Quantitative Structure-Activity Relationships of Fluazinam and Related Fungicidal N-phenylpyridinamines: Preventive Activity against Sphaerotheca fuliginea, Pyricularia oryzae and Rhizoctonia solani

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Quantitative structure-activity relationships (QSAR) analyses of fungicidal activity of 3-chloro-2-[N-(3-substituted-2, 6-dinitro-4-trifluoromethylphenyl)]amino-5-trifluoromethylpyridines against Sphaerotheca fuliginea, Pyricularia oryzae and Rhizoctonia solani were carried out and the results were compared. In the case of S. fuliginea, a usual QSAR equation with Hammett's electronic parameter (σm) and hydrophobicity (π) was obtained, suggesting that the uncoupling mechanism might be involved in the mode of action. In the cases of P. oryzae and R. solani, QSAR equations were consisted of σm, π and the activity rank against Botrytis cinerea as independent variables, indicating both of uncoupling and SH-inhibition were working in the action mechanism. Molecular orbital calculations clarified that the charge delocalization coupled with structural relaxations of the torsion angles between the C–N–C plane and the benzene and pyridine rings were responsible for strong acidity of fluazinam even in the less polar environment, which was reported as an important feature of shuttle-type uncoupling mechanism. More than one mode of action may be working behind the broad fungicidal spectrum of fluazinam.

INTRODUCTION

Fluazinam [3-chloro-N-(3-chloro-5-trifluoromethyl-2-pyridyl)-α,α,α-trifluoro-2, 6-dinitro-p-toluidine, Frowncide®, IKF-1216] is a preventive fungicide which controls wide variety of plant diseases such as Botrytis cinerea, Phytophthora infestans and so on.1,2) Out previous paper revealed that the suitable reactivity of leaving group at the 3-position on the benzene ring was essential for the fungicidal activity against B. cinerea.3) And it was suggested that inhibition of sulfhydryl or some other nucleophilic functional groups might be involved in the mode of fungicidal action.

On the other hand, very powerful uncoupling activity (C200<10^{-8}mol/l; concentration at which the twice rate of respiration as state 4 was observed) of fluazinam was reported in rat-liver mitochondria system.4) Although some analogues without the chlorine atom at the 3-position showed uncoupling activity,5,6) they did not show activity against B. cinerea.7) However, some of them showed fungicidal activity in the case of Sphaerotheca fuliginea, Pyricularia oryzae and Rhizoctonia solani. Although these fungi were not main commercial targets of fluazinam, these phenomena prompted us to investigate similarity and difference in structure-activity relationships among these biological species. In this paper, quantitative structure-activity relationships (QSAR) at the 3-position of the benzene ring of N-phenylpyridinamines were analyzed using the adaptive least-squares (ALS) method. The results were compared with each other and discussion was made on the possibility that the uncoupling activity of fluazinam might participate in the fungicidal mode of action against these species. Molecular orbital calculations were also carried out to investigate structural basis of fluazinam as a preferable protonophore.

MATERIALS AND METHODS

1. Compounds and Fungicidal Activities

3-Chloro-2-[N-(3-substituted-2, 6-dinitro-4-trifluoromethylphenyl)]amino-5-trifluoromethylpyridines were prepared in the previous study.3,4) An aqueous solution of wettable powder containing 20% of the compound at appropriate concentrations was sprayed over the rice (3–5 leaf stage, cv. Chukyoasahi) or cucumber (1 leaf stage, cv. Suyo) seedlings. Twenty-four hours later, the rice plants were inoculated by a spore suspension of P. oryzae containing 5×10^5 spores/ml on the leaves (rice blast) or by rice straws previously incubated with R. solani between leaf sheath portions.
After inoculation the plants were maintained at 25°C (rice blast) or 30°C (rice sheath blight) for 5 days in a moist chamber. The cucumber plants were inoculated by dusting spores of *S. fuliginea* (cucumber powdery mildew) and incubated at 25°C for 10 days. As a degree of disease control, visually assessed area, number or length of lesions in a leaf was used for *S. fuliginea*, *P. oryzae* or *R. solani*, respectively. Each compound was tested at the concentration of 500, 250, 63 and 16 ppm. According to the concentration at which the test solution showed over 90% control of disease, the ranking of fungicidal activity was obtained (Table 1).

### Table 1: Fungicidal activities and ranking.

<table>
<thead>
<tr>
<th>Rank</th>
<th>Concentration (ppm)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&gt; 500</td>
<td>Inactive</td>
</tr>
<tr>
<td>2</td>
<td>250–500</td>
<td>Active</td>
</tr>
<tr>
<td>3</td>
<td>63–250</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>16–63</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>&lt; 16</td>
<td>Highly active</td>
</tr>
</tbody>
</table>

*a* Activity ranks used in QSAR study.

*b* The concentration (ppm) at which the compound showed over 90% control of disease.

2. Substituent Parameters and QSAR Method

Hammett’s electronic parameter ($\sigma_m$), molecular refractivity (MR) and hydrophobicity ($\pi$) were used as physicochemical parameters. The values of these parameters were taken from literatures[^6^, ^7^] or estimated by the method described in the previous paper.[^3^]

QSAR analyses were carried out by using the ALS method.[^8^] Leave-one-out prediction was used as a criterion for the reliability of the equations.

3. Molecular Orbital Calculations

Heats seen in the dissociation of several chemical species in some solvents was calculated by semi-empirical molecular orbital method with AM1 Hamiltonian.[^9^]

Since $H^+$ (naked proton) was unstable and never observed in any aqueous solution, $H_2O^+$ was calculated as counter cation.

$$AH(solv) + H_2O(solv) = A^-(solv) + H_2O^+(solv)$$

(solv = vacuum, octanol, 50% ethanol/water or water)

$H_2O$ and $H_2O^+$ do not exist in real vacuum or bulk octanol. Therefore as for such solvents, the discussion in this study was limited to the relative acidity of the compounds.

The stable conformations of fluazinam and its analogues in vacuum were calculated in the previous study.[^3^]

Eliminating the acidic protons, the initial conformations for the dissociated forms were obtained. Initial coordinates for other chemical species such as $H_2O^+$, DNOC (2, 4-dinitro-6-methylphenol) and so on were prepared on the graphic display using standard bond lengths and angles. All internal coordinates were optimized during energy minimization using semi-empirical molecular orbital calculation (AM1 method). Solvent effects were accounted by conductor-like screening model (COSMO) calculation[^10^, ^11^] of MOPAC 93.[^11^] Dielectric constant was set to 10, 52 and 78 as for octanol, 50% ethanol water (v/v) and water, respectively.

The equation to estimate $\Delta G_{\text{dissoc}}$ values from observed $pK_a$ values was obtained as follows:

$$pK_a = -\log_{10} \frac{[A^-][H_3O^+]}{[AH]}$$

$$\exp \left(-\frac{\Delta G_{\text{dissoc}}}{RT}\right) = \frac{[A^-][H_3O^+]}{[AH][H_2O]}$$

Since $RT$ was equal to 0.59 kcal/mol in 298 K, the following equation was obtained.

$$\Delta G_{\text{dissoc}} = 1.36(pK_a + \log [H_2O])$$

The approximated value of $[H_2O]$ was equal to 1000/18 or 1000/18/2 mol/l in water or 50% ethanol/water, respectively.

### RESULTS AND DISCUSSIONS

1. QSAR Analyses of Fungicidal Activities against *S. fuliginea*, *P. oryzae* and *R. solani*

Fungicidal activities of 3-chloro-2-[N-(3-substituted-2, 6-dinitro-4-trifluoromethylphenyl)] amino-5-trifluoromethylpyridines were listed in Table 2. Biological activity-activity relationships were summarized in Table 3. Tables 2 and 3 clearly showed that structure-activity relationships varied from fungus to fungus.

Structure-activity relationships of fungicidal activity against *B. cinerea* were discussed previously.[^3^] A moderately reactive leaving group such as chlorine or bromine was essential for the activity. In addition, substituent effects other than the 3-position on the benzene ring were also understood by the reactivity of the leaving group at the 3-position. Recently, the inhibition of an SH-containing enzyme by fluazinam was observed, which seemed to be responsible for structure-activity relationships of *B. cinerea* (Mitani et al., unpublished work).

On the contrary, chlorine or bromine was not essential in the case of *S. fuliginea*, *P. oryzae* and *R. solani* as shown in Table 2. Therefore it is likely that the mechanism other than SH-inhibition is working in the mode of action. At first, QSAR equations for fungicidal activity against *S. fuliginea* were examined. The best result was:

$$L(SF) = 1.06 \sigma_m + 0.095 \pi - 1.13 \pi^2 + 3.26$$

$$N = 13, R_s = 0.894, N_{mis} = 5(0), \epsilon = 0.699,$$

leave-one-out $R_s = 0.763, N_{mis} = 6(1)$

In above equation, $L(SF)$, $R_s$, $N_{mis}$ and $\epsilon$ represent...
activity ranks against *S. fuliginea*, Spearman’s correlation coefficient, number of mis-assignment and dispersion of the error, respectively. A contribution factor which is the product of the coefficient and the standard deviation is noted just below the coefficient of each term.

In contrast to the case of *B. cinerea*, satisfactory QSAR equation was obtained using usual physicochemical parameters in this case. The above result suggests that some fungicidal mode of action other than SH-inhibition is dominant. It is not possible to determine the mode of action from this equation only. However, some speculation can be made. Guo *et al.* reported the QSAR study of N-phenylpyridinamines and diphenylamines about uncoupling activity in the rat liver mitochondria. In that case, the QSAR equations consisted of log *P* and p*K*a terms, which were explained to reflect the partition to the inner membrane of mitochondria and the stability of anionic form in the membrane, both effects being important in the shuttle-type mechanism. In addition, the log *P* and p*K*a in the liposomal system, which was the model of biological membrane, were superior descriptor than the log *P* in octanol/water and p*K*a in water. Thus if σm and π terms in Eq. (4) are assumed to be corresponding to p*K*a and log *P*m terms, the Eq. (4) may reflect shuttle-type mechanism of fluazinam in its fungicidal action against *S. fuliginea*. The correlation between p*K*a and σm is discussed in the following section.

In the cases of *P. oryzae* and *R. solani*, the following equations were selected:

\[
L(PO) = 3.338 \sigma_m + 0.0152 \pi - 1.26 \pi^2 + 3.94 \\
(0.101) (0.013) (-1.047)
\]

\[N = 13, R_s = 0.799, N_{mis} = 4(1), \varepsilon = 1.614, \text{ leave-one-out } R_s = 0.564, N_{mis} = 8(2)\]

\[
L(RS) = 0.248 \sigma_m + 0.554 \pi - 1.06 \pi^2 + 3.58 \\
(0.008) (0.469) (-0.879)
\]

\[N = 13, R_s = 0.721, N_{mis} = 5(2), \varepsilon = 1.280, \text{ leave-one-out } R_s = 0.225, N_{mis} = 8(5)\]

In above equations, L(PO) and L(RS) represent activity ranks against *P. oryzae* and *R. solani*, respectively.

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### Table 2 Structure-activity relationships of 3-chloro-2-[N-(3-substituted-2, 6-dinitro-4-trifluoromethylphenyl)] amino-5-trifluoromethylpyridines.

<table>
<thead>
<tr>
<th>Compds.</th>
<th>PO&lt;sup&gt;a&lt;/sup&gt;</th>
<th>RS&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>Obs.</td>
<td>Obs.</td>
</tr>
<tr>
<td>Cl</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Br</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>I</td>
<td>1</td>
<td>2(3)</td>
</tr>
<tr>
<td>NH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>NN&lt;sub&gt;2&lt;/sub&gt;</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>OClH&lt;sub&gt;5&lt;/sub&gt;</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>OC&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;11&lt;/sub&gt;</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>OMeH&lt;sub&gt;7&lt;/sub&gt;</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>OMeH&lt;sub&gt;8&lt;/sub&gt;</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>OCH&lt;sub&gt;3&lt;/sub&gt;CF&lt;sub&gt;3&lt;/sub&gt;</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>SCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>SOCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

<sup>a</sup> Fungicidal activity rank against *B. cinerea* (Ref. 3)).
<sup>b</sup> Fungicidal activity rank against *S. fuliginea*.
<sup>c</sup> Fungicidal activity rank against *P. oryzae*.
<sup>d</sup> Fungicidal activity rank against *R. solani*.
<sup>e</sup> Hammett's electronic parameter.
<sup>f</sup> Hydrophobicity.
<sup>g</sup> Molecular refractivity.

<sup>b</sup> Values in the parentheses are the results of leave-one-out prediction.

### Table 3 Correlation among fungicidal activities.

<table>
<thead>
<tr>
<th></th>
<th>PO&lt;sup&gt;a&lt;/sup&gt;</th>
<th>RS&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
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<tbody>
<tr>
<td>BC&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.234</td>
<td>0.368</td>
</tr>
<tr>
<td>SF</td>
<td>0.483</td>
<td>0.627</td>
</tr>
<tr>
<td>PO</td>
<td>0.492</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Fungicidal activity rank against *S. fuliginea*.
<sup>b</sup> Fungicidal activity rank against *P. oryzae*.
<sup>c</sup> Fungicidal activity rank against *R. solani*.
<sup>d</sup> Fungicidal activity rank against *B. cinerea* (Ref. 3)).
Correlations of above equations were not satisfactory. However, when the compounds with a chlorine or a bromine were discarded, satisfactory correlations were obtained using usual physicochemical parameters ($R_s = 0.889$ or $0.859$ for $P.$ oryzae or $R.$ solani, respectively, using $\sigma_m$, $\sigma_m^2$, and $\pi$. The same parameters as those in Eqs. (4)-(6) gave rather poor correlations). Predicted activities of compounds with a chlorine or a bromine were lower than observed values by two or three ranks. Since only these compounds showed activity in the case of $B.$ cinerea, the underestimation of fungicidal activity of these compounds against $P.$ oryzae or $R.$ solani might be a result of neglect of the factor which was dominant in the case of $B.$ cinerea. Thus we examined QSAR equations which contained physicochemical parameters together with fungicidal activity against $B.$ cinerea as independent variables. The best results were as follows:

$$L(PO) = 25.53 \sigma_m - 66.24 \sigma_m^2 - 2.54 \pi$$
$$\quad + 0.709 L(BC) + 2.82$$
$$N = 13, R_s = 0.906, N_{mis} = 3(1), \varepsilon = 1.248,$$
$$\text{leave-one-out } R_s = 0.762, N_{mis} = 5(2)$$

$$L(RS) = 14.15 \sigma_m - 44.02 \sigma_m^2 - 1.21 \pi$$
$$\quad + 0.547 L(BC) + 2.69$$
$$N = 13, R_s = 0.874, N_{mis} = 5(0), \varepsilon = 0.876,$$
$$\text{leave-one-out } R_s = 0.513, N_{mis} = 7(4)$$

QSAR equations were substantially improved by inclusion of the $L(BC)$ term. Both of Eqs. (7) and (8) were consisted of $\sigma_m$, $\sigma_m^2$, $\pi$ and $L(BC)$ terms. The first three terms may indicate that the uncoupling mechanism assumed in the case of $S.$ fuliginea may be also working in these cases, while the $L(BC)$ term may reflect the SH-inhibition mechanism similar to the case of $B.$ cinerea.

In the case of $S.$ fuliginea, adding $L(BC)$ term to Eq.

(4) resulted worse correlation in leave-one-out prediction. Thus, the SH-inhibition was not detected in this case.

2. Molecular Calculation of Proton-associated and Dissociated Forms

The discussion in the previous section presumed that the $\sigma_m$ and $\sigma_m^2$ terms in Eqs. (4)-(8) reflect proton dissociation in the uncoupling mechanism. This presumption was reasonable since correlations between $pK_a$ and $\sigma$ were reported in many cases. In this section, quantum chemical calculation was carried out to investigate molecular process of proton dissociation further, employing the AM1-COSMO method. In this method of calculation, effect of each solvent is taken into account through dielectric constant ($\varepsilon$) which stabilizes partial charge distribution of the solute molecule. Therefore specific interactions between the solute and the solvent in the solvation shell are neglected.

At first, the enthalpy of dissociation of fluazinam in water and 50% ethanol/water were calculated, while free energy changes were obtained from observed $pK_a$ values in these conditions. From the calculated $\Delta H_{dissoc}$ values and the observed $\Delta G_{dissoc}$ values, the entropic changes were estimated. These results were listed in Table 4. The results were rather satisfactory since the $\Delta S_{dissoc}$ values were almost same magnitude as observed values of $\Delta S_{dissoc}$ of representative weak acid (e.g. $\Delta S_{dissoc} = -21.9$ or $-18.7$ cal/K/mol for acetic acid or benzoic acid, respectively).

The calculated heat of deprotonation in octanol for fluazinam and its analogues were listed in Table 5, together with $\sigma_m$ values of substituents at the 3-position of the benzene ring. Proton dissociation in octanol (i.e. continuous medium of $\varepsilon = 10$ in this study) was interesting because it would reflect the stability of anionic form of the shuttle-type uncoupler in the biological membrane. The regression analysis gave the following excellent correlation:

### Table 4 Calculated and observed thermodynamic parameters of dissociation of fluazinam in 50% ethanol/water and water.

<table>
<thead>
<tr>
<th>Solv.</th>
<th>$\varepsilon$</th>
<th>$\Delta H_{dissoc}$</th>
<th>$pK_a$</th>
<th>$\Delta G_{dissoc}$</th>
<th>$\Delta S_{dissoc}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a)</td>
<td>(b)</td>
<td>b)</td>
<td>(c)</td>
<td>(d)</td>
</tr>
<tr>
<td>50% EtOH</td>
<td>52</td>
<td>7.74</td>
<td>7.22</td>
<td>11.62</td>
<td>-13.02</td>
</tr>
<tr>
<td>Water</td>
<td>78</td>
<td>6.38</td>
<td>7.11</td>
<td>11.76</td>
<td>-13.49</td>
</tr>
</tbody>
</table>

a) Dielectric constant used in the AM1-COSMO calculation.
b) Calculated by the AM1-COSMO method.
c) Estimated by Eq. (3).
d) $\Delta S_{dissoc} = (\Delta H_{dissoc} - \Delta G_{dissoc})/T$
e) Ref. 2.
f) Ref. 13.
g) Ref. 14.
Thus assuming that the entropic changes ($\Delta S_{\text{dissoc}}$) were almost same for the compounds in this study or assuming that $\Delta S_{\text{dissoc}}$ changed in a parallel manner as $\Delta H_{\text{dissoc}}$, it was understandable that $\sigma_m$ and $\sigma_m^2$ terms in Eqs. (4)-(8) reflect proton dissociation in the uncoupling mechanism.

As mentioned above, uncoupling activity of fluazinam in the rat-liver mitochondria system was markedly strong ($C_{200} < 10^{-8}$ mol/l). To clarify structural basis for such a preferable protonophore, the three-dimensional structures, electronic structures and heat of formation of neutral and anionic forms of fluazinam in vacuum, octanol and water were calculated together with DNOC, a representative uncoupler with dinitrophenol skeleton. The results were summarized in Table 6 and Fig. 1. In Table 6, the torsion angles between the C–N–C plane and the benzene and pyridine rings, and Mulliken charges were also listed. Although the estimated acidity of fluazinam is almost as same as that of DNOC in water, the former is more acidic than the latter in octanol.

Table 6 Results of molecular orbital calculation for neutral and anionic forms of fluazinam and DNOC.

<table>
<thead>
<tr>
<th>Form</th>
<th>$\Delta H_{\text{dissoc}}$ (kcal/mol)</th>
<th>$\theta_1$ (deg)</th>
<th>$\theta_2$ (deg)</th>
<th>$\theta_{\text{N/H}}$</th>
<th>$\theta_{\text{P}}$</th>
<th>$\Delta Q_{\text{HN}}$</th>
<th>$\Delta Q_{\text{OH}}$</th>
<th>$\Delta Q_{\text{O}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vacuum</td>
<td>131.29</td>
<td>47.4</td>
<td>32.3</td>
<td>0.01</td>
<td>0.04</td>
<td>-0.06</td>
<td>0.14</td>
<td>0.36</td>
</tr>
<tr>
<td>Anionic</td>
<td>84.5</td>
<td>-0.2</td>
<td>-0.29</td>
<td>-0.10</td>
<td>-0.59</td>
<td>0.31</td>
<td>-0.36</td>
<td>0.41</td>
</tr>
<tr>
<td>Octanol</td>
<td>22.39</td>
<td>46.8</td>
<td>32.4</td>
<td>0.03</td>
<td>0.03</td>
<td>-0.06</td>
<td>23.72</td>
<td>0.05</td>
</tr>
<tr>
<td>Anionic</td>
<td>84.5</td>
<td>-0.2</td>
<td>-0.35</td>
<td>-0.07</td>
<td>-0.57</td>
<td>0.38</td>
<td>-0.47</td>
<td>0.52</td>
</tr>
<tr>
<td>Water</td>
<td>6.38</td>
<td>47.0</td>
<td>32.3</td>
<td>0.03</td>
<td>0.03</td>
<td>-0.06</td>
<td>6.59</td>
<td>0.05</td>
</tr>
<tr>
<td>Anionic</td>
<td>81.9</td>
<td>0.2</td>
<td>-0.35</td>
<td>-0.06</td>
<td>-0.57</td>
<td>0.39</td>
<td>-0.49</td>
<td>0.54</td>
</tr>
</tbody>
</table>

- $\Delta H_{\text{dissoc}}$: Heat of dissociation calculated by the AM1-COSMO method.
- $\theta_1$: Torsion angle between the amino group and the pyridine ring. $\theta_2$: Torsion angle between the amino group and the benzene ring.
- $\theta_{\text{N/H}}$: Torsion angle between the amino group and the pyridine ring.
- $\Delta Q_{\text{HN}}$: Summation of Mulliken charges of atoms in the amino group.
- $\Delta Q_{\text{OH}}$: Summation of Mulliken charges of atoms in the hydroxy group.
- $\Delta Q_{\text{O}}$: Summation of Mulliken charges of atoms on the benzene moiety.
- $\Delta Q_{\text{O}} = Q_{\text{OH}}$ (neutral) $- Q_{\text{OH}}$ (anion).

$\Delta H_{\text{dissoc}} = -8.72(\pm 1.68)\sigma_m + 25.25(\pm 0.46)$

$N = 13$, $R = 0.960$, $S = 0.482$, $F(1, 11) = 128.8$
Since uncouplers are believed to deprotonate in the inner membrane of mitochondria in the shuttle-type mechanism, the strong acidity in the less polar environment plays an important role in uncoupling activity of fluazinam. Figure 1 illustrates the structural change by dissociation of the proton. The torsion angle between the amino group and the benzene ring and that between the amino group and the pyridine ring change as the proton dissociates, enabling delocalization of the partial charge on the nitrogen. These structural relaxations are essentially independent on the polarity of the solvent (Table 6). Thus the charge delocalization coupled with structural relaxation make acidity of fluazinam high even in the less polar environment. Although the electronegativity of the oxygen is partially responsible for high acidity of DNOC in water, the acidity of DNOC is more sensitive to the dielectric constant of the solvent, since charge delocalization is not so large as in the case of fluazinam.

Besides the factors discussed above, the specific interactions in the solvent shell reported by Guo et al.\(^\text{16}\) may be working behind the uncoupling activity of fluazinam.

### Table 7 Mode of fungicidal actions of fluazinam.

<table>
<thead>
<tr>
<th>Mode</th>
<th>B. cinerea(^a)</th>
<th>S. fuliginea</th>
<th>P. oryzae</th>
<th>R. solani</th>
<th>In vitro</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncoupling</td>
<td>-(^b)</td>
<td>+(^c)</td>
<td>+(^d)</td>
<td>+(^e)</td>
<td>+(^f)</td>
</tr>
<tr>
<td>SH-inhibition</td>
<td>+(^g)</td>
<td>-(^h)</td>
<td>+(^i)</td>
<td>+(^j)</td>
<td>+(^k)</td>
</tr>
</tbody>
</table>

\(^a\) Ref. 3).
\(^b\) Not detected in QSAR study.
\(^c\) Suggested in QSAR study.
\(^d\) Observed in rat-liver mitochondria (Ref. 4)).
\(^e\) Mitani et al., unpublished work.

### CONCLUSION

Until now, the studies in vitro system revealed the potential of fluazinam as an SH-inhibitor (Mitani et al., unpublished work) and an uncoupler.\(^4\) The former possibility was already suggested in the previous study in the case of B. cinerea before in vitro experiments were implemented. The latter mechanism was indicated in the present study in the cases of S. fuliginea, P. oryzae and R. solani (Table 7). Molecular calculation in the previous study revealed that the chemical reactivity of fluazinam was suitable as an SH-inhibitor, while in the present study, the charge delocalization coupled with structural relaxation was demonstrated as structural basis for a good uncoupling activity.

It is likely that more than one mode of action are working behind the broad spectrum of fluazinam.

### REFERENCES

フルアツチンおよびその類縁体の定量的構造活性相関：キュウリうどんこ病、イネいちご病およびイネ紋枯病菌に対する予防活性

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フルアツチン [3-chloro-N-(3-chloro-5-trifluoromethyl-2-pyridyl)-a,a,a-trifluoro-2,6-dinitro-p-toluidine, Frowncide(R), IKF-1216] およびその類縁体のうどんこ病，いちご病および紋枯病に対する防除活性についての QSAR 解析（ALS 法）を行ない，灰色かび病での結果（前報）と比較した。灰色かび病の場合と異なり，うどんこ病ではベンゼン環 3 位の置換基効果は電子的，および疎水的パラメータで説明された。作用メカニズムの可能性として，ラット肝ミトコンドリアで報告されている uncoupling 活性などが考えられる。一方，いちご病，紋枯病の場合には電子的，および疎水的パラメータとともに灰色かび病でも見いだされた特異的な効果を考慮することにより良好な結果が得られた。これらの薬では二つの作用メカニズムが働いている可能性が示唆された。溶媒効果を考慮した分子軌道計算（AM1-COSMO 法）の結果，フルアツチンではプロトンの解離によって，構造緩和と電荷の非局在化が起こることによって非極性環境下でも解離しやすくなっていることが確認された。