Chem Biol Drug Des 2011; **78:** 689–694

Research Article

doi: 10.1111/j.1747-0285.2011.01205.x

Design, Synthesis, Biological Activities, and 3D-QSAR of New *N,N'*-Diacylhydrazines Containing 2-(2,4-dichlorophenoxy)propane Moiety

Xing-Hai Liu[1,*], Li Pan[2], Yi Ma[2], Jian-Quan Weng[1], Cheng-Xia Tan[1], Yong-Hong Li[2], Yan-Xia Shi[3], Bao-Ju Li[3], Zheng-Ming Li[2,*] and Yong-Gang Zhang[4]

[1]*Institute of Pesticide, Zhejiang University of Technology, Hangzhou 310014, China*
[2]*State-Key Laboratory of Elemento-Organic Chemistry, Nankai University, Tianjin 300071, China*
[3]*Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing 100014, China*
[4]*Biology Institute of Shandong Academy of Science, Jinan 250014, China*
**Corresponding authors: Xing-Hai Liu, xhliu@zjut.edu.cn; Zheng-Ming Li, nkzml@vip.163.com*
Xing-Hai Liu and Li Pan contributed equally to this work.

A series of new *N,N'*-diacylhydrazine derivatives were synthesized efficiently under microwave irradiation. Their structures were characterized by ^{1}H NMR, MS, and elemental analysis. Various biological activities of these compounds were tested. Most of them exhibited higher herbicidal activities against dicotyledonous weeds than monocotyledonous weeds. In addition, favorable *in vivo* fungicidal activities were also found of these compounds against *Cladosporium cucumerinum*, *Corynespora cassiicola*, *Sclerotinia sclerotiorum*(Lib.)de Bary, *Erysiphe cichoracearum*, and *Colletotrichum orbiculare* (Berk aLMont) Arx. All compounds displayed excellent plant growth regulatory activities: 100% inhibition was achieved against the radicle growth of cucumber. To further investigate the structure–activity relationship, comparative molecular field analysis was performed on the basis of herbicidal activity data, resulting in a statistically reliable model with good predictive power (r^2 = 0.913, q^2 = 0.556). Based on the calculation, five additional novel compounds were designed and synthesized. Satisfyingly, compound 4u displayed excellent herbicidal activity (94.7%) at 1500 g/ha, although it is less active than 2,4-D. Meanwhile, this compound also exhibited good fungicidal activity against *C. orbiculare* (Berk aLMont) Arx (82.16%).

Key words: *N,N'*-diacylhydrazines, 2-(2,4-dichlorophenoxy)propane, microwave synthesis, herbicidal activity, fungicidal activity, plant growth regulatory activity, 3D-QSAR

Received 22 January 2011, revised 17 July 2011 and accepted for publication 23 July 2011

More recently, diacylhydrazine derivatives have become one of the focuses in the development of agrochemicals because of their high biological activities (1–6), especially herbicidal activities as well as fungicidal activities. Some examples of diacylhydrazines are presented in Scheme 1.

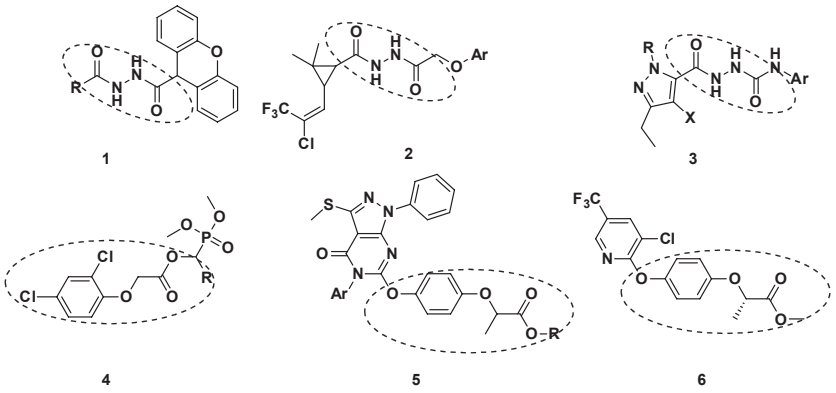
Aryloxyphenoxy-propionates, as an intriguing class of herbicides in the international market (7), have drawn much attention in the past decades. In particular, agrochemists were lured their studies to develop novel modified 2,4-D structures for their remarkable herbicidal activity. A variety of bioactive moieties involving phosphonates (compound **4**) (8), heterocycles (compound **5**) (9) and other types of substituents (compound **6**) (10) were introduced to the aryloxyphenoxy-propionates structural core, which led to higher herbicidal activity where 1-(substituted phenoxyacetoxy) alkylphosphonates was one class of the successful examples (11). Furthermore, some substituted aryloxyacetic acid derivatives also exhibit good plant growth-regulating activity(12). However, unfavorable responses occurred in a few cases; for instance, suppression of leaf growth was observed (13) when the substituted aryloxyacetic acids were used to improve fruit set. Therefore, seeking highly active and effective agents remains a sufficient room.

In line with our continuous efforts to synthesize bioactive lead compounds for crop protection, the title compounds **4a–4u** were designed by introducing 2-(2,4-dichlorophenoxy)propane acid pharmacophore into the diacylhydrazine scaffold. Our original strategy is depicted in Scheme 2.

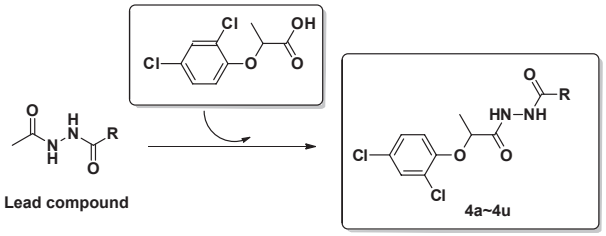
Materials and Methods

Instruments

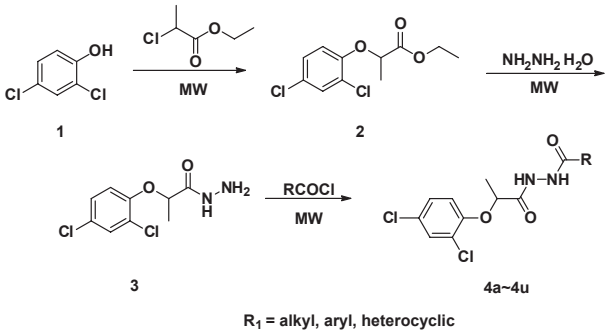
Melting points were determined by an X-4 apparatus (Beijing Tech Instruments Co., Beijing, China) and uncorrected. ^{1}H NMR spectra were measured on a Bruker AC-P500 instrument (300 MHz; Bruker,



Scheme 1: Some representative structures of diacylhydrazine and phenoxy moiety.



Scheme 2: Design strategy of title compounds.



Scheme 3: Synthetic route of title compounds.

Fallanden, Switzerland) using tetramethylsilane (TMS) as an internal standard and DMSO-d_6 as the solvent. Mass spectra were recorded on a Thermo Finnigan LCQ Advantage LC/mass detector instrument. Elemental analyses were performed on a Vario EL elemental analyzer. LWMC-250 domestic microwave oven was used to do microwave reaction. All the reagents are of analytical grade or freshly prepared before use.

General method for preparing title compounds

2,4-dichlorophenol (5 mmol), KI (1 mmol), *N*,*N*-Dimethylformamide (DMF) (1 mL), ethyl 2-chloropropanoate (5 mmol) and tetrabutyl ammonium bromide (TBAB) (0.5 mmol) were placed in a dried round-bottomed flask, and the mixture was irradiated by microwaves (200 W) for 4 min. On completion of the reaction, the mixture was cooled to room temperature and then added to ethanol (10 mL) with constant stirring. After filtering off the inorganic salts, the reaction mixture was added to 85% hydrazine hydrate (5 mmol) and subjected to microwave irradiation (500 W) for an additional 1 min. Then, it was cooled to room temperature and allowed to settle for 1 h, and the precipitates were filtered off and recrystallized from ethanol to afford the pure product **3**. Then, **3** (1 mmol) and substituted acyl chloride (1 mmol) were mixed in tetrahydrofuran (THF). The mixture was put into the microwave oven (495 W) and irradiated for 10 min to produce the crude solid, which on recrystallization with ethanol gave the pure product as shown in Scheme 3. The analysis data of title compounds were found in Supporting Information.

Herbicidal activities assay

The herbicidal activities of **4a–4u** were evaluated using a previously reported procedure (14).

Plant growth regulatory activity assay

The regulatory activity of compounds against the growth of cucumber roots at the same department was evaluated as above. All compounds were tested at a same concentration of 10 mg/L. The detailed procedure was described as follows (3).

Fungicidal activities assay

Fungicidal activity of **4a–4u** against *Cladosporium cucumerinum* (*CCu*), *Corynespora cassiicola* (*CCa*), *Sclerotinia sclerotiorum*(Lib.)de Bary (*SS*), *Erysiphe cichoracearum* (*EC*), and *Colletotrichum orbiculare* (Berk aLMont) Arx. (*CO*) was evaluated according to reference (15).

3D-QSAR analysis

Molecular modeling was performed using SYBYL 6.91 software (Tripos, Inc., San Francisco, CA, USA), and the comparative molecular field analysis (CoMFA) method was carried out according to our previous work (16).

Results and Discussion

Synthesis

The synthetic route of the title compounds was outlined in Scheme 3. At first, 2,4-dichlorophenol (5 mmol), K_2CO_3 (6 mmol), DMF (10 mL), and ethyl 2-chloropropanoate (5 mmol) were placed in a dried round-bottomed flask, and the mixture was stirred at room temperature for overnight. When the reaction reached completion, reaction mixture was poured into water, and compound **2** was afforded by filtration. Next, to a solution of compound **2** (2 mmol) in ethanol (10 mL) was added 85% hydrazine hydrate (3 mmol). After refluxing for 6 h, the reaction mixture was cooled to room temperature, and the precipitates were filtered off and recrystallized from ethanol to afford the pure product **3**. For the last step, **3**(1 mmol), Et_3N (1.1 mmol), and substituted acyl chloride (1 mmol) were mixed in THF and refluxed for 4 h to prepare the crude solid. To optimize the reaction condition and reaction times, microwave irradiation was employed. All these microwave steps are according to the references (17). The key intermediate **2**, **3** can be obtained with excellent yield (>95%) in short reaction time. The processes are shown in the experiment section and Scheme 3.

Herbicidal activity

The herbicidal activities of these compounds were determined *in vivo*. The results for these compounds **4a–u** are summarized in Table 1. As shown in Table 1, all the compounds exhibit excellent herbicidal activity in postemergence against *Amaranthus retroflexus* and *Brassica napus* except **4a**, **4p**, and **4q**. And also, most of these compounds show better herbicidal activities in postemergence treatment than in preemergence treatment against *A. retroflexus* and *B. napus*. Notably, some aryl-substituted compounds show surprisingly higher herbicidal activity against monocotyledon (*Echinochloa crus-galli*) for preemergence than the corresponding alkyl-substituted compounds except **4p**. For the compound **4p**, further bioassay was conducted against *E. crus-galli*, *Digitaria sanguinalis*, *B. napus*, and *Amaranthus retroflerus*. It is indicated from Table 2 that compound **4p** has good (96.9%) herbicidal activity against dicotyledonous weeds *A. retroflerus* at a dose of 375 g/ha, which is comparable with that of the commercial herbicide 2,4-D. However, compound **4p** has weak herbicidal activity against other three plants at the same dosage.

Plant growth regulatory activity

To elucidate why these compounds had excellent herbicidal activity, the cotyledon root of cucumber activity of title compounds was determined. Surprisingly, as listed in Table 1, all the testing compounds showed 100% inhibition on the root cotyledon of cucumber. Such strong inhibition of plant root growth can possibly the reason attributing to the aforementioned excellent herbicidal activity.

Fungicidal activities

The *in vivo* fungicidal results of title compounds are listed in Table 3. Most of the compounds showed promising results in inhib-

Table 1: The herbicidal[a] and plant growth regulatory[a] activity of title compounds

			Ech.		*Bra.*		*Dig.*		*Ama.*	
No.	*R*	CRC	Pre-	Post-	Pre-	Post-	Pre-	Post-	Pre-	Post-
4a	Phenyl	−100	100	8.7	58.0	58.6	37.0	25.0	45.3	56.1
4b	*p*-nitro phenyl	−100	100	22.8	65.7	100	31.5	14.3	74.7	100
4c	*p*-chloro phenyl	−100	100	21.1	63.3	100	59.5	10.7	85.3	100
4d	*p*-fluoro phenyl	−100	39.4	22.8	84.0	100	50.0	28.6	74.7	100
4e	*m*-methyl phenyl	−100	100	11.5	94.7	100	29.6	10.7	78.9	100
4f	*m*-chloro phenyl	−100	24.5	17.2	100	100	72.2	7.1	100	100
4g	*o*-chloro phenyl	−100	20.0	16.6	64.6	63.6	33.3	3.6	93.7	100
4h	2,4-dichloro phenyl	−100	20.7	29.4	100	91.9	46.2	19.6	100	100
4i	*o*-methoxyl phenyl	−100	17.8	26.6	92.1	90.5	55.8	0	85.3	100
4j	4-pyridyl	−100	100	23.9	91.7	100	55.6	14.3	100	100
4k	Isoxazoyl	−100	28.2	13.1	100	100	28.8	0	100	100
4l	1-cycan cyclopropyl	−100	18.4	6.5	100	100	67.3	14.3	73.5	100
4m	Ethyl	−100	36.8	28.5	100	100	71.2	0	100	100
4n	Iso-propyl	−100	4.6	14.5	98.7	100	59.6	0	100	100
4o	Butyl	−100	16.1	11.2	60.5	100	25.0	0	64.7	100
4p	Cyclopropyl	−100	100	23.9	91.7	100	55.6	14.3	100	100
4q	2,4-dichlorophenoxymethyl	−100	0	6.1	21.1	70.8	5.8	0	17.6	6.3
4r	(2-(2,4-dichlorophenoxy)acetyl)propyl	−63.5	19.0	6.1	94.7	83.0	42.4	0	52.9	39.5
4s	*p*-iodo phenyl	−100	14.9	12.6	96.7	91.9	75.0	0	100	100
4t	3-pyridyl	−100	24.1	15.4	88.2	100	71.2	1.8	100	100
4u	*p*-methoxyl phenyl	−100	0.6	13.6	94.7	100	69.2	7.1	91.2	100
	2,4-D	65.7	100	85.4	100	100	100	100	81.1	100

Ech, *Echinochloa crus-galli*; *Dig*, *Digitaria sanguinalis*; *Bra*, *Brassica napus*; *Ama*, *Amaranthus retroflerus*; Pre, preemergence; Post, postemergence.
[a]The test concentration of herbicidal activity is at 1500 g/ha, and the cotyledon root of cucumber (CRC) is at 10 mg/mL.

Table 2: Herbicidal activities of compounds **4p** and **2,4-D** (percent inhibition, %)

		Ech.		*Bra.*		*Dig.*		*Ama.*	
No.	Rate g/ha	Pre-	Post-	Pre-	Post-	Pre-	Post-	Pre-	Post-
4p	375	37.6	0	14.8	58.8	24.1	0	45.1	96.9
	750	44.5	2.5	43.2	60.0	50.4	9.0	92.7	100
2,4-D	375	0	0	0	100	0	0	52.4	100
	750	32.6	32.4	0	100	50.0	0	78.6	100

Table 3: Fungicidal activity of title compounds (percent relative control efficacy) at 500 ppm

No.	CCa	CCu	EC	SS	CO
4a	39.00	55.00	17.25	24.29	63.76
4b	7.00	60.00	50.86	31.23	54.50
4c	38.00	39.00	44.20	53.37	59.81
4d	−4.00	57.00	33.77	22.65	32.71
4e	14.00	0.50	51.30	4.96	78.72
4f	50.00	14.00	21.52	37.94	60.84
4g	34.00	13.00	76.62	−0.43	31.14
4h	68.00	21.00	60.26	27.54	62.75
4i	45.00	40.00	−5.20	6.08	14.93
4j	36.00	18.00	22.85	15.02	56.02
4k	46.00	13.00	14.99	21.41	50.88
4l	52.00	22.00	64.94	27.54	79.34
4m	46.00	11.00	38.21	5.24	67.58
4n	40.00	10.00	71.90	76.78	29.89
4o	15.00	30.00	10.00	24.23	45.11
4p	15.00	4.00	24.03	25.09	71.98
4q	55.00	50.00	−5.19	16.02	80.28
4r	22.00	18.00	53.25	34.09	69.95
4s	62.00	16.00	45.67	41.29	32.82
4t	48.00	19.00	66.74	−6.50	69.36
4u	62.00	50.00	−5.19	−1.08	82.16
a	87.00				
b		99.00			
c			100.00		
d				100.00	
e					100.00

Control pesticide:
a: Daconil 75% WP; b: 40% Fuxing EC; c: Myclobutanil 12.5% EC; d: Dimethachlon 40% WP; e: 25% Prochloraz EC.

iting the mycelial growth of all test fungi at a concentration of 500 μg/mL. Meanwhile, all of these compounds were found safe for the cucumber plants. As shown in Table 3, compound **4q** and **4u** exhibit good control effect against *C. orbiculare* (Berk aLMont) Arx. No fungicidal activities were found of these compounds against *S. sclerotiorum* (Lib.) de Bary, except that compound **4c** (53.37%) and **4n** (76.78%) displayed moderate control effect. Compounds **4h**, **4l**, **4n**, **and 4t** showed fair fungicidal activity (control effect 60–76%) against *E. cichoracearum*. Compounds **4a**, **4b**, **4d**, **4q**, and **4u** held above 50% control effect against *C. cucumerinum*. For the *C. cassiicola*, it was found that most of the halogen-substituted aryl derivatives have higher control effect than the other substituted aryl derivatives. Furthermore, some of the alkyl acylhydrazine had moderate control effect against *C. cassiicola*, such as compounds **4l**, **4m**, and **4q**.

Table 4: Summary of comparative molecular field analysis (CoMFA)

						Contributor (%)	
	q^2	r^2	*S*	*F*	No.	Steric	Electrostatic
CoMFA	0.566	0.913	0.325	97.186	**4f**	82.3	17.7

Comparative molecular field analysis

Comparative molecular field analysis method is widely used in drug design, because it allows for rapid prediction of QSAR of newly designed molecules (16). The results of these computations are summarized in Table 4. The compound **4f** was used as a template to build the other molecular structures. Because these compounds share a common skeleton, 16 atoms marked with an asterisk were used for rms-fitting onto the corresponding atoms of the template structure (Figure 1).

Experimental and predicted activities by CoMFA for all compounds are listed in Table S1. The plots of the predicted versus the actual activity values for all the compounds are shown in Figure S1. As shown in Table 4, a predictive CoMFA model was established with the conventional correlation coefficient r^2 = 0.913 and the cross-validated coefficient q^2 = 0.566. It is shown in Figure 2A,B that the contributions of steric and electrostatic fields ('SD*coeff') are 82.3% and 17.7%, respectively. With the CoMFA, we obtained the isocontour diagrams of the steric and electrostatic field contributions ('SD*coeff'), which is displayed in Figure 2. In Figure 2A, the steric field contours are represented with different colors: the green color at 2 position means that a bulky group here would be favorable for higher herbicidal activity, while the yellow color means oppositely. As shown in Figure 2A, there is a yellow region located around the 4 position of the benzene ring, indicating that the bulky groups at this position will decrease the herbicidal activity. This is in agreement with the actual experimental data: for example, compounds **4b**, **4c**, **and 4d** all have lower herbicidal activity with a

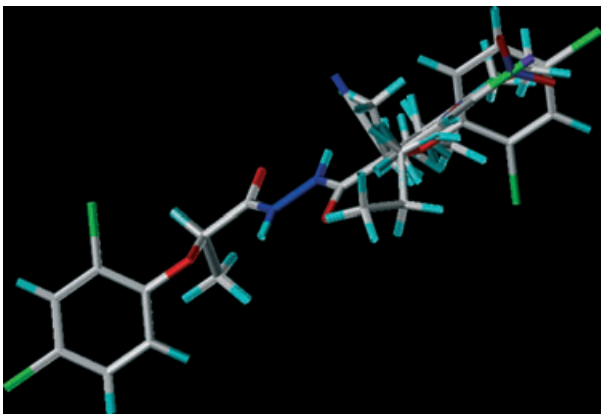


Figure 1: Superposition modes of compounds.

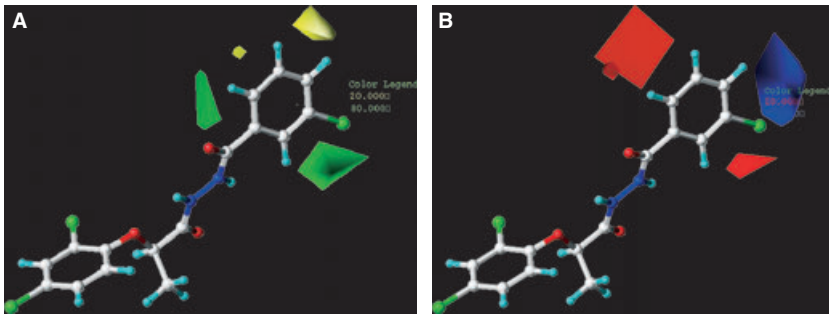


Figure 2: Steric (A) and Electrostatic (B) contribution contour maps of comparative molecular field analysis.

bulky group in this position. In the same figure, the electrostatic contours are displayed in distinguishable colors: blue means that an increase in the positive charge will lead to an increase in the activity, while the red contour defines in the opposite. So, the target compounds bearing an electron-withdrawing group at the 2 position or 3 position of the benzene ring and an electron-donating group at the other positions displayed higher activity. These results provide useful information for further optimization of the compounds. According to the aforementioned CoMFA, compound **4u** (*R* = *p*-OMe Ph) was designed, synthesized, and tested for its herbicidal activity against *B. napus*. The results indicated that the inhibition of compound **4u** is 94.7%, in which inhibition is as good as control. Meanwhile, the compound **4u** exhibited good fungicidal activity against *C. orbiculare* (Berk aLMont) Arx(82.16%).

In conclusion, a series of *N,N'*-diacylhydrazines bearing 2,4-dichlorophenoxy moiety were designed and synthesized *via* conventional and microwave irradiations. The herbicidal tests showed that these compounds have excellent herbicidal activity against the dicotyledon plants along with an interesting plant growth regulatory activity. Additionally, some of them also exhibited excellent fungicidal activities against *C. cassiicola*, *C. cucumerinum*, *E. cichoracearum*, *S. sclerotiorum*(Lib.)de Bary, and *C. orbiculare*(Berk aLMont) Arx.. Based on the biological screening data, these novel *N,N'*-diacylhydrazine derivatives could be the clues for the development of potential herbicides and fungicides. Further investigation of the structure–activity relationship on these molecules is currently in progress

Acknowledgments

This work was supported financially by National Natural Science Foundation of China (No.21002090, 31000008).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. The plots of the predicted versus the actual activity values for all the compounds.

Table S1. The structures, activities and total score of compounds.

Appendix S1. Analytical data for title compounds.

Appendix S2. The detailed biological methods.

Appendix S3. The detailed 3D-QSAR method.

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