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### Photo-controlled Release of Fipronil from a Coumarin Triggered Precursor

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#### ARTICLE INFO

ABSTRACT

Article history: Received Revised Accepted Available online	Developing efficient controlled release system of insecticide can facilitate the better use of insecticide. We described here a first example of photo-controlled release of an insecticide by linking fipronil with photoresponsive coumarin covalently. The generated coumarin-fipronil ( <b>CF</b> ) precursor could undergo cleavage to release free fipronil in the presence of blue light (420 nm) or sunlight. Photophysical studies of CF showed that it exhibited strong fluorescence properties. The CE had no obvious activity against mecanito lawae under dark but it can be
Keywords: Photocaged Fipronil Coumarin Insecticide Light	activated by light inside the mosquito larvae. The released Fip from CF by blue light irradiation <i>in vitro</i> retained its activity to armyworm ( <i>Mythimna separate</i> ) with $LC_{50}$ value of 24.64 µmol L <sup>-1</sup> . This photocaged molecule provided an alternative delivery method for fipronil.
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Direct delivery of pesticide has many uncontrolled adverse effects such as high toxicity, large application amount and decomposition of active ingredients.<sup>1</sup> To address these issues, methods for conditionally controlling release are needed, since controlled release of pesticides has substantial advantages including enhanced bioavailability, prolonged length of activity, improved physiochemical properties, reduced phytotoxicity and lowering of the environment secondary effects.<sup>1</sup> The previously-developed pesticide-release systems took advantage of nanotechnology,<sup>1.6,7</sup> microencapsulation<sup>2-4,8</sup> and polymer science.<sup>5,9</sup> These methodologies provided slow release of active ingredients, but the release process cannot be easily regulated at spatial and temporal resolution. In this context, a more precise controlled technology is desired to the spatiotemporal control over the pesticide release.

The recent fast-developing photo-triggered technology provides possibilities for controlled release which promises better remote, temporal and spatial control than conventional methods.<sup>10,11</sup> To realize such a photochemical process, a photolabile protecting (photocaging) group (PPG) is usually used to covalently couple with the molecule being released, generating a caged precursor that can undergo cleavage under light.<sup>12</sup> The advent of photocaged conception creates great opportunities for spatiotemporal manipulation of a variety of processes in chemical, biological and material science.<sup>10,13</sup> Excellent reviews include light-triggered catalysts,<sup>14</sup> organic surfaces,<sup>15</sup> biomedical materials<sup>13</sup> and photocontrol of cellular chemistry<sup>16-18</sup> or gene expression.<sup>19,20</sup> Normally, such photoresponsive system has blocked function in the caged state and can be irreversibly activated upon irradiation to release the functional ingredients. Due to the encouraging advances, this technology thus far has been well applied for the photo-regulated release of bioactive molecules, such as neurotransmitters, enzyme substrates, pheromones, lipids, and second messengers.<sup>10, 21, 22</sup>

Turning attention back to agrochemical science, the combination of PPG with pesticide provided a promising release method with pioneering work done by N. D. Pradeep Singh *et al.* Example applications included photo-controlled release of 2, 4-D herbicide,<sup>23-25</sup> plant growth regulators,<sup>26</sup> sex pheromone<sup>27</sup> and plant hormone salicylic acid.<sup>28</sup> However, photo-controlled release of an insecticide molecule has not yet been described.

Fipronil (Fip) is a phenylpyrazole insecticide widely used for seed-treatment, sanitary pest control and animal health, but its application was strictly restricted in some districts due to high toxicity to non-target wildlife.<sup>29-32</sup> Fipronil is stable at dark in mildly acidic to neutral water, but is prone to undergo photolysis or biological oxidation or reduction in vivo to form desulfinyl, sulfone, sulfide or amide metabolites.<sup>29,33</sup> Its half-life time of photodegradation is 0.33 day (Florida summer sunlight).<sup>34</sup> In a specific case, the persistence of fipronil reduced significantly when exposed to sunlight.<sup>35</sup> As a consequence, two attempts were previously made for controlling release of fipronil using microencapsulation of in situ polymerization or biocompatible silica nanocapsules. Herein, we demonstrated a photochemical method that releases insecticidal fipronil using pulses of light, which enables more precise control and real-time activation. The fipronil was caged using coumarin as photoremovable protecting group. The caged compound is stable and would release fipronil only upon irradiation.

The existence of an amino group in fipronil provides the possibility for the installation of PPG. Covalent linking of a PPG with an active molecule is a straightforward way to generate a photocaged molecule. Many factors should be taken into account in designing such a photocaged molecule, such as the solubility,

stability, light wavelength, efficiency of desire cleavage reaction, avoidance of photodamage/photodegradation and the toxicity of cleavage product of caging group.<sup>36</sup> Most importantly, a PPG must be subtly selected. Various PPGs have been developed in the past decades. The well-studied and frequently used PPGs include o-nitrobenzyl, coumarin-4-ylmethyl and phydroxylphenacyl.<sup>36</sup> The coumarin phototriggers attract the most attentions recently due to their superior features, such as longer absorption wavelength, large molar coefficients, fast release rates, improved stability, high biocompatibility and fluorescent emitting.<sup>36</sup> Considering the substituent effects on this cages, 7dialkylamino substituted coumarin was selected here since it has the absorption band at biologically benign region. Thus, the diethylamino-coumarin-4-ylmethyl caged fipronil was prepared for our subsequent investigations (Figure 1).



**Figure 1.** Molecular design of photoresponsive coumarin-caged fipronil and its synthetic route. Reagents and condition: a) 1. SeO<sub>2</sub>, Ar, *p*-xylene, reflux, 53 h; 2. NaBH<sub>4</sub>, CH<sub>3</sub>OH, r.t., 4 h, 26%. b) *p*-nitrophenyl chloroformate, DIPEA, Ar, dry dichloromethane, r. t., 20h, 50%. c) fipronil, DMAP, Ar, dry dichloromethane, r. t., 48h, 24%.

The photoresponsive **CF** was prepared from a three-steps route starting from commercially available 7-(diethylamino)-4methyl-2*H*-chromen-2-one **1** (Figure 1). Oxidation of **1** with Selenium dioxide and the following reduction by Sodium borohydride (NaBH<sub>4</sub>) afforded alcohol intermediate **2** (Cou). Alcohol **2** then condensed with *p*-nitrophenyl chloroformate to furnish intermediate **3** under catalysis of DIPEA. Finally, **3** reacted with Fip at presence of DMAP to present the target caged product **CF**.

With successful obtaining of coumarin-caged fipronil, its photophysical properties were studied first. The absorption and emission maximum wavelength, molar absorption coefficient, Stokes shift and fluorescence quantum yield of **CF** were summarized in Table 1. In UV-Vis spectra, **CF** features two obvious absorption bands centered at 390 nm and 245 nm (Figure 2, A), which corresponded to the absorption of coumarin and fipronil fragments, respectively. The maximum emission wavelength of **CF** is about 475 nm and the Stokes shift is 85 nm (Figure 2, B). **CF** has high fluorescence quantum yield ( $\Phi_f = 0.2$ ), which facilitate its application in imaging study of tested targets.

Table 1.UV-Vis and fluorescence data for CF

	UV-Vis		Fluorescence		
compound	$\lambda_{\max}^{a}(nm)$	$\epsilon^{b}(10^{4}M)^{-1}cm^{-1})$	$\lambda_{\max}^{c}(nm)$	stokes shift <sup>d</sup> (nm)	$\phi_{\rm f}^{~e}$
CF	390	1.6	475	85	0.2

<sup>a</sup>Maximum absorption wavelength. <sup>b</sup>Molar absorption coefficient (M<sup>-1</sup>cm<sup>-1</sup>) at the wavelength of 390 nm. <sup>c</sup>Maximum emission wavelength. <sup>d</sup>Difference between wavelengths of the maximal emission and excitation. <sup>e</sup>Fluorescence quantum yield.

To measure the efficiency of the light-induced release of Fip from the cage, we irradiated CF with biologically benign blue light (420 nm, LED). Significant UV-Vis and fluorescence spectra changes were observed at different intervals of time under irradiation (420 nm) (Figure 2, A). The intensity of peak absorption at 390 nm corresponding to the CF gradually decreased and maximum absorption wavelength bathochromically shifted to 395 nm after 50 min irradiation. Similar emission intensity decrease and bathochromic shift were also detected in the fluorescence spectra of CF (Figure 2, B), indicating the chemical reactions occurred upon irradiation. The shift in the peak absorbance and emission to longer wavelength was caused by the generation of photolysis product Cou whose maximum absorption and emission wavelength were 395 nm and 485 nm (Figure 2, D and E), respectively.



**Figure 2.** A: UV-Vis absorption of CF at regular intervals of irradiation in MeOH/H<sub>2</sub>O (50:50) ( $5.6 \times 10^{-5}$  M). B: Emission spectra of CF at regular intervals of irradiation in MeOH/H<sub>2</sub>O (50:50) ( $5.6 \times 10^{-6}$  M). C: UV-Vis absorption of Fip. D: UV-Vis absorption of Cou. E: Emission spectra of Cou.

The UPLC analysis was then conducted to monitor the photoreaction process and identify the photolysis products (Figure 3). The UPLC chart at regular intervals of irradiation time clearly showed the photolysis process of **CF**. The gradual peak decrease at  $R_t = 8.0$  min and increase at  $R_t = 7.0$  min demonstrated the photocleavage of **CF** and generation of Fip, respectively. Besides Fip, two other photolysis products Cou and methylation product of Cou were confirmed by matching the NMR spectra of isolated products with authentic samples. The methylation reaction occurred between Cou and the solvent methanol.

Nearly complete Fip release (95%) was achieved in 80 min and approximately half of the Fip was released in 11 min of light irradiation (Figure 4, A). The Fip release can be turned on and off with the light exposure or not (Figure 4, B), suggesting that the release proceeded only at the presence of light. To exclude other degradation pathway under the biological testing conditions, the hydrolytic and enzymatic stability (see Supplementary Material) of **CF** were also investigated. No significant decomposition (< 5%) of **CF** was observed when **CF** was kept at dark for two weeks or was incubated with homogenized mixture of mosquito larvae, guaranteeing the **CF** can only be activated using light. Together, all the above results indicated that our uncaging strategy have good dark stability and can successfully release insecticidal Fip upon light irradiation.



**Figure 3.** UPLC tracking of photolysis process of CF in CH<sub>3</sub>OH/H<sub>2</sub>O (50:50 v/v) under blue light (420 nm, 1 w) (A) and under sunlight (B).



**Figure 4.** Photo-controlled release of Fip irradiated by blue light. A) Timedependent release of Fip upon blue light (420 nm) irradiation. B) The release process can be turned on and off by switching the light on and off.

To evaluate the efficiency of photo-induced release in living organism and the subsequent toxic effects, the bioassay was conducted using mosquito larvae (Table 2) because of its transparent body structure that allows light easy to penetrate. In the dark, **CF** alone showed low activity against mosquito larva suggesting the inefficiency of the caged compound. Upon exposure to blue light (420 nm), the activity increased

dramatically with nearly 25-folds activity enhancement (LC<sub>50</sub> =  $0.56 \,\mu\text{mol L}^{-1}$ ) (Figure 5, A and B). When subjected to sunlight, a similar activity enhancing trend was observed with a slight higher activity (LC<sub>50</sub> = 0.37  $\mu$ mol L<sup>-1</sup>) than that irradiated by blue light (Figure 5, A and B), indicating that natural sunlight can be used as a clean light source to release the Fip. As comparison, the effect of photolysis product Cou or blue light alone were investigated and no obvious poisoning sign was observed on these two treatments. The increased activity correlated closely with the increased time of light exposure (Figure 5, C). Light do not have any turbulence to the Fip activity as the activities were almost the same at dark or irradiated by blue light or sunlight. Slight decrease of Fip potency under sunlight may partly attribute to its tiny photodecomposition since Fip is sensitive to sunlight. These results proved that the activity was mainly from the released Fip and the CF can only be activated by light irradiation.

**Table 2.** Insecticidal activity of CF, Fip and Cou against

 Aedes albopictus larvae.

	$LC_{50}^{a} (\mu mol L^{-1})$			
Treatment	Aedes larvae <sup>b</sup>	Mythimna separate <sup>c</sup>		
CF-bluelight <sup>d</sup>	0.56	24.64		
CF-sunlight <sup>e</sup>	0.37			
CF-dark	13.73	> 50		
Fip-blue light	0.11			
Fip-sunlight	0.12			
Fip-dark	0.11	6.82		
Cou-blue light	>50	>100		
Cou-sunlight	>50			
Cou-dark	>50	>100		
CK-blue light	no activity	no activity		
CK-dark	no activity	no activity		

<sup>*a*</sup>LC<sub>50</sub> is the median lethal concentration. <sup>*b*</sup>Light was imposed after 1 h treatment of *Aedes* larvae with CF. <sup>*c*</sup>Light was impose to the CF directly before administrated to *Mythimna separate*. <sup>*d*</sup>Irradiated under visible light (420 nm). <sup>*e*</sup>Irradiated under sunlight.



Figure 5. A: The activity of CF, Fip and Cou with or without irradiation against *Aedes albopictus* larvae. B: Mortality of CF at dark and irradiated under visible light (420 nm) or sunlight against *Aedes albopictus* larvae. C:

Time-dependent activity of CF upon blue light (420 nm) irradiation. Each value given as mean  $\pm$  SE of three replicates.

In order to explore the possibility of releasing Fip in larger and opaque insect, activity to armyworm (*Mythimna separate*) was screened using the above described method. However, the activity is low due to the fact that blue light cannot penetrate into the armyworm. Therefore, a modified method was employed alternatively in which **CF** was irradiated by blue light prior to be administrated to armyworm. The released Fip retained its activity with  $LC_{50}$  value of 24.64  $\mu$ mol L<sup>-1</sup>. Such application method has its own rationale, because part of Fip would be released under natural sunlight before the caged Fip reaches the target insects in field application. The **CF**-light-treated armyworms have the similar poisoning signs with that of Fip-treated ones (Figure 6), which suggested that Fip was the main toxic ingredient among the photolysis products of **CF**.



**Figure 6.** Poisoning signs of CF-treated armyworms plus light (A), CF-treated armyworms at dark (B), Fip-treated armyworms (C) and blank control (D).

The blue fluorescence of **CF** makes it possible for the visualization study on the treated insects. The fluorescence and confocal microscopy images were collected as depicted in Figure 7 and 8, respectively. After treatment of mosquito larvae with **CF** at dark, **CF** mainly accumulated in the gastric caeca and guts lumen<sup>37</sup> (Figure 7, A and Figure 8, A). When **CF** and light (blue light or sunlight) were co-applied, the release of Fip led to the death of larvae and the fluorescent **CF** and Cou dispersed throughout the whole body (Figure 7, B and C, and Figure 8, B), indicating the tissue damage caused by Fip. For the Cou-treated mosquito larvae, the Cou was also mainly located in the digestive system.

In conclusion, fast and efficient light-triggered release of fipronil was achieved using coumarin as a cage. To our knowledge, this is the first example of photo-controlled release of an insecticide molecule. The coumarin-fipronil precursor can release fipronil in vivo in mosquito larvae upon blue light or sunlight irradiation, providing the possibility of spatiotemporal insecticide release. Additionally, due to the dual role of CF being a photoresponsive molecule and fluorescent dye, imaging-guided insecticide delivery is an attractive possibility, which might be useful to the toxicology study of an insecticide. The caged Fip would facilitate the understanding of action mechanism of fipronil in living organism with spatiotemporal resolution. The relative shorter absorption wavelength of the current lighttriggered insecticide make it difficult to achieve the in vivo release in opaque insects, but this can be conquered by using auxiliary equipment, such as optical fiber, to pump the light inside the body. Furthermore, structural modifications are needed

to realize the release at longer wavelength of light that have deeper tissue penetration depth.



**Figure 7.** Fluorescence images of *Aedes albopictus* larvae. A: images of *Aedes* larvae treated by CF at dark (20 mg/L); B: images of *Aedes* larvae treated by CF (20 mg/L) and irradiated by blue light (420 nm); C: images of *Aedes* larvae treated by CF (20 mg/L) and irradiated by sunlight; D: images of *Aedes* without any treatment; E: images of *Aedes* larvae treated by Fip (10 mg/L); F: images of *Aedes* larvae treated by Cou (10 mg/L).



**Figure 8.** Confocal microscopy images of *Aedes* larvae treated with CF at dark (10 mg/L) (A), CF (10 mg/L) plus blue light (420 nm) (B) and without any treatment (C).

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