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Synthesis and herbicidal activity of 2-aroyl-3-arylamino/arylthiol cyclohex-2-enones as potential 4-hydroxyphenylpyruvate dioxygenase inhibitors

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ABSTRACT

4-Hydroxyphenylpyruvate dioxygenase (EC 1.13.11.27, HPPD) enzyme is a promising inhibitor in the field of herbicide innovation. To discover this novel selective HPPD inhibitor, to overcome weed resistance problem, a structure-guided design strategy was used for the structural optimization of benzobicyclon, a commercial proherbicide targeting HPPD protein. Three series of 2-aroyl-3-arylamino/arylthiocyclohex-2-enones were designed and synthesized. Two screening methods, petri dish culture method and greenhouse pot culture, were used to evaluate their herbicidal activities. The petri dish test indicated that most of the synthesized target compounds had moderate to excellent inhibitory effects on the roots and shoots of both rape (*Brassica napus*) and barnyard grass (*Echinchloa crusgalli*) at 100 mg/L concentration. Among the 21-synthesized compounds, 11 compounds caused moderate to severe albinism symptoms and the albinism concentration was as low as 1 mg/L for compounds **8a**~**8g**. Moreover, the compounds **8a**~**8g** also showed favourable herbicidal efficacy to test weeds at 200 g ai/ha, indicating that these compounds are promising candidates to develop HPPD inhibitors. Molecular docking revealed that these synthesized compounds formed tight π-π and hydrogen bond interactions with *At*HPPD in the active site.

Key words: 2-aroylcyclohex-2-enone, *Brassica napus*, compound, enzyme, *Echinchloa crusgalli*, herbicidal activity, 4-Hydroxyphenylpyruvate dioxygenase (HPPD), inhibitor, molecular docking, mustard, proherbicide, rape, synthesis.

INTRODUCTION

Herbicides play major role in achieving higher crop yields and ensuring national food security (7). Modern agriculture is facing a major challenge for their sustainability due to spread of herbicide-resistant weeds worldwide (3,4,20).

There are 512 unique cases of herbicide-resistant weeds in 96 crops in 71 countries, involving approximately 266 weeds species (153 monocots and 113 dicots) (8). Weeds have evolved resistance to 21 of the 31 known herbicide sites of action and to 165 different herbicides. The problem of weed resistance to herbicides is compounded by the relatively small number modes of action [acetohydroxyacid synthase (AHAS), acetyl-coenzyme

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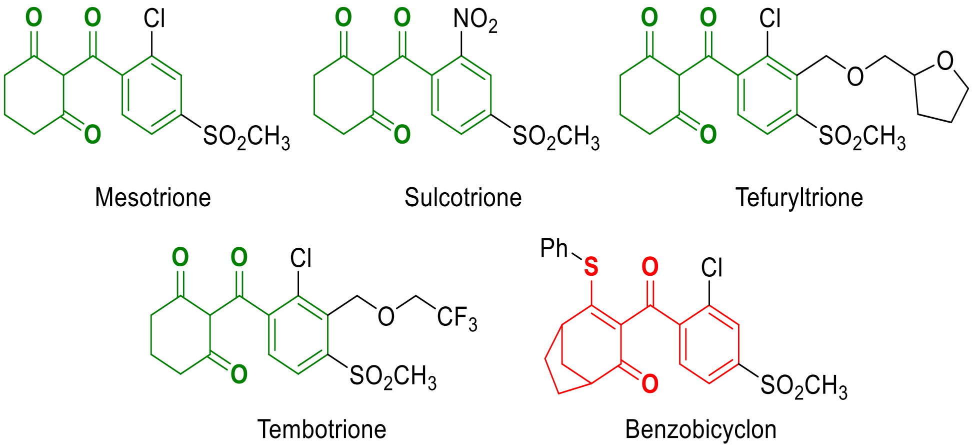
A carboxylase (ACCase), photosystem II (PS II)] of commercial herbicide (11,24,26). Thus, there is an urgent need to discover new herbicides to effectively control the resistant weeds and that have a slow trend of resistance development.

4-Hydroxyphenylpyruvate dioxygenase (EC 1.13.11.27, HPPD) is the one of the most promising herbicide targets as their resistance evolution is relatively slow (19). HPPD is an important non-heme iron (II)-dependent enzyme in the tyrosine metabolic pathway in most aerobic organisms, belonging to the large family of 2-his-1-carboxylate facial triad oxidative enzymes (1). HPPD catalyzes the biotransformation of 4-hydroxyphenylpyruvic acid (HPPA) to homogentisic acid (HGA) (16). In plants, HGA is an essential compound for the biosynthesis of two isoprenoids (plastoquinone and tocopherol), which are elements of the photosynthetic electron transfer chain and are antioxidant compounds, respectively. Inhibition of HPPD leads to the blockage of the natural tyrosine physiological metabolism and a reduction in carotenoid levels, which indirectly affects the photosynthesis of plants. When exposed to sunlight, the plants are severely damaged by ultraviolet light causing necrosis and death (2,12,17,23). Due to their unique molecular mechanisms of action, HPPD-inhibitors have not exhibited cross-resistance with other types of herbicides to date (8). Therefore, HPPD is an important target for herbicide discovery.



Mesotrione

Sulcotrione

Tefuryltrione

Tembotrione

Benzobicyclon

Figure 1. Several commercially available triketone herbicides

To date, there are about 13 commercial HPPD herbicides, classified in 3-categories: triketones, pyrazoles and isoxazoles (diketonitrile). Among them, triketone derivatives [sulcotrione, mesotrione, tefuryltrione, tembotrione, and benzobicyclon (Figure 1) (18,21)] are most widely studied and used in agriculture and their inhibition mechanism is that, the triketone moiety forms a bidentate interaction with Fe(II) in the active site (13). HPPD-inhibiting herbicides have many advantages viz., high efficiency, low resistance evolution risk, low toxicity, high crop safety, broad-spectrum weed control and good environmental compatibility (14,22). Particularly, benzobicyclon as proherbicide has been developed to control broadleaf weeds [*Lindernia attenuate* or *Monochoria vaginalis* and some important grasses in paddy rice (10,25)] resistant to sulfonylurea. Benzobicyclon is characterized by a unique bicyclooctane skeleton with a phenylthio-enol ether structure, which can be easily transformed into the herbicidally active triketone containing the vinylogous acid hydroxyl moiety by the vinylogous phenylthioester hydrolyzation in soil, water, and plants. A proherbicide is a biologically inactive molecule, which requires transformation(s) after its application to become herbicidally active (9). The common biochemical and chemical activation processes of commercial proherbicides mainly are: hydrolysis, dehydrosulfuration and isomerization (Figure 2), among these, hydrolysis activation is most common. Proherbicide strategy offers a unique and modern approach to herbicide discovery, with its own advantages (optimal efficacy, environmental safety, user friendly and economic viability). Therefore, the discovery of a new generation of highly effective HPPD inhibitors as proherbicide for weed management, manage herbicide resistant weeds, has been a research hotspot in herbicide innovation (6).

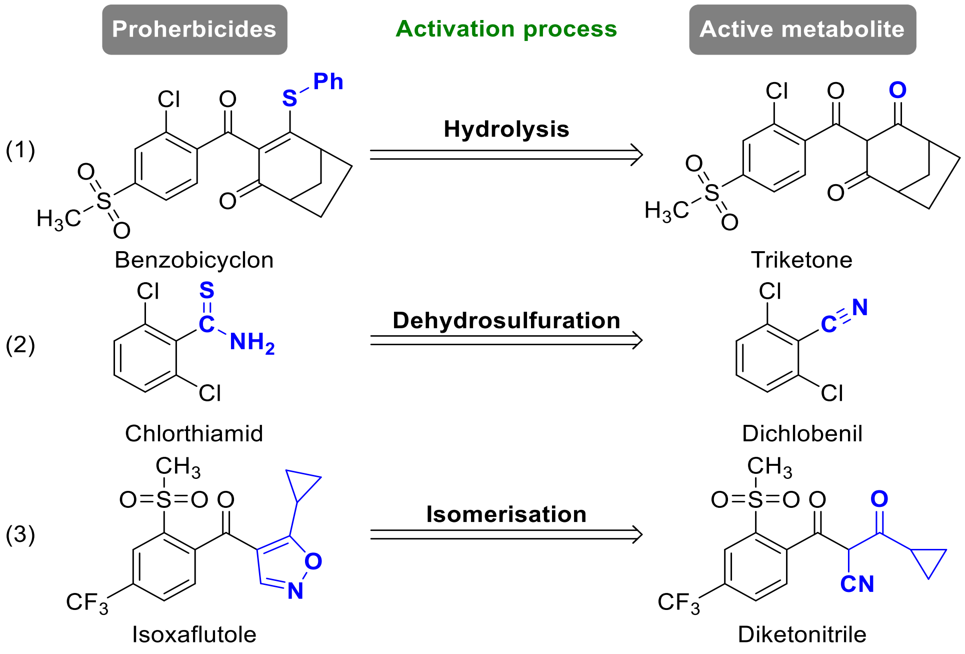


Figure 2. Selection of representative biochemical and chemical activation processes of proherbicides

To discover new HPPD inhibitors as proherbicides, we developed novel and highly potent proherbicides through appropriate structural modifications of the active chemical substructure of commercial HPPD inhibitors, mesotrione and sulcotrione. In this study total of 21 novel 2-aroyl-3-arylamino/arylthiocyclohex-2-enones were designed, synthesized and characterized. These compounds were assessed under laboratory and glasshouse conditions to determine their herbicidal activities. Hence, this study aimed to discover new lead structures for HPPD inhibitor development.

MATERIALS AND METHODS

The study was conducted at Zhejiang Provincial Key Laboratory of Chemical Utilization of Forestry Biomass, Zhejiang A＆F University, Lin'an, Zhejiang Province, China. [30.23 °N, 119.52 °E, Elevation: 1192 m]. The area has a subtropical monsoon climate, with average daily maximum temperature (33 °C) in hot summer and average daily minimum temperature (4 °C) in cold winter, the mean annual rainfall: 1613.9 mm. This study includes design, synthesis, structural characterization and activity test, all studies done for 5 months in 2020.

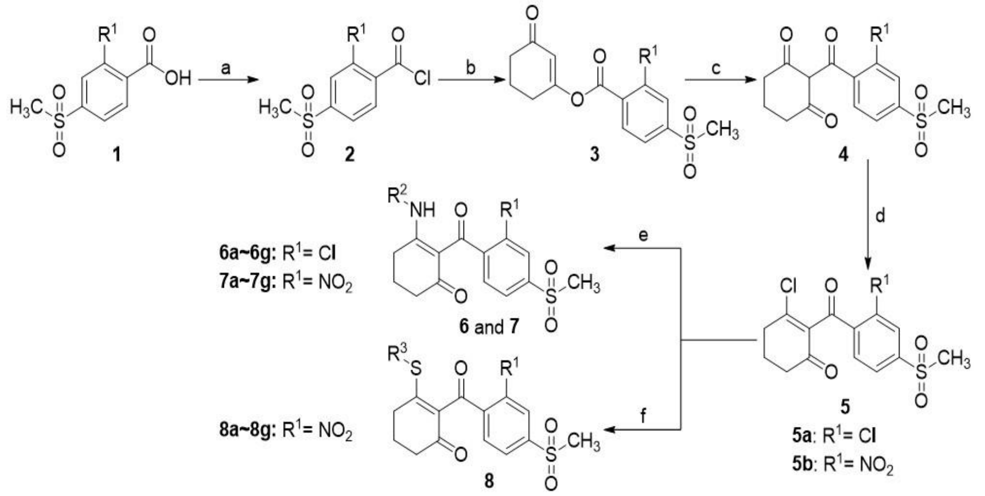
General

^{1}H NMR spectra were recorded with 400/600 MHz spectrometers. Chemical shifts (δ) were reported in ppm quoted relative to internal tetramethylsilane (internal standard, 0.0 ppm) with the coupling constants (J) given in Hz. ^{13}C NMR spectra were recorded with the same spectrometer operating at 100/150 MHz with complete proton decoupling (internal standard $CDCl_3$: 77.0 ppm). Splitting patterns were assigned s = singlet, d = doublet, t =triplet, dd = double doublet, dt = doublet of triplet, q = quartet, etc. Mass spectra were measured with a Finnigan Trace MS spectrometer. High Resolution Mass Spectrometry (HRMS) was performed on Agilent Technologies 6530 Accurate-Mass Q-TOF mass spectrometer. IR measurements were performed on Bruker TENSOR27 using KBr as the substrate. Elementary analysis was taken with a Vario EL III elementary analysis instrument. Melting points (m.p.) were obtained on a digital melting point apparatus (Bibby-Electrothermal) without correction. Unless otherwise noted, materials used were commercially supplied. All reactions were monitored by TLC analysis on silica gel coated plates. Flash column chromatography was performed by using 200-300 mesh silica gel.

Chemical synthesis

(I). Synthesis of intermediates 2a and 2b: As shown in Scheme 1, the 2-chloro/2-nitro-4-methylsulphonyl benzoic acid (**1**) (10 mmol) was added to a 25 mL round-bottomed flask under ice-bath condition, followed by addition of 5 mL dimethyl sulfoxide and 3 drops of *N*, *N*-dimethylformamide (DMF), and stirred for about 30 min, then transferred it to oil bath and reacted at 80 °C for 4 h. At the end of the reaction, dimethyl sulfoxide was evaporated to give 2-chloro or 2-nitro-4-methylsulphonylbenzoic chloride (**2**) as yellow solids.

(II). Synthesis of intermediates 3a and 3b: 1,3-cyclohexanedione (1.12 g, 10 mmol), triethylamine (2.0 g, 10 mmol) and 25 mL anhydrous dichloromethane were successively added to a 50 mL round-bottom flask under ice-bath condition. The mixtures were stirred for 10 min, then anhydrous dichloromethane solution of 2-chloro/2-nitro-4-methylsulphonylbenzoic chloride (**2**, 10 mmol, 10 mL) was slowly added, and then stirred at room temperature for 4 h. After that, the reaction was quenched by adding 20 mL water, extracted with dichloromethane three times (3×50 mL). The combined organic phase was washed successively with saturated $NaHCO_3$ solution (3×100 mL) and saturated saline solution (3×100 mL), dried with anhydrous Na_2SO_4, and concentrated in vacuo to give a residue. The residue was purified by column chromatography on silica gel using a 3:1 petroleum ether/ethyl acetate mixtures to afford intermediate **3** as white solids.



Reagents and conditions: (a) $SOCl_2$, DMF, reflux, 4 h; (b) 1,3-Cyclohexanedione, Et_3N, anhydrous DCM, r. t., 4 h; (c) $AlCl_3$, dry DCM, 0 °C; (d) Oxalyl chloride, DMF, 0 °C - r. t.; (e) R_2NH_2, $CHCl_3$, r. t./reflux; (f) R_3SH, Et_3N, r. t..

Figure 3. Synthesis route of the target compounds.

(III). Synthesis of intermediates 4a and 4b: Intermediate **3** (10 mmol) and 50 mL anhydrous dichloromethane were successively added into a 100 mL round-bottom flask under ice-bath condition, then a drying tube was inserted. The solution was stirred for 30 min, then aluminum trichloride (2.6 g, 20 mmol) was added slowly and continued to react for 3 h. After that, the reaction mixtures were slowly poured into 100 mL of 2 mol/L hydrochloric acid solution, and then extracted with dichloromethane three times (3×50 mL). The combined organic phase was washed with saturated salt (3×100 mL), dried with anhydrous Na_2SO_4 and concentrated in vacuo to give a residue. The residue was purified by column chromatography on silica gel using a 1:3 petroleum ether/ethyl acetate mixtures to afford tritone intermediate **4** as pale-yellow solids.

(IV). Synthesis of intermediates 5a and 5b: Compound **4** (10 mmol, 3.3 g for **4a** and 3.4 g for **4b**), trichloromethane (50 mL) and *N*, *N*-dimethylformamide (1.1 g, 15 mmol) were successively added to a 100 mL round-bottomed flask under ice-bath condition, then oxalyl chloride (1.9 g, 15 mmol) was slowly added and the mixtures continued to react at room temperature for 2 h. After that, the reaction was quenched by 50 mL water, extracted with dichloromethane three times (3×50 mL). The combined organic phase was washed successively with saturated $NaHCO_3$ (3×100 mL) solution and saturated saline solution (3×100 mL), then following the typical purge process as forementioned for intermediate **4** to afford intermediate **5** as pale-yellow solids.

(V). General procedure for the synthesis of target compounds 6a~6g and 7a~7g : 1 mmol compound **5a** (0.35 g) or **5b** (0.36 g), trichloromethane (25 mL) and amine (2.5 mmol) were successively added into a 50 mL round-bottom flask, the mixtures reacted at room temperature for 8 h. After that, the reaction was quenched by 25 mL water, extracted with dichloromethane for three times (3×50 mL). The combined organic phase followed the typical purge process as forementioned for the intermediate **4**, obtaining the target compounds **6a~6g** and **7a~7g** as solids in 59 %-92 % yields.

(VI). General procedure for the synthesis of target compounds 8a~8g : Compound **5b** (345 mg, 1 mmol), *p*-substituted thiophenol (2 mmol), triethylamine (0.2 g, 2 mmol) and 25 mL trichloromethane were successively added into a 50 mL round-bottomed flask. The mixtures reacted at room temperature for 5 h. Then the typical treating process as forementioned for compounds **6~7** was followed to obtain target compounds **8a~8g** as solid in 58 %~76 % yields.

(i). 2-(2-Chloro-4-(methylsulfonyl)benzoyl)-3-(propylamino)cyclohex-2-enone (6a) : As a white solid in 88 % yield, m.p. 155.0-155.8 °C. ^{1}H NMR (600 MHz, $CDCl_3$, ppm): δ 12.20 (s, 1H), 7.84 (s, 1H), 7.75 (d, J = 7.5 Hz, 1H), 7.23 (d, J = 7.9 Hz, 1H), 3.39-3.35 (m, 2H), 3.01 (s, 3H), 2.68 (t, J = 5.8 Hz, 2H), 2.29 (t, J = 5.9 Hz, 2H), 1.97-1.93 (m, 2H), 1.75-1.70 (m, 2H), 1.02 (t, J = 7.3 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$, ppm): δ 193.8, 192.2, 173.9, 149.0, 139.9, 130.2, 128.0, 127.1, 125.6, 108.2, 45.6, 44.6, 37.3, 26.9, 22.4, 19.7, 11.3; IR (KBr, cm^{-1}): 3442, 2945, 2870, 1650, 1584, 1461, 1303, 1154, 971, 855, 756; EI-MS m/z: 334.05 ([M-Cl]$^+$, 100), 334.05(100); Elem. Anal. Calcd. for $C_{17}H_{20}ClNO_4S$: C, 55.20; H, 5.45; N, 3.79; S, 8.67. Found: C, 55.08; H, 5.39; N, 3.92; S, 8.91.

(ii). 2-(2-Chloro-4-(methylsulfonyl)benzoyl)-3-(*o*-tolylamino)cyclohex-2-enone (6b): As a pale-yellow solid in 65 % yield, m.p. 206.7-207.5 °C. ^{1}H NMR (600 MHz, $CDCl_3$, ppm): δ 13.47 (s, 1H), 7.90 (s, 1H), 7.82 (d, J = 7.9 Hz, 1H), 7.35 (d, J = 7.9 Hz, 1H), 7.31 (t, J = 5.4 Hz, 1H), 7.30-7.24 (m, 2H), 7.17 (d, J = 7.1 Hz, 1H), 3.05 (s, 3H), 2.55 (t, J = 6.1 Hz, 2H), 2.37 (t, J = 6.3 Hz, 2H), 2.30 (s, 3H), 1.96-1.88 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$, ppm): δ 194.2, 193.1, 173.6, 148.5, 140.2, 135.2, 134.0, 131.4, 130.2, 128.6, 128.1, 127.3, 127.0, 126.5, 125.8, 109.0, 44.6, 37.6, 28.4, 20.3, 17.9; IR (KBr, cm^{-1}): 3450, 3085, 3013, 2908, 1657, 1568, 1461, 1303, 1158, 1105, 971, 851, 754; EI-MS m/z: 382.11([M-Cl]$^+$, 100), 382.11 (100); Elem. Anal. Calcd. for $C_{21}H_{20}ClNO_4S$: C, 60.35; H, 4.82; N, 3.35; S, 7.67. Found: C, 60.61; H, 4.73; N, 3.62; S, 7.66.

(iii). 2-(2-Chloro-4-(methylsulfonyl)benzoyl)-3-(*m*-tolylamino)cyclohex-2-enone(6c): As a yellow solid in 70 % yield, m.p. 173.7-174.6 °C. ^{1}H NMR (600 MHz, $CDCl_3$, ppm): δ 13.66 (s, 1H), 7.93 (s, 1H), 7.84 (d, J = 7.9 Hz, 1H), 7.36 (t, J = 7.5 Hz, 2H), 7.21 (d, J = 7.6 Hz, 1H), 7.08 (s, 1H), 7.06 (d, J = 12.0 Hz, 1H), 3.08 (s, 3H), 2.73 (t, J = 6.1 Hz, 2H), 2.50 (s, 3H), 2.40 (t, J = 8.0 Hz, 2H), 2.03-1.89 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$, ppm): δ 194.2, 193.0, 173.1, 148.6, 140.2, 139.9, 136.0, 130.3, 129.4, 128.9, 128.1, 127.2, 126.3, 125.7, 122.8, 108.9, 44.6, 37.6, 28.8, 21.3, 20.3; IR (KBr, cm^{-1}): 3441, 3028, 2928, 1653, 1577, 1446, 1303, 1144, 963, 848, 783; EI-MS m/z: 382.03 ([M-Cl]$^+$, 100), 382.03

(100); Elem. Anal. Calcd. for $C_{21}H_{20}ClNO_4S$: C, 60.35; H, 4.82; N, 3.35; S, 7.67. Found: C, 60.45; H, 4.84; N, 3.37; S, 7.51.

(iv). 2-(2-Chloro-4-(methylsulfonyl)benzoyl)-3-(*p*-tolylamino)cyclohex-2-enone (6d) : As a pale-yellow solid in 72 % yield, m.p. 192.5-193.5 °C. ^{1}H NMR (600 MHz, $CDCl_3$, ppm): δ 13.63 (s, 1H), 7.93 (s, 1H), 7.85 (d, J = 7.9 Hz, 1H), 7.35 (d, J = 7.9 Hz, 1H), 7.27 (d, J = 7.8 Hz, 2H), 7.14 (d, J = 8.0 Hz, 2H), 3.08 (s, 3H), 2.71 (t, J = 6.0 Hz, 2H), 2.50 (s, 3H), 2.40 (t, J = 8.0 Hz, 2H), 1.99-1.90 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$, ppm): δ 194.2, 192.9, 173.2, 148.6, 140.2, 138.3, 133.5, 130.3, 130.2, 128.2, 127.2, 125.7, 125.6, 108.9, 44.6, 37.6, 28.7, 21.1, 20.3; IR (KBr, cm^{-1}): 3434, 3027, 2948, 1654, 1567, 1353, 1302,1148, 880, 830, 773; HRMS (ESI) m/z $[M+Na]^+$ Calcd. for $C_{21}H_{20}ClNO_4S$ 440.0694. Found: 440.0707.

(v). 2-(2-Chloro-4-(methylsulfonyl)benzoyl)-3-((4-fluorophenyl)amino)cyclohex-2-enone (6e): As a gray solid in 59 % yield, m.p. 206.9-207.8 °C. ^{1}H NMR (600 MHz, $CDCl_3$, ppm): δ 13.61 (s, 1H), 7.93 (s, 1H), 7.85 (d, J = 7.8 Hz, 1H), 7.35 (d, J = 7.9 Hz, 1H), 7.30 (t, J = 8.0 Hz, 2H), 7.18 (t, J = 8.3 Hz, 2H), 3.08 (s, 3H), 2.68 (t, J = 12.0 Hz, 2H), 2.41 (m, J = 12.0 Hz, 2H), 2.08-1.90 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$, ppm): δ 194.1, 193.2, 173.4, 162.7, 161.0, 148.4, 140.3, 132.2, 130.3, 128.1, 127.8, 127.7, 127.2, 125.7, 116.8, 116.6, 109.1, 44.6, 37.5, 28.6, 20.3; IR (KBr, cm^{-1}): 3431, 3053, 3015, 2958, 1653, 1575, 1512, 1303, 1195, 1146, 970, 847, 788; EI-MS m/z: 386.00 ($[M-Cl]^+$, 100), 386.00 (100); Elem. Anal. Calcd. for $C_{20}H_{17}ClFNO_4S$: C, 56.94; H, 4.06; N, 3.32; S, 7.60. Found: C, 57.12; H, 4.13; N, 3.46; S, 7.77.

(vi). 2-(2-Chloro-4-(methylsulfonyl)benzoyl)-3-((4-chlorophenyl)amino)cyclohex-2-enone (6f): As a white solid in 63 % yield, m.p. 192.9-193.7 °C. ^{1}H NMR (600 MHz, $CDCl_3$, ppm): δ 13.66 (s, 1H), 7.92 (s, 1H), 7.85 (d, J = 7.9 Hz, 1H), 7.46 (d, J = 8.3 Hz, 2H), 7.35 (d, J = 7.9 Hz, 1H), 7.22 (d, J = 8.3 Hz, 2H), 3.08 (s, 3H), 2.70 (t, J = 5.9 Hz, 2H), 2.41 (t, J = 6.2 Hz, 2H), 2.01-1.93 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$, ppm): δ 194.1, 193.3, 173.0, 148.3, 140.3, 134.7, 134.0, 130.3, 129.9, 128.1, 127.2, 127.2, 125.8, 109.2, 44.6, 37.5, 28.7, 20.3; IR (KBr, cm^{-1}): 3429, 3068, 3021, 2936, 1653, 1569, 1494, 1360, 1303, 1146, 1096, 963, 847, 782; EI-MS m/z: 402.06($[M-Cl]^+$, 100), 402.06 (100); Elem. Anal. Calcd. for $C_{20}H_{17}Cl_2NO_4S$: C, 54.80; H, 3.91; N, 3.20; S, 7.32. Found: C, 54.61; H, 4.08; N, 3.56; S, 7.55.

(vii). 3-(Benzylamino)-2-(2-chloro-4-(methylsulfonyl)benzoyl)cyclohex-2-enone (6g) : As a brown solid in 82 % yield, m.p. 184.0-185.1 °C. ^{1}H NMR (600 MHz, $CDCl_3$, ppm): δ 12.51 (s, 1H), 7.86 (s, 1H), 7.77 (d, J = 7.9 Hz, 1H), 7.38 (t, J = 7.3 Hz, 2H), 7.33 (d, J = 7.0 Hz, 1H), 7.29-7.26 (m, 3H), 4.63 (d, J = 5.5 Hz, 2H), 3.01 (s, 3H), 2.71 (t, J = 5.7 Hz, 2H), 2.31 (t, J = 8.0 Hz, 2H), 2.03-1.91 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$, ppm): δ 193.9, 192.5, 174.2, 148.8, 140.0, 135.2, 130.2, 129.2, 128.3, 128.1, 127.2, 127.1, 125.7, 108.7, 47.7, 44.6, 37.2, 27.1, 19.7; IR (KBr, cm^{-1}): 3458, 3182, 2936, 1577, 1469, 1303, 1146, 1088, 955, 848, 739; EI-MS m/z: 382.04($[M-Cl]^+$, 100), 382.04 (100); Elem. Anal. Calcd. for $C_{21}H_{20}ClNO_4S$: C, 60.35; H, 4.82; N, 3.35; S, 7.67. Found: C, 60.11; H, 4.61; N, 3.43; S, 7.81.

(viii). 2-(4-(Methylsulfonyl)-2-nitrobenzoyl)-3-(propylamino)cyclohex-2-enone (7a): As a pale-yellow solid in 89 % yield, m.p. 183.1-183.9 °C. ^{1}H NMR (600 MHz, DMSO, ppm): δ 11.86 (s, 1H), 8.58 (s, 1H), 8.25 (s, 1H), 7.49 (s, 1H), 3.50 (t, J = 12.8 Hz, 2H), 3.39 (s, 3H), 2.85 (t, J = 12.0 Hz, 2H), 2.17 (t, J = 6.2 Hz, 2H), 1.96-1.90 (m, 2H), 1.73-1.64 (m, 2H), 0.93 (t, J = 7.3 Hz, 3H); ^{13}C NMR (150 MHz, DMSO, ppm): δ 193.7, 190.5, 175.0, 145.4, 145.0, 140.4, 132.9, 128.6, 122.9, 106.7, 45.2, 43.5, 37.2, 26.6, 22.4, 19.7, 11.5; IR (KBr, cm^{-1}): 3716, 3077, 2978, 2878, 1585, 1452, 1362, 1303, 1155, 1056, 953, 868, 763, 535; HRMS (ESI) m/z [M+Na]$^+$ Calcd. for $C_{17}H_{20}N_2O_6S$ 403.0934. Found: 403.0943.

(ix). 2-(4-(Methylsulfonyl)-2-nitrobenzoyl)-3-(*o*-tolylamino)cyclohex-2-enone (7b): As a yellow solid in 76 % yield, m.p. 172.1-172.8 °C. ^{1}H NMR (600 MHz, $CDCl_3$, ppm): δ 13.36 (s, 1H), 8.71 (s, 1H), 8.20 (t, J = 7.9 Hz, 1H), 7.45 (d, J = 7.9 Hz, 1H), 7.34-7.30 (m, 3H), 7.21 (d, J = 7.2 Hz, 1H), 3.15 (s, 3H), 2.59 (t, J = 6.0 Hz, 2H), 2.40-2.27 (m, 5H), 1.97-1.89 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$, ppm): δ 194.2, 191.9, 173.9, 145.1, 144.8, 140.9, 135.0, 134.0, 132.4, 131.2, 128.6, 127.8, 126.9, 126.5, 123.3, 107.6, 44.4, 37.3, 28.3, 20.1, 17.8; IR (KBr, cm^{-1}): 3688, 3084, 3027, 1650, 1576, 1457, 1362, 1303, 1154, 962, 757, 540; HRMS (ESI) m/z [M+H]$^+$ Calcd. for $C_{21}H_{20}N_2O_6S$ 429.1115. Found: 429.1113.

(x). 2-(4-(Methylsulfonyl)-2-nitrobenzoyl)-3-(*m*-tolylamino)cyclohex-2-enone (7c): As a yellow solid in 70 % yield, m.p. 137.4-138.5 °C. ^{1}H NMR (600 MHz, $CDCl_3$, ppm): δ 13.48 (s, 1H), 8.71 (s, 1H), 8.19 (d, J = 12.0 Hz, 1H), 7.43 (d, J = 7.9 Hz, 1H), 7.35 (t, J = 7.7 Hz, 1H), 7.21 (d, J = 7.5 Hz, 1H), 7.10 (s, 1H), 7.07 (d, J = 7.9 Hz, 1H), 3.14 (s, 3H), 2.73 (t, J = 6.0 Hz, 2H), 2.41 (s, 3H), 2.34 (t, J = 6.3 Hz, 2H), 1.98-1.90 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$, ppm): δ 193.9, 191.5, 173.0, 144.9, 144.5, 139.6, 139.5, 135.5, 132.0, 129.0, 128.6, 127.4, 126.0, 123.0, 122.4, 107.2, 44.0, 37.0, 28.3, 20.9, 19.9; IR (KBr, cm^{-1}): 3658, 3019, 2928, 1651, 1577, 1454, 1362, 1303, 1154, 1096, 970, 930, 764, 539; HRMS (ESI) m/z [M+H]$^+$ Calcd. for $C_{21}H_{20}N_2O_6S$ 429.1115. Found: 429.1124.

(xi). 2-(4-(Methylsulfonyl)-2-nitrobenzoyl)-3-(*p*-tolylamino)cyclohex-2-enone (7d): As a yellow solid in 89 % yield, m.p. 176.3-177.2 °C. ^{1}H NMR (600 MHz, $CDCl_3$, ppm): δ 13.46 (s, 1H), 8.71 (s, 1H), 8.19 (d, J = 6.0 Hz, 1H), 7.43 (d, J = 7.9 Hz, 1H), 7.27 (d, J = 8.2 Hz, 2H), 7.15 (d, J = 8.1 Hz, 2H), 3.13 (s, 3H), 2.72 (t, J = 6.0 Hz, 2H), 2.41 (s, 3H), 2.33 (t, J = 6.3 Hz, 2H), 2.01-1.88 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$, ppm): δ 194.2, 191.8, 173.5, 145.3, 144.9, 140.0, 138.3, 133.3, 132.4, 130.1, 127.7, 125.5, 123.3, 107.6, 44.4, 37.3, 28.5, 21.0, 20.2; IR (KBr, cm^{-1}): 3682, 3085, 2935, 1643, 1568, 1462, 1354, 1306, 1158, 1056, 955, 828, 756, 524; HRMS (ESI) m/z [M+H]$^+$ Calcd. for $C_{21}H_{20}N_2O_6S$ 429.1115. Found: 429.1119.

(xii). 2-(4-(Methylsulfonyl)-2-nitrobenzoyl)-3-((4-fluorophenyl)amino)cyclohex-2-enone (7e): As a brown solid in 74 % yield, m.p. 181.9-182.7 °C. ^{1}H NMR (600 MHz, $CDCl_3$, ppm): δ 13.45 (s, 1H), 8.73 (s, 1H), 8.20 (d, J = 7.9 Hz, 1H), 7.43 (d, J = 7.9 Hz, 1H), 7.28 (d, J = 6 Hz, 2H), 7.19 (t, J = 8.3 Hz, 2H), 3.14 (s, 3H), 2.69 (t, J = 6.0 Hz, 2H), 2.34 (t, J = 6.3 Hz, 2H), 2.06-1.88 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$, ppm):

δ 193.8, 191.8, 173.2, 162. 3, 160.8, 151.0, 144.7, 144.6, 139.8, 132.1, 131.7, 127.5, 127.3, 122.0, 116.4, 116.3, 107.5, 44.1, 36.9, 28.2, 19.8; IR (KBr, cm^{-1}): 3699, 3086, 3000, 2924, 1643, 1548, 1460, 1343, 1196, 1157, 953, 840, 764; HRMS (ESI) m/z [M+Na]$^+$ Calcd. for $C_{20}H_{17}FN_2O_6S$ 455.0689. Found: 455.0691.

(xiii). 3-((4-Chlorophenyl)amino)-2-(4-(methylsulfonyl)-2-nitrobenzoyl)cyclohex-2-enone (7f): As a yellow solid in 80 % yield, m.p. 200.3-201.2 °C. ^{1}H NMR (600 MHz, $CDCl_3$, ppm): δ 13.52 (s, 1H), 8.74 (s, 1H), 8.21 (d, *J* = 7.4 Hz, 1H), 7.48 (d, *J* = 8.4 Hz, 2H), 7.42 (d, *J* = 7.8 Hz, 1H), 7.26 (s, 1H), 7.24 (d, *J* = 8.4 Hz, 2H), 3.15 (s, 3H), 2.71 (t, *J* = 5.7 Hz, 2H), 2.35 (t, *J* = 12.0 Hz, 2H), 1.98-1.94 (m, 2H); ^{13}C NMR (150 MHz, DMSO, ppm): δ 193.9, 191.1, 173.7, 144.6, 140.4, 135.2, 132.7, 132.2, 129.4, 128.0, 122.6, 107.6, 43.1, 37.0, 28.2, 20.0; IR (KBr, cm^{-1}): 3691, 3078, 2936, 1641, 1570, 1461, 1411, 1354, 1312, 1155, 1095, 954, 838, 764, 574; HRMS (ESI) m/z [M+H]$^+$ Calcd. for $C_{20}H_{17}ClN_2O_6S$ 449.0569. Found: 449.0577.

(xiv). 3-(Benzylamino)-2-(4-(methylsulfonyl)-2-nitrobenzoyl)cyclohex-2-enone (7g): As a yellow solid in 92 % yield, m.p. 156.0-156.8 °C. ^{1}H NMR (600 MHz, DMSO, ppm): δ 12.07 (s, 1H), 8.58 (s, 1H), 8.26 (d, *J* = 8.0 Hz, 1H), 7.52 (d, *J* = 8.0 Hz, 1H), 7.47-7.41 (m, 3H), 7.38 (t, *J* = 6.8 Hz, 1H), 4.82 (d, *J* = 5.7 Hz, 2H), 3.41 (s, 3H), 2.87 (t, *J* = 6.9 Hz, 2H), 2.20 (t, *J* = 7.3 Hz, 2H), 1.90-1.81 (m, 2H); ^{13}C NMR (150 MHz, DMSO, ppm): δ 193.8, 190.6, 175.1, 145.3, 144.9, 140.4, 136.9, 132.9, 129.3, 128.5, 128.2, 127.9, 122.8, 107.0, 47.2, 43.4, 37.0, 26.9, 19.7; IR (KBr, cm^{-1}): 3691, 3160, 3070, 2947, 1651, 1577, 1461, 1353, 1303, 1155, 963, 872, 773, 723, 532; HRMS (ESI) m/z [M+Na]$^+$ Calcd. for $C_{21}H_{20}N_2O_6S$ 451.0934. Found: 451.0918.

(xv). 2-(4-(Methylsulfonyl)-2-nitrobenzoyl)-3-((4-fluorophenyl)thio)cyclohex-2- enone (8a): As a pale-yellow solid in 67 % yield, m.p. 190.3-191.3 °C. ^{1}H NMR (600 MHz, $CDCl_3$, ppm): δ 8.63 (s, 1H), 8.16 (d, *J* = 7.9 Hz, 1H), 7.53 (d, *J* = 8.4 Hz, 2H), 7.42 (d, *J* = 7.9 Hz, 1H), 7.12 (t, *J* = 8.4 Hz, 2H), 3.08 (s, 3H), 2.44 (t, *J* = 6.0 Hz, 2H), 3.32 (t, *J* = 6.3 Hz, 2H), 1.90-1.85 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$, ppm): δ 193.6 , 189.6, 183.4, 164.9, 163.2, 145.2, 143.9, 141.1, 137.3, 137.3, 132.5, 128.4, 127.6, 125.2, 123.2, 117.2, 117.1, 44.4, 37.2, 33.8, 21.5; IR (KBr, cm^{-1}): 3043, 2936, 1668, 1618, 1543, 1477, 1362, 1320, 1220, 1163, 930, 839, 756; HRMS (ESI) m/z [M+Na]$^+$ Calcd. for $C_{20}H_{16}FNO_6S_2$ 472.0295. Found: 472.0024.

(xvi). 3-((4-Chlorophenyl)thio)-2-(4-(methylsulfonyl)-2-nitrobenzoyl)cyclohex-2- enone (8b): As a pale-yellow solid in 58 % yield, m.p. 199.4-200.2 °C. ^{1}H NMR (600 MHz, $CDCl_3$, ppm): δ 8.71 (s, 1H), 8.24 (d, *J* = 7.9 Hz, 1H), 7.56 (d, *J* = 8.2 Hz, 2H), 7.51-7.47 (m, 3H), 3.15 (d, *J* = 14.2 Hz, 3H), 2.53 (t, *J* = 5.8 Hz, 2H), 2.35 (t, *J* = 6.3 Hz, 2H), 1.97-1.88 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$, ppm): δ 193.6, 189.6, 182.7, 145.2, 143.9, 141.1, 137.2, 136.5, 132.6, 130.1, 128.4, 128.1, 127.7, 123.2, 44.4, 37.2, 33.9, 21.5; IR (KBr, cm^{-1}): 3093, 3027, 2928, 1668, 1536, 1477, 1353, 1320, 1145, 1079, 1029, 839, 773; HRMS (ESI) m/z [M+H]$^+$ Calcd. for $C_{20}H_{16}ClNO_6S_2$ 466.0180. Found: 466.0177.

(xvii). 3-((4-Bromophenyl)thio)-2-(4-(methylsulfonyl)-2-nitrobenzoyl)cyclohex-2- enone (8c): As a yellow solid in 65 % yield, m.p. 189.5-190.5 °C. ^{1}H NMR (600 MHz, CDCl$_3$, ppm): δ 8.71 (s, 1H), 8.25 (d, J = 7.9 Hz, 1H), 7.68 (d, J = 8.3 Hz, 2H), 7.58-7.53 (m, 3H), 3.17 (s, 3H), 2.53 (t, J = 6.4 Hz, 2H), 2.36 (t, J = 6.0 Hz, 2H), 1.96-1.91 (m, 2H); ^{13}C NMR (150 MHz, CDCl$_3$, ppm): δ 193.5, 189.5, 182.5, 145.1, 143.8, 141.1, 136.6, 133.0, 132.5, 128.7, 128.3, 127.6, 125.5, 123.2, 44.3, 37.2, 33.8, 21.5; IR (KBr, cm^{-1}): 3073, 2916, 1654, 1540, 1446, 1352, 1296, 1144, 1057, 956, 899, 805, 761; HRMS (ESI) m/z [M+Na]$^+$ Calcd. for $C_{20}H_{16}BrNO_6S_2$ 531.9495. Found: 531.9524.

(xviii). 2-(4-(Methylsulfonyl)-2-nitrobenzoyl)-3-(p-tolylthio)cyclohex-2-enone (8d): As a yellow solid in 73 % yield, m.p. 167.2-168.2 °C. ^{1}H NMR (600 MHz, CDCl$_3$, ppm): δ 8.71 (s, 1H), 8.23 (d, J = 7.9 Hz, 1H), 7.50-7.45 (m, 3H), 7.36-7.26 (m, 2H), 3.15 (s, 3H), 2.54 (t, J = 5.8 Hz, 2H), 2.45 (s, 3H), 2.35 (t, J = 7.6 Hz, 2H), 1.91-1.86 (m, 2H); ^{13}C NMR (150 MHz, CDCl$_3$, ppm): δ 188.7, 184.4, 179.7, 140.1, 139.1, 136.0, 135.9, 129.9, 127.4, 125.5, 123.2, 122.2, 121.1, 118.2, 72.2, 72.0, 71.7, 39.3, 32.2, 28.6, 16.5, 16.3; IR (KBr, cm^{-1}): 3093, 2928, 1660, 1576, 1536, 1477, 1353, 1303, 1162, 922, 888, 780; HRMS (ESI) m/z [M+Na]$^+$ Calcd. for $C_{21}H_{19}NO_6S_2$ 468.0546. Found: 468.0551.

(xix). 3-((4-Ethylphenyl)thio)-2-(4-(methylsulfonyl)-2-nitrobenzoyl)cyclohex-2- enone (8e): As a yellow solid in 71 % yield, m.p. 169.8-170.7 °C. ^{1}H NMR (600 MHz, CDCl$_3$, ppm): δ 8.72 (s, 1H), 8.24 (d, J = 7.7 Hz, 1H), 7.53-7.48 (m, 3H), 7.33 (d, J = 7.7 Hz, 2H), 3.16 (s, 3H), 2.73 (q, J = 7.6 Hz, 2H), 2.54 (t, J = 5.7 Hz, 2H), 2.37 (t, J = 6.4 Hz, 2H), 1.97-1.85 (m, 2H), 1.29 (t, J = 7.6 Hz, 3H); ^{13}C NMR (150 MHz, CDCl$_3$, ppm): δ 193.7, 189.4, 184.8, 147.2, 145.1, 144.2, 140.9, 135.1, 132.5, 129.4, 128.2, 127.1, 126.2, 123.3, 44.3, 37.2, 33.7, 28.5, 21.5, 15.1; IR (KBr, cm^{-1}): 3094, 2961, 2922, 2875, 1658, 1534, 1449, 1354, 1316, 1154, 954, 888, 830, 764; HRMS (ESI) m/z [M+Na]$^+$ Calcd. for $C_{22}H_{21}NO_6S_2$ 482.0702. Found: 482.0739.

(xx). 3-((4-Isopropylphenyl)thio)-2-(4-(methylsulfonyl)-2-nitrobenzoyl)cyclohex-2-enone (8f): As a yellow solid in 73 % yield, m.p. 197.9-198.6 °C. ^{1}H NMR (600 MHz, CDCl$_3$, ppm): δ 8.72 (s, 1H), 8.24 (d, J = 7.2 Hz, 1H), 7.63-7.46 (m, 3H), 7.35 (d, J = 7.8 Hz, 2H), 3.16 (s, 3H), 3.03-2.94 (m, 1H), 2.55 (t, J = 5.7 Hz, 2H), 2.34 (t, J = 6.4 Hz, 2H), 1.98-1.86 (m, 2H), 1.30 (d, J = 6.9 Hz, 6H); ^{13}C NMR (150 MHz, CDCl$_3$, ppm): δ 193.8, 189.5, 185.1, 151.8, 145.0, 144.2, 140.8, 135.0, 132.5, 128.2, 128.1, 128.0, 127.0, 126.2, 123.3, 44.3, 37.2, 33.9, 23.7, 21.5; IR (KBr, cm^{-1}): 3093, 2961, 2920, 1668, 1536, 1460, 1494, 1356, 1308, 1156, 956, 832, 776; HRMS (ESI) m/z [M+Na]$^+$ Calcd. for $C_{23}H_{23}NO_6S_2$ 496.0859. Found: 496.0872.

(xxi). 3-((4-(Tert-butyl)phenyl)thio)-2-(4-(methylsulfonyl)-2-nitrobenzoyl)cyclohex -2-enone (8g): As a yellow solid in 76 % yield, m.p. 172.8-173.6 °C. ^{1}H NMR (600 MHz, CDCl$_3$, ppm): δ 8.71 (s, 1H), 8.24 (d, J = 7.4 Hz, 1H), 7.59-7.41 (m, 5H), 3.16 (s, 3H), 2.56 (t, J = 7.0 Hz, 2H), 2.33 (t, J = 7.4 Hz, 2H), 1.96-1.91 (m, 2H), 1.36 (s, 9H). ^{13}C NMR (150 MHz, CDCl$_3$, ppm): δ 193.7, 189.5, 184.9, 154.1, 145.10, 144.2, 140.9, 134.8, 132.5, 128.2, 127.1, 126.9, 126.0, 123.3, 44.3, 37.3, 34.8, 33.8, 31.0, 21.5; IR

(KBr, cm^{-1}): 3085, 2961, 2870, 1651, 1527, 1461, 1394, 1352, 1312, 1154, 1096, 947, 831, 773; HRMS (ESI) m/z $[M+Na]^+$ Calcd. for $C_{24}H_{25}NO_6S_2$ 510.1016. Found: 510.0997.

Herbicidal activity test of compounds

Herbicidal activities of compounds from 6a to 8g were evaluated by petri dish assay and greenhouse pot culture (5). Seeds of *E. crus-galli*, *B. napus* and *Amaranthus retroflexus* were obtained from the Bioassay Testing and Safety Assessment Center, Zhejiang Research Institute of Chemical Industry.

Petri dish method. Monocotyledon barnyard grass (*E. crus-galli*) and dicotyledon rape (*B. napus*) were selected as model plants. Each compound (2 mg) was dissolved in 100 μL DMF and emulsified with a drop of Tween-80, and the solution was diluted with water to 1000 mg/L concentration, named concentrated solution. Its parts were further diluted to a gradient of 100, 10 and 1 mg/L for experimental use. The solutions of commercial herbicide mesotrione were also made as the positive control, and water was used as a blank control. The seeds were soaked in warm water (25 °C) for 15 h before use. The 9 mL test solutions were added to each Petri dish (9 cm dia) lined with two layers of filter paper on which 10 seeds of each test plant were sown. These petri dishes were kept in an incubator [25 °C, humidity 75 % in dark (1-3 days) followed by the alternating 12/12 diurnal cycle of light (10 Klux)] and dark for another 5 days. The experimental duration for herbicidal activity was 8 days. Each treatment was repeated 3-times. The shoot and root lengths of 5-seedling from each petri dish was measured and their mean inhibition (%) were calculated as under :

$$IR = \frac{\bar{l}_1 - \bar{l}_2}{\bar{l}_1} \times 100\ \%$$

Where, $\bar{l}_1$: Mean length of root/shoot of control and $\bar{l}_2$: Mean length of root/shoot of test compound. Data of root and shoot inhibitory rates were subjected to one-way ANOVA and means were compared by the least significant difference test ($p < 0.05$).

Greenhouse Pot culture: The postemergence herbicidal activity was evaluated in pot culture in greenhouse. The actual weed *Amaranthus retroflexus* instead of rape (*B. napus*) and barnyard grass (*E. crus-galli*) were selected as the model test plants. Mesotrione and the diluted solution of DMF + Tween-80 were used as the positive and blank control. Each target compound was first dissolved in DMF to prepare solution 100 g/L concentration and then diluted with water containing 0.1 % Tween-80 to the desired concentrations and applied to pot-grown plants. Plastic pots 7.5 cm (inner diameter) were filled with 33.3 % garden soil and 66.7 % nursery substrates to a depth of three-fourths. Ten test weed seeds were sown per pot and covered with 0.2 cm soil. The pots were placed in a greenhouse (15-30 °C). Each pot was thinned to 5 plants/pot at 7 days after sowing. The prepared solutions (200 g ai/ha were added @ 0.088 mg per pot) were applied as postemergence treatment at 3-leaf stage. Bleaching herbicidal activity was evaluated visually after 15 days of treatment and the average inhibitions of shoots are calculated as per the above petri dish method. To minimize experimental errors, each treatment was repeated thrice.

Molecular docking. The crystal structure of *At*HPPD (PDB code 5YWG) was downloaded from the Protein Data Bank. The three-dimensional structures of the corresponding active molecule of target compound **8b** or **8g** were generated with the sketch module of SYBYL version 2.0. The docking results were visualized by PyMOL version 1.3 software and Maestro version 12.7 (for 2D interactions).

RESULTS AND DISCUSSION

Petri dish assay

The inhibitory effects of most of synthesized target compounds on roots and shoots of both dicotyledonous rape (*B. napus*) and monocotyledonous barnyard grass (*E. crus-galli*) were higher than mesotrione (Table 1). The compounds **7b**, **7f**, **8c**, **8e** and **8g** at 100 mg/L inhibited root growth of *B. napus* by 93 % to 98 %, while, the compounds **6a**, **8e**, **8f** and **8g** inhibited root growth of *E. crus-galli* from 86 % to 100 %. Moreover, at the test concentrations, the shoot inhibition in monocotyledonous barnyard grass (*E. crus-galli*) were higher than in dicotyledonous rape (*B. napus*) for target compounds **6** and **7** except compounds **6c** and **6e**. Similar results were also obtained for compounds **8a**~**8g** under 100 mg/L concentration. Some compounds, viz., **7a**, **7c**, **7d** and **7g** showed good selectivity and mainly inhibited the shoot elongation of *E. crus-galli*, while, the compound **6e** mainly inhibited the shoot elongation of *B. napus*. The compound **8e** at 100 mg/L concentration completely inhibited the growth of roots and shoots of both weed species. Structure-activity relationship analysis revealed that electronic and steric effects of the target compounds had some effects on herbicidal activities of all target compounds. For example, the ortho-substituted compound **7b** and electron withdrawing chloro-substituted compound **7f** caused greater inhibition in tested weeds than homologous series. Moderate to severe albinism symptoms were observed (Figure 4) for 11 compounds (**6a**, **7e**~**7g**, and **8a**~**8g**) of the 21 synthesized compounds. The albinism concentration for rape (*Brassica napus* L.) was as low as 1 mg/L for compounds **8a**~**8g**, which is similar to mesotrione.

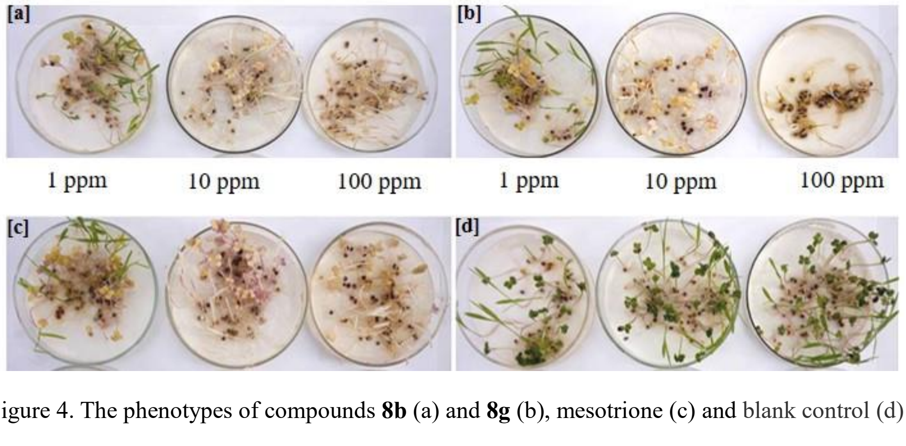


Figure 4. The phenotypes of compounds **8b** (a) and **8g** (b), mesotrione (c) and blank control (d)

Table 1. The results of petri dish herbicidal activity test of cyclohex-2-enones

Compounds	Mean root inhibition (%)^{a}						Mean shoot inhibition (%)^{a}					
	Rape			Barnyard grass			Rape			Barnyard grass		
	100 mg/L	10 mg/L	1 mg/L	100 mg/L	10 mg/L	1 mg/L	100 mg/L	10 mg/L	1 mg/L	100 mg/L	10 mg/L	1 mg/L
6a	69±2e	63±1b	23±1.7j	100±1a^{+}	55±1d	9±1l	40±1.7e	28±1d	0h	76±1c^{+}	45±1.7f	21±1i
6b	62±1.7g	22±1k	34±1h	56±1.7j	66±1b	24±1h	36±1.7f	11±1h	3±1g	65±1d	64±1c	38±1.7e
6c	69±1.7e	27±1j	32±1h	62±1i	28±1.7j	13±1k	28±2h	4±1j	28±1.7c	34±1j	7±1n	0m
6d	72±1.7d	21±1k	34±1.7h	68±1.7g	38±1h	21±1i	40±1e	17±2f	20±1.7f	61±2e	54±1d	44±1.7c
6e	76±2c	34±1h	49±1.7d	79±1d	71±1a	25±1h	62±2c	0k	0h	30±1.7k	31±1i	53±1.7a
6f	60±1.7g	22±2k	23±1j	76±1e	40±1.7g	44±1c	13±1k	40±1b	30±1.7c	81±1.7b	43±2g	43±1d
6g	67±2e	64±1b	52±1.7c	46±2k	38±1.7h	45±1.7c	29±2h	10±1.7h	0h	57±2f	52±1e	37±1e
7a	72±1d	47±1e	55±1b	58±2j	40±2g	36±1e	0n	0k	0h	49±2g	21±1k	23±1i
7b	96±1a	17±1l	52±2c	60±2i	15±2l	13±1k	31±1g	7±1i	4±2g	67±2d	26±1j	26±2h
7c	11±1k	27±2j	0l	27±1m	27±1.7j	11±1k	5±1m	0k	0h	38±2i	24±1j	14±1j
7d	37±2j	47±1e	76±2a	15±2n	32±1i	42±2d	0n	0k	0h	31±2k	46±2f	46±1c
7e	62±2g^{+}	47±1e	40±2f	39±1l	31±2i	32±1f	29±2h^{+}	14±2g	0h	32±2j	34±2h	45±1c
7f	93±2b^{+}	47±2e	37±1g	61±1.7i	20±2k	17±1.7j	49±2d^{+}	0k	0h	56±1f	14±1l	49±2b
7g	66±2f^{+}	32±1h	45±1e	56±2j	42±1g	42±1.7d	0n^{+}	0k	0h	46±2h	4±1o	2±1m
8a	43±1i*	59±2c*	28±2i*	73±2f*	46±1.7f*	35±2f^{+}	20±1.7j*	52±1.7a*	2±1g*	48±2h*	73±2a*	33±1f^{+}
8b	77±1c*	39±1g*	46±2e*	65±1.7h*	42±1g*	37±1.7e^{+}	10±1l*	17±2f*	30±1.7c*	76±1.7c*	22±1k*	29±2g^{+}
8c	98±1a*	91±2a*	42±1f^{++}	77±2d*	38±2h^{+}	47±1b^{+}	65±2b*	32±2c*	33±2b*	76±2c*	14±2l^{+}	10±2k^{+}
8d	62±2g*	37±1g*	49±1.7d*	71±2f*	59±1.7c*	36±2e^{+}	26±1.7i*	40±2b*	33±1.7b*	51±2g*	56±1d*	3±1m^{+}
8e	98±1a*	30±2i*	18±1.7k*	95±1b*	33±1i*	31±2g^{+}	95±2a*	33±1c*	27±2d*	95±2a*	51±1.7e*	6±1l^{+}
8f	78±2c*	51±1.7d*	32±1h^{+}	86±1.7c*	55±1d	38±2e	32±2g*	27±2d*	39±1a^{+}	82±1b*	10±1.7m	10±2k
8g	96±1a*	42±2f^{++}	28±2i*	93±1b*	50±2e*	48±2b^{+}	50±2d*	24±2e^{++}	19±1.7f*	65±1d*	45±2f*	53±1a^{+}
Mesotrione	46±1h*	40±1f*	76±1a*	78±1.7d*	53±1d*	52±1.7a*	22±1.7j*	16±2f*	23±1e*	40±1.7i*	68±2b*	9±1k*
$LSD_{0.05}$	2.55	0.14	-5.21	-1.39	-1.81	-3.58	0.88	0.12	-1.23	3.32	-3.67	2.07

[a] Each value is mean ± SD (n = 3), data were processed by SPSS software and different letters in the same column indicate significant difference among treatments at $P < 0.05$ (LSD test); *indicates severe albinism of the plant, the albino rate was 100 %; + indicates moderate albinism of the plants and the albino rate is between 40 % and 60 %.

Greenhouse Pot culture

Most of the synthesized target compounds showed moderate to good inhibitory effects (50~80 %) on shoots length of *A. retroflexus* and barnyard grass (*E. crus-galli*), and the inhibitory effects were higher on the shoots of *A. retroflexus* (Table 2). However, the concomitant bleaching actions were basically lost for compounds **6a**~**6g** and **7a**~**7g**, but remained for compounds **8a**~**8g** despite the slight decrease in efficacy. These results indicate that only compounds **8a**~**8g** could be transformed into triketones active structure by weeds and have proherbicide properties. Therefore, the efficacy of proherbicide mainly depends on, if it can be transformed into herbicidally active structure by weeds or soil organisms.

Table 2. Herbicidal effects of cyclohex-2-enones at 200 g ai/ha on shoots of test weed species

Compounds	**Inhibition rate (IR, %)** [a]	
	***Amaranthus retroflexus* (shoot)**	***barnyard grass* (shoot)**
6a	30±1i	50±1c
6b	0l	10±1i
6c	35±1h	30±1.7f
6d	45±1f	0j
6e	25±1j	15±1h
6f	30±1i	10±1.7i
6g	50±1.7e	45±1d
7a	20±1k	10±1.7i
7b	70±1.7b	20±1.7g
7c	0l	20±2g
7d	30±2i	0j
7e	60±1.7d^{+}	0j
7f	65±1c^{+}	0j
7g	50±1e^{+}	0j
8a	70 ±2b^{+}	70 ±1.7a^{+}
8b	80±1a^{+}	60±1.7b^{+}
8c	80±2a^{+}	70±1a^{+}
8d	70±1.7b^{+}	60±1.7b^{+}
8e	80±1a^{+}	70±1a^{+}
8f	40±1.7g^{+}	20±1g^{+}
8g	80±1a^{+}	60±1.7b^{+}
mesotrione	51±1e*	42±2e*
LSD$_{0.05}$	-0.22	-1.10

[a] Each value is mean ± SD (n = 3), data were processed by SPSS software and different letters in the same column indicate significant difference among treatments at $P < 0.05$ (LSD test); *indicates severe albinism of the plant, the albino rate was 100 %; + indicates moderate albinism of the plants and the albino rate is between 40 % and 60 %.

To further investigate the interactions between inhibitors and the target enzyme of herbicides, we selected the *At*HPPD (PDB code 5YWG) and compounds **8b** and **8g** as target enzyme and ligands, respectively, for molecular docking experiments. Both compounds **8a** and **8g** were well-matched to the active site of *At*HPPD (Figures 5a and 5d). The carbonyl group attached to the alicyclic hydrocarbon and its bridging carbonyl group of inhibitors **8a** and **8g** cannot form bidentate interactions with the active center Fe^{II} ion (Figures 5b and 5e), which is different to the cocrystal structure of *At*HPPD complexed with the typical HPPD inhibitor mesotrione (15). The π-π interaction was formed between the benzene rings of inhibitors and the two amino acid residues Phe371 and Phe403 (Figures 5c and 5f), which was similar to mesotrione in complex with *At*HPPD. Moreover, the hydrogen bond interactions were also formed between the nitro group and peripheral amino acid residues Gln272 and Gln286 (Figures 5c and 5f).

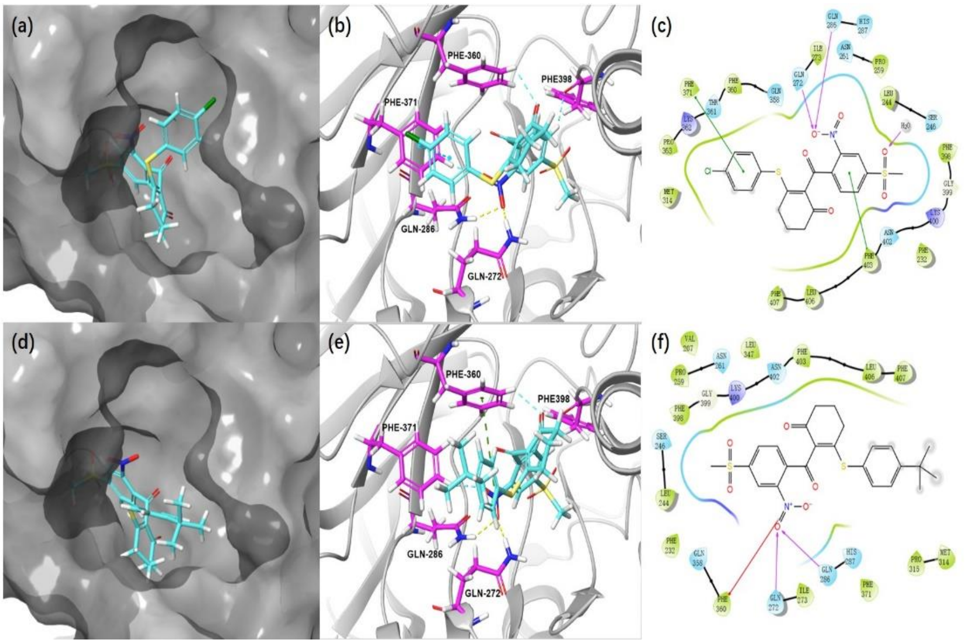


Figure 5. Binding modes of ligands (a~c) compound **8b** and (d~f) compound **8g** with *At*HPPD (PDB code 5YWG) at the active site. The carbon atoms are shown in cyan; Hydrogen atoms are shown in gray; Sulfur atoms are shown in yellow; Oxygen atoms are shown in red; and Nitrogen atoms are shown in blue

Molecular docking indicated that the corresponding active molecules of target compounds and benzobicyclon may share a similar interplay with surrounding residues, which was consistent with the *in-vivo* herbicidal activity test results and corresponding symptoms.

CONCLUSIONS

A series of novel 2-aroyl-3-arylamino/arylthiocyclohex-2-enones were designed and synthesized. Most of the synthesized target compounds (especially 2-aroyl-3-arylthiocyclohex-2-enones **8a**~**8g**), showed outstanding herbicidal activities similar to the commercial herbicide mesotrione, with moderate to excellent inhibitory effects on roots and shoots of test weed plants, and with moderate to severe albinism symptoms. Molecular docking revealed that these synthesized compounds formed tight π-π and hydrogen bond interactions with *At*HPPD in the active site without the bidentate interaction with the active center Fe^{II} ion. These results indicated that the novel 2-aroyl-3-arylamino/ arylthiocyclohex-2-enones might be potential candidates for novel HPPD inhibitors discovery.

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DECLARATION

We declare that all authors of this Ms. have made substantial contributions. We did not exclude any author who substantially contributed to this Ms. We have followed our ethical norms established by our respective institutions.

CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

ETHICAL APPROVAL

The authors declare that the study was carried out following scientific ethics and conduct. However, this study did not involve any use of animals, hence no ethical approval has been obtained from the concerned committee.

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