JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Synthesis and Antifungal Activities of Novel 5-Amino-6-arylamino-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one Derivatives

HONG-QING WANG,*,† WEI-PIMG ZHOU,§ YU-YUAN WANG,† AND CAN-RONG LIN†

College of Chemistry and Chemical Engineering and College of Mathematics and Physics, University of South China, 28 Changsheng Road Hengyang, Hunan 421001, People's Republic of China

A novel approach was developed to regioselectively synthesize new 5-amino-6-arylamino-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one **5** derivatives via a tandem aza-Wittig and annulation reactions of iminophosphorane **2**, aromatic isocyanates, and hydrazine in 52−92% isolated yields. The compounds **5** reacted with triethyl orthoformate to give 1,8-2*H*-pyrazolo[3,4-*d*][1,2,4]triazolo[1,5-*a*]pyrimidin-4-ones **6** in good yields (62−94%). Their structures were clearly confirmed by spectroscopy data (IR, ^{1}H NMR, MS), elemental analysis, or X-ray diffraction crystallography. The results of preliminary bioassay indicated that the compounds **5** and **6** possessed high antifungal activity against *Botrytis cinerea* Pers and *Sclerotinia sclerotiorum*. Compounds **5** showed much better antifungal activities when R was Me instead of $PhCH_2$. Especially, compounds **6c**, **6g**, and **6i** inhibited *Sclerotinia* by 100% at the concentration of 50 mg/L and by 83, 83, and 82% at the dosage of 10 mg/L, respectively.

KEYWORDS: 1*H*-Pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one; aza-Wittig reaction; synthesis; 1,8-2*H*-pyrazolo[3,4-*d*][1,2,4]triazolo[1,5-*a*]pyrimidin-4-one; antifungal

INTRODUCTION

Pyrazolo[3,4-*d*]pyrimidine derivatives have extremely rich biological activities because of their structural similarity to purines (*1*). They are often employed as antimicrobial and antifungal agents (*2–5*), various animal enzyme inhibitors (*6–8*), and agrochemicals (*9–14*). Triazole and its fused heterocycles have been extensively investigated as biologically active compounds, such as substituted triazole derivatives (*15, 16*), triazolo[1,5-*a*]pyrimidine derivatives (*17*), and pyrazolotriazolopyrimidine derivatives (*18*). Our previous paper (*9–11*) reported that a reaction of ethyl 3-alkylthio-1-phenyl-5-triphenylphosphoranoimino-1*H*-pyrazole-4-carboxylate (abbreviation iminophosphorane) **2** with isocycanates and alklyamine could give 6-alkylamino-5-aryl-pyrazolo[3,4-*d*]pyrimidin-4-one derivatives, the nitrogen atoms of which were from isocycanates. Those compounds showed satisfactory antifungicidal activities. Therefore, we have attempted to extend this reaction to hydrazine to synthesize some new antifungicidally active heterocycle compounds containing triazole. However, we obtained 5-amino-6-arylamino-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one derivatives **5** instead of 6-hydrazino-5-aryl-pyrazolo[3,4-*d*]pyrimidin-4-one **7** derivatives. In other words, we got a series of novel 5-amino-6-arylamino-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-ones **5**, having nitrogen atoms from hydrazine, 2*H*-pyrazolo[3,4-*d*][1,2,4]triazolo[1,5-*a*]pyrimidin-4-one derivatives (**Scheme 1**) and a new convenient method of synthesis of compounds **5** and **6**. Their biological activities have been studied also.

MATERIALS AND METHODS

Instruments. The melting points of the products were determined on an X-4 binocular microscope (Beijing Tech Instrument Co., Beijing, China) and are uncorrected. MS was measured on a FinniganTrace Mass 2000 spectrometer at 70 eV. IR was recorded on an Avatar 360 spectrometer as KBr pellets with absorption given in cm^{-1}. ^{1}H NMR spectra were taken on a Varian Mercury 400 (or 300) spectrometer with TMS as the internal reference and DMSO-d_6 or $CDCl_3$ as the solvent. Elemental analysis was taken on a Vario EL elemental analysis instrument. X-ray diffraction was performed with a Brucker Smart 1000.

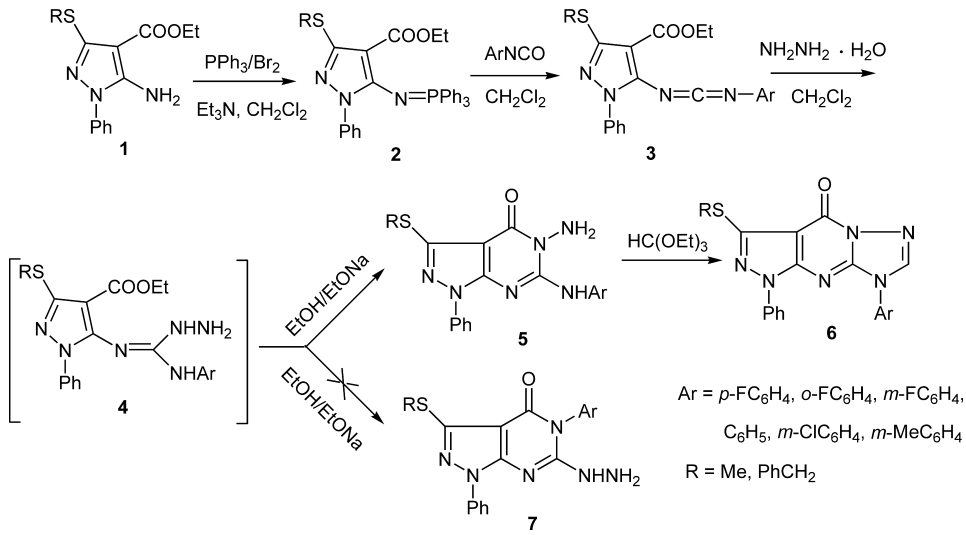
Synthetic Procedures. All of the solvents and materials were of reagent grade and purified as required. Ethyl 5-amino-3-alkylthio-1-phenyl-1*H*-pyrazole-4-carboxylate **1** and iminophosphorane **2** were prepared according to the literature (*19*).

General Synthetic Procedures for 3-Alkylthio-5-amino-6-arylamino-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-ones 5. Aryl isocyanate (0.27 g, 2 mmol) was added to a solution of iminophosphorane **2** (1.07 g, 2 mmol) in dry methylene dichloride (20 mL) under nitrogen atmosphere at room temperature. After the reaction mixture had been stirred for 1.5 h, 0.094 g (2.0 mmol, 85%) of hydrazine hydrate

* Corresponding author (telephone + 86 734 8281710; fax +86 734 8282521; e-mail hqwang2001cn@yahoo.com.cn).
† College of Chemistry and Chemical Engineering.
§ College of Mathematics and Physics.

10.1021/jf801359f CCC: $40.75
Published on Web 08/02/2008

Scheme 1



was added, and the resulting mixture was stirred for an additional 30 min. Then most of the solvent was removed in vacuo, and 25 mL of anhydrous ethanol and 1.5 mL of sodium ethoxide (3 mol/L) in ethanol were added to the mixture. After 3 h of stirring at room temperature, the solution was concentrated under reduced pressure and successively cooled. The crude product was collected by filtration. After recrystallization from DMF/petroleum ether or column chromatography on a silica gel, a white crystal was obtained.

Data for **5a**: white crystal, mp 279−281 °C; IR (KBr) υ (cm^{-1}) 3347, 3311, 3207, 1679, 1596, 1563, 1509, 913, 763, 670; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.74 (s, 1H, *p*-FC_6H_4**NH**), 7.99 (d, 2H, J = 8.7 Hz, Ar), 7.70−7.75 (m, 2H, Ar), 7.46 (d, 2H, J = 7.8 Hz, Ar), 7.18−7.28 (m, 3H, Ar), 5.54 (s, 2H, NH_2), 2.59 (s, 3H, SCH_3); EI-MS (70 eV, *m*/*z* rel intensity) 384 (M + 2, 16), 383 (M + 1, 47), 382 (M^+, 92). Anal. Calcd for $C_{18}H_{15}FN_6OS$: C, 56.53; H, 3.95; N, 21.98. Found: C, 56.41; H, 3.96; N, 22.85.

Data for **5b**: white crystal, mp 238−239 °C; IR (KBr) υ (cm^{-1}) 3352, 3314, 3266, 1721, 1621, 1600, 1542 (Ar), 1455, 922, 768; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.61(s, 1H, *o*-FC_6H_4**NH**), 7.98 (d, 3H, J = 8.4 Hz, Ar), 7.25−7.46 (m, 6H, Ar), 5.65 (s, 2H, NH_2), 2.61 (s, 3H, SCH_3); EI-MS (70 eV, *m*/*z* rel intensity) 384 (M +2, 13), 383 (M + 1, 49), 382 (M^+, 89). Anal. Calcd for $C_{18}H_{15}FN_6OS$: C, 56.53; H, 3.95; N, 21.98. Found: C, 56.39; H, 3.92; N, 22.03.

Data for **5c**: white crystal, mp 246−247 °C; IR (KBr) υ (cm^{-1}) 3314, 3273, 3212, 1702, 1637, 1604, 1560, 1545, 963, 925, 871, 764; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.82 (s, 1H, *m*-FC_6H_4**NH**), 8.04 (d, 2H, J = 7.6 Hz, Ar), 7.88 (d, 1H, J = 12 Hz, Ar), 7.33−7.59 (m, 5H, Ar), 6.95 (t, 1H, J = 2.4 Hz, Ar), 5.59 (s, 2H, NH_2), 2.61 (s, 3H, SCH_3); EI-MS (70 eV, *m*/*z* rel intensity) 384 (M + 2, 21), 382 (M^+, 100). Anal. Calcd for $C_{18}H_{15}FN_6OS$: C, 56.53; H, 3.95; N, 21.98. Found: C, 56.71; H, 4.01; N, 21.85.

Data for **5d**: white crystal, mp 208−209 °C; IR (KBr) υ (cm^{-1}) 3319, 3207, 3025, 2930, 1686,1609, 1595, 1563, 1509, 1390, 1211, 924, 771; ^{1}H NMR (DMSO-d_6, 300 MHz) δ Ä9.76 (br s, 1H, *p*-FC_6H_4**NH**), 8.00 (d, 2H, J = 7.8 Hz, Ar), 7.70−7.75 (m, 2H, Ar), 7.44−7.51 (m, 4H, Ar), 7.19−7.32 (m, 6H, Ar), 5.56 (s, 2H, NH_2), 4.46 (s, 2H, Ph$\mathbf{CH_2}$); EI-MS (70 eV, *m*/*z* rel intensity) 460 (M + 2, 12), 459 (M + 1, 28), 458 (M^+, 50). Anal. Calcd for $C_{24}H_{19}FN_6OS$: C, 62.87; H, 4.18; N, 18.33. Found: C, 62.56; H, 4.22; N, 18.50.

Data for **5e**: white crystal, mp 188−189 °C; IR (KBr) υ (cm^{-1}) 3323, 3274, 3212, 3028, 2924, 1685, 1600, 1553, 1491, 1459, 1390, 1228, 1170, 926, 759; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.61(br s, 1H, *o*-FC_6H_4**NH**), 7.94−7.99 (m, 3H, Ar), 7.26−7.48 (m, 11H, Ar), 5.67 (br s, 2H, NH_2), 4.48 (s, 2H, Ph$\mathbf{CH_2}$); EI-MS (70 eV, *m*/*z*, rel intensity) 458 (M^+, 12). Anal. Calcd for $C_{24}H_{19}FN_6OS$: C, 62.87; H, 4.18; N, 18.33. Found: C, 62.95; H, 4.20; N, 18.21.

Data for **5f**: white crystal, mp 229−230 °C; IR (KBr) υ (cm^{-1}) 3320, 3208, 2919, 1687, 1598, 1556, 1492, 1390, 1171, 945, 774; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.88 (br s, 1H, *m*-FC_6H_4**NH**), 8.05 (d, 2H, J = 8.0 Hz, Ar), 7.84 (d, 1H, J = 12.0 Hz, Ar), 7.25−7.54 (m, 10H, Ar), 6.92 (t, 1H, J = 7.6 Hz, Ar), 5.61 (br s, 2H, NH_2), 4.48 (s, 2H, Ph$\mathbf{CH_2}$); EI-MS (70 eV, *m*/*z*, rel intensity) 458 (M^+, 21). Anal. Calcd for $C_{24}H_{19}FN_6OS$: C, 62.87; H, 4.18; N, 18.33. Found: C, 62.96; H, 4.22; N, 18.26.

Data for **5g**: white crystal, mp 236.2−238.1 °C; IR (KBr) υ (cm^{-1}) 3336, 3267, 1692, 1596, 1554, 909, 770, 743, 688; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.67 (s, 1H, Ph**NH**), 8.03 (d, 2H, J = 7.8 Hz, Ph), 7.73 (d, 2H, J = 7.5 Hz, Ph), 7.46 (d, 2H, J = 7.6 Hz, Ph), 7.36 (t, 2H, J = 7.6 Hz, Ph), 7.25 (t, 1H, J = 7.2 Hz, Ph), 7.11 (t, 1H, J = 7.2 Hz, Ph), 5.57 (br s, 2H, NH_2), 2.60 (s, 3H, SCH_3); EI-MS (70 eV, *m*/*z*, rel intensity) 65 (M + 1, 49), 364 (M^+, 81). Anal. Calcd for $C_{18}H_{16}N_6OS$: C, 59.32; H, 4.43; N, 23.06. Found: C, 59.1; H, 4.37; N, 22.96.

Data for **5h**: white crystal, mp 226.0−227.8 °C; IR (KBr) υ (cm^{-1}) 3319, 1684, 1596, 1554, 927, 759, 689, 501; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.69 (s, 1H, Ph**NH**), 8.04 (d, 2H, J = 8.4 Hz), 7.73 (d, 2H, J = 8.1 Hz), 7.200−7.503 (m, 10H, Ph), 7.12 (t, 1H, J = 7.2 Hz, Ph), 5.58 (s, 2H, NH_2), 4.47 (s, 2H, Ph $\mathbf{CH_2}$); EI-MS (70 eV, *m*/*z*, rel intensity) 442 (M + 2, 2), 441 (M^+, 7). Anal. Calcd for $C_{24}H_{20}N_6OS$: C, 65.44; H, 4.58; N, 19.08. Found: C, 65.52; H, 4.43; N, 19.07.

Data for **5i**: white crystal, mp 245.7−246.4 °C; IR (KBr) υ (cm^{-1}) 3317, 3268, 3212, 1700, 1633, 1591, 1554, 923, 769; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.83 (br s, 1H, NH), 8.16 (s, 1H, Ar), 8.04 (d, 2H, J = 7.5 Hz, Ar), 7.46−7.56 (m, 3H, Ar), 7.26−7.36 (m, 2H, Ar), 7.11 (d, 1H, J = 7.8 Hz, Ar), 5.60 (s, 2H, NH_2), 2.60 (s, 3H, CH_3); EI-MS (70 eV, *m*/*z*, rel intensity) 400 (M + 2, 32), 399 (M + 1, 22), 398 (M^+, 95). Anal. Calcd for $C_{18}H_{15}ClN_6OS$: C, 54.20; H, 3.79; N, 21.07. Found: C, 53.96; H, 3.74; N, 22.27.

Data for **5j**: white crystal, mp 227.0−228.0 °C; IR (KBr) υ (cm^{-1}) 3346, 3302, 3186, 1684, 1599, 1543, 1503, 1398, 930, 773; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.61 (s, 1H, C_6H_4**NH**), 8.08 (d, 2H, J = 8.4 Hz, Ar), 7.80 (s, 1H, Ar), 7.50 (t, 2H, J = 7.8 Hz, Ar), 7.44 (d, 1H, J = 8.0 Hz, Ar), 7.32 (t, 1H, J = 8.0 Hz, Ar), 7.26 (t, 1H, J = 8.0 Hz, Ar), 6.96 (d, 1H, J = 7.2 Hz, Ar), 5.59 (s, 2H, NH_2), 2.61(s, 3H, SCH_3), 2.35 (s, 3H, *m*-$\mathbf{CH_3}C_6H_4$); EI-MS (70 eV, *m*/*z*, rel intensity) 379 (M + 1, 6), 378 (M^+, 27). Anal. Calcd for $C_{19}H_{18}N_6OS$: C, 60.30; H, 4.79; N, 22.21. Found: C, 60.20; H, 4.81; N, 22.42.

General Synthetic Procedures for 3-Alkylthio-8-aryl-phenyl-1,8-dihydropyrazolo[3,4-*d*][1,2,4]triazolo[1,5-*a*]pyrimidin-4-one 6. To a suspension of 5-amino-3-alkylthio-6-arylamino-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one **5** (1.5 mmol) in 25 mL of triethyl orthoformate was added *p*-toluenesulfonic acid (*p*-TsOH) (0.4 g, 2.25 mmol). After the reaction mixture was refluxed for 15 h and then cooled, lots of precipitate appeared. The crude product was collected by filtration and washed with water. After recrystallization from dimethylformamide, a white crystal was obtained.

Data for **6a**: white crystal, mp 267−269 °C; IR (KBr) υ (cm^{-1}) 3139, 2924, 1719, 1583, 1560, 1509, 1400, 1162, 902, 768; ^{1}H NMR

Table 1. Yields of Compounds **5** and **6**

compd	R	Ar	yield of **5**(%)	yield of **6**(%)
5a, **6a**	Me	*p*-FC_6H_4	69	70
5b, **6b**	Me	*o*-FC_6H_4	92	78
5c, **6c**	Me	*m*-FC_6H_4	73	94
5d, **6d**	$PhCH_2$	*p*-FC_6H_4	52	62
5e, **6e**	$PhCH_2$	*o*-FC_6H_4	81	76
5f, **6f**	$PhCH_2$	*m*-FC_6H_4	86	83
5g, 6g	Me	C_6H_5	76	72
5h, 6h	$PhCH_2$	C_6H_5	72	69
5i, **6i**	Me	*m*-ClC_6H_4	64	78
5j, **6j**	Me	*m*-MeC_6H_4	68	79

(DMSO-d_6, 400 MHz) δ 9.30 (s, 1H, triazole-H), 8.05 (d, 2H, J = 8.0 Hz, Ar), 7.94−7.98 (m, 2H, Ar), 7.49−7.56 (m, 4H, Ar), 7.31 (t, 1H, J = 6.8 Hz, Ar), 2.64 (s, 3H, SCH_3); EI-MS (70 eV, *m*/*z*, rel intensity) 392 (M^+, 9). Anal. Calcd for $C_{19}H_{13}FN_6OS$: C, 58.15; H, 3.34; N, 21.42. Found: C, 58.21; H, 3.32; N, 21.51.

Data for **6b**: white crystal, m p 224−225 °C; IR (KBr) υ (cm^{-1}) 3146, 1712, 1607, 1585, 1525, 1509, 1398, 1234, 904, 769, 752; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.23 (s, 1H, triazole-H), 8.00 (d, 2H, J = 8.0 Hz, Ar), 7.92 (t, 1H, J = 7.6 Hz, Ar), 7.44−7.69 (m, 5H, Ar), 7.29 (t, 1H, J = 7.4 Hz, Ar), 2.65 (s, 3H, SCH_3); EI-MS (70 eV, *m*/*z*, rel intensity) 393 (M + 1, 31), 392 (M^+, 63). Anal. Calcd for $C_{19}H_{13}FN_6OS$: C, 58.15; H, 3.34; N, 21.42. Found: C, 58.13; H, 3.32; N, 21.48.

Data for **6c**: white crystal, mp 247−249 °C; IR (KBr) υ (cm^{-1}) 3115, 3064, 2924, 1733, 1608, 1586, 1526, 1510, 1402, 1232, 1159, 905, 850, 746; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.39 (s, 1H, triazole-H), 8.07 (d, 2H, J = 8.0 Hz, Ar), 7.93 (d, 1H, J = 10.0 Hz, Ar), 7.84 (d, 1H, J = 8.4 Hz, Ar), 7.72 (q, 1H, J = 7.5 Hz, Ar), 7.52 (t, 2H, J = 7.8 Hz, Ar), 7.41 (t, 1H, J = 8.4 Hz, Ar), 7.33 (t, 1H, J = 7.2 Hz, Ar), 2.64 (s, 3H, SCH_3); EI-MS (70 eV, *m*/*z*, rel intensity) 392 (M^+, 5). Anal. Calcd for $C_{19}H_{13}FN_6OS$: C, 58.15; H, 3.34; N, 21.42. Found: C, 58.22; H, 3.30; N, 21.26.

Data for **6d**: white crystal, mp 267−268 °C; IR (KBr) υ (cm^{-1}) 3031, 1723, 1616, 1589, 1528, 1508, 1456, 1234, 1128, 901, 843, 766; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.30 (s, 1H, triazole-H), 8.06 (d, 2H, J = 8.8 Hz, Ar), 7.94−7.97 (m, 2H, Ar), 7.26−7.56 (m, 10H, Ar), 4.51 (s, 2H, Ph**CH_2**); EI-MS (70 eV, *m*/*z*, rel intensity) 468 (M^+, 3). Anal. Calcd for $C_{25}H_{17}FN_6OS$: C, 64.09; H, 3.66; N, 17.94. Found: C, 64.20; H, 3.68; N, 17.86.

Data for **6e**: white crystal, mp 231−232 °C; IR (KBr) υ (cm^{-1}) 3133, 1705, 1587, 1558, 1525, 1507, 1398, 1240, 1068, 901, 767, 754; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.23 (s, 1H, triazole-H), 8.00 (d, 2H, J = 8.4 Hz, Ar), 7.92 (t, 1H, J = 8.0 Hz, Ar), 7.25−7.68 (m, 11H, Ar), 4.50 (s, 2H, Ph**CH_2**); EI-MS (70 eV, *m*/*z*, rel intensity) 468 (M^+, 1). Anal. Calcd for $C_{25}H_{17}FN_6OS$: C, 64.09; H, 3.66; N, 17.94. Found: C, 64.15; H, 3.67; N, 17.96.

Data for **6f**: white crystal, mp 217−219 °C; IR (KBr) υ (cm^{-1}) 3134, 1718, 1607, 1582, 1524, 1508, 1400, 1228, 1158, 765; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.37 (s, 1H, triazole-H), 8.07 (d, 2H, J = 7.6 Hz, Ar), 7.93 (d, 1H, J = 8.0 Hz, Ar), 7.84 (d, 1H, J = 7.2 Hz, Ar), 7.69−7.73 (m, 1H, Ar), 7.26−7.55 (m, 9H, Ar), 4.51 (s, 2H, Ph**CH_2**); EI-MS (70 eV, *m*/*z*, rel intensity) 468 (M^+, 2). Anal. Calcd for $C_{25}H_{17}FN_6OS$: C, 64.09; H, 3.66; N, 17.94. Found: C, 64.20; H, 3.65; N, 17.98.

Data for **6g**: white crystal, mp 253−255 °C; IR (KBr) υ (cm^{-1}) 3144, 3081, 2925, 1718, 1605, 1578, 1560, 1527, 1388, 1265, 1165, 768, 685; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.34 (s, 1H, triazole-H), 8.08 (d, 2H, J = 8.4 Hz, Ph), 7.93 (d, 2H, J = 8.0 Hz, Ph), 7.67 (t, 2H, J = 8.2 Hz, Ph), 7.49−7.57 (m, 3H, Ph), 7.31 (t, 1H, J = 6.8 Hz, Ph), 2.65 (s, 3H, SCH_3); EI-MS (70 eV, *m*/*z*, rel intensity) 375 (M + 1, 13), 374 (M^+, 36). Anal. Calcd for $C_{19}H_{14}N_6OS$: C, 60.95; H, 3.77; N, 22.45. Found: C, 61.02; H, 3.75; N, 22.53.

Data for **6h**: white crystal, mp 224−226 °C; IR (KBr) υ (cm^{-1}) 3118, 3064, 2924, 1718, 1608, 1580, 1528, 1389, 1163, 757, 685; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.34 (s, 1H, triazole-H), 8.08 (d, 2H, J = 7.6 Hz, Ph), 7.92 (d, 2H, J = 7.6 Hz, Ph), 7.50−7.67 (m, 7H, Ph), 7.34 (t, 3H, J = 7.4 Hz, Ph), 7.26 (t, 1H, J = 7.2 Hz, Ph), 4.51 (s, 2H, Ph**CH_2**); EI-MS (70 eV, *m*/*z*, rel intensity) 450 (M^+, 8). Anal. Calcd for $C_{25}H_{18}N_6OS$: C, 66.65; H, 4.03; N, 18.65. Found: C, 66.70; H, 4.02; N, 18.71.

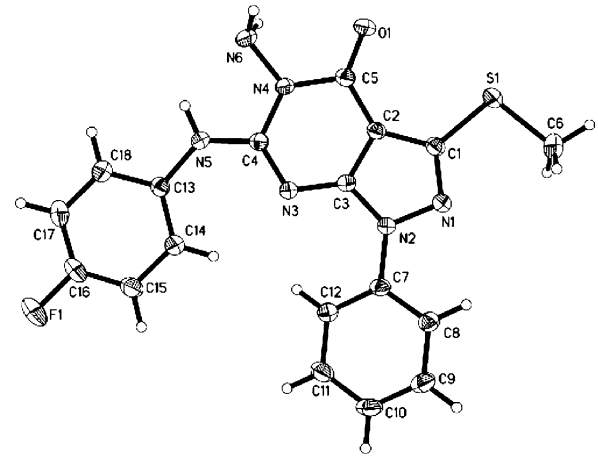


Figure 1. X-ray structure of compound **5a**.

Data for **6i**: white crystal, mp 259.6 −261.0 °C; IR (KBr) υ (cm^{-1}) 3112, 2919, 1735, 1610, 1591, 1525, 1509, 1401, 1121, 800, 764; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.41 (s, 1H, triazole-H), 8.22 (s, 1H, m-Cl**C_6H_4**), 8.08 (d, 2H, J = 8.0 Hz, Ph), 7.93 (d, 1H, J = 8.0 Hz, Ph), 7.69 (t, 1H, J = 7.8 Hz, Ar), 7.61 (d, 1H, J = 8.0 Hz, Ar), 7.50 (t, 2H, J = 7.6 Hz, Ar), 7.32 (t, 1H, J = 7.6 Hz, Ar), 2.64 (s, 3H, SCH_3); EI-MS (70 eV, *m*/*z*, rel intensity) 408 (M^+, 12). Anal. Calcd for $C_{19}H_{13}ClN_6OS$: C, 55.81; H, 3.20; N, 20.55. Found: C, 55.89; H, 3.17; N, 20.61.

Data for **6j**: white crystal, mp 257−259 °C; IR (KBr) υ (cm^{-1}) 3140, 3084, 2930, 1718, 1615, 1586, 1562, 1529, 1510, 1387, 1164, 904, 768; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.34 (s, 1H, triazole-H), 8.09 (d, 2H, J = 7.6 Hz, Ar), 7.85 (s, 1H, Ar), 7.70 (d, 1H, J = 8.0 Hz, Ar), 7.47−7.54 (m, 3H, Ar), 7.30−7.36 (m, 2H, Ar), 2.63 (s, 3H, SCH_3), 2.44 (s, 3H, *m*-CH_3Ph); EI-MS (70 eV, *m*/*z*, rel intensity) 389 (M + 1, 42), 388 (M^+, 76). Anal. Calcd for $C_{20}H_{16}N_6OS$: C, 61.84; H, 4.15; N, 21.63. Found: C, 61.96; H, 4.12; N, 21.68.

Crystal Structure Determination. The crystal structure of the compound **5a** was determined, and X-ray intensity data were recorded on a Bruker SMART 1000 CCD diffraction meter using graphite monochromated Mo Kα radiation (λ = 0.71073 Å). A total of 9306 reflections were measured, of which 3444 were unique (R_{int} = 0.0272) in the range of 2.46 < θ < 26.37° (h, −12 to 17; k, −9 to 8; l, −20 to 20), and 2579 observed reflections with $I > 2\sigma(I)$ were used in the refinement on F^2. The structure was solved by direct methods with the SHELXS-97 program. All of the non-H atoms were refined anisotropically by full-matrix least-squares to give the final R = 0.0362 and wR = 0.0878 ($w = 1/[\sigma^2(Fo^2) + (0.0473P)^2 + 0.3686P]$, where $P = (Fo^2 + 2Fc^2)/3$) with $(\Delta/\sigma)_{max}$ = 0.001 and S = 1.043 by using the SHELXL program. The hydrogen atoms were located from a difference Fourier map and refined isotropically.

Biological Tests: In Vitro Antifungal Activity. The fungi were obtained from the College of Plant Protect, Central China Agriculture University, China. The antifungal activities of compounds **5** and **6** against *Botrytis cinerea* Pers, *Pyricularia oryzae*, *Gibberella zeae*, and *Sclerotinia sclerotiorum* were investigated at dosages of 50 and 10 mg/L using the procedure (*20*).

RESULTS AND DISCUSSION

Iminophosphorane **2**, which was prepared by the reaction of ethyl 5-amino- 3-alkylthio-1-phenyl-1*H*-pyrazole-4-carboxylate **1** with triphenylphosphorane and bromine, reacted with isocyanates to give the key intermediates carbodiimide **3**. Treatment of **3** with hydrazine hydrate gave the guanidine **4** (*9*), which, in the presence of EtONa, proceeded to afford the crude target compounds at room temperature. The crude products were collected by filtration. After recrystallization from DMF/

Table 2. Antifungal Activity of Compounds **5** and **6** (50 mg/L, Inhibitory Rate Percent) [a,b]

	relative inhibition (%)							
	Botrytis		*Pyricularia*		*Gibberella*		*Sclerotinia*	
compd	**5**	**6**	**5**	**6**	**5**	**6**	**5**	**6**
5a, **6a**	45	71	43	9	20	49	94	93
5b, **6b**	93	60	62	5	51	26	97	50
5c, **6c**	87	91	43	29	43	74	96	100
5d, **6d**	55	67	29	43	34	37	83	75
5e, **6e**	64	60	14	14	40	37	71	96
5f, **6f**	69	29	14	0	46	34	82	29
5g, **6g**	13	99	36	80	14	66	84	100
5h, **6h**	60	73	50	29	29	60	72	93
5i, **6i**	99	99	96	57	57	74	98	100
5j, **6j**	71	97	14	70	46	50	86	98
thiabendazole	100		87		100		100	

[a] Mean value for relative inhibition calculated from at least three determinations. [b] *S* (standard deviations) are 1−3.

Table 3. Antifungal Activity of Compounds **5** and **6** (10 mg/L, Inhibitory Rate Percent)[a,b]

	relative inhibition (%)			
	Botrytis		*Sclerotinia*	
compd	**5**	**6**	**5**	**6**
5a, **6a**	9	45	72	70
5b, **6b**	69	31	73	19
5c, **6c**	56	70	75	83
5d, **6d**	12	30	52	32
5e, **6e**	18	27	33	75
5f, **6f**	20	5	40	5
5g, **6g**	3	70	51	83
5h, **6h**	12	49	31	67
5i, **6i**	73	71	76	82
5j, **6j**	36	74	52	73

[a] Mean value for relative inhibition calculated from at least three determinations. [b] *S* (standard deviations) are 1−4.

petroleum ether or column chromatography on silica gel, a white crystal were obtained in 52−92% yield (**Scheme 1**; **Table 1**). The spectral data proved the white crystal was 3-alkylthio-5-amino-6-arylamino-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-ones **5** instead of the isomer 3-alkylthio-6-hydrazine-5-aryl-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-ones **7**. This did not agree with our previous findings (*9–11*) showing the reaction of carbodiimide **3** with alkyl amine gave only an annulation compound, the nitrogen atom of which came from the ArNH group. On refluxing compounds **5** with triethyl orthoformate, compounds **6** were obtained in 62−94% yields in the presence of *p*-TsOH.

The structures of compounds **5** and **6** were deduced from their spectral data (IR, ^{1}H NMR, EI-MS, and elementary analysis). ^{1}H NMR spectra of compounds **5** showed the signal of NH in the ArNH group at 9.61−9.88 ppm, which agreed with the one in PhNH ($\delta > 7.0$) (*21*), and the single signal of NH_2 at 5.54−5.67 ppm. Thus, a combination of chemical shift and couplings allowed the complete and unambiguous assignment of all signals and demonstrated that the major products correspond to structure **5**. The EI-MS spectra of **5** showed the molecular ion peak (M^+, 7−100%). All of the fragmentation ions were consistent with their structures and could be clearly assigned. In addition, the structures of compounds **6** and X-ray single-crystal diffraction of **5a**, which was reported elsewhere (*22*), further verified the proposed structure of compound **5** (**Figure 1**) (*23*).

The antifungal activities of all compounds **5**, **6**, and 2-(4′-thiazoly)benzimidazole (commercial name thiabendazole), a commercially available fungicide, were discovered, as shown in **Tables 2** and **3** by contrasting to distilled water. It is well-known that the incorporation of a fluorine instead of a hydrogen atom can alter biological activity (*24, 25*). By comparing the activities of compounds **5a**−**5f** with those of **5g**−**5j** and the activities of compounds **6a**−**6f** with those of **6g**−**6j**, we came to the conclusion that introducing a fluorine atom into compounds **5** and **6** could not favorably improve their antifungal activities. It was also worthy of note that the compounds **5** showed higher antifungal activities in general when R was substituted for methyl instead of benzyl. Most compounds **5** and **6** possessed a good inhibition effect against *Sclerotinia* (inhibition rates of 72−100% at 50 mg/L) except **6b** and **6f**. For example, compounds **6c**, **6g**, and **6i** held 100% inhibitory rates. Especially, compounds **5c**, **5i**, **6c**, **6g**, and **6i** held by inhibition rates 75%, 76%,83%, 83% and 82% at the dosage of 10 mg/L, respectively. Some compounds **5** and **6** exhibited good inhibition against *Botrytis* at 50 mg/L. For example, the inhibitory rates of compounds **5b**, **5c**, **5i**, **6c**, **6g**, **6i**, and **6j** were 93, 87, 99, 91, 99, 99, and 97%, respectively.

In summary, we have developed a novel approach to regioselectively synthesize 3-alkylthio-5-amino-6-arylamino-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one and 3-alkylthio-8-aryl-1-phenyl-2*H*-pyrazolo[3,4-*d*][1,2,4]triazolo[1,5-*a*]pyrimidin-4 -one derivatives via a tandem aza-Wittig and annulations in good yields. The antifungal tests indicated that most of compounds **5** and **6** possessed excellent antifungal activities and could be further developed as fungicides.

LITERATURE CITED

(1) Bendich, A.; Russell, P. J.; Fox, J. J. The synthesis and properties of 6-chloropurine and purine. *J. Am. Chem. Soc.* **1954**, *76*, 6073–6077.

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

(3) Perumal, R.; Jayachandran, E.; Naragund, L. V. G.; Shivakumar, B.; Swamy, B. H. M. J.; Srinivasa, G. M. Synthesis of 3-substituted N-substituted phenyl pyrazolo[3,4-*d*]pyrimidin-4-ones as antimicrobial agents. *Indian J. Heterocycl. Chem.* **2006**, *15*, 413–414.

(4) Holla, B. S.; Mahalinga, M.; Karthikeyan, M. S.; Akberali, P. M.; Shetty, N. S. Synthesis of some novel pyrazolo[3,4-*d*]pyrimidine derivatives as potential antimicrobial agents. *Bioorg. Med. Chem.* **2006**, *14*, 2040–2047.

(5) Ballell, L.; Field, R. A.; Chung, G. A. C.; Young, R. J. New thiopyrazolo[3,4-*d*]pyrimidine derivatives as anti-mycobacterial agents. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1736–1740.

(6) George, S.; Wang, G.; Fabio, P.; Randy, B.; Robert, M.; Wang, J. Y.; Robert, H.; Megumi, K.; Scott, E.; Nwe, B.; Steven, F. Protein kinase inhibitors WO Patent 2007079164,2007.

(7) Tian, G. H.; Lai, S.; Wang, Z.; Zhu, Y.; Chen, X. J.; Ji, Y. R.; Zhang, J. F.; Jin, W. X.; Lv, H. P.; Liu, J. P.; Wang, W.; Ji, R. Y.; Shen, J. S. Pyrazolopyrimidinone derivatives, their preparation and their use WO Patent 2007056955, 2007.

(8) Wang, X. Q.; Kolasa, T.; El Kouhen, O. F.; Chovan, L. E.; Black-Shaefer, C. L.; Wagenaar, F. L.; Garton, J. A.; Moreland, R. B.; Honore, P.; Lau, Y. Y.; Dandliker, P. J.; Brioni, J. D.; Stewart, A. O. Rapid hit to lead evaluation of pyrazolo[3,4-*d*]pyrimidin-4-one as selective and orally bioavailable mGluR1 antagonists. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4303–4307.

(9) Wang, H. Q.; Ding, M. W.; Liu, Z. J. Synthesis and properties of novel dihydropyrazolopyrimidine derivatives containing sulfur atom. *Heteroatom Chem.* **2004**, *15*, 333–337.

(10) Wang, H. Q.; Ding, M. W.; Liu, Z. J.; Yang, L. M. A new regioselective synthesis and bioactivity of 1*H*-pyrazolo[3,4-*d*]-

pyrimidin-4(5*H*)-one derivatives. *J. Heterocycl. Chem.* **2004**, *41*, 393–397.

(11) Wang, H. Q.; Ding, M. W.; Liu, Z. J. Versatile synthesis and fungicidal activities of 6-alkylamino-3-alkylthio-1,5-diphenyl-1,5-dihydropyrazolo[3,4-*d*]pyrimidine-4-one. *Phosphorus, Sulfur, Silicon Relat. Elem.* **2004**, *179*, 2039–2050.

(12) Liu, H.; Wang, H. Q.; Liu, Z. J. Synthesis and herbicidal activity of novel pyrazolo[3,4-*d*]pyrimidin-4-one derivatives containing aryloxyphenoxy-propionate moieties. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2203–2209.

(13) Poli, T.; Vicentini, C. B.; Brandolini, V. Synthesis and in-vitro antifungal activity of 6-trifluoromethylpyrazolo[3,4-*d*]pyrimidines. *Pestic. Sci.* **1989**, *25*, 161–169.

(14) Sasaki, N.; Hatazawa, M.; Araki, Y.; Inuta, T. Pyrazolopyrimidine derivative and germicide for use in agriculture and gardeningJP Patent 2007008864, 2007.

(15) Cudworth, D. P.; Hegde, V. B.; Yap, M. C. H.; Guenthenspberger, K. A.; Hamilton, C. T.; Pechacek, J. T.; Johnson, P. L.; Bis, S. J.; Tisdell, F. E.; Dripps, J. E. Structure-activity relationship development of dihaloaryl triazole compounds as insecticides and acaricides. 1. Phenyl thiophen-2-yl triazoles. *J. Agric. Food Chem.* **2007**, *55*, 7517–7526.

(16) Arnoldi, A.; Dallavalle, S.; Merlini, L.; Musso, L.; Farina, G.; Moretti, M.; Jayasinghe, L. Synthesis and antifungal activity of a series of *N*-substituted [2-(2,4-dichlorophenyl)-3-(1,2,4-triazol-1-yl)]-propylamines. *J. Agric. Food Chem.* **2007**, *55*, 8187–8192.

(17) Yang, G. F.; Xu, L.; Lu, A. H. Synthesis and bioactivity of novel triazolo[1,5-*a*]pyrimidine derivatives [3]. *Heteroatom Chem.* **2001**, *12*, 491–496.

(18) Russo, F.; Guccione, S.; Romeo, G. Synthesis and pharmacological properties of pyrazolotriazolopyrimidine derivatives. *Eur. J. Med. Chem.* **1992**, *27*, 73–80.

(19) Wang, H. Q.; Liu, H.; Liu, Z. J. Synthesis and biological activity of 1-[2-(2,4-dichlorophenoxylacetyl)]-5-amino-1*H*-pyrazole derivatines. *Chin. J. Org.Chem.* **2004**, *24*, 797–801.

(20) Wilamowski, J.; Kulig, E.; Sepiol, J. J.; Burgiel, Z. J. Synthesis and in vitro antifungal activity of 1-amino-3,4-dialkylnaphthalene-2-carbonitriles and their analogues. *Pest Manag. Sci.* **2001**, *57*, 625–632.

(21) Taylor, E. C.; Patel, M. A simple aza Wittig-mediated pyrimidine annulation reaction. *Heterocycl. Chem.* **1991**, *28*, 1857–1861.

(22) Wang, H. Q.; Zhou, W. P.; Liu, Z. J. Synthesis and structure of 5-amino-6-(4-fluorophenylamino)-3-methylthio-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one. *Chin. Jiegou Huaxue* **2006**, *25*, 1105–1109.

(23) Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication (CCDC 619024).

(24) Liu, H.; Wang, H. Q.; Ding, M. W.; Liu, Z. J.; Xiao, W. J. A novel synthesis and herbicidal activity of fluorine-containing pyrazolo[3,4-*d*]pyrimidin-4-one derivatives. *J. Fluorine Chem.* **2006**, *127*, 1584–1590.

(25) Vicentini, C. B.; Poli, T.; Veronese, A. C.; Brandolini, V.; Manfrini, M.; Guarneri, M.; Giori, P. Synthesis and in-vitro antifungal activity of 6-trifluoromethyipyrazolo[3,4-6*d*]pyrimidin-4(5*H*)-thiones. *Pestic. Sci.* **2006**, *27* (1), 77–83.

Received for review May 1, 2008. Revised manuscript received June 27, 2008. Accepted June 30, 2008. Project supported by Hunan Provincial Natural Science Foundation of China, No. 05JJ30022, and by the Scientific Research Fund of the Hunan Provincial Education Department, No. 06B081.

JF801359F