E Supporting Information

Structure-Activity Relationship Studies Reveals New Astemizole Analogues Active Against *Plasmodium falciparum* In vitro

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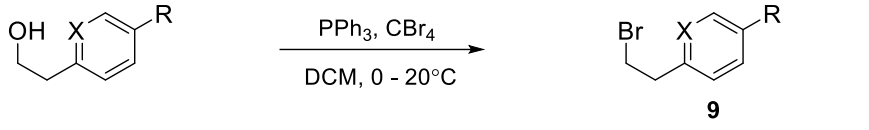
Synthetic Procedures

General Experimental Methods. All commercially available chemicals were purchased from either Sigma-Aldrich (Germany) or Combi-Blocks (United States). All solvents were dried by appropriate techniques. All solvents used were anhydrous, unless otherwise stated. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Spectrometer at 300, 400 or 600 MHz. Analytical thin-layer chromatography (TLC) was performed on aluminum-backed silica-gel 60 F_{254} (70−230 mesh) plates with detection and visualization done using (a) UV lamp (254/366 nm), (b) iodine vapors, or (c) ninhydrin spray reagent. Column chromatography was performed with Merck silica-gel 60 (70−230 mesh). Chemical shifts (δ) are reported in ppm downfield from trimethlysilane (TMS) as the internal standard. Coupling constants (*J*) were recorded in Hertz (Hz). Purity of compounds was determined by an Agilent 1260 Infinity binary pump, Agilent 1260 Infinity diode array detector (DAD), Agilent 1290 Infinity column compartment, Agilent 1260 Infinity standard autosampler, and Agilent 6120 quadrupole (single) mass spectrometer, equipped with APCI and ESI multimode ionization source, and all compounds tested for biological activity were confirmed to have purity of ≥ 95%.

Appel Bromination: Synthesis of alkyl bromides 9a and 9b.[1]



9

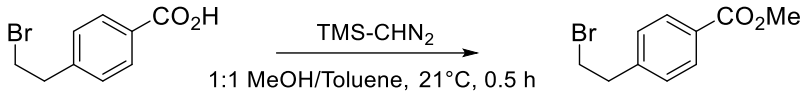
9a, X = CH, R = CN, 81%
9b, X = N, R = H, 70%

To a stirring solution of an appropriate alcohol (1.0 equiv) in DCM at 0 °C, triphenyl phosphine, PPh_3 (1.5 equiv) and carbon tetrabromide, CBr_4 (1.2 equiv) where added successively. The resulting mixture was then allowed to warm to room temperature (18 °C), at which it was stirred for 2 hr. Upon completion (monitored *via* TLC), DCM was evaporated *in vacuo* and diethyl ether (Et_2O) added to the dry residue. Insoluble materials were filtered, and the filtrate dry loaded on silica gel. Products were obtained following purification *via* flash column chromatography, using 20 – 50% EtOAc/Hexanes as an eluent.

4-(2-bromoethyl)-benzonitrile (9a). Following GP1, obtained from 4-(2-hydroxyethyl)benzonitrile (10.0 g, 67.9 mmol) as a white crystalline solid (11.6 g, 81%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.78 (d, *J* = 8.3 Hz, 2H), 7.49 (d, *J* = 8.3 Hz, 2H), 3.78 (t, *J* = 7.0 Hz,

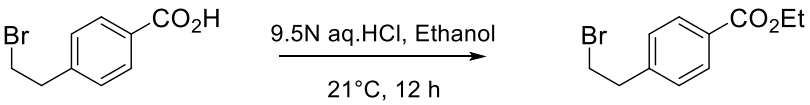
2H), 3.23 (t, *J* = 7.0 Hz, 2H). LC-MS (APCI$^+$/ESI): found *m/z* = 210.0, 212.0 $[M+H]^+$ (cal. For C_9H_8BrN, 208.98, 210.98). Purity: 96%, t_R = 2.753 min.

2-(2-bromoethyl)pyridine (9b). Following GP1, obtained from 2-(yridine-2-yl)ethan-1-ol (0.400 g, 3.25 mmol) as a yellow liquid (0.423 g, 70%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.52 (dd, *J* = 5.0, 1.4 Hz, 1H), 7.73 (td, *J* = 7.8, 1.4 Hz, 1H), 7.32 (dd, *J* = 7.8, 1.3 Hz, 1H), 7.25 (ddd, *J* = 7.8, 4.9, 1.3 Hz, 1H), 3.86 (t, *J* = 6.9 Hz, 2H), 3.28 (t, *J* = 6.9 Hz, 2H). LC-MS (APCI/ESI): found *m/z* = 186.0, 188.0 $[M+H]^+$ (cal. For C_7H_8BrN, 184.9, 186.9). Purity: 96%, t_R = 2.422 min.



9c

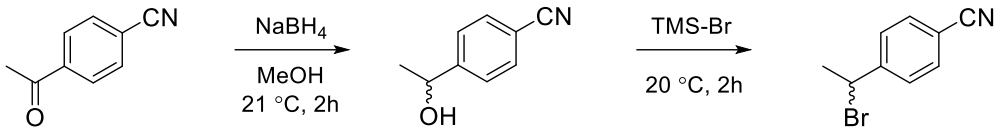
Methyl 4-(2-bromoethyl) benzoate (9c). To a solution of 4-(2-bromoethyl) benzoic acid (7.00 g, 30.6 mmol) in 4:1 toluene/MeOH (60 ml) under ice, trimethylsilyldiazomethane (2M solution in hexane) was added dropwise until the yellow colour no longer dissipated (about 28 ml added in 25 minutes).[2] The mixture was evaporated to dryness *in vacuo*, followed by purification by flash column chromatography using 20% EtOAc/Hexanes as eluent. Product obtained as a colorless oil (7.21 g, 97%). R_f (20% EtOAc/Hexanes) 0.45. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.96 (d, *J* = 8.4 Hz, 2H), 7.20 (d, *J* = 8.4 Hz, 2H), 3.85 (s, 3H), 3.54 (t, *J* = 4.4 Hz, 2H), 3.14 (t, *J* = 4.4 Hz, 2H). LC-MS (APCI$^+$/ESI): found m/z = 242.9, 244.9 $[M+H]^+$ (cal. For $C_{10}H_{11}BrO_2$, 241.99, 243.99). Purity: 99%, t_R = 2.568 min.



9d

Ethyl 4-(2-bromoethyl)benzoate (9d). A solution of 4-(2-bromoethyl) benzoic acid (0.500 g, 0.22 mmol) and 9.5N aqueous HCl (5 ml) in absolute ethanol (10 ml) was refluxed at 80 °C for 2 hr. The mixture was then cooled to room temperature (22 °C) and the solvent evaporated *in vacuo*. The aqueous residue was diluted with 25 ml saturated $NaHCO_3$ and extracted with EtOAc (3 × 25 ml). Combined organic extracts were washed with water (2 × 15 ml), followed by brine (1 × 10 ml), and dried over anhydrous Na_2SO_4. After flash column chromatography using 20% EtOAc/Hexanes as eluent, the product obtained as a yellow oil (0.476 g, 85%). ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.96 (d, *J* = 8.3 Hz, 1H), 7.37 (d, *J* = 8.3 Hz, 2H), 4.35 (q, *J* = 7.1 Hz, 2H),

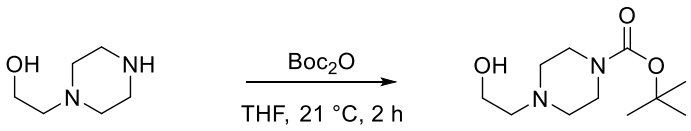
3.79 (t, *J* = 7.0 Hz, 2H), 3.12 (t, *J* = 7.0 Hz, 2H), 1.38 (t, *J* = 7.1 Hz, 3H). LC-MS (APCI$^+$/ESI): found *m/z* = 257.0, 259.0 $[M+H]^+$ (cal. For $C_{11}H_{13}BrO_2$, 256.01, 258.01). Purity: 99%, t_R = 2.543 min.



9e **9f**

4-(1-hydroxyethyl)benzonitrile (9e).[3] Sodium borohydride (0.065 g, 1.72 mmol) was added to a stirring solution of ketone 4-acetylbenzonitrile (0.500 g, 3.44 mmol) in 5 ml MeOH on an ice bath (0 – 3 °C). The reaction mixture was stirred at room temperature (18 °C) for 2 hr. The mixture was diluted with 20 ml water and extracted with DCM (3 × 20 ml). Combined organic phases where further washed with brine (1 × 10 ml), followed by drying over anhydrous Na_2SO_4. The solvent was evaporated under vacuum to give products. Flash column chromatography using 50 – 60 EtOAc/Hexanes afforded product as a light-yellow oil (0.421 g, 83%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.77 (d, *J* = 8.3 Hz), 7.54 (d, *J* = 8.3 Hz, 2H), 5.39 (d, *J* = 4.3 Hz, 1H), 4.80 (qd, *J* = 6.5, 4.3 Hz, 1H), 1.32 (d, *J* = 6.5 Hz, 3H). Purity: 99%, t_R = 2.486 min.

4-(1-bromoethyl)benzonitrile (9f).[4] A mixture of **11a** (0.180 g, 1.22 mmol) and bromotrimethyl silane, TMSBr (0.178 ml, 1.35 mmol) was stirred at room temperature (18 °C) for 2 hr. Upon completion, volatile $(TMS)_2O$ was evaporated under reduced pressure and DCM (10 ml) added. Flash column chromatography was performed using DCM to afford the pure product as a light-yellow oil (0.185 g, 72%). R_f(3% MeOH/DCM) 0.73. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.84 (d, *J* = 8.5 Hz, 2H), 7.70 (d, *J* = 8.5 Hz, 2H), 5.54 (q, *J* = 6.9 Hz, 1H), 1.98 (d, *J* = 6.9 Hz, 3H). Purity: 99%, t_R = 2.782 min.



9g

4-N-Boc-(2-hydroxyethyl) piperazine (9g). To a stirring solution of 1-(2-hydroxyethyl) piperazine (0.50 g, 3.8 mmol) in 7 ml THF at 0^{o}C, di-*tert*-butyl dicarbonate (Boc_2O, 0.922 g, 4.2

mmol) was added and the mixture stirred at 0 °C for 15 min. The mixture was then allowed to warm to ambient temperature (18 °C) and stirred further for 2 hr. The solvent was evaporated to about half the original volume, followed by addition of water (7 ml). The solution was extracted with DCM (3 × 25 ml), combined organic phases washed with brine (1 × 10 ml) and further dried over anhydrous Na2SO_4. Solvent was evaporated under vacuum to give product as a light brown to brick reddish oil (0.866 g, 98%). R_f (10% MeOH/DCM) 0.35. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.56 (t, *J* = 6.9 Hz, 2H), 3.41 (m, *J* = 5.1 Hz, 4H), 2.71 (t, *J* = 6.9 Hz, 2H), 2.44 (m, *J* = 5.0 Hz, 4H), 1.43 (s, 9H).

General Procedure 2 (GP2): Synthesis of Intermediates **5**, **10a – 10c**.

(a) A mixture of 2-chlorobenzimidazole (1.0 equiv), methyl iodide or 4-(bromomethyl)benzonitrile (1.2 equiv) and K_2CO_3 (2.0 equiv) was stirred at room temperature (18 – 22 °C) in acetone for 2 – 12 hours. After completion (monitored *via* TLC), the solvent was removed *in vacuo*. Water was added to the residue, followed by extracting with DCM (×3). Combined organic layers were washed with brine (×3), dried over anhydrous Na_2SO_4, and the solvent evaporated *in vacuo* to give crude residue, which was triturated with *n*-pentane to afford the pure 2-chloro-1-alkyl-benzimidazole products in quantitative yields.

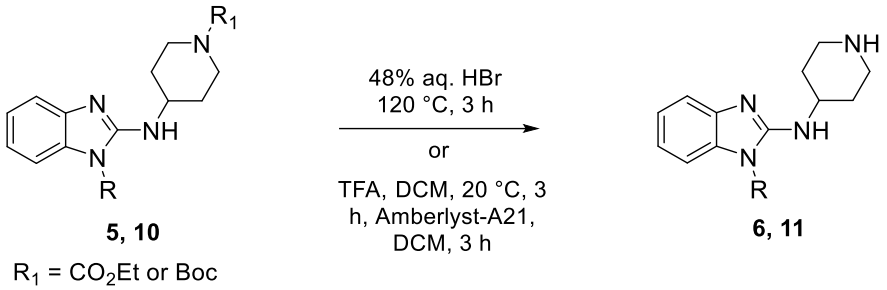
(b) A mixture of 2-chlorobenzimidazole or 2-chloro-1-alkyl-benzimidazole from (a) (1.0 equiv), tert-butyl 4-aminopiperidine-1-carboxylate or ethyl 4-aminopiperidine-1-carboxylate (1.5 equiv), and TEA (2.0 equiv) was stirred at 155 °C in a seal tube for 6 – 36 hours. After completion (monitored *via* TLC), the residue was cooled and diluted with 10% MeOH/DCM. The mixture was washed with saturated $NaHCO_3$ solution (×3), then brine (×1), and dried over anhydrous Na_2SO_4. The solvent was evaporated *in vacuo* to obtain a crude residue which was either triturated with *n*-pentane (or diethyl ether) or purified *via* flash column chromatography to afford the pure products.

*Ethyl 4-((1-(4-fluorobenzyl)-1H-benzo[d]imidazol-2-yl)amino)piperidine-1-carboxylate (**5**).* Following GP2 (b), prepared from 2-Chloro-1-(4-fluorobenzyl)benzimidazole (1.5 g, 5.75 mmol) and ethyl 4-aminopiperidine-1-carboxylate (2.0 g, 11.62 mmol). Obtained as a white solid (2.30 g, 98%). ^{1}H-NMR (400 Hz, $CDCl_3$): δ 7.55 (d, *J* = 8.0 Hz, 2H), 7.22 – 7.11 (m, 3H), 7.10 – 7.00 (m, 4H), 5.14 (s, 2H), 4.22 – 3.90 (m, 3H), 4.11 (q, *J* = 8.0 Hz, 2H), 4.27 – 4.21 (m, 2H), 2.12 – 2.08 (m, 2H), 1.35 – 1.29 (m, 2H), 1.25 (t, *J* = 8.0 Hz, 3H). LC-MS (APCI/ESI)$^+$, found *m/z* = 397.2 $[M+H]^+$ (cal. for $C_{22}H_{25}FN_4O_2$, 396.20).

*Ethyl 4-((1H-benzo[d]imidazol-2-yl)amino)piperidine-1-carboxylate (**10a**)*. Following GP2 (b), prepared from 2-Chlorobenzimidazole (5.50 g, 36.1 mmol) and ethyl 4-aminopiperidine-1-carboxylate (9.3 g, 54 mmol). Obtained as a light brown solid (8.22 g, 79%). ^{1}H NMR (600 MHz, Methanol-d_4) δ 7.18 (dd, *J* = 5.8, 3.2 Hz, 2H), 6.95 (dd, *J* = 5.8, 3.2 Hz, 2H), 4.11 (q, *J* = 7.1 Hz, 2H), 4.09 (m, 2H), 3.80 (tt, *J* = 10.6, 4.0 Hz, 1H), 3.05 – 2.99 (m, 2H), 2.08 – 2.01 (m, 2H), 1.48 – 1.39 (m, 2H), 1.25 (t, *J* = 7.1 Hz, 3H). LC-MS (APCI$^+$/ESI): found *m/z* = 289.0 [M+H] $^+$ (cal. For $C_{15}H_{20}N_4O_2$, 288.16). Purity: 98%, t_R = 2.258 min.

*2-chloro-1-methyl-1H-benzo[d]imidazole (**10b**)*. Following GP2 (a), prepared from 2-chlorobenzimidazole (11.8 g, 77 mmol) and methyl iodide (13.1 g, 92.4 mmol), then reacting product as in GP2 (b) with ethyl 4-aminopiperidine-1-carboxylate (20 g, 116 mmol). Obtained as a brown crystalline solid (19.8 g, 85%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.28 (dd, J = 7.7, 0.9 Hz, 1H), 7.12 (dd, J = 7.9, 1.0 Hz, 1H), 7.04 (ddd, J = 7.7, 7.1, 0.9 Hz, 1H), 6.99 (ddd, J = 8.0, 7.1, 1.1 Hz, 1H), 4.51 – 4.44 (m, 2H), 4.13 (q, J = 7.1 Hz, 2H), 3.92 (tt, J = 11.1, 4.0 Hz, 1H, H^6), 3.51 (s, 3H), 3.07 – 2.95 (m, 2H), 2.09 – 1.99 (m, 2H), 1.50 - 1.51 (m, 2H), 1.27 (t, J = 7.1 Hz, 3H). LC-MS (APCI$^+$/ESI): found m/z = 303.2 [M+H] $^+$ (cal. For $C_{16}H_{22}N_4O_2$, 302.17). Purity: 99%, t_R = 2.381 min.

*Tert-butyl 4-((1-(4-cyanobenzyl)-1H-benzo[d]imidazol-2-yl) amino) piperidine-1-carboxylate (**10c**)*. Following GP2 (a), prepared from 2-chlorobenzimidazole (5.11 g, 33.4 mmol) and 4-(bromomethyl)benzonitrile (7.85 g, 40 mmol), then reacting product as in GP2 (b) with ethyl 4-aminopiperidine-1-carboxylate (9.3 g, 54 mmol). Obtained as a light brown solid (8.67 g, 60%). R_f (10% MeOH/DCM), 0.68. ^{1}H NMR (600 MHz, Methanol-d_4) δ 7.64 (d, *J* = 8.6 Hz, 2H), 7.32 (dd, *J* = 7.9, 1.1 Hz, 1H), 7.22 (d, *J* = 8.6 Hz, 2H), 7.04 (ddd, *J* = 7.9, 7.2, 1.3 Hz, 1H), 6.97 (dd, *J* = 8.2, 1.3 Hz, 1H), 6.93 (ddd, *J* = 8.2, 7.2, 1.1 Hz, 1H), 5.34 (s, 2H), 4.07 – 4.02 (m, 2H), 3.94 (tt, *J* = 11.0, 4.0 Hz, 1H), 2.99 – 2.88 (m, 2H), 2.05 – 2.00 (m, 2H), 1.51 – 1.32 (m, 11H). LC-MS (APCI$^+$/ESI): found *m/z* = 432.2 [M+H] $^+$ (cal. For $C_{25}H_{29}N_5O_2$, 431.23). Purity: 98%, t_R = 2.673 min.



General Procedure 3 (GP3): Preparation of Intermediates **6**, **11a** – **11c**. N-ethyl and N-*tert*-butyl (Boc) carboxylate intermediates were refluxed in 48% HBr solution for 2 – 3 h at 120 °C and stirred in DCM with TFA (10 equiv) for 1 h at 23 °C, respectively.

a) 48% Aqueous HBr workup: After completion, the reaction mixture was cooled and diluted with deionized water, followed by neutralizing with 15% NaOH. The aqueous solution was extracted with DCM, and the combined organic layers washed with brine and dried over anhydrous Na_2SO_4. Evaporating off the solvent *in vacuo* and washing residue in diethyl ether afforded the free amines.

b) TFA workup: After completion, DCM and TFA were evaporated *in vacuo* and the residue taken up 50% MeOH/DCM and stirred with Amberlyst A-21 free base resin at room temperature (23^{o}C) until pH was neutral. The mixture was then filtered, and the filtrate evaporated *in vacuo* to afford free amines.

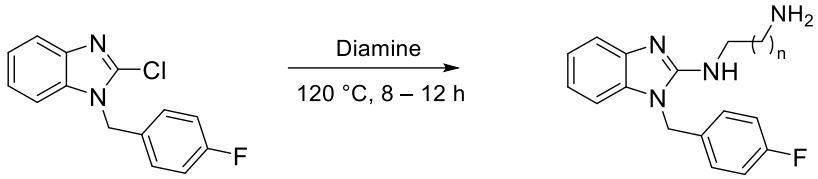
*1-(4-fluorobenzyl)-N-(piperidin-4-yl)-1H-benzo[d]imidazol-2-amine (**6**)*. Obtained from **5**. Orange solid (1.89 g, 85%); ^{1}H-NMR (400 Hz, $CDCl_3$): δ 7.52 (d, *J* = 8.0 Hz, 2H), 7.19 – 7.08 (m, 3H), 7.07-6.98 (m, 4H), 5.07 (s, 2H), 3.04 (dt, *J* = 12.4, 4.8 Hz, 2H), 2.75 (td, *J* = 12.0, 2.8 Hz, 2H), 2.42 (bs, 2H), 2.12 – 2.09 (m, 2H), 1.34 – 1.30 (m, 2H). LC-MS (APCI/ESI)$^+$, found *m/z* = 325.2 $[M+H]^+$ (cal. for $C_{19}H_{21}FN_4$, 324.18), Purity: 95%, t_R = 2.819 min.

*N-(piperidin-4-yl)-1H-benzo[d]imidazol-2-amine (**11a**)*. Obtained from **10a**. White crystalline solid (3.52 g, 58%). ^{1}H NMR (600 MHz, Methanol-d_4) δ 7.43 (dd, *J* = 6.0, 3.1 Hz, 2H), 7.30 (dd, *J* = 6.0, 3.1 Hz, 2H), 4.03 (tt, *J* = 10.6, 4.1 Hz, 1H), 3.59 – 3.54 (m, 2H), 3.30 – 3.24 (m, 2H), 2.39 – 2.33 (m, 2H), 2.04 – 1.96 (m, 2H). LC-MS (APCI$^+$/ESI): found *m/z* = 217.2 $[M+H]^+$ (cal. For $C_{12}H_{16}N_4$, 216.29). Purity: 98%, t_R = 0.621 min.

*1-Methyl-N-(piperidin-4-yl)-1H-benzo[d]imidazol-2-amine (**11b**)*. Obtained from **10b**. White solid (12.2 g, 89%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.27 (dd, *J* = 7.9, 1.2 Hz, 1H), 7.11 (dd,

J = 7.6, 1.5 Hz, 1H), 7.05 – 6.96 (m, 2H), 3.85 (tt, *J* = 11.1, 4.0 Hz, 1H), 3.51 (s, 3H), 3.22 – 3.10 (m, 2H), 2.79 (td, *J* = 12.5, 2.6 Hz, 2H), 2.23 – 2.00 (m, 2H), 1.56 (dtd, *J* = 13.4, 12.0, 4.0 Hz, 2H). LC-MS (APCI$^+$/ESI): found *m/z* = 231.2 [M+H] $^+$ (cal. For $C_{13}H_{18}N_4$, 230.15). Purity: 99%, t_R = 0.703 min.

*4-((2-(piperidin-4-ylamino)-1H-benzo[d]imidazol-1-yl) methyl) benzonitrile (**11c**)*. Obtained from **10c**. Pale yellow solid (6.40 g, 98%). R_f (10% MeOH/DCM), 0.05. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.67 (d, *J* = 8.4 Hz, 2H), 7.32 (dd, *J* = 7.9, 0.9 Hz, 1H), 7.25 (d, *J* = 8.4 Hz, 2H), 7.05 (ddd, *J* = 7.9, 7.1, 1.1 Hz, 1H), 6.99 (dd, *J* = 8.0, 1.5 Hz, 1H), 6.94 (ddd, *J* = 8.0, 7.1, 1.1 Hz, 1H), 5.37 (s, 2H), 3.89 (tt, *J* = 11.1, 4.1 Hz, 1H), 3.07 (dt, *J* = 12.9, 3.6 Hz, 2H), 2.74 (td, *J* = 12.5, 2.6 Hz, 2H), 2.11 – 2.01 (m, 2H), 1.46 (dtd, *J* = 13.3, 11.9, 4.07 Hz, 2H). LC-MS (APCI$^+$/ESI): found *m/z* = 332.20 [M+H] $^+$ (cal. For $C_{20}H_{21}N_5$, 331.18). Purity: 99%, t_R = 0.303 min.

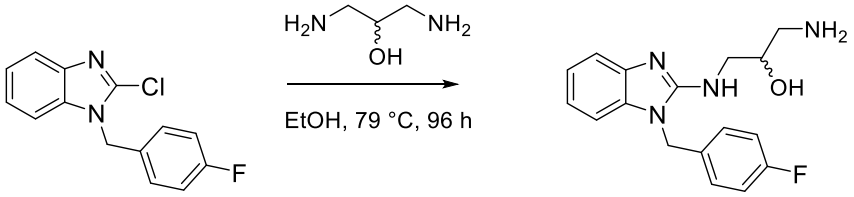


1a, n = 1
1b, n = 2

General Procedure 4 (GP4): Synthesis of Compounds **1a** and **1b**. A mixture of 2-chloro-1-(4-fluorobenzyl)-1H-benzo[d]imidazole and diamine were heated to 90 °C and stirred for 18 h, then at 120 °C for 72 h. After completion of reaction (TLC), the reaction mixture was diluted with ethyl acetate, washed sequentially with sat. $NaHCO_3$ (2 × 15 ml) and brine (2 × 15 ml). The combined EtOAc layers was dried over sodium sulphate and concentrated *in vacuo* to obtain products.

*N-1-(1-(4-fluorobenzyl)-1H-benzo[d]imidazol-2-yl)ethane-1,2-diamine (**1a**)*. Obtained from ethane-1,2-diamine (1.72 g, 1.92 ml, 28.76 mmol). The crude product was washed with diethyl ether to obtain **1a** as a white solid (0.27 g, 92% yield). ^{1}H-NMR (400 Hz, DMSO-d_6): δ 7.31 – 7.22 (m, 4H), 7.09 (d, *J* = 7.9 Hz, 2H), 6.98 – 6.91 (m, 2H), 6.22 (t, *J* = 5.1 Hz, 1H), 6.08 (t, *J* = 6.5 Hz, 2H), 5.26 (s, 2H), 3.54 (m, 2H), 3.09 (m, 2H). LC-MS (APCI/ESI)$^+$: found *m/z* = 285.1 [M+H]$^+$ (cal. for $C_{16}H_{17}FN_4$, 284.14).

*N-1-(1-(4-fluorobenzyl)-1H-benzo[d]imidazol-2-yl)propane-1,3-diamine (**1b**)*. Obtained from propane-1,3-diamine (2.57 g, 2.90 ml, 34.53 mmol). The crude product was washed with diethyl ether to obtain **1b** as a white solid (0.27 g, 85%). ^{1}H-NMR (400 Hz, Methanol-d_4): δ 7.21 (d, *J* = 8.4 Hz, 1H), 7.07-6.99 (two overlapping doublets, *J* = 8.4 Hz, 2H), 6.96 – 6.86 (m, 4H), 6.83 (m, 1H), 5.09 (s, 2H), 3.40 (t, *J* = 6.8 Hz, 2H), 2.58 (t, *J* = 7.2 Hz, 2H), 1.70 (quintet, *J* = 6.8 Hz, 2H). LC-MS (APCI/ESI)$^+$: found m/z = 299.1 $[M + H]^+$ (cal. for $C_{17}H_{19}FN_4$, 298.16).



1c

*1-amino-3-((1-(4-fluorobenzyl)-1H-benzo[d]imidazol-2-yl)amino)propan-2-ol (**1c**)*. A solution of 2-chloro-1-(4-fluorobenzyl)-1H-benzo[d]imidazole (SM, 0.300 g, 1.51 mmol) and 1,3-diaminopropan-2-ol (1.04 g, 11.5 mmol) in ethanol was refluxed (85 °C) for 92 h. After 3 days, LCMS showed only 70% consumption of SM. The reaction mixture was concentrated *in vacuo* and residue was taken in a mixture of 10% MeOH/DCM (50 ml). This mixture was washed with sat. $NaHCO_3$ (1 × 15 ml) and brine (1 × 15 ml), dried over sodium sulphate and concentrated *in vacuo*. The crude product was washed with ethyl acetate to obtain pure compound (0.180 g, 50% yield). ^{1}H-NMR (400 Hz, Methanol-d_4): δ 7.33 (d, *J* = 8.4 Hz, 1H), 7.12 – 7.15 (two overlapping d, *J* = 8.8 Hz, 2H), 7.12 – 7.00 (m, 4H), 6.87 (m, 1H), 5.23 (s, 2H), 3.83 (m, 1H), 3.55 (dd, *J* = 9.6 and 5.2 Hz, 1H), 3.45 (dd, *J* = 13.6 and 6.4 Hz, 1H), 2.74 (dd, *J* = 13.2 and 4.0 Hz, 1H), 2.60 (dd, *J* = 13.6 and 7.2 Hz, 1H); LC-MS (APCI/ESI)$^+$: found *m/z* = 315.1 $[M+H]^+$ (cal. for $C_{17}H_{19}FN_4O$, 314.15).

General Procedure 5 (GP5): Synthesis of Compounds **2** – **4**. A mixture of **1** (**1a** for **2,** and **1b** for **3** and **1c** for **4**, 1.0 equiv), 1-(2-bromoethyl)-4-methoxybenzene (1.05 equiv) and triethylamine (1.1 equiv) in DMF was stirred at ambient temperature (22 °C) for 10 h. Reactions monitored *via* LCMS. The reaction mixture was diluted with DCM (20 ml), washed sequentially with 5% aq. LiCl (5 × 15 ml), NaCl (2 × 15 ml) and water (1 × 15 ml). The organic phase was dried over anhydrous sodium sulphate and concentrated *in vacuo*. The residue was purified by washing in diethyl ether (for **4**) or *via* column chromatography (**2**,**3**) using 2 – 3% MeOH/DCM to obtain desired products.

*N-1-(1-(4-fluorobenzyl)-1H-benzo[d]imidazol-2-yl)-N2-(4-methoxyphenethyl)ethane-1,2-diamine (**2**)*. Oil (0.170 g, 60%); ^{1}H-NMR (400 Hz, Methanol-d_4): δ 7.26 (d, J = 8.0 Hz, 1H), 7.23-7.16 (d, J = 8.8 Hz, 4H), 7.13 – 7.07 (m, 2H), 7.05 (m, 2H), 7.03 – 6.99 (m, 1H), 6.84 (m, J = 8.8 Hz, 2H), 5.26 (s, 2H), 3.74 (s + m, 5H), 3.32 – 3.25 (m, 4H), 3.00 (t, J = 7.2 Hz, 2H). ^{13}C-NMR (101 Hz, Methanol-d_4) δ 163.5, 161.1 (d, J = 242.64 Hz, C-F), 159.0, 154.7, 140.6, 134.2, 132.0, 129.4 (2C), 128.4, 128.3, 128.2, 121.4, 120.1, 115.3, 115.1, 115.0, 114.0 (2C), 108.1, 54.3, 48.7, 48.6, 44.4, 39.7, 31.5; LC-MS (APCI/ESI)$^+$, found *m/z* = 419.2 $[M+H]^+$ (cal. for $C_{25}H_{27}FN_4O$, 418.22). Purity = 95%, t_R = 3.087 min.

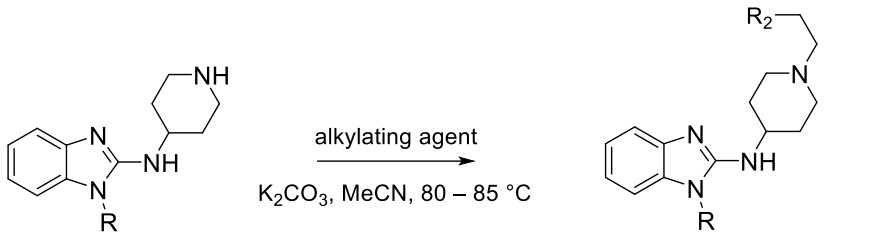
*N-1-(1-(4-fluorobenzyl)-1H-benzo[d]imidazol-2-yl)-N3-(4-methoxyphenethyl)propane-1,3-diamine (**3**)*. Oil (0.180 g, 62%); ^{1}H-NMR (400 Hz, Methanol-d_4): δ 7.34 (d, J = 8.4 Hz, 1H), 7.17 – 7.11 (two overlapping d, J = 8.8 Hz, 2H), 7.09 (d, J = 8.4 Hz, 2H), 7.07 – 6.94 (m, 5H), 6.82 (d, J = 8.4 Hz, 2H), 5.20 (s, 2H), 3.73 (s, 3H), 3.52 (t, J = 6.4 Hz, 2H), 2.81 (m, 2H), 2.77 – 2.65 (m, 4H), 1.87 (quintet, J = 6.8 Hz, 2H). ^{13}C-NMR (101 Hz, Methanol-d_4) δ 163.4, 161.0 (d, J = 242.64 Hz, C-F), 158.4, 154.8, 141.5, 134.2, 132.3, 131.0, 129.2 (2C), 128.3, 128.2, 121.1, 119.5, 115.2, 115.0, 114.7, 113.7 (2C), 107.6, 54.3, 50.6, 46.2, 44.0, 40.5, 33.9, 28.6; LC-MS (APCI/ESI)$^+$, found *m/z* = 433.2 $[M+H]^+$ (cal. for $C_{26}H_{29}FN_4O$, 432.23. Purity: 99%, t_R = 3.990 min.

*1-((1-(4-fluorobenzyl)-1H-benzo[d]imidazol-2-yl)amino)-3-((4-methoxyphenethyl)amino) propan-2-ol (**4**)*. White solid (0.021 g, 15%). ^{1}H-NMR (400 Hz, Methanol-d_4): δ 7.40 (d, J = 8.0 Hz, 1H), 7.30 – 7.24 (two overlapping d, J = 8.4 Hz, 2H), 7.23 – 7.15 (m, 4H), 7.15 – 7.09 (m, 1H), 7.09 – 7.01 (m, 2H), 6.87 (m, J = 8.4 Hz, 2H), 5.36 (s, 2H), 4.22 (m, 1H), 3.76 (s, 3H), 3.69 – 3.52 (m, 2H), 3.27 – 3.25 (m, 3H), 3.11 – 2.30 (m, 3H); ^{13}C-NMR (101 Hz, Methanol-d_4) δ 163.6 and 161.2 (d, J = 242.64 Hz, C-F), 159.0, 153.07, 136.0, 132.8, 131.2, 129.4 (2C), 128.6, 128.5, 128.2, 122.5, 121.5, 115.4, 115.2, 114.0 (2C), 113.5, 109.0, 65.8, 54.3, 49.8, 48.9, 46.3. 44.7, 31.0. LC-MS (APCI/ESI)$^+$, found *m/z* = 449.2 $[M+H]^+$ (cal. for $C_{26}H_{29}FN_4O_2$, 448.23). Purity = 98%, t_R = 3.709 min.

*2-(4-((1-(4-fluorobenzyl)-1H-benzo[d]imidazol-2-yl)amino)piperidin-1-yl)-1-(4-methoxyphenyl)ethan-1-one (**7**)*. A solution of **6** (0.300 g, 0.92 mmol), 2-bromo-1-(4-methoxyphenyl)ethan-1-one (0.220 g, 1.0 mmol) and Et_3N (140 µl, 1.0 mmol) in DCM was stirred at room temperature (25 °C) for 6 h. After the completion of reaction (TLC and LCMS), reaction mixture was diluted with DCM (20 ml), washed with sat. $NaHCO_3$ (2 × 10 ml) solution, dried

over sodium sulphate and concentrated *in vacuo*. The residue was purified by column chromatography using 1 – 2% MeOH/DCM to afford product as a white solid (20 mg, 10% yield). ^{1}H-NMR (400 Hz, Methanol-d_4): δ 8.02 (d, J = 9.2 Hz, 2H), 7.33 (d, J = 8.0 Hz, 1H), 7.18 (d, J = 8.8 Hz, 1H), 7.15 (d, J = 8.8 Hz, 1H), 7.09 – 7.00 (m, 6H), 6.96 (td, J = 8.4, 0.8 Hz, 1H), 5.27 (s, 2H), 3.89 (s, 5H), 3.86 (m, 1H), 3.05 (m, 2H), 2.39 (m, 2H), 2.07 (m, 2H), 1.71 (m, 2H). ^{13}C NMR (101 MHz, Methanol-d_4) δ 195.3, 164.1, 163.4, 161.0 (d, J = 244.64 Hz, C-F), 154.0, 141.6, 134.0, 132.4, 130.1 (2C), 128.8, 128.3, 128.2, 121.1, 119.4, 115.2, 115.0, 114.7, 113.5 (2C), 107.7, 54.7, 52.7 (2C), 49.8, 43.9, 31.4 (2C). LC-MS (APCI/ESI)$^+$, found *m/z* = 473.2 $[M+H]^+$ (cal. for $C_{28}H_{29}FN_4O_2$, 472.23). Purity = 99.8%, t_R = 4.037 min.

*1-(4-((1-(4-fluorobenzyl)-1H-benzo[d]imidazol-2-yl)amino)piperidin-1-yl)-2-(4-methoxyphenyl)ethan-1-one (**8**)*. A solution of **8a** (0.1 g, 0.31 mmol), 2-(4-methoxyphenyl)acetic acid (0.08 g, 0.76 mmol) and *i*-Pr_2NEt (0.12 g, 0.16 ml, 0.93 mmol) was treated with a solution of T_3P (50% in DMF; 0.12 g, 0.24 ml, 0.37 mmol) at 0 °C. The resulting reaction mixture was warmed to room temperature and stirred at ambient temperature (23 °C) for 3 hours. After completion of reaction (TLC and LCMS), the reaction mixture was diluted with DCM and resulting mixture was washed with 5% aq. LiCl (5 × 15 ml), brine (3 × 10 ml) and water (2 × 10 ml). The combined DCM layers was dried over sodium sulphate and concentrated *in vacuo* to afford crude product. This crude product was washed with diethyl ether to obtain product as a white solid (0.300 g, 55%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.33 (dd, J = 7.6 and 0.8 Hz, 1H), 7.19 (d, J = 8.8 Hz, 2H), 7.12 (overlapping d, J = 8.8 Hz, 2H), 7.08 – 7.00 (m, 4H), 7.01 – 6.94 (m, 1H), 6.89 (d, J = 8.4 Hz, 2H), 5.21 (s, 2H), 4.51 (m, 1H), 4.12 – 3.90 (m, 2H), 3.77 (s, 3H), 3.72 (d, J = 12.0 Hz, 2H), 3.22 (m, 1H), 2.95 – 2.80 (m, 1H), 2.15 – 1.92 (m, 2H), 1.55 – 1.34 (m, 1H) and 1.23 (qd, J = 11.8, 4.1 Hz, 1H); ^{13}C-NMR (101 MHz, Methanol-d_4) δ 170.9, 163.4, 161.0 (d, J = 242.64 Hz, C-F), 158.7, 153.8, 141.6, 134.0, 132.3, 129.3 (2C), 128.2, 128.1, 127.0, 121.1, 119.5, 115.2, 114.9, 114.8, 113.8 (2C), 107.8, 54.3, 49.9, 45.1, 43.9, 40.9, 39.3, 32.2, 31.4. LC-MS (APCI/ESI)$^+$, found *m/z* = 473.2 $[M+H]^+$ (cal. for $C_{28}H_{29}FN_4O_2$, 472.23). Purity = 99%, t_R = 3.816 min.



6 or **11** **12 – 14, 27 – 31, 33 – 38**

General Procedure 6 (GP6): Synthesis of Compounds **12** – **14**, **27** – **31**, **33** – **38**. A solution of **6** or **11a** – **c** (1.0 equiv) and K_2CO_3 (1.5 equiv) in MeCN was stirred under reflux at 80 °C for 30 minutes. Thereafter, 1.2 equiv of previously prepared **9a – 9d**, or an appropriate commercially sourced alkylating agent was then added, and the resulting mixture further stirred under reflux at 85 °C for 5 – 24 hours. After completion, MeCN was taken off under reduced pressure, the residue taken up in 10% MeOH/DCM and filtered. The filtrate was adsorbed on silica gel, after which column chromatography was performed using a 3 – 10% MeOH/DCM gradient as eluent, to afford pure final compounds.

*4-(2-(4-((1H-benzo[d]imidazol-2-yl)amino)piperidin-1-yl)ethyl)benzonitrile (**12**)*. Obtained from **11a** (2.50 g, 11.6 mmol) and **9a** (2.91 g, 13.9 mmol) as a pale-yellow solid (3.40 g, 85%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.65 (d, J = 8.3 Hz, 2H), 7.44 (d, J = 8.3 Hz, 2H), 7.20 (dd, J = 5.8, 3.2 Hz, 2H), 6.98 (dd, J = 5.8, 3.2 Hz, 2H), 3.68 (tt, J = 10.7, 4.2 Hz, 1H), 3.12 – 3.02 (m, 2H), 2.99 – 2.88 (m, 2H), 2.74 – 2.65 (m, 2H), 2.35 (td, J = 11.8, 2.6 Hz, 2H), 2.17 – 2.04 (m, 2H), 1.70 – 1.58 (m, 2H). ^{13}C NMR (101 MHz, Methanol-d_4) δ 154.17, 146.02, 137.01, 131.98 (2C), 129.49 (2C), 120.18 (2C), 118.44, 111.29 (2C), 109.68, 59.02, 51.93 (2C), 49.32, 32.73, 31.58 (2C). LC-MS (APCI$^+$/ESI): found m/z = 346.2 [M+H] $^+$ (cal. For $C_{21}H_{23}N_5$, 345.20). Purity: 99%, t_R = 0.196 min.

*4-(2-(4-((1-methyl-1H-benzo[d]imidazol-2-yl)amino)piperidin-1-yl)ethyl) benzonitrile (**13**)*. Obtained from **11b** (0.100 g, 0.43 mmol) and **9a** (0.110 g, 0.52 mmol) as a pale-yellow solid (0.129 g, 84%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.44 (d, J = 8.3 Hz, 2H), 7.23 (d, J = 8.3 Hz, 2H), 7.06 (dd, J = 7.2, 1.1 Hz, 1H), 6.92 (dd, J = 7.5, 1.6 Hz, 1H), 6.85 – 6.76 (m, 2H), 3.57 (tt, J = 11.1, 4.2 Hz, 1H), 3.31 (s, 3H), 2.94 – 2.85 (m, 2H), 2.77 – 2.69 (m, 2H), 2.52 – 2.45 (m, 2H), 2.11 (td, J = 12.0, 2.4 Hz, 1H), 1.97 – 1.88 (m, 2H), 1.47 (dtd, J = 12.2, 11.5, 3.8 Hz, 2H). ^{13}C NMR (101 MHz, Methanol-d_4) δ 154.23, 146.12, 140.98, 134.53 (2C), 131.97 (2C), 129.50, 120.75, 119.31, 118.45, 114.34, 109.65, 106.97, 59.09, 52.28 (2C), 49.87, 32.79, 31.58 (2C),

27.18. LC-MS (APCI$^+$/ESI): found *m/z* = 360.2 [M+H] $^+$ (cal. For $C_{22}H_{25}N_5$, 359.21). Purity: 99%, t_R= 0.212 min.

*4-(2-(4-((1-(4-cyanobenzyl)-1H-benzo[d]imidazol-2-yl) amino) piperidin-1-yl) ethyl) benzonitrile (**14**)*. Obtained from **11c** (0.100 g, 0.30 mmol) and **9a** (0.076 g, 0.36 mmol) as a white solid (0.119 g, 86%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.79 (d, *J* = 8.3 Hz, 2H), 7.73 (d, *J* = 8.3 Hz, 2H), 7.45 (d, *J* = 8.2 Hz, 2H), 7.27 (d, *J* = 8.2 Hz, 2H), 7.21 (dd, *J* = 7.6, 1.3 Hz, 1H), 7.01 (dd, *J* = 7.7, 1.2 Hz, 1H), 6.97 – 6.91 (m, 1H), 6.86 – 6.79 (m, 1H), 6.57 (d, *J* = 6.57 Hz, 1H), 5.38 (s, 2H), 4.01 (tt, *J* = 10.5, 3.7 Hz, 1H), 3.75 – 3.69 (m, 2H), 2.90 – 2.89 (m, 2H), 2.83 – 2.75 (m, 2H), 2.56 – 2.48 (m, 2H), 2.08 – 1.98 (m, 2H), 1.95 – 1.86 (d, *J* = 12.8 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 154.49, 147.47, 144.34, 143.48, 143.30, 134.72, 132.97 (2C), 132.46 (2C), 130.28 (2C), 128.10 (2C), 121.17, 119.50, 118.98, 115.62, 110.56, 109.13, 108.13, 59.12, 52.59 (2C), 50.50, 44.60, 33.39, 32.32(2C). LC-MS (APCI+/ESI): found *m/z* = 461.2 [M+H]$^+$ (cal. For $C_{29}H_{28}N_6$, 460.24). Purity: 98%, t_R = 2.382min.

*4-((2-((1-phenethylpiperidin-4-yl)amino)-benzimidazoyl)methyl)benzonitrile (**27**)*. Obtained from **11c** (0.080 g, 0.24 mmol) and (2-bromoethyl)benzene (0.054 g, 0.30 mmol) as a pale yellow crystalline solid (0.078 g, 75%). ^{1}H NMR (400 MH z, Methanol-d_4) δ δ 7.67 (d, *J* = 8.6 Hz, 2H), 7.34 (dd, *J* = 7.9, 0.9 Hz, 1H), 7.30 – 7.16 (m, 7H), 7.06 (ddd, *J* = 8.6, 7.1, 1.4 Hz, 1H), 7.00 (dd, *J* = 7.9, 1.5 Hz, 1H), 6.95 (ddd, *J* = 7.9, 7.1, 1.1 Hz, 1H), 5.38 (s, 2H), 3.86 (tt, *J* = 11.0, 4.2 Hz, 1H), 3.13 – 3.06 (m, 2H), 2.88 – 2.81 (m, 2H), 2.74 – 2.67 (m, 2H), 2.43 – 2.34 (m, 2H), 2.17 – 2.07 (m, 2H), 1.72 – 1.58 (m, 2H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 154.02, 143.26, 142.00, 136.25, 135.98, 133.31, 131.78 (2C), 128.96 (2C), 128.22 (2C), 127.32 (2C), 122.04, 119.56, 118.65, 114.96, 111.56, 108.09, 60.01, 52.32 (2C), 49.39, 44.56, 31.83, 31.13 (2C). LC-MS (APCI+/ESI): found *m/z* = 435.1 [M+H] $^+$ (cal. For $C_{28}H_{29}N_5$, 435.24). Purity: 99%, t_R = 2.313 min.

*4-((2-((1-(4-(methylsulfonyl) phenethyl) piperidin-4-yl) amino)-1H-benzo[d]imidazol-1-yl) methyl) benzonitrile (**28**)*. Obtained from **11c** (0.080 g, 0.24 mmol) and 1-(2-bromoethyl)-4-(methylsulfonyl)benzene (0.076 g, 0.29 mmol) as a white crystalline solid (0.108 g, 88%). ^{1}H NMR (600 MH z, DMSO-d_6) δ 7.82 (d, *J* = 7.9 Hz, 2H), 7.79 (d, *J* = 8.0 Hz, 2H), 7.52 (d, *J* = 7.9 Hz, 2H), 7.27 (d, *J* = 8.0 Hz, 2H), 7.22 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.01 (dd, *J* = 7.8, 1.3 Hz, 1H), 6.94 (ddd, *J* = 7.8, 7.2, 1.2 Hz, 1H), 6.83 (ddd, *J* = 7.8, 7.2, 1.1 Hz, 1H), 6.58 (d, *J* = 7.43 Hz, 1H), 5.39 (s, 2H), 3.76 (tt, *J* = 10.3, 4.0 Hz, 1H), 3.18 (s, 3H), 2.94 – 2.89 (m, 2H), 2.87 – 2.83

(m, 2H), 2.62 – 2.54 (m, 2H), 2.13 – 2.05 (m, 2H), 2.00 – 1.93 (m, 2H), 1.55 – 1.45 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 154.44, 143.48, 143.27, 138.89, 134.69, 132.97 (2C), 130.04 (2C), 128.07 (2C), 127.27 (2C), 121.13, 119.11, 118.94, 115.58, 110.51, 108.11, 59.27, 52.58, 50.46 (2C), 44.53, 44.07, 33.10, 32.28 (2C), 16.88. LC-MS (APCI+/ESI): found *m/z* = 514.2 $[M+H]^+$ (cal. For $C_{29}H_{31}N_5O_2S$, 513.22). Purity: 99%, t_R = 2.157 min.

*4-((2-((1-(4-methylphenethyl)piperidin-4-yl)amino)-1H-benzo[d]imidazol-1-yl) methyl) benzonitrile (**29**)*. Obtained from **11c** (0.080 g, 0.24 mmol) and 1-(2-bromoethyl)-4-methylbenzene (0.058 g, 0.29 mmol) as a pale yellow crystalline solid (0.082 g, 76%). M.p.: 179 – 181 °C; R_f (10% MeOH/DCM) 0.44. ^{1}H NMR (600 MH z, Methanol-d_4) δ 7.66 (d, *J* = 8.5 Hz, 2H), 7.34 (dd, *J* = 7.9, 1.2 Hz, 1H), 7.25 (d, *J* = 8.5 Hz, 2H), 7.10 (br-s, 4H), 7.06 (ddd, *J* = 8.0, 7.2, 1.3 Hz, 1H), 7.01 (dd, *J* = 7.6, 1.2 Hz, 1H), 6.95 (ddd, *J* = 7.6, 1.5 Hz, 1H), 5.38 (s, 2H), 3.87 (tt, *J* = 10.9, 4.6 Hz, 1H), 3.19 – 3.12 (m, 2H), 2.84 – 2.81 (m, 2H), 2.78 – 2.72 (m, 2H), 2.51 – 2.44 (m, 2H), 2.29 (s, 3H), 2.18 – 2.08 (m, 2H), 1.68 (dtd, *J* = 12.1, 11.1, 3.6 Hz, 2H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 153.84, 142.27, 141.48, 135.86, 135.61, 133.83, 132.24 (2C), 128.80 (2C), 128.14 (2C), 127.09 (2C), 121.34, 119.66, 118.01, 114.83, 111.01, 107.58, 59.63, 52.01 (2C), 49.43, 44.22, 31.79, 30.84 (2C), 19.61. LC-MS (APCI+/ESI): found *m/z* = 450.2 $[M+H]^+$ (cal. For $C_{29}H_{31}N_5$, 449.26). Purity: 99%, t_R = 2.365 min.

*4-((2-((1-(4-(trifluoromethyl) phenethyl) piperidin-4-yl)amino)-1H-benzo[d]imidazol-1-yl) methyl) benzonitrile (**30**)*. Obtained from **11c** (0.080 g, 0.24 mmol) and 1-(2-bromoethyl)-4-(trifluoromethyl)benzene (0.073 g, 0.29 mmol) as a pale yellow crystalline solid (0.095 g, 79%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.66 (d, *J* = 8.4 Hz, 2H), 7.58 (d, *J* = 8.0 Hz, 2H), 7.42 (d, *J* = 8.4 Hz, 2H), 7.33 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.25 (d, *J* = 8.0 Hz, 2H), 7.06 (ddd, *J* = 8.0, 7.3, 1.2 Hz, 1H), 7.00 (dd, *J* = 8.1, 1.3 Hz, 1H), 6.95 (ddd, *J* = 8.1, 7.3, 1.1 Hz, 1H), 5.38 (s, 2H), 3.84 (tt, *J* = 10.9, 4.1 Hz, 1H), 3.08 – 3.02 (m, 2H), 2.94 – 2.89 (m, 2H), 2.72 – 2.64 (m, 2H), 2.36 – 2.30 (m, 2H), 2.13 – 2.05 (m, 2H), 1.63 (dtd, *J* = 11.8, 10.0, 3.8 Hz, 2H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 153.93, 144.51, 142.30, 141.56, 133.84, 132.23 (2C), 128.96 (2C), 128.26 (q, *J* = 22.3 Hz), 127.11 (2C), 124.93 (2C), 123.53, 121.30, 119.57, 118.02, 114.79, 110.99, 107.51, 59.30, 52.12 (2C), 49.85, 44.19, 32.43, 30.77 (2C). LC-MS (APCI+/ESI): found *m/z* = 504.2 $[M+H]^+$ (cal. For $C_{29}H_{28}F_3N_5$, 503.23). Purity: 99%, t_R = 2.633 min.

*Methyl 4-(2-(4-((1-(4-cyanobenzyl)-1H-benzo[d]imidazol-2-yl)amino)piperidin-1-yl) ethyl) benzoate (**31**).* Obtained from **11c** (0.200 g, 0.60 mmol) and **9c** (0.176 g, 0.72 mmol) as a pale-

yellow solid (0.263 g, 89%). ^{1}H NMR (600 MHz, Methanol-d_4) δ 7.94 (d, J = 8.3 Hz, 2H), 7.66 (d, J = 8.4 Hz, 2H), 7.37 – 7.30 (m, 3H), 7.25 (d, J = 8.4 Hz, 2H), 7.06 (ddd, J = 7.7, 7.3, 1.3 Hz, 1H), 7.00 (dd, J = 7.8, 1.3 Hz, 1H), 6.95 (ddd, J = 7.8, 7.3, 1.1 Hz, 1H), 5.37 (s, 2H), 3.88 (s, 3H), 3.83 (tt, J = 10.9, 4.1 Hz, 1H), 3.07 – 3.00 (m, 2H), 2.92 – 2.88 (m, 2H), 2.70 – 2.64 (m, 2H), 2.35 – 2.28 (m, 2H), 2.12 – 2.06 (m, 2H), 1.67 – 1.58 (m, 2H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 167.12, 153.94, 145.77, 142.31, 141.59, 133.85 (2C), 132.23 (2C), 129.33 (2C), 128.53 (2C), 127.94, 127.10, 121.29, 119.55, 118.02, 114.79, 110.99, 107.50, 59.34, 52.13 (2C), 51.08, 49.88, 44.18, 32.67, 31.37 (2C). LC-MS (APCI$^+$/ESI): found m/z = 494.2 [M+H] $^+$ (cal. For $C_{30}H_{31}N_5O_2$, 493.25). Purity: 99%, t_R = 2.330 min.

*Ethyl 4-(2-(4-((1-(4-cyanobenzyl)-1H-benzo[d]imidazol-2-yl) amino) piperidin-1-yl) ethyl) benzoate (**33**)*. Obtained from **11c** (0.150 g, 0.45 mmol) and **9d** (0.140 g, 0.54 mmol) as a pale-yellow solid (0.125 g, 55%); m.p.: 152 – 154 °C; R_f (10% MeOH/DCM) 0.45. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.95 (d, J = 8.2 Hz, 2H), 7.67 (d, J = 8.5 Hz, 2H), 7.36 (d, J = 8.2 Hz, 2H), 7.33 (dd, J = 7.9, 0.9 Hz, 1H), 7.25 (d, J = 8.5 Hz, 2H), 7.06 (ddd, J = 7.9, 7.3, 1.1 Hz, 1H), 7.01 (dd, J = 8.0, 1.1 Hz, 1H), 6.95 (ddd, J = 8.0, 7.3, 1.1 Hz, 1H), 5.38 (s, 2H), 4.35 (q, J = 7.1 Hz, 2H), 3.85 (tt, J = 10.9, 4.1 Hz, 1H), 3.09 – 3.05 (m, 2H), 2.93 – 2.90 (m, 2H), 2.74 – 2.69 (m, 2H), 2.40 – 2.33 (m, 2H), 2.14 – 2.09 (m, 2H), 1.68 – 1.60 (m, 2H), 1.38 (t, J = 7.1 Hz, 3H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 167.54, 154.80, 146.40, 143.20, 142.42, 134.74, 133.14 (2C), 130.20 (2C), 129.41 (2C), 129.21 (2C), 128.00, 122.21, 120.50, 118.92, 115.69, 111.91, 108.43, 61.52, 60.13, 53.01 (2C), 50.68, 45.11, 33.46, 32.16 (2C), 14.08. LC-MS (APCI$^+$/ESI): found m/z = 508.2 [M+H] $^+$ (cal. For $C_{31}H_{33}N_5O_2$, 507.26). Purity: 99%, t_r = 2.419 min.

*Ethyl 4-(2-(4-((1-(4-fluorobenzyl)-1H-benzo[d]imidazol-2-yl)amino)piperidin-1-yl) ethyl) benzoate (**34**)*. Obtained from **6** (0.120 g, 0.371 mmol) and **9d** (0.120 g, 0.45 mmol) as a white solid (0.060 g, 32%). ^{1}H-NMR (Methanol-d_4, 400 MHz) δ 7.98 (d, J = 8.4 Hz, 2H), 7.52 (d, J = 7.6 Hz, 1H), 7.27 (d, J = 8.4 Hz, 2H), 7.20-7.12 (m, 3H), 7.10-6.98 (m, 4H), 5.11 (s, 2H), 4.38 (q, J = 7.2 Hz, 2H), 4.27-4.11 (m, 1H), 4.09-3.98 (m, 1H, 2H), 3.02-2.86 (m, 2H), 2.78-2.59 (m, 2H), 2.39 (t, J = 12.0 Hz, 2H), 2.23-2.10 (m, 2H), 1.70-1.57 (m, 2H), 1.41 (t, J = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, Methanol-d_4) δ 166.5, 163.7, and 161.3 (d, J_F = 244.17), 153.2, 144.9, 142.0, 134.0, 131.2, 129.8 (2C), 128.7 (2C), 128.3, 128.4, 121.7, 119.9, 116.5, 116.3, 116.1, 114.8, 107.3, 60.9, 59.5, 52.1 (2C), 49.4, 45.1, 33.2, 32.0 (2C), and 14.33; LC-MS (APCI/ESI)$^+$, found m/z = 501.2 [M+H]$^+$ (calculated for $C_{30}H_{33}FN_4O_2$, 500.25). Purity: 97%, t_R = 2.565 min.

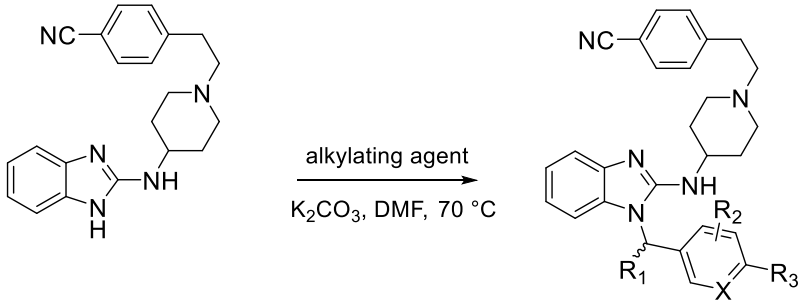
*4-((2-((1-(2-(1H-pyrazyl)ethyl)piperidin-4-yl)amino)-1-benzimidazyl) methyl) benzonitrile (**35**)*. Obtained from **11c** (0.084 g, 0.25 mmol) and 2-bromoethyl-1*H*-pyrazole (0.054 g, 0.30 mmol) as a yellow solid (0.057g, 53%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.79 (d, *J* = 8.3 Hz, 2H), 7.71 (dd, *J* = 7.3, 1.9 Hz, 1H), 7.40 (dd, *J* = 7.3, 1.8 Hz, 1H), 7.27 (d, *J* = 8.3 Hz, 2H), 7.22 (dd, *J* = 7.4, 7.3 Hz, 1H), 7.01 (dd, *J* = 7.5, 1.3, 1H), 6.94 (ddd, *J* = 8.0, 7.5, 1.1 Hz, 1H), 6.82 (dd, *J* = 8.0, 1.2 Hz, 1H), 6.57 (ddd, *J* = 8.0, 7.5, 1.1 Hz, 1H), 6.20 (t, *J* = 2.0 Hz, 1H), 5.38 (s, 2H), 4.21 (t, *J* = 6.6 Hz, 2H), 3.70 (tt, *J* = 10.2, 3.8 Hz, 1H), 2.84 – 2.78 (m, 2H), 2.70 (t, *J* = 6.6 Hz, 2H), 2.17 – 2.05 (m, 2H), 1.93 – 1.85 (m, 2H), 1.55 – 1.41 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 154.49, 143.49, 143.33, 138.76, 134.73, 132.97 (2C), 130.42 (2C), 128.11, 121.15, 118.96, 115.63, 112.43, 110.57, 108.13, 105.22, 57.93, 52.67 (2C), 50.37, 49.59, 44.61, 32.34 (2C). LC-MS (APCI+/ESI): found *m/z* = 426 $[M+H]^+$ (cal. For $C_{25}H_{27}N_7$, 425.23). Purity: 98%, t_R = 2.122 min.

*4-((2-((1-(2-(pyridin-2-yl)ethyl)piperidin-4-yl)amino)-1H-benzo[d]imidazol-1-yl) methyl) benzonitrile (**36**)*. Obtained from **11c** (0.100 g, 0.30 mmol) and **9b** (0.067 g, 0.36 mmol) as an off-white solid (0.101 g, 75%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.45 (dd, *J* = 5.0, 1.9 Hz, 1H), 7.76 (dd, *J* = 7.7, 1.8 Hz, 1H), 7.67 (d, *J* = 8.5 Hz, 2H), 7.37 – 7.32 (m, 2H), 7.29 – 7.23 (m, 3H), 7.06 (ddd, *J* = 7.9, 7.1, 1.5 Hz, 1H), 7.00 (dd, *J* = 8.0, 1.4, 1H), 6.95 (ddd, *J* = 8.1, 7.1, 1.1 Hz, 1H), 5.38 (s, 2H), 3.86 (tt, *J* = 10.9, 4.1 Hz, 1H), 3.11 – 3.06 (m, 2H), 3.05 – 3.00 (m, 2H), 2.88 – 2.82 (m, 2H), 2.45 – 2.36 (m, 2H), 2.15 – 2.08 (m, 2H), 1.70 – 1.59 (m, 2H). ^{13}C NMR (101 MHz, Methanol-d_4) δ 159.50, 153.95, 148.38, 142.32, 141.61, 137.33, 133.90, 132.27 (2C), 127.15 (2C), 123.58, 121.67, 121.33, 119.62, 118.04, 114.88, 111.05, 107.56, 57.71, 52.02 (2C), 49.73, 44.27, 34.19, 31.22 (2C). LC-MS (APCI+/ESI): found *m/z* = 437.2 $[M+H]^+$ (cal. For $C_{27}H_{28}N_6$, 436.24). Purity: 99%, t_R = 2.134 min.

*4-((2-((1-(2-(piperidin-1-yl)ethyl)piperidin-4-yl)amino)-1H-benzo[d]imidazol-1-yl) methyl) benzonitrile (**37**)*. Obtained from **11c** (0.060 g, 0.18 mmol) and 1-(2-bromoethyl)piperidine (0.040 g, 0.22 mmol) as a white solid (0.036 g, 45%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.67 (d, *J* = 8.4 Hz, 2H), 7.33 (dd, *J* = 7.4, 0.9 Hz, 1H), 7.25 (d, *J* = 8.4, 2H), 7.05 (ddd, *J* = 8.2, 7.4, 1.1 Hz, 1H), 7.00 (dd, *J* = 8.1, 1.2 Hz, 1H), 6.94 (ddd, *J* = 8.1, 7.4, 1.1 Hz, 1H), 5.37 (s, 2H), 3.81 (tt, *J* = 10.9, 5.3 Hz, 1H), 2.94 (t, *J* = 3.1 Hz, 2H), 2.60 – 2.52 (m, 4H), 2.50 (m, 4H), 2.25 (dtd, *J* = 12.3, 11.8, 2.6 Hz, 2H), 2.05 – 1.88 (m, 2H), 1.65 – 1.52 (m, 6H), 1.49 – 1.41 (m, 2H). ^{13}C NMR (101 MHz, Methanol-d_4) δ 154.01, 142.35, 141.71, 133.91, 132.25 (2C), 127.15 (2C), 121.28, 119.54, 118.04, 114.86, 111.04, 107.49, 55.83, 54.81, 54.51 (2C), 52.63 (2C), 49.91, 44.24, 31.45 (2C),

25.09 (2C), 23.68. LC-MS (APCI+/ESI): found *m/z* = 443.2 $[M+H]^+$ (cal. For $C_{27}H_{34}N_6$, 442.28). Purity: 98%, t_R = 2.236 min.

*4-((2-((1-(2-morpholinoethyl)piperidin-4-yl)amino)-1H-benzo[d]imidazol-1-yl) methyl) benzonitrile (**38**)*. Obtained from **11c** (0.070 g, 0.21 mmol) and 4-(2-bromoethyl)morpholine (0.047 g, 0.25 mmol) as a pale-yellow solid (0.039 g, 42%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.69 (d, *J* = 8.3 Hz, 2H), 7.34 (dd, *J* = 7.8, 1.0 Hz, 1H), 7.26 (d, *J* = 8.3 Hz, 2H), 7.07 (ddd, *J* = 8.0, 7.1, 1.0 Hz, 1H), 7.02 (dd, *J* = 8.0, 0.9 Hz, 1H), 6.97 (ddd, *J* = 8.0, 7.2, 1.1 Hz, 1H), 5.40 (s, 2H), 3.90 (tt, *J* = 10.6, 5.2 Hz, 1H), 3.73 – 3.69 (m, 4H), 3.27 (t, *J* = 6.9 Hz, 2H), 2.89 (t, *J* = 7.0 Hz, 2H), 2.67 (m, 4H), 2.55 (m, 4H), 2.18 (m, 2H), 1.75 (m, 2H). ^{13}C NMR (101 MHz, Methanol-d_4) δ 142.22, 133.85, 132.28 (2C), 127.13 (2C), 121.44, 119.83, 118.02, 114.91, 112.34, 111.09, 107.71, 66.22, 54.28, 54.01 (2C), 53.44 (2C), 52.22 (2C), 48.89, 44.34, 30.18 (2C). LC-MS (APCI+/ESI): found *m/z* = 445.2 $[M+H]^+$ (cal. For $C_{26}H_{32}N_6O$, 444.26). Purity: 99%, t_R = 2.025 min.



12 **15 – 17** and **19 – 26**

General Procedure 7 (GP7): Synthesis of Compounds **15** – **17** and **19** – **26**. A mixture of **12** (1.0 equiv), alkyl bromide (1.2 equiv) and K_2CO_3 (1.5 equiv) in 5 ml DMF was stirred under nitrogen at 70 °C for 12 hours. After cooling to ambient temperature (23 °C), the mixture was diluted with EtOAc (25 ml). The resulting mixture was washed with water (3 × 30 ml), combined aqueous layers were then extracted with EtOAc (2 × 20 ml). Thereafter, combined EtOAc layers where further washed with 5% LiCl (2 × 10 ml), brine (15 ml), then dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was triturated with Et_2O to obtain products. Further purification was performed by flash chromatography if required.

*4-(2-(4-((1-(4-methoxybenzyl)-1H-benzo[d]imidazol-2-yl)amino)piperidin-1-yl) ethyl) benzonitrile (**15**)*. Obtained from 1-(bromomethyl)-4-methoxybenzene (0.040 g, 0.24 mmol) as a pale-yellow solid (0.082 g, 88%). ^{1}H NMR (600 MHz, Methanol-d_4) δ 7.64 (d, *J* = 8.2 Hz, 2H),

7.42 (d, *J* = 8.0 Hz, 2H), 7.30 (dd, *J* = 7.8, 1.5 Hz), 7.12 – 7.01 (m, 4H), 6.94 (ddd, *J* = 7.8, 7.2, 1.1 Hz, 1H), 6.85 (d, *J* = 8.0 Hz, 2H), 5.19 (s, 2H), 3.83 (tt, *J* = 10.6, 4.0 Hz, 1H), 3.73 (s, 3H), 3.01 – 2.95 (m, 2H), 2.94 – 2.87 (m, 2H), 2.69 – 2.63 (m, 2H), 2.35 – 2.29 (m, 2H), 2.11 – 2.06 (m, 2H), 1.64 (dtd, *J* = 12.9, 10.1, 3.7 Hz, 2H). ^{13}C NMR (151 MHz, Methanol-*d*$_4$) δ 159.26, 153.86, 146.07, 141.40, 134.04, 131.93 (2C), 129.46 (2C), 128.22 (2C), 127.65 (2C), 120.91, 119.34, 118.42, 114.54, 113.76, 109.62, 107.77, 59.05, 54.28 (2C), 51.95, 49.66, 44.08, 32.72, 31.35 (2C). LC-MS (APCI+/ESI): found *m/z* = 466.2 $[M+H]^+$ (cal. for $C_{29}H_{31}N_5O$, 465.25). Purity: 98%, t_R = 2.312 min.

*4-(2-(4-((1-(4-(methylsulfonyl)benzyl)-1H-benzo[d]imidazol-2-yl) amino) piperidin-1-yl) ethyl) benzonitrile (**16**)*. Obtained from 1-(bromomethyl)-4-(methylsulfonyl)benzene (0.060 g, 0.24 mmol) as a pale-yellow solid (0.095 g, 93%). ^{1}H NMR (600 MHz, Methanol-*d*$_4$) δ 7.88 (d, *J* = 8.5 Hz, 2H), 7.64 (d, *J* = 8.2 Hz, 2H), 7.42 (d, *J* = 8.2 Hz, 2H), 7.38 – 7.29 (m, 3H), 7.06 (ddd, *J* = 7.8, 7.3, 1.3 Hz, 1H), 7.01 (dd, *J* = 8.1, 1.5 Hz, 1H), 6.95 (ddd, *J* = 8.1, 7.3, 1.1 Hz, 1H), 5.41 (s, 2H), 3.84 (tt, *J* = 10.9, 4.1 Hz, 1H), 3.07 (s, 3H), 3.05 – 3.00 (m, 2H), 2.94 – 2.86 (m, 2H), 2.70 – 2.64 (m, 2H), 2.33 (td, *J* = 11.9, 2.5 Hz, 2H), 2.13 – 2.06 (m, 2H), 1.68 – 1.58 (m, 2H). ^{13}C NMR (151 MHz, Methanol-*d*$_4$) δ 153.93, 146.00, 143.01, 141.56, 139.92, 133.84 (2C), 131.94 (2C), 129.47 (2C), 127.47 (2C), 127.15, 121.31, 119.58, 118.43, 114.79, 109.63, 107.56, 58.98, 52.09 (2C), 49.85, 44.12, 42.87, 32.69 (2C), 31.35. LC-MS (APCI$^+$/ESI): found *m/z* = 514.2 $[M+H]^+$ (cal. for $C_{29}H_{31}N_5O_2S$, 513.22). Purity: 99%, t_R = 2.093 min.

*Methyl 4-((2-((1-(4-cyanophenethyl) piperidin-4-yl) amino)-1H-benzo[d]imidazol-1-yl) methyl) benzoate (**17**)*. Obtained from methyl 4-(bromomethyl)benzoate (0.055 g, 0.24 mmol) as a pale-yellow solid (0.092 g, 93%). ^{1}H NMR (600 MHz, Methanol-*d*$_4$) δ 7.94 (d, *J* = 8.4 Hz, 2H), 7.64 (d, *J* = 8.0 Hz, 2H), 7.42 (d, *J* = 8.0 Hz, 2H), 7.33 (dd, *J* = 7.7, 1.3 Hz, 1H), 7.20 (d, *J* = 8.4 Hz, 2H), 7.05 (ddd, *J* = 7.9, 7.2, 1.2 Hz, 1H), 7.02 (dd, *J* = 8.1, 1.3 Hz, 1H), 6.95 (ddd, *J* = 8.1, 7.2, 1.1 Hz, 1H), 5.36 (s, 2H), 3.88 – 3.81 (m, 4H), 3.04 – 2.98 (m, 2H), 2.93 – 2.87 (m, 2H), 2.71 – 2.62 (m, 2H), 2.36 – 2.29 (m, 2H,), 2.12 – 2.07 (m, 2H), 1.63 (dtd, *J* = 11.6, 9.5, 3.7 Hz, 2H). ^{13}C NMR (151 MHz, Methanol-*d*$_4$) δ 166.69, 153.93, 146.01, 141.99, 141.48, 133.95 (2C), 131.94 (2C), 129.51 (2C), 129.45 (2C), 129.23, 126.30, 121.18, 119.52, 118.41, 114.71, 109.63, 107.58, 58.99, 52.00 (2C), 51.17, 49.75, 44.29, 32.67 (2C), 31.31. LC-MS (APCI$^+$/ESI): found *m/z* = 494.2 $[M+H]^+$ (cal. for $C_{30}H_{31}N_5O_2$, 493.25). Purity: 98%, t_R = 2.347 min.

*4-(2-(4-((1-(4-(trifluoromethyl) benzyl)-1H-benzo[d]imidazol-2-yl) amino) piperidin-1-yl) ethyl) benzonitrile (**19**)*. Obtained from 1-(bromomethyl)-4-(trifluoromethyl)benzene (0.057 g, 0.24 mmol) as a pale-yellow solid (0.091 g, 90%). ^{1}H NMR (600 MHz, Methanol-d_4) δ 7.64 (d, *J* = 8.2 Hz, 2H), 7.60 (d, *J* = 8.1 Hz, 2H), 7.42 (d, *J* = 8.2 Hz, 2H), 7.33 (dd, *J* = 7.8, 1.3 Hz, 1H), 7.27 (d, *J* = 8.1 Hz, 2H), 7.06 (ddd, *J* = 7.9, 7.2, 1.3 Hz, 1H), 7.01 (dd, *J* = 7.7, 1.2 Hz, 1H), 6.95 (ddd, *J* = 7.7, 7.2, 1.3 Hz, 1H), 5.38 (s, 2H), 3.84 (tt, *J* = 10.9, 4.1 Hz, 1H), 3.06 – 3.00 (m, 2H), 2.94 – 2.88 (m, 2H), 2.71 – 2.64 (m, 2H), 2.32 (td, *J* = 12.0, 2.5 Hz, 2H), 2.13 – 2.07 (m, 2H), 1.67 – 1.58 (m, 2H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 153.95, 146.02, 141.53, 141.06, 133.91, 131.93 (2C), 129.45 (2C), 126.75 (2C), 125.25 (2C), 125.22, 121.23, 119.55, 118.42, 114.74, 109.63, 107.57, 59.01, 52.09 (2C), 49.83, 44.10, 32.71, 31.36 (2C). LC-MS (APCI$^+$/ESI): found *m/z* = 504.2 [M+H] $^+$ (cal. For $C_{29}H_{28}F_3N_5$, 503.23). Purity: 98%, t_R = 2.463 min.

*4-(2-(4-((1-(4-methylbenzyl)-1H-benzo[d]imidazol-2-yl)amino)piperidin-1-yl) ethyl) benzonitrile (**20**)*. Obtained from 1-(bromomethyl)-4-methylbenzene (0.044 g, 0.24 mmol) as a pale-yellow solid (0.079 g, 88%). ^{1}H NMR (600 MHz, Methanol-d_4) δ 7.64 (d, *J* = 8.3 Hz, 2H), 7.42 (d, *J* = 8.1 Hz, 2H), 7.30 (dd, *J* = 7.5, 1.2 Hz, 1H), 7.10 (d, *J* = 8.3 Hz, 2H), 7.07 – 6.97 (m, 4H), 6.93 (ddd, *J* = 8.0, 7.2, 1.5 Hz, 1H), 5.21 (s, 2H), 3.83 (tt, *J* = 10.8, 4.2 Hz, 1H), 3.03 – 2.95 (m, 2H), 2.93 – 2.86 (m, 2H), 2.68 – 2.61 (m, 2H), 2.31 (td, *J* = 11.9, 2.6 Hz, 2H), 2.27 (s, 3H), 2.12 – 2.04 (m, 2H), 1.63 (dtd, *J* = 12.6, 11.0, 3.7 Hz, 2H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 155.65, 147.39, 144.68, 140.52, 138.69, 132.40 (2C), 130.48 (2C), 129.06 (2C), 128.57 (2C), 128.03, 120.57, 119.28, 118.66, 114.35, 110.43, 108.26, 59.68, 51.62 (2C), 49.36, 43.82, 41.86, 33.68 (2C), 30.07. LC-MS (APCI$^+$/ESI): found *m/z* = 450.2 [M+H] $^+$ (cal. for $C_{29}H_{31}N_5$, 449.26). Purity: 99%, t_r = 2.383 min.

*4-(2-(4-((1-((6-(trifluoromethyl)pyridin-3-yl)methyl)-1H-benzo[d]imidazol-2-yl) amino) piperidin-1-yl)ethyl)benzonitrile (**21**)*. Obtained from 5-(bromomethyl)-2-(trifluoromethyl)pyridine (0.058 g, 0.24 mmol) as a white solid (0.075 g, 74%). ^{1}H NMR (600 MHz, Methanol-d_4) δ 8.54 (d, *J* = 2.1 Hz, 1H), 7.75 (d, *J* = 8.2 Hz, 1H), 7.67 – 7.62 (m, 3H), 7.43 (d, *J* = 8.3 Hz, 2H), 7.36 (dd, J = 8.2, 2.1 Hz, 1H), 7.10 – 7.05 (m, 2H), 6.99 (ddd, *J* = 8.0, 7.3, 1.3 Hz, 1H), 5.45 (s, 2H), 3.84 (tt, *J* = 11.0, 4.2 Hz, 1H), 3.05 (m, 2H), 2.95 – 2.90 (m, 2H), 2.71 – 2.68 (m, 2H), 2.37 – 2.32 (m, 2H), 2.14 – 2.09 (m, 2H), 1.67 – 1.59 (m, 2H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 153.85, 148.25, 146.00, 141.61, 136.42, 135.99, 133.71, 131.98 (2C), 129.49 (2C), 128.87 (2C), 128.09 (2C), 121.52, 120.44, 119.78, 118.45, 114.96, 109.67, 107.47,

58.99, 52.13 (2C), 49.91, 42.02, 32.70, 31.37 (2C). LC-MS (APCI/ESI): found *m/z* = 505.1 $[M+H]^+$ (cal. for $C_{28}H_{27}F_3N_6$, 504.22). Purity: 99%, t_R = 2.356 min.

*4-(2-(4-((1-((6-methylpyridin-3-yl)methyl)-1H-benzo[d]imidazol-2-yl) amino) piperidin-1-yl) ethyl) benzonitrile (**22**)*. Obtained from 5-(bromomethyl)-4-methylpyridine (0.045 g, 0.24 mmol) as a pale-yellow solid (0.083 g, 92%). ^{1}H NMR (600 MHz, Methanol-d_4) δ 8.28 (d, *J* = 1.2 Hz, 1H), 7.89 – 7.72 (m, 3H), 7.38 (d, *J* = 8.3 Hz, 2H), 7.29 – 7.11 (m, 2H), 6.98 (dd, *J* = 7.5, 1.2 Hz, 1H), 6.85 (ddd, *J* = 8.0, 7.2, 1.5 Hz, 1H), 6.79 (ddd, J = 8.1, 7.2, 1.3 Hz, 1H), 5.22 (s, 2H), 3.82 (tt, *J* = 10.5, 4.0 Hz, 1H), 2.98 – 2.85 (m, 2H), 2.70 – 2.61 (m, 2H), 2.55 – 2.43 (m, 2H), 2.22 (td, *J* = 12.0, 2.9 Hz, 2H), 2.18 (s, 3H), 2.03 – 1.91 (m, 2H), 1.49 (dtd, *J* = 13.5, 10.2, 4.0 Hz, 2H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 157.55, 148.62, 145.33, 139.78, 138.07, 134.60, 133.89 (2C), 133.18, 131.45, 130.02 (2C), 128.87 (2C), 128.02, 121.21, 119.98, 118.20, 114.39, 110.58, 107.85, 60.87, 50.68 (2C), 49.68, 43.79, 30.55 (2C), 29.97. LC-MS ($APCI^+$/ESI): found *m/z* = 451.2 $[M+H]^+$ (cal. for $C_{28}H_{30}N_6$, 450.25). Purity: 99%, t_R = 2.436 min.

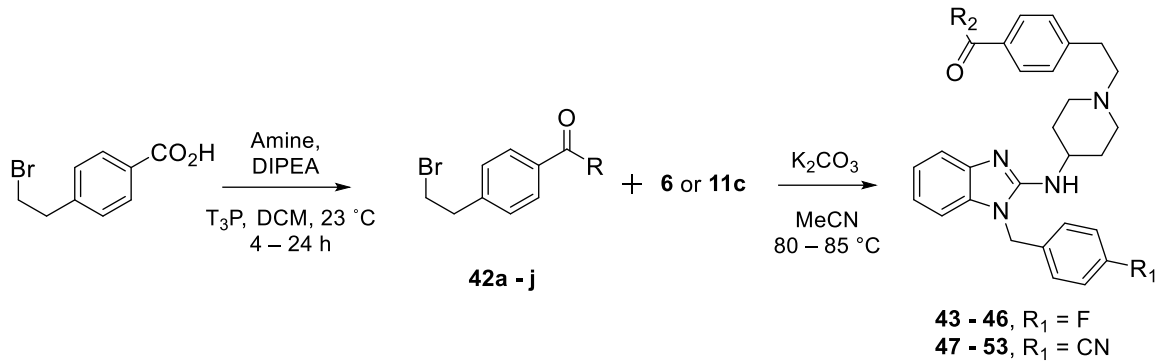
*4-(1-(2-((1-(4-cyanophenethyl)piperidin-4-yl)amino)-1H-benzo[d]imidazol-1-yl) ethyl) benzonitrile (**23**)*. Obtained from **9f** (0.050 g, 0.24 mmol) as a white solid (0.074 g, 78%). ^{1}H NMR (600 MHz, Methanol-d_4) δ 7.72 (d, *J* = 8.5 Hz, 2H), 7.65 (d, *J* = 8.3 Hz, 2H), 7.47 – 7.42 (m, 4H), 7.31 (dd, *J* = 7.8, 0.9 Hz, 1H), 6.99 (ddd, *J* = 7.8, 7.4, 1.1 Hz, 1H), 6.80 (dd, *J* = 8.3, 1.1 Hz, 1H), 6.69 (ddd, *J* = 8.3, 7.4, 0.9 Hz, 1H), 5.85 (q, *J* = 7.1 Hz, 1H), 3.85 (tt, *J* = 11.0, 4.2 Hz, 1H), 3.08 – 3.04 (m, 2H), 2.96 – 2.91 (m, 2H), 2.73 – 2.68 (m, 2H), 2.40 – 2.32 (m, 2H), 2.19 – 2.11 (m, 2H), 1.93 (d, *J* = 7.1 Hz, 3H), 1.71 – 1.62 (m, 2H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 153.70, 146.06, 145.45, 141.98, 132.25, 131.98 (2C), 129.50 (2C), 127.86 (2C), 127.03 (2C), 121.01, 119.17, 118.44, 118.00, 115.04, 111.15, 109.82, 109.68, 59.04, 52.17 (2C), 51.34, 50.04, 32.77, 31.48 (2C), 16.30. LC-MS (APCI+/ESI): found *m/z* = 474.9 $[M+H]^+$ (cal. for $C_{30}H_{30}N_6$, 474.25). Purity: 99%, t_R = 2.441min.

*4-((2-((1-(4-cyanophenethyl)piperidin-4-yl)amino)-1H-benzo[d]imidazol-1-yl) methyl)-2-fluorobenzonitrile (**24**)*. Obtained from 4-(bromomethyl)-2-fluorobenzonitrile (0.054 g, 0.24 mmol) as a white solid (0.077 g, 81%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.68 – 7.58 (m, 3H), 7.46 – 7.40 (m, 3H), 7.33 (ddd, *J* = 7.8, 7.2, 1.1 Hz, 1H), 7.06 (dd, *J* = 7.9, 1.4 Hz, 1H), 7.00 (d, *J* = 7.6 Hz, 1H), 6.98 – 6.93 (m, 1H), 6.89 (d, *J* = 6.7 Hz, 1H), 5.42 (s, 2H), 3.84 (tt, *J* = 11.2, 5.4 Hz, 1H), 3.04 – 2.98 (m, 2H), 2.91 – 2.90 (m, 2H), 2.71 – 2.63 (m, 2H), 2.31 – 2.27 (m, 2H), 2.10 – 2.01 (m, 2H). ^{13}C NMR (101 MHz, Methanol-d_4) δ 161.26, 158.78, 154.01, 146.14, 141.66,

133.71, 131.96, 129.93, 129.79, 129.48, 128.64, 128.42, 121.45 (d, *J* = 22.3 Hz), 119.65, 118.73, 114.89, 112.63, 109.64, 107.40, 59.07, 52.18, 50.45 (2C), 39.12, 32.80, 31.46 (2C). LC-MS (APCI/ESI): found *m/z* = 479.1 $[M+H]^+$ (cal. for $C_{29}H_{27}FN_6$, 478.23). Purity: 99%, t_R = 2.325 min.

*4-((2-((1-(4-cyanophenethyl)piperidin-4-yl)amino)-1H-benzo[d]imidazol-1-yl)methyl)-3-fluorobenzonitrile (**25**)*. Obtained from 4-(bromomethyl)-3-fluorobenzonitrile (0.054 g, 0.24 mmol) as a pale-yellow solid (0.070 g, 73%). ^{1}H NMR (600 MHz, Methanol-d_4) δ 7.69 (dd, *J* = 3.9, 0.6 Hz, 1H), 7.65 (d, *J* = 8.0 Hz, 2H), 7.43 (d, *J* = 8.0 Hz, 2H), 7.34 (dd, *J* = 8.0, 0.8 Hz, 1H), 7.10 – 7.05 (m, 2H), 7.05 – 7.01 (m, 2H), 7.00 – 6.95 (m, 1H), 5.39 (s, 2H), 3.84 (tt, *J* = 11.1, 4.2 Hz, 1H), 3.08 – 2.99 (m, 2H), 2.95 – 2.92 – 2.84 (m, 2H), 2.70 – 2.61 (m, 2H), 2.34 – 2.27 (m, 2H), 2.12 – 2.05 (m, 2H), 1.64 – 1.58 (m, 2H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 164.06, 162.35, 153.87, 145.98, 141.56, 133.85, 131.98 (2C), 128.94 (2C), 128.19, 122.88, 121.50, 119.74, 118.44, 114.92, 114.06 (d, *J* = 21.6 Hz), 113.10, 109.69, 107.62, 99.84, 58.99, 52.13 (2C), 49.89, 43.97, 32.70, 31.36 (2C). LC-MS (APCI+/ESI): found *m/z* = 479.1 $[M+H]^+$ (cal. for $C_{29}H_{27}FN_6$, 478.23). Purity: 99%, t_R = 2.325 min.

*2-((2-((1-(4-cyanophenethyl)piperidin-4-yl)amino)-1H-benzo[d]imidazol-1-yl) methyl)-5-fluorobenzonitrile (**26**)*. Obtained from 2-(bromomethyl)-5-fluorobenzonitrile (0.054 g, 0.24 mmol) as a white solid (0.076 g, 79%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.93 (dd, *J* = 8.5, 2.7 Hz, 1H), 7.72 (d, *J* = 8.2 Hz, 2H), 7.49 – 7.40 (m, 3H), 7.25 (d, *J* = 7.7 Hz, 1H), 6.97 (dd, *J* = 7.7, 5.3 Hz, 1H), 6.83 (ddd, *J* = 7.9, 7.2, 1.1 Hz, 1H), 6.63 – 6.57 (m, 2H), 5.49 (s, 2H), 3.82 (tt, *J* = 10.4, 3.9 Hz, 1H), 2.93 – 2.87 (m, 2H), 2.83 (m, 2H), 2.61 – 2.53 (m, 2H), 2.16 – 2.02 (m, 2H), 1.95 (m, 2H), 1.47 (m, 2H). ^{13}C NMR (101 MHz, Methanol-d_4) δ 164.45, 157.55, 154.23, 145.24, 141.03, 134.88, 130.45, 129.35, 128.88, 128.75, 128.60, 128.32, 121.33, 119.45, 118.21, 115.75 (d, *J* = 19.5 Hz), 112.87, 109.53, 107.37, 59.33, 52.21, 51.65 (2C), 39.33, 32.65, 31.08 (2C). LC-MS (APCI/ESI): found *m/z* = 479.1 $[M+H]^+$ (cal. for $C_{29}H_{27}FN_6$, 478.23). Purity: 99%, t_R = 2.330 min.



General Procedure 8 (GP8): Preparation of Compounds **43** – **53**. A solution of 4-(2-bromoethyl)benzoic acid (1.3 equivalents) was take in DCM and charged with *N*,*N*-Diisopropylethylamine (DIPEA; 3.0 equiv) and propylphosphonic anhydride (T_3P; 1.2 equiv).The resulting solution was stirred for 10 minutes and followed with addition of respective amine (1.0 equiv). The resulting reaction mixture was stirred at ambient temperature for 4-6 hours. After the completion of reaction (LCMS), diluted with DCM, washed with saturated solution of sodium bicarbonate and water, dried over sodium sulphate, and concentrated *in vacuo*. This crude amide product **42** was used in the next step using **GP5** without further purification.

*(4-(2-(4-((1-(4-fluorobenzyl)-1H-benzo[d]imidazol-2-yl)amino)piperidin-1-yl) ethyl) phenyl) (3-hydroxyazetidin-1-yl) methanone (**43**)*. Obtained from **6** (0.150 g, 0.462 mmol) and **42a** (0.158 g, 0.556 mmol) as a white solid (0.080 g, 33%). ^{1}H NMR (600 MHz, DMSO-d_6 at 60 ˚C) δ 7.55 (d, *J* = 8.0 Hz, 2H), 7.33 (d, *J* = 7.9 Hz, 2H), 7.25 – 7.20 (m, 3H), 7.13 (two overlapping doublets, *J* = 8.8 Hz, 2H), 7.05 (d, *J* = 7.7 Hz, 1H), 6.95 (t, *J* = 7.5 Hz, 1H), 6.85 (t, *J* = 7.6 Hz, 1H), 6.43 (d, *J* = 7.4 Hz, 1H), 5.57 (s, 1H), 5.28 (s, 2H), 4.51 (t, *J* = 5.9 Hz, 1H), 4.42 - 4.21 (m, 2H), 4.04-3.76 (m, 3H), 3.08-2.96 (m, 2H), 2.90-2.80 (m, 2H), 2.75-2.65 (m, 2H), 2.43-2.20 (m, 2H), 2.10 – 1.90 (m, 2H), 1.64 (m, 2H); ^{13}C-NMR (151 MHz, DMSO) δ 169.4, 162.7 and 161.1 (d, J_F = 250.0 Hz), 154.4, 143.7, 143.3, 134.8, 133.8, 131.7, 129.4, 129.4, 129.0 (2C), 128.2 (2C), 121.0, 118.9, 115.8, 115.6, 115.6, 108.3, 79.6, 61.0 (2C), 59.0, 52.4, 50.1, 44.4 (2C), 40.8, 32.8 and 31.9; LC-MS (APCI/ESI)$^+$, found *m/z* = 528.2 $[M+H]^+$ (calculated for $C_{31}H_{34}FN_5O_2$, 527.27). Purity: 99.94%, t_R = 2.314 min.

*(4-(2-(4-((1-(4-fluorobenzyl)-1H-benzo[d]imidazol-2-yl)amino)piperidin-1-yl) ethyl) phenyl) (3-hydroxypyrrolidin-1-yl)methanone (**44**)*. Obtained from **6** (0.150 g, 0.462 mmol) and **42b** (0.165 g, 0.556 mmol) as a white solid (0.070 g, 28%). ^{1}H NMR (600 MHz, Methanol-d_4) δ 7.49 (overlapping d, *J* = 7.6 Hz, 2H), 7.34 (m, 3H), 7.19-7.14 (m, 2H), 7.05 (m, 4H), 6.96 (t, *J* = 7.6

Hz, 1H), 5.27 (s, 2H), 4.60 – 4.32 (m, 1H), 3.86 (tt, *J* = 10.9 and 4.1 Hz, 1H), 3.84 – 3.68 (m, 3H), 3.65 – 3.51 (m, 1H), 3.37 (d, *J* = 11.8 Hz, 1H), 3.15-3.03 (m, 2H), 2.98-2.90 (m, 2H), 2.74 – 2.58 (m, 2H), 2.33 (t, *J* = 11.7 Hz, 2H), 2.18 – 2.11 (m, 2H), 2.08-1.94 (m, 1H) and 1.75-1.65 (m, 2H); ^{13}C NMR (151 MHz, Methanol-d_4) δ 170.9, 163.0 and 161.4 (d, J_F = 244.58 Hz), 154.0, 142.6, 141.6, 134.3, 134.0, 132.4, 128.4 (2C), 128.3, 128.2, 127.1, 127.0, 121.1, 119.4, 115.1, 115.0, 114.7, 107.7, 70.1, 68.9, 59.7, 57.1, 54.2, 52.2, 49.9, 44.1, 43.9, 33.9, 32.6, 32.0, 31.4, 29.3. LC-MS (APCI/ESI)$^+$, found *m/z* = 541.7 $[M+H]^+$ (calculated for $C_{32}H_{36}FN_5O_2$, 541.29). Purity: 97%, t_R = 2.330 min.

*(4-(2-(4-((1-(4-fluorobenzyl)-1H-benzo[d]imidazol-2-yl)amino)piperidin-1-yl) ethyl) phenyl) (4-hydroxypiperidin-1-yl)methanone (**45**)*. Obtained from **6** (0.150 g, 0.462 mmol) and **42c** (0.173 g, 0.556 mmol) as a white solid (0.190 g, 74%). ^{1}H-NMR (600 MHz, Methanol-d_4) δ 7.41 – 7.35 (m, 5H), 7.21-7.17 (m, 2H), 7.12-7.17 (m, 4H), 7.00 (t, *J* = 8.3, 1H), 5.31 (s, 2H), 4.33-4.12 (m, 1H), 3.97-3.87 (m, 2H), 3.77-3.63 (m, 1H), 3.41-3.21 (m, 2H), 3.20-3.06 (m, 2H), 3.00-2.90 (m, 2H), 2.82-2.72 (m, 2H), 2.34 (t, *J* = 12.0 Hz, 2H), 2.24-2.14 (m, 2H), 2.04-1.94 (m, 1H), 1.90-1.80 (m, 1H), 1.77-1.66 (m, 2H), 1.66-1.56 (m, 1H) and 1.54-1.44 (m, 1H); ^{13}C NMR (151 MHz, Methanol-d_4) δ 171.1, 163.10 and 161.4 (d, J_F = 252.17), 153.9, 141.9, 141.4, 134.0, 133.7, 132.4, 128.7 (2C), 128.3, 128.2, 126.7 (2C), 121.1, 119.5, 115.1, 115.0, 114.7, 107.8, 66.3, 59.4, 52.1 7 (2C), 49.7, 45.1, 43.9 7 (2C), 39.5, 34.1, 33.3, 32.3 and 31.2; LC-MS (APCI/ESI)$^+$, found *m/z* = 556.3 $[M+H]^+$ (calculated for $C_{33}H_{38}FN_5O_2$, 555.30). Purity: 99%, t_R = 2.365 min.

*(4-(2-(4-((1-(4-fluorobenzyl)-1H-benzo[d]imidazol-2-yl)amino)piperidin-1-yl) ethyl) phenyl) (piperazin-1-yl)methanone (**46**)*. Obtained from **6** (0.150 g, 0.462 mmol) and **42d** (0.165 g, 0.556 mmol) as a white solid (0.100 g, 54%). ^{1}H-NMR (600 MHz, Methanol-d_4) δ 7.45 – 7.40 (m, 4H), 7.37 – 7.33 (m, 1H), 7.20 – 7.15 (m, 1H), 7.11 – 7.03 (m, 5H), 7.00 (t, *J* = 8.2Hz, 1H), 5.31 (s, 1H), 4.01 – 3.87 (m, 1H), 3.87 – 3.57 (m, 4H), 3.33 (m, 2H), 3.11 – 2.94 (m, 8H), 2.78 – 2.68 (t, *J* = 12.0 Hz, 2H), 2.34 – 2.17 (m, 2H), 1.88 – 1.77 (m, 2H); ^{13}C NMR (151 MHz, Methanol-d_4) δ 171.1, 163.0 and 161.4 (d, J_F = 245.20), 153.5, 141.0, 140.9, 133.8, 133.1, 132.3, 128.8 (2C), 128.3, 128.2, 127.2 (2C), 121.3, 119.8, 115.2, 115.0, 114.6, 108.0, 58.4 (2C), 51.8 (2C), 48.9, 44.1, 44.0 (2C), 40.9, 31.4, 30.2 (2C); LC-MS (APCI/ESI)$^+$, found *m/z* = 540.9 $[M+H]^+$ (calculated for $C_{32}H_{37}FN_6O$, 540.3). Purity: 96%, t_R = 2.172 min.

*4-(2-(4-((1-(4-cyanobenzyl)-1H-benzoimidazol-2-yl) amino) piperidin-1-yl) ethyl)-N-methyl benzamide (**47**)*. Obtained from **11c** (0.106 g, 0.319 mmol) and **42e** (0.100 g, 0.415 mmol) as a

white solid (0.019 g, 12%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.33 (d, J = 4.2 Hz, 1H), 7.80 (d, J = 8.2 Hz, 2H), 7.75 (d, J = 8.1 Hz, 2H), 7.31 (d, J = 8.2 Hz, 2H), 7.28 (d, J = 8.2 Hz, 2H), 7.23 (d, J = 7.7 Hz, 1H), 7.02 (d, J = 7.6 Hz, 1H), 6.95 (t, J = 7.6 Hz, 1H), 6.84 (t, J = 7.4 Hz, 1H), 6.61 (d, J = 7.0 Hz, 1H), 5.40 (s, 2H), 3.76 (m, 1H), 2.93 (d, J = 10.3 Hz, 2H), 2.82 – 2.76 (m, 2H), 2.61 – 2.54 (m, 2H), 2.51 (s, 3H), 2.13 (m, 2H), 2.00 – 1.93 (m, 2H), 1.62 – 1.55 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.06, 154.50, 144.31, 143.50, 143.31, 134.73, 132.98 (2C), 132.71, 128.96 (2C), 128.12 (2C), 127.46 (2C), 121.16, 119.12, 118.98, 115.62, 110.56, 108.14, 59.58, 52.60 (2C), 50.46, 44.61, 33.15, 32.24 (2C), 26.65. (APCI/ESI)$^+$, found m/z = 493.2 [M+H]$^+$ (calculated for $C_{30}H_{32}N_6O$, 492.25). Purity: 97%, t_R = 2.669 min.

*4-(2-(4-((1-(4-cyanobenzyl)-1H-benzoimidazol-2-yl)amino)piperidin-1-yl)ethyl)-N,N-dimethyl benzamide (**48**)*. Obtained from **11c** (0.087 g, 0.261 mmol) and **42f** (0.087 g, 0.340 mmol) as a white solid (0.029 g, 22%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.68 (d, J = 8.3 Hz, 2H), 7.42 – 7.31 (m, 5H), 7.27 (d, J = 8.3 Hz, 2H), 7.12 – 7.05 (m, 1H), 7.03 (d, J = 6.6 Hz, 1H), 7.01 – 6.93 (m, 1H), 5.40 (s, 2H), 3.95 – 3.83 (m, 1H), 3.19 – 3.12 (m, 2H), 3.06 (s, 6H), 2.94 (dd, J = 10.5, 5.5 Hz, 2H), 2.91 – 2.80 (m, 2H), 2.53 – 2.49 (m, 2H), 2.20 – 2.15 (m, 2H), 1.75 – 1.69 (m, 2H).^{13}C NMR (101 MHz, Methanol-d_4) δ 172.38, 153.87, 142.28, 141.44, 134.01, 133.88, 132.27 (2C), 128.50 (2C), 127.15 (2C), 126.98 (2C), 121.38, 120.33, 119.70, 118.05, 114.88, 111.04, 107.62, 59.10, 51.98 (2C), 49.50, 44.30, 38.65, 34.30, 32.06, 30.92 (2C). (APCI/ESI)$^+$, found m/z = 507.6 [M+H]$^+$ (calculated for $C_{31}H_{34}N_6O$, 506.23). Purity: 97%, t_R = 3.270 min.

*4-((2-((1-(4-(aziridine-1-carbonyl)phenethyl)piperidin-4-yl)amino)-1H-benzoimidazol-1-yl) methyl)benzonitrile (**49**)*. Obtained from **11c** (0.055 g, 0.167 mmol) and **42g** (0.058 g, 0.217 mmol) as a white solid (0.010 g, 12% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.31 (d, J = 4.1 Hz, 1H), 7.80 (d, J = 8.4 Hz, 2H), 7.73 (d, J = 8.2 Hz, 2H), 7.30 (d, J = 8.4 Hz, 2H), 7.28 (d, J = 8.5 Hz, 2H), 7.23 (dd, J = 7.8, 1.2 Hz, 1H), 7.02 (dd, J = 7.7, 1.3 Hz, 1H), 6.97 – 6.92 (m, 1H), 6.86 – 6.81 (m, 1H), 6.57 (d, J = 7.3 Hz, 1H), 5.40 (s, 2H), 3.80 – 3.70 (m, 1H), 2.93 – 2.88 (m, 2H), 2.86 – 2.83 (m, 1H), 2.81 – 2.75 (m, 2H), 2.57 – 2.52 (m, 2H), 2.09 (t, J = 10.8 Hz, 2H), 1.99 – 1.92 (m, 2H), 1.51 (td, J = 12.4, 3.9 Hz, 2H), 0.72 – 0.66 (m, 2H), 0.59 – 0.54 (m, 2H).^{13}C NMR (101 MHz, DMSO-d_6) δ 167.86, 154.50, 144.51, 143.51, 143.35, 134.74, 132.98 (2C), 132.60, 128.87 (2C), 128.11 (2C), 127.55 (2C), 121.14, 119.11, 118.94, 115.63, 110.56, 108.12, 59.69, 52.64 (2C), 50.54, 44.60, 33.29, 32.36 (2C), 23.46, 6.20 (2C). (APCI/ESI)+, found m/z = 519.2 [M+H]$^+$, (calculated for $C_{32}H_{34}N_6O$, 518.28). Purity: 99 %, t_R = 3.337 min.

*N-allyl-4-(2-(4-((1-(4-cyanobenzyl)-1H-benzoimidazol-2-yl)amino)piperidin-1-yl) ethyl) benzamide (**50**)*. Obtained from **11c** (0.076 g, 0.230 mmol) and **42h** (0.080 g, 0.298 mmol) as a white solid (0.015 g, 53% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.56 (t, *J* = 5.6 Hz, 1H), 7.81 (d, *J* = 8.4 Hz, 2H), 7.78 (d, *J* = 7.9 Hz, 2H), 7.33 (d, *J* = 8.4 Hz, 2H), 7.27 (d, *J* = 7.9 Hz, 2H), 7.23 (dd, *J* = 7.7, 1.5 Hz, 1H), 7.02 (dd, *J* = 7.4, 1.2 Hz, 1H), 6.95 (td, *J* = 7.6, 1.2 Hz, 1H), 6.84 (td, *J* = 7.6, 1.1 Hz, 1H), 6.65 (d, *J* = 6.8 Hz, 1H), 5.89 (dd, *J* = 5.6, 4.2 Hz, 1H), 5.40 (s, 2H), 5.12 (d, *J* = 5.4 Hz, 2H), 3.91 – 3.86 (m, 2H), 3.89 (m, 1H), 3.14 – 2.97 (m, 2H), 2.79 – 2.65 (m, 2H), 2.52 – 2.47 (m, 4H), 2.04 – 1.98 (m, 2H), 1.62 – 1.58 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.39, 154.38, 143.44, 143.15, 135.99, 134.69, 132.99 (2C), 129.00 (2C), 128.10 (2C), 127.72 (2C), 121.23, 119.11, 115.66, 115.54, 110.58, 108.23, 85.09, 68.87, 52.49, 52.24, 52.14 (2C), 44.66, 41.94 (2C), 31.72, 31.59, 31.49. (APCI/ESI)+, found *m/z* = 519.2 $[M+H]^+$, (calculated for $C_{32}H_{34}N_6O$, 518.23). Purity: 97%, t_R = 2.643 min.

*4-(2-(4-((1-(4-cyanobenzyl)-1H-benzoimidazol-2-yl)amino)piperidin-1-yl)ethyl)-N-(2-hydroxyethyl)benzamide (**51**)*. Obtained from **11c** (0.037 g, 0.33 mmol) and **42i** (0.040 g, 0.147 mmol) as a white solid (0.031 g, 53%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.33 (t, *J* = 5.7 Hz, 1H), 7.84 (d, *J* = 8.5 Hz, 2H), 7.80 (d, *J* = 8.0 Hz, 2H), 7.34 (d, *J* = 8.5 Hz, 2H), 7.30 (d, *J* = 8.0 Hz, 2H), 7.25 (dd, *J* = 7.5, 1.2 Hz, 1H), 7.04 (dd, *J* = 7.4, 1.1 Hz, 1H), 6.97 (td, *J* = 7.6, 1.2 Hz, 1H), 6.86 (td, *J* = 7.6, 1.1 Hz, 1H), 6.60 (d, *J* = 7.1 Hz, 1H), 5.42 (s, 2H), 4.71 (t, *J* = 5.2 Hz, 1H), 3.85 – 3.71 (m, 1H), 3.54 (td, *J* = 6.1, 5.2 Hz, 2H), 3.36 (t, *J* = 6.1 Hz, 2H), 2.96 – 2.91 (m, 2H), 2.85 – 2.77 (m, 2H), 2.62 – 2.55 (m, 2H), 2.15 – 2.01 (m, 2H), 1.97 – 1.95 (m, 2H), 1.55 – 1.49 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.74, 154.50, 144.50, 143.51, 143.34, 134.74, 132.98 (2C), 132.70, 128.91 (2C), 128.11 (2C), 127.58 (2C), 121.15, 119.11, 118.95, 115.62, 110.56, 108.12, 60.34, 59.68, 52.65 (2C), 50.54, 44.60, 42.61, 33.28, 32.36 (2C). $(APCI/ESI)^+$, found *m/z* = 523.2 $[M+H]^+$, (calculated for $C_{31}H_{34}N_6O_2$, 522.24). Purity: 98%, t_R = 2.461 min.

*4-(2-(4-((1-(4-cyanobenzyl)-1H-benzoimidazol-2-yl)amino)piperidin-1-yl)ethyl)-N-(2-methoxyethyl)benzamide (**52**)*. Obtained from **11c** (0.100 g, 0.33 mmol) and **42j** (0.127 g, 0.430 mmol) as a white solid (0.025 g, 15%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.44 (s, *J* = 7.2 Hz, 1H), 7.83 (d, *J* = 8.5 Hz, 2H), 7.80 (d, *J* = 7.9 Hz, 2H), 7.36 (d, *J* = 8.5 Hz, 2H), 7.30 (d, *J* = 7.9 Hz, 2H), 7.26 (dd, *J* = 7.5, 1.2 Hz, 1H), 7.05 (dd, *J* = 7.8, 1.3 Hz, 1H), 6.98 (td, *J* = 7.6, 1.1 Hz, 1H), 6.87 (td, *J* = 7.6, 0.9 Hz, 1H), 6.74 – 6.62 (m, 1H), 5.43 (s, 2H), 3.98 – 3.69 (m, 1H), 3.51 – 3.47 (m, 2H), 3.49 – 3.42 (m, 4H), 3.31 – 3.30 (m, 2H), 3.29 (s, 3H), 2.96 – 2.81 (m, 2H), 2.55 – 2.49 (m, 2H), 2.11 – 1.98 (m, 2H), 1.74 – 1.50 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ

166.59, 154.38, 143.46, 134.65, 132.99 (2C), 128.96 (2C), 128.10 (2C), 127.70 (2C), 121.21, 119.64, 119.25, 119.10, 115.65, 110.58, 108.22, 88.67, 71.00 (2C), 63.65, 58.39 (2C), 52.28, 44.65, 33.15, 32.28, 32.10, 27.13, 9.02. (APCI/ESI)+, found *m/z* = 537.2 $[M+H]^+$, (calculated for $C_{32}H_{36}N_6O_2$, 536.28). Purity: 95%, t_R = 2.585 min.

*4-((2-((1-(4-(piperidine-1-carbonyl)phenethyl)piperidin-4-yl)amino)-1H-benzoimidazol-1-yl) methyl)benzonitrile (**53**)*. Obtained from **11c** (0.100 g, 0.33 mmol) and **42k** (0.127 gram, 0.43 mmol) as a white solid (0.025 g, 15 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (d, *J* = 8.3 Hz, 2H), 7.50 (d, *J* = 8.5 Hz, 2H), 7.33 – 7.29 (m, 2H), 7.23 (d, *J* = 8.3 Hz, 2H), 7.21 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.17 – 7.11 (m, 1H), 7.03 (td, *J* = 7.6, 1.1 Hz, 1H), 6.96 (dd, *J* = 7.4, 1.5 Hz, 1H), 6.54 (d, *J* = 6.98 Hz, 1H), 5.23 (s, 2H), 4.07 (m, 1H), 3.76 – 3.55 (m, 2H), 3.40 – 3.25 (m, 2H), 3.10 = 3.06 (m, 2H), 2.97 – 2.84 (m, 2H), 2.84 – 2.72 (m, 2H), 2.47 – 2.42 (m, 2H), 2.20 – 2.16 (m, 2H), 1.76 – 1.72 (m, 2H), 1.35 – 1.16 (m, 4H), 0.93 – 0.82 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.14, 152.96, 141.02, 140.39, 134.81, 134.16, 132.82 (2C), 128.68 (2C), 127.25 (2C), 127.18 (2C), 122.01, 120.22, 118.19, 116.60, 112.15, 107.34, 59.32 (2C), 52.10 (2C), 49.32, 45.35, 32.53, 31.51 (2C), 24.58 (2C), 22.29, 13.99. (APCI/ESI)+, found *m/z* = 547.3 $[M+H]^+$, (calculated for $C_{34}H_{38}N_6O$, 546.31): Purity: 96%, t_R = 2.824 min.

General Procedure 9 (GP9): Synthesis of compounds **39** and **40**. A solution of an appropriate alcohol (1.2 equiv) and TEA (3.0 equiv) in 5 ml anhydrous DCM was cooled to 0 °C, after which mesyl chloride, MsCl (1.0 or 2.0 eq) in 2 ml DCM was added and the reaction mixture stirred at that temperature for 30 min. Amine **11c** (1.0 equiv) and TEA (1.0 equiv) in 4 ml anhydrous DCM was added to the cold mesylate reaction mixture, and the resulting solution stirred at room temperature (23 °C) for 4 h. At completion, reaction mixture was cooled and diluted with 10 ml DCM, washed with saturated $NaHCO_3$ (3 × 10 ml), followed by brine, and dried over anhydrous Na_2SO_4. Products isolated after purification *via* by column chromatography using 4 – 7% MeOH/DCM as the eluent.

*4-((2-((1-(2-(4-(methylsulfonyl)piperazin-1-yl)ethyl)piperidin-4-yl)amino)-1H-benzo[d]imidazol-1-yl)methyl)benzonitrile (**39**)*. Obtained from 2-(piperazinyl)ethanol (0.038 g, 0.29 mmol) as a yellow crystalline solid (0.081 g, 65%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.69 (d, *J* = 8.5 Hz, 2H), 7.41 (dd, *J* = 7.8, 0.9 Hz, 1H), 7.30 (d, *J* = 8.5 Hz, 2H), 7.16 (ddd, *J* = 7.8, 7.2, 1.5 Hz, 1H), 7.12 (dd, J = 8.0, 1.4 Hz, 1H), 7.08 (ddd, 8.0, 7.2, 1.3 Hz, 1H), 5.46 (s, 2H), 4.03 (tt, *J* = 10.7, 4.0 Hz, 1H), 3.64 (m, 2H), 3.45 – 3.31 (m, 6H), 3.24 – 3.18 (m, 2H), 2.86 (s,

3H), 2.82 – 2.77 (m, 2H), 2.66 – 2.48 (m, 4H), 2.32 – 2.27 (m, 2H), 2.06 – 1.93 (m, 2H). ^{13}C NMR (101 MHz, Methanol-d_4) δ 147.22, 135.54, 132.98 (2C), 128.54 (2C), 121.32, 119.09, 117.78, 115.66, 113.76, 110.87, 107.21, 66.34, 54.58, 53.99, 53.05 (2C), 52.01 (2C), 48.33, 44.56 (2C), 35.23, 31.78 (2C). LC-MS (APCI+/ESI): found *m/z* = 522.1 $[M+H]^+$ (cal. for $C_{27}H_{35}N_7O_2S$, 521.2). Purity: 95%, t_R = 0.523 min.

*4-N-Boc-(2-(4-((1-(4-cyanobenzyl)-1H-benzo[d]imidazol-2-yl)amino)piperidin-1-yl)ethyl)piperazine (**40**)*. Obtained from **9g** (0.132 g, 0.58 mmol) as a pale yellow crystalline solid (0.119 g, 46%). ^{1}H NMR (600 MHz, Methanol-d_4) δ 7.67 (d, *J* = 8.4 Hz, 2H), 7.35 (dd, *J* = 7.6, 1.2 Hz, 1H), 7.25 (d, *J* = 8.4 Hz, 2H), 7.08 (ddd, *J* = 7.6, 1.3 Hz, 1H), 7.03 (dd, *J* = 7.9 Hz, 1.3 Hz, 1H), 6.98 (ddd, 7.9, 7.6, 1.3 Hz, 1H), 5.40 (s, 2H), 3.94 (tt, *J* = 10.6, 4.0 Hz, 1H), 3.46 (m, 4H), 3.35 (t, *J* = 6.8 Hz, 2H), 2.98 (t, *J* = 6.8 Hz, 2H), 2.81 (m, 2H), 2.69 (m, 2H), 2.49 (m, 4H, H), 2.21 (m, 2H), 1.80 (m, 2H), 1.46 (s, 9H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 156.37, 155.04, 143.60, 142.65, 135.21, 133.68 (2C), 128.52 (2C), 122.87, 121.27, 119.42, 116.29, 112.45, 111.23, 109.15, 81.34, 66.87, 54.85 (2C), 54.04 (2C), 53.49 (2C), 50.00, 45.72, 31.21 (2C), 28.64 (3C). LC-MS (APCI+/ESI): found *m/z* = 544.2 $[M+H]^+$ (cal. for $C_{31}H_{41}N_7O_2$, 543.33). Purity: 97%, t_R = 2.103 min.

*4-((2-((1-(2-(piperazin-1-yl)ethyl)piperidin-4-yl)amino)-1H-benzo[d]imidazol-1-yl) methyl) benzonitrile (**41**)*. Following GP2(b), obtained from **40** (0.070 g, 0.13 mmol) as a pale-yellow solid (0.054 g, 95%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.70 (d, *J* = 8.5 Hz, 2H), 7.33 (dd, *J* = 7.6, 1.0 Hz, 1H), 7.28 (d, *J* = 8.5 Hz, 2H), 7.09 (ddd, *J* = 8.1, 7.6, 1.0 Hz, 1H), 7.06 (dd, *J* = 8.1, 1.1 Hz, 1H), 6.99 (ddd, *J* = 8.1, 7.5, 1.0 Hz, 1H), 5.39 (s, 2H), 3.88 (tt, *J* = 10.4, 4.9 Hz, 1H), 3.75 – 3.68 (m, 4H), 3.27 (t, *J* = 6.6 Hz, 2H), 2.89 (t, *J* = 6.6 Hz, 2H), 2.65 (m, 4H), 2.53 (m, 4H), 2.17 (m, 2H), 1.79 (m, 2H). ^{13}C NMR (101 MHz, Methanol-d_4) δ 145.67, 134.56, 133.21 (2C), 127.55 (2C), 120.94, 119.50, 117.98, 114.84, 112.54, 111.22, 107.85, 66.87, 54.30, 53.98 (2C), 53.24 (2C), 52.08 (2C), 48.76, 44.47, 30.23 (2C). LC-MS (APCI+/ESI): found *m/z* = 444.2 $[M+H]^+$ (cal. for $C_{26}H_{33}N_7$, 443.28). Purity: 99%, t_R = 0.435 min.

General Procedure 10 (GP10): Synthesis of benzoic acid derivatives **18** and **32**. To a solution of ester (1.0 equiv) in 10 ml methanol, 2M aqueous KOH (5 equiv) was added, and the resulting mixture stirred at 79 °C for 2 hr. Following completion, the reaction mixture was cooled to 0 °C, and acidified to pH 2 while stirring. The product was filtered and obtained after recrystallization in methanol and drying.

*4-((2-((1-(4-cyanophenethyl)piperidin-4-yl)amino)-1H-benzo[d]imidazol-1-yl) methyl) benzoic acid (**18**)*. Obtained from **17** (0.050 g, 0.10 mmol) pale-yellow solid (0.044 g, 90%). ^{1}H NMR (600 MHz, Methanol-d_4) δ 7.85 (d, *J* = 8.4 Hz, 2H), 7.63 (d, *J* = 8.1 Hz, 2H), 7.38 (d, *J* = 8.4 Hz, 2H), 7.25 (dd, *J* = 7.9, 1.2 Hz, 1H), 7.08 (d, *J* = 8.1 Hz, 2H), 6.97 (ddd, *J* = 7.9, 7.0, 1.2 Hz, 1H), 6.46 (dd, *J* = 7.8, 1.2 Hz, 1H), 6.28 (ddd, *J* = 7.8, 7.0, 1.2 Hz, 1H), 5.39 (s, 2H), 3.89 (tt, *J* = 10.8, 4.0 Hz, 1H), 3.04 (td, *J* = 13.2, 3.9 Hz, 2H), 2.90 – 2.83 (m, 2H), 2.68 – 2.59 (m, 2H), 2.29 (td, *J* = 12.3, 3.1 Hz, 2H), 2.10 – 1.97 (m, 2H), 1.66 – 1.52 (m, 2H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 171.56, 149.58, 145.65, 142.20, 141.33, 135.78, 130.98 (2C), 129.28 (2C), 126.56 (2C), 125.80 (2C), 125.32, 121.03, 119.32, 117.26, 114.09, 109.64, 106.57, 60.87, 51.68 (2C), 50.44, 44.39, 32.36, 30.68 (2C). LC-MS (APCI$^+$/ESI): found *m/z* = 480.2 [M+H] $^+$ (cal. for $C_{29}H_{29}N_5O_2$, 479.23). Purity: 98%, t_R = 2.135 min.

*4-(2-(4-((1-(4-cyanobenzyl)-1H-benzo[d]imidazol-2-yl)amino)piperidin-1-yl) ethyl) benzoic acid (**32**)*. Obtained from **31** (0.180 g, 0.37 mmol) as a white solid (0.170 g, 96%); m.p.: 181 – 183 °C; R_f (10% MeOH/DCM), 0.11. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.88 (d, *J* = 8.0 Hz, 2H), 7.79 (d, *J* = 8.1 Hz, 2H), 7.37 (d, *J* = 8.0 Hz, 2H), 7.28 (d, *J* = 8.1 Hz, 2H), 7.23 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.02 (dd, *J* = 7.9, 1.3 Hz, 1H), 6.95 (ddd, *J* = 8.0, 7.3, 1.2 Hz, 1H), 6.84 (ddd, *J* = 7.9, 7.3, 1.2 Hz, 1H), 6.78 (d, *J* = 3.9 Hz, 1H), 5.42 (s, 2H), 3.86 (tt, *J* = 10.3, 4.2 Hz, 1H), 3.17 – 3.10 (m, 2H), 2.96 – 2.90 (m, 2H), 2.86 – 2.79 (m, 2H), 2.45 – 2.52 (m, 2H), overlap with DMSO signal), 2.08 – 2.00 (m, 2H), 1.73 – 1.63 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 167.77, 154.33, 143.45, 143.07, 134.65, 132.97 (2C), 129.90 (2C), 129.85 (2C), 129.42 (2C), 129.29, 128.08, 127.45, 121.21, 119.03, 115.61, 110.52, 108.22, 58.29, 51.96 (2C), 49.52, 44.61, 32.07, 31.05 (2C). LC-MS (APCI$^+$/ESI): found *m/z* = 480.2 [M+H] $^+$ (cal. For $C_{29}H_{29}N_5O_2$, 479.23). Purity: 96%, t_R = 0.155 min.

Solubility and Biological Evaluation

Aqueous Solubility. Solubility was measured from amorphous solid forms of the compounds using the turbidimetric method. Following the dissolution of test compound in DMSO to make a 10 mM stock solution, pre-dilution plate was prepared by taking from each stock solution and serially diluting in triplicate to yield concentrations from 0.25 mM to 10.0 mM on a 96 well plate. From each pre-dilution solution, secondary dilutions of the compounds in both DMSO and 0.01M pH 7.4 PBS where prepared in triplicate on a second 96-well plate. Wells in columns 1-6 would

contain compound in DMSO, while those in columns 7 – 12 would contain samples in PBS at similar nominal concentrations as those in DMSO. The final volume of solvent in each assay plate was 200 µL, prepared by pipetting 4 µL each of solution from the pre-dilution plate to the corresponding well into both DMSO and PBS (both 196 µL). This ensures that the final concentration of DMSO in the PBS aqueous buffer does not exceed 2% v/v. Similarly, a second secondary plate containing compound concentrations of 60, 100 and 120 µM was also prepared. Different concentrations in DMSO were prepared as controls to determine false turbimetric absorbance readings arising from the compounds in solution absorbing incident radiation at the test wavelength. Following preparation, the assay plate was covered and left to equilibrate for 2 h at 25 °C. Afterwards, UV-vis absorbance readings from the plate were measured at 620 nm using a SpectraMax 340PC^{384} microplate reader. Plots of corrected absorbance against compound concentration were computed for a graphical representation of the data using MS Excel. Reserpine and hydrocortisone were used as positive and negative controls, respectively.

In vitro Antiplasmodium Assay. Compounds were tested using parasite lactate dehydrogenase assay as a marker for parasite survival. Briefly, the respective stock solutions of CQ diphosphate and test compounds were prepared to 2 mg.ml^{-1} in distilled water (for CQ) and 100% DMSO for test compounds and then stored at –20 °C, and further dilutions were prepared on the day of the experiment. The cultures were synchronized in the ring stage using 15 ml of 5% (w/v) D-sorbitol in water. Synchronous cultures of *Pf*NF54 (CQ-S) and *Pf*K1 (MDR) in the late trophozoite stage were prepared to 2% parasitemia & 2% hematocrit. Compounds were tested at starting concentrations of 10,000 ng.ml^{-1} (1000 ng.ml^{-1} for CQ), which were then serially diluted 2-fold in complete medium to give 10 concentrations with a final volume of 200 µl in each well. Parasites were incubated in the presence of the compounds at 37 °C under hypoxic conditions (4% CO_2 and 3% O_2 in N_2) for 72 h. After incubation, 100 µl of MalStat reagent and 15 µl of resuspended culture were combined, followed by addition of 25 µl of nitro blue tetrazolium chloride (NBT). The plates were kept in the dark for 10 min in order to fully develop, after which absorbance was measured at 620 nm on a microplate reader. Raw data was processed using GraphPad Prism 4.0 (La Jolla, California, USA) to analyze the dose-response.

In vitro Cytotoxicity Assay. Compounds were screened against Chinese Hamster Ovarian (CHO) mammalian cell lines, using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) assay.[17] Emetine was used as the reference standard. It was prepared to 2 mg/ml in distilled water, while the stock solutions of test compounds were prepared

to 20 mg/ml in DMSO (100%), with the highest concentration of solvent to which the cells were exposed having no measurable effect on the cell viability. The initial concentration of the compounds and control was 100 µg/ml, which was serially diluted in complete medium with 10-fold dilutions to give 6 concentrations, the lowest being 0.001 µg/ml. Plates were incubated for 48 h with 100 µl of test compound and 100 µl of cell suspension in each well and developed afterward by adding 25 µl of sterile MTT (Thermo Fisher Scientific) to each well, followed by 4 h of incubation in the dark. The plates were then centrifuged, the medium aspirated, and 100 µl of DMSO was added to dissolve crystals before reading the absorbance at 540 nm. Data were analyzed, and the sigmoidal dose−response was derived using GraphPad Prism version 4.0 software. All experiments were performed as three independent biological repeats, each with technical triplicates.

In vitro β-hematin Inhibition Assay

Stock solutions of controls (CQ and Pyrimethamine) and test compounds were made to 20 mM in DMSO. A solution containing water/305.5 µM NP40/DMSO at a 70%/20%/10% (v/v) respectively, was added to every well in columns 1 – 11 on a 96–well plate, while 140 µl of water and 40 µl of 305.5 µM NP40 were added to column 12 to mediate the formation of β-hematin. 20 µl of control or test compound (20 mM) was added to column 12, and 100 µl of this solution was serially diluted to column 2, with column 1 left as a blank (no test compound). A 178.8 µl aliquot of hematin stock was suspended in 20 ml of a 1 M acetate buffer at pH 4.9, and 100 µl of this hematin suspension was added into each well. Plates were then incubated for 5 h at 37 °C, after which 32 µl of pyridine solution (20% water, 20% acetone, 10% 2 M HEPES buffer, pH 7.4, 50% pyridine) was added, followed by addition of 60 µl of acetone to all wells. The plates were read at 405 nm, and dose-response curves were plotted in GraphPad Prism version 6.01 (GraphPad Software Inc., La Jolla, California, USA) to obtain IC_{50} values.

Table S1: *In vitro* β-hematin inhibition results of selected analogues

Compound	β-HIA IC_{50} (µM)[a]	Compound	β-HIA IC_{50} (µM)[a]
AST	130.5	**30**	35.4
2	1505	**34**	63.0
3	3203	**43**	5794
4	94.6	**44**	1651

7	4758	**45**	641
8	1621	**46**	173.7
12	168.5	**47**	70.4
14	21.0	**48**	109
15	130.8	**50**	146.2
16	378.6	**51**	200
19	63.0	**52**	>5000
20	127.8	**53**	1318
23	131.7	Chloroquine	23
24	126.0	Amodiaquine	8.63
28	4706		

[a]β-hematin inhibition, expressed as 50% inhibitory concentration, IC_{50} (n = 3, technical triplicates).

In vitro Gametocytocidal Assay

Gametocytes were produced and compounds assayed for gametocytocidal activity following the methods reported by Reader *et al*.[5]

A luciferase reporter assay was used to enable the quantification of the stage-specific activity of compounds for the early- and late-stage gametocyte marker cell line NF54-*Pf*S16-GFP-Luc. The assays were set up on days 5 and 10 to represent >90% of early-stage II/III or late-stage IV/V gametocytes, respectively. In both cases, the assays were set up using a 2 – 3% gametocytemia, 1.5% hematocrit culture, and 48 h drug pressure in a gas chamber containing 90% N_2, 5% O_2, and 5% CO_2 at 37 °C. Luciferase activity was determined in 30 µl parasite lysates by adding 30 µl of the luciferin substrate (Promega Luciferase Assay System) at 25 °C, followed by the detection of resulting bioluminescence at 10s integration constant, with the GloMax® Explorer Detection System using the Instinct® Software.

An ATP bioluminescence assay was used to determine drug activity against mature stage V gametocytes only. Gametocytes were harvested using density gradient centrifugation with NycoPrepTM 1.077 cushions (Axis Shield) at 800x*g* for 20 min at 37°C followed by enrichment using magnetic separation in a glucose-rich medium with MidiMACS magnetic system (Miltenyi Biotec). Gametocytes (~30 000) were treated at 48 h drug pressure in a gas chamber containing 90% N2, 5% O2, and 5% CO2 at 37 °C. The BacTiter-Glo™ Bioluminescence assay (Promega) was performed according to manufacturer's instructions to detect ATP levels integration constant

of 0.5 s with the GloMax®-Multi + Detection System with Instinct® Software. Methylene Blue (5 µM)was included as a positive control. The dual point screens were performed as technical triplicates for a single biological assay.

Table S2: *In vitro* gametocytocidal activity of selected analogues (duo-point and IC_{50}'s).

Compound	Early Gametocytes (EG)[a]			Late Gametocytes (LG)[b]		
	% activity		EG IC_{50} (µM)	% activity		LG IC_{50} (µM)
	1.0 µM	5.0 µM		1.0 µM	5.0 µM	
AST	5	61		0 13[c]	11 52[c]	3.35[c]
2	63	84	6.18	2	39	-
3	84	67		5	49	-
4	6	50	-	17[c]	63[c]	1.08[c]
7	72	96	30.9	0	5	-
8	79	87	14.2	1	12	-
14	0	9	-	12	31	-
15	23	52	-	0	0	-
17	23	38	-	0	0	-
21	0	0	-	1	34	-
23	15	21	-	14	35	-
24	2	2	-	13	39	-
27	5	0	-	23	51	-
28	3	4	-	12	52	-
29	16	62	-	12	0	-
30	0	27	-	36	62	-
31	14	59	-	0	0	-
35	8	0	-	2	13	-
36	4	1	-	10	28	-
37	0	25	-	12	41	-
38	2	2	-	7	16	-
41	11	2	-	6	15	-
43	0	0	-	21	54	-
45	48	43	-	13	45	-
46	42	77	-	65[c]	86[c]	0.6±0.1[c,d]
48	16	49	-	77	43	-

[a]*Pf*NF54 early-stages (I – III) and [b]late-stages (IV – V) gametocytes, obtained at 1.0 µM and 5.0 µM (n = 3, technical triplicates). Methylene blue (EG luc at 1.0 µM = 95% inhibition, EG IC_{50} = 0.2 µM; LG luc at 1.0 µM = 92% inhibition, LG IC_{50} = 0.14 µM).[c]Data generated using ATP bioluminescence assay. [d]n = 3, technical triplicates ± SEM. ND = not determined.

In vitro Microsomal Metabolic Assay

This assay was performed in duplicate using a 96-well microtiter plate. Test compounds (0.1 µM) were incubated at 37 °C in mouse and pooled human liver microsomes with a final protein concentration of 0.4 $mg.ml^{-1}$; XenoTech, Lenexa, KS suspended in 0.1 M phosphate buffer at pH 7.4 for predetermined time points. This was in the presence and absence of cofactor-reduced nicotinamide adenine dinucleotide phosphate (NADPH, 1.0 mM). The reactions were quenched by adding ice-cold MeCN containing an internal standard (Carbamazepine, 0.0236 µg/mL). The samples were centrifuged, and the supernatant was analyzed via liquid chromatography-tandem mass spectrometry (LC–MS/MS) (Agilent Rapid Resolution HPLC, AB SCIEX 4500 MS). The relative loss of the parent compound with time was monitored, and plots were prepared for each compound of Ln% remaining versus time to determine the first-order rate constant for compound depletion. This was used to calculate the degradation half-life and subsequently to predict the *in vitro* intrinsic clearance (CL_{int}) and *in vitro* hepatic extraction ratio (E_H).[6]

Table S3: *In vitro* microsomal metabolic stability and predicted intrinsic clearance (CL_{int}) of selected analogues.

Compound	Metabolic Stability[a]			Predicted CL_{int} ($\mu l.min^{-1}.mg^{-1}$)[b]		
	HLMs	MLMs	RLMs	HLMs	MLMs	RLMs
AST	79.1	88.0	88.4	-	-	-
21	73.6	8.30	36.2	22.5	207.9	84.7
24	41.9	5.40	5.40	72.9	118.3	243.4
30	82.4	36.8	44.7	16.2	83.3	67.4
33	0.58	1.14	0.34	431.3	373.2	475.6
46	92.8	7.45	81.3	<11.0	217.8	17.3

[a]Percentage (%) of test compound remaining after 30 min incubation with human (H), rat (R) or mouse (M) liver microsomes (n = 3, technical replicates)
[b]Predicted intrinsic clearance (CL_{int}) of unbound drug.

In Vitro hERG Assay. A QPatch hERG assay employing a four-point concentration response format was used to carry out hERG inhibition studies by the Metrion Biosciences Ltd, Cambridge, UK. The hERG gene was stably expressed in a CHO K1 cell line from the American Type Culture Collection (ATCC) which was grown and passaged under standard culture conditions. The external (e) and internal (i) recording solutions were of the following compositions (mM): NaCl – 140(e) : 0 (i); KCl – 2 (e): 70 (i); KF – 0 (e) : 60 (i); HEPES – 10 (e) : 10 (i); $MgCl_2$ – 1 (e) : 0 (i); $CaCl_2$ –2 (e) : 0 (i); Glucose – 5 (e) : 0 (i); EGTA - 0 (e) : 5 (i); Mg_2ATP – 0 (e) : 5 (i) and pH – 7.4 (NaOH) (e) : 7.2 (KOH) (i). The external recording solution was regularly prepared and kept at 4°C until required and was maintained at room temperature during recording. The internal recording solution was prepared and kept at –20 °C until required.

The QPatch is a chip-based planar patch clamp which is automated. Using suction, cells added to each well are drawn across a small aperture creating a Giga-ohm seal between the membrane surface and a treated silicon surface. A small volume of bathing solution containing the test compound or control bathing solution is added to a reservoir on the chip which perfuses across the cell through quartz-lined microfluidic channels. The solution is removed by capillary action before the next sample is added. Using the industry +40/–40 voltage protocol, currents were triggered from a holding potential of –90 mV at a stimulus frequency of 0.1 Hz.

By cumulatively adding four escalating concentrations of the test compounds to an individual cell, the concentration response curves were established. This was done by firstly allowing the whole-cell configuration to be achieved followed by the addition of the vehicle (0.1% DMSO v/v in external recording solution) to each well in two bolus additions allowing a two-minute recording time between each addition. This was followed by the addition of four concentrations (0.3 – 10 μM) of test compounds in two bolus additions at 2-minute intervals. The effect on the hERG tail current amplitude was measured during the 4-minute recording time. The concentrations (0.3, 1, 3 and 10 μM) of the test samples were prepared in such a way to have a final concentration of 0.1% of DMSO v/v in the external recording solution. For each compound, the experiments at each concentration were done in triplicate and using a bioinformatics suite developed and running in Pipeline Pilot (Biovia, USA), the percent inhibition, as a reduction in mean peak current relative to the value measured at the end of the vehicle control period, was calculated. Such percent inhibition data were used to construct the concentration-response curves which enabled calculation of the IC_{50} values. For compounds which could not achieve 50% inhibition even at the highest tested concentration of 10 μM, extrapolated IC_{50} values for such are

reported. In this regard, all IC_{50} values above 10 μM reported in this article were extrapolated and should be treated with caution.

^{1}H NMR and ^{13}C NMR Spectra of Selected and Key Target Compounds

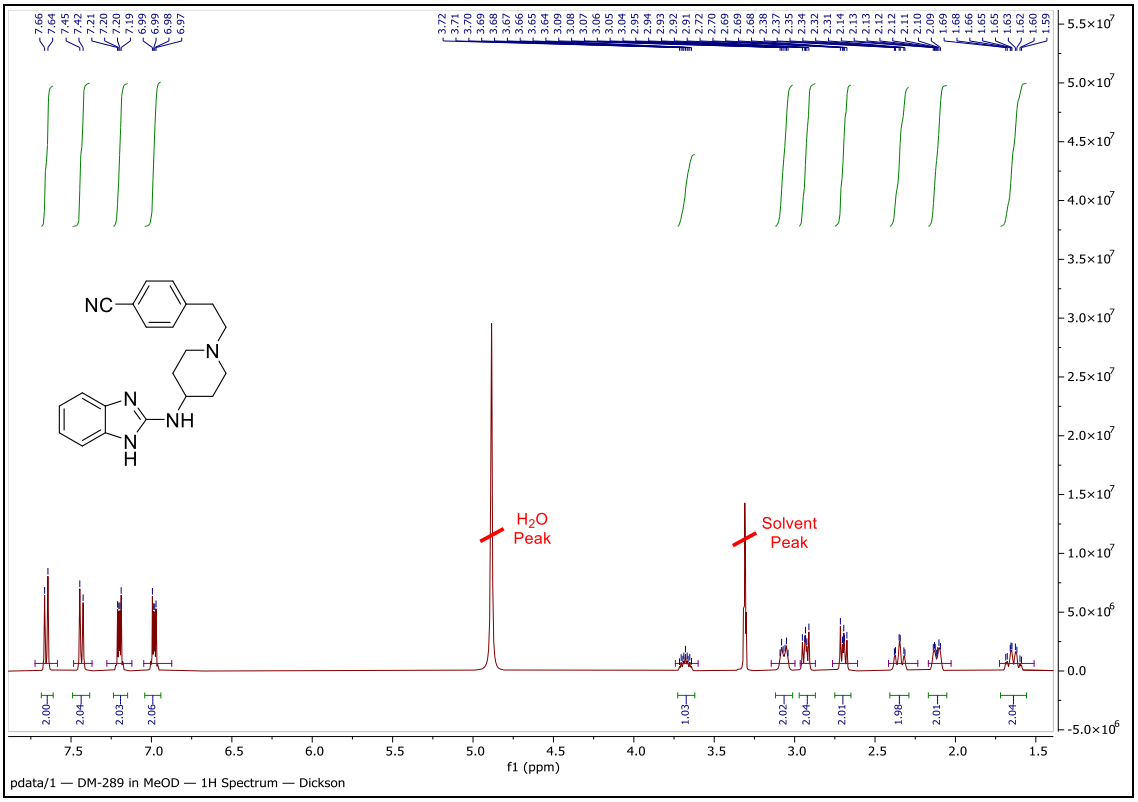


Figure 1: ^{1}H-NMR spectrum of **12** in Methanol-d_4 at 400 MHz

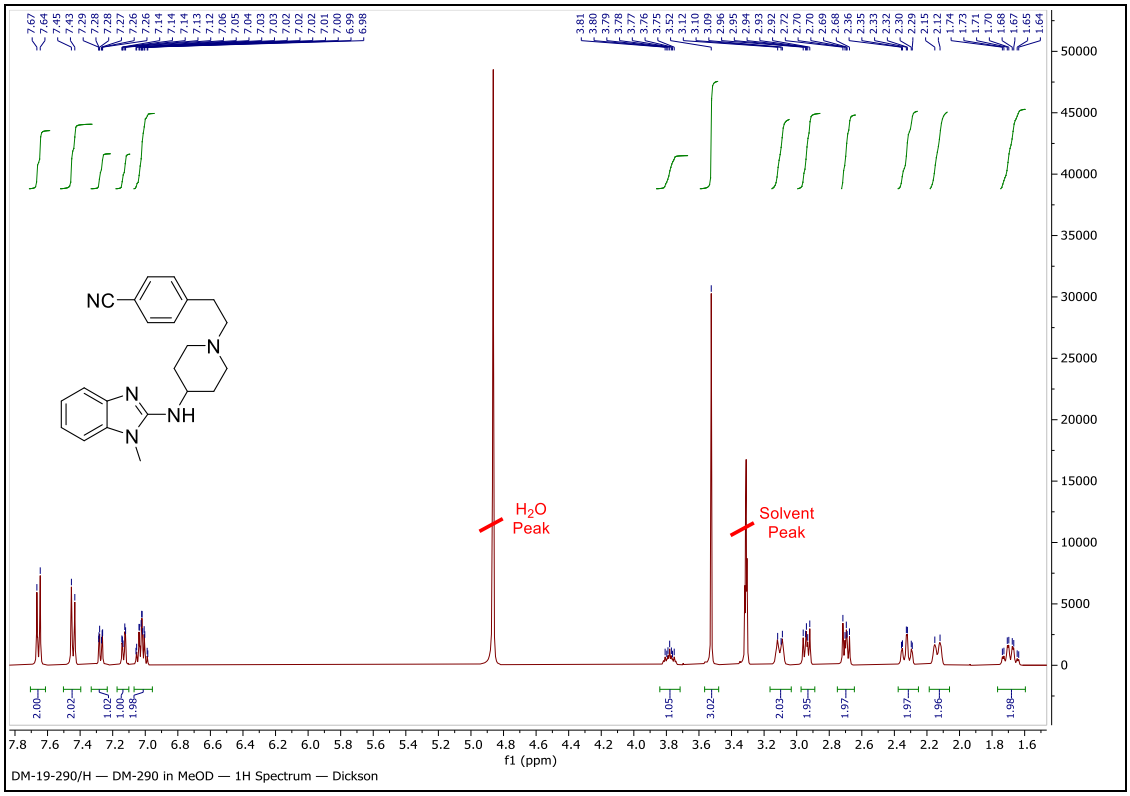


Figure 2: ^{1}H-NMR spectrum of **13** in Methanol-d_4 at 400 MHz

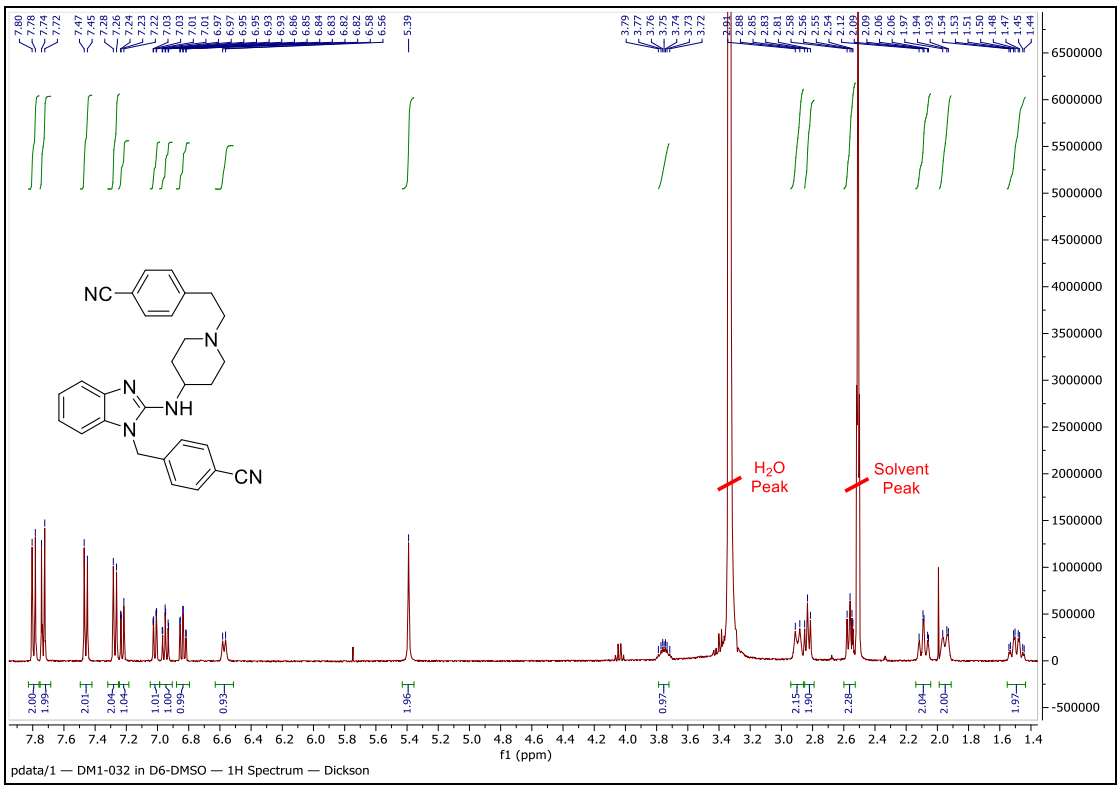


Figure 3: ^{1}H-NMR spectrum of **14** in DMSO-d_6 at 300 MHz

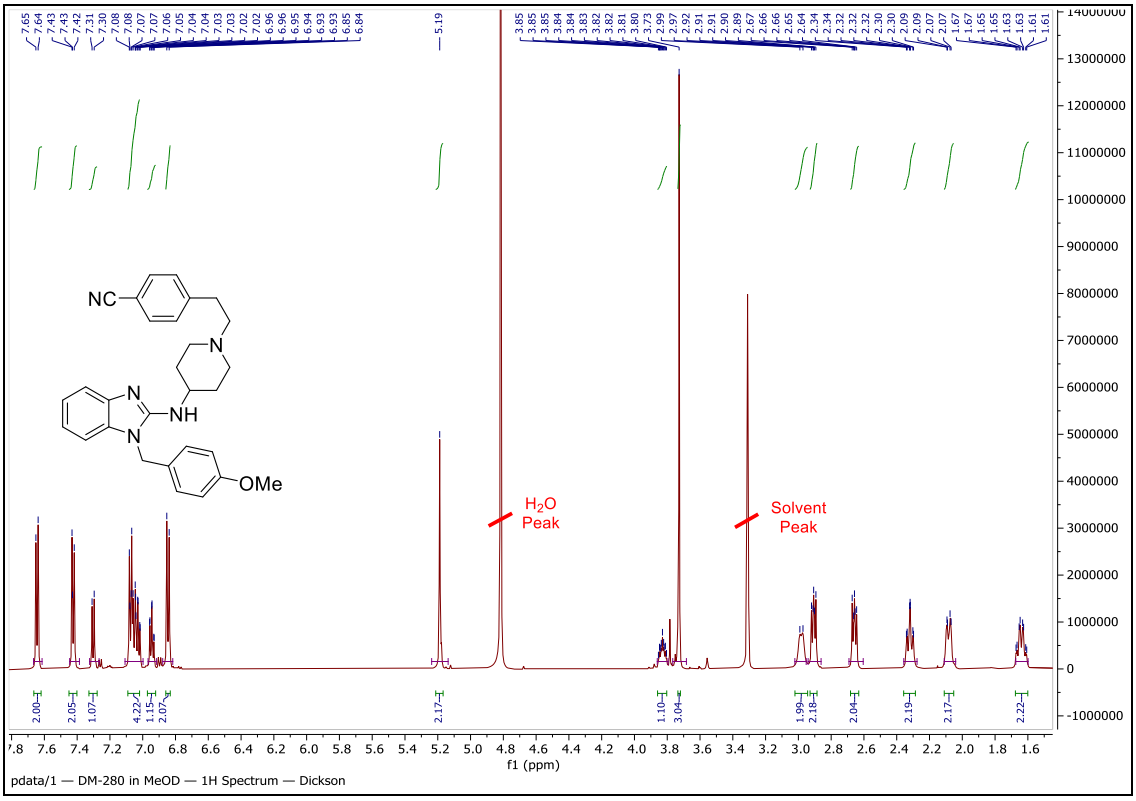


Figure 4: ^{1}H-NMR spectrum of **15** in Methanol-d_4 at 600 MHz

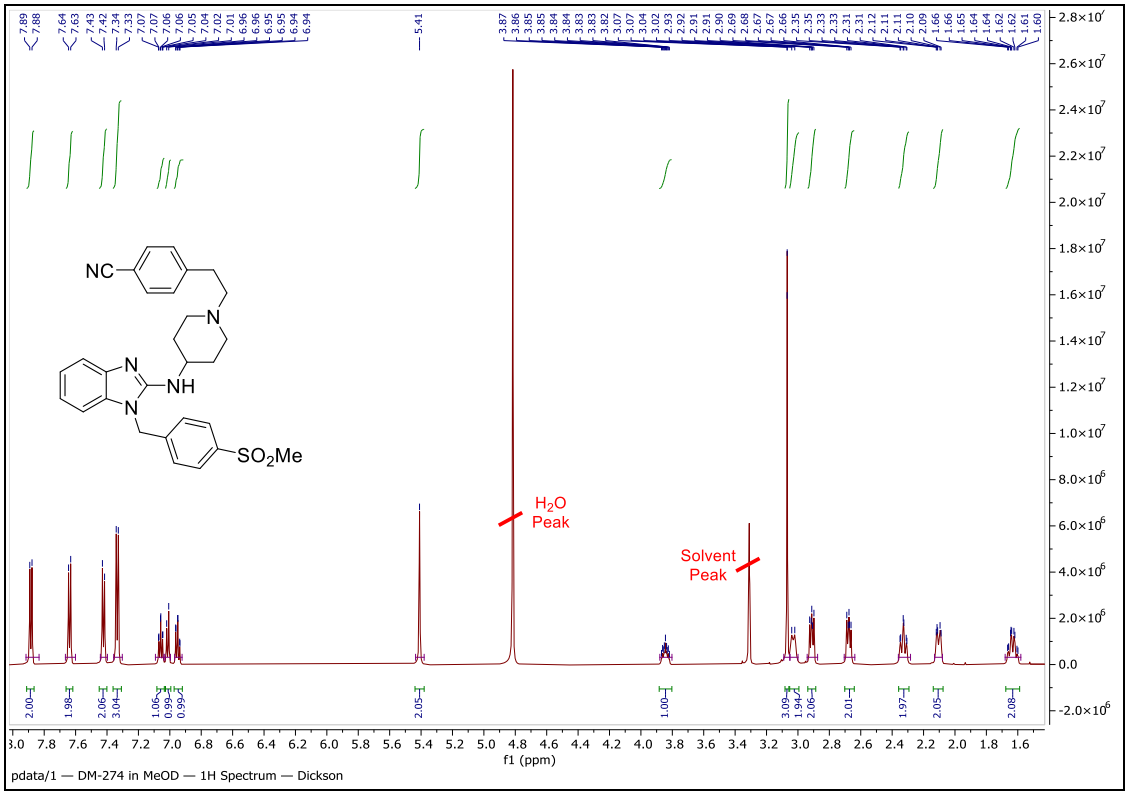


Figure 5: ^{1}H-NMR spectrum of **16** in Methanol-d_4 at 600 MHz

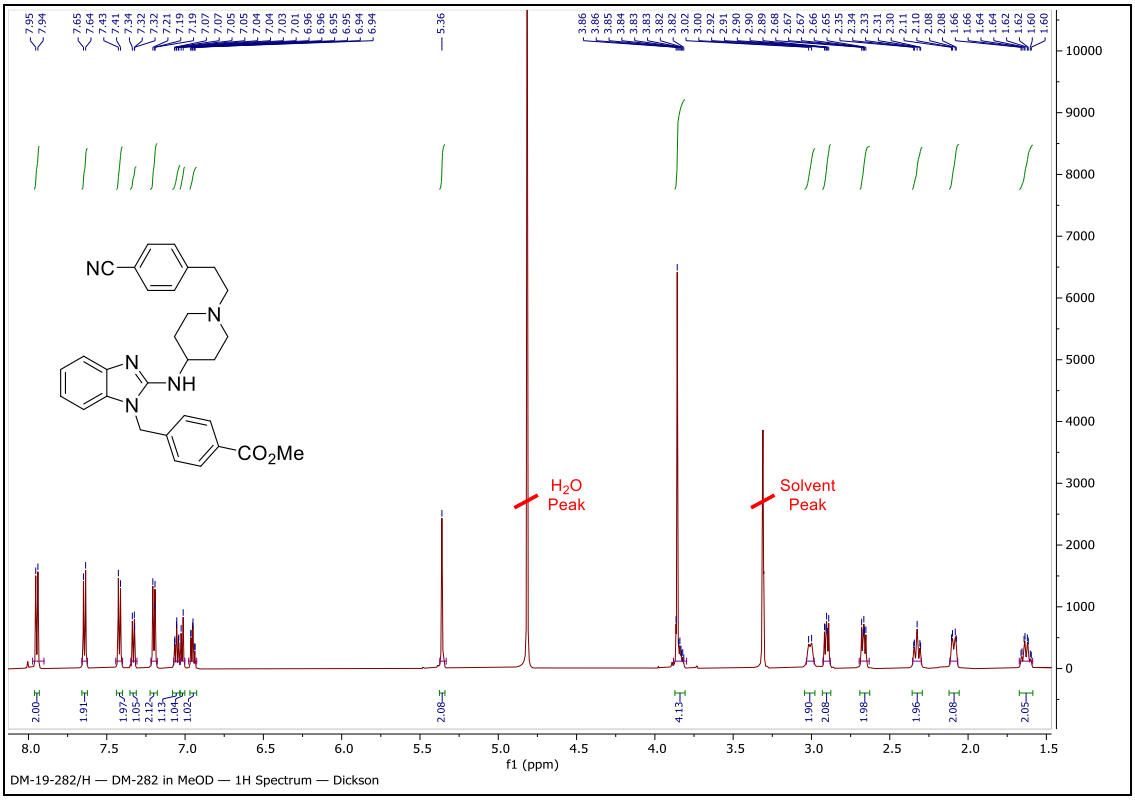


Figure 6: ^{1}H-NMR spectrum of **17** in Methanol-d_4 at 600 MHz

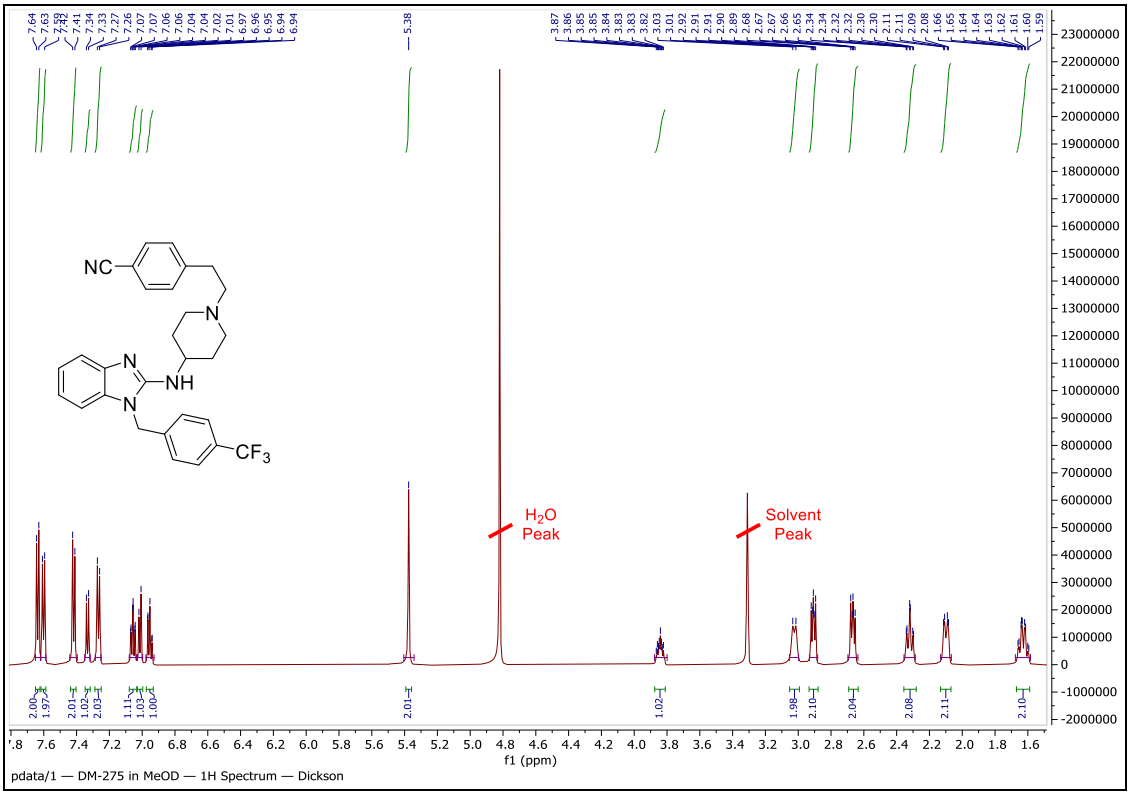


Figure 7: ^{1}H-NMR spectrum of **19** in Methanol-d_4 at 600 MHz

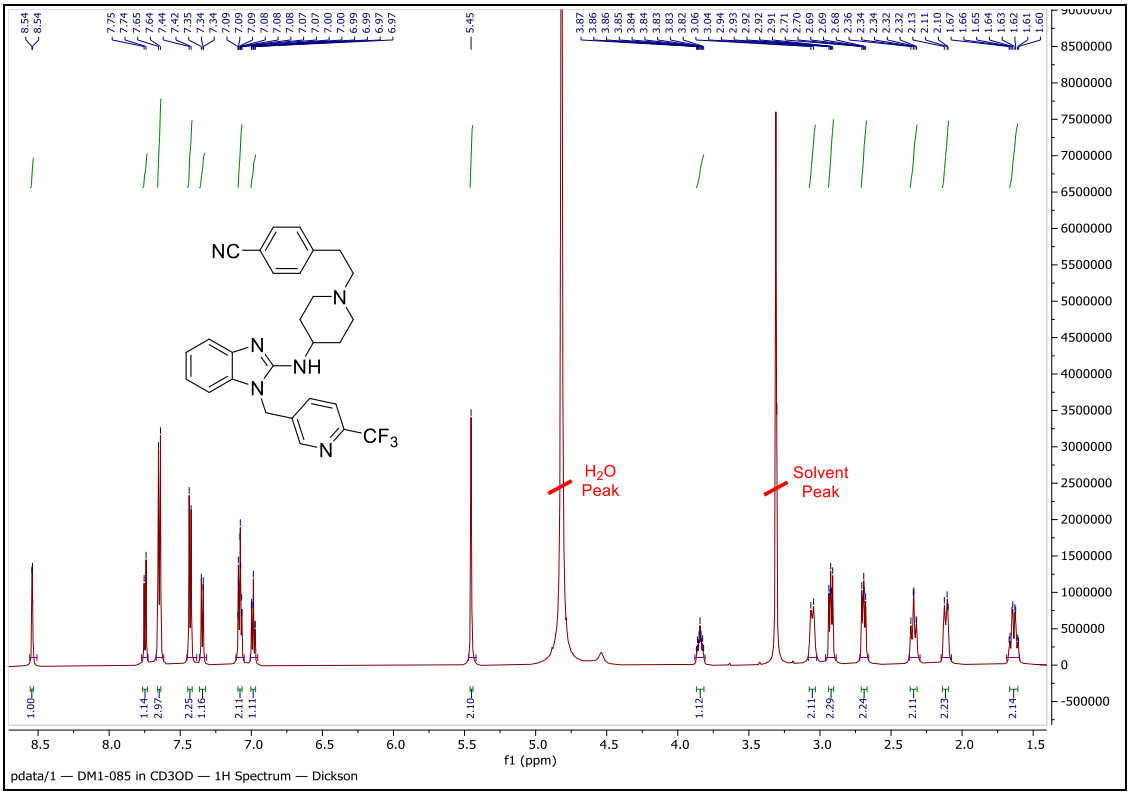


Figure 8: ^{1}H-NMR spectrum of **21** in Methanol-d_4 at 600 MHz

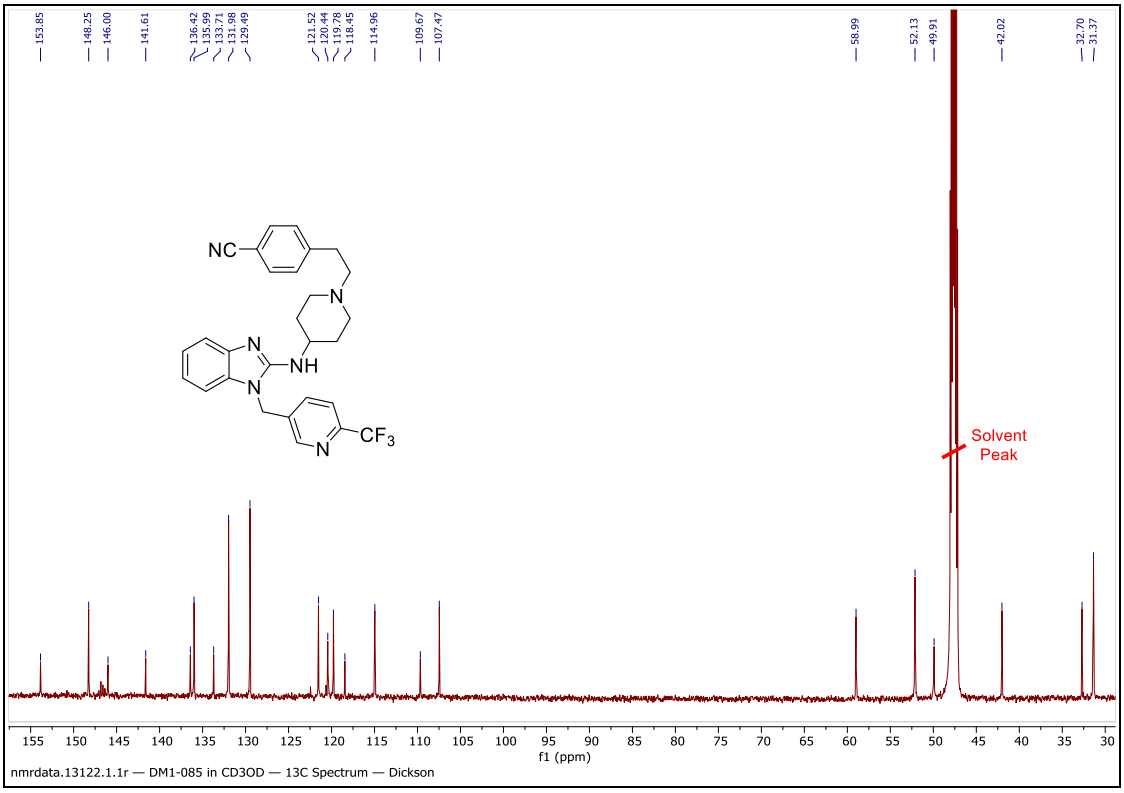


Figure 9: ^{1}13-NMR spectrum of **21** in Methanol-d_4 at 151 MHz

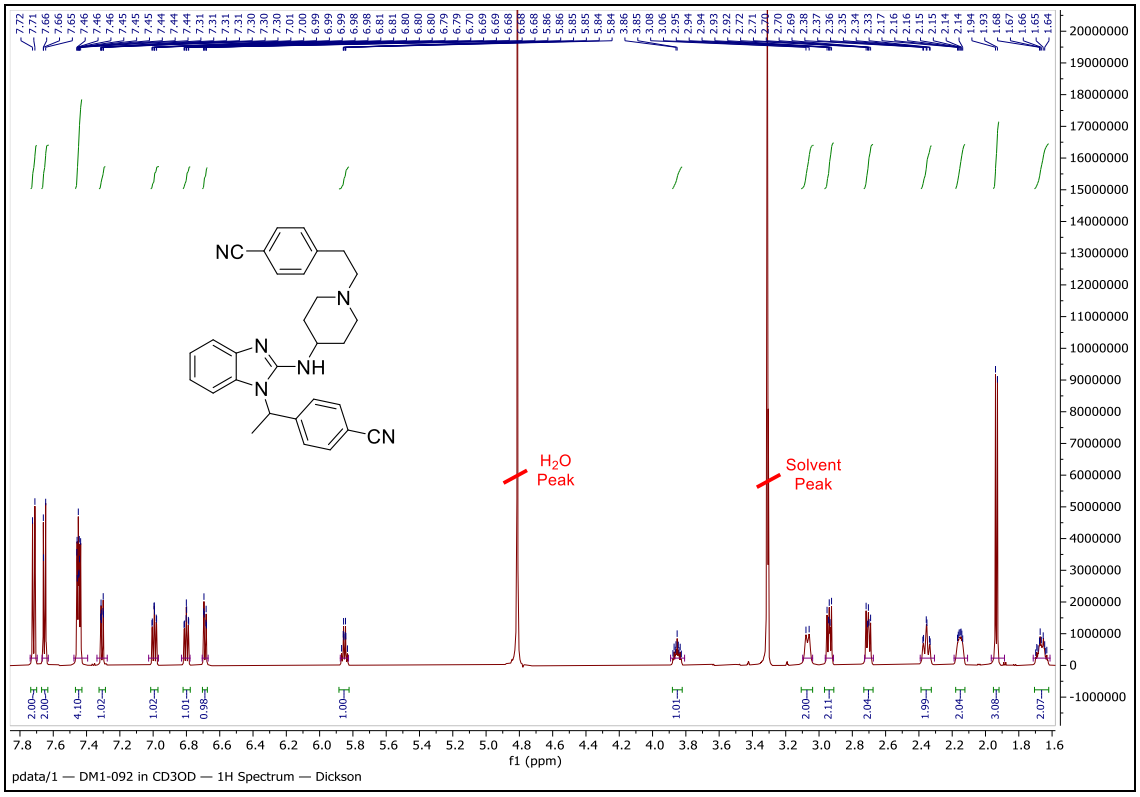


Figure 10: ^{1}H-NMR spectrum of **23** in Methanol-d_4 at 600 MHz

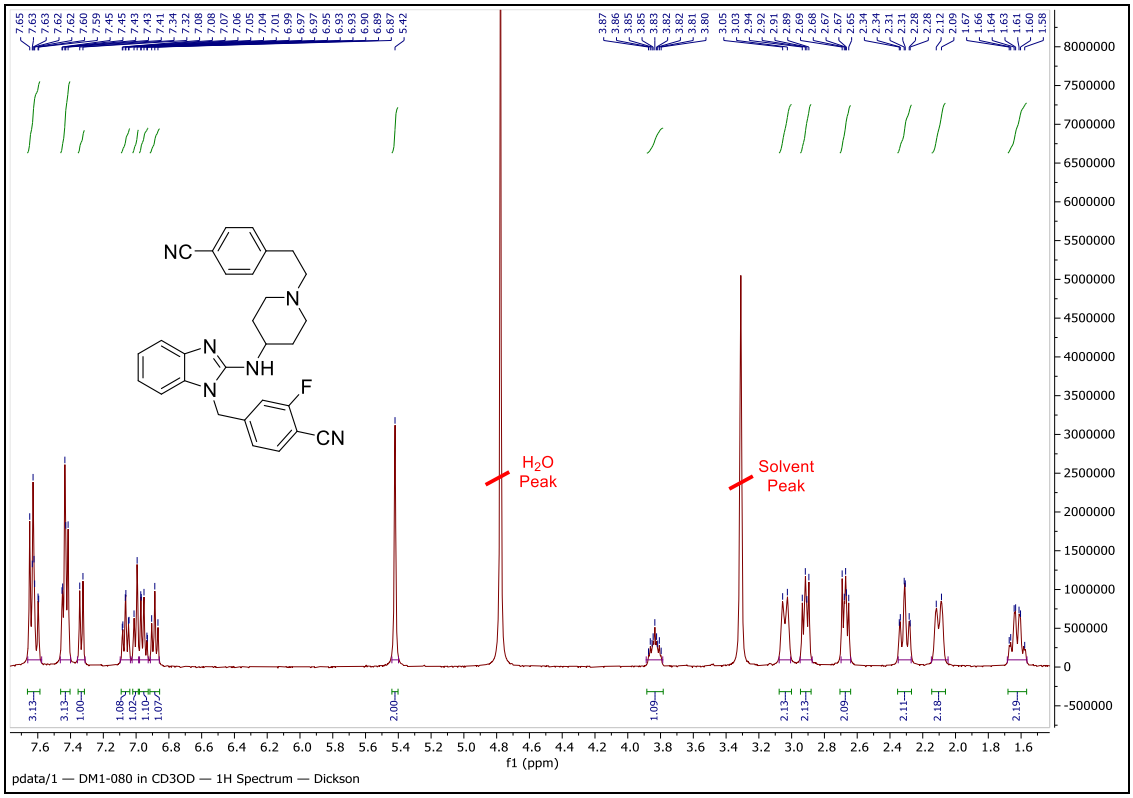


Figure 11: ^{1}H-NMR spectrum of **24** in Methanol-d_4 at 400 MHz

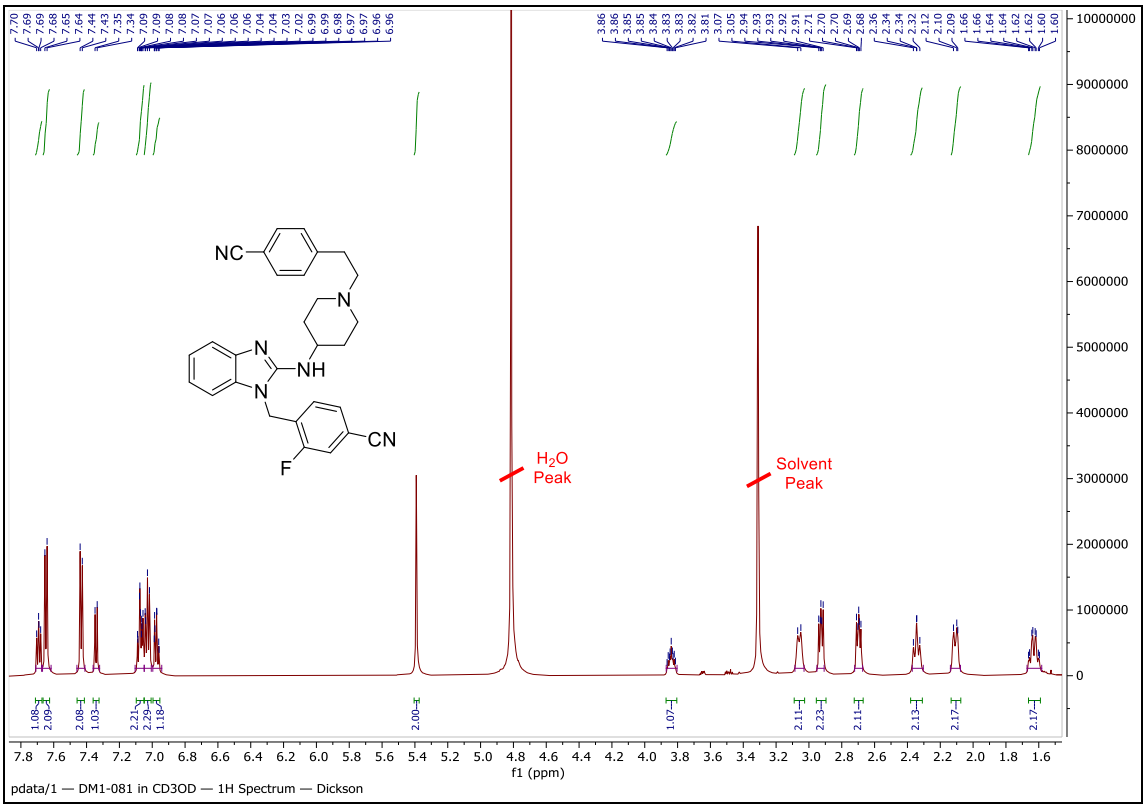


Figure 12: ^{1}H-NMR spectrum of **25** in Methanol-d_4 at 600 MHz

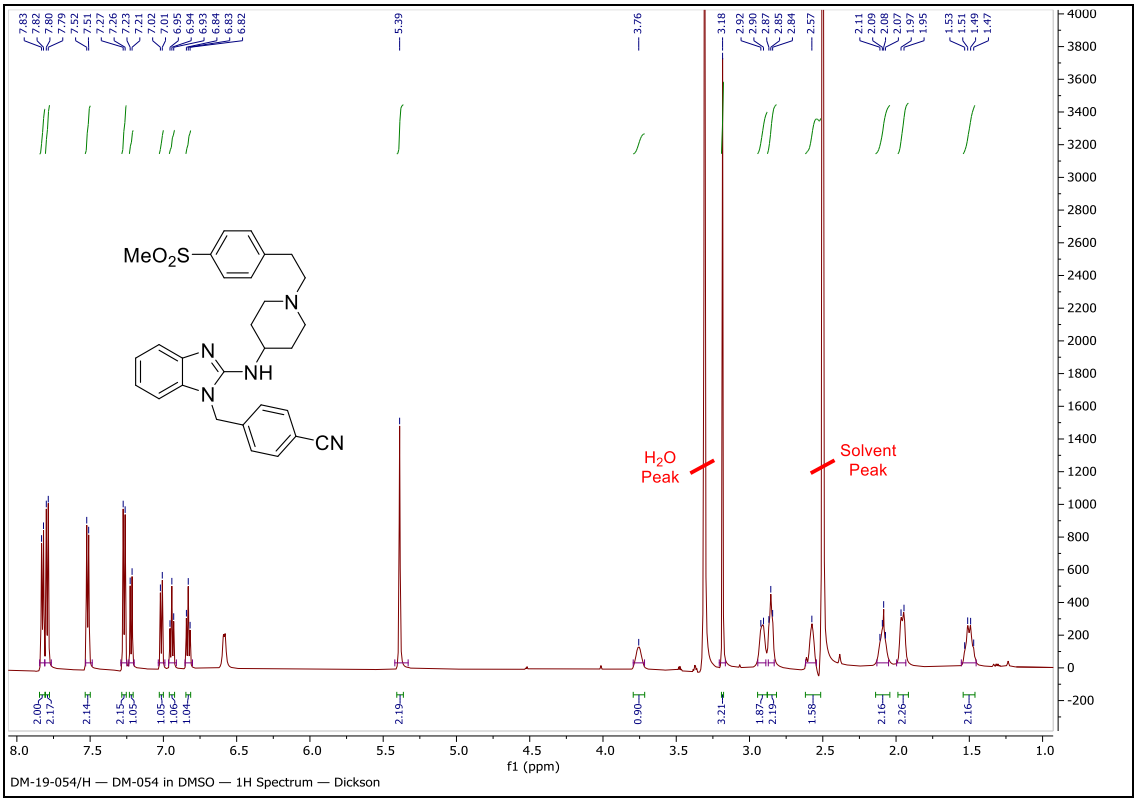


Figure 13: ^{1}H-NMR spectrum of **28** in DMSO-d_6 at 600 MHz

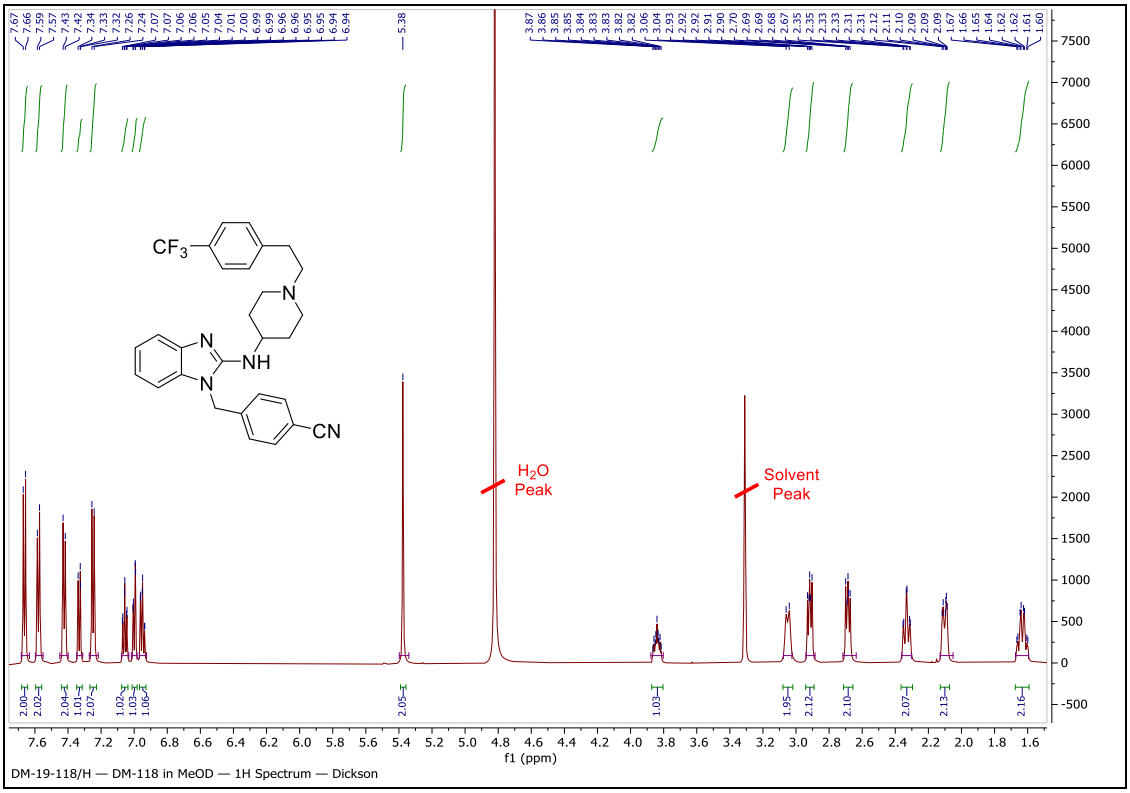


Figure 14: ^{1}H-NMR spectrum of **30** in Methanol-d_4 at 400 MHz

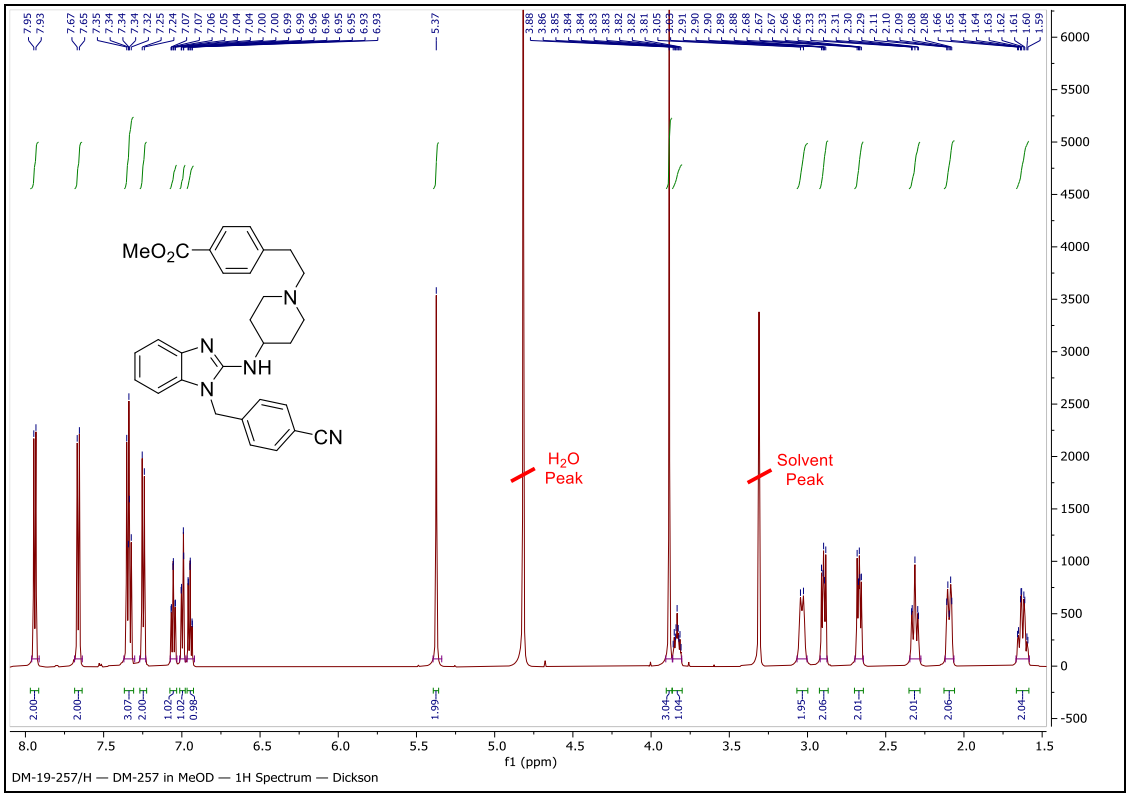


Figure 15: ^{1}H-NMR spectrum of **31** in Methanol-d_4 at 600 MHz

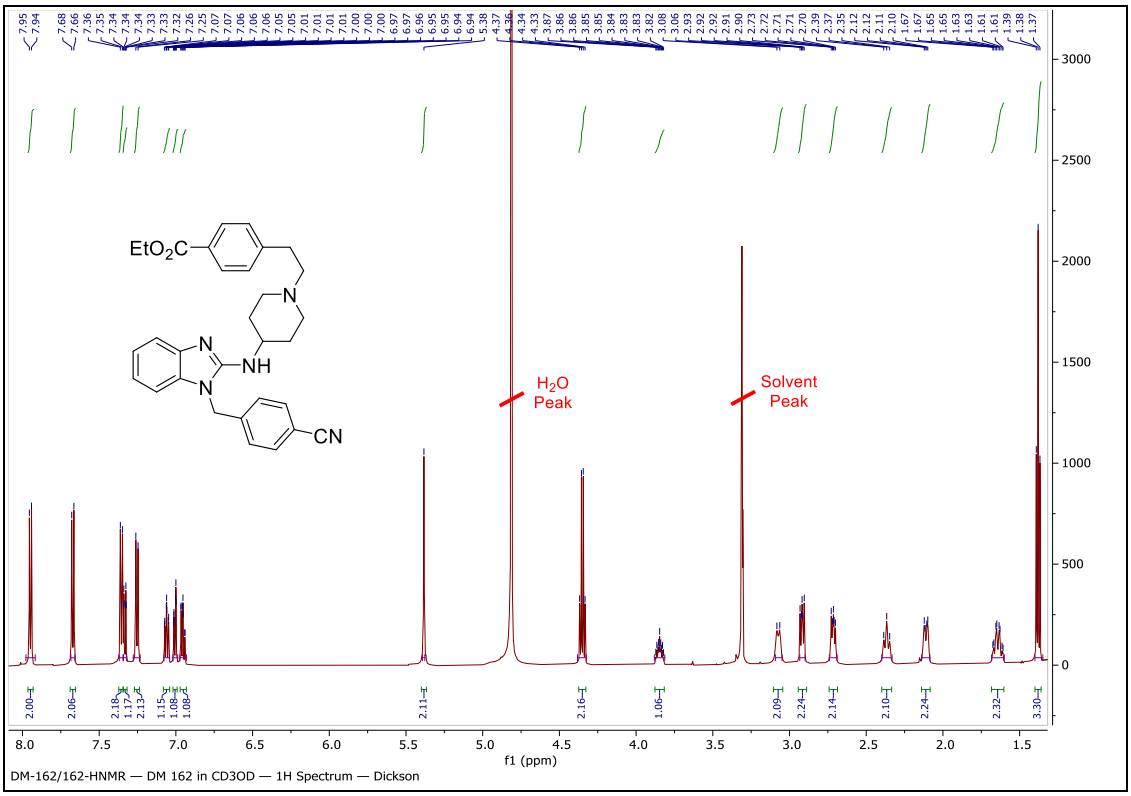


Figure 16: ^{1}H-NMR spectrum of **33** in Methanol-d_4 at 400 MHz

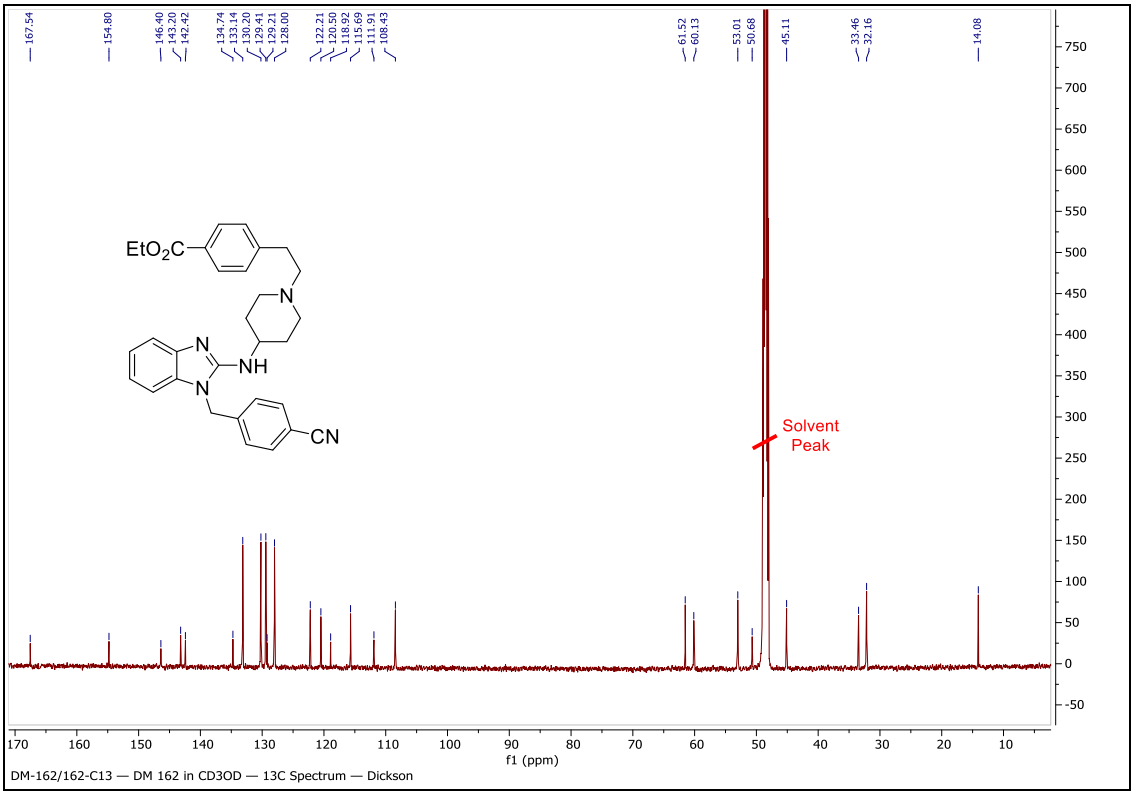


Figure 17: [1]13-NMR spectrum of **33** in Methanol-d_4 at 151 MHz

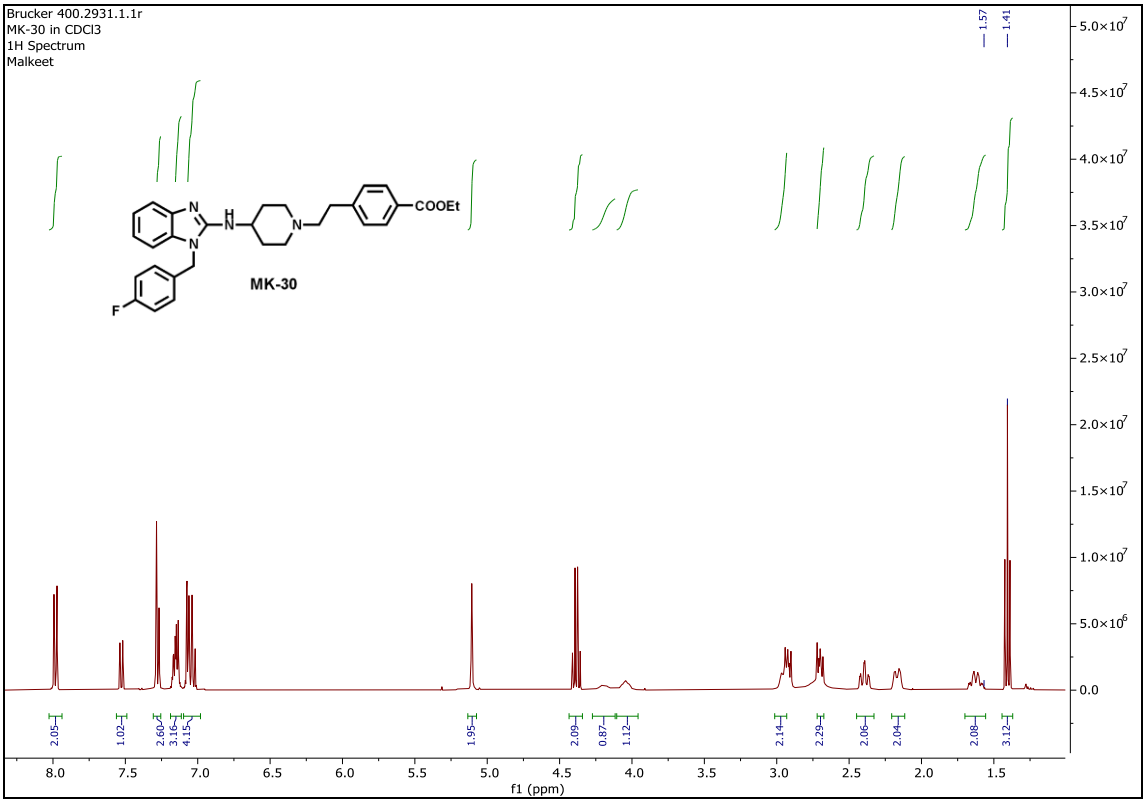


Figure 18: [1]H-NMR spectrum of **34** in Methanol-d_4 at 400 MHz

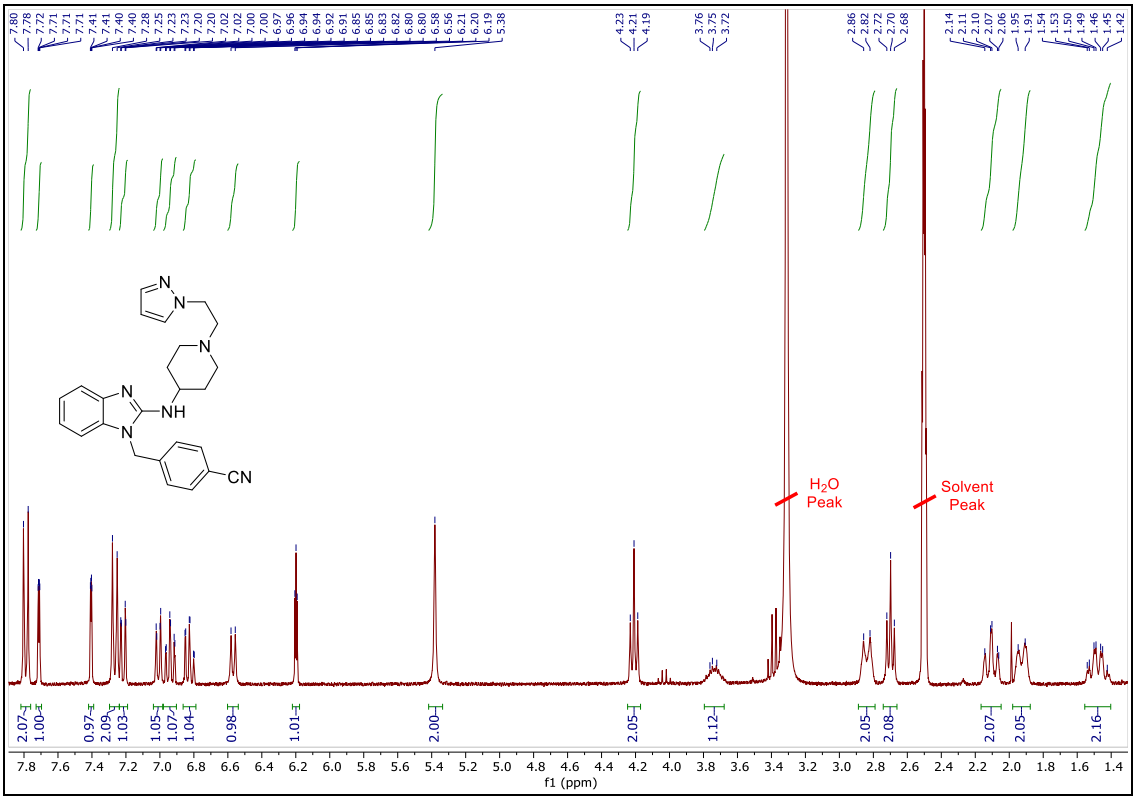


Figure 19: ^{1}H-NMR spectrum of **35** in DMSO-d_6 at 400 MHz

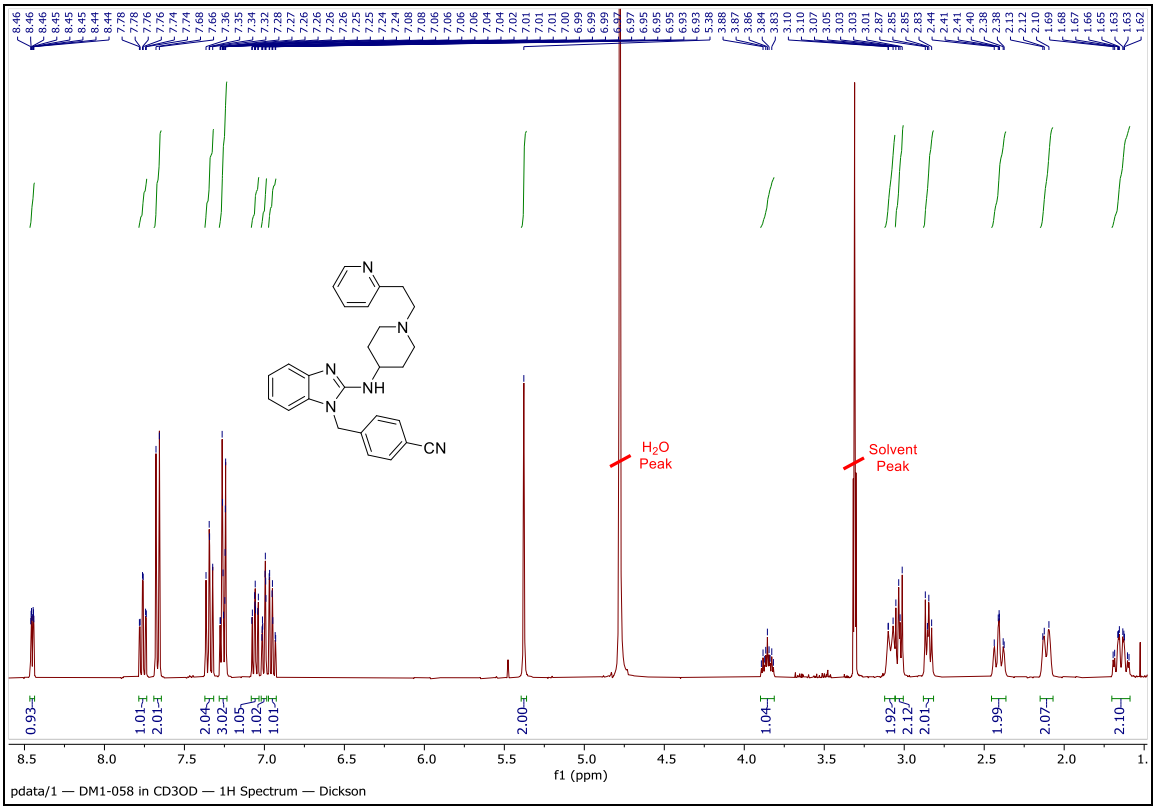


Figure 20: ^{1}H-NMR spectrum of **36** in DMSO-d_6 at 400 MHz

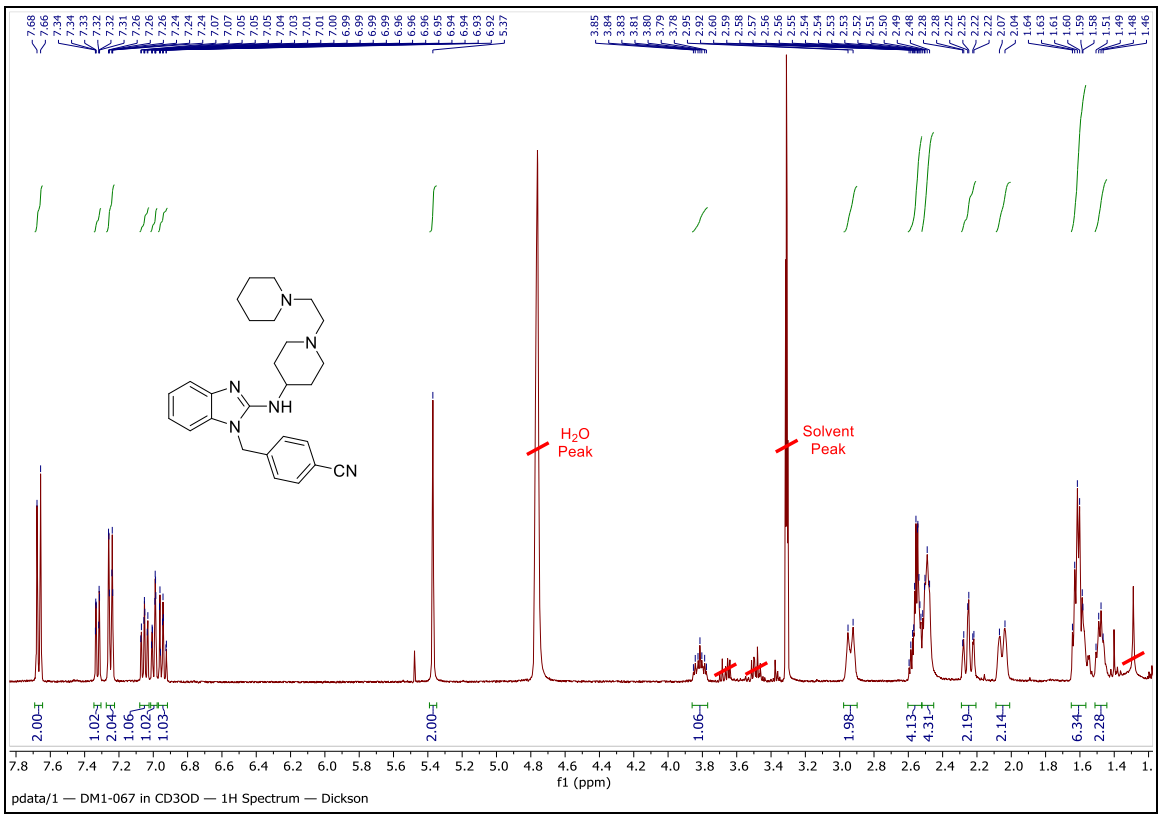


Figure 21: ^{1}H-NMR spectrum of **37** in DMSO-d_6 at 400 MHz

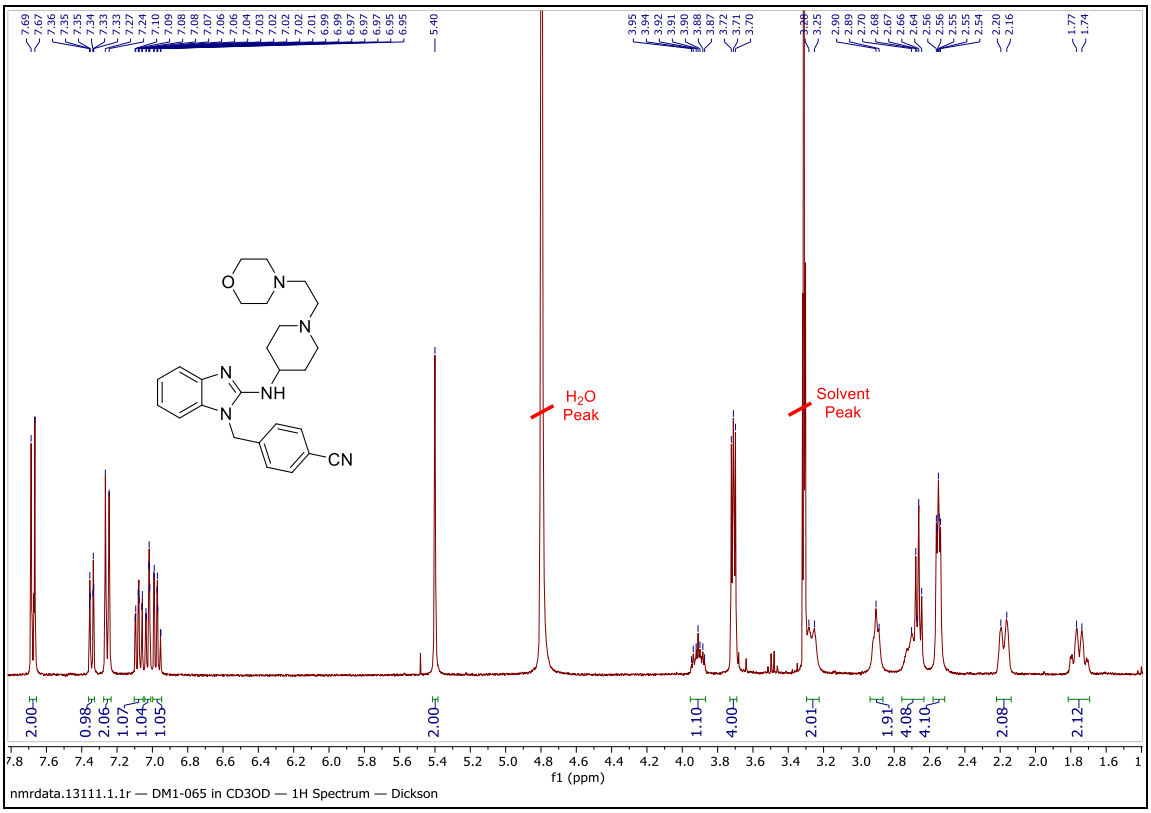


Figure 22: ^{1}H-NMR spectrum of **38** in Methanol-d_4 at 400 MHz

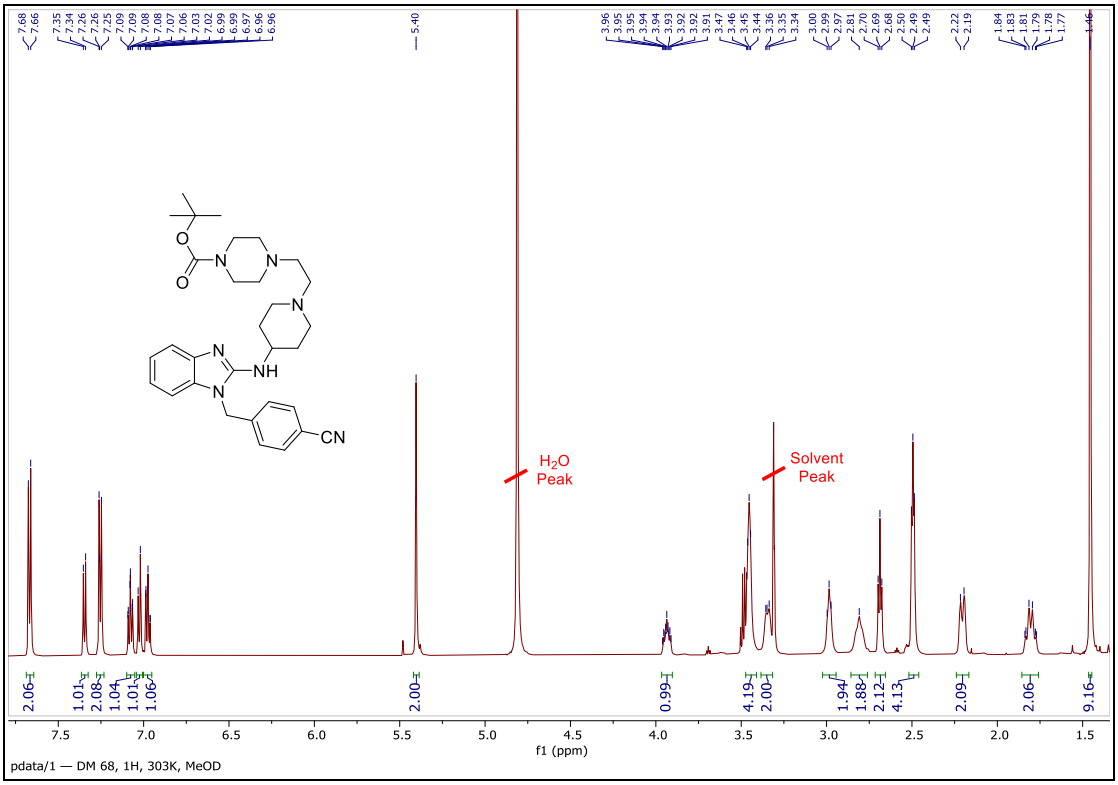


Figure 23: ^{1}H-NMR spectrum of **40** in Methanol-d_4 at 600 MHz

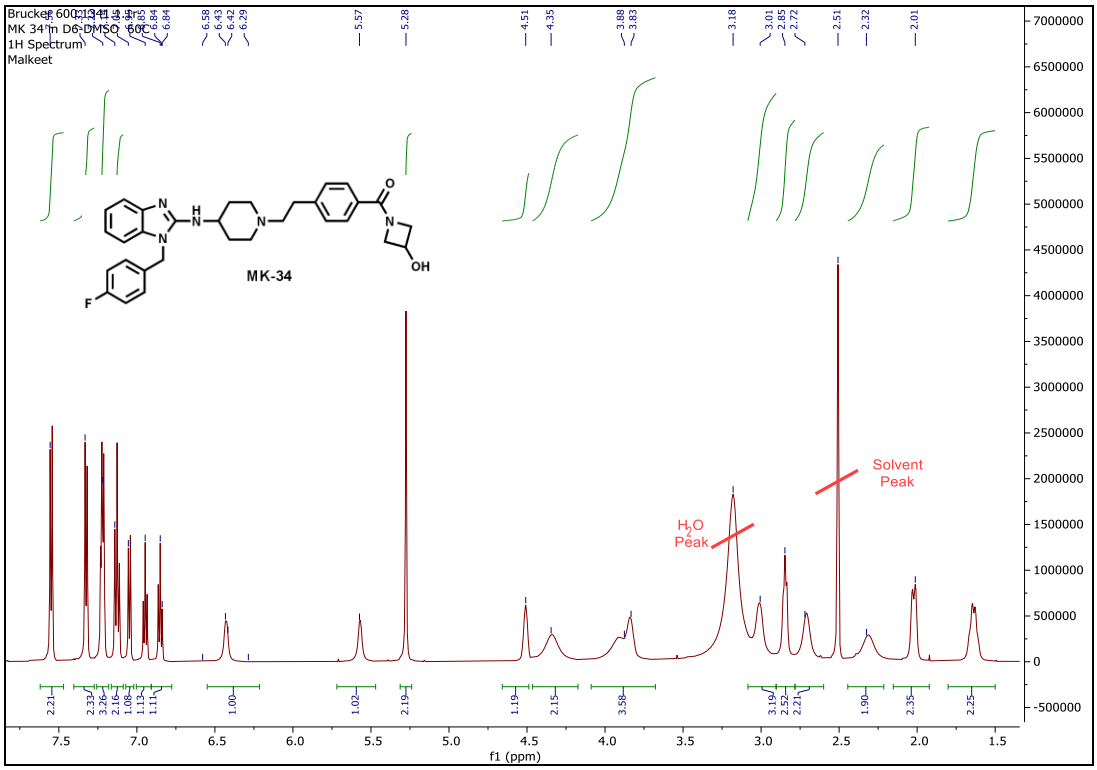


Figure 24: ^{1}H-NMR spectrum of **43** in DMSO-d_6 at 600 MHz

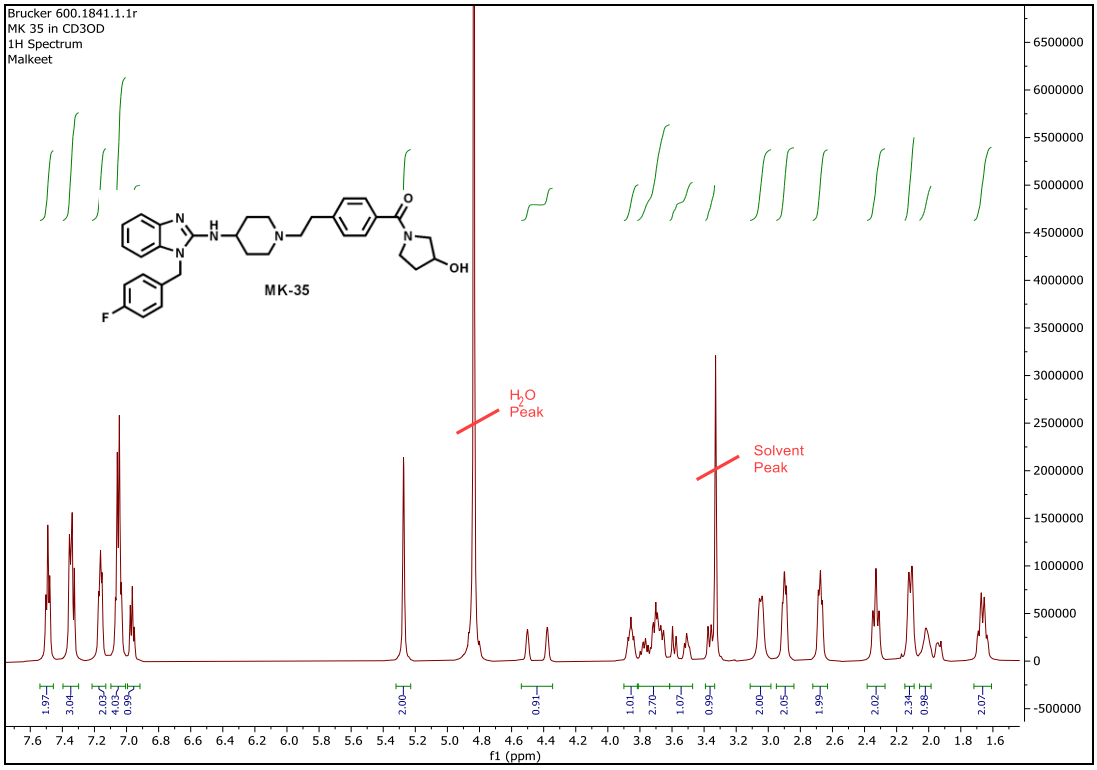


Figure 25: ^{1}H-NMR spectrum of **44** in Methanol-d_4 at 600 MHz

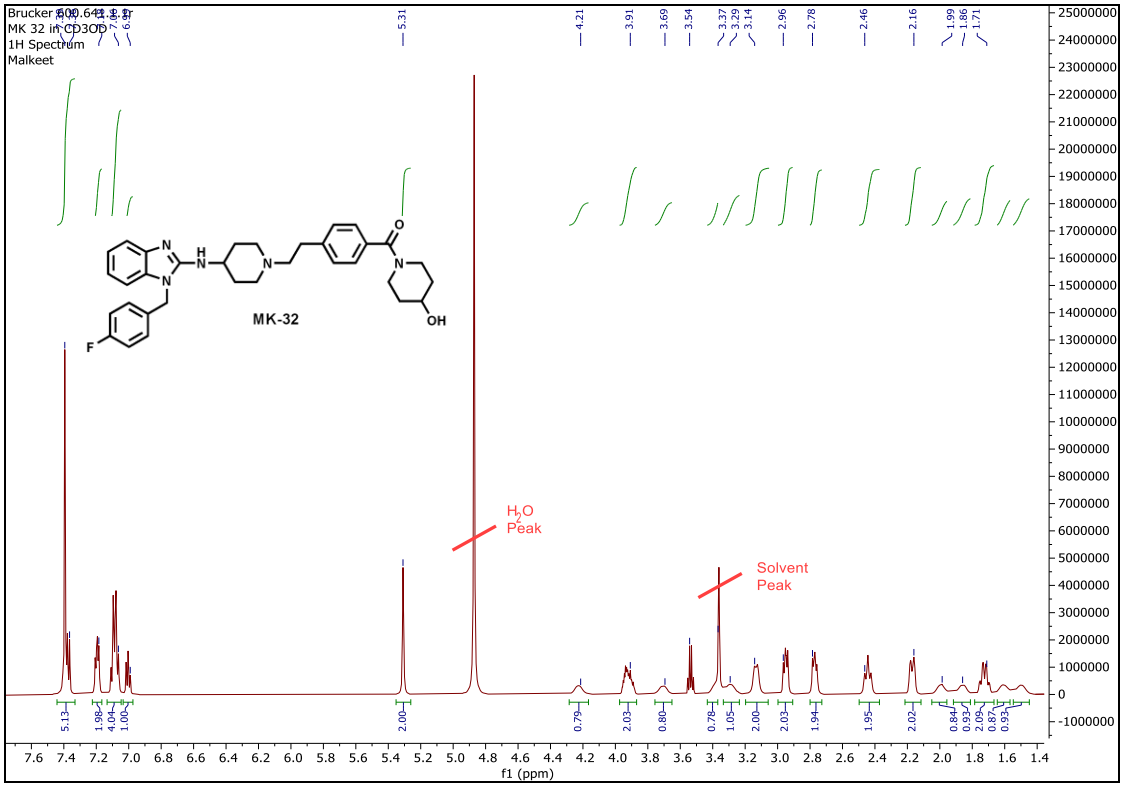


Figure 26: ^{1}H-NMR spectrum of **45** in Methanol-d_4 at 600 MHz

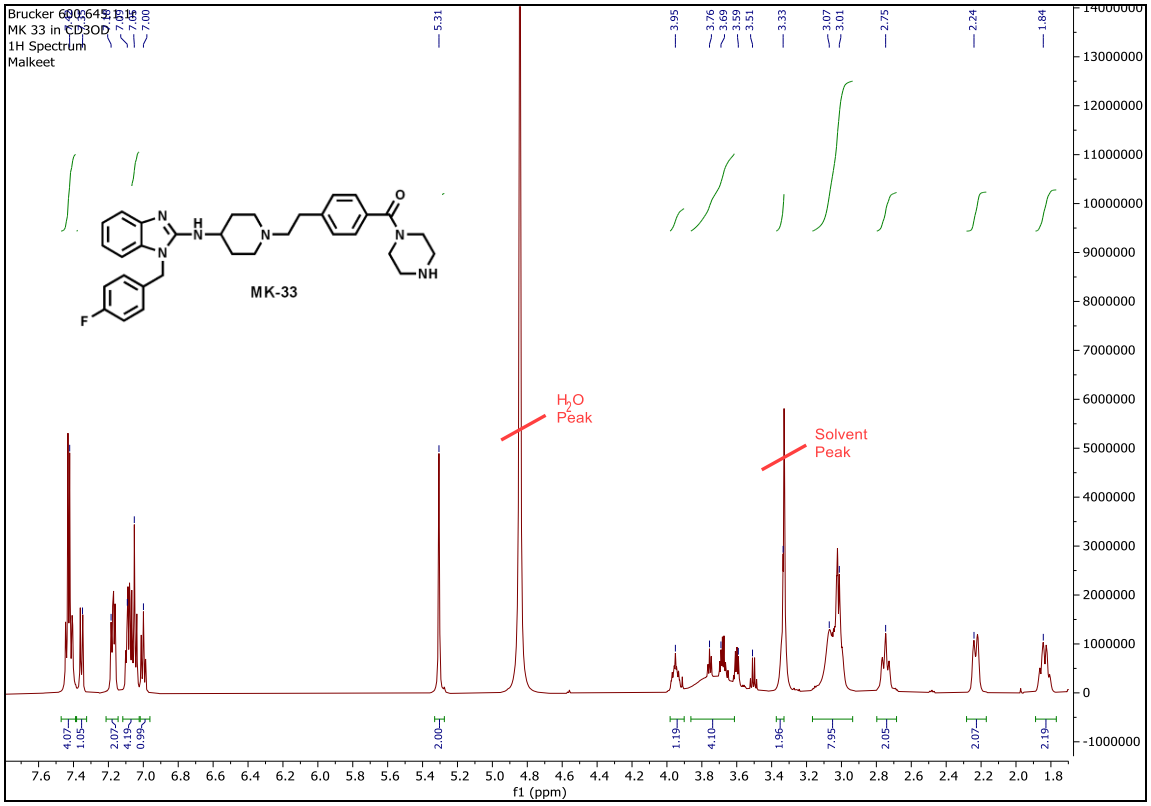


Figure 27: ^{1}H-NMR spectrum of **46** in Methanol-d_4 at 600 MHz

References

(1) Kimura, Tomio; Ohkawa, Nobuyuki; Nakao, Akira; Nagasaki, Takayoshi; Shimozato, T. Preparation of Cyclic Tertiary Amine Compounds That Inhibit Inflammatory Cytokine Production, 2007.

(2) Kühnel, E.; Laffan, D. D. P.; Lloyd-Jones, G. C.; Martínez del Campo, T.; Shepperson, I. R.; Slaughter, J. L. Mechanism of Methyl Esterification of Carboxylic Acids by Trimethylsilyldiazomethane. *Angew. Chemie Int. Ed.* **2007**, *46* (37), 7075–7078.

(3) Rosenberger, N.; Studer, A.; Takatani, N.; Nakajima, H.; Watanabe, Y. Azurin-Poly(N -Isopropylacrylamide) Conjugates by Site-Directed Mutagenesis and Their Thermosensitive Behavior in Electron-Transfer Processes. *Angew. Chemie Int. Ed.* **2009**, *48* (11), 1946–1949.

(4) Ajvazi, N.; Stavber, S. Direct Halogenation of Alcohols with Halosilanes under Catalyst- and Organic Solvent-Free Reaction Conditions. *Tetrahedron Lett.* **2016**, *57* (22), 2430–2433.

(5) Reader, J.; Botha, M.; Theron, A.; Lauterbach, S. B.; Rossouw, C.; Engelbrecht, D.; Wepener, M.; Smit, A.; Leroy, D.; Mancama, D.; Coetzer, T. L.; Birkholtz, L. Nowhere to Hide: Interrogating Different Metabolic Parameters of Plasmodium Falciparum Gametocytes in a Transmission Blocking Drug Discovery Pipeline towards Malaria Elimination. *Malar. J.* **2015**, *14* (1), 213.

(6) Bertrand, M.; Jackson, P.; Walther, B. Rapid Assessment of Drug Metabolism in the Drug

Discovery Process. *Eur. J. Pharm. Sci.* **2000**, *11*, S61–S72.