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Article

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Synthesis and Herbicidal Activity of Pyrido[2,3-*d*]pyrimidine-2,4-dione−benzoxazinone Hybrids as Protoporphyrinogen Oxidase Inhibitors

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ABSTRACT: To search for new protoporphyrinogen oxidase (PPO, EC 1.3.3.4) inhibitors with improved bioactivity, a series of novel pyrido[2,3-*d*]pyrimidine-2,4-dione−benzoxazinone hybrids, **9-13**, were designed and synthesized. Several compounds with improved *tobacco* PPO (*mt*PPO)-inhibiting and promising herbicidal activities were found. Among them, the most potent compound 3-(7-fluoro-3-oxo-4-(prop-2-yn-1-yl)-3,4-dihydro-2*H*-benzo[*b*][1,4] oxazin-6-yl)-1-methylyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione, **11q**, with a K_i value of 0.0074 μM, showed six times more activity than flumioxazin (K_i = 0.046 μM) against *mt*PPO. Compound **11q** displayed a strong and broad spectrum of weed control at 37.5-150 g of active ingredient (ai)/ha by both post- and pre-emergence application, which were comparable to that of flumioxazin. **11q** was safe to maize, soybean, peanut and cotton at 150 g ai/ha, and selective to rice and wheat at 75 g ai/ha by pre-emergence application, indicating potential applicability in these fields.

KEYWORDS: pyrido[2,3-*d*]pyrimidine-2,4-dione, benzoxazinone, protoporphyrinogen oxidase, herbicide, structure−activity relationship

Introduction

During the growing season for agricultural crops, weeds compete with crops for water, nutrients, light and space. If weeds are left uncontrolled, the crop yield losses can be as substantial as 50%.[1, 2] Since the middle of the 20th century, synthetic organic herbicides have played a significant role in the control of weeds. However, the overuse of some herbicides has resulted in significant development of weed resistance and huge negative environmental effects.[3] Therefore, new compounds, covering a broad spectrum of weed control and environmentally friendly, are globally demanded. The protoporphyrinogen oxidase (PPO, EC 1.3.3.4) inhibition compounds can provide these solutions. PPO is the penultimate enzyme in the biosynthesis of chlorophyll and heme, belongs to a large family of flavin adenine dinucleotide (FAD)-containing enzymes, and catalyzes the conversion of protoporphyrinogen IX to protoporphyrin IX.[4-7] Inhibition of PPO in plants can lead to toxic accumulation of protoporphyrin IX in cytoplasm. When exposed to light, protoporphyrin IX will react with oxygen and generate many reactive oxygen species, destroy the cell membranes and cause the rapid burn symptoms in plants (necrosis within one day). PPO-inhibiting herbicides have many attractive properties, such as broad-spectrum weed control (including resistant biotypes), benign environmental characteristics, low toxicity and low application rate.[8-10]

The first PPO herbicide nitrofen was introduced to the market by Dow AgroSciences in 1963. Since then, many diverse chemistries have been discovered, from which about 30 compounds were finally commercialized, which can be further divided into the following types: phenylpyrazoles, triazolinones, *N*-phenyl-phthalimides, oxazolidinediones, diphenyl ethers, oxadiazoles, pyrimidinediones and thiadiazoles. Unlike acetohydroxyacid synthase (AHAS)-

and acetyl-coenzyme A carboxylase (ACCase)-inhibiting herbicides, although PPO herbicides have been used for more than six decades, there has still been no significant development of resistant weeds.[11] One possible reason is that, the structural bases of these herbicides mimic two or three tetrapyrrole rings of the substrate protoporphyrinogen IX.[12, 13] Interestingly, due to some weed biotypes developing resistance to glyphosate and other herbicides, the market shares of PPO herbicides began to increase recently.[14] Flumioxazin can effectively suppress some glyphosate-, AHAS- and triazine-resistant weed populations such as *Amaranthus rudis* Sauer and *Amaranthus palmeri*.[15-17] Saflufenacil has been the most successful herbicide discovered in the past 20 years. By pre-emergence application, saflufenacil can be used for broadleaf weed control in numerous crops, such as cereals, corn, and soybean. It can effectively control more than 90 kinds of dicotyledonous weeds as well as these triazines-, AHAS- and glyphosate-resistant *Amaranthus* biotypes.[18]

Generally, the structures of PPO inhibitors consist of three parts: a heterocyclic moiety, a benzene ring and a hydrophobic chain (Figure 1). The heterocyclic moiety attaches to the benzene ring via a C-N or C-C bond, the hydrophobic chain is placed at the 4- or 5-position of the benzene ring. In most cases, when the 4,5- or 5,6 -position of the benzene ring is fused with a five- or six-membered ring, compounds also show very good herbicidal activities. Examples of this are the *N*-benzothiazolyl compounds[10], benzoxazinones,[19] and benzoxazoles[20] Among these benzene-fused ring heterocyclic inhibitors, three compounds, flumioxazin, thidiazimin and trifludimoxazin, with benzoxazinones were commercialized (Figure 1).[21] Furthermore, researches have proved that modification of the heterocyclic moieties of the PPO inhibitors is an effective strategy for discovering new compounds with improved bioactivities.[22-24]

Quinazoline-2,4-dione is an important heterocyclic scaffold, exiting in many natural products with various biological activities.[25] Previously, we have found that triketone-containing quinazoline-2,4-diones displayed excellent herbicidal activity, and the quinazoline-2,4-dione ring can form π-π stacking interactions with the Phe360 and Phe403 of *Arabidopsis thaliana* 4-hydroxyphenylpyruvate dioxygenase (*At*HPPD).[26-28] It was reported that changing the CH group in the benzene ring to *N* atom can significantly increase the π-π stacking possibility of the target molecule.[29] Based on our molecular simulation studies, the heterocyclic moieties of PPO inhibitors can form π-π stacking interactions with Phe392 of *tobacco* PPO (*mt*PPO).[2, 5], Therefore, we envisioned that pyrido[2,3-*d*]pyrimidine-2,4-dione might be a promising heterocyclic moiety to integrate with benzoxazinone. For this purpose a series of pyrido[2,3-*d*]pyrimidine-2,4-dione−benzoxazinone hybrids were designed. Herein, we report the synthesis, herbicidal activity, *mt*PPO inhibition activity, and structure−activity relationships (SAR) of these compounds.

MATERIALS AND METHODS

Chemicals and instruments. All chemical reagents, such as ethyl bromoacetate, 5-halogen-2-nitrophenols, caesium carbonate, and 4-chloromethylanisole were purchased from Innochem, Science & Technology Co., Ltd. (Beijing, China). Organic solvents, such as dimethylformamide (DMF), petroleum ether (b.p. 60-90 °C), acetic acid, toluene, and methanol were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The solvents were dried and redistilled according to the standard methods before use. ^{1}H NMR and ^{13}C NMR spectra were recorded on a VARIAN Mercury-Plus 400 spectrometer (Bruker Corp.,

Switzerland) in $CDCl_3$ or DMSO-d_6 with trimethyl chlorosilane as the internal reference. High resolution mass spectra (HRMS) were obtained on a 6520 Q-TOF LC/MS (Agilent, Santa Clara, CA). Melting points of the synthesized compounds were taken on a WRS-1B melting point apparatus (Shenguang Instrument Co., Ltd. Shanghai, China) and are uncorrected.

The detailed synthetic routes, physical and spectrum data (NMR, HRMS) of compounds **3-13** are shown in the Supporting Information.

***mt*PPO Inhibitory Experiments.** The expression and purification of *mt*PPO were used the same methods as previously reported.[2,8,10,30] The enzyme product protoporphyrin IX had a maximum excitation and a maximum emission wavelengths at 410 nm and 630 nm, respectively, and the formation of the product can be monitored by using a fluorescence detector with the maximum excitation at 410 nm and maximum emission wavelength at 630 nm. The *mt*PPO reaction rate was assayed by monitoring the speed of formation of the substrate to the product on a 96-well plate using the fluorescence detector. [8]

Sigma Plot software 10.0 (SPSS, Chicago, IL) was used to calculate the kinetic parameters. 50% of the total inhibition (IC_{50}) value of inhibitors was determined by measuring *mt*PPO activity over a range of inhibitor concentrations at a fixed substrate concentration. IC_{50} values were calculated by fitting *v* versus [I] data to a single binding site model (eq 1).

$$y = \min + \frac{\text{max-min}}{1+10^{\log IC_{50} - x}} \quad (1)$$

where y is the percentage of the maximal rate, max and min being the y values at which the curve levels off, x is the logarithm of inhibitor concentration. Calculated inhibition constant of the enzyme reaction (K_i) value can be determined by applying the following relationship among K_i, K_m, and IC_{50} at any saturating substrate concentration (eq 2).

$$K_i = \frac{IC_{50}}{S/K_m + 1} \quad (2)$$

Molecular Simulation and CoMFA study. The crystal structure of *mt*PPO was downloaded from the RSCB protein data bank (PDB ID: 1SEZ). The 3D structure of compounds **9-13** were constructed and optimized using SYBYL 6.9 (Tripos, Inc.,St. Louis, MO).[31] The Gasteiger–Huckel method was used to calculate the charges of all compounds, and the compounds were then optimized with the power method for 5000 steps until a delta energy change lower than 0.005 kcal/mol was reached. The optimized structures were used for docking and subsequent 3D-QSAR studies. The protein and ligand were prepared using the default settings of AutoDockTools4.[32] AutoDock4.2 was used to calculate the interaction of the two molecules, and a total of 258 runs were used for each molecule. The best binding modes were selected by the docking score as well as by comparison with the co-crystal ligand in the *mt*PPO. The SYBYL default parameters were used to calculate the CoMFA steric, descriptors and electrostatic field energies. The parameters used were as follows: an sp^3 carbon probe atom with +1 charge, 2.0 Å grid points spacing, an energy cutoff of 30.0 kcal/mol and a minimum σ (column filting) of 2.0 kcal/mol.[10]

Herbicidal activity. To evaluate the post-emergence activity of compounds **9-13**, six representative broadleaf weeds, *Abutilon juncea* (AJ), *Amaranthus retroflexus* (AR), *Brassica juncea* (BJ), *Chenopodium serotinum* (CS), *Eclipta prostrata* (EP), and *Rumex acetosella* (RA); six representative monocotyledon weeds, *Digitaria sanguinalis* (DS), *Polypogon fugax* (PF), *Echinochloa crusgalli* (EC), *Poa annua* (PA), *Setaria faberii* (SF), and *Alopecurus aequalis* (AA) were tested according to the method as in previously published papers.[26,33,34] To evaluate pre-emergence herbicidal activity of compound **11q** in this present study, we selected four

141 broadleaf weeds, *Amaranthus retroflexus* (AR), *Medicago sativa* (MS), *Eclipta prostrata* (EP),
142 *Ipomoea nil* (IN), and two monocotyledon weeds, *Chloris virgata* (CV)*, Setaria italic* (SI). One
143 of the most widely used PPO herbicide, flumioxazin was used as a control. Before testing,
144 compounds were dissolved in DMF and formulated with Tween-80 as an emulsification reagent
145 to a concentration of 100 g/L. Clay soil, pH 6.5, CEC 12.1 mol/kg, 37.3% clay particles, and
146 1.6% organic matter were used in the experiment. The active ingredient (g ai/ha) was calculated
147 by the total amount of active ingredients in the formulation divided by the surface area of the
148 pot. In the experiment, plastic pots with a diameter of 9 centimeters were filled with clay soil to
149 a depth of 8 centimeters. About 20 of the tested weed seeds were sown in the pots at the depth
150 of 1 to 3 centimeters and grown at 15-30 °C in the greenhouse. During the experiment, the air
151 humidity was kept at about 50%. The diluted formulation solutions were applied for both pre-
152 and post-emergence application. For post-emergence herbicidal activity evaluation,
153 monocotyledon weeds were tested at the one-leaf stage, and broadleaf weeds were tested at the
154 two-leaf stage. Untreated seedlings used the solvent (DMF+Tween-80) as the solvent control
155 group. For pre-emergence herbicidal activity evaluation, after sowing the seeds in the plastic
156 pots, they were directly treated with tested compounds at the rate of 37.5, 75 and 150 g ai/ha,
157 with DMF+Tween-80-treated groups as solvent control. At 30 d post-treatment, the results of
158 herbicidal activity were evaluated visually (Tables 1 and 3), with three replications per
159 treatment.

160 **Crop safety**. To evaluate crop selectivity of the synthesized compounds, six representative
161 crops were tested in the greenhouse experiments: soybean, peanut, cotton, maize, wheat, and
162 rice. The crops were planted in flowerpots (12 cm diameter) and grown at room temperature in

the test soil. Post-emergence crop safety experiments were conducted at the rate of 75 and 150 g ai/ha when the crops had reached the four-leaf stage. Pre-emergence crop experiments were also conducted at 75 and 150 g ai/ha after the crops seeds were sown in the flowerpots. The crop selectivity of tested compounds were evaluated after 30 days of treatment by inhibitors, the data are shown in Table 4, with three replications per experiment.

RESULTS AND DISCUSSION

Synthesis. As shown in Figure 3, all the pyrido[2,3-*d*]pyrimidine-2,4-dione−benzoxazinone hybrids, **9-13**, can be obtained in linear multistep reactions. The key intermediates 6-amino-7-halogen-4-(4-methoxybenzyl)-2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one, **7**, (halogen = F, Cl, or Br) was smoothly synthesized in five steps by using 5-halogen-2-nitrophenol **1** as the starting materials. After reacting with ethyl bromoacetate in DMF as solvent and K_2CO_3 as base, ethyl 2-(5-halogen-2-nitrophenoxy)acetate **3** were obtained in excellent yields (92-95%). Compounds **4** were synthesized in 81-86% yields, by using **3** and iron powder in acetic acid under the reflux. Initially, we tried to do nitration of **4** in 98% H_2SO_4, but unfortunately, some byproducts formed immediately while adding nitric acid to the reaction mixture, even though by the temperature was kept as low as -15 °C. The synthesis of compounds **5** were finally accomplished by using 80% H_2SO_4 as solvent. Then **5** was reacted with protecting group 4-chloromethylanisole in DMF with Cs_2CO_3 as base, 7-halogen-4-(4-methoxybenzyl)-6-nitro-2*H*-benzo[1,4]oxazin-3(4*H*)-one, **6**, were obtained in 78-84% yields. The intermediates **7** were synthesized by using iron powder and NH_4Cl as reducing reagents in 90% ethanol under reflux.

After three consecutive steps of reactions, 3-(7-halogen-4-(4-methoxybenzyl)-3-oxo-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-6-yl)pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione, **8**, were prepared in good yields.[26] Subsequently, compounds **8** reacted with methyl iodide in DMF with Cs_2CO_3 as base to give hybrids **9** in the yields of 87-92%. The 4-methoxybenzyl group of **9** can be easily removed by using CF_3SO_3H and CF_3CO_2H in dichloromethane to give compounds **10**. Finally, **10** reacted with various nucleophilic reagents R^1I or R^1Br; compounds **11-13** were prepared in satisfactory yields.

The structure of all the pyrido[2,3-*d*]pyrimidine-2,4-dione−benzoxazinone hybrids **9-13** were characterized by ^1H and ^{13}C NMR, and HRMS spectrometric data.

PPO inhibition and SAR. As we envisaged, pyrido[2,3-*d*]pyrimidine-2,4-dione−benzoxazinone hybrids might be a promising scaffold for potent PPO inhibitors. In our previous work, we have found that the optimum substituent at the *N*-1 position of quinazoline-2,4-dione was a $–CH_3$ group.[26, 28] In this work we also placed the $–CH_3$ group at the *N*-1 position of pyrido[2,3-*d*]pyrimidine-2,4-dione. Before structural optimization, we investigated the binding modes of a lead compound **10a** with *mt*PPO. As shown in Figure 2, the pyrido[2,3-*d*]pyrimidine-2,4-dione moiety of **10a** can form a π-π stacking interaction with the Phe392 residue. The distance between benzene ring of Phe392 and pyrido[2,3-*d*]pyrimidine-2,4-dione ring is 3.5 Å, indicating the strong interactions of two aromatic rings. The benzoxazinone ring is sandwiched between aliphatic Leu372 and Leu356. Furthermore, the carbonyl group of benzoxazinone can form a 2.6 Å, strong hydrogen bond with the Arg98. On the basis of the above analysis, we decided to synthesize **10a** (Figure 3) and

evaluate its *mt*PPO-inhibiting activity. Though the K_i value of **10a** (K_i = 0.26 μM) was not as potent as flumioxazin (K_i = 0.046 μM) (Table 1), the promising results also suggested that its worth further optimization. Because the *NH* group of **10a** faced to the hydrophobic residues of the open binding pocket, therefore, introducing some nonpolar groups at this position might improve the inhibitory potency of this series of compounds.[35] Bearing this in mind, compounds **11** with different hydrophobic groups at the *N*-1 position of benzoxazinone were synthesized.

As shown in Table 1, introducing a $-CH_3$ group at *N*-1 position of benzoxazinone, compound **11a** (K_i = 0.048 μM) showed five times more potency than the parent lead compound **10a**, nearly comparative to that of flumioxazin. When introducing $-CH_2CH_3$, **11b**, and $-CH_2CH_2CH_3$, **11c**, groups at this position, the *mt*PPO-inhibiting activity of these compounds were further improved. However, when introducing $-CH_2CH_2CH_2CH_3$, **11d**, and $-CH_2CH(CH_3)_2$, **11e**, groups, compared with **11b** the activities did not improve much. Based on the above results, the second round optimization was mainly based on the ethyl group of **11b** and the *n*-propyl group of **11c**. When the terminal hydrogen atom of $-CH_2CH_3$, **11b**, or $-CH_2CH_2CH_3$, **11c**, was replaced by other hydrophobic groups, it was found that electron-withdrawing groups were detrimental to activity (**11b** >**11f**, **11c** > **11j**), and substituting electron-donating groups was favorable for activity (**11i** > **11b**). Furthermore, the fluoro-substituted analogues displayed higher activity than chloro-substituted analogues (**11f** >**11h**, **11g** >**11k**), and the two fluorine atom substituents in **11g** showed better activity than that of singly-substituted **11f**. As for allyl-substituted compounds (**11l-11o**), substituting two terminal hydrogen atoms of the allyl group with two methyl groups was found to be detrimental towards activity, while changing the hydrogen atom on the 2-position of the allyl group to a –

229 CH_3 group led to a 5-fold activity decrease. If the allyl group was changed to a $–CH_2CN$ group,
230 the resulting compound **11p** underwent a more than a 31-fold activity decrease compared with
231 that of **11I**. The possible reason for this is that, $–CH_2CN$ is a hydrophilic group, which causes
232 the repulsion among the hydrophobic residues of *mt*PPO and **11p**.

233 Introducing a propargyl group on the *N*-1 position significantly increased the *mt*PPO
234 inhibitory activity. For example, the activity of compound **11q** (K_i = 0.0074 μM) was 35 times
235 more potent than lead compound **10a,** and 6-fold more potent than flumioxazin. It was also
236 detrimental to activity when the terminal hydrogen atom of the propargyl group was changed to
237 other groups (**11q**>**11r** and **11s**). In addition, if a bigger hydrophobic group such as
238 $-CH_2C_6H_4(4\text{-}OCH_3)$ was introduced at R^1, compound **9a** also displayed good *mt*PPO inhibitory
239 activity. As we have observed previously,[8-10] the fluoro-substituted compounds **11** always
240 demonstrated higher activity than the chloro-substituted **12** and bromo-substituted **13**, while the
241 R^1 groups stayed the same. Furthermore, the SAR observed in the fluoro-substituted
242 compounds **11** can also apply to chloro-substituted **12** and bromo-substituted **13**.
243
244 **CoMFA analysis**. Quantitative structure-activity relationship (QSAR) is an important
245 descriptor widely utilized in understanding chemical-biological interactions in herbicide
246 research.[36] To understand the relationship between chemical structures and *mt*PPO inhibition
247 activities, we performed CoMFA analysis of the synthesized compounds **9-13**. 28 compounds
248 were selected as the training set (Figure 4A), the experimental and calculated *mt*PPO-inhibiting
249 activities are shown in Table 2. The conventional coefficient r^2 of the CoMFA model is 0.949,
250 the cross-validated q^2 value is 0.632, and the predicted non-cross-validated r^2 value is 0.814.

The steric isocontour diagram of CoMFA model is shown in Figure 4B, the yellow regions indicate that a bulk group would be detrimental to the *mt*PPO inhibition activity. For example, the fluoro-containing compound **11q** (X = F, R^1 = -$CH_2C{\equiv}CH$, K_i = 0.0074 μM) exhibited higher *mt*PPO inhibition activity than the chloro-substituted **12f** (X = Cl, R^1 = -$CH_2C{\equiv}CH$, K_i = 0.085 μM) and bromo-substituted **13f** (X = Br, R^1 = -$CH_2C{\equiv}CH$, K_i = 0.099 μM). In contrast, placing a bulk group at the green area was advantageous to activity. For the electrostatic contour map (Figure 4C), the red polyhedra means that, increasing electron-negative groups at this site is favorable, whereas substituting electron-positive groups on the blue contour region will increase activity. As shown in Figure 4C, the red contour mainly locates on the N-1 position of benzoxazinone ring, which is consistent with our experimental results, in which **11q** (R^1 = -$CH_2C{\equiv}CH$, K_i = 0.0074 μM) displayed higher *mt*PPO-inhibiting activity than **11l** (R^1 = -$CH_2CH{=}CH_2$, K_i = 0.016 μM) and **10a** (R^1 = H, K_i = 0.26 μM). The blue contour is located around the terminal alkyl groups, which supports our experimental data, in which placing electron-withdrawing groups on the terminal hydrogen atom of -CH_2CH_3, **11b**, or -$CH_2CH_2CH_3$, **11c**, was detrimental to activity.

Herbicidal activity and SAR. To investigate the herbicidal activity of the synthesized compounds **9-13**, six broadleaf weeds (*E. prostrata*, *R. acetosella, A. retroflexus*, *C. serotinum*, *A. juncea*, *B. juncea*,) and six monocotyledon weeds (*D. sanguinalis*, *P. fugax*, *E.crusgalli*, *P. annua*, *S. faberii*, *A. aequalis*) were selected as representatives for the postemergence herbicidal activity evaluation. The commercial PPO herbicide flumioxazin was selected as a control. In most cases, the herbicidal activities of these compounds were consistent with their *in vitro*

273 *mt*PPO-inhibiting activities (Table 1). For example, when R^1 groups remained the same, the
274 fluoro-substituted compounds always exhibited higher herbicidal activity than the chloro- and
275 bromo-substituted analogues. After treatment with the tested compounds, most of the sensitive
276 weeds showed quick burn symptoms within one or two days, followed by bleaching and death,
277 indicating these compounds are PPO inhibitors. As with most of the PPO herbicides, this series
278 of compounds also displayed strong inhibition against tested broadleaf weeds.[37] As shown in
279 Table 1, most of the fluoro-containing derivatives **11** exhibited over 80% strong inhibition
280 against six test broadleaf weeds. Even at a rate as low as 37.5 g ai/ha, compound **11q** still
281 displayed more than 80% inhibition against the test weeds, which were comparable to that of
282 flumioxazin.

283 As to the fluoro-substituted compounds **9a**, **10a** and **11**, modifying the substituent on the
284 *N*-1 position of benzoxazinone ring had a big impact on the herbicidal activity. Compound **10a**
285 with a hydrogen atom at this position, showed almost no herbicidal activity. However, when
286 changed to a methyl group, **11a** displayed total inhibition against *A. retroflexus*. There were
287 also some variations in herbicidal activity when different hydrophobic alkyl, allyl and
288 propargyl groups were introduced. Substituting an ethyl group in this position can further
289 improve the herbicidal potency; **11b** displayed 100% inhibition against *E. prostrata, A. juncea,*
290 and *A. retroflexus* at 150 g ai/ha, whereas a $-CH_2CH_2CH_3$ group in this position produces a
291 broader spectrum of weed control (**11c** > **11b**). Prolonging the carbon chain of **11c** was found
292 detrimental to herbicidal activity (**11c** > **11d**). For the alkyl halide-substituted analogues,
293 compounds with a fluorine atom on the carbon terminal displayed more improved herbicidal
294 activity than those with a chlorine atom (**11f** > **11h**, **11j** > **11k**). It seemed that, placing an

electron-donating group on the 2-postion of allyl group was favorable for potency (**11n** > **11l** > **11m**). Furthermore, placing a substituent on the 1-position of the propargyl group was found detrimental to herbicidal activity (**11q** > **11s** >**11r**).

It is known that most of the PPO herbicides demonstrate good herbicidal activity when applied both post-emergence and pre-emergence. Compound **11q** had an excellent broad spectrum of post-emergence herbicidal activity, and was selected for further pre-emergence herbicidal activity evaluation. Six representative weeds were tested: *A. retroflexus*, *M. sativa, E. prostrata, I. nil*, *C. Virgata* and *S. italic*, the results are shown in Table 3. At the rate of 150 g ai/ha, **11q** displayed 100% inhibition against *A. retroflexus, I. nil*, *C. virgata* and *S. italic* and over 90% inhibition against *M. sativa* and *E. prostrata*, which is as potent as flumioxazin. Even at a dosage as low as 37.5 g ai/ha, **11q** still exhibited strong inhibition against six of the seven test weeds (> 80% inhibition).

Crop selectivity of 11q. During the herbicide discovery process, crop safety is one of the issues. To evaluate whether **11q** is safe to a target crop or not, we tested its post- and pre-emergence crop selectivity against six representative crops: maize, wheat, rice, soybean, cotton and peanut. As shown in Table 4, both **11q** and flumioxazin were non-selective (inhibition >10%) for six crops at 75 and 150 g of active ingredient (ai)/ha in post-emergence application. The possible reason for the limited post-emergence crop safety of **11q** is that, when **11q** was absorbed by crops, it inhibited the PPO activity in crops and generated a lot of reactive oxygen species in a relatively short time, causing rapid disruption of the membrane. On the basis of the above analysis, we evaluated the pre-emergence crop selectivity of **11q**. Maize,

317 soybean, peanut and cotton displayed high tolerance to **11q** at 150 g ai/ha in pre-emergence
318 application. Furthermore, rice and wheat exhibited good tolerance to **11q** at 75 g ai/ha; however,
319 flumioxazin was non-selective to rice and wheat at the same rate. These promising results
320 suggest that **11q** has potential for development as a pre-emergence herbicide for weed control
321 in maize, soybean, peanut and cotton fields.

322 In summary, in order to obtain new compounds that can form more favorable π-π
323 interactions with Phe392 of *mt*PPO, 37 new pyrido[2,3-*d*]pyrimidine-2,4-dione−benzoxazinone
324 hybrids, **9**-**13**, were designed and synthesized. Several compounds with improved
325 *mt*PPO-inhibiting and good herbicidal activities were found. Among them, the most potent
326 compound 3-(7-fluoro-3-oxo-4-(prop-2-yn-1-yl)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-6-yl)-
327 1-methylpyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione, **11q**, with a K_i value of 0.0074 μM, which
328 demonstrated six-fold more activity than flumioxazin (K_i = 0.046 μM) against *mt*PPO.
329 Compound **11q** displayed a strong and broad spectrum of weed control at 37.5 g ai/ha by both
330 post- and pre-emergence application, which was comparable to that of flumioxazin.
331 Furthermore, **11q** was safe to maize, soybean, peanut and cotton in pre-emergence application
332 at 150 g ai/ha, and selective to rice and wheat at 75 g ai/ha, indicating great applicability in
333 these fields. Our work points out that pyrido[2,3-*d*]pyrimidine-2,4-dione−benzoxazinones may
334 be worth further optimization to attain more applicability, while the structural modifications
335 and field trials of **11q** may be efficient for the development of a new platform for research in
336 the field of herbicides

337

338 **Supporting Information**

Supporting Information includes the detailed synthetic routes, physical and spectrum data (NMR, HRMS) of compounds **3-13**, and the pre-emergence herbicidal activity of compounds **9-13.** These materials are available free of charge via internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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Figure captions:

Figure 1. Structures of Saflufenacil, commercial benzoxazinone-containing PPO herbicides and the synthesized hybrid compounds **10a** and **11**.

Figure 2. Simulated binding mode of lead pyrido[2,3-d]pyrimidine-2,4-dione−benzoxazinone hybrid compound **10a** with *mt***PPO** and the designed compounds **11-13**. **10a** is shown with magenta bonds, and the key residues around it are shown with green bonds.

Figure 3. Synthesis of compounds **11-13**. Reagents and conditions: (a) K_2CO_3, DMF, 50 °C; (b) Fe, acetic acid, reflux; (c) HNO_3, H_2SO_4, 0-room temp.; (d) 4-chloromethylanisole, Cs_2CO_3, DMF, room temp.; (e) Fe, NH_4Cl, C_2H_5OH, reflux; (f) $CO(COCl_3)_2$, Et_3N, 1,4-dioxane, 0 °C to room temp.; (g) methyl 2-aminonicotinate, toluene, reflux; (h) $NaOCH_3$, CH_3OH, room temp.; (i) CH_3I, Cs_2CO_3, DMF, room temp.; (j) CF_3SO_3H, CF_2CO_2H, dichloromethane, room temp.; (k) R^1I or R^1Br, Cs_2CO_3, DMF, room temp..

Figure 4. (A) Alignment of 28 training set molecules. (B) Steric contour map of CoMFA model, **11q** is shown with grey bonds. The yellow contour indicates that, placing a steric bulky group at this site is detrimental to activity, whereas the green contour represents that, increasing the size of a substituent is favorable to activity. (C) Electrostatic contour map of CoMFA model, **11q** is shown in grey stick. The red polyhedra means that, increasing electron-negative charge at this site is favorable; on the contrary, substituting electron-positive charge on the blue contour region will increase activity.

Table 1. Chemical Structure, Post-emergence Herbicidal Activity and *mt*PPO Inhibitory Activities of Compounds **9-13**.

Cmpds	X	R^1	dose (g ai/ha)	AJ/BJ[a]	AR/CS	EP/RA	DS/PF	EC/PA	SF/AA	K_i (μM)[b]
10a	F	H	150	–[c]	–	–	–	–	–	0.26
11a	F	CH_3	150	–	++++	++	–	–	–	0.048
11b	F	CH_2CH_3	150	++++	++++	++++	–	–	–	0.03
			75	+	++++	+	–	–	–	
			37.5	–	++++	–	–	–	–	
11c	F	$CH_2CH_2CH_3$	150	++++	++++	+++	+++	++	++++	0.016
			75	–	++++	+	+	–	+	
			37.5	–	++++	–	–	–	+	
11d	F	$CH_2CH_2CH_2CH_3$	150	++++	++	++	–	–	–	0.022
11e	F	$CH_2CH(CH_3)_2$	150	++++	+++	–	–	–	–	0.051
11f	F	CH_2CH_2F	150	++++	++++	++++	–	–	–	0.038
			75	+++	++++	+++	–	–	–	
			37.5	–	++++	++	–	–	–	
11g	F	CH_2CHF_2	150	++++	++++	+++	–	–	–	0.010
			75	++	++++	+	–	–	–	
			37.5	–	++++	+	–	–	–	
11h	F	CH_2CH_2Cl	150	++++	++++	+++	–	–	–	0.051
			75	+++	++++	+	–	–	–	
			37.5	+	+++	–	–	–	–	
11i	F	$CH_2CH_2OCH_3$	150	++++	++++	++++	–	–	–	0.018
			75	+++	++++	+++	–	–	–	
			37.5	–	++++	+	–	–	–	
11j	F	$CH_2CH_2CH_2F$	150	++++	++++	+++	–	–	–	0.032
			75	+++	+++	+	–	–	–	
			37.5	++	+++	–	–	–	–	
11k	F	$CH_2CH_2CH_2Cl$	150	++++	++++	+++	–	–	–	0.040
			75	+++	++++	+	–	–	–	
			37.5	+	+++	+	–	–	–	
11l	F	$CH_2CH=CH_2$	150	++++	++++	+++	–	–	–	0.016
			75	++	++++	+	–	–	–	
			37.5	+	++++	–	–	–	–	
11m	F	$CH_2CBr=CH_2$	150	++++	–	–	–	–	–	0.014
11n	F	$CH_2C(CH_3)=CH_2$	150	++++	++++	+++	–	–	–	0.084
			75	+++	++++	+	–	–	–	
			37.5	++	++++	–	–	–	–	
11o	F	$CH_2CH=C(CH_3)_2$	150	++++	++++	–	–	–	–	0.027
11p	F	CH_2CN	150	++++	++++	+++	–	–	–	0.50
			75	++	++++	+	–	–	–	
			37.5	+	+++	–	–	–	–	
11q	F	$CH_2C\equiv CH$	150	++++	++++	++++	++++	++++	++++	0.0074

			75	++++	++++	++++	++++	++++	+++	
			37.5	++++	++++	++++	++++	+++	+++	
11r	F	$CH_2C{\equiv}CCH_3$	150	+	++	−	−	−	−	0.027
11s	F	$CH_2C{\equiv}CSi(CH_3)_3$	150	++++	++++	++++	−	−	−	0.053
			75	+++	++++	+++	−	−	−	
			37.5	+	++++	++	−	−	−	
9a	F	$CH_2C_6H_4(4\text{-}OCH_3)$	150	−	−	−	−	−	−	0.028
10b	Cl	H	150	−	−	−	−	−	−	0.74
12a	Cl	CH_3	150	++++	++++	+	−	−	−	0.32
12b	Cl	CH_2CH_3	150	−	−	+++	−	++	−	0.34
12c	Cl	$CH_2CH_2CH_3$	150	++++	++++	−	−	−	−	0.26
12d	Cl	$CH_2CH_2CH_2CH_3$	150	−	−	−	−	−	−	0.12
12e	Cl	$CH_2CH{=}CH_2$	150	−	+++	−	−	−	−	0.38
12f	Cl	$CH_2C{\equiv}CH$	150	−	−	+	−	−	−	0.085
9b	Cl	$CH_2C_6H_4(4\text{-}OCH_3)$	150	−	−	−	−	−	−	0.68
10c	Br	H	150	−	−	−	−	−	−	6.88
13a	Br	CH_3	150	−	−	−	−	−	−	0.75
13b	Br	CH_2CH_3	150	−	−	−	−	−	−	0.17
13c	Br	$CH_2CH_2CH_3$	150	−	−	−	−	−	−	0.6
13d	Br	$CH_2CH_2CH_2CH_3$	150	−	−	−	−	−	−	0.44
13e	Br	$CH_2CH{=}CH_2$	150	−	−	−	−	−	−	1.45
13f	Br	$CH_2C{\equiv}CH$	150	−	−	−	−	−	−	0.099
9c	Br	$CH_2C_6H_4(4\text{-}OCH_3)$	150	−	−	−	−	−	−	23.39
flumioxazin			150	++++	++++	++++	++++	++++	++++	0.046
			75	++++	++++	++++	++++	++++	+++	
			37.5	++++	++++	++++	++++	++++	+	

[a]Abbreviations: AJ: *Abutilon juncea*; BJ: *Brassica juncea*; AR: *Amaranthus retroflexus*; CS: *Chenopodium serotinum*; EP: *Eclipta prostrata*; RA: *Rumex acetosella*; DS: *Digitaria sanguinalis*; PF: *Polypogon fugax*; EC: *Echinochloa crusgalli*; PA: *Poa annua*; SF: *Setaria faberii*; AA: *Alopecurus aequalis*. [b]Inhibition constant of the enzymatic reaction. [c]Rating scale of herbicidal activity (percentage of inhibition): ++++, ≥90%; +++, 80−89%; ++, 60−79%; +, 50−59%; −, <50%.

Table 2. Experimental and Calculated pK_i Values of Compounds **9-13**.

	pK_i[a]			pK_i[a]	
Cmpds	exptl	calcd	Cmpds	exptl	calcd
10a	6.5850	6.6098	**11s**	7.2757	7.2980
11a	7.3188	7.2697	**9a**	7.5528	7.5869
11b	7.5229	7.7300	**10b**	6.1308	5.8226
11c	7.7959	7.7518	**12a**	6.4949	6.4890
11d	7.6576	7.6142	**12b**	6.4685	6.9622
11e	7.2924	7.5178	**12c**	6.5850	6.8230
11f	7.4202	7.3378	**12d**	6.9208	6.8579
11g	8.0000	8.0433	**12e**	6.4202	6.6137
11h	7.2924	7.3666	**12f**	7.0706	7.0744
11i	7.7447	7.7318	**9b**	6.1675	6.0439
11j	7.4949	7.3478	**10c**	5.1624	5.1598
11k	7.3979	7.2704	**13a**	6.1249	5.8233
11l	7.7959	7.3430	**13b**	6.7696	6.3294
11m	7.8539	7.4127	**13c**	6.2218	6.3449
11n	7.0757	7.0382	**13d**	6.3565	6.2214
11o	7.5686	7.3788	**13e**	5.8386	5.9372
11p	6.301	6.5782	**13f**	7.0044	6.4379
11q	8.1308	7.8344	**9c**	4.6310	4.7280
11r	7.5686	7.6708			

[a] pK_i = -logK_i.

Table 3. Pre-emergence Herbicidal Activity Compound **11q** and Flumioxazin.

Cmpds	dose (g ai/ha)	AR[a]	MS	EP	IN	CV	SI
11q	150	++++[b]	++++	++++	++++	++++	++++
	75	++++	++++	+++	++++	++++	++++
	37.5	+++	+++	+	+++	++++	+++
flumioxazin	150	++++	++++	++++	++++	++++	++++
	75	++++	++++	++++	++++	++++	++++
	37.5	++++	++++	+++	++++	++++	++++

[a]Abbreviations: AR: *Amaranthus retroflexus*; MS: *Medicago sativa*; EP: *Eclipta prostrata*; IN: *Ipomoea nil*; CV: *Chloris virgata*; SI: *Setaria italic*. [b]Rating scale of herbicidal activity (percentage of inhibition): ++++, ≥90%; +++, 80−89%; ++, 60−79%; +, 50−59%; −, <50%.

Table 4. Post- and Pre-emergence Crop Selectivity of Compound **11q** and Flumioxazin.

Cmpds	Dose	post-emergence						pre-emergence					
	(g ai/ha)	maize	wheat	rice	soybean	cotton	peanut	maize	wheat	rice	soybean	cotton	peanut
11q	150	30	40	40	80	80	70	0	20	30	5	5	0
	75	20	30	30	70	60	50	0	10	10	0	0	0
flumioxazin	150	50	80	75	100	100	85	0	30	70	0	5	0
	75	30	70	60	100	100	80	0	20	60	0	0	0

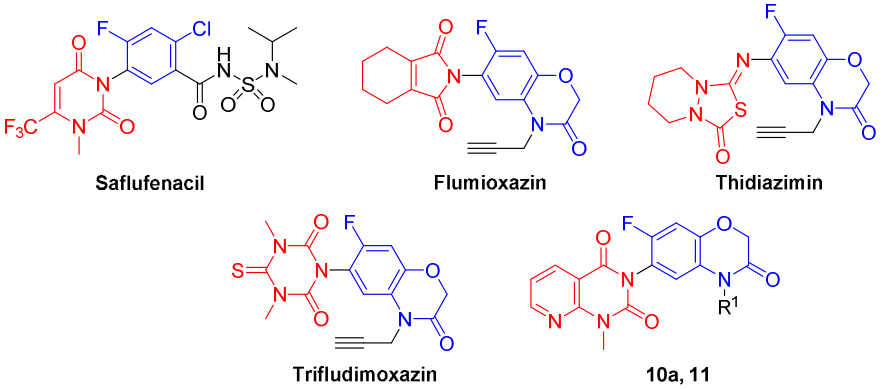
Saflufenacil
Flumioxazin
Thidiazimin
Trifludimoxazin
10a, 11

Figure 1.

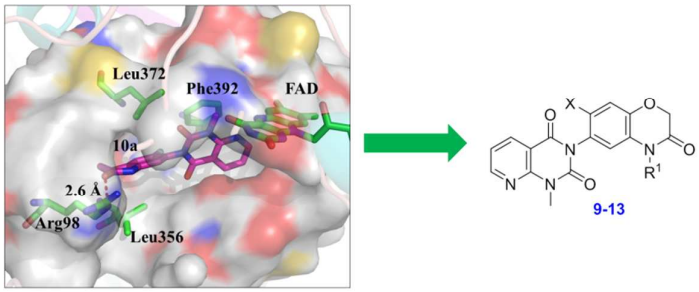

Figure 2.

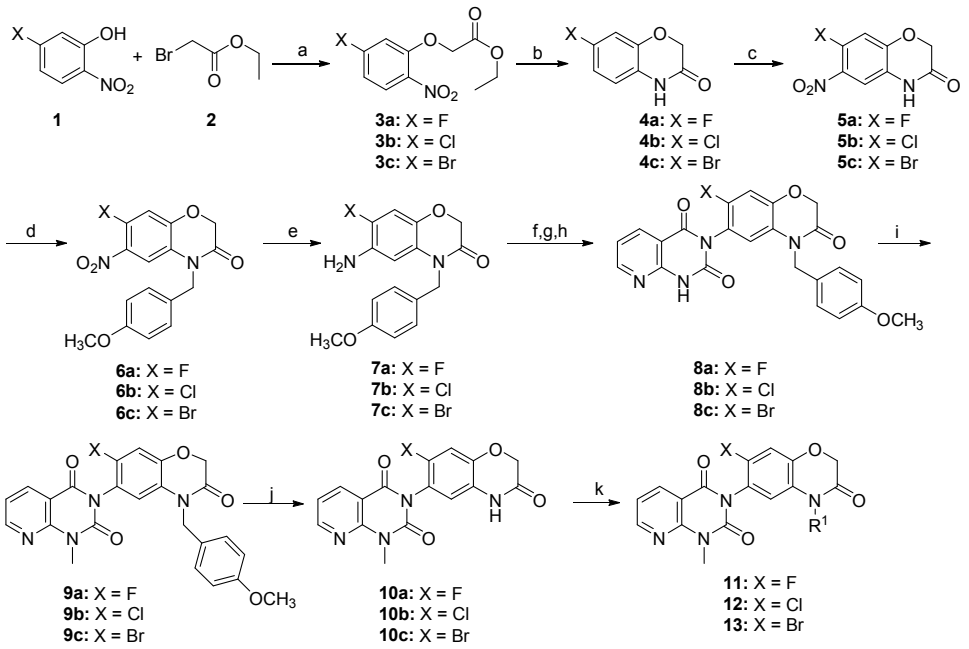
1
2
3a: X = F
3b: X = Cl
3c: X = Br
4a: X = F
4b: X = Cl
4c: X = Br
5a: X = F
5b: X = Cl
5c: X = Br
6a: X = F
6b: X = Cl
6c: X = Br
7a: X = F
7b: X = Cl
7c: X = Br
8a: X = F
8b: X = Cl
8c: X = Br
9a: X = F
9b: X = Cl
9c: X = Br
10a: X = F
10b: X = Cl
10c: X = Br
11: X = F
12: X = Cl
13: X = Br

Figure 3.

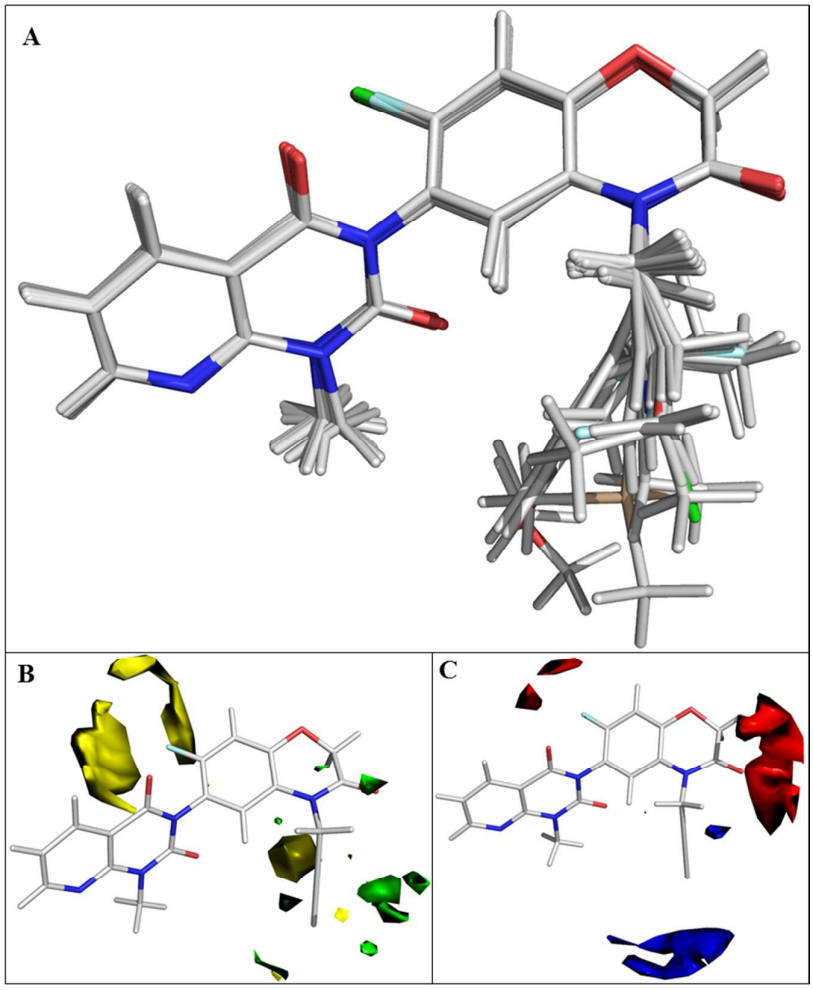

Figure 4.

Graphic for table of contents