Photosynthetic Electron Transport Inhibition by Pyrimidines and Pyridines Substituted with Benzylamino, Methyl and Trifluoromethyl Groups

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The decrease of the number of ring nitrogen atoms of 2-benzylamino-4-methyl-6-trifluoromethyl-1,3,5-triazines on herbicidal activity and inhibition of photosynthetic electron transport (PET) was assayed using thylakoids from *Spinacia oleracea* or atrazine-resistant *Chenopodium album*. Three 2-benzylamino-4-methyl-6-trifluoromethyl-1,3,5-triazines, nine pyrimidines with a benzylamino-, methyl- and trifluoromethyl-group, 2-benzylamino-6-methyl-4-trifluoromethyl- pyridine and *N*-benzyl-3-methyl-5-trifluoromethylaniline were synthesized and assayed. 2-(4-Bromobenzylamino)-4-methyl-6-trifluoromethylpyrimidine exhibited the highest PET inhibitory activity against *Spinacia oleracea* thylakoids of all compounds tested. The 2-benzylaminopyrimidines and 2-methylpyrimidines having a 4-halobenzylamino group exhibited higher PET inhibition than atrazine and 2-trifluoromethylpyrimidines against *Spinacia oleracea* thylakoids. These PET inhibitory active compounds also exhibited a strong and similar inhibition both against atrazine-resistant *Chenopodium album* thylakoids as well as against thylakoids from wild-type *Chenopodium*. The herbicidal activity of 4-(4-bromobenzylamino)-2-methyl-6- trifluoromethylpyrimidine was equivalent to that of known herbicides like simetryne, simazine or atrazine.

Introduction

2,4-Dialkylamino-1,3,5-triazines such as 2-chloro-4-diethylamino-6-isopropylamino-1,3,5- triazine (atrazine) were introduced as herbicides in 1952 by CIBA-GEIGY AG (Uemura *et al.*, 1988) and subsequently widely used around the world. Studies on herbicidal activity, mode of action, quantitative structure activity relationship and so on have been described in many reviews (van Rensen *et al.*, 1993; Trebst, 1987; Mitsutaka *et al.*, 1986). They inhibit photosynthetic electron transport (PET) by displacing plastoquinone from the Q_B-binding niche of the D1 protein in the photosystem-II reaction center (Oettmeier, 1999; Koike *et al.*, 1989; Tietjen *et al.*, 1993). Although the triazine herbicides have contributed to weed control for many years, they are now phasing out due to occurrence of triazine-resistant weeds, 60 plants were found to be triazine-resistant in 1997 (Kohno *et al.*, 2000).

Previously, we have reported on the herbicidal activity of novel 2-benzylamino-1,3,5-triazines (Kuboyama *et al.*, 1998). In our synthetic and bioassay programs, 2-benzylamino-4-methyl-6- trifluoromethyl-1,3,5-triazines, e.g. 2-(4-bromobenzylamino)-4-methyl-6-trifluoromethyl-1,3,5- triazine and 2-(3-chlorobenzylamino)-4-methyl-6-trifluoromethyl-1,3,5-triazine, exhibited both strong PET inhibitory and high herbicidal activity (Kuboyama *et al.*, 1999). Especially they have been found to exhibit a strong PET inhibition against both wild-type and atrazine-resistant thylakoids from *Chenopodium album* (Kohno *et al.*, 2000).

Materials and Methods

Chemicals

2-Benzylamino-4-methyl-6-trifluoromethyl-1,3,5-triazines (**1-3**) were prepared according to Kuboyama *et al.* (1998; 1999). The pyrimidines (**4-12**) were obtained by a nucleophilic replacement reaction of the corresponding chloropyrimidines with corresponding benzylamines. 2-Benzylamino-6-methyl-4-trifluoromethylpyridines (**13**, **14**) were synthesized by a nucleophilic monoamination reaction of 2-chloro-6-methyl-4-trifluoromethylpyridine with benzylamine and 4-chlorobenzylamine, respectively. *N*-Benzyl-3-methyl-5-trifluoromethyl-aniline (**15**) was prepared by reduction of *N*-benzyliden-3-methyl-5-trifluoromethylamiline (**e**) with LiAlH$_4$, which was obtained via reduction of 3-trifluoromethyl-5-nitrobenzoic acid (**a**) with BH$_3$-THF, bromination of 3-trifluoromethyl-5-nitrobenzylalcohol (**b**) with CBr$_4$-TPP, reduction of 3-bromomethyl-5-nitrobenzotrifluoride (**c**) using iron powder and conc. hydrochloride and condensation of 3-amino-5-methylbenzotrifluoride (**d**) with benzaldehyde. However, the product was a (1:1) mixture of *N*-benzyl-3-methyl-5-trifluoromethylaniline and *N*-benzyl-3-methyl-5-difluoromethyl-aniline. In the bioassay we used the mixture. Atrazine was purchased from Wako Pure Chemicals Industries (Osaka, Japan).

All reaction products were purified through recrystallization and/or column chromatography, and their structures confirmed by IR- and NMR spectroscopy. Melting points were not corrected. IR-spectra were recorded on a JASCO FT/IR-420 spectrophotometer and NMR spectra measured in CDCl$_3$ on a JEOL JNM-GX400 FT-NMR (400 Hz). The spectroscopical data of 2-benzylamino-4-methyl-6-trifluoromethyl-1,3,5-triazines (**1–3**) are shown in our previous papers (Kuboyama *et al.*, 1998; Kuboyama *et al.*, 1999). Spectroscopical data of compounds (**4-15**) are documented in Section below.

2-Benzylamino-4-methyl-6-trifluoromethylpyrimidine (**4**)

To a solution of 2-chloro-4-methyl-6-trifluoromethylpyrimidine (590 mg, 3 mmol) in 10 ml of tetrahydrofuran, benzylamine (375 mg, 3.5 mmol) and triethylamine (354 mg, 3.5 mmol) were added.

After stirring at room temperature for 4 h, the mixture was concentrated under reduced pressure. The residue was poured into 10 ml of water and extracted with dichloromethane. 2-Benzylamino-4-methyl-6-trifluoromethylpyrimidine (**4**) was purified by column chromatography over silica gel using ethyl acetate : *n*-hexane = 1 : 6 (v/v). Colorless crystals obtained amounted to 610 mg (76%), m.p. 79–80 °C. IR λ_{MAX} (KBr) cm^{-1} : 1595 (pyrimidine ring). NMR σ_H (CDCl$_3$) ppm : 2.42 (3H, s), 4.66 (2H, d, $J = 6.0$ Hz), 5.60 (1H, br), 6.74 (1H, s), 7.32 (5H, m).

2-(4-Chlorobenzylamino)-4-methyl-6-trifluoromethylpyrimidine (**5**)

This compound was prepared with 88% yield in the above-mentioned method. Colorless crystals showed m.p. 82–83 °C. IR λ_{MAX} (KBr) cm^{-1} : 1604 (pyrimidine ring). NMR σ_H (CDCl$_3$) ppm : 2.49 (3H, s), 4.67 & 4.78 (2H, each br), 5.65 (1H, br), 6.77 (1H, s), 7.35 (4H, m).

2-(4-Bromobenzylamino)-4-methyl-6-trifluoromethylpyrimidine (**6**)

In the same way, (**6**) was obtained with 55% yield. Colorless crystals showed m.p. 83–84 °C. IR λ_{MAX} (KBr) cm^{-1} : 1618 (pyrimidine ring). NMR σ_H (CDCl$_3$) ppm : 2.51 (3H, s), 4.68 & 4.88 (2H, each br), 5.67 (1H, br), 6.79 (1H, s), 7.36 (4H, m).s

4-Benzylamino-2-methyl-6-trifluoromethylpyrimidine (**7**)

Yield: 88%, colorless crystals, m.p. 66–67 °C. IR λ_{MAX} (KBr) cm^{-1} : 1611 (pyrimidine ring). NMR σ_H (CDCl$_3$) ppm : 2.58 (3H, s), 4.60 (2H, br), 5.55 (1H, brs), 6.48 (1H, s), 7.35 (5H, m). NMR σ_C (CDCl$_3$) ppm : 25.9 (s), 45.4 (br), 98.0 (br), 120.8 (q, $J = 275$ Hz), 127.4 (s), 127.8 (s), 128.8 (s), 137.1 (br), 162.8 (br), 169.0 (s)

4-(4-Chlorobenzylamino)-2-methyl-6-trifluoromethylpyrimidine (**8**)

Yield: 64%, colorless crystals, m.p. 68–69 °C. IR λ_{MAX} (KBr) cm^{-1} : 1607 (pyrimidine ring). NMR σ_H (CDCl$_3$) ppm : 2.47 (3H, s), 4.54 & 4.65 (2H, each br), 5.57 (1H, br), 6.49 (1H, s), 7.31 (4H, m).

4-(4-Bromobenzylamino)-2-methyl-6-trifluoromethylpyrimidine (9)

Yield: 68%, colorless crystals, m.p. 76–77 °C. IR λ_{MAX} (KBr) cm^{-1} : 1617 (pyrimidine ring). NMR σ_H (CDCl$_3$) ppm : 2.50 (3H, s), 4.59 & 4.68 (2H, each br), 5.60 (1H, br), 6.51 (1H, s), 7.33 (4H, m).

4-Benzylamino-6-methyl-2-trifluoromethylpyrimidine (10)

Yield: 77%, colorless crystals, m.p. 72–73 °C. IR λ_{MAX} (KBr) cm^{-1} : 1610 (pyrimidine ring). NMR σ_H (CDCl$_3$) ppm : 2.40 (3H, s), 4.55 (2H, br), 5.57 (1H, br), 6.26 (1H, s), 7.34 (5H, m).

4-(4-Chlorobenzylamino)-6-methyl-2-trifluoromethylpyrimidine (11)

Yield: 69%, colorless crystals, m.p. 98–99 °C. IR λ_{MAX} (KBr) cm^{-1} : 1607 (pyrimidine ring). NMR σ_H (CDCl$_3$) ppm : 2.39 (3H, s), 4.48 & 4.58 (2H, each br), 5.50 (1H, br), 6.25 (1H, s), 7.29 (4H, m).

4-(4-Bromobenzylamino)-6-methyl-2-trifluoromethylpyrimidine (12)

Yield: 70%, colorless crystals, m.p. 74–75 °C. IR λ_{MAX} (KBr) cm^{-1} : 1607 (pyrimidine ring). NMR σ_H (CDCl$_3$) ppm : 2.38 (3H, s), 4.48 & 4.58 (2H, each br), 5.50 (1H, br), 6.24 (1H, s), 7.30 (4H, m).

2-Benzylamino-6-methyl-4-trifluoromethylpyridine (13)

To a solution of 2-chloro-6-methyl-4-trifluoromethylpyridine (1.0 g, 0.0051 mol) in 15 ml of tetrahydrofuran, benzylamine (1.1 g, 0.01 mol) and triethylamine (1.04 g, 0.01 mol) were added. The mixture was refluxed for 12 h. The mixture was poured into 20 ml of water and extracted with dichloromethane. 2-Benzylamino-4-methyl-6-trifluoromethylpyrimidine (13) was purified by column chromatography over silica gel using ethyl acetate : *n*-hexane = 1 : 6 (v/v). Yield was 777 mg (77%), colorless crystals, m.p. 80–82 °C. IR λ_{MAX} (KBr) cm^{-1} : 1591 & 1620 (pyridine ring). NMR σ_H (CDCl$_3$) ppm : 2.44 (3H, s), 4.51 (2H, d, J = 5.9 Hz), 5.06 (1H, br), 6.37 (1H, s), 6.65 (1H, s), 7.31 (5H, m). NMR σ_C (CDCl$_3$) ppm : 24.3 (s), 46.2 (br), 99.0 (br), 107.7 (s), 123.1 (q, J = 275 Hz), 127.4 (s), 128.6 (s), 138.4 (s), 140.0 (q, J = 34 Hz), 158.4 (br), 158.7 (s).

2-(4-Chlorobenzylamino)-6-methyl-4-trifluoromethylpyridine (14)

Yield: 64%, colorless crystals, m.p. 70–74 °C. IR λ_{MAX} (KBr) cm^{-1} : 1592 & 1620 (pyridine ring). NMR σ_H (CDCl$_3$) ppm : 2.43 (3H, s), 4.42 & 4.51 (2H, br), 5.06 (1H, br), 6.32 (1H, s), 6.62 (1H, s), 7.32 (5H, m).

N-Benzyl-3-methyl-5-trifluoromethylaniline (15 : See Fig. 1)

3-Trifluoromethyl-5-nitrobenzylalcohol (b : b.p. 139 °C/1.5 mmHg) was synthesized by reduction of 3-trifluoromethyl-5-nitrobenzoic acid (a) with 1M BH$_3$-THF solution, and 3-bromomethyl-5-nitrobenzotrifluoride (c : b.p. 95 °C/1 mmHg) was prepared by bromination of 3-trifluoromethyl-5-nitrobenzylalcohol (b) with CBr$_4$, according to the methods of Honda *et. al.* (1996). 3-Amino-5-methylbenzotrifluoride (d : m.p. 26–28 °C, IR λ_{MAX} (KBr) cm^{-1} : 1625, 3381. NMR σ_H (CDCl$_3$) ppm : 2.31 (3H, s), 3.75 (2H, br), 6.64 (1H, s), 6.71 (1H, s), 6.81 (1H, s).) was obtained generally by reduction of 3-bromomethyl-5-nitrobenzotrifluoride (c) with iron powder and conc. hydrochloride. N-Benzyliden-3-methyl-5-trifluoromethylaniline (e : liquid, IR λ_{MAX} (KBr) cm^{-1} : 1632. NMR σ_H (CDCl$_3$) ppm : 2.45 (3H, s), 7.18 (1H, s), 7.26 (1H, s), 7.30 (1H, s), 7.50 (3H, m), 7.91 (2H, m), 8.45 (1H, s).) was synthesized by a condensation reaction of 3-amino- 5-methyl-benzotrifluoride (d) with benzaldehyde. N-Benzyl-3-methyl-5-trifluoromethylaniline (15 ; (liquid, IR λ_{MAX} (KBr) cm^{-1} : 1614, 3426. NMR σ_H (CDCl$_3$) ppm : 2.27 (3H, s), 3.96 (1H, br), 4.31 (2H, s), 6.46 (2H, m), 6.54 (1H, d), 7.25 (2H, m), 7.34 (3H, m).) was obtained by reduction of N- benzyliden-3-methyl-5-trifluoromethylaniline (e) with LiAlH$_4$. After purification by column chromatography over silica gel, the product was a 1:1 mixture of N-benzyl-3-methyl-5- trifluoromethylaniline and N-benzyl-3-methyl-5-difluoromethylaniline. The mixture was used for the bioassay.

Evaluation of PET inhibitory activity using Spinacia oleracea thylakoids

According to the method of Böger (1993) thylakoids were prepared from *Spinacia oleracea* leaves. Determination of photosynthetic electron
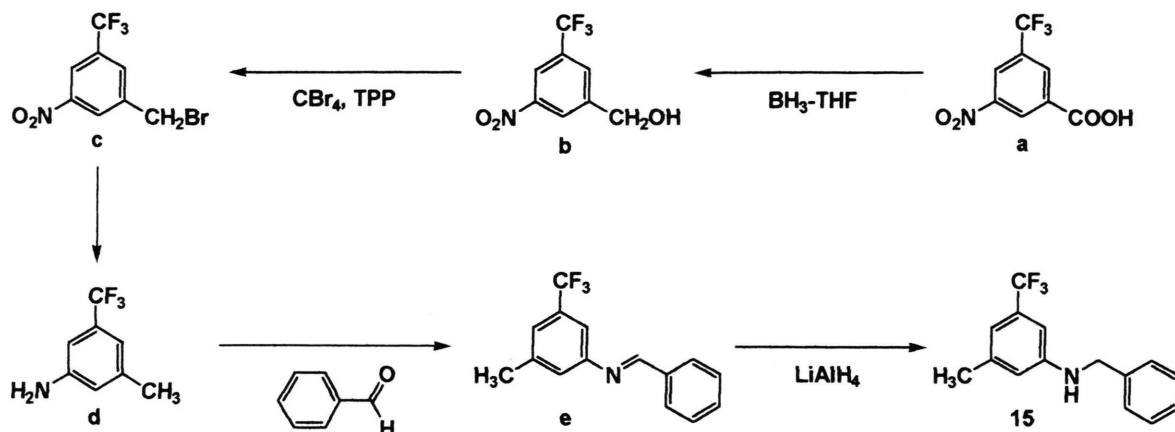
Fig. 1. Synthesis pathway of N-benzyl-3-methyl-5-trifluoromethylaniline (**15**).

transport (PET) inhibition was carried out using the oxygen electrode (Rank Brothers Bottisham, Cambridge, England) and the same methods as in our previous paper (Kuboyama *et al.*, 1999). The activity was determined by the system $H_2O \rightarrow$ potassium ferricyanide, uncoupled by NH_4Cl. The freshly-prepared thylakoid suspension was diluted to a final concentration of 15 μg chlorophyll/ml. The measurement was performed at 20 °C, for 20 sec in the light with 350 W/m^{-2} (approx. 35,000 lux); the final solvent concentration for the compounds added was kept below 1% (v/v). The molar concentration (I_{50}) required for a 50% inhibition was calculated for each compound by the probit method. The pI_{50} value is the logarithm of the reciprocal I_{50}. The test results are shown in Table I.

Evaluation of PET inhibitory activity using thylakoids from atrazine resistant-mutant or wild-type Chenopodium album

Cultivation of *Chenopodium album* either atrazine-resistant or wild-type, and isolation of thylakoids were done according to Jansen *et al.* (1986) and van Rensen *et al.* (1997). Chlorophyll content was measured after Bruinsma (1963), the chlorophyll concentration adjusted to 25 μg chlorophyll/ml. For details see our previous paper (Kohno *et al.*, 2000). The oxygen formed was measured at 25 °C, during a 20 sec illumination with 350 W/m^{-2}; the final solvent concentration for the compounds added was kept below 1% (v/v). The

pI_{50} values of PET inhibitory activity are shown in Table II.
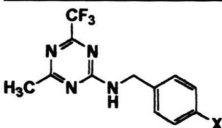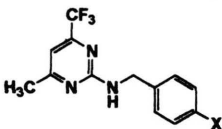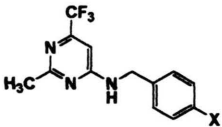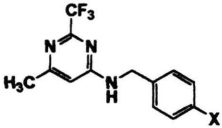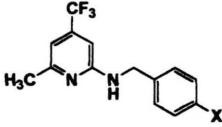
Determination of herbicidal activity
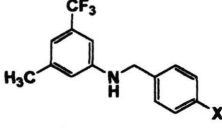
Each compound was formulated as a 10% wettable powder. The determination of herbicidal activity was carried out by three tests, namely paddy application (pre-emergence), field application (pre-emergence) and foliar application (post-emergence). Growth inhibition of compounds was evaluated at 4 or 1 kg a.i./ha. The test plants were *Echinochloa oryzicola*, *Monochoria vaginalis*, *Scirpus juncoides*, *Rotala indica*, *Echinochloa crusgalli*, *Digitaria ciliaris*, *Stellaria media* and *Chenopodium album*. Three weeks after treatment with the test compounds, the herbicidal activity was assayed by visual observation of the treated plants in comparison with the untreated control. The herbicidal potency was assessed by a 0–5 scale where 5 indicates complete kill, and zero indicates no effect. The test results are shown in Table III.

Results and Discussion

PET inhibitory activity of six-membered nitrogen heterocyclic compounds against Spinacia oleracea thylakoids

The effect of six-membered nitrogen heterocyclic compounds with reduced number of nitrogen atom(s) in the 1,3,5-triazine ring on PET inhibitory activity was examined using *Spinacia oleracea* thylakoids (Table I). Assaying compounds with a

Table I. PET inhibitory activity of synthesized six-membered nitrogen heterocyclic compounds against *Spinacia oleracea* thylakoids.

Structural	pI_{50} (*Spinacia oleracea*)					
formulas	No.	X : H	No.	X : Cl	No.	X : Br
	1	6.85	2	6.98	3	6.94
	4	6.15	5	7.17	6	7.20
	7	6.07	8	7.18	9	7.12
	10	4.58	11	6.01	12	6.17
	13	5.31	14	6.64		–
	15	4.76		–		–
Atrazine		6.73				

benzylamino group (**1**, **4**, **7**, **10**, **13**, **15**), 2-benzyl-amino-4-methyl-6-trifluoromethyl-1,3,5-triazine (**1**) was the most potent inhibitor and the pI_{50} value of 6.85 was higher than that of atrazine (pI_{50} = 6.73). The pI_{50} values of three pyrimidines (**4**, **7**, **10**), a pyridine (**13**) and a aniline (**15**), with less nitrogen-atom(s) in the triazine ring decreased when compared with 1,3,5-triazine (**1**). 4-Benzy-lamino-6-methyl-2-trifluoromethylpyrimidine (**10**) as well as *N*-benzyl-3-methyl-5-trifluoromethylanili-line (**15**) exhibited no strong inhibitory activity. The aniline (**15**) was a mixture of *N*-benzyl-3-methyl-5-trifluoromethylaniline and *N*-Benzyl-3-methyl-5-difluoromethylaniline. (See Materials & Methods) We found PET inhibitory activity in the order 1,3,5-triazine (**1**) > the 2-benzylaminopyri-midine (**4**) $\geqq$ the 2-methylpyrimidine (**7**) > the pyridine (**13**) > the aniline (**15**) $\geqq$ the 2-trifluoro-methylpyrimidine (**10**). With the pyrimidine iso-mers, a position-specific effect was observed. The 2-benzylaminopyrimidine (**4**, pI_{50} = 6.15) and the 2-methylpyrimidine (**7**, pI_{50} = 6.07) showed a bet-ter PET inhibitory activity, that of 2-trifluorometh-ylpyrimidine (**10**, pI_{50} = 4.58) was weaker than that of pyrimidines (**4**, **7**) and the pyridine (**13**, pI_{50} = 5.31). These findings indicate that a nitrogen atom in the six-membered heterocyclic ring located be-tween the methyl and benzylamino group may be essential for the highly active PET inhibitors. Compounds having 4-chlorobenzylamine (**2**, **5**, **8**,

11, 14), 2-benzylaminopyrimidine (**5**, pI_{50} = 7.17) and 2-methylpyrimidine (**8**, pI_{50} = 7.18) exhibited a stronger inhibition than atrazine (pI_{50} = 6.73) and 1,3,5-triazine (**2**, pI_{50} = 6.98). A poor activity was found with 2-trifluoromethylpyrimidine (**11**), which has a carbon atom in the six-membered heterocyclic ring between the methyl and benzylamino group. Also in compounds having 4-bromobenzylamine (**3, 6, 9, 12, 15**), introduction of a nitrogen atom produced an excellent PET inhibitory activity, when placed into the six-membered heterocyclic ring between the methyl and benzylamino group. Especially, the pI_{50} value of 2-(4-bromobenzylamino)-4-methyl-6-trifluoromethylpyrimidine (**6**) was the highest of the compounds tested. 2-Benzylaminopyrimidines (**5, 6**) and 2-methylpyrimidines (**8, 9**) having 4-halobenzylamino group exhibited a stronger inhibition than atrazine and the 1,3,5-triazines (**1-3**). Also their activity was about 10 times stronger than that of unsubstituted benzylaminopyrimidines (**4, 7**). These facts indicate that introduction of a halogen at 4-position of the benzene ring in the tested benzylaminopyrimidine isomers may be favorable for enhancing the PET inhibitory activity of unsubstituted benzylaminopyrimidines. Kuboyama *et al.* (1999) reported that the introduction of halogen and trifluoromethyl substituents at 3- and/or 4-position of the benzene ring was advantageous to increase the PET inhibitory activity of the unsubstituted benzylamino- 1,3,5-triazines against *Spinacia oleracea* thylakoids. We are now investigating the effect of additional substituents, at the 2-benzylaminopyrimidines and 2-methylpyrimidines on PET inhibition.

Table II. PET inhibitory activity of synthesized six-membered nitrogen heterocyclic compounds against thylakoids of atrazine resistant or sensitive *Chenopodium album*.

| No.	Structural formulas	pI_{50} (*Chenopodium album*)		R/S*	pI_{50} (*Spinacia oleracea*)
		Resistant type	Sensitive type		
2		6.93	7.19	1.8	6.98
5		6.26	6.44	1.5	7.17
8		5.80	5.87	1.2	7.18
11		5.00	4.98	1.0	6.01
14		5.34	5.53	1.6	6.64
	Atrazine	4.21	6.72	324	6.73

* R/S value: I_{50} (resistant) / I_{50} (sensitive)

PET inhibitory activity of six-membered nitrogen heterocyclic compounds against thylakoids from atrazine-resistant or -sensitive Chenopodium album

Kohno *et al.* (2000) reported that benzylamino-1,3,5-triazines (**1-3**) were highly active in atrazine-resistant plants. However pyrimidine isomers, which are superior to benzylamino-1,3,5-triazines (**1-3**) in PET inhibitory activity against *Spinacia oleracea* thylakoids, have not been investigated yet. To decide whether binding partners (amino acid residues) of the D1 protein for our synthesized compounds are same as for atrazine, PET inhibition of our compounds was checked using thylakoids from atrazine-resistant or sensitive *Chenopodium album*. As shown in Table II, atrazine ($pI_{50} = 6.72$) showed a stronger PET inhibitory activity in sensitive *Chenopodium album* thylakoids than in the resistant-type ones ($pI_{50} = 4.21$). The resistance factor R/S [I_{50} (resistant)/ I_{50} (sensitive)] was 324. PET inhibition of pyrimidines (**5, 8, 11**) and pyridine (**14**) against sensitive-type thylakoids was almost the same as against resistant-type thylakoids, and their R/S values were found around 1. For example, the PET inhibitory activities of 2-(4-chlorobenzylamino)pyrimidine (**5**) were $pI_{50} = 6.26$ and 6.44 for the sensitive and resistant type, respectively. With resistant thyla-

koids, 4-chlorobenzylamino-1,3,5-triazine (**2**) exhibited a 500 times stronger inhibition than atrazine, and 2-(4-chlorobenzylamino)pyrimidine (**5**) yielded an about 100 times stronger inhibition. With pyrimidine isomers, the position-specific effect was also observed as discussed on page 207. PET inhibition of 2-benzylaminopyrimidine (**5**) was the highest with pyrimidine isomers and 2-trifluoromethylpyrimidine (**11**) had no stronger inhibitory activity. This finding implies that a nitrogen atom in the pyrimidine ring between the methyl and benzylamino group may be essential for highly active PET inhibitors. 1,3,5-Triazine (**2**) exhibited almost the same inhibition against thylakoids from *Chenopodium album* or *Spinacia oleracea*. Inhibition by pyrimidine isomers (**5, 8, 11**) and pyridine (**14**) with sensitive *Chenopodium album* thylakoids was about 10 times weaker than with *Spinacia oleracea* thylakoids. These results show that the synthesized compounds in this study may have different binding partners in the same D1 protein binding niche as atrazine, as indicated for 1,3,5-triazines (**1–3**) in our previous paper (Ohki *et al.*, 1999).

Table III. Herbicidal activity of synthesized pyrimidine isomers
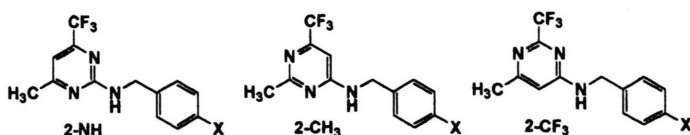
No.	Structural formulas	X	Paddy application test (pre-emergence) [1]					Field application test (pre-emergence) [1]					Foliar application test (post-emergence) [1]				
			Dose[2]	Eo[3]	Mv[3]	Sj[3]	Ri[3]	Dose[2]	Ec[3]	Dc[3]	Sm[3]	Ca[3]	Dose[3]	Ec[3]	Dc[3]	Sm[3]	Ca[3]
4	2-NH	H	4	0		5	3	5	4	nt[4]	nt[4]	nt[4]	nt[4]	4	0	0	3	3
5	2-NH	Cl	8	5	5	3	5	8	5	5	5	5	8	3	5	5	5
6	2-NH	Br	4	3	5	5	5	4	4	5	nt[4]	5	4	0	0	nt[4]	5
7	2-CH₃	H	4	5	5	5	5	4	2	2	3	5	4	2	2	5	5
8	2-CH₃	Cl	8	5	5	5	5	8	5	5	5	5	8	4	4	5	5
9	2-CH₃	Br	8	5	5	5	5	8	4	5	5	5	8	4	5	5	5
11	2-CF₃	Cl	8	4	5	2	4	8	4	1	4	4	8	0	0	5	4
	Simetryne		8	5	5	5	5	–	–	–	–	–	–	–	–	–	–
	Simazine		–	–	–	–	–	8	5	5	5	5	–	–	–	–	–
	Atrazine		–	–	–	–	–	–	–	–	–	–	8	5	5	5	5

[1] Herbicidal activities were visually evaluated by the following score scale : 5 = 91 to 100% control (almost kill), 4 = 76 to 90% control, 3 = 51 to 75% control, 2 = 26 to 50% control, 1 = 1 to 25% control, 0 = zero% control (no effect)
[2] Dose: kg a.i./ha
[3] Eo, *Echinochloa oryzicola*; Mv, *Monochoria vaginalis*; Sj, *Scirpus juncoides*; Ri, *Rotala indica*; Ec, *Echinochloa crus-galli*; Dc, *Digitaria ciliaris*; Sm, *Stellaria media*; Ca, *Chenopodium album*
[4] nt; not tested.

Herbicidal activity of pyrimidine isomers

As shown in Table III, the position-specific effect was also observed as discussed on page ▶▶. 2-Benzylaminopyrimidines (**4**, **5**, **6**) and the 2-methylpyrimidines (**7**, **8**, **9**), which exhibited a higher PET inhibitory activity, showed high herbicidal activity in the pre-emergence paddy test. The activity of the 2-trifluoromethylpyrimidine (**11**), which showed a poor PET inhibition, was weaker. In the pre-emergence field test and post-emergence foliar test, 2-benzylaminopyrimidine (**5**) and the 2-methylpyrimidines (**7**, **8**) showed high herbicidal activity, while the 2-trifluoromethylpyrimidines (**11**) exhibited a poor herbicidal activity. Although 2-(4-bromobenzylamino)-4-methyl-6-trifluoromethylpyrimidine (**6**) had the highest PET inhibitory activity of the compounds tested, herbicidal activity of 2-benzylaminopyrimidine (**6**) was weaker than 2-methylpyrimidines (**8**, **9**). 2-Methylpyrimidines (**8**, **9**) exhibited a herbicidal activity similar to known herbicides, such as simetryne, simazine or atrazine in all three pot tests. We are now investigating the effect of additional substituents of 2-benzylaminopyrimidines and 2-methylpyrimidines on herbicidal activity.

Böger P. (1993), Inhibition of the photosynthetic electron transport system. In: Target Assays for Modern Herbicides and Related Phytotoxic Compounds (P. Böger and G. Sandmann, eds.). Lewis Publ., Boca Raton. FL, pp. 83–91.

Bruinsma J. (1963), The quantitative analysis of chlorophylls a and b in plant extracts. Photochem. Photobiol. **2**, 214–249.

Honda T., Makino T., Nagato T., Kito Y. and Kimura N. (Chugai-Seiyaku Co. Ltd.) (1996), Tokkyo Kokai Koho JP H08–269006.

Jansen M. A. K., Hobé J. H., Wesselius J. H. and van Rensen J. J. S. (1986), Comparison of photosynthetic activity and growth performance in triazine-resistant and susceptible biotypes of *Chenopodium album*. Physiol. Vég. **24**, 475–484.

Kohno H., Ohki A., Ohki S., Koizumi K., Kuboyama N., van den Noort M. E., Rodrigues G. C., van Rensen J. J. S. and Wakabayashi K. (2000), Low resistance against novel 2-benzylamino-1,3,5-triazine herbicides in atrazine-resistant *Chenopodium album* plants. Photosynth. Res., in press.

Koike H., Azami T., Yoshida S., Takahashi N. and Inoue Y. (1989), A new-type photosystem II inhibitor which blocks electron transport in water-oxidation system. Z. Naturforsch. **44c**, 271–279.

Kuboyama N., Koizumi K., Ohki S. and Wakabayashi K. (1998), Synthesis and herbicidal activity of 2-benzylamino-4-methyl-6-trifluoromethyl-1,3,5-triazine derivatives. J. Pestic. Sci. **23**, 268–274.

Kuboyama N., Koizumi K., Ohki A., Ohki S., Kohno H. and Wakabayashi K. (1999), Photosynthetic electron transport inhibitory activity of 2-aralkylamino-4-methyl-6-trifluoromethyl-1,3,5-triazine derivatives. J. Pestic. Sci. **24**, 138–142.

Mitsutaka K., Iwamura H., Shimizu R. and Fujita T. (1986), Quantitative structure-activity relationship of photosystem II inhibitors in chloroplasts and its links to herbicidal action. J. Agric. Food. Chem. **34 (4)**, 725–732.

Ohki A., Kuboyama N., Koizumi K., Tanaka A., Sato Y., Kohno H., Böger P. and Wakabayashi K. (1999), Mode of action of novel 2-(benzylamino)-4-methyl-6-(trifluoromethyl)-1,3,5-triazine herbicides: inhibition of photosynthetic electron transport and binding studies. J. Agric. Food Chem. **47**(10), 4398–4402.

Oettmeier W. (1999), Herbicide resistance and supersensitivity in photosystem II. Cell. Mol. Life Sci. **55**, 1255–1277.

Tietjen K. G., Draber W., Goossens J., Jansen J. R., Kluth J. F., Schindler M., Wroblowsky H. J., Hilp V. and Trebst A. (1993), Binding of triazines and triazinones in the Q_B-binding niche of photosystem II. Z. Naturforsch. **48c**, 205–212.

Trebst A. (1987), The three-dimensional structure of the herbicide binding niche on the reaction center polypeptides of photosystem II. Z. Naturforsch. **42c**, 742–750.

Uemura S., Kawamura H., Tsuji M., Tomita S. and Maeda S. (1988), Dictionary of Toxicity of Agrochemicals (Noyaku dokusei no jiten), Sanseidou Publishing Co. Ltd., Tokyo. van Rensen J. J. S., van den Vet W. and van Vliet W. P. A. (1977), Inhibition and uncoupling of electron transport in isolated chloroplasts by the herbicide 4,6-dinitro-*o*-cresol. Photochem. Photobiol. **25**, 579–583.

van Rensen J. J. S. (1993), Regulation of electron transport at the acceptor side of photosystem II by herbicides, bicarbonate and formate. In: Photosynthesis : Photoreactions to Plant Productivity (Abrol Y. P., Mohanty P., Govindjee K., eds.). Oxford & IBH Publishing Co., New Delhi India, pp.157–180.