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Inhibitory Activities of Griseofulvin and Its Analogues against Phytopathogenic Fungi

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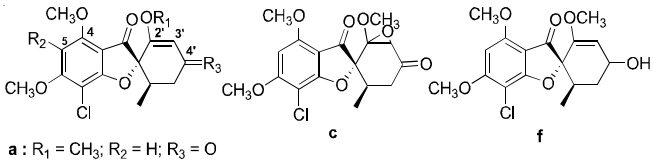
The antifungal activity of griseofulvin was evaluated against phytopathogenic fungi including *Fusarium moniliforme*, *Fusarium solani*, *Fusarium oxysporum* and *Colletotrichum truncatum*. The greenhouse test was also made to evaluate the control efficiency of griseofulvin to eggplant powdery mildew. The results indicated griseofulvin could not only obviously inhibit fungi in laboratory experiments but efficiently control eggplant powdery mildew in greenhouse test. Subsequently, five griseofulvin analogues were synthesized and evaluated for antifungal activities against the four fungi. The results showed that these compounds performed significantly different activities in comparison with griseofulvin. The inhibition mechanism and bioavailability of active analogues were taken to expound their different performance and relations of structure and activity. The solubility in different solvents of analogues and griseofulvin was roughly evaluated and compared.

Key Words: Griseofulvin, Antifungal activity, Greenhouse test, Analogues, Solubility comparison.

INTRODUCTION

Griseofulvin (**a**, Fig. 1 for its structure), first isolated from the mycelium of *Penicillium griseofulvum* Dierckx[1], was a classic antifungal agent against many pathogenic filamentous fungi[2-5]. It had been used clinically for the treatment of dermatomycosesfor years, since it could treat fungus infections caused by tinea organisms of the hair, skin and nails[6-8]. Griseofulvin was also of great use in the treatment of cancer due to that it could block cell-cycle progression at the G_2/M phase and could induce apoptosis in human tumor cells[9,10]. Recently, griseofulvin has become the object of increased interest to its anticancer potential[11]. However, water solubility and bioavailability of griseofulvin were extremely low, leading to the decrease of its effectivity. It also exhibited a number of undesirable side-effects, such as urticaria and fixed drug eruption[12], erythema multiforme[13], *etc*. It had also been reported that griseofulvin was carcinogenioc and teratogenic in animal models[14].

The role of griseofulvin in inhibiting phytopathogenic fungi could contribute to the establishment of it as a specific plant protection against numerous diseases induced by fungi[15,16]. Unfortunately, its use in plant protection was not popularized probably because much attention had been paid to its advantages and disadvantages when used as drug in the treatment of dermatophyte infections.



a : $R_1 = CH_3$; $R_2 = H$; $R_3 = O$

b : $R_1 = R_2 = H$; $R_3 = O$

d : $R_1 = CH_3$; $R_2 = NO_2$; $R_3 = O$

e : $R_1 = CH_3$; $R_2 = H$; $R_3 = N\text{-}OH$

Fig. 1. Chemical structures of compounds. Griseofulvin (**a**); 2'-hydroxylgriseofulvin (**b**); epoxygriseofulvin (**c**); 5-nitrylgriseofulvin (**d**); 4'-hydroxylaminegriseofulvin (**e**) and 4'-hydroxylgriseofulvin (**f**)

Hundreds of papers describing griseofulvin derivative synthesis and relations between structure and biological activities have been published by now[11,17]. Its potential in crop protection still did not receive enough scientific attention. In order to improve the use of griseofulvin in agriculture and the advancement of griseofulvin analogues as antibiotics, the authors conducted both laboratory experiments and greenhouse

test to evaluate its potential in inhibiting phytopathogenic fungi (*Fusarium moniliforme*, *Fusarium solani*, *Fusarium oxysporum* and *Colletotrichum truncatum*) and eggplant powdery mildew. The authors also synthesized five analogues of griseofulvin, tested and compared their fungicidal activity with griseofulvin against the fungi. To our knowledge, there were no reports about the greenhouse test of griseofulvin against powdery mildew or antifungal activities of griseofulvin and its analogues against the fungi included in this study. A few papers have been published described the antifungal activities of griseofulvin against phytopathogenic fungi including *Aspergillus niger*, *Botrytis cinerea*, *Fusarium nivak*, *Glomerella cingulata*[17], *Botrytis allii*, *Botrytis cinerea*[18], *etc*.

The analogues were synthesized by improved methods[11,19]. The correlation of structure and activity was studied. We were interested in determining the influence of the 22, 32, 42 and 5 positions, which were modified to afford our analogues, on the antifungal activities. Effect of analogues on hyphal growth of fungi was evaluated by microscope to determine whether the analogues had the same effect on hyphae as griseofulvin. Low water solubility of griseofulvin tremendously restricted its effectiveness and brought great difficulties in its application. However, the evaluation of its analogues' solubility has seldom been reported. In this study, the solubility of analogues were calculated roughly by the method of saturation and compared to griseofulvin. In this study, we investigated the inhibitory activities of griseofulvin and its analogues against phytopathogenic fungi to provide the basis for the use of griseofulvin in agriculture and improve the development of antibiotics.

EXPERIMENTAL

All the fungi were pathogenic in agriculture and selected for testing from the laboratory collection in Fujian Academy of Agricultural Sciences including *F. moniliforme*, *F. solani*, *F. oxysporum* and *C. truncatum*. Fungi were grown on potato dextrose agar (PDA) in 9 cm petri dishes and incubated at 28 ± 1 ºC.

Griseofulvin (**a**) with purity of 99.9 % was purchased from Shanghai Pharmaceuticals Holding Co., Ltd. (Shanghai, China). Dimethyl sulfoxide (DMSO) was the product of Sigma-Aldrich (St. Louis, MO, USA). The other reagents and solvents were the products of Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) and used without further purification. The water used was redistilled ion-free.

Conidial suspension preparation: Conidia were harvested from 7-day-old cultures by flooding plates with 10 mL of sterile distilled water and dislodging conidia by softly brushing the colonies with aseptic glass spreader. Aqueous conidial suspensions were filtered through sterile gauze to remove hyphae. The suspension was used to test the antifungal activity of compounds.

Mycelial growth assay: The antifungal activity of compounds was evaluated by the mycelial growth assay[20-22], conducted on PDA medium. Prior to the fungal inoculation, 100 µL of different concentrations of the tested compounds were poured and spread on the surface of the agar medium. Dishes were then left opened to enable solvent to evaporate or penetrate. The plates were inoculated in the middle with one drop strain suspension (*ca.* 5 µL). In parallel, control experiments only with DMSO were also tested. The petri dishes were incubated at 28 ± 1 ºC and the fungal colony diameter was measured daily. The inhibition rates were calculated after 7 days of incubation when mycelia in the control experiments completely covered the dishes. It was expressed as an average diameter and calculated using the following equation: Inhibition rate (%) = $(D_c\text{-}D_t)/D_c \times 100$, where D_c and D_t represent mycelia growth diameter in control and treated petri plates, respectively. All the measurements were replicated three times for each treatment. The statistical analysis was performed by one way analysis of variance (ANOVA). IC_{50} values of analogues on the tested fungi were evaluated based on inhibition rates using the China-DPS program[23].

Greenhouse test: The greenhouse test was conducted to evaluate the control efficiency of griseofulvin to eggplant powdery mildew. Water and triadimefon which was a systemic fungicide and often used to control powdery mildew were used as control. Griseofulvin and triadimefon were mixed with water and then sprayed on the eggplants with powdery mildew. The concentrations were 1.25 and 2 mg/mL, respectively. Every treatment included 9 plants. Before spraying, the morbidity of 5 new leaves in 5 plants of each treatment was studied by diagonal sampling method. Then the morbidity was studied 3, 5, 8 and 12 days after spraying. Disease index and control efficiency were calculated according to the guidelines for the field efficacy trials (GB/T17980.22-2000).

Chemistry: The syntheses of 2'-hydroxylgriseofulvin (**b**), epoxygriseofulvin (**c**), 5-nitrylgriseofulvin (**d**), 4'-hydroxylaminegriseofulvin (**e**) and 4'-hydroxylgriseofulvin (**f**) (Fig. 1) were according to the methods as showed in Fig. 2. 2'-Hydroxylgriseofulvin (**b**) was synthesized by treatment of griseofulvin (**a**, 10 mmol) with H_2SO_4 (2 M, 15 mL) in acetic acid (60 mL). Epoxidation of griseofulvin (4 mmol) with H_2O_2 (5 mL) afforded the epoxygriseofulvin (**c**). Nitration of griseofulvin (5 mmol) using nitric acid (3 mL) yielded 5-nitrylgriseofulvin (**d**). Modification of 42-position of griseofulvin gave 4'-hydroxylaminegriseofulvin (**e**) and 4'-hydroxylgriseofulvin (**f**). 4'-hydroxylaminegriseofulvin (**e**) could be synthesized by adding sodium methoxide to the solution of griseofulvin and hydroxylamine hydrochloride in ethanol and DMSO. 4'-Hydroxylgriseofulvin (**f**) was derived from griseofulvin (10 mmol) by reduction with $NaBH_4$ (40 mmol) in tetrahydrofuran (THF, 60 mL) and H_2O (2 mL). The structures of these compounds were established by spectroscopic methods (MS and ^{1}H NMR).

Saturation method for determination of compound solubility: The same amounts (500 mg) of griseofulvin (**a**) and its analogues (compounds **b-f**) were put into isovolumetric solvents (10 mL), respectively and stirred at room temperature. Then the suspensions were filtrated and the residues were collected. The highness or slowness of the solubility of the compounds was preliminarily determined through the weight of the residues. If the weight of the residue of one analogue was larger than that of griseofulvin, the solubility of the analogue was considered as lower than griseofulvin and *vice versa*.

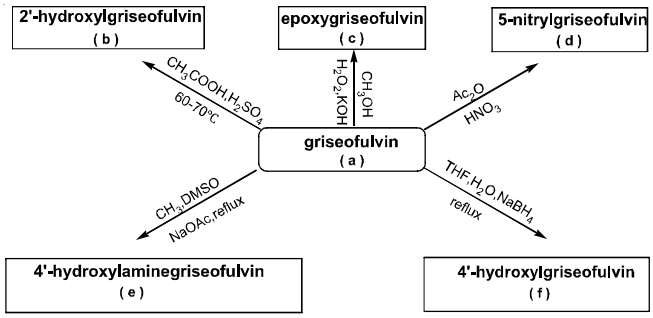


Fig. 2. Brief synthesis methods of (**a**) griseofulvin; (**b**) 2'-hydroxylgriseofulvin; (**c**) epoxygriseofulvin; (**d**) 5-nitrylgriseofulvin; (**e**) 4'-hydroxylaminegriseofulvin and (**f**) 4'-hydroxylgriseofulvin

RESULTS AND DISCUSSION

Antifungal activity of griseofulvin in mycelial growth assay: The antifungal activitiy of griseofulvin was tested by mycelial growth assay against four phytopathogenic fungi: *F. moniliforme*, *F. solani*, *F. oxysporum* and *C. truncatum*. The results were showed in Table-1. The inhibition rates of griseofulvin against four fungi became improved with its concentration increasing. Griseofulvin had highest inhibition rate against *C. truncatum* at low concentration 0.25 mM. According to the IC_{50}, griseofulvin showed the best inhibition efficiency against *C. truncatum* and worst inhibition efficiency against *F. moniliforme*. These results indicated that griseofulvin had inhibition effects against some phytopathogenic fungi.

Greenhouse test of griseofulvin: The control efficiency of griseofulvin was evaluated by greenhouse test. The results were showed in Fig. 3 and Table-2. The pictures in Fig. 3 were took 12 days after spraying. The results indicated that griseofulvin and triadimefon both had control effect to eggplant powdery mildew. Though the effect of triadimefon was a little better than griseofulvin, they did not display a significant difference. This indicated griseofulvin could also exhibit excellent control effect to powdery mildew.

Chemical synthesis of analogues: 2'-Hydroxylgriseofulvin (**b**) was purified as white powder. ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm) 11.84 (2'-OH, H, s), 6.45 (benzene, H, s), 5.32 (3'-CH, H, s), 4.03 (CH_3O-benzene, 3H, s), 3.91 (CH_3O-benzene, 3H, s), 3.42 (6'-CH, H, s), 2.75, 2.50 (5'-CH_2, 2H, m), 0.84 (CH_3, 3H, d, J = 6.2 Hz). ESI-MS: m/z 339.0 (M + H^+).

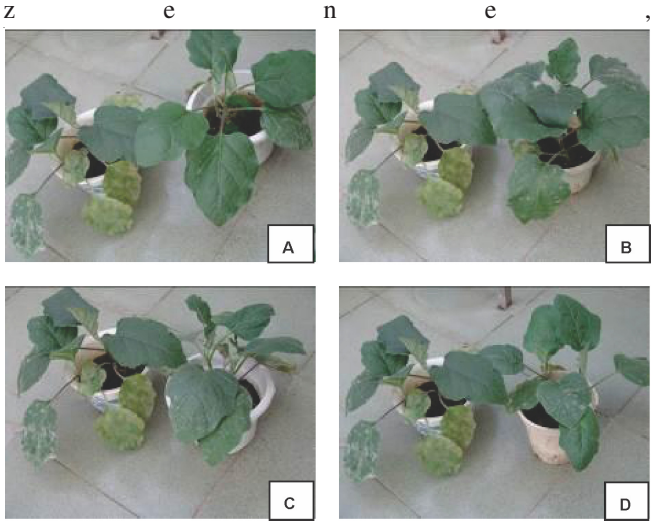


Fig. 3. Control effect of griseofulvin and triadimefon to eggplant powdery mildew. The pictures were taken 12 days after spraying. At pictures A and C, the left were control groups treated with water, the right were 1.25 mg/mL griseofulvin and 1.25 mg/mL triadimefon respectively. At pictures B and D, the left were control groups treated with water, the right were 2 mg/mL griseofulvin and 2 mg/mL triadimefon respectively

Epoxygriseofulvin (**c**) was purified as yellow powder. ^{1}H NMR ($CDCl_3$, 600 MHz): δ (ppm) 6.12 (benzene, 1H, s), 4.00 (CH_3O-benzene, 6H, m), 3.49 (32-CH, H, m), 3.01 (2'-OCH_3, H, m), 2.82 (2'-OCH_3, H, d, J = 6.2Hz), 2.57 (2'-OCH_3, H, m), 2.05 (6'-CH, H, s), 1.27 (5'-CH_2, 2H, d), 0.95 (CH_3, 3H, dd, J = 6.7, 23.3 Hz). ESI-MS: m/z 369.2 (M + H^+).

5-Nitrylgriseofulvin (**d**) was purified as yellow powder. ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm) 5.55 (3'-CH, H, s), 4.04 (CH_3O-benzene, 3H, s), 3.98 (CH_3O-benzene, 3H, s), 3.62 (2'-CH_3O, 3H, s), 3.03 (5'-CH, H, m), 2.84 (52-CH, H, m), 2.43 (6'-CH, H, dd, J = 4.7, 16.7 Hz), 0.96 (CH_3, 3H, d, J = 6.8 Hz). ESI-MS: m/z 398.3 (M + H^+).

4'-Hydroxylaminegriseofulvin (**e**) was purified as light yellow crystals. ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm) 6.20 (benzene, H, s), 5.58 (3'-CH, H, s), 4.02 (CH_3O-benzene, 3H,

TABLE-1
IC_{50} VALUES (mM) OF GRISEOFULVIN TO FOUR FUNGI WITHIN A 95 % CONFIDENCE INTERVAL

Fungi	Inhibition rates (%) at different concentrations (mM)				IC_{50} (mM)
	0.25	1	4	16	
F. moniliforme	5.8 ± 0.7	39.7 ± 2.4	44.8 ± 0.6	52.4 ± 5.0	7.43 ± 1.55
F. solani	26.9 ± 0.1	33.7 ± 1.3	45.2 ± 2.7	53.5 ± 3.5	9.30 ± 7.25
F. oxysporum	23.4 ± 0.6	38.4 ± 0.3	56.9 ± 0.2	59.7 ± 0.3	3.70 ± 1.98
C. truncatum	49.7 ± 3.1	51.1 ± 1.5	54.9 ± 1.0	58.2 ± 0.7	0.39 ± 0.22

TABLE-2
CONTROL EFFECT OF GRISEOFULVIN AND TRIADIMEFON TO EGGPLANT POWDERY MILDEW

Treatment	Initial DI	3 d		5 d		8 d		12 d	
		DI	CE (%)	DI	CE (%)	DI	CE (%)	DI	CE (%)
1.25 mg/mL griseofulvin	19.50	17.25	11.50	28.25	27.73	34.28	26.26	29.70	33.56
2 mg/mL griseofulvin	23.00	18.65	18.90	28.75	26.40	33.92	28.82	28.80	38.48
1.25 mg/mL triadimefon	25.00	18.00	28.00	26.67	31.71	29.64	36.92	25.36	45.83
2 mg/mL triadimefon	11.50	11.56	5.40	25.35	35.8	28.57	41.63	24.90	46.81
Water	20.60	20.87	–	39.06	–	44.64	–	46.82	–

DI = Disease index; CE = Control efficiency.

s), 3.93 (CH_3O-benzene, 3H, s), 3.54 (2'-CH_3O, 3H, m), 2.84 (6'-CH, H, dt, J = 14.6, 12.0 Hz), 2.42 (5'-CH_2, H, m), 1.06 (5'-CH_2, H, t, J = 6.6 Hz), 0.81 (CH_3, 3H, s). ESI-MS: m/z 368.3 ($M + H^+$).

4'-Hydroxygriseofulvin (**f**) was purified as white powder. ^1H NMR ($CDCl_3$, 600 MHz): δ (ppm) 5.34 (32-CH, H, s), 6.15 (benzene, H, s), 3.97, 4.04 (CH_3O-benzene, 6H, s), 3.42 (52-CH, H, s), 3.67 (22-CH_3O, 3H, s), 2.16 (CH_2, 2H, m), 0.84 (CH_3, 3H, d, J = 6.8 Hz). ESI-MS: m/z 377.2 ($M + Na^+$).

Antifungal activity of griseofulvin and analogues in mycelial growth assay and correlation of structure and activity: The antifungal activities of synthesized analogues **b-f** were tested against phytopathogenic fungi *i.e.*, *F. moniliforme*, *F. solani*, *F. oxysporum* and *C. truncatum*. Griseofulvin was employed for comparison purposes as a standard in this test. The results were shown in Table-3. 2'-Hydroxylgriseofulvin (**b**), 5-nitrylgriseofulvin (**d**) and 4'-hydroxylgriseofulvin (**f**) were totally inactive; epoxygriseofulvin (**c**) and 4'-hydroxylaminegriseofulvin (**e**) performed differently against various fungi compared to griseofulvin. The inhibition rates of them were measured and IC_{50} values were calculated.

TABLE-3
IC_{50} VALUES (mM) TO FOUR FUNGI WITHIN A 95 % CONFIDENCE INTERVAL. **a** DENOTES GRISEOFULVIN; **b**, 2'-HYDROXYLGRISEOFULVIN; **c**, EPOXYGRISEOFULVIN; **d**, 5-NITRYLGRISEOFULVIN; **e**, 4'-HYDROXYLAMINEGRISEOFULVIN AND **f**, 4'-HYDROXYLGRISEOFULVIN

Comp.	Fungus			
	F. moniliforme	*F. solani*	*F. oxysporum*	*C. truncatum*
a	7.43 ± 1.55	9.30 ± 7.25	3.70 ± 1.98	0.39 ± 0.22
b	–	–	–	–
c	7.23 ± 2.95	13.25 ± 6.30	3.80 ± 2.82	7.49 ± 0.85
d	–	–	–	–
e	5.90 ± 2.97	3.57 ± 2.50	6.27 ± 4.56	1.66 ± 1.45
f	–	–	–	–

Their inhibition rates even against the same fungi differed and all became improved with the concentrations increasing as showed in Fig. 4. This meant they were dose-dependent. The inhibition effectiveness of griseofulvin (**a**), epoxygriseofulvin (**c**) and 4'-hydroxylaminegriseofulvin (**e**) against four fungi, indicated from IC_{50} values, was listed in Table-3. The antifungal activities of griseofulvin were profoundly altered by changes in chemical structure; and some synthesized analogues were even better than griseofulvin as we expected. The antifungal activities of epoxygriseofulvin (**c**) only increased when against *F. moniliforme* compared to griseofulvin. However, the antifungal activities of 4'-hydroxylaminegriseofulvin (**e**) against *F. moniliforme* and *F. solani* increased phenomenally. Although the antifungal effectiveness of analogue 4'-hydroxylaminegriseofulvin (**e**) were not so good as griseofulvin, its IC_{50} 1.66 mM was also very low which meant they could exhibit considerable inhibitory effects as well.

These results also demonstrated that among the four fungi, griseofulvin (**a**) and 4′-hydroxylaminegriseofulvin (**e**) performed best against *C. truncatum* compared to the other three fungi and epoxygriseofulvin (**c**) performed best against *F. oxysporum*. The effectiveness of griseofulvin against *C. truncatum*

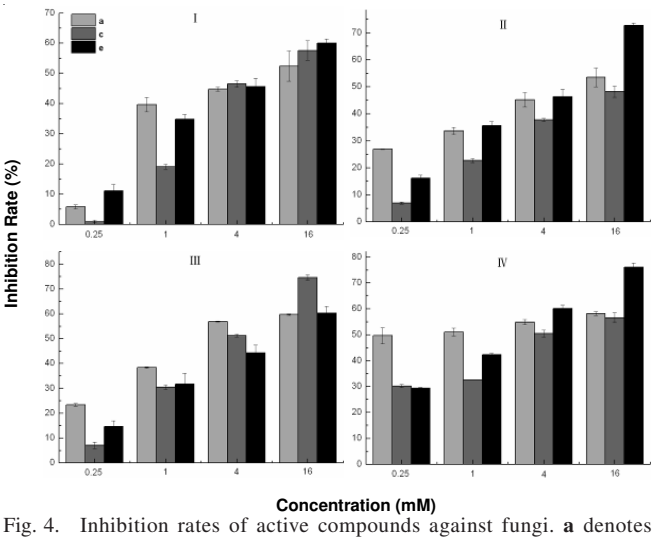


Fig. 4. Inhibition rates of active compounds against fungi. **a** denotes griseofulvin; **c**, epoxygriseofulvin and **e**, 4'-hydroxylaminegriseofulvin. I represents inhibition rates against *F. moniliforme*; II, *F. solani*; III, *F. oxysporum* and IV, *C. truncatum*. The concentrations of these compounds were 0.25, 1, 4 and 16 mM, respectively

was fascinating, although 4'-hydroxylaminegriseofulvin (**e**) also showed its best activity against *C. truncatum*. The effectiveness of epoxygriseofulvin (**c**) against *F. oxysporum* was similar to that of griseofulvin. Grisepfulvin (**a**) and epoxygriseofulvin (**c**) both performed worst against *F. solani*; however, 4'-hydroxylaminegriseofulvin (**e**) could do well against *F. solani*. Besides, the fungicidal activity of 4'-hydroxylaminegriseofulvin (**e**) was better than that of griseofulvin against *F. moniliforme*.

Take all the compounds as a whole, introduction of a nitro at 5 position resulted in no activity, indicating that modification at this position was not tolerated. The 2' position and 2'-3' double bond were altered leading to the synthesis of 2'-hydroxylgriseofulvin (**b**) and epoxygriseofulvin (**c**), the activities of which were quite different even against the same fungus. 2'-Hydroxylgriseofulvin (**b**) showed no activity and this indicated that the alkoxy at the 2' position played an important role in inhibiting fungi. The activity of epoxygriseofulvin (**c**) towards *F. moniliforme* and *F. oxysporum* was comparable to griseofulvin. Besides, epoxygriseofulvin (**c**) was found to be less active than griseofulvin toward *F. solani* and *C. truncatum*. From the modification of 4' position, we got 2 compounds: 4'-hydroxylaminegriseofulvin (**e**) and 4'-hydroxylgriseofulvin (**f**). The activity of 4'-hydroxylaminegriseofulvin (**e**) toward *F. moniliforme* and *F. solani* increased considerably. Nevertheless, when it came to *F. oxysporum* and *C. truncatum*, 4'-hydroxylaminegriseofulvin (**e**) showed a lower activity in comparison with griseofulvin. 4'-Hydroxylgriseofulvin (**f**), available by reduction of griseofulvin, did not show any activity at all. The results from the 4'-position analogues indicated that this position was significant for the antifungal activity as removal of the ketone caused 4'-hydroxylgriseofulvin (**f**) inactive. Combining these data, it suggested that the 4' position should keep its double bond form.

According to Rhodes[16], three specific elements contributed to the antifungal action of griseofulvin in plants: the effect of griseofulvin on the pathogen; the transportation of

griseofulvin within the host; the extent to which chemical degradation occurs in both host and pathogen. As was known, griseofulvin could inhibit fungal mitosis[24] and its analogues probably followed this inhibition mechanism. The prerequisite of their inhibitory effect was that they must reach to their reaction sites, which meant first they had to cross cell walls or to be absorbed by cells. Nevertheless, hyphal walls of the test fungi might be able to show some difference in their requirements for the physical properties of an analogue which could give the best adsorption by and penetration of, the hyphae[17]. Therefore, the diversity of inhibition activity of these compounds including griseofulvin and analogues against the fungi might result from various rates of absorption of analogues by fungi. Also, based on Corvis *et al.*[9], the interactions of griseofulvin with membranes might be important for the mechanism of its biological activity. From this point, the specific membranes of various fungi might be partly responsible for the different performance of griseofulvin to them. But it did not necessarily mean the analogues could exhibit excellent activity even the absorption rate was significantly high because we should not fail to take account of the chemical degradation of analogues. In other words, the susceptibility of fungi to active analogues actually depended on their bioavailability to a great degree. In brief, the above considerations might help to explain the different performance of analogues.

Comparison of solubility: The solubility of compounds was evaluated roughly and compared to that of griseofulvin by the method of saturation. The weights of residues of griseofulvin and analogues were compared. If the weight of the residue of one analogue was larger than that of griseofulvin, the solubility of it was considered as lower than that of griseofulvin and *vice versa*. The results were presented in Table-4. The solubility in methanol of all five analogues was higher than that of griseofulvin. As to the solubility in ethanol, 4'-hydroxylaminegriseofulvin (**e**) was lower than griseofulvin while the others were higher. When it came to the solubility in solution of 10 % NaOH, only 2'-hydroxylgriseofulvin (**b**) and 4'-hydroxylaminegriseofulvin (**e**) were increased. Unfortunately, their solubility in water was not improved evidently compared to griseofulvin. Combined these with the results of antifungal activities, both the activity and solubility of epoxygriseofulvin (**c**) and 4'-hydroxylaminegriseofulvin (**e**) were get improved in certain circumstances.

Modifications on the structure of griseofulvin could afford effective biocides. Epoxygriseofulvin and 4'-hydroxylgriseofulvin synthesized in our project exhibited antifungal activity against phytopathogenic fungi. Their activity and solubility in various solvents got changed and even improved compared with griseofulvin. As the effect of active analogues on hyphal growth showed, they were teratogenic in fungi as well just like griseofulvin. Besides, griseofulvin also had control effect to powdery mildew. All these data showed griseofulvin could be served as a leading compound for novel antifungal agent development. They also enriched the knowledge of biological control of phytopathogenic fungi and other plant diseases.

TABLE-4
COMPARISON OF GRISEOFULVIN AND ANALOGUES ON SOLUBILITY IN DIFFERENT SOLVENTS. **a** DENOTES GRISEOFULVIN; **b**, 2'-HYDROXYLGRISEOFULVIN; **c**, EPOXYGRISEOFULVIN; **d**, 5-NITRYLGRISEOFULVIN; **e**, 4'-HYDROXYLAMINEGRISEOFULVIN AND **f**, 4'-HYDROXYLGRISEOFULVIN

Analogue	Methanol	Ethanol	10 % NaOH	Water
b	+[a]	+	+	–[b]
c	+	+	–	–
d	+	+	–	–
e	+	–	+	–
f	+	+	–	–

[a]Solubility was increased. [b]Solubility was not increased obviously or even decreased.

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