The structure modification of seven-membered aza-bridged neonicotinoids in order to investigate their impact on honey bees

Yuce Chen, Xiaofeng Cao, Xi Chen, Zhong Li and Xiaoyong Xu

Abstract
In order to explore the relationship between the structure and the toxicity to honey bees of seven-membered aza-bridged neonicotinoid analogues, 16 novel seven-membered aza-bridged neonicotinoid analogues are synthesized by replacing the pyridine ring, and changing the substituents on the pyridine ring, the electron-withdrawing group NO2, and the imidazole ring of our previously developed aza-bridged neonicotinoid 1-[(6-chloropyridin-3-yl)methyl]-10-(2,5-dimethylphenyl)-9-nitro-2,3,5,6,7,8-hexahydro-1H,5,8-epiminoimidazo azepine (C-29). The insecticidal bioactivities against cowpea aphid (Aphis craccivora) and the bee toxicities of these compounds are tested. Some of the title compounds present good insecticidal activities against cowpea aphid. The results also show that some of the title compounds exhibit lower bee toxicity than that of C-29 and imidacloprid. This suggests that changing the substituents on the neonicotinoids can influence the toxicity toward honey bees of these analogues.

Keywords
aza-bridged neonicotinoids, bee toxicity, cowpea aphid, insecticidal bioactivity, modification

Introduction
Neonicotinoids have played an important role in the control of pests, particularly in sucking pests, coleopterans, and dipterans.1 They are used worldwide, accounting for more than one-fourth of the global insecticide market.2,3 However, more and more research has found that, after more than 20 years of use, neonicotinoids represent a major threat to bees through increased mortality and decreased colony establishment, a condition which is known as colony collapse disorder (CCD).4–8 Bees contribute to about 80% of insect pollination,9 so it is imperative to verify the causes of bee colony losses, which may threaten plant systems based on the key role of bees in pollination. It has been reported that imidacloprid-treated bees move more actively at first and then become exhausted. In addition, the foraging ability of bees may be reduced by low doses of neonicotinoids, which would lead to the loss of the weight and even death.5–10 At the same time, the survival of worker bees significantly decreased on a large scale.11 These severe effects have resulted in the ban of three kinds of neonicotinoid: imidacloprid, clothianidin, and thiamethoxam in the European Union from May 2018,12 and much more attention has been directed toward studying the toxicity of neonicotinoids to honey bees.13 Thus, it is important to explore neonicotinoids with low bee toxicity. Novel neonicotinoid insecticides with low bee toxicity have gradually been developed, for example, sulfoxaflor14,15 and flupyradifurone16,17 are new members of the neonicotinoid family that act via the same mechanism, that is, as agonists of postsynaptic nicotinic acetylcholine receptors (nAChRs; Figure 1).

The most recent one is reported to be an antagonist of nAChRs.18 Even sulfoxaflor was banned by France in January 2020. Thus, the exploration of the relationship between structure change and bee toxicity is beneficial for finding new insecticidal lead compounds.
In our previous work, seven-membered aza-bridged neonicotinoid analogues were designed and synthesized on the basis of novel seven-membered oxa-bridged compounds and aza-bridged neonicotinoid analogues. Unfortunately, the product with the highest activity of this series (compound C-29, Figure 2), is much more toxic to honey bees than imidacloprid (bee contact toxicity of C-29, LC₅₀ = 0.062 mg L⁻¹; imidacloprid, LC₅₀ = 0.213 mg L⁻¹).

In order to explore the relationship between the structure and bee toxicity and to decrease the bee toxicity by structure modification, we selected seven-membered aza-bridged neonicotinoid C-29 and adopted different strategies to modify its structure (Figure 3). Sixteen seven-membered aza-bridged neonicotinoid analogues were synthesized according to our strategy. We replaced the substituents at position 2 of the pyridine ring (1a, b)²⁰ and the type of heterocycle on the basis of the imidazole ring (2a–c). In addition, a fluoroalkyl chain was used to replace the pyridine ring based on the bioactivity of fluorine atoms (2d–f).²¹ We also tried to modify imidazole ring by expansion or removal (3a, b). In addition, we removed the (heteroarylmethyl) groups (4a–d) and introduced CN and COOEt instead of NO₂ (5a, b) after considering the low bee toxicity of acetamiprid and thiacloprid.²² The insecticidal activities and the bee toxicities of all the compounds were investigated and compared so that we can elucidate structural characteristics that decrease toxicity toward bees.

**Results and discussion**

**Synthesis of the intermediates**

The syntheses of the intermediates are summarized in Scheme 1 and the details are available in the experimental section. Intermediate 7 was obtained by stirring (2-nitroethene-1,1-diyl)bis(methylsulfane) with 1,2-ethylenediamine 6 under reflux conditions (equation (1)). The preparations of intermediates 9–16 were accomplished through nucleophilic substitution reactions using intermediate 7 via nucleophilic attack on the appropriate halogenated reagent 8 (equation (1)). The preparation of intermediate 20 was similar to the preparations of intermediates 9–16, only the starting material 1,2-ethylenediamine was replaced by 1,3-propylenediamine 17 (equation (2)).

To obtain the intermediate 23, (6-chloropyridin-3-yl) methanamine 21 was reacted with bis(methylsulfane) to generate intermediate 22, and amination was conducted with methanamine to result in intermediate 23 (equation (3)).

Intermediates 25–26 were prepared by displacing the methylthiol of bis(methylsulfane) with the appropriate alkyl mercaptan 24 (equation (4)). Intermediate 29 was obtained via the classic Pinner reaction, starting with commercially available nitriles 27 (malononitrile and ethyl cyanoacetate) and ethanol, and then neutralized by Et₃N to give intermediates 29. Cyclization with N1-(6-chloropyridin-3-yl)methyl]ethane-1,2-diamine 30 gave intermediates 31 and 32 (equation (5)).

**General synthesis of the compounds**

The target compounds were obtained by the reaction of the above-mentioned synthetic intermediates (7, 9–16, 18, 20, 23, 25–26, 31–32), 2,5-dimethylaniline and the key reactant succinaldehyde, the latter being obtained by stirring 2,5-dimethoxy-tetrahydrofuran with aqueous acid.²³ The corresponding products were obtained by the following synthetic routes shown in Scheme 2, the details of which are available in the experimental section and the NMR spectra are available in supplement material.

**Insecticidal activity and the bee toxicity of the synthesized compounds**

Compounds 1a, b in which the chloro group at the 6-position of the pyridine ring were replaced by a bromo and a fluoro group, and compounds 2a–f with different substituents on the nitrogen atom of the imidazole ring were synthesized initially. The activity of compounds 1a, b was dependent on the nature of the substituents on the pyridine ring. It is apparent that the electron-withdrawing chloro group contributed to an increase in the bioactivity against cowpea aphid compared with the compounds bearing a bromo group (1a) and a fluoro group (1b). The bioassay also showed that a substituted pyridine ring played an important role in the activity of compounds 2 (Figure 3). The activity of the 2-chlorothiazol-5-yl derivative 2a was lower than that of the bromopyridyl derivative 1a. For compounds 2b and 2c, in which phenyl and tetrahydrofuran-3-yl were attached to the N-methyl group, the bioactivity disappeared totally, which proved the necessity of a pyridine among the four aromatic heterocycles. When fluoroalkyl chains were introduced as replacements for the aromatic heterocycle, the activities of resulting compounds 2d–f were lower than that of C-29 against cowpea aphids; compound 2f (n = 3) had relatively higher activity among compounds 2d–f at 100 mg L⁻¹, the mortality reached 90%, which indicated the importance of the distance between the fluorine atom and the imidazole ring. Unfortunately, when the concentration is
**Figure 3.** The optimization of the C-29.

**Scheme 1.** Synthetic routes to the intermediates.
decreased to 20 mg L\(^{-1}\), the bioactivity of compounds 2d–f totally disappeared (Table 1).

Compounds possessing the feature structures of commercialized neonicotinoids were next synthesized (3a, b, 4a–d). First, hexahydropyrimidine 3a and compound 3b were synthesized after the imidazole ring of C-29 was opened. It was found that these two compounds maintained good activity against cowpea aphid, but still lower than that of C-29. Next, the structures with the 6-chloro-3-pyridyl and 2-chlorothiazol-5-yl substituents of the N terminus of the nitrogen-containing heterocyclic ring removed and only preserving imidazole (4a), hexahydropyrimidine (4b), thiazolidine (4c), and 1,3-thiazinane (4d) were synthesized, respectively. However, these compounds showed no activity against cowpea aphids.

These findings again indicated the importance of the N-[(6-chloro-3-pyridyl)methyl] group. Neonicotinoids bearing a CN group are regarded as insecticides with low bee toxicity,\(^{23}\) so a cyano group 5a and ester group 5b were introduced into C-29 to replace the nitro group. However, this led to a decrease in the activity against cowpea aphids, which further clarified the key role of the nitro group. Although some of the compounds exhibited good insecticidal activities, the activities were much lower compared with that of C-29.

Next, the bee toxicities of the synthesized compounds with higher insecticidal activity were determined. The bee contact toxicities of the compounds are listed in Table 2.

All the tested compounds exhibited lower contact toxicity toward honey bees than C-29, which has an extremely high level of toxicity. First, the Br at the 2-position of the pyridine ring in 1a maintained the bee contact toxicity, which implies that bee toxicity is caused by halogens on the pyridine ring. Next, compounds 3a and 3b, in which the imidazole ring was either expanded or removed completely, showed much lower bee contact toxicity than C-29. This suggested that the change of substituents and imidazole ring contributed to the decrease in bee contact toxicity. In addition, compound 2a also exhibited lower contact toxicity than C-29, which means that replacing the 6-chloropyridine with a 2-chlorothiazole also decreases the bee contact toxicity. In addition, the LD\(_{50}\) values of the bee toxicity of compounds 2a and 3a were also lower than those of imidacloprid at 24 and 48 h, but toxicological grade was still the same (Table 2).
The mortality of the compounds was determined at two concentrations, 100 mg L\(^{-1}\) and 20 mg L\(^{-1}\), and the LC\(_{50}\) value was calculated. The compounds were tested at 20 mg L\(^{-1}\) when the mortality was greater than 50%. The bioactivity of the compounds was evaluated.

### Table 1. Insecticidal activities of the target compounds against cowpea aphids.

| Compounds | Mortality (%) | LC\(_{50}\) |
|-----------|---------------|------------|
|           | 100 mg L\(^{-1}\) | 20 mg L\(^{-1}\) | LC\(_{50}\) |
| C-29      | 100           | 100        | 0.426 mg L\(^{-1}\) |
| 1a        | 100           | 66.97      | 7.216 mg L\(^{-1}\) |
| 1b        | 80            | 0          | – |
| 2a        | 100           | 50.36      | 20.363 mg L\(^{-1}\) |
| 2b        | 0             | 0          | – |
| 2c        | 0             | 0          | – |
| 2d        | 0             | 0          | – |
| 2e        | 80            | 0          | – |
| 2f        | 90            | 0          | – |
| 3a        | 100           | 96.11      | 3.335 mg L\(^{-1}\) |
| 3b        | 100           | 100        | 3.650 mg L\(^{-1}\) |
| 4a        | 0             | 0          | – |
| 4b        | 0             | 0          | – |
| 4c        | 0             | 0          | – |
| 4d        | 0             | 0          | – |
| 5a        | 0             | 0          | – |
| 5b        | 40            | 0          | – |
| Imidacloprid | 100         | 100        | 0.921 mg L\(^{-1}\) |

The insecticidal activities of the synthesized compounds were tested at 100 mg L\(^{-1}\). When the mortality of the compound reached 100% at 20 mg L\(^{-1}\), the concentration was decreased to 20 mg L\(^{-1}\) and the bioactivity was tested again. When the mortality was greater than 50% at 20 mg L\(^{-1}\), the LC\(_{50}\) value was obtained.

The bee oral toxicities of some of the prepared compounds with high insecticidal activity are listed in Table 3. These compounds all exhibited lower bee oral toxicity than C-29. Among them, the toxicity level of compounds 1a and 3a decreased from extremely toxic A to highly toxic B according to the data at 48 h. However, only the bee oral toxicity of compound 3a was lower than that of imidacloprid. In the process of testing of the bee contact toxicity and oral toxicity, the poisoning symptoms of bee were observed, and it was found that the honey bees moved slowly and erratically and the body color of honey bees gradually became black.

By comprehensively comparing the bee contact and oral toxicities of these compounds, there are two, 1a and 3a, that show bee contact toxicities lower than imidacloprid, and only compound 3a has a lower bee oral toxicity than imidacloprid. Moreover, compound 3a maintained relatively good activity against cowpea aphids (Table 1). Thus, compound 3a with a hexahydropyrimidine and a 6-chloropyridine ring is the best candidate compound following modification of C-29. Although we realized the design target to decrease the toxicity of C-29 by modification of the structure, it is not satisfactory that the toxicity level of compound 3a is still very high.

Based on the data of bee contact and oral toxicity at 24 and 48 h, it was found that the bee toxicity at 24 h was lower than that at 48 h. This indicates that the compounds were degraded after they entered the body of the honey bee and the degradation products exhibited higher bee toxicity than the parent compounds. It is known that C-29 is easily degraded into intermediate (E)-2-chloro-5-[2-(nitromethylene)imidazolidin-1-yl]methyl]pyridine (NTN32692), which is a compound with higher bee toxicity (see Scheme 1). This may indicate that the high bee toxicity of NTN32692 might be the cause of the bee toxicity of all the derivatives of NTN32692. The best approach to decrease the bee toxicity is to give up the structural skeleton of NTN32692 and select a novel structural skeleton.

### Conclusion

Sixteen novel seven-membered aza-bridged neonicotinoid analogues are synthesized on the basis of C-29, which has high insecticidal activity but is more toxic to honey bees than imidacloprid. Compound 3a bearing a hexahydropyrimidine moiety retained insecticidal bioactivity comparable to that of C-29 and exhibited toxicity toward honey bee inferior to that of C-29 (bee contact toxicity of C-29, LC\(_{50}\) = 0.062 mg L\(^{-1}\); 3a, LC\(_{50}\) = 17.065 mg L\(^{-1}\)). Insecticidal bioactivity against cowpea aphid of C-29, LC\(_{50}\) = 0.426 mg L\(^{-1}\); 3a, LC\(_{50}\) = 3.335 mg L\(^{-1}\)). In summary, compound 3a exhibits good insecticidal bioactivity against cowpea aphid and lower toxicity toward honey bees, making it a potential lead compound in the discovery of new insecticide.

### Experimental

#### Instrumentation and chemicals

High-resolution mass spectra were recorded under electron impact (70 eV) conditions using a Micromass GCT CA 055 instrument. Melting points were recorded on a Büchi I-540 apparatus and are uncorrected. 1H NMR, 13C NMR and 19F NMR spectra were recorded on a Bruker AM-400 (400 MHz) spectrometer with CDCl\(_3\) or DMSO-d\(_6\) as the solvent and tetramethylsilane (TMS) as the internal standard. Chemical shifts are reported in \(\delta\) (parts per million) values.

Analytical thin-layer chromatography (TLC) was carried out on precoated plates (silica gel 60 F254), and spots were visualized with ultraviolet (UV) light.

Unless otherwise noted, reagents and solvents were purchased from Shanghai Tansoole Chemicals Company, Ltd. (Shanghai, China) and Shanghai Bide Pharmatech Ltd. (Shanghai, China).

#### Insecticidal assay

According to statistical requirements, the bioassay was repeated three times at 25 °C ± 1 °C. All compounds were dissolved in N, N-dimethylformamide and diluted with water containing Triton X-100 (0.1 mg L\(^{-1}\)) to obtain series concentrations of 500.0, 100.0 mg L\(^{-1}\), and others for bioassays.

For cowpea aphids: The insecticidal activities of title compounds against cowpea aphids were tested according to the previously reported procedure.\(^{24}\) Horsebean seedlings with 40–60 healthy apterous adults were dipped in diluted solutions of the chemicals containing Triton X-100 (0.1 mg L\(^{-1}\)) for 5 s, and then the shoots were placed in a conditioned room (25 °C ± 1 °C, 50% relative
humidity (RH) Water containing Triton X-100 (0.1 mg L\(^{-1}\)) was used as control. The mortality rates were assessed after 24 h. Each treatment had three repetitions, and the data were corrected and subjected to probit analysis.

The LC\(_{50}\) values against Cowpea Aphids of low bee toxicity compounds were tested by similar method under different concentrations. The mortality rates of the cowpea aphids were recorded after 72 h. The test data were processed by the SPSS12.0 and obtained the LC\(_{50}\) for 72 h and 95% confidence limit.

### Bee toxicity assay

All compounds were dissolved in acetone and diluted with water to obtain series concentrations for bioassays.

**Contact assay:** Honey bees were put into a dryer and were anesthetized by 5 mL diethyl ether for 3 min before the test. Then, different concentration solutions were dropwise added on the pronotums of the bees by 1.00 µL microdropper. The bees were enclosed in the cage in time before the bees fully recovered and were fed with 33% honey water. The cage was put on the laboratory table and covered by black cloth and acetone was used as control.

**Uptake assay:** Degrease cotton was dipped in diluted solutions of the chemicals, which was added to Tween 80 and diluted by 33% honey water until saturation. Then, degrease cotton was spread on gauze net in the cage and a 50 mL beaker was put on the degrease cotton so that the honey bees could suck up the liquid. The cage was put on the laboratory table and covered by the black cloth. The amount of acetone with Tween 80 was the same as the maximum concentration of diluted solution. It was used as control.

The mortality rates and poisoning symptoms were recorded 24 and 48 h after treatment. The test data were processed by the SPSS12.0 and obtained the LC\(_{50}\) (bee contact toxicity) for 24 and 48 h, LD\(_{50}\) (bee oral toxicity) for 24 and 48 h and 95% confidence limit.

### Table 2. Bee acute contact toxicity of several of the prepared compounds.

| Compound | Time | LD\(_{50}\) (µg bee\(^{-1}\)) | Toxic regression equation | \(R^2\) | Toxicological grade\(^b\) |
|----------|------|-------------------------------|---------------------------|-------|------------------------|
| 3a       | 24   | 0.328                         | \(y = 1.540x + 0.746\)    | 0.981 | B                      |
| 2a       | 24   | 0.320                         | \(y = 1.639x + 0.971\)    | 0.975 | B                      |
| 3b       | 24   | 0.181                         | \(y = 1.176x + 1.596\)    | 0.990 | B                      |
| 1a       | 24   | 0.081                         | \(y = 2.537x + 2.769\)    | 0.947 | B                      |
| NTN32692 | 24   | 0.028                         | \(y = 3.075x + 3.662\)    | 0.985 | B                      |
| Imidacloprid | 24 | 0.213                         | \(y = 3.047x + 3.191\)    | 0.881 | B                      |
| C-29     | 24   | 0.062                         | \(y = 1.569x + 3.331\)    | 0.983 | B                      |

\(^{a}\)Coefficient of determination of the toxic regression equation, which represents the goodness of fit of the toxic regression equation.

\(^{b}\)The grade of the bee toxicity—Extreme toxicity: A; high toxicity: B; moderate toxicity: C; low toxicity: D.

### Table 3. Bee acute oral toxicity of several of the prepared compounds.

| Compound | Time | LC\(_{50}\) (mg L\(^{-1}\)) | Toxic regression equation | \(R^2\) | Toxicological grade\(^b\) |
|----------|------|-----------------------------|---------------------------|-------|------------------------|
| 3a       | 24   | 154.396                     | \(y = 1.078x - 2.359\)    | 0.911 | B                      |
| 2a       | 24   | 8.706                       | \(y = 2.357x - 2.215\)    | 0.949 | B                      |
| 3b       | 24   | 0.483                       | \(y = 1.405x + 0.444\)    | 0.984 | A                      |
| 1a       | 24   | 0.201                       | \(y = 3.151x - 3.882\)    | 0.997 | A                      |
| 2a       | 24   | 0.028                       | \(y = 1.995x + 0.775\)    | 0.977 | A                      |
| C-29     | 24   | 0.024                       | \(y = 2.924x + 3.104\)    | 0.998 | B                      |

\(^{a}\)Coefficient of determination of the toxic regression equation, which represents the goodness of fit of the toxic regression equation.

\(^{b}\)The grade of the bee toxicity—Extreme toxicity: A; high toxicity: B; moderate toxicity: C; low toxicity: D.
General synthetic procedures

Intermediates 9–13. The synthetic procedures of intermediates 9–13 are shown in equation (1). To a solution of (2-nitroethene-1,1-diyl)bis(methylsulfane) (6.5 mmol) in ethanol (50 mL) was added hydrazine 6 dropwise, and the resulting mixture was stirred at reflux for 8 h. After the disappearance of the reactant (monitored by TLC), the reaction mixture was evaporated and the residue treated with water (10 mL). The mixture was extracted with dichloromethane (50 mL × 3), and the combined organic layer was dried over Na₂SO₄, filtered, and concentrated under reduced pressure to yield the crude intermediate 7. The crude intermediate 7 was used in next step.

To a solution of commercially available halogen reagents 8 (2 mmol), respectively, and intermediates 8 (2 mmol) in dimethyl sulfoxide (DMSO; 10 mL) was added KOH (1.5 mmol) and the mixture was stirred at room temperature. After the disappearance of the reactant (monitored by TLC), the reaction mixture was poured into water and extracted with dichloromethane (50 mL). The combined organic layers were washed with saturated aqueous NaCl and dried over sodium sulfate. After evaporation of the solvent, the residue was subjected to a flash chromatography on silica gel to afford the corresponding intermediates.25

Intermediates 14–16. The synthetic procedures of intermediates 14–16 are shown in equation (1). To a solution of the fluorine-substituted brominated hydrocarbons 8 (2 mmol) in acetonitrile (25 mL) was added tetrabutylammonium bromide (TBAB; 2 mmol), intermediates 7 (2 mmol) and Cs₂CO₃ (2 mmol). The resulting mixture was stirred and heated at 82 °C for 8 h. After the disappearance of the reactant (monitored by TLC), the mixture was cooled to room temperature and filtered. The solvent was evaporated and the residue subjected to a flash chromatography on silica gel to afford the crude product. The crude product was purified via flash silica gel chromatography (CH₂Cl₂/MeOH, 20:1) to give the corresponding intermediates.

(E)-1-(4-chlorobenzyl)-2-(nitromethylene)hexahydropyrimidine 20. The synthetic procedures of intermediates 20 are shown in equation (2). To a solution of bis(methylsulfane) (6.5 mmol) in ethanol (50 mL) was added methanediamine 17 dropwise, and the resulting mixture was stirred at reflux for 8 h. After the disappearance of the reactant (monitored by TLC), the reaction mixture was evaporated and the residue treated with water (10 mL). The mixture was extracted with dichloromethane (50 mL × 3), and the combined organic layer was dried over Na₂SO₄, filtered, and concentrated under reduced pressure to yield the crude product. The crude product was purified via flash silica gel chromatography (CH₂Cl₂/MeOH, 15:1) to give the intermediate 20.26

(E)-N-((6-chloropyridin-3-yl)methyl)-N-methyl-2-nitroethene-1,1-diamine 23. The synthetic procedures of intermediate 23 were shown in equation (3). To a solution of bis(methylsulfane) (6.5 mmol) in ethanol (50 mL) was added the prepared intermediate 21 dropwise, and the resulting mixture was stirred at reflux for 8 h. After the disappearance of the reactant (monitored by TLC), the reaction mixture was evaporated and the residue treated with water (10 mL). The mixture was extracted with dichloromethane (50 mL × 3), and the combined organic layer was dried over Na₂SO₄, filtered, and concentrated under reduced pressure to yield the crude product. The crude product was purified via flash silica gel chromatography (CH₂Cl₂/MeOH, 10:1) to give the intermediate 22.

A solution of methylamine in ethanol was added to the prepared intermediate 22 dropwise, and the resulting mixture was stirred at reflux for 8 h. After the disappearance of the reactant (monitored by TLC), the reaction mixture was evaporated, and the residue treated with water. The mixture was extracted with dichloromethane (50 mL × 3), and the combined organic layer was dried over Na₂SO₄, filtered, and concentrated under reduced pressure to yield the crude product. The crude product was purified via flash silica gel chromatography (CH₂Cl₂/MeOH, 15:1) to give the intermediate 23.27

Intermediates 25–26. The synthetic procedures of intermediates 25–26 are shown in equation (4). To bis(methylsulfane) (10 mmol) in ethanol (20 mL) at 78 °C was added commercially available reactants 24 (10 mmol) dropwise, respectively, and the mixture was stirred at reflux 78 °C. After the disappearance of the reactant (monitored by TLC), the reaction mixture was cooled to room temperature during which the product separated out as a solid. The product was filtered and dried to obtain target intermediate.28

Intermediates 31–32. The synthetic procedures of intermediates 31–32 are shown in equation 5. To a flask with three necks was added commercially available reactants 27 (100 mmol), respectively, ethanol (100 mmol) and diethyl ether (30 mL). Then, hydrochloric acid was bubbled into the flask and the formed solid was separated out. After 4 h, the intermediate 28 was filtered out. After the reaction was completed, the mixture was filtered and the residue was dissolved in dichloromethane. Triethylamine was added dropwise to the mixture until the pH became alkaline. The obtained solution could be used directly in the next step.

To a solution of intermediates 29 in dichloromethane (50 mL) was added intermediates 30 (100 mmol, 2.0 mmol mL⁻¹ in dichloromethane) dropwise and the mixture was stirred at reflux for 5–6 h. After the disappearance of the reactant (monitored by TLC), the mixture was cooled to room temperature, and filtered to give the intermediate 31 and 32.29
Target compounds, general procedure

The synthetic procedures of the target compounds are shown in Scheme 2. To a solution of intermediates 7, 9–16, 18, 20, 23, 25–26, 31–32 (2 mmol), respectively, and 2,5-dimethylaniline (5 mmol) in acetonitrile acidified to pH 2 by adding HCl (1 M) was added succinaldehyde (3 mmol) dropwise and the mixture was stirred in an ice bath for 1.5 h. After the disappearance of the reactant (monitored by TLC), the mixture was adjusted to pH 7–8 with saturated aqueous NaHCO₃ and evaporated under reduced pressure. The solution was extracted with dichloromethane (50 mL × 3). The combined organic layer was washed with brine and dried over anhydrous sodium sulfate. After evaporation of the solvent, the residue was purified by silica gel column chromatography to afford the target product.

Succinaldehyde

A mixture of 2,5-dimethoxytetrahydrofuran (0.290 g, 2.2 mmol) and 0.4 mL of 10% aqueous HCl was stirred at room temperature. After 12 h, the pH value of the mixture was adjusted to 2–3 with saturated aqueous NaHCO₃. The obtained solution could be used directly in the next step.²²

1-[[6-(6-Bromopyridin-3-yl)methyl]-1-(2,5-dimethylphenyl)-9-nitro-2,3,5,6,7,8-hexahydro-1H-5,8-epiminooimidazo[1,2-ajazepine (1a): White solid; 68 mg, 43%; m.p. 151–152 °C; ¹H NMR (400 MHz, CDCl₃): δ 8.20 (d, J = 1.6 Hz, 1H), 7.54 (dd, J = 8.2, 2.0 Hz, 1H), 7.37 (d, J = 8.2 Hz, 1H), 7.06 (d, J = 7.6 Hz, 1H), 6.78 (s, 1H), 5.12 (t, J = 6.6 Hz, 1H), 5.08 (d, J = 14.8 Hz, 1H), 4.98 (d, J = 5.6 Hz, 1H), 4.49 (d, J = 15.2 Hz, 1H), 3.69–3.53 (m, 2H), 3.30–3.16 (m, 2H), 2.36 (ddd, J = 15.2, 11.4, 6.0 Hz, 1H), 2.29 (s, 3H), 2.28–2.24 (m, 2H), 2.23 (s, 3H), 2.10–2.00 (m, 1H); ¹³C NMR (100 MHz, CDCl₃): δ 156.4, 149.5, 142.4, 141.9, 138.7, 136.3, 131.9, 131.1, 128.5, 126.6, 124.1, 120.4, 109.6, 73.2, 58.7, 51.6, 48.6, 47.3, 31.9, 31.1, 21.5, 19.1. HRMS (EI): m/z [M-HNO₃]⁺ calcd for C₁₈H₁₈N₄O₂:F 422.1166; found: 422.1108; calcld for C₁₈H₁₈N₄O₂:F 424.1086; found: 424.1090.

10-(2,5-Dimethylphenyl)-1-[[6-(fluoropyridin-3-yl)methyl]-9-nitro-2,3,5,6,7,8-hexahydro-1H-5,8-epiminooimidazo[1,2-ajazepine (1b): Russet solid; 120 mg, 40%; m.p. 162 °C–163 °C; ¹H NMR (400 MHz, DMSO-d₆): δ 8.12 (s, 1H), 7.81 (td, J = 8.2, 2.2 Hz, 1H), 7.09 (dd, J = 8.4, 2.4 Hz, 1H), 7.03 (d, J = 7.6 Hz, 1H), 6.76 (d, J = 7.2 Hz, 1H), 6.64 (s, 1H), 5.14 (d, J = 3.6 Hz, 1H), 4.99 (d, J = 4.0 Hz, 1H), 4.86 (d, J = 15.2 Hz, 1H), 4.67 (d, J = 15.2 Hz, 1H), 3.71 (dd, J = 16.6, 8.2 Hz, 1H), 3.61 (dt, J = 16.0, 8.0 Hz, 1H), 3.42 (t, J = 21.0, 6.4 Hz, 2H), 2.23 (s, 3H), 2.22–2.20 (m, 1H), 2.19 (s, 3H), 2.19–2.01 (m, 2H), 1.90 (dd, J = 17.6, 10.8 Hz, 1H); ¹³C NMR (100 MHz, DMSO-d₆): δ 162.4 (d, JCF = 233 Hz), 155.8, 146.8 (d, JCF = 15 Hz), 142.6, 141.6 (d, JCF = 8 Hz), 135.3, 131.4, 130.6 (d, JCF = 4 Hz), 126.5, 123.2, 119.9, 109.4, 108.4, 72.3, 58.1, 50.4, 48.8, 46.2, 31.2, 31.0, 21.1, 18.6. HRMS (EI): m/z [M⁵⁺] calcd for C₂₂H₂₄N₃O₂:F 409.1914; found: 409.1912.

2-Chloro-5-[[10-(2,5-dimethylphenyl)-9-nitro-2,3,5,6,7,8-hexahydro-1H-5,8-epiminooimidazo[1,2-ajazepin-1-yl]methyl]thiazole (2a): Yellow solid; 69 mg, 38%; m.p. 163 °C–164 °C; ¹H NMR (400 MHz, DMSO-d₆): δ 7.59 (s, 1H), 7.02 (d, J = 7.6 Hz, 1H), 6.74 (d, J = 7.6 Hz, 1H), 6.57 (s, 1H), 5.11 (d, J = 3.6 Hz, 1H), 5.01 (d, J = 4.6 Hz, 1H), 4.90 (q, J = 15.4 Hz, 2H), 3.74–3.59 (m, 2H), 3.58–3.40 (m, 2H), 2.23 (s, 3H), 2.19–2.18 (m, 1H), 2.17 (s, 3H), 2.13–1.84 (m, 3H); ¹³C NMR (100 MHz, DMSO-d₆): δ 155.6, 151.9, 143.0, 141.5, 136.6, 135.7, 131.8, 127.0, 123.8, 120.3, 109.2, 72.8, 54.9, 46.9, 46.4, 31.8, 31.6, 21.5, 19.1. HRMS (EI): m/z [M-HNO₃]⁺ calcd for C₁₈H₁₈N₄S 384.1175; found: 384.1169; calcld for C₁₈H₁₈N₄S 384.1175; found: 386.1148.

2-Benzyl-10-(2,5-dimethylphenyl)-9-nitro-2,3,5,6,7,8-hexahydro-1H-5,8-epiminooimidazo[1,2-ajazepine (2b): Brown solid; 69 mg, 39%; m.p. 196 °C–197 °C; ¹H NMR (400 MHz, CDCl₃): δ 8.04 (s, 1H), 7.27 (s, 1H), 7.03 (d, J = 7.6 Hz, 1H), 6.78 (d, J = 7.6 Hz, 1H), 6.63 (s, 1H), 5.11 (d, J = 5.6 Hz, 1H), 4.95 (d, J = 4.6 Hz, 1H), 3.84–3.54 (m, 4H), 2.46–2.21 (m, 8H), 2.27 (s, 3H), 2.23 (d, J = 9.2 Hz, 3H), 2.16–2.03 (m, 2H); ¹³C NMR (100 MHz, CDCl₃): δ 156.8, 142.6, 136.3, 131.6, 126.9, 123.9, 120.3, 108.5, 72.0, 70.8, 56.3, 53.5, 52.8, 46.8, 45.9, 42.7, 32.2, 29.7, 21.5, 19.0, 7.9. HRMS (EI): m/z [M-HNO₃]⁺ calcd for C₂₁H₂₁N₃O₂ 373.2154; found: 373.2159.
1H), 6.74 (d, J = 7.6 Hz, 1H), 6.65 (s, 1H), 5.13 (d, J = 4.8 Hz, 1H), 4.92 (d, J = 5.2 Hz, 1H), 4.53–4.37 (m, 1H), 4.37–4.25 (m, 1H), 3.79–3.61 (m, 3H), 3.60–3.42 (m, 2H), 3.31–3.18 (m, 1H), 2.23 (s, 3H), 2.19 (m, 2H), 1.81 (s, 3H), 2.17–2.07 (m, 1H), 2.08–1.96 (m, 1H), 1.95–1.73 (m, 3H).

13C NMR (100 MHz, DMSO-d6): δ 155.4, 142.7, 135.2, 131.3, 126.4, 123.1, 120.0, 108.1, 81.6 (d, 1JCP = 161 Hz), 72.2, 58.2, 49.4, 46.6 (3C), 31.2 (d, 1JC = 37 Hz), 28.2 (d, 1JC = 19 Hz), 20.9, 18.6. 19F NMR (376 MHz, DMSO-d6): δ = -218.10 (s). HRMS (EI): m/z [M•] caleld for C16H20N3O2F: 374.2118; found: 374.2116.

1-(6-Chloropyridin-3-ylmethyl)-11-(2,5-dimethylphenyl)-10-nitro-2,3,5,6,7,8-hexahydro-1H,5,8-epiminomimidazolo[1,2-a]azepine (2a): Black oil; 130 mg, 43%; m.p. 128 °C–130 °C; 1H NMR (400 MHz, DMSO-d6): δ 7.02 (d, J = 7.6 Hz, 1H), 6.73 (d, J = 7.6 Hz, 1H), 6.66 (s, 1H), 5.12 (d, J = 5.2 Hz, 1H), 4.91 (d, J = 5.2 Hz, 1H), 4.45 (t, J = 5.6 Hz, 1H), 4.34 (t, 1H), 3.75–3.38 (m, 5H), 3.32–3.16 (m, 1H), 2.23 (s, 3H), 2.20 (m, 1H), 2.18 (s, 3H), 2.13 (td, J = 11.0, 5.6 Hz, 1H), 2.07–1.95 (m, 1H), 1.94–1.81 (m, 1H), 1.65–1.43 (m, 4H). 13C NMR (100 MHz, DMSO-d6): δ 155.3, 142.7, 135.2, 131.3, 126.4, 123.1, 120.0, 108.1, 83.5 (d, 1JCP = 161 Hz), 72.2, 58.3, 54.9, 49.6, 41.6, 46.3, 31.0 (d, 1JC = 34 Hz), 27.0 (d, 1JC = 19 Hz), 23.2 (d, 1JC = 4 Hz), 20.9, 18.6.

19F NMR (376 MHz, DMSO-d6): δ = -218.10 (s). HRMS (EI): m/z [M•] caleld for C80H72N32O4F2: 374.2118; found: 374.2116.

11-(2,5-Dimethylphenyl)-9-nitro-2,3,5,6,7,8-hexahydro-1H,5,8-epiminomimidazolo[1,2-a]azepine (2b): Yellow solid; 93 mg, 41%; m.p. 200 °C–202 °C; 1H NMR (400 MHz, DMSO-d6): δ 8.85 (s, 1H), 7.01 (d, J = 7.6 Hz, 1H), 6.72 (d, J = 7.2 Hz, 1H), 6.56 (s, 1H), 5.11 (d, J = 4.4 Hz, 1H), 4.97 (d, J = 6.0 Hz, 1H), 3.74–3.57 (m, 3H), 3.57–3.44 (m, 1H), 2.28 (ddd, J = 20.4, 14.8, 9.4 Hz, 1H), 2.22 (s, 3H), 2.19 (s, 3H), 2.18–2.04 (m, 2H), 1.94–1.81 (m, 1H).

13C NMR (100 MHz, CDCl3): δ 156.7, 142.6, 136.3, 131.6, 126.9, 123.9, 120.3, 108.5, 72.0, 56.3, 46.8, 42.7, 32.2 (2C), 21.5, 19.0. HRMS (EI): m/z [M•] caleld for C80H72N32O4F2: 374.2118; found: 374.2116.

11-(2,5-Dimethylphenyl)-10-nitro-2,3,5,6,7,8-hexahydro-1H,5,8-epiminomimidazolo[1,2-a]azepine (2c): Light yellow solid; 81 mg, 39%; m.p. 205 °C–206 °C; 1H NMR (400 MHz, DMSO-d6): δ 10.94 (s, 1H), 7.03 (d, J = 7.4 Hz, 1H), 6.78 (d, J = 6.8 Hz, 1H), 6.56 (s, 1H), 5.28 (s, 1H), 4.72 (s, 1H), 3.48–3.26 (m, 4H), 2.35 (s, 3H), 2.27 (s, 3H), 2.14 (ddd, J = 19.2, 10.8 Hz, 2H), 2.02 (ddd, J = 26.8, 6.0 Hz, 4H). 13C NMR (100 MHz, CDCl3): δ 151.8, 142.4, 136.2, 131.7, 127.1, 123.9, 120.2, 109.5, 57.6, 44.0, 38.0, 32.9, 30.9, 21.5, 20.0, 18.9. HRMS (EI): m/z [M•] caleld for C80H72N32O4F2: 374.2118; found: 374.2117.
4.14 (t, J = 5.2 Hz, 1H), 3.35–3.18 (m, 3H), 3.12–3.02 (m, 1H), 2.27 (s, 3H), 2.26 (s, 3H), 2.25–2.00 (m, 4H). 13C NMR (100 MHz, CDCl3): δ 155.5, 151.1, 149.1, 143.5, 138.5, 135.9, 131.4, 130.9, 127.0, 124.6, 123.5, 123.0, 120.7, 71.6, 58.5, 52.5, 46.9 (2C), 44.6, 36.4, 32.3, 21.5, 18.9. HRMS (ESI): m/z [M + H]+ calcd for C23H2435ClN5: 406.1720; found: 406.1798; calcd for C23H2437ClN5: 408.1769; found: 408.1749.

1-[(6-Chloropyridin-3-yl)methyl]-10-(2,5-dimethylphenyl)-2,3,5,6,7,8-hexahydro-1H-5,8-epi minoimidazo[1,2-a]azepine-9-carboxylate (5b): Black oil; 55 mg, 44%; m.p. 114 °C–116 °C; 1H NMR (400 MHz, DMSO-d6): δ 8.25 (d, J = 2.3 Hz, 1H), 7.64 (dd, J = 8.2, 2.4 Hz, 1H), 7.33 (d, J = 8.2 Hz, 1H), 6.98 (d, J = 7.6 Hz, 1H), 4.94 (d, J = 3.6 Hz, 1H), 4.70 (s, 2H), 4.62 (d, J = 4.8 Hz, 1H), 3.94 (q, J = 7.0 Hz, 2H), 3.39–3.34 (m, 1H), 3.32 (dd, J = 8.2, 3.8 Hz, 1H), 3.27 (dd, J = 6.2, 2.4 Hz, 1H), 3.13–3.02 (m, 1H), 2.22 (s, 3H), 2.16 (s, 3H), 2.10 (dd, J = 21.2, 8.8 Hz, 3H), 1.81 (dd, J = 12.8, 5.4 Hz, 1H), 1.08 (t, J = 7.0 Hz, 3H), 13C NMR (100 MHz, DMSO-d6): δ 164.3, 157.6, 149.1, 148.8, 144.1, 139.0, 134.8, 133.5, 131.0, 126.0, 123.8, 122.2, 120.2, 77.8, 70.9, 57.2, 57.0, 50.4, 48.3, 44.7, 35.3, 32.1, 21.1, 18.9, 14.8. HRMS (ESI): m/z [M + H]1+ calcd for C25H3035ClN4O2: 453.2057; found: 453.2053; calcd for C25H3037ClN4O2: 455.2028; found: 455.2033.

Declaration of conflicting interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding
The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was financially supported by the National Natural Science Foundation of China (21672061) and the work was also supported by the Innovation Program of Shanghai Municipal Education Commission (20171070002E00037) and the Fundamental Research Funds for Central Universities. National Key Research Program of China (2018YFD200105, 2017YFD200505).

ORCID iD
Yuce Chen https://orcid.org/0000-0003-3550-5220

Supplemental material
Supplemental material for this article is available online.

References
1. Bass C, Denholm I, Williamson MS, et al. Pestic Biochem Phys 2015; 121: 78.
2. Hladik ML, Main AR and Goulson D. Environ Sci Technol 2018; 52: 3329.
3. Nauen R, Jeschke P and Copping L. Pest Manag Sci 2008; 64: 1081.
4. Daniel C. Nature 2013; 496: 408.
5. Stokstad E. Science 2012; 335: 1555.
6. Whitehorn PR, O’Connor S, Wackers FL, et al. Science 2012; 336: 351.
7. Francis LWR. Science 2010; 327: 152.
8. Juliet LO. Nature 2012; 491: 43.
9. Gill RJ, Ramos-Rodriguez O and Raine NE. Nature 2012; 491: 105.
10. Veerle M, Sofie R, Jana B, et al. Ecotoxicology 2010; 19: 207.
11. Zhu YC, Yao J and Adamczyk J. J Appl Entomol 2019; 143: 118.
12. Gross M. Curr Biol 2018; 28: R1121–R1123.
13. Blake RJ and Copping LG. Pest Manag Sci 2017; 73: 1293.
14. Yuanming Z, Loso MR, Watson GB, et al. J Agric Food Chem 2011; 59: 2950.
15. Zhou H. Acta Entomo Sinica 2017.
16. Nauen R, Jeschke P, Velten R, et al. Pest Manag Sci 2015; 71: 850.
17. Hesselbach H and Scheiner R. Sci Rep 2018; 8: 4954.
18. Onozaki Y, Horikoshi R, Ohno I, et al. J Agric Food Chem 2017; 65: 7865.
19. Xu R, Luo M, Xia R, et al. J Agric Food Chem 2014; 62: 11070.
20. Kagabu S, Murase Y, Imai R, et al. Pest Manag Sci 2007; 63: 75.
21. Kagabu S, Aoki E and Ohno I. J Pestic Sci 2007; 32: 128.
22. Feyerisen R. Curr Biol 2018; 28: R560.
23. Amir N, Motonishi M, Fujita M, et al. Eur J Inorg Chem 2006; 2006: 1041.
24. Zhongzhen T. J Agric Food Chem 2007; 6: 55.
25. Shiokawa K, Toshibe S and Moriee K. Patent JP62048681-A, JP, 1987.
26. Kozo S, Shinzo K and Shinichi T. Patent EP136636-A2, EP, 1985.
27. Cappi MW, Pearson M and Wilson AC. Patent GB2228003-A, GB, 1990.
28. Davies SA, Mankee JB and Mete A. Patent EP623601-A1, EP, 1994.
29. Kagabu S and Medej S. Biosci Biotechnol Biochem 1995; 59: 980.