JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Functional Structure/Activity Relationships

Design and synthesis of novel 4-hydroxyl-3-(2-phenoxyacetyl)-pyran-2-one derivatives for use as herbicides and evaluation of their mode of action

Kang Lei, Pan Li, Xuefang Yang, Shiben Wang, Xuekun Wang, Xuewen Hua, Bin Sun, Lusha Ji, and Xiaohua Xu

J. Agric. Food Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jafc.9b03109 • Publication Date (Web): 27 Aug 2019

Design and synthesis of novel 4-hydroxyl-3-(2-phenoxyacetyl)-pyran-2-one derivatives for use as herbicides and evaluation of their mode of action

Kang Lei,* [a, b,†] Pan Li,[a, †] Xue-Fang Yang,[c] Shi-Ben Wang,[a] Xue-Kun Wang,[a] Xue-Wen Hua,[a, b] Bin Sun,[a, b] Lu-Sha Ji,[a] Xiao-Hua Xu* [b]

[a] School of Pharmacy, Liaocheng University, Liaocheng, China

[b] State Key Laboratory of Elemento-Organic Chemistry, Institute of Elemento-Organic Chemistry, Nankai University, Tianjin, China

[c] College of agriculture, Shanxi Agricultural University, Shanxi, China

[†] Authors contributed equally to this work

[*] Correspondence to: Kang Lei, E-mail: leikang@lcu.edu.cn; Xiao-Hua Xu, E-mail: xiaohuaxu@nankai.edu.cn.

ABSTRACT

In order to develop a novel herbicide containing the *β*-triketone motif, a series of 4-hydroxyl-3-(2-phenoxyacetyl)-pyran-2-one derivatives were designed and synthesized. Bioassay results showed that compound **II15** had good pre-emergent herbicidal activity even at a dosage of 187.5 g ha^{-1}. Moreover, compound **II15** showed a broader spectrum of weed control when compared with a commercial herbicide 2,4-dichlorophenoxyacetic acid (2,4-D), and displayed good crop safety to *Triticum aestivum L.* and *Zea mays Linn.* when applied at 375 g ha^{-1} under pre-emergence conditions, which indicated its great potential as a herbicide. More importantly, studying the molecular mode of action of compound **II15** revealed that the novel triketone structure is a proherbicide of its corresponding phenoxyacetic acid auxin herbicide, which has a herbicidal mechanism similar to that of 2,4-D. The present work indicates that the 4-hydroxyl-3-(2-phenoxyacetyl)-pyran-2-one motif may be a potential lead structure for further development of novel auxin-type herbicides.

Keywords: 4-hydroxyl-3-(2-phenoxyacetyl)-pyran-2-one, *β*-triketone, synthesis, herbicidal activity, auxin herbicide

INTRODUCTION

β-Triketones are known as potential agrochemicals because they exhibit several biological activities, including herbicidal [1-6], antibacterial [7], antifungal [8, 9] and insecticidal activity [10]. Among the various *β*-triketones reported to date, pyran-based diketone lactones have been extensively investigated, and many pyran-based diketone lactones (**Fig. 1**) are reported owing to their effective role as antiviral agents [11], free-radical scavengers [12], and acidichromic colorants [13, 14]. However, relatively little is known about the application of pyran-based diketone lactones in pesticide formulation [15]. In our previous work, it was shown that 3-benzoyl-4-hydroxylcoumarin derivatives (**Fig. 1**), which belong to the family of pyran-based diketone lactones, display herbicidal activity and fungicidal activity [16, 17]. Therefore, this encouraged us to continue to explore the biological activity of pyran-based diketone lactones in pesticide.

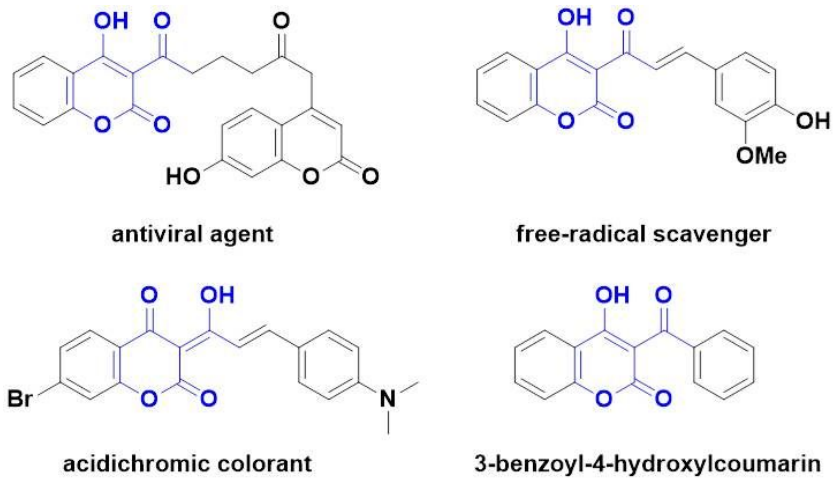


Figure 1. Structures of various pyran-based diketone lactones.

Phenoxyacetic acid and its derivatives are key intermediates in the synthesis of biologically active compounds. Over the last few decades, many

phenoxyacetyl-containing derivatives have been successfully synthesized, and these derivatives display a wide range of biological activity in drug chemistry [18-22]. Moreover, phenoxyacetic acid and its derivatives have also been reported to exhibit herbicidal [23], insecticidal [24], and fungicidal activity [25]. Based on the above-mentioned facts, we envisaged that introducing phenoxyacetyl at the 3rd-position of 4-hydroxylcoumarin and 4-hydroxy-6-methyl-2*H*-pyran-2-one can help construct a variety of 4-hydroxyl-3-(2-phenoxyacetyl)-pyran-2-one derivatives (**Fig. 2**, compounds **I** and **II**), which should possess some interesting biological activity. Therefore, as a continuation of our work on synthesizing and evaluating the biological activity of pyran-based diketone lactones in pesticide formulation, 21 3-(2-phenoxyacetyl)-4-hydroxylcoumarin derivatives (**Fig. 2**, compound **I**) and 21 4-hydroxy-6-methyl-3-(2-phenoxyacetyl)-2*H*-pyran-2-one derivatives (**Fig. 2**, compound **II**) were synthesized, and their herbicidal activities were evaluated to develop a novel herbicide containing *β*-triketone motif. To the best of our knowledge, this is the first report on the herbicidal activity of 4-hydroxyl-3-(2-phenoxyacetyl)-pyran-2-one derivatives.

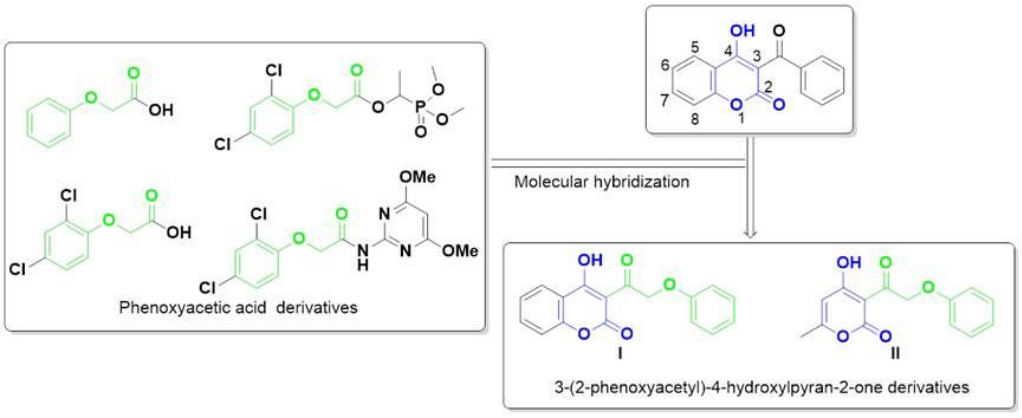
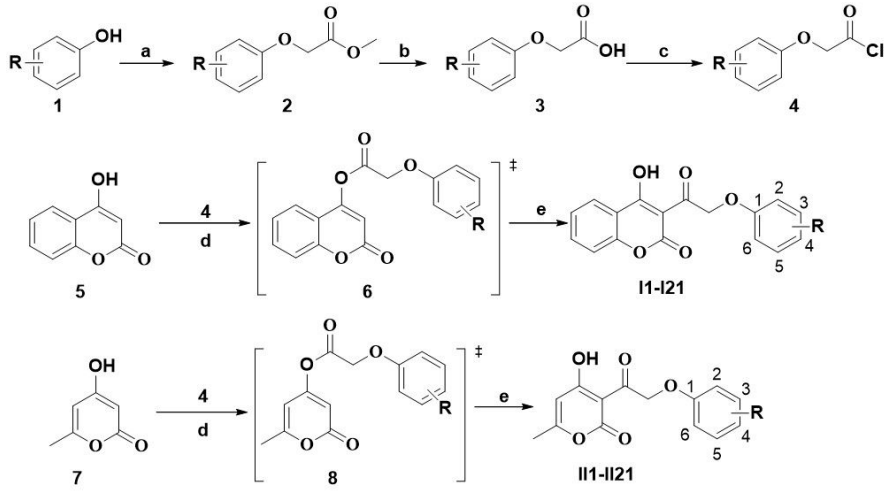


Figure 2. Design strategy used to prepare the target compounds.

MATERIALS AND METHODS

Chemical synthesis procedures

The synthetic pathway used to prepare the target compounds is outlined in **Scheme 1**. The yields were not optimized.



Reagent and conditions: (a) methyl chloroformate, K_2CO_3, DMF, 50 °C, 12 h; (b) LiOH, MeOH/ H_2O, 50 °C, 12 h; (c) oxalyl chloride, DMF, DCM, 25 °C, 12 h; (d) Et_3N, 4-dimethylaminopyridine, DCM, 25 °C, 12 h; (e) KCN, 18-crown-6, Et_3N, DCM, 25 °C, 72 h.

I1/II1 R=H; I2/II2 R=2-Cl; I3/II3 R=3-Cl; I4/II4 R=4-Cl; I5/II5 R=2-Me; I6/II6 R=3-Me; II7/II7 R=4-Me I8/II8 R=2-OMe; I9/II9 R=3-OMe; I10/II10 R=4-OMe; I11/II11 R=2,3-2Cl; I12/II12 R=2,4-2Cl; I13/II13 R=2,5-2Cl I14/II14 R=3,5-2Cl; I15/II15 R=2-Cl-4-F; I16/II16 R=2-Cl-4-Br; I17/II17 R=2-Me-4-F; I18/II18 R=2-Me-4-Cl; I19/II19 R=2-Me-4-Br; I20/II20 R=2-Br-4-F; I21/II21 R=2-Br-4-Cl

Scheme 1. Synthetic route of preparing target compounds **I1-I21** and **II1-II21**.

*General procedure for the synthesis of aryloxyacetyl chloride derivatives **4***

The key intermediate aryloxyacetyl chloride derivatives **4** were synthesized following a reported procedure [5]. The appropriate phenol **1** (20 mmol) and potassium carbonate (40 mmol) were added successively to *N*, *N*-dimethylformamide (100 mL) in a 250 mL flask with stirring, and the mixture was heated up to 50 °C for 1 h. Methyl chloroacetate (20 mmol) was added to the mixture and the suspension was stirred at 50 °C for 12 h. Then, the mixture was cooled to room temperature, poured into water, and stirred for another 0.5 h. To the solution was added 200 mL of ethyl acetate, the ethyl acetate layer was separated and concentrated by rotary evaporation.

Methanol (50 mL) and water (5 mL) were added to the resulting residue, and then lithium hydroxide (100 mmol) was added to the solution with stirring. The solution was heated up to 50 °C for 12 h. After completion of the reaction, 30 mL of methanol was removed by rotary evaporation. The resulting solution was cooled to room temperature and acidified by hydrochloric acid solution (6 M) to pH = 1. The resulting solid was collected by filtration, washed with water, and dried in a vacuum to afford aryloxyacetic acids **3**. Subsequently, aryloxyacetic acids **3** (10 mmol) were dissolved in dichloromethane (20 mL) in a 50 mL flask, oxalyl chloride (20 mmol) and *N*, *N*-dimethylformamide (one drop) were added. The mixture was stirred at room temperature for 12 h. After completion of the reaction, dichloromethane was removed by rotary evaporation to provide aryloxyacetyl chloride derivatives **4**, which used in the next step without purification.

*General procedure for the synthesis of 3-(2-phenoxyacetyl)-4-hydroxylpyran-2-one derivatives (compound **I1-I21** and compound **II1-II21**)*

3-(2-phenoxyacetyl)-4-hydroxylpyran-2-one derivatives were prepared following a reported method [16, 17, 26]. 4-Hydroxylcoumarin **5** (5 mmol) and 20 mL of dichloromethane were added to a 100 mL flask. Aryloxyacetyl chloride derivatives **4** was added to the solution at 0 °C, the solution was stirred for another 30 min, then, triethylamine (10 mmol) and 4-dimethylaminopyridine (0.5 mmol) were added successively to the mixture, and the solution was stirred for 12 h. After completion of the reaction, aqueous hydrochloric acid solution (1 M, 20 mL) was added. The dichloromethane layer was separated and washed with H_2O, saturated sodium chloride

solution, dried by anhydrous sodium sulfate, concentrated by rotary evaporation, and the residue was scratched from ethyl acetate and petroleum ether (1/1 by volume) to give desired product **6**, which used in the next step without further purification.

A solution of compound **6** (2 mmol) in dichloromethane (20 mL) was added to triethylamine (3 mmol), 18-crown-6 (0.2 mmol) and potassium cyanide (1 mmol). The mixture was stirred at room temperature for 72 h. The mixture was poured into water and extracted with dichloromethane. The combined organic phase was dried with anhydrous sodium sulfate, filtered and removed by rotary evaporation. The residue was scratched from ethyl acetate and ethanol (1/1 by volume) to give target compounds **I1-I21**.

Compounds **II1-II21** were synthesized by the similar procedure to compounds **I1-I21**. The data of ^{1}H NMR, ^{13}C NMR, and HRMS of all target compounds is given in the Supporting Information.

X-ray diffraction of compounds I9 and II14

Compound **I9** was recrystallized from a mixture of dichloromethane and methanol (1/1 by volume) to afford a suitable single crystal. Compound **II14** was recrystallized from dichloromethane to afford a suitable single crystal. Crystallographic data for compounds **I9** and **II14** had been deposited with the Cambridge Crystallographic Data Centre as supplementary publications with the deposition numbers 1834640 and 1874641, respectively. The detail data can be obtained free of charge from http://www.ccdc.cam.ac.uk/.

Herbicidal activity evaluation

Based on the reported procedure [26-29], herbicidal activity was evaluated with three replicates per treatment. Mesotrione and 2,4-D were selected as positive control. The preliminary herbicidal activity of target compounds was determined with *Brassica campestris* root test and *Echinochloa crusgalli* cup test. Further herbicidal activity study of target compounds against four species representative of monocotyledonous and dicotyledonous plants (*Brassica campestris*, *Amaranthus retroflexus*, *Echinochloa crusgalli*, *Digitaria sanguinalis*) was performed in the greenhouse. Compound **II15** was selected to study the herbicidal spectra. The procedure is given in the Supporting Information.

Crop selectivity

Based on the reported procedure [5], the crop selectivity of compound **II15** was evaluated with three replicates per treatment. Three representative crops, namely, *Triticum aestivum L.*, *Zea mays Linn.* and *Gossypium spp*, were selected for crop selectivity studies in the greenhouse. The procedure is given in the Supporting Information.

Phenotypic study of *Arabidopsis thaliana*

Arabidopsis thaliana employed in this study is in the Columbia (Col-0) background. Surface-sterilized seeds sown onto Murashige and Skoog (MS) plates at 4°C for 3 days under the darkness to favor vernalization and then grown at 22 °C for 2 days under LD (16h light/8h darkness). Subsequently, plants were transferred to 1/2MS plates supplemented with compound **II15** (from 1 μM to 1.0 nM), 2,4-D (from 1 μM to 1.0 nM), 2-(2-chloro-4-fluorophenoxy) acetic acid (from 1 μM to 0.1 μM),

4-hydroxy-6-methyl-pyran-2-one (1 μM) and mesotrione (1 μM), respectively. The solution of compound **II15**, 2,4-D, 2-(2-chloro-4-fluorophenoxy) acetic acid, 4-hydroxy-6-methyl-pyran-2-one and mesotrione was prepared in 0.1% MeOH and diluted in the 1/2MS to obtain the tested concentrations, respectively. Solvent (0.1% MeOH) was added also to control plates. Plants were grown on vertically oriented plates at 22 °C for 7 days under LD (16h light/8h darkness) for phenotypic investigation and root length measurement.

Quantitative real-time-PCR

Surface-sterilized seeds were sown onto MS plates. The plant was grown on plates for two weeks from day of sowing under LD (16h light/8h darkness) at 22 °C. Subsequently, the plant was carefully placed in a petri dish with filter paper covered. The solution of compound **II15** (10 μM), 2,4-D (10 μM), and mesotrione (10 μM) was added to the petri dish, respectively. Control plants were treated with distilled water. The leaves of seedlings were collected for RNA extraction after 0 h, 12 h, and 24 h, respectively. For quantitative real-time PCR (qRT-PCR), total RNAs were extracted from plant tissues using Trizol reagent (TaKaRa, Japan) according to the manufacturer's instructions. Reverse transcription reactions were performed with the PrimeScript RT reagent kit with gDNA Eraser (Takara, Japan). qRT-PCR reactions were performed on real-time thermal cycling system (Bio-Rad, USA), and the SYBR-Green Ex Taq II kit (TaKaRa, Japan) was used for detecting gene expression abundances. The gene expression levels were normalized with the reference gene *ACTIN-2*. Primers used for PCR are given in the Supporting Information (**Table S1**).

The degradation study of compound II15 in *Arabidopsis thaliana*

Surface-sterilized seeds were sown onto MS plates. Plants were grown on plates at 22 °C for 14 days under LD (16 h light/8 h dark). Subsequently, plants were carefully placed in a petri dish with filter paper covered. The solution of compound **II15** (50 μM) and 2-(2-chloro-4-fluorophenoxy) acetic acid (50 μM) was added to the petri dish, respectively. After 15 min of incubation, the treated plants were transferred to the MS plates. The leaves of seedlings were collected for extraction after 0 h, 1 h, 1.5 h, 2 h, and 2.5 h, respectively. Methanol was used as extraction fluid. The ingredients of extraction were analyzed by HPLC on a Shimadzu HPLC system using a 5 μm C18 column (150 × 4.6 mm; Agilent Zorbax) and 3-indolebutyric acid (IBA) was used as the internal standard. A linear gradient with increasing methanol (solvent A) against double-distilled H_2O (solvent B) at a flow rate of 1 mL min^{-1} over 35 min was used. Both solutions contained 0.1 % phosphoric acid. HPLC conditions were as follows: 0 min, 30 % A; 20 min, 70 % A; 22 min, 30 % A; 35 min, stop. The results are presented at 230 nm.

Statistical analysis

The values shown in each table are mean values ±SD of at least three repeated experiments. SPSS 22.0 (SPSS, Chicago, IL) was used as the statistical software program.

RESULTS AND DISCUSSION

Chemistry

As depicted in **Scheme 1**, the target compounds can be prepared via a five-step

synthetic route using several phenols as the starting material. In the presence of potassium carbonate as base, phenols **1** reacted with methyl chloroacetate, and the corresponding methyl aryloxyacetates **2** were produced. Hydrolysis of methyl aryloxyacetates **2** with lithium hydroxide as a base gave aryloxyacetyl acids **3**. Subsequently, aryloxyacetyl acids **3** were transformed into their corresponding aryloxyacetyl chloride derivatives **4** using oxalyl chloride. Reacting compound **5** with the aryloxyacetyl chloride derivatives **4** in the presence of 4-dimethylaminopyridine and trimethylamine gave intermediate **6**, which was treated with potassium cyanide and 18-crown-6 at room temperature to give target compounds **I1-I21** in 41– 61% yield.

Compounds **II1-II21** were synthesized using a procedure similar to that used for compounds **I1-I21**. The structures of all the target compounds were identified using ^{1}H and ^{13}C NMR spectroscopy, and HRMS. Furthermore, the structures of compound **I9** and compound **II14** were confirmed using X-ray diffraction analysis (CCDC 1834640 and 1874641; **Fig. 3**).

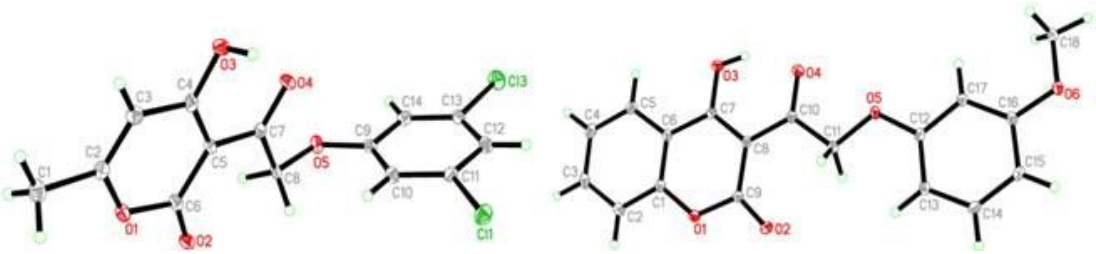
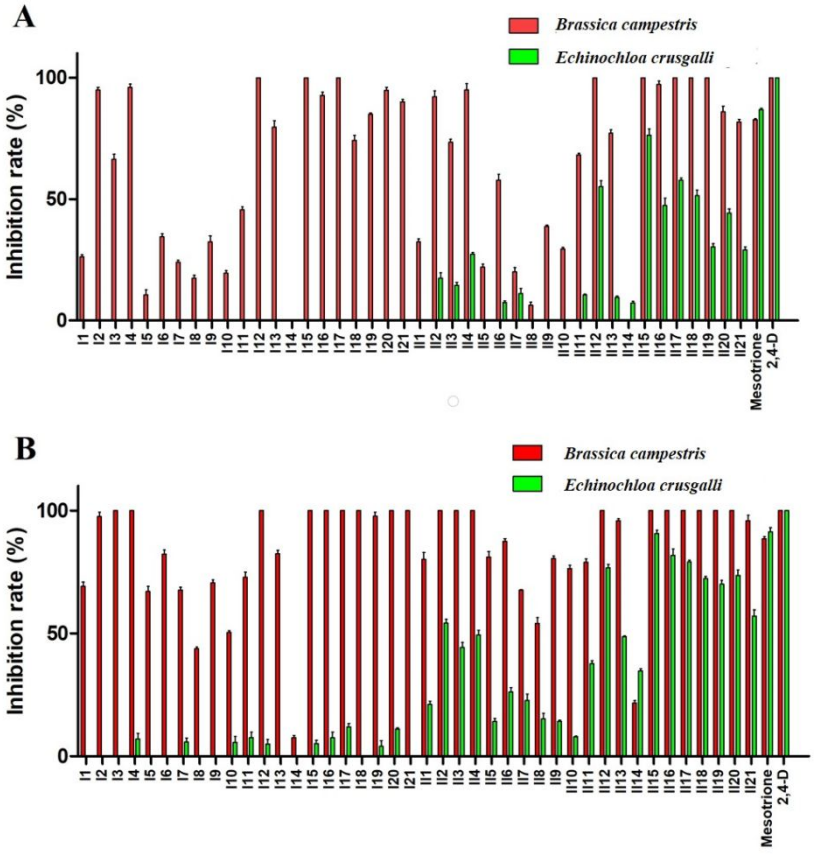


Figure 3. X-ray crystal structures of compounds **I9** (right) and **II14** (left).

***In vitro* herbicidal activity**

The herbicidal activity of the target compounds was preliminarily determined using a *Brassica campestris* root test and *Echinochloa crusgalli* cup test. Mesotrione and

211 2,4-D were selected as positive control samples. As shown in **Fig. 4A**, the target
212 compounds, such as **I2**, **I4**, **I12**, **I15-I17**, **I20**, **I21**, **II2**, **II4**, **II12**, and **II15-II19**,
213 exhibited excellent herbicidal activity against the dicotyledonous plant *Brassica*
214 *campestris* with inhibition >90% even at a dosage of 10 μg mL^{-1}. However, only
215 some of the compound **II**, such as **II12** and **II15-II20**, exhibited >70% control against
216 *Echinochloa crusgalli* at a dosage of 100 μg mL^{-1} (**Fig. 4B**). These preliminary results
217 indicate that the target compounds have good herbicidal activity and exhibit better
218 herbicidal activity against dicotyledonous plants.



219

220 **Figure 4.** Effects (% inhibition) of compounds **I1-I21** and **II1-II21** on mustard root
221 and barnyardgrass seedling growth; (**A**) dosage = 10 μg mL^{-1}; (**B**) dosage = 100 μg
222 mL^{-1}; The error bars represent the standard deviation (s. d.) from three biological
223 replicates.

Molecular mode of action of the target compounds

In order to explore the molecular mode of action of target compounds, compound **II15** was selected to study the herbicidal mechanism with *Arabidopsis thaliana* as a model plant. As shown in **Fig. 5A**, *Arabidopsis thaliana* treated with mesotrione at a dosage of 1.0 μM developed bleaching symptoms, whereas *Arabidopsis thaliana* treated with 2,4-D, 2-(2-chloro-4-fluorophenoxy) acetic acid, 4-hydroxy-6-methyl-pyran-2-one or compound **II15** did not develop any bleaching symptoms, indicating these compounds have a herbicidal mechanism different to that of mesotrione. Subsequently, the phenotypes of *Arabidopsis thaliana* in response to a range of compound **II15** concentrations were investigated. It was found that compound **II15** displays significant inhibitory activity against *Arabidopsis thaliana* root growth with IC_{50} value of 26.42 nM, which comparable with commercial herbicide 2,4-D (IC_{50}=13.55 nM) (Inhibition curve is given in Supporting Information, **Fig. S1**). Interestingly, *Arabidopsis thaliana* treated with compound **II15** at a lower concentration (such as 0.1 and 0.3 μM) exhibited obviously inhibited root elongation and induced in lateral root hairs formation, which consistent with the observation that plants exposed to 2,4-D or 2-(2-chloro-4-fluorophenoxy) acetic acid (**Fig. 5B**). These preliminary phenotypes indicate that compound **II15** may be an auxin-type compound and has a herbicidal mechanism similar to that of 2,4-D.

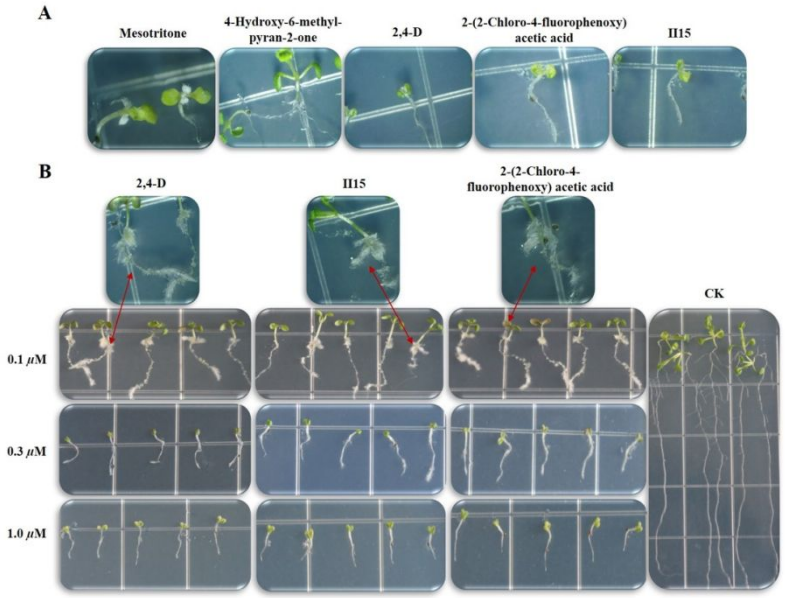


Figure 5. Photographs illustrating seedling and root phenotype of *Arabidopsis thaliana*

To further verify whether compound **II15** is an auxin-type compound, the effect of compound **II15** on the expression levels of auxin related genes was investigated. The auxin induced gene (*IAA5*), auxin regulated gene (*GH3.3*) and auxin transport gene (*AUX1*) were selected for test, since these genes play an important role in auxin early response and transport [30-37]. As shown in **Fig. 6**, the trend in the change observed for the tested genes induced by mesotrione was different to that of 2,4-D. The expression levels of *IAA5*, *GH3.3* and *AUX1* were down-regulated after *Arabidopsis thaliana* treated with mesotrione. Since mesotrione is not an auxin analogue, the downregulation of these genes may be induced by its damage to the plant. To our delight, upon increasing the treatment time with compound **II15**, the expression levels of *IAA5*, *GH3.3* and *AUX1* was up-regulation, and the change trend was consistent with those observed for 2,4-D, which confirmed the auxin-type property of compound **II15**.

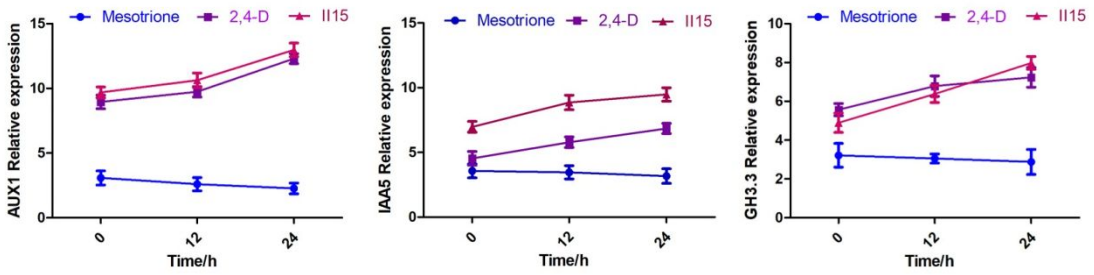


Figure 6. Effect of compound **II15**, mesotrione, and 2,4-D on the expression levels of *IAA5, GH3.3* and *AUX1*; The error bars represent the standard deviation (s. d.) from three biological replicates.

To explore whether the auxin-type property of compound **II15** are due to its degradation products, the degradation of compound **II15** in *Arabidopsis thaliana* was investigated. 2-(2-Chloro-4-fluorophenoxy) acetic acid was selected as a control. It was found that 2-(2-chloro-4-fluorophenoxy) acetic acid and compound **II15** were gradually degraded in the plant (**Fig. 7A and 7B**). Interestingly, with the degradation of compound **II15**, a new peak was observed at 19 min, which is same as the peak time observed for 2-(2-chloro-4-fluorophenoxy) acetic acid (**Fig. 7B**). LC–MS analysis showed that there are two dominant ions m/z 311.17 ($M-H^+$) and m/z 203.14 ($M-H^+$) in negative ionization mode, which corresponds to the molecular weight of compound **II15** (M = 312.02) and 2-(2-chloro-4-fluorophenoxy) acetic acid (M = 204.00) (**Fig. 7C**). This result indicates that compound **II15** is degraded to 2-(2-chloro-4-fluorophenoxy) acetic acid in the plant. It is worth noting that, after 2.5 h, 2-(2-chloro-4-fluorophenoxy) acetic acid was completely degraded, while compound **II15** undergone continuous degradation to release 2-(2-chloro-4-fluorophenoxy) acetic acid, indicating compound **II15** will prolong the lifetime of phenoxycarboxylic acid in the plant and exert a sustained herbicidal effect.

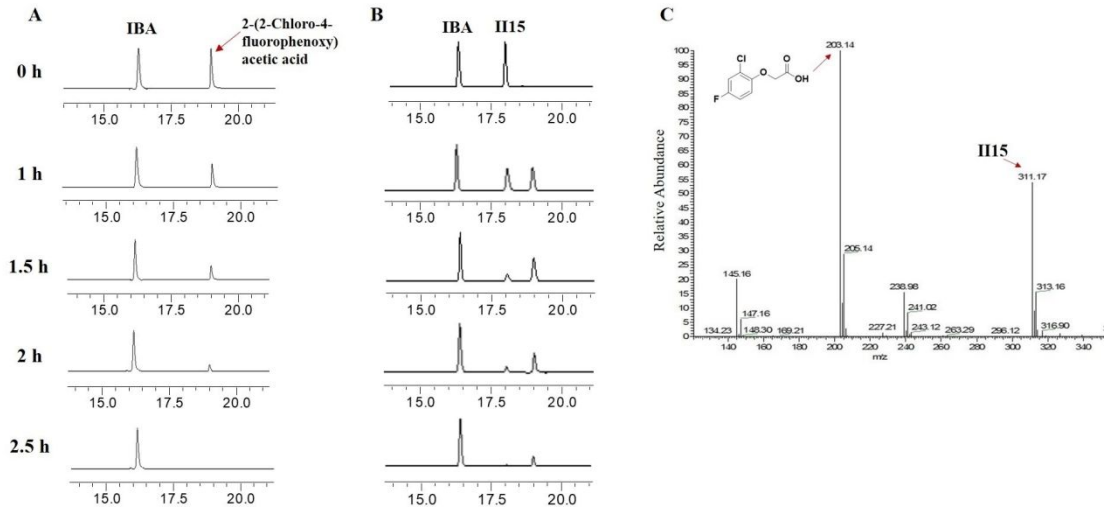
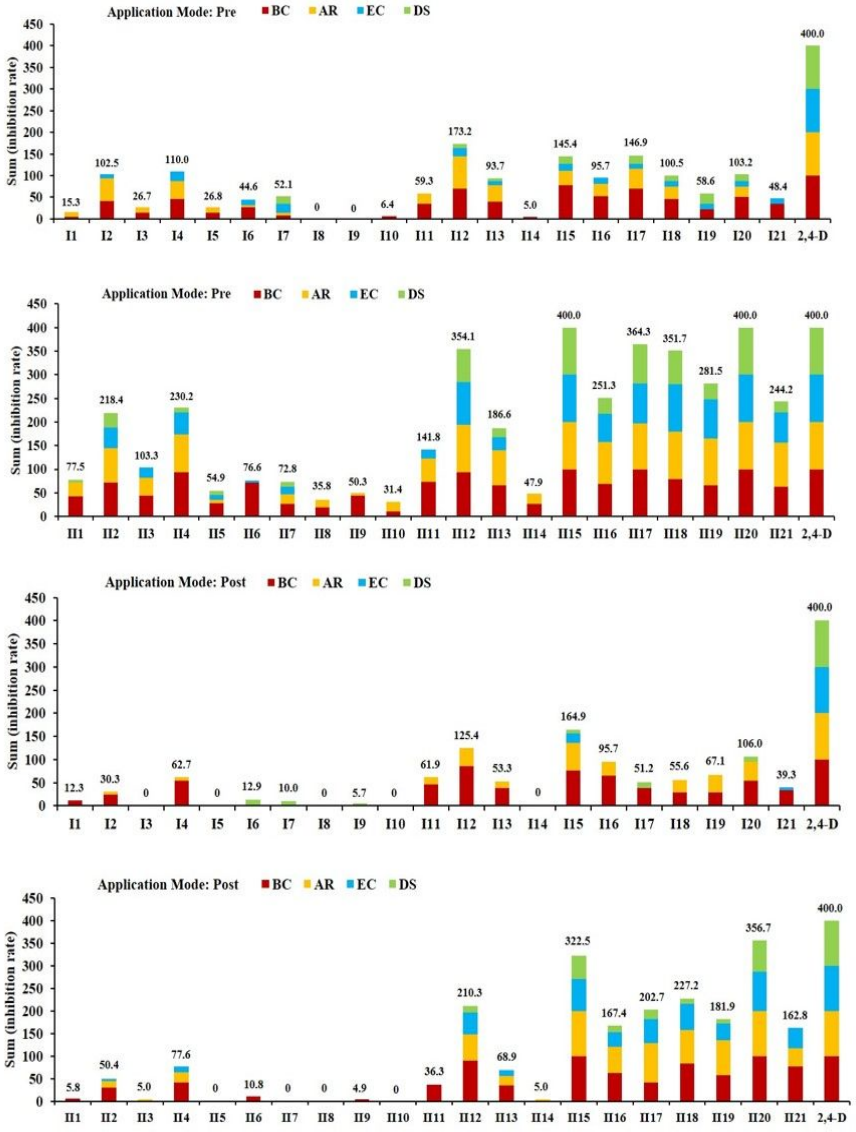


Figure 7. Degradation analysis of 2-(2-chloro-4-fluorophenoxy) acetic acid and compound **II15** in *Arabidopsis thaliana*

Herbicidal activity in greenhouse tests and SARs study

Based on the above preliminary bioassay results, the herbicidal activity of all target compounds was further tested on four species representative of monocotyledonous and dicotyledonous plants at a dosage of 1500 g ha^{-1} located in a greenhouse. 2,4-D was selected as a positive control. As shown in **Figure 8**, most of the target compounds displayed stronger herbicidal activity against dicotyledonous plants when compared to monocotyledonous plants, which was consistent with the preliminary *in vitro* results. Moreover, in most cases, the target compounds had stronger pre-emergent herbicidal activity than post-emergent herbicidal activity against all the weeds tested. This may be attributed to the target compounds being easily absorbed or degraded in the soil when compared to on the surface of the plant. Furthermore, we were encouraged to observe that some of target compound **II**, such as **II15** and **II20**, exhibited 400% sum inhibition against all weeds tested under pre-emergence conditions, which equal to the herbicidal activity of 2,4-D. These bioassay results

296 indicate that compounds **II15** and **II20** are worthy of further study.



297

298 **Figure 8.** Effects (% inhibition) of compounds **I1-I21** and **II1-II21** on loss of plant

299 weight at a dosage of 1500 g ha^{-1} in greenhouse testing; Pre: pre-emergence; Post:

300 post-emergence; BC: *Brassica campestris*; AR: *Amaranthus retroflexus*; EC:

301 *Echinochloa crusgalli*; DS: *Digitaria sanguinalis.*

302 Subsequently, compounds **II15** and **II20** were tested against all weeds to further

303 evaluated their activity using a dose reduction with serial two-fold dilutions. As

304 shown in **Table 1**, the herbicidal activity of target compounds **II15** and **II20** became

progressively lower against the weeds tested at a dosage of 750, 375, and 187.5 g ha^{-1}. In addition, upon decreasing the dosage, the herbicidal activity of compounds **II15** and **II20** under post-emergence conditions decreased faster than that observed under pre-emergence conditions, implying these compounds exhibit enhanced herbicidal activity under pre-emergence conditions than under post-emergence conditions. Compounds **II15** and **II20** exhibit good herbicidal activity against the weeds tested at a dosage of 750 g ha^{-1} under pre-emergence conditions, which is comparable with the activity of 2,4-D. However, compound **II20** displayed lower herbicidal activity than did 2,4-D, when the dosage was reduced to 187.5 g ha^{-1}. To our delight, compound **II15** still displayed good pre-emergent herbicidal activity against *Brassica campestris* and *Digitaria sanguinalis* with 82.7 and 61.8% inhibition at a dosage of 187.5 g ha^{-1}, respectively, which is higher than the herbicidal activity observed for 2,4-D. Meanwhile, compound **II15** displays good pre-emergent herbicidal activity against *Amaranthus retroflexus* with 83.5% inhibition at a dosage of 187.5 g ha^{-1}, which is comparable with the activity observed for 2,4-D. These promising results indicate that compound **II15** has good herbicidal activity under pre-emergence conditions and may be serve as a lead compound for further optimization.

Table 1. Effects (% inhibition) of compounds **II15** and **II20** on loss of plant weight at a dosage of 1500 g ha^{-1} in greenhouse testing [a]

Comp.	Rate (g ha^{-1})	*Brassica campestris*		*Amaranthus retroflexus*		*Echinochloa crusgalli*		*Digitaria sanguinalis*	
		Pre	*Post*	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*

	750	100	100	100	83.3±0.9	77.0±1.2	34.7±1.8	98.3±0.9	31.2±0.9
II15	375	95.5±1.4	41.5±1.2	95.4±0.9	75.9±1.6	62.4±2.0	8.9±1.0	94.7±0.7	15.9±2.1
	187.5	82.7±1.8	27.9±2.3	83.5±2.2	51.9±0.8	23.8±2.5	0	61.8±1.2	0
	750	100	75.0±1.1	100	78.2±1.8	91.8±1.8	29.7±1.3	72.3±2.1	40.4±1.7
II20	375	91.4±0.6	47.8±1.2	95.5±0.7	59.1±1.6	72.4±3.7	9.8±3.1	60.3±1.2	34.7±0.7
	187.5	52.0±1.5	19.1±2.7	56.1±3.2	30.5±1.7	0	0	30.2±1.3	0
	750	100	100	100	100	99.3±0.7	51.1±1.8	100	52.8±2.5
2,4-D	375	94.3±1.2	100	98.5±1.5	100	77.1±2.1	28.3±2.3	61.0±2.5	15.9±1.7
	187.5	47.7±4.6	100	94.4±1.7	100	54.6±1.4	13.8±2.1	20.0±1.7	0

[a] Each value represents the mean ±SD of three experiments

Based on the bioassay data obtained from the greenhouse tests at a dosage of 1500 g ha^{-1} under pre-emergence conditions, the SARs were preliminarily investigated performing parallel activity contrast studies between compounds **I12** and **I15-I21**, and compounds **II12**, and **II15-II21** against *Brassica campestris*, *Amaranthus retroflexus*, *Echinochloa crusgalli*, and *Digitaria sanguinalis*. The results shown in **Fig. 9** indicate that series **II** always displayed higher herbicidal activity against the weeds tested when compared to series **I**. The possible reason for this observation is that the plants have different absorption and/or metabolic capacity toward series **I** and **II**. The preliminary SARs indicate that removing the benzene ring from the 4-hydroxylcoumarin fragment in series **I** was beneficial toward improving the herbicidal activity.

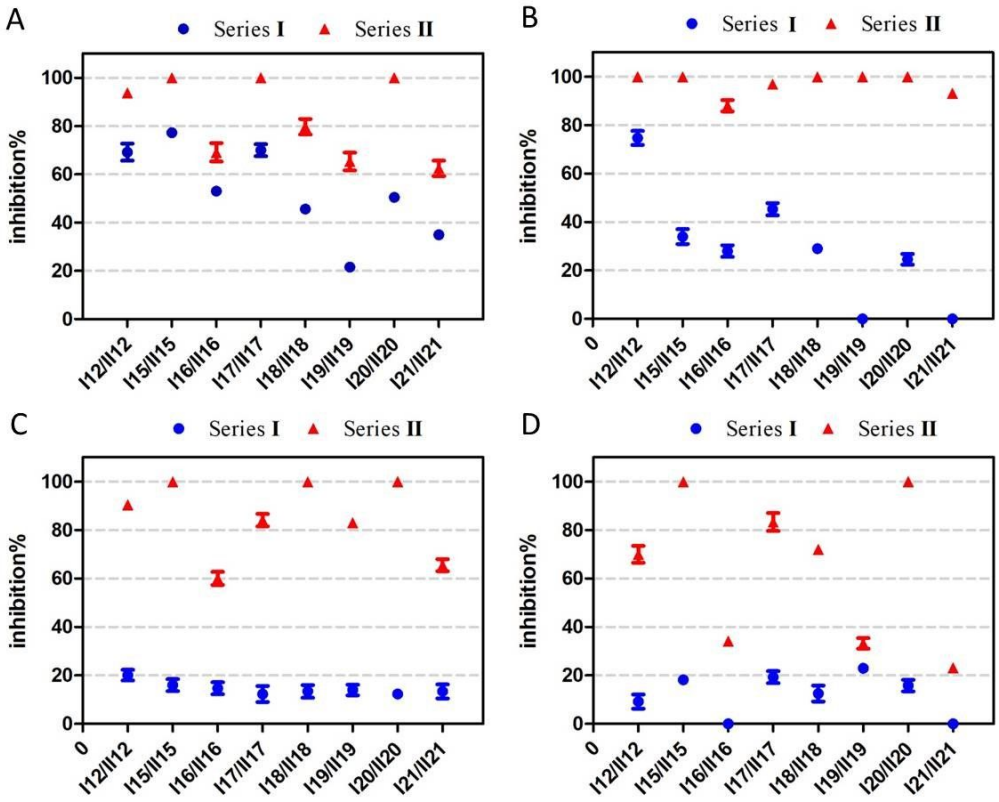


Figure 9. Parallel activity contrast studies between compounds **I12** and **I15-I21** and compounds **II12** and **II15-II21** against *Brassica campestris* (A), *Amaranthus retroflexus* (B), *Echinochloa crusgalli* (C), and *Digitaria sanguinalis* (D). The inhibition activity was tested at 1500 g ha^{-1} and expressed in ordinate.

Analyzing the pre-emergent herbicidal activity against *Brassica campestris* among compounds **II1-II10** reveals that the compounds without a substituent or with an electron-donating group (compounds **II1** and **II5-II10**) generally had lower activities than did the compounds bearing an electron-withdrawing group (compounds **II2** and **II3**). For compounds **II11-II14** with two chlorine substituents on the benzene ring, the herbicidal activity against *Brassica campestris* could be placed in the following order: **II12** (2-Cl-4-Cl) > **II11** (2-Cl-3-Cl) > **II13** (2-Cl-5-Cl) > **II14** (3-Cl-5-Cl), which demonstrates that the 2,4-disubstitution pattern is the most active and the

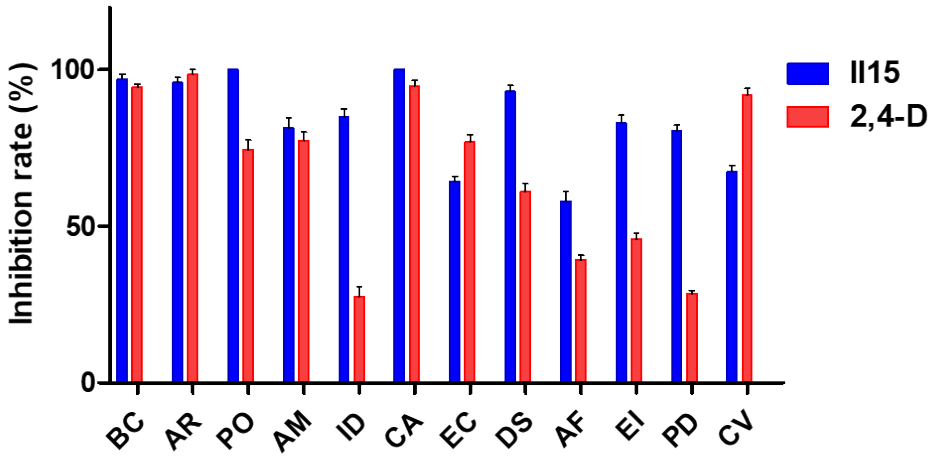
3,5-disubstitution pattern is the least active. These findings indicate that the electron-withdrawing group introduced into benzene ring in compound **II** is beneficial toward improving the herbicidal activity, and the position of the substituent on the benzene ring is very important for herbicidal activity, with the 2,4-disubstitution pattern confirmed as the optimal orientation.

Among the 2,4-disubstituted analogues (compounds **II12** and **II15-II21**), compounds bearing 2-Cl-4-F, 2-Me-4-F, and 2-Br-4-F substituents on the benzene ring display higher herbicidal activity against *Brassica campestris* than do those with other types of substituents at the same positions. For example, the results of obtained for the herbicidal activity of compounds **II12** and **II15-II19** show that their herbicidal activity can be placed in the following order: **II15** (2-Cl-4-F) > **II12** (2-Cl-4-Cl) > **II16** (2-Cl-4-Br); **II17** (2-Me-4-F) > **II18** (2-Me-4-Cl) > **II19** (2-Me-4-Br). These findings indicate that a F atom introduced at the 4-position on the benzene ring of compound **II** is beneficial to improving the herbicidal activity against *Brassica campestris*.

Herbicidal spectrum and crop safety of compound II15

To evaluate whether compound **II15** has the potential to be developed as a herbicide, we tested its herbicidal spectra and crop safety at a dosage of 375 g ha^{-1}. The herbicidal spectrum experiments show that compound **II15** displays >80% inhibition against 9 of the 12 tested weeds at 375 g ha^{-1} (**Fig. 10**). It was worth noting that compound **II15** displays >80% inhibition against *Ixeris denticulate*, *Eleusine indica*, and *Puccinellia distans*, whereas 2,4-D only displays <50% inhibition to these

371 weeds. This finding indicates that compound **II15** has a broader spectrum of weed
372 control than does the commercial herbicide 2,4-D at a dosage of 375 g ha^{-1} under
373 pre-emergence conditions. Subsequently, three representative crops, *Triticum*
374 *aestivum L.*, *Zea mays Linn.* and *Gossypium spp*, were selected for further crop
375 selectivity studies (**Table 2**). The results showed that *Triticum aestivum L.* and *Zea*
376 *mays Linn.* displayed a high tolerance toward compound **II15** at a dosage of 375 g
377 ha^{-1}, but compound **II15** was not selective for *Gossypium spp* (51.3% injury). These
378 promising results indicate that compound **II15** has the potential to be developed as a
379 pre-emergence herbicide for weed control in *Triticum aestivum L.* and *Zea mays Linn.*
380 fields.



Abbreviations: BC, *Brassica campestris*; AR, *Amaranthus retroflexus*; PO, *Portulaca oleracea*; AM, *Abutilon theophrasti Medicus*; ID, *Ixeris denticulate*; CA, *Chenopodium album*; EC, *Echinochloa crusgalli*; DS, *Digitaria sanguinalis*; AF, *Avena fatua*; EI, *Eleusine indica*; PD, *Puccinellia distans*; CV, *Chloris virgate*.

381

382 **Figure 10**. Herbicidal spectrum testing of compounds **II15** under pre-emergence
383 condition at the dosage of 375 g ha^{-1}.

384 **Table 2.** Pre-emergence crop selectivity of compound **II15** at the dosage of 375 g ha^{-1}
385 (injury inhibition) [a]

Comp.	% injury		
	Triticum aestivum L.	*Zea mays Linn.*	*Gossypium spp*
II15	0	0	51.3±2.6
2, 4-D	0	0	0

[a] Each value represents the mean ±SD of three experiments

In summary, a series of 4-hydroxyl-3-(2-phenoxyacetyl)-pyran-2-one derivatives have been designed using molecular hybridization between pyran-based diketone lactones and aryloxyacetic acid moieties. Forty-two 4-hydroxyl-3-(2-phenoxyacetyl)-pyran-2-one derivatives were prepared in moderate to good yield. Our bioassay results showed that compound **II15** displayed good pre-emergent herbicidal activity, even at a dosage of 187.5 g ha^{-1}. Our herbicidal spectrum study revealed that compound **II15** had a broader spectrum of weed control than did the commercial herbicide 2,4-D, and displayed good crop safety against *Triticum aestivum L.* and *Zea mays Linn.* at a dosage of 375 g ha^{-1}, which indicated its great potential as a herbicide for weed control in *Triticum aestivum L.* and *Zea mays Linn.* fields. By investigating the phenotypes of *Arabidopsis thaliana*, detecting the effect on auxin response genes and studying of degradation of compound **II15**, it was found that compound **II15** is metabolized to form an corresponding aryloxy acetic acid in the plant and has a herbicidal mechanism similar to that of 2,4-D, which indicates compound **II15** may be a potential lead structure for the further development of novel auxin-type herbicides. It is worth mentioning that, in our current work, the compounds obtained by molecular hybridization prolong the lifetime of herbicides in the plant, which may

403 exert a sustained herbicidal effect. This is a step forward as less herbicide need be
404 used, which would have a very important meaning in practical applications. Further
405 studies on the structural optimization of compound **II15** are ongoing in our laboratory.

406 **ACKNOWLEDGEMENTS**

407 We are very grateful to the National Natural Science Foundation of China (Grant
408 No. 31701827 and 81803360), the Natural Science Foundation of Shandong Province
409 (Grant No. ZR2017BH037 and ZR2017BC053) and The National Key Research and
410 Development Program of China 2016YFD0200503.

411 **SUPPORTING INFORMATION**

412 Supporting information may be found in the online version of this article.

413 **REFERENCES**

414 1 Beaudegnies, R.; Edmunds, A. J. F.; Fraser, T. E. M.; Hall, R. G.; Hawkes, T. R.;
415 Mitchell, G. et al. Herbicidal 4-hydroxyphenylpyruvate dioxygenase inhibitors-A
416 review of the triketone chemistry story from a Syngenta perspective. *Bioorg. Med.*
417 *Chem.* **2009**, *17*, 4134−4152.
418 2 Wang, D. W.; Lin, H. Y.; Cao, R. J.; Yang, S. G.; Chen, Q.; Hao, G. F.; Yang, W.
419 C.; Yang, G. F. Synthesis and herbicidal evaluation of triketone-containing
420 quinazoline-2, 4-diones. *J. Agric. Food Chem.* **2014***, 62*, 11786−11796.
421 3 Wang, D. W.; Lin, H. Y.; Cao, R. J.; Chen, T.; Wu, F. X.; Hao, G. F.; Chen, Q.;
422 Yang, W. C.; Yang, G. F. Synthesis and herbicidal activity of triketone−quinoline
423 hybrids as novel 4-hydroxyphenylpyruvate dioxygenase inhibitors. *J. Agric. Food*
424 *Chem.* **2015**, *63*, 5587–5596.

425 4 Wang, D. W.; Lin, H. Y.; Cao, R. J.; Ming, Z. Z.; Chen, T.; Hao, G. F.; Yang, W. C.;
426 Yang, G. F. Design, synthesis and herbicidal activity of novel quinazoline-2,
427 4-diones as 4-hydroxyphenylpyruvate dioxygenase inhibitors. *Pest Manag. Sci.*
428 **2015**, *71*, 1122−1132.
429 5 Wang, D.W.; Lin, H. Y.; He, B.; Wu, F. X.; Chen, T.; Chen, Q.; Yang, W. C.; Yang,
430 G. F. An efficient one-pot synthesis of 2-(aryloxyacetyl)cyclohexane-1, 3-diones as
431 herbicidal 4-hydroxyphenylpyruvate dioxygenase inhibitors. *J. Agric. Food Chem.*
432 **2016**, *64*, 8986−8993.
433 6 Li, H. B.; Li, L.; Li, J. X.; Han, T. F.; He, J. L.; Zhu, Y. Q. Novel HPPD inhibitors:
434 triketone 2*H*-benzo[*b*][1, 4]oxazin-3(4*H*)-one analogs. *Pest Manag. Sci.* **2018**, *74*,
435 579–589.
436 7 Cristoph, F.; Kaulfers, P. M.; Stahl-Biskup, E. In vitro evaluation of the
437 antibacterial activity of *β*-triketones admixed to Melaeuca oils. *Planta Med.* **2001**,
438 *67*, 768–771.
439 8 Cristoph, F.; Kaulfers, P. M.; Stahl-Biskup, E. A comparative study of the in vitro
440 antimicrobial activity of tea tree oils with special reference to the activity of
441 β-triketones. *Planta Med.* **2000**, *66*, 556–560.
442 9 Lv, P.; Chen, Y. L.; Zhao, Z.; Shi, T. Z.; Wu, X. W.; Xue, J. Y.; Li, Q. X.; Hua, R.
443 M. Design, synthesis, and antifungal activities of 3-acyl thiotetronic acid
444 derivatives: new fatty acid synthase inhibitors. *J. Agric. Food Chem.* **2018**, *66*,
445 1023−1032.
446 10 Khambay, B. P. S.; Beddie, D. G.; Hooper, A. M.; Simmonds, M. S. J. Isolation,

447 characterization and synthesis of an insecticidal
448 tetramethyltetrahydrochromenedione-spiro-bicyclo[3, 1, 1]-cycloheptane from two
449 species of myrtaceae. *Tetrahedron.* **2003**, *59*, 7131–7133.
450 11 Cacic, M.; Trkovnik, M.; Cacic, F.; Has-Schon, E. Synthesis and antimicrobial
451 activity of some derivatives of (7-hydroxy-2-oxo-2H-chromen-4-yl)-acetic acid
452 hydrazide. *Molecules.* **2006**, *11*, 134–147.
453 12 Jang, S.; Jung, J. C.; Oh, S. Synthesis of 1, 3-diphenyl-2-propen-1-one derivatives
454 and evaluation of their biological activities. *Bioorg. Med. Chem.* **2007**, *15*,
455 4098–4105.
456 13 Lin, C. T.; Shih, J. H.; Chen, C. L.; Yang, D, Y. Synthesis of novel triketone-based
457 acidichromic colorants. *Tetrahedron Lett.* **2005**, *46*, 5033–5037.
458 14 Lin, S. L.; Kuo, P. Y.; Yang, D. Y. Design and synthesis of a coumarin-based
459 acidichromic colorant. *Molecules.* **2007**, *12*, 1316–1324.
460 15 Glade, K. C.; James, M. W.; Richard, J. D.; Mao, C. H. L. Certain
461 4-oxo-3-benzoylvalerolactones and thiolactones. EP249812 A2 **1987**.
462 16 Liu, B.; Xie, L. G.; Xu, X. H.; Li, Y. H. Synthesis, crystal structure and herbicidal
463 activity of 3-benzyol-4-hydroxylcoumarin derivatives. *Chinese J. Org. Chem.* **2011**,
464 *31*, 2067–2073.
465 17 Lei, K.; Sun, D. W.; Hua, X. W.; Tao, Y. Y.; Xu, X. H.; Kong, C. H. Synthesis,
466 fungicidal activity and structure–activity relationships of
467 3-benzoyl-4-hydroxylcoumarin derivatives. *Pest Manag. Sci.* **2006**, *72*, 1381–1389.
468 18 Ferreira, R. S.; Dessoy, M. A.; Pauli, I.; Souza, M. L.; Krogh, R.; Sales, A. I. L.;

469 Oliva, G.; Dias, L. C.; Andricopulo, A. D. Synthesis, biological evaluation, and
470 structure-activity relationships of potent noncovalent and nonpeptidic cruzain
471 inhibitors as anti-trypanosoma cruzi agents. *J. Med. Chem.* **2014**, *57*, 2380–2392.
472 19 Mokale, S. N.; Nevase, M. C.; Sakle, N. S.; Dube, P. N.; Shelke, V. R.; Bhavale, S.
473 A.; Begum, A. Synthesis and in-*vivo* hypolipidemic activity of some novel
474 substituted phenyl isoxazol phenoxy acetic acid derivatives. *Bioorg. Med. Chem.*
475 *Lett.* **2014**, *24*, 2155–2158.
476 20 Lazarow, P. B.; De Duve, C. A fatty acyl-CoA oxidizing system in rat liver
477 peroxisomes; enhancement by clofibrate, a hypolipidemic drug. *Proc. Natl. Acad.*
478 *Sci.* **1976**, *73*, 2043-2046.
479 21 Ali, M. A.; Shaharyar, M. Discovery of novel phenoxyacetic acid derivatives as
480 antimyco-bacterial agents. *Bioorg. Med. Chem.* **2007**, *15*, 1896–1902.
481 22 Yar, M. S.; Siddiqui, A. A.; Ali, M. A. Synthesis and evaluation of phenoxy acetic
482 acid derivatives as anti-mycobacterial agents. *Bioorg. Med. Chem. Lett.* **2006**, *16*,
483 4571–4574.
484 23 Wang, W.; He, H.; Zuo, N.; He, H.; Peng, H.; Tan, X. Synthesis and herbicidal
485 activity of 2-(substituted phenoxyacetoxy)alkyl-5, 5-dimethyl-1, 3,
486 2-dioxaphosphinan-2-one. *J. Agric. Food Chem.* **2012**, *60*, 7581–7587.
487 24 Meng, L.; Shi, D. Q. Synthesis and biological activities of novel
488 *N*-[*cis*-3-(2-chloro-3, 3, 3-trifluoropropenyl)-2,
489 2-dimethylcyclopropylcarbonyl]-*N*'-(aryloxyacetyl) hydrazines. *Chinese J. Org.*
490 *Chem.* **2009**, *29*, 1100–1104.

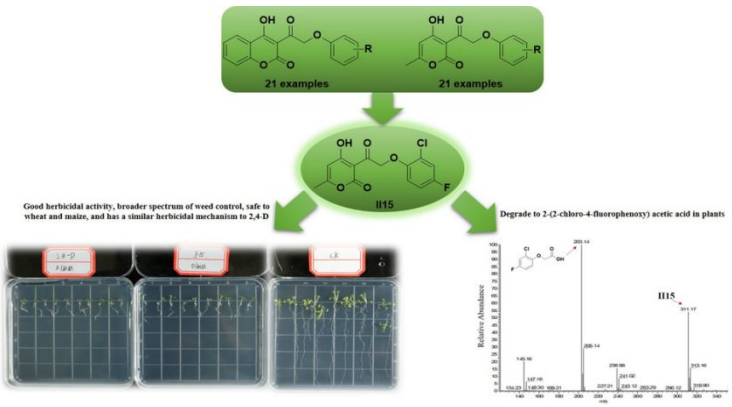
491 25 Buck, W. D.; Raddatz, E. D. Arylcarboxylic acid derivatives, preparation and use
492 thereof. EP 0262393 B1 **1989**.
493 26 Lei, K.; Hua, X. W.; Tao, Y. Y.; Liu, Y.; Liu, N.; Ma, Y.; Li, Y. H.; Xu, X. H.;
494 Kong, C. H. Discovery of (2-benzoylethen-1-ol)-containing 1, 2-benzothiazine
495 derivatives as novel 4-hydroxyphenyl-pyruvate dioxygenase (HPPD)
496 inhibiting-based herbicide lead compounds. *Bioorg. Med. Chem.* **2016**, *24*, 92–103.
497 27 Ogawa, H.; Yamada, I.; Arai, K.; Hirase, K.; Moriyasu, K.; Schneider, C.;
498 Sandmann, G.; Böger, P.; Wakabayashi, K. Mode of bleaching phytotoxicity of
499 herbicidal diphenylpyrrolidinones. *Pest Manag. Sci.* **2001**, *57*, 33–40.
500 28 Wang, B. L.; Duggleby, R. G.; Li, Z. M.; Wang, J. G.; Li, Y. H.; Wang, S. H.;
501 Song H. B. Synthesis, crystal structure and herbicidal activity of mimics of
502 intermediates of the KARI reaction. *Pest Manag. Sci.* **2005**, *61*, 407–412.
503 29 Zhang, M.; Xu, X. H.; Cui, Y.; Xie, L. G.; Kong, C. H. Synthesis and herbicidal
504 potential of substituted aurones. *Pest Manag. Sci.* **2012**, *68*, 1512–1522.
505 30 Raghavan, C.; Ong, E. K.; Dalling, M. J.; Stevenson, T. W. Regulation of genes
506 associated with auxin, ethylene and ABA pathways by 2,4-dichlorophenoxyacetic
507 acid in *Arabidopsis*. *Funct. Integr. Genomics*. **2006**, *6*, 60–70.
508 31 Scherera, G. F. E.; Zahna, M.; Callisb, J.; Jones, A. M. A role for phospholipase A
509 in auxin-regulated gene expression. *FEBS Lett*. **2007**, *581*, 4205–4211.
510 32 Song, Y. L. Insight into the mode of action of 2,4-dichlorophenoxyacetic acid
511 (2,4-D) as an herbicide. *J. Integr. Plant Biol.* **2014**, *56*, 106–113.
512 33 Mallory, A. C.; Bartel, D. P.; Bartelc, B. MicroRNA-directed regulation of

513 *Arabidopsis* auxin response factor17 is essential for proper development and
514 modulates expression of early auxin response genes. *Plant Cell*. **2005**, *17*,
515 1360–1375.
516 34 Yang1, F. X.; Song, Y.; Yang, H.; Liu, Z. B.; Zhu, G. F.; Yang, Y. An
517 auxin-responsive endogenous peptide regulates root development in *Arabidopsis*. *J.*
518 *Integr. Plant Biol.* **2014**, *56*, 635–647.
519 35 Xu, C. X.; Zhang, Y. X.; Yu, Y.; Li, Y.; Wei, S. F. Suppression of *Arabidopsis*
520 flowering by near-null magnetic field is mediated by auxin. *Bioelectromagnetics*.
521 **2018**, *39*, 15–24.
522 36 Dharmasiri, S.; Swarup, R.; Mockaitis, K.; Dharmasiri, N.; Singh, S. K.; Kowalchyk, M.;
523 Marchant, A.; Mills, S.; Sandberg, G.; Bennett, M. J.; Estelle, M. AXR4 is required for
524 localization of the auxin influx facilitator AUX1. *Science*. **2006**, *312*, 1218–1220.
525 37 Marchant, A.; Kargul, J.; May, S. T.; Muller, P.; Delbarre, A.; Perrot-Rechenmann, C.; Bennett,
526 M. J. AUX1 regulates root gravitropism in *Arabidopsis* by facilitating auxin uptake within root
527 apical tissues. *EMBO. J.* **1999**, *18*, 2066–2073.

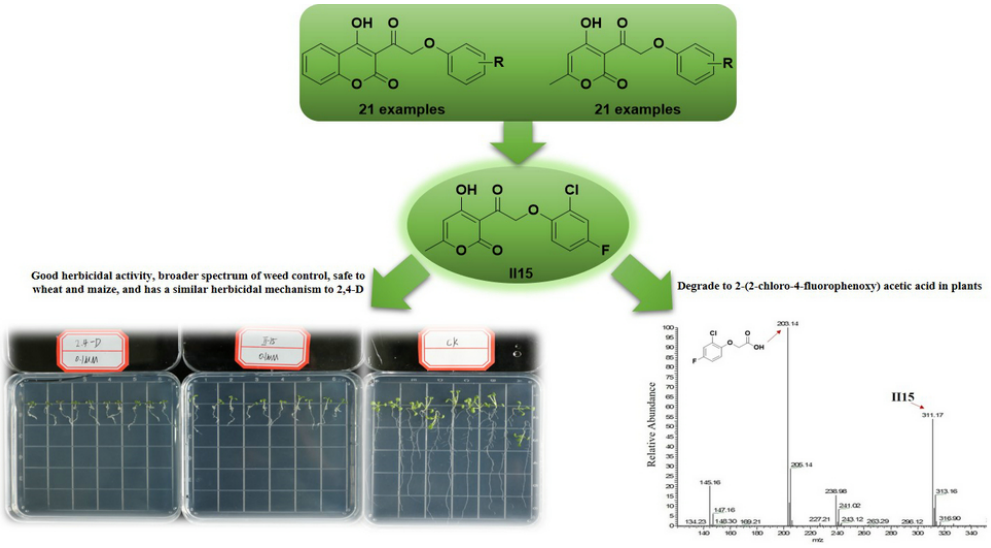
529 **TOC Graphic**

530 **Design and synthesis of novel 4-hydroxyl-3-(2-phenoxyacetyl)-pyran-2-one**

531 **derivatives for use as herbicides and evaluation of their mode of action**



532



164x88mm (150 x 150 DPI)