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Synthesis, Fungicidal Activity, and Structure−Activity Relationship of Spiro-Compounds Containing Macrolactam (Macrolactone) and Thiadiazoline Rings†

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Two series of novel spiro-compounds containing macrolactam or macrolactone and thiadiazoline rings, 1-thia-2-alkylimino-3,4,9-triaza-10-oxospiro[4.15]eicosyl-3-ene (**4F**) and 1-thia-2-alkylimino-3,4-diaza-9-oxa-10-oxospiro[4.15]eicosyl-3-ene (**4G**), were synthesized from 12-oxo-1,15-pentadecanlactam and 12-oxo-1,15-pentadecanlactone, respectively. Their structures were confirmed by elemental analysis, ^{1}H NMR, and ^{13}C NMR. The conformation of compounds **4F** was determined via the crystal structure of a representative compound (**4F$_6$**). The bioassay showed that compounds **4F** have much better fungicidal activity against five fungi (*Botrytis cinerea* Pers., *Sclerotinia sclerotiorum*, *Rhizoctonia solani* Kühn., *Phomopsis asparagi* Sacc., and *Pyricularia oryzae* Cav.) than compounds **4G**. The fact above showed that the presence of a hydrogen-bonding donor for the fungicidal activity of macrocyclic compounds is very important. **4F$_6$** showed excellent fungicidal activity against *P. oryzae*, which is much better than the commercial fungicide isoprothiolane, and **4F$_{13}$** showed excellent fungicidal activity against *P. oryzae* and good fungicidal activity against *P. asparagi*.

KEYWORDS: Spiro-compounds; macrolactam; macrolactone; thiadiazoline; fungicidal activity

INTRODUCTION

In recent years there has been intense investigation of thiadiazole derivatives, some of which are known to possess interesting pesticidal activity such as fungicidal activity (*1*), herbicidal activity (*2*), and insecticidal activity (*3*). Previous research has shown that a series of thiadiazole derivatives (**A**, **Figure 1**) containing thiadiazoline and cyclododecane rings have good fungicidal activity against *Rhizoctonia solani* Kühn and *Verticillium dahliae* (*4*).

It has also been reported that cyclododecanone derivatives, such as 2-oxocyclododecanone oxime ethers (**B**, **Figure 1**) (*5*) and N-substituted-2-oxocyclododecylsulfonamides (**C**, **Figure 1**) (*6*) have good fungicidal activity. Their fungicidal activity was improved by structural derivation employing macrolactam or macrolactone rings to replace cyclododecane ring. That is, alkoxyimino-macrolactams (or alkoxyimino-macrolactones) (**D**, **Figure 1**) (*7*) and 12-alkylsulfonamido-macrolactams (or 12-alkylsulfonamido-macrolactones) (*8*) (**E**, **Figure 1**) have better fungicidal activity than compounds **B** and **C**, which indicated that it may be an effective approach to improve the bioactivity of cyclododecane derivatives to replace the cyclododecane ring employing macrolactam and macrolactone rings.

In this paper the cyclododecane ring of compounds **A** was replaced by macrolactam or macrolactone rings, keeping the thiadiazoline ring, and as a result two series of novel spiro-compounds (**4F** and **4G**) were synthesized. Their fungicidal activity against five fungi (*Botrytis cinerea* Pers., *Sclerotinia sclerotiorum*, *Rhizoctonia solani* Kühn., *Phomopsis asparagi* Sacc., and *Pyricularia oryzae* Cav.) was evaluated in vitro. The synthetic route of compounds **4** is shown in **Scheme 1**.

MATERIALS AND METHODS

General. NMR spectra were recorded in $CDCl_3$ or DMSO-d_6, with a Bruker DPX300 spectrometer, using TMS as internal standard; elemental analysis was performed by the analytical center at the Institute of Chemistry (Beijing), Chinese Academy of Sciences. Melting points were measured on a Yanagimoto melting point apparatus and are uncorrected. The solvents and reagents were used as received or were dried prior to use as needed. Pyrimethanil (purity = 95%) was purchased from Jiangsu Fengdeng Pesticide Co., Ltd., Jiangsu Province, China. Chlorothalonil (purity = 98%) was purchased from Jiangsu Limin Chemical Industry Co., Ltd., Jiangsu Province, China. Thiram (purity = 96%) was purchased from Kerun Pesticide Factory, Tianjin, China. Isoprothiolane 40% emulsifiable concentrates (EC) was purchased from Zhejiang Weierda Chemical Industry Co., Ltd., Zhejiang Province, China.

Chemical Synthesis. Synthesis of compounds followed the outline in **Scheme 1**.

Synthesis of Compounds 1. Compounds **1** were synthesized from 2-nitrocyclododecanone as previously described (*9*, *10*).

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Synthesis of Compounds 2. Compounds **2** were synthesized from amines as previously described (*11*).

Synthesis of Compounds 3. Compounds **3** were synthesized from compounds **1** and **2** as previously described (*12*).

Synthesis of Compounds 4 (4, 13). To a stirred solution of compound **3** (3.0 mmol) in 80 mL of CH_2CL_2 was added MnO_2 (8 g). The mixture was further stirred for 30 min. After filtration, the filtrate was evaporated under reduced pressure to give a crude product, which was purified on silica gel column chromatography with petroleum ether and ethyl acetate to separate the compound **4**.

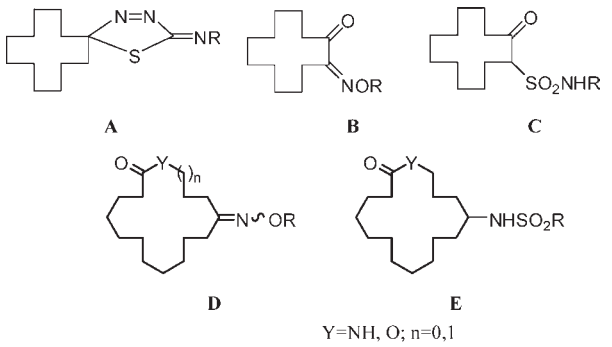
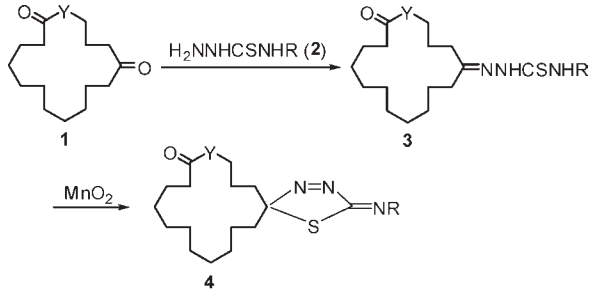


Figure 1. Structures of compounds **A**−**E**.

Scheme 1. Synthetic Route of Compounds **4**



1F, **3F**, **4F**, Y=NH; **1G**, **3G**, **4G**, Y=O.

X-ray Diffraction Analysis of Compound $4F_6$. The crystal of compound $4F_6$ was obtained by slow evaporation from a solution of petroleum ether and ethyl acetate. All measurements were made with a Siemens CCD area detector under graphite monochromatized Mo Kα (λ = 0.071073 nm) radiation at 293 K. The structure was solved by direct method using SHELX (*14*) and refined on F^2 using all data by full-matrix least-squares procedures with SHELXL-97 (*15*).

Bioassay of Fungicidal Activity. *Method.* Fungicidal activity of compounds **4** against *B. cinerea*, *S. sclerotiorum*, *R. solani*, *P. asparagi*, and *P. oryzae* was evaluated using mycelium growth rate test (*16*). All five fungal strains for mycelium growth rate test were provided by the Institute of Plant Protection, Chinese Academy of Agricultural Sciences. *B. cinerea* and *R. solani* were collected from a cucumber field in the suburbs of Beijing, China. *S. sclerotiorum* was collected from a rape field in the suburbs of Tianjin, China. *P. asparagi* was collected from an asparagus field in Anyang, Henan Province, China. *P. oryzae* was collected from a rice field in Songzi, Hubei Province, China.

Fungicidal Activity of Compounds 4. Inhibition of compounds **4** against the five plant pathogenic fungi was tested initially as concentrations of 100, 50, 25, 12.5, and 6.25 μg/mL. The EC_{50} was estimated using logit analysis (*17*). When the EC_{50} values of compounds were < 5 μg/mL, the compounds were then tested at 25, 12.5, 6.25, 3.13, and 1.56 μg/mL. When the EC_{50} was < 1 μg/mL, the compounds were tested at 3.13, 1.56, 0.78, 0.39, and 0.2 μg/mL to determine accurate EC_{50} values. A commercial fungicide commonly used for management of the plant pathogens was used for comparison to the tested compounds. The fungicides included pyrimethanil for *B. cinerea*, chlorothalonil for *S. sclereotiorum* and *P. asparagi*, thiram for *R. solani*, and isoprothiolane for *P. oryzae*.

RESULTS AND DISCUSSION

Synthesis of Compounds 4. As shown in **Scheme 1**, 1-thia-2-alkylimino-3,4,9-triaza-10-oxospiro[4.15]eicosyl-3-ene (**4F**) and 1-thia-2-alkylimino-3,4-diaza-9-oxa-10-oxospiro[4.15]eicosyl-3-ene (**4G**) were synthesized from 12-oxo-1,15-pentadecanlactam (**1F**) and 12-oxo-1,15-pentadecanlactone (**1G**), respectively, by condensation reaction with N-substituted thiosemicarbazide (**2**) to give compounds **3F** and **3G**, which undergo oxidative cyclization on treatment with manganese dioxide. The yields of compounds **4** from **3** are fair to good (64−90%) as shown in **Table 1**. The structures of compounds **4** were confirmed by elemental analysis (**Table 1**), ^{1}H NMR, and ^{13}C NMR (**Table 2**).

Table 1. Physical and Elemental Data of Compounds **4**

				elemental analysis (%)		
compd no.	R	mp (°C)	yield (%)	C (calcd)	H (calcd)	N (calcd)
$4F_1$	C_6H_5	100−102	75	66.24 (65.96)	8.27 (8.05)	13.68 (13.99)
$4F_2$	*o*-$CH_3C_6H_4$	100−102	85	66.23 (66.63)	8.48 (8.27)	13.20 (13.51)
$4F_3$	m-$CH_3C_6H_4$	117−118	71	66.23 (66.63)	8.48 (8.27)	13.20 (13.51)
$4F_4$	*p*-$CH_3C_6H_4$	66−68	76	66.64 (66.63)	8.33 (8.27)	13.15 (13.51)
$4F_5$	*o*-ClC_6H_4	48−49	82	60.71 (60.74)	7.23 (7.18)	12.62 (12.88)
$4F_6$	*p*-ClC_6H_4	67−69	79	61.05 (60.74)	7.30 (7.18)	12.47 (12.88)
$4F_7$	*o*-BrC_6H_4	109−110	70	55.36 (55.11)	6.80 (6.52)	11.92 (11.68)
$4F_8$	*o*-$CH_3OC_6H_4$	38−40	70	64.44 (64.15)	7.80 (7.96)	13.39 (13.01)
$4F_9$	*p*-$CH_3OC_6H_4$	40−41	87	64.63 (64.15)	8.09 (7.96)	12.85 (13.01)
$4F_{10}$	2,3-$(CH_3)_2C_6H_3$	129−131	83	67.34 (67.25)	8.78 (8.47)	13.03 (13.07)
$4F_{11}$	2,4-$(CH_3)_2C_6H_3$	120−122	90	67.01 (67.25)	8.46 (8.47)	13.27 (13.07)
$4F_{12}$	2,5-$(CH_3)_2C_6H_3$	69−70	79	67.22 (67.25)	8.53 (8.47)	13.24 (13.07)
$4F_{13}$	3,4-$Cl_2C_6H_3$	119−120	83	56.42 (56.28)	6.76 (6.44)	11.80 (11.93)
$4F_{14}$	2,5-$Cl_2C_6H_3$	130−131	65	55.94 (56.28)	6.46 (6.44)	11.66 (11.93)
$4F_{15}$	α-naphthyl	115−117	68	69.54 (69.30)	7.75 (7.60)	12.02 (12.43)
$4F_{16}$	benzyl	119−120	89	66.93 (66.63)	7.99 (8.27)	13.28 (13.51)
$4G_1$	*o*-ClC_6H_4	87−89	80	59.20 (60.60)	6.60 (6.94)	9.85 (9.64)
$4G_2$	*p*-ClC_6H_4	91−92	69	60.90 (60.60)	7.07 (6.94)	9.75 (9.64)
$4G_3$[a]	*o*-BrC_6H_4	98−100	71	54.88 (55.00)	6.00 (6.29)	8.96 (8.75)
$4G_4$	*p*-BrC_6H_4	90−92	68	55.18 (55.00)	6.49 (6.29)	8.66 (8.75)
$4G_5$	2,5-$(CH_3)_2C_6H_3$	100−101	64	66.97 (67.10)	8.23 (8.21)	9.90 (9.78)

[a] Its crystal structure was previously reported (*18*).

Table 2. ^{1}H NMR and ^{13}C NMR Data of Compounds **4**

compd no.	^{1}H NMR ($CDCl_3$, δ)	^{13}C NMR ($CDCl_3$, δ)
4F$_1$	1.24−1.69 (m, 19H), 2.16−2.37 (m, 5H), 3.18−3.22 (m, 1H), 3.51−3.55 (m, 1H), 5.73−5.75 (m, 1H), 7.24−7.31 (m, 3H), 7.42−7.48 (m, 2H)	173.6, 173.3, 148.0, 129.4, 126.9, 121.1, 115.2, 39.5, 38.1, 36.9, 34.8, 27.9, 27.6, 27.0, 26.3, 25.8, 25.6, 24.7,24.6, 23.0
4F$_2$	1.21−1.73 (m, 19H), 2.14−2.38 (m, 8H), 3.18−3.23 (m, 1H), 3.49−3.55 (m, 1H), 6.06−6.10 (m, 1H), 7.04−7.07 (m, 1H), 7.12−7.17 (m, 1H), 7.22−7.29 (m, 2H)	174.2, 173.3, 147.8, 130.8, 130.3, 126.6, 126.3, 116.9, 114.5, 39.4, 38.0, 36.8, 34.8, 27.8, 27.6, 26.9, 26.2, 25.7, 25.5, 24.6, 24.5, 22.9, 17.8
4F$_3$	1.22−1.69 (m, 19H), 2.14−2.39 (m, 8H), 3.14−3.21 (m, 1H), 3.46−3.54 (m, 1H), 6.30−6.34 (m, 1H), 7.06−7.10 (m, 3H), 7.29−7.35 (m, 1H)	173.4, 173.3, 147.9, 139.2, 129.1, 127.6, 121.6, 117.6, 115.0, 39.3, 37.9, 36.7, 34.8, 27.7, 27.5, 26.8, 26.2, 25.6, 25.5, 24.6, 24.5, 22.9, 21.3
4F$_4$	1.21−1.68 (m, 19H), 2.17−2.38 (m, 8H), 3.14−3.20 (m, 1H), 3.50−3.57 (m, 1H), 6.06−6.10 (m, 1H), 7.22−7.29 (m, 4H)	173.3, 172.5, 145.0, 137.1, 129.9, 121.4, 115.1, 39.4, 38.0, 36.8, 34.8, 27.8, 27.6, 26.9, 26.2, 25.7, 25.6, 24.6, 24.5, 22.9, 21.1
4F$_5$	1.22−1.70 (m, 19H), 2.18−2.37 (m, 5H), 3.18−3.23 (m, 1H), 3.50−3.57 (m, 1H), 6.10−6.15 (m, 1H), 7.11−7.20 (m, 2H), 7.30−7.35 (m, 1H), 7.47−7.50 (m, 1H)	176.5, 173.4, 146.4, 130.3, 127.7, 126.9, 125.8, 119.2, 115.8, 39.3, 37.9, 36.8, 34.7, 27.8, 27.5, 26.9, 26.2, 25.7, 25.5, 24.6, 24.5, 23.0
4F$_6$	1.23−1.69 (m, 19H), 2.18−2.38 (m, 5H), 3.18−3.22 (m, 1H), 3.51−3.57 (m, 1H), 5.99−6.02 (m, 1H), 7.23−7.29 (m, 2H), 7.39−7.44 (m, 2H)	173.9, 173.4, 146.3, 132.2, 129.5, 122.5, 115.8, 39.3, 37.9, 36.8, 34.7, 27.8, 27.5, 26.9, 26.1, 25.6, 25.5, 24.5, 24.4, 22.9
4F$_7$	1.21−1.70 (m, 19H), 2.17−2.42 (m, 5H), 3.16−3.23 (m, 1H), 3.49−3.58 (m, 1H), 6.08 (s, 1H), 7.07−7.12 (m, 2H), 7.34−7.40 (m, 1H), 7.65−7.68 (m, 1H)	176.5, 173.4, 147.9, 133.4, 128.4, 127.1, 119.0, 115.8, 115.5, 39.4, 38.0, 36.8, 34.7, 27.8, 27.5, 26.9, 26.2, 25.7, 25.5, 24.6, 24.5, 23.0
4F$_8$	1.21−1.68 (m, 19H), 2.15−2.36 (m, 5H), 3.16−3.20 (m, 1H), 3.52−3.58 (m, 1H), 3.88 (s, 3H), 5.55 (s, 1H), 6.98−7.04 (m, 2H), 7.16−7.26 (m, 2H)	173.3, 170.6, 158.9, 139.8, 146.4, 130.3, 127.7, 126.9, 114.5, 55.5, 39.5, 38.1, 37.0, 34.8, 27.9, 27.6, 27.0, 26.3, 25.8, 25.6, 24.7, 24.6, 23.0
4F$_9$	1.21−1.69 (m, 19H), 2.16−2.38 (m, 5H), 3.13−3.20 (m, 1H), 3.52−3.56 (m, 1H), 3.85 (s, 3H), 5.73−5.75 (m, 1H), 6.97−7.02 (m, 2H), 7.38−7.44 (m, 2H)	173.3, 170.6, 158.9, 139.8, 124.0, 115.3, 114.5, 55.5, 39.5, 38.1, 37.0, 34.8, 27.9, 27.6, 27.0, 26.3, 25.8, 25.6, 24.7, 24.6, 23.0
4F$_{10}$	1.24−1.67 (m, 19H), 2.21−2.33 (m, 11H), 3.18−3.23 (m, 1H), 3.50−3.54 (m, 1H), 6.89−6.92 (m, 1H), 7.03−7.06 (m, 1H), 7.12−7.17 (m, 2H)	174.2, 173.3, 147.9, 138.1, 128.9, 127.9, 126.1, 114.7, 114.3, 39.5, 38.1, 36.9, 34.9, 27.9, 27.6, 27.0, 26.3, 25.8, 25.6, 24.7, 24.6, 23.0, 20.2, 13.9
4F$_{11}$	1.21−1.67 (m, 19H), 2.14−2.39 (m, 11H), 3.18−3.23 (m, 1H), 3.50−3.55 (m, 1H), 5.49−5.53 (m, 1H), 7.00−7.11 (m, 3H)	173.2, 173.2, 145.0, 136.4, 131.7, 131.3, 127.1, 117.0, 114.3, 39.6, 38.1, 37.0, 34.9, 27.9, 27.6, 27.0, 26.3, 25.8, 25.6, 24.7, 24.6, 23.0, 21.0, 17.9
4F$_{12}$	1.21−1.70 (m, 19H), 2.16−2.38 (m, 11H), 3.16−3.24 (m, 1H), 3.48−3.58 (m, 1H), 5.74−5.76 (m, 1H), 6.85−6.87 (m, 1H), 6.94−6.97 (m, 1H), 7.15−7.17 (m, 1H)	173.9, 173.3, 147.7, 136.4, 130.7, 127.1, 127.0, 117.5, 114.4, 39.5, 38.1, 36.9, 34.9, 27.9, 27.6. 27.0, 26.3, 25.8, 25.6, 24.7, 24.6, 23.0, 21.0, 17.4
4F$_{13}$	1.21−1.70 (m, 19H), 2.15−2.42 (m, 5H), 3.18−3.25 (m, 1H), 3.48−3.55 (m, 1H), 5.89−5.93 (m, 1H), 7.12−7.16 (m, 1H), 7.38−7.40 (m, 1H), 7.50−7.53 (m, 1H)	174.9, 173.3, 147.5, 133.2, 131.1, 130.2, 122.8, 120.6, 116.5, 39.3, 38.0, 36.8, 34.7, 27.8, 27.5, 26.9, 26.2, 25.7, 25.5, 24.6, 24.6, 23.1
4F$_{14}$	1.24−1.71 (m, 19H), 2.18−2.38 (m, 5H), 3.18−3.21 (m, 1H), 3.50−3.56 (m, 1H), 5.82−6.01 (m, 1H), 7.10−7.17 (m, 2H), 7.40−7.43 (m, 1H)	177.2, 173.4, 147.3, 133.2, 131.3, 126.6, 124.0, 119.4, 116.6, 39.3, 37.9, 36.8, 34.7, 27.8, 27.5, 26.9, 26.2, 25.7, 25.5, 24.6, 24.5, 23.1
4F$_{15}$	1.23−1.68 (m, 19H), 2.14−2.35 (m, 5H), 3.17−3.21 (m, 1H), 3.45−3.50 (m, 1H), 5.88−5.92 (m, 1H), 7.30−7.32 (m, 1H), 7.48−7.55 (m, 3H), 7.74−7.77 (m, 1H), 7.85−7.88 (m, 1H), 8.25−8.28 (m, 1H)	174.4, 173.3, 145.2, 134.1, 127.8, 127.6, 126.9, 126.7, 126.2, 125.5, 123.4, 114.8, 113.2, 39.4, 38.0, 36.8, 34.8, 27.8, 27.6, 26.9, 26.2, 25.7, 25.6, 24.6, 24.5, 23.0
4F$_{16}$	1.18−1.71 (m, 19H), 2.15−2.33 (m, 5H), 3.09−3.16 (m, 1H), 3.51−3.55 (m, 1H), 4.63 (s, 2H), 6.05−6.09 (m, 1H), 7.28−7.43 (m, 5H)	176.4, 173.3, 137.7, 128.5, 127.8, 127.3, 114.8, 62.2, 39.5, 38.0, 36.8, 34.9, 27.8, 27.6, 26.9, 26.3, 25.7, 25.6, 24.7, 24.5, 22.9
4G$_1$	1.27−1.77 (m, 19H), 2.25−2.43 (m, 5H), 4.08−4.21 (m, 2H), 7.10−7.20 (m, 2H), 7.29−7.35 (m, 1H), 7.47−7.50 (m, 1H)	176.2, 173.6, 146.5, 130.4, 127.6, 126.9, 125.9, 119.2, 115.5, 63.0, 38.9, 34.6, 34.6, 27.8, 27.5, 26.6, 26.5, 26.0, 25.2, 25.0, 23.9, 23.4
4G$_2$	1.38−1.76 (m, 19H), 2.27−2.41 (m, 5H), 4.09−4.19 (m, 2H), 7.23−7.28 (m, 2H), 7.39−7.44 (m, 2H)	173.8, 173.7, 146.5, 132.3, 129.5, 122.0, 115.7, 63.0, 39.0, 34.7, 34.7, 27.9, 27.5, 26.7, 26.5, 26.1, 25.3, 25.1, 24.0, 23.4
4G$_3$	1.36−1.75 (m, 19H), 2.30−2.40 (m, 5H), 4.12−4.17 (m, 2H), 7.07−7.12 (m, 2H), 7.34−7.40 (m, 1H), 7.66−7.69 (m, 1H)	176.2, 173.6, 148.1, 133.5, 128.4, 127.1, 119.0, 115.6, 115.5, 62.96, 38.9, 34.6, 34.5, 27.8, 27.5, 26.6, 26.4, 26.0, 25.2, 25.0, 23.9, 23.3
4G$_4$	1.28−1.1.78 (m, 19H), 2.30−2.42 (m, 5H), 4.09−4.19 (m, 2H), 7.16−7.21 (m, 2H), 7.54−7.59 (m, 2H)	173.8, 173.6, 147.0, 132.5, 122.8, 120.1, 115.7, 62.9, 39.0, 34.6, 34.6, 27.8, 27.5, 26.6, 26.5, 26.0, 25.3, 25.1, 24.0, 23.4
4G$_5$	1.37−1.76 (m, 19H), 2.24−2.40 (m, 11H), 4.12−4.17 (m, 2H), 6.86−6.87 (m, 1H), 6.94−6.96 (m, 1H), 7.14−7.17 (m, 1H)	173.6, 173.6, 147.7, 136.3, 130.7, 127.1, 127.0, 117.4, 114.1, 63.0, 39.1, 34.7, 34.6, 27.8, 27.5, 26.6, 26.5, 26.5, 26.0, 25.3, 25.1, 23.9, 23.3, 17.9

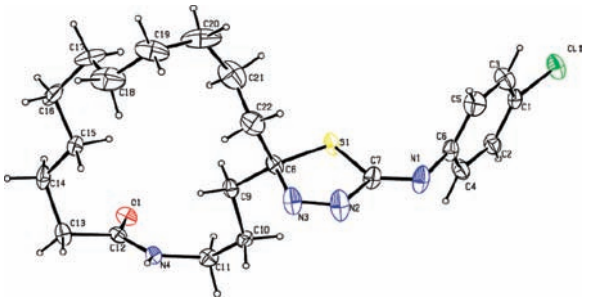


Figure 2. Crystal structure of compound **4F$_6$**.

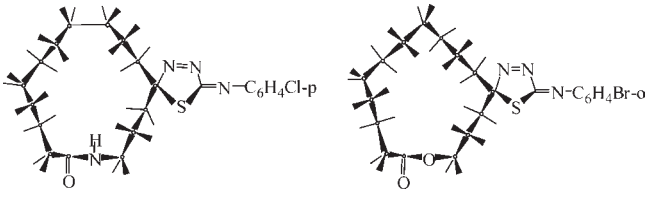


Figure 3. Conformation of **4F$_6$** (left) and **4G$_3$** (right).

Conformation of Compound 4. X-ray diffraction analysis of a representative compound (**4F$_6$**) showed that its large ring skeleton adopts [333133] conformation, in which the carbonyl group is present in a side position and the spiro-carbon atom in a corner position (**Figure 2**). Previous research reported the crystal structure of **4G$_3$** (*18*), the large ring skeleton of which can be described as [33343] conformation with the carbonyl group in a side position and the spiro-carbon atom in a corner position. The conformations of the large ring skeleton of **4F$_6$** and **4G$_3$** are somewhat different from each other. However, they should be similar in the solution due to the dynamic equilibrium (**Figure 3**). [CCDC 739298 contains supplementary crystallographic data for this paper. 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.]

Fungicidal Activity and Structure−Activity Relationship. Compounds **4F** have much better fungicidal activity than compounds **4G** (**Table 3**). Compounds **4F** have fair to excellent fungicidal activity against the five fungi, and there is at least one compound that has good fungicidal activity against one of the five fungi (fair, good, and excellent fungicidal activities indicate that their EC_{50} values are less than 30, 6, and 2 μg/mL, respectively). However, compounds **4G** have only poor fungicidal activity, and the EC_{50} values of almost all compounds are > 30 μg/mL.

In view of stereochemistry, compounds **4F** and **4G** have similar conformations. 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 **4F** and there are two hydrogen-bonding acceptors (−COO− and N=N double bond of diazoline ring) without a hydrogen-bonding donor on the large ring of compounds **4G**. This is the main reason compounds **4F** and **4G** have different fungicidal activities. The results above show that the presence of a hydrogen-bonding donor is very important to the fungicidal activity of macrocyclic compounds, and the rule on the relationship between the fungicidal activity and hydrogen bonding has a general suitability to the macrocyclic compounds (*7*).

Fungicidal Activity of Compounds 4F against B. cinerea. Compounds **4F** have good fungicidal activities against *B. cinerea*. Among them, compounds **4F$_1$**, **4F$_2$**, **4F$_3$**, **4F$_9$**, and **4F$_{11}$**, the EC_{50} values of which were 5.01, 4.21, 6.42, 6.47, and 9.63 μg/mL, respectively, displayed the best fungicidal activities, but they were inferior to the commercial fungicide pyrimethanil, the EC_{50} value of which was 0.14 μg/mL. The compounds with one to two methyl or methoxy groups on the benzene ring have better fungicidal activity (**Tables 1** and **3**).

Table 3. Fungicidal Activity of Compounds **4** against Five Fungi[a]

	EC_{50} (μg/mL)				
compd no.	*B. cinerea*	*S. sclerotiorum*	*R. solani*	*P. asparagi*	*P. oryzae*
4F$_1$	5.01	9.92	42.08	14.18	41.51
4F$_2$	4.21	375.43	124.79	73.79	78.43
4F$_3$	6.42	4.42	30.72	9.67	63.18
4F$_4$	25.10	34.08	71.83	14.52	138.76
4F$_5$	14.89	241.56	31.80	24.48	41.51
4F$_6$	18.67	48.18	39.52	101.63	0.054
4F$_7$	19.86	533.29	56.13	60.32	57.59
4F$_8$	21.85	21.09	37.89	517.04	397.84
4F$_9$	6.47	87.23	93.00	20.56	57.85
4F$_{10}$	22.61	36.53	22.38	14.85	199.48
4F$_{11}$	9.63	55.71	26.75	5.26	30.92
4F$_{12}$	18.58	34.30	5.62	16.42	86.61
4F$_{13}$	34.23	15.03	36.82	9.02	0.72
4F$_{14}$	29.56	109.29	41.77	221.73	1.11
4F$_{15}$	15.73	767.24	17.80	46.15	195.80
4F$_{16}$	14.44	8.32	23.49	21.33	116.85
4G$_1$	43.81	169.20	159.06	116.49	211.36
4G$_2$	49.95	59.91	126.32	42.48	338.37
4G$_3$	36.42	204.57	207.69	53.55	246.10
4G$_4$	49.88	38.30	17.10	29.21	338.37
4G$_5$	126.29	162.84	421.80	208.41	3123.76
pyrimethanil	0.14	nd	nd	nd	nd
chlorothalonil	nd	0.41	nd	4.35	nd
thiram	nd	nd	0.90	nd	nd
isoprothiolane 40% EC	nd	nd	nd	nd	7.19

[a] Regression equations and correlation coefficients are omitted; nd, not determined.

Fungicidal Activity of Compounds 4F against S. sclerotiorum. Compounds **4F** have fair to good fungicidal activities against *S. sclerotiorum*. Among them, compounds **4F$_1$**, **4F$_3$**, and **4F$_{16}$**, the EC_{50} values of which were 9.92, 4.42, and 8.32 μg/mL, respectively, displayed the best fungicidal activities, but they were inferior to the commercial fungicide chlorothalonil (EC_{50} value = 0.41 μg/mL) (**Table 3**). Thus, the compounds without any group on the benzene ring (phenyl) or nonaryl (benzyl) have better fungicidal activity. In addition, the influence of the methyl group on the fungicidal activity should be taken seriously; the compound with the meta-position methyl group on the benzene ring (**4F$_3$**) showed the best fungicidal activity (EC_{50} value = 4.42 μg/mL), but the compound with the para-position methyl group on the benzene ring had only moderate fungicidal activity (EC_{50} value = 34.08 μg/mL), and the compound with the ortho-position methyl group on the benzene ring showed the lowest fungicidal activity (EC_{50} value = 375.43 μg/mL).

Fungicidal Activity of Compounds 4F against R. solani. Compounds **4F** have fair fungicidal activities against *R. solani.* Only individual compound **4F$_{12}$**, with an EC_{50} value of 5.62 μg/mL, displayed good fungicidal activity, but it was inferior to the commercial fungicide thiram (EC_{50} value = 0.90 μg/mL).

Fungicidal Activity of Compounds 4F against P. asparagi. Compounds **4F** have fair to good fungicidal activities against *P. asparagi.* Among them, compounds **4F$_3$**, **4F$_{11}$**, and **4F$_{13}$**, with EC_{50} values of 9.67, 5.26, and 9.02 μg/mL, respectively, displayed the best fungicidal activitues, and they were comparable with the commercial fungicide chlorothalonil (EC_{50} value = 4.35 μg/mL). Some compounds with one to two methyl groups on the benzene ring have better fungicidal activity, and some compounds with

one to two chlorine atoms on the benzene ring have better fungicidal activity. The structure−activity relationship is not clear.

*Fungicidal Activity of Compounds **4F** against P. oryzae*. Compounds **4F** have fair to excellent fungicidal activities against *P. oryzae*. Among them, compounds $\mathbf{4F_6}$, $\mathbf{4F_{13}}$, and $\mathbf{4F_{14}}$, with EC_{50} values of 0.054, 0.72, and 1.11 μg/mL, respectively, displayed the best fungicidal activities, and they were much better than the commercial fungicide isoprothiolane (EC_{50} value = 7.19 μg/mL). The fungicidal activities of compounds $\mathbf{4F_6}$, $\mathbf{4F_{13}}$, and $\mathbf{4F_{14}}$ are 130, 10, and 7 times that of isoprothiolane, repectively. In contrast with the fungicidal activity of compounds **4F** against *B. cinerea*, the compounds with one to two methyl groups on the benzene ring have low fungicidal activity and the compounds with one to two chlorine atoms on the benzene ring have the best fungicidal activity.

In conclusion, most of the compounds **4F** have fair to excellent fungicidal activities against the five fungi mentioned above and have much better fungicidal activity than compounds **4G**. Compound $\mathbf{4F_6}$ showed excellent fungicidal activity against *P. oryzae*, which is an important fungal pathogen causing serious damage to rice production in China. Additionally, compound $\mathbf{4F_{13}}$ showed excellent activity against *P. oryzae*, but it was approximately 10-fold less inhibitory when compared with $\mathbf{4F_6}$. This compound had good fungicidal acitivity against *P. asparagi*, a pathogen of asparagus. Further research is needed to determine if these compounds could be developed into commercial fungicides.

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