



First approach to enhance the physicochemical properties of 2-amino-3*H*-phenoxazin-3-one (APO)

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ABSTRACT

Benzoxazinones and their degradation products are naturally occurring allelochemicals that are present in wheat, corn, rye, other members of the Gramineae, and some non-Gramineae species. 2,4-Dihydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (DIBOA) is the major hydroxamic acid in *Secale cereal* L. This compound is unstable in solution and soils and is transformed into 2-benzoxazolinone (BOA) and other degradation products. Further degradation of this compound yields the tricyclic structures 2-amino-3*H*-phenoxazin-3-one (APO). The synthesis of a wide variety of compounds with an aminophenoxazine skeleton has been studied extensively, mainly due to their pharmaceutical interest rather than for their agronomical use. In this sense, we have prepared a new generation of chemicals that includes eighteen derivatives with the aminophenoxazine skeleton with the aim of improving their phytotoxicity. These compounds were tested in a wheat coleoptile bioassay and with selected standard target species (STS) in the search for the optimal levels for phytotoxicity.

Keywords: Allelochemicals, Aminophenoxazines, Benzoxazinoids, 2-Benzoxazolinone, SAR.

Introduction

In recent years the scientific interest in selective biological weed management has increased significantly (Niemeyer, 1988; Einhelig, 1995). Allelopathy could be an alternative approach to solve this problem and allelochemicals are considered to be promising new resources for agricultural pest management. Moreover, allelochemicals potentially have more specific interactions with target organisms and they may be less environmentally damaging, features that make them attractive agrochemicals (Rimando Agnes and Duke Stephen, 2006). In the past, research into allelochemicals has been mainly focused on their isolation from natural sources, their structural characterization, and the evaluation of their phytotoxic bioactivity in germination and growth bioassays as the final result of bioguided isolation procedures (Vyvyan, 2002). In addition, these compounds may have novel or multiple modes of action, a possibility that could further enhance their effectiveness (Duke et al., 2000).

Benzoxazinones and their degradation products are naturally occurring allelochemicals that are present in wheat, corn, rye, other members of the Gramineae (Niemeyer, 1988) and insome non-

Gramineae species (Pratt et al., 1995; Baumeler et al., 2000). Several types of bioactivity have been described and evaluated for these compounds, including fungistatic (Bravo and Lazo, 1996; Friebe et al., 1998), antifeedant (Klun et al., 1967; Argandoña et al., 1983), and phytotoxic (Barnes and Putnam, 1987; Perez and Ormeno-Nunez, 1991).

2,4-Dihydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (DIBOA) is the major hydroxamic acid in *Secale cereal* L. (Honkanen and Virtanen, 1960). This compound can be detected within the plants as the β -D-glucosides. Upon plant injury the glucoside is enzymatically converted into DIBOA by a β -glucosidase. This aglycone is unstable in solution and soil and is transformed to 2-benzoxazolinone (BOA) and other degradation products. Further degradation of this compound yields the tricyclic structure 2-amino-3*H*-phenoxazin-3-one (APO) (Fomsgaard et al., 2004; Macias et al., 2005b). This metabolite has been reported to be more phytotoxic than 2-benzoxazolinone (BOA) (Gagliardo and Chilton, 1992; Friebe et al., 1996). Transformations in environments such as aqueous solutions, crop soils and microbial cultures have been widely studied (Gagliardo and Chilton, 1992; Zikmundova et al., 2002; Macias et al., 2005b).

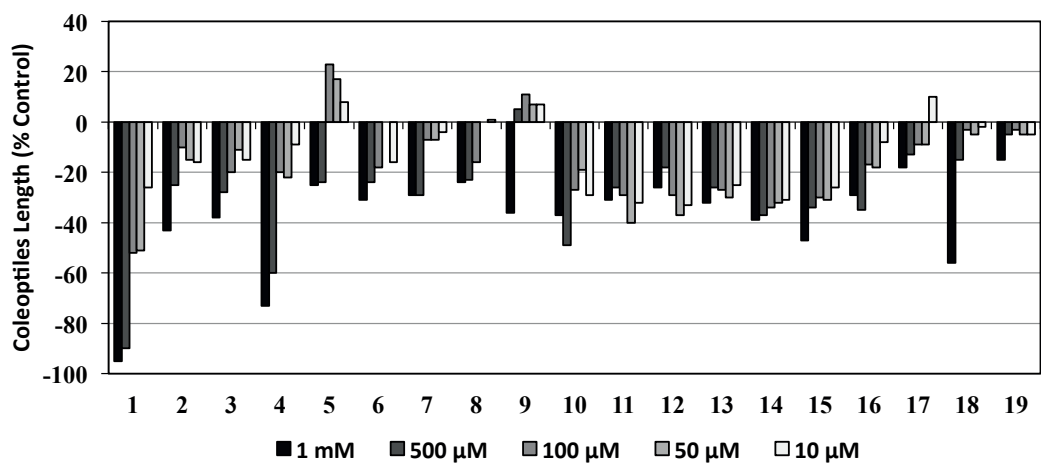


Figure 1. Effects of the assayed compounds in the wheat coleoptile bioassay.

APO displays a wide variety of interesting properties – especially with regard to its phytotoxic activity and the influence that this has on the allelopathic phenomena observed for Poaceae plants. Previously, we evaluated the phytotoxicity of benzoxazinones and related compounds (Tables 1 and 2) on STS and weeds in the search for new compounds that could be applied as part of a strategy directed towards weed control in affected crops (Macias et al., 2005a; 2006). Statistical treatment of the acquired data would enable structure-activity relationships to be highlighted, and these could be useful in the further development of highly phytotoxic compounds based on natural product structures. The results of these studies indicated that 2-amino-3*H*-phenoxazin-3-one (APO) seems to be an optimal candidate for the development of further natural herbicide models for weed management.

The synthesis of a wide variety of compounds with an aminophenoxazine skeleton has been carried out, mainly due to the pharmaceutical interest in these compounds (Tomoda et al., 2011; Takemura et al., 2012) rather than for their agronomical use (Fomsgaard et al., 2004). All of the synthetic methodologies employed to date involve the dimerization of two phenol units to access the tricyclic structure (Kehrmann, 1906; Buckley et al., 1982; Szeverenyi et al., 1991) and they mainly concern the synthesis of 2-amino-3*H*-phenoxacin-3-one (APO).

As part of our ongoing research into the structure-activity relationships of benzoxazinones and their degradation products, a new generation of chemicals that includes eighteen derivatives with the aminophenoxazine skeleton was developed in an effort to enhance the phytotoxicity and aqueous solubility. These compounds were tested in the wheat coleoptile

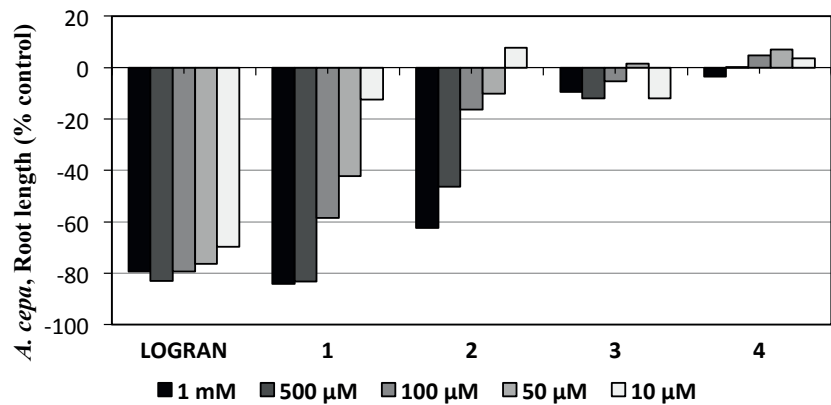


Figure 2. Phytotoxic effects of tested halogenated compounds.

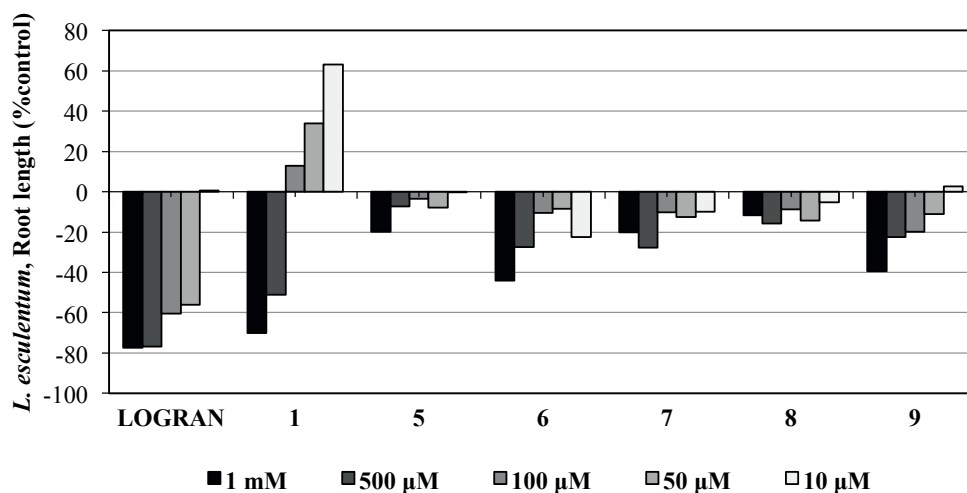


Figure 3. Phytotoxic effects of tested alkenoxy compounds on the root length of the species *Lycopersicon esculentum* W.

bioassay and with selected standard target species (STS) in the search for the optimal aqueous solubility/lipophilicity levels to obtain phytotoxicity. The lipophilicity was increased by incorporating alkyl and alkene groups into the structure. Moreover, an improvement in the aminophenoxazine skeleton by modification of the steric and electronic features was also carried out. These modifications included the addition of halogen substituents to the tricyclic structure.

Materials and methods:

General methods

The purities of the isolated and synthetic compounds were determined by NMR and HPLC analyses and were found to be >98%. ^1H and ^{13}C NMR spectra were recorded using CD_3SOCD_3 as solvent on

a Varian INOVA spectrometer at 399.99 and 100.577 MHz, respectively. The resonance of residual dimethyl sulfoxide (DMSO) was set to δ 2.49. The solvent peak for ^{13}C was set to δ 39.5 as an internal reference. Mass spectra were recorded on a WATERS SYNAPT G2 spectrometer using methanol as solvent. FTIR spectra were obtained on a BX Perkin-Elmer FTIR spectrophotometer. Frequency values are given in cm^{-1} .

Chemicals

The chemicals employed for this study are listed in Table 3. Eighteen derivatives were synthesized and these included aromatic ring modifications at positions C-4, C-6 and C-8 plus the addition of side chains at position C-7 of the aminophenoxazine skeleton. The spectroscopic data are shown in Tables 4, 5 and 6.

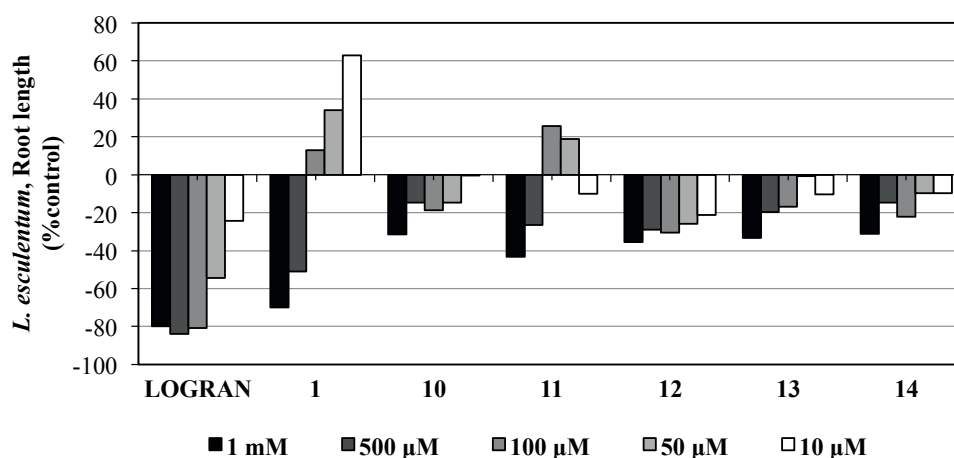


Figure 4. Phytotoxic effects of tested alkyloxy compounds on the root length of the species *Lycopersicon esculentum* W.

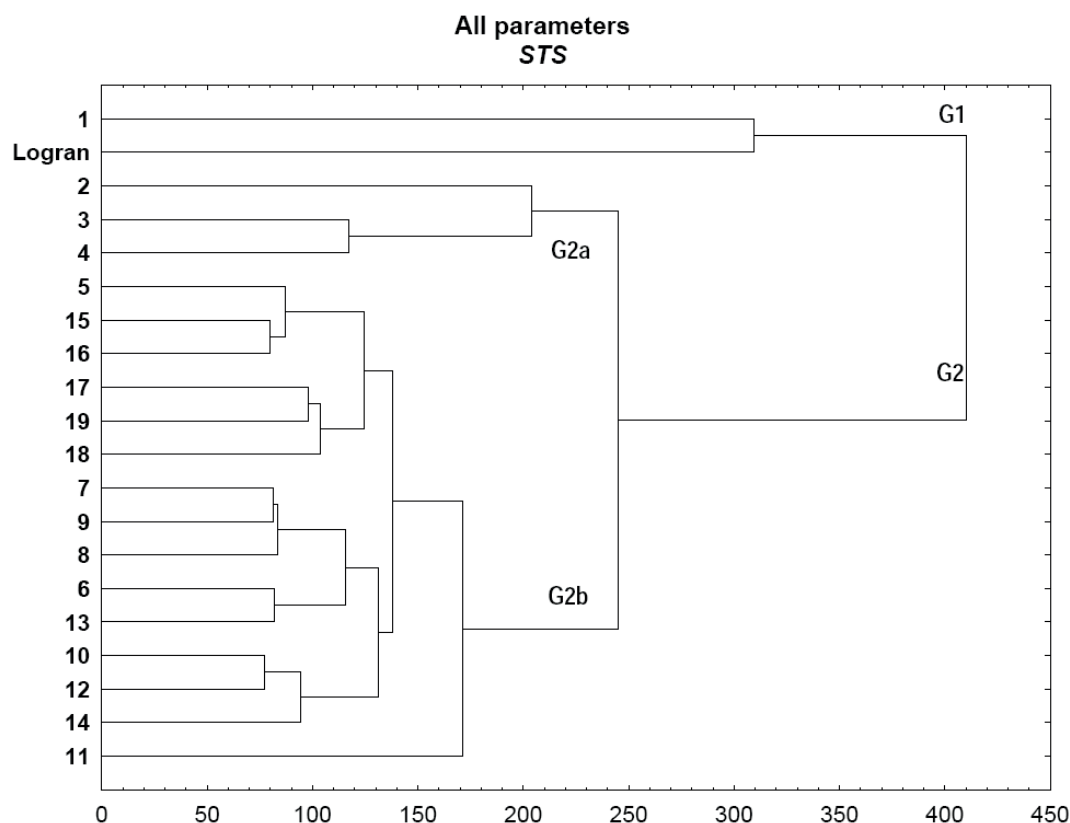


Figure 5. Cluster analysis (phytotoxicity data, germination, and growth parameters, all tested species) of the tested chemicals.

General synthesis procedures

Preparation of 2-amino-3H-phenoxazin-3-one (1). This compound was synthesized according to the literature procedure (Gagliardo and Chilton, 1992).

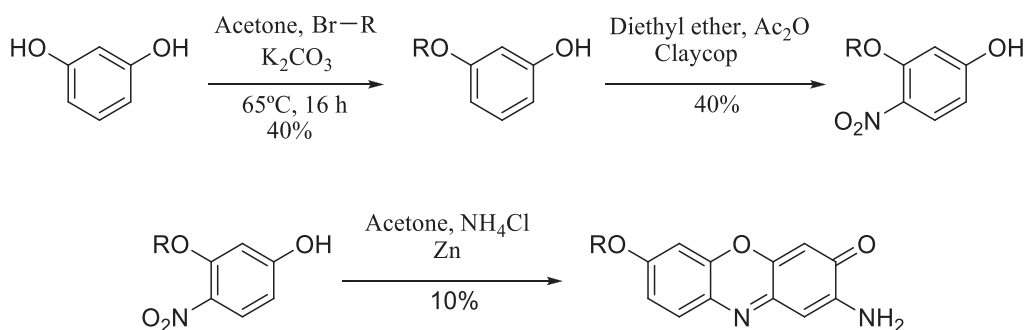
C-7 derivatives. The C-7 derivatives were obtained in a three-step sequence: nucleophilic substitution (Moreno-Manas et al., 2001), nitration employing claycop as the nitrating agent (Dwyer and Holzapfel, 1998) and dimerization to achieve aminophenoxazine ring formation (Scheme 1).

General procedure for nucleophilic substitution: resorcinol (15.1 mmol) was dissolved in acetone. The reaction mixture was stirred under argon at room temperature. The mixture was then heated and K_2CO_3 (23.1 mmol) was added. When the temperature was 68 °C the allyl bromide/iodide/chloride was added. The reaction mixture was stirred at 68 °C for 16 h. After this time the reaction mixture was quenched. The solvent was removed under reduced pressure. The crude product was redissolved in Sørensen buffer and was treated with HCl_{aq} . The product was extracted with dichloromethane (4 × 100 mL). The organic layers were combined and

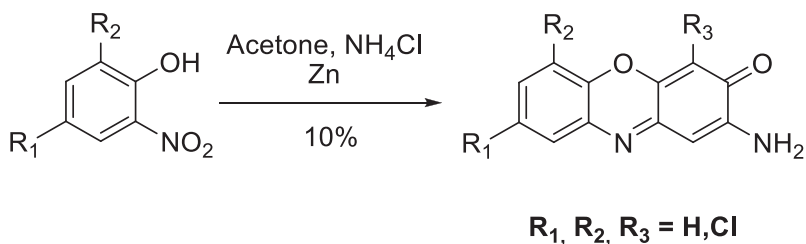
dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure. The product was purified by chromatography (CC, ethyl acetate/hexane).

General procedure for nitration: 3-allylphenol (5 mmol) was dissolved in diethyl ether (25 mL) and acetic anhydride (50 mmol) and the reaction mixture was stirred. Claycop (2.5 g) was added and the reaction mixture was stirred for 2 h. Once the reaction was complete, the suspension was filtered through Celite to remove the claycop and the solvent was removed under reduced pressure. The crude product was purified by chromatography (CC, ethyl acetate/hexane) to give the corresponding derivatives.

General procedure for aminophenoxazine ring formation: 5-allyl-2-nitrophenol (1 mmol) was dissolved in acetone (4.5 mL), and saturated aqueous ammonium chloride (6 mL) and zinc metal (4 mmol) were added. The reaction mixture was stirred for 5 min under ultrasound until the reaction was completed. The evolution of the reaction was monitored by TLC. Once the reaction was complete, the suspension was filtered to remove the zinc metal and the solvent was removed under reduced pressure.



Scheme 1



Scheme 2

The crude product was dissolved in acetone and the solution was allowed to stand at room temperature during 7 days. A brown precipitate was obtained. The suspension was filtered to obtain the corresponding pure derivatives.

Chlorinated derivatives. The chemicals were obtained in one step, as shown in Scheme 2. 4-, 6- or 4,6-(di)chloro-2-nitrophenol (1 mmol) was dissolved in acetone (4.5 mL) and saturated aqueous ammonium chloride (6 mL) and zinc metal (4 mmol) were added. The reaction mixture was stirred for 5 min under ultrasound until the reaction was complete. The evolution of the reaction was monitored by TLC. Once the reaction was complete, the suspension was filtered to remove the zinc metal and the solvent was removed under reduced pressure. The crude product was dissolved in acetone and the solution was allowed to stand at room temperature for 7 d. A brown precipitate was formed. The suspension was filtered to obtain the corresponding pure derivatives.

Calculation of IC_{50}

The phytotoxicity data were fitted to a sigmoidal dose-response model (constant slope) using the Graphpad prism v.4.00 software package (Graphpad, 2003).

General Activity Bioassays

Wheat coleoptile bioassays. Wheat seeds (*Triticum aestivum* L. cv. Catervo) were sown in 15 cm diameter Petri dishes moistened with water (15 mL) and grown in the dark at 22 ± 1 °C for 3 days (Hancock et al., 1964). The roots and caryopses were removed from the shoots and placed in a *Van der Weij* guillotine. The apical 2 mm were cut off and discarded. The next 4 mm of the coleoptiles were removed and used for the bioassay. All manipulations were performed under a green safelight (Nitsch and Nitsch, 1956). Test solutions of each compound were prepared in a phosphate-citrate buffer solution containing 2% sucrose adjusted to pH 5.6. Parallel controls were also run. Five coleoptiles were placed in each test tube containing 2 mL of test or control solutions (three replicates per dilution). The test tubes were kept in constant rotation at 0.25 rpm in a roller tube apparatus for 24 h at 22 °C in the dark. The coleoptiles were then digitally photographed and measured. Data were statistically analyzed using Welch's test (Martín Andrés and Luna del Castillo, 1990). Results are presented as percentage differences from control. Thus, zero represents the control, positive values represent growth stimulation and negative values represent growth inhibition.

Table 1. Natural benzoxazinones and synthetic derivatives mentioned in this study.

R1c1ccc2c(c1)oc(R2)c(=O)n2R3

	R ₁	R ₂	R ₃	Acronym
Natural Allelochemicals (A)	H	O-β-D-Glc	OH	DIBOA-Glc
	H	OH	OH	DIBOA
	OCH ₃	OH	OH	DIMBOA
Degradation Products (B)	H	OH	H	HBOA
	OCH ₃	OH	H	HMBOA
Synthetic analogs (C)	H	H	H	D-HBOA
	OCH ₃	H	H	D-HMBOA
	H	H	OH	D-DIBOA
	OCH ₃	H	OH	D-DIMBOA
	H	H	OAc	ABOA
	OCH ₃	H	OAc	AMBOA

Phytotoxicity bioassays

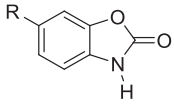
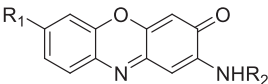
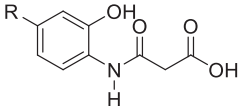
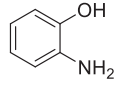
Target Plants. The selection of target plants was based on an optimization process carried out by us in the search for a standard phytotoxicity evaluation bioassay (Macias et al., 2000). This process led to several standard target species (STS) being proposed, including the monocot *Allium cepa* L. (onion) and dicots *Lycopersicon esculentum* Will. (tomato), *Lepidium sativum* L. (cress) and *Lactuca sativa* L. (lettuce), which were assayed for this study.

Methodology. Bioassays were carried out using 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 using 10⁻² M 2-(N-morpholino)ethanesulfonic acid (MES) and the 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 dimethylsulfoxide (DMSO) and then diluted with buffer (5 µL DMSO mL⁻¹ buffer) to give the test concentrations for each compound (1000, 500, 100, 50, 10, 5, 1 µM). This procedure facilitated the solubility of the assayed compounds. Twenty-five seeds were placed in each Petri dish. The treatment control (or internal reference solution) (5 mL) was added to each Petri dish. Four replicates were used for each species.

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 and tomato, and 7 days for onion. After growth, plants were frozen at -10 °C for 24 h to avoid subsequent

Table 2. Benzoxazinone degradation products mentioned in this study.

Benzoxazolinones	R		Acronym
	H		BOA
	OCH ₃		MBOA
Aminophenoxazines	R ₁	R ₂	Acronym
	H	H	APO
	OCH ₃	H	AMPO
	H	OAc	AAPO
	OCH ₃	OAc	AAMPO
	OH	H	AHPO
Malonamic acids	R		Acronym
	H		HPMA
	OCH ₃		HMPMA
Miscellaneous	Acronym		
	APH		

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)aminocarbonyl]benzenesulfonamide (triasulfuron, 0.6%), was used as the internal reference in accordance with a comparison study reported previously (Macias et al., 2000).

Bioassay data acquisition. The evaluated parameters (germination rate, root length, and shoot length) were recorded using a Fitomed system (Castellano et al., 1999), which allowed automatic data acquisition and statistical analysis by its associated software.

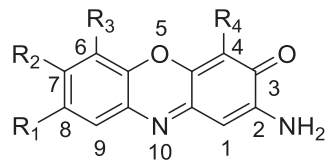
Statistical analysis. Data were statistically analyzed using Welch's test, with significance fixed at 0.01 and 0.05. Results are represented in bar charts in which the zero value represents control, negative values represent inhibition, and positive values represent stimulation of the studied parameter (Macias et al., 2000). 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 behavior and associate the properties with the molecular structure. Complete linkage was used as an amalgamation rule and the distance measurement was based on squared Euclidean distances, 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 using Statistica v.5.0 software (Statsoft Inc.). Germination rate, shoot length, and root length effects for all tested species were included in the analysis to provide 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,

Table 3. Derivatives prepared.



R ₁	R ₂	R ₃	R ₄	COMPUESTO	
H	H	H	H	2-amino-3H-phenoxazin-3-one	1
Cl	H	H	H	2-amino-8-chloro-3H-phenoxazin-3-one	2
H	H	Cl	Cl	2-amino-4,6-dichloro-3H-phenoxazin-3-one	3
Cl	H	Cl	Cl	2-amino-4,6,8-trichloro-3H-phenoxazin-3-one	4
H	-O-CH ₂ -CH ₂ =CH ₂	H	H	7-allyloxy-2-amino-3H-phenoxazin-3-one	5
H	-O-(CH ₂) ₃ =CH ₂	H	H	2-amino-7- butenyloxy-3H-phenoxazin-3-one	6
H	-O-CH ₂ -CH ₂ =CH ₂ -CH ₃	H	H	2-amino-7-crotyloxy-3H-phenoxazin-3-one	7
H	-O-CH ₂ -CH=CH(CH ₃) ₂	H	H	2-amino-7-dimethylallyloxy-3H-phenoxazin-3-one	8
H	-O-CH ₂ -CH(CH ₃)=CH ₂	H	H	2-amino-7-methylallyloxy-3H-phenoxazin-3-one	9
H	-O-CH ₃	H	H	2-amino-7-methoxy-3H-phenoxazin-3-one	10
H	-O-CH ₂ -CH ₃	H	H	2-amino-7-ethoxy-3H-phenoxazin-3-one	11
H	-O-CH ₂ -CH ₂ -CH ₃	H	H	2-amino-7-propoxy-3H-phenoxazin-3-one	12
H	-O-(CH ₂) ₃ -CH ₃	H	H	2-amino-7-butoxy-3H-phenoxazin-3-one	13
H	-O-(CH ₂) ₄ -CH ₃	H	H	2-amino-7-pentyloxy-3H-phenoxazin-3-one	14
H	-O-(CH ₂) ₅ -CH ₃	H	H	2-amino-7-hexyloxy-3H-phenoxazin-3-one	15
H	-O-(CH ₂) ₆ -CH ₃	H	H	2-amino-7-heptyloxy-3H-phenoxazin-3-one	16
H	-O-(CH ₂) ₇ -CH ₃	H	H	2-amino-7-octyloxy-3H-phenoxazin-3-one	17
H	-O-(CH ₂) ₈ -CH ₃	H	H	2-amino-7-nonyloxy-3H-phenoxazin-3-one	18
H	-O-(CH ₂) ₉ -CH ₃	H	H	2-amino-7-decyloxy-3H-phenoxazin-3-one	19

defined by the equation

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

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 the determination coefficient (r²). The adjustment and r² were obtained using GraphPad Prism software v.4.00 (Graphpad, 2003).

Results and discussion

General Bioactivity Profiles

The effects of the assayed compounds on wheat coleoptiles are shown in Figure 1. All of the compounds showed inhibitory profiles for the parameter evaluated, but in general the substitutions performed on APO (1) did not improve the activity. The most

active compound was 4, which showed inhibition values of around 70–75% at a concentration of 1 mM. Compounds 14 and 15 also showed good inhibitory profiles.

Phytotoxic bioactivity profiles

All of the compounds that were active showed inhibitory profiles for all evaluated parameters. In general terms, growth parameters (root and shoot lengths) were more markedly affected than germination rates. Among the growth parameters, root growth was more affected than shoot length, as one would expect for aminophenoxazine derivatives. In general terms, these compounds had a less pronounced phytotoxicity in comparison to 2-amino-3H-phenoxazin-3-one (1). None of the species were significantly affected by the tested chemicals. There was a general decrease in the phytotoxic effects of these compounds in comparison to 2-amino-3H-phenoxazin-3-one (1).

Halogenated derivatives. Among the Standard Target

Table 4. ¹H NMR chemical shifts (400 MHz, signal of residual solvent centered at δ 2.49 ppm for DMSO).

H	2	3	4	5	6	7	8	9	10	11
1	6.36 s	6.41 s	6.98 s	6.34 s	6.41 s	6.47 s	6.35 s	6.34 s	6.35 s	6.35 s
4	6.34 s	--	--	6.32 s	6.37 s	6.40 s	6.32 s	6.32 s	6.32 s	6.33 s
6	7.52 d	--	--	7.11 d	7.16 d	6.87 d	7.10 d	7.10 d	7.11 d	7.10 d
7	7.46 dd	7.70 dd	8.15 d	--	--	--	--	--	--	--
8	--	7.42 t	--	7.02 dd	7.05 dd	6.95 dd	6.99 dd	7.02 dd	7.01 dd	7.01 dd
9	7.73 d	7.64 dd	8.11 d	7.63 d	7.68 d	7.65 d	7.62 d	7.62 d	7.64 d	7.64 d
1'	--	--	--	4.68 dt	4.20 t	4.54 d	4.63-4.58 m	4.59 s	3.85 s	4.14 q
2'	--	--	--	6.05 ddt	2.57 m	5.95-5.88 m	5.47-5.43 m	--	--	1.36 t
3'	--	--	--	5.29 dq 5.43 dq	5.95 ddt	5.95-5.88 m	--	5.07-4.94 m	--	--
4'	--	--	--	--	5.24 ddd	1.78 dd	1.74-1.71 m	--	--	--
5'	--	--	--	--	5.15 dd	--	1.74-1.71 m	1.77 s	--	--

* s: singlet, d: doublet, t: triplet, q: quartet, m: multiplet

Table 5. ¹H NMR chemical shifts (400 MHz, signal of residual solvent centered at δ 2.49 ppm for DMSO).

H	12	13	14	15	16	17	18	19
1	6.36 s	6.34 s	6.35 s	6.45 s	6.45 s	6.45 s	6.46 s	6.46 s
4	6.33 s	6.31 s	6.33 s	6.38 s	6.38 s	6.38 s	6.39 s	6.39 s
6	7.10 d	7.10 d	7.10 d	6.84 d	6.83 d	6.84 d	6.84 d	6.84 d
8	7.01 dd	6.99 dd	7.0 dd	6.92 dd	6.92 dd	6.92 dd	6.93 dd	6.93 dd
9	7.63 d	7.62 d	7.63 d	7.63 d	7.63 d	7.63 d	7.64 d	7.64 d
1'	4.05 t	4.07 t	4.07 t	4.02 t	4.01 t	4.02 t	4.02 t	4.02 t
2'	1.76 tq	1.70 tt	1.74 tt	1.82 tt	1.81 tt	1.81 tt	1.81 tt	1.81 tt
3'	0.99 t	1.43 m	1.38 m	1.46 tt	1.46 tt	1.46 tt	1.46 tt	1.46 tt
4'	--	0.93 t	1.38 m	1.37-1.32 m	1.36-1.31 m	1.38-1.23 m	1.37-1.24 m	1.37-1.24 m
5'	--	--	0.9 t	1.37-1.32 m	1.36-1.31 m	1.38-1.23 m	1.37-1.24 m	1.37-1.24 m
6'	--	--	--	0.9 t	1.36-1.31 m	1.38-1.23 m	1.37-1.24 m	1.37-1.24 m
7'	--	--	--	--	0.88 t	1.38-1.23 m	1.37-1.24 m	1.37-1.24 m
8'	--	--	--	--	--	0.88 t	1.37-1.24 m	1.37-1.24 m
9'	--	--	--	--	--	--	0.87 t	1.37-1.24 m
10'	--	--	--	--	--	--	--	0.87 t

* s: singlet, d: doublet, t: triplet, q: quartet, m: multiplet

Table 6. ¹³C NMR chemical shifts (400 MHz, signal of residual solvent centered at δ 39.5 ppm for DMSO).

C	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	103.7	104.2	104.6	103.3	103.2	104.0	103.2	103.3	103.3	103.3	103.2	103.2	103.1	104.0	104.0	104.0	104.0	104.0
2	149.1	148.9	148.9	148.5	184.5	160.2	148.6	148.6	148.6	148.7	148.6	148.6	148.3	149.2	149.2	149.2	149.2	149.3
3	180.2	176.0	177.4	179.8	179.8	207.0	179.8	179.8	179.8	179.9	179.8	179.8	179.8	184.0	180.1	180.0	180.0	180.1
4	98.0	113.5	113.0	98.8	98.8	100.6	98.8	98.8	98.8	98.9	98.8	98.8	98.5	100.2	100.2	100.2	100.2	100.2
4a	147.8	150.1	149.7	145.7	145.6	145.1	143.2	145.7	145.7	145.6	145.5	145.6	145.6	145.1	145.1	145.1	145.1	145.1
5a	143.2	135.0	139.3	143.1	143.2	144.1	138.0	143.1	143.2	143.3	143.2	143.2	143.2	144.1	144.1	144.1	144.1	144.2
6	115.2	119.8	126.2	100.8	100.5	101.3	100.6	100.8	100.0	100.4	100.4	100.4	100.4	101.3	101.3	101.3	101.3	101.3
7	122.8	128.9	128.2	158.8	159.2	180.1	159.2	158.9	160.0	159.3	159.4	159.4	159.2	160.7	160.7	160.7	160.7	160.7
8	126.6	126.9	127.0	113.9	113.8	114.2	113.9	112.8	113.4	113.8	113.8	113.8	114.0	114.1	114.1	114.1	114.1	114.1
9	117.6	125.7	123.6	128.4	128.3	122.5	120.7	128.4	128.4	128.3	128.3	128.3	128.2	128.7	128.7	128.7	128.7	128.7
9a	140.8	137.7	137.7	129.0	129.0	129.7	128.3	128.9	129.0	129.1	129.0	129.0	129.0	129.6	129.6	129.6	129.6	129.6
10a	148.8	147.2	149.3	146.6	146.5	149.2	146.5	146.6	146.6	146.6	146.5	146.5	146.5	145.8	145.8	145.8	145.8	145.8
1'	--	--	--	69.0	67.5	69.5	65.3	71.6	56.0	64.2	69.8	68.1	68.2	21.5	100.2	68.9	68.9	68.9
2'	--	--	--	133.0	32.8	131.6	119.2	140.3	--	14.5	21.8	30.6	28.0	29.0	31.7	29.3	29.0	29.0
3'	--	--	--	118.1	134.6	125.0	128.9	113.9	--	--	10.3	18.7	27.4	25.6	29.0	26.0	25.6	26.0
4'	--	--	--	--	117.2	17.9	25.4	--	--	--	--	13.6	21.8	22.6	29.0	29.2	29.2	29.3
5'	--	--	--	--	--	--	18.1	19.1	--	--	--	--	13.7	14.0	25.9	29.0	29.5	29.5
6'	--	--	--	--	--	--	--	--	--	--	--	--	--	0.9	22.6	31.8	29.3	29.5
7'	--	--	--	--	--	--	--	--	--	--	--	--	--	--	14.1	22.6	31.8	29.3
8'	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	14.1	22.6	31.9
9'	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	14.1	22.6
10'	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	14.1

Species, onion was the species that was affected the most, whereas lettuce and tomato were the most tolerant plants to the tested treatments. The results obtained indicate that the most active compound was 2-amino-8-chloro-3*H*-phenoxazin-3-one (**2**) on the root length of *Allium cepa* L (Figure 2).

Derivatives with an alkene group at C-7. The compounds considered in this section are 2-amino-7-butenyloxy-3*H*-phenoxazin-3-one (**6**) and 2-amino-7-methylallyloxy-3*H*-phenoxazin-3-one (**9**). Both chemicals caused inhibitions of 40% at 1000 μM for root length in *L. esculentum* W. (Figure 3).

Derivatives with an alkyl group at C-7. As in the case described above, only a slight effect of these compounds on the Standard Target Species was observed for the alkyloxy derivatives. The incorporation of an ether function did not lead to significant increases in the phytotoxicity in comparison to the lead compound. It can be pointed out that the most active compound was 2-amino-7-propoxy-3*H*-phenoxazin-3-one (**12**), which caused an inhibition of 40% at 1000 μM for root length of *Lycopersicon esculentum* L. (Figure 4).

Structure-Activity Relationship (SAR)

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. The results of this analysis are shown

in Figure 5. Cluster analysis of the data allowed us to classify the assayed compounds according to their phytotoxicity into two main groups: G1 and G2 (G1 for the highest activities and G2 for weaker effects). The most active group (G1) includes the compound APO (**1**) and the commercial herbicide Logran, which present the highest activities. Compounds with lower activity are included in G2 and this group includes all of the chemicals synthesized in this work. The chlorinated compounds (**2**, **3** and **4**) were the most active in this group.

Conclusions

The synthesis of derivatives of APO (**1**) has been carried out. Chains were added in the C-7 position with the aim of optimizing the solubility and lipophilicity of these compounds and modifications were also made on the rings by incorporating halogenated substituents in order to improve the stereoelectronic properties of the lead compound. Both the general and phytotoxic activities of these derivatives were evaluated for use in the rational development of model herbicides.

In this sense, the results obtained indicate that the most active compound was 2-amino-4,6,8-trichloro-3*H*-phenoxazin-3-one (**4**) on the root length of *Allium cepa* L. The work described here complements a previous study by examining the effects of further

modifications of the aminophenoxazine skeleton in the search for an accurate description of the chemical selectivity for action. These compounds constitute the first generation of compounds to be tested in this way and further modifications are required to enhance the bioactivity.

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