Supporting information

Structure-activity relationship studies and *Plasmodium* life cycle profiling identifies pan-active N-aryl-3-trifluoromethyl pyrido[1,2-*a*]benzimidazoles that are efficacious in an *in vivo* mouse model of malaria.

Godfrey Mayoka[†], Mathew Njoroge[#], John Okombo[†], Liezl Gibhard[#], Margarida Sanches-Vaz[\], Diana Fontinha[\], Lyn-Marie Birkholtz[∝], Janette Reader[∝], Mariëtte van der Watt[∝], Theresa L Coetzer[▽], Sonja Lauterbach[▽], Alisje Churchyard[▽], Belinda Bezuidenhout[▽], Timothy J. Egan[†, T], Clive Yeates[‖], Sergio Wittlin[Ψ, ‡], Miguel Prudêncio[\] and Kelly Chibale[†, §, T]*

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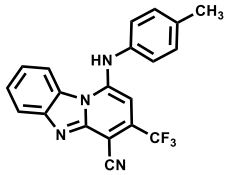
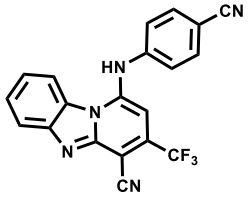
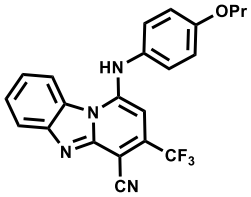
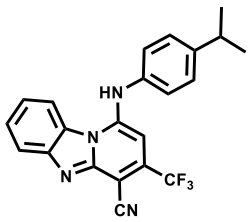
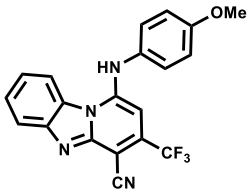
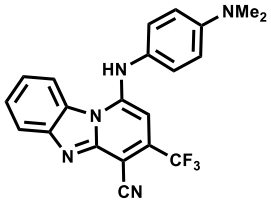
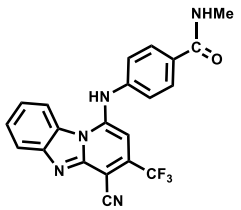
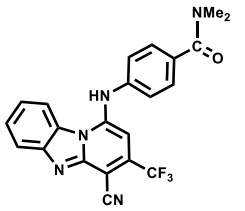
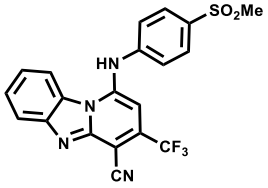
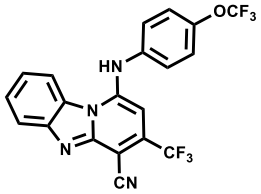
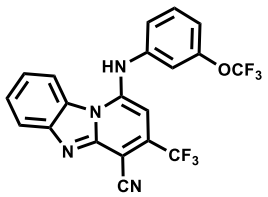
Contents:

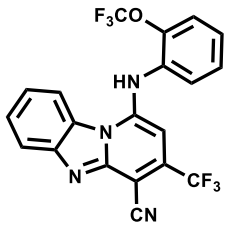
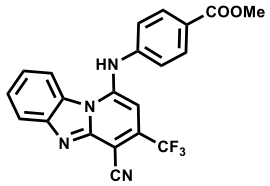
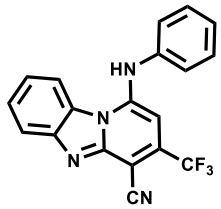
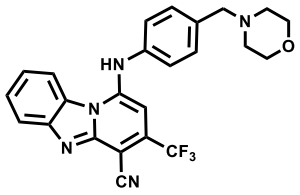
A. Additional spectrometric data (^{1}H NMR, ^{13}C NMR and Mass), experimental procedures, HPLC methods and biological data.

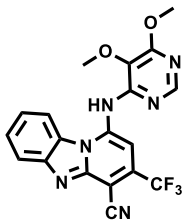
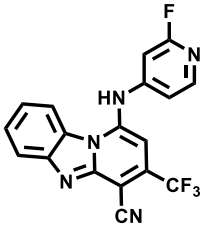
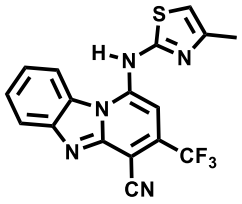
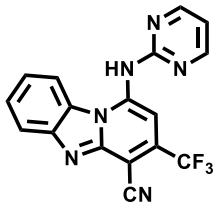
Table S1. Spectrometric, antiplasmodium and Beta hematin inhibition data for Final Compounds

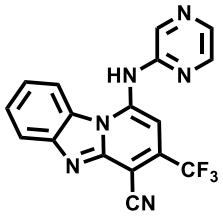
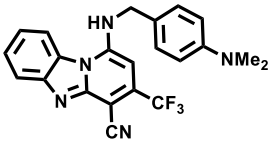
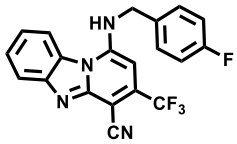
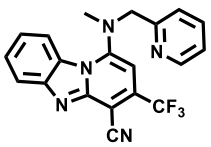
Compound's name and number	Structure	IC_{50} (µM) *Pf*NF54/BHI	MS m/z $[M+H]^+$	^{1}H and ^{13}C NMR
1-(p-tolylamino)-3-(trifluoromethyl) benzo [4,5] imidazo [1,2-a]pyridine-4-carbonitrile **1**		2.14/ 427232	367.1 (calcd for $C_{20}H_{13}F_3N_4$:366.1)	**^{1}H NMR** (400 MHz, DMSO) δ 8.77 (d, *J* = 8.3 Hz, 1H), 7.74 (d, *J* = 7.9 Hz, 1H), 7.60 (t, *J* = 7.7 Hz, 1H), 7.49 – 7.34 (m, 1H), 7.26 (d, *J* = 8.0 Hz, 2H), 7.09 (d, *J* = 6.2 Hz, 2H), 6.25 (s, 1H), 2.35 (s, 3H). **^{13}C NMR** (151 MHz, DMSO) δ 148.42, 147.89, 135.37, 134,52, 131.42, 128.71, 127.33, 125.04 (2C), 124.03, 123.13, 121.97 (2C), 121.30, 119.62, 117.83, 114.91, 112.47, 96.91, 20.94.
1-((4-cyanophenyl)amino)-3-(trifluoromethyl)benzo [4,5]imidazo[1,2-a]pyridine-4-carbonitrile **2**		2.36/596926	378.1 (calcd for $C_{20}H_{10}F_3N_5$:377.1)	**^{1}H NMR** (300 MHz, DMSO) δ 8.80 (d, *J* = 7.9 Hz, 1H), 7.84 (d, *J* = 8.7 Hz, 2H), 7.66 (d, *J* = 7.3 Hz, 1H), 7.63 – 7.56 (m, 1H), 7.49 – 7.36 (m, 1H), 7.21 (d, *J* = 8.5 Hz, 2H), 6.22 (s, 1H). **^{13}C NMR** (101 MHz, DMSO) δ 148.37, 148.11, 135.32, 135.00, 134.41, 132.48, 130.84, 128.61, 127.40, 123.17, 122.96 (2C), 119.80, 118.34, 117.93 (2C), 116.74, 115.21, 105.52, 96.60.
1-((4-propoxyphenyl) amino)-3-(trifluoromethyl) benzo[4,5] imidazo[1,2-a]pyridine-4-carbonitrile **3**		1.16/776	411.1 (calcd for $C_{22}H_{17}F_3N_4O$:410.1)	**^{1}H NMR** (400 MHz, DMSO) δ 8.77 (d, *J* = 8.3 Hz, 1H), 7.78 (d, *J* = 7.2 Hz, 1H), 7.61 (t, *J* = 7.7 Hz, 1H), 7.43 (t, *J* = 7.8 Hz, 1H), 7.18 (s, 2H), 7.04 (d, *J* = 8.7 Hz, 2H), 6.21 (s, 1H), 3.99 (t, *J* = 6.5 Hz, 2H), 1.85 – 1.70 (m, 2H), 1.02 (t, *J* = 7.4 Hz, 3H). **^{13}C NMR** (151 MHz, DMSO) δ 148.38, 144.85, 142.84, 138.92, 132.78, 128.71, 126.86, 123.64, 122.56, 121.82 (2C), 119.92, 117.18, 116.21 (2C), 114.92, 112.36, 110.64, 94.78, 69.98, 22.57, 10.75.

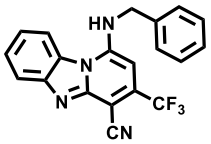
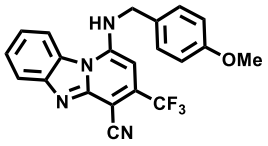
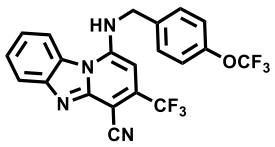
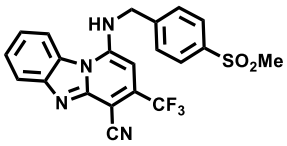
1-((4-isopropylphenyl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile **4**		1.01/43590	395.1 (calcd for $C_{22}H_{17}F_3N_4$:394.1)	**^{1}H NMR** (400 MHz, DMSO) δ 8.78 (d, *J* = 7.9 Hz, 1H), 7.75 (d, *J* = 11.3 Hz, 1H), 7.60 (t, *J* = 7.6 Hz, 1H), 7.42 (t, *J* = 7.8 Hz, 1H), 7.33 (d, *J* = 8.1 Hz, 2H), 7.13 (s, 2H), 6.28 (s, 1H), 3.01 – 2.89 (m, 1H), 1.27 (d, *J* = 6.9 Hz, 6H). **^{13}C NMR** (151 MHz, DMSO) δ 148.60, 145.17, 137.56, 135.40, 134.84, 133.92, 131.78. 129.34, 128.77, 127.87, 127.13, 125.40, 123.65, 122.58 (2C), 121.78, 117.16 (2C), 114.98, 33.58, 24.41 (2C).
1-((4-methoxyphenyl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile **5**		2.26/324434	383.1 (calcd for $C_{20}H_{13}F_3N_4O$:382.1)	**^{1}H NMR** (400 MHz, DMSO) δ 8.78 (d, *J* = 7.3 Hz, 1H), 8.35 (d, *J* = 8.4 Hz, 1H), 7.61 (t, *J* = 7.6 Hz, 1H), 7.44 (t, *J* = 7.9 Hz, 1H), 7.19 (d, *J* = 8.6 Hz, 2H), 7.05 (d, *J* = 8.4 Hz, 2H), 6.18 (s, 1H), 3.80 (s, 3H). **^{13}C NMR** (101 MHz, DMSO) δ 157.43, 149.23, 148.60, 134.78, 128.72, 127.09, 126.83, 124.89 (2C), 124.10, 122.41, 121.38, 118.71, 117.17, 115.60 (2C), 114.89, 112.82, 88.74, 55.92.
1-((4-(dimethylamino)phenyl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile **6**		5.62/687972	396.1 (calcd for $C_{21}H_{16}F_3N_5$:395.1)	**^{1}H NMR** (300 MHz, DMSO) δ 8.75 (d, *J* = 8.4 Hz, 1H), 7.82 (d, *J* = 7.4 Hz, 1H), 7.61 (t, *J* = 7.6 Hz, 1H), 7.43 (t, *J* = 7.9 Hz, 1H), 7.18 (d, *J* = 7.3 Hz, 2H), 6.84 (d, *J* = 8.6 Hz, 2H), 6.15 (s, 1H), 2.95 (s, 6H). **^{13}C NMR** (101 MHz, DMSO) δ 150.01, 149.05, 148.88, 141.89, 135.92, 132.68, 128.73, 126.99 (2C), 125.91, 121.92, 121.35 (2C), 118.86, 117.94, 116.82, 115.32, 113.65, 96.72, 46.58 (2C).
4-((4-cyano-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridin-1-yl)amino)-N-methylbenzamide **7**		>10/295	410.1 (calcd for $C_{21}H_{14}F_3N_5O$:409.1)	**^{1}H NMR** (400 MHz, DMSO) δ 8.81 (d, *J* = 8.2 Hz, 1H), 8.16 (d, *J* = 4.2 Hz, 1H), 7.90 (d, *J* = 8.6 Hz, 2H), 7.70 (d, *J* = 8.1 Hz, 1H), 7.63 – 7.57 (m, 1H), 7.46 – 7.40 (m, 1H), 7.15 (d, *J* = 8.0 Hz, 2H), 6.28 (s, 1H), 2.83 (d, *J* = 4.5 Hz, 3H). **^{13}C NMR** (151 MHz, DMSO) δ 166.84, 148.54, 148.08, 138.26, 134.98, 130.36, 129.19 (2C),

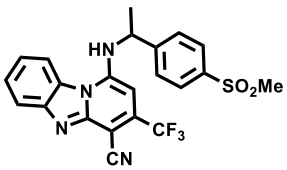
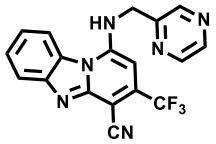
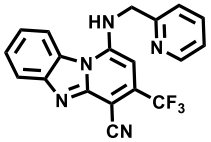
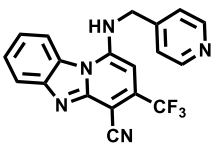
				128.77, 127.24, 125.44, 123.63, 122.97, 121.68 (2C), 117.61 (2C), 115.05, 113.39, 95.97, 26.35.
4-((4-cyano-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridin-1-yl)amino)-N,N-dimethylbenzamide **8**		>2.36/69464	424.1 (calcd for $C_{22}H_{16}F_3N_5O$:423.1)	**^{1}H NMR** (300 MHz, DMSO) δ 8.81 (d, *J* = 8.3 Hz, 1H), 7.67 (d, *J* = 8.3 Hz, 1H), 7.59 (t, *J* = 7.4 Hz, 1H), 7.48 (d, *J* = 8.3 Hz, 2H), 7.42 (t, *J* = 8.2 Hz, 1H), 7.12 (d, *J* = 6.8 Hz, 2H), 6.27 (s, 1H), 3.00 (s, 6H). **^{13}C NMR** (151 MHz, DMSO) δ 170.55, 148.55, 148.21, 135.01, 132.14, 129.21, 128.77, 127.22 (2C), 126.74, 125.37, 123.63, 122.92, 121.79, 120.00, 118.82, 117.72 (2C), 115.04, 96.35, 46.06 (2C).
1-((4-(methylsulfonyl) phenyl) amino)-3-(trifluoromethyl) benzo[4,5]imidazo [1,2-a]pyridine-4-carbonitrile **9**		>2.32/445	431.0 (calcd for $C_{20}H_{13}F_3N_4O_2S$:430.1)	**^{1}H NMR** (300 MHz, DMSO) δ 8.79 (d, *J* = 8.0 Hz, 1H), 7.88 (d, *J* = 8.6 Hz, 2H), 7.62 (d, *J* = 7.9 Hz, 1H), 7.51 – 7.41 (m, 1H), 7.29 – 7.20 (m, 3H), 6.00 (s, 1H), 3.21 (s, 3H). **^{13}C NMR** (101 MHz, DMSO) δ 155.13, 149.37, 133.81, 132.83, 130.01, 129.64, 129.07, 127.68, 125.52, 122.74 (2C), 121.41, 120.45, 117.20 (2C), 115.04, 106.70, 91.45, 86.09, 44.03.
1-((4-(trifluoromethoxy)phenyl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile **10**		0.17/17395	437.1 (calcd for $C_{20}H_{10}F_6N_4O$:436.1)	**^{1}H NMR** (400 MHz, DMSO) δ 8.82 (d, *J* = 8.1 Hz, 1H), 7.69 (d, *J* = 8.0 Hz, 1H), 7.64 – 7.54 (m, 1H), 7.46 – 7.42 (m, 1H), 7.39 (d, *J* = 8.3 Hz, 2H), 7.20 (d, *J* = 8.4 Hz, 2H), 6.24 (s, 1H). **^{13}C NMR** (151 MHz, DMSO) δ 148.53, 144.88, 135.23, 133.74, 128.91, 127.21, 125.36, 123.71, 122.92, 122.71 (2C), 121.81, 121.58, 120.05, 119.68, 117.77 (2C), 115.20, 113.38, 95.93.
1-((3-(trifluoromethoxy)phenyl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile **11**		0.39/-	437.1 (calcd for $C_{20}H_{10}F_6N_4O$:436.1)	**^{1}H NMR** (300 MHz, DMSO-d_6) δ 8.81 (d, *J* = 8.3 Hz, 1H), 7.58 (m, 3H), 7.42 (t, *J* = 7.8 Hz, 1H), 7.19 – 6.96 (m, 3H), 6.23 (s, 1H). **^{13}C NMR** (101 MHz, DMSO) δ 149.82, 148.61, 148.38, 135.72, 133.54, 131.74, 128.58, 127.31, 124.01, 123.11, 121.86, 121.27 (2C), 119.32, 117.88, 115.96, 115.17, 114.61, 112.64, 96.61.

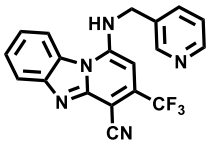
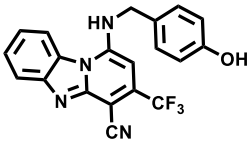
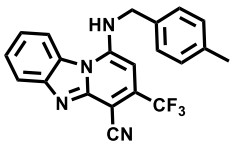
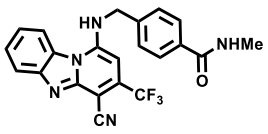
1-((2-(trifluoromethoxy)phenyl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile **12**		0.87/-	437.1 (calcd for $C_{20}H_{10}F_6N_4O$:436.1)	**^{1}H NMR** (300 MHz, DMSO-d_6) δ 8.87 (d, *J* = 8.3 Hz, 1H), 7.75 – 7.52 (m, 2H), 7.52 – 7.34 (m, 3H), 7.22 (m, 2H), 6.10 (s, 1H). **^{13}C NMR** (101 MHz, DMSO) δ 148.65, 141.74, 140.85, 135.03, 134.72, 133.29, 129.07, 128.59, 127.43, 124.84, 123.90, 123.24, 122.01, 121.27, 119.47, 117.73, 115.18, 112.16, 100.00, 97.15.
Methyl 4-((4-cyano-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridin-1-yl)amino)benzoate **13**		1.59/367	411.1 (calcd for $C_{21}H_{13}F_3N_4O_2$:410.1)	**^{1}H NMR** (400 MHz, DMSO) δ 8.80 (d, *J* = 8.2 Hz, 1H), 8.00 (d, *J* = 8.4 Hz, 2H), 7.68 (d, *J* = 8.0 Hz, 1H), 7.60 (t, *J* = 7.6 Hz, 1H), 7.42 (t, *J* = 7.8 Hz, 1H), 7.18 (d, *J* = 8.2 Hz, 2H), 6.27 (s, 1H), 3.87 (s, 3H). **^{13}C NMR** (101 MHz, DMSO) δ 166.47, 148.42, 147.89, 135.37, 131.42 (2C), 128.71, 127.33, 125.04 (2C), 124.03, 123.13, 121.97 (2C), 121.30, 117.83, 114.91, 114.03, 112.47, 96.91, 52.19.
1-(phenylamino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile **14**		>2.83/33266	353.1 (calcd for $C_{19}H_{11}F_3N_4$:352.1)	**^{1}H NMR** (300 MHz, DMSO) δ 8.80 (d, *J* = 8.2 Hz, 1H), 7.70 (d, *J* = 7.0 Hz, 1H), 7.59 (t, *J* = 7.3 Hz, 1H), 7.50 – 7.37 (m, 3H), 7.27 – 7.00 (m, 3H), 6.22 (s, 1H). **^{13}C NMR** (101 MHz, DMSO) δ 148.59, 148.45, 148.29, 136.94, 134.74, 132.38, 130.10, 128.77, 127.17 (2C), 124.55, 124.09, 122.75 (2C), 122.59, 119.85, 117.54, 115.05, 114.40.
1-((4-(morpholinomethyl)phenyl)amino)-3-(trifluoromethyl) benzo[4,5] imidazo[1,2-a]pyridine-4- carbonitrile **15**		>2.21/50	452.1 (calcd for $C_{24}H_{20}F_3N_5O$:451.2)	**^{1}H NMR** (300 MHz, DMSO) δ 8.79 (d, *J* = 8.1 Hz, 1H), 7.61 (d, *J* = 8.1 Hz, 1H), 7.47 –7.38 (m, 3H), 7.22 (t, *J* = 7.4 Hz, 1H), 7.07 (d, *J* = 8.2 Hz, 2H), 5.95 (s, 1H), 3.99 (s, 2H), 3.81-3.61 (m, 4H), 3.02-2.79 (m, 4H). **^{13}C NMR** (101 MHz, DMSO) δ 149.61, 134.71, 132.13 (2C), 130.01, 127.23, 126.46, 125.70, 125.51 (2C), 124.88, 122.47, 121.41, 121.24, 120.48, 117.17 (2C), 115.34, 113.14, 64.99 (2C), 61.03, 52.24 (2C).

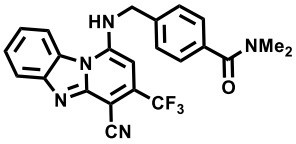
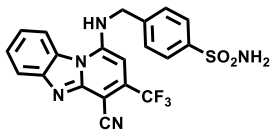
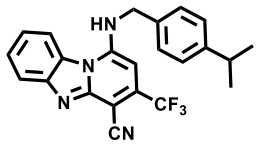
1-((5,6-dimethoxypyrimidin-4-yl)amino)-3-(trifluoromethyl) benzo[4,5] imidazo[1,2-a]pyridine-4- carbonitrile **16**		>2.82/320	415.1 (calcd for $C_{19}H_{13}F_3N_6O_2$:414.1)	**^{1}H NMR** (300 MHz, DMSO) δ 9.08 (d, *J* = 10.1 Hz, 1H), 8.36 (s, 1H), 7.69 (d, *J* = 8.7 Hz, 1H), 7.67 – 7.59 (m, 1H), 7.52 – 7.43 (m, 1H), 7.40 (s, 1H), 4.00 (s, 3H), 3.80 (s, 3H). **^{13}C NMR** (101 MHz, DMSO) δ 163.29, 162.70, 151.38, 147.54, 141.49, 135.42, 135.09, 128.73, 127.71(2C), 124.15, 123.12 (2C), 121.42, 118.52, 114.91, 100.05, 60.64, 54.00.
1-((2-fluoropyridin-4-yl)amino)-3-(trifluoromethyl)benzo [4,5]imidazo[1,2-a]pyridine-4-carbonitrile **17**		>2.52/11580	372.1 (calcd for $C_{18}H_9F_4N_5$:371.1)	**^{1}H NMR** (400 MHz, DMSO) δ 8.78 (d, *J* = 8.3 Hz, 1H), 8.13 (d, *J* = 5.4 Hz, 1H), 7.68 (d, *J* = 8.1 Hz, 1H), 7.57 (t, *J* = 7.6 Hz, 1H), 7.38 (t, *J* = 7.9 Hz, 1H), 6.98 (dt, *J* = 5.4, 1.7 Hz, 1H), 6.72 (s, 1H), 6.23 (s, 1H). **^{13}C NMR** (101 MHz, DMSO) δ 166.34, 164.04, 161.07, 148.68 (2C), 135.88, 128.78, 127.21, 124.13, 122.77, 121.40, 117.91, 116.13, 115.31, 113.38, 101.75, 101.36, 95.71.
1-((4-methylthiazol-2-yl)amino)-3-(trifluoromethyl)benzo [4,5]imidazo[1,2-a]pyridine-4-carbonitrile **18**		>2.82/309	374.1 (calcd for $C_{17}H_{10}F_3N_5S$:373.1)	**^{1}H NMR** (400 MHz, DMSO) δ 9.04 (d, *J* = 8.0 Hz, 1H), 7.83 (d, *J* = 8.1 Hz, 1H), 7.59 (ddd, *J* = 8.3, 7.2, 1.2 Hz, 1H), 7.41 (ddd, *J* = 8.4, 7.2, 1.2 Hz, 1H), 6.97 (s, 1H), 6.84 (d, *J* = 1.2 Hz, 1H), 2.33 (d, *J* = 1.2 Hz, 3H). **^{13}C NMR** (101 MHz, DMSO) δ 150.97, 148.06, 140.91, 137.55, 136.71, 136.39, 131.52, 129.88, 126.78, 121.87, 118.93, 118.18, 117.84, 114.97, 104.81, 100.04, 14.62.
1-(pyrimidin-2-ylamino)-3-(trifluoromethyl)benzo [4,5]imidazo[1,2-a]pyridine-4-carbonitrile **19**		>2.69/42237	355.1 (calcd for $C_{17}H_9F_3N_6$:354.1)	**^{1}H NMR** (300 MHz, DMSO) δ 8.99 (d, *J* = 8.4 Hz, 1H), 8.77 (d, *J* = 5.1 Hz, 2H), 7.83 (d, *J* = 8.0 Hz, 1H), 7.71 (s, 1H), 7.66 – 7.46 (m, 1H), 7.46 – 7.29 (m, 1H), 7.11 (t, *J* = 5.1 Hz, 1H). **^{13}C NMR** (151 MHz, DMSO) δ 158.43, 150.56, 148.30, 143.29, 135.63, 129.69, 126.91, 125.67, 123.86, 122.12, 121.73, 120.42, 118.97, 117.44, 115.05, 112.64, 96.36.

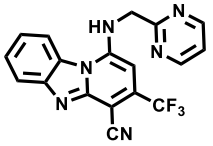
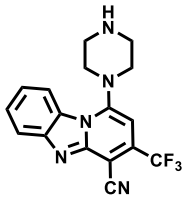
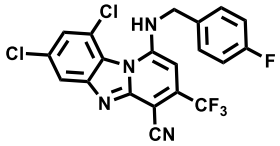
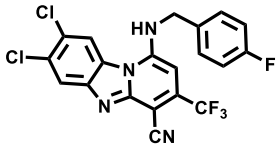
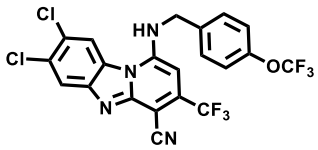
1-(pyrazin-2-ylamino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile **20**		4.42/25130	355.1 (calcd for $C_{17}H_9F_3N_6$:354.1)	**^{1}H NMR** (400 MHz, DMSO) δ 9.08 (d, *J* = 8.4 Hz, 1H), 8.54 (d, *J* = 1.4 Hz, 1H), 8.41 (dd, *J* = 2.7, 1.4 Hz, 1H), 8.25 (d, *J* = 2.7 Hz, 1H), 7.69 (ddd, *J* = 8.1, 1.2, 0.7 Hz, 1H), 7.63 (ddd, *J* = 8.2, 7.3, 1.1 Hz, 1H), 7.57 (s, 1H), 7.47 (ddd, *J* = 8.5, 7.3, 1.3 Hz, 1H). **^{13}C NMR** (101 MHz, DMSO) δ 161.20, 156.43, 149.86, 147.99, 147.30, 142.50 (2C), 138.59, 135.58, 128.83, 127.54, 123.16, 118.91, 118.58, 114.80, 112.86, 99.37.
1-((4-(dimethylamino)benzyl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile **21**		2.98/43519	408.1 $[M-H]^-$ (calcd for $C_{22}H_{18}F_3N_5O$:409.1)	**^{1}H NMR** (300 MHz, DMSO) δ 8.69 (d, *J* = 8.4 Hz, 1H), 8.58 (bs, 1H), 7.92 (d, *J* = 7.8 Hz, 1H), 7.72 – 7.59 (m, 1H), 7.53 – 7.43 (m, 1H), 7.33 (d, *J* = 8.7 Hz, 2H), 6.72 (d, *J* = 8.8 Hz, 2H), 6.36 (s, 1H), 4.77 (s, 2H), 2.87 (s, 6H). **^{13}C NMR** (101 MHz, DMSO) δ 150.34, 149.83, 148.46, 145.44, 137.25, 136.94, 128.60 (2C), 128.28, 127.07, 124.50, 124.11, 121.73, 121.38, 119.35, 115.54, 114.88, 112.88 (2C), 86.70, 46.58 (2C).
1-((4-fluorobenzyl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile **22**		1.27/750786	385.1 (calcd for $C_{20}H_{12}F_4N_4$:384.1)	**^{1}H NMR** (300 MHz, DMSO) δ 8.71 (d, *J* = 8.3 Hz, 1H), 8.63 (bs, 1H), 7.92 (d, *J* = 8.2 Hz, 1H), 7.71 – 7.61 (m, 1H), 7.61 – 7.53 (m, 2H), 7.53 – 7.45 (m, 1H), 7.25 – 7.16 (m, 2H), 6.30 (s, 1H), 4.90 (s, 2H). **^{13}C NMR** (101 MHz, DMSO) δ 163.26, 160.84, 149.97, 148.43, 137.33, 134.34, 133.80, 129.70 (2C), 129.62, 128.48, 127.05, 121.80, 119.35, 115.92 (2C), 115.71, 114.59, 86.63, 46.56.
1-(methyl(pyridin-2-ylmethyl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile **23**		>2.72/747	382.1 (calcd for $C_{20}H_{14}F_3N_5$:381.1)	**^{1}H NMR** (300 MHz, DMSO) δ 8.52 (ddd, *J* = 4.9, 1.8, 0.9 Hz, 1H), 8.42 (d, *J* = 8.4 Hz, 1H), 8.01 (d, *J* = 8.1 Hz, 1H), 7.80 (td, *J* = 7.7, 1.8 Hz, 1H), 7.69 (ddd, *J* = 8.3, 7.3, 1.0 Hz, 1H), 7.53 (ddd, *J* = 8.4, 7.2, 1.2 Hz, 1H), 7.42 (d, *J* = 7.8 Hz, 1H), 7.32 (ddd, *J* = 7.5, 4.9, 1.1 Hz, 1H), 6.85 (s, 1H), 4.76 (s, 2H), 2.98 (s, 3H).

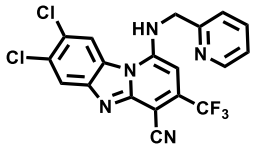
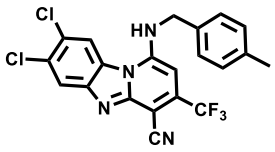
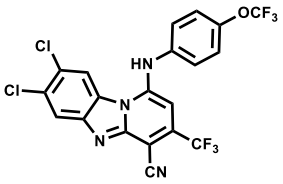
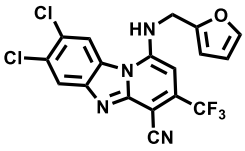
				^{13}C NMR (101 MHz, DMSO) δ 155.73, 154.08, 149.70, 148.00, 145.57, 137.36, 136.33, 136.00, 129.15, 127.40, 123.91, 123.34, 122.74, 119.93, 117.18, 113.64, 96.69, 96.65, 90.06, 58.79.
1-(benzylamino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile **24**		6.11/78048	367.1 (calcd for $C_{20}H_{13}F_3N_4$:366.1)	**^{1}H NMR** (300 MHz, DMSO) δ 8.73 (d, *J* = 8.4 Hz, 1H), 7.91 (d, *J* = 7.6 Hz, 1H), 7.70 – 7.59 (m, 1H), 7.53 – 7.43 (m, 3H), 7.41 – 7.33 (m, 2H), 7.34 – 7.24 (m, 1H), 6.29 (s, 1H), 4.91 (s, 2H). **^{13}C NMR** (101 MHz, DMSO) δ 150.07, 148.58, 145.42, 137.83, 137.22, 129.07 (2C), 128.55, 127.81 (2C), 127.55 (2C), 126.92, 124.17, 121.67, 119.19, 115.70, 114.83, 86.79, 47.41.
1-((4-methoxybenzyl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile **25**		3.18/641	397.1 (calcd for $C_{21}H_{15}F_3N_4O$:396.1)	**^{1}H NMR** (300 MHz, DMSO) δ 8.71 (d, *J* = 8.4 Hz, 1H), 7.91 (d, *J* = 8.1 Hz, 1H), 7.64 (t, *J* = 7.6 Hz, 1H), 7.52 – 7.41 (m, 3H), 6.98 – 6.89 (m, 2H), 6.31 (s, 1H), 4.82 (s, 2H), 3.74 (s, 3H). **^{13}C NMR** (101 MHz, DMSO) δ 159.24, 149.98, 148.55, 145.43, 137.09, 129.58, 128.95 (2C), 128.49, 126.94, 124.02, 121.66, 121.44, 119.22, 115.66, 114.80, 114.65 (2C), 86.76, 55.64, 46.82.
1-((4-(trifluoromethoxy)benzyl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile **26**		0.92/2123	451.1 (calcd for $C_{21}H_{12}F_6N_4O$:450.1)	**^{1}H NMR** (300 MHz, DMSO) δ 8.71 (d, *J* = 8.4 Hz, 1H), 7.92 (d, *J* = 7.7 Hz, 1H), 7.72 – 7.60 (m, 3H), 7.49 (ddd, *J* = 8.4, 7.2, 1.2 Hz, 1H), 7.37 (dd, *J* = 8.8, 0.9 Hz, 2H), 6.31 (s, 1H), 4.95 (s, 2H). **^{13}C NMR** (101 MHz, DMSO) δ 150.00, 148.48, 148.17, 137.28, 135.81, 133.84, 130.28, 129.51, 128.51, 127.00, 126.78, 124.13, 122.84, 121.48 (2C), 119.22, 115.75 (2C), 114.49, 86.73, 46.50.
1-((4-(methylsulfonyl)benzyl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile **27**		12.58/699	445.1 (calcd for $C_{21}H_{15}F_3N_4O_2S$:444.1)	**^{1}H NMR** (300 MHz, DMSO) δ 8.74 (d, *J* = 8.3 Hz, 1H), 7.98-7.88 (m, 3H), 7.79 (d, *J* = 8.3 Hz, 2H), 7.65 (t, *J* = 7.6 Hz, 1H), 7.54 – 7.45 (m, 1H), 6.31 (s, 1H), 5.04 (s, 2H), 3.21 (s, 3H). **^{13}C NMR** (101 MHz, DMSO) δ 150.04, 148.22, 144.13, 140.23, 136.98, 128.56, 128.23 (2C),

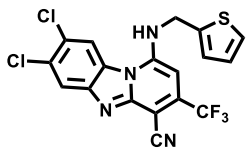
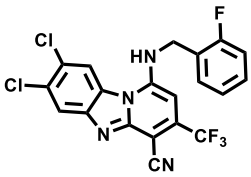
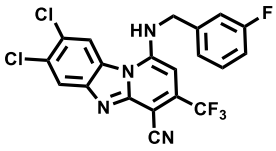
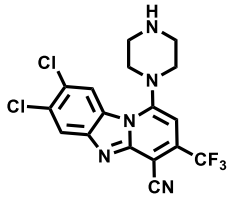
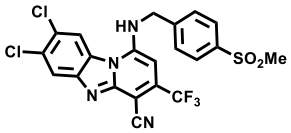
				127.50 (2C), 126.99, 122.24, 121.78, 121.17, 119.38, 119.14, 115.83, 114.75, 86.83, 47.01, 44.10.
1-((1-(4-(methylsulfonyl)phenyl)ethyl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine- 4-carbonitrile **28**		>2.68/287	459.1 (calcd for $C_{22}H_{17}F_3N_4O_2S$:458.1)	**^{1}H NMR** (400 MHz, DMSO) δ 8.65 (d, *J* = 7.74 Hz, 1H), 7.97 – 7.92 (m, 2H), 7.92 – 7.85 (m, 3H), 7.65 (t, *J* = 7.7 Hz, 1H), 7.49 (t, *J* = 7.6 Hz, 1H), 6.23 (s, 1H), 5.30 (q, *J* = 6.8 Hz, 1H), 3.17 (s, 3H), 1.80 (d, *J* = 6.7 Hz, 3H). **^{13}C NMR** (101 MHz, DMSO) δ 149.54, 139.86, 134.27, 127.70 (2C), 126.85, 126.45, 125.70, 125.37, 124.58, 124.22, 121.93, 121.81 (2C), 121.40, 116.78, 116.56, 116.35, 95.84, 54.25, 43.73, 23.00.
1-((pyrazin-2-ylmethyl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile **29**		2.60/224	369.1 (calcd for $C_{18}H_{11}F_3N_6$:368.1)	**^{1}H NMR** (400 MHz, DMSO) δ 8.88 (d, *J* = 1.4 Hz, 1H), 8.71 – 8.65 (m, 2H), 8.61 (d, *J* = 2.6 Hz, 1H), 7.91 (d, *J* = 8.1 Hz, 1H), 7.64 (t, *J* = 7.7 Hz, 1H), 7.53 – 7.42 (m, 1H), 6.47 (s, 1H), 5.09 (s, 2H). **^{13}C NMR** (101 MHz, DMSO) δ 148.16 (3C), 144.89, 143.36, 137.34, 136.34, 134.41, 133.80, 129.07, 124.63, 122.37, 119.81, 115.05 (2C), 106.72, 85.77, 45.34.
1-((pyridin-2-ylmethyl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile **30**		1.16/287012	368.1 (calcd for $C_{19}H_{12}F_3N_5$:367.1)	**^{1}H NMR** (300 MHz, DMSO) δ 8.76 – 8.59 (m, 3H), 7.93 (d, *J* = 8.1 Hz, 1H), 7.85 (td, *J* = 7.7, 1.8 Hz, 1H), 7.65 (t, *J* = 7.7 Hz, 1H), 7.57 (d, *J* = 7.9 Hz, 1H), 7.54 – 7.47 (m, 1H), 7.42 – 7.33 (m, 1H), 6.40 (s, 1H), 4.99 (s, 2H). **^{13}C NMR** (101 MHz, DMSO) δ 156.06, 149.87, 149.43, 148.30, 145.57, 137.68, 137.24, 128.33, 127.10, 124.14, 123.30, 122.38, 122.02, 121.32, 119.55, 115.22, 114.55, 86.72, 48.56.
1-((pyridin-4-ylmethyl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile **31**		>2.72/724	368.1 (calcd for $C_{19}H_{12}F_3N_5$:367.1)	**^{1}H NMR** (300 MHz, DMSO) δ 8.72 (d, *J* = 8.4 Hz, 1H), 8.62 (bs, 1H), 8.55 (d, *J* = 6.0 Hz, 2H), 7.94 (d, *J* = 8.2 Hz, 1H), 7.67 (t, *J* = 7.5 Hz, 1H), 7.58 – 7.39 (m, 3H), 6.26 (s, 1H), 4.98 (s, 2H).

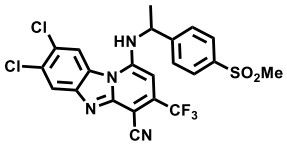
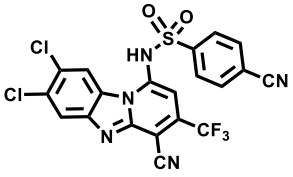
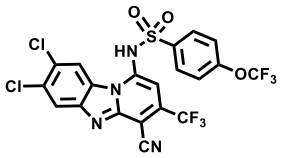
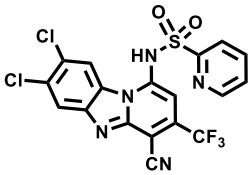
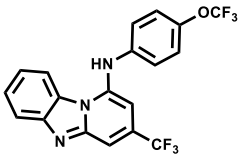
				^{13}C NMR (101 MHz, DMSO) δ 158.18, 150.26 (2C), 148.21, 147.06, 142.92, 133.29, 133.09, 131.24, 128.64, 128.33, 127.12, 122.49 (2C), 121.86, 119.30, 115.78, 114.84, 46.27.
1-((pyridin-3-ylmethyl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile **32**		>2.72/263	368.1 (calcd for $C_{19}H_{12}F_3N_5$:367.1)	**^{1}H NMR** (400 MHz, DMSO) δ 8.75 (d, *J* = 1.6 Hz, 1H), 8.70 (d, *J* = 8.4 Hz, 1H), 8.51 (dd, *J* = 4.8, 1.6 Hz, 1H), 7.98 – 7.86 (m, 2H), 7.70 – 7.59 (m, 1H), 7.53 – 7.43 (m, 1H), 7.40 (ddd, *J* = 7.9, 4.8, 0.8 Hz, 1H), 6.34 (s, 1H), 4.96 (s, 2H). **^{13}C NMR** (101 MHz, DMSO) δ 150.38 (2C), 148.16, 144.89, 143.36, 137.34, 136.34, 134.41, 133.80, 129.07, 124.63, 122.37, 119.81, 115.05 (2C), 106.72, 95.92, 85.77, 45.34.
1-((4-hydroxybenzyl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile **33**		8.74/38063	383.1 (calcd for $C_{20}H_{13}F_3N_4$:382.1)	**^{1}H NMR** (400 MHz, DMSO) δ 8.70 (d, *J* = 8.4 Hz, 1H), 8.57 (bs, 1H), 7.91 (d, *J* = 8.1 Hz, 1H), 7.65 (t, *J* = 7.6 Hz, 1H), 7.48 (t, *J* = 7.7 Hz, 1H), 7.32 (d, *J* = 8.3 Hz, 2H), 6.76 (d, *J* = 8.4 Hz, 2H), 6.31 (s, 1H), 4.78 (s, 2H). **^{13}C NMR** (101 MHz, DMSO) δ 157.17, 149.90, 148.57, 146.13, 145.25, 136.71, 128.91(2C), 128.40, 127.71, 126.97, 121.54, 121.42, 119.21, 115.88(2C), 115.65, 115.02, 86.89, 46.74.
1-((4-methylbenzyl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile **34**		1.49/673	381.1 (calcd for $C_{21}H_{15}F_3N_4$:380.1)	**^{1}H NMR** (300 MHz, DMSO) δ 8.71 (d, *J* = 8.4 Hz, 1H), 8.64 (bs, 1H), 7.93 (d, *J* = 8.1 Hz, 1H), 7.66 (t, *J* = 7.3 Hz, 1H), 7.56 – 7.43 (m, 1H), 7.40 (d, *J* = 8.0 Hz, 2H), 7.18 (d, *J* = 7.8 Hz, 2H), 6.30 (s, 1H), 4.86 (s, 2H), 2.29 (s, 3H). **^{13}C NMR** (101 MHz, DMSO) δ 149.81, 148.37, 145.37, 137.06, 135.43, 134.46, 131.64, 129.69, 128.23, 127.50, 127.03, 121.69 (2C), 119.39 (2C), 115.61, 114.83, 112,74, 86.41, 46.57, 21.14.
4-(((4-cyano-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridin-1-		>2.36/270	424.1 (calcd for $C_{22}H_{16}F_3N_5O$:423.1)	**^{1}H NMR** (300 MHz, DMSO) δ 8.74 (d, *J* = 8.3 Hz, 1H), 8.39 (d, *J* = 4.8 Hz, 1H), 7.90 (d, *J* = 8.0 Hz, 1H), 7.83 (d, *J* = 8.1 Hz, 2H), 7.67-7.54

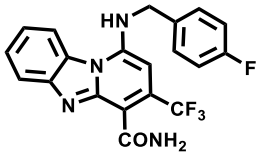
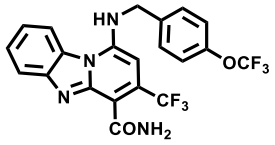
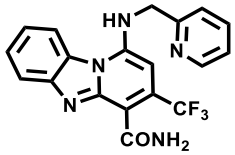
yl)amino)methyl)-N-methylbenzamide **35**				(m, 3H), 7.47 (t, *J* = 7.9 Hz, 1H), 6.24 (s, 1H), 4.95 (s, 2H), 2.78 (d, *J* = 4.5 Hz, 3H). **^{13}C NMR** (101 MHz, DMSO) δ 166.81, 150.10, 148.65, 145.30, 141.15, 134.04, 132.94, 130.76, 128.63, 127.83, 127.32 (2C), 126.85, 121.56 (2C), 119.07, 118.54, 117.48, 115.81, 86.83, 47.17, 26.66.
4-(((4-cyano-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridin-1-yl)amino)methyl)-N,N-dimethylbenzamide **36**		>2.28/499	438.1 (calcd for $C_{23}H_{18}F_3N_5O$:437.1)	**^{1}H NMR** (300 MHz, DMSO) δ 8.77 (d, *J* = 8.4 Hz, 1H), 7.90 (d, *J* = 7.8 Hz, 1H), 7.64 (t, *J* = 7.5 Hz, 1H), 7.57 (d, *J* = 8.2 Hz, 2H), 7.51 – 7.43 (m, 1H), 7.40 (d, *J* = 8.3 Hz, 2H), 6.28 (s, 1H), 4.95 (s, 2H), 2.93 (s, 6H). **^{13}C NMR** (101 MHz, DMSO) δ 170.37, 150.01, 148.66, 145.36, 142.98, 139.28, 135.96, 128.56, 127.74, 127.36 (2C), 126.90, 121.63, 119.10, 118.32, 116.94, 115.82, 115.11(2C), 86.87, 47.05, 46.06 (2C).
4-(((4-cyano-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridin-1-yl)amino)methyl)-benzenesulfonamide **37**		10.20/2294	446.0 (calcd for $C_{20}H_{14}F_3N_5O_2S$:445.1)	**^{1}H NMR** (300 MHz, DMSO) δ 8.73 (d, *J* = 8.4 Hz, 1H), 8.65 (bs, 1H), 7.93 (d, *J* = 7.7 Hz, 1H), 7.83 (d, *J* = 8.5 Hz, 2H), 7.70 (d, *J* = 8.5 Hz, 2H), 7.67 – 7.61 (m, 1H), 7.54 – 7.44 (m, 1H), 7.34 (bs, 2H), 6.28 (s, 1H), 5.00 (s, 2H). **^{13}C NMR** (101 MHz, DMSO) δ 149.97, 148.52, 143.58, 141.90, 137.22, 128.48, 127.90 (2C), 127.03, 126.34 (2C), 124.11, 121.76, 121.32, 119.28, 115.78 (2C), 114.93, 86.42, 46.82.
1-((4-isopropylbenzyl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile **38**		2.40/431	409.1 (calcd for $C_{23}H_{19}F_3N_4$:408.2)	**^{1}H NMR** (300 MHz, DMSO) δ 8.73 (d, *J* = 8.3 Hz, 1H), 7.88 (d, *J* = 7.9 Hz, 1H), 7.62 (t, *J* = 7.5 Hz, 1H), 7.44 (m, 3H), 7.24 (d, *J* = 8.1 Hz, 2H), 6.31 (s, 1H), 4.84 (s, 2H), 2.87 (m, 1H), 1.18 (d, *J* = 6.9 Hz, 6H). **^{13}C NMR** (101 MHz, DMSO) δ 150.10, 148.82, 147.93, 145.37, 136.68, 135.53, 132.84, 128.64, 127.64, 126.95, 126.75, 124.71, 121.46 (2C), 118.99, 115.75 (2C), 115.30, 86.78, 47.29, 33.55, 24.31 (2C).

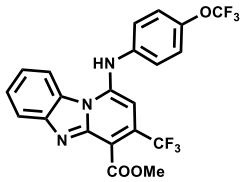
1-((pyrimidin-2-ylmethyl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile **39**		4.80/532264	369.1 (calcd for $C_{18}H_{11}F_3N_6$:368.1)	**^{1}H NMR** (600 MHz, DMSO) δ 8.88 (d, *J* = 4.9 Hz, 2H), 8.70 (d, *J* = 8.4 Hz, 1H), 8.61 (bs, 1H), 7.95 (d, *J* = 8.1 Hz, 1H), 7.67 (t, *J* = 7.6 Hz, 1H), 7.55 – 7.47 (m, 2H), 6.48 (s, 1H), 5.13 (s, 2H). **^{13}C NMR** (151 MHz, DMSO) δ 165.36, 158.23 (2C), 150.27, 148.36, 145.51, 137.33, 131.62, 128.19, 126.91, 123.75, 121.52, 120.30, 119.02, 115.22, 114.57, 86.83, 65.14.
1-(piperazin-1-yl)-3-(trifluoromethyl) benzo[4,5] imidazo[1,2-a]pyridine-4-carbonitrile **40**		10.03/98222	346.1 (calcd for $C_{17}H_{14}F_3N_5$:345.1)	**^{1}H NMR** (300 MHz, Methanol-d_4) δ 8.44 (d, *J* = 8.9 Hz, 1H), 7.96 (d, *J* = 8.4 Hz, 1H), 7.69 (t, *J* = 7.7 Hz, 1H), 7.57 (t, *J* = 7.7 Hz, 1H), 6.87 (s, 1H), 3.60 (d, *J* = 11.7 Hz, 2H), 3.30 – 2.96 (m, 6H). **^{13}C NMR** (101 MHz, DMSO) δ 154.52, 147.89, 145.30, 136.31, 128.95, 127.37 (2C), 122.82, 119.98, 116.67 (2C), 113.72, 95.05, 51.46 (2C), 44.86 (2C).
7,9-dichloro-1-((4-fluorobenzyl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile **41**		0.31/44121	453.0 (calcd for $C_{20}H_{10}Cl_2F_4N_4$:452.0)	**^{1}H NMR** (300 MHz, DMSO-d_6) δ 8.82 (d, *J* = 4.1 Hz, 1H), 8.70 (s, 1H), 7.81 (d, *J* = 6.8 Hz, 1H), 7.56 (dd, *J* = 8.5, 5.5 Hz, 2H), 7.20 (t, *J* = 8.9 Hz, 2H), 6.32 (s, 1H), 4.89 (s, 2H). **^{13}C NMR** (101 MHz, DMSO) δ 163.13, 160.71, 150.15 (2C), 141.56, 137.93, 133.95, 129.83, 129.50 (2C), 126.22, 125.36, 123.06, 121.27, 115.87 (2C), 114.78, 112.78, 88.01, 46.85.
7,8-dichloro-1-((4-fluorobenzyl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile **42**		0.44/480	453.0 (calcd for $C_{20}H_{10}Cl_2F_4N_4$:452.0)	**^{1}H NMR** (300 MHz, DMSO-d6) δ 9.03 (s, 1H), 7.69 (s, 1H), 7.52 (dd, *J* = 8.6, 5.6 Hz, 2H), 7.43 (dd, *J* = 8.4, 5.6 Hz, 2H), 6.00 (s, 1H), 4.59 (s, 2H). **^{13}C NMR** (101 MHz, DMSO) δ 162.97, 160.18, 154.24, 150.99, 145.67, 139.02, 135.82, 134.57, 130.64, 129.58, 125.88, 119.90, 117.50, 116.55, 115.63, 115.57, 115.18, 100.00, 88.62, 43.67.
7,8-dichloro-1-((4-(trifluoromethoxy)benzyl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-		0.44/237	519.0 (calcd for $C_{21}H_{10}Cl_2F_6N_4O$:518.0)	**^{1}H NMR** (300 MHz, DMSO-d_6) δ 9.04 (s, 1H), 7.70 (s, 1H), 7.59 (dd, *J* = 14.0, 8.6 Hz, 2H), 7.38 (t, *J* = 8.8 Hz, 2H), 6.01 (s, 1H), 4.64 (s, 2H, H^5).

a]pyridine-4-carbonitrile **43**				**^{13}C NMR** (101 MHz, DMSO) δ 151.42, 148.67, 148.48, 137.28, 134.58, 132.48, 130.43, 129.51, 128.51, 127.00 (2C), 124.13, 121.48 (2C), 119.22, 116.56, 115.75, 114.49, 112.94, 86.73, 51.67.
7,8-dichloro-1-((pyridin-2-ylmethyl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile **44**		1.42/59	436.0 (calcd for $C_{19}H_{10}Cl_2F_3N_5$:435.0)	**^{1}H NMR** (300 MHz, DMSO-d_6) δ 9.01 (s, 1H), 8.66 – 8.52 (m, 1H), 8.10 (s, 1H), 7.83 (td, *J* = 7.6, 1.8 Hz, 1H), 7.58 (d, *J* = 7.9 Hz, 1H), 7.42 – 7.26 (m, 1H), 6.39 (s, 1H), 4.94 (s, 2H). **^{13}C NMR** (101 MHz, DMSO) δ 150.22, 149.36 (2C), 145.21, 137.52 (2C), 129.15, 128.31, 127.64, 123.18, 122.26 (2C), 119.39, 118.56, 117.09, 116.96, 115.08, 88.03, 49.95.
7,8-dichloro-1-((4-methylbenzyl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile **45**		0.40/470	449.0 (calcd for $C_{21}H_{13}Cl_2F_3N_4$:448.0)	**^{1}H NMR** (300 MHz, DMSO-d_6) δ 9.06 (s, 1H), 7.74 (s, 1H), 7.35 (d, *J* = 8.1 Hz, 2H), 7.20 (d, *J* = 7.9 Hz, 2H), 6.03 (s, 1H), 4.59 (s, 2H), 2.31 (s, 3H). **^{13}C NMR** (101 MHz, DMSO) δ 160.73, 159.00, 155.06, 146.31, 135.62, 134.93, 129.58, 129.21 (2C), 127.62 (2C), 126.38, 121.69, 119.39, 117.52, 116.80, 115.61, 114.83, 88.39, 42.88, 21.08.
7,8-dichloro-1-((4-(trifluoromethoxy)phenyl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile **46**		>1.97/32	505.0 (calcd for $C_{20}H_8Cl_2F_6N_4$:504.0)	**^{1}H NMR** (300 MHz, DMSO-d_6) δ 8.93 (s, 1H), 7.79 (s, 1H), 7.36 – 7.30 (m, 2H), 7.12 – 7.04 (m, 2H), 5.76 (s, 1H). **^{13}C NMR** (101 MHz, DMSO) δ 163.05, 156.19, 148.53, 142.12, 137.62, 128.91, 127.21, 125.36, 123.75 (2C), 122.46 (2C), 120.35, 117.64 (2C), 115.20, 113.50, 102.34, 95.93, 46.29.
7,8-dichloro-1-((furan-2-ylmethyl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile **47**		0.44/1781	425.0 (calcd for $C_{18}H_9Cl_2F_3N_4$:424.0)	**^{1}H NMR** (300 MHz, DMSO-d_6) δ 9.00 (s, 1H), 7.90 (s, 1H), 7.63 (dd, *J* = 1.8, 0.9 Hz, 1H), 6.43 (m, 2H), 6.27 (s, 1H), 4.72 (s, 2H). **^{13}C NMR** (101 MHz, DMSO) δ 155.27, 153.52, 150.99, 145.30, 142.96, 142.30, 134.97, 130.77, 128.78, 123.76, 117.67, 117.45, 114.20, 110.89, 107.15, 94.33, 93.02, 43.95.

7,8-dichloro-1-((thiophen-2-ylmethyl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine- 4-carbonitrile **48**		0.31/902	441.0 (calcd for $C_{18}H_9Cl_2F_3N_4S$:440.0)	**^{1}H NMR** (300 MHz, DMSO-d_6) δ 9.16 (s, 1H), 7.77 (s, 1H), 7.42 (dd, *J* = 5.0, 1.3 Hz, 1H), 7.11 – 7.05 (m, 1H), 7.02 (dd, *J* = 5.0, 3.4 Hz, 1H), 6.05 (s, 1H), 4.80 (s, 2H). **^{13}C NMR** (101 MHz, DMSO) δ 150.91, 145.53, 138.51, 130.23, 129.21, 128.19, 127.02 (2C), 124.34 (2C), 123.52, 123.23, 117.75 (2C), 116.95 (2C), 88.34, 47.38.
7,8-dichloro-1-((2-fluorobenzyl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile **49**		0.17/139	453.0 (calcd for $C_{20}H_{10}Cl_2F_4N_4$:452.0)	**^{1}H NMR** (300 MHz, DMSO-d_6) δ 9.00 (s, 1H), 7.94 (s, 1H), 7.59 (t, *J* = 7.5 Hz, 1H), 7.40 – 7.12 (m, 3H), 6.22 (s, 1H), 4.79 (s, 2H). **^{13}C NMR** (101 MHz, DMSO) δ 161.91, 159.49, 152.40, 150.75, 145.34, 136.58, 132.84, 131.42, 129.92, 129.22, 126.94, 127.87, 124.84, 121.78, 118.14, 117.37 (2C), 116.51, 115.46, 87.88.
7,8-dichloro-1-((3-fluorobenzyl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile **50**		0.19/578	453.0 (calcd for $C_{20}H_{10}Cl_2F_4N_4$:452.0)	**^{1}H NMR** (300 MHz, DMSO-d_6) δ 9.05 (s, 1H), 8.69 (bs, 1H), 8.20 (s, 1H), 7.51 – 7.29 (m, 3H), 7.18 – 7.06 (m, 1H), 6.32 (s, 1H), 4.96 (s, 2H). **^{13}C NMR** (101 MHz, DMSO) δ 161.91, 159.49, 152.40, 150.75, 145.34, 136.58, 131.42, 129.96, 129.22, 127.87, 125.10, 124.70, 121.78, 118.14, 117.37(2C), 116.51, 115.65, 115.46, 87.83.
7,8-dichloro-1-(piperazin-1-yl)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile **51**		8.11/19856	414.0 (calcd for $C_{17}H_{12}Cl_2F_3N_5$:413.0)	**^{1}H NMR** (300 MHz, Methanol-d_4) δ 8.56 (s, 1H), 8.08 (s, 1H), 6.96 (s, 1H), 3.61 (d, *J* = 11.9 Hz, 2H), 3.33 – 3.27 (m, 4H), 3.18 (dd, *J* = 12.7, 6.3 Hz, 2H). **^{13}C NMR** (101 MHz, DMSO) δ 154.20 (2C), 149.71, 144.71, 130.07, 128.22, 124.71, 120.92 (2C), 117.68 (2C), 113.34, 96.37, 51.04 (2C), 44.64 (2C).
7,8-dichloro-1-((4-(methylsulfonyl)benzyl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile **52**		5.34/379	511.0 $[M-H]^-$ (calcd for $C_{21}H_{13}Cl_2F_3N_4O_2S$:512.0)	**^{1}H NMR** (300 MHz, DMSO-d_6) δ 9.11 (bs, 1H), 8.17 (s, 1H), 8.05 (s, 1H), 7.91 (d, *J* = 8.5 Hz, 2H), 7.75 (d, *J* = 8.5 Hz, 2H), 6.24 (s, 1H), 5.00 (s, 2H), 3.21 (s, 3H). **^{13}C NMR** (101 MHz, DMSO) δ 160.38, 150.68, 150.25, 149.66, 146.47, 144.95, 138.07, 135.72,

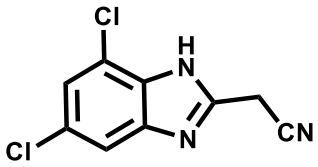
				133.78, 130.50, 128.25, 127.96 (2C), 127.63 (2C), 121.12, 119.42, 117.22, 108.24, 46.22, 44.21.
7,8-dichloro-1-((1-(4-(methylsulfonyl)phenyl)ethyl)amino)-3-(trifluoromethyl)benzo [4,5] imidazo [1,2-a]pyridine-4-carbonitrile **53**		4.97/32	525.0 $[M-H]^-$ (calcd for $C_{22}H_{15}Cl_2F_3N_4O_2S$:526.0)	**^{1}H NMR** (300 MHz, DMSO-d6) δ 8.83 (s, 1H), 8.24 (s, 1H), 7.95 (d, *J* = 8.6 Hz, 2H), 7.88 (d, *J* = 8.4 Hz, 2H), 6.27 (s, 1H), 5.47 – 5.30 (m, 1H), 3.21 (s, 3H), 1.80 (d, *J* = 7.7 Hz, 3H). **^{13}C NMR** (101 MHz, DMSO) δ 162.33, 162.10, 153.77, 148.58, 133.77, 133.21, 130.84, 128.76, 127.70 (2C), 126.87, 126.35, 124.58, 121.81 (2C), 117.96, 116.73, 114.52, 96.89, 63.59, 44.08, 22.97.
4-cyano-N-(7,8-dichloro-4-cyano-3-(trifluoromethyl)benzo [4,5]imidazo[1,2-a]pyridin-1-yl) benzene sulphonamide **54**		5.64/513	507.9 $[M-H]^-$ (calcd for $C_{20}H_8Cl_2F_3N_5O_2S$:509.0)	**^{1}H NMR** (300 MHz, DMSO-d_6) δ 8.91 (s, 1H), 8.14 – 7.88 (m, 5H), 6.77 (s, 1H). **^{13}C NMR** (101 MHz, DMSO) δ 160.90, 158.91, 153.29, 152.32, 150.28, 148.25, 145.11, 141.56, 136.66, 133.55 (2C), 127.19 (2C), 125.74, 122.09, 119.72, 115.15, 114.63, 108.93, 97.85.
N-(7,8-dichloro-4-cyano-3-(trifluoromethyl)benzo [4,5]imidazo[1,2-a]pyridin-1-yl)-4-(trifluoromethoxy)benzenesulfonamide **55**		6.73/78	566.9 $[M-H]^-$ (calcd for $C_{20}H_8Cl_2F_6N_4O_3S$:568.0)	**^{1}H NMR** (300 MHz, DMSO-d_6) δ 8.90 (s, 1H), 8.04 (m, 3H), 7.54 (d, *J* = 8.0, 2H), 6.75 (s, 1H). **^{13}C NMR** (101 MHz, DMSO) δ 152.29, 150.91, 150.78, 147.36, 145.13, 143.23, 140.19, 129.29 (2C), 128.88, 128.81, 122.36, 121.70, 121.57 (2C), 119.14, 118.91, 115.11, 107.89, 91.57.
N-(7,8-dichloro-4-cyano-3-(trifluoromethyl)benzo [4,5]imidazo[1,2-a]pyridin-1-yl)pyridine-2-sulfonamide **56**		>6/255	483.9 $[M-H]^-$ (calcd for $C_{18}H_8Cl_2F_3N_5O_2S$:485.0)	**^{1}H NMR** (400 MHz, DMSO-d_6) δ 9.08 (s, 1H), 8.69 – 8.63 (m, 1H), 8.08 – 7.99 (m, 2H), 7.97 (s, 1H), 7.58 (ddd, *J* = 6.8, 4.7, 2.1 Hz, 1H), 7.04 (s, 1H). **^{13}C NMR** (101 MHz, DMSO) δ 178.80, 164.26, 163.54, 160.73, 152.10, 150.98, 150.27, 148.67, 146.38, 142.05, 131.51, 126.66 (2C), 120.03 (2C), 106.09, 92.81, 89.35.
N-(4-(trifluoromethoxy) phenyl)-3-(trifluoromethyl)benzo [4,5]imidazo[1,2-a]pyridin-1-amine		4.24/-	412.0 (calcd for $C_{19}H_{11}F_6N_3O$:411.1)	**^{1}H NMR** (400 MHz, DMSO-d_6) δ 8.52 (d, *J* = 8.6 Hz, 1H), 7.99 (d, *J* = 8.3 Hz, 1H), 7.95 (s, 1H), 7.77 (t, *J* = 7.8 Hz, 1H), 7.55 (td, *J* = 7.9, 7.3, 1.0 Hz, 1H), 7.47 – 7.35 (m, 4H), 7.00 (s, 1H).

57				**^{13}C NMR** (101 MHz, DMSO) δ 145.99, 144.54, 140.66, 137.63, 135.40, 128.68, 127.43, 124.44, 123.35, 123.08 (2C), 121.72, 121.87 (2C), 119.36, 117.99, 116.00, 103.65, 97.79.
1-((4-fluorobenzyl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carboxamide **58**		>6/-	403.1 (calcd for $C_{20}H_{14}F_4N_4O$:402.1)	**^{1}H NMR** (600 MHz, DMSO-d_6) δ 8.66 (d, *J* = 8.4 Hz, 1H), 8.02 (s, 1H), 7.90 (d, *J* = 8.2 Hz, 1H), 7.81 (s, 1H), 7.65 (bs, 1H), 7.61 (t, *J* = 7.6 Hz, 1H), 7.57 (dd, *J* = 8.5, 5.6 Hz, 2H), 7.48 – 7.42 (m, 1H), 7.20 (t, *J* = 8.9 Hz, 2H), 6.03 (s, 1H), 4.74 (s, 2H). **^{13}C NMR** (151 MHz, DMSO) δ 165.14, 162.09, 160.48, 146.64, 146.09, 144.77, 134.00, 129.11 (2C), 127.62, 125.77, 124.11 (2C), 122.29, 120.67, 118.81, 115.16, 113.47, 84.12, 45.53.
1-((4-(trifluoromethoxy)benzyl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carboxamide **59**		5.16/-	469.1 (calcd for $C_{21}H_{14}F_6N_4O_2$:468.1)	**^{1}H NMR** (300 MHz, DMSO-d_6) δ 8.67 (d, *J* = 8.2 Hz, 1H), 8.03 (s, 2H), 7.90 (d, *J* = 8.1 Hz, 1H), 7.62 – 7.52 (m, 1H), 7.45 – 7.38 (m, 1H), 7.48 (bs, 1H), 7.44 (d, *J* = 7.1 Hz, 2H), 7.37 (d, *J* = 7.7 Hz, 2H), 6.02 (s, 1H), 4.79 (s, 2H). **^{13}C NMR** (151 MHz, DMSO) δ 165.14, 162.09, 160.48, 146.64, 146.09, 144.77, 134.00, 129.11 (2C), 127.62, 127.30, 125.77, 124.11, 122.29, 120.67, 118.81, 115.16 (2C), 113.47, 84.12, 45.53.
1-((pyridin-2-ylmethyl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carboxamide **60**		>10/-	386.1 (calcd for $C_{19}H_{14}F_3N_5O$:385.1)	**^{1}H NMR** (300 MHz, DMSO-d_6) δ 8.69 (d, *J* = 8.2 Hz, 1H), 8.63 (d, *J* = 4.2 Hz, 1H), 8.05 (s, 2H), 7.91 (d, *J* = 8.3 Hz, 1H), 7.82 (td, *J* = 7.7, 1.6 Hz, 1H), 7.66 (d, *J* = 4.2 Hz, 1H), 7.62 (s, 1H), 7.61 – 7.52 (m, 1H), 7.47 (t, *J* = 7.8 Hz, 1H), 7.40 – 7.29 (m, 1H), 6.10 (bs, 1H), 4.85 (s, 2H). **^{1}** **^{3}C NMR** (101 MHz, DMSO) δ 165.65, 157.24, 149.41, 147.19, 146.70, 145.36, 137.55, 128.09, 126.32, 125.12, 123.10, 122.39, 122.14, 121.35, 119.44, 115.51, 113.73, 84.42, 48.57.

Methyl 1-((4-(trifluoromethoxy)phenyl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carboxylate **61**		5.52/-	470.0 (calcd for $C_{21}H_{13}Cl_2F_6N_3O_3$:469.1)	**^{1}H NMR** (400 MHz, DMSO-d_6) δ 8.88 (d, *J* = 8.4 Hz, 1H), 7.81 (d, *J* = 8.1 Hz, 1H), 7.62 – 7.52 (m, 1H), 7.45 – 7.38 (m, 1H), 7.36 (d, *J* = 8.6 Hz, 2H), 7.13 (d, *J* = 8.7 Hz, 2H), 6.35 (s, 1H), 3.89 (s, 3H). **^{13}C NMR** (101 MHz, DMSO) δ 164.08, 148.31, 147.91, 147.17, 144.47, 135.94, 128.01, 126.92, 124.71, 123.25 (2C), 122.85, 122.63 (2C), 121.98, 117.85, 113.40, 113.21, 112.99, 98.53, 52.03.

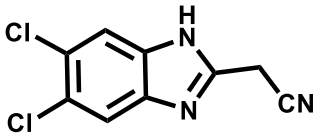
General procedure for the preparation of intermediate **IIb** and **IIc**: DMF (4 ml) was added to a mixture of the appropriately substituted diaminobenzene **I** (1 equiv.) and ethyl cyanoacetate (3 equiv.). The reaction mixture was stirred and heated at 160˚C for 2 hours, cooled to room temperature and extracted using ethyl acetate. The organic layer was washed with 5% LiCl (5 x), distilled water (4 x), brine (5 x) and dried over $MgSO_4$. Organic solvent was removed in vacuo to obtain the product which was occasionally purified by column chromatography.

2-(5, 7-dichloro-1H-benzo[d]imidazol-2-yl) acetonitrile IIb:



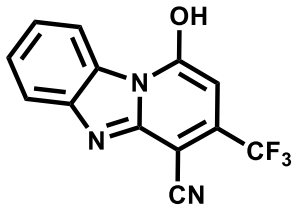
Obtained using 3,5-dichlorobenzene-1,2-diamine **Ia** as a brown solid (61%); R_f (EtOAc:Hexane, 1:1) 0.3; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.63 (d, *J* = 1.8 Hz, 1H), 7.39 (d, *J* = 1.8 Hz, 1H), 4.43 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 147.89 (2C), 127.27, 122.11 (2C), 116.53, 113.57, 55.30, 18.84. MS (EI+) m/z calcd for $C_9H_5Cl_2N_3$:224.99; found, 226.60 (M + 1).

2-(5,6-dichloro-1H-benzo[d]imidazol-2-yl)acetonitrile IIc:



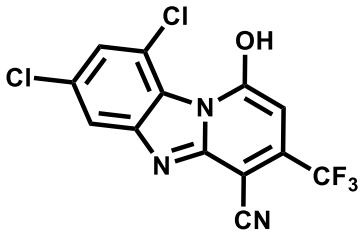
Obtained using 4,5-dichlorobenzene-1,2-diamine **Ib** as a brown solid (76%); R_f (EtOAc:Hexane, 1:1) 0.33; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.90 (s, 1H), 7.83 (s, 2H), 4.41 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 162.19, 148.31 (2C), 143.29, 127.18, 116.57 (2C), 116.13, 26.63. MS (EI+) m/z calcd for $C_9H_5Cl_2N_3$:224.99; found, 225.90 (M + 1).

1-hydroxy-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile IIIa:



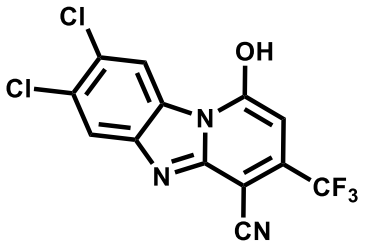
Obtained from **IIa** as a light yellow powder (66%); R_f (EtOAc:Hexane, 7:3) 0.8; ^{1}H NMR (400 MHz, DMSO) δ 14.10 (bs, 1H), 8.60 (d, J = 8.2 Hz, 1H), 7.71 – 7.56 (m, 2H), 7.46 (ddd, J = 8.6, 6.8, 1.8 Hz, 1H), 6.43 (s, 1H); ^{13}C NMR (101 MHz, DMSO) δ 158.05, 148.14, 138.59, 132.21, 127.46, 123.92, 123.48, 121.36, 116.71, 114.62, 112.24, 103.27, 64.56. MS (EI+) m/z calcd for $C_{13}H_6F_3N_3O$:277.05; found, 278.10 (M + 1).

7,9-dichloro-1-hydroxy-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-Carbonitrile IIIb:



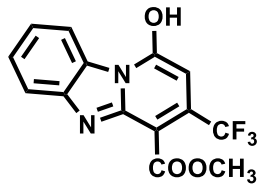
Obtained from **IIb** as a grey solid (28%); R_f (EtOAc:Hexane, 7:3) 0.18; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.48 (d, *J* = 2.0 Hz, 1H), 7.57 (d, *J* = 2.0 Hz, 1H), 5.93 (s, 1H). ^{13}C NMR (101 MHz, DMSO) δ 159.70, 153.00, 139.36, 131.27, 129.32, 127.54, 124.70, 123.47, 120.66, 117.43, 116.91, 114.45, 95.59. MS (EI-) m/z calcd for $C_{13}H_4Cl_2F_3N_3O$:344.97; found, 343.90 (M - 1).

7,8-dichloro-1-hydroxy-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile IIIc:



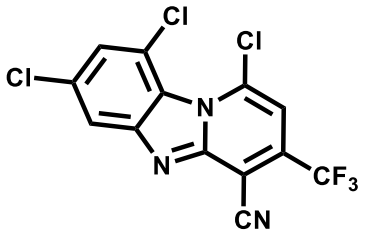
Obtained from **IIc** as a grey powder (49%); R_f (EtOAc:Hexane, 3:2) 0.31; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.64 (s, 1H), 7.83 (s, 1H), 5.85 (s, 1H). ^{13}C NMR (101 MHz, DMSO) δ 172.53, 169.51, 159.85, 154.00, 144.91, 129.71, 127.43, 124.22, 120.92, 117.79, 117.22, 116.56, 94.50. MS (EI-) m/z calcd for $C_{13}H_4Cl_2F_3N_3O$:344.97; found, 343.90 (M - 1).

Methyl 1-hydroxy-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carboxylate **IIId**:



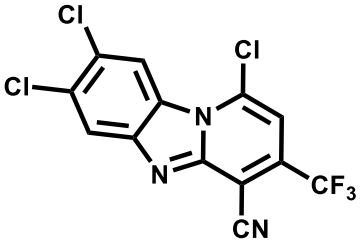
Obtained from **IId** as a light yellow powder (27%); R_f (EtOAc:Hexane, 2:3) 0.38; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.64 (d, *J* = 8.0 Hz, 1H), 7.77 (d, *J* = 8.1 Hz, 1H), 7.58 (td, *J* = 8.4, 7.9, 1.2 Hz, 1H), 7.41 (td, *J* = 7.9, 1.2 Hz, 1H), 6.40 (s, 1H), 3.90 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 163.86, 158.49, 146.50, 137.93, 132.47, 127.51, 123.15, 121.81, 117.09, 116.75, 112.78, 111.53, 104.09, 52.35. MS (EI-) m/z calcd for $C_{14}H_9F_3N_2O_3$:310.06; found, 309.00(M-1).

1,7,9-trichloro-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile IVb:



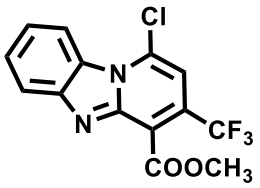
Obtained from **IIIb** as a red-brown solid (63%); R_f (EtOAc:Hexane, 7:3) 0.56; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.77 (d, *J* = 1.7 Hz, 1H), 8.05 (d, *J* = 1.6 Hz, 1H), 7.94 (s, 1H); ^{13}C NMR (101 MHz, DMSO) δ 174.79, 158.66, 150.55, 144.16, 139.79, 133.58, 130.44, 126.23 (2C), 121.26, 115.13 (2C), 100.59. MS (EI+) m/z calcd for $C_{13}H_3Cl_3F_3N_3$:362.93; found, 363.90 (M +1).

1,7,8-trichloro-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile IVc:



Obtained from **IIIc** as a red solid (94%); R_f: (EtOAc:Hexane, 9:1) 0.53; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.86 (s, 1H), 8.36 (s, 1H), 7.86 (s, 1H); ^{13}C NMR (101 MHz, DMSO) δ 158.25, 150.17, 139.16, 132.68, 129.49, 127.76, 124.34, 120.29, 117.42 (2C), 114.55, 112.77, 101.70. MS (EI-) m/z calcd for $C_{13}H_3Cl_3F_3N_3$:362.93; found, 361.90 (M -1).

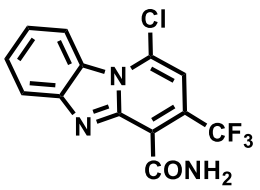
Methyl 1-chloro-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carboxylate IVd:



Synthesised from **IIId** as light yellow powder (90%); R_f (EtoAc:Hexane, 3:7) 0.63; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.65 (dt, J = 8.2, 1.0 Hz, 1H), 7.77 (dt, J = 8.1, 1.0 Hz, 1H), 7.62 – 7.55 (m, 1H), 7.42 (ddd, J = 8.4, 7.4, 1.1 Hz, 1H), 6.40 (s, 1H), 3.90 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 164.01, 145.13, 133.01, 129.64, 127.55, 126.14, 125.80, 123.98, 123.60, 120.61, 116.45, 106.87, 106.83, 54.09. MS (EI+) m/z calcd for $C_{14}H_8ClF_3N_2O_2$:328.02; found, 328.80 (M+1).

1-chloro-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carboxamide VIa:

Synthesised by reacting **IVa** with conc. Sulfuric acid (60 equiv.) at 85 °C with stirring for 2 hours. The reaction mixture was cooled and slowly added to ice-cold water while stirring. The precipitate that formed was filtered and dried at 50 °C in the oven and used without further purifications.



Obtained as light grey powder (89%); R_f (2% MeoH/DCM) 0.14; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.73 (d, J = 8.6 Hz, 1H), 8.14 (s, 1H), 8.03 (d, J = 8.2 Hz, 1H), 8.00 (s, 1H), 7.70 (ddd, J = 8.2, 7.1, 1.0 Hz, 1H), 7.56 (ddd, J = 8.4, 7.2, 1.1 Hz, 1H), 7.51 (s, 1H). ^{13}C NMR (101 MHz, DMSO) δ 164.27, 146.13, 145.05, 130.80, 129.50, 127.33, 126.10, 124.93, 124.30, 123.40, 120.62, 116.28, 107.19. MS (EI+) m/z calcd for $C_{13}H_7ClF_3N_3O$:313.02; found, 313.80 (M+1).

HPLC Methods: Using a Kinetex Core C18 2.6 µm column (30 mm × 2.1 mm), 2 µL injection volume, flow 0.7 mL/min; gradient: t = 0 min: 85% A – 15% B; t = 0.3 min: 85% A – 15% B; t = 1.2 min: 0% A – 100% B; ; t = 4.5 min: 0% A – 100% B. Mobile phase A: 10 mM NH_4OAc in buffer (0.4% acetic acid); Mobile phase B: 10 mM NH_4OAc (0.4% acetic acid) in 90% HPLC grade MeOH in H_2O – APCI/ESI +, UV chromatogram (254, 280 and 290 nm).

B. *In vitro* antiplasmodium assays

1. Asexual blood stage activity using the LDH assay at UCT

The chloroquine-sensitive NF54 strain of *P. falciparum* was used in testing the antiplasmodium asexual blood stage activity of test compounds. The parasites were cultured and maintained according to the approach by Trager and Jensen with slight variations.[1] The antiplasmodial activity was monitored by determining the activity of the parasite lactate dehydrogenase enzyme.

Stock solutions of samples were prepared at 20 mg/ml in 100% DMSO and kept at -20 ℃ prior to analysis. On the assay day, subsequent dilutions of the stock solutions were prepared in medium to give the highest starting concentration of 100 µg/ml. From these, serial dilutions in complete medium were performed to achieve ten concentrations with the concentration range being between 0.2-100 µg/ml. Using these concentrations, a dose response analysis was performed to establish the concentration resulting in inhibition of parasite growth by 50%. Chloroquine and artesunate were employed as controls for all the assays for which their initial testing concentrations was 1000 ng/ml. The dilution approach adopted for the samples and controls was similar. In each case, the final concentration of DMSO had no effects on parasite growth. Non-linear dose-response curves generated in GraphPad Prism v.4.0 software facilitated calculation of the IC_{50} values.

2. Asexual blood stage activity using the modified [^{3}H]-hypoxanthine incorporation assay at Swiss TPH

The modified [^{3}H]-hypoxanthine incorporation assay was used to test compounds against drug-sensitive (NF54) and resistant (K1) strains of *P. falciparum*. The parasites were cultivated in human erythrocytes as previously described[1,2] but with some variations to the medium which comprised RPMI 1640 supplemented with 0.5% ALBUMAX® II, 25 mM Hepes, 25 mM $NaHCO_3$ (pH 7.3), 0.36 mM hypoxanthine, and 100 microg/ml neomycin. Parasite cultures were maintained in an environment of 37 °C, 3% O_2, 4% CO_2, and 93% N_2 in humidified modular chambers.

Test compounds were dissolved in 100% DMSO, by sonication, to give 10 mg/ml stock solutions and diluted in culture medium devoid of hypoxanthine. Infected erythrocytes (100 microliter per well with 2.5% hematocrit and 0.3% parasitemia) were added to each compound titrated in 100 microliter duplicates over a 64-fold range and incubated for 48 h. After this period, 0.5 microCi of [^{3}H] hypoxanthine in 50 microliter media was added and the culture plates allowed to incubate for a further 24 h. From the parasites harvested on glass-fiber filters at the end of the total incubation period, radioactivity was determined using a Betaplate liquid scintillation counter (Wallac, Zurich). The radioactivity was recorded as counts per minute per well at each concentration of test compound and presented as percentage relative to the untreated controls. The concentrations resulting in 50% inhibition (IC_{50}) was determined by linear interpolation.[3]

3. *In vitro* activity against *P. berghei* liver stages.

In vitro activity of test compounds against the liver stage of *P. berghei* infection was performed as described previously [4,5]. Briefly, 1x10^4 Huh-7 cells, a human hepatoma cell line, were seeded in 96-well plates in 1640 RPMI medium supplemented with 10% v/v fetal bovine serum (FBS), 1% v/v non-essential amino acids, 1% v/v penicillin/streptomycin, 1% v/v glutamine and 10 mM HEPES, and incubated overnight at 37 ˚C and 5% CO_2. Stock solutions of 10 mM were prepared by dissolving test compounds in DMSO. Serial dilutions of each compound were then prepared in infection medium, *i.e.* culture medium supplemented with gentamicin (50 µg/mL) and amphotericin B (0.8 µg/mL). On the day of infection, culture medium was replaced by the appropriate compound concentration and incubated for one hour. Next, 1x10^4 firefly luciferase-expressing *P. berghei* sporozoites, freshly obtained through disruption of salivary glands of infected female *Anopheles stephensi* mosquitoes, were added to each well. Plates were then centrifuged at 1,700 x g for 5 minutes and incubated at 37ºC, 5% CO_2 for 48h.

The effect of the compounds on the viability of Huh-7 cells was assessed by the AlamarBlue assay (Invitrogen, U.K.) according to the manufacturer's protocol, followed by measurement of parasite load by a bioluminescence assay (Biotium). Nonlinear regression analysis was employed to fit the normalized results of the dose-response curves, and EC_{50} values were determined using GraphPad Prism 6.0.

4. *In vitro* activity against gametocyte stage.

Two different platforms, ATP- and luciferase reporter line-based assays, were used to determine the gametocytocidal effects of test compounds. In the luciferase assays, two transgenic parasite lines, NF54-PfS16-GFP-Luc (early stage) and NF54-Mal8p1.16-GFP-Luc (late stage), which enable stage-specific assessment of gametocytocidal activity, were utilised. The bioluminescent ATP assay provided an additional independent evaluation of late stage gametocyte activity of test compounds.

Gametocytes were produced as per Reader and co-workers.[6] Experiments were performed on day 5 and 10 (representing >90% of either early stage I/II/III or mature stage IV/V gametocytes, respectively). In each case, assays were set up using a 2 to 3% gametocytaemia, 1.5% haematocrit culture and 48 h drug pressure in a gas chamber (90% N_2, 5% O_2, and 5% CO_2) and temperature maintained at 37 °C. Luciferase activity was determined in 20 µl parasite lysates by adding 50 µl luciferin substrate (Promega Luciferase Assay System) at room temperature and detection of resultant bioluminescence at an integration constant of 10 s with the GloMax®-Multi+ Detection System with Instinct® Software. For the ATP assay, gametocytes representing >90% of late stage IV/V gametocytes (predominantly stage V) were enriched using density gradient centrifugation and magnetic separation. Drug dilutions were placed in triplicate in 96-well plates. Approximately 50 000 gametocytes in glucose-rich complete medium were added to each well in a final volume of 100 µl and the plates incubated for 24 h in a humidified gas chamber (90% N_2, 5% O_2, and 5% CO_2) at 37 °C. ATP levels were determined using the BacTiter-Glo™ assay (Promega) in accordance with manufacturer's instructions, at room temperature in the dark with assay substrate incubated for 10 minutes. Bioluminescence was detected at an integration constant of 0.5 s with the GloMax®- Multi+ Detection System with Instinct® Software.

C. Beta haematin inhibition

Controls and test compounds were reconstituted to 20 mM in 100% DMSO (Fluka, Buchs, Switzerland). In a 96-well plate, columns 1-11 were filled with a solution constituting water/305.5 µM NP40/DMSO at a v/v ratio of 70%/20%/10%, respectively, while to column 12, 140 µLof water and 40 µL of 305.5 µM NP40

were introduced to mediate the formation of β-hematin. To column 12, 20 µL of 20 mM compound stock solution was added and 100 µL of this solution serially diluted to column 2. Column 1 was left as a blank with no compound present (0 µM). Some 100 µL of haematin suspension, prepared by suspending an aliquot (178.8 µL) of haematin stock solution in 20 mL of a 1 M acetate buffer, pH 4.9, was added into each well and the plates left to incubate for 5 hours at 37 °C. Thereafter, pyridine solution (32 µl) comprising 20% water, 20% acetone, 10% 2 M HEPES buffer (pH 7.4) and 50% pyridine was added followed by the addition of 60 µL of acetone to all wells. Optical readings of the plates were taken at 405 nm to give concentration-response values. The concentrations resulting in 50% inhibition of the process were determined using GraphPad Prism v.4.0 software (La Jolla, USA).

D*. In vitro* microsomal metabolic stability

Metabolic stability was conducted using the one-time point assay.[7] Experiments were performed in triplicate in a 96-well microtiter plate using test compounds at a concentration of 0.1 µM, incubated individually in a solution containing 0.35 mg/mL mouse (male mouse BALB/c, Xenotech) or human (mixed gender, Xenotech) liver microsomes. NADPH (1 mM) in phosphate buffer (100 mM) and at pH 7.4 were added to the wells to initiate metabolic reactions and the plates incubated for 30 minutes. After this period, 300 µL of ice-cold acetonitrile containing internal standard (carbamazepine, 0.0236 µg/mL) was added to each well to quench the reactions. The supernatant was centrifuged and filtered after which LC-MS/MS (Agilent Rapid Resolution HPLC, AB SCIEX 4000 QTRAP MS) analysis was performed to determine concentration of test compounds. Differences in the amounts of compounds before and after incubation were therefore determined by LC-MS/MS and results recorded as per cent compound remaining after 30 minutes incubation. Search for products of metabolism was not performed during the metabolic stability assay.

E. Cytotoxicity assay

In vitro cytotoxicity was conducted on the Chinese Hamster ovarian cell lines using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.[8] Test compounds were dissolved in 100% DMSO to yield a 20 mg/mL stock solution while the reference standard, emetine, was dissolved in distilled water to prepare a 2 mg/mL solution. From an initial test compound and control concentration of 100 µg/mL, serial 10-fold dilutions, in complete medium, were performed to achieve six concentrations with 0.001 µg/mL being the lowest concentration. The highest concentration of DMSO exposed to cells

had no effects on cell viability. Plates were incubated for 48 h with 100 µL of drug and 100 µL of cell suspension in each well after which they were developed by the addition of 25 µL of sterile MTT (Thermo Fisher Scientific) to each well and a further incubation in the dark for 4 hours. Thereafter, the plates were centrifuged, the medium aspirated off and DMSO (100 µL) added to dissolve crystals and absorbance readings taken at 540 nM. Nonlinear dose-response curve fitting analysis conducted using GraphPad Prism v.4.0 software (La Jolla, USA), was applied to generate IC_{50} values. The assay was conducted in triplicate and conducted on two separate occasions.

F. hERG inhibition studies

Inhibition potency for test compounds towards the hERG potassium ion channel was carried out using a four-point concentration-response format applying the QPatch hERG assay, Metrion biosciences, Cambridge, United Kingdom.[9,10] Test compounds were dissolved in 100% DMSO to give a 10mM stock solution and thereafter diluted further in DMSO using 0.5-log unit dilutions to achieve the screening concentrations of 0.3, 1, 3 and 10 µM.

Chinese hamster ovarian cell lines stably expressing hERG were used. Electrophysiological recordings of hERG currents were obtained according to in-house procedures and effects on hERG tail current measured. Concentration-response curves were constructed by cumulative double sample additions of each concentration to the same cell. Experiments were carried out in three separate cells and as technical replicates. Inhibition activity was calculated as the reduction in the mean peak current in the test compound relative to the peak current prior to exposure of the cells to the compounds. Percent inhibition values from each cell were used to construct concentration-response curves using the 4-parameter logistic fit with 0% and 100% inhibition levels fixed at very low and very high concentration, respectively and concentrations resulting in a 50% inhibition determined. Verapamil was used as a positive control and was treated in the same way as samples.

G. *In vivo* antimalarial efficacy studies

This work was carried out at the Swiss Tropical and Public Health Institute in accordance with Swiss national and cantonal regulations on animal welfare. *In vivo* antimalarial efficacy studies were conducted in mice harbouring *Plasmodium berghei* infection. A GFP-transfected *P. berghei* ANKA strain (donated by A. P. Waters and C. J. Janse, Glasgow and Leiden Universities respectively), was used to infect three mice and the level of parasitemia determined by flow cytometry, having a detection limit of 0.1% (that is, 1 parasite per 1,000 erythrocytes). A solution or suspension of test compounds in 90/10 Tween80/ethanol

(v/v), diluted 10 times with water, was administered orally as four consecutive doses at 4, 24, 48 and 72 h post-infection. The control group of mice received only the vehicle which has no antimalarial activity. Activity was then determined by calculating the difference between the mean per cent parasitemia for the control and treated groups, presented as a per cent relative to the control group. Mice were considered cured if parasitemia was undetectable on day 30 after the infection, as examined by light microscopy.

H. *In vivo* mouse pharmacokinetic studies

The pharmacokinetic studies were conducted with prior approval of the Animal Ethics Committee of the University of Cape Town (approval number 017/026) in accordance with the South African National Standard (SANS 10386:008) for the Care and Use of Animals for Scientific Purposes,[11] and guidelines from the Department of Health.[12] The studies were performed in male (C57B1/6) mice housed in temperature-controlled rooms and fed a standard diet with free access to water. Test compounds were weighed and prepared freshly before dosing. For intravenous dosing, compounds were prepared in dimethylacetamide, poly- ethylene glycol, and propylene glycol/ethanol mixture 4:1 at a ratio of 1:3:6. An intravenous dose of 2 mg/kg contained in a volume of 50 µL was administered to two mice via the tail vein after anesthetizing the animals.

For oral administration, compounds were suspended in 2mL of 100% hydroxypropyl methylcellulose and given to three mice by gavage at a dose of 20 mg/kg. Blood samples were drawn from the tail of each animal at specified time intervals and collected into microcentrifuge tubes equipped with the anticoagulant lithium heparin S12. Samples were kept on ice and transferred to storage at −80 °C within 1 hour of collection, pending analysis. Samples were subsequently analysed by high-performance liquid chromatography and mass spectrometry (LC-MS/MS) following in-house procedures

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