Supporting Information

Development of Chemical Entities Endowed with Potent Fast-Killing Properties against *Plasmodium Falciparum* Malaria Parasites

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Scheme S1……..…...S2

Experimental Part…….…….S3

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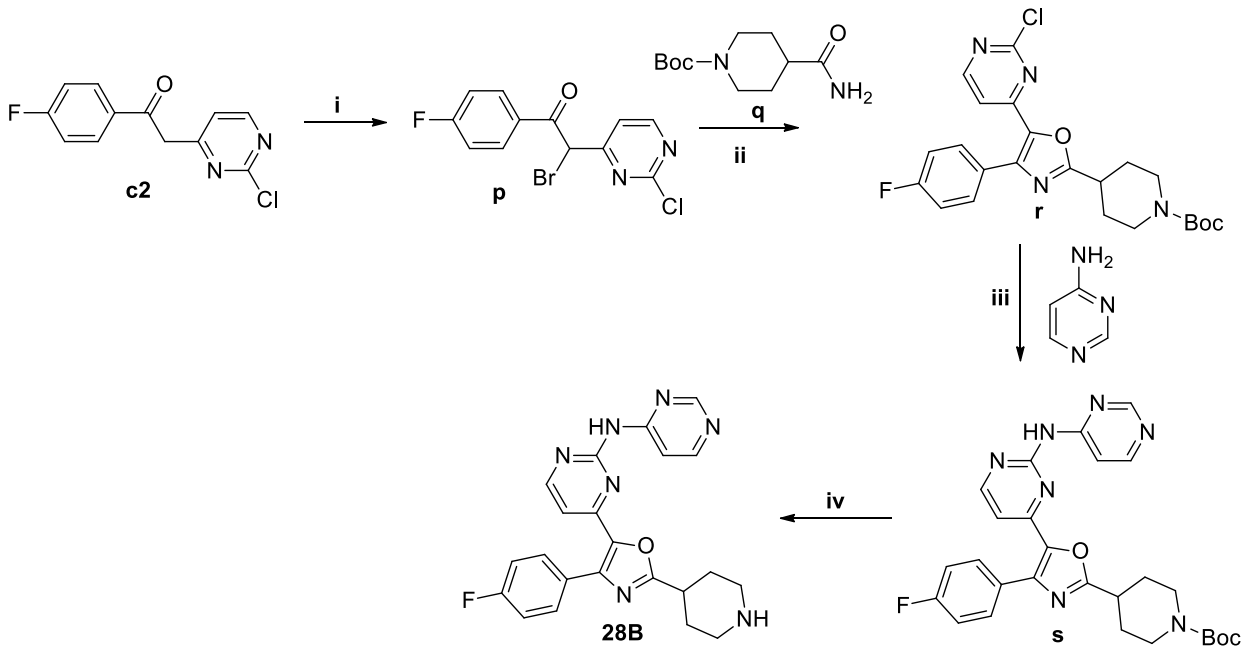
Scheme S1. Synthetic procedure followed for the preparation of the oxazole derivative 28B.



Conditions: i) NBS, CH_2Cl_2, rt, ii) neat, 160 ^oC, iii) $Pd_2(dba)_3$, XantPhos, *t*-BuOK, toluene, reflux, iv) 4M HCl/dioxane, dioxane, rt.

EXPERIMENTAL SECTION

Chemistry. *Materials and Methods.* All starting materials were purchased from commercial sources and used as received or synthesized via literature procedures. Solvents were dried using a commercial solvent purification system and stored under nitrogen. All final compounds were characterized by ^{1}H NMR spectroscopy and LCMS. ^{1}H NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer at 293 K. Purity was determined by HPLC (Acquity UPLC BEH C18 1.7 µ 2.1 mm × 50 mm) at 35 °C. All compounds tested present a purity >95%, except for a couple of derivatives that presented a purity of >90%. Method: acetate NH_4 25 mM + 10% ACN at pH 6.6/ACN, 0−0.2 min 100:0; 0.2−1.0 min 10:90; 1.0−1.8 min 10:90; 1.8−2.0 min 100:0. Flow: 0.8 mL/min. The UV detection wavelength was 254 and 210 nm. Positive ion mass spectra (high resolution mass spectroscopy) was acquired using a QSTAR Elite (AB Sciex Instruments) mass spectrometer, equipped with a turbospray source, over a mass range of 250−700, with a scan time of 1 s. The elemental composition was calculated using Analyst QS 2.0 software.

N-Methoxy-N-methyl 4-fluorobenzamide (compound b, Scheme 1). Triethylamine (1.27 g, 12.60 mmole) was added dropwise to a solution of *N,O*-dimethylhydroxylamine hydrochloride (0.61 g, 6.30 mmol) in CH_2Cl_2 (15 mL) at 0 °C. To this mixture was added slowly 4-fluorobenzoyl chloride (1 g, 6.30 mmol) at 0 °C for 30 min. The reaction mixture was stirred at room temperature for 1 h. Partitioning of the mixture between water and CH_2Cl_2, drying over $MgSO_4$, and the filtering and evaporating of the solvents under reduced pressure gave the desired product (1.09 g, 95.0%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.40 (s, 3H), 3.58 (s, 3H), 7.09 - 7.15 (m, 2H), 7.75 - 7.80 (m, 2H).

Synthesis of 1-(4-fluorophenyl)-2-(2-(methylthio)pyrimidin-4-yl)ethanone (c1) and 2-(2-chloropyrimidin-4-yl)-1-(4-fluorophenyl)ethanone (c2, Scheme 1). To a solution of LDA in hexanes (16.46 mL of 1M solution, 16.46 mmol), 4-methyl-2-(methylthio)pyrimidine (1.54 g, 10.97 mmol) or 2-chloro-4-methylpyrimidine (1.41g, 10.97 mmol) in dry THF (36 mL) was added dropwise at -78 °C and under Ar over 20 min with stirring maintained for further 45 min at the same temperature. A solution of 4-fluoro-*N*-methoxy-*N*-methylbenzamide (2.11 g, 11.58 mmol) in dry THF (10 mL) was added slowly to this mixture at -78 °C over 15 min. On completion of the addition, the resulting mixture was stirred at room temperature for 2 h and then sat. NH_4Cl (5 mL) and water (5 mL) were added followed by EtOAc (25 mL). The two phases were separated, the aqueous phase was washed with EtOAC (25 mL) and the combined organic phases were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was triturated with tert-butylmethyl ether (5 mL) in the ultrasound for 3 min, the solvent was carefully decanted and the residue was dried under vacuum to give the desired compound (quant. yield) as a yellow to red solid which was used in the next step without further purification. Both compounds are in equilibrium in solution with their respective enolates according to the ^{1}H NMR spectra. For **1-(4-fluorophenyl)-2-(2-(methylthio)pyrimidin-4-yl)ethanone (c1):** ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.41-2.62 (s, 3H), 3.00-3.58 (s, 2H), 7.08-7.22 (m, 2H), 7.75-7.98 (m, 2H), 7.99-8.15 (m, 1H), 8.31-8.51 (m, 1H), 14.67 (s, 0.4H). For **2-(2-chloropyrimidin-4-yl)-1-(4-fluorophenyl)ethanone (c2):** ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.38-3.56 (s, 2H), 7.10-7.87 (m, 4H), 8.05-8.21 (m, 1H), 8.39-8.48 (m, 1H), 14.57 (s, 0.4H).

Synthesis of 2-chloro-1-(4-fluorophenyl)-2-(2-(methylthio)pyrimidin-4-yl)ethanone (d1) and 2-chloro-2-(2-chloropyrimidin-4-yl)-1-(4-fluorophenyl)ethanone (d2, Scheme 1). A solution of 1-(4-fluorophenyl)-2-(2-(methylthio)pyrimidin-4-yl)ethanone (**c1**) (3.66 g, 13.96 mmol) or 2-(2-chloropyrimidin-4-yl)-1-(4-fluorophenyl)ethanone (3.5 g, 13.96 mmol) (**c2**, Scheme 2) in dry $CHCl_3$ (75 mL) was treated with a solution of sulfuryl chloride (1.19 mL, 14.66 mmol) in dry $CHCl_3$ (5 mL) dropwise over 10 min at 0 ^oC. After the completion of the addition, the mixture was stirred at room temperature for further 1.5 h. The reaction mixture was concentrated under high vacuum, the residue was washed with cyclohexane (5 mL), the organic phase was carefully decanted and the residue was dried to give the desired product as a brown oil (quant. yield) which was used in the next step without further purification. For **2-chloro-1-(4-fluorophenyl)-2-(2-(methylthio)pyrimidin-4-yl)ethanone (d1):** ^1H NMR (400 MHz, $CDCl_3$) δ 2.53 (s, 3H), 6.35 (s, 1H), 7.15 - 7.24 (m, 2H), 7.30 - 7.37 (m, 1H), 8.11 - 8.16 (m, 2H), 8.64 (d, *J*=5.05 Hz, 1H). For **2-chloro-2-(2-chloropyrimidin-4-yl)-1-(4-fluorophenyl)ethanone (d2):** ^1H NMR (400 MHz, $CDCl_3$) δ 7.22 (s, 1H), 7.29 - 7.49 (m, 2H), 7.82 - 7.94 (m, 1 H), 8.11 - 8.16 (m, 2H), 8.92 (d, *J*=5.05 Hz, 1H).

Synthesis of the intermediates of thiazole derivatives depicted in Table 1.

A) Synthesis of compounds 9 and 10 (Scheme 2).

4-(4-fluorophenyl)-5-[2-(methylthio)pyrimidin-4-yl]-1,3-thiazol-2-amine (g). Compound **d1** (12.40 g, 36 mmol) was dissolved in EtOH (30 mL), thiourea (2.77 g, 36 mmol) was added and the resulting mixture was stirred under reflux overnight for 18 h. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The resulting residue was stirred in saturated $NaHCO_3$ solution (70 mL) and the precipitated solid was filtered, washed with acetone to afford the desired compound as yellow hygroscopic solid. Yield = quant. ^1H NMR (400 MHz, DMSO-d_6) δ 8.20 (d, *J* = 5.51 Hz, 1H), 7.75 (s, 2H), 7.54 (dd, *J* = 5.64 Hz, *J* = 8.66 Hz, 2H), 7.28 (t, *J* = 8.88 Hz, 2H), 6.54 (d, *J* = 5.51 Hz, 1H), 2.43 (s, 3H). MS: m/e 319 (MH^+).

4-[2-chloro-4-(4-fluorophenyl)-1,3-thiazol-5-yl]-2-(methylthio)pyrimidine (h). To a stirred solution of $CuCl_2$ (2.53 g, 18.8 mmol) and *tert*-butyl nitrite (2.61 mL, 22.0 mmol) in anhydrous acetonitrile (120 mL) was added compound **g** (5.0 g, 15.7 mmol) in portions. The reaction mixture was stirred at room temperature for 4 h under N_2 atmosphere. The reaction mixture was diluted with EtOAc, washed with water and brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude material was purified on silica gel cartridge (0-100% EtOAc in cyclohexane) to give the desired compound as a pale yellow solid. Yield = 43%. ^1H NMR (400 MHz, DMSO-d_6) δ 8.51 (d, *J* = 5.30 Hz, 1H), 7.64 (dd, *J* = 5.47 Hz, *J* = 8.84 Hz, 2H), 7.34 (t, *J* = 8.90 Hz, 2H), 6.82 (d, *J* = 5.30 Hz, 1H), 2.49 (s, 3H). MS: m/e 338, 340 (MH^+).

Synthesis of compounds of formula i. First step: To a stirred solution of compound **h** (0.44 mmol) in anhydrous THF (3 mL) at 0 °C was added a solution of morpholine or pyrrolidine (0.89 mmol) in anhydrous THF (1 mL). The reaction mixture was allowed to warm to room temperature and stirred at room temperature for 72 h under N_2 atmosphere. The reaction mixture was added to saturated $NaHCO_3$, extracted with EtOAc, dried over $MgSO_4$, filtered and concentrated under reduced pressure to give the desired compounds which were used without further purification. **4-{4-(4-fluorophenyl)-5-[2-(methylthio)pyrimidin-4-yl]-1,3-thiazol-2-yl}morpholine.** Yellow

solid. Yield = 96%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (d, J = 5.48 Hz, 1H), 7.56 (dd, J = 5.40 Hz, J = 8.80 Hz, 2H), 7.16 (t, J = 8.72 Hz, 2H), 6.58 (d, J = 5.48 Hz, 1H), 3.86 (t, J = 4.94 Hz, 4H), 3.63 (t, J = 4.92 Hz, 4H), 2.57 (s, 3H). MS: m/e 389 (MH^+). **4-[4-(4-fluorophenyl)-2-(pyrrolidin-1-yl)-1,3-thiazol-5-yl]-2-(methylthio)pyrimidine.** Yellow solid. Yield = quant. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, J = 5.52 Hz, 1H), 7.55 (dd, J = 5.44 Hz, J = 8.80 Hz, 2H), 7.16 (t, J = 8.76 Hz, 2H), 6.48 (d, J = 5.52 Hz, 1H), 3.59 (t, J = 6.48 Hz, 4H), 2.58 (s, 3H), 2.13-2.09 (m, 4H). MS: m/e 373 (MH^+). Second step: The previously-mentioned morpholine or pyrrolidine derivatives (0.42 mmol) in anhydrous CH_2Cl_2 (15 mL) were cooled to 0 °C. 3-chloroperbenzoic acid (220 mg, 1.27 mmol) in anhydrous CH_2Cl_2 (15 mL) was added dropwise over 15 min. The reaction mixture was stirred at 0 °C for 2 h and then allowed to warm to room temperature and stirred at room temperature for 48 h. The reaction mixture was diluted with saturated $NaHCO_3$ solution and extracted using CH_2Cl_2 (3 x 20 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure to affording the desired compounds which were used without further purification. **4-{4-(4-fluorophenyl)-5-[2-(methylsulfonyl)pyrimidin-4-yl]-1,3-thiazol-2-yl}morpholine.** Yellow solid. Yield = 90%. MS: m/e 421 (MH^+). **4-[4-(4-fluorophenyl)-2-(pyrrolidin-1-yl)-1,3-thiazol-5-yl]-2-(methylsulfonyl)pyrimidine.** Yellow solid. Yield = quant. MS: m/e 405 (MH^+).

B) Synthesis of the intermediates of compounds 11-18 (Scheme 2).

5-(2-chloropyrimidin-4-yl)-4-(4-fluorophenyl)-1,3-thiazol-2-amine (j). Intermediate **d2** (5.52 g, 17 mmol) was dissolved in EtOH (35 mL), thiourea (1.28 g, 17 mmol) was added and the mixture was stirred under reflux for 5 h. The mixture was cooled to room temperature and concentrated under reduced pressure. Saturated $NaHCO_3$ solution (40 mL) was added. The precipitated solid was filtered and washed with acetone to afford a yellow solid (3.0 g). The filtrate was concentrated under reduced pressure and purified on silica gel cartridge (0-100% EtOAc in cyclohexane) to give a yellow solid (1.60 g). Both solids were combined to furnish the desired compound as a yellow solid. Yield = 90%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.28 (d, J = 5.56 Hz, 1H), 7.93 (s, 2H), 7.56 (dd, J = 5.56 Hz, J = 8.84 Hz, 2H), 7.29 (t, J = 8.92 Hz, 2H), 6.79 (d, J = 5.56 Hz, 1H). MS: m/e 307, 309 (MH^+).

Synthesis of the intermediates of thiazole derivatives depicted in Table 2 (Scheme 1).

Synthesis of ethyl 4-(4-(4-fluorophenyl)-5-(2-(methylthio)pyrimidin-4-yl)thiazol-2-yl)piperidine-1-carboxylate (e1) and ethyl 4-(5-(2-chloropyrimidin-4-yl)-4-(4-fluorophenyl)thiazol-2-yl)piperidine-1-carboxylate (e2). A mixture of *2-chloro-1-(4-fluorophenyl)-2-(2-(methylthio)pyrimidin-4-yl)ethanone* **d1** (3.92 g, 13.22 mmol) or 2-chloro-2-(2-chloropyrimidin-4-yl)-1-(4-fluorophenyl)ethanone **d2** (3.77 g, 13.22 mmol) (Scheme 1) and ethyl 4-carbamothioylpiperidine-1-carboxylate (3.55 g, 16.53 mmol) in abs. EtOH (100 mL) is refluxed for 3 h. Solvent is distilled off and the residue is purified on silica gel cartridge eluted with cyclohexane:EtOAc (5:1) to afford the desired compounds (yields of 47 and 34%, respectively) as sticky yellow solids. For **ethyl 4-(4-(4-fluorophenyl)-5-(2-(methylthio)pyrimidin-4-yl)thiazol-2-yl)piperidine-1-carboxylate (e1)**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.32 (t, J=7.07 Hz, 3H), 1.85 (qd, J=12.38, 4.29 Hz, 2H), 2.20 (br d, J=11.12 Hz, 2H), 2.59 (s, 3H), 2.98 (br t, J=11.49 Hz, 2H), 3.19 - 3.28 (m, 1H), 4.20 (q, J=7.07 Hz, 2H), 4.25 - 4.43

(m, 2H), 6.77 (d, *J*=5.31 Hz, 1H), 7.14 - 7.20 (m, 2H), 7.54 - 7.60 (m, 2H), 8.31 (d, *J*=5.30 Hz, 1 H). **For ethyl 4-(5-(2-chloropyrimidin-4-yl)-4-(4-fluorophenyl)thiazol-2-yl)piperidine-1-carboxylate (e2)**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.06 - 1.22 (m, 4H), 1.58 - 1.70 (m, 2H), 2.06 - 2.15 (m, 2H), 2.99 (br s, 2H), 3.99 - 4.11 (m, 4H), 7.15 (d, *J*=5.31 Hz, 1H), 7.28 - 7.35 (m, 2H), 7.61 - 7.67 (m, 2H), 8.59 (d, *J*=5.30 Hz, 1H).

Synthesis of ethyl 4-(4-(4-fluorophenyl)-5-(2-(methylsulfonyl)pyrimidin-4-yl)thiazol-2-yl)piperidine-1-carboxylate (f). To a suspension of *ethyl 4-(4-(4-fluorophenyl)-5-(2-(methylthio)pyrimidin-4-yl)thiazol-2-yl)piperidine-1-carboxylate* (0.87 g, 1.90 mmol) in a 2:1 mixture of MeOH:H_2O (39 mL) was added OXONE (1.83 g, 5.94 mmol) in portions and the resulting suspension was stirred at room temperature for 48 h. On completion of the reaction, the mixture was concentrated under reduced pressure, the residue was dissolved in EtOAc (60 mL) and washed with water (2 x 20 mL). The organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure to produce the desired compound (96% yield) as a yellow oil that was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.27 - 1.32 (m, 3H), 1.79 - 1.92 (m, 2H), 2.15 - 2.27 (m, 2H), 3.26 (tt, *J*=11.62, 3.79 Hz, 1H), 3.38 (s, 3 H), 4.18 (dq, *J*=15.88, 7.08 Hz, 3H), 4.24 - 4.42 (m, 2H), 7.14 - 7.27 (m, 2H), 7.28 (d, *J*=5.31 Hz, 1H), 7.55 - 7.60 (m, 2H), 8.67 (d, *J*=5.56 Hz, 1H).

General Procedure for the synthesis of *N*-ethyl carbamates of final compounds 19-22 (61-64) and 25-27 (67-69). To a suspension of NaH (60% dispersion in mineral oil, 12.24 mg, 0.31 mmol) in anhydrous THF (0.5 mL), a solution of 1,3,4-thiadiazol-2-amine or 5-methyl-1,3,4-thiadiazol-2-amine or 4-methyl-1,3-thiazole-2-amine or 5-amino-3-methylisoxazole or 2-aminopyridine or aminopyrazine or 3-aminopyridazine (0.11 mmol) in anhydrous THF (1 mL) was added dropwise at room temperature and under N_2. Then, the mixture was stirred at room temperature for 35 min followed by the addition of a solution of ethyl 4-(4-(4-fluorophenyl)-5-(2-(methylsulfonyl)pyrimidin-4-yl)thiazol-2-yl)piperidine-1-carboxylate (0.050 g, 0.10 mmol) in anhydrous THF (1.5 mL). When the addition was finished, the mixture was stirred at room temperature and under N_2 overnight. The reaction was quenched with water (1 mL), and the mixture was extracted with CH_2Cl_2 (3 x 10 mL). The combined organic phase was washed with brine (10 mL), dried over Na_2SO_4, filtered and concentrated. The residues were purified on silica gel cartridge eluted with different ratios of cyclohexane:EtOAc to furnish the desired compounds which were subsequently subjected to deprotection of the ethyl carbamate group.

Ethyl 4-(5-(2-((1,3,4-thiadiazol-2-yl)amino)pyrimidin-4-yl)-4-(4-fluorophenyl)thiazol-2-yl)piperidine-1-carboxylate (61). Yellow solid. Yield = 73%.^{1}H NMR (400 MHz, DMSO-d_6) δ 1.10 - 1.27 (m, 3H), 1.15 - 1.24 (m, 1H), 1.57 - 1.73 (m, 2H), 2.03 - 2.17 (m, 2H), 2.85 - 3.06 (m, 2H), 4.01 - 4.12 (m, 4H), 6.72 (d, *J*=5.31 Hz, 1H), 7.19 - 7.44 (m, 2H), 7.53 - 7.76 (m, 2H), 8.50 (d, *J*=5.30 Hz, 1H), 9.12 (s, 1H), 12.01 - 12.39 (m, 1H). MS: m/e 512 (MH^+).

Ethyl 4-(4-(4-fluorophenyl)-5-(2-((5-methyl-1,3,4-thiadiazol-2-yl)amino)pyrimidin-4-yl)thiazol-2-yl)piperidine-1-carboxylate (62). Yellow solid. Yield = 77%.^{1}H NMR (400 MHz, DMSO-d_6) δ 1.20 (t, *J*=7.07 Hz, 3H), 1.67 (td, *J*=12.06, 8.21 Hz, 2H), 2.13 (br d, *J*=10.86 Hz, 2H), 2.60 (s, 3H), 2.99 (br s, 2H), 4.00 - 4.13 (m, 5H), 6.74 (d, *J*=5.05 Hz, 1H), 7.26 - 7.33 (m, 2H), 7.58 - 7.65 (m, 2H), 8.49 (d, *J*=5.31 Hz, 1H), 12.02 (br s, 1 H). MS: m/e 526 (MH^+).

Ethyl 4-(4-(4-fluorophenyl)-5-(2-((4-methylthiazol-2-yl)amino)pyrimidin-4-yl)thiazol-2-yl)piperidine-1-carboxylate (63). Yellow solid. Yield = 80%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.20 (t, J=7.07 Hz, 3H), 1.52 - 1.59 (m, 2H), 1.62 - 1.73 (m, 2H), 2.09 - 2.15 (m, 2H), 2.23 - 2.30 (m, 3H), 2.97 - 3.04 (m, 1H), 4.03 - 4.12 (m, 4H), 6.61 - 6.66 (m, 1H), 6.70 - 6.75 (m, 1H), 7.07 - 7.12 (m, 1H), 7.27 - 7.35 (m, 2H), 7.60 - 7.66 (m, 2H), 8.42 - 8.48 (m, 1H). MS: m/e 525 (MH^+), 523 (MH^-).

Ethyl 4-(4-(4-fluorophenyl)-5-(2-((3-methylisoxazol-5-yl)amino)pyrimidin-4-yl)thiazol-2-yl)piperidine-1-carboxylate (64). Yellow solid. Yield = 75%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.17 - 1.22 (m, 4H), 1.61 - 1.71 (m, 2H), 2.08 - 2.14 (m, 2H), 2.14 - 2.19 (m, 3H), 2.93 - 3.05 (m, 2H), 4.02 - 4.08 (m, 4H), 6.75 (d, J=5.05 Hz, 1H), 7.12 - 7.38 (m, 3H), 7.59 - 7.64 (m, 2H), 8.47 (d, J=5.31 Hz, 1H), 11.22 - 11.39 (m, 1H). MS: m/e 509 (MH^+), 507 (MH^-).

Ethyl 4-(4-(4-fluorophenyl)-5-(2-(pyridin-2-ylamino)pyrimidin-4-yl)thiazol-2-yl)piperidine-1-carboxylate (67). Yellow solid. Yield = 76%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.17 - 1.23 (m, 3H), 1.23 - 1.25 (m, 1H), 1.57 - 1.74 (m, 2H), 2.08 - 2.17 (m, 2H), 2.89 - 3.10 (m, 2H), 3.96 - 4.17 (m, 4H), 6.66 (d, J=5.05 Hz, 1H), 7.00 (ddd, J=7.26, 4.86, 1.01 Hz, 1H), 7.23 - 7.35 (m, 2H), 7.54 - 7.66 (m, 2H), 7.67 - 7.76 (m, 1H), 8.03 (d, J=8.59 Hz, 1H), 8.22 - 8.32 (m, 1H), 8.38 - 8.47 (m, 1H), 9.84 (s, 1H). MS: m/e 505 (MH^+).

Ethyl 4-(4-(4-fluorophenyl)-5-(2-(pyrazin-2-ylamino)pyrimidin-4-yl)thiazol-2-yl)piperidine-1-carboxylate (68). Yellow solid. Yield = 78%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.16 - 1.22 (m, 4H), 1.47 - 1.72 (m, 2H), 1.72 - 1.86 (m, 1H), 1.98 - 2.18 (m, 2H), 3.54 - 3.66 (m, 1H), 3.99 - 4.13 (m, 4H), 6.69 (d, J=5.31 Hz, 1H), 7.24 - 7.36 (m, 2H), 7.56 - 7.69 (m, 2H), 8.24 (d, J=2.53 Hz, 1H), 8.35 (dd, J=2.65, 1.64 Hz, 1H), 8.45 (d, J=5.05 Hz, 1H), 9.37 (d, J=1.52 Hz, 1H), 10.38 (s, 1 H). MS: m/e 506 (MH^+).

Ethyl 4-(4-(4-fluorophenyl)-5-(2-(pyridazin-3-ylamino)pyrimidin-4-yl)thiazol-2-yl)piperidine-1-carboxylate (69). Yellow solid. Yield = 44%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.20 (t, J=7.07 Hz, 3H), 1.66 (br dd, J=12.38, 3.79 Hz, 2H), 2.11 (br d, J=11.12 Hz, 2H), 2.89 - 3.08 (m, 2H), 3.25 -3.30 (m, 1H), 4.05 (q, J=7.07 Hz, 4H), 6.73 (d, J=5.31 Hz, 1H), 7.26 - 7.34 (m, 2H), 7.55 - 7.66 (m, 1H), 7.58 - 7.64 (m, 1H), 7.59 - 7.59 (m, 1H), 8.22 (dd, J=9.09, 1.52 Hz, 1H), 8.46 (d, J=5.05 Hz, 1H), 8.86 (dd, J=4.67, 1.39 Hz, 1H), 10.60 (s, 1 H). MS: m/e 506 (MH^+), 504 (MH^-).

Synthesis of *N*-ethyl carbamate of final compounds 23 and 24 (65 and 66). Into a microwave vial, ethyl 4-(5-(2-chloropyrimidin-4-yl)-4-(4-fluorophenyl)thiazol-2-yl)piperidine-1-carboxylate (0.12 mmol) is added followed by dry iPrOH (3 mL), 3-amino-1,5-dimethyl-1*H*-pyrazole or *tert*-butyl 4-(4-amino-1*H*-pyrazol-1-yl)piperidine-1-carboxylate (0.18 mmol) and a catalytic amount of 0.4M HCl/ dioxane (60 μL, 0.024 mmol), and the mixture is stirred at 120 ^{o}C in the microwave for 16h. Solvent is distilled off and the residue is used directly to the next step or purified by semipreparative HPLC.

Ethyl 4-(5-(2-((1,5-dimethyl-1H-pyrazol-3-yl)amino)pyrimidin-4-yl)-4-(4-fluorophenyl)thiazol-2-yl)piperidine-1-carboxylate (65). It was used in the next step without further purification. Yellow solid. Yield = 77%. ^{1}H NMR (400 MHz, CDCl3) δ 1.23 - 1.39 (m,

4H), 1.86 (qd, *J*=12.17, 3.66 Hz, 2H), 2.06 - 2.31 (m, 3H), 2.33 (s, 3H), 2.99 (br t, *J*=12.25 Hz, 2H), 3.20 - 3.30 (m, 1H), 3.73 (s, 3H), 4.12 - 4.28 (m, 2H), 4.32 - 4.38 (m, 1H), 6.44 (s, 1H), 6.53 (d, *J*=5.31 Hz, 1H), 7.09 - 7.18 (m, 2H), 7.54 - 7.63 (m, 3H), 8.24 (d, *J*=5.05 Hz, 1H). MS: m/e 522 (MH^+), 520 (MH^-).

Ethyl 4-(4-(4-fluorophenyl)-5-(2-((1-(piperidin-4-yl)-1H-pyrazol-3-yl)amino)pyrimidin-4-yl)thiazol-2-yl)piperidine-1-carboxylate (66). The crude material was purified by semipreparative HPLC. Pale brown solid. Yield = 30%. MS: m/e 577 (MH^+).

General Procedure for the synthesis of *N*-ethyl carbamates of final compounds 28A and 29-39 (70-81). In a microwave vial, ethyl 4-(5-(2-chloropyrimidin-4-yl)-4-(4-fluorophenyl)thiazol-2-yl)piperidine-1-carboxylate (0.059 g, 0.13 mmol), 4-amino-pyrimidine or 4-amino-2-methyl-pyrimidine or 4-amino-2-ethoxy-pyrimidine or 2-methylpyridin-4-amine or 2,6-dimethylpyridine-4-amine or 2-hydroxypyridine-4-amine or 2-methoxypyridine-4-amine or 5-fluoro-6-methylpyridin-2-amine or 4-ethyl-5-fluoro-6-methylpyridin-2-amine[3] or 4-cyclopropyl-5-fluoro-6-methylpyridin-2-amine[24] or 4-cyclobutyl-5-fluoro-6-methylpyridin-2-amine[3] or 4-(aminomethyl)pyridine (0.17 mmol), $Pd_2(dba)_3$ (0.0073 g, 0.008 mmol), Xantphos (0.0076 g, 0.013 mmol), potassium tert-butoxide (0.030 g, 0.26 mmol) and a stirring bar are putted and air is removed in vacuum. Then anhydrous and degassed toluene (3 mL) is added and the reaction mixture is refluxed for 5h under N_2. Reaction is monitored by TLC. Toluene is distilled off under reduced pressure and the resultant mass is purified on silica gel cartridge eluted with different ratios of cyclohexane:EtOAc to furnish the desired compounds which were subsequently subjected to deprotection of the ethyl carbamate group.

Ethyl 4-(4-(4-fluorophenyl)-5-(2-(pyrimidin-4-ylamino)pyrimidin-4-yl)thiazol-2-yl)piperidine-1-carboxylate (70). Yellow solid. Yield = 71%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.20 (t, *J*=7.07 Hz, 3H), 1.23 - 1.25 (m, 1H), 1.54 - 1.74 (m, 2H), 2.10-2.14 (m, 2H), 2.83 - 3.12 (m, 2H), 4.09 (q, *J*=7.07 Hz, 4H), 6.81 (d, *J*=5.31 Hz, 1H), 7.27 - 7.35 (m, 2H), 7.63 (dd, *J*=8.84, 5.56 Hz, 2H), 8.02 (dd, *J*=5.94, 1.39 Hz, 1H), 8.30 - 8.49 (m, 2H), 8.80 - 8.84 (m, 1H), 10.60 (s, 1H). MS: m/e 506 (MH^+).

Ethyl 4-[4-(4-fluorophenyl)-5-{2-[(2-methylpyrimidin-4-yl)amino]pyrimidin-4-yl}-1,3-thiazol-2-yl]piperidine-1-carboxylate (71). Pale yellow solid. Yield = 82%. MS: m/e 521 (MH^+).

Ethyl 4-[5-{2-[(2-ethoxypyrimidin-4-yl)amino]pyrimidin-4-yl}-4-(4-fluorophenyl)-1,3-thiazol-2-yl]piperidine-1-carboxylate (72). Pale orange solid. Yield = 82%. MS: m/e 551 (MH^+).

Ethyl 4-(4-(4-fluorophenyl)-5-(2-((2-methylpyridin-4-yl)amino)pyrimidin-4-yl)thiazol-2-yl)piperidine-1-carboxylate (73). Yellow solid. Yield = 67%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.09 - 1.29 (m, 5H), 1.66 (br dd, *J*=11.75, 3.41 Hz, 2H), 2.07 - 2.19 (m, 2H), 2.41 (s, 3H), 3.28 - 3.30 (m, 1H), 4.02 - 4.08 (m, 4H), 6.66 (d, *J*=5.31 Hz, 1H), 7.22 - 7.40 (m, 2H), 7.48 (dd, *J*=5.56, 2.02 Hz, 1H), 7.57 - 7.70 (m, 3H), 8.21 (d, *J*=5.81 Hz, 1H), 8.44 (d, *J*=5.31 Hz, 1H), 10.13 (s, 1H). MS: m/e 519 (MH^+), 517 (MH^-).

Ethyl 4-(5-(2-((2,6-dimethylpyridin-4-yl)amino)pyrimidin-4-yl)-4-(4-fluorophenyl)thiazol-2-yl)piperidine-1-carboxylate (74). Slightly yellow solid. Yield = 68%. ^{1}H NMR (400 MHz,

DMSO-d_6) δ 1.20 (t, J=7.07 Hz, 3H), 1.58 - 1.71 (m, 2H), 2.09 - 2.21 (m, 2H), 2.36 (s, 6H), 3.00 (br s, 2H), 3.28 - 3.30 (m, 1H), 4.06 (q, J=7.07 Hz, 4H), 6.63 (d, J=5.31 Hz, 1H), 7.27 - 7.35 (m, 2H), 7.45 (s, 2H), 7.60 - 7.67 (m, 2H), 8.43 (d, J=5.31 Hz, 1H), 10.06 (s, 1H). MS: m/e 533 (MH^+), 531 (MH^-).

Ethyl 4-[4-(4-fluorophenyl)-5-{2-[(2-hydroxypyridin-4-yl)amino]pyrimidin-4-yl}-1,3-thiazol-2-yl]piperidine-1-carboxylate (75). Dark brown solid. Yield = quant. MS: m/e 522 (MH^+).

Ethyl 4-[4-(4-fluorophenyl)-5-{2-[(2-methoxy-6-methylpyridin-4-yl)amino]pyrimidin-4-yl}-1,3-thiazol-2-yl]piperidine-1-carboxylate (76). Pale orange solid. Yield = 85%. MS: m/e 550 (MH^+).

Ethyl 4-(5-(2-((5-fluoro-6-methylpyridin-2-yl)amino)pyrimidin-4-yl)-4-(4-fluorophenyl)thiazol-2-yl)piperidine-1-carboxylate (77). Slightly yellow solid. Yield = 71%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.20 (t, J=7.07 Hz, 3H), 1.59 - 1.72 (m, 2H), 2.04 - 2.17 (m, 2H), 2.38 (d, J=3.03 Hz, 3H), 2.98 (br s, 2H), 3.24 - 3.31 (m, 1H), 4.00 - 4.13 (m, 4H), 6.63 (d, J=5.05 Hz, 1H), 7.25 - 7.34 (m, 2H), 7.53 - 7.64 (m, 3H), 7.87 (dd, J=8.97, 3.41 Hz, 1H), 8.40 (d, J=5.05 Hz, 1H), 9.88 (s, 1H). MS: m/e 537 (MH^+), 535 (MH^-).

Ethyl 4-(5-(2-((4-ethyl-5-fluoro-6-methylpyridin-2-yl)amino)pyrimidin-4-yl)-4-(4-fluorophenyl)thiazol-2-yl)piperidine-1-carboxylate (78). Yellow solid. Yield = 81%.^{1}H NMR (400 MHz, DMSO-d_6) δ 1.16 - 1.22 (m, 4H), 1.25 - 1.33 (m, 3H), 1.56 - 1.69 (m, 3H), 2.12 (br d, J=10.36 Hz, 2H), 2.33 - 2.41 (m, 3H), 2.67 (q, J=7.49 Hz, 2H), 4.00 - 4.12 (m, 5H), 6.58 (d, J=5.05 Hz, 1H), 7.27 - 7.36 (m, 2H), 7.59 - 7.66 (m, 2H), 8.00 (d, J=4.80 Hz, 1H), 8.38 (d, J=5.05 Hz, 1H), 9.83 (s, 1H). MS: m/e 565 (MH^+), 563 (MH^-).

Ethyl 4-(5-(2-((4-cyclopropyl-5-fluoro-6-methylpyridin-2-yl)amino)pyrimidin-4-yl)-4-(4-fluorophenyl)thiazol-2-yl)piperidine-1-carboxylate (79). Yellow solid. Yield = 68%.^{1}H NMR (400 MHz, DMSO-d_6) δ 0.85 - 1.04 (m, 2H), 1.12 - 1.25 (m, 6H), 1.65 (br dd, J=12.76, 4.17 Hz, 2H), 2.08 - 2.20 (m, 3H), 2.33 - 2.41 (m, 3H), 2.93 - 3.09 (m, 2H), 4.00 - 4.12 (m, 4H), 6.55 (d, J=5.31 Hz, 1H), 7.27 - 7.37 (m, 2H), 7.57 - 7.71 (m, 3H), 8.36 (d, J=5.31 Hz, 1H), 9.78 (s, 1H). MS: m/e 577 (MH^+), 575 (MH^-).

Ethyl 4-(5-(2-((4-cyclobutyl-5-fluoro-6-methylpyridin-2-yl)amino)pyrimidin-4-yl)-4-(4-fluorophenyl)thiazol-2-yl)piperidine-1-carboxylate (80). White solid. Yield = 69%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.20 (t, J=7.07 Hz, 3H), 1.53 - 1.66 (m, 2H), 1.81 - 1.92 (m, 1H), 2.02 - 2.14 (m, 3H), 2.22 - 2.31 (m, 2H), 2.31 - 2.34 (m, 1H), 2.35 (d, J=3.03 Hz, 3H), 2.37 - 2.42 (m, 1H), 2.99 (br s, 2H), 3.25 - 3.31 (m, 1H), 3.75 (t, J=8.84 Hz, 1H), 4.05 (q, J=7.07 Hz, 4H), 6.56 (d, J=5.31 Hz, 1H), 7.27 - 7.36 (m, 2H), 7.60 - 7.67 (m, 2H), 8.08 (d, J=4.80 Hz, 1H), 8.37 (d, J=5.31 Hz, 1H), 9.80 (s, 1H). MS: m/e 591 (MH^+), 589 (MH^-).

Ethyl 4-[4-(4-fluorophenyl)-5-{2-[(pyridin-4-ylmethyl)amino]pyrimidin-4-yl}-1,3-thiazol-2-yl]piperidine-1-carboxylate trifluoroacetate (81). Yellow solid. Yield = 8%. MS: m/e 519 (MH^+).

General Procedure for the synthesis of *N*-ethyl carbamate of final compound 40 (82). In a solution of ethyl 4-(4-(4-fluorophenyl)-5-(2-(methylsulfonyl)pyrimidin-4-yl)thiazol-2-yl)piperidine-1-carboxylate (0.040 g, 0.08 mmol) in dry DMSO (0.5 mL), a solution of 2-amino-1-(pyridin-4-yl)ethanol (0.16 mmol) in dry DMSO (0.5 mL) is added at room temperature and then the reaction mixture is stirred at 100 ^{o}C overnight under Ar. The mixture is cooled down at room temperature, DMSO is distilled under high vacuum, the residue is triturated with diisopropylether (1 mL) in the ultrasound for 3min, ether is carefully decanted and the residue is used directly to the next step (deprotection of the ethyl carbamate group) without further purification. Pale orange solid. Yield = 34%. MS: m/e 549 (MH^+).

Synthesis of compound 28B (Scheme S1)

2-bromo-2-(2-chloropyrimidin-4-yl)-1-(4-fluorophenyl)ethanone (compound p, Scheme S1). A solution of 2-(2-chloropyrimidin-4-yl)-1-(4-fluorophenyl)ethanone **c2** (0.30 g, 1.20 mmol) in dry CH_2Cl_2 (20 mL) was treated with NBS (0.22 g, 1.23 mmol) in portions over 10 min. On completion of the addition, the mixture was stirred at room temperature for further 1h. The reaction mixture was concentrated under reduced pressure to give the desired product as a brown oil (quant. yield), which was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.17 (s, 1H) 7.19 - 7.34 (m, 2H), 7.72 - 7.87 (m, 1H), 8.14 - 8.19 (m, 2H), 8.97 (d, *J*=5.05 Hz, 1H).

Tert-butyl 4-(5-(2-chloropyrimidin-4-yl)-4-(4-fluorophenyl)oxazol-2-yl)piperidine-1-carboxylate (compound r, Scheme S1). A mixture of 2-bromo-2-(2-chloropyrimidin-4-yl)-1-(4-fluorophenyl)ethanone **p** (0.33 g, 0.99 mmol) and *tert*-butyl 4-carbamoylpiperidine-1-carboxylate **q** (1.13 g, 4.93 mmol) were heated at 160 ^{o}C as a melt for 10 min. The residue was partitioned between CH_2Cl_2 (40 mL) and aq. 2N Na_2CO_3 (20 mL). The aqueous layer was extracted with CH_2Cl_2 (2 x 20 mL) and the combined organic phase was washed with water (30 mL), dried over Na_2SO_4, filtered and concentrated in vacuum to furnish a brown sticky mass which was purified by semipreparative HPLC (70% ACN in water containing ammonium carbonate to 100% ACN) to give the desired compound (0.045g, 10%) as a yellowish solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.52 (s, 9H), 1.87 - 1.99 (m, 2H), 2.17 (br dd, *J*=13.26, 2.91 Hz, 2H), 2.94 - 3.07 (m, 2H), 3.07 - 3.18 (m, 1H), 4.18 (br d, *J*=7.07 Hz, 2H), 7.14 - 7.23 (m, 2H), 7.51 (d, *J*=5.31 Hz, 1H), 8.11 - 8.18 (m, 2H), 8.64 (d, *J*=5.30 Hz, 1H).

Tert-butyl 4-(4-(4-fluorophenyl)-5-(2-(pyrimidin-4-ylamino)pyrimidin-4-yl)oxazol-2-yl)piperidine-1-carboxylate (compound s, Scheme S1). In a microwave vial, tert-butyl 4-(5-(2-chloropyrimidin-4-yl)-4-(4-fluorophenyl)oxazol-2-yl)piperidine-1-carboxylate **r** (0.017 g, 0.036 mmol), 4-amino-pyrimidine (0.0043 g, 0.045 mmol), $Pd_2(dba)_3$ (0.002 g, 0.002 mmol), Xantphos (0.0021 g, 0.004 g), potassium tert-butoxide (0.0081 g, 0.072 mmol) and a stirring bar are putted and air is removed in vacuum. Then, anhydrous and degassed toluene (3 mL) is added and the reaction mixture is refluxed for 5 h under nitrogen. Reaction is monitored by TLC. Toluene is distilled off under reduced pressure and the resultant mass is purified on silica gel cartridge eluted with 100% cyclohexane to 100% EtOAc to furnish the desired compound (0.015 g, 80%) as a slightly yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.52 (s, 9H), 1.81 - 2.01 (m, 2H), 2.11 - 2.31

(m, 2H), 2.97 - 3.08 (m, 2H), 3.08 - 3.21 (m, 1H), 4.10 - 4.30 (m, 2H), 7.09 - 7.20 (m, 2H), 7.24 (d, *J*=5.31 Hz, 1H), 7.80 - 7.90 (m, 3H), 8.28 (d, *J*=6.06 Hz, 1H), 8.68 (d, *J*=5.05 Hz, 1H), 8.92 (d, *J*=1.26 Hz, 1H), 8.97 (s, 1H). MS: m/e 518 (MH^+), 516 (MH^-).

4-(4-(4-fluorophenyl)-2-(piperidin-4-yl)oxazol-5-yl)-N-(pyrimidin-4-yl)pyrimidin-2-amine hydrochloride (28B). Tert-butyl 4-(4-(4-fluorophenyl)-5-(2-(pyrimidin-4-ylamino)pyrimidin-4-yl)oxazol-2-yl)piperidine-1-carboxylate **s** (0.015 g, 0.028 mmol) dissolved in dry dioxane (0.5 mL) is treated with 4N HCl/dioxane (0.071 mL, 0.28 mmol) and the mixture is stirred at room temperature for 1h after which LCMS shows the complete consumption of the starting material. Dioxane is distilled off and the residue is dried in vacuum to give the desired hydrochloride salt. Yellow solid. Yield = quant. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.98 - 2.12 (m, 2H), 2.28 (br dd, *J*=14.15, 3.28 Hz, 2H), 3.03 - 3.14 (m, 2H), 3.29 - 3.43 (m, 4H), 7.22 - 7.35 (m, 2H), 7.41 (d, *J*=5.05 Hz, 1H), 7.91 - 8.00 (m, 3H), 8.39 (d, *J*=6.57 Hz, 1H), 8.80 (d, *J*=5.30 Hz, 1H), 8.99 (s, 1H), 9.05 - 9.14 (m, 1H), 11.50 (br s, 1H). MS: m/e 418 (MH^+). Purity was determined as 98.8 % by HPLC (287 nm). Rt: 0.66 min (Acquity UPLC BEH C18 1.7 μm, 3 mm × 50 mm, CH_3COO^- NH_4^+ 25 mM + 10% acetonitrile at pH 6.6/acetonitrile).

General Procedure for the synthesis of *N*-ethyl carbamates of final compounds 41-57 (83-99, Scheme 1). In a solution of ethyl 4-(4-(4-fluorophenyl)-5-(2-(methylsulfonyl)pyrimidin-4-yl)thiazol-2-yl)piperidine-1-carboxylate (0.040 g, 0.08 mmol) in dry DMSO (0.5 mL), a solution of the respective non-aromatic amine (0.16 mmol) in dry DMSO (0.5 mL) is added at room temperature and then the reaction mixture is stirred at 100 ºC overnight under N_2. The mixture is cooled down to room temperature, DMSO is distilled under high vacuum, the residue is triturated with diisopropylether (1 mL) in the ultrasound for 3 min, ether is carefully decanted and the residue is purified on silica gel cartridge or used directly to the next step (deprotection of the ethyl carbamate group) without further purification.

Ethyl 4-[5-(2-aminopyrimidin-4-yl)-4-(4-fluorophenyl)-1,3-thiazol-2-yl]piperidine-1-carboxylate. Bright yellow solid (83). Yield = 34%. MS: m/e 428 (MH^+).

Ethyl 4-[4-(4-fluorophenyl)-5-{2-[(2-hydroxyethyl)amino]pyrimidin-4-yl}-1,3-thiazol-2-yl]piperidine-1-carboxylate (84). Pale yellow solid. Yield = 54%. MS: m/e 472 (MH^+).

Ethyl 4-[4-(4-fluorophenyl)-5-{2-[(2-methoxyethyl)amino]pyrimidin-4-yl}-1,3-thiazol-2-yl]piperidine-1-carboxylate (85). Sticky yellow solid. Yield = 57%. MS: m/e 486 (MH^+).

Ethyl 4-[4-(4-fluorophenyl)-5-{2-[(tetrahydrofuran-2-ylmethyl)amino]pyrimidin-4-yl}-1,3-thiazol-2-yl]piperidine-1-carboxylate (86). Off-white solid. Yield = 58%. MS: m/e 512 (MH^+).

Ethyl 4-[5-(2-{[2-(dimethylamino)ethyl]amino}pyrimidin-4-yl)-4-(4-fluorophenyl)-1,3-thiazol-2-yl]piperidine-1-carboxylate (87). Sticky yellow solid. Yield = 53%. MS: m/e 499 (MH^+).

Ethyl 4-[5-(2-{[2-cyclopropyl-2-(dimethylamino)ethyl]amino}pyrimidin-4-yl)-4-(4-fluorophenyl)-1,3-thiazol-2-yl]piperidine-1-carboxylate (88). Pale yellow solid. Yield = 57%. MS: m/e 539 (MH^+).

Ethyl 4-[4-(4-fluorophenyl)-5-(2-{[2-(pyrrolidin-1-yl)ethyl]amino}pyrimidin-4-yl)-1,3-thiazol-2-yl]piperidine-1-carboxylate (89). Brown solid. Yield = 53%. MS: m/e 525 (MH^+).

Ethyl 4-[4-(4-fluorophenyl)-5-(2-{[2-(4-methylpiperazin-1-yl)ethyl]amino}pyrimidin-4-yl)-1,3-thiazol-2-yl]piperidine-1-carboxylate (90). Pale yellow solid. Yield = 51%. MS: m/e 554 (MH^+).

Ethyl 4-(4-(4-fluorophenyl)-5-(2-((2-morpholinoethyl)amino)pyrimidin-4-yl)thiazol-2-yl)piperidine-1-carboxylate (91). Yellow solid. Yield = 91%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.04 (d, J=6.06 Hz, 1H), 1.20 (t, J=7.07 Hz, 3H), 1.53 - 1.70 (m, 2H), 2.06 - 2.16 (m, 2H), 2.37 - 2.43 (m, 3H), 2.87 - 2.96 (m, 2H), 3.06 - 3.13 (m, 1H), 3.52 - 3.72 (m, 7H), 3.72 - 3.80 (m, 1H), 3.97 - 4.14 (m, 4H), 6.24 - 6.36 (m, 1H), 7.22 - 7.35 (m, 2H), 7.51 - 7.67 (m, 3H), 8.12 - 8.21 (m, 1H). MS: m/e 541 (MH^+), 539 (MH^-).

Ethyl 4-(4-(4-fluorophenyl)-5-(2-((2-thiomorpholinoethyl)amino)pyrimidin-4-yl)thiazol-2-yl)piperidine-1-carboxylate (92). Yellow solid. Yield = 75%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.20 (t, J=7.07 Hz, 3H), 1.54 - 1.70 (m, 2H), 2.01 - 2.16 (m, 2H), 2.60 - 2.71 (m, 12H), 2.81 - 2.86 (m, 1H), 2.88 - 2.95 (m, 2H), 4.05 (q, J=7.07 Hz, 4H), 7.26 - 7.35 (m, 2H), 7.35 - 7.83 (m, 4H), 8.14 (br d, J=5.31 Hz, 1H). MS: m/e 557 (MH^+), 555 (MH^-).

Ethyl 4-[4-(4-fluorophenyl)-5-{2-[(trans-4-hydroxycyclohexyl)amino]pyrimidin-4-yl}-1,3-thiazol-2-yl]piperidine-1-carboxylate (93). Off-white solid. Yield = 62%. MS: m/e 526 (MH^+).

Ethyl 4-[4-(4-fluorophenyl)-5-{2-[(1-methylpyrrolidin-3-yl)amino]pyrimidin-4-yl}-1,3-thiazol-2-yl]piperidine-1-carboxylate (94). Pale yellow solid. Yield = 32%. MS: m/e 511 (MH^+).

Ethyl 4-{4-(4-fluorophenyl)-5-[2-(pyrrolidin-1-yl)pyrimidin-4-yl]-1,3-thiazol-2-yl}piperidine-1-carboxylate (95). Pale yellow solid. Yield = 69%. MS: m/e 482 (MH^+).

Ethyl 4-{4-(4-fluorophenyl)-5-[2-(3-hydroxypyrrolidin-1-yl)pyrimidin-4-yl]-1,3-thiazol-2-yl}piperidine-1-carboxylate (96). Pale yellow solid. Yield = 49%. MS: m/e 498 (MH^+).

Ethyl 4-[5-{2-[4-(azetidin-1-yl)piperidin-1-yl]pyrimidin-4-yl}-4-(4-fluorophenyl)-1,3-thiazol-2-yl]piperidine-1-carboxylate trifluoroacetate (97). Sticky orange solid. Yield = 45%. MS: m/e 551 (MH^+).

Ethyl 4-(4-(4-fluorophenyl)-5-(2-(4-morpholinopiperidin-1-yl)pyrimidin-4-yl)thiazol-2-yl)piperidine-1-carboxylate (98). Yellow solid. Yield = 87%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.98 - 1.08 (m, 1H), 1.20 (t, J=7.07 Hz, 3H), 1.38 - 1.72 (m, 4H), 1.83 - 1.97 (m, 2H), 2.02 - 2.16 (m, 2H), 2.40 - 2.49 (m, 3H), 2.80 - 2.93 (m, 3H), 2.96 - 3.07 (m, 3H), 3.46 - 3.75 (m, 5H), 3.95 - 4.14 (m, 4H), 4.56 - 4.70 (m, 1H), 7.22 - 7.34 (m, 1H), 7.24 - 7.38 (m, 2H), 7.45 - 7.67 (m, 1H), 7.50 - 7.64 (m, 1H), 8.20 - 8.30 (m, 1H). MS: m/e 581 (MH^+).

Ethyl 4-(4-(4-fluorophenyl)-5-(2-(4-(morpholinomethyl)piperidin-1-yl)pyrimidin-4-yl)thiazol-2-yl)piperidine-1-carboxylate (99). Yellow solid. Yield = 87%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.93 - 1.07 (m, 2H), 1.09 - 1.32 (m, 4H), 1.56 - 1.70 (m, 2H), 1.70 - 1.87 (m, 2H), 2.04 - 2.18 (m, 4H), 2.33 (br d, J=1.77 Hz, 4H), 2.79 - 2.88 (m, 2H), 2.89 - 3.06 (m, 2H), 3.21 - 3.28 (m, 1H), 3.57 (t, J=4.42 Hz, 4H), 3.98 - 4.12 (m, 4H), 4.55 (br d, J=13.14 Hz, 2H), 6.29 (d,

J=5.31 Hz, 1H), 7.20 - 7.34 (m, 2H), 7.57 (dd, *J*=8.84, 5.56 Hz, 2H), 8.20 (d, *J*=5.30 Hz, 1H). MS: m/e 595 (MH^+).

Synthesis of the intermediates of thiazole derivatives depicted in Table 5 (Scheme 3).

Tert-butyl 4-(5-bromothiazol-2-yl)piperidine-1-carboxylate (k). In a microwave vial, tert-butyl 4-carbamothioylpiperidine-1-carboxylate (1.21 g, 4.95 mmol) is dissolved in acetone (15 mL) and a 50% aqueous solution of chloroacetaldehyde (1.56 mL, 9.90 mmol) is added dropwise. The reaction mixture is stirred under reflux overnight. The solvent is distilled off and the residue is purified on silica gel cartridge eluted with cyclohexane:EtOAc 7:3, to furnish *tert*-butyl 4-(thiazol-2-yl)piperidine-1-carboxylate (0.66 g, 50%) as a brownish oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.48 (s, 9H), 1.77 (m, 2H), 2.14 (m, 2H), 2.91 (m, 2H), 3.22 (m, 1H), 4.23 (br d, 2H), 7.26 (d, *J* = 3.3 Hz, 1H), 7.74 (d, *J* = 3.3 Hz, 1H). In a dry flask, tert-butyl 4-(thiazol-2-yl)piperidine-1-carboxylate (0.60 g, 2.24 mmol) is dissolved in dry DMF (12 mL) and to the resulting solution NBS (0.60 g, 3.35 mmol) is added and the mixture is stirred at room temperature overnight. Water (15 mL) is added and the solution is extracted with EtOAc (3 x 20 mL). The combined organic phase is washed with water (3 x 20 mL) and brine (20 mL), dried over Na_2SO_4, filtered and concentrated in vacuum. The residue is purified on silica gel cartridge eluted with cyclohexane:EtOAc 85:15, affording the desired compound **g** (0.50 g, 65%) as a light brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.49 (s, 9H), 1.72 (dq, *J* = 11.9, 4.3 Hz, 2H), 2.08 (d, *J* = 11.7 Hz, 2H), 2.89 (t, *J* = 11.6 Hz, 2H), 3.13 (tt, *J* = 11.5, 3.8 Hz, 1H), 4.20 (br d, *J* = 12.9 Hz, 2H), 7.59 (s, 1H).

Tert-butyl 4-(4-bromothiazol-2-yl)piperidine-1-carboxylate (l). In a dry flask containing LDA solution (0.95 mL, 0.95 mmol) in dry THF (3 mL), a solution of *tert*-butyl 4-(5-bromothiazol-2-yl)piperidine-1-carboxylate **g** (0.100 g, 0.29 mmol) in dry THF (4 mL) is added dropwise at -78 ^{o}C under nitrogen. When the addition is completed, the mixture is stirred at the same temperature for 3h. The reaction mixture is then poured onto water (10 mL), diluted with EtOAc (50 mL), washed with water (20 mL) and brine (20 mL), dried over Na_2SO_4, filtered and concentrated in vacuum. The crude product is purified on silica gel cartridge eluted with cyclohexane:EtOAc 9:1, affording the desired compound **h** (0.080 g, 80%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.46 - 1.53 (m, 9H), 1.74 (dd, *J*=12.88, 4.29 Hz, 2H), 2.04 - 2.16 (m, 2H), 2.89 (br s, 2H), 3.09 - 3.23 (m, 1H), 4.11 - 4.30 (m, 2H), 7.15 (s, 1H).

Synthesis of compounds of formula m (Scheme 3).

Tert-butyl 4-(4-cyclopropylthiazol-2-yl)piperidine-1-carboxylate. In a microwave vial, *tert*-butyl 4-(4-bromothiazol-2-yl)piperidine-1-carboxylate **l** (0.080 g, 0.23 mmol), potassium cyclopropyltrifluoroborate (0.043 g, 0.29 mmol), $Pd(OAc)_2$ (0.0026 g, 0.012 mmol), cataCXium A ligand (0.0062 g, 0.017 mmol), $CsCO_3$ (0.225 g, 0.69 mmol) and a stirring bar are putted and air is removed under reduced pressure. Then, degassed toluene (1 mL) and degassed H_2O (0.1 mL) are added and the reaction mixture is heated at 100 ^{o}C overnight under N_2. Reaction is monitored by TLC and LCMS. The reaction mixture is cooled down to room temperature, solvents are distilled off and the residue is purified on silica gel cartridge eluted with cyclohexane:EtOAc (9:1), affording the desired compound (0.038 g, 54%) as a yellow semisolid. ^{1}H NMR (400 MHz, $CDCl_3$)

δ 0.85 - 0.90 (m, 2H), 0.92 - 0.97 (m, 2H), 1.50 (s, 9H), 1.64 - 1.78 (m, 3H), 2.02 - 2.12 (m, 2H), 2.89 (br t, *J*=12.13 Hz, 2H), 3.06 - 3.18 (m, 1H), 4.20 (br s, 2H), 6.72 (s, 1 H). MS: m/e 309 (MH^+).

Tert-butyl 4-(4-(piperidin-1-ylmethyl)thiazol-2-yl)piperidine-1-carboxylate. In a microwave vial, *tert*-butyl 4-(4-bromothiazol-2-yl)piperidine-1-carboxylate **l** (0.080 g, 0.23 mmol), potassium piperidinylmethyltrifluoroborate[6] (0.059 g, 0.29 mmol), $Pd(OAc)_2$ (0.0026 g, 0.012 mmol), XPhos (0.011 g, 0.023 mmol), Cs_2CO_3 (0.225 g, 0.69 mmol) and a stirring bar are putted and air is removed under reduced pressure. Then, degassed THF (1 mL) and degassed H_2O (0.1 mL) are added and the reaction mixture is heated at 80 ^oC overnight under nitrogen. Reaction is monitored by TLC and LCMS. The reaction mixture is cooled down to room temperature, solvents are distilled off and the residue is purified on silica gel cartridge eluted with cyclohexane:EtOAc (100% cyclohexane to 100% EtOAc), affording the desired compound (0.056 g, 67%) as a yellow oil. ^1H NMR (400 MHz, $CDCl_3$) δ 0.92 – 1.39 (m, 2H), 1.50 (s, 9H), 1.52 - 1.73 (m, 6H), 1.91 (q, *J*=5.05 Hz, 2H), 2.02 - 2.12 (m, 2H), 2.71 (td, *J*=11.81, 3.41 Hz, 2H), 2.89 (br t, *J*=12.13 Hz, 2H), 3.06 - 3.18 (m, 1H), 3.30 (br d, *J*=11.87 Hz, 2H), 4.20 (br s, 2H), 6.72 (s, H). MS: m/e 366 (MH^+).

Synthesis of compounds of formula n (Scheme 3).

Tert-butyl 4-(4-cyclopropyl-5-iodothiazol-2-yl)piperidine-1-carboxylate. In a dry flask, *tert*-butyl 4-(4-cyclopropylthiazol-2-yl)piperidine-1-carboxylate (0.037 g, 0.12 mmol) is dissolved in dry acetonitrile (1 mL) and to the resulting solution trifluoroacetic acid (0.003 mL, 0.036 mmol) and NIS (0.041 g, 0.18 mmol) is added in portions. Then, the mixture is stirred at room temperature for 1.5 h. EtOAc (3 mL) is added, solvents are distilled off and the residue is purified on silica gel cartridge eluted with cyclohexane:EtOAc 85:15, affording the desired compound (0.052 g, quant. yield) as a yellow oil. MS: m/e 366 (MH^+).

Tert-butyl 4-(5-iodo-4-(piperidin-1-ylmethyl)thiazol-2-yl)piperidine-1-carboxylate. In a dry flask, tert-butyl 4-(4-(piperidin-1-ylmethyl)thiazol-2-yl)piperidine-1-carboxylate (0.055 g, 0.15 mmol) is dissolved in dry THF (4 mL) and to the resulting solution *n*-BuLi (0.165 mL, 0.165 mmol) is added dropwise at -78 ^oC. The reaction mixture is stirred at the same temperature for 45 min and then iodine (0.042 g, 0.165 mmol) dissolved in dry THF (1 mL) is added dropwise. The resulting mixture is stirred at the same temperature for 15 min and at room temperature for 1 h. Methanol is added, the mixture is concentrated in vacuum and the residue is purified on silica gel cartridge eluted with 100% cyclohexane to 100% EtOAc, affording the desired compound (0.020 g, 30%) as a yellow oil. MS: m/e 492 (MH^+).

Synthesis of compounds of formula o (Scheme 3).

Tert-butyl 4-(5-(2-chloropyrimidin-4-yl)-4-cyclopropylthiazol-2-yl)piperidine-1-carboxylate. In a microwave vial, a stirring bar, *tert*-butyl 4-(4-cyclopropyl-5-iodothiazol-2-yl)piperidine-1-carboxylate (0.052 g, 0.12 mmol), $Pd(PPh_3)_4$ (0.0069 g, 0.006 mmol), and CuI (0.011 g, 0.06 mmol) are added and air is removed in vacuum. Then, 2-chloro-4-(tributylstannyl)pyrimidine[7] (0.061 g, 0.15 mmol) and dry and degassed DMF (1.5 mL) are added and the resulting mixture is heated at 70 ^oC under nitrogen for 20 min. TLC showed the consumption of the starting material and the formation of a new product. The mixture is cooled down to room temperature, H_2O (3 mL) is added and the mixture is extracted with EtOAc (2 x 25

mL). The combined organic phase is washed with water (15 mL) and brine (15 mL), dried over Na_2SO_4, filtered and concentrated in vacuum. The residue is purified on cartridge containing a mixture of silica and K_2CO_3 in a ratio of 9:1, eluted with cyclohexane:EtOAc (100% cyclohexane to 15% EtOAc in cyclohexane), affording the desired compound (0.040 g, 80%) as a yellow oil. MS: m/e 421 (MH^+), 419 (MH^-).

Tert-butyl 4-(5-(2-chloropyrimidin-4-yl)-4-(piperidin-1-ylmethyl)thiazol-2-yl)piperidine-1-carboxylate. In a microwave vial a stirring bar, *tert*-butyl 4-(5-iodo-4-(piperidin-1-ylmethyl)thiazol-2-yl)piperidine-1-carboxylate (0.019 g, 0.038 mmol), $Pd(PPh_3)_4$ (0.0022 g, 0.002 mmol), and CuI (0.036 g, 0.019 mmol) are added and air is removed by vacuum. Then, 2-chloro-4-(tributylstannyl)pyrimidine[7] (0.019 g, 0.048 mol) and dry and degassed DMF (0.5 mL) are added and the resulting mixture is heated at 80 ºC under nitrogen for 1 h. TLC showed the consumption of the starting material and the formation of a new products. The mixture is cooled down to room temperature, water (0.5 mL) is added and the mixture is extracted with EtOAc (2 x 10 mL). The combined organic phase is washed with water (10 mL) and brine (10 mL), dried over Na_2SO_4, filtered and concentrated in vacuum. The residue is purified on cartridge containing a mixture of silica and K_2CO_3 in a ratio of 9:1, eluted with cyclohexane:EtOAc:MeOH (100% EtOAc to EtOAc:MeOH 9:1), affording the desired compound (0.012 g, 67%) as a yellow oil.

***In vitro* PKG assay.** *a) Expression and purification of recombinant PKG*. Full length *Pf*PKG (NCBI accession code XP_001348520) with native codon usage was cloned into the pTrcHisC plasmid (Life Technologies) that includes an *N*-terminal His-tag as described previously.[54] *Pf*PKG with threonine 618 replaced with a glutamine (*Pf*PKG T618Q) was cloned into the same plasmid as described previously.[12] Recombinant proteins were generated and purified using a protocol based on that described previously.[54] Briefly, freshly transformed *E. coli* RosettaTM 2(DE3) pLysS (Novagen; Cat. No. 71403) were used for expression of recombinant *Pf*PKG. 500 ml cultures in LB Rich Broth (containing 50 µg/ml carbenicillin and 34 µg/ml chloramphenicol) were grown in a shaking incubator at 37 °C until reaching an optical density (O.D.) of 0.6–0.7. The temperature was reduced to 16 °C before induction of expression with 1 mM IPTG. Incubation at 16 °C was continued overnight. The cultures were harvested by centrifugation at 4000×g at 4 °C for 30 min, the supernatant removed and the pellet stored at −80 °C for in excess of 1 hour. The PKGs were purified via the histidine tag on HiTrap TALON (cobalt) columns (GE Healthcare) connected to an AKTA-FPLC as per the manufacturer's instruction. Fractions were analyzed by SDS-PAGE and the main peak concentrated on 10 kDa MWCO concentrators (Amicon). Purified proteins were stored in 50% glycerol at −80 °C in single use aliquots. The final buffer composition of the purified product was: 50 mM Tris/HCl pH 7.5, 0.1 mM EGTA, 150 mM NaCl, 0.1% β-mercaptoethanol, 50% glycerol, 0.03% Brij-35, 1 mM benzamidine and 0.2mM PMSF.

b) PKG inhibitory activity assay. IC_{50} values were determined for test compounds using a microfluidic mobility shift assay. Briefly, compounds were prepared over a 10-well ½ log dilution series in dimethyl sulfoxide (DMSO) in duplicate in 50 µl volumes using 384-well polypropylene U-bottomed plates (Thermo Scientific, UK). The plates contained positive/no inhibitor (DMSO only) and negative (no enzyme) controls in columns 1, 2, and 23, 24. The reaction mix for each well consisted of 20 µl of enzyme/peptide mix (1.25 nM *Pf*PKG, 1.5 µM FAM-labeled PKAtide

[FAM-GRTGRRNSI-NH2, Cambridge Bioscience, UK] in *Pf*PKG assay buffer [25 mM HEPES (pH 7.4), 20 mM β-glycerophosphate, 2 mM DTT, 10 μM cGMP, 0.01% (w/v) BSA, 0.01% (v/v) Triton X-100]) plus 5 μl of compound. Samples were pre-incubated at room temperature for 30 min and reactions were initiated by addition of 25 μl ATP mix [10 mM $MgCl_2$ and ATP, at KM of the enzyme under test (20 μM PfPKG and 90 μM PfPKG T618Q), in water]. Positive controls were complete reaction mixtures with 10% DMSO and negative controls were reaction mixtures with 10% DMSO but lacking enzyme. Reactions were allowed to proceed for 30 min at room temperature, corresponding to conversion of approximately 10% of the substrate in the DMSO controls. Reactions were terminated by addition of 50 μl stop solution (25 mM EDTA in water). Samples were analyzed by electrophoretic separation of substrate and product peaks and fluorescence detection using a Caliper Lab Chip EZ reader (Perkin Elmer, Waltham MA) with 0.2 s sip time, downstream voltage 500 V, upstream voltage 1950 V and pressure 0.5 to 1.5 psi. Substrate and product peak heights were measured and the ratio of the product peak height divided by the sum of the product and substrate peaks were determined using EZ reader software (version 3.0.265.0) to obtain percentage conversion (P) values. P-values were normalized to percentage activity relative to positive and negative controls were % activity = $100 \times (P—P_{neg\ ctrls})/(P_{pos\ ctrls} - P_{neg\ ctrls})$ and fitted to obtain IC_{50} values using a 4-parameter logistical fit (XL-fit, IDBS, Guildford UK). Liquid handling stages were conducted on a Biomek robotic liquid handler (Beckman Coulter).

Male/female gametocyte functional viability assay. *A) Gametocyte production.* Gametocyte cultures were produced as follows: asexual cultures of *P. falciparum* NF54 strain parasites were used to seed gametocyte cultures at 0.5% parasitemia, 4% hematocrit in 50 ml total volume under 5% O_2/5% CO_2/90% N_2 gas. Culture medium (RPMI 25 mM HEPES, 50 mg/l hypoxanthine, 2 g/l $NaHCO_3$ + 5% human serum and 5% Albumax) was replaced daily for 14 days.[28] *B) Dual gamete formation assay.* At day 14, the density of cultures was adjusted to plate 700,000 cells in 50 μL per well in 384-well black microplates with clear bottom, pre-dispensed with test compounds. Plates were incubated for 48 h at 37 ºC (5%O_2/5%CO_2/90% N_2). Gametocyte activation was triggered by reduced temperature and the addition of ookinete medium containing xanthurenic acid supplemented with the antibody anti-*Pf*s25-Cy3 at a final concentration of 1/2000 (from 1mg/ml stock). Plates were analysed to detect exflagellation centres. "Activated" cultures were then incubated (protected from light) at 26°C for 24 h (in a thermo regulated incubator) to increase the fluorescent signal emitted by female gametes. The plates were then analysed to record female activated gametes[28] *C) Measured parameters.* Activation of male gametes: detection is based on light changes provoked by flagella movements which cause movement of surrounding cells. A 10-frame video is taken and then analysed to determine these changes in cell position based on pixels change. The script then determines where exflagellation centers are located, based also on size and intensity of light changes. Activation of female gametes: detection of fluorescent Cy3-Anti Pfs25 antibody (as primary parameter) followed by selection of events according to their size, roundness and the intensity of the fluorescence. Both measurements are performed using an automated inverted microscope Ti-E Nikon using JOBS software. Analysis of images and videos is performed with ICY program.[55]

***In vitro* parasite reduction ratio.** *In vitro* PRR testing was conducted as previously described.[56] The assay used the limiting dilution technique to quantify the number of parasites that remained viable after drug treatment. *P. falciparum* strain 3D7A (Malaria Research and Reference Reagent Resource Center, MR4, BEI Resources; Cat. No. MRA-102) was treated with a drug concentration corresponding to 10× IC_{50}. Conditions of parasites exposed to treatment were identical to those used at GSK in the IC_{50} determination (2% hematocrit, 0.5% parasitemia). Parasites were treated for 120 hours. Drug in culture medium was renewed daily over the entire treatment period. Parasite samples were collected from the treated culture every 24 h (24, 48, 72, 96, and 120 hour time points); drug was washed out of the sample, and parasites were cultured drug-free in 96-well plates by adding fresh erythrocytes and culture medium. To quantify the number of viable parasites after treatment, three-fold serial dilutions were used with the above-mentioned samples after removing the drug. Four independent serial dilutions were performed with each sample to correct experimental variations. The number of viable parasites was determined after 21 and 28 days by counting the number of wells with growth using [^{3}H]-hypoxanthine incorporation. The number of viable parasites was back-calculated by using the formula X^{n-1} where n is the number of wells able to render growth and X the dilution factor (when n = 0, number of viable parasites is estimated as zero).

hERG inhibition determination and cell cytotoxicity assays. hERG Qpatch assay is described in literature.[8] Cell cytotoxicity assays are described in literature.[9]

Intrinsic Clearance (Cli) Assay. Intrinsic clearance (CLi) values were determined in mouse, rat and human liver microsomes. Test compounds (final concentration 0.5 μM) were incubated at 37 °C for 45 min in 50 mM potassium phosphate buffer (pH 7.4) containing 0.5 mg microsomal protein/mL. The reaction was started by addition of cofactor NADPH. The final concentration of solvent was 1% of the final volume. At 0, 5, 15, 30, and 45 min, an aliquot (100 μL) was taken, quenched with acetonitrile containing an appropriate internal standard, and analyzed by HPLC-MS/MS. The intrinsic clearance (CLi) was determined from the first-order elimination constant by nonlinear regression, corrected for the volume of the incubation and assuming 48.0 and 39.7 mg microsomal protein/g liver for mouse, rat and human, respectively. Values for CLi were expressed as mL/min/g liver.

ChromlogD assay. The Chromatographic Hydrophobicity Index (CHI)[10] values were measured using reversed phase HPLC column (50 x 2 mm 3 μM Gemini NX C18, Phenomenex, UK) with fast acetonitrile gradient at starting mobile phase of pHs 2, 7.4 and 10.5. CHI values are derived directly from the gradient retention times by using a calibration line obtained for standard compounds. The CHI value approximates to the volume % organic concentration when the compound elutes. CHI is linearly transformed into ChromlogD[11] by least-square fitting of experimental CHI values to calculated ClogP values for over 20K research compounds using the following formula: ChromlogD = 0.0857CHI - 2.00. The average error of the assay is ±3 CHI unit or ±0.25 ChromlogD.

Chemoproteomics-target identification experiments. Kinobeads were prepared as described.[31,32] Sepharose beads were derivatized with **31** or **50** at a concentration of 1 mM as described.[30] The chemoproteomic affinity capturing experiments were performed as previously

described.[32] Briefly, beads were washed and equilibrated in lysis buffer [50 mM Tris-HCl, pH 7.4, 0.4 % Igepal-CA630, 1.5 mM $MgCl_2$, 5 % Glycerol, 150 mM NaCl, 25 mM NaF, 1 mM Na_3VO_4, 1 mM DTT, and one complete EDTA-free protease inhibitor tablet (Roche) *per* 25 mL]. The beads were incubated at 4°C for 1 hour with 0.1 mL (0.3 mg) *P. falciparum* blood stage extract, which was pre-incubated with compound or DMSO (vehicle control). The experimental set up was such that 10 samples are measured in parallel (TMT 10-plex)[57] to generate values for the affinity of the beads to the bound proteins ("depletion" values, 4 samples) and to generate IC_{50} values (6 samples) in a single experiment. Samples 1 and 2 were the vehicle control, samples 3 and 4 were done in the same way, but while the beads were discarded after the first incubation step the extract was incubated with fresh beads to measure how much protein could rebind to the fresh beads (it was depleted from the extract by first bead-binding). Apparent dissociation constants were determined by taking into account the protein depletion by the beads.[32] Samples 5-10 were used to generate IC_{50} values by adding compound over a range of concentrations (20 µM, 1:3 dilutions). Beads were transferred to Filter plates Durapore (PVDF membrane, Merck Millipore), washed extensively with lysis buffer and eluted with SDS sample buffer.

Proteins were digested according to a modified single pot solid-phase sample preparation (SP3) protocol.[58,59] Briefly, proteins in 2% SDS were bound to paramagnetic beads (SeraMag Speed beads, GE Healthcare, CAT#45152105050250, CAT#651521050502) by addition of Ethanol to a final concentration of 50%. Contaminants were removed by washing 4 times with 70 % Ethanol. Proteins were digested by re-suspending in 0.1 mM HEPES (pH 8.5) containing TCEP, chloroacetamide, Trypsin and LysC following o/n incubation.

Peptides were labeled with isobaric mass tags (TMT10, Thermo FisherScientific, Waltham, MA) using the 10-plex TMT reagents, enabling relative quantification of 10 conditions in a single experiment.[57,60] The labeling reaction was performed in 40 mM triethylammoniumbicarbonate, pH 8.5 at 22°C and quenched with glycine. Labeled peptide extracts were combined to a single sample per experiment, lyophilized and subjected to LC-MS analysis. Dried samples were re-suspended in 0.05% trifluoroacetic acid in water. Half of the sample was injected into an Ultimate3000 nanoRLSC (Dionex) coupled to a Q-Exactive (Thermo Fisher Scientific). Peptides were separated on custom-made 35 cm x 100 um (ID) reversed-phase columns (Reprosil) at 55°C, gradient elution was performed from 3.5 % acetonitrile to 29 % acetonitrile in 0.1% formic acid and 3.5 % DMSO over 120 min. Samples were online injected into Q Exactive Plus mass spectrometer.

LC-MS/MS measurements on Q Exactive Orbitrap or Orbitrap Fusion Lumos mass spectrometers (Thermo Fisher Scientific) was performed as described elsewhere.[61] Mascot 2.4 (Matrix Science, Boston, MA) was used for protein identification by using a 10 parts per million mass tolerance for peptide precursors and 20 mD (HCD) mass tolerance for fragment ions. To create the fasta file for mascot searching, all proteins corresponding to the taxonomy "*Plasmodium falciparum* (isolate 3D7)" were downloaded from Uniprot (release 20170621) and supplemented with common contaminant protein sequences of bovine serum albumin, porcine trypsin and mouse, rat, sheep and dog keratins. To assess the false discovery rate (FDR), "decoy" proteins (reverse of all protein sequences) were created and added to the database, resulting in a database containing a total of 14266 protein sequences, 50% forward, 50% reverse.

Carbamidomethylation of cysteine residues and TMT modification of lysine residues were set as fixed modifications. Methionine oxidation, N-terminal acetylation of proteins and TMT modification of peptide *N*-termini were set as variable modifications.

Unless stated otherwise, we accepted protein identifications as follows: (i) For single-spectrum to sequence assignments, we required this assignment to be the best match and a minimum Mascot score of 31 and a 10× difference of this assignment over the next best assignment. Based on these criteria, the decoy search results indicated <1% false discovery rate (FDR). (ii) For multiple spectrum to sequence assignments and using the same parameters, the decoy search results indicated <0.1% FDR. Quantified proteins were required to contain at least 2 unique peptide matches. FDR for quantified proteins was < 0.1%. Raw data tables for the chemoproteomics experiments can be found in the Supplementary file.

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