Synthesis of Novel Diphenyl Ether Herbicides

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The benzoxazine derivatives are a new chemical family of diphenyl ether herbicides, which exhibit a strong peroxidizing herbicidal activity on mono- and dicotyledonous species in preemergence and postemergence tests. Twenty derivatives were synthesized, and their herbicidal activity was determined to examine structure–activity relationships. Among the compounds investigated, it was found that the fluorine atom introduction into the trifluoromethylbenzene moiety together with an oxazine ring instead of a nitro group led to the most active herbicide.

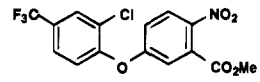
Keywords: *Peroxidizing herbicides; benzoxazine derivatives; protoporphyrin IX*

INTRODUCTION

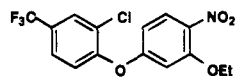
Diphenyl ether herbicides exemplified by acifluorfen methyl and oxyfluorfen (Figure 1) exhibit a broad spectrum of activity to weeds and have been used for the control of annual grasses and broad-leaf weeds. The herbicidal effect has been conjectured to be caused by a light- and oxygen-dependent peroxidation of unsaturated membrane lipids, followed by a destruction of chloroplast membranes and cell constituents (Matsunaka, 1969; Kunert and Böger, 1981). Recently, many reports have been published that these peroxidizing herbicides exert their light-dependent phytotoxic effects by inducing an accumulation of protoporphyrin IX (Matringe and Scalla, 1988; Sandmann and Böger, 1988; Witkowski and Halling, 1988; Becerril and Duke, 1989; Iwata et al., 1992). We have also observed that the accumulation of protoporphyrin IX and further metabolites can be induced by the oxidative condition established by peroxiding herbicides using a liquid culture system of liverwort cells (Iwata et al., 1994).

Thus, the primary mode of action of peroxidizing herbicides is the inhibition of protoporphyrinogen oxidase and the accumulation of protoporphyrin IX, which may occur by nonenzymic oxidation of protoporphyrinogen or be mediated by enzymes present, for example, in the cell membrane (Jacobs et al., 1991). These results also indicate that another mode of phytotoxic action of the herbicide might be the induction of an oxidative condition in plant cells. On the basis of these observations, we sought to develop a new class of diphenyl ether herbicides without a nitro group in the structure, although electron-withdrawing groups such as nitro, cyano, carboxy, and halogen usually have been used in chemical modification to develop useful herbicides of higher potency.

Thus, we focused on some compounds in which the nitro group was reduced to an amino or amido group and had a substituent at the 3-position of the diphenyl ether skeleton, known to increase the activity of diphenyl ether herbicides. In an attempt to test the herbicidal activity of synthesized derivatives, we found that the benzoxazine derivatives (I of Scheme 1) of diphenyl ethers exhibit excellent herbicidal activities with similar phytotoxicity symptoms in the whole plant as *p*-nitro diphenyl ether herbicides. Herein, we report the chemical and biological properties of some aryloxybenzoxazine derivatives.



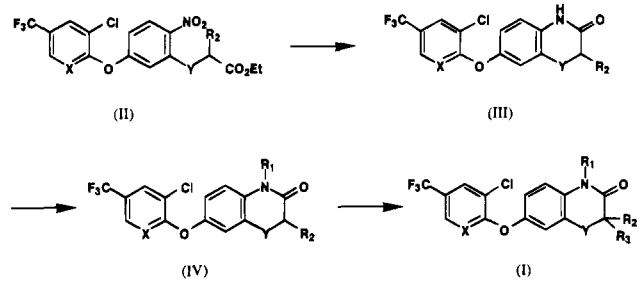
Acifluorfen methyl



Oxyfluorfen

Figure 1. Classical *p*-nitrodiphenyl ether herbicides with peroxidative activity.

Scheme 1. Synthesis Steps of 7-(Aryloxy)-2,4-disubstituted-1,4-benzoxazin-3-ones[a]



[a] See text for details. R and Y are the same as in Table 1.

EXPERIMENTAL PROCEDURES

Synthesis. Melting points were measured by using a Mettler FP-61 (automatic melting apparatus) and are uncorrected. ^{1}H-NMR spectra were recorded on a JEOL JNM-FX 100 spectrometer at 100 MHz using TMS as an internal reference. High-resolution mass spectra were obtained on a JEOL JMX-HX/HX-110A by the FAB ionization method. Analytical data for the compounds are listed in Table 1.

7-(Aryloxy)-2,4-disubstituted-1,4-benzoxazin-3-ones (Scheme 1, Compounds 1–15). Nitro derivatives (II) prepared by usual methods (Foerster et al., 1980) were reduced and successively

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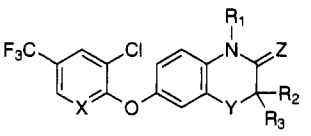
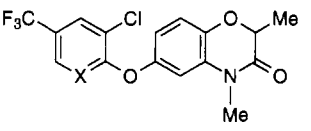
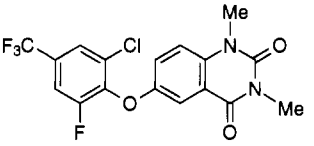
Table 1. Analytical Data for Benzoxazine Derivatives



compd	X	Y	Z	R_1	R_2	R_3	mp, °C	molecular formula	high MS (m/z, M + 1) found	high MS (m/z, M + 1) calcd
1	CH	O	O	H	Me	H	139–140.5	$C_{16}H_{11}NO_3F_3Cl$	357.0370	357.0380
2	CH	O	O	Me	Me	H	81–84	$C_{17}H_{13}NO_3F_3Cl$	371.0530	371.0536
3	CH	O	O	Et	Me	H	67–69	$C_{18}H_{15}NO_3F_3Cl$	385.0719	385.0693
4	CH	O	O	propargyl	Me	H	111–112.5	$C_{19}H_{13}NO_3F_3Cl$	395.0516	395.0536
5	CH	O	O	i-propyl	Me	H	125–127.5	$C_{19}H_{17}NO_3F_3Cl$	399.0862	399.0849
6	CH	O	O	Me	Me	Me	71.5–73	$C_{18}H_{15}NO_3F_3Cl$	385.0667	385.0693
7	CH	O	O	Me	Me	Ph	163–164.5	$C_{23}H_{17}NO_3F_3Cl$	447.0854	447.0849
9	N	O	O	Me	Me	H	77.5–79.5	$C_{16}H_{12}N_2O_2F_3Cl$	372.0509	372.0489
11	CF	O	O	H	Me	H	149.5–151.5	$C_{16}H_{10}NO_3F_4Cl$	375.0254	375.0285
12	CF	O	O	Me	Me	H	62–64	$C_{17}H_{12}NO_3F_4Cl$	389.0465	389.0442
13	CCl	O	O	Me	Me	H	80–81.5	$C_{17}H_{12}NO_3F_3Cl_2$	405.0146	405.0171
14	CF	O	O	CHF_2	Me	H	59–63	$C_{17}H_{10}NO_3F_6Cl$	425.0274	425.0253
15	CF	S	O	Me	Me	H	67–68	$C_{17}H_{12}NO_2SF_4Cl$	405.0211	405.0213
16	CF	O	S	Me	Me	H	81.5–83.5	$C_{17}H_{12}NO_2SF_4Cl$	405.0199	405.0213
17	CF	O	$CHNO_2$	Me	Me	H	164–165	$C_{18}H_{13}N_2O_4F_4Cl$	432.0523	432.0500
18	CF	CH_2	O	Me	H	H	53–55	$C_{17}H_{12}NO_2F_4Cl$	373.0505	373.0493
19	CF	CH_2	O	Me	Me	H	oily	$C_{18}H_{14}NO_2F_4Cl$	387.0656	387.0649



compd	X	mp, °C	molecular formula	high MS (m/z, M + 1) found	high MS (m/z, M + 1) calcd
8	CH	71.5–73	$C_{17}H_{13}NO_3F_3Cl$	371.0527	371.0536
10	N	103–105	$C_{16}H_{12}N_2O_2F_3Cl$	372.0495	372.0489



compd	mp, °C	molecular formula	high MS (m/z, M + 1) found	high MS (m/z, M + 1) calcd
20	154–155.5	$C_{17}H_{11}N_2O_3F_4Cl$	402.0421	402.0394

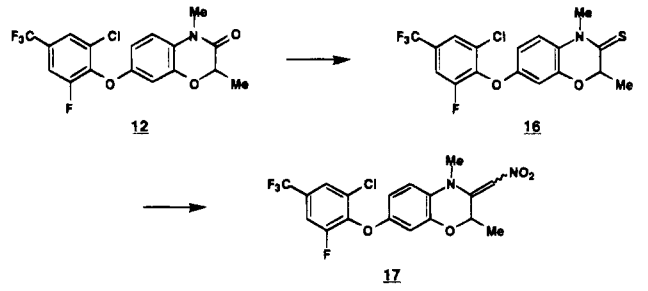
cyclized to benzoxazine derivatives (III). Then, benzoxazine derivatives (III) were N-alkylated to yield the title compounds. We refer to 7-[2-chloro-4-(trifluoromethyl)phenoxy]-2-methyl-2*H*,4*H*-1,4-benzoxazin-3-one (**1**) and 7-[2-chloro-4-(trifluoromethyl)phenoxy]-2,4-dimethyl-2*H*,4*H*-1,4-benzoxazin-3-one (**2**) as typical examples.

7-[2-Chloro-6-fluoro-4-(trifluoromethyl)phenoxy]-2,4-dimethyl-3-(nitromethylene)-2H-1,4-benzoxazine (Scheme 2, Compound 17). The benzoxazinone derivative (**12**) was converted to the corresponding thione (**16**) by Lawesson's reagent (Raucher and Klein, 1980). Compound **17** was obtained by S-methylation, with subsequent nitromethylation (Chorvat et al., 1983).

6-[2-Chloro-6-fluoro-4-(trifluoromethyl)phenoxy]-1,3-dimethyl-1,2,3,4-tetrahydroquinolin-2-one (Scheme 3, Compound 19). Diethyl 2-methylmalonate sodium salt reacted with the benzyl bromide derivative (**19a**) (Bayer et al., 1973) to be converted to the corresponding malonic acid ester derivative (**19b**). The nitro group was reduced to give the tetrahydroquinolinone derivative (**19c**), which was N-methylated and hydrolyzed, followed by decarboxylation under reflux in toluene to obtain the title compound.

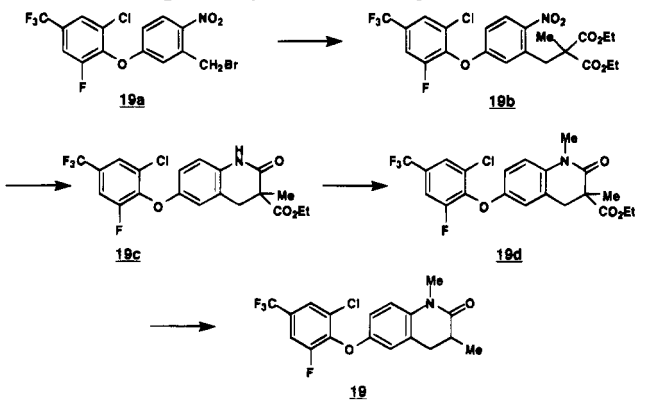
6-[2-Chloro-6-fluoro-4-(trifluoromethyl)phenoxy]-1,3-dimethyl-1H,3H-quinazoline-2,4-dione (Scheme 4, Compound 20). The anthranilate derivative (**20a**) was converted to the corresponding urea derivative (**20b**), which was cyclized by alkaline condition (Lange and Sheibley, 1943) and then N-methylated to the quinazoline derivative (**20**).

Scheme 2. Synthesis of 7-[2-Chloro-6-fluoro-4-(trifluoromethyl)phenoxy]-2,4-dimethyl-3-(nitromethylene)-2*H*-1,4-benzoxazine (17) from 7-[2-Chloro-6-fluoro-4-(trifluoromethyl)phenoxy]-2,4-dimethyl-2*H*,4*H*-1,4-benzoxazin-3-one (12) via 7-[2-Chloro-6-fluoro4-(trifluoromethyl)phenoxy]-2,4-dimethyl-2*H*,4*H*-1,4-benzoxazine-3- thione (16)

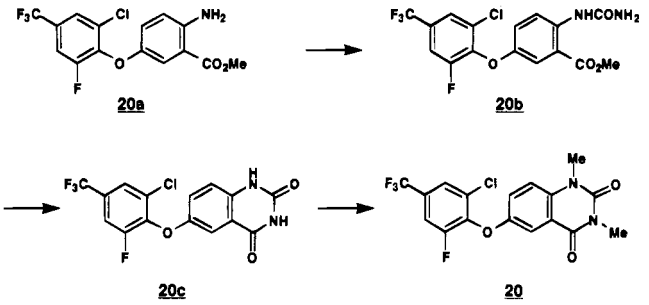


7-[2-Chloro-4-(trifluoromethyl)phenoxy]-2-methyl-2H,4H-1,4-benzoxazin-3-one (Compound 1). Methyl 2-[5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrophenoxy]propionate (1.3 g, 3.1 mmol) was dissolved in EtOH (10 mL), Raney nickel (1.5 mL) was added as a catalyst, and the mixture was hydrogenated for 5 h. After the reaction, the reaction mixture was filtered and evaporated in vacuo to dryness and chromato-

Scheme 3. Synthesis of 6-[2-Chloro-6-fluoro-4-(trifluoromethyl)phenoxy]-1,3-dimethyl-1,2,3,4-tetrahydroquinoline-2-one (19) from 3-[2-Chloro-6-fluoro-4-(trifluoromethyl)phenoxy]-6-nitrobenzyl Bromide (19a)



Scheme 4. Synthesis of 6-[2-Chloro-6-fluoro-4-(trifluoromethyl)phenoxy]-1,3-dimethyl-1*H*,3*H*-quinazoline-2,4-dione (20) from Methyl 2-Amino-5-[2-chloro-6-fluoro-4-(trifluoromethyl)phenoxy]benzoate (20a)



graphed on silica gel eluted by *n*-Hex/AcOEt (1/1) to obtain the title compound as a colorless crystal (0.9 g, 88% yield): ^{1}H NMR ($CDCl_3$) δ 1.60 (d, J = 4 Hz, 3H), 4.68 (q, J = 4 Hz, 1H), 6.50–7.10 (m, 4H), 7.42 (dd, J = 4 and 1 Hz, 1H), 7.70 (d, J = 1 Hz, 1H), 9.30 (s, 1H).

*7-[2-Chloro-4-(trifluoromethyl)phenoxy]-2,4-dimethyl-2H,4H-1,4-benzoxazin-3-one (Compound **2**).* First, sodium hydride (60% oil dispersion, 0.2 g, 5 mmol) was suspended in DMF (5 mL), and a solution of compound **1** (0.4 g, 1.1 mmol) in DMF (5 mL) was added dropwise to the solution, under cooling with ice; the mixture was stirred for 10 min. Then, methyl iodide (0.7 g, 4.9 mmol) was added to the resulting solution and stirred for an additional 10 min. The reaction mixture was poured into water, extracted with AcOEt, washed with water and a saturated NaCl solution, and then dried over anhydrous $MgSO_4$. The solvent was evaporated to dryness, which was purified by silica gel chromatography [eluted by *n*-Hex/AcOEt (3/1)] to give the *N*-methyl derivative as a colorless crystal (0.4 g, 98% yield): ^{1}H NMR ($CDCl_3$) δ 1.54 (d, J = 4 Hz, 3H), 3.36 (s, 3H), 4.64 (q, J = 4 Hz, 1H), 6.60–7.08 (m, 4H), 7.42 (dd, J = 4 and 1 Hz, 1H), 7.70 (d, J = 1 Hz, 1H). The following compounds were prepared similarly.

*7-[2-Chloro-4-(trifluoromethyl)phenoxy]-4-ethyl-2-methyl-2H,4H-1,4-benzoxazin-3-one (Compound **3**):* yield 79.5% (colorless crystals); ^{1}H NMR ($CDCl_3$) δ 1.24 (t, J = 4 Hz, 3H), 1.54 (d, J = 4 Hz, 3H), 3.96 (q, J = 4 Hz, 2H), 4.60 (q, J = 4 Hz, 1H), 6.60–7.04 (m, 4H), 7.42 (dd, J = 4 and 1 Hz, 1H), 7.70 (d, J = 1 Hz, 1H).

*7-[2-Chloro-4-(trifluoromethyl)phenoxy]-2-methyl-4-propargyl-2H,4H-1,4-benzoxazin-3-one (Compound **4**):* yield 62.5% (colorless crystals); ^{1}H NMR ($CDCl_3$) δ 1.58 (d, J = 4 Hz, 3H), 2.27 (t, J = 1 Hz, 1H), 4.40–4.90 (m, 3H), 6.60–6.80 (m, 2H), 7.00 (d,J =4 Hz, 1H), 7.16 (dd, J = 4 and 1 Hz, 1H), 7.46 (dd, J = 4 and 1 Hz, 1H), 7.72 (d, J = 1 Hz, 1H).

*7-[2-Chloro-4-(trifluoromethyl)phenoxy]-4-isopropyl-2-methyl-2H,4H-1,4-benzoxazin-3-one (Compound **5**):* yield 21.4% (colorless crystals); ^{1}H NMR ($CDCl_3$) δ 1.40–1.60 (m, 9H), 4.48 (q, J = 4 Hz, 1H), 4.72 (qui, J = 4 Hz, 1H), 6.60–6.80 (m, 2H), 6.90–7.20 (m, 2H), 7.42 (dd, J = 4 and 1 Hz, 1H), 7.70 (d, J = 1 Hz, 1H).

*7-[2-Chloro-4-(trifluoromethyl)phenoxy]-2,2,4-trimethyl-2H,4H-1,4-benzoxazin-3-one (Compound **6**):* yield 27% (colorless crystals) from methyl 2-[4-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrophenoxy]-2-methylpropionate; ^{1}H NMR ($CDCl_3$) δ 1.50 (s, 6H), 3.34 (s, 3H), 6.60–7.02 (m, 4H), 7.42 (dd, J = 4 and 1 Hz, 1H), 7.70 (d, J = 1 Hz, 1H).

*7-[2-Chloro-4-(trifluoromethyl)phenoxy]-2,4-dimethyl-2-phenyl-2H,4H-1,4-benzoxazin-3-one (Compound **7**):* yield 43% (colorless crystals) from methyl 2-[4-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrophenoxy]-2-phenylpropionate; ^{1}H NMR ($CDCl_3$) δ 1.88 (s, 3H), 3.40 (s, 3H), 6.50–7.00 (m, 4H), 7.16–7.50 (m, 6H), 7.70 (d, J = 1 Hz, 1H).

*6-[2-Chloro-4-(trifluoromethyl)phenoxy]-2,4-dimethyl-2H,4H-1,4-benzoxazin-3-one (Compound **8**):* yield 79% (colorless crystals) from methyl 2-[4-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrophenoxy]propionate; ^{1}H NMR ($CDCl_3$) δ 1.64 (d, J = 4 Hz, 3H), 3.36 (s, 3H), 4.68 (q, J = 4 Hz, 1H), 6.60–7.10 (m, 4H), 7.48 (dd, J = 4 and 1 Hz, 1H), 7.77 (d, J = 1 Hz, 1H).

*7-[3-Chloro-5-(trifluoromethyl)-2-pyridyloxy]-2,4-dimethyl-2H,4H-1,4-benzoxazin-3-one (Compound **9**):* yield 65% (colorless crystals) from methyl 2-[5-[3-chloro-5-(trifluoromethyl)-2-pyridyloxy]-2-nitrophenoxy]propionate; ^{1}H NMR ($CDCl_3$) δ 1.56 (d, J = 4 Hz, 3H), 3.36 (s, 3H), 4.66 (q, J = 4 Hz, 1H), 6.72–7.04 (m, 3H), 7.94 (d, J = 1 Hz, 1H), 8.24 (m, 1H).

*6-[3-Chloro-5-(trifluoromethyl)-2-pyridyloxy]-2,4-dimethyl-2H,4H-1,4-benzoxazin-3-one (Compound **10**):* yield 55% (colorless crystals) from methyl 2-[4-[3-chloro-5-(trifluoromethyl)-2-pyridyloxy]-2-nitrophenoxy]propionate; ^{1}H NMR ($CDCl_3$) δ 1.63 (d, J = 4 Hz, 3H), 3.38 (s, 3H), 4.68 (q, J = 4 Hz, 1H), 6.70–7.20 (m, 3H), 8.02 (d, J = 1 Hz, 1H), 8.30 (m, 1H).

*7-[2-Chloro-6-fluoro-4-(trifluoromethyl)phenoxy]-2-methyl-2H,4H-1,4-benzoxazin-3-one (Compound **11**):* yield 78% (colorless crystals); ^{1}H NMR ($CDCl_3$) δ 1.58 (d, J = 4 Hz, 3H), 4.64 (q, J = 4 Hz, 1H), 6.50–6.80 (m, 3H), 7.40 (dd, J = 4 and 1 Hz, 1H), 7.56 (s, 1H), 8.00 (s,1H).

*7-[2-Chloro-6-fluoro-4-(trifluoromethyl)phenoxy]-2,4-dimethyl-2H,4H-1,4-benzoxazin-3-one (Compound **12**):* yield 90% (colorless crystals); ^{1}H NMR ($CDCl_3$) δ 1.53 (d, J = 4 Hz, 3H), 3.32 (s, 3H), 4.60 (q, J = 4 Hz, 1H), 6.40–7.00 (m, 3H), 7.20–7.60 (m, 2H).

*7-[2,6-Dichloro-4-(trifluoromethyl)phenoxy]-2,4-dimethyl-2H,4H-1,4-benzoxazin-3-one (Compound **13**):* yield 70% (colorless crystals) from methyl 2-[5-[2,6-dichloro-4-(trifluoromethyl)-phenoxy]-2-nitrophenoxy]propionate; ^{1}H NMR ($CDCl_3$) δ 1.56 (d, J = 4 Hz, 3H), 3.33 (s, 3H), 4.62 (q, J = 4 Hz, 1H), 6.40–7.00 (m, 3H), 7.66 (s, 2H).

*7-[2-Chloro-6-fluoro-4-(trifluoromethyl)phenoxy]-4-(difluoromethyl)-2-methyl-2H,4H-1,4-benzoxazin-3-one (Compound **14**).* First, a 60% sodium hydride oil dispersion (0.2 g, 5 mmol) was added to a solution of compound **11** (1.0 g, 2.7 mmol) in DMF (40 mL), and cooled at −60 °C. Chlorodifluoromethane was bubbled into the reaction solution. The mixture was stirred at −60 °C for 5 h and then at room temperature for 10 h. Water was added and the reaction mixture extracted with AcOEt and washed with sodium bicarbonate solution and a saturated NaCl solution. The washed extract was dried over anhydrous $MgSO_4$. The solvent was distilled off to dryness, and then the preparation was chromatographed on silica gel [eluted by *n*-Hex/AcOEt (9/1)] to obtain the title compound (0.3 g, 27% yield) as a colorless crystal: ^{1}H NMR ($CDCl_3$) δ 1.52 (s, 3H), 4.48 (m, 1H), 6.40–6.60 (m, 2H), 7.00–8.20 (m, 4H).

*7-[2-Chloro-6-fluoro-4-(trifluoromethyl)phenoxy]-2,4-dimethyl-2H,4H-1,4-benzothiazin-3-one (Compound **15**).* Reduced iron (1.3g) was suspended in acetic acid (6 mL). A solution of 2-[5-[2-chloro-6-fluoro-4-(trifluoromethyl)phenoxy]-(2-nitrophenyl)-thio]propionic acid (1.5 g, 3.4 mmol) in acetic acid (2 mL) was added to the suspension and the mixture vigorously stirred for 20 h. After the reaction, the mixture was filtered and concentrated in vacuo, and the residue was extracted with AcOEt, washed with water and a saturated NaCl solution, and dried over anhydrous $MgSO_4$. The solvent was evaporated off in vacuo, and the residue was purified by silica gel chromatography [eluted by *n*-Hex/AcOEt (3/1)] to obtain the thiazine

derivative (0.9 g, 69% yield). This compound (0.5 g, 1.28 mmol) was N-methylated according to the same procedure as above, and then the title compound was obtained (0.45 g, 87% yield) as a colorless crystal: ^{1}H NMR ($CDCl_3$) δ 1.44 (d, J = 4 Hz, 3H), 3.41 (s, 3H), 3.48 (q, J = 4 Hz, 1H), 6.70–7.10 (m, 3H), 7.36 (dd, J = 4 and 1 Hz, 1H), 7.56 (s, 1H).

*7-[2-Chloro-6-fluoro-4-(trifluoromethyl)phenoxy]-2,4-dimethyl-2H,4H-1,4-benzoxazine-3-thione (Compound **16**).* Compound **12** (3.0 g, 7.7 mmol) was dissolved in toluene (30 mL), and 2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane 2,4-disulfide (3.4 g, 8.4 mmol) was added; the resulting mixture was refluxed for 2 h. The solvent was distilled off in vacuo, and the residue was purified by silica gel chromatography [eluted by n-Hex/AcOEt (5/1)] to prepare the title compound (2.9 g, 93% yield) as yellow crystals: ^{1}H NMR ($CDCl_3$) δ 1.56 (d, J = 4 Hz, 3H), 3.83 (s, 3H), 5.10 (q, J = 4 Hz, 1H), 6.50–6.70 (m, 2H), 7.02 (d, J = 4 Hz, 1H), 7.20–7.60 (m, 2H).

*7-[2-Chloro-6-fluoro-4-(trifluoromethyl)phenoxy]-2,4-dimethyl-3-(nitromethylene)-2H-1,4-benzoxazine (Compound **17**).* Compound **16** (0.84 g, 2.1 mmol) was dissolved in DMF (10 mL), and methyl trifluoromethanesulfonate (0.26 mL, 2.3 mmol) was added to the solution at 0 °C and then stirred for 30 min. Lithium salt of nitromethane, prepared by the reaction of nitromethane (0.14 mL, 2.6 mmol) with lithium diisopropylamide (2.6 mmol) in DMF at 0 °C, was added to the solution and stirred for 2 h. After the reaction, aqueous ammonium chloride solution was added to the solution, then extracted with AcOEt, washed with saturated saline, and dried over anhydrous $MgSO_4$. The solvent was evaporated off in vacuo, and the residue was purified by silica gel chromatography [eluted by n-Hex/AcOEt (5/1)] to obtain the title compound (0.40 g, 45% yield) as a yellow crystal: ^{1}H NMR ($CDCl_3$) δ 1.44 (d, J = 4 Hz, 3H), 3.30 (s, 3H), 6.30–6.75 (m, 3H), 6.80–7.04 (m, 2H), 7.20–7.60 (m, 2H).

*6-[2-Chloro-6-fluoro-4-(trifluoromethyl)phenoxy]-1-methyl-1,2,3,4-tetrahydroquinolin-2-one (Compound **18**).* A mixture of 6-hydroxy-1,2,3,4-tetrahydroquinolin-2-one (3.16 g, 19.4 mmol) (Mayer et al., 1927), 3-chloro-4,5-difluorobenzotrifluoride (4.15 g, 19.2 mmol) (Cartwright et al., 1983), and powdered KOH (1.08 g, 19.3 mmol) in DMSO (20 mL) was heated at 130 °C for 6 h. The resulting mixture was poured into water, then extracted with AcOEt, washed with saturated NaCl solution, and dried over anhydrous $MgSO_4$. The solvent was evaporated to dryness, which was purified by silica gel chromatography [eluted by n-Hex/AcOEt (1/1)] to obtain 6-[2-chloro-6-fluoro-4-(trifluoromethyl)phenoxy]-1,2,3,4-tetrahydroquinolin-2-one (4.87 g, 70% yield). This compound (1.0 g, 2.8 mmol) was N-methylated according to the same method as mentioned above to give the title compound (0.92 g, 88% yield) as colorless crystals: ^{1}H NMR ($CDCl_3$) δ 2.30–3.00 (m, 4H), 3.36 (s, 3H), 6.70–7.04 (m, 3H), 7.48 (dd, J = 4 and 1 Hz, 1H), 7.68 (s, 1H).

*6-[2-Chloro-6-fluoro-4-(trifluoromethyl)phenoxy]-1,3-dimethyl-1,2,3,4-tetrahydroquinolin-2-one (Compound **19**).* Diethyl 2-methylmalonate (0.57 g, 3.3 mmol) was added to the solution which contained sodium hydride (60% oil dispersion, 0.13 g, 3.3 mmol) and DMF (10 mL) under ice cooling, a solution of 3-[2-chloro-6-fluoro-4-(trifluoromethyl)phenoxy]-6-nitrobenzyl bromide (compound **19a**, 1.12 g, 2.6 mmol) in DMF (4 mL) was added dropwise and stirred for 2 h at room temperature. After the reaction, the resulting mixture was poured into water, then extracted with AcOEt, washed with saturated NaCl, and dried over anhydrous $MgSO_4$. The solvent was evaporated off in vacuo, and the residue was purified by silica gel chromatography [eluted by n-Hex/AcOEt (10/1)] to obtain diethyl 2-[3-[2-chloro-6-fluoro-4-(trifluoromethyl)phenoxy]-6-nitrobenzyl]-2-methylmalonate (compound **19b**, 1.4 g, 96% yield). This compound (1.3 g, 2.5 mmol) was hydrogenated in EtOH (20 mL) in the presence of Raney nickel (1.5 mL). After the reaction, the mixture was filtered and the filtrate evaporated to dryness, which was washed with ethyl ether to give 6-[2-chloro-6-fluoro-4-(trifluoromethyl)phenoxy]-3-(ethoxycarbonyl)-3-methyl-1,2,3,4-tetrahydroquinolin-2-one (compound **19c**, 1.0 g, 90% yield). This compound (0.93 g, 2.1 mmol) was N-methylated according to the same procedure to give 6-[2-chloro-6-fluoro-4-(trifluoromethyl)phenoxy]-3-(ethoxycarbonyl)-1,3-dimethyl-1,2,3,4-tetrahydroquinolin-2-one (compound **19d**, 0.79 g, 82% yield). This ester derivative (0.46 g, 1 mmol) was dissolved in EtOH (4 mL), and 1N NaOH (2 mL) was added and then stirred for 2 h. The resulting solution was acidified with 1 N HCl, then extracted with toluene, washed with water, and dried over anhydrous $MgSO_4$. The toluene solution was refluxed for 6 h. After the reaction, the solution was evaporated in vacuo to dryness, which was chromatographed on silica gel [eluted by n-Hex/AcOEt (3/1)] to obtain the title compound (**19**) (0.32 g, 83% yield) as an oily product: ^{1}H NMR ($CDCl_3$) δ 1.26 (d, J = 4 Hz, 3H), 2.50–3.00 (m, 3H), 3.38 (s, 3H), 6.75–7.05 (m, 3H), 7.48 (dd, J = 4 Hz, 1 Hz, 1H), 7.68 (s, 1H).

*6-[2-Chloro-6-fluoro-4-(trifluoromethyl)phenoxy]-1,3-dimethyl-1H,3H-quinazoline-2,4-dione (Compound **20**).* Methyl 2-amino-5-[2-chloro-6-fluoro-4-(trifluoromethyl)phenoxy]benzoate (compound **20a**, 2.0 g, 5.5 mmol) was dissolved in acetic acid (50 mL), and an aqueous sodium isocyanate (0.4 g, 6.2 mmol) solution (10 mL) was added and then stirred for 2 h. The reaction solution was evaporated off in vacuo, then extracted with AcOEt, washed with water and a saturated NaCl solution, dried over anhydrous $MgSO_4$, and evaporated to dryness. The product was crystallized from ether/n-Hex to give methyl 2-(carbamoylamino)-5-[2-chloro-6-fluoro-4-(trifluoromethyl)-phenoxy]benzoate (compound **20b**, 2.0 g, 89% yield). This urea derivative (2.0 g, 4.9 mmol) was suspended in MeOH (30 mL), and 1 N NaOH (6 mL) was added to the solution. After the addition of 1 N NaOH, the suspended solution became clear and the sodium salt precipitated. The resulting mixture was stirred for 1 h and then acidified with 1 N HCl. The precipitate was filtered and washed with water to give 6-[2-chloro-6-fluoro-4-(trifluoromethyl)phenoxy]-(1*H*,3*H*)-quinazoline-2,4-dione (compound **20c**, 1.83 g, 99% yield). The quinazoline derivative (0.7 g, 1.9 mmol) was dissolved in DMF (10 mL), and sodium hydride (60% oil dispersion, 0.17 g, 4.3 mmol) was added to the solution. After 30 min, methyl iodide (0.58 g, 4.1 mmol) was added to the resulting mixture and then stirred for 1 h. After the reaction, the resulting mixture was poured into water to precipitate the product. The precipitate was filtered, washed with water, and then recrystallized from AcOEt/n-Hex to give the title compound (0.69 g, 92% yield) as colorless crystals: ^{1}H NMR ($CDCl_3$) δ 1.52 (s, 3H), 4.48 (m, 1H), 6.40–6.60 (m, 2H), 7.00–8.20 (m, 4H).

Biological Testing. The pre- and postemergent herbicide evaluations in uplands were conducted with all compounds mentioned above in the greenhouse. The test species included were crabgrass (*Digitaria adscendens*), common purslane (*Portulaca oleracea*), and smartweed (*Polygonum blumei*). An emulsifiable concentrate was prepared by mixing 15 parts of the compound, 65 parts of xylene, and 20 parts of polyoxyethylene alkylaryl ether (w/w). The herbicidal activity of the compound was determined by visible evaluation of the tested plants in comparison with untreated controls (5 = completely killed, 0 = no herbicidal effect).

Preemergent Tests. Seeds of crabgrass, purslane, and smartweed were planted in a seedling case having a size of 6 cm × 15 cm × 10 cm and packed with upland field soil. On the day after seeding, a designed amount of the test compound formulated in an emulsifiable concentrate was diluted with water and then applied to the surface of the soil. Two weeks after the treatment with the test compound, the herbicidal activity was determined (Table 2).

Postemergent Tests. Seeds of crabgrass, purslane, and smartweed were planted in a seedling case having a size of 6 cm × 15 cm × 10 cm and packed with upland field soil. After a 10-day cultivation in the greenhouse, a designed amount of the test compound formulated in an emulsifiable concentrate (see above) was diluted with water and then applied to the surface of the foliage. Two weeks after the treatment with the test compound, the herbicidal activity was determined (Table 3).

Cell Culture and Herbicide Treatments. A cell suspension culture of *Marchantia polymorpha L.* was routinely subcultured every 2 weeks using a modified medium of Murashige and Skoog [see our previous paper (Iwata et al., 1992)] for more than 10 years. The suspension cultures were grown in 100-

Table 2. Preemergent Test at 5 and 1.25 g of Active Ingredient/100 m² [a]

compd	dose, g of ai/100 m²	*D. adscendens*	*P. oleracea*	*P. blumei*
1	5	0	0	0
2	5	2	2.5	2.5
3	5	1	4.5	1
4	5	0	3.5	1.5
5	5	0	0	0
6	5	0	5	2
7	5	0	0	0
8	5	0	0	0
9	5	1	3	1.5
10	5	0	0	0
11	5	2.5	2	2
12	5	5	5	5
	1.25	4.5	4.5	3.5
13	5	0	2	0
14	5	3	5	5
	1.25	1.5	5	1.5
15	5	2	4	2.5
	1.25	0	2	1
16	5	2.5	5	5
17	5	1	5	3
18	5	5	5	4.5
19	5	5	5	5
	1.25	3	5	1.5
20	5	0	1.5	0

[a] The herbicidal activity of the compound was determined by visible evaluation of the tested plants in comparison with untreated controls (5 = completely killed, 0 = no herbicidal effect).

Table 3. Postemergent Test at 5 and 1.25 g of Active Ingredient/100 m² [a]

compd	dose, g of ai/100 m²	*D. adscendens*	*P. oleracea*	*P. blumei*
1	5	0	1	0
2	5	2.5	5	4.5
3	5	1.5	5	3.5
4	5	1	5	1
5	5	0	0	0
6	5	1	5	2
7	5	0	0	0
8	5	0	0	1
9	5	2.5	5	5
10	5	0	0	0
11	5	3	5	4
12	5	5	5	5
	1.25	4.5	5	5
13	5	0	3	1.5
14	5	5	5	5
	1.25	3	5	3
15	5	3	5	5
	1.25	0	5	5
16	5	3	5	5
17	5	2	4.5	4.5
18	5	5	5	5
19	5	5	5	5
	1.25	1.5	5	5
20	5	1	4	2.5

[a] The herbicidal activity of the compound was determined by visible evaluation of the tested plants in comparison with untreated controls (5 = completely killed, 0 = no herbicidal effect).

mL flasks containing 40 mL of medium on a rotary shaker (Model LR-3, Iwashiya K. Sawada Co., Tokyo) at 110 rpm at 25 °C. The cultures were continuously illuminated from the bottom by fluorescent lamps (Matsushita FL40W, "natural white", Matsushita Electric Works, Osaka) with an average light intensity of about 8.7 W/m² at the bottom of the flasks. Suspension cultures were grown in the dark by placing the flasks in black bags. For experimental purposes, 6-day-old cells (cells of exponential growth phase) were adjusted to a cell density of 1 mg of dry weight/mL of culture medium. Aliquots were treated with herbicide at the concentrations indicated and incubated in the light at 25 °C.

Extraction and Determination of Chlorophyll. Cells of 1-mL culture aliquots were collected by centrifugation (5 min at 3000*g*), and the pellet was extracted three times with boiling 90% methanol. Optical densities at 653 nm (A_{653}) and 666 nm (A_{666}) were measured (Model 210A spectrophotometer, Shimadzu, Kyoto), and total chlorophyll concentration was calculated using the equation c (mg/mL) = $23.6A_{653} + 2.57A_{666}$ (Imamura et al., 1970). The cell dry weight was determined as reported (Iwata et al., 1992).

Extraction and Measurement of Protoporphyrin IX (PPIX). Extraction and estimation of PPIX was carried out according to the method of Lee et al. (1991). Cells of 1-mL culture aliquots collected by centrifugation were suspended in 1 mL of water and extracted with 5 mL of a mixture of cold acetone/0.1 N NH_4OH (9:1 v/v) overnight. After centrifugation at 1500*g* for 5 min at 5 °C, the clear supernatant was collected and washed twice with the same volume of hexane. The acetone fraction containing PPIX was measured with a Hitachi F3000 fluorometer, using an excitation wavelength of 410 nm and an emission wavelength of 633 nm.

RESULTS AND DISCUSSION

In general, the series of benzoxazine derivatives was more active on dicotyledonous than monocotyledonous species. The difference was most apparent in postemergent application. All active compounds exhibited peroxidizing herbicidal symptoms characterized by rapid chlorosis, necrosis, or burning of the plant tissue about 2−3 days after spraying in the light. These compounds appear to be contact herbicides with symptoms exhibited by the well-known *p*-nitrodiphenyl ether herbicides.

At first, we investigated compounds having a 4′-(trifluoromethyl)-2′-chlorobenzene moiety (trifluoromethylbenzene moiety) which is usually used in diphenyl ether herbicides. The pre- and postemergence herbicidal activities are summarized in Tables 2 and 3. Among them, compound **1**, which is unsubstituted at the 4-position (nitrogen atom) of the benzoxazine moiety, showed no herbicidal activity at 5 g/a. However, the introduction of a methyl group (compound **2**) gave moderate activity, especially for postapplication against *Portulaca*. Me (**2**), Et (**3**), and propargyl (**4**) exhibited activity, but the isopropyl derivative (**5**) gave a complete loss of activity. Substitution at the 3-position with Me (**2**) and dimethyl (**6**) showed activity, although phenyl (**7**) was not active (Table 2). Compound **9**, having a trifluoromethylpyridine moiety instead of trifluoromethylbenzene, was nearly as active as compounds **2** and **3**. On the other hand, the 6-(aryloxy)benzoxazine derivatives, compounds **8** and **10**, completely lost potency as compared with 7-(aryloxy)benzoxazine derivatives. Compound **10** is a cyclized derivative of pyridyloxyphenoxypropionic acid, which is known as a grass herbicide (Figure 1) (Haga et al., 1987). It should be noted that compound **10** did not show any activity against grass and broad-leaf weeds.

Next, we investigated some substitution of the trifluoromethylbenzene moiety. The introduction of fluorine at the 6′-position (compound **12**) greatly enhanced the herbicidal activity. Compound **12** could control both grass (crabgrass) and broad-leaf weeds at 1.25 g/a either by pre- or postemergence application. The substitution with chlorine (compound **13**) did not increase the activities, showing the same degree as the unsubstituted compound (**2**).

Third, we investigated the conversion of the benzoxazine skeleton. Benzothiazine (**15**) and quinazoline (**20**) lowered activity. Especially, compound **20** decreased remarkably, although we expected it to show increased activity because the carbonyl group of compound **20**

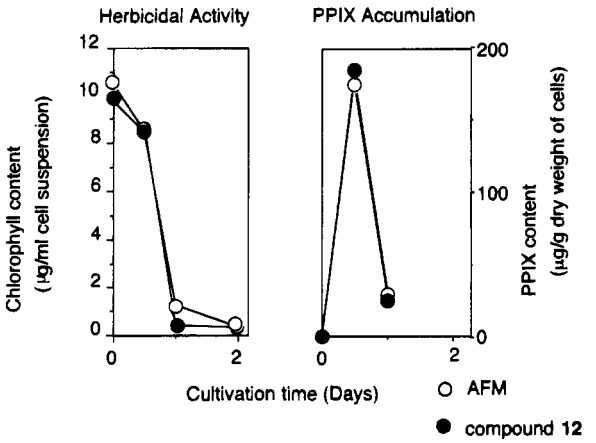


Figure 2. Herbicidal activity and PPIX accumulation. The cell cultures of the exponential growth phase (cells of a 6-day culture) were treated with acifluorfen methyl (AFM) (6 μM) (○) and compound **12** (5 μM) (●). Herbicidal activity was shown by the decrease of chlorophyll in the cell culture suspension during the treatment with herbicides.

corresponded to the carboxyl group of acifluorfen. The electron-withdrawing substituent at this position may inhibit the activities in contrast to nitrodiphenyl ether herbicides. Tetrahydroquinolines (**18** and **19**) retained the activities but were a little weaker than the parent compound (**12**). Both the thione (**16**) and the nitromethylene derivative (**17**) exhibited reduced activities.

To explain the mode of action of these aryloxybenzoxazine derivative herbicides, we also investigated the accumulation of protoporphyrin IX (PPIX) and correlation between the PPIX level and herbicidal damage in the light using liverwort cells treated with the benzoxazine herbicide compound (**12**). Cell cultures of the exponential growth phase were treated with acifluorfen methyl (AFM) and compound **12**, respectively. Figure 2 shows that AFM and compound **12** induced a high PPIX accumulation in the cells during a 1-day light incubation. The level of PPIX accumulation induced by both herbicides was positively correlated with the light-dependent phytotoxic activity on cultured cells. This result suggests that this series of benzoxazine derivatives is a new family of peroxidizing herbicides.

Thus, the benzoxazine herbicides presented here, having a chemical structure without a nitro group, showed the same symptoms with whole plants and mode of action as nitrodiphenyl ether herbicides.

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