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Synthesis of Novel (4, 6-Dimethoxypyrimidin-2-yl) Thiosalicylate Aldoxime Esters as Herbicidal ALS Inhibitors

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Abstract

A series of new pyrimidinylthiosalicylic acid derivatives, (4, 6-dimethoxypyrimidin-2-yl) thiosalicylate aldoxime esters (6a-6x), were designed and synthesized as potential herbicidal acetolactate synthase (ALS) inhibitors. All synthesized new compounds were characterized by ^{1}H NMR, ^{13}C NMR, and high resolution mass spectrometry (HRMS). The herbicidal activity and in vitro ALS enzyme inhibition activity studies were performed to test the synthesized new compounds. The herbicidal activity study shows that all synthesized new compounds exhibit no herbicidal activity against Setaria viridis and Eleusine indica and no harm to rice. Among the tested compounds, 6d, 6i, 6j, 6n, 6o, and 6p exhibit potent herbicidal activity (inhibitory ratio over 85%) against barnyard grass at 10 mg/L, which are better than other synthesized new compounds and Pyriftalid. 6d, 6i, 6j, 6n, 6o, and 6p also exhibit efficient inhibitory activity (IC_{50} 2.63-8.02 mM) against *E.coli* ALS enzyme in in vitro ALS enzyme inhibition activity study. The structure-activity relationship (SAR) analysis reveals that benzaldoxime ester is a highly efficient substituent group for ALS inhibitors, which is better than heteroaromatic aldoxime esters and aliphatic aldoxime ester, and the introduction of -OBoc (6n, 6o), -NH_2 (6p), or -F (6d, 6i, 6j) to the benzaldoxime ester can further enhance ALS enzyme inhibition activity. Molecular docking simulation was performed to gain a better understanding of the probable binding modes of these compounds. Compounds 6d, 6i, 6j, 6n, 6o, and 6p reported in the present work may serve as new candidates for herbicidal ALS inhibitors.

Keywords

Pyrimidylsalicylates, Acetolactate Synthase Inhibitors, Aldoxime Esters, Herbicidal Activity, Molecular Docking Simulation

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1. Introduction

Acetolactate synthase (ALS, EC 4.1.3.18; also known as acetohydroxyacid synthase, EC 2.2.1.6), which is an essential enzyme in the biosynthetic pathways of the branched-chain amino acids, mainly catalyzes the synthesis of 2-aceto-2-hydroxybutyrate in the biosynthetic pathways of isoleucine and 2-acetolactate in the biosynthetic pathways of valine and leucine [1-3]. ALS has been identified as an attractive target for herbicides and antibiotics for many years due to its biological functions [3-5]. Particularly, ALS inhibitors as commercial herbicides achieved great success over the past decades. ALS inhibitors have been a hot research area in herbicide field.

Up to now, several structurally diverse herbicidal ALS inhibitors have been discovered, including sulfonylureas

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(SU), imidazolinones (IM), triazolopyrimidines (TP), sulfonamides (SA), and pyrimidylsalicylates (PS), Figure 1 [6-9]. PS as an important class of herbicidal ALS inhibitors possess a number of features, such as high herbicidal activity, broad herbicidal spectrum (mainly broadleaf weeds and some grass weeds, especially barnyard grass), short residual time in soil, and safe to succeeding crops [10-15]. In general, PS are obtained by the substitution reactions between salicylic acid derivatives and 2-substituted pyrimidine derivatives. Bispyribac-sodium (Figure 1), which belongs to PS herbicides, is usually used to control barnyard grass, the most common weed in rice production. Pyribenzoxim (Figure 1) is modified from Bispyribac-sodium and the dosage of Pyribenzoxim (10-30 g ai ha^{-1}) is lower than that of Bispyribac-sodium (15-45 g ai ha^{-1}), indicating that oxime ester is an effective substituent group for design of more efficient herbicidal ALS inhibitors [10, 16, 17].

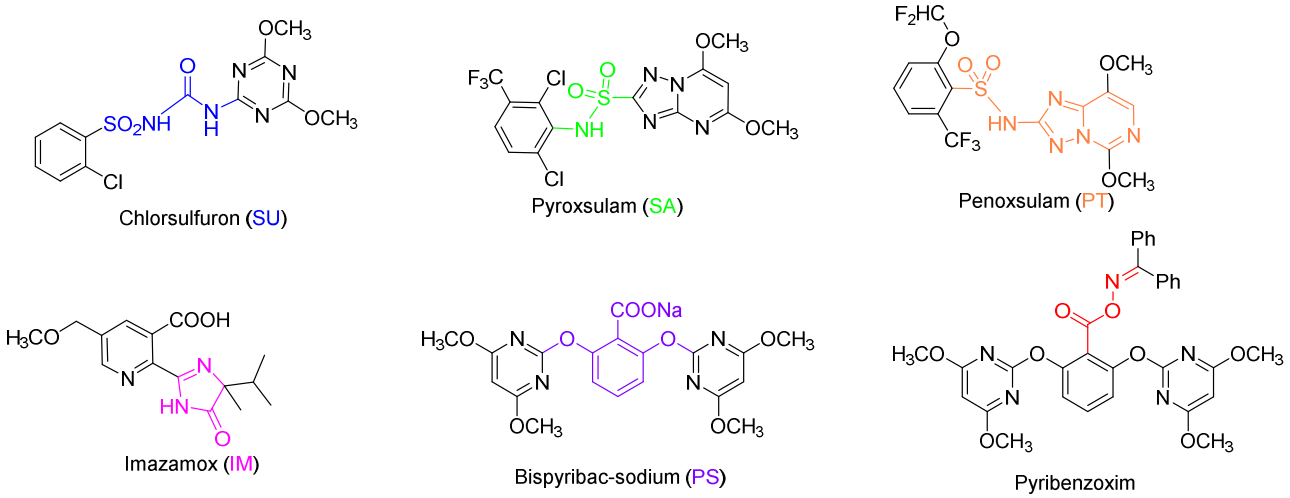


Figure 1. Structures of herbicidal ALS inhibitors.

To discover more promising herbicidal ALS inhibitors, our group focused on structural modifications of pyrimidinylsalicylic acid or pyrimidinylthiosalicylic acid with oximes. In the present work, we designed twenty four (4, 6-dimethoxypyrimidin-2-yl) thiosalicylate aldoxime esters (6a-6x) as potential herbicidal ALS inhibitors and described in detail in the synthesis methods, herbicidal activity, in vitro ALS enzyme inhibition activity, structure-activity relationships (SARs), and probable binding modes of the synthesized new compounds.

2. Materials and Methods

2.1. Materials and Instruments

All reagents, catalysts, and extra-dry solvents were purchased from commercial suppliers Sigma-Aldrich, TCI, Sinopharm, Yonghua Chemicals, and Aladdin Chemicals. All reactions were monitored using thin layer chromatography silica gel plates (Anhui Liangchen Silicon Source, Anhui, China). Melting points were recorded on a model X-4B melting point apparatus (Shanggai Shenguang Instrument, Shanghai, China). ^{1}H and ^{13}C NMR spectra were recorded on an Avance III 300 or 400 spectrometer (Bruker Inc., Switzerland) using DMSO-d_6 or $CDCl_3$ as the solvent and tetramethylsilane (TMS) as the internal reference. The chemical shifts (δ) were given in parts per million (ppm). High resolution mass spectra (HRMS) were determined with a model 6224 time-of-flight liquid chromatograph-mass spectrometer (Agilent Technologies, Santa Clara, CA). The X-ray single-crystal diffraction data of compound 6b (a colorless crystal was obtained directly from CH_2Cl_2/n-hexane) were collected on a SMART APEX DUO CCD area-detector diffractometer (Bruker AXS, Madison, WI) at 173 K using Mo Kα radiation (λ = 0.71073 Å). All of the non-hydrogen atoms of compound 6b were refined with anisotropic displacement parameters. The hydrogen atoms were placed and observed in geometrically idealized positions. The integration of the diffraction profiles and the methods of structural analysis were carried out using SAINT Plus software (Bruker AXS, Madison, WI) and the SHELXS97 program (University of Gottingen, Gottingen, Germany), respectively.

2.2. Synthesis of Compound 3

Compound 1 (64.9 mmol) was dissolved in 40 mL of 80% isopropanol aqueous solution. To the solution, 52 mL of 12.5% NaOH aqueous solution was added under stirring in ice bath. Then, DMSP (2) (64.9 mmol) was added into the above solution and the resulting mixture was heated to 80°C and vigorously stirred for 12-20 h under nitrogen atmosphere. Afterwards, the reaction mixture was cooled to room temperature and 36% HCl aqueous solution was added

dropwisely to adjust the pH to 1~2. The crude product was obtained by freeze crystallization at 4°C. After filtration and dried under vacuum at 50°C, compound 3 as an off-white solid was obtained (yield 79%).

2.3. Synthesis of Compounds 5a-5m and 5q-5x

Hydroxylamine hydrochloride (6 mmol) and anhydrous sodium acetate (6 mmol) were dissolved in CH_3OH (10 mL) /H_2O (5 mL) mixture. To the solution, 4a-4m or 4q-4x (5 mmol) were added under stirring and the resulting mixture was vigorously stirred at room temperature for 2 h. After removing the solvent by vacuum evaporation, the residue was dissolved in CH_2Cl_2 (20 mL) /H_2O (20 mL) mixture. The organic phase was separated. The aqueous phase was extracted by CH_2Cl_2 (15 mL) for three times. The organic phase was merged, dried over anhydrous Na_2SO_4 overnight, and concentrated under vacuum to afford compounds 5a-5m and 5q-5x (yields 92-98%).

2.4. Synthesis of Compounds 5n-5p

The OH or NH_2 groups of compounds 4n-4p were protected by Boc_2O before reacting with hydroxylamine hydrochloride. Briefly, a catalytic amount of 4-dimethylaminopyridine (DMAP) and compounds 4n-4p (5 mmol) were dissolved in CH_2Cl_2 (20 mL) in ice bath. To the solution, Boc_2O (5.5 mmol) was added dropwisely under stirring and the resulting mixture was vigorously stirred at room temperature for 2 h. After removing the solvent by vacuum evaporation, the residue was dissolved in ethyl acetate (20 mL) /H_2O (20 mL) mixture. The organic phase was separated. The aqueous phase was extracted by ethyl acetate (15 mL) for three times. The organic phase was merged, dried over anhydrous Na_2SO_4 overnight, and concentrated under vacuum to afford compounds 7n-7p. Compounds 5n-5p (yields 94−98%) were synthesized from compounds 7n-7p following the steps of 5a-5m and 5q-5x.

2.5. Synthesis of Compounds 6a-6x

Briefly, a catalytic amount of DMAP, dicyclohexylcarbodiimide (DCC, 3.8 mmol), and compounds 3 (3.42 mmol) were dissolved in CH_2Cl_2 (20 mL) and the resulting mixture was stirred for 30 min in ice bath. Then, Compounds 5a-5x dissolved in 10 mL of CH_2Cl_2 were slowly added to the above mixture and the resulting reaction mixture was stirred overnight at room temperature under nitrogen atmosphere. Afterwards, the formed dicyclohexylurea (DCU) was removed by filtration. The filtrate was dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue was purified by flash column chromatography (petroleum ether /ethyl acetate = 20:1～1:1) to provide target compounds 6a-6x (yields 48−93%).

2.6. Greenhouse Herbicidal Assay

The foliar application test was performed to evaluate the postemergence herbicidal activity of compounds 6a-6x. Pyriftalid was used as a positive control. The prepared soil was filled to 3/4 height of flowerpots. Each flowerpot were sown with 10 weeds and maintained at temperatures alternating from 15 to 30°C. When weeds grew to the threeleaf stage, all compounds were dissolved in DMSO and diluted with 0.1% Tween-80 aqueous solution to desired concentrations for use. Mixtures of DMSO and Tween-80 were used as solvent control groups. Each test weed was repeated three times.

2.7. ALS Inhibition Activity Assay

E.coli was cultured in expanded medium at 37°C for a period of time. Then, 0.5 mL of *E.coli* suspension was taken out and added to a mixture of 0.5 M PBS (0.4 mL), 0.1 M $MgCl_2$ (0.1 mL), toluene (0.2 mL), and the tested compounds (0.4 mL). After that, 0.2 M sodium pyruvate (0.2 mL) was added and catalyzed by the ALS enzyme of *E.coli* to generate acetolactic acid. After decarboxylation reaction, acetoin was formed. The formed acetoin was colored by α-naphthol and the absorbance was measured by UV-Vis spectrophotometer (Shanghai Lengguang Technology, Shanghai, China) at 520 nm. According to the standard curve of acetoin, the concentration of acetoin was calculated. *E.coli* without drug treatment was used as control. The inhibitory ratio was calculated as

$$\text{Inhibion Ratio} = \frac{c_1 - c_2}{c_1} \times 100\%$$

Where c_1 and c_2 are the acetoin concentration of the control and the inhibitors, respectively.

2.8. Molecular Docking Simulation Analysis

Molecular docking simulation was performed to study the interactions of 6a-6x and ALS (PDB code: 1YBH) by using Discovery Studio 2017 R2 software. The parameters of the docking model were programmed to the recommended default values. The binding modes were set as flexible docking, which was based on the induced-fit theory. Each docking algorithm had its own scoring function from multiple scoring functions.

3. Results and Discussion

The total synthesis of compounds 6a-6x was given in Figure 2. Firstly, (4, 6-dimethoxypyrimidin-2-yl) thiosalicylic acid (3) was synthesized by substitution reaction between DMSP

(2) and thiosalicylic acid (1). Then, aldoximes (5a-5x) were synthesized by condensation reactions between corresponding aldehydes (4) and hydroxylamine hydrochloride. Finally, (4, 6-dimethoxypyrimidin-2-yl) thiosalicylate aldoxime esters (6a-6x) were obtained by O-acylation of 5 with 5a-5x. To gain the final oxime ester derivatives, two esterification methods could be used: 1) reaction of 5a-5x with 6 in the presence of DCC and DMAP; 2) reaction of 8 with (4, 6-dimethoxypyrimidin-2-yl) thiosalicyloyl halides in the presence of triethylamine (TEA). Method 2) produces more side products than method 1), as determined by TLC. Therefore, method 1) was selected to synthesize compounds 6a-6x. Compounds 6n-6p were prepared according to Figure 3. Aldehydes that having OH or NH_2 were protected by (Boc) $_2$O. Compounds 6a-6x were obtained with yield of 48-93%, Table 1. All synthesized compounds were reported for the first time and were characterized by ^{1}H NMR, ^{13}C NMR, and HRMS. The single-crystal structures of compound 6b were characterized by X-ray single-crystal diffraction as an example, Table 2 and Figure 4.

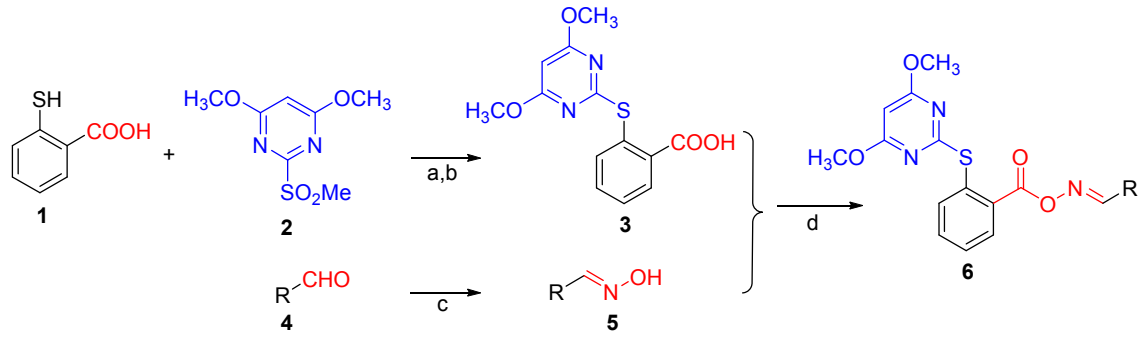


Figure 2. General synthetic routes of compounds 3, 5 and 6. Reagents and conditions: (a) 80% isopropanol aqueous solution, 12.5% NaOH aqueous solution, 80°C; (b) 36% HCl aqueous solution; (c) hydroxylamine hydrochloride, bases; (d) DCC, DMAP, CH_2Cl_2.

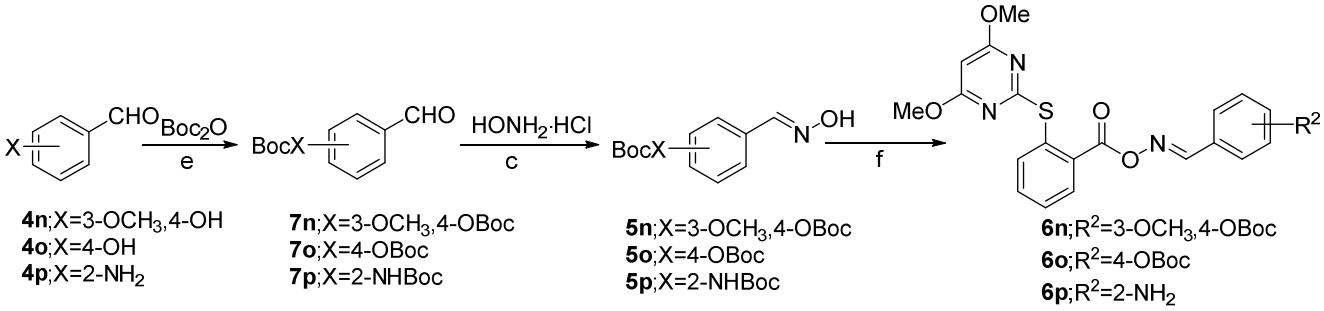
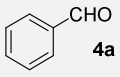
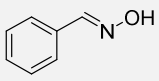
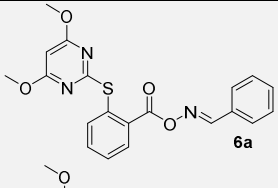
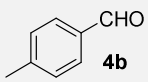
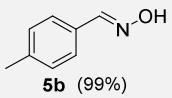
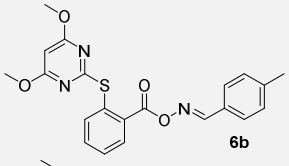
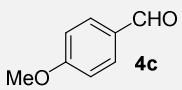
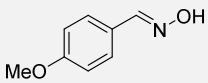
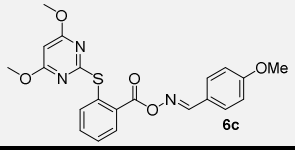
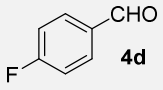
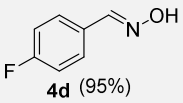
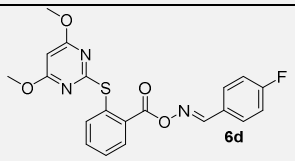
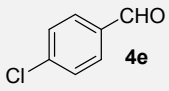
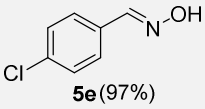
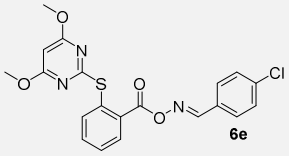
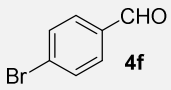
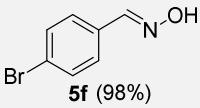
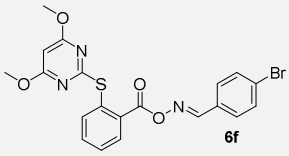
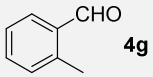
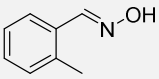
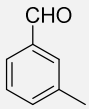
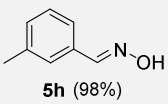
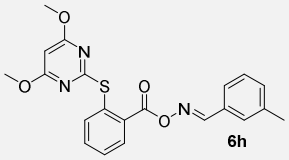
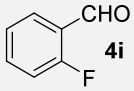
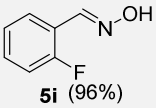
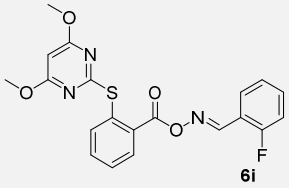
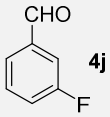
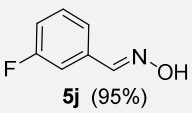
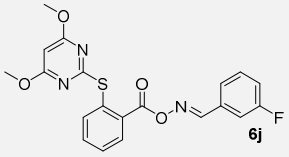
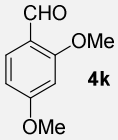
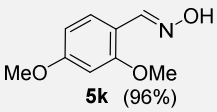
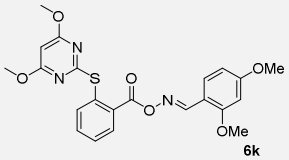
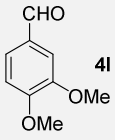
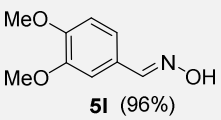
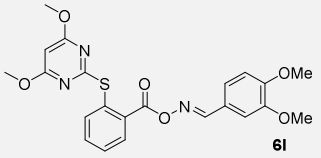
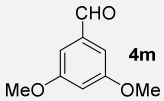
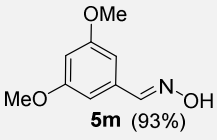
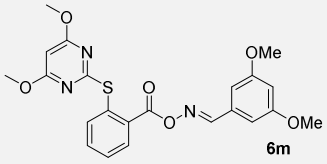
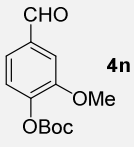
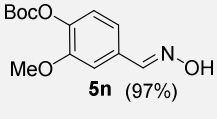
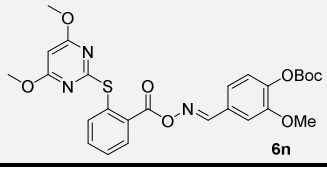


Figure 3. Synthetic routes of compounds 6n, 6o, and 6p. Reagents and conditions: (e) (Boc) $_2$O, DMAP, CH_2Cl_2; (c) hydroxylamine hydrochloride, CH_3COONa, CH_3OH/H_2O (v/v = 2/1); (f) 3, DCC, DMAP, CH_2Cl_2.

Table 1. Chemical structure and yeild of compounds 6a-6x.

Entry	4	5	6	Yield/%
1	 **4a**	 **5a** (98%)	 **6a**	80
2	 **4b**	 **5b** (99%)	 **6b**	85
3	 **4c**	 **5c** (98%)	 **6c**	84

Entry	4	5	6	Yield/%
4				75
5				82
6				83
7				88
8				87
9				77
10				72
11				91
12				92
13				93
14				87

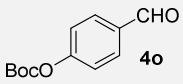
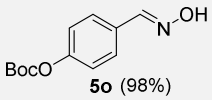
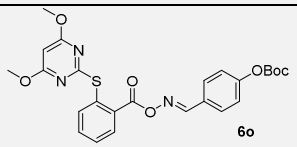
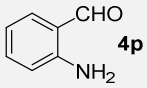
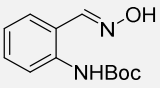
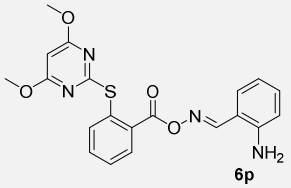
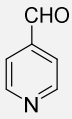
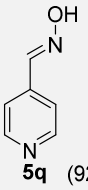
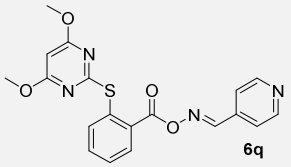
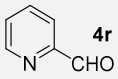
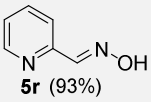
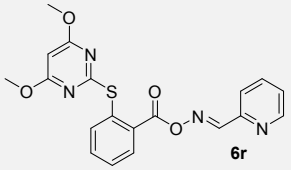
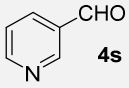
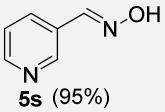
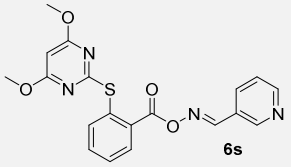
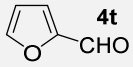
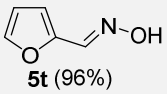
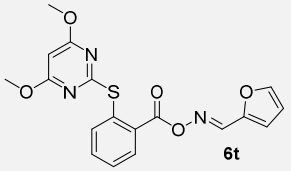
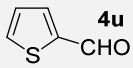
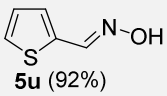
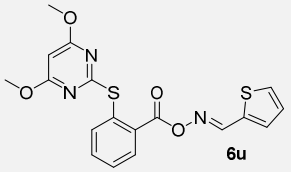
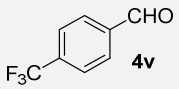
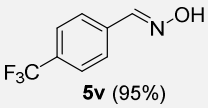
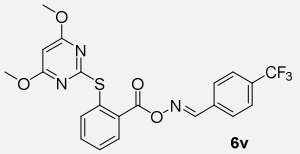
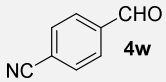
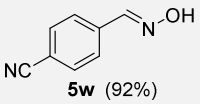
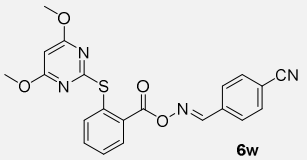
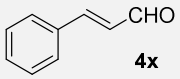
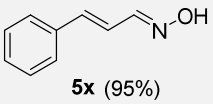
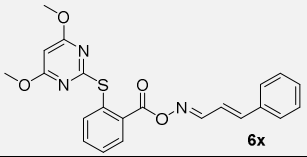
Entry	4	5	6	Yield/%
15	 **4o**	 **5o** (98%)	 **6o**	80
16	 **4p**	 **5p** (94%)	 **6p**	75
17	 **4q**	 **5q** (92%)	 **6q**	85
18	 **4r**	 **5r** (93%)	 **6r**	88
19	 **4s**	 **5s** (95%)	 **6s**	77
20	 **4t**	 **5t** (96%)	 **6t**	71
21	 **4u**	 **5u** (92%)	 **6u**	73
22	 **4v**	 **5v** (95%)	 **6v**	78
23	 **4w**	 **5w** (92%)	 **6w**	52
24	 **4x**	 **5x** (95%)	 **6x**	48

Table 2. The single crystal data of compound 6b.

Name	Value	
Empirical formula	$C_{21}H_{19}N_3O_4S$	
Identification code	A	
Formula weight	409.45	
Temperature	173 K	
Wavelength	0.71073Å	
Crystal system	Triclinic	
Space group	P-1	
Unit cell dimensions	a = 7.8563 (12) Å	α = 80.141 (4) °
	b = 7.9655 (12) Å	β = 88.352 (4) °
	c = 17.767 (3) Å	γ = 63.387 (4) °
Volume	977.9 (3) $Å^3$	
Z	2	
Density (calculated)	1.391 Mg/m^3	
Absorption coefficient	0.199 mm^{-1}	
F (000)	428	
Crystal size	0.190×0.160×0.140 mm^3	
Theta range for data collection	2.330 to 25.009°	
Index ranges	-9<=h<=9, -9<=k<=9, -21<=l<=18	
Reflections collected	6455	
Independent reflections	3411 [R (int) = 0.0420]	
Completeness to theta = 25.009°	99.0%	
Absorption correction	None	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	3411 / 0 / 265	
Goodness-of-fit on F^2	1.024	
Final R indices [I>2sigma (I)]	R1 = 0.0416, wR2 = 0.1047	
R indices (all data)	R1 = 0.0521, wR2 = 0.1108	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.231 and -0.247 e. $Å^{-3}$	

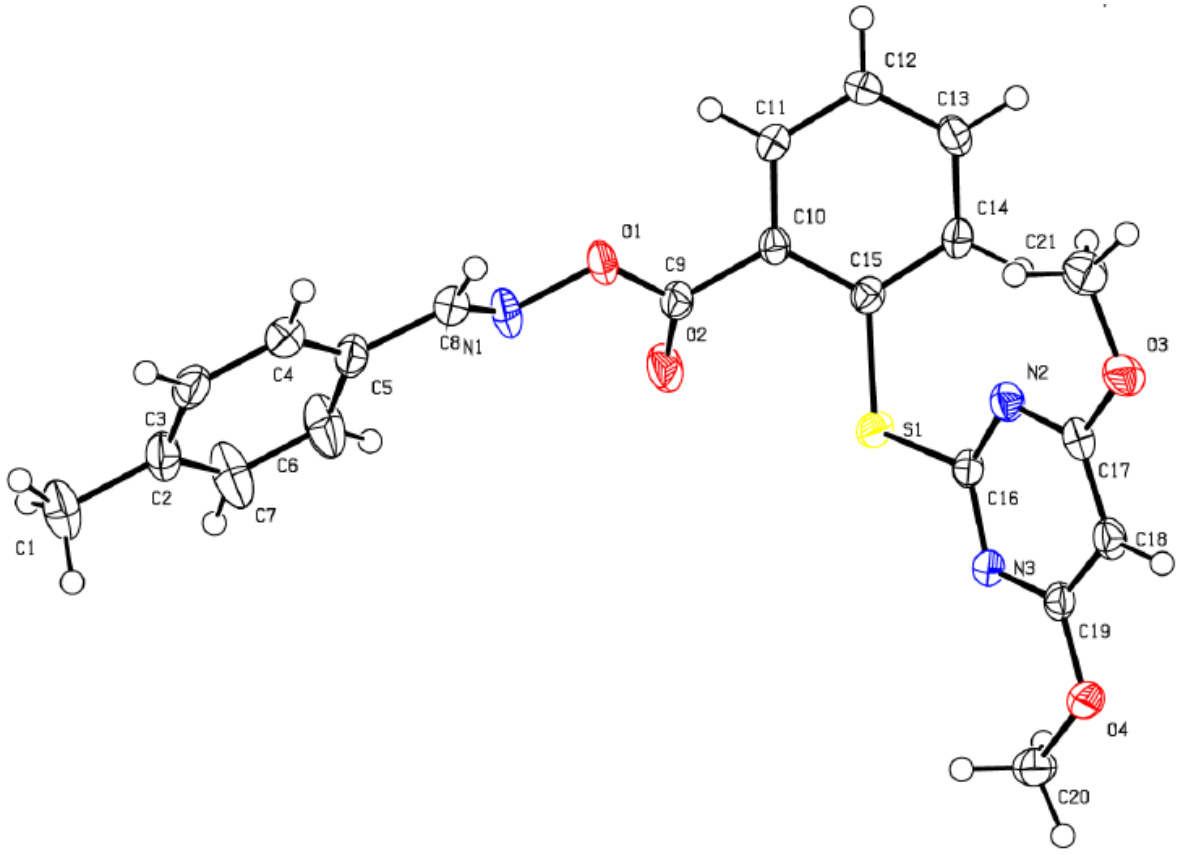


Figure 4. The single crystal structure of compound 6b.

All synthesized new compounds (6a-6x) were evaluated for their herbicidal activity against three weeds (Barnyard grass, Setaria viridis, Eleusine indica) and rice (Safety test). As shown in Table 3, all tested compounds show no growth inhibition effect on barnyard grass at 1 mg/L. When the concentration of the tested compounds is increased to 5 mg/L, 6a, 6d, 6g, 6h, 6i, 6j, 6n, 6o, 6p, 6r, and 6t inhibit the growth of barnyard grass by 20%, 50%, 30%, 20%, 50%, 45%, 60%, 50%, 50%, 35%, and 30%, respectively. When the concentration of the tested compounds is increased to 10 mg/L, all tested compounds show growth inhibition effect on barnyard grass except for 6k, 6l, and 6m, and the inhibitory ratio of 6d, 6i, 6j, 6n, 6o, and 6p are over 85%. All tested compounds show no herbicidal effect on setaria viridis and eleusine indica, and all tested compounds show no harm to rice.

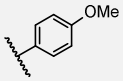
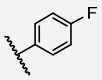
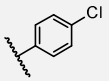
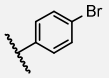
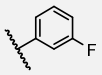
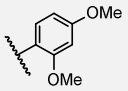
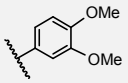
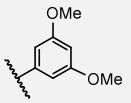
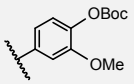
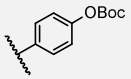
Table 3. Herbicidal activity of compounds 6a-6x.

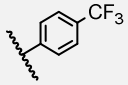
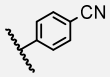
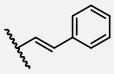
Compound	Inhibitory ratio (%)					
	Barnyard grass[a]			Setaria viridis[a, b]	Eleusine indica[a, b]	Rice[a, b]
	1mg/L	5mg/L	10mg/L			
6a	0	20	60	0	0	0
6b	0	0	70	0	0	0
6c	0	0	40	0	0	0
6d	0	50	95	0	0	0
6e	0	0	20	0	0	0
6f	0	5	30	0	0	0
6g	0	30	65	0	0	0
6h	0	20	55	0	0	0
6i	0	50	90	0	0	0
6j	0	45	85	0	0	0
6k	0	0	0	0	0	0
6l	0	0	0	0	0	0
6m	0	0	0	0	0	0
6n	0	60	95	0	0	0
6o	0	50	90	0	0	0
6p	0	50	90	0	0	0
6q	0	0	10	0	0	0
6r	0	35	40	0	0	0
6s	0	0	20	0	0	0
6t	0	30	60	0	0	0
6v	0	0	50	0	0	0
Pyriftalid	0	0	10	0	0	0

[a]Herbicidal activity of compounds grown under 'upland' conditions.
[b]Inhibitory ratio % at 10 mg/L.

Schloss et al reveal that the "extraneous site inhibitors" is the mechanisms of inhibitors of ALS. The binding sites of ALS inhibitors may not be catalytic or regulatory sites for ALS enzyme [18]. All ALS inhibitors which bind to certain amino acid residues in the substrate channels of ALS block the channels of substrates, leading to inactivation of ALS enzyme [6]. Accordingly, *E.coli* ALS enzyme, which has been studied thoroughly, was selected as the target enzyme to evaluate the inhibitory activity of the synthesized new compounds [19]. All tested compounds were evaluated in vitro for *E.coli* ALS enzyme inhibition activity at the concentration ranging from 1.6 to 12.5 mM. Pyriftalid and Bispyribac-sodium were used as positive controls. As shown in Table 4, all tested compounds show significant inhibitory activity against *E.coli* ALS enzyme at 12.5 mM. Among the tested compounds, the inhibitory activity of Pyriftalid (18.35%) is lower than all synthesized new compounds, the inhibitory activity of Bispyribac-sodium (34.06) is lower than all synthesized new compounds except for 6u (30.18%) and 6x (31.50%), and the inhibitory activity of 14 new compounds (6a, 6b, 6c, 6d, 6g, 6h, 6i, 6j, 6m, 6n, 6o, 6p, 6q, 6r) are over 50%, indicating that aldoxime esters are effective substituent groups for ALS inhibitors. The 14 new compounds with inhibitory ratio over 50% were selected to determine the IC_{50} values. As shown in Table 3, The IC_{50} values of 6a, 6b, 6c, 6d, 6g, 6h, 6i, 6j, 6m, 6n, 6o, 6p, 6q, and 6r are 7.22, 5.43, 7.24, 2.71, 9.88, 9.87, 6.69, 2.63, 7.32, 8.02, 5.34, 4.71, 9.63, and 11.27 mM, respectively.

Table 4. The structures and ALS enzyme inhibition activity of compounds 6a-6x.

Compound	Substituents (R)	Inhibitory ratio (%)[a]	IC_{50} (mM)
6a		62.17	7.22
6b		66.45	5.43
6c		54.50	7.24
6d		73.82	2.71
6e		45.34	ND[b]
6f		43.88	ND
6g		50.69	9.88
6h		52.10	9.87
6i		77.24	6.69
6j		74.76	2.63
6k		39.43	ND
6l		38.06	ND
6m		55.59	7.32
6n		68.12	8.02
6o		66.47	5.34
6p		66.55	4.71
6q		54.56	9.63
6r		58.34	11.27
6s		38.70	ND
6t		40.72	ND
6u		30.18	ND

Compound	Substituents (R)	Inhibitory ratio (%)[a]	IC_{50} (mM)
6v		45.20	ND
6w		38.14	ND
6x		31.50	ND
Pyriftalid		18.35	ND
Bispyribac-sodium		34.06	ND

[a]Inhibitory ratio at 12.5 mM.
[b]ND, not determined.

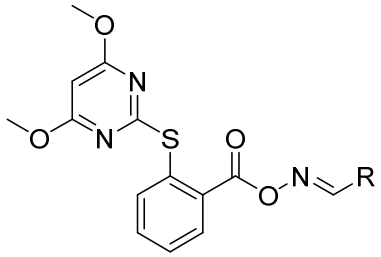


Figure 5. Chemical structure of compounds 6a-6x.

According to the *E.coli* ALS enzyme inhibition activity of the synthesized new compounds, the major SARs can be revealed. The substituted groups R (Figure 5) have significant impact on activity of the compounds. (4, 6-dimethoxypyrimidin-2-yl)-thiosalicylate aldoxime esters with aromatic substituent (6a) shows significantly higher activity (62.17%, IC_{50} = 7.22 mM) than that with aliphatic substituent (6x, 31.50%). Among the aromatic aldoxime esters, benzaldoxime ester 6a shows higher activity than heteroaromatic aldoxime esters 6q-6u. The introduction of electron-withdrawing groups (6e, 6f, 6v, 6w) to the benzaldoxime ester reduce the inhibitory activity against *E.coli* ALS enzyme, whereas the introduction of -OBoc or -NH_2 (6n-6p) to the benzaldoxime ester enhance the inhibitory activity against *E.coli* ALS enzyme. In particular, when the 4-methoxy of 6l (38.06%) is changed to 4-OBoc (6n), the *E.coli* ALS enzyme inhibition activity is recovered (68.12%, IC_{50} = 8.02 mM). The methyl or methoxy substituted benzaldoxime esters (6b, 6c, 6g, 6h, 6k, 6l, 6m) exhibit weakened or slightly enhanced inhibitory activity against *E.coli* ALS enzyme depending on the substituent position. It is worth noting that the fluoro-substituted benzaldoxime esters 6i (2-F), 6j (3-F), and 6d (4-F) also exhibit enhanced inhibitory activity against *E.coli* ALS enzyme, and the inhibitory ratio of 6i, 6j, and 6d were 77.24% (IC_{50} = 6.69 mM), 74.76 (IC_{50} = 2.63 mM), and 73.82 (IC_{50} = 2.71 mM), respectively, indicating that fluorine is an effective substituent group for ALS inhibitors. Fluorine is modestly more lipophilic than hydrogen, which is beneficial for hydrophobic interactions between the drug molecules and the receptor. Moreover, fluorine has small size and hardly produces any steric effect [20, 21]. Those properties of fluorine may contribute to the enhanced activity of fluoro-substituted benzaldoxime esters 6i, 6j, and 6d.

Molecular docking simulation was performed to gain a better understanding of the probable binding modes of 6a-6x to ALS (PDB code: 1YBH) [6]. All amino acid residues of ALS were interacted with compounds 6a-6x and Pyriftalid. As shown in Figure 6, the estimated interaction energies of 6a-6x and Pyriftalid are ranging from -24.11 to -47.12 kcal/mol. 6n and Pyriftalid exhibit the highest and the lowest interaction energies, respectively. In the binding mode, Pyriftalid with binding free energy of -24.11 kcal/mol binds to ALS via a hydrogen bond and a π-cation interaction with ARG377, a carbon hydrogen bond with SER653, and an alkyl interaction with MET490, Figure 7A. In contrast, compound 6n has the best estimated binding free energy of -47.12 kcal/mol and binds superiorly to ALS through three hydrogen bonds, three carbon hydrogen bonds, one π-cation interaction, one lone pair-π interaction, and two σ-π bonds. As shown in Figure 7B and D, 6n significantly binds to seven amino acids, including ARG377, SER653, LEU510, MET513, MET570, GLY511, and VAL485. The docking model reveals that the carbonyl oxygens of 6n form three hydrogen bonds with ARG377 and SER653. The methyl groups on the pyrimidine ring exhibit carbon hydrogen bond interactions with the backbone of LEU510 and VAL485. Those hydrogen bonds may strengthen the interactions of ligand molecules and amino acid residues in the ALS active site. The pyrimidine ring not only interacts with MET513 by a lone pair-π interaction, but also interacts with VAL485 by an σ-π bond. The σ-π bond interaction also occurs between the benzene ring of thiosalicylic acid and MET570. It is worth noting that the benzene ring of aldoxime forms π-cation interaction with

ARG377, justifying the modification of (4, 6-dimethoxypyrimidin-2-yl) thiosalicylic acid with aldoximes. The docking simulation together with herbicidal activity and *E.coli* ALS enzyme inhibition activity study suggest that 6n could be a potential and efficient inhibitor of ALS. Since fluoro-substituted benzaldoxime esters 6i, 6j, and 6d also exhibit high herbicidal activity and *E.coli* ALS enzyme inhibition activity, 6i was analyzed as an example to better understand the probable bind modes of fluoro-substituted benzaldoxime esters. As shown in Figure 7C, 6i binds to ALS through two π-π stacking interactions with TRP547, a hydrogen bond and a π-cation interaction with ARG377, and a halogen (fluorine) bond with ASP376. Halogen bonds are strong, specific, and directional interactions that give rise to well-defined structures. Therefore, the high herbicidal activity and *E.coli* ALS enzyme inhibition activity of fluoro-substituted benzaldoxime esters 6i, 6j, and 6d may be ascribed to the formation of halogen (fluorine) bonds between these three fluoro-substituted compounds and ALS.

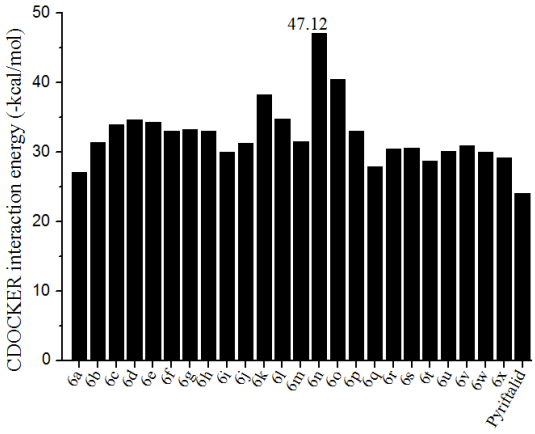


Figure 6. The CDOCKER interaction energy of compounds 6a-6x and Pyriftalid bound to ALS.

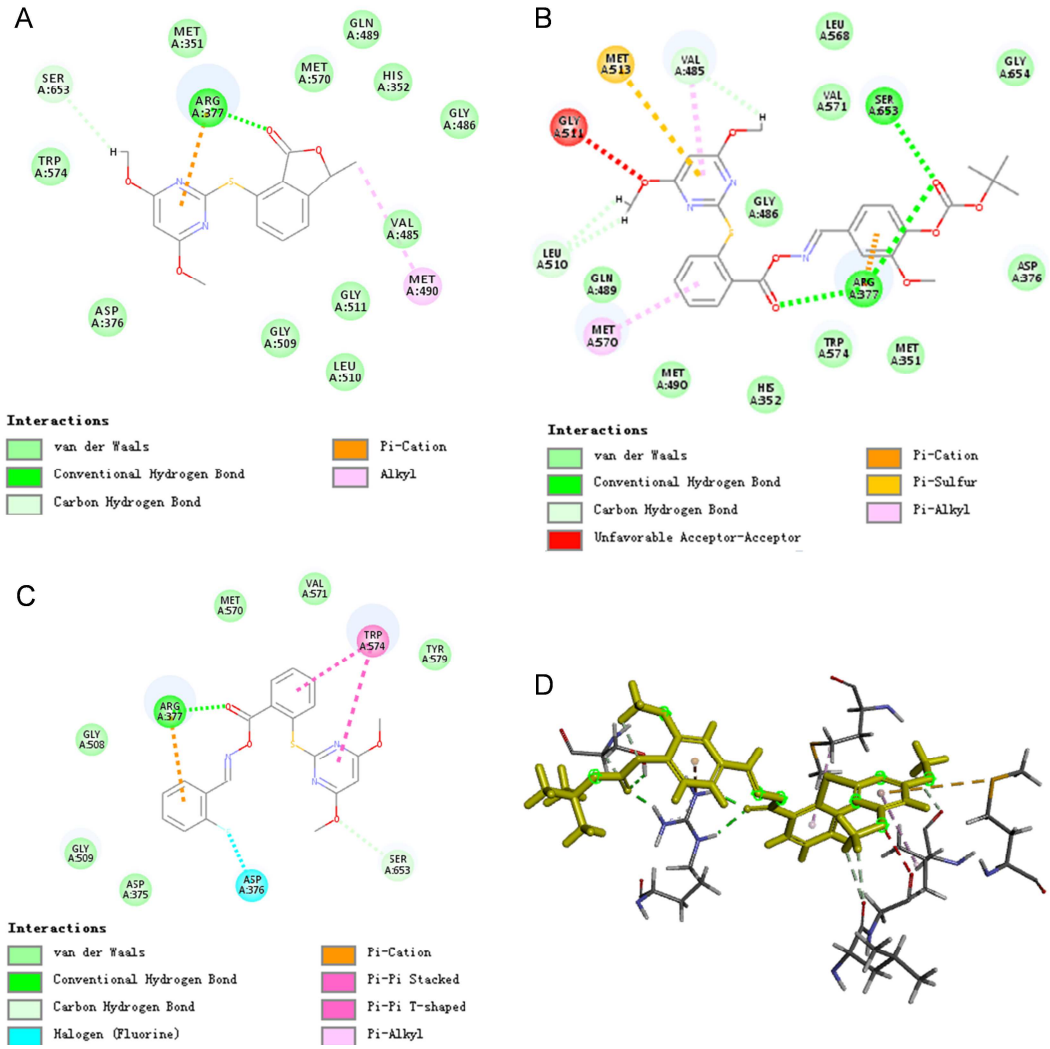


Figure 7. Molecular docking 2D and 3D modeling of Pyriftalid, 6n, and 6i with ALS. (A) Molecular docking 2D modeling of Pyriftalid with ALS. (B) Molecular docking 2D modeling of compound 6n with ALS. (C) Molecular docking 2D modeling of 6i with ALS. (D) Molecular docking 3D modeling of compound 6n with ALS.

4. Conclusion

In summary, a series of novel (4, 6-dimethoxypyrimidin-2-yl) thiosalicylate aldoxime esters were designed and synthesized as potential herbicidal ALS inhibitors. Among the synthesized new compounds, 6d, 6i, 6j, 6n, 6o, and 6p show high herbicidal activity (inhibitory ratio over 85%) against barnyard grass at 10 mg/L. 6d, 6i, 6j, 6n, 6o, and 6p also show efficient inhibitory activity against *E.coli* ALS enzyme. The SAR analysis reveals that benzaldoxime ester is a highly efficient substituent group for ALS inhibitors, which is better than heteroaromatic aldoxime esters and aliphatic aldoxime ester, and the introduction of -OBoc (6n, 6o), -NH_2 (6p), or -F (6d, 6i, 6j) to the benzaldoxime ester can further enhance the inhibitory activity against *E.coli* ALS enzyme. Molecular docking simulation discloses the probable binding modes of these compounds. The present work indicates that 6d, 6i, 6j, 6n, 6o, and 6p may serve as potential herbicidal ALS inhibitors.

Appendix

(4, 6-dimethoxypyrimidin-2-yl) thiosalicylate benzaldoxime ester (6a): Yield 80%. White solid. m.p. 60-63°C. ^{1}H NMR (300MHz, $CDCl_3$) δ 8.37 (s, 1H), 8.03 – 7.96 (m, 1H), 7.79 – 7.76 (m, 1H), 7.75 – 7.70 (m, 2H), 7.56 – 7.41 (m, 5H), 5.68 (s, 1H), 3.71 (s, 6H). ^{13}C NMR (75MHz, $CDCl_3$) δ 171.02, 169.90, 164.73, 157.02, 137.28, 134.61, 131.94, 131.92, 130.87, 130.70, 130.11, 129.14, 129.01, 128.61, 86.56, 54.18. HRMS (ESI) m/z: Calculated for $[M+H]^+$ 396.1013, found 396.1012.

(4, 6-dimethoxypyrimidin-2-yl) thiosalicylate 4-methylbenzaldoxime ester (6b): Yield 85%. White solid. m.p. 96-98°C. ^{1}H NMR (300MHz, $CDCl_3$) δ 8.33 (s, 1H), 8.03 – 7.96 (m, 1H), 7.81 – 7.74 (m, 1H), 7.61 (d, J = 8.2 Hz, 2H), 7.58 – 7.47 (m, 2H), 7.21 (s, 2H), 5.67 (s, 1H), 3.71 (s, 6H), 2.39 (s, 3H). ^{13}C NMR (75MHz, $CDCl_3$) δ 171.01, 169.93, 164.80, 156.99, 142.53, 137.27, 134.72, 131.85, 130.84, 130.66, 129.74, 129.12, 128.59, 127.29, 86.55, 54.17, 21.77. HRMS (ESI) m/z: Calculated for $[M+H]^+$ 410.1169, found 410.1169.

(4, 6-dimethoxypyrimidin-2-yl) thiosalicylate 4-methoxybenzaldoxime ester (6c): Yield 84%. White solid. m.p. 95-97°C. ^{1}H NMR (300MHz, $CDCl_3$) δ 8.30 (s, 1H), 7.97 (dd, *J* = 7.5, 1.8 Hz, 1H), 7.81 – 7.74 (m, 1H), 7.70 – 7.64 (m, 2H), 7.58 – 7.46 (m, 2H), 6.93 (d, *J* = 8.8 Hz, 2H), 5.68 (s, 1H), 3.85 (s, 3H), 3.71 (s, 6H). ^{13}C NMR (75MHz, $CDCl_3$) δ 171.01, 169.96, 164.89, 162.61, 156.58, 137.27, 134.81, 131.81, 130.82, 130.62, 130.36, 129.12, 122.54, 114.49, 86.54, 55.55, 54.18. HRMS (ESI) m/z: Calculated for $[M+H]^+$ 426.1118, found 426.1117.

(4, 6-dimethoxypyrimidin-2-yl) thiosalicylate 4-fluorobenzaldoxime ester (6d): Yield 75%. White solid. m.p. 100-102°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.34 (s, 1H), 7.97 (dd, *J* = 7.5, 1.8 Hz, 1H), 7.80 – 7.70 (m, 3H), 7.60 – 7.48 (m, 2H), 7.12 (t, *J* = 8.6 Hz, 2H), 5.68 (s, 1H), 3.71 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.99, 169.85, 164.94, (*J* = 252 Hz) 164.67, 163.26, 155.76, 137.26, 134.46, 131.96, 130.85, 130.67 (*J* = 9.0 Hz), 129.14, 126.34 (*J* = 3.3 Hz), 116.32 (*J* = 22.3 Hz), 86.53, 54.17. HRMS (ESI) m/z: Calculated for $[M+H]^+$ 414.0918, found 414.0919.

(4, 6-dimethoxypyrimidin-2-yl) thiosalicylate 4-chlorobenzaldoxime ester (6e): Yield 82%. White solid. m.p. 98-101°C. ^{1}H NMR (400MHz, $CDCl_3$) δ 8.34 (s, 1H), 7.97 (dd, *J* = 7.5, 1.7 Hz, 1H), 7.80 – 7.75 (m, 1H), 7.70 – 7.65 (m, 2H), 7.58 – 7.48 (m, 2H), 7.44 – 7.37 (m, 2H), 5.68 (s, 1H), 3.71 (s, 6H). ^{13}C NMR (100MHz, $CDCl_3$) δ 170.97, 169.81, 164.59, 155.81, 138.06, 137.28, 134.37, 132.03, 130.90, 130.63, 129.76, 129.38, 129.18, 128.58, 86.50, 54.23. HRMS (ESI) m/z: Calculated for $[M+H]^+$ 430.0623, found 430.0624.

(4, 6-dimethoxypyrimidin-2-yl) thiosalicylate 4-bromobenzaldoxime ester (6f): Yield 83%. White solid. m.p. 118-119°C. ^{1}H NMR (300MHz, $CDCl_3$) δ 8.32 (s, 1H), 8.04 – 7.94 (m, 1H), 7.77 (dd, *J* = 7.6, 1.4 Hz, 1H), 7.60 – 7.40 (m, 6H), 5.68 (s, 1H), 3.71 (s, 6H). ^{13}C NMR (75MHz, $CDCl_3$) δ 171.03, 169.86, 164.61, 155.91, 137.30, 134.40, 132.36, 132.03, 130.91, 130.75, 129.93, 129.17, 129.06, 126.55, 86.56, 54.20. HRMS (ESI) m/z: Calculated for $[M+H]^+$ 474.0118, found 474.0118.

(4, 6-dimethoxypyrimidin-2-yl) thiosalicylate 2-methylbenzaldoxime ester (6g): Yield 88%. White solid. m.p. 108-111°C. ^{1}H NMR (300MHz, $CDCl_3$) δ 8.61 (s, 1H), 8.00 (dd, *J* = 7.3, 1.9 Hz, 1H), 7.88 – 7.73 (m, 2H), 7.60 – 7.49 (m, 2H), 7.38 – 7.33 (m, 1H), 7.22 (d, *J* = 7.9 Hz, 2H), 5.69 (s, 1H), 3.72 (s, 6H), 2.44 (s, 3H). ^{13}C NMR (75MHz, $CDCl_3$) δ 170.94, 169.92, 164.79, 155.96, 138.35, 137.27, 134.72, 131.93, 131.62, 131.09, 130.91, 130.42, 129.23, 128.43, 128.41, 126.46, 86.46, 54.27, 19.97. HRMS (ESI) m/z: Calculated for $[M+H]^+$ 410.1169, found 410.1169.

(4, 6-dimethoxypyrimidin-2-yl) thiosalicylate 3-methylbenzaldoxime ester (6h): Yield 87%. White solid. m.p. 82-86°C. ^{1}H NMR (300MHz, $CDCl_3$) δ 8.34 (s, 1H), 8.02 – 7.94 (m, 1H), 7.80 – 7.73 (m, 1H), 7.61 (d, *J* = 1.9 Hz, 1H), 7.57 – 7.44 (m, 3H), 7.30 (dd, *J* = 6.5, 0.8 Hz, 2H), 5.68 (s, 1H), 3.72 (s, 6H), 2.38 (s, 3H). ^{13}C NMR (75MHz, $CDCl_3$) δ 171.01, 169.92, 164.75, 157.18, 138.88, 137.29, 134.71, 132.80, 131.90, 130.88, 130.67, 130.01, 129.15, 128.87, 128.73, 126.21, 86.55, 54.21, 21.34. HRMS (ESI) m/z: Calculated for $[M+H]^+$ 410.1169, found 410.1169.

(4, 6-dimethoxypyrimidin-2-yl) thiosalicylate 2-fluorobenzaldoxime ester (6i): Yield 77%. White solid. m.p.

90-93°C. ^{1}H NMR (400MHz, $CDCl_3$) δ 8.35 (s, 1H), 7.98 (d, *J* = 7.5 Hz, 1H), 7.77 (d, *J* = 7.5 Hz, 1H), 7.57 – 7.39 (m, 5H), 7.17 (dd, *J* = 11.2, 4.2 Hz, 1H), 5.68 (s, 1H), 3.71 (s, 6H). ^{13}C NMR (75MHz, $CDCl_3$) δ 171.02, 169.84, 162.96 (*J* = 246 Hz), 164.52, 155.83 (*J* = 3.00Hz), 137.30, 134.40, 132.28 (*J* =7.50 Hz), 132.03, 130.91, 130.73 (*J* = 2.25Hz), 130.60, 129.17, 124.70 (*J* = 3.0 Hz), 118.96 (*J* = 21.0 Hz), 114.85 (*J* = 22.5Hz), 86.56, 54.20. HRMS (ESI) m/z: Calculated for $[M+H]^+$ 414.0918, found 414.0919.

(4, 6-dimethoxypyrimidin-2-yl) thiosalicylate 3-fluorobenzaldoxime ester (6j): Yield 72%. White solid. m.p. 99-102°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.62 (s, 1H), 8.05 – 7.96 (m, 2H), 7.77 (dd, *J* = 7.6, 1.4 Hz, 1H), 7.55 (td, *J* = 7.5, 1.8 Hz, 1H), 7.53 – 7.49 (m, 1H), 7.49 – 7.42 (m, 1H), 7.21 – 7.1 5 (m, 1H), 7.10 (ddd, *J* = 9.5, 8.4, 1.1 Hz, 1H), 5.67 (s, 1H), 3.71 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.00, 169.88, 164.52, 161.78 (*J* = 252.75Hz), 150.68 (*J* = 5.2 Hz), 137.34, 134.39, 133.74 (*J* = 8.6 Hz), 132.05, 130.95, 130.73, 129.19, 127.99 (*J* = 1.50 Hz), 124.75 (*J* = 3.7 Hz), 118.15 (*J* = 10.3 Hz), δ 116.09 (*J* = 20.9 Hz), 86.57, 54.21. HRMS (ESI) m/z: Calculated for $[M+H]^+$ 414.0918, found 414.0919.

(4, 6-dimethoxypyrimidin-2-yl) thiosalicylate 2, 4-dimethoxybenzaldoxime ester (6k): Yield 91%. White oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.63 (s, 1H), 8.13 – 7.87 (m, 2H), 7.82 – 7.73 (m, 1H), 7.59 – 7.44 (m, 2H), 6.51 (dd, *J* = 8.7, 2.3 Hz, 1H), 6.43 (t, *J* = 3.8 Hz, 1H), 5.68 (s, 1H), 3.84 (s, 3H), 3.83 (s, 3H), 3.71 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.97, 170.04, 165.05, 164.19, 160.16, 152.75, 137.33, 135.05, 131.69, 130.77, 130.53, 129.13, 129.10, 111.38, 105.72, 98.29, 86.52, 55.69, 55.66, 54.14. HRMS (ESI) m/z: Calculated for $[M+H]^+$ 456.1224, found 456.1223.

(4, 6-dimethoxypyrimidin-2-yl) thiosalicylate 3, 4-dimethoxybenzaldoxime ester (6l): Yield 92%. White oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.26 (s, 1H), 7.95 (dd, *J* = 7.4, 1.7 Hz, 1H), 7.75 (dd, *J* = 7.5, 1.3 Hz, 1H), 7.52 (ddd, *J* = 10.3, 8.1, 1.6 Hz, 2H), 7.42 – 7.40 (m, 1H), 7.38 (d, *J* = 1.7 Hz, 1H), 7.09 (dd, *J* = 8.2, 1.8 Hz, 1H), 6.86 (d, *J* = 8.3 Hz, 1H), 5.66 (s, 1H), 3.91 (s, 3H), 3.90 (s, 3H), 3.69 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.92, 169.88, 164.82, 156.89, 152.39, 149.47, 137.24, 134.63, 131.80, 130.72, 130.55, 129.08, 124.20, 122.71, 110.64, 108.77, 86.45, 56.19, 56.04, 54.12. HRMS (ESI) m/z: Calculated for $[M+H]^+$ 456.1224, found 456.1223.

(4, 6-dimethoxypyrimidin-2-yl) thiosalicylate 3, 5-dimethoxybenzaldoxime ester (6m): Yield 93%. White oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (s, 1H), 7.98 (dd, *J* = 7.5, 1.6 Hz, 1H), 7.78 (dd, *J* = 7.6, 1.2 Hz, 1H), 7.53 (dtd, *J* = 20.7, 7.5, 1.4 Hz, 2H), 6.85 (d, *J* = 2.3 Hz, 2H), 6.56 (dd, *J* = 5.7, 3.4 Hz, 1H), 5.68 (s, 1H), 3.82 (s, 6H), 3.72 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.97, 169.87, 164.68, 161.08, 157.25, 137.33, 134.50, 131.99, 131.82, 130.89, 130.61, 129.20, 106.30, 104.67, 86.51, 55.75, 54.27. HRMS (ESI) m/z: Calculated for $[M+H]^+$ 456.1224, found 456.1223.

(4, 6-dimethoxypyrimidin-2-yl) thiosalincylate 3-methoxy-4-tert-butyloxy carbonyloxy-benzaldoxime ester (6n): Yield 87%. White solid. m.p. 100-102°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (s, 1H), 7.98 (dd, *J* = 7.5, 1.7 Hz, 1H), 7.77 (dd, *J* = 7.6, 1.4 Hz, 1H), 7.59 – 7.50 (m, 1H), 7.47 (d, *J* = 1.7 Hz, 1H), 7.20 – 7.16 (m, 1H), 7.16 – 7.11 (m, 1H), 5.68 (s, 1H), 3.90 (s, 3H), 3.71 (s, 6H), 1.55 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.97, 169.85, 164.67, 156.43, 151.88, 150.99, 143.29, 137.30, 134.45, 132.01, 130.91, 130.61, 129.20, 128.71, 122.99, 122.90, 110.52, 86.48, 84.05, 56.37, 54.28, 27.72. HRMS (ESI) m/z: Calculated for $[M+H]^+$ 542.1592, found 542.1587.

(4, 6-dimethoxypyrimidin-2-yl) thiosalincylate 4-tert-butyloxycarbonyloxyl benzaldoxime ester (6o): Yield 80%. White solid. m.p. 94-96°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (s, 1H), 7.96 (dd, *J* = 7.5, 1.5 Hz, 1H), 7.74 (dd, *J* = 12.6, 4.9 Hz, 3H), 7.51 (dtd, *J* = 21.4, 7.5, 1.4 Hz, 2H), 7.23 (d, *J* = 8.7 Hz, 2H), 5.66 (s, 1H), 3.69 (s, 6H), 1.54 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.91, 169.76, 164.53, 155.85, 153.66, 151.17, 137.14, 134.36, 131.88, 130.78, 130.63, 129.73, 129.05, 127.47, 121.84, 86.46, 84.13, 54.09, 27.71. HRMS (ESI) m/z: Calculated for $[M+H]^+$ 512.1496, found 512.1500.

(4, 6-dimethoxypyrimidin-2-yl) thiosalicylate 2-aminobenzaldoxime ester (6p): Yield 75%. Yellow soild. m.p. 88-91°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43 (s, 1H), 7.98 (d, *J* = 7.5 Hz, 1H), 7.79 (d, *J* = 7.5 Hz, 1H), 7.54 (ddd, *J* = 19.7, 11.3, 6.9 Hz, 2H), 7.23 (d, *J* = 7.1 Hz, 1H), 7.16 – 7.09 (m, 1H), 6.78 – 6.67 (m, 2H), 5.68 (s, 2H), 3.72 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.99, 169.88, 164.38, 158.93, 153.89, 147.76, 137.34, 134.41, 133.62, 132.44, 131.87, 130.73, 129.10, 116.61, 115.90, 112.03, 86.56, 54.20. HRMS (ESI) m/z: Calculated for $[M+H]^+$ 411.1122, found 411.1119.

(4, 6-dimethoxypyrimidin-2-yl) thiosalicylate pyridine-4-aldoxime ester (6q): Yeild 85%. Lilac soild. m.p. 76-80°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.73 (d, *J* = 5.9 Hz, 2H), 8.36 (s, 1H), 7.99 (dd, *J* = 7.6, 1.6 Hz, 1H), 7.78 (dd, *J* = 7.7, 1.2 Hz, 1H), 7.63 – 7.60 (m, 2H), 7.55 (ddd, *J* = 16.4, 7.6, 1.5 Hz, 2H), 5.68 (s, 1H), 3.71 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.03, 169.73, 164.23, 154.79, 150.47, 137.84, 137.34, 133.92, 132.29, 131.01, 130.88, 129.22, 122.17, 86.58, 54.22. HRMS (ESI) m/z: Calculated for $[M+H]^+$ 397.0965, found 397.0961.

(4, 6-dimethoxypyrimidin-2-yl) thiosalicylate pyridine-2-aldoxime ester (6r): Yeild 88%. White soild. m.p. 87-90°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (ddd, *J* = 4.9, 1.6, 1.0 Hz, 1H), 8.51 (s, 1H), 8.10 (d, *J* = 7.9 Hz, 1H), 7.99 (dd, *J* = 7.6,

1.6 Hz, 1H), 7.81 – 7.73 (m, 2H), 7.54 (dtd, *J* = 21.5, 7.5, 1.5 Hz, 2H), 7.38 – 7.34 (m, 1H), 5.67 (s, 1H), 3.71 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.97, 169.76, 164.39, 157.60, 150.03, 149.96, 137.35, 136.84, 134.15, 132.10, 131.00, 130.85, 129.15, 125.68, 122.32, 86.65, 54.17. HRMS (ESI) m/z: Calculated for $[M+H]^+$ 397.0965, found 397.0961.

(4, 6-dimethoxypyrimidin-2-yl) thiosalicylate pyridine-3-aldoxime ester (6s): Yeild 77%. White soild. m.p. 99-101°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.80 (s, 1H), 8.75 – 8.69 (m, 1H), 8.41 (s, 1H), 8.18 (d, *J* = 8.0 Hz, 1H), 7.98 (dd, *J* = 7.6, 1.5 Hz, 1H), 7.81 – 7.75 (m, 1H), 7.60 – 7.49 (m, 2H), 7.37 (dd, *J* = 7.9, 4.8 Hz, 1H), 5.67 (s, 1H), 3.71 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.02, 169.80, 164.47, 154.19, 152.77, 150.27, 137.30, 134.66, 134.14, 132.17, 130.97, 130.80, 129.20, 126.44, 124.00, 86.58, 54.22. HRMS (ESI) m/z: Calculated for $[M+H]^+$ 397.0965, found 397.0961.

(4, 6-dimethoxypyrimidin-2-yl) thiosalicylate 2-furanaldoxime ester (6t): Yeild 71%. Yellow soild. m.p. 88-91°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (s, 1H), 7.96 (dd, *J* = 7.5, 1.7 Hz, 1H), 7.75 (dd, *J* = 7.6, 1.2 Hz, 1H), 7.57 (dd, *J* = 3.5, 1.6 Hz, 1H), 7.53 (dd, *J* = 7.6, 1.8 Hz, 1H), 7.50 (dd, *J* = 7.5, 1.4 Hz, 1H), 6.92 (d, *J* = 3.5 Hz, 1H), 6.51 (dd, *J* = 3.5, 1.8 Hz, 1H), 5.67 (s, 1H), 3.70 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.89, 169.71, 164.33, 146.27, 146.11, 145.15, 137.15, 134.33, 131.88, 130.82, 130.55, 129.06, 116.54, 112.24, 86.47, 54.07. HRMS (ESI) m/z: Calculated for $[M+H]^+$ 386.0805, found 386.0804.

(4, 6-dimethoxypyrimidin-2-yl) thiosalicylate 2-thenaldoxime ester (6u): Yeild 73%. White soild. m.p. 95-98°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (s, 1H), 7.99 – 7.94 (m, 1H), 7.80 – 7.75 (m, 1H), 7.59 – 7.47 (m, 3H), 7.40 (dd, *J* = 3.7, 1.2 Hz, 1H), 7.10 (dd, *J* = 5.1, 3.6 Hz, 1H), 5.68 (s, 1H), 3.71 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.98, 169.86, 164.41, 151.35, 137.29, 134.48, 133.12, 132.99, 131.91, 130.87, 130.84, 130.64, 129.13, 127.68, 86.53, 54.15. HRMS (ESI) m/z: Calculated for $[M+H]^+$ 402.0587, found 402.0591.

(4, 6-dimethoxypyrimidin-2-yl) thiosalicylate 4-trifluoromethyl benzaldoxime ester (7v): Yield 78%. White solid. m.p. 80-83°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.35 (s, 1H), 7.99 – 7.89 (m, 1H), 7.79 (d, *J* = 8.1 Hz, 2H), 7.75 – 7.69 (m, 1H), 7.62 (d, *J* = 8.2 Hz, 2H), 7.48 (dtd, *J* = 17.7, 7.5, 1.6 Hz, 2H), 5.61 (s, 1H), 3.64 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.01, 169.79, 164.46, 155.48, 137.30, 134.16, 133.57 (*J* = 4.9 Hz), 133.17, 132.15, 130.95, 130.79, 129.19, 128.81, 125.99 (*J* = 3.8 Hz), 121.92, 86.56, 54.20. HRMS (ESI) m/z: Calculated for $[M+H]^+$ 464.0886, found 464.0884.

(4, 6-dimethoxypyrimidin-2-yl) thiosalicylate 4-cyanobenzaldoxime ester (6w): Yield 52%. White solid. m.p. 146-149°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.41 (s, 1H), 7.98 (dd, *J* = 7.5, 1.7 Hz, 1H), 7.85 (d, *J* = 8.4 Hz, 2H), 7.78 (dd, *J* = 7.6, 1.3 Hz, 1H), 7.76 – 7.71 (m, 2H), 7.55 (dtd, *J* = 18.4, 7.5, 1.6 Hz, 2H), 5.68 (s, 1H), 3.71 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.00, 169.74, 164.35, 155.03, 137.32, 134.38, 133.97, 132.75, 132.26, 130.98, 130.80, 129.22, 128.94, 118.17, 115.18, 86.56, 54.23. HRMS (ESI) m/z: Calculated for $[M+H]^+$ 421.0965, found 421.0969.

(4, 6-dimethoxypyrimidin-2-yl) thiosalicylate cinnamaldoxime ester (6x): Yield 48%. White solid. m.p. 95-98°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (dd, J = 6.7, 2.7 Hz, 1H), 7.98 (dd, J = 7.6, 1.6 Hz, 1H), 7.76 (dd, J = 7.6, 1.1 Hz, 1H), 7.55 (td, J = 7.6, 1.7 Hz, 1H), 7.51 (dd, J = 7.5, 1.4 Hz, 1H), 7.47 (dd, J = 7.9, 1.5 Hz, 2H), 7.38 (d, J = 7.3 Hz, 2H), 7.01 – 6.96 (m, 2H), 5.69 (s, 1H), 3.73 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.01, 169.80, 164.79, 158.06, 143.99, 137.06, 135.23, 134.39, 131.96, 130.99, 130.73, 130.03, 129.10, 127.56, 123.73, 120.34, 86.58, 54.18. HRMS (ESI) m/z: Calculated for $[M+H]^+$ 422.1186, found 422.1189.

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Conflict of Interest

The authors declare no conflict of interest.

References

[1] Chen, C. N.; Lv, L. L.; Ji, F. Q.; Chen, Q.; Xu, H.; Niu, C. W.; Xi, Z.; Yang, G. F. (2009). Design and synthesis of N-2, 6-difluorophenyl-5-methoxyl-1, 2, 4-triazolo [1, 5-a]-pyrimidine-2-sulfonamide as acetohydroxyacid synthase inhibitor. *Bioorganic & Medicinal Chemistry*, 17: 3011-3017.

[2] Chen, C. N.; Chen, Q.; Liu, Y. C.; Zhu, X. L.; Niu, C. W.; Xi, Z.; Yang, G. F. (2010). Syntheses and herbicidal activity of new triazolopyrimidine-2-sulfonamides as acetohydroxyacid synthase inhibitor. *Bioorganic & Medicinal Chemistry*, 18: 4897-4904.

[3] Qu, R. Y.; Yang, J. F.; Devendar, P.; Kang, W. M.; Liu, Y. C.; Chen, Q.; Niu, C. W.; Xi, Z.; Yang, G. F. (2017). Discovery of New 2-[(4, 6-Dimethoxy-1, 3, 5-triazin-2-yl) oxy]-6-(substituted phenoxy) benzoic Acids as Flexible Inhibitors of Arabidopsis thaliana Acetohydroxyacid Synthase and Its P197L Mutant. *Journal of Agricultural and Food Chemistry*, 65: 11170-11178.

[4] Bar-Ilan, A.; Balan, V.; Tittmann, K.; Golbik, R.; Vyazmensky, M.; Hübner, G.; Barak, Z. e.; Chipman, D. M. (2001). Binding and Activation of Thiamin Diphosphate in Acetohydroxyacid Synthase. *Biochemistry*, 40: 11946-11954.

[5] Ji, F. Q.; Niu, C. W.; Chen, C. N.; Chen, Q.; Yang, G. F.; Xi, Z.; Zhan, C. G. (2008). Computational design and discovery of conformationally flexible inhibitors of acetohydroxyacid synthase to overcome drug resistance associated with the W586L mutation. *ChemMedChem*, 3: 1203-1206.

[6] Pang, S. S.; Guddat, L. W.; Duggleby, R. G. (2003). Molecular basis of sulfonylurea herbicide inhibition of acetohydroxyacid synthase. *The Journal of Biological Chemistry*, 278: 7639-7644.

[7] Liu, Y. C.; Qu, R. Y.; Chen, Q.; Yang, J. F.; Cong-Wei, N.; Zhen, X.; Yang, G. F. (2016). Triazolopyrimidines as a New Herbicidal Lead for Combating Weed Resistance Associated with Acetohydroxyacid Synthase Mutation. *Journal of Agricultural and Food Chemistry*, 64: 4845-4857.

[8] McCourt, J. A.; Pang, S. S.; King-Scott, J.; Guddat, L. W.; Duggleby, R. G. (2006). Herbicide-binding sites revealed in the structure of plant acetohydroxyacid synthase. *Proceedings of the National Academy of Sciences of the United States of America*, 103: 569.

[9] Garcia, M. D.; Nouwens, A.; Lonhienne, T. G.; Guddat, L. W. (2017). Comprehensive understanding of acetohydroxyacid synthase inhibition by different herbicide families. *Proceedings of the National Academy of Sciences*, 114: E1091.

[10] Koo, S. J.; Kuramochi, H.; Chae, S. H. (2006). Herbicidal efficacy and selectivity of pyribenzoxim in turfgrasses. *Weed Biology and Management*, 6: 96-101.

[11] Chang, H.-R.; Keum, Y. S.; Koo, S.-J.; Moon, J.-K.; Kim, K.; Kim, J.-H. (2011). Metabolism of a New Herbicide, [14C] Pyribenzoxim, in Rice. *Journal of Agricultural and Food Chemistry*, 59: 1918-1923.

[12] Kim, B. H.; Kim, H. J.; Ok, J. H.; Kang, S. H. (2001). Analysis of a new herbicide (pyribenzoxim) residues in soil using direct-extract-injection HPLC with column switching. *Journal of Liquid Chromatography & Related Technologies*, 24: 669-678.

[13] Chauhan, B. S.; Johnson, D. E. (20110. Growth Response of Direct-Seeded Rice to Oxadiazon and Bispyribac-Sodium in Aerobic and Saturated Soils. *Weed Science*, 59: 119-122.

[14] Darren, W. L.; Stephen, E. H. (2006). Seasonal Effects on Annual Bluegrass (Poa annua) Control in Creeping Bentgrass with Bispyribac-Sodium. *Weed Technology*, 20: 722-727.

[15] Seo, J.-S.; Moon, J.-K.; Kim, J.-H. (2012). Photodegradation of pyribenzoxim in water. *Journal of the Korean Society for Applied Biological Chemistry*, 55: 391-396.

[16] Hu, Q.; Lin, G.-S.; Duan, W.-G.; Huang, M.; Lei, F.-H. (2017). Synthesis and Biological Activity of Novel (Z) - and (E) - Verbenone Oxime Esters. *Molecules*, 22: 1678.

[17] Chauhan, B. S.; Abeysekara, A. S. K.; Kulatunga, S. D.; Wickrama, U. B. (2013). Performance of Different Herbicides in a Dry-Seeded Rice System in Sri Lanka. *Weed Technology*, 27: 459-462.

[18] Schloss, J. V.; Ciskanik, L. M.; Dyk, D. E. V. (1988). Origin of the herbicide binding site of acetolactate synthase. *Nature*, 331: 360-362.

[19] Grundman, O.; Khozin-Goldberg, I.; Raveh, D.; Cohen, Z.; Vyazmensky, M.; Boussiba, S.; Shapira, M. (2012). Cloning, mutagenesis, and characterization of the microalga Parietochloris incisa acetohydroxyacid synthase, and its possible use as an endogenous selection marker. *Biotechnology and Bioengineering*, 109: 2340-2348.

[20] Meanwell, N. A. (2018). Fluorine and Fluorinated Motifs in the Design and Application of Bioisosteres for Drug Design. *Journal of Medicinal Chemistry*, 61: 5822-5880.

[21] Hagmann, W. K. (2008). The many roles for fluorine in medicinal chemistry. *Journal of Medicinal Chemistry*, 51: 4359-4369.