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ORIGINAL PAPER

Synthesis of *N*-substituted dimethylmaleimides and their antifungal activities against *Sclerotinia sclerotiorum*

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Abstract Compounds with maleimide, both natural and synthesized, have good biological activities, especially the antifungal activity. In order to investigate the antifungal activity of dimethylmaleimides, 17 *N*-substituted dimethylmaleimides were prepared from the reactions of 2,3-dimethyl maleic anhydride and amines using a facile synthetic method in this paper. These compounds were evaluated for antifungal activities against *Sclerotinia sclerotiorum* by the mycelium growth rate method. They exhibited minimum inhibitory concentrations (MICs) ranging from 0.01–50.0 μg/mL, with *N*-(2-benzimidazole)-3,4-dimethylmaleimide being the most active one with an MIC of 0.01 μg/mL. The structure and activity relationship on these compounds indicated that the hydrophobicity of the *N*-substituents is associated with their antifungal activity. Compared to current antifungals, most of *N*-substituted dimethylmaleimides have a perfect activity for *S. sclerotiorum* control and low toxicity.

Keywords Dimethylmaleimides · *Sclerotinia sclerotiorum* · Antifungal activity · Fungicide

Introduction

Microbial resistance to antimicrobials is an emerging challenge for pesticide industry. The multi-drug-resistant bacteria and fungi are the major causes of failure in the treatment of crop diseases. Developing novel highly effective antibacterial and antifungal agents with low toxicities is critically important for sustainable development of agriculture and environmental protection. Natural products have played an important role in this regard (Ahemad and Khan 2012; Amri et al. 2012; Seyran et al. 2010; Sudisha et al. 2010).

Previous studies have shown that natural products with the maleic anhydride structural moiety possess antifungal activity (Chen et al. 2007). The microbial metabolite tautomycin and related compounds with the maleic anhydride structural moiety also demonstrated strong antifungal activity against *Sclerotinia sclerotiorum* (Lib.) de Bary (Chen et al. 2011; Chen et al. 2010). Various maleimide derivatives have been synthesized and showed antifungal (Li et al. 2012; Sunita et al. 2010; Zicmanis et al. 1997) and antibacterial (Jens et al. 2005; Thomas and Stephan. 2010; Wu and Cheng. 2008; Wael et al. 2010; Frederic and Alain. 2002; David and Emmanuelle. 2010) activities as well as inhibitory effects of several enzymes (Silvia et al. 2005; Manas et al. 2006; Slavica et al. 2007). For example, *N*-(4-fluorophenyl)-dichloromaleimide significantly inhibits microbial growth and thus has been used to control the diseases of apple scab, rice blast, and tomato late blight (Wu and Hu 2009). It has a low toxicity with LD_{50} >15000 mg/kg in mice (Wu and Hu 2009). *N*-butylmaleimide and *N*-(4-phenylbutyl)-maleimide showed potent antifungal activity against ten fungi with minimum inhibitory concentrations (MICs) in the range of 0.48–15.63 μg/mL similar to that of ampicillin, but had little toxicity to human body (Sortino et al. 2011). Mechanistic studies suggest that this class of compounds interact preferably with the hydrophobic domains of the enzymes, based on the fact that the inactivation of sulfhydryl groups (Silvia

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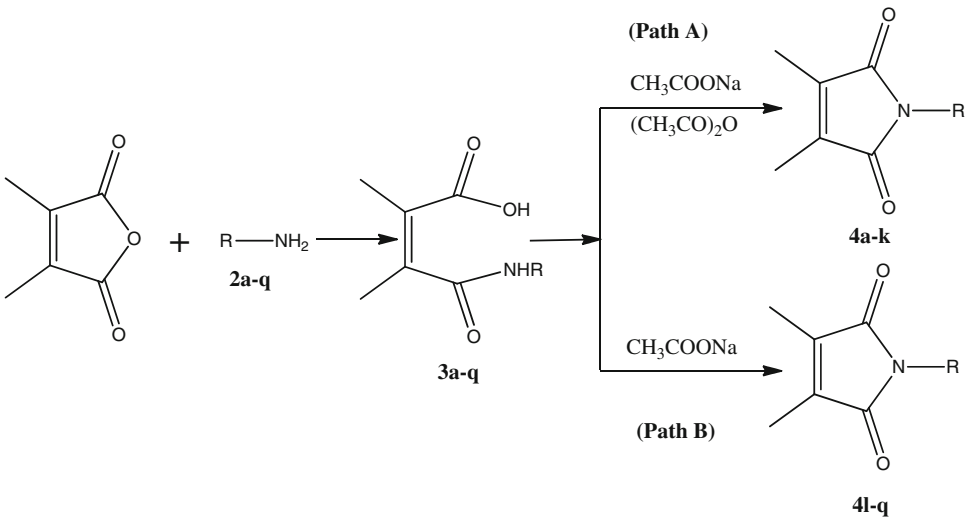

Fig. 1 Synthetic pathways of *N*-substituted dimethylmaleimides

et al. 2005), which is essential for catalytic activities, is affected by the double bond in the maleimide ring. The antimicrobial activity of *N*-ethylmaleimide (NEM) and *N-tert*-butylmaleimide is proved to be associated with the inhibition of β-(1, 3)-glucan synthase (Natalia et al. 2011) In addition, maleimide derivatives have also been extensively studied as potential antianxiety (Jerzy 2003), anti-inflammatory (Nara et al. 2010), anticancer (Khan et al. 2004; Sosabowski et al. 2009), and neuroprotective agents (Khan et al. 2004).

However, it has been noted that previous studies are primarily focused on *N*-substituted maleimides without substituents in positions 3 and 4 of the maleimide ring (Sunita et al. 2010; Daniela and Mircea. 2003). Moreover, most microorganisms researched in the studies were pathogenic human pathogens (Sortino et al. 2011). There are few studies on the synthesis of dimethylmaleimide compounds and their antimicrobial activities, especially in the field of pesticides (Li et al. 2012). In the present work, we report a two-step procedure for the synthesis of a series of *N*-substituted dimethylmaleimides and their inhibitory effects on mycelial growth of *S. sclerotiorum* in vitro. Our goal is to identify new fungicides with high potency against the agricultural pathogen *S. sclerotiorum*.

Materials and methods

Analytical Instruments

Dicloran (96 % purity, reference fungicide) was purchased from Sigma-Aldrich, USA. Other reagents and solvents were reagent grade purchased from the local markets of China. Melting points (Mp) were measured with a WRS-1A melting point apparatus, and were uncorrected. ^{1}H NMR spectra were recorded on a Bruker AVANCE III 500 spectrometer at 500 MHz using tetramethylsilane (TMS) as an internal standard. Electrospray ionization-mass spectra (EIMS) were measured on a mass spectrometer (Thermo Fisher Scientific, LCQ/ADVANTAGE). IR spectrum was recorded in KBr pellets on a Nicolet 6700 infrared spectrophotometer.

Synthesis of compounds

N-substituted dimethylmaleimide derivatives **4a–4q** were synthesized according to an improved procedure based on reported methods (Marcus et al. 1984; Tsou and Barrnentt 1955; Sauers and Middlebush 1962; Torigaoka 1986; Yu et al. 2008) using 2,3-dimethylmaleic anhydride **1** (purity: >98 %) designed in laboratory (Yu et al. 2008) and amines **2** purchased from Internet Aladdin Reagent Database Inc., Shanghai, China, as the starting materials (Fig. 1).

General procedure (Path A) for synthesizing compounds **4a–4k**: 2,3-dimethylmaleic anhydride (2.52 g, 20 mmol) in 15 mL of acetone or toluene was charged into a Pyrex glass flask equipped with a magnetic stirrer, a dropping funnel and a condenser. Then a solution of 19 mmol amine in 10 mL of acetone or toluene was added dropwise from the dropping funnel to the flask over a period of about 10 min. The reaction mixture was stirred at 25–65 °C for 1.5–8.5 h. Then anhydrous sodium acetate (0.08 g, 0.96 mmol), hydroquinone (0.08 g, 0.51 mmol) or cuprous iodide (0.16 g, 0.84 mmol), triethylamine (1.0 mL, 7.2 mmol) and acetic anhydride (3.0 mL, 31.8 mmol) were added sequentially. The reaction mixture was refluxed for additional 3–15 h. Upon completion of the reaction (monitored by TLC), the mixture was separated with silica column chromatography to afford products **4a–4k** in good yields.

General procedure (Path B) for synthesizing compounds **4l–4q**: The first step was the same as Path A except for the solvent for which only toluene was used. For the second step, anhydrous sodium acetate (0.10 g, 1.2 mmol),

hydroquinone (0.15 g, 0.95 mmol) and triethylamine (1.6 mL, 11.5 mmol) were added to the mixture. A high reaction temperature of 101 °C and longer reaction time from 10–24 h were applied, and the resultant water was removed by a water-separator. Upon completion of the reaction (monitored by TLC), the reaction mixture was washed with sodium hydroxide solution, dried over anhydrous sodium sulfate, distillated under reduced pressure and recrystallization in ethyl acetate to afford pure compounds **4l–4q**.

Antifungal activity assays

Test fungus

S. sclerotiorum was isolated from sclerotia of *S. sclerotiorum* collected from a diseased plant of oilseed rape in Zhejiang, People's Republic of China (Chen et al. 2011). The culture medium was potato dextrose agar (PDA).

Inhibition of mycelial growth of S. sclerotiorum by dimethylmaleimides

The in vitro antifungal activities of **4a–4q** against *S. sclerotiorum* were assessed using the mycelium growth rate method (Jiang et al. 2010). The compounds and dicloran were dissolved in 0.2 % (m/v) tween-80 solution were mixed with PDA to generate a series of concentrations in the final test solution at 0.01, 0.1, 1, 5, 10, 50, 100 μg/mL. Fungal cakes (6-mm) were placed at the center of the 9-cm PDA PETRI dishes. The compound-free agar with 0.2 % (m/v) tween-80 solution was used as the blank control. Three dishes were used for each test concentration. The dishes were incubated at 23 °C for 36 h. Then the diameter of each colony by making two measurements at right angles was measured (Chen et al. 2011). The tests were repeated twice. The inhibition rate was calculated using the following formula:

$$\text{Inhibition of growth } (\%) = (D_k - D)/(D_k - 6) \times 100$$

in which D_k is the average colony diameter of the blank control, D is the average diameter of the colony in the presence of test compounds, and 6 is the diameter of the inoculum plug (in mm).

The antifungal activities were assessed with MICs (minimum concentrations that showed the mycelium inhibition).

Statistical analysis

Analysis of variance (ANOVA) (SAS Institute, Cary, NC, USA, Version 8.0, 1999) was employed to determine the statistical significance of differences among treatments in each bioassay. The % data on inhibition of growth of *S. sclerotiorum* in each replicate was arcsine-transformed to angular data prior to ANOVA. Means for different treatments in each bioassay or trial were separated using the Least Significant Difference Test at $P = 0.05$ level.

Results

Synthesis

Seventeen *N*-substituted dimethylmaleimides (**4a–4q**) were synthesized by path A and path B shown in Fig. 1 using 2,3-dimethylmaleic anhydride **1** and amines **2**. The structures of all the compounds were determined by IR, EIMS, ^{1}H NMR and their physical and spectroscopic data are shown below:

N-butyl-3,4-dimethylmaleimide **(4a).** Yield 45.2 %. Yellow oil. IR (KBr) cm^{-1}: 3454, 2960, 2935, 2872, 1709, 1443, 1406, 1378, 1057, 732, 520. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.38 (2H, t, J = 7.0 Hz), 1.89 (6H, s), 1.43–1.48 (2H, m), 1.18–1.26 (2H, m), 0.86 (3H, t, J = 7.4 Hz). EI-MS *m/z* (%): 181 (31) $[M]^+$, 166 (1), 152 (6), 138 (100), 126 (8), 108 (6), 81 (6), 67 (5), 56 (10), 39 (6).

N-iso-butyl-3,4-dimethylmaleimide **(4b).** Yield 39.3 %. Yellow oil. IR (KBr) cm^{-1}: 3457, 2963, 2929, 2874, 1711, 1439, 1408, 1375, 1059, 734, 521. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.29 (2H, d, J = 7.4 Hz), 1.98-2.03 (1H, m), 1.96 (6H, s), 0.88 (3H, d, J = 6.7 Hz). EI-MS *m/z* (%): 181 (32) $[M]^+$, 166 (4), 138 (100), 126 (13), 108 (4), 67 (3), 56 (7), 39 (4).

N-amyl-3,4-dimethylmaleimide **(4c)**. Yield 47.8 %. Yellow oil. IR (KBr) cm^{-1}: 3457, 2958, 2934, 2862, 1701, 1441, 1407, 1373, 1061, 734, 521. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.48 (2H, t, J = 7.3 Hz), 1.97 (6H, s), 1.56 (2H, dt, J = 14.9, 7.5 Hz), 1.22–1.59 (4H, m), 0.88 (3H, t, J = 7.2 Hz). EI-MS *m/z* (%): 195 (37) $[M]^+$, 152 (14), 138 (100), 125 (11), 108 (6), 56 (11).

N-hexyl-3,4-dimethylmaleimide **(4d).** Yield 40.6 %. Yellow oil. IR (KBr) cm^{-1}: 3456, 2957, 2931, 2859, 1702, 1442, 1407, 1377, 1067, 734, 521. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.48 (2H, t, J = 6.7 Hz), 1.96 (6H, s), 1.56 (2H, dt, J = 14.5, 7.3 Hz), 1.26-1.30 (6H, m), 0.87 (3H, t, J = 7.0 Hz). EI-MS *m/z* (%): 209 (40) $[M]^+$, 194 (4), 166 (10), 152 (8), 138 (100), 126 (14), 108 (6), 81 (5), 67 (4), 56 (9), 41 (6).

N-cyclohexyl-3,4-dimethylmaleimide **(4e).** Yield 37.5 %. White crystals. Mp 64.8–65.4 °C. IR (KBr) cm^{-1}: 3446, 2930, 2860, 1703, 1400, 1381, 1089, 733, 524. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.84-3.91 (1H, m), 1.94 (6H, s), 1.26–2.08 (10H, m). EI-MS *m/z* (%): 207 (44) $[M]^+$, 164 (57), 138 (14), 126 (100), 108 (22), 82 (16), 67 (13), 54 (23), 27 (6).

N-octyl-3,4-dimethylmaleimide **(4f).** Yield 50.2 %. Colorless oil. IR (KBr) cm^{-1}: 3457, 2927, 2856, 1707,

1442, 1407, 1375, 1072, 733, 521. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.47 (2H, t, $J = 7.5$ Hz), 1.97 (6H, s), 1.57 (2H, dd, $J = 14.1$, 7.2 Hz), 1.26–1.29 (10H, m), 0.88 (3H, t, $J = 7.0$ Hz). EI-MS *m/z* (%): 237 (26) $[M]^+$, 194 (5), 152 (8), 138 (100), 126 (20), 108 (8), 81 (8), 56 (15), 41 (18).

N-dodecyl-3,4-dimethylmaleimide **(4g).** Yield 43.7 %. Colorless oil. IR (KBr) cm^{-1}: 3458, 2925, 2855, 1710, 1442, 1407, 1375, 1058, 733, 520. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.42 (2H, t, $J = 7.3$ Hz), 1.92 (6H, s), 1.52 (2H, dd, $J = 14.1$, 7.1 Hz), 1.21–1.27 (18H, m), 0.84 (3H, t, $J = 6.9$ Hz). EI-MS *m/z* (%): 293 (100) $[M]^+$, 250 (10), 138 (63), 108 (5), 41 (6).

N-phenyl-3,4-dimethylmaleimide **(4h).** Yield 44.7 %. White crystals. Mp 89.9–90.2 °C. IR (KBr) cm^{-1}: 3450, 1701, 1493, 1395, 1092, 770, 702, 522. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.50 (2H, d, $J = 7.9$ Hz), 7.28–7.32 (2H, m), 7.02 (1H, t, $J = 7.4$ Hz), 2.07 (6H, s). EI-MS *m/z* (%): 201 (100) $[M]^+$, 172 (9), 142 (33), 119 (15), 91 (15), 77 (7), 54 (26), 39 (11).

N-benzyl-3,4-dimethylmaleimide **(4i).** Yield 52.8 %. Yellow crystals. Mp 78.0–79.0 °C. IR (KBr) cm^{-1}: 3457, 3033, 1765, 1703, 1495, 1434, 1405, 1100, 1069, 927, 730, 700, 523. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.30–7.37 (5H, m), 4.66 (2H,s), 1.97 (6H, s). EI-MS *m/z* (%): 215 (100) $[M]^+$, 186 (21), 172 (27), 104 (22), 91 (13), 65 (7), 39 (7).

N-(2-phenylethyl)-3,4-dimethylmaleimide **(4j).** Yield 34.4 %. White crystals. Mp 172.7–174.6 °C. IR (KBr) cm^{-1}: 3449, 3023, 2935, 1702, 1435, 1408, 1357, 1097, 1065, 1000, 734, 704, 522. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.21–7.31 (5H, m), 3.75 (2H, m), 2.91 (2H, t, $J = 7.5$ Hz), 1.96 (6H, s). EI-MS *m/z* (%): 229 (45) $[M]^+$, 138 (100), 104 (45), 28 (6).

N-(3-phenpropyl)-3,4-dimethylmaleimide **(4k).** Yield 40.2 %. Yellow oil. IR (KBr) cm^{-1}: 3454, 3027, 2939, 1703, 1442, 1407, 1372, 1102, 1071, 1025, 732, 700, 520. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.26–7.29 (2H, m), 7.16–7.19 (3H, m), 3.56 (2H, t, $J = 7.1$), 2.64 (2H, t, $J = 7.8$ Hz), 1.94 (6H, s), 1.93 (2H, m). EI-MS *m/z* (%): 243 (48) $[M]^+$, 138 (100), 117 (31), 91 (26), 77 (7), 65 (8), 53 (10).

N-(4-chlorophenyl)-3,4-dimethylmaleimide **(4l).** Yield 59.8 %. Light yellow crystals. Mp 153.9–154.5 °C. IR (KBr) cm^{-1}: 3464, 1709, 1492, 1395, 1089, 831, 731. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.43 (2H, m), 7.34 (2H, m), 2.07 (6H, s). EI-MS *m/z* (%): 235 (100) $[M]^+$, 176 (29), 153 (13), 125 (13), 54 (42), 39 (15).

N-(4-tolyl)-3,4-dimethylmaleimide **(4m).** Yield 57.7 %. White crystals. Mp 113.1–113.9 °C. IR (KBr) cm^{-1}: 3448, 3042, 2921, 1709, 1518, 1397, 1091, 821, 732, 519. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.27 (4H, dd, $J = 20.9$, 8.4 Hz), 7.39 (3H, s), 2.07 (6H, s). EI-MS *m/z* (%): 215 (100) $[M]^+$, 156 (29), 144 (7), 91 (4), 77 (8), 54 (14), 39 (10).

N-(3,5-dichlorophenyl)-3,4-dimethylmaleimide **(4n).** Yield 63.1 %. Light yellow crystals. Mp 181.5–181.7 °C. IR (KBr) cm^{-1}: 3463, 3094, 2926, 1716, 1577, 1454, 1387, 1092, 854, 724, 702, 521. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.40 (2H, d, $J = 2.0$ Hz), 7.34 (1H, t, $J = 2.0$ Hz), 2.08 (6H, s). EI-MS *m/z* (%): 269 (100) $[M]^+$, 124 (17), 54 (59), 39 (15).

N-(2-methyl-3-nitro-phenyl)-3,4-dimethylmaleimide **(4o).** Yield 55.2 %. White crystals. Mp 155.4–157.2 °C. IR (KBr) cm^{-1}: 3463, 3099, 1709, 1534, 1387, 1363, 1094, 730, 715, 519. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.96 (1H, d, $J = 8.1$ Hz), 7.45 (1H, t, $J = 8.0$ Hz), 7.38 (1H, d, $J = 0.5$ Hz), 2.31 (3H, s), 2.10 (6H, s). EI-MS *m/z* (%): 260 (3) $[M]^+$, 243 (100), 230 (7), 215 (29), 188 (96), 171 (20), 159 (20), 77 (63), 54 (96), 39 (48).

N-(2-benzimidazolyl)-3,4-dimethylmaleimide **(4p).** Yield 62.7 %. Light yellow crystals. Mp 205.6–206.2 °C. IR (KBr) cm^{-1}: 3339, 3056, 2920, 1731, 1535, 1440, 1269, 1059, 719, 527. ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.67 (1H, s), 7.54–7.65 (2H, m), 7.23–7.27 (2H, m), 2.05 (6H, s). EI-MS *m/z* (%): 241 (100) $[M]^+$, 212 (9), 184 (15), 159 (50), 131 (11), 77 (5), 54 (14), 39 (8).

N-(3,4,5-trifluorophenyl)-3,4-dimethylmaleimide **(4q).** Yield 59.2 %. White crystals. Mp 152.5–152.7 °C. IR (KBr) cm^{-1}: 3470, 3085, 1712, 1625, 1531, 1451, 1326, 1239, 1091, 1042, 853, 724, 693, 521. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.18–7.21 (2H, m), 2.08 (6H, s). EI-MS *m/z* (%): 255 (100) $[M]^+$, 196 (21), 173 (15), 158 (11), 145 (15), 81 (20), 54 (45).

Antifungal activity

The antifungal testing indicated that most of the 17 dimethylmaleimides showed good inhibitions to the mycelial growth of *S. sclerotiorum* (Table 1). Except for **4b**, **4g**, and **4o** which gave an MIC of 50.0 μg/mL, the synthetic compounds had MICs ranging from 0.01 to 5.00 μg/mL, much more effective than the starting material 2,3-dimethylmaleic anhydride with an MIC of 20.16 μg/mL. Compounds **4d**, **4m**, and **4p**, MICs of which were 0.10, 0.10, and 0.01 μg/mL, respectively, were more effective than the positive control dicloran with an MIC of 1.0 μg/mL, while another six compounds (**4a**, **4c**, **4e**, **4i**, **4n**, and **4q**) exhibited the same potency as dicloran does. However, only compound **4q** achieved 100 % inhibition rate at 50 μg/mL, the same as dicloran, followed by **4n** with an inhibition rate of 92.4 % at the same concentration. Activities of other compounds were less effective than that of dicloran with the inhibition rates ranging from 1.7 to 77.7 % μg/mL. In summary, all synthetic dimethylmaleimides displayed moderate-to-excellent antifungal activities against *S. sclerotiorum*.

On the other hand, microscopic assessments of the mycelium showed that compound **4q** caused mycelium

Table 1 Antifungal activities of compounds **4a–4q** against *S. sclerotiorum* (in vitro)

Compounds	R	MIC (μg/mL)
4a	*n*-butyl	1.00
4b	*iso*-butyl	50.00
4c	*n*-amyl	1.00
4d	*n*-hexyl	0.10
4e	Cyclohexyl	1.00
4f	*n*-octyl	5.00
4g	*n*-dodecyl	50.00
4h	Phenyl	5.00
4i	Benzyl	1.00
4j	2-phenylethyl	0.10
4k	3-phenylpropyl	5.00
4l	4-chlorophenyl	5.00
4m	4-tolyl	0.10
4n	3,5-dichlorophenyl	1.00
4o	2-methyl-3-nitro- phenyl	50.00
4p	2-benzimidazolyl	0.01
4q	3,4,5-trifluorophenyl	1.00
Dimethylmaleic anhydride		20.16
Dicloran		1.00

Dicloran was used as reference fungicide in the tests

swelling (Fig. 2b), while the control hypha had regular mycelium (Fig. 2a). With the increase of the concentration from 1.0 to 50.0 μg/mL, the mycelium became thinner (Fig. 2c), denser (Fig. 2d), and fractured (Fig. 2e).

Discussion

Synthesis

Among the 17 synthetic dimethylmaleimides, six compounds (**4c**, **4f**, **4g**, **4o**, **4p**, and **4q**) were novel and another six (**4a**, **4b**, **4d**, **4e**, **4k,** and **4l**) were simply mentioned in the literature not involved in the biological activities (Rheinfelden et al. 1982; Ohta et al. 1976; Juerg et al. 1984). The remaining five compounds (**4h**, **4i**, **4j**, **4m**, and **4n**) were reported to possess antimicrobial activities against human pathogens (Sortino et al. 2011). However, their activities against plant pathogens, especially, against *S. sclerotiorum* had rarely been evaluated.

As far as the synthetic method involving a condensation reaction between an appropriate amine and maleic anhydride followed by dehydration and ring-closing reaction, path A is the most classical way to prepare *N*-substituted maleimides, especially for *N*-alkyl maleimides. It is noted that toluene is more suitable to serve as a solvent in the reaction than acetone because of its hydrophobicity which allows an easy separation of water produced in the reaction, thereby reducing by-products. Furthermore, the use of acetic anhydride as the dehydrating agent in the second step would produce a large amount of by-products, *N*-substituted acetamide and acetic acid, which might easily cause environmental pollution. To solve this problem, *N*-aryl maleimides (**4l–4q**) were prepared through path B utilizing a water-separator instead of acetic anhydride. An additional benefit is the higher yields for the products when compared to those in path A. Thus, the two-step reaction sequence developed in this study is a facile method to prepare novel antifungal pesticides.

Antifungal activity

Compounds **4h**, **4i**, **4j**, **4m**, and **4n** were reported to possess antimicrobial activities against human pathogens (Sortino et al. 2011). However, their activities against plant pathogens have not been evaluated. The results of antifungal testing on the 17 synthetic dimethylmaleimides against *S. sclerotiorum* indicated that the hydrophobicity of the *N*-alkyl substituents showed a correlation with the antifungal activity. As the hydrophobicity of the side chain increased, the antifungal activities enhanced first, and then decreased as shown for compounds **4a** to **4g** with alkyl substituents (Table 1). *N*-Hexyl-3,4-dimethylmaleimide (**4d**) exhibited the best antifungal activity with an MIC of 0.10 μg/mL within this group. These results were similar to those reported by Watanabe (Watanabe et al. 1992). This might be explained as that maleimides and derivatives could inactivate some enzymes in *S. sclerotiorum*. It was reported that *N*-substituted maleimides could react with cysteine residues of *D*-lactate dehydrogenase (Denicola and Anderson. 1990). With the change of the polarity and chain length of *N*-alkyl substituents, their binding abilities to the enzymes are different. Thus, compounds **4a**–**4g** with different polarities and side chain lengths exhibited different antifungal activities against *S. sclerotiorum.*

As for compounds **4h–4q** with an aromatic substituent on the *N*-side chain, it seemed that there were no obvious correlations between the substituents on the aromatic ring and antifungal activity against *S. sclerotiorum.* However, the greater activity for the halogen-substituted compounds **4n** and **4q** with the same MIC of 1.0 μg/mL when compared to compound **4h** with an MIC 5.0 μg/mL is consistent with the previous study reporting that *N*-aryl maleimides exhibited great activity against most fungi when halogens (**F**, **Cl**, **Br**) were introduced into the 3- and 5-positions of the benzene ring (Tang et al. 1998). This phenomenon needs further studies in the future.

The results of this study have demonstrated the potential of dimethylmaleimides as fungicides against *S.*

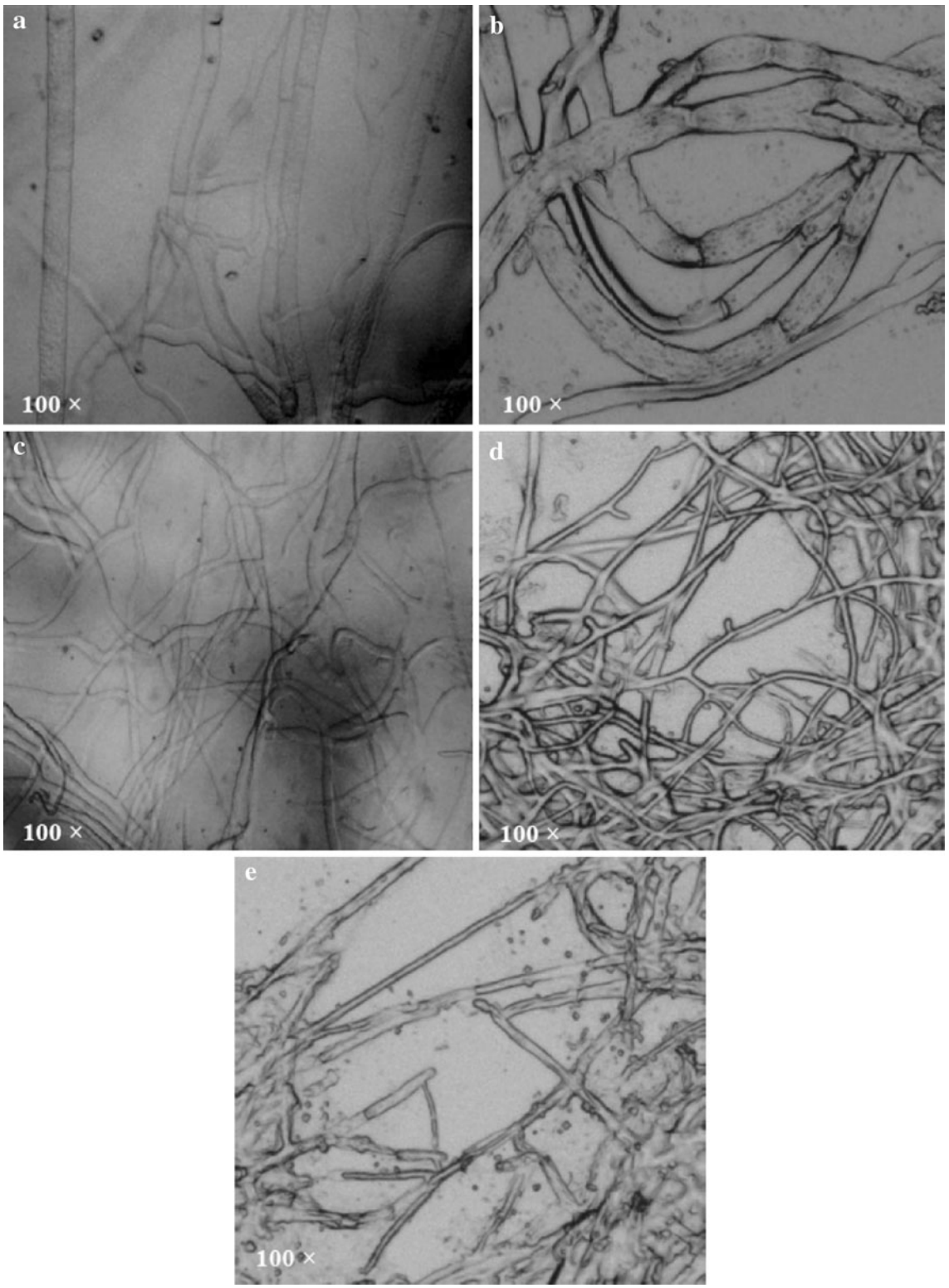

Fig. 2 Morphology of mycelia under the influence of compound (4q). Concentrations of **4q** (μg/mL) **a** 0 (control), **b** 1.00, **c** 5.00, **d** 10.00, and **e** 50.00

sclerotiorum, the causative agent for "white mold" in many plants. Dimethylmaleimides could cause mycelium to become thinner and fractured to achieve the control purpose of the diseases. The preliminary structure-activity relationship information on this class of compounds has laid a foundation for the future work including synthesis

of more derivatives, in-depth structure-activity relationship studies, and dissection of antifungal mechanisms of selected lead compounds to develop novel antifungal pesticides.

A series of dimethylmaleimides had been synthesized and their antifungal activities against *S. sclerotiorum* were also investigated in this work. The results had a profound significance for the control of crop diseases. Therefore, in the next work, more maleimides need to be synthesized to evaluate the activities and to discover the structure-activity relationship, which may give further guidance to the structure modification.

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