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Synthesis and Herbicidal Evaluation of Triketone-Containing Quinazoline-2,4-diones

Da-Wei Wang,[†] Hong-Yan Lin,[†] Run-Jie Cao,[†] Sheng-Gang Yang,[†] Qiong Chen,[†] Ge-Fei Hao,[†] Wen-Chao Yang,*[,†] and Guang-Fu Yang*[,†,‡]

[†]Key Laboratory of Pesticide and Chemical Biology of Ministry of Education, College of Chemistry, Central China Normal University, Wuhan, Hubei 430079, People's Republic of China

[‡]Collaborative Innovation Center of Chemical Science and Engineering, Tianjin 30071, People's Republic of China

Supporting Information

ABSTRACT: Exploring novel 4-hydroxyphenylpyruvate dioxygenase (EC 1.13.11.27, HPPD) inhibitors is one of the most promising research directions in herbicide discovery. To discover new triketone herbicides with broad-spectrum weed control as well as excellent crop selectivity, a series of (total 52) novel triketone-containing quinazoline-2,4-dione derivatives were synthesized and further bioevaluated. The greenhouse testing indicated that many of the newly synthesized compounds showed better or excellent herbicidal activity against broadleaf and monocotyledonous weeds at the dosages of 37.5−150 g of active ingredient (ai)/ha. The structure and activity relationship in this study indicated that the triketone-containing quinazoline-2,4-dione motif has possessed great impact on herbicide activity and may be used for further optimization. Among the new compounds, **III-b** and **VI-a**−**VI-d** displayed a broader spectrum of weed control than mesotrione. In addition, the compound **III-b** also demonstrated comparatively superior crop selectivity to mesotrione, thus possessing great potential for weed control in the field.

KEYWORDS: *4-hydroxyphenylpyruvate dioxygenase, crop selectivity, herbicidal activity, quinazoline-2,4-dione, synthesis*

INTRODUCTION

In agrochemical research, the discovery of new herbicides with broad-spectrum weed control and excellent crop selectivity is still remaining as a challenge. 4-Hydroxyphenylpyruvate dioxygenase (EC 1.13.11.27, HPPD), catalyzing the conversion of 4-hydroxyphenyl pyruvic acid (HPPA) into homogentisic acid (HGA), belongs to the family of non-heme Fe(II)-containing enzymes.[1,2] As an important enzyme in regulating the biosynthesis of tocopherols and plastoquinone in plants, HPPD is an important target for herbicide discovery. HPPD-inhibiting-based herbicides can block photosynthesis, which resulted in unique bleaching symptoms in sunlight and finally caused necrosis and death of treated plants.[3−6] HPPD inhibitors exhibit many advantages, such as excellent crop selectivity, low application rate, low toxicity, broad-spectrum weed control, and benign environmental effects.[7−11]

Thus far, HPPD inhibitors that are widely used in the field as herbicides can be classified into three categories: triketones, pyrazoles (diketonitrile), and isoxazoles. Among the commercialized HPPD herbicides, triketone derivatives are the most deeply studied, owing to their structural diversity. The story of discovering triketone herbicides can be traced back to the 1970s; scientists in California noticed that a few plants grew under *Callistemon citrinus*, and the natural product leptospermone was accordingly extracted.[7,8] Further studies lead to the discovery of the first triketone herbicide sulcotrione by Stauffer in 1991. About 10 years later, the second triketone herbicide mesotrione was successfully launched into the market, which is more potent compared to sulcotrione.[12,13] It can effectively control all of the major broadleaved weeds in a corn field as well as some annual monocotyledon weeds. More importantly, mesotrione could control some of the resistant biotypes, for example, glyphostate-resistant, acetyl coenzyme carboxylase (ACCase) and acetohydroxy acid synthase (AHAS) resistant weeds.[14] In 2009, the market value of mesotrione was about $485 million, and now, it is one of the top five best-selling herbicides worldwide. However, there are still some limitations of mesotrione, such as toxicity to wheat, soybean, rape, cotton, and other crops, because of its poor selectivity. In addition, some grass weeds, for example, *Setaria faberii*, are not sensitive to mesotrione. Therefore, it is necessary to discover a novel triketone herbicide with improved crop selectivity and broader spectrum of weed control (especially against grass weeds).

In our previous work,[15] we have designed and synthesized a series of novel triketone-containing quinazoline-2,4-dione derivatives with a variety of substitutes at the N-1 position of the quinazoline-2,4-dione ring. According to our data of herbicidal activity and crop selectivity, the methyl group turned to be the optimum substitute at the N-1 position. Moreover, the lead compound **I-f** (Table 1) not only showed a broad spectrum of weed control but also possessed better selectivity for maize and wheat at the dosage of 150 g of active ingredient (ai)/ha. In this ongoing study, we performed thorough modification on the triketone-containing quinazoline-2,4-dione motif and synthesized a series of novel triketone-

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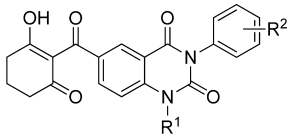
Table 1. Chemical Structures, Postemergence Herbicidal Activities (Inhibition Rating of 0−100) of Compounds I, and Their Inhibitory Activities against *At*HPPD[a]



compound	R^1	R^2	dose (g of ai/ha)	EC[b]	SF[b]	DS[b]	AR[b]	EP[b]	AJ[b]	K_i (nM)[c]
I-a	CH_3	4-Cl	150	90	100	90	90	85	90	21 ± 2
			75	70	60	60	60	50	50	
			37.5	50	50	50	60	0	0	
I-b	CH_3	3-Cl	150	80	100	70	100	80	80	27 ± 5
			75	50	20	40	70	30	20	
			37.5	40	0	0	60	30	20	
I-c	CH_3	2-Cl	150	100	100	100	80	80	85	23 ± 1
			75	90	90	90	75	40	70	
			37.5	60	60	40	60	30	60	
I-d	CH_3	4-CH_3	150	75	75	75	100	75	75	42 ± 1
I-e	CH_3	3-CH_3	150	70	100	75	100	95	70	39 ± 6
I-f	CH_3	2-CH_3	150	100	100	100	100	100	100	32 ± 1
			75	100	100	95	100	87.5	100	
			37.5	95	90	85	97.5	82.5	97.5	
I-g	H	2-CH_3	150	0	10	0	0	0	15	627 ± 42
I-h	CH_2CH_3	2-CH_3	150	100	100	100	100	100	100	5 ± 1
			75	100	100	97.5	100	100	100	
			37.5	100	97.5	60	90	95	100	
I-i	$CH_2CH_2CH_3$	2-CH_3	150	87.5	90	60	95	97.5	100	15 ± 2
			75	80	87.5	50	80	87.5	100	
			37.5	70	65	30	50	70	87.5	
I-j	$CH_2C{\equiv}CH$	2-CH_3	150	50	20	0	90	35	90	33 ± 1
I-k	$CH_2CH_2CH_2CH_3$	2-CH_3	150	0	10	0	30	0	50	19 ± 1
I-l	$CH_2CH(CH_3)_2$	2-CH_3	150	0	10	0	20	20	90	17 ± 2
I-m	$CH_2C_6H_5$	2-CH_3	150	0	10	0	0	0	0	355 ± 9
I-n	CH_2−2-F−C_6H_4	2-CH_3	150	50	50	30	0	90	0	139 ± 8
I-o	CH_2−3-OCH_3−C_6H_4	2-CH_3	150	50	10	25	0	80	0	141 ± 55
mesotrione			150	85	20	95	100	100	100	13 ± 1
			75	75	0	60	100	100	100	
			37.5	30	0	30	100	100	100	

[a]For the method for the preparation of compounds **I**, see ref 15. [b]Abbreviations: EC, *Echinochloa crus-galli*; SF, *Setaria faberii*; DS, *Digitaria sanguinalis*; AR, *Amaranthus retroflexu*; EP, *Eclipta prostrata*; AJ, *Abutilon juncea*. [c]Inhibition constant of the enzymatic reaction.

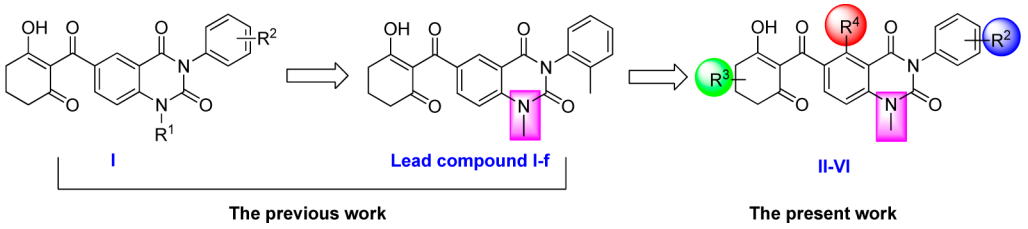


Figure 1. Design of title compounds (**II**−**VI**).

containing quinazoline-2,4-dione derivatives possessing a methyl group at the N-1 position of the quinazoline-2,4-dione ring (Figure 1). The biological test indicated that most newly synthesized compounds showed excellent herbicidal activity against broadleaf and monocotyledonous weeds at the dosages of 37.5−150 g of ai/ha. Notably, some compounds showed significantly improved crop selectivity compared to mesotrione. Herein, we have revealed the detailed synthesis, herbicidal activity, and structure−activity relationship (SAR) of compounds **II**−**VI** as potent herbicide candidates.

MATERIALS AND METHODS

The experimental details and analytical data for all of the intermediates and compounds **II**−**VI** are shown in the Supporting Information.

X-ray Diffraction. Compound **IV-d** was recrystallized from a mixture of chloroform and *n*-hexane to afford a suitable single crystal. Light brown crystals of **IV-d** (0.12 × 0.10 × 0.10 mm) were mounted on a quartz fiber with protection oil. Cell dimensions and intensities were measured at 296 K on a Bruker SMART APEX DUO area detector diffractometer with graphite monochromated Mo Kα radiation (λ = 0.710 73 Å), with θ_{max} = 25.49, 16 744 measured reflections, and 4817 independent reflections (R_{int} = 0.0253). The data sets were integrated and reduced using SAINT Plus Programme.[16]

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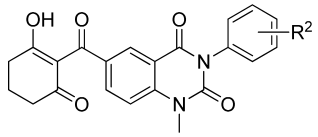
Table 2. Chemical Structures, Postemergent Herbicidal Activity (Inhibition Rating of 0−100) of Compounds II, and Their Inhibitory Activities against *At*HPPD



compound	R^2	dose (g of ai/ha)	EC[a]	SF[a]	DS[a]	AR[a]	EP[a]	AJ[a]	K_i (nM)
II-a	H	150	65	50	65	100	70	80	51 ± 9
II-b	2-F	150	80	100	80	80	80	85	53 ± 3
		75	70	40	60	75	50	50	
		37.5	50	30	40	70	30	0	
II-c	3-F	150	85	80	75	85	80	100	28 ± 4
		75	60	40	60	80	50	30	
		37.5	50	0	30	70	30	0	
II-d	4-F	150	85	100	75	100	95	100	29 ± 1
		75	70	20	60	70	60	40	
		37.5	60	20	20	60	0	20	
II-e	2-Br	150	87.5	90	70	100	82.5	100	27 ± 2
		75	75	82.5	50	100	70	90	
		37.5	45	75	20	100	60	65	
II-f	3-Br	150	85	45	30	95	50	85	16 ± 1
II-g	4-Br	150	85	85	70	100	100	100	17 ± 4
		75	55	60	30	100	100	65	
		37.5	30	45	0	100	95	50	
II-h	2-OCH_3	150	100	100	100	100	82.5	97.5	68 ± 5
		75	100	100	100	100	75	90	
		37.5	92.5	90	100	97.5	60	85	
II-i	3-OCH_3	150	92.5	25	60	95	67.5	100	17 ± 4
II-j	4-OCH_3	150	85	50	75	95	80	60	15 ± 3
II-k	2-CF_3	150	95	100	95	100	85	100	46 ± 5
		75	90	95	90	95	80	97.5	
		37.5	87.5	92.5	65	90	75	90	
II-l	3-CF_3	150	85	80	50	97.5	75	55	65 ± 1
II-m	4-CF_3	150	85	30	60	100	60	100	24 ± 2
II-n	2-OCF_3	150	85	80	85	97.5	85	95	47 ± 2
		75	77.5	80	85	95	65	50	
		37.5	70	50	75	90	50	30	
II-o	4-OCF_3	150	77.5	50	60	95	80	50	12 ± 1
II-p	4-NO_2	150	50	10	40	90	82.5	60	18 ± 4
II-q	2-C_2H_5	150	85	82.5	95	100	85	100	29 ± 1
		75	87.5	90	85	95	0	90	
		37.5	80	87.5	80	90	0	60	
II-r	2-iPr	150	80	97.5	100	90	80	97.5	29 ± 3
		75	82.5	87.5	92.5	95	60	80	
		37.5	70	50	75	90	50	60	
II-s	2,6-di-iPr	150	60	20	55	100	70	35	106 ± 3
II-t	2,6-di-Cl	150	80	40	80	85	50	80	47 ± 2
II-u	2,6-di-CH_3	150	100	100	95	100	70	100	66 ± 5
		75	92.5	100	85	100	65	100	
		37.5	87.5	100	80	100	55	97.5	
II-v	2,4-di-Cl	150	82.5	92.5	65	87.5	20	100	13 ± 3
II-w	2-CH_3-5-Cl	150	85	60	80	90	82.5	85	53 ± 1
		75	85	80	70	100	60	65	
		37.5	70	55	30	100	50	30	
II-x	3,5-di-Cl	150	80	80	70	90	60	100	17 ± 2
mesotrione		150	85	20	95	100	100	100	13 ± 1
		75	75	0	60	100	100	100	
		37.5	30	0	30	100	100	100	

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

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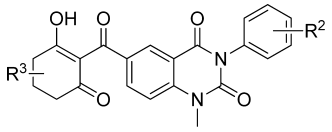
Table 3. Chemical Structures, Postemergent Herbicidal Activity (Inhibition Rating of 0−100) of Compounds III−V, and Their Inhibitory Activities against *At*HPPD



compound	R^2	R^3	dose (g of ai/ha)	EC[a]	SF[a]	DS[a]	AR[a]	EP[a]	AJ[a]	K_i (nM)
III-a	4-Br	5-CH_3	150	80	50	90	95	95	92.5	25 ± 1
			75	70	40	80	90	90	87.5	
			37.5	60	30	65	82.5	80	80	
III-b	2,4-di-Cl	5-CH_3	150	100	100	100	100	95	100	15 ± 4
			75	100	95	95	95	92.5	100	
			37.5	95	90	92.5	90	85	100	
IV-a	H	5,5-di-CH_3	150	77.5	45	85	95	80	100	54 ± 5
IV-b	2-F	5,5-di-CH_3	150	90	100	90	100	90	100	20 ± 3
			75	70	50	50	70	70	60	
			37.5	50	40	50	60	50	40	
IV-c	3-F	5,5-di-CH_3	150	85	80	95	85	85	85	56 ± 5
			75	50	50	50	70	70	30	
			37.5	40	30	50	60	60	30	
IV-d	4-F	5,5-di-CH_3	150	80	95	95	100	90	100	100 ± 9
			75	50	50	30	60	50	50	
			37.5	40	40	30	50	40	40	
IV-e	2-Br	5,5-di-CH_3	150	75	35	95	95	82.5	100	245 ± 21
IV-f	3-Br	5,5-di-CH_3	150	70	50	85	100	100	100	29 ± 3
			75	20	50	65	100	100	100	
			37.5	0	30	20	100	100	100	
IV-g	4-Br	5,5-di-CH_3	150	60	30	92.5	100	87.5	100	24 ± 4
IV-h	2-OCH_3	5,5-di-CH_3	150	85	50	85	95	85	50	287 ± 21
IV-i	3-OCH_3	5,5-di-CH_3	150	60	60	55	80	75	55	103 ± 12
IV-j	4-OCH_3	5,5-di-CH_3	150	80	60	87.5	90	67.5	100	118 ± 19
IV-k	3-CF_3	5,5-di-CH_3	150	50	40	82.5	90	80	92.5	48 ± 1
IV-l	4-CF_3	5,5-di-CH_3	150	40	10	60	80	55	60	42 ± 9
IV-m	4-OCF_3	5,5-di-CH_3	150	65	50	90	95	80	100	24 ± 4
IV-n	4-NO_2	5,5-di-CH_3	150	0	80	25	80	80	100	58 ± 5
IV-o	2-CH_2CH_3	5,5-di-CH_3	150	80	75	60	100	70	100	23 ± 3
IV-p	2-iPr	5,5-di-CH_3	150	55	50	50	100	90	97.5	249 ± 37
IV-q	2,6-di-Cl	5,5-di-CH_3	150	92.5	95	87.5	95	97.5	95	180 ± 10
			75	97.5	85	85	82.5	80	90	
			37.5	90	80	70	70	70	80	
IV-r	2,6-di-CH_3	5,5-di-CH_3	150	85	80	50	100	87.5	100	262 ± 8
			75	87.5	87.5	82.5	80	60	85	
			37.5	75	82.5	75	75	50	80	
IV-s	2,4-di-Cl	5,5-di-CH_3	150	25	30	20	30	50	100	362 ± 39
IV-t	2-CH_3-5-Cl	5,5-di-CH_3	150	80	40	82.5	95	80	65	138 ± 11
IV-u	3,5-di-Cl	5,5-di-CH_3	150	75	85	90	100	80	0	39 ± 3
V	2,6-di-Cl	6,6-di-CH_3	150	95	45	60	97.5	80	100	49 ± 5
mesotrione			150	85	20	95	100	100	100	13 ± 1
			75	75	0	60	100	100	100	
			37.5	30	0	30	100	100	100	

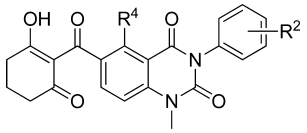
[a]Abbreviations: EC, *Echinochloa crus-galli*; SF, *Setaria faberii*; DS, *Digitaria sanguinalis*; AR, *Amaranthus retroflexu*; EP, *Eclipta prostrata*; AJ, *Abutilon juncea*.

Data were corrected for Lorentz and polarization effects and absorption (T_{max} = 0.9613, and T_{min} = 0.9538). The structure was solved by a direct method using SHELXS97 and refined with SHELXL970.[17] Full-matrix least-squares refinement based on F^2 using the weight of $1/[\sigma^2(F_0^2) + (0.1322P)^2 + 0.6124P]$ gave final values of R_1 = 0.0628, ωR_2 = 0.2008, and GOF(F) = 1.116 for 403 variables, 403 parameters, and 4817 contributing reflections. Maximum shift/error = 0.002, and maximum/minimum residual electron density = 0.679/−0.506 e Å^{-3}. Hydrogen atoms were observed and placed at their ideal positions with a fixed value of their isotropic displacement parameter.

Crystallographic data for compound **IV-d** has been deposited with the Cambridge Crystallographic Data Centre as a supplementary publication with the deposition number 977355. These data can be obtained free of charge from http://www.ccdc.cam.ac.uk/.

Herbicidal Activities. The postemergent herbicidal activities of compounds **I−VI**, against *Echinochloa crus-galli* (EC), *Setaria faberii* (SF), *Digitaria sanguinalis* (DS), *Amaranthus retroflexus* (AR), *Eclipta*

Table 4. Chemical Structures, Postemergent Herbicidal Activity (Inhibition Rating of 0−100) of Compounds III−V, and Their Inhibitory Activities against *At*HPPD



	R^2	R^4	dose (g of ai/ha)	EC[a]	SF[a]	DS[a]	AR[a]	EP[a]	AJ[a]	K_i (nM)
VI-a	2-CH_3	CH_3	150	100	100	100	100	100	100	16 ± 1
			75	100	100	100	100	100	100	
			37.5	100	100	100	100	100	97.5	
VI-b	2-OCH_3	CH_3	150	100	100	100	100	100	100	28 ± 4
			75	100	100	100	100	100	100	
			37.5	97.5	92.5	100	95	95	97.5	
VI-c	2-CF_3	CH_3	150	100	100	100	100	100	100	10 ± 1
			75	97.5	97.5	95	95	95	90	
			37.5	90	95	90	90	90	90	
VI-d	2,6-di-CH_3	CH_3	150	100	100	100	100	100	100	9 ± 1
			75	100	100	100	100	95	100	
			37.5	95	95	100	95	87.5	97.5	
mesotrione			150	85	20	95	100	100	100	13 ± 1
			75	75	0	60	100	100	100	
			37.5	30	0	30	100	100	100	

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

prostrata (EP), and *Abutilon juncea* (AJ) were evaluated according to the above-mentioned procedure;[18−20] the commercially available triketone herbicide mesotrione was selected as a positive control. All test compounds were formulated as 100 g/L emulsified concentrates using *N*,*N*-dimethylformamide (DMF) as the solvent and Tween-80 as the emulsification reagent. The concentrates were diluted with water to the required concentration and applied to pot-grown plants in a greenhouse. Clay soil was used with pH 6.5, 1.6% organic matter, 37.3% clay particles, and cation-exchange capacity (CEC) of 12.1 mol/kg. The rate of application (g of ai/ha) was calculated by the total amount of active ingredient in the formulation divided by the surface area of the pot. Plastic pots with a diameter of 9 cm were filled with soil to a depth of 8 cm. Approximately 20 seeds of selected weeds were sown in soil at the depth of 1−3 cm and grown at the temperature of 15−30 °C in a greenhouse. Atmospheric relative humidity was 50%. The diluted formulation solutions were applied as a postemergence treatment. Broadleaf weeds were treated at the 2-leaf stage, and monocotyledon weeds were treated at the 1-leaf stage. The postemergence application rate was 150 g of ai/ha. Untreated seedlings were used as the control group, and the solvent (DMF + Tween-80)-treated seedlings were used as the solvent control group. Herbicidal activity was evaluated visually after 15 days from post-treatment. The results of herbicidal activities are shown in Tables 1−4, with three replicates per treatment.

Crop Selectivity. The conventional rice, soybean, cotton, wheat, rape, and maize were planted separately in pots (diameter = 12 cm) containing selected soil and grown in a greenhouse at 20−25 °C.[21] After the (crop) plants had reached the 4-leaf stage, new herbicides were applied (spraying) at the dosage of 150 g of ai/ha. The visual injury and growth rate of the individual plants were observed at regular intervals. After 15 days, the final results of crop safety were determined (Table 5), using three replicates per treatment.

Plasmid Construction, Protein Expression and Extraction, HPPD Purification, Activity Assays, and Kinetic Inhibition Studies. *Arabidopsis thaliana* HPPD (*At*HPPD) was constructed by polymerase chain reaction (PCR) using cDNA of HPPD in pMD19-T Simple (Hangzhou BIOSCI Biotechnology Company) as the template. The primers, used here were 5′-CATGCCATGGGCCAC-CAAAACGCCGC-3′ (*Nco*I) and 5′-CGCGGATCCT-CAGTGGTGGTGGTGGTGGTGTCCCACTAACTGTTT-3′ (*Bam*HI). PCR conditions were 35 cycles at 94 °C for 30 s, 55 °C for 30 s, and 68 °C for 1.5 min. The amplion was introduced into the expression vector pET-15b and subsequently transformed into *Escherichia coli* BL21(DE3). The DNA sequences of the positive clones were confirmed by DNA sequencing with Shanghai SANGON Company.

Table 5. Crop Selectivity (Injury Rating of 0−100) of Selected Compounds (Postemergence, 150 g of ai/ha)

compound	soybean	rape	cotton	maize	rice	wheat
II-h	20	0	20	40	50	30
II-k	45	100	25	0	20	35
II-u	55	85	30	0	20	10
III-b	65	45	0	0	20	10
VI-a	100	100	100	15	100	90
VI-b	100	100	86	50	100	60
VI-c	90	100	90	30	100	100
VI-d	100	100	90	70	100	60
mesotrione	55	100	70	10	50	40

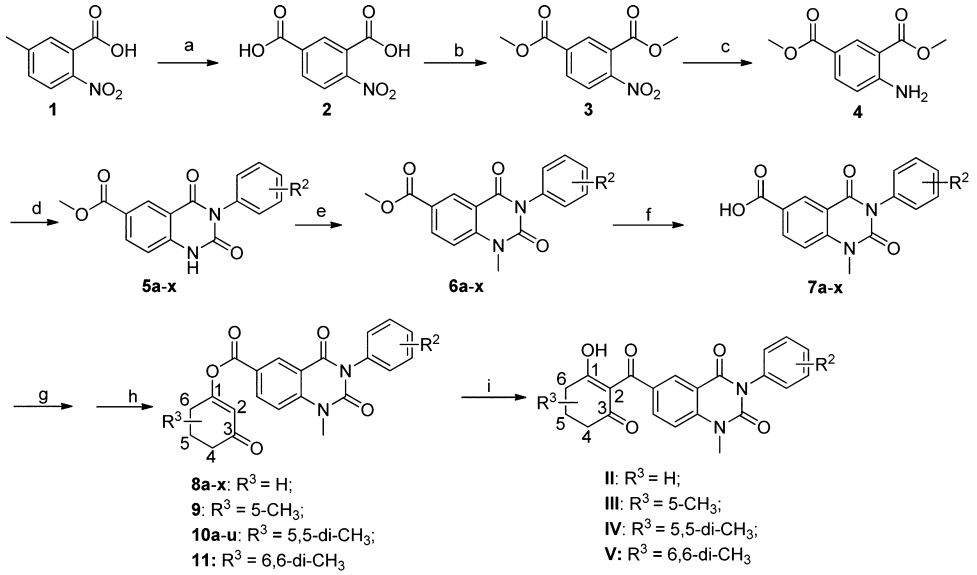
Recombinant *At*HPPD was overexpressed in *E. coli* BL21(DE3) cells with pET-15b-HPPD plasmid. Recombinant HGD from human was overexpressed in *E. coli* BL21(DE3) cells with pET-28a-HGD plasmid. The cells were grown at 37 °C in Luria−Bertani broth supplemented with 50 μg/mL kanamycin (pET-28a plasmid) or 100 μg/mL antimycin (pET-15b plasmid) according to the previous publication.[22] Expression of the *At*HPPD plasmid was incubated at 37 °C for 12 h. HGD was induced by 0.5 mM isopropyl-β-D-thiogalactopyranoside (IPTG) when bacterial growth reached A_{600} of 0.6, and then the cells were incubated for another 40 h at 15 °C. After harvesting by centrifugation (5000*g* for 30 min), the pellet was resuspended in buffer [20 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) and 20 mM NaCl at pH 7.0] and washed twice, followed by sonication using a cell disruptor. A crude cell-free supernatant was obtained by centrifugation at 20000*g* for 30 min. The over-expression and the relative molecular weight of recombinant *At*HPPD were validated by both sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) stained with Coomassie Brilliant Blue-R250 and Simple Western analysis using Simon instrumentation (ProteinSimple, CA)

Table 6. Binding Free Energies (kcal/mol) of Compounds II-k and VI-c

compound	ΔE_{ELE}	ΔE_{VDW}	ΔE_{GAS}	ΔG_{SOL}	ΔE_{MM}	$-T\Delta S$	ΔG_{cal}[a]	ΔG_{exp}[b]
II-k	−128.75 (8.15)	−33.57 (3.46)	−162.31 (8.11)	115.98 (7.74)	−46.33 (4.91)	34.42 (2.65)	−11.91	−10.93
VI-c	−138.94 (7.44)	−31.98 (3.81)	−170.91 (6.57)	124.09 (7.11)	−46.82 (5.31)	37.72 (3.01)	−9.16	−10.02

[a]The results were determined by the MM/PBSA calculations. [b]The experimental values of ΔG_{exp} were derived from the reported experimental K_i values.

Scheme 1. Synthetic Route for the Title Compounds II–V[a]



[a]Reagents and conditions: (a) KOH, $KMnO_4$, HCl; (b) CH_3OH, H_2SO_4, reflux; (c) H_2, 10% Pd/C; (d) (un)substituted phenyl isocyanates, pyridine, 100 °C; (e) Cs_2CO_3, CH_3I, DMF, rt; (f) HOAc, H_2SO_4, H_2O, 100 °C; (g) $SOCl_2$, THF, reflux; (h) substituted 1,3-cyclohexanediones, Et_3N, $CHCl_3$, 0 °C; (i) acetone cyanohydrin, Et_3N, CH_2Cl_2, rt.

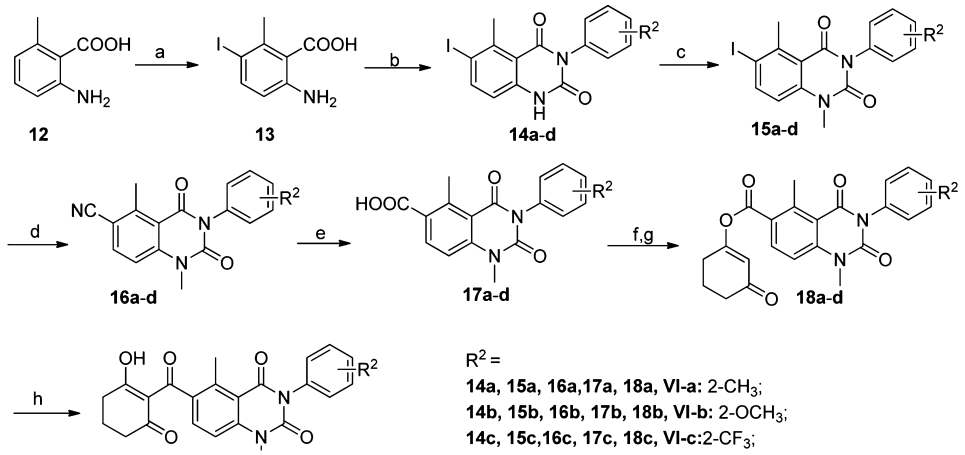
*At*HPPD was purified in two chromatographic steps. The crude cell-free supernatant was loaded onto a nickel–nitrilotriacetic acid (Ni–NTA) column (Qiagen, Canada), equilibrated with 20 mM HEPES at pH 7.0. Then, HPPD was eluted with 20 mM HEPES at pH 7.0, 150 mM NaCl, and 250 mM imidazole. The fractions containing HPPD were concentrated, and the buffer was exchanged for 20 mM HEPES at pH 7.0 by ultrafiltration in Ultrafree filter devices (Millipore, MA). For further purification of the recombinant HPPD, anion-exchange chromatography was carried out on Q resin (Amersham-Pharmacia Biotech, Germany) in 20 mM HEPES at pH 7.0. Elution of the recombinant HPPD was carried out in a linear gradient from 0 to 250 mM NaCl. Again, the HPPD-containing fractions were collected, and the buffer was exchanged to 20 mM HEPES at pH 7.0 by ultrafiltration.

Our coupled enzyme assays for the *in vitro* activity and inhibition of HPPD were measured by a modification of methods previously reported in the literature.[23] Assays were performed in 96-well plates at 30 °C using an ultraviolet/visible plate reader to monitor the formation of maleylacetoacetate at 318 nm (ε_{330} = 13 500 M^{-1} cm^{-1}). The reaction mixture in a total assay volume of 200 μL contained appropriate amounts of HPPA, 100 μM $FeSO_4$, 2 mM sodium ascorbate, 20 mM HEPES buffer (pH 7.0), HPPD, and HGD. Before assays were conducted, all reaction components were pre-equilibrated at 30 °C for at least 10 min. The amount of HGD activity was predetermined to be in a large excess of the HPPD activity to ensure that the reaction was tightly coupled (the K_m of HGD for HGA was 25 μM). Each experiment was repeated at least 3 times, and the values were averaged. HPPD inhibitors were dissolved in dimethyl sulfoxide (DMSO) for stock solution and diluted to various concentrations with reaction buffer just before use. The inhibition constant (K_i), the indication of the potency of an inhibitor, was obtained from the Dixon plot of plotting $1/v$ against the concentration of the inhibitor at certain concentrations of substrate. Bovine serum albumin is usually added up to 0.5% of the total reaction volume for coating of the target enzyme during the incubation. In our assay, no obvious effect from bovine serum albumin on the activity of the compounds has been found, which indicated that the new compounds selectively inhibited the target enzyme but did not interact with bovine serum albumin.

Computational Methods. The crystal structure of *At*HPPD was taken from the Protein Data Bank (PDB ID 1TFZ). Compounds were constructed and optimized using SYBYL 7.0 (Tripos, Inc.), and Gasteiger–Huckel charges were calculated for them. Docking calculations were performed on the two molecules using AutoDock4.0.[24] The protein and ligand structures were prepared with AutoDock Tools. A total of 256 runs were launched for each molecule. Each docked structure was scored by the built-in scoring function and clustered by 0.8 Å of root-mean-square deviation (RMSD) criterions. The best binding modes were determined by docking scores and also the comparison to available complex crystal structure of HPPD and commercial inhibitors (*Streptomyces avermitilis* HPPD complexed with inhibitor NTBC). Standard Amber ff99 force field parameters were assigned to protein, and general AMBER force field (gaff) was assigned to ligands. The partial atomic charges of ligands were calculated using the AM1-BCC method, and the system was solvated in an octahedral box of TIP3P water with the crystallographic water molecules kept. The edge of the box was at least 8 Å from the solute, and appropriate counterions were added to the system to preserve neutrality. In each step, energy minimization was first performed using the steepest descent algorithm for 1000 steps and then the conjugated gradient algorithm for another 2000 steps.

The MD simulation was performed under periodic boundary conditions using the Sander module of the AMBER9 program.[25] The stable MD trajectory was used to perform the binding free energy (ΔG_{bind}) calculation using a modified MM/PBSA method (Table 6). A total of 100 snapshots were taken from the last 500 ps trajectory with an interval of 5 ps to analyze the binding energy, and at the same time,

Scheme 2. Synthetic Route for the Title Compounds VI[a]



[a]Reagents and conditions: (a) ICl, CH_3COOH, rt; (b) substituted phenyl isocyanates, pyridine, 100 °C; (c) Cs_2CO_3, CH_3I, DMF, rt; (d) CuCN, DMF, reflux; (e) H_2SO_4, CH_3COOH, H_2O, 100 °C; (f) $SOCl_2$, THF, reflux; (g) 1,3-cyclohexanediones, Et_3N, $CHCl_3$, 0 °C; (h) acetone cyanohydrin, Et_3N, CH_2Cl_2, rt.

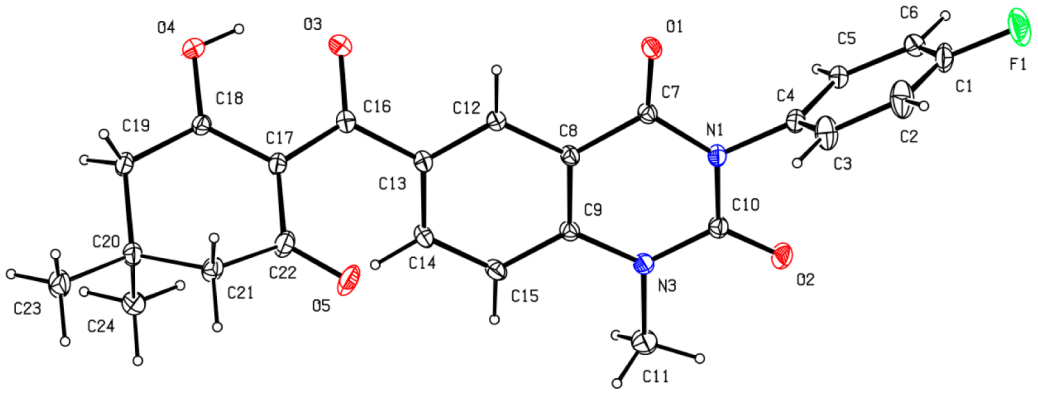


Figure 2. Crystal structure of compound **IV-d**.

the counterions and water molecules (water related to the crucial hydrogen bond was not included) were stripped.

RESULTS AND DISCUSSION

Chemistry. Dependent upon the substituent at R^4, compounds **II**–**VI** were synthesized by two different synthetic routes. When the substituent at R^4 is H, the detailed synthetic routes of compounds **II**–**V** are outlined in Scheme 1. When R^4 is methyl, the detailed synthetic routes of title compounds **VI** are outlined in Scheme 2. As seen in Scheme 1, the target compounds **II**–**V** can be prepared in 10-step synthetic routes using 5-methyl-2-nitrobenzoic acid as the starting material. After oxidation, esterification, and reduction reactions, the key intermediate dimethyl 4-aminoisophthalate (**4**) was obtained in a yield of 83%. According to the reported methods,[26,27] the key intermediate **4** reacted with various (un)substituted phenyl isocyanates in pyridine to afford quinazoline-2,4-dione intermediates **5a**–**5x** in good yields. Then, the intermediates **5a**–**5x** reacted with CH_3I to afford the corresponding intermediates **6a**–**6x** in yields of 81–99%. It was found that the intermediates **6a**–**6x** were very unstable in strong base solution, such as aqueous concentrated NaOH and KOH solution, because of the decomposition of the quinazoline-2,4-dione rings of **6a**–**6x** caused by base. However, to our delight, the quinazoline-2,4-dione rings of intermediates **6a**–**6x** were very stable in acid solution. Finally, using mixed acids (HOAc and H_2SO_4) as the hydrolysis reagents, the hydrolysis of intermediates **6a**–**6x** could smoothly proceed with yields of 78–98%. Subsequently, intermediates **7a**–**7y** reacted with $SOCl_2$ in tetrahydrofuran (THF) to obtain the corresponding acid chlorides that were very unstable. Thus, the acid chlorides were used directly in the next step without further isolation. After the reaction of the acid chlorides with substituted-1,3-cyclohexanediones, the key enol esters (**8a**–**8x**, **9a** and **9b**, **10a**–**10v**, and **11**) were generated. Finally, using acetone cyanohydrin as the Fries rearrangement catalyst, the target compounds **II**–**V** were obtained in yields of 64–94%.

Similarly, the title compounds **VI** can be prepared, in an 8-step synthetic route using 2-amino-6-methylbenzoic acid as the starting material, after an iodination reaction with ICl in acetic acid; the key intermediate 6-amino-3-iodo-2-methylbenzoic acid (**13**) was obtained in a yield of 73%. Intermediates **14a**–**14d** and **15a**–**15d** were obtained using the same methods as the preparation of intermediates **6a**–**6x**. Then, compounds **15a**–**15d** reacted with 2 equiv of CuCN in DMF, and the intermediates **16a**–**16d** were obtained in yields of 69–85%. Because the quinazoline-2,4-dione rings of intermediates **16a**–**16d** were also very sensitive to strong base, the reaction could not be proceeded in aqueous base solution. Using mixed acids (H_2SO_4 and HOAc) as the reactant, the hydrolysis of the cyano group in the intermediates **16a**–**16d** proceeded smoothly. Using the same method as the synthesis of previously enol

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esters, the intermediates **18a−18d** could be prepared from intermediates **17a−17d** in yields of 62−80%. Subsequent Fries rearrangement reaction using acetone cyanohydrin as the catalyst afforded compounds **VI** in yields of 71−78%.

The structures of all of the synthetic triketone-containing compounds were confirmed by ^{1}H and ^{13}C nuclear magnetic resonance (NMR) and high-resolution mass spectrometry (HRMS) spectral data. In addition, the crystal structure of compound **IV-d** was further determined by X-ray diffraction analysis (Figure 2).

Herbicidal Activity and SAR. The postemergence herbicidal activities of all of the target compounds were evaluated against monocotyledon weeds (*E. crus-galli*, *S. faberii*, and *D. sanguinalis*) and broadleaf weeds (*A. retroflexus*, *E. prostrata*, and *A. juncea*) in the greenhouse environment. Mesotrione was used as the positive control, and the results are shown in Tables 1−4. As anticipated, the treated weeds developed unique bleaching symptoms, as typical HPPD herbicides. The herbicidal activity evaluation indicated that most of the synthetic compounds showed between "good" to "excellent" herbicidal activity against the test weeds. Among them, compounds **III-b** and **VI-a−VI-d** showed an even broader spectrum of weed control than mesotrione, because of their superior control efficacy against the monocotyledon weeds to mesotrione. To summarize systematically the SAR of triketone-containing quinazoline-2,4-diones, some of our previously synthesized compounds were also added to the results depicted in Table 1.[15]

Previously, we have found that compound **I-f** was worth considering for further optimization; therefore, a series of substituents with diversity were introduced at the N-1 position of the quinazoline-2,4-dione ring. As shown in Table 1, compound **I-h** with an ethyl group at R^1 displayed almost equipotent herbicidal activity as compound **I-f**. However, compound **I-i** with *n*-Pr at R^1 displayed reduced herbicidal activity compared to compound **I-h**. It seems that too small or sterically bulky substituents at R^1 are detrimental to the activity of target compounds; for example, compounds **I-g** (R^1 = H), **I-k** (R^1 = *n*-Bu), and **I-m** (R^1 = $CH_2C_6H_5$) are almost inactive against six tested weeds. A possible reason for the poor herbicidal activity of these compounds may be due to their uneasily access into the weeds or rapid degradation in the weeds. However, when we placed an electron-withdrawing (**I-n**, F) or electron-donating (**I-o**, OCH_3) group on the benzyl group of **I-m**, the herbicidal activity usually displayed enhanced effects (**I-n** and **I-o** > **I-m**). Therefore, the SAR at this position can be summarized as follows: CH_3 and CH_2CH_3 > *n*-Pr > $CH_2C{\equiv}CH$ > CH_2−2-F−C_6H_4, CH_2−3-OCH_3−C_6H_4, iBu, *n*-Bu, H, and $CH_2C_6H_5$.

On the basis of the above SAR results, we kept the methyl group at R^1 and synthesized a series of novel compounds with different R^2 substitutions to explore further their herbicidal activity (Scheme 1). A variety of substituents were introduced at R^2, including electron-withdrawing (F, Br, OCF_3, CF_3, and NO_2), electron-donating (OCH_3), sterically small (H), or bulky (2-iPr and 2.6-di-iPr) groups, to evaluate the substituent effect of the substituent at this position on herbicidal activity. As depicted in Tables 1 and 2, when a single group was introduced at R^2, in most cases, compounds with substituents at the *ortho* positions would have increased activity and broader spectrum weed control than those with equivalent substitutions on *para* and *meta* positions. For example, the above rule is applied to the chloro-substituted analogues (**I-c** > **I-a** and **I-b**), the methyl-substituted analogues (**I-f** > **I-e** and **I-d**), the methoxyl-substituted analogues (**II-h** > **II-i** and **II-j**), and the trifluoromethyl-substituted analogues (**II-k** > **II-m** and **II-l**). Notably, compounds with multiple substitutions on the phenyl ring showed a decreased herbicidal activity (**II-s−II-x**), even through some of them with substituents at the *ortho* positions. It was also found that compounds with *ortho* substitiutions of electron-donating groups (**I-f**, CH_3; **II-h**, OCH_3) displayed improved herbicidal activity compared to that of the compounds with electron-withdrawing groups (**I-c**, Cl; **II-b**, F; **II-e**, Br; **II-k**, CF_3; and **II-n**, OCF_3). The similar structure−activity trends could also be observed for the compounds with substituents at the *para* positions, although there may be a few exceptions.

The SAR results suggested that substituents in the *ortho* positions of compounds can usually improve their herbicidal activity. Inspired by this, we then synthesized compounds **II-q−II-s** with different sizes of substituents on *ortho* positions to examine the steric effects on herbicidal activity. As shown in Table 2, the steric factors had a significant impact on herbicidal activity. Compounds **II-q** (R^2 = 2-C_2H_5) and **II-r** (R^2 = 2-iPr) showed over 80% control in six deferent weed tests at the rate of 150 g of ai/ha. However, compound **II-s** (R^2 = 2,6-di-iPr) with more sterically bulky substituents in its *ortho* positions displayed decreased effects and was almost inactive toward *S. faberii* and *A. juncea*. A possible explanation for compound **II-s** with lower activity is that the large subsitutuents would prevent making bonds with plant HPPD.[28]

To examine the effect of R^3 substitution on herbicidal activity, we then synthesized another set of compounds (**III−V**). As in Table 3, compounds **III-a** and **III-b**, with a methyl on the 5 position of the 1,3-cyclohexanedione ring, displayed an increased herbicidal effect compared to their non-substituted analogues **II-g** and **II-v**, respectively. Furthermore, compound **III-b** exhibited over 85% control in six deferent weed tests, even at a rate as low as 37.5 g of ai/ha, demonstrating broader spectrum of weed control than mesotrione. However, when another methyl group was also introduced to the 5 position, the herbicidal activity (**IV-g** and **IV-s**) was impaired in most cases. Because of our curiosity, two methyl groups were introduced at 6 positions, with compound **V** displaying an improved herbicidal activity compared to its parent compound **II-t**. One possible reason for this phenomenon is that introducing two methyl groups on 6 posotions would block the metabolism of weeds *in vivo*.[7]

We found that most of the commercial HPPD herbicides have substituents in the *ortho* positions of their benzoyl groups.[7,8,10] It is assumed that placing a substitutent in the *ortho* position would be favorable for increasing their herbicidal activity. In addition, introducing a methyl group in the *ortho* position has been reported, to be able to enhance the herbicidal activity.[9,13] Owning to the structure feature of the triketone-containing quinazoline-2,4-dione motif, we intended to introduce a methyl group in the 5 position of the quinazoline-2,4-dione ring (*ortho* position). To understand whether placing a methyl group in the 5 position is beneficial for herbicidal activity or not, four representative compounds **I-f**, **II-h**, **II-k**, and **II-u** with a promising and broad spectrum of weed control were selected for further optimization. The synthetic methods for these methyl-substituted analogues (**VI-a−VI-d**) are outlined in Scheme 2, the herbicidal activity results are shown in Table 4. Surprisingly, compounds **VI-a−VI-d**, with a methyl group in the *ortho* positions, showed significantly

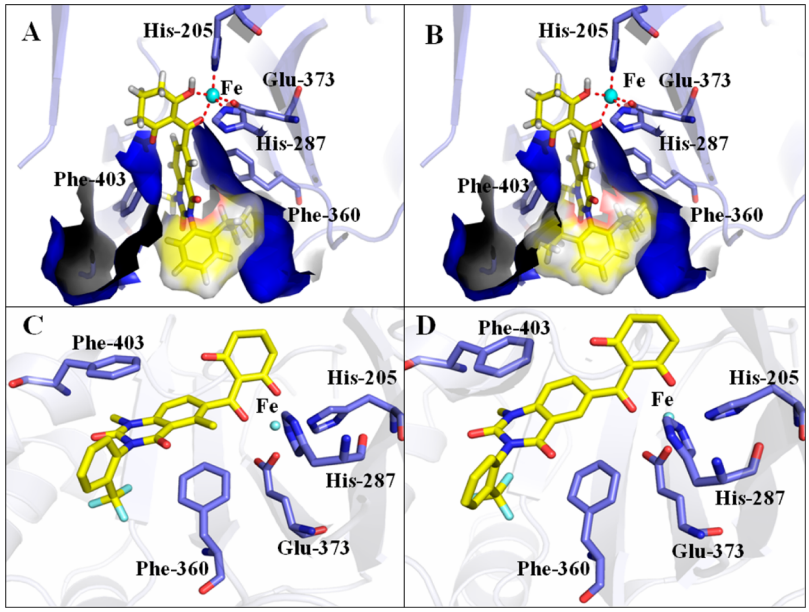

Figure 3. Simulated binding models of four representative compounds (A) **II-r**, (B) **II-s**, (C) **II-k**, and (D) **VI-c** in the active site of *At*HPPD, with panels A and B shown as translucent surfaces and panels C and D shown as directly binding models. Fe(II) is depicted as the aquamarine sphere; the structures of inhibitors are indicated in yellow sticks; and the key residues surrounding the active site are displayed in light blue.

improved herbicidal activity compared to their corresponding parent compounds **I-f**, **II-h**, **II-k**, and **II-u**, respectively. All four methyl-substituted analogues displayed strong inhibition (>85%) and broad spectrum for weed control, even at a dosage as low as 37.5 g of ai/ha. Especially, compound **VI-a** displayed almost 100% inhibition in both tests against monocotyledon and dicotyledon weeds, with its herbicidal potency against dicotyledon weeds (*A. retroflexus*, *E. prostrata*, and *A. juncea*) being almost equipotent to mesotrione and its potency against monocotyledon weeds (*E. crus-galli*, *S. faberii*, and *D. sanguinalis*) being superior to mesotrione.

Crop Selectivity. Crop selectivity is one of the vital concerns in pesticide discovery. Some representative compounds (**II-h**, **II-k**, **II-u**, **III-b**, and **VI-a**–**VI-d**) were chosen for crop selectivity evaluation. The results are listed in Table 5. It is very interesting that compound **III-b** was safe for three crops of cotton, maize, and wheat after postemergence application at a rate of 150 g of ai/ha, whereas mesotrione was not selective for cotton (70% injury) and wheat (40% injury) at the concentration of 150 g of ai/ha. This result indicated that there is a great potential for **III-b** to be used in cotton, maize, and wheat fields to control weeds. Furthermore, **II-u** was safe for maize and wheat, suggesting that **II-u** has potential to develop as a effective herbicide for controlling weeds in maize and wheat fields. In addition, **II-h** was safe for rape at the rate of 150 g of ai/ha, whereas mesotrione displayed 100% injury effect against it at the same conditions, indicating that **II-h** might has potential to develop as a new herbicide in a rape field. Moreover, **II-k** was safe for maize at the rate of 150 g of ai/ha, which indicated that **II-k** can be developed as a herbicide for maize field. Although compounds **VI-a**–**VI-d** displayed excellent herbicidal activity, they were not safe for the six selected crops. A possible explanation of compounds **VI-a**–**VI-d** to improve their herbicidal activity while decreasing crop selectivity is that the methyl group at R^4 would block metabolism of plants at this site.[13]

HPPD Inhibition and SAR. For further understanding, the structural basis for these compounds as HPPD inhibitors and the K_i values of compounds **II**–**VI** against *At*HPPD are shown in Tables 2–4. Mesotrione was used as the positive control. Notably, most of newly synthesized herbicides displayed a strong *At*HPPD inhibitory activity, and some of them showed even better potency than mesotrione. Compound **VI-d** (K_i = 9 nM) displayed the highest HPPD inhibitory activity, showing slightly more potency than mesotrione (K_i = 13 nM). Furthermore, compounds **VI-c** (K_i = 10 nM), **II-o** (K_i = 12 nM), and **II-v** (K_i = 13 nM) also showed excellent inhibitory activity, which are comparable to mesotrione.

On the basis of the *in vitro* activity of those compounds, some SARs can be drawn. As shown in Table 2, when the right side of the benzene ring is single-substituted, compounds with *para* substitutions generally have improved activity compared to those with the same substituents in *meta* and *ortho* positions. With regard to the multi-substituted analogues, the positions of substituents can also affect the HPPD inhibitory activity. Compounds with substituents at 2,4 and 3,5 positions would have enhanced activity compared to those at 2,6 positions (**II-v** and **II-x** > **II-t**). It seemed that the electronic factors did not significantly affect the activity; for example, compounds with either *para* substitutions of strongly electron-donating (**II-j**, OCH_3) groups or electron-withdrawing (**II-p**, NO_2) groups showed almost equal activity (**II-j** = **II-p**). In addition, the steric factors of substituents also have a significant impact on the inhibitory activity. Compounds with sterically bulky groups on the *ortho* position were found detrimental to activity (**II-r** > **II-s**). This was consistent with the herbicide inhibition activity. To understand the structural basis, we investigated the binding modes of the new compounds using compounds **II-r** and **II-s** as two representatives. As shown in panels A and B of Figure 3, the two compounds can form a bidentate association with the active site ferrous ion via its 5′ and 7′ oxygens and the quinazoline-2,4-dione ring can form π–π stacking interaction with the conserved Phe360 and Phe403. In addition, the 2-isopropyl-substituted benzene ring on the quinazoline-2,4-dione moiety of compound **II-r** embedded deep into the hydrophobic cavity and occupied a major part of this pocket.

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However, the 2,6-diisopropyl-substituted benzene ring of compound **II-s** occupied beyond the hydrophobic pocket, showing a strong steric repulsion effect.

It is worth mentioning that, when a methyl group was introduced to the 5 position of the quinazoline-2,4-dione ring, the inhibition of compounds **VI-a**−**VI-d** against *At*HPPD has improved significantly compared to their parent compounds **I-f**, **II-h**, **II-k**, and **II-u**, respectively (Table 4). To investigate why the methyl group at the R^4 position significantly improved the activity of the target compounds, compounds **II-k** and **VI-c** were chosen as the representative inhibitors for computational modeling. The energy component analysis showed that the difference mainly came from the electrostatic interaction, solvation, and entropy effects (Table 5). Compound **II-k** had an electrostatic interaction of −128.75 kcal/mol and a solvation penalty of 115.98 kcal/mol, which result in an enthalpy change of −46.33 kcal/mol. Compound **VI-c** had a lower electrostatic interaction of −138.94 kcal/mol and a higher solvation penalty of 124.09 kcal/mol, which result in a similar enthalpy change of −46.82 kcal/mol. However, a higher entropy penalty of **VI-c** (37.72 kcal/mol compared to 34.42 kcal/mol for **II-k**) resulted in a lower binding affinity (−9.16 kcal/mol compared to −11.91 kcal/mol for **II-k**), which demonstrated that the introduction of the methyl group at the 5 position of the quinazoline-2,4-dione ring can help reduce the entropy penalty and be advantageous for activity improvement.

In summary, a series of novel triketone-containing quinazoline-2,4-diones were designed and subsequently optimized on the basis of a systemic SAR study, leading to the discovery of several novel HPPD inhibitors with the excellent *in vitro* inhibitory potency and greenhouse herbicidal activity. Specifically, compounds **II-h**, **II-k**, **II-u**, **III-b**, and **VI-a**−**VI-d** were found with a potential of displaying a broader spectrum of weed control than mesotrione. More importantly, compound **III-b** was very safe for three crops of cotton, maize, and wheat at the rate of 150 g of ai/ha, showing great applicability in the field. Furthermore, at the dosage of 150 g of ai/ha, compound **II-h** was safe for rape, compound **II-k** was safe for maize, and compound **II-u** was safe for maize and wheat. The promising results suggested that the triketone-containing quinazoline-2,4-dione motif is well worth further optimization. Further structural optimizations and field trials of compounds **II-h**, **II-k**, **II-u**, and **III-b** are still ongoing.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental details and analytical data for all of the intermediates and compounds **II**−**VI**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Authors

*Telephone: +86-27-67867800. Fax: +86-27-67867141. E-mail: tomyang@mail.ccnu.edu.cn.

*Telephone: +86-27-67867800. Fax: +86-27-67867141. E-mail: gfyang@mail.ccnu.edu.cn.

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Notes

The authors declare no competing financial interest.

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