JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Design, Synthesis, Acaricidal Activity, and Mechanism of Oxazoline Derivatives Containing an Oxime Ether Moiety

Yongqiang Li,[†,‡] Chaojie Li,[†] Yanlong Zheng,[†] Xingcun Wei,[†] Qiaoqiao Ma,[†] Peng Wei,[†] Yuxiu Liu,*[,†] Yaoguo Qin,[‡] Na Yang,[†] Yufeng Sun,[‡] Yun Ling,[‡] Xinling Yang,*[,‡] and Qingmin Wang*[,†]

[†]State Key Laboratory of Elemento-Organic Chemistry, Research Institute of Elemento-Organic Chemistry, Nankai University, Tianjin 300071, The People's Republic of China

[‡]Key Laboratory of Pesticide Chemistry and Application, MOA, Department of Applied Chemistry, College of Science, China Agricultural University, The People's Republic of China

ABSTRACT: Two series of novel 2,4-diphenyl-1,3-oxazolines containing an oxime ether moiety were designed and synthesized via the key intermediate *N*-(2-chloro-1-(*p*-tolyl)ethyl)-2,6-difluorobenzamide. The bioassay results showed that the target compounds with an oxime ether substituent at the para position of 4-phenyl exhibited excellent acaricidal activity against *Tetranychus cinnabarinus* in the laboratory. Moreover, all of the target compounds had much higher activities than etoxazole, as the ovicidal and larvicidal activities of the target compounds **I-a**−**I-l** and **II-a**−**II-n** against *T. cinnabarinus* were all over 90% at 0.001 mg L^{-1}, but etoxazole gave only 30% and 40% respectively at the same concentration. The activity order of compounds with regard to acaricidal activity in vivo was almost consistent with their affinity activity with sulfonylurea receptor (SUR) of *Blattella germanica* in vitro, hence, it was supposed that the acaricidal mechanism of action of the target compounds was that they can bind with the site of SUR and therefore inhibit chitin synthesis. Moreover, the eminent effect of the compound **II-l**, [2-(trifluoromethyl)benzaldehyde *O*-(4-(2-(2,6-difluorophenyl)-4,5-dihydrooxazol-4-yl)benzyl) oxime], against *Panonychus citri* and *T. cinnabarinus* in the field indicated that **II-l** exhibited a promising application prospect as a new candicate for controlling spider mites in the field.

KEYWORDS: *2,4-diphenyl-1,3-oxazoline, oxime ether, acaricidal activity, mechanism, sulfonylurea receptor*

INTRODUCTION

With the improvement of living standards and the enhancement of people's awareness of environment protection, the direction of plant protection today is obliged to turn to the rational control of harmful organisms instead of simply "kill (-cide)". The process of chitin synthesis exists only in insect cuticle and fungal cell wall but not in higher plants and mammals, so insect chitin synthesis inhibitors (CSIs),[1] which especially emphasize controlling the pest populations, exhibit high potential on target pests and low toxicity to nontarget organisms, and are therefore safe to humans, livestocks, insects' natural enemies, and the ecological environment.[2] Benzoylphenylureas (Figure 1) are such successful CSIs; in the past 40 years, over twenty benzoylphenylureas were commercialized.[3] 3,4-Diphenyl-1,3-oxazoline derivatives are another class of novel CSIs, of which etoxazole[4,5] is the only commercial ovicide/larvicide with higher efficiency and faster ovicidal/larvicidal action against phytophagous mites than the conventional benzoylphenylureas.

It was reported that similar poisoning symptoms and changes in the chitin content were observed when the larvae of *Spodoptera frugiperda* were exposed to benzoylphenylurea triflumuron and etoxazole, and both compounds could inhibit the incorporation of [^{14}C]GlcNAc into the pieces of isolated integuments in vitro.[6] Likewise, in 2012, Leeuwen and coworkers reported that etoxazole had high bioactivity against the eggs and the nymphs of the two-spotted spider mite, *Tetranychus urticae*, and the offspring of the treated female adults failed to hatch,[7] and the observed symptoms were almost equal to those in *Drosophila melanogaster* that was treated with the benzoylphenylurea diflubenzuron. Furthermore, Leeuwen et al. demonstrated that etoxazole inhibited chitin biosynthesis in *T. urticae* utilizing the technology of calcofluor white (CFW) staining. Therefore, it was strongly suggested that the acaricidal mechanism of action of etoxazole was similar, if not identical, to that of the known benzoylphenylureas.

The structure−activity relationship studies (SARs) also showed that 2,4-diphenyl-1,3-oxazolines and benzoylphenylureas had an intimate relationship. 2,4-Diphenyl-1,3-oxazoline derivatives containing 2,6-difluorophenyl group at the 2-phenyl moiety (part A) always exhibited better acaricidal activity against *T. urticae*, as did the benzoylphenylureas.[8] With respect to the 4-phenyl moiety (part C) of 2,4-diphenyl-1,3-oxazolines, the quantitative structure−activity relationship studies (QSARs) showed that the substituent modification at the ortho and meta positions of the 4-phenyl moiety was unfavorable for ovicidal activity. Moreover, the effects of groups at the para position of the 4-phenyl moiety were also similar to the para-substituent effects at the anilide moiety (part C) of benzoylphenylurea.[9−11]

Based on the similarity in the mechanism of action and QSAR for the 2,4-diphenyl-1,3-oxazolines and benzoylphenylureas,[12,13] a series of compounds **I** containing an oxime ether

Received: January 27, 2014
Revised: March 12, 2014
Accepted: March 13, 2014
Published: March 27, 2014

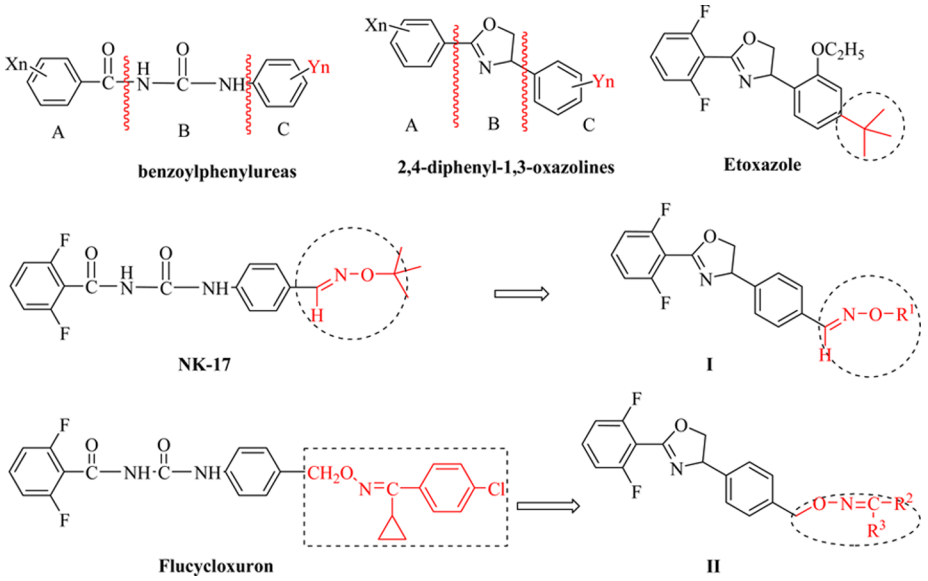
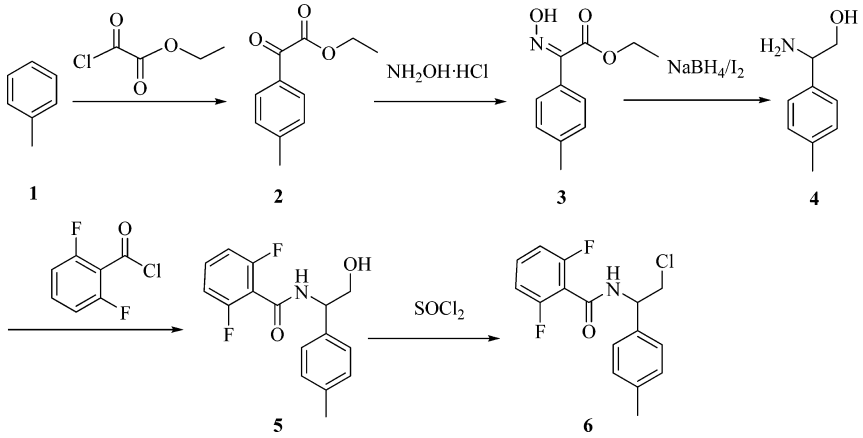


Figure 1. Design of the target compounds **I** and **II**.

Scheme 1. Synthetic Route of 6



group at the 4-phenyl moiety of 2,4-diphenyl-1,3-oxazoline were designed by referring to benzoylphenylurea NK-17, which was discovered by our research group and showed excellent larvicidal activity.[3] Similarly, another series of compounds **II** were designed by referring to the commercial benzoylphenylurea flucycloxuron which was created by Solvay-Duphar B.V. as a CSI and also contained an oxime ether group.[14] The acaricidal activity of all the target compounds (**I-a**−**I-l** and **II-a**−**II-n**) against *T. cinnabarinus* was assayed in the laboratory. Furthermore, the effects of the **II-l** on *T. cinnabarinus* and *Panonychus citri* (*P. citri*) were evaluated in the field.

In 2004, diflubenzuron, a CSI belonging to benzoyphenylurea, was testified to act on the target site of sulfonylurea receptor (SUR) protein by means of the isotope labeling technology,[15] and later the mechanism of NK-17 was also proposed to bind to the same site of SUR by fluorescence polarization (FP) method in our previous report.[16] Since numerous experimental data had indicated that the action mechanism of etoxazole was similar to that of the benzoylphenylureas, we would like to know if etoxazole and the target compounds (**I-a**−**I-l** and **II-a**−**II-n**) that we synthesized in this paper can also bind to the same site of SUR. To raise awareness to the mode of action of the target compounds and help to discover some superior compounds, in this paper, we preliminarily determined the affinity activity of all the target compounds binding to the SUR in vitro by FP method, and the results were discussed.

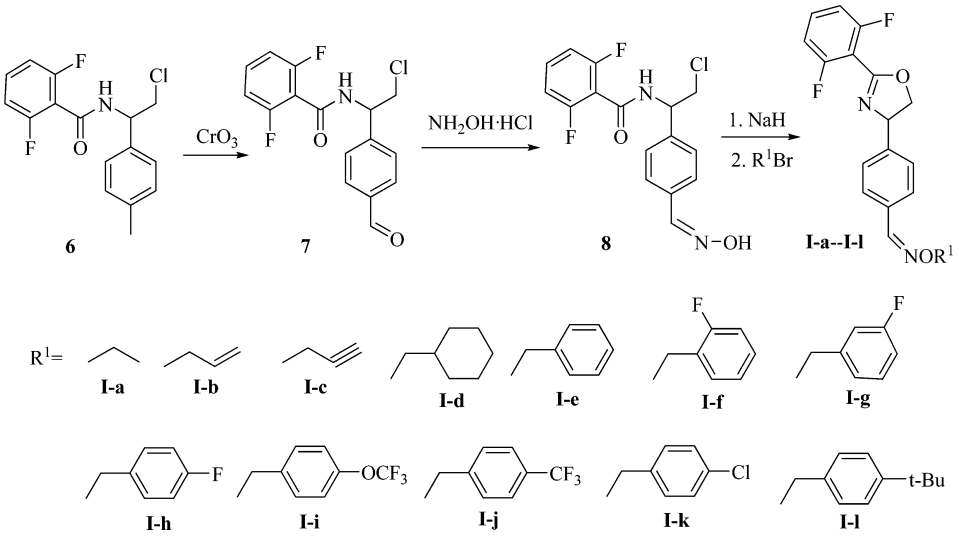
MATERIALS AND METHODS

Instruments and Chemicals. ^{1}H NMR spectra were recorded at 300 or 400 MHz by respectively using a Bruker AV300 spectrometer or a Bruker AV400 spectrometer with $CDCl_3$ or DMSO-d_6 as solvent and tetramethylsilane as the internal standard. Chemical shift values (δ) are reported in parts per million (ppm). High-resolution mass spectrometry (HRMS) was obtained by means of FTICR-MS (Ionspec 7.0T). The melting points were obtained utilizing an X-4 binocular microscope melting point apparatus (Beijing Tech Instruments Co., Beijing, China) and are uncorrected. Yields were not optimized. The values of the FP were obtained by CARY eclipse fluorescence spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). All of the reaction reagents were analytically or chemically pure, which were dried and distilled according to standard methods before use when necessary. All other biochemical reagents used in the FP determination were of the highest purity grade from Sigma-Aldrich Chemical Co., Ltd.

General Synthetic Procedure for Compound 6 (Scheme 1). *Synthesis of Ethyl 2-p-Tolyl-2-oxoacetate (2).* To a solution of ethyl chloroglyoxylate (4.5 g, 33 mmol) in anhydrous dichloromethane (50 mL) was added anhydrous $AlCl_3$ (4.40 g, 33 mmol), and the mixture

dx.doi.org/10.1021/jf500461a | J. Agric. Food Chem. 2014, 62, 3064−3072

Scheme 2. General Synthetic Route of the Target Compounds I-a−I-l



was stirred at room temperature until $AlCl_3$ was dissolved. The mixture was cooled to −10 °C, toluene (2.76 g, 30 mmol) in dichloromethane (50 mL) was added dropwise over a period of 2 h, and then the reaction mixture was stirred at room temperature for another 5 h until the reaction was complete, indicated by TLC. The mixture was poured to ice−water (80 mL), and the value of the pH was adjusted to 2. After the organic phase was separated, the aqueous phase was extracted with dichloromethane. The combined organic phase was washed with saturated brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo to give **2** as a yellow liquid (5.08 g, yield 88%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.91 (d, J = 8.0 Hz, 2H), 7.31 (d, J = 8.0 Hz, 2H), 4.45 (q, J = 7.2 Hz, 2H), 2.45 (s, 3H), 1.42 (t, J = 7.2 Hz, 3H).

*Synthesis of Ethyl 2-(Hydroxyimino)-2-(p-tolyl)acetate (**3**).* To a solution of **2** (86.21 g, 450 mmol) in ethanol (400 mL) was added hydroxylamine hydrochloride (52.98 g, 760 mmol), and the mixture was refluxed for 8 h and monitored by TLC. After removal of ethanol, the solid residue obtained was redissolved in ethyl acetate. The mixture was washed twice with water, dried with anhydrous sodium sulfate, filtered, and concentrated in vacuo to give a crude product, which was purified by recrystallization using carbon tetrachloride and petroleum ether to give **3** (45.80 g). The mother liquid was purified by flash chromatography on silica gel with petroleum ether and ethyl acetate (v/v = 20:1, 10:1) to give another portion of **3** (21.5 g). Overall yield: 72%. Mp: 116−117 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.65 (s, 1H), 7.42 (d, J = 8.1 Hz, 2H), 7.25 (d, J = 8.1 Hz, 2H), 4.35 (q, J = 7.2 Hz, 2H), 2.39 (s, 3H), 1.36 (t, J = 7.2 Hz, 3H).

*Synthesis of 2-Amino-2-(p-tolyl)ethanol (**4**).* To a solution of compound **3** (25.25 g, 120 mmol) in anhydrous tetrahydrofuran (200 mL) under stirring was added sodium borohydride (13.83 g, 370 mmol). Iodine (46.37 g, 180 mmol) in THF (180 mL) was added dropwise over a period of 6.5 h at 0 °C. Afterward, the reaction mixture was refluxed for 5 h. The excess reducing agent was quenched with methanol at 0 °C, and then the solvent was removed. After the residue was added to a solution of 5% sodium hydroxide in water, the mixture was refluxed for 5 h. When it was complete, indicated by TLC, the reaction mixture was cooled to room temperature and extracted by dichloromethane. Then, the organic layer was dried by sodium sulfate, filtered, and concentrated to give **4** as a yellow solid (17.45 g, yield 94%). Mp: 68−70 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.22 (d, J = 8.0 Hz, 2H), 7.16 (d, J = 8.0 Hz, 2H), 4.01 (dd, J = 8.4, 4.4 Hz, 1H), 3.72 (dd, J = 10.8, 4.4 Hz, 1H), 3.53 (dd, J = 10.8, 8.4 Hz, 1H), 2.34 (s, 3H).

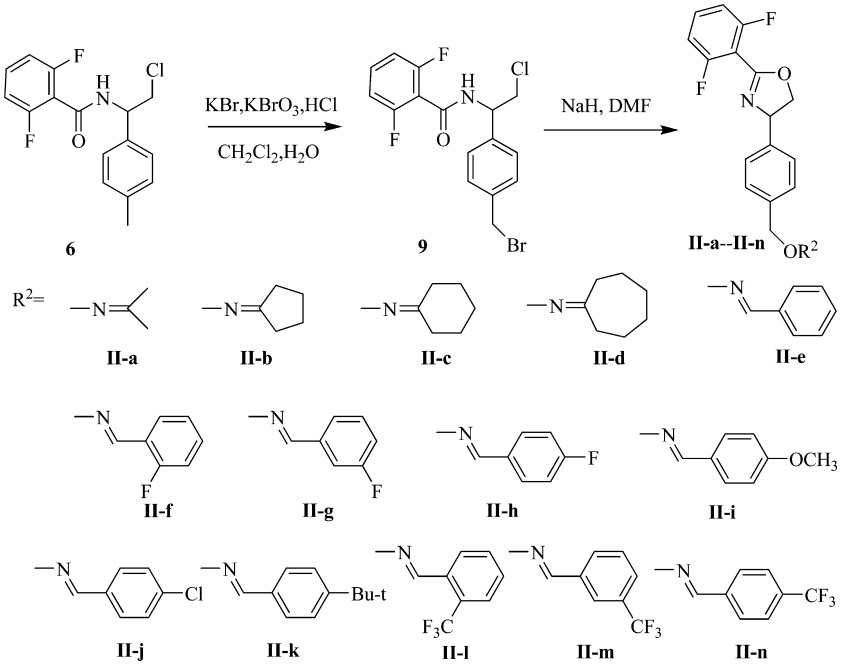
*Synthesis of 2,6-Difluoro-N-(2-hydroxy-1-(p-tolyl)ethyl)-benzamide (**5**).* To a mixture of **4** (5.0 g, 33 mmol) and triethylamine (5.51 g, 40 mmol) in anhydrous tetrahydrofuran (70 mL) at 0 °C was added dropwise a solution of 2,6-difluorobenzoyl chloride (6.14 g, 35 mmol) in tetrahydrofuran (60 mL) over a period of 2.5 h, and then the reaction mixture was warmed to room temperature for 5 h until the reaction was complete. The mixture was filtered, concentrated, and purified by recrystallization using ethyl acetate and petroleum ether to give **5** as a solid (4.57 g). The mother liquid was concentrated and purified by flash chromatography on silica gel with petroleum ether and ethyl acetate (v/v = 2:1) to give another portion of **5** (2.89 g). Overall yield: 77%. Mp: 125−127 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.43−7.35 (m, 1H), 7.28 (d, J = 8.0 Hz, 2H), 7.20 (d, J = 8.0 Hz, 2H), 6.97 (t, J = 8.0 Hz, 2H), 6.55 (d, J = 6.8 Hz, 1H), 5.55 (dd, J = 12.8, 5.2 Hz, 1H), 4.02−3.91 (m, 2H), 2.35 (s, 3H).

*Synthesis of N-(2-Chloro-1-(p-tolyl)ethyl)-2,6-difluorobenzamide (**6**).* To a solution of **5** (41.7 g, 140 mmol) in chloroform (120 mL) was added dropwise thionyl chloride (19.59 g, 165 mmol) in chloroform (60 mL) over a period of 70 min. The reaction mixture was refluxed for 100 min until the reaction was complete. After the value of the pH of the mixture was adjusted to 8 with saturated sodium bicarbonate solution, the organic phase was separated, washed with saturated brine, dried with magnesium sulfate, filtered and concentrated to give **6** as a white solid (41.7 g, yield 94%). Mp: 62−63 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.44−7.37 (m, 1H), 7.29 (d, J = 8.1 Hz, 2H), 7.21 (d, J = 8.1 Hz, 2H), 6.97 (t, J = 8.1 Hz, 2H), 6.53 (d, J = 6.0 Hz, 1H), 5.56 (dd, J = 12.9, 5.1 Hz, 1H), 4.03−3.91 (m, 2H), 2.36 (s, 3H).

General Synthetic Procedure for the Target Compounds I-a−I-l (Scheme 2). *Synthesis of N-(2-Chloro-1-(4-formylphenyl)-ethyl)-2,6-difluorobenzamide (**7**).* To a solution of **6** (10.0 g, 32 mmol) in glacial acetic acid (60 mL) was added dropwise concentrated sulfuric acid (7 mL) over a period of 1 h at the temperature range of −10 °C to −5 °C. Then powdered CrO_3 was added, after which the reaction mixture was stirred for 6 h. The reaction mixture was poured to ice−water (300 mL) and extracted by ethyl acetate (100 mL × 5). The combined organic phase was successively washed with water, saturated sodium bicarbonate, and saturated brine and dried with anhydrous magnesium sulfate. After filtration, the solvent was removed under reduced pressure to give a crude black residue. The residue was added to a mixture of methanol (30 mL) and concentrated hydrochloric acid (10 mL), and then the reaction was stirred under tepidity for 5 h. The reaction mixture was cooled to room temperature, concentrated under reduced pressure, and then extracted by ethyl acetate (50 mL × 3). The organic layer was washed with saturated sodium bicarbonate solution and saturated brine successively, dried with anhydrous magnesium sulfate, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel with petroleum ether and ethyl acetate (v/v = 3:1) to give **7** as a yellow oil (4.1 g, yield 41%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.03 (s, 1H), 7.93 (d, J = 8.4 Hz, 2H), 7.59 (d, J = 8.4 Hz, 2H), 7.47−

dx.doi.org/10.1021/jf500461a | J. Agric. Food Chem. 2014, 62, 3064−3072

Scheme 3. General Synthetic Route of the Target Compounds II-a−II-n



7.40 (m, 1H), 7.00 (t, J = 8.4 Hz, 2H), 6.73 (d, J = 7.6 Hz, 1H), 5.71−5.67 (m, 1H), 4.05 (dd, J = 11.6, 4.4 Hz, 1H), 3.97 (dd, J = 11.6, 4.4 Hz, 1H).

*Synthesis of N-(2-Chloro-1-(4-((hydroxyimino)methyl)phenyl)-ethyl)-2,6-difluorobenzamide (**8**).* To a mixture of **7** (2.43 g, 8 mmol), triethylamine (1.90 g, 19 mmol), and hydroxylamine hydrochloride (1.30 g, 19 mmol) in anhydrous tetrahydrofuran (50 mL) were added molecular sieves (4 Å), and then the mixture was refluxed for 4 h and monitored with TLC. After the reaction was complete, the mixture was filtered to remove molecular sieves, concentrated in vacuo, and purified by flash chromatography on silica gel with petroleum ether and ethyl acetate (v/v = 3:1) to give **8** as a white solid (1.93 g, yield 76%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.26 (s, 1H), 9.47 (d, J = 8.4 Hz, 1H), 8.14 (s, 1H), 7.61 (d, J = 8.4 Hz, 2H), 7.61−7.57 (m, 1H), 7.49 (d, J = 8.4 Hz, 2H), 7.22−7.13 (m, 2H), 5.34−5.29 (m, 1H), 3.95 (dd, J = 11.2, 4.8 Hz, 1H), 3.80 (dd, J = 11.2, 9.6 Hz, 1H).

*Synthesis of 4-(2-(2,6-Difluorophenyl)-4,5-dihydrooxazol-4-yl)-benzaldehyde O-Ethyl Oxime (**I-a**).* To a mixture of **8** (0.82 g, 2.4 mmol) in *N*,*N*-dimethylformamide (DMF) (30 mL) stirred for 10 min at 0 °C was added sodium hydride (0.23 g, 10 mmol), and the reaction mixture was stirred for 1 h. After ethyl iodide (0.57 g, 4 mmol) was added, the reaction mixture was stirred for another 2 h. When the reaction was complete, as indicated by TLC, ethyl acetate (150 mL) was added. The mixture was washed with a large amount of water three times and saturated brine once, dried with anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel with petroleum ether and ethyl acetate (v/v = 12:1) to give the target compound **I-a** as an oil (0.51 g, yield 64%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.07 (s, 1H), 7.60 (d, J = 8.4 Hz, 2H), 7.48−7.40 (m, 1H), 7.35 (d, J = 8.4 Hz, 2H), 7.00 (t, J = 8.0 Hz, 2H), 5.48 (dd, J = 10.4, 8.4 Hz, 1H), 4.83 (dd, J = 10.4, 8.4 Hz, 1H), 4.28 (t, J = 8.4 Hz, 1H), 4.23 (q, J = 7.2 Hz, 2H), 1.33 (t, J = 7.2 Hz, 3H). HRMS (ESI): calcd for $C_{18}H_{16}F_2N_2O_2Na$ $[M + Na]^+$ 353.1072, found 353.1073.

Compounds **I-b**−**I-l** were synthesized according to a method similar to that for **I-a** using the corresponding alkyl bromide instead of ethyl iodide.

*Data for 4-(2-(2,6-Difluorophenyl)-4,5-dihydrooxazol-4-yl)-benzaldehyde O-Allyl Oxime (**I-b**).* Oil; yield 58%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.12 (s, 1H), 7.60 (d, J = 8.0 Hz, 2H), 7.48−7.41 (m, 1H), 7.35 (d, J = 8.0 Hz, 2H), 7.01 (t, J = 8.0 Hz, 2H), 6.10−6.00 (m, 1H), 5.48 (dd, J = 10.4, 8.4 Hz, 1H), 5.36 (dd, J = 17.2, 1.6 Hz, 1H), 5.26 (dd, J = 10.4, 1.2 Hz, 1H), 4.83 (dd, J = 10.4, 8.4 Hz, 1H), 4.68 (d, J = 5.6 Hz, 2H), 4.28 (t, J = 8.0 Hz, 1H). HRMS (ESI): calcd for $C_{19}H_{16}F_2N_2O_2Na$ $[M + Na]^+$ 365.1072, found 365.1075.

*Data for 4-(2-(2,6-Difluorophenyl)-4,5-dihydrooxazol-4-yl)-benzaldehyde O-Prop-2-yn-1-yl Oxime (**I-c**).* Oil; yield 47%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.13 (s, 1H), 7.62 (d, J = 8.1 Hz, 2H), 7.49−7.39 (m, 1H), 7.36 (d, J = 8.1 Hz, 2H), 7.01 (t, J = 8.1 Hz, 2H), 5.49 (dd, J = 10.2, 8.2 Hz, 1H), 4.83 (dd, J = 10.2, 8.2 Hz, 1H), 4.78 (d, J = 2.1 Hz, 2H), 4.27 (t, J = 8.1 Hz, 1H), 2.51 (t, J = 2.4 Hz, 1H). HRMS (ESI): calcd for $C_{19}H_{14}F_2N_2O_2Na$ $[M + Na]^+$ 363.0916, found 363.0912.

*Data for 4-(2-(2,6-Difluorophenyl)-4,5-dihydrooxazol-4-yl)-benzaldehyde O-Cyclohexylmethyl Oxime (**I-d**).* Oil; yield 44%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.07 (s, 1H), 7.59 (d, J = 8.0 Hz, 2H), 7.47−7.42 (m, 1H), 7.34 (d, J = 8.0 Hz, 2H), 7.00 (t, J = 8.0 Hz, 2H), 5.48 (dd, J = 10.0, 8.8 Hz, 1H), 4.82 (dd, J = 10.0, 8.8 Hz, 1H), 4.27 (t, J = 8.0 Hz, 1H), 3.98 (d, J = 6.4 Hz, 2H), 1.82−1.72 (m, 5H), 1.31−1.21 (m, 6H). HRMS (ESI): calcd for $C_{23}H_{24}F_2N_2O_2Na$ $[M + Na]^+$ 421.1698, found 421.1693.

*Data for 4-(2-(2,6-Difluorophenyl)-4,5-dihydrooxazol-4-yl)-benzaldehyde O-Benzyl Oxime (**I-e**).* Yellow oil; yield 50%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.14 (s, 1H), 7.60 (d, J = 8.0 Hz, 2H), 7.47−7.31 (m, 8H), 7.01 (t, J = 8.0 Hz, 2H), 5.48 (dd, J = 12.0, 8.0 Hz, 1H), 5.21 (s, 2H), 4.83 (dd, J = 12.0, 8.0 Hz, 1H), 4.27 (t, J = 8.0 Hz, 1H). HRMS (ESI): calcd for $C_{23}H_{19}F_2N_2O_2$ $[M + H]^+$ 393.1409, found 393.1402.

*Data for 4-(2-(2,6-Difluorophenyl)-4,5-dihydrooxazol-4-yl)-benzaldehyde O-(2-Fluorobenzyl) Oxime (**I-f**).* Oil; yield 63%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.13 (s, 1H), 7.60 (d, J = 8.0 Hz, 2H), 7.49−7.41 (m, 2H), 7.34 (d, J = 8.0 Hz, 2H), 7.32−7.28 (m, 1H), 7.16−7.12 (m, 1H), 7.10−7.05 (m, 1H), 7.01 (t, J = 8.0 Hz, 2H), 5.48 (dd, J = 10.4, 8.0 Hz, 1H), 5.28 (s, 2H), 4.83 (dd, J = 10.4, 8.0 Hz, 1H), 4.27 (t, J = 8.0 Hz, 1H). HRMS (ESI): calcd for $C_{23}H_{17}F_3N_2O_2Na$ $[M + Na]^+$ 433.1134, found 433.1129.

*Data for 4-(2-(2,6-Difluorophenyl)-4,5-dihydrooxazol-4-yl)-benzaldehyde O-(3-Fluorobenzyl) Oxime (**I-g**).* Oil; yield 53%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.15 (s, 1H), 7.60 (d, J = 8.0 Hz, 2H), 7.48−7.40 (m, 1H), 7.35 (d, J = 8.0 Hz, 2H), 7.32−7.30 (m, 1H), 7.19−7.11 (m, 1H), 7.03−6.98 (m, 3H), 5.48 (dd, J = 10.4, 8.4 Hz, 1H), 5.19 (s, 2H), 4.83 (dd, J = 10.4, 8.4 Hz, 1H), 4.27 (t, J = 8.4 Hz,

dx.doi.org/10.1021/jf500461a | J. Agric. Food Chem. 2014, 62, 3064−3072

1H). HRMS (ESI): calcd for $C_{23}H_{17}F_3N_2O_2Na$ $[M + Na]^+$ 433.1134, found 433.1130.

*Data for 4-(2-(2,6-Difluorophenyl)-4,5-dihydrooxazol-4-yl)-benzaldehyde O-(4-Fluorobenzyl) Oxime (**I-h**).* White solid; mp 97−100 °C; yield 69%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.12 (s, 1H), 7.59 (d, J = 8.0 Hz, 2H), 7.48−7.38 (m, 3H), 7.34 (d, J = 8.0 Hz, 2H), 7.07−6.99 (m, 4H), 5.48 (dd, J = 10.4, 8.0 Hz, 1H), 5.16 (s, 2H), 4.83 (dd, J = 10.4, 8.0 Hz, 1H), 4.27 (t, J = 8.0 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.8, 162.5, 162.5, 161.4, 160.0, 159.9, 157.8, 148.7, 143.6, 133.3, 133.3, 132.6, 132.5, 132.4, 131.6, 130.3, 130.3, 127.6, 127.0, 115.4, 115.2, 112.1, 112.1, 112.1, 111.9, 111.9, 75.7, 74.7, 70.0. HRMS (ESI): calcd for $C_{23}H_{17}F_3N_2O_2Na$ $[M + Na]^+$ 433.1134, found 433.1130.

*Data for 4-(2-(2,6-Difluorophenyl)-4,5-dihydrooxazol-4-yl)-benzaldehyde O-(4-(Trifluoromethoxy)benzyl) Oxime (**I-i**).* White solid; mp 102−103 °C; yield 65%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.13 (s, 1H), 7.59 (d, J = 8.4 Hz, 2H), 7.47−7.42 (m, 3H), 7.35 (d, J = 8.0 Hz, 2H), 7.21 (d, J = 8.0 Hz, 2H), 7.00 (t, J = 8.0 Hz, 2H), 5.48 (dd, J = 10.4, 8.0 Hz, 1H), 5.20 (s, 2H), 4.82 (dd, J = 10.4, 8.0 Hz, 1H), 4.27 (t, J = 8.0 Hz, 1H). HRMS (ESI): calcd for $C_{24}H_{17}F_5N_2O_3Na$ $[M + Na]^+$ 499.1052, found 499.1050.

*Data for 4-(2-(2,6-difluorophenyl)-4,5-dihydrooxazol-4-yl)-benzaldehyde O-(4-(Trifluoromethyl)benzyl) Oxime (**I-j**).* White solid; mp 91−93 °C; yield 56%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.15 (s, 1H), 7.62 (d, J = 8.0 Hz, 2H), 7.59 (d, J = 8.0 Hz, 2H), 7.52 (d, J = 8.0 Hz, 2H), 7.47−7.40 (m, 1H), 7.35 (d, J = 8.0 Hz, 2H), 7.00 (t, J = 8.0 Hz, 2H), 5.48 (dd, J = 10.4, 8.0 Hz, 1H), 5.25 (s, 2H), 4.82 (dd, J = 10.4, 8.0 Hz, 1H), 4.27 (t, J = 8.0 Hz, 1H). HRMS (ESI): calcd for $C_{24}H_{17}F_5N_2O_2Na$ $[M + Na]^+$ 483.1102; 483.1104.

*Data for 4-(2-(2,6-Difluorophenyl)-4,5-dihydrooxazol-4-yl)-benzaldehyde O-(4-Chlorobenzyl) Oxime (**I-k**).* Yellow solid; mp 77−80 °C; yield 83%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.33 (s, 1H), 7.72−7.62 (m, 3H), 7.44 (s, 4H), 7.37 (d, J = 8.0 Hz, 2H), 7.30 (t, J = 8.0 Hz, 2H), 5.52 (t, J = 9.2 Hz, 1H), 5.17 (s, 2H), 4.86 (t, J = 9.2 Hz, 1H), 4.20 (t, J = 8.4 Hz, 1H), HRMS (ESI): calcd for $C_{23}H_{18}ClF_2N_2O_2$ $[M + H]^+$ 427.1019, found 427.1020.

*Data for 4-(2-(2,6-difluorophenyl)-4,5-dihydrooxazol-4-yl)-benzaldehyde O-(4-tert-Butylbenzyl) Oxime (**I-l**).* Yellow solid; mp 70−73 °C; yield 75%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.13 (s, 1H), 7.60 (d, J = 8.1 Hz, 2H), 7.49−7.33 (m, 7H), 7.01 (t, J = 8.1 Hz, 2H), 5.48 (dd, J = 10.2, 8.4 Hz, 1H), 5.18 (s, 2H), 4.83 (dd, J = 10.2, 8.7 Hz, 1H), 4.27 (t, J = 8.4 Hz, 1H), 1.32 (s, 9H). HRMS (ESI): calcd for $C_{27}H_{26}F_2N_2O_2Na$ $[M + Na]^+$ 471.1855, found 471.1858.

General Synthetic Procedure for the Target Compounds II-a−II-n (Scheme 3). *Synthesis of N-(1-(4-(Bromomethyl)phenyl)-2-chloroethyl)-2,6-difluorobenzamide (**9**).*[17,18] To a solution of **6** (5.0 g, 16.1 mmol) in dichloromethane (40 mL) were successively added water (40 mL), potassium bromide (1.9 g, 16.1 mmol), and potassium bromate (1.33 g, 8.05 mmol), and then a solution of concentrated hydrochloric acid (6.1 mL) in water (14 mL) was added dropwise at 0 °C. The reaction mixture was stirred for 25 h until reaction was complete. The organic phase was separated and washed with saturated sodium carbonate. The water phase was extracted with dichloromethane. Then the combined organic phase was washed with brine, dried over anhydrous magnesium sulfate, and concentrated to give a white solid, which was purified by recrystallization using toluene and petroleum ether to give **9** as crystals (3.5 g, yield 56%). Mp: 127−128 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.45−7.36 (m, 5H), 6.97 (t, J = 8.0 Hz, 2H), 6.62 (d, J = 7.6 Hz, 1H), 5.61−5.57 (m, 1H), 4.49 (s, 2H), 4.04−3.91 (m, 2H).

*Synthesis of Cyclopentanone O-(4-(2-(2,6-Difluorophenyl)-4,5-dihydrooxazol-4-yl)benzyl) Oxime (**II-b**).* To a mixture of cyclopentanone oxime (0.51 g, 5.15 mmol) in DMF (50 mL) was added sodium hydride (0.49 g, 20.59 mmol). The mixture was stirred for 40 min, and then **9** (1.00 g, 2.57 mmol) was added. The reaction mixture was stirred at 0 °C until TLC showed that the reaction was finished. The reaction mixture was extracted with ethyl acetate, and the organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated to give a reddish brown oil. The crude was purified by flash chromatography on silica gel with petroleum ether and ethyl acetate (v/v = 10:1) to give the target **II-b** as a yellow oil (0.65 g, yield 69%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.49−7.31 (m, 1H), 7.38 (d, J = 8.0 Hz, 2H), 7.32 (d, J = 8.0 Hz, 2H), 7.00 (t, J = 8.0 Hz, 2H), 5.47 (dd, J = 10.0, 8.4 Hz, 1H), 5.07 (s, 2H), 4.81 (dd, J = 10.0, 8.4 Hz, 1H), 4.30 (t, J = 8.4 Hz, 1H), 2.44 (t, J = 6.8 Hz, 2H), 2.36 (t, J = 6.8 Hz, 2H), 1.77−1.71 (m, 4H). HRMS (ESI): calcd for $C_{21}H_{21}F_2N_2O_2$ $[M + H]^+$ 371.1566, found 371.1570.

Compounds **II-a** and **II-c−II-n** were synthesized according to a method similar to that for **II-b**.

*Data for Propan-2-one O-(4-(2-(2,6-Difluorophenyl)-4,5-dihydrooxazol-4-yl)benzyl) Oxime (**II-a**).* Yellow oil; yield 52%. ^{1}H NMR (400 MHz, $CDCl_3$): 7.41−7.46 (m, 1H), 7.37 (d, J = 7.6 Hz, 2H), 7.32 (d, J = 7.6 Hz, 2H), 7.00 (t, J = 8.0 Hz, 2H), 5.49−5.44 (m, 1H), 5.06 (s, 2H), 4.83−4.79 (m, 1H), 4.32−4.28 (m, 1H), 1.88 (s, 3H), 1.87 (s, 3H). HRMS (ESI): calcd for $C_{19}H_{19}F_2N_2O_2$ $[M + H]^+$ 367.1229, found 367.1224.

*Data for Cyclohexanone O-(4-(2-(2,6-Difluorophenyl)-4,5-dihydrooxazol-4-yl)benzyl) Oxime (**II-c**).* Yellow oil; yield 40%. ^{1}H NMR (400 MHz, $CDCl_3$): 7.46−7.41(m, 1H), 7.36 (d, J = 8.0 Hz, 2H), 7.32 (d, J = 8.0 Hz, 2H), 7.00 (t, J = 8.4 Hz, 2H), 5.52−5.42 (m, 1H), 5.05 (s, 2H), 4.85−4.78 (m, 1H), 4.32−4.28 (m, 1H), 2.50 (t, J = 6.2 Hz, 2H), 2.24−2.15 (m, 2H), 1.64−1.66 (m, 4H), 1.59−1.61(m, 2H). HRMS (ESI): calcd for $C_{22}H_{23}F_2N_2O_2$ $[M + H]^+$ 385.1722, found 385.1722.

*Data for Cycloheptanone O-(4-(2-(2,6-Difluorophenyl)-4,5-dihydrooxazol-4-yl)benzyl) Oxime (**II-d**).* Yellow oil; yield 46%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.46−7.41 (m, 1H), 7.37 (d, J = 8.0 Hz, 2H), 7.32 (d, J = 8.0 Hz, 2H), 7.00 (t, J = 8.0 Hz, 2H), 5.47 (dd, J = 10.4, 8.0 Hz, 1H), 5.06 (s, 2H), 4.81 (dd, J = 10.4, 8.4 Hz, 1H), 4.30 (t, J = 8.4 Hz, 1H), 2.59−2.54 (m, 2H), 2.38−2.34 (m, 2H), 1.73−1.56 (m, 8H). HRMS (ESI): calcd for $C_{23}H_{25}F_2N_2O_2$ $[M + H]^+$ 399.1879, found 399.1875.

*Data for Benzaldehyde O-(4-(2-(2,6-Difluorophenyl)-4,5-dihydrooxazol-4-yl)benzyl) Oxime (**II-e**).* Yellow oil; yield 68%. ^{1}H NMR (400 MHz, $CDCl_3$): 8.13 (s, 1H), 7.64−7.54 (m, 2H), 7.45−7.41(m, 3H), 7.41−7.30 (m, 5H), 7.00 (t, J = 8.4 Hz, 2H), 5.53−5.42 (m, 1H), 5.21 (s, 2H), 4.88−4.76 (m, 1H), 4.27−4.31(m, 1H). HRMS (ESI): calcd for $C_{23}H_{19}F_2N_2O_2$ $[M + H]^+$ 393.1409, found 393.1408.

*Data for 2-Fluorobenzaldehyde O-(4-(2-(2,6-Difluorophenyl)-4,5-dihydrooxazol-4-yl)benzyl) Oxime (**II-f**).* Yellow oil; yield 61%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.38 (s, 1H), 7.81 (t, J = 6.6 Hz, 1H), 7.45−7.32 (m, 6H), 7.15−6.96 (m, 4H), 5.48 (dd, J = 10.2, 8.1 Hz, 1H), 5.22 (s, 2H), 4.82 (dd, J = 10.2, 8.4 Hz, 1H), 4.31 (t, J = 8.1 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.1, 162.6, 162.5, 161.7, 160.0, 157.6, 147.93, 147.90, 141.6, 136.8, 134.5, 134.4, 132.5, 132.4, 132.33, 130.28, 130.2, 129.0, 126.8, 123.2, 123.2, 116.9, 116.7, 113.4, 113.2, 112.1, 112.1, 111.8, 76.3, 74.8, 70.1. HRMS (ESI): calcd for $C_{23}H_{18}F_3N_2O_2$ $[M + H]^+$ 411.1315, found 411.1307.

*Data for 3-Fluorobenzaldehyde O-(4-(2-(2,6-Difluorophenyl)-4,5-dihydrooxazol-4-yl)benzyl) Oxime (**II-g**).* Yellow oil; yield 32%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.08 (s, 1H), 7.47−7.26 (m, 8H), 7.09−6.93 (m, 3H), 5.47 (dd, J = 10.2, 8.1 Hz, 1H), 5.20 (s, 2H), 4.80 (dd, J = 10.2, 8.4 Hz, 1H), 4.28 (t, J = 8.1 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.1, 162.6, 162.5, 161.7, 160.0, 157.6, 147.93, 147.90, 141.6, 136.8, 134.5, 134.4, 132.5, 132.4, 132.3, 130.3, 130.2, 129.0, 126.8, 123.25, 123.22, 116.9, 116.7, 113.4, 113.2, 112.1, 112.1, 111.8, 76.3, 74.8, 70.1. HRMS (ESI): calcd for $C_{23}H_{18}F_3N_2O_2$ $[M + H]^+$ 411.1315, found 411.1315.

*Data for 4-Fluorobenzaldehyde O-(4-(2-(2,6-Difluorophenyl)-4,5-dihydrooxazol-4-yl)benzyl) Oxime (**II-h**).* Yellow solid; mp 85−87 °C; yield 33%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.10 (s, 1H), 7.60−7.52 (m, 2H), 7.47−7.39 (m, 3H), 7.35 (d, J = 8.0 Hz, 2H), 7.07−6.98 (m, 4H), 5.48 (dd, J = 10.4, 8.0 Hz, 1H), 5.19 (s, 2H), 4.82 (dd, J = 10.4, 8.4 Hz, 1H), 4.29 (t, J = 8.0 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 147.9, 141.6, 137.0, 132.5, 132.4, 132.3, 128.9, 126.8, 115.9, 115.7, 112.1, 111.8, 76.1, 74.8, 70.1. HRMS (ESI): calcd for $C_{23}H_{18}F_3N_2O_2$ $[M + H]^+$ 411.1315, found 411.1313.

*Data for 4-Methoxybenzaldehyde O-(4-(2-(2,6-Difluorophenyl)-4,5-dihydrooxazol-4-yl)benzyl) Oxime (**II-i**).* Yellow oil; yield 46%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.08 (s, 1H), 7.51 (d, J = 8.8 Hz, 2H), 7.46−7.39 (m, 3H), 7.34 (d, J = 8.0 Hz, 2H), 7.00 (t, J = 8.0 Hz, 2H), 6.88 (d, J = 8.8 Hz, 2H), 5.50− 5.46 (m, 1H), 5.18 (s, 2H), 4.84−4.79

Table 1. Acaricidal Activities of the Target Compounds against *T. cinnabarinus* in the Laboratory

	acaricidal act. (%) at concn (mg L^{-1})											
	eggs of *T. cinnabarinus*						larvae of *T. cinnabarinus*					
compd	10	1	0.1	0.01	0.001	0.0001	10	1	0.1	0.01	0.001	0.0001
I-a	100	100	100	100	100	90	100	100	100	100	100	80
I-b	100	100	100	100	100	88	100	100	100	100	100	95
I-c	100	100	100	100	100	95	100	100	100	100	100	95
I-d	100	100	100	100	100	85	100	100	100	100	100	90
I-e	100	100	100	100	95	57	100	100	100	100	100	75
I-f	100	100	100	100	100	75	100	100	100	100	100	80
I-g	100	100	100	100	100	85	100	100	100	100	100	75
I-h	100	100	100	100	100	75	100	100	100	100	100	85
I-i	100	100	100	100	100	100	100	100	100	100	100	100
I-j	100	100	100	100	100	100	100	100	100	100	100	100
I-k	100	100	100	100	90	60	100	100	100	100	100	70
I-l	100	100	100	100	100	80	100	100	100	100	100	96
II-a	100	100	100	100	100	78	100	100	100	100	100	85
II-b	100	100	100	100	100	0	100	100	100	100	100	90
II-c	100	100	100	100	97	75	100	100	100	100	100	80
II-d	100	100	100	100	100	0	100	100	100	100	100	95
II-e	100	100	100	100	100	90	100	100	100	100	100	96
II-f	100	100	100	100	100	100	100	100	100	100	100	100
II-g	100	100	100	100	100	90	100	100	100	100	100	100
II-h	100	100	100	100	100	85	100	100	100	100	100	100
II-i	100	100	100	100	100	85	100	100	100	100	100	90
II-j	100	100	100	100	100	86	100	100	100	100	100	85
II-k	100	100	100	100	100	75	100	100	100	100	100	100
II-l	100	100	100	100	100	100	100	100	100	100	100	100
II-m	100	100	100	100	100	100	100	100	100	100	100	100
II-n	100	100	100	100	100	95	100	100	100	100	100	100
etoxazole	100	100	78	56	30	0	100	100	80	65	40	0

(m, 1H), 4.31−4.27 (m, 1H), 3.82 (s, 3H). HRMS (ESI): calcd for $C_{24}H_{21}F_2N_2O_3$ $[M + H]^+$ 445.1334, found 445.1335.

*Data for 4-Chlorobenzaldehyde O-(4-(2-(2,6-Difluorophenyl)-4,5-dihydrooxazol-4-yl)benzyl) Oxime (**II-j**).* Yellow solid; mp 78−80 °C; yield 58%. ^{1}H NMR (400 MHz, $CDCl_3$): 8.07 (s, 1H), 7.50 (d, J = 8.0 Hz, 2H), 7.42 (d, J = 7.6 Hz, 3H), 7.38−7.28 (m, 4H), 6.98 (t, J = 9.2 Hz, 2H), 5.49−5.44 (m, 1H), 5.19 (s, 2H), 4.83−4.78 (m, 1H), 4.30−4.26 (m, 1H). HRMS (ESI): calcd for $C_{23}H_{18}ClF_2N_2O_2$ $[M + H]^+$ 427.1019, found 427.1026.

*Data for 4-(tert-Butyl)benzaldehyde O-(4-(2-(2,6-Difluorophenyl)-4,5-dihydrooxazol-4-yl)benzyl) Oxime (**II-k**).* Yellow oil; yield 58%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11 (s, 1H), 7.50 (d, J = 8.0 Hz, 2H), 7.43−7.36 (m, 5H), 7.33 (d, J = 7.6 Hz, 2H), 6.97 (t, J = 8.4 Hz, 2H), 5.45 (t, J = 9.2 Hz, 1H), 5.19 (s, 2H), 4.79 (t, J = 9.2 Hz, 1H), 4.27 (t, J = 8.2 Hz, 1H), 1.31 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.6, 162.5, 160.02, 159.96, 157.6, 153.2, 149.0, 141.5, 137.2, 132.5, 132.4, 132.3, 129.4, 128.9, 126.9, 126.7, 125.7, 112.1, 111.8, 107.5, 107.3, 107.1, 75.9, 74.8, 70.1, 34.8, 31.2. HRMS (ESI): calcd for $C_{27}H_{27}F_2N_2O_2$ $[M + H]^+$ 449.2035, found 449.2032.

*Data for 2-(Trifluoromethyl)benzaldehyde O-(4-(2-(2,6-Difluorophenyl)-4,5-dihydrooxazol-4-yl)benzyl) Oxime (**II-l**).* Yellow oil; yield 70%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.51 (d, J = 2.0 Hz, 1H), 8.02 (d, J = 7.6 Hz, 1H), 7.61 (d, J = 7.6 Hz, 1H), 7.50−7.31 (m, 7H), 6.94 (t, J = 8.4 Hz, 2H), 5.46 (dd, J = 10.0, 8.0 Hz, 1H), 5.23 (s, 2H), 4.78 (dd, J = 10.0, 8.4 Hz, 1H), 4.26 (t, J = 8.4 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.1, 162.6, 162.5, 161.7, 160.0, 157.6, 147.93, 147.90, 141.6, 136.8, 134.5, 134.4, 132.5, 132.4, 132.33, 130.28, 130.2, 129.0, 126.8, 123.25, 123.22, 116.9, 116.7, 113.4, 113.2, 112.1, 112.1, 111.8, 76.3, 74.8, 70.1. HRMS (ESI): calcd for $C_{24}H_{18}F_5N_2O_2$ $[M + H]^+$ 461.1283, found 461.1287.

*Data for 3-(Trifluoromethyl)benzaldehyde O-(4-(2-(2,6-Difluorophenyl)-4,5-dihydrooxazol-4-yl)benzyl) Oxime (**II-m**).* Yellow oil; yield 54%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.15 (s, 1H), 7.84 (s, 1H), 7.74 (d, J = 7.6 Hz, 1H), 7.61 (d, J = 7.6 Hz, 1H), 7.51−7.39 (m, 4H), 7.36 (d, J = 8.0 Hz, 2H), 7.00 (t, J = 8.0 Hz, 2H), 5.48 (dd, J = 10.0, 8.4 Hz, 1H), 5.23 (s, 2H), 4.82 (dd, J = 10.0, 8.4 Hz, 1H), 4.30 (t, J = 8.4 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.1, 162.6, 162.5, 161.7, 160.0, 157.6, 147.93, 147.90, 141.6, 136.8, 134.5, 134.4, 132.5, 132.4, 132.33, 130.28, 130.2, 129.0, 126.8, 123.25, 123.22, 116.9, 116.7, 113.4, 113.2, 112.1, 112.1, 111.8, 76.3, 74.8, 70.1. HRMS (ESI): calcd for $C_{24}H_{18}F_5N_2O_2$ $[M + H]^+$ 461.1283, found 461.1282.

*Data for 4-(Trifluoromethyl)benzaldehyde O-(4-(2-(2,6-difluorophenyl)-4,5-dihydrooxazol-4-yl)benzyl) Oxime (**II-n**).* Yellow oil; yield 53%. ^{1}H NMR (400 MHz, $CDCl_3$): 8.15 (s, 1H), 7.69 (d, J = 8.0 Hz, 2H), 7.61 (d, J = 8.0 Hz, 2H), 7.44−7.42 (m, 3H), 7.36 (d, J = 8.0 Hz, 2H), 7.00 (t, J = 8.0 Hz, 2H), 5.52−5.44 (m, 1H), 5.23 (s, 2H), 4.87−4.78 (m, 1H), 4.27−4.32 (m, 1H). HRMS (ESI): calcd for $C_{24}H_{18}F_5N_2O_2$ $[M + H]^+$ 461.1283, found 461.1285.

Biological Assay. All biological assays were performed on representative test organisms prepared in the laboratory. Each bioassay was replicated three times at 25 ± 1 °C for the statistical requirements. Percentage mortalities were evaluated according to a percentage scale of 0−100, in which 0 indicates no activity and 100 indicates total kill. When the percentage mortality of the blank control was less than 5%, the results of the treats were directly used. However, if the percentage mortality of the blank control was less than 20% and more than 5%, the results were corrected by means of $V = ((X - Y)/X) \times 100$ (V = value of corrected mortality, X = livability of the blank control, Y = livability of the treat). Etoxazole was evaluated using the entirely same procedure as contrast.[19]

Acaricidal Activity against Eggs and Larvae of Spider Mite (*Tetranychus cinnabarinus*). The acaricidal activities of all the target compounds and the contrast etoxazole against eggs and larvae of spider mite (*T. cinnabarinus*) were measured on the basis of previously reported procedure.[20,21]

dx.doi.org/10.1021/jf500461a | J. Agric. Food Chem. 2014, 62, 3064−3072

Table 2. Effect of Acaricidal Program on Spider Mite in the Field in 2013

trial sites	treatments[a]	% effect of II-l 3 days[b]	7 days	14 days	% effect of etoxazole 3 days	7 days	14 days
		P. citri					
Jingzhou, Hubei Province[c]	10	64	81	82			
	15	74	87	86			
	22	74	88	91	55	80	87
Changsha, Hunan Province[d]	10	70	84	88			
	15	77	88	91			
	22	82	91	94	81	90	93
Guilin, Guangxi Autonomous Region[e]	10	79	95[f]	97[g]			
	15	79	95[f]	98[g]			
	22	83	98[f]	99[g]	85	99[f]	97[g]
		T. cinnabarinus					
Tianjin City[h]	10	85	91	89			
	15	88	92	91			
	22	90	93	92	91	94	92

[a]Treatments: the unit of concentration is mg kg^{-1}. [b]The number of days between spraying insecticides and investigating the effects. [c]The amount sprayed is about 1000 g ha^{-1}. [d]The amount sprayed is about 2000 g ha^{-1}. [e]The amount sprayed is about 900 g ha^{-1}. [f]10 days. [g]15 days. [h]The amount sprayed is about 750 g ha^{-1}.

Affinity of the Target Compounds and Etoxazole Binding to SUR. *Preparation of SUR.* According to our previous reported method, the buffer of SUR was prepared from German cockroach (*Blattella germanica*, *B. germanica*) through the process of chopping, homogenization, centrifugation, etc.[15]

Assay of Binding to SUR. The affinity of the target compounds and etoxazole to SUR was tested using a method similar to our previously published method. After the superior concentration of *N*-phenyl-1-naphthylamine (1-NPN) that acted as fluorescence probe was determinated, ligands such as etoxazole and the target compounds in different concentrations were respectively added to a mixture solution containing SUR, buffer, and 1-NPN. After the mixture was incubated for 1 h at room temperature, the binding affinity was tested by FP.[16]

Evaluation of Acaricidal Activity in the Field. The field trials were carried out between April and June in 2013. The trial against *T. cinnabarinus* in tomato field was performed in the trial area of Institute of Plant Protection, Tianjin Academy of Agricultural Science, Tianjin City, China. Moreover, the trials against *Panonychus citri* (*P. citri*) in orange orchard were performed in the trial area of Institute of Plant Protection, Hunan Academy of Agricultural Science, Changsha City; Citrus Institute of Guangxi, Guilin City; and College of Agricuture, Yangtze University, Jingzhou City. The treated plots of tomatoes or oranges were designed in a random block array with four replicates. The untreated plots served as a blank control. The formulation of compound **II-l** and etoxazole (2.5% emulsifiable concentrates (EC)) was prepared in our research group. They were diluted to 10, 15, or 22 mg kg^{-1} before use. The amount sprayed on the field area varied from 750 to 2000 g ha^{-1} at different sites. The control effects expressed as percentages were arcsine transformed to homogenize variances before analysis, which of different spraying treatments were examined using analysis of variance (ANOVA).[22]

RESULTS AND DISCUSSION

Synthesis. *N*-(2-Chloro-1-(*p*-tolyl)ethyl)-2,6-difluorobenzamide (**6**), as a critical intermediate for all the target compounds, was synthesized from toluene (**1**) as shown in Scheme 1. Ethyl 2-oxo-2-(*p*-tolyl)acetate (**2**) was obtained by Friedel–Crafts acylation of toluene with ethyl 2-chloro-2-oxoacetate. **2** was reacted with hydroxylamine hydrochloride to afford hydroxyimino compound **3**, which was further reduced by sodium borohydride and iodine to give α-amino alcohol **4**. After **4** was reacted with 2,6-difluorobenzoyl chloride to give the corresponding benzamide **5**, the hydroxyl in **5** was converted to chloro group in **6** by thionyl chloride using chloroform as solvent.

The target compounds **I-a**–**I-l** were synthesized from **6** as shown in Scheme 2. The methyl group in **6** was oxidized with CrO_3 in the presence of concentrated sulfuric acid and glacial acetic acid to give aldehyde **7**, which was further reacted with hydroxylamine hydrochloride to afford **8** containing an oxime moiety. **8** was treated with sodium hydride and reacted with a series of alkyl or substituted-benzyl bromide to give 2,4-diphenyl-1,3-oxazolines **I-a**–**I-l** containing oxime ether moiety after nucleophilic cyclization and substitution.

The target compounds **II-a**–**II-n** were also synthesized from compound **6** as shown in Scheme 3. The key intermediate **9** was obtained through bromination of **6** according to the reported method using water as solvent, in which the reaction temperature was strictly maintained between 0 °C and 5 °C to minimize the formation of byproduct. A series of oximes were successfully reacted with **9** in the presence of sodium hydride to give the target compounds **II-a**–**II-n** via cyclization and substitution.

Acaricidal Activity against Eggs and Larvae of *T. cinnabarinus* in the Laboratory. Table 1 shows acaricidal activities of the target compounds **I-a**–**I-l** and **II-a**–**II-n** against eggs and larvae of *T. cinnabarinus*, with etoxazole, the only 2,4-diphenyl-1,3-oxazoline acaricide widely used in China, as a contrast. The result showed that all of the target compounds had much higher activities than etoxazole, as the ovicidal and larvicidal activities of the target compounds **I-a**–**I-l** and **II-a**–**II-n** against *T. cinnabarinus* were all over 90% at 0.001 mg L^{-1}, but etoxazole gave only 30% and 40% respectively at the same concentration. Therefore, the modification at the para site of the 4-phenyl moiety of 2,4-diphenyl-1,3-oxazolines was favorable to the acaricidal activity, which was in accordance with our design ideas. Especially, **I-i** and **I-j**, respectively bearing trifluoromethoxyl and trifluoromethyl group at the para position of the benzyl moiety, showed the highest ovicidal and larvicidal activities at 0.0001 mg L^{-1}. Compounds **II-l** and **II-m**, that bear a trifluoromethyl at the ortho and meta positions of the benzene ring, respectively, had obviously higher ovicidal and larvicidal activities than the others.

dx.doi.org/10.1021/jf500461a | J. Agric. Food Chem. 2014, 62, 3064–3072

Evaluation of Acaricidal Activity of II-l in the Field. Based on the comprehensive analysis of bioactivity, physical properties, synthetic procedure, and so on, compound **II-l**, which had exhibited excellent acaricidal activity in the laboratory, was chosen to evaluate the acaricidal activity in the field. 2.5% EC of **II-l** and etoxazole were separately prepared and evaluated for the control of *P. citri* in Jingzhou City (Hubei Province, South China), Changsha City (Hunan Province, South China), and Guilin City (Guangxi Zhuang Autonomous Region, South China) and *T. cinnabarinus* in Tianjin City (North China). Part of the result of the field trials is shown in Table 2. It was shown that **II-l** at the concentration of 22 mg kg^{-1} had a better control effect than etoxazole at the same concentration. The data of the field trial in Jingzhou showed that, 3 days after the spray, the control efficiency of **II-l** against *P. citri* at 22 mg L^{-1} was 75%, whereas that of etoxazole was 55% at the same condition; this phenomenon suggested that the speed of action of **II-l** was faster than that of etoxazole. Fourteen days after the spray, the control efficiency of **II-l** and etoxazole was respectively 91% and 87%. The control efficiency of **II-l** and etoxazole against *P. citri* in Changsha and Guilin was similar, if not identical, to that in Jingzhou. In Tianjin, the control efficiency of both **II-l** and etoxazole against *T. cinnabarinus* at 22 mg L^{-1} was 92% when tested 14 days after the spray. Therefore, it was suggested that **II-l** could be considered as an alternative to the excessively employed acaricide for further development on the basis of the field trials.

Affinity of the Target Compounds Binding to SUR. The binding affinity of each series of the target compounds to the isolated SUR were tested by FP method with etoxazole as a contrast.[23] Since the accurate determination of concentration and stability of the SUR have not been realized up to now, in order to make the data comparable and minimize the errors, the binding affinity was assayed within the same set of experiments and containing 5 replicates. In the paper, that is to say, the target compounds **I-a**–**I-l** and etoxazole were tested with the same vesicle prepared, and so were compounds **II-a**–**II-n**.

In the buffer solution having the most proper concentration of 1-NPN-SUR, each of the target compounds **I-a**–**I-l** and etoxazole in different concentrations was separately added, and the FP data were recorded (Figure 2). From the figure we could find all the FP data were related to the concentration of the added compounds, which meant all these compounds could replace 1-NPN and then bind with SUR by themselves. The affinity with SUR is relevant to the value of the FP: the more decreased polarization was tested, the stronger affinity was exhibited. Thus from the figure, the order of affinity of these compounds was approximately deduced as **I-i** > **I-j** > **I-d** > **I-f** > **I-b** > **I-c** > **I-l** > **I-a** > **I-e** > **I-g** > **I-h** > **I-k** > etoxazole. The result indicated that the binding affinity to SUR of the target compounds **I-a**–**I-l** in vitro was almost consistent with their acaricidal activity against *T. cinnabarinus* in vivo, since **I-i** and **I-j** showed the strongest acaricidal activity against eggs and larvae of *T. cinnabarinus* in vivo, and **I-k** exhibited the lowest activity among the target compounds **I-a**–**I-l**.

Likewise, the FP data against the concentration of **II-a**–**II-n** and etoxazole are shown in Figure 3, from which the affinity order was deduced as **II-m** > **II-l** > **II-f** > **II-g** > **II-k** > **II-n** > **II-h** > **II-j** > **II-e** > **II-i** > **II-a** > **II-c** > **II-d** > **II-b** > etoxazole. The result was also almost consistent with the acaricidal activity against *T. cinnabarinus* in vivo, supported by the fact that **II-m** and **II-l** had the highest acaricidal activity, and **II-a**–**IId** had relatively lower activity in vivo. Therefore, it was verified that

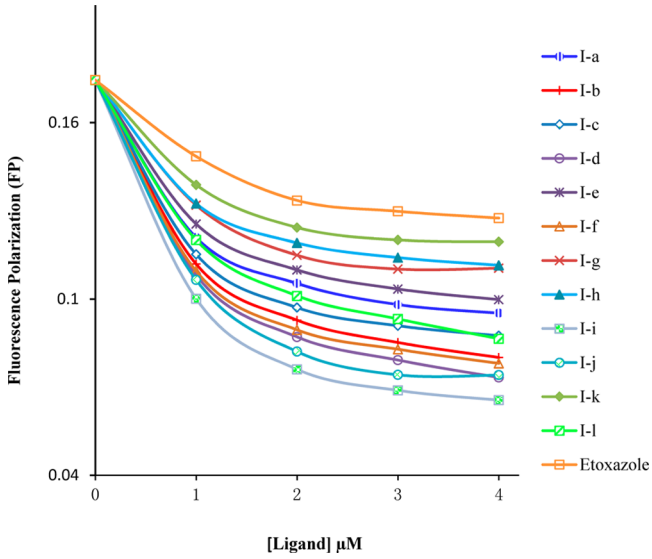


Figure 2. Binding curves of compounds **I-a**–**I-l** and etoxazole to sulfonylurea receptor.

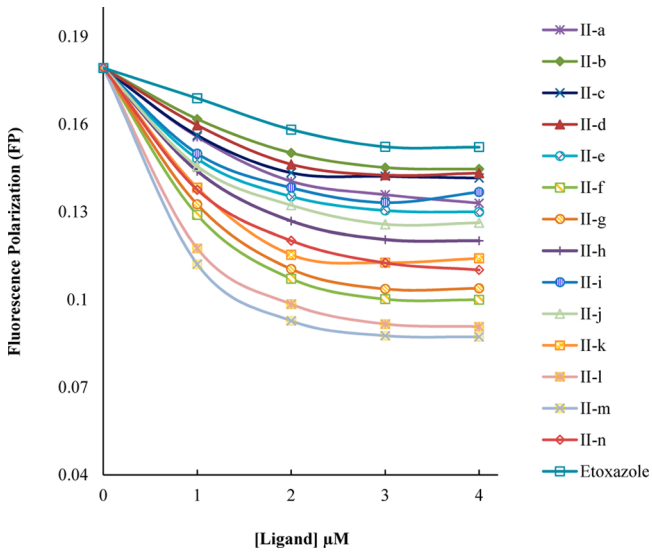


Figure 3. Binding curves of the compounds **II-a**–**II-n** and etoxazole to sulfonylurea receptor.

the target compounds and etoxazole could act on the site of SUR to suppress the chitin synthesis in mite body.

In summary, two series of novel 2,4-diphenyl-1,3-oxazoline compounds both containing an oxime ether group were designed and synthesized on the basis of etoxazole, flucycloxuron, and NK-17. The bioassay results indicated that all of the target compounds exhibited considerable acaricidal activities against *T. cinnabarinus* in the laboratory. Especially, compound **II-l**, 2-(trifluoromethyl)benzaldehyde *O*-(4-(2-(2,6-difluorophenyl)-4,5-dihydrooxazol-4-yl)benzyl) oxime, showed 100% ovicidal activities and 100% larvicidal activities at 0.0001 mg L^{-1}, much higher than etoxazole, thus it was selected to proceed to field trial. The field trial results from four sites in different parts of China clearly showed that **II-l** had nearly equivalent activity with etoxazole against *T. cinnabarinus* and *P. citri* at the same doses. Furthermore, when the fluorescence polarization method was employed to study the action mechanism, the target compounds and etoxazole were all

dx.doi.org/10.1021/jf500461a | J. Agric. Food Chem. 2014, 62, 3064–3072

speculated to act on the SUR then resulting in the suppression of chitin biosynthesis. Because of the easy preparation, good acaricidal activity, and unique mechanism, the new 2,4-diphenyl-1,3-oxazoline compound was expected to be developed as a promising candidate. Further research on acute toxicity, field residues, and their inhibitory activities against resistant mite species is ongoing and will be reported in the future.

AUTHOR INFORMATION

Corresponding Author

*E-mail: liuyuxiu@nankai.edu.cn (Yuxiu Liu); yangxl@cau.edu.cn (Xinling Yang); wangqm@nankai.edu.cn (Qingmin Wang).

Author Contributions

Yongqiang Li, Chaojie Li, Yanlong Zheng, Xingcun Wei, Qiaoqiao Ma, and Peng Wei contributed equally to this work.

Funding

This work was supported by the National Key Project for Basic Research (2010CB126100) and the National Natural Science Foundation of China (21132003, 21372131, 21102077) and the National 12th Five-year Key Technology R&D Program (2011BAE06B08-04).

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Van Daalen, J. J.; Meltzer, J.; Mulder, R. Selective insecticide with a novel mode of action. *Naturwissenschaften* **1972**, *59*, 312−313.

(2) Oberlander, H.; Silhacek, D. L. Mode of action of insect growth regulators in Lepidopteran tissue culture. *Pestic. Sci.* **1998**, *54*, 300−302.

(3) Qian, X. H.; Lee, P. W.; Cao, S. China: Forward to the Green Pesticides via a Basic Research Program. *J. Agric. Food. Chem.* **2010**, *58*, 2613−2623.

(4) Suzuki, J.; Ishida, T.; Shibuya, I.; Toda, k. Development of a new acaricide, Etoxazole. *J. Pestic. Sci.* **2001**, *26*, 215−223.

(5) Suzuki, J.; Ishida, T.; Kikuchi, Y.; Ito, Y.; Morikawa, C.; Tsukidate, Y.; Tanji, I.; Ota, Y.; Toda, K. Synthesis and activity of novel acaricidal/insecticidal 2,4-diphenyl-1,3-oxazolines. *J. Pestic. Sci.* **2002**, *27*, 1−8.

(6) Nauen, R.; Smagghe, G. Mode of action of etoxazole. *Pest Manage. Sci.* **2006**, *62*, 379−382.

(7) Leeuwen, T. V.; Demaeght, P.; Osborne, E. J.; Dermauw, W.; Gohlke, S.; Nauen, R.; Grbic, M.; Tirry, L.; Merzendorfer, H.; Clark, R. M. Population bulk segregant mapping uncovers resistance mutations and the mode of action of a chitin synthesis inhibitor in arthropods. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109* (12), 4407−4412.

(8) Nakagawa, Y.; Izumi, K.; Oikawa, N.; Kurozumi, A.; Iwamura, H.; Fujita, T. Quantitative structure-activity relationships of benzoylphenylurea larvicides: VII. Separation of effects of substituents in the multisubstituted anilide moiety on the larvicidal activity against Chilo suppressalis. *Pestic. Biochem. Physiol.* **1991**, *40* (1), 12−26.

(9) Minakuchi, C.; Suzuki, J.; Toda, K.; Akamatsu, M.; Nakagawa, Y. Estimation of the hydrophobicity of 2,4-diphenyl-1,3-oxaline analogs and QSAR analysis of their ovicidal activity against Tetranycus urticae. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4080−4084.

(10) Suzuki, J.; Tanji, I.; Ota, Y.; Toda, K.; Nakagawa, Y. QSAR of 2,4-diphenyl-1,3-oxazolines for ovicidal activity against the two-spotted spider mite Tetranychus urticae. *J. Pestic. Sci.* **2006**, *31* (4), 409−416.

(11) Roy, K.; Paul, S. Exploring 2D and 3D QSARs of 2,4-Diphenyl-1,3-oxazolines for Ovicidal Activity Against Tetranychus urticae. *QSAR Comb. Sci.* **2009**, *28*, 406.

(12) Liu, Y. X.; Wei, X. C.; Li, Y. Q.; Yang, N.; Wang, Q. M. Design, synthesis and acaricidal/insecticidal activities of etoxazole analogues. *New J. Chem.* **2013**, *37*, 1803−1810.

(13) Lee, C. W.; Chung, J. D. Characteristics and ring-opening isomerization polymerization of 2-(1,3,3-trimethyl-6-azabicyclo[3,2,1]-oct-6-yl)-4,5-dihydro-1,3-oxazoline (TAO). *Polymer (Korea)* **2012**, *36* (3), 262−267.

(14) Yang, X. L.; Wang, D. Q.; Chen, F. H.; Zhang, Z. N. The synthesis and larvicidal activity of N-aroyl-N′-(5-aryl-2-furoyl)ureas. *Pestic. Sci.* **1998**, *52*, 282−286.

(15) Abo-Elghar, G. E; Fujiyoshi, P.; Matsumura, F. Significance of the sulfonylurea receptor (SUR) as the target of diflubenzuron in chitin synthesis inhibition in Drosophila melanogaster and Blattella germanica. *Insect Biochem. Mol. Biol.* **2004**, *34*, 743−752.

(16) Li, Y. Q.; Qin, Y. G.; Yang, N.; Sun, Y. F.; Yang, X. L.; Sun, R. F.; Wang, Q. M.; Ling, Y. Studies on insecticidal activities and action mechanism of novel benzoylphenylurea candidate NK-17. *PLoS One* **2013**, *8* (6), e66251.

(17) Adimurthy, S.; Ghosh, S.; Patoliya, P. U.; Ramachandraiah, G.; Agrawal, M.; Gandhi, M. R.; Upadhyay, S. C.; Ghosh, P. K.; Ranu, B. C. An alternative method for the regio- and stereo selective bromination of alkenes, alkynes, toluene derivatives and ketones using a bromide/bromate couple. *Green Chem.* **2008**, *10*, 232−237.

(18) Kikuchi, D.; Sakaguchi, S.; Ishii, Y. An alternative method for the selective bromination of alkylbenzenes using $NaBrO_3/NaHSO_3$ reagent. *J. Org. Chem.* **1998**, *63*, 6023−6026.

(19) Abbott, W. S. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* **1925**, *18*, 265−267.

(20) Kuhn, D. G.; Kameswaran, V. Insecticidal, acaricidal and molluscicidal 1-(substituted)thioalkylpyrroles. U.S. Patent 5,302,383, 1993.

(21) Wang, Y. J.; Ou, X. M.; Pei, H.; Lin, X. M.; Yu, K. Toxicities of novel insecticide chlorfenpyr against several insects in lab. *Agrochem. Res. Appl.* **2006**, *10*, 20−23.

(22) Siegel, S.; Castellan, N. J., Jr. *Nonparametric statistics for the behavioral sciences*; McGraw-Hill Book Co.: Singapore, 1988.

(23) Harris, A.; Cox, S.; Burns, D.; Norey, C. Miniaturization of fluorescence polarization receptor-binding assays using CyDye-labeled ligands. *J. Biomol. Screen.* **2003**, 8410−8420.