

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

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A novel approach was developed to regioselectively synthesize new 5-amino-6-aryl-amino-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, ¹H 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 PhCH₂. 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 pyrazolotriazolo-pyrimidine 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 isocyanates and alkylamine could give 6-alkylamino-5-aryl-pyrazolo[3,4-*d*]pyrimidin-4-one derivatives, the nitrogen atoms of which were from isocyanates. 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-aryl-amino-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-aryl-amino-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 Finnigan Trace Mass 2000 spectrometer at 70 eV. IR was recorded on an Avatar 360 spectrometer as KBr pellets with absorption given in cm⁻¹. ¹H NMR spectra were taken on a Varian Mercury 400 (or 300) spectrometer with TMS as the internal reference and DMSO-*d*₆ or CDCl₃ as the solvent. Elemental analysis was taken on a Vario EL elemental analysis instrument. X-ray diffraction was performed with a Bruker 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).

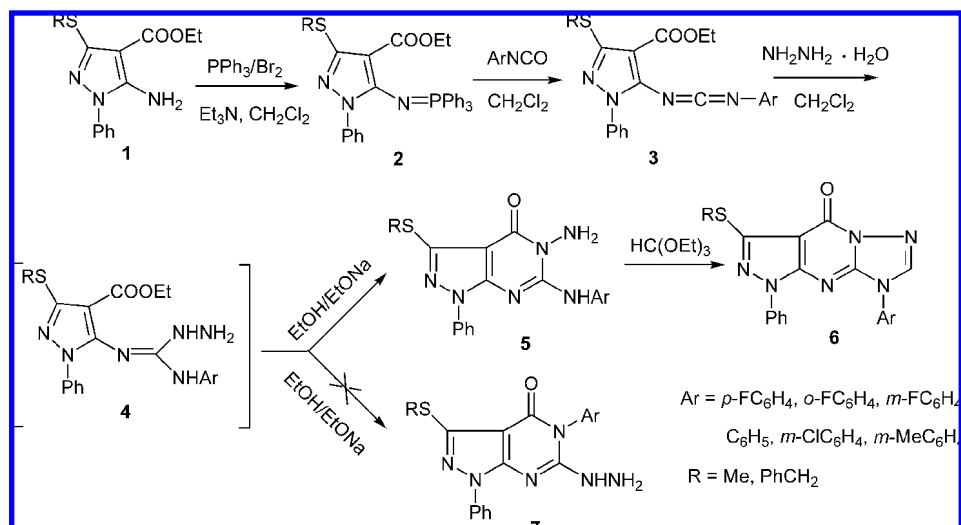
General Synthetic Procedures for 3-Alkylthio-5-amino-6-aryl-amino-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-ones **5.** Aryl isocyanate (0.27 g, 2 mmol) was added to a solution of iminophosphorane **2** (1.07 g, 2 mmol) in dry methylene dichloride (20 mL) under nitrogen atmosphere at room temperature.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

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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) ν (cm⁻¹) 3347, 3311, 3207, 1679, 1596, 1563, 1509, 913, 763, 670; ¹H NMR (DMSO-*d*₆, 300 MHz) δ 9.74 (s, 1H, *p*-FC₆H₄NH), 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, NH₂), 2.59 (s, 3H, SCH₃); EI-MS (70 eV, *m/z* rel intensity) 384 (M + 2, 16), 383 (M + 1, 47), 382 (M⁺, 92). Anal. Calcd for C₁₈H₁₅FN₆O₅: 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) ν (cm⁻¹) 3352, 3314, 3266, 1721, 1621, 1600, 1542 (Ar), 1455, 922, 768; ¹H NMR (DMSO-*d*₆, 400 MHz) δ 9.61 (s, 1H, *o*-FC₆H₄NH), 7.98 (d, 3H, *J* = 8.4 Hz, Ar), 7.25–7.46 (m, 6H, Ar), 5.65 (s, 2H, NH₂), 2.61 (s, 3H, SCH₃); EI-MS (70 eV, *m/z* rel intensity) 384 (M + 2, 13), 383 (M + 1, 49), 382 (M⁺, 89). Anal. Calcd for C₁₈H₁₅FN₆O₅: 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) ν (cm⁻¹) 3314, 3273, 3212, 1702, 1637, 1604, 1560, 1545, 963, 925, 871, 764; ¹H NMR (DMSO-*d*₆, 400 MHz) δ 9.82 (s, 1H, *m*-FC₆H₄NH), 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, NH₂), 2.61 (s, 3H, SCH₃); EI-MS (70 eV, *m/z* rel intensity) 384 (M + 2, 21), 382 (M⁺, 100). Anal. Calcd for C₁₈H₁₅FN₆O₅: 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) ν (cm⁻¹) 3319, 3207, 3025, 2930, 1686, 1609, 1595, 1563, 1509, 1390, 1211, 924, 771; ¹H NMR (DMSO-*d*₆, 300 MHz) δ 9.76 (br s, 1H, *p*-FC₆H₄NH), 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, NH₂), 4.46 (s, 2H, PhCH₂); EI-MS (70 eV, *m/z* rel intensity) 460 (M + 2, 12), 459 (M + 1, 28), 458 (M⁺, 50). Anal. Calcd for C₂₄H₁₉FN₆O₅: 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) ν (cm⁻¹) 3323, 3274, 3212, 3028, 2924, 1685, 1600, 1553, 1491, 1459, 1390, 1228, 1170, 926, 759; ¹H NMR (DMSO-*d*₆, 400 MHz) δ 9.61 (br s, 1H, *o*-FC₆H₄NH), 7.94–7.99 (m, 3H, Ar), 7.26–7.48 (m, 11H, Ar), 5.67 (br s, 2H, NH₂), 4.48 (s, 2H, PhCH₂); EI-MS (70 eV, *m/z* rel intensity) 458 (M⁺, 12). Anal. Calcd for C₂₄H₁₉FN₆O₅: 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) ν (cm⁻¹) 3320, 3208, 2919, 1687, 1598, 1556, 1492, 1390, 1171, 945, 774; ¹H NMR (DMSO-*d*₆, 400 MHz) δ 9.88 (br s, 1H, *m*-FC₆H₄NH), 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, NH₂), 4.48 (s, 2H, PhCH₂); EI-MS (70 eV, *m/z* rel intensity) 458 (M⁺, 21). Anal. Calcd for C₂₄H₁₉FN₆O₅: 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) ν (cm⁻¹) 3336, 3267, 1692, 1596, 1554, 909, 770, 743, 688; ¹H NMR (DMSO-*d*₆, 400 MHz) δ 9.67 (s, 1H, PhNH), 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, NH₂), 2.60 (s, 3H, SCH₃); EI-MS (70 eV, *m/z* rel intensity) 65 (M + 1, 49), 364 (M⁺, 81). Anal. Calcd for C₁₈H₁₆N₆O₅: 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) ν (cm⁻¹) 3319, 1684, 1596, 1554, 927, 759, 689, 501; ¹H NMR (CDCl₃, 300 MHz) δ 9.69 (s, 1H, PhNH), 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, NH₂), 4.47 (s, 2H, Ph CH₂); EI-MS (70 eV, *m/z* rel intensity) 442 (M + 2, 2), 441 (M⁺, 7). Anal. Calcd for C₂₄H₂₀N₆O₅: 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) ν (cm⁻¹) 3317, 3268, 3212, 1700, 1633, 1591, 1554, 923, 769; ¹H 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, NH₂), 2.60 (s, 3H, CH₃); EI-MS (70 eV, *m/z* rel intensity) 400 (M + 2, 32), 399 (M + 1, 22), 398 (M⁺, 95). Anal. Calcd for C₁₈H₁₅ClN₆O₅: 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) ν (cm⁻¹) 3346, 3302, 3186, 1684, 1599, 1543, 1503, 1398, 930, 773; ¹H NMR (DMSO-*d*₆, 400 MHz) δ 9.61 (s, 1H, C₆H₄NH), 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, NH₂), 2.61 (s, 3H, SCH₃), 2.35 (s, 3H, *m*-CH₃C₆H₄); EI-MS (70 eV, *m/z* rel intensity) 379 (M + 1, 6), 378 (M⁺, 27). Anal. Calcd for C₁₉H₁₈N₆O₅: 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-aryl-amino-1-phenyl-1H-pyrazolo[3,4-*d*]pyrimidin-4(5H)-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) ν (cm⁻¹) 3139, 2924, 1719, 1583, 1560, 1509, 1400, 1162, 902, 768; ¹H NMR

Table 1. Yields of Compounds 5 and 6

compd	R	Ar	yield of 5(%)	yield of 6(%)
5a, 6a	Me	<i>p</i> -FC ₆ H ₄	69	70
5b, 6b	Me	<i>o</i> -FC ₆ H ₄	92	78
5c, 6c	Me	<i>m</i> -FC ₆ H ₄	73	94
5d, 6d	PhCH ₂	<i>p</i> -FC ₆ H ₄	52	62
5e, 6e	PhCH ₂	<i>o</i> -FC ₆ H ₄	81	76
5f, 6f	PhCH ₂	<i>m</i> -FC ₆ H ₄	86	83
5g, 6g	Me	C ₆ H ₅	76	72
5h, 6h	PhCH ₂	C ₆ H ₅	72	69
5i, 6i	Me	<i>m</i> -ClC ₆ H ₄	64	78
5j, 6j	Me	<i>m</i> -MeC ₆ H 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, SCH₃); EI-MS (70 eV, *m/z*, rel intensity) 392 (M⁺, 9). Anal. Calcd for C₁₉H₁₃FN₆OS: C, 58.15; H, 3.34; N, 21.42. Found: C, 58.21; H, 3.32; N, 21.51.

Data for **6b**: white crystal, mp 224–225 °C; IR (KBr) ν (cm⁻¹) 3146, 1712, 1607, 1585, 1525, 1509, 1398, 1234, 904, 769, 752; ¹H 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, SCH₃); EI-MS (70 eV, *m/z*, rel intensity) 393 (M + 1, 31), 392 (M⁺, 63). Anal. Calcd for C₁₉H₁₃FN₆OS: 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) ν (cm⁻¹) 3115, 3064, 2924, 1733, 1608, 1586, 1526, 1510, 1402, 1232, 1159, 905, 850, 746; ¹H 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, SCH₃); EI-MS (70 eV, *m/z*, rel intensity) 392 (M⁺, 5). Anal. Calcd for C₁₉H₁₃FN₆OS: 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) ν (cm⁻¹) 3031, 1723, 1616, 1589, 1528, 1508, 1456, 1234, 1128, 901, 843, 766; ¹H 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, PhCH₂); EI-MS (70 eV, *m/z*, rel intensity) 468 (M⁺, 3). Anal. Calcd for C₂₅H₁₇FN₆OS: 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) ν (cm⁻¹) 3133, 1705, 1587, 1558, 1525, 1507, 1398, 1240, 1068, 901, 767, 754; ¹H 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, PhCH₂); EI-MS (70 eV, *m/z*, rel intensity) 468 (M⁺, 1). Anal. Calcd for C₂₅H₁₇FN₆OS: 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) ν (cm⁻¹) 3134, 1718, 1607, 1582, 1524, 1508, 1400, 1228, 1158, 765; ¹H 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, PhCH₂); EI-MS (70 eV, *m/z*, rel intensity) 468 (M⁺, 2). Anal. Calcd for C₂₅H₁₇FN₆OS: 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) ν (cm⁻¹) 3144, 3081, 2925, 1718, 1605, 1578, 1560, 1527, 1388, 1265, 1165, 768, 685; ¹H 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, SCH₃); EI-MS (70 eV, *m/z*, rel intensity) 375 (M + 1, 13), 374 (M⁺, 36). Anal. Calcd for C₁₉H₁₄N₆OS: 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) ν (cm⁻¹) 3118, 3064, 2924, 1718, 1608, 1580, 1528, 1389, 1163, 757, 685; ¹H 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,

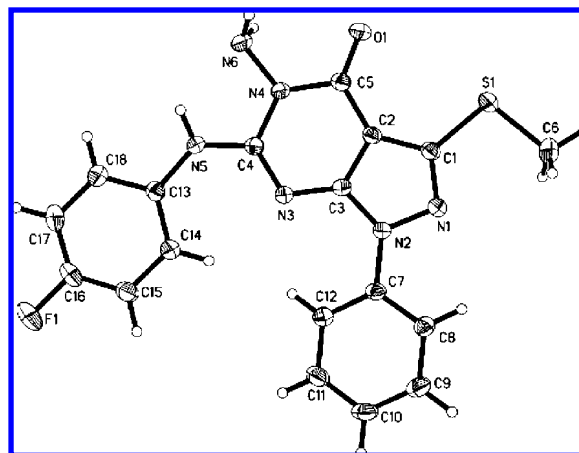


Figure 1. X-ray structure of compound 5a.

PhCH₂); EI-MS (70 eV, *m/z*, rel intensity) 450 (M⁺, 8). Anal. Calcd for C₂₅H₁₈N₆OS: C, 66.65; H, 4.03; N, 18.65. Found: C, 66.70; H, 4.02; N, 18.71.

Data for **6i**: white crystal, mp 259.6–261.0 °C; IR (KBr) ν (cm⁻¹) 3112, 2919, 1735, 1610, 1591, 1525, 1509, 1401, 1121, 800, 764; ¹H NMR (DMSO-*d*₆, 400 MHz) δ 9.41 (s, 1H, triazole-H), 8.22 (s, 1H, *m*-ClC₆H₄), 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, SCH₃); EI-MS (70 eV, *m/z*, rel intensity) 408 (M⁺, 12). Anal. Calcd for C₁₉H₁₃ClN₆OS: 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) ν (cm⁻¹) 3140, 3084, 2930, 1718, 1615, 1586, 1562, 1529, 1510, 1387, 1164, 904, 768; ¹H 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, SCH₃), 2.44 (s, 3H, m-CH₃Ph); EI-MS (70 eV, *m/z*, rel intensity) 389 (M + 1, 42), 388 (M⁺, 76). Anal. Calcd for C₂₀H₁₆N₆OS: 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 K α 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 $2.46 < \theta < 26.37^\circ$ (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 ($w = 1/[\sigma^2(\text{Fo}^2) + (0.0473P)^2 + 0.3686P]$, where $P = (\text{Fo}^2 + 2\text{Fc}^2)/3$) with $(\Delta/\sigma)_{\text{max}}$ = 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}

compd	relative inhibition (%)							
	<i>Botrytis</i>		<i>Pyricularia</i>		<i>Gibberella</i>		<i>Sclerotinia</i>	
	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.

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, ¹H NMR, EI-MS, and elementary analysis). ¹H 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 NH₂ 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

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

compd	relative inhibition (%)			
	<i>Botrytis</i>		<i>Sclerotinia</i>	
	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.

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.

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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