# Biochemical and Physiological Effects of Benzheterocycles and Related Compounds

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The biochemical and physiological effects of 18 benzheterocycles and 1 tetrahydrophthalimide-substituted indolin-2(3*H*)-one were investigated. Eighteen of the compounds were inhibitors of protoporphyrinogen oxidase (Protox) *in vitro*, with $I_{50}$ values ranging from 25 to 650 nM. There was a good correlation between the activity of these compounds as *in vitro* Protox inhibitors and their *in vivo* effects on protoporphyrin IX (Proto IX) accumulation. There was also a good correlation between the amount of Proto IX caused to accumulate and the light-induced cellular damage. However, several of the compounds had phytotoxic activity in darkness, indicating that they have mechanisms of action in addition to inhibition of Protox. Greenhouse results generally agreed with laboratory results. The structure/activity relationships for inhibition of Protox are discussed.

**Keywords:** *Benzheterocycles; herbicide; peroxidizing herbicides; protoporphyrinogen oxidase; protoporphyin IX*

## INTRODUCTION

Certain diphenyl ether herbicides exert their effect by causing rapid peroxidative photobleaching and desiccation of plant tissues (Kunert et al., 1987). They are known to be potent competitive inhibitors of protoporphyrinogen oxidase (Protox), the last common enzyme in the biosynthesis of both heme and chlorophylls (Matringe et al., 1989, 1992; Witkowski and Halling, 1989; Duke et al., 1990, 1991a,b; Scalla et al., 1990; Varsano et al., 1990; Camadro et al., 1991; Nandihalli et al., 1992a; Nandihalli and Duke, 1993; Scalla and Matringe, 1994). Paradoxically, inhibition of Protox causes massive accumulation of protoporphyrin IX (Proto IX), the product of Protox (Matringe and Scalla, 1988; Becerril and Duke, 1989b; Matsumoto and Duke, 1990; Sherman et al., 1991a). This puzzling phenomenon has recently been explained by the existence of a herbicide-resistant Protox in the plasma membrane (PM) (Jacobs et al., 1991; Jacobs and Jacobs, 1993; Lee et al., 1993; Duke et al., 1994; Lee and Duke, 1994). When plastid Protox is inhibited by a diphenyl ether herbicide, protoporphyrinogen IX (Protogen IX), the substrate of Protox, is exported from the plastid (Jacobs and Jacobs, 1993) and is rapidly oxidized to Proto IX by PM-bound herbicide-resistant Protox (Jacobs et al., 1991; Lee et al., 1993; Lee and Duke, 1994). Proto IX, which is relatively lipophilic, accumulates in the PM and/or other extraplastidic membranes (Nandihalli et al., 1992b; Lee et al., 1993). In the presence of light and molecular oxygen, Proto IX generates singlet oxygen, subsequently causing rapid membrane peroxidation and cellular death (Duke et al., 1990, 1991a; Scalla et al., 1990).

Strong correlations have been reported between Protox inhibition, level of Proto IX accumulation, and resulting herbicidal damage caused by several groups of Protox inhibitors (Becerril and Duke, 1989b; Sherman et al., 1991a,b; Nandihalli et al., 1992b,c, 1994). A new group of herbicides, phenoxy-substituted benzheterocycles (Condon et al., 1993; Karp et al., 1993; Karp, 1992; Wepplo et al., 1993), with all of the structural prerequisites of Protox inhibitors (Nandihalli and Duke, 1993, 1994), was recently disclosed (Karp, 1992). In this study, we examine 18 phenoxy-substituted benzheterocycles and one tetrahydrophthalimide substituted indolin-2(3*H*)-one (Figure 1) to determine if they exert their herbicidal activity through inhibition of Protox. We demonstrate that some of these compounds are potent Protox inhibitors and that their herbicidal activities at the tissue and whole plant levels correlate well with their capacities to cause Protox inhibition and Proto IX accumulation.

## MATERIALS AND METHODS

**Plant Materials.** For tissue section and *in vitro* experiments, seeds of barley (*Hordeum vulgare* L. var. Morex) were germinated in flats in a commercial greenhouse substrate (Jiffy-Mix; JPA, West Chicago, IL) and watered with distilled water. Plants were grown at 25 °C under white light of 500 $\mu$mol $m^{-2}$ $s^{-1}$ photosynthetically active radiation (PAR) and >90% relative humidity before tissues were harvested for use at 7 days of growth. Some barley plants were grown in darkness at 25 °C for 7 days.

For whole plant studies seeds of velvetleaf (*Abutilon theophrasti*), *Amaranthus* species (pigweed), ragweed (*Ambrosia artemisiifolia* L.), *Ipomoea* species (morningglory), and soybean (*Glycine max*) were planted in a 12 cup flat, with each cup containing ca. 200 $cm^3$ of artificial potting media (METRO 350) and grown in a greenhouse maintained at 32 °C days and 21 °C nights with a 14-h photoperiod. Plants were grown for 10–14 days prior to treatment, at which time they had two to four leaves.

**Herbicide Treatment.** In tissue section experiments, tissues were treated with the phenoxy-substituted compounds depicted in Figure 1 (technical grade, >95% purity) as before (Kenyon et al., 1985) by cutting 50 4-mm barley leaf sections (approximately 0.2 g of fresh weight) with a razor blade and then placing them in a 6-cm diameter polystyrene Petri dish in 5 mL of 1% sucrose and 1 mM 2-(*N*-morpholino)ethanesulfonic acid (MES, pH 6.5) with or without the test compound

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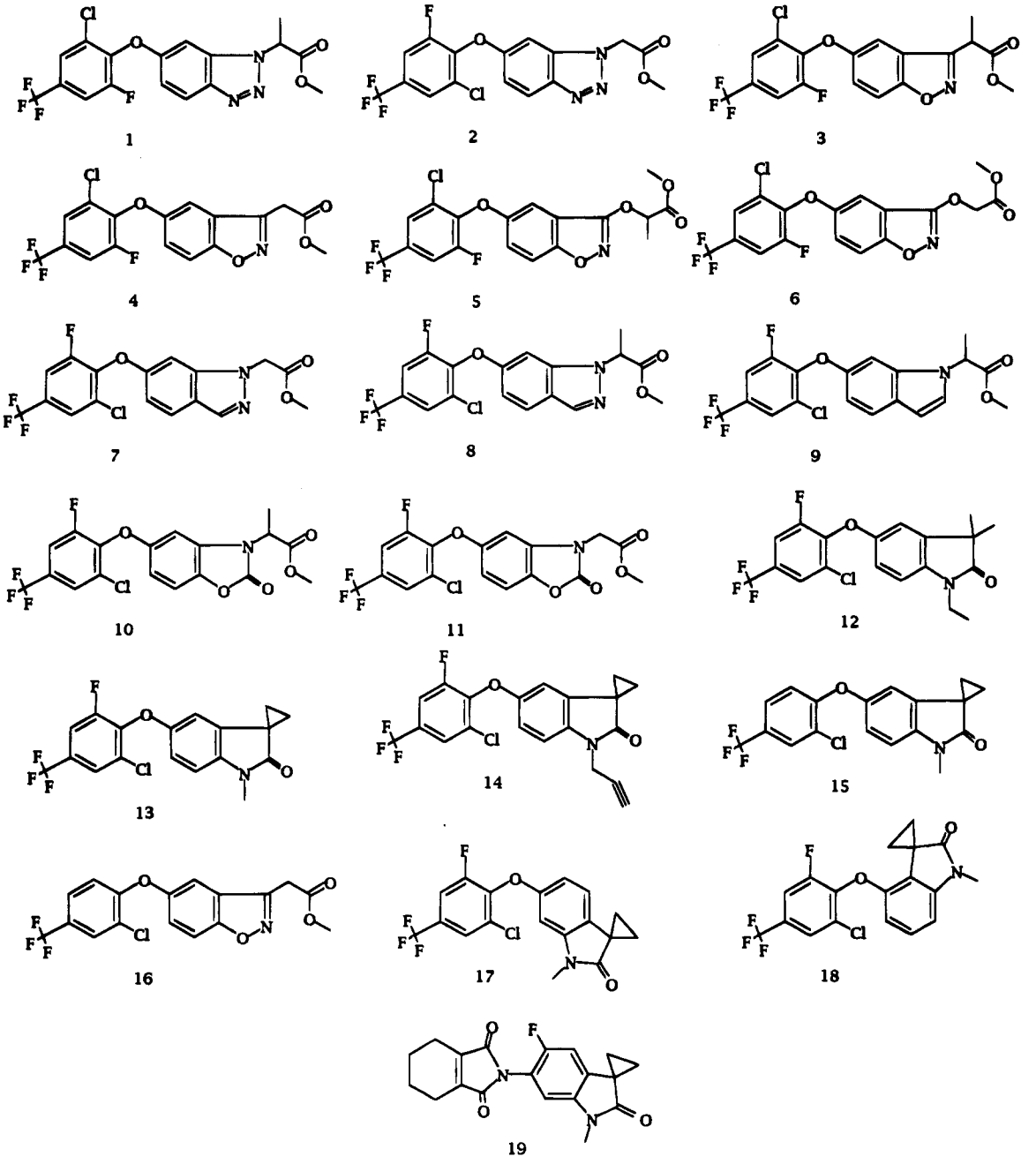


**Figure 1.** Structures of herbicides used in these studies.

dissolved in acetone or dimethyl sulfoxide (DMSO). Controls contained the same amount of acetone or DMSO without the test compounds. The final concentration of acetone or DMSO in the dishes was 1–2% (v/v). The tissues were incubated at 25 °C in darkness for 20 h and then exposed to 500 $\mu$mol $m^{-2}$ $s^{-1}$ PAR for up to 24 h.

In whole plant studies, plants were sprayed with technical grade herbicide in a 50:50 acetone/water solution containing 1% (v/v) Sun-it II spray adjuvant, using a laboratory belt sprayer delivering 400 L/ha spray volume. Flats were returned to the same greenhouse conditions after application and allowed to grow for 10 days before being evaluated visually.

**Herbicidal Activity.** Cellular damage was measured by detection of electrolyte leakage into the bathing medium with a conductivity meter with the capability of assaying 1 mL of bathing medium and returning it to the dish as before (Kenyon et al., 1985). Conductivity was monitored for 20 h in darkness, followed by 24 h of continuous light. Because of differences in background conductivity of different treatment solutions, results were expressed as change in conductivity after initial measurement at the beginning of the dark period. All treatments for electrolyte leakage measurements were triplicated.

Visual herbicide efficacy ratings of intact sprayed plants were made using a 0–9 scale with 0 equal to no plant injury and 9 being equivalent to plant death. Acceptable crop injury is considered to be a rating of less than 2, and acceptable weed injury is a rating of greater than 7.

**Porphyrin Determinations.** All extractions for HPLC determinations of Proto IX, Mg-Proto IX, Mg-Proto IX monomethyl ester (ME), and protochlorophyllide (PChlide) were made under a dim, green light source after 20 h of incubation in darkness at 25 °C. Samples (approximately 0.2 g of barley leaf section) were homogenized in 3 mL of HPLC grade methanol/0.1 N $NH_4OH$ (9:1 v/v) with a Brinkman Polytron at full speed for 15 s. The homogenate was centrifuged at 30000*g* for 10 min at 4 °C and the supernatant saved. The pellet was resuspended in 3 mL of HPLC grade basic methanol, sonicated for 5 min, and centrifuged at 30000*g* for 10 min at 4 °C. Supernatants were combined and evaporated to dryness at 40 °C with a rotary evaporator. The residue was dissolved in 2 mL of HPLC grade basic methanol by sonication for 5 min and filtered through a 0.2-$\mu$m nylon syringe filter. Samples were stored in light-tight, aluminum foil-wrapped, glass vials at −20 °C until analysis by HPLC.

HPLC determinations for porphyrins were made according to the method of Matsumoto and Duke (1990). The HPLC system was composed of Waters Associate components which included two Model 510 pumps, a Model 712 autosampler, a Maxima 820 controller, and a Model 990 photodiode spectrophotometric detector. A Model 470 fluorescence detector preceded the Model 990 detector. The column was a 250 × 4.6 mm (i.d.) Spherisorb 5-$\mu$m ODS-1 reversed-phased column preceded by a Bio-Rad ODS-5S guard column. The solvent gradient was composed of 0.1 M ammonium phosphate (pH 5.8) (solvent A) and HPLC grade methanol (solvent B) at a flow rate of 1.4 mL/min. The solvent delivery program was as follows: 20% A in B from 0 to 10 min, a linear transition from 20 to 0% A in B from 10 to 18 min, and B only from 18 to 35 min. The injection volume was 50 $\mu$L. Commercial standards of Proto IX (Sigma Chemical Co.), Mg-Proto IX, and Mg-Proto IX ME (Porphyrin Products, Inc.) were used. PChlide was obtained by extraction from etiolated tissues, quantified spectrophotometrically as before (Becerril and Duke, 1989a), and injected into the HPLC for calibration of the spectrofluorometric detector. Porphyrin detection was performed with fluorescence detector excitation and emission wavelength settings of 400 and 630 nm, respectively, for Proto IX; 415 and 595 nm, respectively, for Mg-Proto IX and Mg-Proto IX ME; and 440 and 630 nm, respectively, for PChlide. The photodiode array detector scanned from 300 to 700 nm to confirm all peaks. All porphyrin compound levels were expressed on a molar basis per gram of fresh weight. All treatments for porphyrin determinations were triplicated.

**Etioplast Preparation.** Crude etioplasts were prepared for *in vitro* experiments as before (Lee et al., 1993). All procedures were conducted under a dim, green light source. Leaves of dark-grown barley seedlings were cut into small pieces and homogenized with a Sorvall Omni-Mixer twice for 5 s at full speed using a fresh weight/volume ratio of 1:3. Homogenization buffer consisted of 50 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES, pH 7.8), 330 mM sucrose, 1 mM $MgCl_2$, 1 mM ethylenediaminetetraacetic acid (EDTA), 2.5 mM dithiothreitol (DTT), and 0.1% bovine serum albumin (BSA, fatty acid free). The homogenate was filtered through one layer of Miracloth and was centrifuged at 200 *g* for 5 min at 4 °C to remove crude cell debris. The resulting supernatant was centrifuged at 1500*g* for 20 min at 4 °C. The pelleted crude etioplasts were gently resuspended in a suspension buffer using a small paintbrush. The suspension buffer was composed of 50 mM HEPES (pH 7.8) and 330 mM sucrose. The etioplasts were stored at −80 °C until use.

**Protox Assays.** Before assay, the extracts of etioplasts were thawed and sonicated twice for 5 s at 0 °C. Protein concentration was determined according to the method of Bradford (1976) with BSA used for a standard, and the extracts were adjusted to 4 mg of protein/mL in the suspension buffer. When the test compounds (Figure 1) were utilized, they were added in a volume of 2 $\mu$L of acetone or DMSO to 200 $\mu$L of the extract. Acetone or DMSO was added to control treatments. The extracts were allowed to incubate on ice for 15 min with or without the test compound.

Protogen IX was prepared according to the procedure of Jacobs and Jacobs (1982) with the following modifications. Proto IX stock solution (0.5 mM in 20% ethanol containing 10 mM KOH) was reduced to Protogen IX with approximately one-eighth volume of freshly ground sodium amalgam. The resulting colorless solution was adjusted to pH 8.0 by addition of an equal volume of (5× strength) assay buffer, consisting of 500 mM HEPES (pH 7.5) and 25 mM EDTA. Residual amalgam and porphyrin aggregates were removed by passing the solution through a 0.2-$\mu$m nylon syringe filter. DTT was added to the Protogen IX solution in a light-tight glass to a final concentration of 2 mM. The resulting preparation was stable in dim light at room temperature for at least 2 h.

Protox was assayed according to the procedure of Sherman et al. (1991a). The assay mixture consisted of 100 mM HEPES (pH 7.5), 5 mM EDTA, 2 mM DTT, and approximately 2 $\mu$M Protogen IX. The reaction was initiated by addition of 0.1 mL of extract with or without the test compound to 0.9 mL of assay mixture and monitored for 2 min at 30 °C. Fluorescence was monitored directly from the assay mixture using a Shimadzu RF-5000U, temperature-controlled, recording spectrofluorometer with excitation and emission wavelengths set at 395 and 622 nm, respectively. The reaction rate was essentially constant over a 2-min period. Autoxidation of Protogen IX to Proto IX in the presence of heat-inactivated (80 °C for 15 min) extract was negligible. All treatments for Protox assays were triplicated.

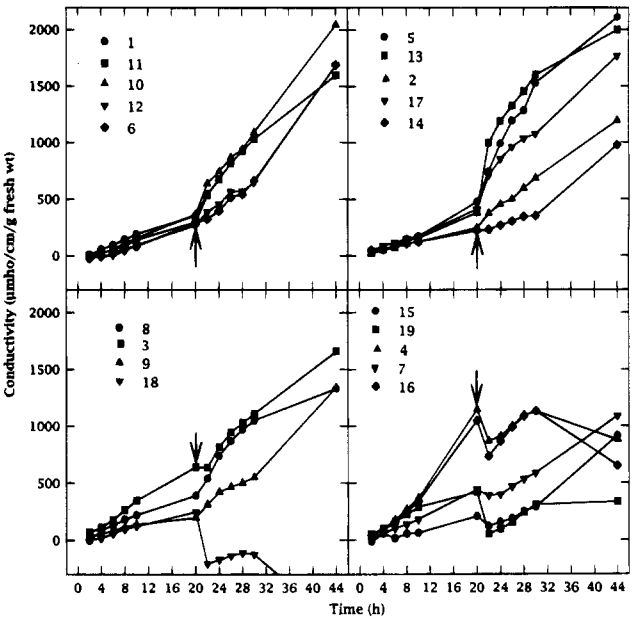


**Figure 2.** Effects of 100 $\mu$M of each of 19 compounds of Figure 1 on electrolyte leakage of barley leaf sections on the solutions for 20 h in darkness, followed by exposure to continuous light (arrows indicate beginning of illumination) for 24 h. Values are averages of differences between treated and untreated (control) tissues from three measurements.

## RESULTS

**Herbicidal Activity.** Cellular damage measured by electrolyte leakage from green barley leaf sections varied greatly with the test compounds used (Figure 2). For example, compounds **5**, **10**, and **13** were the most active, whereas compounds **18** and **19** were not herbicidal with this assay. Most of the compounds caused some cellular damage during 20 h of dark incubation, and two (**4** and **16**) were quite active in darkness. The most rapid light-caused cellular leakage was seen in sections treated with compounds **5** and **13**. The kinetics of electrolyte leakage was close to linear for all compounds in darkness, except the two most active compounds in darkness (**4** and **16**). The kinetics in the light was extremely variable, ranging from initial rapid leakage that later slowed for the most active compounds to more linear rates for less active compounds. The rate of leakage was more rapid in the light than darkness for 13 of the compounds. The electrolyte leakage in darkness caused by the two compounds with greatest activity in darkness was reduced when the tissues were exposed to light. A slight reduction in electrolyte leakage after light exposure was measured with a few other compounds. Considering that other Protox inhibitor herbicides are not known to cause significant cellular damage in darkness (Duke et al., 1984; Sherman et al., 1991a), these compounds might have a different or an additional mechanism of action from that of compounds that only act as Protox inhibitors.

In whole plant studies, most of the compounds killed or caused severe phytotoxicity to the four weed species

at 32 g/ha (Table 1). Velvetleaf and pigweed were particularly vulnerable to the herbicides. None of the compounds killed soybean at this application rate. However, several of them caused unacceptable herbicide injury at this rate, and others had useful selectivity with soybean.

**Porphyrin Accumulation.** There was no significant Proto IX accumulation in control tissues after 20 h in darkness (Table 2). All of the compounds except **18** caused Proto IX to accumulate in darkness. Proto IX accumulation caused by the different compounds ranged from 1.81 to 11.62 nmol/g of fresh weight (11–73-fold the control level). Levels of PChlide were almost unaffected or changed insignificantly by the test compounds (data not shown). Mg-Proto IX and Mg-Proto IX ME could not be found in any of the treatments because they were below the limit of the fluorescence detector used.

**Protox Inhibition.** All of the test compounds inhibited Protox activity in an *in vitro* assay. Typical dose–response curves for Protox inhibition by the test compounds are shown in Figure 3. The $I_{50}$ values of the test compounds ranged from 25 to approximately 100 000 000 nM (Table 2).

**Correlation of Proto IX Accumulation with Protox Inhibition and Herbicidal Damage.** A significant (95% level) correlation was found between *in vitro* Protox inhibition and *in vivo* Proto IX accumulation caused by the 18 compounds that caused Proto IX to accumulate and also inhibited Protox (Figure 4A). Compound **18** was not included in this analysis because it had neither activity. The light-induced herbicidal damage caused by the test compounds correlated very well (99% level) with the Proto IX that accumulated (Figure 4B).

We found poor correlations between whole plant activity of the herbicides (Table 1) and barley Protox inhibition (Table 2) (data not shown). However, those compounds with low Protox $I_{50}$ values were very herbicidal, and the compound that was not a Protox inhibitor (**18**) was the only compound that was not herbicidal on any species, even at 500 g/ha.

## DISCUSSION

We describe in this paper the herbicidal and inhibitory properties of an array of Protox inhibitors belonging to the phenoxy-substituted benzheterocycle class. The relationships between *in vitro* Protox-inhibiting activity, capacity to cause Proto IX accumulation, and herbicidal activities were reasonably clear. In previous work with 24 diphenyl ether compounds, there were no such correlations (Nandihalli et al., 1992a); however, in earlier studies with fewer, but active, diphenyl ether compounds, we found good correlations between Proto IX accumulation and herbicidal activity (Becerril and Duke, 1989b). In a study of 10 Protox inhibitors, each from a different chemical class, there was a poor correlation between herbicidal activity and the Protox $I_{50}$; however, there was a good correlation between the amount of Proto IX induced by the compounds and the herbicidal damage (Reddy et al., 1995). In studies with pyrazole phenyl ethers (Sherman et al., 1991b) and *O*-phenylpyrrolidino- and piperidinocarbamate herbicides (Nandihalli et al., 1992c), good correlations were found between *in vitro* effects on Protox, Proto IX accumulation, and herbicidal damage. Lack of correlations between *in vitro* and *in vivo* results can be due to uptake, metabolism, bioactivation, or secondary site of

**Table 1. Herbicidal Activities of Different Application Rates of Each of 18 Compounds on Velvetleaf, Pigweed, Ragweed, Morningglory, and Soybean**

| | | herbicidal injury[b] | | | | |
|---|---|---|---|---|---|---|
| compd | rate[a] (g/ha) | velvetleaf | pigweed | ragweed | morningglory | soybean |
| **1** | 63 | 9 | 9 | 9 | 9 | 8 |
| | 32 | 9 | 9 | 9 | 9 | 4 |
| **2** | 63 | 9 | 9 | 9 | 9 | 7 |
| | 32 | 6 | 9 | 9 | 8 | 7 |
| **3** | 125 | 9 | 9 | 9 | 9 | 5 |
| | 63 | 9 | 9 | 9 | 9 | 5 |
| | 32 | 8 | 9 | 8 | 8 | 4 |
| **4** | 125 | 9 | 9 | 9 | 9 | 8 |
| | 63 | 9 | 9 | 9 | 9 | 8 |
| | 32 | 9 | 9 | 9 | 9 | 8 |
| **5** | 500 | – | – | – | 9 | 6 |
| | 250 | 9 | 9 | 9 | 9 | 5 |
| | 125 | 9 | 9 | 9 | 9 | 3 |
| | 63 | 9 | 9 | 9 | 9 | 4 |
| | 32 | 9 | 9 | 9 | 9 | 3 |
| **6** | 125 | – | – | – | 9 | – |
| | 63 | – | 9 | 9 | 9 | 6 |
| | 32 | – | 9 | 9 | 9 | 3 |
| **7** | 500 | 9 | 9 | 9 | 9 | 7 |
| | 250 | 9 | 9 | 9 | 9 | 6 |
| | 125 | 8 | 9 | 9 | 8 | 5 |
| | 63 | 9 | 9 | 8 | 8 | 5 |
| | 32 | 8 | 9 | 8 | 9 | 3 |
| **8** | 500 | 9 | 9 | 9 | 9 | 8 |
| | 250 | 9 | 9 | 9 | 9 | 7 |
| | 125 | 9 | 9 | 9 | 9 | 5 |
| | 63 | 9 | 9 | 9 | 9 | 5 |
| | 32 | 9 | 9 | 9 | 8 | 5 |
| **9** | 500 | – | 9 | 9 | 9 | 3 |
| | 250 | – | 9 | 5 | 7 | 3 |
| | 125 | – | 9 | 5 | 9 | 3 |
| | 63 | – | 8 | 6 | 6 | 3 |
| | 32 | – | 8 | 6 | 5 | 3 |
| **10** | 125 | 9 | 9 | 9 | 9 | 7 |
| | 63 | 9 | 9 | 9 | 9 | 7 |
| | 32 | 9 | 9 | 8 | 6 | 3 |
| **11** | 125 | 9 | – | 9 | 9 | 8 |
| | 63 | 9 | – | 9 | 9 | 8 |
| | 32 | 7 | – | 9 | 9 | 7 |
| **12** | 500 | 9 | 9 | 8 | 9 | 4 |
| | 250 | 9 | 9 | 8 | 9 | 4 |
| | 125 | 9 | 9 | 7 | 9 | 5 |
| | 63 | 9 | 9 | 7 | 8 | 3 |
| | 32 | 8 | 4 | 9 | 8 | 3 |
| **13** | 250 | 9 | 9 | 9 | 9 | 4 |
| | 125 | 9 | 9 | 8 | 8 | 4 |
| | 63 | 9 | 9 | 7 | 8 | 6 |
| | 32 | 8 | 9 | 6 | 8 | 5 |
| **14** | 500 | 9 | 9 | 9 | 9 | 3 |
| | 250 | 9 | 9 | 8 | 9 | 3 |
| | 125 | 8 | 9 | 7 | 8 | 3 |
| | 63 | 9 | 9 | 7 | 8 | 1 |
| | 32 | 8 | 7 | 8 | 8 | 0 |
| **15** | 500 | 9 | 9 | 4 | 9 | 3 |
| | 250 | 9 | 9 | 5 | 7 | 4 |
| | 125 | 9 | 9 | 4 | 8 | 2 |
| | 63 | 9 | 9 | 4 | 7 | 3 |
| | 32 | 7 | 4 | 3 | 7 | 2 |
| **16** | 250 | 9 | 9 | 9 | 9 | 8 |
| | 125 | 9 | 9 | 9 | 9 | 9 |
| | 63 | 9 | 9 | 9 | 9 | 5 |
| | 32 | 8 | 9 | 9 | 9 | 4 |
| **17** | 500 | 9 | 9 | 6 | 9 | 6 |
| | 250 | 7 | 9 | 5 | 9 | 3 |
| | 125 | 7 | 9 | 4 | 7 | 2 |
| | 63 | 5 | 9 | 5 | 7 | 1 |
| | 32 | 7 | 7 | 5 | 4 | 1 |
| **18** | 500 | 0 | 0 | 0 | 0 | 0 |
| **19** | 500 | 9 | 9 | 8 | 8 | 4 |
| | 250 | 9 | 9 | 7 | 7 | 4 |
| | 125 | 9 | 9 | 7 | 8 | 3 |
| | 63 | 9 | 9 | 7 | 7 | 2 |
| | 32 | 9 | 9 | 5 | 7 | 2 |

[a] Those rates at which all species were given scores of 9 are deleted. [b] A rating of 9 represents complete mortality and 0 indicates no observed phytotoxicity. Intermediate ratings are described under Materials and Methods. A dash indicates missing data.

**Table 2. Effects of 100 μM of Each of 19 Compounds on Proto IX Accumulation in Barley Leaf Sections Floated on the Test Solution for 20 h in Darkness and Protox $I_{50}$ Values for the Compounds, Using Barley Etioplasts as a Protox Source**

| compd | Proto IX content (nmol/g of fresh wt ± SE) | Protox $I_{50}$ (nM) |
|---|---|---|
| **1** | 8.74 ± 0.51 | 25 |
| **2** | 6.59 ± 0.59 | 70 |
| **3** | 8.39 ± 0.56 | 45 |
| **4** | 5.39 ± 0.29 | 55 |
| **5** | 5.89 ± 0.31 | 150 |
| **6** | 6.51 ± 0.70 | 250 |
| **7** | 8.44 ± 0.25 | 55 |
| **8** | 11.17 ± 0.61 | 50 |
| **9** | 7.29 ± 0.63 | 320 |
| **10** | 11.48 ± 1.11 | 30 |
| **11** | 8.30 ± 0.59 | 35 |
| **12** | 5.44 ± 0.19 | 250 |
| **13** | 11.62 ± 1.07 | 30 |
| **14** | 6.75 ± 0.41 | 70 |
| **15** | 1.81± 0.32 | 150 |
| **16** | 2.31 ± 0.20 | 650 |
| **17** | 9.23 ± 0.55 | 70 |
| **18** | 0.15 ± 0.02 | 100 000 000 |
| **19** | 2.15 ± 0.22 | 150 |
| control | 0.16 | |

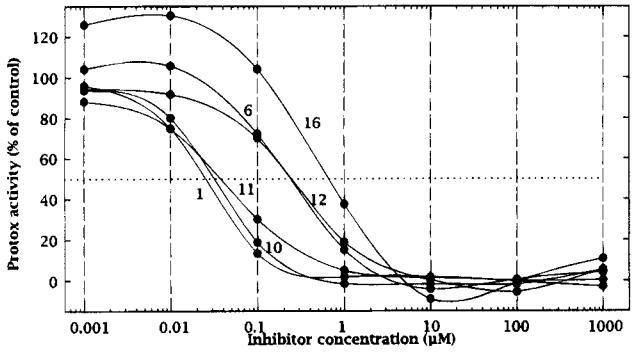


**Figure 3.** Typical dose–response curves for inhibition of Protox by herbicides used in these studies.

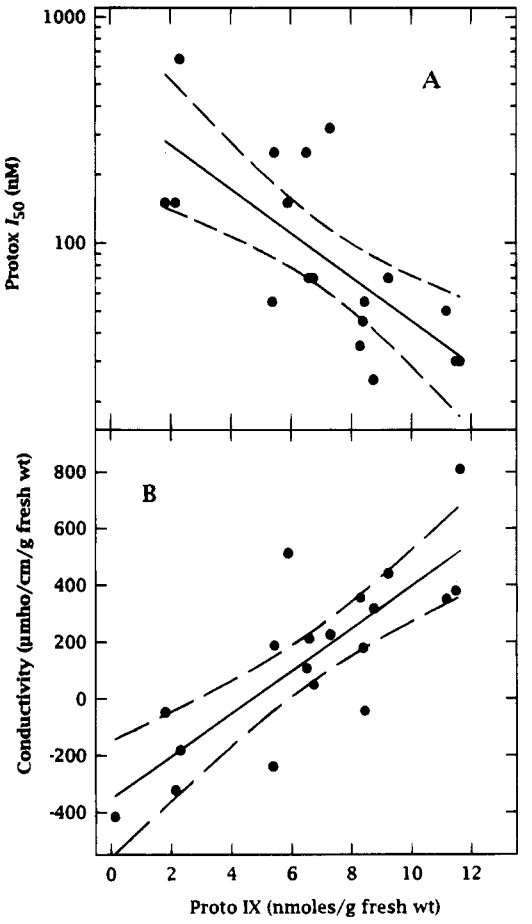


**Figure 4.** Relationships between Proto IX accumulated after a 20-h dark period after treatment with 18 (A) or 19 (B) different herbicides and (A) *in vitro* Protox $I_{50}$ values or (B) light-induced electrolyte leakage as measured by the conductivity of the bathing solution after 4 h of light minus the conductivity at the beginning of the dark period.

action causes. Some of these compounds would likely be hydrolized *in vivo*, resulting in either a more or a less active Protox inhibitor. The somewhat different kinetics of induced electrolyte leakage for the 19 compounds tested here (Figure 2) will account for some of the variability in the correlation analysis of Figure 4B. Greenhouse data with four weed and one crop species (Table 1) agreed only in a very general way with our laboratory results with barley (Table 2). This is the best one could expect, because the greenhouse data did not cover a range of effects, except in soybeans, and soybeans are known to rapidly detoxify compounds with similar structure [*e.g.*, Frear et al. (1983)].

The finding that several of the compounds used in this study were quite phytotoxic in darkness suggests that some members of this chemical family may have another primary site of action. There was no correlation between dark phytotoxicity and effects on Protox or on Proto IX accumulation. The diphenyl ethers are also known to have several primary sites of action other than Protox, including effects on carotenoid synthesis, electron transport (both respiratory and photosynthetic), and chloroplast ATPase activity (Kunert et al., 1987).

Except for one compound (**18**), the Protox $I_{50}$ values were within a relatively narrow range (25–650 nM). In the two previous studies in which we found correlations between Protox $I_{50}$ values and *in vivo* effects, the range of Protox $I_{50}$ values was much larger (Sherman et al., 1991b; Nandihalli et al., 1992c). Since the log of the Protox $I_{50}$ values correlated with physiological effects, a very wide range of values enhances the capacity to find a correlation, if it does exist.

With this limited number of compounds and perhaps two mechanisms of action, conclusions regarding structure/activity relationships cannot be confidently made. However, a few generalizations are suggested from our data. For good activity at the molecular level, chlorine and fluorine at the 2- and 6-positions, respectively, of the phenyl ring are required (compare activities of **4** with **16** and **13** with **15**). The position of the phenoxy group on the indolin-2(3*H*)-one is critical (compare **13** and **18**). Analogs that are 5- and 6-aryloxy are active, whereas 4-aryloxy analogs are inactive. Heterocyclic structures other than indole provide good activity (e.g., **1**, a 6-aryloxy-2*H*-benzotriazole; **3**, a 5-aryloxybenzisoxazole; **7**, a 6-aryloxyindazole; and **10**, a 5-aryloxybenzoxazolone). Structure–activity interpretations become more difficult at the phytotoxicity level (Figure 2). The two compounds with a high level of dark activity (**7** and **16**) differ by only a fluorine subsitution on the phenyl ring and the nature of the benzheterocycle (an aryloxyindazole and an aryloxybenzisoxazole, respectively). Compounds **5** and **6** differ from **4** in having an oxygen atom linking the side chain to the 3-position of the benzisoxazole ring. Unlike compound **4**, the ester linkage of these substitutions in **5** and **6** would suggest that they might be cleaved *in vivo*.

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