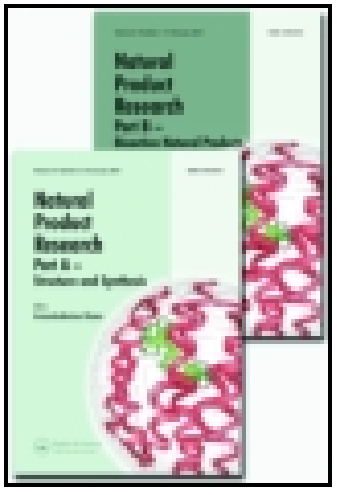

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Synthesis and antifungal activity evaluation of new heterocycle containing amide derivatives

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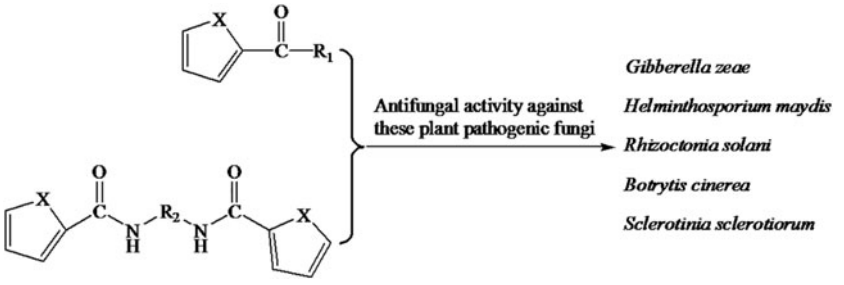

A series of heterocycle containing amide derivatives (**1**–**28**) were synthesised by the combination of acyl chlorides (**1a**, **2a**) and heterocyclic/homocyclic ring containing amines, and their *in vitro* antifungal activity was evaluated against five plant pathogenic fungi, namely *Gibberella zeae*, *Helminthosporium maydis*, *Rhizoctonia solani*, *Botrytis cinerea* and *Sclerotinia sclerotiorum*. Results of antifungal activity analysis indicated that some of the products showed good to excellent antifungal activity, as compound **2** showed excellent activity against *G. zeae* and *R. solani* and potent activity against *H. maydi, B. cinerea* and *S. sclerotiorum*, and compounds **1**, **8** and **10** also displayed excellent antifungal potential against *H. maydi*, *B. cinerea* and *S. sclerotiorum* and good activity against *R. solani* when compared with the standard carbendazim.

Keywords: synthesis; amide derivatives; plant pathogenic fungi; antifungal activity

1. Introduction

Amide fungicides have played an important role in the history of pesticide science. The first amide fungicide, carboxin, was discovered in 1966 (Schmeling & Kulka 1966). After this, numerous amide fungicides with novel structures have successively emerged such as benodanil, furcarbanil and mebenil (Yang et al. 2008). Presently, amide fungicides are extensively used to control diseases caused by plant pathogenic fungi and bacteria (Raffa et al. 2002; Narayana et al. 2004; Wen et al. 2005; Priya et al. 2006; Ertan et al. 2007). Fungicides inhibit the growth of pathogens and cause their eventual death by interfering in pathogen's respiration (Leroux 1996; Huang 2004). Furthermore, amide fungicides are usually efficient, safe and environmental friendly (Smiley et al. 1990; Kataria et al. 1993).

Heterocycle compounds are very important for the development of fungicides. They have various bioactivities including antifungal and antibacterial (Raffa et al. 1999; El-masry et al.

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2000; Charris et al. 2002; Mahran et al. 2003; Sundari & Valliappan 2004). When they are used as fungicides, they usually possess the following advantages: good selectivity (Xu et al. 2007), excellent activity (Nakib et al. 1991; Laldhar et al. 1996; Ganesabaskaran et al. 2006), low toxicity (Huang et al. 2003) and special mode of action (Kuhn 1989). Therefore, cyclic compounds have huge potential in the agricultural chemistry field.

In this study, a series of heterocycle containing amides have been designed in accordance with the principle of connecting bioactive substructures together. Their syntheses were based on amine derivatives and furan-2-carboxylic acid or thiophene-2-carboxylic acid to find new fungicides or lead compounds with high efficacy and low toxicity as well as safety to non-target organisms. So, amides **1**–**28** were synthesised, and their antifungal activities against *Gibberella zeae*, *Helminthosporium maydis*, *Rhizoctonia solani*, *Botrytis cinerea* and *Sclerotinia sclerotiorum* were also evaluated.

2. Results and discussion

2.1. *Chemistry*

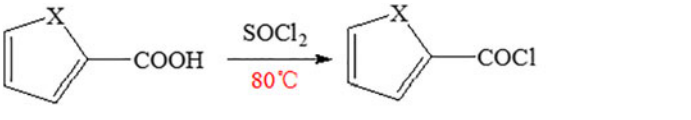
The acyl chlorides **1a** and **2a** used in the synthesis of amide derivatives were synthesised by the reaction described in Figure 1. They were used without further purification in the subsequent amides synthesis reactions, which are shown in Figure 2.

In the synthesis of compounds **1**–**22**, the ratio of the acyl chlorides and amine derivatives was 1:1, while in the synthesis of compounds **23**–**28**, the ratio was 2:1. Compounds **9**, **15** and **17** were obtained as liquid, whereas the rest were obtained as solid. Compounds **23**–**28** were re-crystallised with the mixture of dimethyl sulphoxide (DMSO) and water (15:1), and the rest solids were re-crystallised with anhydrous ethanol. The products are listed in Table 1. To date, compounds **3**, **6**, **21**–**22** and **24**–**28** have not been reported.

2.2. *Antifungal activity*

Compared with the efficient fungicide carbendazim, the synthesised compounds **1**–**28** were evaluated for their antifungal potential against *G. zeae, H. maydis, R. solani, B. cinerea* and *S. sclerotiorum* fungi, and the activity results are shown in Table 2. Compounds **1**–**4**, **6**, **8** and **10** showed good to excellent anti-fungal activity. Among them, compound **2** showed the best antifungal activity against all the five under examine fungi such as it showed potent activity against *H. maydis, B. cinerea* and *S. sclerotiorum* whereas moderate activity against *G. zeae* and *R. solani* when compared with the standard carbendazim. Compound **1** exhibited potent activity against *H. maydis* and excellent activity against *G. zeae*, *R. solani*, *B. cinerea* and *S. sclerotiorum* as compared to the standard carbendazim. Compounds **8** and **10** displayed remarkable activity against *H. maydis* and *B. cinerea*, whereas they displayed good activity against *G. zeae* and *S. sclerotiorum* as compared to carbendazim.

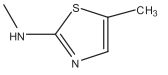
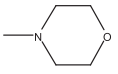
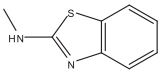
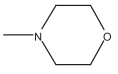
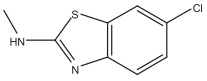
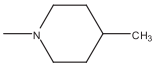
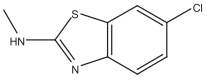
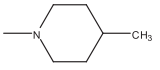
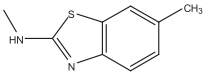
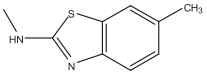
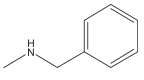
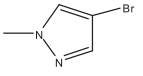
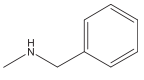
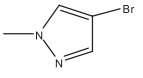
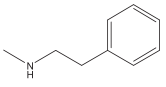
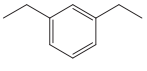
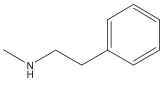
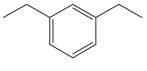
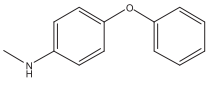
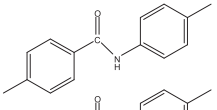
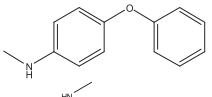
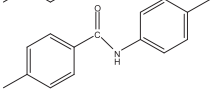
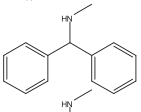
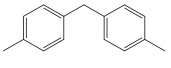
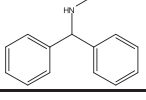
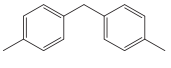
The activity results indicated that thiazole, benzothiazole, benzyl and 2-phenylethyl ring in products possibly contributed to the anti-fungal activity because the compounds containing such ring structures indicated better activity. Results further showed that when the benzothiazole



1a, 2a (1a: X = O; 2a: X = S)

Figure 1. Preparation of acyl chlorides (**1a**, **2a**).

Table 1. Synthesised heterocycle containing amide derivatives.

Compound	X	R_1	R_2	Compound	X	R_1	R_2
1	S			**15**	O		
2	O			**16**	S		
3	O			**17**	O		
4	S			**18**	S		
5	O			**19**	O		
6	S			**20**	S		
7	O			**21**	O		
8	S			**22**	S		
9	O			**23**	O		
10	S			**24**	S		
11	O			**25**	O		
12	S			**26**	S		
13	O			**27**	O		
14	S			**28**	S		

rings have a substituent at position-6, their antifungal activity decreased remarkably irrespective of whether the substituent was electron denoting or electron withdrawing. This possibly implies that when the benzothiazole ring has an appropriate electronic density, the compound could have good activity. The activity results further showed that the products having sulphur atom in the five-membered ring had better activity than those having oxygen atom in the five-membered ring. The comparison of activity results of compounds **7**–**10** indicated that the methylene added to the carbon chains did not have a great influence on the activity.

Table 2. Anti-fungal activity results of compounds **1**–**28** at 100 mg/L.

Compound	Inhibition of growth[a] (%)				
	G. zeae	*H. maydis*	*R. solani*	*B. cinerea*	*S. sclerotiorum*
1	93.1 ± 2.3	93.2 ± 1.8	83.4 ± 1.7	90.6 ± 1.4	91.5 ± 2.1
2	100.0 ± 0	100.0 ± 0	100.0 ± 0	100.0 ± 0	100.0 ± 0
3	35.1 ± 1.9	75.3 ± 1.4	32.5 ± 2.5	56.0 ± 1.6	60.6 ± 2.3
4	47.2 ± 2.4	78.6 ± 2.3	48.1 ± 1.8	57.3 ± 2.2	65.5 ± 3.1
5	33.3 ± 1.2	45.5 ± 1.9	38.6 ± 1.1	44.4 ± 2.1	51.6 ± 3.0
6	56.1 ± 2.7	80.6 ± 1.3	49.7 ± 2.5	58.9 ± 1.1	58.3 ± 1.5
7	35.5 ± 1.8	48.6 ± 2.2	32.6 ± 2.5	56.0 ± 1.2	52.3 ± 2.7
8	71.4 ± 1.5	91.8 ± 2.1	57.5 ± 2.9	93.7 ± 2.4	91.9 ± 2.2
9	37.0 ± 1.2	48.4 ± 1.3	33.3 ± 2.0	56.4 ± 3.1	51.6 ± 1.6
10	73.1 ± 1.9	92.7 ± 1.5	59.6 ± 2.1	94.5 ± 1.8	91.4 ± 1.4
11	43.2 ± 2.3	43.3 ± 2.7	21.0 ± 1.3	43.5 ± 2.1	41.4 ± 1.9
12	36.2 ± 1.0	44.6 ± 2.5	26.4 ± 1.3	41.2 ± 2.6	39.1 ± 1.7
13	44.1 ± 1.5	43.1 ± 1.3	28.5 ± 2.4	39.4 ± 2.3	40.3 ± 1.9
14	38.6 ± 1.3	41.5 ± 2.2	23.4 ± 1.7	44.6 ± 2.4	37.2 ± 1.8
15	41.2 ± 1.1	42.4 ± 1.7	30.3 ± 2.3	36.5 ± 2.0	39.3 ± 2.5
16	39.3 ± 1.4	34.6 ± 2.3	25.0 ± 1.5	41.2 ± 2.4	43.1 ± 2.9
17	37.3 ± 2.1	25.4 ± 2.3	23.6 ± 1.9	39.0 ± 3.1	36.2 ± 1.2
18	36.2 ± 1.7	43.5 ± 3.2	24.6 ± 3.0	41.1 ± 1.4	36.4 ± 1.6
19	44.2 ± 2.3	37.5 ± 1.6	22.1 ± 2.1	38.8 ± 2.5	35.3 ± 1.7
20	38.4 ± 1.5	41.2 ± 1.9	28.0 ± 1.4	32.1 ± 2.3	32.3 ± 2.7
21	40.0 ± 2.0	38.8 ± 1.2	23.4 ± 1.5	39.3 ± 2.5	30.4 ± 1.3
22	35.5 ± 2.2	29.6 ± 1.3	29.1 ± 2.4	39.7 ± 1.9	28.5 ± 1.2
23	31.8 ± 2.3	31.3 ± 1.6	26.6 ± 2.8	34.3 ± 1.4	29.3 ± 2.6
24	29.3 ± 1.8	43.9 ± 1.1	27.7 ± 1.5	29.6 ± 2.5	30.3 ± 1.7
25	32.5 ± 1.2	34.5 ± 1.9	26.8 ± 2.3	28.6 ± 1.4	27.4 ± 2.6
26	26.9 ± 1.9	37.3 ± 2.5	22.0 ± 2.3	36.2 ± 2.8	31.5 ± 1.3
27	29.2 ± 1.8	40.8 ± 1.2	31.2 ± 1.6	24.4 ± 2.0	32.6 ± 2.3
28	31.3 ± 2.3	43.2 ± 1.7	29.2 ± 3.1	33.3 ± 2.9	34.4 ± 1.4
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.

Although a definite structure–activity relationship could not be found by means of the synthesised compounds, the interesting results obtained can be used for further designing and synthesising more similar compounds to study their quantitative structure–activity relationship, so that more bioactive compounds or bioactive lead compounds may be discovered.

3. Experimental

3.1. *General*

All the chemicals and solvents were purchased from commercial sources and used as such. The fungi *G. zeae*, *H. maydis*, *R. solani*, *B. cinerea* and *S. sclerotiorum* were obtained from the Chinese Academy of Agricultural Sciences and they were preserved at 4°C. NMR (^{1}H and ^{13}C) spectra were taken on a Bruker 300 MHz spectrometer in deuterated-dimethyl sulphoxide (DMSO-d_6). Melting points were determined using an X-4B micro-melting point apparatus (Shanghai Precision Instruments Co., Ltd., Shanghai, China) and were not corrected.

3.2. *Preparation of carboxylic acid chlorides (1a and 2a)*

Thionyl chloride (15 mL) was added to 0.01 mol corresponding acids. The mixture was refluxed at 80°C for 2 h in a tube filled with anhydrous calcium chloride. The reaction was monitored by

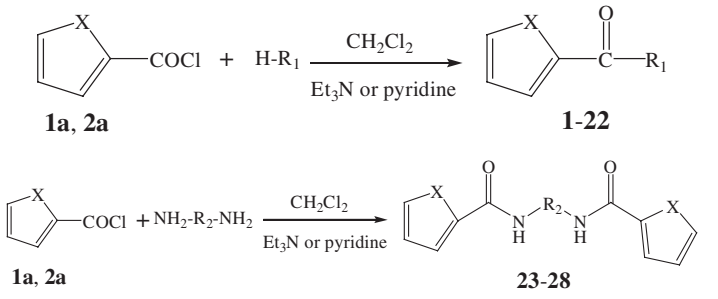


Figure 2. Synthesis of amides derivatives **1**–**28**.

periodic thin layer chromatography (TLC). When the reaction was completed, excess thionyl chloride was removed under reduced pressure. The crude products were used in the subsequent reaction (Figure 1).

3.3. *General procedure for target compounds 1–28*

Compounds H-R_1 (0.01 mol) or NH_2–R_2–NH_2 (0.005 mol) were completely dissolved in CH_2Cl_2.Triethylamine (Et_3N) or pyridine (3 mL) was added to the solution (Figure 2). Under stirring, the carboxylic acid chloride (**1a** or **2a**) was added drop by drop to the mixture at room temperature. Afterwards, the reaction mixture was further stirred for 5 h at 20–70°C. The reaction was monitored by periodic TLC. After the completion of the reaction, the reaction mixture was washed with HCl (10%) and NaOH (10%) in turn. The solvent was removed under reduced pressure. Compounds **9**, **15** and **17** were obtained pure directly by this method. The crude products of compounds **23**–**28** were re-crystallised with the mixture of DMSO and water (15:1), and the rest were re-crystallised with anhydrous ethanol. The purity of the synthesised compounds was checked by TLC.

3.4. *Assay of antifungal activity*

The antifungal activity of compounds **1**–**28** against *G. zeae*, *H. maydis*, *R. solani*, *B. cinerea* and *S. sclerotiorum* was determined using the plate growth rate method (Huang & Yang 2006).

Each synthesised compound was dissolved in DMSO. The solution was then diluted with 0.1% Tween-80 solution and then added to the sterile culture medium (PDA) at 45°C. The mixture was homogenised and transferred to a sterile Petri dish to solidify. For primary screening, compounds were used at a concentration of 100 mg/L. At the same time, carbendazim (standard fungicide, purity 90%) and 1 equiv. of DMSO were used as positive and negative controls, respectively. Afterwards, a mycelium agar disc (5 mm diameter) of the target fungi was placed in the centre of the PDA plates, and then the plates were incubated at 28°C in the dark until the target fungi used as the negative control covered the plate's surface. Then the diameters of all fungi in the cultures were measured and the results were reported as the inhibition of the growth following Abbott's formula. Each compound was tested three times.

4. Conclusion

A series of new heterocycle containing amide derivatives have been synthesised and their antifungal activity was evaluated against *G. zeae*, *H. maydis*, *R. solani*, *B. cinerea* and *S. sclerotiorum*. The results showed that compound **2** had remarkable antifungal activity and its activity against *H. maydis*, *B. cinerea* and *S. sclerotiorum* surpassed the standard carbendazim.

Compounds **1**, **8** and **10** also exhibited excellent antifungal activity against the above-mentioned five fungi. The results acquired in this study are promising and beneficial for further developing and making researches on novel and more effective fungicides in the agricultural chemistry field.

Supplementary material

Supplementary material relating to this paper is available online, alongside Figures S1–S56. http://dx.doi.org/10.1080/14786419.2015.1041137

Disclosure statement

No potential conflict of interest was reported by the authors.

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