Synthesis and biological activity of a novel fungicide, flutianil

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Flutianil was discovered as a novel fungicide chemically characterized as a cyano-methylene thiazolidine. This chemical showed particularly high fungicidal activities against powdery mildew on various crops. Optimization of the fungicidal performance of the series of synthesized chemicals finally led to the identification of flutianil, which could control powdery mildew diseases. In this report, details of the structure–activity relationships from the lead compound to flutianil are described.

Keywords: flutianil, OK-5203, fungicide, structure–activity relationship, cyano-methylene thiazolidine.

Introduction

Powdery mildew is one of the most important crop diseases and is an obligate biotrophic plant pathogen that can only grow and reproduce on living host cells. It causes devastating damage to various crops.1–3 Powdery mildew such as Blumeria graminis and Podosphaera xanthii is considered a high-resistance-risk pathogen by the Fungicide Resistance Action Committee (FRAC)4 because it has many of the characteristics of a high-risk pathogen, such as large population size, airborne spores, short life cycles, and sexual reproduction. Regarding fungicides currently available to control powdery mildew, such as benzimidazoles, 2-amino-pyrimidines, quinone outside inhibitors (QOIs), and demethylation inhibitors (DMIs), it is reported that powdery mildew resistance to these fungicides appears within 2 to 7 years after a launch.5 Therefore, farm producers desire fungicides with novel and different modes of action against powdery mildew compared to the existing powdery mildew fungicides.

To discover a new lead compound to control powdery mildew, we evaluated the biological activity of original compounds in an in-house library, as well as compounds possessing unique chemical structures that were introduced from external libraries using random screening approaches. We found that the compound containing a cyano-methylene thiazolidine ring (Fig. 1) shows good activity against P. xanthii. Because of its excellent fungicidal activity and unique chemical structure, we selected it as a lead compound. Since the lead compound has a novel skeleton in its chemical structure, it was expected to have a novel mode of action. It is of interest to note that several compounds, including the lead compound show fungicidal activity against only powdery mildew (details will be described in a separate paper). Optimization of the fungicidal activity against powdery mildew of a series of compounds finally led to the identification of flutianil. Flutianil has excellent activities against powdery mildew on various vegetables in the field and is registered for fruits, vegetables, and ornamental plants in Japan, Korea, the USA, and the EU. It has been granted reduced-risk status by the US EPA because of its low impact on human health, low lower toxicity to nontarget organisms, low potential for ground-water contamination, low use rates, low pest resistance potential, and compatibility with integrated pest management (IPM) practices when compared to other registered fungicides. Here we describe the discovery and the structure–activity relationship of this cyano-methylene thiazolidine through the evaluation of its control activity against cucumber powdery mildew and wheat powdery mildew.

Materials and Methods

1. General

The chemical structure was confirmed via 1H-NMR spectroscopy using a BRUKER DPX300NMR spectrometer with tetramethylsilane as an internal standard. Melting points were measured with a Büchi Melting Point B-545.

2. Synthesis

All cyano-methylene thiazolidine derivatives were synthesized according to the general procedure described previously.6 We described in more detail with one of the derivative compounds, 2-[2-fluoro-5-(trifluoromethyl)phenylthio]-2-[3-(2-methoxyphenyl)-1,3-thiazolidin-2-ylidene] acetonitrile (2e, flutianil) below as an example. 2-Methoxyphenylisothiocyanate...
1.65 g, 10 mmol) and 2.35 g (10 mmol) of 2-fluoro-5-trifluoromethylphenylthioacetonitrile dissolved in 10 mL of dimethylformamide were added portionwise with stirring at 0°C to a suspension of 0.84 g of sodium hydride (60% in oil, 21 mmol). The mixture was stirred for 1 hr, and 1.87 g (10 mmol) of 1,2-dibromoethane was added dropwise at room temperature followed by stirring for another 3 hr. To the reaction mixture were added 50 mL of water and 30 mL of ether, followed by stirring. The precipitated crystals were collected by filtration, giving 2.67 (105 conidia/mL) of, which had been maintained on plants in a P. xanthii and a temperature of 15°C. For plants were kept in a growth chamber under a 16 hr photoperiod for 7 days. Cucumber seeds (Cucumis sativus L. cv. Chikugoizumi) were sown in 128-cell (59.5 × 30 × 50 mm) plastic trays filled with culture soil (Aisai 1-go, Katakura & Co-Op Agri, Tokyo, Japan) and placed in a greenhouse for 21 days. Each compound was prepared as 4000 mg/L spray solution of 0.84 g (1.65 g) of 2-fluoro-5-trifluoromethylphenylthioacetonitrile and a suspension of 0.84 g of sodium hydride (60% in oil, 21 mmol). The mixture was stirred for 1 hr, and 1.87 g (10 mmol) of 1,2-dibromoethane was added dropwise at room temperature followed by stirring for another 3 hr. To the reaction mixture were added 50 mL of water and 30 mL of ether, followed by stirring. The precipitated crystals were collected by filtration, giving 2.67 g of the title compound as being solid (yield 63%). Melting point 172–173°C. 1H-NMR (δ ppm/CDCl3): 3.1–3.4 (2H, m), 4.0 (3H, s), 4.1–4.2 (1H, m), 4.3–4.4 (1H, m), 7.0–7.2 (3H, m), 7.2–7.6 (4H, m). The other derivatives were synthesized in a similar way.

### Results and Discussion

From external libraries, using random screening approaches, we selected (Z)-2-(3-phenylthiazolidin-2-ylidine)-2-(phenylthio)acetonitrile as a lead compound (Fig. 1) that showed good activity against P. xanthii. To develop a robust pharmacophoric model and understand the basis for the fungidal activity of cyano-methylene thiazolidine, its structure can be subdivided into five key structural moieties: S-phenyl (A), N-phenyl (B), thiazolidine (C), cyano-methylene (D), and sulfide (E) (Fig. 1). Each structural moiety can be modified independently in order to facilitate the systematic refinement of the search for an increasingly effective lead compound. For part A, a phenyl or cyclohexyl group was preferable. For part B, only phenyl was preferable; and for part C, only ethylene (thiazolidine ring) was preferable; for part D, the CN group was preferable; and for part E, S was preferable. This indicated that the structure of the lead compound is the most appropriate.

Regarding the substitution patterns of N-phenyl moiety (Xω, Table S1), only the mono-ortho-chloro analog is active against P. xanthii (EC50 = 19.1 mg/L). Regarding the substitution patterns of the S-phenyl moiety (Yω, Table S2), the introduction of Cl at the 2,5-position (S2f) greatly enhanced the activity against P. xanthii (EC50 = 0.5 mg/L). The 2-Cl (S2a), 3-Cl (S2b), 2,3-diCl (S2d), and 3,5-diCl (S2i) showed moderate fungidal activities against P. xanthii. However, the introduction of Cl at the para-position and additional ortho-position was unfavorable; 4-Cl (S2c), 2,4-diCl (S2e), 2,6-diCl (S2g), and 3,4-diCl (S2h), showed the most significant activity against P. xanthii.
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A wide range of fungicidal activities with the most active analogs containing 5-CF₃, i.e., 1o, 1q, and 1r (EC₅₀ = 1.3, 8.2, and 0.8 mg/L, respectively against *P. xantii* and EC₅₀ = 140.9 mg/L, respectively, against *B. graminis* f. sp. *tritici*), while the compound with 2-OMe-5-CF₃ (1p) was inactive. Compound 1r, containing both 2-F and 5-CF₃ (1r) was the most active against *P. xantii* (EC₅₀ = 0.8 mg/L) and *B. graminis* f. sp. *tritici* (EC₅₀ = 1.3 mg/L). Replacing the CF₃ group with F at the 5-position resulted in a loss of fungicidal activity against *P. xantii* and *B. graminis* f. sp. *tritici* (EC₅₀ > 200 mg/L); 1a vs. 1r, 1h vs. 1o.

Introducing a CF₃ group is known to improve the hydrophobicity of a compound, which appears to contribute positively to the fungicidal activity of the 2,5-disubstituted compound against both *P. xantii* and *B. graminis* f. sp. *tritici*. Replacing F with OMe at the 2-position (1j, 1k, and 1l) resulted in a loss of activity. The introduction of an OMe group at the 2-position was detrimental to the activity except for 1g.

The ortho-position of the N-phenyl moiety was variously substituted as shown in Table 1 (2a–2o), where the S-phenyl moiety was substituted with 2-F-5-CF₃. Among them, the compounds

### Table 1. Fungicidal activity of cyano-methylene thiazolidine derivatives against *Podosphaera xanthii* and *Blumeria graminis* f. sp. *tritici.*

| Compound No. | Xₙ | Yₘ | mp (°C) | Activity EC₅₀ (mg/L) | P. xanthii | B. graminis f. sp. tritici |
|--------------|----|----|--------|----------------------|-----------|----------------------------|
| lead compound | H  | H  | 157–158 | 35.4 | >200 | >200 |
| 1a           | H  | 2,5-diF | 149–150 | >200 | >200 | |
| 1b           | H  | 2,5-diBr | 192–195 | >200 | >200 | |
| 1c           | H  | 2-Me-5-Me | 148–150 | 9.5 | 140.9 | |
| 1d           | H  | 2-Me-5-Cl | 168–169 | 1.8 | >200 | |
| 1e           | H  | 2-Cl-5-Me | 179–180 | 20.2 | >200 | |
| 1f           | H  | 2-Cl-5-OMe | 148–149 | >200 | >200 | |
| 1g           | H  | 2-OMe-5-OMe | 134–136 | 39.0 | 167.5 | |
| 1h           | H  | 2-Me-5-F | 150–151 | 230.7 | >200 | |
| 1i           | H  | 2-F-5-Me | 149–150 | 11.3 | >200 | |
| 1j           | H  | 2-OMe-5-Me | 175–177 | >200 | >200 | |
| 1k           | H  | 2-OMe-5-Cl | 178–180 | >200 | >200 | |
| 1l           | H  | 2-OMe-5-F | 178–180 | >200 | >200 | |
| 1m           | H  | 2-Me-5-Br | 173–179 | >200 | >200 | |
| 1n           | H  | 2-Me-5-t-Bu | 118–119 | 4.9 | >200 | |
| 1o           | H  | 2-Me-5-CF₃ | 148–150 | 1.3 | 32.4 | |
| 1p           | H  | 2-OMe-5-CF₃ | 181–183 | >200 | >200 | |
| 1q           | H  | 2-Cl-5-CF₃ | 160–161 | 8.2 | 54.9 | |
| 1r           | H  | 2-F-5-CF₃ | 157–158 | 0.8 | 1.3 | |
| 2a           | 2-F | 2-F-5-CF₃ | 185–186 | 1.0 | >200 | |
| 2b           | 2-Cl | 2-F-5-CF₃ | 165–166 | 0.8 | >200 | |
| 2c           | 2-Me | 2-F-5-CF₃ | 160–161 | 0.4 | >200 | |
| 2d           | 2-Et | 2-F-5-CF₃ | 153–154 | >200 | >200 | |
| 2e           | 2-OMe | 2-F-5-CF₃ | 172–173 | 0.2 | 1.2 | |
| 2f           | 2-OEt | 2-F-5-CF₃ | 146–147 | >200 | >200 | |
| 2g           | 2-CN | 2-F-5-CF₃ | 185–186 | 5.5 | >200 | |
| 2h           | 2-CF₂ | 2-F-5-CF₃ | 174–175 | 1.6 | >200 | |
| 2i           | 2-OCF₃ | 2-F-5-CF₃ | 149–150 | 11.1 | >200 | |
| 2j           | 2-CO₂Me | 2-F-5-CF₃ | 167–168 | >200 | >200 | |
| 2k           | 2-SMe | 2-F-5-CF₃ | 167–170 | 1.2 | >200 | |
| 2l           | 2-SOMe | 2-F-5-CF₃ | 199–201 | >200 | >200 | |
| 2m           | 2-SO₂Me | 2-F-5-CF₃ | 154–155 | >200 | >200 | |
| 2n           | 2-NO₂ | 2-F-5-CF₃ | 183–184 | >200 | >200 | |
| 2o           | 2-NMe₂ | 2-F-5-CF₃ | 172–173 | 7.9 | >200 | |
with 2-F (2a), 2-Cl (2b), 2-Me (2c), 2-OMe (2e), 2-CF₃ (2h), and 2-SMe (2k) showed good activity against *P. xanithii*. The compounds with 2-CN (2g), 2-OCF₃ (2i), and 2-NMe₂ (2o) were moderately active (EC₅₀ = 5.5, 11.1, and 7.9 mg/L, respectively), but compounds with 2-ethyl (2d), 2-OEt (2f), 2-CO₂Me (2j), 2-SOMe (2l), 2-SO₂Me (2m), and 2-NO₂ (2n) were inactive against *P. xanithii* (EC₅₀ > 200 mg/L). However, most of the compounds with substituents at the 2-position of *N*-phenyl except for OMe (2e) were inactive against *B. graminis f. sp. tritici*. Interestingly, H (1r) and 2-OMe (2e) were very potent against *B. graminis f. sp. tritici*. Through further examination and various field trials, compound 2e was finally selected as a compound for development. The common name of this compound is flutianil.

**Conclusion**

Flutianil was discovered as a new chemical possessing a cyano-methylene thiazolidine structure, which showed high fungicidal activities specifically against powdery mildew at a very low concentration. To investigate a robust pharmacophoric model and understand the basis for the fungicidal activity of cyano-methylene thiazolidine, the structure of the lead compound showed the most activity against *P. xanithii*. The structure–activity relationship of *N*-phenyl suggested that the control activity against *P. xanithii* was affected by the substituent effect at the 2-position and also that the 2-OMe substituent resulted in excellent activity against both *P. xanithii* and *B. graminis f. sp. tritici*. These results on structure–activity relationships through the evaluation of control activity against *P. xanithii* and *B. graminis f. sp. tritici* demonstrate that flutianil is the most effective molecular configuration against powdery mildew.

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**References**

1) K. Amano: “Host Range and Geographical Distribution of the Powdery Mildew Fungi,” Japan Scientific Societies Press, Tokyo, 1986.
2) S. E. Perfect and J. R. Green: *Mol. Plant Pathol.* 2, 101–108 (2001).
3) R. P. Oliver and H. G. Hewitt: “Fungicides in Crop Protection,” 2nd Ed. CAB International, Wallingford, 2014.
4) K. J. Brent and D. W. Hollomon: “Fungicide Resistance: The Assessment of Risk,” Crop Life International, FRAC, Brussels, 2007.
5) H. Ishii and D. W. Hollomon: “Fungicide Resistance in Plant Pathogens In Principles and a Guide to Practical Management,” Springer, Tokyo, 2015.
6) M. Hayashi, Y. Endo and T. Komura (Otsuka Chemical Co., Ltd.); *Jpn. Kokai Tokkyo Koho* JP2000-319270 (2000).