JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Phytoene Desaturase Inhibition by *O*-(2-Phenoxy)ethyl-*N*-aralkylcarbamates

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O-[1-Ethyl-2-(3-trifluoromethylphenoxy)]ethyl-*N*-benzylcarbamate exhibits a marked inhibition of carotenoid biosynthesis. Forty-one analogues were synthesized and assayed for plant-type phytoene desaturase (PDS) and ζ-carotene desaturase (ZDS) inhibition in a cell-free system using recombinant enzymes obtained from *Escherichia coli* transformants. The target enzyme of all carbamates synthesized in this study is PDS and not ZDS; no inhibition of ZDS was observed using a 10^{-4} M inhibitor concentration. Four compounds, *O*-[1-ethyl-2-(3-trifluoromethylphenoxy)]ethyl-*N*-(2-phenylethyl)carbamate (**23**), *O*-[1-ethyl-2-(3-trifluoromethylphenoxy)]ethyl-*N*-(2-chlorobenzyl)carbamate (**25**), *O*-[1-ethyl-2-(3-trifluoromethylphenoxy)]ethyl-*N*-(2-chlorobenzyl)carbamate (**26**), and *O*-[1-methyl-2-(3-trifluoromethylphenoxy)]ethyl-*N*-benzylcarbamate (**30**), were the most potent PDS inhibitors. Their pI_{50} values, the negative logarithms of the molar concentration that produces a 50% inhibition, were 7.5, representing the same inhibitory activity as norflurazon. With respect to a structure−activity relationship the oxygen atom of the phenoxy group and a carbamate structure in *O*-(1-ethyl-2-phenoxy)ethyl-*N*-aralkylcarbamates studied were found to be essential for strong PDS inhibitors. Also, introduction of an ethyl group at the α-position of the ethylene bridge between the phenoxy group and the carbamate was important for a strong PDS inhibitor. Substituents at the 2- and/or 3-position of the phenoxybenzene ring were found to be favorable to a strong PDS inhibition of the analogues.

KEYWORDS: Phytoene desaturase inhibitors; *O*-(2-phenoxy)ethyl-*N*-aralkylcarbamates

INTRODUCTION

The carotenoid biosynthesis pathway is an important site of herbicide actions. Heterocyclic compounds, such as norflurazon, flurochloridone, or LS80707, prevent the formation of carotenoids, and some of them have been already commercialized as herbicides (see **Figure 1**) (*1*). Most of such herbicides belonging to different chemical classes, namely, diphenylpyridones, diphenylpyridines, phenoxynicotinamides, phenylpyridazinones, and others (*2*−*4*), were reported as phytoene desaturase (PDS) inhibitors. Recently, 1,1′-biphenyl derivatives (*5*), diphenylpyrimidines (*6*), and diphenylpyrrolidinones (*7*) have been characterized as new PDS inhibitors. Mitchell (*8*) proposed a model of the herbicidal binding site of PDS using superimposition with five structurally different inhibitors. Although structure−activity relationship (SAR) studies have been carried out (*4*, *9*), it is still not known which structural elements of the inhibitors are essential for potent PDS inhibition. In the long run, such an SAR study should be performed using PDS inhibitors belonging to different chemical classes and the PDS inhibitory data of the compounds obtained by a cell-free assay.

O-(2-Phenoxy)ethyl-*N*-benzylcarbamates were developed by Imperial Chemical Industry Ltd. (*10*) and Ciba-Geigy AG (*11*). Their compounds showed good herbicidal activity in a preemergence application against *Avena fatua*, *Echinochloa crus-galli*, *Setaria viridis*, *Ipomoea purpurea*, *Abutilon theophrasti*, and *Brassica kaber*. In our preliminary mode of action studies, *O*-[1-ethyl-2-(3-trifluoromethylphenoxy)]ethyl-*N*-benzylcarbamate exhibited a marked inhibition of carotenoid biosynthesis. Accordingly, we synthesized a number of analogues and assayed their inhibition of PDS and ζ-carotene desaturase (ZDS) using recently developed cell-free assays (*12*, *13*) to clarify their mode of action and to obtain more information for the molecular design of new carotenoid biosynthesis inhibitors.

MATERIALS AND METHODS

Syntheses. The carbamate and benzoate derivatives synthesized in this study are listed in **Tables 1**−**3**. 2-Phenoxyethanols (*10*, *11*), 2-(3-chlorophenylthio)-1-ethylethanol (*14*), 2-ethyl-2-(3-trifluoromethylphenoxy)ethanol (*15*), and 2-(*N*-acylanilino)-1-ethylethanols (*16*) were

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10.1021/jf0262413 CCC: $25.00
Published on Web 04/10/2003

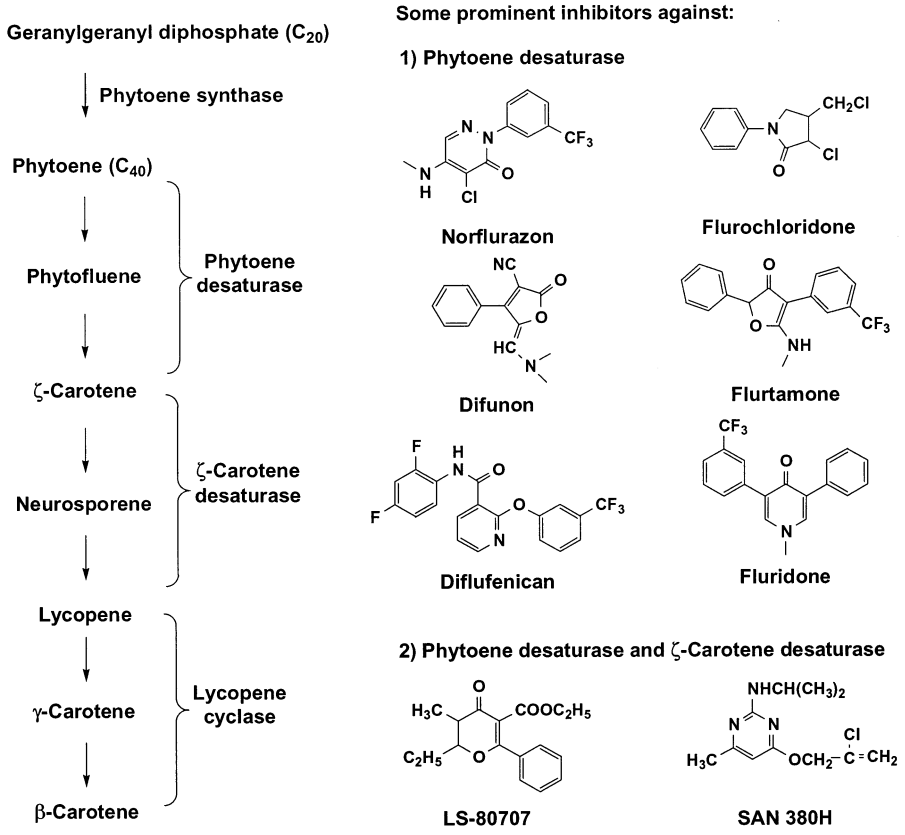


Figure 1. Structures of inhibitors that affect desaturation of phytoene and ζ-carotene and prevent the formation of colored carotenoids.

prepared according to standard methods. Corresponding chloroformates were obtained by reaction of the appropriate alcohols with excess phosgene in an inert solvent, such as dichloromethane, in the presence of triethylamine. The product carbamates were synthesized by reaction of the chloroformate intermediates with the appropriate amines in an inert solvent using a base such as a tertiary amine or pyridine (*10*, *11*). [2-(3-Chlorophenoxy)-1-ethyl]ethyl benzoate was prepared by the reaction of [2-(3-chlorophenoxy)-1-ethyl]ethanol with benzoyl chloride in the presence of triethylamine.

All reaction products were purified through recrystallization and/or flash column chromatography, and their structures were confirmed by NMR, mass spectroscopy, and elemental analysis. Melting points are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were obtained using a Bruker AC250 FT-NMR at 250 and 62.5 MHz, respectively. Mass spectra data were measured by a Varian MAT 312 for EI techniques. Elemental analyses (C, H, N) were performed by an elementar Vario eL, and the results obtained were within ±0.3 of the calculated percentage.

[2-(3-Chlorophenoxy)-1-ethyl]ethanol. 3-Chlorophenol (12.0 g, 90 mmol), 1,2-butylene oxide (7.2 g, 100 mmol), and lithium hydroxide monohydrate (0.4 g, 10 mmol) were heated in a sealed tube (pressure reactor) for 16 h to 160 °C. After the reactor had cooled, the reaction mixture was poured into water (50 mL) and extracted with ethyl acetate (3 × 50 mL). The organic phase was dried over anhydrous sodium sulfate and concentrated. The residue was flash-chromatographed on silica gel with ethyl acetate/petroleum ether (9:1, v/v): yield, 13.7 g (76%); liquid; ^{1}H NMR (250 MHz, DMSO) δ 0.90 (3H, t, J_H = 7.4 Hz), 1.40 and 1.51(2H, m), 3.67 (1H, m), 3.84 (2H, m), 4.85 (1H, d, J_H = 5.1 Hz), 6.94 (3H, m), 7.28 (1H, t, J_H = 8.1 Hz).

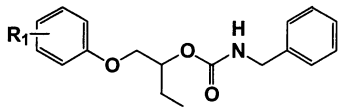
[2-(3-Chlorophenylthio)-1-ethyl]ethanol. To 1,2-butylene oxide (2.9 g, 40 mmol) was added 3-chlorothiophenol (5.0 g, 35 mmol) and lithium hydroxide monohydrate (0.2 g, 5 mmol) at 0 °C, and the mixture was stirred for 2 h at room temperature. The reaction mixture was poured onto ice−water (50 mL) and extracted with ethyl acetate (3 × 50 mL). The organic phase was dried over anhydrous sodium sulfate and concentrated. The desired compound was separated using silica gel flash chromatography with ethyl acetate/petroleum ether (10:1, v/v): yield, 6.0 g (85%); liquid; ^{1}H NMR (250 MHz, DMSO) δ 0.86 (3H, t, J_H = 7.4 Hz), 1.29−1.64 (2H, m), 3.00 (2H, dd, J_H = 2.0 and 6.0 Hz), 3.53 (1H, m), 4.94 (1H, d, J_H = 5.3 Hz), 7.14−7.37 (4H, m).

[2-(N-Acetylanilino)-1-ethyl]ethanol. [2-(3-Chlorophenylamino)-1-ethyl]ethanol (yield, 56%; mp, 32−35 °C) was prepared according to the same synthetic method of [2-(3-chlorophenoxy)-1-ethyl]ethanol. To a solution of [2-(3-chloroanilino)-1-ethyl]ethanol (2.0 g, 10 mmol) in tetrahydrofuran (10 mL) was added acetic anhydride (2.0 g, 20 mmol) at 5 °C. The reaction mixture was stirred for 4 h at room temperature and concentrated by evaporation. Ethyl acetate (50 mL) was added to the residue and washed with sodium hydrogencarbonate solution (3 × 50 mL) and saturated NaCl solution (3 × 50 mL). The organic phase was dried over anhydrous sodium sulfate and evaporated. Flash chromatography of the residue on silica gel with ethyl acetate/petroleum ether (1:3, v/v) provided the desired compound in a pure state: yield, 1.3 g (54%); liquid; ^{1}H NMR (250 MHz, DMSO) δ 0.81 (3H, t, J_H = 7.3 Hz), 1.15−1.40 (2H, m), 1.75 (3H, s), 3.45 (2H, m), 3.62 (1H, m), 4.63 (1H, s), 7.32−7.53 (4H, m).

[1-Ethyl-1-(3-trifluoromethylphenoxy)]ethanol. Ethyl 2-(3-trifluoromethylphenoxy)butanoate was synthesized by the reaction of 3-trifluoromethylphenol with ethyl 2-bromobutanoate according to the method of Wimmer et al. (*15*). 2-(3-Trifluoromethylphenoxy)butanoic acid was prepared by a hydrolysis of ethyl 2-(3-trifluoromethylphenoxy)butanoate using sodium hydroxide in methanolic solution. 2-(3-Trifluoromethylphenoxy)butanoic acid (6.9 g, 30 mmol) in diethyl ether (30 mL) was added to a suspension of $LiAlH_4$ (1.2 g, 30 mmol) in dry diethyl ether (30 mL) at 0 °C. The reaction mixture was stirred for 3 h at room temperature and refluxed for 3 h. Excess $LiAlH_4$ was decomposed by dropwise addition of water, and the resulting white suspension was filtered. After concentration of the filtrate, pure [1-ethyl-1-(3-trifluoromethylphenoxy)]ethanol was obtained: yield, 5.5 g (78.3%); liquid; ^{1}H NMR (250 MHz, DMSO) δ 0.89 (3H, t, J_H = 7.4 Hz), 1.63 (2H, m), 3.54 (2H, t, J_H = 5.3 Hz), 4.33 (1H, m), 4.86 (1H, t, J_H = 5.7 Hz), 7.15−7.32 (3H, m), 7.51 (1H, t, J_H = 12.5 Hz).

*O-[2-(3-Chlorophenoxy)-1-ethyl]ethyl-N-benzylcarbamate (**4**).* A solution of [2-(3-chlorophenoxy)-1-ethyl]ethanol (6.0 g, 30 mmol) and triethylamine (3.0 g, 31 mmol) in tetrahydrofuran (15 mL) was added

Table 1. Physical Data of *O*-(1-Ethyl-2-phenoxy)ethyl-*N*-benzylcarbamates and Their Phytoene Desaturase and ζ-Carotene Desaturase Inhibitory Activities

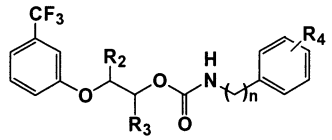


no.	R_1	mp (°C)	pI_{50} (PDS)	pI_{50} (ZDS)	^{1}H NMR (δ, DMSO)	elem anal. found [calcd] (%)
1	3-CF_3	60–61	6.9	<4.0	0.90 (3H, t, J_H = 7.4 Hz), 1.64 (2H, m), 4.04 (2H, d, J_H = 6.3 Hz), 4.18 (2H, d, J_H = 6.1 Hz), 4.91 (1H, m), 6.88–6.97 (3H, m), 7.01–7.32 (6H, m), 7.78 (1H, t, J_H = 6.2 Hz)	C, 62.22; H, 5.38; N, 3.75 [C, 62.12; H, 5.49; N, 3.81]
2	H	62–63	7.1	<4.0	0.91 (3H, t, J_H = 7.4 Hz), 1.66 (2H, m), 4.01 (2H, d, J_H = 5.1 Hz), 4.19 (2H, d, J_H = 6.2 Hz), 4.90 (1H, m), 6.90–6.95 (3H, m), 7.18–7.33 (7H, m), 7.78 (1H, t, J_H = 6.2 Hz)	C, 72.79; H, 7.32; N, 4.13 [C, 72.22; H, 7.07; N, 4.68]
3	2-Cl	71–72	7.0	<4.0	0.91 (3H, t, J_H = 7.4 Hz), 1.70 (2H, m), 4.09 (2H, d, J_H = 10.5 Hz), 4.18 (2H, d, J_H = 6.5 Hz), 4.92 (1H, m), 6.94 (1H, t, J_H = 7.6 Hz), 7.14–7.31 (7H, m), 7.41 (1H, d, J_H = 7.9 Hz), 7.78 (1H, t, J_H = 6.2 Hz)	C, 64.75; H, 6.03; N, 4.20 [C, 64.77; H, 6.04; N, 4.20]
4	3-Cl	60–61	7.3	<4.0	0.90 (3H, t, J_H = 7.4 Hz), 1.64 (2H, m), 4.04 (2H, d, J_H = 6.3 Hz), 4.18 (2H, d, J_H = 6.1 Hz), 4.91 (1H, m), 6.88–6.97 (3H, m), 7.01–7.32 (6H, m), 7.78 (1H, t, J_H = 6.2 Hz)	C, 64.80; H, 5.95; N, 4.10 [C, 64.77; H, 6.04; N, 4.20]
5	4-Cl	81–82	5.5	<4.0	0.89 (3H, t, J_H = 7.4 Hz), 1.63 (2H, m), 4.03 (2H, m), 4.17 (2H, d, J_H = 6.2 Hz), 4.88 (1H, m), 6.94 (2H, d, J_H = 8.9 Hz), 7.20–7.32 (7H, m), 7.77 (1H, t, J_H = 6.2 Hz)	C, 64.73; H, 6.16; N, 3.97 [C, 64.77; H, 6.04; N, 4.20]
6	3-F	63–64	7.2	<4.0	0.90 (3H, t, J_H = 7.4 Hz), 1.64 (2H, m), 3.99–4.19 (4H, m), 4.89 (1H, m), 6.72–6.84 (3H, m), 7.20–7.34 (6H, m), 7.78 (1H, t, J_H = 6.1 Hz)	C, 68.00; H, 6.42; N, 4.25 [C, 68.12; H, 6.35; N, 4.41]
7	3-Br	67–68	7.1	<4.0	0.90 (3H, t, J_H = 7.4 Hz), 1.65 (2H, m), 4.06 (2H, m), 4.18 (2H, d, J_H = 6.1 Hz), 4.89 (1H, m), 6.94 (2H, d, J_H = 8.1 Hz), 7.10–7.32 (7H, m), 7.77 (1H, t, J_H = 6.2 Hz)	C, 57.20; H, 5.50; N, 3.40 [C, 57.15; H, 5.33; N, 3.70]
8	3-CN	90–91	7.3	<4.0	0.91 (3H, t, J_H = 7.4 Hz), 1.66 (2H, m), 4.05–4.19 (4H, m), 4.92 (1H, m), 7.20–7.33 (6H, m), 7.38–7.51 (3H, m), 7.77 (1H, t, J_H = 6.2 Hz)	C, 70.31; H, 6.17; N, 8.65 [C, 70.35; H, 6.21; N, 8.64]
9	3-NO_2	76–77	7.1	<4.0	0.92 (3H, t, J_H = 7.4 Hz), 1.67 (2H, m), 4.12–4.26(4H, m), 4.92 (1H, m), 7.20–7.27 (5H, m), 7.40 (1H, dd, J_H = 2.1 and 8.1 Hz), 7.56 (1H, t, J_H = 8.2 Hz), 7.71–7.83 (3H, m)	C, 64.74; H, 5.82; N, 7.83 [C, 62.78; H, 5.85; N, 8.13]
10	3-CH_3	75–76	6.9	<4.0	0.90 (3H, t, J_H = 7.5 Hz), 1.66 (2H, m), 2.26 (3H, s), 3.98 (2H, m), 4.19 (2H, d, J_H = 6.2 Hz), 4.89 (1H, m), 6.70–6.75 (3H, m), 7.11–7.33 (6H, m), 7.78 (1H, t, J_H = 6.2 Hz)	C, 72.73; H, 7.50; N, 4.28 [C, 72.82; H, 7.40; N, 4.47]
11	3-C_3H_7-iso	56–57	6.9	<4.0	0.91 (3H, t, J_H = 7.4 Hz), 1.17 (6H, d, J_H = 6.9 Hz), 1.65 (2H, m), 2.83 (1H, m), 4.01 (2H, m), 4.19 (2H, d, J_H = 6.2 Hz), 4.88 (1H, m), 6.71–6.82 (3H, m), 7.14–7.32 (6H, m), 7.77 (1H, t, J_H = 6.1 Hz)	C, 73.85; H, 7.87; N, 3.85 [C, 73.87; H, 7.97; N, 4.10]
12	3-OCH_3	48–49	6.8	<4.0	0.90 (3H, t, J_H = 7.3 Hz), 1.65 (2H, m), 3.71 (3H, s), 4.02 (2H, m), 4.18 (2H, d, J_H = 6.1 Hz), 4.88 (1H, m), 6.48–6.51 (3H, m), 7.12–7.32 (6H, m), 7.77 (1H, t, J_H = 5.9 Hz)	C, 69.16; H, 7.12; N, 4.00 [C, 69.28; H, 7.04; N, 4.25]
13	3-$COOC_2H_5$	oil	6.9	<4.0	0.91 (3H, t, J_H = 7.4 Hz), 1.31 (3H, t, J_H = 7.1 Hz), 1.67 (2H, m), 4.03–4.20 (4H, m), 4.30 (2H, q, J_H = 7.1 Hz), 4.91 (1H, m), 7.18–7.22 (6H, m), 7.39–7.46 (2H, m), 7.54 (1H, m), 7.76 (1H, t, J_H = 6.1 Hz)	C, 67.70; H, 6.71; N, 3.46 [C, 67.91; H, 6.78; N, 3.77]
14	3-COOH	170–172	<5.0	<4.0	0.91 (3H, t, J_H = 7.4 Hz), 1.46 (2H, m), 4.00–4.19 (4H, m), 4.91 (1H, m), 7.16–7.43 (8H, m), 7.53 (1H, d, J_H = 7.7 Hz), 7.77 (1H, t, J_H = 6.2 Hz), 13.02 (1H, br)	C, 66.36; H, 6.12; N, 3.92 [C, 66.46; H, 6.16; N, 4.08]
15	3-$NHCOCH_3$	139–140	5.4	<4.0	0.91 (3H, t, J_H = 7.4 Hz), 1.65 (2H, m), 2.02 (3H, s), 3.99 (2H, m), 4.19 (2H, d, J_H = 6.1 Hz), 6.60 (1H, d, J_H = 8.0 Hz), 7.05–7.32 (8H, m), 7.77 (1H, t, J_H = 6.0 Hz), 9.90 (1H, br)	C, 67.29; H, 6.69; N, 7.70 [C, 67.40; H, 6.79; N, 7.86]
16	2,3-Cl_2	104–105	6.9	<4.0	0.91 (3H, t, J_H = 7.4 Hz), 1.69 (2H, m), 4.08–4.26 (4H, m), 4.92 (1H, m), 7.15–7.33 (8H, m), 7.77 (1H, t, J_H = 6.0 Hz)	C, 58.62; H, 5.27; N, 3.50 [C, 58.71; H, 5.20; N, 3.80]
17	2,4-Cl2	72–73	5.3	<4.0	0.91 (3H, t, J_H = 7.4 Hz), 1.69 (2H, m), 4.05–4.26 (4H, m), 4.92 (1H, m), 7.18–7.36 (7H, m), 7.55 (1H, d, J_H = 2.5 Hz), 7.78 (1H, t, J_H = 6.1 Hz)	C, 58.60; H, 5.28; N, 3.58 [C, 58.71; H, 5.20; N, 3.80]
18	2,5-Cl2	90–91	5.4	<4.0	0.91 (3H, t, J_H = 7.4 Hz), 1.67 (2H, m), 4.08–4.26 (4H, m), 4.93 (1H, m), 7.02 (1H, dd, J_H = 1.8 and 8.5 Hz), 7.09–7.31 (6H, m), 7.44 (1H, d, J_H = 8.3 Hz), 7.77 (1H, t, J_H = 6.1 Hz)	C, 58.60; H, 5.28; N, 3.58 [C, 58.71; H, 5.20; N, 3.80]
19	2,6-Cl_2	99–100	<5.0	<4.0	0.94 (3H, t, J_H = 7.4 Hz), 1.73 (2H, m), 4.02–4.27 (4H, m), 4.87 (1H, m), 7.13–7.32 (6H, m), 7.47 (1H, d, J_H = 8.1 Hz), 7.78 (1H, t, J_H = 6.1 Hz)	C, 58.67; H, 5.05; N, 3.80 [C, 58.71; H, 5.20; N, 3.80]
20	3,4-Cl2	58–59	6.5	<4.0	0.90 (3H, t, J_H = 7.4 Hz), 1.63 (2H, m), 4.08 (2H, m), 4.18 (2H, d, J_H = 6.2 Hz), 4.89 (1H, m), 6.95 (1H, dd, J_H = 2.9 and 8.9 Hz), 7.20–7.33 (6H, m), 7.50 (1H, t, J_H = 8.9 Hz), 7.77 (1H, d, J_H = 6.2 Hz)	C, 58.68; H, 5.48; N, 3.52 [C, 58.71; H, 5.20; N, 3.80]
21	3,5-Cl_2	88–89	6.5	<4.0	0.90 (3H, t, J_H = 7.4 Hz), 1.63 (2H, m), 4.04–4.24 (4H, m), 4.88 (1H, m), 7.00–7.33 (8H, m), 7.77 (1H, t, J_H = 6.1 Hz)	C, 58.74; H, 5.51; N, 3.49 [C, 58.71; H, 5.20; N, 3.80]
norflurazon			7.5	<4.0		
flurtamone			6.7	<4.0		
flurochloridone			6.1	<4.0		
SAN 380H			5.1	4.3		

to diphosgene (3.6 g, 31 mmol) at 0 °C and stirred for 3 h at 0−25 °C. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure. [2-(3-Chlorophenoxy)-1-ethyl]ethyl chloroformate was obtained in quantitative yield. The product could be used in the subsequent reaction without further purification. To the chloroformate was added a solution of benzylamine (3.3 g, 30 mmol) and pyridine (2.5 g, 31 mmol) in tetrahydrofuran (20 mL) at 5 °C. The reaction mixture was stirred for 24 h at room temperature. After evaporation, the residue was flash-chromatographed on silca gel with ethyl acetate/petroleum ether (1:10, v/v). The isolated compound was further purified by recrystallization from a mixture of dichloromethane and petroleum ether: yield, 7.0 g (70%); solid; mp, 60−61 °C; ^{1}H NMR (250 MHz, DMSO) δ 0.91 (3H, t, J_H = 7.4 Hz), 1.70 (2H, m), 4.09 (2H, d, J_H = 10.5 Hz), 4.18 (2H, d, J_H = 6.5 Hz), 4.92 (1H, m), 6.94 (1H, t, J_H = 7.6 Hz), 7.14−7.31 (7H, m), 7.41 (1H, d, J_H = 7.9 Hz), 7.78 (1H, t, J_H = 6.2 Hz); ^{13}C NMR (62.5 MHz, DMSO) δ 9.56 (1C, s), 24.03 (1C, s), 45.06 (1C, s), 68.99 (1C, s), 73.84 (1C, s), 113.03−115.16 (5C, m), 127.13−128.64 (5C, m), 121.13 (1C, s), 138.40 (1C, s), 159.45 (1C, s); MS (EI), *m*/*z* 335 (M + 2, 32.5%), 333 (M^+, 100%), 206 ($M^+ - C_6H_4ClO$). Anal. Calcd for $C_{18}H_{20}ClNO_3$: C, 64.77; H, 6.04; N, 4.20. Found: C, 64.80; H, 5.95; N, 4.05.

*[2-(3-Chlorophenoxy)-1-ethyl]ethyl benzoate (**41**).* Benzoyl chloride (700 mg, 5 mmol) in tetrahydrofuran (10 mL) was added to a solution of [2-(3-chlorophenoxy)-1-ethyl]ethanol (800 mg, 4 mmol) and triethylamine (500 mg, 5 mmol) in tetrahydrofuran (10 mL). The reaction mixture was stirred for 5 h at 5−25 °C and heated for 30 min at 40 °C. After evaporation of the reaction mixture, ethyl acetate (50 mL) was added. The solution was washed with NaCl solution (3 × 50 mL). The organic phase was dried over anhydrous sodium sulfate and then concentrated to a syrup. The desired compound was separated using flash chromatography on silica gel with ethyl acetate/petroleum ether (1:15, v/v): yield, 740 mg (61%); oil; ^{1}H NMR (250 MHz, DMSO) δ 0.94 (3H, t, J_H = 7.4 Hz), 1.80 (2H, m), 4.24 (2H, d, J_H = 5.0 Hz), 5.29 (1H, m), 6.89−6.99 (2H, m), 7.06 (1H, t, J_H = 2.2 Hz), 7.27 (1H, t, J_H = 8.1 Hz), 7.51 (2H, t, J_H = 7.7 Hz), 7.65 (1H, t, J_H = 7.4 Hz), 7.94 (2H, d, J_H = 7.0 Hz); MS (EI), *m*/*z* 306 (M + 2, 32.5%), 304 (M^+, 100%), 177 ($M^+ - C_6H_4ClO$). Anal. Calcd for $C_{17}H_{17}ClO_3$: C, 67.00; H, 5.62. Found: C, 66.92; H, 5.67.

Table 2. Physical Data of *O*-[2-(3-Trifluoromethylphenoxy)]ethylcarbamates and Their Phytoene Desaturase and ζ-Carotene Desaturase Inhibitory Activities



no.	R_2	R_3	*n*	R_4	mp (°C)	pI_{50} (PDS)	pI_{50} (ZDS)	^{1}H NMR (δ, DMSO)	elem anal. found [calcd] (%)
1	H	C_2H_5	1	H	60–61	6.9	<4.0		
22	H	C_2H_5	0	H	73–74	7.3	<4.0	0.96 (3H, t, J_H = 7.4 Hz), 1.76 (2H, m), 4.21 (2H, m), 5.03 (1H, m), 6.97 (1H, t, J_H = 7.0 Hz), 7.22–7.29 (5H, m), 7.45–7.54 (3H, m), 9.71 (1H, s)	C, 61.11; H, 5.31; N, 3.76 [C, 61.19; H, 5.13; N, 3.96]
23	H	C_2H_5	2	H	39–41	7.5	<4.0	0.89 (3H, t, J_H = 7.4 Hz), 1.64 (2H, m), 2.70 (2H, t, J_H = 7.2 Hz), 3.20 (2H, q, J_H = 6.7 Hz),), 4.11 (2H, q, J_H = 5.8 Hz), 4.87 (1H, m), 7.12–7.30 (9H, m), 7.51 (1H, t, J_H = 4.7 Hz)	C, 62.80; H, 5.88; N, 3.42 [C, 62.98; H, 5.81; N, 3.67]
24	H	C_2H_5	3	H	oil	7.1	<4.0	0.90 (3H, t, J_H = 7.4 Hz), 1.53–1.73 (4H, m), 2.53 (2H, m), 2.99 (2H, q, J_H = 6.3 Hz), 4.10 (2H, m), 4.89 (1H, m), 7.12–7.17 (3H, m), 7.23–7.28 (6H, m), 7.49 (1H, t, J_H = 8.0 Hz)	C, 63.83; H, 6.17; N, 3.28 [C, 63.79; H, 6.12; N, 3.54]
25	H	C_2H_5	1	2-Cl	72–73	7.5	<4.0	0.92 (3H, t, J_H = 7.5 Hz), 1.68 (2H, m), 4.15 (2H, m), 4.25 (2H, d, J_H = 6.0 Hz), 4.93 (1H, m), 7.16–7.30 (6H, m), 7.37–7.52 (2H, m), 7.83 (1H, t, J_H = 6.0 Hz)	C, 56.68; H, 4.93; N, 3.16 [C, 56.79; H, 4.77; N, 3.49]
26	H	C_2H_5	1	3-Cl	61–62	7.5	<4.0	0.91 (3H, t, J_H = 7.4 Hz), 1.65 (2H, m), 4.06–4.20 (4H, m), 4.93 (1H, m), 6.96–7.36 (7H, m), 7.50 (1H, t, J_H = 8.3 Hz), 7.82 (1H, t, J_H = 6.1 Hz)	C, 56.78; H, 5.14; N, 2.99 [C. 56.79; H, 4.77; N, 3.49]
27	H	C_2H_5	1	4-Cl	68–69	7.1	<4.0	0.90 (3H, t, J_H = 7.4 Hz), 1.64 (2H, m), 4.06–4.19 (4H, m), 4.89 (1H, m), 7.22–7.37 (7H, m), 7.51 (1H, t, J_H = 8.3 Hz), 7.81 (1H, t, J_H = 6.2 Hz)	C, 56.65; H, 4.77; N, 3.46 [C, 56.79; H, 4.77; N, 3.49]
28	C_2H_5	H	1	H	40–42	4.7	<4.0	0.92 (3H, t, J_H = 7.4 Hz), 1.67 (2H, m), 4.14–4.18 (4H, m), 4.60 (1H, m), 7.13–7.28 (8H, m), 7.49 (1H, t, J_H = 7.8 Hz), 7.78 (1H, t, J_H = 6.0 Hz)	C, 61.58; H, 5.41; N, 3.61 [C, 62.12; H, 5.49; N, 3.81]
29	H	H	1	H	80–81	7.1	<4.0	4.17–4.32 (6H, m), 7.20–7.33 (8H, m), 7.52 (1H, t, J_H = 7.8 Hz), 7.85 (1H, t, J_H = 6.1 Hz)	C, 60.18; H, 4.80; N, 3.80 [C, 60.18; H, 4.75; N, 4.13]
30	H	CH3	1	H	80–82	7.5	<4.0	1.27 (3H, d, J_H = 6.5 Hz), 4.04–4.19 (4H, m), 5.03 (1H, m), 7.21–7.32 (8H, m), 7.51 (1H, t, J_H = 8.2 Hz), 7.77 (1H, t, J_H = 6.2 Hz)	C, 61.59; H, 5.46; N, 4.02 [C, 61.19; H, 5.13; N, 3.96]
31	H	CH_2Cl	1	H	77–79	7.0	<4.0	3.91 (2H, m), 4.18–4.31 (4H, m), 5.19 (1H, m), 7.21–7.33 (8H, m), 7.52 (1H, t, J_H = 8.2 Hz), 8.00 (1H, t, J_H = 6.2 Hz)	C, 55.82; H, 4.60; N, 3.48 [C, 55.75; H, 4.42; N, 3.61]

Biological Assays. *Substrate and Enzymes.* Phytoene was produced by transformed *Escherichia coli* JM101, which included the plasmid pACCRT-EB with the genes for geranylgeranyl diphosphate synthase and phytoene synthase (*17*). ζ-Carotene was prepared from an *E. coli* transformant with the plasmid pACCRT-EBP according to the method of Linden et al. (*18*). The two enzymes were obtained from *E. coli* transformants with plasmid pG-pds (*12*) for the expression of phytoene desaturase or pQE30zds (*13*, *19*) for ζ-carotene desaturase. The first enzyme is produced by the plant-type pds-gene from the cyanobacterium *Synechococcus* PCC7942; the zds-gene for the second enzyme is from *Capsicum annuum*.

Cell-Free Assays. Determination of PDS inhibitory activity was performed according to the method of Sandmann et al. (*12*). The assay reaction mixture contained 5 μL of l-α-phosphatidylcholine (0.6 g/mL), 500 μL of enzyme solution in tris(hydroxymethyl)aminomethane/HCl buffer (0.1 M, pH 8.0), 500 μL of phytoene (70 μg/mL), and 10 μL of the synthesized compound in methanol. After incubation at 28 °C for 8 h, the assay was terminated by the addition of ethanol (4 mL). The produced ζ-carotene was extracted with 2 mL of diethyl ether/petroleum ether (1:9, v/v). An upper organic phase was evaporated in a stream of N_2 and resuspended in 2 mL of diethyl ether/petroleum ether (1:9, v/v). The amount of ζ-carotene formulation was quantitated from the optical absorbance spectra at 400 and 424 nm.

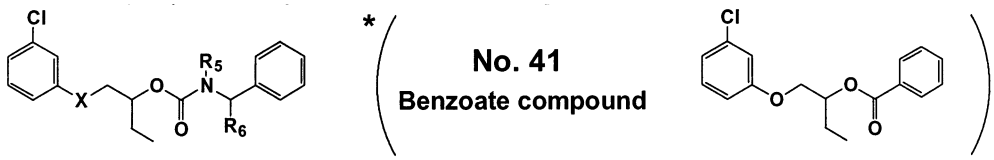
A cell-free assay of ZDS was carried out according to the methods of Breitenbach et al. (*13*). Placed in a test tube were 5 μL of L-α-phosphatidylcholine (0.3 g/mL), 700 μL of enzyme solution in potassium phosphate buffer (0.2 M, pH 7.8), 300 μL of ζ-carotene (10 μg/mL), and 10 μL of a synthesized compound in methanol. The reaction mixture was incubated for 8 h at 28 °C, and then 4 mL of acetone was added to stop the assay. After extraction of the produced lycopene with 3 mL of diethyl ether/petroleum ether (1:9, v/v), the upper organic phase was evaporated in a stream of N_2. The residue was resuspended in 1 mL of acetone and the amount of lycopene formed measured by its absorbance at 465 nm.

I_{50} values of the compounds assayed were calculated from Dixon plots by linear regression analysis with five inhibitor concentrations as shown for three examples in **Figure 2**. Inhibitory activities of the compounds on PDS and ZDS are documented as pI_{50} values, the negative logarithms of the molar concentration that produced a 50% inhibition. The experimental error of the pI_{50} value was within ±0.1.

RESULTS AND DISCUSSION

Target Site of *O*-[1-Ethyl-2-(3-trifluoromethylphenoxy)]-ethyl-*N*-benzylcarbamate (1). Herbicidal activity of *O*-[1-ethyl-2-(3-trifluoromethylphenoxy)]ethyl-*N*-benzylcarbamate (**1**) was reported by Baker (*10*). In a forerunning experiment, bleaching was observed by the trifluoromethyl analogue (**1**) against *Echinochloa crus-galli* in pre-emergence pot tests (data not shown). Compound **1** showed a strong PDS inhibitory activity

Table 3. Physical Data of *O*-(1-Ethyl-2-substituted)ethyl-*N*-aralkylcarbamates and Their Phytoene Desaturase and ζ-Carotene Desaturase Inhibitory Activities



no.	X	R_5	R_6	mp (°C)	pI_{50} (PDS)	pI_{50} (ZDS)	^{1}H NMR (δ, DMSO)	elem anal. found [calcd] (%)
4	O	H	H	60–61	7.3	<4.0		
32	S	H	H	oil	5.3	<4.0	0.87 (3H, t, J_H = 7.8 Hz), 1.63 (2H, m), 3.20 (2H, m), 4.18 (2H, d, J_H = 6.2 Hz), 4.72 (1H, m), 7.18–7.41 (9H, m), 7.71 (1H, t, J_H = 6.2 Hz)	C, 61.92; H, 5.80; N, 3.54 [C, 61.79; H, 5.76; N, 4.00]
33	$NCOCH_3$	H	H	69–70	<5.0	<4.0	0.81 (3H, t, J_H = 7.3 Hz), 1.48 (2H, m), 1.71 (3H, s), 3.73 (2H, m), 4.15 (2H, d, J_H = 4.9 Hz), 4.73 (1H, m), 7.20–7.42 (9H, m), 7.56 (1H, br)	C, 64.04; H, 6.36; N, 7.39 [C, 64.08; H, 6.18; N, 7.47]
34	NCOPh	H	H	109–110	<5.0	<4.0	0.87 (3H, t, J_H = 7.3 Hz), 1.54 (2H, m), 3.81 (2H, m), 4.11 (2H, m), 4.98 (1H, m), 7.00–7.30 (14H, m), 7.73 (1H, t, J_H = 6.1 Hz)	C, 68.70; H, 5.62; N, 6.43 [C, 68.72; H, 5.77; N, 6.41]
35	O	C_2H_5	H	oil	7.2	<4.0	0.97 (6H, m), 1.67 (2H, m), 3.16 (2H, m), 4.09 (2H, m), 4.42 (2H, m), 4.95 (1H, br), 6.89–7.02 (3H, m), 7.22–7.32 (6H, m)	C, 66.37; H, 6.67; N, 3.64 [C, 66.38; H, 6.69; N, 3.87]
36	O	COCH3	H	oil	7.1	<4.0	0.76 (3H, t, J_H = 7.5 Hz), 1.65 (2H, m), 2.47 (3H, s), 4.08 (2H, m), 4.81 (2H, m), 5.04 (1H, m), 6.87 (1H, dd, J_H = 2.3 and 9.4 Hz), 6.98–7.01 (2H, m), 7.18–7.32 (6H, m)	C, 63.85; H, 5.51; N, 3.49 [C, 63.91; H, 5.90; N, 3.73]
37	O	COH	H	oil	7.3	<4.0	0.82 (3H, t, J_H = 7.5 Hz), 1.69 (2H, m), 4.14 (2H, m), 4.69 (2H, q, J_H = 15.2 Hz), 5.14 (1H, m), 6.86 (1H, dd, J_H = 1.7 and 9.2 Hz), 6.98–7.02 (2H, m), 7.15–7.31 (6H, m), 9.26 (1H,s)	C, 62.99; H, 5.88; N, 3.51 [C, 63.07; H, 5.57; N, 3.87]
38	O	H	CH_3 (racemic)	50–52	7.2	<4.0	0.88 (3H, td, J_H = 7.5 and 16.1 Hz), 1.32 (3H, d, J_H = 7.0 Hz), 1.62 (2H, m), 4.08 (2H, m), 4.65 (1H, m), 4.82 (1H, m), 6.91–7.00 (3H, m), 7.18–7.32 (6H, m), 7.70 (1H, br)	C, 65.60; H, 6.28; N, 3.82 [C, 65.61; H, 6.38; N, 4.03]
39	O	H	CH_3 (*R*-form)	50–52	7.1	<4.0	0.88 (3H, td, J_H = 7.5 and 16.1 Hz), 1.32 (3H, d, J_H = 7.0 Hz), 1.62 (2H, m), 4.08 (2H, m), 4.65 (1H, m), 4.82 (1H, m), 6.91–7.00 (3H, m), 7.18–7.32 (6H, m), 7.70 (1H, br)	C, 65.43; H, 6.28; N, 3.72 [C, 65.61; H, 6.38; N, 4.03]
40	O	H	CH_3 (*S*-form)	51–52	7.0	<4.0	0.88 (3H, td, J_H = 7.5 and 16.1 Hz), 1.32 (3H, d, J_H = 7.0 Hz), 1.62 (2H, m), 4.08 (2H, m), 4.65 (1H, m), 4.82 (1H, m), 6.91–7.00 (3H, m), 7.18–7.32 (6H, m), 7.70 (1H, br)	C, 65.62; H, 6.30; N. 3.86 [C, 65.61; H, 6.38; N, 4.03]
41		benzoate compd*			6.1	<4.0	0.94 (3H, t, J_H = 7.4 Hz), 1.80 (2H, m), 4.24 (2H, d, J_H = 5.0 Hz), 5.29 (1H, m), 6.89–6.99 (2H, m), 7.06 (1H, t, J_H = 2.2 Hz), 7.27 (1H, t, J_H = 8.1 Hz), 7.51 (2H, t, J_H = 7.7 Hz), 7.65 (1H, t, J_H = 7.4 Hz), 7.94 (2H, d, J_H = 7.0 Hz)	C, 66.92; H, 5.62 [C, 67.00; H, 5.62]

against cell-free PDS, with a pI_{50} (PDS) value of 6.9. The inhibition was higher than that of flurtamone (pI_{50} = 6.7) or flurochloridone (pI_{50} = 6.1) and 4 times weaker than that of norflurazon (pI_{50} = 7.5). The reciprocal values of ζ-carotene formation were plotted in a Dixon plot against various inhibitor concentrations. With all compounds tested, straight lines were obtained and the regression coefficients obtained were >0.90 (see **Figure 2**). PDS inhibitory activities of 41 compounds synthesized were measured (see **Tables 1**−**3**). Among them, compounds with pI_{50} values >7.3, such as **4**, **8**, **22**, **23**, **25**, **26**, **30**, and **37**, were the strongest PDS inhibitors, the inhibition of which is similar to that of norflurazon. Compounds **1**−**3**, **6**, **7**, **9**−**13**, **16**, **24**, **27**, **29**, **31**, **35**, **36**, and **38**−**40** had strong PDS inhibitory activities, with pI_{50} > 6.8. A moderate activity, pI_{50} > 6, was exhibited by compounds **20**, **21**, and **41** (one reference benzoate compound, changed to an ester from the carbamate structure), corresponding to those of flurtamone and flurochloridone. Compounds **5**, **15**, **17**, **18**, **28**, and **32** had weak inhibitory activities (pI_{50} > 5). Even less activity (pI_{50} < 5.0) was observed with compounds **14**, **19**, **33**, and **34**. A few PDS inhibitors such as SAN 380H and RH-1965 [5-ethyl-2-phenyl-3-propyn-2-yl-6-trifluoromethyl-4(*3H*)-pyrimidinone] have been reported to inhibit both PDS and ZDS (*1*, *20*). However, with carbamates of this study, no inhibition of ZDS was observed using even a 10^{-4} M inhibitor concentration. The target enzyme of these carbamates is PDS, not ZDS.

Effect of Substitutents at the Phenoxybenzene Ring on PDS Inhibition. The effect of substitutents at benzene ring of

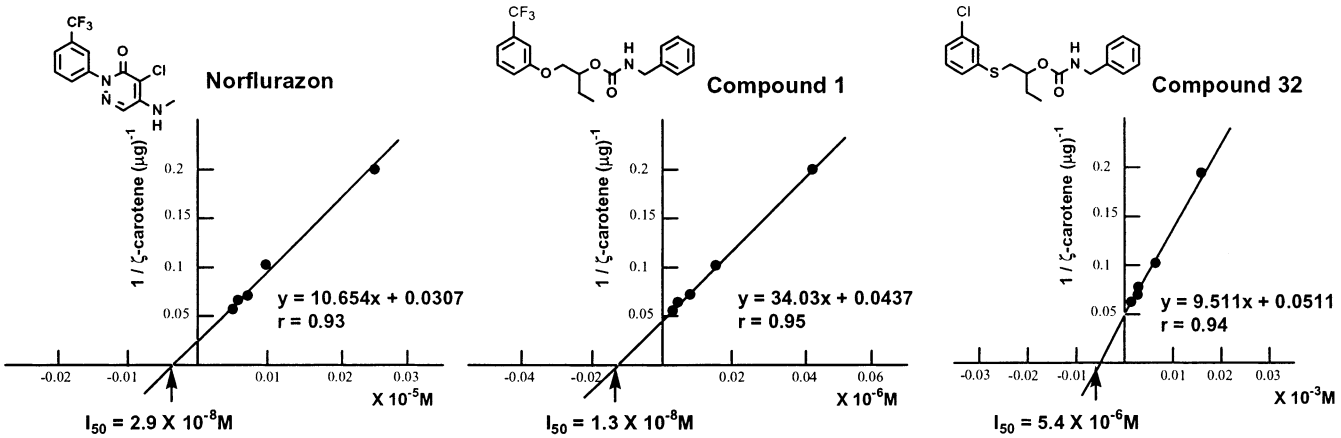


Figure 2. Dixon plots to determine I_{50} values for phytoene desaturase inhibitors.

the phenoxy group of *O*-(1-ethyl-2-phenoxy)ethyl-*N*-benzylcarbamates on PDS inhibition was investigated. A nonsubstituted analogue (compound **2**; pI_{50} value = 7.1) exhibited a high PDS inhibitory activity, matching that of the trifluoromethyl analogue (**1**; pI_{50} = 6.9). Ogawa et al. (*7*) reported a PDS inhibitory activity of 4-alkyl-1,3-diphenylpyrrolidin-2-ones using the same cell-free assay. The nonsubstituted phenyl compound, 4-ethyl-1,3-diphenylpyrrolidin-2-one, showed no PDS inhibition at 10^{-4} M concentration. Substituted phenyl compounds, such as 4-ethyl-3-(3-fluorophenyl)-1-phenylpyrrolidin-2-one (pI_{50} = 6.77) and 4-ethyl-3-(3-fluorophenyl)-1-(3-trifluoromethylphenyl)pyrrolidin-2-one (pI_{50} = 7.30), gave excellent PDS inhibition. With many PDS inhibitors, substituents at the 3-position of the phenoxybenzene moiety increased their herbicidal activity and/or inhibition of ζ-carotene formation in cell (*4*). These results indicate that *O*-(1-ethyl-2-phenoxy)ethyl-*N*-benzylcarbamate must be also a favorable structure for newly designed potent PDS inhibitors. The PDS inhibitory activity of carbamate analogues depended on the position of the substituents at the phenoxybenzene moeity. In the case of the monochloro analogues (**3**−**5**), the 3-chloro analogue (**4**; pI_{50} = 7.3) and the 2-chloro analogue (**3**; pI_{50} = 7.0) were apparently stronger inhibitors than the 4-chloro analogue (**5**; pI_{50} = 5.5). A similar positional effect has been observed with other above-mentioned PDS inhibitors. For dichloro analogues (**16**−**21**), the 2,3-dichloro compound (**16**; pI_{50} = 6.9) showed the best inhibitory activity. 3,4-Dichloro (**20**; pI_{50} = 6.5) and 3.5-dichloro (**21**; pI_{50} = 6.5) analogues were moderate PDS inhibitors. 2,4-Dichloro (**17**; pI_{50} = 5.3) and 2,5-dichloro (**18**; pI_{50} = 5.4) analogues exhibited a weak inhibition, and the 2,6-dichloro analogue (**19**; pI_{50} < 5.0) had little inhibition. Introduction of a substituent(s) at the 2- and/or 3-position of the phenoxybenzene ring was favorable to keeping the strong PDS inhibition. With 3-substituted phenoxy derivatives (**4** and **6**−**13**) a strong PDS inhibition with a pI_{50} value of ∼7 was found, except for the carboxylic acid (**14**; pI_{50} < 5.0) and the acetylamino analogue (**15**; pI_{50} = 5.4). Introduction of an electron-withdrawing group such as fluorine (**6**; pI_{50} = 7.2) or the cyano group (**8**; pI_{50} = 7.3) produced a somewhat higher inhibition than that of an electron-donating group such as the methyl group (**10**; pI_{50} = 6.9).

Effect of the Methylene Chain at Aralkylamino Groups on PDS Inhibition. As another structural modification, the length of the methylene chain at aralkylamino groups was changed. Four *O*-[1-ethyl-2-(3-trifluoromethylphenoxy)]ethylcarbamates investigated (**1** and **22**−**24** in **Table 2**) showed a strong PDS inhibitory activity. Their activity order was as follows: phenylethyl (**23**; pI_{50} = 7.5), phenyl (**22**; pI_{50} = 7.3), phenylpropyl (**24**; pI_{50} = 7.1), and benzyl (**1**; pI_{50} = 6.9). The phenylethyl analogue (**23**; pI_{50} = 7.5) exhibited the highest PDS inhibitory activity in this study, although it was interesting that the benzyl analogue (**1**; pI_{50} = 6.9) exhibited the lowest activity in this series. Apparently arylcarbamates with an even number (including 0) of the methylene chain were more active than aralkylcarbamates with an odd number.

Effect of the Substituent at the Benzene Ring of *N*-Benzylcarbamates. 2-Chloro (**25**; pI_{50} = 7.5) and 3-chloro (**26**; pI_{50} = 7.5) derivatives showed the highest PDS inhibitory activities (see **Table 2**). The pI_{50} value of the 4-chloro derivative was 7.1, which is about the same activity of the nonsubstituted analogue (**1**; pI_{50} = 6.9). Introduction of chlorine into the 2- or 3-position at the benzene ring of the *N*-benzylcarbamate moiety was favorable to enhancing the PDS inhibitory activity.

Introduction of an Alkyl Group at the Phenoxyethyl Moiety and PDS Inhibitory Activity. The methyl analogue (**30**; pI_{50} = 7.5) showed a 4 times stronger activity than the ethyl analogue (**1**; pI_{50} = 6.9). Elimination (**29**; pI_{50} = 7.1) of the ethyl group kept the strong PDS inhibition. PDS inhibitory activity of the *O*-[2-ethyl-2-(3-trifluoromethylphenoxy)]ethyl analogue (**28**; pI_{50} = 4.7) was 100 times weaker than that of the *O*-[1-ethyl-2-(3-trifluoromethylphenoxy)]ethyl analogue (**1**). Introduction of a short-chain alkyl group at the α-position of the ethylene bridge between the phenoxy group and the carbamate is of importance to enhancing the PDS inhibitory activity.

Importance of Oxygen at the Phenoxy Group for PDS Inhibition. Exchange of the oxygen bridge at the phenoxyethyl moiety by sulfur or nitrogen led to a complete loss of inhibitory activity (**32**; pI_{50} = 5.3) and (**33** or **34**; pI_{50} < 5.0), respectively. The phenoxy moiety is essential to producing a strong PDS inhibition, and the oxygen atom may be necessary to bind the inhibitor to the target enzyme by hydrogen bonding.

Other Modifications and PDS Inhibition. The *N*-ethyl (**35**; pI_{50} = 7.2), *N*-acetyl (**36**; pI_{50} = 7.1), and *N*-formyl (**37**; pI_{50} = 7.3) analogues exhibited a potent inhibition, with almost the same inhibitory activity as that of the mother NH compound (**4**; pI_{50} = 7.3). The influence of the absolute configuration of the chiral center at the 2-phenylethylamino group was also investigated (see **Table 3**). Racemic (**38**; pI_{50} = 7.2), *R*-(+)-form (**39**; pI_{50} = 7.1), and *S*-(−)-form (**40**; pI_{50} = 7.0) analogues showed almost the same PDS inhibitory activity, apparently indicating independence of chirality for PDS inhibition. A reference benzoate compound (**41**; pI_{50} = 6.1), changed to ester from the carbamate structure of compound (**4**; pI_{50} = 7.3), was ∼15 times less active than the mother compound (**4**), indicating that the carbamate moiety must be essential for a strong PDS inhibitory activity.

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Received for review December 20, 2002. Revised manuscript received March 2, 2003. Accepted March 6, 2003. A scholarship from Deutscher Akademischer Austauschdienst (DAAD) for S.O. is gratefully acknowledged.

JF0262413