RSC Advances

PAPER

View Article Online
View Journal | View Issue

Cite this: *RSC Adv.*, 2017, 7, 3145

Synthesis and fungicidal activity of 3,4-dichloroisothiazole based strobilurins as potent fungicide candidates†

Lai Chen,[a] Xiao-Feng Guo,[a] Zhi-Jin Fan,*[ab] Nai-Lou Zhang,[a] Yu-Jie Zhu,[a] Zhi-Ming Zhang,[a] Inna Khazhieva,[c] Morzherin Y. Yurievich,[c] Nataliya P. Belskaya[c] and Vasiliy A. Bakulev*[c]

Received 20th October 2016
Accepted 30th November 2016

DOI: 10.1039/c6ra25520e

www.rsc.org/advances

A series of 3,4-dichloroisothiazole based novel strobilurin analogs were designed and synthesized, and their structures were elucidated by NMR and HRMS, and the typical crystal structure was determined by X-ray diffraction for validation. Results from different biological assays suggested that most target compounds displayed very good fungicidal activity against one or multiple plant pathogens *in vitro* and *in vivo*. Among them, compounds **6d**, **6g** and **8d** showed a broad spectrum of fungicidal activity. Further field experiments indicated that compound **8d** displayed better efficacy against *Sphaerotheca fuliginea* than commercial standards azoxystrobin and trifloxystrobin, and better efficacy against *Pseudoperonospora cubensis* than trifloxystrobin. Overall, a new fungicidal candidate for plant disease management was discovered in this study.

Introduction

A sustainable food supply for an increasing global demand requires new innovations in crop protection technology.[1] Continuous global population growth and a shift in consumers' food preference call for an increased crop yield under fixed/shrinking arable lands.[2] Agrochemicals have been one of the most effective tools to meet this need. However, pest resistance, pest shifts and an ever evolving regulatory landscape mean a high pressure for current agrochemicals. Innovative agrochemicals that can overcome resistance are always welcome for farmers.[3]

Resistance of plant pathogens to some strobilurin analogs needs innovative products. The strobilurin fungicides are the second largest group of launched fungicides, which act through inhibition of mitochondrial respiration by blocking electron transfer within the respiratory chain, thus in turn causes important cellular biochemical processes to be severely disrupted, and results in cessation of fungal growth.[4] Strobilurin fungicides with a broad spectrum are highly efficacious and are suitable for a wide range of crops.[5] However, since the first launch of this class of fungicide, widespread applications have led to pathogen resistance.[6] Resistant cases of *Septoria* in European wheat and the U.S. turf market were good examples.[7] Therefore, new strobilurins are possibly needed for future markets.

Heterocyclic compounds exhibit wide spectrum of biological activity.[8] Isothiazoles constitute a relatively novel class of heterocyclic compounds. As one of their members, 3,4-dichloroisothiazoles possess a broad spectrum of biological activity such as insecticide, fungicide and potential systemic acquired resistance activities, 3,4-dichloroisothiazole-5-carboxylic acid and its' derivatives manifest fungicidal activity;[9] isotianil being as its' derivative was developed as a novel fungicide with activating defence responses against a wide range of plant pathogens.[10]

The purpose of this study was to use strobilurin A as a template to design and synthesize new strobilurin analogs (Fig. 1) with a active substructure of 3,4-dichloroisothiazole. Extensive biological assays demonstrated that newly synthesized compounds displayed good to excellent activity against one or multiple plant pathogens. Field trials suggested that the fungicidal activity of the best candidate **8d** was better than commercial standards.

Results and discussion

Chemistry

The synthetic route of **3a** and **3c** was outlined in Scheme 1. Compound **2** was obtained in a high yield from compound **1** by ref. 11. Then compound **3a** was obtained from compound **2** through substitution reaction. Compound **3b** was prepared

---

[a] *State Key Laboratory and Institute of Elemento-Organic Chemistry, Nankai University, Tianjin 300071, P. R. China. E-mail: fanzj@nankai.edu.cn; Tel: +86-13920714666*

[b] *Collaborative Innovation Center of Chemical Science and Engineering (Tianjin), Nankai University, Tianjin 300071, P. R. China*

[c] *The Ural Federal University Named after the First President of Russia B. N. Yeltsin, Yeltsin UrFU 620002, Ekaterinburg, Russia. E-mail: v.a.bakulev@urfu.ru*

† Electronic supplementary information (ESI) available: X-ray crystal structure data of compound **6d**, characterization and copies of NMR spectra for all target compounds **6** and **8**. CCDC 1509531. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c6ra25520e

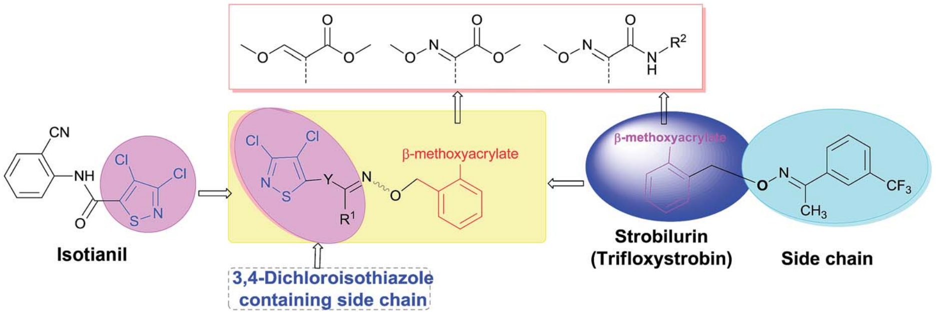


Fig. 1 Design of the title compounds.

from the oxidation of 3,4-dichloro-5-hydroxylmethyl isothiazole. Compound **3c** was obtained by aldol condensation reaction between compound **3b** and acetone.

For the target compounds synthesis, compounds **6a–6h** were prepared by intermediates **3a–3d** reacting with compounds **5a** or **5b** respectively (Table 1).[12] Compounds **8a–8r** were synthesized according to the method described in Table 2.

Compounds **7** series were obtained by hydrolytic reaction from the corresponding compounds **6b**, **6f** or **6h**, and then compounds **8** series were obtained by the reaction of the corresponding compound **7** with a corresponding amino compound. The structures of the synthesized compounds were confirmed by ^{1}H NMR, ^{13}C NMR, HRMS or elemental analyses.

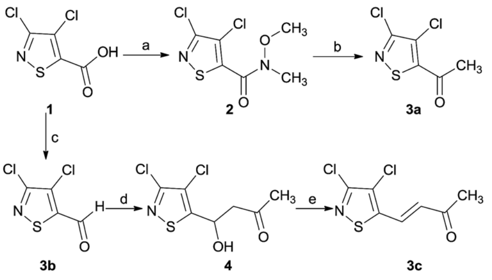
Fungicidal activity

The result of *in vitro* fungicidal activity determination of all synthesized compounds and the positive control azoxystrobin against 9 fungi was assessed at 50 μg mL^{-1}. The results were shown in Table 3.

For each fungus, most of the synthesized compounds were more active than the positive control azoxystrobin under the same condition. Especially for BC, SS and RC, majority of synthesized compounds exhibited better activity than azoxystrobin. For PI, most compounds showed similar or better activity than azoxystrobin except for compounds **8m, 8o** and compounds with a sulfur atom in the substitution of R^2. Besides, for compounds **8a–8r**, it was also found that the compounds with a methyl group at R^2 exhibited better activity. Compounds **8d**, **8j** and **8p** exhibited 100% inhibition activity against GZ, while they had higher activity than azoxystrobin against RC. Furthermore, compounds **6d–6g** and **8d** showed a broad spectrum of fungicidal activity *in vitro*. Overall, the *in vitro* fungicidal potency and spectrum are dependent on the individual structures, or substitutions (*e.g.*, X, Y, R^1, or R^2) in the structures of the compounds **6** or **8** series.

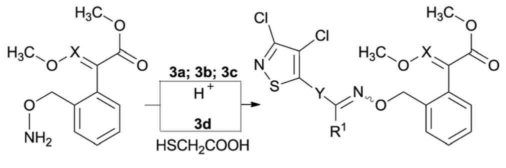
In order to explore the fungicidal potency, precision toxicity determination for the EC$_{50}$ of compounds **6d–6g**, **8d** and **8p** with a broader fungicidal spectrum described above were further conducted. The results in Table 4 indicated that compound **8d** exhibited excellent activity with EC$_{50}$ of 0.07 μg mL^{-1} and 0.49 μg mL^{-1} against RC and PI, respectively; they were at the same level as that of the positive control azoxystrobin. Besides, compound **8d** showed higher activity against GZ and BC with much lower EC$_{50}$ than azoxystrobin. Compound **8d** was confirmed with broad spectrum of fungicide activity by this precision toxicity experiments.

The *in vivo* fungicidal activity of all compounds and positive control (*i.e.*, azoxystrobin) against *P. cubensis*, *E. graminis*, *P. sorghi* Schw and *C. lagenarium* were further assessed at 400 μg mL^{-1} and the results were listed in Table 5. Most of the synthesized compounds were more active than azoxystrobin. For *P. cubensis*, the compounds **6a, 6d, 6f, 8d** and **8r** exhibited



Scheme 1 Reagents: (a) EDCI, $CH_3NHOCH_3\cdot HCl$, CH_2Cl_2, rt; (b) CH_3MgBr, THF, −30 °C; (c) (i): $SOCl_2$, CH_3OH; (ii): $NaBH_4$, CH_3OH; (iii): PCC; (d) acetone/H_2O, NaOH; (e) $SOCl_2$, DMF, 25 °C.

Table 1 General synthetic route of title compounds **6a–6h**



5a(X=CH),5b(X=N)   6a-6h (X=CH,N)

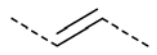
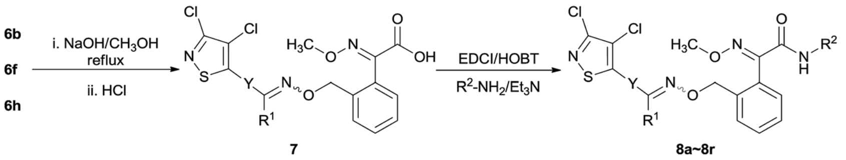
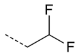
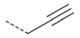
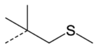
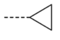
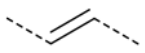
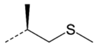
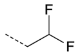
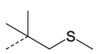
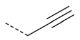
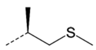
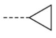
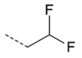
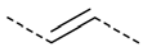
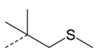
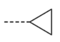
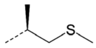
Compd	X	R^1	Y
6a	CH	···CH_3	Covalent bond
6c	CH	···H	Covalent bond
6e	CH	···NH_2	Covalent bond
6g	CH	···CH_3	
6b	N	···CH_3	Covalent bond
6d	N	···H	Covalent bond
6f	N	···NH_2	Covalent bond
6h	N	···CH_3	

Table 2 General synthetic route of title compounds **8a–8r**



Compd	Y	R^1	R^2	Compd	Y	R^1	R^2
8a	Covalent bond	$\cdots CH_3$		**8j**		$\cdots CH_3$	$\cdots CH_3$
8b	Covalent bond	$\cdots CH_3$		**8k**		$\cdots CH_3$	
8c	Covalent bond	$\cdots CH_3$		**8l**		$\cdots CH_3$	
8d	Covalent bond	$\cdots CH_3$	$\cdots CH_3$	**8m**	Covalent bond	$\cdots NH_2$	
8e	Covalent bond	$\cdots CH_3$		**8n**	Covalent bond	$\cdots NH_2$	
8f	Covalent bond	$\cdots CH_3$		**8o**	Covalent bond	$\cdots NH_2$	
8g		$\cdots CH_3$		**8p**	Covalent bond	$\cdots NH_2$	$\cdots CH_3$
8h		$\cdots CH_3$		**8q**	Covalent bond	$\cdots NH_2$	
8i		$\cdots CH_3$		**8r**	Covalent bond	$\cdots NH_2$	

almost 100% activity which were better than azoxystrobin (with only 85% of activity). Besides, the compounds **6a–6g**, **8d**, **8g**, **8h**, **8p** and azoxystrobin showed the similar 100% activity against *E. graminis*. Even though azoxystrobin showed no activity against *P. sorghi* Schw, the compounds **6b**, **6d**, **6f**, **6g**, **8a** and **8b** exhibited 100% activity. Furthermore, almost all of the compounds except for **6b**, **6h** and **8e** had similar to or better activity than azoxystrobin against *C. lagenarium*. Thus, the compounds **6a–6g**, **8b** and **8d** were confirmed as with broad-spectrum of fungicidal activity *in vivo* too. Afterwards, **6a**, **6d**, **6g**, **6f**, **8b** and **8d** with good activity were further validated at concentrations of 50 μg mL^{-1}, 12.5 μg mL^{-1}, 3.13 μg mL^{-1}, 0.78 μg mL^{-1} and 0.20 μg mL^{-1} *in vivo*, respectively. The results were shown in Table 6.

As can be seen from Table 6, compounds **6a**, **6d**, **6f**, **8b** and **8d** exhibited 90–100% inhibition activity against *E. graminis* even at 0.20 μg mL^{-1}, which were similar to that of positive control trifloxystrobin (with inhibition of 95%) and were much better than enestroburin (with inhibition of 30%). The compound **6a** exhibited best activity against *P. sorghi* Schw as the same as positive control trifloxystrobin, they both had 95% of fungicidal activity at 0.2 μg mL^{-1}; their activities were much better than that of enestroburin (only 30% at 0.20 μg mL^{-1}). The compounds **6a** and **8d** exhibited best activity against *C. lagenarium* as the same as positive control trifloxystrobin and enestroburin, they all had about 65% of activity even at 0.20 μg mL^{-1}. Most importantly, the compound **8d** also exhibited very good activity against *P. cubensis* with 20% of inhibition at 0.20 μg mL^{-1}, while the two positive controls trifloxystrobin and enestroburin had no activity against *P. cubensis* even at 12.5 μg mL^{-1}. Our *in vivo* confirmation studies indicated, most of the active compounds discovered not only kept its' highly activity, but also acted to a broad spectrum of fungi tested. Compound **8d** deserved for further novel fungicide development studies as a candidate.

Field efficacy evaluation of compound 8d

The results of the field experiments were shown in Table 7. Studies indicated that the compound **8d** exhibited significantly better efficacy (78.62%) against cucumber *S. fuliginea* than commercial standards (*i.e.*, azoxystrobin and trifloxystrobin) at the same application rate of 37.5 g ai per hm^2 at 21 days after spraying application. At an application rate of 75 g ai per hm^2, the compound **8d** showed similar activity against *P. cubensis* as one commercial standard pyraclostrobin, but significantly better than another commercial standard trifloxystrobin. These results suggested that the compound **8d** could be considered as an alternative to control *S. fuliginea* and *P. cubensis*, it is under further novel pesticide development studies.

Experimental

Equipment and materials

Melting points of all compounds were determined on an X-4 binocular microscope (Gongyi Tech. Instrument Co., Henan, China) and the thermometer was not corrected. Proton NMR spectra were obtained using a Bruker AVANCE-400 MHz spectrometer and chemical shift values (δ) were reported in ppm with deutero-chloroform ($CDCl_3$) as a solvent and tetramethylsilane (TMS) as an internal standard. High resolution mass spectrometry (HRMS) data were obtained on an FTICR-MS Varian 7.0T FTICR-MS instrument. Elemental analyses were taken on a Vario EL III elemental analysis instrument. Crystal structure was recorded by Bruker SMART 1000 CCD diffraction meter. All solvents and reagents were an analytical reagent grade. Column chromatography purification was carried out on silica gel.

General procedure for the synthesis of compound 3a

3,4-Dichloro-*N*-methoxy-*N*-methylisothiazole-5-carboxamide **2** can be prepared from 3,4-dichloroisothiazole-5-carboxylic acid **1** according to the revising procedures of ref. 11. To a solution of the compound **2** (3.00 g, 12.44 mmol) in anhydrous tetrahydrofuran (45 mL) at −30 °C under N_2 atmosphere was added dropwise with a solution of methyl magnesium bromide in Et_2O (3 mol L^{-1}, 5.81 mL, 17.42 mmol). Then the mixture was allowed to stir at −30 °C for 1 h and room temperature for another 1 h. When the reaction completed, the reaction mixture was worked-up by the sat. aq. NH_4Cl (50 mL). After removal of the tetrahydrofuran under vacuum, the aqueous phase was extracted with ethyl acetate (3 × 50 mL). The organic layers were combined, washed with water (50 mL) and saturated brine (50 mL), and then dried over anhydrous sodium sulfate. After filtration, the solvent was evaporated. The residue was then purified by column chromatography on silica gel using ethyl acetate and petroleum ether (60–90 °C) with 1 : 9 of *v*/*v* as an eluent to obtain yellow oil **3a** (1.56 g) in a yield of 63.8%. ^1H NMR (400 MHz, $CDCl_3$) δ 2.65 (s, 3H, O=C–CH_3).

Table 3 *In vitro* fungicidal activity of compounds **6a–6h** and **8a–8r** at 50 μg mL^{-1}

Compd	AS	CA	GZ	PP	BC	SS	RC	PS	PI
6a	43	67	52	78	100	80	100	65	53
6b	47	50	41	53	100	100	85	77	79
6c	57	67	74	78	100	53	100	65	58
6d	100	92	71	62	100	90	98	69	95
6e	57	100	61	87	100	100	100	59	47
6f	53	75	50	71	100	98	89	77	89
6g	57	50	74	74	100	100	100	71	79
6h	36	50	61	48	100	87	81	59	47
8a	37	43	79	40	54	84	88	20	80
8b	29	36	100	49	52	100	87	73	80
8c	28	44	64	61	47	80	61	57	100
8d	48	68	100	72	100	67	100	89	100
8e	27	46	52	43	63	29	76	21	29
8f	16	13	33	33	46	53	76	15	17
8g	34	36	45	30	53	53	85	35	90
8h	41	52	82	65	47	80	100	78	100
8i	48	60	64	72	58	73	71	68	100
8j	54	84	100	65	84	73	85	89	100
8k	5	13	24	40	32	41	58	15	11
8l	13	13	61	58	43	53	62	21	20
8m	13	17	33	40	38	53	62	24	23
8n	8	20	55	19	42	47	66	46	100
8o	21	28	64	23	37	73	85	19	33
8p	34	44	100	40	58	67	100	84	100
8q	16	13	21	28	39	53	62	15	17
8r	9	17	45	11	36	53	54	21	11
Azoxystrobin	75	81	71	100	91	100	100	81	100

Table 4 EC_{50} of compounds **6d–6g** and **8d**

Compd	AS	CA	GZ	BC	SS	RC	PI
6d	33.34[a]	14.47	nd[b]	2.94	5.21	0.13	3.10
6e	nd	58.25	nd	12.15	20.06	0.21	nd
6f	nd	nd	nd	1.62	7.53	nd	nd
6g	nd	nd	nd	3.93	4.47	0.11	nd
8d	nd	nd	1.75	0.15	nd	0.07	0.49
8p	nd	nd	1.81	nd	nd	0.69	3.84
Azoxystrobin	185.42	2.50	6.92	6.31	4.04	0.06	0.40

[a] Determined values based on the results shown in Table 3. [b] nd, not detect.

General procedure for the synthesis of compound 3b

A solution of (3,4-dichloroisothiazol-5-yl)methanol (1.37 g, 7.44 mmol), which can be prepared from 3,4-dichloroisothiazole-5-carboxylic acid, in 50 mL CH_2Cl_2 was added to the suspension of PCC (2.40 g, 11.16 mmol) and diatomite (2.40 g) in 50 mL CH_2Cl_2 at 0 °C, then the reaction mixture was allowed to stir at

Table 5 Preventive fungicidal *in vivo* activities of compounds **6a–6h** and **8a–8r** at 400 μg mL^{-1}

Compd	*P. cubensis*	*E. graminis*	*P. sorghi* Schw	*C. lagenarium*
6a	100	100	0	98
6b	0	100	100	100
6c	85	100	0	98
6d	99	100	100	100
6e	85	100	85	50
6f	98	100	100	80
6g	85	100	100	100
6h	30	0	50	0
8a	75	50	100	98
8b	85	100	100	100
8c	0	30	70	85
8d	100	100	0	80
8e	70	80	40	0
8f	40	0	0	85
8g	0	100	40	85
8h	0	100	40	100
8i	0	0	60	98
8j	0	60	0	100
8k	85	0	0	80
8l	60	0	60	100
8m	80	0	0	98
8n	60	0	80	100
8o	85	0	40	80
8p	70	100	80	85
8q	85	0	0	100
8r	100	0	0	80
Azoxystrobin	85	100	0	80

room temperature for overnight. The reaction mixture was filtrated and the solvent was evaporated. The residue was then purified by column chromatography on silica gel using ethyl acetate and petroleum ether (60–90 °C) with 1 : 10 of *v*/*v* as an eluent to afford the white solid **3b** (0.89 g) in a yield of 65.9%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.09 (s, O═CH).

General procedure for the synthesis of compound 3c

To a solution of **3b** (0.10 g, 0.05 mmol) in 9 mL acetone was added 1 mL water, then 0.15 mL of aqueous NaOH (2%) was added dropwise within 30 s at ice bath. The reaction mixture was stirred at this temperature for about 2 min and then was quenched with 0.50 mL dilute hydrochloric acid (1 mol L^{-1}). After removal of the acetone under vacuum, water (20 mL) was added and the aqueous phase was extracted with ethyl acetate (2 × 10 mL). The organic layers were combined and washed with saturated brine (50 mL), dried over anhydrous sodium sulfate. After filtration, the solvent was evaporated. The residue was then purified by column chromatography on silica gel using ethyl acetate and petroleum ether (60–90 °C) with 1 : 10 of *v*/*v* as an eluent to afford the white solid intermediate **4** (0.10 g) in a yield of 75.9%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.43 (d, J = 8.8 Hz, 1H, O–CH), 4.00 (s, 1H, OH), 3.14 (d, J = 17.7 Hz, 1H, O═C–CH_2), 2.81 (dd, J = 18.1, 9.6 Hz, 1H, O═C–CH_2), 2.24 (s, 3H, O═C–CH_3).

Table 6 Fungicidal activity validation studies *in vivo*

Compd	*C* (μg mL^{-1})	*P. cubensis*	*E. graminis*	*P. sorghi* Schw	*C. lagenarium*
6a	0.2	0	100	95	65
	0.78	0	100	95	70
	3.13	0	100	98	80
	12.5	0	100	100	90
	50	0	100	100	98
6d	0.2	0	100	0	0
	0.78	0	100	0	0
	3.13	0	100	30	0
	12.5	0	100	45	15
	50	0	100	70	40
6g	0.2	0	30	0	0
	0.78	0	45	0	0
	3.13	0	65	0	0
	12.5	0	90	0	0
	50	0	100	30	0
6f	0.2	0	100	0	0
	0.78	0	100	0	0
	3.13	0	100	20	0
	12.5	0	100	55	0
	50	0	100	80	0
8b	0.2	0	95	0	0
	0.78	0	100	0	0
	3.13	0	100	0	0
	12.5	0	100	0	0
	50	0	100	0	0
8d	0.2	20	90	10	60
	0.78	30	95	45	70
	3.13	45	100	85	85
	12.5	55	100	90	95
	50	65	100	95	100
Trifloxystrobin	0.2	0	95	95	65
	0.78	0	100	98	85
	3.13	0	100	100	98
	12.5	0	100	100	98
	50	20	100	100	100
Enestroburin	0.2	0	30	30	65
	0.78	0	65	75	85
	3.13	0	95	98	95
	12.5	0	98	100	98
	50	0	100	100	100

To a solution of intermediate **4** (0.10 g, 0.42 mmol) in anhydrous CH_2Cl_2 (20 mL), and $SOCl_2$ (0.1 g, 0.84 mmol) was added and followed by a catalytic amount of DMF. The reaction mixture was allowed to stir at 25 °C for 24 h. The solvent was evaporated under reduced pressure and the residue was then purified by column chromatography on silica gel using ethyl acetate and petroleum ether (60–90 °C) with 1 : 5 of *v*/*v* as an eluent to afford the white solid **3c** (0.08 g) in a yield of 88.9%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 (d, J = 16.4 Hz, 1H, CH═CH), 6.71 (d, J = 16.3 Hz, 1H, CH═CH), 2.39 (s, 3H, C═C–CH_3).

General procedure for the synthesis of compounds 6a–6d and 6g–6h

Compounds **5a** and **5b** can be prepared following the procedure reported by ref. 13. A solution of compound **5a** or **5b** (1.12 mmol) in 15 mL ethanol was add to the solution of intermediates **3a**, **3b** or **3c** (1.02 mmol) in ethanol (15 mL), and the reaction mixture was added the catalytic amount of 2 mol L^{-1} hydrochloric acid and stirred at room temperature for overnight. The solvent was evaporated under reduced pressure and the residue was then purified by recrystallization in ethanol or column chromatography on silica gel using ethyl acetate and petroleum ether (60–90 °C) with 1 : 9 to 1 : 4 of *v*/*v* as an eluent to obtain the desired derivatives **6** (Table 1).

General procedure for the synthesis of compounds 6e and 6f

Compounds **6e** and **6f** can be prepared following the procedure reported by ref. 13. A solution of compounds **5a** or **5b** (1.22 mmol) in 15 mL ethanol was added to the solution of commercially available 3,4-dichloroisothiazole-5-carbonitrile **3d** (0.20 g, 1.11 mmol) in ethanol (15 mL), 2-mercaptoacetic acid (0.10 g, 1.09 mmol) was added to the reaction mixture for stirring at room temperature for overnight. The solvent was evaporated under reduced pressure and the residue was then purified by recrystallization in ethyl acetate or column chromatography on silica gel using ethyl acetate and petroleum ether (60–90 °C) with 1 : 4 to 1 : 9 of *v*/*v* as an eluent to give the desired derivatives (Table 1).

General procedure for the synthesis of compounds 7

Intermediates **6b**, **6f** or **6h** (2.49 mmol) was dissolved in 15 mL methanol, the solution of sodium hydroxide (0.30 g, 7.47 mmol) in 15 mL methanol was added to the reaction mixture for 30 min of refluxing. After completing of the reaction, the solvent was evaporated under reduced pressure and the residue was added 15 mL water. The aqueous phase was adjusted to pH 2–3 with dilute hydrochloric acid (3 mol L^{-1}). The aqueous layer was extracted with ethyl acetate (2 × 15 mL). The organic layers were combined and washed with saturated brine (50 mL), dried over sodium sulfate. After filtration, the solvent was evaporated to obtain the compounds **7** as a white solid.

General procedure for the synthesis of compounds 8a–8r

The reaction mixture of the compound **7** (0.75 mmol), EDCI (0.17 g, 0.90 mmol), HOBT (0.11 g, 0.77 mmol) in dichloromethane (25 mL) was stirred for 15 min in ice bath. A solution of amine in dichloromethane (25 mL) was added and followed by Et_3N (0.09 g, 0.90 mmol), the reaction mixture was stirred for further 16 h. After completion of the reaction, the organic layer was successively washed with water (2 × 30 mL) and saturated brine (40 mL), dried over $MgSO_4$ and concentrated under vacuum. After filtration, the solvent was evaporated. The residue was then purified by column chromatography on silica gel using ethyl acetate and petroleum ether (60–90 °C) with 1 : 2 to 1 : 5 of *v*/*v* as an eluent to give the desired derivatives (Table 2).

Crystal structure determination for compound 6d

The crystal of compound **6d** was obtained by recrystallization from ethanol (Fig. 2). X-ray intensity data were recorded on a Bruker SMART 1000 CCD diffraction meter using graphite-monochromatic Mo Kα radiation ($\lambda = 0.71073$ Å). A total of 13 757 reflections were measured, of which 3004 were unique ($R_{int} = 0.0635$) in the range of $3.06° \le \theta \le 25.02°$ (h, −13 to 13; k, −9 to 9; l, −22 to 19), and 2884 observed reflections with $I > 2\sigma(I)$ were used in the refinement on F^2. The structure was solved by direct methods with the SHELXS-97 program. All of the non-H atoms were refined anisotropically by full-matrix least-squares to give the final $R = 0.0698$ and $wR = 0.2049$ ($w = 1/[\sigma^2((F_o^2) + (0.1200P))^2 + 6.9197P]$, where $P = (F_o^2 + 2F_c^2)/3$ with $(\Delta/\sigma)_{max} = 0.981$ and $S = 1.061$ by using the SHELXL program. The hydrogen atoms were located from a difference Fourier map and refined isotropically.

Fungicidal assay

The preliminary *in vitro* fungicidal activities of newly synthesized compounds against *Alternaria solani* (AS), *Botrytis cinerea* (BC), *Cercospora arachidicola* (CA), *Gibberella zeae* (GZ), *Phytophthora infestans* (Mont) de Bary (PI), *Physalospora piricola* (PP), *Pellicularia sasakii* (PS), *Sclerotinia sclerotiorum* (SS), and *Rhizoctonia cerealis* (RC) were tested according to ref. 14. Precision toxicity studies were conducted for the median effective concentration (EC_{50}) calculation according to ref. 14.

The preventive *in vivo* activities of the target compounds against *Pseudoperonospora cubensis*, *Erysiphe graminis*, *Puccinia sorghi* Schw and *Colletotrichum lagenarium* were tested in green house according to the ref. 15. For the active compounds, different lower concentration tests were also conducted for validation.

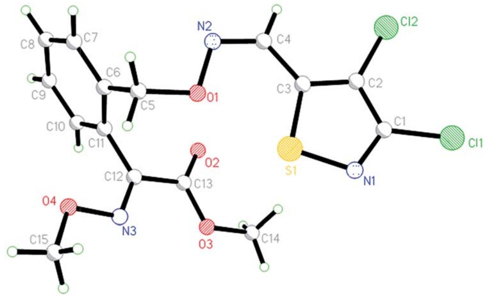


Fig. 2 X-ray diffraction structure of the title compound **6d**.

Evaluation of field efficacy of compound 8d

Due to well laboratory performance against fungi *in vitro* and *in vivo*, the compound **8d** was employed to evaluate its efficacy in the cucumber field. A 9.60% EC of **8d** was prepared for the efficacy against *S. fuliginea* and *P. cubensis* in Wuqing County, Tianjin, P. R. China. Commercial available products of 250 g L^{-1} azoxystrobin SC, 50% trifloxystrobin WG, 250 g L^{-1} pyraclostrobin EC were chosen as positive control. An application dosages of **8d** and positive standards was 37.5 and 75 g. ai per ha for *S. fuliginea* and *P. cubensis*, respectively. Disease index was evaluated by formula as $DI = \sum(A \times B) \times 100/(C \times 9)$; *A*-means the number of disease leaf; *B*-means the corresponding grade of *A*; *C*-means the total number of investigation leaf. Prevention efficacy was calculated with a formula efficacy (%) = $[1 - CK_0 \times PT_1/(CK_1 \times PT_0)] \times 100\%$; CK_0-means DI of control

Table 7 Efficacy of compound **8d** against two diseases in cucumber field (2015)

Disease	Compd	Amount (g ai per hm^2)	Base DI[a]	After DI[b]	Efficacy (%)	DD[d] 5%	DD[d] 1%
S. fuliginea	9.60% **8d** EC	37.5	1.98	8.01	78.62 ± 4.38	a	A
	250 g L^{-1} azoxystrobin SC	37.5	1.66	9.59	70.19 ± 2.91	b	B
	50% trifloxystrobin WG	37.5	1.64	10.04	68.02 ± 5.58	b	B
	CK	nd[c]	1.07	20.63	nd	nd	nd
P. cubensis	9.60% **8d** EC	75	3.71	5.39	79.18 ± 1.09	a	A
	50% trifloxystrobin WG	75	3.73	7.24	72.02 ± 2.15	b	B
	250 g L^{-1} pyraclostrobin EC	75	3.57	5.78	77.02 ± 1.77	a	AB
	CK	nd[c]	3.58	24.96	nd	nd	nd

[a] Base DI, base disease index. [b] After DI, disease index after compounds application. [c] nd, not need to detect. [d] DD, distinct difference.

group before applying water; PT_0 means DI of treatment group before applying compound; CK_1 means DI of control group after applying water; PT_1 means DI of treatment group after applying compound. Duncan's new multiple range (DMRT) statistic method was used for data analysis.

Conclusion

A series of strobilurin analogues with a substructure of 3,4-dichloroisothiazole were synthesized and characterized by NMR, HRMS and X-ray diffraction. These compounds were assessed for biological activity against a few important plant fungi *in vitro* and *in vivo*. The fungicidal potency and spectrum varied with compounds and fungi. Most of synthesized compounds displayed good to excellent fungicidal activity against one or multiple plant fungi, some of these compounds showed better activity against one or multiple plant fungi than commercial standard such as azoxystrobin. Further field experiment suggested that compound **8d** showed better efficacy against cucumber *S. fuliginea* at than two commercial standards azoxystrobin and trifloxystrobin at the same application rate. Moreover, the compound **8d** showed similar efficacy against *P. cubensis* as that of pyraclostrobin, but significantly better than that of trifloxystrobin. In summary, an excellent fungicide candidate which reached commercially biological levels against some plant pathogen was developed in this study for further development.

Acknowledgements

This research was in part financial supported by the International Science & Technology Cooperation Program of China (Grant no. 2014DFR41030), the National Natural Science Foundation of China (No. 21372132 and 31571991), the "111" Project of Ministry of Education of China (Project No. B06005) and Tianjin Natural Science Foundation (No.: 14JCYBJC20400). V. A. Bakulev thanks The Ministry of Education and Science of the Russian Federation (State task 4.1626.2014/K).

Notes and references

1 B. A. Chalmers, H. Xing, S. Houston, C. Clark, S. Ghassabian, A. Kuo, B. Cao, A. Reitsma, C. E. P. Murray and J. E. Stok, *Angew. Chem., Int. Ed.*, 2016, **55**, 3580–3585.

2 Anonymous, *Nature*, 2010, **466**, 531–532.

3 P. Jeschke, *Pest Manage. Sci.*, 2016, **72**, 433–455.

4 (*a*) D. W. Bartlett, J. M. Clough, J. R. Godwin, A. A. Hall, M. Hamer and B. Parr-Dobrzanski, *Pest Manage. Sci.*, 2002, **58**, 649–662; (*b*) P. M. Wood and D. W. Hollomon, *Pest Manage. Sci.*, 2003, **59**, 499–511; (*c*) J. O. Obuya and G. D. Franc, *Eur. J. Plant Pathol.*, 2016, **146**, 817–827.

5 (*a*) H. B. Christensen and K. Granby, *Food Addit. Contam.*, 2001, **18**, 866–874; (*b*) S. Tu, L.-H. Xu, L.-Y. Ye, X. Wang, Y. Sha and Z.-Y. Xiao, *J. Agric. Food Chem.*, 2008, **56**, 5247–5253; (*c*) Y. Wang, Y.-B. Duan and M.-G. Zhou, *Can. J. Plant Pathol.*, 2014, **36**, 354–359; (*d*) L. Li, M. Li, H. Chi, J. Yang, Z. Li and C. Liu, *J. Fluorine Chem.*, 2016, **185**, 173–180.

6 (*a*) E. Flampouri, S. Mavrikou, A.-C. Mouzaki-Paxinou and S. Kintzios, *Biochem. Pharmacol.*, 2016, **113**, 97–109; (*b*) J. Leiminger, B. Adolf and H. Hausladen, *Plant Pathol.*, 2014, **63**, 640–650.

7 U. Gisi, H. Sierotzki, A. Cook and A. McCaffery, *Pest Manage. Sci.*, 2002, **58**, 859–867.

8 (*a*) Y. J. Zhu, X. F. Guo, Z. J. Fan, L. Chen, L. Y. Ma, H. X. Wang, Y. Wei, X. M. Xu, J. P. Lin and V. A. Bakulev, *RSC Adv.*, 2016, **6**, 112704–112711; (*b*) S. Pulya, Y. Kommagalla, D. G. Sant, S. U. Jorwekar, S. G. Tupe, M. V. Deshpande and C. V. Ramana, *RSC Adv.*, 2016, **6**, 11691–11701; (*c*) B. Varun, G. Divya, A. Vikrant, D. Saurabh and S. Poonam, *RSC Adv.*, 2015, **5**, 15233–15266; (*d*) N. P. Belskaya, V. A. Bakulev and Z. J. Fan, *Chem. Heterocycl. Compd.*, 2016, **52**, 627–636; (*e*) Z. Jin, *Nat. Prod. Rep.*, 2006, **23**, 464–496.

9 (*a*) F. Clerici, M. L. Gelmi, S. Pellegrino and D. Pocar, *Top. Heterocycl. Chem.*, 2007, **9**, 179–264; (*b*) R. V. Kaberdin and V. I. Potkin, *Russ. Chem. Rev.*, 2002, **71**, 673–694; (*c*) X. Y. Chen, L. Y. Dai, Y. D. Li, W. T. Mao, Z. Fang, J. J. Li, D. Wang, K. Tatiana and Z. J. Fan, *Chin. J. Pestic. Sci.*, 2013, **15**, 140–144.

10 (*a*) Y. Bektas and T. Eulgem, *Front. Plant Sci.*, 2015, **5**, 804; (*b*) X. Y. Chen, D. Wang, J. Huang, Y. Huang and Z. J. Fan, *J. China Agrochem.*, 2012, **56**, 31–34.

11 D. K. Friel, M. L. Snapper and A. H. Hoveyda, *J. Am. Chem. Soc.*, 2008, **130**, 9942–9951.

12 F. Al-Saffar, S. Berlin, T. Musil and S. Sivadasan, WO Pat., 2006/090153, 2006.

13 P. Strazzolini and A. Pavsler, *Ind. Eng. Chem. Res.*, 2005, **44**, 1625–1626.

14 Z. J. Fan, Z. K. Yang, H. K. Zhang, N. Mi, H. Wang, F. Cai, X. Zuo, Q. X. Zheng and H. B. Song, *J. Agric. Food Chem.*, 2010, **58**, 2630–2636.

15 Y. Q. Xie, Y. B. Huang, J. S. Liu, L. Y. Ye, L. M. Che, S. Tu and C. L. Liu, *Pest Manage. Sci.*, 2015, **71**, 404–414.