Design, Synthesis and Evaluate *In Vitro* Antifungal Activity of Novel Benzamide Derivatives

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Summary: A series of novel benzamide derivatives according to fluopicolide were designed and synthesized following the rule of combination carboxylic acid amides and amines derivatives together. The antifungal activity of the 15 new compounds were evaluated *in vitro* against five pathogenic fungi, including *Sclerotinia sclerotiorum*, *Gibberella zeae*, *Rhizoctonia solani*, *Helminthosporium maydis* and *Botrytis cinerea*. Almost all the structure have not been reported, except compounds **3**, **5** and **6**. A surprising finding is that all the five tested fungi breed faster than negative controls when supplementary with compound **7–15** , respectively.

Keywords: Design, Synthesis, Fluopicolide, Benzamide derivatives, Antifungal activity.

Introduction

Carboxylic acid amide fungicides play an important role in the fields of pesticide. Since carboxin was firstly been discovered by Schmeling and Kulla [1], numerous carboxylic acid amide fungicides with new structures have emerged consecutively [2]. One of representatives is fluopicolide (Fig. 1), which is developed by Bayer Company, Germany. Ever since fluopicolide was invented as a new fungicide, it has occupied crucial adjective role in the agricultural field and has various applications [3–5]. In consistent with other carboxylic acid amide fungicides, fluopicolide has the common peculiarity that almost all of them can affect the growth of pathogens and cause their eventually death by interfering with the respiration of pathogens [6, 7].

On the other hand, heterocyclic/homocyclic compounds are very important in the course of researches and developments of fungicides. They have a great diversity of antimicrobial activity, including antifungal and antibacterial activity [8–12]. When used as fungicides, these heterocyclic/homocyclic compounds possess many advantages, such as good selectivity [13–17], excellent activity [18– 21], low toxicity and special mode of action [6]. Therefore, heterocyclic/homocyclic compounds have huge potentials in development novel carboxylic acid amide fungicides.
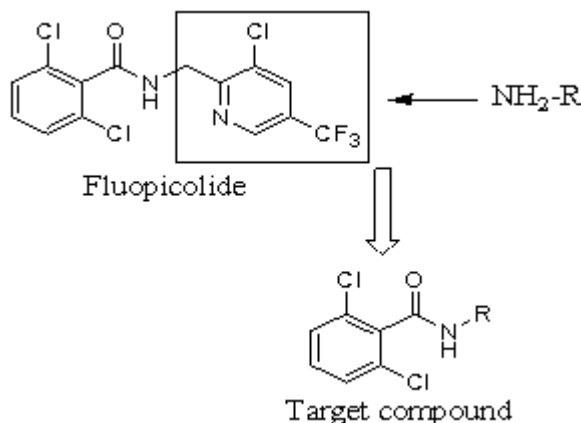

Fig. 1:   Design route of novel benzamide derivatives

As the reason of fungicide resistance [22], in order to discover new fungicides which possess high efficacy, low toxicity and safety to non-target organisms, a series of heterocyclic/homocyclic benzamide derivatives based on fluopicolide have been designed according to the rule of connecting bioactive substructures together (Fig. 1) [23–26]. In this study, 15 novel compounds are synthesized by using heterocyclic/homocyclic ring amines derivatives and chlorobenzoic acids as bioactive substructures. In the meantime, their antifungal activity was tested against *Gibberella zeae, Helminthosporium maydis, Rhizoctonia solani, Botrytis cinerea* and *Sclerotinia sclerotiorum* in laboratory.

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Experimental

General

Gibberella zeae, *Helminthosporium maydis*, *Rhizoctonia solani*, *Botrytis cinerea* and *Sclerotinia sclerotiorum* 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. NMR spectra (^{1}H NMR and ^{13}C NMR) were taken on a Bruker 300 MHz spectrometer using deuteron-dimethyl sulfoxide (DMSO-d_6) as the solvent. Melting points were determined using an X-4B micro-melting point apparatus and were not corrected.

*Preparation of carboxylic acid chlorides (**1a**, **2a** and **3a**)*

Thionyl chloride (15 mL) was added to 0.01 mol corresponding acids. The mixture was heated under reflux at 80 °C for 2 h with the help of a drying tube filled with anhydrous calcium chloride. The reaction was monitored by thin layer chromatography (TLC). When the reaction was complete, the excess thionyl chloride was removed under reduced pressure. The crude products were used in the subsequent reaction without further purification (Fig. 2).
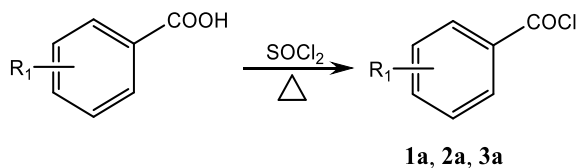
(**1a**: R_1 = 2,6-Cl_2; **2a**: R_1 = 2,4-Cl_2; **3a**: R_1 = 2-Cl)

Fig. 2: The syntheszing reaction of intermediates (**1a**, **2a**, **3a**).

*General procedure for target compounds **1–15***

Compounds **H-R₂** (0.01 mol) or **NH₂-R₃-NH₂** (0.005 mol) were completely dissolved in CH_2Cl_2. Triethylamine (Et₃N) or pyridine (3 mL) was added to the mixture (Fig. 3). Under stirring, carboxylic acid chloride (**1a**, **2a** or **3a**) was added drop by drop into the mixture at the room temperature. Afterwards, the mixture was stirred for another 3 h at 20–70 °C. The reaction was monitored by thin layer chromatography (TLC). When the reaction was complete, the mixture was washed with HCl (10%) and NaOH (10%) in turn. The solvent was removed under reduced pressure. The crude products of compounds **7–15** were re-crystallized with dimethyl sulfoxide (DMSO) and water (15:1), and the rest were re-crystallized with anhydrous ethanol. The purity of the synthesized compounds was checked by TLC. The pure compounds obtained are summarized in Table-1.
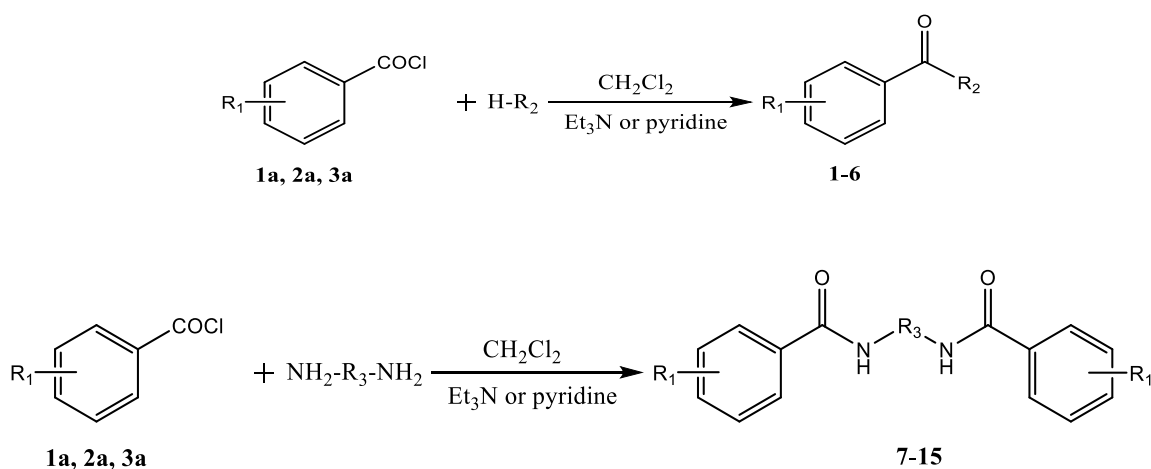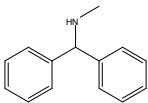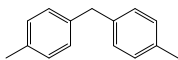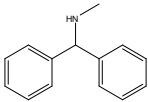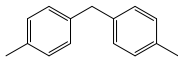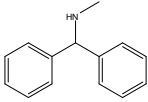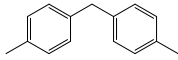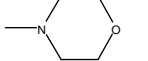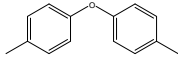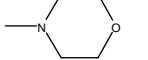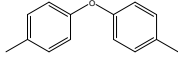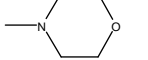

Fig. 3: The synthesizing reaction of target compounds **1–15.**

Table-1:　The novel benzamide derivatives.

Compound	R_1	R_2	Compound	R_1	R_3
1	2,6-Cl$_2$		10	2,6-Cl$_2$	
2	2,4-Cl$_2$		11	2,4-Cl$_2$	
3	2-Cl		12	2-Cl	
4	2,6-Cl$_2$		13	2,6-Cl$_2$	
5	2,4-Cl$_2$		14	2,4-Cl$_2$	
6	2-Cl		15	2-Cl	
7	2,6-Cl$_2$				
8	2,4-Cl$_2$				
9	2-Cl				

Assay of antifungal activity

　　　　The antifungal activity of compounds **1–15** against *Gibberella zeae*, *Helminthosporium maydis*, *Rhizoctonia solani*, *Botrytis cinerea* and *Sclerotinia sclerotiorum* was determined using the plate growth rate method [27].

　　　　The synthesized compounds and carbendazim (purity 90%) were dissolved in DMSO, respectively. The solution obtained was diluted by water with 0.1% Tween−80. For primary screenings, they were determined at a concentration of 100 mg L^{-1}. The solution was added to the sterile culture medium (PDA) at 45 °C, mixed to homogeneity and transferred to sterile Petri di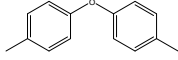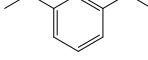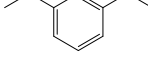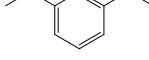shes to solidify. A mycelium agar disc (5 mm in diameter) of the target fungi was placed in the center of PDA plates. They were incubated at 28 °C in the dark until the target fungi used as negative controls covered the surface of these plates moderately. Negative controls groups were treated with the corresponding solution without the synthesized compound or carbendazim. Posotive controls groups were treated with the corresponding

solution with carbendazim. The diameter of the fungi in the cultures was measured and the inhibition of growth was calculated according to the formula of Abbott. Every experiment was replicated three times (Table-2).

Results and Discussion

Chemistry

　　　　In the present study, the carboxylic acid chlorides **1a**, **2a** and **3a** were synthesized and used in the next reaction without further purification.

　　　　In processing of synthesizing compounds **1–6**, the ratio of the carboxylic acid chlorides and heterocyclic/homocyclic ring amines derivatives was 1:1, whereas in synthesizing compounds **7–15**, the ratio was 2:1. The crude compounds **7–15** were re-crystallized in the mixture of dimethyl sulfoxide (DMSO) and water (15:1). However, the rest crude compounds were re-crystallized with anhydrous ethanol. Up to now, compounds **1**, **2**, **4** and **7–15** were been reported firstly.

Table-2:   The antifungal activity results of compounds **1 − 15** at 100 mg L^{-1}.

Compound	Inhibition of growth[a] (%)				
	Gibberella zeae	*Helminthosporium maydis*	*Rhizoctonia solani*	*Botrytis cinerea*	*Sclerotinia sclerotiorum*
1	38.5 ± 3.3	35.4 ± 3.3	34.4 ± 3.8	34.2 ± 2.6	32.7 ± 2.9
2	39.8 ± 1.7	36.7 ± 3.3	32.7 ± 3.5	31.4 ± 2.4	37.5 ± 3.8
3	33.4 ± 2.5	30.2 ± 3.5	26.5 ± 3.5	31.6 ± 3.2	35.4 ± 1.8
4	32.5 ± 1.5	26.8 ± 3.3	25.6 ± 2.9	33.3 ± 2.5	37.7 ± 3.4
5	35.5 ± 2.5	33.2 ± 3.7	25.7 ± 1.8	34.9 ± 3.3	38.8 ± 3.7
6	36.7 ± 2.3	33.9 ± 1.5	25.8 ± 3.0	31.7 ± 2.8	37.2 ± 3.5
7	-14.2 ± 2.4	-11.8 ± 3.4	-14.9 ± 3.5	-18.7 ± 3.5	-13.4 ± 3.3
8	-16.9 ± 3.3	-16.2 ± 2.3	-17.3 ± 2.5	-13.3 ± 1.5	-17.5 ± 2.4
9	-18.4 ± 1.5	-10.5 ± 3.3	-11.5 ± 3.4	-10.4 ± 2.5	-13.3 ± 3.3
10	-24.2 ± 2.3	-20.3 ± 3.7	-23.5 ± 3.1	-26.4 ± 3.3	-21.7 ± 1.8
11	-21.7 ± 3.4	-25.5 ± 1.7	-22.1 ± 3.6	-21.2 ± 2.9	-27.5 ± 3.3
12	-21.2 ± 2.8	-22.1 ± 1.5	-21.4 ± 3.2	-25.4 ± 3.5	-29.3 ± 3.1
13	-24.4 ± 3.3	-21.8 ± 3.5	-23.3 ± 2.3	-28.3 ± 3.3	-21.2 ± 3.0
14	-22.7 ± 1.7	-23.3 ± 2.4	-21.2 ± 1.3	-25.3 ± 2.6	-25.8 ± 2.3
15	-21.3 ± 3.4	-26.2 ± 2.4	-22.7 ± 2.8	-27.4 ± 3.6	-20.4 ± 1.7
Carbendazim	100.0 ± 0	87.5 ± 1.6	100.0 ± 0	91.3 ± 1.9	95.0 ± 1.7

[a] Data are given as mean of triplicates ± SD.

Antifungal activity

The new synthesized compounds were submitted to laboratory to determine their antifungal activity against *Gibberella zeae, Botrytis cinerea*, *Helminthosporium maydis, Rhizoctonia solani* and *Sclerotinia sclerotiorum*. Compared with the high efficacy fungicide carbendazim, the antifungal activity results of the synthesized compounds are listed in Table-2. Unfortunately, the preliminary antifungal activity results show that all the compounds do not reveal any antifungal activity against the 5 tested pathogenic fungi. Although the antifungal activity results of synthesized compounds are not satisfactory, an interesting finding is obtained. When compared to negative controls, all the 5 tested fungi, with supplementary compound **7–15** respectively, grow faster than negative controls at the concentration of 100 mg L^{-1}.

As shown in Table-2, it can be found that morpholine ring and benzene ring (single or double) without any substituent group, including electrophilic/nucleophilic substitution, possibly no contribute to antifungal activity, because the compounds merely containing these rings do not reveal any antifungal activity. This maybe imply that when compound containing electrophilic/nucleophilic substitution on these rings and keep compound at an appropriate electronic density, the compound could have good activity. At the same time, the compounds **7–15** maybe serve as nutriments to supply C source and N source for fungi breeding, because the inhibition ratio of these compounds are minus when compared to negative controls.

Although the definite relationship between structure and activity has not been found, an interesting finding has been obtained. These results can further be used for designing and synthesis carboxylic acid amide fungicides, so that more bioactive compounds or bioactive lead compounds can be discovered.

Conclusion

Fifteen benzamide derivatives with novel strcutures have been successfully synthesized and tested their antifungal activity against *Gibberella zeae, Helminthosporium maydis, Rhizoctonia solani, Botrytis cinerea* and *Sclerotinia sclerotiorum* in laboratory. Regrettably, the results do not obtain any anti-fungi pesticides at the concentration of 100 mg L^{-1}. On the other side, an interesting finding has been obtained. The benzamide derivatives with morpholine or benzene rings reveal no antifungi activity. But these compounds can afford the nutrients for fungi breeding, such as C source and N source. Regardless the relationship between structure and activity has not been clarify clearly, some valuable conclusions have been obtained. Therefore, these preliminary results have advantages in developing new and high efficiency carboxylic acid amide fungicides.

Acknowledgement

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