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Article

Synthesis and Antifungal Activity of 5-[2-(Alkylamino)pyrimidin-4-yl]-4-phenylthiazol-2-cycloalkylamine Derivatives on *Phytophthora capsici*

Seok-Woo Nam[1], In-Young Choi[2], and Won-Sik Choi[1]*

[1]*Department of Biotechnology, Soonchunhyang University, Asan 336-745, Republic of Korea*
[2]*Dongbu Hitek Co., Ltd. 103-2 Moonji-Dong, Daeduck Science Town, Daejon 305-708, Republic of Korea*

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Fungicidal activity against phytopathogenic fungi of diarylthiazole compound of *N*-[4-(4-fluoro) phenyl-2-(*N*-methyl)piperidin-4-yl-thiazol-5-yl]pyrimidin-2-yl-*N*-(3-hydroxy-methyl)phenylamine (I) have been determined to be a superior and compound I was used as the leading compounds in this study. Furthermore, the synthesis of this compound was conducted by reacting them with four functional groups, *N*-cyclopropyl, *N*-cyclopentyl, *N*-cyclohexyl, and *N*-isopropylamine instead of the phenylamine. Also, 2-aminothiazole, 2-(*N*-ethoxycarbonyl)piperidin-4-yl, and 2-piperidin-4-yl-thiazole were introduced as the leads instead of the 2-aminothiazole group of compound I. From this scheme, VIII-1~VIII-4 and XII-1~XIV-4 compounds were newly synthesized and their structures were confirmed by ^{1}H-NMR-spectrum. The fungicidal activities of all the synthesized compounds against *Phytophthora capsici* were examined using the whole plant method. While the EC_{50} value of the commercial fungicide dimethomorph and I was 4.26 mM and that of *N*-[4-(4-fluoro)phenyl-2-(*N*-methyl)piperidin-4-yl-thiazol-5-yl]pyrimidin-2-yl-cyclopropylamine (XIV-2) on *P. capsici* was 0.84 mM. Among the XII-1~XIV-4 chemicals, XIV-2 showed the most potential antifungal activity *in vivo*. Therefore, XIV-2 can be considered as a viable candidate for the control of phytopathogenic diseases characterized by *P. capsici* infection, and further studies will be conducted on the mode of action XIV-2.

Key words: fungicidal activity, *N*-[4-(4-fluoro)phenyl-2-(*N*-methyl)piperidin-4-yl-thiazol-5-yl]pyrimidin-2-yl-cycloalkylamine, *Phytophthora capsici*

Phytophthora blight of pepper caused by *Phytophthora capsici* is the most important fungal disease found in peppers growing across the globe [Ristaino *et al*., 1991; Rajkumar *et al*., 2005], and root, crown, and fruit rot caused by *P. capsici* are the limiting factors for the production of peppers, tomatoes, and cucurbit crops [Sujkowski *et al*., 2000; Lamour and Hausbeck, 2002]. Like many species in the genus *Phytophthora*, *P. capsici* has the potential to undergo a rapid polycyclic disease development from a limited amount of initial inoculum [Ristaino *et al*., 1991]. The pathogen is soil-borne and causes blight on single or groups of plants in the field, especially in soil saturated with water after irrigation or rainfall. In the early stages, the first signs of disease are brown necrotic areas found on the root and crown of plants, afterwards the disease develops rapidly, causing plants to wilt and die [Black *et al*., 1991]. This infection can be readily found in pepper and tomato plants in southern Korea [Rajkumar *et al*., 2005]. Similar sightings have also been reported in Europe, the Western hemisphere, and in Asia [Hurtado-Gozales *et al*., 2008]. Recently, several studies demonstrated a method by which this fungus can be controlled in the pepper fields using the process of rotating non-susceptible crops, planting with fresh, clean seeds, and the development of cultivars that are resistant to *P. capsici* [Granke *et al*., 2009]. In plants infected by *P. capsici*, numerous cytological studies have already been performed [Grangeon and Coulomb, 1977; Durand and Salle G, 1981; Coulomb *et al*., 1985]. To reduce the failure of fungal disease management by the currently used fungicides, new compounds with high efficacy and selectivity against target species are desirable [Huang *et al*., 2007]. Several studies on the synthesis of thiazole derivatives have already been published, reflecting the growing interest in the chemical and biological significance of these chemicals [Xu *et al*., 2004]. The objective of present study is to develop a new

*Corresponding author
Phone: +82-41-530-1351; Fax: +82-41-530-1351
E-mail: wschoi@sch.ac.kr

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environmentally-sound fungicide to control pepper late blight by optimizing novel scaffolds for commercial use. Recently, bio-pesticides were intensively studied [Hodges *et al*., 1993; Zhou and Boland, 1998] but its efficacy is lower than synthetic pesticides and fluctuates extensively, which pronounces a possibility to be an ecosystem hazard. Derivatives of *N*-[4-(4-fluoro)phenyl-2-(*N*-methyl) piperidin-4-yl-thiazol-5-yl]pyrimidin-2-yl-*N*-(3-hydroxymethyl) phenylamine showed potential antifungal activity *in vivo* [Choi *et al*., 2010]. Therefore, in the present study, based on the structural features of thiazole, compounds that substituted phenylamine of **I** with *N*-cyclopropyl, *N*-cyclopentyl, *N*-cyclohexyl, *N*-isopropylamine were designed and synthesized. To these compounds, 2-aminothiazole, 2-(*N*-ethoxycarbonyl)piperidin-4-yl, and 2-piperidin-4-yl-thiazole were adapted instead of the amine of piperidine groups containing *N*-methyl group (**XII-1~XIV-4**), and investigated their bioactivities and fungicidal activities on *P. capsici.*

Materials and Methods

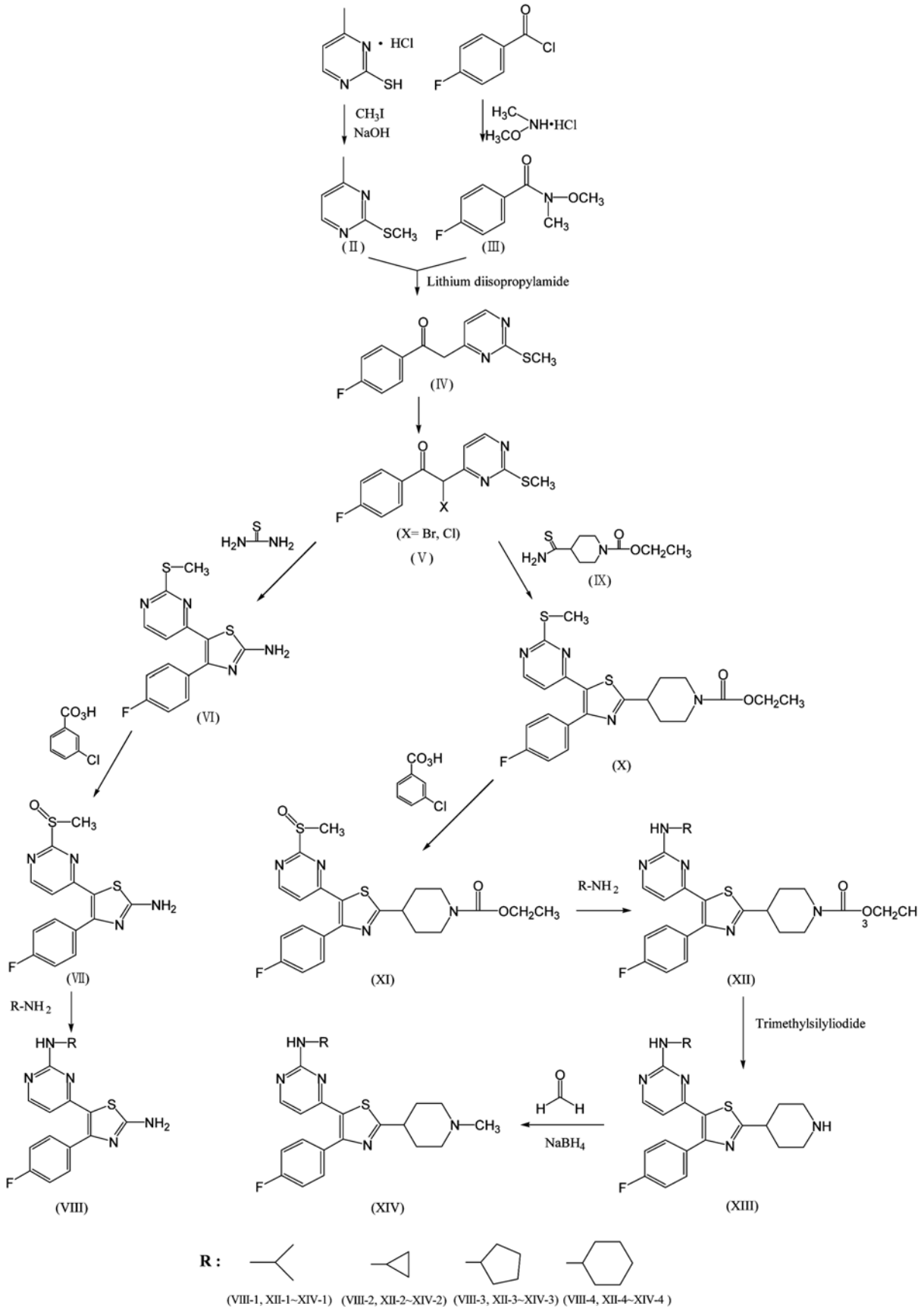
Phytopathogen. *P. capsici* was produced and supplied by the National Institute of Agricultural Science and Technology (NIAST, Suwon, Korea). This species was sub-cultured on potato dextrose agar (PDA) (3.9 g) in water (100 mL) at 15 atm and 121°C for 15 min and then uniformly sprayed on a petri dish. *P. capsici* was inoculated onto the medium at intervals of 5 mm and was then used to test for antifungal activity for 4~7 days at 20~25 and 70% humidity in a darkroom.

Materials. 2-Mercapto-4-methylpyrimidine hydrochloride (98%), iodomethane (99%), *N,O*-dimethylhydroxylamine hydrochloride (98%), 4-fluorobenzoyl chloride (98%), lithium diisopropylamide (2.0 M), bromine (99.5%), thiourea (99%), sulfuryl chloride (97%), isonipecotamide (97%), lawesson's reagent (97%), 3-chloroperoxybenzoic acid (77%), 3-aminobenzyl alcohol (99%), cyclopropylamine (98%), cyclopentylamine (99%), cyclohexylamine (99%), trimethylsilyliodide (97%), formaldehyde solution (37%), and sodium borohydride (98%) were obtained from Aldrich Chemical (St. Louis, MO). PDA was supplied from Difco (Sparks, MD). Dimethomorph was supplied from Dongbu-hannong Chemical Co (Daejeon, Korea). ^{1}H-NMR spectroscopy was carried out on a Bruker 400 NMR spectrometer (Bruker, Ettlingen, Germany), The IR spectra were recorded on a Jasco Fourier Transform (FT)/ IR-4100 Fourier-transform spectrometer (JASCO, Tokyo, Japan).

***In vitro* fungicidal activity.** The *in vitro* test determines the inhibition of mycelium of the compound under investigation in the agar culture medium [Li *et al*., 2005]. A bioindicator (Table 1) was established and used in the test. The suspended solution was prepared at the concentrations needed to obtain 40 mg/L of the studied substance after dilution with the agar culture medium (PDA). Petri scale pans were used, into which the agar culture medium and the studied substance were poured. As the culture medium set, the infectious material of the tested fungus, in the form of agar disks overgrown with mycelium, was placed at three sites on its surface. All other chemicals were of reagent grade. The linear growth of the mycelium colony was measured after 7 days, depending on the mycelium culture [Kim *et al*., 2004]. The compound's action was determined by the percentage of mycelium growth inhibition as compared with the control using the equation $J=[(C-T)/C]\times100$, where J is the percentage of colony growth inhibition, C is the zone of fungus colony growth in the control combination (millimeters), and T is the zone of fungus colony growth in combination with the compound (millimeters). The inhibitory responses were classified as follows: very strong response, ++++, J 91-100%; strong response, +++, J 71-90%; moderate response, ++, J 51-70%; weak response, +, J 41-50%; no response, –, J <40%.

***In vivo* fungicidal activity.** The plant disease evaluated was *Phytophthora* blight caused by *P. capsici.* The fungi were routinely kept and monitored on PDA slants and V-8 agar slants, and kept for stock at 4°C. The fungicidal activity of the test sample was determined by the whole plant method in a greenhouse, as previously described [Lee *et al*., 2001]. The initial concentration of the test solution was 330 mg/L, and the tests employed a dilution sequence of 40, 20, and 12.5 mg/L. To prepare the test solutions, 330 mg/L of the test sample was dissolved in 10 mL of acetone, followed by dilution with 90 mL water containing Tween 20 (330 mg/mL). Fifty milliliters of each test sample solution was simultaneously sprayed into two pots on a turntable. The treated plants were kept in a greenhouse for 1 day, before inoculation with the pathogen. The controls were sprayed with the Tween 20 solution. All tests were replicated three times. In a test with *Phytophthora* blight caused by *P. capsici*, red pepper plants at the first leaf stage (one plant per pot) were sprayed with each test solution. The red pepper was inoculated by spraying the leaves with conidia (1×10^4 spores/mL) of *P. capsici* incubated on a PDA medium at 23°C for 2 days and was then placed in a chamber (23°C) for 4-5 days. The control effect of the test sample on disease was evaluated with a control value (CV) calculated using the formula $CV\ (\%)=[(A-B)/A]\times100$, where A and B represent the disease area on the untreated and treated plants, respectively.

Statistical analysis. Analysis of variance was performed



Scheme 1. Synthesis of *N*-[4-(4-fluoro)phenylthiazol-5-yl]pyrimidin-2-yl-alkylamine derivatives (VIII-1~VIII-4, XII-1~XIV-4).

with the procedure formulated by SAS (SAS Institute, Cary, NC). If $p>F$ was less than 0.01, the means were separated with the least significant difference (LSD) test at the p=0.05 level.

Synthesis. Synthesis of compounds designated as **VIII-1~VIII-4, XII-1~XIV-4** are shown in Scheme 1. **VII** compounds were synthesized by the method of Laszlo *et al*. [2004], II by the method of Kenneth *et al*. [2003], **I**, **V III**, and **XI** by the method of Choi *et al.* [2010], and **IX** by the method of James *et al*. [2005].

***N*-[4-(4-Fluoro)phenyl-2-aminothiazol-5-yl]pyrimidin-2-yl-alkylamine derivatives (VIII-1~VIII-4).** A mixture of 2-methylsulfinyl-[4-(4-fluoro)phenyl-2-aminothiazol-5-yl] pyrimidine (**VII**) (1.0 g, 2.99 mmole) in isopropylamine (1.76 g, 29.90 mmole), cyclopropylamine (1.70 g, 29.90 mmole), cyclopentylamine (2.54 g, 29.90 mmole), and cyclohexylamine (2.96 g, 29.90 mmole) was stirred at 100°C for 2 h. The mixture was poured into 5% HCl solution (5 mL) and extracted with ethyl acetate (5 mL). The combined organic phase was dried (magnesium sulfate) and concentrated. The residue was re-crystallized from ethyl acetate and hexane to give **VIII-1** (0.50 g, 50.8%), **VIII-2** (0.51 g, 53%), **VIII-3** (0.65 g, 62%), and **VIII-4** (0.71 g, 65%) as yellow solids.

VIII-1; IR (cm^{-1}): 3312, 1451, ^{1}H-NMR ($CDCl_3$, ppm): 1.14-1.21 (d, 3H, -CH**C**$\mathbf{H_3}$), 1.26-1.28 (d, 3H, -CH**C**$\mathbf{H_3}$), 1.87-1.90 (m, 1H, -NH**C**$\mathbf{H}(CH_3)_2$), 4.99-5.02 (d, 1H, -**NH**CH-), 5.36 (s, 2H, -$\mathbf{NH_2}$), 6.31-6.33 (d, 1H, -**C**$\mathbf{H}$=CH-N), 7.09-7.14 (m, 4H, aromatic J=7.29 Hz), 7.54-7.59 (m, 4H, aromatic), 8.06-8.08 (d, 1H, -CH=**C**$\mathbf{H}$-N).

VIII-2; IR (cm^{-1}): 3314, 2931, ^{1}H-NMR ($CDCl_3$, ppm): 0.55-0.57 (q, 2H, -NHCH**C**$\mathbf{H_2}$-), 0.81-0.84 (q, 2H, -NHCH**C**$\mathbf{H_2}$-), 1.31 (quin, 1H, -NH**C**$\mathbf{H}CH_2$-), 4.97-5.03 (d, 1H, -**NH**CH-), 5.34 (s, 2H, -$\mathbf{NH_2}$), 6.30-6.35 (d, 1H, -**C**$\mathbf{H}$=CH-N), 7.11-7.15 (m, 4H, aromatic J=7.29 Hz), 7.56-7.63 (m, 4H, aromatic), 8.08-8.12 (d, 1H, -CH=**C**$\mathbf{H}$-N).

VIII-3; IR (cm^{-1}): 3315, 2942, ^{1}H-NMR ($CDCl_3$, ppm): 1.47-1.51 (quin, 4H, -NHCHCH$_2$**C**$\mathbf{H_2}$-), 1.62-1,67 (q, 4H, -NHCH**C**$\mathbf{H_2}CH_2$-), 2.03 (quin, 1H, -NH**C**$\mathbf{H}CH_2$-), 4.95-5.01 (d, 1H, -**NH**CH-), 5.31 (s, 2H, -$\mathbf{NH_2}$), 6.30-6.33 (d, 1H, -**C**$\mathbf{H}$=CH-N), 7.11-7.13 (m, 4H, aromatic J=7.29 Hz), 7.52-7.61 (m, 4H, aromatic), 8.05-8.07 (d, 1H, -CH=**C**$\mathbf{H}$-N).

VIII-4; IR (cm^{-1}): 3275, 2892, ^{1}H-NMR ($CDCl_3$, ppm): 1.46-1.52 (q, 4H, -NHCHCH$_2$**C**$\mathbf{H_2}$-), 1.62-1.65 (quin, 2H, -CH$_2$CH$_2$**C**$\mathbf{H_2}$-), 1.67-1.75 (m, 4H, -NHCH**C**$\mathbf{H_2}$-), 2.03-2.07 (quin, 1H, -NH**C**$\mathbf{H}$-), 5.22 (s, 2H, -$\mathbf{NH_2}$), 6.37-6.39 (d, 1H, -**C**$\mathbf{H}$=CH-N), 7.32-7.35 (m, 4H, aromatic J=7.29 Hz), 7.56-7.58 (m, 4H, aromatic), 8.12-8.14 (d, 1H, -CH=**C**$\mathbf{H}$-N).

***N*-[4-(4-Fluoro)phenyl-2-(*N*-ethoxycarbonyl)piperidin-4-yl-thiazol-5-yl]pyrimidin-2-yl-alkylamine derivatives (XII-1~XII-4).** A mixture of 2-methylsulfinyl-[4-(4-fluoro) phenyl-2-(*N*-ethoxy-carbonyl)piperidin-4-yl-thiazol-5-yl] pyrimidine (**XI**) (1.0 g, 2.10 mM) in isopropylamine (1.24 g, 21.07 mM), cyclopropylamine (1.20 g, 21.07 mM), cyclopentylamine (1.79 g, 21.07 mM), and cyclohexylamine (2.08 g, 21.07 mM) was stirred at 100°C for 2 h. Application of the **VIII** method described above afforded **XII-1** (0.86 g, 87.0%), **XII-2** (0.86 g, 88.0%), **XII-3** (0.88 g, 85.0%), and **XII-4** (0.91 g, 85.0%) as yellow solids.

XII-1; IR (cm^{-1}): 3277, 1753, ^{1}H-NMR ($CDCl_3$, ppm): 1.14-1.21 (d, 3H, -NHCH**C**$\mathbf{H_3}$), 1.23-1.29 (t, 3H, -OCH$_2$**C**$\mathbf{H_3}$), 1.26-1.28 (d, 3H, -NHCH**C**$\mathbf{H_3}$), 1.31 (septet, 1H, -NH**C**$\mathbf{H}$-), 1.97-2.18 (q, 4H, -CH**C**$\mathbf{H_2}CH_2$N- J=2.5 Hz), 2.73-2.81 (quin, 1H, -**C**$\mathbf{H}CH_2CH_2$N-), 2.98-3.02 (t, 4H, -CH$_2$**C**$\mathbf{H_2}$N- J=12.1 Hz), 4.10-4.17 (q, 2H, -O**C**$\mathbf{H_2}CH_3$ J=7.5 Hz), 5.51 (s, 1H, -**NH**CH-), 6.38-6.39 (d, 1H, -**C**$\mathbf{H}$=CH-N J= 1.6 Hz), 7.09-7.14 (m, 4H, aromatic J=8.1 Hz), 7.54-7.59 (m, 4H, aromatic J=8.5 Hz), 8.12-8.14 (d, 1H, -CH=**C**$\mathbf{H}$-N J=1.8 Hz).

XII-2; IR (cm^{-1}): 3317, 1673, ^{1}H-NMR ($CDCl_3$, ppm): 0.55-0.57 (q, 2H, -NHCH**C**$\mathbf{H_2}$-), 0.81-0.84 (q, 2H, -NHCH**C**$\mathbf{H_2}$-), 1.23-1.29 (t, 3H, -OCH$_2$**C**$\mathbf{H_3}$), 1.31 (quin, 1H, -NH**C**$\mathbf{H}$-), 1.97-2.18 (q, 4H, -CH**C**$\mathbf{H_2}CH_2$N- J=2.5 Hz), 2.73-2.81 (quin, 1H, -**C**$\mathbf{H}CH_2CH_2$N-), 2.98-3.02 (t, 4H, -CH$_2$**C**$\mathbf{H_2}$N- J=12.1 Hz), 4.10-4.17 (q, 2H, -O**C**$\mathbf{H_2}CH_3$ J=7.5 Hz), 4.99 (d, 1H, -**NH**CH-), 6.38-6.39 (d, 1H, -**C**$\mathbf{H}$=CH-N J=1.6 Hz), 7.09-7.14 (m, 4H, aromatic J=8.1 Hz), 7.54-7.59 (m, 4H, aromatic J=8.5 Hz), 8.12-8.14 (d, 1H, -CH=**C**$\mathbf{H}$-N J=1.8 Hz).

XII-3; IR (cm^{-1}): 3252, 1711, ^{1}H-NMR ($CDCl_3$, ppm): 1.23-1.29 (t, 3H, -OCH$_2$**C**$\mathbf{H_3}$), 1.31 (quin, 1H, -NH**C**$\mathbf{H}$-), 1.47-1.51 (quin, 4H, -NHCHCH$_2$**C**$\mathbf{H_2}$-), 1.62-1,67 (q, 4H, -NHCH**C**$\mathbf{H_2}CH_2$-), 1.97-2.18 (q, 4H, -CH**C**$\mathbf{H_2}CH_2$N- J=2.5 Hz), 2.73-2.81 (quin, 1H, -**C**$\mathbf{H}CH_2CH_2$N-), 2.98-3.02 (t, 4H, -CH$_2$**C**$\mathbf{H_2}$N- J=12.1 Hz), 4.10-4.17 (q, 2H, -O**C**$\mathbf{H_2}CH_3$ J=7.5 Hz), 4.99 (d, 1H, -**NH**CH-), 6.38-6.39 (d, 1H, -**C**$\mathbf{H}$=CH-N J=1.6 Hz), 7.09-7.14 (m, 4H, aromatic J=8.1 Hz), 7.54-7.59 (m, 4H, aromatic J=8.5 Hz), 8.12-8.14 (d, 1H, -CH=**C**$\mathbf{H}$-N J=1.8 Hz).

XII-4; IR (cm^{-1}): 3211, 1677, ^{1}H-NMR ($CDCl_3$, ppm): 1.23-1.29 (t, 3H, -OCH$_2$**C**$\mathbf{H_3}$), 1.46-1.52 (q, 4H, -NHCHCH$_2$**C**$\mathbf{H_2}$-), 1.62-1.65 (quin, 2H, -CH$_2$CH$_2$**C**$\mathbf{H_2}$-), 1.67-1.75 (m, 4H, -NHCH**C**$\mathbf{H_2}$-), 2.03-2.07 (quin, 1H, -NH**C**$\mathbf{H}$-), 1.97-2.18 (q, 4H, -CH**C**$\mathbf{H_2}CH_2$N- J=2.5 Hz), 2.73-2.81 (quin, 1H, -**C**$\mathbf{H}CH_2CH_2$N-), 2.98-3.02 (t, 4H, -CH$_2$**C**$\mathbf{H_2}$N- J=12.1 Hz), 4.10-4.17 (q, 2H, -O**C**$\mathbf{H_2}CH_3$ J=7.5 Hz), 4.99 (d, 1H, -**NH**CH-), 6.38-6.39 (d, 1H, -**C**$\mathbf{H}$=CH-N J=1.6 Hz),

7.09-7.14 (m, 4H, aromatic *J*=8.1 Hz), 7.54-7.59 (m, 4H, aromatic *J*=8.5 Hz), 8.12-8.14 (d, 1H, -CH=**CH**-N *J*=1.8 Hz).

***N*-[4-(4-Fluoro)phenyl-2-piperidin-4-yl-thiazol-5-yl] pyrimidin-2-yl-alkylamine derivatives (XIII-1~XIII-4).** Trimethylsilyliodide (1.27 g, 6.36 mM) was added to a solution of **XII-1** (1.0 g, 2.12 mM), **XII-2** (0.99 g, 2.12 mM), **XII-3** (1.05 g, 2.12 mM), and **XII-4** (1.08 g, 2.12 mM) in chloroform (10 mL) was added trimethylsilyliodide (1.27 g, 6.36 mM). After the addition was completed, the resulting mixture was stirred at 60°C for 4 h. Subsequently, then 10 mL of 6 M HCl in isopropanol and 10 mL of 2M NaOH solution were added to the mixture. The organic phase was then separated, dried (magnesium sulfate), and allowed to evaporate to yield a residue. The residue was re-crystallized from methylene chloride and ether to give **XIII-1** (0.26 g, 32%), **XIII-2** (0.46 g, 55.0%), **XIII-3** (0.52 g, 58.0%), and **XIII-4** (0.57 g, 62.0%) as yellow solids.

XIII-1; IR (cm^{-1}): 3277, 1440, ^{1}H-NMR ($CDCl_3$, ppm): 1.14-1.21 (d, 3H, -NHCH**CH_3**), 1.26-1.28 (d, 3H, -NHCH**CH_3**), 1.31 (septet, 1H, -NH**CH**-), 1.97-2.18 (q, 4H, -CH**CH_2**CH_2N-*J*=2.5 Hz), 2.73-2.81 (quin, 1H, -**CH**CH_2CH_2N-), 2.98-3.02 (t, 4H, -CH_2**CH_2**N- *J*=12.1 Hz), 5.51 (s, 1H, -**NH**CH-), 6.38-6.39 (d, 1H, -**CH**=CH-N *J*=1.6 Hz), 7.09-7.14 (m, 4H, aromatic *J*=8.1 Hz), 7.54-7.59 (m, 4H, aromatic *J*=8.5 Hz), 8.12-8.14 (d, 1H, -CH=**CH**-N *J*=1.8 Hz).

XIII-2; IR (cm^{-1}): 3101, 1413, ^{1}H-NMR ($CDCl_3$, ppm): 0.55-0.57 (q, 2H, -NHCH**CH_2**-), 0.81-0.84 (q, 2H, -NHCH**CH_2**-), 1.31 (quin, 1H, -NH**CH**-), 1.97-2.18 (q, 4H, -CH**CH_2**CH_2N-*J*=2.5 Hz), 2.73-2.81 (quin, 1H, -**CH**CH_2CH_2N-), 2.98-3.02 (t, 4H, -CH_2**CH_2**N- *J*=12.1 Hz), 4.99 (d, 1H, -**NH**CH-), 6.38-6.39 (d, 1H, -**CH**=CH-N *J*=1.6 Hz), 7.09-7.14 (m, 4H, aromatic *J*=8.1 Hz), 7.54-7.59 (m, 4H, aromatic *J*=8.5 Hz), 8.12-8.14 (d, 1H, -CH=**CH**-N *J*=1.8 Hz).

XIII-3; IR (cm^{-1}): 3217, 1401, ^{1}H-NMR ($CDCl_3$, ppm): 1.31 (quin, 1H, -NH**CH**-), 1.47-1.51 (quin, 4H, -NHCHCH$_2$**CH_2**-), 1.62-1.67 (q, 4H, -NHCH**CH_2**CH_2-), 1.97-2.18 (q, 4H, -CH**CH_2**CH_2N-*J*=2.5 Hz), 2.73-2.81 (quin, 1H, -**CH**CH_2CH_2N-), 2.98-3.02 (t, 4H, -CH_2**CH_2**N- *J*=12.1 Hz), 4.99 (d, 1H, -**NH**CH-), 6.38-6.39 (d, 1H, -**CH**=CH-N *J*=1.6 Hz), 7.09-7.14 (m, 4H, aromatic *J*=8.1 Hz), 7.54-7.59 (m, 4H, aromatic *J*=8.5 Hz), 8.12-8.14 (d, 1H, -CH=**CH**-N *J*=1.8 Hz).

XIII-4; IR (cm^{-1}): 3211, 1401, ^{1}H-NMR ($CDCl_3$, ppm): 1.46-1.52 (q, 4H, -NHCHCH$_2$**CH_2**-), 1.62-1.65 (quin, 2H, -CH_2CH_2**CH_2**-), 1.67-1.75 (m, 4H, -NHCH**CH_2**-), 2.03-2.07 (quin, 1H, -NH**CH**-), 1.97-2.18 (q, 4H, -CH**CH_2**CH_2N-*J*=2.5 Hz), 2.73-2.81 (quin, 1H, -**CH**CH_2CH_2N-), 2.98-3.02 (t, 4H, -CH_2**CH_2**N- *J*=12.1 Hz), 4.99 (d, 1H, -**NH**CH-), 6.38-6.39 (d, 1H, -**CH**=CH-N *J*=1.6 Hz), 7.09-7.14 (m, 4H, aromatic *J*=8.1 Hz), 7.54-7.59 (m, 4H, aromatic *J*=8.5 Hz), 8.12-8.14 (d, 1H, -CH=**CH**-N *J*=1.8 Hz).

***N*-[4-(4-Fluoro)phenyl-2-(*N*-methyl)piperidin-4-yl-thiazol-5-yl]pyrimidin-2-yl-alkylamine derivatives (XIV-1~XIV-4).** Formaldehyde (0.13 g, 5.02 mM) and sodium borohydride (0.16 g, 5.02 mM) were added to a solution of **XIII**-1 (1.0 g, 2.51 mM), **XIII**-2 (0.99 g, 2.51 mM), **XIII**-3 (1.06 g, 2.51 mM), and **XIII**-4 (1.09 g, 2.51 mM) in methanol (10 mL) After accumulation, the resulting mixture was stirred at room temperature for 30 min, followed by the addition of 10 mL water and 10 mL ethyl acetate. The organic phase was then separated, dried (magnesium sulfate), and evaporated to yield residue. The residue was re-crystallized from methylene chloride and ether to give **XIV-1** (0.25 g, 25.0%), **XIV-2** (0.53 g, 52%), **XIV-3** (0.52 g, 48.0%), and **XIV-4** (0.48 g, 43.0%) as yellow solids.

XIV-1; IR (cm^{-1}): 3347, 1352, ^{1}H-NMR ($CDCl_3$, ppm): 1.14-1.21 (d, 3H, -NHCH**CH_3**), 1.26-1.28 (d, 3H, -NHCH**CH_3**), 1.31 (septet, 1H, -NH**CH**-), 1.94-1.98 (q, 4H, -**CH_2**CH_2NCH_3 *J*=10.3 Hz), 2.09-2.19 (t, 4H, -CH_2**CH_2**NCH_3 J=12.0 Hz), 2.36 (s, 3H, -N**CH_3**), 2.99-3.04 (quin, 1H, -**CH**CH_2CH_2N-*J*=2.5 Hz), 6.53-6.55 (d, 1H, -**CH**=CH-N *J*=1.5 Hz), 7.08-7.19 (m, 5H, aromatic *J*=7.7 Hz), 7.32-7.34 (m, 4H, aromatic *J*=8.2 Hz), 8.20-8.22 (d, 1H, -CH=**CH**-N *J*=1.5 Hz).

XIV-2; IR (cm^{-1}): 3221, 1242, ^{1}H-NMR ($CDCl_3$, ppm): 0.55-0.57 (q, 2H, -NHCH**CH_2**-), 0.81-0.84 (q, 2H, -NHCH**CH_2**-), 1.31 (quin, 1H, -NH**CH**-), 1.97-2.18 (q, 4H, -CH**CH_2**CH_2N-*J*=2.5 Hz), 2.34 (s, 3H, -N**CH_3**), 2.73-2.81 (quin, 1H, -**CH**CH_2CH_2N-), 2.98-3.02 (t, 4H, -CH_2**CH_2**N- *J*=12.1 Hz), 4.99 (d, 1H, -**NH**CH-), 6.38-6.39 (d, 1H, -**CH**=CH-N *J*=1.6 Hz), 7.09-7.14 (m, 4H, aromatic *J*=8.1 Hz), 7.54-7.59 (m, 4H, aromatic *J*=8.5 Hz), 8.12-8.14 (d, 1H, -CH=**CH**-N *J*=1.8 Hz).

XIV-3; IR (cm^{-1}): 3299, 1277, ^{1}H-NMR ($CDCl_3$, ppm): 1.31 (quin, 1H, -NH**CH**-), 1.47-1.51 (quin, 4H, -NHCHCH$_2$**CH_2**-), 1.62-1,67 (q, 4H, -NHCH**CH_2**CH_2-), 1.97-2.18 (q, 4H, -CH**CH_2**CH_2N- *J*=2.5 Hz), 2.34 (s, 3H, -N**CH_3**), 2.73-2.81 (quin, 1H, -**CH**CH_2CH_2N-), 2.98-3.02 (t, 4H, -CH_2**CH_2**N- *J*=12.1 Hz), 4.99 (d, 1H, -**NH**CH-), 6.38-6.39 (d, 1H, -**CH**=CH-N *J*=1.6 Hz), 7.09-7.14 (m, 4H, aromatic *J*=8.1 Hz), 7.54-7.59 (m, 4H, aromatic *J*=8.5 Hz), 8.12-8.14 (d, 1H, -CH=**CH**-N *J*=1.8 Hz).

XIV-4; IR (cm^{-1}): 3285, 1311, ^{1}H-NMR ($CDCl_3$, ppm): 1.46-1.52 (q, 4H, -NHCHCH$_2$**CH_2**-), 1.62-1.65 (quin, 2H, -CH_2CH_2**CH_2**-), 1.67-1.75 (q, 4H, -NHCH**CH_2**-), 2.03-2.07 (quin, 1H, -NH**CH**-), 1.97-2.18 (q, 4H, -CH**CH_2**CH_2N-*J*=2.5 Hz), 2.31 (s, 3H, -NCH_3), 2.73-2.81 (quin, 1H, -**CH**CH_2CH_2N-), 2.98-3.02 (t, 4H, -CH_2**CH_2**N- *J*=12.1 Hz), 4.99 (d, 1H, -**NH**CH-), 6.38-6.39 (d, 1H, -**CH**=CH-N *J*=1.6 Hz), 7.09-7.14 (m, 4H, aromatic *J*=8.1 Hz), 7.54-7.59 (m, 4H, aromatic *J*=8.5 Hz), 8.12-8.14 (d, 1H, -CH=**CH**-N *J*=1.8 Hz).

Results and Discussion

N-[4-(4-Fluoro)phenyl-2-aminothiazol-5-yl]pyrimidin-2-yl-*N*-(3-hydroxymethyl)phenylamine and *N*-[4-(4-fluoro) phenyl-2-aminothiazol-5-yl]pyrimidin-2-yl-*N*-3-(1-hydroxyethyl) phenylamine are known to have good antifungal activity [Laszlo *et al*., 2004]. *N*-[4-(4-Fluoro)phenyl-2-(*N*-methyl) piperidin-4-yl-thiazol-5-yl]pyrimidin-2-yl-*N*-(3-hydroxymethyl) phenylamine (**I**), which introduced amine of piperidine groups containing *N*-methyl group instead of the 2-aminothiazole of compounds synthesized by the method of Laszlo *et al*. [2004], was shown have good antifungal activity against *P. capsici* [Choi *et al*., 2010]. Table 1 shows fungicidal activity of **I** against *P. capsici*. At 40 mg/L, **I** exerted a strong inhibitory action on the growth of *P. capsici*, inhibiting growth in at least 80% of the growth zone. In the whole plant experiments, the EC_{50} values of was 1.03, whereas that of the commercial fungicide dimethomorph was 4.26 mM (Table 3). **I** demonstrated a potent fungicidal effect on *P. capsici*, and appears suitable for use as a leading compound for further synthesis [Choi *et al*., 2010]. To obtain new synthesized compounds (**VIII-1~VIII-4**, **XII-1~XIV-4**) from **I**, compounds, in which phenylamine of **I** was replaced with *N*-cyclopropyl, *N*-cyclopentyl, *N*-cyclohexyl, and *N*-isopropylamine, were synthesized. In addition, 2-aminothiazole, 2-(*N*-ethoxycarbonyl)piperidin-4-yl-thiazole, and 2-piperidin-4-yl-thiazole were introduced as the leads instead of the 2-(*N*-methyl)piperidin-4-yl-thiazole group of **I**. Structure of the compounds (**VIII-1~VIII-4**, **XII-1~XIV-4**) were confirmed by ^{1}H-NMR spectroscopy. *N*-[4-(4-Fluoro)phenyl-2-(*N*-methyl)piperidin-4-yl-thiazol-5-yl]pyrimidin-2-yl-cyclopropylamine, designated as **XIV-2**, was synthesized. ^{1}H-NMR analysis revealed a peak displayed as a doublet at δ 8.12 and 8.14 corresponding to CH of -CH=**CH**-N, a multiplet at δ 7.09 and 7.59 corresponding to the four hydrogens of a phenyl group, a doublet at δ 6.38 and 6.39 corresponding to CH of **-CH**=CH-N, a doublet at δ 4.99 corresponding to CH of -NH**CH-**, a triplet at δ 2.98 and 3.02 corresponding to CH_2-N of -CH_2**CH_2**NCH_3, a quintet at δ 2.73-2.81 corresponding to CH of **-CH**CH_2-, a singlet at δ 2.34 corresponding to CH_3 of -N**CH_3**, a quartet at δ 1.97 and 2.18 corresponding to CH_2 of **-CH_2**CH_2NCH_3, a quintet at δ 1.31 corresponding to CH of -NH-**CH-**, a quartet at δ 0.55 and 0.84 corresponding to the four hydrogen of a cyclopropyl group. The others were also confirmed using the same method. A series of new compounds that possessed the cycloalkane (**XIV-2** and **-4**) showed potent fungicidal effects on *P. capsici* (Table 1). **XIV-2**, **XIV-3**, and **XIV-4** had EC_{50} values of 1.01, 3.21, and 4.06, respectively

Table 1. Fungicidal activities of *N*-[4-(4-fluoro)phenyl-2-(*N*-methyl)piperidin-4-yl-thiazol-5-yl]pyrimidin-2-yl-(3-hydroxymethyl)phenylamine (I) and *N*-[4-(4-fluoro) phenylthiazol-5-yl]pyrimidin-2-yl-alkylamine derivatives (VIII-1~VIII-4, XII-1~XIV-4) against *P. capsici* obtained from plate culture method

Compounds (40.0 mg/L)	Inhibition Zone (Diameter, mm[a)])
VIII-1	-
VIII-2~VIII-4	+
XII-1~XII-4	-
XIII-1	+
XIII-2	+++
XIII-3	++
XIII-4	++
XIV-1	+
XIV-2	++++
XIV-3	+++
XIV-4	+++
I	+++
Dimethomorph	++

[a)] $p<0.05$

Table 2. Fungicidal activities of *N*-[4-(4-fluoro)phenyl-2-(*N*-methyl)piperidin-4-yl-thiazol-5-yl]pyrimidin-2-yl-(3-hydroxymethyl)phenylamine (I) and *N*-[4-(4-fluoro)phenylthiazol-5-yl]pyrimidin-2-yl-cycloakylamine derivatives (XIII-2, XIV-2~XIV-4) against *P. capsici* obtained from whole plant experiments

Compounds	Inhibition Activity (%)[a)]				
	40.0 mg/L	35.0 mg/L	25.0 mg/L	20.0 mg/L	12.5 mg/L
XIII-2	71.5±0.31	81.5±1.01	63.2±1.55	45.3 ±0.13	15.3±0.02
XIV-2	98.3±0.02	103.1±2.34	98.2±0.11	91.5±0.04	73.2±0.45
XIV-3	95.3±0.01	101.2±2.56	98.1±0.01	88.5±0.01	68.2±0.01
XIV-4	93.2±0.03	98.4±1.33	90.1±0.02	86.7±0.01	63.5±0.01
I	98.1±1.43	105.2±2.11	93.7±2.13	85.5±0.03	59.1±0.05
Dimethomorph	75.4±0.01	83.2±0.03	69.2±0.02	50.2±0.05	17.1±0.12

[a)] $p<0.05$

Values are means±SD of three replicated tests.

Table 3. EC_{50} and EC_{90} values of fungicidal activities of *N*-[4-(4-fluoro)phenyl-2-(*N*-methyl)piperidin-4-yl-thiazol-5-yl]pyrimidin-2-yl-(3-hydroxymethyl)phenylamine (I) and *N*-[4-(4-fluoro)phenylthiazol-5-yl]pyrimidin-2-yl-cycloalkylamine derivatives (XIII-2, XIV-2~XIV-4) against *P. capsici* obtained from whole plant experiments

Compounds	EC_{50} (mM)	EC_{90} (mM)
XIII-2	5.07±0.11	17.87±0.18
XIV-2	1.01±0.11	6.31±0.09
XIV-3	3.21±0.15	9.54±0.07
XIV-4	4.06±0.05	12.37±0.17
I	1.03±0.09	8.26±0.06
Dimethomorph	4.26±0.02	14.72±0.05

$p<0.05$

Values are means±SD of three replicated tests.

(Table 3). These EC_{50} values were much stronger than that of the commercial fungicide dimethomorph (EC_{50} value, 4.26 mM), and there was a pattern in the activity. The structure-activity relationship in compounds **XIV-2**, **XIV-3**, and **XIV-4** were as follows: **XIV-2**, **XIV-3**, and **XIV-4** with *N*-methyl piperidine manifested strong fungicidal activity, and the replacement of the *N*-cycloamine significantly enhanced the fungicidal activity. **XIV-3** and **XIV-4** had also more improved fungicidal activity against *P. capsici* than that of the dimethomorph, but were less active than **I**. These compounds showed a gradual increase in fungicidal activity in the following order: **XIV-4**, **XIV-3**, **I** and **XIV-2** (Tables 2 and 3). Compound **I** showed fungicidal activity on *P. capsici* with the protection rate of 85.5% at 20 mg/mL; this compound had EC_{50} and EC_{90} values of 1.03 and 8.26, respectively. Taken together, all results confirmed our original scheme: thiazole compounds mixed with cycloamine groups on the pyrimidine ring could increase the fungicidal activity. In the present study, all newly synthesized compounds containing cycloamine groups displayed potent fungicidal activity against *P. capsici*. With regard to structure-activity relationship of the newly synthesized compounds **XIV-2** to **XIV-4**, the functional group on the pyrimidine ring of compounds such as cycloamine group may play an important role in the fungicidal activity against *P. capsici*. A dramatic fungicidal effect was found in the compounds that possessed the cyclopropylamine group on the pyrimidine ring designated as **XIV-2.** On the other hand, amine of piperidine groups containing *N*-methyl group among the newly synthesized compounds also showed fungicidal activity on *P. capsici*, and **XIV-2** containing cyclopropylamine group increased fungicidal activities against *P. capsici* at the protection rate of 91.5% and 20 mg/mL. These data may be extremely useful for developing new fungicides from compounds that include a cycloamine ring.

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