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Design, synthesis and antifungal activity evaluation of coumarin-3-carboxamide derivatives

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ABSTRACT

A series of coumarin-3-carboxamides/hydrazides have been designed and synthesized, all the target compounds were evaluated in vitro for their antifungal activity against *Botrytis cinerea*, *Alternaria solani*, *Gibberella zeae*, *Rhizoctorzia solani*, *Cucumber anthrax* and *Alternaria* leaf spot, some of the designed compounds **4a**-**4g** exhibited potential activity in the primary assays, this highlighted by the compounds **4a**, **4d**, **4e** and **4f**, EC_{50} values of which against *Rhizoctorzia solani* were as low as 1.80 μg/mL, 2.50 μg/mL, 2.25 μg/mL and 2.10 μg/mL, respectively, exhibiting more effective control with that of the positive control than Boscalid. Furthermore, compounds **4a** and **4e** represented equivalent antifungal activity with Boscalid against *Botrytis cinerea*.

1. Introduction

With readily synthetic methods and excellent chemical/physical characteristics, coumarins have received extensive attention and show great practical values in many fields, such as medicine discovery, dye chemistry, materials chemistry and so on [1–3]. Actually, over 1300 kinds of coumarins have been identified, chiefly as secondary metabolites in green plants [4,5], fungi and bacteria [6–9]. The active ingredient coumarins also can be found in many traditional Chinese herbal medicines, such as the former Hu, founder Ma, Cnidium, pepper, Qin Pekin, etc., and they usually possess a broad scope of pharmacological and biological activities [10].

As the structural core, coumarin is widely used as a scaffold in medicinal and agricultural chemicals (Fig. 1). Such as Warfarin and Acenocoumarol, both are anticoagulant agents that function as vitamin K antagonists, Coumoxystrobin is a strobilurin fungicide bearing a coumarin subunit, it displays a broad spectrum of antifungal activity, and Osthole with a coumarin scaffold has been using as a fungicide for a long history in China. Inspired by these facts, our group designed and synthesized several coumarin derivatives with high antifungal activity [11–13], such as Osthole derivatives, coumarino[8,7-e][1,3]oxazine, furo[3,2-*c*]coumarin, and pyrano[3,2-c] chromene-2,5-dione [8,14–16] (Fig. 1). However, the discovery of coumarin-based fungicides with high activity still has a long way to go.

In the field of fungicide research, the development of carboxylic acid amide (CAA) fungicides has received a lot of attention. Since the early report of amide fungicide carbendazim (carboxin) in 1966, amide fungicides have been used for nearly 50 years [17], and a number of commercial amide fungicides have been launched to the market. During the late 1990s, by the introduction of fluorine atom and active heterocycle to the target molecules, the research on amide fungicides won a big step forward. New structures of amide fungicides were reported with detailed understanding of their mode of action [18]. The newly developed amide fungicides such as Fluopyram, Bixafen, Sedaxane, Isopyrazam, Penthiopyrad and Boscalid [19,20] (Fig. 2) with high activity and broad antifungal spectrum were successfully used to control a variety of plant diseases with very limited cross-resistance to most of the main fungal diseases.

In addition to amide group, molecules with hydrazide scaffolds also displayed a broad scope of pharmacological and biological activities [21,22] and have been widely used as frameworks in drug design. For example, the hydrazide derivative maleic hydrazide [23] has been found a temporary and inhibiting effect on plant growth, and the indole-coumarin [24] exhibited good antiproliferative profile against colon HT-2cell line, and the hydrazide derivative tebufenozide [25] exhibited moderate insecticidal activity as a kind of insect growth regulators (IGRs) (Fig. 3).

Considering these facts, we envisioned that the combination of the coumarin framework with carboxamides/hydrazide units may afford the desired high performance fungicides. Based on our previous work on structural modification and bioscreening of coumarin based fungicides, which exhibited effective control to certain phytopathogenic diseases [8,14,16,26], herein we report the design, synthesis and antifungal activities of novel coumarin carboxamides/hydrazides. It was luckily to discover that a new category of coumarin based fungicides with excellent performance such as high antifungal activities and low

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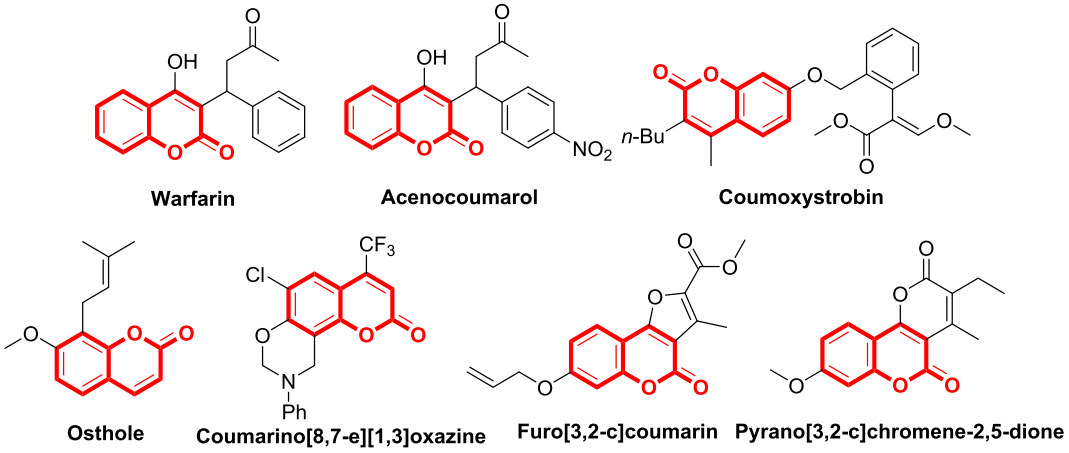


Fig. 1. Sample structures of coumarin-containing drugs and fungicides.

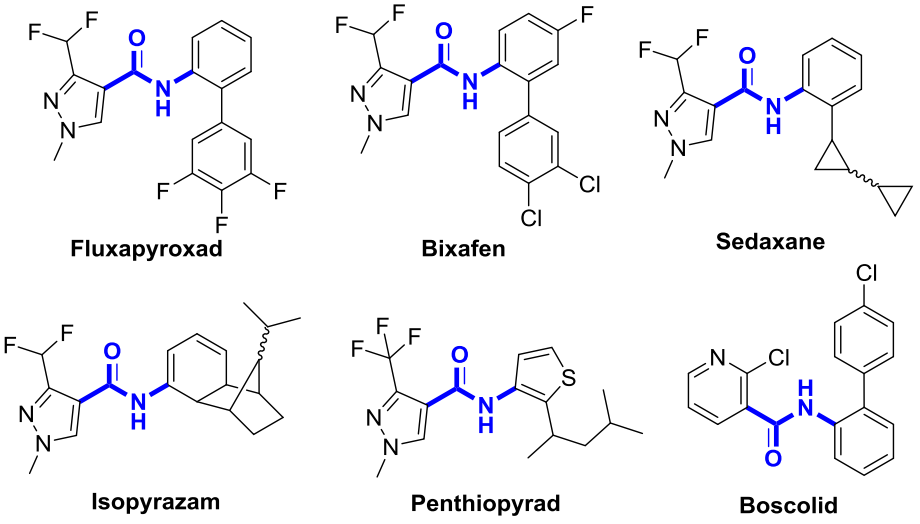


Fig. 2. Representative structures of carboxylic acid amide fungicides.

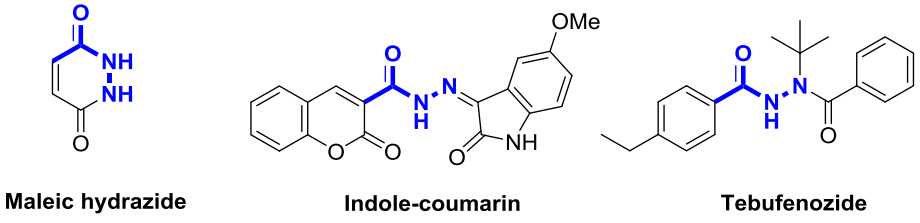


Fig. 3. Representative structures of hydrazide.

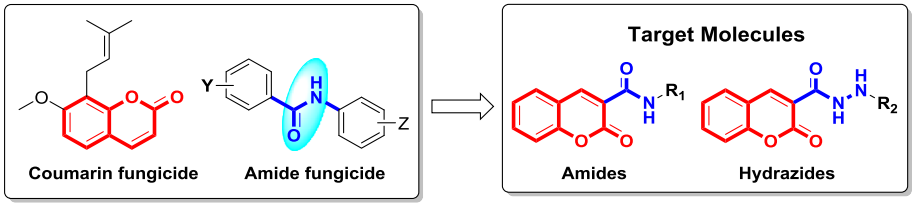


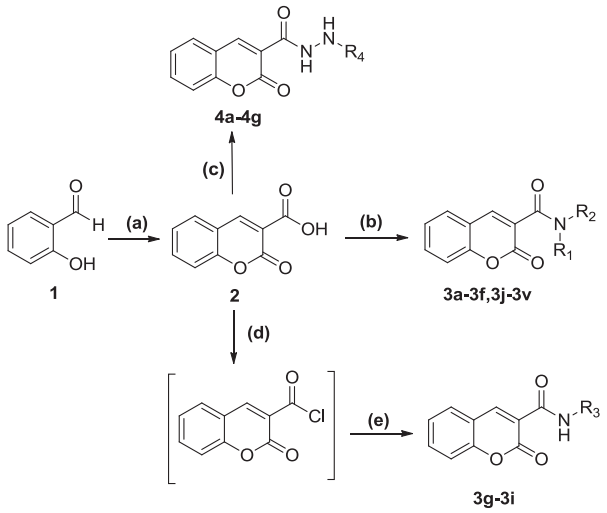
Fig. 4. Design strategy for target molecules.

drug resistance effect was successfully developed (Fig. 4).

2. Materials and methods

2.1. Chemicals and instruments

All commercially available reagents including substituted salicylaldehydes were purchased from Crystal Chemicals and used without



(a) Meldrum's acid, K_2CO_3, H_2O, rt; (b) T_3P, DIEA, DMF, 0 °C; (c) EDCI, DMAP, CH_2Cl_2, 0 °C; (d) $(COCl)_2$, DMF, CH_2Cl_2, rt; (e) DIEA, CH_2Cl_2, 0 °C.

Scheme 1. Synthetic routes for the target compounds.
(a) Meldrum's acid, K_2CO_3, H_2O, rt.; (b) T_3P, DIEA, DMF, 0 °C; (c) EDCI, DMAP, CH_2Cl_2, 0 °C; (d) $(COCl)_2$, DMF, CH_2Cl_2, rt.; (e) DIEA, CH_2Cl_2, 0 °C.

further purification unless otherwise stated. The melting points of the amide of coumarin derivatives were determined on an X-4 apparatus (uncorrected), which was purchased from Shanghai Tech. Infrared (IR) spectra were recorded on a Bruker Tensor 27 spectrometer, and samples were prepared as KBr plates. ^{1}H NMR and ^{13}C NMR spectra were obtained using a Bruker Avance 400 or 300 MHz spectrometer in $CDCl_3$, DMSO-d_6, Acetone-d_6 or TFA-d solution with TMS as an internal standard. HR-MS (ESI) spectra were carried out with a Thermo Exactive spectrometer, and X-rays were measured at 296 K on a Bruker SMART APEX2 CCD area detector diffractometer. Most reaction yields were not optimized.

*2.1.1. General procedure for the preparation of compounds **3a-3f, 3j-3q** (Scheme 1)*

To compound **2** (5.0 mmol, 0.95 g) dissolved in dry DMF (30 mL) aniline (6.0 mmol, 1 mL) was added and the mixture was stirred at iced water. Once **2** was completely dissolved, DIEA (15.0 mmol, 1.94 g) and T_3P (propyl phosphoric acid anhydride, 5.5 mmol, 1.75 g) were added gradually with stirring and cooling for 40 min. After mixed with the appropriate water, the solution was extracted with ethyl acetate, and then washed with water for three times, and the product was purified by column chromatography to give a white solid.

*2.1.2. General procedure for the preparation of compounds **4a-4g** (Scheme 1)*

To compound **2** (5.0 mmol, 0.95 g) dissolved in anhydrous CH_2Cl_2 (30 mL) (2-chlorophenyl) hydrazine hydrochloride (6.0 mmol, 1.08 g) was added and the mixture was stirred at iced water. Once **2** was completely dissolved, DMAP (4-dimethylaminopyridine, 77.2 mmol, 1.53 g) and EDCI (carbodiimide hydrochloride, 6.0 mmol, 1.15 g) were added gradually with stirring and cooling for 4 h. The reaction mixture was extracted with saturated brine for three times. After took off the solvent, the resulting precipitate was purified by column chromatography to give an orange solid.

*2.1.3. General synthetic procedure for the preparation of compounds **3g-3i** (Scheme 1)*

To a solution of **2** (5.0 mmol, 0.95 g) in anhydrous CH_2Cl_2 (10 mL) in a 100 mL two-neck flask, was followed by a few drops of DMF. After stirring for 10 min, 1 mL of oxalyl chloride in anhydrous CH_2Cl_2 (10 mL) was added dropwise during 30 min, and the mixture was stirred for 1 h at room temperature. The reaction mixture was took off the solvent, and then dissolved in dry DMF (30 mL) 4-chloroaniline (6 mmol, 1 mL) was added and the mixture was stirred at iced water, extracted with water for three times until the ether layer was colorless. And then the organic phase layer was took off the solvent, the resulting precipitate was filtered off and recrystallized from ethanol to give a green solid.

*2.1.3.1. Coumarin-3-carboxylic acid **(2)**.* A white solid; mp: 193.2–193.5 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ 12.13 (s, 1H), 8.98 (s, 1H), 8.06 (dd, J = 8.1, 1.5 Hz, 1H), 7.89 (ddd, J = 9.0, 7.5, 1.6 Hz, 1H), 7.69–7.40 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.45, 157.15, 154.93, 148.85, 134.74, 130.65, 125.27, 118.79, 118.45, 116.59; IR (KBr) ν (cm^{-1}) 3057, 1670, 1206; HR-MS (ESI): m/z calcd for $C_{10}H_6O_4$ ([M + H]$^+$) 191.0344, found 191.0336.

*2.1.3.2. 2-Oxo-2H-chromene-3-carboxylic acid phenylamide **(3a)**.* A green solid; mp: 255.4–256.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.87 (s, 1H), 9.05 (s, 1H), 7.74 (dd, J = 19.1, 7.7 Hz, 4H), 7.60–7.34 (m, 4H), 7.19 (t, J = 7.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.84, 159.30, 154.49, 148.97, 137.67, 134.38, 129.96, 129.08, 125.50, 124.83, 120.57, 118.72, 118.65, 116.74; IR (KBr) ν (cm^{-1}) 3221, 3030, 1705, 1203; HR-MS (ESI): m/z calcd for $C_{16}H_{11}NO_3$ ([M + H]$^+$) 266.0817, found 266.0808.

*2.1.3.3. 2-Oxo-2H-chromene-3-carboxylic acid o-tolylamide **(3b)**.* A green solid; mp: 232.5–233.3 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.82 (s, 1H), 9.07 (s, 1H), 8.28 (d, J = 8.0 Hz, 1H), 7.81–7.69 (m, 2H), 7.51–7.41 (m, 2H), 7.30 (d, J = 8.1 Hz, 1H), 7.26 (d, J = 7.6 Hz, 1H), 7.13 (t, J = 7.2 Hz, 1H), 2.45 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.00, 159.30, 154.48, 148.97, 136.14, 134.34, 130.50, 129.96, 128.59, 126.75, 125.49, 124.97, 121.90, 118.86, 118.75, 116.74, 18.12; IR (KBr) ν (cm^{-1}) 3276, 3051, 2920, 1699, 1201; HR-MS (ESI): m/z calcd for $C_{17}H_{13}NO_3$ ([M + H]$^+$) 280.0974, found 280.0968.

*2.1.3.4. 2-Oxo-2H-chromene-3-carboxylic acid p-tolylamide **(3c)**.* A green solid; mp: 234.7–235.4 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.80 (s, 1H), 9.05 (s, 1H), 7.81–7.69 (m, 2H), 7.65 (d, J = 8.4 Hz, 2H), 7.53–7.36 (m, 2H), 7.21 (d, J = 8.3 Hz, 2H), 2.37 (s, 3H);^{13}C NMR (100 MHz, TFA-d) δ 164.47, 154.16, 152.40, 138.20, 136.07, 131.92, 130.34, 129.61, 126.44, 122.79, 118.39, 116.42, 19.09; IR (KBr) ν

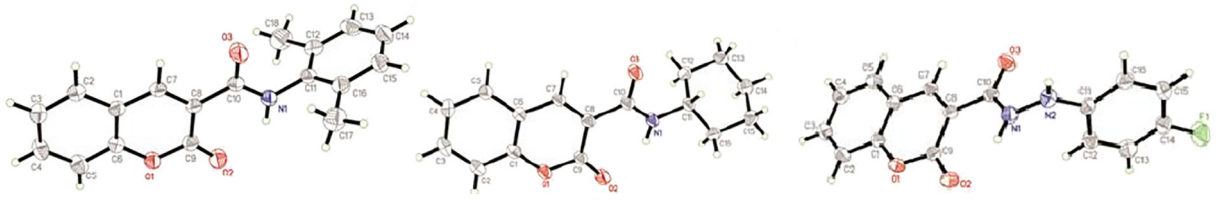


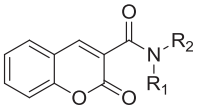
Fig. 5. X-ray single crystal structures of compounds **3d**, **3t** and **4f.**

Table 1
Antifungal activity of target compounds (inhibitory rate, %).

Species[a]		BOT	ALT	GIB	RHI	CUC	ALS
Compd	Rate (μg/mL)	100	100	100	100	100	100
2		76[b]	15	10	22	13	10[c]
3a		51	10	10	37	10	10
3b		58	10	10	30	10	10
3c		51	10	10	20	10	10
3d		51	10	10	14	10	11
3e		13	10	10	10	10	10
3f		34	10	10	17	11	10
3g		12	10	10	10	10	10
3h		10	10	10	10	10	10
3i		10	10	10	10	10	10
3j		59	10	10	32	10	10
3k		47	10	10	22	10	10
3l		41	10	10	39	30	22
3m		59	10	10	52	10	10
3n		29	10	10	10	10	10
3o		32	15	10	10	10	10
3p		11	10	10	31	10	10
3q		64	10	12	41	13	10
3r		75	10	20	34	11	13
3s		64	10	10	20	10	25
3t		54	10	10	30	10	10
3u		37	10	10	20	10	10
3v		47	10	10	17	10	10
4a		**100**[d]	15	10	**85**	**90**	10
4b		64	37	21	**88**	10	10
4c		68	53	28	72	74	30
4d		**92**	**82**	10	**100**	**100**	14
4e		**82**	75	10	**100**	10	30
4f		**82**	**100**	28	72	74	62
4g		57	29	28	52	10	79
Osthole		66	30	67	70	92	51
Boscalid		100	73	23	92	10	90

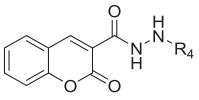
[a] BOT, Botrytis cinerea; ALT, Alternaria solani; GIB, Gibberella zeae; RHI, Rhizoctorzia solani; CUC, Cucumber anthrax; ALS, Alternaria leaf spot.
[b] All the data was the average value of three replications.
[c] 10 indicate the data 10% and below 10% inhibitory.
[d] The bold means data equal to or above 80% inhibitory.

Table 2
Structures and yields of coumarin-3-carboxamides.



Compound	R_1	R_2	Yield (%)
3a	H	Ph	48
3b	H	*o*-Methylphenyl	56
3c	H	*p*-Methylphenyl	52
3d	H	2,6-Dimethylphenyl	58
3e	H	*m*-Methoxyphenyl	56
3f	H	*p*-Methoxyphenyl	58
3g	H	*o*-Chlorophenyl	38
3h	H	*m*-Chlorophenyl	35
3i	H	*p*-Chlorophenyl	42
3j	H	2-Biphenyl	64
3k	H	1-Naphthyl	68
3l	H	Bn	60
3m	H	4-Methylbenzyl	61
3n	H	4-Methoxybenzyl	63
3o	H	2,4-Dimethoxybenzyl	59
3p	H	4-Fluorobenzyl	47
3q	Bn	Bn	71
3r	H	Me	54
3s	Et	Et	58
3t	H	Cyclohexyl	75
3u	H	Adamantyl	55
3v	H	1-Methyl-1*H*-pyrazol-3-yl	42

Table 3
Structures and yields of coumarin-3-carboxhydrazides.



Compound	R_4	Yield (%)
4a	*o*-Chlorophenyl	68
4b	*m*-Chlorophenyl	54
4c	*p*-Chlorophenyl	48
4d	*o*-Fluorophenyl	52
4e	*m*-Fluorophenyl	43
4f	*p*-Fluorophenyl	72
4g	*p*-Methoxyphenyl	66

(cm^{-1}) 3271, 2918, 1701, 1200; HR-MS (ESI): *m/z* calcd for $C_{17}H_{13}NO_3$ ($[M + H]^+$) 280.0974, found 280.0966.

*2.1.3.5. 2-Oxo-2H-chromene-3-carboxylic acid (2,6-dimethyl-phenyl)-amide (**3d**).* A white solid; mp: 222.6–223.8 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.20 (s, 1H), 9.06 (s, 1H), 7.79–7.69 (m, 2H), 7.45 (ddd, J = 11.7, 8.5, 4.7 Hz, 2H), 7.20–7.12 (m, 3H), 2.32 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.89, 160.38, 154.42, 147.77, 135.36, 134.86, 134.61, 130.70, 128.27, 127.26, 125.68, 120.44, 118.95, 116.69, 18.71; IR (KBr) ν (cm^{-1}) 3306, 3098, 2930, 1701, 1155; HR-MS (ESI): m/z calcd for $C_{18}H_{15}NO_3$ ($[M + H]^+$) 294.1130, found 294.1123.

*2.1.3.6. 2-Oxo-2H-chromene-3-carboxylic acid (3-methoxy-phenyl)-amide (**3e**).* A yellow solid; mp: 186.6–187.3 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.66 (s, 1H), 8.93 (s, 1H), 8.02 (d, J = 7.7 Hz, 1H), 7.85–7.74 (m, 1H), 7.57 (d, J = 8.3 Hz, 1H), 7.48 (t, J = 7.5 Hz, 1H), 7.43 (s, 1H), 7.33–7.22 (m, 2H), 6.74 (dd, J = 7.9, 1.1 Hz, 1H), 3.78 (s, 3H); ^{13}C NMR (100 MHz, TFA-d) δ 164.45, 162.29, 161.86, 161.78, 161.49, 161.42, 161.35, 160.98, 158.41, 154.15, 152.59, 136.27, 136.10, 130.33, 130.19, 130.09, 126.42, 116.37, 108.93, 55.27; IR (KBr) ν (cm^{-1}) 3273, 3046, 2962, 1699, 1234; HR-MS (ESI): *m/z* calcd for $C_{17}H_{13}NO_4$ ($[M + Na]^+$) 318.0743, found 318.0733.

*2.1.3.7. 2-Oxo-2H-chromene-3-carboxylic acid (4-methoxy-phenyl)-amide (**3f**).* A yellow solid; mp: 221.4–221.9 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ

Table 4
EC_{50} determination of compounds 4a-4g.

Pathogen	Compound	Toxic regression	R	EC_{50} (μg/mL)	95% Confidence interval
Botrytis cinerea	**4a**	Y = 4.7001 + 1.5332x	0.9998	1.5690	1.4508–1.6968
	4b	Y = 3.5532 + 2.0429x	0.9948	5.1074	4.0956–6.3691
	4c	Y = 3.7370 + 1.3387x	0.9945	8.7792	3.7370–6.9622
	4d	Y = 4.0105 + 1.9952x	0.9938	3.1327	2.2587–4.3450
	4e	Y = 4.8562 + 0.6595x	0.9993	1.6523	1.4167–1.9271
	4f	Y = 4.4011 + 1.2183x	0.9961	3.1014	2.8115–3.4212
	4g	Y = 3.3664 + 1.7554x	0.9986	8.5235	7.9700–9.1154
	Osthole	Y = 3.7194 + 1.1772x	0.9959	12.2400	10.3776–14.4366
	Boscalid	Y = 4.0833 + 1.7989x	0.9814	0.5096	0.3924–0.6269
Alternaria solani	**4d**	Y = 4.6597 + 0.7402x	0.9955	2.8822	2.2341–3.7182
	4e	Y = 4.8753 + 0.4022x	0.9978	2.0424	1.7411–2.3959
	4f	Y = 2.4088 + 3.2917x	1.0000	6.1265	6.0764–6.1771
	Boscalid	Y = 3.5223 + 1.0390x	0.9996	25.4399	25.5210–27.3918
Rhizoctorzia solani	**4a**	Y = 4.7709 + 0.8944x	0.9996	1.8034	1.6129–2.0164
	4b	Y = 4.7849 + 0.4068x	0.9997	3.3787	3.1774–3.5927
	4c	Y = 4.8026 + 0.3899x	0.9998	3.2079	3.1945–3.2214
	4d	Y = 4.1872 + 2.0445x	0.9962	2.4977	2.07533.0061
	4e	Y = 4.3434 + 1.8676x	0.9923	2.2468	1.6879–2.9908
	4f	Y = 4.7826 + 0.6765x	0.9960	2.0961	1.5970–2.7513
	Boscalid	Y = 4.0772 + 1.9480x	0.9950	2.9767	2.4754–3.5796
Cucumber anthrax	**4a**	Y = 3.9617 + 1.1657x	0.9908	7.7756	5.6352–10.7289
	4d	Y = 4.3293 + 1.7802x	0.9955	2.3810	2.1493–2.6376
	4e	Y = 4.1668 + 1.3828x	0.9727	3.9468	2.8761–5.4656
	4f	Y = 2.2553 + 2.3373x	0.9909	1.1743	1.0858–1.2627
	Boscalid	Y = −1.0626 + 1.7594x	0.9887	172.9260	95.0799–314.5081
Alternaria leaf spot	**4f**	Y = −4.1368 + 1.4440x	0.9974	3.9606	3.6468–4.3014
	4g	Y = −3.4533 + 1.4168x	0.9949	12.3503	10.3579–14.7260
	Boscalid	Y = 5.5725 + 1.2370x	0.9966	0.3445	0.3013–0.3939

All the data was the average value of three replications.

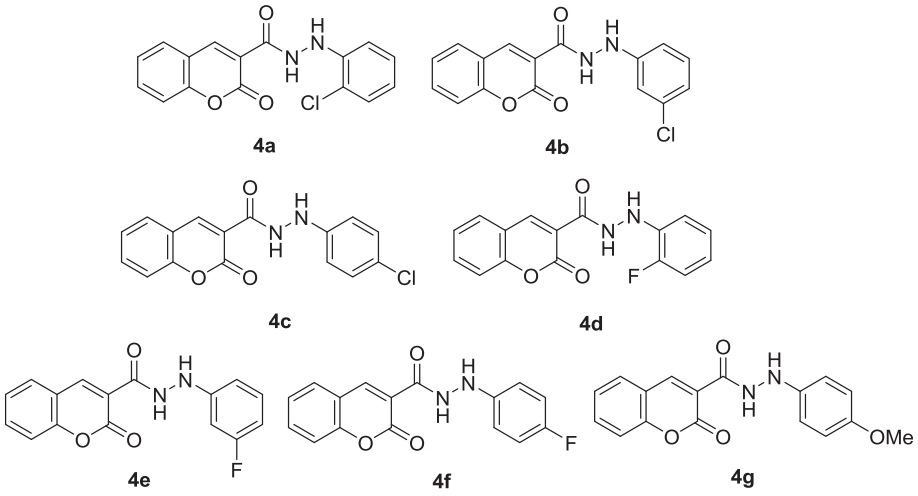


Fig. 6. Structures of the most active synthetic compounds.

10.75 (s, 1H), 9.03 (s, 1H), 7.79–7.70 (m, 2H), 7.68 (d, J = 9.0 Hz, 2H), 7.45 (dd, J = 18.2, 8.0 Hz, 2H), 6.94 (d, J = 8.9 Hz, 2H), 3.84 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.93, 161.75, 160.76, 154.36, 148.04, 135.70, 135.67, 134.56, 130.73, 129.98, 129.89, 125.59, 119.51, 118.93, 116.60, 115.65, 115.44, 42.51; IR (KBr) ν (cm^{-1}) 3277, 3054, 2840, 1698, 1237; HR-MS (ESI): m/z calcd for $C_{17}H_{13}NO_4$ ($[M + H]^+$) 296.0923, found 296.0915.

*2.1.3.8. 2-Oxo-2H-chromene-3-carboxylic acid (2-chloro-phenyl)-amide (**3g**).* A white solid; mp: 234.9–235.2 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.36 (s, 1H), 9.05 (s, 1H), 8.65–8.53 (m, 1H), 7.75 (dd, J = 17.4, 7.7 Hz, 2H), 7.51–7.41 (m, 3H), 7.35 (t, J = 7.8 Hz, 1H), 7.16–7.09 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.55, 159.63, 154.61, 149.21, 149.17, 135.03, 134.53, 134.50, 130.00, 129.37, 127.52, 125.48, 125.46, 125.22, 124.22, 122.32, 118.64, 118.59, 116.82; IR (KBr) ν (cm^{-1}) 3190, 3052, 1706, 643; HR-MS (ESI): m/z calcd for $C_{16}H_{10}ClNO_3$ ($[M + H]^+$) 300.0427, found 300.0417.

*2.1.3.9. 2-Oxo-2H-chromene-3-carboxylic acid (3-chloro-phenyl)-amide (**3h**).* A green solid; mp: 237.0–238.1 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.92 (s, 1H), 9.04 (s, 1H), 7.93 (s, 1H), 7.76 (dd, J = 17.0, 7.7 Hz, 2H), 7.58 (d, J = 8.2 Hz, 1H), 7.51–7.42 (m, 2H), 7.31 (dd, J = 15.8, 7.7 Hz, 2H), 7.16 (d, J = 8.0 Hz, 1H); ^{13}C NMR (100 MHz, TFA-d) δ 164.60, 154.18, 152.74, 136.17, 135.97, 135.06, 130.33, 129.93, 127.00, 126.47, 122.53, 120.41, 116.4; IR (KBr) ν (cm^{-1}) 3182, 3056, 1693, 1201, 645; HR-MS (ESI): m/z calcd for $C_{16}H_{10}ClNO_3$ ($[M + H]^+$) 300.0427, found 300.0416.

*2.1.3.10. 2-Oxo-2H-chromene-3-carboxylic acid (4-chloro-phenyl)-amide (**3i**).* A green solid; mp: 261.6–262.4 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ

10.90 (s, 1H), 9.04 (s, 1H), 7.75 (dd, J = 17.8, 8.2 Hz, 4H), 7.46 (dd, J = 16.1, 8.2 Hz, 2H), 7.37 (d, J = 8.8 Hz, 2H); ^{13}C NMR (100 MHz, TFA-d) δ 164.60, 154.17, 152.66, 136.16, 133.38, 132.94, 130.33, 129.12, 126.48, 123.62, 118.35, 116.40; IR (KBr) ν (cm^{-1}) 3191, 3052, 1694, 639; HR-MS (ESI): m/z calcd for $C_{16}H_{10}ClNO_3$ ($[M + H]^+$) 300.0427, found 300.0416.

2.1.3.11. 2-Oxo-2H-chromene-3-carboxylic acid biphenyl-2-ylamide ***(3j)***. A yellow solid; mp: 200.9–201.6 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.62 (s, 1H), 8.99 (s, 1H), 8.39 (d, J = 7.5 Hz, 1H), 8.04–7.97 (m, 1H), 7.78–7.70 (m, 1H), 7.52–7.37 (m, 8H), 7.33–7.20 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.93, 160.32, 160.12, 154.36, 147.97, 139.54, 134.78, 130.78, 130.31, 125.76, 120.30, 118.96, 116.72, 112.62, 110.27, 106.10, 55.57; IR (KBr) ν (cm^{-1}) 3225, 3052, 1702, 1203; HR-MS (ESI): m/z calcd for $C_{22}H_{15}NO_3$ ($[M + H]^+$) 342.1130, found 342.1122.

2.1.3.12. 2-Oxo-2H-chromene-3-carboxylic acid naphthalen-1-ylamide ***(3k)***. A yellow solid; mp: 228.6–229.0 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.52 (s, 1H), 9.13 (s, 1H), 8.45 (d, J = 7.0 Hz, 1H), 8.21 (d, J = 8.5 Hz, 1H), 7.92 (d, J = 8.1 Hz, 1H), 7.82–7.72 (m, 3H), 7.64 (ddd, J = 8.4, 6.9, 1.3 Hz, 1H), 7.60–7.44 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 148.66, 130.96, 129.17, 127.14, 126.76, 126.32, 126.23, 125.86, 119.13; IR (KBr) ν (cm^{-1}) 3248, 3055, 1703, 1207; HR-MS (ESI): m/z calcd for $C_{20}H_{13}NO_3$ ($[M + H]^+$) 316.0974, found 316.0966.

2.1.3.13. 2-Oxo-2H-chromene-3-carboxylic acid benzylamide ***(3l)***. A yellow solid; mp: 139.4–140.2 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.21 (s, 1H), 8.98 (s, 1H), 7.71 (dd, J = 16.5, 7.7 Hz, 2H), 7.47–7.34 (m, 6H), 4.66 (dd, J = 24.9, 6.1 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.55, 161.43, 154.45, 148.61, 137.89, 134.12, 129.84, 128.73, 127.73, 127.48, 125.33, 118.64, 118.41, 116.66, 43.88; IR (KBr) ν (cm^{-1}) 3329, 3055, 1700, 1244; HR-MS (ESI): m/z calcd for $C_{17}H_{13}NO_3$ ($[M + H]^+$) 280.0974, found 280.0965.

2.1.3.14. 2-Oxo-2H-chromene-3-carboxylic acid 4-methyl-benzylamide ***(3m)***. A white solid; mp: 136.8–137.9 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.05 (t, J = 5.8 Hz, 1H), 8.87 (s, 1H), 8.03–7.92 (m, 1H), 7.80–7.66 (m, 1H), 7.55–7.36 (m, 2H), 7.23 (d, J = 7.9 Hz, 2H), 7.13 (d, J = 7.9 Hz, 2H), 4.48 (d, J = 5.9 Hz, 2H), 2.26 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.62, 154.36, 148.00, 136.56, 136.30, 134.57, 130.74, 129.43, 127.91, 125.61, 119.56, 118.97, 116.62, 42.98, 21.16; IR (KBr) ν (cm^{-1}) 3318, 3053, 2920, 1704, 1244; HR-MS (ESI): m/z calcd for $C_{18}H_{15}NO_3$ ($[M + Na]^+$) 316.0950, found 316.0941.

2.1.3.15. 2-Oxo-2H-chromene-3-carboxylic acid 4-methoxy-benzylamide ***(3n)***. A white solid; mp: 145.1–145.7 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.04 (t, J = 5.4 Hz, 1H), 8.88 (s, 1H), 7.99 (d, J = 7.7 Hz, 1H), 7.76 (t, J = 7.8 Hz, 1H), 7.51 (d, J = 8.4 Hz, 1H), 7.45 (t, J = 7.5 Hz, 1H), 7.29 (d, J = 8.2 Hz, 2H), 6.91 (d, J = 8.2 Hz, 2H), 4.47 (d, J = 5.8 Hz, 2H), 3.74 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.53, 160.84, 158.83, 154.34, 147.99, 134.55, 131.25, 130.72, 129.36, 125.60, 119.52, 118.95, 116.60, 114.27, 55.53, 42.71; IR (KBr) ν (cm^-1) 3311, 3060, 2838, 1721, 1248; HR-MS (ESI): m/z calcd for $C_{18}H_{15}NO_4$ ($[M + Na]^+$) 332.0899, found 332.0893.

2.1.3.16. 2-Oxo-2H-chromene-3-carboxylic acid 2,4-dimethoxy-benzylamide ***(3o)***. A white solid; mp: 142.9–143.7 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.03 (s, 1H), 8.89 (s, 1H), 7.99 (d, J = 7.7 Hz, 1H), 7.76 (t, J = 7.8 Hz, 1H), 7.51 (d, J = 8.3 Hz, 1H), 7.45 (t, J = 7.5 Hz, 1H), 7.17 (d, J = 8.2 Hz, 1H), 6.60 (s, 1H), 6.49 (d, J = 8.2 Hz, 1H), 4.43 (d, J = 5.6 Hz, 2H), 3.84 (s, 3H), 3.75 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.27, 161.05, 160.56, 158.72, 154.39, 148.14, 133.85, 130.28, 129.74, 125.18, 118.81, 118.68, 118.56, 116.57, 103.86, 98.61, 55.39, 55.27, 39.38; IR (KBr) ν (cm^{-1}) 3333, 3054, 2834, 1703, 1160; HR-MS (ESI): m/z calcd for $C_{19}H_{17}NO_5$ ($[M + Na]^+$) 362.1005, found 362.0993.

2.1.3.17. 2-Oxo-2H-chromene-3-carboxylic acid 4-fluoro-benzylamide ***(3p)***. A white solid; mp: 175.6–176.4 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.15 (s, 1H), 8.88 (s, 1H), 7.99 (d, J = 7.6 Hz, 1H), 7.76 (t, J = 7.8 Hz, 1H), 7.52 (d, J = 8.3 Hz, 1H), 7.48–7.36 (m, 3H), 7.17 (t, J = 8.6 Hz, 2H), 4.53 (d, J = 5.9 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.51 (d, J = 13.1 Hz), 154.45, 148.69, 134.20, 133.76 (d, J = 3.2 Hz), 129.85, 129.44 (d, J = 8.1 Hz), 125.36, 118.60, 118.30, 116.66, 115.65, 115.44, 43.14; IR(KBr) ν (cm^{-1}) 3328, 3051, 2944, 1700, 1157; HR-MS (ESI): m/z calcd for $C_{17}H_{12}FNO_3$ ($[M + H]^+$) 298.0879, found 298.0871.

2.1.3.18. 2-Oxo-2H-chromene-3-carboxylic acid dibenzylamide ***(3q)***. A white solid; mp: 143.6–144.2 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.29 (s, 1H), 7.73 (dd, J = 7.8, 1.4 Hz, 1H), 7.65 (ddd, J = 8.8, 7.4, 1.6 Hz, 1H), 7.34 (dddd, J = 11.7, 11.0, 7.7, 5.6 Hz, 10H), 7.22–7.16 (m, 2H), 4.51 (d, J = 20.0 Hz, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 142.24, 136.90, 136.89, 133.12, 129.42, 129.07, 129.01, 128.02, 127.89, 127.05, 125.47, 125.29, 118.84, 116.79, 51.60, 47.10; IR (KBr) ν (cm^{-1}) 3031, 2923, 1709, 1226; HR-MS (ESI): m/z calcd for $C_{24}H_{19}NO_3$ ($[M + H]^+$) 370.1443, found 370.1436.

2.1.3.19. 2-Oxo-2H-chromene-3-carboxylic acid methylamide ***(3r)***. A white solid; mp: 168.7–169.8 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.85 (s, 1H), 8.62 (d, J = 4.3 Hz, 1H), 7.98 (dd, J = 7.7, 1.6 Hz, 1H), 7.78–7.67 (m, 1H), 7.44 (ddd, J = 11.2, 8.4, 4.7 Hz, 2H), 2.84 (d, J = 4.8 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.04, 160.72, 154.31, 147.71, 134.45, 130.70, 125.56, 119.45, 118.93, 116.57, 26.79; IR (KBr) ν (cm^{-1}) 3352, 3057, 2943, 1692, 1242; HR-MS (ESI): m/z calcd for $C_{11}H_9NO_3$ ($[M + H]^+$) 204.0661, found 204.0654.

2.1.3.20. 2-Oxo-2H-chromene-3-carboxylic acid diethylamide ***(3s)***. A white solid; mp: 69.6–71.1 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, J = 5.1 Hz, 1H), 7.60–7.55 (m, 1H), 7.53 (d, J = 7.7 Hz, 1H), 7.37–7.27 (m, 2H), 3.55 (q, J = 7.1 Hz, 2H), 3.31 (q, J = 7.1 Hz, 2H), 1.26 (t, J = 7.1 Hz, 3H), 1.18 (t, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.15, 158.28, 153.80, 141.28, 132.96, 129.37, 125.96, 125.32, 118.84, 116.76, 42.99, 39.08, 14.48, 13.17; IR (KBr) ν (cm^{-1}) 3056, 2943, 1711, 1210; HR-MS (ESI): m/z calcd for $C_{14}H_{15}NO_3$ ($[M + H]^+$) 246.1130, found 246.1121.

2.1.3.21. 2-Oxo-2H-chromene-3-carboxylic acid cyclohexylamide ***(3t)***. A white solid; mp: 176.2–177.3 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.87 (s, 1H), 8.62 (d, J = 7.7 Hz, 1H), 8.00 (dd, J = 7.8, 1.2 Hz, 1H), 7.84–7.66 (m, 1H), 7.52 (d, J = 8.3 Hz, 1H), 7.45 (t, J = 7.5 Hz, 1H), 3.82 (d, J = 4.3 Hz, 1H), 1.86 (d, J = 9.5 Hz, 2H), 1.69 (dd, J = 9.2, 4.0 Hz, 2H), 1.57 (d, J = 11.9 Hz, 1H), 1.50–0.84 (m, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.14, 160.52, 154.29, 147.81, 134.50, 130.71, 125.61, 119.51, 118.97, 116.60, 48.22, 32.54, 25.56, 24.67; IR (KBr) ν (cm^{-1}) 3320, 3051, 2851, 1703, 1248; HR-MS (ESI): m/z calcd for $C_{16}H_{17}NO_3$ ($[M + H]^+$) 272.1287, found 272.1279.

2.1.3.22. 2-Oxo-2H-chromene-3-carboxylic acid adamantan-1-ylamide ***(3u)***. A white solid; mp: 214.5–215.0 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.88 (s, 1H), 8.69 (s, 1H), 7.67 (dd, J = 14.7, 7.8 Hz, 2H), 7.40 (dd, J = 17.0, 8.2 Hz, 2H), 2.16 (s, 9H), 1.80–1.69 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.65, 159.92, 154.37, 147.68, 133.73, 129.69, 125.17, 119.63, 118.79, 116.54, 52.32, 41.41, 36.41, 29.45; IR (KBr) ν (cm^{-1}) 3353, 3055, 2900, 1694, 1242; HR-MS (ESI): m/z calcd for $C_{20}H_{21}NO_3$ ($[M + Na]^+$) 346.1420, found 346.1409.

2.1.3.23. 2-Oxo-2H-chromene-3-carboxylic acid (1-methyl-1H-pyrazol-3-yl)-amide ***(3v)***. A green solid; mp: 248.0–249.1 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.89 (s, 1H), 8.96 (s, 1H), 8.02 (dd, J = 7.8, 1.5 Hz, 1H),

7.78 (ddd, J = 8.8, 7.4, 1.6 Hz, 1H), 7.64 (d, J = 2.2 Hz, 1H), 7.57–7.42 (m, 2H), 6.60 (d, J = 2.2 Hz, 1H), 3.77 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.44, 158.84, 154.38, 148.35, 146.16, 134.89, 132.02, 130.88, 125.79, 119.18, 119.00, 116.72, 97.19; IR (KBr) ν (cm^{-1}) 3262, 3132, 1693, 1693, 1123; HR-MS (ESI): m/z calcd for $C_{14}H_{11}O_3N_3$ ($[M + Na]^+$) 292.0699, found 292.0688.

2.1.3.24. 2-Oxo-2H-chromene-3-carboxylic acid N′-(2-chloro-phenyl)-hydrazide ***(4a)***. A yellow solid; mp: 198.2–199.0 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.24 (s, 1H), 8.81 (s, 1H), 8.01 (dd, J = 7.7, 1.2 Hz, 1H), 7.79 (dd, J = 10.6, 3.4 Hz, 2H), 7.55 (d, J = 8.3 Hz, 1H), 7.47 (t, J = 7.6 Hz, 1H), 7.33 (dd, J = 7.9, 1.1 Hz, 1H), 7.18 (t, J = 7.4 Hz, 1H), 6.98–6.91 (m, 1H), 6.84–6.76 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.31, 160.09, 154.30, 153.40, 147.35, 142.97, 134.51, 130.58, 125.60, 120.23, 118.79, 116.66, 114.73, 114.41, 55.73, 31.15; IR (KBr) ν (cm^{-1}) 3348, 3312, 3045, 1718, 1177; HR-MS (ESI): m/z calcd for $C_{16}H_{11}ClN_2O_3$ ($[M + Na]^+$) 337.0356, found 337.0345.

2.1.3.25. 2-Oxo-2H-chromene-3-carboxylic acid N′-(3-chloro-phenyl)-hydrazide ***(4b)***. An orange solid; mp: 230.2–231.7 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.37 (s, 1H), 8.95 (s, 1H), 7.75 (ddd, J = 7.4, 4.2, 1.5 Hz, 2H), 7.53–7.40 (m, 2H), 7.19 (t, J = 8.0 Hz, 1H), 6.93 (dd, J = 7.0, 5.2 Hz, 2H), 6.86–6.80 (m, 1H), 6.44 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.59, 159.93, 154.36, 150.88, 147.47, 134.59, 134.03, 130.89, 130.62, 125.62, 120.27, 118.78, 118.75, 116.69, 112.11, 111.52; IR (KBr) ν (cm^{-1}) 3398, 3230, 3067, 1726, 1160; HR-MS (ESI): m/z calcd for $C_{16}H_{11}ClN_2O_3$ ($[M + H]^+$) 315.0536, found 315.0529.

2.1.3.26. 2-Oxo-2H-chromene-3-carboxylic acid N′-(4-chloro-phenyl)-hydrazide ***(4c)***. A yellow solid; mp: 207.4–208.3 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.14 (d, J = 1.7 Hz, 1H), 8.79 (s, 1H), 8.26 (d, J = 1.9 Hz, 1H), 7.99 (d, J = 7.7 Hz, 1H), 7.76 (q, J = 7.7 Hz, 1H), 7.54 (d, J = 8.4 Hz, 1H), 7.46 (t, J = 7.5 Hz, 1H), 7.21 (d, J = 8.3 Hz, 2H), 6.84 (d, J = 8.3 Hz, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.53, 159.93, 154.35, 148.23, 147.46, 134.59, 130.61, 129.01, 125.63, 122.65, 120.24, 118.76, 116.70, 114.36; IR (KBr) ν (cm^{-1}) 3306, 3226, 3041, 1724, 1169, 642; HR-MS (ESI): m/z calcd for $C_{16}H_{11}ClN_2O_3$ ($[M + Na]^+$) 337.0356, found 337.0346.

2.1.3.27. 2-Oxo-2H-chromene-3-carboxylic acid N′-(2-fluoro-phenyl)-hydrazide ***(4d)***. An orange solid; mp: 208.8–209.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.37 (s, 1H), 8.95 (s, 1H), 7.78–7.71 (m, 2H), 7.45 (ddd, J = 10.3, 8.3, 4.9 Hz, 2H), 7.04 (qdd, J = 15.4, 7.9, 1.3 Hz, 3H), 6.89 (dddd, J = 9.5, 7.3, 4.9, 2.0 Hz, 1H), 6.58 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.54, 160.00, 154.37, 147.62, 136.79 (d, J = 10.6 Hz), 134.64, 130.66, 125.65, 125.02 (d, J = 3.1 Hz), 120.13, 119.62 (d, J = 6.8 Hz), 118.76, 116.71, 115.40 (d, J = 17.7 Hz), 114.42; IR (KBr) ν (cm^{-1}) 3333, 3043, 1710, 1191; HR-MS (ESI): m/z calcd for $C_{16}H_{11}FN_2O_3$ ($[M + H]^+$) 299.0832, found 299.0825.

2.1.3.28. 2-Oxo-2H-chromene-3-carboxylic acid N′-(3-fluoro-phenyl)-hydrazide ***(4e)***. An orange solid; mp: 218.1–219.2 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.15 (d, J = 2.6 Hz, 1H), 8.79 (s, 1H), 8.38 (d, J = 2.4 Hz, 1H), 7.99 (dd, J = 7.8, 1.4 Hz, 1H), 7.82–7.71 (m, 1H), 7.54 (d, J = 8.4 Hz, 1H), 7.49–7.43 (m, 1H), 7.19 (dd, J = 14.9, 8.1 Hz, 1H), 6.66 (dd, J = 8.1, 1.5 Hz, 1H), 6.60 (dt, J = 11.7, 2.2 Hz, 1H), 6.52 (td, J = 8.3, 2.1 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.90, 162.51, 159.92, 154.34, 151.49 (d, J = 10.4 Hz), 147.35, 134.55, 130.84 (d, J = 9.9 Hz), 130.59, 125.62, 120.38, 118.77, 116.70, 108.89, 105.35 (d, J = 21.3 Hz), 99.38 (d, J = 25.8 Hz); IR (KBr) ν (cm^{-1}) 3288, 3051, 1703, 1176; HR-MS (ESI): m/z calcd for $C_{16}H_{11}FN_2O_3$ ($[M + H]^+$) 299.0832, found 299.0822.

2.1.3.29. 2-Oxo-2H-chromene-3-carboxylic acid N′-(3-fluoro-phenyl)-hydrazide ***(4f)***. An orange solid; mp: 196.1–196.5 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.15 (d, J = 2.6 Hz, 1H), 8.79 (s, 1H), 8.38 (d, J = 2.4 Hz, 1H), 7.99 (dd, J = 7.8, 1.4 Hz, 1H), 7.82–7.71 (m, 1H), 7.54 (d, J = 8.4 Hz, 1H), 7.49–7.43 (m, 1H), 7.19 (dd, J = 14.9, 8.1 Hz, 1H), 6.66 (dd, J = 8.1, 1.5 Hz, 1H), 6.60 (dt, J = 11.7, 2.2 Hz, 1H), 6.52 (td, J = 8.3, 2.1 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.52, 159.97, 157.68, 155.35, 154.33, 147.36, 145.75, 134.55, 130.59, 125.63, 120.32, 118.78, 116.69, 115.80, 115.58, 114.11 (d, J = 7.6 Hz); IR (KBr) ν (cm^{-1}) 3353, 3320, 3052, 1704, 1203; HR-MS (ESI): m/z calcd for $C_{16}H_{11}ClN2O_3$ ($[M + H]^+$) 299.0832, found 299.0820.

2.1.3.30. 2-Oxo-2H-chromene-3-carboxylic acid N′-(4-methoxy-phenyl)-hydrazide ***(4g)***. An orange solid; mp: 173.2–174.5 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.07 (d, J = 3.6 Hz, 1H), 8.78 (s, 1H), 7.99 (dd, J = 7.8, 1.2 Hz, 1H), 7.80–7.74 (m, 2H), 7.53 (d, J = 8.3 Hz, 1H), 7.46 (t, J = 7.5 Hz, 1H), 6.80 (s, 4H), 3.68 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.43, 159.97, 154.38, 147.74, 144.65, 134.69, 130.69, 129.69, 128.25, 125.66, 120.37, 120.02, 118.75, 117.90, 116.71, 113.77, 31.17; IR (KBr) ν (cm^{-1}) 3331, 3285, 3057, 2839, 1709, 1030; HR-MS (ESI): m/z calcd for $C_{17}H_{14}N_2O_4$ ($[M + Na]^+$) 333.0852, found 333.0840.

2.2. X-ray diffraction analysis

Among the synthesized compounds, the crystal structures of compounds **3d**, **3t** and **4f** were determined by X-ray diffraction analysis. The crystallographic data have been deposited with the Cambridge Crystallographic Data Centre (CCDC) as supplementary publication number: CCDC 1511231 (compound **3d**), CCDC 1511235 (compound **3t**), CCDC 1511236 (compound **4f**), copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB12 1EZ, UK (Fax +441,223,336,033 or E-mail: deposit@ccdc.cam.ac.uk). The structures are shown in Fig. 5.

2.3. Biological assays

The antifungal activities of the synthesized compounds were tested in vitro against six phytopathogenic fungi (*Botrytis cinerea*, *Alternaria solani*, *Gibberella zeae*, *Rhizoctorzia solani*, *Cucumber anthrax*, *Alternaria* leaf spot) at the concentration of 100 μg/mL using mycelia growth inhibitory rate methods on PDA, with Osthole and Boscalid used as the positive control. The six phytopathogenic fungi were provided by the Laboratory of Plant Disease Control, Nanjing Agricultural University, and they are representative fungal strains in the outbreaks region in China and USA, the detailed procedure of experimental methods for the antifungal activity refers to the paper from Department of Plant Pathology, Nanjing Agriculture University [27]. The compounds were dissolved in DMF to generate a stock solution. The compounds possessing good activity (inhibitory rate>70% at 100 μg/mL) were further evaluated using different concentration by diluting the above solution. DMF served as the negative control. The antifungal results of all the compounds against *Botrytis cinerea*, *Alternaria solani*, *Gibberella zeae*, *Rhizoctorzia solani*, *Cucumber anthrax* and *Alternaria* leaf spot are listed in Table 1.

3. Results and discussion

3.1. Synthetic chemistry

Due to the problems of time consuming, not environment friendly, and complex post-processing, most conventional carboxylic acid and amine acylation reactions are not satisfactory. In recent years, however, there are plenty of condensation reagents becoming more and more popular in modern combinatorial and medicinal chemistry, such as T_3P and EDCI. T_3P has the good selectivity, mild conditions, simple post-

processing and pollution-free which is our first choice to generate compounds **3a-3f**, **3j-3v**. Nonetheless, to increase yield, EDCI is used to generate compounds **4a-4g**. Furthermore, compared with the modern condensation reagents, conventional acylation reaction reactivity is higher. It is beneficial for synthesis of carboxylic acid and aniline bearing electron-withdrawing groups (compounds **3g-3i**).

With the efficient method, we synthesized all target compounds listed in Tables 2–3, and gave satisfactory analytical and spectroscopic data (^{1}H NMR, ^{13}C NMR, HRMS, IR), which were full accordance with their depicted structures, and the structures of three typical compounds **3d**, **3t** and **4f** were further confirmed by X-ray crystallographic diffraction analysis (Fig. 5).

3.2. Antifungal activity and the structure-activity relationships (SAR)

The results of the biological testing against six phytopathogenic fungi are given in Table 1. For the purposes of the structure-activity relationship analysis, the antifungal activity of all target compounds were compared with the positive controls, including Osthole, and Boscalid, a broad spectrum fungicide used for protecting plants and food crops from fungal diseases.

In general, data presented in Table 1 indicated that the synthesized compounds exhibit certain activities at 100 μg/mL, while most of the target compounds showed rather poor activity against all fungi.

As seven compounds **4a-4 g** showed relatively effective control against *Botrytis cinerea*, *Colletotrichum capsica*, *Rhizoctorzia solani*, *Cucumber anthrax and Alternaria* leaf spot, therefore, we further tested the EC_{50} values of these compounds together with Boscalid. As shown in Table 4, it was noticed that the EC_{50} values of compounds **4a** and **4e** were as low as 1.57 μg/mL and 1.65 μg/mL, respectively against *Botrytis cinerea*, which proves they are equivalent to Boscalid (0.51 μg/mL). Meanwhile, compound **4a** (1.80 μg/mL) exhibited much more effective activity than the control Boscalid (2.98 μg/mL) against *Rhizoctorzia solani*.

Although it is difficult to extract clear structure-activity relationships from the presented biological data, the conclusion still can be drawn is that the spectrum of antifungal activity is improved by replacing the amide bond by the hydrazide bond, or even displayed a broader spectrum of activity. Firstly, these coumarin amide compounds were noticeably more active against *Botrytis cinerea* and *Rhizoctorzia solani*, but they usually lacked potency against *Alternaria solani*, *Gibberella zeae*, *Cucumber anthrax* and *Alternaria* leaf spot. Secondly, it is noticeable that almost all the electron-withdrawing groups substituted on the phenylhydrazine of compounds **4a-4f** are inactive, while electron-donating group substituted compound **4g** is active against *Alternaria* leaf spot. Furthermore, the introduction of meta-F/Cl substituted phenylhydrazine to the coumarin core afforded active drugs such as compounds **4e** and **4e** against *Cucumber anthrax*.

Totally, compounds **4a** and **4e** showed effective control to *Botrytis cinerea*, and compounds **4a-4g** displayed a broader spectrum of activity in vitro (Fig. 6).

4. Conclusions

In conclusion, a series of coumarin-3-carboxamides/hydrazides were designed, synthesized and evaluated for their antifungal activity against six important phytopathogens. The in vitro assessment of target compounds revealed that most of compounds showed certain biological activity, compounds **4a-4g** were identified as the most active ones among the target compounds (Fig. 4), the EC_{50} values of these active compounds together with Boscalid were further tested. Seven most active compounds **4a-4g** were identified as the most promising candidates for further study. Further structural optimization of coumarin-based hydrazides is well under way, alongside more detailed biological testing of the most active compounds, aiming to improve their levels of antifungal activity.

Conflict of interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

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