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SYNTHESIS AND BIOLOGICAL ACTIVITIES OF SOME NOVEL STROBILURIN DERIVATIVES CONTAINING 1, 2, 4- TRIAZOLE MOIETY

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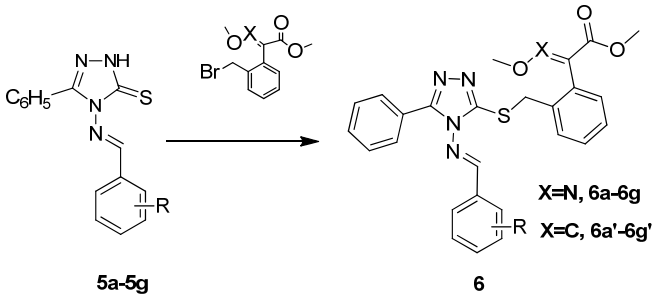
In this study, a series of strobilurin derivatives containing 1, 2, 4-triazole Schiff base side chain were designed and synthesized. Their structures were confirmed by IR, ^{1}H NMR, ^{13}C NMR, and HRMS. The antifungal tests indicated that compounds **6d′** displayed modest antifungal activity against *Rhizoctonia solani*, *Botrytis cinereapers*, and *Fusarium graminearum*. In addition, compounds **6g′**, **6f′**, **6e**, and **6e′** exhibited better fungicidal activities against *Blumeria graminis* at a concentration of 50 μg/mL, with the inhibitory rates of 83.20%, 85.85%, 92.09%, and 100%, respectively.



INTRODUCTION

Strobilurin fungicides are some of the most potent and successful agrochemicals of recent decades owing to their broad spectrum of activity, long duration, high efficiency, and low toxicity.[1-3] Azoxystrobin and kresoxim-methyl (Figure 1) were launched as the first commercialized products in 1996.[4] Subsequently, a significant number of strobilurin analogs have been synthesized[5-8] and at least ten of them have already been commerciallized.[9,10] However, strobilurin fungicides develop fungicide resistance after being used for long term necessitating the development of new types of strobilurin.[11, 12]

Many 1, 2, 4-triazole derivatives possess potent pesticidal,[13] antifungal,[14-16] and herbicidal[17] activities, attributing to "1, 2, 4-triazol" unit as the key to their bioactivities. Triadimenol and tebuconazole are representatives of this class of fungicides (Figure 1).

As shown in Figure 2, it is hypothesized that the 1, 2, 4-triazolo units and the strobilurin pharmacophore moiety are two critical components imparting activities to the designed compounds, and the combination of these two critical components would further improve their biological activities. With this in mind, utilizing the intermediate derivatization method based on the active substructure combination and bioisosteric replacement, the methoxyiminoacetate pharmacophore of trifloxystrobin and methoxyacrylate pharmacophore of azoxystrobin were introduced into the substituted 1, 2, 4-triazolo structure,and a series

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of novel strobilurin analogues **6** were designed and synthesized. In this study, the synthesis, fungicidal activity, and the structure-activity relationship of new strobilurins containing 1, 2, 4-triazolo Schiff base are described.

RESULTS AND DISCUSSION

Synthetic Chemistry

Scheme 1 shows the synthetic route for the target compounds. Compound **4** was reacted with appropriate aromatic aldehydes and 2 to 3 drops of glacial acetic acid, affording **5** in moderate yield according to the similar method reported in the literatures.[18] The strobilurin derivatives of **6** were obtained by the reaction of derivatives **5** with (*E*)-methyl 2-(2-(bromomethyl) phenyl)-2-(methoxyimino)acetate or (*E*)-methyl 2-(2-(bromomethyl) phenyl)-3-methoxyacrylate in the presence of base according to the literature procedure.[19] The structures of the desired target compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR, and ESI-HRMS.

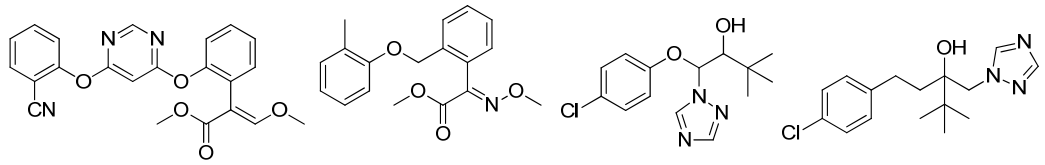


Fig. 1 – Structures of azoxystrobin, kresoxim-methyl, Triadimenol, and Tebuconazole.

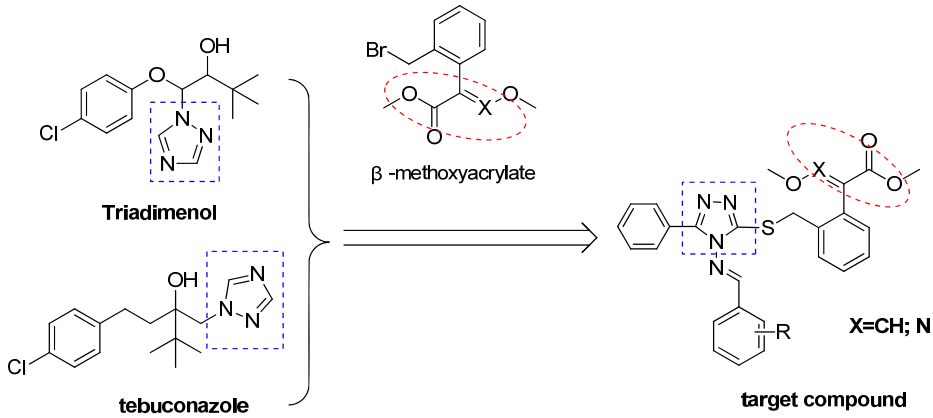
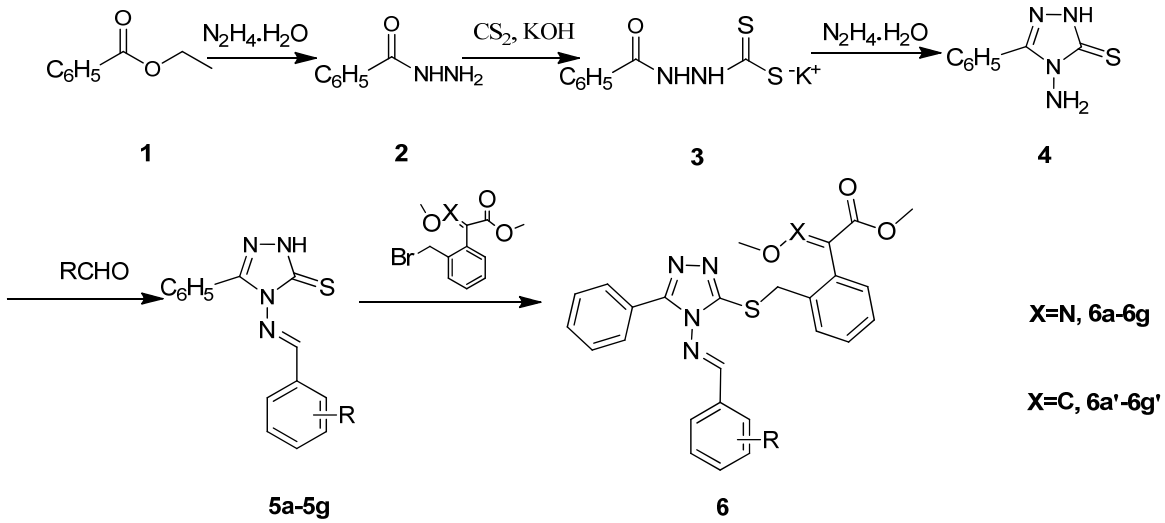


Fig. 2 – Design strategy of the proposed compounds.



R = 6a, 6a′. 2-CH_3; 6b, 6b′. 3-CH_3; 6c, 6c′. 2,4-$(OCH_3)_2$; 6d, 6d′. 2,6-Cl_2; 6e, 6e′. 2,4-F_2; 6f, 6f′. 3,4-F_2; 6g, 6g′. 3-Cl-4-F.

Biological evaluation

The fungicidal activities of compounds **6** were tested at a concentration of 50 μg/mL by the modified method. The four fungi, *Rhizoctonia solani*, *Botrytis cinereapers*, *Fusarium graminearum*, and *Cotton rhizoctoniosis* used in the fungicidal bioassay were tested by the mycelium growth rate method, and *Blumeria graminis* was tested by the pot culture test method. The commercial agricultural fungicide kresoxim-methyl was used as the standards. The results of initial bioassays are summarized in Table 1.

As listed in Table **1**, none of the designed compounds exhibited good fungicidal activity against *Cotton Rhizoctoniosis* at a concentration of 50 μg/mL. Compound **6d′** exhibited promising antifungal activity, inhibiting growth of *Rhizoctonia solani* at 52.85%, *Botrytis cinereapers* at 68.63%, and *Fusarium graminearum* at 66.35% However, its antifungal activity is still less than that of kresoxim-methyl (65.32% against *Rhizoctonia solani*, 81.69% against *Botrytis cinereapers*, and 73.36% against *Fusarium graminearum* at 50 μg/mL). In addition, under the same test conditions, compounds **6e**, **6f′** and **6g′** exhibited slightly better fungicidal activities against *Blumeria graminis* with the inhibition rates of 92.09%, 85.85%, and 83.20% respectively. In particular, compound **6e′** showed 100.00% inhibition rate against *Blumeria graminis*, which was about equal to the control medicament kresoxim-methyl.

These results also demonstrated that the introduction of 1, 2, 4-substituted triazole rings to the strobilurin fungicides might improve their fungicidal activities. Structural optimization of compounds **6** was carried out by the modification of two primary substructures: R and X moieties. When R was kept and X was changed from N to CH, the fungicidal activity of the corresponding compounds increased slightly, as shown by the following order, **6d′** > **6d**, **6e′** > **6e**, **6f′** > **6f**.

When X was constant, the fungicidal activity of the synthesized compounds **6** was influenced by the nature and position of the substituted group R in the benzene ring. The modification of R from electron-donating groups (CH_3, **6a′**; OCH_3, **6c′**) to electron-withdrawing group (Cl; F, **6e′**, **6g′**) improved the fungicidal activities against *Blumeria graminis*. e.g., **6e**> **6c**, **6e′** > **6c′**. In contrast, when the position of substituent R in the phenyl group changed, from *ortho* to *meta*, the fungicidal activity improved, **6a** > **6b** and **6a′** > **6b′**.

Table 1

Fungicidal activities of **6a-6g**, **6a′-6g′** (inhibition rate /%, 50 μg/mL)

Entry	*Rhizoctonia solani*	*Botrytis cinereapers*	*Fusarium graminearum*	*Cotton Rhizoctoniosis*	*Blumeria graminis*
6a	21.42	27.45	26.24	23.46	24.03
6b	16.66	23.57	16.45	18.56	22.04
6c	23.43	32.02	13.45	16.46	42.12
6d	41.01	53.24	48.98	15.64	45.76
6e	24.96	38.13	21.78	23.44	92.09
6f	34.32	23.75	25.16	22.34	63.58
6g	18.42	23.53	17.54	19.78	72.18
6a′	33.21	30.36	35.36	29.22	39.52
6b′	21.15	28.12	32.58	26.32	36.36
6c′	25.23	35.25	28.46	23.63	43.32
6d′	52.85	68.63	66.35	29.42	49.31
6e′	45.36	39.82	35.62	36.23	100.00
6f′	40.23	26.36	32.10	35.36	85.85
6g′	25.24	26.38	21.32	36.57	83.20
kresoxim-methyl	65.32	81.69	73.36	61.56	100.00

EXPERIMENTAL

General procedures

All melting points were determined on an XT-4A apparatus and are uncorrected. The IR spectra (KBr disks) were taken on a Bruker Quinox 55 spectrophotometer. The ^{1}H NMR spectra were measured on a Bruker Advance 600 spectrometer for DMSO-*d6* solutions using TMS as internal standard. Elemental analyses were determined on a Flash-1112 series elemental analyzer. All the reagents used were AR grade. Molecular weights of monomers were determined by high resolution mass spectroscopy (ESI-HRMS, Bruker Daltonics ApexUltra 7.0 Tesla Fourier Transform Ion Cyclotron Resonance Mass Spectrometer).The completion of reactions was monitored by TLC.

***General procedure for the synthesis of benzohydrazide* (2)** [20]

A mixture of Ethyl benzoate **1** (0.1 mol) and hydrazine hydrate (0.1mol) in 30 mL of ethanol was stirred vigorously for 6 h at room temperature. The mixture was filtered, and the solid was washed with cold water and dried. After drying, the solid was recrystallized from ethanol to give intermediate **2**.

***General method for the synthesis of potassium dithiocarbazinate* (3)** [21]

Potassium hydroxide (0.15 mol) was dissolved in absolute ethanol (100 mL). To the above solution, benzohydrazide **2** (0.1 mol) was added and cooled the solution in ice. Then, carbon disulfide (0.15 mol) was added drop wise, and the reaction mixture was stirred for 15 h at room temperature. The precipitated potassium dithiocarbazinate was collected by filtration. The precipitate was further washed with anhydrous ether (100 mL). After drying, it was used for the next reaction directly without purification.

***Synthesis of 4-amino-3-phenyl-5-thiol-1, 2, 4-triazole* (4)** [22]

The above potassium dithiocarbazinate 3 (0.5 mol) was taken into hydrazine hydrate (0.15 mol) and refluxed for 6 h with occasional shaking. The color of the reaction mixture changed to green with the evolution of hydrogen sulfide gas (lead acetate paper and odor). The reaction mixture was cooled to room temperature and diluted with water. On acidification with concentrated hydrochloric acid, the required triazole was precipitated. It was filtered, washed thoroughly with cold water, and recrystallized from ethanol to give 4-amino-3-phenyl -5- thiol - 1, 2, 4-triazole **4**. White crystal, Yield: 63.1%, m.p.: 201- 202 ^{o}C; IR: (KBr, cm^{-1}): 3412, 3070, 2667, 1640; ^{1}H NMR (600 MHz, DMSO-*d6*) δ: 13.90 (s, 1H, triazole-NH), 7.52-8.01 (m, 5H, Ar-H), 5.81 (s, 2H, NH_2).

***General method for the preparation of (E)-3-thiol-4-arylideneamino-5- phenyl - 4H -1, 2, 4- triazole* (5a-5g)**.

To a solution of compound 4 (l0 mmol) dissolved in absolute alcohol (30 mL), the appropriate benzaldehyde (l0 mmol) and 2 to 3 drops of glacial acetic acid were added. The mixture was refluxed for 4 h with stirring, the solid that was obtained upon cooling was filtered, washed with cold water, dried and recrystallized from alcohol to give the Schiff bases **5a-5g**.

***(E) - 3-thiol-4-(2-methylbenzylideneamino)-5-phenyl-4H-1, 2, 4-triazole* (5a)**

White solid, Yield: 70.1%, m.p.: 212-213 ^{o}C; IR: (KBr, cm^{-1}): 3115, 2968, 1613, 1514, 1511, 1479, 1270; ^{1}H NMR (600 MHz, DMSO-*d6*) δ: 2.40(s, 3H, CH_3), 7.21-7.46 (m, 2H, Ar-H), 7.63 (t, *J* =12.0 Hz, 1H, Ar-H), 7.56-7.62 (m, 3H, Ar-H), 7.71- 7.92(m, 3H, Ar-H), 9.50(s, 1H, CH=N), 14.19 (s, 1H, triazole-NH); ^{13}C NMR(125 MHz, DMSO-*d6*) δ: 121.11, 128.00, 128.21, 128.25, 128.37, 128.64, 130.86, 131.75, 137.95, 144.84, 154.78, 165.16; Anal. calcd for $C_{16}H_{14}N_4S$; C, 65.28; H, 4.79; N, 19.03; Found: C, 65.31; H, 4.70; N, 18.98.

***(E) - 3-thiol-4-(3-methylbenzylideneamino)-5-phenyl-4H-1, 2, 4-triazole* (5b)**

White solid, Yield: 61.8%, m.p.: 224-225 ^{o}C; IR: (KBr, cm^{-1}): 3116, 2988, 1623, 1519, 1508, 1480, 1279; ^{1}H NMR (600 MHz, DMSO-*d6*) δ: 2.42 (s, 3H, CH_3), 7.16 (t, *J* = 14.2, 1H, Ar-H), 7.30 (t, *J* = 14.6 Hz, 1H, Ar-H), 7.51-7.42 (m, 3H, Ar-H), 7.81-7.64 (m, 4H, Ar-H), 9.71 (s, 1H, CH=N), 14.19 (s, 1H, triazole-NH); ^{13}C NMR(125 MHz, DMSO-*d6*) δ: 21.09, 128.32, 128.24, 128.32, 128.39, 128.57, 130.79, 131.87, 137.95, 144.86, 154.71, 165.18; Anal. calcd for $C_{16}H_{14}N_4S$; C, 65.28; H, 4.79; N, 19.03; Found: C, 65.26; H, 4.72; N, 19.15.

***(E)-3-thiol-4-(2,4-dimethoxybenzylideneamino)-5-phenyl-4H-1,2,4-triazole* (5c)**

White needle crystal, Yield: 79.0%, m.p.: 206-207 ^{o}C; IR: (KBr, cm^{-1}): 3101, 2971, 1613, 1541, 1532, 1484, 1278; ^{1}H NMR (600 MHz, DMSO-*d6*) δ: 3.84(s, 3H, OCH_3), 3.86(s, 3H, OCH_3), 7.37-7.60(m, 4H, Ar-H), 7.69-7.88 (m, 3H, Ar-H), 8.34 (s, 1H, Ar-H), 9.39 (s, 1H, CH=N), 14.28 (s, 1H, triazole-NH); ^{13}C NMR(125 MHz, DMSO-*d6*) δ: 56.04, 56.67, 97.67, 109.01, 114.63, 128.21, 129.87, 130.86, 144.84, 147.42, 161.26, 165.14; Anal. calcd for $C_{17}H_{16}N_4O_2S$; C, 59.98; H, 4.74; N, 16.40; Found: C, 60.03; H, 4.69; N, 16.44.

***(E) - 3-thiol-4- (2,6-dichlorobenzylideneamino) -5-phenyl-4H-1, 2, 4-triazole* (5d)**

Pale yellow needle crystal, Yield: 73.7%, m.p.: 210-211 ^{o}C; IR: (KBr, cm^{-1}): 3112, 2984, 1624, 1560, 1525, 1482, 1276; ^{1}H NMR (600 MHz, DMSO-*d6*) δ: 7.38 (t, *J* = 12.6, 1H, Ar-H), 7.48-7.60 (m, 3H, Ar-H), 7.82-7.98 (m, 4H, Ar-H), 9.76(s, 1H, CH=N), 14.29 (s, 1H, triazole-NH); ^{13}C NMR(600 MHz, DMSO-*d6*) δ: 128.22, 128.25, 128.30, 128.32, 128.43, 128.44, 130.87, 132.97, 133.52, 134.94, 144.86, 153.92, 165.14; Anal. calcd for $C_{15}H_{10}Cl_2N_4S$; C, 51.59; H, 2.89; N, 16.04; Found: C, 51.62; H, 2.95; N, 15.98.

***(E)-3-thiol-4-(2,4-difluorobenzylideneamino)-5-phenyl-4H-1,2,4-triazole* (5e)**

White needle crystal, Yield: 78.6 %, m.p.: 208-209 ^{o}C; IR: (KBr, cm^{-1}): 3117, 2975, 1616, 1533, 1522, 1476, 1271; ^{1}H NMR (600 MHz, DMSO -*d6*) δ: 7.45 (t, *J* =12.6 Hz, 1H, Ar-H), 7.51-7.64(m, 4H, Ar-H), 7.68-7.76(m, 1H, Ar-H), 7.96 (dd, *J* = 14.6, 12.4 Hz, 2H, Ar-H), 9.77(s, 1H, CH=N), 14.34(s, 1H, triazole-NH); ^{13}C NMR(600 MHz, DMSO-*d6*) δ: 128.23, 128.24, 128.12, 128.22, 128.24, 128.45, 130.84, 132.93, 133.54, 134.95, 144.81, 153.95, 165.12; Anal. calcd for $C_{15}H_{10}F_2N_4S$; C, 56.95; H, 3.19; N, 17.71; Found: C,57.02; H, 3.23; N, 17.65.

***(E)-3-thiol-4-(3,4-difluorobenzylideneamino)-5-phenyl-4H-1,2,4-triazole* (5f)**

White needle crystal, Yield: 69.8 %, m.p.: 197-198 ^{o}C; IR: (KBr, cm^{-1}): 3119, 2973, 1617, 1531, 1525, 1476, 1274; ^{1}H NMR (600 MHz, DMSO -*d6*) δ: 7.43 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.54-761 (m, 2H, Ar-H), 7.82 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.85-7.98 (m, 2H, Ar-H), 9.64 (s, 1H, CH=N), 14.32 (s, 1H, triazole-NH); ^{13}C NMR(125 MHz, DMSO-*d6*) δ: 126.83, 128.03, 128.16, 128.22, 128.23, 128.42, 130.86, 132.97, 133.55, 134.97, 144.83, 155.95, 165.12; Anal. calcd for $C_{15}H_{10}F_2N_4S$; C, 56.95; H, 3.19; N, 17.71; Found: C,56.92; H, 3.22; N, 17.76.

(E)-3-thiol-4-(3-chloro-4-fluorobenzylideneamino)-5-phenyl-4H-1,2,4-triazole **(5g)**

White needle crystal, Yield: 76.0 %, m.p.: 216-218 °C; IR: (KBr, cm^{-1}): 3120, 2989, 1623, 1535, 1523 , 1487, 1271; ^{1}H NMR (600 MHz, DMSO -*d*6) *δ*:7.32 ((dd, *J* =16.2, 15.8 Hz, 1H, Ar-H), 7.45- 7.51 (m, 3H, Ar-H), 7.73-7.88 (m, 3H, Ar-H), 7.88 (dd, *J* = 9.8, 3.2 Hz, 1H), 9.78 (s, 1H, CH=N), 14.36 (s, 1H, triazole-NH); ^{13}C NMR(125 MHz, DMSO-d_6) *δ*: 56.05, 56.64, 97.67, 109.04, 114.67, 128.26, 129.84, 130.85, 144.92, 147.41, 161.28, 165.17; Anal. calcd for $C_{15}H_{10}ClFN_4S$; C, 54.14; H, 3.03; N, 16.84; Found: C, 54.17; H, 3.07; N, 16.81.

General procedure for the synthesis of target compounds (6)

(*E*)-3-thiol-4-arylideneamino-5- phenyl - 4H -1, 2, 4-triazole **5** (1.75 mmol) was dissolved in 15 mL of DMF, and anhydrous potassium carbonate (1.75 mmol) was added to the solution. The solution was stirred for 0.5 h and (*E*)-methyl 2-(2-(bromomethyl)phenyl)- 2- (methoxyimino) acetate (0.50 g, 1.75 mmol) was then added. The reaction mixture was heated to 80 °C and monitored by TLC. After 1 h, the mixture was cooled, diluted with 30 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 1:3 (v/v) mixture of ethyl acetate and petroleum ether (boiling point range 60-90 °C) as the eluting solution to obtain compound **6a-6g**.

(E)-methyl-2-(2-(((4-((E)-(2-methylbenzylidene)amino)-5-phenyl- 4H-1, 2, 4-triazol-3-yl)thio) methyl) phenyl) - 2-(methoxyimino)acetate **(6a)**

White solide, Yield: 67.6%, m.p.: 108-109 °C; IR: (KBr, cm^{-1}): 3112, 2959, 1655, 1600, 1565, 1526, 1482, 1281; ^{1}H NMR (600 MHz, DMSO -*d*6) *δ*: 2.44 (s, 3H, CH_3), 3.64 (s, 3H, $COOCH_3$), 3.93 (s, 3H, =N-OCH_3), 4.35 (s, 2H, CH_2), 7.20-7.29 (m, 5H, ArH), 7.32-7.41(m, 4H, ArH), 7.46-7.52 (m, 2H, ArH), 7.65-8.03 (m, 2H, ArH), 8.66 (s, 1H, CH=N); ^{13}C NMR(125 MHz, DMSO-*d*6) *δ*: 21.24, 33.73, 52.01, 60.45, 128.03, 128.22, 128.32, 128.31, 128.65, 128.63, 128.71, 130.66, 130.74, 130.92, 131.14, 131.73, 137.92, 140.58, 149.72, 152.43, 158.56, 159.53, 163.53; ESI-HRMS: calcd. for $C_{27}H_{26}N_5O_3S$ $[M+H]^+$ 500.17509, found 500.17529.

(E)-methyl-2-(2-(((4-((E)-(3-methylbenzylidene)amino)-5-phenyl-4H-1,2,4-triazol-3-yl)thio)methyl) phenyl) -2-(methoxyimino)acetate **(6b)**

White solide, Yield: 60.6%, m.p.: 115-116 °C; IR: (KBr, cm^{-1}): 3111, 2962, 1657, 1600, 1562, 1523, 1485, 1289; ^{1}H NMR (600 MHz, DMSO -*d*6) *δ*: 2.42 (s, 3H, CH_3), 3.62 (s, 3H, $COOCH_3$), 3.94 (s, 3H, =N-OCH_3), 4.32 (s, 2H, CH_2), 7.16-7.30 (m, 3H, ArH), 7.32-7.56(m, 5H, ArH), 7.64 (d, *J* = 14.9 Hz, 1H, ArH), 7.69-7.80 (m, 4H, ArH), 8.75 (s, 1H, CH=N); ^{13}C NMR(125 MHz, DMSO-*d*6) *δ*: 21.32, 33.76, 52.14, 60.49, 128.08, 128.14, 128.37, 128.39, 128.62, 128.68, 128.78, 130.62, 130.79, 130.95, 131.16, 131.75, 137.98, 140.67, 149.77, 152.46, 158.54, 159.59, 163.53; ESI-HRMS: calcd. for $C_{27}H_{26}N_5O_3S$ $[M+H]^+$ 500.17509, found 500.17519.

(E)-methyl-2-(2-(((4-((E)-(2,4-dimethoxybenzylidene)amino)-5-phenyl-4H-1,2,4-triazol-3-yl) thio) methyl) phenyl)-2-(methoxyimino)acetate **(6c)**

White solide,Yield: 77.2%, m.p. : 131-132 °C; IR: (KBr, cm^{-1}): 3104, 2961, 1652, 1612, 1557, 1521, 1484, 1296; ^{1}H NMR (600 MHz, DMSO -*d*6) *δ*: 3.63 (s, 3H, $COOCH_3$), 3.82 (s, 3H, Ar-OCH_3), 3.84 (s, 3H, Ar-OCH_3), 3.92 (s, 3H, =N-OCH_3), 4.34 (s, 2H, CH_2), 7.01 (d, *J* = 12.6 Hz, 2H, ArH), 7.23-7.62 (m, 5H, ArH), 7.77-8.05 (m, 5H, ArH), 8.63 (s, 1H, CH=N); ^{13}C NMR(125 MHz, DMSO-d_6) *δ*: 33.70, 52.07, 56.83, 60.42, 111.41, 113.30, 123.47, 128.20, 128.33, 128.34, 128.34, 128.67, 128.77, 130.65, 130.77, 130.96, 131.15, 140.65, 149.75, 151.21, 151.66, 152.45, 158.54, 160.01, 163.52; HRMS calcd. for $C_{28}H_{28}N_5O_5S$ $[M+H]^+$ 546.18057, found 546.18068.

(E)-methyl-2-(2-(((4-((E)-(2,6-dichlorobenzylidene)amino)-5-phenyl-4H-1,2,4- triazol-3- yl) thio)methyl) phenyl)-2-(methoxyimino)acetate **(6d)**

Pale yellow solide, Yield: 78.2%, m.p.: 121-122 °C; IR: (KBr, cm^{-1}): 3102, 2963, 1645, 1611, 1562, 1516, 1484, 1272; ^{1}H NMR (600 MHz, DMSO -*d*6) *δ*: 3.65 (s, 3H, $COOCH_3$), 3.94 (s, 3H, =N-OCH_3), 4.41 (s, 2H, CH_2), 7.21-7.32 (m, 2H, ArH), 7.33-7.44 (m, 4H, ArH), 7.46-7.60 (m, 3H, ArH), 7.77 (dd, *J* = 15.0, 4.9 Hz, 1H, ArH), 8.00-8.09 (m, 2H, ArH), 8.92 (s, 1H, CH=N); ^{13}C NMR(125 MHz, DMSO-d6) δ: 33.71, 52.08, 60.31, 128.26, 128.32, 128.36, 128.33, 128.64, 128.68, 129.52, 129.75, 129.94, 130.65, 130.77, 130.97, 131.12, 134.58, 134.71, 140.66, 149.73, 152.42, 155.12, 158.54, 163.55; ESI-HRMS: calcd. for $C_{26}H_{22}Cl_2N_5O_3S$ $[M+H]^+$ 554.08149, found 554.08158.

(E)-methyl-2-(2-(((4-((E)-(2,4-difluorobenzylidene)amino)-5-phenyl-4H-1,2,4- triazol-3- yl) thio) methyl) phenyl)-2-(methoxyimino)acetate **(6e)**

Pale yellow solide, Yield: 78.0%, m.p.: 134-135°C; IR: (KBr, cm^{-1}): 3098, 2966, 1646, 1607, 1564, 1516, 1487, 1275; ^{1}H NMR (600 MHz, DMSO -*d*6) *δ*: 3.63 (s, 3H, $COOCH_3$), 3.93 (s, 3H, =N-OCH_3), 4.36 (s, 2H, CH_2), 7.15 (d, *J* = 8.6 Hz, 1H, ArH), 7.35-7.43 (m, 2H, ArH), 7.56-7.67 (m, 4H, ArH), 7.65 (d, *J* = 7.8 Hz, 1H, ArH), 7.84 (d, *J* = 6.8 Hz, 2H, ArH), 7.88 (s, 1H, ArH), 8.06 (d, *J* = 8.8 Hz, 1H, ArH), 8.91 (s, 1H, CH=N); ^{13}C NMR(125 MHz, DMSO-*d*6) *δ*: 33.71, 52.06, 60.31, 128.28, 128.35, 128.39, 128.43, 128.62, 128.67, 129.55, 129.74, 129.92, 130.63, 130.79, 130.95, 131.16, 134.59, 134.74, 140.65, 149.72, 152.45, 155.07, 158.57, 163.55; ESI-HRMS: calcd. for $C_{26}H_{22}F_2N_5O_3S$ $[M+H]^+$ 522.14115, found 522.14121.

(E)-methyl-2-(2-(((4-((E)-(3,4-difluorobenzylidene)amino)-5-phenyl-4H-1,2,4-triazol-3-yl) thio)methyl) phenyl)-2-(methoxyimino)acetate **(6f)**

Pale yellow solide,Yield: 70.2%, m.p.: 121-122 °C; IR: (KBr, cm^{-1}): 3114, 2959, 1655, 1604, 1546, 1531, 1499, 1275; ^{1}H NMR (600 MHz, DMSO -*d*6) *δ*: 3.59 (s, 3H, $COOCH_3$), 3.91 (s, 3H, =N-OCH_3), 4.31(s, 2H, CH_2), 7.19 (d, *J* = 8.2 Hz, 1H, ArH), 7.33-7.45 (m, 2H, ArH), 7.45-7.56 (m, 4H, ArH), 7.80-7.87 (m, 2H, ArH), 7.89 (d, *J* = 8.4 Hz, 2H, ArH), 8.12(s, 1H, ArH), 8.79(s, 1H, CH=N); ^{13}C NMR(125 MHz, DMSO-*d*6) *δ*: 33.72, 52.03, 60.35, 128.32, 128.34, 128.36, 128.34, 128.68, 128.65, 129.55, 129.77, 129.94, 130.62, 130.74, 130.95, 131.16, 134.59, 134.70, 140.65, 149.72, 152.46, 155.03, 158.54, 163.57; ESI-HRMS: calcd. for $C_{26}H_{22}F_2N_5O_3S$ $[M+H]^+$ 522.14115, found 522.14106.

(E)-methyl-2-(2-(((4-((E)-(3-chloro-4-fluorobenzylidene)amino)-5-phenyl-4H-1,2,4-triazol-3-yl) thio) methyl) phenyl)-2-(methoxyimino)acetate **(6g)**

Pale yellow solide, Yield: 72.6%, m.p. : 130-131°C ; IR: (KBr, cm^{-1}): 3118, 2959, 1654, 1612, 1557, 1525, 1498, 1278; ^{1}H NMR (600 MHz, DMSO -*d*6) *δ*: 3.70 (s, 3H, $COOCH_3$), 3.94 (s, 3H, =N-OCH_3), 4.35 (s, 2H, CH_2), 7.16 (t, *J* = 16.8

Hz, 1H, ArH), 7.36 (t, *J* = 14.9 Hz, 2H, ArH), 7.42-7.48 (m, 1H, ArH), 7.52 (d, *J* = 5.2 Hz, 4H, ArH),7.58 (d, *J* = 7.6 Hz, 1H, ArH), 7.62-7.73 (m, 1H, ArH), 7.86 (d, *J* = 6.6 Hz, 2H, ArH), 8.85 (s, 1H, CH=N); ^{13}C NMR(125 MHz, DMSO-d_6) *δ*: 163.54, 158.53, 158.15, 155.06, 152.45, 149.75, 140.68, 136.43, 132.64, 131.17, 130.96, 130.73, 130.64, 128.76, 128.66, 128.36, 128.33, 128.19, 126.84, 117.56, 114.85, 60.42, 52.08, 33.75. HRMS calcd. for $C_{26}H_{22}ClFN_5O_3S$ $[M+H]^+$ 538.11104, found 538.11093.

(*E*)-3-thiol-4-arylideneamino-5- phenyl - 4H -1, 2, 4-triazole **5** (1.75 mmol) was dissolved in 15 mL of DMF, and anhydrous potassium carbonate (0.24 g, 1.75 mmol) was added to the solution. The solution was stirred for 0.5 h and (*E*)-methyl 2-(2- (bromomethyl) phenyl) -3- methoxyacrylate (0.50 g, 1.75 mmol) was then added. The reaction mixture was heated to 80 ˚C and monitored by TLC. After 1h, the mixture was cooled, diluted with 30 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 1:3 (v/v) mixture of ethyl acetate and petroleum ether (boiling point range 60-90 ˚C) as the eluting solution to obtain compound **6a'-6g'**.

***(E)-methyl-3-methoxy-2-(2-(((4-((E)-(2-methylbenzylidene)amino)-5-phenyl-4H-1,2,4-triazol- 3-yl)thio) methyl) phenyl)acrylate* (6a')**

White solide, Yield: 65.2%, m.p.: 102-103 °C; IR: (KBr, cm^{-1}): 3119, 2932, 1651, 1612, 1561, 1520, 1493, 1278; ^{1}H NMR (600 MHz, DMSO -*d*6) *δ*: 2.34 (s, 3H, CH_3), 3.65 (s, 3H, $COOCH_3$), 3.96 (s, 3H, =C-OCH_3), 4.32 (s, 2H, CH_2), 7.11-7.30 (m, 5H, ArH), 7.33-7.40(m, 4H, ArH), 7.47-7.51 (m, 2H, ArH), 7.63(s, 1H, =CHOC), 7.61-7.98 (m, 2H, ArH), 8.60 (s, 1H, CH=N); ^{13}C NMR(125 MHz, DMSO-*d*6) *δ*: 21.22, 33.71, 52.11, 60.48, 128.12, 128.25, 128.35, 128.33, 128.68, 128.61, 128.76, 130.61, 130.78, 130.97, 131.16, 131.84, 137.85, 140.49, 149.69, 152.37, 158.51, 159.52, 163.39; ESI-HRMS: calcd. for $C_{28}H_{27}N_4O_3S$ $[M+H]^+$ 499.17984, found 499.17972.

***(E)-methyl-3-methoxy-2-(2-(((4-((E)-(3-methylbenzylidene)amino)-5-phenyl-4H-1,2,4-triazol-3-yl) thio) methyl)phenyl)acrylate* (6b')**

White solide, Yield: 61.0%, m.p.: 110-112 °C; IR: (KBr, cm^{-1}): 3118, 2967, 1652, 1598, 1565, 1527, 1481, 1291; ^{1}H NMR (600 MHz, DMSO -*d*6) *δ*: 2.42 (s, 3H, CH_3), 3.52 (s, 3H, $COOCH_3$), 3.85 (s, 3H, =C-OCH_3), 4.29 (s, 2H, CH_2), 7.12-7.31 (m, 3H, ArH), 7.41-7.58(m, 5H, ArH), 7.64 (s, 1H, =CHOC), 7.75 (d, *J* = 13.2 Hz, 1H, ArH), 7.84-7.92 (m, 4H, ArH), 8.76 (s, 1H, CH=N); ^{13}C NMR(125 MHz, DMSO-*d*6) *δ*: 21.29, 33.71, 52.12, 60.46, 128.03, 128.15, 128.36, 128.36, 128.67, 128.61, 128.75, 130.46, 130.74, 130.87, 131.14, 131.78, 138.10, 140.69, 149.72, 152.41, 158.56, 159.63, 163.51; ESI-HRMS: calcd. for $C_{28}H_{27}N_4O_3S$ $[M+H]^+$ 499.17984, found 499.17993.

***(E)-methyl-2-(2-(((4-((E)-(2,4-dimethoxybenzylidene)amino)-5-phenyl-4H-1,2,4-triazol-3-yl) thio) methyl) phenyl)-3-methoxyacrylate* (6c')**

White solide,Yield: 71.6%, m.p. : 125-126°C; IR: (KBr, cm^{-1}): 3100, 2967, 1655, 1610, 1552, 1527, 1488, 1292; ^{1}H NMR (600 MHz, DMSO -*d*6) *δ*: 3.62 (s, 3H, $COOCH_3$), 3.85 (s, 3H, Ar-OCH_3), 3.87 (s, 3H, Ar-OCH_3), 3.93 (s, 3H, =C-OCH_3), 4.33 (s, 2H, CH_2), 6.97 (d, *J* = 12.0 Hz, 2H, ArH), 7.18-7.53 (m, 5H, ArH), 7.61 (s, 1H, =CHOC), 7.75-8.07 (m, 5H, ArH), 8.62 (s, 1H, CH=N); ^{13}C NMR(125 MHz, DMSO-d_6) *δ*: 163.49, 160.13, 158.48, 152.54, 151.58, 151.22, 149.74, 140.60, 131.11, 130.92, 130.70, 130.61, 128.78, 128.77, 128.41, 128.37, 128.32, 128.26, 123.51, 113.36, 111.42, 60.44, 56.87, 52.05, 33.74; ESI-HRMS calcd. for $C_{29}H_{29}N_4O_5S$ $[M+H]^+$ 545.18532, found 545.18547.

***(E)-methyl-2-(2-(((4-((E)-(2,6-dichlorobenzylidene)amino)-5-phenyl-4H-1,2,4-triazol-3-yl) thio)methyl) phenyl)-3-methoxyacrylate* (6d')**

Pale yellow solide, Yield: 75.8%, m.p.: 114-115 °C; IR: (KBr, cm^{-1}): 3105, 2962, 1641, 1622, 1568, 1511, 1487, 1276; ^{1}H NMR (600 MHz, DMSO -*d*6) *δ*: 3.62 (s, 3H, $COOCH_3$), 3.96 (s, 3H, =C-OCH_3), 4.44 (s, 2H, CH_2), 7.01-7.15 (m, 2H, ArH), 7.23-7.37 (m, 4H, ArH), 7.44-7.58 (m, 3H, ArH), 7.62 (s, 1H, =CHOC), 7.75 (dd, *J* = 14.8, 5.6Hz, 1H, ArH), 8.02-8.13 (m, 2H, ArH), 8.86 (s, 1H, CH=N); ^{13}C NMR(125 MHz, DMSO-d6) *δ*: 33.68, 52.05, 60.33, 128.22, 128.38, 128.32, 128.31, 128.68, 128.60, 129.55, 129.70, 129.91, 130.68, 130.79, 130.98, 131.13, 134.50, 134.74, 140.62, 149.70, 152.41, 155.10, 158.57, 163.54; ESI-HRMS: calcd. for $C_{27}H_{23}Cl_2N_4O_3S$ $[M+H]^+$ 553.08624, found 553.08612.

***(E)-methyl-2-(2-(((4-((E)-(2,4-difluorobenzylidene)amino)-5-phenyl-4H-1,2,4-triazol-3-yl) thio) methyl) phenyl) -3-methoxyacrylate* (6e')**

Pale yellow solide, Yield: 74.8%, m.p.: 125-126°C; IR: (KBr, cm^{-1}): 3105, 2968, 1641, 1603, 1560, 1516, 1483, 1278; ^{1}H NMR (600 MHz, DMSO -*d*6) *δ*: 3.61 (s, 3H, $COOCH_3$), 3.78(s, 3H, =C-OCH_3), 4.32 (s, 2H, CH_2), 7.12 (d, *J* = 7.8 Hz, 1H, ArH), 7.31-7.46 (m, 2H, ArH), 7.52-7.67 (m, 4H, ArH), 7.63(s, 1H, =CHOC), 7.69 (d, *J* = 7.8 Hz, 1H, ArH), 7.84 (d, *J* = 6.8 Hz, 2H, ArH), 7.88 (s, 1H, ArH), 8.02 (d, *J* = 8.4 Hz, 1H, ArH), 8.93 (s, 1H, CH=N); ^{13}C NMR(125 MHz, DMSO-*d*6) *δ*: 33.73, 52.02, 60.36, 128.25, 128.32, 128.38, 128.40, 128. 612, 128.67, 129.51, 129.76, 129.90, 130.66, 130.81, 130.96, 131.18, 134.60, 134.78, 140.61, 149.75, 152.46, 155.02, 158.53, 163.56; ESI-HRMS: calcd. for $C_{27}H_{23}F_2N_4O_3S$ $[M+H]^+$ 521.14589, found 521.14571.

***(E)-methyl-2-(2-(((4-((E)-(3,4-difluorobenzylidene)amino)-5-phenyl-4H-1,2,4-triazol-3-yl) thio) methyl) phenyl)-3-methoxyacrylate* (6f')**

Pale yellow solide,Yield: 72.0%, m.p.: 112-113 °C; IR: (KBr, cm^{-1}): 3110, 2961, 1652, 1606, 1542, 1531, 1503, 1276; ^{1}H NMR (600 MHz, DMSO -*d*6) *δ*: 3.62 (s, 3H, $COOCH_3$), 3.78 (s, 3H, =C-OCH_3), 4.33(s, 2H, CH_2), 7.21 (d, *J* = 7.8 Hz, 1H, ArH), 7.30-7.42 (m, 2H, ArH), 7.44-7.56 (m, 4H, ArH), 7.64 (s, 1H, =CHOC), 7.78-7.89 (m, 2H, ArH), 7.91 (d, *J* = 8.0 Hz, 2H, ArH), 8.14(s, 1H, ArH), 8.76(s, 1H, CH=N); ^{13}C NMR(125 MHz, DMSO-*d*6) *δ*: 33.75, 52.06, 60.33, 128.31, 128.36, 128.40, 128.45, 128.66, 128.71, 129.53, 129.74, 129.98, 130.60, 130.78, 130.93, 131.12, 134.60, 134.76, 140.62, 149.70, 152.45, 155.06, 158.56, 163.59; ESI-HRMS: calcd. for $C_{27}H_{23}F_2N_4O_3S$ $[M+H]^+$ 521.14589, found 521.14602.

***(E)-methyl-2-(2-(((4-((E)-(3-chloro-4-fluorobenzylidene)amino)-5-phenyl-4H-1,2,4-triazol-3-yl) thio) methyl) phenyl)- 3-methoxyacrylate* (6g')**

Pale yellow solide,Yield: 69.6%, m.p.: 125-126°C ; IR: (KBr, cm^{-1}): 3112, 2963, 1653, 1615, 1552, 1529, 1493, 1274; ^{1}H NMR (600 MHz, DMSO -*d*6) *δ*: 3.72 (s, 3H, $COOCH_3$), 3.98 (s, 3H, =C-OCH_3), 4.35 (s, 2H, CH_2), 7.14 (t, *J* = 14.2 Hz, 1H, ArH), 7.36 (t, *J* = 13.6 Hz, 2H, ArH), 7.40-7.52 (m, 4H, ArH), 7.55 (d, *J* = 7.3 Hz, 1H, ArH), 7.58 (d, *J* = 7.6 Hz, 1H, ArH), 7.63 (s, 1H, =CHOC), 7.66-7.78 (m, 2H, ArH), 7.86 (d, *J* = 6.6 Hz, 1H, ArH), 8.85 (s, 1H, CH=N); ^{13}C NMR(125 MHz, DMSO-d_6) *δ*: 163.56, 158.50, 158.21, 155.12, 152.51,

149.70, 140.69, 136.40, 132.61, 131.18, 130.91, 130.78, 130.66, 128.71, 128.60, 128.32, 128.28, 128.18, 126.80, 117.51, 114.88, 60.40, 52.09, 33.78. ESI-HRMS calcd. for $C_{27}H_{23}ClFN_4O_3S$ $[M+H]^+$ 537.11579, found 537.11592.

Antimicrobial activity assessment

The *in vitro* fungicidal activities of the title compounds **6** against *Rhizoctonia solani*, *Botrytis cinereapers*, *Fusarium graminearum*, and *Cotton rhizoctoniosis* were evaluated by the mycelium growth rate test as reported previously.[23] The antifungal activity was determined by measuring the diameter of the inhibition zone. The growth inhibition rates were calculated by using the following equation: $I = [(C–T)/C]\times100\%$, where I is the growth inhibition rate (%), C is the control settlement radius (mm), and T is the treatment group fungi settlement radius (mm).

The *in vivo* fungicidal preventive activities of compounds **6** against *Blumeria graminis* was tested according to the procedure described previously.[24] Wheat plants were grown under greenhouse conditions (T = 22 °C, 60 (±5) % relative humidity and a 12 h light cycle). The plants were maintained in plastic pots (6 cm diameter × 10 cm height) and inoculated by a spore suspension of *Blumeria graminis* (1.0×10^5 spores mL^{-1}). After one day, the test compounds were sprayed over the plants. After one or two weeks, symptoms were examined. Each bioassay was conducted in triplicate, and the biological effect was reported as the average of the triplicates. The results are the percentage disease control compared with the untreated check, where 100 is complete disease control and 0 is no disease control.

CONCLUSIONS

In summary, a series of novel strobilurin derivatives containing 1,2,4-triazole moiety were synthesized, and their structures were confirmed *via* melting point, IR, ^{1}H NMR, ^{13}C NMR, and HRMS. The antifungal tests indicated that compound **6d′** displayed modest antifungal activity against *Rhizoctonia solani*, *Botrytis cinereapers*, and *Fusarium graminearum*. In addition, compounds **6e**, **6f′**, and **6g′** exhibited slightly better fungicidal activities against *Blumeria graminis*. Especially, Compound **6e′** could be promising lead structures for the development of novel fungicides towards *Blumeria graminis*. Further investigation of this type of compound is in progress now and will be reported in the near future.

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