JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Novel Protoporphyrinogen Oxidase Inhibitors: 3*H*-Pyrazolo[3,4-d][1,2,3]triazin-4-one Derivatives

HUA-BIN LI, YOU-QUAN ZHU, XIAO-WEI SONG, FANG-ZHONG HU, BIN LIU, YONG-HONG LI, ZI-XIA NIU, PEI LIU, ZHI-HONG WANG, HAI-BIN SONG, XIAO-MAO ZOU,* AND HUA-ZHENG YANG*

State Key Laboratory of Elemento-organic Chemistry, Institute of Elemento-organic Chemistry, Nankai University, Tianjin 300071, People's Republic of China

A series of 3*H*-pyrazolo[3,4-d][1,2,3]triazin-4-one derivatives were synthesized as candidate herbicides by diazotization of different 5(3)-amino-*N*-phenyl-1*H*-pyrazole-4-carboxamide derivatives prepared by the reaction of substituted 5(3)-amino-pyrazole-4-carbonyl chloride with a substituted aniline. Their structures were identified by ^{1}H NMR and elemental analyses. The isomers **D** and **E** were isolated, and their structures were identified by two-dimensional NMR analyses (heteronuclear single quantum coherence and heteronuclear multiple-bond correlation) and single-crystal X-ray diffraction analysis. The bioassay results showed that some of the title compounds exhibited both excellent herbicidal activity at a dose of 93.75 g/ha and strong inhibition against protoporphyrinogen oxidase activity in vitro. The structure−activity relationship showed that **D16** possessed the highest activities both in vivo and in vitro when the N-substituted group of the pyrazole ring was allyl and the N-substituted group of benzooxazinone was propargyl.

KEYWORDS: 3*H*-Pyrazolo[3,4-d][1,2,3]triazin-4-one; PPO inhibitor; SAR; herbicidal activity

INTRODUCTION

The protoporphyrinogen oxidase (PPO, E.C. 1.3.3.4) is the last enzyme in the common tetrapyrrole biosynthesis pathway before the pathway branches toward chlorophyll and heme synthesis. The enzyme is the target of many classes of herbicides including diphenyl ether, cyclic imides, and thiadiazolidines. The application of PPO-inhibiting herbicides to plant leads to the peroxidative destruction of cellular membrane and bleaching of tissues in the presence of light. In contrast to other herbicides, PPO inhibitors have some general characteristics: (i) PPO inhibitors give long-lasting control for up to 30 days after the application. (ii) PPO inhibitors are effective on currently difficult to control weeds. (iii) PPO inhibitors are more rapid than many other herbicides, causing necrosis within 24 h and death in 2−5 days. (iv) PPO inhibitors in combination with other herbicides can provide one-shot weed control with a wide window of application. For these characteristics, PPO inhibitors constitute a kind of important herbicide (*1*). Generally, commercial cyclic imides possess the following structural features: (i) a heterocycle structure with one or more nitrogen atoms; (ii) a polysubstituted benzene ring that links with the nitrogen atom of the heterocycle ring (*2*−*8*). It was noticed that when the polysubstituted benzene ring was replaced by a benzo-heterocycle ring, the corresponding compounds also possessed excellent PPO inhibitory activity and herbicidal activities, such as flumioxazin (*2*) (**Figure 1**). In our previous work (*9*), the imidazotetrazinone moiety of mitozolomide **A** and temozolomide **B** with antineoplastic activity (**Figure 1**) (*10, 11*) was modified into pyrazolotetrazinone **C**, and some of these compounds provided more than 80% control of *Brassica campestris* at 10 μg/mL. To further improve their herbicidal activity and find valuable lead compounds, according to the bioisosteric principle, novel 3*H*-pyrazolo[3,4-d][1,2,3]triazin-4-one derivatives **D** and **E** were designed (**Figure 1**). In this paper, their synthesis, herbicidal activities in vivo, and inhibition activities in vitro against PPO have been described.

MATERIALS AND METHODS

Synthetic Procedures. ^{1}H NMR spectra were obtained on 300 MHz (a Bruker AV300 spectrometer), 400 MHz (Varian Mercury Plus400 spectrometer), or 600 MHz (a Bruker AV600) in $CDCl_3$ solution with tetramethylsilane as the internal standard. Chemical shift values (δ) are given in ppm. Elemental analyses were determined on a Yanaca CHN Corder MT-3 elemental analyzer. Melting points were taken on a Thomas-Hoover melting point apparatus and are uncorrected. Yields were not optimized. Solvents were dried according to standard methods and distilled prior to use.

General Synthetic Procedure for F and H. Compounds **F** and **H** were synthesized as the literature described (*12*−*14*).

General Synthetic Procedure for D1−13 (*15, 16*). After the mixture of **F** (10 mmol) in THF−MeOH (50 mL, V:V = 1:1) with 2.5 N NaOH (25 mL) was heated at 60 °C for 4 h, the solvent was removed under reduced pressure, and the residue was acidified with 6 N HCl at 0 °C. A gray solid was precipitated, filtered, and washed with water. Then, the

* To whom correspondence should be addressed. Tel: +86-22-23503799. Fax: +86-22-23503627. E-mail: nk_yanghz@126.com or zyq8165@nankai.edu.cn.

10.1021/jf801774k CCC: $40.75
Published on Web 09/23/2008

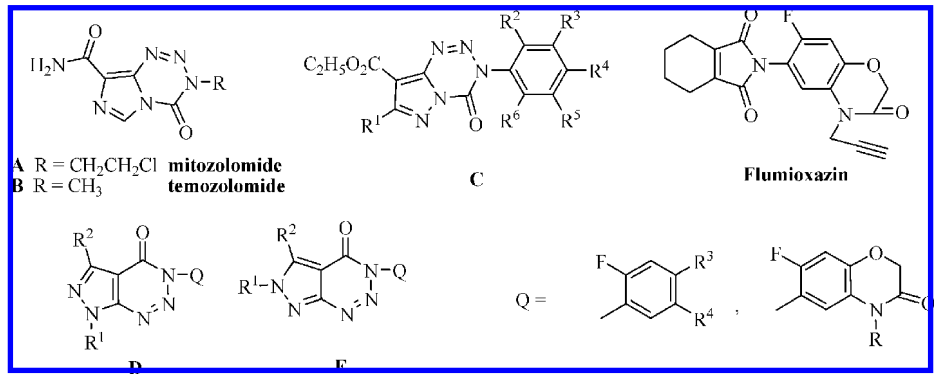
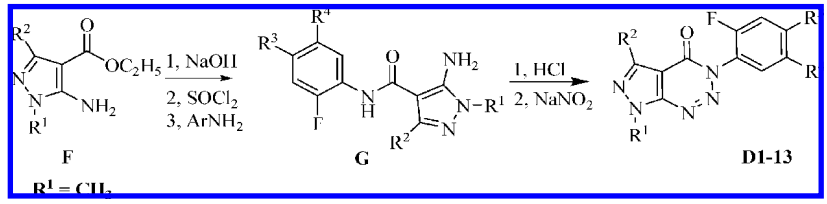
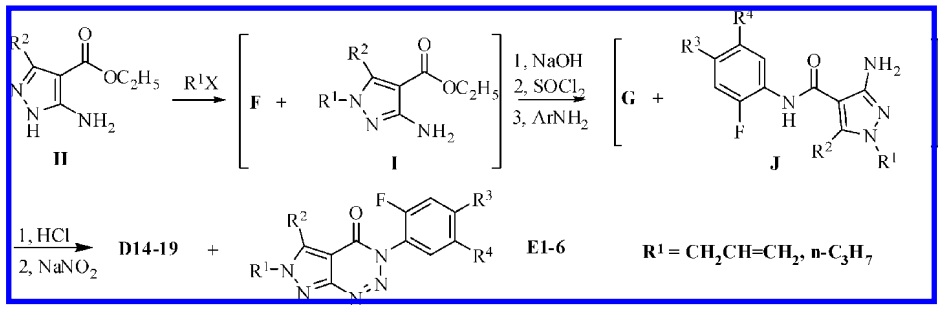


Figure 1. Chemical structures of **A**−**E** and flumioxazin.

Scheme 1



Scheme 2



dry solid was stirred with $SOCl_2$ at room temperature for 3 h, and the corresponding acid chloride was obtained by vacuo to remove the excess of $SOCl_2$. The substituted aniline (10 mmol) in CH_2Cl_2 (50 mL) and pyridine (5 mL) was mixed with the acid chloride at 0 °C and stirred at room temperature overnight and concentrated, and the residue was washed with 0.5 N HCl, saturated $NaHCO_3$ solution, and brine and was dried in vacuo to obtain **G** used for next step without further purification.

The solution of **G** (1 mmol) in 10 mL of 6 N HCl and 1 mL of MeOH was stirred for 10 h and then cooled down below 0 °C, sodium nitrite (3 mmol) in 2 mL of water was added dropwise and stirred for 2.5 h, and then, ice water (10 mL) was added and extracted with ethyl acetate (15 mL × 3). The organic layer was washed with brine and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure, and the residue was purified by flash column chromatography on silica gel using ethyl acetate−petroleum ether as an eluent to afford the products **D1**−**13** (**Scheme 1**).

General Synthetic Procedure for D14−**19 and E1**−**6.** NaH (60%, 11 mmol) was added slowly to a stirred solution of **H** (10 mmol) in 50 mL of dry dimethyl formamide (DMF) at 0 °C. After 0.5 h, the appropriate alkyl halide (11 mmol) was added dropwise and stirred for 4 h at room temperature. Then, the mixture was slowly poured into ice water (200 mL) and extracted with ethyl acetate. The organic layer was washed with brine and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to give a mixture of **F** and **I** (*17*), and the mixture was directly used for the preparation of **G** and **J** without isolation according to the procedure of **Scheme 1**.

As polarities of **G** and **J** are very similar, the mixture of these compounds was used to prepare the target products **D14**−**19** and **E1**−**6** according to the procedure of **Scheme 1**. Their structures were identified by two-dimensional (2D) NMR analyses [heteronuclear single quantum coherence (HSQC) and heteronuclear multiple-bond correlation (HMBC)] and single-crystal X-ray diffraction analysis (**Scheme 2**).

General Synthetic Procedure for D20−**28.** A solution of **D3** (0.68 mmol) in 10 mL of DMF was cooled down below 0 °C, and 0.5 mL of NaOH (1.4 mmol) solution was added slowly. The cooling bath was removed after addition, and the solution was stirred at room temperature for 2 h. The reaction mixture was poured slowly into 50 mL of ice water, the pH was adjusted to 6, and the mixture was extracted with ethyl acetate. The organic layer was washed with brine and dried over anhydrous Na_2SO_4. The solvent was removed in vacuo, and the residue was purified by flash column chromatography on silica gel, using ethyl acetate−petroleum ether as the eluent to afford the pure target product **D20** (*18*).

Different halides were reacted with **D20** (0.34 mmol) and K_2CO_3 (0.35 mmol) in 10 mL of DMF to afford the pure target products **D21**−**28**, respectively (**Scheme 3**). The melting points, yields, and elemental analyses of compounds **D1**−**28** and **E1**−**6** are listed in **Table 1**−**3**, and the data of their ^{1}H NMR are listed in **Table 4**.

Bioassays. For comparative purposes, the herbicidal activities of the title compounds **D**, **E**, and flumioxazin {2-(7-fluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-4,5,6, 7-tetrahydro-2*H*-isoindole-1,3-dione} were evaluated using a previously reported procedure (*19*). All treatments were triplicated.

Inhibitory Activity in Vitro against PPO. The pI_{50} values against PPO of compounds **D** and **E** were assayed according to the procedure reported (*20*−*25*). Etioplasts were prepared from the etiolated leaves of corn. Seven day old leaves of dark-grown corn seedlings were homogenized with an FSH-2A Variable High Speed Homogenizer for 20 s at 15000 rpm using a fresh weight to volume ratio of 1:5. Homogenization buffer consisted of 50 mM Hepes (pH 7.8 at 25 °C), 500 mM sucrouse, 1 mM EDTA, 1 mM $MgCl_2$, 1 mM dithiothreitol (DTT), and 0.2% bovine serum albumin (BSA). Homogenate was filtered through two layers of fabric, and crude cell debris was removed by centrifugation at 800*g* for 2 min at 4 °C. Etioplasts were collected

Scheme 3

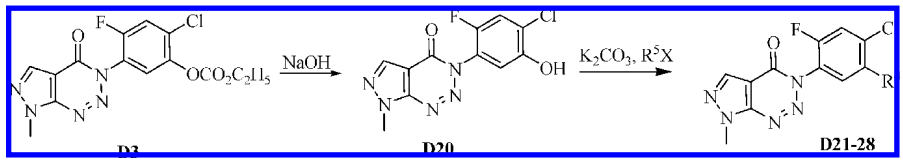


Table 1. List of Compounds **D** and **E**

no.	R^1	R^2	R^3	R^4	no.	R^1	R^2	R^3	R^4
D1	CH_3	H	Cl	$OCH_2C'CH$	**D18**	n-C_3H_7	H	R^3R^4=$OCH_2CONCH_2C'CH$	
D2	CH_3	H	Cl	$OCH_2CH=CH_2$	**D19**	n-C_3H_7	H	R^3R^4=$OCH_2CONCH_2CH=CH_2$	
D3	CH_3	H	Cl	$OCO_2CH_2CH_3$	**D20**	CH_3	H	Cl	OH
D4	CH_3	CF_3	Cl	$OCH_2C'CH$	**D21**	CH_3	H	Cl	OCH_3
D5	CH_3	CH_3	Cl	$OCH_2C'CH$	**D22**	CH_3	H	Cl	OC_2H_5
D6	CH_3	CH_3	Cl	$OCH_2CH=CH_2$	**D23**	CH_3	H	Cl	O(n-C_4H_9)
D7	CH_3	H	R^3R^4=$OCH_2CONCH_2C'CH$		**D24**	CH_3	H	Cl	O(n-C_5H_{11})
D8	CH_3	H	R^3R^4=$OCH_2CONCH_2CH=CH_2$		**D25**	CH_3	H	Cl	$OCH_2CH=CHCl(E)$[a]
D9	CH_3	H	R^3R^4=$OCH_2CON(n\text{-}C_3H_7)$		**D26**	CH_3	H	Cl	$OCH_2C(Cl)=CH_2$
D10	CH_3	CF_3	R^3R^4=$OCH_2CONCH_2C'CH$		**D27**	CH_3	H	Cl	OCH_2CN
D11	CH_3	CF_3	R^3R^4=$OCH_2CONCH_2CH=CH_2$		**D28**	CH_3	H	Cl	$OCH_2OC_2H_4OCH_3$
D12	CH_3	CH_3	R^3R^4=$OCH_2CONCH_2C'CH$		**E1**	allyl	H	Cl	$OCH_2C'CH$
D13	CH_3	CH_3	R^3R^4=$OCH_2CONCH_2CH=CH_2$		**E2**	allyl	H	Cl	$OCH_2CH=CH_2$
D14	allyl	H	Cl	$OCH_2C'CH$	**E3**	allyl	H	R^3R^4=$OCH_2CONCH_2C'CH$	
D15	allyl	H	Cl	$OCH_2CH=CH_2$	**E4**	allyl	H	R^3R^4=$OCH_2CONCH_2CH=CH_2$	
D16	allyl	H	R^3R^4=$OCH_2CONCH_2C'CH$		**E5**	n-C_3H_7	H	R^3R^4=$OCH_2CONCH_2C'CH$	
D17	allyl	H	R^3R^4=$OCH_2CONCH_2CH=CH_2$		**E6**	n-C_3H_7	H	R^3R^4=$OCH_2CONCH_2CH=CH_2$	

[a] It showed the configuration of the alkenyl substituent.

Table 2. Melting Points and Yields of Compounds **D** and **E**

no.	mp (°C)	yield (%)	no.	mp (°C)	yield (%)	no.	mp (°C)	yield (%)
D1	215−216	27.8[a]	**D13**	212−213	17.6[a]	**D25**	182−184	97.3
D2	191−193	34.2[a]	**D14**	147−149	20.0[b]	**D26**	155−157	91.9
D3	182−184	48.9[a]	**D15**	149−151	9.9[b]	**D27**	137−139	71.7
D4	144−145	29.0[a]	**D16**	193−195	16.2[b]	**D28**	115−117	67.8
D5	192−194	16.5[a]	**D17**	136−137	11.5[b]	**E1**	169−170	30.0[b]
D6	123−124	18.0[a]	**D18**	173−175	18.4[b]	**E2**	132−134	14.9[b]
D7	245−246	47.7[a]	**D19**	124−126	19.1[b]	**E3**	189−191	24.3[b]
D8	235−237	60.9[a]	**D20**	235−237	99.7	**E4**	162−163	17.2[b]
D9	208−210	52.2[a]	**D21**	220−222	79.4	**E5**	215−217	27.6[b]
D10	261−263	27.2[a]	**D22**	204−205	99.5	**E6**	196−198	28.6[b]
D11	238−239	20.5[a]	**D23**	162−163	84.1			
D12	235−237	19.5[a]	**D24**	144−145	89.0			

[a] Yield calculated from starting material **F**. [b] Yield calculated from starting material **H**.

Table 3. Elemental Analysis Data of Compounds **D** and **E**

	elemental analysis (%, calcd)				elemental analysis (%, calcd)		
no.	C	H	N	no.	C	H	N
D1	50.21 (50.39)	2.92 (2.72)	20.77 (20.99)	**D18**	56.35 (56.54)	4.09 (3.95)	22.17 (21.98)
D2	49.93 (50.09)	3.39 (3.30)	20.82 (20.86)	**D19**	56.04 (56.25)	4.59 (4.46)	21.81 (21.86)
D3	45.72 (45.73)	2.99 (3.02)	18.99 (19.05)	**D20**	44.40 (44.69)	2.37 (2.39)	23.48 (23.69)
D4	44.79 (44.85)	2.19 (2.01)	17.29 (17.43)	**D21**	46.28 (46.54)	3.07 (2.93)	22.41 (22.61)
D5	51.91 (51.81)	3.31 (3.19)	20.01 (20.14)	**D22**	48.00 (48.23)	3.43 (3.43)	21.40 (21.63)
D6	51.31 (51.51)	3.97 (3.75)	20.11 (20.02)	**D23**	51.19 (51.22)	4.26 (4.30)	19.81 (19.91)
D7	54.39 (54.24)	3.17 (3.13)	23.48 (23.72)	**D24**	52.40 (52.54)	4.65 (4.68)	19.01 (19.15)
D8	53.71 (53.93)	3.52 (3.68)	23.62 (23.59)	**D25**	45.16 (45.43)	2.95 (2.72)	18.92 (18.92)
D9	53.62 (53.63)	4.07 (4.22)	23.21 (23.45)	**D26**	45.18 (45.43)	2.84 (2.72)	18.68 (18.92)
D10	48.46 (48.35)	2.62 (2.39)	19.96 (19.90)	**D27**	46.42 (46.65)	2.48 (2.41)	25.06 (25.11)
D11	48.28 (48.12)	2.72 (2.85)	19.64 (19.81)	**D28**	46.70 (46.95)	3.96 (3.94)	18.38 (18.25)
D12	55.29 (55.44)	3.77 (3.56)	22.60 (22.82)	**E1**	53.25 (53.42)	3.20 (3.08)	19.31 (19.47)
D13	54.99 (55.13)	4.11 (4.08)	22.60 (22.69)	**E2**	53.08 (53.12)	3.40 (3.62)	19.47 (19.36)
D14	53.41 (53.42)	3.21 (3.08)	19.22 (19.47)	**E3**	56.54 (56.84)	3.62 (3.45)	21.86 (22.10)
D15	53.22 (53.12)	3.90 (3.62)	19.10 (19.36)	**E4**	56.50 (56.54)	4.08 (3.95)	22.09 (21.98)
D16	56.70 (56.84)	3.56 (3.45)	21.92 (22.10)	**E5**	56.58 (56.54)	3.93 (3.95)	22.09 (21.98)
D17	56.64 (56.54)	4.07 (3.95)	21.70 (21.98)	**E6**	56.21 (56.25)	4.31 (4.46)	21.92 (21.86)

from this supernatant by centrifugation at 17000*g* for 6 min at 4 °C. The extracts were resuspended in assay buffer (50 mM Tris, 2 mM EDTA, and 20% glycerol, pH 7.3, at 25 °C) and stored at −80 °C until use.

Protoporphyrinogen IX (protogen IX) was prepared according to Jacobs (*23*) with the following modifications. Protoporphyrin IX (Proto IX) stock solution (0.5 mM in 20% ethanol containing 10 mM KOH) was reduced to protogen IX with approximately one-eighth volume of

Table 4. ^{1}H NMR of Compounds **D** and **E**

no.	δ (ppm)
D1 (400 MHz)	2.57 (t, 1H, J = 2.2 Hz, ’CH), 4.31 (s, 3H, NCH_3), 4.79 (d, 2H, J = 2.2 Hz, OCH_2), 7.21 (d, 1H, J = 6.3 Hz, Ph), 7.38 (d, 1H, J = 8.9 Hz, Ph), 8.25 (s, 1H, pyrazole-H)
D2 (300 MHz)	4.31 (s, 3H, NCH_3), 4.61 (d, 2H, J = 5.1 Hz, OCH_2), 5.32−5.50 (m, 2H, $=CH_2$), 5.99−6.15 (m, 1H, CH), 7.02 (d, 1H, J = 6.4 Hz, Ph), 7.37 (d, 1H, J = 9.0 Hz, Ph), 8.24 (s, 1H, pyrazole-H)
D3 (300 MHz)	1.41 (t, 3H, J = 7.1 Hz, CH_3), 4.31 (s, 3H, NCH_3), 4.37 (q, 2H, J = 7.1 Hz, OCH_2), 7.44 (d, 1H, J = 9.0 Hz, Ph), 7.46 (d, 1H, J = 6.7 Hz, Ph), 8.24 (s, 1H, pyrazole-H)
D4 (400 MHz)	2.57 (t, 1H, J = 2.2 Hz, ’CH), 4.36 (s, 3H, NCH_3), 4.79 (d, 2H, J = 2.2 Hz, OCH_2), 7.20 (d, 1H, J = 6.3 Hz, Ph), 7.39 (d, 1H, J = 8.9 Hz, Ph)
D5 (400 MHz)	2.57 (t, 1H, J = 1.9 Hz, ’CH), 2.65 (s, 3H, CCH_3), 4.21 (s, 3H, NCH_3), 4.78 (d, 2H, J = 1.9 Hz, OCH_2), 7.19 (d, 1H, J = 6.3 Hz, Ph), 7.37 (d, 1H, J = 9.0 Hz, Ph)
D6 (400 MHz)	2.65 (s, 3H, CCH_3), 4.21 (s, 3H, NCH_3), 4.60 (d, 2H, J = 5.1 Hz, OCH_2), 5.32−5.48 (m, 2H, $=CH_2$), 6.00−6.09 (m, 1H, CH), 7.00 (d, 1H, J = 6.4 Hz, Ph), 7.37 (d, 1H, J = 9.0 Hz, Ph)
D7 (300 MHz)	2.28 (t, 1H, J = 2.4 Hz, ’CH), 4.32 (s, 3H, NCH_3), 4.70 (d, 2H, J = 2.4 Hz, OCH_2), 4.74 (s, 2H, OCH_2), 6.99 (d, 1H, J = 9.7 Hz, Ph), 7.29 (d, 1H, J = 6.7 Hz, Ph), 8.25 (s, 1H, pyrazole-H)
D8 (300 MHz)	4.31 (s, 3H, NCH_3), 4.54−4.56 (m, 2H, NCH_2), 4.74 (s, 2H, OCH_2), 5.18−5.27 (m, 2H, $=CH_2$), 5.78−5.90 (m, 1H, CH), 6.97 (d, 1H, J = 9.7 Hz, Ph), 7.04 (d, 1H, J = 6.8 Hz, Ph), 8.24 (s, 1H, pyrazole-H)
D9 (400 MHz)	0.95 (t, 3H, J = 7.4 Hz, CH_3), 1.65−1.74 (m, 2H, CH_2), 3.87 (t, 2H, J = 7.4 Hz, NCH_2), 4.31 (s, 3H, NCH_3), 4.69 (s, 2H, OCH_2), 6.96 (d, 1H, J = 9.7 Hz, Ph), 7.02 (d, 1H, J = 6.8 Hz, Ph), 8.25 (s, 1H, pyrazole-H)
D10 (400 MHz)	2.28 (t, 1H, J = 2.4 Hz, ’CH), 4.36 (s, 3H, NCH_3), 4.69 (d, 2H, J = 2.3 Hz, OCH_2), 4.74 (s, 2H, OCH_2), 6.98 (d, 1H, J = 9.7 Hz, Ph), 7.28 (d, 1H, J = 6.7 Hz, Ph)
D11 (400 MHz)	4.35 (s, 3H, NCH_3), 4.54−4.55 (m, 2H, NCH_2), 4.74 (s, 2H, OCH_2), 5.18−5.27 (m, 2H, $=CH_2$), 5.78−5.88 (m, 1H, CH), 6.96 (d, 1H, J = 9.7 Hz, Ph), 7.03 (d, 1H, J = 6.8 Hz, Ph)
D12 (400 MHz)	2.27 (t, 1H, J = 2.4 Hz, ’CH), 2.65 (s, 3H, CCH_3), 4.21 (s, 3H, NCH_3), 4.68 (d, 2H, J = 2.4 Hz, CH_2), 4.73 (s, 2H, OCH_2), 6.97 (d, 1H, J = 9.6 Hz, Ph), 7.27 (d, 1H, J = 7.8 Hz, Ph)
D13 (400 MHz)	2.65 (s, 3H, CCH_3), 4.21 (s, 3H, NCH_3), 4.54−4.55 (m, 2H, NCH_2), 4.73 (s, 2H, OCH_2), 5.18−5.26 (m, 2H, $=CH_2$), 5.79−5.88 (m, 1H, CH), 6.95 (d, 1H, J = 9.7 Hz, Ph), 7.03 (d, 1H, J = 6.8 Hz, Ph)
D14 (400 MHz)	2.57 (t, 1H, J = 2.1 Hz, ’CH), 4.78 (d, 2H, J = 2.1 Hz, OCH_2), 5.25 (d, 2H, J = 5.9 Hz, NCH_2), 5.34−5.39 (m, 2H, $=CH_2$), 6.07−6.17 (m, 1H, CH), 7.21 (d, 1H, J = 6.3 Hz, Ph), 7.38 (d, 1H, J = 8.9 Hz, Ph), 8.28 (s, 1H, pyrazole-H)
D15 (400 MHz)	4.60−4.61 (m, 2H, OCH_2), 5.24−5.26 (m, 2H, NCH_2), 5.32−5.48 (m, 4H, $2=CH_2$), 6.00−6.15 (m, 2H, 2CH), 7.01 (d, 1H, J = 6.3 Hz, Ph), 7.37 (d, 1H, J = 8.6 Hz, Ph), 8.27 (s, 1H, pyrazole-H)
D16 (400 MHz)	2.28 (t, 1H, J = 2.4 Hz, ’CH), 4.69 (d, 2H, J = 2.4 Hz, NCH_2), 4.74 (s, 2H, OCH_2), 5.26 (d, 2H, J = 6.0 Hz, NCH_2), 5.35−5.40 (m, 2H, $=CH_2$), 6.08−6.18 (m, 1H, CH), 6.98 (d, 1H, J = 9.7 Hz, Ph), 7.29 (d, 1H, J = 6.7 Hz, Ph), 8.28 (s, 1H, pyrazole-H)
D17 (400 MHz)	4.54−4.55 (m, 2H, NCH_2), 4.74 (s, 2H, OCH_2), 5.18−5.26 (m, 4H, $2CH_2$), 5.34−5.39 (m, 2H, $=CH_2$), 5.79−5.88 (m, 1H, CH), 6.07−6.17 (m, 1H, CH), 6.96 (d, 1H, J = 9.7 Hz, Ph), 7.04 (d, 1H, J = 6.8 Hz, Ph), 8.27 (s, 1H, pyrazole-H)
D18 (400 MHz)	1.00 (t, 3H, J = 7.4 Hz, CH_3), 2.04−2.12 (m, 2H, CH_2), 2.27 (t, 1H, J = 1.9 Hz, ’CH), 4.61 (t, 2H, J = 7.2 Hz, NCH_2), 4.69 (d, 2H, J = 1.9 Hz, NCH_2), 4.73 (s, 2H, OCH_2), 6.98 (d, 1H, J = 9.6 Hz, Ph), 7.29 (d, 1H, J = 6.7 Hz, Ph), 8.25 (s, 1H, pyrazole-H)
D19 (400 MHz)	1.00 (t, 3H, J = 7.4 Hz, CH_3), 2.04−2.10 (m, 2H, CH_2), 4.54−4.55 (m, 2H, NCH_2), 4.60 (t, 2H, J = 7.1 Hz, NCH_2), 4.74 (s, 2H, OCH_2), 5.19−5.26 (m, 2H, $=CH_2$), 5.80−5.88 (m, 1H, CH), 6.96 (d, 1H, J = 9.8 Hz, Ph), 7.04 (d, 1H, J = 6.7 Hz, Ph), 8.24 (s, 1H, pyrazole-H)
D20 (300 MHz)	4.31 (s, 3H, NCH_3), 5.60 (s, 1H, OH), 7.16 (d, 1H, J = 6.5 Hz, Ph), 7.33 (d, 1H, J = 8.8 Hz, Ph), 8.25 (s, 1H, pyrazole-H)
D21 (400 MHz)	3.91 (s, 3H, OCH_3), 4.31 (s, 3H, NCH_3), 7.00 (d, 1H, J = 6.3 Hz, Ph), 7.37 (d, 1H, J = 8.9 Hz, Ph), 8.25 (s, 1H, pyrazole-H)
D22 (400 MHz)	1.48 (t, 3H, J = 6.9 Hz, CH_3), 4.09 (q, 2H, J = 6.9 Hz, OCH_2), 4.30 (s, 3H, NCH_3), 6.99 (d, 1H, J = 6.3 Hz, Ph), 7.36 (d, 1H, J = 8.9 Hz, Ph), 8.24 (s, 1H, pyrazole-H)
D23 (400 MHz)	0.98 (t, 3H, J = 7.4 Hz, CH_3), 1.49−1.57 (m, 2H, CH_2), 1.79−1.86 (m, 2H, CH_2), 4.02 (t, 2H, J = 6.4 Hz, OCH_2), 4.30 (s, 3H, NCH_3), 6.99 (d, 1H, J = 6.4 Hz, Ph), 7.35 (d, 1H, J = 9.0 Hz, Ph), 8.24 (s, 1H, pyrazole-H)

Table 4. Continued

no.	δ (ppm)
D24 (400 MHz)	0.93 (t, 3H, J = 7.2 Hz, CH_3), 1.36−1.50 (m, 4H, $2CH_2$), 1.81−1.88 (m, 2H, CH_2), 4.01 (t, 2H, J = 6.5 Hz, OCH_2), 4.30 (s, 3H, NCH_3), 6.99 (d, 1H, J = 6.4 Hz, Ph), 7.35 (d, 1H, J = 9.0 Hz, Ph), 8.24 (s, 1H, pyrazole-H)
D25 (300 MHz)	4.31 (s, 3H, NCH_3), 4.58 (dd, 2H, J_1 = 1.4 Hz, J_2 = 5.8 Hz, OCH_2), 6.14−6.22 (m, 1H, CH), 6.44−6.49 (m, 1H, CH), 7.01 (d, 1H, J = 6.4 Hz, Ph), 7.39 (d, 1H, J = 8.9 Hz, Ph), 8.25 (s, 1H, pyrazole-H)
D26 (400 MHz)	4.31 (s, 3H, NCH_3), 4.64 (s, 2H, OCH_2), 5.49−5.50 (m, 1H, $=CH_2$), 5.65−5.66 (m, 1H, $=CH_2$), 7.02 (d, 1H, J=6.3 Hz, Ph), 7.39 (d, 1H, J=8.9 Hz, Ph), 8.25 (s, 1H, pyrazole-H)
D27 (300 MHz)	4.32 (s, 3H, NCH_3), 4.86 (s, 2H, OCH_2), 7.25 (d, 1H, J = 5.4 Hz, Ph), 7.44 (d, 1H, J = 8.9 Hz, Ph), 8.26 (s, 1H, pyrazole-H)
D28 (300 MHz)	3.35 (s, 3H, OCH_3), 3.55 (t, 2H, J = 3.3 Hz, OCH_2), 3.88 (t, 2H, J = 3.0 Hz, OCH_2), 4.31 (s, 3H, NCH_3), 5.35 (s, 2H, OCH_2), 7.36 (d, 1H, J = 9.0 Hz, Ph), 7.41 (d, 1H, J = 6.6 Hz, Ph), 8.24 (s, 1H, pyrazole-H)
E1 (400 MHz)	2.57 (t, 1H, J = 2.4 Hz, 'CH), 4.79 (d, 2H, J = 2.4 Hz, OCH_2), 5.05 (d, 2H, J = 6.3 Hz, NCH_2), 5.43−5.50 (m, 2H, $=CH_2$), 6.07−6.17 (m, 1H, CH), 7.23 (d, 1H, J = 6.4 Hz, Ph), 7.36 (d, 1H, J = 9.0 Hz, Ph), 8.26 (s, 1H, pyrazole-H)
E2 (400 MHz)	4.60−4.62 (m, 2H, OCH_2), 5.04−5.06 (m, 2H, NCH_2), 5.31−5.50 (m, 4H, $2=CH_2$), 6.00−6.17 (m, 2H, 2CH), 7.03 (d, 1H, J = 6.4 Hz, Ph), 7.35 (d, 1H, J = 9.0 Hz, Ph), 8.26 (s, 1H, pyrazole-H)
E3 (400 MHz)	2.27 (t, 1H, J = 1.8 Hz, 'CH), 4.69 (d, 2H, J = 1.8 Hz, NCH_2), 4.72 (s, 2H, OCH_2), 5.06 (d, 2H, J = 5.6 Hz, NCH_2), 5.43−5.50 (m, 2H, $=CH_2$), 6.07−6.17 (m, 1H, CH), 6.96 (d, 1H, J = 9.6 Hz, Ph), 7.30 (d, 1H, J=6.7 Hz, Ph), 8.27 (s, 1H, pyrazole-H)
E4 (400 MHz)	4.54−4.56 (m, 2H, NCH_2), 4.72 (s, 2H, OCH_2), 5.05 (d, 2H, J = 6.3 Hz, NCH_2), 5.18−5.26 (m, 2H, $=CH_2$), 5.42−5.50 (m, 2H, $=CH_2$), 5.79−5.88 (m, 1H, CH), 6.07−6.17 (m, 1H, CH), 6.94 (d, 1H, J = 9.7 Hz, Ph), 7.06 (d, 1H, J = 6.8 Hz, Ph), 8.26 (s, 1H, pyrazole -H)
E5 (400 MHz)	1.00 (t, 3H, J = 7.4 Hz, CH_3), 2.04−2.13 (m, 2H, CH_2), 2.27 (t, 1H, J = 2.4 Hz, 'CH), 4.40 (t, 2H, J = 7.0 Hz, NCH_2), 4.69 (d, 2H, J = 2.1 Hz, NCH_2), 4.72 (s, 2H, OCH_2), 6.96 (d, 1H, J = 9.7 Hz, Ph), 7.30 (d, 1H, J = 6.8 Hz, Ph), 8.23 (s, 1H, pyrazole-H)
E6 (400 MHz)	1.00 (t, 3H, J = 7.4 Hz, CH_3), 2.05−2.11 (m, 2H, CH_2), 4.40 (t, 2H, J = 6.9 Hz, NCH_2), 4.54−4.55 (m, 2H, NCH_2), 4.72 (s, 2H, OCH_2), 5.18−5.26 (m, 2H, $=CH_2$), 5.79−5.87 (m, 1H, CH), 6.94 (d, 1H, J = 9.6 Hz, Ph), 7.06 (d, 1H, J = 6.8 Hz, Ph), 8.22 (s, 1H, pyrazole-H)

freshly ground sodium amalgam. Residual amalgam and porphyrin aggregates were removed by filtration. The resulting colorless filtrate was adjusted to pH 8 with 10% HCl and diluted with triplication volume of assay buffer, consisting of 100 mM Tris (pH 7.5) with 1 mM EDTA and 4 mM DTT. The resulting preparation was stable for at least 1 week at −80 °C. All operations were conducted under dim light.

The assay of PPO inhibitory activity was carried out as follows. The reaction mixture (1 mL) contained 100 mM Tris (pH 7.5), 1 mM EDTA, 4 mM DTT, 2−5 μM protogen IX, and 0.3−0.6 mg of etioplasts. The reaction was initiated by the addition of protogen IX with or without test compound. After incubation for 30 min at 30 °C in darkness, the mixture was transferred to 2.0 mL of 100 mM Tris (pH 7.8) buffer that contained 1 mM EDTA, 5 mM DTT, and 1% (V/V) Tween 80, and then, fluorescence at 630 nm (with excitation at 410 nm) was immediately measured with a fluorescence spectrophotometer (960MC Shanghai Lengguang Corp.). Heat-denatured etioplasts were used in the control experiments. Data were fit to a one-site competition model (*26*) using GraphPad Prism versions 4.03 for Windows (D. Radushev, Graph Pad Software Inc. Trial), and the pI_{50} values were obtained (**Table 5**).

Treatment. The emulsions of purified compounds were prepared by dissolving them in 100 μL of *N*,*N*-dimethylformamide with the addition of a little Tween 20 and proper water. There were three replicates for each treatment. The mixture of the same amount of water, *N*,*N*-dimethylformamide, and Tween 20 was used as the control.

Pre-emergence. Sandy clay (100 g) in a plastic box (11 cm × 7.5 cm × 6 cm) was wetted with water. Fifteen sprouting seeds of the weed under test were planted in fine earth (0.6 cm depth) in the glasshouse and sprayed with the test compound solution.

Postemergence. Seedlings (one leaf and one stem) of the weed were sprayed with the test compounds at the same rate as used for the pre-emergence test. For both methods, the fresh weights were determined 15 days later, and the percentage inhibition relative to the controls was calculated. The herbicidal activity is summarized in **Tables 6** and **7**.

RESULTS AND DISCUSSION

Synthesis and Structure Characterization. The intermediate pyrazole derivatives **F** and **H** were synthesized as the literature described (*12*−*14*). Compound **F** was hydrolyzed and then converted to its acid chloride, which reacted directly with substituted aniline in CH_2Cl_2 in the presence of pyridine to afford **G**. Suggs reported that the cyclization temperature of **G** to **D1**−**13** was below −25 °C (*16*); however, a higher temperature (90 ± 5 °C) and stronger acid atmosphere were more

Table 5. pI_{50} of Compounds **D** and **E** against PPO in Corn

no.	pI_{50}	SE	no.	pI_{50}	SE	no.	pI_{50}	SE
D1	7.12	0.08	**D13**	6.28	0.07	**D25**	6.55	0.07
D2	6.33	0.11	**D14**	7.94	0.07	**D26**	6.58	0.02
D3	6.31	0.04	**D15**	7.19	0.01	**D27**	6.64	0.04
D4	5.71	0.05	**D16**	8.02	0.01	**D28**	6.09	0.01
D5	6.49	0.02	**D17**	7.78	0.01	**E1**	4.51	0.06
D6	6.10	0.04	**D18**	7.87	0.03	**E2**	<4.50	
D7	7.85	0.03	**D19**	7.75	0.05	**E3**	5.27	0.02
D8	7.34	0.06	**D20**	6.23	0.06	**E4**	4.83	0.05
D9	7.37	0.11	**D21**	6.12	0.03	**E5**	5.00	0.01
D10	5.74	0.07	**D22**	6.53	0.01	**E6**	5.04	0.03
D11	5.41	0.10	**D23**	6.61	0.03	Flumioxazin	8.49 [reference value (*27*): 8.50]	0.03
D12	6.63	0.07	**D24**	6.32	0.07			

Table 6. Herbicical Activity of Compounds (Percent Inhibition) (Rate = 1500g/ha)[a]

	B. campestris		*A. retroflexus*		*E. crus-galli*		*D. sanguinalis*	
no.	pre	post	pre	post	pre	post	pre	post
D1	12.7	9.2	0	0	0	20.8	0	19.6
D2	7.4	1.2	10.6	0	11.0	28.0	0	17.9
D3	0	0	0	7.7	19.8	37.7	0	0
D4	21.6	32.7	15.5	5.5	0	2.4	6.7	0
D5	9.5	0	0	0	0	0	38.3	0
D6	7.8	7.5	0	14.7	17.2	21.0	0	16.6
D7	98.8	45.6	100	71.8	100	58.4	100	39.2
D8	17.5	20.9	30.6	20.3	24.4	9.1	0	27.0
D9	100	20.5	100	54.1	94.8	46.9	94.1	47.5
D10	0	20.8	0	0	3.0	8.2	8.3	0
D11	0	12.0	1.2	0	0	13.6	0	0
D12	44.4	0	30.0	0	15.8	0	57.0	0
D13	34.6	0	50.0	0	22.3	0	47.7	0
D14	7.4	18.5	87.0	88.6	0	38.3	37.4	41.3
D15	30.5	10.3	60.1	100	30.1	32.3	10.5	23.1
D16	100	100	100	100	100	85.2	100	32.1
D17	100	100	100	100	96.5	89.3	92.3	31.2
D18	98.1	90.7	100	99.6	95.4	91.5	100	33.9
D19	100	97.5	100	100	86.4	20.1	90.7	52.8
D20	0	27.9	23.7	25.1	3.5	13.2	0	9.2
D21	0	28.3	40.5	37.5	11.3	1.0	0	20.6
D22	29.8	21.5	0	70.6	12.4	20.2	0	0
D23	4.8	16.1	0	16.6	0	0	0	0
D24	13.9	6.1	0	23.2	1.1	15.3	0	0
D25	0	2.9	0	14.0	9.7	11.1	1.4	0
D26	0	0	11.0	54.1	15.9	31.1	0	0
D27	0	31.3	40.5	100	17.5	32.6	2.3	0
D28	0	28.0	51.8	74.9	5.6	43.6	2.3	0
E1	65.8	70.3	100	100	40.9	20.3	79.6	36.1
E2	50.7	60.8	100	100	39.6	35.8	18.0	60.7
E3	100	70.9	100	100	100	38.7	80.7	32.7
E4	100	95.6	100	100	100	30.8	90.0	0
E5	75.1	27.9	100	100	37.8	40.9	51.6	43.8
E6	88.7	50.3	100	100	60.4	11.2	78.6	0

[a] Post, postemergence; pre, pre-emergence; and −, not measured.

helpful to salify **G** to afford **D1**, **D2**, **D4**, **D10**, and **D11**. To make the substituent R^4 more representative, **D21**−**28** were synthesized via the intermediate **D20** as shown in **Scheme 3**. Approximately a 1:1 mixture of the **F** and **I** was obtained by alkylation of H.

Because the polarities of **F** and **I** are very similar, the mixture was directly used for the next step without further isolation. For the same reason, the mixture of **G** and **J** obtained was reacted with hydrogen chloride and $NaNO_2$ (**Scheme 1**). After general workup, a two-component mixture was obtained, and the target products were isolated by flash column chromatography on silica gel. A combination of 2D NMR analyses (HSQC and HMBC) of **D16** and **E3** not only confirmed the position of allyl group but also allowed complete hydrogen and carbon assignments. Some of the major long-range correlations (J^2 and J^3) observed in the HMBC contour plots of compounds **D16** and **E3** were outlined in the structure (**Figure 2**). **Tables 1**−**3** summarized the chemical structures, physical constants, yields, and elemental analysis data of the new compounds **D** and **E**. ^{1}H NMR data were listed in **Table 4**. To further validate their structures, their starting material of the mixture of **F** and **I** (R^1 = allyl; R^2 = H) (**Scheme 2**) was hydrolyzed, and 1-allyl-3-amino-1*H*-pyrazole-4-carboxylic acid was obtained by crystallizing with ethanol. Its structure was determined by ^{1}H NMR and X-ray analysis (**Figure 3**). Then, it was reacted with $SOCl_2$, arylamine, HCl, and $NaNO_2$, respectively, according to **Scheme 1**, and the final product was confirmed to be **E3**.

Structure−**Activity Relationship.** As shown in ref *1*, many commercial heterocycle PPO inhibitors always contain a 2-fluoro-4-chloro-5-alkoxybenzene ring, and the polysubstituted benzene ring was crucial for their herbicidal activities. In our

Table 7. Herbicical Activity of Compounds (Percent Inhibition)[a]

		B. campestris		*A. retroflexus*		*E. crus-galli*		*D. sanguinalis*	
no.	rate (g/ha)	pre	post	pre	post	pre	post	pre	post
D7	750	97.1	−	100	−	100	−	100	−
	375	95.6	−	96.3	−	94.8	−	100	−
	187.5	77.7	−	66.0	−	48.4	−	80.0	−
	93.75	47.3	−	60.2	−	47.1	−	26.8	−
D9	750	79.9	−	95.3	−	91.2	−	87.8	−
	375	57.1	−	93.2	−	69.6	−	65.4	−
	187.5	38.5	−	89.5	−	28.4	−	14.2	−
	93.75	32.2	−	83.8	−	0	−	3.9	−
D14	750	0	7.4	41.0	41.5	0	17.1	17.4	19.4
	375	0	3.4	27.9	12.2	0	11.2	13.0	0
	187.5	0	0	21.3	7.3	0	7.3	6.5	0
D15	750	16.4	2.9	34.4	100	16.9	22.0	4.3	11.1
	375	11.9	0	27.9	85.4	10.0	18.0	0	0
	187.5	9.0	0	1.6	12.2	6.5	2.4	0	0
D16	750	100	100	100	100	98.9	51.6	100	16.7
	375	100	100	100	83.5	97.3	43.5	100	4.2
	187.5	100	72.9	100	5.8	63.7	27.8	77.6	0
	93.75	100	18.5	100	0	53.4	7.0	44.9	0
	45	66.7	−	86.2	−	34.0	−	2.4	−
	22.5	22.3	−	76.6	−	25.1	−	0	−
D17	750	100	100	100	85.1	89.2	49.9	67.4	4.2
	375	100	95.3	100	53.7	47.4	23.2	51.0	0
	187.5	93.3	55.6	100	30.6	34.4	14.2	26.5	0
	93.75	60.4	19.6	100	19.0	10.0	0.2	20.4	0
	45	40.7	−	82.8	−	7.4	−	17.1	−
	22.5	12.7	−	3.4	−	5.4	−	4.9	−
D18	750	82.1	70.3	100	87.8	87.0	61.0	93.5	16.7
	375	67.9	64.6	73.8	41.5	80.1	19.0	91.3	5.6
	187.5	63.4	35.4	37.7	14.6	43.7	0.5	80.4	0
D19	750	100	78.9	100	100	62.8	7.3	71.7	33.3
	375	82.8	45.1	77.0	100	55.0	2.3	67.4	25.0
	187.5	26.9	12.6	67.2	48.8	42.9	1.5	37.0	8.3
E1	750	37.3	36.6	93.4	100	19.5	9.3	47.8	13.9
	375	25.4	18.9	90.2	100	0	0.5	6.5	8.3
	187.5	16.4	2.9	41.0	22.0	0	0	2.2	0
E2	750	23.9	32.6	100	100	22.1	18.0	8.7	44.4
	375	13.4	8.6	100	100	18.4	16.1	4.3	33.3
	187.5	3.0	6.3	100	36.6	0	14.1	0	22.2
E3	750	100	50.4	100	100	95.7	23.6	67.4	0
	375	100	38.9	100	48.8	49.1	17.6	24.5	0
	187.5	90.8	0	100	40.5	13.3	14.7	16.3	0
	93.75	26.0	0	100	30.6	3.5	12.1	6.1	0
E4	750	100	88.5	100	100	89.7	18.1	75.5	0
	375	100	60.8	100	83.5	27.9	17.2	40.8	0
	187.5	95.9	12.3	100	45.5	18.2	2.8	16.3	0
	93.75	51.2	10.2	100	28.9	8.2	0	6.1	0
E5	750	55.2	19.4	100	100	16.9	25.9	32.6	22.2
	375	45.5	18.9	100	100	5.6	11.2	26.1	16.7
	187.5	16.4	11.4	100	56.1	0	10.2	0	13.9
E6	750	69.4	33.7	100	100	35.9	5.4	54.3	0
	375	55.2	29.1	100	85.4	16.0	2.4	39.1	0
	187.5	13.4	0	27.9	7.3	10.8	0	4.3	0
flumioxazin	187.5	100	−	100	−	100	−	100	−
	93.75	100	−	100	−	100	−	100	−
	45	100	−	100	−	71.4	−	100	−
	22.5	92.6	−	100	−	40.9	−	85.4	−

[a] Post, postemergence; pre, pre-emergence; -, not measured.

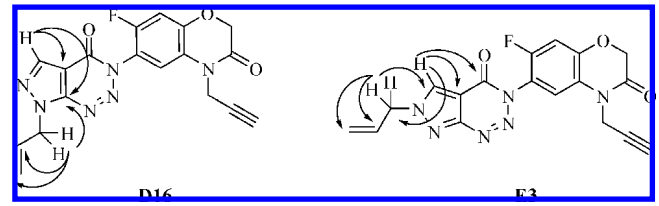


Figure 2. Selected long-range HMBC correlations found in the corresponding contour plots of compounds **D16** and **E3**.

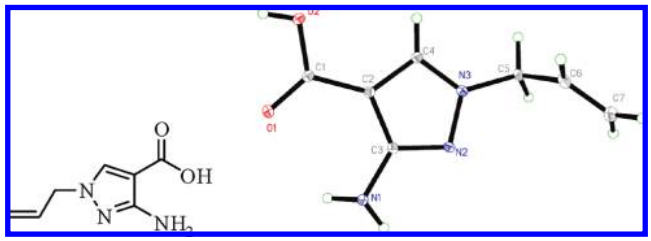


Figure 3. Crystal strucure of 1-allyl-3-amino-1*H*-pyrazole-4-carboxylic acid.

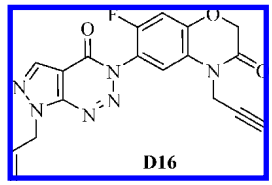


Figure 4. Chemical structure of **D16**.

previous paper (*9*), compound **C** ($R^1 = CH_3$) exhibited better herbicidal activity when the imidazotetrazinone moiety was modified into pyrazolotetrazinone. On the basis of the above, the imidazotetrazinone moiety was modified into pyrazolotriazinone, and a series of compounds was designed and synthesized. As shown in **Table 5**, the pI_{50} was affected greatly by the variance of R^1, R^2, R^3, and R^4. When R^3 was chloro (**D1**−**6**, **D14**−**15**, **D20**−**28**, and **E1**−**2**), only **D14** showed better inhibitory activity ($pI_{50} = 7.94$). When the benzene ring was modified into benzoxazinone ($R^1 = CH_3$, $CH_2CH{=}CH_2$, or n-C_3H_7; $R^2 = H$, CH_3, or CF_3) and R^1 was methyl, allyl, or propyl group and R^2 was hydrogen (**D7**−**9**, **D16**−**19**), their pI_{50} values increased greatly. Especially, compound **D16** (**Figure 4**) showed similar inhibitory activity against PPO with flumioxazin ($pI_{50} = 8.49$). When R^2 was CH_3 or CF_3 (**D10**−**13**), the pI_{50} values changed slightly. When the position of R^1 was changed, their inhibitory activity against PPO decreased sharply (**E1**−**6**). The result showed that (i) the inhibitory activity in vitro was related with the position of R^1, while allyl and propyl groups are more suitable than the methyl group for R^1, and (ii) that the H atom is more satisfactory for increasing pI_{50} value than trifluoromethyl group and methyl group on R^2.

All of the title compounds were tested at 1500 g/ha dosage. As shown in **Table 6**, only four compounds with a four-substituted benzene ring (**D14**, **D15**, **E1**, and **E2**) exhibited better herbicidal activities against *A. retroflexus*. When the four-substituted benzene ring was modified into 2*H*-benzo[b][1,4]-oxazin-3(4*H*)-one, most of these derivatives expressed excellent herbicidal activities on dicotyledon and monocotyledon weeds in both pre- and postemergence treatment (**D7**, **D9**, **D16**−**19**, and **E3**−**6**) and their injury symptoms included leaf cupping, crinkling, bronzing, and necrosis, typical of PPO inhibitor herbicides (*28*). This indicated that the introduction of the structure unit 2*H*-benzo[b][1,4]oxazin-3(4*H*)-one was helpful to improve their herbicidal activity.

Thus, some compounds with higher inhibition rates at a dosage of 1500 g/ha were further bioassayed. From the biological assay results in **Table 7**, most of the compounds exhibited better herbicidal activity on dicotyledon weeds than that on monocotyledon weeds and better in pre-emergence than in postemergence. Compounds **D16**, **D17**, **E3**, and **E4** showed excellent herbicidal activity at 93.75 g/ha in pre-emergence treatments against *A. retroflexus*. Compound **D16** had excellent herbicidal activity on dicotyledon weeds at 93.75 g/ha in pre-emergence treatments. Compounds **E2** and **E5** showed good selectivity at 187.5 g/ha in pre-emergence treatments against dicotyledon weeds.

Compound **D**'s herbicidal activities correlated well with their PPO inhibitory activity. Although the pI_{50} values of the isomers **D** and **E** differed greatly, **E1**−**6** showed nearly as good herbicidal activity as **D14**−**19**. This suggested that the R^1 position has important influence on the combination of the target compounds with PPO, and compounds **E** may share other type of mode of action against the weeds besides PPO. In addition, the pI_{50} value of **D16** increased slightly as compared to **D18** and **D7**. However, the herbicidal activity increased greatly. Compound **D16** showed the highest herbicidal activity. Therefore, the changing R^2 group from methyl group to allyl or propyl group can increase the herbicidal activity of the target compounds greatly.

In this paper, the synthesis and herbicidal evaluation of a series of 3*H*-pyrazolo[3,4-d][1,2,3]triazin-4-one derivatives as a novel class of PPO inhibitors were described. Their herbicidal activities were optimized. When the R^3R^4 moiety of the title compounds is 4-(prop-2-ynyl)-2*H*-1,4-oxazin-3(4*H*)-one, the R^2 moiety is an H atom, and when the R^1 moiety is a methyl, allyl, or propyl group, they have better herbicidal activities. The bioassay data show that some of them, for example, **D16**, **D17**, and **E3**, have promising herbicidal activities. They are chosen as lead compounds, and further investigation on lead optimization, herbicidal activity, and crop selectivity in vivo for the compounds is underway in our group.

LITERATURE CITED

(1) Hirai, K.; Uchida, A.; Ohno, R. *Herbicide Classes in Development*; Böger, P., Wakabayashi, K., Hirai, K., Eds.; Springer-Verlag: Berlin, Heidelberg, 2002; pp 255−274.

(2) Yoshida, R.; Sakaki, M.; Sato, R.; Nagano, E.; Oshio, H.; Kamoshita, H. S-53482, a new phthalimide herbicide. *Proceedings of the Brighton Crop Protection Conference—Weeds*; BCPC, Farnham: Surrey, United Kingdom, 1991; pp 69−75.

(3) Nagano, E.; Hashimoto, S.; Yoshida, R.; Matsumoto, H.; Kamoshita, K. (Sumitomo Chemical Company). U.S. Patent 4670046, 1987.

(4) Grossmann, K.; Schiffer, H. Protoporphyrinogen oxidase inhibiting activity of the new, wheat-selective isoindoldione herbicide, cinidon-ethyl. *Pestic. Sci.* **1999**, *55*, 687–695.

(5) Dickmann, R.; Melgarejo, J.; Loubiere, P.; Montagnon, M. Oxadiargyl: A novel herbicide for rice and sugarcane. *Proceedings of the Brighton Crop Protection Conference—Weeds*; BCPC, Farnham: Surrey, United Kingdom, 1997; pp 51−57.

(6) Auti, K.; Trombini, A.; Giammarusti, L.; Sbriscia, C.; Harder, H.; Gabard, J. Azafenidin: A new low use rate herbicide for weed control in perennial crops, industrial weed control and forestry. *Proceedings of the Brighton Crop Protection Conference—Weeds*; BCPC, Farnham: Surrey, United Kingdom, 1997; pp 59−66.

(7) Van Saun, W. A.; Bahr, J. T.; Crosby, G. A.; Fore, Z. Q.; Guscar, H. L.; Harnish, W. N.; Hooten, R. S.; Marques, M. S.; Parrish, D. S.; Theodoridis, G.; Tymonko; J. M.; Wilson, K. R.; Wyle, M. J. F6285-A new herbicide for the post-emergence selective control of broad-leaved weeds soybeans. *Proceedings of the Brighton Crop Protection Conference—Weeds*; BCPC, Farnham: Surrey, United Kingdom, 1991; pp 77−82.

(8) Van Saun, W. A.; Bahr, J. T.; Bordouxhe, L. J.; Gargantiel, F. J.; Hotzman, F. W.; Shires, S. W.; Sladen, N. A.; Tutt, F. S.; Wilson, K. R. F8426-A new rapidly acting, low-rate herbicide for the postemergence selective control of broad-leaved weeds in cereals. *Proceedings of the Brighton Crop Protection Conference—Weeds*; BCPC, Farnham: Surrey, United Kingdom, 1993; pp 19−22.

(9) Zhu, Y.-Q.; Wu, C.; Li, H.-B.; Zou, X.-M.; Si, X.-K.; Hu, F.-Z.; Yang, H.-Z. Design, synthesis, and quantitative structure-activity relationship study of herbicidal analogues of pyrazolo[5,1-

d][1,2,3,5]tetrazin-4(3H)ones. *J. Agric. Food Chem.* **2007**, *55*, 1364–1369.
(10) Barraja, P.; Diana, P.; Lauria, A.; Montalbano, A.; Almerico, A. M.; Dattolo, G.; Cirrincione, G. Synthesis and antiproliferative activity of [1,2,3,5]tetrazino[5,4-a]indoles, a new class of azolotetrazinones. *Biol. Med. Chem.* **2004**, *13* (2), 295–300.
(11) Diana, P.; Barraja, P.; Lauria, A.; Almerico, A. M.; Dattolo, G.; Cirrincione, G. Pyrrolo[2,1-d][1,2,3,5]tetrazines, a new class of azolotetrazines related to the antitumor drug Temozolomide. *Synthesis* **1999**, *12*, 2082–2086.
(12) Marina, K.; Jean-Charles, L.; Patrick, D.; Sylvain, R. Synthesis of novel pyrazolopyrrolopyrazines, potential analogs of sildenafil. *J. Heterocycl. Chem.* **2001**, *38*, 1045–1050.
(13) Yoshida, K.; Matsuo, S.; Kitashima, T.; Tomiya, K.; Kodaka, K. 5-Aminopyrazole-4-carboxylate derivative and process for preparing the same. EP 1067121, 2001.
(14) Jiang, H.-J.; Sun, R.-Y.; Cai, J.-P. Synthesis of ethyl-3 (5)-aminopyrazole-4- carboxylate. *Huagong Shikan* **2005**, *19*, 12–13, in Chinese.
(15) Dyckman, A.; Das, J.; Leftheris, K.; Liu, C.-J.; Zhao, R.-L.; Chen, B.-C.; Wrobleski, S. T. Aryl-substituted pyrazole-amide compounds useful as kinase inhibitors. WO 099156, 2004.
(16) Suggs, J. A.; Srivastava, P. C. Synthesis and biodistribution of *p*-iodophenyl analogues of a naturally occurring imidazole ribonucleoside. *J. Heterocycl. Chem.* **1988**, *25*, 1331–1335.
(17) Baraldi, P. G.; Cacciari, B.; Romagnoli, R.; Spalluto, G.; Moro, S.; Klotz, K. N.; Leung, E.; Varani, K.; Gessi, S.; Merighi, S.; Borea, P. A. Pyrazolo[4,3-e]1,2,4-triazolo[1,5-c]pyrimidine derivatives as highly potent and selective human A3 adenosine receptor antagonists: Influence of the chain at the N8 pyrazole nitrogen. *J. Med. Chem.* **2000**, *43*, 4768–4780.
(18) Nagano, E.; Hashimoto, S.; Yoshida, R.; Matsumoto, H.; Oshio, H.; Kamoshita, K. Tetrahydrophthalimides, and their use. EP 0061741, 1982.
(19) Zhu, Y.-Q.; Zou, X.-M.; Hu, F.-Z.; Yao, C.-S.; Liu, B.; Li, Y.-H.; Yang, H.-Z. Synthesis and herbicidal evaluation of novel 3-(α-hydroxy-substitutedbenzylidine) pyrrolidine-2,4-diones. *J. Agric. Food Chem.* **2005**, *53*, 9566–9570.
(20) Witkowak, D. A.; Halling, B. P. Inhibition of plant protoporphyrinogen oxidase by the herbicide acifluorfen-methyl. *Plant Physiol.* **1989**, *90*, 1239–1242.
(21) Lee, H.; Duke, M. V.; Duke, S. O. Cellular localization of protoporphyrinogen-oxidizing activities of etiolated barley (*Hordeum vulgare L.*) leaves. *Plant Physiol.* **1993**, *102*, 881–889.
(22) Lee, H. J.; Duke, S. O. Protoporphyrinogen IX-oxidizing activities involved in the mode of action of peroxidizing herbicides. *J. Agric. Food Chem.* **1994**, *42*, 2610–2618.
(23) Böger, P.; Wakabayashi, K. *Peroxidizing Herbicides*; Springer-Verlag: Berlin, Herdelberg, 1999; pp 279−291.
(24) Jacobs, N. J.; Jacobs, J. M. Assay for enzymatic protoporphyrinogen oxidation, a late step in heme synthesis. *Enzyme* **1982**, *28*, 206–217.
(25) Li, H.-B.; Li, Y.-H.; Liu, B.; Wang, Y.-F.; Wu, C.; Li, B.; Zou, X.-M.; Yang, H.-Z. Study of method for measuring the inhibition of protoporphyrinogen oxidase activity *in vitro*. *Plant Physiol. Commun* **2007**, *43*, 1152–1156, in Chinese.
(26) Shepherd, M.; Dailey, H. A. A continuous fluorimetric assay for protoporphyrinogen oxidase by monitoring porphyrin accumulation. *Anal. Biochem.* **2005**, *344*, 115–121.
(27) Ishida, S.; Hirai, K.; Kohno, H.; Sato, Y.; Kubo, H.; Böger, P.; Wakabayashi, K. Protoporphyrinogen-IX oxidase inhibition by N-(2,4,5-trisubstituted phenyl)-3,4,5,6-tetrahydrophthalimides. *J. Pestic. Sci.* **1997**, *22*, 299–302.
(28) Johnson, W. O.; Kollman, G. E.; Swithenbank, C.; Yih, R. Y. RH-6201 (Blazer): A new broad spectrum herbicide for postemergence use in soybean. *J. Agric. Food Chem.* **1978**, *26*, 285–286.

Received for review June 11, 2008. Revised manuscript received August 5, 2008. Accepted August 5, 2008. We gratefully acknowledge the support of this work by the National Key Project for Basic Research (2003CB114400), the National Natural Science Foundation of China (20572054; 20772066), and the Research Foundation for the Doctoral Program of Higher Education (20050055042).

JF801774K