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

Ana M.F. Oliveira-Campos^{*a)}, Aravind Sivasubramanian^{a)}, Lúcia M. Rodrigues^{a)}, Julio A. Seijas^{b)}, M. Pilar Vázquez-Tato^{b)}, Francisco Peixoto^{c)}, Carlos G. Abreu^{d)}, Honorina Cidade^{e)}, Ana Elizabete Oliveira^{e)}, and Madalena Pinto^{e)}

^{a)} Centro de Química, Universidade do Minho, 4710-057, Braga, Portugal
(fax: +351253604382; e-mail:amcampos@quimica.uminho.pt)

^{b)} Departamento de Química Orgânica, Facultad de Ciencias, Universidad de Santiago de Compostela, 27080-Lugo, Spain

^{c)} CECAV, UTAD, 5001-801 Vila Real, Portugal

^{d)} Dept. Plant Protect. UTAD, 5001-801, Vila Real, Portugal

^{e)} CEQOFFUP, Laboratório de Química Orgânica, Faculdade de Farmácia, Universidade do Porto, 4050-047 Porto, Portugal

Abstract

A simple and efficient method has been developed for the synthesis of various pyrazolo[3,4-*d*]pyrimidines 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, 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 pyrazolopyrimidines [12]. The pyrazole and pyrimidine derivatives have attracted the organic chemists interest due to their biological importance. Pyrazolopyrimidines and related fused heterocycles are known to exhibit several pharmacological activities such as CNS depressant [13], neuroleptic [14], tuberculostatic [15], antibacterial and antifungal [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 pyrazolopyrimidines, we decided to synthesise 4-aminopyrazolopyrimidines 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 potassium tert-butoxide 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, benzyl cyanide (phenylacetone nitrile), 3-cyanophenyl nitrile, nicotinonitrile, isonicotinonitrile, 2-thiophenecarbonitrile and 2-furanecarbonitrile.

A typical experiment involved mixing the pyrazole the nitrile and Kt-BuO in a 1:1.1:0.2 ratio followed by irradiation at 300Watt in an open vessel for 10 minutes, 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 1*) are synthesized by vigorously heating *o*-aminonitriles with nitriles in a sealed tube at elevated temperatures for extended time periods [23].

INSERT SCHEME 1

For example, when 1-phenyl-5-amino-4-cyanopyrazole was subjected to microwaves in the presence of 2-phenylacetonitrile and *Kt*-BuO, the reaction occurred smoothly in 10 minutes giving **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 protons were in consonance with the expected values, for example, the proton at position 3 of all the compounds was found between 8.28 and 8.78 ppm as a sharp singlet. The signal for NH₂ appears as a broad singlet between 7.37 and 8.22 ppm. For compounds **2a-2g** and **3a-3g** the doublets for the *para* 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 7.79, 6.76, 7.52 and 7.57, for H₃, NH₂ and the phenyl ring protons, respectively. The ¹³C 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 linked to an amino group. Another evidence is the disappearance of the cyano absorption band at 2230 cm⁻¹, corresponding to the starting material, for all compounds except **1c**, **2c** and **3c**.

All the 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.

INSERT FIG. 1

2. *X-ray analysis.* The structure of compounds **1a** and **1b** was also confirmed by X-ray crystallography, from their crystal data. The crystals, obtained from dimethyl sulfoxide, were mounted on glass fibers and diffraction data were collected at 100 K with Mo-K α radiation ($k = 0.71073 \text{ \AA}^\circ$) with *APEX2* v2.1-4 (Bruker AXS B.V., 2007); 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 hydrogen bonds as shown in *fig. 2*.

INSERT FIG. 2

In the X-ray from **1b** there is a third hydrogen bond involving the pyrazole ring from a third molecule (*fig. 3*).

INSERT FIG. 3

Compound **1a**; C₁₈H₁₅N₅, $Mr = 301.350$, Monoclinic, group *P21/n*, $a = 14.3727 (2) \text{ \AA}$, $b = 7.72200 (10) \text{ \AA}$, $c = 14.4919 (2) \text{ \AA}$, $\beta = 108.8630 (10)^\circ$, $V = 1522.02 (4) \text{ \AA}^3$, $Z = 4$, $D_x = 1.315 \text{ Mg m}^{-3}$, $\lambda = 0.71073 \text{ \AA}$, Mo-K α radiation, $\lambda = 0.7107 \text{ \AA}$; cell parameters from 9265 reflections, $\theta = 2.42\text{--}28.21^\circ$, $\mu = 0.083 \text{ mm}^{-1}$, $T = 296 (2) \text{ K}$, Prism colourless $0.26 \times 0.25 \times 0.12 \text{ mm}$.¹

Compound **1b**, 2(C₁₇H₁₃N₅), $Mr = 574.65$, Monoclinic, *P21/c*, $a = 10.1482 (2) \text{ \AA}$, $b = 12.8286 (2) \text{ \AA}$, $c = 21.5776 (4) \text{ \AA}$, $\beta = 102.1050 (10)^\circ$, $V = 2746.67 (9) \text{ \AA}^3$,

¹ CCDC 665130 contains the supplementary crystallographic data for **1a**. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

$Z = 4$, $\lambda = 0.71073 \text{ \AA}$, Mo-K α radiation, $\lambda = 0.7107 \text{ \AA}$, $\mu = 0.09 \text{ mm}^{-1}$, $T = 100 \text{ (2)}$
K, Prism yellow $0.52 \times 0.35 \times 0.16 \text{ mm}$.²

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 its IC₅₀ values (*Table 1*). The results obtained for these compounds revealed a weak inhibitory effect.

INSERT TABLE 1

3.2. *Antifungal Activity*. The compounds were screened for their antifungal activity, against *Alternaria* sp. isolated from grape vine (n° UTAD 175), *Botrytis* spp. isolated from apple (n° UTAD 158), *Septoria nodorum* isolated from triticales (n° UTAD 35) and *Phytophthora cinnamomi* isolated from chestnut trees (n° UTAD 107 and IMI n°340340).

Pyrazolopyrimidines **1** exhibited the highest activity against the four fungi but mainly for *Botrytis*; the best results are shown in the *Table 2*. *P. cinamomi* 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.

² CCDC 665129 contains the supplementary crystallographic data for **1b**. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Therefore, it is now included as oomycetes in Chromista kingdom which differ from true fungi by their unique molecular systems.

INSERT TABLE 2

Conclusion

A total of twenty one pyrazolopyrimidines, eighteen of which new, were prepared by a microwave 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 the compounds were also screened for their antifungal activity and pyrazolopyrimidines **1** exhibited the highest activity against the four fungi but the results were more significant *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*). Melting points (m.p.): *Gallenkamp* apparatus; uncorrected. UV Spectra: *Hitachi U-2000* apparatus; $\lambda_{\max}$ (log ϵ) in nm. IR Spectra: *Perkin Elmer FTIR-1600*; in cm^{-1} . NMR Spectra: *Varian Unity Plus Spectrometer* apparatus; at 300 MHz (^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 are based on 2-D NMR experiments (HMQC, HMBC). EI and HR-EI-MS: *VG AutoSpecE* mass spectrometer. Elemental analyses: *Leco CHNS-932* apparatus.

Column chromatography was performed with mixtures of light petroleum and ethyl acetate of increasing polarity, unless other conditions are described.

Microwave experiments were conducted using a CEM Discover monomode oven operating at 2450 MHz monitored by a PC computer and temperature 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- 300W; Solvent-No; Ramp time- 3 min; Hold time- 10 min; Stirring- On; Temperature- 145° C.

Usual Procedure:

To the pyrazole (0.5 mmol) taken in the special open glass vessel, was added the nitrile (0.6 mmol) and potassium *tert*-butoxide (10 mg, 0.089 mmol). The mixture was thoroughly mixed and the tube was then subjected to microwave irradiation following the above protocol. The reaction mixture was purified either by column chromatography in light petroleum/ethyl acetate or by recrystallisation from ethanol.

1,6-Diphenyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (1a). The title compound was obtained as an off-white solid (85%). M.p. 224-226° (lit. 71%, m.p. 224-225° [23]). IR (Nujol): 3381, 2726, 2671, 1648, 1588, 1566, 1168, 971, 774, 722. ¹H-NMR: 8.47-8.40 (*m*, H-C(2'') and H-C(6'')); 8.36 (*s*, H-C(3)); 8.32 (*d*, *J* = 9.0, H-C(2') and H-C(6')); 8.08-7.76 (*br. s*, NH₂); 7.59 (*t*, *J* = 8.2, H-C(3') and H-C(5')); 7.51-7.47 (*m*, H-C(3''), H-C(5'') and H-C(4'')); 7.35 (*t*, *J* = 7.2, H-C(4')). ¹³C-NMR: 161.95; 158.33; 154.61; 139.26; 138.04; 134.25; 130.51; 129.32 (2C);

128.42 (2C); 128.19 (2C); 126.04; 120.51 (2C); 100.37. HR-EI-MS: 287.1171 (M^+ , $C_{17}H_{13}N_5$; calc. 287.1172).

6-Benzyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**1b**). The title compound was obtained as an off-white solid (83%). M.p. 220-222° (lit. 57%, m.p. 220-222° [23]). IR (Nujol): 3278, 2781, 2674, 1588, 1566, 1175, 971, 774. 1H -NMR: 8.28 (s, H-C(3)); 8.20 (dt, $J = 7.5$ and 1.5, H-C(2') and H-C(6')); 7.64-7.96 (br. s, NH_2); 7.51 (t, $J = 7.5$, H-C(3') and H-C(5')); 7.35-7.22 (m, H-C(2''), H-C(3''), H-C(4''), H-C(5'') and H-C(6'')); 7.17 (tt, $J = 7.2$ and 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 (2C); 129.09 (2C); 128.30 (2C); 126.23; 126.00; 120.44 (2C); 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-1H-pyrazolo[3,4-d]pyrimidin-6-yl)benzonitrile (**1c**). Compound **1c** was obtained as a yellow solid (41%). M.p. 238-239°. IR (Nujol): 3182, 2737, 2242, 1662, 1578, 1561, 1314, 1167, 987, 819, 792, 719. 1H -NMR: 8.66-8.73 (m, H-C(4'') and H-C(2'')); 8.38 (s, H-C(3)); 8.26 (dd, $J = 8.7$ and 1.2, H-C(2') and H-C(6')); 8.05-8.22, (br. s, NH_2); 7.96 (dt, $J = 7.5$ and 1.5, H-C(6'')); 7.73 (t, $J = 8.8$, H-C(5'')); 7.59 (t, $J = 8.0$, H-C(3') and H-C(5')); 7.36 (tt, $J = 7.5$ and 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 (2C); 126.23; 120.74 (2C); 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)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**1d**). The title compound was obtained as a light yellow solid (64%). M.p. 239-241° (lit. 71%, m.p. 239-240° [23]). IR (Nujol): 3174, 2726, 2672, 1649, 1586, 1063, 972, 772. 1H -NMR: 9.54 (d, $J = 2.1$, H-C(2'')); 8.67-8.64 (m, H-C(4'') and H-C(6'')); 8.39

(*s*, H-C(3)); 8.30 (*dd*, $J = 8.7$ and 1.2 , H-C(2') and H-C(6')); 8.16 and 7.96 (2 br. *s*, NH₂); 7.52-7.64 (*m*, H-C(3'), H-C(4'), H-C(5')); 7.36 (*t*, $J = 7.5$, H-C(5'')). ¹³C-NMR: 160.21; 158.38; 154.21; 151.06; 149.40; 139.08; 135.42; 134.30; 133.40; 129.33 (2C); 126.15; 123.63; 120.57 (2C); 100.53. HR-EI-MS: 288.1123 (M^+ , C₁₆H₁₂N₆; calc. 288.1122).

1-Phenyl-6-(pyridin-4-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (1e). A light yellow solid was obtained (70%). M.p. 289-290°. IR (Nujol): 3187, 2735, 2678, 1654, 1568, 978, 785. ¹H-NMR: 8.74 (br. *d*, $J = 6.0$, H-C(2'') and H-C(6'')); 8.41 (*s*, H-C(3)); 8.29 (*m*, H-C(2') and H-C(6')); 8.27 (*m*, H-C(3') and H-C(5'')); 8.18 and 8.01 (2 br. *s*, NH₂); 7.60 (*tt*, $J = 7.5$ and 2.0 , H-C(3') and H-C(5'')); 7.36 (*tt*, $J = 7.2$ and 1.2 , H-C(4')). ¹³C-NMR: 159.89; 158.42; 154.13; 150.20 (2 C); 145.23; 138.98; 134.25; 129.29 (2C); 126.17; 121.96 (2C); 120.59 (2C); 100.89. HR-EI-MS: 288.1123 (M^+ , C₁₆H₁₂N₆; calc. 288.1122). Anal. calc. for C₁₆H₁₂N₆: C 66.65, H 4.2, N 29.15; found: C 66.7, H 4.,; N 29.0.

6-(Thiophen-2-yl)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (1f). The title compound was obtained as a off-white solid (47%). M.p. 178-180°. IR (Nujol): 3184, 2726, 1646, 1586, 1225, 1212, 970, 823, 786, 720, 703. ¹H-NMR: 8.33 (*s*, H-C(3)); 7.80-8.06 (br. *s*, NH₂); 8.30 (*dd*, $J = 7.5$ and 1.5 , H-C(2') and H-C(6')); 7.93 (*dd*, $J = 3.6$ and 1.2 , H-C(3'')); 7.69 (*dd*, $J = 5.3$ and 1.5 , H-C(5'')); 7.57 (*t*, $J = 7.5$, H-C(3') and H-C(5'')); 7.34 (*t*, $J = 7.5$, H-C(4')); 7.18 (*dd*, $J = 5.0$ and 3.9 Hz, H-C(4'')). ¹³C-NMR: 158.59; 158.14; 154.03; 143.98; 139.16; 134.31; 130.09; 129.20 (2C); 128.46; 128.18; 125.93; 120.23 (2C), 100.11. HR-EI-MS: 293.0735 (M^+ , C₁₅H₁₁N₅S; calc. 293.0736). Anal. calc. for C₁₅H₁₁N₅S: 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-1H-pyrazolo[3,4-d]pyrimidin-4-amine (1g). A light brown solid was obtained (40%). M.p. 204-206°. IR (Nujol): 3278, 2781, 2674, 1588, 1566, 1175, 965, 889, 823, 780. ¹H-NMR: 8.92 (*d*, *J* = 7.8, H-C(2') and 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') and H-C(5')); 7.87 (*br. s*, NH₂); 8.40-7.76 (*m*, H-C(4') and H-C(3'')); 7.10 (*dd*, *J* = 3.6 and 1.5, H-C(4'')). ¹³C-NMR: 159.41; 156.43; 155.45; 154.09, 145.48; 140.67; 134.22; 129.74 (2C); 126.60; 121.34 (2C); 113.81; 112.68; 101.31. HR-EI-MS: 277.0964 (*M*⁺, C₁₅H₁₁N₅O; calc. 277.0965).

1-(4-Chlorophenyl)-6-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (2a). Light yellow crystals were obtained (74%). M.p. 231-233°. IR (Nujoll): 3378, 2753, 2693, 1662, 1588, 1536, 1181, 972, 774, 724. ¹H-NMR: 8.46-8.41 (*m*, H-C(2'') and H-C(6'')); 8.38 (*d*, *J* = 8.7, H-C(2') and H-C(6')); 8.30 (*s*, H-C(3)); 8.10-7.84 (*br. s*, NH₂); 7.65 (*d*, *J* = 9.0, H-C(3') and H-C(5'')); 7.54-7.47 (*m*, H-C(3''), H-C(4'') and H-C(5'')). ¹³C-NMR: 161.99; 158.23; 154.65; 138.02; 137.84; 134.56; 130.47; 129.88; 128.30 (2C); 129.20 (2C); 128.14 (2C); 121.76 (2C); 100.33. HR-EI-MS: 321.0781 (*M*⁺, C₁₇H₁₂³⁵ClN₅; calc. 321.0769). Anal. calc. for C₁₇H₁₂Cl N₅: C 63.46; H 3.76; N 21.77; Cl 11.02; found: C 63.49; H 3.72; N 21.86; Cl 11.04.

6-Benzyl-1-(4-chlorophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (2b). An off-white solid was obtained (42%). M.p. 226-228°. IR (Nujol): 3278, 2781, 2674, 1588, 1566, 1175, 971, 774. ¹H-NMR: 8.29 (*s*, H-C(3)); 8.27 (*d*, *J* = 7.0, H-C(2') and H-C(6')); 7.85 (*br. s*, NH₂); 7.58 (*d*, *J* = 7.0, H-C(3') and H-C(5'')); 7.28 (*t*, *J* = 7.5, H-C(3'') and H-C(5'')); 7.14- 7.38 (*m*, H-C(2'') and H-C(6'')); 7.18 (*t*, *J* = 7.2, H-C(4'')); 4.01 (*s*, CH₂Ar). ¹³C-NMR: 167.80; 158.32; 154.44; 138.83;

138.04; 134.41; 129.81; 129.11 (2C); 129.03 (2C); 128.22 (2C); 126.17; 121.61 (2C); 99.80; 45.42 (CH₂). HR-EI-MS: 335.0944 (M^+ , C₁₈H₁₄³⁵ClN₅; calc. 335.0938).

3-(4-Amino-1-(4-chlorophenyl)-1H-pyrazolo[3,4-d]pyrimidin-6-yl)benzonitrile (**2c**). The title compound was obtained as a yellow solid (51%). M.p. 243-245°. IR (Nujol): 3157, 2716, 2274, 1662, 1589, 1559, 1311, 1172, 978, 817, 784, 726. ¹H-NMR: 8.66-8.64 (*m*, H-C(2'') and H-C(4'')); 8.39 (*s*, H-C(3)); 8.32 (*dd*, *J* = 7.2 and 2.1, H-C(2') and H-C(6')); 8.20 and 8.00 (2 br. *s*, NH₂); 7.95 (*dt*, *J* = 7.8 and 1.5, H-C(6'')); 7.73 (*t*, *J* = 7.8, H-C(5'')); 7.65 (*dd*, *J* = 6.9 and 2.1, H-C(3') and H-C(5')). ¹³C-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 (2C); 122.03 (2C); 118.72(CN); 111.60; 100.65. HR-EI-MS: 346.0734 (M^+ , C₁₈H₁₁N₆³⁵Cl; calc. 346.0739).

1-(4-Chlorophenyl)-6-(pyridin-3-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**2d**). An off-white solid was obtained (67%). M.p. 241-243°. IR (Nujol): 3187, 2735, 2678, 1654, 1568, 978, 785. ¹H-NMR: 9.5 (br. *s*, H-C(2'')); 8.65-8.73 (*m*, H-C(4'') and H-C(6'')); 8.39 (*s*, H-C(3)); 8.36 (*d*, *J* = 9.0, H-C(2') and H-C(6')); 8.16 and 8.00 (2 br. *s*, NH₂); 7.65 (*d*, *J* = 9.0, H-C(3') and H-C(5')); 7.50-7.58 (*m*, H-C(5'')). ¹³C-NMR: 160.37; 158.38; 154.35; 151.14; 137.93; 135.50; 135.42; 134.70; 133.28; 130.12; 129.33 (2C); 123.62; 121.97 (2C); 100.61. HR-EI-MS: 322.0734 (M^+ , C₁₆H₁₁N₆³⁵Cl; calc. 322.0734). Anal. calc. for C₁₆H₁₁N₆ Cl: 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)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**2e**). The compound was obtained as yellow crystals (69%). M.p. 267-269°. IR (Nujol): 3174, 2726, 2672, 1649, 1586, 1063, 972, 772. ¹H-NMR: 8.74 (*d*, *J* = 6.3,

H-C(2'') and H-C(6'')); 8.42 (s, H-C(3)); 8.34 (d, $J = 9.0$, H-C(2') and H-C(6')); 8.28 (d, $J = 6.3$, H-C(3'') and H-C(5'')); 8.20 and 8.00 (2 br. s, NH₂); 7.66 (d, $J = 6.5$, H-C(3') and H-C(5')). ¹³C-NMR: 160.06; 158.43; 157.42; 150.23 (2C); 145.10; 137.82; 134.67; 130.20; 129.32 (2C); 122.03 (4C); 100.96. HR-EI-MS: 322.0745 (M^+ , C₁₆H₁₁N₆³⁵Cl; calc. 322.0734).

1-(4-Chlorophenyl)-6-(thiophen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine

(2f). An off-white solid was isolated (40%). M.p. 190-192°. IR (Nujol): 3176, 2732, 1654, 1586, 1233, 1212, 970, 853, 786, 755, 712. ¹H-NMR: 8.34 (d, $J = 9.0$, H-C(2') and H-C(6'')); 8.34 (s, H-C(3)); 7.94 (dd, $J = 3.9$ and 1.2 , H-C(3'')); 7.70 (dd, $J = 5.1$ and 1.2 , H-C(5'')); 7.62 (d, $J = 9.3$, H-C(3') and H-C(5')); 7.90-8.04 (br. s, NH₂); 7.18 (dd, $J = 5.1$ and 3.9 , H-C(4'')). ¹³C-NMR: 158.87; 158.27; 154.29; 143.88; 138.10; 134.84; 130.38; 130.09; 129.35 (2C); 128.84; 128.39; 121.82 (2C); 100.25. HR-EI-MS: 327.0345 (M^+ , C₁₅H₁₀³⁵ClN₅S; calc. 327.0353).

1-(4-Chlorophenyl)-6-(furan-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (2g).

An off-white solid was isolated (45%). M.p. 206-208°. IR (Nujol): 3269, 2781, 2685, 1587, 1586, 1175, 957, 889, 831, 772. ¹H-NMR: 8.53 (d, $J = 9.3$, H-C(2') and H-C(6'')); 8.35 (s, H-C(3)); 8.0 (br. s, NH₂); 7.70-7.90 (m, H-C(4'')); 7.62 (d, $J = 9.3$, H-C(3') and H-C(5'')); 7.34 (dd, $J = 3.3$ and 0.9 , H-C(3'')); 6.66 (dd, $J = 3.3$ and 1.8 , H-C(5'')). ¹³C-NMR: 159.41; 156.60; 153.99; 145.60; 141.80; 139.48; 134.65; 131.32; 129.80 (2C); 122.58 (2C); 114.01; 112.74; 101.34. HR-EI-MS: 311.0574 (M^+ , C₁₅H₁₀³⁵ClN₅O; calc. 311.0570).

1-(4-Bromophenyl)-6-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (3a).

Light yellow crystals were obtained (84%). M.p. 238-239°. IR (Nujol): 3378, 2753, 2693, 1662, 1588, 1536, 1181, 972, 774, 724. ¹H-NMR: 8.47-8.39 (m, H-C(2'))'

and H-C(6'')); 8.38 (*s*, H-C(3)); 8.31 (*d*, $J = 9.0$, H-C(2') and H-C(6')); 8.12-7.80 (br. *s*, 2H, NH₂); 7.78 (*d*, $J = 9.0$, H-C(3') and H-C(5')); 7.54-7.47 (*m*, H-C(3''), H-C(4'') and H-C(5'')). ¹³C-NMR: 162.10; 158.31; 154.75; 138.49; 137.87; 134.70; 132.23 (2C); 130.61; 128.43 (2C); 128.24 (2C); 122.23 (2C); 118.26; 100.42. HR-EI-MS: 365.0263 (M^+ , C₁₇H₁₂⁷⁹BrN₅; calc. 365.0276).

6-Benzyl-1-(4-bromophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine **(3b).**

Colourless needles were obtained (48%). M.p. 225-227°. IR (Nujol): 3282, 2787, 2669, 1588, 1578, 1183, 989, 778. ¹H-NMR: 8.33 (*s*, H-C(3)), 8.22 (*d*, $J = 8.7$, H-C(2') and H-C(6')); 7.95-7.74 (br. *s*, NH₂); 7.71 (*d*, $J = 8.7$, H-C(3') and H-C(5')); 7.36-7.23 (*m*, H-C(2''), H-C(3''), H-C(5''), H-C(6'')); 7.18 (*tt*, $J = 6.9$ and 1.2, H-C(4'')); 4.02 (*s*, CH₂Ar). ¹³C-NMR: 167.79; 158.32; 154.46; 138.81; 138.46; 134.53; 132.00 (2C); 129.02 (2C); 128.20 (2C); 126.15; 121.90 (2C); 118.03; 99.82; 45.41. HR-EI-MS: 379.0447 (M^+ , C₁₈H₁₁⁷⁹BrN₅; calc. 379.0433). Anal. calc. for C₁₈H₁₁ Br N₅: 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)-1H-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. ¹H-NMR: 8.72-8.66 (*m*, H-C(2'') and H-C(4'')); 8.38 (*s*, H-C(3)); 8.26 (*d*, $J = 8.7$, H-C(2') and H-C(6'')); 8.22-8.13 (br. *s*, NH₂); 7.95 (*dt*, $J = 7.5$ and 1.2, H-C(6'')); 7.76 (*d*, $J = 8.7$, H-C(3') and H-C(5'')); 7.71 (*t*, $J = 8.1$, H-C(5'')). ¹³C-NMR: 159.93; 158.33; 154.34; 139.00; 138.25; 134.69; 133.88; 132.60; 132.21 (2C); 131.47; 129.87; 122.37 (2C); 118.77; 118.44 (CN); 111.63; 100.71. HR-EI-MS: 390.0229 (M^+ , C₁₈H₁₁⁷⁹BrN₆; calc. 390.0231).

1-(4-Bromophenyl)-6-(pyridin-3-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine

(3d). The titled compound was obtained as yellow crystals (80%). M.p. 243-245°. IR (Nujol): 3168, 2757, 2675, 1653, 1586, 1076, 972, 775. ¹H-NMR: 9.56 (*d*, *J* = 2.1, H-C(2'')); 8.68 (*d*, *J* = 7.0, H-C(4'') and H-C(6'')); 8.41 (*s*, H-C(3)); 8.31 (*d*, *J* = 9.0, H-C(3') and H-C(5')); 8.02 and 8.17 (2 br. *s*, NH₂); 7.79 (*d*, *J* = 9.0, H-C(2') and H-C(6')); 7.58-7.52 (*m*, H-C(5')). ¹³C-NMR: 160.34; 158.33; 154.33; 151.12; 149.41; 138.30; 135.43; 134.70; 133.22; 132.21 (2C); 123.56; 122.23 (2C); 118.32; 100.60. HR-EI-MS: 366.0233 (*M*⁺, C₁₆H₁₁⁷⁹BrN₆; calc. 366.0229).

1-(4-Bromophenyl)-6-(pyridine-4-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine

(3e). An off-white powder was obtained (79%). M.p. 269-271°. IR (Nujol): 3168, 2757, 2675, 1653, 1586, 1069, 972, 777. ¹H-NMR: 8.74 (*d*, *J* = 4.7, H-C(2'') and H-C(6'')); 8.41 (*s*, H-C(3)); 8.29 (*d*, *J* = 8.7, H-C(2') and H-C(6')); 8.26 (*d*, *J* = 4.8, H-C(3'') and H-C(5'')); 8.22 and 8.04 (2 br. *s*, NH₂); 7.77 (*d*, *J* = 8.7, H-C(3') and H-C(5')). ¹³C-NMR: 160.02; 158.40; 154.26; 150.19 (2C); 145.08; 138.22; 134.67; 132.18 (2C); 122.27 (2C); 121.99 (2C); 118.42; 118.42. HR-EI-MS: 366.0230 (*M*⁺, C₁₆H₁₁⁷⁹BrN₆; calc. 366.022).

1-(4-Bromophenyl)-6-(thiophen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine

(3f). The title compound was obtained as a yellow solid (57%). M.p. 192-194°. IR (Nujol): 3182, 2731, 1639, 1587, 1225, 1202, 970, 834, 783, 717, 709. ¹H-NMR (Acetone-*d*₆): 8.47 (*d*, *J* = 9.0, H-C(2') and H-C(6')); 8.34 (*s*, H-C(3)); 8.06 (*dd*, *J* = 3.9 and 1.2, H-C(3'')); 7.65 (*dd*, *J* = 5.1 and 1.2, H-C(5'')); 7.78 (*d*, *J* = 9.0, H-C(3') and H-C(5')); 7.37 (br. *s*, NH₂); 7.20 (*dd*, *J* = 5.1 and 3.9, H-C(4'')). ¹³C-NMR (Acetone-*d*₆): 160.10; 159.29; 155.64; 145.16; 139.89; 134.80; 132.81

(2C); 130.45; 129.55; 128.69; 122.85 (2C); 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)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (3g).

A dark brown solid was obtained (45%). M.p. 206-208°. IR (Nujol): 3282, 2787, 2674, 1582, 1568, 1169, 959, 891, 823, 776. 1H -NMR: 8.35 (s, H-C(3)); 8.28 (d, J = 9.3, H-C(2') and 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') and H-C(5')); 7.26 (dd, J = 3.3 and 0.9, H-C(3'')); 6.67 (dd, J = 3.3 and 1.5, H-C(4'')). ^{13}C -NMR: 158.23; 155.25; 154.16; 152.40; 145.20; 138.39; 134.65; 132.07 (2C); 122.00 (2C); 118.15; 113.21; 112.18; 100.17. HR-EI-MS: 355.0062 (M^+ , $C_{15}H_{10}^{79}BrN_5O$; calc. 355.00687).

Biological Assays.

Xanthine Oxidase Assay. The reaction mixture containing 50 mM potassium dihydrogen phosphate buffer, pH 7.4, xanthine oxidase (0.066 U/mL), and a solution of test compounds in DMSO was incubated at room temperature, during 15 minutes. The reaction was started by addition of xanthine (100 μ M) in the presence of EDTA (1 mM), and uric acid formation was followed by measure of absorbance at 295 nm during 2 minutes. 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 (n° UTAD

175), *Botrytis* spp. isolated from apple (n° UTAD 158), *Septoria nodorum* isolated from triticale (n° UTAD 35) and *Phytophthora cinnamomi* isolated from chestnut trees (n° UTAD 107 and IMI n°340340), based on the method of Jasso de Rodríguez and co-workers [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°C 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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Table 1. *Inhibitory effects on xanthine oxidase*

Compound	IC ₅₀
1e	106,6 ± 6,2
2b	76,6 ± 2,0
3e	78,1 ± 6,6

Results are expressed as means ± SEM of 3 independent observations performed in triplicate. Allopurinol was used as positive control (% Inhibition (100 µM) = 96.3 ± 0.3, IC₅₀ = 24.4 ± 0.5 µM)

Table 2. *Antifungal activity data*

Compound	(% inhibition)							
	<i>Alternaria</i>		<i>Botrytis</i>		<i>S. nodorum</i>		<i>P. cinnamomi</i>	
	100ppm	200ppm	100ppm	200ppm	100ppm	200ppm	100ppm	200ppm
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

R = 1) C₆H₅, 2) *p*-Cl C₆H₄, 3) *p*-Br C₆H₄

R' = a) phenyl, b) benzyl, c) 3-cyanophenyl, d) 3-pyridyl, e) 4-pyridyl, f) 2-thiophenyl, g) 2-furanyl.

Scheme 1. Synthesis of pyrazolo[3,4-*d*]pyrimidines

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

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

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

Graphical illustration