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Design, Synthesis and Fungicidal Activity of Novel Sclerotiorin Derivatives

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Sclerotiorin, a chlorine-containing azaphilone-type natural product, was first isolated from *Penicillium sclerotiorum* and has been reported to exhibit weak fungicidal activity. Optimization of the substituents at the 3- and 5-positions of the sclerotiorin framework was investigated with the aim of discovering novel fungicides with improved activity. The design of sclerotiorin analogues involved replacing the diene side chain with a phenyl group or an aromatic- or heteroaromatic-containing aliphatic side chain. The designed compounds were synthesized by cycloisomerization and subsequent oxidation of suitable 2-alkynylbenzaldehydes, in which a variety of substituents were introduced using a Sonogashira coupling reaction. The structures of these newly prepared compounds were confirmed by ^{1}H and ^{13}C NMR spectroscopy, HRMS and single-crystal X-ray analysis. The antifungal activity of the synthesized compounds was evaluated against seven phytopathogenic species. Compounds 3, 9g and 9h were found to have a broad spectrum of fungicidal activity, and these structurally simpler products can be recognized as lead compounds for further optimization.

Key words: antifungal activity, azaphilone, natural product, sclerotiorin, structural modification

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Sclerotiorin was first isolated in 1940 from *Penicillium sclerotiorum* as a chlorine-containing fungal pigment (1). It belongs to the class of natural products known as azaphilones, which have been isolated from a variety of fungal species (2–5) and feature a highly oxygenated bicyclic core and a chiral quaternary centre that breaks the aromaticity of the ring system (6–10). To date, over 170 different natural azaphilones have been identified (11). They have been reported to exhibit a wide range of biological activities, such as inhibition of monoamine oxidase (12), inhibition of the formation of the P53–MDM2 complex (13), inhibition of the gp120–CD4 binding reaction (14), inhibition of fatty acid synthase (15), inhibition of lipoxygenase (16), as well as having antifungal activity (17,18). Although various potentially beneficial biological activities have been discovered, the relationship between structure and activity amongst the azaphilones, as well as their mechanism of action, remains unclear. Furthermore, most of the azaphilones have been isolated as secondary metabolites of fungal species, and little attention has been paid to the modification of structure for the optimization of antifungal activity. As we know, natural product leads offer an efficient approach for the discovery and optimization of new agrochemicals for the control of plant diseases. As part of our programme for developing fungicides with novel scaffolds (19–22), we envisioned that sclerotiorin, which had shown weak antifungal activity in Syngenta's screens, might be a useful lead for the discovery of novel agricultural fungicides.

Although the nine-carbon aliphatic diene side chain on the bicyclic core containing a chiral quaternary centre is the common characteristic of sclerotiorin, some recent research has reported that sclerotiorin analogues with substitution other than the long diene chain displayed interesting biological activities (23–25). For example, mitorubrinic acid (23), in which a carboxyvinyl substituent replaces the diene chain, has been shown to induce formation of chlamydospore-like cells in fungi and also to inhibit trypsin. Furthermore, bulgarialactone B and related compounds (24) bearing a simple aliphatic group at the 3-position showed inhibitory activity against heat shock protein 90 (Hsp90) (Figure 1). These results indicate that the character of the substitution at 3-position of sclerotiorin and other azaphilones plays an important role in their observed biological activity and provides us with an opportunity to optimize the structure of sclerotiorin with the aim of discovering novel fungicides. Thus, compound **1**, in which the diene side chain has been removed and replaced with a hydrogen atom, was first designed and synthesized to determine the effect of the substitution on biological activity (Figure 1). Compounds **2** and **3**, in which the long aliphatic side chain has been replaced by a phenyl ring, were also designed and synthesized. In addition, analogues of the type **4**, in which a chain containing oxygen or sulphur atoms links the bicyclic core with a terminal aromatic ring, were designed and prepared (the longer side chain in these analogues was expected to mimic the long aliphatic side chain of sclerotiorin). Finally, examples were prepared in which the phenyl ring of compounds **2** and **3** was replaced with the biologically interesting heterocycles pyrimidine or pyrimidinone. For all of these designed compounds, the bicyclic core

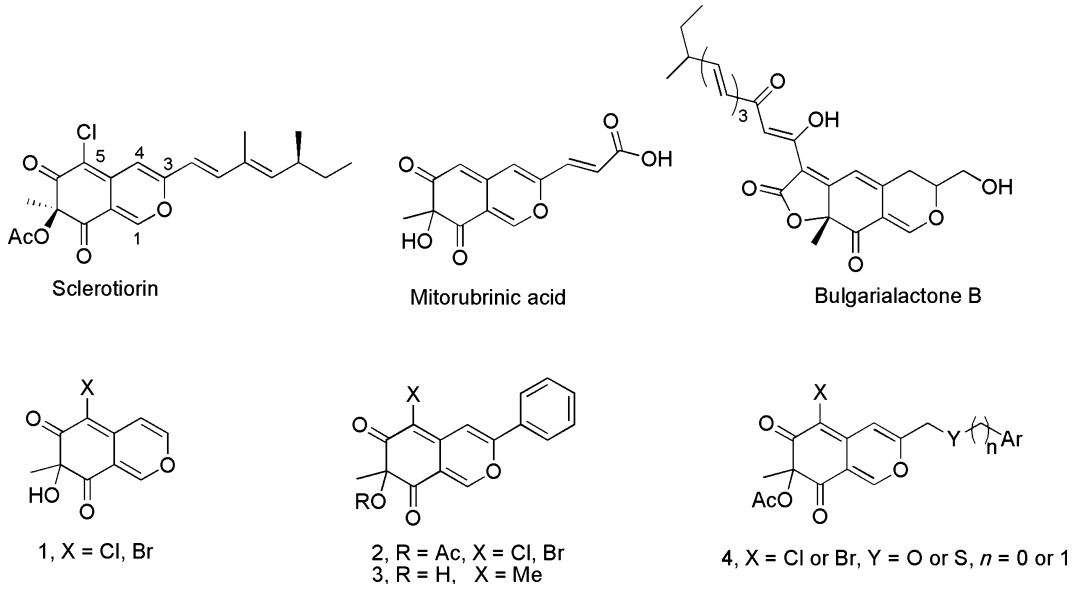


Figure 1: The structures of sclerotiorin, mitorubrinic acid, bulgarialactone B and the designed analogues.

of sclerotiorin was retained to mimic the natural parent structure, and racemic compounds were prepared, ignoring the absolute stereochemistry of the chiral centre of the bicyclic system. Herein, we report the synthesis and characterization of these sclerotiorin analogues as well as an assessment of their antifungal activities.

Materials and Methods

General techniques

All chemical reagents were commercially available and treated with standard methods before use. Solvents were dried and redistilled before use. ^{1}H NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on a Varian Mercury 400/600 spectrometer, and chemical shifts (δ) are given in ppm relative to tetramethylsilane. ^{13}C NMR spectra were recorded in $CDCl_3$ on a Varian Mercury 600 (150 MHz) spectrometer and (δ) are given in ppm relative to the centre line of a triplet at 77.0 ppm of chloroform-d. The following abbreviations are used to designate multiplicities: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad. HRMS were obtained on a Waters MALDI SYNAPT G2 HDMS equipped with an electrospray source (Manchester, UK).

Preparation of the designed compounds

General procedure for the microwave-assisted Sonogashira coupling reaction

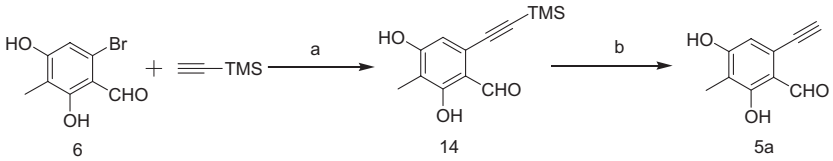
To a mixed solution of 1,4-dioxane (4 mL) and H_2O (1 mL) were successively added the 2-bromobenzaldehyde **6** (92.4 mg, 0.40 mmol), $Pd(PPh_3)_2Cl_2$ (14.0 mg, 0.02 mmol), CuI (3.8 mg, 0.02 mmol), NEt_3 (1.6 mmol, 162 mg) and the appropriate alkyne (0.48 mmol). The resulting mixture was sealed in a microwave tube and irradiated at 100 °C for the indicated period of time. The reactant was cooled to room temperature, diluted with water and neutralized with 1.0 N aqueous HCl. The mixture was extracted with ethyl acetate, and the combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4 and filtered and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel to provide the desired product.

General procedure for the conventional Sonogashira coupling reaction

To a mixture of the 2-bromobenzaldehyde **6** (92.4 mg, 0.40 mmol), an alkyne (0.48 mmol), $Pd(PPh_3)_2Cl_2$ (14.0 mg, 0.02 mmol) and CuI (3.8 mg, 0.02 mmol) in 5 mL of anhydrous DMF was added NEt_3 (1.2 mmol, 121.2 mg) under an argon atmosphere. The resulting mixture was heated in an oil bath until TLC analysis indicated that the starting material had disappeared. The reactant was cooled to room temperature, diluted with water, neutralized with 1.0 N aqueous HCl and extracted with ethyl acetate. The combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4 and filtered and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel to provide the desired product.

Preparation of 6-ethynyl-2,4-dihydroxy-3-methylbenzaldehyde 5a

To a mixture of the 2-bromobenzaldehyde **6** (924 mg, 4 mmol), $PdCl_2(PPh_3)_2$ (140 mg, 0.2 mmol) and CuI (38 mg, 0.2 mmol) in 40 mL anhydrous NEt_3 was added ethynyltrimethylsilane (0.49 g, 5 mmol) (Scheme 1). The resulting mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with water, neutralized with 1.0 N aqueous HCl and extracted with ethyl acetate. The combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4 and filtered and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (petroleum ether/-EtOAc 12:1) to provide the trimethylsilylethynylbenzaldehyde **14**. The



Scheme 1: Reagents and conditions: (A) 5 mol % $PdCl_2(PPh_3)_2$, 5 mol% CuI, Et_3N, room temperature, 2h, 92% (B) 1.5 equiv. K_2CO_3, MeOH, rt, 45 min, 81%.

obtained compound **14** (4 mmol) was dissolved in 20 mL of dry methanol, and dry K_2CO_3 (828 mg, 6 mmol) was added. The resulting mixture was stirred at room temperature until TLC analysis indicated that the starting material had disappeared. The reaction mixture was diluted with water, neutralized with 1.0 N aqueous HCl and extracted with ethyl acetate. The combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4 and filtered and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (petroleum ether/EtOAc 10:1) to provide the product **5a**.

Data for **14**, ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.26 (s, 9H), 1.97 (s, 3H), 6.60 (s, 1H), 10.06 (s, 1H), 11.11 (br, 1H), 12.26 (s, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 193.9, 162.8, 162.2, 124.8, 112.7, 112.5, 112.3, 100.8, 99.9, 7.4, −0.39; HRMS (MALDI) calcd. for $C_{13}H_{16}O_3Si$ $[M + H]^+$ 249.0947, found 249.0944.

Data for **5a**, m.p.109–111 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 2.13 (s, 3H), 3.36 (s. 1H), 5.68 (br, 1H), 6.61 (s, 1H), 10.24 (s, 1H), 12.34 (s, 1H).

General procedure for the preparation of azaphilones 8a∼8g

To a mixture of the alkynylbenzaldehyde **5** (0.5 mmol) and $AgNO_3$ (4.25 mg, 0.025 mmol) or $Au(OAc)_3$ (9.35 mg, 0.025 mmol) were added 2.0 mL 1,2-dichloroethane and 200 μL trifluoroacetic acid, and the mixture was stirred at room temperature until TLC monitoring indicated the disappearance of the material **5** (about 5 min). To the resulting mixture was added 2-iodoxybenzoic acid (IBX, 155 mg, 0.55 mmol) and tetrabutylammonium iodide (9.25 mg, 0.025 mmol), and the reaction mixture was stirred at room temperature for a further 1 h and then quenched with saturated $Na_2S_2O_3$ and extracted three times with ethyl acetate. The organic extracts were combined, washed with brine, dried over Na_2SO_4 and concentrated. Purification by flash chromatography on silica gel afforded compounds **8a∼8g**.

Data for **8a**, m.p. 147–149 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.57 (s, 3H), 3.65 (br, 1H), 5.60 (s, 1H), 6.35 (d, 1H, J = 5.4 Hz), 7.12 (d, 1H, J = 5.4 Hz), 7.89 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 28.3, 83.7, 106.4, 112.6, 116.5, 142.2, 148.4, 152.6, 195.5, 196.3; HRMS (MALDI) calcd. for $C_{10}H_8O_4$ $[M + Na]^+$ 215.0320, found 215.0340.

Data for **8b**, m.p. 129–131 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.57 (s, 3H), 3.89 (br, 1H), 4.75 (s, 2H), 5.62 (s, 1H), 6.50 (s, 1H), 6.95–6.96 (m, 2H), 7.04–7.07 (m, 1H), 7.33–7.34 (m, 2H), 7.90 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 28.2, 65.2, 83.6, 106.9, 109.3, 114.6, 116.1, 122.2, 129.7, 142.5, 152.3, 156.6, 157.3, 195.3, 196.2; HRMS (MALDI) calcd. for $C_{17}H_{14}O_5$ $[M + Na]^+$ 321.0739, found 321.0763.

Data for **8c**, m.p. 95–97 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.55 (s, 3H), 3.87 (br, 1H), 4.22 (s, 2H), 4.64 (s, 2H), 5.59 (s, 1H), 6.40 (s, 1H), 7.34–7.40 (m, 5H), 7.86 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 28.3, 67.1, 73.4, 83.5, 106.4, 108.9, 116.0, 127.8, 128.2, 128.6, 136.5, 142.9, 152.4, 158.1, 195.4, 196.2; HRMS (MALDI) calcd. for $C_{18}H_{16}O_5$ $[M + Na]^+$ 335.0895, found 335.0877.

Data for **8d**, m.p. 177–178 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.54 (s, 3H), 2.30 (s, 3H), 2.62 (s, 3H), 4.91 (dd, J = 16.2 Hz, 12.6 Hz, 2H), 5.59 (s, 1H), 6.24 (s, 1H), 6.27 (s, 1H), 7.83 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 22.9, 23.5, 28.2, 83.7, 107.5, 110.1, 110.5, 116.1, 141.9, 152.1, 154.2, 158.0, 161.5, 163.2, 195.0, 196.1; HRMS (MALDI) calcd. for $C_{17}H_{16}N_2O_5$ $[M + Na]^+$ 351.0957, found 351.0946.

Data for **8e**, m.p. 111–113 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.26 (s, 3H), 2.74 (s, 3H), 3.91 (br, 1H), 4.97 (s, 2H), 5.62 (s, 1H), 6.37 (s, 1H), 6.79 (s, 1H), 7.83 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 23.3, 44.5, 83.8, 107.8, 110.8, 111.2, 116.0, 119.1, 120.9, 141.7, 151.2, 151.4, 152.2, 153.0, 161.0, 161.4, 195.1, 196.3; HRMS (MALDI) calcd. for $C_{17}H_{13}F_3N_2O_5$ $[M + Na]^+$ 405.0674, found 405.0591.

Data for **8f**, m.p. 176–178 °C, ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.54 (s, 3H), 2.46 (s, 6H), 4.12 (dd, J = 15.6 Hz, 16.8Hz, 2H), 4.59 (br, 1H), 5.54 (s, 1H), 6.51 (s, 1H), 6.82 (s, 1H), 7.89 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 23.7, 28.4, 31.6, 83.5, 105.9, 110.1, 115.6, 116.7, 143.6, 153.0, 158.3, 167.6, 168.0, 195.5, 196.3; HRMS (MALDI) calcd. for $C_{17}H_{16}N_2O_4S$ $[M + Na]^+$ 367.0728, found 367.0660.

Data for **8g**, m.p. 164–166 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.60 (s, 3H), 3.98 (br, 1H), 5.70 (s, 1H), 6.78 (s, 1H), 7.54–7.50 (m, 3H), 7.75 (d, J = 6.6 Hz 2H), 8.04 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 196.1, 195.5, 157.3, 152.6, 143.8, 131.6, 130.0, 129.1, 125.6, 115.8, 106.8, 106.2, 83.5, 28.5; HRMS (MALDI): Calcd for $C_{16}H_{12}O_4$ $[M + Na]^+$ 291.0633, Found 291.0638.

General procedure for the bromination of azaphilones 8a∼8g

To a solution of one of the azaphilones **8a∼8g** (0.4 mmol) in 5 mL of CH_2Cl_2 was added NBS (106.8 mg, 0.6 mmol). The resulting mixture was stirred at room temperature until TLC monitoring indicated the disappearance of starting material. The reaction mixture was washed with water, dried over anhydrous Na_2SO_4 and filtered and concentrated *in vacuo*. Purification by flash chromatography on silica gel afforded the brominated azaphilones.

Data for **1b**, m.p. 128–130 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.60 (s, 3H), 3.86 (br, 1H), 6.86 (d, J = 5.4 Hz, 1H), 7.35 (d, J = 5.4 Hz,

1H), 7.91 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 28.4, 84.2, 101.5, 112.3, 116.9, 140.4, 150.3, 151.8, 190.4, 194.0; HRMS (MALDI) calcd. for $C_{10}H_7BrO_4$ $[M + Na]^+$ 292.9425, found 292.9473.

Data for **9b**, m.p.: 95–97 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.25 (s, 3H), 4.83 (s, 2H), 6.97 (s, 1H), 6.98–6.99 (m, 2H), 7.05–7.07 (m, 1H), 7.33–7.36 (m, 2H), 7.91 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 28.3, 65.5, 84.2, 102.3 109.3, 114.7, 115.5, 122.4, 129.8, 140.7, 151.6, 157.3, 158.8, 190.3, 193.8; HRMS (MALDI) calcd. for $C_{17}H_{13}BrO_5$ $[M + Na]^+$ 398.9844, found 398.9841.

Data for **9d**, m.p. 104–106 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.57 (s, 3H), 3.94 (br, 1H), 4.29 (s, 2H), 4.67 (s, 2H), 6.88 (s, 1H), 7.34–7.38 (m, 5H), 7.86 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 28.3, 67.2, 73.6, 84.1, 101.7, 108.8, 116.4, 127.9, 128.3, 128.6, 136.4, 141.0, 151.6, 160.3, 190.3, 193.9; HRMS (MALDI) calcd. for $C_{18}H_{15}BrO_5$ $[M + Na]^+$ 413.0001, found 413.0030.

Data for **9f**, m.p. 139–141 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.56 (s, 3H), 2.47 (s, 6H), 4.17 (dd, J = 15.0 Hz, 25.8 Hz, 2H), 6.82 (s, 1H), 7.07 (s, 1H), 7.90 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 23.8, 28.5, 31.9, 84.1, 101.0, 110.4, 116.1, 116.8, 141.6, 152.4, 160.4, 167.7, 168.0, 190.3, 193.9; HRMS (MALDI) calcd. for $C_{17}H_{15}BrN_2O_4S$ $[M + Na]^+$ 444.9834, found 444.9834.

Data for **9h**, m.p.: 142–144 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.63 (s, 3H), 3.96 (br, 1H), 7.26 (s, 1H), 7.59–7.53 (m, 3H), 7.83 (d, J = 7.2 Hz, 2H), 8.05 (s, 1H); HRMS (MALDI): Calcd for $C_{16}H_{11}BrO_4$ $[M + Na]^+$ 368.9738, found 368.9748.

General procedure for the chlorination of azaphilones 8a∼8g

To a solution of one of the azaphilones **8a**∼**8g** (0.4 mmol) in 5 mL CH_3CN was added NCS (80.1 mg, 0.6 mmol). The resulting mixture was stirred at 30 °C until TLC analysis indicated the disappearance of the starting material. The reaction mixture was diluted with water and extracted with ethyl acetate, and the combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4 and filtered and concentrated *in vacuo*. Purification by flash chromatography on silica gel afforded the chlorinated azaphilones.

Data for **1a**, m.p. 127–129 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.61 (s, 3H), 3.88 (br, 1H), 6.82 (d, J = 6.0 Hz, 1H), 7.33 (d, J = 6.0 Hz, 1H), 7.93 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 28.4, 84.2, 109.7, 109.9, 116.1, 138.0, 149.9, 151.8, 190.1, 193.8; HRMS (ESI) calcd. for $C_{10}H_7ClO_4$ $[M + H]^+$ 227.0111, found 227.0128.

Data for **9a**, m.p. 61–63 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.61 (s, 3H), 4.83 (s, 2H), 6.97 (s, 1H), 6.98–6.99 (m, 2H), 7.06–7.09 (m, 1H), 7.35–7.37 (m, 2H), 7.95 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 28.4, 65.4, 84.2, 106.7 110.6, 114.7, 115.8, 122.4, 129.8, 138.4, 151.6, 157.2, 158.5, 190.1, 193.7; HRMS (ESI) calcd. for $C_{17}H_{13}ClO_5$ $[M + H]^+$ 333.0530, found 333.0550.

Data for **9c**, m.p. 57–59 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.58 (s, 3H), 4.28 (s, 2H), 4.67 (s, 2H), 6.85 (s, 1H), 7.36–7.40 (m, 5H), 7.89 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 28.2, 67.2, 73.5, 84.1, 106.2, 110.0, 115.6, 127.9, 128.3, 128.6, 136.4, 138.6, 151.6, 160.0, 190.0, 193.8; HRMS (MALDI) calcd. for $C_{18}H_{15}ClO_5$ $[M + Na]^+$ 369.0506, found 369.0508.

Data for **9e**, m.p.: 76–78 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.56 (s, 3H), 2.47 (s, 6H), 4.16 (dd, J = 15.0 Hz, 18.0 Hz, 2H), 6.81 (s, 1H), 7.05 (s, 1H), 7.92 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 23.7, 28.4, 31.9, 84.0, 107.7, 109.3, 115.3, 116.7, 139.3, 152.3, 160.1, 167.6, 168.0, 190.0, 193.8; HRMS (ESI) calcd. for $C_{17}H_{15}ClN_2O_4S$ $[M + H]^+$ 379.0519, found 379.0554.

Data for **9g**, ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.63 (s, 3H), 3.96 (br, 1H), 7.22 (s, 1H), 7.58–7.53 (m, 3H), 7.83–7.82 (m, 2H), 8.08 (s, 1H); HRMS (MALDI): Calcd for $C_{16}H_{11}ClO_4$ $[M + Na]^+$ 325.0244, Found 325.0240.

General procedure for the preparation of acetylated azaphilones 4

To a stirred solution of an azaphilone **9** (0.4 mmol) and acetic anhydride (1.6 mL) in 5 mL CH_2Cl_2 were added Et_3N (0.8 mmol) and 4-(dimethylamino)pyridine (0.4 mmol) at 0 °C. The resulting mixture was stirred at 0 °C until TLC analysis indicated the disappearance of the starting material. The solvent was removed under reduced pressure. The residue was purified by flash chromatography on silica gel to provide the acetylated product **4**.

Data for **4a**, m.p. 157–159 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.57 (s, 3H), 2.18 (s, 3H), 4.82 (s, 2H), 6.97–6.99 (m, 2H), 7.02 (s, 1H), 7.06–7.08 (m, 1H), 7.34–7.37 (m, 2H), 7.92 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 20.0, 22.2, 65.4, 84.5, 104.4, 109.9, 114.7, 115.8, 122.3, 129.8, 139.6, 152.7, 157.3, 158.3, 170.0, 186.4, 191.5; HRMS (ESI): calcd. for $C_{19}H_{15}BrO_6$ $[M + H]^+$ 419.0130, found 419.0160.

Data for **4b**, m.p. 155–157 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.56 (s, 3H), 2.18 (s, 3H), 4.28 (s, 2H), 4.67 (s, 2H), 6.87 (s, 1H), 7.37–7.40 (m, 5H), 7.90 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.0, 22.2, 67.2, 73.4, 84.6, 106.7, 111.7, 115.0, 127.9, 128.3, 128.6, 136.5, 137.5, 152.7, 159.4, 170.0, 186.3, 191.4; HRMS (MALDI) calcd. for $C_{20}H_{17}ClO_6$ $[M + Na]^+$ 411.0611, found 411.0623.

Data for **4c**, m.p. 173–175 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.56 (s, 3H), 2.18 (s, 3H), 4.28 (s, 2H), 4.67 (s, 2H), 6.92 (s, 1H), 7.34–7.41 (m, 5H), 7.88 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.0, 22.3, 67.2, 73.5, 84.5, 104.1, 109.5, 115.8, 128.0, 128.4, 128.7, 136.5, 139.9, 152.8, 159.7, 170.1, 186.4, 191.7; HRMS (ESI) calcd. for $C_{20}H_{17}BrO_6$ $[M + H]^+$ 433.0287, found 433.0323.

Data for **4d**, m.p. 159–161 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.53 (s, 3H), 2.16 (s, 3H), 2.48 (s, 6H), 4.16 (dd, J = 6.6 Hz, 15.0 Hz, 2H), 6.80 (s, 1H), 7.09 (s, 1H), 7.90 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.0, 22.3, 23.8, 31.9, 84.5, 103.4, 110.8, 115.4, 116.7, 140.5, 153.4, 159.8, 167.6, 168.1, 170.1, 186.4, 191.6; HRMS (ESI) calcd. for $C_{19}H_{17}BrN_2O_5S$ $[M + H]^+$ 465.0120, found 465.0130.

Preparation of 3-bromo-4,6-dihydroxy-5-methyl-2-(2-phenylethynyl)benzaldehyde 12

To a solution of compound **5g** (0.252 g, 1 mmol) in 4 mL of acetonitrile was added NBS (0.25 g, 1.4 mmol). The reaction mixture was

stirred for 2 h at room temperature (progress of the reaction was monitored by TLC), and 30 mL of ethyl acetate was added. The resulting mixture was washed with water (2 × 20 mL) and brine and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure, and the residue was purified by flash chromatography on silica gel to give **12** as a yellow solid (0.298 g, 90%).

^{1}H NMR (600 MHz, DMSO-d_6) δ 13.31 (s, 1H), 10.73 (br, 1H), 10.22 (s, 1H), 7.65–7.63 (m, 2H), 7.46–7.44 (m, 3H), 2.09 (s, 3H); HRMS (MALDI): Calcd for $C_{16}H_{11}BrO_3$ $[M + H]^+$ 330.9970, Found 330.9941.

Preparation of 2,4-dihydroxy-3,5-dimethyl-6-(2-phenylethynyl)benzaldehyde 13

Compound **12** (66 mg, 0.20 mmol), $Pd(PPh_3)_4$ (35 mg, 0.03 mmol) and $Sn(CH_3)_4$ (108 mg, 0.60 mmol) were suspended in 3 mL of DMF under argon. The vessel was sealed and heated at 140 °C by irradiation with microwaves for 20 min. The resulting mixture was poured into water and extracted three times with ethyl acetate. The combined organic extracts were washed with brine and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure, and the residue was purified by flash chromatography on silica gel to give **13** as a yellow solid (35.7 mg, 67%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 12.38 (s, 1H), 10.39 (s, 1H), 7.56–7.55 (m, 2H), 7.40–7.38 (m, 3H), 5.53 (s, 1H), 2.41 (s, 3H), 2.17 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 194.8, 161.0, 160.8, 131.1, 128.5, 128.2, 124.1, 122.3, 120.6, 112.9, 111.6, 98.8, 83.7, 13.6, 7.7; HRMS (MALDI): Calcd for $C_{17}H_{14}O_3$ $[M + H]^+$ 267.1021, Found 267.0984.

Preparation of 7-hydroxy-5,7-dimethyl-3-phenylisochroman-6,8-dione 3

A mixture of **13** (16 mg, 0.06 mmol), $Au(OAc)_3$ (3 mg, 0.008 mmol) and trifluoroacetic acid (1.5 mL) in 1,2-dichloroethane (1.5 mL) was stirred for 10 min at room temperature. Then IBX (18.6 mg) and TBAI (2 mg) were added. The progress of the reaction was monitored by TLC, and when it was complete, the mixture was quenched by the addition of aqueous $Na_2S_2O_3$, and it was extracted three times with ethyl acetate. The combined organic extracts were washed successively with water and brine and then dried with anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure, and the residue was purified by flash chromatography on silica gel to give **3** as a yellow solid (10 mg, 59%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 1.58 (s, 3H), 2.18 (s, 3H), 4.05 (br, 1H), 6.83 (s, 1H), 7.53–7.49 (m, 3H), 7.76 (d, J = 7.2 Hz, 2H), 7.93 (s, 1H); ^{13}C NMR (150 MHz $CDCl_3$): δ 196.4, 195.6, 156.8, 150.8, 138.8, 131.4, 130.7, 129.1, 125.6, 116.0, 112.5, 104.3, 83.2, 28.7, 10.1; HRMS (MALDI): Calcd for $C_{17}H_{14}O_4$ $[M + Na]^+$ 305.0790, Found 305.0769.

Biological testing

All testing were undertaken in 96-well microtitre plates. For the leaf piece assays, 200–300 μL water agar was dispensed into each well of the assay plates. Leaf pieces (6 mm diameter for tomato and bean, 6 mm length for wheat) were transferred onto the surface of the agar. Samples (10 μL) at the appropriate concentration (200 ppm and 60 ppm for *Phytophthora infestans*, 100 ppm for *Septoria tritici* and *Uromyces viciae-fabae*) were dispensed onto the surface of each leaf piece. Each rate was carried out in duplicate or triplicate.

Spore suspensions of the pathogen species were made up to the necessary rate (approximately 150 000 sporangia/mL for *Phytophthora infestans*, 1 000 000 conidia/mL for *Septoria tritici* and 0.3 mg spores/mL for *Uromyces viciae-fabae*) and applied to the treated leaf pieces using a handheld spray gun. Lids were placed on the plates, and they were stored under appropriate controlled environment conditions for between 5 and 14 days, depending on the test species.

For the artificial media assays, stock cultures of the target species were grown in appropriate conditions on artificial media in 90-mm petri dishes. The test pathogens, except for the *Pythium dissimile* spore suspensions, were prepared in water from these stock plates, and they were made into a 3% nutrient agar (final spore concentrations of 10 000 sp/mL for *Gibberella zeae*, 15 000 sp/mL for *Botryotinia fuckeliana* and 20 000 sp/mL for *Alternaria solani*). For *Pythium dissimile*, a water suspension of fragments of surface mycelia was prepared from the stock cultures and made into a 3% agar as for the other species, adjusted to a final optical density reading of 0.5 at 425 nm.

The formulated samples were transferred to the assay plates using a liquid handling robot. The spore/agar suspensions (90 μL) were dispersed into the individual wells of each plate using an automated liquid handling system to provide the final rates in the media of 20 ppm (high rate) or 2 ppm (low rate). The plates were then stored in controlled environments set to appropriate conditions, until assessment between 5 and 14 days later, depending on the species. Two replicates were carried out for each rate. The average scores were used to determine the overall activity level of the compound.

Result and Discussion

Chemistry

Although various studies on the synthesis of azaphilones have been reported, some with stereoselective chemistry (26–28) and others focused on racemic analogues (13,29–33), this deceptively simple-looking bicyclic structure still presents significant challenges, especially when the requirement is to prepare analogues with diverse substituents at the 3-position. Recently, Porco's (34) group reported an efficient gold-catalysed cycloisomerization of 2-alkynylbenzaldehydes to give 2-benzopyrylium salts that can subsequently be oxidized with IBX to form the azaphilone ring system. In contrast to the earlier approaches, Porco's method uses readily available alkynes to construct the key intermediate 2-alkynylbenzaldehydes, which can be then transformed into the corresponding azaphilones with diverse side chains at the 3-position. We realized that this method would enable us to investigate the effect of modifications at this position on the activity of sclerotiorin analogues. Thus, we envisioned preparing the core structures **1**∼**4** by oxidation of the

appropriate benzopyrylium salts **11**, which may be derived from the alkynylbenzaldehyde **5**. Furthermore, the key intermediate alkynylbenzaldehydes **5** should be readily accessible using Sonogashira coupling (Figure 2).

The intermediate 2-bromobenzaldehyde **6** was synthesized according to Porco's (34) method, with minor modifications on the larger scale. With this in hand, the Sonogashira coupling reaction with ethynyltrimethylsilane was investigated, and it proceeded smoothly under 5 mol% Pd/Cu co-catalysis at room temperature to give the product **14** in 92% isolated yield (Scheme 1). Removal of the trimethylsilyl group was then carried out using K_2CO_3 in methanol at room temperature and provided the desired product **5a**. However, when 1-(prop-2-ynyloxy)benzene (**7b**) was selected as the terminal alkyne for the coupling reaction under similar conditions, the yield of the desired product **5b** decreased to 18%, even when the reaction time was extended to 15 h. Similarly, when 2-methyl-1-(prop-3-ynyl)-4-trifluoromethyl-pyrimidin-6-one **7e** was used in the coupling reaction, only traces of the expected product **5e** could be isolated. Thus, alkyne **7b** was chosen as a model substrate and was used in the coupling reaction with the 2-bromobenzaldehyde **6** to optimize the reaction conditions. Initially, we investigated the effect of the catalyst and solvent on the reaction under conventional heating, but none of these efforts provided satisfactory results (Table 1, entries 1–3). Microwave-assisted organic synthesis is now widely recognized as a valuable tool for increasing the rates and improving the yields of organic reactions, so we next examined the effect of microwave irradiation on this Sonogashira coupling reaction. The solvent effect on the reaction was first evaluated. We found that THF and 1,4-dioxane were poor solvents because the temperature could not be raised to the desired 100 °C. However, the yield improved to 45% when the coupling reaction was conducted in DMF. As the pure solvents did not give satisfactory yields, mixtures of solvents were considered. When a mixture of THF/DMF (V/V = 4:1) was used, a complex reaction mixture was observed (Table 1, entry 7), but the yield of **5b** increased to 60% when a mixture of 1,4-dioxane and water in a 4:1 ratio was chosen as the solvent. Furthermore, increasing the amount of alkyne **7b** from 1.2 to 3 equiv. resulted in a useful increase in the yield to 85%. However, when the catalyst was changed from $Pd(PPh_3)_2Cl_2$ to $Pd(CH_3CN)_2Cl_2$, only traces of the product were detected (Table 1, entry 11). Thus, we chose the following conditions as optimum for all the subsequent coupling reactions: 5 mol % $PdCl_2(PPh_3)_2$, 5 mol% CuI, 3 equiv. Et_3N and 3 equiv. alkyne under microwave irradiation at 100 °C for 15 min.

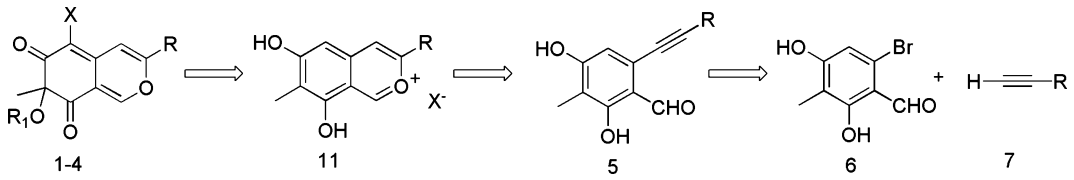
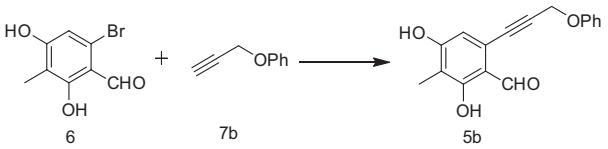


Figure 2: Retrosynthetic analysis of the designed sclerotiorin analogues.

Table 1: Optimization of the coupling reaction conditions[a]



Entry	**7b** (equiv.)	Catalyst (5 mol %)	Solvent	Time (min)	Yield (%)[b]
1[c]	1.2	$PdCl_2(PPh_3)_2$	DMF	900	18
2[c]	1.2	$PdCl_2(PPh_3)_2$	THF	600	10
3[d]	1.2	Pd $(PPh_3)_4$	DMF	900	20
4	1.2	$PdCl_2(PPh_3)_2$	THF	–[e]	–
5	1.2	$PdCl_2(PPh_3)_2$	1,4-dioxane	–[e]	–
6	1.2	$PdCl_2(PPh_3)_2$	DMF	10	45
7	1.2	$PdCl_2(PPh_3)_2$	THF/DMF (4:1)	10	Complex
8	1.2	$PdCl_2(PPh_3)_2$	1,4-dioxane/H_2O	10	60
9	1.2	$PdCl_2(PPh_3)_2$	1,4-dioxane/H_2O	15	65
10	3.0	$PdCl_2(PPh_3)_2$	1,4-dioxane/H_2O	15	85
11	3.0	$PdCl_2(CH_3CN)_2$	1,4-dioxane/H_2O	15	Trace

[a]The reaction was conducted using 5 mol % $PdCl_2(PPh_3)_2$, 5 mol% CuI and three equiv. Et_3N under microwave irradiation at 100 °C unless otherwise noted.
[b]Isolated yield.
[c]The reaction was conducted using 5 mol % $PdCl_2(PPh_3)_2$, 5 mol% CuI and three equiv. Et_3N under conventional heating at 100 °C.
[d]The reaction was conducted using 5 mol % $PdCl_2(PPh_3)_2$, 5 mol% CuI and three equiv. Et_3N under conventional heating at 100 °C.
[e]The reaction was stopped by the reactor system because the temperature could not reach the setting value.

With the optimum reaction conditions in hand, we next conducted the Sonogashira coupling reaction between 2-bromobenzaldehyde **6** and several functionalized terminal acetylenes (Table 2) and found that aryloxy-, benzyloxy- and heterocycle-substituted acetylenes all tolerated the reaction conditions and afforded the desired coupling products in good yields.

The subsequent cycloisomerization with catalytic Lewis acid ($AgNO_3$ or $Au(OAc)_3$) in a mixed solvent of 1,2-dichloroethane and trifluoroacetic acid (10:1) afforded the pyrylium salts, which were oxidized without isolation using *o*-iodoxybenzoic acid (IBX) to give the azaphilones **8** in moderate yields (Table 3). Generally, the intermediate 2-alkynylbenzaldehydes **5** were converted smoothly into the corresponding azaphilones, and the nature of the substituents on the carbon–carbon triple bond did not dramatically affect the yield of azaphilones. However, it is worth noting that a very low yield was observed when the simple 2-ethynylbenzaldehyde **5a** was used as the starting material under similar reaction conditions. In this case, we changed the catalyst from $AgNO_3$ to $Au(OAc)_3$ in the cycloisomerization step, and a satisfactory yield was obtained.

Compound **8** was then converted into the corresponding 5-halogenated product **9** by treating it with NCS or NBS in dichloromethane at room temperature, and some of the halogenated compounds were transformed into the acetylated derivatives by treatment with acetic anhydride in the presence of triethylamine and DMAP (Table 4). The structure of compounds **8** and their equivalent halogenated compounds **9** and the acetylated compounds **4** were confirmed by their NMR spectra and HRMS analysis. The structures of **4a** and **8e** were further confirmed by carrying out an X-ray structure analysis (Figure 3).

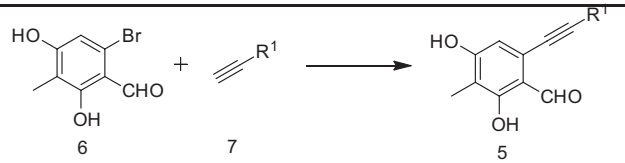
During this research, we noticed that some halogenation reactions suffered from the problem of low yield, especially for the preparation of the compounds **1a**, **1b** and **9a**. We therefore designed an alternative route to circumvent this problem, as shown in Scheme 2. In this route, the halogen atom was introduced earlier in the sequence, prior to the cycloisomerization and oxidation steps, and led to the desired products in higher yields. Thus, the isolated yields of **1a**, **1b** and **9a** were improved from 15%, 32% and 30% to 49%, 50% and 53%, respectively.

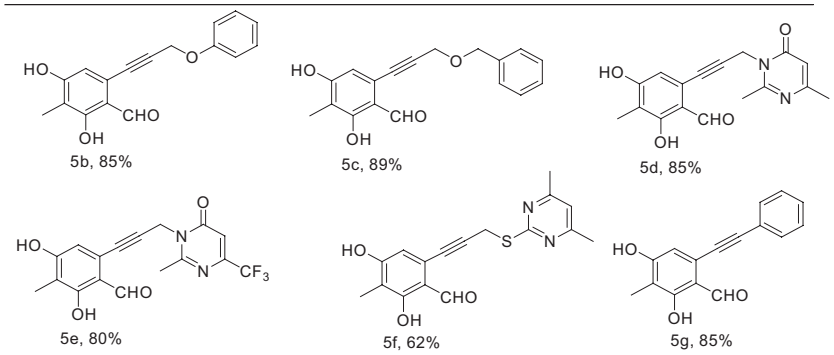
Synthesis of the analogues **3** with a methyl group at the 5-position and an additional phenyl substituent was accomplished to further probe the structure–activity relationships. We initially planned to construct the skeleton of compound **3** by direct methylation of **9h**. However, treatment of **9h** with tetramethyl stannane in the presence of a palladium catalyst failed to produce compound **3**. Thus, an alternative synthetic route was designed and is illustrated in Scheme 3. The synthesis began with the bromination of the 2-alkynylbenzaldehyde **5g** by treating it with four equivalents of aqueous HBr in the presence of IBX to give the congested alkynylbenzaldehyde **12**. Palladium-catalysed cross-coupling with tetramethyl stannane then successfully led to compound **13**, which underwent the cycloisomerization and oxidation procedure to produce the required product **3**.

Fungicidal activity

The fungicidal activity of compounds **1**–**4** and their precursors **8b-g** and **9a-h** were evaluated against seven phytopathogenic species, in a combination of assays conducted in artificial media or on leaf pieces (Table 5). In general, any activity observed was restricted to the assays in artificial media and rarely extended to pathogens tested on leaf pieces. Furthermore, although the most active compounds were effective against a range of species, the activity tended to lack potency and was rarely observed at the lower rates tested.

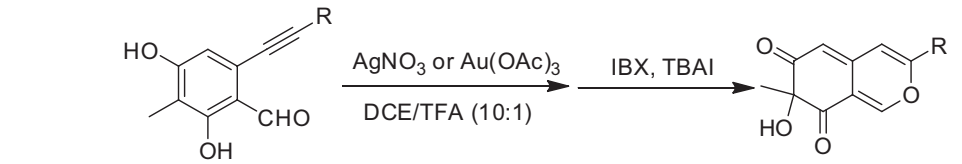
Table 2: Microwave-assisted synthesis of 2-alkynylbenzaldehydes **5**[a]





[a]The reaction was run using 5 mol % $PdCl_2(PPh_3)_2$, 5 mol% CuI and three equiv. Et_3N under microwave irradiation at 100 °C.

Table 3: Synthesis of 8**a**–**f** via cycloisomerization and oxidation



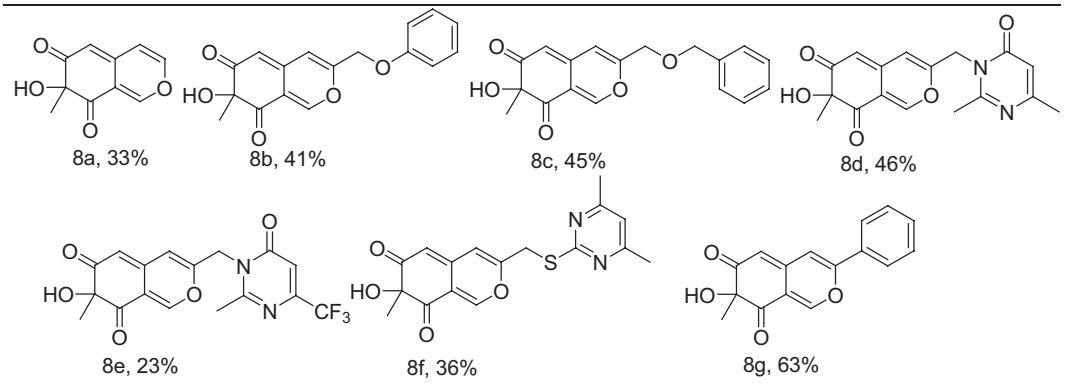
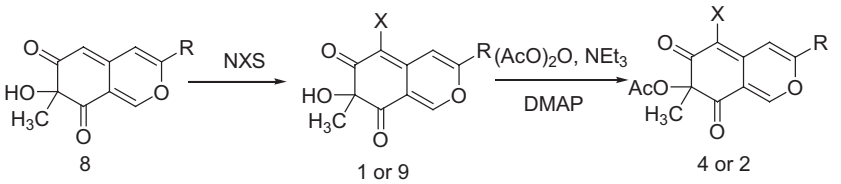


Table 4: Synthesis of compounds **3** and **9** via halogenation and acetylation[a]



Entry	R	X	Yield (%) **1** or **9**	**4** or **2**
1	H	Cl	**1a**, 15	–[b]
2	H	Br	**1b**, 32	–
3	C_6H_5-OCH_2-	Cl	**9a**, 30	–
4	C_6H_5-OCH_2-	Br	**9b**, 71	**4a**, 45
5	C_6H_5-CH_2OCH_2-	Cl	**9c**, 76	**4b**, 62
6	C_6H_5-CH_2OCH_2-	Br	**9d**, 68	**4c**, 60
7	(4,6-di-Me-pyrimidin-2-yl)-SCH_2-	Cl	**9e**, 59	–
8	(4,6-di-Me-pyrimidin-2-yl)-SCH_2-	Br	**9f**, 43	**4d**, 65
9	C_6H_5-	Cl	**9g**, 60	**2a**, 50
10	C_6H_5-	Br	**9h**, 70	**2b**, 56

[a]The halogenation was conducted by employing 1.5 equiv. NBS in dichloromethane at room temperature or 1.5 equiv. NCS in acetonitrile at 30 °C.
[b]–indicates not synthesized.

It is difficult to draw a detailed structure–activity relationship for the synthesized azaphilone derivatives on the basis of the present biological data; however, some interesting results have been observed. Most importantly, the spectrum of antifungal activity was improved by the incorporation of a halogen (chlorine or bromine) or methyl group at the 5-position and a phenyl group at the 3-position of the sclerotiorin framework. The resulting compounds, **3**, **9g** and **9h,** all gave strong antifungal activity in artificial media against *Pythium dissimile*, *Alternaria solani* and *Gibberella zeae*, with **3** and **9g** in particular displaying activity at the low rate of 2 ppm against *G. zeae* and *P. dissimile,* respectively. Compound **3** was also active on the leaf piece assay against *Uromyces viciae-fabae*. In contrast, **2a** and **2b**, the *O*-acetylated products of **9g** and **9h**, were much less active than their corresponding unprotected analogues, which demonstrates that the free hydroxyl group plays an important role in the observed activity.

Compound **8g**, the non-halogenated precursor of **9g** and **9h**, also showed a much narrower spectrum of antifungal activity, which may be attributed to the absence of a substituent at the 5-position.

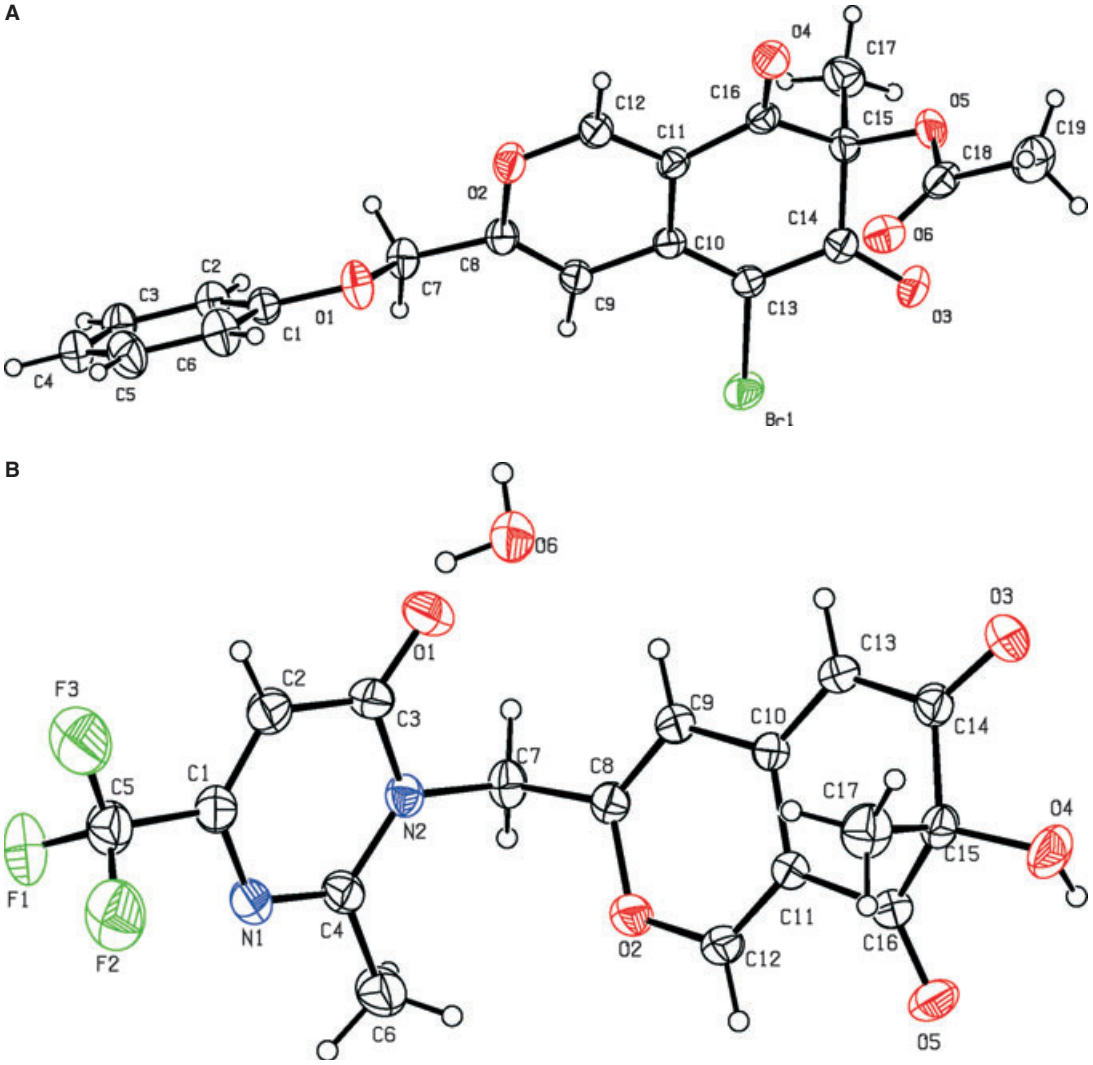
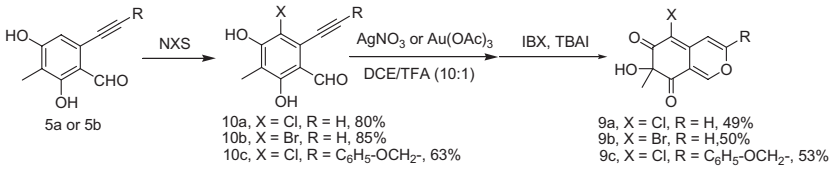


Figure 3: X-ray structures of **4a** and **8e**.



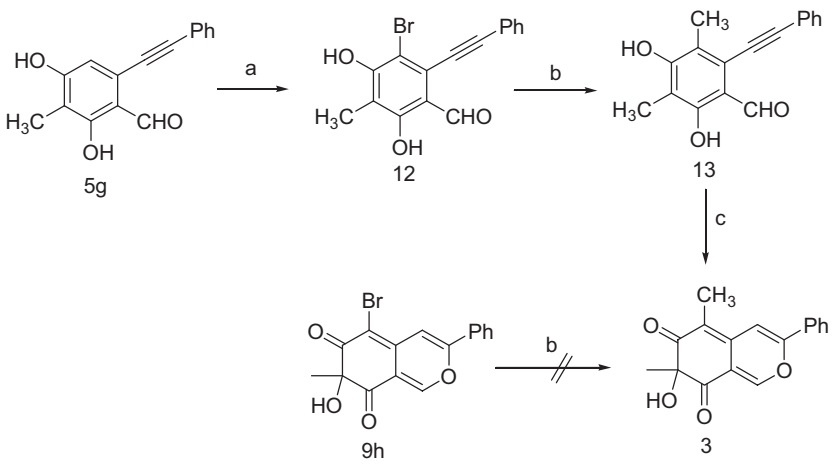
Scheme 2: Synthesis of azaphilones **9a**, **9b** and **9c**.

Furthermore, compound **1b**, the analogue of **9h** without the phenyl group at the 3-position, did not exhibit any antifungal activity in these assays, demonstrating the importance of the substituent at the 3-position for activity.

Compounds **9a**–**9f**, which have substituents at the 3-position such as -CH_2OPh, -CH_2OCH_2Ph or chains terminating in heterocycles such as pyrimidine or pyrimidinone, were found to be far less active than the simple phenyl-bearing compounds **9g** and **9h**, and it follows that these more complex side chains do not represent useful starting points for further structural modification.

Conclusion

In summary, we have synthesized a series of novel azaphilone analogues by replacement of the diene side chain of sclerotiorin with hydrogen, phenyl and aliphatic substituents. A practical approach to



Scheme 3: Reagents and conditions: (A) four equiv. HBr (aq.), 1.5 equiv. IBX, r.t., 80% (B) $SnMe_4$, $Pd(PPh_3)_4$, DMF, MW, 140 °C, 20min, 67% c) (i) $Au(OAc)_3$, DCE/TFA (10:1) (ii) IBX, TBAB, 40% over two steps.

Table 5: Fungicidal activity of the synthesized compounds[a]

	Pi[b]	St	Uvf	Pd	As	Bf	Gz		Pi	St	Uvf	Pd	As	Bf	Gz
No	200/60[c]	100	100	20/2	20/2	20/2	20/2	No	200/60[c]	100	100	20/2	20/2	20/2	20/2
2a	0/–[a]	99	0	99/77	0/0	0/0	0/0	**8g**	0/0	0	0	99/49	0/0	0/0	0/0
2b	0/–	49	0	99/99	0/0	0/0	0/0	**1b**	0	0	27	0/0	0/0	0/0	0/0
4a	0	0	27	0/0	0/0	0/0	0/0	**9a**	99	18	0	0/0	0/0	0/0	0/0
4b	0	0	0	0/0	0/0	0/0	0/0	**9b**	49	0	0	0/0	0/0	0/0	0/0
4c	0	0	27	0/0	0/0	0/0	0/0	**9d**	49	0	0	0/0	0/0	0/0	0/0
4d	0	0	0	0/0	0/0	0/0	0/0	**9e**	0	0	0	0/0	0/0	0/0	0/0
8b	0	0	0	99/49	0/0	0/0	0/0	**9f**	0	0	0	0/0	0/0	0/0	0/0
8c	0	0	0	99/27	0/0	0/0	0/0	**9g**	0/0	0	0	99/99	99/27	27/0	99/0
8d	0	0	0	0/0	0/0	0/0	0/0	**9h**	0/0	0	0	99/77	77/0	0/0	99/0
8e	0	0	27	0/0	0/0	0/0	0/0	**3**	0/0	0	99	99/0	27/0	99/0	99/99
8f	0	0	0	0/0	0/0	0/0	0/0	Scl[d]	0/0	0	NCH	99/0	0/0	0/0	0/0

[a]Mean scores across replicates, where 0 means 0–49% control of pathogen; 55 means 50–80% control of pathogen; 99 means 81–100 control of pathogen; and '–' indicates that the compound was untested at that rate. NCH indicates that no assessment was possible because of herbicidal effects on the leaf piece.
[b]Key: Pi, *Phytophthora infestans* (tested on tomato leaf pieces); St, *Septoria tritici* (tested on wheat leaf pieces); Uvf, *Uromyces viciae-fabae* (tested on bean leaf pieces); Pd, *Pythium dissimile*; As, *Alternaria solani*; Bf, *Botryotinia fuckeliana*; Gz, *Gibberella zeae* (all tested on artificial media).
[c]Rates in ppm.
[d]Sclerotiorin.

these compounds has been developed by employing metal-catalysed cycloisomerization and subsequent oxidation of suitable 2-alkynylbenzaldehydes, in which a variety of desired substituents were introduced using a Sonogashira coupling reaction.

The antifungal activity of the synthesized compounds was assessed. Where activity was observed, it tended to be in the assays conducted on artificial media. While several of the test compounds (notably **2a**, **2b**, **8b**, **8c**, **8g**) showed good activity against *Pythium dissimile*, it was the compounds **3**, **9g** and **9h** that gave the broadest spectrum of activity. The following conclusions can be drawn: (i) Activity is improved by the presence of a halogen or methyl substituent at the 5-position and a phenyl group at the 3-position. (ii) The free hydroxyl group at the 7-position is important for maintaining antifungal activity. (iii) Aliphatic- or heterocycle-containing aliphatic side chains at the 3-position appear to be detrimental to antifungal activity.

Compounds **3**, **9g** and **9h**, which have much better antifungal activity and simpler structures than sclerotiorin, are inspiring leads for further optimization of fungicidal activity. Further structural modifications to the sclerotiorin framework are underway, with a view to developing a full understanding of the structural requirements for optimal fungicidal activity.

Acknowledgments

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