Substituted Pyrazolo[3,4-*d*]pyrimidines: Microwave-Assisted, Solvent-Free Synthesis and Biological Evaluation

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A simple and efficient method has been developed for the synthesis of various pyrazolo[3,4-*d*]pyrimidines by using microwave irradiation under solvent-free conditions. The advantages of applying microwave irradiation compared with the classical method were demonstrated. The structures of all the compounds were confirmed by the usual techniques and, in two cases, by X-ray analysis. The compounds did not display appreciable ability to inhibit xanthine oxidase activity. Screening for antifungal activity showed that some derivatives were active against four fungi, with more significant results for *Botrytis*.

Introduction. – The development of simple and eco-friendly synthetic procedures constitutes an important goal in organic synthesis. Microwave-assisted organic synthesis (MAOS) is a fast-growing area of research, due to the generally short reaction times, and high purities and yields of the resulting products when compared to conventional methods [1].

Microwave irradiation has been used to effect organic reactions such as pericyclic [2], cyclization [3], aromatic substitution [4], oxidation, [5] alkylation [6], decarboxylation [7], radical reactions [8], condensation [9], and peptide synthesis [10].

Solvent-free reactions under microwave irradiation are the subject of constant development because of its ease of set-up, mild conditions, and increased yields of products, cost efficiency, and environment friendliness compared to their solution counterparts [11].

As part of our ongoing research program on heterocyclic compounds which may serve as leads for designing novel chemotherapeutic agents, we were particularly interested in pyrazolo-pyrimidines [12]. The pyrazole and pyrimidine derivatives have attracted the organic chemists' interest due to their biological importance. Pyrazolo-pyrimidines and related fused heterocycles are known to exhibit several pharmacological activities such as CNS depressant [13], neuroleptic [14], tuberculostatic [15], antibacterial, and antifungal activities [16]. Pyrazolo[3,4-*d*]pyrimidines were also

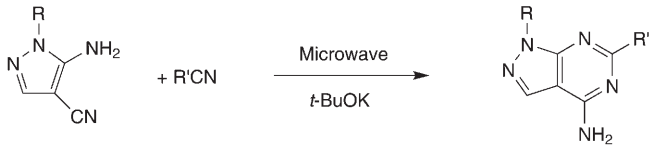
identified as a general class of adenosine receptors [17]. Furthermore, their ability to inhibit the activity of xanthine oxidase was recently described by our group [18].

Thus, in search of an innovative and reliable method for the synthesis of pyrazolo-pyrimidines, we decided to synthesise 4-aminopyrazolo-pyrimidines by the *Taylor* modification of *von Niementowski* reaction [19] using microwave irradiation. The validity of MAOS to build the 4-aminopyrimidine moiety of the 4-aminoquinazoline nucleus was already established by *Seijas et al.* under solvent-free conditions and *t*-BuOK as base [20], which proved to be a valuable tool for the preparation of this type of compounds [21][22].

Results and Discussion. – 1. *Chemistry.* The preparation of the pyrazolo[3,4-*d*]pyrimidines involved the reaction of the respective pyrazoles with aromatic nitriles under microwave irradiation in a *CEM Discover* monomode oven. Three pyrazoles were studied with different substitution in the *N*-aryl group; these were condensed with different nitriles such as benzonitrile, phenylacetonitrile, 3-cyanobenzonitrile, nicotinonitrile (= pyridine-3-carbonitrile), isonicotinonitrile (= pyridine-4-carbonitrile), thiophene-2-carbonitrile, and furane-2-carbonitrile.

A typical experiment involved mixing 1*H*-pyrazole, nitrile, and *t*-BuOK in a 1:1.1:0.2 ratio, followed by irradiation at 300 W in an open vessel for 10 min. The reactions were carried out in absence of solvent, and yields ranged from good to moderate. The improvement regarding the conventional conditions was very clear, since pyrazolo[3,4-*d*]pyrimidines of the type discussed here (*Scheme*) are synthesized by vigorously heating *o*-amino nitriles with nitriles in a sealed tube at elevated temperatures for extended time periods [23].

Scheme. *Synthesis of Pyrazolo[3,4-*d*]pyrimidines*



1a – 1g, 2a – 2g, 3a – 3g

1 R = Ph
2 R = 4-Cl–C_6H_4
3 R = 4-Br–C_6H_4

a R' = Ph
b R' = Ph–CH_2
c R' = 3-cyanophenyl
d R' = pyridin-3-yl
e R' = pyridin-4-yl
f R' = thiophen-2-yl
g R' = furan-2-yl

For example, when 5-amino-1-phenyl-1*H*-pyrazole-4-carbonitrile was subjected to microwaves in the presence of 2-phenylacetonitrile and *t*-BuOK, the reaction occurred smoothly in 10 min to give **1b** in 83% yield. Overall yields ranged from 40–85%.

The formation of the pyrazolo[3,4-*d*]pyrimidine system was unequivocally established after analysis of NMR data of the products (*Fig. 1* shows the numbering of the atoms). The chemical shifts and multiplicity of the H-atom signals were in accordance with the expected values, for example, the H-atom at C(3) of all the compounds was

found between 8.28 and 8.78 ppm as a sharp *singlet*. The signal for NH_2 appears as a broad *singlet* between 7.37 and 8.22 ppm. For compounds **2a**–**2g** and **3a**–**3g**, the *doublet*s for the 4-substituted phenyl ring are well separated, for compound **2b**, for example, they appear at 8.27 and 7.58 ppm. The corresponding signals for the pyrazole precursor of compounds **2** [18] are at 7.79, 6.76, 7.52, and 7.57 ppm, for H–C(3), NH_2, and the phenyl ring H-atoms, respectively. The ^{13}C-NMR data confirm also that the expected products were obtained; for example, C(4) (73.49 for pyrazole) shows a very high chemical shift (159.29–156.60 ppm), as it is expected for a C-atom linked to an NH_2 group. Another evidence is the disappearance of the CN absorption band at 2230 cm^{-1}, corresponding to the starting material, for all compounds except **1c**, **2c**, and **3c**.

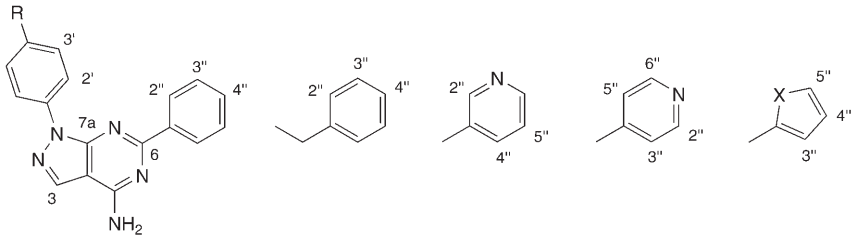


Fig. 1. *Numbering of the atoms of compound structures* **1**–**3**

All new compounds were characterized by spectroscopic methods, and elemental analysis or high-resolution mass spectrometry (see *Exper. Part*). Other techniques such as HMQC and HMBC were also used.

2. *X-Ray Analysis.* The structure of compounds **1a** and **1b** was also confirmed by X-ray crystallography. The crystals obtained from DMSO were mounted on glass fibers, and diffraction data were collected at 100 K with MoK_α radiation ($k = 0.71073$ Å) with *APEX2* v2.1–4 (*Bruker AXS B.V.*, 2007); the structure was solved with program SIR97 [24]; and refinement was carried out with *SHELXL97* [25].

In the crystal structure of compound **1a**, molecules are paired by two H-bonds as shown in *Fig. 2*.

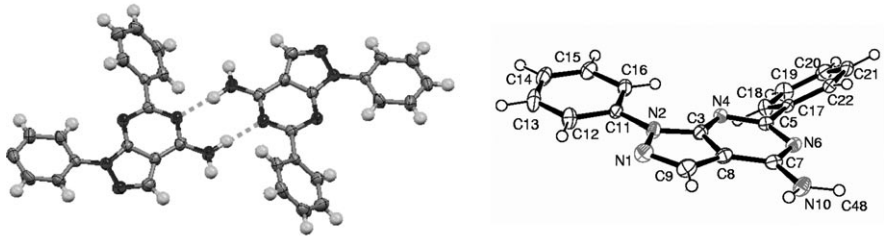


Fig. 2. *X-Ray structure of compound* **1a**

In the X-ray crystal structure of **1b**, there is a third H-bond involving the pyrazole ring from a third molecule (*Fig. 3*).

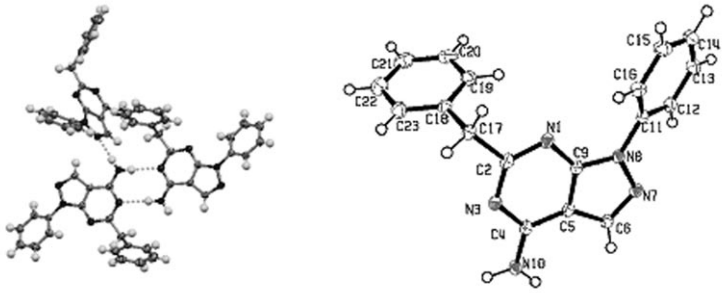


Fig. 3. *X-Ray structure of compound* **1b**

Compound **1a**: $2(C_{17}H_{13}N_5)$, $M_r = 574.65$, monoclinic, $P21/c$, $a = 10.1482(2)$, $b = 12.8286(2)$, $c = 21.5776(4)$ Å, $\beta = 102.1050(10)^\circ$, $V = 2746.67(9)$ Å^3, $Z = 4$, $\lambda = 0.71073$ Å, MoK_α radiation, $\mu = 0.09$ mm^{-1}, $T = 100(2)$ K, yellow prism $0.52 \times 0.35 \times 0.16$ mm[1]).

Compound **1b**: $C_{18}H_{15}N_5$, M_r 301.350, monoclinic, group $P21/n$, $a = 14.3727(2)$, $b = 7.72200(10)$, $c = 14.4919(2)$ Å, $\beta = 108.8630(10)^\circ$, $V = 1522.02(4)$ Å^3, $Z = 4$, $D_x = 1.315$ Mg m^{-3}, $\lambda = 0.71073$ Å, MoK_α radiation; cell parameters from 9265 reflections, $\theta = 2.42 - 28.21^\circ$, $\mu = 0.083$ mm^{-1}, $T = 296(2)$ K, colorless prism $0.26 \times 0.25 \times 0.12$ mm[1]).

3. *Biological Activity.* 3.1. *Effects of Compounds on Xanthine Oxidase.* The inhibition of xanthine oxidase activity by pyrazolo[3,4-*d*]pyrimidines **1**–**3** was examined at the maximum concentration of 120 μM. No appreciable activity was observed, except for compounds **1e**, **2b**, and **3e**. These pyrazolo[3,4-*d*]pyrimidines were further tested at a wide range of concentrations in order to determine their IC_{50} values (*Table 1*). The results obtained for these compounds revealed a weak inhibitory effect.

Table 1. *Inhibitory Effects on Xanthine Oxidase*[a])

Compound	IC_{50}
1e	106.6 ± 6.2
2b	76.6 ± 2.0
3e	78.1 ± 6.6

[a]) Results are expressed as means ± SEM of three independent observations, performed in triplicate. Allopurinol was used as positive control (% inhibition (100 μM) = 96.3 ± 0.3, $IC_{50} = 24.4 \pm 0.5$ μM).

3.2. *Antifungal Activity.* The compounds were screened for their antifungal activity, against *Alternaria* sp. isolated from grape vine (No. UTAD 175), *Botrytis* spp. isolated from apple (No. UTAD 158), *Septoria nodorum* isolated from triticale (No. UTAD 35), and *Phytophthora cinnamomi* isolated from chestnut trees (No. UTAD 107 and IMI No. 340340).

[1]) The crystallographic data can be obtained free of charge from *The Cambridge Crystallographic Data Centre via* www.ccdc.cam.ac.uk/data_request/cif. Deposition No. CCDC-665130 contains the supplementary crystallographic data for **1a**, and deposition No. CCDC-665129 the data for **1b**.

Pyrazolo-pyrimidines **1** exhibited the highest activity against the four fungi but mainly for *Botrytis*; the best results are shown in *Table 2*. *P cinnamomi* is only highly sensitive to compound **1c** (200 ppm) and slightly-to-moderately sensitive for all the other compounds tested. These results could be explained by the phylogenetic sense, since *P. cinnamomi* can no longer be classified as fungi. Therefore, it is now included as oomycetes in Chromista kingdom which differ from true fungi by their unique molecular systems.

Table 2. *Antifungal Activity Data* [% inhibition]

Compound	*Alternaria*		*Botrytis*		*S. nodorum*		*P. cinnamomi*	
	100 ppm	200 ppm	100 ppm	200 ppm	100 ppm	200 ppm	100 ppm	200 ppm
1a	35.0	37.0	52.0	76.0				
1b	57.5	64.9	40.0	66.0	31.0	69.0		
1c	42.5	48.6	60.0	78.0	33.3	52.4	63.6	75.0
1f	57.5	64.9	72.0	78.0	38.1	54.8		
1g					14.3	50.0		
2a	23.0	43.0				14.0		21.0
2b	22.5	43.2	16.0	60.0				
2e							31.8	56.8
2f			34.0	60.0				
2g			50.0	46.0				
3d			34.0	66.0				

Conclusions. – A total of 21 pyrazolo-pyrimidines, 18 of which are new, were prepared by a microwave-assisted solvent-free reaction in moderate-to-good yields. The structures of all the compounds were confirmed by the usual techniques and, in two cases, by X-ray analysis. Considering biological activities, no substantial ability to inhibit xanthine oxidase was observed.

All of the compounds were also screened for their antifungal activity and pyrazolo-pyrimidines **1** exhibited the highest activity against the four fungi, but the results were more significant for *Botrytis*.

Experimental Part

General. Petroleum ether (PE) had a b.p. range of 40–60°. Column chromatography (CC): silica gel *60* (70–230 mesh; *Merck*); elution with mixtures of light petroleum and AcOEt of increasing polarity, unless other conditions are described. M.p.: *Gallenkamp* apparatus; uncorrected. UV Spectra: *Hitachi U-2000* apparatus; λ_{max} (log ε) in nm. IR Spectra: *Perkin Elmer FTIR-1600*; in cm^{-1}. NMR Spectra: *Varian Unity Plus Spectrometer* apparatus; at 300 (^1H) and 75.4 MHz (^{13}C) in DMSO (unless noted otherwise); δ in ppm rel. to solvent peak or Me_4Si, J in Hz; NMR assignments based on 2D-NMR experiments (HMQC, HMBC). EI- and HR-EI-MS: *VG AutoSpecE* mass spectrometer; in m/z. Elemental analyses: *Leco CHNS-932* apparatus.

Microwave experiments were conducted using a *CEM Discover* monomode oven operating at 2450 MHz monitored by a PC computer, and temp. was maintained at a constant value by power modulation (0–300 W). Stirring was provided by an *in situ* magnetic stirrer. Reactions were performed in open glass vessels (capacity 10 ml). Reaction conditions: power 300 W; no solvent; ramp time 3 min; hold time 10 min; stirring on; temp. 145°.

General Procedure. To pyrazole (0.5 mmol) taken in the special open glass vessel, was added nitrile (0.6 mmol) and *t*-BuOK (10 mg, 0.089 mmol). The mixture was thoroughly mixed, and the tube was then subjected to microwave irradiation according to the above protocol. The reaction mixture was purified either by CC with light petroleum/AcOEt or by recrystallisation from EtOH.

*1,6-Diphenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine* (**1a**). Off-white solid (85%). M.p. 224–226° ([23]: 71%, m.p. 224–225°). IR (Nujol): 3381, 2726, 2671, 1648, 1588, 1566, 1168, 971, 774, 722. ^{1}H-NMR: 8.47–8.40 (*m*, H–C(2″), H–C(6″)); 8.36 (*s*, H–C(3)); 8.32 (*d*, *J* = 9.0, H–C(2′), H–C(6′)); 8.08–7.76 (br. *s*, NH_2); 7.59 (*t*, *J* = 8.2, H–C(3′), H–C(5′)); 7.51–7.47 (*m*, H–C(3″), H–C(5″), H–C(4″)); 7.35 (*t*, *J* = 7.2, H–C(4′)). ^{13}C-NMR: 161.95; 158.33; 154.61; 139.26; 138.04; 134.25; 130.51; 129.32 (2 C); 128.42 (2 C); 128.19 (2 C); 126.04; 120.51 (2 C); 100.37. HR-EI-MS: 287.1171 (M^+, $C_{17}H_{13}N_5^+$; calc. 287.1172).

*1-Phenyl-6-(phenylmethyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine* (**1b**). Off-white solid (83%). M.p. 220–222° ([23]: 57%, m.p. 220–222°). IR (Nujol): 3278, 2781, 2674, 1588, 1566, 1175, 971, 774. ^{1}H-NMR: 8.28 (*s*, H–C(3)); 8.20 (*dt*, *J* = 7.5, 1.5, H–C(2′), H–C(6′)); 7.64–7.96 (br. *s*, NH_2); 7.51 (*t*, *J* = 7.5, H–C(3′), H–C(5′)); 7.35–7.22 (*m*, H–C(2″), H–C(3″), H–C(4″), H–C(5″), H–C(6″)); 7.17 (*tt*, *J* = 7.2, 1.5, H–C(4′)); 4.01 (*s*, CH_2). ^{13}C-NMR: 167.72; 158.39; 154.39; 139.22; 138.99; 134.06; 129.22 (2 C); 129.09 (2 C); 128.30 (2 C); 126.23; 126.00; 120.44 (2 C); 99.82; 45.51 (CH_2). HR-EI-MS: 300.1249 ($[M-1]^+$, $C_{18}H_{15}N_5^+$; calc. 300.1253).

*3-(4-Amino-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-yl)benzonitrile* (**1c**). Yellow solid (41%). M.p. 238–239°. IR (Nujol): 3182, 2737, 2242, 1662, 1578, 1561, 1314, 1167, 987, 819, 792, 719. ^{1}H-NMR: 8.66–8.73 (*m*, H–C(4″), H–C(2″)); 8.38 (*s*, H–C(3)); 8.26 (*dd*, *J* = 8.7, 1.2, H–C(2′), H–C(6′)); 8.05–8.22, (br. *s*, NH_2); 7.96 (*dt*, *J* = 7.5, 1.5, H–C(6″)); 7.73 (*t*, *J* = 8.8, H–C(5″)); 7.59 (*t*, *J* = 8.0, H–C(3′), H–C(5′)); 7.36 (*tt*, *J* = 7.5, 1.2, H–C(4′)). ^{13}C-NMR: 159.82; 158.37; 154.24; 139.17; 139.02; 134.30; 133.80; 132.55; 131.48; 129.92; 129.35 (2 C); 126.23; 120.74 (2 C); 118.82 (CN); 111.63; 100.65. HR-EI-MS: 312.1123 (M^+, $C_{18}H_{12}N_6^+$; calc. 312.1122).

*1-Phenyl-6-(pyridin-3-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine* (**1d**). Light yellow solid (64%). M.p. 239–241° ([23]: 71%, m.p. 239–240°). IR (Nujol): 3174, 2726, 2672, 1649, 1586, 1063, 972, 772. ^{1}H-NMR: 9.54 (*d*, *J* = 2.1, H–C(2″)); 8.67–8.64 (*m*, H–C(4″), H–C(6″)); 8.39 (*s*, H–C(3)); 8.30 (*dd*, *J* = 8.7, 1.2, H–C(2′), H–C(6′)); 8.16, 7.96 (2 br. *s*, NH_2); 7.52–7.64 (*m*, H–C(3′), H–C(4′), H–C(5′)); 7.36 (*t*, *J* = 7.5, H–C(5″)). ^{13}C-NMR: 160.21; 158.38; 154.21; 151.06; 149.40; 139.08; 135.42; 134.30; 133.40; 129.33 (2 C); 126.15; 123.63; 120.57 (2 C); 100.53. HR-EI-MS: 288.1123 (M^+, $C_{16}H_{12}N_6^+$; calc. 288.1122).

*1-Phenyl-6-(pyridin-4-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine* (**1e**). Light yellow solid (70%). M.p. 289–290°. IR (Nujol): 3187, 2735, 2678, 1654, 1568, 978, 785. ^{1}H-NMR: 8.74 (br. *d*, *J* = 6.0, H–C(2″), H–C(6″)); 8.41 (*s*, H–C(3)); 8.29 (*m*, H–C(2′), H–C(6′)); 8.27 (*m*, H–C(3′), H–C(5″)); 8.18, 8.01 (2 br. *s*, NH_2); 7.60 (*tt*, *J* = 7.5, 2.0, H–C(3′), H–C(5′)); 7.36 (*tt*, *J* = 7.2, 1.2, H–C(4′)). ^{13}C-NMR: 159.89; 158.42; 154.13; 150.20 (2 C); 145.23; 138.98; 134.25; 129.29 (2 C); 126.17; 121.96 (2 C); 120.59 (2 C); 100.89. HR-EI-MS: 288.1123 (M^+, $C_{16}H_{12}N_6^+$; calc. 288.1122). Anal. calc. for $C_{16}H_{12}N_6$: C 66.65, H 4.2, N 29.15; found: C 66.7, H 4.1, N 29.0.

*1-Phenyl-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine* (**1f**). Off-white solid (47%). M.p. 178–180°. IR (Nujol): 3184, 2726, 1646, 1586, 1225, 1212, 970, 823, 786, 720, 703. ^{1}H-NMR: 8.33 (*s*, H–C(3)); 7.80–8.06 (br. *s*, NH_2); 8.30 (*dd*, *J* = 7.5, 1.5, H–C(2′), H–C(6′)); 7.93 (*dd*, *J* = 3.6, 1.2, H–C(3″)); 7.69 (*dd*, *J* = 5.3, 1.5, H–C(5″)); 7.57 (*t*, *J* = 7.5, H–C(3′), H–C(5′)); 7.34 (*t*, *J* = 7.5, H–C(4′); 7.18 (*dd*, *J* = 5.0, 3.9 Hz, H–C(4″)). ^{13}C-NMR: 158.59; 158.14; 154.03; 143.98; 139.16; 134.31; 130.09; 129.20 (2 C); 128.46; 128.18; 125.93; 120.23 (2 C), 100.11. HR-EI-MS: 293.0735 (M^+, $C_{15}H_{11}N_5S^+$; calc. 293.0736). Anal. calc. for $C_{15}H_{11}N_5S$: C 61.42, H 3.7, N 23.87, S 10.93; found: C 61.42, H 3.69, N 23.78, S 11.13.

*6-(Furan-2-yl)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine* (**1g**). Light brown solid (40%). M.p. 204–206°. IR (Nujol): 3278, 2781, 2674, 1588, 1566, 1175, 965, 889, 823, 780. ^{1}H-NMR: 8.92 (*d*, *J* = 7.8, H–C(2′), H–C(6′)); 8.78 (*s*, H–C(3)); 8.25–8.20 (*m*, H–C(5″)); 8.03 (*t*, *J* = 8.0, H–C(3′), H–C(5′)); 7.87 (br. *s*, NH_2); 8.40–7.76 (*m*, H–C(4′), H–C(3″)); 7.10 (*dd*, *J* = 3.6, 1.5, H–C(4″)). ^{13}C-NMR: 159.41; 156.43; 155.45; 154.09; 145.48; 140.67; 134.22; 129.74 (2 C); 126.60; 121.34 (2 C); 113.81; 112.68; 101.31. HR-EI-MS: 277.0964 (M^+, $C_{15}H_{11}N_5O^+$; calc. 277.0965).

*1-(4-Chlorophenyl)-6-phenyl-1*H-*pyrazolo[3,4-*d*]pyrimidin-4-amine* (**2a**). Light yellow crystals (74%). M.p. 231–233°. IR (Nujol): 3378, 2753, 2693, 1662, 1588, 1536, 1181, 972, 774, 724. ^{1}H-NMR: 8.46–8.41 (*m*, H–C(2″), H–C(6″)); 8.38 (*d*, *J* = 8.7, H–C(2′), H–C(6′)); 8.30 (*s*, H–C(3)); 8.10–7.84 (br. *s*, NH_2); 7.65 (*d*, *J* = 9.0, H–C(3'), H–C(5′)); 7.54–7.47 (*m*, H–C(3″), H–C(4″), H–C(5″)). ^{13}C-NMR: 161.99; 158.23; 154.65; 138.02; 137.84; 134.56; 130.47; 129.88; 128.30 (2 C); 129.20 (2 C); 128.14 (2 C); 121.76 (2 C); 100.33. HR-EI-MS: 321.0781 (M^+, $C_{17}H_{12}{}^{35}ClN_5^+$; calc. 321.0769). Anal. calc. for $C_{17}H_{12}ClN_5$: C 63.46, H 3.76, Cl 11.02, N 21.77; found: C 63.49, H 3.72, Cl 11.04, N 21.86.

*1-(4-Chlorophenyl)-6-(phenylmethyl)-1*H-*pyrazolo[3,4-*d*]pyrimidin-4-amine* (**2b**). Off-white solid (42%). M.p. 226–228°. IR (Nujol): 3278, 2781, 2674, 1588, 1566, 1175, 971, 774. ^{1}H-NMR: 8.29 (*s*, H–C(3)); 8.27 (*d*, *J* = 7.0, H–C(2′), H–C(6′)); 7.85 (br. *s*, NH_2); 7.58 (*d*, *J* = 7.0, H–C(3′), H–C(5′)); 7.28 (*t*, *J* = 7.5, H–C(3″), H–C(5″)); 7.14–7.38 (*m*, H–C(2″), H–C(6″)); 7.18 (*t*, *J* = 7.2, H–C(4″)); 4.01 (*s*, CH_2Ar). ^{13}C-NMR: 167.80; 158.32; 154.44; 138.83; 138.04; 134.41; 129.81; 129.11 (2 C); 129.03 (2 C); 128.22 (2 C); 126.17; 121.61 (2 C); 99.80; 45.42 (CH_2). HR-EI-MS: 335.0944 (M^+, $C_{18}H_{14}{}^{35}ClN_5^+$; calc. 335.0938).

*3-[4-Amino-1-(4-chlorophenyl)-1*H-*pyrazolo[3,4-*d*]pyrimidin-6-yl]benzonitrile* (**2c**). Yellow solid (51%). M.p. 243–245°. IR (Nujol): 3157, 2716, 2274, 1662, 1589, 1559, 1311, 1172, 978, 817, 784, 726. ^{1}H-NMR: 8.66–8.64 (*m*, H–C(2″), H–C(4″)); 8.39 (*s*, H–C(3)); 8.32 (*dd*, *J* = 7.2, 2.1, H–C(2′), H–C(6′)); 8.20, 8.00 (2 br. *s*, NH_2); 7.95 (*dt*, *J* = 7.8, 1.5, H–C(6″)); 7.73 (*t*, *J* = 7.8, H–C(5″)); 7.65 (*dd*, *J* = 6.9, 2.1, H–C(3′), H–C(5′)). ^{13}C-NMR: 159.90; 158.30; 154.30; 139.00; 137.80; 134.63; 133.84; 132.55; 131.43; 130.14; 129.83; 129.27 (2 C); 122.03 (2 C); 118.72 (CN); 111.60; 100.65. HR-EI-MS: 346.0734 (M^+, $C_{18}H_{11}{}^{35}ClN_6^+$; calc. 346.0739).

*1-(4-Chlorophenyl)-6-(pyridin-3-yl)-1*H-*pyrazolo[3,4-*d*]pyrimidin-4-amine* (**2d**). Off-white solid (67%). M.p. 241–243°. IR (Nujol): 3187, 2735, 2678, 1654, 1568, 978, 785. ^{1}H-NMR: 9.5 (br. *s*, H–C(2″)); 8.65–8.73 (*m*, H–C(4″), H–C(6″)); 8.39 (*s*, H–C(3)); 8.36 (*d*, *J* = 9.0, H–C(2′), H–C(6′)); 8.16, 8.00 (2 br. *s*, NH_2); 7.65 (*d*, *J* = 9.0, H–C(3′), H–C(5′)); 7.50–7.58 (*m*, H–C(5″)). ^{13}C-NMR: 160.37; 158.38; 154.35; 151.14; 137.93; 135.50; 135.42; 134.70; 133.28; 130.12; 129.33 (2 C); 123.62; 121.97 (2 C); 100.61. HR-EI-MS: 322.0734 (M^+, $C_{16}H_{11}N_6{}^{35}Cl^+$; calc. 322.0734). Anal. calc. for $C_{16}H_{11}N_6Cl$: C 59.54, H 3.44, N 26.04, Cl 10.98; found: C 59.56, H 3.44, N 26.14, Cl 10.92.

*1-(4-Chlorophenyl)-6-(pyridin-4-yl)-1*H-*pyrazolo[3,4-*d*]pyrimidin-4-amine* (**2e**). Yellow crystals (69%). M.p. 267–269°. IR (Nujol): 3174, 2726, 2672, 1649, 1586, 1063, 972, 772. ^{1}H-NMR: 8.74 (*d*, *J* = 6.3, H–C(2″), H–C(6″)); 8.42 (*s*, H–C(3)); 8.34 (*d*, *J* = 9.0, H–C(2′), H–C(6′)); 8.28 (*d*, *J* = 6.3, H–C(3″), H–C(5″)); 8.20, 8.00 (2 br. *s*, NH_2); 7.66 (*d*, *J* = 6.5, H–C(3′), H–C(5′)). ^{13}C-NMR: 160.06; 158.43; 157.42; 150.23 (2 C); 145.10; 137.82; 134.67; 130.20; 129.32 (2 C); 122.03 (4 C); 100.96. HR-EI-MS: 322.0745 (M^+, $C_{16}H_{11}N_6{}^{35}Cl^+$; calc. 322.0734).

*1-(4-Chlorophenyl)-6-(thiophen-2-yl)-1*H-*pyrazolo[3,4-*d*]pyrimidin-4-amine* (**2f**). Off-white solid (40%). M.p. 190–192°. IR (Nujol): 3176, 2732, 1654, 1586, 1233, 1212, 970, 853, 786, 755, 712. ^{1}H-NMR: 8.34 (*d*, *J* = 9.0, H–C(2′), H–C(6′)); 8.34 (*s*, H–C(3)); 7.94 (*dd*, *J* = 3.9, 1.2, H–C(3″)); 7.70 (*dd*, *J* = 5.1, 1.2, H–C(5″)); 7.62 (*d*, *J* = 9.3, H–C(3′), H–C(5′)); 7.90–8.04 (br. *s*, NH_2); 7.18 (*dd*, *J* = 5.1, 3.9, H–C(4″)). ^{13}C-NMR: 158.87; 158.27; 154.29; 143.88; 138.10; 134.84; 130.38; 130.09; 129.35 (2 C); 128.84; 128.39; 121.82 (2 C); 100.25. HR-EI-MS: 327.0345 (M^+, $C_{15}H_{10}{}^{35}ClN_5S^+$; calc. 327.0353).

*1-(4-Chlorophenyl)-6-(furan-2-yl)-1*H-*pyrazolo[3,4-*d*]pyrimidin-4-amine* (**2g**). Off-white solid (45%). M.p. 206–208°. IR (Nujol): 3269, 2781, 2685, 1587, 1586, 1175, 957, 889, 831, 772. ^{1}H-NMR: 8.53 (*d*, *J* = 9.3, H–C(2′), H–C(6′)); 8.35 (*s*, H–C(3)); 8.0 (br. *s*, NH_2); 7.70–7.90 (*m*, H–C(5″)); 7.62 (*d*, *J* = 9.3, H–C(3′), H–C(5′)); 7.34 (*dd*, *J* = 3.3, 0.9, H–C(3″)); 6.66 (*dd*, *J* = 3.3, 1.8, H–C(4″)). ^{13}C-NMR: 159.41; 156.60; 153.99; 145.60; 141.80; 139.48; 134.65; 131.32; 129.80 (2 C); 122.58 (2 C); 114.01; 112.74; 101.34. HR-EI-MS: 311.0574 (M^+, $C_{15}H_{10}{}^{35}ClN_5O^+$; calc. 311.0570).

*1-(4-Bromophenyl)-6-phenyl-1*H-*pyrazolo[3,4-*d*]pyrimidin-4-amine* (**3a**). Light yellow crystals (84%). M.p. 238–239°. IR (Nujol): 3378, 2753, 2693, 1662, 1588, 1536, 1181, 972, 774, 724. ^{1}H-NMR: 8.47–8.39 (*m*, H–C(2″), H–C(6″)); 8.38 (*s*, H–C(3)); 8.31 (*d*, *J* = 9.0, H–C(2′), H–C(6′)); 8.12–7.80 (br. *s*, NH_2); 7.78 (*d*, *J* = 9.0, H–C(3′), H–C(5′)); 7.54–7.47 (*m*, H–C(3″), H–C(4″), H–C(5″)). ^{13}C-NMR: 162.10; 158.31; 154.75; 138.49; 137.87; 134.70; 132.23 (2 C); 130.61; 128.43 (2 C); 128.24 (2 C); 122.23 (2 C); 118.26; 100.42. HR-EI-MS: 365.0263 (M^+, $C_{17}H_{12}{}^{79}BrN_5^+$; calc. 365.0276).

*1-(4-Bromophenyl)-6-(phenylmethyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine* (**3b**). Colorless needles (48%). M.p. 225–227°. IR (Nujol): 3282, 2787, 2669, 1588, 1578, 1183, 989, 778. ^{1}H-NMR: 8.33 (*s*, H–C(3)), 8.22 (*d*, *J* = 8.7, H–C(2′), H–C(6′)); 7.95–7.74 (br. *s*, NH_2); 7.71 (*d*, *J* = 8.7, H–C(3′), H–C(5′)); 7.36–7.23 (*m*, H–C(2″), H–C(3″), H–C(5″), H–C(6″)); 7.18 (*tt*, *J* = 6.9, 1.2, H–C(4″)); 4.02 (*s*, $ArCH_2$). ^{13}C-NMR: 167.79; 158.32; 154.46; 138.81; 138.46; 134.53; 132.00 (2 C); 129.02 (2 C); 128.20 (2 C); 126.15; 121.90 (2 C); 118.03; 99.82; 45.41. HR-EI-MS: 379.0447 (*M*$^+$, $C_{18}H_{11}{}^{79}BrN_5^+$; calc. 379.0433). Anal. calc. for $C_{18}H_{11}BrN_5$: C 55.75, H 3.3, N 19.18, Br, 10.98; found: C 55.76, H 3.32, N 19.21, Br 11.01.

*3-(4-Amino-1-(4-bromophenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-yl)benzonitrile* (**3c**). Light yellow solid (54%). M.p. 245–246°. IR (Nujol): 3185, 2726, 2233, 1659, 1589, 1561, 1305, 1169, 982, 817, 784, 722. ^{1}H-NMR: 8.72–8.66 (*m*, H–C(2″), H–C(4″)); 8.38 (*s*, H–C(3)); 8.26 (*d*, *J* = 8.7, H–C(2′), H–C(6′)); 8.22–8.13 (br. *s*, NH_2); 7.95 (*dt*, *J* = 7.5, 1.2, H–C(6″)); 7.76 (*d*, *J* = 8.7, H–C(3′), H–C(5′)); 7.71 (*t*, *J* = 8.1, H–C(5″)). ^{13}C-NMR: 159.93; 158.33; 154.34; 139.00; 138.25; 134.69; 133.88; 132.60; 132.21 (2 C); 131.47; 129.87; 122.37 (2 C); 118.77; 118.44 (CN); 111.63; 100.71. HR-EI-MS: 390.0229 (*M*$^+$, $C_{18}H_{11}{}^{79}BrN_6^+$; calc. 390.0231).

*1-(4-Bromophenyl)-6-(pyridin-3-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine* (**3d**). Yellow crystals (80%). M.p. 243–245°. IR (Nujol): 3168, 2757, 2675, 1653, 1586, 1076, 972, 775. ^{1}H-NMR: 9.56 (*d*, *J* = 2.1, H–C(2″)); 8.68 (*d*, *J* = 7.0, H–C(4″), H–C(6″)); 8.41 (*s*, H–C(3)); 8.31 (*d*, *J* = 9.0, H–C(2′), H–C(6′)); 8.02, 8.17 (2 br. *s*, NH_2); 7.79 (*d*, *J* = 9.0, H–C(3′), H–C(5′)); 7.58–7.52 (*m*, H–C(5″)). ^{13}C-NMR: 160.34; 158.33; 154.33; 151.12; 149.41; 138.30; 135.43; 134.70; 133.22; 132.21 (2 C); 123.56; 122.23 (2 C); 118.32; 100.60. HR-EI-MS: 366.0233 (*M*$^+$, $C_{16}H_{11}{}^{79}BrN_6^+$; calc. 366.0229).

*1-(4-Bromophenyl)-6-(pyridin-4-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine* (**3e**). Off-white powder (79%). M.p. 269–271°. IR (Nujol): 3168, 2757, 2675, 1653, 1586, 1069, 972, 777. ^{1}H-NMR: 8.74 (*d*, *J* = 4.7, H–C(2″), H–C(6″)); 8.41 (*s*, H–C(3); 8.29 (*d*, *J* = 8.7, H–C(2′), H–C(6′)); 8.26 (*d*, *J* = 4.8, H–C(3″), H–C(5″)); 8.22, 8.04 (2 br. *s*, NH_2); 7.77 (*d*, *J* = 8.7, H–C(3′), H–C(5′)). ^{13}C-NMR: 160.02; 158.40; 154.26; 150.19 (2 C); 145.08; 138.22; 134.67; 132.18 (2 C); 122.27 (2 C); 121.99 (2 C); 118.42; 118.42. HR-EI-MS: 366.0230 (*M*$^+$, $C_{16}H_{11}{}^{79}BrN_6^+$; calc. 366.022).

*1-(4-Bromophenyl)-6-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine* (**3f**). Yellow solid (57%). M.p. 192–194°. IR (Nujol): 3182, 2731, 1639, 1587, 1225, 1202, 970, 834, 783, 717, 709. ^{1}H-NMR ((D_6)acetone): 8.47 (*d*, *J* = 9.0, H–C(2′), H–C(6′)); 8.34 (*s*, H–C(3)); 8.06 (*dd*, *J* = 3.9, 1.2, H–C(3″)); 7.65 (*dd*, *J* = 5.1, 1.2, H–C(5″)); 7.78 (*d*, *J* = 9.0, H–C(3′), H–C(5′)); 7.37 (br. *s*, NH_2); 7.20 (*dd*, *J* = 5.1, 3.9, H–C(4″)). ^{13}C-NMR ((D_6)acetone): 160.10; 159.29; 155.64; 145.16; 139.89; 134.80; 132.81 (2 C); 130.45; 129.55; 128.69; 122.85 (2 C); 119.12; 101.34. HR-EI-MS: 370.9840 (*M*$^+$, $C_{15}H_{10}{}^{79}BrN_5S^+$; calc. 370.9845).

*1-(4-Bromophenyl)-6-(furan-2-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine* (**3g**). Dark brown solid (45%). M.p. 206–208°. IR (Nujol): 3282, 2787, 2674, 1582, 1568, 1169, 959, 891, 823, 776. ^{1}H-NMR: 8.35 (*s*, H–C(3)); 8.28 (*d*, *J* = 9.3, H–C(2′), H–C(6′)); 8.10–7.91 (br. *s*, NH_2); 7.90–7.87 (*m*, H–C(5″)); 7.75 (*d*, *J* = 9.0, H–C(3′), H–C(5′)); 7.26 (*dd*, *J* = 3.3, 0.9, H–C(3″)); 6.67 (*dd*, *J* = 3.3, 1.5, H–C(4″)). ^{13}C-NMR: 158.23; 155.25; 154.16; 152.40; 145.20; 138.39; 134.65; 132.07 (2 C); 122.00 (2 C); 118.15; 113.21; 112.18; 100.17. HR-EI-MS: 355.0062 (*M*$^+$, $C_{15}H_{10}{}^{79}BrN_5O^+$; calc. 355.0069).

Biological Assays. Xanthine Oxidase Assay. The reaction mixture containing 50 mM KH_2PO_4 buffer, pH 7.4, xanthine oxidase (0.066 U/ml), and a soln. of test compounds in DMSO was incubated at r.t., for 15 min. The reaction was started by addition of xanthine (100 μM) in the presence of EDTA (1 mM), and uric acid formation was followed by measuring the absorbance at 295 nm during 2 min. Each study corresponds to three experiments, performed in triplicate. Final concentrations of DMSO (1%) did not interfere with enzyme activity. Allopurinol was used as positive control.

Antifungal assays. The compounds, resuspended in DMSO, were screened for their antifungal activity, against *Alternaria* sp. isolated from grape vine (No. UTAD 175), *Botrytis* spp. isolated from apple (No. UTAD 158), *Septoria nodorum* isolated from triticale (No. UTAD 35), and *Phytophthora cinnamomi* isolated from chestnut trees (No. UTAD 107 and IMI No. 340340), based on the method of *Jasso de Rodríguez et al.* [26]. All isolates were transferred from UTAD stock cultures to establish fresh PDA agar cultures in *Petri* dishes. The test plates were incubated at 25° after placing a 8 mm agar disc

containing the mycelium of fungi. When the mycelium of fungi reached the edges of the control plate (with DMSO only), the antifungal index was calculated as follows:

$$\text{Antifungal index } [\%] = (1 - D_a/D_b) \times 100,$$

where D_a is the diameter of the growth zone in the test plates, and D_b is the diameter of growth zone in the control plate. Each experiment was performed three times, and the data were averaged.

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