

SUPPORTING INFORMATION S1

Benzimidazole derivatives are potent against multiple life cycle stages of *Plasmodium falciparum* malaria parasites

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Supporting information contents:

Supporting Figure S1

Chemical and spectroscopic information on new compounds

An additional Excel file with a summary of all data for all *in vitro* experiments is provided (supporting information file S2).

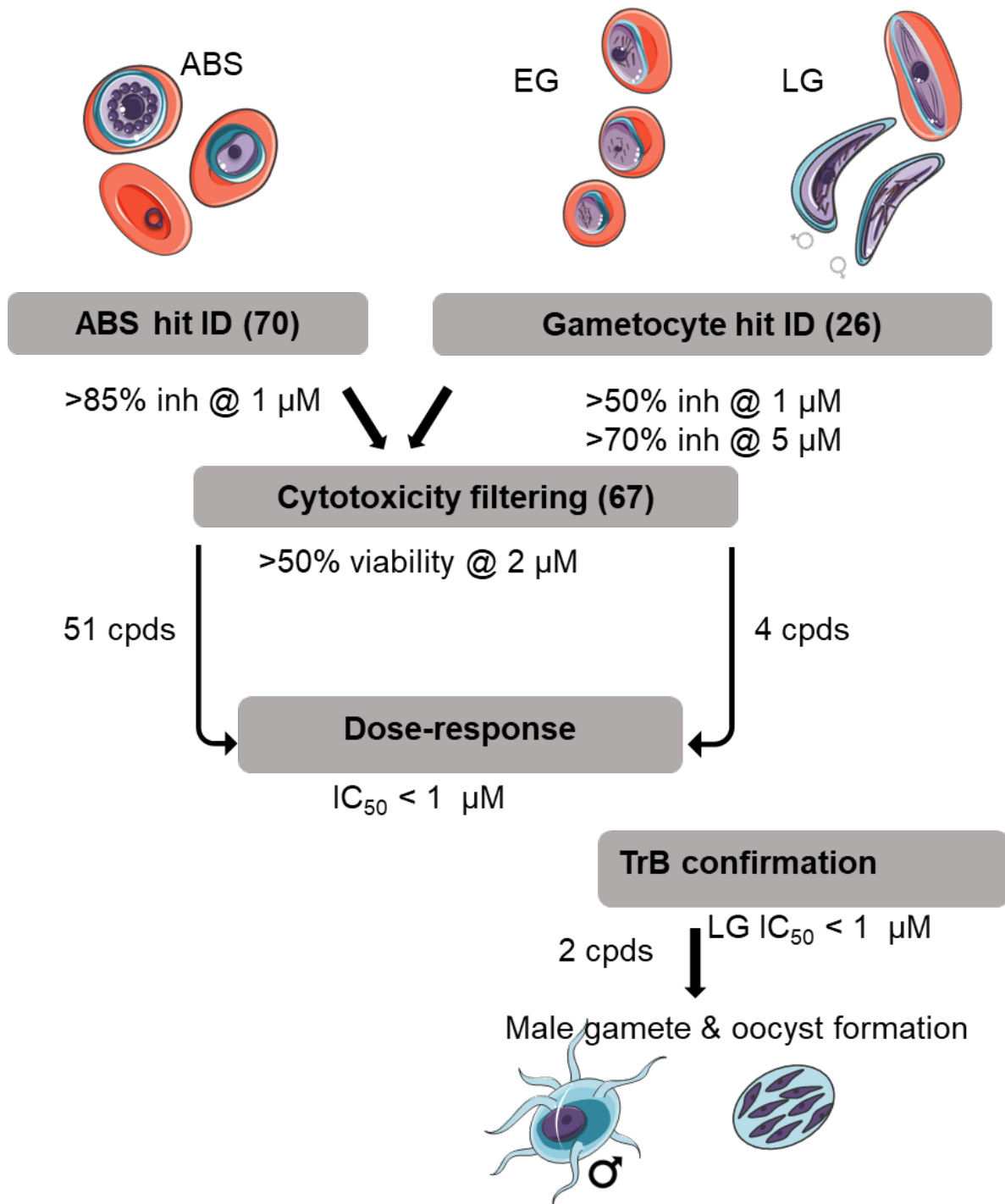


Figure S1. Screening cascade followed for screening of the benzimidazole which includes primary hit confirmation and cytotoxicity filtering, activity profiling and transmission-blocking confirmation. The number of compounds in each series is indicated in parentheses.

Chemical and spectroscopic information on new compounds.

1-(4-(4-fluorobenzyl)piperazin-1-yl)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile (**PBI.20**)

Yellow solid (0.86 g, 86%); m.p. 254 – 256 °C; *Rf* (EtOAc:Hex, 3:7) 0.40; ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.31 (dd, *J* = 8.3, 1.0 Hz, 1H), 7.98 (dd, *J* = 8.3, 1.1 Hz, 1H), 7.67 (ddd, *J* = 8.2, 7.1, 1.0 Hz, 1H), 7.54 (dd, *J* = 8.6, 5.6 Hz, 2H), 7.22 (ddd, *J* = 8.2, 7.2, 1.2 Hz, 1H), 7.11 (dd, *J* = 9.8, 8.5 Hz, 2H), 6.85 (s, 1H), 3.64 (s, 2H), 3.52 - 3.44 (m, 4H), 2.98 - 2.77 (m, 4H); HPLC-MS (APCI/ESI): Purity = 97%, *t_R* = 3.209 min, *m/z* [M+H]⁺ = 454.2.

1-(4-benzylpiperazin-1-yl)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile (**PBI.21**)

Yellow solid (0.81 g, 81%); m.p. 267 – 269 °C; *Rf* (EtOAc:Hex, 3:7) 0.30; ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.31 (dd, *J* = 8.4, 1.0 Hz, 1H), 7.98 (dd, *J* = 8.3, 1.1 Hz, 1H), 7.67 (ddd, *J* = 8.2, 7.2, 1.0 Hz, 1H), 7.54 (ddd, *J* = 8.1, 7.3, 1.0 Hz, 1H), 7.42 - 7.35 (m, 2H), 7.29 - 7.23 (m, 3H), 6.85 (s, 1H), 3.65 (s, 1H, 2H), 3.09 - 3.02 (m, 4H), 2.67 - 2.52 (m, 4H); HPLC-MS (APCI/ESI): Purity = 98%, *t_R* = 3.038 min, *m/z* [M+H]⁺ = 436.1.

1-(4-(4-fluorophenyl)piperazin-1-yl)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile (**PBI.22**)

Yellow solid (0.83 g, 83%); m.p. 244 – 246 °C; *Rf* (EtOAc:Hex, 3:7) 0.33; ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.40 (dd, *J* = 8.3, 1.1 Hz, 1H), 8.00 (dd, *J* = 8.3, 1.0 Hz, 1H), 7.68 (ddd, *J* = 8.2, 7.1, 1.1 Hz, 1H), 7.55 (ddd, *J* = 8.2, 7.2, 1.2 Hz, 1H), 7.14 (dd, *J* = 9.8, 8.5 Hz, 2H), 7.09 (dd, *J* = 8.6, 5.5 Hz, 2H), 6.92 (s, 1H), 3.67 - 3.50 (m, 4H), 3.18 - 3.07 (m, 4H); HPLC-MS (APCI/ESI): Purity = 98%, *t_R* = 3.034 min, *m/z* [M+H]⁺ = 440.1.

1-(4-benzoylpiperazin-1-yl)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile (**PBI.23**)

Yellow solid (0.83 g, 83%); m.p. 255 – 257 °C; *Rf* (EtOAc:Hex, 2:3) 0.30; ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.42 (dd, *J* = 8.4, 1.0 Hz, 1H), 8.03 - 7.96 (m, 2H), 7.69 (ddd, *J* = 8.2, 7.1, 1.1 Hz, 1H), 7.56 (ddd, *J* = 8.3, 7.1, 1.2 Hz, 1H), 7.51 - 7.46 (m, 3H), 7.44 (ddd, *J* = 8.2, 7.1, 1.2 Hz, 1H), 6.94 (s, 1H), 3.64 - 3.57 (m, 4H), 3.16 - 3.08 (m, 4H); HPLC-MS (APCI/ESI): Purity = 98%, *t_R* = 2.832 min, *m/z* [M+H]⁺ = 450.2.

1-(4-phenethylpiperazin-1-yl)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile (**PBI.24**)

Yellow solid (0.90 g, 90%); m.p. 248 – 250 °C; *Rf* (EtOAc:Hex, 1:1) 0.48; ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.34 (dd, *J* = 8.4, 1.0 Hz, 1H), 8.00 (dd, *J* = 8.2, 1.2 Hz, 1H), 7.69 (ddd, *J* = 8.3, 7.1,

1.1 Hz, 1H), 7.56 (ddd, $J = 8.5, 7.1, 1.2$ Hz, 1H), 7.44 - 7.38 (m, 2H), 7.34 - 7.27 (m, 3H), 6.87 (s, 1H), 3.62 - 3.55 (m, 4H), 3.11 - 3.05 (m, 4H), 2.84 (t, $J = 7.4$ Hz, 2H), 2.59 (t, $J = 7.2$ Hz, 2H); HPLC-MS (APCI/ESI): Purity = 98%, $t_R = 3.081$ min, m/z $[M+H]^+ = 450.2$.

1-(4-(p-tolyl)piperazin-1-yl)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile
(**PBI.25**)

Yellow solid (0.67 g, 67%); m.p. 269 – 271 °C; R_f (EtOAc: Hex, 2:3) 0.34; 1H NMR (400 MHz, DMSO- d_6) δ 8.42 (dd, $J = 8.4, 1.0$ Hz, 1H), 8.01 (dd, $J = 8.2, 1.1$ Hz, 1H), 7.69 (ddd, $J = 8.3, 7.1, 1.1$ Hz, 1H), 7.56 (ddd, $J = 8.3, 7.1, 1.4$ Hz, 1H), 7.10 (d, $J = 7.9$ Hz, 2H), 6.98 (d, $J = 8.1$ Hz, 2H), 6.90 (s, 1H), 3.19 - 3.12 (m, 4H), 3.07 - 3.00 (m, 4H), 2.26 (s, 3H); HPLC-MS (APCI/ESI): Purity = 97%, $t_R = 3.115$ min, m/z $[M+H]^+ = 436.2$.

3-(trifluoromethyl)-1-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile (**PBI.26**)

Yellow solid (0.75 g, 75%); m.p. 254 – 256 °C; R_f (EtOAc: Hex, 1:1) 0.49; 1H NMR (400 MHz, DMSO- d_6) δ 8.44 (dd, $J = 8.3, 1.0$ Hz, 1H), 8.01 (dd, $J = 8.3, 1.0$ Hz, 1H), 7.70 (ddd, $J = 8.3, 7.2, 1.0$ Hz, 1H), 7.62 - 7.54 (m, 3H), 7.21 (d, $J = 8.1$ Hz, 2H), 6.92 (s, 1H), 3.15 - 2.98 (m, 8H); HPLC-MS (APCI/ESI): Purity = 97%, $t_R = 3.080$ min, m/z $[M+H]^+ = 490.1$.

1-(4-cyclohexylpiperazin-1-yl)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile
(**PBI.27**)

Yellow solid (0.67 g, 67%); m.p. 256 – 258 °C; R_f (EtOAc: Hex, 2:3) 0.40; 1H NMR (400 MHz, DMSO- d_6) δ 8.34 (dd, $J = 8.5, 1.0$ Hz, 1H), 8.00 (dd, $J = 8.2, 1.0$ Hz, 1H), 7.73 (ddd, $J = 8.3, 7.2, 1.1$ Hz, 1H), 7.57 (ddd, $J = 8.3, 7.1, 1.2$ Hz, 1H), 6.83 (s, 1H), 3.16 - 3.11 (m, 4H), 3.07 - 3.01 (m, 5H), 1.85 - 1.79 (m, 4H), 1.69 - 1.63 (m, 2H), 1.31 - 1.23 (m, 4H); HPLC-MS (APCI/ESI): Purity = 97%, $t_R = 2.645$ min, m/z $[M+H]^+ = 428.2$.

1-(4-(2-fluorobenzyl)piperazin-1-yl)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile (**PBI.28**)

Yellow solid (0.93 g, 93%); m.p. 269 – 271 °C; R_f (EtOAc: Hex, 2:3) 0.50; 1H NMR (600 MHz, DMSO- d_6) δ 8.33 (dd, $J = 8.3, 1.0$ Hz, 1H), 7.99 (dd, $J = 8.2, 1.1$ Hz, 1H), 7.68 - 7.53 (m, 2H), 7.45 (ddd, $J = 8.2, 7.1, 1.2$ Hz, 1H), 7.29 (ddd, $J = 8.2, 7.1, 1.0$ Hz, 1H), 7.22 - 7.14 (m, 2H), 6.87 (s, 1H), 3.72 (s, 2H), 3.32 - 3.26 (m, 4H), 3.16 - 3.08 (m, 4H); HPLC-MS (APCI/ESI): Purity = 99%, $t_R = 3.325$ min, m/z $[M+H]^+ = 454.2$.

1-(4-(3-fluorobenzyl)piperazin-1-yl)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile (**PBI.29**)

Yellow solid (0.90 g, 90%); m.p. 259 – 261 °C; *Rf* (EtOAc: Hex, 2:3) 0.44; ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.35 (dd, *J* = 8.2, 1.0 Hz, 1H), 7.99 (dd, *J* = 8.1, 1.1 Hz, 1H), 7.68 - 7.55 (m, 2H), 7.44 (ddd, *J* = 8.2, 7.1, 1.2 Hz, 1H), 7.25 (ddd, *J* = 8.2, 7.1, 1.1 Hz, 1H), 7.19 - 7.13 (m, 2H), 6.85 (s, 1H), 3.72 (s, 2H), 3.27 - 3.21 (m, 4H), 3.19 - 3.08 (m, 4H); HPLC-MS (APCI/ESI): Purity = 96%, *t_R* = 3.319 min, *m/z* [M+H]⁺ = 454.1

1-(4-(4-methylbenzyl)piperazin-1-yl)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-*a*]pyridine-4-carbonitrile (**PBI.30**)

Yellow solid (0.78 g, 86%); m.p. 254 – 256 °C; *Rf* (EtOAc: Hex, 2:3) 0.50; ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.35 (dd, *J* = 8.4, 1.0 Hz, 1H), 7.92 (dd, *J* = 8.3, 1.1 Hz, 1H), 7.68 (ddd, *J* = 8.2, 7.1, 1.1 Hz, 1H), 7.28 (ddd, *J* = 8.2, 7.1, 1.1 Hz, 1H), 7.17 (d, *J* = 7.8 Hz, 2H), 7.12 (d, *J* = 7.9, 2H), 6.34 (s, 1H), 3.67 (s, 2H), 3.30 - 3.23 (m, 4H), 3.18 - 3.09 (m, 4H), 2.31 (s, 3H); HPLC-MS (APCI/ESI): Purity = 98%, *t_R* = 3.370 min, *m/z* [M+H]⁺ = 450.2.

1-(4-(4-fluorobenzyl)piperazin-1-yl)-3-propylbenzo[4,5]imidazo[1,2-*a*]pyridine-4-carbonitrile (**PBI.31**)

Yellow solid (0.88 g, 88%); m.p. 246 – 248 °C; *Rf* (EtOAc: Hex, 2:3) 0.50; ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.29 (dd, *J* = 8.3, 1.1 Hz, 1H), 7.87 (dd, *J* = 8.1, 1.1 Hz, 1H), 7.56 (ddd, *J* = 8.2, 7.2, 1.1 Hz, 1H), 7.47 (dd, *J* = 8.5, 5.1 Hz, 2H), 7.43 (ddd, *J* = 8.2, 7.2, 1.2 Hz, 1H), 7.15 (dd, *J* = 9.8, 8.6 Hz, 2H), 6.64 (s, 1H), 3.64 (s, 2H), 3.52 - 3.44 (m, 4H), 3.10 (t, *J* = 7.0 Hz, 2H), 3.01 - 2.87 (m, 4H), 1.86 - 1.79 (m, 2H), 1.23 (t, *J* = 7.3 Hz, 3H); HPLC-MS (APCI/ESI): Purity = 98%, *t_R* = 3.150 min, *m/z* [M+H]⁺ = 428.2.

7,8-dichloro-1-(4-cyclohexylpiperazin-1-yl)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-*a*]pyridine-4-carbonitrile (**PBI.32**)

Yellow solid (0.66 g, 66%); m.p. 264 – 266 °C; *Rf* (EtOAc: Hex, 2:3) 0.50; ¹H NMR (300 MHz, DMSO-*d*₆) δ 8.83 (s, 1H), 8.34 (s, 1H), 6.78 (s, 1H), 3.16 - 3.11 (m, 4H), 3.07 - 3.01 (m, 5H), 1.87 - 1.79 (m, 4H), 1.68 - 1.63 (m, 2H), 1.37 - 1.31 (m, 4H); HPLC-MS (APCI/ESI): Purity = 96%, *t_R* = 3.052 min, *m/z* [M+H]⁺ = 496.1.

1-(4-(pyridin-2-yl)piperazin-1-yl)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-*a*]pyridine-4-carbonitrile (**PBI.33**)

Yellow solid (0.91 g, 91%); m.p. 275 – 277 °C; *Rf* (EtOAc: Hex, 1:1) 0.32; ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.45 (dd, *J* = 8.4, 1.0 Hz, 1H), 8.18 (dd, *J* = 8.1, 1.1 Hz, 1H), 8.01 (dd, *J* = 8.5, 1.1 Hz, 1H), 7.70 (ddd, *J* = 8.0, 7.2, 1.1 Hz, 1H), 7.61 (ddd, *J* = 8.3, 7.1, 1.0 Hz, 1H), 7.55 (ddd, *J* = 8.6, 7.1, 1.4 Hz, 1H), 6.99 (ddd, *J* = 8.1, 7.1, 1.2 Hz, 1H), 6.92 (dd, *J* = 8.0, 1.2 Hz, 1H), 6.89 (s, 1H), 3.47 - 3.40 (m, 4H), 3.22 - 3.18 (m, 4H); HPLC-MS (APCI/ESI): Purity = 97%, *t_R* = 2.916 min, *m/z* [M+H]⁺ = 423.2.

1-(4-(pyrazin-2-yl)piperazin-1-yl)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile (**PBI.34**)

Yellow solid (0.63 g, 63%); m.p. 256 – 258 °C; *Rf* (EtOAc: Hex, 2:3) 0.50; ¹H NMR (600 MHz, DMSO-*d*₆) δ 8.39 (dd, *J* = 8.3, 1.0 Hz, 1H), 8.18 (d, *J* = 8.0 Hz, 1H), 8.14 - 8.11 (m, 2H), 8.01 (dd, *J* = 8.3, 1.1 Hz, 1H), 7.94 (ddd, *J* = 8.3, 7.1, 1.0 Hz, 1H), 7.70 (ddd, *J* = 8.2, 7.1, 1.0 Hz, 1H), 6.94 (s, 1H), 3.69 - 3.58 (m, 4H), 3.18 - 3.08 (m, 4H); HPLC-MS (APCI/ESI): Purity = 98%, *t_R* = 3.008 min, *m/z* [M+H]⁺ = 424.1.

1-(4-(4-chlorophenyl)piperazin-1-yl)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile (**PBI.35**)

Yellow solid (0.77 g, 77%); m.p. 265 – 267 °C; *Rf* (EtOAc: Hex, 2:3) 0.44; ¹H NMR (600 MHz, DMSO-*d*₆) δ 8.41 (dd, *J* = 8.4, 1.0 Hz, 1H), 8.01 (dd, *J* = 8.3, 1.1 Hz, 1H), 7.69 (ddd, *J* = 8.2, 7.0, 1.0 Hz, 1H), 7.56 (ddd, *J* = 8.4, 7.0, 1.2 Hz, 1H), 7.25 (d, *J* = 8.0 Hz, 2H), 7.07 (d, *J* = 8.1 Hz, 2H), 6.93 (s, 1H), 3.19 - 3.14 (m, 4H), 3.06 - 3.02 (m, 4H); HPLC-MS (APCI/ESI): Purity = 98%, *t_R* = 3.375 min, *m/z* [M+H]⁺ = 456.1.

3-(trifluoromethyl)-1-(4-(5-(trifluoromethyl)pyridin-2-yl)piperazin-1-yl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile (**PBI.36**)

Yellow solid (0.65 g, 65%); m.p. 266 – 268 °C; *Rf* (EtOAc: Hex, 2:3) 0.43; ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.50 (s, 1H), 8.46 (dd, *J* = 8.4, 1.0 Hz, 1H), 8.02 (d, *J* = 8.0 Hz, 1H), 7.89 (dd, *J* = 8.3, 1.1 Hz, 1H), 7.70 (ddd, *J* = 8.3, 7.1, 1.1 Hz, 1H), 7.56 (ddd, *J* = 8.4, 7.1, 1.2 Hz, 1H), 7.05 (d, *J* = 8.1 Hz, 1H), 6.94 (s, 1H), 3.28 - 3.25 (m, 4H), 3.22 - 3.16 (m, 4H); HPLC-MS (APCI/ESI): Purity = 98%, *t_R* = 3.227 min, *m/z* [M+H]⁺ = 491.1.

1-(4-(2,4-difluorophenyl)piperazin-1-yl)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile (**PBI.37**)

Yellow solid (0.84g, 84%); m.p. 270 – 272 °C; *Rf* (EtOAc: Hex, 2:3) 0.48; ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.43 (dd, *J* = 8.4, 1.0 Hz, 1H), 8.02 (dd, *J* = 8.2, 1.0 Hz, 1H), 7.70 (ddd, *J* = 8.3, 7.1, 1.0 Hz, 1H), 7.60 (ddd, *J* = 8.4, 7.1, 1.1 Hz, 1H), 7.32 - 7.24 (m, 2H), 7.09 – 7.04 (m, 1H), 6.97 (s, 1H), 3.65 - 3.57 (m, 4H), 3.15 - 3.07 (m, 4H); HPLC-MS (APCI/ESI): Purity = 98%, *t_R* = 3.188 min, *m/z* [M+H]⁺ = 458.1.

1-(4-(4-(methylsulfonyl)benzyl)piperazin-1-yl)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile (**PBI.38**)

Yellow solid (0.73 g, 73%); m.p. 260 – 262 °C; *Rf* (EtOAc: Hex, 2:3) 0.40; ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.35 (dd, *J* = 8.3, 1.0 Hz, 1H), 8.00 (dd, *J* = 8.2, 1.1 Hz, 1H), 7.90 (d, *J* = 8.2 Hz, 2H), 7.70 (d, *J* = 8.1 Hz, 2H), 7.65 (ddd, *J* = 8.3, 7.1, 1.0 Hz, 1H), 7.54 (ddd, *J* = 8.3, 7.2, 1.4 Hz, 1H), 6.86 (s, 1H), 3.56 (s, 2H), 3.42 - 3.36 (m, 4H), 3.20 (s, 3H), 3.12 - 2.97 (m, 4H); HPLC-MS (APCI/ESI): Purity = 98%, *t_R* = 2.990 min, *m/z* [M+H]⁺ = 514.1.

7,8-difluoro-1-((2-fluoropyridin-4-yl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile (**PBI.75**)

Yellow solid (0.098 g, 78%), m.p > 300 °C; *Rf* (EtOAc : Hexane, 3 : 7) 0.18. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.2 (d, *J* = 7.7 Hz, 1H), 8.4 (dd, *J* = 10.9, 7.4 Hz, 1H), 7.8 (dd, *J* = 7.8, 6.6 Hz, 1H), 6.8 (d, *J* = 7.6 Hz, 1H), 6.5 (d, *J* = 7.3 Hz, 1H), 6.2 (s, 1H); HPLC-MS (APCI/ ESI): Purity = 96%, *t_R* = 3.03 min, *m/z* [M-H]⁻ = 409.0

1-((5,6-dimethoxyimidin-4-yl)amino)-7,8-difluoro-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile (**PBI.76**)

Yellow solid (0.093 g, 46%), m.p 299 – 302 °C; *Rf* (EtOAc : Hexane, 3 : 7) 0.18. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.4 (s, 1H), 8.3 (dd, *J* = 10.7, 7.5 Hz, 1H), 8.3 (dd, *J* = 8.7, 6.9 Hz, 1H), 6.1 (s, 1H), 4.2 (s, 3H), 4.0 (s, 3H); HPLC-MS (APCI/ ESI): Purity = 97%, *t_R* = 3.17 min, *m/z* [M+H]⁺ = 451.1

7,8-difluoro-1-((1-(4-(methylsulfonyl)phenyl)ethyl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile (**PBI.104**)

Yellow solid (0.063 g, 46%), m.p 261 – 263 °C; *Rf* (EtOAc : Hexane, 0.5 : 9.5) 0.18. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.7 (dd, *J* = 11.3, 7.1 Hz, 1H), 8.4 (s, 1H), 8.0 (dd, *J* = 10.9, 7.0 Hz, 1H), 8.0 (d = 8.8 Hz, 2H), 7.9 (d, *J* = 8.5 Hz, 2H), 6.3 (s, 1H), 5.4 (q, *J* = 7.5 Hz, 1H), 3.2 (s, 3H), 1.8 (d, *J* = 6.7 Hz, 3H); HPLC-MS (APCI/ ESI): Purity = 99%, *t_R* = 3.43 min, *m/z* [M-H]⁻ = 493.0.

7,8-difluoro-1-((1-(*p*-tolyl)ethyl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile (**PBI.111**)

Orange solid (0.074 g, 82%), m.p 215 – 217 °C; *Rf* (DCM) 0.27. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.68 (dd, *J* = 11.3, 7.2 Hz, 1H), 8.3 (d, *J* = 5.7 Hz, 1H), 8.0 (dd, *J* = 11.0, 7.6 Hz, 1H), 7.5 (d, *J* = 7.8 Hz, 2H), 7.2 (d, *J* = 7.8 Hz, 2H), 6.3 (s, 1H), 5.1 (m, 1H), 2.3 (s, 3H), 1.8 (d, *J* = 6.7 Hz, 3H); HPLC-MS (APCI/ ESI): Purity = 98%, *t_R* = 3.07 min, *m/z* [M+H]⁺ = 431.1.

(*S*)-1-((1-(2-chlorophenyl)ethyl)amino)-7,8-difluoro-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile (**PBI.125**)

Yellow solid (0.101 g, 74%), m.p 231 - 233 °C; *Rf* (DCM) 0.23. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.8 (dd, *J* = 11.5, 7.4 Hz, 1H), 8.4 (s, 1H), 7.8 (dd, *J* = 11.0, 7.0 Hz, 1H), 7.8 (dd, *J* = 8.9, 3.3 Hz, 1H), 7.6 (m, 1H), 7.5-7.3 (m, 2H), 5.9 (s, 1H), 5.2 (q, *J* = 6.7 Hz, 1H), 1.8 (d, *J* = 7.1 Hz, 3H); HPLC-MS (APCI/ ESI): Purity = 97%, *t_R* = 3.15 min, *m/z* [M-H]⁻ = 451.0.

1-((3-((ethylamino)methyl)-4-hydroxyphenyl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile (**PBI.126**)

Brown solid (26 mg, 37%), m.p. 380–382 °C, *R*_f = 0.48 (20% MeOH/DCM); ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.15 (s, 1H), 8.79 (d, *J* = 8.8 Hz, 1H), 8.79 – 8.77 (m, 1H), 7.69 (d, *J* = 7.2 Hz, 1H), 7.55 (t, *J* = 7.9 Hz, 1H), 7.36 (t, *J* = 7.9 Hz, 1H), 7.25 – 7.07 (m, 1H), 7.07 – 6.93 (m, 1H), 6.11 (s, 1H), 4.09 (s, 2H), 2.97 (q, *J* = 7.7 Hz, 2H), 1.23 (t, *J* = 7.2 Hz, 3H); HPLC-MS (ESI⁺): Purity 96%, *t*_R = 3.502 min, *m/z* [M+H]⁺ = 426.2.

1-((3-((diethylamino)methyl)-4-hydroxyphenyl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-*a*]pyridine-4-carbonitrile (**PBI.127**)

Brown solid (26 mg, 37%), m.p. 244 – 245 °C, *R*_f = 0.838 (20% MeOH/DCM); ¹H NMR (600 MHz, DMSO-*d*₆) δ 8.81 (dd, *J* = 8.1, 0.9 Hz, 1H), 7.58 (dd, *J* = 8.1, 1.0 Hz, 1H), 7.36 (td, *J* = 8.2, 1.3 Hz, 1H), 7.14 (td, *J* = 8.3, 1.2 Hz, 1H), 7.02 – 7.01 (m, 1H), 6.94 – 6.93 (m, 2H), 5.84 (s, 1H), 4.16 (s, 2H), 3.07 (q, *J* = 7.2 Hz, 4H), 1.28 – 1.19 (m, 6H); HPLC-MS (ESI⁺): Purity 97%, *t*_R = 2.366 min, *m/z* [M+H]⁺ = 454.2.

1-((4-((ethylamino)methyl)-3-hydroxyphenyl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-*a*]pyridine-4-carbonitrile (**PBI.128**)

Brown solid (16.9 mg, 23%), m.p. 274 – 276 °C, *R*_f = 0.15 (10% MeOH/DCM); ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.81 (d, *J* = 8.2 Hz, 1H), 7.62 (d, *J* = 8.2 Hz, 1H), 7.42 (t, *J* = 7.8 Hz, 1H), 7.30 (d, *J* = 7.8 Hz, 1H), 7.21 (t, *J* = 7.9 Hz, 1H), 6.60–6.63 (m, 2H), 6.00 (s, 1H), 4.20 (s, 2H), 3.15 (q, *J* = 7.2 Hz, 2H), 1.38 (t, *J* = 7.3 Hz, 3H); HPLC-MS (ESI⁺): Purity 96%, *t*_R = 2.384 min, *m/z* [M+H]⁺ = 426.2.

1-((3-((ethylamino)methyl)-4-fluorophenyl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-*a*]pyridine-4-carbonitrile (**PBI.129**)

Orange-brown solid (34 mg, 34%), m.p. 235–237 °C, *R*_f = 0.63 (20% MeOH/DCM); ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.80 (dd, *J* = 8.1, 1.1 Hz, 1H), 7.57 (dd, *J* = 7.9, 1.8 Hz, 1H), 7.34 (td, *J* = 7.2, 1.2 Hz, 1H), 7.30 – 7.24 (m, 1H), 7.17 (dd, *J* = 6.8, 2.7 Hz, 1H), 7.11 (td, *J* = 7.4, 1.2 Hz, 1H), 7.05 (ddd, *J* = 8.7, 4.7, 2.6 Hz, 1H), 5.72 (s, 1H), 4.20 (s, 2H), 3.05 (q, *J* = 7.3 Hz, 2H), 1.23 (t, *J* = 7.3 Hz, 3H); HPLC-MS (ESI⁺): Purity 97%, *t*_R = 2.382 min, *m/z* [M+H]⁺ = 428.1.

1-((3-((ethylamino)methyl)-2-hydroxyphenyl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-*a*]pyridine-4-carbonitrile (**PBI.130**)

Yellow solid (31.8 mg, 26%). m.p. 275–277 °C, *R*_f = 0.31 (15% MeOH/DCM); ¹H NMR (600 MHz, DMSO-*d*₆) δ 8.83 (dd, *J* = 8.1, 1.8 Hz, 1H), 7.56 (dd, *J* = 8.1, 1.8 Hz, 1H), 7.34 (td, *J* = 8.2, 1.3 Hz, 1H), 7.11 (td, *J* = 8.2, 1.2 Hz, 1H), 7.01 (dd, *J* = 7.6, 1.6 Hz, 1H), 6.97 (dd, *J* = 7.7, 1.6 Hz, 1H), 6.85 (t, *J* = 7.7 Hz, 1H), 5.76 (s, 1H), 4.11 (s, 2H), 3.00 (q, *J* = 7.2 Hz, 2H), 1.22 (t, *J* = 7.3 Hz, 3H); HPLC-MS (ESI⁺): Purity 97%, *t*_R = 2.460 min, *m/z* [M+H]⁺ = 426.2.

1-((3-((ethylamino)methyl)-4-hydroxyphenyl)amino)-3-methylbenzo[4,5]imidazo[1,2-

a]pyridine-4-carbonitrile (**PBI.131**)

Yellow solid (42 mg, 33%). m.p. 227–228 °C, *Rf* = 0.09 (10% MeOH/DCM); ¹H NMR (600 MHz, DMSO-*d*₆) δ 8.47 (d, *J* = 8.3 Hz, 1H), 7.72 (d, *J* = 8.3 Hz, 1H), 7.47 (t, *J* = 7.7 Hz, 1H), 7.30 (s, 1H), 7.25 (t, *J* = 8.4 Hz, 1H), 7.17 (m, 3H), 7.01 (d, *J* = 8.5 Hz, 1H), 5.95 (s, 1H), 4.02 (s, 2H), 2.91 (q, *J* = 7.3 Hz, 2H), 2.38 (s, 3H), 1.19 (t, *J* = 7.2 Hz, 3H); HPLC-MS (ESI⁺): Purity 98%, *t_R* = 2.208 min, *m/z* [M+H]⁺ = 372.1.

1-((3-((ethylamino)methyl)-2-hydroxy-5-methylphenyl)amino)-3-methylbenzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile (**PBI.132**)

Yellow solid (33 mg, 25%). m.p. 217–218 °C, *Rf* = 0.1 (10% MeOH/DCM), ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.52 (d, *J* = 8.1 Hz, 1H), 7.57 – 7.49 (m, 2H), 7.48 (t, *J* = 7.6 Hz, 1H), 7.37 – 7.27 (m, 2H), 5.86 (s, 1H), 4.16 (s, 1H), 3.06 (q, *J* = 7.2 Hz, 2H), 2.34 (s, 3H), 1.27 (t, *J* = 7.2 Hz, 3H); HPLC-MS (ESI⁺): Purity 98% *t_R* = 2.326 min, *m/z* [M+H]⁺ = 386.1.

1-((3-((ethylamino)methyl)-4-hydroxyphenyl)amino)-7,8-difluoro-3-methylbenzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile (**PBI.133**)

Yellow solid (33 mg, 22%). m.p. 269–270 °C, *Rf* = 0.12 (10 % MeOH/DCM); ¹H NMR (600 MHz, DMSO-*d*₆) δ 8.58 (dd, *J* = 11.6, 7.6 Hz, 1H), 7.70 (dd, *J* = 11.2, 7.7 Hz, 1H), 7.23 (d, *J* = 2.6 Hz, 1H), 7.13 (dd, *J* = 8.7, 2.6 Hz, 1H), 6.98 (d, *J* = 8.6 Hz, 1H), 5.84 (s, 1H), 4.03 (s, 2H), 2.93 (q, *J* = 7.3 Hz, 2H), 2.33 (s, 3H), 1.19 (t, *J* = 7.2 Hz, 3H); HPLC-MS (ESI⁺): Purity 98%, *t_R* = 2.263 min, *m/z* [M+H]⁺ = 408.2.

1-((3-((ethylamino)methyl)-2-hydroxy-5-methylphenyl)amino)-7,8-difluoro-3-methylbenzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile (**PBI.134**)

Yellow solid (45 mg, 29%). m.p. 257–258 °C, *Rf* = 0.47 (10% MeOH/DCM); ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.77 (dd, *J* = 11.8, 7.4 Hz, 1H), 7.88 – 7.79 (m, 1H), 7.23 (d, *J* = 2.3 Hz, 1H), 6.89 (d, *J* = 2.4 Hz, 1H), 5.60 (s, 1H), 4.15 (s, 2H), 3.05 (q, *J* = 7.2 Hz, 2H), 2.44 (s, 3H), 2.39 (s, 3H), 1.27 (t, *J* = 7.3 Hz, 3H); HPLC-MS (ESI⁺): Purity 98%, *t_R* = 2.406 min, *m/z* [M+H]⁺ = 422.2.

1-((3-((ethylamino)methyl)-4-hydroxyphenyl)amino)-7,8-difluoro-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile (**PBI.135**)

Yellow solid (25 mg, 18%). m.p. 265–266 °C, *Rf* = 0.13 (10% MeOH/DCM); ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.80 (dd, *J* = 11.5, 7.7 Hz, 1H), 7.79 (dd, *J* = 10.9, 7.4 Hz, 1H), 7.24 (d, *J* = 2.6 Hz, 1H), 7.14 (dd, *J* = 8.6, 2.6 Hz, 1H), 7.04 (d, *J* = 8.5 Hz, 1H), 6.04 (s, 1H), 4.10 (s, 2H), 3.15 – 3.04 (m, 2H), 1.23 (t, *J* = 7.3 Hz, 3H); HPLC-MS (ESI⁺): Purity 98%, *t_R* = 2.402 min, *m/z* [M+H]⁺ = 462.1.

1-((3-((ethylamino)methyl)-2-hydroxy-5-methylphenyl)amino)-7,8-difluoro-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile (**PBI.136**)

Yellow solid (17 mg, 12%). m.p. >300 °C, *Rf* = 0.41 (10% MeOH/DCM); ¹H-NMR (600 MHz, DMSO-*d*₆) δ 8.75 (dd, *J* = 11.6, 8.1 Hz, 1H), 7.54 (dd, *J* = 11.1, 7.4 Hz, 1H), 6.84 (s, 1H), 6.79 (s, 1H), 5.69 (s, 1H), 4.06 (s, 2H), 3.00 – 2.96 (m, 2H), 2.20 (s, 3H), 1.20 (t, *J* = 7.3 Hz, 3H); HPLC-MS (ESI+): Purity 98%, *t_R* = 2.607 min, *m/z* [M+H]⁺ = 476.1.

1-((3-((diethylamino)methyl)-2-hydroxy-5-methylphenyl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile (**PBI.137**)

Yellow solid (41.3 mg, 26%). M.P. 268–269 °C *Rf* = 0.54 (9% MeOH/DCM); ¹H NMR (600 MHz, DMSO-*d*₆) δ 8.85 (d, *J* = 8.2 Hz, 1H), 7.63 (d, *J* = 8.0 Hz, 1H), 7.46 (t, *J* = 7.7 Hz, 1H), 7.25 (t, *J* = 7.7 Hz, 1H), 7.00 (d, *J* = 2.1 Hz, 1H), 6.88 (d, *J* = 2.1 Hz, 1H), 5.80 (s, 1H), 4.24 (s, 2H), 3.12 (q, *J* = 7.2 Hz, 4H), 2.26 (s, 3H), 1.27 (t, *J* = 7.2 Hz, 6H); HPLC-MS (ESI+): Purity 98%, *t_R* = 2.151 min, *m/z* [M+H]⁺ = 468.2.

1-((3-((diethylamino)methyl)-4-hydroxyphenyl)amino)-3-methylbenzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile (**PBI.138**)

Yellow solid (46 mg, 34%). m.p. 243–244 °C, *Rf* = 0.48 (9% MeOH/DCM); ¹H-NMR (600 MHz, DMSO-*d*₆) δ 10.10 (s, 1H), 8.52 (d, *J* = 8.4 Hz, 1H), 7.78 (d, *J* = 8.3 Hz, 1H), 7.59 (t, *J* = 7.8 Hz, 1H), 7.49 (d, *J* = 2.7 Hz, 1H), 7.38 (t, *J* = 7.9 Hz, 1H), 7.28 (dd, *J* = 8.6, 2.7 Hz, 1H), 7.13 (d, *J* = 8.6 Hz, 1H), 6.27 (s, 1H), 4.17 (d, *J* = 5.2 Hz, 2H), 3.11 – 2.97 (m, 4H), 2.46 (s, 3H), 1.23 (t, *J* = 7.2 Hz, 6H); HPLC-MS (ESI+): Purity 98%, *t_R* = 2.071 min, *m/z* [M+H]⁺ = 400.3.

1-((3-((diethylamino)methyl)-2-hydroxy-5-methylphenyl)amino)-3-methylbenzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile (**PBI.139**)

Yellow solid (55 mg, 39%). m.p. 234–235 °C, *Rf* = 0.48 (10% MeOH/DCM); ¹H-NMR (600 MHz, DMSO-*d*₆) δ 10.12 (s, 1H), 8.68 (d, *J* = 8.4 Hz, 1H), 7.76 (d, *J* = 8.2 Hz, 1H), 7.59 (t, *J* = 7.7 Hz, 1H), 7.38 (t, *J* = 7.8 Hz, 1H), 7.36 (s, 1H), 7.13 (s, 1H), 4.25 (s, 2H), 3.10 – 3.02 (m, 4H), 2.40 (s, 3H), 2.24 (s, 3H), 1.26 (t, *J* = 7.2 Hz, 6H); HPLC-MS (ESI+): Purity 98%, *t_R* = 2.393 min, *m/z* [M+H]⁺ = 414.2.

1-((3-((diethylamino)methyl)-4-hydroxyphenyl)amino)-7,8-difluoro-3-methylbenzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile (**PBI.140**)

Yellow solid (45 mg, 29%). m.p. 289–290 °C, *Rf* = 0.44 (10% MeOH/DCM); ¹H-NMR (600 MHz, DMSO-*d*₆) δ 8.55 (dd, *J* = 11.4, 7.3 Hz, 1H), 7.82 (dd, *J* = 11.0, 7.6 Hz, 1H), 7.43 (d, *J* = 2.7 Hz, 1H), 7.28 (dd, *J* = 7.7, 2.6 Hz, 1H), 7.08 (d, *J* = 8.6 Hz, 1H), 6.03 (s, 1H), 4.18 (s, 2H), 3.06 (q, *J* = 7.24 Hz, 4H), 2.41 (s, 3H), 1.23 (t, *J* = 7.2 Hz, 6H); HPLC-MS (ESI+): Purity 98%, *t_R* = 2.322 min, *m/z* [M+H]⁺ = 436.2.

1-((3-((diethylamino)methyl)-2-hydroxy-5-methylphenyl)amino)-7,8-difluoro-3-methylbenzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile (**PBI.141**)

Yellow solid (41 mg, 25%). m.p. 205–206 °C, *R*_f = 0.47 (10% MeOH/DCM); ¹H-NMR (600 MHz, DMSO-*d*₆) δ 8.69 (dd, *J* = 11.6, 7.3 Hz, 1H), 7.79 (dd, *J* = 11.6, 7.3 Hz, 1H), 7.27 (s, 1H), 7.14 (s, 1H), 5.59 (s, 1H), 4.23 (s, 2H), 3.08 (q, *J* = 7.2 Hz, 4H), 2.35 (s, 3H), 2.24 (s, 3H), 1.24 (t, *J* = 7.2 Hz, 6H); HPLC-MS (ESI⁺): Purity 98%, *t*_R = 2.461 min, *m/z* [M+H]⁺ = 450.2.

1-((3-((diethylamino)methyl)-4-hydroxyphenyl)amino)-7,8-difluoro-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile (**PBI.142**)

Yellow solid (8.7 mg, 12%). m.p. 150–151 °C, *R*_f = 0.49 (10% MeOH/DCM); ¹H-NMR (600 MHz, DMSO-*d*₆) δ 8.70 (dd, *J* = 11.5, 8.2 Hz, 1H), 7.51 (dd, *J* = 11.4, 7.5 Hz, 1H), 7.01 (d, *J* = 2.5 Hz, 1H), 6.93 (d, *J* = 8.5 Hz, 1H), 6.89 (dd, *J* = 8.5, 2.5 Hz, 1H), 5.70 (s, 1H), 4.17 (s, 2H), 3.08 (q, *J* = 7.2 Hz, 4H), 1.21 (t, *J* = 7.2 Hz, 6H); HPLC-MS (ESI⁺): Purity 98%, *t*_R = 2.445 min, *m/z* [M+H]⁺ = 490.2.

1-((3-((diethylamino)methyl)-2-hydroxy-5-methylphenyl)amino)-7,8-difluoro-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-a]pyridine-4-carbonitrile (**PBI.143**)

Yellow solid (19 mg, 25%). m.p. 235–236 °C, *R*_f = 0.41 (10% MeOH/DCM); ¹H-NMR (600 MHz, DMSO-*d*₆) δ 8.72 (dd, *J* = 11.5, 8.1 Hz, 1H), 7.51 (dd, *J* = 11.4, 7.4 Hz, 1H), 6.86 (s, 1H), 6.80 (s, 1H), 5.63 (s, 1H), 4.20 (s, 2H), 3.10 (q, *J* = 7.2 Hz, 4H), 2.20 (s, 3H), 1.23 (t, *J* = 7.2 Hz, 6H); HPLC-MS (ESI⁺): Purity 98%, *t*_R = 2.635 min, *m/z* [M+H]⁺ = 504.2.