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Synthesis and Bioactivity of *N*-(1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)-amide Derivatives

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A series of novel *N*-(1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)-amide derivatives were synthesized by a simple approach from 1,8-naphthalic anhydride and their organic acids in high yield. The chemical structures of the products were elucidated by NMR and HR-ESI-MS, and their purity were checked with the melting points. Their plant growth regulating activity and fungicidal activity were also evaluated. Seed germination tests showed that the seed germination rate of wheat and cucumber can be promoted significantly by these six compounds. The maximal seed germination promotion rates were observed at the concentration of 25 mg/L and 12.5 mg/L for wheat and cucumber, respectively. In addition, 4a, 4b and 4d exhibited the best promoting effects on wheat seedling growth, with the best performance on primary roots, moderate promoting effects on lateral roots. They also exhibited higher cucumber seedling growth activities than DA-6 at 12.5 mg/L. Meanwhile, 4a-d showed a high fungicidal activity against *Alternaria solani, Fusarium graminearum, Phytophthora Infestans* and *Phytophthora capsici* at 125 mg/L. All these results revealed that the bioactivity of *N*-(1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)-amide derivatives can improve notably with the introduction of phenyl group.

1. Introduction

1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione derivatives has been continuously investigated in the past few decades for their application in anti-tumor agents (Mukherjee et al., 2010), fluorescence probe (Mahato et al., 2012) and fluorescent sensor (Yordanova et al., 2014) due to their high photostability and large Stokes shift in fluorescence emission. In addition, their starting materials 1,8-naphthalic anhydride of some 1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione derivatives has been demonstrated to protect plant from the phytotoxicity of herbicides (Barrett and Harwood, 1998). Based on these merits mentioned above, a series of 1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione derivatives were synthesized from 1,8-naphthalic anhydride in our previous research. Plant growth regulating bioassay showed that some compounds exhibited significantly promoting effect on wheat seed germination and seedling growth. Fungicidal activity test showed that some compound had fungicidal activities against mycelial growth of five fungicides.

Recent studies suggest that imide substructure is an excellent pharmacophore and its derivatives show good performance in pesticide fields due to their unique biological properties. However, few reports about that *N*-(1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)-amide derivatives could be used as insecticides, fungicides, herbicides or plant growth regulators (León et al., 2003).

Organic acids naphthalene-1-acetic acid, cinnamic acid, 2,4-dichloro-cinnamic acid, sorbic acid and n-hexanoic acid (and their derivatives) show diverse plant growth regulating activity in different bioassays (Heikal, 2010). Salicylic acid is a natural phenolic product that plays an important role in many biochemical processes. One of its derivatives acetylsalicylic acid (aspirin) is a successful and widely used medicine (Vlot et al., 2009).

Therefore, as a continuation of our research and in order to obtain information about their plant growth regulating activity and fungicidal activity, *N*-(1,3-dioxo-1*H*-benzo[de]isoquinolin-2(3*H*)-yl)-amide derivatives were synthesized from the active units1,8-naphthalic anhydride and organic acids by a convenient method (Figure 1). The preliminary plant growth regulating activity against wheat and cucumber were tested at the dosage ranging from 6.25 to 125 mg/L, fungicidal activity against *Alternaria solani*, *Fusarium graminearum*,

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Phytophthora Infestans and *Phytophthora capsici* were also evaluated at 125 mg/L. These are examples of a new class of materials that will open up a door for a series of new studies encompassing topics in botany and agronomy.

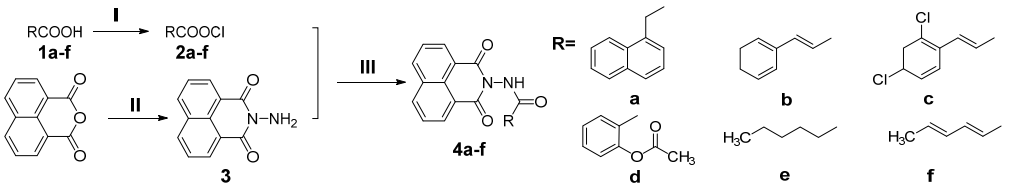

Figure 1: General synthetic route for compounds 4a-f. Reagents and conditions: (I) $SOCl_2$, reflux 3 h; (II) $N_2H_4 \cdot H_2O$, reflux 4 h; (III) toluene, reflux 2-3 h

2. Experimental

2.1 Instrument and chemicals

Melting points were determined on a SGW X-4 microscope melting point apparatus (Shanghai INESA Scientific Instrument Co., Ltd., China) without being corrected. ^{1}H NMR and ^{13}C NMR spectra were obtained on a Bruker Avance spectrometer (500 MHz) with tetramethylsilane (TMS) as internal standard and DMSO-d_6 as solvent at room temperature. Chemical shift values (δ) are given in parts per million. HR-ESI-MS were recorded on a Thermo LTQ Orbitrap XL spectrometer. Progress of the following reactions was monitored by TLC. Silica gel GF254 for TLC was produced by Qingdao Marine Chemical Company, China. All reagents were commercial products purchased from Sinopharm Chemical Reagent Co., Ltd., China.

2.2 General methods for the preparation of the intermediates acyl chlorides (2a-f)

To a stirred solution of organic acid (1a-d, 10 mmol) in toluene (20 mL), 1e-f (10 mmol) in dichloromethane (20 mL), thionyl chloride (3.57 g, 2.18 mL, 30 mmol) was slowly added over 30 min at room temperature. Then, the reaction was stirred for 3 h under reflux. The solution and excess thionyl chloride were removed at atmospheric pressure to give the corresponding acyl chlorides 2a-f as intermediates at yields of more than 95%. The intermediates were kept in toluene for further use.

2.3 Synthesis of 2-amino-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (3)

To a suspension of 1,8-naphthalic anhydride (6 g, 30.26 mmol) in distilled water (250 mL), 80% aqueous solution of hydrazine hydrate (3.79 g, 3.71 mL, 60.52 mmol) was added dropwisely. Then, the mixture was heated to reflux for 4 h. A lot of yellow precipitates appeared. The reaction mixture was cooled to room temperature, and the precipitates were filtered and washed three times with water. Then, the product was dried in oven at 54ºC. The melting point and physicochemical spectral data of the compound were consistent with the data reported in the reference (Reddy et al., 2012).

Yellow powder, yield 96.3%, m.p. 262-264ºC, ^{1}H NMR (500 MHz, DMSO-d_6) δ 5.80 (s, 2H, NH_2), 7.88 (t, 2H, J = 7.6 Hz, Ar-H), 8.47 (d, 2H, J = 8.2 Hz, Ar-H), 8.52 (d, 2H, J = 7.2 Hz, Ar-H).

2.4 Synthesis of *N*-(1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)-amides (4a-f)

To a stirred solution of 2-amino-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (3, 2.12 g, 10 mmol) in toluene (25 mL), the toluene solution of 2a-f (12 mmol) was slowly added over 30 min at room temperature, respectively. Then the reaction was stirred for 2-3 h under reflux. Upon the completion of acylation, the precipitates were filtered under reduced pressure, washed with toluene and recrystallized with toluene to give pure 4a-f as white or light yellow powders.

N-(1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)-2-(naphthalen-1-yl)acetamide (4a)

White powder, yield 89%, m.p. 280-282ºC, ^{1}H NMR (500 MHz, DMSO-d_6) δ 4.23 (s, 2H, CH_2), 7.51 (t, 1H, J = 7.6 Hz, Ar-H), 7.55 (t, 1H, J = 7.4 Hz, Ar-H), 7.59-7.64 (m, 2H, Ar-H), 7.86-7.92 (m, 3H, Ar-H), 7.95 (d, 1H, J = 8.1 Hz, Ar-H), 8.25 (d, 1H, J = 8.4 Hz, Ar-H), 8.51-8.55 (m, 4H, Ar-H), 11.17 (s, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d_6) δ 37.9, 122.2, 124.8, 126.0, 126.2, 126.6, 127.7, 127.9, 128.3, 128.8, 131.9, 132.4, 133.8, 135.6, 162.2, 169.7. HR-ESI-MS *m/z* 381.1226 $[M+H]^+$ (requires for $C_{24}H_{17}N_2O_3$ 381.1234).

N-(1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)cinnamamide (4b)

Light yellow powder, yield 88%, m.p. 242-244ºC, ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.98 (d, 1H, J = 15.9 Hz, CH), 7.43-7.49 (m, 3H, Ar-H), 7.63-7.66 (m, 2H, Ar-H), 7.70 (d, 1H, J = 7.1 Hz, CH), 7.88-7.94 (m, 2H, Ar-H), 8.50-8.57 (m, 4H, Ar-H), 10.96 (s, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d_6) δ 119.1, 122.2, 127.7, 127.9,

128.4, 129.1, 129.5, 130.6, 132.0, 134.9, 135.6, 142.1, 162.2, 164.5. HR-ESI-MS *m/z* 343.1073 $[M+H]^+$ (requires for $C_{21}H_{15}N_2O_3$ 343.1077), 365.0891 $[M+Na]^+$ (requires for $C_{21}H_{14}N_2O_3Na$ 365.0897).

(*E*)-3-(2,4-dichlorophenyl)-*N*-(1,3-dioxo-1*H*-benzo[d*e*]isoquinolin-2(3*H*)-yl)acrylamide (4c)

White powder, yield 85%, m.p. 295-297ºC, ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.99 (d, 1H, *J* = 15.9 Hz, CH), 7.56-7.58 (m, 1H, Ar-H), 7.77-7.78 (m, 1H, Ar-H), 7.85 (d, 1H, *J* = 15.8 Hz, Ar-H), 7.90-7.93 (m, 2H, Ar-H), 7.95 (d, 1H, *J* = 7.7 Hz, CH), 8.57 (t, 4H, *J* = 6.5 Hz, Ar-H), 11.10 (s, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d_6) δ 122.2, 122.8, 127.7, 127.9, 128.7, 129.8, 130.0, 131.7, 132.0, 134.9, 135.7, 136.1, 162.1, 163.9. HR-ESI-MS *m/z* 411.0288 $[M+H]^+$ (requires for $C_{21}H_{13}Cl_2N_2O_3$ 411.0298), 433.0108 $[M+Na]^+$ (requires for $C_{21}H_{12}Cl_2N_2O_3Na$ 433.0117).

2-(1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-ylcarbamoyl)phenyl acetate (4d)

Light yellow powder, yield 86%, m.p. 226-228ºC, ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.32 (s, 3H, CH_3), 7.32 (d, 1H, *J* = 8.1 Hz, Ar-H), 7.47 (t, 1H, *J* = 7.5 Hz, Ar-H), 7.66 (t, 1H, *J* = 7.3 Hz, Ar-H), 7.81 (d, 1H, *J* = 7.3 Hz, Ar-H), 7.94 (t, 2H, *J* = 7.7 Hz, Ar-H), 8.52-8.60 (m, 4H, *J* = 7.3 Hz, Ar-H), 11.07 (s, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d_6) δ 21.4, 122.1, 124.4, 126.7, 127.1, 127.7, 128.0, 130.1, 131.3, 132.1, 133.0, 135.0, 135.9, 148.9, 162.0, 164.3, 169.5. HR-ESI-MS *m/z* 397.0790 $[M+Na]^+$ (requires for $C_{21}H_{14}N_2O_5Na$ 397.0795).

N-(1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)hexanamide (4e)

White powder, yield 86%, m.p. 159-161ºC, ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.90 (t, 3H, *J* = 6.9 Hz, CH_3), 1.30-1.41 (m, 4H, $2CH_2$), 1.60-1.65 (m, 2H, CH_2), 2.35 (t, 2H, *J* = 7.3 Hz, CH_2), 7.91 (t, 2H, *J* = 7.8 Hz, Ar-H), 8.52-8.54 (m, 4H, Ar-H), 10.66 (s, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d_6) δ 14.3, 22.4, 25.2, 31.2, 33.6, 122.2, 127.7, 127.9, 131.9, 135.5, 162.1, 171.7. HR-ESI-MS *m/z* 311.1384 $[M+H]^+$ (requires for $C_{18}H_{19}N_2O_3$ 311.1390), 333.1202 $[M+Na]^+$ (requires for $C_{18}H_{18}N_2O_3Na$ 333.1210).

(2*E*,4*E*)-*N*-(1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)hexa-2,4-dienamide (4f)

Light yellow powder, yield 89%, m.p. 224-226ºC, ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.85 (d, 3H, *J* =6.6 Hz, CH_3), 6.17-6.28 (m, 2H, CH), 6.37 (t, 1H, *J* = 13.1 Hz, CH), 7.17-7.22 (m, 1H, CH), 7.92 (t, 2H, *J* = 7.8 Hz, Ar-H), 8.53-8.55 (m, 4H, Ar-H), 10.80 (s, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d_6) δ 18.9, 119.7, 122.2, 127.9, 130.3, 132.0, 135.6, 139.4, 142.6, 162.2, 164.8. HR-ESI-MS *m/z* 307.1073 $[M+H]^+$ (requires for $C_{18}H_{15}N_2O_3$ 307.1077), 329.0891 $[M+Na]^+$ (requires for $C_{18}H_{14}N_2O_3Na$ 329.0897).

2.5 Plant growth regulating activity assay

Plant growth regulating activity of the synthesized compounds 4a-f against wheat (*Triticum aestivum* L., a monocotyledonous plant) and cucumber (*Cucumis sativus* L., a dicotyledonous plant) were investigated. 50 g/L suspension concentrate (SC) formulations of compounds 4a-f were prepared, then diluted with distilled water to obtain the test solutions of different concentrations (6.25, 12.5, 25, 50 and 125 mg/L). Seeds treated with water and DA-6 (2-(diethylamino)ethyl hexanoate) were used as control groups. Each experiment was performed three times.

2.5.1 Seed germination test

Thirty wheat seeds were sterilized with EtOH for 5 min and washed with running tap water thoroughly. The sterilized seeds were soaked in test solutions (6.25-125 mg/L) for 5 h. Then, the seeds were placed on double-layered filter paper moistened with distilled water in a 9 cm Petri dish, and allowed to germinate under continuous dark for 20 h at 25 ± 1ºC. The percentage of seeds that showed radicle emergence was calculated (Todoroki et al., 2011; Aguilar et al., 2015). The effects of compounds 4a-f on seed germination of cucumber were tested by the same method (Li et al., 2016).

2.5.2 Wheat seedling growth test

During the period of wheat germinating, five seedlings (about 6-7 mm height) were chosen and transplanted to a 20 mL beaker containing 5% agar culture medium (Rodriguez-Furlán et al., 2016). When the roots of wheat grew to close to the bottom of the beaker, primary roots length, lateral roots length and shoots height were measured.

2.5.3 Cucumber cotyledon root generation test

The sterilized cucumber seeds were incubated under continuous dark for 72 h at 25 ± 1ºC, and 10 cotyledons were cut off. The experiments were carried out in 9 cm Petri dishes with two sheets of filter paper. To each dish, 0.3 mL of the test solution at a concentration of 12.5 mg/L was added, followed by 3 mL water and the above cotyledons. Assays were carried out under continuous dark for 5 days at 25 ± 1ºC. Then the number of roots was counted.

2.6 Fungicidal activity bioassay

The fungicidal activities of compounds 4a-f against *Alternaria solani*, *Fusarium graminearum*, *Phytophthora Infestans* and *Phytophthora capsici* were tested by mycelium growth rate method (Zhang et al., 2016). A test solution of 4a-f (1 mL, 125 mg/L) was added into a sterile Petri dish (9 cm diameter), and agar culture medium (9 mL) was added. Sterile water (1 mL) and agar culture medium (9 mL) were used as control groups. 4 mm diameter agar disks containing active growing mycelium obtained from the margin of a ten-day colony were placed in the center of the above Petri dish, and incubated at 25 ± 1°C. Each experiment was performed three times. The diameter of the mycelium was measured after 72 h.

3. Results and discussion

3.1 Synthesis

Acyl chlorides (2a-f) were synthesized from organic acid and thionyl chloride as shown in Figure 1. 1,8-naphthalic anhydride was treated with 80% aqueous solution of hydrazine hydrate in water to yield 2-amino-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (3). The target compounds *N*-(1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)-amide derivatives (4a-f) were conveniently synthesized via condensation reaction from acyl chlorides (2a-f) and 2-amino-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (3) under reflux in toluene. Completion of the reaction afforded the target compounds in 85-89% yield. It is noteworthy that 2-amino-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (3) could be efficiently prepared by using distilled water as solvent rather than using ethanol (Sharma et al., 2012), chloroform (Reddy et al., 2012) as solvent mentioned in previous literature. It was difficult to obtain the target compounds by traditional acylation with triethylamine as catalyst in ice-water bath or at room temperature because of the high steric hindrance of 2-amino-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione. Instead, by the method presented in this paper, the target compounds could be easily obtained in high yields, and toluene could be reused to protect the environment. The experimental results showed that the method presented in this paper could prepare the target compounds efficiently and eco-friendly, and was considered to be an excellent method.

3.2 Effects on seed germination

The effects of the target compounds 4a-f and DA-6 on seed germination of wheat and cucumber were evaluated at a concentration ranging from 6.25 to 125 mg/L.

The maximal wheat seed germination promotion rates of 4a-f were observed at the concentration of 25 mg/L for all the six compounds (Table 1), and in particular, 4a, 4b and 4d showed rate enhancement 36%, 33% and 38%, respectively. In addition, all six compounds had promoting rates varied from 12% to 27% at 6.25, 12.5 and 50 mg/L. While at 125 mg/L, all compounds performed promotion rates of less than 10%. Under the same condition, DA-6 showed the maximal promoting rate of 19% at 25 mg/L.

Table 1: Wheat seed germination promotion rates (%) of 4a-f at different concentrations

Compd.	6.25 mg/L	12.5 mg/L	25 mg/L	50 mg/L	125 mg/L
4a	17	25	36	28	10
4b	16	21	33	23	9
4c	14	20	29	21	7
4d	19	24	38	27	10
4e	12	15	25	18	6
4f	14	18	24	19	8
DA-6	5	13	19	15	7

The same trend as that of wheat was shown on cucumber seed germination except the maximal promotion rates were at the concentration of 12.5 mg/L. 4a, 4b and 4d provided the maximal promoting rates of 34%, 35% and 37%, respectively. Additionally, all the treatments did not cause any germination rate decrease comparing to the control. In brief, most compounds possess higher activities than DA-6, and could significantly promote the seed germination of wheat and cucumber.

3.3 Effects on wheat seedling growth

In order to gain more insight into the effects on wheat seedling growth, the primary roots length and lateral roots length were evaluated. The primary root length and lateral root length of the blank control were 42.3 mm and 28.4 mm, respectively. The promotion rates of primary roots length increased at first with the increasing of concentrations of 4a-f (Figure 2A), however it started to decrease when further increasing the concentration.

At 25 mg/L, 4a-f had the maximal promotion rates of 30.7%, 35.2%, 29.3%, 37.8%, 24.8% and 25.5%, respectively. At 12.5 and 50 mg/L, 4a-d exhibited moderate promotion rates varied from 19.9% to 27.4%, and in particular, 4d showed promoting effect of 27.4% at 50 mg/L. At 125 mg/L, the promotion rates were less than that at 12.5 mg/L and slightly higher than that at 6.25 mg/L for all compounds. Overall, compounds 4a-d significantly promoted the growth of primary roots of wheat.

All compounds showed similar promoting effects on lateral root with the concentration of the optimum promotion rate at 25 mg/L for 4b and 4d, and 50 mg/L for 4a, 4c, 4e and 4f. Therefore, 4a, 4b and 4d exhibited the best promotion rate of 29.9%, 27.5% and 30.6%, respectively (Figure 2B).

All compounds had promoting rates of less than 20% on shoots height (data not shown).

Based on these results, all six novel compounds can possess plant growth promoting activity against wheat. It showed the most effectively promoting effects on primary roots, moderate promoting effects on lateral roots, while the weakest promoting effects on shoots. Compounds 4a-d, especially 4a, 4b and 4d processed higher promoting effects on wheat seedling growth than those of 4e-f.

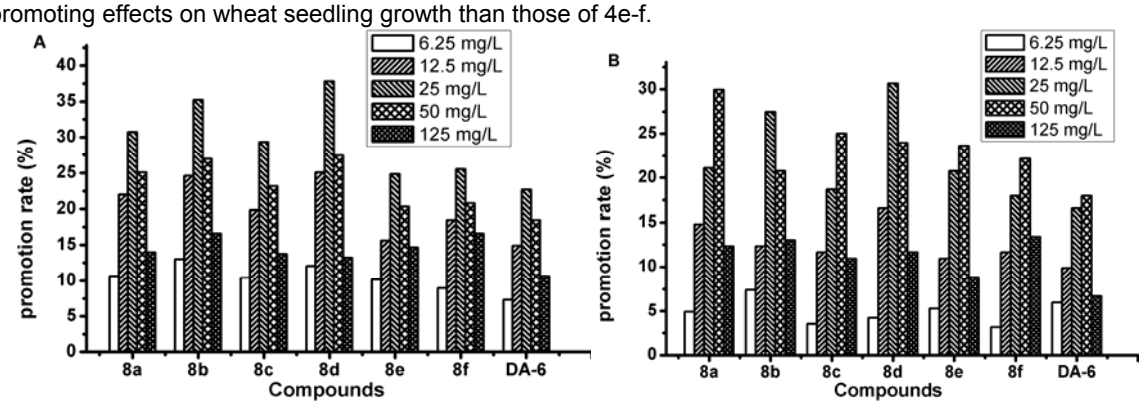

Figure 2: Promotion rates of wheat primary roots length (A) and lateral roots length (B) of 4a-f

3.4 Effects on cucumber seedling growth

The promoting effects on the radicle growth of cucumber at 12.5 mg/L were also investigated and their promotion rates shown in Table 2. All compounds exhibited good stimulating effects on radicle growth of cucumber (>18%). and in particular, 4a, 4b and 4d had stimulating effect of 34%, 31% and 41%, respectively, which were higher than that of DA-6 (13%).

Table 2: Effects of 4a-f on cucumber seedling growth at 12.5 mg/L and fungicidal activities at 125 mg/L

Compd.	Promotion rate (%)	Fungicidal activity (%)			
		A. solani	*F. graminearum*	*P. Infestans*	*P. capsici*
4a	34	37.5	34.9	29.5	27.9
4b	31	16.1	29.3	12.7	16.7
4c	25	20.9	31.6	16.9	19.6
4d	41	35.3	39.7	37.8	38.1
4e	19	15.8	22.1	6.9	13.3
4f	18	12.9	19.4	11.3	14.6
DA-6	13	-	-	-	-

3.5 Fungicidal activities

The fungicidal activities of compounds 4a-f against *Alternaria solani*, *Fusarium graminearum*, *Phytophthora Infestans* and *Phytophthora capsici* were evaluated at 125 mg/L. The results (Table 2) indicated that all six compounds had fungicidal activities, and 4a-d exhibited relatively higher activities than 4e-f on inhibiting mycelial growth of four fungicides. 4d exhibited the highest activity among the six compounds.

Overall, the results of Table 1, Table 2 and Figure 2 indicated that 4a-d had higher activities than 4e-f, which revealed that the introduction of phenyl group could improve bioactivities of *N*-(1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)-amide derivatives compared with aliphatic group.

4. Conclusions

In summary, six novel *N*-(1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)-amide derivatives were synthesized by an efficient and eco-friendly method with a high yields. The preliminary bioassay indicated that six new compounds significantly promoted the seed germination of wheat and cucumber in the range of 6.25-125 mg/L. The maximal seed germination promotion rates were observed at the concentration of 25 mg/L and 12.5 mg/L for wheat and cucumber, respectively. For wheat seedling growth, all six compounds showed the best promoting effects on primary roots, moderate promoting effects on lateral roots, the weakest effects on shoots. For cucumber seedling growth, 4a, 4b and 4d exhibited higher activities than DA-6 at 12.5 mg/L. Meanwhile, 4a-d showed high fungicidal activities against *Alternaria solani*, *Fusarium graminearum*, *Phytophthora Infestans* and *Phytophthora capsici* at 125 mg/L. The results revealed that the introduction of phenyl group improved the bioactivities of *N*-(1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)-amide derivatives. These derivatives could potentially be used as plant growth regulators and fungicides.

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