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SYNTHESIS AND ANTIFUNGAL ACTIVITY OF CUMINIC ACID DERIVATIVES

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*A series of benzamide derivatives (compounds **1–29**) was synthesized based on the lead compound cuminic acid. The crystal structure of compound **11** was characterized by single crystal X-ray diffraction. The antifungal activity of the synthesized compounds was determined against seven plant pathogenic fungi, namely* Rhizoctonia solani*,* Gibberella zeae*,* Helminthosporium maydis*,* Sclerotinia sclerotiorum*,* Botrytis cinerea*,* Coniothyrium diplodiella*, and* Coniothyrium lagenarium*. Preliminary results indicated that most of them revealed significant antifungal activity. Among them, compound **22** showed the strongest activity and possessed better antifungal activity against* H. maydis*,* S. sclerotiorum*, and* B. cinerea *than carbendazim.*

Keywords: cuminic acid, synthesis, crystal structure, antifungal activity.

Cuminic acid (*p*-isopropylbenzoic acid) is a natural product found in the seed of *Cuminum cyminum* [1, 2]. It can also be obtained by chemical synthesis. It has various biological activities, including insecticidal and antifungal activity [3–6]. At the same time, its structure is simple and synthesis is easy, so its structure may be further optimized and modified to synthesize and discover more active compounds or lead compounds with new structures, which will lay a good foundation for its application in the agricultural chemistry field.

Carboxylic acid amide fungicides have always played an important role in the history of pesticide science. Since the first carboxylic acid amide fungicide carboxin was discovered in 1966 [7], numerous carboxylic acid amide fungicides with novel structures have been successively reported [8–11]. At present, these carboxylic acid amide fungicides are mostly used to control diseases caused by plant pathogenic fungi and bacteria [12–16]. They can inhibit the growth of pathogens and cause their eventual death by interfering with the pathogen′s respiration [17]. In addition, carboxylic acid amide fungicides are usually efficient, safe, and environmentally friendly [18, 19].

In the present study, a series of cuminic acid derivatives has been designed in accordance with its structural features and synthesized using cuminic acid and its derivatives and amines as raw materials to find new fungicides or lead compounds with high efficacy and low toxicity as well as safety to non-target organisms. In the meantime, their antifungal activities against *R. solani*, *G. zeae*, *H. maydis*, *S. sclerotiorum*, *B. cinerea*, *C. diplodiella*, and *C. lagenarium* were evaluated in the laboratory.

According to the method shown in Scheme 1, compounds **1–29** were successfully synthesized. Their structures were confirmed by ^{1}H NMR and ^{13}C NMR. The results showed that their NMR spectra were in agreement with the proposed structures. To date, all the compounds have not been reported except **3**, **7**, **11**, **16**, **19**, **22**, **23**, and **24**.

The molecular structure of compound **11** is shown by X-ray determination in Fig. 1. The crystal data and structure refinement for compound **11** are displayed in Table 1. Its crystallographic information file is deposited at the Cambridge Crystallographic Data Center (CCDC No. 1415261).

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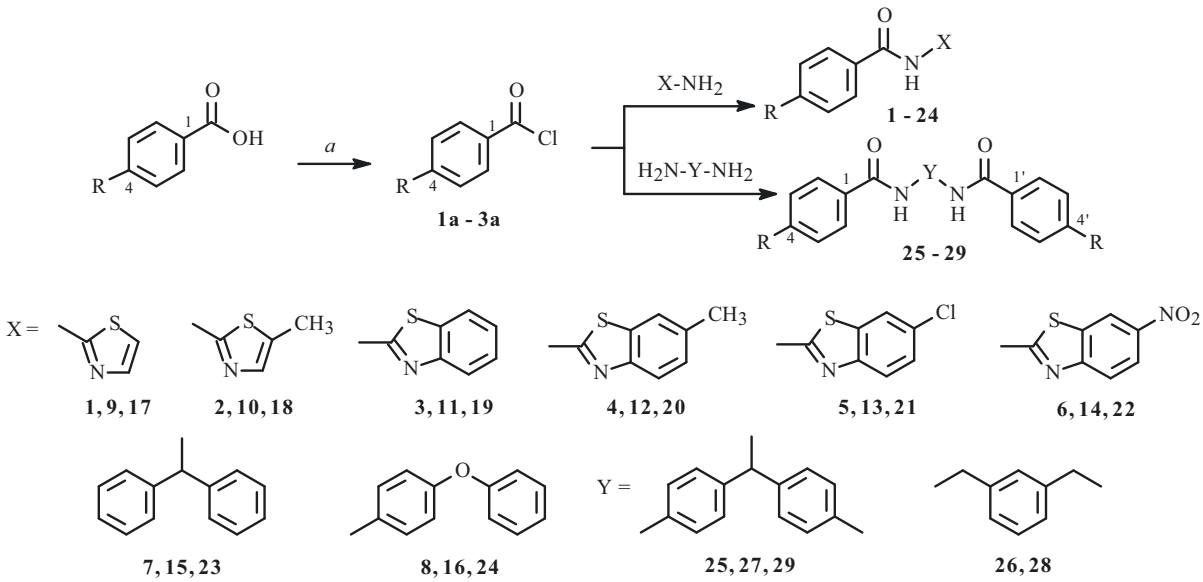
TABLE 1. Crystal Data and Structure Refinement of Compound **11**

Empirical formula	$C_{18}H_{18}N_2OS$	Absorption coefficient/mm^{-1}	0.204
Formula weight	310.40	F(000)	656.0
Temperature/K	293.15	Crystal size/mm^3	0.3 × 0.2 × 0.2
Crystal system	Monoclinic	Wavelength/Å	0.71073
Space group	$P2_1/c$	θ range for data collection/°	6.22–52.74
A/Å	6.1288 (2)	Reflections collected	6892
B/Å	18.8208 (8)	Independent reflections (R_{int})	3299(0.0229)
C/Å	14.1610 (5)	Data/restraints/parameters	3299/0/202
α/°	90.00	Good-of-fit on F^2	0.984
β/°	99.544 (4)	Final R indexes [$I > 2\sigma(I)$]	$R_1 = 0.0477$, $wR_2 = 0.1044$
γ/°	90.00	Final R indexes (all data)	$R_1 = 0.0767$, $wR_2 = 0.1127$
Volume/Å^3	1610.85 (11)	Largest diff. peak/hole/e Å^{-3}	0.21/–0.21
Z	4	Index ranges	$-7 \le h \le 7$, $-23 \le k \le 15$, $-13 \le l \le 17$
D_c/(g cm^{-3})	1.280		



1 – 8, 25, 26: R = $CH(CH_3)_2$; **9 – 16, 27, 28:** R = $C(CH_3)_3$; **17 – 24, 29:** R = CF_3

a. $SOCl_2$, reflux

Scheme 1

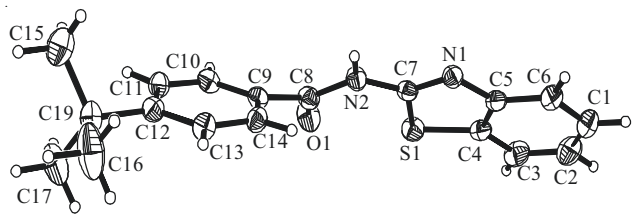


Fig. 1. Molecular structure of compound **11**.

Compared with the efficient fungicide carbendazim, the synthesized compounds were submitted to laboratorial bioassay using *R. solani*, *G. zeae*, *H. maydis*, *S. sclerotiorum*, *B. cinerea*, *C. diplodiella*, and *C. lagenarium* as targets. The results are presented in Table 2. Compound **22** possessed the best antifungal activity against the seven fungi, and its activity against *H. maydis*, *S. sclerotiorum*, and *B. cinerea* was superior to carbendazim. Compounds **1** and **4** showed excellent activity against *R. solani*, *H. maydis*, and *B. cinerea*, with their inhibition rate reaching above 90% at 100 mg/L and their activity against *H. maydis* and *B. cinerea* exceeding carbendazim. Compounds **9**, **12**, **17**, and **20** displayed good activity against *R. solani*, *H. maydis*, and *B. cinerea*, and their inhibition rate reached above 80% at 100 mg/L. Similarly, the inhibition rate of compounds **15**, **27**, and **29** against *H. maydis* and *B. cinerea* also surpassed 80% at 100 mg/L. Compounds **11** and **16** revealed good activity against *B. cinerea*.

TABLE 2. Antifungal Activity of Compounds **1–29** at 100 mg L^{-1} (inhibition of growth[a], %)

Compound	*R. solani*	*G. zeae*	*H. maydis*	*S. sclerotiorum*	*B. cinerea*	*C. diplodiella*	*C. lagenarium*
1	91.8 ± 2.1	66.8 ± 2.4	92.7 ± 1.1	86.3 ± 2.3	93.4 ± 1.9	83.8 ± 2.5	24.7 ± 2.6
2	44.7 ± 1.3	19.6 ± 1.7	47.4 ± 1.5	36.8 ± 2.0	53.7 ± 1.8	33.3 ± 1.4	11.4 ± 1.6
3	36.9 ± 1.8	31.5 ± 1.5	44.3 ± 2.1	36.1 ± 2.2	48.2 ± 2.6	35.8 ± 2.3	19.2 ± 1.3
4	90.7 ± 2.1	62.3 ± 1.9	93.1 ± 1.8	88.2 ± 0.7	98.6 ± 1.3	80.4 ± 2.4	24.9 ± 2.2
5	36.1 ± 2.6	28.1 ± 2.2	44.1 ± 1.6	33.4 ± 1.4	47.8 ± 1.9	31.8 ± 1.8	18.2 ± 1.2
6	29.3 ± 2.3	18.4 ± 2.7	35.7 ± 2.1	21.1 ± 1.8	43.1 ± 2.4	19.3 ± 1.4	14.6 ± 1.5
7	31.3 ± 2.1	15.8 ± 1.8	33.4 ± 2.0	26.3 ± 2.4	37.8 ± 2.5	23.3 ± 2.3	13.9 ± 1.9
8	42.5 ± 1.8	34.6 ± 1.6	44.3 ± 1.9	38.1 ± 1.5	49.4 ± 0.7	35.6 ± 1.1	–17.4 ± 0.9
9	82.7 ± 1.4	64.2 ± 2.1	85.3 ± 2.3	80.2 ± 1.3	87.4 ± 1.3	73.5 ± 2.8	21.5 ± 2.2
10	35.3 ± 2.5	28.1 ± 0.9	40.7 ± 1.1	32.8 ± 2.7	45.4 ± 1.5	31.1 ± 1.4	19.6 ± 3.8
11	61.7 ± 1.2	24.8 ± 1.4	67.1 ±1.7	42.5 ± 1.1	88.3 ± 1.9	36.7 ± 1.6	18.3 ± 2.6
12	81.7 ± 1.5	57.9 ± 2.1	84.2 ± 2.6	78.9 ± 1.9	90.4 ± 2.2	64.4 ± 1.1	34.9 ± 1.3
13	36.9 ± 1.9	23.4 ± 1.7	41.9 ± 2.2	31.1 ± 2.6	47.3 ± 1.5	30.7 ± 2.7	18.2 ± 2.5
14	36.0 ± 2.2	19.5 ± 2.7	40.3 ± 2.7	30.3 ± 2.3	45.3 ± 2.9	21.9 ± 1.1	9.3 ± 1.4
15	71.1 ± 2.7	30.6 ± 2.4	84.3 ± 2.5	64.7 ± 2.0	86.7 ± 1.7	51.6 ± 2.2	7.3 ± 2.6
16	69.3 ± 1.5	42.1 ± 1.6	73.5 ± 2.8	58.8 ± 2.4	87.8 ± 1.9	53.3 ± 2.5	19.7 ± 2.9
17	88.7 ± 2.1	30.4 ± 2.5	89.7 ± 0.9	72.1 ± 2.3	90.4 ± 1.8	37.2 ± 2.0	14.3 ± 1.3
18	38.7 ± 1.0	28.5 ± 1.8	42.6 ± 1.6	33.1 ± 2.8	53.5 ± 3.4	31.8 ± 1.5	13.5 ± 1.9
19	38.5 ± 1.7	29.1 ± 2.6	43.4 ± 1.6	33.2 ± 2.2	59.4 ± 1.8	31.4 ± 2.7	27.9 ± 1.4
20	80.7 ± 1.1	64.2 ± 2.1	87.1 ± 2.2	71.6 ± 1.7	90.0 ± 1.9	65.2 ± 1.9	36.9 ± 2.0
21	31.9 ± 2.2	18.5 ± 0.7	38.1 ± 2.5	28.4 ± 0.9	43.6 ± 2.7	21.8 ± 1.8	17.3 ± 1.1
22	100.0 ± 0	87.1 ± 1.1	100.0 ± 0	100.0 ± 0	100.0 ± 0	100.0 ± 0	41.9 ± 1.5
23	34.7 ± 1.4	22.1 ± 1.9	36.4 ± 1.2	31.4 ± 2.9	39.2 ± 1.7	25.4 ± 2.6	14.8 ± 2.9
24	31.3 ± 2.2	17.2 ± 0.7	35.6 ± 1.8	25.8 ± 1.5	37.2 ± 2.7	19.8 ± 1.5	13.6 ± 1.8
25	37.3 ± 1.2	22.1 ± 2.3	39.1 ± 0.8	31.8 ± 2.0	48.8 ± 1.5	28.9 ± 2.5	12.5 ± 1.2
26	39.7 ± 0.9	30.6 ± 1.7	43.3 ± 1.2	36.6 ± 1.9	47.8 ± 2.7	34.2 ± 1.1	19.4 ± 2.3
27	70.6 ± 2.0	30.2 ± 1.2	81.9 ± 1.9	61.3 ± 2.1	89.4 ± 2.1	37.8 ± 2.2	20.9 ± 2.7
28	30.7 ± 1.8	22.9 ± 1.3	33.8 ± 2.0	28.7 ± 1.6	37.8 ± 0.9	25.6 ± 1.7	14.6 ± 2.7
29	73.4 ± 1.7	35.3 ± 1.3	85.3 ± 1.7	50.7 ± 1.4	86.7 ± 2.2	43.3 ± 0.7	13.3 ± 1.5
Cuminic acid	26.3 ± 1.5	27.4 ± 1.1	57.6 ± 2.4	17.0 ± 3.3	73.1 ± 1.8	29.6 ± 1.7	18.3 ± 2.4
Carbendazim	100 ± 0	100 ± 0	87.5 ± 1.6	95.0 ± 1.7	91.3 ± 1.9	100 ± 0	100 ± 0

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

As shown in Table 2, compounds **1–29** showed inhibitory activity against the seven fungi in the following order: *B. cinerea* > *H. maydis* > *R. solani* > *S. sclerotiorum* > *C. diplodiella* > *G. zeae* > *C. lagenarium*. If the activity of compounds **1**, **9**, and **17** were compared with that of compounds **2**, **10**, and **18**, respectively, it is obvious that the methyl group at position 5 of the thiazole ring causes their activity to decrease remarkably, which needs to be further studied. In accordance with the results of compounds **3–6**, it could be found that when the hydrogen atom at position 6 of the benzothiazole ring was substituted by CH_3, Cl, and NO_2 group, respectively, their antifungal activity exhibited the following order: $CH_3 > H > Cl > NO_2$. Compounds **11–14** also produced the same results, whereas the antifungal activity order of compounds **19–22** was $NO_2 > CH_3 > H > Cl$, which also needs to be further investigated. It was noteworthy that although compound **8** displayed inhibitory activity against *R. solani*, *G. zeae*, *H. maydis*, *S. sclerotiorum*, *B. cinerea*, and *C. diplodiella*, it showed a promoting effect against *C. lagenarium*.

Although a definite structure–activity relationship could not be found through the target compounds, the interesting results obtained can be used for further designing and synthesizing more similar compounds to study their quantitative structure–activity relationship (QSAR) so that more bioactive compounds or lead compounds may be discovered.

EXPERIMENTAL

All chemicals and solvents were purchased from commercial sources unless otherwise specified. *R. solani*, *G. zeae*, *H. maydis*, *S. sclerotiorum*, *B. cinerea*, *C. diplodiella*, and *C. lagenarium* were obtained from the Chinese Academy of Agricultural Sciences. They were preserved at 4°C. NMR spectra (^{1}H and ^{13}C NMR) were taken on a Bruker 300 MHz spectrometer using deuterated dimethyl sulfoxide (DMSO-d_6) as solvent. Melting points were determined using an X-4B micro-melting point apparatus and were not corrected.

Preparation of Acyl Chlorides (1a, 2a, and 3a). Thionyl chloride (15 mL) was added to 0.01 mol of the 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 completed, excess thionyl chloride was removed under reduced pressure. The crude products were used in the subsequent reaction without further purification (Scheme 1).

General Procedure for Target Compounds 1–29. Compound H_2N-X (0.01 mol) or NH_2-Y-NH_2 (0.005 mol) was completely dissolved in CH_2Cl_2. Triethylamine (Et_3N) or pyridine (3 mL) was added to the mixture (Scheme 1). Under stirring, the acyl chloride (**1a**, **2a**, or **3a**) was slowly added to the mixture at room temperature. Afterwards, the reaction mixture was stirred for another 5 h at 20–70°C. After the reaction was completed, the mixture was washed with HCl (10%) and NaOH (10%) in turn. The solvent was removed under reduced pressure. The progress of the reactions was monitored by TLC. The crude products of compounds **7**, **8**, and **25–27** were recrystallized with dimethyl sulfoxide (DMSO) and water (15:1), and the rest were recrystallized from anhydrous ethanol.

Determination of Crystal Structure of Compound 11. The pure compound **11** was dissolved in anhydrous ethanol and heated under reflux for 1 h. The solution obtained was kept at room temperature for 2 days. A light brown prismatic single crystal was obtained. A suitable crystal was selected and mounted on an Xcalibur Eos diffractometer. The crystal was kept at 293.15 K during data collection. Using Olex2 [20], the structure was solved with the Superflip [21–23] structure solution program using charge flipping and refined with the ShelXL [24] refinement package using least squares minimization.

Assay of Antifungal Activity. The antifungal activity of compounds **1–29** against *R. solani*, *G. zeae*, *H. maydis*, *S. sclerotiorum*, *B. cinerea*, *C. diplodiella*, and *C. lagenarium* was determined using the plate growth rate method [25].

The synthesized compounds and carbendazim (purity 90%) and cuminic acid were dissolved in DMSO, respectively. The solution obtained was diluted with 0.1% Tween-80 solution. For primary screenings, they were determined at a concentration of 100 mg/L. The solution was added to the sterile potato dextrose agar (PDA) medium at 45°C, mixed to homogeneity, and transferred to sterile Petri dishes 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 controls covered the surface of these plates. Control groups were treated with the corresponding solution without the synthesized compound or 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.

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