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Research and Development of Macrocyclic Compounds as Fungicides

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1. Introduction

"The latest economical development in agriculture calls for an increasing demand for food and feed on optimized plant production. If we are to intensify crop production, the efficient control of plant disease is essential. At present the most reliable means of doing this is by the use of fungicides" (Dehne, 2007). Unfortunately, resistance to these fungicides has always been observed, thus, leading to the continuing need of further research to discover new classes of fungicides, especially those with novel modes of action. One strategy for discovering new fungicides is to mimick the chemistry of biologically active natural products. Naturally occurring macrocyclic compounds, especially macrolactam and macrolactone have attracted considerable interest of chemists and the natural products community because they display a diverse range of biological activity including pesticidal activity. Several examples include avermectins, a group of 16-membered ring lactones with anthelmintic, insecticidal and acaricidal activities (Fisher, 1990 and Green, 1991), spinosyns, a group of 12-membered ring lactones with insecticidal activity (Sparks *et al*, 1998; Crouse *et al*, 2001), epilachnene, a kind of structurally novel azamacrolide with antifeedant activity (Attygalle *et al*, 1993), pyrenophorol, a macrodiolide with herbicidal activity (Kastanise and Chrysayi-Tokousbalides, 2000), and maltophilin, a novel macrolactam, produced by strains of *Stenotrophomonas maltophilia* R3089 isolated from the rhizosphere of rape plants (*Brassica napus L.*), which exhibited biological activity against a broad spectrum of fungi (Jakobi *et al*, 1996).

This chapter will describe the discovery of fungicidal novel lead compounds, especially those macrocyclic compounds which have relative simple structure and can be synthesized easily from readily available raw materials, by mimicking the structure of natural occurring macrolactone and macrolactam, and the development of the compounds with high fungicidal activity.

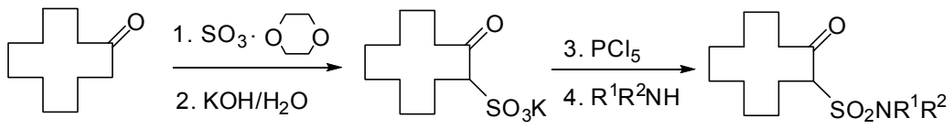
2. Research of the cyclododecanone derivatives

Cyclododecanone is an important intermediate in the organic synthesis and can be prepared from cyclododecatriene which can easily be produced in the large scale from petrochemical product butadiene (Wilke & Muller, 1958 and Weber *et al*, 1965). In this section, synthesis and fungicidal activity of several series of cyclododecanone derivatives are described.

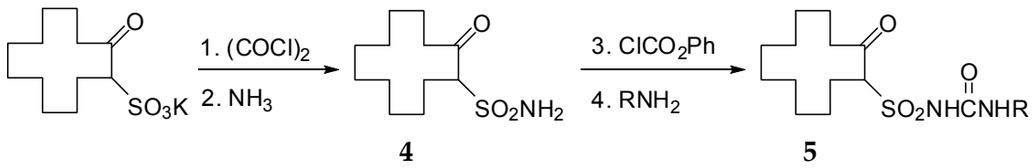
2.1 Synthesis and fungicidal activity of 2-oxocyclododecylsulfonamides (3) and 2-oxocyclododecylsulfonylureas (5)

Wang and Wang (1997) reported the synthesis and fungicidal activity of 2-oxocyclododecylsulfonamides (**3**). Compounds **3** were synthesized by amination of 2-oxocyclododecylsulfonic acid chloride, obtained from the readily available cyclododecanone (**1**) via 2-oxocyclododecylsulfonate (**Scheme 1**).

Bioassay showed that compounds **3** are active against *G. zeae* (*Gibberella zeae* Petch). Among them, compounds only having one substituent on the nitrogen of sulfonic acid amide group are more active than those having two substituents on the nitrogen of sulfonic acid amide group. This may mean the importance of hydrogen-bonding donor in the structure. In addition, QSAR study (CoMFA) (Xie *et al*, 1999) showed that 2-oxocyclododecylsulfonylureas (**5**) may have higher predicted fungicidal activity although some types of sulfonylureas are high efficient chemical herbicides. Thus, a series of compounds **5** were synthesized (Li *et al*, 2005) (**Scheme 2**).

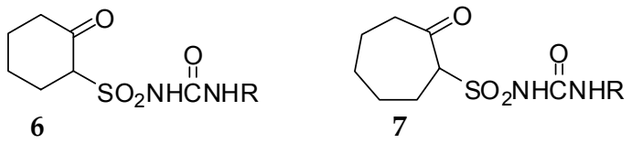


Scheme 1. Synthetic route of 2-oxocyclododecylsulfonamides (**3**)



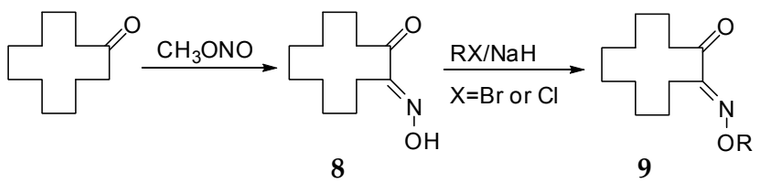
Scheme 2. Synthetic route of 2-oxocyclododecylsulfonylureas (**5**)

Compound **2** was allowed to react with oxalyl chloride to give corresponding sulfonyl chloride, which was converted into sulfonamide (**4**) using NH_3. The reaction of **4** with phenyl chloroformate and amines successively afforded desired compounds **5**. Bioassay showed that compounds **5** exhibited some fungicidal activity against *G. zeae* but do not fully accord with the prediction of CoMFA. The fungicidal activity of a representative compounds, N-(2,5-dichlorophenyl)-N'-(2-oxocyclododecylsulfonyl)urea against seven fungi [(*G. zeae*, *B. cinerea* (*Botrytis cinerea* Pers), *C. orbiculare* (*Colletotrichum orbiculare* Arx), *P. aphanidermatum* (*Pythium aphanidermatum* Fitzp), *F. oxysporum* (*Fusarium oxysporum* Schl.f.sp Vasinfectum), *R. solani* (*Rhizoctonia solani* Kuhn), and *V. dahliae* (*Verticillium dahliae* Kled)] was further evaluated. The results showed that it has better fungicidal activity against *C. orbiculare* and *P. aphanidermatum* than the commercial fungicide carbendazim. In addition, corresponding 2-oxocyclohexylsulfonylureas (**6**) and 2-oxocycloheptylsulfonylureas (**7**) were also synthesized and their fungicidal activity against *G. zeae* was evaluated. The result showed that compounds containing 12-membered ring (**5**) are more active than those containing 6- or 7-membered ring (**6**, **7**), which indicated that 2-oxocyclododecyl may be an active group showing pesticidal activity and is worth to pay attention to in research and development of novel pesticides.



2.2 Synthesis and fungicidal activity of (*E*)-α-oxocyclododecanone oxime ethers (9)

Li *et al* (2006) reported the synthesis and fungicidal activity of (*E*)-α-oxocyclododecanone oxime ethers (**9**). As shown in **Scheme 3**, compounds **9** were synthesized by oximation of cyclododecanone followed by etherification. Configuration of α-oxocyclododecanone oxime (**8**) was determined to be *E* isomer via the Beckmann reaction which gives 11-cyanoundodecanoic acid (Hou *et al*, 1999). Its *E* configuration was further confirmed by single crystal X-ray diffraction analysis of a representative of compound **9** (R = 4-$FC_6H_4CH_2$) (Li *et al*, 2006).

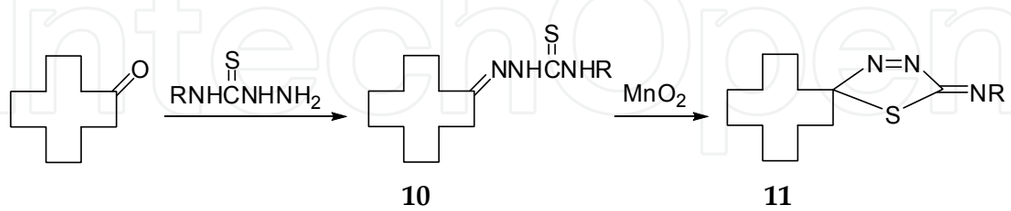


Scheme 3. Synthetic route of (*E*)-α-oxocyclododecanone oxime ethers (**9**)

Bioassay showed that most of compounds **9** present good fungicidal activity against *R. solani*, *C. cucumerinum (Cladosporium cucumerinum* Ell.et Arthur), *C. orbiculare*, *B. cinerea*, *F. oxysporum*, and *P. asparagi (Phomopsis asparagi* Bubak). Although their activity against *R. solani, C. cucumerinum, B. cinerea, F. oxysporum* and *P. asparagi* are lower than commercial fungicides carbendazim, but the activity of individual compound (e.g. R=$CH_2CO_2CH_3$) against *C. orbiculare* is better than carbendazim.

2.3 Synthesis and fungicidal activity of 2-(1,11-undecylidene)-5-substituted imino-Δ^3-1,3,4-thiadiazolines (11)

Zhang *et al* (2001) and Chen *et al* (2002) reported the synthesis and fungicidal activity of 2-(1,11-undecylidene)-5-substituted imino-Δ^3-1,3,4-thiadiazolines (**11**).



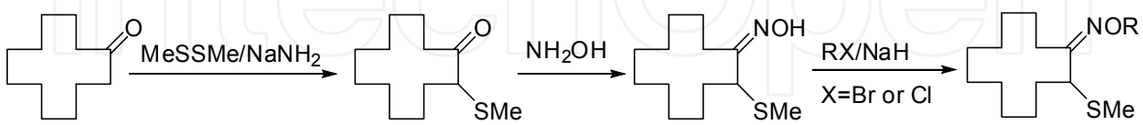
Scheme 4. Synthetic route of 2-(1,11-undecylidene)-5-substituted imino-Δ^3-1,3,4-thiadiazolines (**11**)

As shown in **Scheme 4**, Compounds **11** were synthesized by oxidative cyclisation of N-substituted cyclododecanone thiosemicarbazones (**10**), prepared by condensation reaction of cyclododecanone and *N*-substituted thiosemicarbazide, on treatment with manganese dioxide. The conformation of compounds **11** was analyzed by NMR, molecular mechanic calculation and X-ray diffraction study (Wang *et al*, 2002). Bioassay showed that most of

compounds **11** have some fungicidal activity against *R. solani* and *V. dahlide* and individual compound exhibit good activity against *R. solani.*

Synthesis and fungicidal activity of α–methylthiocyclododecanone oxime ethers (14)

Song *et al* (2005) reported the synthesis and fungicidal activity of α–methylthiocyclododecanone oxime ethers (**14**). As shown in **Scheme 5**, compounds **14** were synthesized by alkylation of α–methylthiocyclododecanone oxime, which was prepared by methylthiolation of cyclododecanone followed by oximation.

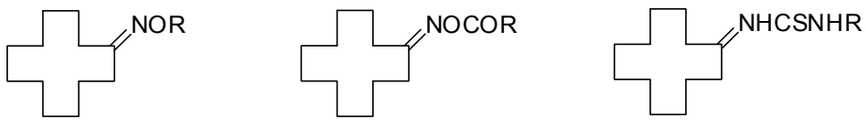


12 **13** **14**

Scheme 5. Synthetic route of α–methylthiocyclododecanone oxime ethers (**14**)

Bioassay showed that some of compounds **14** have good fungicidal activity against *R. solani and B. cinerea.*

Apart from mentioned above compounds (compounds **3**, **5**, **9**, **11** and **14**) carrying two substituents on the cyclododecane ring (carbonyl group and side chain, and thiadiazoline ring of compounds **11** may be considered as two substituents on the spiro carbon atom), monosubstituted cyclododecane derivatives, for example, **15**, **16** and **17**, were synthesized and were found to be completely ineffective as fungicides (Huang *et al*, 2007) (**Figure 1**). It is suggested that the coexistence of two polarizable groups on the cyclododecane ring is necessary for fungicidal activity of the cyclododecanone class of compounds. The results showed that it should be very useful for designing new classes of macrocyclic fungicides, especially those with novel modes of action.



15 **16** **17**

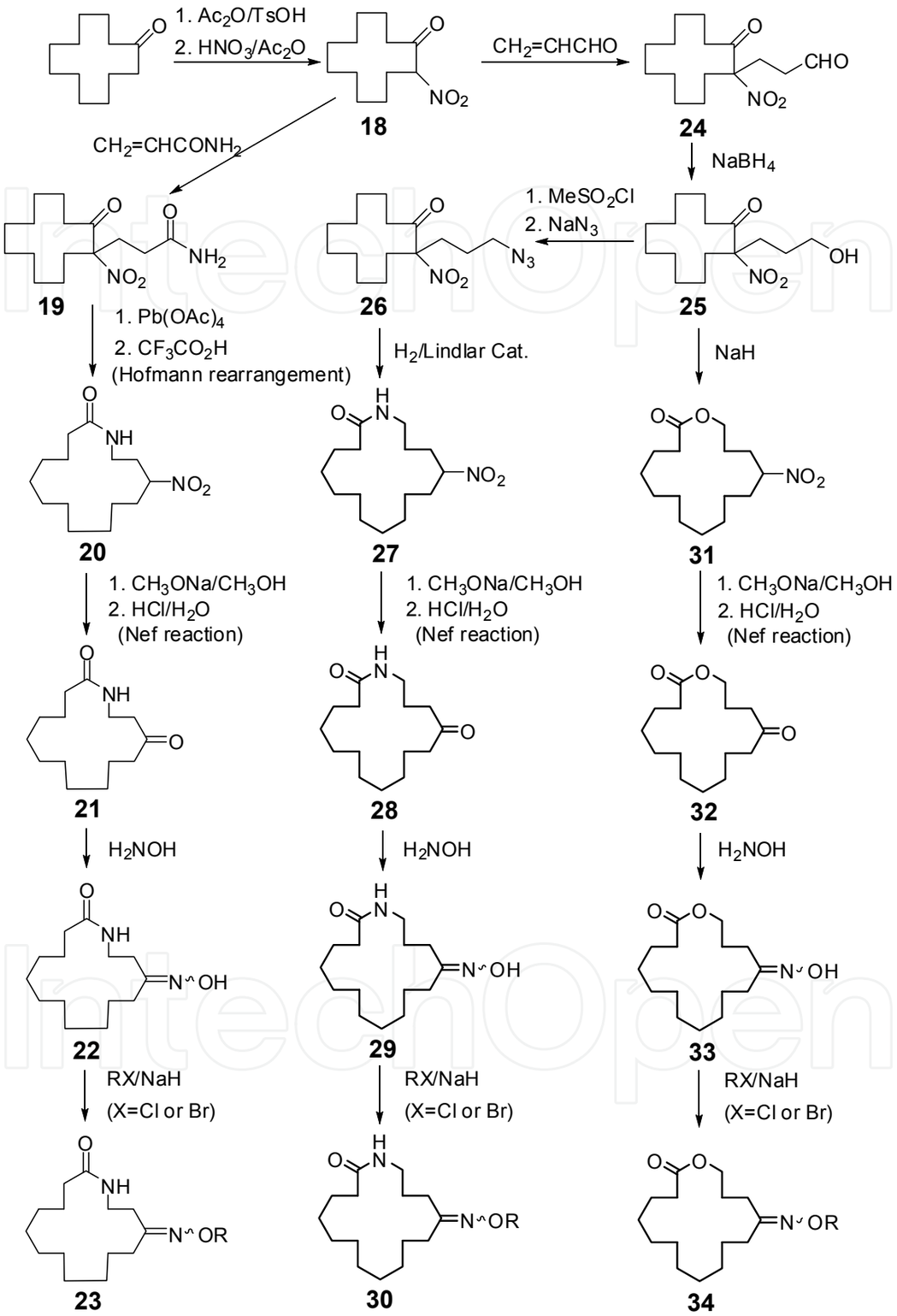
Fig. 1. Chemical structure of several monosubstituted cyclododecane derivatives

3. Research of macrolactam and macrolactone derivatives

In this section, the improvement of the fungicidal activity of the compounds with higher fungicidal activity (compounds **3**, **9** and **11**) in section 1 through structural derivation was described. The approach is to replace the cyclododecane ring in the cyclododecanone derivatives by macrolactam or macrolactone rings.

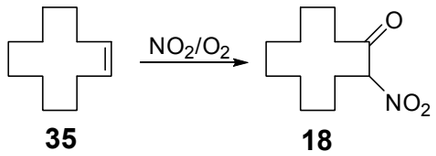
3.1 Synthesis and fungicidal activity of macrolactams and macrolactones with an oxime ether side chain (23, 30 and 34)

The improvement of the fungicidal activity of compounds **9** through the replacement of cyclododecane ring by macrolactam or macrolactone rings was described (Huang *et al*, 2007). Compounds 12-alkoxyiminotetradecanlactam (**23**), 12-alkoxyiminopentadecanlactam (**30**) and 12-alkoxyiminopentadecanlactone (**34**) were designed.



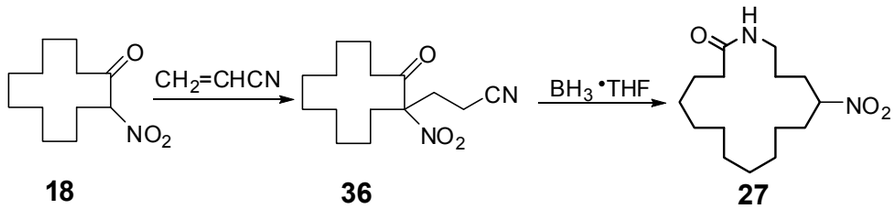
Scheme 6. Synthetic route of macrolactams and macrolactones with an oxime ether side chain

Compounds **23**, **30** and **34** were synthesized by oximation of compound **21**, **28** and **32** followed by etherification respectively. The compound **21** was synthesized from 2-nitrocyclododecanone (**18**), prepared from cyclododecanone, via Michael addition to acrylamide followed by Hofmann rearrangement, ring enlargement and Nef reaction (Pan and Wang, 1993; Jia *et al*, 2007). Compound **28** was synthesized from compound **18** via Michael addition to acrolein, selective reduction of aldehyde group followed by the conversion of hydroxyl group to amino group, ring enlargement and Nef reaction (Huang *et al*, 2004). Compound **32** was synthesized from compound **25** by ring enlargement and Nef reaction (Zhang *et al*, 2003) (**Scheme 6**).
Compound **18** was also synthesized from cyclododecene (**35**) by nitrooxidation (Fang *et al*, 2007) (**Scheme 7**).



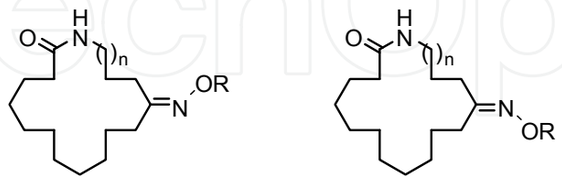
Scheme 7. Synthetic route of 2-nitrocyclododecanone (**18**)

Compound **27** was also synthesized from compound **18** via Michael addition to acrylnitrile followed by selective reduction of nitrile group, ring enlargement (Hou *et al,* 2006) (**Scheme 8**).



Scheme 8. Synthetic route of 12-nitro-1,15-pentadecanlactam (**27**)

There exist two isomers (*Z* and *E* isomers) for compounds **23**, **30** and **34**. The *Z* and *E* isomers of compounds **23** and **30** were isolated by column chromatography (**Figure 2**), but the two isomers of compounds **34** could not be separated due to the insufficient polarity difference in their structures.



n=0 for compounds **23**, n=1 for compounds **30**

Fig. 2. *Z* and *E* isomers of compounds **23** and **30**

As shown in **Table 1**, compounds **23** have fair to good fungicidal activity against *R. solani*. In general, the following structure-activity relationship in compounds **23** was observed: (1) The compounds with C3-C4 straight chain alkyl and benzyl without any substituent have better

fungicidal activity; (2) *E* isomers are more active than *Z* isomers, especially the compounds **23a** (*E*), **23c** (*E*), and **23f** (*E*), the EC_{50} values of which were 9.11, 7.21 and 7.24 μg/mL respectively, displayed higher fungicidal activity than corresponding *Z* isomers. The replacement of tetradecanlactam ring with pentadecanlactam ring (compounds **30**) results in significantly improved fungicidal activity. For example the EC_{50} values of the compounds **30a** (*E*), **30c** (*E*), **30c** (*Z*), **30h** (*Z*) were 3.62, 2.34, 3.97 and 2.34 μg/mL respectively. The pentadecanlactone derivatives (**34**) have somewhat improved fungicidal activity against *R. solani* than that of **23**, but less active than **30**. Namely, in the order of **23**, **34** and **30**, the compounds have a gradual increase of fungicidal activity. The results confirmed the original judgment in section 1: macrocyclic compounds with two polarizable groups on the ring may have certain fungicidal activity. It can also be seen from **Table 1**, all of tetradecanlactam and pentadecanlactam derivatives containing two polarizable groups –CONH- and =N-O- on the ring (**23** and **30**), and pentadecanlactone derivatives containing –COO- and =N-O- on the ring (**34**) displayed fair to excellent fungicidal activity against *R. solani*. In the macrocyclic derivatives with two polarizable groups on the ring, the compounds in which there is a three methylene distance ($CH_2CH_2CH_2$) between the two polarizable groups (**30**, **34**) are more active than those in which there is a two methylene distance (CH_2CH_2) between the two polarizable groups (**23**). The fact that compounds **30** have higher fungicidal activity than compounds **34** indicates that the macrocyclic derivatives with a hydrogen-bonding acceptor (here is =N-O-) and a hydrogen-bonding donor (here is -CONH-) have the best fungicidal activity among the macrocyclic derivatives with two polarizable groups and a three methylenes distance between these groups.

R	Compds No.	EC_{50} (μg /mL)	Compds No.	EC_{50} (μg /mL)	Compds No.*	EC_{50} (μg /mL)
$CH_3CH_2CH_2$	**23a** (Z)	22.10	**30a** (Z)	13.14	**34**a	8.08
	23a (E)	9.11	**30a** (E)	3.62		
CH_2=$CHCH_2$	**23b** (Z)	44.03	**30b** (Z)	45.61	**34**b	12.76
	23b (E)	21.76	**30b** (E)	15.92		
$CH_3(CH_2)_2CH_2$	**23c** (Z)	55.10	**30c** (Z)	3.97	**34**c	13.36
	23c (E)	7.21	**30c** (E)	2.34		
CH_3 $(CH_2)_5CH_2$	**23d** (Z)	209.43	**30d** (Z)	19.25	**34**d	9.68
	23d (E)	50.35	**30d** (E)	27.88		
CH_3 $(CH_2)_{14}CH_2$	**23e** (Z)	135.04	**30e** (Z)	47.75	**34**e	40.39
	23e (E)	127.22	**30e** (E)	51.48		
C_6H_5 CH_2	**23f** (Z)	18.15	**30f** (Z)	5.84	**34**f	45.13
	23f (E)	7.24	**30f** (E)	8.28		
4-$ClC_6H_4CH_2$	**23g** (Z)	97.25	**30g** (Z)	68.37	**34**g	19.61
	23g (E)	84.79	**30g** (E)	164.28		
2-Cl-6-$FC_6H_3CH_2$	**23h** (Z)	1734.74	**30h** (Z)	2.34	**34**h	51.27
	23h (E)	42.99	**30h** (E)	6.21		

* A mixture of *Z* and *E* isomers

Table 1. Fungicidal activity of compounds **23, 30** and **34** against *R. Solani*

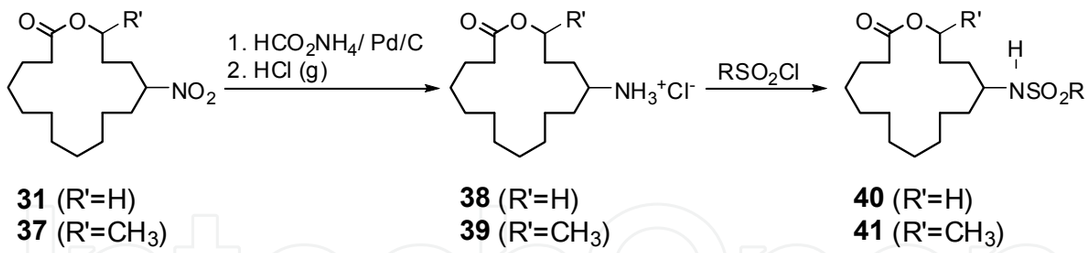
Furthermore, compound **30a** (a mixture of *Z* and *E* isomers) was selected as a representative to evaluated its fungicidal spectrum. The result is listed in **Table 2**. It can be seen from **Table 2** that compound **30a** has a broad spectrum of fungicidal activities, and especially has excellent fungicidal activities against *A. kikuchiana* (*Alternaria kikuchiana*), *P. physaleos* (*Phyllospicpa physaleos* Sacc), *R. solani* and *B. cinerea*. The EC_{50} values were 1.2, 1.9, 4.6 and 8.6 μg/mL respectively.

Pathogen	EC_{50} (μg/mL)
Pear black spot (*Alternaria kikuchiana*)	1.2
Tomoto southern blight (*Phyllospicpa physaleos Sacc*)	1.9
Cotton rhizoctonia rot (*Rhizoctonia solani Kuhn*)	4.6
Cucumber grey mold (*Botrytis cinerea Pers*)	8.6
Asparagus stem blight (*Phomopsis asparagi Sacc*)	12.0
Apple ring spot (*Physalospora piricola Nose*)	13.8
Cotton verticillium wilt (*Vercicillium alboatrum Reinke et Berthold*)	19.3
Cucumber anthracnose (*Colletotrichum Lagenarium*)	23.9
Cotton fusarium wilt (*Fusarium vasinfectum Atkimson*)	29.0
Cotton damping-off (*Pythium aphanibermatum (Eds.) Fipzp*)	33.3
Tomoto early blight (*Alternaria solani Jones et Grout*)	44.2
Tomoto leaf mold (*Cladosporium fulvum Cooke*)	53.1
Peppers fruit rot (*Phytophthora capsici Len*)	57.8

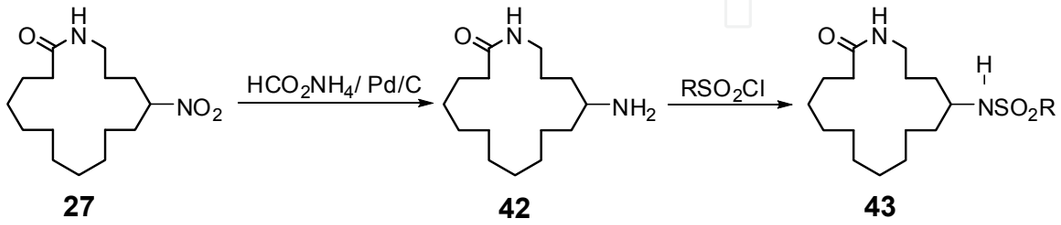
Table 2. Fungicidal spectrum of **30a** (a mixture of *Z* and *E* isomer)

3.2 Synthesis and fungicidal activity of macrolactones and macrolactams with a sulfonamide side chain (40, 41, 43 and 47)

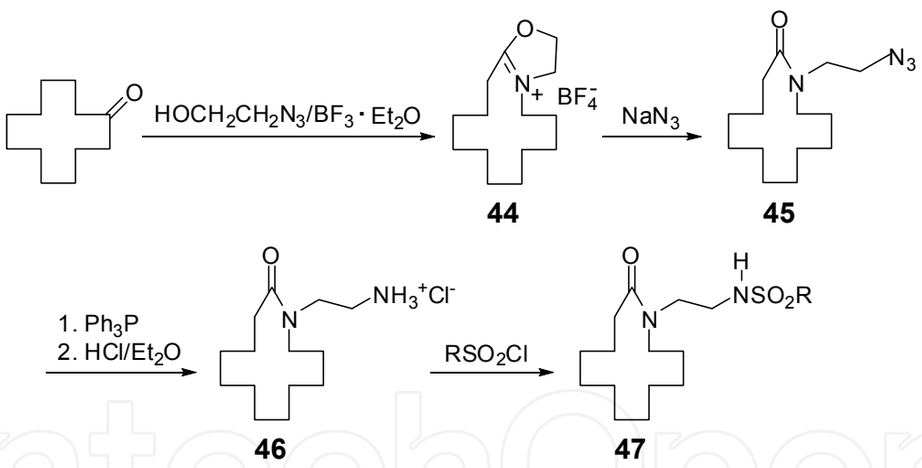
Based on the result obtained in section 3.1 and the structural feature of compounds **3** with certain fungicidal activity, Zhu *et al* (2008) introduced a sulfonamide group into pentadecanlactone, designing a series of novel 12-alkylsulfonamido-1,15-pentadecanlactones (**40**), which retain a hydrogen-bonding acceptor (here, it is –CO-O-) and a hydrogen-bonding donor (here, it is –NH-SO_2-) on the large ring and still have a three methylenes distance between two polarizable groups, and expect that the compounds have a better fungicidal activity than compounds **30** or comparable fungicidal activity with compounds **30**. In order to investigate whether the rule on the relationship between the activity and hydrogen-bonding has a general suitability to the macrocyclic compounds, further structural derivation on the compounds **40** was carried out: (a) A methyl group was introduced at C15 position and the 12-alkylsulfonamido-15-methyl-1,15- pentadecanlactones (**41**) were designed. (b) The lactone ring was replaced by lactam ring and the 12-alkylsulfonamido-1,15-pentadecanlactams (**43**) were designed. (c) The sulfonamide group was transferred to the terminal of side chain and still kept a suitable distance between the two polarizable groups, and the *N*-(alkylsulfonamidoethyl)-1, 12-dodecanlactams (**47**) were designed.



Scheme 9. Synthetic route of macrolactones with a sulfonamide side chain (**40** and **41**)



Scheme 10. Synthetic route of macrolactams with a sulfonamide side chain (**43**)



Scheme 11. Synthetic route of macrolactams with a sulfonamidoethyl side chain (**47**)

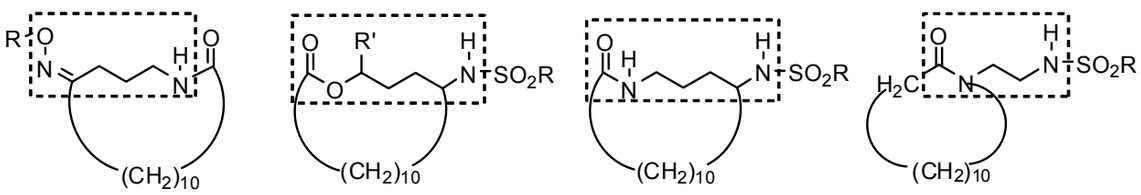
Compounds **40** and **41** were synthesized from intermediates **31** and **37** respectively by transfer hydrogenation using ammonium formate and palladium on carbon followed by sulfonylation with alkylsulfonyl chloride (**Scheme 9**). The compound **37** could be synthesized from nitrocyclododecanone (**18**) according to the method of synthesizing compound **31** just using methyl vinyl ketone instead of acrolein. Compounds **43** were synthesized from intermediate **27** according to the method of synthesizing compounds **40** (**Scheme 10**). The synthetic route of compounds **47** is shown in **Scheme 11**. Schmidt reaction (Li, 2006) of cyclododecanone with 2-azidoethanol followed by treating with sodium azide (Gracias *et al*, 1996; Gracias *et al*, 1997) give *N*-(2-azidoethyl)dodecanlactam (**45**), which was reduced and sulfonylated to afford compounds **47**.

As shown in **Table 3**, compounds **40**, **41**, **43** and **47** displayed fair to excellent fungicidal activity against *R. solani* and have a gradual increase of fungicidal activity in the order of **41**, **43**, **47**, and **40**. Compounds **40** displayed well to excellent activity except individual compound **40k**. Among them, compounds **40a**, **40b** and **40c**, the EC_{50} values of which were 2.4, 3.7 and 3.3 μg/mL respectively, displayed excellent fungicidal activity and were comparable with compounds **30**.

R	Compds No.	EC_{50} (μg/mL)	Compds No.	EC_{50} (μg/mL)	Compds No.	EC_{50} (μg/mL)	Compds No.	EC_{50} (μg/mL)
C_6H_5	**40a**	2.4	**41a**	51.3	**43a**	55.3	**47a**	11.6
4-MeC_6H_4	**40b**	3.7	**41b**	57.0	**43b**	13.4	**47b**	8.8
4-ClC_6H_4	**40c**	3.3	**41c**	69.6	**43c**	19.6	**47c**	18.7
3-$O_2NC_6H_4$	**40d**	18.7	**41d**	86.0	**43d**	27.6	**47d**	10.7
2,5-$Cl_2C_6H_3$	**40e**	28.4	**41e**	30.0	**43e**	30.4	**47e**	5.3
4-FC_6H_4	**40f**	4.5	**41f**	25.1	**43f**	36.7		
2-ClC_6H_4	**40g**	25.0	**41g**	37.9	**43g**	59.5		
2-$O_2NC_6H_4$	**40h**	22.3	**41h**	93.3	**43h**	21.5		
2-($MeCO_2$)C_6H_4	**40i**	20.9	**41i**	53.4	**43i**	12.8		
	**40j**	5.1	**41j**	20.3	**43j**	18.3		
$C_6H_5CH_2$	**40k**	52.4	**41k**	28.2	**43k**	8.1		
CH_3	**40l**	12.2	**41l**	56.4	**43l**	45.1		

Table 3. Fungicidal activity of compounds **40, 41, 43** and **47** against *R. solani*

As mentioned above the designed idea of compounds **40** was originated from compounds **30**. In view of the hydrogen-bonding acceptor and hydrogen-bonding donor, compounds **40** and **30** (**Figure 2**) have similar structural characteristics although compounds **40** are macrolactones with a sulfonamide side chain and compounds **30** are macrolactams with an oxime ether side chain. The results showed that the rule on the relationship between the fungicidal activity and hydrogen-bonding has a general suitability to the macrocyclic compounds. Compounds **47** have somewhat lower fungicidal activity against *R. solani* than that of compounds **40**. Although the former is 13-membered lactam derivatives and latter 16-membered lactone derivatives, they are similar in the chemical structure (active moiety is similar and all of lipophilic moiety are ten to eleven methylenes of large rings). The difference is that greater part of active moiety of compounds **47** is out of large ring as the skeleton of side chain which is flexible instead of rigid. The flexible characteristics of active moiety reduce the reactivity of molecule in combination with target enzyme. This may be the reason why the fungicidal activity of compounds **47** is somewhat lower than that of compounds **40**.



30 **40, 41** **43** **47**

Fig. 3. Comparison of structures of compounds **30**, **40**, **41**, **43** and **47**. The structures of active moiety of compounds in the square frames are similar.

Compounds **41** have a much lower fungicidal activity against *R. solani* (the EC_{50} values of all compounds **41** are larger than 20 μg/mL) than that of compounds **40**. However, their difference in chemical structure is only that there is a methyl group on the C15 for compounds **41** and none but hydrogen atom on the C15 for compounds **40**, which indicated that methyl group plays an inhibitory role to the fungicidal activity. May be the existence of methyl group with a great volume between two polarizable groups will interferes in the interaction of pesticide molecules with target enzyme as shown in **Figure 3**. In the molecule of compounds **43**, carbonyl group of amide can play the role as hydrogen-bonding acceptor, therefore they have good fungicidal activity, but the active hydrogen on the nitrogen atom adjacent carbonyl would interfere in the interaction of pesticide molecules and target enzyme. This may be the reason why fungicidal activity of compounds **43** is lower than compounds **40**.

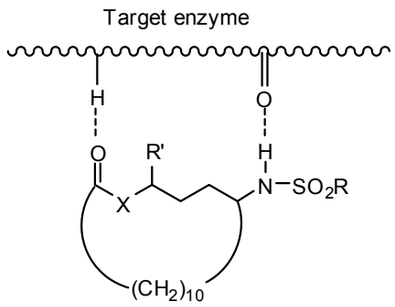


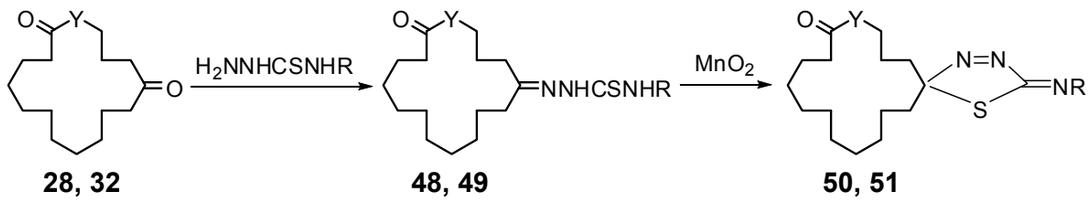
Fig. 4. Sketch map of the interaction of compounds **40**, **41** and **43** with target enzyme: Pesticide molecules combine with target enzyme by hydrogen-bondings. Interaction of pesticide molecule with target enzyme is interfered by methyl group for compounds **41** (R' is methyl group and X is O), and the interaction is interfered by active hydrogen on nitrogen atom for compounds **43** (X is NH and R' is H).

3.3 Synthesis and fungicidal activity of spiro-compounds containing macrolactam (macrolactone) and thiadiazoline rings (50, 51)

The result, from sections 3.1 and 3.2, indicated that it may be an effective approach to improve the bioactivity of cyclododecane derivatives to replace cyclododecane ring by macrolactam and macrolactone rings. Li *et al* (2010) designed and synthesized a series of spiro-compounds containing macrolactam and thiadiazoline rings (**50**), which retain a hydrogen-bonding donor (CO-NH) by replacing the cyclododecane ring of compounds **11** using macrolactam. For comparison, several spiro-compounds containing macrolactone and thiadiazoline rings (**51**) were also designed and synthesized.

Compounds **50** and **51** were synthesized by oxidative cyclisation of intermediates **48** and **49**, prepared by condensation reaction of 12-oxo-1,15-pentadecanlactam or 12-oxo-1,15-pentadecanlactone and *N*-substituted thiosemicarbazide, on treatment with manganese dioxide (**Scheme 12**)

Bioassay showed that compounds **50** have much better fungicidal activity than that of compounds **51** (**Table 3**). Compounds **50** have fair to excellent fungicidal activity against five fungi. However, compounds **51** have only poor fungicidal activity and the EC_{50} values of almost all compounds are greater than 30 μg/mL. It is worth notice that compound **50f** showed excellent fungicidal activity against *P. oryzae*, which is an important fungal pathogen causing serious damage to rice production in China.



28, **48** and **50**, Y=NH; **32**, **49** and **51**, Y=O

Scheme 12. Synthetic route of spiro-compounds containing macrolactam (macrolactone) and thiadiazoline rings (**50**, **51**)

X-ray diffraction analysis of two representative compounds (**50f**, R= p-ClC_6H_4; **51c**, R= o-BrC_6H_4) showed that their large ring skeleton can be described as [333133] and [33343] conformation respectively (CCDC 739298; Li *et al*, 2007). The conformation of the large ring skeleton of two compounds is somewhat different from each other. However, they should be similar in the solution due to the dynamic equilibrium (**Fig. 4**). Namely, in view of the conformation, compounds **50** and **51** have similar structural characteristics (**Fig. 5**). However, there are a hydrogen-bonding donor (-CONH-) and a hydrogen-bonding acceptor (N=N double bond of diazoline ring) on the large ring of compounds **50** and there are two hydrogen-bonding acceptor (-COO- and N=N double bond of diazoline ring) without hydrogen-bonding donor on the large ring of compounds **51**.

R	Compds No.	*B. cinerea*	*S. sclerotiorum*	*R. solani*	*P. asparagi*	*P. oryzae*
C_6H_5	**50a**	5.01	9.92	42.08	14.18	41.51
o-$CH_3C_6H_4$	**50b**	4.21	375.43	124.79	73.79	78.43
m-$CH_3C_6H_4$	**50c**	6.42	4.42	30.72	9.67	63.18
p-$CH_3C_6H_4$	**50d**	25.10	34.08	71.83	14.52	138.76
o-ClC_6H_4	**50e**	14.89	241.56	31.80	24.48	41.51
p-ClC_6H_4	**50f**	18.67	48.18	39.52	101.63	0.054
o-BrC_6H_4	**50g**	19.86	533.29	56.13	60.32	57.59
o-$CH_3OC_6H_4$	**50h**	21.85	21.09	37.89	517.04	397.84
p-$CH_3OC_6H_4$	**50i**	6.47	87.23	93.00	20.56	57.85
2,3-$(CH_3)_2C_6H_3$	**50j**	22.61	36.53	22.38	14.85	199.48
2,4-$(CH_3)_2C_6H_3$	**50k**	9.63	55.71	26.75	5.26	30.92
2,5-$(CH_3)_2C_6H_3$	**50l**	18.58	34.30	5.62	16.42	86.61
3,4-$Cl_2C_6H_3$	**50m**	34.23	15.03	36.82	9.02	0.72
2,5-$Cl_2C_6H_3$	**50n**	29.56	109.29	41.77	221.73	1.11
α-naphythyl	**50o**	15.73	767.24	17.80	46.15	195.80
benzyl	**50p**	14.44	8.32	23.49	21.33	116.85
o-ClC_6H_4	**51a**	43.81	169.20	159.06	116.49	211.36
p-ClC_6H_4	**51b**	49.95	59.91	126.32	42.48	338.37
o-BrC_6H_4	**51c**	36.42	204.57	207.69	53.55	246.10
p-BrC_6H_4	**51d**	49.88	38.30	17.10	29.21	338.37
2,5-$(CH_3)_2C_6H_3$	**51e**	126.29	162.84	421.80	208.41	3123.76

Table 4. Fungicidal activity of compounds **50** and **51** against five fungi (EC_{50}, μg/mL)

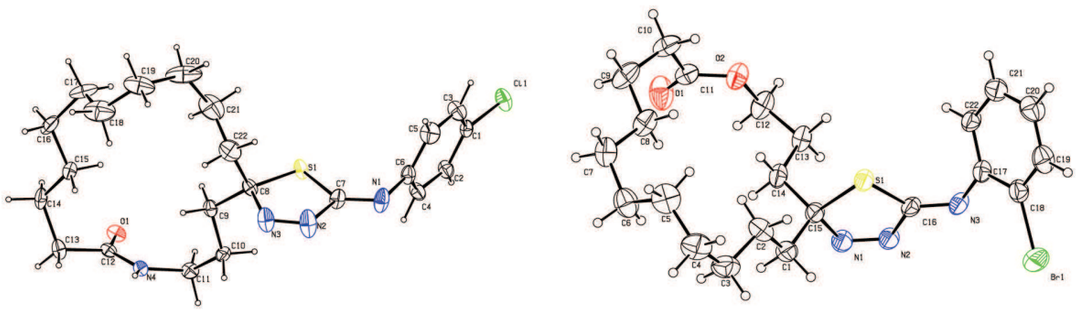


Fig. 5. The crystal structure of compound **50f** (left) and **51c** (right)

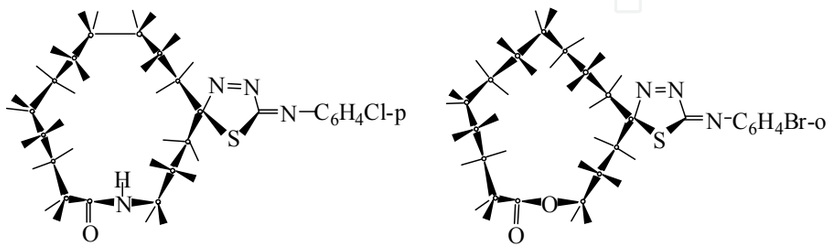
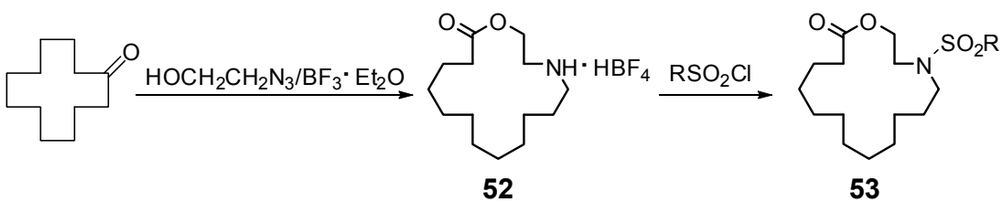


Fig. 6. The conformation of **50f** (left) and **51c** (right)

The results above showed that the presence of hydrogen-bonding donor to the fungicidal activity of macrocyclic compounds is very important and the rule on the relationship between the fungicidal activity and hydrogen-bonding has a general suitability to the macrocyclic compounds.

3.4 Synthesis and fungicidal activity of 16-oxo-1-oxa-4-azoniacyclohexadecan-4-ium tetrafluoroborate

Under the guidance of the rule on the relationship between the fungicidal activity and hydrogen-bonding, and following the principle of the simplicity of structure and the ease of synthesis, Dong *et al* (2008) designed and synthesized a novel azamacrolactone with a hydrogen donor (NH) and a hydrogen acceptor (C=O) on the ring and two methylene groups between these two polarizable groups, 16-oxo-1-oxa-4-azoniacyclohexadecan-4-ium tetrafluoroborate (**52**). And for further verifying the role of hydrogen donor, several sulfonyl-derivatives (**53**) of **52** with only one polarizable group (C=O) were also synthesized. Compound **52** was synthesized by intramolecular Schmidt reaction (Li, 2006) of cyclododecanone with 2-azidoethanol. And sulfonylation of **52** afford compounds **53** (**Scheme 13**).



Scheme 13. Synthetic route of 16-oxo-1-oxa-4-azoniacyclohexadecan-4-ium tetrafluoroborate (**52**) and its sulfonyl derivatives (**53**)

The fungicidal activity of compounds **52** and **53** against six fungus species in vitro was evaluated. The result showed that compound **52** has good fungicidal activity against these fungi, especially has excellent fungicidal activity against *R. solani* and is much better than compounds **53** which indicate that the presence of hydrogen-bonding donor to the fungicidal activity of macrocyclic compounds is indeed very important. In view of the ease of synthesis and the high fungicidal activity against *R. solani,* compound **52** will be expected to develop as a useful fungicide (see next section).

R	Compds No.	Inhibition rate (%)					
		Rhizoctonia solani Kuhn	*Fulvia fulva* (Cooke) Ciferri	*Colletotrichum orbiculare* (Berk. et Mont.) Arx	*Verticilliu m dahliae* Kleb.	*Sclerotinia sclerotiorum* (Lib.) de Bary	*Alterneria kikuchiarna* Tanaka
	52	96.0	89.3	73.2	86.9	85.7	85.7
C_6H_5	**53a**	0.0	63.6	56.3	61.9	61.3	66.6
p-MeC_6H_4	**53b**	0.0	50.1	46.4	50.7	39.5	52.5
o-Cl C_6H_4	**53c**	0.0	59.3	43.8	50.7	49.3	55.6
p-Cl C_6H_4	**53d**	0.0	45.0	0.0	44.5	39.5	55.6
o-$O_2NC_6H_4$	**53e**	0.0	45.0	43.8	34.6	36.0	52.5
m-$O_2NC_6H_4$	**53f**	0.0	45.0	35.4	50.7	46.2	46.2
p-$O_2NC_6H_4$	**53g**	7.5	45.0	29.6	44.5	39.5	55.6
p-(MeCO-NH)C_6H_4	**53h**	27.1	39.8	32.5	41.3	32.4	32.4

Table 5**.** Inhibition rate of the compounds **52** and **53** against six fungus species at 50 μg/mL

4. Development of the compounds with high fungicidal activity.

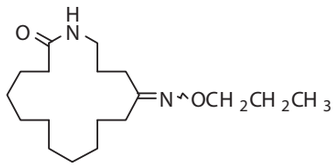
In this section, the development of two compounds (**30a** and **52**) with high fungicidal activity will be described.

4.1 The development of compound 30a

As described in section 3.1, 12-propoxyimino-1,15-pentadecanlactam (**30a**) (**Fig. 5**) exhibited a broad spectrum of fungicidal activity and especially excellent fungicidal activity against *A. kikuchiana*, *P. physaleos, R. solani* and *B. cinerea in vitro.* The EC_{50} values were 1.2, 1.9, 4.6 and 8.6 μg mL^{-1} respectively.

The diseases caused by *A. kikuchiana* and *P. physaleos* are not common in China. However, *R. solani* is an important agricultural fungus which causes serious decrease in yield especially in major cotton-growing regions in China (Chen *et al*, 1998; Gong *et al*, 2004; Deng *et al*, 2006), and grey mould caused by *B. cinerea* has become one of the most widely distributed diseases in vegetable (cucumber, tomato, calabash, eggplant, onion *etc*) and fruit (apple, grape, strawberry *etc*) growing regions and seriously affects crop production in China (Zhang *et al*, 2005; Xu *et al*, 2005; Chen *et al*, 2006; Wang *et al*, 2006).

In this section, the development of compound **30a** as a fungicide controlling cotton sheath blight (*R. solani*) and vegetable (cucumber and tomato) grey mould (*B. cinerea*) through pot culture and field efficacy trials, its dynamic distribution in cotton plants, and its toxicology was described (Huang *et al*, 2009. Original code name of **30a** was **7B3**)



30a

Fig. 7. Chemical structure of compound **30a**

4.1.1 Control effect of 30a against *R. solani*

Compound **30a** showed good control against *R. solani* on cucumber in the pot culture test (**Table 6**). The control effect of **30a** reached 53.5% at a rate of 315 g ai ha^{-1}, and was better than commercial fungicide thiram at a rate of 450 g ai ha^{-1} (42.1%). The control effect of **30a** reached 73.6% at a rate of 504 g ai ha^{-1} and is much better than thiram at a rate of 450 g ai ha^{-1}.

Treatment	Dosage, g ai ha^{-1}	Control effect (%)
30a 28% WP	168	39.0
30a 28% WP	315	53.5
30a 28% WP	504	73.6
thiram 50% WP	450	42.1

Table 6. Control effect of **30a** against *R. solani* on cucumber in pot culture test (Soil drenching)

In two-year two-place field efficacy trials, the control effect of **30a** against *R. solani* on cotton reached 64~92% at a rate of 140 g ai ha^{-1} and was better than or comparable to commercial fungicide carbendazin at the same rate (**Table 7**).

Treatment	Dosage		Control effect (%)	
	g, WP/1 kg seed	g, ai ha^{-1}	First year	Second year
Site: Jinan, Shandong Province, China				
30a 25% WP	4	93.3	88.9	65.3
30a 25% WP	6	140	91.5	74.7
carbendazin 50% WP	3	140	82.1	58.3
Site: Yuncheng, Shanxi Province, China				
30a 25% WP	4	93.3	69.2	61.9
30a 25% WP	6	140	72.7	64.4
carbendazin 50% WP	3	140	73.1	63.3

Table 7. Control effect of **30a** against *R. solani* on cotton in field efficacy trials (Seed dressing)

4.1.2 Control effect of 30a against *B. cinerea*

The control effect of **30a** against *B. cinerea* on tomato in the pot culture test reached 85.8% at a rate of 315 g ai ha^{-1} and 87.5% at a rate of 504 g ai ha^{-1} respectively, which is not as good as commercial fungicide huimeike [a mixed preparation of diethofencarb and chlorothalonil (1:1.8, w/w)], but is comparable to iprodione (**Table 8**). However, in a one-year one-place field efficacy trial (**Table 9**) **30a** displayed excellent fungicidal activity against *B. cinerea* on

cucumber. The control effect reached 95.1% at a rate of 450 g ai ha^{-1}, and was comparable to huimeike (control effect was 96.1% at a rate of 504 g ai ha^{-1}).

Treatment	Dosage, g ai ha^{-1}	Control effect (%)
30a 28% WP	168	80.7
30a 28% WP	315	85.8
30a 28% WP	504	87.5
huimeike 28% WP	315	96.6
Iprodione 50% WP	563	90.4

Table 8. Control effect of **30a** against *B. cinerea* on tomato in pot culture test (foliar spraying)

Treatment	Dosage, g, ai ha^{-1}	Control effect (%)
30a 25% WP	281	89.5
30a 25% WP	450	95.1
huimeike 28% WP	504	96.1

Table 9. Field efficacy trials of **30a** against *B. cinerea* on cucumber (Foliar spraying)

4.1.3 Dynamic distribution of 30a in cotton plant

The study showed that the concentration of **30a** reached 54.44 μg g^{-1} in roots of cotton plants 4 h after treatment with 500 μg mL^{-1} of **30a** (**Table 10**), which showed that **30a** can penetrate through the epidermis of the root and be absorbed by cotton plants. The content of **30a** in roots, stems and leaves all increased with extension of treatment time and the concentration of **30a** reached 137.05, 5.12 and 2.53 μg g^{-1} respectively, 48 h after treatment. However, the concentration of **30a** in roots was almost 27 and 54 times of the concentration in stems and leaves respectively, which revealed that **30a** has almost no acropetal translocation and systemic activity in cotton plant. The results provide a theoretical basis for an application method of **30a** for soil treatment or seed coating treatment.

Treatment Time (h)	Content of **30a** in the different parts of cotton plants (μg g^{-1})		
	root	stem	leave
4	54.44 ± 1.64	0.19 ± 12.30	0.04 ± 5.52
8	66.69 ± 2.81	0.87 ± 1.91	0.11 ± 2.52
16	96.99 ± 1.29	2.71 ± 2.08	0.33 ± 4.87
24	98.02 ± 2.08	4.27 ± 2.93	0.89 ± 2.48
36	116.25 ± 2.13	8.95 ± 6.84	2.93 ± 6.50
48	137.05 ± 4.39	5.12 ± 3.84	2.53 ± 5.80

Table 10. Distribution of **30a** in cotton plants when the chemical applied as root treatment

4.1.4 Toxicological test of 30a

The results of toxicological tests indicated that **30a** was a low toxicological compound （LD_{50} > 5000 mg kg^{-1} for acute oral and LD_{50} > 2000 mg kg^{-1} for acute dermal） based on classification standard procedure of People's Republic of China (People's Republic of China, 1995). The teratogenesis, mutagenesis and carcinogenesis tests were negative; therefore **30a** is safe for human being (**Table 11**).

Tested item	Results
Acute oral (Rats) (LD_{50} ,mg /kg^{-1})	> 5000
Acute dermal (Rats) (LD_{50} ,mg /kg^{-1})	> 2000
Eye irritation (Rabbits)	slight irritation
Skin irritation (Rabbits)	not irritation
Mutagenesis	negative
Teratogenesis	negative
Carcinogenesis	negative

Table 11. Toxicological profile of **30a**

4.1.5 Conclusion

30a has very weak systemic activity and is suitable for controlling cotton sheath blight with soil treatment or seed coating treatment. The field efficacy trials showed that the control effect of **30a** against *R. solani* on cotton is better or comparable to carbendazin and against *B. cinerea* on cucumber is comparable to huimeike. Therefore, **30a** may be expected to be further developed as a practical fungicide due to the high control effect, low toxicological properties and novel structure. However, the disadvantage is its long synthetic route from cyclododecanone (see **Scheme 6**).

4.2 The development of compound 52

As decribed in section 3.4, compound **52** (16-oxo-1-oxa-4-azoniacyclohexadecan-4-ium tetrafluoroborate) against six fungi (*R. solani* and so on) has good fungicidal activity, especially has excellent fungicidal activity against *R. solani*, an important agricultural fungus in China.

In this section, the development of compound **52** as a fungicide controlling cotton sheath blight (*R. solani*) through pot culture and field efficacy trials, and its toxicology was described (Dong *et al*, 2008. Original code name of **52** was **2a** or **D1**)

4.2.1 Control effect of 52 against *R. solani* on cotton

The results of potted test (**Table 12**) and field efficacy trials showed that compound **52** against *R. solani* on cotton was more active than commercial fungicide carbendazim.

Treatment	Dosage		Control effect (%)
	g, WP/1 kg seed	g, ai ha^{-1}	
52, 25% WP	2	47	81.8
52, 25% WP	3	70	87.2
Carbendazim, 50% WP	3	140	79.2

Table 12. Control effect of **52** against *R. solani* on cotton in pot culture test (seed dressing)

Treatment	Dosage		Control effect (%)	
	g, WP/1 kg seed	g, ai ha^{-1}	First year	Second year
52, 25% WP	4	93.3	55.8	48.9
52, 25% WP	6	140	61.9	63.6
carbendazin 50% WP	3	140	49.6	53.4

Table 13. Control effect of **52** against *R. solani* on cotton in field efficacy trials (seed dressing, site: Yuncheng, Shanxi Province, China)

4.2.2 Toxicological test of 52

The results of toxicological tests indicated that **52** was a low toxicological compound (LD_{50} > 3160 mg kg^{-1} for acute oral and LD_{50} > 2000 mg kg^{-1} for acute dermal)based on classification standard procedure of People's Republic of China (People's Republic of China, 1995). The mutagenesis, teratogenesis and carcinogenesis tests were negative; therefore **52** is safe for human being (**Table 14**).

Tested item	results
Acute oral (Rats) (LD_{50}, mg/kg^{-1})	> 3160 (male) > 3160 (female)
Acute dermal (Rats)(LD_{50}, mg/kg^{-1})	> 2000mg kg^{-1} (male) > 2000mg kg^{-1} (female)
Eye irritation (Rabbits)	slight irritation
Skin irritation (Rabbits)	not irritation
Mutagenesis	negative
Teratogenesis*	negative
Carcinogenesis*	negative

Table 14. Toxicological profile of **52**
*Unpublished results

4.2.3 Conclusion

The bioassay showed that compound **52** has excellent fungicidal activity against *R. solani* than commercial fungicide carbendazim. In addition, low toxicological property, short synthetic route and green synthetic technology with high atom economy (all of the atoms of raw materials including boron trifluoride were almost fully utilized except one nitrogen molecule and part of boron atoms lost, see section 3.4), indicated that compound **52** may be expected to further develop as a useful fungicide.

5. The biochemical mode of action of compounds 30a and 52 against *R. solani*

In this section, effects of compounds **30a** and **52** on ultrastructure of hyphae, cell membrane and respiration of mycelia cell suspension were described (Yan *et al*, 2010).

5.1 Effect of 30a and 52 on morphology and ultrastructure transformation of *R. solani*

Mycelia of *R. solani* grew smoothly along the surface of culture media without **30a** or **52**, and the shape of the whole colony appeared to be radiated from its central point. The fringe of

the colony was round and regular. However, the growth of mycelia was seriously depressed when it was cultured in the media with 50 μg mL^{-1} **30a** or **52**. The fringe of the colony was concavo-convex, irregular, and is not as smooth and regular as that of control mycelia.
SEM images indicated that the mycelial grew well in control media (Mu *et al*, 2006; Butler and Bracker, 1970) and it was of low density, fresh and had a fine structure, and most of the mycelia ramification occurred in right angle (**Fig. 8 A, D**). However, there were similar morphology changes in mycelia of *R. solani* when mycelia were cultured in culture media with **30a** or **52** of 50 μg mL^{-1}. Mycelia grew abnormally with comparatively high density of colony, the ramification was not in right angle any more, the distance between ramifications decreased and some mycelia were entangled each other (**Fig. 8 B, C**). In the presence of **30a** or **52**, the surface of mycelia was rough (**Fig. 8 B, E**) and mycelia were irregularly ramified and formed irregular ramification or abnormal configuration producing a "beaded" morphology with some parts of mycelia contracted and some parts swelled (**Fig. 8 C**).

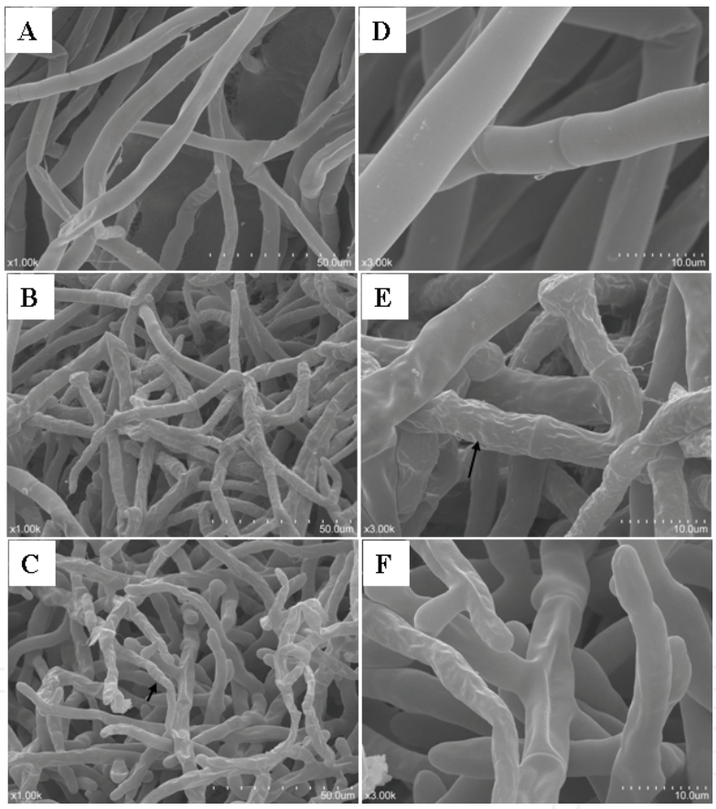


Fig. 8. Scanning electron micrographs of the hyphae from the colony of *R. solani* (A, D) 1000 (A) and 3000 (D) sections of *R. solani* hyphae grown on PDA medium in the absence of **30a** or **52** (control) (the mycelium was low density, fresh and had a fine structure); (B, E) 1000 (B) and 3000 (E) sections of *R. solani* hyphae grown on PDA medium containing 50 μg mL^{-1} **30a** (the mycelium was comparatively high density of colony and the surface of mycelium was rough (arrowhead)); (C, F) 1000 (C) and 3000 (F) sections of *R. solani* hyphae grown on PDA medium containing 50 μg mL^{-1} **52** (the amount of ramification increased and formed irregular ramification or abnormal configuration ("beaded" morphology) on the mycelium tip (arrowhead)). Bars: (A, B, C) 50.0 μm; (D, E, F) 10.0 μm

R. solani mycelial tips (5 mm) from the margin of actively growing colony on PDA medium were examined by TEM (**Fig. 9**). The cell walls and septa of the hyphae from the untreated control were uniform (**Fig. 9 B**, **C**). There were abundant organelles in cytoplasm such as vacuole (V), mitochondria (M) and lipid body (L) (**Fig. 9 A**). The dolipore septa and septal pore caps (SPCs) were obviously visible in control mycelia (**Fig. 9 D**). Following fungicides treatment, different ultrastructural modifications occurred in the hyphae (**Fig. 9 G**, **H** ultrastructure treated with **30a**; **Fig. 9 E**, **F**, **I**, **J** ultrastructure treated with **52**). The cell walls of the hyphae became considerably thicker following exposure to either **30a** or **52** (**Fig. 9 F**, **G**). The walls of the septa were also abnormally thickened (**Fig. 9 I**).

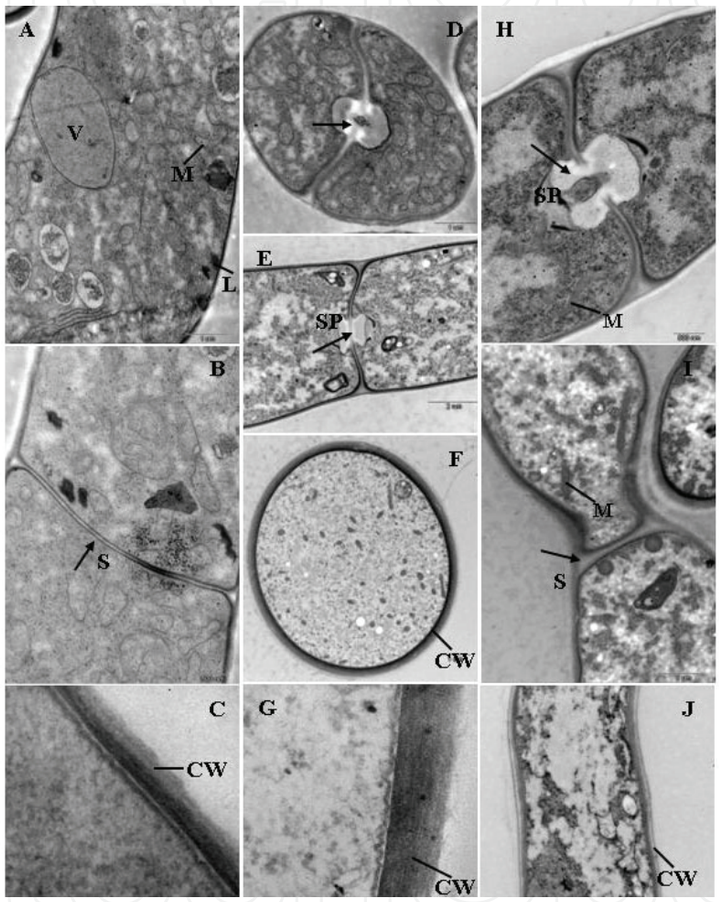


Fig. 9. Transmission electron micrographs of *R. solani* hyphae: (A, B, C, D) TEM of the hyphae of *R. solani* in the untreated control ((A) longitudinal of control hyphae; many organelles were observed such as vacuole (V), mitochondria (M) and lipid body (L); (B) uniform spectra (S) of control hyphae; (C) cell wall of control hyphae; (D) transverse of control hyphae and septal pore caps was visible); (E, F, I, J) TEM of the hyphae of *R. solani* treated with 50 µg mL^{-1} **52** ((E) longitudinal of **52**-treated hyphae (the septal pore caps disappeared); (F) transverse of **52**-treated hyphae (cell wall thickening); (I) longitudinal of **52**-treated hyphae (septum of hyphae thickening); (J) longitudinal of **52**-treated hyphae (cell wall thickening)); (G, H) TEM of the hyphae of *R. solani* treated with 50 µg mL^{-1} **30a** ((G) cell wall thickening of **30a**-treated hyphae; (H) longitudinal of **30a**-treated hyphae (the septal pore caps were almost unaffected)). Bars: (A, D, E, F, I, J) 1.0 µm; (B, H) 500 nm; (C, G) 100 nm.

Under the treatment of **30a** and **52**, the organelles became disorganized and decreased in the hyphae cytoplasm (**Fig. 9 F**, **G**). Another striking characteristics was the disappearance of septal pore caps of **52**-treated hyphae (**Fig. 9 E**), while the septal pore caps was almost not affected in **30a**-treated hyphae (**Fig. 9 H**).

SEM and TEM observations revealed that growth inhibition of *R. solani* as a response to **30a** and **52** was accompanied with marked morphological and cytological changes, including irregular ramification and a "beaded" morphology, excessive branching, irregular thickening of hyphae cell walls and necrosis or degeneration of hyphae cytoplasm. These changes were very similar to those occurring in some other fungi treated with chitosan and antibiotics, which inhibited fungi cell wall (Vesentini *et al*, 2007; Debono & Gordee, 1994). The cell wall of fungi is a sturdy structure providing physical protection and osmotic support, which is considered as that complex of macromolecules with chitin, glucan and mannose interconnected by covalent bonds. Hyphae growth, branching, cell fusion and other morphogenetic events all depended on a balance between decomposition and extension of the hyphae wall, as well as on synthesis and incorporation of new wall material (Wessels, 1993; Kang *et al*, 2001). In the present study, the hyphae walls of *R. solani* were thickened irregularly and the excessive branching of the hyphae, which were very similar to the phenomena induced by EBI (ergosterol biosynthesis inhibitor) fungicides (Kang *et al*, 2001) although there are great differences in chemical structure between **30a** or **52** and EBI fungicides. So it was also assumed that the thickness of cell wall and excessive branching might result from the changes of the activity of enzymes involved in wall synthesis. However, another phenomenon observed in TEM study was the increasing of thickness of septa and disappearance of septal pore caps of **52** treated hyphae. More studies should be done to interpret whether the phenomenon was in relation to cell wall associated enzymes.

5.2 The effect of compounds 30a and 52 on cell membrane of *R. solani*

The effect of **30a** and **52** on cell membrane was examined by measuring electrical conductivity of mycelia suspension. The conductivity of mycelia suspension treated with **30a**, **52** and triadimefon all increased extremely comparing with the conductivity of control mycelia along with all the time of treatment. **52** induced more significant electrolyte leakage from hyphae than **30a**, and similar change with triadimefon. Thus it was proposed that **30a** and **52** were all related to the impairment of cell membrane.

Electrolyte leakage was used as an indicator of cell membrane permeability of hyphae exposed to various fungicides. The alteration of conductivity induced by **30a** and **52** resembled with the alteration caused by triadimefon, one of a class of azole compounds which can inhibit ergosterol biosynthesis and damage the permeability of cell membrane (Yoshida *et al*, 1990). These results indicated that both **30a** and **52** caused damage to mycelia cell membrane system, induced electrolyte leakage from the cell, and as a result, the conductivity of solution was increased. Moreover, the presumption can also interpret the morphological and cytological alterations of the hyphae. Sterols are required for growth and reproduction of eukaryotic organisms and serve as architectural components of membranes. Thus, the thickening of hyphae walls was most likely to be associated with biochemical changes in the plasmalemma induced by **30a** and **52**. In addition, the phenomenon which **52** induced more significant grade in the morphological and cytological alterations and conductivity alteration than **30a** probably can be explained by the difference of solubility between **52** and **30a**, because **52** was a tetrafluoroborate possessing better solubility in water than **30a**.

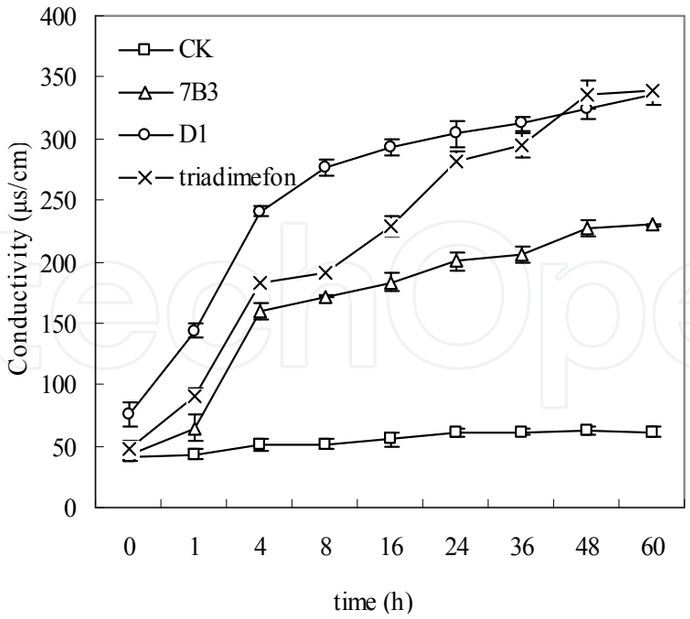


Fig. 10. Electrolyate leakage from *R. solani* suspensions during different time exposure to different fungicides. CK (□), **30a** (Δ), **52** (○), triadimefon (×). The conductivity of the solutions was measured using DDS-11C model conductivity detector at different treatment time after the addition of 1g fresh mycelia into 50 μg mL^{-1} **30a**, **52** and triadimefon solution, respectively.

5.3 Effect on respiration of intact mycelia

As shown in **Table 15**, both **30a** and **52** almost did not affect oxygen consumption of intact mycelia while azoxystrobin, a respiration inhibitor, exhibited the strong effect on oxygen consumption of intact mycelia. These results proved that either **30a** or **52** was not a respiration inhibitor and indicated that they did not disturb the energy generation system of *R. solani*.

Inhibitor	Concentration (μg mL^{-1})	R_0 (μmol O_2 g^{-1} min^{-1})	R_1 (μmol O_2 g^{-1} min^{-1})	Inhibition rate (%)
30a	10	27.03 ± 1.39	26.30 ± 0.85	3.39 ± 0.62
	100	27.03 ± 1.39	23.95 ± 7.49	12.05 ± 5.50
52	10	27.03 ± 1.39	26.77 ± 1.39	1.67 ± 1.02
	100	27.03 ± 1.39	26.41 ± 2.45	3.00 ± 1.80
azoxystrobin	10	27.03 ± 1.39	4.12 ± 1.80	84.86 ± 1.33

Table 15. Respiratory inhibition of intact mycelia of *R. solani* by **30a** and **52**

5.4 Conclusion

Both **30a** and **52** caused marked changes of hyphae with a "beaded" morphology, excessive branching, irregular thickening of hyphae cell walls and necrosis or degeneration of hyphae

cytoplasm and electrolyte leakage of membrane. In addition, neither **30a** nor **52** affected the respiration of mycelia. These results suggested that **30a** and **52** had the similar mode of action against *R. solani* relating to the impairment of biosynthesis of cell wall or membrane,

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CCDC 739298 contains supplementary crystallographic data for compound 50f. These data can be obtained free of charge from the CCDC, 12 Union Road, Cambridge CB2 1EZ,U.K. Telephone (44) 01223 762910; e-mail deposit@ccdc.cam.ac.uk

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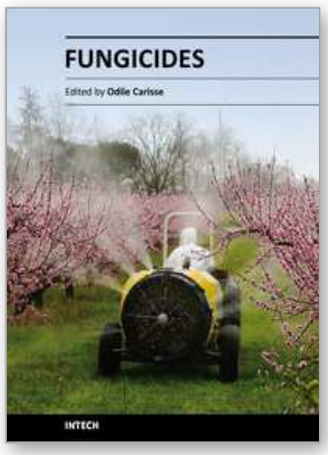

Fungicides

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Plant and plant products are affected by a large number of plant pathogens among which fungal pathogens. These diseases play a major role in the current deficit of food supply worldwide. Various control strategies were developed to reduce the negative effects of diseases on food, fiber, and forest crops products. For the past fifty years fungicides have played a major role in the increased productivity of several crops in most parts of the world. Although fungicide treatments are a key component of disease management, the emergence of resistance, their introduction into the environment and their toxic effect on human, animal, non-target microorganisms and beneficial organisms has become an important factor in limiting the durability of fungicide effectiveness and usefulness. This book contains 25 chapters on various aspects of fungicide science from efficacy to resistance, toxicology and development of new fungicides that provides a comprehensive and authoritative account for the role of fungicides in modern agriculture.

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