

Optimization and Antifungal Activity of Amide Analogues

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Using salicylic acid as a lead compound, a series of its analogues (compounds **1-16**) were designed and synthesized. Their activity of anti-pathogenic fungi of plants has been evaluated in the laboratory. The results showed that these compounds had certain antifungal activity against *Sclerotinia sclerotiorum* and *Bipolaris maydis* (Nisikado et Miyake) Shoem. Among them, the inhibition of growth for 2-(3-fluorophenylcarbamoyl)phenyl acetate (**1**) and 2-(3-chlorophenylcarbamoyl)phenyl acetate (**2**) reached 91.1 %, 92.8 % and 90.1 %, 90.1 % at a concentration of 100 mg L⁻¹, respectively.

Key Words: Antifungal activity, Amide analogues, Synthesis.

INTRODUCTION

In agriculture, crop disease is the major issues in agricultural production. Crop yields cutting down more than 10 % are caused by them. Meanwhile, fungal diseases are about 80 % in the plant disease^{1,2}. Many kinds of fungicides are chemical fungicides. However, their over use has brought about side effects, such as the contamination of the environment and occurrence of resistance³. Botanical fungicides play an important role in controlling pathogenic fungi of plants⁴⁻⁶. Nevertheless, they have obvious drawbacks such as low yield, instability, complex components and limited antifungal spectrum. Salicylic acid (Fig. 1) is a natural compound existing in many plants. It can help plants to disease resistance⁷⁻¹⁸. Thus, salicylic acid used as a lead structure to synthesize new fungicides is meaningful.

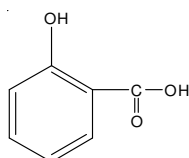


Fig. 1. Chemical structure of salicylic acid

EXPERIMENTAL

Sclerotinia sclerotiorum and *Bipolaris maydis* (Nisikado et Miyake) Shoem were obtained from the Chinese Academy of Agricultural Sciences. They were preserved at 4 °C. All chemicals and solvents were purchased from commercial

sources unless specified otherwise. IR spectra were recorded on a Thermofisher Nicolet-6700 spectrophotometer. ¹H NMR spectra were taken on a Varian Unity Inova-400 instrument using deuterium chloro-form as the solvent.

Synthesis of target compounds: The target compounds were synthesized according to the route shown in Fig. 2 and their yield was not optimized. Benzenamine or its derivatives (30 mmol) and pyridine (30 mmol) were dissolved in CH₂Cl₂ (10 mL). The mixture was stirred and heated to 30 °C. 2-(Chlorocarbonyl)phenyl acetate or 2-methoxybenzoyl chloride (30 mmol) were dissolved in CH₂Cl₂ (10 mL) and it was slowly mix under stirring 3-5 h until the reaction was complete. The precipitate was filtered and washed with distilled water. The pure compounds **1-16** were obtained by re-crystallization in anhydrous ethanol.

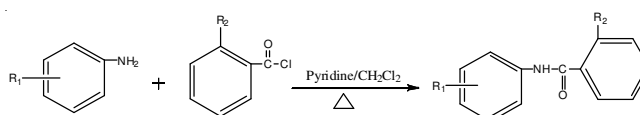


Fig. 2. Synthesis of target compounds **1-16**

Compound 1: White crystal, yield: 20 %, m.p. 116-117 °C; IR (KBr, $\nu_{\max}$, cm⁻¹): 3296, 3261, 3187, 3077, 1770, 1743, 1666, 1593, 1537, 1484, 1450, 1368, 1316, 1227, 1162, 1135, 785, 775, 751, 696, 680, 583; ¹H NMR (400 MHz, CDCl₃) δ (ppm): 2.32(s, 3H), 7.13 (t, J = 8.4 Hz, 2H), 7.26 (t, J = 8.0 Hz, 1H), 7.33 (t, J = 8.0 Hz, 1H), 7.40 (d, J = 8.0 Hz, 1H), 7.51 (t, J = 7.2 Hz, 1H), 7.74 (s, 1H), 7.79 (d, J = 8.0 Hz, 1H), 8.14 (s, 1H).

Compound 2: White crystal, yield: 27 %, m.p. 126-127 °C; IR (KBr, $\nu_{\max}$, cm^{-1}): 3296, 3261, 3187, 3115, 3077, 1770, 1743, 1676, 1666, 1593, 1537, 1484, 1450, 1368, 1316, 1203, 1162, 1135, 785, 775, 751, 696, 583; ^1H NMR (400 MHz, CDCl_3) δ (ppm): 2.31 (s, 3H), 7.12 (t, $J = 8.0$ Hz, 2H), 7.23-7.27 (m, 1H), 7.31 (t, $J = 8.0$ Hz, 1H), 7.39 (d, $J = 8.0$ Hz, 1H), 7.49 (t, $J = 8.0$ Hz, 2H), 7.76 (t, $J = 8.0$ Hz, 1H), 8.18 (s, 1H).

Compound 3: White crystal, yield: 30 %, m.p. 119-120 °C; IR (KBr, $\nu_{\max}$, cm^{-1}): 3293, 3258, 3118, 3076, 1768, 1742, 1666, 1609, 1589, 1480, 1449, 1367, 1318, 1192, 1134, 1095, 783, 774, 751, 703, 679, 583; ^1H NMR (400 MHz, CDCl_3) δ (ppm): 2.31 (s, 3H), 7.13 (d, $J = 8.0$ Hz, 1H), 7.19 (t, $J = 8.0$ Hz, 1H), 7.26 (t, $J = 6.4$ Hz, 1H), 7.31 (t, $J = 8.0$ Hz, 1H), 7.45-7.52 (m, 2H), 7.77 (d, $J = 8.0$ Hz, 1H), 7.87 (s, 1H), 8.15 (s, 1H).

Compound 4: White solid, yield: 32 %, m.p. 147-148 °C; IR (KBr, $\nu_{\max}$, cm^{-1}): 3325, 3272, 3130, 3099, 1737, 1675, 1608, 1524, 1487, 1446, 1353, 1333, 1229, 1199, 1134, 814, 758, 737, 677, 590; ^1H NMR (400 MHz, CDCl_3) δ (ppm): 2.35 (s, 3H), 7.17 (d, $J = 8.0$ Hz, 1H), 7.35 (t, $J = 8.0$ Hz, 1H), 7.50-7.56 (m, 2H), 7.80 (t, $J = 6.8$ Hz, 2H), 7.81 (d, $J = 8.0$ Hz, 1H), 8.34 (s, 1H), 8.45 (s, 1H).

Compound 5: White solid, yield: 75 %, m.p. 95-96 °C; IR (KBr, $\nu_{\max}$, cm^{-1}): 3338, 3200, 3074, 3000, 2838, 1651, 1597, 1524, 1465, 1323, 1250, 1185, 1184, 1102, 1028, 753, 690, 595; ^1H NMR (400 MHz, CDCl_3) δ (ppm): 4.05 (s, 3H), 7.03 (d, $J = 8.4$ Hz, 1H), 7.11-7.15 (m, 2H), 7.36 (t, $J = 8.0$ Hz, 2H), 7.47-7.51 (m, 1H), 7.68 (d, $J = 8.0$ Hz, 2H), 8.28-8.30 (m, 1H), 9.81 (m, 1H).

Compound 6: Light yellow needle crystal, yield: 70 %, m.p. 97-98 °C; IR (KBr, $\nu_{\max}$, cm^{-1}): 3329, 3284, 3075, 3027, 2842, 1647, 1601, 1579, 1464, 1317, 1287, 1210, 1181, 1094, 1022, 831, 759, 638, 591; ^1H NMR (400 MHz, CDCl_3) δ (ppm): 4.05 (s, 3H), 7.02-7.07 (m, 3H), 7.13 (t, $J = 8.0$ Hz, 1H), 7.48-7.52 (m, 1H), 7.62-7.65 (m, 2H), 8.27-8.29 (m, 1H), 9.78 (s, 1H).

Compound 7: White needle crystal, yield: 80 %, m.p. 92-93 °C; IR (KBr, $\nu_{\max}$, cm^{-1}): 3345, 3107, 3031, 2983, 2843, 1670, 1650, 1593, 1463, 1399, 1316, 1245, 1181, 1022, 1014, 825, 759, 689, 619, 590; ^1H NMR (400 MHz, CDCl_3) δ (ppm): 4.05 (s, 3H), 7.03 (d, $J = 8.4$ Hz, 1H), 7.13 (t, $J = 8.0$ Hz, 1H), 7.30 (t, $J = 8.8$ Hz, 2H), 7.48-7.52 (m, 1H), 7.63 (d, $J = 8.8$ Hz, 2H), 8.26-8.28 (m, 1H), 9.83 (s, 1H).

Compound 8: White powder, yield: 65 %, m.p. 124-125 °C; IR (KBr, $\nu_{\max}$, cm^{-1}): 3334, 3080, 3006, 2932, 2835, 1655, 1589, 1518, 1465, 1316, 1248, 1163, 1025, 1013, 819, 757, 629, 618; ^1H NMR (400 MHz, CDCl_3) δ (ppm): 4.05 (s, 3H), 7.03 (d, $J = 8.4$ Hz, 1H), 7.13 (t, $J = 7.2$ Hz, 1H), 7.46 (t, $J = 6.8$ Hz, 2H), 7.49-7.52 (m, 1H), 7.58 (d, $J = 8.8$ Hz, 2H), 8.26-8.28 (m, 1H), 9.83 (s, 1H).

Compound 9: Golden yellow crystal, yield: 58 %, m.p. 157-159 °C; IR (KBr, $\nu_{\max}$, cm^{-1}): 3280, 3256, 3074, 2987, 2842, 1668, 1580, 1464, 1425, 1343, 1273, 1148, 1118, 1045, 1014, 826, 745, 741, 686; ^1H NMR (400 MHz, CDCl_3) δ (ppm): 4.14 (s, 3H), 7.06 (d, $J = 8.0$ Hz, 1H), 7.12 (t, $J = 8.0$ Hz, 1H), 7.17-7.21 (m, 1H), 7.51-7.56 (m, 1H), 7.64-7.68 (m, 1H), 8.19-8.21 (m, 1H), 8.26-8.28 (m, 1H), 8.98-9.00 (m, 1H), 12.12 (s, 1H).

Compound 10: White crystal, yield: 83 %, m.p. 66-67 °C; IR (KBr, $\nu_{\max}$, cm^{-1}): 3348, 3068, 3015, 2833, 1660, 1556, 1513, 1462, 1414, 1322, 1243, 1174, 1047, 1017, 833, 827, 753, 665, 600; ^1H NMR (400 MHz, CDCl_3) δ (ppm): 3.80 (s, 3H), 4.02 (s, 3H), 6.89 (d, $J = 8.8$ Hz, 2H), 7.01 (d, $J = 8.4$ Hz, 1H), 7.12 (t, $J = 8.0$ Hz, 1H), 7.45-7.49 (m, 1H), 7.57-7.60 (m, 2H), 8.27-8.29 (m, 1H), 9.69 (s, 1H).

Compound 11: Light purple solid, yield: 60 %, m.p. 77-78 °C; IR (KBr, $\nu_{\max}$, cm^{-1}): 3348, 3068, 2960, 2833, 1660, 155, 1513, 1462, 1243, 1174, 1047, 1017, 833, 827, 753, 665, 600; ^1H NMR (400 MHz, CDCl_3) δ (ppm): 3.80 (s, 3H), 4.02 (s, 3H), 6.89 (d, $J = 8.8$ Hz, 2H), 7.01 (d, $J = 8.4$ Hz, 1H), 7.12 (t, $J = 8.0$ Hz, 1H), 7.45-7.49 (m, 1H), 7.57-7.60 (m, 2H), 8.27-8.29 (m, 1H), 9.69 (s, 1H).

Compound 12: White needle crystal, yield: 88 %, m.p. 144-145 °C; IR (KBr, $\nu_{\max}$, cm^{-1}): 3351, 3071, 3032, 2940, 1669, 1587, 1534, 1485, 1463, 1408, 1319, 1272, 1186, 1126, 1023, 823, 755, 654, 592; ^1H NMR (400 MHz, CDCl_3) δ (ppm): 2.59 (s, 3H), 4.08 (s, 3H), 7.05 (d, $J = 8.8$ Hz, 1H), 7.15 (t, $J = 7.2$ Hz, 1H), 7.50-7.55 (m, 1H), 7.78 (d, $J = 8.8$ Hz, 2H), 7.97 (d, $J = 8.8$ Hz, 2H), 8.27-8.29 (m, 1H), 10.05 (s, 1H).

Compound 13: Light yellow powder, yield: 85 %, m.p. 68-69 °C; IR (KBr, $\nu_{\max}$, cm^{-1}): 3447, 3392, 3072, 3029, 2847, 1669, 1604, 1492, 1457, 1326, 1243, 1146, 1072, 1016, 768, 746, 680; ^1H NMR (400 MHz, CDCl_3) δ (ppm): 4.05 (s, 3H), 6.79-6.84 (m, 1H), 7.03 (d, $J = 8.4$ Hz, 1H), 7.11-7.15 (m, 1H), 7.25-7.29 (m, 2H), 7.48-7.52 (m, 1H), 7.67-7.70 (m, 1H), 8.26-8.28 (m, 1H), 9.89 (s, 1H).

Compound 14: White needle crystal, yield: 87 %, m.p. 78-79 °C; IR (KBr, $\nu_{\max}$, cm^{-1}): 3446, 3333, 3082, 3049, 2839, 1669, 1593, 1484, 1456, 1316, 1230, 1128, 1048, 1024, 791, 747, 684, 606; ^1H NMR (400 MHz, CDCl_3) δ (ppm): 4.06 (s, 3H), 7.03 (d, $J = 8.4$ Hz, 1H), 7.10 (t, $J = 6.0$ Hz, 1H), 7.14 (d, $J = 8.0$ Hz, 1H), 7.48-7.53 (m, 2H), 7.80 (s, 1H), 8.26-8.28 (m, 1H), 9.85 (s, 1H).

Compound 15: White needle crystal, yield: 85 %, m.p. 79-80 °C; IR (KBr, $\nu_{\max}$, cm^{-1}): 3438, 3341, 3069, 2843, 1665, 1598, 1485, 1458, 1311, 1236, 1181, 1129, 1068, 1016, 779, 756, 681; ^1H NMR (400 MHz, CDCl_3) δ (ppm): 4.06 (s, 3H), 7.03 (d, $J = 8.4$ Hz, 1H), 7.13 (t, $J = 8.0$ Hz, 1H), 7.18-7.26 (m, 2H), 7.48-7.53 (m, 1H), 7.58-7.60 (m, 1H), 7.92 (d, $J = 1.6$ Hz, 1H), 8.25-8.28 (m, 1H), 9.84 (s, 1H).

Compound 16: Light yellow crystal, yield: 80 %, m.p. 145-146 °C; IR (KBr, $\nu_{\max}$, cm^{-1}): 3312, 3203, 3123, 2986, 2843, 1669, 1552, 1531, 1486, 1467, 1340, 1258, 1236, 1130, 1073, 1020, 804, 750, 738, 676; ^1H NMR (400 MHz, CDCl_3) δ (ppm): 4.11 (s, 3H), 7.07 (d, $J = 8.4$ Hz, 1H), 7.16 (t, $J = 8.0$ Hz, 1H), 7.49-7.56 (m, 2H), 7.95-7.97 (m, 1H), 8.27-8.30 (m, 1H), 8.49 (t, $J = 2.0$ Hz, 1H), 10.07 (s, 1H).

Assay of antifungal activity: All the synthesized compounds were screened for their activity against *Sclerotinia sclerotiorum* and *Bipolaris maydis* (Nisikado et Miyake) Shoem using the plate growth rate method¹⁹.

Each tested compound and carbendazim (purity 90 %) were dissolved in dimethyl sulfoxide, respectively. They were added to the sterile culture medium (PDA) at 45 °C, mixed to homogeneity and transferred to sterile Petri dishes to solidify.

For primary screening, each compound was used at a concentration of 100 mg L⁻¹. A mycelium agar disc (5 mm in diameter) of the target fungi was placed in the center of PDA plates. They were incubated at 28 °C in the dark until the target fungi used as the controls covered the surface of these plates. Control groups were treated with the corresponding solutions without the tested compound or carbendazim. The diameter of the fungi in the cultures was measured and the inhibition of growth was calculated according to Abbott's formula. Each compound was replicated three times.

RESULTS AND DISCUSSION

All the synthesized analogues of amide were screened for their activity against *Sclerotinia sclerotiorum* and *Bipolaris maydis* (Nisikado et Miyake) Shoem. The results are presented in Table-1. Some of them were active against the two different pathogenic fungi at a concentration of 100 mg L⁻¹. Among them, the inhibition rate of compound **1** and compound **2** reached 91.1 %, 92.8 % and 90.1 %, 90.1 %, respectively, which was close to carbendazim.

TABLE-1
ANTIFUNGAL ACTIVITY AGAINST *Sclerotinia sclerotiorum* AND *Bipolaris maydis*(Nisikado et Miyake) Shoem **1-16** AT 100 mg L⁻¹

Comp.	R1	R2	Inhibition of growth (%)*	
			<i>Sclerotinia sclerotiorum</i>	<i>Bipolaris maydis</i> (Nisikado et Miyake) Shoem
1	3-F	OCOCH ₃	91.1	90.1
2	3-Cl	OCOCH ₃	92.8	90.1
3	4-Br	OCOCH ₃	77.7	85.0
4	3-NO ₂	OCOCH ₃	92.8	74.1
5	H	OCH ₃	88.8	50.9
6	4-F	OCH ₃	62.0	87.6
7	4-Cl	OCH ₃	23.4	63.5
8	4-Br	OCH ₃	-2.6	17.7
9	4-NO ₂	OCH ₃	-0.3	9.7
10	4-CH ₃	OCH ₃	74.2	69.4
11	4-OCH ₃	OCH ₃	49.2	65.4
12	4-COCH ₃	OCH ₃	1.0	14.8
13	3-F	OCH ₃	44.4	57.56
14	3-Cl	OCH ₃	56.6	54.5
15	3-Br	OCH ₃	65.1	37.1
16	3-NO ₂	OCH ₃	11.97	13.46
Carbendazim			99.8	91.0

*Based on the mean of triplicates

A series of analogues of amide have been successfully synthesized and tested for their antifungal activity against *Sclerotinia sclerotiorum* and *Bipolaris maydis* (Nisikado et Miyake) Shoem. The preliminary results suggested that the design and synthesis of these compounds may be conducive to the antifungal activity of analogues of amide. They are also promising and beneficial to further studies in developing new and more effective fungicides in the agricultural chemistry field. However, there is more work to be done. A number of analogues of amide should be further synthesized for screening and surveying quantitative structure-activity relationships so as to find novel fungicides with high effect and low toxicity. The structural modification and optimization of compound **1** and compound **2** need to be done. Meanwhile, its mode of action and its safety to humans and non-target organisms also need to be investigated.

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