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Supplementary Materials for

Specific activation of the TLR1-TLR2 heterodimer by small-molecule agonists

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This PDF file includes:

Supplementary Materials

General Methods

NMR spectra were acquired on a Bruker 400 spectrometer, running at 400 MHz for ^{1}H and 101 MHz for ^{13}C, respectively. ^{1}H NMR spectra were recorded at 400 MHz in $CDCl_3$, $(CD_3)_2SO$ using residual $CHCl_3$ (7.28 ppm), and $(CH_3)_2SO$ (2.50 ppm) as the internal standards. ^{13}C NMR spectra were recorded at 101 MHz in $CDCl_3$, $(CD_3)_2CO$ or $(CD_3)_2SO$ using residual $CHCl_3$ (77.16 ppm), $(CH_3)_2CO$ (29.84 and 206.26 ppm), $(CH_3)_2SO$ (39.97 ppm) and CH_3CN (1.32 and 118.26 ppm), as internal references. Thin layer chromatography was performed on Merck Kieselgel 60 Å F254 or Silicycle 60Å F254 plates eluting with the solvent indicated, visualized by a 254 nm UV lamp, and stained with an ethanolic solution of 12-molybdophosphoric acid. Compounds were purified using flash chromatography (FC) (Silica gel 60, 200 - 400 mesh, Sorbent Tech.) or recrystalization. Mass spectrometry was performed at the mass spectrometry facility of the Department of Chemistry and Biochemistry at University of Colorado at Boulder on a double focusing high resolution mass spectrometer.

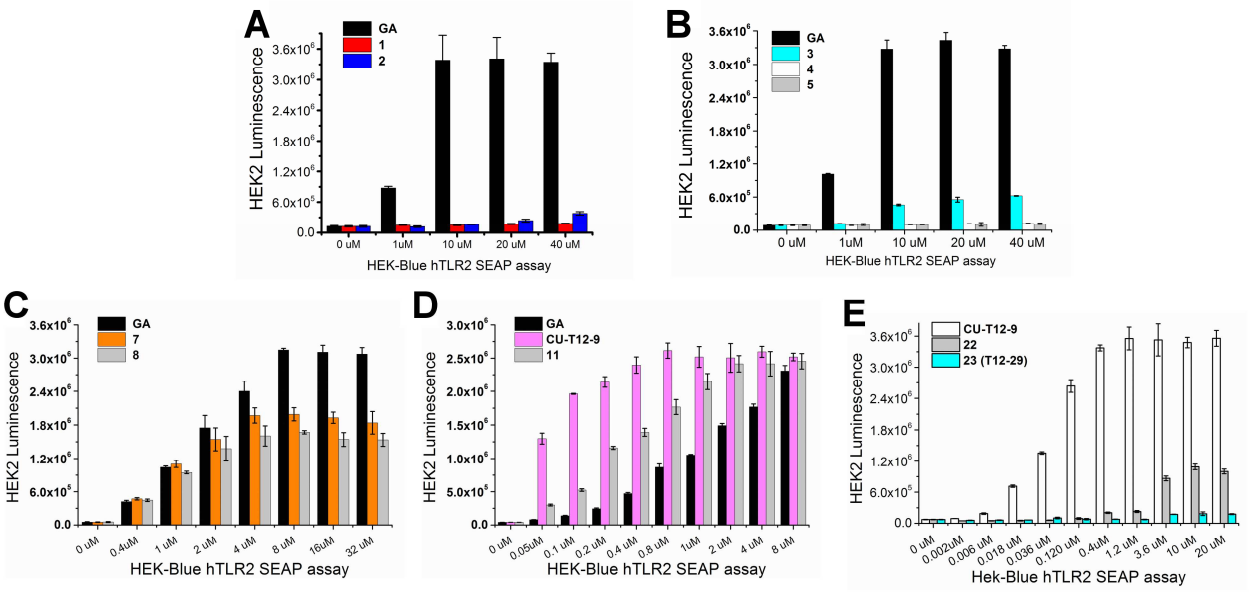


Fig. S1. Dose-dependent activation of SEAP signaling by analogs in HEK-Blue hTLR2 cells after 24 hours. (A) Compounds **1** and **2** (Supplementary Table S1) compared to GA by SEAP activity indicates the importance of the nitro group in the potency of GA. (B) Compounds **3**, **4**, and **5**, compared to GA by SEAP activity indicates the importance of the aliphatic chain length in potency of GA. (C) Compounds **7** and **8** are compared to GA. SEAP activity indicates the electron-donating groups are not tolerated. (D) Compounds CU-T12-9 and **11** show increased potency compared to GA as seen by SEAP activity. (E) The SEAP secretion of CU-T12-9, **22** and T12-29 were compared in the same experiment and show that the ester group or carboxylic acid group was not necessary for activity. Data are shown as mean ± s.d. of triplicates and representative of three independent experiments showing similar results.

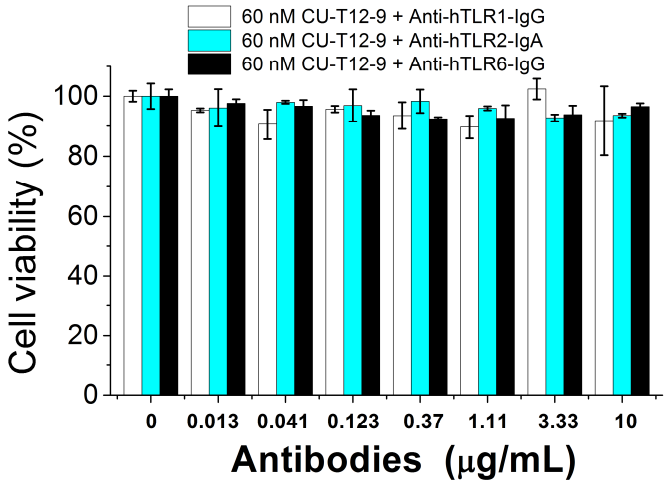


Fig. S2. The MTT cell viability of HEK-Blue hTLR2 cells after 24 hours of incubation with CU-T12-9 and antibodies. The result showed no significant toxicity for all three antibodies at concentrations up to 10 μg/mL.

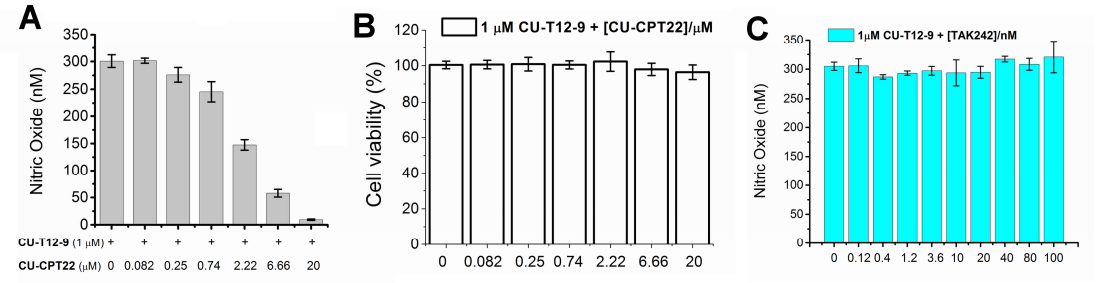


Fig. S3. NO activation of CU-T12-9 can be suppressed by a TLR1/2 antagonist, but not by a TLR4 antagonist. (A) CU-T12-9 shows strong nitric oxide activation at 1 μM in Raw 264.7 macrophage cells. Addition of a TLR1/2-specific antagonist (CU-CPT22) shows a dose-dependent inhibition of CU-T12-9 induced NO activation. (B) The MTT cell viability assay indicates that the inhibition was not due to toxicity of CU-CPT22. (C) TAK-242, a known TLR4 antagonist, is unable to inhibit CU-T12-9 activation.

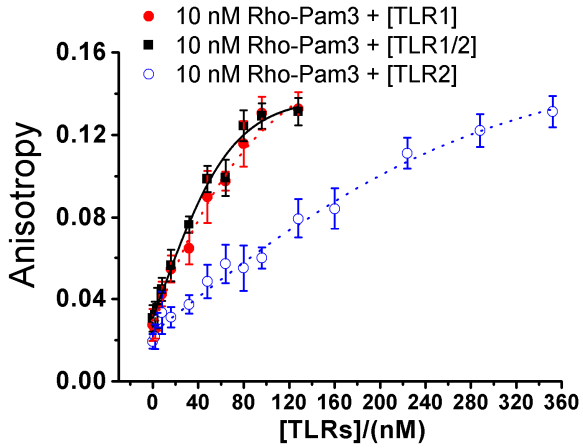


Fig. S4. Anisotropy assays for TLR1, TLR2, or TLR1/2 protein binding to rhodamine-labeled Pam_3CSK_4 (Rho-Pam3). Indicated concentrations of protein is titrated in 500 μL PBS buffer (pH = 7.4) including 10 nM (20 ng/mL) Rho-Pam3 (Ex: 549nm; Em: 566nm), and the fluorescence anisotropy were tested after the protein added and incubated for 3 minutes. The anisotropy experiment results showed that K_d of Rho-Pam3 binding to TLR1 is 41.64±2.36 nM (red), to TLR2 is 125.08±3.43 nM (blue), and to the TLR1/2 complex is 34.97±1.98 nM (black).

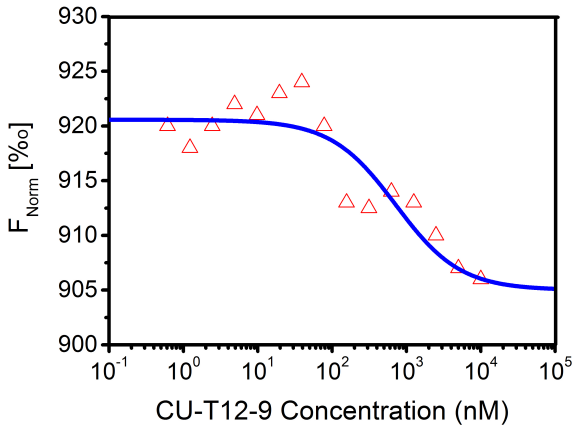


Fig. S5. Binding of CU-T12-9 to TLR1 by MST. For performing experiments with the TLR1 protein a fluorescent label (NT-647) was covalently attached to the protein (NHS coupling). In the MST experiment we have kept the concentration of NT-647 labeled TLR1 constant at 10 nM, while the concentration of the non-labeled CU-T12-9 was varied between 0.61 nM-10 µM. The assay was performed in PBS + 0.05% Tween20 buffer. After a short incubation the samples were loaded into MST NT.115 hydrophilic glass capillaries and the MST analysis was performed using the Monolith NT.115. Concentrations on the x-axis are plotted in nM. A K_d of 229 nM ± 66 nM was determined for this interaction. F_{Norm} = F1/F0; F_{Norm}: normalized fluorescence; F0: initial fluorescence or fluorescence after temperature-jump; F1: fluorescence after thermodiffusion.

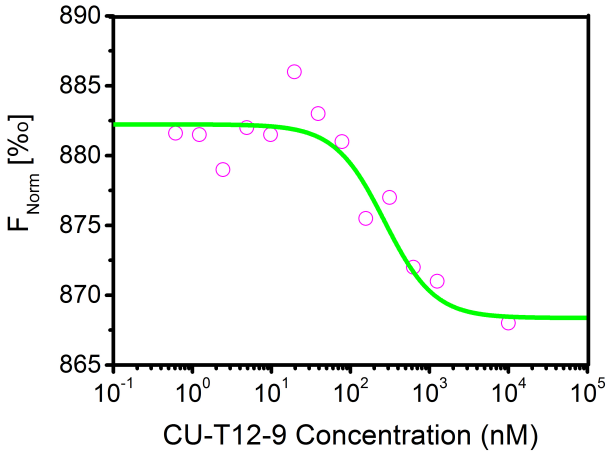


Fig. S6. Binding of CU-T12-9 to TLR2 by MST. For performing experiments with the TLR2 protein a fluorescent label (NT-647) was covalently attached to the protein (NHS coupling). In the MST experiment we have kept the concentration of NT-647 labeled TLR2 constant at 10 nM, while the concentration of the non-labeled CU-T12-9 was varied between 0.31 nM-10 µM. The assay was performed in PBS + 0.05% Tween20 buffer. After a short incubation the samples were loaded into MST NT.115 hydrophilic glass capillaries and the MST analysis was performed using the Monolith NT.115. Concentrations on the x-axis are plotted in nM. A K_d of 494 nM ± 114 nM was determined for this interaction. F_{Norm} = F1/F0; F_{Norm}: normalized fluorescence; F0: initial fluorescence or fluorescence after temperature-jump; F1: fluorescence after thermodiffusion.

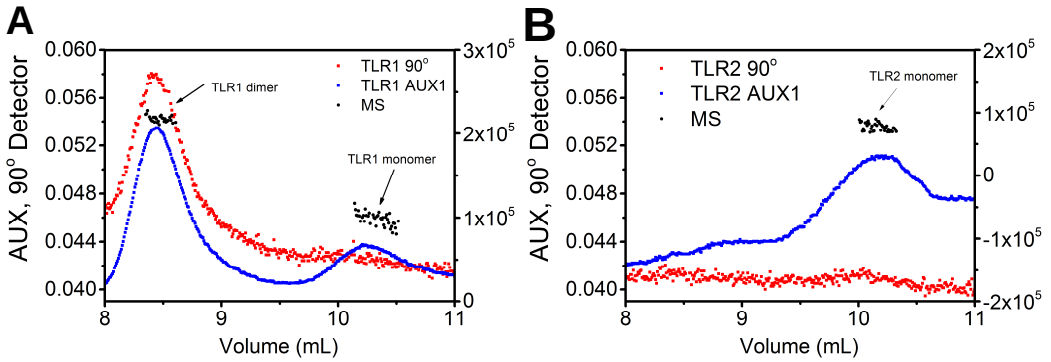


Fig. S7. TLR1 and TLR2 oligomeric states as seen by SEC-LS. (A) TLR1 protein exists in both monomeric and dimeric states with the majority of the protein existing as a dimer. (B) TLR2 protein exists preferentially in the monomeric state.

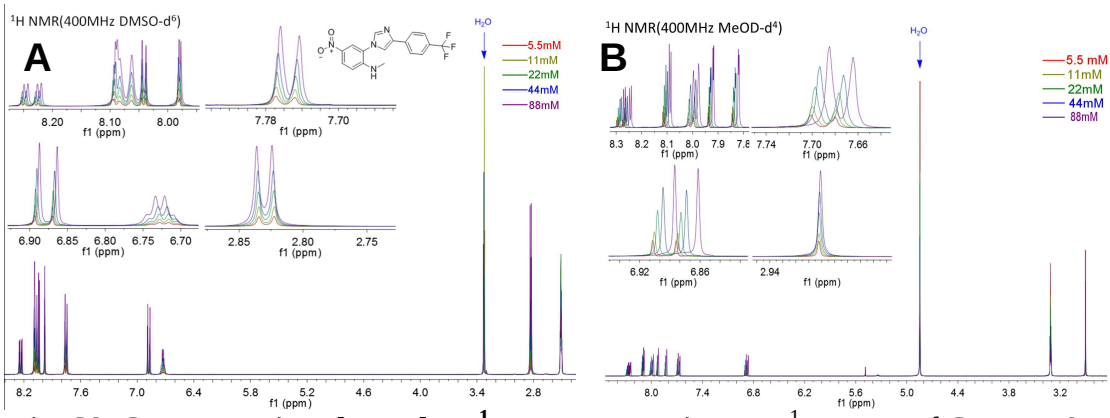


Fig. S8. Concentration-dependent ^{1}H NMR experiments. ^{1}H NMR of CU-T12-9 in $(CD_3)_2SO$ (A) and CD_3OH (B) at various concentrations ranging from 5.5 mM to 88 mM. The chemical shifts showed minimal changes upon dilution of CU-T12-9, indicating that there is neglectable oligomer formation in solution.

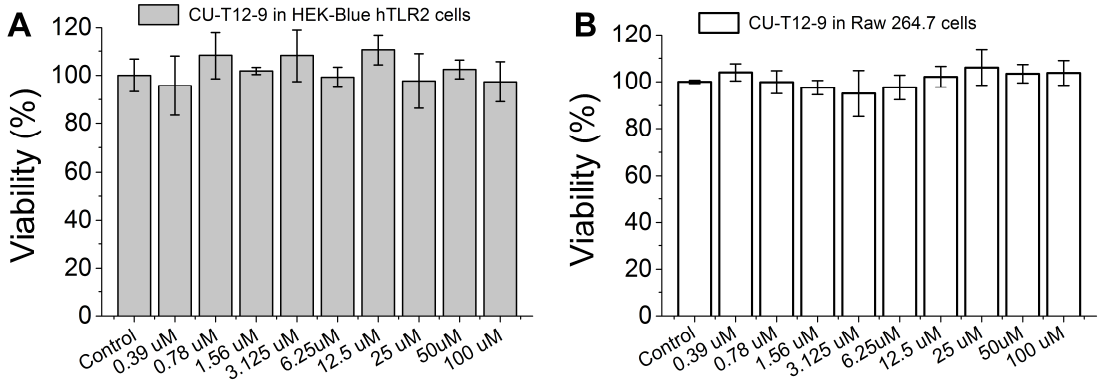


Fig. S9. HEK-Blue hTLR2 and Raw 264.7 cell viability upon CU-T12-9 treatment. Cell lines were treated with different concentrations of CU-T12-9 (0-100 μM) for 24 h. (A) No toxicity was seen by MTT assay in HEK-Blue hTLR2 cells up to 100 μM of CU-T12-9. (B) No toxicity was seen in Raw 264.7 cells up to 100 μM of CU-T12-9.

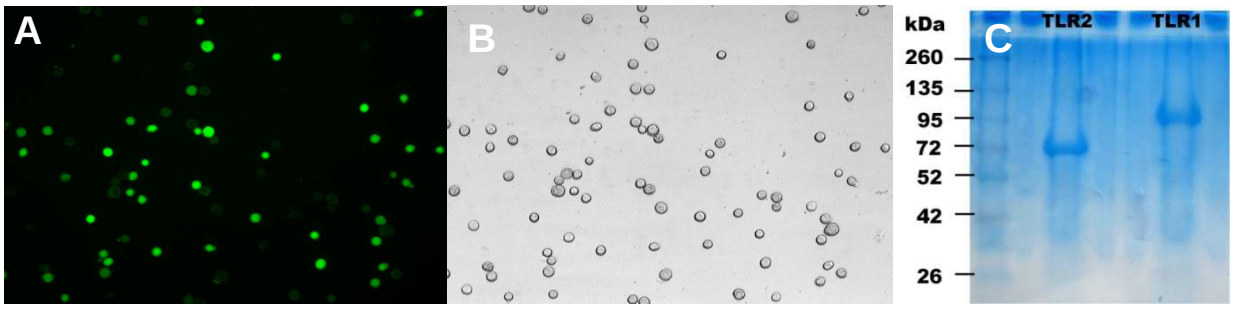
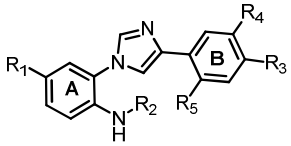
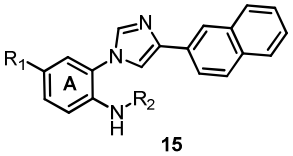


Fig. S10. Protein expression and characterization. (A) The recombinant virus was prepared by cotransfected TLR2 cDNA and Bright Baculovirus DNA (contains the GFP gene) in sf9 cells for 3 days. Then, the amplified recombinant virus was added into high 5 cells for protein expression. After a 3 day incubation with the GFP contained recombinant virus, the high five cells exhibit green fluorescence. (B) Bright field image of Hi 5 cells. (C) Characterization of the purified TLR1 and TLR2 protein. TLR1 and TLR2 protein were confirmed by coomassie brilliant blue stain after purification.

Table S1. SAR studies of the GA analogs in activation of SEAP signaling in HEK-Blue hTLR2 cells.



GA, 1-8, 10-24: R_5 = H
9: R_5 = F



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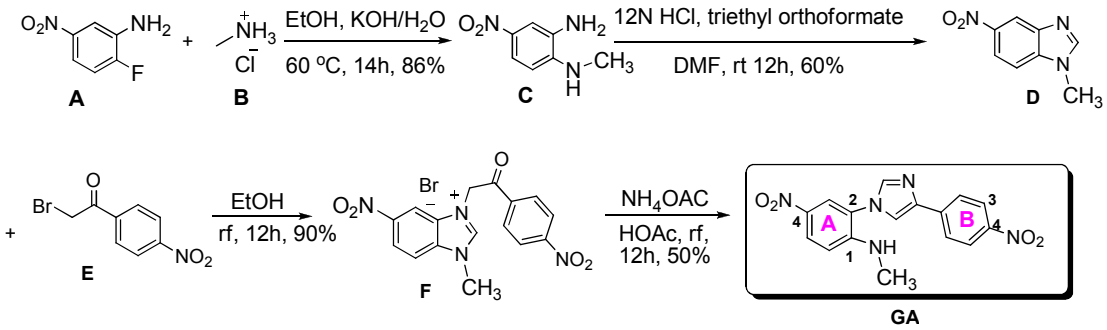
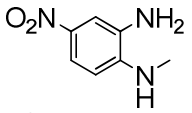
Compound	R_1	R_2	R_3	R_4	EC_{50} (nM) [*]
GA	NO_2	CH_3	NO_2	H	2510± 420
1	H	CH_3	NO_2	H	ND[†]
2	NO_2	CH_3	H	H	ND
3	NO_2	CH_2CH_3	NO_2	H	>100000[‡]
4	NO_2	*n*-Bu	NO_2	H	ND
5	NO_2	benzyl	NO_2	H	ND
6	NO_2	CH_3	F	H	2780± 810
7	NO_2	CH_3	CH_3	H	ND
8	NO_2	CH_3	OCH_3	H	ND
9	NO_2	CH_3	F	F	>100000[‡]
10	NO_2	CH_3	H	NO_2	ND
11	NO_2	CH_3	CN	H	210±30
12 (CU-T12-9)	NO_2	CH_3	CF_3	H	52.9 ± 6.2
13	NO_2	CH_3	Ph	H	84.7±1.2
14	NO_2	CH_3	cyclohexyl	H	120±20
15	NO_2	CH_3	-	-	420±50
16	NO_2	CH_3	*t*-Bu	H	150± 20
17	NO_2	CH_3	*n*-Bu	H	230± 30
18	NO_2	CH_3	$COOCH_3$	H	990± 190
19	NO_2	CH_3	COOH	H	ND
20	CF_3	CH_3	CF_3	H	240±40
21	NH_2	CH_3	CF_3	H	ND
22	$COOCH_3$	CH_3	CF_3	H	>100000[‡]
23(T12-29)	COOH	CH_3	CF_3	H	ND
24	CN	CH_3	NO_2	H	10960± 2120[‡]

[*] EC_{50} and corresponding SD values are determined from at least three independent repeats. [†] No activity was detected at the tested concentrations up to 100 μM. [‡] The highest SEAP activation observed is at least 50% lower than the highest activation of GA.

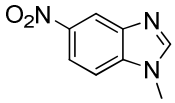
General Method I



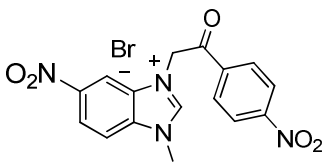
Supplementary Scheme 1: General synthesis of TLR1/2 agonist GA.



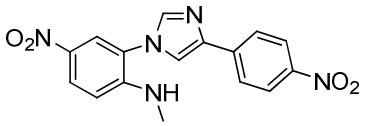
***N*^{1}-methyl-4-nitrobenzene-1,2-diamine** (**C**). Loosely following the precedent of Piersanti [52] 2-fluoro-5-nitroaniline (468 mg, 3.00 mmol) and methylamine hydrochloride (486 mg, 9.00 mmol) were dissolved in 15 mL EtOH and stirred at room temperature for 10 min. An aqueous solution of potassium hydroxide (1.009 g, 18.0 mmol) in 5 mL H_2O was introduced and the mixture was stirred at reflux at 60 ^oC overnight. Then poured into water (100 mL) and precipitate formed was extracted with ethyl acetate (3 × 30 mL). The organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel, using ethyl acetate/hexane (2:1) as eluent, and gave *N*^{1}-methyl-4-nitrobenzene-1,2-diamine as red solid (429 mg, 86 %); ^{1}H NMR (400 MHz, DMSO) δ 7.56 (dd, *J* = 8.8, 2.7 Hz, 1H), 7.41 (d, *J* = 2.7 Hz, 1H), 6.43 (d, *J* = 8.9 Hz, 1H), 6.12 (s 1H), 5.08 (s, 2H), 2.85 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 144.09, 136.99, 134.89, 116.46, 107.42, 106.94, 30.12; LRMS (ESI): calcd for: $C_7H_9N_3O_2$ $[M+H]^+$ = 168.2, obsd $[M+H]^+$ = 168.2.



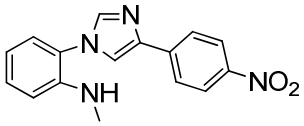
1-methyl-5-nitro-1*H*-benzo[*d*]imidazole (**D**). Hydrochloric acid (12 N solution, 167 µL) was added to a solution of **C** (251 mg, 1.50 mmol) in triethyl orthoformate (10 mL) and *N*,*N*-dimethylformamide (added with stirring until the turbidity disappeared) [47]. The mixture was stirred at room temperature for 16 h, under a nitrogen atmosphere. The solvent was evaporated under reduced pressure and the brown oily residue was purified by flash column chromatography on silica gel, using ethyl acetate/hexane (5:1) as eluent, and gave 1-methyl-5-nitro-1*H*-benzo[*d*]imidazole as light yellow solid (187 mg, 70 %): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.75 (dd, *J* = 2.1, 0.4 Hz, 1H), 8.30 (dd, *J* = 8.9, 2.1 Hz, 1H), 8.08 (s, 1H), 7.49 (dd, *J* = 8.9, 0.5 Hz, 1H), 3.96 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 149.23, 143.15, 142.88, 139.43, 118.32, 116.00, 111.41, 31.69; LRMS (ESI): calcd for: $C_8H_7N_3O_2$ $[M+H]^+$ = 178.2, obsd $[M+H]^+$ = 178.2.



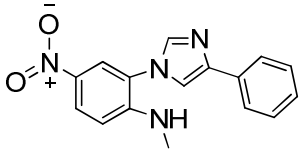
Compound **D** (177 mg, 1.00 mmol) and 2-bromo-1-(4-nitrophenyl)ethanone (**E**) (244 mg, 1.00 mmol) were dissolved in MeOH (10 mL) and was stirred at reflux under nitrogen for 12h (monitored by TLC) [53]. The solvent was concentrated under reduced pressure. The residue was washed by acetone (3 × 2 mL) to give the white solid product **F** in 92 % yield: ^{1}H NMR (400 MHz, DMSO) δ 9.91 (s, 1H), 9.36 – 9.24 (m, 1H), 8.59 (ddd, *J* = 9.2, 2.0, 0.9 Hz, 1H), 8.50 (d, *J* = 8.2 Hz, 2H), 8.41 – 8.31 (m, 3H), 6.57 (s, 2H), 4.28 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 190.69, 151.04, 148.26, 146.46, 138.90, 135.61, 132.08, 130.37, 124.52, 122.16, 115.69, 111.90, 54.73, 34.67; LRMS (ESI): calcd for: $C_{16}H_{13}BrN_4O_5$ $[M+H]^+$ = 422.2, obsd $[M+H]^+$ = 422.2.



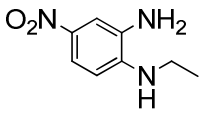
***N*-methyl-4-nitro-2-(4-(4-nitrophenyl)-1*H*-imidazol-1-yl)aniline** (**GA**). A solution of **F** (211 mg, 0.500 mmol) and 308 mg (4.00 mmol) of ammonium acetate in 2 ml of glacial acetic acid was refluxed for 12 h, after which the mixture was dropped into 20 ml of water. The resulting yellow precipitate is filtered off, washed with water (3 × 3 mL) and dried under high vacuum condition to give the product *N*-methyl-4-nitro-2-(4-(4-nitrophenyl)-1*H*-imidazol-1-yl)aniline (124 mg, 73 %); ^{1}H NMR (400 MHz, DMSO) δ 8.24 (dt, *J* = 8.9, 6.9 Hz, 4H), 8.11 (d, *J* = 8.7 Hz, 2H), 8.07 – 7.99 (m, 2H), 6.86 (d, *J* = 9.3 Hz, 1H), 6.77 (s, 1H), 2.82 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 150.69, 146.06, 141.31, 139.97, 139.92, 135.51, 127.25, 125.46, 124.63, 124.28, 121.37, 121.06, 110.50, 30.26; LRMS (ESI): calcd for: $C_{16}H_{13}N_5O_4$ $[M+H]^+$ = 340.1, obsd $[M+H]^+$ = 340.1. The compound can be further purified by flash silica gel column chromatography, using ethyl acetate/hexane (1:1) as eluent, if necessary.



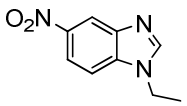
***N*-methyl-2-(4-(4-nitrophenyl)-1*H*-imidazol-1-yl)anilin**e (**1**). Following the general method I, using 1-methyl-1*H*-benzo[*d*]imidazole (132 mg, 1 mmol) instead of **D**, gave an final orange solid *N*-methyl-2-(4-(4-nitrophenyl)-1*H*-imidazol-1-yl)aniline with overall yield 60 %: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 – 8.20 (m, 2H), 7.99 – 7.90 (m, 2H), 7.72 (d, *J* = 1.2 Hz, 1H), 7.54 (d, *J* = 1.2 Hz, 1H), 7.43 – 7.37 (m, 1H), 7.16 (dd, *J* = 8.0, 1.6 Hz, 1H), 6.85 – 6.78 (m, 2H), 2.87 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 146.48, 144.36, 140.58, 140.09, 139.04, 130.72, 126.92, 125.08, 124.24, 122.33, 118.61, 116.70, 111.25, 30.22; HRMS (ESI): calcd for: $C_{16}H_{14}N_4O_2$ $[M+H]^+$ = 295.1190, obsd $[M+H]^+$ = 295.1188.



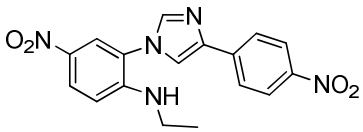
***N*-methyl-4-nitro-2-(4-phenyl-1*H*-imidazol-1-yl)aniline** (**2**). Following the general method I, using 2-bromo-1-phenylethanone (199 mg, 1 mmol) instead of **E**, gave an final orange solid *N*-methyl-4-nitro-2-(4-phenyl-1*H*-imidazol-1-yl)aniline with overall yield 62 %: ^{1}H NMR (400 MHz, DMSO) δ 8.27 – 8.17 (m, 1H), 8.03 – 7.97 (m, 1H), 7.94 – 7.79 (m, 4H), 7.39 (dd, *J* = 9.3, 4.6 Hz, 2H), 7.28 – 7.18 (m, 1H), 6.87 (d, *J* = 9.0 Hz, 1H), 6.76 – 6.60 (m, 1H), 2.82 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 150.71, 141.92, 138.84, 135.55, 134.59, 128.95, 127.04, 127.01, 124.95, 124.07, 121.77, 117.54, 110.41, 30.29; HRMS (ESI): calcd for: $C_{16}H_{14}N_4O_2$ $[M+H]^+$ = 295.1190, obsd $[M+H]^+$ = 295.1197.



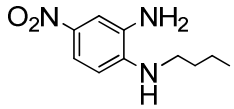
***N*^{1}-ethyl-4-nitrobenzene-1,2-diamine**. Following the general method I, using ethylamine (597 μL, 9 mmol) instead of methylamine hydrochloride, gave an red solid *N*^{1}-ethyl-4-nitrobenzene-1,2-diamine (451 mg, 83 %): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 – 7.79 (m, 1H), 7.70 – 7.58 (m, 1H), 6.57 (t, *J* = 9.6 Hz, 1H), 4.27 (s, 2H), 3.33 – 3.21 (m, 2H), 1.27 (t, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 144.96, 138.13, 131.79, 119.39, 112.44, 108.15, 38.23, 14.51.



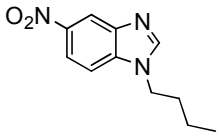
1-ethyl-5-nitro-1*H*-benzo[*d*]imidazole. Following the general method I, using *N*^{1}-ethyl-4-nitrobenzene-1,2-diamine (271 mg, 1.5 mmol) instead of **C**, gave an white solid 1-ethyl-5-nitro-1*H*-benzo[*d*]imidazole (218 mg, 76 %): ^{1}H NMR (400 MHz, DMSO) δ 8.59 (s, 1H), 8.55 (d, *J* = 2.1 Hz, 1H), 8.21 – 8.13 (m, 1H), 7.87 (d, *J* = 9.0 Hz, 1H), 4.37 (m, 2H), 1.50 – 1.36 (m, 3H); ^{13}C NMR (101 MHz, DMSO) δ 148.23, 143.15, 143.08, 138.50, 118.29, 116.13, 111.49, 40.21, 15.58.



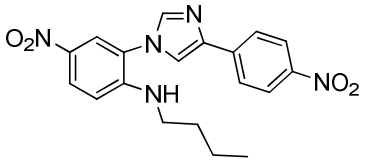
***N*-ethyl-4-nitro-2-(4-(4-nitrophenyl)-1*H*-imidazol-1-yl)aniline** (**3**). Following the general method I, using 1-ethyl-5-nitro-1*H*-benzo[*d*]imidazole (191 mg, 1 mmol) instead of **D**, gave a white solid under reduced pressure. The intermediate product was washed by acetone (3 × 2 mL) and followed by the reflux in 4 mL of glacial acetic acid with 612 mg ammonium acetate (4 mmol) for 12 h. Then the mixture was dropped into 20 ml of water. The resulting yellow precipitate is filtered off, washed with water (3 × 3 mL) and dried under high vacuum condition to give the product *N*-ethyl-4-nitro-2-(4-(4-nitrophenyl)-1*H*-imidazol-1-yl)aniline (198 mg, 56 %): ^{1}H NMR (400 MHz, DMSO) δ 8.28 (d, *J* = 8.9 Hz, 2H), 8.25 – 8.17 (m, 2H), 8.11 (d, *J* = 8.9 Hz, 2H), 8.03 (dd, *J* = 3.4, 1.8 Hz, 2H), 6.96 (d, *J* = 9.4 Hz, 1H), 6.81 – 6.70 (m, 1H), 3.33 – 3.23 (m, 2H), 1.13 (t, 3H); ^{13}C NMR (101 MHz, DMSO) δ 149.75, 146.08, 141.31, 139.97, 139.93, 135.43, 127.13, 125.48, 124.61, 124.53, 121.42, 120.99, 110.71, 37.72, 14.18; HRMS (ESI): calcd for: $C_{17}H_{15}N_5O_4$ $[M+H]^+$ = 354.1197, obsd $[M+H]^+$ = 354.1194.



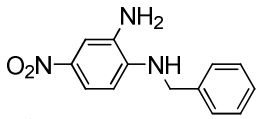
***N*^{1}-butyl-4-nitrobenzene-1,2-diamine**. Following the *N*^{1}-ethyl-4-nitrobenzene-1,2-diamine synthesis method, using butylamine (889 μL, 9 mmol) instead of ethylamine, gave an red solid *N*^{1}-butyl-4-nitrobenzene-1,2-diamine (546 mg, 87 %): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 – 7.80 (m, 1H), 7.64 (d, *J* = 2.5 Hz, 1H), 6.55 (d, *J* = 8.9 Hz, 1H), 4.34 (s, 1H), 3.37 (s, 2H), 3.24 (m, 2H), 1.70 (m, 2H), 1.48 (m, 2H), 1.00 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 145.17, 137.90, 131.75, 119.49, 112.49, 108.07, 43.34, 31.26, 20.27, 13.84.



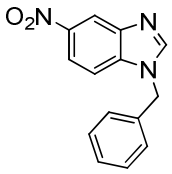
1-butyl-5-nitro-1*H*-benzo[*d*]imidazole. Following the general method I, using *N*^{1}-butyl-4-nitrobenzene-1,2-diamine (329 mg, 1.5 mmol) instead of **C**, gave an white solid 1-butyl-5-nitro-1*H*-benzo[*d*]imidazole (266 mg, 81 %): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.75 – 8.70 (m, 1H), 8.25 (dd, *J* = 8.9, 2.2 Hz, 1H), 8.09 (s, 1H), 7.48 (m, 1H), 4.25 (t, 2H), 2.00 – 1.82 (m, 2H), 1.50 – 1.27 (m, 2H), 0.99 (t, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 146.32, 143.65, 143.12, 137.95, 118.68, 117.16, 109.68, 45.37, 31.85, 19.98, 13.49.



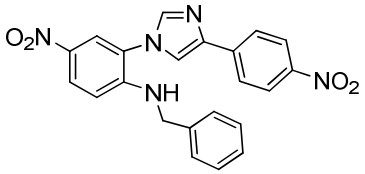
***N*-butyl-4-nitro-2-(4-(4-nitrophenyl)-1*H*-imidazol-1-yl)aniline** (**4**). Following the general synthesis method of **3**, using 1-butyl-5-nitro-1*H*-benzo[*d*]imidazole (218 mg, 1 mmol) instead of 1-ethyl-5-nitro-1*H*-benzo[*d*]imidazole, gave an white solid *N*-butyl-4-nitro-2-(4-(4-nitrophenyl)-1*H*-imidazol-1-yl)aniline (252 mg, 66 %): ^{1}H NMR (400 MHz, DMSO) δ 8.28 (d, *J* = 9.0 Hz, 2H), 8.19 (dd, *J* = 9.8, 1.9 Hz, 2H), 8.11 (d, *J* = 9.0 Hz, 2H), 8.02 (dd, *J* = 6.9, 1.9 Hz, 2H), 6.95 (d, *J* = 9.4 Hz, 1H), 6.75 (dd, *J* = 7.6, 4.2 Hz, 1H), 3.23 (m, 2H), 1.52 (m, 2H), 1.41 – 1.26 (m, 2H), 0.89 (t, 3H); ^{13}C NMR (101 MHz, DMSO) δ 149.92, 146.05, 141.32, 139.97, 139.91, 135.30, 127.13, 125.44, 124.63, 124.59, 121.35, 121.04, 110.73, 42.63, 30.54, 20.01, 14.19; HRMS (ESI): calcd for: $C_{19}H_{19}N_5O_4$ $[M+H]^+$ = 382.1510, obsd $[M+H]^+$ = 382.1508.



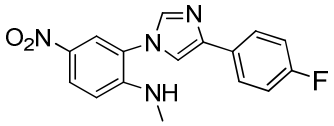
***N*^{1}-benzyl-4-nitrobenzene-1,2-diamine**. Following the general method I, using benzylamine (655 μL, 9 mmol) instead of methylamine hydrochloride, gave an red solid *N*^{1}-benzyl-4-nitrobenzene-1,2-diamine (613 mg, 84 %): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (ddd, *J* = 8.9, 2.5, 0.5 Hz, 1H), 7.67 (d, *J* = 2.5 Hz, 1H), 7.44 – 7.32 (m, 5H), 6.60 (d, *J* = 8.9 Hz, 1H), 4.66 (s, 1H), 4.46 (d, 2H), 3.41 (s, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 144.48, 137.59, 132.14, 128.91, 127.83, 127.54, 126.99, 119.16, 112.53, 108.87, 47.96.



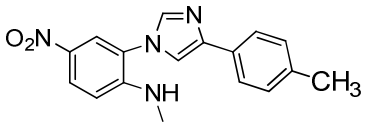
1-benzyl-5-nitro-1*H*-benzo[*d*]imidazole. Following the general method I, using *N*^{1}-benzyl-4-nitrobenzene-1,2-diamine (365 mg, 1.5 mmol) instead of **C**, gave an white solid 1-benzyl-5-nitro-1*H*-benzo[*d*]imidazole (312 mg, 82 %):^{1}H NMR (400 MHz, DMSO) δ 8.75 (s, 1H), 8.57 (d, *J* = 2.1 Hz, 1H), 8.16 (dd, *J* = 9.0, 2.2 Hz, 1H), 7.78 (d, *J* = 9.0 Hz, 1H), 7.43 – 7.23 (m, 5H), 5.62 (s, 2H); ^{13}C NMR (101 MHz, DMSO) δ 148.83, 143.35, 143.20, 138.53, 136.71, 129.29, 128.46, 127.91, 118.59, 116.25, 111.85, 48.52.



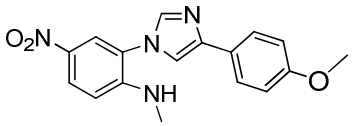
***N*-benzyl-4-nitro-2-(4-(4-nitrophenyl)-1*H*-imidazol-1-yl)aniline** (**5**). Following the general synthesis method of **3**, using 1-benzyl-5-nitro-1*H*-benzo[*d*]imidazole (253 mg, 1 mmol) instead of 1-ethyl-5-nitro-1*H*-benzo[*d*]imidazole, gave an white solid *N*-benzyl-4-nitro-2-(4-(4-nitrophenyl)-1*H*-imidazol-1-yl)aniline (261mg, 63 %): ^{1}H NMR (400 MHz, DMSO) δ 8.37 – 8.24 (m, 3H), 8.20 – 8.04 (m, 5H), 7.45 – 7.30 (m, 5H), 7.26 (ddd, J = 6.2, 5.0, 2.4 Hz, 1H), 6.75 (d, J = 9.3 Hz, 1H), 4.50 (s, 2H); ^{13}C NMR (101 MHz, DMSO) δ 149.80, 146.10, 141.34, 140.07, 140.02, 138.69, 135.94, 128.97, 127.45, 127.23, 126.95, 125.51, 124.65, 124.55, 121.73, 121.12, 111.38, 46.16; HRMS (ESI): calcd for: $C_{22}H_{17}N_5O_4$ $[M+H]^+$ = 416.1354, obsd $[M+H]^+$ = 416.1349.



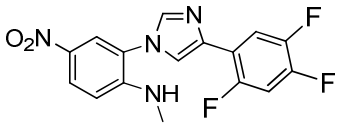
2-(4-(4-fluorophenyl)-1*H*-imidazol-1-yl)-*N*-methyl-4-nitroaniline (**6**). Following the general method I, using 2-bromo-1-(4-fluorophenyl)ethanone (191 mg, 1 mmol) instead of **E**, gave a white solid under reduced pressure. The intermediate product was washed by acetone (3 × 2 mL) and followed by the reflux in 4 mL of glacial acetic acid with 612 mg ammonium acetate (4 mmol) for 12 h. Then the mixture was dropped into 20 ml of water. The resulting yellow precipitate is filtered off, washed with water (3 × 3 mL) and dried under high vacuum condition to give the product 2-(4-(4-fluorophenyl)-1*H*-imidazol-1-yl)-*N*-methyl-4-nitroaniline (172 mg, 55 %):^{1}H NMR (400 MHz, DMSO) δ 8.34 – 8.12 (m, 1H), 8.10 – 7.57 (m, 5H), 7.37 – 7.08 (m, 2H), 6.94 – 6.79 (m, 1H), 6.76 – 6.58 (m, 1H), 2.81 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 162.78, 160.36, 150.70, 141.06, 138.89, 135.55, 131.20, 127.03, 126.81, 124.07, 121.71, 117.37, 115.89, 115.68, 110.40, 31.13; HRMS (ESI): calcd for: $C_{16}H_{13}FN_4O_2$ $[M+H]^+$ = 313.1096, obsd $[M+H]^+$ = 313.1101.



***N*-methyl-4-nitro-2-(4-*p*-tolyl-1*H*-imidazol-1-yl)aniline** (**7**). Following the general synthesis method of **6**, using 2-bromo-1-*p*-tolylethanone (213 mg, 1 mmol) instead of **E**, gave a white solid *N*-methyl-4-nitro-2-(4-*p*-tolyl-1*H*-imidazol-1-yl)aniline (188 mg, 61 %):^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (dd, *J* = 9.2, 2.6 Hz, 1H), 8.09 (t, *J* = 2.7 Hz, 1H), 7.61 (dd, *J* = 5.5, 2.6 Hz, 3H), 7.28 – 7.11 (m, 3H), 6.76 (dd, *J* = 9.2, 2.9 Hz, 1H), 5.27 (s, 1H), 3.00 (s, 3H), 2.39 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 149.81, 143.70, 137.51, 137.15, 137.01, 130.32, 129.34, 126.95, 124.83, 123.76, 121.48, 115.05, 109.42, 29.97, 21.23; HRMS (ESI): calcd for: $C_{17}H_{16}N_4O_2$ $[M+H]^+$ = 309.1347, obsd $[M+H]^+$ = 309.1347.

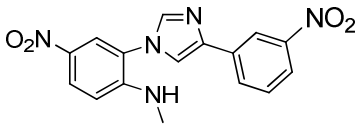


2-(4-(4-methoxyphenyl)-1*H*-imidazol-1-yl)-*N*-methyl-4-nitroaniline (**8**). Following the general synthesis method of **6**, using 2-bromo-1-(4-methoxyphenyl)ethanone (229 mg, 1 mmol) instead of **E**, gave a white solid 2-(4-(4-methoxyphenyl)-1*H*-imidazol-1-yl)-*N*-methyl-4-nitroaniline (221 mg, 68 %): ^{1}H NMR (400 MHz, DMSO) δ 8.21 (ddd, *J* = 5.3, 4.6, 1.7 Hz, 1H), 7.99 (dd, *J* = 3.8, 2.7 Hz, 1H), 7.91 – 7.58 (m, 4H), 7.08 – 6.90 (m, 2H), 6.89 – 6.77 (m, 1H), 6.68 (dd, *J* = 5.1, 2.0 Hz, 1H), 3.78 (s, 3H), 2.82 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 158.62, 150.70, 141.91, 138.55, 135.55, 127.33, 126.93, 126.24, 123.99, 121.86, 116.20, 114.38, 110.36, 55.51, 30.28; HRMS (ESI): calcd for: $C_{17}H_{16}N_4O_3$ $[M+H]^+$ = 325.1296, obsd $[M+H]^+$ = 325.1299.

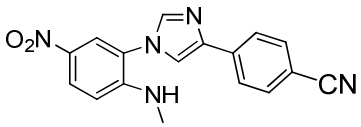


***N*-methyl-4-nitro-2-(4-(2,4,5-trifluorophenyl)-1*H*-imidazol-1-yl)aniline** (**9**). Following the general synthesis method of **6**, using 2-bromo-1-(2,4,5-trifluorophenyl)ethanone (253 mg, 1 mmol) instead of **E**, gave a white solid *N*-methyl-4-nitro-2-(4-(2,4,5-trifluorophenyl)-1*H*-imidazol-1-yl)aniline (188 mg, 54 %): ^{1}H NMR (400 MHz, DMSO) δ 8.22 (dd, *J* = 9.3, 2.4 Hz, 1H), 8.13 – 7.91 (m, 3H), 7.73 (d, *J* = 3.3 Hz, 1H), 7.70 – 7.58 (m, 1H), 6.95 – 6.78 (m, 1H), 6.70 (d, *J* = 4.8 Hz, 1H), 2.82 (s, 3H); 13C NMR (101 MHz, DMSO) δ 150.84, 139.12, 135.50, 133.77, 127.21, 124.44, 121.31, 121.18, 121.04, 114.69,

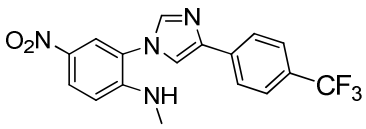
114.48, 110.46, 107.21, 106.93, 106.72, 30.23; HRMS (ESI): calcd for: $C_{16}H_{11}FN_4O_2$ $[M+H]^+$ = 349.0907, obsd $[M+H]^+$ = 349.0909.



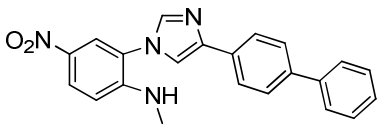
***N*-methyl-4-nitro-2-(4-(3-nitrophenyl)-1*H*-imidazol-1-yl)aniline** (**10**). Following the general synthesis method of **6**, using 2-bromo-1-(3-nitrophenyl)ethanone (244 mg, 1 mmol) instead of **E**, gave a white solid *N*-methyl-4-nitro-2-(4-(3-nitrophenyl)-1*H*-imidazol-1-yl)aniline (214 mg, 63 %): ^{1}H NMR (400 MHz, DMSO) δ 8.74 – 8.61 (m, 1H), 8.32 – 8.21 (m, 2H), 8.19 (d, *J* = 1.2 Hz, 1H), 8.10 (ddd, *J* = 8.2, 2.3, 0.9 Hz, 1H), 8.05 (d, *J* = 2.7 Hz, 1H), 8.01 (d, *J* = 1.2 Hz, 1H), 7.71 (t, *J* = 8.0 Hz, 1H), 6.88 (d, *J* = 9.4 Hz, 1H), 6.74 (d, *J* = 4.7 Hz, 1H), 2.82 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 150.66, 148.82, 139.75, 139.50, 136.48, 135.53, 131.13, 130.64, 127.17, 124.18, 121.56, 121.48, 119.54, 119.00, 110.49, 30.28; HRMS (ESI): calcd for: $C_{16}H_{13}N_5O_4$ $[M+H]^+$ = 340.1041, obsd $[M+H]^+$ = 340.1042.



4-(1-(2-(methylamino)-5-nitrophenyl)-1*H*-imidazol-4-yl)benzonitrile (**11**). Following the general synthesis method of **6**, using 4-(2-bromoacetyl)benzonitrile (224 mg, 1 mmol) instead of **E**, gave a white solid 4-(1-(2-(methylamino)-5-nitrophenyl)-1*H*-imidazol-4-yl)benzonitrile (211 mg, 66 %): ^{1}H NMR (400 MHz, DMSO) δ 8.23 (dd, *J* = 9.2, 2.3 Hz, 1H), 8.15 (s, 1H), 8.03 (dd, *J* = 13.1, 8.0 Hz, 4H), 7.86 (d, *J* = 8.2 Hz, 2H), 6.87 (d, *J* = 9.3 Hz, 1H), 6.74 (d, *J* = 4.6 Hz, 1H), 2.81 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 150.69, 140.26, 139.67, 139.20, 135.55, 133.09, 127.19, 125.39, 124.23, 121.44, 120.26, 119.65, 110.48, 109.04, 30.26; HRMS (ESI): calcd for: $C_{17}H_{13}N_5O_2$ $[M+H]^+$ = 320.1142, obsd $[M+H]^+$ = 320.1147.

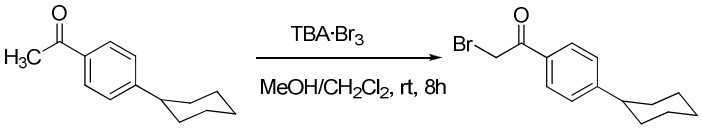


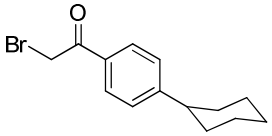
***N*-methyl-4-nitro-2-(4-(4-(trifluoromethyl)phenyl)-1*H*-imidazol-1-yl)aniline** (**12**). Following the general synthesis method of **6**, using 2-bromo-1-(4-(trifluoromethyl)phenyl)ethanone (267 mg, 1 mmol) instead of **E**, gave a light yellow solid *N*-methyl-4-nitro-2-(4-(4-(trifluoromethyl)phenyl)-1*H*-imidazol-1-yl)aniline (257 mg, 71 %): ^{1}H NMR (400 MHz, DMSO) δ 8.24 (dd, *J* = 9.3, 2.3 Hz, 1H), 8.18 – 8.02 (m, 4H), 7.98 (s, 1H), 7.76 (d, *J* = 8.2 Hz, 2H), 6.87 (d, *J* = 9.2 Hz, 1H), 6.74 (s, 1H), 2.82 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 150.70, 140.46, 139.45, 138.63, 135.56, 127.14, 125.98, 125.94, 125.30, 124.19, 121.54, 119.48, 110.46, 30.26; HRMS (ESI): calcd for: $C_{17}H_{13}F_3N_4O_2$ $[M+H]^+$ = 363.1064, obsd $[M+H]^+$ = 363.1062.



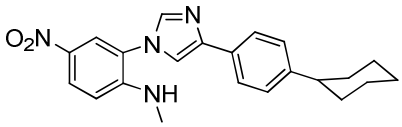
2-(4-(biphenyl-4-yl)-1*H*-imidazol-1-yl)-*N*-methyl-4-nitroaniline (**13**). Following the general synthesis method of **6**, using 1-(biphenyl-4-yl)-2-bromoethanone (275 mg, 1 mmol) instead of **E**, gave a light yellow solid 2-(4-(biphenyl-4-yl)-1*H*-imidazol-1-yl)-*N*-methyl-4-nitroaniline (300 mg, 81 %): ^{1}H NMR (400 MHz, DMSO) δ 8.24 (dd, *J* = 9.2, 2.5 Hz, 1H), 8.03 (d, *J* = 2.7 Hz, 1H), 7.95 (ddd, *J* = 6.4, 4.1, 1.6 Hz, 4H), 7.72 (dd, *J* = 4.9, 3.7 Hz, 4H), 7.54 – 7.43 (m, 2H), 7.40 – 7.33 (m, 1H), 6.88 (d, *J* = 9.4 Hz, 1H), 6.78 – 6.68 (m, 1H), 2.84 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 150.70, 141.58, 140.27, 138.98,

138.56, 135.57, 133.80, 129.38, 127.73, 127.17, 127.02, 126.96, 126.80, 125.51, 124.07, 121.76, 117.79, 110.43, 30.29; HRMS (ESI): calcd for: $C_{22}H_{18}N_4O_2$ $[M+H]^+$ = 371.1503, obsd $[M+H]^+$ = 371.1501.

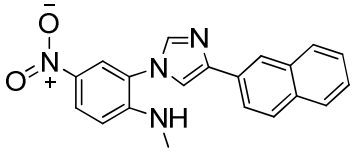




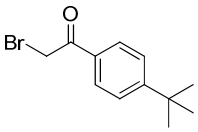
2-bromo-1-(4-cyclohexylphenyl)ethanone. 2-bromo-1-(4-cyclohexylphenyl)ethanone was synthesized according to literature procedure [54]. Briefly, 1-(4-cyclohexylphenyl)ethanone (1.213 g, 6.0 mmol), CH_2Cl_2 (30 mL), CH_3OH (12 mL), and tetrabutylammonium tribromide ($TBA{\cdot}Br_3$) (2.893 g, 6.0 mmol) were added to a 100-mL flask. The mixture was stirred at room temperature for 2-12 h until the red color disappeared. Then the solvent was removed under vacuum and the residue was diluted with water (20 mL) and extracted with hexane (3 × 20 mL). The organic layer was then dried over Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel, using ethyl acetate/hexane (1:20) as eluent, and gave 2-bromo-1-(4-cyclohexylphenyl)ethanone as a clean oil (1.130 g, 67 %): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (d, J = 8.4 Hz, 2H), 7.38 – 7.31 (m, 2H), 4.46 (s, 2H), 2.60 (m, 1H), 1.98 – 1.84 (m, 4H), 1.83 – 1.73 (m, 1H), 1.53 – 1.36 (m, 4H), 1.36 – 1.19 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.91, 154.81, 131.81, 129.16, 127.36, 44.77, 34.03, 30.91, 26.68, 26.00.



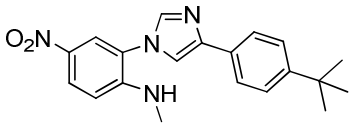
2-(4-(4-cyclohexylphenyl)-1H-imidazol-1-yl)-N-methyl-4-nitroaniline (**14**). Following the general synthesis method of **6**, using 2-bromo-1-(4-cyclohexylphenyl)ethanone (281 mg, 1 mmol) instead of **E**, gave a light yellow solid 2-(4-(4-cyclohexylphenyl)-1H-imidazol-1-yl)-N-methyl-4-nitroaniline (290 mg, 77 %): ^{1}H NMR (400 MHz, DMSO) δ 8.22 (dd, *J* = 9.3, 2.6 Hz, 1H), 7.99 (d, *J* = 2.7 Hz, 1H), 7.88 (d, *J* = 1.3 Hz, 1H), 7.81 (d, *J* = 1.3 Hz, 1H), 7.79 – 7.72 (m, 2H), 7.24 (d, *J* = 8.2 Hz, 2H), 6.86 (d, *J* = 9.4 Hz, 1H), 6.67 (d, *J* = 4.9 Hz, 1H), 3.34 (s, 1H), 2.82 (s, 3H), 1.81 (m, 4H), 1.72 (m, 1H), 1.51 – 1.31 (m, 4H), 1.31 – 1.16 (m, 1H); ^{13}C NMR (101 MHz, DMSO) δ 150.66, 146.40, 142.08, 138.66, 135.56, 132.25, 127.18, 126.94, 125.01, 123.96, 121.83, 116.95, 110.41, 43.96, 34.44, 30.29, 26.85, 26.10; HRMS (ESI): calcd for: $C_{22}H_{24}N_4O_2$ $[M+H]^+$ = 377.1973, obsd $[M+H]^+$ = 377.1975.



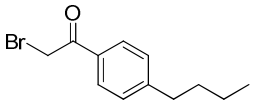
***N*-methyl-2-(4-(naphthalen-2-yl)-1*H*-imidazol-1-yl)-4-nitroaniline** (**15**). Following the general synthesis method of **6**, using 2-bromo-1-(naphthalen-2-yl)ethanone (249 mg, 1 mmol) instead of **E**, gave a light yellow solid *N*-methyl-2-(4-(naphthalen-2-yl)-1*H*-imidazol-1-yl)-4-nitroaniline (296 mg, 86 %): ^{1}H NMR (400 MHz, DMSO) δ 8.41 (s, 1H), 8.30 – 8.16 (m, 1H), 8.07 – 8.00 (m, 3H), 7.99 – 7.86 (m, 4H), 7.56 – 7.40 (m, 2H), 6.88 (d, J = 9.3 Hz, 1H), 6.75 (dd, J = 3.4, 1.2 Hz, 1H), 2.83 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 150.72, 141.89, 139.12, 135.55, 133.82, 132.57, 132.14, 128.45, 128.39, 128.30, 128.15, 128.08, 127.05, 126.74, 125.87, 124.16, 124.12, 122.64, 121.75, 118.23, 110.42, 30.30; HRMS (ESI): calcd for: $C_{20}H_{16}N_4O_2$ $[M+H]^+$ = 345.1347, obsd $[M+H]^+$ = 345.1350.



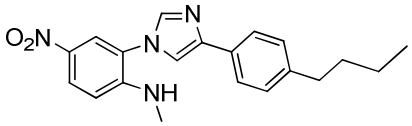
2-bromo-1-(4-*tert*-butylphenyl)ethanone. Following the general synthesis method of 2-bromo-1-(4-cyclohexylphenyl)ethanone, gave a colorless oil 2-bromo-1-(4-*tert*-butylphenyl)ethanone (1.026 g, 67 %): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 – 7.91 (m, 2H), 7.58 – 7.48 (m, 2H), 4.46 (s, 2H), 1.37 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.90, 157.90, 131.40, 128.92, 125.83, 35.25, 31.02, 30.88.



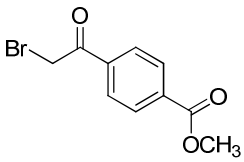
2-(4-(4-*tert*-butylphenyl)-1*H*-imidazol-1-yl)-*N*-methyl-4-nitroaniline (**16**). Following the general synthesis method of **6**, using 2-bromo-1-(4-*tert*-butylphenyl)ethanone (350 mg, 1 mmol) instead of **E**, gave a light yellow solid 2-(4-(4-*tert*-butylphenyl)-1*H*-imidazol-1-yl)-*N*-methyl-4-nitroaniline (242 mg, 69 %): ^{1}H NMR (400 MHz, DMSO) δ 8.22 (dd, *J* = 9.3, 2.7 Hz, 1H), 8.00 (d, *J* = 2.7 Hz, 1H), 7.89 (d, *J* = 1.3 Hz, 1H), 7.82 (d, *J* = 1.3 Hz, 1H), 7.80 – 7.74 (m, 2H), 7.50 – 7.31 (m, 2H), 6.87 (d, *J* = 9.4 Hz, 1H), 6.74 – 6.62 (m, 1H), 2.82 (s, 3H), 1.31 (s, 9H); ^{13}C NMR (101 MHz, DMSO) δ 150.66, 149.43, 142.00, 138.68, 135.57, 131.83, 126.94, 125.63, 124.78, 123.98, 121.83, 117.02, 110.42, 34.71, 31.63, 30.30; HRMS (ESI): calcd for: $C_{20}H_{22}N_4O_2$ $[M+H]^+$ = 351.1816, obsd $[M+H]^+$ = 351.1813.



2-bromo-1-(4-butylphenyl)ethanone. Following the general synthesis method of 2-bromo-1-(4-cyclohexylphenyl)ethanone, gave a colorless oil 2-bromo-1-(4-butylphenyl)ethanone (964 mg, 63 %): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 – 7.90 (m, 2H), 7.32 (dd, *J* = 8.0, 0.5 Hz, 2H), 4.46 (s, 2H), 2.75 – 2.65 (m, 2H), 1.64 (m, 2H), 1.45 – 1.31 (m, 2H), 0.96 (t, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.92, 149.90, 131.68, 129.07, 128.90, 35.76, 33.12, 30.91, 22.31, 13.87.

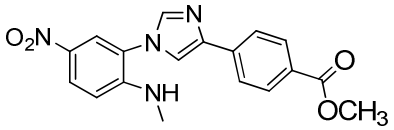


2-(4-(4-butylphenyl)-1*H*-imidazol-1-yl)-N-methyl-4-nitroaniline (**17**). Following the general synthesis method of **6**, using 2-bromo-1-(4-butylphenyl)ethanone (255 mg, 1 mmol) instead of **E**, gave a light yellow solid 2-(4-(4-butylphenyl)-1*H*-imidazol-1-yl)-N-methyl-4-nitroaniline (256 mg, 73 %): ^{1}H NMR (400 MHz, DMSO) δ 8.22 (dd, *J* = 9.3, 2.6 Hz, 1H), 7.99 (d, *J* = 2.7 Hz, 1H), 7.88 (d, *J* = 1.3 Hz, 1H), 7.82 (d, *J* = 1.3 Hz, 1H), 7.76 (d, *J* = 8.2 Hz, 2H), 7.21 (d, *J* = 8.3 Hz, 2H), 6.86 (d, *J* = 9.4 Hz, 1H), 6.68 (dt, *J* = 9.3, 2.4 Hz, 1H), 2.82 (s, 3H), 2.64 – 2.54 (m, 2H), 1.65 – 1.49 (m, 2H), 1.32 (m, 2H), 0.91 (t, 3H); ^{13}C NMR (101 MHz, DMSO) δ 150.69, 142.06, 141.09, 138.67, 135.56, 132.09, 128.85, 126.96, 124.95, 124.00, 121.82, 116.96, 110.40, 35.03, 33.56, 30.29, 22.19, 14.26; HRMS (ESI): calcd for: $C_{20}H_{22}N_4O_2$ $[M+H]^+$ = 351.1816, obsd $[M+H]^+$ = 351.1813.

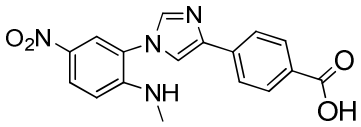


2-bromo-1-(4-butylphenyl)ethanone. Following the general synthesis method of 2-bromo-1-(4-cyclohexylphenyl)ethanone, gave a colorless oil 2-bromo-1-(4-butylphenyl)ethanone (940 mg, 61 %):

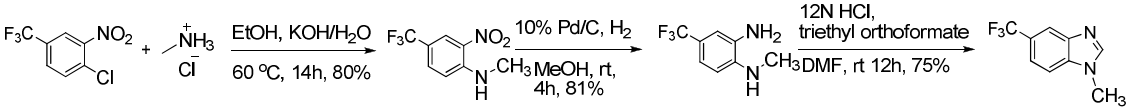
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 – 8.13 (m, 2H), 8.08 – 8.01 (m, 2H), 4.49 (s, 2H), 3.97 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.79, 165.91, 137.15, 134.61, 129.99, 128.84, 52.54, 30.66.

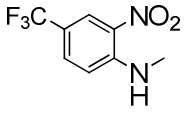


4-(1-(2-(methylamino)-5-nitrophenyl)-1*H*-imidazol-4-yl)benzoate (**18**). Following the general synthesis method of **6**, using 2-bromo-1-(4-butylphenyl)ethanone (257 mg, 1 mmol) instead of **E**, gave a light yellow solid methyl 4-(1-(2-(methylamino)-5-nitrophenyl)-1*H*-imidazol-4-yl)benzoate (250 mg, 71 %): ^{1}H NMR (400 MHz, DMSO) δ 8.26 – 8.20 (m, 1H), 8.10 – 8.07 (m, 1H), 8.04 (dd, *J* = 5.0, 2.1 Hz, 1H), 8.02 – 7.94 (m, 5H), 6.91 – 6.82 (m, 1H), 6.77 – 6.68 (m, 1H), 3.86 (s, 3H), 2.81 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 166.59, 150.71, 140.82, 139.46, 139.28, 135.54, 130.08, 127.83, 127.14, 124.90, 124.18, 121.54, 119.60, 110.46, 52.44, 30.27; HRMS (ESI): calcd for: $C_{18}H_{16}N_4O_4$ $[M+H]^+$ = 353.1245, obsd $[M+H]^+$ = 353.1251.

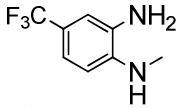


4-(1-(2-(methylamino)-5-nitrophenyl)-1*H*-imidazol-4-yl)benzoic acid (**19**). To a mixture of **18** (169 mg, 0.5 mmol) and LiOH (11.5 mg, 1.5 mmol) in THF (3 mL) was added MeOH (0.3 mL) and H_2O (1 mL), stirred at room temperature for 8 h [24]. Then HCl (1 M) was added to the reaction mixture to pH = 4, and extracted with EtOAc (3 × 15 mL), wash with H_2O (3 × 20 mL). The organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. Purified by flash chromatography on silica gel, using ethyl acetate/hexane (4:1) as eluent, give 4-(1-(2-(methylamino)-5-nitrophenyl)-1*H*-imidazol-4-yl)benzoic acid as white powder (137 mg, 81 %): ^{1}H NMR (400 MHz, DMSO) δ 12.78 (s, 1H), 8.24 (dd, *J* = 9.3, 2.7 Hz, 1H), 8.05 (dd, *J* = 10.4, 2.0 Hz, 2H), 7.97 (t, *J* = 1.1 Hz, 5H), 6.87 (d, *J* = 9.4 Hz, 1H), 6.73 (d, *J* = 4.8 Hz, 1H), 2.82 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 167.75, 150.72, 140.98, 139.38, 138.77, 135.51, 130.21, 127.14, 124.72, 124.19, 121.57, 119.34, 110.45, 99.98, 30.27; HRMS (ESI): calcd for: $C_{17}H_{14}N_4O_4$ $[M+H]^+$ = 339.1087, obsd $[M+H]^+$ = 339.1089.



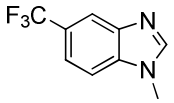


***N*-methyl-2-nitro-4-(trifluoromethyl)aniline**. Following the general method I, using 1-chloro-2-nitro-4-(trifluoromethyl)benzene (677 mg, 3 mmol) instead of **A**, gave an yellow solid *N*-methyl-2-nitro-4-(trifluoromethyl)aniline (528 mg, 80 %): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49 (dd, *J* = 2.0, 0.8 Hz, 1H), 8.29 (s, 1H), 7.67 (dd, *J* = 9.0, 2.2 Hz, 1H), 6.96 (d, *J* = 9.0 Hz, 1H), 3.11 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 147.68, 132.18, 132.14, 124.87, 124.83, 114.05, 29.86.

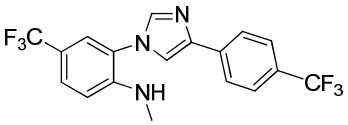


***N*^{1}-methyl-4-(trifluoromethyl)benzene-1,2-diamine**. After three vacuum/H_2 cycles to remove air from the reaction tube, the stirred mixture of the *N*-methyl-2-nitro-4-(trifluoromethyl)aniline (440 mg, 2 mmol) and 10 % Pd/C catalyst (44 mg) in MeOH (4 mL) was hydrogenated under ambient pressure

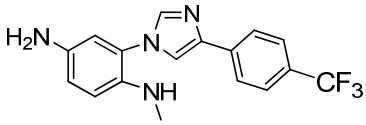
(balloon) at room temperature for 4h. The reaction mixture was filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel, using ethyl acetate as eluent, and gave N^1-methyl-4-(trifluoromethyl)benzene-1,2-diamine as red solid (308 mg, 81 %); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.15 (ddd, *J* = 8.3, 2.0, 0.9 Hz, 1H), 6.98 – 6.89 (m, 1H), 6.66 (d, *J* = 8.2 Hz, 1H), 3.81 – 3.10 (m, 3H), 2.92 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 141.97, 133.24, 126.29, 123.60, 118.42, 112.98, 109.42, 30.60.



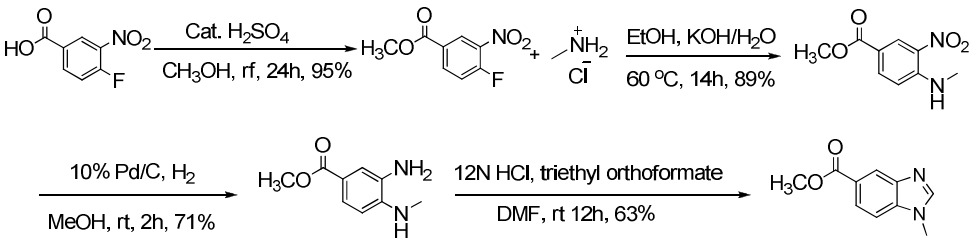
1-methyl-5-(trifluoromethyl)-1*H*-benzo[*d*]imidazole. Following the general method I, using N^1-methyl-4-(trifluoromethyl)benzene-1,2-diamine (285 mg, 1.5 mmol) instead of **C**, gave an light yellow solid 1-methyl-5-(trifluoromethyl)-1*H*-benzo[*d*]imidazole (225 mg, 75 %): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 – 8.08 (m, 1H), 7.99 (s, 1H), 7.59 (dd, *J* = 8.5, 1.5 Hz, 1H), 7.49 (d, *J* = 8.5 Hz, 1H), 3.91 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 145.32, 143.17, 136.44, 124.90, 119.92, 119.88, 118.09, 118.05, 31.24.

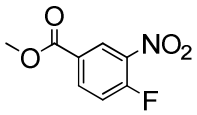


***N*-methyl-4-(trifluoromethyl)-2-(4-(4-(trifluoromethyl)phenyl)-1*H*-imidazol-1-yl)aniline** (**20**). Following the general synthesis method of **12**, using 1-methyl-5-(trifluoromethyl)-1*H*-benzo[*d*]imidazole (200 mg, 1 mmol) instead of 1-methyl-5-nitro-1*H*-benzo[*d*]imidazole, gave a light yellow solid *N*-methyl-4-(trifluoromethyl)-2-(4-(4-(trifluoromethyl)phenyl)-1*H*-imidazol-1-yl)aniline (212 mg, 55 %): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (dd, *J* = 8.7, 0.7 Hz, 2H), 7.70 – 7.59 (m, 4H), 7.46 – 7.40 (m, 2H), 6.83 (d, *J* = 8.7 Hz, 1H), 4.35 – 4.25 (m, 1H), 2.92 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 147.01, 141.95, 138.29, 136.77, 127.84, 127.81, 125.71, 125.68, 125.64, 124.93, 124.46, 124.42, 121.82, 116.96, 110.56, 29.97; HRMS (ESI): calcd for: $C_{18}H_{13}F_6N_3$ $[M+H]^+$ = 386.1087, obsd $[M+H]^+$ = 386.1086.

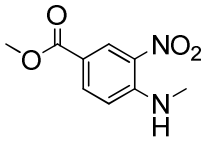


***N*^{1}-methyl-2-(4-(4-(trifluoromethyl)phenyl)-1*H*-imidazol-1-yl)benzene-1,4-diamine as white solid** (**21**). After three vacuum/H_2 cycles to remove air from the reaction tube, the stirred mixture of the **12** (181 mg, 0.5 mmol) and 10 % Pd/C catalyst (18 mg) in MeOH (1 mL) was hydrogenated under ambient pressure (balloon) at room temperature for 6h. The reaction mixture was filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel, using MeOH/$CHCl_3$ (1:20) as eluent, and gave N^1-methyl-2-(4-(4-(trifluoromethyl)phenyl)-1*H*-imidazol-1-yl)benzene-1,4-diamine as white solid (99 mg, 60 %); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (d, *J* = 8.1 Hz, 2H), 7.77 – 7.61 (m, 3H), 7.48 (d, *J* = 1.2 Hz, 1H), 6.81 (dd, *J* = 8.6, 2.6 Hz, 1H), 6.69 (d, *J* = 8.6 Hz, 1H), 6.62 (d, *J* = 2.6 Hz, 1H), 3.44 (s, 3H), 2.79 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 141.23, 138.44, 137.43, 137.28, 137.20, 128.94, 128.62, 125.68, 125.64, 124.88, 123.59, 122.99, 117.66, 117.25, 114.51, 112.98, 31.06; HRMS (ESI): calcd for: $C_{17}H_{15}F_3N_4$ $[M+H]^+$ = 333.1322, obsd $[M+H]^+$ = 333.1320.

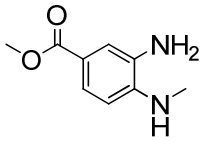




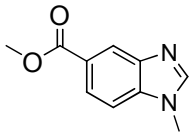
Methyl 4-fluoro-3-nitrobenzoate. 4-fluoro-3-nitrobenzoic acid (1.110 g, 6 mmol) was dissolved in MeOH (30 mL). Then, conc. H_2SO_4 (298 µL, 12 mmol) was added to the solution and was stirred at reflux for 24h (monitored by TLC) [26]. The solvent was concentrated under reduced pressure. After extraction with EtOAc (50 mL), the solution was washed with distilled water (3 × 20 mL) and saturated $NaHCO_3$ (20 mL), and dried over Na2SO4. The solution was evaporated and purified by flash chromatography on silica gel, using ethyl acetate/hexane (1:8) as eluent, to give methyl 4-fluoro-3-nitrobenzoate as a yellow solid (1.135 g, 95 %): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.76 (dd, *J* = 7.2, 2.2 Hz, 1H), 8.34 (ddd, *J* = 8.7, 4.2, 2.2 Hz, 1H), 7.41 (dd, *J* = 10.2, 8.7 Hz, 1H), 4.00 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.07, 159.41, 156.71, 136.44, 127.83, 127.23, 118.67, 52.89.



Methyl 4-(methylamino)-3-nitrobenzoate. Following the general method I, using methyl 4-fluoro-3-nitrobenzoate (587 mg, 3 mmol) instead of **A**, gave an yellow solid methyl 4-(methylamino)-3-nitrobenzoate (561 mg, 89 %): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.89 (d, *J* = 2.1 Hz, 1H), 8.37 (s, 1H), 8.09 (ddd, *J* = 9.0, 2.1, 0.7 Hz, 1H), 6.88 (d, *J* = 9.0 Hz, 1H), 3.91 (s, 3H), 3.10 (d, *J* = 5.1 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.62, 148.49, 136.38, 131.35, 129.41, 117.18, 113.12, 52.07, 29.90.

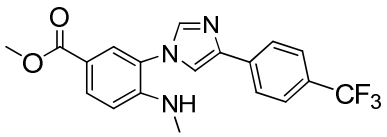


Methyl 3-amino-4-(methylamino)benzoate. Following the general synthesis method of *N*^{1}-methyl-4-(trifluoromethyl)benzene-1,2-diamine, using methyl 4-(methylamino)-3-nitrobenzoate (420 mg, 2 mmol) instead of *N*-methyl-2-nitro-4-(trifluoromethyl)aniline, gave an red solid methyl 3-amino-4-(methylamino)benzoate (292 mg, 81 %): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (dd, *J* = 8.3, 1.9 Hz, 1H), 7.42 (d, *J* = 1.9 Hz, 1H), 6.61 (d, *J* = 8.3 Hz, 1H), 3.87 (s, 3H), 3.52 (s, 2H), 2.93 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.55, 144.01, 132.17, 124.34, 118.81, 117.88, 108.82, 51.56, 30.44.

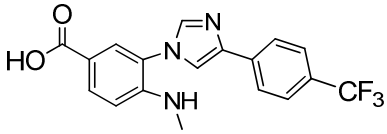


Methyl 1-methyl-1*H*-benzo[*d*]imidazole-5-carboxylate. Following the general method I, using methyl 3-amino-4-(methylamino)benzoate (270 mg, 1.5 mmol) instead of **C**, gave an white solid methyl 1-methyl-1*H*-benzo[*d*]imidazole-5-carboxylate (225 mg, 79 %): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54 (dd, *J* = 1.5, 0.6 Hz, 1H), 8.08 (dd, *J* = 8.5, 1.5 Hz, 1H), 7.99 (s, 1H), 7.44 (dd, *J* = 8.5, 0.6 Hz, 1H), 3.97 (s,

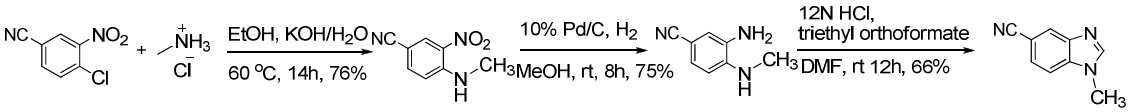
3H), 3.90 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.56, 145.19, 143.29, 137.69, 124.57, 124.49, 122.73, 109.08, 52.08, 31.22.

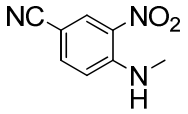


Methyl 4-(methylamino)-3-(4-(4-(trifluoromethyl)phenyl)-1*H*-imidazol-1-yl)benzoate (**22**). Following the general synthesis method of **12**, using 1-methyl-1*H*-benzo[*d*]imidazole-5-carboxylate (190 mg, 1 mmol) instead of 1-methyl-5-nitro-1*H*-benzo[*d*]imidazole, gave a yellow solid methyl 4-(methylamino)-3-(4-(4-(trifluoromethyl)phenyl)-1*H*-imidazol-1-yl)benzoate (266 mg, 61 %): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (ddd, *J* = 8.7, 2.0, 0.5 Hz, 1H), 7.97 – 7.85 (m, 3H), 7.68 (d, *J* = 1.2 Hz, 2H), 7.66 (s, 1H), 7.44 (d, *J* = 1.3 Hz, 1H), 6.78 (d, *J* = 8.7 Hz, 1H), 4.37 (s, 1H), 3.90 (s, 3H), 2.93 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.32, 147.99, 141.88, 138.43, 136.92, 132.53, 128.82, 125.76, 125.72, 125.68, 125.64, 124.96, 121.70, 118.21, 117.02, 110.02, 51.89, 29.96; HRMS (ESI): calcd for: $C_{19}H_{18}F_3N_3O_2$ $[M+H]^+$ = 376.1268, obsd $[M+H]^+$ = 376.1270.

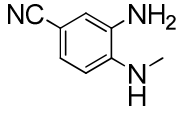


4-(methylamino)-3-(4-(4-(trifluoromethyl)phenyl)-1*H*-imidazol-1-yl)benzoic acid (**23**). Following the synthesis method of **19**, using **22** (187 mg, 0.5 mmol) instead of **18**, gave a white solid 4-(methylamino)-3-(4-(4-(trifluoromethyl)phenyl)-1*H*-imidazol-1-yl)benzoic acid (105 mg, 58 %): ^{1}H NMR (400 MHz, DMSO) δ 9.17 (s, 1H), 8.44 (s, 1H), 8.19 (d, J = 8.2 Hz, 2H), 7.96 (dd, J = 8.7, 2.0 Hz, 1H), 7.88 (dd, J = 18.1, 5.1 Hz, 3H), 6.85 (d, J = 8.9 Hz, 1H), 6.39 (s, 1H), 2.77 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 167.02, 148.52, 139.14, 133.22, 129.65, 126.50, 126.47, 126.43, 126.39, 126.22, 121.29, 120.55, 117.45, 110.93, 29.94; HRMS (ESI): calcd for: $C_{18}H_{14}F_3N_3O_2$ $[M+H]^+$ = 362.1111, obsd $[M+H]^+$ = 362.1111.



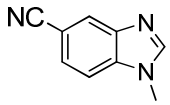


4-(methylamino)-3-nitrobenzonitrile. Following the synthesis method of *N*-methyl-2-nitro-4-(trifluoromethyl)aniline, using 4-chloro-3-nitrobenzonitrile (548 mg, 3 mmol) instead of 1-chloro-2-nitro-4-(trifluoromethyl)benzene, gave an yellow solid 4-(methylamino)-3-nitrobenzonitrile (468 mg, 88 %): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (d, *J* = 2.0 Hz, 1H), 8.44 (s, 1H), 7.66 (ddd, *J* = 9.0, 2.0, 0.7 Hz, 1H), 6.94 (d, *J* = 9.0 Hz, 1H), 3.13 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 147.96, 137.72, 132.08, 131.49, 117.97, 114.43, 98.11, 29.91.

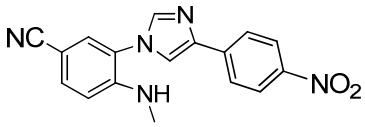


3-amino-4-(methylamino)benzonitrile. Following the synthesis method of N^1-methyl-4-(trifluoromethyl)benzene-1,2-diamine, using 4-(methylamino)-3-nitrobenzonitrile (354 mg, 2 mmol) instead of *N*-methyl-2-nitro-4-(trifluoromethyl)aniline, gave a red solid 3-amino-4-

(methylamino)benzonitrile (233 mg, 79 %): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.19 (dd, J = 8.2, 1.9 Hz, 1H), 6.94 (d, J = 1.9 Hz, 1H), 6.58 (d, J = 8.3 Hz, 1H), 3.38 (s, 2H), 2.92 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 143.47, 133.05, 126.75, 120.65, 119.00, 109.45, 99.16, 30.29.



1-methyl-1*H*-benzo[*d*]imidazole-5-carbonitrile. Following the synthesis method of 1-methyl-5-(trifluoromethyl)-1*H*-benzo[*d*]imidazole, using 3-amino-4-(methylamino)benzonitrile (220 mg, 1.5 mmol) instead of N^1-methyl-4-(trifluoromethyl)benzene-1,2-diamine, gave an light yellow solid 1-methyl-1*H*-benzo[*d*]imidazole-5-carbonitrile (120 mg, 51 %): ^{1}H NMR (400 MHz, DMSO) δ 8.43 (s, 1H), 8.21 (d, J = 0.8 Hz, 1H), 7.79 (dd, J = 8.4, 0.4 Hz, 1H), 7.67 (dd, J = 8.4, 1.5 Hz, 1H), 3.90 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 147.90, 143.24, 137.93, 126.04, 124.81, 120.38, 112.36, 104.21, 31.47.



4-(methylamino)-3-(4-(4-nitrophenyl)-1*H*-imidazol-1-yl)benzonitrile (**24**). Following the general synthesis method of **3**, using 1-methyl-1*H*-benzo[*d*]imidazole-5-carbonitrile (157 mg, 1 mmol) instead of 1-ethyl-5-nitro-1*H*-benzo[*d*]imidazole, gave an white solid 4-(methylamino)-3-(4-(4-nitrophenyl)-1*H*-imidazol-1-yl)benzonitrile (172 mg, 54 %): ^{1}H NMR (400 MHz, DMSO) δ 8.33 – 8.22 (m, 2H), 8.18 (s, 1H), 8.14 – 8.05 (m, 2H), 8.00 – 7.91 (m, 1H), 7.74 (dd, *J* = 8.7, 1.8 Hz, 1H), 7.68 (d, *J* = 2.0 Hz, 1H), 6.89 – 6.79 (m, 1H), 6.28 (s, 1H), 2.74 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 148.62, 146.03, 141.38, 139.89, 139.80, 134.92, 131.55, 125.42, 124.61, 122.45, 121.00, 119.87, 111.64, 96.20, 29.97; HRMS (ESI): calcd for: $C_{17}H_{13}N_3O_2$ $[M+H]^+$ = 320.1142, obsd $[M+H]^+$ = 320.1147.