アリール（4-置換ピリジン-3-イル）メチルカーバメート類の合成と除草活性

<table>
<thead>
<tr>
<th>項目</th>
<th>内容</th>
</tr>
</thead>
<tbody>
<tr>
<td>誌名</td>
<td>Journal of pesticide science</td>
</tr>
<tr>
<td>ISSN</td>
<td>1348589X</td>
</tr>
<tr>
<td>巻/号</td>
<td>371</td>
</tr>
<tr>
<td>掲載ページ</td>
<td>p. 45-55和文抄録（p.102）</td>
</tr>
<tr>
<td>発行年月</td>
<td>2012年2月</td>
</tr>
</tbody>
</table>

農林水産省 農林水産技術会議事務局筑波事務所
Tsukuba Office, Agriculture, Forestry and Fisheries Research Council Secretariat
Synthesis of novel aryl(4-substituted pyridin-3-yl)methyl carbamates and their herbicidal activity

Hitoshi NAKAYAMA,†,‡‡,* Tohru KOYANAGI,† Hiroshi KIKUGAWA,† Makiko SANO,† Ken OHNO,† Kuni FUSHIKIDA† and Jun'ichi UENISHI††

† Ishihara Sangyo Kaisha, Ltd., 2-3-1 Nishi-shibukawa, Kusatsu 525-0025, Japan
†† Department of Pharmaceutical Chemistry, Kyoto Pharmaceutical University, Misasagi, Yamashina, Kyoto 607-8412, Japan

(Received September 11, 2011; Accepted October 20, 2011)

A certain number of aryl(4-substituted pyridin-3-yl)methyl carbamates and related compounds were synthesized, and their herbicidal activities against weeds and phytotoxicity against transplanted rice were evaluated. The herbicidal efficacy varied with the structures of the aryl group, carbamoyl group, and substituent on the 4-position of the pyridine ring. It was revealed that the combination of the 4-(trifluoromethyl)pyridin-3-yl group and the naphthalen-1-yl group is favorable to achieve superior herbicidal activity without showing phytotoxicity in transplanted rice. Among these analogs, naphthalen-1-yl[4-(trifluoromethyl)pyridin-3-yl]methyl N,N-dimethylcarbamate 2a showed an excellent herbicidal efficacy as a new candidate for paddy rice herbicide. A (-)-enantiomer of 2a exhibited higher activity than its (+)-enantiomer. The mechanism of herbicidal action would be considered to be inhibition of obtusifoliol 14α-methyl demethylase P450.

Keywords: 4-(trifluoromethyl)pyridine; naphthalen-1-yl; carbamate; herbicidal activity; paddy field; CYP51.

Introduction

Cytochrome-P450-dependent 14α-methyl demethylase (DM) is known to convert obtusifoliol to phytosterols in plants. Inhibitors of this pathway deprive organisms of important sterols needed for cell membranes, making them potential herbicide candidates. In fact, some DM inhibitors have been reported to possess herbicidal activity. However, unfortunately, none of them has been commercialized yet. We have been interested in the development of new DM inhibitors for herbicides with high potential and wide spectrum that may reduce the risk of herbicide cross resistance.

Although sulfonylurea herbicides, such as bensulfuron-methyl, pyrazosulfuron-ethyl, and imazosulfuron, have been widely used to regulate the growth of weeds in paddy fields, their herbicidal activities are generally inadequate against barnyard grasses of the Echinochloa species. To overcome this disadvantage, many efforts have been made to develop mixed herbicides with agents that are active against Echinochloa species. In 1997, Chin et al. reported that bis[4-(trifluoromethyl)pyridin-3-yl]methyl N,N-dimethylcarbamate 1 (Fig. 1) and its derivatives exhibit potent activity against the Echinochloa species as well as some other broadleaf weeds.

Later, Bargar et al. reported that the activity of 1 is due to inhibition of DM. However, no selective herbicidal activity between transplanted rice and weeds was found in 1 in the paddy field based on our own investigation. Therefore, we have attempted a structural modification of 1 to design new analogs possessing a better selective profile with a wider herbicidal spectrum against weeds and less phytotoxicity to transplanted rice in paddy fields.

In this paper, we describe the synthesis of aryl(4-substituted pyridin-3-yl)methyl carbamates 2-10 and their biological evaluation including herbicidal activity and phytotoxicity to transplanted rice.

Materials and Methods

1. Synthesis and instrumental analysis

Melting points were determined on a Yanagimoto micro-melt-
To a stirred solution of diisopropylamine (3.3 g, 33 mmol) in dry THF (60 mL) was added 1.56 mol/L hexane solution of n-ButLi (20.2 mL, 31.5 mmol) dropwise at −40°C, and the mixture was stirred at −15°C for 1 hr. Then, 2-chloro-4-(trifluoromethyl)pyridine (5.5 g, 30 mmol) was added to the mixture at −50°C, and the stirring was continued for 1 hr at the same temperature. After the reaction mixture was cooled to −78°C, a corresponding aldehyde (30 mmol) was added dropwise. After the mixture was stirred for an additional 2 hr at the same temperature, the reaction mixture was quenched with saturated aqueous NH₄Cl (100 mL). The mixture was condensed in vacuo and extracted with EtOAc (100 mL×2). The combined extract was washed with brine and dried over MgSO₄. The crude product was purified by column chromatography on silica gel to afford 11a.

1.1.1. 2-Chloro-4-(trifluoromethyl)pyridin-3-yl(phenyl)methanol (11a)

Compound 11a was obtained from 1-naphthaldehyde in 79% yield. White solid, mp 74–77°C. Rᵢ = 0.26 (20% EtOAc in hexane). ¹H NMR δₜ: 2.99 (1H, bs, OH), 6.89 (1H, d, J = 7.6 Hz, CH-O), 6.97 (1H, m, naphthalene 2-H), 7.33 (1H, t, J = 7.6 Hz, ArH), 7.51–7.63 (3H, m, ArH), 7.84 (1H, d, J = 8.4 Hz, ArH), 7.89 (1H, m, ArH), 8.38 (1H, d, J = 8.4 Hz, ArH), 8.43 (1H, d, J = 5.2 Hz, pyridine 6-H). EI-MS m/z 337 (M⁺). EI-HRMS m/z (M⁺): Calcd. for C₁₆H₁₄ClF₃N₂O₃: 364.9833, Found: 364.9830.

1.1.2. 5-Chloro-1,3-benzodioxol-4-yl[2-chloro-4-(trifluoromethyl)pyridin-3-yl]methanol (11c)

Compound 11c was obtained from 6-chloro-2,3- (methyleneoxy)benzaldehyde in 85% yield. White powder, mp 113–115°C. Rᵢ = 0.38 (50% EtOAc in hexane). ¹H NMR δₜ: 3.69 (1H, d, J = 7.6 Hz, OH), 5.80 (2H, dd, J = 7.8 and 1.4 Hz, CH₂-O), 6.53 (1H, d, J = 7.1 Hz, Ar-H), 6.73 (1H, d, J = 8.3 Hz, Ar-H), 6.91 (1H, d, J = 8.3 Hz, CH-O), 7.58 (1H, d, J = 5.0 Hz, pyridine 5-H), 8.52 (1H, d, J = 5.0 Hz, pyridine 6-H). EI-MS m/z 365 (M⁺). EI-HRMS m/z (M⁺): Calcd. for C₁₇H₁₄ClF₃N₂O₃: 364.9833, Found: 364.9830.

1.1.3. Acenaphthen-5-yl[2-chloro-4-(trifluoromethyl)pyridin-3-yl]methanol (11d)

Compound 11d was obtained from 5-acenaphthenecarboxaldehyde in 37% yield. Pale yellow solid, mp 166–173°C. Rᵢ = 0.39 (33% EtOAc in hexane). ¹H NMR δₜ: 3.27 (1H, d, J = 10.0 Hz, OH) 3.29–3.37 (4H, m, CH₂-CH₂), 6.77 (1H, d, J = 6.8 Hz, acenaphthene 4-H), 6.92 (1H, d, J = 10.0 Hz, CH-O), 7.09 (1H, d, J = 7.2 Hz, acenaphthene 8-H), 7.35 (1H, d, J = 6.8 Hz, acenaphthene 3-H), 7.55 (1H, dd, J = 8.4 and 7.2 Hz, acenaphthene 7-H), 7.65 (1H, d, J = 5.2 Hz, pyridine 5-H), 8.10 (1H, d, J = 8.4 Hz, acenaphthene 6-H), 8.60 (1H, d, J = 5.2 Hz, pyridine 6-H). EI-MS m/z 363 (M⁺). EI-HRMS m/z (M⁺): Calcd. for C₁₇H₁₄ClF₃N₂O₃: 363.0638, Found: 363.0645.

1.1.4. 2-Chloro-4-(trifluoromethyl)pyridin-3-yl(quinolin-4-yl)methanol (11e)

Compound 11e was obtained from 4-quinolinecarboxaldehyde in 16% yield. Amorphous solid. Rᵢ = 0.18 (50% EtOAc in hexane). ¹H NMR δₜ: 3.95 (1H, bs, OH), 6.97 (1H, s, CH-O), 7.02 (1H, d, J = 4.8 Hz, quinoline 3-H), 7.61 (1H, m, ArH), 7.69 (1H, d, J = 5.2 Hz, pyridine 5-H), 7.76 (1H, m, ArH), 8.21 (1H, d, J = 8.4 Hz, ArH), 8.27 (1H, d, J = 8.4 Hz, ArH), 8.64 (1H, d, J = 5.2 Hz, pyridine 6-H), 8.79 (1H, d, J = 4.8 Hz, quinoline 2-H). EI-MS m/z 338 (M⁺). EI-HRMS m/z (M⁺): Calcd. for C₂₀H₁₅ClF₃N₂O: 338.0434, Found: 338.0441.

1.2. General synthesis of aryl[4-(trifluoromethyl)pyridin-3-yl]methanol (12a-d)

A mixture of 11 (10 mmol), triethylamine (1.2 g, 12 mmol) and 5% palladium on carbon (0.4 g) in MeOH (70 mL) was stirred vigorously under an atmosphere of hydrogen for 15 hr at room temperature. The reaction mixture was filtered off through celite pad by suction and the pad was washed with MeOH (30 mL). Evaporation of solvent and the residue was purified by trituration with 20% EtOAc in hexane or column chromatography on silica gel to give 12.
To an ice-cooled solution of alcohol (12–17) (2.0 mmol) in dry THF (15 mL) was added 60% NaH (0.16 g, 4.0 mol) by several portions. After the evolution of hydrogen gas ceased, N,N-dimethylcarbamoyl chloride (0.20 g, 3.6 mmol) was added at 5°C. The mixture was stirred for 5 hr at room temperature. Ice water (40 mL) and EtOAc (60 mL) were added to the mixture. The organic layer was separated, and the aqueous layer was extracted with EtOAc (40 mL). The combined extract was washed with brine and dried over MgSO₄. The solvent was removed in vacuo and the residue was purified by column chromatography on silica gel to give the corresponding carbamate.

1.3.1. Naphthalen-1-yl[4-(trifluoromethyl)pyridin-3-yl]methyl N,N-dimethylcarbamate (2a)

Compound 2a was obtained from 12a in 80% yield. White powder, mp 133–134°C. Rₛ = 0.33 (50% EtOAc in hexane). ¹H NMR δ(H): 2.93 (3H, s, CH₃-N), 2.99 (3H, s, CH₃-N), 5.92 (1H, d, J=1.2 Hz, CHF), 5.95 (1H, d, J=1.2 Hz, CHF), 6.69 (1H, m, 2,3-(methyleneoxy)benzene 5-H), 6.78 (1H, d, J=5.2 Hz, ArH), 6.78 (1H, d, J=3.6 Hz, ArH), 7.24 (1H, s, CH-O), 7.53 (1H, d, J=5.2 Hz, pyridine 5-H), 7.82 (1H, d, J=5.2 Hz, pyridine 6-H), 9.05 (1H, s, pyridine 2-H). EI-MS m/z 368 (M⁺), 280 (M⁻→OCON(CH₃)₂). EI-HRMS m/z (M⁺): Calcd. for C₁₀H₈F₂N₂O₂: 368.0894, Found: 368.0890.

1.3.2. 2,3-(Methyleneoxy)phenyl[4-(trifluoromethyl)pyridin-3-yl]methyl N,N-dimethylcarbamate (2b)

Compound 2b was obtained from 12b in 50% yield. Amorphous solid, Rₛ = 0.27 (50% EtOAc in hexane). ¹H NMR δ(H): 2.91 (3H, s, CH₃-N), 2.99 (3H, s, CH₃-N), 5.92 (1H, d, J=1.2 Hz, CHF), 5.95 (1H, d, J=1.2 Hz, CHF), 6.69 (1H, m, 2,3-(methyleneoxy)benzene 5-H), 6.78 (1H, d, J=5.2 Hz, ArH), 6.78 (1H, d, J=3.6 Hz, ArH), 7.24 (1H, s, CH-O), 7.53 (1H, d, J=5.2 Hz, pyridine 5-H), 7.82 (1H, d, J=5.2 Hz, pyridine 6-H), 9.05 (1H, s, pyridine 2-H). EI-MS m/z 368 (M⁺), 280 (M⁻→OCON(CH₃)₂). EI-HRMS m/z (M⁺): Calcd. for C₁₀H₈F₂N₂O₂: 368.0894, Found: 368.0890.
J=8.4Hz, J=4.4Hz, 384.0473, Found: 384.0466.

A mixture of 12a (0.61 g, 2.0mmol), triethylamine (0.31mL, 2.2mmol) and N,N'-carbonyldimidazole (CDI) (0.34g, 2.1mmol) in dry 1,2-dichloroethane (20mL) was heated at 55°C overnight. After cooling, the mixture was condensed in vacuo and purified by column chromatography on silica gel eluted with 30% EtOAc in hexane to give 5 (0.58 g) in 80% yield. White powder, mp 96–103°C. Rf=0.25 (33% EtOAc in hexane). 

EI-MS m/z 402 (M+), 286 (M+-OCON(CH3)2), EI-HRMS m/z (M+): Calcd. for C19H15BrN2O2: 402.1555, Found: 402.1549.

1.3.7. 4-Bromopyrpyridin-3-yl(naphthalen-1-yl)methyl N,N-dimethylcarbamate (6)

Compound 6 was obtained from 13 in 65% yield. Pale yellow powder, mp 115–123°C. Rf=0.15 (33% EtOAc in hexane). 

EI-MS m/z 384 (M+), 296 (M+-OCON(CH3)2). EI-HRMS m/z (M+): Calcd. for C19H15BrN2O2: 384.0473, Found: 384.0466.

1.3.8. 4-Chloropyrpyridin-3-yl(naphthalen-1-yl)methyl N,N-dimethylcarbamate (7)

Compound 7 was obtained from 14 in 78% yield. Pale yellow powder, mp 115–116°C. Rf=0.32 (50% EtOAc in hexane). 

EI-MS m/z 320 (M+), 286 (M+-OCON(CH3)2). EI-HRMS m/z (M+): Calcd. for C19H15ClN2O2: 320.0978, Found: 340.0984.

1.3.9. 4-Methoxypyridin-3-yl(naphthalen-1-yl)methyl N,N-dimethylcarbamate (8)

Compound 8 was obtained from 15 in 80% yield. White needles, mp 156–165°C. Rf=0.25 (EtOAc). 

EI-MS m/z 336 (M+), 248 (M+-OCON(CH3)2). EI-HRMS m/z (M+): Calcd. for C20H14N2O2: 336.1474, Found: 336.1480.

1.3.10. 4-Methylpyrpyridin-3-yl(naphthalen-1-yl)methyl N,N-dimethylcarbamate (9)

Compound 9 was obtained from 16 in 85% yield. White powder, mp 91–93°C. Rf=0.05 (EtOAc). 

EI-MS m/z 375 (M+), 287 (M+-OCON(CH3)2). EI-HRMS m/z (M+): Calcd. for C20H20N2O2: 375.1194, Found: 375.1186.

1.3.11. 4-Cyanopyrpyridin-3-yl(naphthalen-1-yl)methyl N,N-dimethylcarbamate (10)

Compound 10 was obtained from 17 in 46% yield. White powder, mp 119–120°C. Rf=0.41 (66% EtOAc in hexane). 

EI-MS m/z 331 (M+), 242 (M+-OCON(CH3)2). EI-HRMS m/z (M+): Calcd. for C20H19F3N2O2: 331.1321, Found: 331.1312.

1.4. Naphthalen-1-yl(3-(trifluoromethyl)pyridin-3-yl)methyl N-methylcarbamate (3)

To a stirred solution of 12a (0.61 g, 2.0mmol) in dry dichloromethane (15mL) were added dibutyltin dilaurate (2 drops) and methyl isocyanate (0.34 g, 4.0mmol) at 10°C. The mixture was stirred for 15hr at room temperature. Then, the reaction mixture was condensed in vacuo and the residue was purified by column chromatography on silica gel eluted with 40% EtOAc in hexane to give 3 (0.58 g) in 80% yield. White powder, mp 96–103°C. Rf=0.30 (50% EtOAc in hexane).
was refluxed for 2 hr under removing of water by Dean-Stark
to Stark apparatus. After cooling, the reaction mixture was filtered.
Evaporation of solvent in vacuo and purification of the crude product by column chromatography on silica gel eluted with 20% EtOAc in hexane gave 18 (13.8 g) in 82% yield. Pale yellow prisms, mp 98–103°C. Rf = 0.43 (20% EtOAc in hexane). 1H NMR δH: 7.43 (1H, t, J = 7.6 Hz, ArH), 7.50 (1H, m, ArH), 7.63 (1H, m, ArH), 7.68–7.71 (2H, m, ArH), 7.94 (1H, d, J = 8.4 Hz, ArH), 8.09 (1H, d, J = 8.4 Hz, ArH), 8.76 (1H, s, pyridine 2-H), 8.93 (1H, d, J = 5.2 Hz, pyridine 6-H), 8.99 (1H, d, J = 8.8 Hz, ArH). EI-MS m/z 301 (M+). EI–HRMS m/z (M+): Calcd. for C16H17F3N2O2: 301.0714, Found: 301.0705.

1.8. Preparation of optically active material
1.8.1. (+)-Naphthalen-1-yl[4-(trifluoromethyl)pyridin-3-yl]methanol ((+)-12a)

To a solution of (1R,2S)-(+)-1-amino-2-indanol (0.15 g, 1.0 mmol) in dry THF (10 mL) was added trimethyl borate (0.13 g, 1.2 mmol) at 5°C, and the mixture was stirred for 1 hr at 10°C. A 90% borane dimethyl sulfide complex (1.7 g, 20 mmol) was then added dropwise for 10 min at 5°C. A solution of 18 (3.0 g, 10 mmol) in dry THF (10 mL) was added to the mixture at 5°C and this mixture was stirred for 2 hr at 20°C. To an ice-cooled reaction mixture, CH2Cl2 (30 mL) and 10% hydrochloric acid (30 mL) were added, and the mixture was stirred overnight at room temperature. The pH of the mixture was then adjusted to 8 by the addition of aq. NaOH at 5°C. The organic layer was separated and dried over Na2SO4. Evaporation of solvent and purification of the crude product by column chromatography on silica gel eluted with 20% EtOAc in hexane gave (−)-12a in 17% yield. White powder, mp 138–143°C. [α]D = −41° (c 0.25, CHCl3). Enantiomeric purity was determined to be 88%ee by chiral HPLC analysis. Solvent; 17% 2-propanol in hexane, flow rate; 1.0 mL/min, temp; 20°C, UV detector; 254 nm, retention time; (+)-12a tR = 5.0 min and (−)-12a tR = 6.4 min.

1.8.2. (−)-Naphthalen-1-yl[4-(trifluoromethyl)pyridin-3-yl]methanol (−)-12a

Enantiomer (−)-12a was obtained in 14% yield with 91%ee from 18 by using (1S,2R)-(−)-1-amino-2-indanol. White powder, mp 142–146°C. [α]D = +45° (c 0.25, CHCl3).

1.8.3. (−)-Naphthalen-1-yl[4-(trifluoromethyl)pyridin-3-yl]methyl N,N-dimethylcarbamate (−)-2a

Compound (−)-2a was obtained from (−)-12a by the same procedure described for the synthesis of 2a in 55% yield. White prisms, mp 86–89°C. [α]D = −42° (c 1.0, CHCl3). Enantiomeric purity was determined to be 90%ee by chiral HPLC analysis. Solvent; 17% 2-propanol in hexane, flow rate; 1 mL/min, temp; 20°C, UV detector; 254 nm, retention time; (−)-2a tR = 11.4 min and (−)-2a tR = 20.1 min.

1.8.4. (−)-Naphthalen-1-yl[4-(trifluoromethyl)pyridin-3-yl]methyl N,N-dimethylcarbamate (−)-2a

Compound (−)-2a was obtained from (−)-12a in 33% yield. White prisms mp 86–89°C. [α]D = +41° (c 1.0, CHCl3). Enantiomeric purity was determined to be 91%ee by chiral HPLC analysis.

2. Biological evaluation

2.1. Herbicidal efficacy against Echinochloa oryzicola and Scirpus juncoides in a greenhouse

Test compounds were formulated as either 100 g/kg wettable powders or 100 g/L emulsifiable concentrates in accordance with the usual preparation methods. Each formulation was diluted with water to the required application rate. The diluted solution was uniformly applied using a pipette to water the
surface of the pots. The rate of application (g/ha) was calculated as the total amount of active ingredient included in the diluted solution converted from the surface area of the pot.

Biological evaluation tests were conducted in a greenhouse maintained at 20–25°C under natural and supplementary artificial lighting, Echinocloa oryzicola (ECHOR) and Scirpus juncoides Roxb (SCPJO) were used in the experiments and grown under flooded conditions.

A ceramic pot (0.01 m³) was filled with paddy soil (400 mL) and kept wet by watering. About 20 seeds of ECHOR were sown in the soil at a depth of 5 mm and grown at 22–25°C for a few days. At emergence or the 2.5-leaf stage, the diluted formulation was applied using the method reported above. In a separate paddy pot, about 20 seeds of SCPJO were sown uniformly on the soil and the upper layer of the soil was gently padded. The plants were then grown at 20–25°C for 5–7 days. When SCPJO had grown to the 0.5-leaf stage, the diluted formulation was applied.

The herbicidal activity was judged visually 14–20 days later and evaluated on a scale of zero (no damage to weeds) to 100 (complete killing of weeds). Herbicidal data are reported as ED₅₀; ED₃₀ shows the dosage in grams of the active ingredient per ha (g.a.i./ha) causing visual damage of 80%.

2.2. Phytotoxicity to transplanted rice
The phytotoxicity to transplanted rice (Oryza sativa L. cv. Nipponbare) was evaluated under the same experimental conditions as the herbicidal efficacy test. A ceramic pot filled with paddy soil was flooded, seeds of ECHOR, and SCPJO were sown in the upper layer of the soil was gently padded. Young rice plants at the 2-leaf stage were then transplanted to the soil at a depth of 3 cm. Four days after transplanting, the water depth was adjusted to 3.5 cm. The diluted formulation was then applied according to the method described above. After 20–25 days, phytotoxicity was evaluated on a scale of zero (no damage to rice) to 100 (complete killing of rice). Phytotoxicity data to transplanted rice are reported as ED₅₀; ED₃₀ shows the dosage causing visual damage of 20%.

2.3. Test in concrete pot outside a greenhouse
A concrete pot (0.36 m²) filled with paddy soil was flooded with water and the soil was paddled to make flat. Just after paddling, seeds of SCPJO, Monochoria vaginalis (MOOVA), bloodleaf weeds (BL; Linderia pyxidaria, Rotala indica, and Elatine triandra), and ECHOR were sown on the soil surface. On the next day, rice (Oryza sativa L. cv. Kinukihari) at the 3–4 leaf stages was transplanted. The plants were then grown for several days, and a diluted solution of a wettable powder of the test compound was uniformly applied using a pipette to water the surface of the pots at the timing of the 0, 1.6, and 2.3-leaf stage of ECHOR. Twenty-six days later, the herbicidal activity and phytotoxicity were evaluated on a scale of zero (inactive/ non-phytotoxic to rice) to 100 (complete inhibition of growth) by visual observation. For comparison, cafestrol, one of the typical paddy field herbicides for ECHOR, was used as a positive control in this test.

3. Interaction with cytochrome P450 monoxygenase

3.1. Construction of Sorghum halepense CYP51 expression vector
An expression vector, ShCYP51/ptac-g10L, which contains Sorghum halepense CYP51 cDNA fused with the leader sequence of T7 bacteriophage gene 10 under the regulation of the tac promoter, was constructed as follows. Total RNA was prepared from etiolated seedlings of Sorghum halepense and cDNA was synthesized using a ThermoScript RT-PCR System. Sorghum halepense CYP51 cDNA was PCR-amplified from the total cDNA with following primers, each designed according to the CYP51 mRNA sequence of Sorghum bicolor (GenBank accession number U74319): 5'-GCTCTCGCTGATATCCCACAACAGC (F1 primer) and 5'-TTAGTGATGATGATGATGATGTGGTGTCCACAA-CAGCTTC (R1 primer, nucleotides for C-terminal 6x histidine tag are indicated by italics). PCR was conducted with Pyrobest DNA Polymerase (TaKaRa Bio, Japan). Sorghum halepense CYP51 cDNA was first cloned into a pET28a+ expression vector (Novagen) digested with NcoI/Xhol and blunt-ended using Klenow Fragment (TaKaRa Bio, Japan), resulting in the plasmid ShCYP51/pET28a+. Second, the plasmid pGEX-Mut-Xba was created by introducing an Xhol site into the upstream of the start codon for the GST tag of the pGEX-6P-1 expression vector (GE Healthcare). The following primers were used to introduce an Xhol site into pGEX-6P-1: 5’-CGTATAATGTTGCTAGTGGACGGAT (F1 primer) and 5’-TATCCGCTCCTCTAGAACAACATTACGG (R2 primer). Sorghum halepense CYP51 cDNA fused with the T7 bacteriophage gene 10 leader sequence was PCR-amplified from the ShCYP51/pET28a+ and further cloned into pGEX-Mut-Xba digested with Xhol/Smal, resulting in the expression vector ShCYP51/ptac-g10L.

3.2. Expression and purification of recombinant Sorghum halepense CYP51 protein
E. coli strain JM109 transformed with ShCYP51/ptac-g10L was cultured overnight in 10 mL LB broth containing 100 μg/mL ampicillin, diluted into 1 L of Terrific broth supplemented with 100 μg/mL ampicillin and cultured at 37°C. When the optical density at 600 nm reached 0.8, 0.5 mM IPTG and 1 mM δ-aminolevulinic acid were added, and protein expression was induced at 28°C for 24 hr. The cell pellet from the 1 L culture was re-suspended in 50 mL buffer A (50 mM Tris-HCl pH 8.0 and 0.25 M sucrose) and 0.1 mg/mL of lysozyme was added. The spheroplast was pelleted at 10,000 g for 15 min and frozen at -80°C. The pellet thawed at room temperature was resuspended in buffer B (100 mM potassium phosphate pH 7.4, 150 mM KCl, 20% glycerol and 2–mercaptoethanol) and sonicated. The cell lysate was centrifuged at 10,000 g and 4°C for 20 min, and the supernatant was further centrifuged at 35,000 rpm and 4°C for 1 hr in Beckman Type 50.2 Ti rotor (Beckman Coulter). The pellet was suspended in buffer B containing 1% CHAPS and 0.1% Triton X-100 and incubated at 4°C for 1 hr. Insoluble materials were removed by centrifugation.
Aryl(4-substituted pyridin-3-yl)methyl carbamates and herbicidal activity

LDA, THF

ArCHO

CF3

a: Ar = naphthalen-1-yl
b: Ar = 1,3-benzodioxol-4-yl
c: Ar = 5-chloro-1,3-benzodioxol-4-yl
d: Ar = acenaphthen-5-yl
e: Ar = quinolin-4-yl

MeOH

Cat.

Bu2Sn(OOCCH2CH3)2

MeNCO, CH2Cl2

Et3N, CDI

2,4

Fig. 3. Synthesis of aryl(4-CF3-pyridin-3-yl)methyl carbamates (2-5).

at 35,000 rpm and 4°C for 30 min in the rotor described above, and the supernatant fraction was applied to Ni Sepharose 6 Fast Flow (GE Healthcare) equilibrated with buffer B containing 0.5% CHAPS and 0.1% Triton X-100. The recombinant Sorghum halepense CYP51 protein was eluted with buffer B containing 0.1% Triton X-100 and 200 mM imidazole. The amount of cytochrome P450 was determined using the extinction coefficient 91 mM⁻¹ cm⁻¹ for the CO-bound reduced P450 species.

3.3. Spectral binding titrations

The titration experiments were carried out using a Shimadzu UV-2550 UV-visible spectrophotometer at 25°C in tandem 1 mL cuvettes each containing 1 μM ShCYP51 protein in buffer B. To each cuvette, the tested compounds dissolved in DMSO or DMSO only were added in 0.5 μL aliquots, and titration data were generated by subtraction of the spectrum of compound-free enzyme from that of the enzyme added with compounds. Titration data were linearized by plotting [Compound] for the x-axis against [Compound]/ΔA for the y-axis, where [Compound] is the total concentration of added compound and ΔA is the change in absorption (peak-trough). The spectral dissociation constant was calculated from the intercept of the linear plot with the y-axis.

Results and Discussion

1. Synthesis

1.1. Synthesis of 2-10

We designed compounds 2-10 according to the following directions: i) replacement of one of the two CF3-pyridine rings of 1 with other aromatic ring, ii) replacement of N,N-dimethyl carbamate with other carbamate and iii) modification of C-4 position with other substituent group instead of the CF3 group.

The synthesis of compounds 2-5 is shown in Fig. 3. The synthesis commenced from 2-chloro-4-(trifluoromethyl)pyridine (2,4-CTF). Lithiation of 2,4-CTF with LDA and the resulting 3-lithiopyridine was quenched with arylcarboxaldehyde to give 11. Hydrogenolysis of a chlorine bond at the C-2 position of 11 was performed under H2/palladium-carbon conditions in methanol to give 12. Finally, 12 was reacted with N,N-dimethylcarbamoyl chloride to give 2 (R=NMe2; 2a: Ar=naphthalen-1-yl, 2b: Ar=1,3-benzodioxol-4-yl, 2c: Ar=5-chloro-1,3-benzodioxol-4-yl, 2d: Ar=acenaphthen-5-yl and 2e: Ar=quinolin-4-yl) and 4. Compounds 3 and 5 were prepared by the reaction with MeNCO and CDI.

On the other hand, compounds 6-10 were synthesized in two or five steps from 4-bromopyridine, 4-chloropyridine, or 4-methoxypyridine, via their hydroxy intermediates 13-17 as shown Fig. 4, respectively.

Compounds 13-15 were prepared by the same manner described for the synthesis of 11. N,N-dimethyl carbamoylation of 13-17 with N,N-dimethylcarbamoyl chloride gave compounds 6-10 in good to excellent yields.

1.2. Stereoselective synthesis of (-)-2a and (+)-2a

Since the herbicidal activity was examined by the use of racemic 2a, we were interested in comparing the herbicidal potency of two enantiomers, (-)-2a and (+)-2a. Their synthesis is shown in Fig. 5.

![Fig. 4. Synthesis of naphthalen-1-yl(4-substituted pyridin-3-yl)methyl N,N-dimethylcarbamates (6-10).](image-url)
MnO₂ oxidation of racemic 12a gave ketone 18 in 82% yield. Asymmetric reduction of 18 was achieved by the use of a chiral borane reagent. Treatment of 18 with the chiral reagent generated by the borane dimethyl sulfoxide complex with a combination of trimethyl borate and \((1R,2S)\)-1-amino-2-indanol in THF at 20°C for 2 hr gave \((-)\)-12a in 17% yield with 88% ee. Conversion of \((-)\)-12a to \((-)\)-2a was performed following the same procedure described for the synthesis of 2a.

### Table 1. Herbicidal efficacy and phytotoxicity to transplanted rice of 1-10

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound No.</th>
<th>Substituents</th>
<th>ED₅₀ (g a.i./ha)</th>
<th>ECHOR[^d]</th>
<th>SCPIO[^d]</th>
<th>ED₅₀ (g a.i./ha) Transplanted rice[^d]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>X</td>
<td>R</td>
<td>Ar</td>
<td>E</td>
<td>2.5 L</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>CF₃</td>
<td>NMe₂</td>
<td>i</td>
<td>63</td>
<td>nt</td>
</tr>
<tr>
<td>2</td>
<td>2a</td>
<td>CF₃</td>
<td>NMe₂</td>
<td>ii</td>
<td>31</td>
<td>125</td>
</tr>
<tr>
<td>3</td>
<td>2b</td>
<td>CF₃</td>
<td>NMe₂</td>
<td>iii</td>
<td>63</td>
<td>nt</td>
</tr>
<tr>
<td>4</td>
<td>2c</td>
<td>CF₃</td>
<td>NMe₂</td>
<td>iv</td>
<td>63</td>
<td>500</td>
</tr>
<tr>
<td>5</td>
<td>2d</td>
<td>CF₃</td>
<td>NMe₂</td>
<td>v</td>
<td>500</td>
<td>&gt;500</td>
</tr>
<tr>
<td>6</td>
<td>2e</td>
<td>CF₃</td>
<td>NMe₂</td>
<td>vi</td>
<td>125</td>
<td>nt</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>CF₃</td>
<td>NHMe</td>
<td>ii</td>
<td>63</td>
<td>250</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>CF₂</td>
<td>NMe₂</td>
<td>ii</td>
<td>31</td>
<td>250</td>
</tr>
<tr>
<td>9</td>
<td>5</td>
<td>CF₂</td>
<td>NEt₂</td>
<td>ii</td>
<td>&gt;500</td>
<td>nt</td>
</tr>
<tr>
<td>10</td>
<td>6</td>
<td>Br</td>
<td>NMe₂</td>
<td>ii</td>
<td>31</td>
<td>250</td>
</tr>
<tr>
<td>11</td>
<td>7</td>
<td>Cl</td>
<td>NMe₂</td>
<td>ii</td>
<td>63</td>
<td>&gt;500</td>
</tr>
<tr>
<td>12</td>
<td>8</td>
<td>OMe</td>
<td>NMe₂</td>
<td>ii</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>13</td>
<td>9</td>
<td>Me</td>
<td>NMe₂</td>
<td>ii</td>
<td>500</td>
<td>nt</td>
</tr>
<tr>
<td>14</td>
<td>10</td>
<td>CN</td>
<td>NMe₂</td>
<td>ii</td>
<td>63</td>
<td>250</td>
</tr>
</tbody>
</table>

[^d]: ECHOR: *Echinochloa oryzicola*. E: emergence stage, 2.5L; 2.5 leaf stage. SCPIO: *Scirpus Juncoides*. 0.5L; 0.5 leaf stage. Phytotoxicity to rice (*Oryza sativa* L cv. Nipponbare) that was planted at a depth of 3 cm from the surface of soil. nt: Not tested.
to give the crystalline compound (−)-2a in 55% yield with 90% ee. A similar reduction of 18 by the use of (S,S,2R)-(−)-1-amino-2-indanol as a chiral ligand followed by carbamoylation gave the other enantiomer (+)-2a with 91% ee.

2. Herbicidal activity against weeds and phytotoxicity to transplanted rice

Aryl(4-substituted pyridin-3-yl)methyl carbamates 2-10 were subjected to biological tests for herbicidal activity against weeds in paddy fields and phytotoxicity to transplanted rice. The results are shown in Table 1.

2.1. Modification of aryl moiety

We speculated that only one of the CF₃-pyridine rings in 1 must be essential as a DM inhibitor, where a nitrogen atom of the pyridine ring coordinates with an iron atom in the porphyrin. If our speculation is correct, one of two pyridine rings is unnecessary for herbicidal activity. Indeed, one CF₃-pyridine ring in 1 could be replaced with a benzene ring, reported by Chin et al. (Zeneca Ltd.), and the compound indicated potent herbicidal activity with phytotoxicity against rice. In comparison with 1, 1-naphthyl derivative 2a exhibited twice as much activity against ECHOR at the emergence stage, with an ED₅₀ value of 31 g a.i./ha, though the activity against SCPJ0 is weaker than 1 (entries 1 and 2). On the other hand, the phytotoxicity to transplanted rice (depth of the root: 3 cm) was dramatically decreased. ED₅₀ of 2a indicated 2000 g a.i./ha vs. that of 1 < 250 g a.i./ha. Meanwhile, 1,3-benzodioxol-4-yl derivative (2b) exhibited similar potency in herbicidal activity against ECHOR in comparison with 2a, but its phytotoxicity to transplanted rice was severer than that of 2a (entries 2 and 3). Introduction of a chlorine to 2b at the 5-position instead of hydrogen decreased the herbicidal activity against SCPJ0 (entry 4). However, the selectivity between the rice plant and paddy weeds of compound 2c was better than that of 2b. The ace-naphthen-5-yl derivative 2d showed weak herbicidal activity against the weeds (entry 5). These results indicated that the naphthalen-1-yl group showed the most favorable performance in both herbicidal activity and phytotoxicity to transplanted rice. In the case of 2e, the more electrophilic N-atom of the quinoline ring is suggested to coordinate with the iron atom in the porphyrin ring, resulting in loss of potency relative to 2a.

2.2. Modification of the carbamoyl group

Secondly, the N,N-dimethylamino group of the carbamate moiety in 2a was modified. Compounds with the N-alkyl-carbamoyl group 3 and 4 exhibited excellent herbicidal activity against ECHOR at the emergence stage (entries 7 and 8). However, in comparison to 2a, the efficacy of 3 and 4 to ECHOR at the 2.5-leaf stage and SCPJ0 did not exceed that of 2a. The compound having the imidazole group (5) exhibited poor activity against ECHOR even at the emergence stage (entry 9). Based on these results, the N,N-dimethylcarbamoyl group is the most appropriate both in herbicidal activity and phytotoxicity to transplanted rice on the carbamoyl moieties.

2.3. Modification of the 4-position on the pyridine ring

The CF₃ group of 2a was replaced with halides, methoxy, methyl, and cyano groups. Compound 6 (X=Br) exhibited the same herbicidal activity as 2a against ECHOR at the emergence stage, however at the 2.5-leaf stage, the activity was 2-fold lower than that of 2a (entry 10). Compound 7 (X=Cl) showed inferior activity to 2a against ECHOR at the emergence stage (ED₅₀=63 g a.i./ha) but superior activity against SCPJ0 at the 0.5-leaf stage (ED₅₀ value of 63 g a.i./ha) to that of 2a (entry 11). On the other hand, compounds 8 and 9, which have electron-donating groups (methoxy and methyl, respectively), exhibited poor herbicidal activity (entries 12 and 13). Finally, compound 10 (X=CN) showed moderate herbicidal activity against ECHOR and SCPJ0 (entry 14).

2.4. Herbicidal spectrum of 2a in outdoor concrete pot test, and comparison with cafenstrole

To reveal the herbicidal spectrum of 2a in a paddy field, experiments in concrete pots outside a greenhouse were conducted (Table 2). The results indicate that 2a possesses excellent herbicidal activity against ECHOR, MOOVA, and annual broad-

<table>
<thead>
<tr>
<th>Compound No. (Name)</th>
<th>Application time</th>
<th>Rate g a.i./ha</th>
<th>Herbicidal efficacy</th>
<th>Phytotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>ECHOR</td>
<td>SCPJ0</td>
</tr>
<tr>
<td>2a</td>
<td>0</td>
<td>200</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td></td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td></td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>2a</td>
<td>1.6</td>
<td>100</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>2a</td>
<td>300</td>
<td></td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>2a</td>
<td>300</td>
<td></td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>2a</td>
<td>200</td>
<td></td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>2a</td>
<td>200</td>
<td></td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>Cafenstrole</td>
<td>1.6</td>
<td>300</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>Cafenstrole</td>
<td>2.3</td>
<td>300</td>
<td>++++</td>
<td>++++</td>
</tr>
</tbody>
</table>

Table 3. Herbicidal efficacy of stereoisomers of 2a

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>ECHOR (g a.i./ha)</th>
<th>SCPJO (0.5 L)</th>
<th>MOOVA (g a.i./ha)</th>
<th>BL (1–2.2 L)</th>
<th>MOOVA : Monochoria vaginalis. 1.5 L; 1.5 leaf stage. BL: Average efficacy against annual broadleaf weeds such as Linderia pyxidaria, Rotala indica and Illatine triandra (1–2.2 leaf stage). nt: Not tested.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-)-2a</td>
<td>&lt;31</td>
<td>&lt;63</td>
<td>125</td>
<td>&lt;100</td>
<td>500</td>
</tr>
<tr>
<td>(+)-2a</td>
<td>125</td>
<td>nt (a)</td>
<td>&gt;500</td>
<td>nt</td>
<td>1000</td>
</tr>
<tr>
<td>2a</td>
<td>31</td>
<td>125</td>
<td>250</td>
<td>&lt;100</td>
<td>2000</td>
</tr>
</tbody>
</table>

2.5. Herbicidal activity and stereochemistry of 2a

Since good crystals were not obtained for (-)-2a and (+)-2a, we could not determine their absolute stereochemistry. In comparison with the data of enantiomers shown in Table 3, (+)-2a is less active in all cases. The racemic compound was evaluated between the two enantiomers.

3. Interaction of 2a, 2d, 5 and 9 with cytochrome P450 mono-oxygenase

Based on the structural similarity to compound 1, we assumed that the newly synthesized aryl(4-substituted pyridin-3-yl) methyl carbamates also have inhibitory activity against obtusifoliol DM and analyzed their binding affinity for E. coli-expressed plant CYP51. Addition of compound 2a to the recombinant Sorghum halepense CYP51 protein induced a type II spectral response, which is characteristic of P450 inhibitors with a nitrogen-containing aliphatic or aromatic moiety, suggesting a direct coordination of pyridyl nitrogen of compound 2a to the heme iron (Fig. 6). To investigate the relationships between the herbicidal efficacy and the binding affinity for obtusifoliol DM, the dissociation constant $K_d$ for compound 2a was compared to those of the other synthesized compounds (Table 4). Among the selected compounds 2d, 5 and 9, all of which showed markedly decreased herbicidal efficacy against tested weeds, compounds 2d and 5 exhibited obviously larger $K_d$ than that of compound 2a. On the other hand, the $K_d$ value for compound 9 was comparable to that of compound 2a, suggesting that herbicidal activity in a paddy field may not depend entirely on the binding affinity for obtusifoliol DM but that other factors such as soil persistence and translocation in plants, may be partly operative.

Table 4. Binding affinity of each compound for Sorghum halepense CYP51 protein

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>$K_d$ (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2a</td>
<td>$6.3 \times 10^{-7}$</td>
</tr>
<tr>
<td>2d</td>
<td>$9.4 \times 10^{-7}$</td>
</tr>
<tr>
<td>5</td>
<td>$3.4 \times 10^{-6}$</td>
</tr>
<tr>
<td>9</td>
<td>$6.5 \times 10^{-7}$</td>
</tr>
</tbody>
</table>
**Conclusion**

New herbicidal carbamates 2–10 were synthesized by the structural modification of 1 and their herbicidal activity in a paddy field and phytotoxicity to transplanted rice were examined. The study revealed that naphthalen-1-yl[4-(trifluoromethyl)-pyridin-3-yl]methyl N,N-dimethylcarbamate 2a exhibited the most favorable characteristics for herbicidal activity against ECHOR, MOOVA, SCPJO and some broadleaf weeds without showing phytotoxicity to transplanted rice. In comparison with two enantiomeric isomers, one optical isomer (−)-2a was more active than (+)-2a. Finally, it can be considered that the herbicidal mechanism of 2a would be due to the inhibition of obtusifoliol DM. The influence on its enzymatic activity and its contribution to the herbicidal activity remain to be further elucidated.

**Acknowledgement**

The authors thank to Drs. T. Haga, S. Mizukoshi, Y. Hayase, Ms. S. Miyashita and Mr. S. Nagayama for their kind suggestions and supports on this work.

**References**

アリール(4-置換ビリジン-3-イル)メチル カーパメート類
の合成と除草活性

中野仁志, 小柳 淳, 菊川弘司, 佐野真喜子, 
大野 研, 伏木田地, 上西潤一

既存の除草剤とは異なる新たな作用機構をもつ水田用除草剤を創製することを目的に、アルコール部分で総合環とビリジン環とがメチル基に置換した構造をとる種々の新規カルバミン酸エステル誘導体を合成し、除草活性およびイネに対する薬害を検討した。その結果、ナフタレン-1-イル[4-(トリフルオロメチル)ビリジン-3-イル]メチル N,N-ジメチルカーパメート(2a)が水田で多発するタイヌビエや各種の広葉雑草に対して優れた除草活性を有すると同時に、移植イネに対し薬害を示すこを見出した。また一方の光学異性体(-)-2aがもう一方の光学異性体(+)-2aより高い除草活性を有することが判った。さらに、今回合成した除草活性合物は、オプシフィオール14a-脱メチル化酵素の阻害剤として作用し、殺草作用を発現していると思われる。