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Proinsecticide candidates *N*-(5-methyl-2-oxo-1, 3-dioxol-4-yl)methyl derivatives of imidacloprid and 1-chlorothiazolylmethyl-2-nitroimino-imidazolidine

Ikuya Ohno,[a] Koichi Hirata,[b] Chiharu Ishida,[b] Makoto Ihara,[b] Kazuhiko Matsuda[b] and Shinzo Kagabu[a,*]

[a]*Department of Chemistry, Faculty of Education, Gifu University, Gifu 501-1193, Japan*
[b]*Department of Applied Biological Chemistry, School of Agriculture, Kinki University, 3327-204 Nakamachi, Nara 631-8505, Japan*

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Abstract—Prodrugs of imidacloprid and the thiazolylmethyl analog masked with oxodioxolylmethyl group on the *N*3 site were prepared. The prodrugs decomposed in a buffer solution of pH 8.3 and in a physiological salt solution with half-lives of 10–15 h, releasing the parent insecticides. Being consistent with this, an inward current was evoked in dissociated cockroach neurons treated with the masked compound solutions, which were maintained for 24 h after preparation, as measured using patch–clamp electrophysiology, whereas no response was observed in neurons when the solutions were challenged immediately after preparation. The insecticidal test on the American cockroach showed that the minimum lethal dose for each compound at 24 h after injection was 6.4×10^{-8} mol, which was similar to that for imidacloprid and the thiazolyl derivative. This result strongly suggested a regeneration of the active ingredients in vivo.

Imidacloprid (**1**), the current top-selling insecticide, exhibits insecticidal performance by acting selectively on nicotinic acetylcholine receptors (nAChR).[1–3] Since the finding of the extraordinary potency of the structure composed of a chloronicotinyl group and the nitroimino-imidazolidine moiety, manifold modifications have been attempted to explore a product that can discriminate from the prototype in insecticidal spectrum, selectivity, toxicity, persistence, mobility or other marketing respects. Up to now six new insecticides referred to as neonicotinoids have been developed with their own prominence.[4] The hitherto structure modification strategy includes replacement of the pyridine ring with a thiazole or other bioisosteric heterocyclic rings, substitution of the nitroimino group with other electron-withdrawing groups, and transformation of the imidazolidine moiety to acyclic equivalents.[5] However, no attempts have been made to modify the structure of imidacloprid from the prodrug view point although a few examples were reported with other neonicotinoids.[6–9]

Prerequisites for a proinsecticide would be sufficient stability during the formulations and the regeneration property of the original ingredient in vivo within a measurable time-span. Masking the free proton of imidacloprid at the 3-*N* site is a possible design for a prodrug of imidacloprid. Although carbamates and *N*-acyloxymethyl derivatives are representative prodrugs and have been applied to versatile active amines,[10–12] the 3-*N* moiety of imidacloprid is not basic due to the conjugated powerful electron-withdrawing $=NNO_2$ group.[13] Accordingly, the introduction of these major prosthetic groups to this site will yield derivatives that would be labile in the nucleophilic in vitro milieu. *N*-Alkylation is another conventional masking. However, 3-*N* alkyl derivatives are so stable that no demethylation metabolism occurs for 3*N*-methyl-imidacloprid in an insect enzyme system.[14] In exploring a pro-moiety that permits regeneration of the active species during an adequate time-span, we have found (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl moiety[15,16] meets this prerequisite. Herein we report on the first prodrugs of imidacloprid, 3-*N*-(5-methyl-2-oxo-1,3-dioxol-4-yl)methyl imidacloprid (**3**) and the thiazolylmethyl derivative (**4**) (Fig. 1), and the in vivo and in vitro hydrolysis.

Keywords: Imidacloprid; Prodrug; *N*-(Oxodioxolyl)methyl derivative; Patch–clamp electrophysiology.
* Corresponding author. E-mail: kagabus@gifu-u.ac.jp

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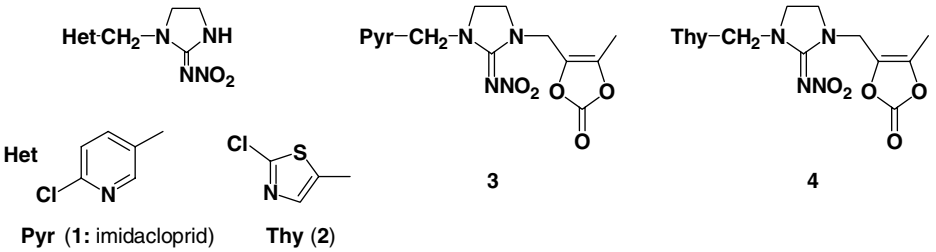

Figure 1. Imidacloprid (**1**), the thiazolyl analog (**2**), and their (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl derivatives (**3**) and (**4**).

Preparation of 3-*N*-(5-methyl-2-oxo-1,3-dioxol-4-yl)-methyl derivatives (**3** and **4**) was carried out by the coupling reaction of **1** or **2** with 5-methyl-2-oxo-1,3-dioxol-4-yl-methyl bromide in the presence of potassium carbonate in refluxing acetonitrile for 3 h (longer reaction time reduced the yields drastically). The reaction mixture was subjected to SiO_2 chromatography with ethyl acetate/hexane and the following recrystallization afforded the analytical samples.[17]

Masked imidacloprid (**3**) decomposed with half-lives of 15.4 and 11.4 h in alkaline and physiological salt solutions, respectively, releasing imidacloprid quantitatively (Table 1). Thiazolyl derivative (**4**) unveiled a little faster in both solutions. From the plausible dissociation mechanism depicted in Figure 2, the susceptibility of this prosthetic group toward the basic milieu is understandable. The marked decrease in the yields for the last step to compounds **3** and **4** on prolonged reaction time in the presence of a base also manifests itself. The mechanism accounts for, on the other hand, why the derivatives should be stable under acidic conditions as shown by the fact that no decomposition of the derivatives was detected at pH 5.4 during 48 h.

The actions of the masked compounds **3** and **4** on dissociated American cockroach neurons were investigated using whole-cell patch–clamp electrophysiology. The cockroach neurons were prepared as previously described.[18] The compounds were applied using a U-tube and the membrane currents recorded via a microelectrode were digitized to be stored on a personal

Table 1. The half-lives and the insecticidal potencies of tested and reference compounds

Compound	Half-lives (min)[a]			Insecticidal potency (MLD) (mol/insect)[b]
	Acidic	Alkaline	Physiological	
1				1.1×10^{-9}[c]
2				3.8×10^{-9}[c]
3	>2880	928	693	6.4×10^{-8}
4	>2880	635	630	6.4×10^{-8}

[a] Min unless otherwise stated. Hydrolysis measurements: three standard solutions were prepared at 25 °C as follows: an alkaline buffer solution (4 ml of a solution dissolved of 1.237 g of H_3BO_4 and 1.491 g of KCl in 20 ml of deionized water was mixed with 10 ml of 0.1 M NaOH, and the whole was diluted with 20 ml of water and then with 34 ml of acetonitrile; pH 8.3); an acidic buffer solution (a mixture of 2 M NaOAc and 2 M HOAc 6.95:3.05, v/v and diluted in 100 ml of water. A mixture of 35 ml of the buffer and 15 ml of acetonitrile; pH 5.4); an physiological SOS solution (NaCl 100 mM, KCl 2.4 mM, $CaCl_2$ 1.8 mM, and Hepes 5.0 mM; pH 7.6). An 0.02% (w/v) solution of a compound containing 0.02% (w/v) of benzonitrile as the internal standard for each standard solution was kept at 25 °C and an aliquot was injected at 1, 2, 4, 8 h into an HPLC system (Jasco UVDECK-100-VI, 270 nm) with an ODS column (Merck, LiChrosorb RP-18) using acetonitrile/water (30:70, v/v) as the mobile phase. The mean half-lives were calculated from two runs. he retention times of benzonitrile, compounds **1**, **2**, **3**, and **4** are 8.72, 4.98, 4.32, 3.68, and 4.33 min, respectively.

[b] Biological test: various volumes (1–10 μl) of each compound dissolved in dimethyl sulfoxide (DMSO) containing various amounts of methanol were injected without synergists into the abdomen of a male adult American cockroach, *Periplaneta americana* L. Organic solvents alone in this range had no toxic effect. The doses varied by every 0.1 in log units. Three insects were used to test each dose of each compound and were kept at 24–26 °C for 24 h after injection. The minimum lethal doses (MLD; mol/insect) were determined for each compound. The MLD values are indicated as means of at least two experimental runs with a deviation within 0.2 in log unit.

[c] Cited from Ref. 19.

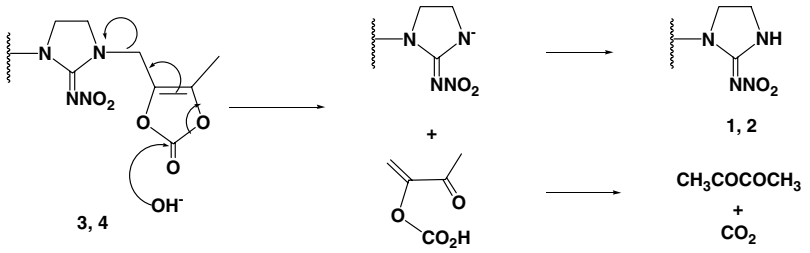

Figure 2. Dissociation mechanism of *N*-oxo-dioxolylmethyl compounds (**3** and **4**).

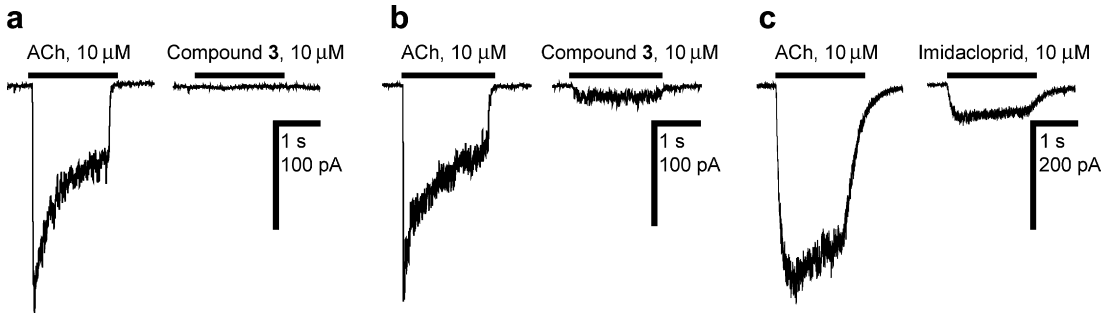

Figure 3. Actions of *N*-oxo-dioxolylmethyl compound **3** on American cockroach neurons as recorded using whole-cell patch–clamp electrophysiology. (a) Inward currents induced in a cockroach neuron by the application of freshly prepared solutions of acetylcholine (ACh) (left trace, 10 μM) and compound **3** (right trace). (b) Inward currents induced by ACh (left trace, 10 μM), which was tested immediately after preparation, and the solution of compound **3** (right trace), which was tested after incubation at 25 °C for 24 h. (c) Inward currents induced by Ach (left trace, 10 μM) and imidacloprid at 10 μM (right trace). Imidacloprid solution was applied 24 h after incubation at 25 °C for 24 h. In (a–c) ACh was applied for 2 s and washed for 1 min with physiological saline, and then compound **3** or imidacloprid was applied for 2 s. All experiments were repeated ($n = 3$) and reproducibility of the data was confirmed.

computer using Digidata 2000 (Molecular Devices).[18] Compound **3** did not evoke any inward current when applied immediately after its preparation (Fig. 3a); however, an inward current was observed (Fig. 3b) when its solution, which was maintained at 25 °C for 24 h, was applied via a U-tube ($n = 3$), suggesting that decomposition of the compound yielded the active ingredient imidacloprid. Nevertheless, the compound does not seem to decompose completely because the response of the cockroach neuron to the compound solution (Fig. 3b) was smaller to that induced by imidacloprid at 10 μM (Fig. 3c), which was applied 24 h after preparation of its solution ($n = 3$). A similar result was the case for compound **4** (data not shown).

The insecticidal activity of the masked compounds was evaluated against American cockroaches, *Periplaneta americana* L., by injection as reported earlier without synergists (Table 1). Although obvious intoxication symptoms were not observed during the initial few hours, all the tested species died eventually after 24 h, and the minimum lethal (MLD) dose was 6.4×10^{-8} mol for each, very close to 1.1×10^{-9} and 3.8×10^{-9} mol for the unmasked ingredients **1** and **2**, respectively.[19] The result reflects the dissociation of the prodrugs with half-lives in a physiological solution. Imidacloprid is poor in integument penetration and less effective on lepidopteran insects, while some other neonicotinoids with higher hydrophobicity are effective on them.[4] The prodrugs are expected to exhibit a broader insecticidal spectrum due to the enhanced lipophilicity, thereby improving the insect dermal permeability.

In summary, this study demonstrates a proinsecticide for two current highly evaluated products of a unique structure by masking the acidic imidazolidine NH site with a unique functional group. Insecticidal assessment under greenhouse conditions is in progress.

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17. Compound **3**: isolated yield: 7.6%; mp 102 °C; IR (KBr) cm^{-1}: 1815, 1560, 1450, 1255, 1220; ^{1}H NMR δ ($CDCl_3$): 2.18 (3H, s), 3.63 (2H, m), 3.88 (2H, m), 4.31 (2H, s), 4.49 (2H, s), 7.36 (1H, d, J = 7.3 Hz), 7.68 (1H, dd, J = 7.3/2.5 Hz), 8.32 (1H, d, J = 2.5 Hz); ^{13}C NMR δ ($CDCl_3$): 9.2, 39.7, 45.3, 46.9, 47.5, 124.9, 128.6, 131.8, 139.0, 139.8, 149.3, 151.8, 151.9, 160.4; HR-EIMS for $C_{14}H_{14}ClN_5O_5$: Calcd 367.0683. Found: 367.0714.
Compound **4**: yield: 8.5%; mp 106 °C; IR (KBr) cm^{-1}: 1820, 1560, 1420, 1260, 1220; ^{1}H NMR δ ($CDCl_3$): 2.17 (3H, s), 3.67 (2H, m), 3.86 (2H, m), 4.29 (2H, s), 4.58 (2H, s), 7.48 (1H, s); ^{13}C NMR δ ($CDCl_3$): 9.2, 39.8, 43.2, 45.2, 47.0, 131.9, 132.7, 140.0, 141.5, 151.8, 154.2, 159.7. Anal. for $C_{12}H_{12}ClN_5O_5S$: Calcd C, 38.56; H, 3.24; N, 18.74. Found: C, 38.45; H, 3.19; N, 18.40.
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