Electronic Supplementary Material (ESI) for MedChemComm.


**Supporting Information**

# 2-Pyridylquinolone Antimalarials with Improved Antimalarial Activity and Physicochemical Properties

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## Synthetic methods and procedures

### General

Air- and moisture-sensitive reactions were carried out in oven-dried glassware sealed with rubber septa under nitrogen from a balloon. Sensitive liquids and reagents were transferred via syringe. Reactions were stirred using Teflon-coated magnetic stir bars. All commercial reagents were used without further purifications. Organic solutions were concentrated under vacuum using Buchi rotary evaporator.

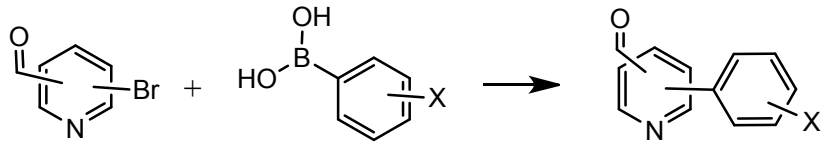
Anhydrous solvents were either purchased from reliable commercial sources or distilled from a still prior to use under inert gas atmosphere. THF was distilled from Na with benzophenone as an indicator. DCM was distilled from $CaH_2$. All reagents were purchased from reliable commercial sources and were used without any purification unless otherwise indicated. TLC analysis was performed to confirm the reagents purity.

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 Brucker 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. Elemental analysis (%C, %H, %N) was performed in the University of Liverpool microanalysis laboratory. All melting points were determined with Gallenkamp melting point apparatus and were uncorrected.

**Experimental procedures and compound characterisation**

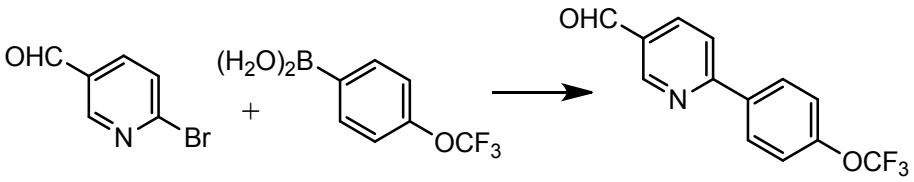
**General procedure 1**




**2**

To a solution of selected aromatic aldehyde (1.0 eq), $Pd(PPh_3)_4$ (0.08 eq) and potassium carbonate (3.3 eq) in THF (25 mL) and $H_2O$ (10 mL) under $N_2$ atmosphere was added phenylboronic acids (1.1 eq). The reaction was allowed to stir and heated to reflux condition (80 $^oC$) overnight. After the mixture was allowed to cool to room temperature, water (20 mL) was poured into the mixture. Extraction with EtOAc (3 x 30 mL), washing with brine and drying over $MgSO_4$ gave the crude after solvent evaporation. Purification was performed by column chromatography (eluting with 10-30% EtOAc/*n*-Hexane).

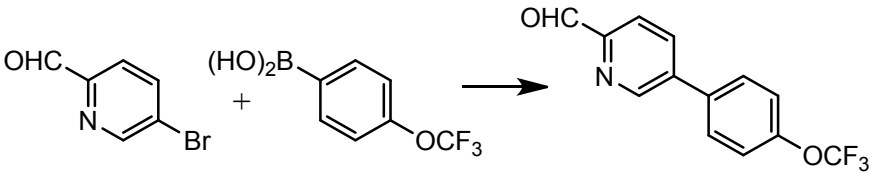
Preparation of **6-(4-(trifluoromethoxy)phenyl)nicotinaldehyde, 2a**




**2a**

6-Bromonicotinaldehyde (835 mg, 5 mmol) was treated following **the General procedure 1** gave **2a** (1.06 g, 88%) as a pale yellow solid. $^1H$ NMR (400 MHz, $CDCl_3$) δ 10.16 (s, 1H,COH), 9.14 (d, *J* = 1.5 Hz, 1H), 8.26 (dd, *J* = 8.2, 2.2 Hz, 1H), 8.14 (d, *J* = 8.9 Hz, 2H), 7.90 (d, *J* = 8.3 Hz, 1H), 7.37 (d, *J* = 8.1 Hz, 2H);$^{13}C$ NMR (100 MHz, $CDCl_3$) δ 190.78, 161.07, 152.82, 137.17, 136.88, 130.43, 129.57, 121.60, 120.91. ESI-HRMS: m/z calculated for $C_{14}H_{13}NO_3F_3$ ($[M+MeOH+H]^+$) 300.0848, found 300.0849.

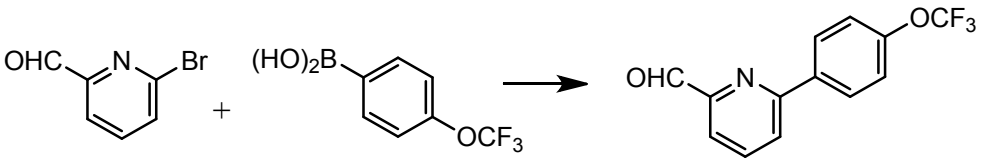
Preparation of **5-(4-(trifluoromethoxy)phenyl)picolinaldehyde, 2b**




**2b**

5-Bromopicolinaldehyde (930 mg, 5 mmol) was treated following **the General procedure 1** gave **2b** (1.30 g, 98%) as a pale yellow solid. $^{1}$H NMR (400 MHz, $CDCl_3$) δ 10.14 (s, 1H), 9.00 (dd, *J* = 1.9, 1.0 Hz, 1H), 8.09 – 8.00 (m, 2H), 7.68 (d, *J* = 8.7 Hz, 2H), 7.38 (d, *J* = 8.0 Hz, 2H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 193.31, 152.23, 148.98, 139.74, 135.64, 129.36, 128.09, 122.26, 122.14. ESI-HRMS: m/z calculated for $C_{13}H_9NO_2F_3$ ($[M+H]^+$) 268.0585, found 268.0587.

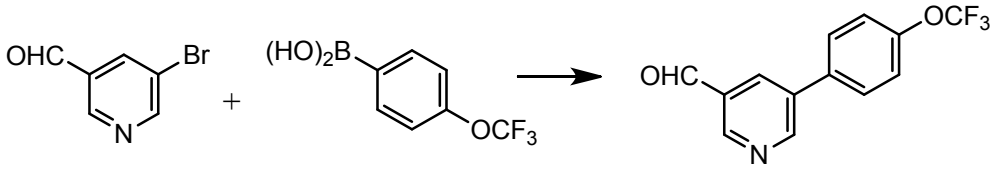
Preparation of **6-(4-(trifluoromethoxy)phenyl)picolinaldehyde, 2c**




**2c**

6-Bromopicolinaldehyde (930 mg, 5 mmol) was treated following the **General procedure 1** gave **2c** (1.20 g, 89%) as yellow needles. $^{1}$H NMR (400 MHz, $CDCl_3$) δ 10.16 (d, *J* = 0.6 Hz, 1H), 8.18 – 8.10 (m, 2H), 7.97 – 7.90 (m, 3H), 7.36 (dd, *J* = 8.9, 0.9 Hz, 2H).$^{13}$C NMR (101 MHz, $CDCl_3$) δ 194.03, 156.90, 153.22, 150.79, 150.77, 138.41, 137.10, 128.96, 124.68, 121.61, 120.45. ESI-HRMS: m/z calculated for $C_{13}H_8NO_2F_3Na$ ($[M+Na]^+$) 290.0405, found 290.0406.

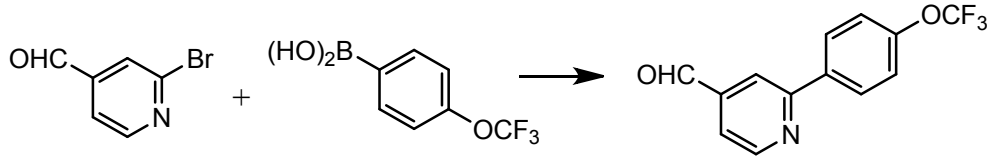
Preparation of **5-(4-(trifluoromethoxy)phenyl)nicotinaldehyde, 2d**




**2d**

5-Bromonicotinaldehyde (2.4 g, 13 mmol) was treated following the **General procedure 1** gave **2d** (3.4 g, 99%) as a white solid. $^{1}$H NMR (400 MHz, $CDCl_3$) δ 10.21 (s, 1H), 9.09 (d, *J* = 1.7 Hz, 1H), 9.07 (d, *J* = 2.2 Hz, 1H), 8.34 (t, *J* = 2.1 Hz, 1H), 7.67 (d, *J* = 8.8 Hz, 2H), 7.38 (d, *J* = 8.0 Hz, 2H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 190.92, 153.38, 151.41, 150.24, 136.48, 135.40, 134.04, 131.82, 129.17, 122.18.ESI-HRMS: m/z calculated for $C_{14}H_{13}NO_3F_3$ ([(M+MeOH)+H]$^+$) 300.0848, found 300.0847.

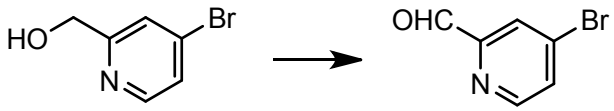
Preparation of **2-(4-(trifluoromethoxy)phenyl)isonicotinaldehyde, 2e**




**2e**

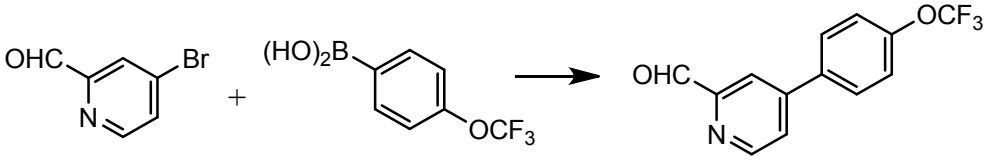
2-Bromoisonicotinaldehyde (2.6 g, 14 mmol) was treated following the **General procedure 1** gave **2e** (3.52 g, 94%) as a colourless oil. $^{1}$H NMR (400 MHz, $CDCl_3$) δ 10.14 (s, 1H), 8.94 (dd, *J* = 4.9, 0.6 Hz, 1H), 8.17 – 8.02 (m, 3H), 7.65 (dd, *J* = 4.9, 1.4 Hz, 1H), 7.34 (d, *J* = 8.0 Hz, 2H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 191.74, 158.01, 151.60, 150.82, 150.81, 143.00, 137.13, 128.95, 121.55, 121.44, 118.94. ESI-HRMS: m/z calculated for $C_{14}H_{13}NO_3F_3$ ([(M+MeOH)+H]$^+$) 300.0848, found 300.0853.

Preparation of **4-bromopicolinaldehyde**




Wet DCM (10 mL) was added slowly to the stirring solution of (4-bromopyridin-2-yl)methanol (658 g, 3.5 mmol) and DMP (1.5 eq) in DCM (15 mL). The cloudy mixture was left for an hour then diluted with ether, concentrated on rotavap. The residue was then taken up in 30 mL of ether and then washed with 20 mL of 1:1 mixture of 10% $Na_2S_2O_3$ and sat.$NaHCO_3$, followed by water and brine. The aqueous washings were back-extracted with ether. The combined organic layers were dried over $MgSO_4$ and evaporated to dryness. Purification was achieved by column chromatography (eluting with 40% EtOAc/*n*-Hexane) to afford 4-bromopicolinaldehyde (432 mg, 66%) as a yellow oil. $^1$H NMR (400 MHz, $CDCl_3$) δ 10.04 (s, 1H), 8.61 (dd, *J* = 5.2, 0.4 Hz, 1H), 8.12 (dd, *J* = 1.9, 0.5 Hz, 1H), 7.69 (dd, *J* = 5.2, 2.0 Hz, 1H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 192.42, 154.06, 151.27, 134.57, 131.31, 125.52.

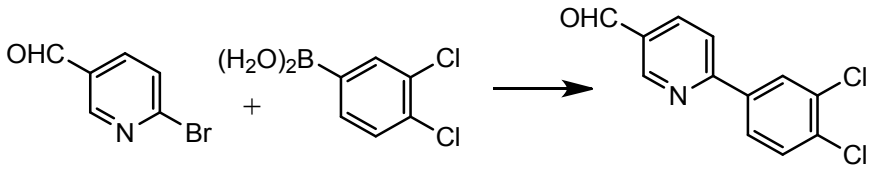
Preparation of **4-(4-(trifluoromethoxy)phenyl)picolinaldehyde, 2f**




**2f**

4-Bromopicolinaldehyde (550 mg, 2.96 mmol) was treated following the **General procedure 1** gave **2f** (610 mg, 83%) as a yellow solid. $^1$H NMR (400 MHz, $CDCl_3$) δ 10.15 (s, 1H), 8.85 (dd, *J* = 5.1, 0.7 Hz, 1H), 8.17 (dd, *J* = 1.9, 0.7 Hz, 1H), 7.75 – 7.67 (m, 3H), 7.37 (d, *J* = 8.0 Hz, 2H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 193.73, 151.23, 148.68, 129.06, 125.76, 122.03, 119.70, 117.74. ESI-HRMS: m/z calculated for $C_{13}H_8NO_2F_3Na$ ($[M+Na]^+$) 290.0405, found 290.0409.

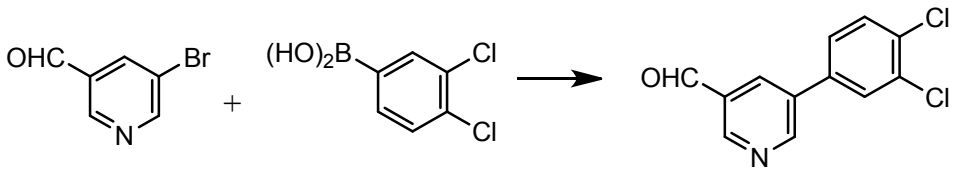
Preparation of **6-(3,4-dichlorophenyl)nicotinaldehyde, 2g**




**2g**

6-Bromonicotinaldehyde (986 mg, 5.3 mmol) and 3,4-dichlorobenzeneboronic acid (1.09 g, 5.7 mmol) was treated following the **General procedure 1** gave **2g** (1.04 g, 78%) as a white solid. $^{1}$H NMR (400 MHz, $CDCl_3$) δ 10.15 (s, 1H), 9.13 (d, *J* = 2.1 Hz, 1H), 8.28 – 8.21 (m, 2H), 7.93 (dd, *J* = 8.4, 2.1 Hz, 1H), 7.88 (d, *J* = 8.2 Hz, 1H), 7.59 (d, *J* = 8.4 Hz, 1H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 190.54, 159.94, 152.72, 138.20, 137.28, 135.16, 133.89, 131.36, 130.74, 129.85, 126.89, 120.81. MS (CI): m/z calculated for $C_{12}H_8{}^{35}Cl_2NO$ ($[M+H]^+$) 252.0, found 252.1(100%), 254.0(79), 256.2(11).

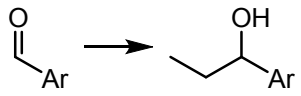
Preparation of **5-(3,4-dichlorophenyl)nicotinaldehyde, 2h**




**2h**

5-Bromonicotinaldehyde (930 mg, 5 mmol) and 3,4-dichlorobenzeneboronic acid (1.07 g, 5.5 mmol) was treated following the **General procedure 1** gave **2h** (987 mg, 78%) as a yellow solid. $^{1}$H NMR (400 MHz, $CDCl_3$) δ 10.21 (s, 1H), 9.09 (d, *J* = 0.8 Hz, 1H), 9.05 (d, *J* = 1.6 Hz, 1H), 8.31 (t, *J* = 2.2 Hz, 1H), 7.73 (d, *J* = 2.1 Hz, 1H), 7.60 (d, *J* = 8.3 Hz, 1H), 7.47 (dd, *J* = 8.3, 2.2 Hz, 1H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 190.73, 179.85, 153.27, 151.83, 136.72, 134.14, 133.89, 133.85, 131.76, 129.48, 128.08, 126.78. MS (CI): m/z calculated for $C_{12}H_8Cl_2NO$ ($[M+H]^+$) 252.0, found 252.2(100%), 254.2(66), 256.2(11).

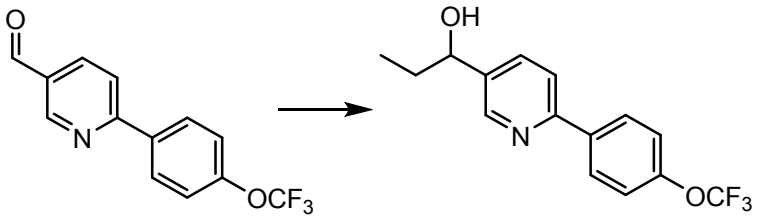
**General procedure 2.**




**2** **3**

Aldehyde **2** (1.0 eq) was dissolved in THF (30 mL) and cooled down to 0 $^{o}$C under $N_2$ atmosphere. 1.0 M Ethylmagnesium bromide solution (1.5 eq) was added dropwise to the mixture and the reaction was allowed to stir at 0 $^{o}$C for 1 hour. The solution was then quenched with 1M HCl (30 mL) and extracted with ether (3 x 30 mL). The combined organic layer was washed with brine and dried over $MgSO_4$. The solution was then evaporated under vacuum. The purification was performed using column chromatography eluted with 10% increasing to 40% EtOAc/*n*-Hexane.

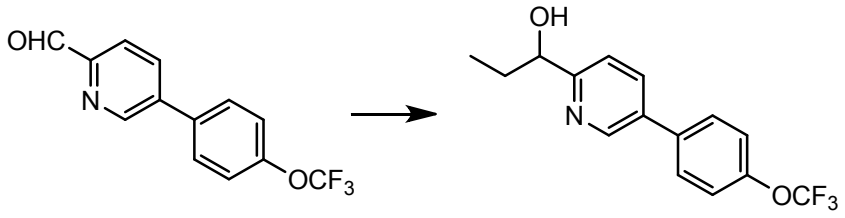
Preparation of **1-(6-(4-(trifluoromethoxy)phenyl)pyridin-3-yl)propan-1-ol, 3a**




**2a** **3a**

**2a** (1.28 g, 4.8 mmol) was treated following the **General procedure 2** to give **3a** as a white solid (954 mg, 67%). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 8.64 (d, *J* = 2.1 Hz, 1H), 8.02 (d, *J* = 9.0 Hz, 2H), 7.79 (ddd, *J* = 8.2, 2.3, 0.5 Hz, 1H), 7.71 (dd, *J* = 8.2, 0.6 Hz, 1H), 7.32 (dd, *J* = 8.9, 0.9 Hz, 2H), 4.73 (td, *J* = 6.8, 3.0 Hz, 1H), 1.93 – 1.75 (m, 2H), 0.97 (t, *J* = 7.4 Hz, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 155.76, 148.37, 138.17, 134.97, 128.72, 121.49, 120.62, 73.89, 32.35, 10.32. ESI-HRMS: m/z calculated for $C_{15}H_{15}NO_2F_3$ ($[M+H]^+$) 298.1055, found 298.1060.

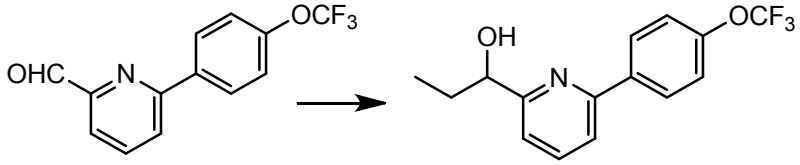
Preparation of **1-(5-(4-(trifluoromethoxy)phenyl)pyridin-2-yl)propan-1-ol, 3b**




**2b** **3b**

**2b** (1.20 g, 4.49 mmol) was treated following the **General procedure 2** to give **3b** as a yellow oil (503 mg, 38%). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 8.75 (d, *J* = 1.8 Hz, 1H), 7.86 (dd, *J* = 8.1, 2.3 Hz, 1H), 7.60 (d, *J* = 8.8 Hz, 2H), 7.39 – 7.29 (m, 3H), 4.76 (s, 1H), 4.03 (d, *J* = 4.6 Hz, 1H), 1.94 (dqd, *J* = 14.8, 7.4, 4.6 Hz, 1H), 1.83 – 1.70 (m, 1H), 0.99 (t, *J* = 7.4 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 161.70, 146.92, 136.74, 135.51, 134.47, 128.93, 121.99, 120.78, 74.14, 31.76, 9.84. ESI-HRMS: m/z calculated for $C_{15}H_{15}NO_2F_3$ ($[M+H]^+$) 298.1055, found 298.1053.

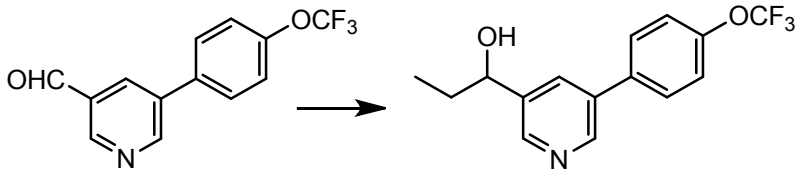
Preparation of **1-(6-(4-(trifluoromethoxy)phenyl)pyridin-2-yl)propan-1-ol, 3c**




**2c** **3c**

**2c** (1.0 g, 3.74 mmol) was treated following the **General procedure 2** to give **3c** as a yellow oil (816 mg, 73%). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 8.05 (d, *J* = 8.8 Hz, 2H), 7.78 (t, *J* = 7.8 Hz, 1H), 7.63 (d, *J* = 7.8 Hz, 1H), 7.33 (d, *J* = 8.0 Hz, 2H), 7.21 (d, *J* = 7.7 Hz, 1H), 4.76 (dd, *J* = 11.7, 5.0 Hz, 1H), 4.46 (d, *J* = 5.5 Hz, 1H), 1.95 (dqd, *J* = 14.8, 7.4, 4.6 Hz, 1H), 1.82 – 1.69 (m, 1H), 0.98 (t, *J* = 7.4 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 162.21, 154.70, 150.36, 138.03, 137.85, 128.77, 121.49, 119.58, 119.24, 73.89, 31.80, 9.77. ESI-HRMS: m/z calculated for $C_{15}H_{14}NO_2F_3Na$ ($[M+Na]^+$) 320.0874, found 320.0877.

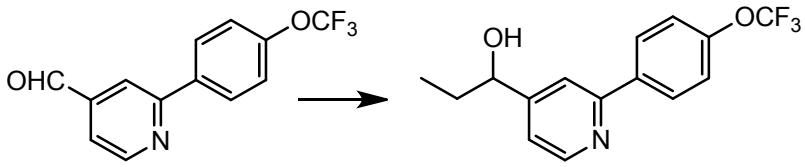
Preparation of **1-(5-(4-(trifluoromethoxy)phenyl)pyridin-3-yl)propan-1-ol, 3d**




**2d** **3d**

**2d** (4.0 g, 15.0 mmol) was treated following the **General procedure 2** to give **3d** as a yellow oil (3.11 g, 77%). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 8.73 (s, 1H), 8.57 (s, 1H), 7.88 (s, 1H), 7.62 (d, *J* = 8.8 Hz, 2H), 7.34 (d, *J* = 8.5 Hz, 2H), 4.77 (t, *J* = 6.2 Hz, 1H), 2.11 (s, 1H), 1.96 – 1.76 (m, 2H), 0.98 (t, *J* = 7.4 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 149.68, 147.70, 147.47, 140.20, 136.90, 135.62, 132.34, 129.05, 121.97, 73.92, 32.52, 10.35. ESI HRMS: m/z calculated for $C_{15}H_{15}NO_2F_3$ ($[M+H]^+$) 298.1055, found 298.1051.

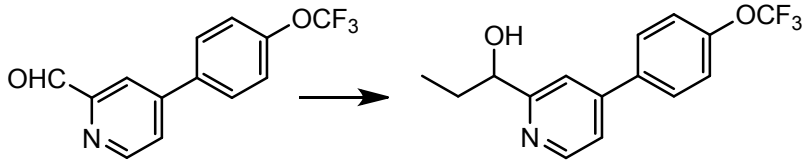
Preparation of **1-(2-(4-(trifluoromethoxy)phenyl)pyridin-4-yl)propan-1-ol, 3e**




**2e** **3e**

**2e** (3.62 g, 13.5 mmol) was treated following the **General procedure 2** to give **3e** as a yellow oil (2.17 g, 54%).$^{1}$H NMR (400 MHz, $CDCl_3$) δ 8.57 (dd, *J* = 5.1, 0.7 Hz, 1H), 7.97 (d, *J* = 8.9 Hz, 2H), 7.68 – 7.62 (m, 1H), 7.28 (dd, *J* = 8.9, 0.9 Hz, 2H), 7.17 (ddd, *J* = 5.1, 1.5, 0.5 Hz, 1H), 4.65 (t, *J* = 6.3 Hz, 1H), 2.83 (s, 1H), 1.85 – 1.70 (m, 2H), 0.95 (t, *J* = 7.4 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 156.59, 155.08, 150.29, 149.99, 138.33, 128.86, 121.39, 120.22, 118.14, 74.72, 32.18, 10.11. ESI HRMS: m/z calculated for $C_{15}H_{15}NO_2F_3$ ($[M+H]^+$) 298.1055, found 298.1056.

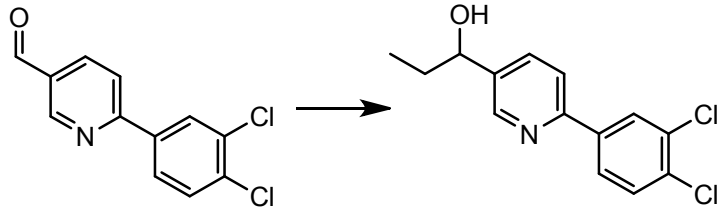
Preparation of **1-(4-(4-(trifluoromethoxy)phenyl)pyridin-2-yl)propan-1-ol, 3f**




**2f** **3f**

**2f** (640 mg, 2.39 mmol) was treated following the **General procedure 2** to give **3f** as a yellow oil (221 mg, 31%).$^{1}$H NMR (400 MHz, $CDCl_3$) δ 8.61 (d, *J* = 5.2 Hz, 1H), 7.66 (d, *J* = 8.8 Hz, 2H), 7.44 (s, 1H), 7.39 (dd, *J* = 5.2, 1.6 Hz, 1H), 7.34 (d, *J* = 8.1 Hz, 2H), 4.84 – 4.70 (m, 1H), 4.01 (s, 1H), 1.94 (dqd, *J* = 14.8, 7.5, 4.8 Hz, 1H), 1.85 – 1.71 (m, 1H), 0.99 (t, *J* = 7.4 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 163.35, 149.21, 148.15, 137.31, 129.03, 121.88, 120.73, 118.61, 117.74, 74.47, 31.84, 9.86. ESI HRMS: m/z calculated for $C_{15}H_{15}NO_2F_3$ ($[M+H]^+$) 298.1055, found 298.1064.

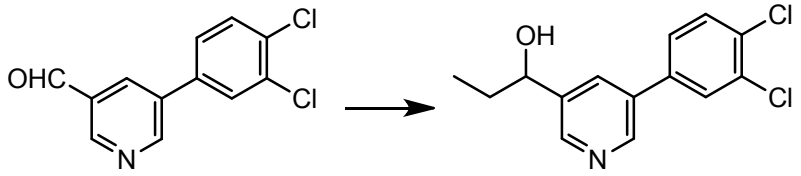
Preparation of **1-(6-(3,4-dichlorophenyl)pyridin-3-yl)propan-1-ol, 3g**




**2g** **3g**

**2g** (1.0 g, 3.97 mmol) was treated following the **General procedure 2** to give **3g** as a colourless oil (934 mg, 83%). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 8.63 (d, *J* = 2.2 Hz, 1H), 8.13 (d, *J* = 2.1 Hz, 1H), 7.83 (dd, *J* = 8.4, 2.1 Hz, 1H), 7.79 (ddd, *J* = 8.2, 2.3, 0.4 Hz, 1H), 7.69 (dd, *J* = 8.2, 0.6 Hz, 1H), 7.53 (d, *J* = 8.4 Hz, 1H), 4.73 (td, *J* = 6.6, 3.6 Hz, 1H), 1.96 (d, *J* = 3.6 Hz, 1H), 1.85 (qd, *J* = 13.7, 7.4 Hz, 2H), 0.97 (t, *J* = 7.4 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 154.60, 148.43, 139.46, 139.32, 135.02, 133.48, 131.08, 129.15, 126.28, 120.49, 117.73, 73.82, 32.36, 10.25. ESI-HRMS: m/z calculated for $C_{14}H_{14}NO^{35}Cl_2$ ($[M+H]^+$) 282.0452, found 282.0445.

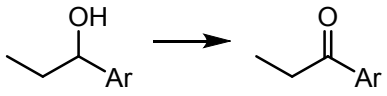
Preparation of **1-(5-(3,4-dichlorophenyl)phenyl)pyridin-3-yl)propan-1-ol, 3h**




**2h** **3h**

**2h** (673 mg, 2.67 mmol) was treated following the **General procedure 2** to give **3h** as a yellow oil (490 mg, 65%). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 8.71 (d, *J* = 2.2 Hz, 1H), 8.58 (d, *J* = 2.0 Hz, 1H), 7.86 (t, *J* = 1.9 Hz, 1H), 7.68 (d, *J* = 2.1 Hz, 1H), 7.56 (d, *J* = 8.3 Hz, 1H), 7.43 (dd, *J* = 8.3, 2.2 Hz, 1H), 4.77 (td, *J* = 6.7, 2.7 Hz, 1H), 2.02 (d, *J* = 3.1 Hz, 1H), 1.93 – 1.78 (m, 2H), 0.99 (t, *J* = 7.4 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 147.48, 147.11, 134.24, 133.35, 131.76, 131.07, 129.03, 127.69, 126.39, 73.45, 32.16, 9.89. ESI-HRMS: m/z calculated for $C_{14}H_{14}NO^{35}Cl_2$ ($[M+H]^+$) 282.0452, found 282.0447.

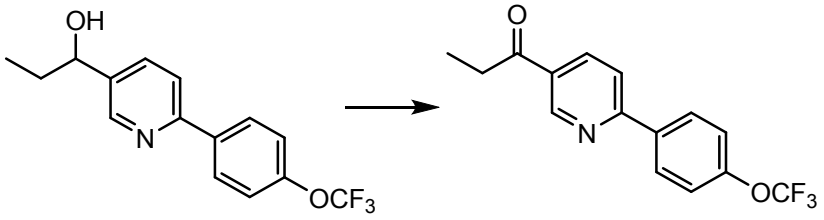
**General procedure 3.**




**3** **4**

To a stirred solution of alcohol **3** (1.0 eq) in DCM (30 mL), PCC (1.5 eq) was added and the mixture was allowed to stir under $N_2$ atmosphere for 2 hours at ambient temperature. The reaction was then quenched and diluted by adding ether (60 mL). The solution was passed through a silica pad to get rid of any precipitate. The filtrate was concentrated under vacuum to yield the crude product as clear yellow oil which was further purified by column chromatography (eluting with 10% EtOAc/*n*-Hexane) to give the product.

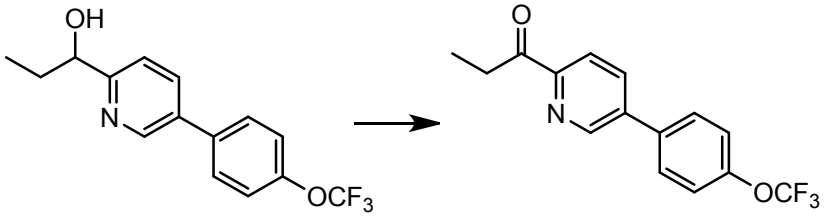
Preparation of **1-(6-(4-(trifluoromethoxy)phenyl)pyridin-3-yl)propan-1-one, 4a**




**3a** **4a**

**3a** (954 mg, 3.21 mmol) was oxidised according to the **General procedure 3** to give **4a** as a pale yellow solid (654 mg, 69%). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 9.24 (d, *J* = 1.4 Hz, 1H), 8.32 (dd, *J* = 8.3, 2.0 Hz, 1H), 8.11 (d, *J* = 8.8 Hz, 2H), 7.82 (d, *J* = 8.3 Hz, 1H), 7.35 (d, *J* = 8.1 Hz, 2H), 3.06 (q, *J* = 7.2 Hz, 2H), 1.28 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 199.55, 159.62, 151.00, 150.16, 137.14, 136.81, 130.97, 129.30, 122.12, 121.54, 120.47, 32.60, 8.35. ESI-HRMS: m/z calculated for $C_{15}H_{13}NO_2F_3$ ($[M+H]^+$) 296.0898, found 296.0899.

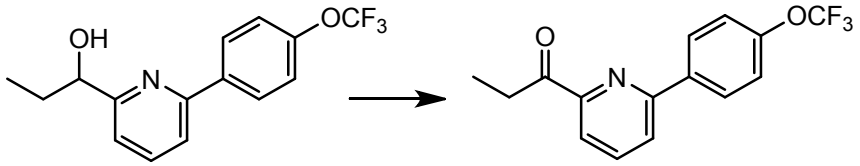
Preparation of **1-(5-(4-(trifluoromethoxy)phenyl)pyridin-2-yl)propan-1-one, 4b**




**3b** **4b**

**3b** (474 mg, 1.59 mmol) was oxidised according to the **General procedure 3** to give **4b** as a pale yellow solid (457 mg, 97 %). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 8.88 (dd, *J* = 2.3, 0.6 Hz, 1H), 8.14 (dd, *J* = 8.1, 0.7 Hz, 1H), 7.99 (dd, *J* = 8.1, 2.3 Hz, 1H), 7.66 (d, *J* = 8.8 Hz, 2H), 7.37 (d, *J* = 8.8 Hz, 2H), 3.28 (q, *J* = 7.3 Hz, 2H), 1.25 (t, *J* = 7.3 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 202.55, 152.77, 150.16, 147.67, 138.79, 136.06, 135.47, 129.21, 122.32, 122.09, 31.62, 8.40. ESI-HRMS: m/z calculated for $C_{15}H_{12}NO_2F_3Na$ ($[M+Na]^+$) 318.0718, found 318.0718.

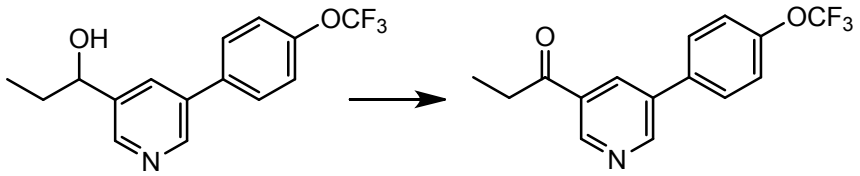
Preparation of **1-(5-(4-(trifluoromethoxy)phenyl)pyridin-2-yl)propan-1-one, 4c**




**3c** **4c**

**3c** (815 mg, 2.74 mmol) was oxidised according to the **General procedure 3** to give **4c** as a pale yellow solid (767 mg, 95 %). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 8.14 (d, *J* = 8.9 Hz, 2H), 8.00 (dd, *J* = 6.1, 2.7 Hz, 1H), 7.94 – 7.89 (m, 2H), 7.36 (dd, *J* = 8.9, 0.9 Hz, 2H), 3.37 (q, *J* = 7.3 Hz, 2H), 1.26 (t, *J* = 7.3 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 200.56, 153.68, 138.29, 128.81, 123.67, 121.58, 120.60, 31.56, 8.40. ESI-HRMS: m/z calculated for $C_{15}H_{12}NO_2F_3Na$ ($[M+Na]^+$) 318.0718, found 318.0722.

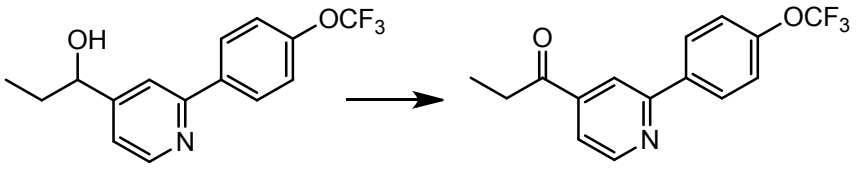
Preparation of **1-(5-(4-(trifluoromethoxy)phenyl)pyridin-3-yl)propan-1-one, 4d**




**3d** **4d**

**3d** (963 mg, 3.24 mmol) was oxidised according to the **General procedure 3** to give **4d** as a pale yellow solid (725 mg, 76 %). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 9.17 (d, *J* = 2.0 Hz, 1H), 8.98 (d, *J* = 2.3 Hz, 1H), 8.41 (t, *J* = 2.2 Hz, 1H), 7.65 (d, *J* = 8.8 Hz, 2H), 7.37 (d, *J* = 8.0 Hz, 2H), 3.10 (q, *J* = 7.2 Hz, 2H), 1.29 (t, *J* = 7.2 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 199.73, 152.00, 150.05, 148.85, 135.97, 135.91, 133.88, 132.48, 129.14, 122.11, 32.84, 8.32. ESI HRMS: m/z calculated for $C_{15}H_{13}NO_2F_3$ ($[M+H]^+$) 296.0898, found 296.0899.

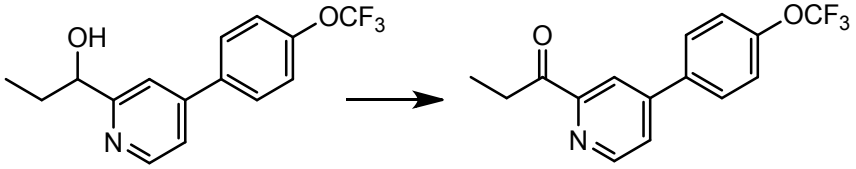
Preparation of **1-(2-(4-(trifluoromethoxy)phenyl)pyridin-4-yl)propan-1-one, 4e**




**3e** **4e**

**3e** (2.0 g, 6.73 mmol) was oxidised according to the **General procedure 3** to give **4e** as a pale yellow solid (1.21 g, 61 %). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 8.86 (dd, *J* = 5.0, 0.8 Hz, 1H), 8.18 – 8.15 (m, 1H), 8.09 (d, *J* = 8.9 Hz, 2H), 7.68 (dd, *J* = 5.0, 1.5 Hz, 1H), 7.35 (d, *J* = 8.0 Hz, 2H), 3.07 (q, *J* = 7.2 Hz, 2H), 1.27 (t, *J* = 7.2 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 200.43, 157.77, 151.29, 144.23, 137.65, 128.95, 121.56, 120.23, 118.07, 32.75, 8.21. ESI HRMS: m/z calculated for $C_{15}H_{13}NO_2F_3$ ($[M+H]^+$) 296.0898, found 296.0895.

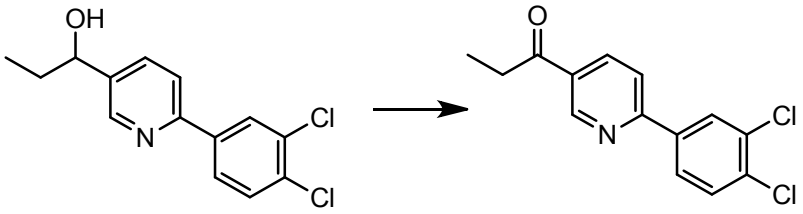
Preparation of **1-(4-(4-(trifluoromethoxy)phenyl)pyridin-2-yl)propan-1-one, 4f**




**3f** **4f**

**3f** (280 mg, 0.94 mmol) was oxidised according to the **General procedure 3** to give **4f** as a pale yellow solid (184 mg, 66 %). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 8.73 (dd, *J* = 5.1, 0.7 Hz, 1H), 8.25 (dd, *J* = 1.9, 0.7 Hz, 1H), 7.72 (d, *J* = 8.9 Hz, 2H), 7.65 (dd, *J* = 5.1, 1.9 Hz, 1H), 7.36 (dd, *J* = 8.8, 0.9 Hz, 2H), 3.29 (q, *J* = 7.3 Hz, 2H), 1.25 (t, *J* = 7.3 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 202.94, 169.50, 154.52, 150.01, 148.37, 136.57, 129.02, 124.89, 121.95, 119.87, 117.73, 31.69, 8.39.ESI HRMS: m/z calculated for $C_{15}H_{12}NO_2F_3Na$ ($[M+Na]^+$) 318.0718, found 318.0724.

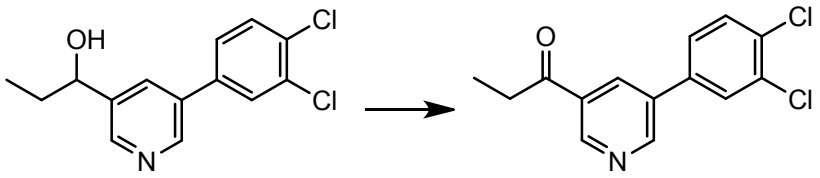
Preparation of **1-(6-(3,4-dichlorophenyl)pyridin-2-yl)propan-1-one, 4g**




**3g** **4g**

**3g** (512 mg, 2.13 mmol) was oxidised according to the **General procedure 3** to give **4g** as a pale yellow solid (354 mg, 69 %). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 9.23 (dd, *J* = 2.2, 0.8 Hz, 1H), 8.31 (dd, *J* = 8.3, 2.3 Hz, 1H), 8.21 (d, *J* = 2.1 Hz, 1H), 7.90 (dd, *J* = 8.4, 2.1 Hz, 1H), 7.81 (dd, *J* = 8.3, 0.8 Hz, 1H), 7.57 (d, *J* = 8.4 Hz, 1H), 3.06 (q, *J* = 7.2 Hz, 2H), 1.28 (t, *J* = 7.2 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 199.01, 169.12, 158.13, 149.78, 138.08, 136.50, 133.39, 130.93, 130.89, 129.25, 126.31, 120.00, 32.25, 7.95. ESI-HRMS: m/z calculated for $C_{14}H_{12}NO^{35}Cl_2$ ($[M+H]^+$) 280.0296, found 280.0290.

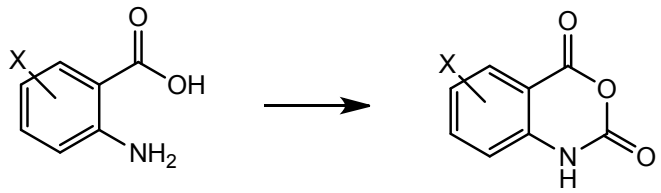
Preparation of **1-(5-(3,4-dichlorophenyl)pyridin-2-yl)propan-1-one, 4h**




**3h** **4h**

**3h** (490 mg, 1.73 mmol) was treated following the **General procedure 3** to give **4h** as a brown solid (160 mg, 33%). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 9.17 (s, 1H), 8.97 (s, 1H), 8.38 (s, 1H), 7.71 (d, *J* = 2.1 Hz, 1H), 7.59 (d, *J* = 8.3 Hz, 1H), 7.46 (dd, *J* = 8.3, 2.1 Hz, 1H), 3.09 (q, *J* = 7.2 Hz, 2H), 1.29 (t, *J* = 7.2 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 199.17, 169.12, 151.41, 148.82, 136.82, 133.62, 133.36, 133.20, 131.27, 129.09, 126.42, 32.46, 7.92. ESI-HRMS: m/z calculated for $C_{14}H_{12}NO^{35}Cl_2$ ($[M+H]^+$) 280.0296, found 282.0294.

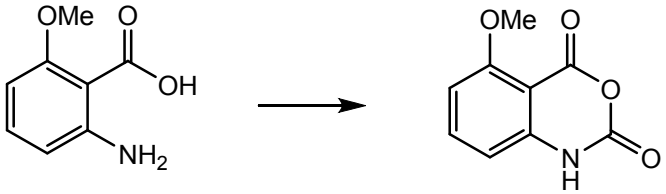
**General procedure 4.**



**5**

To a stirred solution of substituted anthranilic acid (1.0 eq) in THF (20 mL) and $H_2O$ (20 mL), $CCl_3COCl$ (1.5 eq) was added dropwisely at $^o$0 C. After the addition, the reaction was then allowed to raise the temperature to room temperature and stirred for 2 hours. The reaction mixture was evaporated to remove THF. The precipitate was filtered, washed successively with water, collected and dried under vacuum to give the desire product **5.**

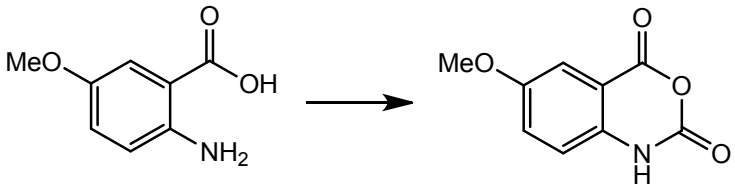
Preparation of **5-methoxy-1H-benzo[d][1,3]oxazine-2,4-dione, 5a**



**5a**

2-Amino-6-methoxybenzoic acid (2.50 g, 15 mmol) was reacted as in the **General procedure 4** to give **5a** as pale brown crystal (1.78 g, 61%). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.01 (s, 1H), 7.59 (t, *J* = 8.3 Hz, 1H), 6.74 (d, *J* = 8.5 Hz, 1H), 6.56 (dd, *J* = 8.1, 0.8 Hz, 1H), 4.01 (s, 3H).

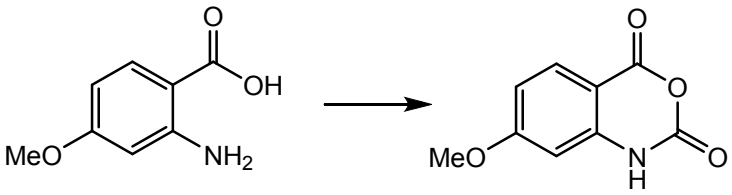
Preparation of **6-methoxy-1H-benzo[d][1,3]oxazine-2,4-dione, 5b**



**5b**

2-Amino-5-methoxybenzoic acid (2.50 g, 15 mmol) was treated as the **General procedure 4** to give **5b** as pale yellow solid (1.92 g, 66%). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.76 (s, 1H), 7.51 (d, *J* = 2.9 Hz, 1H), 7.29 (dd, *J* = 8.9, 2.9 Hz, 1H), 6.96 (d, *J* = 8.8 Hz, 1H), 3.87 (s, 3H).

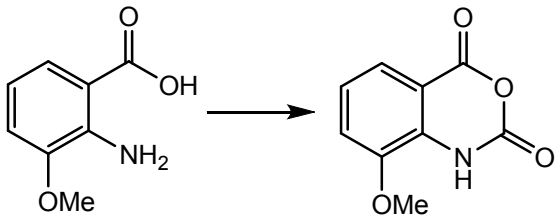
Preparation of **7-methoxy-1*H*-benzo[*d*][1,3]oxazine-2,4-dione, 5c**




**5c**

2-Amino-4-methoxybenzoic acid (2.50 g, 15 mmol) was reacted as in the **General procedure 4** to give **5c** as a pale brown solid (2.03 g, 70%). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.67 (s, 1H), 8.01 (d, *J* = 8.9 Hz, 1H), 6.82 (dd, *J* = 8.9, 2.3 Hz, 1H), 6.46 (d, *J* = 2.3 Hz, 1H), 3.92 (s, 3H). Elemental Analysis Calculated for $C_9H_7NO_4$: C, 55.96; H, 3.65; N, 7.25. Found: C, 53.36; H, 3.92; N, 6.88.

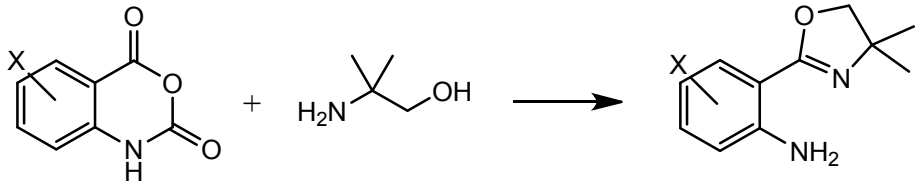
Preparation of **8-methoxy-1H-benzo[d][1,3]oxazine-2,4-dione, 5d**




**5d**

2-Amino-3-methoxybenzoic acid (2.50 g, 15 mmol) was reacted as in the **General procedure 4** to give **5d** as an off-white solid (2.12 g, 73%). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.02 (s, 1H), 7.67 (ddd, *J* = 7.2, 2.0, 0.6 Hz, 1H), 7.24 – 7.15 (m, 2H), 3.98 (s, 3H).

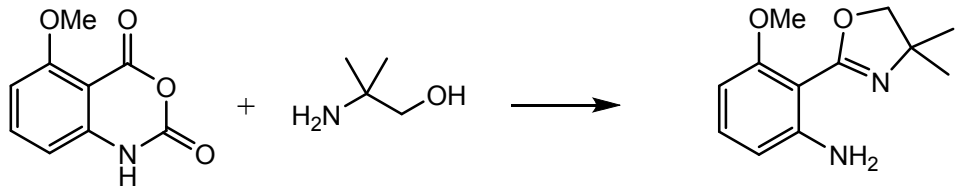
**General procedure 5.**



**5** **6**

The isatoic anhydride **5** (1.0 eq) was dissolved in chlorobenzene under nitrogen and stirred for 5 minutes. 2-amino-2-methylpropan-1-ol (1.5 eq) was added to the mixture, followed by addition of anhydrous zinc chloride (20 mol%). The resulting mixture was slowly heated to remove gas and then to reflux at 130 $^{o}$C for 24 hours. The mixture was allowed to cool to room temperature and the solvent was removed under vacuum. EtOAc (30 mL) was added to the residue and washed with brine. The aqueous layer was then extracted with EtOAc thrice. The combined organic layer was dried over $MgSO_4$, and the solvent was evaporated to yield dark brown oil which was further purified by column chromatography (eluting with 20% EtOAc/*n*-Hexane)

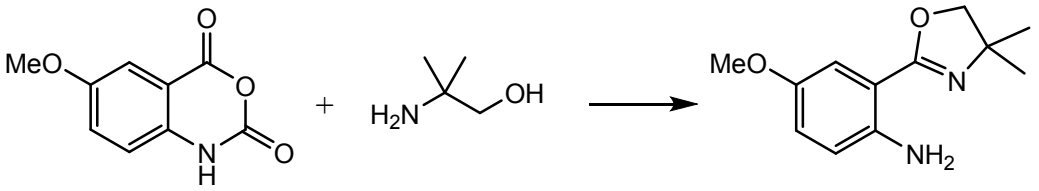
Preparation of **2-(4,4-dimethyl-4,5-dihydrooxazol-2-yl)-3-methoxyaniline, 6a**



**5a** **6a**

**5a** (1.28 g, 4.8 mmol) was treated following the **General procedure 5** but eluted the column with 50% EtOAc in *n*-Hexane to give **6a** as a yellow oil (1.66 g, 98%). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 7.76 (s, 2H), 7.08 (t, *J* = 8.2 Hz, 1H), 6.32 (dd, *J* = 8.2, 1.0 Hz, 1H), 6.21 (dd, *J* = 8.2, 0.7 Hz, 1H), 3.86 (s, 3H), 3.66 (s, 2H), 1.37 (s, 6H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 169.22, 159.02, 151.61, 132.21, 111.46, 105.74, 99.72, 71.39, 56.58, 56.42, 25.38.

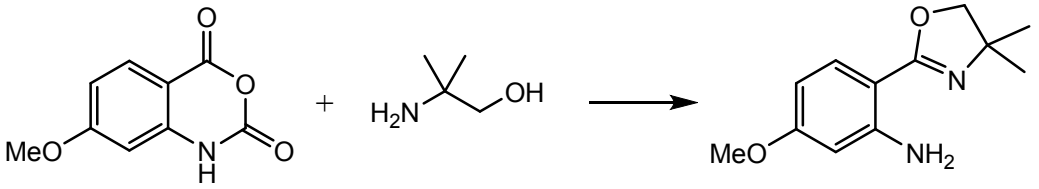
Preparation of **2-(4,4-dimethyl-4,5-dihydrooxazol-2-yl)-4-methoxyaniline, 6b**



**5b** **6b**

**5b** (1.93 g, 9.9 mmol) was treated following the **General procedure 5** to afford **6b** as pale-yellow solid (1.06 g, 48.4%). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 7.21 (d, *J* = 3.0 Hz, 1H), 6.86 (dd, *J* = 8.8, 3.0 Hz, 1H), 6.66 (d, *J* = 8.8 Hz, 1H), 5.76 (s, 2H), 4.00 (s, 2H), 3.76 (s, 3H), 1.37 (s, 6H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 162.22, 150.98, 143.41, 120.80, 117.60, 112.71, 109.86, 68.37, 56.32, 29.13. Elemental Analysis Calculated for $C_{12}H_{16}N_2O_2$: C, 65.43; H, 7.32; N, 12.72. Found: C, 65.45; H, 7.46; N, 12.65.

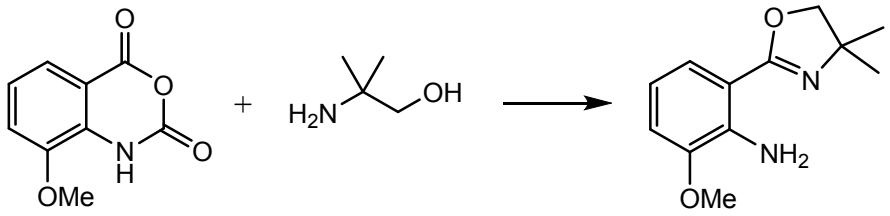
Preparation of **2-(4,4-dimethyl-4,5-dihydrooxazol-2-yl)-5-methoxyaniline, 6c**



**5c** **6c**

**5c** (2.0 g, 10.3 mmol) was treated as in the **General procedure 5** to give **6c** as a white solid (454 mg, 19.9%). $^{1}$H NMR (400 MHz, $CDCl_3$) δ $^{1}$H NMR (400 MHz, $CDCl_3$) δ 7.59 (d, *J* = 8.8 Hz, 1H), 6.25 (dd, *J* = 8.8, 2.5 Hz, 1H), 6.18 (d, *J* = 2.4 Hz, 1H), 6.14 (s, 2H), 3.96 (s, 2H), 3.78 (s, 3H), 1.35 (s, 6H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 162.94, 162.29, 150.57, 131.39, 103.87, 103.49, 99.63, 68.02, 55.54, 29.19. ESI-HRMS: m/z calculated for $C_{12}H_{17}N_2O_2$ ($[M+H]^+$) 221.1285, found 221.1285.

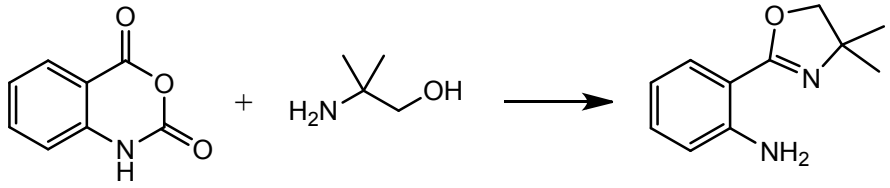
Preparation of **2-(4,4-dimethyl-4,5-dihydrooxazol-2-yl)-6-methoxyaniline, 6d**



**5d** **6d**

**5d** (2.13 g, 11.0 mmol) was treated as in the **General procedure 5** to give **6d** as a yellow solid (740 mg, 30.5%). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 7.30 (dd, *J* = 8.1, 1.3 Hz, 1H), 6.81 (dd, *J* = 7.9, 1.2 Hz, 1H), 6.59 (t, *J* = 8.0 Hz, 1H), 6.31 (s, 2H), 3.99 (s, 2H), 3.87 (s, 3H), 1.37 (s, 6H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 162.56, 147.16, 139.84, 121.44, 114.99, 111.91, 109.15, 68.21, 56.15, 29.17. Elemental Analysis Calculated for $C_{12}H_{16}N_2O_2$: C, 65.43; H, 7.32; N, 12.72. Found: C, 65.46; H, 7.42; N, 12.63.

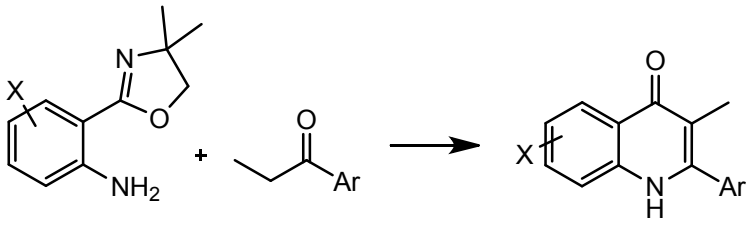
Preparation of **2-(4,4-dimethyl-4,5-dihydrooxazol-2-yl)aniline, 6e**



**6e**

Isatoic anhydride (10 g, 61 mmol) which is commercially available was treated as in the **General procedure 5** to give **6e** as an off-white solid (3.62 g, 31.2%) $^{1}$H NMR (400 MHz, $CDCl_3$) δ 7.67 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.22 – 7.15 (m, 1H), 6.69 (dd, *J* = 8.2, 1.0 Hz, 1H), 6.65 (ddd, *J* = 8.0, 7.2, 1.1 Hz, 1H), 6.08 (s, 2H), 3.99 (s, 2H), 1.36 (s, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 162.44, 148.88, 132.25, 129.85, 116.41, 116.00, 109.73, 68.23, 29.15. Elemental Analysis calculated for $C_{11}H_{14}N_2O$: C, 69.45; H, 7.42; N, 14.73. Found: C, 69.49; H, 7.42; N, 14.77.

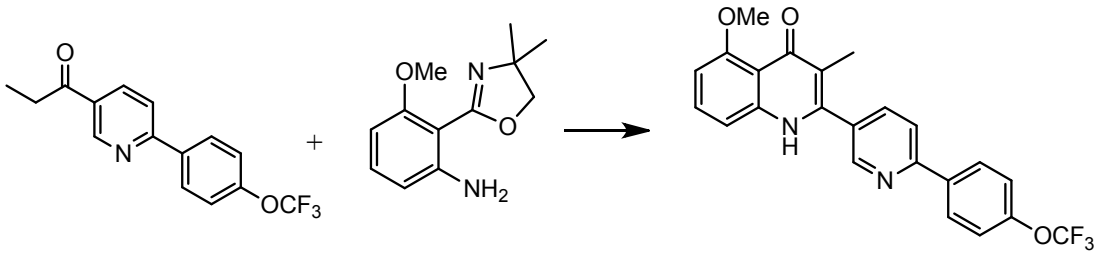
**General procedure 6.**



**6** **4** **7 - 22**

To a solution of *o*-oxazoline-substituted anilines **6** (1.1 eq, 1.1 mmol) and ketones **4** (1.0 eq, 1.0 mmol) in dry *n*-butanol (15 mL) was added trifluoromethane sulfonic acid (20 mol %) and the mixture was allowed to stir under reflux (135 $^{o}$C) for 24 hours. The reaction was cooled to room temperature and the solvent evaporated under vacuum. Saturated sodium carbonate solution (20 mL) was added. The aqueous solution was extracted with EtOAc (3 x 20 mL), washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The crude mixture was purified either trituration with EtOAC or by column chromatography to give quinolones.

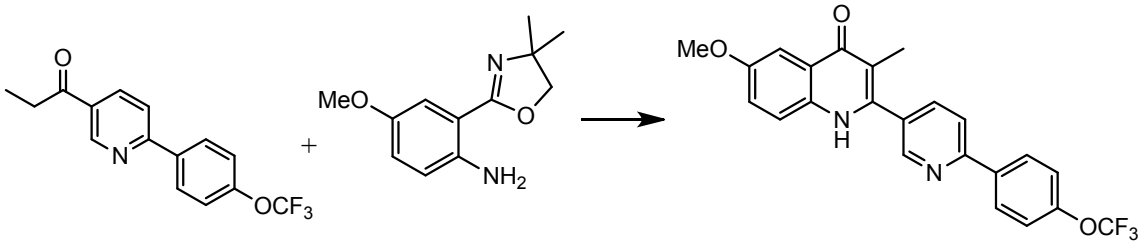
Preparation of **5-methoxy-3-methyl-2-(6-(4-(trifluoromethoxy)phenyl)pyridin-3-yl)quinolin-4(1H)-one, 7**



**4a** **6a** **7**

**4a** (309 mg, 1.04 mmol) was reacted with **6a** (235 mg, 1.2 mmol) as in the **General procedure 6** eluted with 10% MeOH in DCM to give **7** as a light brown powder (18 mg, 4%). $^{1}$H NMR (400 MHz, DMSO) δ 11.40 (s, 1H), 8.86 (s, 1H), 8.33 (d, *J* = 8.8 Hz, 2H), 8.22 (d, *J* = 8.1 Hz, 1H), 8.12 (dd, *J* = 8.1, 2.1 Hz, 1H), 7.54 (d, *J* = 8.0 Hz, 2H), 7.48 (t, *J* = 8.1 Hz, 1H), 7.10 (d, *J* = 8.1 Hz, 1H), 6.73 (d, *J* = 7.9 Hz, 1H), 3.82 (s, 3H), 1.86 (s, 3H). ES HRMS: m/z calculated for $C_{23}H_{18}N_2O_3F_3$ ($[M+H]^+$) 427.1270, found 427.1271

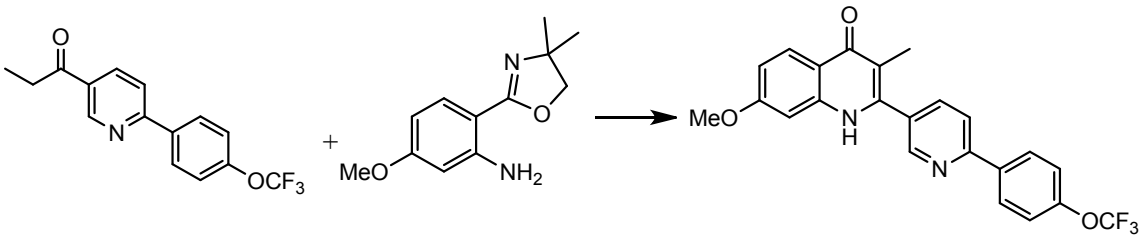
Preparation of **6-methoxy-3-methyl-2-(6-(4-(trifluoromethoxy)phenyl)pyridin-3-yl)quinolin-4(1H)-one, 8**



**4a** **6b** **8**

**4a** (309 mg, 1.04 mmol) was reacted with **6b** (235 mg, 1.2 mmol) as in the **General procedure 6** eluted with 5% MeOH in DCM to give **8** as a white solid (119 mg, 22%). mp = 312 - 314 $^{o}$C. $^{1}$H NMR (400 MHz, DMSO) δ 11.77 (s, 1H), 8.89 (s, 1H), 8.34 (d, *J* = 8.9 Hz, 2H), 8.24 (d, *J* = 8.1 Hz, 1H), 8.16 (dd, *J* = 8.1, 2.3 Hz, 1H), 7.56 (m, 4H), 7.31 (dd, *J* = 9.0, 3.0 Hz, 1H), 3.86 (s, 3H), 1.97 (s, 3H); $^{13}$C NMR (100 MHz, DMSO) δ 176.24, 155.70, 155.44, 149.77, 149.70, 143.99, 138.52, 137.47, 134.71, 130.37, 129.16, 124.43, 122.59, 121.73, 120.34, 114.45, 104.26, 55.69, 12.54. ES HRMS: m/z calculated for $C_{23}H_{18}N_2O_3F_3$ $[M+H]^+$ requires 427.1270, found 427.1248.

Preparation of **7-methoxy-3-methyl-2-(6-(4-(trifluoromethoxy)phenyl)pyridin-3-yl)quinolin-4(1H)-one, 9**

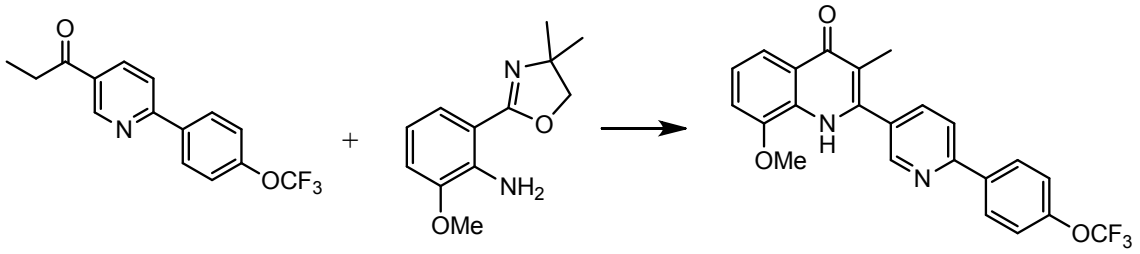


**4a** **6c** **9**

**4a** (253 mg, 0.86 mmol) was reacted with **6c** (126 mg, 0.57 mmol) as in the **General procedure 6** eluted with 50-80% EtOAc in DCM to give **9** as a pale yellow solid (126 mg, 52%). mp = 324 - 325 $^{o}$C. $^{1}$H NMR (400 MHz, DMSO) δ 11.60 (s, 1H), 8.88 (d, *J* = 1.9 Hz, 1H), 8.34 (d, *J* = 8.8 Hz, 2H), 8.24 (d, *J* = 8.2 Hz, 1H), 8.15 (dd, *J* = 8.2, 2.2 Hz, 1H), 8.04 (d, *J* = 8.9 Hz, 1H), 7.55 (d, *J* = 8.3 Hz, 2H), 6.99 (d, *J* = 2.3 Hz, 1H), 6.93 (dd, *J* = 9.0, 2.3 Hz, 1H), 3.84 (s, 3H), 1.93 (s, 3H). $^{13}$C NMR (101 MHz, DMSO) δ 162.06, 155.43, 149.75, 138.46, 130.34, 129.16, 129.16, 121.74,

121.74, 120.36, 118.03, 115.22, 113.59, 99.02, 55.72, 12.32. ES HRMS: m/z calculated for $C_{23}H_{18}N_2O_3F_3$ ($[M+H]^+$) 427.1270, found 427.1287.

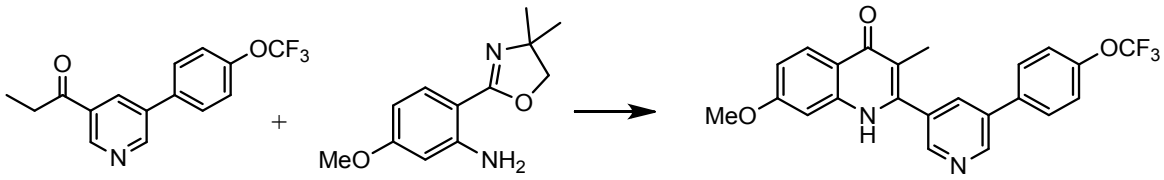
Preparation of **8-methoxy-3-methyl-2-(6-(4-(trifluoromethoxy)phenyl)pyridin-3-yl)quinolin-4(1H)-one, 10**



**4a** **6d** **10**

**4a** (350 mg, 1.2 mmol) was reacted with **6d** (317 mg, 1.4 mmol) as in the **General procedure 6** eluted with 80% EtOAc in DCM to give **10** as a pale yellow solid (124 mg, 25%). mp = 182 - 185 $^oC$. $^1H$ NMR (400 MHz, DMSO) δ 11.11 (s, 1H), 8.78 (d, *J* = 1.6 Hz, 1H), 8.33 (d, *J* = 8.9 Hz, 2H), 8.18 (dd, *J* = 8.2, 0.5 Hz, 1H), 8.05 (dd, *J* = 8.2, 2.3 Hz, 1H), 7.72 (dd, *J* = 7.8, 1.6 Hz, 1H), 7.54 (d, *J* = 8.1 Hz, 2H), 7.27 (t, *J* = 7.8 Hz, 1H), 7.23 (dd, *J* = 7.9, 1.6 Hz, 1H), 3.94 (s, 3H), 1.89 (s, 3H). ES HRMS: m/z calculated for $C_{23}H_{18}N_2O_3F_3$ ($[M+H]^+$) 427.1270, found 427.1268

Preparation of **7-methoxy-3-methyl-2-(5-(4-(trifluoromethoxy)phenyl)pyridin-3-yl)quinolin-4(1H)-one, 11**

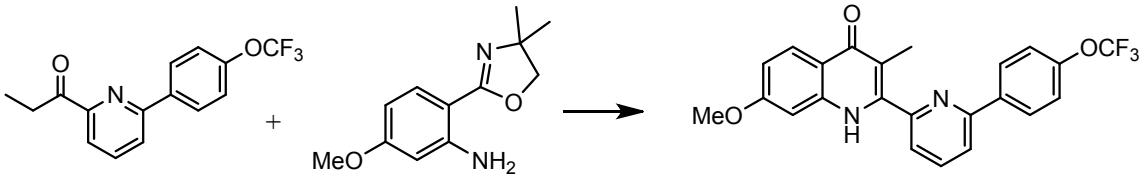


**4d** **6c** **11**

**4d** (295 mg, 1.0 mmol) was reacted with **6c** (242 mg, 1.1 mmol) as in the **General procedure 6** eluted with 5% MeOH in DCM to give **11** as a pale yellow solid (145 mg, 34%).; mp = 255 - 258 $^oC$; $^1H$ NMR (400 MHz, DMSO) δ 11.57 (s, 1H), 9.10 (d, *J* = 2.2 Hz, 1H), 8.80 (d, *J* = 2.0 Hz, 1H), 8.35 (t, *J* = 2.2 Hz, 1H), 8.05 (d, *J* = 9.0 Hz, 1H), 8.01 (d, *J* = 8.9 Hz, 2H), 7.55 (d, *J* = 7.9 Hz, 2H), 6.97 (d, *J* = 2.3 Hz, 1H), 6.93 (dd, *J* = 8.9, 2.4 Hz, 1H), 3.84 (s, 3H), 1.94 (s, 3H). $^{13}C$ NMR (101

MHz, DMSO) δ 176.55, 162.10, 148.91, 148.65, 144.18, 141.70, 136.05, 135.12, 134.25, 131.44, 129.57, 127.30, 122.10, 118.06, 115.27, 113.58, 99.03, 55.73, 12.31. ESI HRMS: m/z calculated for $C_{23}H_{18}N_2O_3F_3$ ([M+H]$^+$) 427.1270, found 427.1272.

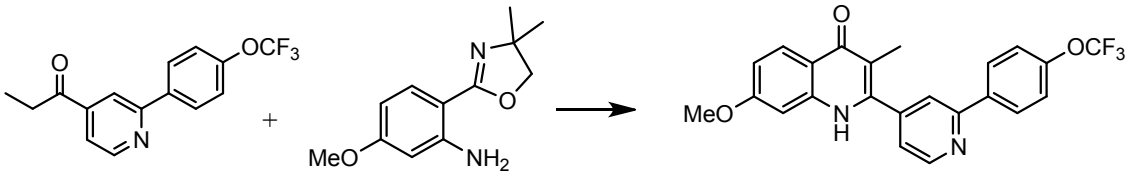
Preparation of **7-methoxy-3-methyl-2-(6-(4-(trifluoromethoxy)phenyl)pyridin-2-yl)quinolin-4(1H)-one, 12**




**4c** **6c** **12**

**4c** (205 mg, 0.69 mmol) was reacted with **6c** (205 mg, 0.93 mmol) as in the **General procedure 6** eluted with 100% EtOAc to give **12** as an off-white solid (118 mg, 40%).; mp = 200 - 204 $^o$C; $^1$H NMR (400 MHz, DMSO) δ 11.51 (s, 1H), 8.34 (d, *J* = 8.9 Hz, 2H), 8.21 – 8.13 (m, 2H), 8.05 (d, *J* = 9.0 Hz, 1H), 7.76 (dd, *J* = 7.2, 1.2 Hz, 1H), 7.55 (d, *J* = 8.1 Hz, 2H), 7.14 (d, *J* = 2.4 Hz, 1H), 6.93 (dd, *J* = 9.0, 2.4 Hz, 1H), 3.85 (s, 3H), 2.03 (s, 3H). $^{13}$C NMR (101 MHz, DMSO) δ 162.12, 155.14, 153.17, 148.43, 145.48, 142.92, 141.59, 138.72, 137.69, 129.37, 127.24, 121.65, 121.29, 113.66, 99.20, 55.73, 12.22. ESI HRMS: m/z calculated for $C_{23}H_{18}N_2O_3F_3$ ([M+H]$^+$) 427.1270, found 427.1282.

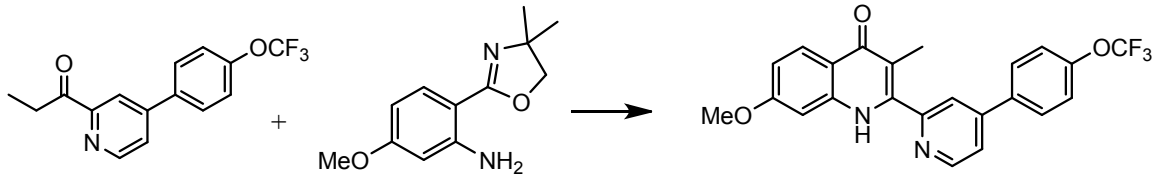
Preparation of **7-methoxy-3-methyl-2-(2-(4-(trifluoromethoxy)phenyl)pyridin-4-yl)quinolin-4(1H)-one, 13**




**4e** **6c** **13**

**4e** (295 mg, 1.0 mmol) was reacted with **6c** (242 mg, 1.1 mmol) as in the **General procedure 6** eluted with 100% EtOAc to give **13** as a white solid (147 mg, 34%).; mp = 245 - 247 $^{o}$C; $^{1}$H NMR (400 MHz, DMSO) δ 11.56 (s, 1H), 8.89 (d, *J* = 5.0 Hz, 1H), 8.34 (d, *J* = 8.9 Hz, 2H), 8.22 (s, 1H), 8.06 (d, *J* = 9.0 Hz, 1H), 7.60 (dd, *J* = 5.0, 1.5 Hz, 1H), 7.53 (d, *J* = 8.0 Hz, 2H), 7.00 (d, *J* = 2.4 Hz, 1H), 6.94 (dd, *J* = 9.0, 2.4 Hz, 1H), 3.85 (s, 4H), 1.93 (s, 3H). $^{13}$C NMR (101 MHz, DMSO) δ 176.55, 162.14, 155.48, 150.40, 144.93, 144.15, 141.66, 137.67, 129.18, 127.29, 123.24, 121.61, 120.71, 118.07, 114.70, 113.66, 111.41, 99.11, 55.74, 12.18. ESI HRMS: m/z calculated for $C_{23}H_{18}N_2O_3F_3$ ([M+H]$^{+}$) 427.1270, found 427.1266.

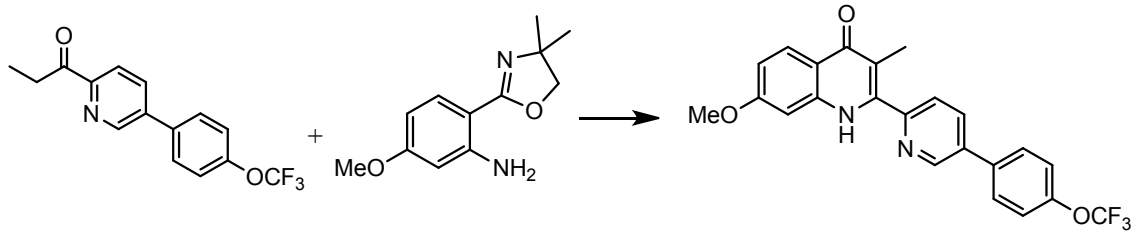
Preparation of **7-methoxy-3-methyl-2-(4-(4-(trifluoromethoxy)phenyl)pyridin-2-yl)quinolin-4(1H)-one, 14**




**4f** **6c** **14**

**4f** (184 mg, 0.62 mmol) was reacted with **6c** (151 mg, 0.68 mmol) as in the **General procedure 6** eluted with 100% EtOAc to give **14** as a white solid (113 mg, 42%).; mp = 188 - 190 $^{o}$C; $^{1}$H NMR (400 MHz, DMSO) δ 11.51 (s, 1H), 8.89 (d, *J* = 4.9 Hz, 1H), 8.12 – 8.03 (m, 4H), 7.93 (dd, *J* = 5.2, 1.8 Hz, 1H), 7.57 (d, *J* = 8.1 Hz, 2H), 7.13 (d, *J* = 2.4 Hz, 1H), 6.93 (dd, *J* = 9.0, 2.4 Hz, 1H), 3.85 (s, 3H), 2.03 (s, 3H). $^{13}$C NMR (101 MHz, DMSO) δ 176.96, 154.22, 150.65, 146.84, 145.47, 141.58, 136.32, 129.68, 127.25, 122.74, 122.09, 118.04, 115.03, 113.52, 99.27, 55.72, 12.17. ESI HRMS: m/z calculated for $C_{23}H_{18}N_2O_3F_3$ ([M+H]$^{+}$) 427.1270, found 427.1284.

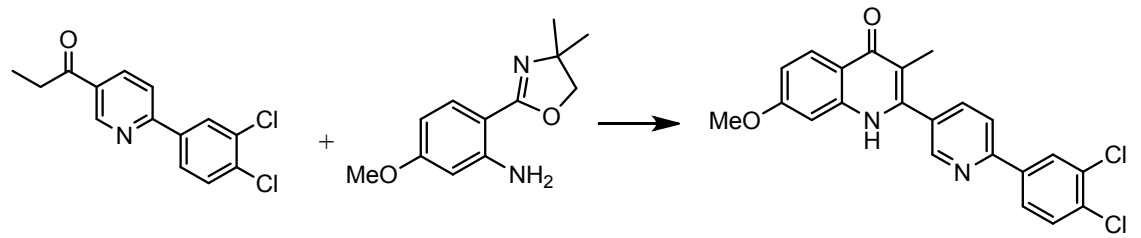
Preparation of **7-methoxy-3-methyl-2-(5-(4-(trifluoromethoxy)phenyl)pyridin-2-yl)quinolin-4(1H)-one, 15**



**4b** **6c** **15**

**4b** (265 mg, 0.90 mmol) was reacted with **6c** (268 mg, 1.22 mmol) as in the **General procedure 6** eluted with 100% EtOAc to give **15** as a pale yellow solid (237 mg, 62%).; mp = 255 - 258 $^{o}$C; $^{1}$H NMR (400 MHz, DMSO) δ 11.52 (s, 1H), 9.16 (dd, *J* = 2.4, 0.7 Hz, 1H), 8.36 (dd, *J* = 8.2, 2.4 Hz, 1H), 8.05 (d, *J* = 9.0 Hz, 1H), 8.01 (d, *J* = 8.8 Hz, 2H), 7.89 (dd, *J* = 8.2, 0.7 Hz, 1H), 7.57 (d, *J* = 8.0 Hz, 2H), 7.17 (d, *J* = 2.4 Hz, 1H), 6.93 (dd, *J* = 9.0, 2.4 Hz, 1H), 3.85 (s, 3H), 2.04 (s, 3H). $^{13}$C NMR (101 MHz, DMSO) δ 176.94, 162.12, 160.44, 152.40, 148.00, 145.09, 141.61, 136.03, 135.40, 134.83, 129.55, 127.22, 125.48, 122.17, 118.00, 114.96, 113.51, 99.35, 55.72, 12.27. ES HRMS: m/z calculated for $C_{23}H_{18}N_2O_3F_3$ ([M+H]$^{+}$) 427.1270, found 427.1281.

Preparation of **7-methoxy-3-methyl-2-(6-(3,4-dichlorophenyl)pyridin-3-yl)quinolin-4(1H)-one, 16**

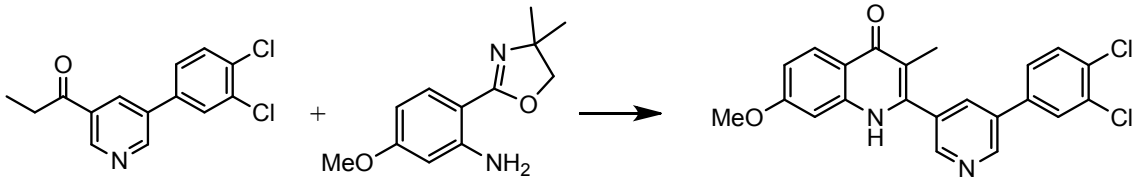


**4g** **6c** **16**

**4g** (195 mg, 0.70 mmol) was reacted with **6c** (126 mg, 0.66 mmol) as in the **General procedure 6** eluted with EtOAc to give **16** as a pale yellow solid (63 mg, 23%).; mp = 268 - 270 $^{o}$C; $^{1}$H NMR (400 MHz, DMSO) δ 11.56 (s, 1H), 8.89 (d, *J* = 1.7 Hz, 1H), 8.47 (d, *J* = 2.1 Hz, 1H), 8.31 (d, *J* = 8.0 Hz, 1H), 8.22 (dd, *J* = 8.5, 2.1 Hz, 1H), 8.15 (dd, *J* = 8.2, 2.3 Hz, 1H), 8.05 (d, *J* = 9.0

Hz, 1H), 7.83 (d, *J* = 8.5 Hz, 1H), 6.99 (d, *J* = 2.2 Hz, 1H), 6.94 (dd, *J* = 9.0, 2.4 Hz, 1H), 3.85 (s, 3H), 1.93 (s, 3H). ES HRMS: m/z calculated for $C_{22}H_{17}N_2O_2{}^{35}Cl_2$ ([M+H]$^+$) 411.0667, found 411.0683.

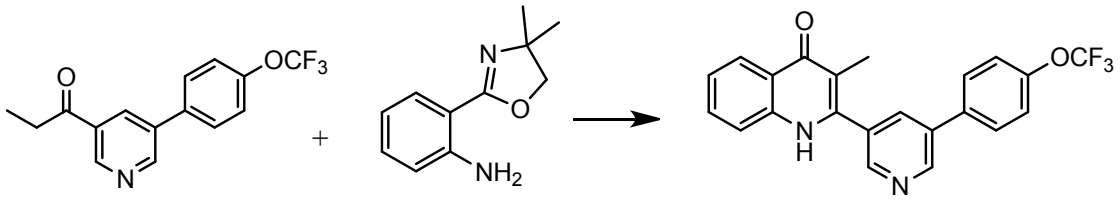
Preparation of **7-methoxy-3-methyl-2-(5-(3,4-dichlorophenyl)pyridin-3-yl)quinolin-4(1H)-one, 17**




**4h** **6c** **17**

**4h** (168 mg, 0.60 mmol) was reacted with **6c** (146 mg, 0.66 mmol) as in the **General procedure 6** washed with EtOAc to give **17** as a pale yellow solid (90 mg, 36%).; mp = 290 - 292 $^o$C; $^1$H NMR (400 MHz, DMSO) δ 11.59 (s, 1H), 9.15 (d, *J* = 2.2 Hz, 1H), 8.81 (d, *J* = 1.9 Hz, 1H), 8.43 (t, *J* = 2.1 Hz, 1H), 8.22 (d, *J* = 2.2 Hz, 1H), 8.05 (d, *J* = 8.9 Hz, 1H), 7.91 (dd, *J* = 8.4, 2.2 Hz, 1H), 7.82 (d, *J* = 8.5 Hz, 1H), 6.98 (d, *J* = 2.3 Hz, 1H), 6.94 (dd, *J* = 8.9, 2.4 Hz, 1H), 3.85 (s, 3H), 1.93 (s, 3H). ESI HRMS: m/z calculated for $C_{22}H_{17}N_2O_2{}^{35}Cl_2$ ([M+H]$^+$) 411.0667, found 411.0668.

Preparation of **3-methyl-2-(5-(4-(trifluoromethoxy)phenyl)pyridin-3-yl)quinolin-4(1H)-one, 18**

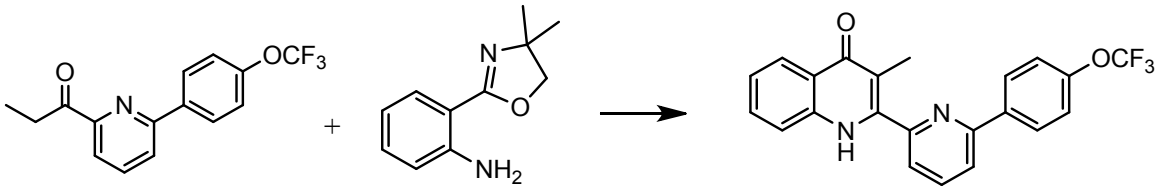



**4d** **6e** **18**

**4d** (238 mg, 0.81 mmol) was reacted with **6c** (217 mg, 1.14 mmol) as in the **General procedure 6** eluted with 100% EtOAc and 5% MeOH in EtOAc to give **18** as a pale yellow solid (172 mg, 54%).; mp = 217 - 220 $^o$C; $^1$H NMR (400 MHz, DMSO) δ 11.77 (s, 1H), 9.12 (d, *J* = 2.3 Hz, 1H), 8.82 (d, *J* = 2.0 Hz, 1H), 8.38 (t, *J* = 2.1 Hz, 1H), 8.16 (dd, *J* = 8.1, 1.2 Hz, 1H), 8.01 (d, *J* = 8.8 Hz, 2H), 7.66 (ddd, *J* = 8.3, 6.9, 1.5 Hz, 1H), 7.59 (d, *J* = 7.9 Hz, 1H), 7.56 (d, *J* = 8.0 Hz, 2H),

7.34 (ddd, *J* = 8.1, 6.9, 1.1 Hz, 1H), 1.96 (s, 3H). $^{13}$C NMR (101 MHz, DMSO) δ 176.99, 148.91, 148.72, 144.73, 139.94, 136.04, 135.18, 134.24, 131.88, 131.39, 129.59, 125.39, 123.50, 123.25, 122.13, 118.48, 115.63, 12.44. ES HRMS: m/z calculated for $C_{22}H_{16}N_2O_2F_3$ ([M+H]$^+$) 397.1164, found 397.1169.

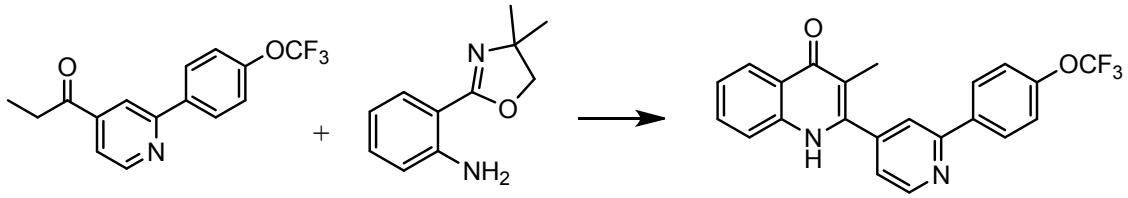
Preparation of **3-methyl-2-(6-(4-(trifluoromethoxy)phenyl)pyridin-2-yl)quinolin-4(1H)-one, 19**



**4c** **6e** **19**

**4c** (303 mg, 1.0 mmol) was reacted with **6e** (224 mg, 1.1 mmol) as in the **General procedure 6** eluted with 100% EtOAc and 5% MeOH in EtOAc to give **19** as an off-white solid (225 mg, 55%).; mp = 187 - 192 $^o$C; $^1$H NMR (400 MHz, DMSO) δ 11.74 (s, 1H), 8.33 (d, *J* = 8.9 Hz, 2H), 8.21 (dd, *J* = 8.0, 1.1 Hz, 1H), 8.19 – 8.13 (m, 2H), 7.78 (dd, *J* = 7.3, 1.1 Hz, 1H), 7.71 (d, *J* = 7.7 Hz, 1H), 7.66 (ddd, *J* = 8.3, 6.7, 1.5 Hz, 1H), 7.54 (d, *J* = 8.1 Hz, 2H), 7.33 (ddd, *J* = 8.1, 6.7, 1.3 Hz, 1H), 2.03 (s, 3H). $^{13}$C NMR (101 MHz, DMSO) δ 177.40, 155.13, 153.18, 146.02, 139.81, 138.77, 137.65, 131.88, 129.35, 125.37, 124.41, 123.47, 123.19, 121.67, 121.34, 118.72, 115.13, 12.27. ES HRMS: m/z calculated for $C_{22}H_{16}N_2O_2F_3$ ([M+H]$^+$) 397.1164, found 397.1179.

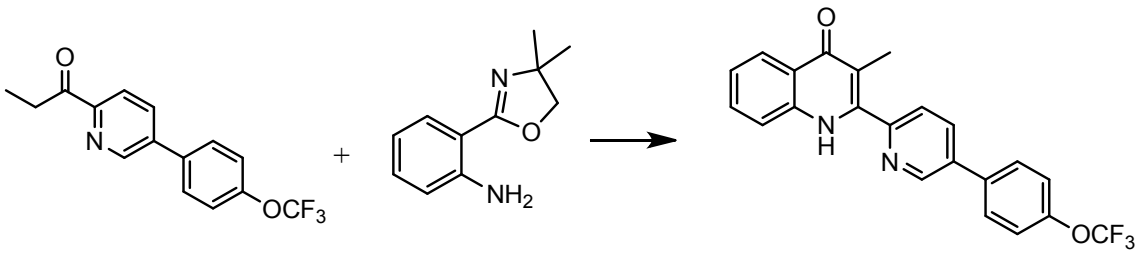
Preparation of **3-methyl-2-(2-(4-(trifluoromethoxy)phenyl)pyridin-4-yl)quinolin-4(1H)-one, 20**



**4e** **6e** **20**

**4e** (135 mg, 0.56 mmol) was reacted with **6e** (121 mg, 0.64 mmol) as in the **General procedure 6** eluted with 100% EtOAc to give **20** as a white solid (101 mg, 56%).; mp = 244 - 246 $^{o}$C; $^{1}$H NMR (400 MHz, DMSO) δ 11.78 (s, 1H), 8.90 (dd, *J* = 4.9, 0.7 Hz, 1H), 8.35 (d, *J* = 8.9 Hz, 2H), 8.26 (s, 1H), 8.16 (dd, *J* = 8.2, 1.1 Hz, 1H), 7.67 (ddd, *J* = 8.3, 6.8, 1.5 Hz, 1H), 7.63 (dd, *J* = 5.0, 1.5 Hz, 1H), 7.60 (d, *J* = 7.8 Hz, 1H), 7.53 (d, *J* = 8.1 Hz, 2H), 7.34 (ddd, *J* = 8.1, 6.8, 1.2 Hz, 1H), 1.95 (s, 3H). $^{13}$C NMR (101 MHz, DMSO) δ 150.42, 144.11, 139.89, 131.93, 129.18, 125.38, 123.52, 123.32, 123.28, 123.24, 121.64, 120.74, 118.56, 114.98, 12.30. ES HRMS: m/z calculated for $C_{22}H_{16}N_2O_2F_3$ ($[M+H]^+$) 397.1164, found 397.1171.

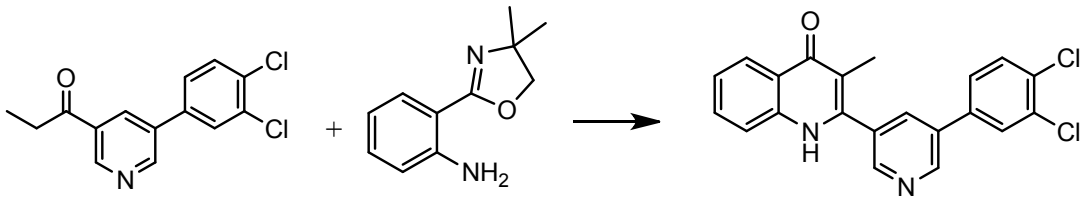
Preparation of **3-methyl-2-(5-(4-(trifluoromethoxy)phenyl)pyridin-2-yl)quinolin-4(1H)-one, 21**




**4b** **6e** **21**

**4b** (298 mg, 1.0 mmol) was reacted with **6e** (213 mg, 1.1 mmol) as in the **General procedure 6** eluted with 100% EtOAc to give **21** as a pale yellow solid (248 mg, 62%).; mp = 239 - 241 $^{o}$C; $^{1}$H NMR (400 MHz, DMSO) δ 11.74 (s, 1H), 9.18 (d, *J* = 1.7 Hz, 1H), 8.37 (dd, *J* = 8.2, 2.4 Hz, 1H), 8.15 (dd, *J* = 8.1, 1.3 Hz, 1H), 8.01 (d, *J* = 8.8 Hz, 2H), 7.91 (d, *J* = 8.2 Hz, 1H), 7.72 (d, *J* = 8.1 Hz, 1H), 7.65 (ddd, *J* = 8.4, 6.9, 1.5 Hz, 1H), 7.58 (d, *J* = 8.0 Hz, 2H), 7.33 (ddd, *J* = 8.0, 6.9, 1.1 Hz, 1H), 2.05 (s, 3H). $^{13}$C NMR (101 MHz, DMSO) δ 152.42, 148.07, 145.65, 139.86, 136.04, 135.44, 134.90, 131.88, 129.57, 125.56, 125.34, 123.43, 123.17, 122.21, 118.74, 115.25, 12.36. ES HRMS: m/z calculated for $C_{22}H_{16}N_2O_2F_3$ ($[M+H]^+$) 397.1164, found 397.1178.

Preparation of **3-methyl-2-(5-(3,4-dichlorophenyl)pyridin-3-yl)quinolin-4(1H)-one, 22**



**4h** **6e** **22**

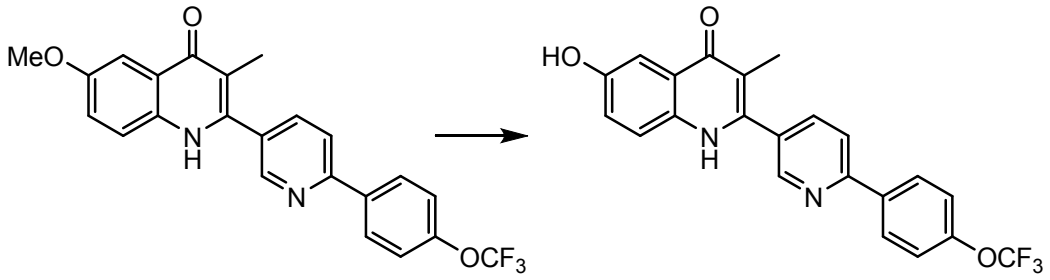
**4h** (168 mg, 0.60 mmol) was reacted with **60e** (126 mg, 0.66 mmol) as in the **General procedure 6** washed with EtOAc to give **22** as a pale yellow solid (58 mg, 25%).; mp = 270 - 272 $^{o}$C; $^{1}$H NMR (400 MHz, DMSO) δ 11.75 (s, 1H), 9.15 (d, *J* = 2.3 Hz, 1H), 8.82 (d, *J* = 2.0 Hz, 1H), 8.45 (t, *J* = 2.1 Hz, 1H), 8.22 (d, *J* = 2.1 Hz, 1H), 8.15 (dd, *J* = 8.0, 0.8 Hz, 1H), 7.91 (dd, *J* = 8.4, 2.2 Hz, 1H), 7.81 (d, *J* = 8.4 Hz, 1H), 7.66 (ddd, *J* = 8.3, 6.9, 1.5 Hz, 1H), 7.59 (d, *J* = 8.0 Hz, 1H), 7.33 (ddd, *J* = 8.0, 6.9, 1.1 Hz, 1H), 1.95 (s, 3H). HRMS (CI): m/z calculated for $C_{21}H_{14}N_2O^{35}Cl_2$ ($[M+H]^+$) 381.0556, found 381.0553.

**General procedure 7**

**8 - 10** **23 -25**

To a solution of methoxy quinolone (1.0 eq) in anhydrous DCM at 0 $^{o}$C was added 1M solution of $BBr_3$ in DCM (0.6 ml, 3.0 eq), the reaction mixture was allowed to warm to room temp and continued stirring for 24 hr. The reaction mixture was quenched with cold water and extracted with ethyl acetate. The combined organic extracts were washed with brine and dried over $MgSO_4$ and concentrated *in vacuo* to yield a brown solid which was purified by column chromatography (5% to 10% MeOH in DCM) to give hydroxyl analogue.

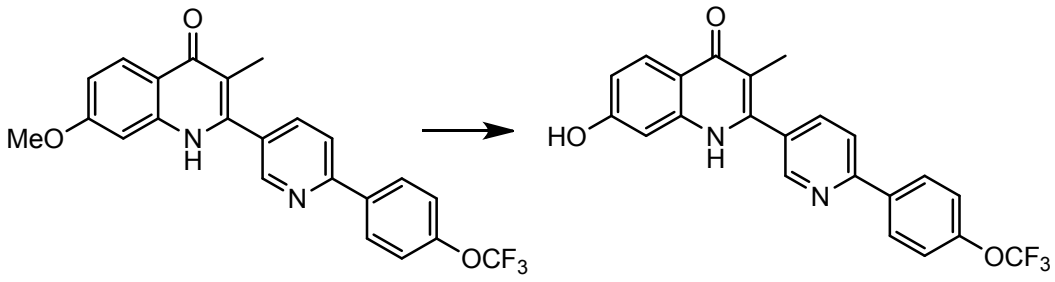
Preparation of **6-hydroxy-3-methyl-2-(6-(4-(trifluoromethoxy)phenyl)pyridin-3-yl)quinolin-4(1H)-one, 23**




**8** **23**

**8** (79 mg, 0.18 mmol) was treated as in the **General procedure 7** to give **23** as a white solid (10 mg, 10%). mp = decomposed at > 250 $^{o}$C. $^{1}$H NMR (400 MHz, DMSO) δ 11.61 (s, 1H), 9.62 (s, 1H), 8.87 (dd, *J* = 2.3, 0.8 Hz, 1H), 8.33 (d, *J* = 8.9 Hz, 2H), 8.22 (d, *J* = 7.6 Hz, 1H), 8.13 (dd, *J* = 8.2, 2.3 Hz, 1H), 7.54 (d, *J* = 8.0 Hz, 2H), 7.48 (d, *J* = 8.9 Hz, 1H), 7.46 (d, *J* = 2.8 Hz, 1H), 7.16 (dd, *J* = 8.9, 2.8 Hz, 1H), 1.93 (s, 3H). ES HRMS: m/z calculated for $C_{22}H_{16}N_2O_3F_3$ ($[M+H]^+$) 413.1113, found 413.1117.

Preparation of **7-hydroxy-3-methyl-2-(6-(4-(trifluoromethoxy)phenyl)pyridin-3-yl)quinolin-4(1H)-one, 24**

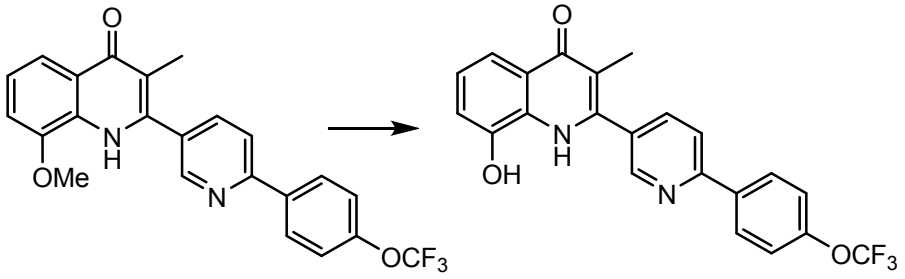



**9** **24**

**9** (82 mg, 0.18 mmol) was treated as in the **General procedure 7** to give **24** as a white solid (55 mg, 68%). $^{1}$H NMR (400 MHz, MeOD) δ 8.81 (dd, *J* = 2.2, 1.0 Hz, 1H), 8.33 – 8.20 (m, 3H), 8.17 – 8.13 (m, 1H), 8.11 (dd, *J* = 8.2, 1.0 Hz, 1H), 8.08 (dd, *J* = 8.2, 2.2 Hz, 1H), 7.45 (dd, *J* = 8.9, 0.9 Hz, 2H), 6.92 (dd, *J* = 8.9, 2.3 Hz, 1H), 6.89 (m, 1H), 2.06 (s, 3H); $^{13}$C NMR (101 MHz,

MeOD) δ 199.27, 178.62, 161.39, 156.56, 155.27, 150.26, 149.03, 145.49, 141.82, 137.97, 137.26, 130.10, 128.66, 126.87, 120.95, 120.26, 117.19, 115.18, 114.82, 112.86, 100.31, 11.01; ES HRMS: m/z calculated for $C_{22}H_{16}N_2O_3F_3$ ($[M+H]^+$) 413.1113, found 413.1102.

Preparation of **8-hydroxy-3-methyl-2-(6-(4-(trifluoromethoxy)phenyl)pyridin-3-yl)quinolin-4(1H)-one, 25**




**10** **25**

**10** (90 mg, 0.18 mmol) was treated as in the **General procedure 7** to give **25** as a light brown solid (35 mg, 40%). mp = 154 - 156 $^oC$ $^1H$ NMR (400 MHz, DMSO) δ 11.00 (s, 1H), 10.53 (s, 1H), 8.79 (d, *J* = 1.8 Hz, 1H), 8.33 (d, *J* = 8.8 Hz, 2H), 8.17 (d, *J* = 7.8 Hz, 1H), 8.06 (dd, *J* = 8.1, 2.2 Hz, 1H), 7.59 (dd, *J* = 8.2, 1.2 Hz, 1H), 7.54 (d, *J* = 8.1 Hz, 2H), 7.14 (t, *J* = 7.8 Hz, 1H), 7.05 (dd, *J* = 7.7, 1.4 Hz, 1H), 1.89 (s, 3H). ES HRMS: m/z calculated for $C_{22}H_{16}N_2O_3F_3$ ($[M+H]^+$) 413.1113, found 413.1109

## Biological methods

### *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.

### *Drug sensitivity assay*

Drug-sensitivity phenotypes of *P. falciparum* strains 3D7, W2, and TM90C2B (Thailand) have been described previously[3-5]. *In vitro* antimalarial activity of quinolones 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 frozen at -20°C overnight. Growth proliferation was determined by SYBR Green method. The half maximal inhibitory concentration ($IC_{50}$) was calculated using 'ic50' package in R programming software.

***Solubility assay***

***Test compounds***

The compounds have so far been tested in pH 7.4 (phosphats) and pH 1 (FIXANAL) buffers and in culture media. 20µl of 10mM stock compound in DMSO was added to 980 µl of each medium in Eppendorf's. This gives a final concentration of 200µM compound and 2% DMSO. Blanks were also made using 20µl of DMSO in 980µl media. For best results the experiment carries out in triplicate. The samples were rotated at room temperature over night to allow equilibration.

Using a needle the compounds were drawn up into a small syringe and passed through a 0.22 µm MILLEX GP PES membrane syringe end filter. The PES membrane in the filter is important to reduce the binding of the test compound. 200 µl of the resulting solution was transferred to a well in a UV 96 well plate (see materials).The spectrum was then read every 2nM between 200 and 400nM and the blank for each buffer was deducted.

***Calibration curve***

Two calibration curves of the test compounds were made using 50% DMSO and 50% buffer. pH 1 buffer was used for the pH1 samples and pH 7.4 buffer was used for the pH 7.4 and the media samples. (NB- once the DMSO was added the pH was readjusted using HCl and NaOH to counteract any variation from the dilution.)

In a UV 96 well plate a dilution series was made up for each compound using 200µM as the top concentration with 1 in 3 dilutions i.e. 200µm, 66.66µM, 22.22µM, 7.41µM, 2.50µM, 0.82µM, 0.27µM, 0.091µM, 0µM. The final volume in each well was 200µl. This was again read on the speck between 200 and 400nM every 2nM. The blank was deducted and a peak was selected from the graph. The absorbances at the peak's wavelength were plotted against concentration to produce a calibration curve. The maximum concentration of the compounds in the media and buffer solutions was read off the off the calibration curve using the absorbance at the corresponding wavelength.

***Metabolic stability***

Pooled human liver microsomes (pooled male and female) were purchased from a reputable commercial supplier. Alternative species and strains are available upon request. Microsomes are stored at -80°C prior to use.

Microsomes (final protein concentration 0.5mg/mL), 0.1M phosphate buffer pH7.4 and test compound (final substrate concentration = 3µM; final DMSO concentration = 0.25%) are pre-incubated at 37°C prior to the addition of NADPH (final concentration = 1mM) to initiate the reaction. The final incubation volume is 25µL. A control incubation is included for each compound tested where 0.1M phosphate buffer pH7.4 is added instead of NADPH (minus NADPH). Two control compounds are included with each species. All incubations are performed singularly for each test compound.

Each compound is incubated for 0, 5, 15, 30 and 45min. The control (minus NADPH) is incubated for 45min only. The reactions are stopped by the addition of 50µL methanol containing internal standard at the appropriate time points. The incubation plates are centrifuged at 2,500rpm for 20min at 4 °C to precipitate the protein. Following protein precipitation, the sample supernatants are combined in cassettes of up to 4 compounds and analysed using LC-MS/MS conditions.

From a plot of ln peak area ratio (compound peak area/internal standard peak area) against time, the gradient of the line is determined. Subsequently, half-life and intrinsic clearance are calculated using the equations below:

Elimination rate constant (k) = (- gradient)

Half-life ($t_{1/2}$) (min) = 0.693/k

Intrinsic Clearance ($CL_{int}$) (µL/min/mg protein) = V x 0.693/$t_{1/2}$ where V=Incubation volume µL/mg microsomal protein.

***Bovine $bc_1$ counterscreen***[6]

Cytochrome $bc_1$ complex from bovine heart was isolated from mitochondrial membranes as described previously[7]. Cytochrome *c* reductase activity measurements were assayed in 50 mM potassium phosphate, pH 7.5, 2 mM EDTA, 10 mM KCN, and 30 µM equine cytochrome *c* (Sigma Chemical, Poole, Dorset, UK) at room temperature[8]. Cytochrome *c* reductase activity was initiated by the addition of decylubiquinol (50 µM). Reduction of cytochrome *c* was monitored in a Cary 4000 UV-visible spectrophotometer (Varian, Inc., Palo Alto, CA) at 550 versus 542 nm. Initial rates (computer-fitted as zero-order kinetics) were measured as a function of decylubiquinol concentration. The cytochrome *b* content of membranes was determined from the dithionite-reduced minus ferricyanide-oxidized difference spectra, using $\epsilon_{562\text{-}575}$ = 28.5 $mM^{-1}\,cm^{-1}$.[9] Turnover rates of cytochrome *c* reduction were determined using $\epsilon_{550\text{-}542}$ = 18.1 $mM^{-1}\,cm^{-1}$. Inhibitors of $bc_1$ activity were added without prior incubation. DMSO in the assays did not exceed 0.3% (v/v). Data were collected and analyzed using an Online Instrument Systems Inc. computer interface and software.

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