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Synthesis and Fungicidal Activity of (*E*)-5-[1-(2-Oxo-1-oxaspiro[4,5]dec/non-3-en-3-yl)ethylidene]-2-aminoimidazolin-4-one Derivatives

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The novel fungicidal agents, (*E*)-5-[1-(2-oxo-1-oxaspiro[4,5]dec/non-3-en-3-yl)ethylidene]-2-aminoimidazolin-4-one derivatives, were designed and synthesized in moderate to excellent yields in four steps using α-hydroxyketone and diketene as raw materials and characterized by HR-ESI-MS, ^{1}H NMR and X-ray diffraction. The preliminary bioassay showed that some of these compounds, such as **5e**, **6a**, **6e**, and **7h** exhibit 87.8%, 91.3%, 89.9% and 87.8% inhibition rates against *Sclerotinia scleotiorum*, **3b**, **3c**, **4c** and **7h** exhibit 96.4%, 92.5%, 90.3% and 76.9% inhibition rates against *Phytophthora capsici* at the concentration of 50 μg/mL, respectively. These compounds exhibited significant fungicidal activities against *S. scleotiorum* and *P. capsici* with EC_{50} values of 2.56 −11.60 μg/mL, and compounds **6e** and **7h** exhibited weak inhibition against the spore germination of *S. scleotiorum*, while the spore germination of *P. capsici* was strongly inhibited by compound **7h** solution. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) observation indicated that compound **7h** had a significant impact on the structure and function of the hyphal cell wall of *P. capsici* mycelium.

Keywords (*E*)-5-[1-(2-oxo-1-oxaspiro[4.5]dec/non-3-en-3-yl)ethylidene]-2-aminoimidazolin-4-one, synthesis, fungicidal activities

Introduction

In the development process of potential pesticides, some natural tetramic and tetronic acid derivatives were found to display fungicidal and herbicidal activities,[1-3] the further modification in 5-position with spirocycles unexpectedly led to discovery of new class of insecticides such as spirodiclofen, spiromesifen and spirotetramat (Scheme 1), which indicated that the 5-spirotetramic and 5-spirotetronic acid moieties change the mode of action and improve the activities greatly. In recent years, much more attention was paid to the synthesis and biological activity evaluation of 5-spirotetramic and 5-spirotetronic acid derivatives.[4-11] Furthermore, the novel synthetic strategy of 2-aminoimidazolinone derivatives has been developed in several decades[12-14] and provided the diversity of chemical structures and the possibility of discovery of new lead compounds, because various imidazolinone-containing compounds have been found in nature or synthesized and reported to display a wide range of biological activities such as fenamidone (Scheme 1) having antimicrobial and fungicidal activities.[15-18] In order to find new lead compounds of fungicidal agents, several 5-cyclohexylidene-2-aminoimidazolin-4-one and 5-(5,5-dimethylbutenolide-3-ethylidene)-2-aminoimidazolin-4-one derivatives have been prepared in our laboratory and showed significant fungicidal activities against several important agricultural phytopathgens.[19] Based on the above results, we desired to introduce the spirocycle scaffold into the structure of 5-(butenolide-3-ethylidene)-2-aminoimidazolin-4-one and confirm if these novel types of chemicals can improve the fungicidal activity (Scheme 2). Continuing our pursuit of novel biologically active 2-aminoimidazolin-4-one heterocyclic compounds, a series of 5-(5-spirocyclicbutenolide-3-ethylidene)-2-aminoimidazolin-4-one derivatives were designed and synthesized (Scheme 3), and their fungicidal activities and the structure-activity relationships were explored.

Experimental

All reactions were performed with magnetic stirring under an N_2 atmosphere. Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Organic solvents were concentrated under reduced pressure using a rotary

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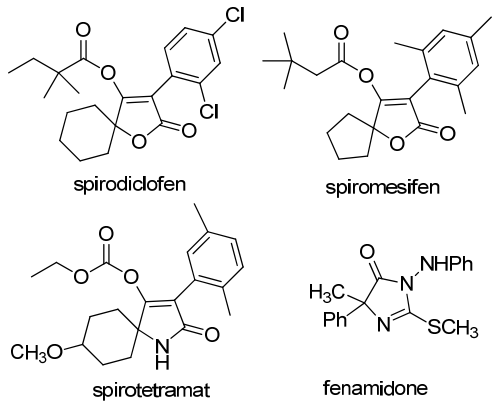
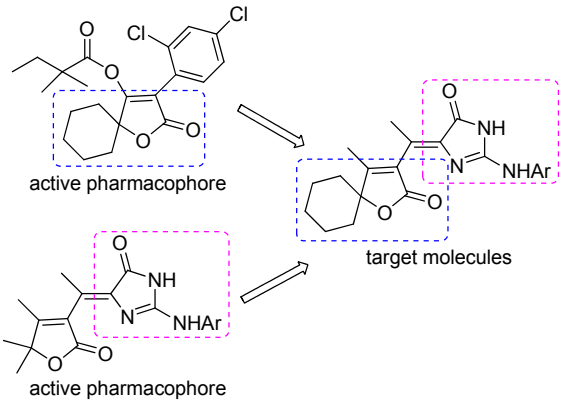
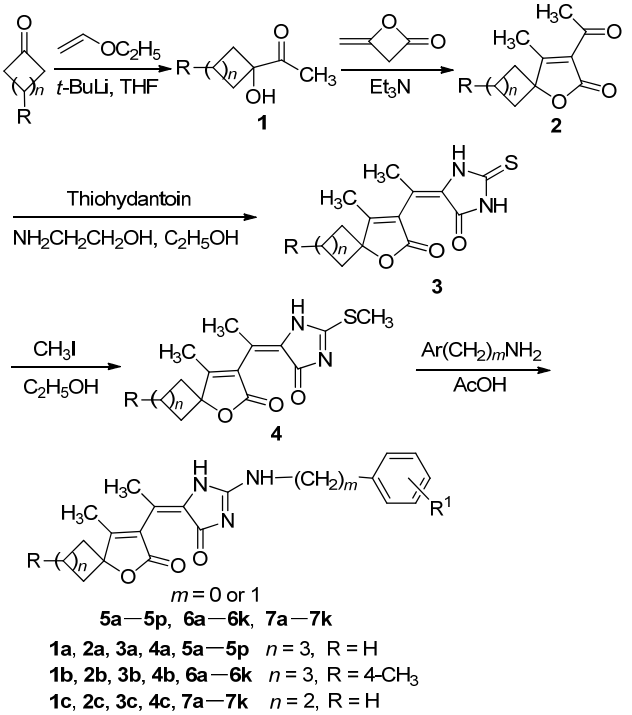
Scheme 1 Typical insecticides of 5-spirotetramic and -tetronic acid derivatives and fungicide fenamidone



Scheme 2 Design strategy of novel spirocyclic 2-aminoimidazolin-4-one derivatives



Scheme 3 Synthetic route of 5-(5-spirocyclicbutenolide-3-ethylidene)-2-aminoimidazolin-4-one



evaporator or oil pump. Column chromatography was performed using Qingdao Haiyang flash silica gel (200—300 mesh). Melting points were measured on a Yanagimoto apparatus and uncorrected. IR (KBr plate) spectra were recorded on a Perkin Elmer FTS-40 in rotary strument. ^{1}H NMR spectra were obtained on a Bruker DPX 300 spectrometer with $CDCl_3$ or DMSO-d_6 as the solvent and TMS as the internal standard. High resolution mass spectral analysis was performed on an LTQ Orbitrap instrument. SEM observation was performed on a Hitachi S-3400N scanning electron microscope, and TEM observation on a JEOL-1230 transmission electron microscope.

Synthesis of 3-acetyl-4-methyl-5,5-spirobutenolide 2a—2c

The synthesis of the intermediates **2a**—**2c** was carried out according to the literature protocols in two steps using ketone and vinyl ethyl ether as starting materials, and their spectral data were identical with those in the references.[10,20]

2a: colorless solid, overall yield 51%; m.p. 98—99 ℃; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.57 (s, 3H), 2.33 (s, 3H), 1.81—1.22 (m, 10H).

2b: colorless solid, yield 61%; m.p. 36—37 ℃; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.56 (s, 3H), 2.41 (s, 1.5H), 2.36 (s, 1.5H), 2.09—1.37 (m, 9H), 1.06 (d, J=6.0 Hz, 1.5H), 0.98 (d, J=6.0 Hz, 1.5H).

2c: colorless solid, yield 60%; m.p. 59—60 ℃; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.57 (s, 3H), 2.35 (s, 3H), 2.05—1.86 (m, 8H).

Synthesis of 5-[4-methyl-5,5-spirobutenolide-3-ethylidene]-2-thiohydantoin 3a—3c

The synthesis of the intermediates **3a**—**3c** was performed following the processes in our previous paper[19] by reaction of **2a**—**2c** with thiohydantoin.

3a: white solid, yield 72%; m.p. 295—297 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 12.11 (s, 1H), 11.96 (s, 1H), 2.02 (s, 3H), 1.85 (s, 3H), 1.82—1.24 (m, 10H); HR-ESI-MS calcd for $C_{15}H_{19}O_3N_2S$ $[M+H]^+$ 307.1111, found 307.1102.

3b: white solid, yield 54%; m.p. 273—275 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 12.20—12.12 (m, 1H), 11.96—11.71 (m, 1H), 2.22—1.23 (m, 15H), 1.03—0.92 (m, 3H); HR-ESI-MS calcd for $C_{16}H_{21}O_3N_2S$ $[M+H]^+$ 321.1267, found 321.1259.

3c: white solid, yield 56%; m.p. 265—267 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 12.08 (s, 1H), 11.73 (s, 1H), 2.24—1.67 (m, 14H); HR-ESI-MS calcd for $C_{14}H_{17}O_3N_2S$ $[M+H]^+$ 293.0954, found 293.0944.

Synthesis of 5-[4-methyl-5,5-spirobutenolide-3-ethylidene]-2-methylthioimidazo-4-one 4a—4c

The synthesis of the intermediates **4a**—**4c** was performed according to the methods in our previous paper[19] by reaction of **3a**—**3c** with methyl iodide.

4a: white solid, yield 90%; m.p. 151—152 ℃; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 10.04—9.79 (m, 1H), 2.62

−2.52 (m, 3H), 2.46−2.36 (m, 3H), 1.88−1.22 (m, 13H); HR-ESI-MS calcd for $C_{16}H_{21}N_2O_3S$ $[M+H]^+$ 321.1267, found 321.1257.

4b: white solid, yield 86%; m.p. 212−214 ℃; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 9.97−9.65 (m, 1H), 2.62−2.52 (m, 3H), 2.46−2.37 (m, 3H), 2.07−1.47 (m, 12H), 1.06−0.97 (m, 3H); HR-ESI-MS calcd for $C_{17}H_{23}N_2O_3S$ $[M+H]^+$ 335.1424, found 335.1414.

4c: white solid, yield 85%; m.p. 186−188 ℃; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 10.02−9.76 (m, 1H), 2.62−2.55 (m, 3H), 2.48−2.39 (m, 3H), 2.02−1.81 (m, 11H); HR-ESI-MS calcd for $C_{15}H_{19}N_2O_3S$ $[M+H]^+$ 307.1111, found 307.1102.

General procedure for the synthesis of compounds 5−7

To a stirred solution of 1.0 mmol of **4a**−**4c** in 20 mL of acetic acid, the amines were added at ambient temperature and heated to reflux for 10−24 h. The reactions were monitored by TLC. After completion, the solvents were removed under reduced pressure. The residues were purified by silica gel chromatography using CH_2Cl_2/acetone as eluents to afford compounds **5**−**7**.

(*E*)-5-[1-(4-Methyl-2-oxo-1-oxaspiro[4,5]dec-3-en-3-yl)ethylidene]-2-phenylamino-3,5-dihydro-4*H*-imidazol-4-one (**5a**): white solid, yield 95%; m.p. 202−204 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.41 (s, 1H), 9.66 (s, 1H), 7.71−6.99 (m, 5H), 2.11 (s, 3H), 1.99−1.25 (m, 13H); IR *v*: 3426, 3215, 3054, 2924, 1685, 1646, 1605, 1574, 1496 cm^{-1}; HR-ESI-MS calcd for $C_{21}H_{24}N_3O_3$ $[M+H]^+$ 366.1812, found 366.1801.

(*E*)-5-[1-(4-Methyl-2-oxo-1-oxaspiro[4,5]dec-3-en-3-yl)ethylidene]-2-(4-flurophenyl)amino-3,5-dihydro-4*H*-imidazol-4-one (**5b**): white solid, yield 68%; m.p. 227−229 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.50 (s, 1H), 9.63 (s, 1H), 7.69−7.04 (m, 4H), 2.10 (s, 3H), 1.94−1.23 (m, 13H); IR *v*: 3435, 3216, 3034, 2938, 1688, 1654, 1602, 1575, 1498 cm^{-1}; HR-ESI-MS calcd for $C_{21}H_{23}FN_3O_3$ $[M+H]^+$ 384.1718, found 384.1706.

(*E*)-5-[1-(4-Methyl-2-oxo-1-oxaspiro[4,5]dec-3-en-3-yl)ethylidene]-2-(4-methylphenyl)amino-3,5-dihydro-4*H*-imidazol-4-one (**5c**): white solid, yield 53%; m.p. 196−198 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.37 (s, 1H), 9.49 (s, 1H), 7.59−7.03 (m, 4H), 2.26 (s, 3H), 2.10 (s, 3H), 1.95−1.25 (m, 13H); IR *v*: 3431, 3210, 3045, 2945, 1680, 1651, 1607, 1578, 1492 cm^{-1}; HR-ESI-MS calcd for $C_{22}H_{26}N_3O_3$ $[M+H]^+$ 380.1969, found 380.1957.

(*E*)-5-[1-(4-Methyl-2-oxo-1-oxaspiro[4,5]dec-3-en-3-yl)ethylidene]-2-(4-methoxyphenyl)amino-3,5-dihydro-4*H*-imidazol-4-one (**5d**): white solid, yield 58%; m.p. 235−237 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.39 (s, 1H), 9.46 (s, 1H), 7.62−6.90 (m, 4H), 3.73 (s, 3H), 2.09 (s, 3H), 1.94−1.24 (m, 13H); HR-ESI-MS calcd for $C_{22}H_{26}N_3O_4$ $[M+H]^+$ 396.1918, found 396.1908.

(*E*)-5-[1-(4-Methyl-2-oxo-1-oxaspiro[4,5]dec-3-en-3-yl)ethylidene]-2-(2-flurophenyl)amino-3,5-dihydro-4*H*-imidazol-4-one (**5e**): yellow solid, yield 76%; m.p. 159−161 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.50 (s, 1H), 9.53 (s, 1H), 7.24−7.06 (m, 4H), 2.12 (s, 3H), 1.93 − 1.23 (m, 13H); HR-ESI-MS calcd for $C_{21}H_{23}FN_3O_3$ $[M+H]^+$ 384.1718, found 384.1706.

(*E*)-5-[1-(4-Methyl-2-oxo-1-oxaspiro[4,5]dec-3-en-3-yl)ethylidene]-2-(2-chlorophenyl)amino-3,5-dihydro-4*H*-imidazol-4-one (**5f**): yellow solid, yield 50%, m.p. 213−215 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.69 (s, 1H), 9.54 (s, 1H), 7.44−7.01 (m, 4H), 2.09 (s, 3H), 1.95 − 1.24 (m, 13H); HR-ESI-MS calcd for $C_{21}H_{23}ClN_3O_3$ $[M+H]^+$ 400.1422, found 400.1411.

(*E*)-5-[1-(4-Methyl-2-oxo-1-oxaspiro[4,5]dec-3-en-3-yl)ethylidene]-2-(2-methylphenyl)amino-3,5-dihydro-4*H*-imidazol-4-one (**5g**): pink solid, yield 63%; m.p. 216−218 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.37 (s, 1H), 9.54 (s, 1H), 7.59−6.99 (m, 4H), 2.22 (s, 3H), 2.08 (s, 3H), 1.90−1.24 (m, 13H); HR-ESI-MS calcd for $C_{22}H_{26}N_3O_3$ $[M+H]^+$ 380.1969, found 380.1957.

(*E*)-5-[1-(4-Methyl-2-oxo-1-oxaspiro[4,5]dec-3-en-3-yl)ethylidene]-2-(2-methoxyphenyl)amino-3,5-dihydro-4*H*-imidazol-4-one (**5h**): yellow solid, yield 61%; m.p. 167−169 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.15 (s, 1H), 9.09 (s, 1H), 7.07−6.95 (m, 4H), 3.89 (s, 3H), 2.13 (s, 3H), 1.94−1.22 (m, 13H); HR-ESI-MS calcd for $C_{22}H_{26}N_3O_4$ $[M+H]^+$ 396.1918, found 396.1904.

(*E*)-5-[1-(4-Methyl-2-oxo-1-oxaspiro[4,5]dec-3-en-3-yl)ethylidene]-2-(3-trifluromethylphenyl)amino-3,5-dihydro-4*H*-imidazol-4-one (**5i**): white solid, yield 65%; m.p. 210−212 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.72 (s, 1H), 9.95 (s, 1H), 8.34−7.30 (m, 4H), 2.12 (s, 3H), 1.99 − 1.30 (m, 13H); HR-ESI-MS calcd for $C_{22}H_{23}F_3N_3O_3$ $[M+H]^+$ 434.1686, found 434.1673.

(*E*)-5-[1-(4-Methyl-2-oxo-1-oxaspiro[4,5]dec-3-en-3-yl)ethylidene]-2-benzylamino-3,5-dihydro-4*H*-imidazol-4-one (**5j**): white solid, yield 69%; m.p. 146−148 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.58 (s, 1H), 9.84 (s, 1H), 7.35−7.26 (m, 5H), 4.50 (s, 2H), 2.13 (s, 3H), 1.97 − 1.24 (m, 13H); HR-ESI-MS calcd for $C_{22}H_{26}N_3O_3$ $[M+H]^+$ 380.1969, found 380.1958.

(*E*)-5-[1-(4-Methyl-2-oxo-1-oxaspiro[4,5]dec-3-en-3-yl)ethylidene]-2-(4-flurobenzyl)amino-3,5-dihydro-4*H*-imidazol-4-one (**5k**): yellow solid, yield 58%; m.p. 141−143 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.76 (s, 1H), 9.89 (s, 1H), 7.37−7.13 (m, 4H), 4.48 (s, 2H), 2.12 (s, 3H), 1.97−1.25 (m, 13H); HR-ESI-MS calcd for $C_{22}H_{25}FN_3O_3$ $[M+H]^+$ 398.1874, found 398.1862.

(*E*)-5-[1-(4-Methyl-2-oxo-1-oxaspiro[4,5]dec-3-en-3-yl)ethylidene]-2-(4-chlorobenzyl)amino-3,5-dihydro-4*H*-imidazol-4-one (**5l**): yellow solid, yield 58%; m.p. 240−242 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.58 (s, 1H), 9.89 (s, 1H), 7.48−7.29 (m, 4H), 4.54 (s, 2H), 2.08 (s, 3H), 1.95−1.31 (m, 13H); HR-ESI-MS calcd for $C_{22}H_{25}ClN_3O_3$ $[M+H]^+$ 414.1579, found 414.1566.

(*E*)-5-[1-(4-Methyl-2-oxo-1-oxaspiro[4,5]dec-3-en-3-yl)ethylidene]-2-(4-methoxybenzyl)amino-3,5-dihydro-

4*H*-imidazol-4-one (**5m**): yellow solid, yield 61%; m.p. 211—213 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.55 (s, 1H), 9.90 (s, 1H), 7.49—6.89 (m, 4H), 4.43 (s, 2H), 3.73 (s, 3H), 2.08 (s, 3H), 1.99—1.25 (m, 13H); HR-ESI-MS calcd for $C_{23}H_{28}N_3O_4$ $[M+H]^+$ 410.2074, found 410.2062.

(*E*)-5-[1-(4-Methyl-2-oxo-1-oxaspiro[4,5]dec-3-en-3-yl)ethylidene]-2-(2-flurobenzyl)amino-3,5-dihydro-4*H*-imidazol-4-one (**5n**): white solid, yield 66%; m.p. 210—213 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.57 (s, 1H), 9.83 (s, 1H), 7.45—7.17 (m, 4H), 4.52 (s, 2H), 2.07 (s, 3H), 1.91—1.20 (m, 13H); HR-ESI-MS calcd for $C_{22}H_{25}FN_3O_3$ $[M+H]^+$ 398.1874, found 398.1864.

(*E*)-5-[1-(4-Methyl-2-oxo-1-oxaspiro[4,5]dec-3-en-3-yl)ethylidene]-2-(2-methoxybenzyl)amino-3,5-dihydro-4*H*-imidazol-4-one (**5o**): yellow solid, yield 54%; m.p. 138—140 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.50 (s, 1H), 9.77 (s, 1H), 7.57—6.91 (m, 4H), 4.46 (s, 2H), 3.83 (s, 3H), 2.10 (s, 3H), 1.94—1.24 (m, 13H); HR-ESI-MS calcd for $C_{23}H_{28}N_3O_4$ $[M+H]^+$ 410.2074, found 410.2062.

(*E*)-5-[1-(4-Methyl-2-oxo-1-oxaspiro[4,5]dec-3-en-3-yl)ethylidene]-2-(3-trifluromethylbenzyl)amino-3,5-dihydro-4*H*-imidazol-4-one (**5p**): white solid, yield 54%; m.p. 138—140 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.56 (s, 1H), 9.95 (s, 1H), 8.02—7.57 (m, 4H), 4.54 (s, 2H), 2.10 (s, 3H), 1.92—1.24 (m, 13H); HR-ESI-MS calcd for $C_{23}H_{25}F_3N_3O_3$ $[M+H]^+$ 448.1843, found 448.1832.

(*E*)-5-[1-(4,8-Dimethyl-2-oxo-1-oxaspiro[4,5]dec-3-en-3-yl)ethylidene]-2-phenylamino-3,5-dihydro-4*H*-imidazol-4-one (**6a**): pink solid, yield 48%; m.p. 217—219 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.43 (s, 1H), 9.67 (s, 1H), 7.69—6.99 (m, 5H), 2.11 (s, 3H), 1.99—0.93 (m, 15H); IR ν: 3442, 3225, 3058, 2944, 1690, 1656, 1601, 1575, 1498 cm^{-1}; HR-ESI-MS calcd for $C_{22}H_{26}N_3O_3$ $[M+H]^+$ 380.1969, found 380.1957.

(*E*)-5-[1-(4,8-Dimethyl-2-oxo-1-oxaspiro[4,5]dec-3-en-3-yl)ethylidene]-2-(4-flurophenyl)amino-3,5-dihydro-4*H*-imidazol-4-one (**6b**): yellow solid, yield 60%; m.p. 208—210 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.52 (s, 1H), 9.64 (s, 1H), 7.70—7.13 (m, 4H), 2.09 (s, 3H), 2.00—0.93 (m, 15H); IR ν: 3436, 3223, 3057, 2934, 1685, 1647, 1603, 1573, 1495 cm^{-1}; HR-ESI-MS calcd for $C_{22}H_{25}FN_3O_3$ $[M+H]^+$ 398.1874, found 398.1865.

(*E*)-5-[1-(4,8-Dimethyl-2-oxo-1-oxaspiro[4,5]dec-3-en-3-yl)ethylidene]-2-(4-methylphenyl)amino-3,5-dihydro-4*H*-imidazol-4-one (**6c**): yellow solid, yield 38%; m.p. 210—212 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.39 (s, 1H), 9.62 (s, 1H), 7.59—7.11 (m, 4H), 2.26 (s, 3H), 2.10 (s, 3H), 2.00—0.93 (m, 15H); IR ν: 3423, 3235, 3066, 2938, 1687, 1644, 1606, 1575, 1497 cm^{-1}; HR-ESI-MS calcd for $C_{23}H_{28}N_3O_3$ $[M+H]^+$ 394.2125, found 394.2113.

(*E*)-5-[1-(4,8-Dimethyl-2-oxo-1-oxaspiro[4,5]dec-3-en-3-yl)ethylidene]-2-(4-methoxyphenyl)amino-3,5-dihydro-4*H*-imidazol-4-one (**6d**): yellow solid, yield 61%, m.p. 197—199 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.40 (s, 1H), 9.61 (s, 1H), 7.62—6.90 (m, 4H), 3.73 (s, 3H), 2.08 (s, 3H), 1.94—0.93 (m, 15H); HR-ESI-MS calcd for $C_{23}H_{28}N_3O_4$ $[M+H]^+$ 410.2074, found 410.2061.

(*E*)-5-[1-(4,8-Dimethyl-2-oxo-1-oxaspiro[4,5]dec-3-en-3-yl)ethylidene]-2-(4-triflurometylphenyl)amino-3,5-dihydro-4*H*-imidazol-4-one (**6e**): yellow solid, yield 49%; m.p. 198—200 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.66 (s, 1H), 9.97 (s, 1H), 7.87—7.60 (m, 4H), 2.09 (s, 3H), 1.99—0.93 (m, 15H); HR-ESI-MS calcd for $C_{23}H_{25}F_3N_3O_3$ $[M+H]^+$ 448.1843, found 448.1834.

(*E*)-5-[1-(4,8-Dimethyl-2-oxo-1-oxaspiro[4,5]dec-3-en-3-yl)ethylidene]-2-(2-chlorophenyl)amino-3,5-dihydro-4*H*-imidazol-4-one (**6f**): yellow solid, yield 51%; m.p. 174—176 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.67 (s, 1H), 9.54 (s, 1H), 7.44—7.01 (m, 4H), 2.16 (s, 3H), 1.94—0.94 (m, 15H); HR-ESI-MS calcd for $C_{22}H_{25}ClN_3O_3$ $[M+H]^+$ 414.1579, found 414.1568.

(*E*)-5-[1-(4,8-Dimethyl-2-oxo-1-oxaspiro[4,5]dec-3-en-3-yl)ethylidene]-2-(2-methoxyphenyl)amino-3,5-dihydro-4*H*-imidazol-4-one (**6g**): yellow solid, yield 61%; m.p. 185—187 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.15 (s, 1H), 9.82 (s, 1H), 7.07—6.82 (m, 4H), 3.89 (s, 3H), 2.13 (s, 3H), 1.99—0.92 (m, 15H); HR-ESI-MS calcd for $C_{23}H_{28}N_3O_4$ $[M+H]^+$ 410.2074, found 410.2061.

(*E*)-5-[1-(4,8-Dimethyl-2-oxo-1-oxaspiro[4,5]dec-3-en-3-yl)ethylidene]-2-(3-triflurometylphenyl)amino-3,5-dihydro-4*H*-imidazol-4-one (**6h**): yellow solid, yield 43%; m.p. 209—211 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.73 (s, 1H), 9.90 (s, 1H), 8.43—7.34 (m, 4H), 2.12 (s, 3H), 1.96—0.93 (m, 13H); HR-ESI-MS calcd for $C_{23}H_{25}F_3N_3O_3$ $[M+H]^+$ 448.1843, found 448.1833.

(*E*)-5-[1-(4,8-Dimethyl-2-oxo-1-oxaspiro[4,5]dec-3-en-3-yl)ethylidene]-2-benzylamino-3,5-dihydro-4*H*-imidazol-4-one (**6i**): yellow solid, yield 56%; m.p. 143—145 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.58 (s, 1H), 9.83 (s, 1H), 7.34—7.26 (m, 5H), 4.51 (s, 2H), 2.12 (s, 3H), 1.95—0.92 (m, 15H); HR-ESI-MS calcd for $C_{23}H_{28}N_3O_3$ $[M+H]^+$ 394.2125, found 394.2113.

(*E*)-5-[1-(4,8-Dimethyl-2-oxo-1-oxaspiro[4,5]dec-3-en-3-yl)ethylidene]-2-(4-chlorobenzylamino-3,5-dihydro-4*H*-imidazol-4-one (**6j**): yellow solid, yield 56%; m.p. 143—145 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.64 (s, 1H), 9.91 (s, 1H), 7.42—7.27 (m, 4H), 4.50 (s, 2H), 2.12 (s, 3H), 1.91—0.93 (m, 15H); HR-ESI-MS calcd for $C_{23}H_{27}ClN_3O_3$ $[M+H]^+$ 394.2125, found 394.2113.

(*E*)-5-[1-(4,8-Dimethyl-2-oxo-1-oxaspiro[4,5]dec-3-en-3-yl)ethylidene]-2-(2-methoxybenzyl)amino-3,5-dihydro-4*H*-imidazol-4-one (**6k**): yellow solid, yield 52%; m.p. 137—139 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.43 (s, 1H), 9.76 (s, 1H), 7.20—6.92 (m, 4H), 4.48 (s, 2H), 3.83 (s, 3H), 2.12 (s, 3H), 1.95—0.92 (m, 15H); HR-ESI-MS calcd for $C_{24}H_{30}N_3O_4$ $[M+H]^+$

424.2231, found 424.2216.

(*E*)-5-[1-(4-Methyl-2-oxo-1-oxaspiro[4.5]non-3-en-3-yl)ethylidene]-2-phenylamino-3,5-dihydro-4*H*-imidazol-4-one (**7a**): pink solid, yield 46%; m.p. 244－246 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.43 (s, 1H), 9.66 (s, 1H), 7.71－6.99 (m, 5H), 2.13 (s, 3H), 2.08－1.46 (m, 11H); IR *v*: 3441, 3225, 3059, 2944, 1688, 1642, 1606, 1575, 1493 cm^{-1}; HR-ESI-MS calcd for $C_{20}H_{22}N_3O_3$ $[M+H]^+$ 352.1656, found 352.1644.

(*E*)-5-[1-(4-Methyl-2-oxo-1-oxaspiro[4.5]non-3-en-3-yl)ethylidene]-2-(4-flurophenyl)amino-3,5-dihydro-4*H*-imidazol-4-one (**7b**): yellow solid, yield 38%; m.p. 174－176 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.71 (s, 1H), 9.64 (s, 1H), 7.68－7.10 (m, 4H), 2.12 (s, 3H), 2.03－1.44 (m, 11H); IR *v*: 3446, 3233, 3052, 2937, 1685, 1649, 1602, 1571, 1498 cm^{-1}; HR-ESI-MS calcd for $C_{20}H_{21}FN_3O_3$ $[M+H]^+$ 370.1561, found 370.1549.

(*E*)-5-[1-(4-Methyl-2-oxo-1-oxaspiro[4.5]non-3-en-3-yl)ethylidene]-2-(4-methylphenyl)amino-3,5-dihydro-4*H*-imidazol-4-one (**7c**): yellow solid, yield 36%; m.p. 176－178 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.56 (s, 1H), 9.61 (s, 1H), 7.54－6.97 (m, 4H), 2.27 (s, 3H), 2.12 (s, 3H), 2.08－1.45 (m, 11H); IR *v*: 3436, 3227, 3061, 2944, 1692, 1656, 1603, 1582, 1497 cm^{-1}; HR-ESI-MS calcd for $C_{21}H_{24}N_3O_3$ $[M+H]^+$ 366.1812, found 366.1802.

(*E*)-5-[1-(4-Methyl-2-oxo-1-oxaspiro[4.5]non-3-en-3-yl)ethylidene]-2-(4-methoxyphenyl)amino-3,5-dihydro-4*H*-imidazol-4-one (**7d**): yellow solid, yield 71%; m.p. 190－192 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.41 (s, 1H), 9.61 (s, 1H), 7.62－6.81 (m, 4H), 3.73 (s, 3H), 2.10 (s, 3H), 2.05－1.48 (m, 11H); IR *v*: 3438, 3237, 3060, 2948, 1690, 1658, 1601, 1583, 1492 cm^{-1}; HR-ESI-MS calcd for $C_{21}H_{24}N_3O_4$ $[M+H]^+$ 382.1761, found 382.1750.

(*E*)-5-[1-(4-Methyl-2-oxo-1-oxaspiro[4.5]non-3-en-3-yl)ethylidene]-2-(2-flurophenyl)amino-3,5-dihydro-4*H*-imidazol-4-one (**7e**): yellow solid, yield 54%; m.p. 160－162 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.46 (s, 1H), 9.67 (s, 1H), 7.84－6.95 (m, 4H), 2.09 (s, 3H), 2.00 － 1.43 (m, 11H); HR-ESI-MS calcd for $C_{20}H_{21}FN_3O_3$ $[M+H]^+$ 370.1561, found 370.1550.

(*E*)-5-[1-(4-Methyl-2-oxo-1-oxaspiro[4.5]non-3-en-3-yl)ethylidene]-2-(2-chlorophenyl)amino-3,5-dihydro-4*H*-imidazol-4-one (**7f**): yellow solid, yield 50%: m.p. 168－170 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.68 (s, 1H), 9.64 (s, 1H), 7.44－7.01 (m, 4H), 2.12 (s, 3H), 2.02 － 1.43 (m, 11H); HR-ESI-MS calcd for $C_{20}H_{21}ClN_3O_3$ $[M+H]^+$ 386.1266, found 386.1254.

(*E*)-5-[1-(4-Methyl-2-oxo-1-oxaspiro[4.5]non-3-en-3-yl)ethylidene]-2-(2-methoxyphenyl)amino-3,5-dihydro-4*H*-imidazol-4-one (**7g**): orange solid, yield 40%; m.p. 175－177 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.18 (s, 1H), 9.89 (s, 1H), 7.69－6.97 (m, 4H), 3.83 (s, 3H), 2.12 (s, 3H), 2.08－1.45 (m, 11H); HR-ESI-MS calcd for $C_{21}H_{24}N_3O_4$ $[M+H]^+$ 382.1761, found 382.1750.

(*E*)-5-[1-(4-Methyl-2-oxo-1-oxaspiro[4.5]non-3-en-3-yl)ethylidene]-2-(3-trifluromethylphenyl)amino-3,5-dihydro-4*H*-imidazol-4-one (**7h**): yellow solid, yield 91%; m.p. 159－161 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.74 (s, 1H), 9.92 (s, 1H), 8.32－7.31 (m, 4H), 2.14 (s, 3H), 2.04－1.43 (m, 11H); HR-ESI-MS calcd for $C_{21}H_{21}F_3N_3O_3$ $[M+H]^+$ 420.1530, found 420.1518.

(*E*)-5-[1-(4-Methyl-2-oxo-1-oxaspiro[4.5]non-3-en-3-yl)ethylidene]-2-benzylamino-3,5-dihydro-4*H*-imidazol-4-one (**7i**): yellow solid, yield 33%; m.p. 115－117 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.03 (s, 1H), 9.44 (s, 1H), 7.41－7.25 (m, 5H), 4.50 (s, 2H), 2.14 (s, 3H), 1.99－1.42 (m, 11H); HR-ESI-MS calcd for $C_{21}H_{24}N_3O_3$ $[M+H]^+$ 366.1812, found 366.1802.

(*E*)-5-[1-(4-Methyl-2-oxo-1-oxaspiro[4.5]non-3-en-3-yl)ethylidene]-2-(4-chlorobenzylamino-3,5-dihydro-4*H*-imidazol-4-one (**7j**): yellow solid, yield 45%; m.p. 199－201 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.63 (s, 1H), 9.88 (s, 1H), 7.42－7.28 (m, 4H), 4.48 (s, 2H), 2.13 (s, 3H), 2.01－1.43 (m, 11H); HR-ESI-MS calcd for $C_{21}H_{23Cl}N_3O_3$ $[M+H]^+$ 400.1422, found 400.1412.

(*E*)-5-[1-(4-Methyl-2-oxo-1-oxaspiro[4.5]non-3-en-3-yl)ethylidene]-2-(2-methoxybenzyl)amino-3,5-dihydro-4*H*-imidazol-4-one (**7k**): yellow solid, yield 48%; m.p. 128－130 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.40 (s, 1H), 9.70 (s, 1H), 7.27－6.91 (m, 4H), 4.47 (s, 2H), 3.83 (s, 3H), 2.12 (s, 3H), 2.00－1.44 (m, 11H); HR-ESI-MS calcd for $C_{22}H_{26}N_3O_4$ $[M+H]^+$ 396.1918, found 396.1910.

Bioassay

Fungicidal activities of the compounds against *S. scleotiorum*, *A. solani*, *B. cinerea*, *R. solani*, *F. graminearum* and *P. capsici* were evaluated using the mycelial growth rate and spore germination tests.[19,21] The culture media with known concentration of the test compounds were obtained by mixing the solution in DMSO with potato dextrose agar (PDA), on which fungus cakes were placed. The blank test was made using DMSO and chlorothalonil, carbendazim and fenamidone were used as the positive control. The culture was incubated at (25±0.5) ℃. Three replicates were performed. After the mycelia in the blank grew completely, the diameter of the mycelia was measured, and the inhibition rate was calculated according to the formula in reference[19] from the three replicates. The EC_{50} values were determined from the inhibition rates of five different concentrations based on the statistics method for the compounds which had more than 70% inhibition rates.

Scanning electron microscopy (SEM)

P. capsici mycelial tips (5 mm) of an actively growing colony on PDA medium amended with 0, 10, 50, and 100 μg/mL compound **7h** were cut from the edge of the colony cultured for 96 h. The tips were treated with 2% glutaraldehyde at 4 ℃, followed by rinsing with 0.1 mol•L^{-1} phosphate buffer (pH 7.3) and fixed with 1.0

g•mL^{-1} osmium tetraoxide solution. Rinsed with 0.1 mol•L^{-1} phosphate buffer three times, the mycelial tips were dehydrated using a series of ethanol solutions in the order of concentration 30%, 50%, 70%, 80%, 90% and 100%. The processes of drying at critical point, mounting, and gold spraying were completed at last and examined with a Hitachi S-3400N scanning electron microscope with an accelerating voltage of 18—20 kV.

Transmission electron microscopy (TEM)

The mycelial tips were prepared according to the method given above. After dehydrating with acetone and embedding in SPURR resin, thin sections were cut with LEICAUCi machine, and double-stained with uranyl acetate and lead citrate. The grids were examined with a JEOL-1230 transmission electron microscope.

Results and Discussion

As shown in Scheme 3, the intermediates **2a**, **2b** and **2c** were prepared in two steps by one-pot manner following the literature procedures[22,23] by the reaction of cycloketone with vinyl ethyl ether to afford *α*-hydroxyketone, followed by reacting with diketene in 50%—60% overall yields. The intermediates **2a**—**2c** reacted with thiohydantion catalyzed by 2-aminoethanol to afford **3a**—**3c** in 54%—72% yields, respectively,[19] then methylations of **3a**—**3c** with methyl iodide were carried out at ambienent temperature to give the key intermediates **4a**—**4c** in 85%—90% yields.[19,20] Finally, the intermediates **4a**—**4c** reacted with anilines or benzyl amines under reflux to produce the target compounds **5a**—**5p**, **6a**—**6k** and **7a**—**7k** in moderate to excellent yields.[19] In the ^{1}H NMR spectra, the chemical shifts of the methyl groups in compounds **2a**—**2c** and **3a**—**3c** were easily assigned, while the complex peaks of the methyl signals and broad active NH proton peaks were observed due to the existance of tautomerism isomers of the 2-aminoimidazolin-4-one moiety in the solution after they transfered into compounds **4a**—**4c** and **5**—**7**. The ^{1}H NMR spectra of **5a**—**5p**, **6a**—**6k** and **7a**—**7k** were similar to those of compounds in the literatures.[19,20] In the previous papers, the configuration of C=C double bond for title compounds was not ascertained.[19] Now, good crystal solid suitable for X-ray diffraction analysis was obtained, and the X-ray structure of compound **5g** was unambiguously confirmed and depicted in Figure 1, which indicated that one methanol molecule was bonded with **5g** molecule via hydrogen bond.[24] The X-ray structure of compound **5g** clearly indicated that the configuration of C=C double bond for this class of compounds was *E*-configuration, which was the same as that reported in literatures.[20,25] The parent skeleton of imidazolinone moiety was determined to be 2-amino-1,5-dihydro-4*H*- imidazolin-4-one (**A**), rather than 2-amino-3,5-dihydro-4*H*-imidazolin-4-one (**B**) and 2-iminoimidazolidin-4-one (**C**) as shown in Figure 2, in which the parent structure of 2-amino-3,5-dihydro-4*H*-imidazol-4-one was consistent with X-ray structures of the other similar compounds in literatures.[12,26]

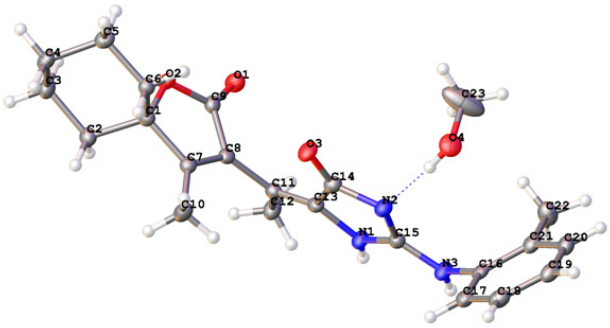


Figure 1 The X-ray structure of compound **5g**.

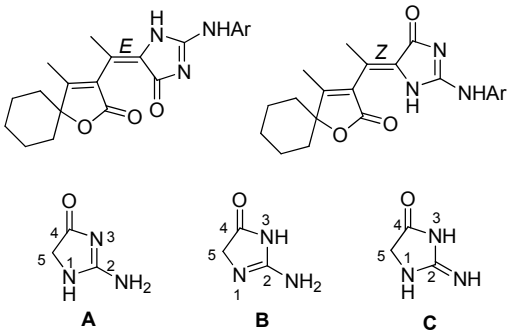


Figure 2 Two possible configurations of C=C double bond and three parent skeleton structures.

The data in Table 1 showed that the intermediates **3b**, **3c** and **4c** exhibited excellent activities against *P. capsici* with 96.4%, 92.5% and 90.3% inhibition rates *in vitro* evaluation while they had no significant inhibition against *S. scleotiorum*, *R. solani*, *A. Solani*, *B. cinerea* and *F. graminearum*. So further structure modification was performed with reaction of **4a**—**4c** with various amines to afford novel 2-aminoimidazolin-4-one derivatives **5**—**7** with spirocyclic scaffold. The data in Table 1 indicated that these 2-aminoimidazolin-4-one derivatives have significant fungicidal activities against *S. scleotiorum*, *A. solani*, *B. cinerea* and *P. capsici* compared with those of the intermediates **4a**—**4c**, but no activities against *R. Solani* and *F. graminearum*. In comparison with the results in the previous paper,[19c] most of the compounds, such as **5e**, **6a**, **6d**, **6e**, **6f**, **6g**, **6h**, **6j**, **7f**, **7g** and **7h**, exhibited excellent inhibitory activities against *S. scleotiorum*, but not against *R. Solani*, this probably means the spirocyclic part in butenolide scaffold changes target fungi and improves the activities greatly, and the aromatic amine derivatives have much better fungicidal activities against *S. scleotiorum* than the benzyl amine derivatives. At the same time, only compound **7h** showed excellent inhibitory activities against *P. capsici*. These results indicated that the electron-withdrawing groups at *othro*-, *meta*- and

Table 1 Fungicidal activities of compounds **3**—**7** against phytopathogens (inhibitory rate/%, 50 μg/mL)[a]

Compd.	*m*	R^1	*S.S*	*R.S*	*A.S*	*B.C*	*P.C*	*F.G*
3a			7.4	8.4	39.0	6.8	50.4	17.8
3b			13.8	0.0	0.0	26.2	**96.4**	32.4
3c			6.6	5.4	0.0	0.0	**92.5**	16.4
4a			19.0	18.6	39.6	35.6	58.8	43.1
4b			50.6	15.6	21.9	40.5	48.0	35.7
4c			0.0	0.0	0.0	9.8	**90.3**	7.1
5a	0	H	34.6	3.6	22.6	27.3	34.6	31.6
5b	0	4-F	49.9	7.0	35.6	41.2	49.9	50.9
5c	0	4-CH_3	21.2	13.0	0.0	48.5	39.6	13.0
5d	0	4-OCH_3	46.8	1.8	24.4	39.8	50.3	29.2
5e	0	2-F	**87.8**	21.9	36.4	45.0	57.1	62.2
5f	0	2-Cl	56.8	30.9	26.4	36.0	20.6	54.5
5g	0	2-CH_3	37.4	3.6	50.1	33.3	37.2	16.5
5h	0	2-OCH_3	46.3	24.9	37.6	22.5	0.0	54.0
5i	0	3-CF_3	41.3	14.7	29.8	45.9	35.6	33.2
5j	1	H	67.0	12.2	0.0	0.0	3.2	33.8
5k	1	4-F	0.0	0.0	4.3	24.5	27.3	30.1
5l	1	4-Cl	1.7	7.6	62.6	39.9	20.9	28.7
5m	1	4-OCH_3	24.4	2.7	17.5	34.1	53.1	22.6
5n	1	2-F	36.7	13.8	21.3	40.5	32.3	14.8
5o	1	2-OCH_3	17.6	0.0	31.5	21.9	23.5	35.5
5p	1	3-CF_3	31.1	3.6	8.9	20.3	8.9	12.1
6a	0	H	**91.3**	12.0	33.4	59.4	60.5	62.6
6b	0	4-F	40.6	0.0	15.5	48.6	30.8	40.2
6c	0	4-CH_3	60.4	3.1	29.2	47.6	36.8	39.6
6d	0	4-OCH_3	**73.6**	28.6	56.4	47.6	46.9	63.5
6e	0	3-CF_3	**89.9**	6.1	**72.6**	46.0	63.0	35.5
6f	0	2-Cl	**83.5**	28.6	53.6	40.3	47.7	55.5
6g	0	2-OCH_3	**83.3**	8.3	8.2	69.2	61.9	58.2
6h	0	3-CF_3	**73.6**	1.5	8.8	57.1	23.8	40.2
6i	1	H	30.3	10.3	3.7	7.1	45.1	34.9
6j	1	4-Cl	**79.1**	2.3	31.6	14.5	42.2	22.6
6k	1	2-OCH_3	17.0	0.0	0.0	7.1	43.9	36.1
7a	0	H	13.0	3.8	0.0	21.2	17.2	36.4
7b	0	4-F	30.5	6.8	69.9	31.5	9.5	19.4
7c	0	4-CH_3	32.5	4.6	19.4	9.4	8.7	16.1
7d	0	4-OCH_3	5.3	7.6	14.9	29.9	20.9	33.7
7e	0	2-F	67.4	10.3	0.0	16.0	0.8	47.3
7f	0	2-Cl	**72.6**	19.3	11.6	19.3	7.1	38.4
7g	0	2-OCH_3	**74.5**	18.7	4.4	21.3	0.0	45.2
7h	0	3-CF_3	**87.8**	29.7	0.8	45.6	**76.9**	47.3
7i	1	H	55.3	10.9	0.0	0.0	0.0	39.2
7j	1	4-Cl	10.3	7.6	29.8	36.0	21.4	23.2
7k	1	2-OCH_3	26.4	14.9	3.7	0.0	0.0	37.3
Chlorothalonil (CT)			100	99.9	100	100	99.1	95.8
Carbendazim (CD)			81.0	100	100	4.2	34.7	100
Fenamidone (FA)			97.8	96.4	93.5	57.1	79.8	71.9

[a] *S.S*: *Sclerotinia scleotiorum*; *R.S*: *Rhizoctonia solani*; *A.S*: *Alternaria Solani*; *B.C*: *Botrytis cinerea*; *P.C*: *Phytophthora capsici*; *F.G*: *Fusarium graminearum*.

para-positions in the benzene ring of aromatic amines would be beneficial to improve the activities of this class of compounds.

The EC_{50} values were determined further for these compounds having inhibition rates more than 70% in Table 2. For *S. scleotiorum*, the EC_{50} values of seven compounds were smaller than that of carbendazim and those of five compounds were smaller than that of fenamidone, especially **6a**, **6e**, **6f**, **6g** and **6h** exhibited remarkable fungicidal activities with EC_{50} values of 2.56—3.65 μg/mL. For *A. solani*, only **6e** with EC_{50} value of 3.20 μg/mL had almost equal inhibitory activity with fenamidone (EC_{50} 1.99 μg/mL). For *P. capsici*, compound **7h** was the only one which showed much better activity (EC_{50} 6.50 μg/mL) than those of carbendazim (EC_{50} 77.80 μg/mL) and fenamidone (EC_{50} 9.32 μg/mL), also the intermediates **3b** (EC_{50} 2.45 μg/mL), **3c** (EC_{50}7.42 μg/mL) and **4c** (EC_{50} 4.82 μg/mL) had better activities than those of carbendazim and fenamidone. Based on the above results, the strategy of introduction of spirocyclic butenolide scaffold into 2-aminoimidazolin-4-one derivatives was confirmed to be rational, and compounds **6e** and **7h** can be used as lead compounds to optimize further.

Table 2 The EC_{50} (μg/mL) values for compounds **5**—**7**

Compd.	*S.S*	*A.S*	*P.C*	Compd.	*S.S*	*A.S*	*P.C*
3b			2.45	**6f**	3.65		
3c			7.42	**6g**	3.17		
4c			4.82	**6h**	2.86		
5e	9.29			**6j**	124.70		
6a	2.66			**7f**	10.41		
6d	6.30			**7g**	11.60		
6e	2.56	3.20		**7h**	6.50		7.51
CD	7.87	0.05	77.80				
FA	5.55	1.99	9.32				

In order to observe their action on *S. scleotiorum* and *P. capsici*, the spore germination inhibition experiments were carried out. Compounds **6e** and **7h** exhibited very weak inhibition activities against the spore germination of *S. scleotiorum* when treated with 50 μg/mL solution, while the spore germination of *P. capsici* was strongly inhibited when treated with 50 μg/mL compound **7h** solution. The mycelian variances of *P. capsici* treated with compound **7h** were observed by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The results by SEM showed that the excessive branching mycelium occurred, and the surface of hyphae was irregularly swelling (Figure 3, N), while the results by TEM indicated that the hyphal cell wall became thickening irregularly, the vacuole and the internal structure were damaged, and the membrane was also changed (Figure 3, P). These results suggested that this compound had a significant impact on the structure and function of the hyphal cell wall of *P. capsici* mycelium cell.[26] The further mode of action is to be made

clear with more experimental evidences in our laboratory.

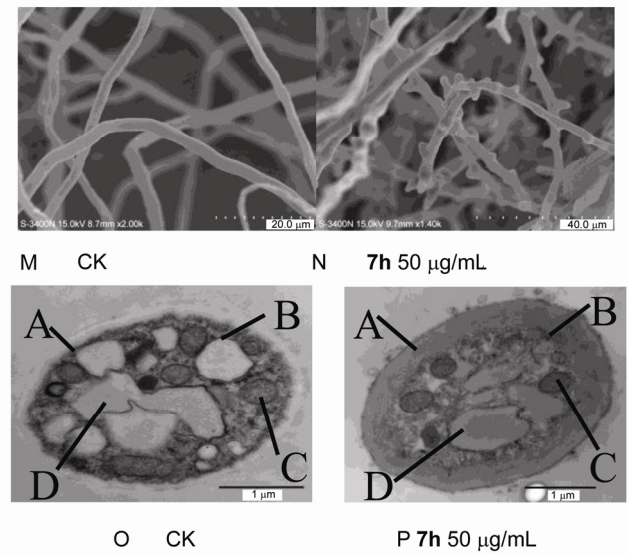


Figure 3 Mycelium growth inhibition and cell variance of *P. capsici* treated with **7h**.

Conclusions

The novel fungicidal agents (*E*)-5-[1-(2-oxo-1-oxaspro[4.5]dec/non-3-en-3-yl)ethylidene]-2-aminoimidazolin-4-one derivatives, were designed and synthesized in moderate to excellent yields in four steps using α-hydroxyketone and diketene as raw materials and characterized by HR-ESI-MS,^{1}H NMR and X-ray diffraction. These compounds showed significant fungicidal activities against *S. scleotiorum* and *P. capsici* with EC_{50} values of 2.56—11.60 μg/mL, and compounds **6e** and **7h** exhibited very weak inhibition activities against the spore germination of *S. scleotiorum*, while the spore germination of *P. capsici* was strongly inhibited when treated with compound **7h** solution. This compound had a significant impact on the structure and function of the hyphal cell wall of *P. capsici* mycelium cell.

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