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An Efficient One-Pot Synthesis of 2-(Aryloxyacetyl)cyclohexane-1,3-diones as Herbicidal 4-Hydroxyphenylpyruvate Dioxygenase Inhibitors

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Supporting Information

ABSTRACT: 4-Hydroxyphenylpyruvate dioxygenase (EC 1.13.11.27, HPPD) is an important target for new bleaching herbicides discovery. As a continuous work to discover novel crop selective HPPD inhibitor, a series of 2-(aryloxyacetyl)-cyclohexane-1,3-diones were rationally designed and synthesized by an efficient one-pot procedure using *N*,*N*′-carbonyldiimidazole (CDI), triethylamine, and acetone cyanohydrin in CH_2Cl_2. A total of 58 triketone compounds were synthesized in good to excellent yields. Some of the triketones displayed potent in vitro *Arabidopsis thaliana* HPPD (*At*HPPD) inhibitory activity. 2-(2-((1-Bromonaphthalen-2-yl)oxy)acetyl)-3-hydroxycyclohex-2-en-1-one, **II-13**, displayed high, broad-spectrum, and postemergent herbicidal activity at the dosage of 37.5−150 g ai/ha, nearly as potent as mesotrione against some weeds. Furthermore, **II-13** showed good crop safety against maize and canola at the rate of 150 g ai/ha, indicating that **II-13** might have potential as a herbicide for weed control in maize and canola fields. **II-13** is the first HPPD inhibitor showing good crop safety toward canola.

KEYWORDS: *one-pot synthesis, 4-hydroxyphenylpyruvate dioxygenase, triketone, structure−activity relationship, herbicidal activity*

INTRODUCTION

4-Hydroxyphenylpyruvate dioxygenase (EC 1.13.11.27, HPPD) is an important enzyme in the tyrosine metabolic pathway, belonging to the large family of 2-his-1-carboxylate facial triad oxidative enzymes.[1−3] HPPD catalyzes the biotransformation of 4-hydroxyphenylpyruvic acid (HPPA) to homogentisic acid (HGA). In planta, homogentisic acid is a very essential compound for the biosynthesis of plastoquinone and tocopherols. Once HPPD is inhibited, the photosynthesis of plants will be blocked. When exposed to sunlight, the plants will be severely damaged by ultraviolet light, developing unique bleaching symptoms, leading to necrosis and death. Therefore, HPPD is an important target for herbicide discovery. HPPD herbicides have many advantages, such as low toxicity, high herbicidal activity, excellent crop selectivity, wide-spectrum weed control, inclusive of resistant biotypes, and benign environment effects.[4−6]

To date, several categories of HPPD-inhibiting compounds have been discovered and used in agriculture, such as triketone derivatives, isoxazoles (diketonitriles), pyrazoles, and others.[7,8] Among them, about 13 compounds are widely used in agriculture for weed control. Mesotrione, sulcotrione, topramezone, pyrasulfotole, and bicyclopyrone are popular HPPD herbicides with excellent weed control and good crop selectivity. According to the crystal structures of HPPD, the catalytic center of this enzyme is Fe^{II}.[9] Fe^{II} can form an octahedral complex with three water molecules, one glutamic acid, and two histidines. During the catalytic processes of HPPD, the substrate HPPA first chelates to Fe^{II} and is then oxidized to homogentisic acid in the presence of oxygen. HPPD inhibitors can compete with HPPA chelating Fe^{II}; therefore, bearing an Fe^{II} chelating group is the minimum requirement of these compounds. As reported, the minimum substructure of almost all of the HPPD-inhibiting compounds is mainly based on 2-heteroaroylethen-1-ol or 2-benzoylethen-1-ol. To make the binding affinity of the inhibitors tighter to HPPD, the benzoyl or heteroaroyl moieties were generally modified with hydrophobic groups. Currently, several HPPD inhibitors with new benzoyl and heteroaroyl moieties have been discovered and patented, including uracil-5-carboxamides, 6-acyl-1,2,4-triazine-3,5-diones, and pyridine ketones.[10−13]

Generally, the synthetic methods for triketone-containing HPPD inhibitors consist of two reaction steps.[14,15] First, activated aroyl acids are reacted with 1,3-cyclohexanediones to form O-acylated intermediates, followed by O−C rearrangement in the presence of appropriate catalysts to afford the triketone compounds. Various catalysts, for example, 1,2,4-triazole, cyanide, aluminum chloride, and potassium fluoride, can be used for this isomerization process.[16] Many studies have proven that modification of the benzoyl side chain of triketones is an effective way to get new potent herbicides. Little effort has been made on modifying the carbon−carbon bond between the triketones and side chains. We are very interested in the

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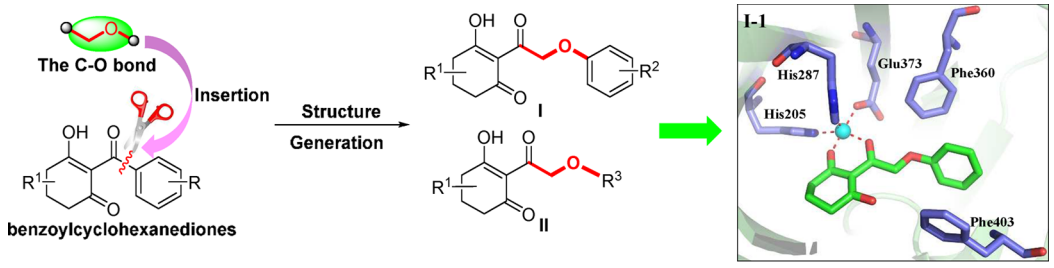

Figure 1. Design strategy of 2-(aryloxyacetyl)cyclohexane-1,3-diones and the simulated binding mode of compound **I-1** with *At*HPPD. The key residues in the active site are shown in blue sticks, the Fe^{II} is shown as a cyan sphere, and **I-1** is shown in green sticks.

herbicidal activity of triketone analogues and performed work on new HPPD inhibitor identification based on the triketone scaffold. Previously, we have reported the herbicidal and HPPD inhibitory activities of triketone-containing quinazoline-2,4-diones and quinolines.[17−20] In our continuing effort to search for novel HPPD-inhibiting compounds, we inserted a carbon−oxygen bond between the triketones and side chains in the current study (Figure 1). Through this strategy, we were hoping the aryl group in the extended side chain could form a more favorable sandwich $\pi-\pi$ interaction with residues Phe360 and Phe403 of *Arabidopsis thaliana* HPPD (*At*HPPD) in the active pocket (Figure 1). Additionally, an efficient one-pot synthesis of 2-(aryloxyacetyl)cyclohexane-1,3-diones **I** and **II** was successfully developed to prepare the title compounds. Fortunately, a candidate with promising herbicidal activity and crop selectivity was identified in the primary bioevaluation.

MATERIALS AND METHODS

Reagents and Procedures. All chemical reagents (Thermo Fisher Scientific, Waltham, MA, USA; J&K Scientific Ltd., Beijing, China) were commercially available and prepared with standard methods before use. Organic solvents were redistilled according to the standard method before experiment. A thin layer chromatography (TLC) plate (Qingdao Makall Group Co., Ltd., Qingdao, China) was monitored at 254 nM. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury-Plus 600/400 spectrometer (Varian Inc., Palo Alto, CA, USA) in $CDCl_3$ or DMSO-d_6 with TMS as the internal reference. High-resolution mass spectra (HRMS) were taken on an Agilent 6224 TOF LC/MS (Agilent Technologies, Santa Clara, CA, USA). Melting points were obtained on a Buchi B-545 melting point apparatus (Büchi Labortechnik, Flawil, Switzerland) and are uncorrected.

*General Method for the Prepare of Aryloxyacetic Acid Derivatives **3** and **5**.* The key intermediate aryloxyacetic acids **3** and **5** were synthesized following a reported procedure.[21−23] The appropriate phenol **1** or **4** (20 mmol), DMSO (80 mL), and K_2CO_3 (30 mmol) were added successively to a 200 mL flask with stirring, and the mixture was heated to 50 °C for 0.5 h. Ethyl bromoacetate (5.01 g) was added to the mixture in 10 min, and the suspension was stirred for 6 h. After completion of the reaction according to TLC detection, the mixture was cooled to 25 °C, poured into water, and stirred for another 0.5 h. To the solution was added 200 mL of ethyl acetate, and the ethyl acetate layer was separated and concentrated by rotary evaporation. Fifty milliliters of acetone and 50 mL of water were added to the resulting residue, and then NaOH (1.6 g) was added to the solution with stirring. The solution was heated to 50 °C for 3 h. After completion of the reaction based on TLC detection, acetone was removed by rotary evaporation. The resulting solution was cooled to 25 °C and acidified by HCl solution (12 mol/L) to pH 1−2. The resulting solid was collected by filtration, washed with water, and dried in a vacuum to afford acids **3** and **5**.

*General Method for the Synthesis of 2-(Aryloxyacetyl)-cyclohexane-1,3-diones **I** and **II**.* Aryloxyacetic acid **3** or **5** (2 mmol) and 40 mL of CH_2Cl_2 were added to a 150 mL flask. Three millimoles of *N*,*N*′-carbonyldiimidazole (CDI) was added to the solution at 25 °C, the solution was stirred for another 30 min, then substituted 1,3-cyclohexanediones (0.12 g), Et_3N (0.4 g), and acetone cyanohydrin (0.02 g) were added successively to the mixture, and the solution was stirred for 12 h. After completion of the reaction according to TLC detection, aqueous HCl solution (1 mol/L, 30 mL) was added and the solution stirred vigorously for 0.5 h. The CH_2Cl_2 layer was separated by a separating funnel and washed with saturated NaCl solution, dried by Na_2SO_4, concentrated by rotary evaporation, and recrystallized from methanol to afford the corresponding triketone series **I** and **II** compounds.

The detailed ^{1}H NMR, ^{13}C NMR, and HRMS data for triketone series **I** and **II** compounds are shown in the Supporting Information.

X-ray Diffraction. **I-31** was recrystallized from methanol to afford a suitable single crystal. White crystals of **I-31** (0.12 mm × 0.10 mm × 0.10 mm) were mounted on a quartz fiber with protection oil. Cell dimensions and intensities were measured at 298 K on a Bruker SMART APEX DUO area detector diffractometer (Bruker AXS, Madison, WI, USA) with graphite monochromated Mo Kα radiation (λ = 0.71073 Å); θ_{max} = 30.00; 13815 measured reflections; 4058 independent reflections (R_{int} = 0.0319). The data sets were integrated and reduced using SAINT Plus Programme.[24] Data were corrected for Lorentz and polarization effects and for absorption (T_{max} = 0.9723; T_{min} = 0.9669). The structure was solved by direct method using SHELXS97 and refined with SHELXL970.[25] Full-matrix least-squares refinement based on F^2 using the weight of $1/[\sigma^2(Fo^2) + (0.0774P)^2 + 0.1971P]$ gave final values of R_1 = 0.0429, ωR_2 = 0.1337, and GOF(F) = 1.045 for 202 variables, 202 parameters, and 4058 contributing reflections. Maximum shift/error = 0.002, and maximum/minimum residual electron density = 0.278 per −0.211 e Å^{-3}. Hydrogen atoms were observed and placed at their ideal positions with a fixed value of their isotropic displacement parameter.

We have deposited the crystallographic data for compound **I-31** with the Cambridge Crystallographic Data Centre (CCDC) with deposition no. 1040196. The detailed information can be taken free of charge via http://www.ccdc.cam.ac.uk/.

***At*HPPD Inhibitory Experiments.** We constructed the full-length *At*HPPD (1−445) by polymerase chain reaction (PCR) amplification from cDNA of HPPD in pMD19-T Simple (Hangzhou BIOSCI Biotechnology Co., Hangzhou, China). The primers we have used were 5′-CGCGGATCCTCAGTGGTGGTGGTGGTGGTGTCC-CACTAACTGTTT-3′ (*Bam*HI) and 5′-CATGCCATGGGCCACC-AAAACGCCGC-3′ (*Nco*I). PCR conditions were 30 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 3 min. We introduced the amplicon into the expression vector pET-15b and then transformed it into *Escherichia coli* JM109. The positive clones of the DNA sequences were verified by DNA sequencing by Genewiz Biotechnology Co., Ltd., Suzhou, China. The recombinant human homogentisate 1,2-dioxygenase (*h*HGD) was constructed by using a similar method.

Recombinant pET-15b-HPPD plasmid and human homogentisate 1,2-dioxygenase pET-28a-homogentisate 1,2-dioxygenase plasmid were overexpressed in *E. coli* BL-21 (DE3) cells. The cells were incubated at 37 °C in Luria−Bertani broth containing 100 μg/mL ampicillin (pET-15b plasmid) or 50 μg/mL of kanamycin (pET-28a plasmid).[26] When the *E. coli* grown reached an A_{600} of 0.6, isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to the concentration of 0.2 mM, and the cells were then grown for another 14 h at 20 °C. The

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Table 1. Reaction Yields, Postemergence Herbicidal Activity (150 g ai/ha) of Compounds I, and Their Inhibitory Activity against *At*HPPD

				% inhibition						
compd	R^1	R^2	yield (%)	AJ[a]	AR[a]	EP[a]	EC[a]	DS[a]	SF[a]	*At*HPPD inhibition K_i[b] (μM)
I-1	H	H	86	0	0	0	0	0	0	1.243 ± 0.070
I-2	5,5-diCH_3	H	87	0	0	30	0	0	0	5.571 ± 0.383
I-3	H	2-CH_3	88	0	0	0	0	0	0	0.894 ± 0.061
I-4	5,5-diCH_3	2-CH_3	82	0	0	0	0	0	0	1.596 ± 0.120
I-5	H	3-CH_3	87	0	0	0	0	0	0	0.623 ± 0.076
I-6	H	4-CH_3	88	0	0	0	0	0	0	0.988 ± 0.028
I-7	H	4-OCH_3	86	0	0	0	0	0	0	0.301 ± 0.076
I-8	H	2-SCH_3	87	0	0	0	0	0	0	0.598 ± 0.010
I-9	H	2-Cl	84	0	0	0	0	0	0	1.143 ± 0.035
I-10	5,5-diCH_3	2-Cl	81	0	0	0	0	0	0	2.141 ± 0.099
I-11	H	3-Cl	95	0	0	0	0	0	0	0.075 ± 0.001
I-12	H	4-Cl	90	0	30	30	0	0	0	0.029 ± 0.001
I-13	H	2-CF_3	80	100	50	90	30	30	40	0.134 ± 0.004
I-14	H	3-CF_3	73	0	0	0	0	0	0	0.561 ± 0.011
I-15	H	4-CF_3	73	0	0	0	0	0	0	1.052 ± 0.095
I-16	H	2-NO_2	68	0	0	75	0	0	0	0.045 ± 0.007
I-17	H	4-SO_2CH_3	86	0	0	0	0	0	0	0.354 ± 0.033
I-18	5-CH_3	4-SO_2CH_3	68	0	0	0	0	0	0	0.544 ± 0.021
I-19	H	2,3-diCl	72	0	0	0	0	0	0	0.113 ± 0.007
I-20	H	2,4-diCl	72	30	80	80	0	0	0	0.043 ± 0.008
I-21	H	2,5-diCl	65	0	0	0	0	0	0	0.121 ± 0.026
I-22	H	2,6-diCl	68	0	0	0	0	0	0	0.045 ± 0.015
I-23	H	3,4-diCl	80	0	0	0	0	0	0	0.267 ± 0.001
I-24	H	3,5-diCl	73	0	0	0	0	40	0	0.956 ± 0.019
I-25	5-CH_3	2,4-diCl	68	0	80	80	0	0	0	0.197 ± 0.010
I-26	5,5-diCH_3	2,4-diCl	70	50	90	90	20	0	20	4.803 ± 0.248
I-27	4,4-diCH_3	2,4-diCl	78	0	80	100	0	0	0	0.242 ± 0.024
I-28	H	2,4-diBr	77	30	50	85	0	0	0	0.066 ± 0.003
I-29	5,5-diCH_3	2,4-diBr	78	30	70	100	0	0	0	0.088 ± 0.008
I-30	H	2-CH_3-4-F	73	30	30	30	30	30	30	0.208 ± 0.004
I-31	H	2-CH_3-4-Cl	85	0	35	80	0	0	0	0.031 ± 0.003
I-32	5-CH_3	2-CH_3-4-Cl	75	40	60	85	0	0	0	0.037 ± 0.005
I-33	5,5-diCH_3	2-CH_3-4-Cl	70	0	35	70	0	0	0	0.110 ± 0.004
I-34	4,4-diCH_3	2-CH_3-4-Cl	72	100	50	50	30	70	30	2.001 ± 0.006
I-35	H	2-CH_3-4-Br	82	0	0	40	0	0	0	0.078 ± 0.003
I-36	H	2-CH_3-4-NO_2	73	0	40	30	0	0	0	0.077 ± 0.010
I-37	H	2-Cl-4-F	70	0	0	0	0	0	0	0.547 ± 0.004
I-38	H	2-Cl-4-NO_2	65	0	40	0	0	40	0	0.034 ± 0.002
I-39	H	2-F-4-Cl	73	0	0	30	0	30	30	0.351 ± 0.011
I-40	H	2-NO_2-3-CH_3	83	0	0	0	0	0	0	0.069 ± 0.004
I-41	H	3,5-diF-4-CN	74	0	0	0	0	0	0	0.082 ± 0.004
I-42	H	2,4,6-tri-Cl	64	0	30	40	0	0	0	0.184 ± 0.009
I-43	H	2,3,4,5,6-5F	66	0	0	75	0	0	0	0.055 ± 0.006
mesotrione				100	100	100	95	80	20	0.013 ± 0.001

[a]Abbreviations: AJ, *Abutilon juncea*; AR, *Amaranthus retroflexus*; EP, *Eclipta prostrata*; EC, *Echinochloa crus-galli*; DS, *Digitaria sanguinalis*; SF, *Setaria faberii*. [b]Inhibition constant of the enzyme reaction.

cells containing HPPD were harvested by centrifugation (5000*g*, 10 min), and then the cell precipitate was resuspended in buffer (150 mM NaCl, 20 mM HEPES, pH 7.0) and washed twice. The cells were disrupted by sonication using a cell disruptor. The crude HPPD was obtained after centrifugation at 20000*g* for 50 min.

To obtain purified HPPD, the crude supernatant containing recombinant HPPD was first loaded onto a Ni-NTA column (Invitrogen), equilibrated with 150 mM NaCl and 20 mM HEPES, pH 7.0. Then, HPPD was purified with imidazole gradient and eluted from the column with 250 mM imidazole. Finally, anion exchange chromatography was carried out on Q resin (Amersham-Pharmacia Biotech, Freiburg, Germany) combined with fast protein liquid chromatography (GE Healthcare, Fairfield, CT, USA) eluted with a 0−1 M NaCl gradient. The HPPD-containing fractions exhibiting the highest purity protein as determined by 15% SDS-PAGE were collected and concentrated by ultrafiltration tubes (Millipore, Billerica, MA, USA).

The in vitro *At*HPPD inhibiting activity was determined by a continuous enzyme assay method with slight modification of the published method.[27] *At*HPPD inhibitory activity was tested by monitoring the production of maleylacetoacetate at 318 nm (ε_{330} = 13,500/M/cm) in 96-well plates at 30 °C using a UV/visible plate reader (Biotech, Winooski, VT, USA). The total assay volume of the reaction mixture was 200 μL containing appropriate amounts of

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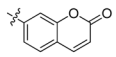
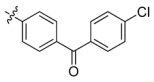
Table 2. Reaction Yields, Postemergence Herbicidal Activity (150 g ai/ha) of Compounds II, and Their Inhibitory Activity against *At*HPPD[a]

				% inhibition						
compd	R^1	R^3	yield (%)	AJ[a]	AR[a]	EP[a]	EC[a]	DS[a]	SF[a]	*At*HPPD inhibition K_i(μM)
II-1	H	1-naphthyl	66	0	0	0	0	0	0	0.215±0.011
II-2	5-CH_3	1-naphthyl	90	0	0	0	0	30	30	1.298±0.059
II-3	5,5-diCH_3	1-naphthyl	73	30	30	90	30	30	30	3.556±0.155
II-4	4,4-diCH_3	1-naphthyl	80	0	40	30	50	30	40	1.133±0.088
II-5	H	2-naphthyl	62	0	0	30	0	0	0	0.253±0.009
II-6	5-CH_3	2-naphthyl	76	0	30	50	30	30	70	3.615±0.031
II-7	5,5-diCH_3	2-naphthyl	79	50	40	50	30	30	0	3.662±0.118
II-8	4,4-diCH_3	2-naphthyl	73	0	40	30	30	40	30	0.079±0.001
II-9	H	6-CN-2-naphthyl	60	30	0	30	0	30	0	0.815±0.067
II-10	5,5-diCH_3	6-CN-2-naphthyl	65	0	0	0	0	0	0	2.335±0.257
II-11	H	6-Br-2-naphthyl	63	0	0	0	0	0	0	2.831±0.068
II-12	5,5-diCH_3	6-Br-2-naphthyl	78	0	0	0	0	0	0	3.930±0.128
II-13	H	1-Br-naphthalen-2-yl	70	100	100	100	50	80	70	0.189±0.040
II-14	H		60	0	60	30	40	30	30	3.152±0.122
II-15	H		58	0	30	30	30	0	30	0.369±0.002
mesotrione				100	100	100	95	80	20	0.013±0.001

[a]Abbreviations: AJ, *Abutilon juncea*; AR, *Amaranthus retroflexus*; EP, *Eclipta prostrata*; EC, *Echinochloa crus-galli*; DS, *Digitaria sanguinalis*; SF, *Setaria faberii*.

Table 3. Postemergent Herbicidal Activity Spectrum of Compound II-13[a]

		% inhibition											
compd	dose (g ai/ha)	DS	EC	SF	CB	BJ	EP	BN	AR	AS	AJ	BT	CS	SM
II-13	150	80	50	70	80	60	100	80	100	75	100	80	100	85
	75	70	40	40	70	30	100	70	100	50	100	70	95	80
	37.5	60	30	30	50	20	100	50	100	40	100	70	90	80
mesotrione	150	80	95	20	95	100	100	100	100	100	100	100	100	100
	75	70	90	0	85	100	95	100	85	100	100	100	100	100
	37.5	50	85	0	80	100	90	95	80	100	100	100	100	100

[a]Abbreviations: DS, *Digitaria sanguinalis*; EC, *Echinochloa crus-galli*; SF, *Setaria faberii*; CB, *Commelina bengalensis*; BJ, *Brassica juncea*; EP, *Eclipta prostrata*; BN, *Boehmeria nivea*; AR, *Amaranthus retroflexus*; AS, *Amaranthus spinosus*; AJ, *Abutilon juncea*; BT, *Bidens tripartite*; CS, *Chenopodium serotinum*; SM, *Stellaria media*.

HPPA, 2 mM sodium ascorbate, 20 mM HEPES buffer (pH 7.0), 100 μM $FeSO_4$, human homogentisate 1,2-dioxygenase, and HPPD. Before detection, the reaction components should equilibrate at 30 °C for about 10 min. The amount of human homogentisate 1,2-dioxygenase added must be far more than HPPD to keep the reaction proceeding successfully (the K_m of human homogentisate 1,2-dioxygenase for homogentisic acid was 25 μM). Before testing, the inhibitors were dissolved in DMSO as concentrates and then diluted with testing buffer to various concentrations. The inhibition constant (K_i) was calculated by Dixon plot $1/v$ according to the methods reported by us.[17−20] Each experiment was three replicates and averaged.

Molecular Modeling. A representative *At*HPPD cocrystallized with NTBC (PDB ID: 5CTO) reported by us was taken from the PDB data bank. The 3D structures of representative compounds **I-1**, **I-31**, and **II-13** were constructed and optimized according to the standard methods by using SYBYL 7.0 (Tripos Inc.).[17] AutoDock 4.2 was used to dock inhibitor to the active site of *At*HPPD. Before docking, the structures of *At*HPPD and inhibitor were prepared according to the standard method using AutoDock Tools. For each ligand, the docking runs were set to 256. On the basis of the internal docking score of the software, the best binding mode was selected as the starting structure in the following optimizations. The energy minimizations were performed with the Sander module of AMBER 9. The AM1-BCC (bond charge corrections) method was used to calculate the charge of inhibitor. The force fields used for protein and inhibitor were Amber ff99 and gaff, respectively. After energy minimization, PYMOL was used to analyze the binding modes.

Herbicidal Activities. All of the synthesized series **I** and **II** compounds were evaluated against monocotyledon weeds *Setaria faberi* (SF), *Digitaria sanguinalis* (DS), and *Echinochloa crus-galli* (EC) and broadleaf *Eclipta prostrata* (EP), *Amaranthus retroflexus* (AR), and *Abutilon juncea* (AJ) by postemergence application.[18] *Commelina bengalensis* (CB), *Brassica juncea* (BJ), *Boehmeria nivea* (BN), *Amaranthus spinosus* (AS), *Bidens tripartite* (BT), *Chenopodium serotinum* (CS), and *Stellaria media* (SM) were also evaluated in herbicidal spectrum activity experiments. All of the inhibitors were first dissolved in DMF and then diluted with Tween-80 to 100 g/L. The solutions were diluted with water to the appropriate concentrations

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before using. The soil used was a mixed soil ($V_{vegetable\ garden\ soil}$:$V_{seedling\ substrate}$ = 1:2). Flowerpots with an inner diameter of 7.5 cm were filled with the above soil to three-fourths of their height. About 20 seeds of each weed were sown in the pot and covered with soil to a thickness of 0.2 cm and grown at temperatures from 15 to 30 °C. When the weeds had grown to about the three-leaf stage, they were treated by the inhibitors at the required concentrations (g ai/ha). The solvent control group weeds were treated by (DMF + Tween-80). After 15 days of treatment by inhibitors, the herbicidal activity was evaluated visually (Tables 1−3), with three duplicates per experiment.

Crop Selectivity. Six representative crops, rice, wheat, maize, soybean, cotton, and canola, were selected for further crop selectivity studies in the greenhouse experiment. The crops were planted in flowerpots (12 cm diameter) and grown at room temperature in the test soil. Crop safety experiments were conducted at the rate of 150 g ai/ha when the crops had reached the four-leaf stage. After 15 days of treatment by inhibitors, the crop selectivity was evaluated (Table 4), with three duplicates per experiment.

Table 4. Postemergence Crop Selectivity of Compound II-13 (150 g ai/ha)

	% injury					
compd	maize	rice	wheat	soybean	cotton	canola
II-13	2.5	40	80	50	100	10
mesotrione	0	50	40	55	80	100

RESULTS AND DISCUSSION

Chemistry. The commercially unavailable acids can be smoothly synthesized by following the appropriate reactions depicted in Figures 2 and 3. In the presence of K_2CO_3 as base, phenol **1** or **4** reacted with ethyl bromoacetate, and the corresponding ethyl aryloxyacetylacetates were prepared. The aryloxyacetyl acids **3** and **5** were synthesized by hydrolyzing the corresponding ethyl aryloxyacetylacetates with NaOH as a base.

Initially, we tried to prepare the 2-(aryloxyacetyl)-cyclohexane-1,3-diones by using the traditional O-acylation and O−C rearrangement. However, we found that when the aryloxyacetyl acid chlorides reacted with 1,3-cyclohexanediones, the yields of enol esters were very low, and some of the enol esters were not obtained due to their transformation to the 2-(aryloxyacetyl)cyclohexane-1,3-diones in the presence of Et_3N. In addition, the reaction rate can further be accelerated by using 0.1 equivalence of acetone cyanohydrin as a catalyst. Encouraged by this observation, we intended to synthesize series **I** and **II** compounds in a one-pot procedure. Because it is inconvenient to prepare the aryloxyacetyl acid chlorides, we tried to use some coupling reagents to make the one-pot procedure easier to handle. We optimized the standard conditions of reaction with phenoxyacetic acid and 1,3-cyclohexanedione. The effects of solvents, bases, coupling reagents, time, and temperature were optimized for the one-pot reaction. The reaction was first carried out with CH_2Cl_2 as solvent, Et_3N as base, and DCC as the coupling reagent. After

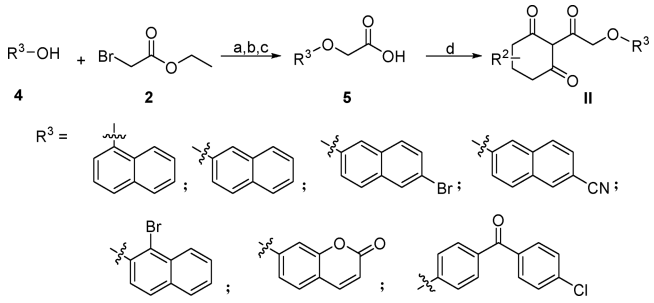

Figure 3. Synthesis of compounds **II**. Reagents and condition: (a) K_2CO_3, DMSO, 50 °C; (b) acetone, H_2O, NaOH, 50 °C; (c) concentrated HCl solution, (d) 1,3-cyclohexanediones, CDI, Et_3N, CH_2Cl_2, room temperature.

reacting at room temperature for 12 h, we isolated compound **I-1** with a yield of 58%. This promising result indicated that it was feasible to synthesize the 2-(aryloxyacetyl)cyclohexane-1,3-diones by one-pot reaction. Next, we intended to further optimize the reaction conditions such as coupling reagent, solvent, and base. It was found that CDI was a better coupling reagent for this one-pot reaction than DCC and EDCI. To further improve the reaction yield, we evaluated different solvents. The experimental results indicated that CH_2Cl_2, DCE, and $CHCl_3$ provided higher yields than toluene, THF, and CH_3CN. The effects of several other bases on yields were evaluated as well. The result showed that Et_3N was a relatively better base for this reaction. Furthermore, it was found that the base was crucial for this reaction. The reaction cannot proceed without base. Interestingly, the base can deprotonate 1,3-cyclohexanedione and improve its nucleophilic affinity. Moreover, adding 0.1 equiv of acetone cyanohydrin to the reaction further improved the reaction yield. Under nitrogen atmosphere, no changes in yields were observed.

Under the optimized reaction conditions, we explored the scope of the one-pot reaction for various aryloxyacetyl acids and 1,3-cyclohexanediones, and the results are shown in Tables 1 and 2. As a general trend, phenyl groups bearing either electron-donating (CH_3, OCH_3, SCH_3) or electron-withdrawing groups (Cl, CF_3, NO_2, SO_2CH_3) were tolerated under the one-pot reaction conditions, with the final compounds (**I-3**−**I-18**) in good to excellent yields (68−95%) (Table 1). It was detrimental to reactivity when the ortho position of the benzene ring was introduced to strong electron-withdrawing groups; for example, the yield of compound **I-16** was just 68%. Variations of the numbers and positions of the substituents on the benzene ring of the acids were explored as well. For example, when the bis-substituted acids were used as the reactants, the corresponding triketones **I-19**−**I-40** can be conveniently synthesized in yields of 65−85%; when trisubstituted acids were used as substrates, the corresponding triketones **I-41** and **I-42** were obtained in 74 and 64% yields, respectively; when a fully substituted acid was used as substrate, the corresponding triketone **I-43** was formed in 66% yield. In

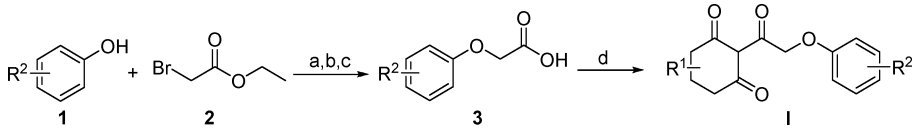

Figure 2. Synthesis of compounds **I**. Reagents and condition: (a) K_2CO_3, DMSO, 50 °C; (b) acetone, H_2O, NaOH, 50 °C; (c) concentrated HCl solution; (d) 1,3-cyclohexanediones, CDI, Et_3N, CH_2Cl_2, room temperature.

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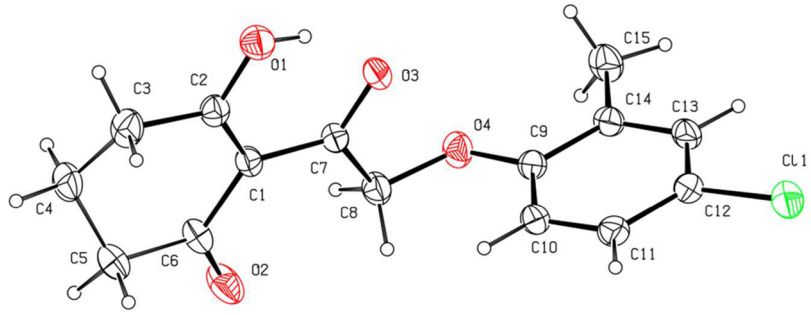

Figure 4. Crystal structure of compound **I-31**.

most cases, the substituents on 1,3-cyclohexanediones did not have much effect on the reaction yields. Then we extended this methodology to other aryloxyacetyl acids, 2-((2-oxo-2*H*-chromen-7-yl)oxy)acetic acid and 2-(4-(4-chlorobenzoyl)-phenoxy)acetic acid, and the reactions proceeded well with 1,3-cyclohexanediones, giving the corresponding products **II-1**—**II-13**, **II-14**, and **II-15** in yields of 60—60, 60, and 58%, respectively.

The structures of all 2-(aryloxyacetyl)cyclohexane-1,3-diones were confirmed by ^{1}H and ^{13}C NMR and HRMS data. Furthermore, the structure of compound **I-31** was verified by X-ray diffraction analysis (Figure 4).

***At*HPPD Inhibition and Structure—Activity Relationships (SARs).** The K_i values of series **I** and **II** compounds against *At*HPPD were evaluated. The data are shown in Tables 1 and 2. Some of the synthesized compounds displayed strong inhibition against *At*HPPD, comparable to that of the commercial herbicide mesotrione (K_i = 0.013 μM). For example, the K_i values for compounds **I-31**, **I-32**, and **I-38** are 0.031, 0.037, and 0.034 μM, respectively.

Initially, we performed molecular modeling studies of compound **I-1** with *At*HPPD (PDB entry: 5CTO). The result showed that there were mainly two interactions of *At*HPPD with **I-1**; one was the bidentate coordination of the triketone part with Fe^{II}, and another was the $\pi-\pi$ interaction of the benzene ring with Phe403 and Phe360. The K_i value of **I-13** for *At*HPPD was 0.134 μM, well worth further optimization. Therefore, we synthesized a series of 2-(phenoxyacetyl)-cyclohexane-1,3-diones by the one-pot reaction protocol. It was found that when electron-withdrawing groups were introduced on the benzene ring, compounds showed better HPPD inhibitory activity than compounds with electron-donating groups. Interestingly, when a methyl group was placed on the ortho position and another electron-withdrawing group was introduced to the para position of benzene ring, compounds showed significantly enhanced HPPD inhibitory activity. For example, the K_i value of **I-3** was 0.894 μM. When a chlorine atom was introduced on the para position of benzene ring, the K_i value of **I-31** was significantly improved to be 0.031 μM, >28-fold more active than the parent compound. With the bis-substituted analogues (**I-19**—**I-24**), substituents on the 2,4-positions of benzene ring displayed higher HPPD inhibitory activity than the other two positions. It seemed that substituents introduced on the cyclohexane-1,3-dione were detrimental to activity. The possible reason is that the methyl groups increase the repulsions of the compounds with the amino acid backbone of *At*HPPD.[18,19]

Furthermore, it was unfavorable to the activity of the target compounds when the benzene ring was replaced by other bigger ring systems. To investigate the molecular basis for this, molecular docking studies were performed on the two representative compounds **I-31** and **II-13**. As shown in Figure 5, there are no significant differences in binding modes of **I-31**

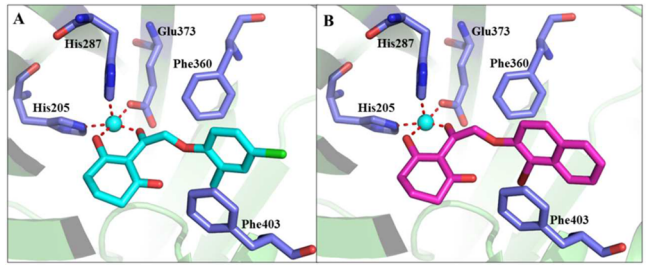

Figure 5. Simulated binding mode of compounds **I-31** and **II-13** with *At*HPPD. The key residues in the active site are shown in blue sticks, and Fe^{II} is shown as a cyan sphere. (A) Binding mode of **I-31** with *At*HPPD. **I-31** is shown in cyan sticks. (B) Binding mode of **II-13** with *At*HPPD. **II-13** is shown in magenta sticks.

and **II-13**. The triketone part of **I-31** and **II-13** can form bidentate interaction with Fe^{II}, whereas the benzene and naphthalene rings can form $\pi-\pi$ interaction with Phe360 and Phe403. When the distances of $\pi-\pi$ interaction were compared, we found that the distance of the benzene ring of **I-31** with Phe360 is 4.71 Å and that with Phe403 is 3.93 Å; the distance of the naphthalene ring of **II-13** with Phe360 is 4.95 Å and that with Phe403 is 4.95 Å. The increased distances of the naphthalene ring from Phe360 and Phe403 may be responsible for the decreased HPPD inhibitory activity of **II-13**.

Herbicidal Activity and SARs. The postemergence herbicidal activity of the target compounds **I** and **II** were evaluated in the greenhouse experiments. Six kinds of representative weeds were tested. HPPD herbicide mesotrione was selected as a positive control, and the results are shown in Tables 1 and 2. Furthermore, when exposed to light, some of the tested weeds have shown unique bleaching symptoms, indicating that these compounds were HPPD inhibitors. In most cases, the herbicidal activities were in accordance with *At*HPPD-inhibiting activity. Several of the synthesized compounds showed >70% control against some of tested weeds at the dosage of 150 g ai/ha. Compound **II-13** displayed >80% control against two-thirds of tested weeds at the dosage of 150 g ai/ha. Its herbicidal potency against three tested dicot weeds even showed the same activity as mesotrione.

As can be seen from Table 1, most of the mono- or unsubstituted analogues **I-1**—**I-18** did not show promising herbicidal activity. To our surprise, compound **I-13** with a trifluoromethyl group on the ortho position of the benzene ring

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displayed >90% inhibition against *A. juncea* and *E. prostrata*; compound **I-16** with a nitrogen group on the ortho position of the benzene ring showed 75% inhibition against *E. prostrata*. This promising result indicated that the introduction of an electron-withdrawing group at the ortho position of the benzene ring appears to favor herbicidal activity. For compounds (**I-19**−**I-24**) with two chlorine substituents on the benzene ring, compound **I-20** with a chlorine atom at 2,4-positions displayed higher herbicidal activity than those with substituents on the other positions. When different substituents were introduced to the 1,3-cyclohexanedione ring, it was found that the addition of two methyl groups at position C4 of the ring favors herbicidal activity. Among the 2,4-disubstituted analogues, compounds with 2,4-dichloro, 2-CH_3-4-chloro, and 2,4-dibromo substituents on the benzene ring displayed higher herbicidal activity than those with other types of substituents at the same positions. It was interesting that the more powerful electron-withdrawing groups on the benzene ring were detrimental to herbicidal activity. The possible reason for this observation is that when more electron-withdrawing groups were introduced in the benzene ring, the pK_a values of compounds decreased. The instability of these compounds increased as well. The electron-deficient benzene rings made the ether bonds in these compounds very weak, indicating an easy metabolism of the compounds when absorbed by plants.

To study the herbicidal activity of other ring systems, we synthesized compounds **II**. As shown in Table 2, compared to their benzene-substituted analogues, compounds **II-2** and **II-7** with naphthalene rings displayed more enhanced herbicidal activity than compound **I-2**. To our surprise, compound **II-13** with a 1-bromonaphthalen-2-yl group displayed significantly higher herbicidal activity than the phenyl-substituted analogues. At the dosage of 150 g ai/ha, **II-13** displayed complete control against the tested broadleaf weeds (*E. prostrata*, *A. retroflexus*, and *A. juncea*), 80% inhibition against *D. sanguinalis*, and 70% inhibition against *S. faberii*.

Herbicidal Spectrum and Crop Safety of II-13. The herbicidal spectrum experiments indicated that compound **II-13** displayed >70% inhibition against 11 of the 13 tested weeds at the dasage of 150 g ai/ha (Table 3). It is worth noting that, at the rate of 150 g ai/ha, **II-13** displayed 70% inhibition ratio against *S. faberii*, whereas mesotrione was almost inactive to it at the same rate. Even at a rate of 37.5 g ai/ha, **II-13** still displayed 100% control of *E. prostrata*, *A. retroflexus*, and *A. juncea*, >80% inhibition of *C. serotinum* and *S. media*, and 60% control of *D. sanguinalis*, which were nearly as potent as mesotrione against these six weeds. To evaluate whether **II-13** has the potential to be developed as a herbicide or not, we tested its crop safety (Table 4). The results indicated that canola and maize displayed high tolerance to **II-13** at the rate of 150 g ai/ha, whereas mesotrione was not selective for canola (100% injury), indicating that **II-13** has the potential to be developed as a postemergence herbicide for weed control in maize and canola fields.

In summary, a series of novel 2-(aryloxyacetyl)cyclohexane-1,3-diones were rationally designed by inserting a carbon−oxygen bond between the triketone part and aroyl moieties. An efficient one-pot procedure for synthesis of the designed triketones by using CDI, triethylamine, and acetone cyanohydrin in CH_2Cl_2 was developed. Fifty-eight triketone compounds were prepared in good to excellent yields. Several of the synthesized compounds displayed potent *At*HPPD inhibitory activity, which were nearly as potent as the commercial herbicide mesotrione. Compound **II-13** displayed a strong and broad spectrum of weed control at dosages of 37.5−150 g ai/ha by postemergence application, which was similar to the potency of mesotrione in controlling the growth of some weeds. Furthermore, **II-13** was selective for canola and maize at the dosage of 150 g ai/ha, showing that **II-13** could be developed as a herbicide for canola and maize fields. **II-13** is the first HPPD inhibitor showing crop safety to canola. The promising finding will provide a new insight for the future design of selective HPPD inhibitors for canola fields. Further structural optimization and field trial experiments of **II-13** are ongoing.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.6b04110.

Detailed analytical ^{1}H NMR, ^{13}C NMR, and HRMS data for triketone series **I** and **II** compounds (PDF)

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Notes

The authors declare no competing financial interest.

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