JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Synthesis, Fungicidal Activity, and 3D-QSAR of Pyridazinone-Substituted 1,3,4-Oxadiazoles and 1,3,4-Thiadiazoles

XIA-JUAN ZOU,† LU-HUA LAI,*,† GUI-YU JIN,‡ AND ZU-XING ZHANG‡

State Key Laboratory for Structural Chemistry Studies of Stable and Unstable Species, Institute of Physical Chemistry, College of Chemistry and Molecular Engineering, Peking University, Beijing, P. R. China, 100871 and National Key Laboratory of Elemento-Organic Chemistry, Institute of Elemento-Organic Chemistry, Nankai University, Tianjin, P. R. China, 300071

A series of novel 5-[1-aryl-1,4-dihydro-6-methylpyridazin-4-one-3-yl] -2-arylamino-1,3,4-oxadiazoles, fungicidally active, were synthesized based on bioisosterism and tested in vivo against wheat leaf rust, *Puccinia recondita*. These compounds were shown to be fungicidally active, and their activity was influenced by the nature of the substituents. By using the three-dimensional quantitative structure−activity relationships (3D-QSAR) method of comparative molecular field analysis (CoMFA), we have studied the structure and activity relationship of the compounds containing both pyridazinone-substituted 1,3,4-thiadiazoles and pyridazinone-substituted 1,3,4-oxadiazoles. The 3D-QSAR modes gave good correlation between the variations on percent inhibition and the steric-electrostatic properties. The results are consistent with a common mode of action for the pyridazinone-substituted 1,3,4-thiadiazoles and the pyridazinone-substituted 1,3,4-oxadiazoles, which further confirms that the 1,3,4-oxadiazole ring is a bioisosteric analogue of the 1,3,4-thiadiazole ring. These offer important structural insights into designing highly active compounds prior to their synthesis.

KEYWORDS: Synthesis; pyridazinoneoxadiazoles; pyridazinonethiadiazoles; fungicides; wheat leaf rust; 3D-QSAR; CoMFA

INTRODUCTION

The idea of bioisosterism is one of the most successful techniques of bioactive compound design (*1*). The substitution of sulfur for oxygen in the heterocyclic ring represents an example of an approach that is commonly known as bioisosterism. The 1,3,4-oxadiazole ring is a bioisosteric analogue of the 1,3,4-thiadiazole ring. In previous papers, we reported that pyridazinone-substituted 1,3,4-thiadiazole exhibited highly fungicidal activity against wheat leaf rust, *Puccinia recondita* (*2, 3*). On the other hand, 1,3,4-oxadiazoles exhibit a broad spectrum of biological activity (*4*−*6*). In view of these facts and in continuation of our interest on the chemistry of pyridazinones (*7*−*9*), we contemplated undertaking the synthesis of an, as yet, unreported novel di-heterocyclic compound containing both pyridazinone and 1,3,4-oxadiazole moieties in order to obtain compounds possessing better biological activity.

The preliminary bioassay indicated that pyridazinone-substituted 1,3,4-oxadiazoles exhibited fungicidal activity against wheat leaf rust, *Puccinia recondita*, just as that of pyridazinone-substituted 1,3,4-thiadiazoles. Prompted by these results, and in an attempt to establish an agrophore model and find more potent analogues, three-dimensional quantitative structure−activity relationships (3D-QSAR) analysis has been performed on both pyridazinone-substituted 1,3,4-thiadiazoles and 1,3,4-oxadiazoles by using comparative molecular field analysis (CoMFA) (*10*−*13*).

MATERIALS AND METHODS

Synthetic Procedures. Melting points were measured on a Yanaco melting point apparatus and were uncorrected. Infrared spectra (IR) (potassium bromide) were recorded by a Shimadzu IR-435. ^{1}H NMR spectra were recorded on a JEOL FX-90Q spectrometer, and tetramethysilane was used as an internal standard (chemical shifts are in δ values). Elemental analyses (C, H, and N) were carried out on a MT-3 analyzer.

5-[1-Aryl-1,4-dihydro-6-methylpyridazin-4-one-3-yl]-2-arylamino-1,3,4-oxadiazoles (**4**). To a solution of compound **2** (0.5 mmol) in ethanol (20 mL) was added $Hg(OAc)_2$ (0.5 mmol). The reaction mixture was refluxed for 3 h and concentrated under reduced pressure. The solid was dissolved in hot *N*,*N*-dimethylformamide (DMF) and filtered. The filtrate was concentrated under reduced pressure and recrystallized from ethanol/DMF. The physical constants and spectral analyses of these substituted 1,3,4-oxadiazoles are listed in **Tables 1** and **2**.

Biological Assay. The antifungal activity was tested in vivo on wheat leaf rust, *Puccinia recondita*.

The local susceptible cultivated Ming-Xian 169 wheat plants (10 germinating seeds) were grown under greenhouse condition (T = 15−

* To whom correspondence should be addressed. E-mail: lai@mdl.ipc.pku.edu.cn. Tel: 086-10-62757520. Fax: 086-10-62751725.
† State Key Laboratory for Structural Chemistry Studies of Stable and Unstable Species.
‡ National Key Laboratory of Elemento-Organic Chemistry.

10.1021/jf0201677 CCC: $22.00
Published on Web 05/25/2002

Table 1. Physical Data of New Compounds **4**

compd.	R_1	R_2	yield (%)	mp (°C)	molecular	analysis (%, calcd.) C	H	N
4a	*o*-Cl	*m*-CF_3	90	142–4	$C_{20}H_{13}ClF_3N_5O_2$	53.56 (53.62)	2.85 (2.90)	15.62 (15.70)
4b	*o*-Cl	*o*-F	82.5	252–3	$C_{19}H_{13}ClFN_5O_2$	57.30 (57.41)	3.15 (3.27)	17.51 (17.70)
4c	*o*-Cl	H	84	266–9	$C_{19}H_{14}ClN_5O_2$	59.80 (60.05)	3.75 (3.68)	18.51 (18.51)
4d	H	*o*-F	80	245–6	$C_{19}H_{14}FN_5O_2$	62.57 (62.77)	3.95 (3.85)	19.32 (19.35)
4e	2,6-Cl_2	*o*-F	95	262–3	$C_{19}H_{12}Cl_2FN_5O_2$	52.43 (52.77)	2.74 (2.77)	16.47 (16.27)
4f	*p*-Cl	*m*-CF_3	90	278–80	$C_{20}H_{13}ClF_3N_5O_2$	53.52 (53.62)	3.25 (2.90)	15.78 (15.70)
4g	*p*-Cl	*o*-F	90	257–8	$C_{19}H_{13}ClFN_5O_2$	57.12 (57.33)	3.47 (3.27)	17.49 (17.68
4h	2,4,5-Cl_3	*o*-F	90	268–9	$C_{19}H_{11}Cl_3FN_5O_2$	48.85 (48.87)	2.40 (2.36)	15.25 (15.07)
4i	2,4–2CH_3	*m*-CF_3	83	251–2	$C_{22}H_{18}F_3N_5O_2$	59.60 (59.84)	3.98 (4.08)	15.97 (15.93)
4j	2,4–2CH_3	*o*-F	77.6	195–6	$C_{21}H_{18}FN_5O_2$	64.26 (64.41)	4.96 (4.60)	17.78 (17.97)
4k	*p*-Cl	*p*-OCH_3	78	268–70	$C_{20}H_{16}ClN_5O_3$	58.70 (58.58)	3.83 (3.90)	16.82 (17.16)

Table 2. Spectral Analyses of New Compounds **4**

compd.	IR(ν/cm^{-1})	δ ($DMSOd_6$, ppm)
4a	1627 (C═O)	2.04(s, 3H, CH_3), 6.80(s,1H, pyridazinone), 7.20∼8.00(m, 8H, $2C_6H_4$), 11.08(bs, NH)
4b	1620(C═O), 3206(NH)	2.04(s, 3H, CH_3), 6.80(s,1H, pyridazinone), 6.88∼8.20(m, 8H, $2C_6H_4$), 10.20(bs, NH)
4c	1634(C═O), 3242(NH)	2.04(s, 3H, CH_3), 6.64(s,1H, pyridazinone), 6.96∼7.84(m, 9H, C_6H_5, C_6H_4), 10.20(bs, NH)
4d	1645(C═O), 3285(NH)	2.00(s, 3H, CH_3), 6.36(s,1H, pyridazinone), 6.80∼8.00 (m, 9H, C_6H_5, C_6H_4), 10.80(bs, NH)
4e	1619(C═O), 3198(NH)	2.04(s, 3H, CH_3), 6.80(s,1H, pyridazinone), 6.88∼8.20(m, 7H, C_6H_3, C_6H_4), 10.10(bs, NH)
4f	1655(C═O), 3151(NH)	2.20(s, 3H, CH_3), 6.80(s,1H, pyridazinone), 7.20∼8.04(m, 8H, $2C_6H_4$), 11.08(bs, NH)
4g	1631(C═O),	2.20(s, 3H, CH_3), 6.80(s,1H, pyridazinone), 6.88∼8.20(m, 8H, $2C_6H_4$), 10.64(bs, NH)
4h	1641(C═O), 3260(NH)	2.20(s, 3H, CH_3), 6.80(s,1H, pyridazinone), 6.88∼8.40(m, 6H, C_6H_2, C_6H_4), 10.96(bs, NH)
4i	1630(C═O), 3267(NH)	2.04(s, 6H, $2CH_3$), 2.40(s, 3H, CH_3), 6.68(s,1H, pyridazinone), 7.12∼8.00(m, 7H, C_6H_3, C_6H_4),
4j	1628(C═O)	2.04(s, 6H, $2CH_3$), 2.20(s, 3H, CH_3), 6.68(s,1H, pyridazinone), 7.04∼8.40(m, 7H, C_6H_3, C_6H_4)
4k	1631(C═O)	2.20(s, 3H, CH_3), 3.68(s, 3H, CH_3), 6.68(s,1H, pyridazinone), 6.80∼7.68(m, 8H, $2C_6H_4$), 10.40(bs, NH)

20 °C). The tested compounds were dissolved in water/DMF (5:1 by volume, containing Sorpal-144) to 0.001 M solutions, and these solutions were applied to the wheat leaves as foliar sprays using a hand-held spray gun.

The wheat leaves were inoculated with the uredospore (and talcum powder, 1:10 by volume) of *Puccinia recondita* (the causal fungus of the wheat leaf rust). The plants were placed immediately in a moist chamber for 24 h. When the leaves were just dry, the solutions containing tested compounds (10 mL) were sprayed into each pot. The plants were placed in a greenhouse. The percentage of disease control in the treated pots was compared to that of pots with a treatment in the absence of the tested compounds, and fungicidal activity was evaluated 10 days after treatment. Three replicates were included in the evaluation. For comparative purposes, the commercial fungicide Triadimefon was tested under the same condition as the title compounds (at concentration of 100 ppm, it gave 100% control).

Computational Approaches. All molecular modeling techniques and CoMFA studies were performed on SGI indigo 2 workstations using SYBYL v. 6.4 molecular modeling software from Tripos Inc. (St. Louis, MO).

In this study, one of the most active compounds, **3m**, was used as a template to construct the three-dimensional models of all the compounds by replacing phenyl substitutents or modifying the heterocycle. The 3D structures of the entire set of these compounds were built using the SKETCH option and fully geometry-optimized using the standard Tripos force field, including the electrostatic term calculated from Gasteiger and Hückel atomic charges. The methods of steepest descent and conjugate gradients were used successively for energy minimization until the gradient value was smaller than 0.001 kcal/mol. The search routine of Sybyl was then employed for the systematic conformational search to find the global energy-minimum conformation.

The CoMFA studies were performed with the QSAR module of SYBYL for each combination of the two molecular fields: (Ste) steric and (Ele) electrostatic. The molecules were placed one at a time into a three-dimensional cubic lattice with 0.2-nm spacing. The steric (van der Waals) and electrostatic (Coulombic) fields were calculated at each grid point using an sp^3 carbon probe with a +1.0 charge. Calculated steric and electrostatic energies were set at 10 kcal/mol. Column filtering was set at 2 kcal/mol. The method of partial least squares (PLS) implemented in the QSAR module of SYBYL was used to construct and validate the models. Cross-validation was performed with the leave-one-out procedure. The optional number of components, *N*, retained for final PLS analyses was defined as the one that yielded the highest cross-validated q^2. The robustness of the models was internally evaluated by calculating the r^2, *s*, and *F* test values from the training set.

Data Set for Analysis. The pyridazinone-substituted 1,3,4-thiadiazoles and 1,3,4-oxadiazoles share a common core structure but differ from each other by the heterocyclic ring and the phenyl substituents. We selected 16 compounds from pyridazinone-substituted 1,3,4-thiadiazoles (*3*) and 11 compounds from pyridazinone-substituted 1,3,4-oxadiazoles to construct the CoMFA models. The compounds and activity data are given in **Table 3**.

RESULTS AND DISCUSSION

Synthesis. In our previous paper, we reported the synthesis of hydrazide **1** (*8*), thiosemicarbazides **2** (*9*), and pyridazinone-substituted 1,3,4-thiadiazoles **3** (*3*). Treatment of compounds **2** with $Hg(OAc)_2$ yields the corresponding 1,3,4-oxadiazoles **4** (**Scheme 1**).

Scheme 1

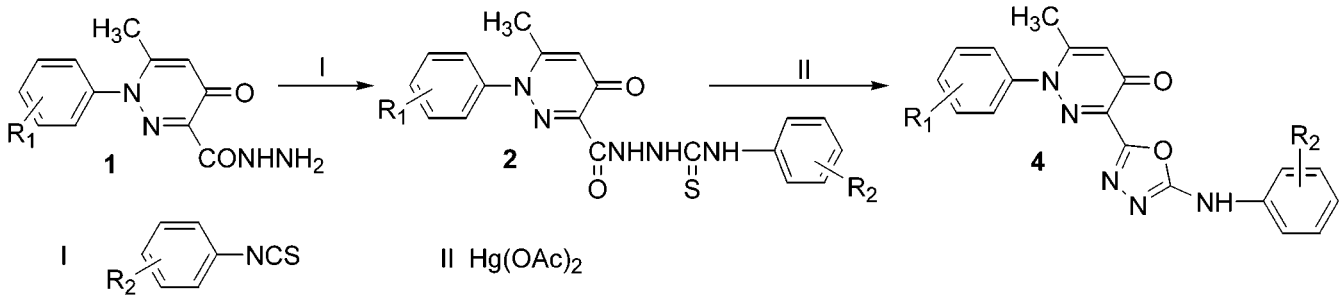

Table 3. Experimental and Calculated Activities and Residual Values of the Title Compounds

compd.	R_1	R_2	X	EA[a]	CoMFA	PA[b]	residue[c]
3a	*o*-Cl	*m*-CF_3	S	0.7	185	0.838	–0.138
3b	*o*-Cl	*o*-F	S	0.1	172	0.099	0.001
3c	*o*-Cl	H	S	0.1	165	0.032	0.068
3d	H	*o*-F	S	0.5	164	0.609	–0.109
3e	H	*m*-CF_3	S	0.6	180	0.544	0.056
3f	2,6-Cl_2	*m*-CF_3	S	0.5	184	0.489	0.011
3g	2,6-Cl_2	*o*-F	S	0.3	174	0.364	–0.064
3h	*p*-Cl	*m*-CF_3	S	0.6	192	0.574	0.026
3i	*p*-Cl	*o*-F	S	0.5	169	0.532	–0.032
3k	2,4,5-Cl_3	*o*-F	S	0.6	180	0.619	–0.019
3l	2,4,5-Cl_3	*m*-CF_3	S	0.7	179	0.681	0.019
3m	2,4–$2CH_3$	*m*-CF_3	S	0.9	199	0.909	–0.009
3n	2,4–$2CH_3$	*o*-F	S	0.9	181	0.800	0.100
3o	2,4-Cl_2	*o*-F	S	0.8	172	0.680	0.120
3p	2,4-Cl_2	*m*-CF_3	S	0.9	185	0.851	0.049
3q	2,4-Cl_2	H	S	0.2	171	0.280	–0.080
4a	*o*-Cl	*m*-CF_3	O	0.9	181	0.770	0.130
4b	*o*-Cl	*o*-F	O	0.8	165	0.768	0.032
4c	*o*-Cl	H	O	0.1	161	0.128	–0.028
4d	H	*o*-F	O	0.9	161	0.949	–0.049
4e	2,6-Cl_2	*o*-F	O	0.8	172	0.828	–0.028
4f	*p*-Cl	*m*-CF_3	O	0.9	172	0.967	–0.067
4g	*p*-Cl	*o*-F	O	0.7	160	0.681	0.019
4h	2,4,5-Cl_3	*o*-F	O	0.9	181	0.881	0.019
4i	2,4–$2CH_3$	*m*-CF_3	O	0.7	196	0.727	–0.027
4j	2,4–$2CH_3$	*o*-F	O	0.8	185	0.792	0.008
4k	*p*-Cl	*p*-OCH_3	O	0.8	187	0.810	–0.010

[a] Experimental activities (percent inhibition × 10^{-2}). [b] Predictive activities (percent inhibition × 10^{-2}). [c] Residue = EA − PA.

Fungicidal Activity. The fungicidal screening results of the compounds studied are summarized in **Table 3**. The results indicate that pyridazinone-substituted 1,3,4-oxadiazoles exhibited the same fungicidal activity as that of pyridazinone-substituted 1,3,4-thiadiazoles. The fungicidal activity varies with the substituents of the phenyl moiety.

3D-QSAR Analyses. *Alignment Rule.* Structural alignment is perhaps the most subjective, yet critical, step in a CoMFA study, inasmuch as experience shows that the resulting CoMFA model is often sensitive to the particular alignment scheme. In this study, we selected compound **3m** as the template molecule because of its scaffold being involved in all compounds and its high activity. Nine features were selected for the alignment of all compounds. These atoms are numbered 1−9 in **Figure 1**. The alignments of the bioactive conformation are showed in **Figure 2**.

CoMFA Analyses. The 3D-QSAR models gave a good cross-validated value of 0.584 with an optimized component of 5, the conventional correlation coefficient is $r^2 = 0.943$, $F = 69.281$, and the estimated standard error is 0.071. The contributions from the calculated steric and electrostatic fields in explaining the variations in the percent inhibition are 0.549 and 0.451, respectively. The predictive activity versus the experimental activity is graphically represented in **Figure 3**. The analyses results are presented in **Table 3**.

A unique feature of CoMFA is its ability to generate color-coded three-dimensional contour maps depicting regions in space around the molecules where variations in steric and electrostatic

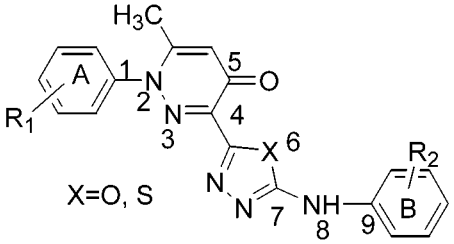

Figure 1. Superposition modes.

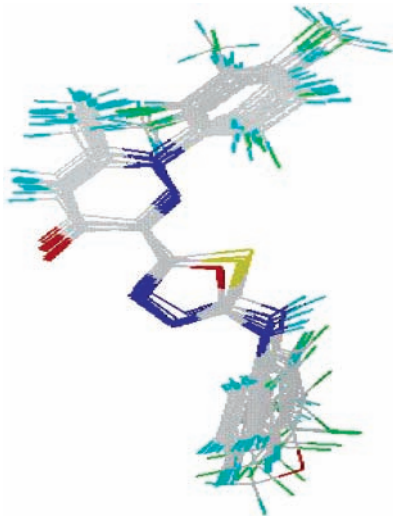

Figure 2. Alignment of 27 compounds.

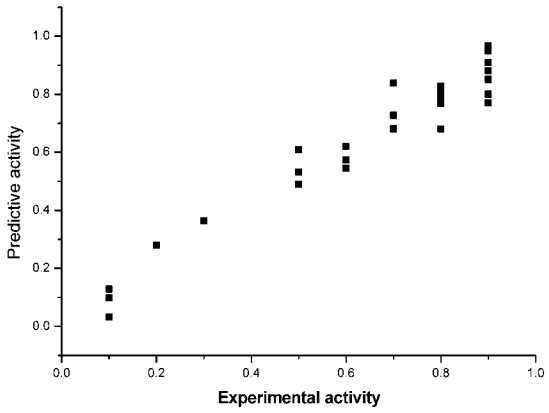

Figure 3. Predictive and experimental activities for the CoMFA model.

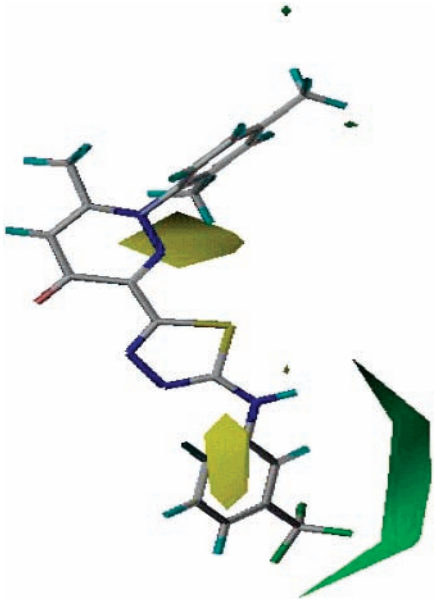

Figure 4. Steric field contour map of CoMFA.

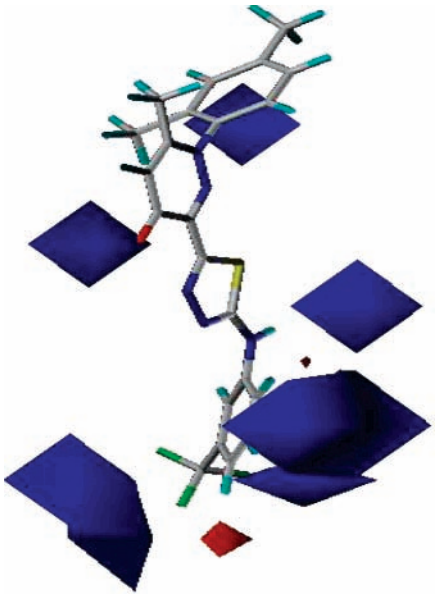

Figure 5. Electrostatic field contour map of CoMFA.

fields are most strongly correlated with variations in the percent inhibition. These CoMFA maps can serve as visual guides for designing novel and more potent analogues. The CoMFA contour maps of this series of compounds are presented in **Figures 4** and **5**, respectively. The green−yellow steric contour in **Figure 4** describes that region in space where increasing (green) or decreasing (yellow) steric bulk is consistent with enhanced percent inhibition. The red−blue electrostatic contour in **Figure 5** illustrates that the large blue areas are the regions where positive charge is favorable for the percent inhibition; red areas are unfavorable. One red polyhedra near the *para*-phenyl ring B moiety indicates that electron rich groups are beneficial to the activity. Six blue contours in the molecules suggest that positive-charged substituents are favorable to increase the activity.

In summary, 3D-QSAR analyses have been performed on both 16 pyridazinone-substituted 1,3,4-thiadiazoles and 11 pyridazinone-substituted 1,3,4-oxadiazoles. The 3D-QSAR models gave good correlation between the variations on the percent inhibition and the steric-electrostatic properties. These results are consistent with a common mode of action for the pyridazinone-substituted 1,3,4-thiadiazoles and the pyridazinone-substituted 1,3,4-oxadiazoles, which further confirms that the 1,3,4-oxadiazole ring is a bioisosteric analogue of the 1,3,4-thiadiazole ring.

LITERATURE CITED

(1) Lipinski, C. A. Bioisosterism in drug design. *Annu. Rep. Med. Chem.* **1986**, *21*, 283.

(2) Zou, X.-J.; Jin, G.-Y. Synthesis of pyridazinonethiadiazoles as possible antifungal agents. *Chin. Chem. Lett.* **2001**, *12* (5), 419−420.

(3) Zou, X.-J.; Jin, G.-Y.; Zhang, Z.-X. Synthesis, funigicidal activity, and QSAR of pyridazinonethiadiazoles. *J. Agric. Food Chem.* **2002**, *50* (6), 1451−1454.

(4) Holla, B. S.; Gonsalves, R.; Shenoy, S. Synthesis and antibacterial studies of a new series of 1,2-bis(1,3,4-oxadiazole-2-yl) ethanes and 1,2-bis(4-amino-1,2,4-triazole-3-yl) ethanes. *Eur. J. Med. Chem.* **2000**, *35* (2), 267.

(5) Chen, H.; Li, Z.; Han, Y. Synthesis and Fungicidal Activity against *Rhizoctonia solani* of 2-Alkyl(Alkylthio)-5-pyrazolyl-1,3,4-oxadiazoles (Thiodiazoles). *J. Agric. Food Chem.* **2000**, *48*, 5312−5315.

(6) Ashour, F. A.; El-Hawash, S. A. M.; Mahran, M. A.; et al. Synthesis, antibacterial and antifungal activity of some new 1,3,4-oxadiazoles and 2-substituted amino-1,3,4-oxadiazoles containing benzimidazole moiety. *Bull. Pharm. Sci., Assiut Univ.* **1994**, *17* (1), 17.

(7) Zou, X.-J.; Jin, G.-Y.; Yang, Z. Synthesis of the hydrazides of 1-aryl-1,4-dihydro-3-carboxy-6-methyl-4-pyridazinone and their antiviral activity against TMV. *Chin. J. Appl. Chem.* **2001**, *18* (8), 599−601.

(8) Zou, X.-J.; Jin, G.-Y.; Yang, Z. Synthesis of the thiosemicarbazides of 1-aryl-1,4-dihydro-3-carboxy-6-methyl-4-pyridazinone and their antiviral activity against TMV. *Chem. J. Chin. Univ.* **2002**, *23* (3), 404−406.

(9) Zou, X.-J.; Jin, G.-Y. 1-Aryl-1,4-dihydro-3-Acylhydrazinocarbonyl-6-Methylpyridazin-4-one and Their Biological Activity. *Chin. J. Pestic. Sci.* **2001**, *3* (1), 17.

(10) Cramer, R. D.; Pattern, D. E.; Bunce, J. D. Comparative molecular field analysis (CoMFA). 1. Effect of shape on binding of steroids to carrier proteins. *J. Am. Chem. Soc.* **1988**, *110*, 5959.

(11) Chavatte, P.; Yous, S.; Marot, C.; Baurin, N.; Lesieur, D. Three-dimensional quantitative structure−activity relationships of cyclooxygenase-2 (COX-2) inhibitors: a comparative molecular field analysis. *J. Med. Chem.* **2001**, *44*, 3223.

(12) Collantes, E. R.; Xing, L.; Miller, P. C.; Welsh. W. J.; Salvatore Profeta, J. Comparative Molecular Field Analysis as a Tool to Evaluate Mode of Action of Chemical Hybridization agents. *J. Agric. Food Chem.* **1999**, *47*, 5245.

(13) Wang, R.-X.; Gao, Y.; Liu, L.; Lai, L.-H. All-orientation search and all-placement search in comparative molecular field analysis. *J. Mol. Model.* **1998**, *4*, 276.

Received for review February 7, 2002. Revised manuscript received April 12, 2002. Accepted April 17, 2002. Financial support of this work by the National Natural Science Foundation of China (project 29832050) is gratefully acknowledged.

JF0201677