

# アリアル(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

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Original Article

# Synthesis of novel aryl(4-substituted pyridin-3-yl)methyl carbamates and their herbicidal activity

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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 obtusifoliosyl 14 $\alpha$ -methyl demethylase P450. © Pesticide Science Society of Japan

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

## Introduction

Cytochrome-P450-dependent 14 $\alpha$ -methyl demethylase (DM) is known to convert obtusifoliosyl to phytosterols in plants.<sup>1)</sup> 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.<sup>2,3)</sup> 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,<sup>4)</sup> pyrazosulfuron-ethyl,<sup>5)</sup> and imazosulfuron,<sup>6)</sup> 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<sup>7-9)</sup> 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

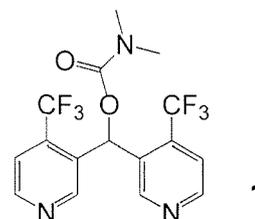


Fig. 1. Structure of **1**.

*Echinochloa* species as well as some other broadleaf weeds.<sup>10,11)</sup> Later, Bargar *et al.* reported that the activity of **1** is due to inhibition of DM.<sup>12)</sup> 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-

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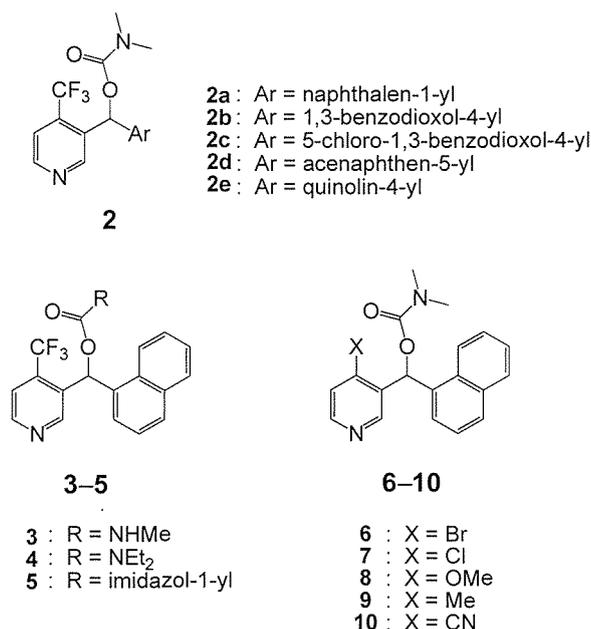


Fig. 2. Structures of aryl(4-substituted pyridin-3-yl)methyl carbamates 2–10.

ing point apparatus and were not corrected. <sup>1</sup>H NMR spectra were recorded on a JEOL JNM-GSX 400 (400 MHz) spectrometer in CDCl<sub>3</sub> with tetramethylsilane as an internal standard. Column chromatography was carried out using Merck silica gel 60 (70–230 mesh). Low- and high-resolution mass spectra were recorded on a JEOL JMS-GC MATE. Enantiomeric purity was determined by HPLC connected with a chiral column (Daicel CHIRALCEL OJ-H 4.6×150 mm).

#### 1.1. General synthesis of aryl[2-chloro-4-(trifluoromethyl)pyridin-3-yl]methanol (**11a–d**)

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*-BuLi (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 NH<sub>4</sub>Cl (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 MgSO<sub>4</sub>. The crude product was purified by column chromatography on silica gel to afford **11**.

##### 1.1.1. 2-Chloro-4-(trifluoromethyl)pyridin-3-yl(naphthalen-1-yl)methanol (**11a**)

Compound **11a** was obtained from 1-naphthaldehyde in 79% yield. White solid, mp 74–77°C. *R*<sub>f</sub>=0.26 (20% EtOAc in hexane). <sup>1</sup>H NMR δ<sub>H</sub>: 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<sup>+</sup>). EI-HRMS *m/z* (M<sup>+</sup>): Calcd. for C<sub>17</sub>H<sub>11</sub>ClF<sub>3</sub>NO: 337.0481, Found: 337.0477.

##### 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-(methylenedioxy)benzaldehyde in 85% yield. White powder, mp 113–115°C. *R*<sub>f</sub>=0.38 (50% EtOAc in hexane). <sup>1</sup>H NMR δ<sub>H</sub>: 3.69 (1H, d, *J*=7.6 Hz, OH), 5.80 (2H, dd, *J*=7.8 and 1.4 Hz, CH<sub>2</sub>-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<sup>+</sup>). EI-HRMS *m/z* (M<sup>+</sup>): Calcd. for C<sub>14</sub>H<sub>8</sub>Cl<sub>2</sub>F<sub>3</sub>NO<sub>3</sub>: 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. *R*<sub>f</sub>=0.39 (33% EtOAc in hexane). <sup>1</sup>H NMR δ<sub>H</sub>: 3.27 (1H, d, *J*=10.0 Hz, OH), 3.29–3.37 (4H, m, CH<sub>2</sub>-CH<sub>2</sub>), 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<sup>+</sup>). EI-HRMS *m/z* (M<sup>+</sup>): Calcd. for C<sub>19</sub>H<sub>13</sub>ClF<sub>3</sub>NO: 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. *R*<sub>f</sub>=0.18 (50% EtOAc in hexane). <sup>1</sup>H NMR δ<sub>H</sub>: 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<sup>+</sup>). EI-HRMS *m/z* (M<sup>+</sup>): Calcd. for C<sub>16</sub>H<sub>10</sub>ClF<sub>3</sub>N<sub>2</sub>O: 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% Et<sub>2</sub>O in hexane or column chromatography on silica gel to give **12**.

##### 1.2.1. Naphthalen-1-yl[4-(trifluoromethyl)pyridin-3-yl]methanol (**12a**)

Compound **12a** was obtained from **11a** in 87% yield after purification by column chromatography on silica gel eluted with 30% EtOAc in hexane. Pale yellow powder, mp 123–125°C.

$R_f=0.20$  (33% EtOAc in hexane).  $^1\text{H NMR } \delta_{\text{H}}$ : 3.88 (1H, bs, OH), 6.95 (1H, s, CH-O), 7.37 (1H, d,  $J=6.4$  Hz, ArH), 7.41 (1H, d,  $J=8.0$  Hz, ArH), 7.43–7.49 (2H, m, ArH), 7.55 (1H, d,  $J=4.8$  Hz, pyridine 5-H), 7.81 (1H, d,  $J=7.6$  Hz, ArH), 7.84–7.90 (2H, m, ArH), 8.60 (1H, d,  $J=4.8$  Hz, pyridine 6-H), 8.73 (1H, s, pyridine 2-H). EI-MS  $m/z$  303 ( $\text{M}^+$ ). EI-HRMS  $m/z$  ( $\text{M}^+$ ): Calcd. for  $\text{C}_{17}\text{H}_{12}\text{F}_3\text{NO}$ : 303.0871, Found: 303.0866.

#### 1.2.2. 6-Chloro-2,3-(methylenedioxy)phenyl[4-(trifluoromethyl)pyridin-3-yl]methanol (**12c**)

Compound **12c** was obtained from **11c** in 85% yield after purification by column chromatography on silica gel eluted with 50% EtOAc in hexane. White powder,  $R_f=0.38$  (50% EtOAc in hexane).  $^1\text{H NMR } \delta_{\text{H}}$ : 3.29 (1H, bs, OH), 5.93 (2H, dd,  $J=14$  and 1.4 Hz,  $\text{CH}_2\text{-O}$ ), 6.58 (1H, s, CH-O), 6.76 (1H, d,  $J=8.5$  Hz, Ar-H), 6.91 (1H, d,  $J=8.5$  Hz, Ar-H), 7.56 (1H, d,  $J=4.9$  Hz, pyridine 5-H), 8.73 (1H, d,  $J=4.9$  Hz, pyridine 6-H), 8.91 (1H, s, pyridine 2-H). EI-MS  $m/z$  331 ( $\text{M}^+$ ). EI-HRMS  $m/z$  ( $\text{M}^+$ ): Calcd. for  $\text{C}_{14}\text{H}_9\text{ClF}_3\text{NO}_3$ : 331.0223, Found: 331.0231.

#### 1.2.3. Acenaphthen-5-yl[4-(trifluoromethyl)pyridin-3-yl]methanol (**12d**)

Compound **12d** was obtained from **11d** in 66% yield. White powder, mp 148–151°C (EtOAc–hexane).  $R_f=0.36$  (50% EtOAc in hexane).  $^1\text{H NMR } \delta_{\text{H}}$ : 2.97 (1H, bs, OH), 3.28–3.34 (4H, m,  $\text{CH}_2\text{-CH}_2$ ), 6.81 (1H, s, CH-O), 7.16 (1H, t,  $J=7.2$  Hz, acenaphthene 4-H), 7.21–7.25 (2H, m, ArH), 7.36 (1H, m, ArH), 7.46 (1H, d,  $J=5.2$  Hz, pyridine 5-H), 7.54 (1H, d,  $J=8.4$  Hz, acenaphthene 6-H), 8.59 (1H, d,  $J=5.2$  Hz, pyridine 6-H), 8.77 (1H, s, pyridine 2-H). EI-MS  $m/z$  329 ( $\text{M}^+$ ). EI-HRMS  $m/z$  ( $\text{M}^+$ ): Calcd. for  $\text{C}_{19}\text{H}_{14}\text{F}_3\text{NO}$ : 329.1027, Found: 329.1031.

#### 1.2.4. Quinolin-4-yl[4-(trifluoromethyl)pyridin-3-yl]methanol (**12e**)

Compound **12e** was obtained from **11e** in 75% yield. Pale yellow powder,  $R_f=0.11$  (EtOAc).  $^1\text{H NMR } \delta_{\text{H}}$ : 6.96 (1H, s, CH-O), 7.26 (1H, d,  $J=4.5$  Hz, quinoline 3-H), 7.64–7.73 (4H, m, ArH), 8.17 (1H, m, ArH), 8.56 (1H, s, pyridine 2-H), 8.78 (1H, d,  $J=5.4$  Hz, pyridine 6-H), 9.01 (1H, d,  $J=4.5$  Hz, quinoline 2-H). EI-MS  $m/z$  304 ( $\text{M}^+$ ). EI-HRMS  $m/z$  ( $\text{M}^+$ ): Calcd. for  $\text{C}_{16}\text{H}_{11}\text{F}_3\text{N}_2\text{O}$ : 304.0823, Found: 304.0831.

#### 1.3. General synthesis of aryl[4-(trifluoromethyl)pyridin-3-yl]methyl *N,N*-dimethylcarbamate (**2a–e**, **4**, and **6–10**)

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 mmol) 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  $\text{MgSO}_4$ . 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.  $R_f=0.33$  (50% EtOAc in hexane).  $^1\text{H NMR } \delta_{\text{H}}$ : 2.91 (3H, s,  $\text{CH}_3\text{-N}$ ), 2.93 (3H, s,  $\text{CH}_3\text{-N}$ ), 7.07 (1H, d,  $J=6.8$  Hz, naphthalene 2-H), 7.38 (1H, t,  $J=7.6$  Hz, ArH), 7.48–7.55 (2H, m, ArH), 7.59 (1H, d,  $J=5.2$  Hz, pyridine 5-H), 7.84 (1H, d,  $J=8.0$  Hz, ArH), 7.88 (1H, m, ArH), 7.94 (1H, s, CH-O), 8.00 (1H, m, ArH), 8.78 (1H, d,  $J=5.2$  Hz, pyridine 6-H), 8.85 (1H, s, pyridine 2-H). EI-MS  $m/z$  374 ( $\text{M}^+$ ), 286 ( $\text{M}^+ - \text{OCON}(\text{CH}_3)_2$ ). EI-HRMS  $m/z$  ( $\text{M}^+$ ): Calcd. for  $\text{C}_{20}\text{H}_{17}\text{F}_3\text{N}_2\text{O}_2$ : 374.1242, Found: 374.1245.

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

Compound **2b** was obtained from **12b** in 50% yield. Amorphous solid,  $R_f=0.27$  (50% EtOAc in hexane).  $^1\text{H NMR } \delta_{\text{H}}$ : 2.91 (3H, s,  $\text{CH}_3\text{-N}$ ), 2.99 (3H, s,  $\text{CH}_3\text{-N}$ ), 5.92 (1H, d,  $J=1.2$  Hz, CHH), 5.95 (1H, d,  $J=1.2$  Hz, CHH), 6.69 (1H, m, 2,3-(methylenedioxy)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), 8.72 (1H, d,  $J=5.2$  Hz, pyridine 6-H), 9.05 (1H, s, pyridine 2-H). EI-MS  $m/z$  368 ( $\text{M}^+$ ), 280 ( $\text{M}^+ - \text{OCON}(\text{CH}_3)_2$ ). EI-HRMS  $m/z$  ( $\text{M}^+$ ): Calcd. for  $\text{C}_{17}\text{H}_{15}\text{F}_3\text{N}_2\text{O}_4$ : 368.0984, Found: 368.0980.

#### 1.3.3. 6-Chloro-2,3-(methylenedioxy)phenyl[4-(trifluoromethyl)pyridin-3-yl]methyl *N,N*-dimethylcarbamate (**2c**)

Compound **2c** was obtained from **12c** in 37% yield. White prisms, mp 124–127°C.  $R_f=0.31$  (50% EtOAc in hexane).  $^1\text{H NMR } \delta_{\text{H}}$ : 2.94 (3H, s,  $\text{CH}_3\text{-N}$ ), 2.97 (3H, s,  $\text{CH}_3\text{-N}$ ), 5.84 (1H, d,  $J=1.2$  Hz, CHH), 5.90 (1H, d,  $J=1.2$  Hz, CHH), 6.74 (1H, d,  $J=8.0$  Hz, 2,3-(methylenedioxy)benzene 4-H), 6.90 (1H, d,  $J=8.0$  Hz, 2,3-(methylenedioxy)benzene 5-H), 7.47 (1H, s, CH-O), 7.58 (1H, d,  $J=5.2$  Hz, pyridine 5-H), 8.75 (1H, d,  $J=5.2$  Hz, pyridine 6-H), 8.88 (1H, s, pyridine 2-H). EI-MS  $m/z$  402 ( $\text{M}^+$ ), 367 ( $\text{M}^+ - \text{Cl}$ ), 314 ( $\text{M}^+ - \text{OCON}(\text{CH}_3)_2$ ). EI-HRMS  $m/z$  ( $\text{M}^+$ ): Calcd. for  $\text{C}_{17}\text{H}_{14}\text{ClF}_3\text{N}_2\text{O}_4$ : 402.0594, Found: 402.0603.

#### 1.3.4. Acenaphthen-5-yl[4-(trifluoromethyl)pyridin-3-yl]methyl *N,N*-dimethylcarbamate (**2d**)

Compound **2d** was obtained from **12d** in 80% yield. White powder, mp 140–143°C.  $R_f=0.30$  (50% EtOAc in hexane).  $^1\text{H NMR } \delta_{\text{H}}$ : 2.95 (3H, s,  $\text{CH}_3\text{-N}$ ), 2.97 (3H, s,  $\text{CH}_3\text{-N}$ ), 3.27–3.45 (4H, m,  $\text{CH}_2\text{-CH}_2$ ), 6.99 (1H, d,  $J=7.2$  Hz, acenaphthene 3-H), 7.18 (1H, d,  $J=7.2$  Hz, ArH), 7.33 (1H, d,  $J=7.2$  Hz, ArH), 7.52 (1H, dd,  $J=8.4$  and 7.2 Hz, acenaphthene 7-H), 7.60 (1H, d,  $J=5.2$  Hz, pyridine 5-H), 7.76 (1H, d,  $J=8.4$  Hz, acenaphthene 6-H), 7.88 (1H, s, CH-O), 8.80 (1H, d,  $J=5.2$  Hz, pyridine 6-H), 8.97 (1H, s, pyridine 2-H). EI-MS  $m/z$  400 ( $\text{M}^+$ ), 311 ( $\text{M}^+ - \text{OCON}(\text{CH}_3)_2$ ). EI-HRMS  $m/z$  ( $\text{M}^+$ ): Calcd. for  $\text{C}_{22}\text{H}_{19}\text{F}_3\text{N}_2\text{O}_2$ : 400.1398, Found: 400.1404.

#### 1.3.5. Quinolin-4-yl[4-(trifluoromethyl)pyridin-3-yl]methyl *N,N*-dimethylcarbamate (**2e**)

Compound **2e** was obtained from **12e** in 41% yield. White powder, mp 92–94°C.  $R_f=0.25$  (EtOAc).  $^1\text{H NMR } \delta_{\text{H}}$ : 2.92

(3H,s, CH<sub>3</sub>-N), 2.93 (3H, s, CH<sub>3</sub>-N), 7.07 (1H, d, *J*=4.4 Hz, quinoline 3-H), 7.55 (1H, m, ArH), 7.62 (1H, d, *J*=5.2 Hz, pyridine 5-H), 7.72 (1H, m, ArH), 7.89 (1H, s, CH-O), 7.90 (1H, m, ArH), 8.15 (1H, d, *J*=8.4 Hz, ArH), 8.75 (1H, s, pyridine 2-H), 8.80 (1H, d, *J*=5.2 Hz, pyridine 6-H), 8.88 (1H, d, *J*=4.4 Hz, quinoline 2-H). EI-MS *m/z* 375 (M<sup>+</sup>), 287 (M<sup>+</sup>-OCON(CH<sub>3</sub>)<sub>2</sub>). EI-HRMS *m/z* (M<sup>+</sup>): Calcd. for C<sub>19</sub>H<sub>16</sub>F<sub>3</sub>N<sub>3</sub>O<sub>2</sub>: 375.1194, Found: 375.1186.

1.3.6. *Naphthalen-1-yl[4-(trifluoromethyl)pyridin-3-yl]methyl N,N-diethylcarbamate (4)*

Compound **4** was prepared from **12a** with *N,N*-diethylcarbamoyl chloride instead of *N,N*-dimethylcarbamoyl chloride in 80% yield. Amorphous solid. *R*<sub>f</sub>=0.39 (33% EtOAc in hexane). <sup>1</sup>H NMR δ<sub>H</sub>: 0.90–0.95 (3H, m, CH<sub>3</sub>-C), 1.00–1.11 (3H, m, CH<sub>3</sub>-C), 3.16–3.28 (4H, m, (CH<sub>2</sub>)<sub>2</sub>N), 6.99 (1H, d, *J*=7.2 Hz, naphthalene 2-H), 7.31 (1H, t, *J*=7.6 Hz, ArH), 7.43–7.48 (2H, m, ArH), 7.54 (1H, d, *J*=5.6 Hz, pyridine 5-H), 7.77–7.83 (2H, m, ArH), 7.89 (1H, s, CH-O), 7.91–7.94 (1H, m, ArH), 8.72 (1H, d, *J*=4.8 Hz, pyridine 6-H), 8.80 (1H, s, pyridine 2-H). EI-MS *m/z* 402 (M<sup>+</sup>), 286 (M<sup>+</sup>-OCON(C<sub>2</sub>H<sub>5</sub>)<sub>2</sub>). EI-HRMS *m/z* (M<sup>+</sup>): Calcd. for C<sub>22</sub>H<sub>21</sub>F<sub>3</sub>N<sub>2</sub>O<sub>2</sub>: 402.1555, Found: 402.1549.

1.3.7. *4-Bromopyridin-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. *R*<sub>f</sub>=0.15 (33% EtOAc in hexane). <sup>1</sup>H NMR δ<sub>H</sub>: 2.86 (6H, s, (CH<sub>3</sub>)<sub>2</sub>N), 7.16 (1H, d, *J*=6.0 Hz, naphthalene 2-H), 7.33 (1H, m, ArH), 7.44–7.49 (3H, m, ArH), 7.54 (1H, s, CH-O), 7.78–7.81 (2H, m, ArH), 8.03 (1H, d, *J*=8.4 Hz, ArH), 8.28 (1H, d, *J*=5.2 Hz, pyridine 6-H), 8.50 (1H, s, pyridine 2-H). EI-MS *m/z* 384 (M<sup>+</sup>), 296 (M<sup>+</sup>-OCON(CH<sub>3</sub>)<sub>2</sub>). EI-HRMS *m/z* (M<sup>+</sup>): Calcd. for C<sub>19</sub>H<sub>17</sub>BrN<sub>2</sub>O<sub>2</sub>: 384.0473, Found: 384.0466.

1.3.8. *4-Chloropyridin-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. *R*<sub>f</sub>=0.32 (50% EtOAc in hexane). <sup>1</sup>H NMR δ<sub>H</sub>: 2.94 (3H, s, CH<sub>3</sub>-N), 2.95 (3H, s, CH<sub>3</sub>-N), 7.23 (1H, d, *J*=5.6 Hz, pyridine 5-H), 7.39–7.42 (2H, m, ArH), 7.50–7.54 (2H, m, ArH), 7.84–7.89 (2H, m, ArH), 7.86 (1H, s, CH-O), 8.08 (1H, d, *J*=8.4 Hz, ArH), 8.48 (1H, d, *J*=5.6 Hz, pyridine 6-H), 8.62 (1H, s, pyridine 2-H). EI-MS *m/z* 340 (M<sup>+</sup>), 252 (M<sup>+</sup>-OCON(CH<sub>3</sub>)<sub>2</sub>). EI-HRMS *m/z* (M<sup>+</sup>): Calcd. for C<sub>19</sub>H<sub>17</sub>ClN<sub>2</sub>O<sub>2</sub>: 340.0978, 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. *R*<sub>f</sub>=0.25 (EtOAc). <sup>1</sup>H NMR δ<sub>H</sub>: 2.82 (3H, s, CH<sub>3</sub>-N), 2.86 (3H, s, CH<sub>3</sub>-N), 3.70 (3H, s, CH<sub>3</sub>-O), 6.67 (1H, d, *J*=5.6 Hz, pyridine 5-H), 7.31 (1H, d, *J*=5.2 Hz, naphthalene 2-H), 7.34–7.44 (3H, m, ArH), 7.70 (1H, m, ArH), 7.75 (1H, d, *J*=7.6 Hz, ArH), 7.79 (1H, s, CH-O), 8.09 (1H, d, *J*=8.4 Hz, ArH), 8.34 (1H, d, *J*=5.6 Hz, pyridine 6-H), 8.41 (1H, s, pyridine 2-H). EI-MS *m/z* 336 (M<sup>+</sup>), 248 (M<sup>+</sup>

-OCON(CH<sub>3</sub>)<sub>2</sub>). EI-HRMS *m/z* (M<sup>+</sup>): Calcd. for C<sub>20</sub>H<sub>20</sub>N<sub>2</sub>O<sub>3</sub>: 336.1474, Found: 336.1480.

1.3.10. *4-Methylpyridin-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. *R*<sub>f</sub>=0.45 (EtOAc). <sup>1</sup>H NMR δ<sub>H</sub>: 2.23 (3H, s, CH<sub>3</sub>-pyridine), 2.93 (6H, s, (CH<sub>3</sub>)<sub>2</sub>N), 7.09 (1H, d, *J*=5.2 Hz, pyridine 5-H), 7.20 (1H, d, *J*=7.2 Hz, naphthalene 2-H), 7.37 (1H, d, *J*=7.6 Hz, ArH), 7.47–7.54 (2H, m, ArH), 7.74 (1H, s, CH-O), 7.81 (1H, d, *J*=8.4 Hz, ArH), 7.86 (1H, d, *J*=8.0 Hz, ArH), 8.08 (1H, d, *J*=8.0 Hz, ArH), 8.41 (1H, d, *J*=5.2 Hz, pyridine 6-H), 8.56 (1H, s, pyridine 2-H). EI-MS *m/z* 320 (M<sup>+</sup>), 231 (M<sup>+</sup>-OCON(CH<sub>3</sub>)<sub>2</sub>). EI-HRMS *m/z* (M<sup>+</sup>): Calcd. for C<sub>20</sub>H<sub>20</sub>N<sub>2</sub>O<sub>2</sub>: 320.1525, Found: 320.1519.

1.3.11. *4-Cyanopyridin-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. *R*<sub>f</sub>=0.41 (66% EtOAc in hexane). <sup>1</sup>H NMR δ<sub>H</sub>: 2.94 (3H, s, CH<sub>3</sub>-N), 3.00 (3H, s, CH<sub>3</sub>-N), 7.43–7.46 (2H, m, ArH), 7.48–7.54 (3H, m, ArH), 7.73 (1H, s, CH-O), 7.84–7.89 (2H, m, ArH), 8.06 (1H, m, ArH), 8.68 (1H, d, *J*=4.0 Hz, pyridine 6-H), 8.77 (1H, s, pyridine 2-H). EI-MS *m/z* 331 (M<sup>+</sup>), 242 (M<sup>+</sup>-OCON(CH<sub>3</sub>)<sub>2</sub>). EI-HRMS *m/z* (M<sup>+</sup>): Calcd. for C<sub>20</sub>H<sub>17</sub>N<sub>3</sub>O<sub>2</sub>: 331.1321, Found: 331.1312.

1.4. *Naphthalen-1-yl[4-(trifluoromethyl)pyridin-3-yl]methyl N-methylcarbamate (3)*

To a stirred solution of **12a** (0.61 g, 2.0 mmol) in dry dichloromethane (15 mL) were added dibutyltin dilaurate (2 drops) and methyl isocyanate (0.34 g, 4.0 mmol) at 10°C. The mixture was stirred for 15 hr 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. *R*<sub>f</sub>=0.30 (50% EtOAc in hexane). <sup>1</sup>H NMR δ<sub>H</sub>: 2.76 (3H, d, *J*=6.0 Hz, CH<sub>3</sub>-N), 4.99 (1H, d, *J*=6.0 Hz, NH), 7.05 (1H, d, *J*=6.8 Hz, naphthalene 2-H), 7.35 (1H, t, *J*=7.6 Hz, ArH), 7.47–7.53 (2H, m, ArH), 7.56 (1H, d, *J*=4.8 Hz, ArH), 7.81–7.88 (2H, m, ArH), 7.94 (1H, s, CH-O), 8.00 (1H, d, *J*=7.6 Hz, ArH), 8.75 (1H, d, *J*=4.8 Hz, pyridine 6-H), 8.85 (1H, s, pyridine 2-H). EI-MS *m/z* 360 (M<sup>+</sup>), 286 (M<sup>+</sup>-OCONHCH<sub>3</sub>). EI-HRMS *m/z* (M<sup>+</sup>): Calcd. for C<sub>19</sub>H<sub>15</sub>F<sub>3</sub>N<sub>2</sub>O<sub>2</sub>: 360.1085, Found: 360.1077.

1.5. *Naphthalen-1-yl[4-(trifluoromethyl)pyridin-3-yl]methyl imidazol-1-ylcarboxylate (5)*

A mixture of **12a** (0.61 g, 2.0 mmol), triethylamine (0.31 mL, 2.2 mmol) and *N,N'*-carbonyldiimidazole (CDI) (0.34 g, 2.1 mmol) in dry 1,2-dichloroethane (20 mL) 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** (0.73 g) in 92% yield. White powder, mp 128–132°C. *R*<sub>f</sub>=0.24 (33% EtOAc in hexane). <sup>1</sup>H NMR δ<sub>H</sub>: 7.05 (1H, d, *J*=6.8 Hz, naphthalene 2-H), 7.30–7.52 (5H, m, ArH), 7.66 (1H, m, ArH), 7.83 (1H, s, CH-O), 7.84–7.92 (2H, m, ArH), 8.09–8.16 (2H, m, ArH),

8.72 (1H, d,  $J=4.8$  Hz, pyridine 6-H), 8.83 (1H, s, pyridine 2-H). EI-MS  $m/z$  397 ( $M^+$ ), 286 ( $M^+ - \text{OCO} - 1\text{-imidazolyl}$ ). EI-HRMS  $m/z$  ( $M^+$ ): Calcd. for  $C_{21}H_{14}F_3N_3O_2$ : 397.1038, Found: 397.1030.

#### 1.6. Naphthalen-1-yl(4-substituted pyridin-3-yl)methanol (13–15)

Preparation of **13**, **14** and **15** was performed by the same method as described for the general synthesis of aryl[4-(trifluoromethyl)pyridin-3-yl]methanol in 1.1.

##### 1.6.1. 4-Bromopyridin-3-yl(naphthalen-1-yl)methanol (13)

Compound **13** was prepared from 4-bromopyridine and 1-naphthaldehyde in 80% yield. Yellow powder, mp 157–163°C.  $R_f=0.20$  (33% EtOAc in hexane).  $^1\text{H NMR } \delta_{\text{H}}$ : 6.84 (1H, s, CH-O), 7.32 (1H, m, ArH), 7.43 (1H, m, ArH), 7.46–7.52 (3H, m, ArH), 7.82 (1H, d,  $J=8.0$  Hz, ArH), 7.88 (1H, m, ArH), 8.00 (1H, m, ArH), 8.26 (1H, d,  $J=5.2$  Hz, pyridine 6-H), 8.54 (1H, s, pyridine 2-H). EI-MS  $m/z$  313 ( $M^+$ ). EI-HRMS  $m/z$  ( $M^+$ ): Calcd. for  $C_{16}H_{12}\text{BrNO}$ : 313.0102, Found: 313.0109.

##### 1.6.2. 4-Chloropyridin-3-yl(naphthalen-1-yl)methanol (14)

Compound **14** was prepared from 4-chloropyridine and 1-naphthaldehyde in 71% yield. Pale yellow powder, mp 154–158°C.  $R_f=0.25$  (50% EtOAc in hexane).  $^1\text{H NMR } \delta_{\text{H}}$ : 6.91 (1H, s, CH-O), 7.33 (1H, d,  $J=5.2$  Hz, pyridine 5-H), 7.41–7.46 (2H, m, ArH), 7.47–7.53 (2H, m, ArH), 7.83 (1H, d,  $J=8.0$  Hz, ArH), 7.88 (1H, m, ArH), 8.01 (1H, m, ArH), 8.42 (1H, d,  $J=5.2$  Hz, pyridine 6-H), 8.61 (1H, s, pyridine 2-H). EI-MS  $m/z$  269 ( $M^+$ ), 252 ( $M^+ - \text{OH}$ ). EI-HRMS  $m/z$  ( $M^+$ ): Calcd. for  $C_{16}H_{12}\text{ClNO}$ : 269.0607, Found: 269.0616.

##### 1.6.3. 4-Methoxypyridin-3-yl(naphthalen-1-yl)methanol (15)

Compound **15** was prepared from 4-methoxypyridine and 1-naphthaldehyde in 35% yield by the same method except the use of PhLi instead of LDA at  $-10^\circ\text{C}$ . White powder, mp 188–197°C.  $R_f=0.29$  (EtOAc).  $^1\text{H NMR } \delta_{\text{H}}$ : 3.91 (3H, s,  $\text{CH}_3\text{-O}$ ), 6.83 (1H, s, CH-O), 6.84 (1H, d,  $J=6.0$  Hz, pyridine 5-H), 7.42–7.48 (3H, m, ArH), 7.57 (1H, d,  $J=6.8$  Hz, ArH), 7.81 (1H, d,  $J=8.4$  Hz, ArH), 7.86 (1H, m, ArH), 7.98 (1H, d,  $J=6.0$  Hz, ArH), 8.19 (1H, s, pyridine 2-H), 8.42 (1H, d,  $J=6.0$  Hz, pyridine 6-H). EI-MS  $m/z$  265 ( $M^+$ ). EI-HRMS  $m/z$  ( $M^+$ ): Calcd. for  $C_{17}H_{15}\text{NO}_2$ : 265.1103, Found: 265.1110.

##### 1.7. 3-(1-Naphthoyl)-4-(trifluoromethyl)pyridine (18)

To a stirred solution of **12a** (17.0 g, 56 mmol) in toluene (300 mL) was added 88%  $\text{MnO}_2$  (50 g, 0.22 mol). The mixture was refluxed for 2 hr under removing of water by Dean-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.  $R_f=0.43$  (20% EtOAc in hexane).  $^1\text{H NMR } \delta_{\text{H}}$ : 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  $C_{17}H_{10}F_3\text{NO}$ : 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 (1*R*,2*S*)-(+)-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  $5^\circ\text{C}$ , and the mixture was stirred for 1 hr at  $10^\circ\text{C}$ . A 90% borane dimethyl sulfide complex (1.7 g, 20 mmol) was then added dropwise for 10 min at  $5^\circ\text{C}$ . A solution of **18** (3.0 g, 10 mmol) in dry THF (10 mL) was added to the mixture at  $5^\circ\text{C}$  and this mixture was stirred for 2 hr at  $20^\circ\text{C}$ . To an ice-cooled reaction mixture,  $\text{CH}_2\text{Cl}_2$  (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  $5^\circ\text{C}$ . The organic layer was separated and dried over  $\text{Na}_2\text{SO}_4$ . 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.  $[\alpha]_{\text{D}}^{26} -41^\circ$  ( $c$  0.25,  $\text{CHCl}_3$ ). 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;  $20^\circ\text{C}$ , UV detector; 254 nm, retention time; (+)-**12a**  $t_{\text{R}}=5.0$  min and (–)-**12a**  $t_{\text{R}}=6.4$  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 (1*S*,2*R*)-(–)-1-amino-2-indanol. White powder, mp 142–146°C.  $[\alpha]_{\text{D}}^{26} +45^\circ$  ( $c$  0.25,  $\text{CHCl}_3$ ).

##### 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.  $[\alpha]_{\text{D}}^{24} -42^\circ$  ( $c$  1.0,  $\text{CHCl}_3$ ). Enantiomeric purity was determined to be 90% ee by chiral HPLC analysis. Solvent; 17% 2-propanol in hexane, flow rate; 1 mL/min, temp;  $20^\circ\text{C}$ , UV detector; 254 nm, retention time; (+)-**2a**  $t_{\text{R}}=11.4$  min and (–)-**2a**  $t_{\text{R}}=20.1$  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.  $[\alpha]_{\text{D}}^{24} +41^\circ$  ( $c$  1.0,  $\text{CHCl}_3$ ). Enantiomeric purity was determined to be 91% ee by chiral HPLC analysis.

## 2. Biological evaluation

### 2.1. Herbicidal efficacy against *Echinochloa oryzicola* and *Scirpus juncooides* 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. *Echinochloa oryzicola* (ECHOR) and *Scirpus juncooides* Roxb (SCPJO) were used in the experiments and grown under flooded conditions.

A ceramic pot (0.01 m<sup>2</sup>) 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 paddled. 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 ED<sub>80</sub>; ED<sub>80</sub> 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 condition as the herbicidal efficacy test. A ceramic pot (0.01 m<sup>2</sup>) was filled with paddy soil (400 mL) and kept wet by watering, and the upper layer of the soil was paddled. 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 ED<sub>20</sub>; ED<sub>20</sub> shows the dosage causing visual damage of 20%.

### 2.3. Test in concrete pot outside a greenhouse

A concrete pot (0.36 m<sup>2</sup>) 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), broadleaf weeds (BL; *Lindera pyxidaria*, *Rotala indica*, and *Elatine triandra*), and ECHOR were sown on the soil surface. On the next day, rice (*Oryza sativa* L. cv. Kinuhikari) 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, cafenstrole,<sup>7)</sup> one of the typical paddy field herbicides for ECHOR, was used as a positive control in this test.

## 3. Interaction with cytochrome P450 monooxygenase

### 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 (Invitrogen). *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'-TTAGTGATGATGATGATGATGGTTGTCCACAA-CAAGCTTC (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/XhoI* 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 *XbaI* 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 *XbaI* site into pGEX-6P-1: 5'-CGTATAATGTGTTCTAGAGTGAGCGGATAA (F2 primer) and 5'-TTATCCGCTCACTCTAGAACACATTATACG (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 *XbaI/SmaI*, 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<sup>13)</sup> 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 6,000 *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 10 mM 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

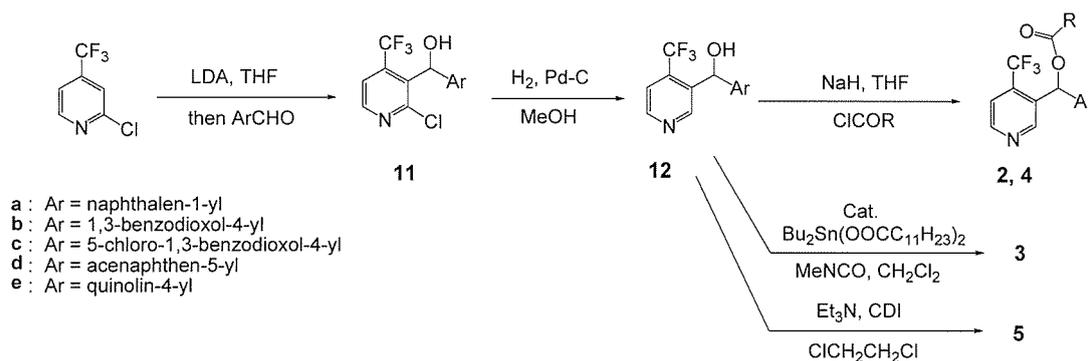


Fig. 3. Synthesis of aryl(4-CF<sub>3</sub>-pyridin-3-yl)methyl carbamates (2–5).

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<sup>-1</sup> cm<sup>-1</sup> for the CO-bound reduced P450 species.<sup>14</sup>

### 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]/Δ*A* for the *y*-axis, where [Compound] is the total concentration of added compound and Δ*A* is the change in absorption (peak-trough). The spectral dissociation constant was calculated from the intercept of the linear plot with the *x*-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 CF<sub>3</sub>-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 CF<sub>3</sub> 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 H<sub>2</sub>/palladium-carbon conditions in methanol to give **12**. Finally, **12** was reacted with *N,N*-dimethylcarbamoyl chloride to give **2** (R=NMe<sub>2</sub>; **2a**: Ar=naphthalen-1-yl, **2b**: Ar=1,3-benzodioxol-4-yl, **2c**: Ar=5-chloro-1,3-benzodioxol-4-yl, **2d**: Ar=5-acenaphthyl 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.

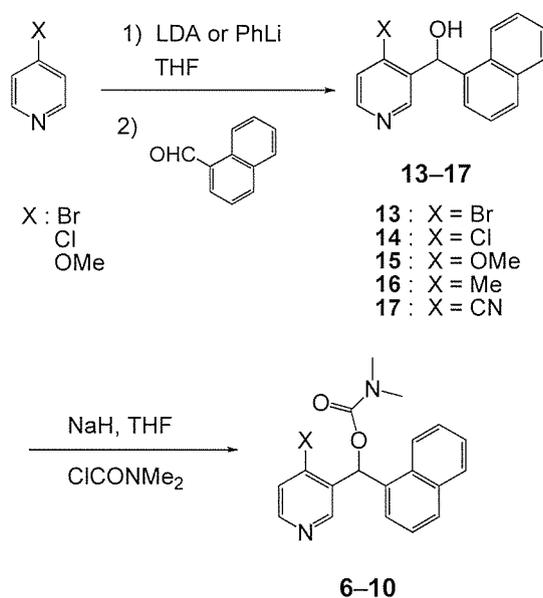


Fig. 4. Synthesis of naphthalen-1-yl(4-substituted pyridin-3-yl)methyl *N,N*-dimethylcarbamates (**6–10**).

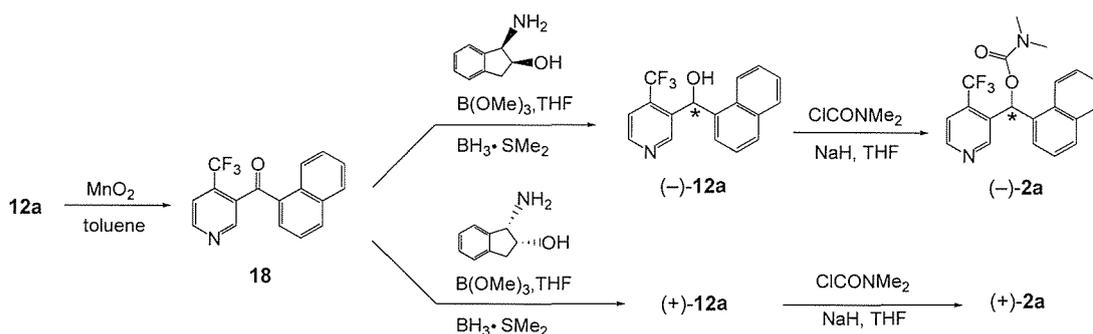


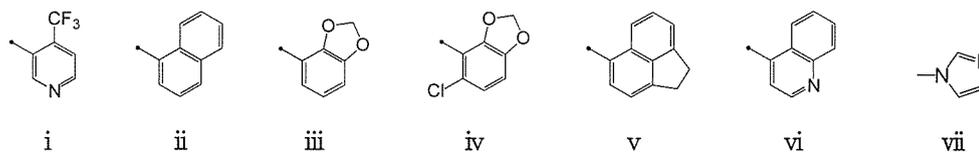
Fig. 5. Synthesis of (-)-2a and (+)-2a.

MnO<sub>2</sub> oxidation of racemic **12a** gave ketone **18** in 82% yield. Asymmetric reduction of **18** was achieved by the use of a chiral borane reagent.<sup>15)</sup> Treatment of **18** with the chiral reagent generated by the borane dimethyl sulfide complex with a

combination of trimethyl borate and (1*R*,2*S*)-(+)-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

Entry	Compound No.	Substituents			ED <sub>50</sub> (g a.i./ha)			ED <sub>20</sub> (g a.i./ha) Transplanted rice <sup>c)</sup>
		X	R	Ar	ECHOR <sup>a)</sup>		SCPJO <sup>b)</sup>	
					E	2.5 L	0.5 L	
1	1	CF <sub>3</sub>	NMe <sub>2</sub>	i	63	nt <sup>d)</sup>	63	<250
2	2a	CF <sub>3</sub>	NMe <sub>2</sub>	ii	31	125	250	2000
3	2b	CF <sub>3</sub>	NMe <sub>2</sub>	iii	63	nt	250	500
4	2c	CF <sub>3</sub>	NMe <sub>2</sub>	iv	63	500	500	>2000
5	2d	CF <sub>3</sub>	NMe <sub>2</sub>	v	500	>500	>1000	>2000
6	2e	CF <sub>3</sub>	NMe <sub>2</sub>	vi	125	nt	500	2000
7	3	CF <sub>3</sub>	NHMe	ii	63	250	500	2000
8	4	CF <sub>3</sub>	NEt <sub>2</sub>	ii	31	250	1000	>2000
9	5	CF <sub>3</sub>	vii	ii	>500	nt	>1000	nt
10	6	Br	NMe <sub>2</sub>	ii	31	250	250	2000
11	7	Cl	NMe <sub>2</sub>	ii	63	>500	63	1000
12	8	OMe	NMe <sub>2</sub>	ii	>1000	>1000	>1000	>2000
13	9	Me	NMe <sub>2</sub>	ii	500	nt	>1000	2000
14	10	CN	NMe <sub>2</sub>	ii	63	250	250	>1000



<sup>a)</sup> ECHOR: *Echinochloa oryzicola*. E; emergence stage. 2.5 L; 2.5 leaf stage. <sup>b)</sup> SCPJO: *Scirpus juncooides*. 0.5 L; 0.5 leaf stage. <sup>c)</sup> Phytotoxicity to rice (*Oryza sativa* L. cv. Nipponbare) that was planted at a depth of 3 cm from the surface of soil. <sup>d)</sup> nt: Not tested.

to give the crystalline compound (–)-**2a** in 55% yield with 90%ee. A similar reduction of **18** by the use of (1*S*,2*R*)-(–)-1-amino-2-indanol as a chiral ligand followed by carbamylation gave the another 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 CF<sub>3</sub>-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 CF<sub>3</sub>-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.<sup>11)</sup> In comparison with **1**, 1-naphthyl derivative **2a** exhibited twice as much activity against ECHOR at the emergence stage, with an ED<sub>80</sub> value of 31 g a.i./ha, though the activity against SCPJO 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. ED<sub>20</sub> 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 SCPJO (entry 4). However, the selectivity between the rice plant and paddy weeds of compound **2c** was better than that of **2b**. The acenaphthen-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 perfor-

mance 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 SCPJO 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 CF<sub>3</sub> 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 (ED<sub>80</sub>=63 g a.i./ha) but superior activity against SCPJO at the 0.5-leaf stage (ED<sub>80</sub> 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 SCPJO (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 2. Herbicidal spectrum and phytotoxicity of **2a** in outdoor concrete pot test

Compound No. (Name)	Application time <sup>a)</sup>	Rate g a.i./ha	Herbicidal efficacy <sup>b)</sup>							Phytotoxicity <sup>c)</sup> Transplanted rice	
			ECHOR	SCPJO	MOOVA	BL					
<b>2a</b>	0	200	+++++	+++++	+	+++	+++++	+++++	+++++	+++++	+++++
		100	+++++	+++++	+	++	+++++	+++++	+++++	+++++	+++++
<b>2a</b>	1.6	200	++++	+++++	+	+	+++++	+++++	+++++	+++++	+
		100	++++	+++++	+	+	++	+++++	+++++	+++++	+
Cafenstrole <sup>d)</sup>	1.6	300	+++++	+++++	+	+	++	++	+++++	+++++	+
<b>2a</b>	2.3	300	+++++	++++	+	++	+++++	+++++	+++++	+++++	++
		200	++++	++++	+	++	++++	++++	+++++	+++++	+
Cafenstrole <sup>d)</sup>	2.3	300	+++++	++++	+	+	++	+	+++++	++++	+

<sup>a)</sup> Leaf stage of ECHOR. <sup>b)</sup> +++++: complete inhibition, ++++: 99–95% inhibition, +++: 94–90%, ++: 89–50%, +: 49–0%. <sup>c)</sup> +: 0–5% inhibition, ++: 6–10%, +++: 11–15%, ++++: 16–30%. <sup>d)</sup> Positive control.

Table 3. Herbicidal efficacy of stereoisomers of 2a

Compound No.	ED <sub>80</sub> (g a.i./ha)					ED <sub>20</sub> (g a.i./ha) Transplanted rice
	ECHOR		SCPJO 0.5L	MOOVA <sup>a)</sup> 1.5 L	BL <sup>b)</sup> 1–2.2 L	
	E	2.5 L				
(–)-2a	<31	<63	125	<100	<63	500
(+)-2a	125	nt <sup>c)</sup>	>500	nt	nt	1000
2a	31	125	250	<100	100	2000

<sup>a)</sup> MOOVA : *Monochoria vaginalis*. 1.5 L; 1.5 leaf stage. <sup>b)</sup> BL: Average efficacy against annual broadleaf weeds such as *Lindera pyxidaria*, *Rotala indica* and *Elatine triandra* (1–2.2 leaf stage). <sup>c)</sup> nt: Not tested.

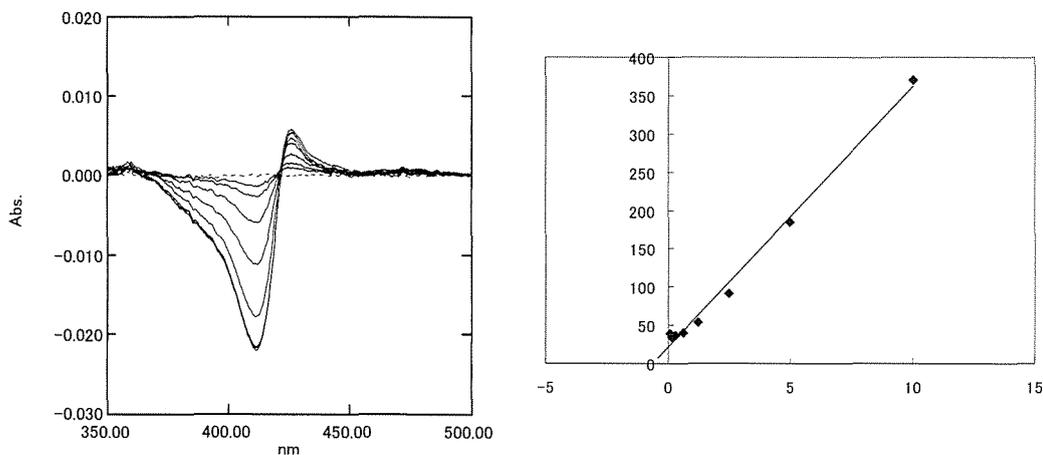


Fig. 6. Binding of compound 2a to *Sorghum halepense* CYP51 protein. Left, spectrophotometric titration of *Sorghum halepense* CYP51 protein with compound 2a. Right, a plot of compound 2a concentration/absorbance difference  $\Delta A$  (y-axis) against compound 2a concentration (x-axis).

leaf weeds (BL; described previously) at the dosage of 100 g/ha at the early stage (at the 0–1.6 leaf stage of ECHOR) but is not effective against perennial weeds, such as SCPJO. At the timing of the 2.3-leaf stage of ECHOR, 2a exhibited moderate herbicidal efficacy against weeds at the dosage of 200 g/ha. Based on the data, 2a possesses superior characteristics to cafenstrole.<sup>7)</sup>

### 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 monooxygenase

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, sug-

gesting 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

Compound No.	$K_d$ (M)
2a	$6.3 \times 10^{-7}$
2d	$9.4 \times 10^{-7}$
5	$3.4 \times 10^{-6}$
9	$6.5 \times 10^{-7}$

### 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

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### 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

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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, アルコール部分で縮合環とピ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)ピリジン-3-イル]メチル *N,N*-ジメチルカーバメート (**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さないことを見出した. 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化化合物は, オブツシフォリオール14 $\alpha$ -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.