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New Herbicide Models from Benzoxazinones: Aromatic Ring Functionalization Effects

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The utility of benzoxazinones and some of their synthetic derivatives in the search for new leads for herbicide model development has been widely discussed. As the benzoxazinone skeleton contains three different potential areas for functionalization (C-2, N-4, and aromatic protons H-5, H-6, H-7, and H-8), and the first two have already been optimized, the main objective of this work was the substitution of aromatic protons for different substituent types and the study of the effects of the prepared chemicals on selected standard target species (STS) and weeds. Thus, different combinations of aromatic substituents, including methoxy, methoxycarbonyl, fluorine, chlorine, and trifluoromethyl, were introduced at different positions. Phytotoxicity results were successfully correlated with steric and electronic molecular parameters, the resulting molecular volume (*V*) and dipole moment (μ) being the most influential ones. Halogenations at C-6 and fluorination at C-7 were the most successful modifications. Compounds 6-fluoro-(2*H*)-1,4-benzoxazin-3(4*H*)-one (6F-D-DIBOA), 7-fluoro-(2*H*)-1,4-benzoxazin-3(4*H*)-one (7F-D-DIBOA), and 6-chloro-(2*H*)-1,4-benzoxazin-3(4*H*)-one (6Cl-D-DIBOA) had the highest phytotoxic activities. The dose−response profiles on wheat and two of its most common weeds (*Lolium rigidum* Gaud. and *Avena fatua* L.) were compared by means of a proposed selectivity index, which displayed 7F-D-DIBOA as the most selective of the tested benzoxazinones.

KEYWORDS: Benzoxazinones; halobenzoxazinones; dipole moment; structure−activity relationship (SAR); herbicide models; phytotoxicity

INTRODUCTION

The preparation of derivatives from natural products as leads for new bioactive chemicals has been a commonly followed strategy in recent times (*1*). In most of the cases, the design of these new chemicals has been carried out by following two different strategies: employment of the molecular fragments responsible for the observed bioactivity of the natural product or functionalization of the basic molecular backbone. These simplifications are necessary in most of the cases, as the structures of bioactive natural products are usually complicated, mainly due to the presence of one or more stereogenic centers. Their synthetic preparation at the multigram scale, needed for greenhouse and field bioassays, is therefore difficult and expensive.

In the context of new herbicide model development from benzoxazinones, their unusual chemical simplicity, in comparison to most secondary metabolites, eliminates the need to use molecular fragments. Functionalization led to several synthetic benzoxazinone analogues with various substituents at C-2, N-4, and some aromatic protons. A preliminary structure−activity relationship study with these derivatives along with natural benzoxazinones and their degradation metabolites was a first successful approach with respect to enhancing the phytotoxicity of benzoxazinones and discovering the potential ecological role of the degradation products (*2*−*4*). These works provided the optimal heterocyclic structure for phytotoxicity enhancement. Taking advantage of the hydroxyl group at N-4 present in the most active models, and by systematic esterification, some interesting derivatives with optimal lipophilicity were obtained (*5*). The different modification possibilities for benzoxazinone model 2,4-dihydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (D-DIBOA) are summarized in **Figure 1**. The most relevant structural features of D-DIBOA were the lack of substitution at C-2 (especially with regard to the role of hydroxyl group of DIBOA and DIMBOA in their degradability) and the presence of the hydroxyl group at N-4 (preserving the integrity of the natural hydroxamic acid moiety). In general terms the presence of aromatic substituents lowered the phytotoxic effects of the tested chemicals.

Research on the chemistry and bioactivity of benzoxazinones yielded a wide variety of natural and synthetic derivatives, with

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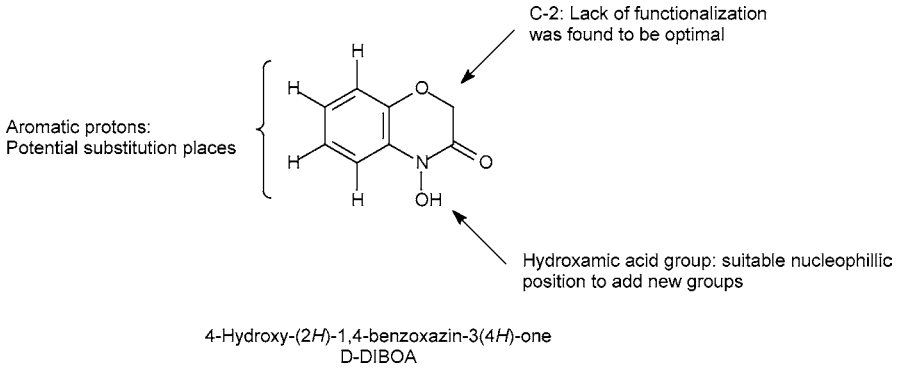
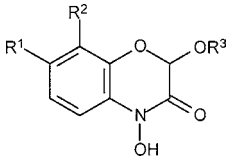


Figure 1. Structure and potential modification places for 4-hydroxy-(2*H*)-2,4-benzoxazin-3(4*H*)-one (D-DIBOA).

Table 1. Natural Benzoxazinones Substituted at C-7 and/or C-8



R^1	R^2	R^3	name
H	H	H	DIBOA
H	H	Glc	DIBOA-Glc
CH_3O	H	H	DIMBOA
CH_3O	H	Glc	DIMBOA-Glc
CH_3O	CH_3O	H	DIM_2BOA
CH_3O	CH_3O	Glc	DIM_2BOA-Glc
OH	H	H	TRIBOA

different substituent combinations in the aromatic ring. In addition to the natural 7-methoxybenzoxazinone DIMBOA and its glucoside, there are a few natural benzoxazinones containing aromatic methoxy groups at C-8 (*6*), such as DIM_2BOA and its glucoside (**Table 1**), which have been isolated from maize. Although it is not widely distributed on benzoxazinone producer plans, Le-Van and Wratten (*7*) isolated a 5-chloro-substituted benzoxazinone (Cl-HMBOA-Glc), which was demonstrated to be naturally occurring (**Figure 2**).

There are several studies on the chemistry and potential utilities of some other halo-substituted benzoxazinones, obtained by means of synthetic procedures as they are not naturally occurring chemicals. For example, 7-fluoro-4-hydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (7F-D-DIBOA) (**Table 2**) was synthesized by Geng et al. (*8*) by adapting the method previously described by Quiroz and Niemeyer, (*9*) which afforded several chloro-substituted derivatives. These methods were based on direct chlorinations of synthetic benzoxazinones D-DIBOA and 4-hydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (D-DIMBOA). Chlorination at C-5 was achieved for D-DIMBOA, whereas D-DIBOA was functionalized at C-5 and C-7. Logically, these procedures were needed for the previous synthesis of the starting benzoxazinones (*10*).

The antialgal, antifungal, and antimicrobial activities of 7F-D-DIBOA were evaluated on several organisms (*Chlorella xanthella*, *Candida albicans*, *Escherichia coli*, and *Staphylococcus aureus*), with medium or low activities, depending on the organism tested (*11*, *12*).

Other relevant contributions to chlorobenzoxazinone's chemistry were made by Hashimoto et al. (*13*). In this case, a suitable side chain was added to readily available chloronitrophenols, yielding a chlorobenzoxazinone [6-chloro-4-hydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one, 6Cl-D-DIBOA, **Table 2**] after reductive cyclization.

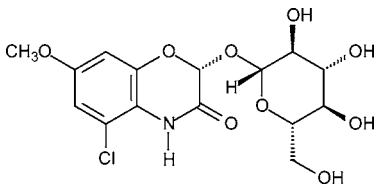


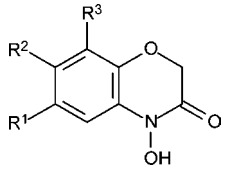
Figure 2. Molecular structure for 2-*O*-β-D-glucopyranosyl-5-chloro-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (5-Cl-HMBOA-Glc).

No bioactivities were described for this benzoxazinone chloride. Its isomer, 8-chloro-4-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one, was synthesized by employing the latter procedure and using 6-chloro-2-nitrophenol as starting material, being further evaluated from the point of view of its antifungal activity (*14*). Soon after this patent, Özden and collaborators (*15*) synthesized this derivative in an analogous manner, employing the Zn/NH_4Cl reductive cyclization procedure, and evaluated its antifungal activity. It was described as slightly active. With regard to polyhalogenated benzoxazinones, the derivative 6,8-dichloro-4-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one (6,8-diCl-D-DIBOA, **Table 2**) was synthesized in previous studies about plant growth regulators chemistry (*16*). This work is older than the discovery of natural benzoxazinones DIBOA and DIMBOA, and the synthesis is made in the context of the preparation of several derivatives of aryloxyalkanecarboxylic acids such as 2,4-dichlorophenoxyacetic acid (the herbicide 2,4-D). The catalytic hydrogenation of the 6-nitro derivative of 2,4-D with Raney nickel afforded the desired substituted benzoxazinone. This chemical has also been patented as a fungicide (*17*).

There are some precedents about the usage of benzoxazinone derivatives as active ingredients of commercial herbicides. The benzoxazinyltetrahydrobenzotriazol oxide derivatives (*18*) and the benzylpyridones (*19*) were proposed as candidates for it. The radicals at position N-4 were defined as alkyl or alkenyl with one or more halogen atoms and successfully tested on several weed species. There is also a commercial herbicide belonging to the phenylphthalymides group, featuring a benzoxazinone heterocycle as a substituent (Flumioxazin) (*20*).

Taking into consideration the functionalization possibilities of benzoxazinones (**Figure 1**), further research on the effects of modifications to the aromatic substitution should help to complete our knowledge about the optimal structural requirements for maximum phytotoxicity, in addition to yielding new derivatives with potential utility in the development of natural herbicide models, with a minimum modification of the natural benzoxazinone moiety. Thus, 12 benzoxazinones with different combinations of functional groups linked to the aromatic ring

Table 2. Benzoxazinone Derivatives Tested



R^1	R^2	R^3	compound
H	H	H	4-hydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (D-DIBOA)
H	CH_3O	H	4-hydroxy-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (D-DIMBOA)
CH_3O	H	H	4-hydroxy-6-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (6-MeO-D-DIMBOA)
H	CH_3O	CH_3O	4-hydroxy-7,8-dimethoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (7,8-diMeO-D-DIMBOA)
CH_3OCO	H	H	6-methoxycarbonyl-(2*H*)-1,4-benzoxazin-3(4*H*)-one (6-MeOCO-D-DIMBOA)
H	CH_3OCO	H	7-methoxycarbonyl-(2*H*)-1,4-benzoxazin-3(4*H*)-one (7-MeOCO-D-DIMBOA)
F	H	H	6-fluoro-(2*H*)-1,4-benzoxazin-3(4*H*)-one (6F-D-DIMBOA)
H	F	H	7-fluoro-(2*H*)-1,4-benzoxazin-3(4*H*)-one (7F-D-DIMBOA)
H	H	F	8-fluoro-(2*H*)-1,4-benzoxazin-3(4*H*)-one (8F-D-DIMBOA)
H	F	F	7,8-difluoro-(2*H*)-1,4-benzoxazin-3(4*H*)-one (7,8-diF-D-DIMBOA)
CF_3	H	H	6-trifluoromethyl-(2*H*)-1,4-benzoxazin-3(4*H*)-one (6CF_3-D-DIMBOA)
Cl	H	H	6-chloro-(2*H*)-1,4-benzoxazin-3(4*H*)-one (6Cl-D-DIMBOA)
H	H	Cl	8-chloro-(2*H*)-1,4-benzoxazin-3(4*H*)-one (8Cl-D-DIMBOA)
Cl	H	Cl	6,8-dichloro-(2*H*)-1,4-benzoxazin-3(4*H*)-one (6,8-diCl-D-DIMBOA)

(methoxy, methoxycarbonyl, fluorine, trifluoromethyl, and chlorine) were synthesized according to the previously described methodology (*10*). They were evaluated on standard target species (STS) *Lepidium sativum* L. (cress), *Allium cepa* L. (onion), *Lactuca sativa* L. (lettuce), *Lycopersicon esculentum* Mill. (tomato), and *Triticum aestivum* L. (wheat), in addition to the common wheat weeds *Lolium rigidum* Gaud. (rigid ryegrass) and *Avena fatua* L. (wild oat). Their effects, structure−activity relationships, and their correlation with molecular parameters of the tested chemicals are discussed.

MATERIALS AND METHODS

General Methods. The purity of the isolated and synthetic compounds was determined by ^{1}H NMR and HPLC analyses and was found to be >98%. ^{1}H and ^{13}C NMR spectra were recorded using MeOH-d_4 or $CDCl_3$ as solvent in a Varian INOVA spectrometer at 399.99 or 100.577 MHz, respectively. The resonance of residual methanol for ^{1}H was set to δ 3.30 and that for residual chloroform to δ 7.25. The solvent peak for ^{13}C was set to δ 49.00 (methanol) or δ 77.00 (chloroform) and used as internal reference. Mass spectra were recorded by means of a Varian 1200L quadrupole MS/MS detector. The physical data for all tested chemicals can be found in the Supporting Information.

Chemicals. The chemicals employed for this study are summarized in **Table 2**. They can be classified into four groups: methoxy derivatives (with methoxy groups at C-6 and C-7 or two groups at C-7 and C-8), methoxycarbonyl derivatives (C-6 and C-7), fluorine derivatives (monosubstituted at C-6, C-7, and C-8 or disubstituted at C-7 and C-8 and a trifluoromethyl group at C-6), and chlorine derivatives (monosubstituted at C-6 and C-8 and disubstituted at C-6 and C-8). The commercial herbicide Logran was used as internal standard for the phytotoxicity study according to a previously reported bioassay methodology (*21*). The starting compound for herbicide models based on benzoxazinones, D-DIBOA, was also included in the study. It does not have any aromatic substituents.

Synthesis of Aromatic Ring-Modified Benzoxazinones. The chemicals were obtained according to a previously reported methodology (**Figure 3**) (*10*) by a sequence of nucleophillic substitutions (side-chain linkage) and reductive cyclization (benzoxazinone ring formation). The starting materials employed were 2-nitrophenols substituted in the adequate positions: 5-methoxy-2-nitrophenol and 5,6-dimethoxyphenol were synthesized from 3-methoxyphenol and 2,3-dimethoxyphenol (Sigma-Aldrich Co.), respectively, by following the previously reported procedure for aromatic nitration (*22*). These methods afforded D-DIMBOA and 6-MeO-D-DIBOA, respectively. The remaining chemicals were obtained directly by transforming the suitable nitrophenols, as they were commercially available.

General Procedure for Nucleophilic Substitution. The starting 2-nitrophenol was dissolved in a solution of 0.1 M potassium hydroxide in absolute ethanol (1 mol equiv of KOH). After 1 h, the solvent was removed at reduced pressure. The resulting alkoxide was redissolved in *N*,*N*-dimethylformamide (50 mL/g starting material), and 1.2 mol equiv of ethyl 2-bromoacetate was added. The reaction mixture was stirred under argon for 24 h. After this time, 50 mL/g starting material of ethyl acetate was added, and the resulting organic solutions were washed with five portions of distilled water. The organic layers were combined and dried over anhydrous sodium sulfate, and the solvent was distilled at reduced pressure. Reaction crude was chromatographed (column chromatography, ethyl acetate/hexane, 1:4) to obtain the ethyl 2-[2′-nitrophenoxy]acetates in quantitative yield.

General Procedure for Reductive Cyclization. Palladium on carbon (10% Pd, 10% w/w proportion from starting nitrophenoxyacetate) was suspended in an aqueous solution of 1,4-dioxane (1:1) (100 mL/g starting material). After this, sodium borohydride (2 mol equiv) was added, and the solution was vigorously stirred. To this stirred suspension as added dropwise a solution of the nitrophenoxyacetate in 1,4-dioxane (0.5 g/mL). The reaction evolution was controlled by thin-layer chromatography. Once the reaction was completed, the suspension was vacuum filtered through Celite to remove the catalyst, and the filtrate was treated with 10% hydrochloric acid until pH 2 was reached. This

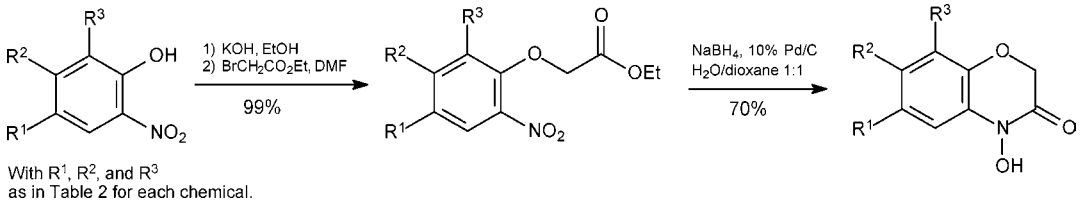


Figure 3. General procedure for tested chemicals' synthesis.

solution was further extracted with ethyl acetate (three times). The organic layers were combined and dried over anhydrous sodium sulfate, and the solvent was distilled at reduced pressure. The obtained residue was chromatographed (column chromatography, ethyl acetate/hexane, increasing polarity) to obtain the corresponding benzoxazinones.

Bioactivity Evaluation and Statistical Analysis. *Target Plants*. Selection of target plants is based on an optimization process made by us in the search for a standard phytotoxicity evaluation bioassay (*21*). After this process, several STS were proposed, including monocots wheat and onion and dicots tomato, cress, and lettuce, which were assayed for this study. The weeds rigid ryegrass and wild oat were also tested by employing the same methodology.

Methodology. Bioassays used Petri dishes (90 mm diameter) with one sheet of Whatman no. 1 filter paper as substrate. Germination and growth were conducted in aqueous solutions at controlled pH by using 10^{-2} M 2-[*N*-morpholino]ethanesulfonic acid (MES) and 1 M NaOH (pH 6.0). Compounds to be assayed were dissolved in dimethyl sulfoxide (DMSO) at different concentrations (0.2, 0.1, 0.02, 0.01, and 0.002 M), and these solutions were diluted with buffer (5 μL of DMSO solution/mL of buffer) so that test concentrations for each compound (10^{-3}, 5×10^{-4}, 10^{-4}, 5×10^{-5}, and 10^{-5} M) were reached. Additional treatments for 5×10^{-6}, 10^{-6}, 5×10^{-7}, 10^{-7}, and 5×10^{-8} M were prepared the same way for the most active chemicals to calculate accurate IC_{50} values (concentrations at which the observed parameter is stimulated or inhibited at 50% from control). This procedure facilitated the solubility of the assayed compounds. The number of seeds in each Petri dish depended on the seed size. The numbers of seeds that were used for tomato, lettuce, cress, and onion were 25, 15 for rigid ryegrass, and 10 were used for wheat and wild oat. Treatments, negative controls (buffered aqueous solutions with DMSO and without any tested compound), or internal reference solutions [commercial herbicide Logran, selected after a comparison study previously reported (*21*)] were added (5 mL) to each Petri dish. Four replicates were used for tomato, cress, onion, lettuce, and rigid ryegrass; 10 replicates were used for wheat and wild oat. The same number of replicates was used for treatments, negative controls, and internal references.

After the addition of seeds and aqueous solutions, Petri dishes were sealed with Parafilm to ensure closed-system models. Seeds were further incubated at 25 °C in a Memmert ICE 700 controlled-environment growth chamber, in the absence of light. Bioassays took 4 days for cress, 5 days for lettuce, tomato, rigid ryegrass, wild oat, and wheat, and 7 days for onion. After growth, plants were frozen at −10 °C for 24 h to avoid subsequent growth during the measurement process. This facilitated the handling of the plants and allowed a more accurate measurement of root and shoot lengths.

Bioassay Data Acquisition. Evaluated parameters (germination rate, root and shoot length) were recorded by using a Fitomed system (*23*), 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 and presented as percentage differences from control. Zero represents control, positive values represent stimulation of the studied parameter, and negative values represent inhibition. The cluster analysis (joining/tree clustering) was obtained by using Statistica v. 5.0 software (*24*), and it is presented as a horizontal hierarchical tree plot. Germination rate, shoot length, and root length average activity values for each concentration, for all tested species and all chemicals, were included in the analysis to acquire an overall view of the phytotoxicity and its relation with chemical structure.

IC_{50} values were obtained after the phytotoxicity data and concentrations (logarithmic scale) had been adjusted to a constant slope sigmoidal dose−response curve, defined by the equation

$$Y = Y_{min} + \frac{Y_{max} - Y_{min}}{1 + 10^{\log IC_{50} - X}}$$

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 determination coefficient (R^2). The adjustment and the R^2 were obtained by using GraphPad Prism software v. 4.00 (*25*).

Molecular Modeling and QSAR Calculations. Three-dimensional models for the tested chemicals were obtained from AM1 calculations performed by Hyperchem 7.01 software (*26*). Dipole moments, partial charges, polarizabilities, and molecular volumes were obtained by employing the algorithms implemented in this software. Molecular parameters−activity correlations were performed by means of the suitable Microsoft Office Excel 2003 spreadsheets (*27*). Statistical significance, given as probability value for correlation (P), was calculated for volume and dipole moment adjustments to phytotoxicity, by employing the Pearson product moment correlation method, as implemented in SigmaStat 3.1 software (*28*).

RESULTS AND DISCUSSION

General Bioactivity Profiles. All compounds, when active, showed inhibitory profiles for all evaluated parameters. Growth parameters (root and shoot length) were more affected than germination rates in most of the cases, although 6-Cl-D-DIBOA provoked very powerful inhibitions also in this parameter (IC_{50} = 20.4 μM, R^2 = 0.8103, *L. esculentum*; and 10.3 μM, R^2 = 0.9754, *L. sativa*). Among growth parameters, root length was more affected than shoot length. All tested species were significantly affected by the tested chemicals. Among STS, cress and onion were the most affected, whereas lettuce and wheat were the plants most tolerant to the tested treatments. The effects provoked by certain chemicals such as 6-Cl-D-DIBOA and 6-F-D-DIBOA forced us to test them at lower concentrations (see above). Results for both compounds on all tested species (root lengths) are shown in **Figure 4**.

In general terms, these compounds had a more pronounced phytotoxicity compared to the previously tested benzoxazinones (*2*−*4*). To relate phytotoxic effects with structural features and to compare the aromatic ring substituted benzoxazinones with the previously tested ones, a cluster analysis was performed (**Figure 5**).

The analysis, based on more than 1500 activity data, yielded four clusters (G1, G2, G3, and G4). The latter three are grouped in a cluster of higher order, indicating the chemicals from the first one (6-Cl- and 6-F-D-DIBOA) to be very different from the other ones in terms of bioactivity profiles. Groups G2 and G3 belong to the same cluster, as similarities among the chemicals grouped in them, in terms of bioactivity, may exist. Chemicals are ordered by their average phytotoxic effects from top to bottom. Thus, G1 groups the most phytotoxic compounds, G2 contains highly phytotoxic chemicals, G3 is formed by medium or low phytotoxicity compounds, and the last group, G4, is formed by slightly active or nonactive chemicals.

6-Cl- and 6-F-D-DIBOA provoked highly significant inhibitions at concentrations lower than the other chemicals. This behavior can be quantified by calculating the corresponding IC_{50} values (micromolar), shown in **Table 3**. Blank spaces indicate a bad fit to the dose−response model ($R^2 < 0.85$).

Both chemicals provoked on root length with IC_{50} values close to or below 1 μM, as recorded for *A. cepa* and *L. sativum*. The effects on the weed *A. fatua* are especially relevant. None of their IC_{50} values are above 60 μM. Their structures have a halogen atom at C-6 in common.

With regard to the chemicals placed in group G2, all of them have in common the presence of fluorine in their structures, this atom being placed at C-7 and C-8 or as a trifluoromethyl group at C-6. IC_{50} values for these chemicals varied over a wide range of concentrations. Wheat was the less sensitive plant (248.3−735.1 μM), whereas the most pronounced inhibitions were obtained for onion and cress with IC_{50} values below 50 μM. It is interesting to compare the effects on wheat and its

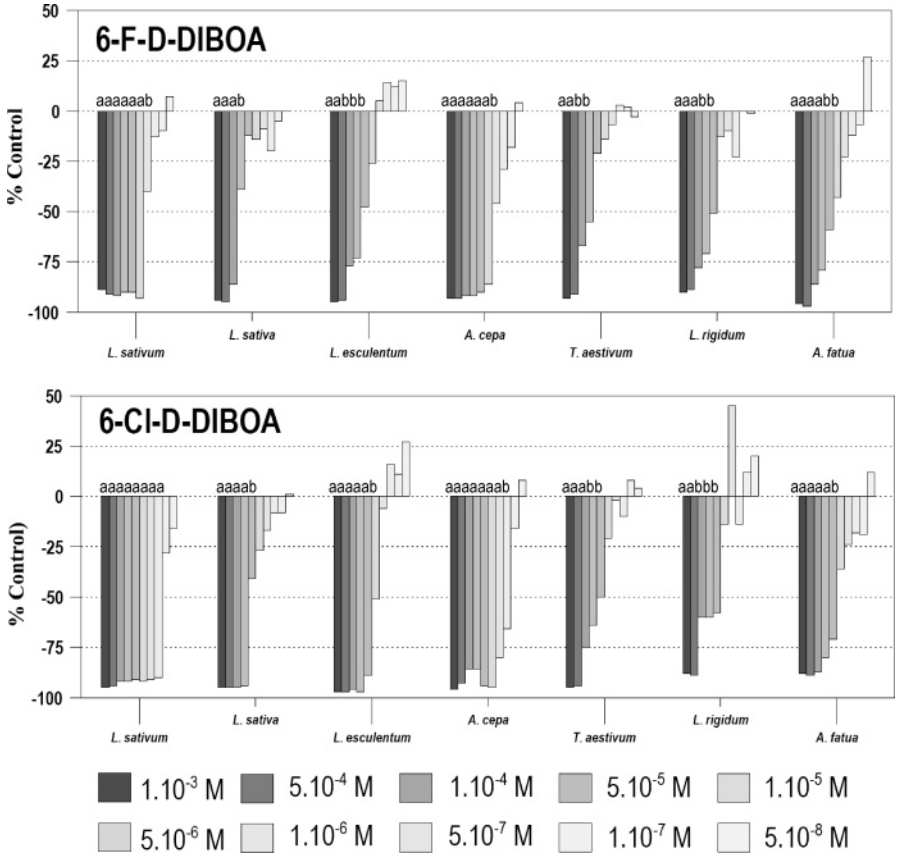


Figure 4. Phytotoxic effects of 6-chloro-4-hydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (6-Cl-D-DIBOA) and 6-fluoro-4-hydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (6-F-D-DIBOA) on standard target species and common wheat weeds (root length, percent of control). If it is not indicated, $P > 0.05$ for Welch's test: (a) values significantly different at $P < 0.01$; (b) values significantly different at $0.01 < P < 0.05$.

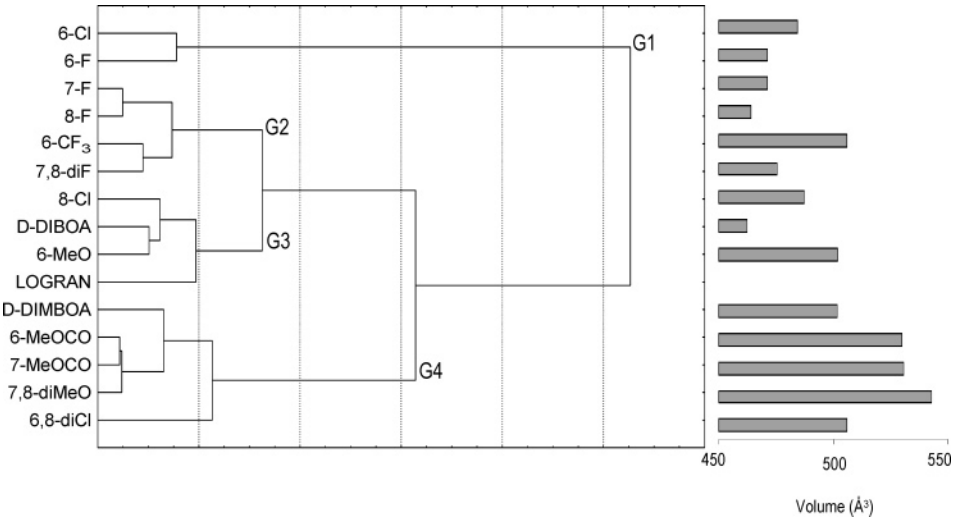


Figure 5. Cluster analysis (phytotoxicity data, germination and growth parameters, all tested species) and molecular volumes of the tested chemicals.

common weeds, as the effects recorded for both species (*A. fatua* and *L. rigidum*) are higher. A quantitative description of this selective behavior is provided below.

The phytotoxicity of some chemicals belonging to group G3 has been already described (*3*, *4*). Their relative phytotoxic character is maintained also when they are compared with much more phytotoxic halobenzoxazinones. It is interesting to point out the effects of 6-MeO-D-DIBOA, as the situation of the methoxy group greatly affects the bioactivity: 7-methoxybenzoxazinones were already found to be less active than the nonmethoxylated ones (*3*, *4*). The 6-methoxy derivative is placed between D-DIMBOA and D-DIBOA, being closer in its effects to the latter one as they belong to the G3 cluster.

All chemicals belonging to group G4 provoked very low effects on the tested plants. All IC_{50} values for root lengths are above 100 μM, especially for methoxycarbonyl, dimethoxy, and dichloride derivatives.

***A. fatua* Wheat Selectivity.** After the bioactivity data had been acquired and the corresponding IC_{50} values obtained, *A. fatua* and wheat IC_{50} were compared to find the most selective chemicals of the tested series. Herein we propose the employ-

Table 3. Selected IC_{50} Values (Micromolar, Root Length), with Indication of Clustering Groups

		A. fatua	*L. sativum*	*A. cepa*	*L. sativa*	*L. rigidum*	*L. esculentum*	*T. aestivum*
G1	6Cl-D-DIBOA	10.9	0.1	0.5	12.2	29.0	4.9	25.0
	6F-D-DIBOA	8.7	1.1	0.9	55.7	28.1	8.6	43.6
G2	7F-D-DIBOA	69.7	8.9	48.8	167.6	220.1	304.2	735.1
	8F-D-DIBOA	148.4	40.4	41.7	190.2	195.0	389.0	318.4
	6CF_3-D-DIBOA	250.9		18.8	64.4	194.9	162.9	248.3
	7,8-diF-D-DIBOA	108.8		28.7	191.6	159.3	89.5	453.4
G3	8Cl-D-DIBOA	154.7	128.9	374.0	2681.0	176.0	55.2	
	D-DIBOA	194.3	147.9	100.0	862.1	465.9	605.5	892.3
	6MeO-D-DIBOA	428.4	129.5	218.8	835.4			914.1
	Logran	242.2	1481.0	10.5	390.3	58.8		951.3
	D-DIMBOA	149.2	151.8	212.1	1092.0	288.4	211.9	942.5
G4	6-MeOCO-D-DIBOA	709.7	578.9	362.2		776.2		
	7-MeOCO-D-DIBOA	1140.0	1796.0	977.8			947.1	
	7,8-diMeO-D-DIBOA	642.4	627.1			636.9	730.1	935.5
	6,8-diCl-D-DIBOA	1009.0	114.1	266.1		319.8	691.8	

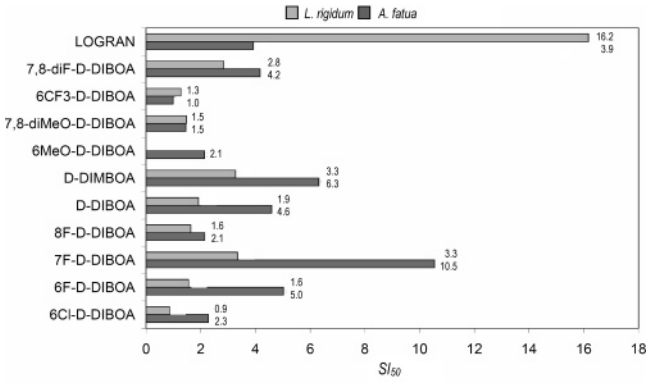


Figure 6. Selectivity index (SI_{50}) for selected chemicals on *Avena fatua* and *Lolium rigidum.*

ment of the quotient of both IC_{50} values as a "selectivity index" (SI):

$$SI_{50} = \frac{IC_{50}^{\text{wheat}}}{IC_{50}^{\text{wheat weed}}}$$

Thus, the higher the value, the more selective toward the weed the compound is. This parameter was calculated for all of the chemicals with values fitting the dose−response model correctly for both species (**Figure 6**).

The commercial herbicide Logran was found to be the most selective treatment on *L. rigidum.* Among the tested benzoxazinones, the most remarkable behavior was the one recorded for 7F-D-DIBOA and D-DIMBOA. SI_{50} values on *A. fatua* are higher, and the most selective benzoxazinones were 7F-D-DIBOA, D-DIMBOA, and 6F-D-DIBOA. Thus, 7F-D-DIBOA has special interest as its effects on wheat are very low (IC_{50} = 735.1 μM) (*29*). Its effects are the most remarkable from the point of view of both weed species tested. Further research will be needed to clarify the selectivity of the tested chemicals in an agricultural environment, but we propose the SI_{50} quotient as a useful and easy-to-calculate parameter to select or discard chemicals for further modifications and/or higher level bioassays (greenhouse, field).

Quantitative Structure−Activity Relationships (QSAR). To find the structural requirements for maximum phytotoxicity, the activity data were correlated with several molecular property descriptors. For that purpose, all tested chemicals were modeled by means of AM1 calculations, and their three-dimensional structures were obtained.

Steric Effects. Molecular volume is one of the most immediate parameters for the description of a bioactive chemical, because it determines the possibility to interact with the corresponding molecular target site of action. The presence of substituents at the benzoxazinone aromatic ring provokes a volume change, which was correlated with phytotoxicity data. A qualitative description of the volume effects is shown in **Figure 5**.

As the molecular volume increases, the phytotoxicity decays. An approach to a quantitative analysis is shown in **Figure 7**. This correlation displays a close relationship of activity with molecular volume, as two zones can be found: one for groups G1 and G2, where low volume changes cause high activity decreases, and other one for groups G3 and G4, in which changes in volume do not affect the phytotoxic effects. Thus, molecular volume seems to be a determinant parameter for groups G1 and G2.

According to the cluster analysis shown above, all chemicals substituted at C-6 are more active than their respective isomers with substituents placed at C-7 and/or C-8. Then, not only the overall molecular volume but the location of the different substituents seem to affect bioactivity. This general tendency is less clear with fluorine derivatives, as the steric hindrances provided by fluorine and hydrogen atoms are very similar. These differences are much clearer for chlorine, methoxy, and methoxycarbonyl derivatives. Then, no good correlations could be observed by treating the chemicals all together, but discarding for correlation the ones substituted at C-7 or C-8 (**Figure 8**).

These data fitted correctly a second-order polynomial function, in which the decay of the phytotoxic effect with volume is clearly displayed. In conclusion, a general correlation of the activity with molecular volume could be observed, the location of the substituents being also crucial. Compounds substituted at C-7 and C-8 were less active than the ones substituted just at C-6, the size of the radical at this position being also determinant in the activity observed.

Electronic Effects. A relationship between benzoxazinone's bioactivity and the electron density at N-4 has been suggested (*13*). Thus, to evaluate the role of molecular electronic distribution, some electronic densities for heterocycle atoms were calculated (**Table 4**). No significant variation (see standard deviation, σ) was found for any of these chemicals, which included active and nonactive. Thus, the aromatic substitution pattern seems not to affect the electron density of the heterocycle and the differences in activity cannot be attributed to these parameters. Then, a representative of the overall electronic

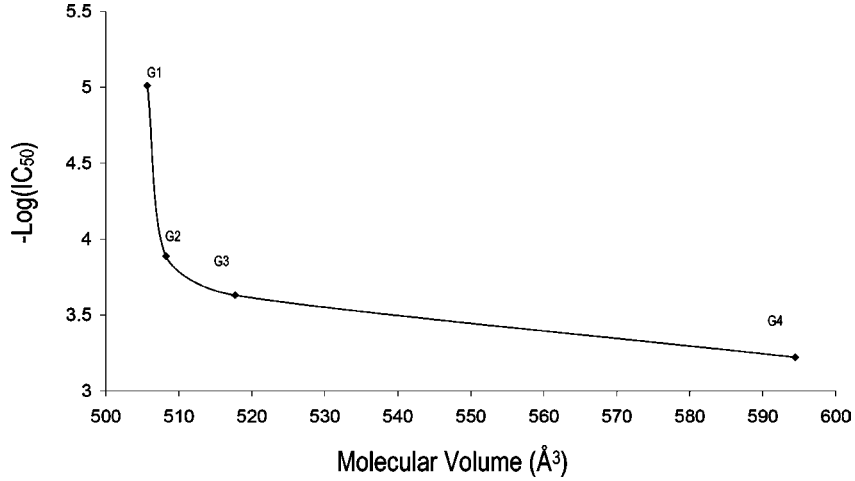


Figure 7. Phytotoxicity (*Avena fatua*, root length) versus average molecular volume for each cluster.

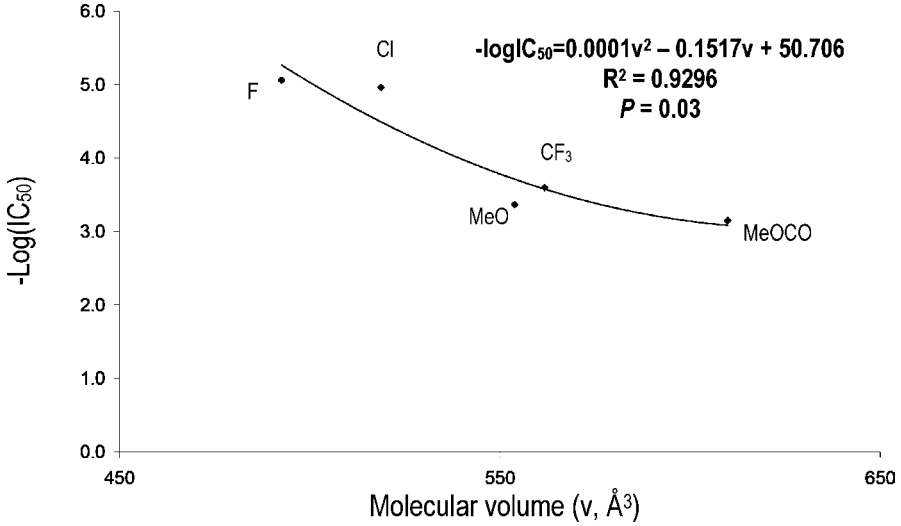
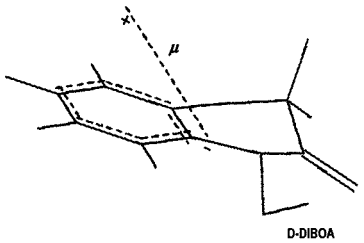


Figure 8. Phytotoxicity–molecular volume correlation. R^2, determination coefficient; *P*, statistical significance for correlation (variables correlated if $P < 0.05$).

Table 4. Electron Densities for Selected Atoms of 6-Substituted Benzoxazinones

	N-4	O (hydroxamic acid)	H (hydroxamic acid)	C-3	O (carbonyl)
D-DIBOA	0.552	–0.259	0.215	0.346	–0.773
6-MeO-D-DIBOA	0.554	–0.258	0.215	0.344	–0.774
6-F-D-DIBOA	0.553	–0.260	0.216	0.346	–0.772
6-Cl-D-DIBOA	0.551	–0.259	0.215	0.348	–0.771
6-CF_3-D-DIBOA	0.549	–0.260	0.215	0.349	–0.770
6-MeOCO-D-DIBOA	0.553	–0.260	0.216	0.346	–0.772
σ	0.002	0.001	0.001	0.002	0.001



μ (Debye)	
6Cl-D-DIBOA	2.06
6F-D-DIBOA	2.07
7F-D-DIBOA	1.65
8F-D-DIBOA	2.52
6CF3-D-DIBOA	2.87
7,8-diF-DIBOA	2.42
8Cl-D-DIBOA	2.38
D-DIBOA	2.50
6-MeO-D-DIBOA	3.58
7-MeO-D-DIBOA	2.05
6MeOCO-D-DIBOA	3.16
7MeOCO-D-DIBOA	2.22
7,8diMeO-D-DIBOA	3.04
6,8diCL-D-DIBOA	1.73

Figure 9. Representative direction and module values of dipole moment (μ) for the tested chemicals.

population of the molecule was needed to correlate effects with electronic parameters.

Dipole moment (μ) is a frequently used parameter for QSAR studies (*30*). It describes the overall polarity of the molecule, which is closely related to transport phenomena and interactions with target sites of action. Thus, the dipole moment modules for all chemicals tested were calculated by using their three-dimensional structures (**Figure 9**), with the purpose of correlating its magnitude with the phytotoxic effects observed.

Dipole moments were obtained from the three-dimensional structures found for the chemicals by means of AM1 calculations. Compounds belonging to group G4 were discarded as the effects on the activity provoked by steric factors could distort the dipole moment−activity correlation. Moreover, 7F-D-DIBOA and 6-CF3-D-DIBOA data were not used for the correlation as highly significant changes were observed for the dipole moment orientation. From the point of view of its dipole moment module, this chemical could belong to the group formed by 6-F- and 6-Cl-D-DIBOA, but its orientation is more similar to the lower activity and higher dipole moment module chemicals (**Figure 10**).

Taking into consideration the approaches made above, IC_{50} data were correlated with dipole moment modules (**Figure 11**). According to this, slight variation in dipole moment in the region

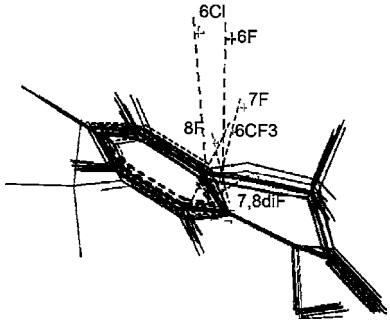


Figure 10. Directions of dipole moments for selected chemicals.

from $\mu = 2$ to $\mu = 2.5$ (G1 and first cluster of G2) has a great influence on activity. In addition to this, if polarizability (p) calculations are performed for each of the chemicals, the most active are the less polarizable ones, the ones in which transformations in dipole moments caused by the electronic environment are lower. The average value of this parameter for each of the clusters is shown in **Figure 12**.

The overall charge distribution in the benzoxazinones tested is a fundamental parameter to the understanding of their phytotoxic effects. When discarding the effects of the higher volume molecules, a quadratic dependence of activity with dipole moment modules is observed, the most active chemicals sharing a similar dipole moment orientation pattern. The changes provoked by the electronic environment to the dipole moments, quantified by means of polarity calculations, are relevant for the most active chemicals, as the three most active groups show similar values for this parameter.

The correlations observed for topological and electronic QSAR descriptors show a strong dependence of activity with volume and dipole moment and are closely related to polarizability. Taking into consideration the results shown above, the optimal value ranges for these parameters are

parameter	optimal value
molecular volume (V)	470–520 Å^3
dipole moment (μ)	2.0–2.5 Db
polarizability (p)	16.3–17.5 Å^3

Minimum values for the three parameters favored phytotoxic activity. Including selectivity comparisons, the best leads for natural herbicide model development are the chemicals placed in groups G1 and G2. 6-Chloro-4-hydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (6-Cl-D-DIBOA) and 6-fluoro-4-hydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (6-F-D-DIBOA) (G1) were the most phytotoxic chemicals and fitted the optimal structure requirements in terms of volume and dipole moment. 7-Fluoro-4-hydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (7F-D-DIBOA) was the most selective chemical toward *A. fatua* and fitted the optimal values for the three parameters, although the differences of overall electronic distribution, caused by changes in dipole moment orientation, probably affect its phytotoxicity.

These results, in combination with those obtained in the previous studies on structure−activity relationships for benzoxazinones and related compounds (*2*−*5*), provide an overview

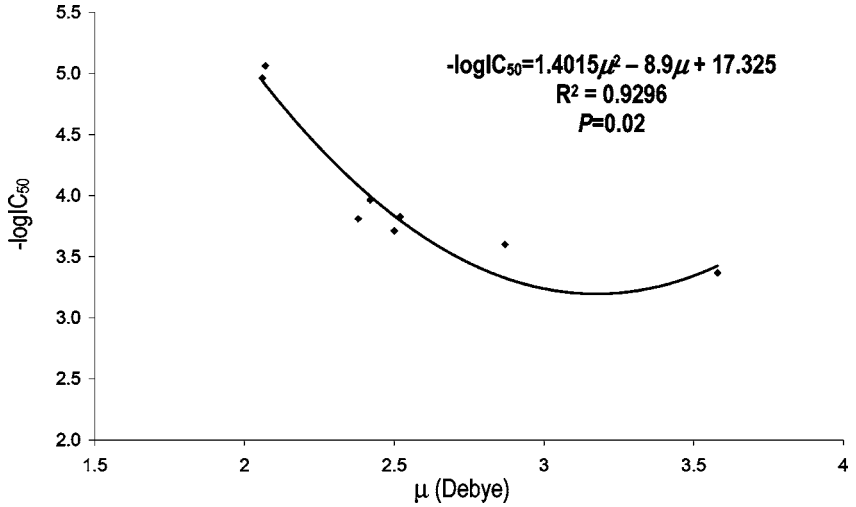


Figure 11. Phytotoxicity–dipole moment correlation. R^2, determination coefficient; P, statistical significance for correlation (variables correlated if $P < 0.05$).

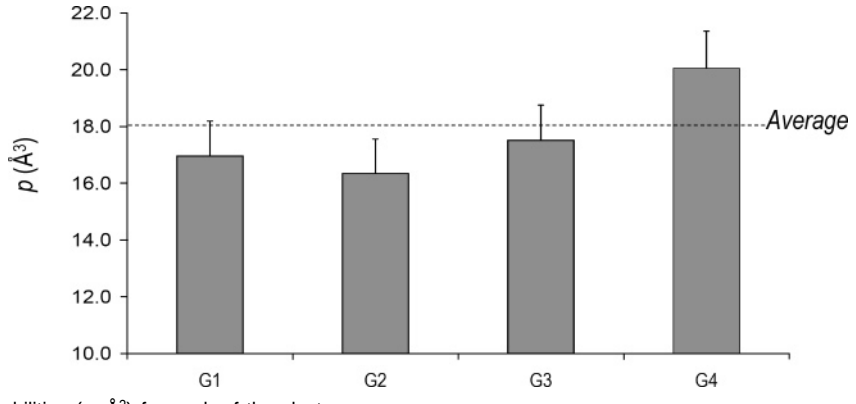


Figure 12. Average polarizabilities (p, Å^3) for each of the clusters.

of the thermodynamic, steric, electronic, and functionalization requirements for benzoxazinones' phytotoxic effects.

Supporting Information Available: Physical data for all chemicals tested, phytotoxic activity values for all chemicals and all plant species, and complete tables for all calculated QSAR parameters. This material is available free of charge via the Internet at http://pubs.acs.org.

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