



Original article

Design, synthesis and insecticidal activity of spiro heterocycle containing neonicotinoid analogs

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ABSTRACT

Spiro heterocycles frequently occur in bioactive molecules. In the pursuit of neonicotinoids with spiro heterocycles, three types of novel neonicotinoids with spirobenzofuranone, spirooxindole or spiroacenaphthylenone framework were designed and synthesized. Insecticidal evaluation showed that some of spirobenzofuranone containing neonicotinoids exhibited moderate activity against cowpea aphid, armyworm or brown planthopper.

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1. Introduction

Spiro heterocycles often exist in a number of natural or synthetic molecules [1–3]. A spiro compound in which the spiro carbon is part of cyclic ring has many unique properties, such as spiroconjugation, anomeric effect and axial chirality [4–7]. Therefore, spiro heterocyclic scaffolds have broad applications in many areas [8–12] and are widely used as building blocks for generating biological and pharmaceutical relevance [13–15].

Neonicotinoid is the largest insecticide now [16–18], but its superiority is being challenged due to resistance [19] and severe bee toxicity [20,21]. Thus, there is an urgent need for the development of novel, effective, neonicotinoid replacements. As part of a project aiming at exploring functionality of the spiro heterocycle core in neonicotinoids, we herein report our investigation in spiro heterocycle containing neonicotinoids.

2. Experimental

2.1. Chemistry

Melting points (mp) were recorded on Büchi B540 apparatus (Büchi Labortechnik AG, Flawil, Switzerland) and are uncorrected. The ¹H NMR and ¹³C NMR spectra were recorded on Bruker

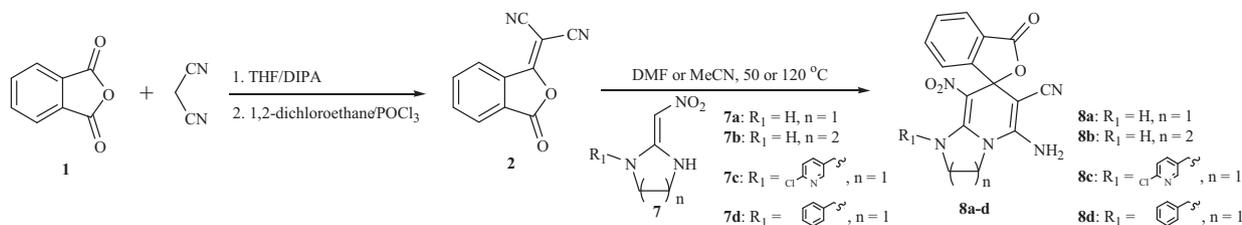
AM-400 (400 MHz) spectrometer with DMSO-*d*₆ as the solvent and TMS as the internal standard. Chemical shifts are reported in δ (parts per million) values. High-resolution mass spectra were recorded under electron impact (70 eV) conditions using a MicroMass GCT CA 055 instrument. Analytical thin-layer chromatography (TLC) was carried out on precoated plates (silica gel 60 F254), and spots were visualized with ultraviolet (UV) light.

The general synthetic methods for compounds **8a–d**, **9a–g** and **10a** and **10b** are depicted in Schemes 1–3. Unless otherwise noted, reagents and solvents were used as received from commercial suppliers. Yields were not optimized. All reactions were carried out under a protective atmosphere of drying nitrogen or utilizing a calcium chloride tube.

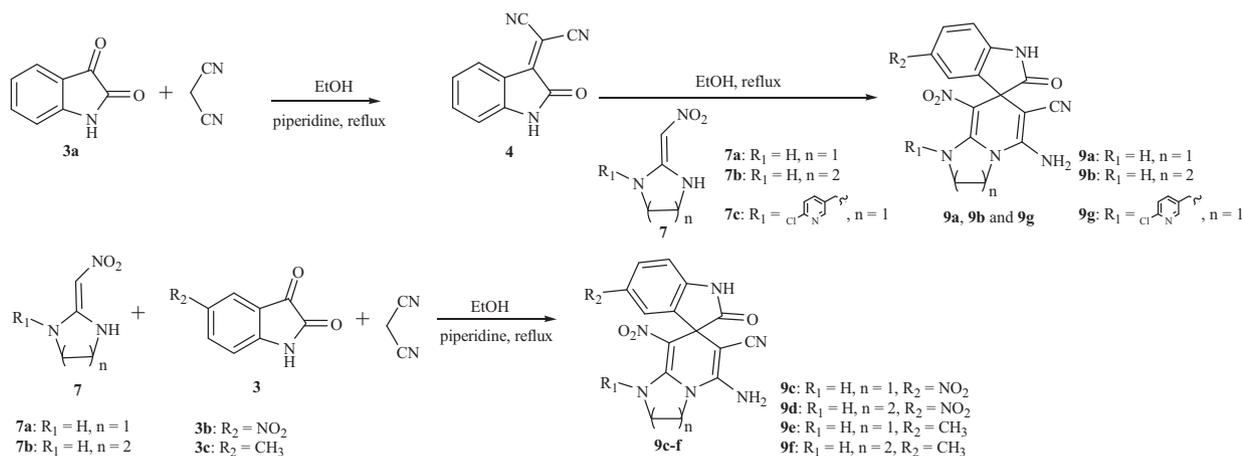
General synthetic procedure for **8a–d**: Knoevenagel adduct **2** was synthesized according to the reported procedure [8]. A mixture of phthalic anhydride (1.480 g, 10 mmol) and malononitrile (0.66 g, 11 mmol) in THF (25 mL) were stirred at room temperature. Then, diisopropylamine (DIPA) (2.02 g, 20 mmol) was added dropwise. The resulting mixture was stirred at room temperature for 8 h, and then was filtered and the precipitate was washed with THF (10 mL) to afford the white solid. Then, to a solution of the obtained white solid in 1,2-dichloroethane (20 mL) was added POCl₃ (10 mmol) dropwise at room temperature. After completion, the mixture was refluxed and the progress of the reaction was monitored by TLC. At last, the reaction mixture was neutralized by saturated dicarbonate solution to pH 7, extracted by dichloromethane (30 mL \times 3), concentrated and the residue was purified by flash chromatography eluting with dichloromethane to afford compound **2** (yield 40%).

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Scheme 1. Synthetic routes for neonicotinoids containing spiro heterocycles 8a–d.



Scheme 2. Synthetic routes for neonicotinoids containing spiro heterocycles 9a–g.

Then, a solution of compound **2** (0.190 g, 1 mmol) and compounds **7a–d** (1 mmol) in DMF (4 mL) was heated at 50 °C or 120 °C with the progress of the reaction monitored by TLC. After completion, the mixture was concentrated and the residue was purified by recrystallization with ethanol to afford the pure products **8a–d**.

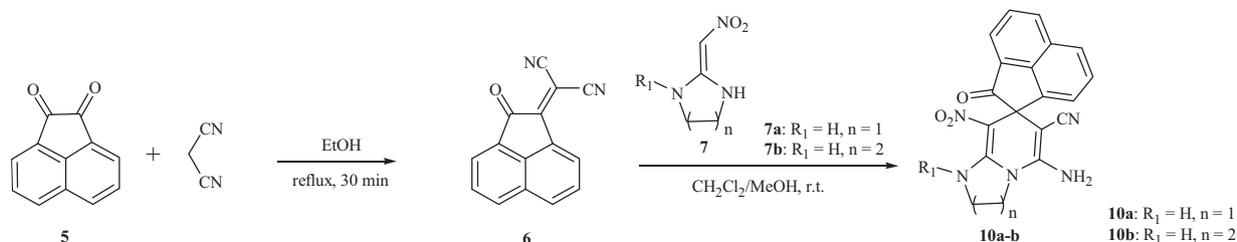
General synthetic procedure for **9a–g**: Method 1 (for **9a**, **9b** and **9g**): A mixture of isatin (1.470 g, 10 mmol), malononitrile (0.60 g, 10 mmol) and piperidine (10 mol%) in absolute ethanol (10 mL) was refluxed for 1 h. The cooled mixture was filtered and the precipitate was washed with ethanol (4 mL) to afford compound **4** (yield 80%). Then, a solution of compound **4** (0.189 g, 1 mmol) and compound **7a–c** (1 mmol) in ethanol (4 mL) was refluxed for 8 h. The mixture was filtered and the precipitate washed with cold ethanol (4 mL) affording the pure products **9a**, **9b** and **9g**. Method 2 (one-pot procedure for **9c–9f**): A solution of **3b** or **3c** (1 mmol), malononitrile (0.060 g, 1 mmol) and piperidine (10 mol%) was refluxed in ethanol (4 mL) for 1 h. Then, **7a** or **7b** was added simultaneously. The resulted mixture was stirred for 8 h under refluxing condition with the progress of the reaction monitored by TLC. After completion, the cooled mixture was filtered and the precipitate was washed with cold ethanol (4 mL) to afford the desired products **9c–9f**.

General synthetic procedure for **10a** and **10b**: A mixture of acenaphthylene-1,2-dione (1.820 g, 10 mmol) and malononitrile (0.60 g, 10 mmol) in absolute ethanol (20 mL) was refluxed for 30 min. The cooled mixture was filtered and the precipitate was washed with cold ethanol (10 mL) to afford compound **6** (yield 85%). Then, a solution of compound **6** (0.230 g, 1 mmol) and **7a** and **7b** (1 mmol) in 5 mL of mixed solvent of dichloromethane and methanol (4:1, v/v) was stirred for 2 h at room temperature. At last, the mixture was filtered and the precipitate was washed with methanol (4 mL) to afford the pure products **10a** and **10b**.

Analytical data of the target compounds are listed in Supporting information.

2.2. Biological assay

All bioassays were performed on representative test organisms grown in the laboratory. The bioassay was repeated at $(25 \pm 1)^\circ\text{C}$ according to statistical requirements. All compounds were dissolved in *N,N*-dimethylformamide (AP, Shanghai Chemical Reagent Co., Ltd., Shanghai, China) and diluted with distilled water containing Triton X-100 (0.1 mg L^{-1}) to obtain a series of concentrations of 500.0, 250.0, 125.0 mg L^{-1} and others for bioassays. The results of bioassay are depicted in Table 1.



Scheme 3. Synthetic routes for neonicotinoids containing spiro heterocycles 10a–b.

Table 1

Insecticidal activity of compounds **8a–d**, **9a–g** and **10a** and **b** against cowpea aphid (*Aphis craccivora*), armyworm (*Mythimna separata*) and brown planthopper (*Nilaparvata lugens*).

Compound	Mortality (%; 500 mg L ⁻¹)		
	<i>Aphis craccivora</i>	<i>Mythimna separata</i>	<i>Nilaparvata lugens</i>
8a	0	100	0
8b	0	20	0
8c	90	0	95
8d	0	80	0
9a	0	0	0
9b	0	0	0
9c	0	0	0
9d	0	0	0
9e	0	0	0
9f	0	0	0
9g	0	0	0
10a	0	0	0
10b	0	0	0
Imidacloprid	100	–	100

Insecticidal test for cowpea aphids (*Aphis craccivora*): The activities of insecticidal compounds against cowpea aphids were tested by leaf-dip method. The leaves of the horsebean plant with 40–60 apterous adults were dipped in diluted solutions of the chemicals containing Triton X-100 (0.1 mg L⁻¹) for 5 s and the excess solution was sucked out with filter paper, and the burgeons were placed in the conditioned room ((25 ± 1) °C, 50% RH). Water containing Triton X-100 (0.1 mg L⁻¹) was used as a control. The mortality rates were evaluated 24 h after treatment. Each treatment had three repetitions and the data were adjusted and subjected to probit analysis as before.

Insecticidal test for Armyworm (*Mythimna separata*): The insecticidal activity against armyworm was tested by foliar application. Individual corn (*Zea mays*) leaves were placed on moistened pieces of filter paper in Petri dishes. The leaves were then sprayed with the sample solutions and exposed to dry. The dishes were infested with 10 s-instar larvae and placed in the conditioned room. The mortality rates were evaluated 48 h after treatment. Each treatment had three repetitions and the data were adjusted and subjected to probit analysis as before.

Insecticidal test for brown planthopper (*Nilaparvata lugens*): The activities of insecticidal compounds against *Nilaparvata lugens* were tested using the dipping method. Rice plants at tillering to booting stage were pulled out and the rice stems (about 10 cm lengths) with roots were cut and air dried to remove excess water. Three rice stems were dipped in appropriate solutions of tested compound for 30 s. After rice stems had been air dried, the rice roots was wrapped with moistened cotton and then placed into a tumbler. Thirty *N. lugens* were introduced into the tumbler with the treated insects were maintained at a temperature of (27 ± 1) °C. Only distilled water only was used as control for each tested chemical. Each process was repeated for 3 times and the mortality rates were evaluated 48 h after treatment. The data were adjusted and subjected to probit analysis as before.

3. Results and discussion

3.1. Synthesis

The introduction of a spiro heterocycle into neonicotinoid molecule was accomplished by reactions of nitroenamine **7**, a neonicotinoid precursor, and Knoevenagel adducts (KAs) **2**, **4** and **6**. Nitroenamines have highly polarized push–pull ethylene systems, making them reactive intermediates with a vast variety of electrophiles [22–24]. In a previous study, we found that nitroenamine could react with KAs benzylidenemalononitriles

affording tetrahydroimidazo[1,2-a]pyridine derivatives as high active neonicotinoids [25]. Enlightened by this, the spiro heterocycle might be embodied in parental neonicotinoid by the corresponding spirocyclization using cyclic KAs. Therefore, cyclic KAs **2**, **4** and **6** were prepared here by condensation of malononitrile and cyclic ketones according to reported procedures [8,26].

Reactions of **7** with KAs **2**, **4** and **6** afforded spirobenzofuranones **8a–d**, spirooxindoles **9a–g** and spiroaceraaphthylenones **10a** and **10b**, respectively, as spiro heterocycle containing neonicotinoids. In further studies, we found that compounds **9a–g** could be easily obtained in high yields by a one-pot method from nitroenamine **7**, ketone **3** and malononitrile. The reaction solvents of **7a** with **6** were screened. The reaction proceeded very well in the mixed solvent of dichloromethane and methanol (4:1), while no reactions were detected in the single solvents, such as dichloromethane, methanol, ethanol, pyridine and acetonitrile. Spirocyclization of **7c** with **6** was unsuccessful probably due to steric effects. Attempts to synthesize corresponding products using other cyclic KAs 2-(2,3-dihydro-1*H*-inden-1-ylidene)malononitrile and 2-cyclohexylidene-malononitrile failed in varied solvents, such as acetonitrile, dichloromethane, methanol and DMF.

3.2. Insecticidal activity

The insecticidal activity of the target compounds against cowpea aphids (*A. craccivora*), armyworm (*M. separata*) and brown planthopper (*N. lugens*) was evaluated. Spirobenzofuranone analogs **8a**, **8b** and **8d** have some activity against armyworm with 100%, 20% and 80% mortality, respectively, while **8c** showed moderate activity to hemiptera insects such as cowpea aphids and brown planthopper. The other two types of spiro heterocyclic neonicotinoids **9a–g** and **10a** and **10b** were inactive to these three tested insects. The poor activity of these compounds might be caused by their low solubility in water and the large size of the spirocycles which made it difficult to bind within the receptor pocket.

4. Conclusion

The first introduction of a spiro heterocycle into the neonicotinoid molecule was accomplished. Using cyclic KAs, neonicotinoids bearing spirobenzofuranone, spirooxindole and spiroaceraaphthylenone were successfully constructed and their insecticidal activity was screened. Some compounds had moderate insecticidal activity, and ongoing research is focused on improving the efficacy through further analog generation. These novels compounds are expected to provide a basis for designing new spiro heterocycle containing neonicotinoids.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ccllet.2013.12.004>.

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