JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Synthesis and Antiviral Activities of Pyrazole Derivatives Containing an Oxime Moiety

GUIPING OUYANG, XUE-JIAN CAI, ZHUO CHEN, BAO-AN SONG,*
PINAKI S. BHADURY, SONG YANG, LIN-HONG JIN, WEI XUE, DE-YU HU, AND SONG ZENG

Center for Research and Development of Fine Chemicals, Key Laboratory of Green Pesticide and Agricultural Bioengineering, Ministry of Education, Guizhou University, Guiyang 550025, People's Republic of China

Target compounds **4a**−**n** were obtained by the reaction of 1-substituted phenyl-3-methyl-5-substituted phenylthio-4-pyrazolaldoximes (**3**) with chloromethylated heterocyclic compounds ($ClCH_2$-R_3) under reflux conditions in ethanol. Subsequently, the oxidation of **4a**−**e** with $KMnO_4$ in HOAc at room temperature afforded eight new compounds, **5a**−**h**. The synthesized compounds were characterized by physical constants, and the structures of the title compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR, and elemental analysis. The bioassay revealed that the compounds possessed antiviral activities. It was found that title compounds **4a** and **4g** had the same inactivation effects against TMV (EC_{50} = 58.7 and 65.3 μg/mL) as the commercial product Ningnanmycin (EC_{50} = 52.7 μg/mL). To the best of our knowledge, this is the first report on the antiviral activity of pyrazole derivatives containing an oxime moiety.

KEYWORDS: Pyrazole; oxime ethers moiety; antiviral activity; synthesis

INTRODUCTION

Pyrazole and its derivatives, a class of hererocyclic compounds, occupy an important position in medicinal and pesticide chemistry with a wide range of bioactivities. As medicines, many of them display antibacterial (*1*), antimicrobial (*2*), anticancer (*3*), antiinflammatory (*4*), and selective enzyme inhibitory activities (*5*). As pesticides, they are used as insecticides (*6*), fungicides (*7, 8*), and herbicides (*9*−*12*). As shown in **Figure 1**, for example, Pyrazophos, the first fungicide of this class to be commercialized, was put on the market by Hoechst AG in 1974 to control powdery mildew in vegetables, and more than 10 pyrazole derivatives are now commercially available. The advantages, such as a novel mode of action, wide spectrum, low toxicity toward mammalian cells, and favorable profiles to humans, have prompted chemists to design and synthesize novel pyrazole derivatives. Recently, pyrazole compounds, such as Penthiopyrad (Mitsui Toatsu Chemicals, 1995) and Pyraclostrobin (BASF, 2001), have been found to have potential antifungal activities for the control of some plant diseases. With growing applications on their synthesis and bioactivity, chemists and biologists in recent years have directed considerable attention to the research of pyrazole derivatives (*13*).

Tobacco mosaic virus (TMV) infection is very widely distributed and can cause serious damage and large economic loss. It has been found that in certain fields, 90−100% of the plants show mosaic by harvest time. In view of the unsatisfactory curatives (30−60%) cure rate obtained by common antiviral agents (Ningnanmycin or Virus A) and widespread economic loss of tobacco, further research needs to be conducted in this area for the development of a highly efficient, novel, environmentally benign antiviral agent.

Modification of the structural profile by the change of substituents at the 1-, 3-, or 4-position in pyrazole ring can bring about significant change in bioactivity. However, pyrazolaldoxime ethers bearing other heterocyclic moieties are scarcely evaluated for their activities (*14, 15*). It has been demonstrated that the pyridine and pyrazole groups are important pharmacophores of many fungicides. Linking the pyridine and pyrazole group with a structurally diverse side chain is an effective way to obtain new heterocyclic derivatives with high fungicidal activities (*13, 16*). We assumed that if the pyridine and pyrazole pharmacophores were introduced into the pyrazole scaffold, the resulting compounds should have an interesting lead structure for antiviral agent development (**Figure 2**), which will be

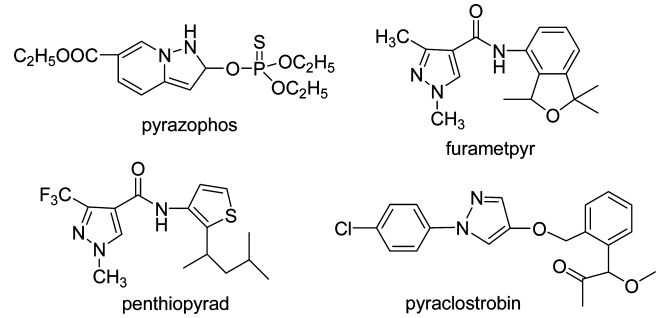


Figure 1. The structures of commercial fungicides.

10.1021/jf802489e CCC: $40.75
Published on Web 10/22/2008

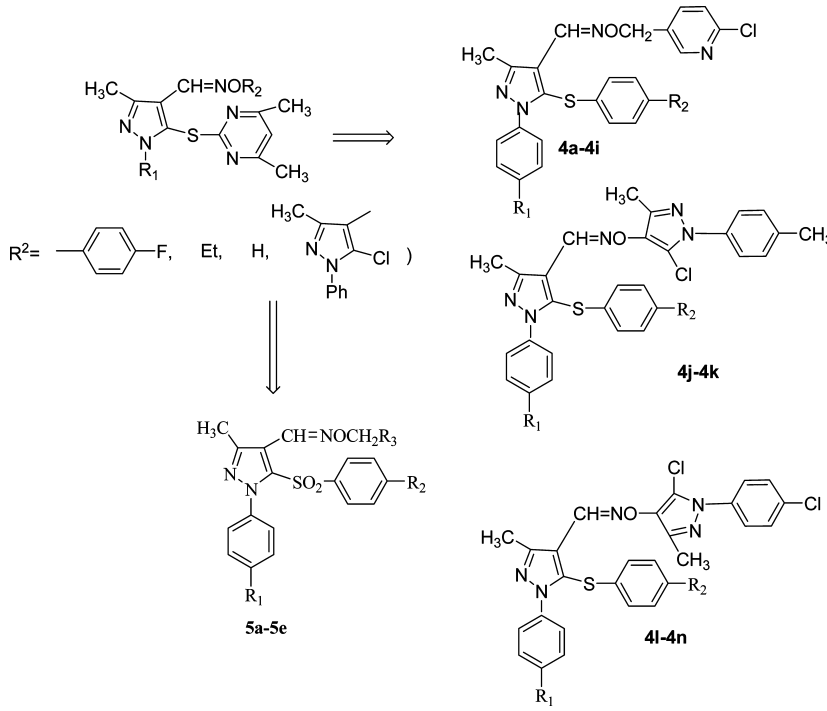


Figure 2. Design of the target compounds.

expected to exhibit interesting features due to the coexistence of two kinds of fungicidal pharmacophores with different action mechanisms. In continuation of our research program of the discovery of novel lead compounds (*17*), we describe herein the synthesis and antiviral activity of a series of new pyrazolaldoxime ether analogues (**3**). Meanwhile, as a control, we also synthesized and measured their antiviral activities in a series of pyrazolaldoxime ether analogues (**4**). The synthetic route is shown in **Scheme 1**. Starting from the key intermediate, 1-substitutedphenyl-3-methyl-5-substituted phenylthio-4-pyrazolaldoximes **3**, the title compounds **4** and **5** are synthesized by etherification with chloromethylated heterocyclic compound followed by potassium permangante oxidation in acetic acid. The structures of **4** and **5** were firmly established by well-defined IR, ^{1}H NMR, ^{13}C NMR, and elemental analysis. Preliminary bioassay tests showed that some compounds possess a certain degree of antiviral activity against TMV at 500 mg/L in vivo as shown in **Tables 1** and **2**, however, with a degree of variation. The bioassay results showed that title compounds **4a**,**g** possess high inactivation activities against TMV, and the EC_{50} values range from 58.7 to 65.3 μg/mL.

MATERIAL AND METHODS

Instruments. The melting points of the products were determined on a XT-4 binocular microscope (Beijing Tech Instrument Co., China) and were not corrected. The IR spectra were recorded on a Bruker VECTOR 22 spectrometer in KBr disk. ^{1}H, ^{13}C, and ^{31}P NMR (solvent DMSO-d_6) spectra were performed on a JEOL-ECX 500 NMR spectrometer at room temperature using tetramethylsilane as an internal standard. Elemental analysis was performed on an Elementar Vario-III CHN analyzer. Analytical thin-layer chromatography was performed on silica gel GF_{254}. Column chromatographic purification was carried out using silica gel. All reagents were of analytical reagent grade or chemically pure. All solvents were dried, deoxygenated, and redistilled before use.

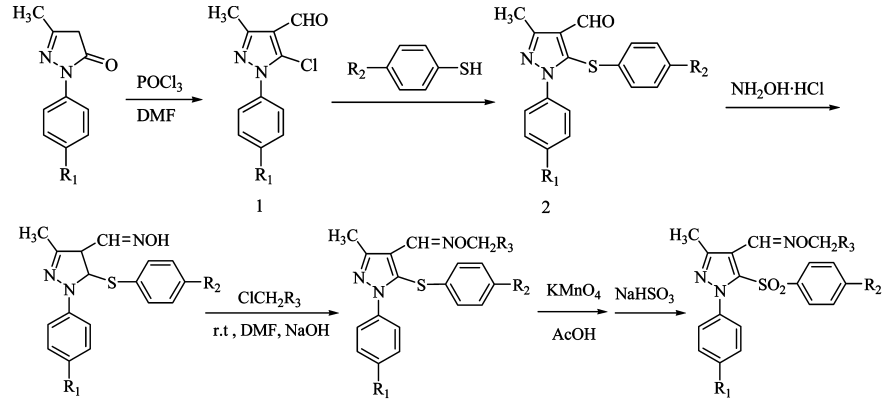
Synthetic Procedures. 1-Aryl-3-methyl-1*H*-4-pyrazoleylformaldehydes and 1-(4-chlorophenyl)-3-methyl-1*H*-4-pyrazoleylformaldehydes were prepared according to the literature method as described (*18, 19*). Intermediates 1-aryl-3-methyl-5-chloro-1*H*-4-pyrazolylformaldehydes **1**−**3** were prepared according to the reported methods (*20, 21*).

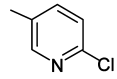
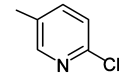
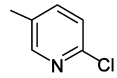
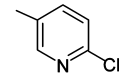
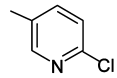
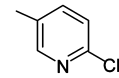
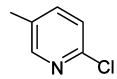
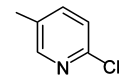
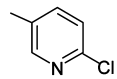
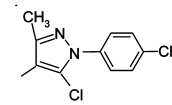
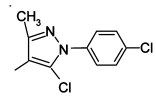
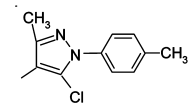
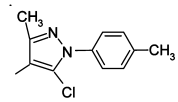
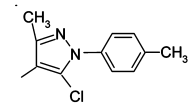
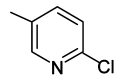
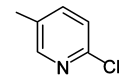
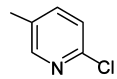
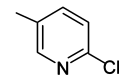
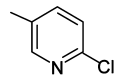
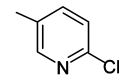
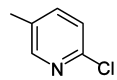
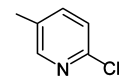
General Procedure for the Preparation of Title Compounds 4a−n. A 100 mL round-bottomed flask equipped with a magnetic stirrer was charged with **3** (10 mmol), dimethylformamide (DMF) (50 mL), and NaOH (12 mmol). The flask was stirred at room temperature for 10 min, and then, R_3CH_2Cl (0.012mol) was added dropwise over a period of 10 min and stirred at room temperature for 4 h. The mixture was poured into ice water and filtered. The white solid that resulted was washed with distilled water, dried under vacuum, and recrystallized from ethanol to give the title compounds **4a**−**n** in 54−87% yields.

*Data for 1-Phenyl-3-methyl-5-(4-methylphenylthio)-4-pyrazolaldoxime-(2-chloropyridine-5-ylmethyl) Ether (***4a***).* White crystal; mp 95−96 °C; yield, 81%. IR (KBr, cm^{-1}): ν 3063 (Ar−H), 2923, 2867 (CH_3 + CH_2), 1593 (*C*═N), 1495, 1458 (Ar skeleton vibration), 1216 (*C*═N−N), 845 (*p*-disubstituted benzene), 655 (C−S−C) cm^{-1}. ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 8.42 (d, J = 2.30 Hz, 1H, Py-6-H), 8.25 (s, 1H, CH-H), 7.70 (dd, J = 2.30 Hz, J = 2.30 Hz, 1H, Py-4-H), 7.38−7.40 (m, 6H, 1-Ph-H, Py-3-H), 6.99 (d, J = 8.55 Hz, 2H, S-Ph-2, 6-H), 6.83 (d, J = 8.60 Hz, 2H, S-Ph-3, 5-H), 5.12 (s, 2H, CH_2−H), 2.52 (s, 3H, pyrazole-CH_3-H), 2.26 (s, 3H, Ph-CH_3-H). ^{13}C NMR (DMSO-d_6, 125 MHz, ppm): δ 151.1, 149.9, 148.9, 144.0, 139.2, 138.9, 1136.9, 133.0, 132.4, 131.2, 130.2, 128.8, 128.4, 127.8, 125.6, 124.1, 118.7, 77.1, 21.0, 14.9. Anal. calcd for $C_{24}H_{21}ClN_4OS$ (448.5): C, 64.24%; H, 4.66%; N, 12.46%. Found: C, 64.32%; H, 4.38%; N, 12.60%.

*1-Phenyl-3-methyl-5-(4-methoxyphenylthio)-4-pyrazolaldoxime-(2-chloropyridine-5-ylmethyl) Ether (***4b***).* White crystal; mp 95−96 °C; yield, 81%. IR (KBr, cm^{-1}): ν 3094 (Ar−H), 2963, 2829 (CH_3 + CH_2), 1593 (*C*═N), 1591, 1386 (Ar skeleton vibration), 1246 (*C*═N−N), 827 (*p*-disubstituted benzene), 665 (C−S−C). ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 8.46 (d, J = 2.30 Hz, 1H, Py-6-H), 8.23 (s, 1H, CH-H), 7.88 (dd, J = 2.30 Hz, J = 2.30 Hz, 1H, Py-4-H), 7.44−7.53 (m, 6H, 1-Ph-H, Py-3-H), 6.91 (d, J = 9.20 Hz, 2H, S-Ph-3,5-H), 6.81 (d, J = 9.15 Hz, 2H, S-Ph-2, 6-H), 5.17 (s, 2H, CH_2−H), 3.68 (s, 3H, O-CH_3−H), 2.37 (s, 3H, CH_3−H). ^{13}C NMR (DMSO-d_6, 125 MHz, ppm): δ 169.3, 160.9, 150.4, 150.3, 143.6, 140.5, 138.9, 134.1, 133.3, 130.9, 129.4, 128.9, 126.1, 124.5, 117.7, 116.6, 115.7, 72.4, 55.7, 14.8. Anal. calcd for $C_{24}H_{21}ClN_4O_2S$

Scheme 1. Synthetic Route to Pyrazole Analogues **4** and **5** Containing Oxime



Compd.	R_1	R_2	R_3	Compd.	R_1	R_2	R_3
4a	H	Me		**4b**	H	OMe	
4c	H	F		**4d**	3-Cl	F	
4e	4-Cl	Me		**4f**	4-Cl	MeO	
4g	4-Cl	F		**4h**	4-Me	F	
4i	4-Me	Me		**4j**	4-Me	F	
4k	H	F		**4l**	4-Me	Me	
4m	4-Me	F		**4n**	4-Cl	Me	
5a	H	Me		**5b**	H	OMe	
5c	H	F		**5d**	3-Cl	F	
5e	4-Cl	Me		**5f**	4-Cl	MeO	
5g	4-Cl	F		**5h**	4-Me	F	

(464.5): C, 62.00%; H, 4.52%; N, 12.05%. Found: C, 61.89%; H, 4.20%; N, 11.90%.

*1-Phenyl-3-methyl-5-(4-fluorophenylthio)-4-pyrazolaldoxime-(2-chloropyridine-5-ylmethyl) Ether (***4c***).* White crystal; mp 95−96 °C; yield, 81%. IR (KBr, cm^{-1}): ν 3055 (Ar−H), 2925 (CH_3), 2974 (CH_3), 1591 (*C*═N), 1492 (Ar, skeleton vibration), 1225 (*C*═N−N), 821(*p*-disubstituted benzene), 652 (C−S−C). ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 8.44 (d, J = 2.30 Hz, 1H, Py-6-H), 8.26 (s, 1H, CH-H), 7.70 (dd, J = 2.30 Hz, J = 2.30 Hz, 1H, Py-4-H), 7.27−7.39 (m, 6H, 1-Ph-H, Py-3-H), 6.90−6.84 (m, 4H, S-Ph-2, 3, 5, 6-H), 5.14 (s, 2H, CH_2−H), 2.48 (s, 3H, CH_3−H). ^{13}C NMR (DMSO-d_6, 125 MHz, ppm): δ 162.9, 160.9, 150.9, 149.9, 149.1, 143.6, 139.2, 132.3, 130.2, 130.1, 128.8, 128.5,125.6, 124.1, 118.5, 116.6, 116.4, 72.7,14.8. Anal. calcd for $C_{23}H_{18}ClFN_4OS$ (452.5): C, 60.95%; H, 3.96%; N, 12.38%. Found: C, 60.85%; H, 3.79%; N, 12.10%.

*1-(3-Chlorophenyl-3-methyl-5-(4-fluorophenylthio)-4-pyrazolaldoxime-(2-chloro-pyridine-5-ylmethyl) Ether (***4d***).* White crystal; mp 95−96 °C; yield, 81%. IR (KBr, cm^{-1}): ν 3041 (Ar−H), 2855 (CH_3 + CH_2), 16411 (*C*═N), 1589, 1493 (Ar, skeleton vibration), 1226 (*C*═N−N), 831(*p*-disubstituted benzene), 676 (C−S−C). ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 8.43 (d, J = 2.25 Hz, 1H, Py-6-H), 8.24 (s, 1H, CH-H), 7.69 (dd, J = 2.85 Hz, J = 2.30 Hz, 1H, Py-4-H), 7.39 (s, 1H, 1-Ph-2-H), 7.26−7.36 (m, 5H, 1-Ph-H, Py-3-H), 6.88−6.70 (m, 4H, S-Ph-H), 5.12 (s, 2H, CH_2−H), 2.49 (s, 3H, pyrazole-CH_3−H). ^{13}C NMR (DMSO-d_6, 125 MHz, ppm): δ 162.9, 161.0, 151.1, 149.9, 149.3, 143.4, 137.2, 134.2, 132.8, 132.2, 130.1, 129.1, 129.0, 124.1, 119.1, 118.5,116.7,116.5,72.8,14.8. Anal. calcd for $C_{23}H_{17}Cl_2FN_4OS$(487.0): C, 56.65%; H, 3.45%; N, 11.46%. Found: C, 56.47%; H, 3.60%; N, 11.28%.

Table 1. Protection Effect, Inactivation Effect, and Curative Effect of the New Compounds against TMV in Vivo[a]

agents	concentration (μg/mL)	protection effect (%)	inactivation effect (%)	curative effect (%)
4a	500	31.3*	90.4**	47.0*
4b	500	16.4	71.2**	26.6*
4c	500	18.9*	65.9*	24.0*
4d	500	28.6*	61.7*	34.3*
4e	500	37.7*	61.2*	26.0*
4f	500	22.0*	34.5*	12.9
4g	500	43.5*	80.6**	37.3*
4h	500	31.0*	56.6*	22.0
4i	500	15.7	61.0*	32.1*
4j	500	19.0	43.0*	31.9
4k	500	32.0*	55.9*	35.6*
4l	500	44.8*	51.2*	38.9*
4m	500	41.0*	50.9**	22.0*
4n	500	21.0	36.7*	18.9
5a	500	28.0*	45.0*	9.0a
5b	500	29.8*	30.1**	15.6*
5c	500	40.2*	20.6	21.7
5d	500	12.9	30.0*	11.0
5e	500	31.3	33.3	9.2
5f	500	16.4	30.9	3.6
5g	500	42.2*	44.1*	7.7*
5h	500	28.2	11.0	22.0
Ningnamycin	500	57.0*	98.0**	54.8*

[a] $n = 3$ for all groups; *$P < 0.05$, and **$P < 0.01$.

Table 2. Antiviral Activities in Vivo (%) of Compounds **4a**,**b**,**g**

TMV concentration (μg mL^{-1})	inactivation effect 500	250	125	62.5	31.8	EC_{50} (μg mL^{-1})
4a	90.4	76.0	69.2	55.1	40.1	58.7
4b	71.0	65.0	48.9	41.0	32.0	115.0
4g	80.6	65.7	53.0	51.2	40.6	65.3
Ningnamycin	98.0	81.1	72.0	59.9	44.0	52.7

*1-(4-Chlorophenyl-3-methyl-5-(4-methylphenylthio)-4-pyrazolaldoxime-(2-chloropyridine-5-ylmethyl) Ether (***4e***).* White crystal; mp 103−105 °C; yield, 87%. IR (KBr, cm^{-1}): ν 3007 (Ar−H), 2978, 2887 (CH_3 + CH_2), 1615 (*C*═N), 1579, 1489 (Ar, skeleton vibration), 1216 (*C*═N−N), 845 (*p*-disubstituted benzene), 653 (C−S−C). ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 8.42 (d, J = 2.30 Hz, 1H, Py-6-H), 8.24 (s, 1H, CH-H), 7.69 (dd, J = 2.30 Hz, J = 2.30 Hz, 1H, Py-4-H), 7.26−7.34 (m, 5H, 1-Ph-H, Py-3-H), 6.99 (d, J = 8.55 Hz, 2H, S-Ph-2, 6-H), 6.81 (d, J = 8.60 Hz, 2H, S-Ph-3, 5-H), 5.12 (s, 2H, CH_2−H), 2.47 (s, 3H, pyrazole-CH_3-H), 2.27 (s, 3H, Ph-CH_3-H). ^{13}C NMR (DMSO-d_6, 125 MHz, ppm): δ 149.8, 149.2, 143.7, 139.1, 137.3, 137.0, 132.2, 130.8, 130.1, 128.9, 127.6, 126.6, 124.0, 127.6, 124.1, 119.8, 117.7, 116.7, 77.2, 72.6, 20.9, 14.8. Anal. calcd for $C_{24}H_{20}Cl_2N_4OS$ (483.0): C, 59.63%; H, 4.14%; N, 11.55%. Found: C, 59.45%; H, 4.02%; N, 11.48%.

*1-(4-Chlorophenyl-3-methyl-5-(4-methoxyphenylthio)-4-pyrazolaldoxime-(2-chloropyridine-5-ylmethyl) Ether (***4f***).* White crystal; mp 103−105 °C; yield, 87%. IR (KBr, cm^{-1}): ν 3041 (Ar−H), 2921, 2855 (CH_3 + CH_2), 1611 (*C*═N), 1589, 1493 (Ar, skeleton vibration), 1226 (*C*═N−N), 831 (*p*-disubstituted benzene), 676 (C−S−C). ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 8.43 (d, J = 2.25 Hz, 1H, Py-6-H), 8.24 (s, 1H, CH-H), 7.69 (dd, J = 2.85 Hz, J = 2.30 Hz, 1H, Py-4-H), 7.39 (s, 1H, 1-Ph-2-H), 7.26−7.36 (m, 4H, 1-Ph-3, 5, 6-H, Py-3-H), 6.88−6.70 (m, 4H, S-Ph-H), 5.12 (s, 2H, CH_2-H), 3.68(s, 3H, O-CH_3-H), 2.49 (s, 3H, pyrazole-CH_3-H). ^{13}C NMR (DMSO-d_6, 125 MHz, ppm): δ 162.9, 161.0, 151.1, 149.9, 149.3, 143.4, 137.2, 134.2, 132.8, 132.2, 130.1, 129.1, 129.0 124.1, 119.1, 118.5, 116.7, 116.5, 72.8, 5.7, 14.8. Anal. calcd for $C_{24}H_{20}Cl_2N_4O_2S$ (499.0): C, 57.75%; H, 4.00%; N, 11.22%. Found: C, 57.52%; H, 4.16%; N, 11.08%.

*1-(4-Chlorophenyl-3-methyl-5-(4-fluorophenylthio)-4-pyrazolaldoxime-(2-chloropyridine-5-ylmethyl) Ether (***4g***).* White crystal; mp 103−105 °C; yield, 87%. IR (KBr, cm^{-1}): ν 3041 (Ar−H), 2921, 2855 (CH_3 + CH_2), 1611 (*C*═N), 1589, 1493 (Ar, skeleton vibration), 1226 (*C*═N−N), 831 (*p*-disubstituted benzene), 676 (C−S−C). ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 8.43 (d, J = 2.25 Hz, 1H, Py-6-H), 8.24 (s, 1H, CH-H), 7.69 (dd, J = 2.85 Hz, J = 2.30 Hz, 1H, Py-4-H), 7.26−7.36 (m, 5H, 1-Ph-H, Py-3-H), 6.88 (m, 4H, S-Ph-H), 5.12 (s, 2H, CH_2-H), 2.49 (s, 3H, pyrazole-CH_3-H). ^{13}C NMR (DMSO-d_6, 125 MHz, ppm): δ 162.9, 161.0, 151.1, 149.9, 149.3, 143.4, 137.2, 134.2, 132.8, 132.2, 130.1, 129.1, 129.0 124.1, 119.1, 116.7, 116.5, 72.8,14.8. Anal. calcd for $C_{23}H_{17}Cl_2FN_4OS$ (487.0): C, 56.67%; H, 3.49%; N, 11.50%. Found: C, 56.50%; H, 3.60%; N, 11.30%.

*1-(4-Methylphenyl-3-methyl-5-(4-fluorophenylthio)-4-pyrazolaldoxime-(2-chloropyridin-5-ylmethyl) Ether (***4h***).* White crystal; mp 129−130 °C; yield, 78%. IR (KBr, cm^{-1}): ν 3023 (Ar−H), 2935, 2867 (CH_3 + CH_2), 1611 (*C*═N), 1598, 1497 (Ar, skeleton vibration), 1228 (*C*═N−N), 846 (*p*-disubstituted benzene), 658 (C−S−C). ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 8.43 (d, J = 2.25 Hz, 1H, Py-6-H), 8.16 (s, 1H, CH-H), 7.87 (dd, J = 2.30 Hz, J = 2.90 Hz, 1H, Py-4-H), 7.52 (d, J = 8.00 Hz, 1H, Py-3-H), 7.30 (d, J = 8.00 Hz, 2H, 1-Ph-2, 6-H), 7.25 (d, J = 8.60 Hz, 2H, 1-Ph-3, 5-H), 6.98 (t, J = 1.70 Hz, J = 2.90 Hz, 2H, S-Ph-2, 6-H), 6.95 (dd, J = 2.30 Hz, J = 2.90 Hz, 2H, S-Ph-3,5-H), 5.14 (s, 2H, CH_2-H), 2.49 (s, 3H, pyrazole-CH_3-H), 2.37 (s, 3H, Ph-CH_3-H). ^{13}C NMR (DMSO-d_6, 125 MHz, ppm): δ 162.4, 161.0, 151.1, 149.9, 149.3, 143.4, 137.2, 134.2, 132.8, 132.2, 130.1, 129.1, 129.0, 124.5, 119.1, 117.9, 117.2, 72.1, 21.1, 14.7. Anal. calcd for $C_{24}H_{20}ClFN_4OS$ (466.5): C, 61.74%; H, 4.27%; N, 12.00%. Found: C, 61.55%; H, 4.21%; N, 11.85%.

*1-(4-Methylphenyl-3-methyl-5-(4-methylphenylthio)-4-pyrazolaldoxime-(2-chloropyridine-4-ylmethyl) Ether (***4i***).* White crystal; mp 106−108 °C; yield, 81%. IR (KBr, cm^{-1}): ν 3031 (Ar−H), 2937, 2868 (CH_3 + CH_2), 1623 (*C*═N), 1587, 1487 (Ar, skeleton vibration), 1221 (*C*═N−N), 843 (*p*-disubstituted benzene), 656 (C−S−C). ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 8.42 (d, J = 2.20 Hz, 1H, Py-6-H), 8.12 (s, 1H, CH-H), 7.86 (dd, J = 2.30 Hz, J = 2.85 Hz, 1H, Py-4-H), 7.51 (d, J = 8.00 Hz, 1H, Py-3-H), 7.31 (d, J = 8.60 Hz, 2H, 1-Ph-2, 6-H), 7.25 (d, J = 8.60 Hz, 2H, 1-Ph-3, 5-H), 7.06 (d, J = 8.05 Hz, 2H, S-Ph- 2, 6-H), 6.82 (d, J = 8.60 Hz, 2H, S-Ph-3, 5-H), 5.14 (s, 2H, CH_2−H), 2.37 (s, 3H, pyrazole-CH_3-H), 2.33, 2.21 (2s, 6H, Ph-CH_3-H). ^{13}C NMR (DMSO-d_6, 125 MHz, ppm): δ 150.4, 148.2, 143.5, 140.5, 138.6, 136.5, 133.2, 132.6, 131.4, 130.7, 129.8, 127.5, 125.8, 124.5, 117.2, 72.3,21.1, 20.9, 14.8. Anal. calcd for $C_{25}H_{23}ClN_4OS$ (462.5): C, 64.86%; H, 4.97%; N, 12.11%. Found: C, 64.70%; H, 4.85%; N, 11.99%.

*1-(4-Methylphenyl-3-methyl-5-(4-fluorophenylthio)-4-pyrazolaldoxime-(5-chloro-3-methyl-1-(4-chlorophenyl)pyrazol-4-ylmethyl Ether (***4j***).* White crystal; mp 108−109 °C; yield, 58%. IR (KBr, cm^{-1}): ν 3045 (Ar−H), 2924, 886 (CH_3 + CH_2), 1623 (*C*═N), 1599, 1499 (Ar, skeleton vibration), 1221 (*C*═N−N), 874 (*p*-disubstituted benzene), 660 (C−S−C). ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 8.24 (s, 1H, CH-H), 7.50 (d, J = 8.60 Hz, 2H, pyrazole-Ph-2, 6-H), 7.43 (d, J = 9.15 Hz, 2H, pyrazole-Ph-3, 5-H), 7.23 (d, J = 8.00 Hz, 2H, 1-Ph-2, 6-H), 7.16 (d, J = 8.60 Hz, 2H,1-Ph-3, 5-H), 6.92−6.85 (m, 4H, S-Ph-H), 5.05 (s, 2H, CH_2-H), 2.54 (s, 3H, pyrazole-CH_3-H), 2.39 (s, 3H, Ph-CH_3-H), 2.36 (s, 3H, Cl-pyrazole-CH_3-H). Anal. calcd for $C_{29}H_{24}Cl_2FN_5OS$ (580.0): C, 60.00%; H, 4.14%; N, 12.07%. Found: C, 60.38%; H, 4.30%; N, 12.15%.

*1-Phenyl-3-methyl-5-(4-fluorophenylthio)-4-pyrazolaldoxime-(5-chloro-3-methyl-1-(4-chlorophenyl)-pyrazol-4-ylmethyl Ether (***4k***).* White crystal; mp 105−106 °C; yield, 56%. IR (KBr, cm^{-1}): ν 3045 (Ar−H), 2924, 886 (CH_3 + CH_2), 1599, 1499 (Ar, skeleton vibration), 874 (*p*-disubstituted benzene), 660 (C−S−C). ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 8.25 (s, 1H, CH-H), 7.50 (dd, J = 2.30 Hz, J = 1.75 Hz, 2H, pyrazole-Ph-2, 6-H), 7.43 (dd, J = 1.80 Hz, J = 1.75 Hz, 2H, pyrazole-Ph-3, 5-H), 7.33−7.48 (m, 5H, 1-Ph-H), 6.97 (d, J = 8.00 Hz, 2H, S-Ph-2, 6-H), 6.83 (d, J = 8.60 Hz, 2H, S-Ph-3,5-H), 5.05 (s, 2H, CH_2-H), 2.55 (s, 3H, pyrazole-CH_3-H), 2.38 (s, 3H, Ph-CH_3-H), 2.24 (s, 3H, Cl-pyrazole-CH_3-H). Anal. calcd for $C_{28}H_{22}Cl_2FN_5OS$ (566.0): C, 59.36%; H, 3.89%; N, 12.37%. Found: C, 59.26%; H, 3.76%; N, 12.42%.

*1-(4-Methylphenyl-3-methyl-5-(4-methylphenylthio)-4-pyrazolaldoxime-(5-chloro-3-methyl-1-(4-methylphenyl)pyrazol-4-ylmethyl Ether (***4l***).*

White crystal; mp 115−117 °C; yield, 55%. IR (KBr, cm^{-1}): ν 3035 (Ar−H), 2964, 730 (CH_3 + CH_2), 1609, 1488 (Ar, skeleton vibration),1213 (*C*═N−N), 834 (*p*-disubstituted benzene), 673 (C−S−C). ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 8.23 (s, 1H, CH-H), 7.40 (d, J = 8.60 Hz, 2H, pyrazole-Ph-2, 6-H), 7.25 (d, J = 6.30 Hz, 4H, pyrazole-Ph-3, 5-H, 1-Ph-2, 6-H), 7.15 (d, J = 8.00 Hz, 2H, S-Ph-2, 6-H), 6.98 (d, J = 8.00 Hz, 1-Ph-3, 5-H), 6.84 (d, J = 8.55 Hz, 2H, S-Ph-3, 5-H), 5.04 (s, 2H, CH_2−H), 2.55 (s, 3H, pyrazole-CH_3-H), 2.35, 2.37, 2.39 (3s, 9H, Ph-CH_3-H), 2.24 (s, 3H, Cl-pyrazole-CH_3-H). Anal. calcd for $C_{31}H_{30}ClN_5OS$ (555.5): C, 66.96%; H, 5.40%; N, 12.60%. Found: C, 67.21%; H, 5.68%; N, 12.86%.

*1-(4-Methylphenyl-3-methyl-5-(4-fluorophenylthio)-4-pyrazolaldoxime-(5-chloro-3-methyl-1-(4-methylphenyl)pyrazol-4-ylmethyl Ether (***4m***).* White crystal; mp 118−119 °C; yield, 54%. IR (KBr, cm^{-1}): ν 3035 (Ar−H), 2925, 783 (CH_3 + CH_2), 1590, 1488 (Ar, skeleton vibration),1230 (*C*═N−N), 841 (*p*-disubstituted benzene), 670 (C−S−C). ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 8.24 (s, 1H, CH-H), 7.39 (d, J = 8.60 Hz, 2H, pyrazole-Ph-2, 6-H), 7.25−7.20 (m, 4H, pyrazole-Ph-3, 5-H, 1-Ph-2, 6-H), 7.16 (d, J = 8.05 Hz, 2H, S-Ph-2, 6-H), 6.92−6.85 (m, 4H, 1-Ph-3, 5-H, S-Ph-3, 5-H), 5.06 (s, 2H, CH_2-H), 2.54 (s, 3H, pyrazole-CH_3-H), 2.39, 2.40 (2s, 6H, Ph-CH_3-H), 2.36 (s, 3H, Cl-pyrazole-CH_3-H). Anal. calcd for $C_{30}H_{27}ClFN_5OS$ (559.5): C, 64.34%; H, 4.83%; N, 12.51%. Found: C, 64.54%; H, 5.00%; N, 12.63%.

*1-(4-Chlorophenyl-3-methyl-5-(4-methylphenylthio)-4-pyrazolaldoxime-(5-chloro-3-methyl-1-(4-methylphenyl)pyrazol-4-ylmethyl Ether (***4n***).* White crystal; mp 109−110 °C; yield, 66%. IR (KBr, cm^{-1}): ν 3017 (Ar−H), 2923, 865 (CH_3 + CH_2), 1593, 1495 (Ar, skeleton vibration), 1235 (*C*═N−N), 834 (*p*-disubstituted benzene), 672 (C−S−C). ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 8.24 (s, 1H, CH), 7.39 (d, J = 8.60 Hz, 2H, pyrazole-Ph-2, 6-H), 7.25−7.19 (m, 4H, pyrazole-Ph-3,5-H, 1-Ph-2, 6-H), 7.16 (d, J = 8.05 Hz, 2H, S-Ph-2, 6-H), 6.85−6.92 (m, 4H, 1-Ph-3, 5-H, S-Ph-3, 5-H), 5.06 (s, 2H, CH_2-H), 2.54 (s, 3H, pyrazole-CH_3-H), 2.39, 2.40 (2s, 6H, Ph-CH_3-H), 2.36 (s, 3H, Cl-pyrazole-CH_3-H). Anal. calcd for $C_{30}H_{27}Cl_2N_5OS$ (576.0): C, 62.50%; H, 4.69%; N, 12.15%. Found: C, 62.75%; H, 4.78%; N, 12.32%.

General Procedure for the Preparation of Title Compounds 5a−h. A 50 mL round-bottomed flask equipped with a magnetic stirrer was charged with **4** (5 mmol) dissolved in ice HOAc (30 mL). The flask was stirred at room temperature for 10 min, and then, potassium permanganate (1.0 g) was added and stirred at room temperature for 2 h. Sodium bisulfite was then added to turn the mixture colorless again and filtered. The white solid that resulted was washed with distilled water, dried under vacuum, and recrystallized from ethanol to give the title compounds **5a−h** in 50−58% yields.

*Data for 1-Phenyl-3-methyl-5-(4-methylphenylsulfonyl)-4-pyrazolaldoxime-(2-chloropyridine-5-ylmethyl) Ether (***5a***).* White crystal; mp 120−121 °C; yield, 56%. IR (KBr, cm^{-1}): ν 3069 (Ar−H), 2926, 2877 (CH_3 + CH_2), 1605 (*C*═N), 1587, 1488 (Ar skeleton vibration), 1344, 1152 (SO_2 vibration), 1230 (*C*═N−N), 845 (p-disubstituted benzene) cm^{-1}. ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 8.75 (s, 1H, CH-H), 8.46 (d, J = 2.35 Hz, 1H, Py-6-H), 7.60 (d, J = 2.30 Hz, 1H, Py-4-H), 7.48, ∼7.38 (m, 6H, 1-Ph-H, Py-3-H), 7.37 (dd, J = 8.60 Hz, J = 8.00 Hz, 2H, SO_2-Ph-2, 6-H), 7.10 (t, J = 8.55 Hz, J = 8.60 Hz, 2H, SO_2Ph-3, 5-H), 5.24 (s, 2H, CH_2−H), 2.52 (s, 3H, pyrazole-CH_3-H), 2.25 (s, 3H, Ph-CH_3-H). Anal. calcd for $C_{24}H_{21}ClN_4O_3S$ (480.5): C, 59.93%; H, 4.37%; N, 11.65%. Found: C, 59.92%; H, 4.39%; N, 11.50%.

*1-Phenyl-3-methyl-5-(4-methoxyphenylsulfonyl)-4-pyrazolaldoxime-(2-chloropyridine-5-ylmethyl) Ether (***5b***).* White crystal; mp 125−126 °C; yield, 53%. IR (KBr, cm^{-1}): ν 3098 (Ar−H), 2928, 2889 (CH_3 + CH_2), 1613 (*C*═N), 1598, 1398 (Ar skeleton vibration), 1349, 1155 (SO_2 vibration), 1250 (*C*═N−N), 847 (p-disubstituted benzene). ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 8.46 (d, J = 2.30 Hz, 1H, Py-6-H), 8.23 (s, 1H, CH-H), 7.88 (dd, J = 2.30 Hz, J = 2.30 Hz, 1H, Py-4-H), 7.44−7.53 (m, 6H, 1-Ph-H, Py-3-H), 7.37−7.28 (m, 5H, 1-Ph-H), 6.91 (d, J = 9.20 Hz, 2H, S-Ph-3,5-H), 6.81 (d, J = 9.15 Hz, 2H, S-Ph-2, 6-H), 5.17 (s, 2H, CH_2-H), 3.68 (s, 3H, O-CH_3-H), 2.37 (s, 3H, CH_3-H). ^{13}C NMR (DMSO-d_6, 125 MHz, ppm): δ 169.3, 160.9, 150.4, 150.3, 143.6, 140.5, 138.9, 134.1, 133.3, 130.9, 129.4, 128.9, 126.1, 124.5, 117.7, 116.6, 115.7, 72.4, 55.7, 14.8. Anal. calcd for $C_{24}H_{21}ClN_4O_4S$ (496.5): C, 58.00%; H, 4.22%; N, 11.28%. Found: C, 57.79%; H, 4.32%; N, 11.50%.

*1-Phenyl-3-methyl-5-(4-fluorophenylsulfonyl)-4-pyrazolaldoxime-(2-chloropyridine-5-ylmethyl) Ether (***5c***).* White crystal; mp 125−126 °C; yield, 50%. IR (KBr, cm^{-1}): ν 3076 (Ar−H), 2929, 2873 (CH_3 + CH_2), 1615 (*C*═N), 1590, 1488 (Ar, skeleton vibration), 1343, 1149 (SO_2 vibration), 1235 (*C*═N−N), 840 (*p*-disubstituted benzene). ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 8.89 (s, 1H, CH-H), 8.45 (d, J = 2.30 Hz, 1H, Py-6-H), 7.46−7.39 (m, 4H, Py-4-H + 1-Ph-3, 4, 5-H), 7.35 (dd, J = 8.60 Hz, J = 8.00 Hz, 2H, SO_2-Ph-2, 6-H), 7.27 (d, J = 8.560 Hz, 2H, 1-Ph-2, 6-H), 7.14 (d, J = 8.00 Hz, 1H, Py-3-H), 7.05 (t, J = 8.55 Hz, J = 8.60 Hz, 2H, SO_2Ph-3, 5-H), 5.22 (s, 2H, CH_2-H), 2.41 (s, 3H, pyrazole-CH_3-H). Anal. calcd for $C_{23}H_{18}ClFN_4O_3S$ (484.5): C, 56.96%; H, 3.71%; N, 11.56%. Found: C, 57.10%; H, 3.90%; N, 11.69%.

*1-(3-Chlorophenyl-3-methyl-5-(4-fluorophenylsulfonyl)-4-pyrazolaldoxime-(2-chloropyridine-5-ylmethyl) Ether (***5d***).* White crystal; mp 120−122 °C; yield, 58%. IR (KBr, cm^{-1}): ν 3076 (Ar−H), 2929, 2873 (CH_3 + CH_2), 1590, 1488 (Ar, skeleton vibration), 1343, 1149 (SO_2 vibration), 1235 (*C*═N−N), 840 (*p*-disubstituted benzene). ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 8.89 (s, 1H, CH-H), 8.45 (d, J = 2.30 Hz, 1H, Py-6-H), 7.46−7.51 (m, 1H, Py-4-H), 7.41−7.40 (m, 2H, 1-Ph-4, 6-H), 7.35 (dd, J = 8.60 Hz, J = 8.00 Hz, 2H, S O_2-Ph-2, 6-H), 7.27 (s, 1H, 1-Ph-2-H), 7.14 (d, J = 7.90 Hz, 1H, Py-3-H), 7.05 (t, J = 8.55 Hz, J = 8.60 Hz, 2H, SO_2-Ph-3, 5-H), 6.97 (t, J = 2.3 Hz, J = 1.75 Hz, 1H, 1-Ph-5-H), 5.22 (s, 2H, CH_2-H), 2.41 (s, 3H, pyrazole-CH_3-H). Anal. calcd for $C_{23}H_{17}Cl_2FN_4O_3S$ (519.0): C, 53.18%; H, 3.28%; N, 10.79%. Found: C, 53.28%; H, 3.22%; N, 10.66%.

*1-(4-Chlorophenyl-3-methyl-5-(4-methylphenylsulfonyl)-4-pyrazolaldoxime-(2-chloropyridine-5-ylmethyl) Ether (***5e***).* White crystal; mp 138−140 °C; yield, 54%. IR (KBr, cm^{-1}): ν 3045 (Ar−H), 2924, 2865 (CH_3 + CH_2), 1615 (*C*═N), 1592, 1459 (Ar, skeleton vibration), 1338, 1149 (SO_2 vibration), 1235 (*C*═N−N), 840 (*p*-disubstituted benzene). ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 8.89 (s, 1H, CH-H), 8.46 (d, J = 2.30 Hz, 1H, Py-6-H), 7.75−7.34 (m, 1H, Py-4-H), 7.35 (d, J = 8.05 Hz, 1H, Py-3-H), 7.32 (d, J = 8.55 Hz, 2H, SO_2-Ph-2, 6-H), 7.26 (d, J = 8.60 Hz, 2H, 1-Ph-2, 6-H), 7.14 (d, J = 8.55 Hz, 2H, 1-Ph-3, 5-H), 7.07 (d, J = 8.60 Hz, 2H, SO_2-Ph-3, 5-H), 5.20 (s, 2H, CH_2-H), 2.38 (s, 3H, pyrazole-CH_3-H), 2,33, (s, H, Ph-CH_3-H). Anal. calcd for $C_{24}H_{20}Cl_2N_4O_3S$ (515.0): C, 55.92%; H, 3.88%; N, 10.87%. Found: C, 56.08%; H, 3.78%; N, 10.69%.

*1-(4-Chlorophenyl)-3-methyl-5-(4-methoxylphenylsulfonyl)-4-pyrazolaldoxime-(2-chloropyridine-5-ylmethyl) Ether (***5f***).* White crystal; mp 139−140 °C; yield, 58%. IR (KBr, cm^{-1}): ν 3033 (Ar−H), 2933, 2852 (CH_3 + CH_2), 1615 (*C*═N), 1591, 1463 (Ar, skeleton vibration), 1315, 1148 (SO_2 vibration), 1264 (*C*═N−N), 843 (*p*-disubstituted benzene), 809, 771 (*m*-disubstituted benzene). ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 8.89 (s, 1H, CH-H), 8.46 (d, J = 2.30 Hz, 1H, Py-6-H), 7.75−7.39 (m, 1H, Py-4-H), 7.36 (d, J = 8.05 Hz, 2H, SO_2-Ph-2, 6-H), 7.32 (dd, J = 5.30 Hz, J = 1.75 Hz, 1H, Py-3-H), 7.29 (d, J = 8.55 Hz, 2H, 1-Ph-3, 5-H), 7.08 (dd, J = 2.25 Hz, J = 2.30 Hz, 2H, 1-Ph-2, 6-H), 6.80 (d, J = 8.55 Hz, 2H, SO_2-Ph-3, 5-H), 5.20 (s, 2H, CH_2-H), 3.84 (s, 3H, O-CH_3-H), 2.39 (s, 3H, pyrazole-CH_3-H). Anal. calcd for $C_{24}H_{20}Cl_2N_4O_4S$ (531.0): C, 54.24%; H, 3.77%; N, 10.54%. Found: C, 54.36%; H, 3.60%; N, 10.42%.

*1-(4-Chlorophenyl)-3-methyl-5-(4-fluorophenylsulfonyl)-4-pyrazolaldoxime-(2-chloropyridine-5-ylmethyl) Ether (***5g***).* White crystal; mp 144−145 °C; yield, 87%. IR (KBr, cm^{-1}): ν 3041 (Ar−H), 2921, 2855 (CH_3 + CH_2), 1611 (*C*═N), 1589, 1493 (Ar, skeleton vibration), 1315, 1148 (SO_2 vibration), 1226 (*C*═N−N), 831 (*p*-disubstituted benzene). ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 8.89 (s, 1H, CH-H), 8.43 (d, J = 2.30 Hz, 1H, Py-6-H), 8.24 (s, 1H, CH-H), 7.71 (d, J = 2.30 Hz, 1H, Py-4-H), 7.32 (d, J = 8.50 Hz, 2H, SO_2-Ph-2, 6-H), 7.36 (d, J = 8.05 Hz, 1H, Py-3-H), 7.29 (d, J = 8.60 Hz, 2H, 1-Ph-2, 6-H), 7.21 (d, J = 8.50 Hz, 2H, 1-Ph-3, 5-H), 7.17 (d, J = 8.60 Hz, 2H, SO_2-Ph-3, 5-H), 5.19 (s, 2H, CH_2-H), 2.40 (s, 3H, pyrazole-CH_3-H). Anal. calcd for $C_{23}H_{17}Cl_2FN_4O_3S$ (519.0): C, 53.17%; H, 3.27%; N, 10.78%. Found: C, 53.00%; H, 3.21%; N, 10.71%.

*1-(4-Methylphenyl-3-methyl-5-(4-fluorophenylsulfonyl)-4-pyrazolaldoxime-(2-chloropyridine-5-ylmethyl) Ether (***5h***).* White crystal; mp 112−114 °C; yield, 52%. IR (KBr, cm^{-1}): ν 3045 (Ar−H), 2924, 2865

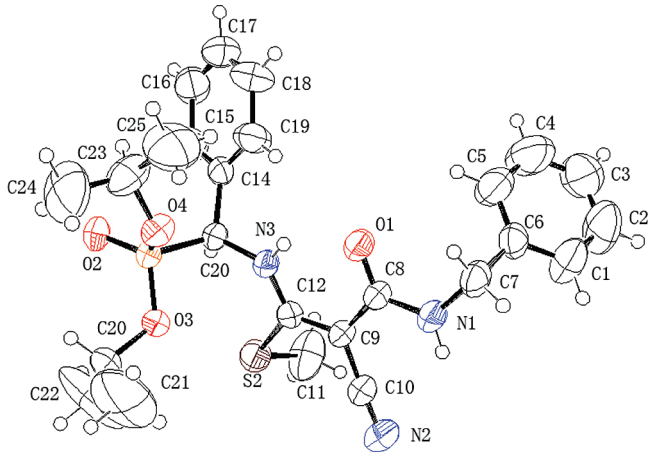


Figure 3. Molecular structure of **4b**.

($CH_3 + CH_2$), 1615 (C=N), 1592, 1459 (Ar, skeleton vibration), 1338, 1149 (SO_2 vibration), 1213 (C=N−N), 840 (*p*-disubstituded benzene). ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 8.90 (s, 1H, CH-H), 8.46 (d, J = 2.30 Hz, 1H, Py-6-H), 7.75−7.65 (m, 1H, Py-4-H), 7.35 (d, J = 8.05 Hz, 1H, Py-3-H), 7.32 (d, J = 8.55 Hz, 2H, SO_2-Ph-2, 6-H), 7.31 (d, J = 8.60 Hz, 2H, 1-Ph-2, 6-H), 7.25 (d, J = 8.60 Hz, 2H, 1-Ph-3, 5-H), 7.07 (d, J = 8.60 Hz, 2H, SO_2-Ph-3,5-H), 5.20 (s, 2H, CH_2-H), 2.39 (s, 3H, pyrazole-CH_3-H), 2.39 (s, 3H, Ph-CH_3-H). Anal. calcd for $C_{24}H_{20}ClFN_4O_3S$ (498.5): C, 57.73%; H, 4.01%; N, 11.23%. Found: C, 57.60%; H, 3.97%; N, 11.45%.

X-ray Diffraction. Colorless blocks of **4b** (0.20 mm × 0.19 mm × 0.18 mm) were counted on a quartz fiber with protection oil. Cell dimensions and intensities were measured at 293 K on a Bruker SMART CCD area detector diffractometer with graphite monochromated Mo Kα radiation (λ = 0.71073 Å), θ_{max} = 25.00, 19528 measured reflections, and 2498 independent reflections (R_{int} = 0.1763) of which 4595 had $I > 2\delta(I)$. Data were corrected for Lorentz and polarization effects and for absorption (T_{min} = 0.7680; T_{max} = 0.8237). The structure was solved by direct methods using SHELXS-97; all other calculations were performed with Bruker SAINT System and Bruker SMART programs. Full-matrix least-squares refinement based on $F2$ using the weight of $1/[\sigma^2(F_0^2) + (0.0850P)^2 + 0.0458P]$ gave final values of R = 0.0517, ωR = 0.1631, and GOF(F) = 1.011 for 453 variables and 4595 contributing reflections. The maximum shift/error = 0.001, and max/min residual electron density = 0.400/−0.322 e Å^{-3}. Hydrogen atoms were observed and refined with a fixed value of their isotropic displacement parameter.

Antiviral Biological Assay. *Purification of TMV.* Using Gooding's method (*22*), the upper leaves of *Nicotiana tabacum* L. inoculated with TMV were selected, ground in phosphate buffer, and then filtered through double layer pledget. The filtrate was centrifuged at 10000*g*, treated twice with poly(ethylene glycol), and centrifuged again. The whole experiment was carried out at 4 °C. Absorbance values were estimated at 260 nm using an ultraviolet spectrophotometer.

$$\text{virus concn} = (A_{260} \times \text{dilution ratio})/E_{1\text{cm}}^{0.1\%,260\text{nm}}$$

Protective Effects of Compounds against TMV in Vivo. The compound solution was smeared on the left side, while the solvent served as the control on the right side of growing *N. tabacum* L. leaves of the same ages. The leaves were then inoculated with the virus after 12 h. A brush was dipped in TMV of 6 × 10^{-3} mg/mL to inoculate the leaves, which were previously scattered with silicon carbide. The leaves were then washed with water and rubbed softly along the nervature once or twice. The local lesion numbers appearing 3−4 days after inoculation were counted (*23*). Three repetitions were conducted for each compound.

Inactivation Effect of Compounds against TMV in Vivo. The virus was inhibited by mixing with the compound solution at the same volume for 30 min. The mixture was then inoculated on the left side of the leaves of *N. tabacum* L., while the right side of the leaves was inoculated with the mixture of solvent and the virus for control. The local lesion numbers were recorded 3−4 days after inoculation (*23*). Three repetitions were conducted for each compound.

Curative Effect of Compounds against TMV in Vivo. Growing leaves of *N. tabacum* L. of the same ages were selected. The TMV (concentration of 6 × 10^{-3}mg/mL) was dipped and inoculated on the whole leaves. Then, the leaves were washed with water and dried. The compound solution was smeared on the left side, and the solvent was smeared on the right side for control. The local lesion numbers were then counted and recorded 3−4 days after inoculation (*23*). For each compound, three repetitions were measured. The inhibition rate of the compound was then calculated according to the following formula ("av" means average).

$$\text{inhibition rate (\%)} = \frac{\text{av local lesion numbers of control (not treated with compound)} - \text{av local lesion numbers smeared with drugs}}{\text{av local lesion numbers of control (not treated with compound)}} \times 100\%$$

RESULTS AND DISCUSSION

Synthesis. The synthetic route designed for the oxime ester analogues **4** and **5** is summarized in **Scheme 1**. Starting from 1-substituted phenyl 3-methyl-pyrazolone derivative, the intermediate **1**, 1-substituted phenyl-3-methyl-4-formyl-5-chloropyrazole was prepared by chlorination reaction with $POCl_3$ in DMF. Then, treatment of **1** with substituted thiophenol afforded 1-substituted phenyl-3-methyl-5-substituted-phenylthio-4-pyrazol-aldehyde **2**. Reaction of **2** with hydroxylamine hydrochloride gave 1-substituted phenyl-3-methyl-5-substituted phenylthio-4-pyrazolaldoximes **3**. The title compounds, 1-substituted phenyl-3-methyl-5-substituted phenylthio-4-pyrazolaldoxime ethers **4** were synthesized by the etherification reaction of **3** with chloromethylated heterocyclic compounds ($ClCH_2R_3$). 1-Substituted phenyl-3-methyl-5-substituted phenyl- sulfonyl-4-pyrazolaldoxime ether derivatives **5** were then obtained from the oxidation of **4** with potassium permanganate in HOAc solution at room temperature. While the compounds **4a**−**n** were obtained in high yields (54−87%), the compounds **5a**−**h** were obtained in good yields (50−58%). The X-ray single structure of typical **4b** has been shown in **Figure 3**, and all compounds were characterized unequivocally by spectroscopic data and elemental analysis as described in the Materials and Methods.

Antiviral Activity and Structure−Activity Relationship. To make a judgment of the antiviral potency of the synthesized compounds **4a**−**n** and **5a**−**h**, the commercially available plant virucide Ningnanmycin (*24*), perhaps the most successful registered antiplant viral agent available in China, was used as the control. The antiviral bioassay against TMV is assayed by the reported method (*22, 23*), and the antiviral results of all of the compounds against TMV are listed in **Table 1**. The results showed that most of our designed compounds had moderate antiviral activities at 500 mg/L against TMV in vivo.

The title compounds **4a**−**n** and **5a**−**h** exhibited protection activities of 15.7−44.8% at 500 mg/L. Compound **4g** (R_1 is 4-Cl, R_2 is F, and R_3 is 2-chloropyridine-5-ylmethyl), **4l** [R_1 is 4-Me, R_2 is Me, and R_3 is 5-chloro-3-methyl-1-(4-methylphenyl)-pyrazol-4-ylmethyl], **4m** [R_1 is 4-Me, R_2 is F, and R_3 is 5-chloro-3-methyl-1-(4-methylphenyl)-pyrazol-4-ylmethyl], **5c** (R_1 is H, R_2 is F, and R_3 is 2-chloropyridine-5-ylmethyl), and **5g** (R_1 is 4-Cl, R_2 is F, and R_3 is 2-chloropyridine-5-ylmethyl) had moderate protection activities (43.5, 44.8, 41.0, 40.2, and 42.2, respectively), comparable to that that of the standard reference (59.9%). In addition, the other compounds **4c**−**f**,**h**−**k** and **5a**,**b**,**d**−**f**,**h** showed less than 40% protection activities at

500 mg/L. From the data presented in **Table 1**, it can be observed that the title compounds **4a**−**n** possess potential inactivation bioactivities, with values of 90.4, 71.2, 65.9, 61.7, 61.2, 34.5, 80.6, 56.6, 61.0, 43.0, 55.9, 51.2, 50.9, and 36.7% at 500 μg/mL, respectively. Among these compounds, **4a** and **4g**are appreciably more active than the rest, with inactivation rates of 90.4 and 80.6%, respectively, which are similar to that of Ningnanmycin (98.0%) against TMV at 500 μg/mL. The data also indicate that all compounds have a relatively lower curative activity than that of Ningnanmycin. At this moment, however, it is difficult to provide a rational account of structure−activity relationships on the basis of steric, electronic, and hydrophobic effects. The diversity of the structures was limited by the availability of the reagents and the ease with which para-substituted (for R_1 and R_2 groups) products could be obtained as compared to those with ortho- and meta-substituted ones. The electronic factor does not seem to play a significant role as both the compounds **4a** (R_1 as H and R_2 Me) and **4g** (R_1 as Cl and R_2 as F) with electron-rich and electron-poor substituents, respectively, were found to display good bioactivity. Comparison of biological activities among **4a**−**n** and **5a**−**h** confirms that the functional groups with alkylthio are potentially more active than those with sulfonyl groups at the 5-position of pyrazole, which is necessary for inactivation activity to occur.

In addition, as shown in **Table 2**, compounds **4a**,**b**,**g** were found to display good antiviral activities. These compounds were bioassayed further to investigate their inactivation activities at different concentrations with Ningnanmycin serving as the commercial control. As shown in **Table 2**, the inactivation effects against TMV of compounds **4a**,**b**,**g** are significant. The EC_{50} values were 58.7, 115.0, and 65.3 μg/mL, respectively. Among these compounds, **4a** displayed more potent antiviral activity than the others, being similar to that of Ningnanmycin (EC_{50}= 52.7 μg/mL) against TMV.

In summary, a series of new pyrazole derivatives containing oxime moieties **4a**−**n** were designed and synthesized by the thioetherification reaction of 1-substituted phenyl-3-methyl-5-substituted phenylthio-4-pyrazolaldoximes **3** with chloromethylated heterocyclic compounds ($ClCH_2R_3$) and NaOH in DMF. The in vivo tests indicated that compounds **4a**,**g** exhibited a very similar inactivation bioactivity level as that of Ningnanmycin against TMV. Therefore, the present work demonstrates that the antiviral activity of pyrazole derivatives was significantly improved via the introduction of the oxime moiety. Although structure−activity relationships could not be successfully established due to lack of structural diversity, the present work paves the way toward the synthesis and antiviral studies of new pyrazole derivatives containing oxime moieties. Effects of steric, hydrophobic, electronic, and electrostatic parameters on structure−activity relationships and structural modification studies for identifying lead bioactive compounds are currently underway.

ACKNOWLEDGMENT

We thank the National Key Program for Basic Research (2003CB114404) and the National Natural Science Foundation of China (20672024 and 20762002) for the financial support.

LITERATURE CITED

(1) Liu, X. H.; Cui, P.; Song, B. A.; Bhadury, P. S.; Zhu, H. L.; Wang, S. F. Synthesis, structure and antibacterial activity of novel 1-(5-substituted-3-substituted-4,5-dihydropyrazol-1-yl) ethanone oxime ester derivatives. *Bioorg. Med. Chem.* **2008**, *16*, 4075–4082.

(2) Velaparthi, S.; Brunsteiner, M.; Uddin, R.; Wan, B.; Franzblau, S. G.; Petukhov, P. A. 5-*tert*-Butyl-*N*-pyrazol-4-yl-4,5,6,7-tetrahydrobenzo[d]isoxazole-3-carboxamide deriva- tives as novel potent inhibitors of Mycobacterium tuberculosis pantothenate synthetase: initiating a quest for new antitubercular drugs. *J. Med. Chem.* **2008**, *51*, 1999–2002.

(3) Magedov, I. V.; Manpadi, M.; Van slambrouck, S.; Steelant, W. F. A.; Rozhkova, E.; Przheval'skii, N. M.; Rogelj, S.; Kornienko, A. Discovery and investigation of antiproliferative and apoptosis-iInducing properties of new heterocyclic podophyllo- toxin analogues accessible by a one-step multicomponent synthesis. *J. Med. Chem.* **2007**, *50*, 5183–5192.

(4) Rovnyak, G. C.; Millonig, R. C.; Schwartz, J.; Shu, V. Synthesis and antiinflammatory activity of hexahydrothiopyrano[4,3-c]pyrazoles and related analogs. *J. Med. Chem.* **1982**, *25*, 1482–1488.

(5) Wachter, G. A.; Hartmann, R. W.; Sergejew, T.; Grun, G. L.; Ledergerber, D. Tetrahydronaphthalenes: Influence of heterocyclic substituents on inhibition of steroidogenic enzymes P450 arom and P450 17. *J. Med. Chem.* **1996**, *39*, 834–841.

(6) Colliot, F.; Kukorowski, K. A.; Hawkins, D. W.; Roberts, D. A. Fipronil: A new soil and foliar broad spectrum insecticide. *Brighton Crop Prot. Conf. Pests Dis.* **1992**, *1*, 29–34.

(7) Chen, H. S.; Li, Z. M.; Han, Y. F. 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.

(8) Vicentini, C. B.; Romagnoli, C.; reotti, E.; Mares, D. Synthetic pyrazole derivatives as growth inhibitors of some phytopathogenic fungi. *J. Agric. Food Chem.* **2007**, *55* (25), 10331–10338.

(9) Vicentini, C. B.; Mares, D.; Tartari, A.; Manfrini, M.; Forlani, G. Synthesis of pyrazole derivatives and their evaluation as photosynthetic electron transport inhibitors. *J. Agric. Food Chem.* **2004**, *52*, 1898–1906.

(10) Waldrep, T. W.; Beck, J. R.; Lynch, M. P.; Wright, F. L. Synthesis and herbicidal activity of 1-aryl-5-halo and 1-aryl-5-(trifluoromethyl)-1H-pyrazole-4-carboxamides. *J. Agric. Food Chem.* **1990**, *38*, 541–544.

(11) Vicentini, C. B.; Guccione, S.; Giurato, L.; Ciaccio, R.; Mares, D.; Forlani, G. Pyrazole derivatives as photosynthetic electron transport inhibitors: new leads and structure-activity relationship. *J. Agric. Food Chem.* **2005**, *53*, 3848–3855.

(12) Clark, R. D. Synthesis and QSAR of herbicidal 3-pyrazolyl α,α,α-trifluorotolyl ethers. *J. Agric. Food Chem.* **1996**, *44*, 3643–3652.

(13) Li, Y.; Zhang, H. Q.; Liu, J.; Yang, X. P.; Liu, Z. J. Stereoselective synthesis and antifungal activities of (*E*)-α-(methoxyimino)benzeneacetate derivatives containing 1,3,5-substituted pyrazole ring. *J. Agric. Food. Chem.* **2006**, *54*, 3636–3640.

(14) Huang, R. Q.; Song, J.; Feng, L. Synthesis and bioactivity of 1, 3, 5-substituted pyrazol-4-methylene cyclopropane carboxylate. *Chem. J. Chin. Univ.* **1996**, *17*, 1089–1091.

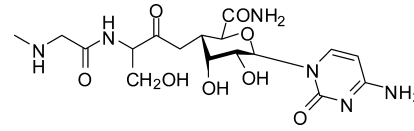
(15) Liu, Y.; Ren, J.; Jin, G.-Y. Synthesis and biological activities of 1-phenyl (methyl)-3-methyl-5-(4,6-dimethyl-2-thio)pyrimidyl-4-pyrazolyl oxime ethers (esters). *Chin. J. Pestic. Sci.* **2001**, *3*, 12–16.

(16) Minakata, S.; Hamada, T.; Komatsu, M.; Tsuboi, H.; Kikuta, H.; Ohshiro, Y. Synthesis and biological activity of 1*H*-pyrrolo[2,3-b]pyridine derivatives: correlation between inhibitory activity against the fungus causing rice blast and ionization potential. *J. Agric. Food Chem.* **1997**, *45*, 2345–2348.

(17) Cui, P.; Liu, X. H.; Zhi, L. P.; Wang, X. H.; Song, B. A.; Zuo, R. B. Synthesis and biological activity of novel 5-aryl-*N*-pyrazole oxime ester derivatives. *Chin. J. Appl. Chem.* **2008**, *25* (7), 820–824.

(18) Jin, G. Y.; Cao, C. Y.; Li, L. T.; Ren, J. J.; Zhao, G. F. Synthesis and biological activities of substituted pyrazolo-[4,5-e][1,3]thiazine derivatives. *Chin. J. Pestic. Sci.* **1999**, *1*, 15–19.

(19) Li, Z. G. *Preparation of Organic Intermediates*; Chemical Industry Press: Beijing, 2001; pp 8, 152, and 445 (in Chinese).
(20) Xu, K. X. *Handbook of Materials and Intermediates of Fine Organic Chemical Industry*; Chemical Industry Press: Beijing, 1998; pp 4−39 (in Chinese).
(21) (a) Xiao, H. Q.; Ouyang, G. P.; Sun, X. D.; Yao, X. D.; Bao, G. Q.; Qi, C. Z. Synthesis of pyrazole oxime esters. *Chin. J. Syn. Chem.* **2005**, *13*, 600–602. (b) Ouyang, G. P. Synthesis and antiviral and antitumor activities of novel pyrazole derivatives. Ph.D. Dissertation, Guizhou University, Guiyang, China, 2008.
(22) Gooding, G. V., Jr.; Hebert, T. T. A simple technique for purification of tobacco mosaic virus in large quantities. *Phytopathology* **1967**, *57*, 1285–1290.
(23) Song, B. A.; Zhang, H. P.; Wang, H.; Yang, S.; Jin, L. H.; Hu, D. Y.; Pang, L. L.; Xue, W. Synthesis and antiviral activity of novel chiral cyanoacrylate derivatives. *J. Agric. Food Chem.* **2005**, *53*, 7886–7891.
(24) Ningnanmycin is produced by *Strepcomces noursei* var. *xichangensisn* var. Ningnanmycin is now commercially available in China as a registered plant virucide. However, its action mechanism is not yet clear. Its structure is as follows.



Received for review August 12, 2008. Revised manuscript received September 17, 2008. Accepted September 17, 2008.

JF802489E