JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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Azobenzene-diamides as Photopharmacological Ligands for Insect Ryanodine Receptor

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Cite This: J. Agric. Food Chem. 2020, 68, 14409−14416 Read Online

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ABSTRACT: Photoresponsive ligands are powerful tool compounds for studying receptor function with spatiotemporal resolution. However, to the best of our knowledge, such a ligand is not available for the ryanodine receptor (RyR). Herein, we present a photochromic ligand (PCL) for insect RyR by decorating chlorantraniliprole (CHL) with photoswitchable azobenzene (AB). We demonstrated that one potent ligand, named ABCHL13, shows light-induced reversible trans−cis isomerization and 3.5-fold insecticidal activity decrease toward oriental armyworm (*Mythimna separata*) after UV-light irradiation, that is, *trans*-ABCH13 has higher activity than the *cis*-ABCH13. ABCHL13 enables optical control over intracellular Ca^{2+} release in dorsal unpaired median (DUM) neurons of *M. separata* and American cockroach (*Periplaneta americana*) and cardiac function of *P. americana*. Our results provide a first photopharmacological toolkit that is applicable to light-dependent regulation of RyR and heart beating.

KEYWORDS: *photopharmacology, ryanodine receptor, azobenzene, chlorantraniliprole, insect*

INTRODUCTION

Ryanodine receptors (RyRs) are intracellular homotetrameric nonvoltage-gated calcium channels that are located in the sarcoplasmic or endoplasmic reticulum (SR or ER) of both vertebrates and invertebrates.[1−4] RyRs play an essential role in mediating the release of Ca^{2+} in many cell types, such as neurons, epithelial cells, and muscle cells. Defective function of RyRs is associated with heart failure, muscular dystrophy, malignant hyperthermia, central core disease, and neurodegenerative disease.[1−4] Currently, several mammalian and invertebrate RyR crystal structures have been elucidated.[5−7] RyRs respond to an array of antagonists (e.g., ryanodine, dantrolene, and ruthenium red)[8] and activators (e.g., *p*-chlorocresol, suramin, caffeine, ATP, and cyclic ADP ribose).[9] These ligands are basic tools for deciphering their role in gating and regulation.

Insect RyRs are good models for physiological function exploration and have attracted intensive attention recently. Previous studies on RyRs from fruit flies, houseflies, cockroaches, and diamondback moths indicate that insect RyRs show a high degree of homology in amino acid sequence with mammalian receptors.[7,10] RyRs have three mammalian isoforms (RyR1 to RyR3), while in insects, RyRs only have a single isoform showing 47% identity at the amino acid level with RyR2.[7] Insect RyRs are the targets of a class of newly developed insecticides called diamides whose global annual sales approach US$2 billion.[11,12] Diamide can activate the receptor to induce the excessive release of Ca^{2+} from intracellular stores located in SR, leading to insect feeding cessation, lethargy, paralysis, and eventual death.[13]

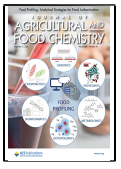
Photopharmacology is a fast-growing photonics-based technology that relies on photoresponsive ligands to manipulate biological functions.[14−16] This technology provides control over bioactivity or biological events with a spatiotemporal resolution that other technologies cannot confer. Various photopharmacological ligands have been developed toward biologically important targets, opening a new door for the research community to stimulate or silence the function of target proteins.[17] In calcium channel photopharmacology, a couple of photochromic ligands (PCLs) were developed, allowing for optical control over cardiac function exemplified by photoswitchable diltiazem[18] and muscarinic agonist.[19] However, the implementation of photopharmacology in the pesticide arena is neglected. We recently developed a series of photoresponsive pesticides, such as photocaged fipronil[20] and spirotetramat-enol,[21] and photoswitchable azobenzene-modified imidacloprid and benzoylphenylurea analogues.[22,23] Owing to the lack of photoresponsive ligands for RyR, we report herein the first example of photochromic insect RyR ligand by blending azobenzene (AB) with chlorantraniliprole (CHL) and demonstrate its ability in optical control over neuron and cardiac function of living cockroach.

MATERIALS AND METHOD

Synthetic Routes and Characterization. The chemicals and instruments used, the detailed synthetic procedures, and ^{1}H NMR, ^{13}C NMR, and HRMS data are provided in the Supporting Information.

Optical Properties. The target compound in acetonitrile (20 μM) in a quartz cuvette (1 cm × 1 cm) was irradiated with ultraviolet

Received: May 24, 2020
Revised: September 11, 2020
Accepted: November 13, 2020
Published: November 30, 2020



https://dx.doi.org/10.1021/acs.jafc.0c03272
J. Agric. Food Chem. 2020, 68, 14409−14416

(365 nm) at room temperature. The absorbance changes at different time intervals were recorded with a UV−vis spectrophotometer until they no longer changed. The ratio of cis/trans-isomers was measured by ultraperformance liquid chromatography. The fatigue resistance was determined by monitoring the absorbance change at the maximum absorption through alternative irradiation with UV (λ = 365 nm) and blue light (λ = 430 nm). For the half-life test, the target compound in acetonitrile (20 μM) was irradiated with UV to reach a photostable state. The solution is stored in the dark and the absorbance change at the maximum absorption wavelength was measured by a UV−vis spectrophotometer at different time intervals. All the operations have six repetitions.

Insecticidal Activity against *M. separate* and *Aedes albopictus* Larvae. *Insecticidal Test Method for Aedes albopictus Larvae.* The fourth-instar larvae of *A. albopictus* were provided by Shanghai Southern Pesticide Creation Center. Two groups of aqueous solutions with the same concentration gradient were prepared: one was irradiated with UV (λ = 365 nm) for 30 min and the other group was stored in the dark. Ten Aedes larvae of the same size were selected and placed in a centrifugation tube. Then, the solution containing different concentrations of chemicals was added to the centrifugation tube, and the tube was placed in the dark in a conditioned room (25 ± 1 °C, 50% relative humidity (RH)). After 24 h treatment, the mortality of *A. albopictus* larvae was counted; the larvae were considered to be dead unless they moved when touched with a syringe. Water was used as a control. Each treatment had three repetitions, and the data were adjusted and subjected to probit analysis as before.

Insecticidal Test for M. separate. The *M. separate* larvae were reared in our laboratory, and the insecticidal activity was tested by the leaf-dipping method. Two groups of solutions with the same concentration gradient were prepared: one was irradiated with UV (λ = 365 nm) for 30 min and the other group was stored in the dark. The corn (*Zea mays*) leaves were well-immersed in the above-prepared solutions, dried naturally in the dark, then placed in clean culture dishes. Ten third instar *M. separate* larvae that were starved for 2 h were introduced into each dish. Then, the dishes were placed in the dark for 72 h in a conditioned room (25 ± 1 °C, 50% RH). The mortality of the larvae was counted. The larvae were considered to be dead unless they moved when touched with a syringe. Water was used as a control. Each treatment had three repetitions, and the data were adjusted and subjected to probit analysis as before.

***Periplaneta americana* Dorsal Unpaired Median (DUM) Neuron Preparation.** *Physiological Solutions.* A mixture of physiological solution A, 185 mM NaCl, 3.0 mM KCl, 4 mM $MgCl_2$, 10 mM D-glucose, 10 mM *N*-(2-hydroxyethyl)piperazine-*N*′-ethanesulfonic acid (HEPES) was adjusted to pH 7.2 with NaOH and stored at 4 °C. Physiological solution B was prepared by adding type IA collagenase 1.5 mg mL^{-1} to solution A. Physiological solution C was prepared by adding 5 mM $CaCl_2$, fetal calf serum (10% by volume), and double antibody [1% penicillin (50 IU mL^{-1})/streptomycin (50 mg mL^{-1})] to solution A.

Preparation of DUM Neurons of P. americana. The body surface of male *P. americana* adults (purchased from Tengfei Cockroach Company, Anhui, China) was disinfected with 75% alcohol. Under a dissecting microscope, the body wall was cut along the midline and fixed in a wax plate. The impurities such as trachea, digestive tract, and fat body were carefully removed and the ganglion was dissected in physiological solution A. The whole process is operated on a clean bench. The obtained ganglion was transferred to physiological solution B and hydrolyzed at 37 °C for 20 min. After the enzymatic hydrolysis was completed, the ganglion was transferred to physiological solution C, and the digestion was terminated by rinsing three times with physiological solution C. The ganglion was repeatedly blown into physiological solution C to disperse the cells in the ganglion. The obtained cell suspension was filtered through a 200-mesh filter into a Petri dish containing physiological solution C at 28 °C.

Intracellular Ca^{2+} Analysis in *Pa*DUM Neurons. *Intracellular Ca^{2+} Analysis in the Presence of Extracellular Ca^{2+}.* ABCHL13 was dissolved in dimethyl sulfoxide (DMSO) to give a certain concentration of mother solution. The prepared solutions were divided into two groups: one of which was irradiated with ultraviolet (365 nm) for 30 min and the other group was stored in the dark. Ten microliters of each solution was added to 1 mL of DUM neuron cell-culture medium. After 6 h of incubation, 5 μM fluorescent dye $DiBAC_4(3)$ was added for further incubation for 30 min, then the dye was washed away. A 24-well plate containing DUM neuron cells was immobilized on a laser confocal microscope stage to detect changes in the fluorescence intensity of DUM neurons before and after illumination. The excitation wavelength was 488 nm and the emission wavelength was 525 nm, and each experiment was repeated three times.

Intracellular Ca^{2+} Analysis in the Absence of Extracellular Ca^{2+}. *Pa*DUM neurons in physiological solution C (1 mL) were placed in four glass-bottom cell-culture dishes (35 mm). To the above four dishes, 1 μL of DMSO, 1 μL of *trans*-ABCHL13 in DMSO without irradiation, 1 μL of *trans*-ABCHL13 plus ruthenium red (10 μM), and 1 μL of ABCHL13 irradiated by UV light were added, respectively, and then incubated for 60 min at 28 °C. The incubated neurons were washed with phosphate-buffered saline (PBS), added with Fluo 3-AM (10 μM), and incubated for 30 min at 28 °C. After incubation, the neurons were photographed by a confocal laser scanning microscope (Nikon Inc., Melville, NY). Emission was collected at 525 nm upon excitation at 488 nm to observe changes in the fluorescence intensity in DUM neuron cells. Three separate experiments were performed, each with triplicate samples.

Intracellular Ca^{2+} Analysis in *Mythimna separata*. *Isolation of M. separata Neurons.* *M. separata* Walker were initially obtained from shallot fields in Tianjin, China, and reared indoors in climatic chambers on an agar-based semisynthetic diet at 27 ± 1 °C, 75 ± 5% relative humidity, and 16:8 h LD photocycle. The insects were reared for two generations prior to the experiment. Third instar larvae of *M. separata* were first anesthetized with 70% ethanol and their thoracic and abdomen ganglia were removed and placed in saline. The thoracic and abdomen ganglia were transferred to a solution containing 0.3% trypsin for 6 min at 28 °C, plated into a 35 mm culture dish containing 1 mL of improved L-15 Leibovitz culture medium supplemented with fetal calf serum (15%, v−v), and then mechanically dissociated using a fire-polished Pasteur pipette. The cultures were maintained at 28 °C for 2 h to allow the cell to adhere to the dish. All procedures were carried out under sterile conditions.

Intracellular Ca^{2+} Analysis. Calibration of the fluorescence signal was achieved using the method reported by Takahashi et al. with modifications. Briefly, the attached neurons were rinsed twice in standard physiological saline [(mM): NaCl 150, KCl 4, $MgCl_2$ 2, $CaCl_2$ 2, HEPES 10], buffered to pH 7.0 and then incubated in the dark for 30 min at 28 °C in standard external saline containing the dye Fluo 3-AM (10 μM) or incubated for 2 h with Fluo 5-N AM (10 μM). After dye loading, the cells were again rinsed in physiological saline twice. Calcium-free extracellular fluid has the following composition (mM), NaCl 150, KCl 4, $MgCl_2$ 2, EGTA 2, HEPES 10, buffered to pH 7.0. The new compound was applied after 3 min of fluorescence recording. The original compound is eluted with calcium-free extracellular fluid before the next compound is applied. Calcium ratio imaging studies were conducted using the imaging system coupled to an inverted fluorescence microscope with a Fluor 40 × oil immersion objective (Olympus IX71). The cells were excited at 488 nm and the 530 nm fluorescence emission acquired using a CCD (Image Pro-6.0). Each experiment was repeated at least six times. The data were analyzed using GraphPad Prism on 7.0. The results were expressed as mean ± SD (n = number of cells). Fluorescence values were expressed as $F/F0$, $F0$ being the resting (or baseline) fluorescence and F the change in fluorescence from baseline after the drug application.

Photomodulation of *P. americana* Heartbeat. Four male *P. americana* were fixed by two pins with back up and their wings and legs were cut off. Then, their first and second terga were sequentially lifted up and cut off, and the fat was carefully removed and washed off by physiological saline (pH = 7.2). The separated terga with the heart

https://dx.doi.org/10.1021/acs.jafc.0c03272
J. Agric. Food Chem. 2020, 68, 14409−14416

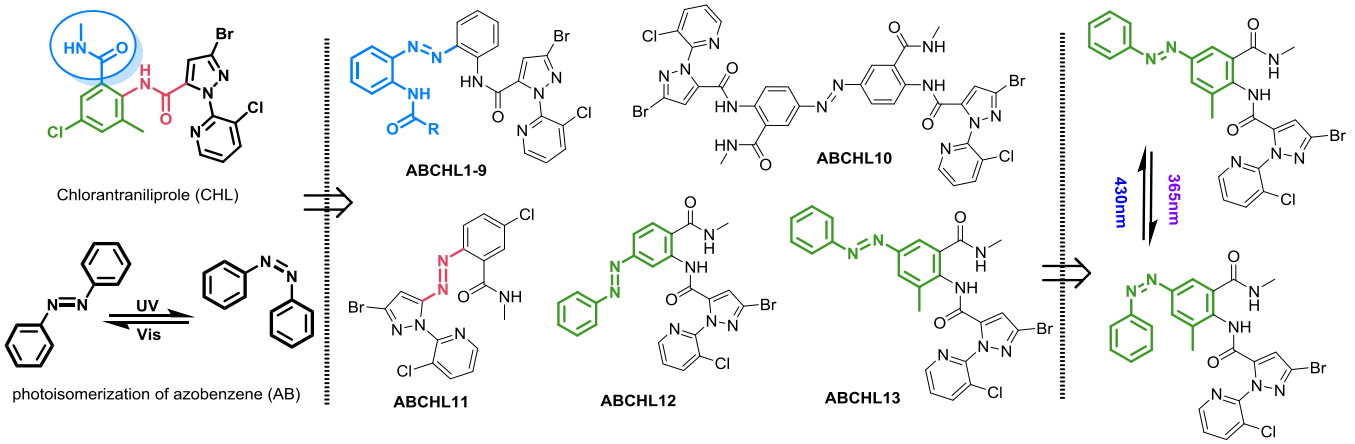


Figure 1. RyR PCLs are derived from RyR activator chlorantraniliprole and photoswitch azobenzene. Ligands ABCHL1−9 and ABCHL11 are designed by the replacement of amide bond with azobenzene. ABCHL10 is a divalent compound linked by azobenzene. ABCHL12−13 are generated by the exterior attachment of azobenzene.

was transferred to a cell-culture dish containing physiological solution (NaCl 200 μM, KCl 10.7 μM, $MgSO_4$ 0.1 μM, $NaHCO_3$ 2.1 μM, NaH_2PO_4 0.08 μM, and HEPES 10 g·L^{-1}) (3 mL) and incubated for 30 min at 25 °C. Then, the separated terga were treated with DMSO (3 μL), *trans*-ABCHL13 (100 μM), *trans*-ABCHL13 (100 μM), and UV irradiation (10 min) and chlorantraniliprole (100 μM), respectively, incubated for 10 min at 25 °C. Then, the heartbeat rates in 1 min were calculated after 5, 10, 20, and 30 min treatment. Each experiment was repeated six times.

RESULTS AND DISCUSSION

Molecular Design and Synthesis. Diamides are a new chemotype of RyR activator. The currently developed diamides are divided into two categories: phthalic diamides exemplified by flubendiamide[13] and anthranilic diamides represented by cyantraniliprole and CHL.[24,25] CHL is now the largest insecticide with sales volume over US$1 billion. CHL has a high binding affinity with insect RyR.[26] We therefore used CHL as a prototype for structural modification by introducing photoswitchable azobenzene at different positions to confer photosensitivity. Bioisosteric replacement and azo-extension strategy were employed in our molecular modification. CHL contains three structural segments: benzene ring, two amides, and pyridine−pyrazole. Previous investigations proved that aryl benzamide is a bioisosteric group of azobenzene that has been successfully applied for putting bioactive molecules under optical control.[27,28] We first replaced two amide groups in CHL with azobenzene-generating compounds ABCHL1−9 and ABCHL10, respectively. We then linked two CHL molecules together by diazene unit giving a divalent compound ABCHL11. Attaching azobenzene at the exterior of bioactive molecule is also an effective strategy.[29] Therefore, we prepared analogues ABCHL12 and ABCHL13 by introducing azobenzene at the phenyl ring of CHL (Figure 1).

The synthetic routes of target compounds are depicted in Figure 2. Compounds ABCHL1−9 were prepared from *o*-diaminobenzene. ABCHL10 was prepared from intermediates 6 and 9. Divalent ABCHL11 and monovalent ABCHL12 were synthesized by four-step and five-step procedures, respectively. Finally, ABCHL13 was obtained through a seven-step procedure using 2-amino-3-methylbenzoic acid as the starting material. All of the compounds were well-characterized by ^{1}H NMR, ^{13}C NMR, and HRMS.

Photophysiochemical Properties. With the target compounds in hand, we set out to investigate their photophysiochemical properties using UV−vis spectroscopy and high-performance liquid chromatography (HPLC) analysis to verify whether they are suitable for further biological tests. The half-lives of azobenzene analogues were calculated based on the change in absorbance at 336 nm ($\lambda_{\text{trans-max }\pi-\pi^*}$) over time. The isosbestic points of the trans- and cis-structure were determined by UV−vis spectroscopy, which are taken as the detection wavelength of HPLC to calculate the percentages of both isomers. The maximum absorbance wavelength (λ_{max}, nm), the ratio of photoisomers, and the thermal relaxation half-lives are summarized in Table 1. All of the compounds show typical azobenzene absorption band and photoswitching behaviors with the maximum absorption wavelength at around 365 nm. ABCHL1−9 showed poor photoisomerization efficiency (39−54% conversion from trans to cis configuration) and their cis-isomers were not stable enough with 1−2 h thermal relaxation half-lives. The thermal instability of a cis-isomer was probably caused by substituent effects of *ortho*-amide as the substituents at the ortho-position would attenuate cis-isomer's stability.[30,31] ABCHL10 and ABCHL11 have improved isomerization efficiency but their cis forms still have low thermal stability. ABCHL12 and ABCHL13 can gradually transform to their cis forms (>90% conversion rates) upon UV irradiation (Figure 3A) and thermal back half-lives were 26 and 11 h, respectively, indicating suitable photoswitching properties for further biological studies. ABCHL13 is resistant to photobleaching and could be switched back and forth more than 15 times (Figure 3B).

Insecticidal Activity. To identify the performance of our RyR PCLs, we evaluated their insecticidal activities using armyworm (*M. separate*) and mosquito larvae (*A. albopictus*). We initially screened the insecticidal activity of trans-isomers. Toward *M. separate*, *trans*-ABCHL1−12 almost lost insecticidal activity (Table 2). Fortunately, ABCHL13 showed 100% mortality at 500 mg L^{-1}. These results demonstrate that the replacement of aryl amide with azobenzene or preparing divalent ligand is not applicable for maintaining activity. As *cis*-ABCHL1−9 have low thermal stability,it is difficult to test their accurate activities. We therefore selected ABCHL10−13 to further investigate the insecticidal activities of both photoisomers. No obvious insecticidal activities were observed

https://dx.doi.org/10.1021/acs.jafc.0c03272
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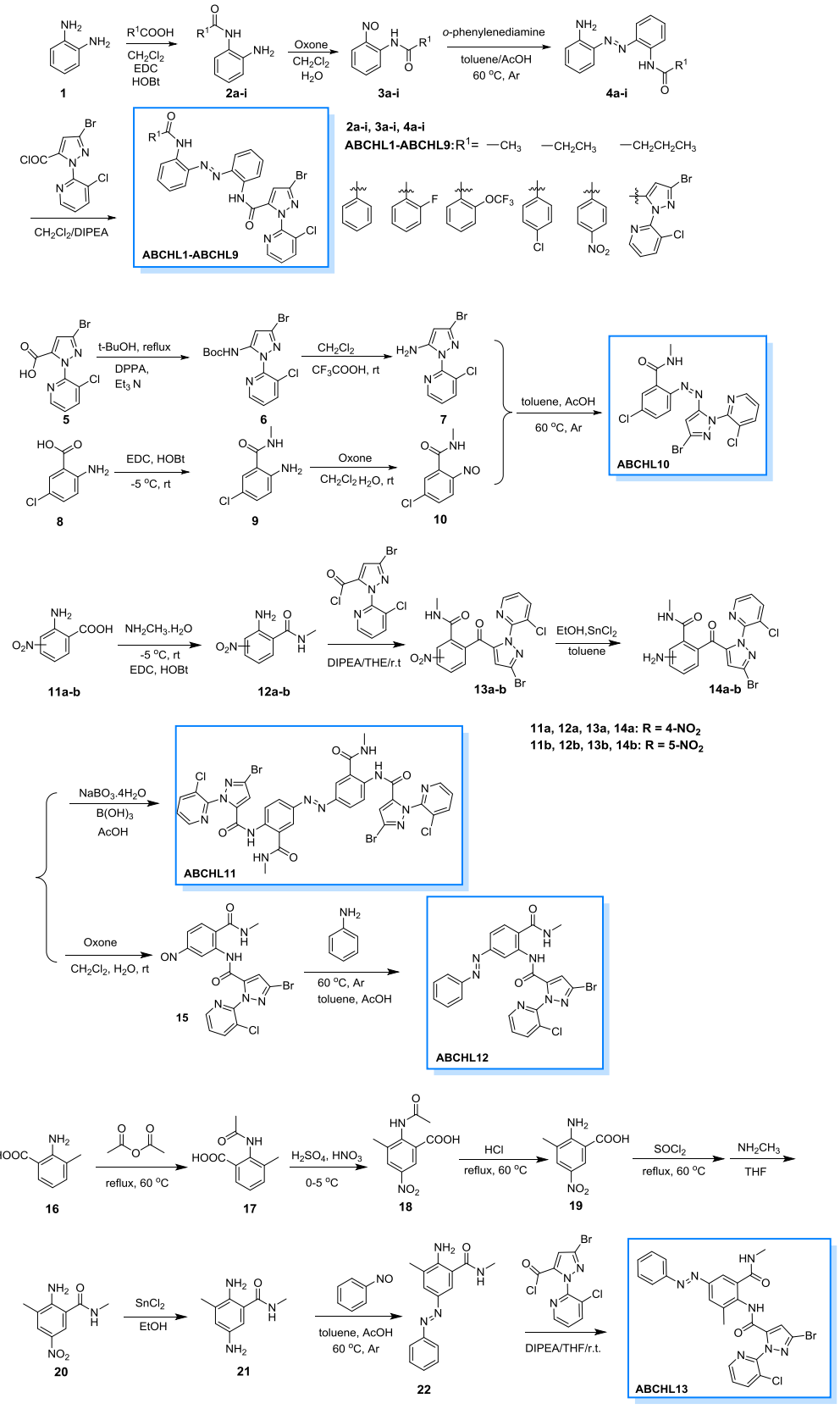

Figure 2. Synthesis routes of ABCHLs.

for ABCHL10−12 before and after irradiation. Both isomers of ABCHL13 have almost the same activity against mosquito larvae, while they exhibited 3.5-fold activity difference toward *M. separate* (Figure 4A), indicating that ABCHL13 can be photoinactivated upon light irradiation. In control experiments, light has no perturbance on the activity of the precursor CHL. Recently, bee toxicity has become a big concern. Therefore, we

https://dx.doi.org/10.1021/acs.jafc.0c03272
J. Agric. Food Chem. 2020, 68, 14409−14416

Table 1. Maximum Absorbance Wavelength (λ_{max}, nm), the Ratio of Trans- and Cis-Isomers and the Thermal Relaxation Half-Lives of ABCHLs

compound	nonirradiated trans:cis	irradiated trans:cis	$t_{1/2}$ (h)
ABCHL1	93: 7	46: 54	1.5
ABCHL2	81:19	50:50	1.4
ABCHL3	81:19	46: 54	1.0
ABCHL4	80:20	54:46	2.0
ABCHL5	82:18	60:40	1.8
ABCHL6	95:5	63:37	1.6
ABCHL7	88:12	56:44	1.8
ABCHL8	80:20	55:45	1.3
ABCHL9	93:7	61:39	1.6
ABCHL10	100:0	38:62	3.6
ABCHL11	100:0	21:79	56
ABCHL12	97:3	5:95	26
ABCHL13	100:0	9:91	11

evaluated bee toxicity of ABCHL13 using BeeTox,[32,33] and the results showed that it is not toxic (87.22% negative) to bees.

ABCHL13 Enables Optical Modulation of Neurons. Having identified the in vivo activity difference, we sought to investigate its ability for optically modulating insect RyR using *M. separate* neurons and American cockroach (*P. americana*) DUM neurons. We used fluorescence dye Fluo 3-AM as an indicator for monitoring ratiometric Ca^{2+} inside the cells. Fluo 3-AM is an intracellular Ca^{2+} fluorescent probe that can enter the cytoplasm and be enzymatically hydrolyzed into Fluo 3. The free Fluo 3 has a weak fluorescence. However, when Fluo 3 chelates Ca^{2+}, its fluorescence intensity enhances the proportionally to Ca^{2+} concentration. Diamide insecticides can keep the ryanodine receptor open, resulting in a sharp increase in Ca^{2+} in the cytoplasm. When *M. separate* neurons were loaded with Fluo 3-AM and treated with *trans*-ABCHL13 (5 μM), the peak value of $F/F0$ (%) was 102.0 ± 0.41 (n = 12). After irradiation, the peak value of $F/F0$ (%) decreased to 99.8 ± 0.15 (n = 12) (Figure 4B), which is almost equal to that of the control. From this observation, we concluded that *trans*-ABCHL13 can be photoinactivated to induce the opening of the calcium channel in an on−off manner. The same trend was also observed for neurons treated with 20 μM ABCHL13 (Figure 4C). After the neurons of *M. separate* were incubated with ryanodine for 5 min and then treated with *trans*-ABCHL13 (20 μM) and light, the peak value of $F/F0$ (%) decreased from 106.1 ± 1.61 (n = 12) to 104.63 ± 1.87 (n = 12, Figure 4D), indicating that ryanodine can perturb the binding affinity of our ligand. These experiments implied that RyRs in the neuron ER are the possible targets of *trans*-ABCHL13 (Table 3).

Table 2. Insecticidal Activity of ABCHLs against *A. albopictus* and *M. separate*

	insecticidal activity (mortality at 500 mg L^{-1})	
compound	*A. albopictus*	*M. separate*
ABCHL1	100	80 ± 1
ABCHL2	100	50 ± 3
ABCHL3	100	80 ± 4
ABCHL4	80 ± 2	70 ± 2
ABCHL5	100	60 ± 1
ABCHL6	90 ± 3	80 ± 2
ABCHL7	100	70 ± 1
ABCHL8	100	80 ± 1
ABCHL9	20 ± 2	30 ± 2
ABCHL10	0	20 ± 5
ABCHL11	0	0
ABCHL12	0	0
ABCHL13	100	100
CHL	100	100
azobenzene	0	

Next, we used $DiBAC_4(3)$ to monitor the intracellular cation changes in *Pa*DUM neurons. When *Pa*DUM neurons were subjected to *trans*-ABCHL13 (10 μM) and the dye, strong fluorescence was observed (fluorescence intensity, 49.6). Upon irradiation, 1.5-fold fluorescence intensity reduction (32.9) was detected (Figure 5A). To further identify our ligand acts on insect RyRs, we used ruthenium red (RuR) as the RyR inhibitor[8] and Fluo 3-AM as a probe to measure the Ca^{2+}-releasing ability in acute separation *P. americana* DUM neurons. RuR is a noncompetitive inhibitor of the ryanodine receptor. In the extracellular absence of Ca^{2+}, the *Pa*DUM neurons treated with RuR and Fluo 3-AM do not emit fluorescence. After adding *trans*-ABCHL13 to the above-treated neurons, the fluorescence gradually resumed with an intensity of 16.9 (Figure 5B), indirectly indicating that our ligand acts on insect RyR. While the corresponding fluorescence intensity recovered by *cis*-ABCHL13 was 5.46, suggesting a 3.42-fold decrease in activity toward RyR. The above results implied that ABCHL13 alone can open the channel and cause an increase in the intracellular Ca^{2+} concentration.

ABCHL13 Enables Optical Modulation of Heart Pacing. After establishing the light-dependent inactivation of

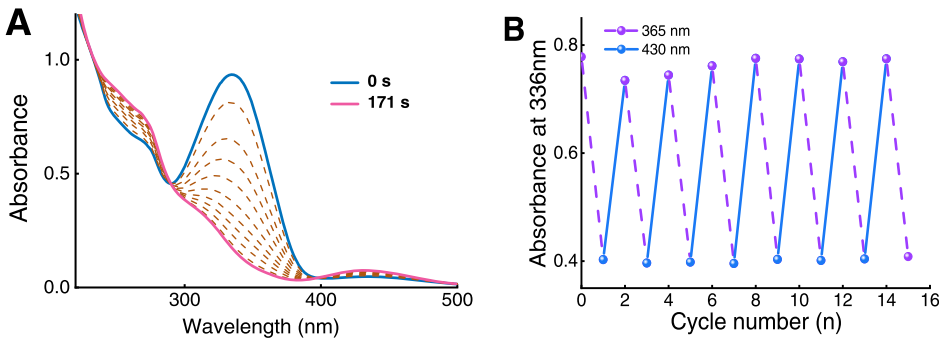

Figure 3. UV−vis spectroscopy analysis. (A) UV−vis absorption spectra change of ABCHL13 (20 μM) upon UV (356 nm) irradiation. (B) Fatigue resistance of ABCHL13 (20 μM) by alternative irradiation of UV light and (purple lines) and blue light (blue lines).

https://dx.doi.org/10.1021/acs.jafc.0c03272
J. Agric. Food Chem. 2020, 68, 14409−14416

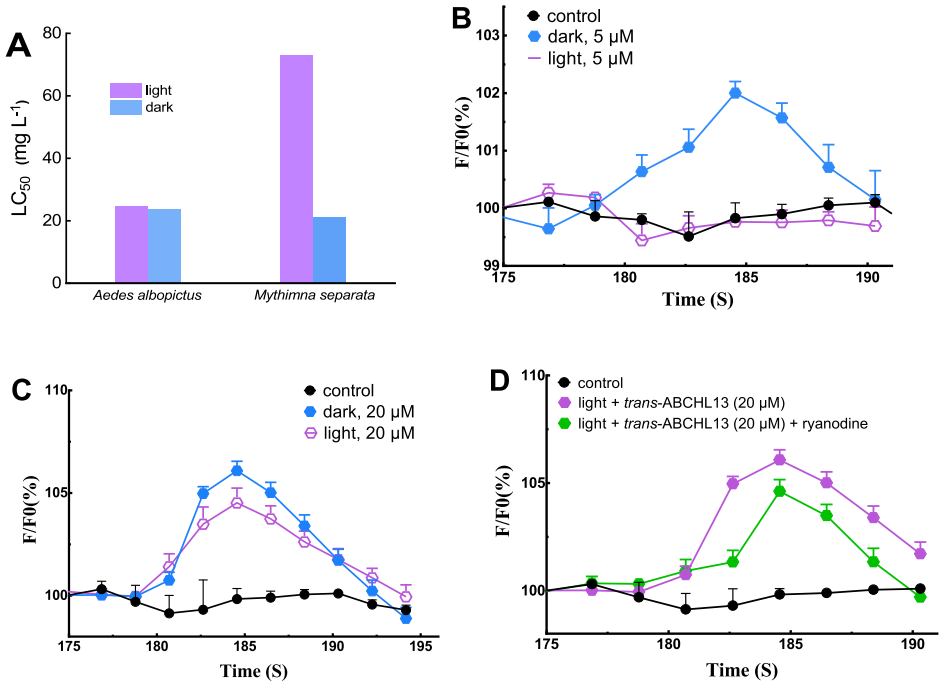

Figure 4. Insecticidal activity and Ca^{2+}-releasing ability of ABCHL13. (A) Insecticidal activity ABCHL13 toward *A. albopictus* and *M. separate* before and after irradiation. (B–D) Ca^{2+}-releasing ability of ABCHL13 in *M. separate* neurons using fluorescence dye Fluo 3-AM as an indicator at 5 μM (B) and 20 μM (C) and in the presence of ryanodine (D).

Table 3. Insecticide Activity of ABCHLs against *A. albopictus* and *M. separate* before and after UV Irradiation

		A. albopictus		*M. separate*	
compound		LC_{50} (mg L^{-1})	$y = ax + b$	LC_{50} (mg L^{-1})	$y = ax + b$
ABCHL10	light	n.a.[a]		n.a.	
	dark	n.a.		n.a.	
ABCHL11	light	n.a.		n.a.	
	dark	n.a.		n.a.	
ABCHL12	light	n.a.		n.a.	
	dark	n.a.		n.a.	
ABCHL13	light	23.71	$y = 2.6252x + 1.7275$	73.09	$y = 3.4436x + 0.8348$
	dark	24.58	$y = 2.6884x + 1.6627$	21.13	$y = 4.0840x + 0.6914$
CHL	light			0.21	$y = 5.7001x + 1.0385$
	dark			0.21	$y = 5.7801x + 1.1408$
CK		n.a.		n.a.	

[a]n.a. = no activity.

insect RyR in neurons, we turned to investigate its ability to optically control cardiac functions. Diamides regulate the release of stored intracellular calcium and play a critical role in muscle contraction and insect heart beating. *P. americana* has an open circulatory system with a heart (an elongated contractile tube) lying along the mid-dorsal line beneath the terga. The heart contraction was controlled by alary muscles. The back of *P. americana* containing the heart was cut off and stored in physiological solution followed by quick addition of ABCHL13. After 5 min of incubation, the heartbeat rate was calculated every 5 min (Figure 5C). After the application of *trans*-ABCHL13, the heartbeat rate reduced about 71% ($n = 5$ hearts) in comparison with untreated control. Upon illumination with UV light, the heartbeat rate reduced to 65% (Figure 5D), that is, the cis-isomer has a lower ability to reduce heartbeat rate than the trans-isomer. After 30 min of treatment, the difference in the heart pacing between *trans*- and *cis*-ABCHL13 was 1.13-fold, which is consistent with the difference observed in calcium-releasing ability. Both isomers had a higher influence on the heartbeat rate than CHL, although the in vivo insecticidal activity of ABCHL13 was lower than that of CHL. The above observations demonstrated that ABCHL13 enables remote and optical control of heart rate in the cockroach.

Cardiac pacing plays an important role in the circulatory system, and its malfunction relates to many serious diseases. Cardiac rhythm control drug is the most commonly used strategy to steady the heartbeat and ease symptoms. Cardiac function is controlled by the nervous system and cardiac muscle. For optical control of cardiac function, photoswitchable diltiazem[18] and muscarinic agonist[19] were developed toward L-type Ca^{2+} channels and muscarinic acetylcholine receptors, respectively. Our results established here for the first time that RyR is an alternative target for optical control of cardiac function. As RyRs exist both in vertebrates and invertebrates, the information gained from invertebrates may

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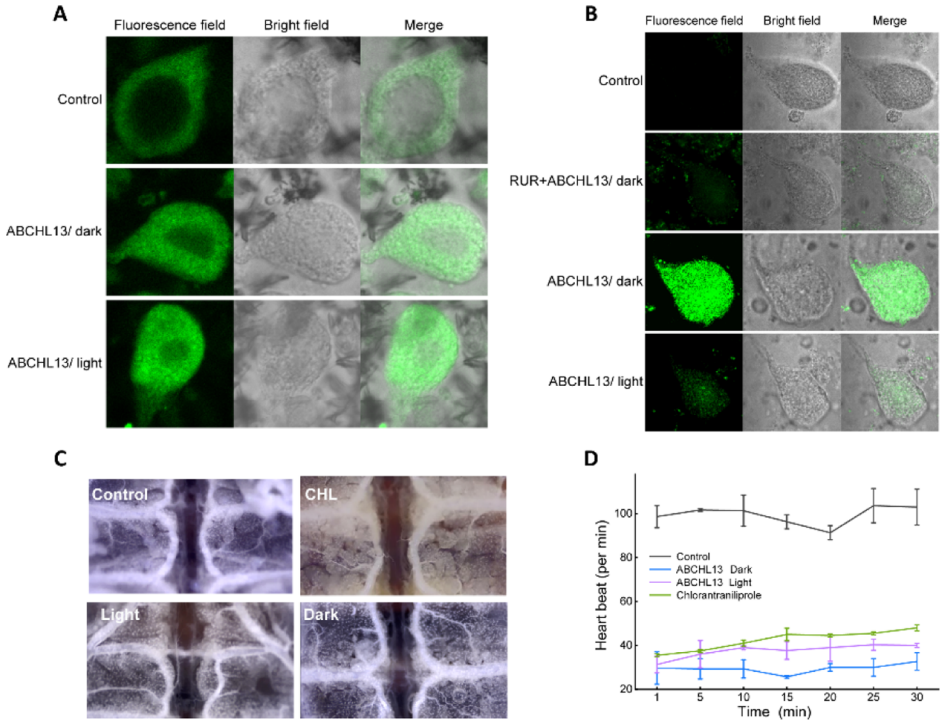

Figure 5. (A) Fluorescence images of *Pa*DUM neuron treated with ABCHL13 and Fluo 3-AM. (B) Fluorescence images of *Pa*DUM neuron treated with RuR, ABCHL13, and Fluo 3-AM. (C) *P. americana* heart of the control and insect treated with CHL, ABCHL13 alone, and ABCHL13 plus light. (D) Heartbeat rate of *P. americana* treat with CHL and ABCHL13 before and after irradiation.

provide guidelines for developing new therapeutic agents in vertebrates.

In conclusion, we present a first example of the photochromic RyR ligand via coupling of azobenzene with diamide CHL. ABCHL13 can be photoinactivated upon irradiation and showed differential insecticidal activity toward *M. separate* and different Ca^{2+}-releasing ability in *M. separate* and *Pa*DUM neurons by acting on insect RyR. Furthermore, ABCHL13 can be used for real-time modulation of the heartbeat of cockroach. Although the difference in the activity was not high enough, our results implied that RyR PCLs are applicable for cardiac function control. We hope the information we gained here would provide guidelines for the future development of PCLs toward mammalian RyR to enable spatiotemporal control over cardiac function.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.0c03272.

Synthetic procedures and ^{1}H NMR, ^{13}C NMR, and HRMS of new compounds (PDF)

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Funding

This work was financially supported by the National Key Research and Development Program of China (2018YFD0200100), the National Natural Science Foundation of China (No. 21877039), and the Innovation Program of Shanghai Municipal Education Commission (2017-01-07-00-02-E00037).

Notes

The authors declare no competing financial interest.

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https://dx.doi.org/10.1021/acs.jafc.0c03272
J. Agric. Food Chem. 2020, 68, 14409−14416