無水マレイン酸またはジメチルマレイミド構造を含む化合物群の灰色かび病菌(Botrytis cinerea)に対する殺菌活性

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Original Article

Antifungal activity of simple compounds with maleic anhydride or dimethylmaleimide structure against *Botrytis cinerea*

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Compounds with maleic anhydride or maleimide presented considerable antifungal activity, and several maleimide derivatives have been reported to possess marginal or null zootoxic activity. In order to research the antifungal activity of the maleic anhydride or maleimide structure in these compounds, 44 related compounds were purchased or synthesized, and their MICs against *Botrytis cinerea* were investigated. The results showed that most maleic anhydrides and maleimides presented antifungal activity against *B. cinerea*. 2,3-Dimethyl maleic anhydride had the highest activity (MIC=3 μg/mL) among the 27 simple compounds with maleic anhydride. Meanwhile, *N*-3,5-dichloroaniline-3,4-dimethylmaleimide was the best (MIC=0.1 μg/mL) of the 17 maleimide derivatives, with activities nearly equal to those of dichloran. Furthermore, the data in this article reveals that the polarity and steric hindrance of maleic anhydride derivatives could affect their antifungal activities.

Keywords: maleic anhydride, maleimide, *Botrytis cinerea*, antifungal activity.

Introduction

The *Botrytis* species are a group of serious plant pathogens; a representative one in this group is *B. cinerea*, which damages economically important crops. Many commercial fungicides have been used in an effort to eliminate *B. cinerea*. Until recently, fungicides basically belonged to three groups: carbamates, benzimidazoles, and cyclic imides. Due to the extreme variability observed within the pathogen, *B. cinerea* has developed resistance to some commercial fungicides.[1] Therefore, treatments with these fungicides rapidly became inefficient. Consequently, there is a great interest in developing novel and effective antifungal agents to combat this particularly damaging and drug-resistant fungus.[2,3]

Natural products with a maleic anhydride structure are valuable antifungal agents, enzyme inhibitors, and herbicides.[4] Among them, tautomycin **1**,[5,6] tautomycetin **2**,[7] viburspiran **3**,[8] phomoidride A **4**,[9,10] phomoidride B **5**,[9,10] zopfiellin **6**,[11,12] heveadride **7**,[13,14] and scytalidin **8**[15–17] (Fig. 1) have good antifungal activities. Tautomycin and tautomycetin were discovered in the screening of soil microorganisms for new antibiotics against *Sclerotinia sclerotiorum* in China.[5,7] Their MICs for *B. cinerea* were 125 μg/mL and 12.5 μg/mL, respectively. Viburspiran was found to have an eight-membered ring with two maleic anhydride units, and it exhibited antifungal activity against both *Microbotryum violaceum* and *B. cinerea*.[8] Zopfiellin was discovered in the 1990s in Japan. It suppresses the growth of several fungi, bacterial, and yeast species and is especially effective against *B. cinerea* (MIC=0.78 μg/mL[12]). Heveadride and its derivatives were discovered while screening for new antifungal substances from fungal sources against pathogenic filamentous fungi. They showed strong antifungal activity against various filamentous fungi.[13] Scytalidin inhibited the growth of stain and decay fungi associated with deterioration of pulpwood chips in outside storage.[16–18] These substances were comprised of at least one maleic anhydride structure.

Natural products with a maleimide structure also have high biological activities, such as showdomycin **9**,[19,20] aqabamycins **10**,[21] and arcyriaflavins **11**,[22] also have high biological activities (Fig. 2). All of them possess antifungal and anti-bacterial activity. In addition, *N*-(4-fluorophenyl)-dichloromaleimide, *N*-butylmaleimide, *N*-phenylbutylmaleimide, and other unnatural compounds with maleimide have been reported to possess excellent antifungal activity.[22,23] In particular, these maleimide derivatives had been shown to be a potent antifungal compound with low zoo-toxicity in mice. The LD_{50} of *N*-(4-fluorophenyl)-dichloromaleimide in mice was more than 15,000 mg/kg,[22]

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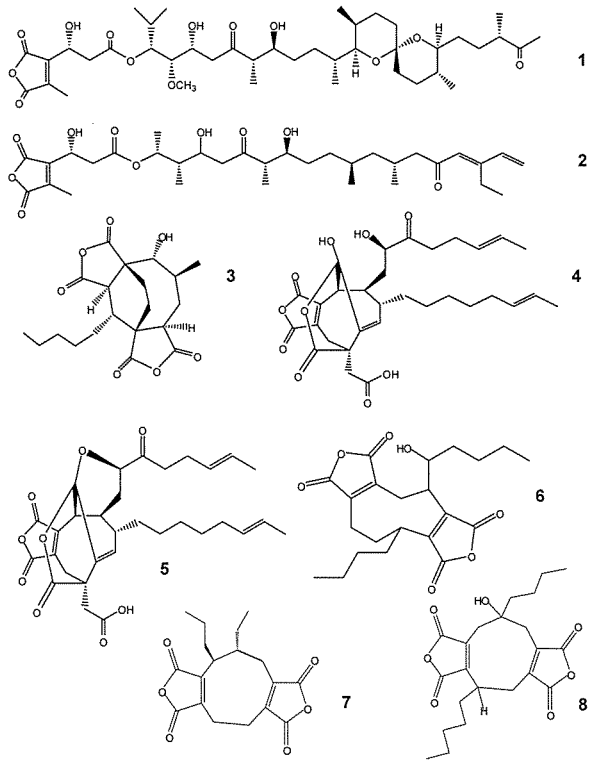


Fig. 1. Natural products with maleic anhydride.

whereas that of dichloran was approximately 1,500 mg/kg. Given that maleimide derivatives were as active as those zootoxic fungicides, maleimide derivatives would be more favorable. To obtain several maleimide derivatives with high activity, in this study, we synthesized a series of compounds and investigated their antifungal activity against *B. cinerea*. In addition, the initial structure-activity relationship could be obtained, which might facilitate further research.

Materials and Methods

1. Chemicals and chemical analysis

Maleic anhydride and phthalic anhydride (**12**) were both purchased locally. Trimellitic anhydride (**13**) was purchased from Alfa Aesar. 4-Methylphthalic anhydride (**14**), 3-fluorophthalic anhydride (**15**), 3,6-difluorophthalic anhydride (**16**), tetrafluorophthalic anhydride (**17**), 4,5-dichlorophthalic anhydride (**18**), 3,4-dichlorophthalic anhydride (**19**), 3,6-dichlorophthalic anhydride (**20**), 2-bromomaleic anhydride (**21**), phenylmaleic anhydride (**22**), diphenylmaleic anhydride (**23**), and dichloromaleic anhydride (**24**) were all purchased from Sigma(Fig. 3). The others, including **25–37**, were prepared according to the literatures[24–28] and confirmed to be the target compounds with ^{1}H-NMR. Their structures and synthetic methods were summarized in Table 1.

Seventeen *N*-substituted dimethylmaleimide derivatives, including **38–54** (Fig. 4), were synthesized according to improved procedures based upon reported methods.[29] All of the maleimide derivatives were synthesized using **25** and amines as the starting materials. The general procedure was as follows: a solution of amines of acetone or toluene was added dropwise to a solution of **25** of acetone or toluene at room temperature. The reaction mixture was allowed to reach 25–65°C and stirred for 1.5–8.5 hr. Then, after proper addition of anhydrous sodium acetate, iodinated and copper, triethylamine, and acetic anhydride into the mixture of the first step, the reaction mixture was refluxed for 3–15 hr more. The products were obtained with good yields following separation. The structures were elucidated by spectroscopic methods, such as ^{1}H-NMR, IR, and EIMS.

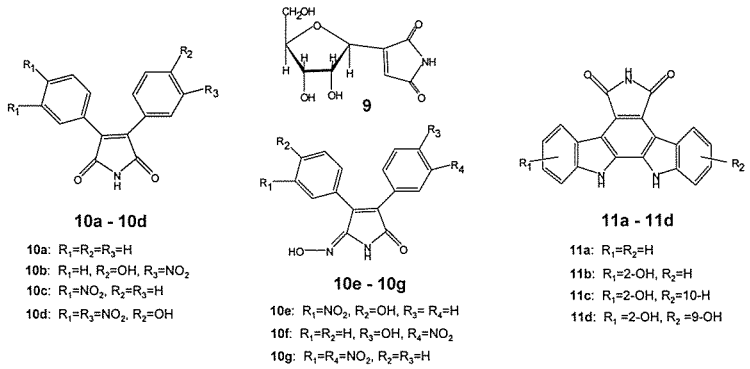


Fig. 2. Natural products with maleimide structure.

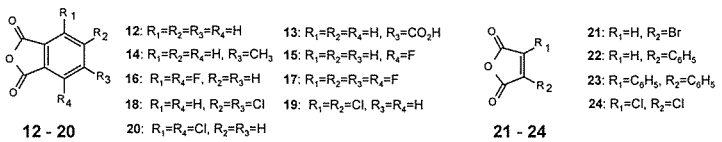
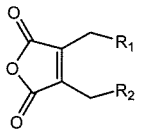


Fig. 3. Purchased compounds with maleic anhydride structure.

Table 1. Important synthetic applications of the derivatives of maleic anhydride



Starting material	Reaction	Product	Reference
Maleic anhydride	i) 2-amino pyridine, AcOH, reflux; ii) 4 N H_2SO_4, reflux, 2 hr	**25** (R_1=H, R_2=H)	[24]
25	NBS, BPO, CCl_4, reflux, 10 hr	**26** (R_1=H, R_2=Br)	[25]
26	i) 4 N, KOH, r. t., 5 hr; ii) H_2SO_4, r.t., 0.5 hr	**27** (R_1=H, R_2=OH)	[26]
26	i) diethyl malonate, NaH, C_6H_6, r.t., 8 hr; ii) HCl, r.t., 0.5 h	**28** (R_1=H, R_2=CH(COOEt)$_2$)	[25]
28	18% HCl, reflux, 12 hr	**29** (R_1=H, R_2=CH_2COOH)	[25]
25	NBS, BPO, CCl_4, reflux, 40 hr	**30** (R_1=Br, R_2=Br)	[27]
26	CH_3CH_2MgX, Et_2O, HMPA, CuI, −5 to 0°C, 8 hr	**31** (R_1=H, R_2=CH_2CH_3)	[28]
26	$CH_3(CH_2)_3MgX$, Et_2O, HMPA, CuI, −5 to 0°C, 8 hr	**32** (R_1=H, R_2=$(CH_2)_3CH_3$)	[28]
26	$CH_3(CH_2)_4MgX$, Et_2O, HMPA, CuI, −5 to 0°C, 8 hr	**33** (R_1=H, R_2=$(CH_2)_4CH_3$)	[28]
26	$CH_3(CH_2)_8MgX$, Et_2O, HMPA, CuI, −5 to 0°C, 8 hr	**34** (R_1=H, R_2=$(CH_2)_8CH_3$)	[28]
27	CH_3COOH, DCC/DMAP	**35** (R_1=H, R_2=CH_3COO)	[24]
27	C_6H_5COOH, DCC/DMAP	**36** (R_1=H, R_2=C_6H_5COO)	[25]
26	3,5-dichloro-benzenamine, DCC/DMAP	**37** (R_1=H, R_2=3,5-dichlorobenzenamine)	[26]

2. Biological materials

B. cinerea strain ACCC 30387 was provided by Agricultural Culture Collection of China, the strain was maintained on potato dextrose agar (PDA) at 21°C.

3. Bioassays

The antifungal activity of compounds with maleic anhydride or maleimide structure against *B. cinerea* was measured on a solid PDA medium. Compounds were dissolved in a Tween 80 aqueous solution. The pH of the medium was adjusted to 7.0 with 1 M NaOH or 1 M HCl solution. The solution was mixed with PDA to obtain final concentrations of 10, 50, 100, 150, 200, 500 and 1000 μg/mL. A mixture of PDA and Tween 80 aqueous solution was used as the control sample. The medium was poured into 9-cm diameter Petri dishes (10 mL/plate), which were then inoculated with a 6 mm diameter mycelial plug with 3-day-old *B. cinerea*. Plates in three replicates were used for each experiment. All plates were incubated in the dark at 21°C for 3 days, the time by which the growth of the control would have reached the edge of the plate. Growth inhibition was calculated as the percentage of inhibition of radial growth relative to the control. Each test was repeated three times.

Results and Discussion

1. Antifungal activities of compounds with maleic anhydride against B. cinerea

The antifungal activities of 27 kinds (dichloran and **12–37**) of chemical compounds were evaluated. The results are shown in Table 2.

The antifungal activity of **25** was good (3 μg/mL). However, when the compounds were not symmetrical, their activities decreased. Taking **31**, for example, its MIC reached 25 μg/mL, almost 10 times as high as that of **25**, although the former had only one more ethyl than the latter. When there were no groups (maleic anhydride) or only one group on maleic anhydride, the activities against *B. cinerea* were very low.

The compounds with maleic anhydride and the phenyl group, such as **12–20** (Fig. 3), had no obvious antifungal activities against *B. cinerea* (MICs>150 μg/mL). The explanation that the phenyl ring is more stable than the maleic anhydride ring, which may cause the double bond on the phenyl ring to become more inactive than that on the maleic anhydride, is one possibility. This results in no obvious antifungal activity against *B. cinerea* for these compounds.

The polarity of two alkyl groups was also a very important

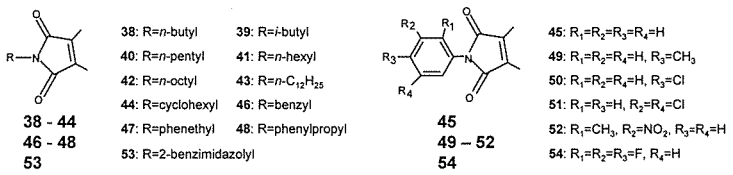


Fig. 4. Synthesized compounds with maleimide structure.

Table 2. Effect of maleic anhydride derivatives on *in vitro* mycelial growth of *B. cinerea*

Compound with maleic anhydride	MIC (μg/mL)	Compound with maleic anhydride	MIC (μg/mL)
Maleic anhydride	250	Dichloran	0.1
12	>1000	**25**	3
13	>1000	**26**	10
14	>1000	**27**	>1000
15	500	**28**	8
16	150	**29**	80
17	500	**30**	125
18	500	**31**	25
19	>1000	**32**	5
20	>1000	**33**	4
21	1000	**34**	3
22	150	**35**	20
23	20	**36**	15
24	100	**37**	50

factor influencing the antifungal activity. Weak polarity enhanced the activity, as in the case of **31**–**34** (Table 1). With the same methyl group at the third site, the length of the carbon-chain at the second site determined their polarity. The longer alkyl group resulted in less polarity and higher activity. This result suggests that a compound with less polarity may more easily pass through a cell membrane.

The steric hindrance was another factor to affecting the activity. The activity of **23** was lower than that of **25**. Compared to that of **25**, the substituent of **23** was larger, which could more easily bring about the steric hindrance.

2. *Antifungal activities of compounds with maleimide against* B. cinerea

In further research, 17 compounds (**38**–**54**) with a maleimide unit were synthesized. Most of them had higher activity than that of maleic anhydride derivatives (Table 3).

Both **45** and **54** could inhibit the growth of *B. cinerea* at the concentration of 1 μg/mL, and the activity of **51** (MIC=0.1 μg/mL) was equal to that of dichloran. Therefore, it was potentially utilizable for replacing dichloran. On the condition in which potentiality is developed, postharvest crop diseases could be controlled efficiently. Regretfully, we could not get the structure activity relationship from Table 3. Much more study will be required to obtain the activity structure relationship.

Conclusions

Taking into account heavy yield losses caused by the gray mol, *B. cinerea* was chosen as a working system to target the identification of novel antifungal compounds. In the study, 27 simple compounds with a maleic anhydride structure were tested for antifungal activity against *B. cinerea*. Some compounds with maleic anhydride proved to have antifungal activity against *B. cinerea*, and 2,3-dimethyl maleic anhydride (**25**) was the best one (MIC=3 μg/mL). In this study, we initially determined that the activity of maleic anhydride derivatives was related to the steric hindrance and polarity. Through preliminary screening of *N*-substituted dimethylmaleimides, a series of compounds with high activity was obtained. To a certain degree, both maleic anhydride and dimethymaleimide derivatives possess antifungal activity against *B. cinerea*. However, the structure-activity relationship was not obvious from the data shown in Table 3. In addition, the action mechanisms of these compounds were not studied. Therefore, considerable work remains for the screening of maleimide derivatives with higher antifungal activity. This is a promising area that deserves further research.

Table 3. Effect of compounds with dimethylmaleimide on *in vitro* mycelial growth of *B. cinerea*

Compound with dimethylmaleimide	MIC (μg/mL)	Compound with dimethylmaleimide	MIC (μg/mL)
38	5	**47**	75
39	50	**48**	10
40	5	**49**	5
41	5	**50**	10
42	50	**51**	0.1
43	50	**52**	10
44	10	**53**	10
45	1	**54**	1
46	5	Dichloran	0.1

Acknowledgements

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英文編掲載報文・短報等の要旨

報　文

種の感受性分布を用いた育苗箱施用殺虫剤の生態リスク比較

永井孝志，横山淳史

水田における殺虫剤使用が湛水散布から育苗箱施用に切り替わった場合の生態リスクの違いを評価した．箱施用剤の代表としてイミダクロプリドとフィプロニル，湛水散布の代表としてフェニトロチオンを評価対象とした．種の感受性分布の手法を用いて，河川水中と田面水中の生態リスクを「影響を受ける割合」を指標として定量化した．フェニトロチオンの生態リスクが最も高く，箱施用剤への切り替えはリスクを下げると予測された．また，メソコスム試験の報告と比較することにより評価結果の検証を行った．影響を受ける種の割合とメスコスム中での生物群集への影響の大きさは良く対応していた．50%以上の種が影響を受けると評価される場合であっても，その影響は一時的であり実際の生物群集は強い回復性を有していることが明らかとなった．

界面活性剤を利用した土壌洗浄によるメチルパラチオンの除去およびその後の生物的排水処理

Luis G. Torres, Fany Ramos, Marco A. Avila, Irmene Ortiz

本研究の目的は，メチルパラチオン（MP）汚染土壌を，界面活性剤を加えて土壌洗浄するとともに，その結果生じた廃液を好気性（浸液）バイオフィルターで処理する総合的な工程を示すことにある．MPを約0.4 mg/kgまたは13 mg/kg含有する土壌を，陰イオン性，非イオン性，両性，および天然系の界面活性剤を加えて洗浄したところ，初期濃度に対して63～98%のMPが除去された．この土壌洗浄によって生じた廃液に天然系の界面活性剤であるローカストビーンガムを0.01% w/wになるように加え，好気性バイオフィルターの実験に使用した．その結果，廃液中MP濃度は6日間で初期値の0.78 mg/Lから0.05 mg/Lまで低下し，本法の実効性が示された．（文責：編集事務局）

無水マレイン酸またはジメチルマレイミド構造を含む化合物群の灰色かび病菌（*Botrytis cinerea*）に対する殺菌活性

Wei Li, Yongxian Fan, Zhenzhong Shen, Xiaolong Chen, Yinchu Shen

無水マレイン酸やマレイミド構造を含む化合物が顕著な抗菌活性を示すこと，および，マレイミド誘導体の多くは動物毒性をほとんど示さないことが知られている．そこで，44関連化合物を購入または合成し，*Botrytis cinerea*に対する抗菌活性を検討したところ，ほとんどの化合物で活性が認められた．2,3-ジメチル無水マレイン酸は，無水マレイン酸構造をもつ27化合物中で最強の殺菌活性（MIC = 3 μg/mL）を示した．一方，17のマレイミド誘導体の中では，*N*-3,5-ジクロロアニリン-3,4-ジメチルマレイミドが最強の活性（MIC = 0.1 μg/mL）を示し，それはジクロランに匹敵するものであった．更に本実験データから，無水マレイン酸誘導体における極性および立体障害がその活性に影響を与えることも示された．（文責：編集事務局）

キュウリ果実およびメタノール・水で抽出された土壌中ディルドリン濃度の関係

清家伸康, 酒井美月, 村野宏達, 岡本真理, 齋藤　隆, 成田伊都美, 橋本良子, 池田悠里, 遠藤昌伸, 大谷　卓

12圃場で4品種のキュウリを栽培し，果実中ディルドリン濃度と50%(v/v)メタノール・水で抽出した株元土壌中ディルドリン濃度との関係を解析した．その結果，いずれの品種においても両者の間には有意（$p<0.01$）な正の相関関係が認められ，果実中濃度の品種間差は回帰式の傾きで表現された．果実中濃度を土壌中濃度（全量抽出）で除した値（FCF）は夏採り＞冬採りの関係にあり，収穫時期が異なるとキュウリ果実中濃度が変動する可能性がある．以上の結果から，50%(v/v)メタノール・水で抽出した土壌中濃度からキュウリ果実中ディルドリン濃度の予測値を精度良く得るには，同一品種を栽培し，かつ，同一の収穫時期において同一部位からキュウリ果実を採取する必要があることがわかった．

短　報

クロルフェナピルの効力増強キャリアーとして用いる分散性シリカナノ粒子

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長年にわたり安価に販売されてきた分散性シリカナノ粒子をキャリアーとして用いて，クロルフェナピルのナノ製剤を試製した．クロルフェナピルを充填したシリカ粒子の大きさは50～200 nmで，有効成分含量は39.78%である．室内および圃場試験において，ナノレベルの製剤の殺虫力は，マイクロレベル製剤の2倍であることが示された．本論文は，今後のナノ製剤の実用化に向けた重要な基礎的データを提示する