell, D. R.; Stevens, M. F. G.; Matthews, C. S.; Bradshaw, T. D.; Westwell, A. D. Synthesis and Biological Properties of Benzothiazole, Benzoxazole, and Chromen-4-one Analogues of the Potent Antitumor Agent 2-(3,4-Dimethoxyphenyl)-5-fluorobenzothiazole. *J. Med. Chem.* **2008**, *51*, 5135−5139 and references therein..

(21) Inomata, H.; Toh, A.; Mitsui, T.; Fukuzawa, S. *N*-Heterocyclic carbene copper(I) complex-catalyzed direct C−H thiolation of benzothiazoles. *Tetrahedron Lett.* **2013**, *54*, 4729−4731.

(22) (a) Wang, X.; Li, Y.; Yuan, Y. The Direct Thiolation of Benzothiazole Catalyzed by Copper Complex in Superbase System. *Synthesis* **2013**, *45*, 1247−1255. (b) Correa, A.; Carril, M.; Bolm, C. Iron-Catalyzed S-Arylation of Thiols with Aryl Iodides. *Angew. Chem., Int. Ed.* **2008**, *47*, 2880−2883.

(23) Ranjit, S.; Lee, R.; Heryadi, D.; Shen, C.; Wu, J.; Zhang, P.-F.; Huang, K.-W.; Liu, X.-G. Copper-Mediated C−H Activation/C−S Cross-Coupling of Heterocycles with Thiols. *J. Org. Chem.* **2011**, *76*, 8999−9007.

(24) Murru, S.; Ghosh, H. K.; Sahoo, S. K.; Patel, B. Intra- and Intermolecular C−S Bond Formation Using a Single Catalytic System: First Direct Access to Arylthiobenzothiazoles. *Org. Lett.* **2009**, *11*, 4254−4257.

(25) Meites, L.; Zuman. P. *Handbook Series in Organic Electrochemistry*; CRC Press. New York, 1976; Vol. *1*.

(26) Tierney, J. P.; Lidstrom, P. *Microwave Assisted Organic Synthesis*; Blackwell Publishing: Oxford, UK, 2005.

(27) (a) Loupy, A. *Microwaves in Organic Synthesis*; Wiley-VCH, Verlag GmbH & Co. KGaA, Weinheim, Germany, 2006. (b) Kappe, C. O. Controlled Microwave Heating in Modern Organic Synthesis. *Angew. Chem., Int. Ed.* **2004**, *43*, 6250−6284.

(28) Ravichandran, S.; Karthikeyan, E. Microwave Synthesis —A Potential Tool for Green Chemistry. *Int. J. Chem. Technol. Res.* **2011**, *3*, 466−470.

(29) Herrera Cano, N.; Santiago, A. N. Different methodologies by arylations of arylchlorides. A convenient preparation of new triazolic fungicides. *Tetrahedron* **2014**, *70*, 280−285.

(30) Huang, W.; Yang, G. F. Microwave-assisted, one-pot syntheses and fungicidal activity of polyfluorinated 2-benzylthiobenzothiazoles. *Bioorg. Med. Chem.* **2006**, *14*, 8280−8285.

(31) Soria-Castro, S. M.; Caminos, D. A.; Peñéñory, A. B. An expedient route to heterocycles through α-arylation of ketones and arylamides by microwave induced thermal $S_{RN}1$ reactions. *RSC Adv.* **2014**, *4*, 17490−17497.

(32) Choquer, M.; Fournier, E.; Kunz, C.; Levis, C.; Pradier, J. M.; Simon, A.; Viaud, M. *Botrytis cinerea* virulence factors: new insights into a necrotrophic and polyphageous pathogen. *FEMS Microbiol. Lett.* **2007**, *277*, 1−10.

(33) Soylu, E. M.; Kurt, S.; Soylu, S. In vitro and in vivo antifungal activities of the essential oils of various plants against tomato grey mould disease agent *Botrytis cinerea*. *Int. J. Food Microbiol.* **2010**, *143*, 183−189.

DOI: 10.1021/acs.jafc.5b00150
J. Agric. Food Chem. 2015, 63, 3681−3686