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Design, Synthesis, and Herbicidal Activity of Biaryl-Pyridazinone/Phthalimide Derivatives as Novel Protoporphyrinogen Oxidase Inhibitors

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ABSTRACT: In this study, a series of biaryl-pyridazinone/phthalimide derivatives were designed and synthesized as novel protoporphyrinogen IX oxidase (PPO) inhibitors. Herbicidal activity and crop safety assessments revealed that some compounds exhibited excellent herbicidal activity and crop safety profiles. For instance, at 37.5 g ai/ha, compound **7m** inhibited *Amaranthus retroflexus* (*AR*), *Abutilon theophrasti* (*AT*), *Medicago sativa* (*MS*), *Echinochloa crus-galli* (*EC*), and *Digitaria sanguinalis* (*DS*) with 90% to 100% efficacy, comparable to the commercial herbicide saflufenacil (SAF). Compound **7m** still showed effective weed control against the test broadleaf weeds at a lower dose of 9.375 g ai/ha. Additionally, **7m** demonstrated excellent safety for wheat and corn at dosages up to 150 g ai/ha. *In vitro* experiments revealed that the representative compounds exhibited significant inhibitory activity against *Arabidopsis thaliana* PPO (*At*PPO). Molecular docking of *Nicotiana tabacum* PPO (*Nt*PPO) and **7m** showed significant hydrogen bonding, $\pi-\pi$ stacking, and π-alkyl interactions between **7m** and residues, such as Cys-177, Thr-176, Arg-98, Phe-392, and Leu-372. Furthermore, **7m** caused a notable reduction in chlorophyll (Chl) content in weeds. **7m** is a promising candidate for the development of novel herbicides.

KEYWORDS: *PPO inhibitor, biaryl-pyridazinone/phthalimide derivatives, herbicidal activity, crop safety*

INTRODUCTION

Weeds compete with crops for nutritional resources, leading to a significant decrease in food production and causing substantial economic losses in agriculture.[1,2] This competition poses a serious threat to agricultural productivity.[3] Additionally, weeds serve as carriers of viruses and pests, further worsening their negative impact.[4] Consequently, effective weed control is crucial for ensuring stable and increased crop yields. Currently, chemical methods are the preferred choice for weed control due to their simplicity, efficiency, and low cost.[5,6] However, due to the use of high doses of pesticides and single-mode pesticides, serious weed resistance issues have arisen.[7,8] As a result, developing herbicides with novel targets and structures has become a primary focus for pesticide researchers.

Protoporphyrinogen IX oxidase (PPO, EC 1.3.3.4) is the final enzyme in the tetrapyrrole biosynthetic pathway, regulating the synthesis of chlorophyll (Chl) and heme.[9] PPO catalyzes the conversion of protoporphyrinogen IX to protoporphyrin IX.[10] In plants, protoporphyrin IX is a precursor to chlorophyll and is typically converted to chlorophyll in the presence of magnesium chelatase.[11−13] When PPO is inhibited, photosensitive protoporphyrin IX accumulates, leading to the buildup of reactive oxygen species (ROS), which ultimately causes plant death.[2,14,15] The critical functional characteristics make PPO a significant target for developing new herbicides. PPO inhibitors exhibit broad-spectrum, high-efficiency, low-resistance, and safety advantages for nontarget organisms, playing an important role in weed management in crop fields.[16−18] The first PPO herbicide, nitrofen, was commercialized in the early 1960s.[19,20] PPO herbicides were categorized as *N*-phenylimides, diphenyl ethers, *N*-phenyltriazolinones, and *N*-phenyl-oxadiazolones, according to the herbicide mechanism, with more than 20 compounds included.[21] However, certain PPO herbicides exhibit limited crop selectivity, restricting their postemergence application in crop fields. For instance, saflufenacil (SAF), a widely used PPO inhibitor, is limited to be used as a pre-emergence herbicide for certain crops or as a herbicide for noncultivated fields due to its poor crop selectivity.[22,23] Additionally, the long-term and extensive use of traditional PPO herbicides has led to resistance in certain weed species, further complicating their effectiveness.[24] These challenges underscore the necessity of developing new PPO inhibitors with high herbicidal activity and excellent crop selectivity to address these limitations and enhance weed management strategies.

In 2003, Gerhard Hamprecht et al. reported the phenyl-pyridines exhibit good herbicidal activity.[25] On this basis, our

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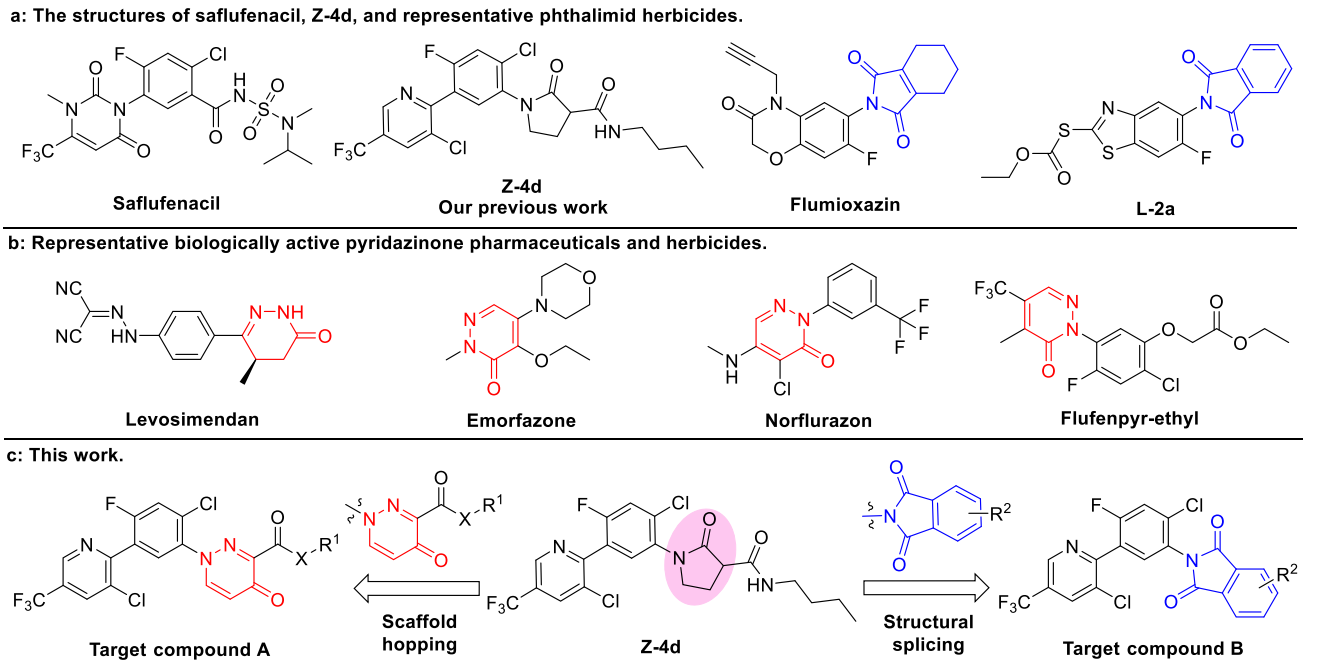
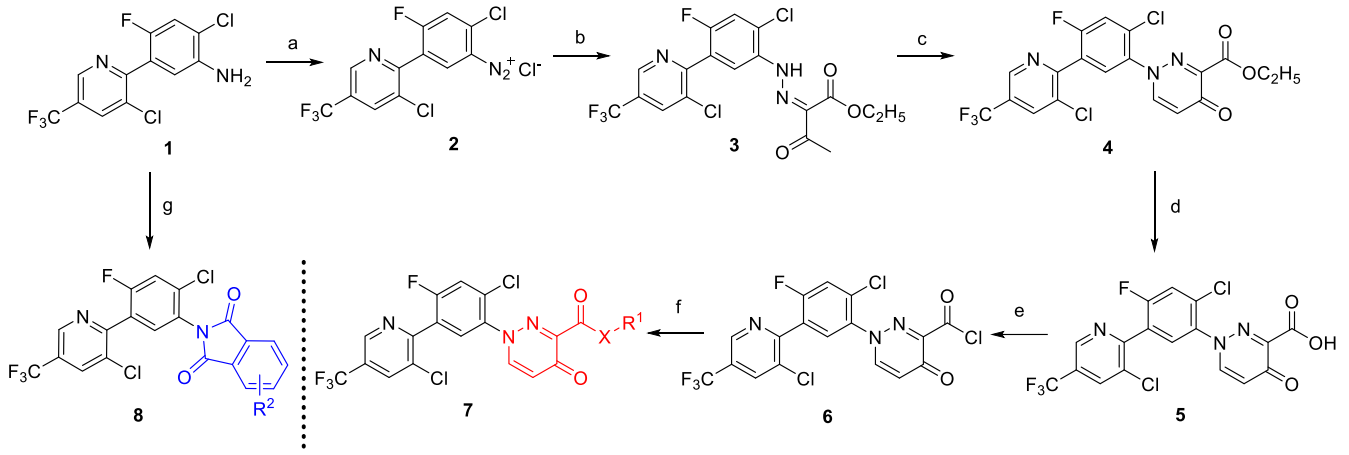


Figure 1. Design strategy of target compounds.

Scheme 1. Synthesis of Compounds 2–8[i]



[i]Reagents and conditions: (a) $NaNO_2$, HCl, 0 °C; (b) ethyl acetoacetate, EtOH/H_2O, KOAc, 0 °C; (c) DMF-DME, 80 °C; (d) LiOH, MeOH/H_2O = 1/1, r.t.; (e) oxalyl chloride, DCM, DMF, r.t.; (f) R^1NH_2 or R^1OH, Et_3N, DCM, 0 °C−r.t.; (g) (substituted) phthalic anhydride, AcOH, 80 °C.

research group developed a novel fluorinated phenylpyridine skeleton with excellent herbicidal activity by introducing trifluoromethylpyridine fragments to the halogen-substituted phenyl moiety of saflufenacil through a structure splicing strategy.[26] Subsequently, we designed and synthesized a series of 2-phenylpyridine derivatives containing pyrrolidone. Herbicidal activity and crop safety assessments indicated that compound **Z-4d** exhibited excellent herbicidal activity and high crop selectivity within the dosage range of 9.375 to 150 g ai/ha (Figure 1a).[27] It is noteworthy that the pyridazinone fragment is frequently used in pharmaceutical and agrochemical molecules, displaying excellent biological activities such as anticancer, anti-inflammatory, antibacterial, and herbicidal properties.[28−33] For instance, levosimendan and emorfazone are used as positive inotropic drugs for treating heart failure and as nonsteroidal anti-inflammatory drugs (NSAIDs), respectively.[32,34] Norflurazon is a phytoene desaturase (PDS) inhibitor with a pyridazinone structure that blocks the biosynthesis of carotenoids and chlorophyll in weeds, leading to leaf bleaching and death.[35] Flufenpyr-ethyl, developed by Sumitomo Chemical Company, is a high-efficiency PPO inhibitor containing a pyridazinone structure that quickly bleaches weed leaves and causes death after application (Figure 1b).[36] Additionally, in the field of agrochemicals, compounds containing a phthalimide structure are widely used in PPO inhibitor herbicides (Figure 1a, right).[37−39] For example, flumioxazin, developed by Sumitomo Chemical Company, is a highly effective herbicide containing *N*-aryl-3,4,5,6-tetrahydrophthalimide, providing extended weed control duration.[40] In 2011, Yang et al. reported that compound **L-2a**, which contains a phthalimide fragment, exhibits a higher inhibitory activity against PPO than sulfentrazone (Figure 1a).[41]

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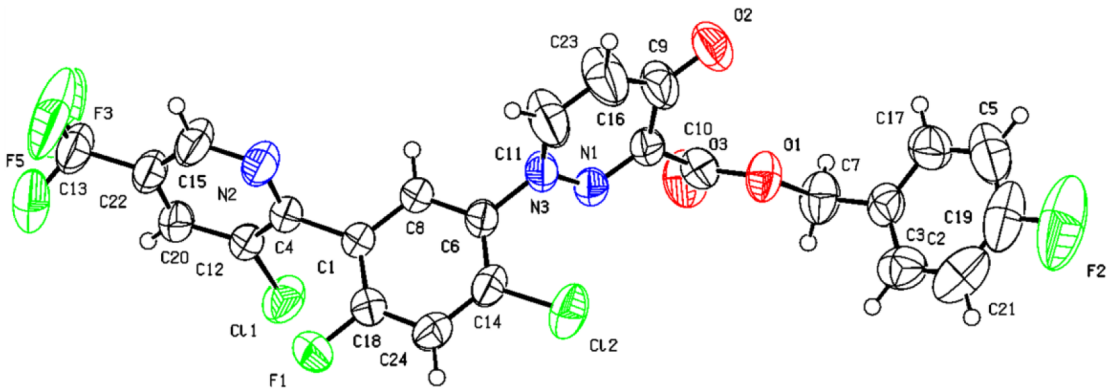

Figure 2. X-ray crystal structure of compound **7s**.

Based on the above analysis, we used pyridazinone and phthalimide pharmacophores to replace the five-membered ring in compound **Z-4d**, guiding by scaffold hopping and structural splicing strategies. Consequently, we designed and synthesized a series of new biaryl-pyridazinone derivatives and biaryl-phthalimide derivatives (Figure 1c). Systematic post-emergence herbicidal activity tests and crop safety studies of these compounds were conducted. In addition, the *Arabidopsis thaliana* PPO (*At*PPO) inhibitory activity of some representative compounds was evaluated *in vitro*. The results indicated that several synthesized target compounds exhibited excellent herbicidal activity and crop safety, with some also showing good activity against *At*PPO. Furthermore, chlorophyll content testing and molecular docking studies were performed to gain a deeper understanding of the inhibitory mechanism. This study suggests that biaryl-pyridazinone/phthalimide derivatives may serve as potential lead structures for the development of novel PPO inhibitors.

MATERIALS AND METHODS

Chemicals and Instruments. Commercially available materials purchased from Leyan (Shanghai, China), Bide (Shanghai, China), or Energy Chemistry (Anhui, China) were used directly in the experiment without further purification. The ^{1}H, ^{19}F, and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance III 400 MHz spectrometer (Bruker BioSpin AG, Germany) in deuterated chloroform ($CDCl_3$) with trimethylchlorosilane as the internal reference. High-resolution mass spectrometry (HRMS) was obtained on a Thermo Fisher Q Exactive mass spectrometer (Thermo Fisher Scientific, USA). The melting points of all the compounds were measured using a WGX-4B binocular microscopic melting point apparatus (Nanjing Weiguang Instrument and Equipment Co., Ltd., China).

Synthetic Procedures for Compounds 1−8. The synthesis of compound **1** followed the method reported in our previous report.[26] The synthetic routes for compounds **2**−**8** are shown in Scheme 1. The detailed synthetic methods and characterization data for these compounds, including ^{1}H, ^{13}C, and ^{19}F NMR spectra, HRMS, as well as melting points, are outlined in the Supporting Information.

X-ray Diffraction. The single crystal of compound **7s** was obtained through a process of slow evaporation from a chloroform-*d* solution. The supplementary crystallographic data for **7s** have been submitted and deposited in the Cambridge Crystallographic Data Centre (CCDC, http://www.ccdc.cam.ac.uk/) under deposition number 2364414. The crystal structure of **7s** is shown in Figure 2.

Herbicidal Activity Assay. The postemergence herbicidal activities of the target compounds **4**, **7**, and **8** against *Amaranthus retroflexus* (*AR*, AMARE), *Abutilon theophrasti* (*AT*, ABUTH) and *Medicago sativa* (*MS*, MEDSA), *Echinochloa crus-galli* (*EC*, ECHCR), *Digitaria sanguinalis* (*DS*, DIGSA) and *Lolium perenne* (*LP*, LOLPE) were evaluated. The herbicidal activities of all compounds were tested at a dose of 150 g ai/ha using methods similar to previous reports.[13,27] Before testing, weed seeds were sown in pots with an inner diameter of 7 cm and cultivated in a greenhouse at a temperature of 28 ± 1 °C and a relative humidity of 60% for 2 weeks. Weeds with uniform growth were selected as test subjects. Subsequently, compounds **4**, **7k**−**7n**, and **7p** were tested at the dose of 37.5−75 g ai/ha, and compounds **4**, **7k**, **7l**, and **7m** were further tested for herbicidal activity at 9.375 and 18.75 g ai/ha, respectively. SAF served as a positive control. Meanwhile, the solvent control group weeds were treated with an emulsion (*N*,*N*-dimethylformamide, DMF + Tween-80). After 14 days, the herbicidal activity of the compounds was evaluated by visually assessing the extent of weed damage.

In addition, to evaluate the herbicidal spectrum of compound **7m**, we conducted postemergence herbicidal activity tests against various weed species at a dose of 75 g ai/ha, including *Amaranthus tricolor* (AMATR), *Galium aparine* (GALAP), *Bassia scoparia* (BASSC), *Clinopodium polycephalum* (CLIPO), *Chenopodium album* (CHEAL), *Veronica polita* (VERPO), *Leonurus japonicus* (LEOJA), *Solanum nigrum* (SOLNI), *Portulaca oleracea* (POROL), *Amaranthus hybridus* (AMAHY), *Setaria viridis* (SETVI), *Eleusine indica* (ELEIN).

Crop Selectivity. Compounds **4**, **7k**, **7l**, and **7m** with excellent herbicidal activity were selected for crop safety testing. Three representative monocotyledonous plants (rice, wheat, and corn) and three representative dicotyledonous plants (cotton, soybean, and peanut) were selected as test subjects for crop safety studies in a greenhouse. Compounds were tested at dosages ranging from 37.5 to 150 g ai/ha, and SAF was selected as a positive control. The test methods were consistent with those we previously reported.[13,27] After 14 days of application, the crop safety of the compounds was evaluated according to the degree of crop damage.

***At*PPO Inhibitory Experiments.** The *in vitro* inhibitory activity against *At*PPO of the representative compounds was evaluated using a plant PPO enzyme-linked immunosorbent

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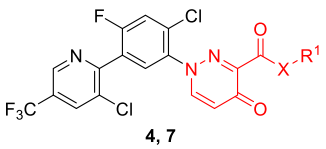
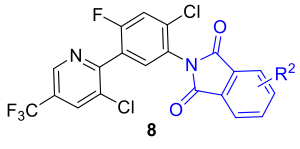
Table 1. Chemical Structure of Compounds 4, 7, and 8



4, 7



8

compd	X	R^1	compd	X	R^1	compd	R^2
4	O	CH_2CH_3	**7j**	NH	$CH_2C_6H_4$(4-Cl)	**8a**	H
7a	NH	CH_2CH_3	**7k**	O	CH_3	**8b**	4-CH_3
7b	NH	$(CH_2)_2CH_3$	**7l**	O	$(CH_2)_2CH_3$	**8c**	4-F
7c	NH	$(CH_2)_3CH_3$	**7m**	O	$(CH_2)_3CH_3$	**8d**	4-Cl
7d	NH	$CH(CH_3)_2$	**7n**	O	$CH(CH_3)_2$	**8e**	4-NO_2
7e	NH	$C(CH_3)_3$	**7o**	O	$C(CH_3)_3$	**8f**	4-$NHCOCH_3$
7f	NH	$CH_2C{\equiv}CH$	**7p**	O	$CH_2COOCH_2CH_3$	**8g**	5-OCH_3
7g	NH	$CH_2C_6H_5$	**7q**	O	$CH_2C_6H_5$	**8h**	5-$C(CH_3)_3$
7h	NH	$CH_2C_6H_4$(4-CH_3)	**7r**	O	$CH_2C_6H_4$(4-CH_3)	**8i**	5-F
7i	NH	$CH_2C_6H_4$(4-F)	**7s**	O	$CH_2C_6H_4$(4-F)	**8j**	5-Cl
						8k	5,6-diF
						8l	5,6-diCl
						8m	4,7-diCl
						8n	4,5,6,7-tetraCl

Table 2. Postemergence Herbicidal Activity of Compounds 4, 7, and 8

		% inhibition[a]								% inhibition					
compd	dosage g ai/ha	*AR*[b]	*AT*	*MS*	*EC*	*DS*	*LP*	compd	dosage g ai/ha	*AR*	*AT*	*MS*	*EC*	*DS*	*LP*
4	150	10	10	10	10	10	7		37.5	10	10	10	8	8	2
	75	10	10	10	10	10	6	**7o**	150	10	9	9	4	5	4
	37.5	10	10	10	9	10	5	**7p**	150	10	10	10	9	10	5
	18.75	10	10	9	8	9	2		75	10	10	10	9	10	5
	9.375	10	9	9	4	8	1		37.5	10	8	10	9	10	3
7a	150	10	10	9	1	1	0	**7q**	150	10	10	10	6	9	4
7b	150	10	10	9	2	3	1	**7r**	150	8	3	3	1	1	3
7c	150	10	10	9	2	3	1	**7s**	150	10	10	9	9	10	4
7d	150	10	10	9	3	5	2	**8a**	150	9	9	9	1	2	1
7e	150	9	7	9	1	2	1	**8b**	150	6	5	1	2	1	0
7f	150	10	9	9	1	2	2	**8c**	150	9	5	2	1	1	1
7g	150	10	8	8	1	2	1	**8d**	150	8	4	2	1	1	2
7h	150	10	6	7	0	2	0	**8e**	150	10	10	9	1	2	0
7i	150	10	9	9	1	2	1	**8f**	150	10	10	5	2	2	0
7j	150	10	9	8	1	2	0	**8g**	150	10	5	2	2	1	1
7k	150	10	10	10	10	10	7	**8h**	150	10	8	2	0	1	0
	75	10	10	10	9	10	5	**8i**	150	10	9	5	1	1	3
	37.5	10	10	10	9	9	4	**8j**	150	10	9	7	1	1	0
	18.75	10	9	9	8	9	2	**8k**	150	10	9	1	1	1	0
	9.375	10	8	9	4	8	1	**8l**	150	10	6	1	2	1	1
7l	150	10	10	10	10	10	7	**8m**	150	10	9	3	5	1	1
	75	10	10	10	10	10	7	**8n**	150	8	8	2	2	1	1
	37.5	10	10	10	9	10	4	**Z-4d**	150	10	10	10	8	8	6
	18.75	10	10	9	5	9	2		75	10	10	10	7	7	5
	9.375	10	9	9	4	8	1		37.5	10	10	9	5	7	4
7m	150	10	10	10	10	10	7		18.75	10	10	9	2	4	2
	75	10	10	10	10	10	7		9.375	10	10	9	1	4	1
	37.5	10	10	10	9	10	7	SAF[c]	150	10	10	10	10	10	9
	18.75	10	10	9	8	9	2		75	10	10	10	10	10	9
	9.375	10	9	9	5	8	1		37.5	10	10	10	9	10	8
7n	150	10	10	10	9	9	4		18.75	10	10	10	9	9	5
	75	10	10	10	9	9	4		9.375	10	10	10	9	8	3

[a]Rating scale of inhibition percent in relation to the untreated control: 10, 100%; 9, 99−90%; 8, 89−80%; 7, 79−70%; 6, 69−60%; 5, 59−50%; 4, 49−40%; 3, 39−30%; 2, 29−20%; 1, 19−0%. [b]Abbreviations: *AR, Amaranthus retroflexus*; *AT, Abutilon theophrasti*; *MS, Medicago sativa*; *EC, Echinochloa crus-galli*; *DS, Digitaria sanguinalis*; *LP, Lolium perenne*. [c]Saflufenacil (SAF).

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assay (ELISA) kit that produced by Jiangsu Meimian Industrial Co., Ltd. (Jiangsu Province, China). The kit employs a double-antibody sandwich method to measure the PPO levels in samples.[42] The experimental procedure was similar to that previously reported.[27,42,43] Before the assay, the standard PPO enzyme was diluted to concentrations within the detection range of the kit and plated on a cell culture plate with 100 μL per well. Solutions of tested compounds with five different concentrations were prepared and submitted to interact with the PPO enzyme, while dimethyl sulfoxide (DMSO) was used as the blank control. The samples were incubated at 37 °C for 2 h, after which the supernatant was transferred to centrifuge tubes and centrifuged to collect the supernatant for further testing. The enzyme content in the samples was then measured using the kit according to the manufacturer's instructions. The half maximal inhibitory concentration (IC_{50}) value was calculated by the linear regression equation established based on the inhibition rates and the logarithms of the corresponding concentrations.

Molecular Docking. The crystal structure of *Nicotiana tabacum* PPO (*Nt*PPO, PDB code: 1SEZ) was obtained from the Protein Data Bank (PDB) and used as the receptor for molecular docking studies. Compound **7m** was constructed and optimized using Chem3D 20.0 before its use. AutoDockTools 1.5.6 was employed to prepare the ligand and receptor, and AutoDock 4.2 was used to predict the binding mode of compound **7m** with *Nt*PPO. The interaction results between the compound and receptor were visualized using PYMOL 1.8.6.[13]

Determination of the Chl Content. The Chlorophyll Content Determination Kit (BC0995; Solarbio) was employed to assess the Chl content in the leaves of *AT*. Specifically, compound **7m** was applied at a dose of 37.5 g ai/ha to *AT* leaves at the fourth leaf stage, and leaf tissue was collected 48 h postapplication. Chl content was determined according to the specific experimental methods outlined in the kit's instructions.[44,45]

RESULTS AND DISCUSSION

Synthesis. As shown in Scheme 1 and Table 1, a total of 34 target compounds were synthesized. These compounds can be divided into two categories (compounds **4**, **7** and **8**) based on their structures. Detailed synthetic procedures and NMR characterization data, including ^{1}H, ^{13}C, and ^{19}F spectra, are provided in the Supporting Information. Compound **1** was synthesized following the method previously reported by our group.[26]

Synthesis of Compound 4 and 7. Aniline **1** underwent diazotization with sodium nitrite ($NaNO_2$) and concentrated hydrochloric acid (HCl) to form a diazonium salt intermediate (**2**). The intermediate **2** then reacted with ethyl acetoacetate in the presence of potassium acetate (KOAc) to produce compound **3**. Compound **3** further reacted with *N*,*N*-dimethylformamide dimethyl acetal (DMF-DME) to yield compound **4**. Subsequently, compound **4** was dissolved in a methanol−water mixture and hydrolyzed in the presence of lithium hydroxide (LiOH). Carboxylic acid **5** then reacted with oxalyl chloride in dichloromethane (DCM) to form acyl chloride **6**.[46] Finally, acyl chloride **6** reacted with alcohols or amines in DCM in the presence of triethylamine (Et_3N) to afford the target compounds **7a** to **7s**,[47] with yields ranging from 54% to 89%.

Synthesis of Compound 8. Compounds **8a** to **8n** were prepared by reacting aniline **1** with (substituted) phthalic anhydride in glacial acetic acid (AcOH) under 80 °C for 2 h,[41] with yields ranging from 41% to 87%.

Herbicidal Activity. The herbicidal activities of 34 target compounds against three typical broadleaf weeds and three grass weeds were evaluated in a greenhouse environment. The PPO herbicide SAF was selected as the positive control. Notably, the weeds exhibited injury symptoms after treatment with our compounds, similar to the fading green whitening and drying out symptoms caused by SAF. This supports our compounds act as PPO inhibitors. As shown in Table 2, most compounds exhibited significant herbicidal activity at an application dose of 150 g ai/ha. Specifically, they demonstrated excellent herbicidal activity against broadleaf weeds, with marked differences in efficacy against grass weeds. Compounds **4**, **7k**, **7l**, **7m**, **7n**, and **7p** showed markedly higher herbicidal activity than other compounds. Thus, their herbicidal activity was further evaluated at doses of 75 and 37.5 g ai/ha, respectively. Compounds **4** and **7k**−**7n** maintained 100% inhibition against *AR*, *AT*, and *MS* at a dose of 37.5 g ai/ha, showing comparable efficacy to compound **Z-4d** (100%, 100%, 90%) and the positive control SAF (100%, 100%, 100%). Meanwhile, compounds **4**, **7l**, and **7m** exhibited herbicidal activity above 90% against *EC* and *DS*, superior to **Z-4d** (50%, 70%) and similarly effective as the positive control SAF (90%, 100%). However, herbicidal activity against *LP* was slightly lower compared to SAF. At a lower dose of 9.375 g ai/ha, compounds **4**, **7k**, **7l**, and **7m** achieved 100% inhibition against *AR*, equivalent to SAF. In addition, these compounds demonstrated 80% inhibition of *DS*, which is comparable with SAF (80%) and superior to **Z-4d** (40%). These findings underscored the promising herbicidal potential of compounds **4**, **7k**, **7l**, **7m**, **7n**, and **7p**, suggesting their suitability for further development as effective herbicides.

Structure−Activity Relationship (SAR). As shown in Table 2 and Figure 3, compounds **4**, **7**, and **8** exhibited higher inhibition rates against broadleaf weeds than grass weeds. Compound **4** and **7** that contain a pyridazinone fragment exhibited significantly higher herbicidal activity than com-

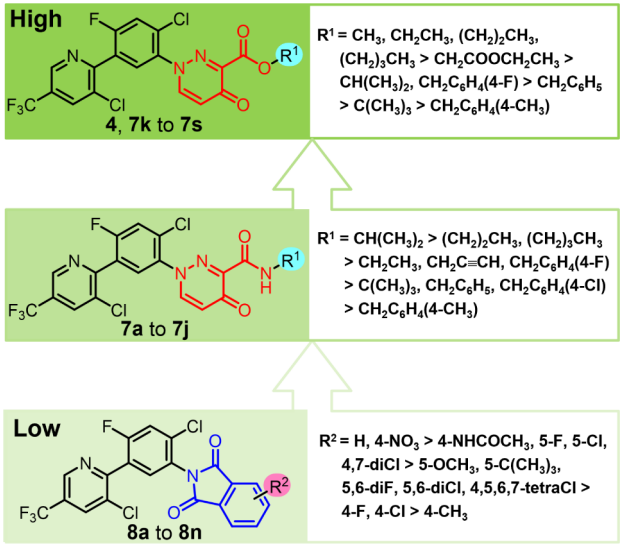

Figure 3. Relationship between structure and herbicidal activity of compounds **4**, **7**, and **8**.

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pound **8** which contains a phthalimide structure. Different pyridazinone substituents and nitrogen heterocycles exhibited varying herbicidal activities. Additionally, we found that replacing the pyrrolidone structure with a pyridazinone structure slightly decreased the herbicidal activity against *AT* but significantly improved the herbicidal activity against *EC* and *DS*. As indicated in Table 2 and Figure 3, the substituents on the X and R^1 positions significantly affected the herbicidal activity of pyridazinone derivatives **4** and **7**. Pyridazinone derivatives containing the O group (X = O) generally showed higher herbicidal activity than NH-substituted compounds (X = NH). When X = O and R^1 were ethyl (**4**), methyl (**7k**), propyl (**7l**), or butyl (**7m**), the compounds exhibited excellent herbicidal activity. The butyl-substituted compound (**7m**) was the most potent herbicide among these. Switching R^1 to isopropyl (**7n**) or $CH_2COOCH_2CH_3$ (**7p**) slightly decreased the herbicidal activity. The herbicidal activity of **7p** was superior to that of **7n**. Herbicidal activity significantly decreased with *tert*-butyl substitution at R^1 (**7o**). When benzyl occupied the R^1 position (**7q**), the herbicidal activity of benzyl alcohol ester was lower than that of isopropyl substitution (**7n**). Electron-donating substituents at the 4-position (**7r**) led to a substantial decrease in herbicidal activity. In contrast, electron-withdrawing group at the same position (**7s**) gave higher herbicidal activity than that of the benzyl (**7q**) substituent and second only to the $CH_2COOCH_2CH_3$ (**7p**) substituent. When X = NH and R^1 were ethyl (**7a**), propyl (**7b**), or butyl (**7c**), the compounds exhibited limited herbicidal activity. Furthermore, herbicidal activity improved when R^1 was isopropyl (**7d**) and was the highest among NH-substituted compounds. Similar to the ester derivative (**7o**), the herbicidal activity decreased with *tert*-butyl substitution at R^1 (**7e**). When R^1 was propargyl (**7f**), herbicidal activity was slightly lower than butyl (**7c**). Switching R^1 to benzyl (**7g**) led to decrease the herbicidal activity. Introducing an electron-donating group at the 4-position of the benzene ring (**7h**) further reduced herbicidal activity. In contrast, introducing electron-withdrawing groups (**7i**, **7j**) at the same position enhanced herbicidal activity against broadleaf weeds. For compound **8**, when the *N*-heterocycle linked to the biaryl group was a phthalimide, some compounds exhibited good herbicidal activity against *AR* and *AT* at a dose of 150 g ai/ha, but showed weak herbicidal activity against grass weeds.

Weeding Spectrum Experiments. To investigate the herbicidal spectrum of compound **7m**, a postemergence herbicidal activity assay against 18 weed species was conducted at a dose of 75 g ai/ha. SAF was used as a positive control. As shown in Figure 4, compound **7m** exhibited inhibition rates ranging from 70% to 100% across the tested weed species, while SAF showed inhibition rates of 90% to 100%. The herbicidal activity of compound **7m** against broadleaf weeds was comparable to that of SAF. The activity against LOLPE, SETVI, and ELEIN was lower than that of SAF.

Crop Selectivity. To further evaluate the potential of our synthesized target compounds as candidate herbicides, postemergence crop safety tests of representative compounds such as **4**, **7k**, **7l**, and **7m** were conducted at dosages ranging from 37.5 to 150 g ai/ha. Six representative crops (rice, wheat, corn, soybean, cotton, and peanut) were selected for safety evaluation. As shown in Table 3, at a dosage of 150 g ai/ha, compound **7l** exhibited excellent safety for rice, wheat, and corn, significantly outperforming the commercial herbicide SAF. Compounds **4**, **7k**, and **7m** also demonstrated excellent

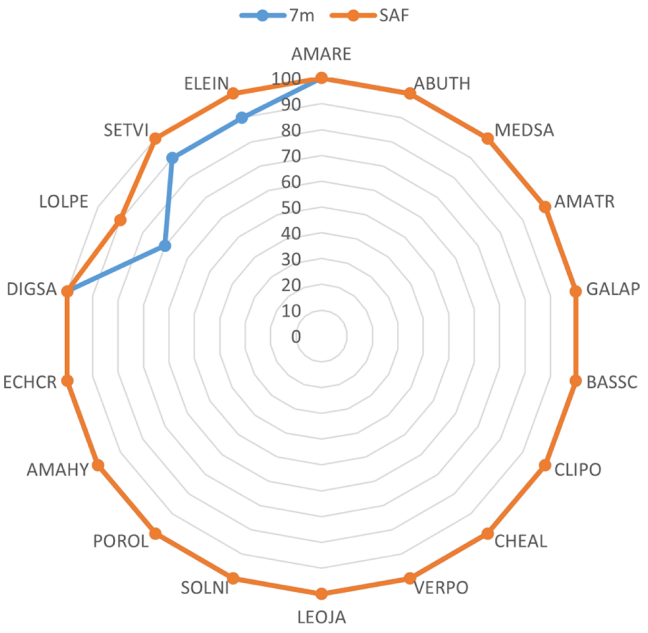

Figure 4. Postemergence herbicidal spectrum of compounds **7m** and SAF.

Table 3. Postemergence Crop Selectivity of Compounds 4, 7k, 7l, and 7m

		% inhibition[a]					
compd	dosage g ai/ha	rice	wheat	corn	soybean	cotton	peanut
4	150	6	3	3	10	9	8
	75	4	3	2	9	9	7
	37.5	2	1	1	9	7	3
7k	150	6	4	2	10	9	8
	75	4	3	1	9	9	7
	37.5	2	0	0	9	8	3
7l	150	2	2	1	8	9	6
	75	2	1	1	8	8	3
	37.5	2	1	0	8	8	2
7m	150	6	2	1	8	8	8
	75	3	2	1	8	8	7
	37.5	1	0	1	8	7	3
SAF	150	8	5	7	10	10	8
	75	7	5	6	10	10	7
	37.5	6	4	4	10	10	5

[a]Rating scale of inhibition percent in relation to the untreated control: 10, 100%; 9, 99−90%; 8, 89−80%; 7, 79−70%; 6, 69−60%; 5, 59−50%; 4, 49−40%; 3, 39−30%; 2, 29−20%; 1, 19−10, 0, 9−0%.

safety for wheat and corn. Notably, compounds **7l** and **7m** caused only about 10% injury to corn even at 150 g ai/ha, far less than the 70% injury rate caused by SAF. At dosages of 37.5 and 75 g ai/ha, these compounds demonstrated superior safety for rice, wheat, and corn compared to SAF, with compound **7l** also showing excellent safety for peanuts. When applied at 37.5 g ai/ha, these compounds caused minimal injury to peanuts. However, within the dosage range of 37.5 to 150 g ai/ha, soybean and cotton were severely damaged by compounds **4**, **7k**, **7l**, and **7m**. At dosages of 75 and 150 g ai/ha, peanuts also exhibited high sensitivity to compounds **4**, **7k**, and **7m**, with injury rates comparable to those caused by SAF. Despite the limited selectivity of compounds **7l** and **7m** for soybean and cotton, their selectivity for these crops was still significantly

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higher than that of SAF. Therefore, the results indicated that compound **7l** has potential as a herbicide for rice, wheat, and corn fields at a dosage of 150 g ai/ha, and for peanut fields at 75 g ai/ha. Compounds **4**, **7k**, and **7m** have potential as herbicides for rice, wheat, and corn fields at a dosage of 75 g ai/ha, and they can be used in peanut fields at a dosage of 37.5 g ai/ha.

PPO Inhibitory Activity. To evaluate the inhibitory effects of our compounds on *At*PPO, representative compounds were subjected to *in vitro* experiments, as shown in Figure 5. The

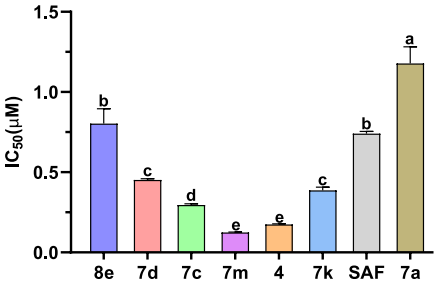

Figure 5. *At*PPO inhibitory activity of representative compounds and SAF. Based on one-way ANOVA ($p < 0.05$), distinct lowercase letters signify values with significant differences between treatment groups.

results indicated that some compounds exhibit superior inhibition activity compared to the commercial herbicide SAF (IC_{50} = 0.741 ± 0.014 μM). For instance, compounds **4** (IC_{50} = 0.175 ± 0.003 μM), **7c** (IC_{50} = 0.296 ± 0.008 μM), **7d** (IC_{50} = 0.452 ± 0.008 μM), **7k** (IC_{50} = 0.387 ± 0.020 μM), and **7m** (IC_{50} = 0.124 ± 0.003 μM) all demonstrated higher PPO inhibitory activity than SAF (IC_{50} = 0.741 ± 0.014 μM). Notably, compound **7m** exhibited the lowest IC_{50} value among all tested synthesized compounds, indicating the highest inhibition activity for *At*PPO, consistent with the herbicidal activity data. These findings supported the view that the synthesized compounds function as PPO inhibitors.

Molecular Docking. To gain insights into the inhibition mechanism of the highly active herbicidal compound at the molecular level, we performed molecular docking studies on the interaction between compound **7m** and *Nt*PPO. As depicted in Figure 6, the binding mode of compound **7m** with *Nt*PPO involved hydrogen bonding, $\pi-\pi$ stacking, and π-alkyl interactions. The trifluoromethyl group on compound **7m** formed a 4.0 Å hydrogen bond with Cys-177, the carbonyl group of the pyridazinone moiety formed a 2.9 Å hydrogen bond with Thr-176, and the ester group formed a 3.2 Å hydrogen bond with Arg-98. Additionally, the phenyl ring of Phe-392 engaged in a $\pi-\pi$ stacking interaction with the pyridine ring of compound **7m**, with a distance of 4.3 Å. In contrast, the trifluoromethyl group on the commercial herbicide SAF did not form a hydrogen bond with Cys-177. However, a stronger $\pi-\pi$ stacking interaction was observed between the pyridine ring and the phenyl ring of Phe-392, with a distance of 3.8 Å. Additionally, the carbonyl and sulfonyl groups of SAF formed hydrogen bonds with Arg-98, with the bond lengths being 2.8 and 3.1 Å, respectively. Both compound **7m** and SAF exhibited π-alkyl interactions with Leu-372. Overall, compound **7m** and SAF interacted with residues in the active site pocket through similar types of interaction. Finally, the binding energies between *Nt*PPO and compound **7m** and SAF were calculated to be −10.5 and −8.8 kcal/mol, respectively, confirming the above conclusion. This result further emphasized the potential of compound **7m** as an efficient PPO inhibitor.

Determination of the Chl Content. To investigate the inhibitory effect of compound **7m** on Chl in weeds, the Chl content was measured on *AT* leaves after being treated with **7m**. As shown in Figure 7, compared to the blank control

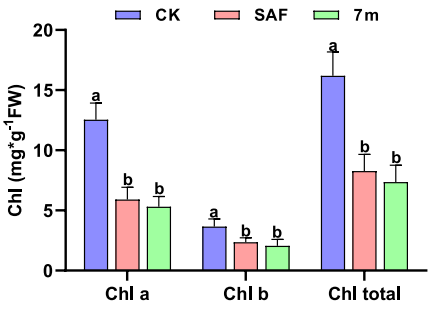

Figure 7. Chl content of compound **7m** and SAF treated *AT* after 48 h. Based on one-way ANOVA ($p < 0.05$), distinct lowercase letters signify values with significant differences between treatment groups.

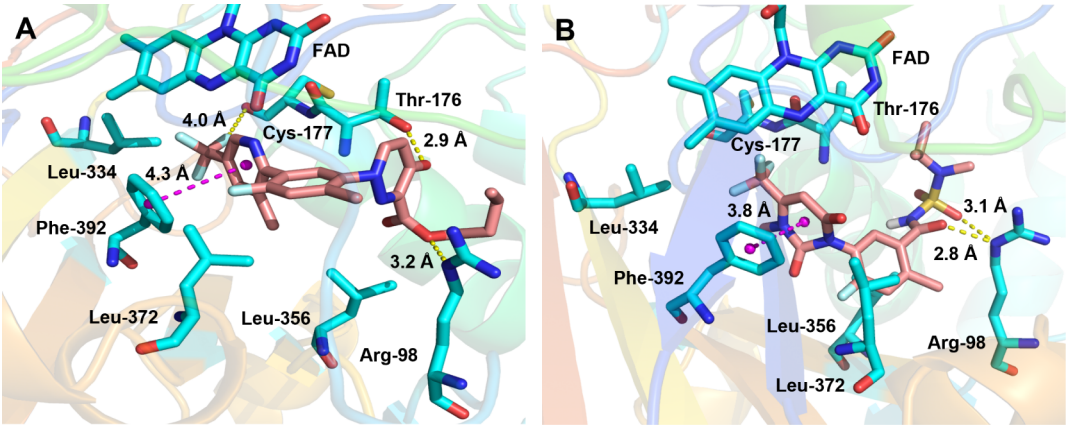

Figure 6. Molecular docking of compound **7m** and SAF with *Nt*PPO. The compound **7m** and SAF are shown as rose gold sticks. The key residues in the active pocket are shown in cyan sticks, the hydrogen bond is depicted as yellow dashed lines, and the $\pi-\pi$ stacking is depicted as magenta dashed lines. (A) Simulated binding modes of **7m** with *Nt*PPO. (B) Simulated binding modes of SAF with *Nt*PPO.

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(CK), significant reductions in Chl a and Chl b content were observed in *AT* leaves after being treated with **7m**, with inhibition rates being 57.7% and 43.8%, respectively. These are comparable to the positive control SAF (52.9%, 35.3%). These results revealed that our synthesized compounds effectively inhibit the Chl in weeds, supporting their role as PPO inhibitors.

In summary, we have developed two series of compounds: biaryl-pyridazinone derivatives (compounds **4** and **7a** to **7s**) and biaryl-phthalimide derivatives (compounds **8a** to **8n**) as novel PPO inhibitors. A systematic herbicidal activity evaluation of 34 synthesized compounds revealed significant efficacy. At a dosage of 150 g ai/ha, compounds **4**, **7k**, **7l**, and **7m** exhibited 100% inhibition against *AR, AT, MS, EC, and DS*, comparable to the commercial formulation SAF. Notably, at a dosage of 9.375 g ai/ha, these compounds maintained an 80% to 100% inhibition rate against *AR, AT, MS,* and *DS*. Crop safety tests indicated that compound **7l** can be applied at 150 g ai/ha in rice, wheat, and corn fields. Compounds **4**, **7k**, and **7m** are suitable for rice, wheat, and corn fields at dosages of 75 g ai/ha. *In vitro* inhibition experiments indicated that most tested compounds exhibited superior *At*PPO inhibition compared to SAF, with compound **7m** exhibiting the highest efficacy. Molecular docking analysis highlighted significant hydrogen bonding, $\pi-\pi$ stacking, and π-alkyl interactions between compound **7m** and amino acid residues (Cys-177, Thr-176, Arg-98, Phe-392, and Leu-372). Furthermore, compound **7m** led to a significant reduction of the Chl content in the weeds. Our research findings have identified compound **7m** as a promising candidate for developing novel herbicides. Additionally, this study suggests that the pyridazinone/phthalimide scaffold can serve as valuable pharmacophores for developing efficient PPO inhibitors.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.4c07526.

Detailed synthetic routes, characterization data, ^{1}H, ^{13}C, ^{19}F NMR spectra, HRMS spectra for the target compounds, and the crystal structure of **7s** (PDF)

CIF of **7s** (CIF)

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Notes

The authors declare no competing financial interest.

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