Synthesis and Biological Activity of New (*E*)-α-(Methoxyimino)benzeneacetate Derivatives Containing a Substituted Pyrazole Ring[†]

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Strobilurins are one of the most important classes of agricultural fungicides. To discover new strobilurin analogues with high activity, a series of new strobilurin derivatives containing a substituted pyrazole in the side chain were synthesized and their biological activities were tested. The compounds were identified by ^{1}H nuclear magnetic resonance, infrared, and elemental analysis. The test results indicated that the compounds exhibited strong fungicidal activities against *Pyricularia oryzae*, *Phytophthora infestans*, *Pseudoperonospora cubensis*, and *Erysiphe graminis*. The relationship between structure and biological activity is discussed in terms of the effects of the substituents on the pyrazole ring. The present work demonstrates that strobilurin analogues with a 3-(substituted phenyl)-1*H*-pyrazol-5-oxy side chain can be used as possible lead compounds for the development of potential agrochemicals.

KEYWORDS: Strobilurin; pyrazole; fungicide

INTRODUCTION

The strobilurins are a class of fungicidal compounds modeled after natural compounds isolated from several basidomycete species that inhabit decaying plant material in woodland soils, and they have, therefore, been applied to agricultural disinfectants in many countries (*1*, *2*). These compounds, which contain the (*E*)-β-methoxyacrylate pharmacophore, act through inhibition of mitochondrial respiration by blocking electron transfer at the ubiquinol oxidation center (Q$_o$ site) of the cytochrome *bc*1 complex (complex III) (*3*). The related methyl (*E*)-methoxyimino-acetate pharmacophore (*4*) is represented in the synthetic strobilurin kresoxim-methyl. Substituted pyrazole ring derivatives exhibit a broad spectrum of biological activities such as antimicrobial (*5*), herbicidal (*6*), antitumor (*7*), and antiinflammatory (*8*) activities. For example, the fungicide pyraclostrobin contains a 1*H*-pyrazol-3-oxy side chain and exhibits excellent biological activity (*2*). Additionally, the strobilurin derivatives containing both a β-methoxyacrylate and a substituted pyrazole in the side chain display excellent fungicidal and acaricidal activities (*9*). In this study we synthesized a series of strobilurin analogues containing methyl (*E*)-methoxyiminoacetate and *N*-methyl (*E*)-methoxyiminoacetamide with a 1*H*-pyrazol-5-oxy side chain. We found that compounds **5i** and **6l** displayed excellent fungicidal activity against *Pseudoperonospora cubensis* at the concentration of 6.25 mg L^{-1}.

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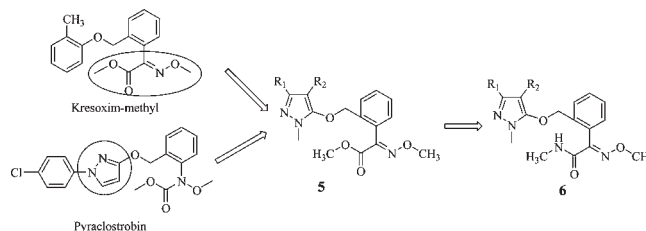
MATERIALS AND METHODS

All starting materials and reagents were commercially available and used without further purification except as indicated. Melting points were determined on a Büchi melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded with a Mercury 300 (Varian, 300 MHz) spectrometer with CDCl$_3$ as the solvent and TMS as the internal standard. Infrared spectra were measured with KBr disks using a PF-983G instrument (Perkin-Elmer). Mass spectra were recorded using a JEOL JMS-700 mass spectrometer.

According to known methods, (*10*) substituted β-keto esters (**2**) were prepared from substituted ketones (**1**) [substituted aceto-phenones (**1**: R$_2$ = H) and propiophenones (**1**: R$_2$ = Me)] and dimethyl carbonate. The ester (**2**) and methyl hydrazine were dissolved in methanol, and the mixture was heated to reflux to obtain the substituted 5-hydroxy-1*H*-pyrazole (**3**). The strobilurin analogues (**5**) were prepared by reacting the intermediate pyrazoles (**3**) with (*E*)-methyl 2-(2-(bromomethyl)phenyl)-2-methoxyiminoacetate (**4**) under basic conditions. The pyrazole derivatives (**6**) were prepared by reaction of the compounds

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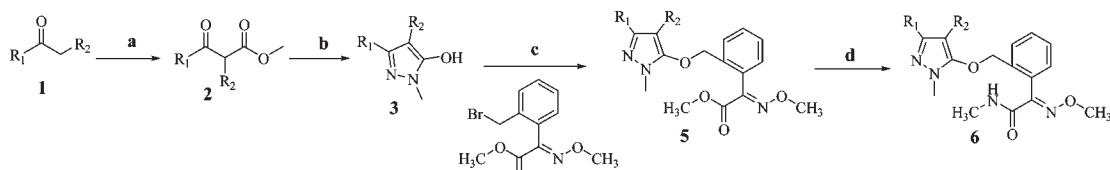
Scheme 1[a]

[a] Reagents and conditions: (a) NaH/(CH$_3$O)$_2$CO in THF, reflux; (b) CH$_3$NHNH$_2$ in CH$_3$OH, reflux; (c) NaH in DMF, 80°C; (d) CH$_3$NH$_2$ in THF, reflux.

(**5**) with aqueous methylamine in tetrahydrofuran (THF) as shown in **Scheme 1**.

General Procedure for the Synthesis of Intermediate β-Keto Esters (2). A suspension of 60% sodium hydride (0.10 mol, washed with petroleum ether) in a mixture of dimethyl carbonate (0.05 mol) and 100 mL of tetrahydrofuran was heated to reflux for 0.5 h. A solution of the substituted phenyl ketones (**1**) (0.05 mol) in 100 mL of tetrahydrofuran was added dropwise over 0.5 h while refluxing continued. When the reaction mixture became clear, it was refluxed for an additional 4−5 h. Then, the mixture was cooled and acidified with 36.5% hydrochloric acid and filtered. The filtrate was poured into a large amount of water, extracted three times with ethyl acetate. The combined extracts were washed with brine, dried, and concentrated under vacuum to obtain the crude oily product.

General Procedure for the Synthesis of Intermediate Pyrazoles (3). Each of the β-keto esters (**2**) was dissolved in methanol and heated to reflux. Methyl hydrazine was added dropwise to the reaction solution. The process of the reaction was monitored by thin-layer chromatography (TLC), and upon completion the reaction solution was evaporated under vacuum and cooled. The filtered solid was washed with methanol, dried, and used directly in the preparation of compounds **5**.

Procedure for the Synthesis of Compound 5i. 3-(4-Chlorophenyl)-1,4-dimethyl-1*H*-pyrazol-5-ol (10.10 mmol) was dissolved in 5 mL of DMF, and 60% sodium hydride (19.00 mmol, washed with petroleum ether) was added to the solution. The solution was stirred for 0.5 h, and (*E*)-methyl 2-(2-(bromomethyl)phenyl)-2-methoxyiminoacetate (10.30 mmol) was added. The reaction mixture was heated to 80 °C and monitored by TLC. At completion of the reaction (after 3 h), the mixture was added to 50 mL of brine and extracted three times with 100 mL of ethyl acetate. The combined organic extracts were dried and concentrated to obtain the crude product. It was purified via silica gel column chromatography, using a 1:4 (v/v) mixture of ethyl acetate and petroleum ether (boiling range = 60−90 °C) as the eluting solution to obtain compound **5i** as viscous oil.

Procedure for the Synthesis of the Compounds 6. The compounds **5** (3.00 mmol) were dissolved in 5 mL of THF, and a slight excess of a methylamine solution (25−30%) was added dropwise to the solution. The mixture was refluxed for 1 h (the reaction was monitored by TLC) and then concentrated. Water was added to the residue and extracted three times with 50 mL of ethyl acetate, and the combined organic extracts were dried and concentrated. The crude product was purified via silica gel column chromatography to obtain the compounds **6**.

Example data of **5i** and **6l** are shown as follows, whereas data for the other compounds can be found in the Supporting Information.

Data for 5i: yield 75.3% of oil; IR (KBr) ν 2945 (s, C−H), 1730 (s, C=O), 1490 (s, C=N), 1440 (s, CH$_3$), 1310 (m, C−N), 1210 (s, C−O), 770, 700 (s, Ph−H) cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.59−7.61 (m, 1H, Ph-6-H), 7.40−7.46 (m, 4H, 3-Ph-2,3,5,6-4H), 7.26−7.30 (m, 2H, Ph-3,5-2H), 7.19−7.21 (m, 1H, Ph-4-H), 5.15 (s, 2H, CH$_2$), 4.05 (s, 3H, OCH$_3$), 3.85 (s, 3H, CO$_2$CH$_3$), 3.61 (s, 3H, NCH$_3$), 1.84 (s, 3H, Py-CH$_3$). Anal. Calcd: C, 61.75; H, 5.18; N, 9.82. Found: C, 61.54; H, 5.27; N, 10.03.

Data for 6l: yield 87.5% of a white solid; mp 163−165 °C; IR (KBr) ν 3240(s, NH), 2920 (s, C−H), 1660 (s, C=O), 1500 (s, C=N), 1400 (s, CH$_3$), 1295(m, C−N), 1190 (s, C−O), 820, 750 (s, Ph−H) cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.59−7.61 (m, 1H, Ph-6-H), 7.39−7.41 (m, 2H, Ph-3,5-2H), 7.20 (m, 1H, Ph-4-H), 7.04−7.08 (m, 3H, 3-Ph-3,5,6-3H), 6.76 (m, 1H, NH), 5.15 (s, 2H, CH$_2$), 3.96 (s, 3H, OCH$_3$), 3.41 (s, 3H, NCH$_3$), 2.87−2.89 (d, *J* = 4.8 Hz, 3H, NHCH$_3$), 2.37 (s, 3H, 2-CH$_3$), 2.09 (s, 3H, 4-CH$_3$), 1.70 (s, 3H, Py-4-CH$_3$).

Biological Assay. Evaluations of biological activities of the reported compounds were performed as previously described (*11*−*13*). Biological data were reported as the range from 0 (indicates no control) to 100% (complete control).

RESULTS AND DISCUSSION

Synthetic Chemistry. According to procedures exemplified above, strobilurin derivatives were synthesized mostly with good overall yields of 60−75% (**5**) and 70−90% (**6**), as shown in **Tables 1** and **2** (for 3-substituted-phenyl-1-methyl-1*H*-pyrazol-5-yloxy analogues, **5a**−**5h** and **6a**−**6h**) and in **Tables 1** and **2** (for 3-substituted-phenyl-1,4-dimethyl-1*H*-pyrazol-5-yloxy analogs, **5i**−**5r** and **6i**−**6r**). The synthesized compounds were characterized by ^{1}H NMR, IR, and elemental analyses. All spectral and analytical data were consistent with the assigned structures. The IR spectra of compounds showed C−H and C=O stretching bands at 2920−2960 and 1700−1720 cm^{-1}, respectively.

Fungicidal Activities. As indicated in **Tables 1** and **2**, many of the synthesized compounds exhibit potent activity against *Pyricularia oryzae*, *Phytophthora infestans*, *P. cubensis*, and *Erysiphe graminis*. Methylpyrazolyloxy analogues (abbreviated as the Me series, hereafter) seem to be slightly less potent than the corresponding equivalent dimethylpyrazolyloxy analogues (abbreviated as the Me$_2$ series). However, there are a few exceptions such as the pair of 2,4-(CH$_3$)$_2$-Ph analogues (**e** and **l**). For both the oximinoacetates **5** and the oxinoacetamides **6**, under equivalent dosage conditions of 25 or 400 mg L^{-1}, most of the two series of analogues show 100% inhibition against *P. oryzae*, *P. infestans*, *P. cubensis*, and *E. graminis*. Therefore, the fungicidal activity was measured at a lower dose range.

Because of the paucity of the dose-potency data, detailed structure−activity discussion for the fungicidal activity is almost impossible. There seems to be an optimal hydrophobicity and/or a sterically acceptable limit of phenyl substituents (R$_1$) as shown for the mono and dimethyl phenyl substituents in compounds **e**, **j**, **k**, **l**, and **m** in **Tables 1** and **2**. This structure−activity relationship appears to apply for the activity against *P. cubensis* and *E. graminis*. The most potent compounds at the lowest doses are, in general, the most active against *P. oryzae* and *P. infestans* as shown in **Tables 1** and **2**. Due to the lack of a wide range of phenyl substituents, it is difficult to conclude if there is an electron-withdrawing effect. For example, in the Me series of **Tables 1** and **2**, 4-Cl−Ph (**b**) versus 4-CH$_3$O−Ph (**f**) and 2-Cl−Ph (**h**) versus 2-CH$_3$O−Ph (**g**) provide substantially similar activities. In the Me$_2$ series of **Table 1** 4-Cl−Ph (**i**) is more active than 4-CH$_3$O−Ph (**p**), whereas in **Table 2** they are about equivalent in activity. In this study, compounds **5i**, **5j**, **5l**, **6l**, and **6m** of the Me$_2$ series are the most active and possess a broader spectrum of fungicidal activity than compounds of the Me series.

Insecticidal Activity. Some of the synthesized compounds exhibit insecticidal and acaricidal activities. The compounds were tested against *Leucania venalba*, *Myzus persicae*, *Culex pipiens* pallens, and *Tetranychus cinnabarinus* at 600 mg L^{-1}. Compounds **5m** and **6p** exhibited 100% control against *L. venalba*, whereas compounds **5a**, **5d**, **5e**, **5m**, **6b**, **6d**, **6g**, and **6m** exhibited 100% control against *M. persicae*. Compounds **5b** and **6b**

Table 1. Fungicidal Activity of the Synthesized Compounds **5** (Percent Control)

	pathogen				P. oryzae		P. infestans		P. cubensis			E. graminis		
compd	R_1	R_2	mp (°C)	yield	25	0.92	25	0.92	400	25	6.25	400	6.25	1.56
5a	Ph	H	oil	72.1	0	/[a]	0	/	95	**70**	/	70	/	/
5b	4-Cl−Ph	H	102−105	68.7	80	/	80	50	100	90	80	100	40	/
5c	4-C(CH$_3$)$_3$−Ph	H	86−88	75.0	100	80	100	60	95	0	/	0	/	/
5d	3,4-(CH$_3$)$_2$−Ph	H	117−118	69.4	100	50	100	80	98	40	/	100	30	/
5e	2,4-(CH$_3$)$_2$−Ph	H	oil	70.3	100	0	100	100	95	90	65	100	65	/
5f	4-CH$_3$O−Ph	H	oil	71.2	0	/	100	0	98	50	/	100	10	/
5g	2-CH$_3$O−Ph	H	oil	66.9	100	50	0	/	50	/	/	100	90	40
5h	2-Cl−Ph	H	77.5	77.5	100	0	100	0	80	30	/	100	75	45
5i	4-Cl−Ph	CH$_3$	oil	75.3	50	/	100	100	100	100	100	100	100	98
5j	4-CH$_3$−Ph	CH$_3$	oil	67.6	50	/	100	100	95	60	/	100	100	100
5k	3,4-(CH$_3$)$_2$−Ph	CH$_3$	130−132	64.7	100	80	100	100	100	75	70	100	100	60
5l	2,4-(CH$_3$)$_2$−Ph	CH$_3$	oil	76.1	100	80	100	100	100	100	98	100	100	75
5m	2,5-(CH$_3$)$_2$−Ph	CH$_3$	oil	72.0	100	100	100	60	100	80	40	100	40	20
5n	4-C$_2$H$_5$−Ph	CH$_3$	oil	70.5	50	/	100	80	98	70	50	100	100	100
5o	4-C(CH$_3$)$_3$−Ph	CH$_3$	118−120	73.4	50	/	0	/	40	/	/	70	/	/
5p	4-CH$_3$O−Ph	CH$_3$	oil	68.2	100	80	100	50	100	55	15	100	85	30
5q	4-C$_2$H$_5$O−Ph	CH$_3$	98−100	71.6	100	50	100	50	100	40	/	100	100	50
5r	4-CF$_3$CH$_2$O−Ph	CH$_3$	oil	66.0	100	80	50	/	80	0	/	100	70	15
azoxystrobin					100	/	/	/	100	100	95	100	100	60
kresoxim-methyl					100	/	100	/	/	35	0	100	100	100

[a] /, no data.

Table 2. Fungicidal Activity of the Synthesized Compounds **6** (Percent Control)

	pathogen				P. oryzae		P. infestans		P. cubensis			E. graminis		
compd	R_1	R_2	mp (°C)	yield	25	0.92	25	0.92	400	25	6.25	400	6.25	1.56
6a	Ph	H	oil	69.6	0	/[a]	/	/	100	100	70	50	/	/
6b	4-Cl−Ph	H	132−134	71.4	0	/	/	/	100	100	60	100	40	0
6c	4-C(CH$_3$)$_3$−Ph	H	172−174	90.3	100	50	100	50	80	0	/	0	/	/
6d	3,4-(CH$_3$)$_2$−Ph	H	109−111	88.6	100	50	100	80	100	100	85	100	100	40
6e	2,4-(CH$_3$)$_2$−Ph	H	115−117	89.4	100	50	100	100	100	100	70	100	100	30
6f	4-CH$_3$O−Ph	H	oil	91.0	100	80	100	0	98	30	/	100	0	/
6g	2-CH$_3$O−Ph	H	oil	89.1	100	0	0	/	75	10	/	100	100	45
6h	2-Cl−Ph	H	oil	87.5	0	/	100	0	90	40	/	100	90	55
6i	4-Cl−Ph	CH$_3$	oil	90.6	0	/	100	/	80	40	/	100	100	85
6j	4-CH$_3$−Ph	CH$_3$	oil	89.9	0	/	100	100	65	/	/	100	95	40
6k	3,4-(CH$_3$)$_2$−Ph	CH$_3$	184−186	86.8	100	80	100	100	100	100	40	100	100	55
6l	2,4-(CH$_3$)$_2$−Ph	CH$_3$	163−165	87.5	100	50	100	100	100	100	100	100	100	65
6m	2,5-(CH$_3$)$_2$−Ph	CH$_3$	134−136	87.8	100	100	100	100	100	100	50	100	40	0
6n	4-C$_2$H$_5$−Ph	CH$_3$	oil	85.9	100	0	100	80	50	/	/	100	100	90
6o	4-C(CH$_3$)$_3$−Ph	CH$_3$	oil	88.2	100	100	100	40	95	20	/	100	0	0
6p	4-CH$_3$O−Ph	CH$_3$	135−137	87.3	100	50	80	50	98	85	55	100	100	75
6q	4-C$_2$H$_5$O−Ph	CH$_3$	142−144	88.1	100	100	100	50	98	20	/	100	100	70
6r	4-CF$_3$CH$_2$O−Ph	CH$_3$	oil	90.2	80	50	100	50	100	80	30	100	100	40
azoxystrobin					100	/	/	/	100	100	95	100	100	60
kresoxim-methyl					100	/	100	/	/	35	0	100	100	100

[a] /, no data.

exhibited 100% control against *C. pipiens*, and compounds **5d**, **5q**, and **6d** exhibited 100% control against *T. cinnabarinus*.

Because the bioactivity was measured in vivo, effects of metabolism and/or biotransformation within the plant/fungus may make some contribution to the apparent potency variations. The inference that the electron-withdrawing substituents of the phenyl moiety seem to enhance the potency may be related to the fact that they tend to retard mechanisms of oxidative metabolism occurring on (or close to) the benzene ring (*14*). Moreover, a higher electronic (negative) charge within the ring system may facilitate oxidative metabolism as hydroxylation of the benzene ring.

The fungicidal activity of two commercial fungicides, azoxystrobin and kresoxim-methyl, is shown in **Tables 1** and **2**. The fungicidal activity of the compounds of this study is equal to or slightly better than those of these two commercial standards.

Conclusion. Strobilurin analogues synthesized by introducing various substituted-phenyl groups (R_1) into the 1*H*-pyrazol-5-oxy ring of the designed skeletal lead, **5** and **6** were fungicidally active in planta, against *P. cubensis* and *E. graminis*. Some of these new strobilurins were more potent than the reference strobilurin compounds azoxystrobin and kresoxim-methyl. Variations in the in vivo potency may be affected by hydrophobic, steric, and electronic effects of the phenyl substituents (R_1) and can also be additionally influenced by metabolic detoxification. All of these combined factors result in complex structure−activity relationships. Further analogue syntheses and structure optimization studies are in progress.

Supporting Information Available: ^{1}H NMR, IR, melting point, and element analysis data for the target compounds. This information is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED

(1) Sauter, H.; Steglich, W.; Anke, T. Strobilurins: evolution of a new class of active substances. *Angew. Chem., Int. Ed.* **1999**, *38*, 1328–1349.

(2) Bartlett, D. W.; Clough, J. M.; Godwin, J. R.; Hall, A. A.; Hamer, M.; Parr-Dobrzanski, B. The strobilurin fungicides. *Pestic. Manage. Sci.* **2002**, *58*, 649–662.

(3) Becker, W. F.; Jagow, V. G.; Anke, T.; Steglich, W. Oudemansin, strobilurin A, strobilurin B and myxothiazol: New inhibitors of the *bc*1 segment of the respiratory chain with an *E-β*-methoxyacrylate system as common structural element. *FEBS Lett.* **1981**, *132*, 329–333.

(4) Rossi, R.; Carpita, A.; Pazzi, P.; Mannina, L.; Valensin, D. A novel method for the efficient synthesis of methyl 2-oxo-2-arylacetates and its application to the preparation of fungicidal methyl (*E*)-*o*-methyloximino-2-aryl acetates and their (*Z*)-stereoisomers. *Tetrahedron* **1999**, *55*, 11343–11364.

(5) Chen, H.; Li, Z.; Han, Y. Synthesis and fungicidal activity against *Rhizoctonia solani* of 2-alkyl(alkylthio)-5-pyrazolyl-1,3,4-oxadiazoles (thiadiazoles). *J. Agric. Food Chem.* **2000**, *48*, 5312–5315.

(6) Meazza, G.; Bettarini, F.; Porta, L. P.; Piccardi, P.; Signorini, E.; Portoso, D.; Fornara, L. Synthesis and herbicidal activity of novel heterocyclic protoporphyrinogen oxidase inhibitors. *Pest Manag. Sci.* **2004**, *60*, 1178–1188.

(7) Park, H.; Lee, K.; Park, S.; Ahn, B.; Lee, J.; Cho, H. Y.; Lee, K. Identification of antitumor activity of pyrazole oxime ethers. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3307–3312.

(8) Bekhit, A. A.; Ashour, H. M. A.; Guemei, A. A. Novel pyrazole derivatives as potential promising antiinflammatory antimicrobial agents. *Arch. Pharm.* **2005**, *338*, 167–174.

(9) Li, M.; Liu, C. L.; Li, L.; Yang, H.; Li, Z. N.; Zhang, H. Li, Z. M. Design, synthesis and biological activities of new strobilurin derivatives containing substituted pyrazoles. *Pest Manag. Sci.* **2009**, submitted for publication.

(10) Morrison, G. C. 2,4-Dihydro-5-((substituted)phenyl)-4,4-disubstituted-3*H*-pyrazol-3-ones, 2,4-dihydro-5-((substituted)phenyl)-4,4-disubstituted-3*H*-pyrazol-3-thiones, and a process for producing the same. Eur. Patent Appl. EP 0126651, Nov 28, 1984.

(11) Ross, R.; Fujimoto, T. T.; Shaber, S. H. Benzyloxy substituted aromatics and their use as fungicides and insecticides. Eur. Patent Appl. EP 0811614, Dec 10, 1997.

(12) Li, M.; Zhang, J. B.; Yang, J. C; Li, Z. N.; Liu, C. L; Li, Z. M. Design, synthesis and bioactivity of new *N*-methoxycarbamate containing pyrazole. *Chinese J. Pestic.* **2009**, *30*, 1348–1352.

(13) Li, H. C.; Liu, C. L.; Chai, B. S.; Li, M.; Li, Z. N.; Yang, J. C. Design, synthesis and fungicidal activity of novel strobilurin analogues containing substituted *N*-phenylpyrimidin-2-amines. *Nat. Prod. Commun.* **2009**, *4*, 1209–1214.

(14) Nakagawa, Y.; Kitahara, K.; Nishioka, T.; Iwamura, H.; Fujita, T. Quantitative structure−activity studies of benzoylphenylurea larvicides (1). *Pestic. Biochem. Physiol.* **1984**, *21*, 309–325.