Chinese Chemical Letters 27 (2016) 635–639

Contents lists available at ScienceDirect

Chinese Chemical Letters

journal homepage: www.elsevier.com/locate/cclet

Original article

Azopyridine-imidacloprid derivatives as photoresponsive neonicotinoids

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ARTICLE INFO

Article history:
Received 24 February 2016
Received in revised form 15 March 2016
Accepted 18 March 2016
Available online 31 March 2016

Keywords:
Azopyridine
Photoresponsive
Imidacloprid
Solubility
Insecticide

ABSTRACT

A series of imidacloprid derivatives containing an azopyridine motif as a photoswitchable functional group were designed and synthesized. The new version of photoresponsive imidacloprid analogues showed improved solubility in comparison with their azobenzene analogues. 1.2 to 2-fold activity difference was observed for these azopyridine-imidacloprids against house fly (*Musca domestica*) and cowpea aphid (*Aphis craccivora*) upon irradiation.

1. Introduction

The development of photoswitches provides enormous potential for applications in chemistry, biology and material science [1–7]. With an increasing number of examples, photopharmacology is rapidly developing recently [8,9]. The photocontrol of the bioactivity has many advantages over the conventional method, such as precise manipulation of activity of interest, antiresistance and avoidance of side effects. Thus, many photoswitchable pharmaceuticals have been elaborately designed and achieved by attaching a light-responsive moiety to a bioactive molecules, such as using an optical mechanism to control antibacterial activity [10], coupling a photoswitch with the propofol analogue [11,12], optical control of insulin release with photoswitchable sulfonylurea [13], photoswitchable acetylcholinesterase inhibitors [14], nociception regulators [15], mast cell activation inhibitors [16] and microtubule formation inhibitors [17].

The optical control of activity was well addressed in the pharmaceutical area but seldom used in pesticide design. We recently reported a first example of photoswitchable neonicotinoids by merging imidacloprid (IMI) with azobenzene, which facilitated the remote regulation of insecticide performance with light [18]. Activity variation upon irradiation was achieved in the above examples, but the difference was not large enough and the relatively poor solubility limited their applications.

The azobenzene is a most commonly used photoswitched motif, but it has poor solubility in water. The recently developed azopyridines (**AP**) revealed a novel type of photoswitchable molecules with excellent properties, such as quantitative rates of photoisomerization, good water solubility and slow thermal isomerization [19,20].

Enlightened by the above descriptions, therefore, to develop a novel photoswitchable version of imidacloprid, our strategy here is trying to replace the chloropyrindyl part with **AP** by sharing a common pyridine fragments, generating the target compounds (Fig. 1). Besides, we hope the introduction of the **AP** fragment would lead to the improvement of the water solubility and insecticidal activity.

2. Experimental

2.1. Chemicals and instrumentations

Melting points were recorded on a Büchi B540 apparatus (Büchi Labortechnik AG, Flawil, Switzerland) and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AM-400 (400 MHz) spectrometer with $CDCl_3$ or DMSO-d_6 as the solvent and TMS as the internal standard. Chemical shifts are reported in δ (parts per million) values. Electrospray ionization (ESI) mass spectrometry was performed in an HP 1100 LC-MS spectrometer.

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http://dx.doi.org/10.1016/j.cclet.2016.03.033

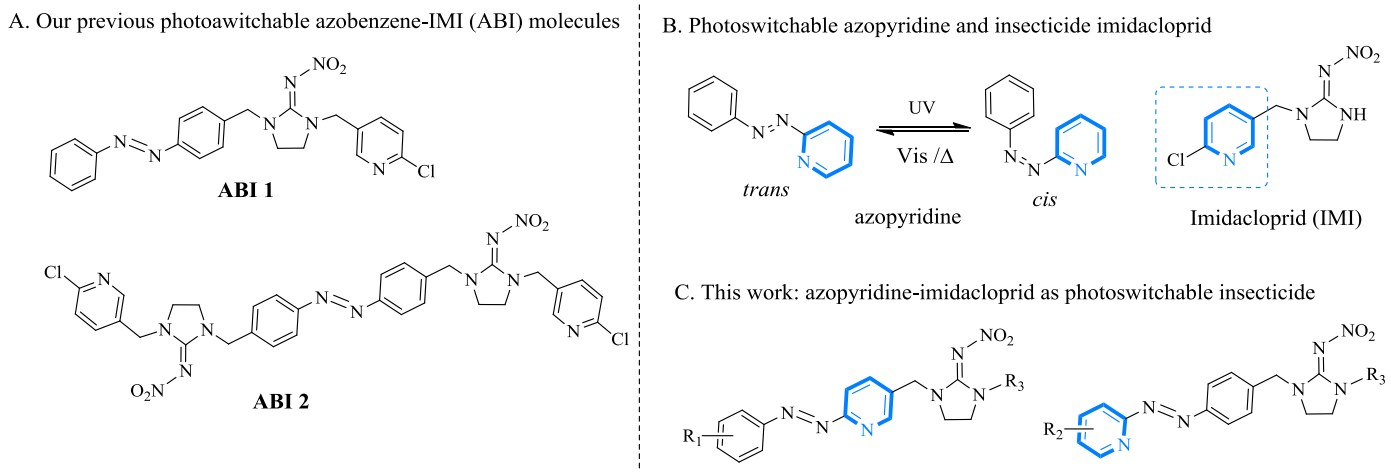


Fig. 1. Molecular design of azopyridine-imidacloprid as photoswitchable neonicotinoids.

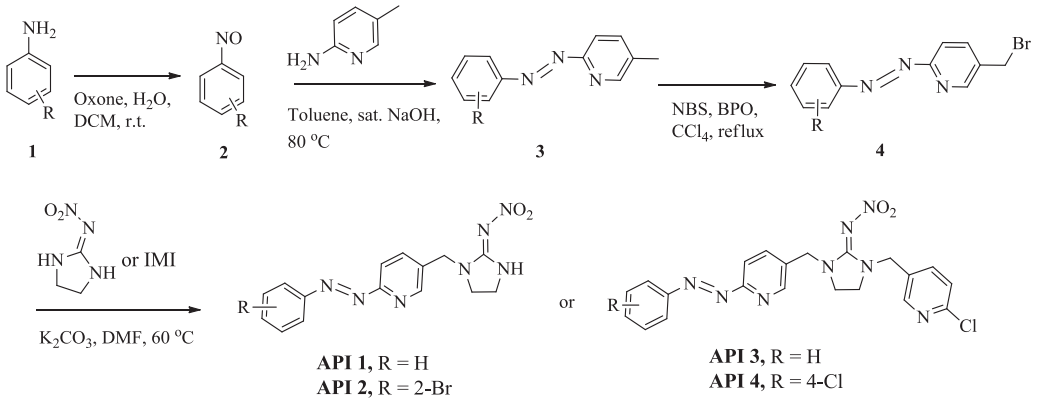
Analytical thinlayer chromatography (TLC) was carried out on precoated plates (silica gel 60 F254), and spots were visualized under ultraviolet (UV) light. Column chromatography was performed using 200–300 mesh silica gel (Hailang, Qingdao). The water solubility of the compounds was determined by SiriusT3 (Sirius Analytical Ltd, UK). 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.

2.2. Synthesis the target compounds

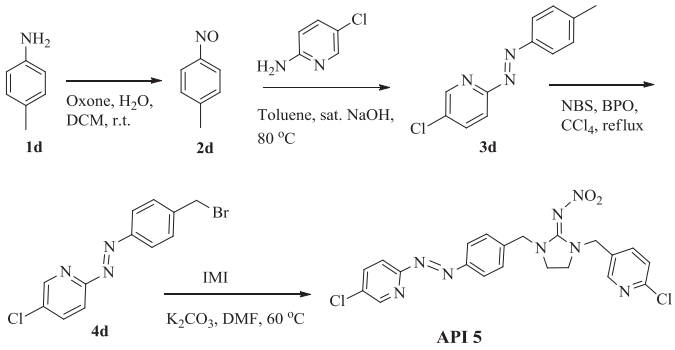
Compounds **API 1–API 4** were synthesized starting from anilines (Scheme 1). Oxidation of anilines by Oxone generated nitrosobenzenes, which then reacted with 2-amino-5-methylpyridine to construct the corresponding azopyridine. Bromination of azopyridine by NBS/BPO afforded bromomethyl-intermediate, which coupled with *N*-(imidazolidin-2-ylidene)nitramide or IMI to provide the final products (Scheme 1). **API 5** was prepared by the similar procedure from *p*-toluidine and 2-amino-5-chloropyridine (Scheme 2). The synthetic procedure of **API 1** as representative is given as follow and the detailed syntheses for **API 2–5** are provided in the Supporting information.

Synthesis of methyl-2-phenyldiazenylpyridine (**3a**): Aniline (**1a**) (33.1 mmol, 1.0 equiv.) was dissolved in 100 mL of dichloromethane. To this solution was added potassium peroxymonosulfate (Oxone) (66.2 mmol, 2.0 equiv.) in 400 mL of water. The solution was stirred under nitrogen at room temperature until TLC monitoring indicated the complete consumption of the starting material (0.5 h). After separation of the layers, the aqueous layer was extracted with dichloromethane twice. The combined organic layers were washed with 1 mol/L HCl, saturated sodium bicarbonate solution, water, brine, dried (magnesium sulfate) and evaporated to dryness affording the crude nitrosobenzene (**2a**). Nitrosobenzene is directly used for the next step without further purification. Crude nitrosobenzene (3.49 mmol, 1.2 equiv.) was dissolved in 25 mL of toluene. To this solution was added 2-amino-5-methylpyridine (2.9 mmol, 1.0 equiv.). Then to this solution was added saturated aqueous solution of sodium hydroxide (12 mmol, 4.0 equiv.). The resulting mixture was stirred at 60 °C for 30 min. Then 15 mL of water was added in the mixture, after separation of the layers, the aqueous layer was extracted with ethyl acetate twice. The combined organic layers were washed with brine, dried (magnesium sulfate) and evaporated to dryness. Purification by chromatography (petroleum ether/ethyl acetate = 10:1, silica gel) yielded the product as an orange solid. Yield 43%, ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.64 (d, 1H, J = 2.0 Hz), 8.08–8.01 (m, 2H), 7.87 (dd, 1H, J = 8.2, 2.3 Hz), 7.72 (d, 1H, J = 8.2 Hz), 7.57–7.52 (m, 3H), 2.54 (s, 2H).

Synthesis of 5-bromomethyl-phenyldiazenylpyridine (**4a**): **3a** (10 mmol, 1.0 equiv.) was dissolved in 25 mL of carbon tetrachloride. To this solution was added benzoyl peroxide (BPO) (1 mmol, 0.1 equiv.). Then *N*-bromosuccinimide (NBS) (11 mmol, 1.1 equiv.) was partially added to this solution. The solution was stirred under nitrogen at 70 °C for 12 h. The precipitate was separated by filtration; the filtrate was washed with brine, dried (magnesium sulfate) and evaporated to dryness. Purification by chromatography



Scheme 1. Synthesis of **API 1–API 4**.



Scheme 2. Synthesis of **API 5**.

(petroleum ether/ethyl acetate = 20:1, silica gel) afforded the pure product as an orange solid. Compound **4a**: Yield 73%, ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.74 (d, 1H, J = 2.0 Hz), 8.08–8.01 (m, 2H), 7.94 (dd, 1H, J = 8.2, 2.3 Hz), 7.82 (d, 1H, J = 8.2 Hz), 7.57–7.52 (m, 3H), 4.55 (s, 2H).

Synthesis of N-(1-((6-((E)-(2-bromophenyl)diazenyl)pyridin-3-yl)methyl)imidazolidin-2-ylidene)nitramide (**API 1**): *N*-nitroiminoimidazolidine (14 mmol, 1.4 equiv.) and K_2CO_3 (14 mmol, 1.4 equiv.) were added to DMF (5 mL) and the mixture was stirred for 10 min, then compound **4a** (10 mmol, 1.0 equiv.) was added to this solution. The resulting mixture was stirred at 60 °C under Ar for 8 h. The precipitate was separated by filtration. The solvent was removed *in vacuo*. Purification by chromatography (dichloromethane/ethyl acetate = 3:1, silica gel) yielded **API 1** as an orange solid. Yield 56%, m.p. 153.0–154.3 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.00 (s, 1H), 8.67 (d, 1H, J = 1.9 Hz), 8.03–7.92 (m, 3H), 7.75 (d, 1H, J = 8.2 Hz), 7.67–7.62 (m, 3H), 4.60 (s, 2H), 3.71–3.62 (m, 2H), 3.59–3.52 (m, 2H); ^{13}C NMR (101 MHz, DMSO-d_6): δ 162.27, 160.39, 151.83, 148.75, 138.24, 134.16, 132.49, 129.60, 122.94, 113.35, 45.16, 44.99, 41.61, HRMS (ESI): m/z calcd. for $C_{15}H_{15}N_7O_2$ $[M+Na]^+$ 324.1185, found 348.1186.

2.3. Photoisomerization

Absorption spectra were recorded on a Lambda 25 UV/vis spectrometer (PerkinElmer, Shanghai). Target compounds were dissolved in CH_3CN using a microcuvette. Photochromism is irradiated by a hand-held MQK-WFH-204B ultraviolet lamp (365 nm, 10 mW /cm^2, MQK, Shanghai). UV–vis absorption spectroscopy was used to detect the change of absorption profiles of target compounds upon 365 nm irradiation. The isomerization of the **API 1** and **API 4** analogues was also investigated in detail by ^{1}H NMR spectroscopy. The corresponding UV–vis and ^{1}H NMR spectra were provided in supporting information. The proportions of *trans*/*cis* isomer at the photostationary states before and after irradiation were determined by HPLC analysis. The *trans*/*cis* ratios were calculated at the isosbestic points. The half-lives of thermal relaxations under dark condition are shown in Table 1.

2.4. Insecticidal activity assay

Insecticidal test of **APIs** was done with cowpea aphids (*Aphis craccivora*). The plant leaves of horsebean with about 50 apterous adults were dipped in corresponding **APIs** solutions containing Triton X-100 (0.1 mg/L) for 5 s and the excess solution was sucked out with a filter paper. Then burgeons were positioned in the conditioned room (25 ± 1 °C). Water with Triton X-100 (0.1 mg/L) was used as control. Twenty-four hours after treatment, the mortality rate was measured. Each treatment had three repetitions and the data were subjected to probit analysis. Insecticidal test for house fly (*Musca domestica*). House fly adults were anesthetized with carbon dioxide for 10 min and then were treated with the **APIs** dissolved in distilled water or 10% DMSO aqueous solution by intrathoracic injection (0.4 μL for each fly). Twenty flies were treated for each dosage with duplicate samples. The mortality rate was measured 24 h after treatment. Each treatment had three repetitions and the data were subjected to probit analysis.

3. Results and discussion

Previously, we merged the IMI with azobenzene to successfully generate photoswitchable insecticidal molecules. Here, a similar strategy was employed using azopyridine as a replacement. Imidacloprid was a successful neonicotinoid in the past decades with annual sales approaching to one billion US dollars [21,22]. The SAR study indicated that the pyridine is an indispensable pharmacophore by interacting with the nicotinic acetylcholine receptors through cation–π interactions [23]. Thus, we incorporated the azopyridine into the IMI by sharing a common pyridine or into the imidazoline ring of IMI to generate the photoswitchable azopyridine-imidacloprid (**API**) derivatives.

The isomerization of the **API** analogues was investigated in detail using ^{1}H NMR spectroscopy, UV–vis spectroscopy and HPLC analysis. The *trans*-isomers showed a typical azobenzene absorption at around 325 nm (319–330 nm) belonging to the π–π^* transition band. Irradiation with 365 nm UV light isomerizes the azopyridine part to its metastable *cis*-form with the appearance of absorption at around 430 nm corresponding to n–π^* transition band, indicating the initiation of *trans*-to-*cis* photoisomerization (Fig. 2). The *trans*/*cis* content was determined by HPLC analysis at the photostationary state. The *trans*-to-*cis* isomerization showed high transformation rate of photoisomerization ranging from 24:76 to 10:90. The *cis*-to-*trans* change can be achieved upon irradiation at 430 nm and the half-life of the thermal relaxation of the *cis*-isomer was more than 120 h, which guaranteed the stability of *cis*-isomers for biological test.

Then the water solubility of the compounds was studied. All the compounds except **API 5** have higher solubility than the azobenzene analogue **ABI 1**. **API 5** had very poor water solubility as a *trans*–isomer, indicating that appending azopyridine in such a way was unfavourable to the solubility. Interestingly, almost a 2-fold solubility increase was observed for compound **API 1**-**API 5**

Table 1
Maximum absorbance wavelength (nm), ratio of *trans* and *cis* isomers, the rate of thermal relaxation and the water solubility of **APIs**.

Compd.	π–π^*	Nonirradiated (*trans*:*cis*)	n–π^*	Irradiated (*trans*:*cis*)	$t_{1/2}$ (h)	Water solubility (mg/L)	
						trans	*cis*
API 1	320	96:4	432	16:84	158.2	12	27
API 2	324	78:22	435	25:75	140.0	12	26
API 3	320	83:17	445	16:84	127.6	17	37
API 4	320	98:2	435	24:76	155.5	16	31
API 5	327	96:4	441	10:90	183.5	5	11
ABI 1	321	95:5	432	17:83	342.1	6	11

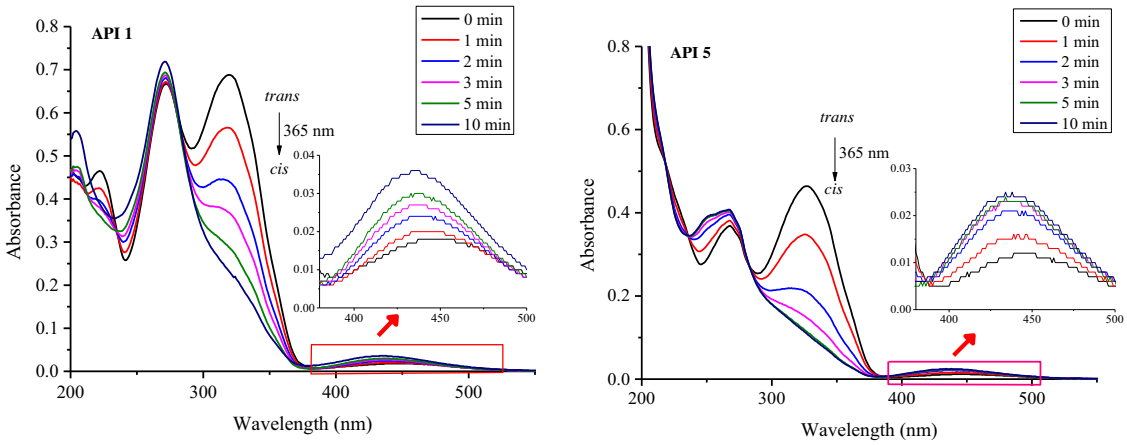


Fig. 2. UV–vis spectrum of **API 1** and **API 5** upon irradiation at 365 nm at varied times.

and **ABI 1** upon irradiation, suggesting that the bend form of azopyridine would enhance the solubility (Table 1). Pyridine fragment in the AP is a hydrophilic group in comparison with benzene in the azobenzene, which leads to the higher water solubility of azopyridine-imidacloprid analogues.

Having identified the suitable photochemical properties, the insecticidal performance of these novel photoresponsive insecticides was evaluated against house fly (*Musca domestica*) by the intrathoracic injection and cowpea aphid (*Aphis craccivora*) by the leaf-dip method, respectively. The results are summarized in Table 2. **API 1** and **API 2** showed very low insecticidal efficacy both to *Musca* and cowpea aphid ($LD_{50} > 10$ μg/g and $LC_{50} > 500$ mg/L, respectively) for the two photoisomers. **API 3**, **4** and **5** exhibit almost the same level of activity to the two insects with LD_{50} values of around 2 μg/g for *Musca* and LC_{50} of 150–181 mg/L for aphid, respectively. 2-Chloropyridine is most important pharmacophore in the IMI, the removal of 2-chloropyridine will cause a sharp drop in insecticidal activity found in previous SAR study. We replaced the chloropyrindyl part with AP not only to increase the solubility in water but also wished to share a common pyridine fragment, so we synthesized **API 1** and **API 2**. However, **API 1** and **API 2** showed very low insecticidal efficacy, possibly because AP is not a good pharmacophore as 2-chloropyridine. **API 1** and **2** had no activity, possibly caused by the remove of 2-chloropyridine, while in compounds **API 3–5**, 2-chloropyridine were retained. To our disappointment, only slight activity difference was observed for **API 3** before and after irradiation. In comparison with azobenzene analogues **ABI 1**, the **API 3–5** showed nearly 3-fold activity increase along with increased solubility, upon irradiation, a 2-fold activity difference was achieved for **API 4** and **API 5**. All the compounds had lower activity than the imidacloprid, indicating that such chemical modifications were not tolerated to the activity enhancement (Table 2).

Table 2
Insecticidal activity of **APIs** against house fly (*Musca domestica*) and cowpea aphid (*Aphis craccivora*).

Compd.	*Musca domestica* (*in vivo*, LD_{50}, μg/g)		*Aphis craccivora* (*in vivo*, LC_{50}, mg/L)	
	Nonirradiated	Irradiated	Nonirradiated	Irradiated
API 1	>10	>10	>500	>500
API 2	>10	>10	>500	>500
API 3	1.9	1.7	181	152
API 4	2.2	1.1	151	132
API 5	2.7	2.1	156	143
ABI 1	6.5	9.2	298	316
ABI 2	2.8	0.5	207	129
Azobenzene	>20	>20	>1000	>1000
IMI	0.05		5.8	

4. Conclusion

In conclusion, we prepared a novel type of photoswitchable imidacloprid derivatives by incorporating the photoisomerizable azopyridine unit based on our previous azobenzene-imidacloprid analogues. The azopyridine-imidacloprid analogues had higher insecticidal activity and water solubility than the corresponding azobenzene-imidacloprid analogues.

Acknowledgments

This work was financial supported by National Natural Science Foundation of China (Nos. 21372079, 21472046), Shanghai Pujiang Program (No. 14PJD012) and the Fundamental Research Funds for the Central Universities (No. 222201414015).

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.cclet.2016.03.033.

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