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

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Abstract

In order to explore the relationship between the structure and the toxicity to honey bees of seven-membered aza-bridged neonicotinoids, 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 NO_2 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-1*H*-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

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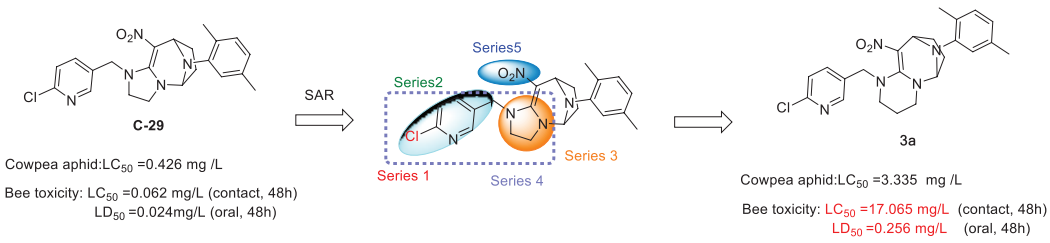

Introduction

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

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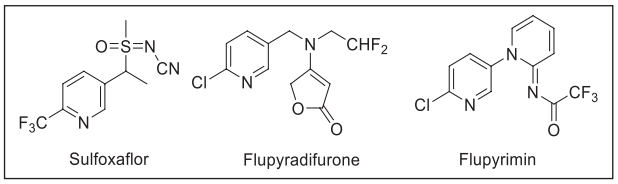


Figure 1. Some commercial nAChRs compounds.

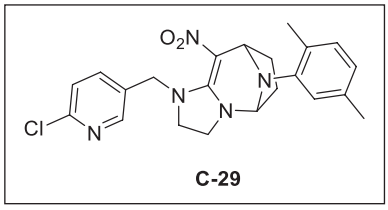


Figure 2. An aza-bridged compound.

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, sulfoxaflor[14,15] and flupyradifurone[16,17] 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.[19] 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**, LC_{50} = 0.062 mg L^{-1}; imidacloprid, LC_{50} = 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 (heteroaryl)methyl groups (**4a–d**) and introduced CN and COOEt instead of NO_2 (**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 Et_3N to give intermediates **29**. Cyclization with *N*1-[(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

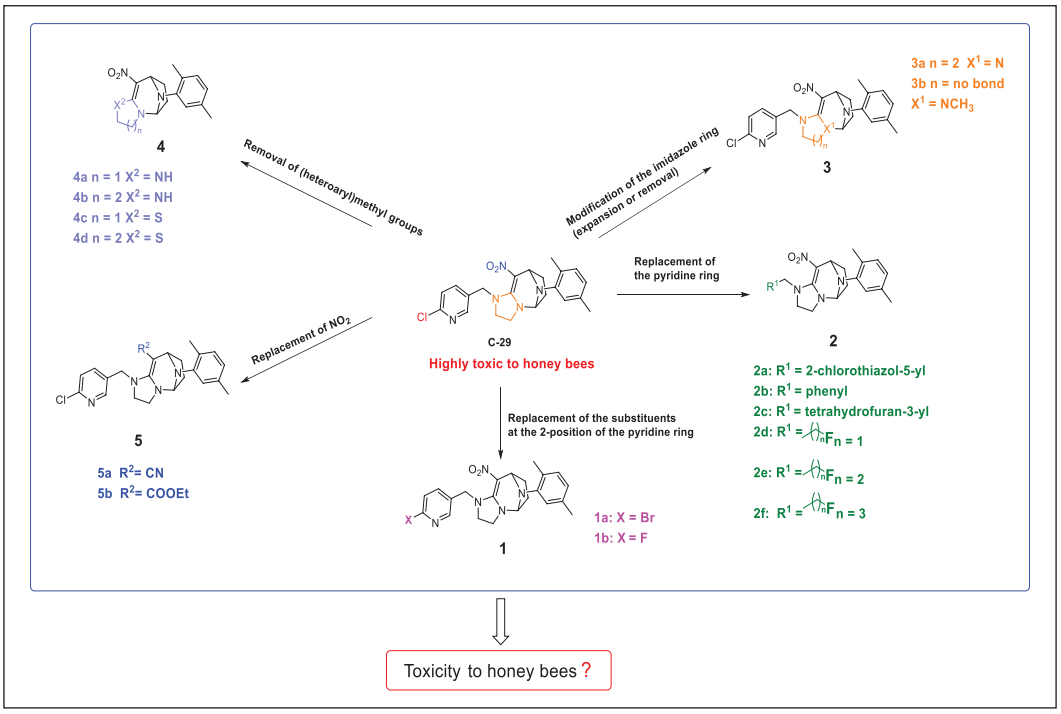
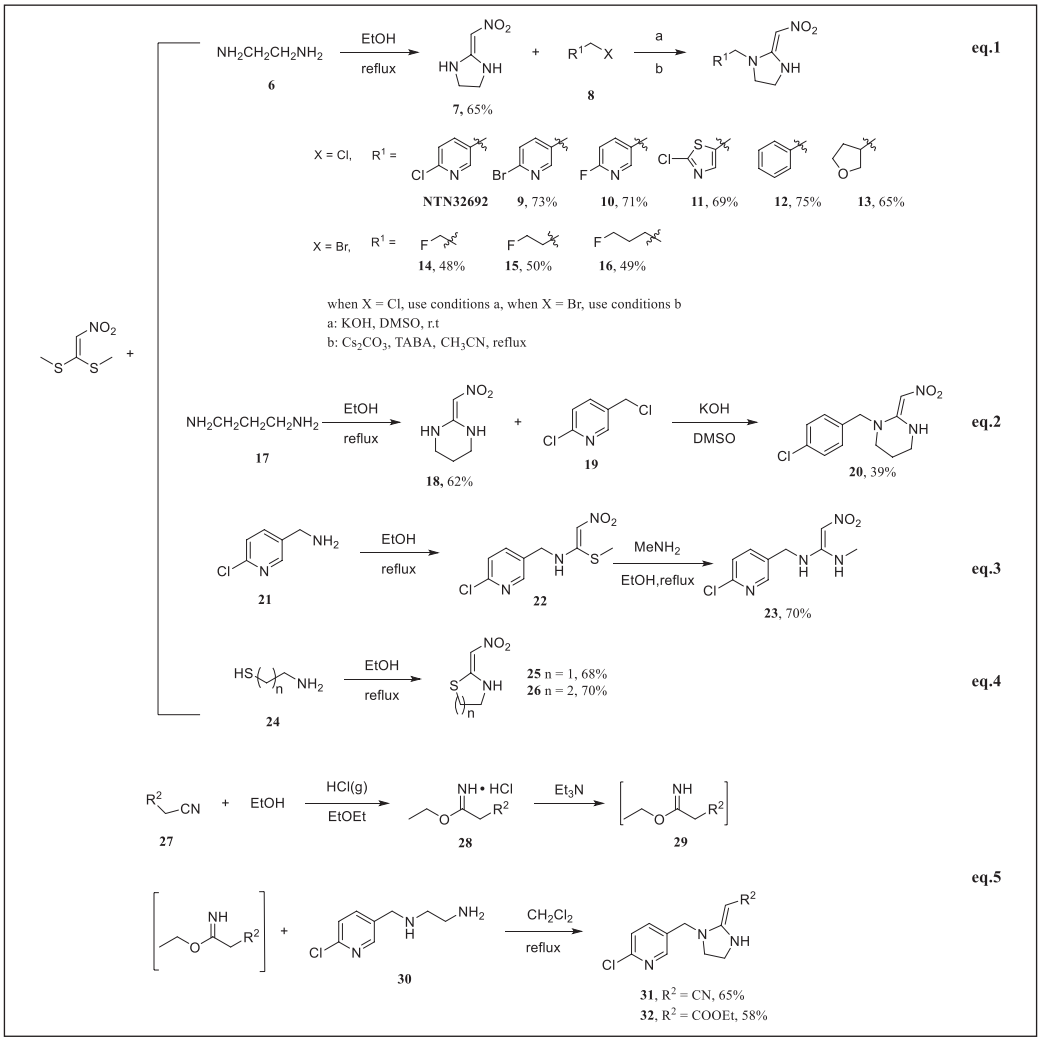
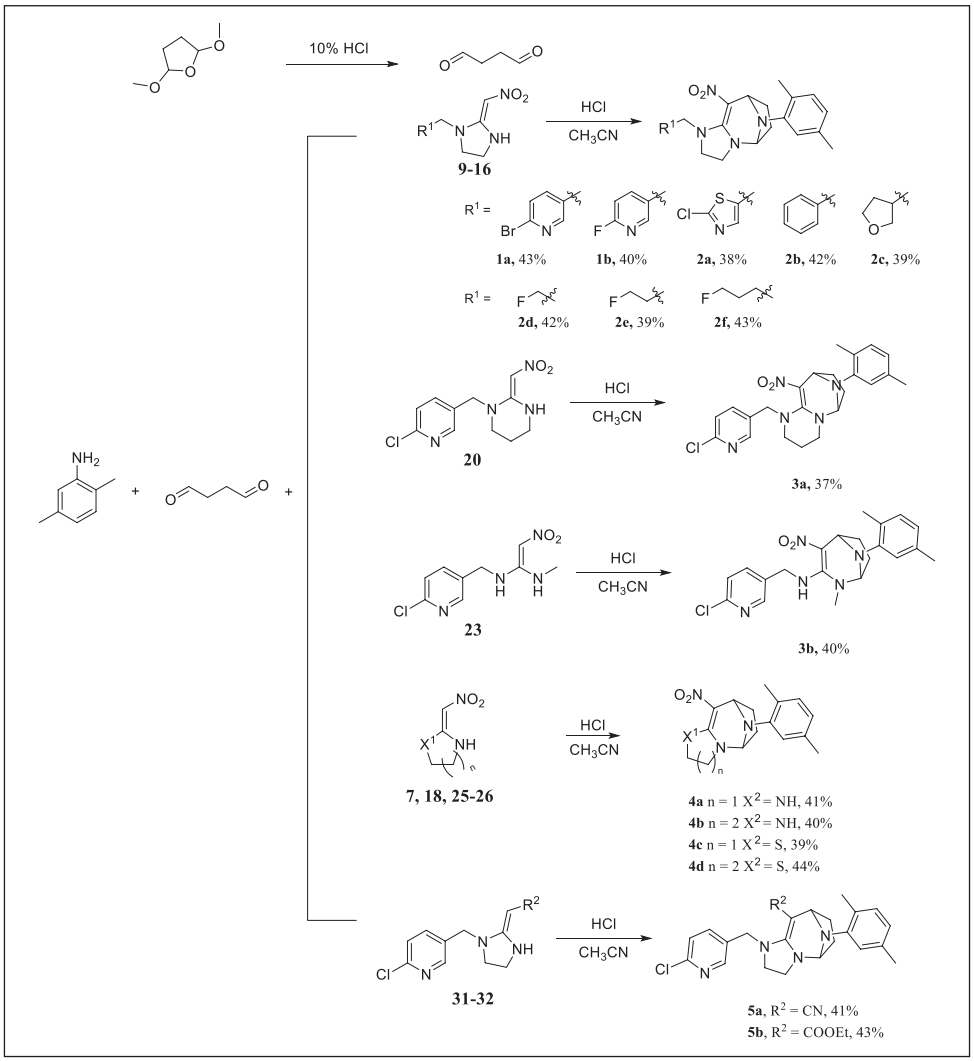


Figure 3. The optimization of the **C-29.**



Scheme 1. Synthetic routes to the intermediates.



Scheme 2. General synthesis for target compounds.

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 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

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

Compounds	Mortality (%)		
	100 mg L^{-1}	20 mg L^{-1}	LC_{50}
C-29	100	100	LC_{50} = 0.426 mg L^{-1}
1a	100	66.97	LC_{50} = 7.216 mg L^{-1}
1b	80	0	–
2a	100	50.36	LC_{50} = 20.363 mg L^{-1}
2b	0	0	–
2c	0	0	–
2d	0	0	–
2e	80	0	–
2f	90	0	–
3a	100	96.11	LC_{50} = 3.335 mg L^{-1}
3b	100	100	LC_{50} = 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	LC_{50} = 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%, 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}, LC_{50} value was obtained.

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 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 two 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, 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. ^{1}H NMR, ^{13}C NMR and ^{19}F 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 δ (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

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

Compound	Time	LD_{50} (μg bee^{-1})	Toxic regression equation	R^{2a}	Toxicological grade[b]
3a	24	0.328	y = 1.540x + 0.746	0.981	B
	48	0.256	y = 1.639x + 0.971	0.975	
2a	24	0.320	y = 0.702x + 0.347	0.965	B
	48	0.191	y = 1.074x + 0.773	0.966	
3b	24	0.181	y = 1.559x + 1.157	0.999	B
	48	0.125	y = 1.765x + 1.596	0.990	
1a	24	0.081	y = 2.537x + 2.769	0.947	B
	48	0.028	y = 2.357x + 3.662	0.985	
NTN32692	24	0.028	y = 2.047x + 3.191	0.881	B
	48	0.023	y = 2.485x + 4.097	0.972	
Imidacloprid	24	0.213	y = 0.637x + 0.428	0.962	B
	48	0.076	y = 0.837x + 0.935	0.988	
C-29	24	0.062	y = 1.769x + 3.331	0.983	B
	48	0.024	y = 1.858x + 3.689	0.996	

[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^{2a}	Toxicological grade[b]
3a	24	154.396	y = 1.078x − 2.359	0.911	B
	48	17.065	y = 3.151x − 3.882	0.997	
1a	24	8.706	y = 2.357x − 2.215	0.949	B
	48	2.594	y = 4.111x − 1.702	0.981	
2a	24	0.483	y = 1.405x + 0.444	0.984	A
	48	0.201	y = 1.952x + 1.360	0.991	
3b	24	0.409	y = 1.995x + 0.775	0.977	A
	48	0.328	y = 2.010x + 0.973	0.984	
Imidacloprid	48	10.560	y = 1.142x − 1.169	0.926	B
C-29	24	0.214	y = 3.061x + 2.051	0.987	A
	48	0.087	y = 2.924x + 3.104	0.998	

[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.

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.

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 Na_2SO_4, 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 × 3). 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 target 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 Cs_2CO_3 (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 was subjected to a flash chromatography on silica gel (CH_2Cl_2/EtOH, 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 Na_2SO_4, filtered, and concentrated under reduced pressure to yield the crude product. The crude product was purified via flash silica gel chromatography (CH_2Cl_2/MeOH, 18:1) to give intermediate **18**.

To a solution of 2-chloro-5-(chloromethyl)pyridine **19** (2 mmol) and intermediate **18** (2 mmol) in 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 × 3). 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 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 Na_2SO_4, filtered, and concentrated under reduced pressure to yield the crude product. The crude product was purified via flash silica gel chromatography (CH_2Cl_2/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 evaporated the solvent and quenched with water. The mixture was extracted with dichloromethane (50 mL × 3), and the combined organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure to yield the crude product. The crude product was purified via flash silica gel chromatography (CH_2Cl_2/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 time 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 $NaHCO_3$ 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 $NaHCO_3$. The obtained solution could be used directly in the next step.[22]

*1-[(6-Bromopyridin-3-yl)methyl]-10-(2,5-dimethylphenyl)-9-nitro-2,3,5,6,7,8-hexahydro-1*H*-5,8-epiminoimidazo[1,2-*a*]azepine* **(1a):** White solid; 68 mg, 43%; m.p. 151 °C–152 °C; ^{1}H NMR (400 MHz, $CDCl_3$): 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), 6.83 (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); ^{13}C NMR (100 MHz, $CDCl_3$): 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* $[M\text{-}HNO_2]^+$calcd for $C_{22}H_{23}{}^{79}BrN_4$: 422.1106; found: 422.1108; calcd for $C_{22}H_{23}{}^{81}BrN_4$: 424.1086; found: 424.1090.

*10-(2,5-Dimethylphenyl)-1-[(6-fluoropyridin-3-yl)methyl]-9-nitro-2,3,5,6,7,8-hexahydro-1*H*-5,8-epiminoimidazo[1,2-*a*]azepine* **(1b):** Russet solid; 120 mg, 40%; m.p. 162 °C–163 °C; ^{1}H NMR (400 MHz, DMSO-d_6): 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 (tt, *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). ^{13}C NMR (100 MHz, DMSO-d_6): δ 162.4 (d, $^1J_{CF}$ = 233 Hz), 155.8, 146.8 (d, $^2J_{CF}$ = 15 Hz), 142.6, 141.6 (d, $^3J_{CF}$ = 8 Hz), 135.3, 131.4, 130.6 (d, $^3J_{CF}$ = 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, 21.0, 18.6. ^{19}F NMR (376 MHz, DMSO-d_6): δ −70.59 (d, *J* = 7.5 Hz). HRMS (EI): *m/z* $[M^+]$ calcd for $C_{22}H_{24}N_5O_2F$: 409.1914; found: 409.1912.

*2-Chloro-5-{[10-(2,5-dimethylphenyl)-9-nitro-2,3,5,6,7,8-hexahydro-1*H*-5,8-epiminoimidazo[1,2-*a*]azepin-1-yl]methyl}thiazole* **(2a):** Yellow solid; 69 mg, 38%; m.p. 163 °C–164 °C; ^{1}H NMR (400 MHz, DMSO-d_6): *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). ^{13}C NMR (100 MHz, DMSO-d_6): 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, 58.5, 49.3, 46.9, 46.4, 31.8, 31.6, 21.5, 19.1. HRMS (EI): *m/z* $[M\text{-}HNO_2]^+$ calcd for $C_{20}H_{21}{}^{35}ClN_4S$: 384.1175; found: 384.1169; calcd for $C_{20}H_{21}{}^{37}ClN_4S$: 386.1146; found: 386.1148.

*1-Benzyl-10-(2,5-dimethylphenyl)-9-nitro-2,3,5,6,7,8-hexahydro-1*H*-5,8-epiminoimidazo[1,2-*a*]azepine* **(2b):** Yellow solid; 69 mg, 42%; m.p. 132 °C–133 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.29–7.22 (m, 3H), 7.14 (dd, *J* = 6.0, 2.4 Hz, 2H), 7.05 (d, *J* = 7.6 Hz, 1H), 6.78 (d, *J* = 7.6 Hz, 1H), 6.69 (s, 1H), 5.17 (d, *J* = 4.0 Hz, 1H), 5.01 (d, *J* = 4.8 Hz, 1H), 4.85–4.74 (m, 2H), 3.75–3.61 (m, 1H), 3.61–3.49 (m, 1H), 3.43–3.27 (m, 2H), 2.24 (s, 3H), 2.20 (m, 1H), 2.19 (s, 3H), 2.17–1.94 (m, 2H), 1.90 (dd, *J* = 18.0, 10.8 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 156.0, 142.6, 136.6, 135.4, 131.5, 128.5 (2C), 127.4, 127.3 (2C), 126.3, 123.2, 120.0, 108.3, 72.2, 58.2, 53.2, 48.3, 46.3, 31.2, 30.7, 21.1, 18.7. HRMS (EI): *m/z* $[M\text{-}HNO_2]^+$ calcd for $C_{23}H_{25}N_3$: 343.2048; found: 343.2049.

*10-(2,5-Dimethylphenyl)-9-nitro-1-[(tetrahydrofuran-3-yl)methyl]-2,3,5,6,7,8-hexahydro-1*H*-5,8-epiminoimidazo[1,2-*a*]azepine* **(2c):** Brown solid; 69 mg, 39%; m.p. 196 °C–197 °C; ^{1}H NMR (400 MHz, $CDCl_3$): 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). ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.7, 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* $[M\text{-}HNO_2]^+$ calcd for $C_{21}H_{27}N_3O$: 337.2154; found: 337.2159.

*10-(2,5-Dimethylphenyl)-1-(2-fluoroethyl)-9-nitro-2,3,5,6,7,8-hexahydro-1*H*-5,8-epiminoimidazo[1,2-*a*]azepine* **(2d):** Orange solid; 78 mg, 42%; m.p. 143 °C–144 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.03 (d, *J* = 7.4 Hz, 1H), 6.78 (d, *J* = 8.0 Hz, 2H), 5.06 (d, *J* = 4.2 Hz, 1H), 4.94 (d, *J* = 6.0 Hz, 1H), 4.81–4.79 (m, 1H), 4.70–4.52 (m, 1H), 4.03–3.74 (m, 3H), 3.66–3.44 (m, 2H), 3.18 (dd, *J* = 19.6, 10.0 Hz, 1H), 2.44–2.16 (m, 3H), 2.28 (s, 3H), 2.23 (s, 3H), 2.08–1.96 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.2, 142.5, 136.3, 131.6, 126.6, 124.0, 120.6, 109.4, 83.8 (d,

$^{1}J_{CF}$ = 161 Hz), 73.3, 58.7, 51.8 (d, $^{2}J_{CF}$ = 19 Hz), 51.6 (d, $^{3}J_{CF}$ = 2 Hz), 47.9, 31.9, 31.0, 21.2, 18.8. ^{19}F NMR (376 MHz, $CDCl_3$): δ −217.20 (s). HRMS (EI): *m/z* [M^+] calcd for $C_{18}H_{23}N_4O_2F$: 346.1805; found: 346.1810.

*10-(2,5-Dimethylphenyl)-1-(3-fluoropropyl)-9-nitro-2,3,5,6,7,8-hexahydro-1*H*-5,8-epiminoimidazo[1,2-*a*]azepine* **(2e):** Orange solid; 86 mg, 39%; m.p. 156 °C–157 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.02 (d, *J* = 7.6 Hz, 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, 1H), 2.18 (s, 3H), 2.17–2.07 (m, 1H), 2.08–1.96 (m, 1H), 1.95–1.73 (m, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 155.4, 142.7, 135.2, 131.3, 126.4, 123.1, 120.0, 108.1, 81.6 (d, $^{1}J_{CF}$ = 161 Hz), 72.2, 58.2, 49.4, 46.6 (3C), 31.2 (d, $^{2}J_{CF}$ = 37 Hz), 28.2 (d, $^{3}J_{CF}$ = 19 Hz), 20.9, 18.6. ^{19}F NMR (376 MHz, DMSO-d_6): δ −218.10 (s). HRMS (EI): *m/z* [M^+] calcd for $C_{19}H_{25}N_4O_2F$: 360.1962; found: 360.1960.

*10-(2,5-Dimethylphenyl)-1-(4-fluorobutyl)-9-nitro-2,3,5,6,7,8-hexahydro-1*H*-5,8-epiminoimidazo[1,2-*a*]azepine* **(2f):** Orange solid; 86 mg, 43%; m.p. 128 °C–130 °C; ^{1}H NMR (400 MHz, DMSO-d_6): 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). ^{13}C NMR (100 MHz, DMSO-d_6): δ 155.3, 142.7, 135.2, 131.3, 126.4, 123.1, 120.0, 108.1, 83.5 (d, $^{1}J_{CF}$ = 161 Hz), 72.2, 58.3, 54.9, 49.6, 49.1, 46.6, 31.0 (d, $^{2}J_{CF}$ = 34 Hz), 27.0 (d, $^{3}J_{CF}$ = 19 Hz), 23.2 (d, $^{4}J_{CF}$ = 4 Hz), 20.9, 18.6. ^{19}F NMR (376 MHz, DMSO-d_6): δ −218.61 (s). HRMS (EI): *m/z* [M^+] calcd for $C_{20}H_{27}N_4O_2F$: 374.2118; found: 374.2116.

1-[(6-Chloropyridin-3-yl)methyl]-11-(2,5-dimethylphenyl)-10-nitro-1,2,3,4,6,7,8,9-octahydro-6,9-epiminopyrimido[1,2-a]azepine **(3a):** Black oil; 130 mg, 37%; m.p. 133 °C–134 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.17 (d, *J* = 2.2 Hz, 1H), 7.46 (dd, *J* = 8.2, 2.4 Hz, 1H), 7.37 (d, *J* = 8.2 Hz, 1H), 7.08 (d, *J* = 7.6 Hz, 1H), 6.81 (d, *J* = 7.6 Hz, 1H), 6.76 (s, 1H), 5.11 (d, *J* = 5.8 Hz, 1H), 4.89 (d, *J* = 2.8 Hz, 1H), 4.49 (d, *J* = 15.6 Hz, 1H), 4.31 (d, *J* = 15.4 Hz, 1H), 3.56–3.44 (m, 1H), 3.15–3.06 (m, 1H), 2.92–2.73 (m, 2H), 2.36–2.28 (m, 1H), 2.26 (s, 3H), 2.15 (s, 3H), 2.10 (d, *J* = 7.6 Hz, 1H), 2.01 (dt, *J* = 14.0, 7.2 Hz, 2H), 1.82 (ddd, *J* = 14.8, 10.0, 5.6 Hz, 1H), 1.70–1.60 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 154.6, 149.5, 149.3, 142.6, 139.2, 135.5, 131.8, 131.6, 126.4, 123.9, 123.3, 120.2, 108.1, 77.1, 59.8, 51.98, 46.1, 44.6, 31.3, 29.8, 21.3, 18.7, 7.1. HRMS (EI): *m/z* [M-HNO_2]$^+$ calcd for $C_{23}H_{25}{}^{35}ClN_4$: 392.1768; found: 392.1766; calcd for $C_{23}H_{25}{}^{37}ClN_4$: 394.1738; found: 394.1731.

N-[(6-Chloropyridin-3-yl)methyl]-8-(2,5-dimethylphenyl)-2-methyl-4-nitro-2,8-diazabicyclo[3.2.1]oct-3-en-3-amine **(3b):** Brown solid; 93 mg, 40%; m.p. 133 °C–134 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.90 (t, *J* = 5.8 Hz, 1H), 8.26 (d, *J* = 2.2 Hz, 1H), 7.57 (d, *J* = 8.2 Hz, 1H), 7.42 (d, *J* = 8.4 Hz, 1H), 7.03 (d, *J* = 7.6 Hz, 1H), 6.76 (d, *J* = 7.6 Hz, 1H), 6.52 (s, 1H), 5.12 (d, *J* = 4.2 Hz, 1H), 4.98 (d, *J* = 3.2 Hz, 1H), 4.61 (d, *J* = 6.0 Hz, 2H), 3.09 (s, 3H), 2.21 (s, 3H), 2.17 (m, 1H), 2.14 (s, 3H), 2.12–1.95 (m, 3H), 1.89 (t, *J* = 8.6 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 157.5, 149.4, 148.6, 142.3, 138.4, 135.4, 133.3, 131.4, 126.4, 124.2, 123.3, 119.6, 112.7, 77.4, 57.1, 45.4, 37.8, 31.5, 30.4, 20.9, 18.6. HRMS (EI): *m/z* [M^+] calcd for $C_{21}H_{24}N_5O_2{}^{35}Cl$: 413.1619; found: 413.1617; calcd for $C_{21}H_{24}N_5O_2{}^{37}Cl$: 415.1589; found: 415.1598.

*10-(2,5-Dimethylphenyl)-9-nitro-2,3,5,6,7,8-hexahydro-1*H*-5,8-epiminoimidazo[1,2-*a*]azepine* **(4a):** Yellow solid; 93 mg, 41%; m.p. 200 °C–202 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.55 (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). ^{13}C NMR (100 MHz, $CDCl_3$): 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^+] calcd for $C_{16}H_{20}N_4O_2$: 300.1586; found: 300.0629.

*11-(2,5-Dimethylphenyl)-10-nitro-1,2,3,4,6,7,8,9-octahydro-6,9-epiminopyrimido[1,2-*a*]azepine* **(4b):** Light yellow solid; 99 mg, 40%; m.p. 212 °C–213 °C; ^{1}H NMR (400 MHz, $CDCl_3$): 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 (dd, *J* = 19.2, 10.8 Hz, 2H), 2.02 (dd, *J* = 26.8, 6.0 Hz, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): 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^+] calcd for $C_{17}H_{22}N_4O_2$: 314.1743; found: 314.1739.

*10-(2,5-Dimethylphenyl)-9-nitro-2,3,5,6,7,8-hexahydro-5,8-epiminothiazolo[3,2-*a*]azepine* **(4c):** Light yellow solid; 81 mg, 39%; m.p. 205 °C–206 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.05 (d, *J* = 7.6 Hz, 1H), 6.76 (d, *J* = 7.6 Hz, 1H), 6.47 (s, 1H), 5.30 (d, *J* = 4.0 Hz, 1H), 5.01 (d, *J* = 5.6 Hz, 1H), 4.06–3.91 (m, 2H), 3.25 (ddd, *J* = 11.3, 8.4, 5.8 Hz, 1H), 3.20–3.08 (m, 1H), 2.31 (ddd, *J* = 16.0, 10.6, 5.8 Hz, 1H), 2.25 (s, 3H), 2.24–2.20 (m, 2H), 2.19 (s, 3H), 2.03–1.93 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.9, 142.4, 135.3, 131.5, 126.6, 123.5, 119.6 (2C), 73.5, 56.1, 52.8, 32.9, 32.7, 27.6, 21.1, 18.7. HRMS (EI): *m/z* [M^+] calcd for $C_{16}H_{20}N_3O_2S$: 317.1198; found: 317.1196.

*11-(2,5-Dimethylphenyl)-10-nitro-3,4,6,7,8,9-hexahydro-2*H*-6,9-epimino[1,3]thiazino[3,2-*a*]azepine* **(4d):** Light yellow solid; 55 mg, 44%; m.p. 192 °C–193 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.04 (d, *J* = 7.6 Hz, 1H), 6.79 (d, *J* = 7.6 Hz, 1H), 6.54 (s, 1H), 5.22 (d, *J* = 4.0 Hz, 1H), 4.72 (d, *J* = 4.0 Hz, 1H), 3.61–3.44 (m, 1H), 3.31 (dt, *J* = 13.2, 6.6 Hz, 1H), 2.80 (dtd, *J* = 17.8, 12.6, 5.4 Hz, 2H), 2.33 (dd, *J* = 14.4, 4.4 Hz, 2H), 2.29 (s, 3H), 2.25 (s, 3H), 2.23–2.07 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.4, 142.2, 136.3, 131.6, 127.3, 124.3, 121.6, 120.3, 80.3, 58.7, 47.8, 33.4, 32.7, 26.7, 22.1, 21.5, 18.7. HRMS (EI): *m/z* [M^+] calcd for $C_{17}H_{21}N_3O_2S$: 331.1354; found: 331.1351.

*1-[(6-Chloropyridin-3-yl)methyl]-10-(2,5-dimethylphenyl)-2,3,5,6,7,8-hexahydro-1*H*-5,8-epiminoimidazo[1,2-*a*]azepine-9-carbonitrile* **(5a):** Brown oil; 55 mg, 44%; m.p. 192 °C–193 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.22 (d, J = 2.0 Hz, 1H), 7.54 (dd, J = 8.2, 2.4 Hz, 1H), 7.20 (d, J = 8.2 Hz, 1H), 7.05 (d, J = 7.6 Hz, 1H), 6.91 (s, 1H), 6.81 (d, J = 7.6 Hz, 1H), 4.90 (d, J = 16.0 Hz, 1H), 4.79 (d, J = 4.4 Hz, 1H), 4.43 (d, J = 16.0 Hz, 1H), 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). ^{13}C NMR (100 MHz, $CDCl_3$): 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 $C_{23}H_{24}{}^{35}ClN_5$: 406.1720; found: 406.1798; calcd for $C_{23}H_{24}{}^{37}ClN_5$: 408.1769; found: 408.1749.

*1-[(6-Chloropyridin-3-yl)methyl]-10-(2,5-dimethylphenyl)-2,3,5,6,7,8-hexahydro-1*H*-5,8-epiminoimidazo[1,2-*a*]azepine-9-carboxylate* **(5b):** Black oil; 55 mg, 44%; m.p. 114 °C–116 °C; ^{1}H NMR (400 MHz, DMSO-d_6): 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), 6.76 (s, 1H), 6.69 (d, J = 7.2 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). ^{13}C NMR (100 MHz, DMSO-d_6): 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* $[M + H]^+$ calcd for $C_{25}H_{30}{}^{35}ClN_4O_2$: 453.2057; found: 453.2053; calcd for $C_{25}H_{30}{}^{37}ClN_4O_2$: 455.2028; found: 455.2033.

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Supplemental material

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