INTRODUCTION

The intensive use of synthetic herbicides during the past 50 years has provoked different problems mainly connected to environmental impact (1, 2) and weed resistance to herbicides (3, 4). In the context of an increasing demand for high-quality feeding products and environmental concerns, it is urgent to change the philosophy applied to the research and development of strategies useful in crop protection. Chemical control of crop pests and diseases is one of the areas in which new methodologies mainly focused on the understanding and application of ecological phenomena should contribute to solve the problems created in the past (5).

The employment of allelochemicals (natural plant toxins) as templates for the discovery of new phytotoxic agents useful in agriculture has developed into a convenient approach to control weeds and diseases (6). In the context of an increasing demand for high-quality feeding products and environmental concerns, it is urgent to change the philosophy applied to the research and development of strategies useful in crop protection. Chemical control of crop pests and diseases is one of the areas in which new methodologies mainly focused on the understanding and application of ecological phenomena should contribute to solve the problems created in the past (5).

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The employment of allelochemicals (natural plant toxins) as templates for the discovery of new phytotoxic agents useful in agriculture has developed into a convenient approach to chemical low-dose treatments with less environmental impact, higher selectivity, and alternative modes of action, which could decisively contribute to solve the problems associated with chemical weed control (6, 7). Commercial herbicides such as cinmethyliso (a derivative from natural monoterpene 1,4-cineole, which is widely distributed in plants) (8) and the herbicide family of triketones, which derive from leptospernone, a natural product from the plant genus Callistemon (9), exemplify the potential utility of plant allelochemicals for weed management.

Hydroxamic acids with a (2H)-1,4-benzoxazin-3(4H)-one skeleton have attracted the attention of phytochemistry and allelopathic researchers since the discovery of 2,4-dihydroxy-(2H)-1,4-benzoxazin-3(4H)-one (DIBOA) (10) and 2,4-dihydroxy-7-methoxy-(2H)-1,4-benzoxazin-3(4H)-one (DIMBOA) (11) (Figure 1). They were first isolated from rye and maize, respectively, being preserved as β-D-glucosides prior to their release (12–14). Soon after their discovery, they were found in a wide variety of plants mainly belonging to Gramineae, Ranunculaceae, and Scrophulariaceae families (15). Their antifungal (16), antimicrobial (17), and phytotoxic effects (18–22), in addition to their interesting ecological role (23), raised interest in their isolation and synthesis in the search for an accurate explanation of the defense mechanisms of benzoxazinone producer plants. Moreover, the discovery of microbial degradation phenomena (24, 25), which led to the detection of new chemicals with different structures, effects, ecological roles, and potential utilities, increased the interest in the development of analytical methods (26) and the design of experiments in
which all of the chemicals present in the plant—plant interaction involving benzoxazinones could be present (23). Finally, the availability of all those instruments makes benzoxazinones some of the most attractive groups of natural products to be employed in the design of new chemicals with potential use in weed management (27).

In the context of the new methodologies for bioactive compounds’ design, quantitative structure—activity relationships studies (QSAR) constitute the key for a systematic analysis of structure and bioactivity properties. An adequate correlation of them to search for the most significant structural requirements is needed to achieve the desired physical, chemical, and biological properties (26) with the aid of combinatorial chemistry techniques (29).

QSAR methodology has been extensively employed for drug discovery, and its applications on new agrochemicals’ design are starting to rise, as the potential applicability of the physical—chemical parameters employed in QSAR design of pharmaceuticals has been discussed (30). According to those studies, four parameters should be taken into consideration to generate leads for herbicide development: molecular mass, number of hydrogen-bond donors, number of hydrogen-bond acceptors, and log P (logarithm of the octanol/water partition coefficient), which constitutes a measurement of the aqueous solubility—lipophilicity ratio for a given chemical. On the basis of an empirical study on more than 2000 pharmaceuticals, Lipinski et al. proposed the optimal values for those parameters. Log P, previously employed to analyze the assimilation capacity of oral usage drugs (30), could also help to determine the bioavailability of a given organic chemical for a plant, as was stated by Tice (31), after the fitting of those parameters to the herbicidal and insecticidal activity requirements, and the addition of a new parameter (number of rotatable bonds). The optimal values of those parameters are shown in Table 1. These studies have been greatly influential as they provide useful clues for drug and agrochemical development on the basis of physicochemical parameters, which are easy to calculate.

The barriers that a crop protection agent must cross to reach its molecular target site may vary: Postemergence herbicides must cross the leaf cuticle to reach the whole plant, having more potent effects when being phloem mobile (32). On the other hand, root and shoot uptake and xylem mobility are more important for pre-emergence herbicides (33). Systemic transport would be favored by a higher aqueous solubility, but some lipophilicity will be necessary to cross cell membranes and to reach the target site of action.

In this work, the algorithm included in OSIRIS Property Explorer (34) for cLog P calculation (35) was found to be the most adequate one, as it fits the previously reported values for the empirical rules mentioned above.

Herein we report the correlation of phytotoxic effects of two series of benzoxazinone ester derivatives with increasing lipophilicity in the search for the optimal value for log P. The lipophilicity was increased by preparing esters with increasing side chain length. This was found to be the most chemically convenient way, taking advantage of the hydroxyl group at N-4 present in the hydroxamic acids with a (2H)-1,4-benzoxazin-3(4H)-one skeleton selected for this study (Figure 1). In our previous papers on structure—activity relationship studies on benzoxazinones and related compounds (20–22), several requisites for phytotoxicity enhancement were displayed: the lack of functional groups at the aromatic ring, the lack of a hydroxyl group at C-2, and the presence of a hydroxamic acid moiety. 4-Hydroxy-(2H)-1,4-benzoxazin-3(4H)-one (D-DIBOA, Figure 1) was proposed as the optimal template for herbicide model development. Its derivative, 4-hydroxy-6-methoxy-(2H)-1,4-benzoxazin-3(4H)-one (6-MeO-D-DIBOA, Figure 1), was included in the study to compare the effects on transport modulation in two structurally related chemicals, in order to assume or discard intrinsic phytotoxic effects from the ester side chains. Thus, 14 ester derivatives of D-DIBOA (Figure 2) were prepared and tested on the same species as in our previous studies. Their correlation to Tice’s modification to Lipinski’s rule of 5 (31), in the context of Hansch’s transport theory (37), is discussed. These benzoxazinones constitute a second generation of chemicals from previously reported SAR studies of benzoxazinones and related compounds (20–22).

### MATERIALS AND METHODS

**General Experimental Procedures.** The purity of the compounds to test was determined by 1H NMR and HPLC analyses and found to
be >98%. 1H and 13C NMR spectra were recorded using CDCl3 as solvent in 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 13C was set to δ 77.00 (chloroform) and used as internal reference. UV–vis spectra were acquired by means of a Varian Cary 50 BIO spectrophotometer, chloroform being used as solvent. Mass spectra (EIMS) were recorded by means of a Voyager Termostofig equipment. FTIR spectra were obtained by means of a Spectrum BX Perkin-Elmer FTIR system. Frequency values are given in cm⁻¹.

Starting Material. 4-Hydroxy-(2H)-1,4-benzoazin-3(4H)-one (D-DIBOA, Figure 1) was synthetically obtained as previously reported (25). Acyl chlorides were purchased from Sigma-Aldrich Co. and used as received, except undecanoyl and tridecanoyl chlorides; these were synthesized as described below. 6-Methoxy-D-DIBOA was synthesized by using 4-methoxy-2-nitrophenol (purchased from Acros Organics, used as received) as starting material and following the same procedures as for D-DIBOA synthesis.

Undecanoyl and Tridecanoyl Chloride Obtention. These acyl chlorides were obtained by means of the typical thionyl chloride procedure. Five hundred milligrams of undecanoyl or tridecanoyl acid (purchased from Acros Organics Inc. and Sigma-Aldrich Co., respectively, used as received) were dissolved in thionyl chloride and refluxed for 4 h. After this, the excess thionyl chloride was distilled at reduced pressure, and the oily residue, containing the corresponding pure acyl chloride, was dissolved in dry THF (0.1 N assuming complete conversion) prior to their use.

General Esterification Method. One hundred milligrams of D-DIBOA or 6-methoxy-D-DIBOA was dissolved in 25 mL of dry pyridine, and 1.2 mol equiv of the suitable acyl chloride (commercial or readily synthesized and dissolved in THF for undecanoyl and tridecanoyl) was added dropwise at 0 °C (ice bath) and under a dry argon atmosphere. After the addition, the flask was allowed to warm to room temperature; 12 h later, 25 mL of EtOAc was added, and the mixture was transferred to an extraction funnel and washed with 50 mL of 0.1 N HCl (3 times) and 50 mL of 0.1 N NaHCO3 (three times). The organic phases were further combined and dried over anhydrous magnesium sulfate, and the solvent was removed (rotatory evaporator). The dry residue was chromatographed (CC, SiO2, EtOAc/hexane 1:10) to obtain the benzoxazinone ester derivatives.

Calculation of IC50 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 (34). cLog P data were acquired by using the OSIRIS property explorer (35). This software uses the Chou and Jurs algorithm, based on computed atom contributions (36).

Bioassays. Target Plants. Selection of target plants is based on an optimization process made by us in the search for a standard phytotoxicity evaluation bioassay (38). After this process several standard target species (STS) were proposed, including monocots Triticum aestivum L. (wheat) and Allium cepa L. (onion) and dicots Lepidium sativum L. (cress), Lepidium esculentum Will. (tomato), Lepidium sativum L. (cress), and Lactuca sativa L. (lettuce), which were assayed for this study. The weeds Lolium rigidum Gaud. (rigid ryegrass) and Avena fatua L. (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 10⁻³ M 2-[N-morpholino]ethanesulfonic acid (MES) and 1 M NaOH (pH 6.0). Compounds to be assayed were dissolved in DMSO (0.2, 0.1, 0.02, 0.01, and 0.002 M), and these solutions were diluted with buffer (5 mL 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. This procedure facilitated the solubility of the assayed compounds. The number of seeds in each Petri dish depended on the seed size; 25 seeds were used for tomato, lettuce, cress, and onion, 15 seeds were used for rigid ryegrass, and 10 seeds were used for wheat and wild oat. Five milliliters of treatment, control, or internal reference solution was added 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.

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 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-dimethylpropyl)-N'-(N,N-dimethyl)-1,3,5-triazine-2,4-diamine (Terbutryn, 59.4%), and 2-(2-chloroethoxy)-N,N-dimethyl-1,3,5-triazin-2-ylaminocarbonyl]benzenesulfonamide (Triasulfuron, 66.8%), was used as internal reference, according to a comparison study previously reported (38). It was used at the same concentrations (10⁻³, 5 × 10⁻⁴, 10⁻⁴, 5 × 10⁻⁵, and 10⁻⁵ M) and in the same conditions as the compounds in study. Negative control samples (buffered aqueous solutions with DMSO and without any tested compound) were used for all of the plant species assayed.

Bioassay Data Acquisition. Evaluated parameters (germination rate, root length, and shoot length) were recorded by using a Fitomed system (39), 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 (39). Statistical significance is expressed by means of letters, ‘‘a’’ meaning significantly different from control with 0.01 confidence and ‘‘b’’ meaning significantly different from control with a confidence from 0.01 to 0.05. The absence of a letter indicates no significant difference from control values. 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 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 (41), given by the equation

\[ d(x,y) = \sum_i (x_i - y_i)^2 \]

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 by using Statistica v. 5.0 software (41). 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.

EC50 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

\[ Y = Y_{\text{min}} + \frac{Y_{\text{max}} - Y_{\text{min}}}{1 + 10^\left(\text{LogEC50} - x\right)} \]

where \( X \) indicates the logarithm of concentration, \( Y \) indicates the response (phytotoxicity), and \( Y_{\text{max}} \) and \( Y_{\text{min}} \) 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 by using GraphPad Prism software v. 4.00 (39).

RESULTS AND DISCUSSION

Synthesis of D-DIBOA, 6-Methoxy-D-DIBOA, and Their Esters. D-DIBOA was obtained according to the previously reported methodology. 6-Methoxy-D-DIBOA was obtained following the same methodology with 4-methoxy-2-nitrophenol as starting material, with a 65% yield after two reaction steps. Quantitative yield was achieved in all esterification reactions.
Fitting to Lipinski’s Rule of 5 (Tice Approach for Agrochemical Design). The parameters included in Lipinski’s rule of 5, as they were reinvestigated by Tice as a useful approach for agrochemicals design, are summarized in Table 2 for all of the obtained chemicals. All tested compounds after Dec-D-DIBOA did not fit the rule, as they have cLog P values > 5, which agrees with Lipinski’s requirements. In addition to this, Tridec- and Mir-D-DIBOA exceeded the number of rotatable bonds. Compounds from D-DIBOA to Oct-D-DIBOA have molecular properties adequate to reach good phytotoxicity values, according to Tice’s optimal cLog P range.

Phytotoxic Activity. All tested compounds, when active, showed inhibitory profiles. The esters were more or less active than D-DIBOA depending on the tested plant and the evaluated parameter (germination rate, root length, and shoot length). The persistence, the increase or decrease of the phytotoxic effect with cLog P, was also plant and parameter dependent. In general terms, root length was the most affected parameter in all species. A qualitative general comparison of the esters with D-DIBOA, with regard to plant species and parameters, is given in Table 3. The addition of an ester function to D-DIBOA provoked a significant phytotoxicity enhancement on cress (with increasing germination rate, root length, and shoot length inhibition), as the most affected STS, and the weeds rigid ryegrass (in which root length inhibition increases) and especially wild oat (with a drastic difference on germination inhibition rate and highly significant effects on root length).

Cress was more affected by chemicals of medium polarity of the series: Valeryl and heptanoyl D-DIBOA derivatives strongly inhibited root and shoot length. Low values of IC50 were reached, especially on shoot length inhibition [IC50 = 82.4 and 87.0 µM for valeryl and heptanoyl derivatives, respectively, with excellent fitting to the dose–response model (R2 > 0.99 in both cases)]. Valeryl D-DIBOA also provoked a significant effect on germination rate (IC50 = 580 µM, R2 = 0.9374), maintaining significant activity values at all concentrations when D-DIBOA was found to be active only at the most concentrated treatment.

With regard to the phytotoxicity on weeds, D-DIBOA and ABOA were the most active chemicals on L. rigidum if joint effects on roots and shoots were considered. Nevertheless, heptanoyl and octanoyl derivatives were also active on roots, with IC50 values around 100 µM (123.4 and 107.4 µM, respectively, with R2 > 0.95 in both cases). No significant effects were observed for germination rate.

Among the different weed species tested to date, Avena fatua (wild oat) has been the one most affected by benzoazinone treatments, according also to our previous research (21, 23). Wild oat is the only plant of all that were studied that showed a significant effect on its germination rate, this effect being closely related to lipophilicity (Figure 3). The effects drastically increase from Pr-D-DIBOA (cLog P = 1.65) to Hept-D-DIBOA (cLog P = 3.50). A loss of the effect is observed from the octanoyl derivative to the end of the series, this behavior being in good accordance with the empiric rules of Tice (29). Optimal values of cLog P are in the propanoyl–valeryl interval (1.65–2.58). Compounds outside the cLog P values that fit the rule (Table 2) did not provoke any significant effect on germination rate. Pr-D-DIBOA affected root length like no other chemical of the series, preserving an almost complete inhibition effect in the first three concentrations, which provides a very low IC50 (27.8 µM, R2 = 0.9772). Without presentation of those so potent values, Val-D-DIBOA is the only chemical to provoke consistent effects on the three parameters for this plant, the effects on shoot being of medium potency (IC50 = 309.6 µM, R2 = 0.9800). The consistency of the effects of the tested compounds on wild oat makes this plant the optimal one to attempt a mathematical correlation of lipophilicity, expressed as cLog P, with phytotoxicity values (see next section).

Table 2. Molecular Parameter Values Found for D-DIBOA and Its Esters.

<table>
<thead>
<tr>
<th></th>
<th>molecular weight</th>
<th>rotatable bonds</th>
<th>H-bond donors</th>
<th>H-bond acceptors</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-DIBOA</td>
<td>175.15</td>
<td>0.42</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>ABOA</td>
<td>207.19</td>
<td>1.18</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Pr-D-DIBOA</td>
<td>221.21</td>
<td>1.65</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Bu-D-DIBOA</td>
<td>235.23</td>
<td>2.11</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Val-D-DIBOA</td>
<td>249.25</td>
<td>2.58</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Hex-D-DIBOA</td>
<td>263.27</td>
<td>3.04</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Hept-D-DIBOA</td>
<td>279.23</td>
<td>3.5</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Oct-D-DIBOA</td>
<td>291.31</td>
<td>3.97</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Dec-D-DIBOA</td>
<td>305.33</td>
<td>4.43</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Dec-D-DIBOA</td>
<td>319.35</td>
<td>4.89</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Undec-D-DIBOA</td>
<td>333.37</td>
<td>5.36</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>Undec-D-DIBOA</td>
<td>345.39</td>
<td>5.82</td>
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<td>0</td>
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<tr>
<td>Tridec-D-DIBOA</td>
<td>361.41</td>
<td>6.29</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>Mir-D-DIBOA</td>
<td>375.43</td>
<td>6.75</td>
<td>14</td>
<td>0</td>
</tr>
</tbody>
</table>

* Values outside the optimal ranges for activity are shown in boldface type.
* Number of donors was found to be pH-dependent, value at pH 6.0 being displayed.

Table 3. Comparison of Phytotoxic Effects of D-DIBOA and Its Esters.

<table>
<thead>
<tr>
<th></th>
<th>germination rate</th>
<th>root length</th>
<th>shoot length</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. sativum</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A. cepa</td>
<td>0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>L. esculentum</td>
<td>–</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>L. salvia</td>
<td>0</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>T. aestivum</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>L. rigidum</td>
<td>0</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>A. fatua</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

The structures of all synthesized chemicals were confirmed by spectroscopy methods (see Supporting Information).

Strongly inhibited root and shoot length. Low values of IC50 were reached, especially on shoot length inhibition [IC50 = 82.4 and 87.0 µM for valeryl and heptanoyl derivatives, respectively, with excellent fitting to the dose–response model (R2 > 0.99 in both cases)]. Valeryl D-DIBOA also provoked a significant effect on germination rate (IC50 = 580 µM, R2 = 0.9374), maintaining significant activity values at all concentrations when D-DIBOA was found to be active only at the most concentrated treatment.

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It is interesting to point out the effects of these chemicals on wheat. The IC50 (root length) for the commercial herbicide Logran was 7268 µM (R2 = 0.9410) and 7026 µM for D-DIBOA (R2 = 0.9798). They are much higher than the highest tested dose, so they were obtained by extrapolation. All IC50 values for D-DIBOA esters are >1 mM, the maximum tested dose. A detailed analysis of the dose–response curves for Pr-D-DIBOA on A. fatua and wheat (root length) revealed a highly relevant result from the point of view of the selectivity (see below).

Lipophilicity–Activity Correlations. Taking into consideration effects on all parameters and species, a cluster analysis was made to classify the tested chemicals according to their phytotoxicity and properties. The results are shown in Figure 4. Two main groups of compounds can be discerned according to their phytotoxicity profiles: G1, in which the commercial herbicide Logran, D-DIBOA, and their esters from acetyl to heptanoyl are included, and G2, containing D-DIBOA esters from octanoyl to miristoyl. G1 is formed by the most phytotoxic chemicals on most of the tested species. This result clearly agrees with the proposed dependence of phytotoxicity with cLog P, as the chemicals fitting the proposed optimal cLog P interval are grouped as the most active ones, except the octanoyl derivative, which is still active in some cases (IC50 = 316.8
microM, \( R^2 = 0.9570 \), A. fatua, root length), but with a phytotoxicity profile much closer to the lipophilic side of the series. Those differences are very clearly displayed in several cases: germination rate on A. fatua and germination and root and shoot lengths on L. sativa and L. esculentum (all parameters, but on root length especially). This loss of phytotoxicity with lipophilicity could agree with the Lipinski and Tice models, as compounds with lipophilicities higher than that of octanoyl-D-DIBOA (cLog \( P \) 3.97) do not fit the optimal requirements for phytotoxicity (Table 2). This fact makes difficult a mathematical correlation of cLog \( P \) values with phytotoxicity results, at least if the most lipophilic chemicals are included. These kinds of correlations have been made recently though, yielding a lipophilicity-activity relationship and an effect on activity related to the even or odd character of side chains as the most interesting results (42). In that paper, the authors employ the wheat coleoptile bioassay as a phytotoxicity prediction, finding good phytotoxicity-Lipinski cLog \( P \) correlations. They also detect some influence on the activity, depending on the odd or even nature of the ester. Herein we present a similar correlation made by means of the results found for a whole plant, A. fatua, which was the tested weed in which higher phytotoxicity (21, 22) for benzoxazinones was found, seemed to be adequate for this study. The phytotoxicity [expressed as log(1/IC\(_{50}\))] was correlated with cLog \( P \) as shown in Figure 5. Two zones can be clearly separated: the lower cLog \( P \) zone (fitting the Lipinski cLog \( P \) requirements for optimal bioactivity), in which some correlations can be made, and the zone of higher cLog \( P \) values, in which the phytotoxicity...
effect of the chemicals could not be adjusted to a parabolic equation, as Hansch’s transport model proposes (37).

The phytotoxicity data for the lower cLog \( P \) zone did not fit the quadratic model correctly when treated all together, but the separation of even and odd side chains provided a couple of excellent fittings. This effect was previously found and discussed (42), so that a relationship with the capacity of the esters’ side chains to interact with the cell membrane lipidic bilayer was proposed to be the cause for this phenomenon. Even and odd side chains reasonably could behave differently, on the basis of the side-chain lipidic bilayer van der Waals interactions that occur. In this case, as in the previous paper, odd esters are more active than the even ones. This could be a result of a stronger van der Waals interaction with the lipidic bilayer of the even ones, resulting in a low access to the corresponding target site(s) of action. Thus, a good permeability through the lipidic bilayer (measured in terms of Log \( P \)), but weak interactions with it, favor the phytotoxic effect. Logically, the closer the side chains are to the cell membrane fatty acid length (and lipophilicity), the better the interaction, resulting in a retention of the phytotoxins and the subsequent lower effects. The tendency of both even and odd series helps to explain the disposition of butyric ester in the cluster analysis (Figure 4), which seems not to be ordered. As can be seen, IC\(_{50}\) values of Bu-D-DIBOA are very close to the ones for hexanoyl, heptanoyl, and also octanoyl D-DIBOA, located in the cLog \( P \) region in which the distance from one curve to another is narrower. This puts Bu-D-DIBOA in the middle of both clusters. In addition to this, the even–odd effect is also observed in the cluster analysis, if compounds other than the butyric ester are grouped following this behavior in the G1 group. The most lipophilic side of the series, in which the transport model has no application (G2), does not follow this pattern. The QSAR correlations of even and odd esters separately resulted in the following equations:

odd esters

\[
\log(1/\text{IC}_{50}) = 0.151(\text{cLog} \ P)^2 - 1.1685(\text{cLog} \ P) + 3.0612, \\
R^2 = 0.9929
\]

even esters

\[
\log(1/\text{IC}_{50}) = 0.138(\text{cLog} \ P)^2 - 1.0626(\text{cLog} \ P) + 2.473, \\
R^2 = 0.9640
\]

Figure 6. Phytotoxic effects of 6-MeO-D-DIBOA esters on A. fatua L. (root and shoot lengths). 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 \( 0.01 < P < 0.05 \).

Lipophilicity is not the only molecular parameter modified by the systematic esterification of D-DIBOA. The increasing
ester length provides a larger number of rotatable bonds, whereas a new H-bond acceptor (carboxyl group) is added and a H-bond donor (hydroxylic proton of the hydroxamic acid moiety) is lost. The numbers of H-bond donors and acceptors, which are relevant in terms of H-bond interaction of the phytotoxin with the target site of action, remains within the model limits throughout the series. The increasing number of rotatable bonds lowers the conformational stability of the molecule, making it difficult to fit the target site of action. Nevertheless, some conformational flexibility is needed for this purpose, as compounds with 12 or fewer rotatable bonds fit the rule. Thus, the ester derivatives of D-DIBOA provide conformational flexibility to the molecule (which lacks rotatable bonds) in addition to lipophilicity. All of the tested chemicals fit the model in terms of molecular weight.

6-Methoxy-D-DIBOA Esters. Once the lipophilicity–phytotoxicity relationships have been determined, and in the context of Hansch’s transport theory (37), in which bioactivity is described as a function of transport phenomena and “intrinsic” bioactivity, it would be interesting to discover if the phytotoxic effects observed for the D-DIBOA esters are provoked for a higher bioavailability of the active part of the molecule, or the ‘intrinsic’ phytotoxicity of the fatty acid residues attached to D-DIBOA could have. If side chains were responsible for those phytotoxicity values, a change in the “active” part of the molecule would not significantly affect the phytotoxicity. 6-Methoxy-D-DIBOA (Figure 1), a benzoxazinone derivative with phytotoxicity values very similar to those found for D-DIBOA (see Supporting Information), is examined through the same methodology as D-DIBOA on A. fatua. Phytotoxicity results are shown on Figure 6 (growth parameters). The zone of the series in which the maximum root length effects are obtained is slightly displaced to the lipophilic side in comparison to the D-DIBOA series (which could be caused by the higher aqueous solubility of the 6-methoxy derivative, with cLog P = 0.32). Neither acetyl nor propanoyl derivatives affected root length significantly. Moreover, shoot length is the most affected parameter instead of root length. The phytotoxic effect of the more lipophilic compounds could be due to their accumulation in the seed surface during the experiment, avoiding the seed water uptake (as could happen with Mir-D-DIBOA), rather than a “true” phytotoxic effect in which the active chemical reaches the corresponding molecular target site. Thus, 6-methoxy-D-DIBOA derivatives modulate the transport phenomena for this compound, enhancing the phytotoxic effect in the case of A. fatua shoot length, but in the context of the intrinsic activity of this chemical, in which the methylation of the aromatic ring makes it less active, as in the case of the previously reported 7-methoxy derivative (20, 22).

Selective Action of D-DIBOA Esters: Pr-D-DIBOA. Pr-D-DIBOA was the most active tested chemical on A. fatua root length (IC50 = 27.8 μM, R2 = 0.9772). The introduction of an ester function in D-DIBOA provokes a modulation of the transport phenomena, resulting in an increase or decrease of phytotoxicity. This effect could be different, depending on the tested plant, as their morphological structures, then the barriers the chemical needs to cross to reach the target site of action, could be different.

One of the determinant parameters in the optimization of herbicides is the selectivity: A herbicidal treatment, when made to a crop, involves necessarily a loss in the crop production due to the phytotoxicity of the treatment. Thus, it is necessary to compare the effects that a herbicide candidate can produce on crops and their common weeds. As A. fatua is a common
The systematic esterification of D-DIBOA was effective for the purpose of studying the influence of transport phenomena in the phytotoxic effect of this chemical, a useful lead for natural herbicide model development (20–22). These derivatives displayed a modulated phytotoxicity, which could be related to their interactions with cell membrane lipidic bilayers, and the subsequent capability of reaching the D-DIBOA target site of action. Propanoyl and valeryl derivatives resulted as the most interesting ones from the point of view of their effects, establishing the optimal $\text{clog}_P$ in the range from 1.65 to 2.58. These lipophilic D-DIBOA derivatives could constitute a second generation of leads for natural herbicide development (43), in which lipophilicity, a determinant parameter in drug and agrochemical design (30–31), is optimized. Pr-D-DIBOA also possesses a much higher selectivity of action than D-DIBOA, being then potentially able to control a common wheat weed (A. fatua) population without significantly affecting the crop. This is the first time in which this kind of analysis is reported with allochemical derivatives, by using whole plants as target sites. This work also sheds light on the usefulness of QSAR analysis, more likely applied in drug design, to the development of phytotoxins useful in the search for new herbicide models.

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Supporting Information Available: Physical data for all tested chemicals (FTIR, $\text{H}$ NMR, $\text{13C}$ NMR, EIMS); phytotoxicity data for D-DIBOA series chemicals on all species; and phytotoxicity data for 6-MeO-D-DIBOA series on A. fatua. This material is available free of charge via the Internet at http://pubs.acs.org.

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