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ORIGINAL ARTICLE

Synthesis of pyrazolo[1,5-*a*]pyrimidine derivatives and their antifungal activities against phytopathogenic fungi in vitro

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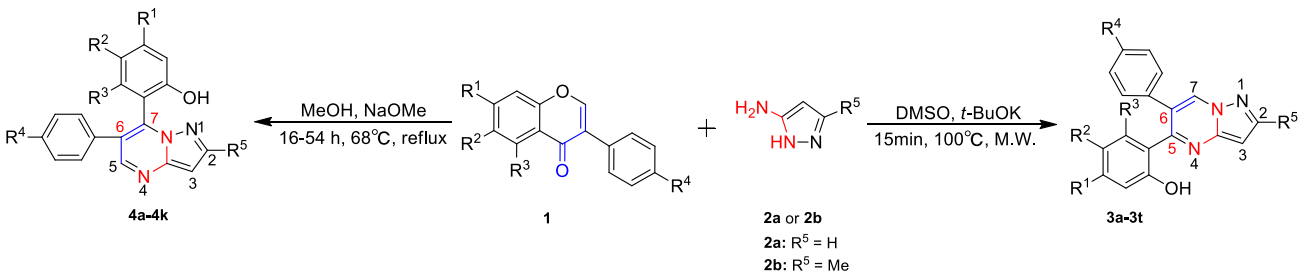
Abstract 5,6-Diarylpyrazolo[1,5-*a*]pyrimidines (**3**) and 6,7-diarylpyrazolo[1,5-*a*]pyrimidines (**4**) were chemoselectively synthesized by the condensation of isoflavone (**1**) and 3-aminopyrazole (**2**). 5,6-Diarylpyrazolo[1,5-*a*]pyrimidines (**3**) were obtained via microwave irradiation, and 6,7-diarylpyrazolo[1,5-*a*]pyrimidines (**4**) were obtained *via* conventional heating. In addition, the pyrimidine derivatives **3** and **4** were evaluated against five phytopathogenic fungi (*Cytospora* sp., *Colletotrichum gloeosporioides*, *Botrytis cinerea*, *Alternaria solani*, and *Fusarium solani*) using the mycelium growth rate method. Some of them were effective in inhibiting the growth of the five phytopathogenic fungi. For instance, 6,7-diarylpyrazolo[1,5-*a*]pyrimidines (**4j**) inhibited the growth of *A. solani* with an IC_{50} value of 17.11 μg/mL, and 6,7-diarylpyrazolo[1,5-*a*]pyrimidines (**4h**) inhibited the growth of both *Cytospora* sp. and *F. solani* with IC_{50} values of 27.32 and 21.04 μg/mL, respectively.

Graphical Abstract A chemoselective synthesis of 5,6-pyrazolo[1,5-*a*]pyrimidines **3** derivatives in excellent yields was performed under microwave irradiation and 6,7-pyrazolo [1,5-*a*]pyrimidines **4** were also prepared using heating method. The antifungal properties of **3** and **4** were tested against *Cytospora* sp., *Colletotrichum gloeosporioides*, *Botrytis cinerea*, *Alternaria solani*, and *Fusarium solani*.



R^1 = H, OMe, O^{i}Pr; R^2 = H, F; R^3 = H, OMe; R^4 = H, Me, OMe, F, CF_3.

Keywords Pyrazolo[1, 5-*a*]pyrimidines · Chemoselectivity · Synthesized · Phytopathogenic fungi · Antifungal

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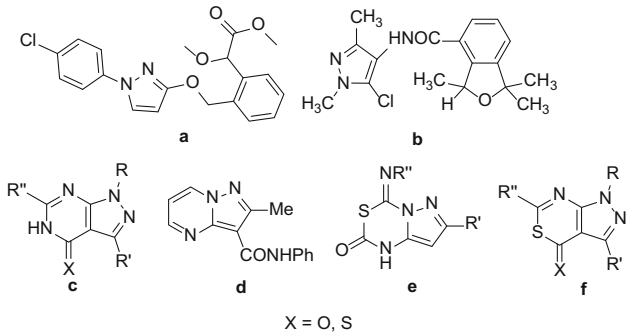
Abbreviations

HRMS	High-resolution mass spectrometry
TLC	Thin-layer chromatography
mp	Melting point
IC_{50}	Half-maximal inhibitory concentration
SAR	Structure–activity relationship
PDA	Potato dextrose agar

Introduction

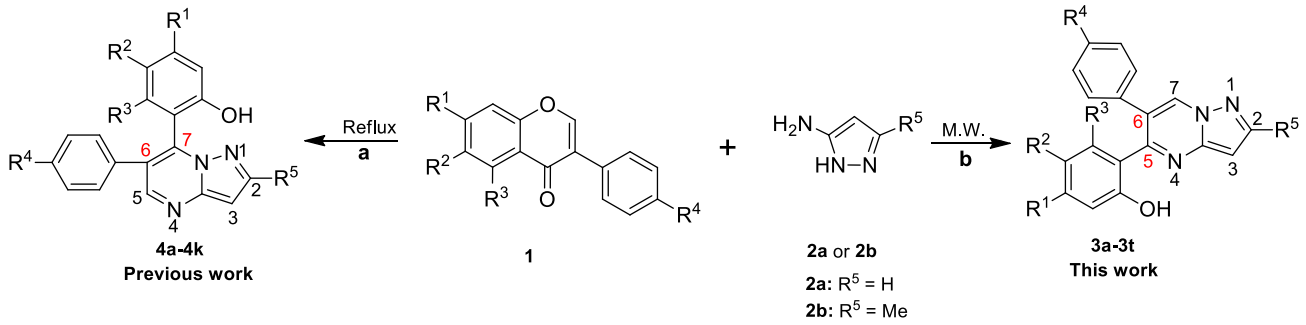
Phytopathogenic fungi cause severe yield losses and quality decrease of crop production when they infect tissues at any growth stage of a plant [1]. Moreover, phytopathogenic fungi can produce mycotoxins that can be severely harmful to animal and human health [2]. As a result, scientists have developed a variety of different antifungal agents to control those fungi [3]. Although fungicides have played a vital role in preventing and treating this problem, several drawbacks, such as severe drug resistance, serious drug interactions, drug-related toxicity, and nonoptimal pharmacokinetics, still make the current antifungal agents far from ideal for antifungal therapy [4,5]. Therefore, new, safer, and more effective antifungal agents are still urgently needed.



Scheme 1 Examples of pyrazole-based fungicides

Pyrazolo[1,5-*a*]pyrimidines have a wide range of biological and pharmaceutical activities, which have been patented for herbicide, insecticide, and sterilization uses [6]. They have also been reported to be antitrichomonal agents and effectively inhibit trichophyton mentagrophytes [7,8]. In addition, they are also known to act as HIV reverse transcriptase inhibitors [9] and antifungal agents [10]. Pyrazole antifungals such as pyraclostrobin and furametpyr (Scheme 1a, b) were used to control plant fungi. Among them, pyraclostrobin showed a broad spectrum of antifungal activities because it is a strobilurin-mitochondrial electron transport inhibitor [11]. Furametpyr was reported to control rice sheath blight, which was caused by *Rhizoctonia solani* [12]. Moreover, a large number of polycyclic pyrazole fungicides have also been synthesized. For example, pyrazolo[3,4-*d*]pyrimidine-4(5*H*)-thiones and 2-methyl-*N*-phenylpyrazolo[1,5-*a*]pyrimidine-3-carboxamide (Scheme 1c, d) exhibited remarkable inhibition activities against *Rhizoctonia solani* and *Pythium ultimum* [13,14]. Pyrazolo[1,5-*c*][1,3,5]thiadiazin-4-one/thione and pyrazolo[3,4-*d*][1,3]thiazin-4-one/thione derivatives (Scheme 1e, f) were tested for antifungal activity against *Magnaporthe grisea* to control rice blast disease, and some of these compounds effectively inhibited fungal growth [15].

Based on pyrazolo[1,5-*a*]pyrimidines' broad spectrum of pharmacological and medicinal properties, several synthesis of pyrazolo[1,5-*a*]pyrimidines have been reported. Among such approaches, the most common one is the condensation of aminopyrazoles with 1,3-bis-electrophilic compounds (i.e., α, β-unsaturated carbonyl compounds) [16,17]. In our previous work, 6,7-diarylpyrazolo[1,5-*a*] pyrimidines **4** were synthesized by the condensation of isoflavone **1** and 3-aminopyrazole **2** under conventional heating (Scheme 2) [18]. However, this approach suffered from a long reaction time and low yields. In order to address these limitations, microwave irradiation was used instead [19–21]. Surprisingly, unexpected 5,6-diarylpyrazolo[1,5-*a*]pyrimidine



Scheme 2 General synthetic route for compounds **3** and **4**

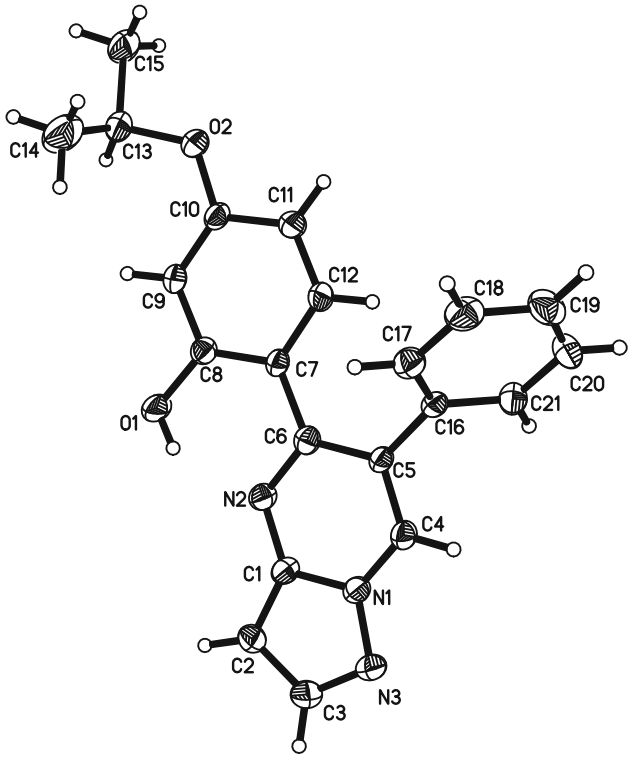


Fig. 1 Crystal structure of **3a**

derivatives **3** were obtained with high chemoselectivity (Scheme 2). We herein report the preparation of derivates **3** and **4** and their antifungal activities.

Results and discussion

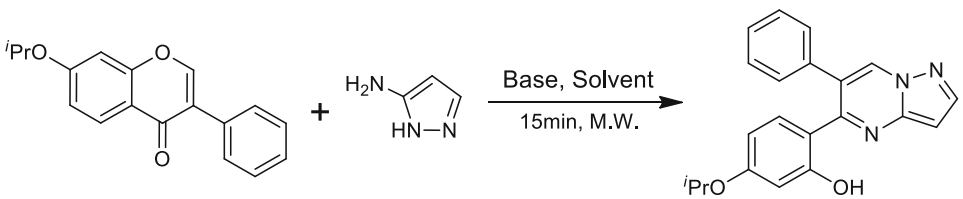
Chemistry

In order to improve the formation of 6,7-diarylpyrazolo[1,5-*a*]pyrimidine **4a**, MeONa (2.5 mmol) was added as a base for the condensation of 7-isopropoxyisoflavone (**1a**; 1 mmol) and 3-aminopyrazole (**2a**; 1.5 mmol) in methanol (20 mL) under microwave irradiation. To our surprise, 5,6-diarylpyrazolo[1,5-*a*]pyrimidine **3a** was obtained instead (confirmed by single-crystal X-ray diffraction analysis) (Fig. 1).

Optimization of the reaction conditions for the preparation of **3a** is shown in Table 1. Different bases were tested and it was found that $(CH_3)_3COK$ was the best choice (entry 4). Further investigation on solvents (entries 4–6) and equivalents of reactants (entries 6–11) revealed that DMSO provided the best results when the equivalent ratio of reactants was 1:1.5:2.5 (**1a**: **2a**: $(CH_3)_3COK$; entry 8).

Under our optimized conditions, a variety of 5,6-diarylpyrazolo[1,5-*a*]pyrimidines **3a**–**3t** were synthesized

Table 1 Optimization of cyclocondensation of isoflavone **1a** with 3-aminopyrazole **2**



1a **2a** **3a**

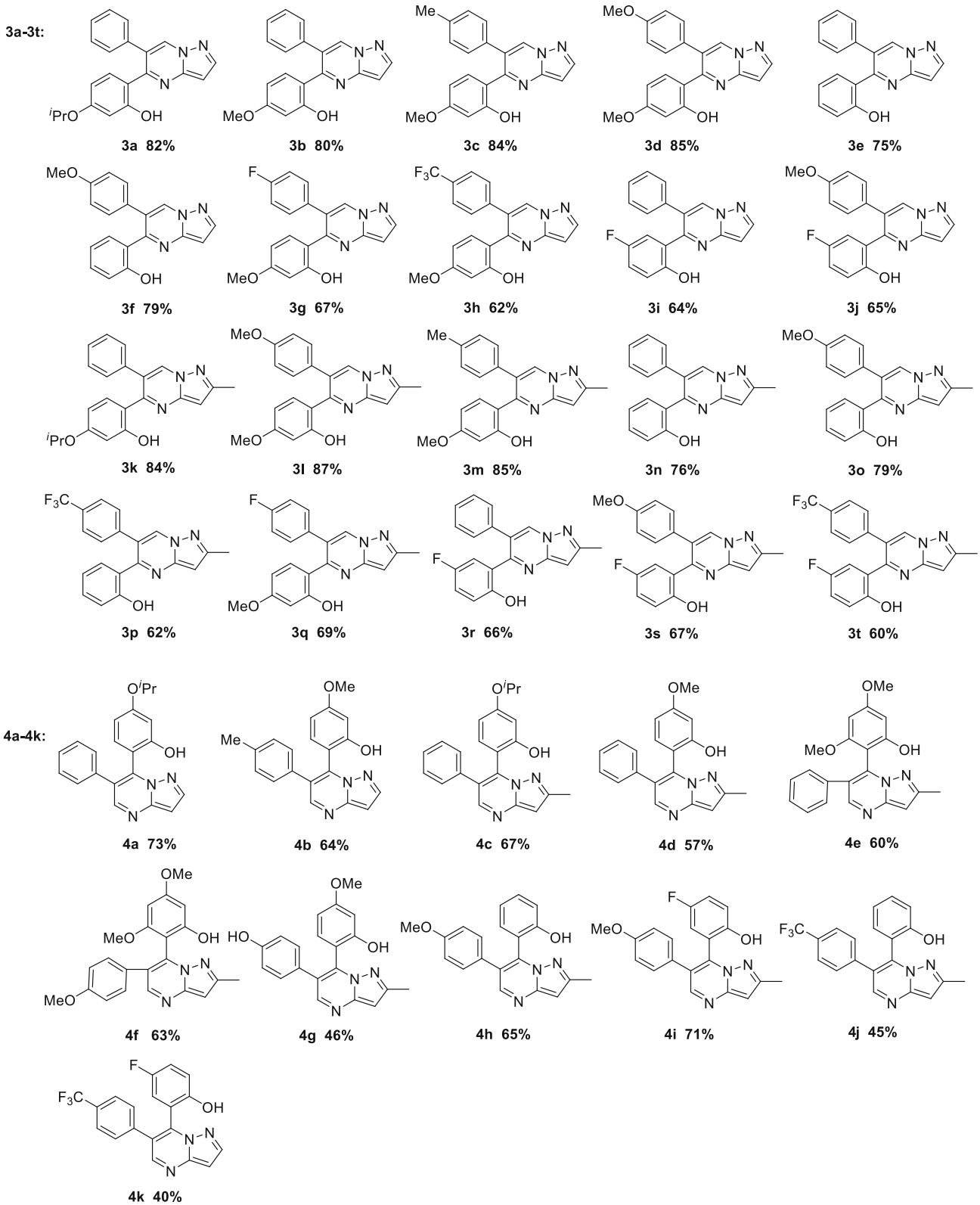
Entry	Solvent	Base	Molar ratios 1a/2/base	*T* (°C)	**3a** yield (%)[a]
1	CH_3OH	CH_3ONa	1:2:2.5	68	27
2	CH_3OH	K_2CO_3	1:2:2.5	68	0
3	CH_3OH	NaOH	1:2:2.5	68	20
4	CH_3OH	$(CH_3)_3COK$	1:2:2.5	68	65
5	CH_3CH_2OH	$(CH_3)_3COK$	1:2:2.5	79	63
6	DMSO	$(CH_3)_3COK$	1:2:2.5	120	81
7	DMSO	$(CH_3)_3COK$	1:1:2.5	120	70
8	DMSO	$(CH_3)_3COK$	1:1.5:2.5	120	83
9	DMSO	$(CH_3)_3COK$	1:1.5:2	120	63
10	DMSO	$(CH_3)_3COK$	1:1.5:3	120	67
11	DMSO	$(CH_3)_3COK$	1:1.5:2.5	100	82[b]
12	DMSO	$(CH_3)_3COK$	1:1.5:2.5	90	72

All reactions were carried out on 1 mmol scale of **1a** in 20 mL of solvent

[a] Isolated yield on the basis of isoflavone **1a**

[b] Microwave irradiation for 10 min; yield of **3a** was 65 %

Table 2 Synthesized **3** and **4** analogs



Reaction times for the preparation of **4a**–**4k** were 16–54 h. Isolated yield after column chromatography

from isoflavones **1** and 3-aminopyrazoles **2** (Table 2). 6,7-Diarylpyrazolo[1,5-*a*]pyrimidine derivatives (**4a**–**4i**) were prepared using our conventional heating method (Table 2) [19]. While these results show the importance of the heating source, the reason for the observed chemoselectivity is not clear. As a general trend, substrates bearing electron-donating groups afforded the corresponding products in higher yields than those with electron-withdrawing groups.

Antifungal activity

Twenty 5,6-diarylpyrazolo[1,5-*a*]pyrimidines (**3a**–**3t**) and eleven 6,7-diarylpyrazolo[1,5-*a*] pyrimidines (**4a**–**4k**) were screened at 50 μg/mL primarily to determine their antifungal performance against *Cytospora* sp., *Colletotrichum gloeosporioides*, *Botrytis cinerea*, *Alternaria solani*, and *Fusarium solani* (Table 3). The mycelial growth inhibition rates of compounds **3f**, **3p**, **4a**, **4h**, **4j**, and **4k** were over 60 % and they were selected to obtain their IC_{50} values (Table 4) [22].

As shown in Table 4, compounds **3f**, **3p**, **4a**, **4h**, **4j**, and **4k** exhibited different antifungal activities against the five tested fungi. These analogs performed better than the positive control hymexazol against *Cytospora* sp., *C. gloeosporioides*, *A. solani*, and *F. Solani*; however, they were almost inactive to *Botrytis cinerea* compared to hymexazol (IC_{50} = 7.23 ± 0.33 μg/mL). Of special interest was that analog **4** exhibited better antifungal activity than **3**. Analogs **4a** and **4k** exhibited IC_{50} values of 19–33 μg/mL against *Cytospora* sp., *C. gloeosporioides*, *A. solani*, and *F. Solani*.

When the 4′-isopropoxyl on the 7-phenyl group of **4a** and the 5′-fluoro on the 7-phenyl group of **4k** were included, their antifungal activities and broad-spectrum antifungal properties were enhanced. Compound **4h**, bearing a 4′-methoxy group on the 6-phenyl group, showed higher growth inhibition against *Cytospora* sp. and *F. Solani* (IC_{50} = 27.32±0.31 μg/mL and 21.40±0.37 μg/mL, respectively). Notably, **4j** with a 4′-trifluoromethyl unit on the 6-phenyl group had a significant effect toward inhibiting *A. solani* (IC_{50} = 17.11±0.41 μg/mL) proving to be much better than the control hymexazol (IC_{50} > 50μg/mL). Similarly, **4k**, having a 4′-trifluoromethyl group, was also effective against *A. solani* (IC_{50} = 20.64 ± 1.05 μg/mL). These results indicate that the trifluoromethyl group of **4** could be a key group necessary to inhibit the growth of *A. solani*.

In the case of 5,6-diarylpyrazolo[1,5-*a*]pyrimidines, compound **3f** showed antifungal activity against *Cytospora* sp. with an IC_{50} of 44.83 ± 0.53 μg/mL, and **3p** against *C. gloeosporioides* and *F. Solani* with an IC_{50} of 57.61 ± 1.74 μg/mL and 35.21 ± 3.76 μg/mL, respectively.

Table 3 Preliminary antifungal activity of compounds **3** and **4** at 50 μg/mL

Entry	Compd.	Average antifungal rate (%)				
		C.s.	C.g.	B.c.	A.s.	F.s.
1	**3a**	0.41	5.30	15.64	0.62	3.49
2	**3b**	0.82	17.42	26.38	4.32	2.82
3	**3c**	40.82	44.70	30.98	23.46	40.35
4	**3d**	6.12	24.24	23.31	6.17	20.91
5	**3e**	48.98	57.58	41.72	45.06	37.67
6	**3f**	59.85	54.59	38.65	34.57	46.38
7	**3g**	0.41	0.61	15.64	0.25	0.27
8	**3h**	6.63	13.64	11.04	10.49	14.21
9	**3i**	47.96	51.52	41.72	54.32	54.42
10	**3j**	36.73	53.03	35.58	32.10	39.68
11	**3k**	4.59	6.82	11.04	0.49	3.49
12	**3l**	26.53	25.76	23.31	19.75	22.25
13	**3m**	0.00	5.30	27.91	0.74	4.16
14	**3n**	34.18	40.91	35.58	27.78	25.60
15	**3o**	1.02	8.33	21.78	0.00	3.49
16	**3p**	50.00	59.09	35.58	49.38	63.81
17	**3q**	26.53	30.30	23.31	17.28	28.95
18	**3r**	1.53	22.73	11.04	2.47	0.54
19	**3s**	4.08	18.18	11.04	7.41	22.25
20	**3t**	51.02	50.76	32.52	41.98	49.73
21	**4a**	54.72	60.61	35.58	70.37	71.85
22	**4b**	51.61	52.46	14.00	45.27	41.55
23	**4c**	32.65	33.33	29.45	38.27	55.76
24	**4d**	51.61	56.56	12.00	56.08	45.07
25	**4e**	52.69	39.34	8.00	51.35	39.44
26	**4f**	26.34	36.89	8.00	33.78	14.08
27	**4g**	29.57	21.31	4.00	18.24	15.49
28	**4h**	72.45	43.94	35.58	46.91	85.25
29	**4i**	22.58	42.62	44.00	19.59	20.42
30	**4j**	56.45	66.39	30.00	69.59	48.59
31	**4k**	70.43	68.03	40.00	72.97	75.35
32	**Hy**	23.74	18.18	100.00	56.37	42.36

A. s., *Alternaria solani*; B. c., *Botrytis cinerea*; C. g., *Colletotrichum gloeosporioides*; C. s. *Cytospora* sp.; F. s., *Fusarium solani*; Hy, hymexazol

Conclusions

In summary, we report the chemoselective synthesis of 5,6-pyrazolo[1,5-*a*]pyrimidine (**3a**–**3t**) derivatives in excellent yields under microwave irradiation. Pyrazolo[1,5-*a*] pyrimidines **4** were also selectively prepared using conventional heating. The antifungal profiles of compounds **3** and **4** against *Cytospora* sp., *C. gloeosporioides, Alternaria solani, and F. Solani* were obtained. Among these analogs, **4h** exhibited higher inhibition toward fungi *Cytospora* sp. and *F. Solani* (IC_{50} = 27.32 ± 0.31 μg/mL and

Table 4 Antifungal activity of selected compounds

Compd	$IC_{50} \pm SD/(\mu g/mL)$				
	C.s.	C.g.	B.c.	A.s.	F.s.
3f	44.83 ± 0.53	>50	>100	>100	>50
3p	>50	57.61 ± 1.74	>50	>50	35.21 ± 3.76
4a	27.08 ± 0.72	21.99 ± 0.22	>50	21.12 ± 0.64	28.59 ± 0.95
4h	27.32 ± 0.31	>50	>50	>50	21.40 ± 0.37
4j	>50	40.71 ± 0.64	>50	17.11 ± 0.41	>50
4k	25.22 ± 0.93	33.56 ± 1.41	>50	20.64 ± 1.05	19.54 ± 0.37
Hy	>100	>100	7.23 ± 0.33	52.38 ± 4.38	38.45 ± 2.50

$21.40 \pm 0.37 \mu g/mL$, respectively), **4j** had strong antifungal activity toward *Alternaria solani* ($IC_{50} = 17.11 \pm 0.41 \mu g/mL$), and they all perform better than fungicide hymexazol. Compounds **4a**, **4h**, **4j**, and **4k** offer a great opportunity as starting points for further optimization toward improved antifungal agrochemicals.

Experimental procedures

Instruments

Microwave irradiation was carried out using the commercial microwave oven MCR-3 (Gongyi Yuhua Instrument Corp. Ltd., China). Melting points were measured using X-5 melting point apparatus and were uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AM 400 or 600 instrument using either $CDCl_3$ or DMSO-d_6 as a deuterated solvent. Chemical shifts are given in δ relative to tetramethylsilane (TMS) as an internal standard, and coupling constants J are given in Hz. Abbreviations used for ^{1}H NMR signals are *s* = singlet, *d* = doublet, *m* = multiplet, *b* = broad, and *dd* = doublet of doublet. High-resolution mass spectrometry (HRMS) was performed on a Bruker Maxis instrument using electrospray ionization quadrupole time-of-flight (ESI-Q-TOF) technique and IR spectra were recorded on a Nicolet 170SX FT-IR spectrophotometer with KBr pellets. The crystal diffraction data were collected on a Bruker Smart-1000 CCD diffractometer. Thin-layer chromatography (TLC) used silica gel 60 GF254 plate. Silica gel (size 200–300 mesh) used for column chromatography was purchased from Qingdao Haiyang Chemistry Plant (China). All starting materials were obtained from commercial sources and used without purification.

Fungal material

Five species of fungi *Cytospora* sp., *Colletotrichum gloeosporioides*, *Botrytis cinerea*, *Alternaria solani*, and *Fusarium solani* were all kindly provided by the Institute of Pesticides, Northwest A&F University. The method used for culturing them was general fungi culture technique, i.e., inoculating and culturing on PDA (potato dextrose agar) at 28 °C for 5–7 days.

General procedure for the synthesis of 5,6-diarylpyridine[1,5-*a*]pyrimidines (3a–3t)

The corresponding isoflavone (**1**; 1 mmol), 3-aminopyrazole (**2a**; 1.5 mmol) or 3-amino-5-methylpyrazole (**2b**; 1.5 mmol), and $(CH_3)_3COK$ (280 mg, 2.5 mmol) were dissolved in 20 mL of anhydrous DMSO (20 mL), and the solution was subsequently stirred and irradiated under microwave irradiation for 15 min. Then, the reaction mixture was poured onto brine (100 mL) and adjusted to pH 6–7 by the addition of 20 % HCl. The resulting yellow precipitate was filtered and purified via column chromatography on silica gel (petroleum ether) to give **3** in moderate to good yields.

*5-(2-Hydroxy-4-isopropoxyphenyl)-6-phenylpyrazolo[1,5-a]pyrimidine (***3a***)*

Yellow solid; mp, 162.3–163.4 °C; IR (KBr) ν (cm^{-1}) 3545, 3513, 3420, 2024, 1620, 1390, 1178, 826, 612; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 9.70 (s, 1H), 9.04 (s, 1H), 8.24 (d, J = 2.4 Hz, 1H), 7.28 (s, 5H), 7.09 (d, J = 8.4 Hz, 1H), 6.72 (d, J = 2.0, 1H), 6.36 (dd, J = 8.4, 2.4 Hz, 1H), 6.23 (d, J = 2.0 Hz, 1H), 4.51 (m, 1H), 1.24(m, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 159.1, 157.2, 156.3, 146.5, 145.0, 136.1, 133.9, 131.3, 129.0, 127.7, 127.1, 123.5, 118.3, 106.1, 102.5, 95.9, 69.1, 21.7. HRMS (ESI): m/z $[M+H]^+$ calculated for $C_{21}H_{20}N_3O_2$: 368.1375; found: 368.1365.

*5-(2-Hydroxy-4-methoxy)phenyl-6-pyrazolo[1,5-a]pyrimidine (***3b***)*

Yellow solid; mp, 192.5–195.7 °C; IR (KBr) ν (cm^{-1}) 3559, 3423, 3239, 2961, 2029, 1618, 1486, 1409, 1238, 1139, 819, 632; ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm) 9.72 (s, 1H), 9.02 (s, 1H), 8.22 (s, 1H), 7.26 (s, 5H), 7.10 (d, J = 8.4 Hz, 1H), 6.71 (s, 1H), 6.37 (d, J= 8.4 Hz, 1H), 6.24 (s, 1H), 3.67 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 161.4, 157.5, 156.6, 147.0, 145.7, 136.7, 134.7, 131.8, 129.0, 128.5,

127.8, 123.9, 119.0, 105.2, 101.5, 96.1, 55.5. HRMS (ESI): m/z $[M+H]^+$ calculated for $C_{19}H_{16}N_3O_2$: 340.1062; found: 340.1048.

*5-(2-Hydroxy-4-methoxy)phenyl-6-(4-methyl)phenylpyrazolo[1,5-a]pyrimidine (**3c**)*

Yellow solid; mp, 178.5–180.2 °C; IR (KBr) ν (cm^{-1}) 3557, 3478, 3417, 3238, 2959, 2026, 1620, 1388, 1240, 1108, 838, 629; ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm) 9.74 (s, 1H), 8.98 (s, 1H), 8.23 (d, J = 2.4 Hz, 1H), 7.16 (m, 2H), 7.11 (m, 3H), 6.71 (d, J = 1.8 Hz, 1H), 6.40 (dd, J = 8.4, 1.8 Hz, 1H), 6.27 (d, J = 1.8 Hz, 1H), 3.71 (m, 3H), 2.28 (m, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 160.9, 157.0, 156.1, 146.3, 145.1, 136.5, 134.0, 133.3, 131.3, 130.0, 128.8, 128.64, 123.4, 118.6, 113.6, 104.7, 101.0, 95.5, 55.0, 20.6. HRMS (ESI): m/z $[M+Na]^+$ calculated for $C_{20}H_{17}N_3O_2Na$: 354.1218; found: 354.1218.

*5-(2-Hydroxy-4-methoxy)phenyl-6-(4-methoxy)phenylpyrazolo[1,5-a]pyrimidine (**3d**)*

Yellow solid; mp, 176.4–180.2 °C; IR (KBr) ν (cm^{-1}) 3566, 3512, 3430, 2836, 2026, 1615, 1384, 1242, 1117, 1029, 959, 816, 634; ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm) 9.76 (s, 1H), 8.97 (s, 1H), 8.22 (d, J = 2.4 Hz, 1H), 7.19 (m, 2H), 7.11 (d, J = 9.0 Hz, 1H), 6.86 (d, J = 9.0 Hz, 1H), 6.70 (d, J = 2.4 Hz, 1H), 6.40 (dd, J = 8.4, 2.4 Hz, 1H), 6.29 (m, 1H), 3.74 (m, 3H), 3.73 (m, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 160.8, 158.5, 157.1, 156.2, 146.3, 145.0, 133.8, 131.3, 130.1, 128.3, 123.1, 118.5, 113.5, 104.7, 101.1, 95.4, 55.0, 55.0. HRMS (ESI): m/z $[M+Na]^+$ calculated for $C_{20}H_{17}N_3O_3Na$: 370.1168; found: 370.1148.

*5-(2-Hydroxyphenyl)-6-phenylpyrazolo[1,5-a]pyrimidine (**3e**)*

Yellow solid; mp, 146.6–149.3 °C; IR (KBr) ν (cm^{-1}) 3577, 3442, 3354, 3032, 2025, 1616, 1489, 1404, 1247, 1156, 1068, 751, 641; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 9.45 (s, 1H), 9.08 (s, 1H), 8.27 (d, J = 2.4 Hz, 1H), 7.26 (s, 5H), 7.23 (d, J = 8.0 Hz, 1H), 7.16 (m, 1H), 6.80 (m, 1H), 6.75 (s, 1H), 6.69 (d, J = 8.0 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 157.2, 154.4, 146.6, 145.2, 135.8, 133.9, 130.3, 130.0, 129.0, 127.9, 127.3, 126.1, 123.5, 118.6, 115.5, 95.8. HRMS (ESI): m/z $[M+Na]^+$ calculated for $C_{18}H_{13}N_3ONa$: 310.0956; found: 310.0943.

*5-(2-Hydroxyphenyl)-6-(4-methoxyphenyl)pyrazolo[1,5-a]pyrimidine (**3f**)*

Yellow solid; mp, 166.2–169.3 °C; IR (KBr) ν (cm^{-1}) 3522, 3452, 3413, 2960, 2031, 1639, 1256, 1026, 808, 657; ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm) 9.45 (s, 1H), 9.02 (s, 1H), 8.24 (d, J = 2.4 Hz, 1H), 7.22(d, J = 7.8 Hz, 1H), 7.18 (m, 3H), 6.82 (m, 3H), 6.73 (m, 2H), 3.72 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 158.6, 157.4, 154.5, 146.5, 145.1, 133.6, 130.3, 130.2, 130.0, 128.0, 126.3, 123.3, 118.6, 115.5, 113.4, 95.7, 55.0. HRMS (ESI): m/z $[M+Na]^+$ calculated for $C_{19}H_{15}N_3O_2Na$: 340.1062; found: 340.1047.

*5-(2-Hydroxy-4-methoxy)phenyl-6-(4-fluorine)phenylpyrazolo[1,5-a]pyrimidine (**3g**)*

Yellow solid; mp, 158.3–162.5 °C; IR (KBr) ν (cm^{-1}) 3554, 3483, 3452, 2960, 2026, 1622, 1359, 1073, 823, 629; ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm) 9.65 (s, 1H), 9.04 (s, 1H), 8.22 (d, J = 2.4 Hz, 1H), 7.27 (m, 2H), 7.12 (m, 3H), 6.70 (d, J = 1.8 Hz, 1H), 6.40 (dd, J= 8.4, 2.4 Hz, 1H), 6.23 (d, J = 1.8 Hz, 1H), 3.68 (m, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 162.01 (^1J = 243 Hz), 161.5, 157.5, 156.4, 147.1, 145.8, 134.6, 133.1, 133.1, 131.9, 131.5 (^3J = 8.4 Hz), 123.0, 119.0, 115.3 (^2J = 21.6 Hz), 105.3, 101.4, 96.1, 55.5. HRMS (ESI): m/z $[M+Na]^+$ calculated for $C_{19}H_{14}FN_3O_2Na$: 358.0968; found: 358.0946.

*5-(2-Hydroxy-4-methoxy)phenyl-6-(4-trifluoromethyl)phenylpyrazolo[1,5-a]pyrimidine (**3h**)*

Yellow solid; mp, 165.2–167.1 °C; IR (KBr) ν (cm^{-1}) 3568, 3502, 3439, 3235, 2945, 2026, 1618, 1480, 1407, 1327, 1282, 1155, 1109, 1065, 843. 641; ^{1}H NMR [600 MHz, DMSO-d_6] δ (ppm) 9.60 (s, 1H), 9.16 (s, 1H), 8.28 (d, J = 2.4 Hz, 1H), 7.67 (m, 2H), 7.49 (m, 2H), 7.26 (d, J = 8.4 Hz, 1H), 6.76 (d, J = 2.4 Hz, 1H), 6.47 (dd, J = 8.4, 2.4 Hz, 1H), 6.23 (d, J = 2.4 Hz, 1H), 3.71 (m, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 161.2, 156.5, 155.7, 146.8, 145.6, 140.7, 134.6, 131.5, 129.6, 127.6 (^2J = 31.35 Hz), 124.7 (^3J = 3.45 Hz), 124.3 (^1J = 270.3 Hz), 122.2, 118.4, 105.0, 100.9, 95.8, 55.0. HRMS (ESI): m/z $[M+Na]^+$ calculated for $C_{20}H_{14}F_3N_3O_2Na$: 408.0936; found: 408.0920.

*5-(2-Hydroxy-5-fluorine)phenyl-6-phenylpyrazolo[1,5-a]pyrimidine (**3i**)*

Yellow solid; mp, 154.6–157.4 °C; IR (KBr) ν (cm^{-1}) 3567, 3501, 3439, 3235, 2960, 2026, 1618, 1493, 1412, 1248, 1172, 1074, 974, 902, 831, 635; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 9.43 (s, 1H), 9.12 (s, 1H), 8.28 (d, J = 2.4 Hz, 1H), 7.29 (s, 5H), 7.11 (d, J = 8.4 Hz, 1H), 7.01 (m, 1H), 6.77 (s, 1H), 6.67 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 155.9, 154.9 (^1J = 233 Hz), 150.8, 146.6, 145.4, 135.5, 134.1, 129.1, 127.9, 127.4, 127.0 (^3J = 7.6 Hz), 123.4, 116.4 (^2J = 23.7 Hz), 116.4 (^2J = 21.9 Hz), 116.5 (^3J = 8.5 Hz), 96.0. HRMS (ESI): m/z $[M+Na]^+$ calculated for $C_{18}H_{12}FN_3ONa$: 328.0862; found: 328.0845.

*5-(2-Hydroxy-5-fluorine)phenyl-6-(4-methoxy)phenylpyrazolo[1,5-a]pyrimidine (**3j**)*

Yellow solid; mp, 150.8–153.7 °C; IR (KBr) ν (cm^{-1}) 3581, 3511, 3446, 3380, 2960, 2071, 1641, 1489, 1252, 1024, 845, 662; ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm) 9.43 (s, 1H), 9.06 (s, 1H), 8.26 (d, J = 2.4 Hz, 1H), 7.21 (m, 2H), 7.11 (dd, J = 8.4, 2.4 Hz, 1H), 7.03 (m, 1H), 6.85 (m, 2H), 6.76 (d, J = 2.4 Hz, 1H), 6.69 (dd, J = 8.4, 2.4 Hz, 1H), 3.73 (m, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 158.6, 156.1, 154.9 (^1J = 233.0 Hz), 150.8, 146.4, 145.2, 133.7, 130.3, 127.7, 127.2 (^3J = 7.5 Hz), 123.2, 116.5 (^3J = 233.0 Hz), 116.4 (^2J = 233.0 Hz), 116.3 (^2J = 22.7 Hz), 113.4, 95.9, 55.0. HRMS (ESI): m/z $[M+Na]^+$ calculated for $C_{19}H_{14}FN_3O_2Na$: 358.0968; found: 358.0952.

*2-Methyl-5-(2-hydroxy-4-isopropoxy)phenyl-6-phenylpyrazolo[1,5-a]pyrimidine (**3k**)*

Yellow solid; mp, 154.5–155.1 °C; IR (KBr) ν (cm^{-1}) 3570, 3512, 3423, 2026, 1618, 1420, 1294, 1198, 1155, 804, 602; ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm) 9.79 (s, 1H), 8.90 (s, 1H), 7.27 (m, 5H), 7.05 (d, J = 8.4 Hz, 1H), 6.50 (s, 1H), 6.34 (dd, J = 8.4, 2.4 Hz, 1H), 6.24 (d, J = 2.4 Hz, 1H), 4.51 (m, 1H), 2.45 (s, 3H), 1.24 (s, 3H), 1.23 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 159.1, 156.5, 156.3, 154.7, 147.1, 136.4, 133.7, 131.3, 128.9, 128.0, 127.1, 122.4, 118.0, 106.2, 102.6, 94.6, 69.1, 21.7, 14.3. HRMS (ESI): m/z $[M+Na]^+$ calculated for $C_{22}H_{21}N_3O_2Na$: 382.1531; found: 382.1512.

*2-Methyl-5-(2-hydroxy-4-methoxy)phenyl-6-(4-methoxy)phenylpyrazolo[1,5-a]pyrimidine (**3l**)*

Yellow solid; mp, 208.7–210.9 °C; IR (KBr) ν (cm^{-1}) 3557, 3483, 3438, 2961, 2027, 1619, 1387, 1281, 1127, 1074, 827, 634; ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm) 9.83 (s, 2H), 8.84 (s, 2H), 7.16 (m, 2H), 7.08 (m, 2H), 6.85 (m, 2H), 6.48 (br, 1H), 6.38 (dd, J = 8.4, 2.2 Hz, 1H), 6.28 (m, 2H), 3.74 (s, 3H), 3.70 (s, 3H), 2.45 (s, 3H);^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 160.8, 158.5, 156.6, 156.2, 154.5, 147.0, 133.4, 131.3, 130.1, 128.5, 122.2, 118.4, 113.5, 104.7, 101.1, 94.5, 55.0, 55.0, 14.2. HRMS (ESI): m/z $[M+Na]^+$ calculated for $C_{21}H_{19}N_3O_3Na$: 384.1324; found: 384.1307.

*2-Methyl-5-(2-hydroxy-4-methoxy)phenyl-6-(4-methyl)phenylpyrazolo[1,5-a]pyrimidine (**3m**)*

Yellow solid; mp, 208.4–211.2 °C; IR (KBr) ν (cm^{-1}) 3564, 3440, 3233, 2960, 2025, 1620, 1389, 1123, 823, 634; ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm) 9.80 (s, 1H), 8.85 (s, 1H), 7.14 (m, 2H), 7.09 (m, 3H), 6.49 (br, 1H), 6.37 (dd, J = 8.4, 2.2 Hz, 1H), 6.27 (d, J = 2.4 Hz, 1H), 3.70 (s, 3H), 2.45 (s, 3H), 2.28 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 160.8, 156.5, 156.2, 154.6, 147.0, 136.3, 133.49, 133.4, 131.3, 128.8, 128.7, 122.4, 118.5, 104.6, 101.0, 94.6, 55.0, 20.6, 14.3; HRMS (ESI): m/z $[M+Na]^+$ calculated for $C_{21}H_{19}N_3O_2Na$: 368.1375; found: 368.1359.

*2-Methyl-5-(2-hydroxy)phenyl-6-phenylpyrazolo[1,5-a]pyrimidine (**3n**)*

Yellow solid; mp, 158.2–159.7 °C; IR (KBr) ν (cm^{-1}) 3453, 3420, 2956, 2027, 1621, 1393, 1075, 849, 631; ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm) 9.45 (s, 1H), 8.95 (s, 1H), 7.25 (s, 5H), 7.20 (d, J = 7.8 Hz, 1H), 7.16 (m, 1H), 6.80 (m, 1H), 6.69 (d, J = 7.8 Hz, 1H), 6.53 (br, 1H), 2.47 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 156.8, 154.7, 154.5, 147.3, 136.0, 133.4, 130.3, 129.9, 129.0, 127.9, 127.1, 126.1, 122.7, 118.6, 115.5, 94.9, 14.3. HRMS (ESI): m/z $[M+Na]^+$ calculated for $C_{19}H_{15}N_3ONa$: 324.1113; found: 324.1098.

*2-Methyl-5-(2-hydroxy)phenyl-6-(4-methoxy)phenylpyrazolo[1,5-a]pyrimidine (**3o**)*

Yellow solid; mp, 208.8–210.0 °C; IR (KBr) ν (cm^{-1}) 3440, 3383, 2961, 2027, 1620, 1395, 1075, 829, 629; ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm) 9.44 (s, 1H), 8.89 (s, 1H), 7.19–7.15 (m, 4H), 6.80 (m, 3H), 6.72 (d, J = 7.8 Hz, 1H), 6.5 (br, 1H), 3.72 (s, 3H), 2.46 (s, 3H);^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 158.5, 156.9, 154.5, 154.5, 147.2, 133.1, 130.3, 130.1, 129.9, 128.2, 126.3, 122.4, 118.6, 115.5, 113.4, 94.8, 55.0, 14.3. HRMS (ESI): m/z $[M+Na]^+$ calculated for $C_{20}H_{17}N_3O_2Na$: 354.1218; found: 354.1201.

*2-Methyl-5-(2-hydroxy)phenyl-6-(4-trifluoromethyl)phenylpyrazolo[1,5-a]pyrimidine (**3p**)*

Yellow solid; mp, 215.3–217.5 °C; IR (KBr) ν (cm^{-1}) 3484, 3240, 2960, 2028, 1624, 1391, 1326, 1121, 840, 627; ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm) 9.43 (s, 1H), 9.07 (s, 1H), 7.62 (m, 2H), 7.46 (m, 2H), 7.31 (d, J = 7.8 Hz, 1H), 7.19 (m, 1H), 6.86 (m, 1H), 6.67 (d, J = 7.8 Hz, 1H), 6.57 (br, 1H), 2.48 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 156.3, 155.2, 154.3, 147.5, 140.5, 133.9, 131.7, 130.5, 129.7, 127.6 (^2J = 33.25 Hz), 125.9 (^1J = 217.65 Hz), 125.8, 124.6 (^3J = 3.75 Hz), 121.4, 118.9, 115.5, 95.2, 14.3. HRMS (ESI): m/z $[M+Na]^+$ calculated for $C_{20}H_{14}F_3N_3ONa$: 392.0987; found: 392.0968.

*2-Methyl-5-(2-hydroxy-4-methoxy)phenyl-6-(4-fluorine)phenylpyrazolo[1,5-a]pyrimidine (**3q**)*

Yellow solid; mp, 160.2–165.4 °C; IR (KBr) ν (cm^{-1}) 3489, 3415, 2960, 2027, 1620, 1388, 1078, 828, 630; ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm) 9.73 (s, 1H), 8.91 (s, 1H), 7.27 (m, 2H), 7.12 (m, 3H), 6.50 (s, 1H), 6.40 (m, 1H), 6.26

(s, 1H), 3.70 (s, 3H), 2.45 (s, 3H);^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 161.5 (^1J = 241.5 Hz), 160.9, 156.5, 156.0, 154.7, 147.2, 133.6, 132.8, 131.4, 130.9 (^3J = 7.8 Hz), 121.6, 118.4, 114.8 (^2J = 21.15 Hz), 104.8, 101.0, 94.7, 55.0, 14.2. HRMS (ESI): m/z [M+Na]$^+$ calculated for $C_{20}H_{16}FN_3O_2Na$: 372.1124; found: 372.1105.

*2-Methyl-5-(2-hydroxy-5-fluorine)phenyl-6-phenylpyrazolo[1,5-a]pyrimidine (***3r***)*

Yellow solid; mp, 185.3–187.0 °C; IR (KBr) ν (cm^{-1}) 3487, 3378, 2960, 2026, 1622, 1491, 1395, 1275, 1073, 834, 629; ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm) 9.42 (s, 1H), 8.99 (s, 1H), 7.27 (s, 5H), 7.08 (dd, J = 8.4, 2.4 Hz, 1H), 7.00 (m, 1H), 6.67 (dd, J = 8.4, 2.4 Hz, 1H), 6.56 (s, 1H), 2.47 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 155.4, 154.9, 154.8 (^1J = 233 Hz), 150.8, 147.2, 135.7, 133.5, 129.0, 127.9, 127.3, 127.1 (^3J = 7.8 Hz), 122.6, 116.4 (^3J = 8.1 Hz), 116.4 (^2J = 23.3 Hz), 116.3 (^2J = 22.7 Hz), 95.1, 14.3. HRMS (ESI): m/z [M+Na]$^+$ calculated for $C_{19}H_{14}FN_3ONa$: 342.1019; found: 342.1001.

*2-Methyl-5-(2-hydroxy-5-fluorine)phenyl-6-(4-methoxy)phenylpyrazolo[1,5-a]pyrimidine (***3s***)*

Yellow solid; mp, 171.2–173.4 °C; IR (KBr) ν (cm^{-1}) 3574, 3442, 3378, 2961, 1620, 1498, 1385, 1241, 1170, 828, 637; ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm) 9.43 (s, 1H), 8.92 (s, 1H), 7.18 (d, J = 8.4 Hz, 2H), 7.07 (dd, J = 8.4, 2.4 Hz, 1H), 7.01 (m, 1H), 6.84 (m, 2H), 6.69 (dd, J = 8.4, 2.4 Hz, 1H), 6.53 (s, 1H), 3.73 (s, 3H), 2.46 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 158.6, 155.6, 154.9 (^1J = 233.0 Hz), 154.7, 150.8, 147.1, 133.2, 131.6 (^2J = 33.9 Hz), 130.2, 128.6, 127.9, 127.2 (^3J = 7.5 Hz), 122.3, 116.5 (^3J = 7.2 Hz), 116.2 (^2J = 22.1 Hz), 113.4, 94.9, 55.0, 14.2. HRMS (ESI): m/z [M+Na]$^+$ calculated for $C_{20}H_{16}FN_3O_2Na$: 372.1124; found: 372.1111.

*2-Methyl-5-(2-hydroxy-5-fluorine)phenyl-6-(4-trifluoromethyl)phenylpyrazolo[1,5-a]pyrimidine (***3t***)*

Yellow solid; mp, 193.8–195.4 °C; IR (KBr) ν (cm^{-1}) 3581, 3442, 3345, 3234, 2936, 2026, 1618, 1498, 1327, 1274, 1126, 1067, 834, 626; ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm) 9.43 (s, 1H), 9.10 (s, 1H), 7.65 (m, 2H), 7.49 (m, 2H), 7.20 (dd, J = 8.4, 2.4 Hz, 1H), 7.04 (m, 1H), 6.65 (dd, J = 8.4, 2.4 Hz, 1H), 6.59 (s, 1H), 2.48 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 155.3, 155.0 (^1J = 233.1 Hz), 155.0, 150.6, 147.4, 140.2, 134.0, 129.7, 127.9, 127.6, 126.6 (^3J = 7.7 Hz), 124.7, 124.2 (^1J = 270.5 Hz), 121.3, 116.7 (^2J = 23.0 Hz), 116.6 (^2J = 24.5 Hz), 116.4 (^3J = 8.3 Hz), 95.4, 14.4. HRMS (ESI): m/z [M+H]$^+$ calculated for $C_{20}H_{14}N_3O$: 388.1073; found: 388.1061.

General procedure for the synthesis of 6,7-diarylpyridine[1,5-*a*]pyrimidines (4a–4k)

4a–**4k** were prepared according to the literature [19]. The corresponding isoflavone (**1**; 1 mmol), 3-aminopyrazole (**2a**; 1.3 mmol) or 3-amino-5-methylpyrazole (**2b**; 1.3 mmol), and $(CH_3)_3COK$ (280 mg, 2 mmol) were stirred and refluxed in dried MeOH (20 mL) at 68 °C for 16–54 h. All reactions were monitored by TLC until **1** was fully consumed. The reaction mixture was then poured onto ice water (100 mL) and adjusted to pH 6–7 by the addition of 20 % HCl. The resulting yellow precipitate was filtered and purified via column chromatography on silica gel (petroleum ether) to give products **4a-4k**. The analytical data for compounds **4a**–**4i** are in agreement with the literature [19].

*2-Methyl-6-(4-trifluoromethyl)-7-(2-hydroxy)phenylpyrazolo[1,5-a]pyrimidine (***4j***)*

Yellow solid; mp, >300 °C; IR (KBr) ν (cm^{-1}) 3068, 2927, 1600, 1515, 1456, 1397, 1323, 1282, 1163, 1121, 1062, 837, 747; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 9.87 (s, 1H), 8.60 (s, 1H), 7.66 (m, 2H), 7.49 (m, 2H), 7.28 (m, 1H), 7.15 (d, J = 7.6 Hz, 1H), 6.89 (d, J = 8.4 Hz, 1H), 6.82 (m, 1H), 6.65 (s, 1H), 2.37 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 155.8, 154.9, 150.2, 149.1, 142.8, 140.1, 132.2, 130.6, 129.1, 128.2 (^2J = 31.90 Hz), 125.5 (^3J = 3.50 Hz), 124.0 (^1J = 270.90 Hz), 120.5, 119.4, 118.0, 116.2, 96.2, 14.9. HRMS (ESI): m/z [M+H]$^+$ calculated for $C_{20}H_{15}N_3O$: 370.1167; found: 370.1163.

*6-(4-trifluoromethyl)-7-(2-hydroxy-5-fluorine)phenylpyrazolo[1,5-a]pyrimidine (***4k***)*

Yellow solid; mp, >300 °C; IR (KBr) ν (cm^{-1}) 3043, 1610, 1517, 1443, 1323, 1271, 1171, 1121, 1059, 1011, 845, 783; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 9.89 (s, 1H), 8.70 (s, 1H), 8.21 (d, J = 2.3 Hz, 1H), 7.72 (m, 2H), 7.55 (m, 2H), 7.18 (m, 2H), 6.89–6.85 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 155.3 (^1J = 233.10 Hz), 152.3, 150.7, 148.4, 145.5, 141.7, 139.6, 130.7, 128.5 (^2J = 31.6 Hz), 125.6 (^3J = 3.6 Hz), 124.6 (^1J = 270.40 Hz), 121.3, 118.5 (^2J = 24.00 Hz), 118.4 (^3J = 8.2 Hz), 117.7 (^2J = 24.3 Hz), 117.3 (^3J = 8.1 Hz), 97.1. HRMS (ESI): m/z [M+H]$^+$ calculated for $C_{19}H_{13}F_4N_3O$: 374.0916; found: 374.0914.

Antifungal bioassay

Mycelium growth rate method [22] was adopted to evaluate the antifungal activities of the new pyrazolo[1,5-*a*]pyrimidine derivatives (**3** and **4**) against five different phytopathogenic fungi in *vitro*, namely *Cytospora* sp., *Colletotrichum gloeosporioides*, *Botrytis cinerea*, *Alternaria*

solani, and *Fusarium solani*. All of the five kinds of fungi were incubated on PDA and cultured for 7 days at 28 °C until their mycelia grew well. An acetone solution of each sample (**3** and **4**) was added into sterilized Petri dishes that contained about 20 mL sterile molten PDA agar medium and mixed to the final certain concentration. Every kind of mycelium with medium was cut to 4 mm diameter, placed in the center of the dishes with aseptic technique, and grew at 28 °C for 4 days. All of the antifungal tests for compounds **3** and **4** were performed in triplicate. Acetone was used as a negative control and the commercial fungicide hymexazol as a positive control.

The diameters of the mycelia were measured after complete growth. The mycelial growth inhibition rate was calculated by the following formula:

$$\text{Mycelia growth inhibition rate } (\%) = (C - T)/(C - 4) \times 100\ \%,$$

where C stands for the average diameter of mycelia of negative control test and T stands for the average diameter of mycelia on other treated PDA, which were expressed in mm [23].

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