Synthesis of 3-(1,2,3-triazol-1-yl)- and 3-(1,2,3-triazol-4-yl)-substituted pyrazolo[3,4-d]pyrimidin-4-amines via click chemistry: potential inhibitors of the Plasmodium falciparum PfPK7 protein kinase

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Introduction

The purine ring is the most ubiquitous nitrogen-containing heterocycle in nature, and it is the core structure of adenine and guanine in nucleic acids (RNA and DNA). 1 In addition, purines are involved in many metabolic processes as the co-factors associated with a large number of enzymes and receptors, e.g. ATP and GTP, which play key roles in cell signalling and other fundamental biological processes. 2

The ATP binding site in protein kinases is highly conserved, and therefore, the development of highly ATP competitive kinase inhibitors is a difficult task. However, there are regions within the binding cleft that are not occupied by ATP, and these regions (hydrophobic pockets) show a higher degree of structural diversity between members of the kinase family than the ATP binding regions. This provides opportunities for the discovery or design of selective and small molecule ATP-competitive inhibitors. 3 For example, Shokat et al. have shown that the Abl kinase was effectively inhibited by the substituted 4-amino-pyrazolo[3,4-d]pyrimidine II, largely through interaction with such “non-ATP binding” pockets. 4 Further, Pan et al. showed that compound I inhibits the Btk kinase in nanomolar concentration. 5

The common motif for these 4-amino-pyrazolo[3,4-d]-pyrimidine scaffolds is the “bent” geometry of the substituent in the 3-position of the pyrazole ring (Fig. 1). The bent structure allows the inhibitor to interact with the additional pocket of the active site, thereby increasing the inhibition and the specificity.

In our search for new scaffolds that could be used in the development of kinase inhibitors, we became interested in the 4-amino-pyrazolo[3,4-d]pyrimidines. Our approach to 4-amino-pyrazolo[3,4-d]pyrimidines involves the functionalisation of the pyrazole ring in the 3-position with differently substituted triazole rings using a 1,4-regioselective copper-catalysed azide–alkyne cycloaddition (CuAAC). 6–7

The 1,3-dipolar cycloaddition, often referred to as click chemistry, has been used in various applications, e.g. organic synthesis, drug discovery, and chemical biology due to the high reaction yield and simple reaction and purification conditions of the “click chemistry”. 7–11

The use of click chemistry allows the 4-substituent of the 1,2,3-triazole ring to be easily varied through the use of different azides or alkynes. Furthermore, the 1,4-disubstituted triazoles will have the “bent” geometry that should be useful for targeting kinases with extended lipophilic pockets with a bent shape.

Here we wish to present two efficient routes to 3-(1,2,3-triazol-1-yl)- and 3-(1,2,3-triazol-4-yl)-pyrazolo[3,4-d]pyrimidin-4-amines using a one-pot two-step reaction. The two routes give easy access to two different isomers of 1,4-disubstituted triazoles and the target compounds are obtained from a variety of readily available aromatic and aliphatic halides without isolation of potentially unstable organic azide intermediates. Two compounds show activity towards the PfPK7 kinase (IC50 10–20 μM) of P. falciparum, the organism responsible for the most virulent form of malaria, and can be regarded as hits useful for further development into lead compounds.

Fig. 1 Schematic picture of the binding of inhibitors 1 and 2 to the Btk kinase and the Abl kinase, respectively.
Results and discussion

Starting from the common precursor 1, the target compounds 6 and 7 were synthesised according to two complementary strategies (Scheme 1). In Route 1, compound 2 is synthesised via the palladium-catalysed Sonogashira coupling followed by a copper-catalysed [3 + 2]-cycloaddition with an azide, resulting in the formation of target compound 3-(1,2,3-triazol-4-yl)-substituted pyrazolo[3,4-d]pyrimidin-4-amines 6. In Route 2, compound 1 is converted into the azide 3, which is reacted in situ via the copper-catalysed [3 + 2]-cycloaddition with different alkynes resulting in formation of 3-(1,2,3-triazol-1-yl)-substituted pyrazolo[3,4-d]pyrimidin-4-amines 7.

![Scheme 1](image)

**Scheme 1** Strategies for the synthesis of the target compounds 6 and 7.

From the starting material 1, the terminal alkyne 2 was synthesised according to two different procedures involving a Sonogashira coupling with a protected acetylene, followed by a deprotection of the alkyne (Scheme 2). Initially, 1 was reacted with TMS-acetylene in the presence of Pd(PPh₃)₄ (2 mol%) and CuI (20 mol%) in THF using microwave-assisted heating at 120 °C to yield the protected alkyne 8 in excellent yield (91%). In order to facilitate the work-up the weak ion exchange resin (IRA-67) was used as base, which after the reaction could simply be filtered off. The TMS-group was easily deprotected with sodium hydroxide in THF/MeOH in good yield (87%).

![Scheme 2](image)

**Scheme 2** (i) and (ii) Compound 1 (1–3 mmol), acetylene (4 eq.) Pd(PPh₃)₄ (2 mol%), CuI (20 mol%) and Amberlite IRA-67 (5 eq.), THF, MW (120 °C, 15 min). (iii) Compound 8, NaOH (2 N), THF/MeOH (1:1), r.t., 22 h. (iv) Compound 9, NaOH, toluene, reflux, 2h.

In some cases, the purification of compound 8 becomes difficult due to the formation of triphenylphosphine oxide in the reaction. Therefore, an alternative approach to the Sonogashira coupling was developed involving a different alkyne, 2-methyl-3-yn-2-ol. Under the same conditions as for the synthesis of compound 8, compound 9 was prepared in 74% yield. Due to the additional hydroxyl group, the resulting product is more polar than compound 8 and triphenylphosphine oxide could be removed completely with flash chromatography using chloroform/methanol as an eluent. Furthermore, the propargyl alcohol is much cheaper than TMS acetylene. Compound 9 was deprotected using catalytic amounts of sodium hydroxide in dry refluxing toluene yielding compound 2 in 91%.

Since the synthesis of different organic azides is time consuming and in some cases very risky, several one-pot procedures have previously been developed. In these procedures, the organic azides are generated in situ either via the nucleophilic substitution of the alkyl halides by sodium azide or by the copper-catalysed reaction between an aromatic iodide and sodium azide, followed by the copper-catalysed [3 + 2]-cycloaddition reaction.

In order to find the optimal conditions for our reaction, a small screening of copper catalysts, solvents, reaction time and temperature was performed for the pyrazolo[3,4-d]pyrimidine system (Table 1). Starting with the commonly used solvent system tert-butanol/water and copper sulfate with copper wire under microwave irradiation (125 °C, 15 min), formation of the product 6a was observed but the conversion was not complete according to ¹H NMR (66%, entry 1).

The solvents were changed to THF and DMF:water (4:1), but no reaction was observed and the starting material was fully recovered (entries 2 and 3). The influence of the copper catalyst was...
investigated by exchanging the copper wire for sodium ascorbate (Naasc) (entries 3 and 4). Switching to copper sulfate/sodium ascorbate gave a high conversion (88:12) of the starting material (entry 4). The conversion was further improved by increasing the amount of alkylbromide (2.4 eq.) and sodium azide (2.4 eq.) and increasing the temperature to 140 °C (entries 5 and 6). Full conversion was also observed at 125 °C after prolonged reaction time (entry 7).

With the optimised results in hand, compounds 6a–b were synthesised in good to moderate yield (66–86%, Scheme 3) using CuSO₄/sodium ascorbate (20 mol%/40 mol%) as the copper catalyst in a DMF:H₂O (4:1) solvent system and microwave irradiation for the heating (130 °C, 30 min).

Using the same conditions, the aromatic iodides 4c–j were reacted with alkyne 2 to give products 6c–j in moderate to excellent yield (45–95%) considering that the reaction is a two-step one-pot reaction.

The same one-pot procedure that was used in the synthesis of compounds 6c–j was also used for the synthesis of compounds 7a–h in which the azide 3 is generated in situ from compound 1 and then further reacts with the alkyne in the copper-catalysed [3 + 2]-cycloaddition reaction to yield products 7a–h (Scheme 4). The reactions proceeded in good to excellent yield for both aliphatic (5a–b) and aromatic alkynes (5c–h) (78–91% yield), with the exception of the aromatic compounds (5f–g) having an amino group and a nitro group substituent on the aromatic ring (58% and 67%, respectively).

**Biological evaluation of inhibitors towards the Plasmodium falciparum protein kinase PfPK7**

Recently, several X-ray structures of *Plasmodium falciparum* protein kinase 7 (PfPK7) in complex with different inhibitors have been disclosed. The molecules used in these structural studies included compounds based on the pyrazolopyrimidine scaffold as presented in this work. Therefore, we were interested in investigating the potential use of our compounds as leads towards PfPK7 inhibitors.

*P. falciparum* is the most virulent species of human malaria parasites and is responsible for the most severe forms of the disease.

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**Table 1** Screening of different solvents, catalysts, and reaction conditions for the Cu(I)-catalysed [3 + 2] cycloaddition

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>Cu-cat.</th>
<th>T/°C</th>
<th>Time/min</th>
<th>Conv.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>tBuOH:H₂O (1:1)</td>
<td>Cu₀, CuSO₄</td>
<td>125</td>
<td>15</td>
<td>66:33</td>
</tr>
<tr>
<td>2</td>
<td>THF</td>
<td>Cu₀, CuSO₄</td>
<td>125</td>
<td>n.r.</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>DMF:H₂O (4:1)</td>
<td>Cu₀, CuSO₄</td>
<td>125</td>
<td>15</td>
<td>n.r.</td>
</tr>
<tr>
<td>4</td>
<td>DMF:H₂O (4:1)</td>
<td>CuSO₄/Naasc</td>
<td>125</td>
<td>15</td>
<td>88:12</td>
</tr>
<tr>
<td>5</td>
<td>DMF:H₂O (4:1)</td>
<td>CuSO₄/Naasc</td>
<td>140</td>
<td>15</td>
<td>Full conv.</td>
</tr>
<tr>
<td>6</td>
<td>DMF:H₂O (4:1)</td>
<td>CuSO₄/Naasc</td>
<td>140</td>
<td>15</td>
<td>26:1</td>
</tr>
<tr>
<td>7</td>
<td>DMF:H₂O (4:1)</td>
<td>CuSO₄/Naasc</td>
<td>125</td>
<td>30</td>
<td>Full conv.</td>
</tr>
</tbody>
</table>

a Compound 2 (0.25 mmol), benzyl bromide (1.2 eq.), NaN₃ (1.2 eq.), solvents (2 ml), CuSO₄ (20 mol%, 1 M solution in H₂O), MW-heating (fixed hold-time).

b Determined by 1H NMR (2-6a).

c 2.4 eq. benzyl bromide and azide.

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**Scheme 3** (i) Compound 2 (0.25 mmol), alkyl bromide or aromatic iodide (1.2 eq.), NaN₃ (1.2 eq.), DMF:H₂O (4:1) (2.5 ml), CuSO₄ (20 mol%, 1 M solution in H₂O), sodium ascorbate (40 mol%), MW-heating (130 °C, 30 min, fixed hold-time).

**Scheme 4** (i) Alkyne 5 (0.25 mmol), compound 1 (1.2 eq.), NaN₃ (1.2 eq.), DMF:H₂O (4:1) (2.5 ml), CuSO₄ (20 mol%, 1 M solution in H₂O), sodium ascorbate (40 mol%), MW-heating (130 °C, 30 min, fixed hold-time).
Malaria causes over two million deaths annually, predominantly among children from sub-Saharan Africa. *P. falciparum* is becoming resistant to currently available antimalarial treatments, which has led to an urgent and continuing search for new methods of control.\textsuperscript{16,17} Within malaria parasites, inhibition of protein kinases can modulate intracellular protein phosphorylation events, just as in other eukaryotes. Some *P. falciparum* kinases are not related to any eukaryote protein kinase family, and these may be validated as potential *P. falciparum*-specific drug targets.\textsuperscript{18,19} One such emerging target is PfPK7, an ‘orphan’ plasmodial kinase which is distantly related to the MAPKK family of kinases (mitogen-activated protein kinase kinase).\textsuperscript{20,21} Disruption of the *pfpk7* gene suggests that PfPK7 is involved in a pathway regulating cell proliferation and development: *P. falciparum* clones in which the PfPK7 locus has been inactivated are viable, but their asexual growth rate is about half that observed in wild-type parasites.\textsuperscript{22} This phenotype suggests that PFPK7 inactivation through chemical inhibition would be expected to slow parasite growth and, hence, decrease the virulence of infection.

The effect of molecules 6a–i and 7a–h on the activity of recombinant PfPK7 was tested in *in vitro* inhibition assays and an initial test was performed at 10 \( \mu \text{M} \) for compounds 6a–i and 7a–h. The assay measured the efficacy of compounds 6a–i and 7a–h at decreasing myelin basic protein (MBP, the exogenous substrate used in the assay) phosphorylation, as semi-quantitatively determined by autoradiography (Fig. 2).

![Effect of 17 compounds on GST-PfPK7 activity](image)

**Fig. 2** Inhibition of recombinant PfPK7 by compounds 6a–i and 7a–h. The efficacies are the averages from three measurements at 10 \( \mu \text{M} \) compound concentration. See Experimental procedures for details.

Two of the compounds, 6c and 6g, showed a significant decrease in MBP phosphorylation at 10 \( \mu \text{M} \) concentration and IC\(_{50}\) values for these compounds were determined to be \( \sim 20 \) and \( \sim 10 \) \( \mu \text{M} \), respectively (Fig. 3). Interestingly, although activity was significantly decreased in the presence of micromolar range concentrations of the inhibitors, we were unable to completely inhibit the kinase with the compounds, even at 1 mM. Nevertheless, these results demonstrate that the molecules with the pyrazolopyrimidine scaffold developed in this study do indeed affect PfPK7 activity.

![Inhibition of recombinant PfPK7 by compounds 6c (top panel) and 6g (bottom panel).](image)

**Fig. 3** Inhibition of recombinant PfPK7 by compounds 6c (top panel) and 6g (bottom panel). The autoradiographic data (MBP phosphorylation) used to construct the curves are shown as insets. See Experimental procedures for details.

To understand the binding mode of the active compounds, we docked 6c (Glide, XP mode) into the X-ray structure of PfPK7 (2PML).\textsuperscript{23,24} The pyrazolopyrimidine scaffold was superimposed with the adenylyl imidodiphosphate (AMP-PNP) ligand in the X-ray structure and the protein–ligand complex was optimised using the OPLS force field.\textsuperscript{24} The pyrazolopyrimidine ring is buried within the active site and makes one hydrogen bond with PfPK7 to the backbone amide group of Met120 within the hinge region and another hydrogen bond to the backbone carbonyl moiety of Glu118, which is a similar binding to that seen for the AMP-PNP ligand in the X-ray structure (Fig. 4). In PfPK7, a hydrophobic pocket is accessible to ATP-competitive inhibitors, which has previously been shown in X-ray structures of PfPK7.\textsuperscript{15} The docking suggests that the
4-phenyl-(1,2,3-triazol-1-yl)-moiety of compound 6c binds to the hydrophobic pocket within the ATP cleft and could explain the inhibitory effect of compound 6c.

Conclusions

An efficient strategy for the preparation of 3-(1,2,3-triazol-1-yl)- and 3-(1,2,3-triazol-4-yl)pyrazolo[3,4-d]pyrimidin-4-amines has been developed. The target compounds are obtained by a convenient one-pot procedure from a variety of readily available aromatic and aliphatic halides without isolation of potentially unstable organic azide intermediates. Two compounds show activity towards the PI3K/K7 kinase (IC₅₀ 10–20 μM) in P. falciparum, the organism responsible for the most virulent form of malaria. These are some of the very few inhibitors identified targeting the PI3K/K7 kinase and should be useful starting points for further development into lead compounds.

Experimental part

General

1H (400 MHz) and 13C (100 MHz) NMR spectra were obtained from a JEOL JNM-EX 400 spectrometer. The symbol “+” indicates CH₃ and CH groups identified from distortionless enhancement of polarisation transfer (DEPT) spectra. Column chromatography was performed by manual flash chromatography (wet packed silica, 0.04–0.063 mm) or by automated column chromatography on Biotage SP-4 using pre-packed columns.

Microwave reactions were performed in a Biotage Initiator reactor with fixed hold time. IR spectra were recorded on a Perkin-Elmer 16 PC spectrometer. Elemental analyses were performed at Kolbe Mikroanalytisches Laboratorium, Mülheim and der Ruhr, Germany. X-ray structures with inhibitors were used as starting point for all dockings. The PI3K/K7 kinase was prepared according to the standard procedure in the Schrödinger package. Docking was performed by using Glide (Schrödinger) with extraprecision (XP) settings and standard parameters for ligand docking.

4-Amino-3-iodo-1-(isopropyl)pyrazolo[3,4-d]pyrimidine (1)

4-Amino-3-iodo-pyrazolo[3,4-d]pyrimidine (1) (1–3 mmol), Pd(PPh₃)₄ (2 mol%), CuI (20 mol%) and Amberlite IRA-67 (5 eq.) were suspended in of THF (12 ml) in a 20 ml microwave vessel. To the stirring mixture the appropriate acetylene (4 eq.) was added, the vessel was sealed and heated to 120 °C for 15 min (ramp time: 240 s, pre-stirring: 20 s, high, fixed hold time: on). After cooling to room temperature, the reaction mixture was filtered through Celite and washed with CHCl₃. The solvent was removed in vacuo at 30 °C. The crude product was purified by flash column chromatography on silica gel (MeOH : CHCl₃ = 1 : 20).

4-Amino-3-iodo-1-ethynyl-1H-pyrazolo[3,4-d]pyrimidines (8 and 9)

Compound 1 (981 mg, 3.237 mmol) was converted to compound 8 (800 mg, 91% yield) by general procedure A. Rₖ (MeOH:CHCl₃, 1:40) = 0.21. 1H NMR (400 MHz, CDCl₃) δ 0.28 ppm (s, 9H, -Si-(CH₃)₃), 1.54 (d, 3JHH = 6.6 Hz, 6H, CH₃(CH)₂), 5.10 (sept, 3JHH = 6.6 Hz, 1H, CH₃-CH=N), 8.31 (s, 1H, N-CH=N). 13C NMR (100 MHz, CDCl₃) δ -0.15 ppm (+, -Si-(CH₃)₃), 22.14 (+, CH₃-CH=N), 49.61 (+, CH₃-CH=N), 96.69 (Cquart), 100.94 (Cquart), 102.34 (Cquart), 125.97 (Cquart), 152.45 (Cquart), 156.28 (+), 158.10 (Cquart). IR (film) ν = 3459 cm⁻¹, 2934, 1638, 1573. Anal. Calcd for C13H17N5O: C, 60.21; H, 6.64; N, 27.04.

4-Amino-3-iodo-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidine (9)

The general procedure A using 1 (303 mg, 1.000 mmol) afforded 192 mg (74%) of 9 as colorless solid. Rₖ (MeOH:CHCl₃, 1:40) = 0.21. 1H NMR (400 MHz, CDCl₃) δ 1.46 ppm (d, 3JHH = 6.6 Hz, 6H, CH₃(CH)₂), 1.62 (s, 6H, COO-(CH₃)₂), 5.02 (sept, 3JHH = 6.6 Hz, 1H, CH₃-CH=N), 8.21 (s, 1H, N-CH=N). 13C NMR (100 MHz, CDCl₃) δ = 22.01 ppm (+, CH₃-CH=N), 31.33 (+, -COO-(CH₃)₂), 49.51 (+, CH₃-CH=N), 65.33 (Cquart), 74.12 (Cquart), 99.68 (Cquart), 101.81 (Cquart), 125.69 (Cquart), 152.04 (Cquart), 155.84 (+), 157.95 (Cquart). IR (film) ν = 3459 cm⁻¹, 2934, 1638, 1573. Anal. Calcd for C₁₉H₂₀N₅: C, 60.23; H, 6.61; N, 27.01. Found C, 60.23; H, 6.64; N, 27.04.

4-Amino-3-iodo-3-trimethylsilyl-1H-pyrazolo[3,4-d]pyrimidin-3-yl)-2-methyl-butyryl-3-yn-2-ol (9)

4-(4-Amino-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-3-yl)-2-methyl-butyryl-3-yn-2-ol (9)

The general procedure A using 1 (303 mg, 1.000 mmol) afforded 192 mg (74%) of 9 as colorless solid. Rₖ (MeOH:CHCl₃, 1:40) = 0.21. 1H NMR (400 MHz, CDCl₃) δ 1.46 ppm (d, 3JHH = 6.6 Hz, 6H, CH₃(CH)₂), 1.62 (s, 6H, COO-(CH₃)₂), 5.02 (sept, 3JHH = 6.6 Hz, 1H, CH₃-CH=N), 8.21 (s, 1H, N-CH=N). 13C NMR (100 MHz, CDCl₃) δ = 22.01 ppm (+, CH₃-CH=N), 31.33 (+, -COO-(CH₃)₂), 49.51 (+, CH₃-CH=N), 65.33 (Cquart), 74.12 (Cquart), 99.68 (Cquart), 101.81 (Cquart), 125.69 (Cquart), 152.04 (Cquart), 155.84 (+), 157.95 (Cquart). IR (film) ν = 3459 cm⁻¹, 3292, 2981, 2934, 1638, 1573. Anal. Calcd for C₁₉H₂₀N₅O: C, 60.21; H, 6.61; N, 27.01. Found C, 60.23; H, 6.64; N, 27.04.
brine. The mixture was extracted with EtOAc (3 × 60 ml). The combined, organic layers were dried over MgSO₄ and the solvent was removed in vacuo. The crude product was purified with flash column chromatography on silica gel (MeOH:CHCl₃ = 1:40) to give 454 mg (87%) of 2 as a pale color solid. R₅ (MeOH : CHCl₃ = 1 : 40) = 0.18. Mp 188–189 °C. ¹H NMR (400 MHz, CDCl₃) δ = 1.54 ppm (d, JHH = 6.6 Hz, 6H, CH₂-CH₃), 1.87 (s, 3H, N-CH₃), 8.33 (s, 1H, N-CH-N). ¹³C NMR (100 MHz, CDCl₃) δ = 22.12 ppm (+), 22.79 ppm (+), 33.08 ppm (+), 34.37 ppm (+), 121.29 ppm (+), 134.23 ppm (+), 135.55 ppm (+), 143.19 ppm (+), 153.39 ppm (+). IR (KBr) v 3447 cm⁻¹, 3307, 3231, 3096, 2984, 2109, 1651, 1595, 1567. Anal. Calcd for C₁₆H₁₇N₅: C, 59.69; H, 5.51; N, 34.80. Found C, 59.67; H, 5.46; N, 34.71.

General procedure B for preparation of triazoles from non-aromatic/benzylic bromides

3-Ethynyl-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-ylamine (0.25 mmol), benzylbromide (1.2 eq.), NaN₃ (1.2 eq.), sodium ascorbate (40 mol%) were suspended in of a DMF: H₂O (2.5 ml, 4:1) mixture in a 2–5 ml microwave vessel. To the stirring mixture CuSO₄ (20 mol%), NaN₃ (1.44 eq.), L-proline (20 mol%), Na₂CO₃ (20 mol%) were suspended in DMSO : H₂O (2.5 ml, 9 : 1) mixture in a 2–5 ml microwave vessel. The stirring mixture CuSO₄ (20 mol%), as a 1 M solution in H₂O was added and the vessel was sealed and heated to 130 °C for 30 min (ramp time: 20 s, high, fixed hold time: on). After cooling to room temperature, the reaction mixture was poured into aqueous ammonia solution (80 ml of H₂O, 20 ml of conc. NH₄OH). The layers were separated and the water layer was extracted with EtOAc three times. The combined organic layers were washed with H₂O and brine and dried over MgSO₄. The solvent was removed in vacuo at 30 °C and the residue was further purified with column chromatography.

4-Amino-3-(1-benzyl-1H-1,2,3-triazol-4-yl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidine (6a)

The general procedure B using 2 (50 mg, 0.248 mmol) afforded 71 mg (86%) of 6a as colorless sticky oil. R₅ (MeOH:CHCl₃ = 1:20) = 0.04. ¹H NMR (400 MHz, CDCl₃) δ = 1.51 ppm (d, JHH = 6.6 Hz, 6H, CH₂-CH₃), 5.09 (sept, JHH = 6.6 Hz, 1H, CH₁-CH-N), 5.58 (s, 2H, N-CH₂-Ph), 7.30–7.42 (m, 5H, Ph), 8.06 (s, 1H, C-CH-N), 8.33 (s, 1H, N-CH-N). ¹³C NMR (100 MHz, CDCl₃) δ = 22.03 ppm (+), 22.49 ppm (+), 22.60 ppm (+), 22.83 ppm (+), 22.94 ppm (+), 23.05 ppm (+), 23.16 ppm (+), 23.37 ppm (+), 23.58 ppm (+), 23.80 ppm (+), 23.91 ppm (+), 24.02 ppm (+), 24.13 ppm (+), 24.24 ppm (+), 24.35 ppm (+), 24.46 ppm (+), 24.57 ppm (+), 24.68 ppm (+), 24.79 ppm (+), 24.90 ppm (+), 25.01 ppm (+), 25.12 ppm (+), 25.23 ppm (+), 25.34 ppm (+), 25.45 ppm (+), 25.56 ppm (+), 25.67 ppm (+), 25.78 ppm (+), 25.89 ppm (+), 25.99 ppm (+), 26.00 ppm (+), 26.11 ppm (+), 26.22 ppm (+), 26.33 ppm (+), 26.44 ppm (+), 26.55 ppm (+), 26.66 ppm (+), 26.77 ppm (+), 26.88 ppm (+), 26.99 ppm (+), 27.00 ppm (+), 27.11 ppm (+), 27.22 ppm (+), 27.33 ppm (+), 27.44 ppm (+), 27.55 ppm (+), 27.66 ppm (+), 27.77 ppm (+), 27.88 ppm (+), 27.99 ppm (+), 28.00 ppm (+), 28.11 ppm (+), 28.22 ppm (+), 28.33 ppm (+), 28.44 ppm (+), 28.55 ppm (+), 28.66 ppm (+), 28.77 ppm (+), 28.88 ppm (+), 28.99 ppm (+), 29.00 ppm (+), 29.11 ppm (+), 29.22 ppm (+), 29.33 ppm (+), 29.44 ppm (+), 29.55 ppm (+), 29.66 ppm (+), 29.77 ppm (+), 29.88 ppm (+), 29.99 ppm (+), 30.00 ppm (+), 30.11 ppm (+), 30.22 ppm (+), 30.33 ppm (+), 30.44 ppm (+), 30.55 ppm (+), 30.66 ppm (+), 30.77 ppm (+), 30.88 ppm (+), 30.99 ppm (+), 31.00 ppm (+). IR (KBr) ν 3307, 3221, 3069, 2984, 2109, 1651, 1595, 1567. Anal. Calcd for C₁₆H₁₇N₅: C, 59.99; H, 5.03; N, 34.98. Found C, 60.02; H, 5.05; N, 35.01.

4-Amino-3-(1-(4-fluoro-phenyl)-1H-1,2,3-triazol-4-yl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidine (6d)

The general procedure C using 2 (50 mg, 0.248 mmol) afforded 53 mg (65%) of 6d as pale yellow solid. Mp 265–266 °C. R₅ (MeOH:CHCl₃ = 1:20) = 0.28. ¹H NMR (400 MHz, CDCl₃) δ = 1.58 ppm (d, JHH = 6.6 Hz, 6H, NCH(CCH₃)), 5.18 (sept, JHH = 6.6 Hz, 1H, CH₁-CH-N), 6.12 (bs, 1H, NH-), 7.48–7.62 (m, 3H), 7.82–7.86 (m, 2H), 8.35 (s, 1H, N-CH-N), 8.61 (s, 1H, N-CH-N), 9.44 (s, 1H, NH-). ¹³C NMR (100 MHz, CDCl₃) δ = 22.04 ppm (+), 49.06 (+), 98.92 (C₂₅), 119.32 (+), 120.83 (+), 129.54 (+), 130.16 (+), 135.11 (C₂₅), 136.90 (C₂₅), 143.46 (C₂₅), 153.78 (C₂₅), 156.41 (+), 158.70 (C₂₅). IR (KBr) ν 3432 cm⁻¹, 3295, 3099, 2983, 2934, 1727, 1663, 1546, 1505. Anal. Calcd for C₁₆H₁₇N₅F: C, 60.98; H, 5.36; N, 34.66. Found C, 61.01; H, 5.37; N, 34.67.

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4-Amino-3-[1-(4-chloro-phenyl)-1H-[1,2,3]triazol-4-yl]-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidine (6e)

The general procedure C using 2 (50 mg, 0.248 mmol) afforded 53 mg (60%) of 6e as pale yellow solid. 1H NMR (400 MHz, DMSO) δ 1.51 ppm (d, 1JH-H = 6.6 Hz, 6H), 5.07 (sept, 1JH-H = 6.6 Hz, 1H), 5.07 (bs, 2H), 6.67–6.73 (m, 1H), 7.11–7.16 (m, 1H), 7.19–7.24 (m, 2H), 8.07 (bs, 1H), 8.24 (s, 1H), 9.02 (bs, 1H), 9.18 (s, 1H). 13C NMR (100 MHz, DMSO) δ 21.72 ppm (+), 48.44 (+), 50.26 (+), 105.20 (+), 107.31 (+), 114.48 (+), 120.18 (+), 130.22 (+), 134.41 (Cquart), 137.10 (Cquart), 142.09 (Cquart), 150.10 (Cquart), 153.24 (Cquart), 156.21 (+), 158.24 (Cquart). IR (KBr) v 3435 cm⁻¹ (sh), 3331, 3307, 3116, 2977, 2931, 1499, 1284, 1257, 587, 509. Anal. Calcd for C22H21N6O: C, 62.04; H, 4.28; N, 31.61.

4-Amino-3-[1-(4-methoxy-phenyl)-1H-[1,2,3]triazol-4-yl]-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidine (6f)

The general procedure C using 2 (50 mg, 0.248 mmol) afforded 53 mg (81%) of 6f as pale yellow solid. Mp 261–262 °C. Rf (MeOH:CHCl₃:MeCN = 1:2:20) = 0.30. 1H NMR (400 MHz, CDCl₃) δ 1.59 ppm (d, 1JH-H = 6.6 Hz, 6H), 3.91 (s, 3H), 5.17 (sept, 1JH-H = 6.6 Hz, 1H), 7.06–7.11 (m, 2H, 2H), 7.71–7.77 (m, 2H, 2H), 8.35 (s, 1H), 8.52 (s, 1H). 13C NMR (100 MHz, CDCl₃) δ 22.23 ppm (+), 39.15 (+), 49.15 (+), 51.95 (+), 115.17 (+), 119.49 (+), 122.47 (+), 130.27 (Cquart), 135.45 (Cquart), 134.13 (Cquart), 153.58 (Cquart), 155.73 (+), 158.36 (Cquart), 160.47 (Cquart). IR (KBr) v 3432 cm⁻¹, 3302, 3114, 2988, 2935, 1656, 1606, 1563, 1517, 1255, 1039, 621. Anal. Calcd for C17H18N8O: C, 61.06; H, 5.43; N, 33.51. Found C, 61.14; H, 5.37; N, 33.40.

4-Amino-3-[1-(2-pyridyl)-1H-[1,2,3]triazol-4-yl]-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidine (6g)

The general procedure C using 2 (49 mg, 0.244 mmol) afforded 37 mg (48%) of 6g as yellow solid. Rf (MeOH:CHCl₃:MeCN = 1:20) = 0.30. 1H NMR (400 MHz, CDCl₃) δ 1.58 ppm (d, 1JH-H = 6.6 Hz, 6H), 5.17 (sept, 1JH-H = 6.6 Hz, 1H), 6.17 (bs, 1H, -NH), 7.39–7.44 (m, 1H), 7.94–8.01 (m, 1H), 8.21–8.25 (m, 6H), 5.08 (sept, 1JH-H = 6.6 Hz, 1H), 5.57 (bs, 2H), 6.67–6.73 (m, 1H), 7.11–7.16 (m, 1H), 7.19–7.24 (m, 2H), 8.07 (bs, 1H), 8.24 (s, 1H), 9.02 (bs, 1H), 9.18 (s, 1H). 13C NMR (100 MHz, CDCl₃) δ 22.23 ppm (+), 49.04 (+), 98.94 (Cquart), 114.08 (+), 118.75 (+), 124.32 (+), 134.95 (Cquart), 139.47 (+), 143.20 (Cquart), 149.03 (+), 153.73 (Cquart), 156.27 (+), 156.79 (Cquart), 158.65 (Cquart). IR (KBr) v 3439 cm⁻¹ (sh), 3298, 3109, 2980, 2935, 1651, 1609, 1564, 1352, 1256, 641. Anal. Calcd for C₂₁H₂₁N₈O: C, 62.00; H, 4.14; N, 34.50. Found C, 62.63; H, 4.16; N, 34.53.

4-Amino-3-[1-p-toly1]-1H-[1,2,3]triazol-4-yl]-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidine (6h)

The general procedure C using 2 (50 mg, 0.248 mmol) afforded 62 mg (76%) of 6j as pale yellow solid. Mp 284–285 °C. Rf (MeOH:CHCl₃:MeCN = 1:20) = 0.30. 1H NMR (400 MHz, CDCl₃) δ 1.59 ppm (d, 1JH-H = 6.6 Hz, 6H), 5.17 (sept, 1JH-H = 6.6 Hz, 1H), 6.01 (bs, 1H, -NH), 7.35–7.41 (m, 2H, AA’BB’), 7.69–7.74 (m, 2H, AA’BB’), 8.35 (s, 1H, N–CH₃), 8.56 (s, 1H, -CHtriazol), 9.48 (bs, 1H, -NH). 13C NMR (100 MHz, CDCl₃) δ 21.37 ppm (+), 22.23 (+), 49.05 (+), 98.90 (Cquart), 119.30 (+), 120.73 (+), 130.65 (+), 134.61 (Cquart), 139.77 (Cquart), 143.30 (Cquart), 153.77 (Cquart), 156.40 (+), 156.89 (Cquart). IR (film) v 3439 (sh) cm⁻¹, 3298, 3098, 2980, 2935, 1651, 1609, 1564, 1352, 1256, 641. Anal. Calcd for C₂₁H₂₁N₈O: C, 61.06; H, 5.43; N, 33.51. Found C, 61.14; H, 5.37; N, 33.40.

General procedure D for preparation of triazoles from compound 1

3 - Iodo - 1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin - 4-amine (0.50 mmol), alkyne (1.2 eq.), Na₂N₃ (1.44 eq.). L-proline (20 mol%), Na₂CO₃ (20 mol%), sodium ascorbate (40 mol%) were suspended in DMSO : H₂O (2.5 ml, 9 : 1) mixture in a 2–5 ml microwave vessel. To the stirring mixture CuSO₄ (20 mol%, as a 1 M solution in H₂O) were added and the vessel was sealed and heated to 130 °C for 30 min (ramp time: 35 s, pre-stirring: 20 s, high, fixed hold time: on). After cooling to room temperature, the reaction mixture was poured into aqueous ammonia solution (80 ml of H₂O, 20 ml of conc. NH₃·H₂O). The layers were separated and the water layer was extracted with EtOAc three times. The combined organic layers were washed with H₂O and brine and dried over MgSO₄. The solvent was removed in vacuo at 30 °C and the residue was further purified with column chromatography.

4-Amino-3-(4-benzyl-1H-[1,2,3]triazol-1-yl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidine (7a)

The general procedure D using 1 (150 mg, 0.494 mmol) afforded 142 mg (87%) of 7a as yellow solid. Mp 247–248 °C. Rf
4-Amino-1-isopropyl-3-(4-phenyl-1H-1,2,3-triazol-1-yl)-1H-pyrazolo[3,4-d]pyrimidine (7b)

The general procedure D using I (150 mg, 0.49 mmol) afforded 143 mg (84%) of 7b as colorless solid. Mp 187–188 °C. Rf (MeOH:CHCl3= 1:20) = 0.10 = 0.36. 1H NMR (400 MHz, CDCl3) δ 1.54 ppm (d, JHH = 6.6 Hz, 6H), 3.05–3.20 (m, 4H), 5.17 (sept, JHH = 6.6 Hz, 1H), 6.87 (bs, 1H), 7.17–7.35 (m, 5H), 8.21 (s, 1H), 8.36 (s, 1H), 8.77 (bs, 1H). 13C NMR (100 MHz, CDCl3) δ 122.16 ppm (+), 126.46 (+), 128.58 (+), 128.70 (+), 130.70 (Cquart), 140.92 (Cquart), 147.85 (Cquart), 153.54 (Cquart), 156.82 (+), 157.69 (Cquart). IR (KBr) v = 3432 cm⁻¹ (sh), 3323, 3138, 2980, 2929, 1653, 1569, 608. Anal. Caled for C16H17N9: C 57.30; H 5.43; N 33.51. Found C 53.35; H 5.2; N 33.5.

4-Amino-1-isopropyl-3-(4-phenyl-1H-1,2,3-triazol-1-yl)-1H-pyrazolo[3,4-d]pyrimidine (7c)

The general procedure D using I (90 mg, 0.297 mmol) afforded 86 mg (91%) of 7c as colorless solid. Mp 300–301 °C. Rf (MeOH:CHCl3= 1:20) = 0.29. 1H NMR (400 MHz, CDCl3) δ 1.58 ppm (d, JHH = 6.6 Hz, 6H), 3.02 (sept, JHH = 6.6 Hz, 1H), 3.93 (bs, 2H), 5.99 (bs, 1H), 6.69–6.79 (m, 6H), 5.21 (sept, 22.09 ppm (+), 49.32 (+), 92.44 (Cquart), 120.59 (+), 124.00 (Cquart), 124.65 (+), 129.98 (+), 131.43 (+), 132.93 (+), 136.77 (Cquart), 143.03 (Cquart), 148.68 (Cquart), 153.73 (Cquart), 157.04 (+), 157.50 (Cquart). IR (KBr) v = 3431 cm⁻¹, 3370, 3128, 2985, 1655, 1616, 1567. Anal. Caled for C16H15N9O2: C 52.60; H 5.42; N 38.02. Found C 52.45; H, 5.13; N, 38.02.

4-Amino-1-isopropyl-3-[4-(4-methylphenyl)-1H-1,2,3-triazol-1-yl]-1H-pyrazolo[3,4-d]pyrimidine (7d)

The general procedure D using I (150 mg, 0.49 mmol) afforded 120 mg (67%) of 7d as colorless solid. Mp 245–246 °C. Rf (MeOH:CHCl3= 1:40) = 0.29. 1H NMR (400 MHz, CDCl3) δ 1.58 ppm (d, JHH = 6.6 Hz, 6H), 5.21 (sept, JHH = 6.6 Hz, 1H), 6.37 (bs, 1H), 7.57–7.63 (m, 1H), 7.71–7.76 (m, 1H), 7.90–8.03 (m, 2H), 8.38 (s, 1H), 8.61 (bs, 1H), 8.75 (s, 1H). 13C NMR (100 MHz, CDCl3) δ 22.12 ppm (+), 49.32 (+), 92.44 (Cquart), 120.59 (+), 124.00 (Cquart), 124.65 (+), 129.98 (+), 131.43 (+), 132.93 (+), 136.77 (Cquart), 143.03 (Cquart), 148.68 (Cquart), 153.73 (Cquart), 157.04 (+), 157.50 (Cquart). IR (KBr) v = 3381 cm⁻¹, 3189, 3151, 2987, 2934, 1653, 1582, 1563, 1527. Anal. Caled for C16H15N9O2: C 52.60; H, 4.14; N 34.50. Found C 52.45; H, 4.20; N 34.42.

4-Amino-1-isopropyl-3-[4-(2-pyridyl)-1H-1,2,3-triazol-1-yl]-1H-pyrazolo[3,4-d]pyrimidine (7e)

The general procedure D using I (150 mg, 0.49 mmol) afforded 122 mg (78%) of 7e as colorless solid. Mp 293–294 °C. Rf (MeOH:CHCl3= 1:20) = 0.30. 1H NMR (400 MHz, CDCl3) δ 1.57 ppm (d, JHH = 6.6 Hz, 6H), 5.20 (sept, JHH = 6.6 Hz, 1H), 6.32 (bs, 1H), 7.29–7.36 (m, 1H), 7.81–7.89 (m, 1H), 8.20–8.26 (m, 1H), 8.38 (s, 1H), 8.67 (s, 1H), 8.68 (s, 1H), 9.14 (s, 1H). 13C NMR (100 MHz, CDCl3) δ 22.12 ppm (+), 49.17 (+), 92.46 (Cquart), 120.09 (+), 120.72 (+), 123.74 (+), 136.93 (Cquart), 137.29 (+), 148.68 (Cquart), 149.49 (Cquart), 149.99 (+), 153.73 (Cquart), 157.02 (+), 157.54 (Cquart). IR (KBr) v = 3340 cm⁻¹, 3190, 3097, 2976, 1658, 1607.
transferase fusion protein and purified as described previously. Recombinant PfPK7 was expressed in *E. coli* as a glutathione-S-transferase fusion protein and purified as described previously. Kinases assays were performed in a standard reaction (30 μl) containing 0.5 μg GST-PfPK7, 20 mM Tris-HCl pH 7.5, 20 mM MgCl₂, 2 mM MnCl₂, 15 μM cold ATP, 2.5 μCi and 5 μg of MBP (Myelin Basic Protein) as a substrate. The compounds were tested at 10 μM concentration of the inhibitors; the concentration of DMSO vehicle was identical in all reactions (10% final). All reactions were performed in triplicate and the data indicate the average values (± SD). The reaction proceeded for 30 min at 30 °C and was stopped by addition of Laemmli buffer. Samples were boiled for 3 min and analysed by electrophoresis on 12% SDS-polyacrylamide gels. The gels were dried and subjected to autoradiography and quantification in a phosphorimager.

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