Modified Benzoxazinones in the System *Oryza sativa*–*Echinochloa crus-galli*: An Approach to the Development of Biorational Herbicide Models

Francisco A. Macías,* Nuria Chinchilla, Rosa M. Varela,
José M. G. Molinillo, David Marín, and João M. De Siqueira[†]

Allelopathy Group, Department of Organic Chemistry, University of Cádiz, Avenida República
Saharaui s/n, 11510 Puerto Real, Cádiz, Spain

The utility of benzoxazinones and some of their synthetic derivatives in the search for new leads for herbicide model development has been explored. The work described focuses on obtaining derivatives that present selectivity in the system *Oryza sativa*–*Echinochloa crus-galli*. To achieve this goal the influence of lipophilicity in this system has been studied by preparing 14 ester derivatives at the N-4 position of D-DIBOA along with other compounds with different functionalization and chain lengths at position C-2. These compounds have been tested in the aforementioned system, and the dose–response profiles have been compared. The most active compound was 2-ethyl-4-hydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one, which presented higher selectivity than the specific herbicide Cotanil-35. These results confirm the potential of D-DIBOA as a lead herbicide for the control of *Echinochloa* spp. in rice crops.

KEYWORDS: Rice; *Echinochloa***; barnyardgrass; benzoxazinoids; phytotoxicity; lipophilicity; herbicides; SAR**

INTRODUCTION

The use of modified natural products in the field of rational pest control has been a prominent research area in recent times (*1*). Natural products offer several advantages for the design of new molecules with potential utility in pest control because they have high bioactivity levels as organisms release them in very small quantities. In addition, as these compounds are already present in the environment, they would be expected to cause less environmental damage (*2, 3*).

In the context of new methodologies for the design of bioactive molecules, quantitative structure–activity relationship (QSAR) studies are among the key approaches for a systematic analysis of the properties of chemical structure and bioactivity (*4*). A suitable correlation of the two factors may provide a successful approach to predict the physical, chemical, and biological properties for the bioactive molecule being designed. Lipophilicity is a key factor in the absorption of any bioactive compound as it determines the bioavailability in the cell. Furthermore, lipophilicity is usually expressed through the logarithm of the octanol–water partition coefficient (log *P*) (*5, 6*) and is present in many QSAR equations because an optimum equilibrium between water, the usual carrier, solubility, and lipophilicity (related to the ability to cross cell membranes) must

exist. Hence, log *P* is an important parameter in the design of pesticides because it is related to the transport of the xenobiotics in plants (*7–9*).

The potential utility of modified benzoxazinones in the development of chemical tools for weed control has been extensively described in recent research (*10–13*). Benzoxazinones are natural products released as root exudates by Gramineae species such as wheat and rye, and empowering modifications of these compounds have been investigated in the search for pest control methods for common weeds in wheat. The effects of natural benzoxazinones, some of their immediate degradation products, and some simple synthetic analogues have been reported (*14*).

Although benzoxazinones have been extensively studied using common wheat weeds as models (*15*), mostly due to the origin of these compounds, there is another interesting area to be explored: the use of benzoxazinones as templates for the development of herbicides that can be used in other systems. This new field is illustrated by our exploration of the effects of natural benzoxazinones, some of their degradation products, and a selection of immediate synthetic analogues on *Echinochloa crus-galli* L. P. /Beauv. (barnyardgrass).

Previously, we evaluated the phytotoxicity of benzoxazinones and related compounds on barnyardgrass in the search for new compounds that could be applied as part of a strategy directed at weed control on barnyardgrass-affected crops (*16*). Statistical treatment of the acquired data would enable structure–activity relationships to be highlighted, and these could be useful in the

* Author to whom correspondence should be addressed (telephone +34-956.016.370; fax +34-956.016.193; famacias@uca.es).
† Permanent address: CCO Dona Lindu, Universidade Federal de Sao João Del Rei, 35501-296, Divinopolis, MG, Brazil.
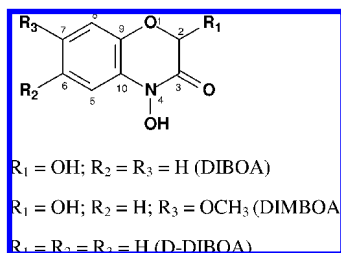
9942 *J. Agric. Food Chem.*, Vol. 56, No. 21, 2008

Macías et al.

Figure 1. Molecular structures of benzoxazinones DIBOA, DIMBOA, and D-DIBOA.

Table 1. Molecular Parameters Found To Be Relevant in Pharmaceutical and Herbicidal Activities, with Their Optimal Values

parameter	optimal values	
	Lipinski (pharmaceuticals)	Tice (postemergence herbicides)
H-bond donors	≤5	≤3
H-bonds acceptors	≤10	≥2 and ≤12
mol wt	≥500	≥150 and ≤500
cLog P	≤5	≤4
rotable bonds		≤12

Table 2. Molecular Parameters for D-DIBOA Esters

	mol wt	cLog P	rotatable bonds	H-bond donors	H-bond acceptors
D-DIBOA	166.15	0.42	0	1	4
ABOA	207.19	1.18	2	0	5
Pr-D-DIBOA	221.21	1.65	3	0	5
Bu-D-DIBOA	235.23	2.11	4	0	5
Val-D-DIBOA	249.25	2.58	5	0	5
Hex-D-DIBOA	263.27	3.04	6	0	5
Hept-D-DIBOA	277.29	3.5	7	0	5
Oct-D-DIBOA	291.31	3.97	8	0	5
Non-D-DIBOA	305.33	**4.43**	9	0	5
Dec-D-DIBOA	319.35	**4.89**	10	0	5
Undec-D-DIBOA	333.37	**5.36**	11	0	5
Lau-DIBOA	347.39	**5.82**	12	0	5
Tridec-D-DIBOA	361.41	**6.29**	**13**	0	5
Mir-D-DIBOA	375.43	**6.75**	**14**	0	5

Figure 2. Structures and acronyms of the tested benzoxazinone esters at N-4.

Table 3. Molecular Parameters for D-DIBOA Modified at C-2

	mol wt	cLog P	rotatable bonds	H-bond donors	H-bond acceptors
D-DIBOA	165	0.42	1	4	1
2-Me-D-DIBOA	179	0.90	2	4	1
2-Et-D-DIBOA	193	1.17	3	4	1
2-Pr-D-DIBOA	207	1.64	4	4	1
2-Bu-D-DIBOA	221	2.10	5	4	1
2-Hex-D-DIBOA	249	3.03	7	4	1
2-Ph-D-DIBOA	241	1.74	2	4	1
2-EtOCO	237	0.24	5	6	1
2-Me-2-EtOCO	251	0.58	6	6	1

further development of highly phytotoxic compounds based on natural product structures. The results of these studies indicated that 4-hydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (D-DIBOA, **Figure 1**) seems to be an optimal candidate for the development of further natural herbicide models for *E. crus-galli* management. The hydroxyl group at N-4 is a structural requirement for bioactivity, as the absence of this group provided only moderate or zero effect. In terms of the functionalization at the C-2 position, phytotoxic effects increased in the absence of this group in the case of DIBOA and D-DIBOA. This result is similar to those obtained in STS evaluation (*16*) and confirms the utility of D-DIBOA in the search for new natural herbicide models.

In our ongoing research into the structure−activity relationships of benzoxazinones and the structural requirements for their phytotoxicity enhancement, and after characterization of the optimal structural features, a new generation of chemicals with enhanced lipophilicity was developed. These compounds were tested on selected standard target species and weeds (*17*) in a search for the optimal aqueous solubility−lipophilicity levels for phytotoxicity. This physical parameter is known to be crucial in modern drug and agrochemical design strategies. The lipophilicity was increased in two ways. The first approach involved the preparation of esters with increasing chain length. This was found to be the most chemically convenient way,
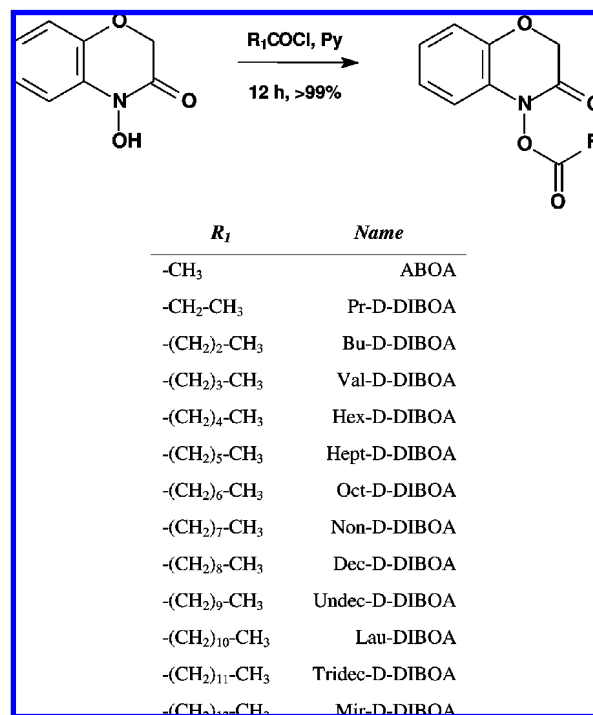
taking advantage of the hydroxyl group at N-4 in the hydroxamic acid in the (²H)-1,4-benzoxazin-3(4*H*)-one skeleton selected for this study. The second approach involved the introduction of chains of increasing length at position C-2. This method allowed us to increase the lipophilicity of these compounds without changing the hydroxamic acid moiety. The new compounds obtained in this way had interesting phytotoxicity profiles and, in some cases, the phytotoxic effect of the starting benzoxazinone template was enhanced.

This paper constitutes a logical continuation of our previous research by examining the effects of further modifications of the benzoxazinone skeleton on barnyardgrass and comparing those effects to the results related to rice, in the search for an accurate description of the chemical selectivity of action.

The phytotoxic effects of some benzoxazinones, together with those of some of their degradation products and analogues, have already been described by us, and these constitute the first generation of tested compounds. The work presented here continues that research by adding different structural modifications. Comparison of the activity that these compounds have on barnyardgrass with the effects they have on rice was also made in the search for a selective behavior for the maximum applicability of the chemicals in the field.

A total of 13 ester derivatives at the N-4 position (**Figure 2**) and 8 derivatives at the C-2 position of D-DIBOA (**Figure 3**)
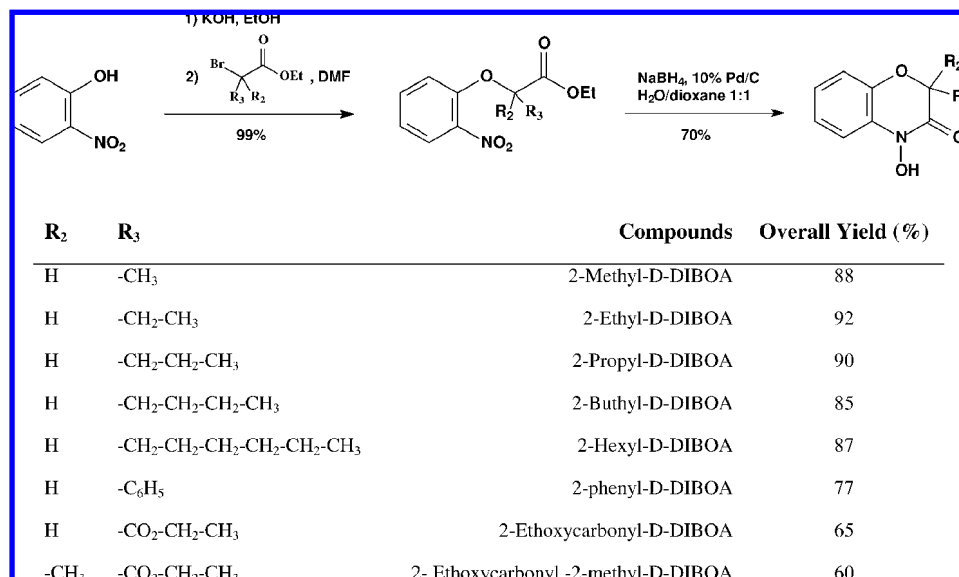
Modified Benzoxazinones in the *Oryza−Echinochloa* System

J. Agric. Food Chem., Vol. 56, No. 21, 2008   **9943**

R$_2$	R$_3$	Compounds	Overall Yield (%)
H	-CH$_3$	2-Methyl-D-DIBOA	88
H	-CH$_2$-CH$_3$	2-Ethyl-D-DIBOA	92
H	-CH$_2$-CH$_2$-CH$_3$	2-Propyl-D-DIBOA	90
H	-CH$_2$-CH$_2$-CH$_2$-CH$_3$	2-Buthyl-D-DIBOA	85
H	-CH$_2$-CH$_2$-CH$_2$-CH$_2$-CH$_2$-CH$_3$	2-Hexyl-D-DIBOA	87
H	-C$_6$H$_5$	2-phenyl-D-DIBOA	77
H	-CO$_2$-CH$_2$-CH$_3$	2-Ethoxycarbonyl-D-DIBOA	65
-CH$_3$	-CO$_2$-CH$_2$-CH$_3$	2- Ethoxycarbonyl -2-methyl-D-DIBOA	60

Figure 3. Structures and acronyms of the tested benzoxazinone modified at C-2.
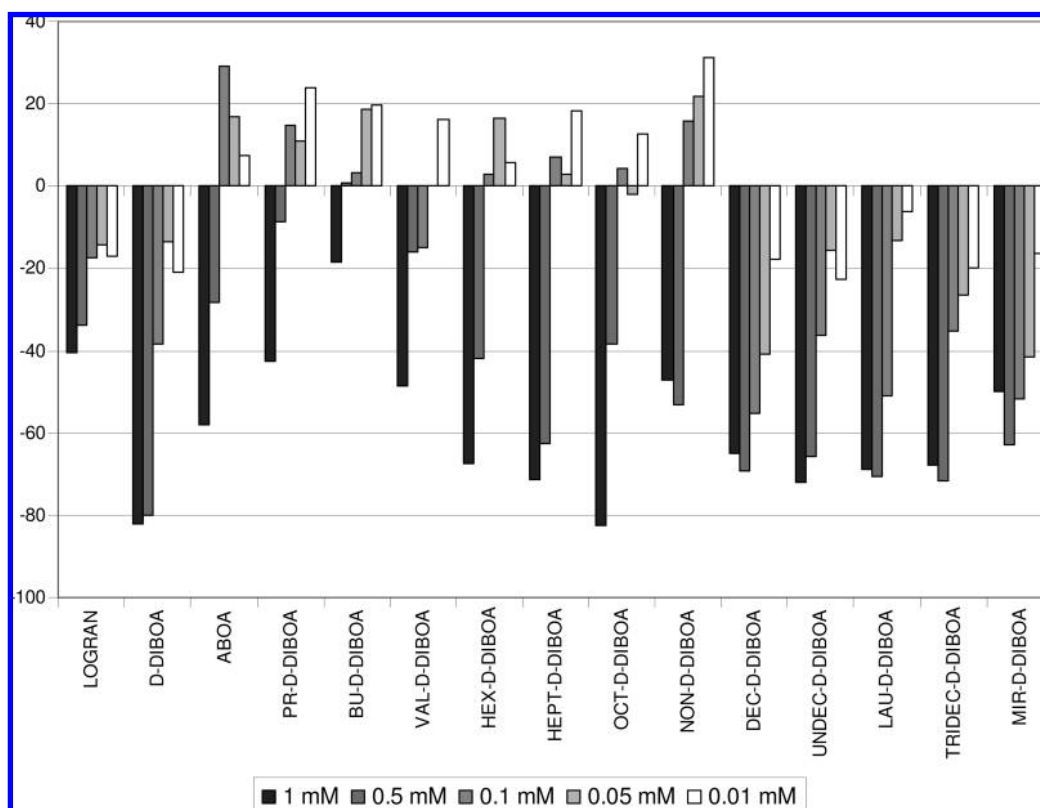
Figure 4. Phytotoxicity bioassay results for N-4 (root length, percent from control) for *Echinochloa crus-galli* L.

were prepared and tested on the species *Echinochloa crus-galli* and *Oryza sativa* L. The correlation to Tice's modification to Lipinski's rule of 5 (*18*) is discussed in the context of Hansch's transport theory (*19*). These benzoxazinones constitute a second generation of chemicals developed from previously reported SAR studies on benzoxazinones and related compounds (*15, 16, 20*).

MATERIALS AND METHODS

General Experimental Procedures. The purities of the compounds to be tested were determined by ^{1}H NMR and HPLC analyses and found to be >98%. ^{1}H and ^{13}C NMR spectra were recorded using CDCl$_3$ as solvent on a Varian INOVA spectrometer at 399.99 and 100.577 MHz, respectively. The resonance of residual chloroform was set to δ 7.25. The solvent peak for ^{13}C was set to δ 77.00 (chloroform),

and this was used as internal reference. UV−vis spectra were obtained using a Varian Cary 50 BIO spectrophotometer, with chloroform as the solvent. Mass spectra (EIMS) were recorded using a Voyager Termoquest spectrometer. FTIR spectra were obtained on a Spectrum BX Perkin-Elmer FTIR system. Frequency values are given in cm^{-1}.

Preparation of N-4 Derivatives. 4-Hydroxy-(2*H*)-1,4-benzoxazin-3(^{4}H)-one (D-DIBOA) was obtained as described previously (*21*). Acyl chlorides were purchased from Sigma-Aldrich Co. and were used as received, except undecanoyl and tridecanoyl chlorides; these were synthesized as previously reported (*17*).

Preparation of C-2 Derivatives. The starting 2-nitrophenol (500 mg) was dissolved in a solution of 0.1 M KOH in absolute ethanol (35.9 mL). After 30 min, the solvent was removed under reduced pressure. The resulting alkoxide was redissolved in dry DMF (50 mL),
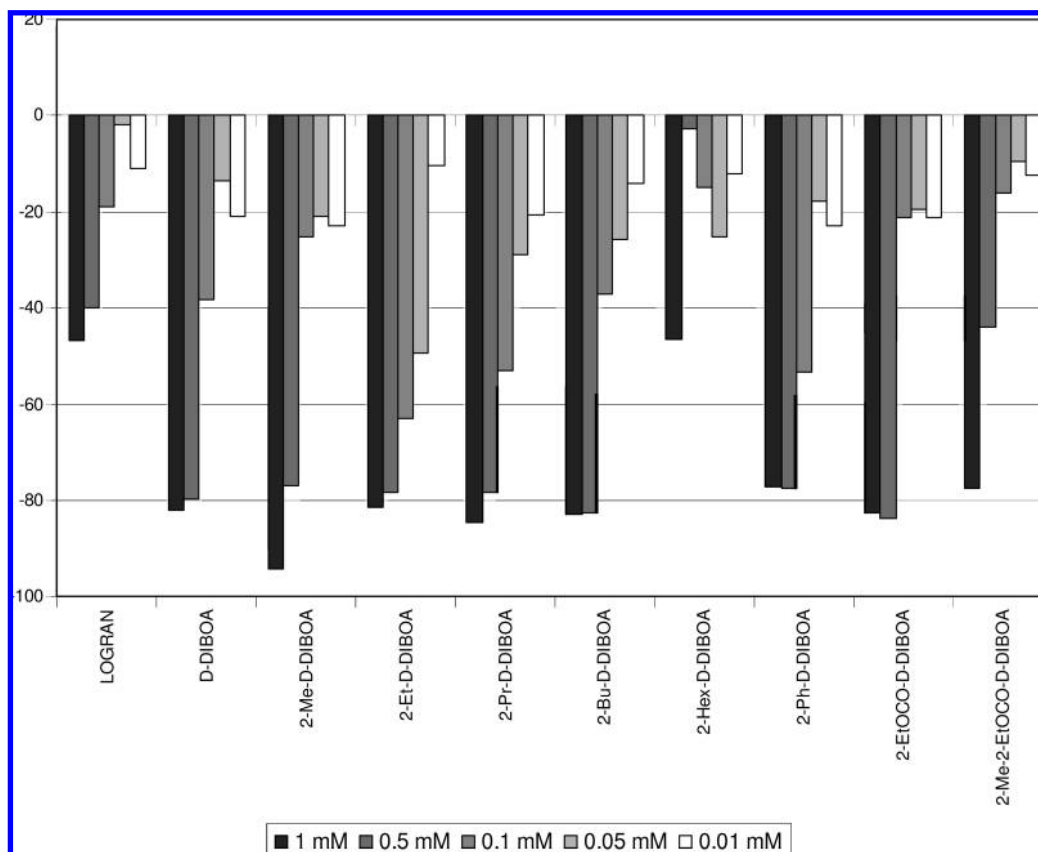
Figure 5. Phytotoxicity bioassay results for C-2 (root length, percent from control) for *Echinochloa crus-galli* L.

and the bromopropanoate (464 μL) was added. The reaction mixture was stirred under argon for 16 h. After this time, ethyl acetate (100 mL) was added and the resulting organic solution was washed with five portions of distilled water and dried over anhydrous sodium sulfate. The solvent was distilled under reduced pressure to yield ethyl 2-[2′-nitrophenoxy]propanoate in quantitative yield. Pd/C (10%) (9.6 mg) was suspended in an aqueous solution of 1,4-dioxane (1:1) (20 mL). Sodium borohydride (95.5 mg) was added, and the solution was vigorously stirred. To this stirred suspension was added dropwise a solution of ethyl 2-[2′-nitrophenoxy]propanoate (500 mg) in 1,4-dioxane (1 mL). The reaction was complete in 1 h. The suspension was filtered through Celite to remove the catalyst, and the filtrate was treated with 0.1 N HCl to give a pH of 4. This solution was extracted with ethyl acetate (3 × 50 mL). The organic layers were combined and dried over anhydrous sodium sulfate. The solvent was distilled off under reduced pressure. 2-Methyl-D-DIBOA was obtained in 88% yield. A similar procedure was carried out to afford the corresponding C-2 derivatives.

Calculation of IC$_{50}$ and Log *P*. The phytotoxicity data were fitted to a sigmoidal dose−response model (constant slope) by employing the GraphPad Prism v. 4.00 software package (*22*). cLog *P* values were estimated using the OSIRIS property explorer (*23*). This software uses the Chou and Jurs algorithm, based on computed atom contributions (*24*).

Phytotoxicity Bioassays. *Methodology.* Bioassays were carried out using Petri dishes (90 mm diameter) with one sheet of Whatman no. 1 filter paper as support. Germination and growth were conducted in aqueous solutions at controlled pH using 10^{-2} M 2-[*N*-morpholino]-ethanesulfonic acid (MES) and addition of 1 M NaOH to give a pH of 6.0. Solutions (0.2, 0.1, 0.02, 0.01, and 0.002 M) of the compounds to be assayed were prepared in DMSO and then diluted with buffer (5 μL DMSO/mL of buffer) to give the test concentrations for each compound (1, 0.5, 0.1, 0.05, and 0.01 mM).

E. crus-galli seeds were purchased from Herbiseed Co. (Twyford, U.K.) and were used as received. The number of seeds in each Petri dish was 25, and 5 mL of treatment, control, or internal reference solution was added to each Petri dish. Four replicates were used (100

seeds). *O. sativa* L. seeds were purchased from Dow AgroSciences (Sevilla, Spain) and were used as received. The number of seeds in each Petri dish was 10, and 5 mL of treatment, control, or internal reference solution were added to each Petri dish. Ten replicates were used (100 seeds).

After the addition of seeds and aqueous solutions, the Petri dishes were sealed with Parafilm to ensure closed-system models. Seeds were subsequently incubated at 25 °C in a Memmert ICE 700 controlled environment growth chamber in the absence of light. The bioassay took 5 days for *E. crus-galli* and 7 days for *O. sativa* L. After growth, plants were frozen at −10 °C for 24 h to avoid subsequent growth during the measurement process. This helped in the handling of the plants and allowed a more accurate measurement of root and shoot lengths.

The commercial herbicide Logran, a combination of *N*-(1,1-dimethylethyl)-*N*-ethyl-6-(methylthio)-1,3,5-triazine-2,4-diamine (terbutryn, 59.4%) and 2-(2-chloroethoxy)-*N*-{[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl}benzenesulfonamide (triasulfuron, 0.6%), was used as the internal reference in accordance with a comparison study reported previously (*25*). To give an approximate selectivity of assayed compounds in the system *E. crus-galli*−*O. sativa*, the commertial herbicide Cotanil-35 was employed. This herbicide contains *N*-(3,4-dichloropropionanilide) (propanil, 35%), as active material. Both herbicides were used at the same concentrations (1, 0.5, 0.1, 0.05, and 0.01 mM) and under the same conditions as the studied compounds. Buffered aqueous solutions with DMSO and without any tested compound were used as control samples.

Bioassay Data Acquisition. Evaluated parameters (germination rate, root length, and shoot length) were recorded using a Fitomed system (*26*), which allowed automatic data acquisition and statistical analysis by its associated software.

Statistical Analysis. Data were statistically analyzed using Welch's test, with significance fixed at 0.01 and 0.05. Results are expressed in bar charts in which the null value represents control, negative values represent inhibition, and positive values represent stimulation of the studied parameter (*26*). Phytotoxic activities expressed in this way can be found in the Supporting Information for all chemicals and species. Once the germination and growth data had been acquired, cluster
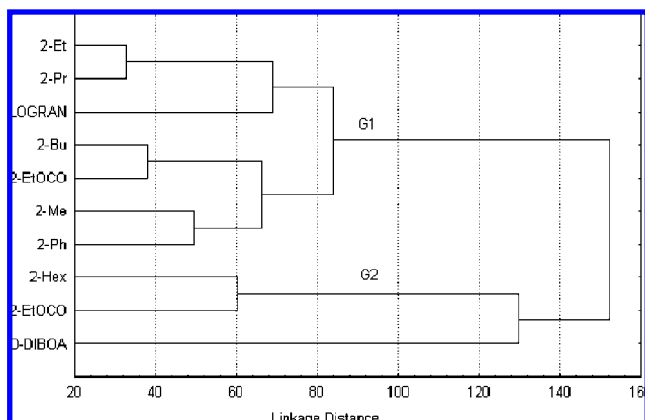
Modified Benzoxazinones in the *Oryza−Echinochloa* System

J. Agric. Food Chem., Vol. 56, No. 21, 2008 **9945**

Figure 6. Cluster analysis for D-DIBOA derivatives in C-2, made on the basis of the growth effects for *Echinochloa crus-galli.*
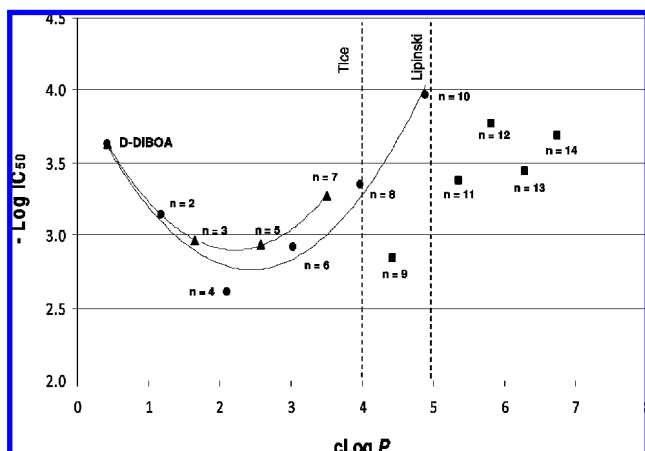

Figure 7. QSAR correlations of phytotoxic effects with lipophilicity of N-4 esters. *n* indicates number of carbon atoms in the ester chain. Vertical lines indicate maximum lipophilicity according to Tice and Lipinski models.
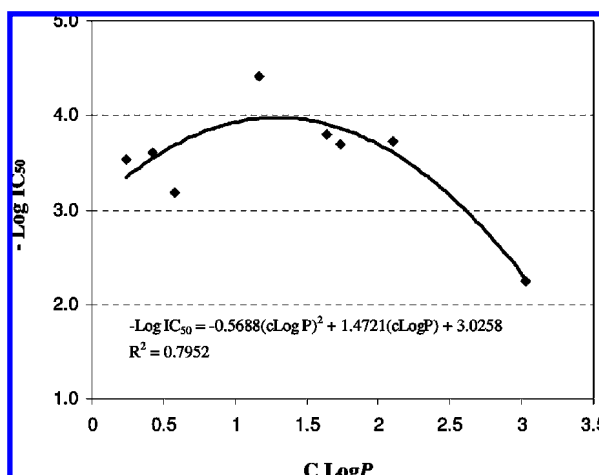

Figure 8. QSAR correlations of phytotoxic effects with lipophilicity of C-2 derivatives. *n* indicates number of carbons of chains.

analysis was used to group compounds with similar phytotoxicity behaviors and associate them with their molecular structure. Complete linkage was used as an amalgamation rule, and the distance measurement was based on squared Euclidean distances (*27*), given by the equation where d(*x,y*) is the squared Euclidean distance (*i*-dimensional), *i* represents the number of variables, and *x* and *y* are the observed values. The cluster was obtained using Statistica v. 5.0 software (*27*). Germination rate, shoot length, and root length effects, for all tested species, were included in the analysis to acquire an overall view of the

phytotoxicity and its relationship with chemical structure. IC_{50} values were obtained after the phytotoxicity data had been adjusted to concentration (logarithmic scale), to a constant slope sigmoidal dose−response curve, defined by the equation where *X* indicates the logarithm of concentration, *Y* indicates the response (phytotoxicity), and Y_{max} and Y_{min} are the maximum and minimum values of the response, respectively. Goodness of fit is described by the determination coefficient (r^2). The adjustment and the r^2 were obtained using GraphPad Prism software v. 4.00 (*26*).

RESULTS AND DISCUSSION

Fitting to Lipinski's Rule of 5 (Tice Approach for Agrochemical Design). The parameters included in Lipinski's rule of 5, as reinvestigated by Tice as a useful approach for the design of agrochemicals, are summarized in **Table 1** for all of the compounds obtained. For the N-4 derivatives (**Table 2**), all compounds tested after Dec-D-DIBOA did not fit the rule as they have cLog *P* values >5, a situation that is consistent with Lipinski's requirements. As far as the C-2 derivatives are concerned (**Table 3**), all compounds fitted Tice's rule. Therefore, compounds from D-DIBOA to Oct-D-DIBOA and all C-2 derivatives have appropriate molecular properties to give good phytotoxicity values, according to Tice's optimal cLog *P* range.

Phytotoxic Activity. The incorporation of an ester function did not lead to significant increases in the phytotoxicity in comparison to the lead compound. All N-4 esters were active and showed inhibitory profiles for all of the parameters evaluated (**Figure 4**). The parameters root and shoot length were affected more than germination. In general terms, length was the parameter that was most affected. Longer chain esters led to the highest root inhibition. This is particularly clear for the decanoyl and lauroyl derivatives, which gave values of $IC_{50} = 107$ μM ($r^2 = 0.9742$) and 170 μM ($r^2 = 0.9274$), respectively, and had activities very similar to that found for D-DIBOA.

Regarding C-2 derivatives, these were either as active or more active than the commercial herbicide used as positive control. The highest concentration provoked inhibition effects in the root length close to 100% (**Figure 5**). Taking into consideration the effects on all parameters and species, a cluster analysis was carried out to classify the tested chemicals according to their phytotoxicity. Cluster analysis of the data allows us to classify the assayed compounds according to their phytotoxicity into two main groups: G1 and G2. The results of this analysis are shown in **Figure 6**. G1 includes the commercial herbicide and those compounds that present higher activity. It can be pointed out that the most active compounds were ethyl and propyl derivatives, which had IC_{50} values of 39 μM ($r^2 = 0.9665$) and 162 μM ($r^2 = 0.9827$) for root length, respectively. Compounds with lower activity are included in G2, but in this case the activities are similar to that of the commercial herbicide. The activity of 2-ethyl-D-DIBOA stands out because it maintains its activity (close to 50%) on root length at the 50 μM concentration and presents an IC_{50} value almost 3 times lower than that of propanil (114 μM, $r^2 = 0.9985$), which is used for the control of *E. crus-galli.*

Lipophilicity−Activity Correlations. The parameters included in Lipinski's rule of 5, as reinvestigated by Tice as a useful approach for agrochemical design, are summarized in **Tables 2** and **3** for all of the compounds obtained. N-4 derivatives from Dec-D-DIBOA above did not fit the rule as they have cLog *P* values higher than 4, a situation that is consistent with Tice's rule. In addition to this, Tridec- and Mir-D-DIBOA exceeded the number of rotatable bonds. Nevertheless, these compounds are the most active ones along with
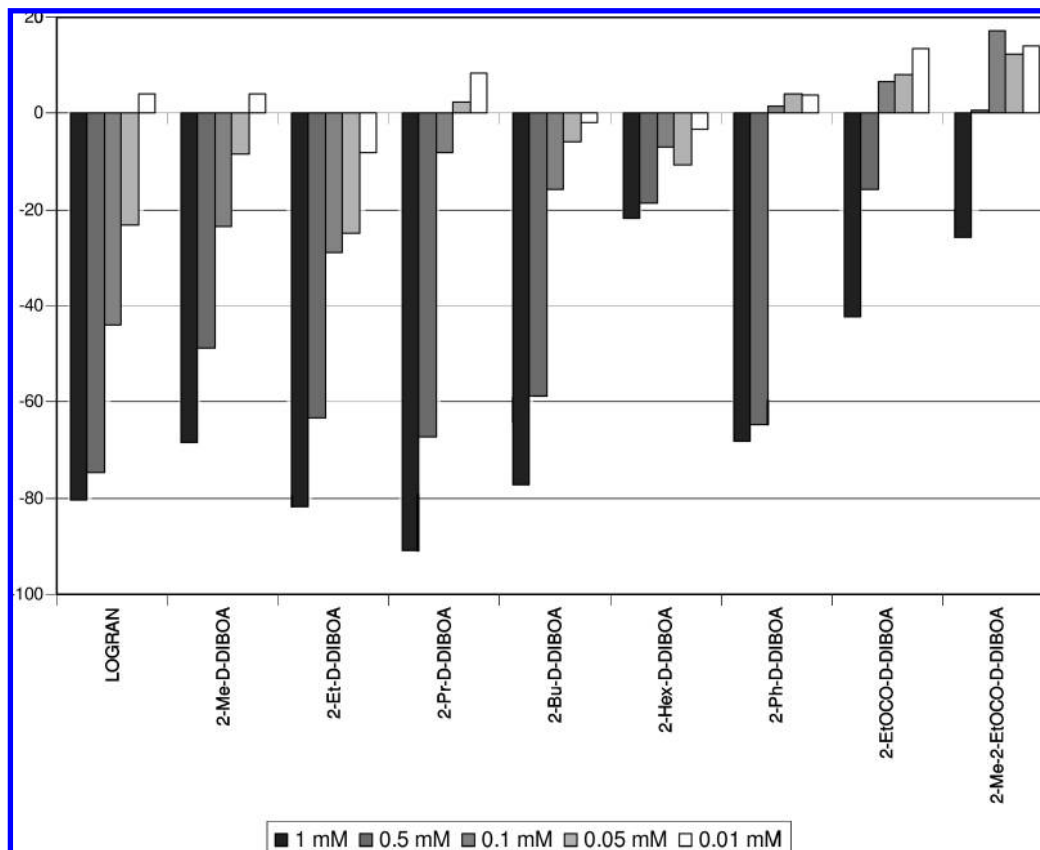
Figure 9. Phytotoxicity bioassay results for C-2 (root length, percent from control) for *Oryza sativa* L.
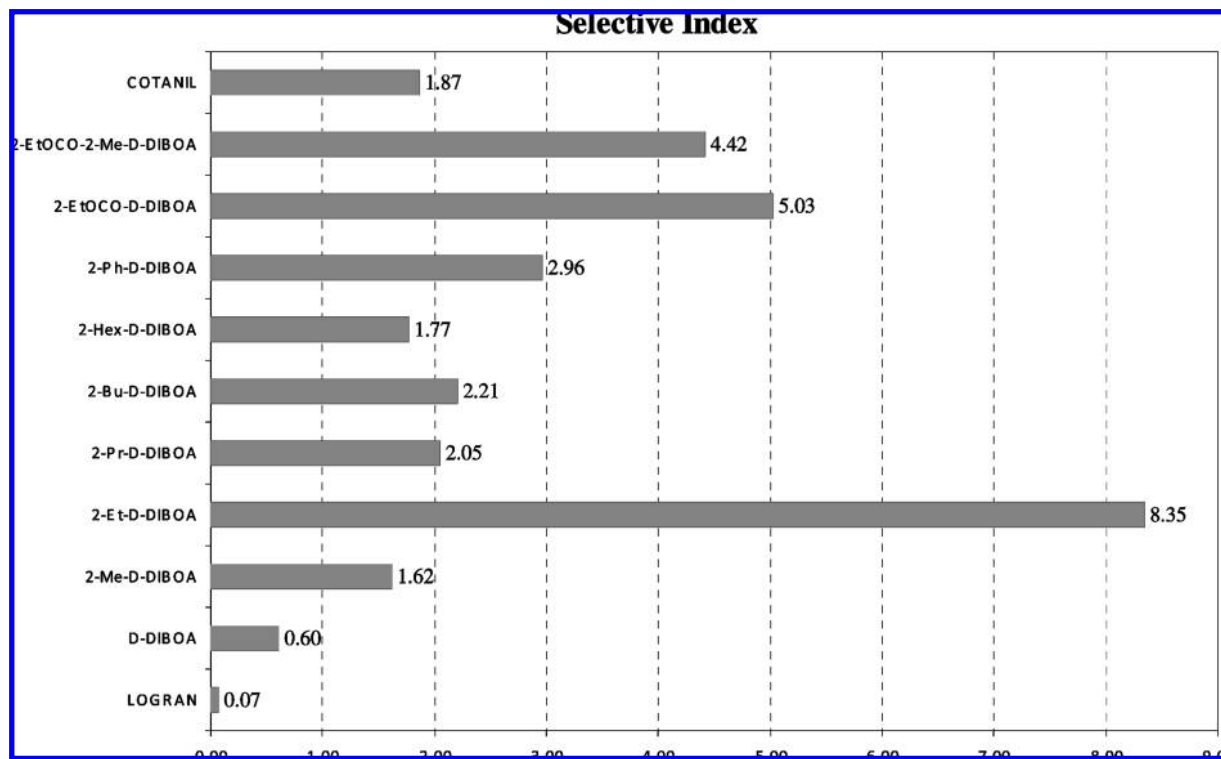

Figure 10. Selectivity index (SI$_{50}$) calculated for tested chemicals.

D-DIBOA. This finding does not agree with the lipophilicity models and could be explained by the accumulation of the compounds on the seed obstructing its access to water and nutrients rather than being a real phytotoxic effect. The effect of lipophilicity on the activity is shown in **Figure 7**, where IC$_{50}$ is represented versus cLog P for the search compound. If one

considers the even/odd chain effect of the ester (*28*), we can distinguish two clear zones in the graph. In the first zone the compounds fit Tice's rule, whereas in the second zone the compounds do not. In the first zone correlations were obtained with a high Pearson's coefficient of determination. In the other zone, however, the phytotoxic effects of chemicals could not
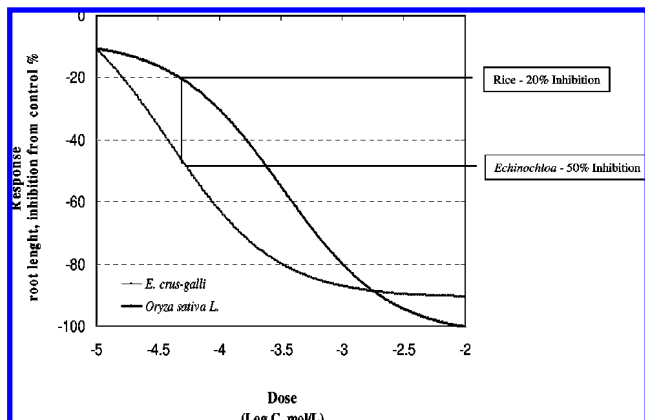
Figure 11. Dose−response curves for 2-Et D-DIBOA on rice and *E. crus-galli* (root length).

be adjusted to the parabolic equation proposed by Hansch's transport model (**Figure 7**).

$$\text{even esters: } \text{Log}(1/\text{IC}_{50}) = 0.21(\text{cLog } P)^2 - 1.022(\text{cLog } P) + 4.008 \quad r^2 = 0.9520 \quad (1)$$

$$\text{odd esters: } \text{Log}(1/\text{IC}_{50}) = 0.226(\text{cLog } P)^2 - 1.002(\text{cLog } P) + 4.010 \quad r^2 = 0.9990 \quad (2)$$

An even−odd effect has been previously been found (*28*) and was related with the capacity of ester side chains to interact with the cell membrane lipidic bilayer.

Regarding the C-2 derivatives, the behavior could be fitted to a quadratic model (**Figure 8**), but the coefficient of determination was not as good as that previously described for N-4 esters.

Lipophilicity is not the only molecular parameter that is modified by the introduction of hydrocarbon chains and functionalization at C-2. The increasing chain length leads to a large number of rotatable bonds and a new H-bond acceptor (carbonyl group) in the esters. The number of H-bond acceptors and the volume are relevant in terms of interactions of the phytotoxin with the target site of action. The number of rotatable bonds introduces conformational flexibility, which is needed for fitting to the target site, and conformational instability, which makes this fitting more difficult. Optimal values of Log *P* are in the range of 1−2, which agrees with the proposal in Tice's rule that cLog *P* must be lower than 4.

Selective Action of C-2 Derivatives of D-DIBOA. One of the determinant parameters in the optimization of herbicides is the selectivity. It is therefore necessary to compare the effects that a herbicide candidate can produce on crops and their common weeds. As *E. crus-galli* is a common rice weed, and based on results obtained previously, we evaluated the phytotoxicity of compounds that have different moieties at C-2 on seeds of *O. sativa* var. Puntal (**Figure 9**). This should allow us to measure the selectivity of these compounds to estimate their possible use as herbicides to control *Echinochloa* spp. in rice crops. The influence of D-DIBOA and its derivatives was studied for both plants by comparison of their dose−response curves.

The most affected growth parameter was root length, with 2-ethyl-D-DIBOA having $\text{IC}_{50} = 326$, $r^2 = 0.9882$. To find the most selective chemicals in the series tested, we employed the selectivity index as the quotient of IC_{50} values for rice and banyardgrass (*29*). Thus, the higher this value, the more selective toward the weed the compound is. This parameter was calculated

for all chemicals using the IC_{50} calculated for the root length. The results are shown in **Figure 10**. All chemicals have values higher than 1, except D-DIBOA and the commercial herbicide based on terbutryn and triasulfuron. The result obtained for 2-ethyl-D-DIBOA is noteworthy as this presents an IC_{50} value for *Echinochloa* (39 μM) that is almost 10 times lower than that of D-DIBOA, 2-methyl and 2-hexyl, with *O. sativa* (326 μM). Moreover, most chemicals have SI values that are higher than that of the commercial herbicide commonly used for rice protection (Propanil).

The SI values given here must be considered as a laboratory estimation. They are affected by the limitations inherent to laboratory bioassay methods. As propanil has a mode of action related to photosynthesis (PSII inhibitor), its bioactivity should be underestimated by a bioassay conducted in the darkness. This would also modify the selectivity calculation results. Nevertheless, for this approach it has been the preferred herbicide used in the field to treat the problem under study (barnyardgrass occurrence in rice fields). A more accurate description of the selectivity (and the bioactivity) of the tested materials will be needed for the mode of action evaluation of the most interesting tested chemicals, and the corresponding greenhouse and field experiments will be conducted in the near future. We believe this selectivity description to be highly valuable to select the compounds that will be submitted to those future screenings. In any case, the comparison to commercial herbicides is given to complete the description of the real potential of these novel molecules. Any variation in propanil's bioactivity under any other assay conditions would not vary our diagnosis on which of the chemicals shown herein are better herbicide model leads.

The influence of D-DIBOA and its derivatives was studied for both plants through a comparison of their dose−response curves. Dose−response curves for D-DIBOA and its ethyl derivative (2-Et-D-DIBOA) on rice and *E. crus-galli* (root length) are shown in **Figure 11**. It can be seen that the doses of 2-ethyl-D-DIBOA required for 20% inhibition of root growth for *O. sativa* (48 μM) caused a 50% of inhibition of *E. crus-galli*. These data indicate a significant difference in toxicity for both plant species. The selection of the optimal doses to be applied in crops needs a selectivity and bioactivity description with adult plants under bioassay models is closer to the real crop conditions.

These results confirm the potential of D-DIBOA as a lead compound for herbicides, and this substance can be proposed for the control of *Echinochloa* spp. in rice crops.

LITERATURE CITED

(1) Copping, L. G.; Duke, S. O. Natural products that have been used commercially as crop protection agents. *Pest Manag. Sc.* **2007**, *63*, 524–554.

(2) Sullivan, T. P.; Sullivan, D. S. Vegetation management and ecosystem disturbance: impact of glyphosate herbicide on plant and animal diversity in terrestrial systems. *Environ. Rev.* **2003**, *11*, 37–59.

(3) Wang, Y. S. Environmental impact of herbicide use in the subtropics. *Food Sci. Agric. Chem.* **1999**, *1*, 165–179.

(4) Kubinyi, H. Strategies and recent technologies in drug discovery. *Pharmazie* **1995**, *50*, 647–662.

(5) Mannhold, R.; Rekker, R. F.; Dross, K.; Biljoo, G.; de Vries, G. The lipophilic behavior of organic compounds: 1. An updating

9948 *J. Agric. Food Chem.,* Vol. 56, No. 21, 2008

Macías et al.

of the hydrophobic fragmental constant approach. *Quant. Struct.–Act. Relat.* **1998**, *17*, 517–536.

(6) Rekker, R. F.; Mannhold, R.; Biljoo, G.; de Vries, G.; Dross, K. The lipophilic behaviour of organic compounds: 2. The development of an aliphatic hydrocarbon/water fragmental system via interconnection with octanol–water partitioning data. *Quant. Struct.–Act. Relat.* **1998**, *17*, 537–548.

(7) Sicbaldi, F.; Sacchi, G. A.; Trevisan, M.; Del Re, A. A. M. Root uptake and xylem translocation of pesticides from different chemical classes. *Pestic. Sci.* **1997**, *50*, 111–119.

(8) Inoue, J.; Chamberlain, K.; Bromilow, R. H. Physicochemical factors affecting the uptake by roots and translocation to shoots of amine bases in barley. *Pestic. Sci.* **1998**, *54*, 8–21.

(9) Satchivi, N. M.; Stoller, E. W.; Wax, L. M.; Briskin, D. P. A nonlinear dynamic simulation for xenobiotic transport and whole plant allocation following foliar application II. *Pestic. Biochem. Physiol.* **2000**, *68*, 85–95.

(10) Honkanen, E.; Virtanen, A. I. Synthesis of some 1,4-benzoxazine derivatives and their antimicrobial activity. *Acta Chem. Scand.* **1960**, *14*, 1214–1217.

(11) Escobar, C. A.; Sicker, D.; Niemeyer, H. M. Evaluation of DIMBOA analogues as antifeedants and antibiotics towards the aphid *Sitobion avenae* in artificial diets. *J. Chem. Ecol.* **1999**, *25*, 1543–1554.

(12) Schulz, M.; Friebe, A.; Kueck, P.; Seipel, M.; Schnabl, H. Allelopathic effects of living quack grass (*Agropyron repens* L.). Identification of inhibitory allelochemicals exuded from rhizome borne roots. *Angew. Bot.* **1994**, *68*, 195–200.

(13) Wolf, R. B.; Spencer, G. F.; Plattner, R. D. Benzoxazolinone, 2,4-dihydroxy-1,4-benzoxazin-3-one, and its glucoside from *Acanthus mollis* seeds inhibit velvetleaf germination and growth. *J. Nat. Prod.* **1985**, *48*, 59–63.

(14) Macías, F. A.; Marín, D.; Oliveros-Bastidas, A.; Varela, R. M.; Simonet, A. M.; Carrera, C.; Molinillo, J. M. G. Allelopathy as a new strategy for sustainable ecosystems development. *Biol. Sci. Space* **2003**, *17*, 18–23.

(15) Macías, F. A.; Marín, D.; Oliveros-Bastidas, A.; Castellano, D.; Simonet, A. M.; Molinillo, J. M. G. Structure–activity relationship (SAR) studies of benzoxazinones, their degradation products, and analogues. phytotoxicity on problematic weeds *Avena fatua* L. and *Lolium rigidum* Gaud. *J. Agric. Food Chem.* **2006**, *54*, 1040–1048.

(16) Macías, F. A.; Chinchilla, N.; Varela, R. M.; Oliveros, A.; Marín, D.; Molinillo, J. M. G. Structure–activity relationship studies of benzoxazinones and related compounds. phytotoxicity on *Echinochloa crus-galli* (L.) P. Beauv. *J. Agric. Food Chem.* **2005**, *53*, 4373–4380.

(17) Macías, F. A.; Marín, D.; Oliveros-Bastidas, A.; Molinillo, J. M. G. Optimization of benzoxazinones as natural herbicide models by lipophilicity enhancement. *J. Agric. Food Chem.* **2006**, *54*, 9357–9365.

(18) Tice, C. M. Selecting the right compounds for screening: does Lipinski's rule of 5 for pharmaceuticals apply to agrochemicals? *Pest Manag. Sci.* **2001**, *57*, 3–16.

(19) Hansch, C.; Leo, A. J. *Substituent Constants for Correlation Analysis in Chemistry*; Wiley: New York, 1979.

(20) Macías, F. A.; Marínv, D.; Oliveros-Bastidas, A.; Castellano, D.; Simonet, A. M.; Molinillo, J. M. G. Structure–activity relationship (SAR) studies of benzoxazinones, their degradation products, and analogues. Phytotoxicity on problematic weeds *Avena fatua* L. and *Lolium rigidum* Gaud. *J. Agric. Food Chem.* **2006**, *54*, 1040–1048.

(21) Macías, F. A.; Marín, D.; Oliveros-Bastidas, A.; Chinchilla, D.; Simonet, A. M.; Molinillo, J. M. G. Isolation and synthesis of allelochemicals from Gramineae: benzoxazinones and related compounds. *J. Agric. Food Chem.* **2006**, *54*, 991–1000.

(22) *PRISM 4.0*; GraphPad Software: San Diego, CA, 2003.

(23) Sander, T. OSIRIS Property Explorer. Actelion Property Explorer; 2001; http://www.chemexper.com/tools/propertyExplorer/main.html.

(24) Chou, J.; Jurs, P. *J. Chem. Inf. Comput. Sci.* **1979**, *19*, 172.

(25) De Prado, R.; Franco, A. R. Cross-resistance and herbicide metabolism in grass weeds in Europe: biochemical and physiological aspects. *Weed Sci.* **2004**, *52*, 441–447.

(26) Castellano, D.; Macías, F. A.; Castellano, M.; Cambronero, R. M. FITOMED (automated system for measurement of variable lengths). Sp. Patent P9901565.

(27) *Statistica for Windows 5.0*; Statsoft: Tulsa, OK, 1984–1995.

(28) Macías, F. A.; Velasco, R. F.; Castellano, D.; Galindo, J. C. G. Application of Hansch's model to guaianolide ester derivatives: a quantitative structure–activity relationship study. *J. Agric. Food Chem.* **2005**, *53*, 3530–3539.

(29) Macías, F. A.; De Siqueira, J.; Chinchilla, N.; Varela, R. M.; Marín, D.; Molinillo, J. M. G. New herbicide models from benzoxazinones: aromatic ring functionalization effects. *J. Agric. Food Chem.* **2006**, *54*, 9843–9851.