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Synthesis and Antifungal Activity of 2-Chloromethyl-1*H*-benzimidazole Derivatives against Phytopathogenic Fungi in Vitro

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Supporting Information

ABSTRACT: A series of 35 benzimidazole derivatives were synthesized from 2-chloromethyl-1*H*-benzimidazole in good yields. Their structures were characterized by ^{1}H and ^{13}C NMR and HRESIMS. Antifungal activities of all of the synthesized compounds were evaluated against five phytopathogens fungi (*Cytospora* sp., *Colletotrichum gloeosporioides*, *Botrytis cinerea*, *Alternaria solani*, and *Fusarium solani*) using the mycelium growth rate method. Compound **4m** displayed strong growth inhibition of *C. gloeosporioides*, *A. solani*, and *F. solani* with IC_{50} of 20.76, 27.58, and 18.60 μg/mL, respectively. Selective inhibition of *B. cinerea* instead of the other fungal pathogenes was observed with **7f** (IC_{50} of 13.36 μg/mL), comparable to that of positive control, a commercial agricultural fungicide hymexazol (IC_{50} of 8.92 μg/mL). Compound **5b** exhibited remarkable antifungal properties against *Cytospora* sp., *C. gloeosporioides*, *B. cinerea*, and *F. solani* with IC_{50} values of 30.97, 11.38, 57.71, and 40.15 μg/mL, respectively; among the target fungi, **5b** was the most active compound and superior to the reference against *C. gloeosporioides* alone. Structure−activity relationship (SAR) data of these compounds are as follows: (1) introduction of the chlorine atom on para-position in the benzene ring help to increase activity (**4f** vs **4c**; **7f** vs **7n**), (2) the sulfonyl group is critical for the inhibition of *C. gloeosporioides* (**5b** and **5c** vs **5a**), and (3) the unsubstituted benzene ring improve activity (**4m** vs **4n**, **4e** and **4a**). Thus, compounds **5b**, **4m**, and **7f** emerged as a new leading structure for the development of new fungicides.

KEYWORDS: *synthesis, 2-chloromethyl-1H-benzimidazole derivatives, antifungal activity, phytopathogenic fungi, structure−activity relationship*

INTRODUCTION

Plant diseases are caused primarily by fungal and bacterial pathogens, leading to severe losses to agriculture and horticulture crop production worldwide and constitute an emerging threat to the global food security.[1] Considerable postharvest losses of fruits and vegetables occur by decay caused by fungal pathogens, which, in addition to causing rot, may produce mycotoxins capable of the harmful consequences on animal and human health.[2−5] As a result, several important fungal plant pathogens of the genera *Colletotrichum*, *Botrytis*, *Alternaria*, and *Fusarium* have received great attention due to their typical infected disease symptoms (e.g., postharvest diseases of fruits such as anthracnose caused by *Colletotrichum* species).[3,4] Many of the currently available antifungal agents have several drawbacks, such as drug related toxicity, severe drug resistance, nonoptimal pharmacokinetics, and serious drug interactions.[6] Therefore, there is a growing need to develop new antifungal agents to effectively control those agricultural diseases.

Benzimidazole is one of the commonly used chemical scaffolds because it plays an important role in medicinal chemistry and agrochemicals; it has earned an essential place in the list of chemotherapeutic agents. During the past 5−10 years, many condensation products of benzimidazole have been patented for a variety of biological activities, such as antitumor, antiinflammatory, antiviral, and antifungal.[7−9] Extensive biochemical and pharmacological studies have confirmed that benzimidazole molecules are effective against various strains of microorganisms.[8] Especially, benzimidazole is a core substructure of systemic fungicides currently in use for controlling mycoses in humans.[7] Similarly, 2-substituted benzimidazole and its derivatives have been found to be potent biologically active compounds as well. The activity and structural diversity exhibited by compounds containing the benzimidazole moiety has led to the development of a series of novel and useful bioactive benzimidazole analogs.[9]

In our previous work, acetophenone derivatives were designed and synthesized,[10] and some compounds showed diverse and promising bioactivities such as fungicidal and antitumor activities. In continuation of our investigation on the design and synthesis of bioactive compounds, a series of novel analogs of 2-chloromethyl-1*H*-benzimidazole were designed and synthesized from the intermediates 2-chloromethyl-1*H*-benzimidazole and 2-chloromethyl-1-methyl-5-nitro-1*H*-benzimid-azole via an efficient method (Scheme 1). Their antifungal effects on selected five target phytopathogenic fungi were investigated, and their structure-antifungal activity relationships were also discussed. To our knowledge, the antifungal activities of all the synthetic derivatives including known compounds were reported for the first time.

MATERIALS AND METHODS

General Experimental Procedures. The melting points of the products were determined on an X-4 apparatus (Beijing Tech Instrument Co., Beijing, P. R. China) and are uncorrected. Nuclear magnetic resonance

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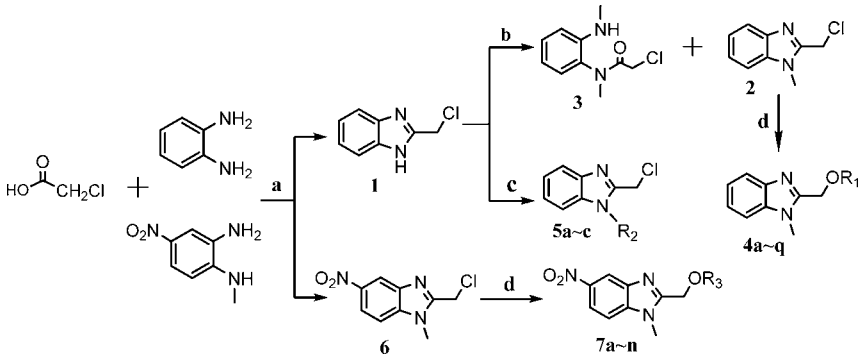
Scheme 1. Synthetic Route of 2-Chloromethyl-1*H*-benzimidazole Derivatives[a]



[a]Reagents and conditions: (a) HCl, reflux; (b) toluene, $(CH_3O)_2SO_2$, reflux; (c) triethylamine, acetone, acyl chloride, 0 °C; (d) K_2CO_3, acetone, phenols, reflux, 60 °C.

R_1=	R_2=
4a: $-C_6H_4-COCH_3$ (m)	**5a:** −Ac
4b: $-C_6H_4-COCH_3$ (o)	**5b:** $-O_2SCH_3$
4c: $-C_6H_4-COCH_3$ (p)	**5c:** $-O_2SC_6H_4Cl$(p)
4d: $-C_6H_4-COOCH_2CH_2CH_3$ (p)	R_3=
4e: $-C_6H_4-F$ (p)	**7a:** $-C_6H_4-COCH_3$ (m)
4f: $-C_6H_4-Cl$ (p)	**7b:** $-C_6H_4-COCH_3$ (o)
4g: $-C_6H_4-CHO$ (p)	**7c:** $-C_6H_4-COCH_3$ (p)
4h: $-C_6H_3-OCH_3$ (*o*)-$CH_2CH{=}CH_2$(p)	**7d:** $-C_6H_4-COOCH_2CH_2CH_3$ (p)
4i: −naphthylene (a)	**7e:** $-C_6H_4-F$ (p)
4j: $-C_6H_4-COOCH_2CH_3$ (p)	**7f:** $-C_6H_4-Cl$ (p)
4k: $-C_6H_4-COOCH_3$ (p)	**7g:** $-C_6H_4-CHO$ (p)
4l: $-C_6H_4-C(CH_3)_3$ (p)	**7h:** $-C_6H_3-OCH_3$ (*o*)-$CH_2CH{=}CH_2$(p)
4m: $-C_6H_5$	**7i:** −naphthylene (a)
4n: $-C_6H_3-(-OCH_2O-)$(m,p)	**7j:** $-C_6H_4-COOCH_2CH_3$ (p)
4o: $-C_6H_3-OCH_3$ (*o*)-CHO(p)	**7k:** $-C_6H_4-COOCH_3$ (p)
4p: $-C_6H_3-OCH_3$ (*o*)-$OCCH_3$(p)	**7l:** $-C_6H_4-C(CH_3)_3$ (p)
4q: $-C_6H_4-NO_2$ (p)	**7m:** $-C_6H_5$
	7n: $-C_6H_3-(-OCH_2O-)$(m,p)

(NMR) spectra were recorded on a Bruker Avance spectrometer (Unity plus 400 or 500 MHz) (Bruker Bios pin, Rheinstetten, Germany) with tetramethylsilane (TMS) as internal standard. Chemical shift values (δ) were given in parts per million (ppm). Thin-layer chromatography (TLC) was performed on silica gel 60 F_{254} (Qingdao Marine Chemical Ltd., P. R. China). Column chromatography (CC) was performed over silica gel (200−300 mesh, Qingdao Marine Chemical Ltd.), High resolution electrospray ionization mass spectrometry (HRESIMS) data were recorded on LCMS-IT-TOF (Shimadzu, Kyoto, Japan). Commercial solvents and reagents were of reagent grade.

General Synthetic Procedure for the Key Intermediates. Intermediates **1** and **6** were synthesized from *o*-phenylenediamine using the reported procedure.[11−15] Intermediate **2** was synthesized according to reported literature.[11]

*Synthesis of 2-Chloromethyl-1H-benzimidazole (**1**) by Conventional Method.* A mixture of *o*-phenylenediamine (7.56 g, 80 mmol) and chloroacetic acid (7.56 g, 70 mmol) was taken in a solution of 5 N HCl (60 mL), refluxed in oil bath for 8 h. The reaction mixture was then cooled to room temperature and neutralized with ammonia, till neutral to pH. A yellow residue was obtained by filtration and washed with water.[11,12] Purification by CC on silica gel eluting with petroleum ether (PE)-acetone (3:1) gave **1** as a yellow solid (9.23 g, yield 79.2%). mp, 152−154 °C (lit.[10] 154−155 °C); MS (+ESI) m/z 166.96 $[M+H]^+$.

*Synthesis of 2-Chloromethyl-1-methyl-benzimidazole (**2**) by Conventional Method.* To a solution of compound **1** (1.01 g, 6.07 mmol) in anhydrous toluene (10 mL), dimethyl sulfate (0.63 mL, 6.67 mmol) was added dropwise at room temperature. The reaction mixture was refluxed in oil bath for 3 h. After cooling, water (10 mL) was added and the mixture was basified with ammonia.[11] After filtration, the solution was extracted with $CHCl_3$ (3 × 20 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated. Purification by CC on silica gel eluting with PE−acetone (4:1) gave the major product **2** as a light yellow solid (0.55 g, yield 50%) and the minor product **3** as a white crystal (0.29 g, yield 9%). Compound **2**: HR-MS (ESI): m/z calcd. for $C_9H_9ClN_2$: 181.0527; found: 181.0514 $[M+H]^+$; Compound **3**: mp, 126 − 128 °C; ^1H NMR (500 MHz, $CDCl_3$) δ 7.24 (d, J = 7.5 Hz, 1H, ArH), 6.99 (d, J = 7.8 Hz, 1H, ArH), 6.67 (t, J = 6.7 Hz, 2H, ArH), 4.23 (d, J = 3.2 Hz, 1H, −NH−), 3.84 (q, J = 13.7 Hz, 2H, $-CH_2Cl-$), 3.18 (s, 3H, −NMe−H), 2.85 (d, J = 4.8 Hz, 3H, −HNMe−H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.7 (−C═O), 145.0 (−C−NH−), 130.4 (ArC), 128.0 (ArC), 127.0 (ArC), 117.1 (ArC), 111.3 (ArC), 42.3 ($-CH_2Cl-$), 36.1 (−NMe−C), 30.2 (−HNMe−C); HR-MS (ESI): m/z calcd. for $C_{10}H_{13}ClN_2O$: 213.0789; found: 213.0765 $[M+H]^+$.

*Synthesis of 2-Chloromethyl-1-methyl-5-nitro-1H-benzimidazole (**6**) by Conventional Method.* A mixture of 4-nitro-2-amino-1-*N*-methylaniline (3.52 g, 21 mmol) and chloroacetic acid (2.58 g, 25.2 mmol) was refluxed for 4 h in 45 mL of 4 N HCl and then cooled to room temperature and adjusted to pH = 7 with ammonia. A gray residue was obtained.[13−15] Purification by column chromatography on neutral Al_2O_3 eluting with $CHCl_3$−MeOH (100:0.8) gave compound **6** as a yellow solid (3.37 g, yield 71%), mp, 180−182 °C (lit.[13] 191−192 °C). ^1H NMR (500 MHz, $CDCl_3$) δ 8.66 (s, 1H, ArH), 8.28 (d, J = 8.9 Hz, 1H, ArH), 7.44 (d, J = 8.9 Hz, 1H, ArH), 4.87 (s, 2H, $-CH_2O-$), 3.96 (s, 3H, Me−H); ^{13}C NMR (125 MHz, $CDCl_3$) δ

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152.9 (ArC), 144.2 (ArC), 141.4 (−N−C═N−), 140.2 (ArC), 119.5 (ArC), 117.2 (ArC), 109.8 (ArC), 36.5 (−CH_2Cl−), 31.0 (Me-C). HR-MS (ESI): *m/z* calcd. for $C_9H_8ClN_3O_2$: 226.0378; found: 226.0352 $[M+H]^+$.

General Procedure To Synthesize Compounds 4a−q by Conventional Method. To a solution of compound **2** (90.25 mg, 0.5 mmol) and different phenols (0.55 mmol) in dry acetone (30 mL), anhydrous potassium carbonate (103.5 mg, 0.75 mmol) was added. The mixture was refluxed in oil bath at 60 °C for 8 h. After cooling and filtration, the solution was concentrated under vacuum and then purified by CC on silica gel eluting with $CHCl_3$−MeOH (9:1−95:5) to obtain the title compounds **4a−q**.

*1-(3-((1-Methyl-1H-benzo[d]imidazol-2-yl)methoxy)phenyl)-ethanone (**4a**).* Yield, 81%; white solid; mp, 128−130 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82−7.75 (m, 1H, ArH), 7.70−7.64 (m, 1H, ArH), 7.57 (d, *J* = 7.6 Hz, 1H, ArH), 7.41−7.34 (m, 2H, ArH × 2), 7.30 (dq, *J* = 7.0, 5.4 Hz, 3H, ArH × 3), 5.42 (s, 2H, −CH_2O−), 3.88 (s, 3H, Me-H), 2.58 (s, 3H, Me-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 197.9 (−C═O), 158.2 (ArC), 149.0 (ArC), 142.3 (ArC), 138.8 (−N═C−), 136.3 (ArC), 130.0 (ArC), 123.5 (ArC), 122.6 (ArC), 122.0 (ArC), 120.3 (ArC), 119.9 (ArC), 114.3 (ArC), 109.6 (ArC), 63.5 (−CH_2O−), 30.4 (−NMe−C), 26.9 (−Me−C). HR-MS (ESI): *m/z* calcd. for $C_{17}H_{16}N_2O_2$: 281.1285; found: 281.1264 $[M+H]^+$.

*1-(2-((1-Methyl-1H-benzo[d]imidazol-2-yl)methoxy)phenyl)-ethanone (**4b**).* Yield, 83%; white solid; mp, 122−124 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (dd, *J* = 6.9, 1.3 Hz, 1H, ArH), 7.68 (dd, *J* = 7.7, 1.8 Hz, 1H, ArH), 7.46 (ddd, *J* = 8.6, 7.4, 1.8 Hz, 1H, ArH), 7.40−7.36 (m, 1H, ArH), 7.36−7.28 (m, 3H, ArH × 3), 7.08−6.99 (m, 1H, ArH), 5.48 (s, 2H, −CH_2O−), 3.90 (s, 3H, Me−H), 2.55 (s, 3H, Me−H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.6 (−C═O), 156.9 (ArC), 148.8 (ArC), 142.1 (−N═C−), 136.3 (ArC), 133.8 (ArC), 130.5 (ArC), 129.0 (ArC), 123.7 (ArC), 122.7 (ArC), 121.8 (ArC), 120.3 (ArC), 113.3 (ArC), 109.7 (ArC), 63.8 (−CH_2O−), 31.8 (−NMe−C), 30.5 (Me−C). HR-MS (ESI): *m/z* calcd. for $C_{17}H_{16}N_2O_2$: 281.1285; found: 281.1266 $[M+H]^+$.

*1-(4-((1-Methyl-1H-benzo[d]imidazol-2-yl)methoxy)phenyl)-ethanone (**4c**).* Yield, 86%; white solid; mp, 186−188 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00−7.86 (m, 2H), 7.78 (dd, *J* = 6.8, 1.5 Hz, 1H, ArH), 7.38−7.27 (m, 3H, ArH × 3), 7.19−7.08 (m, 2H, ArH × 2), 5.44 (s, 2H, −CH_2O−), 3.87 (s, 3H, Me−H), 2.53 (s, 3H, Me−H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.8 (−C═O), 161.7 (ArC), 148.8 (ArC), 142.3 (−N═C−), 136.3 (ArC), 131.3 (ArC), 130.8 (ArC × 2), 123.6 (ArC), 122.6 (Ph- C), 120.3 (ArC), 114.7 (ArC × 2), 109.6 (ArC), 63.5 (−CH_2O−), 30.4 (−NMe−C), 26.5 (Me−C). HR-MS (ESI): *m/z* calcd. for $C_{17}H_{16}N_2O_2$: 281.1285; found: 281.1265 $[M+H]^+$.

*Propyl 4-((1-methyl-1H-benzo[d]imidazol-2-yl)methoxy)-benzoate (**4d**).* Yield, 80%; white solid; mp, 96−98 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10−7.92 (m, 2H, ArH × 2), 7.82−7.75 (m, 1H, ArH), 7.42−7.24 (m, 3H, ArH × 3), 7.20−7.04 (m, 2H, ArH × 2), 5.44 (s, 2H, −CH_2O−), 4.23 (t, *J* = 6.7 Hz, 2H, −CH_2O−), 3.88 (s, 3H, Me-H), 1.85−1.68 (m, 2H, −CH_2−), 1.00 (t, *J* = 7.4 Hz, 3H, Me−H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.3 (ArC), 161.5 (ArC), 148.8 (ArC), 142.2 (ArC), 136.3 (ArC), 131.8 (ArC × 2), 124.2 (ArC), 123.6 (ArC), 122.6 (ArC), 120.3 (ArC), 114.6 (ArC × 2), 109.6 (ArC), 66.5 (−CH_2O−), 63.5 (−CH_2O−), 30.5 (−NMe−C), 22.3 (−CH_2−), 10.6 (Me-C). HR-MS (ESI): *m/z* calcd. for $C_9H_9ClN_2$: 325.1547; found: 325.1526 $[M+H]^+$.

*2-((Benzo[d][1,3]dioxol-5-yloxy)methyl)-1-methyl-1H-benzo[d]-imidazole (**4n**).* Yield, 80%; light pink solid; mp, 138−140 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (dd, *J* = 6.9, 1.5 Hz, 1H, ArH), 7.32 (dtd, *J* = 9.2, 7.1, 1.5 Hz, 3H, ArH × 3), 6.69 (d, *J* = 8.5 Hz, 1H), 6.62 (d, *J* = 2.5 Hz, 1H, ArH), 6.51 (dd, *J* = 8.5, 2.5 Hz, 1H, ArH), 5.90 (s, 2H, −OCH_2O−), 5.30 (d, *J* = 1.8 Hz, 2H, −CH_2O−), 3.87 (d, *J* = 2.1 Hz, 3H, −Me−H−). ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.4 (ArC), 148.5 (ArC), 147.5 (ArC), 141.6 (ArC), 141.3 (−C−), 135.3 (ArC), 122.4 (ArC), 121.5 (ArC), 119.2 (ArC), 108.5 (ArC), 107.2 (ArC), 105.3 (ArC), 100.4 (ArC), 97.6 (−OCH_2O−), 63.5 (−CH_2O−), 29.4 (−NMe−C). HR-MS (ESI): *m/z* calcd. for $C_{16}H_{14}N_2O_3$: 283.1077; found: 283.1059 $[M+H]^+$.

*3-Methoxy-4-((1-methyl-1H-benzo[d]imidazol-2-yl)methoxy)-benzaldehyde (**4o**).* Yield, 88%; light yellow solid; mp, 144 − 146 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.83 (s, 1H, ArH), 7.77 (dd, *J* = 6.9, 1.4 Hz, 1H, ArH), 7.40 (dd, *J* = 5.6, 1.7 Hz, 2H, ArH × 2), 7.36 (d, *J* = 8.7 Hz, 2H, ArH × 2), 7.34 − 7.26 (m, 2H, ArH × 2), 5.54 (s, 2H, −CH_2O−), 3.92 (s, 3H, −OMe−H−), 3.91 (s, 3H, −Me−H−). ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.0 (−CHO), 152.7 (ArC), 150.3 (ArC), 148.6 (ArC), 142.2 (ArC), 136.4 (ArC), 131.2 (ArC), 126.8 (ArC), 123.6 (ArC), 122.6 (ArC), 120.3 (ArC), 113.4 (ArC), 109.6 (ArC), 109.4 (ArC), 64.5 (−CH_2O−), 56.1 (−OMe−C), 30.5 (−NMe−C). HR-MS (ESI): *m/z* calcd. for $C_{17}H_{16}N_2O_3$: 297.1234; found: 297.1220 $[M+H]^+$.

General Synthetic Procedure for Compounds 5a−c by a Slightly Modified Method. To a solution of compound **1** (166.5 mg, 1 mmol) and triethylamine (0.13, 0.9 mmol) in dry acetone (10 mL), three different acyl chlorides including acetyl chloride, methanesulfonyl chloride and 4-chlorobenzenesulfonyl chloride (0.9 mmol) was added dropwise. The mixture was stirred at 0 °C for 12 h.[16−18] The mixture was concentrated under vacuum and then purified by CC on silica gel eluting with PE−EtOAc (3:1−5:1) to obtain the corresponding compounds **5a−c**.

*2-(Chloromethyl)-1-((4-chlorophenyl)sulfonyl)-1H-benzo[d]-imidazole (**5c**).* Yield, 68%; white solid; mp, 150−152 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09−8.01 (m, 2H, ArH × 2), 7.92 (t, *J* = 5.5 Hz, 1H, ArH), 7.78−7.73 (m, 1H, ArH), 7.50 (dd, *J* = 9.1, 2.1 Hz, 2H, ArH × 2), 7.45−7.37 (m, 2H, ArH × 2), 5.16 (s, 2H, −CH_2−). ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.0 (C), 142.0 (ArC), 141.7 (ArC), 136.2, 132.9 (ArC), 130.1 (ArC × 2), 129.1 (ArC × 2), 126.5 (ArC), 125.6 (ArC), 121.3 (ArC), 113.6 (ArC), 37.9 (−CH_2−). HR-MS (ESI): *m/z* calcd. for $C_{14}H_{10}Cl_2N_2O_2S$: 340.9913; found: 340.9904 $[M+H]^+$.

General Procedure To Synthesize Compounds 7a−n by Conventional Method. The method to synthesize this series of compounds is the same as the compound **4**.

*1-(3-((1-Methyl-5-nitro-1H-benzo[d]imidazol-2-yl)methoxy)-phenyl)ethanone (**7a**).* Yield, 82%; white solid; mp, 200−202 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.62 (d, *J* = 2.0 Hz, 1H, ArH), 8.19 (dd, *J* = 8.9, 2.1 Hz, 1H, ArH), 7.60 (s, 1H, ArH), 7.53 (d, *J* = 7.7 Hz, 1H, ArH), 7.41 − 7.31 (m, 2H, ArH × 2), 7.22 (dd, *J* = 8.2, 2.6 Hz, 1H, ArH), 5.39 (s, 2H, −CH_2O−), 3.90 (s, 3H, Me-H), 2.53(s, 3H, Me). ^{13}C NMR (100 MHz, $CDCl_3$) δ 197.7 (−C═O), 158.0 (ArC), 153.0 (ArC), 144.0 (ArC), 141.6 (−N═C−), 140.3 (ArC), 138.9 (ArC), 130.2 (ArC), 122.5 (ArC), 119.9 (ArC), 119.3 (ArC), 117.1 (ArC), 114.0 (ArC), 109.7 (ArC), 63.4 (−CH_2O−), 31.0 (−NMe−C), 26.9 (−O═C−Me−C). HR-MS (ESI): *m/z* calcd. for $C_{17}H_{15}N_3O_4$: 326.1135; found: 326.1126 $[M+H]^+$.

*1-(2-((1-Methyl-5-nitro-1H-benzo[d]imidazol-2-yl)methoxy)-phenyl)ethanone (**7b**).* Yield, 81%; white solid; mp, 202−204 °C; ^{1}H NMR (400 MHz, Pyr) δ 8.89 (d, *J* = 2.1 Hz, 1H, ArH), 8.30 (dd, *J* = 9.0, 2.2 Hz, 1H, ArH), 7.88 (dd, *J* = 7.7, 1.7 Hz, 1H, ArH), 7.52 (dd, *J* = 8.3, 3.4 Hz, 2H, ArH × 2), 7.50 − 7.44 (m, 1H, 1H, ArH), 7.09 − 7.02 (m, 1H, 1H, ArH), 5.70 (s, 2H, −CH_2O−), 3.88 (s, 3H, Me−H), 2.62 (s, 3H, Me−H). ^{13}C NMR (100 MHz, C_5D_5N) δ 198.8 (−C═O), 157.2 (ArC), 153.6 (ArC), 143.9 (ArC), 141.9 (−N═C−), 140.7 (ArC), 133.7 (ArC), 130.5 (ArC), 129.3 (ArC), 121.8 (ArC), 118.8 (ArC), 116.8 (ArC), 113.8 (ArC), 110.6 (ArC), 63.5 (−CH_2O−), 31.6 (−NMe−C), 30.6 (−O═C−Me−C). HR-MS (ESI): *m/z* calcd. for $C_{17}H_{15}N_3O_5$: 326.1135; found: 326.1128 $[M+H]^+$.

*1-(4-((1-Methyl-5-nitro-1H-benzo[d]imidazol-2-yl)methoxy)-phenyl)ethanone (**7c**).* Yield, 85%; white solid; mp, 254−256 °C; ^{1}H NMR (400 MHz, C_5D_5N) δ 8.92 (d, *J* = 2.2 Hz, 1H, ArH), 8.31 (dd, *J* = 8.9, 2.2 Hz, 1H, ArH), 8.09 (d, *J* = 8.9 Hz, 2H, ArH × 2), 7.50 (d, *J* = 9.0 Hz, 1H, ArH), 7.34 (d, *J* = 8.9 Hz, 2H, ArH × 2), 5.69 (s, 2H, −CH_2O−), 3.81 (s, 3H, Me−H), 2.51 (s, 3H, Me-H). HR-MS (ESI): *m/z* calcd. for $C_{17}H_{15}N_3O_6$: 326.1135; found: 326.1128 $[M+H]^+$.

*Propyl-4-((1-methyl-5-nitro-1H-benzo[d]imidazol-2-yl)methoxy)-benzoate (**7d**).* Yield, 83%; white solid; mp, 140−142 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (d, *J* = 2.1 Hz, 1H, ArH), 8.25 (dd, *J* = 9.0, 2.1 Hz, 1H,ArH), 8.05−7.98 (m, 2H, ArH × 2), 7.43 (d, *J* = 9.0 Hz, 1H, ArH), 7.14−7.06 (m, 2H, ArH × 2), 5.47 (s, 2H, −CH_2O−), 4.24 (t, *J* = 6.7 Hz, 2H, −OCH_2Et), 3.96 (s, 3H, Me−H), 1.76 (dq, *J* = 14.2, 7.2 Hz, 2H, −CH_2Me), 1.00 (t, *J* = 7.4 Hz, 3H, Me−H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.2 (−C═O), 161.2 (ArC), 152.7

(ArC), 144.1 (ArC), 141.6 (−N═C−), 140.3 (ArC), 131.9 (ArC × 2), 124.6 (ArC), 119.3 (ArC), 117.1 (ArC), 114.5 (ArC × 2), 109.7 (ArC), 66.6 ($-CH_2O-$), 63.3 ($-CH_2Et$), 31.1 (−NMe−C), 22.2 ($-CH_2Me$), 10.6 (Me-C). HR-MS (ESI): *m/z* calcd. for $C_{19}H_{19}N_3O_5$: 370.1397; found: 370.1384 $[M+H]^+$.

*2-((4-Fluorophenoxy)methyl)-1-methyl-5-nitro-1H-benzo[d]-imidazole (**7e**).* Yield, 80%; light yellow solid; mp, 224−226 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 8.69 (d, *J* = 2.1 Hz, 1H,ArH), 8.27 (dd, *J* = 8.9, 2.1 Hz, 1H, ArH), 7.43 (d, *J* = 9.0 Hz, 1H, ArH), 7.07−6.93 (m, 4H, ArH × 4), 5.38 (s, 2H, $-CH_2O-$), 3.96 (s, 3H, Me−H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.3 (ArC), 153.8 (ArC), 153.2 (ArC), 144.1 (ArC), 141.60 (−N═C−), 140.4 (ArC), 119.3 (ArC), 117.1 (ArC), 116.5 (ArC), 116.3 (ArC), 116.1 (ArC), 116.1 (ArC), 109.6 (ArC), 64.0 ($-CH_2O-$), 31.1 (−NMe−C). HR-MS (ESI): *m/z* calcd. for $C_{15}H_{12}FN_3O_3$: 302.0935; found: 302.0925 $[M+H]^+$.

*2-((4-Chlorophenoxy)methyl)-1-methyl-5-nitro-1H-benzo[d]-imidazole (**7f**).* Yield, 81%; white solid; mp, 256−258 °C; ^1H NMR (400 MHz, C_5D_5N) δ 8.90 (d, *J* = 2.1 Hz, 1H, ArH), 8.30 (dd, *J* = 8.9, 2.2 Hz, 1H, ArH), 7.48 (d, *J* = 9.0 Hz, 1H, ArH), 7.41−7.32 (m, 2H, ArH × 2), 7.24−7.21 (m, 2H, ArH × 2), 5.56 (s, 2H, $-CH_2O-$), 3.78 (s, 3H, Me−H). HR-MS (ESI): *m/z* calcd. for $C_{15}H_{12}ClN_3O_3$: 318.0640; found: 318.0633 $[M+H]^+$.

*4-((1-Methyl-5-nitro-1H-benzo[d]imidazol-2-yl)methoxy)-benzaldehyde (**7g**).* Yield, 83%; white solid; mp, 248−250 °C; ^1H NMR (400 MHz, C_5D_5N) δ 10.02 (s, 1H, −CHO), 8.92 (d, *J* = 2.1 Hz, 1H, ArH), 8.31 (dd, *J* = 9.0, 2.2 Hz, 1H, ArH), 7.96 (d, *J* = 8.8 Hz, 2H, ArH × 2), 7.50 (d, *J* = 9.0 Hz, 1H, ArH), 7.38 (d, *J* = 8.7 Hz, 2H, ArH × 2), 5.70 (s, 2H, $-CH_2O-$), 3.81 (s, 3H, Me-H). HR-MS (ESI): *m/z* calcd. for $C_{16}H_{13}N_3O_4$: 312.0979; found: 312.0968 $[M+H]^+$.

*2-((4-Allyl-2-methoxyphenoxy)methyl)-1-methyl-5-nitro-1H-benzo[d] imidazole (**7h**).* Yield, 83%; light yellow solid; mp, 114−116 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 8.66 (d, *J* = 2.1 Hz, 1H, ArH), 8.24 (dd, *J* = 8.9, 2.1 Hz, 1H, ArH), 7.41 (d, *J* = 9.0 Hz, 1H, ArH), 7.03 (d, *J* = 8.1 Hz, 1H, ArH), 6.77−6.67 (m, 2H, ArH × 2), 5.93 (ddt, *J* = 16.9, 10.1, 6.7 Hz, 1H, $-CH_2CH-$), 5.41 (s, 2H, $-CH_2O-$), 5.15 − 5.01 (m, 2H, $-═CH_2$), 4.02 (s, 3H, MeO-H), 3.84 (s, 3H, Me−H), 3.32 (d, *J* = 6.7 Hz, 2H, $-CH_2CH-$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.8 (ArC), 150.1 (ArC), 145.3 (ArC), 143.9 (ArC), 141.6 (−N═C−), 140.5 (ArC), 137.4 (ArC), 135.5 (ArC), 120.8 (ArC), 119.1 (ArC), 117.00 (ArC), 116.1 ($=CH_2$), 115.9 (ArC), 112.6 (ArC), 109.5 (ArC), 65.2 ($-CH_2O-$), 55.9 (MeO-C), 40.0 ($-CH_2-$), 31.0 (−NMe−C). HR-MS (ESI): *m/z* calcd. for $C_{19}H_{19}N_3O_4$: 354.1448; found: 354.1436 $[M+H]^+$.

*1-Methyl-2-((naphthalen-1-yloxy)methyl)-5-nitro-1H-benzo[d]-imidazole (**7i**).* Yield, 82%; pink solid; mp, 200−202 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 8.78 − 8.67 (m, 1H, ArH), 8.34 − 8.17 (m, 2H, ArH × 2), 7.82 (d, *J* = 7.7 Hz, 1H, ArH), 7.63−7.46 (m, 3H, ArH × 3), 7.40 (dd, *J* = 18.9, 8.6 Hz, 2H, ArH × 2), 7.11 (d, *J* = 7.6 Hz, 1H, ArH), 5.62 (s, 2H, $-CH_2O-$), 4.00 (s, 3H, Me−H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.4 (ArC), 153.3 (ArC), 144.0 (ArC), 141.7 (−N═C−), 140.5 (ArC), 134.7 (ArC), 127.9 (ArC), 126.8 (ArC), 125.9 (ArC), 125.9 (ArC), 125.4 (ArC), 121.9 (ArC), 121.5 (ArC), 119.2 (ArC), 117.1 (ArC), 109.6 (ArC), 105.6 (ArC), 63.5 ($-CH_2O-$), 31.2 (−NMe−C). HR-MS (ESI): *m/z* calcd. for $C_{19}H_{15}N_3O_3$: 334.1186; found: 334.1177 $[M+H]^+$.

*Ethyl 4-((1-methyl-5-nitro-1H-benzo[d]imidazol-2-yl)methoxy)-benzoate (**7j**).* Yield, 83%; white solid; mp, 190−192 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 8.68 (d, *J* = 2.0 Hz, 1H, ArH), 8.26 (dd, *J* = 8.9, 2.1 Hz, 1H, ArH), 8.01 (d, *J* = 8.8 Hz, 2H, ArH × 2), 7.43 (d, *J* = 9.0 Hz, 1H, ArH), 7.10 (d, *J* = 8.9 Hz, 2H, ArH × 2), 5.47 (s, 2H, $-CH_2O-$), 4.34 (q, *J* = 7.1 Hz, 2H, $-OCH_2CH_3-$), 3.96 (s, 3H, Me−H), 1.36 (t, *J* = 7.1 Hz, 3H,Me−H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.2 (ArC), 161.2 (ArC), 152.7 (ArC), 144.1 (ArC), 141.56 (−N═C−), 140.3 (ArC), 131.9 (ArC × 2), 124.6 (ArC), 119.3 (ArC), 117.1 (ArC), 114.5 (ArC × 2), 109.7 (ArC), 63.3 ($-CH_2O-$), 61.0 ($-OCH_2Me$), 31.1 (−NMe−C), 14.48 (Me−C). HR-MS (ESI): *m/z* calcd. for $C_{18}H_{17}N_3O_5$: 356.1241; found: 356.1231 $[M+H]^+$.

*Methyl 4-((1-methyl-5-nitro-1H-benzo[d]imidazol-2-yl)methoxy)-benzoate (**7k**).* Yield, 81%; white solid; mp, 246−248 °C; ^1H NMR (400 MHz, C_5D_5N) δ 8.94−8.89 (m, 1H, ArH), 8.30 (dd, *J* = 8.9, 2.2 Hz, 1H, ArH), 8.24−8.12 (m, 2H, ArH × 2), 7.49 (d, *J* = 9.0 Hz, 1H, ArH), 7.42−7.30 (m, 2H, ArH × 2), 5.67 (s, 2H, $-CH_2O-$), 3.80 (s, 6H, Me × 2-H). HR-MS (ESI): *m/z* calcd. for $C_{17}H_{15}N_3O_5$: 342.1084; found: 342.1079 $[M+H]^+$.

*2-((4-(tert-Butyl)phenoxy)methyl)-1-methyl-5-nitro-1H−benzo-[d]imidazole (**7l**).* Yield, 85%; yellow solid; mp, 158−160 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 8.69 (d, *J* = 2.0 Hz, 1H, ArH), 8.25 (dd, *J* = 8.9, 2.1 Hz, 1H, ArH), 7.42 (d, *J* = 9.0 Hz, 1H, ArH), 7.39−7.27 (m, 2H, ArH × 2), 7.06−6.97 (m, 2H, ArH × 2), 5.40 (s, 2H, $-CH_2O-$), 3.99 − 3.93 (m, 3H, Me−H), 1.29 (s, 9H, Me × 3-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.4 (ArC), 153.7 (ArC), 145.0 (ArC), 144.0 (ArC), 141.5 (−N═C−), 140.4 (ArC), 126.7 (ArC 2), 119.2 (ArC), 117.0 (ArC), 114.3 (ArC × 2), 109.6 (ArC), 71.0 ($-CH_2O-$), 34.3 (−C−Me_3), 31.6 (Me × 3-C), 31.1 (−NMe−C). HR-MS (ESI): *m/z* calcd. for $C_{17}H_{15}N_3O_5$: 340.1656; found: 340.1642 $[M+H]^+$.

*1-Methyl-5-nitro-2-(phenoxymethyl)-1H-benzo[d]imidazole (**7m**).* Yield, 82%; light yellow solid; mp, 234−236 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 8.70 (d, *J* = 2.1 Hz, 1H, ArH), 8.27 (dd, *J* = 9.0, 2.1 Hz, 1H, ArH), 7.43 (d, *J* = 9.0 Hz, 1H, ArH), 7.32 (dd, *J* = 8.5, 7.5 Hz, 2H, ArH × 2), 7.07 (d, *J* = 8.0 Hz, 2H, ArH × 2), 7.02 (t, *J* = 7.4 Hz, 1H, ArH), 5.42 (s, 2H, $-CH_2O-$), 3.97 (s, 3H, Me−H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.6 (ArC), 153.3 (ArC), 143.9 (ArC), 141.5 (−N═C−), 140.3 (ArC), 129.8 (ArC × 2), 122.1 (ArC), 119.1 (ArC), 116.9 (ArC), 114.7 (ArC × 2), 109.5 (ArC), 63.2 ($-CH_2O-$), 31.0 (−NMe−C). HR-MS (ESI): *m/z* calcd. for $C_{15}H_{13}N_3O_3$: 284.1030; found: 284.1016 $[M+H]^+$.

*2-((Benzo[d][1,3]dioxol-5-yloxy)methyl)-1-methyl-5-nitro-1H-benzo[d]imidazole (**7n**).* Yield, 81%; light yellow solid; mp, 246−248 °C; ^1H NMR (400 MHz, C_5D_5N) δ 8.88 (d, *J* = 2.1 Hz, 1H, ArH), 8.29 (dd, *J* = 8.9, 2.2 Hz, 1H, ArH), 7.47 (d, *J* = 8.9 Hz, 1H, ArH), 6.98 (d, *J* = 2.5 Hz, 1H, ArH), 6.86 (d, *J* = 8.5 Hz, 1H, ArH), 6.74 (dd, *J* = 8.5, 2.5 Hz, 1H, ArH), 5.94 (s, 2H, $-OCH_2O-$), 5.52 (s, 2H, CH_2O), 3.80 (s, 3H, Me-H). HR-MS (ESI): *m/z* calcd. for $C_{16}H_{13}N_3O_5$: 328.0928; found: 328.0921 $[M+H]^+$.

For spectral data of known compounds **1**, **2**, **4e−m**, **4p−q**, **5a−b**, and **6** see the Supporting Information.

Antifungal Bioassay. According to our previously reported methods,[10,19−21] fungicidal activities of the synthetic compounds were tested in vitro against five plant pathogenic fungi (*Cytospora* sp., *Colletotrichum gloeosporioides*, *Botrytis cinerea*, *Alternaria solani*, and *Fusarium solani*) by using the mycelium growth rate method. All of the fungi were provided by the Institute of Pesticides, Northwest A&F University. The strains were retrieved from the storage tube and cultured for 2 weeks at 28 °C on potato dextrose agar (PDA).

Antifungal activity was assessed as follows: the synthesized compounds were screened in vitro for their antifungal activities against the five phytopathogenic fungi. PDA medium was prepared in the flasks and sterilized. Those compounds were dissolved in acetone before mixing with molten agar at 55 °C, and final concentrations of the stock solutions of the tested compounds dissolved in acetone were 100, 50, 25, 12.5, and 6.25 μg/mL. The medium was then poured into sterilized Petri dishes. All types of fungi were incubated in PDA at 28 °C for 1 week to get new mycelium for the antifungal assays, and a mycelia disk of approximately 4 mm diameter cut from culture medium was picked up with a sterilized inoculation needle and inoculated in the center of the PDA Petri dishes.[5,14] The inoculated Petri dishes were incubated at 28 °C for 3−4 d. Acetone was served as the control, while commercially available agricultural fungicide hymexazol was used as a positive control. Each sample was measured by three replicate, each colony diameter of the three replicate was measured 4 times by cross bracketing method. After the mycelia grew completely, the diameters of the mycelia were measured and the inhibition rate was calculated according to the formula

$$\text{inhibition rate } (\%) = (C - T)/(C - 4\text{mm}) \times 100\%$$

where *C* is the average diameter of mycelia in the blank test, and *T* is the average diameter of mycelia on treated PDA with those compounds. The inhibition ratio of those compounds at the concentration of 100 μg/mL is summarized in Table 1. The IC_{50} (median inhibitory concentration) values of some compounds were determined, and the results were listed in Table 2.

Table 1. Preliminary Antifungal Activities of Compounds at 100 μg/mL[a]

compound	average values of inhibition rate (%) to five pathogens				
	C. s.	C. g.	B. c.	A. s.	F. s.
1	<5.00	67.57	53.22	25.29	6.59
2	45.40	69.57	91.53	51.56	28.76
3	<5.00	41.27	64.39	25.29	30.11
4a	55.46	41.27	82.46	64.52	78.49
4b	64.52	71.43	64.91	65.52	64.73
4c	87.07	38.10	65.41	55.67	81.85
4d	48.28	20.63	<5.00	63.62	42.20
4e	80.60	62.67	47.37	85.22	83.87
4f	76.29	90.48	83.05	77.83	64.07
4g	20.98	49.21	<5.00	27.75	11.96
4h	49.71	38.10	47.37	40.07	60.35
4i	62.64	22.22	<5.00	50.74	52.96
4j	79.89	71.74	35.90	39.08	21.37
4k	31.03	<5.00	6.43	64.62	48.25
4l	28.16	63.84	64.81	23.65	<5.00
4m	84.20	87.30	29.82	75.37	84.54
4n	80.60	80.95	88.30	63.98	74.46
4o	10.20	<5.00	15.20	40.89	24.73
4p	26.01	14.29	59.06	17.90	<5.00
4q	19.54	41.27	<5.00	11.33	8.60
5a	8.05	43.87	57.26	11.33	21.37
5b	100.00	100.00	79.86	67.98	79.17
5c	27.44	74.60	57.26	31.86	28.09
6	100.00	100.00	48.70	100.00	100.00
7a	51.87	<5.00	<5.00	<5.00	<5.00
7b	<5.00	<5.00	<5.00	13.79	<5.00
7c	<5.00	<5.00	38.60	33.50	<5.00
7d	10.92	<5.00	<5.00	8.05	19.35
7e	22.41	<5.00	<5.00	<5.00	9.95
7f	10.20	6.35	100.00	30.21	<5.00
7g	9.48	<5.00	<5.00	16.26	<5.00
7h	<5.00	<5.00	23.98	35.14	25.40
7i	<5.00	<5.00	<5.00	19.54	<5.00
7j	<5.00	<5.00	<5.00	11.33	<5.00
7k	<5.00	59.13	32.20	<5.00	18.01
7l	<5.00	30.16	57.26	30.21	<5.00
7m	<5.00	<5.00	7.89	10.51	5.91
7n	12.36	<5.00	91.23	7.22	34.81
Hy	38.94	49.21	100.00	80.30	72.45

[a]A.s., *Alternaria solani*; B.c., *Botrytis cinerea*; C.g., *Colletotrichum gloeosporioides*; C.s. *Cytospora* sp; F.s., *Fusarium solani*; Hy, hymexazol.

The data of the fungicidal activities were statistically analyzed using Excel software from Windows to give the results of IC_{50} values. The results were expressed as the mean ± SD of triplicate experiments

RESULTS AND DISCUSSION

Chemistry. The synthetic routes of the title compounds are outlined in Scheme 1. Reaction of *o*-phenylenediamine with chloroacetic acid under reflux gave compound **1** in 79% yield, which was then treated with dimethyl sulfate in dried toluene to introduce a methyl group at N-1 position, affording the corresponding benzimidazole **2** as a major product in 50% yield and 2-chloro-*N*-(2-(methylamino)phenyl)acetamide **3** as a minor one. A possible formation mechanism of **3** was shown in Scheme 2. A formation of benzimidazole was thought to be reversible,[22] so a primary amine group of the resulting amide intermediate proceeds a first nucelophilic substitution reaction with dimethyl sulfate followed by a second nucelophilic substitution to form **3**. Subsequent Williamson reaction of the resulting intermediate **2** with various substituted phenols in dry acetone in anhydrous K_2CO_3 produced the desired compounds **4a−q** in good yields (>80%). Furthermore, treatment of **1** in the presence of triethylamine with three acetyl chloride and two sulfonyl chlorides yielded the corresponding products **5a−c** in ca. 70% yield. Similarly, the target compounds **7a−n** were prepared from **6** in good yields (>80%). At the beginning of this study, we made attempts to prepare **6** from **2** through nitration reaction, but an inseparable mixture of two isomers, **6** and its 6-nitro-1*H*-benzimidazole, was obtained by column chromatography.

The structures of the synthetic compounds were confirmed by ^{1}H NMR, ^{13}C NMR and MS, as well as HRESIMS spectroscopic data. Out of the 35 synthesized derivatives, 22 compounds (**3**, **4a−d**, **4n**, **4o**, **5c**, and **7a−n**) were new. It should be noted that the synthetic approach for this family of compounds used in the current study is a simple, efficient and low-cost method with high yields.

Antifungal Activity. Preliminary in vitro screening results of the title compounds for antifungal activities against five phytopathogenic fungi, *Cytospora* sp., *Colletotrichum gloeosporioides*, *Botrytis cinerea*, *Alternaria solani*, and *Fusarium solani*, are listed in Table 1. The results indicated that some of these synthetic compounds exhibited over 65% growth inhibition against mycelial growth of these tested fungi at concentrations of 100 μg/mL. Here, the inhibition rates were for only IC_{50} values for further comparison.

Encouraged by these preliminary findings, we planned further SAR studies on the title compounds. We determined their IC_{50} (median inhibitory concentration) values by using mycelial growth inhibitory rate method. As shown in Table 2, the tested compounds presented different fungicidal activity against *Cytospora* sp. and *C. gloeosporioides*, superior to the positive control hymexazol (IC_{50} > 100 μg/mL), whereas they were inactive or less active than hymexazol against *B. cinerea* and *A. solani* (IC_{50} = 8.92 and 14.03 μg/mL, respectively). Specifically, a fungicidal activity of the intermediate **6**, bearing a $-NO_2$ group, was 2−3 fold of **1** and **2** against the five fungal species (except *B. cinerea*). Notably, **6** showed remarkable antifungal activities against *Cytospora* sp., *C. gloeosporioides*, *A. solani*, and *F. solani* (IC_{50} = 22.48, 28.03, 27.95, and 23.34 μg/mL, respectively). The results revealed that the presence of the NO_2 group at the 5-position of benzimidazole ring in **6** could be critical for antifungal activity.

In case of the 4-series, compound **4m**, with nonsubstituents in the benzene ring displaying increased effects, showed higher growth inhibition of the three fungi *C. gloeosporioides*, *A. solani*, and *F. solani* with IC_{50} values of 20.76, 27.58, and 18.60 μg/mL (Table 2), respectively, than other 4-series derivatives such as **4e** and **4l** with a fluorine or *t*-butyl group, but had no activity toward *B. cinerea*. This indicated that para- or ortho-substitution of the benzene ring is not beneficial for the activity. Notably, **4m** showed a fungicidal activity against *F. solani* which was 2-fold of the control hymexazol (IC_{50} of 38.49 μg/mL). We reasoned that *F. solani* was not sensitive to the Cl atom (**4f** vs **4m** and **4e**). Furthermore, within the 4-series derivatives, compound **4f**, having a chlorine atom on para-position of the benzene ring, was the best compound in inhibiting both *Cytospora* sp. and *B. cinerea* with IC_{50} values of 24.91 and 26.82 μg/mL, respectively, in particular, with inhibition of **4f** on *Cytospora* sp. being much higher than that of the control hymexazol. This suggested the presence of this

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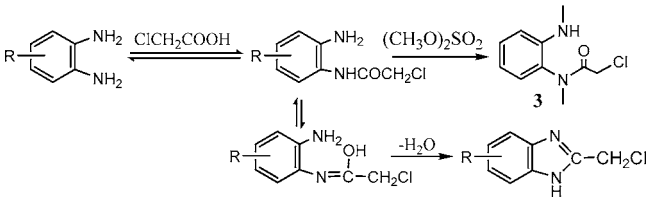
Table 2. Antifungal Activity of Some Compounds against Five Phytopathogens[a]

compound	IC_{50} ± SD/ (μg/mL)				
	C. s.	C. g.	B. c.	A. s.	F. s.
1	NA	83.15 ± 12.63	>100	>100	NA
2	>100	80.29 ± 2.03	68.11 ± 3.70	>100	>150
4a	>100	>120	68.88 ± 5.90	>100	82.36 ± 5.54
4b	>100	70.62 ± 0.81	>100	>100	>100
4c	50.68 ± 1.17	>120	89.58 ± 9.54	>100	39.05 ± 7.08
4e	58.25 ± 1.34	>100	>120	69.35 ± 4.51	31.52 ± 1.41
4f	24.91 ± 0.34	41.26 ± 2.25	26.82 ± 2.77	62.46 ± 1.10	>100
4j	52.58 ± 1.53	49.71 ± 3.58	>150	>150	>150
4m	50.04 ± 1.03	20.76 ± 0.67	>150	27.58 ± 0.59	18.60 ± 1.10
4n	54.30 ± 1.44	55.69 ± 0.18	63.72 ± 10.03	>100	72.97 ± 0.84
5a	NA	>150	50.91 ± 2.65	NA	NA
5b	30.97 ± 1.12	11.38 ± 0.90	57.71 ± 3.51	>100	40.15 ± 0.76
5c	NA	22.29 ± 0.69	>100	NA	NA
6	22.48 ± 0.44	28.03 ± 1.69	>100	27.95 ± 3.52	23.34 ± 2.08
7f	NA	NA	13.36 ± 1.35	NA	NA
7n	NA	NA	69.10 ± 2.57	NA	NA
Hy	>100	>100	8.92 ± 0.44	14.03 ± 0.14	38.49 ± 6.95

[a]NA, not active.

Scheme 2. Possible Mechanism for the Formation of 3



chlorine atom on para-position in the benzene ring to contribute to enhancing activity. Additionally, compared to intermediate **2**, displacement of the chlorine atom of **2** by varying phenoxyl groups resulted in the general trend of improving inhibitory activity of 4-series compounds to the five fungi due to lower IC_{50} data (Table 2). For example, inhibitory potency of **4m** to *C. gloeosporioides* increased by approximately 4-fold of **2** with an IC_{50} of 80.29 μg/mL.

In case of the 5-series, **5b** with a methanesulfonyl group addition exhibited moderate to strong antifungal effects on *Cytospora* sp., *C. gloeosporioides*, *B. cinerea*, and *F. solani* with IC_{50} values of 30.97, 11.38, 57.71, and 40.15 μg/mL, respectively, while **5c** selectively inhibited growth of *C. gloeosporioides* with an IC_{50} value of 22.29 μg/mL but was almost inactive toward the other fungal pathogens. Notably, **5b** was 2-fold more active against *C. gloeosporioides* (IC_{50} = 11.38 μg/mL) than **5c** with a 4-chlorobenzenesulfonyl moiety, which were both superior to the reference (IC_{50} > 100 μg/mL). In addition, **5a** gave moderate activity toward *B. cinerea* with an IC_{50} value of 50.91 ± 2.65 μg/mL but had no antifungal effects on other fungal pathogens. These findings indicated that introduction of a small bulky sulfonyl group at the NH-position of the benzimidazole ring appeared to be more favorable for increasing activity against *C. gloeosporioides* (**5b** and **5c** vs **5a**). These structures provide new templates for the potential treatment and management of anthracnose diseases.

However, only two phenoxyl ether derivatives **7f** and **7n** of intermediate **6** selectively inhibited the mycelial growth of *B. cinerea* (IC_{50} of 13.36 and 69.10 μg/mL, respectively), with **7f** being 5-fold stronger than **7n** and being comparable to hymexazol (IC_{50} = 8.92 μg/mL) in antifungal potency (Table 2). Both **7f** and **7n** presented much higher inhibitory activity against *B. cinerea* than the parent compound **6**. This suggested that the chlorine atom at para-position in the phenyl ring of **7f** plays an important role in the fungicidal activity to *B. cinerea*. Intriguingly, in comparison with **6**, **7f**, and **7n** had no fungicidal activity against the other pathogenic species tested *Cytospora* sp., *C. gloeosporioides*, *A. solani*, and *F. solani* since **7f** and **7n** were devoid of the chlorine group of **6**. These findings indicated that *B. cinerea* is more susceptible to **7f** than to **7n** and that the chlorine atom of **6** to be a structural feature important for improved antifungal activity to the other organisms.

In conclusion, we have reported the synthesis of a series of 2-chloromethyl-1*H*-benzimidazole derivatives as well as the ability of these compounds to inhibit growth of five fungal phytopathogenes tested. Many of the synthetic compounds tested, including intermediate **6**, were found to have significant antifungal activity against certain phytopathogenic fungi in vitro. Compounds **4m** and **6** displayed higher antifungal activity against *Cytospora* sp., *C. gloeosporioides*, *A. solani*, and *F. solani*. **5c** and **7f** also exhibited strong antifungal properties with relatively high selectivity against *C. gloeosporioides* and *B. cinerea* with IC_{50} values of 22.29 and 13.36 μg/mL, respectively. Compound **5b** gave substantially strong antifungal effects on *C. gloeosporioides* with an IC_{50} value of 11.38 μg/mL, which is much better than that of hymexazol, a well-known commercial fungicide. Taken together, compounds **4m**, **5b**, and **7f** were demonstrated to possess the most potent inhibition of the fungal plant pathogens tested in the present study and could be potential lead structures for further discovery of novel antifungal agrochemicals.

■ ASSOCIATED CONTENT

Supporting Information

HRMS and NMR spectra of new synthetic compounds, and (HR)MS and NMR data of known compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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