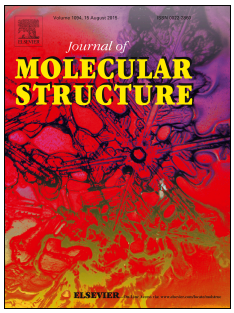

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CRediT author statement

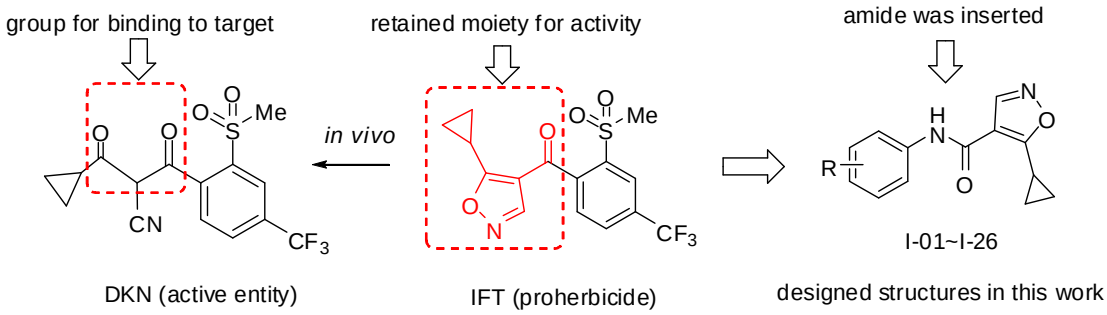
Xinli Sun: Methodology, Software

Zhenmeng Ji: Visualization, Investigation.

Shaopeng Wei: Data curation, Writing- Original draft preparation.

Zhiqin Ji: Conceptualization, Validation, Writing- Reviewing and Editing.

Graphic Abstract



Design, Synthesis and Herbicidal Activity of 5-cyclopropyl-*N*-phenylisoxazole-4-carboxamides

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Abstract: 4-Hydroxyphenylpyruvate dioxygenase (HPPD) inhibitors are a type of important herbicides, and they cause bleaching symptoms by indirectly inhibiting the biosynthesis of carotenoids. In this study, thirty isoxazolamide compounds were designed based on the structure of Isoxaflutole, a commercial HPPD herbicide. Starting from 1,1-dimethoxy-*N*,*N*-dimethyl-methanamine and methyl 3-cyclopropyl-3-oxo-propanoate, the title compounds were readily prepared and their structures were determined by MS and NMR analysis. In Petri dish tests, most of the title compounds showed strong inhibitory effect on the root and stem growth of both monocotyledon and dicotyledon weeds, and it was clearly different from the symptoms caused by HPPD inhibitors. However, several of them, especially **I-17,** showed characteristic bleaching symptoms of HPPD herbicides and good post-emergence herbicidal activity on tested weeds in glasshouse assay. These compounds are prodrugs, and compounds undergo conversion to the active entity diketonitrile (DKN) in plant and soil. The result of molecular docking analysis revealed that the DKN moiety of **I-17** excellently binds to the active sites of HPPD. The 1,3-diketone can form bidentate interaction with Fe^{II}, and the benzene ring can form π-π interaction with Phe 360 and Phe 403. These results indicated that the title compounds bears other herbicidal mechanism except for HPPD inhibitor. Therefore, a lead compound for the discovery of novel multi-target herbicides is provided.

KEYWORDS: *4-hydroxyphenylpyruvate dioxygenase; herbicidal activity; isoxazole; phenylamine derivatives*

1. Introduction

With the widely use of agro-chemicals, weeds resistance to commercial herbicides has become a major concern to crop production worldwide[1]. Although numerous studies have demonstrated that rational application of herbicide groups is helpful for delaying the evolution of herbicide-resistant weeds, the development of herbicides with new mode of action is the eventual solution to address the problem[2, 3]. 4-Hydroxyphenylpyruvate dioxygenase (HPPD) is a relative new target for herbicides discovered in 1990s[4]. In plants, HPPD catalyzes the biotransformation of 4-hydroxyphenylpyruvic acid (HPPA) to homogentisic acid (HGA), which is an intermediate in the biosynthesis of plastidquinone[5]. Plastoquinone is a co-factor of phytoene desaturase, and the inhibition of HPPD finally results in a depletion of carotenoids and an absence of chloroplast development in emerging foliar tissues, which is followed by necrosis and death[6]. Up to now, more than a dozen of HPPD inhibitors such as Sulcotrione, Mesotrione, Topramezone, Pyrazolynate and others have been used in the management of weeds[7-12]. HPPD herbicides exhibit high herbicidal activity against a variety of broadleaf and grass weeds both in pre- and post-emergence treatments, and have low mammalian toxicity. More important, only few weed species are resistant to HPPD herbicides[13, 14]. These good features attract more attention from pesticide industry. Isoxaflutole (IFT) is a HPPD herbicide developed by Rhône-Poulenc Agriculture Limited, and its herbicidal mechanism, root uptake and translocation, as well as metabolism in soil and plants, have been well clarified in previous study[15]. IFT itself is a prodrug, and IFT undergoes conversion to the active entity diketonitrile (DKN) in plant and soil. Although IFT is a highly effective herbicide, its complex structure results in a longer synthesis route and high cost for production[16, 17]. Furthermore, the weed spectrum and crop selectivity of IFT are also not perfect enough[18]. In view of its promising activity, screening novel herbicidal

compounds by the modification on the structure of IFT is an interesting program. We firstly analyzed the interaction between the active DKN and its target. HPPD is a non-haem Fe^{II}-containing dioxyganase, the chelating 1,3-diketone moiety of the DKN is responsible for the binding to active site[19]. The ortho-Me-SO_2 substituted at phenyl ring provides additionally support for the interaction. In our strategy, the carbonyl between phenyl ring and isoxazole is replaced with amide while retaining the crucial group for the binding to target (**Figure 1**). The reason for this is because various structural types of amides possess good herbicidal activity in previous studies [20]. We hope that this change can provide herbicidal candidates with good activity and low cost.

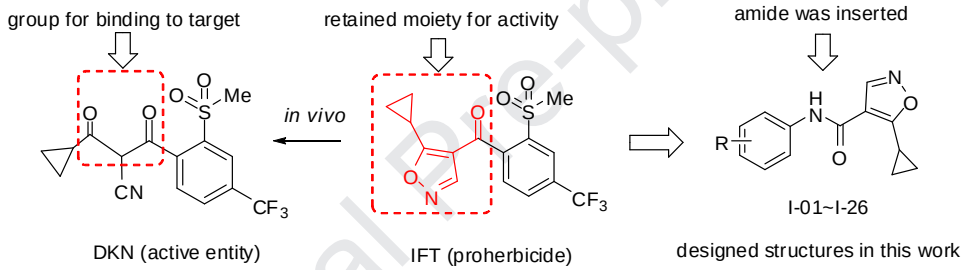


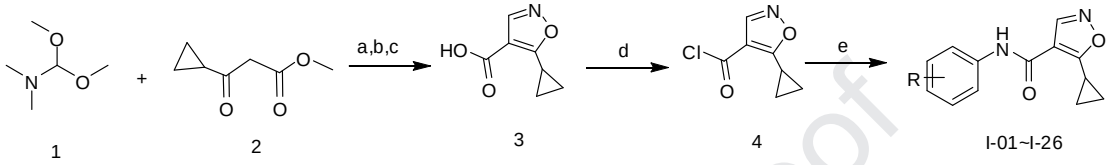
Figure. 1. Design strategy for the title compounds

2. Result and discussion

2.1. Synthesis

The synthetic routes for **I-01~I-26** are illustrated in **Scheme 1**. The key intermediate, 5-cyclopropylisoxazole-4-carboxylic acid **(3),** is prepared from 1,1-dimethoxy-*N*,*N*-dimethyl-methanamine (**1**) and methyl 3-cyclopropyl-3-oxo-propanoate (**2**) according to the procedure disclosed in the patent[21]. The yield of final product was strongly affected by the reaction temperature in the last step. We found that the optimum temperature for the reaction was 100 ^{o}C, and the yield of final product was above 80%. For the preparation of 5-cyclopropylisoxazole-4-carbonyl chloride, we examined the effect of temperature on the yield, and found that the yield of acyl chloride was close to 100% at room

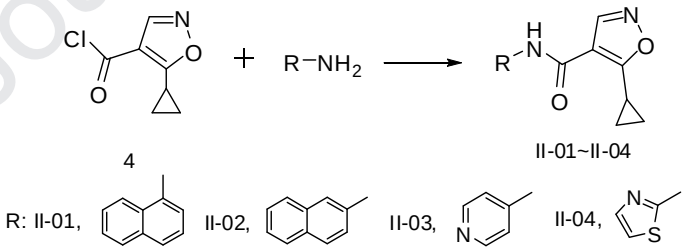
temperature. The preparation of **I-01**~**I-26** was carried out under ice bath condition. Because the ring of isoxazole is readily opened in strong alkaline conditions, the yield of final products was strongly affected by the types of alkalis and adding order of reactants used in the acylation reaction. After examining the effect of different conditions on the yield, we found that adding acid chloride and pyridine simultaneously to the solution of phenylamines was helpful for the stability of isoxazole.



Reagents and conditions: (a) 60 ^{o}C, 20h; (b) H_2NOH-HCl, H_2O, MeOH, 90 min, 60 °C;(c) concentrated HCl, AcOH, 4 h, 100 ^{o}C; (d) oxalyl chloride, CH_2Cl_2, 30min, room temperature; (e) substituted benzylamines, pyridine, CH_2Cl_2, 30min, 0 °C.

Scheme 1. Synthetic routes for I-01~I-26

Starting from naphthalen-1-amine, naphthalen-2-amine, pyridin-4-amine and thiazol-2-amine, other analogues, **II-01~II-04**, were prepared by the procedure described above (**Scheme 2**).



Scheme 2. Synthetic routes for II-01~II-04

2.2. Herbicidal activity

2.2.1. Petri dish tests and structure-activity relationship analyses

The herbicidal activities of **I-01~I-26** and **II-01~II-04** against monocotyledon weeds such as *Echinochloa crusgalli* (EC), *Digitaria sanguinalis* (DS), and dicotyledon weeds such as *Amaranthus*

retroflexus (AR), *Portulaca oleracea* (PO), *Abutilon theophrasti* (AT) and *Chenopodium album* (CA) were evaluated by Petri dish tests as described in literature[22]. The herbicidal activity of the title compounds at 100 mg/L and 10 mg/L against tested weeds are listed in **Tables 1** and **2**, respectively. As shown in **Table 1**, most of the title compounds exhibited strong inhibitory effect against the root and stem growth of the tested weeds. As for 5-cyclopropyl-*N*-phenylisoxazole-4-carboxamides, **I-16~I-24** showed good herbicidal activity against both monocotyledon and dicotyledon weeds, and several of them exhibited nearly 100% inhibition against tested weeds. **I-08~I-15** had stronger activity against dicotyledon weeds than monocotyledon weeds. **I-01~I-07** showed weak to moderate inhibitory effect against the tested weeds, and monocotyledon weeds were less sensitive to these compounds compared to dicotyledon weeds. **Table 2** reports the effects of the title compounds, at the dose of 10 mg/L, on the root and stem growth of 6 species of weeds. The data clearly showed that **I-22~I-24** had stronger inhibitory effect than other phenylamine derivatives. As for other four analogues, **II-04** had better herbicidal activity than **II-01~II-03**, and its inhibitory rate on the tested weeds are equivalent to those of **I-22~I-24**.

Table 1. Inhibitory effect of the title compounds on the growth of weeds in Petri dish tests (100 mg/L)

No.	R	inhibition rate (%)											
		EC[a]		DS[a]		AR[a]		PO[a]		AT[a]		CA[a]	
		root	stem	root	stem	root	stem	root	stem	root	stem	root	stem
I-01	H	40	30	30	30	60	50	80	70	70	60	60	70
I-02	2-CH_3	40	40	50	40	40	50	70	60	60	70	50	40
I-03	3-CH_3	30	40	30	20	50	60	60	70	80	70	60	50
I-04	4-CH_3	40	30	50	40	60	40	60	60	70	80	60	70
I-05	3-OCH_3	50	50	40	40	50	50	70	60	60	50	50	60
I-06	4-OCH_3	40	30	30	40	50	50	70	70	70	70	50	60
I-07	4-$C(CH_3)_3$	20	30	20	20	40	60	70	50	60	50	40	30
I-08	2-F	40	40	50	30	60	50	80	80	70	80	60	50
I-09	3-F	50	40	70	60	70	60	100	90	90	80	80	70
I-10	4-F	80	70	80	60	100	80	100	100	100	90	100	90
I-11	2-Cl	30	40	20	40	70	60	90	80	70	60	80	80

I-12	3-Cl	50	50	60	70	80	90	90	100	80	100	100	90
I-13	4-Cl	60	60	80	90	90	100	100	100	100	100	90	80
I-14	2-Br	40	50	60	50	60	70	80	70	70	80	70	70
I-15	3-Br	70	60	80	70	80	70	100	80	80	80	90	80
I-16	3-CF_3	90	100	100	100	90	100	90	100	90	80	80	70
I-17	4-CF_3	80	80	100	90	100	100	100	90	90	90	100	90
I-18	4-NO_2	70	60	80	100	80	100	80	90	100	100	90	80
I-19	2,4-diF	90	70	70	80	90	90	100	100	60	70	70	80
I-20	2,4-diCl	80	60	70	70	100	90	100	80	70	60	80	70
I-21	3,4-diCl	70	80	70	60	90	80	90	100	70	70	80	80
I-22	3-Cl-4-F	90	90	100	90	100	80	100	90	90	80	100	90
I-23	3-CF_3-4-F	100	80	100	90	100	80	90	90	100	100	100	90
I-24	3-CF_3-4-Cl	100	90	80	100	100	100	80	100	80	80	70	80
I-25	3-CF_3-4-Br	60	50	70	60	60	50	70	80	70	60	90	80
I-26	2-Br-4-CF_3	70	70	60	70	80	80	80	90	60	70	80	60
II-01	-	50	60	40	30	40	20	50	60	50	40	30	30
II-02	-	40	30	60	50	50	40	60	50	60	50	50	40
II-03	-	60	50	70	60	80	80	80	70	70	80	60	60
II-04	-	100	90	100	100	80	90	100	100	90	100	90	100
Isoxaflutole		30	20[b]	30	20[b]	20	30[b]	30	10[b]	30	20[b]	30	20[b]
Butachlor		100	90	90	100	80	80	80	70	80	80	70	80

[a]Abbreviations: EC for *Echinochloa crusgalli*; DS for *Digitaria sanguinalis*; AR for *Amaranthus retroflexus*; PO for *Portulaca oleracea* , AT for *Abutilon theophrasti* and CA for *Chenopodium album.* [b]Exhibit bleaching symptoms.

Table 2. Inhibitory effect of the title compounds on the growth of weeds in Petri dish tests (10 mg/L)

No.	R	inhibition rate (%)											
		EC[a]		DS[a]		AR[a]		PO[a]		AT[a]		CA[a]	
		root	stem	root	stem	root	stem	root	stem	root	stem	root	stem
I-01	H	0	0	0	10	0	10	20	10	30	20	30	30
I-02	2-CH_3	10	0	10	0	20	20	30	30	10	10	20	10
I-03	3-CH_3	10	10	10	0	30	20	30	20	20	20	30	20
I-04	4-CH_3	0	10	0	10	30	30	20	20	30	30	30	30
I-05	3-OCH_3	10	0	0	10	20	30	30	10	30	20	20	20
I-06	4-OCH_3	0	10	10	10	20	20	20	20	40	20	30	40
I-07	4-$C(CH_3)_3$	0	0	10	0	10	20	20	10	20	30	20	10
I-08	2-F	10	20	20	30	20	30	30	40	50	40	20	30
I-09	3-F	30	40	20	10	30	30	30	30	50	40	40	30
I-10	4-F	30	30	30	20	40	40	40	40	40	50	50	50
I-11	2-Cl	20	40	30	40	30	30	20	20	30	30	30	30
I-12	3-Cl	30	30	20	30	30	20	20	30	40	20	20	20
I-13	4-Cl	20	30	30	30	40	40	40	40	60	50	40	50
I-14	2-Br	30	20	20	20	20	30	30	20	20	30	30	30

I-15	3-Br	30	30	10	0	40	50	30	30	30	40	40	50
I-16	3-CF_3	20	30	30	20	40	40	40	50	70	60	50	60
I-17	4-CF_3	40	50	20	10	60	60	50	60	60	70	50	60
I-18	4-NO_2	20	30	30	30	30	30	40	50	40	50	40	60
I-19	2,4-diF	20	30	30	40	30	40	30	40	40	40	40	50
I-20	2,4-diCl	30	20	20	20	40	50	50	60	50	60	60	60
I-21	3,4-diCl	40	50	30	40	40	40	40	50	60	60	50	60
I-22	3-Cl-4-F	70	70	60	70	60	50	70	80	80	70	80	70
I-23	3-CF_3-4-F	70	80	80	80	70	70	80	70	80	60	80	60
I-24	3-CF_3-4-Cl	70	90	70	60	70	80	70	80	80	80	80	70
I-25	3-CF_3-4-Br	20	30	30	30	40	50	40	50	20	30	50	50
I-26	2-Br-4-CF_3	30	20	40	30	30	40	30	40	20	30	40	30
II-01	-	20	10	10	20	10	20	20	20	30	20	10	20
II-02	-	20	0	20	20	20	20	30	20	40	30	30	20
II-03	-	30	20	30	30	20	30	40	30	40	40	40	40
II-04	-	60	70	60	70	50	60	60	60	70	60	60	70
Isoxaflutole		30	20[b]	30	20[b]	20	30[b]	30	10[b]	30	20[b]	30	20[b]
				90		80		80		80	80	70	80
Butachlor		100	90		100		80		70				

[a]Abbreviations: EC for *Echinochloa crusgalli*; DS for *Digitaria sanguinalis*; AR for *Amaranthus retroflexus*; PO for *Portulaca oleracea* , AT for *Abutilon theophrasti* and CA for *Chenopodium album*. [b]Exhibit bleaching symptoms.

Based on the analysis of chemical structures of **I-01~I-26**, it was found that their herbicidal activity was significantly affected by the types of substituents introduced at the benzene ring. Firstly, we examined the influence of the electronic effect and position of the substituents on the activity. Generally, the herbicidal activities of **I-08~I-26** were stronger than those of **I-02~I-07**. It indicates that introducing electron withdrawing groups at benzene ring is more beneficial for the herbicidal activity than electron donating groups. **I-08**, **I-11**, **I-14** showed weaker activity than other halogenated compounds, which implies that the halogen atoms substituted at *meta*- and *para*-positions of benzene ring are better for the activity than at *ortho*-position. The herbicidal activity of **II-03** on dicotyledon weeds was comparable to those of **I-01**, but it showed stronger inhibition than **I-01** on monocotyledon weeds. It reveals that the replacement of benzene with pyridine broaden the weed spectrum. Finally,

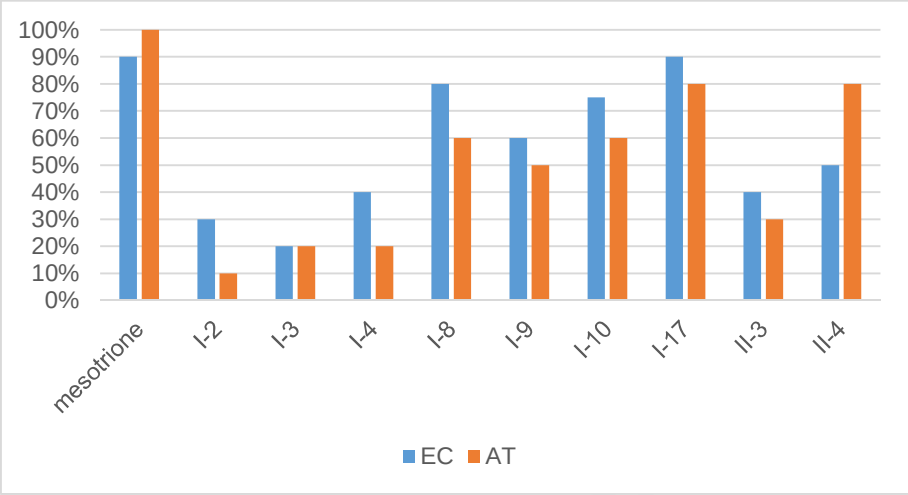
II-04 showed better activity than most of other title compounds, which implies that five-membered heterocyclic moiety might be a more promising structure in the follow-up study.

Surprisingly, the weeds treated by the title compounds showed clearly different symptoms from those treated by IFT in Petri dish tests. IFT caused characteristic bleaching symptoms, but only slightly inhibitory effect on the growth of weeds was observed. As shown in **Tables 1** and **2**, the inhibitory rate of IFT on the growth of weeds ranged from 10% to 30%. Generally, all the synthesized compounds only showed inhibition against tested weeds, and some of them have better herbicidal activity than Butachlor against broadleaf weeds. Unfortunately, we have not observed bleaching symptoms in the weeds treated by the title compounds.

2.2.2. Pre- and post-emergence herbicidal activity and structure-activity relationship analyses

Pre- and post-emergence herbicidal activity of the title compounds against *E. crusgalli* and *A. theophrasti* were evaluated in green house tests according to a procedure reported previously[22]. All title compounds showed no pre-emergence herbicidal activity on the weeds at the application rate of 150 g ai/ha. However, **I-02**~**I-04**, **I-08**~**I-10**, **I-17**, **II-03** and **II-04** exhibited post-emergence herbicidal activity against both *E. crusgalli* and *A. theophrasti*, and the results are illustrated as **Figure 2**. The inhibition rate of **I-08**, **I-10** and **I-17** on *E. crusgalli* were above 70%. **I-17** and **II-04** had excellent herbicidal activity on *A. theophrasti*, and the inhibition rate were around 80%. The herbicidal activity of **I-17** on *E. crusgalli* was comparable to Mesotrione. The SAR revealed that the size and steric hindrance of the substituted groups at benzene ring play important roles, and introducing small groups such as CH_3 and F are beneficial to the activity. Furthermore, the difference in activity between **II-04** and **II-03** remind us that five-member ring may be more promising than six-member ring. Because the activities of **II-08**~**II-10** were stronger than those of **II-02**~**II-04**, it can be concluded that the

introduction of electron withdrawing groups at benzene ring is beneficial for the herbicidal activity. The effect of the substituted positions at benzene ring on the activity is insignificant.



EC for *E. crusgalli*; AT for *A. theophrasti*

Figure 2. Post-emergence herbicidal activity of several compounds on *E. crusgalli* and *A. theophrasti*

More importantly, the active title compounds caused characteristic bleaching symptoms similar to that of Mesotrione. This result indicated that the title compounds take effect as HPPD inhibitors in post-emergence treatments.

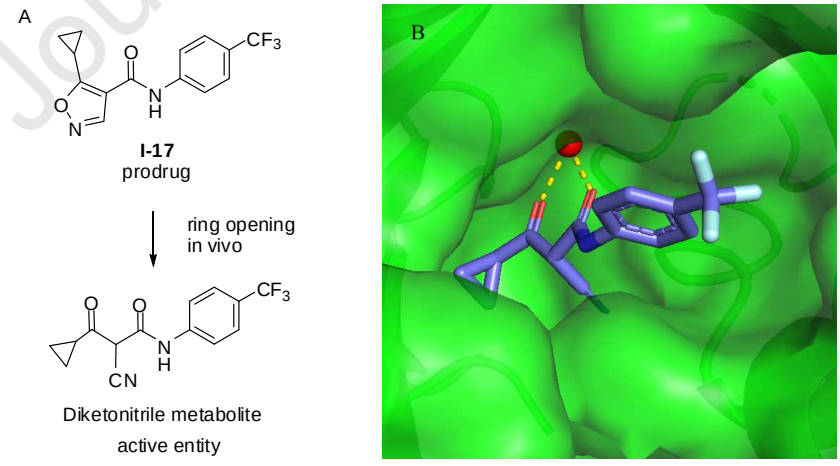
2.3. Molecular modeling

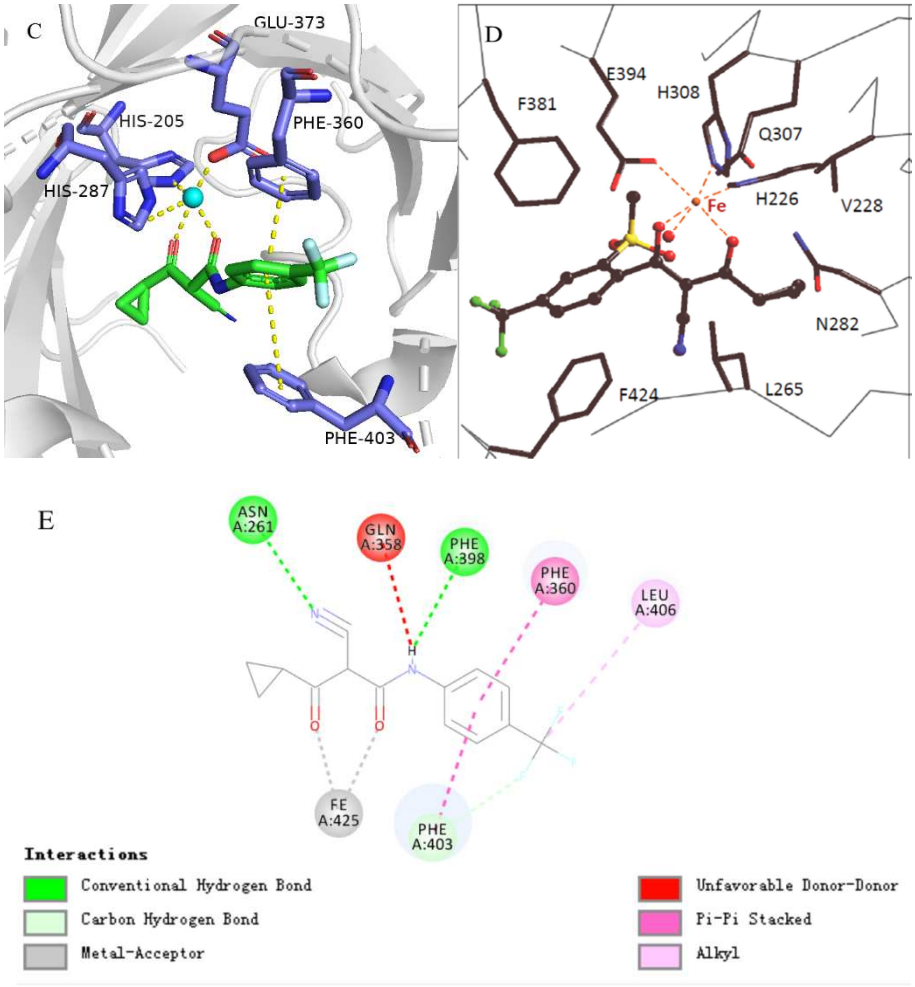
The interaction between **I-17** with HPPD was studied *via* molecular docking method by using Discovery Studio 4.0. Because **I-17** is a prodrug, and the DKN formed by the ring opening of isoxazole is the active entity. The 3D structure of the DKN derivative of **I-17** was constructed by using ChemBiodraw ultra 10.0 (**Figure 3A)**. The compound was then opened in Discovery studio 4.0 and energy minimization was carried out by CHARMm force field using ligand partial charge method CFF (Consistent Force Field)[23]. The original ligand, 1TFZ, was firstly docked into *At*HPPD to check the docking reliability. Consequently, the DKN derivative of **I-17** was docked into the same active site, and twenty conformations were obtained through CDOCKER[24]. **Figure 3B** shows the spatial binding of the molecule in the active cavity. It was found that the molecule does not face repulsions with the

amino acid backbone and thus are able to occupy the binding pocket. As illustrated in **Figure 3C**, the DKN moiety of **I-17** form bidentate interaction with Fe^{II}, and the benzene ring can form π-π interaction with Phe 360 and Phe 403.

The results of molecular docking are partly consist with that of IFT. The two compounds have in common is the chelating of the 1,3-diketone moiety with Fe^{II} in the active site of HPPD. The difference is that the ortho-$MeSO_2$ in the structure of IFT additionally supports the interaction by forming an H-bridge to a molecule of water, which interacts with Fe^{II} at the same time (**Figure D**), whereas the π-π interaction between benzene ring with Phe 360 and Phe 403 provides the additional supports for the interaction in **I-17**.

We can get more information from the **2D** diagram(**Figure E**). Except for the two binding forces mentioned above, the hydrogen atom on the amino group and the nitrogen atom on the cyano group of **I-17** form hydrogen bond with Phe398 and Asn261, respectively.





(A) Active DKN formed *via* ring opening. (B) **I-17** was found to fully embed into the active pocket. (C) Simulated binding mode of **I-17** with *At*HPPD. The key residues in the active site are shown in blue sticks, and Fe^{II} is shown as a cyan sphere, **I-17** is shown in green sticks. (D) DKN derivative of IFT and its molecular interaction with HPPD[19]. (E) 2D diagram simulated binding mode of **I-17** with *At*HPPD.

Figure 3. The receptor-ligand interaction of **I-17** with the HPPD active site

In general, the objective of this study is to screen high activity and low cost herbicidal compounds, and the results are partly accomplished the expected goal. Firstly, the title compound is easier to synthesize than IFT. Secondly, the title compounds exhibited two distinct symptoms in Petri dish test and post-emergence herbicidal activity experiment, which implies that they are multi-target herbicides. This characteristic might be beneficial to delay the emergence of weeds resistance to the title

compounds. The disadvantage is that the two herbicidal mechanism can not take effect at the same time. We suspect that this phenomena may be caused by differences in target site sensitivity, uptake and translocation effects or metabolism of the chemicals in plants.

3. Conclusions

In summary, thirty compounds were designed based on the chemical structure of Isoxaflutole. The title compounds were prepared *via* a simple procedure, and their structures were determined by NMR and MS analysis. All the compounds were evaluated for their herbicidal activities against a panel of weeds, and the SAR of them was analyzed. The post-emergence herbicidal activity of **I-17** against *E.crusgalli* and *A. theophrasti* is comparable to that of Mesotrione. Furthermore, the title compounds bear two distinct herbicidal mechanism. In spite of several weaknesses, the research is conducive to the development of novel herbicides.

4. Experimental section

4.1. General chemistry methods

All chemical reagents were commercially available and used without further purification. Precoated silica gel plates (Si_{60} GF_{254}, Merck Chemical Co. Ltd) were used to monitor the progress of reaction. Purification of target compounds was performed on silica gel column chromatography (200~300 mesh, Qingdao Marine Chemical Co. Ltd, China). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance III-500 NMR spectrometer, and the residual solvent signals were used as reference. Mass spectral analysis was carried out on a Finnegan LCQ Advantage MAX LC/MS spectrometer equipped with an ESI source. IR spectra were recorded on a Nicolet FT-IR 750 spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). The melting points were conducted on a WRS-3 apparatus, and are uncorrected.

4.2. Synthesis of 5-Cyclopropylisoxazole-4-carboxylic acid

1,1-Dimethoxy-*N*,*N*-dimethylmethanamine (**1**, 24g, 0.2 mol) and methyl 3-cyclopropyl-3-oxo-propanoate (**2**, 28g, 0.2 mol) were mixed and heated for 20 h at 60 °C. The obtained yellow oil was firstly dissolved in methanol (200 mL) and water (100 mL), and then hydroxylamine hydrochloride (14g, 0.2 mol) was added. The solvents were evaporated under vacuum after the mixture was heated for 90 min at 60 °C. The residue was dissolved in the mixture of acetic acid (100 mL) and concentrated HCl (100mL) and refluxed for 4h. The reaction mixture was diluted with water (500 mL) and extracted with ethyl acetate (200 mL×3). The organic layer was combined and washed with brine, and then dried with anhydrous sodium sulfate. Ethyl acetate was evaporated under vacuum. The residue was subjected to a silica gel column and eluted with the mixture of ethyl acetate and petroleum ether at the ratio of 1:3 (v/v) to afford **3**. Compound **3** was obtained as white solid (yield 80.13%), mp, 163-165 $^{\circ}$C; ^{1}H NMR (500 MHz, Chloroform-*d*): δ 8.53 (s, 1H), 2.91 (m, 1H), 1.38 (m, 2H), 1.3 (m, 2H). ^{13}C NMR (126 MHz, Chloroform-*d*): δ 179.91, 167.63, 150.62, 108.36, 10.86, 8.87. ESI-MS: *m/z* 152, $[M-H]^-$.

4.3. General procedure for the synthesis of 5-cyclopropyl-N-phenylisoxazole-4-carboxamides (I-01~I-26 , II-01~II-04)

To a solution of substituted phenylamine (1mmol) in 20 mL of anhydrous dichloromethane, **4** (1 mmol) and pyridine (1 mmol) previously dissolved in 5 mL of anhydrous dichloromethane were added dropwise at 0 ^{0}C. The mixture was continuously stirred at 0 ^{0}C for 30 min. After completion of the reaction based on TLC detection, the solution was washed with water (30 mL), saturated sodium chloride solution (30 mL) and brine (30 mL), successively. The organic layer was dried over anhydrous sodium sulfate. After the solvent was removed under vacuum, the residue was subjected to silica gel column and eluted by ethyl acetate/petroleum ether (1:5) afford **I-01**.

4.3.1. 5-Cyclopropyl-N-phenylisoxazole-4-carboxamide(I-01)

Yield 92.10%; yellow solid; mp, 89.3-91.4 °C; IR (KBr, cm^{-1}) *v* 3444 (-NH-), 1649 (C=O), 1533-1443 (C=C), 1092 (C-O); ^{1}H NMR (500 MHz, Chloroform-*d*): *δ* 8.52 (s, 1H), 8.30 (s, 1H), 7.60-7.51 (m, 2H), 7.34 (t, *J* = 7.9 Hz, 2H), 7.20-7.14 (m, 1H), 2.86 (tt, *J* = 8.4, 5.1 Hz, 1H), 1.28 (tt, *J* = 6.0, 3.3 Hz, 2H), 1.25-1.18 (m, 2H). ^{13}C NMR (126 MHz, Chloroform-*d*): *δ* 177.05, 160.03, 148.47, 137.28, 129.06, 125.04, 121.00, 111.96, 10.07, 8.61. ESI-MS: *m/z* 229, $[M+H]^+$.

4.3.2. 5-Cyclopropyl-N-o-tolylisoxazole-4-carboxamide (I-02)

Yield 78.51%; Yellow solid; m.p, 125.9-127.1 °C; IR (KBr, cm^{-1}) *v* 3443 (-NH-), 1639 (C=O), 1543-1453 (C=C), 1122 (C-O);^{1}H NMR (500 MHz, Chloroform-*d*): *δ* 8.44 (s, 1H), 7.81 (s, 1H), 7.40 (s, 1H), 7.29 (d, *J* = 7.5 Hz, 2H), 7.22-7.16 (m, 1H), 2.83 (tt, *J* = 8.5, 5.1 Hz, 1H), 2.36 (s, 3H), 1.38-1.33 (m, 2H), 1.28 (dt, *J* = 8.9, 3.5 Hz, 2H). ESI-MS: *m/z* 243, $[M+H]^+$.

4.3.3. 5-Cyclopropyl-N-m-tolylisoxazole-4-carboxamide (I-03)

Yield: 82.64%; white solid; m.p, 119.6-122.0 °C; IR (KBr, cm^{-1}) *v* 3444 (-NH-), 1637 (C=O), 1546-1449 (C=C), 1103-1078 (C-O); ^{1}H NMR (500 MHz, Chloroform-*d*): *δ* 8.47 (s, 1H), 7.62 (s, 1H), 7.47 (s, 1H), 7.37 (dt, *J* = 8.1, 2.6 Hz, 1H), 7.30-7.25 (m, 1H), 7.03 (dd, *J* = 7.6, 3.9 Hz, 1H), 2.86 (m, 1H), 2.40 (q, *J* = 4.1 Hz, 3H), 1.37-1.24 (m, 4H). ESI-MS: *m/z* 243, $[M+H]^+$.

4.3.4. 5-Cyclopropyl-N-p-tolylisoxazole-4-carboxamide(I-04)

Yield: 80.35%; Yellow solid; m.p, 117.1-120.0 °C; IR (KBr, cm^{-1}) *v* 3445 (-NH-), 1634 (C=O), 1556-1451 (C=C), 1108-1088 (C-O); ^{1}H NMR (500 MHz, Chloroform-*d*): *δ* 8.47 (s, 2H), 7.55-7.42 (m, 3H), 7.19 (q, *J* = 8.3 Hz, 3H), 2.86 (tt, *J* = 8.3, 5.1 Hz, 1H), 2.41-2.34 (m, 3H), 1.33 (m, 2H), 1.29-1.23 (m, 2H). ESI-MS: *m/z* 243, $[M+H]^+$.

4.3.5. 5-Cyclopropyl-N-(3-methoxyphenyl)isoxazole-4-carboxamide (I-05)

Yield: 87.21%; Yellow wax; IR (KBr, cm^{-1}) *v* 3445 (-NH-), 1647 (C=O), 1556-1451 (C=C), 1104 (C-O); ^{1}H NMR (500 MHz, Chloroform-*d*): *δ* 8.55 (d, *J* = 9.4 Hz, 2H), 7.32-7.23 (m, 1H), 7.18 (t, *J* = 8.1 Hz, 1H), 7.06 (dd, *J* = 8.0, 2.0 Hz, 1H), 6.68 (dd, *J* = 8.3, 2.5 Hz, 1H), 3.74 (s, 3H), 2.86 (tt, *J* = 8.4, 5.1 Hz, 1H), 1.28-1.22 (m, 2H), 1.22-1.16 (m, 2H). ESI-MS: *m/z* 259, $[M+H]^+$.

4.3.6. 5-Cyclopropyl-N-(4-methoxyphenyl)isoxazole-4-carboxamide (I-06)

Yield: 77.52%; Reddish brown solid; m.p, 122.6-125.1 °C; IR (KBr, cm^{-1}) *v* 3444 (-NH-), 1640 (C=O), 1545-1453 (C=C), 1107-1071 (C-O); ^{1}H NMR (500 MHz, Chloroform-*d*): *δ* 8.48 (s, 1H), 7.58

(s, 0H), 7.47 (q, *J* = 8.8 Hz, 2H), 6.97-6.85 (m, 2H), 3.86-3.82 (m, 3H), 2.87 (tt, *J* = 8.5, 5.1 Hz, 1H), 1.35-1.19 (m, 4H). ESI-MS: *m/z* 259, $[M+H]^+$.

4.3.7. N-(4-tert-butylphenyl)-5-cyclopropylisoxazole-4-carboxamide (I-07)

Yield: 86.27%; yellow solid; m.p, 121.8-123.4 °C; IR (KBr, cm^{-1}) *v* 3443 (-NH-), 1629 (C=O), 1548-1451 (C=C), 1100-1088 (C-O); ^{1}H NMR (500 MHz, Chloroform-*d*): *δ* 8.48 (s, 1H), 7.64 (d, *J* = 51.0 Hz, 1H), 7.55-7.49 (m, 2H), 7.44-7.39 (m, 2H), 2.86 (m, 1H), 1.36 (d, *J* = 2.0 Hz, 9H), 1.35-1.25 (m, 4H). ESI-MS: *m/z* 285, $[M+H]^+$.

4.3.8. 5-Cyclopropyl-N-(2-fluorophenyl)isoxazole-4-carboxamide (I-08)

Yield: 89.43%; yellow solid; m.p, 113.2-115.1 °C; IR (KBr, cm^{-1}) *v* 3445 (-NH-), 1636 (C=O), 1552-1467 (C=C), 1086 (C-O); ^{1}H NMR (500 MHz, Chloroform-*d*): *δ* 8.53 (s, 1H), 8.38 (t, *J* = 7.8 Hz, 1H), 7.89 (s, 1H), 7.24-7.12 (m, 3H), 2.77 (tt, *J* = 8.2, 5.2 Hz, 1H), 1.38-1.28 (m, 4H). ESI-MS: *m/z* 247, $[M+H]^+$.

4.3.9. 5-Cyclopropyl-N-(3-fluorophenyl)isoxazole-4-carboxamide (I-09)

Yield: 89.43%; white solid; m.p, 113.2-115.1 °C; IR (KBr, cm^{-1}) *v* 3445 (-NH-), 1641 (C=O), 1552-1459 (C=C), 1113-1082 (C-O); ^{1}H NMR (500 MHz, Chloroform-d) δ 8.50 (d, J = 8.1 Hz, 1H), 7.94 (d, J = 115.6 Hz, 1H), 7.54 (m, 1H), 7.37-7.26 (m, 2H), 6.89 (qd, J = 8.0, 2.6 Hz, 1H), 2.86 (td, J = 8.4, 4.1 Hz, 1H), 1.33 (m, 2H), 1.28 (m, 2H). ESI-MS: *m/z* 247, $[M+H]^+$.

4.3.10. 5-Cyclopropyl-N-(4-fluorophenyl)isoxazole-4-carboxamide (I-10)

Yield: 99.59%; yellow solid; m.p, 77.5-79.6 °C; IR (KBr, cm^{-1}) *v* 3445 (-NH-), 1649 (C=O), 1527-1453 (C=C), 1126-1073 (C-O); ^{1}H NMR (500 MHz, Chloroform-*d*): *δ* 8.49 (d, *J* = 6.4 Hz, 1H), 7.90 (d, *J* = 130.3 Hz, 1H), 7.53 (tq, *J* = 8.2, 4.3, 3.7 Hz, 2H), 7.07 (dt, *J* = 16.7, 8.7 Hz, 2H), 2.91-2.80 (m, 1H), 1.32 (m, 2H), 1.26 (m, 2H). ESI-MS: *m/z* 247, $[M+H]^+$.

4.3.11. N-(2-chlorophenyl)-5-cyclopropylisoxazole-4-carboxamide (I-11)

Yield: 93.65%; yellow solid; m.p, 99.4-101.4 °C; IR (KBr, cm^{-1}) *v* 3445 (-NH-), 1644 (C=O), 1541-1458 (C=C), 1113 (C-O); ^{1}H NMR (500 MHz, Chloroform-*d*): *δ* 8.52 (s, 1H), 8.47 (td, *J* = 7.6, 7.0, 1.5 Hz, 1H), 8.12 (s, 1H), 7.45 (m, 1H), 7.36 (tq, *J* = 8.1, 1.5 Hz, 1H), 7.17-7.09 (m, 1H), 2.80 (m, 1H), 1.36 (dq, *J* = 6.3, 3.3 Hz, 2H), 1.31 (m, 2H). ESI-MS: *m/z* 263, $[M+H]^+$.

4.3.12. N-(3-chlorophenyl)-5-cyclopropylisoxazole-4-carboxamide (I-12)

Yield: 95.42%; Reddish brown solid; m.p, 114.6-117.0 °C; IR (KBr, cm^{-1}) *v* 3445 (-NH-), 1646 (C=O), 1537-1432 (C=C), 1114-1079 (C-O); ^{1}H NMR (500 MHz, Chloroform-*d*): *δ* 8.54-8.48 (m, 1H), 7.99 (d, *J* = 155.8 Hz, 1H), 7.74-7.65 (m, 1H), 7.46 (dt, *J* = 8.5, 2.7 Hz, 1H), 7.33-7.25 (m, 1H), 7.16 (m, 1H), 2.87 (m, 1H), 1.36-1.31 (m, 2H), 1.28 (m, 2H). ESI-MS: *m/z* 263, $[M+H]^+$.

4.3.13. N-(4-chlorophenyl)-5-cyclopropylisoxazole-4-carboxamide (I-13)

Yield: 93.28%; white solid; m.p, 79.7-81.8 °C; IR (KBr, cm^{-1}) *v* 3445 (-NH-), 1647 (C=O), 1539-1435 (C=C), 1098-1067 (C-O); ^{1}H NMR (500 MHz, Chloroform-*d*): *δ* 8.49 (s, 1H), 7.84-7.74 (m, 1H), 7.59-7.53 (m, 2H), 7.38-7.33 (m, 2H), 2.86 (tt, *J* = 8.3, 5.1 Hz, 1H), 1.34 (m, 2H), 1.28 (m, 2H). ESI-MS: *m/z* 263, $[M+H]^+$.

4.3.14. N-(2-bromophenyl)-5-cyclopropylisoxazole-4-carboxamide (I-14)

Yield: 96.40%; Reddish brown solid; m.p, 107.8-109.2 °C; IR (KBr, cm^{-1}) *v* 3445 (-NH-), 1649 (C=O), 1543-1453 (C=C), 1082 (C-O); ^{1}H NMR (500 MHz, Chloroform-*d*): *δ* 8.53 (s, 1H), 8.49 (dd, *J* = 8.3, 1.5 Hz, 1H), 8.09 (s, 1H), 7.63 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.44-7.38 (m, 1H), 7.08 (td, *J* = 7.8, 1.6 Hz, 1H), 2.85 (tt, *J* = 8.5, 5.1 Hz, 1H), 1.39 (dt, *J* = 5.5, 3.2 Hz, 2H), 1.33 (dt, *J* = 8.5, 3.1 Hz, 2H). ESI-MS: *m/z* 307, $[M+H]^+$.

4.3.15. N-(3-bromophenyl)-5-cyclopropylisoxazole-4-carboxamide (I-15)

Yield: 53.92%; Reddish brown solid; m.p, 112.2-124.3 °C; IR (KBr, cm^{-1}) *v* 3443 (-NH-), 1644 (C=O), 1541-1438 (C=C), 1097 (C-O); ^{1}H NMR (500 MHz, Chloroform-*d*): *δ* 8.50 (d, *J* = 6.9 Hz, 1H), 7.95-7.81 (m, 1H), 7.82-7.70 (m, 1H), 7.52 (dd, *J* = 8.2, 2.2 Hz, 1H), 7.35-7.31 (m, 1H), 7.28-7.23 (m, 1H), 2.86 (tt, *J* = 8.1, 5.1 Hz, 1H), 1.34 (dq, *J* = 5.6, 2.5 Hz, 2H), 1.29 (m, 2H). ESI-MS: *m/z* 307, $[M+H]^+$.

4.3.16. 5-Cyclopropyl-N-(3-(trifluoromethyl)phenyl)isoxazole-4-carboxamide (I-16)

Yield: 67.57%; Reddish brown solid; m.p, 66.9-69.0 °C; IR (KBr, cm^{-1}) *v* 3445 (-NH-), 1639 (C=O), 1553-1423 (C=C), 1103-1087 (C-O); ^{1}H NMR (500 MHz, Chloroform-*d*): *δ* 8.57-8.50 (m, 1H), 7.94-7.87 (m, 1H), 7.81 (dt, *J* = 8.6, 2.5 Hz, 1H), 7.54-7.40 (m, 2H), 2.88 (tt, *J* = 8.3, 5.1 Hz, 1H), 1.35 (m, 2H), 1.32-1.25 (m, 1H). ESI-MS: *m/z* 297, $[M+H]^+$.

4.3.17. 5-Cyclopropyl-N-(4-(trifluoromethyl)phenyl)isoxazole-4-carboxamide (I-17)

Yield: 95.63%; White solid; m.p, 121.5-124.1 °C; IR (KBr, cm^{-1}) *v* 3445 (-NH-), 1641 (C=O), 1540-1453 (C=C), 1107-1077 (C-O); ^{1}H NMR (500 MHz, Chloroform-*d*): *δ* 8.51 (d, *J* = 3.6 Hz, 1H),

7.95-7.62 (m, 5H), 2.87 (tt, *J* = 8.4, 5.1 Hz, 1H), 1.37 (qt, *J* = 5.1, 1.9 Hz, 2H), 1.34- 1.28 (m, 2H). ESI-MS: *m/z* 297, $[M+H]^+$.

4.3.18. 5-Cyclopropyl-N-(4-(trifluoromethyl)phenyl)isoxazole-4-carboxamide (I-18)

Yield: 50.36%; Yellow solid; m.p, 117.0-120.2 °C; IR (KBr, cm^{-1}) *v* 3443 (-NH-), 1649 (C=O), 1543-1459 (C=C), 1108-1076 (C-O); ^{1}H NMR (500 MHz, Chloroform-*d*): *δ* 8.57 (s, 1H), 8.33-8.27 (m, 2H), 7.89-7.83 (m, 2H), 6.70-6.63 (m, 1H), 2.90 (tt, *J* = 8.4, 5.1 Hz, 1H), 1.38 (dt, *J* = 5.4, 3.0 Hz, 2H), 1.34 (dt, *J* = 8.3, 2.9 Hz, 2H). ESI-MS: *m/z* 275, $[M+H]^+$.

4.3.19. 5-Cyclopropyl-N-(2,4-difluorophenyl)isoxazole-4-carboxamide (I-19)

Yield: 81.44%; white solid; m.p,87.5-89.3 °C; IR (KBr, cm^{-1}) *v* 3445 (-NH-), 1647 (C=O), 1543-1440 (C=C), 1124-1067 (C-O); ^{1}H NMR (500 MHz, Chloroform-*d*): *δ* 8.52 (s, 1H), 8.36-8.28 (m, 1H), 7.75 (d, J = 6.3 Hz, 1H), 6.96 (m, 2H), 2.76 (tt, J = 8.3, 5.2 Hz, 1H), 1.38-1.29 (m, 4H). ESI-MS: *m/z* 265, $[M+H]^+$.

4.3.20. 5-Cyclopropyl-N-(2,4-dichlorophenyl)isoxazole-4-carboxamide (I-20)

Yield: 80.36%; white solid; m.p, 85.2-87.4 °C; IR (KBr, cm^{-1}) *v* 3445 (-NH-), 1641 (C=O), 1547-1431 (C=C), 1104 (C-O); ^{1}H NMR (500 MHz, Chloroform-d): *δ* 8.51 (s, 1H), 8.45 (dd, *J* = 8.9, 5.6 Hz, 1H), 8.05 (s, 1H), 7.47 (t, *J* = 2.6 Hz, 1H), 7.34 (dt, *J* = 8.9, 2.7 Hz, 1H), 2.79 (tt, *J* = 8.3, 5.1 Hz, 1H), 1.40-1.35 (m, 2H), 1.32 (m, 2H). ESI-MS: *m/z* 297, $[M+H]^+$.

4.3.21. 5-Cyclopropyl-N-(3,4-dichlorophenyl)isoxazole-4-carboxamide (I-21)

Yield: 96.28%; Brown solid; m.p, 88.4-90.3 °C; IR (KBr, cm^{-1}) *v* 3443 (-NH-), 1643 (C=O), 1530-1434 (C=C), 1122-1089 (C-O); ^{1}H NMR (500 MHz, Chloroform-*d*): *δ* 8.51 (s, 1H), 8.02 (s, 1H), 7.82 (d, *J* = 2.3 Hz, 1H), 7.47-7.40 (m, 2H), 2.87 (tt, *J* = 8.3, 5.2 Hz, 1H), 1.34 (dt, *J* = 5.5, 2.9 Hz, 2H), 1.30 (dt, *J* = 8.3, 3.0 Hz, 2H). ESI-MS: *m/z* 297, $[M+H]^+$.

4.3.22. N-(3-chloro-4-fluorophenyl)-5-cyclopropylisoxazole-4-carboxamide (I-22)

Yield: 97.65%; yellow solid; m.p, 83.7-85.2 °C; IR (KBr, cm^{-1}) *v* 3442 (-NH-), 1642 (C=O), 1540-1440 (C=C), 1110 (C-O); ^{1}H NMR (500 MHz, Chloroform-*d*): *δ* 8.51 (d, *J* = 8.0 Hz, 1H), 8.08 (d, *J* = 106.3 Hz, 1H), 7.73 (m, 1H), 7.42 (m, 1H), 7.13 (dt, *J* = 11.8, 8.7 Hz, 1H), 2.87 (td, *J* = 8.4, 4.2 Hz, 1H), 1.32 (m, 2H), 1.27 (m, 1H). ESI-MS: *m/z* 281, $[M+H]^+$.

4.3.23. 5-Cyclopropyl-N-(4-fluoro-3-(trifluoromethyl)phenyl)isoxazole-4-carboxamide (I-23)

Yield: 79.62%; white solid; m.p, 88.7-90.6 °C; IR (KBr, cm^{-1}) *v* 3443 (-NH-), 1632 (C=O), 1527-1430 (C=C), 1100-1079 (C-O); ^{1}H NMR (500 MHz, Chloroform-d): *δ* 8.49 (s, 1H), 7.87 (dd, *J* = 6.1, 2.7 Hz, 1H), 7.82 (dt, *J* = 8.8, 3.5 Hz, 1H), 7.76 (s, 1H), 7.25 (t, *J* = 9.3 Hz, 1H), 2.88 (tt, *J* = 8.3, 5.1 Hz, 1H), 1.36 (dt, *J* = 5.7, 3.1 Hz, 2H), 1.32 (dt, *J* = 8.6, 3.0 Hz, 2H). ESI-MS: *m/z* 315, $[M+H]^+$.

4.3.24. *N*-(4-chloro-3-(trifluoromethyl)phenyl)-5-cyclopropylisoxazole-4-carboxamide (I-24)

Yield: 60.60%; white solid; m.p, 127.2-129.5 °C; IR (KBr, cm^{-1}) *v* 3445 (-NH-), 1645 (C=O), 1542-1443 (C=C), 1117-1087 (C-O); ^{1}H NMR (500 MHz, Chloroform-*d*): *δ* 8.51 (s, 1H), 7.96-7.87 (m, 2H), 7.82 (dd, *J* = 8.7, 2.6 Hz, 1H), 7.52 (d, *J* = 8.7 Hz, 1H), 2.88 (tt, *J* = 8.3, 5.2 Hz, 1H), 1.35 (dt, *J* = 5.5, 2.9 Hz, 2H), 1.31 (m, 2H). ESI-MS: *m/z* 331, $[M+H]^+$.

4.3.25. N-(4-bromo-3-methylphenyl)-5-cyclopropylisoxazole-4-carboxamide (I-25)

Yield: 62.50%; Yellow solid; m.p, 97.3-99.8 °C; IR (KBr, cm^{-1}) *v* 3443 (-NH-), 1635 (C=O), 1537-1434 (C=C), 1083 (C-O); ^{1}H NMR (500 MHz, Chloroform-*d*): *δ* 8.48 (s, 1H), 7.75 (s, 1H), 7.55-7.50 (m, 2H), 7.28 (dd, *J* = 8.6, 2.7 Hz, 1H), 2.86 (tt, *J* = 8.4, 5.1 Hz, 1H), 2.42 (s, 3H), 1.34 (tt, *J* = 5.8, 2.6 Hz, 2H), 1.29 (m, 2H). ESI-MS: *m/z* 321, $[M+H]^+$.

4.3.26. N-(2-bromo-4-(trifluoromethyl)phenyl)-5-cyclopropylisoxazole-4-carboxamide (I-26)

Yield: 53.48%; white solid; m.p, 110.2-111.5 °C; IR (KBr, cm^{-1}) *v* 3442 (-NH-), 1642 (C=O), 1540-1443 (C=C), 1112 (C-O); ^{1}H NMR (500 MHz, Chloroform-d): δ 8.69 (d, J = 8.7 Hz, 1H), 8.53 (s, 1H), 8.22 (s, 1H), 7.90 (d, J = 2.0 Hz, 1H), 7.67 (dd, J = 8.7, 2.0 Hz, 1H), 2.85 (tt, J = 8.4, 5.1 Hz, 1H), 1.41 (tt, J = 5.9, 2.8 Hz, 2H), 1.36 (m, 2H). ESI-MS: *m/z* 375, $[M+H]^+$.

4.3.27. 5-Cyclopropyl-N-(naphthalen-1-yl)isoxazole-4-carboxamide (II-01)

Yield: 85.64%; Reddish brown solid; m.p, 115.8-117.2 °C; IR (KBr, cm^{-1}) ν 3413 (-NH-), 1600 (C=O), 1537-1437 (C=C), 1130-1100 (C-O); ^{1}H NMR (500 MHz, DMSO-d_6): *δ* 10.25 (s, 1H), 9.20 (s, 1H), 8.07-8.02 (m, 1H), 8.00-7.95 (m, 1H), 7.87 (d, *J* = 8.1 Hz, 1H), 7.63 (d, *J* = 7.3 Hz, 1H), 7.57 (qd, *J* = 7.8, 7.3, 4.6 Hz, 3H), 3.00-2.92 (m, 1H), 1.22 (m, 2H), 1.17 (dt, *J* = 5.3, 3.0 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6): *δ* 176.99, 160.68, 149.92, 133.45, 129.46, 128.58, 126.88, 126.63, 126.56, 126.02, 124.28, 123.78, 111.96, 10.28, 8.80. ESI-MS: *m/z* 279, $[M+H]^+$.

4.3.28. 5-Cyclopropyl-N-(naphthalen-2-yl)isoxazole-4-carboxamide (II-02)

Yield: 79.13%; white solid; m.p, 78.2-81.0 °C; IR (KBr, cm^{-1}) *v* 3444 (-NH-), 1600 (C=O), 1537-1430 (C=C), 1126 (C-O); ^{1}H NMR (500 MHz, Chloroform-*d*): *δ* 8.86 (s, 1H), 8.61 (s, 1H), 8.13

(d, *J* = 2.2 Hz, 1H), 7.78-7.73 (m, 1H), 7.71 (d, *J* = 8.8 Hz, 1H), 7.69-7.64 (m, 1H), 7.52 (dd, *J* = 8.8, 2.2 Hz, 1H), 7.47-7.40 (m, 2H), 2.89 (tt, *J* = 8.4, 5.1 Hz, 1H), 1.24 (tt, *J* = 6.0, 3.5 Hz, 2H), 1.20-1.13 (m, 2H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 177.11, 160.08, 148.20, 134.48, 133.32, 130.60, 128.44, 127.29, 126.31, 125.11, 120.44, 118.01, 111.57, 9.94 , 8.41. ESI-MS: *m/z* 279, $[M+H]^+$.

4.3.29. 5-Cyclopropyl-N-(pyridin-4-yl)isoxazole-4-carboxamide(II-03)

Yield: 70.35%; yellow solid; m.p, 89.8-91.8°C; IR (KBr, cm^{-1}) *v* 3447 (-NH-), 1633 (C=O), 1504-1414 (C=C), 1123 (C-O); ^{1}H NMR (500 MHz, Chloroform-*d*): δ 8.59 (d, J = 5.4 Hz, 2H), 8.54 (s, 1H), 7.98 (s, 1H), 7.65-7.58 (m, 2H), 2.92-2.85 (m, 1H), 1.37 (dt, *J* = 5.4, 3.0 Hz, 2H), 1.33 (dt, *J* = 8.6, 3.1 Hz, 2H). ESI-MS: *m/z* 230, $[M+H]^+$.

4.3.30. 5-Cyclopropyl-N-(thiazol-2-yl)isoxazole-4-carboxamide (II-04)

Yield: 55.32%; white solid; m.p, 157.9-159.9 °C; IR (KBr, cm^{-1}) *v* 3445 (-NH-), 1596 (C=O), 1443 (C=C), 1128-1076 (C-O); ^{1}H NMR (500 MHz, Chloroform-*d*):δ 8.61 (s, 1H), 7.37 (d, *J* = 3.6 Hz, 1H), 7.31 (s, 1H), 7.09 (d, *J* = 3.6 Hz, 1H), 3.04 (tt, *J* = 8.4, 5.1 Hz, 1H), 1.40 (tt, *J* = 6.0, 3.6 Hz, 2H), 1.32 (tt, *J* = 6.3, 2.5 Hz, 2H). ^{13}C NMR (126 MHz, Chloroform-*d*): δ 179.22, 159.86, 159.39, 148.31, 136.55, 114.24, 110.15, 10.77, 8.99. ESI-MS: *m/z* 236, $[M+H]^+$.

4.4. Herbicidal activity assay

4.4.1. Petri dish tests

Seeds of monocotyledon weeds such as *Echinochloa crusgalli* and *Digitaria sanguinalis*, and dicotyledon weeds such as *Amaranthus retroflexus*, *Portulaca oleracea* and *Abutilon theophrasti* were collected from campus of Northwest A&F University in 2017. The germinated seeds were placed in Petri dishes (90 mm diameter) containing two layers of filter paper, and impregnated with 5 mL of the solutions of tested compounds at 100 mg/L and 10 mg/L, respectively. Water was used as blank control, isoxaflutole and butachlor were used as positive control. Then the Petri dishes were placed in a

light incubator at 25 °C, light intensity of 300 Lux. The growth inhibition rate of root and stem were observed after 5 days.

4.4.2 Pre- and post-emergence herbicidal activity

Pre- and post-emergence herbicidal activities of the title compounds against *E. crusgalli* and *A. theophrasti* were evaluated in glasshouse according to a procedure reported previously[22]. All tested compounds were firstly dissolved in DMSO to the concentration of 100 g/L. The solutions were then diluted with 0.1% Tween-80 to desired concentrations before using. The soil used was a mixed soil (33.3% garden soil and 66.7% seedling substrate). Plastic pots with an inner diameter of 7.5 cm were filled with the above soil to three-fourths of their height. About 20 seeds of the tested weeds were sown in the pot and covered with soil to a thickness of 0.2 cm and grown at temperatures from 15 to 30 °C in a glasshouse. For pre-emergence treatments, the diluted test solutions (150 g ai/ha) were sprayed on the surface of soil 24 h after the seeds were sown. For post-emergence treatment, the weeds were treated with the solutions of tested compounds (150 g ai/ha) at three-leaf stage. The seedlings treated with the diluted solution of DMSO and Tween-80 were used as the control groups. Each treatment was performed in 4 replicates. IFT were used as positive control. After 15 days of treatment, the herbicidal activity was evaluated visually.

4.5. Molecular docking protocol

The 3D structure of the DKN derivative of **I-17**, the representative compound, was constructed by using ChemBiodraw ultra 10.0. It was then opened in Discovery studio 4.0 and energy minimization was carried out by CHARMm force field using ligand partial charge method CFF (Consistent Force Field)[23].Minimization was carried out until energy gradient of 0.01 was reached. The CDOCKER was used for docking of all compounds. A representative *At*HPPD co-crystallized with NTBC (PDB ID:

1TFZ) was taken from the PDB data bank. The water molecules were deleted and hydrogen atoms were added. Finally protein was refined with CHARMm in DS 4.0 at physiological pH. To validate the docking reliability, co-crystalized ligand (DAS869) was first re-docked to the binding site of HPPD. Consequently, the DKN derivative of **I-17** was docked into the same active site, and twenty conformations of it were obtained through CDOCKER[24]. The conformation with lowest energy was selected as the most probable binding conformation. PYMOL was used to analyze the binding mode.

Declare of Competing Interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary material

Supplementary data to this article can be found online.

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Highlights

- Strong inhibitory effect on the growth of weeds were observed in Petri dish tests
- Characteristic bleaching effect was observed in post-emergence treatments
- Excellent binding with HPPD was observed in molecular docking analysis
- A potential lead compound for multi-target herbicides

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: