Amino- and Urea-Substituted Thiazoles Inhibit Photosynthetic Electron Transfer

Franck E. Dayan,*[,†] Armelle C. Vincent,[†] Joanne G. Romagni,[†] Stacy N. Allen,[†] Stephen O. Duke,[†] Mary V. Duke,[‡] John J. Bowling,[§] and Jordan K. Zjawiony[§]

USDA-ARS Natural Products Utilization Research Unit, P.O. Box 8048, University, Mississippi 38677, USDA-ARS Southern Weed Science Research Unit, P.O. Box 350, Stoneville, Mississippi 38776, and Department of Pharmacognosy, NCNPR, RIPS, School of Pharmacy, University of Mississippi, University, Mississippi 38677

Amino- and urea-substituted thiazoles exhibited in vivo herbicidal activity on duckweed (*Lemna paucicostata* Hegelm. strain 6746) cultures and appeared to act via inhibition of photosynthetic electron transport system. A small number of the thiazole derivatives tested were active but only at relatively high concentrations. The most active structures were the amino-substituted thiazoles with isopropyl and *n*-butyl side chains and the urea-substituted thiazole with *p*-chlorophenyl side chain. Decreasing the length of the side chain had a negative effect on the PSII inhibitory activity. The urea-substituted series was as a group less active than the amino series, and the free acid series had no biological activity. The most active compounds competed for the same binding site as atrazine on PSII. Computer modeling highlighted the structural similarities between some of the thiazoles and the commercial herbicides diuron and atrazine.

Keywords: *Thiazole; substituted ureas; photosynthetic inhibitors; binding; computer modeling*

INTRODUCTION

Many molecules inhibit photosynthetic electron transport (PET) by binding to the D-1 protein of photosystem II (PSII) (Tischer and Strotmann, 1977; Pfister et al., 1979; Astier et al., 1984; Mets and Thiel, 1989). These herbicides apparently compete with the natural electron acceptor plastoquinone for the Q_B binding site. This site appears to be sensitive to many classes of chemicals with widely different structural characteristics including triazines (Egner et al., 1992), substituted ureas (Camillari et al., 1987), and natural and synthetic quinones (Draber et al., 1995; Rimando et al., 1998). While all of these compounds bind to the Q_B binding site, it has been shown that they interact with different amino acids within the binding pocket (Astier et al., 1984). The amino acids involved in the binding of the natural substrate (Sobolov et al., 1995) and the inhibitors (Kluth et al., 1991) have been determined.

A new group of amino- and urea-substituted thiazoles (Zjawiony et al., in preparation), synthesized for their peptidomimetic characteristics, has recently been disclosed (Table 1). These compounds possess some of the structural features of PET inhibitors. In particular, parts of the amino-substituted thiazoles resembled the commercial triazine herbicides such as atrazine, and parts of the urea-substituted thiazoles resembled the phenylurea herbicides such as diuron (Camilleri et al., 1987; Bowyer et al., 1990). Thiazole and thiazole-like derivatives have been studied previously for their PSII inhibitory activity (Yoshida, 1990; Draber et al., 1991; Kluth et al., 1991; Tiejen et al., 1991). While no thiazole

* To whom correspondence should be addressed (fax, 662-232-1035; e-mail, fdayan@ag.gov).
† USDA-ARS Natural Products Utilization Research Unit.
‡ USDA-ARS Southern Weed Science Research Unit.
§ University of Mississippi.

Table 1. Structures of the Thiazoles Tested and Their Relative Inhibitory Activity on Oxygen Evolution from Isolated Spinach Thylakoid Membranes

code	R group	$\Delta \log P^a$	series 1	series 2	series 3
			relative PET activity at 100 μM		
a	H	0	90	95	$-^b$
b	methyl	0.35	96	—	—
c	ethyl	0.69	89	—	76
d	*n*-propyl	1.09	55	—	77
e	isopropyl	0.89	5	—	85
f	*n*-butyl	1.45	8	—	38
g	*tert*-butyl	1.12	56	—	—
h	cyclohexyl	1.42	—	—	74
i	phenyl	1.75	93	101	—
j	*o*-chlorophenyl	2.27	28	100	101
k	*m*-chlorophenyl	2.4	39	101	64
l	*p*-chlorophenyl	2.38	26	101	8
m	*o*-methoxyphenyl	1.27	63	125	—
n	*m*-methoxyphenyl	1.31	—	106	92
o	*p*-methoxyphenyl	1.26	—	107	95

a Change in log *P* as evaluated according to the method of Alkorta and Villar (1992) to illustrate the contribution of the R group. *b* (−) indicates that the compounds were not available.

has been developed as a PET-inhibiting herbicide, many commercial herbicides targeting this molecular site have been developed. Of the numerous herbicides available, atrazine and its triazine analogues are among the most widely used herbicides in the world. We demonstrate that these new herbicidally active thiazole derivatives also inhibit PET and compete for the same binding site on Q_B as other synthetic PSII inhibitors. We also present

3690 *J. Agric. Food Chem.*, Vol. 48, No. 8, 2000

Dayan et al.

a modeling study that demonstrates the structural similarities between the active thiazole derivatives and several known PS II-inhibiting herbicides.

MATERIALS AND METHODS

In Vivo Activity of the Thiazole Derivatives on Duckweed Cultures. Cultures of duckweed (*Lemna paucicostata* Hegelm. strain 6746) were grown according to Becerril et al. (1992). The thiazole derivatives were dissolved in acetone and tested at 10, 33, and 100 μM. Controls received similar amounts of solvent as the treated samples (0.3% v/v). Cultures were grown for 1 week under continuous illumination (150 μmol m^{-2} s^{-1}) at 25 °C. Fresh weight and chlorophyll concentrations were measured at the end of the experiment according to Arnon (1949) as modified by Hiscox and Israelstam (1979).

Extraction of Photosynthetic Thylakoid Membranes. Thylakoid membranes were isolated from spinach leaves (*Spinacea oleracea* L.) as described by Nimbal et al. (1997). For the oxygen evolution experiments, the thylakoid membranes were diluted to 4 mg of chlorophyll/mL.

Oxygen Evolution Assays. O_2 evolution was initiated under saturating light conditions (10 mmol m^{-2} s^{-1} PAR) using a fiber optic light source and measured polarographically using a computer-controlled Hansatech DW1 oxygen probe. The reaction assay buffer consisted of 800 mM sucrose, 50 mM Mes-NaOH buffer (pH 6.2), 15 mM CaCl$_2$, and 1 mM K$_3$Fe-(CN)$_6$. All assays were performed at 30 °C. The inhibitory activity of the thiazole derivatives was studied in groups. Test compounds were diluted in ethanol or acetone, and control treatments received the same concentration of solvent (less than 1% v/v). Membrane preparations were incubated with test compounds (0–100 μM) on ice for 20 min prior to the assay. The assay was initiated by addition of thylakoid membranes to the reaction assay buffer, and the rate of oxygen evolution was measured for 20 s over the linear portion of the curve. Data are expressed as relative activity.

Binding Studies. Binding of [^{14}C]atrazine to spinach thylakoid membranes in the presence or absence of test compounds was determined according to Tischer and Strotmann (1977) as modified by Dayan et al. (1997). Thylakoid membranes (100 μg of chlorophyll/mL) were suspended in a 1-mL reaction solution consisting of 330 mM sorbitol, 100 mM HEPES buffer (pH 7.7), 1 mM EDTA, and 1 mM MgCl$_2$. A half-log dilution series (33–0.03 μM) of ^{14}C-labeled atrazine (specific activity of 20.1 mCi mmol^{-1}) plus 10 μM selected nonlabeled thiazoles were added. The suspensions were thoroughly mixed and incubated for 15 min on ice. The samples were centrifuged (6 min, 12000g, 4 °C). The supernatant was transferred to vials and mixed with 18 mL of premixed scintillation cocktail (Ultima Gold, Packard Instrument) for radioactivity measurements. The inner walls of tubes were dried with cotton swabs without disturbing the pellets to remove excess [^{14}C]atrazine. A 100-μL aliquot of tissue solubilizer (Soluene, Packard Instrument) was added to the pellets and heated in a water bath at 50 °C for 15 min. The slurry was neutralized with 50 μL of 1 M Tris-HCl buffer (pH 7.0) and transferred to scintillation vials. Ethanol (20 μL) used to wash the inner walls of each tube was combined with the slurry before radioactivity measurements. The amount of bound [^{14}C]atrazine was calculated from the radioactivity in the pellets. Binding for atrazine and the selected thiazoles was determined from double-reciprocal plots of bound atrazine vs free atrazine (Tischer and Strotmann, 1977). All regressions and intercepts were calculated using the raw data imported in the SAS software.

Computer Modeling of Selected Thiazole Derivatives. The molecular properties of thiazoles were calculated using Sybyl 6.4 (Tripos, Inc., St. Louis, MO) molecular modeling software on a Silicon Graphics O$_2$ 250 MHz R10000. The basic three-dimensional structure of the aminothiazole template was built using standard atoms and fragments available in the Sybyl library. A low-energy form of the structure was obtained using MAXIMIN2; Powell gradient and conformation was
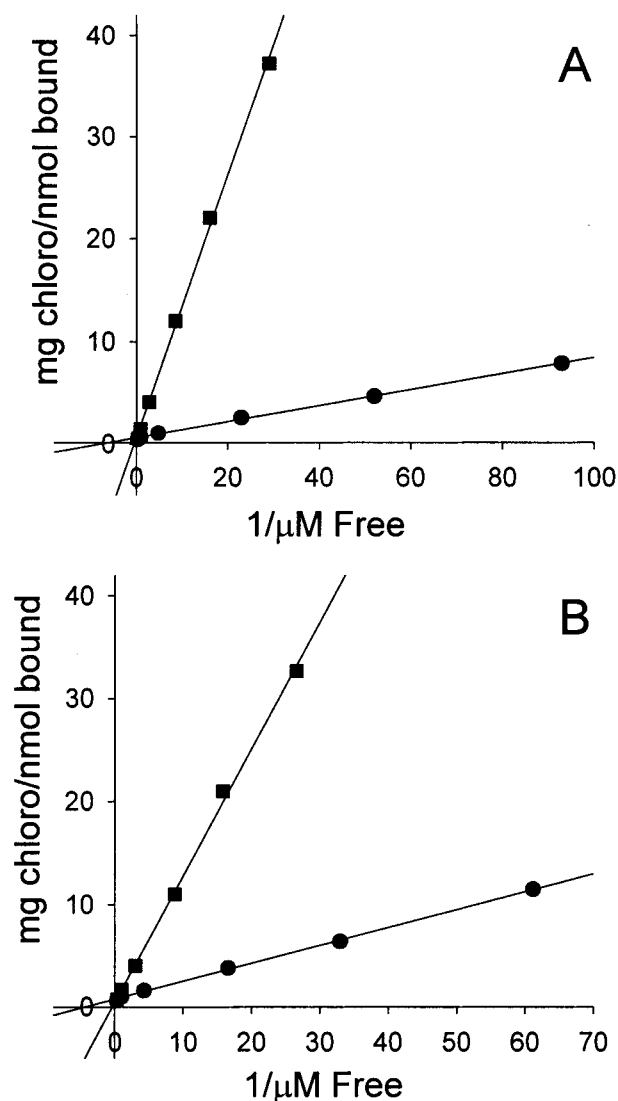

Figure 1. Competitive binding of selected thiazole derivatives on isolated thylakoid membranes. (A) Double-reciprocal plot of the binding of [^{14}C]atrazine alone (●) and with 100 μM amino-substituted **1f** (■). (B) Comparison of the displacement of [^{14}C]atrazine with the active methyl ester **1k** (■) and inactive free acid **2k** (●) amino-substituted thiazoles.

optimized using the Gridsearch module on the unrestrained carboxymethyl and isopropyl bonds on the thiazole backbone. Structures of the other thiazoles used in the study (including the urea series) were derived from this optimized structure. All of the structures were then subjected to full semiempirical geometry optimization with MOPAC 6.0 (QCPE 560, Department of Chemistry, Indiana University, Bloomington, IN) using AM1 (Austin Model) parametrization. Appropriate parameters were used for the amide linkages. The entire database was aligned on their amino−thiazole backbone. Partial charges of selected active and inactive molecules were calculated using the Gasteiger methods, and electrostatic and lipophilic surfaces were generated. The log P of each analogue in series 1 was evaluated according to the method of Alkorta and Villar (1992). The X-ray crystallography coordinates of the commercial PS II inhibitors diuron and atrazine were obtained from samples deposited with the Cambridge Crystallographic Database (Allen and Kennard, 1993).

RESULTS AND DISCUSSION

In Vivo Activity of the Thiazole Derivatives on Duckweed Cultures. The two series of compounds tested were the methyl esters of the amino-substituted
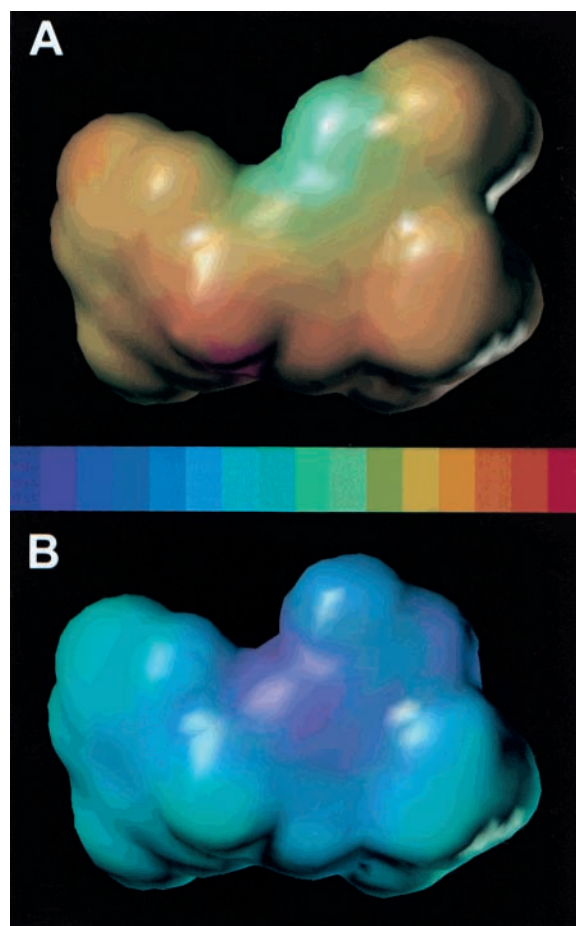
Figure 2. Electrostatic surface maps of (A) the active methyl ester (**1e**) and (B) inactive free acid (**2k**) amino-substituted thiazoles. Molecule **2k** is depicted as a carboxylate anion. The color ramp ranges from −130 (blue) to 20 eV (red).

thiazoles (series 1) and of urea-substituted thiazoles (series 3). The overall effect of these compounds was greater on the weight of the duckweed colonies than on the chlorophyll concentration in the fronds (data not shown).

Compounds **1f**, **1k**, **1l**, **1m**, and **3d** caused 43, 84, 75, 50, and 70% reduction in biomass at the highest concentration tested, relative to the controls, respectively. However, some compounds (i.e., **1f** or **1g**) caused

an increase in the weight of the colonies at the lowest concentration tested, whereas compounds **1c** or **1o** caused an increase in the biomass of the colonies at the higher concentrations.

Some of the active compounds consistently decreased the amount of chlorophyll in duckweed fronds (i.e., **1k**, **1l**, and **3d**). Others, such as **1e** and **3a**, also caused a reduction in chlorophyll levels when tested at 100 μM, although they induced an initial increase in chlorophyll levels at the lower concentrations.

Inhibition of Photosynthetic Oxygen Evolution. All the thiazole derivatives available have been tested for their ability to inhibit PET on isolated spinach thylakoids (Table 1). Overall, the methyl ester forms of the amino-substituted thiazoles (series 1) were most active, whereas the free carboxylic acid of amino-substituted thiazoles (series 2) were totally inactive. Finally, the methyl esters of urea-substituted thiazoles (series 3) were slightly active, except for the relatively more active **3l**. The data are expressed as relative activity at 100 μM compound.

The most active compounds in the series 1 are **1e**, **1f**, **1k**, and **1l**, which decreased the photosynthetic electron activity to 5%, 8%, 39%, and 26% of the activity observed in the control, respectively (Table 1). Lipophilicity, as modulated by the length of the hydrocarbon side chain, has a great impact on activity (Table 1). While compounds with hydrogen, methyl, and ethyl substituents are essentially not active, an increase in the number of carbons tends to yield more active derivatives. The *n*-propyl side chain provided sufficient lipophilicity to the thiazoles of series 1 to allow penetration of the membrane in order to reach the Q_B binding site, whereas maximum activity was obtained with the isopropyl and *n*-butyl side chains. The compound with the *tert*-butyl side chain was not as active as the one with the *n*-butyl, which had an activity similar to the *n*-propyl derivative. This suggests that, in addition to lipophilicity, steric parameters may also play a role in the ability of the thiazoles to bind to Q_B.

None of the compounds of the series 2 were active on PSII activity (Table 1), although they possessed identical side chains. The lack of activity of these molecules may be due to the highly polar carboxyl function found on this series. At physiological pH, this acidic function is most likely anionic. This is particularly true in the generally high pH of the stroma resulting from the
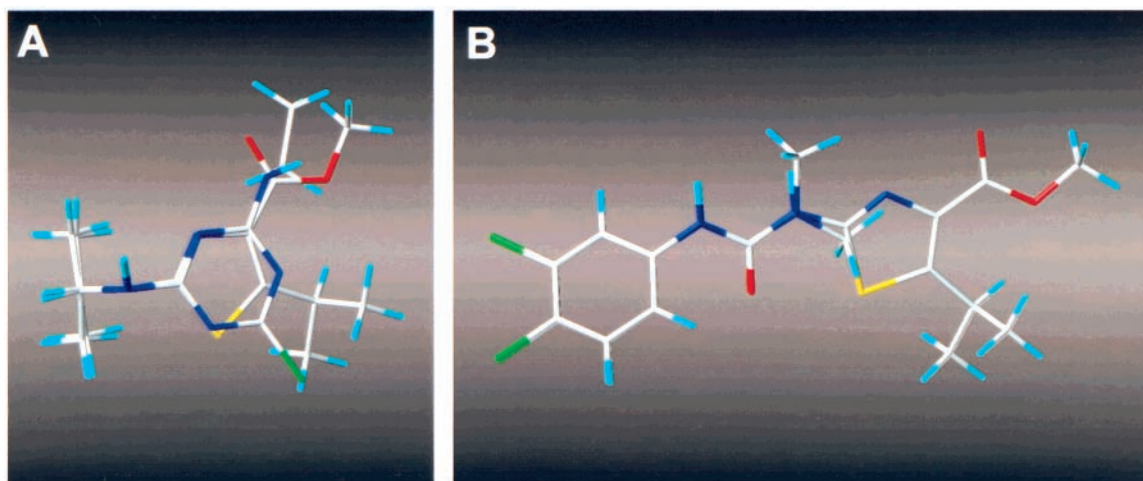
Figure 3. Overlay of (A) **1f** with atrazine and (B) **3l** with diuron. Note the structural similarities between the thiazole derivatives and their respective commercial herbicide analogues.

3692 *J. Agric. Food Chem.,* Vol. 48, No. 8, 2000

Dayan et al.

proton gradient occurring in plants exposed to light. The Fujita and Hansch π-value of the carboxylate substitution is -4.36, whereas this value is -0.01 for the carboxymethyl substitution (Hansch et al., 1995), illustrating the large hydrophilic contribution of the free acid side chain to the entire thiazole structure. Thus, the molecules in series 2 may not have the lipophilic properties necessary for reaching the Q_B binding site within the thylakoid membranes, even when the most lipophilic side chains are present.

When compared to their analogues in series 1, **3e** and **3f** were less active, indicating that the urea substituent behaves differently than the amino functionality. The most active urea-substituted thiazole was **3l** with a *p*-chlorophenyl side chain. Our data indicate that the position of the chloro substituent has a large effect on the activity of this group of thiazoles, with relative efficacy increasing in the order of ortho, meta, and para arrangement.

Competitive Binding on Isolated Thylakoid Membranes. The decrease of photosynthetic oxygen evolution obtained with some of the thiazoles is consistent with the inhibition of PET. The binding study confirmed that the active analogues were competitive inhibitors of PSII. Compound **1f** (Figure 1A) seems to have the lipophilic characteristics to move across from thylakoid membrane in order to reach the binding site and has the structural features required to compete for the binding site of plastoquinone. As expected, the active analogue was able to displace the commercial herbicide atrazine (Figure 1A). Analysis of the double reciprocal plot suggests that **1f** competes for the same binding site as atrazine.

A pairwise comparison of the inactive free carboxylic acid **2k** and the more active carboxymethyl analogue (**1k**) translated the underlying differences between the two series. Compound **2k**, the inactive carboxylic acid analogue of **1k**, did not displace atrazine (Figure 1B). The difference in activity between **1k** and **2k** is most likely due to the much more electronegative nature of the **2k** derivative, relative to its methyl ester analogue **1k**.

Molecular Modeling of Selected Thiazole Derivatives. The presence of the free carboxylic acid function in series 2 affected the partial charge distribution of this group, relative to the series 1. Figure 2A,B contrasts the electrostatic properties of the active **1k** and inactive **2k** *m*-chlorophenyl analogues. The electronegative potential surrounding the carboxylic function of **2k** renders the molecule much more polar than **1k**.

Computer modeling was also useful in highlighting the similarities between the structural features of the isopropylaminothiazole (**1e**) and atrazine (Figure 3A). A significant portion of **1e** (e.g., the entire isopropyl-amino side chain) is found in atrazine. This similarity may account for the highest level of activity associated with this analogue. Furthermore, the most active compound from series 3 (**3l**) possesses a *p*-chlorophenyl side chain that resembles a large portion of the phenylurea herbicide diuron (Figure 3B).

CONCLUSIONS

There is some discrepancy between in vivo and in vitro activity of the thiazole series tested. For example, while **1k** has the highest in vivo activity on the weight of duckweed colonies and **3d** caused the greatest de-

crease in chlorophyll concentration, our in vitro assay shows that **1e**, **1n**, and **3l** were the most active at inhibiting PET. Although more work remains to be done to fully account for these observations, some aspects of the activity of the three series of thiazoles may be explained from the data. It is clear that the conditions of the assays affect the results. It is possible that the isopropyl and *n*-butyl side chains of **1e** and **1f** are optimal for inhibition of PET in isolated thylakoid membranes, but these side chains might render the structures too lipophilic for in vivo activity, causing the molecules to be compartmentalized in the membrane. Under the conditions of the in vivo assay, it appears that the *p*-chlorophenyl substitution of **1l** is better.

A full QSAR study will be useful in determining the structural characteristics necessary to impart better biological activity to the thiazole derivatives. We are currently synthesizing more analogues to continue this work.

LITERATURE CITED

Alkorta, I.; Villar, H. O. Quantum mechanical parametrization of a conformationally dependent hydrophobic index. *Int. J. Quantum Chem.* **1992,** *44*, 203-218.

Allen, F. H.; Kennard, O. 3D Search and Research Using the Cambridge Structural Database. *Chem. Des. Autom. News* **1993**, *8*, 31-37.

Arnon D. I. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris. Plant Physiol.* **1949**, *24*, 1-15.

Astier, C.; Boussac, A.; Etienne, A. L. Evidence for different binding sites on the 33 kDa protein for DCMU, atrazine, and Q_B. *FEBS Lett.* **1984**, *167*, 321-326.

Becerril, J. M.; Duke, M. V.; Nandihalli, U. B.; Matsumoto, H.; Duke, S. O. Light control of porphyrin accumulation in acifluorfen-methyl treated *Lemna paucicostata. Physiol. Plant.* **1992**, *86*, 6-16.

Bowyer, J.; Hilton, M.; Whitelegge, J.; Jewess, P.; Camilleri, P.; Crofts, A.; Robinson, H. Molecular modelling studies on the binding of phenylurea inhibitors to the D1 protein of photosystem II. *Z. Naturforsch.* **1990**, *45*, 379-387.

Camilleri, P.; bowyer, J. R.; Gilkerson, T.; Odell, B.; Weaver, R. C. Structure-activity relationships in the Hill inhibitory activity of substituted phenylureas. *J. Agric. Food Chem.* **1987**, *35*, 479-483.

Dayan, F. E.; Duke, S. O.; Reddy, K. N.; Hamper, B. C.; Leschinsky, K. L. Effects of isoxazole herbicides on protoporphyrinogen oxidase and porphyrin physiology. *J. Agric. Food Chem.* **1997**, *45*, 967-975.

Draber, W.; Tietjen, K.; Kluth, J. F.; Trebst, A. Herbicides in photosynthesis research. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1621-1633.

Draber, W.; Trebst, A.; Walter, O. Structure-activity relationships of quinone and acridone photosystem II inhibitors. In *Classical and three-dimensional QSAR in Agrochemistry*; ACS Symposium Series; American Chemical Society: Washington, DC, 1995; pp 186-198.

Egner, U.; Hoyer, G.-A.; Saenger, W. The binding of triazine herbicides to the photosynthetic reaction center of *Rhodopseudomonas viridis. Eur. J. Biochem.* **1992,** *206*, 685-690.

Hansch, C.; Leo, A.; Hoekman, D. Exploring QSAR: Hydrophobic, electronic, and steric constants. *Am. Chem. Soc. Prof. Ref.* **1995**.

Hiscox J. D.; Israelstam G. F. A method for the extraction of chlorophyll from leaf tissue without maceration. *Can. J. Bot.* **1979**, *57*, 1332-1334.

Kluth, J. F.; Tietjen, K. G.; Andree, R.,; Ewald, R.; Oettmeier, W.; Trebst, A. Thiazoles that inhibit photosynthetic reaction centres both in purple bacteria and chloroplasts. *Pestic. Sci.* **1991**, 424-427.

Mets, L.; Thiel A. Biochemistry and genetic control of the photosystem II herbicide target site. In *Target Sites of*

Herbicide Action; Böger, P., Sandmann, G., Eds.; CRC Press: Boca Raton, FL, 1989; pp 1−24.

Nimbal, C. I.; Yerkes, C. N.; Weston, L. A.; Weller, S. C. Herbicidal activity and site of action of the natural product sorgoleone. *Pestic. Biochem. Physiol.* **1997**, *54*, 73−83.

Pfister, W.; Radosevich, S. R.; Arntzen, C. G. Modification of herbicide binding to photosystem II in two biotypes of *Senecio vulgaris* L. *Plant Physiol.* **1979**, *64*, 995−999.

Rimando, A. M.; Dayan, F. E.; Czarnota, M. A.; Weston, L. A.; Duke, S. O. A new photosystem II electron-transfer inhibitor from *Sorghum bicolor* (L.). *J. Nat. Prod.* **1998**, *61*, 927−930.

Sobolev, V.; Edelman, M. Modeling the quinone B binding site of the photosystem II reaction center using notions of complementarity and contact-surface between atoms. *Proteins* **1995**, *21*, 214−225.

Tietjen, K. G.; Kluth, J. F.; Andree, R.; Haug, M.; Lindig, M.; Müller, K. H.; Wroblowsky, H. J.; Trebst, A. The herbicide binding niche of photosystem II—A model. *Pestic. Sci.* **1991**, *31*, 65−72.

Tischer, W.; Strotmann, H. Relationship between inhibitor binding by chloroplasts and inhibition of photosynthetic electron transport. *Biochim. Biophys. Acta* **1977**, *460*, 113−125.

Yoshida, S. Heuristic approaches to finding new herbicides active in the chloroplast. *Z. Naturforsch.* **1990**, *45C*, 329−335.

Zjawiony, J. K.; Yu, D.; Bartyzel, P.; Bowling, J. An efficient synthesis of 2-amino-5-isopropyl-4-thiazolecarboxylic acid derivatives. *Synthesis*, in preparation.