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Design, Synthesis and Biological Activity of Novel Diazabicyclo Derivatives as Safeners

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ABSTRACT

Herbicide safeners selectively protect crops from herbicide damage without reducing the herbicidal efficiency on target weed species. The title compounds were designed by intermediate derivatization approach and fragment splicing in order to exploit novel potential safeners. A total of thirty-one novel diazabicyclo derivatives were synthesized by microwave-assistant method using isoxazole-4-carbonyl chloride and diazabicyclo derivatives. All synthetic compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR, HRMS. The bioassay results demonstrated that most of the title compounds could reduce the nicosulfuron phytotoxicity on maize. The glutathione-S-transferase (GST) activity *in vivo* was assayed and compound **4 (S15)** revealed an inspiring safener activity comparable to commercialized safeners isoxadifen-ethyl and BAS-145138. The molecular docking model exhibited that the competition at the active sites of target enzymes between compound **4 (S15)** and nicosulfuron was investigated with respect to herbicide detoxification. The current work not only provided a powerful supplement to the intermediate derivatization approach and fragment splicing in design pesticide bioactive molecules, but also assisted safener development and optimization.

Keywords: *Fragment splicing, Intermediate derivatization approach, N-substituted diazabicyclo, Microwave-assistant Synthesis, Herbicide safener*

INTRODUCTION

The history of agriculture has shown that humans must effectively control weeds all times. One of the most effective and modern methods of controlling weeds in growing crops is the use of herbicides.[1] The development of chemical herbicides began with the discovery of selective herbicide 2, 4-D. So far, herbicides have entered an efficient development stage, and the variety has been continuously updated.[2] Herbicides mainly used in wheat, rice, potato, cotton, and maize fields around the world, which greatly improve the crop production and agricultural productivity.[3,4]

Sulfonylurea herbicides were the most widely used herbicides in the world with the widest range of applications and the deepest research. The sulfonylurea herbicide nicosulfuron is a broad-spectrum, flexible and inner-absorption conducting herbicide, which is used to control annual grasses and certain broadleaf weeds in maize or other field crops.[5-7] Nicosulfuron has a wide maize safety margin, but due to large amounts of its application and toxigenic potential, certain maize hybrids and inbreds can be severely injured by nicosulfuron.[8,9] Some researches showed that maize seedlings can be severely inhibited after nicosulfuron treatment from 7 to 14 days.[10] Nicosulfuron is easy to cause phytotoxicity to sensitive crops and may have adverse effects on later crops.[11] For sensitive maize, it caused severe injury to lipids and changed the ascorbate-glutathione cycle, with symptoms ranging from severe stunting of plant growth to death.[12] In order to solve these serious problems, many methods have been reported, including the development of new herbicides and so on.[13-15] Other than these methods, using herbicide safeners, is also an effective solution.

Herbicide safeners selectively protect crops from certain herbicides without affecting weed control effectiveness.[16,17] Since the first phenomenon of herbicide safening was discovered, the mechanisms of action of safeners have received considerable attention. Nevertheless, due to the complexity of the interactions between the safeners and the molecular mechanisms and the expression of the enzyme, there is no conclusive mechanism explaining action of the available safeners.[18,19] It is widely accepted that safeners can improve crop metabolism and detoxification by upregulating cytochrome-P450-monooxygenases (CYPs), glutathione-S-transferases (GSTs), glutathione (GSH) and ATP-binding casette (ABC) transporter proteins that detoxify herbicides.[20-23] The use of a suitable safener can reduce or eliminate herbicide damage to crop without affecting the herbicidal activity. As a result, it is highly urgent to develop new safeners to provide new solutions to weed control problems.

Isoxadifen-ethyl was the first safener launched by Bayer CropScience AG.[16] It can protect maize from herbicide damage with various modes of action, which could increase the absorption, translocation and detoxification of sulfonylurea herbicides in maize.[24] Isoxadifen-ethyl can effectively alleviate the phytotoxicity of nicosulfuron to maize and increase the plant's metabolism of nicosulfuron through non-P450-catalyzed routes.[25] BAS-145138, diazabicyclo herbicide safener, can protect sorghum and reduce herbicide toxicity by enhancing the metabolism of lauric acid.[26] Also, BAS-145138 can accelerate the metabolic rate of chlorimuron ethyl in leaves and roots of maize without affecting the absorption of chlorimuron ethyl in

maize.[27] It was shown that the concentration of safety agent was linear with GSH content and GST activity.

The Intermediate Derivatization Methods (IDM) is a new approach for discovering and developing novel patentable leads or target compounds by chemical reaction utilizing various intermediates.[28] The key to the success of the IDM is to find the right intermediate. Successful examples such as the development of herbicides pyribenzoxim (derivatives of bispyribac-sodium), butafenacil (derivatives of flupropacil), and herbicide safener fenchlorazole-ethyl.[29-31] Fragment splicing is also the main strategy to design and optimize of new skeleton structures with target biological activity. For instance, the new fungicide fluopyram was designed by fragment splicing of fluopicolide and flutolanil.[32] Combining the structural features of diphenylmethoxyacetic acid and the experimental Monsanto safener benzhydryloxy acetic acid led to the strong maize safener isoxadifen-ethyl.[33] In connection with the facts mentioned above, the attempt to design novel compounds with safener activity will be valuable for alleviating the toxity of herbicides. Based on our work on synthesis of nitrogen-containing heterocyclic compound,[34,35] herein we use *N*-substituted diazabicyclo as the key intermediate, then designed a series of novel substituted diazabicyclo derivatives utilizing the IDM combined with fragment splicing (**Scheme 1**). The bioassay was also conducted for evaluating the safener activity of the title compounds. Furthermore, molecular structure comparisons and molecular docking were performed to determine the possible detoxifcation mechanism for the safener and to design more effective new safeners.

MATERIALS AND METHODS

Equipment and Materials

The melting points were obtained on a Beijing Taike X-4 stage apparatus and uncorrected. Infrared (IR) spectra were measured as KBr pellets on ALPHA-T. The NMR spectra were recorded on a Bruker AV-400 spectrometer, using TMS as an internal standard. The high-resolution mass spectrum (HRMS) were recorded on a Xevo TQ spectrometer. X-ray diffraction data were collected on a Rigaku R-AXIS RAPID diffractometer. The four isoxazole-4-carbonyl chlorides (5-methyl-3-phenylisoxazole-4-carbonyl chloride, 3-(2-chloro-6-fluorophenyl)-5-methylisoxazole-4-carbonyl chloride, 3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carbonyl chloride and 3-(2-chlorophenyl)-5-methylisoxazole-4-carbonyl chloride) were purchased from Hubei Xinmingtai Chemical Co., Ltd. Purification by column chromatography on silica gel. Microwave activation was performed using an XH-100A focused microwave.

General Procedure for the Synthesis of Compounds 4

Diamine (0.05 mol), ethyl levulinate (0.05 mol) (or ethyl 4-acetobutyrate) and montmorillonite k10 (3 g) were added to 100mL three-necked flask sequentially, the reaction was stirred for 12 min under the microwave conditions (300 W, 25℃). The products obtained above without reprocessing and anhydrous K_2CO_3 (1.5 eq) were dissolved in $CHCl_3$ at room temperature. The isoxazole-4-carbonyl chloride (1.5 eq) was added to the mixture slowly at 0℃ and the reaction was monitored by TLC.

After the reaction was over, the solution was washed with water. The organic phase was dried over anhydrous $MgSO_4$. After the solvent was removed off under reduced pressure, the crude products were purified by column chromatography on silica gel eluting with ethyl acetate and petroleum ether (6:1) or recrystallized with ethyl acetate and light petroleum ether.

3-phenyl-4-(5-methyl-8-oxa-1,4-diazabicyclo[3.3.0]octane)-5-methyl-isoxazolec arboxamide ***4 (S1)***. Yield: 65%; m.p. 169.9-171.0℃; ^{1}H NMR ($CDCl_3$): δ 7.69-7.28 (m, 5H, Ar-H), 3.92-3.87 (m, 2H, N-$\underline{CH_2}$-CH_2-N), 3.21-2.94 (m, 2H, N-CH_2-$\underline{CH_2}$-N), 2.76-2.67 (m, 2H, N-CH_2-CH_2-N), 2.52 (S, 3H, CH_3), 2.49-2.43 (m, 2H, N-CH_2-CH_2-N), 1.73 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 175.21, 169.12, 160.90, 159.23, 130.69, 129.16, 129.16, 128.36, 127.11, 127.11, 112.64, 83.53, 47.52, 38.95, 34.06, 32.15, 22.81, 11.35; HRMS (ESI) calcd for $C_{18}H_{19}N_3O_3$ ($[M+H]^+$) 326.1499, found 326.1495.

3-(2'-chloro-phenyl)-4-(5-methyl-8-oxa-1,4-diazabicyclo[3.3.0]octane)-5-methyl -oxazole carboxamide ***4 (S2)***. Yield: 51%; m.p. 219.5-220.6℃; ^{1}H NMR ($CDCl_3$): δ 7.55-7.39 (m, 4H, Ar-H), 3.97-3.40(m, 2H, N-$\underline{CH_2}$-CH_2-N), 3.26-3.05 (m, 2H, N-CH_2-$\underline{CH_2}$-N), 2.70-2.67 (m, 2H, C-$\underline{CH_2}$-CH_2-C=O), 2.58 (s, 3H, CH_3), 2.54-2.30 (m, 2H, C-CH_2-$\underline{CH_2}$-C=O), 1.66 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 175.60, 169.00, 160.40, 158.86, 132.67, 131.63, 131.43, 130.27, 127.81, 127.40, 114.25, 83.74, 47.33, 39.26, 33.59, 32.11, 22.97, 12.37; HRMS (ESI) calcd for $C_{18}H_{18}ClN_3O_3$ ($[M+H]^+$) 360.1109, found 360.1107.

3-(2',6'-dichloro-phenyl)-4-(5-methyl-8-oxa-1,4-diazabicyclo[3.3.0]octane)-5-methyl-isoxazole carboxamide ***4 (S3)***. Yield: 57%; m.p. 197.4-198.3℃; ^{1}H NMR ($CDCl_3$): δ 7.48-7.38 (m, 3H, Ar-H), 4.05-3.56 (m, 2H, N-$\underline{CH_2}$-CH_2-N), 3.45-3.10 (m, 2H, N-CH_2-$\underline{CH_2}$-N), 2.81-2.67 (m, 2H, C-$\underline{CH_2}$-CH_2-C=O), 2.60 (s, 3H, CH_3), 2.54-2.32 (m, 2H, C-CH_2-$\underline{CH_2}$-C=O), 1.62 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 175.66, 168.48, 159.72, 157.00, 135.71, 135.04, 131.75, 128.65, 128.20, 127.19, 114.52, 83.81, 47.20, 39.33, 33.60, 32.09, 22.80, 12.75; HRMS (ESI) calcd for $C_{18}H_{17}Cl_2N_3O_3$ ($[M+H]^+$) 394.0720, found 394.0719.

3-(2'-fluoro-6'-chloro-phenyl)-4-(5-methyl-8-oxa-1,4-diazabicyclo[3.3.0]octane)-5-methyl-isoxazolecarboxamide ***4 (S4)***. Yield: 50%; m.p. 166.7-167.8℃; ^{1}H NMR ($CDCl_3$): δ 7.47-7.34 (m, 3H, Ar-H), 4.02-3.50 (d, 2H, N-$\underline{CH_2}$-CH_2-N), 3.40-2.59 (d, 2H, N-CH_2-$\underline{CH_2}$-N), 2.80-2.64 (m, 2H, C-$\underline{CH_2}$-CH_2-C=O), 2.55-2.34 (m, 2H, C-CH_2-$\underline{CH_2}$-C=O), 2.59 (S, 3H, CH_3), 1.64 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 175.62, 168.58, 161.87, 159.86, 159.35, 153.68, 134.68, 132.16, 125.99, 116.75, 114.67, 83.73, 47.22, 39.26, 33.61, 32.08, 22.76, 12.47; HRMS (ESI) calcd for $C_{18}H_{17}ClFN_3O_3$ ($[M+H]^+$) 378.1015, found 378.1019.

3-phenyl-4-(5-methyl-9-oxa-1,4-diazabicyclo[3.4.0]nonane)-5-methyl-isoxazolecarboxamide ***4 (S5)***. Yield: 74%; m.p. 152.9-154.5℃; ^{1}H NMR ($CDCl_3$): δ 7.61-7.29 (m, 5H, Ar-H), 3.97-3.94 (d, 2H, N-$\underline{CH_2}$-C-CH_2-N), 3.15-2.81 (m, 4H, N-CH_2-C-CH_2-N), 2.53 (S, 3H, CH_3), 2.52-1.76 (m, 4H, N-CH_2-CH_2-N), 1.69 (s, 3H, CH_3), 1.36-1.32 (d, 2H, N-CH_2-C-$\underline{CH_2}$-N); ^{13}C NMR ($CDCl_3$): δ 172.81, 172.74, 172.72, 172.67, 163.56, 163.54, 160.13, 160.11, 130.52, 128.98, 128.15, 127.83,

112.21, 43.99, 34.64, 33.99, 29.25, 22.53, 20.53, 11.89; HRMS (ESI) calcd for $C_{19}H_{21}N_3O_3$ ($[M+Na]^+$) 362.1477, found 362.1475.

3-(2'-chloro-phenyl)-4-(5-methyl-9-oxa-1,4-diazabicyclo[3.4.0]nonane)-oxazole carboxamide ***4 (S6)***. Yield: 66%; m.p. 181.7-183.1℃; ^{1}H NMR ($CDCl_3$): δ 7.53-7.40 (m, 4H, Ar-H), 4.04-2.71 (m, 4H, N-$\underline{CH_2}$-CH_2-$\underline{CH_2}$-N), 2.58 (s, 3H, CH_3), 2.39-1.71 (m, 4H, C-CH_2-CH_2-C=O), 1.63 (s, 3H, CH_3), 1.51-1.47 (m, 2H, N-CH_2-$\underline{CH_2}$-CH_2-N); ^{13}C NMR ($CDCl_3$): δ 172.67, 163.64, 132.91, 131.55, 131.43, 131.43, 130.14, 130.14, 127.71, 127.38, 127.38, 113.40, 44.58, 35.00, 33.79, 29.25, 23.37, 19.37, 12.25; HRMS (ESI) calcd for $C_{19}H_{20}ClN_3O_3$ ($[M+H]^+$) 374.1266, found 374.1268.

3-(2',6'-dichloro-phenyl)-4-(5-methyl-9-oxa-1,4-diazabicyclo[3.4.0]nonane)-5-methyl-isoxazole carboxamide ***4 (S7)***. Yield: 71%; m.p. 200-201℃; ^{1}H NMR ($CDCl_3$): δ 7.47-7.34 (m, 3H, Ar-H), 3.98-2.67 (m, 4H, N-$\underline{CH_2}$-CH_2-$\underline{CH_2}$-N), 2.60 (s, 3H, CH_3), 2.39-2.25 (m, 2H, C-$\underline{CH_2}$-CH_2-C=O), 1.57 (s, 3H, CH_3), 1.51-1.49 (m, 2H, C-CH_2-$\underline{CH_2}$-C=O), 0.85-0.81 (m, 2H, N-CH_2-$\underline{CH_2}$-CH_2-N); ^{13}C NMR ($CDCl_3$): δ 172.59, 163.37, 156.56, 135.53, 135.37, 131.80, 131.80, 128.80, 128.80, 128.27, 127.04, 113.63, 44.46, 35.05, 33.64, 29.21, 23.51, 19.17, 12.43; HRMS (ESI) calcd for $C_{19}H_{19}Cl_2N_3O_3$ ($[M+H]^+$) 408.0876, found 408.0873.

3-(2'-fluoro-6'-chloro-phenyl)-4-(5-methyl-9-oxa-1,4-diazabicyclo[3.4.0]nonane)-5-methyl-isoxazolecarboxamide ***4 (S8)***. Yield: 60%; m.p. 183.0-183.8℃; ^{1}H NMR ($CDCl_3$): δ 7.47-7.12 (m, 3H, Ar-H), 4.02-3.98 (m, 2H, N-$\underline{CH_2}$-C-CH_2-N), 3.44-3.20 (m, 2H, N-$\underline{CH_2}$-C-CH_2-N), 2.85-2.69 (m, 2H, N-CH_2-C-$\underline{CH_2}$-N), 2.62 (S,

3H, CH_3), 2.36-1.67 (m, 4H, N-CH_2-CH_2-N), 1.61 (s, 3H, CH_3), 1.54-1.51 (m, 2H, N-CH_2-C-$\underline{CH_2}$-N); ^{13}C NMR ($CDCl_3$): δ 172.55, 163.48, 161.98, 159.44, 134.81, 134.77, 132.30, 128.01, 125.98, 114.72, 114.50, 113.71, 44.49, 36.00, 33.70, 29.19, 23.47, 19.13, 12.28; HRMS (ESI) calcd for $C_{19}H_{19}ClFN_3O_3$ ($[M+Na]^+$) 414.0989, found 414.0991.

3-phenyl-4-(2 or 3,5-dimethyl-8-oxa-1,4-diazabicyclo[3.3.0]octane)-5-methyl-isoxazole formamide ***4 (S9)***. Yield: 61%; m.p. 156.5-157.5℃; ^{1}H NMR ($CDCl_3$): δ 7.69-7.48 (m, 5H, Ar-H), 4.07-4.05 (m, 1H, N-$\underline{CH}$-CH_2-N), 2.90-2.86 (m, 2H, N-CH-$\underline{CH_2}$-N), 2.74-2.67 (m, 2H, C-$\underline{CH_2}$-CH_2-C=O), 2.53 (s, 3H, CH_3), 2.44-2.32 (m, 2H, C-CH_2-$\underline{CH_2}$-C=O), 1.78 (s, 3H, CH_3), 0.96-0.94 (d, *J*=8, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 175.47, 169.48, 160.98, 159.45, 130.68, 129.18, 129.18, 127.35, 127.35, 127.35, 112.58, 83.83, 54.66, 47.91, 36.53, 32.54, 24.57, 20.81, 11.78; HRMS (ESI) calcd for $C_{19}H_{21}N_3O_3$ ($[M+H]^+$) 340.1656, found 340.1653.

3-(2'-chloro-phenyl)-4-(2 or 3,5-dimethyl-8-oxa-1,4-diazabicyclo[3.3.0]octane)-5-methyl-isoxazolecarboxamide ***4 (S10)***. Yield: 42%; m.p. 170.4-172.3℃; ^{1}H NMR ($CDCl_3$): δ 7.56-7.38 (m, 4H, Ar-H), 4.08-4.04 (m, 1H, N-$\underline{CH}$-CH_2-N), 3.63-3.09 (m, 2H, N-CH-$\underline{CH_2}$-N), 2.77-2.61 (m, 2H, C-$\underline{CH_2}$-CH_2-C=O), 2.59 (s, 3H, CH_3), 2.56-2.29 (m, 2H, C-CH_2-$\underline{CH_2}$-C=O), 1.70 (s, 3H, CH_3), 1.06 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 175.76, 169.51, 160.50, 158.79, 132.88, 131.63, 131.47, 130.32, 127.89, 127.39, 114.00, 83.96, 54.49, 48.21,

36.13, 32.55, 24.52, 20.63, 12.25; HRMS (ESI) calcd for $C_{19}H_{20}ClN_3O_3$ ($[M+H]^+$) 374.1266, found 374.1262.

3-(2',6'-dichloro-phenyl)-4-(2 or 3,5-dimethyl-8-oxa-1,4-diazabicyclo[3.3.0]octane Alkyl)-5-methyl-isoxazolecarboxamide ***4 (S11)***. Yield: 55%; m.p. 162.1-163.8℃; ^{1}H NMR ($CDCl_3$): δ 7.52-7.40 (m, 3H, Ar-H), 4.03-4.00 (m, 1H, N-CH-CH_2-N), 3.81-3.14 (m, 2H, N-CH-CH_2-N), 2.63 (s, 3H, CH_3), 2.48-2.40 (m, 2H, C-CH_2-CH_2-C=O), 1.94-1.62 (m, 2H, C-CH_2-CH_2-C=O), 1.41 (s, 3H, CH_3), 0.99 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 170.46, 168.39, 159.66, 155.51, 136.30, 135.45, 131.84, 131.84, 128.70, 128.45, 127.26, 115.12, 78.84, 52.82, 46.22, 35.01, 30.08, 21.64, 17.55, 12.08; HRMS (ESI) calcd for $C_{19}H_{19}Cl_2N_3O_3$ ($[M+H]^+$) 408.0876, found 408.0881.

3-(2'-fluoro-6'-chloro-phenyl)-4-(2 or 3,5-dimethyl-8-oxa-1,4-diazabicyclo[3.3.0]octane)-5-methyl-isoxazolecarboxamide ***4 (S12)***. Yield: 39%; m.p. 187.3-187.9℃; ^{1}H NMR ($CDCl_3$): δ 7.48-7.14 (m, 3H, Ar-H), 4.08-4.17 (m, 1H, N-CH-CH_2-N), 3.73-3.20 (m, 2H, N-CH-CH_2-N), 2.61 (s, 3H, CH_3), 1.60-1.15 (m, 4H, C-CH_2-CH_2-C=O), 1.68 (s, 3H, CH_3), 1.13 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 175.81, 169.21, 160.01, 132.30, 132.21, 126.07, 126.04, 114.72, 114.50, 83.93, 54.47, 48.28, 36.01, 32.56, 30.99, 28.29, 24.37, 20.73, 12.36; HRMS (ESI) calcd for $C_{19}H_{19}ClFN_3O_3$ ($[M+H]^+$) 392.1172, found 392.1175.

3-phenyl-4-(3,3,6-trimethyl-9-oxa-1,5-diazabicyclo[3.4.0]nonane)-5-methyl-isox azole formamide ***4 (S13)***. Yield: 81%; m.p. 135.1-137℃; ^{1}H NMR ($CDCl_3$): δ

7.64-7.45 (m, 5H, Ar-H), 3.75-3.02 (m, 2H, N-$\underline{CH_2}$-CH_2-N), 2.79-2.76 (m, 2H, N-CH_2-$\underline{CH_2}$-N), 2.53 (s, 3H, CH_3), 2.52-2.48 (m, 2H, C-$\underline{CH_2}$-CH_2-C=O), 2.44-2.38 (m, 2H, C-CH_2-$\underline{CH_2}$-C=O), 1.60 (s, 3H, CH_3), 0.81-0.49 (m, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 172.23, 165.19, 130.51, 129.08, 129.08, 129.08, 128.23, 127.58, 112.55, 55.68, 46.09, 35.31, 30.31, 30.31, 29.66, 24.50, 21.54, 21.52, 21.39, 17.67, 11.70, 11.70; HRMS (ESI) calcd for $C_{21}H_{25}N_3O_3$ ($[M+H]^+$) 368.1969, found 368.1966.

3-(2'-chloro-phenyl)-4-(3,3,6-trimethyl-9-oxa-1,5-diazabicyclo[3.4.0]nonane)-5-methyl-isoxazolecarboxamide ***4 (S14)***. Yield: 70%; m.p. 174.9-176.3℃; ^{1}H NMR ($CDCl_3$): δ 7.53-7.37 (m, 4H, Ar-H), 3.75-3.11 (m, 2H, N-$\underline{CH_2}$-CH_2-N), 2.93-2.82 (m, 2H, N-CH_2-$\underline{CH_2}$-N), 2.54 (s, 3H, CH_3), 2.51-2.30 (m, 4H, C-CH_2-CH_2-C=O), 1.52 (s, 3H, CH_3), 0.86 (s, 3H, CH_3), 0.48 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 172.34, 164.96, 133.05, 131.58, 131.58, 130.44, 127.79, 127.33, 113.93, 77.26, 77.20, 55.92, 46.25, 34.80, 30.43, 29.63, 24.58, 24.58, 21.20, 17.39, 12.23; HRMS (ESI) calcd for $C_{21}H_{24}ClN_3O_3$ ($[M+H]^+$) 402.1579, found 402.1577.

3-(2',6'-dichloro-phenyl)-4-(3,3,6-trimethyl-9-oxa-1,5-diazabicyclo[3.4.0]nonan e alkyl)-5-methyl-isoxazolecarboxamide ***4 (S15)***. Yield: 78%; m.p. 218.2-219.2℃; ^{1}H NMR ($CDCl_3$): δ 7.48-7.35 (m, 3H, Ar-H), 3.77-3.21 (m, 2H, N-$\underline{CH_2}$-CH_2-N), 2.92-2.85 (m, 2H, N-CH_2-$\underline{CH_2}$-N), 2.57 (s, 3H, CH_3), 2.53-2.26 (m, 4H, C-CH_2-CH_2-C=O), 1.48 (s, 3H, CH_3), 0.92 (s, 3H, CH_3), 0.61 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 172.35, 169.49, 169.47, 164.45, 156.56, 136.14, 135.18, 131.74, 128.93, 128.27, 127.22, 114.21, 55.86, 46.37, 34.87, 30.52, 29.60, 24.59, 21.37, 17.29, 12.50; HRMS (ESI) calcd for $C_{21}H_{23}Cl_2N_3O_3$ ($[M+H]^+$) 436.1189, found 436.1187.

3-(2'-fluoro-6'-chloro-phenyl)-4-(3,3,6-trimethyl-9-oxa-1,5-diazabicyclo[3.4.0]nonane)-5-methyl-isoxazole carboxamide ***4 (S16)***. Yield: 62%; m.p. 198.6-200.3℃; ^{1}H NMR ($CDCl_3$): δ 7.45-7.11 (m, 3H, Ar-H), 3.78-3.21 (m, 2H, N-$\underline{CH_2}$-CH_2-N), 2.92-2.86 (m, 2H, N-CH_2-$\underline{CH_2}$-N), 2.58 (s, 3H, CH_3), 2.56-2.27 (m, 4H, C-CH_2-CH_2-C=O), 1.50 (s, 3H, CH_3), 0.92 (s, 3H, CH_3), 0.64 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 172.34, 164.56, 135.01, 132.19, 132.10, 126.20, 126.16, 114.68, 114.46, 114.38, 77.26, 77.20, 55.96, 46.28, 34.85, 30.48, 29.61, 24.66, 21.56, 17.19, 12.34; HRMS (ESI) calcd for $C_{21}H_{23}ClFN_3O_3$ ($[M+H]^+$) 420.1485, found 420.1486.

3-phenyl-4-(6-methyl-9-oxa-1,4-diazabicyclo[4.3.0]nonane)-5-methyl-isoxazolecarboxamide ***4 (S17)***. Yield: 63%; m.p. 127.4-128.7℃; ^{1}H NMR ($CDCl_3$): δ 7.71-7.46 (m, 5H, Ar-H), 4.24-3.18 (m, 2H, N-$\underline{CH_2}$-CH_2-N), 3.13-2.93 (m, 2H, N-CH_2-$\underline{CH_2}$-N), 2.52 (S, 3H, CH_3), 2.49-1.86 (m, 4H, N-CH_2-CH_2-N), 1.71 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 168.93, 167.57, 161.33, 159.26, 130.65, 129.12, 129.12, 128.39, 127.19, 127.19, 113.12, 78.42, 45.35, 39.86, 32.90, 30.47, 22.18, 17.52, 11.78; HRMS (ESI) calcd for $C_{19}H_{21}N_3O_3$ ($[M+H]^+$) 340.1656, found 340.1656.

3-(2'-chloro-phenyl)-4-(6-methyl-9-oxa-1,4-diazabicyclo[4.3.0]nonane) oxazole carboxamide ***4 (S18)***. Yield: 48%; m.p. 172.6-174.1℃; ^{1}H NMR ($CDCl_3$): δ 7.55-7.38 (m, 4H, Ar-H), 4,26-3.11 (m, 4H, N-CH_2-CH_2-N), 3.04-3.01 (m, 2H, C-CH_2-CH_2-$\underline{CH_2}$-C=O), 2.57 (s, 3H, CH_3), 2.52-1.81 (m, 2H, C-$\underline{CH_2}$-CH_2-CH_2-C=O), 1.65 (s, 3H, CH_3), 1.60-1.53 (m, 2H, C-CH_2-$\underline{CH_2}$-CH_2-C=O); ^{13}C NMR ($CDCl_3$): δ 168.81, 167.67, 160.83, 159.05, 132.67, 131.59, 131.48, 130.19, 127.81, 127.35,

114.64, 78.53, 45.30, 40.05, 32.81, 30.53, 22.03, 17.47, 12.39; HRMS (ESI) calcd for $C_{19}H_{20}ClN_3O_3$ ($[M+H]^+$) 374.1266, found 374.1269.

3-(2',6'-dichloro-phenyl)-4-(6-methyl-9-oxa-1,4-diazabicyclo[4.3.0]nonane)-5-methyl-isoxazole carboxamide ***4 (S19)***. Yield: 54%; m.p. 244.1-245.6℃; ^{1}H NMR ($CDCl_3$): δ 7.48-7.35 (m, 3H, Ar-H), 4,35-3.19 (m, 4H, N-CH_2-CH_2-N), 3.01-2.97 (m, 2H, C-CH_2-CH_2-$\underline{CH_2}$-C=O), 2.60 (s, 3H, CH_3), 2.51-1.88 (m, 2H, C-$\underline{CH_2}$-CH_2-CH_2-C=O), 1.59 (s, 3H, CH_3), 1.56-1.48 (m, 2H, C-CH_2-$\underline{CH_2}$-CH_2-C=O); ^{13}C NMR ($CDCl_3$): δ 168.47, 167.66, 160.16, 157.11, 135.95, 134.80, 131.71, 128.81, 127.97, 127.22, 114.91, 78.58, 45.23, 40.10, 32.91, 30.56, 21.87, 17.46, 12.74; HRMS (ESI) calcd for $C_{19}H_{19}Cl_2N_3O_3$ ($[M+H]^+$) 408.0876, found 408.0872.

3-(2'-fluoro-6'-chloro-phenyl)-4-(6-methyl-9-oxa-1,4-diazabicyclo[4.3.0]nonane)-5-methyl-isoxazolecarboxamide ***4 (S20)***. Yield: 39%; m.p. 236.3-237.8℃; ^{1}H NMR ($CDCl_3$): δ 7.45-7.12 (m, 3H, Ar-H), 4.35-3.52 (m, 2H, N-$\underline{CH_2}$-CH_2-N), 3.26-3.22 (m, 2H, N-CH_2-$\underline{CH_2}$-N), 3.03-3.00 (m, 2H, C-CH_2-CH_2-$\underline{CH_2}$-C=O), 2.60 (s, 3H, CH_3), 2.49-1.91 (m, 2H, C-$\underline{CH_2}$-CH_2-CH_2-C=O), 1.63 (s, 3H, CH_3), 1.54-1.53 (m, 2H, C-CH_2-$\underline{CH_2}$-CH_2-C=O); ^{13}C NMR ($CDCl_3$): δ 168.54, 167.69, 160.36, 153.82, 134.74, 134.70, 132.20, 132.10, 125.98, 125.94, 114.66, 78.54, 45.19, 40.08, 32.93, 30.58, 21.85, 17.48, 12.49; HRMS (ESI) calcd for $C_{19}H_{19}ClFN_3O_3$ ($[M+H]^+$) 392.1172, found 392.1176.

3-phenyl-4-(6-methyl-10-oxa-1,5-diazabicycl[4.4.0]decane)-5-methyl-isoxazolecarboxamide ***4 (S21)***. Yield: 59%; m.p. 163.5-164.9℃; ^{1}H NMR ($CDCl_3$): δ 7.67-7.50 (m, 5H, Ar-H), 4.50-2.60 (m, 4H,

N-$\underline{CH_2}$-CH_2-$\underline{CH_2}$-N), 2.52 (s, 3H, CH_3), 2.33-1.42 (m, 6H, C-CH_2-CH_2-CH_2-C=O), 1.28 (s, 3H, CH_3), 1.06-0.69 (m, 2H, N-CH_2-$\underline{CH_2}$-CH_2-N); ^{13}C NMR ($CDCl_3$): δ 173.55, 161.98, 160.18, 130.50, 130.43, 129.07, 129.07, 128.98, 128.95, 127.81, 113.40, 111.19, 36.10, 33.46, 32.00, 29.73, 29.57, 16.92, 12.89, 11.79; HRMS(ESI) calcd for $C_{20}H_{23}N_3O_3$ ([M+H]$^+$) 354.1812, found 354.1819.

3-(2'-chloro-phenyl)-4-(6-methyl-10-oxa-1,5-diazabicyclo[4.4.0]decane) oxazole carboxamide ***4 (S22)***. Yield: 43%; m.p. 197.7-198.7℃; ^{1}H NMR ($CDCl_3$): δ 7.55-7.40 (m, 4H, Ar-H), 4.61-2.98 (m, 4H, N-$\underline{CH_2}$-CH_2-$\underline{CH_2}$-N), 2.90-2.79 (m, 2H, C-CH_2-CH_2-$\underline{CH_2}$-C=O), 2.61 (s, 3H, CH_3), 2.55-2.35 (m, 2H, C-$\underline{CH_2}$-CH_2-CH_2-C=O), 1.79-1.64 (m, 2H, N-CH_2-$\underline{CH_2}$-CH_2-N), 1.44-1.27 (m, 2H, C-CH_2-$\underline{CH_2}$-CH_2-C=O), 1.88 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 170.31, 169.01, 162.37, 159.42, 133.04, 131.60, 131.46, 130.02, 130.02, 127.90, 127.43, 114.74, 43.21, 33.81, 32.61, 31.73, 24.68, 22.67, 16.90, 12.30; HRMS (ESI) calcd for $C_{20}H_{22}ClN_3O_3$ ([M+H]$^+$) 388.1422, found 388.1427.

3-(2',6'-dichloro-phenyl)-4-(6-methyl-10-oxa-1,5-diazabicyclo[4.4.0]decane)-5-methyl-isoxazole carboxamide ***4 (S23)***. Yield: 47%; m.p. 232.1-233.5℃; ^{1}H NMR ($CDCl_3$): δ 7.48-7.38 (m, 3H, Ar-H), 4.61-2.92 (m, 4H, N-$\underline{CH_2}$-CH_2-$\underline{CH_2}$-N), 2.61 (s, 3H, CH_3), 2.41-2.23 (m, 4H, C-$\underline{CH_2}$-CH_2-$\underline{CH_2}$-C=O), 1.88 (s, 3H, CH_3), 1.66-1.65 (m, 2H, N-CH_2-$\underline{CH_2}$-CH_2-N), 1.60-1.58 (m, 2H, C-CH_2-$\underline{CH_2}$-CH_2-C=O); ^{13}C NMR ($CDCl_3$): δ 169.42, 161.74, 157.01, 135.87, 135.76, 135.48, 131.62, 128.61, 128.55, 128.20, 127.32, 115.01, 43.62, 33.89, 32.82, 31.90, 25.25, 22.89, 16.91, 12.55; HRMS (ESI) calcd for $C_{20}H_{21}Cl_2N_3O_3$ ([M+H]$^+$) 422.1033, found 422.1035.

3-(2'-fluoro-6'-chloro-phenyl)-4-(6-methyl-10-oxa-1,5-diazabicyclo[4.4.0]decan e)-5-methyl-isoxazolecarboxamide ***4 (S24)***. Yield: 31%; m.p. 183.7-185.6℃; ^{1}H NMR ($CDCl_3$): δ 7.44-7.14 (m, 3H, Ar-H), 4.64-3.54 (m, 4H, N-$\underline{CH_2}$-CH_2-$\underline{CH_2}$-N), 2.59 (s, 3H, CH_3), 3.14-2.86 (m, 4H, C-$\underline{CH_2}$-CH_2-$\underline{CH_2}$-C=O), 2.42-2.16 (m, 2H, C-CH_2-$\underline{CH_2}$-CH_2-C=O), 1.88 (s, 3H, CH_3), 1.82-1.21 (m, 2H, N-CH_2-$\underline{CH_2}$-CH_2-N); ^{13}C NMR ($CDCl_3$): δ 170.49, 169.28, 162.04, 161.89, 159.53, 153.94, 134.97, 132.10, 125.87, 115.15, 114.67, 114.45, 43.38, 33.88, 32.69, 31.88, 24.98, 22.78, 16.85, 12.34; HRMS (ESI) calcd for $C_{20}H_{21}ClFN_3O_3$ ($[M+H]^+$) 406.1328, found 406.1331.

3-(2'-chloro-phenyl)-4-(2 or 3,5-dimethyl-9-oxa-1,4-diazabicyclo[4.3.0]nonane)-5-methyl-isoxazolecarboxamide ***4 (S25)***. Yield: 33%; m.p. 223.3-224.5℃; ^{1}H NMR ($CDCl_3$): δ 7.56-7.39 (m, 4H, Ar-H), 4.34-4.32 (m, 1H, N-$\underline{CH}$-CH_2-N), 3.45-3.11 (m, 2H, N-CH-$\underline{CH_2}$-N), 3.04-3.01 (m, 2H, C-CH_2-CH_2-$\underline{CH_2}$-C=O), 2.58 (s, 3H, CH_3), 2.38-1.89 (m, 2H, C-$\underline{CH_2}$-CH_2-CH_2-C=O), 1.71 (s, 3H, CH_3), 1.15 (s, 3H, CH_3), 1.48-1.17 (m, 2H, C-CH_2-$\underline{CH_2}$-CH_2-C=O); ^{13}C NMR ($CDCl_3$): δ 169.07, 168.16, 161.12, 158.95, 132.82, 131.60, 131.50, 130.25, 127.82, 127.36, 114.56, 78.78, 51.49, 49.53, 33.30, 29.91, 24.43, 20.37, 16.85, 12.26; HRMS (ESI) calcd for $C_{20}H_{22}ClN_3O_3$ ($[M+H]^+$) 388.1422, found 388.1428.

3-(2',6'-dichloro-phenyl)-4-(2 or 3,5-dimethyl-9-oxa-1,4-diazabicyclo[4.3.0]nonane alkyl)-5-methyl-isoxazolecarboxamide ***4 (S26)***. Yield: 38%; m.p. 187.3-189.3℃; ^{1}H NMR ($CDCl_3$): δ 7.50-7.40 (m, 3H, Ar-H), 4.03-4.00 (m, 1H, N-$\underline{CH}$-CH_2-N),

3.81-3.15 (m, 3H, N-CH-$\underline{CH_2}$-N), 2.69-2.64 (m, 2H, C-CH_2-CH_2-$\underline{CH_2}$-C=O), 2.63 (s, 3H, CH_3), 2.62-1.62 (m, 4H, C-$\underline{CH_2}$-$\underline{CH_2}$-CH_2-C=O), 1.41 (s, 3H, CH_3), 0.99-1.01 (d, *J*=8, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 170.47, 168.38, 159.67, 155.51, 136.29, 135.45, 131.84, 131.84, 128.70, 128.45, 127.26, 115.12, 78.84, 52.82, 46.24, 30.01, 30.09, 21.66, 17.56, 12.09; HRMS (ESI) calcd for $C_{20}H_{21}Cl_2N_3O_3$ ($[M+H]^+$) 422.1033, found 422.1035.

3-(2'-fluoro-6'-chloro-phenyl)-4-(2 or 3,5-dimethyl-9-oxa-1,4-diazabicyclo[4.3.0]nonane)-5-methyl-isoxazole carboxamide ***4 (S27)***. Yield: 21%; m.p. 226.5-227.7℃; ^{1}H NMR ($CDCl_3$): δ 7.44-7.14 (m, 3H, Ar-H), 4.35-4.34 (m, 1H, N-$\underline{CH}$-CH_2-N), 3.68-3.19 (m, 2H, N-CH-$\underline{CH_2}$-N), 3.01-2.98 (m, 2H, C-CH_2-CH_2-$\underline{CH_2}$-C=O), 2.60 (s, 3H, CH_3), 2.47-1.84 (m, 2H, C-$\underline{CH_2}$-CH_2-CH_2-C=O), 1.44-1.25 (m, 2H, C-CH_2-$\underline{CH_2}$-CH_2-C=O), 1.68 (s, 3H, CH_3), 1.23 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 169.05, 161.94, 160.72, 159.42, 153.74, 134.78, 132.32, 126.04, 116.75, 114.84, 114.68, 78.91, 51.46, 50.01, 33.28, 29.77, 24.13, 20.32, 16.74, 12.44; HRMS (ESI) calcd for $C_{20}H_{21}ClFN_3O_3$ ($[M+H]^+$) 406.1328, found 406.1331.

3-phenyl-4-(3,3,6-trimethyl-10-oxa-1,5-diazabicyclo[4.4.0]decane)-5-methyl-iso xazole formamide ***4 (S28)***. Yield: 79%; m.p. 178.8-179.5℃; ^{1}H NMR ($CDCl_3$): δ 7.70-7.44 (m, 5H, Ar-H), 4.42-2.63 (m, 2H, N-$\underline{CH_2}$-CH_2-N), 2.49 (s, 3H, CH_3), 2.43-2.31 (m, 2H, C-CH_2-CH_2-$\underline{CH_2}$-C=O), 2.04-1.94 (m, 2H, N-CH_2-$\underline{CH_2}$-N), 2.01-1.91 (m, 4H, C-$\underline{CH_2}$-$\underline{CH_2}$-CH_2-C=O), 2.05 (s, 3H, CH_3), 0.89-0.39 (m, 6H, CH_3); ^{13}C NMR ($CDCl_3$): δ 169.09, 130.44, 129.12, 129.10, 129.04, 128.53, 127.55, 127.49,

346 113.50, 54.47, 54.44, 54.38, 54.35, 46.00, 33.63, 33.63, 32.20, 26.64, 25.71, 17.11,
347 11.76, 11.71; HRMS (ESI) calcd for $C_{22}H_{27}N_3O_3$ ($[M+H]^+$) 382.2125, found
348 382.2123.

349 *3-(2'-chloro-phenyl)-4-(3,3,6-trimethyl-10-oxa-1,5-diazabicyclo[4.4.0]decane)-5*
350 *-methyl-isoxazolecarboxamide* ***4 (S29)***. Yield: 65%; m.p. 111.5-113.5℃; ^{1}H NMR
351 ($CDCl_3$): δ 7.54-7.35 (m, 4H, Ar-H), 4.43-2.92 (m, 2H, N-$\underline{CH_2}$-CH_2-N), 2.55 (s, 3H,
352 CH_3), 2.52-1.99 (m, 6H, C-CH_2-CH_2-CH_2-C=O), 1.95 (s, 3H, CH_3), 1.69-1.50 (m, 2H,
353 N-CH_2-$\underline{CH_2}$-N), 0.86-0.83 (d, *J*=12, 6H, CH_3); ^{13}C NMR ($CDCl_3$): δ 169.41, 162.66,
354 158.21, 133.40, 131.37, 131.37, 130.38, 130.38, 127.92, 127.03, 127.03, 115.05,
355 55.07, 45.84, 33.30, 32.46, 32.06, 26.67, 25.87, 25.25, 16.95, 11.87; HRMS (ESI)
356 calcd for $C_{22}H_{26}ClN_3O_3$ ($[M+H]^+$) 416.1735, found 416.1732.

357 *3-(2',6'-dichloro-phenyl)-4-(3,3,6-trimethyl-10-oxa-1,5-diazabicyclo[4.4.0]decan*
358 *e alkyl)-5-methyl-isoxazolecarboxamide* ***4 (S30)***. Yield: 72%; m.p. 177.2-178.6℃; ^{1}H
359 NMR ($CDCl_3$): δ 7.48-7.37 (m, 3H, Ar-H), 4.46-3.08 (m, 2H, N-$\underline{CH_2}$-CH_2-N), 2.59 (s,
360 3H, CH_3), 2.56-2.08 (m, 6H, C-CH_2-CH_2-CH_2-C=O), 1.95 (s, 3H, CH_3), 1.64-1.39 (m,
361 2H, N-CH_2-$\underline{CH_2}$-N), 0.95-0.89 (d, *J*=24, 6H, CH_3); ^{13}C NMR ($CDCl_3$): δ 170.15,
362 169.58, 162.18, 155.92, 136.07, 135.57, 131.77, 131.77, 128.73, 128.27, 127.46,
363 115.34, 76.43, 55.35, 45.83, 33.30, 32.33, 26.94, 26.02, 25.81, 16.91, 12.08; HRMS
364 (ESI) calcd for $C_{22}H_{25}Cl_2N_3O_3$ ($[M+H]^+$) 450.1346, found 450.1348.

365 *3-(2'-fluoro-6'-chloro-phenyl)-4-(3,3,6-trimethyl-10-oxa-1,5-diazabicyclo[4.4.0]*
366 *decane)-5-methyl-isoxazole carboxamide* ***4 (S31)***. Yield: 54%; m.p. 120.9-121.9℃;
367 ^{1}H NMR ($CDCl_3$): δ 7.48-7.12 (m, 3H, Ar-H), 4.47-3.00 (m, 2H, N-$\underline{CH_2}$-CH_2-N),

2.58 (s, 3H, CH_3), 2.55-2.00 (m, 6H, C-CH_2-CH_2-CH_2-C=O), 1.95 (s, 3H, CH_3), 1.66-1.43 (m, 2H, N-CH_2-$\underline{CH_2}$-N), 0.94-0.87 (d, J=28, 6H, CH_3); ^{13}C NMR ($CDCl_3$): δ 169.53, 162.22, 159.49, 153.31, 135.33, 132.26, 125.93, 125.89, 117.08, 116.90, 115.43, 114.37, 55.20, 45.83, 33.21, 32.57, 32.27, 26.75, 25.97, 25.42, 16.91, 12.03; HRMS (ESI) calcd for $C_{22}H_{25}ClFN_3O_3$ ([M+H]$^+$) 434.1641, found 434.1638.

X-ray Crystal Structure of Compound 4 (S14)

A crystal of compound **4 (S14)**, with dimensions of 0.15 mm × 0.12 mm × 0.10 mm, was measured at 293 K on a Rigaku R-AXIS-RAPID area detector diffractometer (Japan) with graphite-monochromated Mo-Kα radiation (λ=0.71073 Å). θ_{max}=28.318; 22323 measured reflections; 5167 independent reflections (R_{int}=0.0227). The structure was solved by direct methods using *SHELXS 97* and refined with *SHELXL 97*. Full-matrix least-squares refinement based on F^2 using the weight of $\omega=1/[\sigma^2(F^2_o)+(0.0702P)^2+0.0736P]$ gave final values of R_1=0.0585, ωR_2=0.1696 with $I>2\sigma(I)$, $\Delta\rho_{max}$=1.053 e Å^3 and $\Delta\rho_{min}$=-0.493e Å^3. Crystallographic data has been deposited at the Cambridge Crystallographic Data Centre as supplementary publication number 1912712.

Biological Assay

Maize seeds (Kennian 1, Shuangji Seed Industry Co., Ltd., Jilin) were moistened with warm water for 30 min, and then soaked with 0.6% carbendazim about 30 min. The seeds were immersed in a solution comprising a mixture of the title compounds (10 mg/kg) at 26.5 °C for 12 h, and then germinated in an incubator for 24 h. The injury recovery rate (IRR) of the growth indices (root length, plant height, root and

plant fresh weights) were calculated after treated with nicosulfuron for 7 d. IRR was calculated as follows:

$$\text{Injury Recovery Rate (\%)} = \frac{\text{Treated with safener and nicosulfuron - Treated with nicosulfuron}}{\text{Contrast - Treated with nicosulfuron}} \times 100\%$$

GST Enzyme Extraction and Assay *in vivo*

The GST was extracted and determined as described by Jablnkai.[36] All test procedures were carried out at 0-4°C. To measure the GST activity, 200 mg frozen maize seedling tissue was ground into powder under liquid nitrogen in 1 mL of enzyme extracting solution. The homogenate was centrifuged for 20 minutes, and the supernatant was taken. 1 mM CDNB and 1 mM GSH were added to the enzyme to be measured. The reaction mixture was measured through spectrophotometry at 340 nm for 6 minutes. The absorbance of the crude enzyme solution at 595 nm was determined by Coomassie Brilliant Blue method.[37]

Statistical Analysis

Each reported value represents the mean±standard deviation (SD) of data from at least three replicates of each assay. For the determinations of growth indexes, three different plant batches were repeated. Statistical analyses were conducted in SPSS 20 (IBM Corp., Armonk, NY, USA). Significant differences between treatment means were identified by Duncan's tests at $p < 0.05$.

Docking Study

The docking method was based on the reported method in references.[32,35] The three-dimensional structure of compound **4 (S15)** was generated in the Sketch module of SYBYL-X 2.0. Gasteiger-Huckel charges were calculated and the molecule was

optimized. The crystal structure of acetolactate synthase (ALS) was adopted from the Protein Data Bank (PDB ID 3EA4). Docking modeling used the "CDOCKER" method in Accelrys Discovery Studio 3.0. Before docking, the CHARMm force field was applied to the protein structure and water and certain co-crystallized small molecules were removed. The active site for the docking studies was determined with a subset region 13.0 Å from the center of the known ligand. The Top Hits was set to 100, the remaining parameters were used "CDOCKER" as the default value.[38] The binding energy of the small molecule-receptor protein complex was used as an evaluation index, with the largest negative representation of the most stable conformation. Based on the molecular docking results, the essential amino acids in the binding pocket were then confirmed by site-directed mutagenesis.

RESULTS AND DISCUSSION

Chemistry

The synthetic route of compounds **4** was outlined in **Scheme 2**. The synthesis of compounds **4** was performed by reacting isoxazole-4-carbonyl chloride with diazabicyclo derivatives in $CHCl_3$ for stirring 1-2 h with 21%~81% yields (**Table 1**). As shown in **Table 1**, the structure of the substituent greatly affected the yields. The yields of compounds **4 (S1)-4 (S16)** which were obtained by the ethyl levulinate were significantly better than those of compounds **4 (S17)-4 (S31)** with ethyl acetobutylate as the raw material. For compounds **4 (S1)-4 (S16)**, when the substituent R^1 was five-membered ring, the target compounds with substitution at R^2 being -$(CH_2)_3$- showed better yields than the substituent being -$(CH_2)_2$-. So the yields of compounds

434 **4 (S5)-4 (S7)** were better than others, which were 66%-74%, this may due to the
435 six-membered ring had a more stable structure than the five-membered ring. Notably,
436 the yields of compound **4 (S4)**, with F substituent at R^3 and Cl substituent at R^4, were
437 the lowest among all compounds. This was because the five-membered ring was
438 unstable, and the more obvious steric hindrance effect caused by
439 electron-withdrawing group F. When the substituents R^1 and R^2 were the same, the
440 yields of compounds **4 (S9)-4 (S12)** were generally higher than those of compounds **4**
441 **(S13)-4 (S16)**. Presumably, the six-membered ring was more stable. For dihalogen
442 substituted compounds, the yields of **4 (S12)** and **4 (S16)**, with F atom at R^3 and Cl
443 atom at R^4, were the lowest among all compounds with 39% and 62%, respectively.
444 This was most likely because introduction of halogen, the electron-withdrawing
445 groups such as F, Cl had a large steric hindrance, resulting in decreased yields.
446 Meanwhile, when the R^3 and R^4 positions were replaced by different halogen groups,
447 which made the structure not symmetrical and had poor stability, thus the yields were
448 lower.

449 Data presented in **Table 1** revealed that when the substituent $-(CH_2)_3-$ was at R^1
450 position could find the same conclusion. Compounds with no substituents (R^3=R^4=H)
451 showed the highest yields. However, the yields of the compounds decreased when the
452 substituent was halogen. For example, the yield of compound **4 (S28)** was 79%, but
453 compound **4 (S31)** was only obtained in 54% yield. The main reason for this
454 phenomenon may probably because the H atom with a small steric effect, which was
455 favorable for the progress of the reaction. As a general trend, phenyl groups bearing

electron-withdrawing groups (Cl; F), in the final compounds gave bad yields. Interestingly, when Cl atoms were placed both on the R^3 position and R^4 position of benzene ring, compounds showed greatly enhanced yields. For example, the yield of compound **4 (S30)** increased to 72%. The result showed that when R^3 position and R^4 position were both replaced by the same atom, the structure of the compound had good symmetry and can improve the reaction yield.

Biological Activity and Structure-Activity Relationships (SARs).

The safener activity of the target compounds **4** were evaluated in the greenhouse environment. All the novel compounds were evaluated for their protection of maize (Kennian 1) *in vivo* against the injury of nicosulfuron at the concentration of 60 g.a.i/hm^2 (**Table 2**). The results indicated that BAS-145138 showed better recovery rates than isoxadifen-ethyl. Most of the target compounds showed excellent growth index recovery, indicating the successful design of the title compounds.

As shown in **Table 2** most of the synthesized compounds improved recovery rate of growth index. Among the series, compound **4 (S15)** exhibited the best activity against nicosulfuron, even better than both two commercialized safeners BAS-145138 and isoxadifen-ethyl. The IRR of compound **4 (S15)** on root length, root fresh weight, plant height and plant fresh weight were 88.4%, 81.8%, 76.6% and 71.6%, respectively, which verified that compound **4 (S15)** was the best among all target compounds. The substituent on benzene ring played a key role in the biological activity. Herein, we found that different substituents exhibited diverse biological activity. For the same substitutions of R^1 and R^2, the target compounds with

478 substitution at R^3 and R^4 being Cl displayed better biological activity than those with
479 H at R^3 and R^4 positions. For example, compound **4 (S23)** exhibited 2.3 times higher
480 recovery of root length than its corresponding compound **4 (S21)**. In terms of overall
481 biological activity, the introduction of F atom was not conducive to the increase in
482 safety activity. For example, compounds **4 (S4)** and **4 (S8)** showed lower biological
483 activity in the same series of compounds. In addition, it was also found that the
484 difference in root length injury recovery rate was more obvious in biological
485 indicators. For example, the IRR of root length of compound **4 (S22)** (R^3=H; R^4=Cl)
486 was about 3.5 times that of compound **4 (S24)** (R^3=F; R^4=Cl). The IRR of root length
487 was only 0.4% for compound **4 (S31)** (R^3=F; R^4=Cl), while it was 27.2% for
488 compound **4 (S29)** (R^3=H; R^4=Cl). The results indicated that the F atom on the
489 benzene ring induced the biological activity descend.

490 For the same substituents in the benzene ring (R^3 and R^4), the target compounds
491 with different substitutions of the *N*-substituted diazabicyclo affected the biological
492 activity considerably. To be specific, R^1 and R^2 affected the safening activities
493 significantly. The target compounds **4** with ethyl at the R^1 position revealed a better
494 biological activity compared with the corresponding methyl substitution. As
495 anticipated, the recovery of root length of compound **4 (S28)** was 84.0%, which was
496 better than that of compound **4 (S13)** with 20.6%. Compounds with
497 3,3-dimethylpentane groups at R^2 exhibited better biological activity than that of the
498 compound with an isopentane group. Replacement of propyl group by ethyl group
499 diminished the protective effects of compounds **4**.

The SAR at the R^2 can be summarized as following **(Scheme 3)**: 3,3-dimethylpentane>propyl>isopentane>ethyl. The SAR results suggested that the structure of compound **4 (S15)** was more similar to the combination of isoxadifen-ethyl and BAS-145138, indicating that the structure-activity correlations were useful methods for discovering bioactivity because they provided favorable information about substituents that are necessary for the required biological activity.

GST Activity

GST is present in all plant tissues and plays an important role in the process of safener detoxification. Here we aimed to investigate the specific effect of compounds **4** on GST activity, compound **4 (S15)** with the best biological activity was selected for the experimental study on detoxification mechanism, and commercialized safener BAS-145138 was used as control. It turned out that GST activity increased obviously *in vivo* (**Table 3**).

The GST activity of maize treated by compound **4 (S15)** was similar to that of BAS-145138. There was a significant effect on GST activity after treatment with BAS-145138 and compound **4 (S15)**. The GST activity reached the maximum value when the concentration of BAS-145138 was 5 mg/kg, and then gradually decreased. It turned out that high concentrations were not conducive to GST activity. Similarly, compound **4 (S15)** also exhibited a negative correlation at 50 mg/kg. This is due to the high concentration, compound **4 (S15)** acted as xenobiotics against maize. When the concentration of compound **4 (S15)** was 10 mg/kg, the largest GST activity value in the root was attained. Therefore, it can be inferred that compound **4 (S15)** can induce

the GST activity to the greatest extent, thereby enhancing its detoxification effect on nicosulfuron in maize.

Molecular Structure Comparisons

Molecular structure comparisons provided an increased understanding of lead compounds and demonstrated promise in drug discovery. Comparing the physicochemical properties of compound **4 (S15)**, isoxadifen-ethyl and BAS-145138 (**Table 4**), it was observed that log *p* and aromatic rings (ARs) of compound **4 (S15)** were similar to that of isoxadifen-ethyl, rotatable bonds (RBs) and the electronegativity of compound **4 (S15)** were similar to BAS-145138. Moreover, hydrogen bond acceptors (HBAs) and hydrogen bond donors (HBDs) of compound **4 (S15)** were similar to both kinds of safeners. According to the investigated features, the compound **4 (S15)**/safener BAS-145138 were quite similar at the molecular level. As shown in Figure **2**, the visual assessment of the molecular overlay revealed BAS-145138 and compound **4 (S15)** were perfectly aligned in common skeleton. This indicated that, on the basis of its physicochemical properties, compound **4 (S15)** and BAS-145138 may share the similar mechanism of action. Compound **4 (S15)** has great potential for the future development of novel safener.

Molecular Docking Studies

Molecular docking is a perfect way for exploring the binding modes of two interacting molecules.[39,40] To further investigate the interaction between these safeners and target enzyme of herbicides, molecular docking experiment was conducted (**Figure 3**). The representative compound **4 (S15)**, the most potent

compound of this series, revealed excellent shape complementarity between ligand and the binding pocket. As shown in **Figure 3**, among all three safeners interact with ARG-377 and TRP-574, compound **4 (S15)** had the binding mode similar to that of BAS-145138 as we expected. BAS-145138 and TRP-574 have one π-alkyl interaction, while compound **4 (S15)** has three π-alkyl interactions, which suggested that compound **4 (S15)** had a stronger binding effect and was more likely to occupy the acetolactate synthase (ALS) binding site. The molecular docking models showed that nicosulfuron prevented the substrate from binding to the active site by effectively blocking the active pocket inlet.[41,42] Nicosulfuron had three H-bond interactions and a π-π interaction with ARG-377, and had a π-π interaction with TRP-574, which in turn occupied the ALS binding site. (**Figure 3D**). In contrast, compound **4 (S15)** only partially blocked the entrance of the channel, and small substrate molecules could still enter the channel and catalyzed the active site. To our delight and amazement, the minimum distance between the oxygen atom and residue of nicosulfuron was 2.0 Å, while the corresponding distances of compound **4 (S15)** was only 1.8 Å. (**Figure 4A** and **4B**). The shorter the distance between the oxygen atom and the binding residue in compound, the easier it is to combine. This result indicated that when compound **4 (S15)** was combined or previously applied with nicosulfuron, it can compete with nicosulfuron on the active site of ALS by preventing herbicide action or by reaching the target active site. Based on docking research and combined with biometric results, compound **4 (S15)** might be as a potential candidate for novel safeners discovery.

CONCLUSIONS

In conclusion, this series of novel diazabicyclo derivatives with different substituents were designed and synthesized *via* intermediate derivatization methods and fragment splicing. The bioassay showed that most of target compounds exhibited different safening activity to sensitive maize from nicosulfuron injury. In particular, compound **4 (S15)** displayed the best activity against nicosulfuron and improved the tolerance of maize by enhancing GST activity. The molecular structure comparisons indicated that compound **4 (S15)** was quite similar to safener BAS-145138 at molecular level. Molecular docking revealed that the promising safener efficacy may be attributed to the competition between compound **4 (S15)** and nicosulfuron in the active pocket of ALS. The current work will provide a new insight for the design of new safeners in the future.

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Supporting Information - Brief descriptions in non-sentence format listing the contents of the files supplied as Supporting Information.

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715 activity of enzyme in vivo. *J Agric Food Chem.* **2019**, *67*, 11568-11576.

716

Figure captions:

Scheme 1. Design of the target compounds.

Scheme 2. Route for the synthesis of title compounds.

Scheme 3. Sequence of activity with different substitution.

Figure 1. X-ray crystal structure for compound **4 (S14)**.

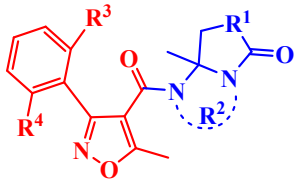
Figure 2. Superimposed molecular structure modeling. The structure of BAS-145138 is shown in green, and compound **4 (S15)** is shown in red.

Figure 3. The docking modeling of isoxadifen-ethyl (A), BAS-145138 (B) and compound **4 (S15)** (C) and nicosulfuron (D) with ALS. Yellow, light orange, red, green and light purple represent carbon atoms, sulfur atoms, oxygen atoms, chlorine atoms and nitrogen atoms respectively.

Figure 4. Compounds **4 (S15)** (A) and nicosulfuron (B) interact with receptor-ligands of amino acid residues at the active sites: yellow dotted line indicates hydrogen bonds.

Tables

Table 1. The structures of target compounds **4 (S1)-4 (S31)**



Compound	R^1	R^2	R^3	R^4
4 (S1)	$-CH_2-$	$-(CH_2)_2-$	H	H
4 (S2)	$-CH_2-$	$-(CH_2)_2-$	H	Cl
4 (S3)	$-CH_2-$	$-(CH_2)_2-$	Cl	Cl
4 (S4)	$-CH_2-$	$-(CH_2)_2-$	F	Cl
4 (S5)	$-CH_2-$	$-(CH_2)_3-$	H	H
4 (S6)	$-CH_2-$	$-(CH_2)_3-$	H	Cl
4 (S7)	$-CH_2-$	$-(CH_2)_3-$	Cl	Cl
4 (S8)	$-CH_2-$	$-(CH_2)_3-$	F	Cl
4 (S9)	$-CH_2-$		H	H
4 (S10)	$-CH_2-$		H	Cl
4 (S11)	$-CH_2-$		Cl	Cl
4 (S12)	$-CH_2-$		F	Cl
4 (S13)	$-CH_2-$		H	H
4 (S14)	$-CH_2-$		H	Cl
4 (S15)	$-CH_2-$		Cl	Cl
4 (S16)	$-CH_2-$		F	Cl
4 (S17)	$-(CH_2)_2-$	$-(CH_2)_2-$	H	H
4 (S18)	$-(CH_2)_2-$	$-(CH_2)_2-$	H	Cl
4 (S19)	$-(CH_2)_2-$	$-(CH_2)_2-$	Cl	Cl
4 (S20)	$-(CH_2)_2-$	$-(CH_2)_2-$	F	Cl
4 (S21)	$-(CH_2)_2-$	$-(CH_2)_3-$	H	H
4 (S22)	$-(CH_2)_2-$	$-(CH_2)_3-$	H	Cl
4 (S23)	$-(CH_2)_2-$	$-(CH_2)_3-$	Cl	Cl
4 (S24)	$-(CH_2)_2-$	$-(CH_2)_3-$	F	Cl
4 (S25)	$-(CH_2)_2-$		H	Cl
4 (S26)	$-(CH_2)_2-$		Cl	Cl
4 (S27)	$-(CH_2)_2-$		F	Cl
4 (S28)	$-(CH_2)_2-$		H	H
4 (S29)	$-(CH_2)_2-$		H	Cl
4 (S30)	$-(CH_2)_2-$		Cl	Cl
4 (S31)	$-(CH_2)_2-$		F	Cl

Table 2. Protective effect of target compounds on the growth indexes of maize[i, ii, iii]

Compound	Root Length IRR (%)	Plant Height IRR (%)	Root Fresh Weight IRR (%)	Plant Fresh Weight IRR (%)
isoxadifen-ethyl	21.0±0.9^{cde}	15.5±0.7^{cde}	50.9±0.7^{c}	39.7±0.5^{cde}
BAS-145138	40.8±1.4^{cde}	46.8±0.9^{b}	48.0±0.8^{bcde}	46.0±0.4^{abcde}
4(S1)	19.6±1.1^{de}	17.9±0.7^{cde}	43.8±0.6^{d}	38.7±1.0^{cde}
4(S2)	48.5±0.8^{bcd}	3.4±0.4^{e}	38.6±1.0^{d}	22.4±0.4^{de}
4(S3)	31.0±1.4^{cde}	35.8±0.8^{cde}	-5.9±0.9^{e}	26.1±0.6^{de}
4(S4)	13.3±0.7^{de}	22.8±0.8^{cde}	27.1±0.7^{b}	31.6±1.3^{de}
4(S5)	28.0±1.2^{cde}	73.5±1.0^{ab}	32.4±0.4^{b}	67.0±0.8^{abc}
4(S6)	27.9±0.7^{cde}	19.2±0.3^{cde}	25.0±0.8^{b}	18.2±0.6^{e}
4(S7)	27.3±0.9^{cde}	37.2±0.6^{cde}	26.8±0.8^{b}	35.3±0.9^{de}
4(S8)	-0.1±0.2^{e}	24.8±0.6^{cde}	14.8±0.5^{de}	20.6±1.1^{de}
4(S9)	61.0±0.8^{abc}	61.0±1.6^{ab}	66.0±1.4^{bc}	69.7±0.5^{abc}
4(S10)	26.0±0.9^{cde}	48.9±1.2^{cd}	30.6±1.1^{d}	45.7±0.6^{bcde}
4(S11)	22.9±1.3^{cde}	57.5±0.4^{bc}	44.4±1.4^{d}	34.9±0.7^{de}
4(S12)	35.6±0.8^{cde}	21.8±0.6^{cde}	28.1±0.7^{d}	15.6±0.6^{e}
4(S13)	20.6±0.5^{cde}	54.9±0.8^{bc}	31.7±0.5^{d}	45.3±0.9^{bcde}
4(S14)	10.4±0.4^{de}	59.6±1.3^{ab}	19.7±0.8^{de}	48.1±1.0^{bcde}
4(S15)	88.4±1.1^{a}	76.6±0.8^{ab}	81.8±0.6^{ab}	78.1±1.1^{ab}
4(S16)	25.2±0.9^{cde}	60.9±0.7^{ab}	32.7±1.1^{d}	71.6±1.3^{abc}
4(S17)	23.5±0.7^{cde}	70.9±1.4^{ab}	95.4±1.0^{a}	73.7±0.3^{abc}
4(S18)	24.3±0.4^{cde}	64.8±0.7^{ab}	54.8±0.8^{bc}	64.7±0.5^{abc}
4(S19)	46.8±1.0^{bcd}	36.0±0.4^{cde}	39.3±0.5^{d}	41.4±1.1^{cde}
4(S20)	48.3±0.9^{bcd}	5.9±0.3^{e}	34.5±0.9^{d}	28.6±1.0^{de}
4(S21)	33.0±0.8^{cde}	54.9±0.8^{bc}	77.5±1.4^{abc}	44.8±1.3^{bcde}
4(S22)	80.3±1.7^{ab}	10.9±0.9^{de}	27.8±1.0^{d}	31.7±0.5^{de}
4(S23)	77.3±0.9^{ab}	67.7±1.2^{ab}	27.0±0.9^{d}	65.4±0.8^{abc}
4(S24)	23.2±0.8^{cde}	44.0±1.4^{cde}	66.5±1.2^{bc}	38.1±0.7^{cde}
4(S25)	67.7±1.1^{ab}	17.3±0.8^{cde}	37.5±0.7^{d}	27.9±0.9^{de}
4(S26)	29.3±0.5^{cde}	95.3±1.2^{ab}	49.3±0.5^{c}	89.2±1.6^{a}
4(S27)	22.1±0.8^{cde}	99.4±1.1^{a}	38.9±0.7^{d}	74.5±1.0^{abc}
4(S28)	84.0±1.4^{ab}	42.7±0.9^{cde}	53.9±0.9^{bc}	56.8±0.6^{abcd}
4(S29)	27.2±1.3^{cde}	70.5±1.1^{ab}	30.1±0.3^{d}	73.6±0.4^{abc}
4(S30)	76.2±0.6^{ab}	57.3±0.5^{bc}	67.1±1.1^{bc}	64.1±0.7^{abc}
4(S31)	0.4±0.2^{e}	94.8±1.0^{ab}	29.4±0.9^{d}	79.9±1.2^{ab}

[i]Data are means of three replicates

[ii]Water treated was used as contrast

[iii]SPSS 20 software (IBM Corp., Armonk, NY, USA) was used for the statistical analyses of the data, and different lowercase letters in the figure indicate significant difference ($p < 0.05$)

717 **Table 3.** Effect of compound **4 (S15)** and BAS-145138 on GST activity[i, ii, iii]

Compound	Concentration	GST vitality (μmol min^{-1} mg^{-1} protein)	Increase in GST activity (%)
CK	-	9.17±0.57[a]	-
0	-	6.93±0.38[ab]	-
BAS-145138	1mg/kg	8.27±0.61[a]	23.67±0.47[abc]
	5mg/kg	10.20±0.57[a]	53.07±1.14[a]
	10mg/kg	8.63±0.45[a]	26.70±0.79[ab]
	25mg/kg	7.93±0.82[a]	15.97±0.56[abc]
	50mg/kg	2.87±0.66[c]	-49.27±1.04[bc]
compound 4(S15)	1mg/kg	7.43±0.54[ab]	12.57±0.61[abc]
	5mg/kg	8.83±1.10[a]	32.53±1.03[ab]
	10mg/kg	10.17±0.62[a]	47.57±0.42[a]
	25mg/kg	7.67±0.76[ab]	10.73±0.56[abc]
	50mg/kg	4.13±0.37[bc]	-36.87±0.66[bc]

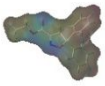
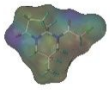
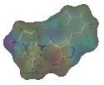
[i]Data are means of three replicates

[ii]CK (Control check) was treated by water

[iii]SPSS 20 software (IBM Corp., Armonk, NY, USA) was used for the statistical analyses of the data, and different lowercase letters in the figure indicate significant difference ($p < 0.05$)

718 **Table 4.** Chemical properties comparisons of compound **4 (S15)**, isoxadifen-ethyl,

719 and BAS-145138

Compound	log *p*[a]	RBs[a]	ARs[a]	HBAs[a]	HBDs[a]	Electronegativity[b]
Isoxadifen-ethyl	3.338	5	2	4	0	
BAS-145138	0.378	1	0	2	0	
4 (S15)	3.385	2	2	3	0	

[a]The parameters were calculated by Accelrys Discovery Studio 3.0. (Catalyst, Version 4.10. Accelrys Inc., San Diego, CA, USA, 2005).

[b]The electronegativity was predicted by SYBYL-X 2.0 (Tripos Inc., St. Louis, MO, USA).

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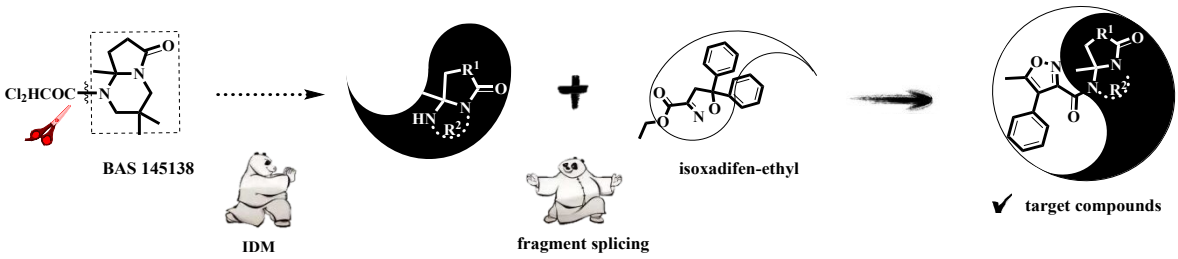
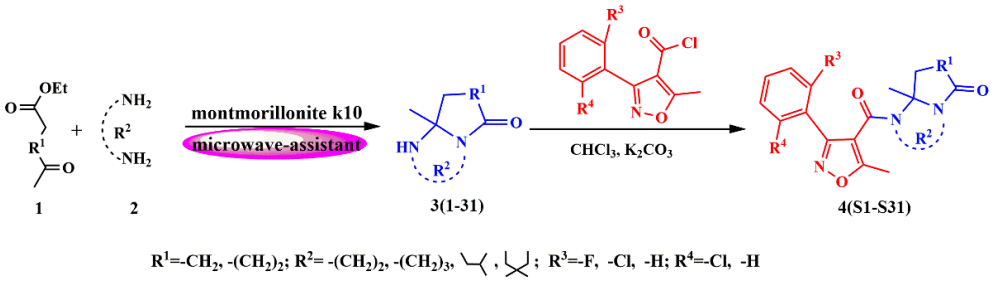
Figures

Scheme 1

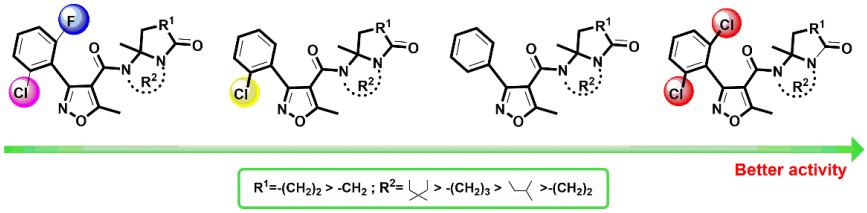


Scheme 2



R^1=-CH_2, -$(CH_2)_2$; R^2= -$(CH_2)_2$, -$(CH_2)_3$, , ; R^3=-F, -Cl, -H; R^4=-Cl, -H

Scheme 3



721

Figure 1

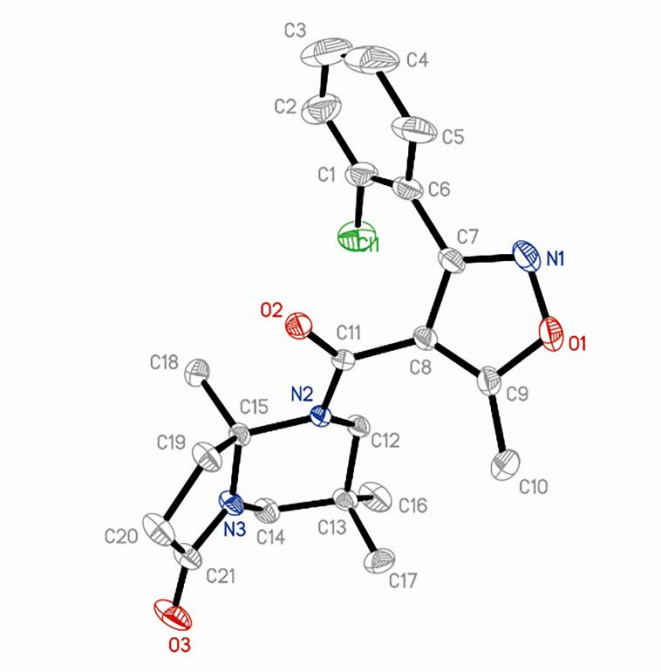
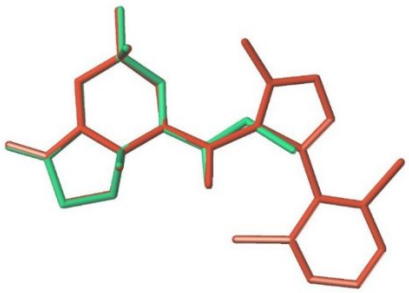


Figure 2



722

Figure 3

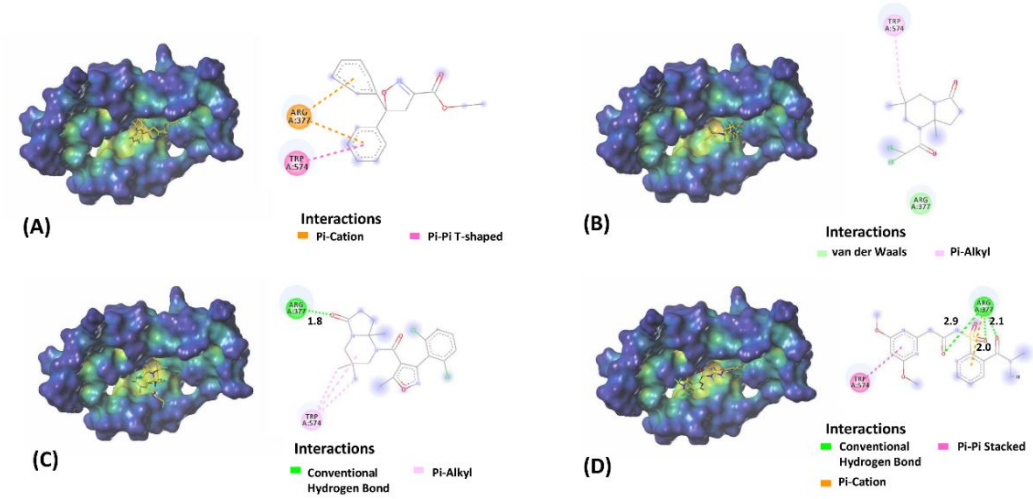
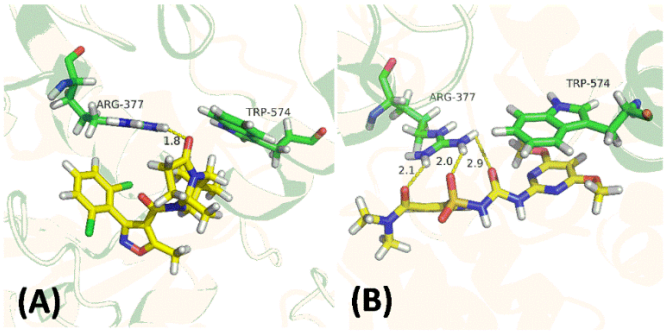
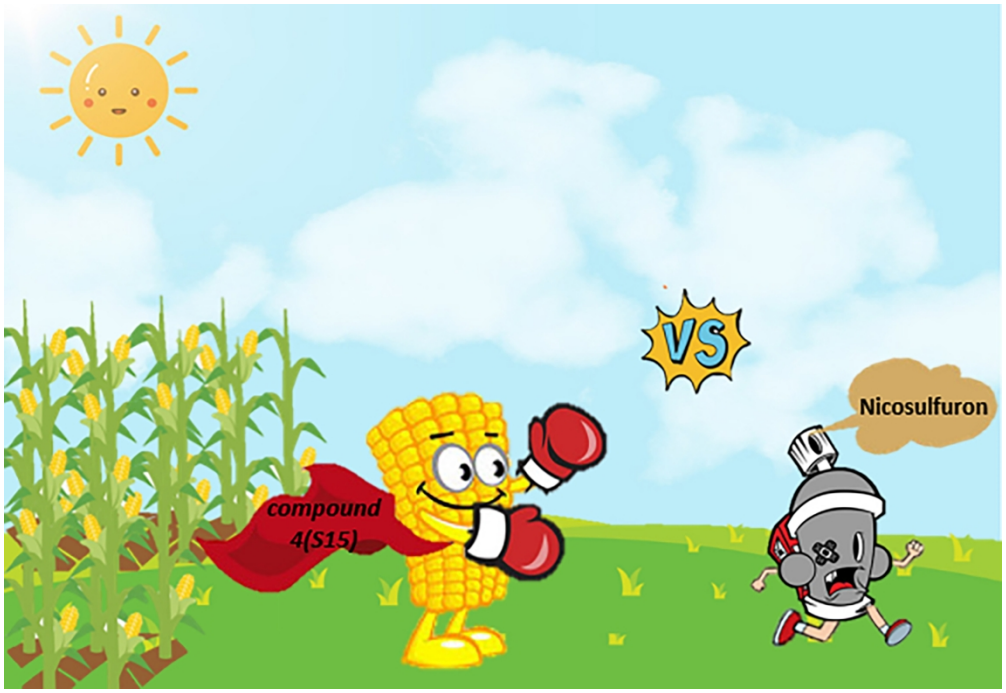


Figure 4





safener

202x138mm (300 x 300 DPI)

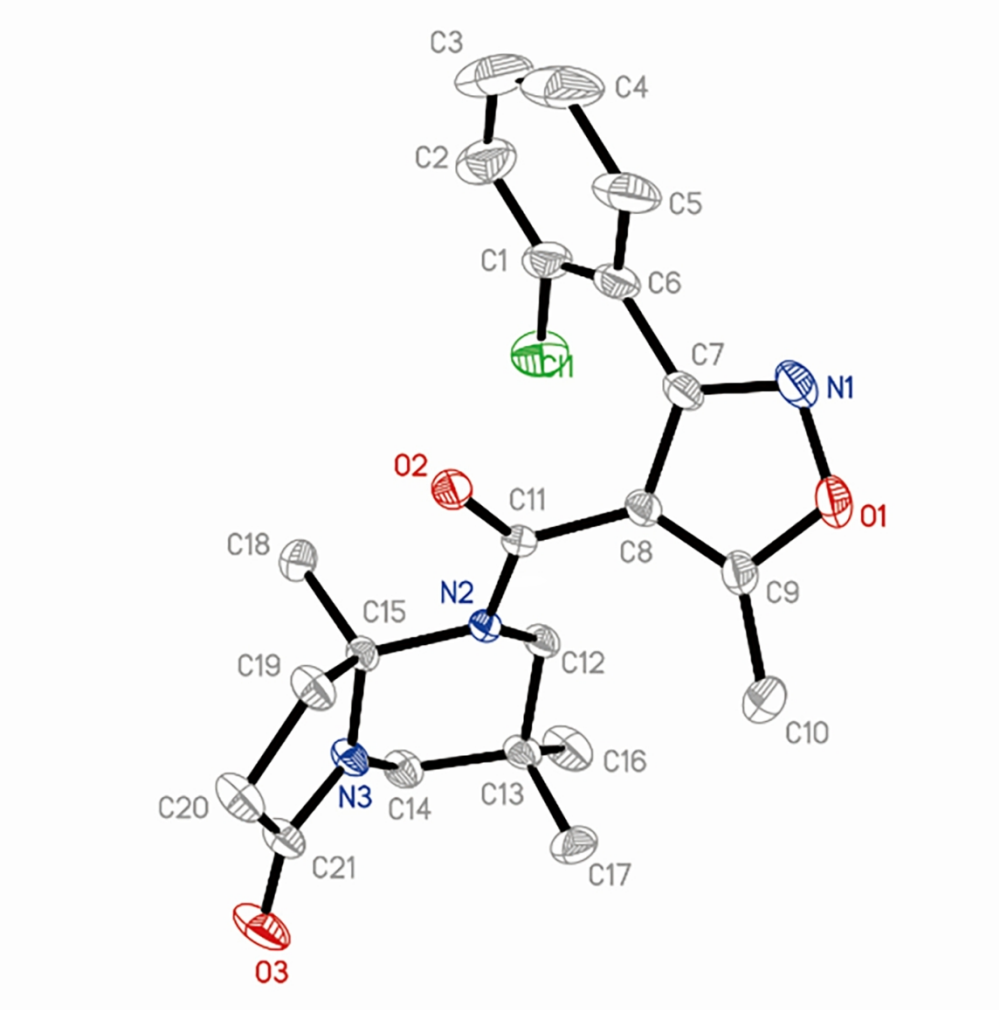


Figure 1. X-ray crystal structure for compound **4(S14)**.

117x118mm (300 x 300 DPI)

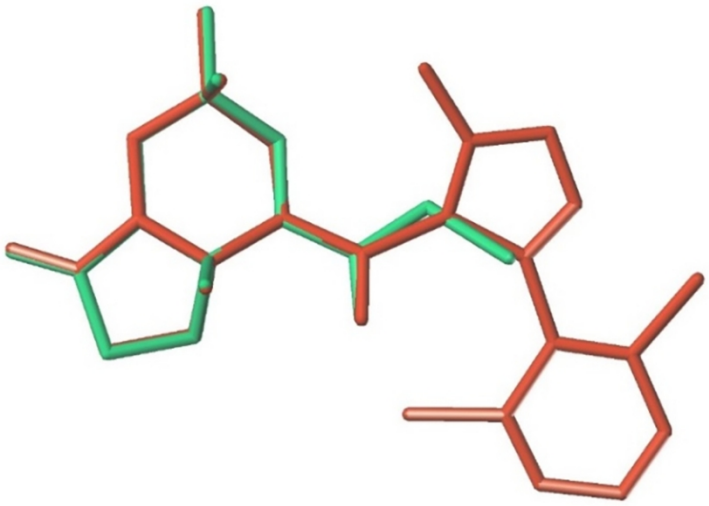


Figure 2. Superimposed molecular structure modeling. The structure of BAS-145138 is shown in green, and compound **4(S15)** is shown in red.

106x70mm (300 x 300 DPI)

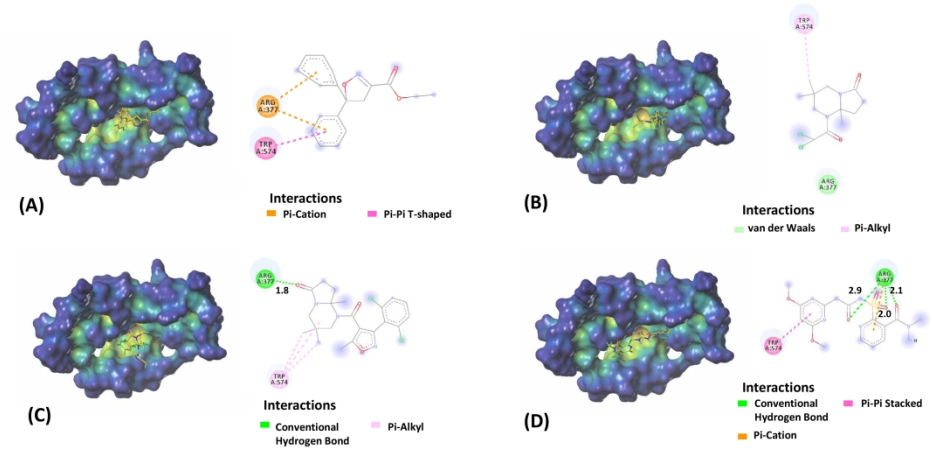


Figure 3. The docking modeling of isoxadifen-ethyl (A), BAS-145138 (B) and compound **4(S15)** (C) and nicosulfuron (D) with ALS. Yellow, light orange, red, green and light purple represent carbon atoms, sulfur atoms, oxygen atoms, chlorine atoms and nitrogen atoms respectly.

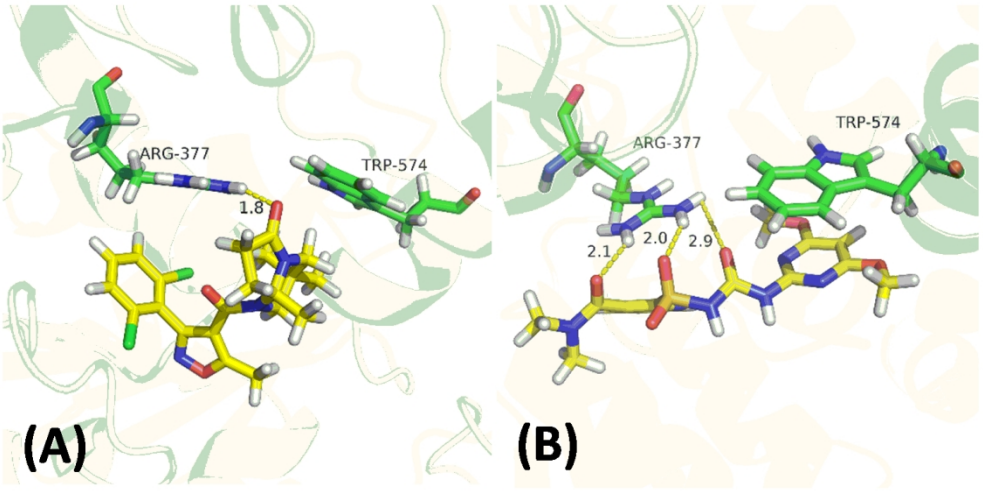
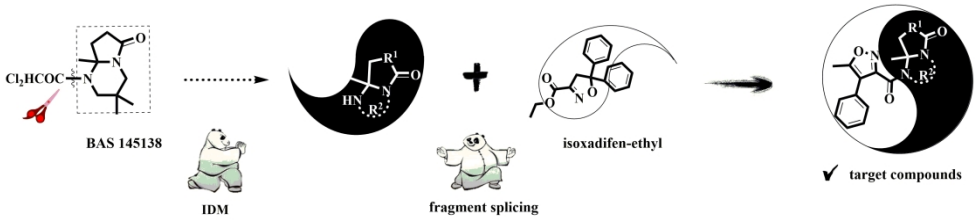


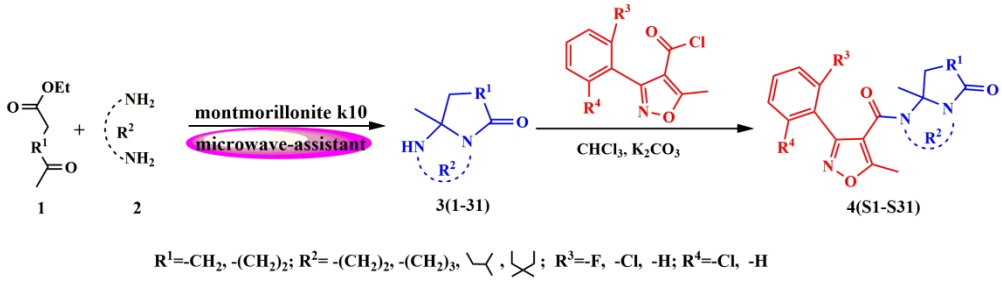
Figure 4. Compounds **4(S15)** (A) and nicosulfuron (B) interact with receptor-ligands of amino acid residues at the active sites: yellow dotted line indicates hydrogen bonds.

132x67mm (300 x 300 DPI)

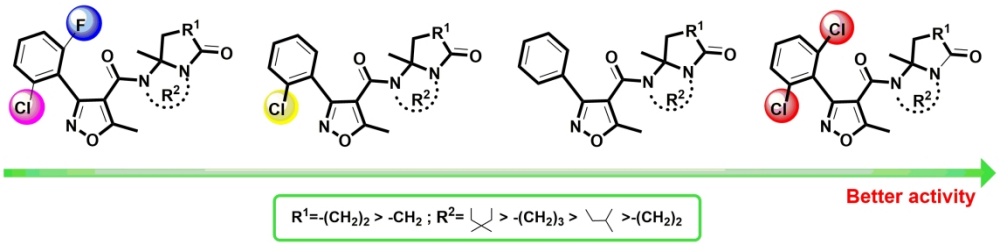


Scheme 1. Design of the target compounds.

288x71mm (300 x 300 DPI)



Scheme 2. Route for the synthesis of title compounds.



Scheme 3. Sequence of activity with different substitution.

233x56mm (300 x 300 DPI)