Medicinal Chemistry Research
https://doi.org/10.1007/s00044-018-2199-4

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and anti-*Plasmodium falciparum* evaluation of novel pyrazolopyrimidine derivatives

Flávia F. Silveira[1,2] · Lívia M. Feitosa[1,2] · João C. M. Mafra[1,2] · Maria de Lourdes G. Ferreira[1] · Kamilla R. Rogerio[1,2,3] · Leonardo J. M. Carvalho[3] · Nubia Boechat[1] · Luiz C. S. Pinheiro[1]

Received: 8 February 2018 / Accepted: 29 May 2018

Abstract

Nine 1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine derivatives with different substituents in the 4-position of the phenyl group and benzenesulfonamide moiety were synthesized and evaluated against *Plasmodium falciparum*. Six compounds exhibited activity in vitro against the chloroquine-resistant clone W2 with IC_{50} values ranging from 5.13 to 12.22 µM. The most active derivative with substituents $R_1 = F / R_2 = CH_3$ exhibited an IC_{50} value of 5.13 µM and an IS value of 62.90, which was higher than that of the control drug sulfadoxine. For this reason, it is possible to conclude that the 1*H*-pyrazolo[3,4-*d*] pyrimidine system is promising as a prototype for further studies of antimalarial candidates.

Keywords Malaria · *Plasmodium falciparum* · Pyrazolopyrimidine · Sulfonamide

Introduction

The data from the World Health Organization (WHO) revealed a significant reduction in the number of cases of malaria across the globe. In 2010, there were an estimated 237 million cases of malaria compared with 211 million cases in 2015. However, in 2016, an estimated 5 million more malaria cases occurred globally compared to 2015 (WHO 2017).

Monotherapy is no longer used to treat malaria due to the high parasitic resistance exhibited by *Plasmodium falciparum* to most available drugs. Instead, the WHO recommends the use of artemisinin-combined therapies (ACTs) (WHO 2015). Combination therapy with the simultaneous use of drugs having different modes of action helps prevent recrudescence and is employed to slow the development of parasite resistance (WHO 2015). Artemisinin derivatives are fast acting against intraerythrocytic asexual blood-stage malaria parasites; however, the disadvantage is their very short *in vivo* half-lives. Therefore, they are coadministered with longer half-life drugs (WHO 2015). Due to the resistance against quinoline derivatives and artemisinin derivatives, there is an urgent need for novel antimalarials with better safety profiles than current medicines (White 2004; Wells et al. 2015; Tilley et al. 2016).

In the recent literature, a number of new antimalarial compounds in different stages of preclinical and clinical development have been described (Schrader et al. 2012; Barnett and Guy 2014; Anthony et al. 2012; Biamonte et al. 2013; Teixeira et al. 2014). Quinoline derivatives are still the predominant class of antimalarial compounds (Vandekerckhove and D'hooghe 2015; Mushtaque and Shahjahan 2015; Kaur et al. 2010). However, due to the resistance to this class of drugs, the search for other analogous compounds is extremely important.

In our previous search for new drugs against malaria, we demonstrated the importance of quinolone (Varotti et al. 2008; Boechat et al. 2014; Pinheiro et al. 2015; Carvalho et al. 2016) and non-quinolone derivatives (Boechat et al. 2012; Silva et al. 2016; Azeredo et al. 2017).

We have recently synthesized a new series of quinoline-sulfadoxine hybrids resulting from molecular hybridization

✉ Nubia Boechat
nubia.boechat@far.fiocruz.br

✉ Luiz C. S. Pinheiro
luiz.pinheiro@far.fiocruz.br

[1] Departamento de Síntese de Fármacos, Instituto de Tecnologia em Fármacos, Farmanguinhos - FIOCRUZ, Fundação Oswaldo Cruz, Rua Sizenando Nabuco 100, Manguinhos, Rio de Janeiro, RJ 21041-250, Brazil

[2] Programa de Pós-Graduação em Química, PGQu Instituto de Química, Universidade Federal do Rio de Janeiro - UFRJ, Rio de Janeiro, RJ, Brazil

[3] Laboratório de Pesquisa em Malária, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brazil

Published online: 20 June 2018

between the quinoline ring and the benzenesulfonamide moiety present in chloroquine and sulfadoxine, respectively, which are drugs used in the treatment of malaria. All compounds in this series were active in vitro against the *P. falciparum* W2 chloroquine-resistant clone, and none of these compounds were toxic to mammalian BGM cells. The majority presented IC_{50} values lower than chloroquine with values ranging from 0.05 to 0.40 µM. The most active hybrids have a structural relationship among the four methylene groups that are used as linkers between the quinoline ring and the benzenesulfonamide moiety. Four compounds exhibited selectivity index (SI) values (3386.0–1031.3) higher than chloroquine (834.74). When evaluated against *P. berghei* malaria, compound **I** (Fig. 1) was the most active and inhibited parasitemia by 49% on day 5 after inoculation, contributing to the discovery of new prototypes with antimalarial activity (Pinheiro et al. 2015).

In an effort to find new non-quinoline compounds with anti-*P. falciparum* activity, we used precursor compound **I** to design the new derivatives *N*-(4-((1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)amino)butyl)benzenesulfonamides **(1–9)** (Fig. 2). The 7-chloroquinoline moiety was replaced by the 1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine system by ring isosterism. An *N*-(4-aminobutyl)benzenesulfonamide moiety was attached to the 4-position of the heterocyclic ring.

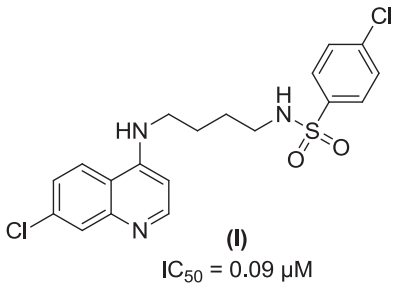


Fig. 1 Structure of compound **I**

The 1*H*-pyrazolo[3,4-*d*]pyrimidine ring remains separated from the benzenesulfonamide moiety by the linker containing four methylene groups, similar to that found in the individual molecular framework of precursor **I**. The anti-*P. falciparum* activities of the quinoline and 1*H*-pyrazolo[3,4-*d*]pyrimidine systems were then compared.

Chemistry

The synthetic route for preparing the *N*-(4-((1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)amino)butyl)benzenesulfonamides (**1–9**) is shown in Scheme 1.

The 5-amino-1-phenyl-1*H*-pyrazole-4-carbonitrile compounds **(10a–c)** can easily be prepared in 61–80% yield from the reaction of the appropriate phenylhydrazine (0.001 mol) and 2-(ethoxymethylene)malononitrile (0.001 mol) in ethanol under reflux for 2 h (Santos et al. 2012).

The 1-phenyl-1H-pyrazolo[3,4-*d*]pyrimidin-4-ols **(11a–c)** can be prepared in 73–89% yield from the reaction of suitable 5-amino-1-phenyl-1H-pyrazole-4-carbonitriles **(10ab–c)** (0.01 mol) and formic acid (20 mL) under reflux for 12 h (Soliman et al. 2012).

Derivatives **11a–c** (0.004 mol) were refluxed with phosphorous oxychloride (10 mL) for 24 h to produce 4-chloro-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidines **(12a–c)** in 78–97% yield (Bernardino et al. 2006).

The N^1-(1-phenyl-1*H*-pyrazolo[3,4-d]pyrimidin-4-yl) butane-1,4-diamine compounds **(13a–c)** were synthesized in 23–36% yield by the nucleophilic substitution reaction between the 4-chloro-1-phenyl-1H-pyrazolo[3,4-d]pyrimidines **(12a–c)** (0.004 mol) and butane-1,4-diamine

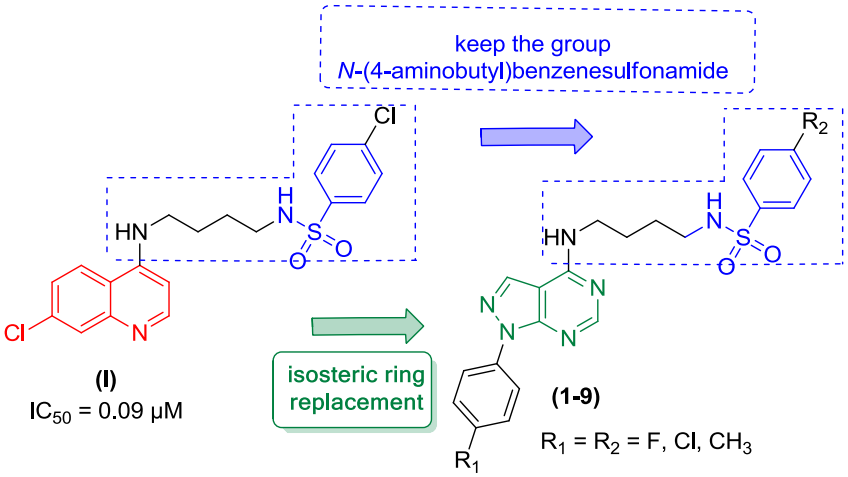
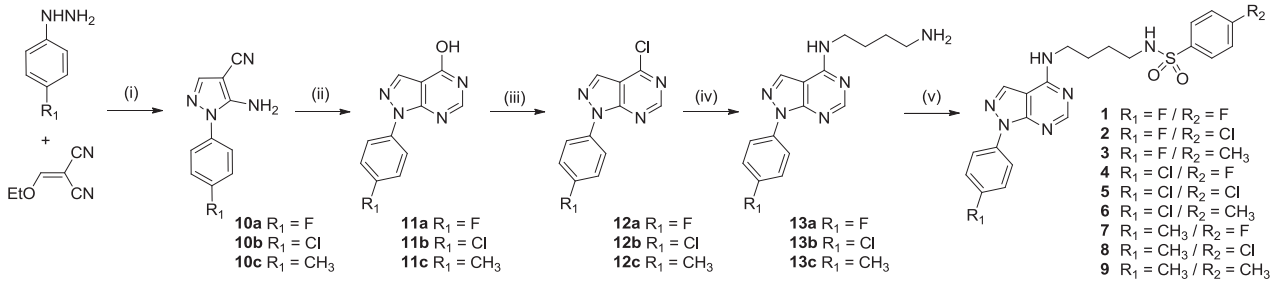


Fig. 2 Rational approach to the design of compounds **1–9**



Reagents and conditions: (i) EtOH, reflux, 2 h, 61-80%; (ii) HCOOH, reflux, 12 h, 73-89%; (iii) $POCl_3$, reflux, 24 h, 78-97%; (iv) butane-1,4-diamine, CH_3CN, 25 °C, 24 h, 23-36%; (v) appropriate sulfonyl chloride, DMF, TEA, 90 °C, 24 h, 25-79%.

Scheme 1 Synthetic route used to prepare compounds **1–9**

(0.004 mol) in CH_3CN (80 mL) at 25 °C for 24 h (Pinheiro et al. 2015; Silva et al. 2016).

The addition-elimination reaction between the amines **(13a–c)** (0.001 mol) and the appropriate sulfonyl chloride (4.5 eq) was performed in DMF (10 mL) and triethylamine (TEA) (1 eq) at 90 °C for 24 h to obtain the target compounds *N*-(4-((1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl) amino)butyl)benzenesulfonamides (**1**–**9**) in 25–79% yield (Pinheiro et al. 2015).

Material and methods

All reagents and solvents used were analytical grade. The 1 H, 13 C and 19 F nuclear magnetic resonance (NMR) spectra were obtained at 400.00, 100.00 and 376.00 MHz, respectively, using a BRUKER Avance instrument equipped with a 5-mm probe. Tetramethylsilane was used as an internal standard. The chemical shifts (δ) are reported in ppm, and the coupling constants (J) are reported in Hertz. Electron-ionization mass spectra (EI-MS, scan ES + capillary (3.0 kV)/cone (30 V)/extractor (1 V)/RF lens (1.0 V)/source temperature (150 °C)/desolvation temperature (300 °C)) were recorded using a Micromass/Waters Spectrometer (model: ZQ-4000). High Resolution Mass Spectrometry (HRMS) data were obtained using an LC-MS Bruker Daltonics MicroTOF (time of flight analyzer). Fourier transform infrared (FT-IR) absorption spectra were recorded on a Shimadzu mode IR Prestige-21 spectrophotometer through KBr reflectance. The melting points (m.p.) were determined using a Büchi model B-545 apparatus. TLC (thin layer chromatography) was performed using a silica gel F-254 glass plate (20 × 20 cm). Column chromatography was performed using silica gel 60 (0.040–0.063 mm). The analysis by high performance liquid chromatography (HPLC) was performed on Shimadzu liquid chromatography LC-10AD using Hypersil BDS C18 column (5μm 250 × 4.6 mm).

General procedure for preparing 5-amino-1-phenyl-1H-pyrazole-4-carbonitriles (10a–c)

A mixture of the appropriate phenylhydrazine (0.001 mol) and 10 mL of ethanol was stirred and allowed to reflux. Then, 2-(ethoxymethylene)malononitrile (0.001 mol) dissolved in 10 mL of ethanol was slowly added. The reaction mixture was refluxed for 2 h. The reaction mixture was poured into 50 mL of ice-cold water. The precipitate was collected by filtration and washed with water to provide **10a–c** in 61–80% yield.

5-amino-1-(4-fluorophenyl)-1*H*-pyrazole-4-carbonitrile (10a)

Yield: 61%. MP: 173–174 °C. IR (cm^{-1}): 3297–3183; 2225; 1662; 1568; 1222. ^{1}H NMR (400 MHz, DMSO-d_6, TMS, δ in p.p.m.): 7.31–7.25; (m; 2 H; H3′, H5′); 7.54–7.49; (m; 2 H; H2′, H6′); 7.67; (s; 1 H; H3). ^{13}C NMR (100 MHz, DMSO-d_6, TMS, δ in ppm): 73.2 (C4); 114.8 (CN); 116.3 (d; $J = 22.8$ Hz; C3′, C5′); 126.9 (d; $J = 8.9$ Hz; C2′, C6′); 133.7 (d; $J = 2.8$ Hz; C1′) 141.7 (C5); 151.4 (C3); 161.2 (d; $J = 243.6$ Hz; C4′). ^{19}F NMR (376 MHz, DMSO-d_6, TMS, δ in p.p.m.): -114.26. EI $[M + 1]^+$ 203.07.

5-amino-1-(4-chlorophenyl)-1*H*-pyrazole-4-carbonitrile (10b)

Yield: 78%. MP: 164–166 °C. IR (cm^{-1}): 3295–3174; 2229; 1663; 1562; 828. ^{1}H NMR (400 MHz, DMSO-d_6, TMS, δ in ppm): 7.50; (d; 2 H; $J = 8.9$ Hz H3′, H5′); 7.55; (d; 2 H; $J = 8.9$ Hz; H2', H6′); 7.68; (s; 1 H; H3). ^{13}C NMR (100 MHz, DMSO-d_6, TMS, δ in ppm): 73.5 (C4); 114.6 (CN); 126.0 (C2′, C6′); 129.4 (C3', C5′); 132.2 (C4′) 136.3 (C1′); 142.0 (C5); 151.4 (C3). EI $[M + 1]^+$ 219.07.

5-amino-1-(*p*-tolyl)-1*H*-pyrazole-4-carbonitrile (10c)

Yield: 80%. MP: 147–149 °C. IR (cm^{-1}): 3357–3314; 2214; 1660; 1564. ^1H NMR (400 MHz, DMSO-d_6, TMS, δ in ppm): 2.36 (s; 3 H; CH_3); 6.60 (s; 2 H; NH_2); 7.32; (d; 2 H; $J = 8.9$ Hz; H2′, H6′); 7.36; (d; 2 H; $J = 8.9$ Hz; H3′, H5′); 7.75; (s; 1 H; H3). ^{13}C NMR (100 MHz, DMSO-d_6, TMS, δ in ppm): 20.6 (CH_3); 73.2 (C4); 114.8 (CN); 124.1 (C2′, C6′); 129.8 (C3′, C5′); 134.9 (C4′) 137.4 (C1′); 141.4 (C5); 151.1 (C3). EI $[M + 1]^+$ 199.09.

General procedure for preparing 1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ols (11a–c)

A mixture of 5-amino-1-phenyl-1*H*-pyrazole-4-carbonitrile **(10a–c)** (0.01 mol) and 20 mL of formic acid was stirred and allowed to reflux for 12 h. The reaction mixture was poured into 50 mL of ice-cold water. The precipitate was collected by filtration and washed with water to afford **11a–c** in 73–89% yield.

1-(4-fluorophenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ol (11a)

Yield: 89%. MP: > 300 °C. IR (cm^{-1}): 3113; 1724; 1597; 1510; 1233. ^1H NMR (400 MHz, DMSO-d_6, TMS, δ in p.p.m.): 7.44–7.40 (m; 2 H; H3′, H5′); 8.08–8.04 (m; 2 H; H2′, H6′); 8.21 (d; 1 H; H6; $J = 3.8$ Hz); 8.33 (s; 1 H; H3); 12.47 (s; 1 H; OH). ^{13}C NMR (100 MHz, DMSO-d_6, TMS, δ in p.p.m.): 107.5 (C3a); 116.1 (d; $J = 22.9$ Hz; C3′, C5′); 123.8 (d; $J = 8.6$ Hz; C2′, C6′); 134.6 (d; $J = 2.7$ Hz; C1′); 136.0 (C3); 148.9 (C6); 151.7 (C7a); 157.1 (C4); 160.6 (d; $J =$ 242.8 Hz; C4′)^{19}F NMR (376 MHz, DMSO-d_6, TMS, δ in ppm): -114.88. EI $[M + 1]^+$ 231.07.

1-(4-chlorophenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ol (11b)

Yield: 73%. MP: > 300 °C. IR (cm^{-1}): 3097; 1795; 1667; 1597; 824. ^1H NMR (400 MHz, DMSO-d_6, TMS, δ in p.p.m.): 7.66 (d; 2 H; $J = 6.8$ Hz; H3′, H5′); 8.13 (d; 2 H; $J =$ 6.8 Hz; H2′, H6′); 8,22 (d; 1 H; $J = 3.8$ Hz; H6); 8.36 (s; 1 H; H3); 12.51 (s; 1 H; OH). ^{13}C NMR (100 MHz, DMSO-d_6, TMS, δ in ppm): 107.7 (C3a); 123.0 (C2′, C6′); 129.2 (C3′, C5′); 131.2 (C4′); 136.3 (C3); 137.1 (C1′); 149.0 (C6); 151.9 (C7a); 157.1 (C4). EI $[M + 1]^+$ 247.07.

1-(p-tolyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ol (11c)

Yield: 80%. MP: 275–277 °C. IR (cm^{-1}): 3094; 1667; 1589; 1510; 1397. ^1H NMR (400 MHz, DMSO-d_6, TMS, δ in p.p.m.): 2.37 (s; 3 H; CH_3); 7.36 (d; 2 H; $J = 8.4$ Hz; H3', H5'); 7.91 (d; 2 H; $J = 8.4$ Hz; H2', H6'); 8.18 (s; 1 H; H6); 8.30 (s; 1 H; H3); 12.42 (s; 1 H; OH). ^{13}C NMR (100 MHz, DMSO-d_6, TMS, δ in p.p.m.): 20.5 (CH_3); 107.4 (C3a); 121.6 (C2′, C6′); 129.5 (C3′, C5′); 135.7 (C4′); 135.8 (C3); 136.5 (C1′); 148.6 (C6); 151.6 (C7a); 157.2 (C4). EI $[M + 1]^+$ 227,07.

General procedure for preparing 4-chloro-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidines (12a–c)

To 0.004 mol of derivatives **11a–c** was added 10 mL of phosphorus oxychloride. The mixture was stirred under reflux for 24 h. Excess solvent was removed under reduced pressure, and the resulting material was carefully added to 50 mL of crushed ice. The mixture was then basified to pH 7 with NaOH (6 M aq.) and stirred for 40 min. The mixture was diluted with water (30 mL) and extracted with chloroform (3×30 mL). The combined organic solutions were washed with water (3×50 mL), dried (anhydrous sodium sulfate), filtered, and concentrated under vacuum to provide **12a–c** in 78–97% yield.

4-chloro-1-(4-fluorophenyl)-1H-pyrazolo[3,4-*d*] pyrimidine (12a)

Yield: 87%. MP: 158–160 °C (dg). IR (cm^{-1}): 3113; 1588; 1508; 1197; 838. ^1H NMR (400 MHz, DMSO-d_6, TMS, δ in p.p.m.): 7.50–7.45 (m; 2 H; H3′, H5′); 8.18–8.14 (m; 2 H; H2′, H6′); 8.76 (s; 1 H; H3); 8.98 (s; 1 H; H6). ^{13}C NMR (100 MHz, DMSO-d_6, TMS, δ in p.p.m.): 114.4 (C3a); 116.4 (d; $J = 22.8$ Hz; C3′, C5′); 123.4 (d; $J =$ 8.6 Hz; C2′, C6′); 133.9 (C3); 134.1 (d; $J = 2.8$ Hz C1′); 152.4 (C7a); 154.1 (C4). 155.3 (C6); 160.6 (d; $J = 243$ Hz; C4'). ^{19}F NMR (376 MHz, DMSO-d_6, TMS, δ in p.p.m.): −114.37. EI $[M + 1]^+$ 249.07.

4-chloro-1-(4-chlorophenyl)-1*H*-pyrazolo[3,4-*d*] pyrimidine (12b)

Yield: 97%. MP: 140–142°C (dg). IR (cm^{-1}): 3110; 1698; 1541; 854; 824. ^1H NMR (400 MHz, DMSO-d_6, TMS, δ in p.p.m.): 7.67 (d; 2 H; $J = 6.8$ Hz; H3′, H5′); 8.19 (d; 2 H; J = 6.8 Hz; H2′, H6′); 8.76 (s; 1 H; H3); 8.99 (s; 1 H; H6). ^{13}C NMR (100 MHz, DMSO-d_6, TMS, δ in ppm): 114.7 (C3a); 122.4 (C2′, C6′); 129.3 (C3′, C5′); 131.3 (C4′); 134.2 (C3); 136.5 (C1′); 152.5 (C7a); 154.1 (C4). 155.4 (C6). EI $[M + 1]^+$ 265.07.

4-chloro-1-(*p*-tolyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine (12c)

Yield: 78%. MP: 128–130 °C (dg). IR (cm^{-1}): 3094; 1677; 1539; 1351; 815. ^1H NMR (400 MHz, DMSO-d_6, TMS, δ in ppm): 2.39 (s; 3 H; CH_3); 7.43 (d; 2 H; $J = 8.4$ Hz; H3′,

H5'); 8.02 (d; 2 H; $J = 8.4$ Hz; H2', H6′); 8.74 (s; 1 H; H3); 8.97 (s; 1 H; H6). ^{13}C NMR (100 MHz, DMSO-d_6, TMS, δ in ppm): 20.5 (CH_3); 114.4 (C3a); 121.2 (C2′, C6′); 129.7 (C3′, C5′); 133.6 (C3); 135.3 (C4′); 136.8 (C1′); 152.3 (C7a); 154.0 (C4). 155.2 (C6). EI $[M + 1]^+$ 245.05.

General procedure for preparing N^1-(1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)butane-1,4-diamines (13a–c)

A solution of 0.004 mol of 4-chloro-1-phenyl-1*H*-pyrazolo [3,4-*d*]pyrimidines **(12a–c)** in 40 mL of CH_3CN was added dropwise to a solution of 0.004 mol of butane-1,4-diamine in 40 mL of CH_3CN. To avoid dimer formation, the reaction mixture was stirred at 25 °C for 24 h. The progress of the reaction was monitored using TLC ($CHCl_3$/MeOH 9:1). The solvent was evaporated to dryness and was added to ice-cold water (50 mL). The precipitate was collected by filtration and washed with water to afford **13a–c** in 23–36% yield.

N^1-(1-(4-fluorophenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)butane-1,4-diamine (13a)

Yield: 23%. MP: 178–180 °C. IR (cm^{-1}): 3211; 2949–2874; 1578–1507; 1207. ^{1}H NMR (400 MHz, DMSO-d_6, TMS, δ in ppm): 1.69–1.61 (m; 4 H; H9, H10); 2.77 (t; 2 H; $J = 7.0$ Hz; H11); 3.54 (m; 2 H; H8); 7.41–7.37 (m; 2 H; H3′, H5′); 8.22–8.19 (m; 2 H; H2′, H6′); 8.37 (s; 1 H; H6); 8.48 (s; 1 H; H3); 8.72 (s; 1 H; NH). ^{13}C NMR (100 MHz, DMSO-d_6, TMS, δ in p.p.m.): 26.2 (C9); 29.8 (C10); 39.7 (C8); 40.9 (C11); 101.5 (C3a); 115.8 (d; $J = 22.6$ Hz; C3′, C5′); 122.4 (d; $J = 8.3$ Hz; C2′, C6′); 133.8 (C3); 135.2 (d; $J = 2.3$ Hz; C1′); 152.5 (C7a); 156.4 (C6); 156.5 (C4); 159,8 (d; $J = 241,5$ Hz; C4′). ^{19}F NMR (376 MHz, DMSO-d_6, TMS, δ in p.p.m.): −116.29. HRMS (ESI) calc. for $C_{15}H_{17}FN_6$ 300.1499; found $[M + 1]^+$ 301.1583. HPLC: 95,4%.

N^1-(1-(4-chlorophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-yl)butane-1,4-diamine (13b)

Yield: 35%. MP: 138–139°C. IR (cm^{-1}): 3235–3102; 2942–2861; 1589–1499; 825. ^{1}H NMR (400 MHz, DMSO-d_6, TMS, δ in p.p.m.): 1.52–1.45 (m; 2 H; H10); 1.66–1.64 (m; 2 H; H9); 2.64 (t; 2 H; $J = 6.8$ Hz; H11); 3.54 (t; 2 H; $J = 6.8$ Hz; H8); 7.59 (d; 2 H; $J = 6.8$ Hz; H3′, H5′); 8.26 (d; 2 H; $J = 6.8$ Hz; H2′, H6′); 8.37 (s; 1 H; H6); 8.41 (s; 1 H; H3). ^{13}C NMR (100 MHz, DMSO-d_6, TMS, δ in ppm): 26.1 (C9); 29.2 (C10); 39.7 (C8); 40.6 (C11); 101.7 (C3a); 121.7 (C2′, C6′); 129.0 (C3′, C5′); 129.9 (C4′); 134.0 (C3); 137.7 (C1′); 152.8 (C7a); 156.5 (C6); 157.3 (C4). HRMS (ESI) calc. for $C_{15}H_{17}ClN_6$ 316.1203; found $[M + 1]^+$ 317.1278. HPLC: 96,2%.

N^1-(1-(p-tolyl)-1H-pyrazolo[3,4-d]pyrimidin-4-yl) butane-1,4-diamine (13c)

Yield: 36%. MP: 114-116 °C. IR (cm^{-1}): 3342–3292; 2938–2870; 1599; 1312; 1162. ^{1}H NMR (400 MHz, DMSO-d_6, TMS, δ in p.p.m.): 1.48–1.48 (m; 2 H; H10); 1.68–1.60 (m; 2 H; H9); 2.36 (s; 3 H; CH_3); 2.64 (t; 2 H; $J = 6.9$ Hz; H11); 3.52 (t; 2 H; $J = 6.9$ Hz; H8); 7.34 (d; 2 H; $J = 8.2$ Hz; H3', H5'); 8.05 (d; 2 H; $J = 8.2$ Hz; H2', H6'); 8.34 (s; 1 H; H6); 8.36 (s; 1 H; H3). ^{13}C NMR (100 MHz, DMSO-d_6, TMS, δ in p.p.m.): 20.4 (CH_3); 26.2 (C9); 30.5 (C10); 39.8 (C8); 41.2 (C11); 101.5 (C3a); 120.4 (C2′, C6′); 129.3 (C3′, C5′); 133.2 (C3); 135.2 (C4′); 136.5 (C1′); 152.4 (C7a); 156.3 (C6); 156.4 (C4). HRMS (ESI) calc. for $C_{16}H_{20}N_6$ 296.1749; found $[M + 1]^+$ 297.1832. HPLC: 96,2%.

General procedure for preparing N-(4-((1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)amino)butyl) benzenesulfonamides (1–9)

The amines **(13a–c)** (0.001 mol) were treated with the appropriate sulfonyl chloride (0.0045 mol) in 10 mL of DMF as the solvent and TEA (1 eq.). The reaction mixture was maintained under stirring at 90 °C for 24 h. The progress of the reaction was monitored using TLC ($CHCl_3$/MeOH 9:1), and the reaction mixture was poured into ice-cold water (50 mL). The precipitate was filtered and dried. The residual crude product was purified via silica gel column chromatography using a gradient mixture of $CHCl_3$/MeOH. Compounds **1–9** were obtained as white solids in 25–79% yield.

4-fluoro-N-(4-((1-(4-fluorophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-yl)amino)butyl)benzenesulfonamide (1)

Yield: 38%. MP: 119 °C. IR (cm^{-1}): 3275–3217; 1510–1414; 1316; 1153; 1075. ^{1}H NMR (400 MHz, DMSO-d_6, TMS, δ in p.p.m.): 1.50–1.42 (m; 2 H; H10); 1.63–1.56 (m; 2 H; H9); 2.79 (t; 2 H; $J = 6.8$ Hz; H11); 3.47 (s; 2 H; H8); 7.42–7.37 (m; 4 H; H3′, H5′, H3′′, H5′′); 7.69 (s; 1 H; SO_2NH); 7.85–7.81 (m; 2 H, H2′′, H6′′); 8.23–8.18 (m; 2 H; H2′, H6′); 8.34 (s; 1 H; H6); 8.38 (s; 1 H; H3); 8.51 (s; 1 H; NH). ^{13}C NMR (100 MHz, DMSO-d_6, TMS, δ in p.p.m.): 25.8 (C9); 26.4 (C10); 39.3 (C8); 42.2 (C11); 101.5 (C3a); 115.8 (d; $J = 22.6$ Hz; C3′, C5′); 116.1 (d; $J = 22.5$ Hz; C3′′, C5′′); 122.4 (d; $J = 8.3$ Hz; C2′, C6′); 129.3 (d; $J = 9.4$ Hz; C2′′, C6′′); 133.6 (C3); 135.2 (d; $J = 2.7$ Hz; C1′); 136.8 (d; $J = 2.8$ Hz; C1′′); 152.5 (C7a); 156.4 (C4); 156.5 (C6); 159.8 (d; $J = 241.5$ Hz; C4′); 163.8 (d; $J = 249.0$; C4′′). ^{19}F NMR (376 MHz, DMSO-d_6, TMS, δ in p,p,m,): −107.34; −116.30. HRMS (ESI) calc. for

$C_{21}H_{20}F_2N_6O_2S$ 458.1337, found $[M + 1]^+$ 459.1420. HPLC: 89.4%.

4-chloro-N-(4-((1-(4-fluorophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-yl)amino)butyl)benzenesulfonamide (2)

Yield: 49%. MP: 174–175 °C. IR (cm^{-1}): 3360–3118; 1512–1475; 1318; 1220; 1092; 843. ^1H NMR (400 MHz, DMSO-d_6, TMS, δ in ppm): 1.50–1.43 (m; 2 H; H10); 1.61–1.56 (m; 2 H; H9); 2.83–2.78 (m; 2 H; H11); 3.50–3.45 (m; 2 H; H8); 7.39 (t; 2 H; $J = 8.8$ Hz; H3′, H5′); 7.64 (d; 2 H; $J = 8.5$ Hz; H3′′, H5′′) 7.73 (t; 1 H; $J = 5.6$ Hz SO_2NH); 7.78 (d; 2 H, $J = 8.5$ Hz; H2′′, H6′′); 8.22–8.19 (m; 2 H; H2′, H6′); 8.35 (s; 1 H; H6); 8.37 (s; 1 H; H3); 8.47 (s; 1 H; NH). ^{13}C NMR (100 MHz, DMSO-d_6, TMS, δ in ppm): 25.8 (C9); 26.4 (C10); 39.3 (C8); 42.1 (C11); 101.5 (C3a); 115.8 (d; $J = 22.6$ Hz; C3′, C5′); 122.4 (d; $J = 8.3$ Hz; C2′, C6′); 128.2 (C2′′, C6′′); 129.2 (C3′′, C5′′); 133.6 (C3); 135.2 (C1′); 137.0 (C4′′); 139.3 (C1′′); 152.4 (C7a); 156.2 (C4); 156.3 (C6); 159.8 (d; $J = 241.5$ Hz; C4′). ^{19}F NMR (376 MHz, DMSO-d_6, TMS, δ in p.p.m.): −116.23. HRMS (ESI) calc. for $C_{21}H_{20}ClFN_6O_2S$ 474.1041, found $[M + 1]^+$ 475.0600. HPLC: 89.0%.

N-(4-((1-(4-fluorophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-yl)amino)butyl)-4-methylbenzenesulfonamide (3)

Yield: 40%. MP: 105–107 °C. IR (cm^{-1}): 3370–3065; 1510–1427; 1318; 1216. ^1H NMR (400 MHz, DMSO-d_6, TMS, δ in p.p.m.): 1.49–1.42 (m; 2 H; H10); 1.62–1.55 (m; 2 H; H9); 2.75 (t; 2 H; $J = 6.8$ Hz; H11); 3.49–3.44 (m; 2 H; H8); 7.41–7.34 (m; 4 H; H3′, H5′, H3′′, H5′′); 7.54 (s; 1 H; SO_2NH); 7.65 (d; 2 H, $J = 8.5$ Hz; H2′′, H6′′); 8.22–8.19 (m; 2 H; H2′, H6′); 8.34 (s; 1 H; H6); 8.39 (s; 1 H; H3); 8.51 (s; 1 H; NH). ^{13}C NMR (100 MHz, DMSO-d_6, TMS, δ in p.p.m.): 25.8 (C9); 26.4 (C10); 39.3 (C8); 42.1 (C11); 101.5 (C3a); 115.8 (d; $J = 22.6$ Hz; C3′, C5′); 122.4 (d; $J = 8.3$ Hz; C2′, C6′); 126.3 (C2′′, C6′′); 129.4 (C3′′, C5′′); 133.6 (C3); 135.2 (C1′); 137.5 (C4′′); 142.3 (C1′′); 152.5 (C7a); 156.4 (C4); 156,5 (C6); 159,8 (d; $J = 241,5$ Hz; C4′) ^{19}F NMR (376 MHz, DMSO-d_6, TMS, δ in p.p.m.): −116.31. HRMS (ESI) calc. for $C_{22}H_{23}FN_6O_2S$ 454.1587, found $[M + 1]^+$ 455.1664. HPLC: 95.2%.

N-(4-((1-(4-chlorophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-yl)amino)butyl)-4-fluorobenzenesulfonamide (4)

Yield: 45%. MP: 202 °C. IR (cm^{-1}): 3089–2952; 1599–1492; 1148; 1090; 826. ^1H NMR (400 MHz, DMSO-d_6, TMS, δ in p.p.m.): 1.53–1.47 (m; 2 H; H10); 1.65–1.60 (m; 2 H; H9); 2.82–2.77 (m; 2 H; H11); 3.55–3.51 (m; 2 H; H8); 7.40 (t; 2 H; $J = 8.5$ Hz; H3′′, H5′′); 7.44 (s; 1 H; $J = 5.6$ Hz; SO_2NH); 7.64 (d; 2 H; $J = 8.8$ Hz; H3', H5′); 7.86–7.83 (m; 2 H, H2′′, H6′′); 8.19 (d; 2 H; $J = 8.8$ Hz; H2′, H6′); 8.42 (s; 1 H; H6); 8.67 (s; 1 H; H3); 9.66 (s; 1 H; NH). ^{13}C NMR (100 MHz, DMSO-d_6, TMS, δ in p.p.m.): 25.4 (C9); 26.2 (C10); 40.4 (C8); 42.1 (C11); 101.6 (C3a); 116.1 (d; $J = 22.5$ Hz; C3′′, C5′′); 122.4 (C2′, C6′); 129.1 (C3′, C5′); 129.3 (d; $J = 9.4$ Hz; C2′′, C6′′); 130.7 (C4′); 135.2 (C3); 137.0 (C1′); 136.8 (d; $J = 2.8$ Hz; C1′′); 151.5 (C7a); 152.8 (C6); 153.4 (C4); 163.8 (d; $J = 249.0$; C4′′). ^{19}F NMR (376 MHz, DMSO-d_6, TMS, δ in p.p.m.): -107.24. HRMS (ESI) calc. for $C_{21}H_{20}ClFN_6O_2S$ 474.1041, found $[M + 1]^+$ 475.1112. HPLC: 97.1%.

4-chloro-N-(4-((1-(4-chlorophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-yl)amino)butyl)benzenesulfonamide (5)

Yield: 30%. MP: 233 °C. IR (cm^{-1}): 3223–3094; 1582–1498; 1327–1238; 1090; 825. ^1H NMR (400 MHz. DMSO-d_6. TMS. δ in p.p.m.): 1.48–1.44 (m; 2 H; H10); 1.61–1.57 (m; 2 H; H9); 2.82–2.77 (m; 2 H; H11); 3.49–3.45 (m; 2 H; H8); 7.64–7.60 (m; 4 H; H3′, H5′; H3", H5′′); 7.73 (t; 1 H; $J = 5.6$ Hz; SO_2NH); 7.77 (d; 2 H; $J = 8.5$ Hz; H2′′, H6′′); 8.27 (d; 2 H; $J = 8.8$ Hz; H2′, H6'); 8.36 (s; 1 H; H6); 8.41 (s; 1 H; H3); 8.50 (t; 1 H; $J = 5.2$ Hz; NH). ^{13}C NMR (100 MHz, DMSO-d_6, TMS, δ in p.p.m.): 25.8 (C9); 26.4 (C10); 39.3 (C8); 42.1 (C11); 101.7 (C3a); 121.7 (C2′, C6′); 128.2 (C2′′, C6′′); 129.0 (C3′, C5′), 129.1 (C3′′, C5′′); 129.9 (C4′); 134.0 (C3); 137.0 (C4′′); 137.7 (C1′); 139.3 (C1′′); 152.8 (C7a); 156.4 (C4); 156.5 (C6). HRMS (ESI) calc. for $C_{21}H_{20}Cl_2N_6O_2S$ 490.0746, found $[M + 1]^+$ 491.0833. HPLC: 93.5%.

N-(4-((1-(4-chlorophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-yl)amino)butyl)-4-methylbenzenesulfonamide (6)

Yield: 33%. MP: 190 °C. IR (cm^{-1}): 3383–3263; 1563–1499; 1374; 1305–1290; 1089; 833. ^1H NMR (400 MHz, DMSO-d_6, TMS, δ in ppm): 1.49–1.42 (m; 2 H; H10); 1.61–1.55 (m; 2 H; H9); 2.75 (t; 2 H; $J = 6.8$ Hz; H11); 3.49–3.44 (m; 2 H; H8); 7.35 (d; 2 H; $J = 8.5$ Hz; H3′′, H5′′); 7.55 (s; 1 H; SO_2NH); 7.61 (d; 2 H; $J = 8.8$ Hz; H3′, H5′); 7.65 (d; 2 H, $J = 8.5$ Hz; H2′′, H6′′); 8.27 (d; 2 H; $J = 8.8$ Hz; H2′, H6′); 8.36 (s; 1 H; H6); 8.42 (s; 1 H; H3); 8.54 (t; 1 H; $J = 5.2$ Hz; NH). ^{13}C NMR (100 MHz, DMSO-d_6, TMS, δ in p.p.m.): 20.7 (CH_3); 25.8 (C9); 26.4 (C10); 39.3 (C8); 42.1 (C11); 101.7 (C3a); 121.7 (C2′′, C6′); 126.3 (C2′′, C6′′); 129.0 (C3′, C5′); 129.4 (C3′′, C5′

′); 129.9 (C4′); 134.0 (C3); 137.5 (C1′); 137.7 (C4′′); 142.3 (C1′′); 152.8 (C7a); 156.4 (C4); 156.5 (C6). HRMS (ESI) calc. for $C_{22}H_{23}ClN_6O_2S$ 470.1292, found $[M + 1]^+$ 471.1375. HPLC: 89.1%.

4-fluoro-N-(4-((1-(p-tolyl)-1H-pyrazolo[3,4-d] pyrimidin-4-yl)amino)butyl)benzenesulfonamide (7)

Yield: 71%. MP: 211–212 °C. IR (cm^{-1}): 3295–3277; 1511–1492; 1434; 1321–1289; 1220; 1074. ^{1}H NMR (400 MHz, DMSO-d_6, TMS, δ in p.p.m.): 1.53–1.45 (m; 2 H; H10); 1.67–1.60 (m; 2 H; H9); 2.38 (s; 3 H; CH_3); 2.80 (q; 2 H; $J = 6.7$ Hz; H11); 3.49 (q; 2 H; $J = 6.7$ Hz; H8); 7.44–7.38 (m; 4 H; H3′–H5′, H3′′–H5′′); 7.69 (t; 1 H; $J = 5.8$ Hz; SO_2NH); 7.87–7.83 (m; 2 H; H2′′–H6′′); 7.94 (d; 2 H; $J = 8.4$ Hz; H2′–H6′); 8.47 (s; 1 H; H6); 8.52 (s; 1 H; H3). ^{13}C NMR (100 MHz, DMSO-d_6, TMS, δ in ppm): 20.4 (CH_3); 25.2 (C9); 26.3 (C10); 40.7 (C8); 42.0 (C11); 101.2 (C3a); 116.2 (d; $J = 22.5$ Hz; C3′′, C5′′); 121.3 (C2′, C6′); 129.3 (d; $J = 9.3$ Hz; C2′′, C6′′); 129.6 (C3′, C5′); 134.5 (C3); 135.5 (C4′); 136.6 (C1′); 136.8 (d; $J = 2.9$ Hz; C1′′); 150.5 (C7a); 151.0 (C6); 152.5 (C4); 163.9 (d; $J = 249.1$ Hz; C4′′) ^{19}F NMR (376 MHz, DMSO-d_6, TMS, δ in p.p.m.): −107.15. HRMS (ESI) calc. for $C_{22}H_{23}FN_6O_2S$ 454.1587, found $[M + 1]^+$ 455.1676. HPLC: 99.6%.

4-chloro-*N*-(4-((1-(*p*-tolyl)-1*H*-pyrazolo[3,4-*d*] pyrimidin-4-yl)amino)butyl)benzenesulfonamide (8)

Yield: 79%. MP: 231–233 °C. IR (cm^{-1}): 3295–3276; 1587–1491; 1320–1215; 1086; 814. ^{1}H NMR (400 MHz, DMSO-d_6, TMS, δ in ppm): 1.53–1.45 (m; 2 H; H10); 1.67–1.60 (m; 2 H; H9); 2.81 (q; 2 H; $J = 6.7$ Hz; H11); 3.49 (q; 2 H; $J = 6.7$ Hz; H8); 7.61 (d; 2 H; $J = 9.0$ Hz; H3′′–H5′′); 7.65 (d; 2 H; $J = 8.4$ Hz; H3′–H5); 7.75 (t; 1 H; $J = 5.8$ Hz; SO_2NH); 7.81 (d; 2 H; $J = 9.0$ Hz; H2′′–H6′′); 7.94 (d; 2 H; $J = 8.4$ Hz; H2′–H6′); 8.46 (s; 1 H; H6); 8.51 (s; 1 H; H3). ^{13}C NMR (100 MHz, DMSO-d_6, TMS, δ in p.p.m.): 20.4 (CH_3); 25.2 (C9); 26.3 (C10); 40.6 (C8); 42.0 (C11); 101.2 (C3a); 121.3 (C2′, C6′); 128.2 (C2′′, C6′′); 129.2 (C3′, C5′); 129.5 (C3′′, C5′′); 134.4 (C3); 135.5 (C4′); 136.5 (C1′); 137.0 (C4′′); 139.2 (C1′′), 150.6 (C7a); 151.2 (C6); 152.6 (C4). HRMS (ESI) calc. for $C_{22}H_{23}ClN_6O_2S$ 470.1292, found $[M + 1]^+$ 471.1375. HPLC: 99.6%.

4-methyl-*N*-(4-((1-(*p*-tolyl)-1*H*-pyrazolo[3,4-*d*] pyrimidin-4-yl)amino)butyl)*benzenesulfonamide* (9)

Yield: 25%. MP: 213–215 °C. IR (cm^{-1}): 3371–3274; 1034; 1560–1512; 1305–1213; 1437; 1356. ^{1}H NMR (400 MHz, DMSO-d_6, TMS, δ in p.p.m.): 1.49–1.41 (m; 2 H; H10); 1.62–1.55 (m; 2 H; H9); 2.34 (s; 3 H; CH_3′′); 2.36 (s; 3 H; CH_3’); 2.75 (t; 2 H; $J = 6.8$ Hz; H11); 3.48–3.44 (m; 2 H; H8); 7.35–7.33 (m; 4 H; H3′, H5′, H3′′, H5′′); 7.53 (s; 1 H; SO_2NH); 7.64 (d; 2 H, $J = 8.5$ Hz; H2′′, H6′′); 8.05 (d; 2 H; $J = 8.8$ Hz; H2′, H6′); 8.33 (s; 1 H; H6); 8.35 (s; 1 H; H3); 8.41 (t; 1 H; $J = 5.2$ Hz; NH). ^{13}C NMR (100 MHz, DMSO-d_6, TMS, δ in p.p.m.): 20.4 (CH_3′); 20.7 (CH_3′′); 25.9 (C9); 26.4 (C10); 39.3 (C8); 42.1 (C11); 101.5 (C3a); 120.4 (C2′, C6’′; 126.3 (C2′′, C6′′); 129.0 (C3′, C5′); 129.4 (C3′′, C5′′); 135.2 (C4′); 133.2 (C3); 137.5 (C4′′); 142.3 (C1′′); 136.5 (C1′); 152.4 (C7a); 156.3 (C6); 156.4 (C4). HRMS (ESI) calc. for $C_{23}H_{26}N_6O_2S$ 450.1838, found $[M + 1]^+$ 451.1928. HPLC: 94.3%.

Biological evaluation

Continuous cultures of *P. falciparum*-infected erythrocytes and *in vitro* assays

The cultivation of the *P. falciparum* W2 strain (chloroquine resistant) was performed according to the protocol of Trager & Jensen using human erythrocytes (A^+) in RPMI 1640 medium supplemented with human serum (Trager and Jensen 1976). Parasites were synchronized with sorbitol to obtain the young (ring) parasite forms (Lambros and Vanderberg 1979). Parasitemia was adjusted to 0.05%; and hematocrit, to 1.5%. Subsequently, cultures were placed in microplates together with the drugs to be tested in serial dilutions (50 – 0.78 µM) along with positive (chloroquine and sulfadoxine) and negative (no drug) controls and were incubated for 72 h. The microplates were then subjected to three cycles of freezing and thawing, and parasite growth was measured through quantification of histidine-rich protein II (HRPII) using specific monoclonal antibodies in sandwich ELISA (Silva et al. 2016; Noedl et al. 2002). Absorbance values were read at 450 nm using a SpectraMax 190 spectrophotometer, and IC_{50} determinations were performed using dose–response curves with a nonlinear regression function (Origin software, OriginLab Corporation, Northampton, MA, USA).

Cell cultures and cytotoxicity tests

BGM cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) at a concentration of 5×10^3 cells per milliliter. Cells were added together with the test compounds in serial dilution. The microplates were incubated for 24 h at 37 °C. Subsequently, MTT salt at a concentration of 3 mg/mL was added after 3 h, the supernatant was removed, and the dye present on the bottom of the plate wells was dissolved in DMSO in a volume of 100 µL/well. The microplates were then read using a SpectraMax 190 spectrophotometer with a 570-nm

Table 1 *In vitro* growth inhibitory activity against *P. falciparum* parasites (W2 clone) resistant to chloroquine, cytotoxicity tests in the BGM normal monkey kidney cell line and the selectivity index (SI) values of compounds **1–9** and **13a–c**, chloroquine and sulfadoxine

Compounds		Anti-*P. falciparum* activity IC_{50} (μM)	Cytotoxicity to BGM cells MLD_{50} (μM)	SI
1	$R_1 = F$ / $R_2 = F$	28.20 ± 9.20	260	9.21
2	$R_1 = F$ / $R_2 = Cl$	21.15 ± 0.45	928	43.87
3	$R_1 = F$ / $R_2 = CH_3$	5.13 ± 1.86	323	62.90
4	$R_1 = Cl$ / $R_2 = F$	43.40 ± 3.80	551	12.69
5	$R_1 = Cl$ / $R_2 = Cl$	a	ND	ND
6	$R_1 = Cl$ / $R_2 = CH_3$	a	ND	ND
7	$R_1 = CH_3$ / $R_2 = F$	42.12 ± 0.32	>1000	>23.80
8	$R_1 = CH_3$ / $R_2 = Cl$	a	ND	ND
9	$R_1 = CH_3$ / $R_2 = CH_3$	12.22 ± 0.02	676	55.31
13a	$R_1 = F$	21.63 ± 9.43	>1000	>46.23
13b	$R_1 = Cl$	13.15 ± 0.25	>1000	>76.04
13c	$R_1 = CH_3$	18.19 ± 2.99	>1000	>54.96
chloroquine	0.55	385.00	700.00	
sulfadoxine	15.00	310.00	20.70	

IC_{50}inhibitory concentration for 50% of parasite growth, evaluated in three different experiments for each test (±standard deviation (SD)). Inactive Minimal lethal dose for 50% of cells (MLD_{50}), used to calculate the selectivity index (SI) [MLD_{50} / IC_{50}]

ND not determine

[a]$IC_{50} > 50$ μM

filter (Denizot and Lang 1986). MLD_{50} determinations were performed using dose–response curves with a nonlinear regression function (Origin software, OriginLab Corporation, Northampton, MA, USA). Selectivity index (SI) was defined as the ratio of the MLD_{50} to the IC_{50} values of the active compounds (Denizot and Lang 1986).

Results and discussion

The nine derivatives of 1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (**1–9**) that were synthesized with different substituents at the 4-position of the phenyl group and the benzenesulfonamide moiety were tested for their efficacy against the W2 chloroquine- and sulfadoxine-resistant *P. falciparum* clone and for cytotoxicity (Table 1).

The values are summarized in Table 1. Sulfonamides **1–4**, **7**, **9** and amines **13a–c** showed *in vitro* growth inhibitory activity against the W2 chloroquine-resistant *P. falciparum* clone with IC_{50} values ranging from 5.13 to 43.40 μM in the anti-HPR2 assay and low toxicity to BGM cells.

Among the 1*H*-pyrazolo[3,4-*d*]pyrimidine derivatives **(1–9)**, the best performance was observed for compound **3** ($R_1 = F$ / $R_2 = CH_3$), which had an IC_{50} value of 5.13 μM. It is interesting to note that in the previous works by our group (Pinheiro et al. 2015; Silva et al. 2016), the compounds with $R = CH_3$ at the 4-position of the benzenesulfonamide moiety were among the more potent.

Compound **9** ($R_1 = CH_3$ / $R_2 = CH_3$) exhibited an IC_{50} value of 12.22 μM, which, along with the value of compound **3**, was below that of the control drug sulfadoxine ($IC_{50} = 15.00$ μM). The two compounds also showed better SI values of 62.90 and 55.31. In addition, based on the activity values, intermediate **13b** ($R_1 = Cl$), with an IC_{50} value of 13.15 μM and the highest SI value of 76.04, exhibited greater potency than sulfonamides **1**, **2**, **4** and **7**. The 1*H*-pyrazolo[3,4-*d*]pyrimidine derivatives were not more active than the control drug chloroquine ($IC_{50} = 0.55$ μM) or the quinoline prototype **I** ($IC_{50} = 0.09$ μM).

However, the 1*H*-pyrazolo[3,4-*d*]pyrimidine system is promising as a prototype for further studies of antimalarials, as all compounds of the series except derivative **4** had IS values (23.80 - 76.04) higher than sulfadoxine (SI = 20.70).

Conclusions

Among the 1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidines **(1–9)** synthesized with different substituents at the 4-position of the phenyl group and benzenesulfonamide moiety, six compounds exhibited anti-*P. falciparum* activity *in vitro* against chloroquine-resistant parasites, and none of those were toxic to BGM cells. The quinoline prototype **I** is still more potent than the 1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine derivatives. However, this study shows that three compounds, **3**, **9**, and **13b**, presented IC_{50} values of 5.13, 12.22 and 13.15 μM, respectively, which were lower than that of the control drug sulfadoxine ($IC_{50} = 15.00$ μM) in the anti-HRPII assay. In addition, the compounds in this series (except derivative **4**) have higher SI values than

sulfadoxine. It is possible to conclude that the 1*H*-pyrazolo [3,4-*d*]pyrimidine system is promising as a prototype for further studies of antimalarial candidates with the objective of overcoming the burden of resistance in *P. falciparum*.

Acknowledgements We thank the Coordination of Improvement of Higher Education (CAPES) and the National Council of R&D of Brazil (CNPq) for the fellowships granted to the authors. We also thank the Foundation for Research of the State of Rio de Janeiro (FAPERJ), Technological Development Program on Products for Health (PDTIS), for financial support. LJMC, LCSP and NB are recipients of research productivity fellowships from the CNPq and from FAPERJ ("*Cientista do Nosso Estado*").

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Anthony MP, Burrows JN, Duparc S, JMoehrle J, Wells TNC (2012) The global pipeline of new medicines for the control and elimination of malaria. Malar J 11:316

Azeredo LFSP, Coutinho JP, Jabor VAP, Feliciano PR, Nonato MC, Kaiser CR, Menezes CMS, Hammes ASO, Caffarena ER, Hoelz LVB, Souza NB, Pereira GAN, Ceravolo IP, Krettli AU, Boechat N (2017) Evaluation of 7-arylaminopyrazolo[1,5-a]pyrimidines as anti-*Plasmodium falciparu*m, antimalarial, and *P*f-dihydroorotate dehydrogenase inhibitors. Eur J Med Chem 126:72–83

Barnett DS, Guy RK (2014) Antimalarials in development in 2014. Chem Rev 114:11221–11241

Bernardino AMR, Pinheiro LCS, Rodrigues CR, Loureiro NI, Castro HC, Lanfredi-Rangel A, Sabatini-Lopes J, Borges JC, Carvalho JM, Romeiro GA, Ferreira VF, Frugulhettic ICPP, Vannier-Santos MA (2006) Design, synthesis, SAR, and biological evaluation of new 4-(phenylamino)thieno[2,3-*b*]pyridine derivatives. Bioorg Med Chem 14:5765–5770

Biamonte MA, Wanner J, Le Roch KG (2013) Recent advances in malaria drug discovery. Bioorg Med Chem Lett 23:2829–2843

Boechat N, Ferreira MLG, Pinheiro LCS, Jesus AML, Leite MMM, Aguiar ACC, Andrade IM, Krettli AU (2014) New compounds hybrids 1*H*-1,2,3-triazole-quinoline against *Plasmodium falciparum*. Chem Biol Drug Des 84:325–332

Boechat N, Pinheiro LCS, Silva TS, Aguiar ACC, Carvalho AS, Bastos MM, Costa CCP, Pinheiro S, Pinto AC, Mendonça JS, Dutra KDB, Valverde AL, Santos-Filho OA, Ceravolo IP, Krettli AU (2012) New trifluoromethyl triazolopyrimidines as anti-*Plasmodium falciparum* agents. Molecules 17:8285–8302

Carvalho RCC, Martins WA, Silva TP, Kaiser CR, Bastos MM, Pinheiro LCS, Krettli AU, Boechat N (2016) New pentasubstituted pyrrole hybrid atorvastatin–quinoline derivatives with antiplasmodial activity. Bioorg Med Chem Lett 26:1881–1884

Denizot F, Lang R (1986) Rapid colorimetric assay for cell growth and survival. Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. J Immunol Methods 89:271–277

Kaur K, Jain M, Reddy RP, Jain R (2010) Quinolines and structurally related heterocycles as antimalarials. Eur J Med Chem 45:3245–3264

Lambros C, Vanderberg JP (1979) Synchronization of *Plasmodium falciparum* erythrocytic stages in culture. J Parasitol 65:418–420

Mushtaque M, Shahjahan (2015) Reemergence of chloroquine (CQ) analogs as multi-targeting antimalarial agents: A review. Eur J Med Chem 90:280–295

Noedl H, Wongsrichanalai C, Miller RS, Myint KSA, Looareesuwan S, Sukthana Y, Wongchotigul V, Kollaritsch H, Wiedermann G, Wernsdorfer WH (2002) *Plasmodium falciparum*: effect of antimalarial drugs on the production and secretion characteristics of histidine-rich protein II. Exp Parasitol 102:157–163

Pinheiro LCS, Boechat N, Ferreira MLG, Junior CCS, Jesus AML, Leite MMM, Souza N, Krettli AU (2015) Anti-*Plasmodium falciparum* activity of quinoline–sulfonamide hybrids. Bioorg Med Chem 23:5979–5984

Santos MS, Bernardino AMR, Pinheiro LCS, Canto-Cavalheiro MM, Leon LL (2012) An efficient synthesis of new 5-(1-Aryl-1*H*-pyrazole-4-yl)-1*H*-tetrazoles from 1-Aryl-1*H*-pyrazole-4-carbonitriles via [3 + 2]Cycloaddition reaction. J Heterocycl Chem 49:1425–1428

Schrader FC, Barho M, Steiner I, Ortmann R, Schlitzer M (2012) The antimalarial pipeline-an update. Int J Med Microbiol 302:165–171

Silva TB, Bernardino AMR, Ferreira MLG, Rogerio KR, Carvalho LJM, Boechat N, Pinheiro LCS (2016) Design, synthesis and anti-*P. falciparum* activity of pyrazolopyridine–sulfonamide derivatives. Bioorg Med Chem 24:4492–4498

Soliman AM, Sultan AA, El Remaily MAA, Abdel-Ghany H (2012) Synthesis of some novel fused azole derivatives. Synth Comm 42:2748–2762

Teixeira C, Vale N, Pérez B, Gomes A, Gomes JRB, Gomes P (2014) "Recycling" classical drugs for malaria. Chem Rev 114:11164–11220

Tilley L, Straimer J, Gnädig NF, Ralph SA, Fidock DA (2016) Artemisinin action and resistance in *Plasmodium falciparum*. Trends Parasitol 32:682–696

Trager W, Jensen JB (1976) Human malaria parasites in continuous culture. Science 193:673–675

Vandekerckhove S, D'hooghe M (2015) Quinoline-based antimalarial hybrid compounds. Bioorg Med Chem 23:5098–5119

Varotti FP, Botelho ACC, Andrade AA, Paula RC, Fagundes EMS, Valverde A, Mayer LMU, Mendonca JS, Souza MVN, Boechat N, Krettli AU (2008) Synthesis, antimalarial activity, and intracellular targets of MEFAS, a new hybrid compound derived from mefloquine and artesunate. Antimicrob Agents Chemother 52:3868–3874

Wells TN, Hooft van Huijsduijnen R, Van Voorhis WC (2015) Malaria medicines: a glass half full? Nat Rev Drug Discov 14:424–442

White NJ (2004) Antimalarial drug resistance. J Clin Invest 113:1084–1092

World Health Organization (WHO) (2015) Guidelines for the Treatment Of Malaria 3rd ed, 2015. http://www.who.int/malaria/publications/atoz/9789241549127/en/ Accessed 14 Jan 2018

World Health Organization (WHO) (2017) World Malaria Report 2017. http://www.who.int/malaria/publications/world_malaria_report/en/ Accessed 14 Jan 2018