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Discovery of flufenoxystrobin: Novel fluorine-containing strobilurin fungicide and acaricide

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ABSTRACT

To discover new strobilurin analogues with high activity, a series of new fluorine-containing strobilurin derivatives were synthesized utilizing intermediate derivatization methods (IDM). The compounds were identified by ^{1}H and ^{19}F nuclear magnetic resonance (NMR), IR and elemental analysis. Preliminary bioassays in greenhouse indicated that compounds **2a** (SYP-3759, flufenoxystrobin) and **2c** exhibited excellent fungicidal activities against *Erysiphe graminis* protecting wheat at 1.56 mg L^{-1} concentration and compounds **2a** showed a moderately high acaricidal activity at 10 mg L^{-1}. Field trials showed the fungicidal activities of compounds **2a** and **2c** is almost equivalent to that of pyraclostrobin, higher than that of triadimefon and the acaricidal activity of compound **2a** is almost equivalent to that of pyidaben, but lower than that of fluacrypyrim. The preliminary mammalian toxicology results showed compound **2a** was a low-toxicity compound. In conclusion compound **2a** is a promising candidate for further development; mammalian toxicology and ecotoxicology with compound **2a** are in progress.

1. Introduction

The strobilurins are known as one of the most important classes of agricultural fungicides with broad fungicidal spectrum, lower toxicity towards mammalian cells and environmentally benign characteristics. Although many strobilurin fungicides have already been commercialized, we postulated that new strobilurin analogues could be discovered using the Intermediate Derivatization Methods (IDM), a useful three-pronged approach for agrochemical discovery that includes the Common Intermediate Method (CIM), Terminal Group Replacement Method (TRM), and the Active Compound Derivatization Method (ADM) [1–16]. In this paper, we present our results from the application of TRM to generate new strobilurin derivatives.

The strobilurin derivatives containing *meta*-trifluoromethyl substituted phenyl, pyridine and pyrimidine in the side chain display excellent fungicidal and acaricidal activity, such as fungicides picoxystrobin [17] and acaricide fluacrypyrim [18]. In order to discover new strobilurin analogues with higher biological activity, we initially designed general structure (**1**) by combining structural components taken from highly potent picoxystrobin and acaricidal fluacrypyrim as shown in Fig. 1. The candidate structure (**1**) encompasses the strobilurin moieties, "methyl *(E)*-β-methoxyacrylate", "methyl (*E*)-methoxyiminoacetate", "*N*-methyl (*E*)-methoxyiminoacetamide" and "methyl *N*-methoxycarbamate", and the *meta*-trifluoromethyl substituted phenol in the side chain.

3-Trifluoromethylphenol is also an important intermediate in synthesis of herbicides picolinafen and diflufenican. Therefore, we hypothesized that the 3-trifluoromethyl phenol moiety and the strobilurin moieties are two critical components in their activities and by replacing the pyridine or pyrimidine moiety in picoxystrobin and fluacrypyrim with 3-trifluoromethylphenol would improve their biological activities further (see Fig. 1). Using this approach, we synthesized a number of compounds of the type **1** and screened them for fungicidal activity.

In order to further optimize our lead compound (**1**), we observed that the diphenyl ether herbicides fomesafen, oxyfluorfen, lactofen, acifluorfen, ethoxyfen-ethyl and fluoroglycofen-ethyl each share the 2-chloro-4-(trifluoromethyl)phenol (**7a**) moiety which is by-product in synthesis process of oxyfluorfen (Figs. 2 and 3). Our next step was to replace the 3-trifluoromethylphenol moiety in the designed structure (**1**) with the 2-chloro-4-(trifluoromethyl)phenol moiety, common to the above listed herbicides, to obtain compounds shown as design structure (**2**) [19]. We also prepared similar analogues with pyridine as shown in design structure (**3**).

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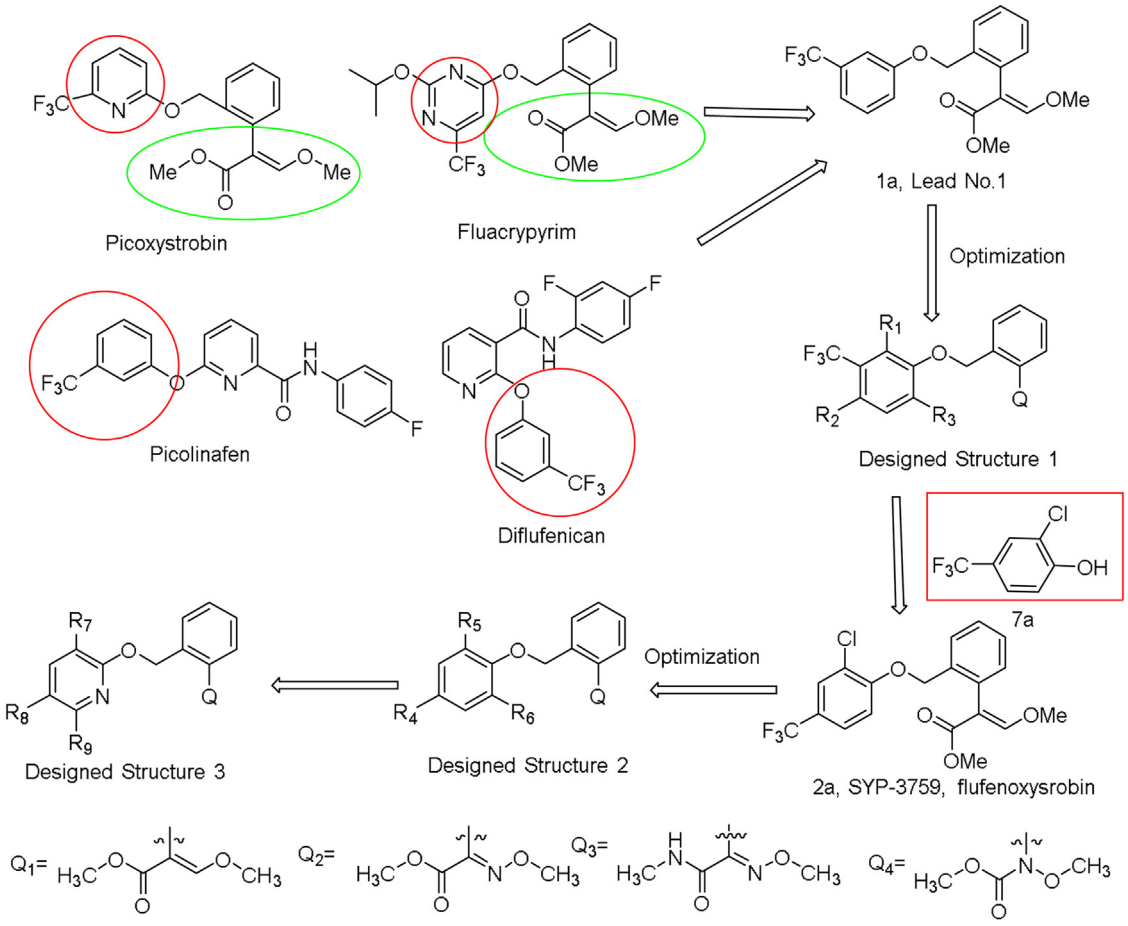


Fig. 1. Design of skeleton.

A series of new strobilurin derivatives containing phenyl and pyridine in the side chain were synthesized and bioassayed. We have found that some compounds display an excellent fungicidal activity against *Erysiphe graminis* (*E. graminis*) protecting respective crops as compared to such standard fungicides as azoxystrobin, kresoxim-methyl and pyraclostrobin (Fig. 4). In addition to its strong fungicidal activity, compound **2a** (SYP-3759, flufenoxystrobin) exhibits an acaricidal activity against *Tetranychus*

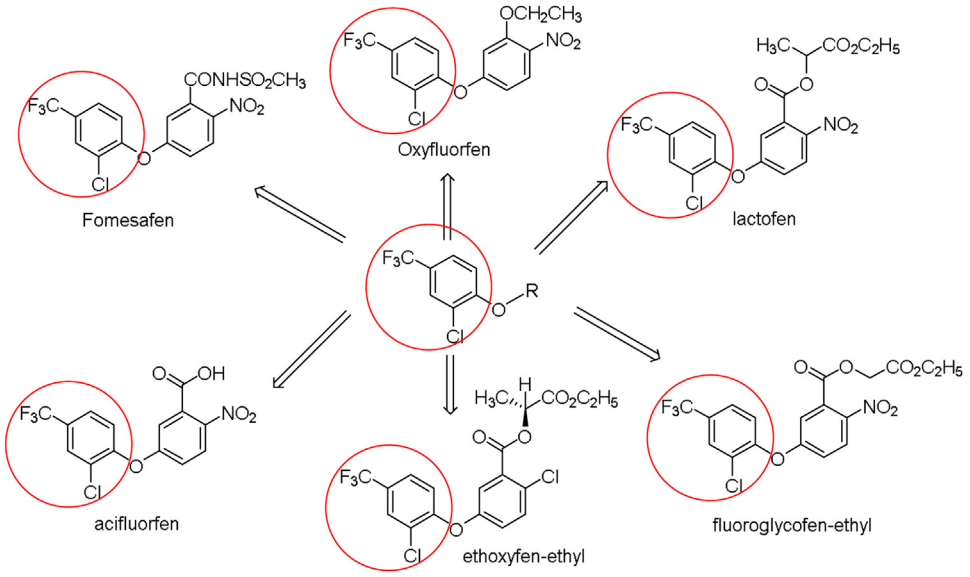


Fig. 2. Agrochemicals containing 2-chloro-4-trifluoromethylphenol.

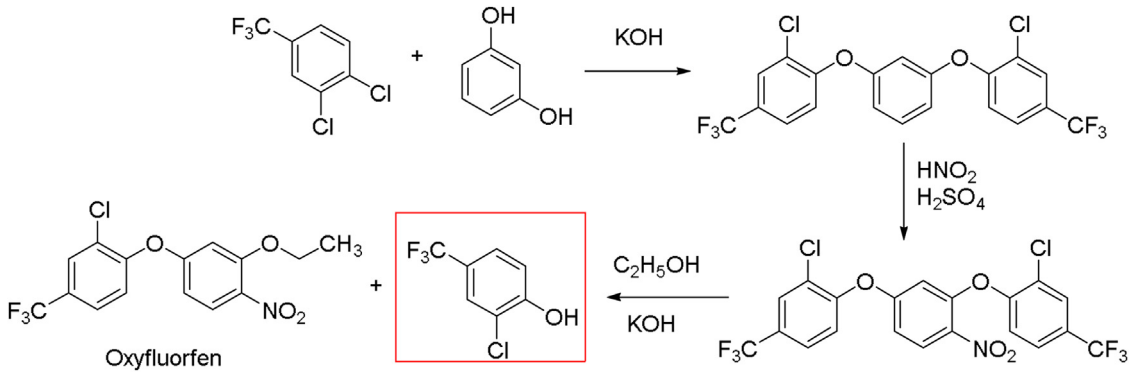


Fig. 3. Synthesis routes of oxyfluorfen and by-product (**7a**).

cinnabarinus (*T. cinnabarinus)* comparable with the reference acaricides fluacrypyrim and pyridaben (Fig. 4). Compound **2a** is a promising candidate for further development.

2. Results and discussion

2.1. Synthesis

According to the Schemes 1–3 , strobilurin derivatives were synthesized generally in good yield of 70–90%, as shown in Table 1 (for *meta*-CF_3-phenol analogues **1a**-**1i**), Table 2 (for other substituted-phenol **2a**–**2k**) and Table 3 (for substituted pyridin-2-ol **3a**–**3f**). The synthesized compounds were characterized by ^{1}H NMR, ^{19}F NMR, IR and elemental analyses. All spectral and analytical data were consistent with the assigned structures.

2.2. Fungicidal activity

The fungicidal activity of the above compounds against *E. graminis* in vivo was deter-mined at the concentration ranging from 0.39 to 25 mg/L, using azoxystrobin, kresoxim-methyl and pyraclostrobin which is the similar structure in the series as the reference standards, in accordance to the methods described in Section 3.2 and the results are listed in Tables 1–3. Some of the synthesized compounds exhibited potential activity against *E. graminis*. Compounds **2a**–**2c**, having strong electron withdrawing group CF_3 on 4-position displayed best fungicidal activity (85, 80 and 85% respectively control at 0.39 mg/L), much higher than azoxystrobin, kresoxim-methyl and pyraclostrobin (30, 0 and 70% at 0.39 mg/L).

2.3. Discovery of the lead compounds *1a* and *2a*

Considering that many fluorine-containing compounds exhibit significant agricultural bioactivities, owing to the unique properties of the fluorine atom, such as high thermal stability and lipophilicity, 3-trifluoromethylphenol and 2-chloro-4-(trifluoromethyl)phenol were introduced into strobilurin derivatives to obtain compounds **1a** and **2a,** respectively. Both compounds exhibited good activities against *E. graminis*, particularly compound **2a** with 2-chloro-4-trifluoromethyl-phenol substituent displayed significant control of 100% against *E. graminis* at 1.56 mg L^{-1}, much higher than azoxystrobin (Table 2). Encouraged by this finding, it was decided to make further structural modifications around these two lead compounds **1a** and **2a** in order to discover new compounds with higher activity.

2.4. Optimization of compound *1a*

Using compound **1a** as lead compound for further optimization, we turned our attention to introducing the Cl atom on the 2, 4 and 6 positions of 3-CF_3-phenyl ring. First, we introduced the Cl atom on the 2 position and kept the 4 and 6 positions fixed as H. We synthesized two compounds **1b** with Q1 and **1c** with Q2 respectively. The bioassay results showed that compounds **1b** and **1c** were less efficacious than lead compound **1a** (60 and 65 versus 100% at 25 mg L^{-1}), indicating that introducing the Cl atoms on the 2 position has a negative effect on bioactivity. Then, we introduced the Cl atom on the 4 position and kept the 2 and 6 positions fixed as H. We synthesized three compounds **1d**,**1e** and **1f** with Q1, Q2 and Q3 respectively. To our surprise, compounds

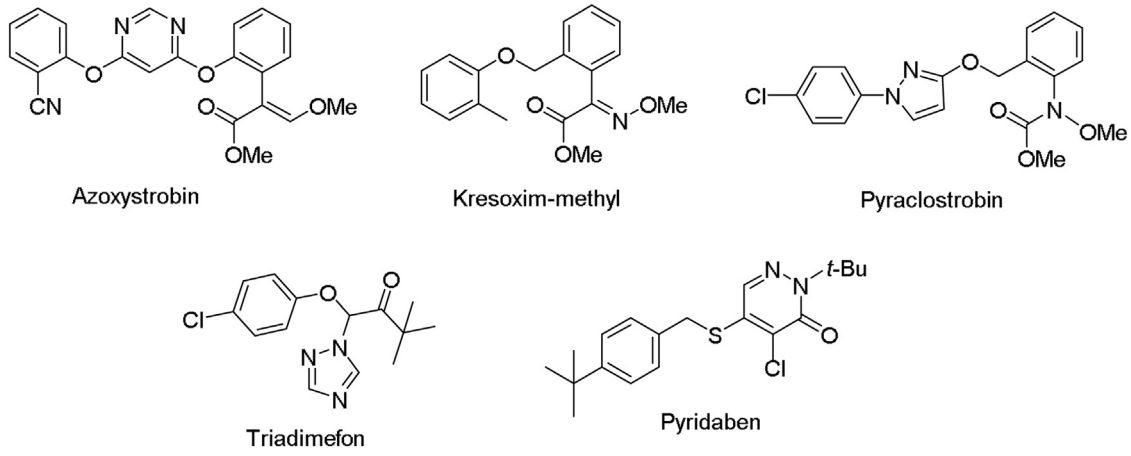
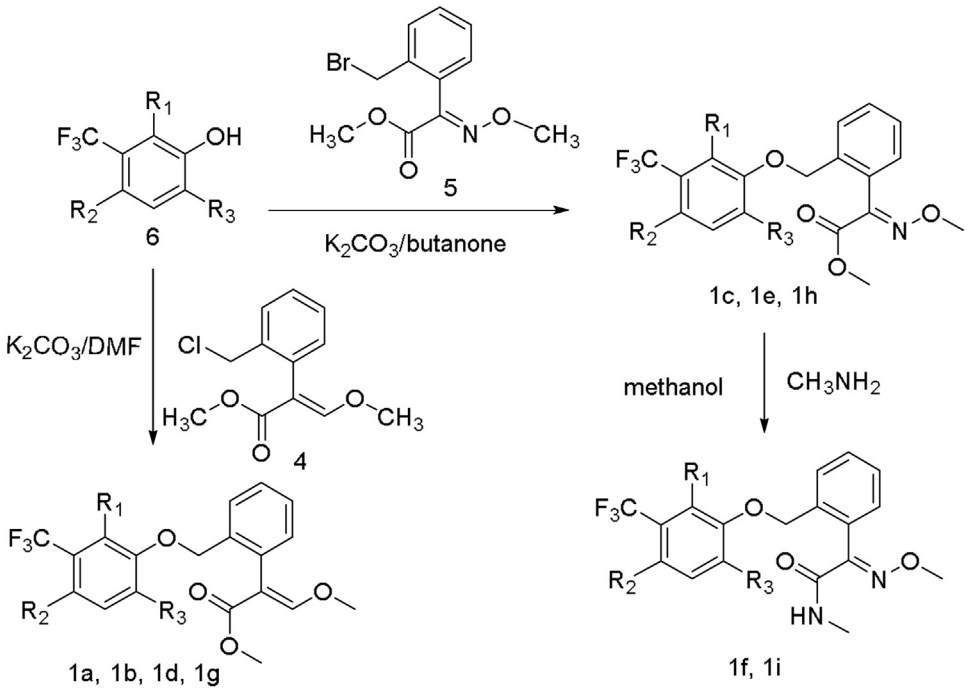
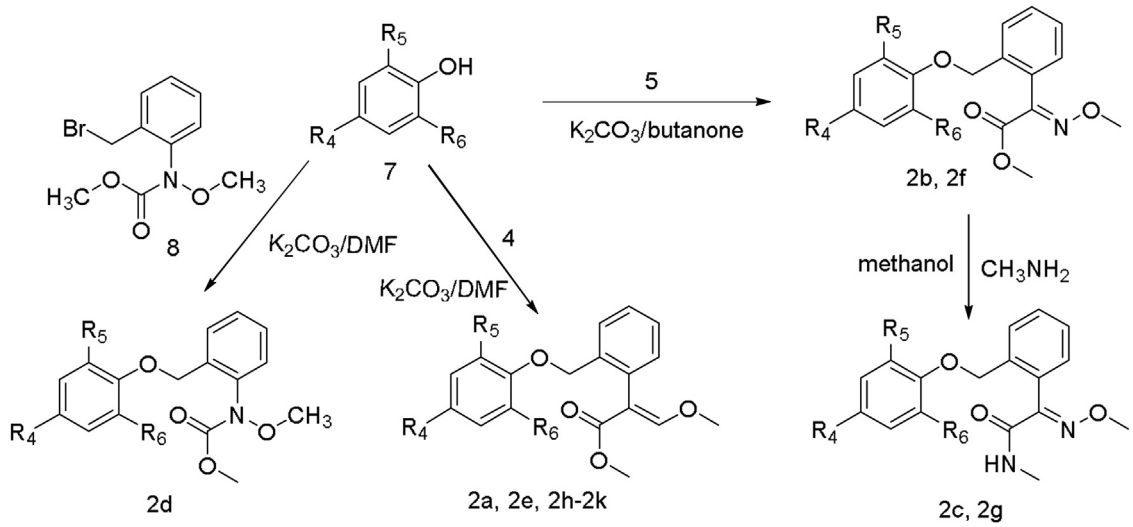


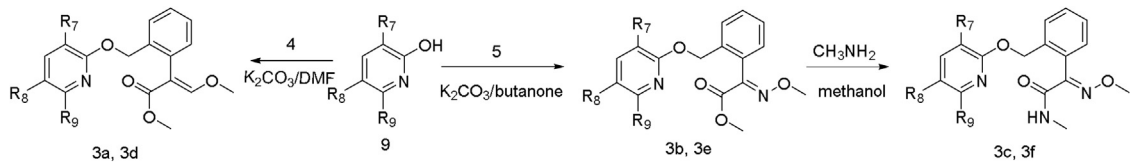
Fig. 4. Reference compounds.



Scheme 1. Synthesis schemes of compound **1**.

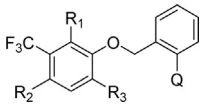


Scheme 2. Synthesis schemes of compound **2**.



Scheme 3. Synthesis schemes of compound **3**.

Table 1
Physical properties and fungicidal activity against *E. graminis* of *meta*-substituted-phenol analogues **1**.



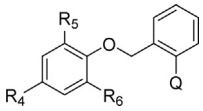
Compound	R_1	R_2	R_3	Q	mp. (°C)	Yield (%)	% control at given concentration mg L^{-1}			
							25	6.25	1.56	0.39
1a	H	H	H	Q1	oil	71.6	100	60	50	40
1b	Cl	H	H	Q1	84-86	70.5	60	0	0	0
1c	Cl	H	H	Q2	100–102	75.3	65	20	0	0
1d	H	Cl	H	Q1	oil	76.1	100	78	70	20
1e	H	Cl	H	Q2	oil	74.9	100	90	60	15
1f	H	Cl	H	Q3	oil	75.6	100	100	80	30
1g	H	H	Cl	Q1	72-78	73.4	100	82	50	10
1h	H	H	Cl	Q2	92-94	73.2	90	45	0	0
1i	H	H	Cl	Q3	132–134	88.5	100	100	70	30
Azoxystrobin							100	100	60	30
Kresoxim-methyl							100	98	40	0
Pyraclostrobin							100	100	100	70

1d–**1f** exhibited 60–80% control of *E. graminis* at 1.56 mg L^{-1}. All of these compounds were more efficacious than compound **1a** which only showed 50% control at 1.56 mg L^{-1} and nearly equivalent to or a little higher than that of azoxystrobin and kresoxim-methyl which gave 60 and 40% control respectively at 1.56 mg L^{-1}. Finally, we introduced the Cl atom on the 6 position and kept the 2 and 4 positions fixed as H and synthesized three compounds **1g**, **1h** and **1i** with Q1, Q2 and Q3 respectively. The fungicidal activity results showed that compound **1i** was more efficacious than the other compounds **1g**, **1h**, lead compound **1a** and azoxystrobin at 1.56 mg L^{-1}, but lower than compound **1f** which share the same moiety Q3. Based on the structure-potency data, introducing the Cl atom on the 4 position (compounds **1d**–**1f**) are most potent among 2, 4 and 6 position of phenyl. The sequence of the substituents R_1, R_2 and R_3 of *meta*-CF_3-phenyl are 4-Cl (R_2 = Cl) > 6-Cl (R_3 = Cl) > 2-Cl (R_1 = Cl) and the sequence of the substituent Q is Q3 > Q2 and Q1.

2.5. Optimization of compound ***2a***

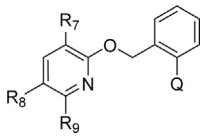
2-Chloro-4-(trifluoromethyl)phenol is an important intermediate as introduced above, so we fixed 2-chloro-4-(trifluoromethyl)phenol moiety and replaced Q1 with Q2, Q3 and Q4 to obtain compounds **2b**, **2c** and **2d**. Fortunately, the results showed that compounds **2b** and **2c** exhibited better activities than **2d** and similar to compound **2a**, which displayed significant control of 100% against *E. graminis* at 1.56 mg L^{-1} (Table 2). More importantly,

Table 2
Physical properties and fungicidal activity against *E. graminis* of *para*-substituted-phenol analogues **2**.



Compound	R_4	R_5	R_6	Q	mp. (°C)	Yield (%)	% control at given concentration mg L^{-1}			
							25	6.25	1.56	0.39
2a	CF_3	Cl	H	Q1	104–106	70.8	100	100	100	85
2b	CF_3	Cl	H	Q2	115–116	72.9	100	100	100	80
2c	CF_3	Cl	H	Q3	92–94	87.5	100	100	100	85
2d	CF_3	Cl	H	Q4	oil	71.3	100	95	65	10
2e	CH_3	Cl	H	Q1	oil	72.3	100	85	50	40
2f	CH_3	Cl	H	Q2	oil	75.6	100	95	60	20
2g	CH_3	Cl	H	Q3	oil	80.1	100	40	20	0
2h	CN	Cl	H	Q1	86–88	75.6	100	100	15	0
2i	CN	Cl	Cl	Q1	84–86	77.3	50	0	0	0
2j	CN	Br	Br	Q1	94–96	73.4	60	20	0	0
2k	NO_2	H	H	Q1	98–100	70.8	90	50	0	0
Azoxystrobin							100	100	60	30
Kresoxim-methyl							100	98	40	0
Pyraclostrobin							100	100	100	70

Table 3
Physical properties and fungicidal activity against *E. graminis* of pyridinol analogues **3**.



Compound	R_7	R_8	R_9	Q	mp. (°C)	Yield (%)	% control at given concentration mg L^{-1} 25	6.25	1.56	0.39
3a	Cl	CF_3	H	Q1	108–110	74.8	100	100	90	40
3b	Cl	CF_3	H	Q2	107–109	73.5	100	100	90	30
3c	Cl	CF_3	H	Q3	103–105	76.4	100	100	80	20
3d	Cl	Cl	Cl	Q1	96–98	72.8	100	100	100	40
3e	Cl	Cl	Cl	Q2	89–91	74.1	100	100	100	45
3f	Cl	Cl	Cl	Q3	88–90	71.9	100	100	98	20
Azoxystrobin							100	100	60	30
Kresoxim-methyl							100	98	40	0
Pyraclostrobin							100	100	100	70

compounds **2a**–**2c** showed better fungicidal activity than the commercial fungicides azoxystrobin and kresoxim-methyl at 0.39 mg L^{-1} (85, 80 and 85% respectively, versus 30, 0 and 70% for azoxystrobin, kresoxim-methyl and pyraclostrobin). Therefore, compounds **2a** and **2c** could be selected as the promising candidate for further commercial development. We next considered changing the substituent CF_3 of compound **2a** to CH_3 (compounds **2e**–**2g**), unfortunately these compounds did not exhibit excellent bioactivity compared with the compounds containing substituent CF_3 according to the pairs of **2a** and **2e**, **2b** and **2f**, **2c** and **2g**.

Because the activities were measured in vivo, effects of metabolism and/or biotransformation inside the bodies of plant/fungus and acarids result in the apparent potency variations. The inference that the electron-withdrawing substituents of the phenyl moiety may enhance the potency could be related to the fact that they tend to retard mechanisms of oxidative metabolism occurring on (or close to) the benzene ring [20,21]. The higher the electronic (negative) charge within the ring system, the easier would occur such an oxidative detoxication metabolism as hydroxylation of the benzene ring. So we introduced strong electronic group CN (**2h**–**2j**) and NO_2 (**2k**) into the phenyl ring in order to discover higher active compound, but these compounds did not show more efficacious than compounds **2a**–**2c** as expected.

On the whole, under equivalent dosage (25 mg L^{-1}) most of the strobilurin derivatives containing meta and *para*-substituted phenol show 100% control against *E. graminis* but their fungicidal activity varies in the lower concentrations. Generally, *meta*-CF_3-phenol analogues in Table 1 (R_1 = Cl) seem to be less potent than corresponding *para*-CF_3-phenol analogues in Table 2 (R_4 = CF_3) sharing common substituted Cl, for example the pair of meta and *para*-CF_3-phenol analogues **1b** and **2a**, **1c** and **2b**.

Additionally, 3-chloro-5-(trifluoromethyl)pyridin-2-ol and 3,5,6-trichloro-pyridin-2-ol attracted our attention because it is a popular heterocycle intermediate in the field of pesticide. We designed and synthesized pyridine analogues **3a**–**3f** (Table 3), however these compounds showed lower fungicidal activities than compounds **2a**–**2c**.

2.6. Field trials with compounds *2a* and *2c* against E. graminis

In this study **2a** and **2c** were two of the most potent compounds with fungicidal activity against *E. graminis*. We carried out field trials of these two compounds against *E. graminis* in 2008 as summarized in Table 4. The fungicidal activities of compounds **2a** and **2c** as 20% suspension concentrate (SC) is almost equivalent to that of pyraclostrobin as 25% SC at equivalent doses, and higher than that of triadimefon as 15% wettable powder (WP) at 135 mg L^{-1}.

2.7. Acaricidal activity and field trials with compound *2a*

The target compounds were tested for control of *T. cinnabarinus*, however the compounds showed no acaricidal activity at 600 mg L^{-1} dosages except compounds **2a** and **2d** which exhibited 100% mortality. As indicated in Table 5, compound **2a** was further tested for control of *T. cinnabarinus* and showed a moderately high acaricidal activity at 10 mg L^{-1}, similar to the reference acaricide fluacrypyrim.

Field trials were carried out in Pulandian, Liaoning Province in 2008 as summarized in Table 6. The acaricidal activity of compound **2a** against the red spider mite, *Panonychus ulmi* (Koch), is almost equivalent to that of pyidaben at 50–100 mg L^{-1}, and lower than that of fluacrypyrim.

2.8. Mammalian toxicology of compounds *2a* and *2c*

The primary mammalian toxicology tests of compounds **2a** and **2c** were studied, as shown in Table 7. The following results were

Table 4
Field trial results for compounds **2a** and **2c** in Liaoning against *E. graminis*.

Compound	Doses (mg L^{-1})	Disease index	Control (%)
2a 200 g L^{-1} SC	135	2.7	93.1
	45	5.9	84.9
	30	7.1	81.8
2c 200 g L^{-1} SC	135	3.2	91.7
	45	7.5	81.0
	30	8.4	78.3
Pyraclostrobin 250 g L^{-1} SE	135	3.5	91.0
	45	5.1	86.8
	30	4.9	87.5
Triadimefon 150 g L^{-1} WP	135	5.2	86.6

Table 5
Acaricidal activity against *T. cinnabarinus* of compound **2a**.

Compound	% mortality at given concentration mg L^{-1}		
	150	40	10
2a 200 g L^{-1} SC	100	95	95
Fluacrypyrim	100	100	95

determined compounds **2a** and **2c** were all low-toxicity compounds.

3. Experimental

All starting materials and reagents were commercially available and used without further purification except as indicated. Melting points were determined on a Büchi melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded with Mercury 300 (Varian, 300 MHz) spectrometer with $CDCl_3$ as the solvent and TMS as the internal standard. ^{19}F NMR spectra were obtained on a Mercury 300 (Varian, 300 MHz) spectrometer using CF_3COOH (TFA) as an external standard, positive for downfield shift. Infrared spectra were measured with KBr discs using a PF–983 G instrument (Perkin-Elmer). Elemental analyses were performed on a Vario EL elemental analyzer. These compounds were tested for controlling wheat powdery mildew (*Erysiphe graminis*) on "Liaochun No.10" wheat and spider mites (*Tetranychus cinnabarinus*) on Kidney bean obtained from the Agrochemical Discovery Group in Shenyang Research Institute of Chemical Industry.

The general synthesis routes for the title compounds are shown in Schemes 1–3. Representative procedures are given below and reaction yields were not optimized. New compounds were identified and verified by ^{1}H NMR, ^{19}F NMR, IR, MS and elemental analysis.

3.1. Synthesis of target compounds (*1a–1i*, *2a–2k* and *3a–3f*)

3.1.1. Synthesis of (E)-methyl 2-(2-((2-chloro-4-(trifluoromethyl) phenoxy)methyl)phenyl)-3-methoxyacrylate (*2a*, SYP-3759, flufenoxystrobin; general procedure for the compounds *1a*, *1b*, *1d*, *1g*, *2d*, *2e*, *3a* and *3d*)

2-Chloro-4-(trifluoromethyl)phenol (**7a**) (0.39 g, 1.98 mmol) was dissolved in 15 mL of DMF, and anhydrous potassium carbonate (0.55 g, 3.99 mmol) was added to the solution. The solution was stirred for 0.5 h, and methyl (*E*)-methyl 2-(2-(chloromethyl)phenyl)-3-methoxyacrylate (0.48 g, 2.00 mmol) was added. The reaction mixture was heated to 80 °C and was monitored by TLC. At completion (after 3 h) the mixture was partitioned with 50 mL of brine, and extracted 3 times with 100 mL of ethyl acetate. The combined organic extracts were dried, and concentrated to obtain the crude product. It was further purified via silica gel column chromatography, using a 1:4 (v/v) mixture of ethyl acetate and petroleum ether (boiling point range: 60–90 °C) as the eluting solution to obtain **2a** as a white solid 0.51 g.

IR(KBr)ν: 2950 (s, C—H), 1690 (s, C=O), 1630 (s, C=C), 1500, 1430, 1410 (s, CH_3), 1320, 1270 (m, C—N), 1120 (s, C—O), 990, 890, 810, 770, 740 (s, Ph-H) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δppm 7.62 (s, 2H, CH + Ph-3-H), 7.53(m, 1H, Ph'-6-H), 7.35(m, 3H, Ph-5-H + Ph'-3,5-2H), 7.20(m, 1H, Ph'-4-H), 6.89(d, *J* = 8.4 Hz, 1H, Ph-6-H), 5.12(s, 2H, OCH_2), 3.83(s, 3H, $COOCH_3$), 3.71(s, 3H, OCH_3); ^{19}F NMR($CDCl_3$, TFA): δppm −6.75(s, 3F, CF_3); Anal. calcd (%) for $C_{19}H_{16}ClF_3O_4$: C, 56.94; H, 4.02. Found: C, 56.99; H, 4.00.

Table 6
Field trial results for compound **2a** in Pulandian against *Panonychus ulmi* (Koch).

Compound	Doses (mg L^{-1})	Mortality at days after spraying (%)			
		3 d	7 d	14 d	21 d
2a 200 g L^{-1} SC	50	89.6	91.1	89.2	77.7
	100	93.1	96.5	95.0	87.8
	150	96.1	97.1	95.9	91.2
Fluacrypyrim	50	99.7	100.0	98.2	94.8
	100	99.5	100.0	99.1	95.3
Pyidaben	50	91.9	95.9	96.8	78.8
	100	95.9	95.5	97.1	78.2

3.1.1. Synthesis of (E)-methyl 2-(2-((2-chloro-4-(trifluoromethyl) phenoxy)methyl)phenyl)-2-(methoxyimino)acetate (*2b*; general procedure for the compounds *1c*, *1e*, *1h*, *2f*, *3b* and *3e*)

2-Chloro-4-(trifluoromethyl)phenol (0.43 g, 2.19 mmol) was dissolved in 15 mL of butanone, and anhydrous potassium carbonate (0.60 g, 4.35 mmol) was added to the solution. The solution was stirred for 0.5 h, and methyl (*E*)-methyl 2-(2-(bromomethyl)phenyl)-2-(methoxyimino)acetate (0.63 g, 2.20 mmol) was then added. The reaction mixture was heated to 80 °C and monitored by TLC. After five hours, the mixture was cooled, diluted with 50 mL water and extracted with ethyl acetate (3 × 100 mL). The combined extracts were washed with brine, dried (anhydrous magnesium sulfate), and filtered. The filtrate was evaporated and the crude product was purified via silica gel column chromatography, using a 1:2 (v/v) mixture of ethyl acetate and petroleum ether (boiling point range: 60–90 °C) as the eluting solution to obtain compound **2b** as a white solid: 0.67 g (72.9%), mp 115–116 °C.

^{1}H NMR(300 MHz, $CDCl_3$): δppm 7.63(s, 1H, Ph-3-H), 7.58(m, 1H, Ph'-6-H), 7.45(m, 3H, Ph'-3,4,5-3H), 7.22(d, *J* = 8.4 Hz, 1H, Ph-5-H), 6.93(d, *J* = 8.4 Hz, 1H, Ph-6-H), 5.09(s, 2H, OCH_2), 4.04(s, 3H, $NOCH_3$), 3.88(s, 3H, $COOCH_3$); ^{19}F NMR($CDCl_3$, TFA): δppm −6.88(s, 3F, CF_3); Anal. calcd (%) for $C_{18}H_{15}ClF_3NO_4$: C, 53.81; H, 3.76; N, 3.49. Found: C, 53.86; H, 3.74; N, 3.46.

3.1.2. Synthesis of (E)-2-(2-((2-chloro-4-(trifluoromethyl)phenoxy) methyl)phenyl)-2-(methoxyimino)-N-methylacetamide (*2c*; general procedure for the compounds *1f*, *1i*, *2g*, *3c* and *3f*)

Compound **2b** (0.40 g, 0.96 mmol) was dissolved in 10 mL of methanol, and methylamine (0.08 g, 2.57 mmol) was added to the solution. The solution was stirred at room temperature and monitored by TLC. After three hours, the mixture was concentrated, diluted with 50 mL water and extracted with ethyl acetate (3 × 100 mL). The combined extracts were washed with brine, dried (anhydrous magnesium sulfate), and filtered. The filtrate was evaporated and the crude product was purified via silica gel column chromatography, using a 1:1 (v/v) mixture of ethyl acetate and petroleum ether (boiling point range: 60–90 °C) as the eluting solution to obtain compound **2c** as a white solid: 0.35 g (87.5%).

^{1}H NMR(300 MHz, $CDCl_3$): δppm 7.62(m, 1H, Ph-3-H), 7.51(m, 1H, Ph'-6-H), 7.42(m, 3H, Ph'-3,4,5-3H), 7.24 (m, 1H, Ph-5-H), 6.93–6.96(d, *J* = 8.4 Hz, 1H, Ph-6-H), 6.78(bs, 1H, CONH), 5.12(s, 2H,

Table 7
Toxicity results.

Test	Compound **2a**	Compound **2c**
Acute oral	$LD_{50} \geq 5000$ mg kg^{-1}	$LD_{50} > 4640$ mg kg^{-1}
Acute percutaneous	$LD_{50} \geq 5000$ mg kg^{-1}	$LD_{50} \geq 2150$ mg kg^{-1}
Skin irritant	No irritation	No irritation
Eye irritant	No irritation	No irritation
Ames	Negative	Negative

OCH_2), 3.92(s, 3H, $NOCH_3$), 2.92(d, J = 5.4 Hz, 3H, $CONHCH_3$); ^{19}F NMR($CDCl_3$, TFA): δppm −6.65(s, 3F, CF_3); Anal. calcd (%) for $C_{18}H_{16}ClF_3N_2O_3$: C, 53.94; H, 4.02; N, 6.99. Found: C, 53.86; H, 4.05; N, 7.01.

3.2. Fungicidal assay

Each of the test compounds (4 mg) was first dissolved in 5 mL mixture of acetone and methanol (1:1 by volume), then 5 mL of water containing 0.1% Tween 80 was added to generate a 10 mL stock solution of concentration 400 mg L^{-1}. Serial test solutions were prepared by diluting the above solution. A 1:1:2 (v/v/v) mixture containing 2‰ Tween 80 of acetone, methanol and water was used as untreated control.

Evaluations of fungicidal activities of the synthesized compounds against *E. graminis* were performed as follows: briefly, a whole plant is used in this test, and the testing solution is sprayed to the host plant by a special plant sprayer. The plant is inoculated with fungus after 24 h. According to the infecting characteristics of fungus, the plant is stored in a humidity chamber and then transferred into a greenhouse after infection is finished. The other plants are placed in a greenhouse directly. The activity of each compound was estimated by visual inspection after 7 days, and screening results were reported as a range from 0% (no control) to 100% (complete control).

3.3. Acaricidal assay

Each of the test compounds was first dissolved in a mixture of acetone and water, and water containing 0.1% Tween 80 was then added to make the stock solution. Serial test solutions were prepared by using acetone + water (9 + 1 by volume). Kidney bean plants with one true leaf were infested with *T. cinnabarinus* prior to spraying. An airbrush was used for spraying the compound solutions, and three replicates were used for each treatment. After the plants were dried, they were transferred to a maintaining room for observation. The mortality of spider mites was scored as percentage of control by visual inspection 48 h after the spray treatment.

4. Conclusions

As described above, 26 strobilurin analogues synthesized by introducing the CF_3 group into the phenyl ring of the designed skeletal lead were indeed active fungicidally against *E. graminis*. Some of them were not only more potent than such reference strobilurin compounds azoxystrobin and krezoxim-methyl but were also acaricidal though a little less potent against *T. cinnabarinus* than the reference acaricide fluacrypyrim. Compounds **2a** and **2c** are active fungicidally against *E. graminis* at 1.56 mg L^{-1} concentration and compound **2a** showed a moderately high acaricidal activity at 10 mg L^{-1}. Compound **2a** is a promising candidate for further development. This study demonstrates the effectiveness of IDM approach to the discovery of highly bioactive compounds. More field trials, mammalian toxicology and ecotoxicology of compound **2a** are in progress.

Supporting information description

^1H NMR, IR and elemental analyses data for all synthesized compounds can be found in the Supporting Information. This material is available free of charge via the Internet at http://www.sciencedirect.com.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jfluchem.2016.03.013.

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