Supporting information

Potent antimalarial 2-pyrazoyl quinolone *bc$_1$* (Q_i) inhibitors with improved drug-like properties

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S1. Synthetic methods, procedures and chemical analysis data

General

Air- and moisture-sensitive reactions were carried out in oven-dried glassware sealed with rubber septa under a balloon of nitrogen. Sensitive liquids and reagents were transferred via syringe. Reactions were allowed to stir using a Teflon-coated magnetic stirring bar. Organic solutions were concentrated under vacuum using a Buchi rotary evaporator.

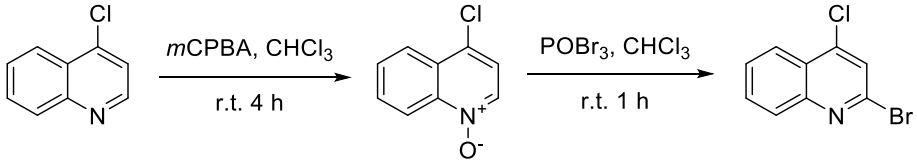
Anhydrous solvents were either purchased from reliable commercial sources or distilled from a still prior to use under an inert gas atmosphere. All reagents were purchased from reliable commercial sources and were used without any purification unless otherwise indicated.

TLC was performed on 0.25 mm thickness Merck silica gel 60 with fluorescent indicator at 254 nm and visualised under UV light. UV inactive compounds were stained and visualised using iodine, *p*-anisaldehyde, or potassium permanganate solution followed by gentle heating. Flash column chromatography was performed using normal phase silica gel purchased from Sigma-Aldrich.

NMR spectra were recorded in a solution of $CDCl_3$ or DMSO-d_6 on a Bruker AMX400 spectrometer (^{1}H 400 MHz, ^{13}C 100 MHz). Chemical shifts (δ) were expressed in ppm relative to tetramethylsilane (TMS) used as an internal standard. *J* coupling constants are in hertz (Hz) and the multiplicities were designed as follows: s, singlet; d, doublet; t, triplet; q, quartet; dd, double of doublet; m, multiplet. Mass spectra were recorded on either a Micromass LCT Mass Spectrometer using electrospray ionisation (ESI) or Trio-1000 Mass Spectrometer using chemical ionisation (CI). Reported mass values are within error limits of ±5 ppm.

Purity determination was performed by HPLC analysis using Agilent 1200 solvent delivery system. The HPLC methods used the following conditions: ZORBAX Eclipse Plus C18 (4.6 mm x 100 mm, 3.5 µm) at 25^{o}C with 1.0 mL/min flow rate. Solvents – A) water containing 0.05% trifluoroacetic acid and B) acetonitrile containing 0.05% trifluoroacetic acid; Method (Acidic, 2-98%): Run time: 15 min, gradient: 2% B hold to 2 min, 2-98% B in 10 min, then hold at 98% B to 15 min. All melting points were determined with Gallenkamp melting point apparatus and were uncorrected.

Preparation of 2-bromo-4-chloroquinoline (2a)

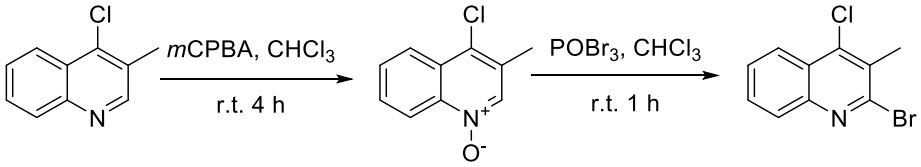


To a 4-chloroquinoline (0.99 g, 6.0 mmol) solution in $CHCl_3$ (50 mL), *m*CPBA (1.24 g, 7.2 mmol, 1.2 eq.) was added. The resulting mixture was allowed to stir at room temperature for 4 hours. After this period of time, the reaction solution was washed with sat. $NaHCO_3$(aq) to remove the *m*-chloro benzoic acid side product. The water layer was extracted with DCM (25 mL X 2). All

organic layers were combined, washed with brine and dried over $MgSO_4$. After removal of all solvents, the first step product was obtained as a yellow solid and used directly in the next step without further purification.

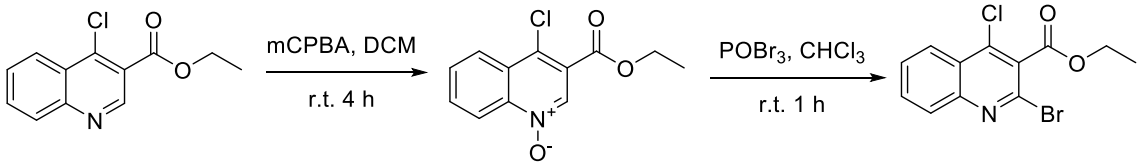
To a solution of the *N*-oxide in $CHCl_3$ (25 mL), $POBr_3$ (1.72 g, 6.0 mmol, 1.0 eq.) was added. The resulting mixture was allowed to stir at room temperature for 90 minutes. After that, the reaction was quenched with ice-water and neutralized to pH7 by Na_2CO_3(sat.). The organic layer was separated, and the water layer was extracted with DCM (30 mL X 2). All organic layers were combined, washed with brine and dried over $MgSO_4$. After removal of the solvents, the crude product was isolated as a yellow solid. The crude product was purified by flash column chromatograph eluting with 10% EtOAc in hexane to give the title product as an off-white solid (1.04 g, 72% for two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (d, J = 7.1 Hz, 1H), 8.07 (d, J = 7.9 Hz, 1H), 7.80 (t, J = 7.1 Hz, 1H), 7.72 – 7.64 (m, 2H).

Preparation of 2-bromo-4-chloro-3-methylquinoline (2e)



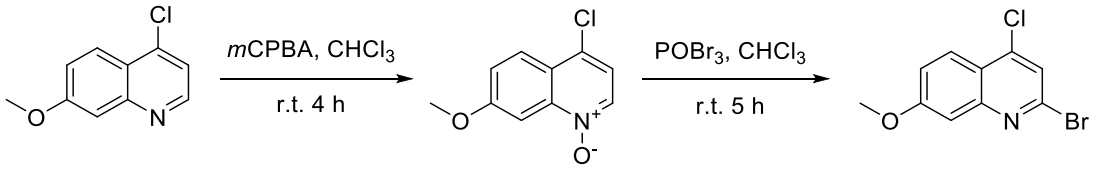
The reaction, work-up and purification procedure of the title compound followed a similar procedure to that described for 2-bromo-4-chloroquinoline (**2a**). The title product was obtained as a colorless crystalline solid in 72% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (d, J = 7.0 Hz, 1H), 8.04 (d, J = 7.3 Hz, 1H), 7.82 (t, J = 7.1 Hz, 1H), 7.65 (t, J = 7.3 Hz, 1H), 2.55 (s, 3H).

Preparation of ethyl 2-bromo-4-chloroquinoline-3-carboxylate (2f)



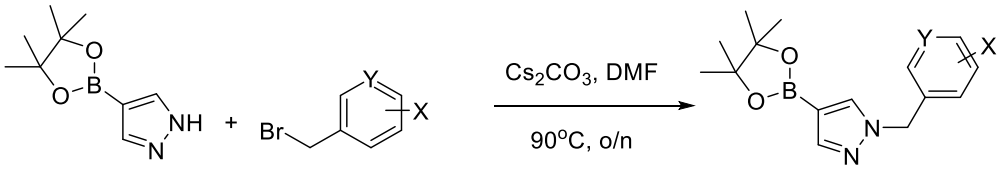
The reaction, work-up and purification procedure of the title compound followed a similar procedure to that described for the preparation of 2-bromo-4-chloroquinoline (**2a**). The title product was isolated as a colorless crystalline solid in 75% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (d, J = 8.4 Hz, 1H), 8.08 (d, J = 8.4 Hz, 1H), 7.84 (dd, J = 8.4, 7.0 Hz, 1H), 7.72 (dd, J = 8.3, 7.0 Hz, 1H), 4.55 (q, J = 7.1 Hz, 2H), 1.47 (t, J = 7.1 Hz, 3H).

Preparation of 2-bromo-4-chloro-7-methoxyquinoline (2g)



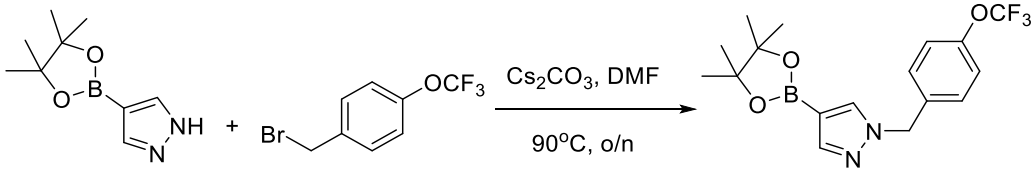
The reaction, work-up and purification procedure of the title compound followed a similar procedure to that described for 2-bromo-4-chloroquinoline (**2a**). The title product was obtained as a colorless crystalline solid in 71% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (d, J = 9.2 Hz, 1H), 7.50 (s, 1H), 7.37 (d, J = 2.5 Hz, 1H), 7.29 (dd, J = 9.2, 2.5 Hz, 1H), 3.95 (s, 3H).

General Procedure A – Preparation of Pyrazole side chain boronic acid pinocol ester



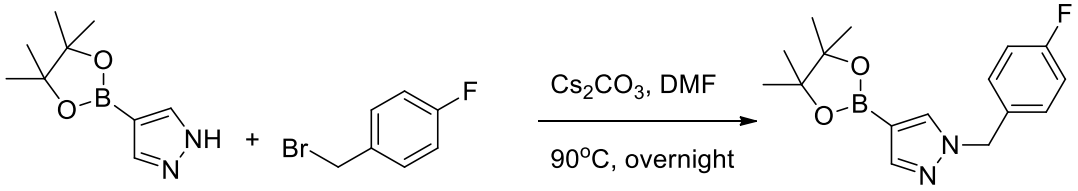
To a solution of 4-pyrazole boronic acid pinacol ester (5.0 mmol) in DMF (10 mL), the corresponding benzyl bromide (1.1 eq.) and Cs_2CO_3 (1.1 eq.) were added. The resulting mixture was allowed to stir at at 90 ^{o}C overnight. DMF was removed *in vacuo*. The residue was dissolved in water, and extracted with DCM (25 mL X 3). All organic layers were combined, washed with brine and dried with $MgSO_4$. The solvent was evaporated to give the crude product which was purified by column chromatography using 20% EtOAc in DCM to give the desired compound.

Preparation of 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1-(4-(trifluoromethoxy)benzyl)-1H-pyrazole



The reaction, work-up and purification procedure of title compound followed General Procedure A. The title product was obtained as a colorless oil in 81%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.70 (s, 1H), 7.28 – 7.22 (m, 2H), 7.18 (d, J = 8.8 Hz, 2H), 5.31 (s, 2H), 1.31 (s, 12H).

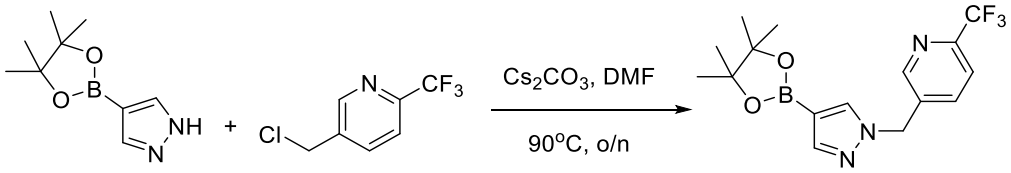
Preparation of 1-(4-fluorobenzyl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole



The reaction, work-up and purification procedure of title compound followed General Procedure A. The title product was obtained as a pale yellow oil in 82% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ

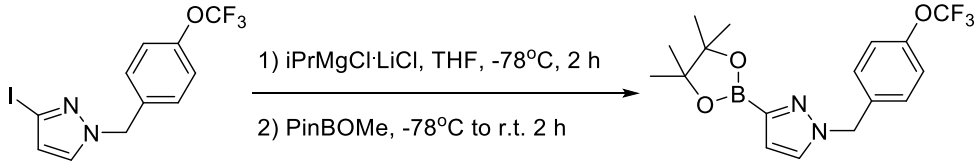
7.81 (s, 1H), 7.66 (s, 1H), 7.25 – 7.16 (m, 2H), 7.09 – 6.98 (m, 2H), 5.27 (s, 2H), 1.30 (s, 12H).

Preparation of 5-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazol-1-yl)methyl)-2-(trifluoromethyl)pyridine



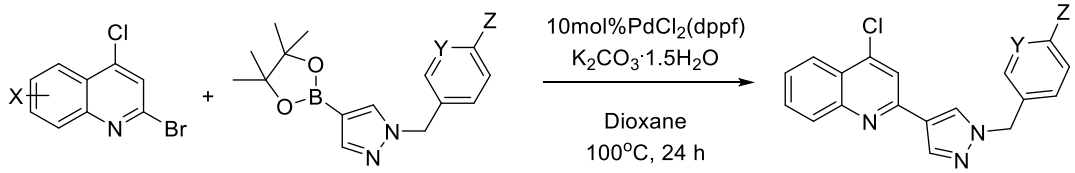
The reaction, work-up and purification procedure of title compound followed General Procedure A. The title product was obtained as a pale yellow oil in 75% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.63 (s, 1H), 7.85 (s, 1H), 7.75 (s, 1H), 7.71 – 7.62 (m, 2H), 5.42 (s, 2H), 1.32 (s, 12H).

Preparation of 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1-(4-(trifluoromethoxy) benzyl)-1H-pyrazole



A solution of 3-iodo-1-(4-(trifluoromethoxy)benzyl)-1H-pyrazole(0.74 g, 2.0 mmol) in THF (8 mL) was cooled to -78°C, a solution of iPrMgCl·LiCl (1.3M in THF, 1.6 mL, 2.1 mmol) was then added dropwise. After addition, the resulting solution was kept stirring at -78°C for 2 hours. After this, 2-methoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (0.36 g, 0.38 mL, 2.2 mmol) was added, and the reaction temperature was allowed to rise to room temperature slowly. After 2 hours at room temperature, the reaction was quenched with NH_4Cl (sat.) (1mL). The reaction mixture was diluted with Et_2O (20 mL) and H_2O (9 mL). The organic layer was separated, and the water layer was extracted with Et_2O (10 mL X 2). The organic layers were combined, washed with brine, dried over Na_2SO_4 and evaporated to give the crude product. The crude material was purified by flash column chromatography eluting with 10 to 20% EtOAc in DCM to give the title product as a colorless oil in 58% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (d, J = 2.3 Hz, 1H), 7.22 (d, J = 8.6 Hz, 2H), 7.16 (d, J = 8.2 Hz, 2H), 6.72 (d, J = 2.3 Hz, 1H), 5.43 (s, 2H), 1.37 (s, 12H).

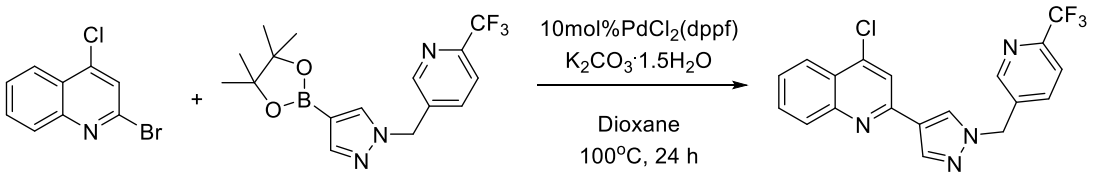
General Procedure B - Preparation of 2-Substituted Quinoline Derivatives



To a solution of 2-bromo-4-chloroquinoline (1.0 mmol) in Dioxane (20 mL), the boronic acid pinacol ester (1 eq.), $PdCl_2$(dppf) (0.10 eq.) and K_2CO_3•1.5H_2O (2.5 eq.) were added. The reaction mixture was degassed and refilled with N_2, the reaction was then heated to reflux for 24 hours. After 24h the resulting mixture was filtered through a pad of silica to remove the Pd catalyst and the inorganic salts. The filtrate was evaporated to give the crude product which was purified by

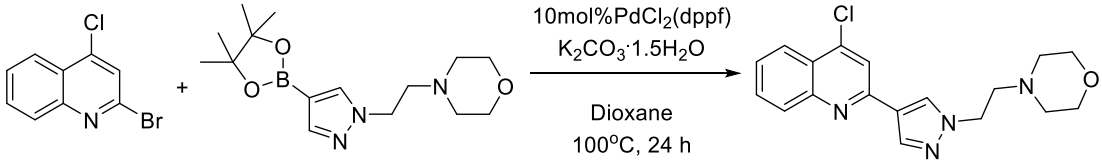
column chromatography using 10% EtOAc in DCM to give the desired compound.

Preparation of 4-chloro-2-(1-((6-(trifluoromethyl)pyridin-3-yl)methyl)-1H-pyrazol-4-yl)quinoline (3a)



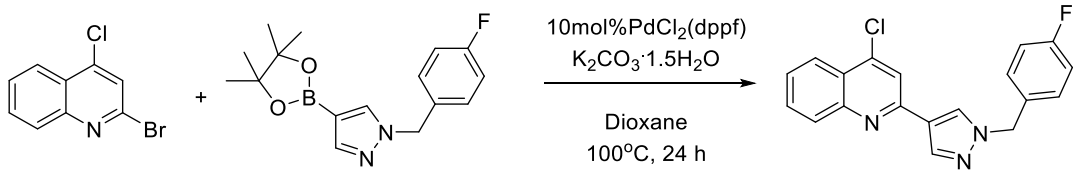
The reaction, work-up and purification procedure of the title compound followed General Procedure B. The title product was obtained as a pale yellow crystalline solid in 51% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.73 (s, 1H), 8.26 – 8.12 (m, 3H), 8.02 (d, J = 8.3 Hz, 1H), 7.71 – 7.76 (m, 2H), 7.69 (t, J = 8.0 Hz, 1H), 7.61 (d, J = 8.6 Hz, 1H), 7.49 (t, J = 7.5 Hz, 1H), 5.50 (s, 2H).

Preparation of 4-(2-(4-(4-chloroquinolin-2-yl)-1H-pyrazol-1-yl)ethyl)morpholine (3b)



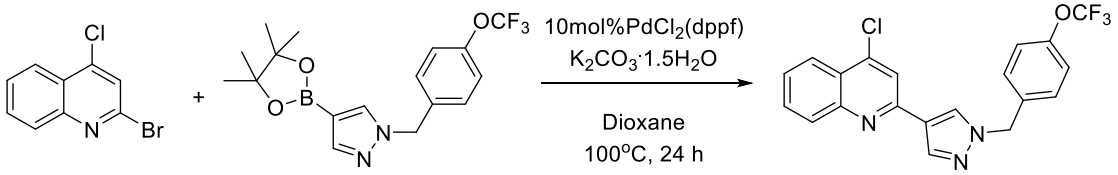
The reaction, work-up and purification procedure of the title compound followed General Procedure B. The title product was obtained as a pale yellow crystalline solid in 93% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 – 8.15 (m, 2H), 8.10 (s, 1H), 8.04 (d, J = 8.5 Hz, 1H), 7.73 (d, J = 7.0 Hz, 1H), 7.69 (s, 1H), 7.56 (t, J = 7.1 Hz, 1H), 4.32 (t, J = 6.6 Hz, 2H), 3.76 – 3.69 (m, 4H), 2.89 (t, J = 6.7 Hz, 2H), 2.57 – 2.49 (m, 4H).

Preparation of 4-chloro-2-(1-(4-fluorobenzyl)-1H-pyrazol-4-yl)quinoline (3c)



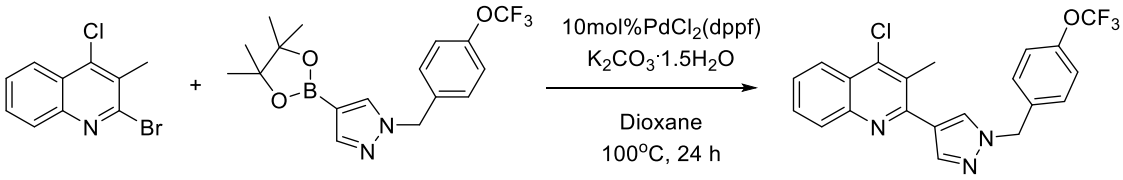
The reaction, work-up and purification procedure of the title compound followed General Procedure B. The title product was obtained as an off-white solid in 36% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 – 8.12 (m, 2H), 8.09 (s, 1H), 8.02 (d, J = 8.5 Hz, 1H), 7.73 (dd, J = 8.4, 6.9 Hz, 1H), 7.67 (s, 1H), 7.55 (dd, J = 8.1, 6.9 Hz, 1H), 7.30 (dd, J = 8.5, 5.3 Hz, 2H), 7.06 (t, J = 8.6 Hz, 2H), 5.35 (s, 2H).

Preparation of 4-chloro-2-(1-(4-(trifluoromethoxy)benzyl)-1H-pyrazol-4-yl)quinoline (3d)



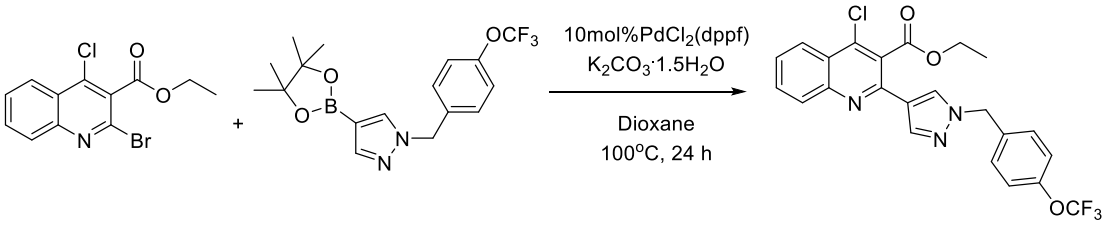
The reaction, work-up and purification procedure of the title compound followed General Procedure B. The title product was obtained as a pale yellow solid (63%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 – 8.11 (m, 3H), 8.02 (d, J = 8.5 Hz, 1H), 7.73 (dd, J = 8.4, 6.9 Hz, 1H), 7.69 (s, 1H), 7.56 (dd, J = 8.2, 6.9 Hz, 1H), 7.33 (d, J = 8.8 Hz, 2H), 7.22 (d, J = 8.0 Hz, 2H), 5.39 (s, 2H).

Preparation of 4-chloro-3-methyl-2-(1-(4-(trifluoromethoxy)benzyl)-1H-pyrazol-4-yl)quinoline (3e)



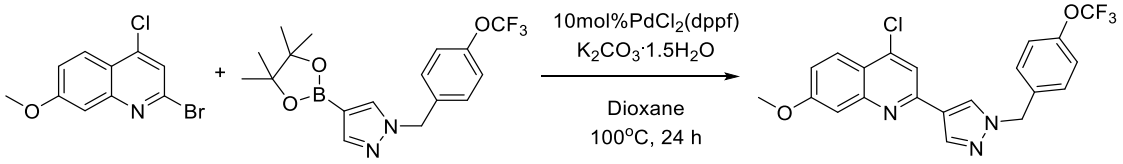
The reaction, work-up and purification procedure of the title compound followed General Procedure B. The title product was obtained as a pale yellow crystalline solid in 38% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (d, J = 8.4 Hz, 1H), 8.05 (s, 1H), 8.02 (d, J = 8.4 Hz, 1H), 7.98 (s, 1H), 7.69 (dd, J = 8.4, 6.9 Hz, 1H), 7.57 (dd, J = 8.2, 6.9 Hz, 1H), 7.35 (d, J = 8.6 Hz, 2H), 7.22 (d, J = 8.1 Hz, 2H), 5.40 (s, 2H), 2.73 (s, 3H).

Preparation of ethyl 4-chloro-2-(1-(4-(trifluoromethoxy)benzyl)-1H-pyrazol-4-yl)quinoline-3-carboxylate (3f)



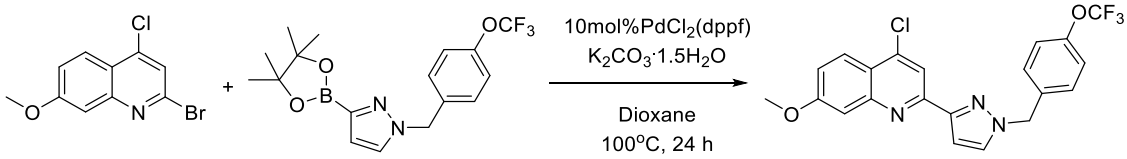
The reaction, work-up and purification procedure of the title compound followed General Procedure B using K_3PO_4·1.5H_2O instead of K_2CO_3. The title product was obtained as a pale yellow crystalline solid in 68% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (dd, J = 8.4, 0.9 Hz, 1H), 8.06 (d, J = 8.4 Hz, 1H), 8.01 (s, 1H), 7.95 (s, 1H), 7.79 (dd, J = 8.4, 6.9 Hz, 1H), 7.63 (dd, J = 8.2, 6.9 Hz, 1H), 7.33 (d, J = 8.7 Hz, 2H), 7.22 (d, J = 9.0 Hz, 2H), 5.36 (s, 2H), 4.39 (q, J = 7.2 Hz, 2H), 1.31 (t, J = 7.2 Hz, 3H).

Preparation of 4-chloro-7-methoxy-2-(1-(4-(trifluoromethoxy)benzyl)-1H-pyrazol-4-yl)quinoline (3g)



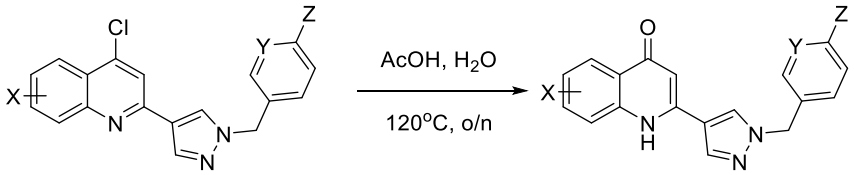
The reaction, work-up and purification procedure of the title compound followed General Procedure B. The title product was obtained as an off-white solid in 53% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (s, 1H), 8.09 (s, 1H), 8.05 (d, J = 9.2 Hz, 1H), 7.54 (s, 1H), 7.36 – 7.29 (m, 3H), 7.24 – 7.15 (m, 3H), 5.38 (s, 2H), 3.96 (s, 3H).

Preparation of 4-chloro-7-methoxy-2-(1-(4-(trifluoromethoxy)benzyl)-1H-pyrazol-3-yl)quinoline (3h)



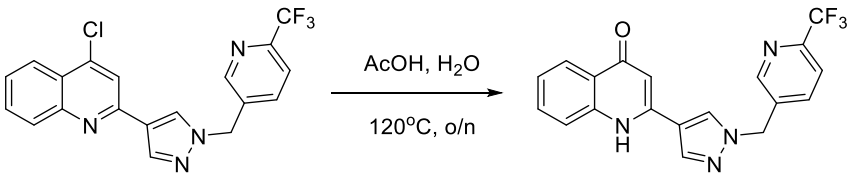
The reaction, work-up and purification procedure of title compound followed General Procedure B. The title product was obtained as a pale yellow solid in 52% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 – 8.04 (m, 2H), 7.48 (dd, J = 6.0, 2.4 Hz, 2H), 7.32 – 7.27 (m, 2H), 7.25 – 7.18 (m, 3H), 7.08 (d, J = 2.4 Hz, 1H), 5.44 (s, 2H), 3.97 (s, 3H).

General Procedure C - Preparation of Quinolin-4(1H)-one Analogues



To a suspended 4-chloroquinoline starting material (0.5 mmol) in water (2 mL), AcOH (8 mL) was added. The resulting mixture was heated to 120 °C and was stirred overnight. Saturated NH_4•H_2O(aq) was used to basify the resulting solution. During the basification, some precipitate was formed. The precipitate was separated by filtration to give the crude product which was purified by flash column chromatograph eluting with 5-10% MeOH in DCM to give the desired compound.

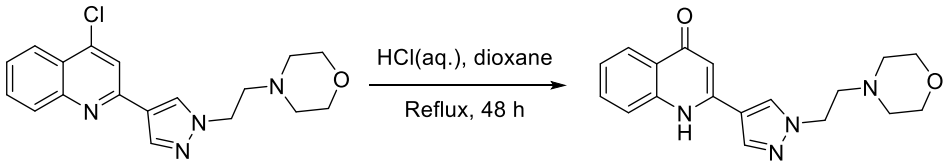
Preparation of 2-(1-((6-(trifluoromethyl)pyridin-3-yl)methyl)-1H-pyrazol-4-yl)quinolin-4(1H) -one (4a)



The reaction, work-up and purification procedure of title compound followed General Procedure C. The title product was obtained as a white solid in 53% yield. Melting point: 272 – 273 °C. ^{1}H NMR (400 MHz, DMSO) δ 11.43 (s, 1H), 8.78 (s, 1H), 8.64 (s, 1H), 8.24 (s, 1H), 8.05 (d, J = 7.7 Hz,

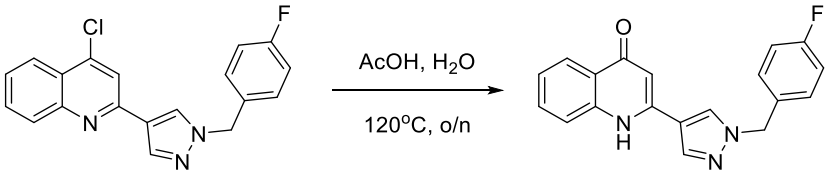
1H), 8.01 – 7.92 (m, 2H), 7.71 – 7.59 (m, 2H), 7.30 (t, J = 7.1 Hz, 1H), 6.41 (s, 1H), 5.64 (s, 2H); ^{13}C NMR (101 MHz, DMSO) δ 178.58, 162.41, 149.99, 149.05, 148.44, 140.71, 138.87, 138.12, 136.82, 132.00, 130.76, 126.56, 125.25, 125.03, 123.33, 121.21, 118.52, 105.29, 52.55. ES HRMS: m/z found 371.1115, $C_{19}H_{14}N_4OF_3$ $[M+H]^+$ requires 371.1120; Purity HPLC 97.8%, R_t = 7.65 min.

Preparation of 2-(1-(2-morpholinoethyl)-1H-pyrazol-4-yl)quinolin-4(1H)-one (4b)



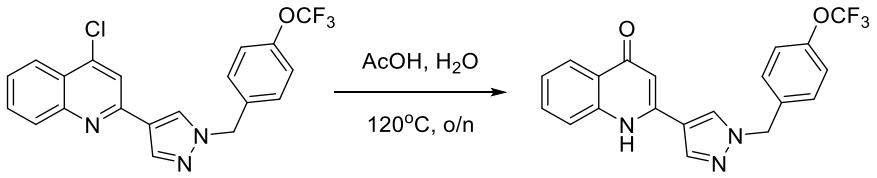
To a solution of 4-(2-(4-(4-chloroquinolin-2-yl)-1H-pyrazol-1-yl)ethyl)morpholine (150 mg, 0.44 mmol) in dioxane (10 mL), a solution of HCl (10 mL, 2M) in H_2O was added. The resulting mixture was heated to reflux with stirring for 48 hours. Subsequently, all solvents in the reaction mixture were evaporated. The residue was basified with NaOH(aq.). The water in the resulting mixture was removed *in vacuo* and the residue was redissolved in 30% MeOH in DCM. The precipitate formed was removed by filtration and the filtrate was concentrated to give the crude product as a dark oil. The crude product was purified by flash column chromatograph eluting with 10%-20% MeOH in DCM to give the title product (130mg, 90%) as a white solid. Melting point: 238 – 239 ^{o}C. ^{1}H NMR (400 MHz, DMSO) δ 11.41 (s, 1H), 8.50 (s, 1H), 8.16 (s, 1H), 8.05 (d, J = 8.1 Hz, 1H), 7.70 (d, J = 8.1 Hz, 1H), 7.64 (t, J = 8.2 Hz, 1H), 7.29 (t, J = 7.9 Hz, 1H), 6.38 (s, 1H), 4.31 (t, J = 6.5 Hz, 2H), 3.63 – 3.49 (m, 4H), 2.76 (t, J = 6.5 Hz, 2H), 2.46 – 2.39 (m, 4H); ^{13}C NMR (101 MHz, DMSO) δ 177.03, 143.60, 140.64, 137.74, 131.94, 130.42, 125.28, 125.03, 123.26, 118.46, 116.28, 104.97, 66.52, 57.87, 53.48, 49.44. ES HRMS: m/z found 325.1653, $C_{18}H_{21}N_4O_2$ $[M+H]^+$ requires 325.1665; Purity HPLC 98.9%, R_t = 5.47 min.

Preparation of 2-(1-(4-fluorobenzyl)-1H-pyrazol-4-yl)quinolin-4(1H)-one (4c)



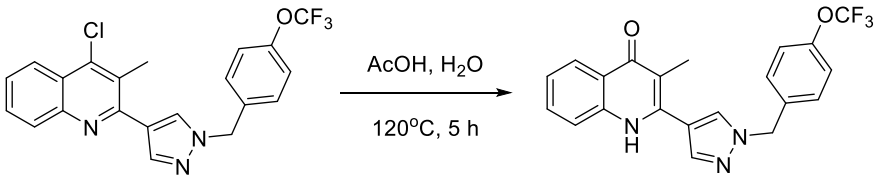
The reaction, work-up and purification procedure of title compound followed General Procedure C. The title product was obtained as a white solid in 79% yield. Melting point: 308-310 ^{o}C. ^{1}H NMR (400 MHz, DMSO) δ 11.39 (s, 1H), 8.57 (s, 1H), 8.20 (s, 1H), 8.05 (d, J = 8.3 Hz, 1H), 7.72 – 7.59 (m, 2H), 7.45 – 7.34 (m, 2H), 7.29 (t, J = 7.1 Hz, 1H), 7.26 – 7.18 (m, 2H), 6.40 (s, 1H), 5.42 (s, 2H); ^{13}C NMR (101 MHz, DMSO) δ 177.05, 160.93, 143.37, 140.63, 138.37, 133.43, 131.98, 130.46, 130.17, 125.28, 125.04, 123.28, 118.44, 116.89, 115.73, 105.16, 54.85. ES HRMS: m/z found 320.1188, $C_{19}H_{15}N_3OF$ $[M+H]^+$ requires 320.1199; Purity HPLC 98.7%, R_t = 7.72 min.

Preparation of 2-(1-(4-(trifluoromethoxy)benzyl)-1H-pyrazol-4-yl)quinolin-4(1H)-one (4d)



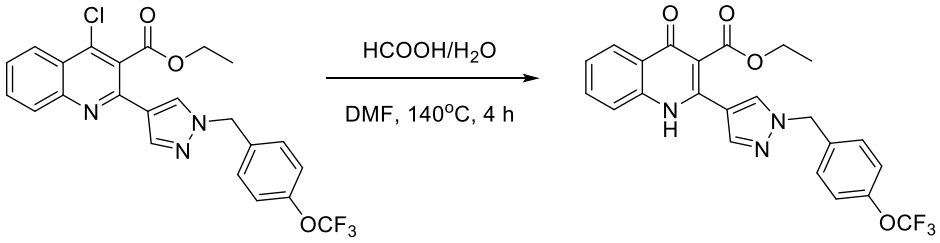
The reaction, work-up and purification procedure of title compound followed General Procedure C. The title product was obtained as a white solid in 67% yield. Melting point: 300-301 °C. ^{1}H NMR (400 MHz, DMSO) δ 11.40 (s, 1H), 8.60 (s, 1H), 8.21 (s, 1H), 8.05 (d, J = 7.8 Hz, 1H), 7.69 – 7.60 (m, 2H), 7.47 – 7.36 (m, 4H), 7.30 (ddd, J = 8.1, 5.7, 2.4 Hz, 1H), 6.40 (s, 1H), 5.48 (s, 2H); ^{13}C NMR (101 MHz, DMSO) δ 176.65, 147.86, 142.92, 141.41, 140.23, 138.10, 136.33, 131.59, 130.01, 129.71, 124.88, 124.65, 122.89, 121.25, 118.04, 116.58, 104.81, 54.35. ES HRMS: m/z found 386.1106, $C_{20}H_{15}N_3O_2F_3$ $[M+H]^+$ requires 386.1116; Purity HPLC 95.1%, R_t = 8.64 min.

Preparation of 3-methyl-2-(1-(4-(trifluoromethoxy)benzyl)-1H-pyrazol-4-yl)quinolin-4(1H)-one (4e)



The reaction, work-up and purification procedure of title compound followed General Procedure C. The title product was obtained as a white solid in 47% yield. Melting point: 193-195°C. ^{1}H NMR (400 MHz, DMSO) δ 11.28 (s, 1H), 8.44 (s, 1H), 8.08 (d, J = 8.1 Hz, 1H), 7.96 (s, 1H), 7.66 – 7.54 (m, 2H), 7.46 (d, J = 8.6 Hz, 2H), 7.39 (d, J = 8.5 Hz, 2H), 7.26 (t, J = 7.0 Hz, 1H), 5.50 (s, 2H), 2.09 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 176.79, 148.21, 147.86, 147.44, 140.46, 139.90, 137.01, 131.82, 131.49, 130.09, 125.32, 123.17, 122.80, 121.62, 118.27, 116.24, 114.25, 54.48, 12.52. ES HRMS: m/z found 400.1261, $C_{21}H_{17}N_3O_2F_3$ $[M+H]^+$ requires 400.1273; Purity HPLC 98.4%, R_t = 9.09 min.

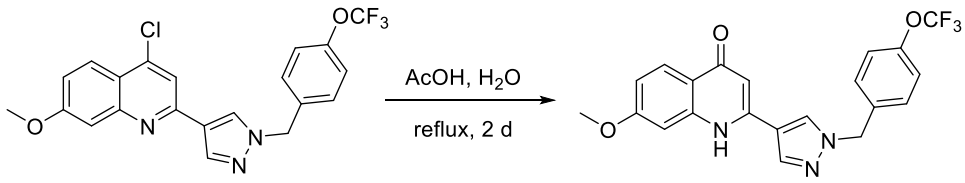
Preparation of ethyl 4-oxo-2-(1-(4-(trifluoromethoxy)benzyl)-1H-pyrazol-4-yl)-1,4-dihydroquinoline-3-carboxylate (4f)



To a solution of 4-chloro-2-(1-(4-(trifluoromethoxy)benzyl)-1H-pyrazol-4-yl)quinoline-3-carboxylate (75 mg, 0.16 mmol) in DMF (6 mL), HCOOH (85% in H_2O, 2 mL) was added. The resulting mixture was heated to 140°C for 4 hours. After that, all solvents and HCOOH were removed *in vacuo* to give the crude product. The crude product was purified by flash column chromatograph eluting with 5% MeOH in DCM to give the title compound (52mg, 71%) as a white solid. Melting point: 208-210°C. ^{1}H NMR (400 MHz, DMSO) δ 11.75 (s, 1H), 8.26 (s, 1H), 8.07 (d, J = 8.7 Hz, 1H), 7.87 (s, 1H), 7.73 – 7.65 (m, 2H), 7.45 (d, J = 8.7 Hz, 2H), 7.42 – 7.34 (m, 3H), 5.50 (s, 2H), 4.09 (q, J = 7.1 Hz, 2H), 1.05 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, DMSO) δ

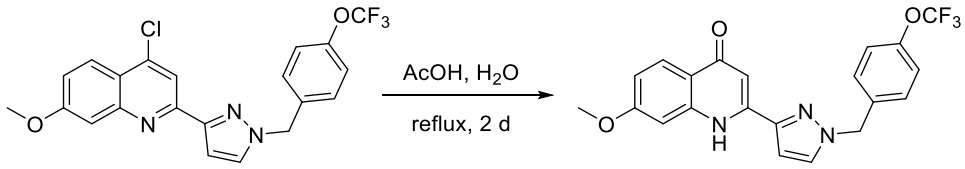
173.97, 167.36, 156.87, 148.28, 141.10, 140.05, 139.18, 136.74, 132.74, 131.20, 130.23, 125.15, 124.60, 124.14, 121.64, 118.85, 115.06, 114.72, 61.01, 54.50, 14.06. ES HRMS: m/z found 480.1156, $C_{23}H_{18}N_3O_4F_3Na$ $[M+Na]^+$ requires 480.1147; Purity HPLC 95.1%, R_t = 9.44 min.

Preparation of
7-methoxy-2-(1-(4-(trifluoromethoxy)benzyl)-1H-pyrazol-4-yl)quinolin-4(1H)-one (4g)



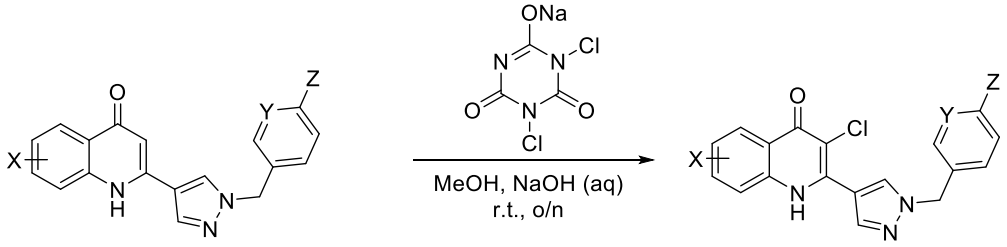
The reaction, work-up and purification procedure of title compound followed General Procedure C. The title product was obtained as an off-white solid in 89% yield. Melting point: 266-268°C. ^{1}H NMR (400 MHz, DMSO) δ 11.23 (s, 1H), 8.56 (s, 1H), 8.18 (s, 1H), 7.95 (d, J = 8.9 Hz, 1H), 7.44 (d, J = 8.8 Hz, 2H), 7.38 (d, J = 6.0 Hz, 2H), 7.07 (d, J = 2.4 Hz, 1H), 6.89 (dd, J = 8.9, 2.4 Hz, 1H), 6.32 (d, J = 1.7 Hz, 1H), 5.48 (s, 2H), 3.87 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 176.67, 162.17, 150.28, 148.28, 142.96, 142.35, 141.40, 138.30, 136.74, 134.38, 130.12, 126.86, 121.65, 119.58, 117.05, 113.17, 104.96, 55.77, 54.76. ES HRMS: m/z found 416.1234, $C_{21}H_{17}N_3O_3F_3$ $[M+H]^+$ requires 416.1222; Purity HPLC 96.3%, R_t = 8.87 min.

Preparation of
7-methoxy-2-(1-(4-(trifluoromethoxy)benzyl)-1H-pyrazol-3-yl)quinolin-4(1H)-one (4h)



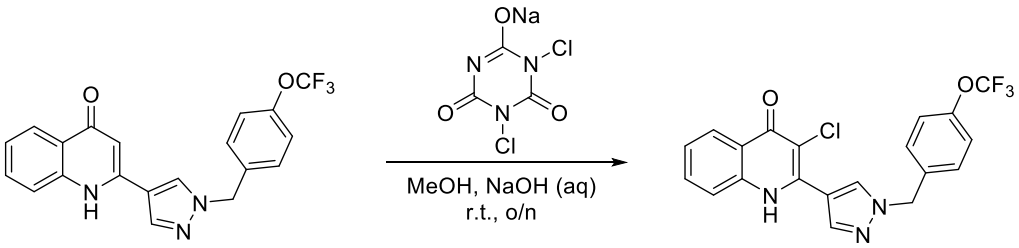
The reaction, work-up and purification procedure of title compound followed General Procedure C. The title product was obtained as an off-white solid in 92% yield. Melting point: 199-200°C. ^{1}H NMR (400 MHz, DMSO) δ 11.41 (s, 1H), 8.08 (d, *J* = 2.3 Hz, 1H), 7.96 (d, *J* = 8.9 Hz, 1H), 7.44 – 7.34 (m, 5H), 7.05 (d, *J* = 2.3 Hz, 1H), 6.90 (dd, *J* = 8.9, 2.4 Hz, 1H), 6.49 (d, *J* = 1.5 Hz, 1H), 5.54 (s, 2H), 3.85 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 177.05, 162.36, 148.32, 145.92, 142.87, 142.47, 137.06, 133.67, 129.83, 126.86, 121.77, 120.06, 119.24, 113.38, 105.76, 105.63, 100.54, 55.83, 54.86. ES HRMS: m/z found 416.1217, $C_{21}H_{17}N_3O_3F_3$ $[M+H]^+$ requires 416.1217; Purity HPLC 95.8%, R_t = 9.32 min.

General Procedure D – Chlorination of 3-H quinolone analogues



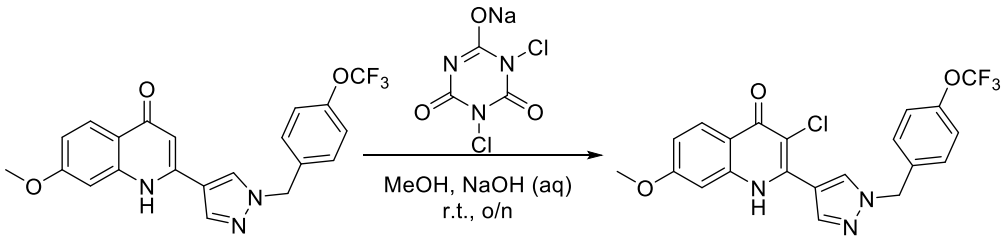
To a suspension of quinolin-4(1H)-one (0.17 mmol) in MeOH (5 mL) and NaOH(aq.) (1M in H_2O, 1 mL), sodium dichloroisocyanurate (0.54 eq.) was added. The reaction mixture was stirred overnight at room temperature (followed by TLC). The reaction was quenched by acidification with HCl(aq.) (2M in H_2O, 0.5 mL), and was filtered. The filter cake was washed thoroughly with MeOH/DCM (1:1, 40 mL). The filtrate and the wash solution were combined and concentrated *in vacuo* to give the crude product. The crude product was purified by flash column chromatograph eluting with 10% MeOH in DCM to give the desired compound.

Preparation of 3-chloro-2-(1-(4-(trifluoromethoxy)benzyl)-1H-pyrazol-4-yl)quinolin-4(1H)-one (5a)



The reaction, work-up and purification procedure of title compound followed General Procedure D. The title product was obtained as a white solid in 56% yield. Melting point: 255-257^{o}C. ^{1}H NMR (400 MHz, DMSO) δ 11.81 (s, 1H), 8.69 (s, 1H), 8.15 (s, 1H), 8.13 (d, J = 7.8 Hz, 1H), 7.75 – 7.65 (m, 2H), 7.48 (d, J = 8.7 Hz, 2H), 7.43 – 7.33 (m, 3H), 5.53 (s, 2H);^{13}C NMR (101 MHz, DMSO) δ 171.16, 156.30, 147.81, 140.55, 139.93, 138.86, 136.33, 132.45, 131.95, 129.73, 124.94, 123.55, 123.00, 121.17, 118.25, 113.72, 112.22, 54.06. ES HRMS: m/z found 420.0727 and 422.0714, $C_{20}H_{14}N_3O_2F_3Cl$ $[M+H]^+$ requires 420.0727 and 422.0697; Purity HPLC 98.4%, R_t = 9.49 min.

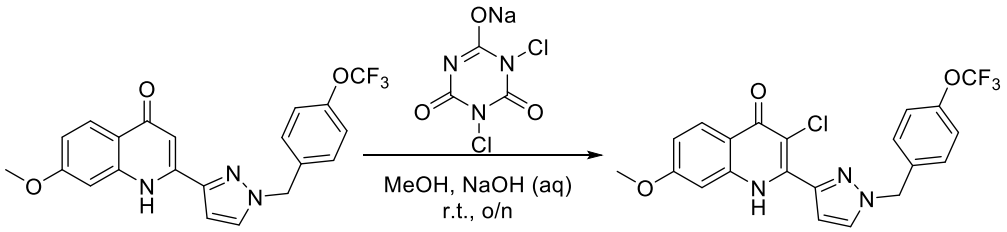
Preparation of 3-chloro-7-methoxy-2-(1-(4-(trifluoromethoxy)benzyl)-1H-pyrazol-4-yl)quinolin-4(1H)-one (5b)



The reaction, work-up and purification procedure of title compound followed General Procedure D. The title product was obtained as a pale yellow solid in 65% yield. Melting point: 234-236 ^{o}C. ^{1}H NMR (400 MHz, DMSO) δ 11.60 (s, 1H), 8.67 (s, 1H), 8.13 (s, 1H), 8.01 (d, J = 9.0 Hz, 1H), 7.47 (d, J = 8.7 Hz, 2H), 7.40 (d, J = 8.1 Hz, 2H), 7.12 (d, J = 2.4 Hz, 1H), 6.97 (dd, J = 9.0, 2.4 Hz, 1H), 5.53 (s, 2H), 3.86 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 170.77, 162.02, 147.86, 140.74, 140.09, 139.80, 137.30, 136.39, 132.34, 129.76, 126.87, 121.23, 118.74, 117.45, 113.85, 111.98, 99.06, 55.46, 54.13. ES HRMS: m/z found 450.0844 and 452.0823, $C_{21}H_{16}N_3O_3F_3Cl$ $[M+H]^+$ requires 450.0832 and 452.0803; Purity HPLC 98.3%, R_t = 9.62 min.

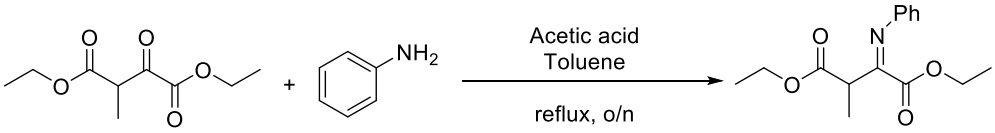
Preparation of 3-chloro-7-methoxy-2-(1-(4-(trifluoromethoxy)benzyl)-1H-pyrazol-3-yl)quinolin-

4(1H)-one (5c)



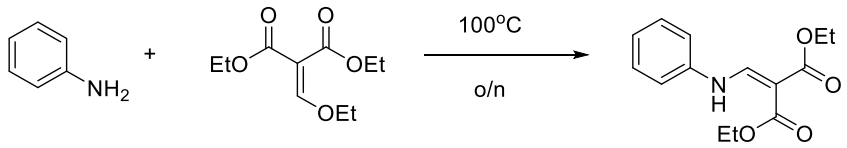
The reaction, work-up and purification procedure of title compound followed General Procedure D. The title product was obtained as a pale yellow solid in 72% yield. Melting point: 176-177^{o}C. ^{1}H NMR (400 MHz, DMSO) δ 11.73 (s, 1H), 8.15 (d, J = 2.4 Hz, 1H), 8.02 (d, J = 9.0 Hz, 1H), 7.46 (d, J = 8.7 Hz, 2H), 7.43 – 7.37 (m, 3H), 7.11 (d, J = 2.4 Hz, 1H), 6.97 (dd, J = 9.0, 2.4 Hz, 1H), 5.58 (s, 2H), 3.86 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 171.06, 162.14, 147.89, 143.51, 140.70, 139.66, 136.34, 132.40, 129.53, 126.80, 121.30, 117.64, 114.06, 112.88, 108.83, 104.13, 99.66, 55.46, 54.42. ES HRMS: m/z found 450.0827 and 452.0815, $C_{21}H_{16}N_3O_3F_3Cl$ $[M+H]^+$ requires 450.0832 and 452.0803; Purity HPLC 99.0%, R_t = 10.39 min.

Preparation of diethyl 2-methyl-3-(phenylimino)succinate



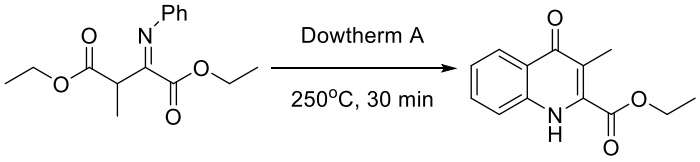
To a solution of diethyl oxalpropionate (2.02 g, 10.0 mmol) in toluene (20 mL), aniline (4.9 g, 53 mmol) and acetic acid (0.2 mL) were added. The resulting solution was heated to reflux overnight in a Dean-Stark condenser to remove the water generated. When the reaction was completed, the solvent was removed *in vacuo* to give the crude product as a yellow oil. The crude product was purified by flash column chromatograph eluting with 10% EtOAc in hexane to give the title compound (1.85 g, 67%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.17 (s, 1H), 7.25 (dd, J = 8.4, 7.5 Hz, 2H), 7.05 (t, J = 7.4 Hz, 1H), 6.98 (d, J = 7.5 Hz, 2H), 4.22 (q, J = 7.1 Hz, 2H), 4.16 (q, J = 7.1 Hz, 2H), 1.85 (s, 3H), 1.32 (t, J = 7.1 Hz, 3H), 1.10 (t, J = 7.1 Hz, 3H).

Preparation of diethyl 2-((phenylamino)methylene)malonate



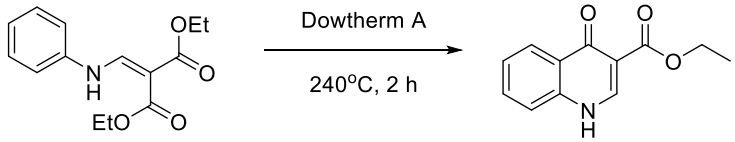
A mixture of aniline (2.13 mL, 23.2 mmol) and diethyl-ethoxymethylonemalonate (5.07 g, 23.2 mmol) was stirred and heated to 100^{o}C overnight. The EtOH side-product was removed *in vacuo* to give the crude product which was purified by flash column chromatograph eluting with 20% EtOAc in hexane to give the title compound (5.22 g, 85%) as a pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.02 (d, J = 13.2 Hz, 1H), 8.54 (d, J = 13.7 Hz, 1H), 7.38 (t, J = 8.0 Hz, 2H), 7.20 – 7.11 (m, 3H), 4.32 (q, J = 7.1 Hz, 2H), 4.25 (q, J = 7.1 Hz, 2H), 1.39 (t, J = 7.1 Hz, 3H), 1.33 (t, J = 7.1 Hz, 3H).

Preparation of ethyl 3-methyl-4-oxo-1,4-dihydroquinoline-2-carboxylate



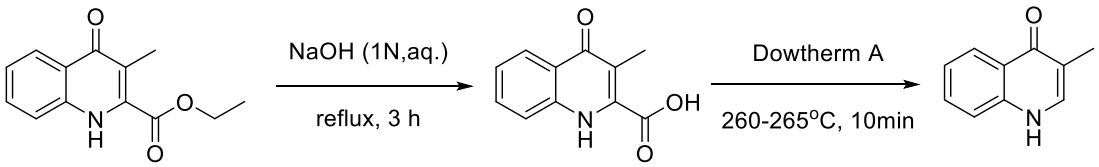
A solution of diethyl 2-methyl-3-(phenylimino)succinate (1.65 g, 6.0 mmol) in Dowtherm A (15 mL) was kept at 250 °C for 30 min. The resulting mixture was cooled to room temperature anddiluted with hexane (60 mL). The resulting precipitate was collected by filtration, washed with Et_2O and dried *in vacuo* to give the title compound (1.0 g, 72%) as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO) δ 11.71 (s, 1H), 8.10 (d, J = 8.2 Hz, 1H), 7.81 (d, J = 8.1 Hz, 1H), 7.67 (dd, J = 8.5, 6.9 Hz, 1H), 7.33 (dd, J = 8.1, 6.9 Hz, 1H), 4.46 (q, J = 7.1 Hz, 2H), 2.22 (s, 3H), 1.40 (t, J = 7.1 Hz, 3H).

Preparation of ethyl 4-oxo-1,4-dihydroquinoline-3-carboxylate



The solution of diethyl 2-((phenylamino)methylene)malonate (2.63 g, 10.0 mmol) in Dowtherm A (10 mL) was kept at 240 °C for 2 hours. After this, that resulting mixture was cooled to room temperature and diluted with hexane (60 mL).The precipitate formed was separated by filtration, washed with Et_2O and dried *in vacuo* to give the title compound (0.82 g, 38%) as an off-white solid. ^{1}H NMR (400 MHz, DMSO) δ 8.55 (s, 1H), 8.16 (d, J = 8.1 Hz, 1H), 7.72 (t, J = 7.6 Hz, 1H), 7.61 (d, J = 8.2 Hz, 1H), 7.43 (t, J = 7.5 Hz, 1H), 4.21 (q, J = 7.1 Hz, 2H), 1.27 (t, J = 7.1 Hz, 3H).

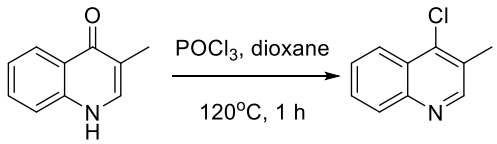
Preparation of 3-methylquinolin-4(1H)-one



To a solution of NaOH in H_2O (1M, 15 mL), ethyl 3-methyl-4-oxo-1,4-dihydroquinoline-2-carboxylate (0.95 g, 4.1 mmol) was added. The suspension was heated to reflux for 3 hours. After that, HCl (2M in H_2O, 10 mL) was used to acidify the reaction solution, and a precipitate was produced. The precipitate was collected by filtration, washed with EtOH and dried *in vacuo* to give the first step product, 3-methyl-4-oxo-1,4-dihydroquinoline-2-carboxylic acid as a white solid. This product was used directly in the next step without further purification.

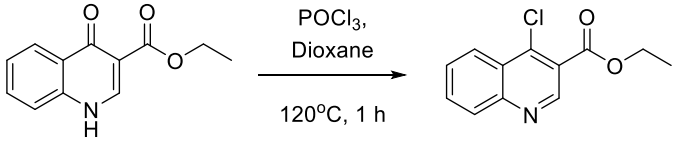
A suspension of 3-methyl-4-oxo-1,4-dihydroquinoline-2-carboxylic acid in Dowtherm A (5 mL) was kept at 260-265 °C for 10 min. The resulting mixture was cooled to room temperature anddiluted with hexane. The precipitate formed was collected by filtration, washed with Et_2O and dried *in vacuo* to give the title compound (0.43 g, 66%) as an off-white solid. ^{1}H NMR (400 MHz, DMSO) δ 11.64 (s, 1H), 8.11 (d, J = 8.1 Hz, 1H), 7.89 (d, J = 4.0 Hz, 1H), 7.60 (dd, J = 8.4, 6.9 Hz, 1H), 7.50 (d, J = 7.8 Hz, 1H), 7.28 (dd, J = 8.1, 6.9 Hz, 1H), 1.99 (s, 3H).

Preparation of 4-chloro-3-methylquinoline



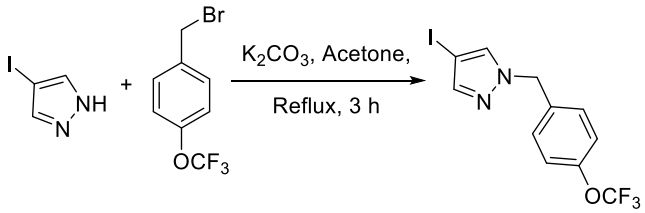
To a suspension of 3-methylquinolin-4(1H)-one (0.40 g, 2.5 mmol) in dioxane (5 mL), $POCl_3$ (0.28 mL, 3.0 mmol) was added slowly. The mixture was allowed to stir at 120 ^oC for 1 hour. The reaction mixture was then cooled to room temperature and poured into ice water (~15 mL). The resulting mixture was neutralized by K_2CO_3 (sat.) to pH7. The neutralized solution was extracted by DCM (20 mL X 3). The organic layers were combined, washed with brine and dried over Na_2SO_4. After removed all organic solvents, the title compound was obtained as a pale yellow crystalline solid. This product was used in the next step directly without further purification.

Preparation of ethyl 4-chloroquinoline-3-carboxylate



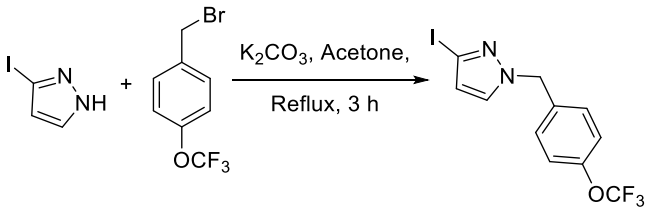
The reaction, work-up and purification procedure of the title compound followed a similar procedure as described previously for the preparation of 4-chloro-3-methylquinoline. The title product was obtained as a white crystalline solid in 57% yield. ^1H NMR (400 MHz, CDCl3) δ 9.22 (s, 1H), 8.42 (d, J = 8.5 Hz, 1H), 8.15 (d, J = 8.4 Hz, 1H), 7.86 (dd, J = 8.4, 6.9 Hz, 1H), 7.72 (dd, J = 8.3, 6.9 Hz, 1H), 4.51 (q, J = 7.1 Hz, 2H), 1.47 (t, J = 7.1 Hz, 3H).

Preparation of 4-iodo-1-(4-(trifluoromethoxy)benzyl)-1H-pyrazole



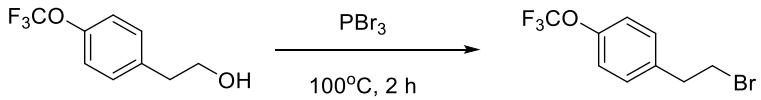
To a stirred suspension of 4-iodo-1H-pyrole (1.94 g, 10.0 mmol) and K_2CO_3 (3.46 g, 25 mmol) in acetone (50 mL), 4-(trifluoromethoxy)benzyl bromide (1.71 mL, 20.5 mmol) was added. The resulting mixture was heated to reflux for 3 hours. The reaction mixture was then cooled to room temperature and filtered to remove the insoluble salt. The filtrate was concentrated to give the crude product as a pale yellow oil. The crude product was purified by flash column chromatograph eluting with 10-20% EtOAc in hexane to give the title product (3.6 g, 99%) as a colorless oil. ^1H NMR (400 MHz, $CDCl_3$) δ 7.56 (s, 1H), 7.43 (s, 1H), 7.24 (d, J = 8.3 Hz, 2H), 7.20 (d, J = 8.3 Hz, 2H), 5.30 (s, 2H).

Preparation of 3-iodo-1-(4-(trifluoromethoxy)benzyl)-1H-pyrazole



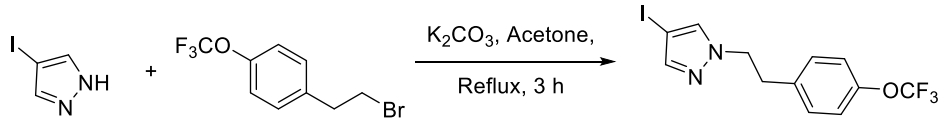
The reaction, work-up and purification procedure of title compound followed a similar procedure as described previously for the preparation of 4-iodo-1-(4-(trifluoromethoxy)benzyl)- 1H-pyrazole. The title product was obtained as a pale yellow solid in 71% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 – 7.17 (m, 5H), 6.46 (d, J = 2.3 Hz, 1H), 5.32 (s, 2H).

Preparation of 1-(2-bromoethyl)-4-(trifluoromethoxy)benzene



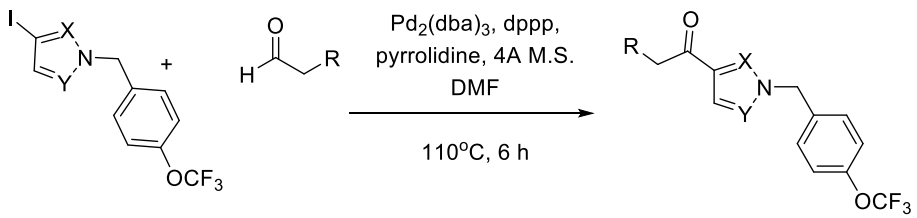
To neat 2-[4-(trifluoromethoxy)phenyl] ethanol (2.10 g, 10.0 mmol) which was cooled by a cold water bath, PBr_3 (0.58 mL, 6.0 mmol) was added dropwise. After addition, the reaction mixture was heated to 100°C for 2 hours. The reaction was quenched by adding the resulting mixture into ice-water dropwise. The resulting suspension was extracted with hexane (30 mL X 2). The organic layers were combined and dried with $MgSO_4$. After removal of all solvents *in vacuo*, the title compound (2.6 g, 97%) was obtained as a colorless oil. This product was used directly in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 (d, J = 8.6 Hz, 2H), 7.17 (d, J = 8.0 Hz, 2H), 3.56 (t, J = 7.4 Hz, 2H), 3.17 (t, J = 7.4 Hz, 2H).

Preparation of 4-iodo-1-(4-(trifluoromethoxy)phenethyl)-1H-pyrazole



The reaction, work-up and purification procedure of the title compound followed a similar procedure as described previously for the preparation of 4-iodo-1-(4-(trifluoromethoxy)benzyl)-1H-pyrazole. The title product was obtained as a pale yellow solid in 65% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (s, 1H), 7.22 (s, 1H), 7.13 (d, J = 8.0 Hz, 2H), 7.07 (d, J = 8.7 Hz, 2H), 4.32 (t, J = 7.2 Hz, 2H), 3.15 (t, J = 7.2 Hz, 2H).

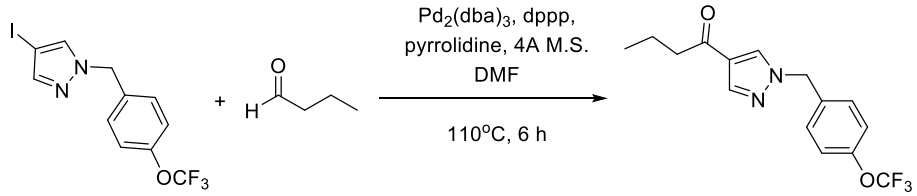
General Procedure E – Formation of ketone side chain



To a suspension of $Pd_2(dba)_3$ (0.046 mmol, 0.01 eq.), dppp (0.03 eq.) and 4Å M.S. (5 g) in DMF (20 mL), iodo-pyrazole (4.6 mmol), corresponding aldehyde (5 eq.) and pyrrolidine (2 eq.) were added. The resulting mixture was degassed and heated to 110°C for 6 hours under N_2 (followed

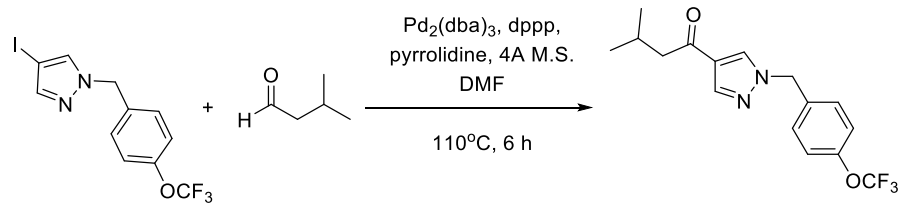
by TLC). The reaction mixture was diluted with 40% EtOAc in hexane (20 mL) and filtered through a pad of silica. The silica pad was washed further with 40% EtOAc in hexane (100 mL). The solvent was removed to give the crude product which was purified by flash column chromatograph eluting with 40-60% EtOAc in hexane to give the desired compound.

Preparation of 1-(1-(4-(trifluoromethoxy)benzyl)-1H-pyrazol-4-yl)butan-1-one (10a)



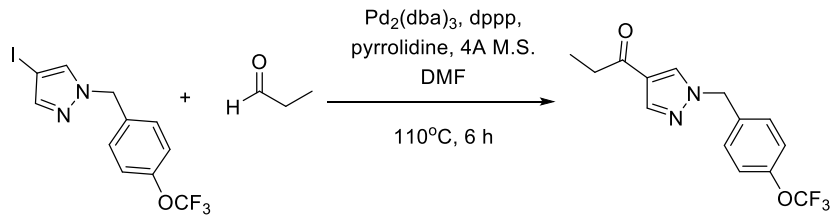
The reaction, work-up and purification procedure of title compound followed General Procedure E. The title product was obtained as a pale yellow solid in 53% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (s, 1H), 7.89 (s, 1H), 7.29 (d, J = 8.0 Hz, 2H), 7.22 (d, J = 7.9 Hz, 2H), 5.32 (s, 2H), 2.71 (t, J = 7.4 Hz, 2H), 1.79 – 1.66 (m, 2H), 0.97 (t, J = 7.4 Hz, 3H).

Preparation of 3-methyl-1-(1-(4-(trifluoromethoxy)benzyl)-1H-pyrazol-4-yl)butan-1-one (10b)



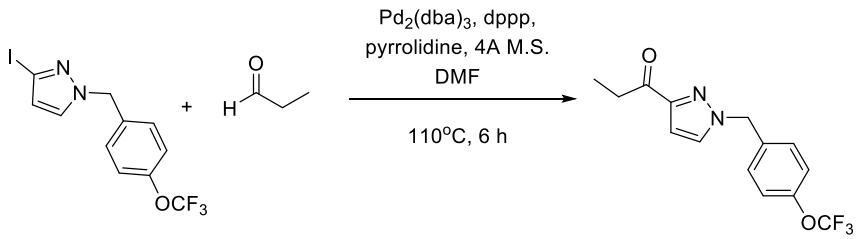
The reaction, work-up and purification procedure of title compound followed General Procedure E. The title product was obtained as a pale yellow solid in 55% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (s, 1H), 7.89 (s, 1H), 7.29 (d, J = 8.8 Hz, 2H), 7.22 (d, J = 8.6 Hz, 2H), 5.32 (s, 2H), 2.60 (d, J = 7.0 Hz, 2H), 2.31 – 2.18 (m, 1H), 0.97 (d, J = 6.7 Hz, 6H).

Preparation of 1-(1-(4-(trifluoromethoxy)benzyl)-1H-pyrazol-4-yl)propan-1-one (10c)



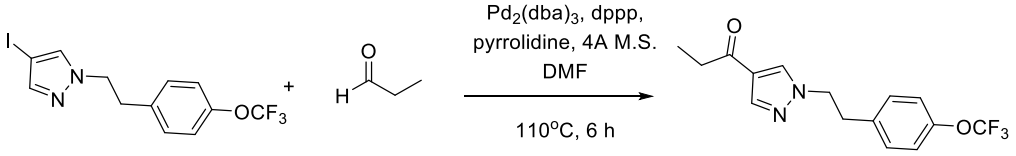
The reaction, work-up and purification procedure of title compound followed General Procedure E. The title product was obtained as a brown solid in 37% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (s, 1H), 7.90 (s, 1H), 7.28 (d, J = 8.7 Hz, 2H), 7.22 (d, J = 8.7 Hz, 2H), 5.32 (s, 2H), 2.78 (q, J = 7.3 Hz, 2H), 1.18 (t, J = 7.3 Hz, 3H).

Preparation of 1-(1-(4-(trifluoromethoxy)benzyl)-1H-pyrazol-3-yl)propan-1-one (10i)



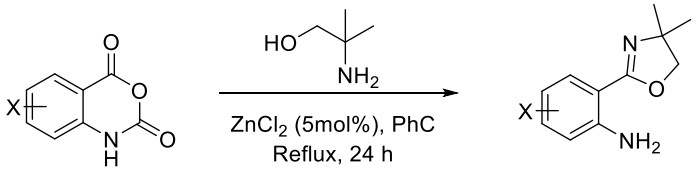
The reaction, work-up and purification procedure of title compound followed General Procedure E. The title product was obtained as a pale yellow oil in 41% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (d, J = 2.4 Hz, 1H), 7.29 – 7.16 (m, 4H), 6.82 (d, J = 2.4 Hz, 1H), 5.36 (s, 2H), 3.03 (q, J = 7.4 Hz, 2H), 1.21 (t, J = 7.4 Hz, 3H).

Preparation of 1-(1-(4-(trifluoromethoxy)phenethyl)-1H-pyrazol-4-yl)propan-1-one (10j)



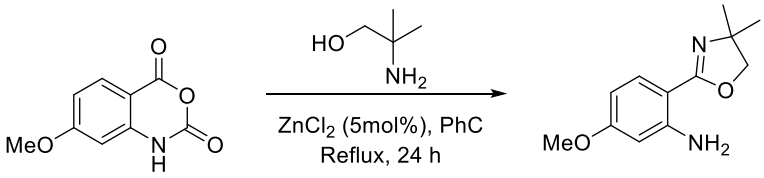
The reaction, work-up and purification procedure of title compound followed General Procedure E. The title product was obtained as a pale yellow solid in 26% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (s, 1H), 7.63 (s, 1H), 7.12 (d, J = 8.7 Hz, 2H), 7.07 (d, J = 8.7 Hz, 2H), 4.34 (t, J = 7.1 Hz, 2H), 3.20 (t, J = 7.1 Hz, 2H), 2.71 (q, J = 7.4 Hz, 2H), 1.16 (t, J = 7.4 Hz, 3H).

General Procedure F – Preparation of oxazoline 7



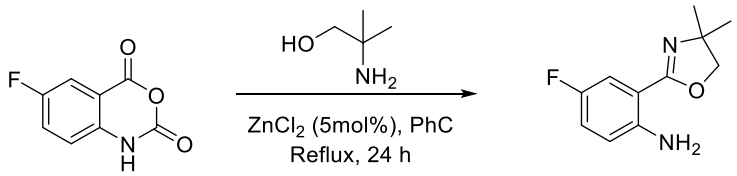
Isatoic anhydride (17.6 mmol) was suspended in anhydrous chlorobenzene (50 mL) under nitrogen. 2- Methyl-2-amino-1-propanol (1.3 eq.) was added to the suspension followed by anhydrous $ZnCl_2$ (0.13 eq.), and the mixture was heated to reflux for 24 h (followed by TLC). The reaction was allowed to cool to room temperature. The solvent was removed under reduced pressure, the residue was added to ethyl acetate and the resulting solution was washed with brine. The aqueous layer was extracted with ethyl acetate (50 mL x 2) and the combined organic layer was dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure to give the crude product. Purification by column chromatography using 10% ethyl acetate in hexane gave the desired compound.

Preparation of 2-(4,4-dimethyl-4,5-dihydrooxazol-2-yl)-5-methoxyaniline (7c)



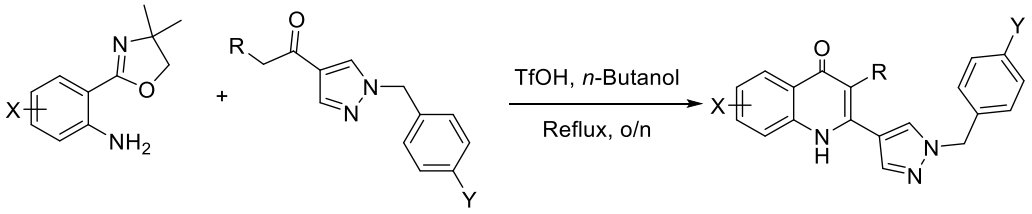
The reaction, work-up and purification procedure of title compound followed General Procedure F. The title product was obtained as a pale yellow solid in 60% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (d, J = 8.8 Hz, 1H), 6.24 (dd, J = 8.8, 2.4 Hz, 1H), 6.17 (d, J = 2.4 Hz, 1H), 6.13 (br. s, 2H), 3.96 (s, 2H), 3.77 (s, 3H), 1.34 (s, 6H).

Preparation of 2-(4,4-dimethyl-4,5-dihydrooxazol-2-yl)-4-fluoroaniline (7d)



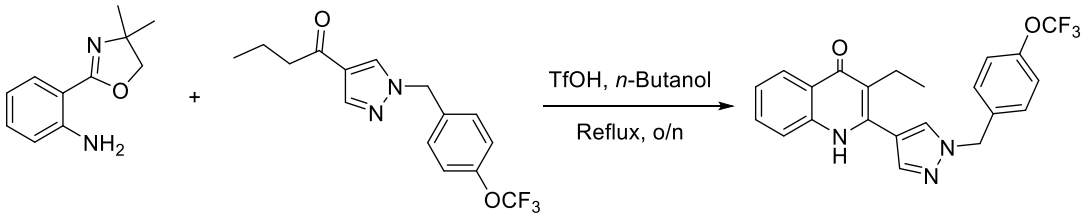
The reaction, work-up and purification procedure of title compound followed General Procedure F. The title product was obtained as a pale yellow solid in 60% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (dd, J = 9.8, 3.1 Hz, 1H), 6.94 (ddd, J = 8.9, 7.9, 3.1 Hz, 1H), 6.63 (dd, J = 8.9, 4.6 Hz, 1H), 5.92 (s, 2H), 4.00 (s, 2H), 1.37 (s, 6H).

General Procedure G - Preparation of quinolones through cyclisation of oxazoline and ketone



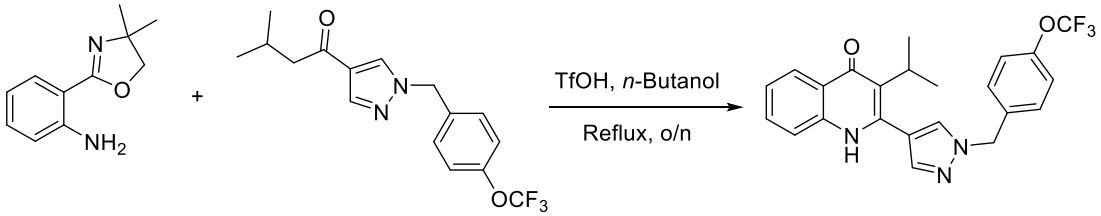
To a solution of oxazoline (1 mmol, 1 eq.) and the respective ketone **10** (1 eq.) in anhydrous *n*-butanol (10 mL) was added trifluoromethanesulfonic acid (20 mol%). The mixture was heated at 135^{o}C for 24 h (followed by TLC). The reaction was cooled to room temperature and the solvent was removed *in vacuo*. Saturated $NaHCO_3$ (aq) (20 mL) was added and the aqueous solution was extracted with ethyl acetate (20 mL x 3), the combined organic layers were washed with water and brine, dried over $MgSO_4$, filtered and concentrated to a solid. The crude product was triturated with diethyl ether or purified by column chromatography to give the desired quinolone.

Preparation of 3-ethyl-2-(1-(4-(trifluoromethoxy)benzyl)-1H-pyrazol-4-yl)quinolin-4(1H)-one (11a)



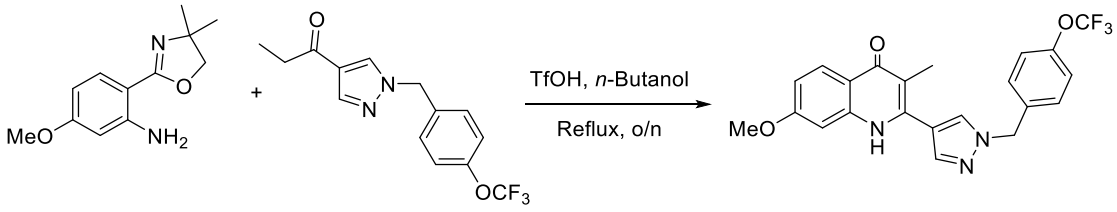
The reaction, work-up and purification procedure of title compound followed General Procedure G. The title product was obtained as a white solid in 65% yield. Melting point: 142-144 ^{o}C. ^{1}H NMR (400 MHz, DMSO) δ 11.29 (s, 1H), 8.37 (s, 1H), 8.08 (d, J = 8.0 Hz, 1H), 7.88 (s, 1H), 7.60 – 7.58 (m, 2H), 7.47 (d, J = 8.7 Hz, 2H), 7.40 (d, J = 8.4 Hz, 2H), 7.26 (dd, J = 8.1, 4.6 Hz, 1H), 5.51 (s, 2H), 2.60 – 2.43 (m, 2H), 1.05 (t, J = 7.3 Hz, 3H); ^{13}C NMR (101 MHz, DMSO) δ 176.37, 161.96, 148.21, 140.28, 139.93, 139.61, 137.07, 131.56, 131.23, 130.10, 125.29, 123.67, 122.81, 121.62, 121.05, 118.27, 115.86, 54.42, 19.61, 14.47. ES HRMS: m/z found 414.1432, $C_{22}H_{19}N_3O_2F_3$ $[M+H]^+$ requires 414.1429; Purity HPLC 97.9%, R_t = 9.47 min.

Preparation of 3-isopropyl-2-(1-(4-(trifluoromethoxy)benzyl)-1H-pyrazol-4-yl)quinolin-4(1H)-one (11b)



The reaction, work-up and purification procedure of title compound followed General Procedure G. The title product was obtained as a white solid in 63% yield. Melting point: 210-212 ^{o}C. ^{1}H NMR (400 MHz, DMSO) δ 11.23 (s, 1H), 8.30 (s, 1H), 8.06 (d, J = 9.2 Hz, 1H), 7.78 (s, 1H), 7.60 – 7.46 (m, 4H), 7.41 (d, J = 8.0 Hz, 2H), 7.25 (dd, J = 8.1, 6.6 Hz, 1H), 5.49 (s, 2H), 3.01 – 2.88 (m, 1H), 1.32 (d, J = 6.9 Hz, 6H); ^{13}C NMR (101 MHz, DMSO) δ 176.71, 160.70, 148.21, 140.24, 139.68, 137.92, 137.03, 131.49, 131.20, 130.28, 125.20, 124.80, 123.73, 122.74, 121.63, 118.07, 116.02, 55.29, 29.13, 20.74. ES HRMS: m/z found 428.1566, $C_{23}H_{21}N_3O_2F_3$ $[M+H]^+$ requires 428.1586; Purity HPLC 98.9%, R_t = 10.08 min.

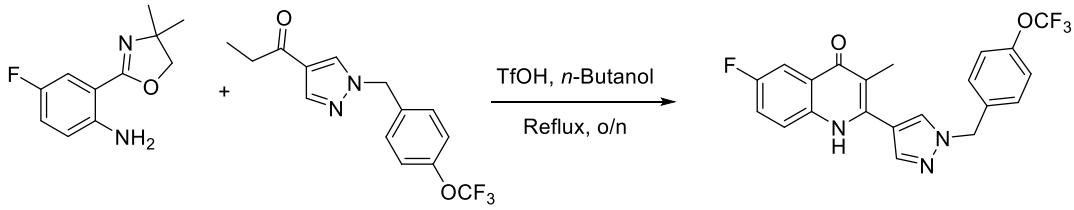
Preparation of 7-methoxy-3-methyl-2-(1-(4-(trifluoromethoxy)benzyl)-1H-pyrazol-4-yl)quinolin-4(1H)-one (11c)



The reaction, work-up and purification procedure of title compound followed General Procedure

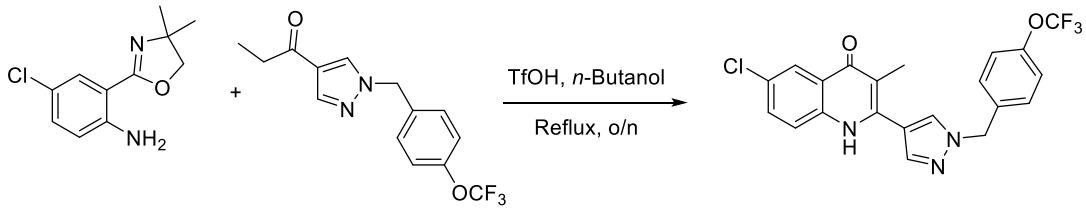
G. The title product was obtained as a white solid in 42% yield. Melting point: 171-173 °C. ^{1}H NMR (400 MHz, DMSO) δ 11.12 (s, 1H), 8.43 (s, 1H), 7.98 (d, J = 8.9 Hz, 1H), 7.95 (s, 1H), 7.46 (d, J = 8.7 Hz, 2H), 7.40 (d, J = 8.2 Hz, 2H), 7.03 (d, J = 2.4 Hz, 1H), 6.87 (dd, J = 8.9, 2.4 Hz, 1H), 5.50 (s, 2H), 3.83 (s, 3H), 2.07 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 175.94, 161.37, 147.74, 141.13, 139.46, 139.31, 136.59, 131.23, 129.59, 126.72, 121.19, 118.67, 117.21, 115.87, 113.30, 112.66, 98.45, 55.19, 54.01, 11.95. ES HRMS: m/z found 430.1392, $C_{22}H_{19}N_3O_3F_3$ $[M+H]^+$ requires 430.1379; Purity HPLC 97.3 %, R_t = 10.68 min.

Preparation of 6-fluoro-3-methyl-2-(1-(4-(trifluoromethoxy)benzyl)-1H-pyrazol-4-yl)quinolin-4(1H)-one (11d)



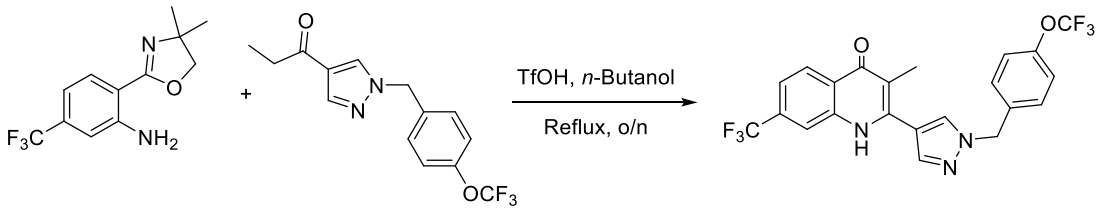
The reaction, work-up and purification procedure of title compound followed General Procedure G. The title product was obtained as a white solid in 47% yield. Melting point: 210-212 °C. ^{1}H NMR (400 MHz, DMSO) δ 11.46 (s, 1H), 8.46 (s, 1H), 7.97 (s, 1H), 7.75 – 7.65 (m, 2H), 7.53 (td, J = 8.7, 3.0 Hz, 1H), 7.46 (d, J = 8.8 Hz, 2H), 7.40 (d, J = 8.4 Hz, 2H), 5.51 (s, 2H), 2.09 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 175.98, 159.51, 148.20, 140.75, 139.91, 136.99, 136.60, 131.90, 130.09, 124.12, 121.64, 121.10, 120.60, 120.34, 116.03, 113.72, 109.20, 54.48, 12.50. ES HRMS: m/z found 418.1190, $C_{21}H_{16}N_3O_2F_4$ $[M+H]^+$ requires 418.1179; Purity HPLC 95.9%, R_t = 9.45 min.

Preparation of 6-chloro-3-methyl-2-(1-(4-(trifluoromethoxy)benzyl)-1H-pyrazol-4-yl)quinolin-4(1H)-one (11e)



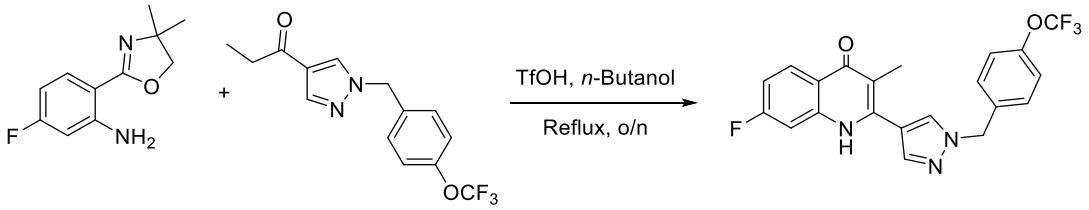
The reaction, work-up and purification procedure of title compound followed General Procedure G. The title product was obtained as a white solid in 48% yield. Melting point: 228-230 °C. ^{1}H NMR (400 MHz, DMSO) δ 11.47 (s, 1H), 8.46 (s, 1H), 8.01 (dd, J = 1.9, 1.0 Hz, 1H), 7.97 (s, 1H), 7.69 – 7.60 (m, 2H), 7.46 (d, J = 8.8 Hz, 2H), 7.39 (d, J = 8.1 Hz, 2H), 5.50 (s, 2H), 2.09 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 175.62, 163.90, 148.21, 140.91, 139.93, 138.45, 136.96, 131.95, 131.69, 130.09, 127.40, 124.19, 124.07, 121.63, 120.80, 115.95, 114.73, 54.50, 12.51. ES HRMS: m/z found 456.0691 and 458.0677, $C_{21}H_{15}N_3O_2F_3ClNa$ $[M+Na]^+$ requires 456.0703 and 458.0673; Purity HPLC 97.8%, R_t = 9.96 min.

Preparation of 3-methyl-2-(1-(4-(trifluoromethoxy)benzyl)-1H-pyrazol-4-yl)-7-(trifluoromethyl)-quinolin-4(1H)-one (11f)



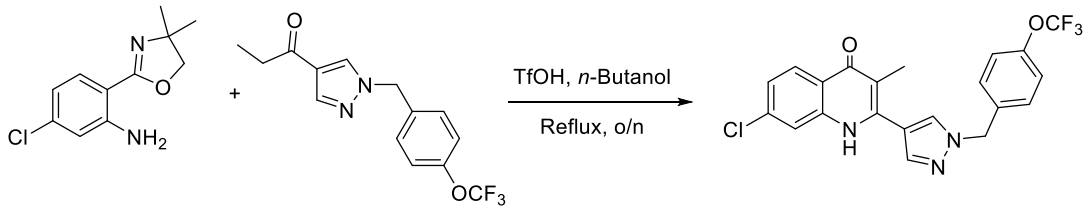
The reaction, work-up and purification procedure of title compound followed General Procedure G. The title product was obtained as a white solid in 51% yield. Melting point: 231-233 ^{o}C. ^{1}H NMR (400 MHz, DMSO) δ 11.55 (s, 1H), 8.49 (s, 1H), 8.28 (d, J = 8.4 Hz, 1H), 8.05 – 7.97 (m, 2H), 7.55 (dd, J = 8.5, 1.5 Hz, 1H), 7.47 (d, J = 8.8 Hz, 2H), 7.40 (d, J = 8.8 Hz, 2H), 5.52 (s, 2H), 2.13 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 176.14, 164.96, 162.99, 148.23, 141.45, 139.91, 139.33, 136.92, 134.88, 132.01, 130.10, 127.28, 125.61, 124.96, 121.65, 118.40, 115.92, 115.65, 54.54, 12.50. ES HRMS: m/z found 490.0971, $C_{22}H_{15}N_3O_2F_6Na$ $[M+Na]^+$ requires 490.0966; Purity HPLC 98.2%, R_t = 10.52 min.

Preparation of 7-fluoro-3-methyl-2-(1-(4-(trifluoromethoxy)benzyl)-1H-pyrazol-4-yl)quinolin-4(1H)one (11g)



The reaction, work-up and purification procedure of title compound followed General Procedure G. The title product was obtained as a white solid in 55% yield. Melting point: 214-216 ^{o}C. ^{1}H NMR (400 MHz, DMSO) δ 11.33 (s, 1H), 8.45 (s, 1H), 8.13 (dd, J = 9.0, 6.5 Hz, 1H), 7.96 (s, 1H), 7.46 (d, J = 8.7 Hz, 2H), 7.40 (d, J = 8.2 Hz, 2H), 7.32 (dd, J = 10.4, 2.5 Hz, 1H), 7.12 (td, J = 8.8, 2.5 Hz, 1H), 5.51 (s, 2H), 2.08 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 176.27, 162.69, 148.23, 145.17, 141.06, 140.83, 139.83, 136.96, 131.83, 130.09, 128.77, 121.63, 120.25, 116.04, 114.51, 111.84, 103.00, 54.50, 12.36. ES HRMS: m/z found 440.1014, $C_{21}H_{15}N_3O_2F_4Na$ $[M+Na]^+$ requires 440.0998; Purity HPLC 96.6%, R_t = 9.63 min.

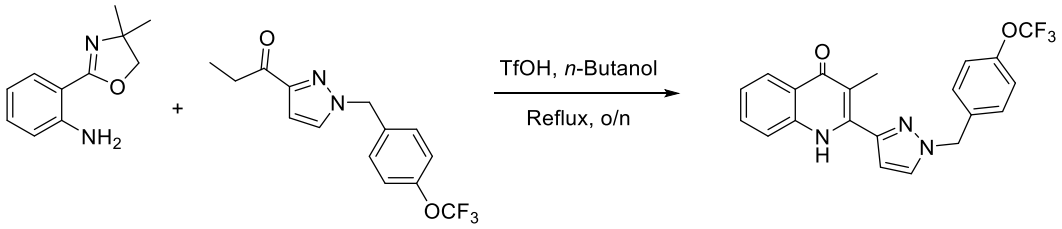
Preparation of 7-chloro-3-methyl-2-(1-(4-(trifluoromethoxy)benzyl)-1H-pyrazol-4-yl)quinolin-4(1H)-one (11h)



The reaction, work-up and purification procedure of title compound followed General Procedure G. The title product was obtained as a white solid in 59% yield. Melting point: 223-225 ^{o}C. ^{1}H NMR (400 MHz, DMSO) δ 11.33 (s, 1H), 8.46 (s, 1H), 8.08 (d, J = 8.7 Hz, 1H), 7.97 (s, 1H), 7.65 (d, J = 2.0 Hz, 1H), 7.46 (d, J = 8.8 Hz, 2H), 7.40 (d, J = 8.1 Hz, 2H), 7.28 (dd, J = 8.7, 2.0 Hz, 1H), 5.51 (s, 2H), 2.08 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 176.26, 162.24, 148.23, 140.81, 140.55, 139.87, 136.94, 136.07, 131.90, 130.11, 127.75, 123.21, 121.73, 121.64, 117.32, 115.99, 114.96, 54.51, 12.41. ES HRMS: m/z found 456.0683 and 458.0667, $C_{21}H_{15}N_3O_2F_3ClNa$ $[M+Na]^+$ requires

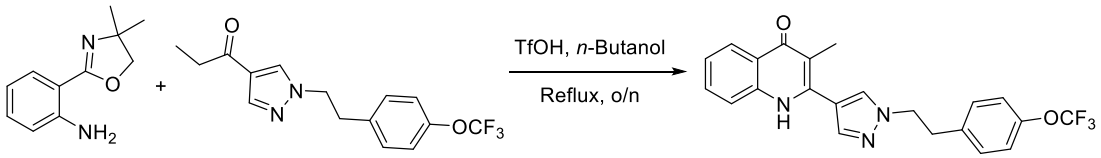
456.0703 and 458.0673; Purity HPLC 95.4%, R_t = 10.09 min.

Preparation of 3-methyl-2-(1-(4-(trifluoromethoxy)benzyl)-1H-pyrazol-3-yl)quinolin-4(1H)-one (11i)



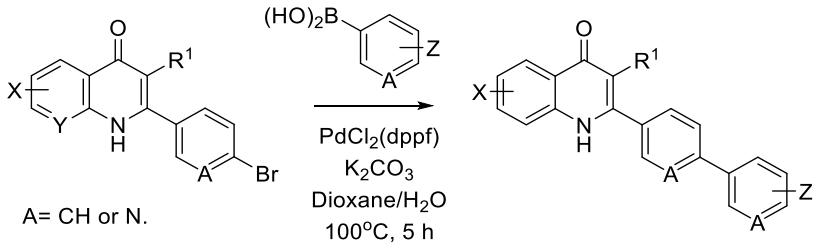
The reaction, work-up and purification procedure of title compound followed General Procedure G. The title product was obtained as a white solid in 52% yield. Melting point: 63-65 ^{o}C. ^{1}H NMR (400 MHz, DMSO) δ 11.38 (s, 1H), 8.13 (d, J = 2.3 Hz, 1H), 8.10 (d, J = 8.1 Hz, 1H), 7.76 (d, J = 8.4 Hz, 1H), 7.60 (t, J = 7.0 Hz, 1H), 7.45 (d, J = 8.7 Hz, 2H), 7.39 (d, J = 8.4 Hz, 2H), 7.27 (t, J = 7.5 Hz, 1H), 6.82 (d, J = 2.3 Hz, 1H), 5.55 (s, 2H), 2.13 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 177.20, 159.88, 148.21, 146.08, 140.39, 139.81, 136.99, 132.40, 131.61, 129.96, 125.27, 123.23, 122.90, 121.66, 118.69, 115.18, 108.12, 54.65, 12.29. ES HRMS: m/z found 400.1291, $C_{21}H_{17}N_3O_2F_3$ $[M+H]^+$ requires 400.1273; Purity HPLC 97.2%, R_t = 9.72 min.

Preparation of 3-methyl-2-(1-(4-(trifluoromethoxy)phenethyl)-1H-pyrazol-4-yl)quinolin-4(1H)-one (11j)



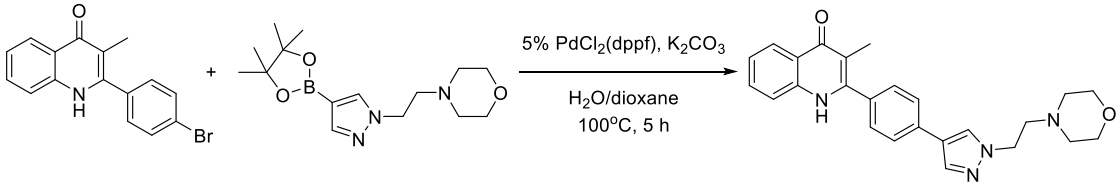
The reaction, work-up and purification procedure of title compound followed General Procedure G. The title product was obtained as a white solid in 84% yield. Melting point: 210-212 ^{o}C. ^{1}H NMR (400 MHz, DMSO) δ 11.25 (s, 1H), 8.12 (s, 1H), 8.07 (d, J = 7.7 Hz, 1H), 7.93 (s, 1H), 7.66 – 7.55 (m, 2H), 7.36 – 7.22 (m, 5H), 4.49 (t, J = 7.1 Hz, 2H), 3.22 (t, J = 7.1 Hz, 2H), 1.96 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 176.75, 147.38, 140.57, 139.87, 139.21, 138.06, 131.64, 131.46, 130.95, 125.30, 123.14, 122.78, 121.71, 121.36, 118.25, 115.52, 114.11, 52.76, 35.46, 12.27. ES HRMS: m/z found 414.1427, $C_{22}H_{19}N_3O_2F_3$ $[M+H]^+$ requires 414.1429; Purity HPLC 95.8%, R_t = 9.17 min.

General Procedure H – Synthesis of quinolones 13a and 13b



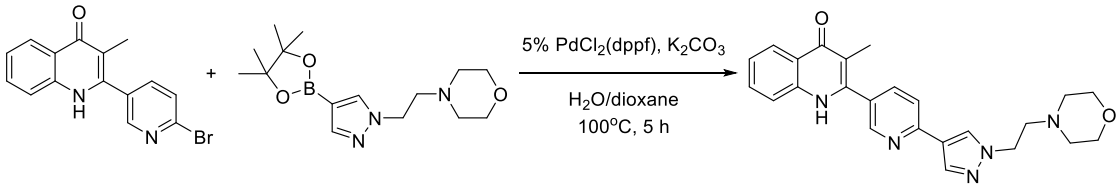
Quinolone (1 mmol, 1.0 eq.), $PdCl_2$(dppf) (5 mol%) and potassium carbonate (3 mmol, 3 eq.) in anhydrous 1,4-dioxane (9 mL) and water (1 mL) were allowed to stir for 5 min under N_2. Boronic acid (2 mmol, 2 eq.) was added. The reaction was evacuated and backfilled with N_2 (x3). The reaction mixture was heated to 100^{o}C for 5 h (followed by TLC). The mixture was cooled to room temperature, diluted with ethyl acetate and filtered through a pad of $MgSO_4$-silica. The silica pad was further washed with ethyl acetate. The filtrate was evaporated and the crude material was purified by flash chromatography on silica gel to give the desired quinolone.

Preparation of 3-methyl-2-(4-(1-(2-morpholinoethyl)-1H-pyrazol-4-yl)phenyl)quinolin-4(1H)-one (13a)



The reaction, work-up and purification procedure of title compound followed General Procedure H. The title product was obtained as a white solid in 87% yield. Melting point: 118 – 120 ^{o}C. ^{1}H NMR (400 MHz, DMSO) δ 11.58 (s, 1H), 8.33 (s, 1H), 8.13 (d, J = 8.0 Hz, 1H), 8.00 (s, 1H), 7.77 (d, J = 8.2 Hz, 2H), 7.63 – 7.60 (m, 2H), 7.55 (d, J = 8.2 Hz, 2H), 7.34 – 7.25 (m, 1H), 4.27 (t, J = 6.6 Hz, 2H), 3.63 – 3.50 (m, 4H), 2.76 (t, J = 6.6 Hz, 2H), 2.48 – 2.37 (m, 4H), 1.94 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 176.58, 147.40, 139.39, 136.22, 133.70, 132.32, 131.09, 129.46, 127.80, 124.82, 124.67, 122.93, 122.48, 120.78, 118.02, 114.19, 66.08, 57.58, 53.04, 48.78, 12.13. ES HRMS: m/z found 415.2122, $C_{25}H_{27}N_4O_2$ $[M+H]^+$ requires 415.2134; Purity HPLC 96.7%, R_t = 6.40 min.

Preparation of 3-methyl-2-(6-(1-(2-morpholinoethyl)-1H-pyrazol-4-yl)pyridin-3-yl) quinolin-4(1H)-one (13b)



The reaction, work-up and purification procedure of title compound followed General Procedure H. The title product was obtained as a white solid in 92% yield. Melting point: 132 – 133 ^{o}C. ^{1}H NMR (400 MHz, DMSO) δ 11.68 (s, 1H), 8.70 (d, J = 2.3 Hz, 1H), 8.46 (s, 1H), 8.17 – 8.10 (m, 2H),

8.00 (dd, J = 8.2, 2.3 Hz, 1H), 7.85 (d, J = 7.6 Hz, 1H), 7.68 – 7.56 (m, 2H), 7.32 (dd, J = 8.1, 6.6 Hz, 1H), 4.30 (t, J = 6.5 Hz, 2H), 3.61 – 3.51 (m, 4H), 2.77 (t, J = 6.5 Hz, 2H), 2.47 – 2.40 (m, 4H), 1.95 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 176.51, 152.38, 149.04, 144.77, 139.46, 137.37, 137.30, 131.28, 129.63, 127.79, 124.89, 123.00, 122.67, 121.90, 118.53, 118.01, 114.93, 66.09, 57.51, 53.03, 48.49, 11.95. ES HRMS: m/z found 416.2086, $C_{24}H_{26}N_5O_2$ $[M+H]^+$ requires 416.2087; Purity HPLC 96.6%, R_t = 5.80 min.

S2. Biological testing methods and procedures

Parasite culture

Laboratory strains of *P. falciparum* were cultured in human erythrocytes following Trager and Jensen method[1] with modifications[2]. The parasites were retrieved from cryopreserved stock by thawing in water bath at 37°C until completion. 1 ml of 3.5% NaCl solution was gently added to thawed blood. The solution was centrifuged at slow speed and supernatant was removed. The culture was then initialised by adding 10 mL of 10% serum based culture medium (RPMI-1640 supplemented with 25 mM HEPES and 4 μg/ml gentamicin). The parasites were maintained in fresh human erythrocytes at 37°C under a low oxygen atmosphere (3% CO_2, 4% O_2, and 93% N_2). The culture was daily evaluated for parasitemia and parasite stages using Giemsa-stained microscopy method.

***In vitro* antimalarial activity**

Drug-sensitivity phenotypes of *P. falciparum* strains 3D7, W2, and TM90C2B (Thailand) have been described previously[3-5]. *In vitro* antimalarial activity of compounds described in the manuscript was assessed by the SYBR Green I fluorescence-based method[2]. The assay was set up in 96-well plates by Hamilton Star robotic platform with two-fold dilutions of each drug across the plate at a final concentration of 2% parasitemia at 0.5% haematocrit (v/v). The dilution series was initiated at a concentration of 1 μM ranging to 0.61 nM. ATQ and CQ were used as positive control (IC_{50} (3D7) = 0.9 and 11 nM, respectively). The plates were incubated for 48 hours under a culture condition. The assay was terminated by freezing at -20°C overnight. Growth proliferation was determined by the SYBR Green method. The half maximal inhibitory concentration (IC_{50}) was calculated using the four-parameter logistic method (Grafit program; Erithacus Software, United Kingdom).

***Pf*NDH2 enzymatic activity assessment**

Recombinant *Pf*NDH2 was prepared from the *Escherichia coli* heterologous expression strain F571. *Pf*NDH2 activities were measured as described previously.[6]

DMPK properties assays

The DMPK properties data described in the manuscript were measured through a high through-put platform kindly provided by AstraZeneca U.K. The methods of the four assays, including aqueous solubility, plasma protein binding, microsome and hepatocyte clearance measurements have been reported previously.[7-8]

Hep G2 cell toxicity assay

Hep G2 cells were obtained from the European Collection of Authenticated Cell Cultures. Dulbecco's modified Eagle's medium (DMEM), *L*-glutamine, sodium pyruvate, foetal bovine serum (FBS), and penicillin/streptomycin were obtained from ThermoFisher Scientific. Rotenone, tamoxifen, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), 3-(4,5-Dimethyl-2-thiazolyl)-2,5- diphenyl-2H-tetrazolium bromide (MTT) and dimethyl sulfoxide (DMSO) were obtained from Sigma-Aldrich. Hep G2 cell toxicities were determined as described in Warman et al.[9] In brief, Hep G2 cells cultured in media (DMEM containing 25 mM D-glucose

and 1 mM sodium pyruvate and supplemented with 5 mM HEPES, 10% FBS and 500 µg/mL pen-strep) were added to clear-bottomed 96-well plates (100 µL and $1x10^4$ cells/well) and incubated for 24 h at 37°C. After incubation, various concentration of test compounds, rotenone or tamoxifen (100 µM – 1 nM) or DMSO (0.001 – 1 % v/v) were added to a final volume of 200 µL/well, and incubated for another 24 h at 37°C. The plates were subsequently incubated in the presence of 1 mg/mL MTT for 2 hr at 37 °C. After incubation, the media was removed and DMSO added (100 µL/well). The plates were shaken for 5 min before well absorbance at 560 mm was measured using a PHERAstar FS plate reader (BMG Labtech). Raw absorbance values were expressed as percentage cell viability, using the non-treated and tamoxifen-treated cells (100 µM) mean values as negative and positive controls, respectively. Data analysis was performed using GraphPad Prism, v6.0 software (GraphPad Software, San Diego, CA, USA). Concentration-response data were fitted to curves using a four-parameter logistic equation. Data represent means ± s.e.m. of three independent experiments performed in duplicate.

Figure S1. *P. falciparum* bc_1 enzyme inhibition of 11c (WDH-2G-7)

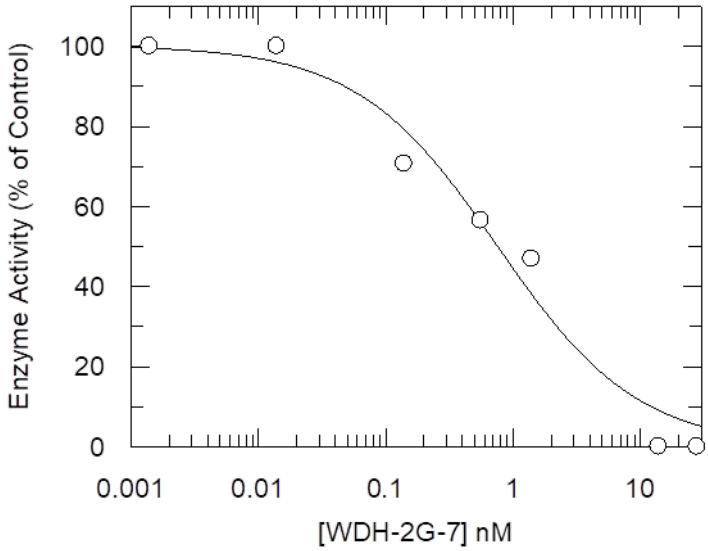


Table S1. Human Microsomes Intrinsic Clearance, Rat Hepatocytes Intrinsic Clearance and Human Plasma Protein Binding for Selected 2-Pyrazolyl Quinolones.

Compound	Human Microsomes Intrinsic Clearance (µl/min/mg)	Rat Hepatocytes Intrinsic Clearance (µl/min/10^6 cells)	Human Plasma Protein Binding (%)
4e	4.44	7.6	99.9
5a	5.05	8.11	> 99.96
11a	8.74	8.74	99.6
11c	10.28	4.98	99.75
11i	5.75	7.21	99.8

S3. Cytochrome *bc1* preparation and crystallography

***Cytochrome bc_1* preparation**

Cytochrome bc_1 was purified as previously described[10]. Briefly, bovine mitochondria were isolated from bovine hearts and solubilised in dodecyl-maltoside (DDM). The suspension was centrifuged at 200,000 g for an hour at 4°C. The supernatant was loaded onto a 50 mL DEAE sepharose CL6B (GE Healthcare) column pre-equilibrated and washed with 50 mM KPi buffer pH7.5, 250 mM NaCl, 0.5 mM EDTA, 0.01% DDM. Protein was eluted along a linear gradient from 250 to 500 mM NaCl. Cytochrome bc_1 fractions were concentrated and loaded onto a 120 mL Sephacryl S300 column (GE Healthcare) pre-equilibrated with 20 mM KMOPS buffer pH7.2, 100 mM NaCl, 0.5 mM EDTA, 0.01% DDM. Cytochrome bc_1 fractions were collected and concentrated to 40mg/mL. PEG fractionation with increased concentrations of PEG4000 was used to precipitate cytochrome bc_1. The protein start precipitating at 2%PEG4000, pure protein was in the fractions with 2.5-4% of PEG4000. Obtained pellet was re-suspended in 25 mM KPi buffer pH7.5, 100 mM NaCl, 3mM NaN_3, 0.015% DDM and dialysed against the same buffer to remove PEG contamination. The inhibitors were dissolved in DMSO at the concentration of 30 mM. 5 uM cytochrome bc_1 was incubated at 4°C for 12 hours with 30 mkM inhibitor (six-fold molar excess).

***Cytochrome bc_1* crystallography**

The inhibitor-bound cytochrome bc_1 was concentrated to 40 mg/mL and mixed with 1.6% HECAMEG, 2 mkl of final protein solution was mixed with 2 mkl of reservoir solution (50 mM KPi pH 6.8, 100 mM NaCl, 3 mM NaN_3, 10-13% PEG4000). The crystals were grown by hanging drop method at 4°C. The red crystals appear overnight and reach their maximal size in a few days. Prior to flash freezing in liquid nitrogen, crystals were soaked stepwise in 50% ethylene glycol in reservoir solution. The data were collected at 100K using 0.92819 Å wavelength with a Pilatus3 6M detector at I04 beamline, Diamond Light Source, UK. Data were processed in iMosflm[11] and scaled by Aimless[12]. Inhibitor free isomorphous bovine cytochrome bc_1 structure (PDB: 5OKD) was used as a starting model for the refinement. Jelly body and TLS refinements were carried out with Refmac5[13]. The model was manually rebuild in COOT[14] between the refinement cycles. The inhibitor molecules were produced with Jligand[15] software and modelled into the omit F_o-F_c electron density present in Q_i site. Data collection and refinement statistics are shown in the Table S1.

***Plasmodium falciparum* homology model**

Plasmodium falciparum (*Pf*) cytochrome b homology model was generated using bovine cytochrome b (5OKD) template and Pf cytochrome b sequence (Q02768) with web-based SwissModel software[16-18]. The model was built based on target-template alignment using ProMod3[19]. The conserved coordinates between bovine and *Pf* bc_1 were preserved to the model, while other side chains were re-modelled using a fragment library. The final model was regularised by a force field and assessed its quality using the QMEAN scoring[20]. The *Pf* homology model used in this paper has QMEAN score of -5.67 and 41.71% sequence identity.

Molecular docking

The compound **4e** and **11c** docking was carried out by web-based SwissDock software[21] based on

EADock DSS using the CHARMM force field[22]. The docking was performed within defined interest region of Pf Q_i site, using the "Accurate" parameter, allowing flexibility for side chain within 5Å in its reference binding mode. The final solution for **11c** was determined based on binding pose corresponding to the ligand pose in bovine crystal structure with highest Fullfitness score, while **4e** position was judged using *Fullfitness* score only.

Figure S2. The *P. falciparum* homology model (magenta) aligned to bovine crystal structure showing possible model of binding for compound **11c** (teal) in the Q_i site. Steric clashes are illustrated in red dashes. The residue Phe30 involved in steric clash shown by red circle and labelled in red. H-bonds are indicated by black dash lines. The surface of the Q_i site is colored in grey.

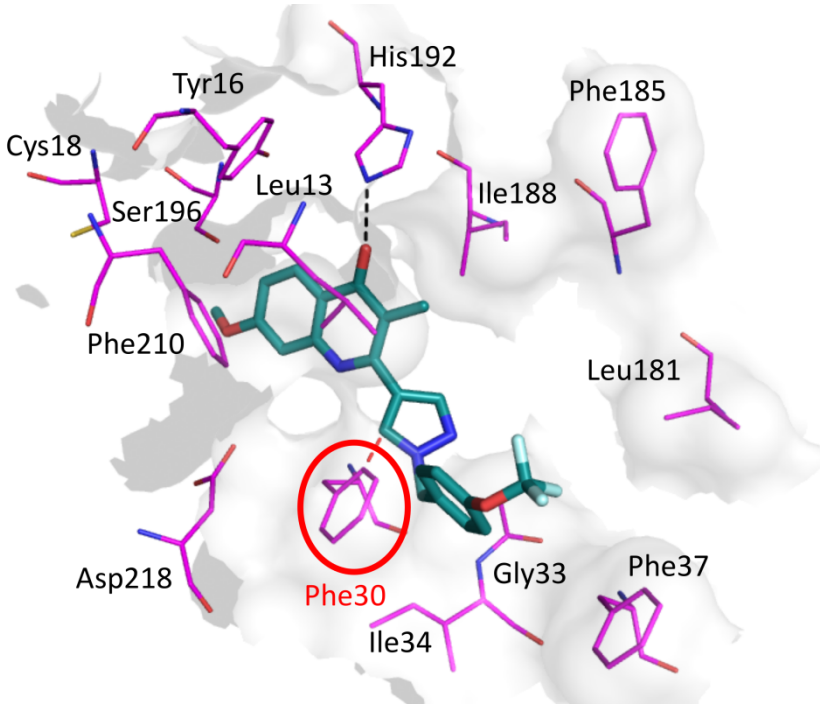


Table S2. Data collection and refinement statistics for inhibitor-bound cytochrome *bc1*

Data collection	**Cyt.bc_1- compound 11c**
Space group	$P6_522$
Unit-cell dimensions (a,b,c) (Å) α, β, γ (^{o})	212.69, 212.69, 347.07 90^o, 90^o, 120^o
Resolution (last shell) (Å)	92.10-3.45 (3.55-3.45)
R_{merge} (last shell) (%)	16.1 (112.5)
R_{pim} (last shell) (%)	7.4 (53.4)
CC(1/2) (last shell)	0.998 (0.315)
I/σ (last shell)	10.1 (2.1)
Completeness (last shell) (%)	90.2 (91.4)
Redundancy	10.2 (10.1)
No. of unique reflections	54,993
Rwork/Rfree	21.13/24.52
Non-hydrogen atoms	
Protein	15,424
Inhibitor	31
waters	29
Other ligands	568
B factor (Å^2)	
Protein	139.48
Inhibitor	135.80
Other ligands	149.38
Waters	99.86
R.M.S deviations	
Bond length (Å)	0.008
Bond angles (°)	1.410
PDB code	6HAW

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