

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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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 are presented. 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.

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-aminopyrazolo[3,4-*d*]pyrimidines. Our approach to 4-aminopyrazolo[3,4-*d*]pyrimidines involves the functionalisation

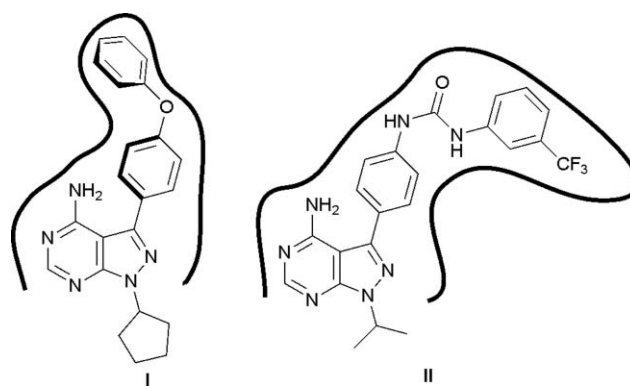


Fig. 1 Schematic picture of the binding of inhibitors **1** and **2** to the Btk kinase and the Abl kinase, respectively.

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.

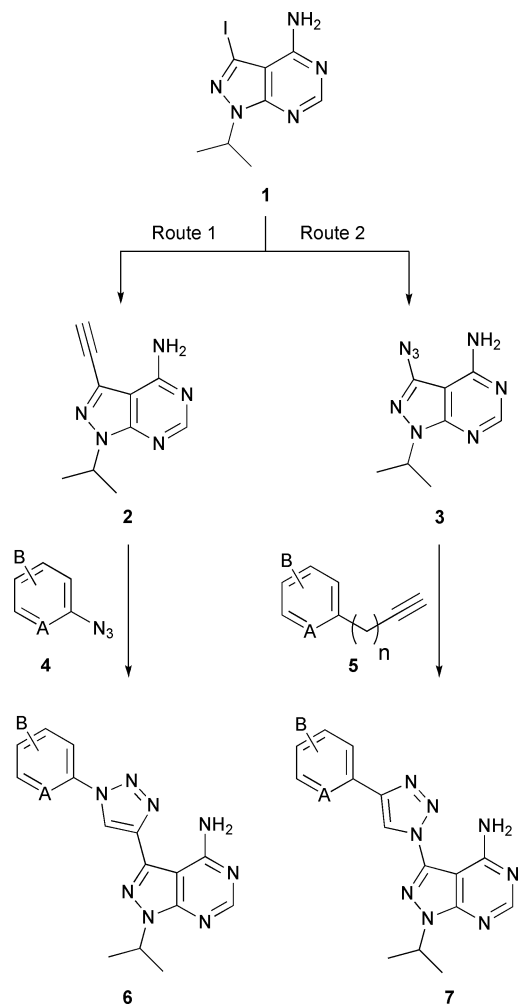
Here we wish to present two efficient routes to 3-(1,2,3-triazol-1-yl)- and 3-(1,2,3-triazol-4-yl)-pyrazolo[3,4-*d*]pyrimidin-4-amines using a one-pot two-step reaction. The two routes developed give easy access to both 1- and 4-substituted triazole rings in the 3-position of the pyrazole ring. Two compounds show activity in micromolar concentration (IC₅₀ 10–20 μM) towards the PfPK7 kinase in *P. falciparum*, the organism responsible for the most virulent form of malaria, and can be regarded as hits useful for further developments into lead compounds.

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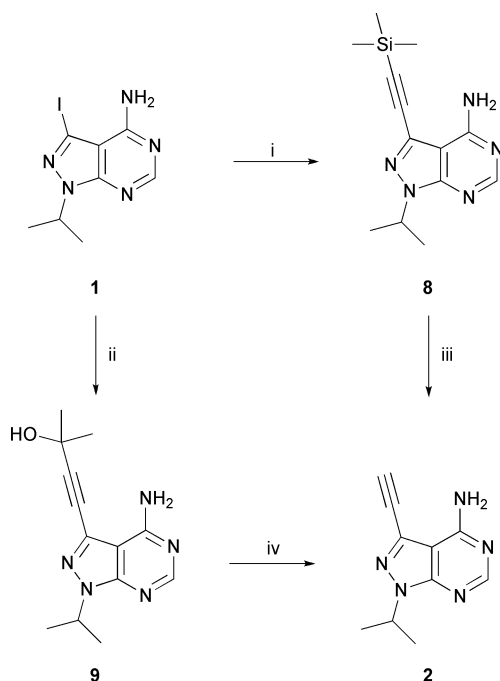
Results and discussion

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 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 (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.

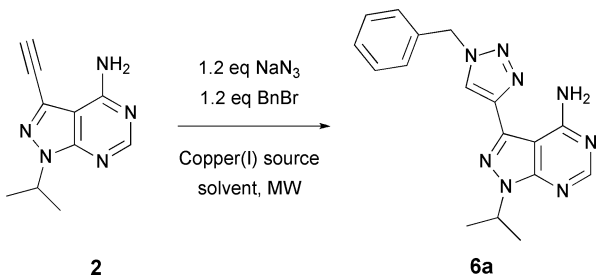
In some cases, the purification of compound **8** becomes difficult due to the formation of triphenylphosphine oxide in the reaction. Therefore, an alternative approach to the Sonogashira coupling was developed involving a different alkyne, 2-methyl-3-yn-2-ol. Under the same conditions as for the synthesis 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.^{12,13} 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

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



Entry	Solvent	Cu-cat.	T/°C	Time/min	Conv. ^b
1	^t BuOH:H ₂ O (1:1)	Cu ⁰ , CuSO ₄	125	15	66:33
2	THF	Cu ⁰ , CuSO ₄	125	15	n.r.
3	DMF:H ₂ O (4:1)	Cu ⁰ , CuSO ₄	125	15	n.r.
4	DMF:H ₂ O (4:1)	CuSO ₄ /Naasc	125	15	88:12
5	DMF:H ₂ O (4:1)	CuSO ₄ /Naasc	140	15	Full conv.
6	DMF:H ₂ O (4:1)	CuSO ₄ /Naasc	140	15	26:1 ^c
7	DMF:H ₂ O (4:1)	CuSO ₄ /Naasc	125	30	Full conv.

^a Compound **2** (0.25 mmol), benzylbromide (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. benzylbromide and azide.

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).

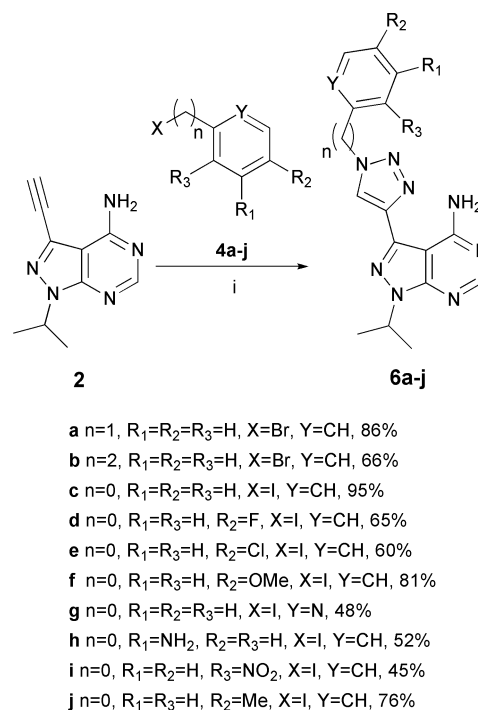
With the optimised results in hand, compounds **6a–b** were synthesised in good to moderate yield (66–86%, Scheme 3) using CuSO₄/sodium ascorbate (20 mol%/40 mol%) as the copper catalyst in a DMF:H₂O (4:1) solvent system and microwave irradiation for the heating (130 °C, 30 min).

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.

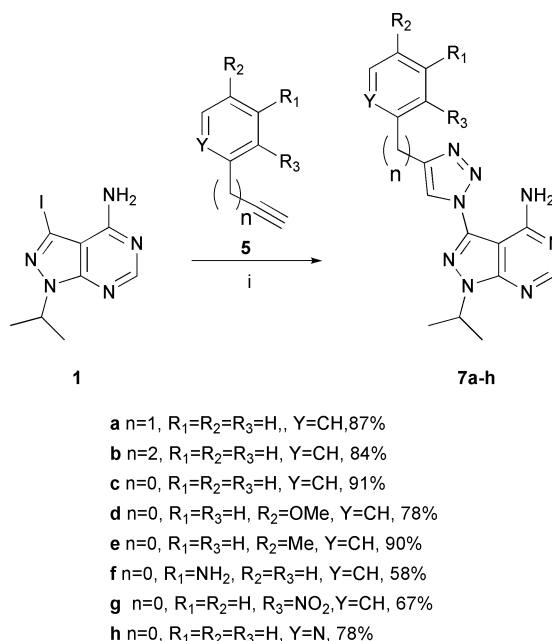
The same one-pot procedure that was used in the synthesis of compounds **6c–j** was also used for the synthesis of compounds **7a–h** in which the azide **3** is generated *in situ* from compound **1** and then further reacts with the alkyne in the copper-catalysed [3 + 2]-cycloaddition reaction to yield 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 amine 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* (*P. falciparum*) protein kinase 7 (PfPK7) in complex with different inhibitors have been disclosed. The molecules used in these



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).

structural studies included compounds based on the same pyrazolopyrimidine scaffold as presented in this work.^{14,15} 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.

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.^{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.^{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).^{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.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 μM 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).

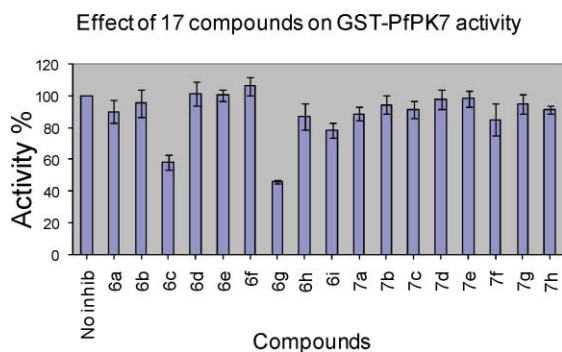


Fig. 2 Inhibition of recombinant PfPK7 by compounds **6a–i** and **7a–h**. The efficacies are the averages from three measurements at 10 μM compound concentration. See Experimental procedures for details.

Two of the compounds, **6c** and **6g**, showed a significant decrease in MBP phosphorylation at 10 μM concentration and IC_{50} values for these compounds were determined to be ~ 20 and ~ 10 μM , 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.

To understand the binding mode of the active compounds, we docked **6c** (Glide, XP mode) into the X-ray structure of PfPK7 (2PML).^{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.²⁴

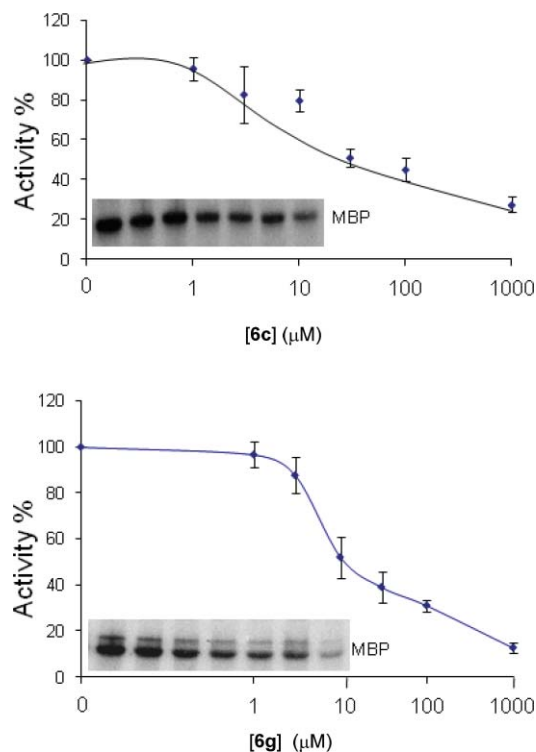


Fig. 3 Inhibition of recombinant PfPK7 by compounds **6c** (top panel) and **6g** (bottom panel). The autoradiographic data (MBP phosphorylation) used to construct the curves are shown as insets. See Experimental procedures for details.

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 AMPNP 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 PfPK7.¹⁵ The docking suggests that the

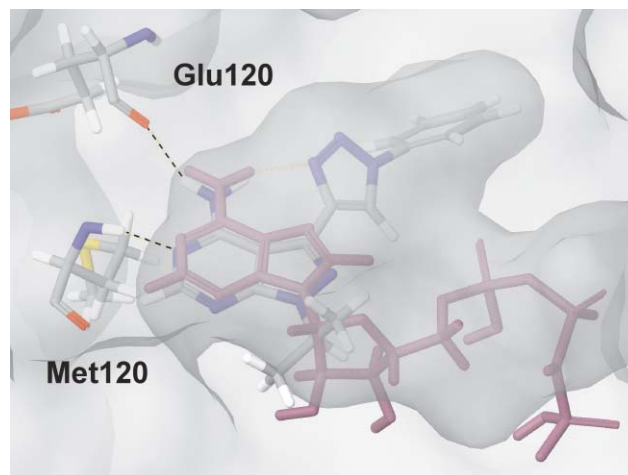


Fig. 4 Docking of compound **6c** into the ATP-pocket of PfPK7 (purple: AMP-PNP). Compound **6c** is suggested to have the same binding mode as ATP and to also bind to the additional hydrophobic part of the ATP pocket.

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 PfPK7 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 PfPK7 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 PfPK7 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 (2000 mg, 7.66 mmol) and K₂CO₃ (2115 mg, 15.32 mmol) were suspended in 12 ml of dry DMF in a 20 ml microwave vessel. To this mixture, 2-chloropropane (0.77 ml, 662 mg, 8.426 mmol) were added and the sealed tube was heated to 200 °C for 5 min. (20 s of pre-stirring and fixed hold time: on). After cooling of the reaction mixture to room temperature, additional 2-chloropropane (0.35 ml, 301 mg, 3.83 mmol) was added and the microwave vessel was heated again to 200 °C for 5 min. After cooling to ambient temperature, the reaction mixture was diluted with DMF and filtered. The solvent was removed *in vacuo* at 80 °C and the residue was co-distilled with toluene three times. The crude product was purified by flash column chromatography on silica gel (MeOH : CHCl₃ = 1 : 20) to give 2205 mg (95%) of **2** as fine yellow needles. R_f (MeOH : CHCl₃ = 1 : 40) = 0.29. Mp 203–204 °C. ¹H NMR (400 MHz, CDCl₃) δ 1.55 ppm (d, ³J_{HH} = 6.6 Hz, 6H), 5.09 (sept, ³J_{HH} = 6.6 Hz, 1H), 6.17 (bs, 2H, -NH₂), 8.32 (s, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 22.27 ppm (+), 49.89 (+), 85.62 (C_{quart}), 104.25 (C_{quart}), 153.17 (C_{quart}), 155.88 (+), 157.63 (C_{quart}). Anal. Calcd for

C₈H₁₀IN₅ (302.9981): C, 31.70; H, 3.33; N, 23.11. Found C, 31.84; H, 3.40; N, 23.19.

General procedure A for preparation of 3-ethynyl substituted 4-amino-1-isopropyl-1H-pyrazolo[3,4-*d*]pyrimidines (8 and 9)

4-Amino-3-iodo-1-isopropyl-1H-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 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-1-isopropyl-3-trimethylsilylethynyl-1H-pyrazolo[3,4-*d*]pyrimidine (8)

Compound **1** (981 mg, 3.237 mmol) was converted to compound **8** (800 mg, 91% yield) by general procedure A. R_f (MeOH:CHCl₃ 1:40) = 0.21. ¹H NMR (400 MHz, CDCl₃) δ 0.28 ppm (s, 9H, -Si-(CH₃)₃), 1.54 (d, ³J_{HH} = 6.6 Hz, 6H, CH₃-CH), 5.10 (sept, ³J_{HH} = 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), 49.61 (+, CH₃-CH-N), 96.69 (C_{quart}), 100.94 (C_{quart}), 102.34 (C_{quart}), 125.97 (C_{quart}), 152.45 (C_{quart}), 156.28 (+), 158.10 (C_{quart}). IR (KBr) ν = 3453 cm⁻¹, 3326, 3113, 2975, 2163, 1657, 1595, 1574, 1252, 862, 846. Anal. Calcd for C₁₃H₁₉N₅Si: C, 57.11; H, 7.00; N, 25.61. Found C, 57.24; H, 7.10; N, 25.48.

4-(4-Amino-1-isopropyl-1H-pyrazolo[3,4-*d*]pyrimidin-3-yl)-2-methyl-but-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. R_f (MeOH:CHCl₃ = 1:40) = 0.21. ¹H NMR (400 MHz, CDCl₃) δ 1.46 ppm (d, ³J_{HH} = 6.6 Hz, 6H, CHN-(CH₃)₂), 1.62 (s, 6H, CCO-(CH₃)₂), 5.02 (sept, ³J_{HH} = 6.6 Hz, 1H, (CH₃)₂-CH-N), 8.21 (s, 1H, N-CH-N). ¹³C NMR (100 MHz, CDCl₃) δ = 22.01 ppm (+, CHN-(CH₃)₂), 31.33 (+, -CCO-(CH₃)₂), 49.51 (+, CH₃-CH-CH₃), 65.33 (C_{quart}), 74.12 (C_{quart}), 99.68 (C_{quart}), 101.81 (C_{quart}), 125.69 (C_{quart}), 152.04 (C_{quart}), 155.84 (+), 157.95 (C_{quart}). IR (film) ν 3459 cm⁻¹, 3329, 2981, 2934, 1638, 1573. Anal. Calcd for C₁₃H₁₇N₅O: C, 60.21; H, 6.61; N, 27.01. Found C, 60.23; H, 6.64; N, 27.04.

4-Amino-1-isopropyl-3-ethynyl-1H-pyrazolo[3,4-*d*]pyrimidine (2)

Method 1. Alcohol **9** (151 mg, 0.582 mmol) was suspended in 5 ml of dry toluene (dried over Na/benzophenone) and crushed NaOH (84 mg, 2.10 mmol) was added. The reaction mixture was stirred at reflux for 2h. The solvent was removed *in vacuo* at 30 °C and the residue was purified by flash column (MeOH:CHCl₃ = 1:80) to give 106 mg (91%) of **2** as a pale brownish solid.

Method 2. Compound **8** (713 mg, 2.61 mmol) was dissolved in THF (20 ml) and MeOH (15 ml) and 2 N NaOH was added and the reaction mixture was stirred at room temperature. After 22 h, the reaction was quenched by the addition of 30 ml of

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 colored solid. R_f (MeOH : CHCl₃ = 1 : 40) = 0.18. Mp 188–189 °C. ¹H NMR (400 MHz, CDCl₃) δ = 1.54 ppm (d, ³J_{HH} = 6.6 Hz, 6H, CH₃-CH), 3.49 (s, 1H, C_{sp}-H), 5.11 (sept, ³J_{HH} = 6.6 Hz, 1H, CH₃-CH-N), 8.33 (s, 1H, N-CH-N). ¹³C NMR (100 MHz, CDCl₃) δ = 22.12 ppm (+, CH₃-CH), 49.67 (+, CH₃-CH-N), 76.19 (+), 82.51 (C_{quart}), 124.93 (C_{quart}), 152.45 (C_{quart}), 156.08 (+), 158.02 (C_{quart}). IR (film) ν 3447 cm⁻¹, 3307, 3221, 3069, 2984, 2109, 1651, 1595, 1567. Anal. calcd for C₁₀H₁₁N₅: 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-1*H*-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%, as a 1 M solution in H₂O) was added and the vessel was sealed and heated to 130 °C for 30 min (ramp time: 40 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. 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-1*H*-[1,2,3]triazol-4-yl)-1-isopropyl-1*H*-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. R_f (MeOH:CHCl₃= 1:80) = 0.04. ¹H NMR (400 MHz, CDCl₃) δ 1.51 ppm (d, ³J_{HH} = 6.6 Hz, 6H, CH₃-CH), 5.09 (sept, ³J_{HH} = 6.6 Hz, 1H, CH₃-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 (+, CH₃-CH), 48.87 (+, CH₃-CH-N), 54.68 (–, N-CH₂-Ph), 98.77 (C_{quart}), 121.17 (+), 128.39 (+), 129.18 (+), 129.41 (+), 134.19 (C_{quart}), 135.50 (C_{quart}), 143.09 (C_{quart}), 153.39 (C_{quart}), 156.32 (+), 158.60 (C_{quart}). IR (film) ν 3439 cm⁻¹, 3296, 3105, 2981, 2934, 1661, 1560. Anal. Calcd for C₁₇H₁₈N₈: C, 61.06; H, 5.43; N, 33.51. Found C, 60.94; H, 5.40; N, 33.36.

4-Amino-1-isopropyl-3-(1-phenethyl-1*H*-[1,2,3]triazol-4-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidine (**6b**)

The general procedure B using **2** (50 mg, 0.248 mmol) afforded 57 mg (66%) of **6b** as colorless solid. Mp 197–198 °C. R_f (MeOH:CHCl₃= 1:20) = 0.30. ¹H NMR (400 MHz, CDCl₃) δ 1.54 ppm (d, ³J_{HH} = 7.0 Hz, 6H, NCH-(CH₃)₂), 3.29 (t, ³J_{HH} = 7.7 Hz, 2H), 4.66 (t, ³J_{HH} = 7.7 Hz, 2H), 5.13 (sept, ³J_{HH} = 7.0 Hz, 1H, (CH₃)₂-CH-N), 6.36 (bs, 1H, NH-), 7.13–7.19 (m, 2H), 7.23–7.35 (m, 3H), 8.01 (s, 1H, N-CH-C), 8.32 (s, 1H, N-CH-N), 9.48 (bs, 1H). ¹³C NMR (100 MHz, CDCl₃) δ

22.16 ppm (+), 36.79 (–), 48.91 (+), 52.16 (–), 98.76 (C_{quart}), 121.41 (+), 127.44 (+), 128.79 (+), 129.11 (+), 135.43 (C_{quart}), 136.76 (C_{quart}), 142.68 (C_{quart}), 153.63 (C_{quart}), 156.28 (+), 158.77 (C_{quart}). IR (KBr) ν 3439 cm⁻¹, 3282, 3117, 2978, 2932, 1652, 1601, 1561. Anal. Calcd for C₁₈H₂₀N₈: C, 62.05; H, 5.79; N, 32.16. Found C, 62.18; H, 5.71; N, 32.04.

General procedure C for preparation of triazoles from aromatic iodides

3-Ethynyl-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamine (0.25 mmol), aromatic iodide (1.2 eq.), NaN₃ (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. 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-1-isopropyl-3-(1-phenyl-1*H*-[1,2,3]triazol-4-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidine (**6c**)

The general procedure C using **2** (50 mg, 0.248 mmol) afforded 75 mg (95%) of **6c** as colorless solid. Mp 251–252 °C. R_f (MeOH:CHCl₃= 1:20) = 0.30. ¹H NMR (400 MHz, CDCl₃) δ 1.58 ppm (d, ³J_{HH} = 6.6 Hz, 6H, NCH-(CH₃)₂), 5.18 (sept, ³J_{HH} = 6.6 Hz, 1H, (CH₃)₂-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-C), 9.44 (bs, 1H, NH-). ¹³C NMR (100 MHz, CDCl₃) δ 22.24 ppm (+), 49.06 (+), 98.92 (C_{quart}), 119.32 (+), 120.83 (+), 129.54 (+), 130.16 (+), 135.11 (C_{quart}), 136.90 (C_{quart}), 143.46 (C_{quart}), 153.78 (C_{quart}), 156.41 (+), 158.70 (C_{quart}). IR (KBr) ν 3432 cm⁻¹, 3295, 3099, 2983, 2934, 1727, 1663, 1564, 1505. Anal. Calcd for C₁₆H₁₆N₈: 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)-1*H*-[1,2,3]triazol-4-yl]-1-isopropyl-1*H*-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. R_f (MeOH:CHCl₃= 1:20) = 0.28. ¹H NMR (400 MHz, CDCl₃) δ 1.58 ppm (d, ³J_{HH} = 6.6 Hz, 6H, NCH-(CH₃)₂), 5.17 (sept, ³J_{HH} = 6.6 Hz, 1H, (CH₃)₂-CH-N), 6.09 (bs, 1H, NH-), 7.25–7.32 (m, 2H), 7.79–7.85 (m, 2H), 8.35 (s, 1H, N-CH-N), 8.56 (s, 1H, N-CH-C), 9.38 (bs, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 22.24 ppm (+, -CH₃, isopropyl), 49.08 (+, -CH, isopropyl), 98.90 (C_{quart}), 117.19 (d, ²J_{CF} = 23.1 Hz, “ortho-C”, +), 119.48 (+), 122.87 (d, ³J_{CF} = 9.2 Hz, “meta-C”, +), 133.18 (d, ⁴J_{CF} = 2.3 Hz, “para-C”, C_{quart}), 134.96 (C_{quart}), 143.62 (C_{quart}), 153.80 (C_{quart}), 156.42 (+), 158.66 (C_{quart}), 162.97 (d, ¹J_{CF} = 250.6 Hz, “ipso-C”, C_{quart}). IR (KBr) ν 3432 cm⁻¹, 3296, 3149, 2982, 2934, 1659, 1565, 1516, 1235, 622. Anal. Calcd for C₁₆H₁₅FN₈: C, 56.80; H, 4.47; N, 33.12. Found C, 56.88; H, 4.42; N, 33.19.

4-Amino-3-[1-(4-chloro-phenyl)-1*H*-[1,2,3]triazol-4-yl]-1-isopropyl-1*H*-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, ³J_{HH} = 6.6 Hz, 6H), 5.08 (sept, ³J_{HH} = 6.6 Hz, 1H), 7.69–7.75 (m, 2H, AA'BB') 8.09 (bs, 1H), 8.11–8.18 (m, 2H, AA'BB') 8.24 (s, 1H), 8.96 (bs, 1H), 9.42 (s, 1H). ¹³C NMR (100 MHz, DMSO) δ 21.77 ppm (+), 48.47 (+), 97.83 (C_{quart}), 120.75 (+), 122.34 (+), 129.92 (+), 133.64 (C_{quart}), 134.25 (C_{quart}), 135.15 (C_{quart}), 142.47 (C_{quart}), 153.24 (C_{quart}), 156.21 (+), 158.24 (C_{quart}). IR (KBr) ν 3453 cm⁻¹(sh), 3331, 3307, 3116, 2977, 2931, 1654, 1565, 1499, 1284, 1257, 587, 509. Anal. Calcd for C₁₆H₁₅ClN₈: C, 54.16; H, 4.26; N, 31.58. Found C, 54.20; H, 4.28; N, 31.61.

4-Amino-3-[1-(4-methoxy-phenyl)-1*H*-[1,2,3]triazol-4-yl]-1-isopropyl-1*H*-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. R_f (MeOH:CHCl₃ = 1:20) = 0.30. ¹H NMR (400 MHz, CDCl₃) δ 1.59 ppm (d, ³J_{HH} = 6.6 Hz, 6H), 3.91 (s, 3H), 5.17 (sept, ³J_{HH} = 6.6 Hz, 1H), 7.06–7.11 (m, 2H, AA'BB'), 7.71–7.77 (m, 2H, AA'BB'), 8.35 (s, 1H), 8.52 (s, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 22.23 ppm (+, CHN-(CH₃)₂), 49.15 (+, CH₃-CH-CH₃), 55.91 (+), 115.17 (+), 119.49 (+), 122.47 (+), 130.27 (C_{quart}), 135.45 (C_{quart}), 143.14 (C_{quart}), 153.58 (C_{quart}), 155.73 (+), 158.36 (C_{quart}), 160.47 (C_{quart}). IR (KBr) ν 3432 cm⁻¹, 3302, 3114, 2988, 2935, 1656, 1606, 1563, 1517, 1255, 1039, 621. Anal. Calcd for C₁₇H₁₈N₈O: C 58.27; H 5.18; N 31.98. Found C, 58.30; H, 5.20; N, 32.01.

4-Amino-3-[1-(2-pyridyl)-1*H*-[1,2,3]triazol-4-yl]-1-isopropyl-1*H*-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. R_f (MeOH:CHCl₃ = 1:20) = 0.30. ¹H NMR (400 MHz, CDCl₃) δ 1.58 ppm (d, ³J_{HH} = 6.6 Hz, 6H), 5.17 (sept, ³J_{HH} = 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, 1H), 8.35 (s, 1H), 8.54–8.59 (m, 1H), 9.18 (s, 1H), 9.37 (bs, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 22.23 ppm (+), 49.04 (+), 98.94 (C_{quart}), 114.08 (+), 118.75 (+), 124.32 (+), 134.95 (C_{quart}), 139.47 (+), 143.20 (C_{quart}), 149.03 (+), 153.73 (C_{quart}), 156.27 (+), 156.79 (C_{quart}), 158.65 (C_{quart}). IR (KBr) ν 3439 cm⁻¹(sh), 3295, 3111, 2977, 2936, 2874, 1663, 1563, 1471, 1031, 775. Anal. Calcd for C₁₅H₁₅N₉ (321.1450): C 56.07; H 4.71; N 39.23. Found C, 56.10; H, 4.73; N, 39.26.

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

The general procedure C using **2** (49 mg, 0.244 mmol) afforded 42 mg (52%) of **6h** as pale brownish solid. Mp 262–263 °C. ¹H NMR (400 MHz, DMSO) δ 1.51 ppm (d, ³J_{HH} = 6.6 Hz, 6H), 5.07 (sept, ³J_{HH} = 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, DMSO) δ 21.72 ppm (+), 48.44 (+), 105.20 (+), 107.31 (+), 114.48 (+), 120.18 (+), 130.22 (+), 134.41 (C_{quart}), 137.10 (C_{quart}), 142.09 (C_{quart}), 150.10 (C_{quart}),

153.19 (C_{quart}), 156.15 (+), 158.25 (C_{quart}). IR (KBr) ν 3439 cm⁻¹, 3370, 2980, 1635, 1563, 1500. Anal. Calcd for C₁₆H₁₇N₉: C, 57.30; H, 5.11; N, 37.59. Found C, 57.34; H, 5.13; N, 38.02.

4-Amino-3-[1-(2-nitrophenyl)-1*H*-[1,2,3]triazol-4-yl]-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (6i)

The general procedure C using **2** (49 mg, 0.244 mmol) afforded 40 mg (45%) of **6i** as yellow solid. Mp 256–257 °C. R_f (MeOH:CHCl₃ = 1:20) = 0.35. ¹H NMR (400 MHz, CDCl₃) δ 1.57 ppm (d, ³J_{HH} = 6.6 Hz, 6H), 5.17 (sept, ³J_{HH} = 6.6 Hz, 1H), 6.07 (bs, 1H, -NH), 7.67–7.72 (m, 1H), 7.75–7.81 (m, 1H), 7.83–7.89 (m, 1H), 8.12–8.19 (m, 1H), 8.36 (s, 1H), 8.48 (s, 1H), 9.26 (bs, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 22.23 ppm (+), 49.10 (+), 98.92 (C_{quart}), 122.67 (+), 126.07 (+), 127.92 (+), 130.11 (C_{quart}), 131.51 (+), 134.18 (+), 134.60 (C_{quart}), 143.56 (C_{quart}), 144.75 (C_{quart}), 153.80 (C_{quart}), 156.41 (+), 158.59 (C_{quart}). IR (KBr) ν 3432 cm⁻¹(sh), 3299, 3104, 2980, 2935, 1651, 1609, 1564, 1538, 1352, 1256, 641. Anal. Calcd for C₁₆H₁₅N₉O₂: C, 52.60; H, 4.14; N, 34.50. Found C, 52.63; H, 4.16; N, 34.53.

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

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. R_f (MeOH:CHCl₃ = 1:20) = 0.30. ¹H NMR (400 MHz, CDCl₃) δ 1.59 ppm (d, ³J_{HH} = 6.6 Hz, 6H, CHN-(CH₃)₂), 5.17 (sept, ³J_{HH} = 6.6 Hz, 1H, (CH₃)₂-CH-N), 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-CH-N), 8.56 (s, 1H, -CH_{triazol}), 9.48 (bs, 1H, -NH). ¹³C NMR (100 MHz, CDCl₃) δ 21.37 ppm (+), 22.23 (+), 49.05 (+), 98.90 (C_{quart}), 119.30 (+), 120.73 (+), 130.65 (+), 134.61 (C_{quart}), 135.22 (C_{quart}), 139.77 (C_{quart}), 143.30 (C_{quart}), 153.77 (C_{quart}), 156.40 (+), 158.69 (C_{quart}). IR (film) ν 3439 (sh) cm⁻¹, 3298, 3098, 2980, 1663, 1560, 1520. Anal. Calcd for C₁₇H₁₈N₈: 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-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (0.50 mmol), alkyne (1.2 eq.), NaN₃ (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. 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-(4-benzyl-1*H*-[1,2,3]triazol-1-yl)-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (7a)

The general procedure D using **1** (150 mg, 0.49 mmol) afforded 142 mg (87%) of **7a** as yellow solid. Mp 247–248 °C. R_f

(MeOH:CHCl₃ = 1:20) = 0.30. ¹H NMR (400 MHz, CDCl₃) δ 1.53 ppm (d, ³J_{HH} = 6.6 Hz, 6H), 4.20 (s, 2H), 5.16 (sept, ³J_{HH} = 6.6 Hz, 1H), 6.47 (bs, 1H), 7.22–7.39 (m, 5H), 8.19 (s, 1H), 8.35 (s, 1H), 8.77 (s, 1H). ¹³C NMR (100 MHz, CDCl₃) δ = 22.04 ppm (+), 32.35 (–), 49.12 (+), 92.32 (C_{quart}), 119.68 (+), 127.02 (+), 128.99 (+), 129.02 (+), 137.28 (C_{quart}), 138.46 (C_{quart}), 148.18 (C_{quart}), 153.58 (C_{quart}), 156.90 (+), 157.59 (C_{quart}). IR (KBr) ν = 3453 cm^{–1} (sh), 3349, 3098, 2978, 2928, 1654, 1568, 617. Anal. Calcd for C₁₇H₁₈N₈: C 61.06; H 5.43; N 33.51. Found C 61.20; H 5.49; N 33.39.

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

The general procedure D using **1** (150 mg, 0.49 mmol) afforded 143 mg (84%) of **7b** as colorless solid. Mp 187–188 °C. R_f (MeOH:CHCl₃ = 1:10) = 0.36. ¹H NMR (400 MHz, CDCl₃) δ 1.54 ppm (d, ³J_{HH} = 6.6 Hz, 6H), 3.05–3.20 (m, 4H), 5.17 (sept, ³J_{HH} = 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 22.03 ppm (+), 27.56 (–), 35.42 (–), 49.04 (+), 92.30 (C_{quart}), 119.21 (+), 126.46 (+), 128.58 (+), 128.70 (+), 137.30 (C_{quart}), 140.92 (C_{quart}), 147.85 (C_{quart}), 153.54 (C_{quart}), 156.82 (+), 157.69 (C_{quart}). IR (KBr) ν 3432 cm^{–1} (sh), 3332, 3138, 2980, 2929, 1653, 1569, 608. Anal. Calcd for C₁₈H₂₀N₈: C 62.05; H 5.79; N 32.16. Found C, 62.09; H, 5.81; N, 32.17.

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

The general procedure D using **1** (90 mg, 0.297 mmol) afforded 86 mg (91%) of **7c** as colorless solid. Mp >300 °C. R_f (MeOH:CHCl₃ = 1:20) = 0.30. ¹H NMR (400 MHz, CDCl₃) δ 1.59 ppm (d, ³J_{HH} = 6.6 Hz, 6H, CHN-(CH₃)₂), 5.22 (sept, ³J_{HH} = 6.6 Hz, 1H, (CH₃)₂-CH-N), 6.03 (bs, 1H, -NH), 7.39–7.54 (m, 3H), 7.95–7.99 (m, 2H), 8.38 (s, 1H, N-CH-N), 8.75 (bs, 1H, -NH), 8.75 (s, 1H, CH_{triazol}). ¹³C NMR (100 MHz, CDCl₃) δ = 22.13 ppm (+), 49.24 (+), 92.45 (C_{quart}), 117.49 (+), 126.23 (+), 129.16 (+), 129.28 (+), 129.70 (C_{quart}), 137.14 (C_{quart}), 148.19 (C_{quart}), 153.77 (C_{quart}), 157.09 (+), 157.54 (C_{quart}). IR (KBr) ν 3448 cm^{–1}, 3339, 3101, 1658, 1567, 1584. Anal. Calcd for C₁₆H₁₆N₈: C, 59.99; H, 5.03; N, 34.98. Found C, 60.02; H, 5.05; N, 35.01.

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

The general procedure D using **1** (150 mg, 0.49 mmol) afforded 134 mg (78%) of **7d** as colorless solid. Mp 282–283 °C. R_f (MeOH:CHCl₃ = 1:20) = 0.30. ¹H NMR (400 MHz, CDCl₃) δ 1.59 ppm (d, ³J_{HH} = 6.6 Hz, 6H), 3.89 (s, 3H), 5.21 (sept, ³J_{HH} = 6.6 Hz, 1H), 5.93 (bs, 1H), 7.01–7.06 (m, 2H, AA'BB'), 7.86–7.92 (m, 2H, AA'BB'), 8.38 (s, 1H), 8.66 (s, 1H), 8.77 (bs, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 22.13 ppm (+), 49.20 (+), 55.62 (+), 92.44 (C_{quart}), 114.69 (+), 116.63 (+), 122.35 (C_{quart}), 127.59 (+), 137.19 (C_{quart}), 148.07 (C_{quart}), 153.78 (C_{quart}), 157.13 (+), 157.53 (C_{quart}), 160.42 (C_{quart}). IR (KBr) ν 3424 cm^{–1} (sh), 3340, 3103, 2989, 2936, 1650, 1569, 1492, 1251. Anal. Calcd for C₁₇H₁₈N₈O: C 58.27; H 5.18; N 31.98. Found C, 58.29; H, 5.20; N, 32.01.

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

The general procedure D using **1** (150 mg, 0.49 mmol) afforded 148 mg (90%) of **7e** as colorless solid. Mp 293–294 °C. R_f (MeOH:CHCl₃ = 1:20) = 0.30. ¹H NMR (400 MHz, CDCl₃) δ 1.58 ppm (d, ³J_{HH} = 6.6 Hz, 6H), 5.21 (sept, ³J_{HH} = 6.6 Hz, 1H), 2.43 (s, 3H), 5.94 (bs, 1H), 7.29–7.34 (m, 2H, AA'BB'), 7.83–7.88 (m, 2H, AA'BB'), 8.38 (s, 1H), 8.71 (s, 1H), 8.76 (bs, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 21.60 ppm (+), 22.13 (+), 49.21 (+), 91.83 (C_{quart}), 117.12 (+), 126.14 (+), 126.87 (C_{quart}), 129.96 (+), 137.18 (C_{quart}), 139.17 (C_{quart}), 148.28 (C_{quart}), 153.77 (C_{quart}), 157.11 (+), 157.53 (C_{quart}). IR (KBr) ν 3439 cm^{–1}, 3342, 3155, 3100, 2984, 2932, 1658, 1579 (sh), 1567, 612. Anal. Calcd for C₁₇H₁₈N₈: C 61.06; H 5.43; N 33.51. Found C, 61.06; H, 5.42; N, 33.54.

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

The general procedure D using **1** (150 mg, 0.49 mmol) afforded 95 mg (58%) of **7f** as greyish solid. Mp 287–288 °C. ¹H NMR (400 MHz, CDCl₃) δ 1.58 ppm (d, ³J_{HH} = 6.6 Hz, 6H), 5.21 (sept, ³J_{HH} = 6.6 Hz, 1H), 3.83 (bs, 2H), 5.99 (bs, 1H), 6.69–6.79 (m, 1H), 7.20–7.38 (m, 3H), 8.38 (s, 1H), 8.70 (s, 1H), 8.76 (bs, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 22.13 ppm (+), 49.22 (+), 92.44 (C_{quart}), 112.57 (+), 115.83 (+), 116.54 (+), 117.51 (+), 130.25 (+), 130.62 (C_{quart}), 137.16 (C_{quart}), 147.25 (C_{quart}), 148.31 (C_{quart}), 153.76 (C_{quart}), 157.06 (+), 157.50 (C_{quart}). IR (KBr) ν 3451 cm^{–1}, 3370, 3128, 2985, 1655, 1616, 1567. Anal. Calcd for C₁₆H₁₇N₉: C 57.30; H 5.11; N 37.59. Found C, 57.32; H, 5.13; N, 38.02.

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

The general procedure D using **1** (150 mg, 0.49 mmol) afforded 120 mg (67%) of **7g** as colorless solid. Mp 245–246 °C. R_f (MeOH:CHCl₃ = 1:40) = 0.29. ¹H NMR (400 MHz, CDCl₃) δ 1.58 ppm (d, ³J_{HH} = 6.6 Hz, 6H), 5.21 (sept, ³J_{HH} = 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 22.09 ppm (+), 49.32 (+), 92.44 (C_{quart}), 120.59 (+), 124.00 (C_{quart}), 124.65 (+), 129.98 (+), 131.43 (+), 132.93 (+), 136.77 (C_{quart}), 143.03 (C_{quart}), 148.68 (C_{quart}), 153.73 (C_{quart}), 157.04 (+), 157.48 (C_{quart}). IR (KBr) ν 3381 cm^{–1}, 3189, 3151, 2987, 2934, 1653, 1582, 1563, 1527. Anal. Calcd for C₁₆H₁₅N₉O₂: C 52.60; H 4.14; N 34.50. Found 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 (7h)

The general procedure D using **1** (150 mg, 0.49 mmol) afforded 122 mg (78%) of **7h** as colorless solid. Mp 247–248 °C. R_f (MeOH:CHCl₃ = 1:40) = 0.29. ¹H NMR (400 MHz, CDCl₃) δ 1.57 ppm (d, ³J_{HH} = 6.6 Hz, 6H), 5.20 (sept, ³J_{HH} = 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 (C_{quart}), 120.09 (+), 120.72 (+), 123.74 (+), 136.93 (C_{quart}), 137.29 (+), 148.68 (C_{quart}), 149.49 (C_{quart}), 149.99 (+), 153.73 (C_{quart}), 157.02 (+), 157.54 (C_{quart}). IR (KBr) ν 3340 cm^{–1}, 3190, 3097, 2976, 1658,

1579, 1565, 1528. Anal. Calcd C₁₅H₁₅N₉: C 56.07; H 4.71; N 39.23. Found C 56.15; H 4.12; N 39.16.

PfPK7 inhibition assays

Recombinant PfPK7 was expressed in *E. coli* as a glutathione-S-transferase fusion protein and purified as described previously.²⁰ Kinases assays were performed in a standard reaction (30 µl) containing 0.5 µg GST-PfPK7, 20mM Tris-HCl pH 7.5, 20mM MgCl₂, 2mM 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 in the first instance. To determine IC₅₀ values for compounds **6c** and **6g**, kinase reactions were performed with a range of concentrations 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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