Electronic Supplementary Material (ESI) for RSC Advances.
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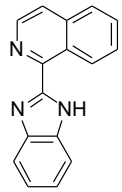
Supporting Information

Contents

1. Chemistry

All chemicals were purchased from Sigma Aldrich, Merck, A K Scientific, or Ark Pharm Inc. BODIPY®630/650-SE was purchased from Life Technologies. Reactions were carried out at room temperature (rt) unless otherwise stated. Thin layer chromatography (t.l.c.) was performed on 0.2 mm aluminium-backed silica gel plates 60 F_{254} and visualised under UV light at λ = 254 and 365 nM, and with ninhydrin and/or $KMnO_4$ dip. Flash silica gel column chromatography was performed using 40-63 µm silica. An Agilent 1260 Infinity system was used for reverse phase high-performance liquid chromatography (RP-HPLC), with a YMC C8 5 µm (150 x 10 mm) semi-preparative or YMC C8 5 µm (150 x 4.6 mm) analytical column. RP-HPLC solvents were A: H_2O (0.05% TFA) and B: 9:1 MeCN:H_2O (0.05% TFA). Analytical RP-HPLC retention times are reported using following method - 5% solvent B 1 min, gradient of 5-95% solvent B 1-27 mins, 95% solvent B 27-28 mins, gradient of 95-5% solvent B 28-30 mins, 5% solvent B 30-34 mins. TFA salts of RP-HPLC purified compounds were neutralised using an Amberlyst A21 ion exchange resin before biological testing. Analytical RP-HPLC was used to confirm purity (> 95%) at 254 and 380 nm for all compounds biologically tested. High resolution electrospray ionization mass spectra (HRMS-ESI) were recorded on a Bruker microTOF mass spectrometer. NMR spectra were obtained on a Varian 400-MR or Varian 500 MHz AR Premium Shielded spectrometer. Chemical shifts are listed in ppm (δ), calibrated using residual undeuterated solvent as the internal standard, and coupling constants (*J*) are recorded in hertz (Hz). Note - not all magnetically non-equivalent carbons were observed in ^{13}C NMR spectrum for all compounds.

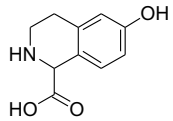
Synthesis of 1-(1*H*-Benzimidazol-2-yl)isoquinoline (1)



Compound was synthesised according to a previously reported literature synthesis of **1**.[1] ^{1}H NMR spectroscopy data in DMSO-d_6 was similar to this literature report[1]. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.22 – 7.36 (m, 2H, ArH benzimidazole), 7.59 – 7.66 (m, 1H, ArH benzimidazole), 7.80 – 7.91 (m, 3H, ArH benzimidazole and ArH benzimidazole), 7.96 – 8.03 (m, 1H, ArH isoquinoline), 8.03 – 8.13 (m, 1H, ArH isoquinoline), 8.70 (d, 1H, J = 5.5, ArH isoquinoline), 10.09 – 10.13 (m, 1H, ArH isoquinoline), 13.22 (s, 1H, NH). ^{13}C NMR (101 MHz, DMSO-d_6) δ 112.05, 119.72, 121.94, 122.34, 123.63, 125.95, 127.21, 127.82, 128.61, 130.61, 134.02, 136.77, 141.64, 144.07, 146.64, 151.17.
^{1}H NMR (400 MHz, Methanol-d_4) δ 7.28 – 7.40 (m, 2H, ArH benzimidazole), 7.65 (s, 1H, ArH benzimidazole), 7.75 – 7.87 (m, 3H, ArH benzimidazole and ArH isoquinoline), 7.90 (d, 1H, J = 5.9 Hz, ArH isoquinoline), 7.97 – 8.05 (m, 1H, ArH isoquinoline), 8.64 (d, 1H, J = 5.6 Hz, ArH isoquinoline), 9.45 – 9.54 (m, 1H, ArH isoquinoline). ^{13}C NMR (101 MHz, Methanol-d_4) δ 123.62, 127.91, 128.25, 128.71, 129.59, 131.88, 138.67, 142.83, 149.04, 152.34. HRMS calculated for $C_{16}H_{12}N_3$ $(M + H)^+$, 246.1026; found, 246.1029. Analytical RP-HPLC R_t = 12.93 min.

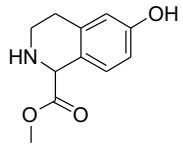
1.1 Experimental details for scheme 1

6-Hydroxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid (5)



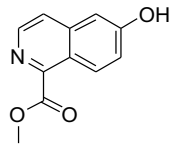
2-(3-Methoxyphenyl)ethan-1-amine was converted to **4** according to literature.[2] ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.74 – 2.81 (m, 2H, CH_2), 2.95 – 3.04 (m, 2H, CH_2), 6.60 – 6.75 (m, 3H, ArH), 7.03 – 7.17 (m, 1H, ArH), 7.80 (br s). Amine **4** was then cyclised to give **5** according to literature.[3] ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.66 – 2.79 (m, 1H, C**H***HCH$_2$), 2.79 – 2.93 (m, 1H, CH**H***CH$_2$), 3.01 – 3.14 (m, 1H, CH$_2$C**H***H), 3.26 – 3.34 (m, 1H, CH$_2$CH**H***), 4.33 (s, 1H, C**H**CO$_2$H), 6.49 (d, J = 2.5 Hz, 1H, ArH), 6.60 (dd, J = 2.6 Hz,1H, 8.5, ArH), 7.50 (d, J = 8.5 Hz,1H, ArH), 8.80 (br m, 2H, NH, OH), 9.37 (br s, 1H, OH). (* designates diastereotopic protons). ^{13}C NMR (101 MHz, DMSO-d_6) δ 25.25, 57.82, 113.57, 113.91, 121.19, 129.06, 132.69, 155.88, 167.15. HRMS calculated for $C_{10}H_{10}NO_3$ $(M - H)^-$, 192.0652; found, 192.0666.

Methyl 6-hydroxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylate (6)



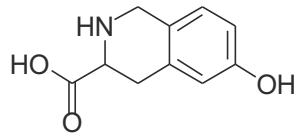
$SOCl_2$ (7.5 mL, 102.95 mmol) was added dropwise to a 0 °C suspension of **5** (6.63 g, 34.32 mmol) in MeOH (150 mL), then the mixture was warmed to rt and refluxed for 15 h. Solvent was removed under reduced pressure and the resulting solid was dissolved in THF (30 mL) and neutralised with Et_3N (15 mL). The reaction mixture was filtered to remove $Et_3N.HCl$ and the filtrate evaporated to give **6** (7.11 g, 34.31 mmol, yield quantitative) as a yellowish solid. ^{1}H NMR (400 MHz, MeOD-d_4) δ 2.66 – 2.79 (m, 2H, C**H**$_2^*$CH$_2$), 2.93 – 3.02 (m, 1H, CH$_2$C**H***H), 3.21 – 3.30 (m, 1H, CH$_2$CH**H***), 3.74 (s, 3H, OC**H**$_3$), 4.60 (s, 1H, C**H**CO$_2$CH$_3$), 6.54 (d, J = 2.6 Hz,1H, ArH), 6.60 (dd, J = 2.6, 8.4 Hz, 1H, ArH), 7.13 (d, J = 8.46 Hz, 1H, ArH). (* designates diastereotopic protons). ^{13}C NMR (101 MHz, MeOD-d_4) δ 29.49, 41.18, 52.65, 58.98, 114.60, 116.22, 123.45, 129.77, 137.57, 157.64, 174.89. HRMS calculated for $C_{11}H_{14}NO_3$ $(M + H)^+$, 208.0973; found, 208.0975. The synthesis of **6** has previously been reported by Ma *et al*[4] but without spectroscopic data of **6**.

Methyl 6-hydroxyisoquinoline-1-carboxylate (7)



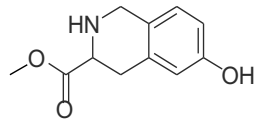
To a solution of **6** (0.17 g, 0.83 mmol) in 1,4-dioxane:THF (10mL 1:1, *v*:*v*) at 45 °C was added DDQ (0.38 g, 1.67 mmol) and the reaction was stirred vigorously at 45 °C for 5 h with the mouth of the flask open to the atmosphere to allow mixing of air. 1,4-Dioxane (10 mL) was added and the reaction mixture filtered, the filtrate was diluted with EtOAc and washed three times with sat aq. $NaHCO_3$ solution. The organic washings were combined, washed once with water, brine solution, then dried over $MgSO_4$, concentrated under reduced pressure and purified by silica gel flash column chromatography (30 to 50% EtOAc/hexane) to provide **7** (84 mg, 0.413 mmol, yield 49 %) as an off-white solid. ^{1}H NMR (400 MHz, MeOD-d_4) δ 4.04 (s, 3H, OCH$_3$), 7.16 (d, J = 2.5 Hz,1H, ArH), 7.28 (dd, 1H, J = 2.5, 9.3 Hz, ArH), 7.73 (d, 1H, J = 5.7 Hz, ArH), 8.30 (d, 1H, J = 5.7 Hz, ArH), 8.52 (d, 1H, J = 9.3 Hz, ArH). ^{13}C NMR (101 MHz, MeOD-d_4) δ 53.24, 108.72, 122.70, 122.89, 124.25, 129.49, 141.07, 141.83, 149.28, 161.18, 167.61. HRMS calculated for $C_{11}H_{10}NO_3$ $(M + H)^+$, 204.0655; found, 204.0647. To our knowledge, isoquinoline **7** has only been reported once before in the literature in a Japanese patent[5] but no spectroscopic data was provided.

6-Hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (9)



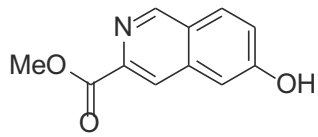
(+/-)-*m*-Tyrosine was converted to **9** according to literature.[6] ^{1}H NMR (400 MHz, deuterium oxide[#]) δ 2.73 (dd, 1H, *J* = 11.0, 16.4 Hz, C**H**[*]HCHNH), 2.92 (dd, 1H, *J* = 4.5, 16.5 Hz, CH**H**[*]CHNH), 3.38 (dd, 1H, *J* = 4.5, 11.0 Hz, CH), 3.73 – 3.9 6 (m, 2H, NHC**H$_2$**[*]), 6.40 – 6.51 (m, 2H), 6.87 (d, 1H, *J* = 8.3 Hz) ([*] designates diastereotopic protons). ^{13}C NMR (101 MHz, deuterium oxide*[#]) δ 32.03, 45.96, 58.01, 117.30, 118.14, 120.55, 127.30, 135.26, 164.38, 181.23. [#]Due to insolubility of compound in MeOD-d_4, DMSO-d_6 and deuterium oxide, NMR was obtained by dissolving 31 mg of **9** in a solution of deuterium oxide (20 mg KOH in 0.8 mL deuterium oxide). HRMS calculated for $C_{10}H_{10}NO_3$ $(M - H)^-$, 192.0651; found, 192.0666.

Methyl 6-hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (10)



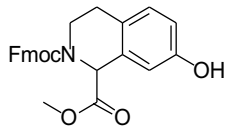
To a solution of **9** (2.0 g, 10.35 mmol) in MeOH (50 mL) was added H_2SO_4 (96%, 1 mL) and the reaction refluxed for 12 h, cooled, neutralised with $NaHCO_3$ solution and extracted with EtOAc (3 × 100 mL). The EtOAc layer was washed with brine solution (50 mL), dried over $MgSO_4$ and the solvent removed under reduced pressure to give **10** (2.05 g, 9.90 mmol, 96% yield) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.80 – 3.03 (m, 2H, NHCHC**H$_2$**[*]), 3.68 – 3.74 (m, 1H, NHC**H**), 3.76 (s, 3H, OCH_3), 3.93 – 4.10 (m, 2H, NHC**H$_2$**[*]), 4.68 (br s, 2H, OH, NH), 6.48 (d, 1H, *J* = 2.6 Hz, ArH), 6.60 (dd, 1H, *J* = 2.6, 8.3 Hz, ArH), 6.85 (d, 1H, *J* = 8.3 Hz, ArH). [*] designates diastereotopic protons. ^{13}C NMR (101 MHz, $CDCl_3$) δ 31.48, 46.66, 52.45, 55.73, 114.30, 115.62, 125.78, 127.36, 134.12, 154.91, 173.44. HRMS calculated for $C_{11}H_{14}NO_3$ $(M + H)^+$, 208.0968; found, 208.0950. The synthesis of methyl ester **10** from **9** has previously been reported[7-11] however none of these reports include spectroscopic data for **10**.

Methyl 6-hydroxyisoquinoline-3-carboxylate (11)



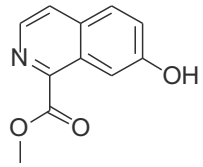
Following the procedure described for **7**, a mixture of **10** (1.2 g, 5.79 mmol) and DDQ (2.63 g, 11.58 mmol) gave **11** (0.6g, 2.95 mmol, 52% yield) as an off-white solid. ^{1}H NMR (400 MHz, MeOD-d_4) δ 3.99 (s, 3H, OC**H$_3$**), 7.23 (d, *J* = 2.3 Hz, 1H, ArH), 7.34 (dd, *J* = 8.9, 2.4 Hz, 1H, ArH), 8.01 (d, *J* = 8.9 Hz, 1H, ArH), 8.37 (s, 1H, ArH), 9.04 (s, 1H, ArH). ^{13}C NMR (101 MHz, MeOD-d_4) δ 53.01, 109.88, 123.57, 123.97, 126.30, 131.11, 139.25, 141.89, 152.66, 162.04, 167.28. HRMS calculated for $C_{11}H_9NNaO_3$ $(M + Na)^+$, 226.0475; found, 226.0462. The synthesis of **11** from **10** has previously been reported[11] but without spectroscopic data of **11**.

2-(9H-Fluoren-9-yl)methyl 1-methyl 7-hydroxy-1,2,3,4-tetrahydroisoquinoline-1,2-dicarboxylate (13)



4-(2-aminoethyl)phenol was converted to (9H-fluoren-9-yl)methyl N-[2-(4-hydroxyphenyl)ethyl]carbamate **12** according to modified literature procedure.[3] ^{1}H NMR (400 MHz, MeOD-d_4) δ 2.67 (t, 2H, J = 7.3 Hz, NHCH$_2$C**H$_2$**), 3.27 (t, 2H, J = 7.3 Hz, NHC**H$_2$**CH$_2$), 4.18 (t, 1H, J = 6.8 Hz, C**H** Fmoc), 4.32 (d, 2H, J = 6.9 Hz, C**H$_2$** Fmoc), 6.63 – 6.73 (m, 2H, ArH), 7.00 (d, 2H, J = 8.1 Hz, ArH), 7.25 – 7.35 (m, 2H, ArH Fmoc), 7.35 – 7.44 (m, 2H, ArH Fmoc), 7.62 (d, 2H, J = 7.5 Hz, ArH Fmoc), 7.79 (d, 2H, J = 7.5 Hz, ArH Fmoc). HRMS calculated for $C_{23}H_{21}NNaO_3$ $(M + Na)^+$, 382.1414; found, 382.1387. To a solution of **12** (19.7 g, 54.85 mmol) in AcOH/H_2SO_4 (200 mL, 3:1 *v*:*v*) was added a solution of glyoxylic acid monohydrate (5.5 g, 92.1 mmol) and stirred for 24 h. The reaction mixture was poured slowly into ice/water (caution: exothermic reaction) and the precipitate formed was separated and dried under vacuum oven (60°C, 300 mbar) for 12 h to give a pinky white solid. This solid was dissolved in DCM and washed with water, brine, dried over $MgSO_4$ and evaporated under reduced pressure to give 2-{[(9H-fluoren-9-yl)methoxy]carbonyl}-7-hydroxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid (previously reported by Maillard *et al*[3]) as a pinky white solid (18.4 g) which was used in the next reaction without further purification. To a solution of 2-{[(9H-fluoren-9-yl)methoxy]carbonyl}-7-hydroxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid (18.2 g, 43.81 mmol) in MeOH (200 mL) at 0^o C was added dropwise $SOCl_2$ (6.4 mL, 87.62 mmol). The reaction mixture was then heated to 60° C and stirred for 12 h. The reaction mixture was cooled to rt then concentrated under reduced pressure to give a syrupy residue. This residue was dissolved in EtOAc, washed with sat aq. $NaHCO_3$ solution, brine solution, dried over $MgSO_4$ and purified by silica gel flash column chromatography (30 to 40% EtOAc/hexane) to give **13** (4.3 g, 10.01 mmol, 18% yield from **12**) as a white foamy solid. Rt and high temperature NMR spectra revealed the presence of two rotamers in an 2:3 ratio (labelled rotamer A and B). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.56 – 2.79 (m, 2H, C**H$_2$**CH$_2$NFmoc rotamer A and B), 3.45 – 3.72 (m, 4H, OC**H$_3$**, C**H**CO$_2$CH$_3$ rotamer A and B), 4.23 – 4.51 (m, 2H, CH$_2$C**H$_2$**NFmoc rotamer A and B), 5.13 – 5.45 (m, 1H, CHFmoc rotamer A and B) 6.61 – 6.71 (m, 1H, ArH rotamer A and B), 6.81 (dd, J = 2.5, 37.4 Hz, 1H, ArH rotamer A and B), 7.00 (t, J = 8.5, 8.5 Hz, 1H, ArH rotamer A and B), 7.26 – 7.38 (m, 2H, ArH rotamer A and B), 7.38 – 7.48 (m, 2H, ArH rotamer A and B), 7.57 – 7.71 (m, 2H, ArH rotamer A and B), 7.85 – 7.95 (m, 2H, ArH rotamer A and B), 9.39 – 9.46 (m, 1H, OH rotamer A and B). HRMS calculated for $C_{26}H_{23}NNaO_5$ $(M + Na)^+$, 452.1468; found, 452.1443.

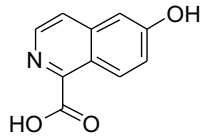
Methyl 7-hydroxyisoquinoline-1-carboxylate (14)



According to a modified literature procedure[12] - tetrahydroisoquinoline **13** (4.1 g, 9.55 mmol), DMSO (20 mL) and MeOH (20 mL) were stirred at 60 °C for 12 h, diluted with EtOAc, filtered, the filtrate washed with water and dried over $MgSO_4$. The solvent was evaporated under reduced pressure to give the Fmoc-deprotected product methyl 7-hydroxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylate, which was then aromatised according to the procedure for **7**, using DDQ (4.4 g, 19.09 mmol) and 1,4-dioxane:THF (1:1 *v*:*v*, 100 mL). The crude material was purified by silica gel flash column chromatography (30 to 50% EtOAc/hexane) to provide **14** (1.2 g, 5.90 mmol, 61% yield) as an off-white solid. ^{1}H NMR (500 MHz, MeOD-d_4) δ 4.03 (s, 3H, OC**H**$_3$), 7.39 (dd, 1H, J = 2.4 Hz, 8.9, ArH), 7.84 – 7.90 (m, 2H, ArH), 8.01 – 8.06 (m, 1H, ArH), 8.31 (d, 1H, J = 5.5 Hz, ArH). ^{13}C NMR (126 MHz, MeOD-d_4) δ 53.11, 107.81, 125.21, 125.86, 129.76, 130.23, 133.68, 139.16, 147.02, 159.46, 167.59. HRMS calculated for $C_{11}H_9NNaO_3$ $(M + Na)^+$, 226.0475; found, 226.0481.

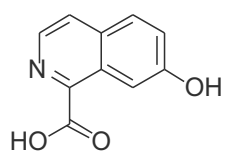
1.1 Experimental details for scheme 2

6-Hydroxyisoquinoline-1-carboxylic acid (15)



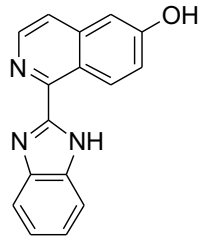
To a solution of **7** (2.6g, 12.79 mmol) in THF (20 mL) at 0°C was added a solution of LiOH (0.92 g, 38.38 mmol) in water (15 mL). The reaction mixture was warmed to rt and stirred for another 12 h. The solvent was evaporated under reduced pressure and the residue was acidified (pH 2.0-3.0) with 2.0 N aq. HCl , which gave a yellow precipitate that was collected by filtration, washed with 5.0 N aq. HCl (50 mL) and dried to give **15** (2.3 g, 12.15 mmol, 96% yield) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.24 (d, 1H, J = 2.5 Hz, ArH), 7.33 (dd, 1H, J = 2.5, 9.2 Hz, ArH), 7.85 (dd, 1H, J = 0.8, 6.0 Hz, ArH), 8.33 (d, J = 5.8 Hz,1H, , ArH), 8.65 (d, 1H, J = 9.2 Hz, ArH), 10.78 (br s, 1H, OH). ^{13}C NMR (101 MHz, DMSO-d_6) δ 107.77, 120.06, 121.75, 122.26, 129.11, 139.05, 139.42, 149.60, 159.96, 165.99. HRMS calculated for $C_{10}H_7NNaO_3$ $(M + Na)^+$, 212.0318; found, 212.0323.

7-Hydroxyisoquinoline-1-carboxylic acid (16)



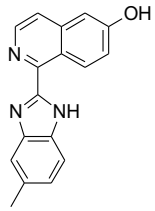
According to the procedure for **15**, a solution of **14** (1.0 g, 4.92 mmol) and LiOH (0.235 g, 9.84 mmol) gave **16** (0.65 g, 3.43 mmol, 70% yield) as yellowish solid. ^{1}H NMR (500 MHz, D_2O) δ 7.24 – 7.28 (m, 1H, ArH), 7.33 (dd, 1H, *J* = 2.4, 8.9 Hz, ArH), 7.68 (d, 1H, *J* = 5.8 Hz, ArH), 7.80 (d, 1H, *J* = 9.0 Hz, ArH), 8.09 (d, 1H, *J* = 5.7 Hz, ArH). ^{13}C NMR (126 MHz, D_2O) δ 110.27, 123.79, 128.39, 128.88, 131.25, 133.19, 139.13, 158.22, 163.05, 177.87. HRMS calculated for $C_{10}H_8NO_3$ $(M + H)^+$, 190.0499; found, 190.0494.

1-(1H-1,3-Benzodiazol-2-yl)isoquinolin-6-ol (17)



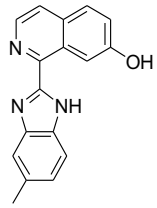
According to a modified literature procedure,[1] a mixture of **15** (0.1 g, 0.49 mmol), *o*-phenylenediamine (0.08 g, 0.74 mmol) and PPA (polyphosphoric acid, ≥83% phosphate as P_2O_5 basis) (~5 g) were heated at 250 °C for 6 h. The resulting viscous black liquid was slowly basified (pH = 8.0 - 10.0) with KOH at 0 °C (caution:exothermic) and extracted with EtOAc. The EtOAc layer was washed with sat aq. $NaHCO_3$ solution, water, brine solution, dried over $MgSO_4$ and the residue was purified by silica gel flash column chromatography (10 to 50% EtOAc/hexane) to give **17** (27 mg, 0.10 mmol, 21% yield) as an off-white solid. ^{1}H NMR (400 MHz, MeOD-d_4) δ 7.19 (d, 1H, *J* = 2.5 Hz, ArH isoquinoline), 7.27 – 7.38 (m, 3H, ArH isoquinoline, benzimidazole), 7.55 – 7.88 (m, 3H, ArH isoquinoline, benzimidazole), 8.46 (d, 1H, *J* = 5.8 Hz, ArH isoquinoline), 9.25 (d, 1H, *J* = 9.3 Hz, ArH isoquinoline). ^{13}C NMR (101 MHz, MeOD-d_4) δ 101.42, 108.77, 122.12, 122.31, 123.03, 130.86, 141.11, 142.86, 148.67, 152.44, 160.97. HRMS calculated for $C_{16}H_{12}N_3O$ $(M + H)^+$, 262.0975; found, 262.0967. Analytical RP-HPLC R_t = 11.61 min.

1-(5-Methyl-1H-1,3-benzodiazol-2-yl)isoquinolin-6-ol (18)



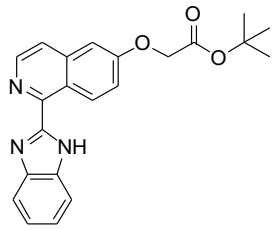
According to the procedure for **17**, a mixture of **15** (0.1 g, 0.49 mmol) and 3,4-diaminotoluene (0.07g, 0.59 mmol) were reacted to give **18** (24 mg, 0.08 mmol, 18% yield) as an off-white solid. ^{1}H NMR (400 MHz, MeOD-d_4) δ 2.48 (s, 3H, C**H**$_3$), 7.06 – 7.20 (m, 2H, ArH isoquinoline and ArH benzimidazole), 7.24 – 7.32 (m, 1H, ArH isoquinoline), 7.47 (br s, 1H, benzimidazole), 7.53 – 7.69 (m, 2H, ArH isoquinoline and ArH benzimidazole), 8.40 (d, 1H, *J* = 5.7 Hz, ArH isoquinoline), 9.20 (d, 1H, *J* = 9.3 Hz, ArH isoquinoline). ^{13}C NMR (101 MHz, MeOD-d_4) δ 21.81, 108.74, 121.98, 122.16, 122.94, 125.91, 130.87, 134.42, 141.02, 142.76, 148.70, 152.04, 160.82. HRMS calculated for $C_{17}H_{14}N_3O$ $(M + H)^+$, 276.1131; found, 276.1118. Analytical RP-HPLC R_t = 13.26 min.

1-(5-Methyl-1H-1,3-benzodiazol-2-yl)isoquinolin-7-ol (19)



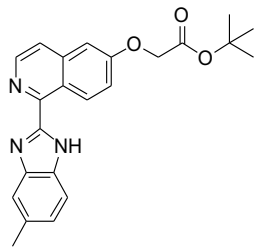
According to the procedure described for **17**, a mixture of **16** (0.2 g, 1.05 mmol) and 3,4-diaminotoluene (0.13 g, 1.05 mmol) were reacted to give **19** (72 mg, 0.26 mmol, 24% yield) as an off-white solid. ^{1}H NMR (500 MHz, MeOD-d_4) δ 2.51 (s, 3H, CH_3), 7.16 (d, 1H, J = 8.2 Hz, ArH benzimidazole), 7.40 (dd, 1H, J = 2.4, 8.9 Hz, ArH isoquinoline), 7.49 (br s, 1H, ArH benzimidazole), 7.60 (br s, 1H, ArH benzimidazole), 7.78 (d, 1H, J = 5.5 Hz, ArH isoquinoline), 7.88 (d, 1H, J = 8.9 Hz, ArH isoquinoline), 8.44 (d, 1H, J = 5.5 Hz, ArH isoquinoline), 8.74 (d, 1H, J = 2.4 Hz, ArH isoquinoline). ^{13}C NMR (126 MHz, MeOD-d_4) δ 21.82, 49.00, 109.34, 123.37, 124.61, 129.65, 129.98, 133.56, 140.13, 147.22, 158.93. HRMS calculated for $C_{17}H_{14}N_3O$ $(M + H)^+$, 276.1131; found, 276.1138. Analytical RP-HPLC R_t = 12.69 min.

***tert*-Butyl 2-{[1-(1H-1,3-benzodiazol-2-yl)isoquinolin-6-yl]oxy}acetate (20)**



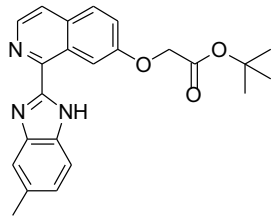
Isoquinoline **17** (45 mg, 0.172 mmol) and K_2CO_3 (48 mg, 0.0.34 mmol) in anhydrous THF (5 mL) were stirred at 60 °C for 20 min, followed by addition of a solution of *tert*-butyl bromoacetate (40 µL, 0.26 mmol) in anhydrous THF (1 mL). The reaction was stirred at 60 °C for 12 h. The solvent was removed under reduced pressure and the residue was partitioned between EtOAc and saturated aq. NH_4Cl, the EtOAc layer was washed further with brine solution, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography (20 to 30% EtOAc/hexane) to give **20** (60 mg, 0.16 mmol, 93% yield) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.51 (s, 9H, C(C**H**$_3$)$_3$), 4.69 (s, 2H, CH_2), 7.03 (d, 1H, J = 2.6 Hz, ArH benzimidazole), 7.28 – 7.34 (m, 2H, ArH benzimidazole), 7.37 – 7.54 (m, 2H, ArH isoquinoline and ArH benzimidazole), 7.58 (d, 1H, J = 5.6 Hz, ArH isoquinoline), 7.94 (br s, 1H, ArH benzimidazole), 8.47 (d, 1H, J = 5.6 Hz, ArH isoquinoline), 10.17 (d, 1H, J = 9.5 Hz, ArH isoquinoline), 11.48 (br s, 1H, NH). ^{13}C NMR (101 MHz, $CDCl_3$) δ 28.20, 65.77, 82.98, 105.71, 111.28, 120.68, 121.41, 121.82, 122.63, 122.95, 124.24, 131.02, 133.35, 139.31, 141.95, 144.95, 146.40, 151.48, 159.28, 167.46. HRMS calculated for $C_{22}H_{22}N_3O_3$ $(M + H)^+$, 376.1656; found, 376.1628.

***tert*-Butyl 2-{[1-(5-methyl-1H-1,3-benzodiazol-2-yl)isoquinolin-6-yl]oxy}acetate (21)**



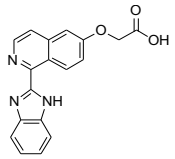
According to the procedure described for **20**, a mixture of **18** (150 mg, 0.54 mmol), K_2CO_3 (151 mg, 1.09 mmol) and *tert*-butyl bromoacetate (0.12 mL, 0.82 mmol) were reacted to give **21** (203 mg, 0.521 mmol, 96% yield) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.51 (s, 9H, C(C**H**$_3$)$_3$), 2.50 (s, 3H, CH_3), 4.69 (s, 2H, CH_2), 7.03 (d, 1H, *J* = 2.7 Hz, ArH isoquinoline), 7.10 – 7.17 (m, 1H, ArH benzimidazole), 7.32 (br s, 1H, ArH benzimidazole), 7.48 (dd, 1H, *J* = 2.7, 9.5 Hz, ArH isoquinoline), 7.57 (d, 1H, *J* = 5.6 Hz, ArH isoquinoline), 7.64 – 7.89 (br s, 1H, ArH benzimidazole), 8.45 (d, 1H, *J* = 5.6 Hz, ArH isoquinoline), 10.16 (d, 1H, *J* = 9.5 Hz, ArH isoquinoline), 11.24 (br s, 1H, NH). ^{13}C NMR (101 MHz, $CDCl_3$) δ 21.94, 28.20, 65.78, 82.96, 105.71, 111.19, 120.20, 121.33, 121.63, 122.88, 124.56, 131.09, 133.85, 139.30, 141.89, 146.47, 151.14, 159.27, 167.47. HRMS calculated for $C_{23}H_{24}N_3O_3$ $(M + Na)^+$, 390.1812; found, 390.1783.

***tert*-Butyl 2-{[1-(5-methyl-1H-1,3-benzodiazol-2-yl)isoquinolin-7-yl]oxy}acetate (22)**



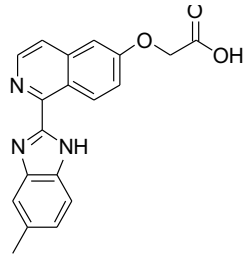
According to the procedure described for **20**, a mixture of **19** (45 mg, 0.16 mmol), K_2CO_3 (68 mg, 0.49 mmol) and *tert*-butyl bromoacetate (0.025 mL, 0.17 mmol) were reacted to give **22** (42 mg, 0.11 mmol, 66% yield) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.52 (s, 9H, C(C**H**$_3$)$_3$), 2.52 (s, 3H, CH_3), 4.88 (s, 2H, CH_2), 7.09 – 7.21 (m, 1H, ArH benzimidazole), 7.28 – 7.45 (m, 1H, ArH benzimidazole), 7.52 (dd, 1H, *J* = 2.6, 9.0 Hz, ArH isoquinoline), 7.66 (d, 1H, *J* = 5.4 Hz, ArH isoquinoline), 7.70 – 7.86 (m, 2H, ArH isoquinoline and ArH benzimidazole), 8.46 (d, 1H, *J* = 5.4 Hz, ArH isoquinoline), 9.67 – 9.75 (m, 1H, ArH isoquinoline), 10.78 (br s, 1H, NH). ^{13}C NMR (101 MHz, $CDCl_3$) δ 22.05, 28.27, 65.77, 82.72, 107.06, 110.63*, 110.98*, 120.20*, 120.42*, 122.20, 124.08, 124.19*, 125.81*, 127.92, 128.68, 133.47, 134.34, 140.03, 143.24, 145.19, 151.39, 157.82, 167.83 (*designates carbons linked to broadened benzimidazole protons, as determined by HSQC, gHMBC experiment). HRMS calculated for $C_{23}H_{23}N_3NaO_3$ $(M + Na)^+$, 412.1632; found, 412.1602.

2-{[1-(1H-1,3-Benzodiazol-2-yl)isoquinolin-6-yl]oxy}acetic acid (23)



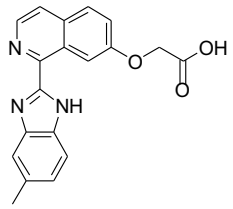
To a solution of **20** (50 mg, 0.13 mmol) in DCM (5 mL) at 0 °C was added trifluoroacetic acid (TFA) (1 mL, 13.32 mmol) and the mixture stirred for 5 h. The DCM and TFA were removed under reduced pressure to provide an oil which solidified to a yellow solid upon co-evaporation with $CHCl_3$/hexane. This solid was washed with cold MeOH to give **23** (27 mg, 0.08 mmol, 64% yield) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.91 (s, 2H, CH_2), 7.19 – 7.35 (m, 2H, ArH benzimidazole), 7.42 (d, 1H, J = 2.7 Hz, ArH isoquinoline), 7.52 (dd, 1H, J = 2.7, 9.5 Hz, ArH isoquinoline), 7.61 (d, 1H, J = 7.8 Hz, ArH benzimidazole), 7.83 (d, 1H, J = 8.0 Hz, ArH benzimidazole), 7.87 (d, 1H, J = 5.6 Hz, ArH isoquinoline), 8.59 (d, 1H, J = 5.6 Hz, ArH isoquinoline), 10.04 (d, 1H, J = 9.5 Hz, ArH isoquinoline), 13.17 (br m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 64.64, 105.98, 112.05, 119.66, 121.12, 121.59, 121.82, 121.93, 123.57, 129.87, 133.99, 138.90, 142.21, 144.02, 146.10, 151.25, 158.74, 169.65. HRMS calculated for $C_{18}H_{14}N_3O_3$ $(M + H)^+$, 320.10297; found, 320.1008.

2-{[1-(5-Methyl-1H-1,3-benzodiazol-2-yl)isoquinolin-6-yl]oxy}acetic acid (24)



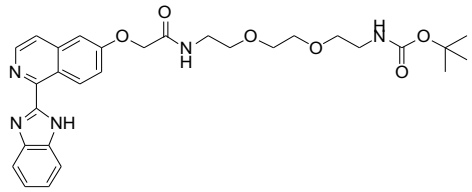
According to the procedure described for **23**, a mixture of **21** (196 mg, 0.503 mmol) and TFA gave **24** (172 mg, 0.51 mmol, quantitative yield) as a yellow solid. ^{1}H NMR (400 MHz, MeOD-d_4) δ 2.53 (s, 3H, CH_3), 4.90 (s, 2H, CH_2), 7.21 – 7.32 (m, 1H, ArH benzimidazole), 7.35 (d, 1H, J = 2.6 Hz, ArH isoquinoline or ArH benzimidazole), 7.48 (dd, 1H, J = 2.6, 9.4 Hz, ArH isoquinoline), 7.56 (s, 1H, ArH benzimidazole), 7.66 (d, 1H, J = 8.3 Hz, ArH isoquinoline or ArH benzimidazole), 7.85 (d, 1H, J = 5.6 Hz, ArH isoquinoline), 8.56 (d, 1H, J = 5.7 Hz, ArH isoquinoline), 9.00 (d, 1H, J = 9.4 Hz, ArH isoquinoline). ^{13}C NMR (101 MHz, MeOD-d_4) δ 21.79, 65.95, 107.39, 115.10, 115.56, 123.63, 123.70, 124.56, 128.61, 128.81, 133.62, 135.34, 137.94, 140.84, 143.65, 144.56, 149.01, 161.33, 171.68. HRMS calculated for $C_{19}H_{15}N_3NaO_3$ $(M + Na)^+$, 356.1006; found, 356.0997. Analytical RP-HPLC R_t = 13.00 min.

2-{[1-(5-Methyl-1H-1,3-benzodiazol-2-yl)isoquinolin-7-yl]oxy}acetic acid (25)



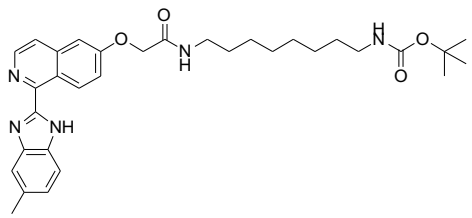
According to the procedure described for **23**, a mixture of **22** (350 mg, 0.90 mmol) and TFA gave **25** (179 mg, 0.54 mmol, 60% yield) as a yellow solid. This compound was used as such for next reaction without further purification.

***tert*-Butyl N-(2-{2-[2-(2-{[1-(1H-1,3-benzodiazol-2-yl)isoquinolin-6-yl]oxy}acetamido)ethoxy]ethoxy}-ethyl)carbamate (26)**



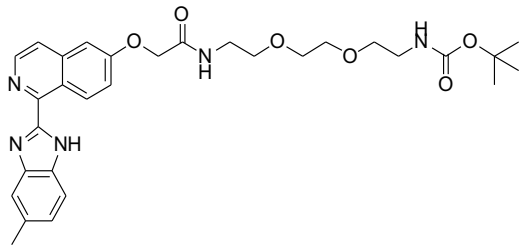
To a solution of **23** (25 mg, 0.08 mmol) and HATU (31 mg, 0.08 mmol) in anhydrous DMF (1 mL), was added DIPEA (30 µL) and the mixture was stirred for 15 min. A solution of *tert*-butyl {2-[2-(2-aminoethoxy)ethoxy]ethyl}carbamate (prepared according to literature procedure[13]) (40 mg, 0.16 mmol) in DMF (1 mL) was then added and the mixture stirred for 4 h. The solvent was removed under reduced pressure and the resulting residue partitioned between EtOAc and water. The EtOAc layer was washed three times with NH_4Cl solution, once with water and brine solution, dried over $MgSO_4$ and the solvelnt evaporated. The residue was purified by silica gel flash column chromatography (50% EtOAc/hexane to 2% MeOH/EtOAc) to provide **26** as pale yellow oil (23 mg, 0.04 mmol, 55% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.41 (s, 9H, C(C**H**$_3$)$_3$), 3.28 (q, 2H, J = 5.4 Hz, CONHC**H**$_2$ or C**H**$_2$NHBoc), 3.44 – 3.70 (m, 10H, CH_2 PEG), 4.61 (s, 2H, OCH_2CO), 5.06 (s, 1H, NH), 7.05 (s, 1H, ArH isoquinoline), 7.12 (s, 1H, NH), 7.29 – 7.44 (m, 3H, ArH isoquinoline or ArH benzimidazole), 7.62 (d, 1H, J = 5.6 Hz, ArH isoquinoline), 7.73 – 7.83 (m, 2H, ArH isoquinoline or ArH benzimidazole), 8.36 – 8.49 (m, 1H, ArH isoquinoline), 9.77 (d, 1H, J = 9.2 Hz, ArH isoquinoline). ^{13}C NMR (126 MHz, $CDCl_3$) δ 28.54, 29.83, 39.15, 40.44, 67.30, 69.70, 70.24, 70.29, 70.38, 79.53, 106.11, 115.86, 121.64, 122.46, 122.70, 124.41, 130.57, 139.46, 141.55, 145.01, 149.70, 156.20, 158.78, 167.71. HRMS calculated for $C_{29}H_{35}N_5NaO_6$ $(M + Na)^+$, 572.2480; found, 572.2456.

***tert*-Butyl N-[8-(2-{[1-(5-methyl-1H-1,3-benzodiazol-2-yl)isoquinolin-6-yl]oxy}acetamido)octyl]-carbamate (27)**



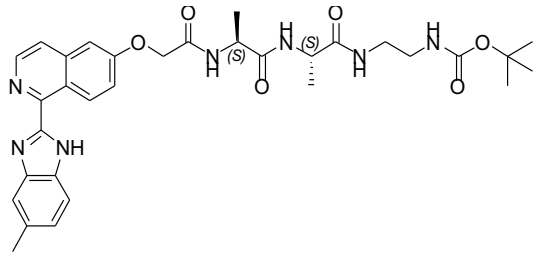
According to the procedure described for **26**, a mixture of **24** (32 mg, 0.09 mmol), *tert*-butyl (8-aminooctyl)carbamate (prepared according to literature procedure[14]) (70 mg, 0.29 mmol), HATU (38 mg, 0.10 mmol) and DIPEA (30 µL, 0.19 mmol) gave a residue that was purified by silica gel flash column chromatography (20 to 50% EtOAc/hexane) to give **27** (37 mg, 0.06 mmol, yield 69%) as a clear oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.08 – 1.32 (m, 8H, $CONHCH_2CH_2(C\mathbf{H_2})_4$), 1.44 (s, 13H, 4H of $CONHCH_2C\mathbf{H_2}(CH_2)_4C\mathbf{H_2}$ and 9H of $C(C\mathbf{H_3})_3$), 2.50 (s, 3H, CH_3), 3.05 (q, 2H, J = 6.8 Hz, $CONHC\mathbf{H_2}$ or $C\mathbf{H_2}NHBoc$), 3.35 (q, 2H, J = 6.8 Hz, $CONHC\mathbf{H_2}$ or $C\mathbf{H_2}NHBoc$), 4.53 (t, 1H, J = 6.0 Hz, NH), 4.66 (s, 2H, OCH_2CO), 6.59 (t, 1H, J = 6.0 Hz, NH), 7.09 (d, 1H, J = 2.7 Hz, ArH isoquinoline), 7.11 – 7.18 (m, 1H, ArH benzimidazole), 7.43 (dd, 1H, J = 2.6, 9.5 Hz, ArH isoquinoline), 7.50 – 7.75 (m, 3H, ArH isoquinoline and ArH benzimidazole), 8.48 (d, 1H, J = 5.6 Hz, ArH isoquinoline), 10.18 (d, 1H, J = 9.4 Hz, ArH isoquinoline), 11.16 (b s, 1H, NH benzimidazole). ^{13}C NMR (101 MHz, $CDCl_3$) δ 21.94, 26.79, 26.88, 28.58, 29.25, 29.27, 29.63, 30.11, 39.26, 40.68, 67.39, 79.15, 105.92, 120.96, 121.68, 122.97, 124.79, 131.38, 139.28, 142.27, 146.49, 151.01, 156.14, 158.33, 167.41. HRMS calculated for $C_{32}H_{42}N_5O_4$ $(M + H)^+$, 560.3231; found, 560.3196.

***tert*-Butyl N-(2-{2-[2-(2-{[1-(5-methyl-1H-1,3-benzodiazol-2-yl)isoquinolin-6-yl]oxy}acetamido)ethoxy]-ethoxy}ethyl)carbamate (28)**



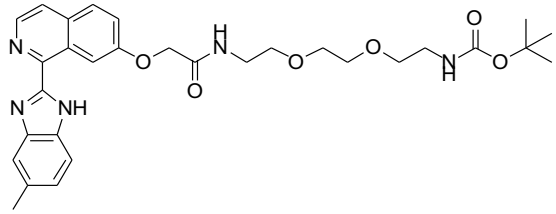
According to the procedure described for **26**, a mixture of **24** (60 mg, 0.18 mmol), *tert*-butyl {2-[2-(2-aminoethoxy)ethoxy] ethyl}carbamate (134 mg, 0.54 mmol), HATU (72 mg, 0.19 mmol) and DIPEA (70 µL, 0.36 mmol) gave a residue that was purified by silica gel flash column chromatography (50% EtOAc/hexane to 4% MeOH/EtOAc) to give **28** (52 mg, 0.09 mmol, yield 52%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 (s, 9H, $C(C\mathbf{H_3})_3$), 2.48 (s, 3H, CH_3), 3.25 – 3.32 (m, 2H, $CONHC\mathbf{H_2}$ or $C\mathbf{H_2}NHBoc$), 3.45 – 3.63 (m, 10H, CH_2 peg), 4.65 (s, 2H, OCH_2CO), 5.06 (br s, 1H, NH), 6.97 – 7.18 (m, 3H, NH, ArH benzimidazole, ArH isoquinoline), 7.28 – 7.79 (m, 4H, ArH benzimidazole, ArH isoquinoline), 8.46 (d, 1H, J = 5.6 Hz, ArH isoquinoline), 10.15 (d, 1H, J = 9.4 Hz, ArH isoquinoline), 11.32 (br s, 1H, NH benzimidazole). ^{13}C NMR (101 MHz, $CDCl_3$) δ 21.90, 28.51, 39.03, 40.39, 67.34, 69.78, 70.19, 70.30, 70.35, 79.40, 105.90, 120.92, 121.63, 122.93, 124.96, 131.31, 133.71, 139.23, 142.18, 146.56, 151.00, 156.08, 158.33, 167.61. HRMS calculated for $C_{30}H_{38}N_5O_6$ $(M + H)^+$, 564.2817; found, 564.2798. Analytical RP-HPLC R_t = 16.42 min.

***tert*-Butyl N-{2-[(2S)-2-[(2S)-2-(2-{[1-(5-methyl-1H-1,3-benzodiazol-2-yl)isoquinolin-6-yl]oxy}acetamido)propanamido]propanamido]ethyl}carbamate (29)**



Fmoc-Ala-Ala-CH_2-CH_2-NH-Boc was prepared in 3 steps according to standard step-wise solution-phase peptide synthesis using Fmoc-*L*-Ala-OH, *N*-Boc-ethylenediamine, HBTU, HOBt, DIPEA and diethylamine. This was then Fmoc-deprotected using diethylamine to afford Ala-Ala-CH_2-CH_2-NH-Boc as white solid. According to the procedure described for **26**, a mixture of **24** (45 mg, 0.13 mmol), Ala-Ala-CH_2-CH_2-NH-Boc (122 mg, 0.40 mmol), HATU (54 mg, 0.14 mmol), DIPEA (50 µL, 0.27 mmol) gave a residue that was purified by silica gel flash column chromatography (70% EtOAc/hexane to 4% MeOH/EtOAc) to give **29** (24 mg, 0.04 mmol, 29% yield) as a pale yellow solid. ^{1}H NMR (500 MHz, MeOD-d_4) δ 1.29 – 1.48 (m, 15H, 2×3H of C**H**$_3$ belonging to alanines and 9H of C(C**H**$_3$)$_3$), 2.50 (s, 3H, CH_3 benzimidazole), 3.10 – 3.18 (m, 2H, C**H**$_2$$CH_2$NHBoc or CH_2C**H**$_2$NHBoc), 3.20 – 3.28 (m, 2H, C**H**$_2$$CH_2$NHBoc or CH_2C**H**$_2$NHBoc), 4.25 – 4.35 (m, 1H, C**H**CONH), 4.48 (q, 1H, *J* = 7.1 Hz, C**H**CONH), 4.78 (d, 2H, *J* = 3.0 Hz, OCH_2CO), 7.16 (d, 1H, *J* = 8.4 Hz, ArH benzimidazole), 7.32 – 7.36 (m, 1H, ArH isoquinoline), 7.46 – 7.54 (m, 2H, ArH benzimidazole, ArH isoquinoline), 7.60 (br s, 1H, ArH benzimidazole), 7.78 (d, 1H, *J* = 5.69 Hz ArH isoquinoline), 8.52 (d, 1H, *J* = 1.6, 5.6 Hz, ArH isoquinoline), 9.42 (d, 1H, *J* = 9.4 Hz, ArH isoquinoline). ^{13}C NMR (126 MHz, MeOD-d_4) δ 18.00, 21.83, 28.75, 40.65, 40.79, 50.51, 50.67, 68.05, 80.14, 107.21, 107.22, 122.18, 122.21, 122.83, 123.91, 125.75, 130.91, 131.05, 140.70, 143.45, 148.59, 152.01, 158.49, 160.39, 170.43, 174.47, 175.07. HRMS calculated for $C_{32}H_{40}N_7O_6$ $(M + H)^+$, 618.3035; found, 618.3078.

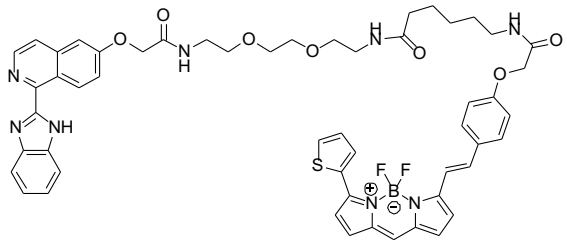
***Tert*-Butyl N-(2-{2-[2-(2-{[1-(5-methyl-1H-1,3-benzodiazol-2-yl)isoquinolin-7-yl]oxy}acetamido)-ethoxy]ethoxy}ethyl)carbamate (30)**



According to the procedure described for **26**, a mixture of **25** (20 mg, 0.06 mmol), *tert*-butyl {2-[2-(2-aminoethoxy)ethoxy]ethyl}carbamate (45 mg, 0.18 mmol), HATU (24 mg, 0.06 mmol), DIPEA (20 µL, 0.12 mmol) gave a residue that was purified by silica gel flash column chromatography (50% EtOAc/hexane to 3% MeOH/EtOAc) to give **30** (14 mg, 0.02 mmol, 43% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 (s, 9H, C(C**H**$_3$)$_3$)), 2.50 (s, 3H, CH_3), 3.27 (q, 2H, *J* = 5.5 Hz, m, 2H, C**H**$_2$NH), 3.41 – 3.67 (m, 10H, CH_2 peg), 4.85 (s, 2H, OCH_2CO), 5.04 (m, 1H, *J* = 6.7 Hz, NH), 7.14 (dd, 1H, *J* = 1.5, 8.3 Hz, ArH benzimidazole), 7.19 (br s, 1H, NH), 7.36 – 7.56 (m, 2H, ArH isoquinoline, ArH benzimidazole), 7.65 (d,

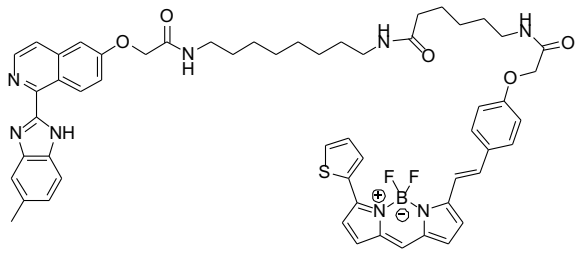
2H, *J* = 5.5 Hz, ArH isoquinoline, ArH benzimidazole), 7.80 (d, 1H, *J* = 9.0 Hz, ArH isoquinoline), 8.46 (d, 1H, *J* = 5.4 Hz, ArH isoquinoline), 9.73 (s, 1H, ArH isoquinoline). ^{13}C NMR (101 MHz, $CDCl_3$) δ 21.91, 28.53, 39.06, 40.41, 67.71, 69.94, 70.21, 70.30, 70.32, 79.46, 108.44, 122.14, 123.32, 125.20, 127.78, 128.89, 133.55, 140.06, 145.21, 150.80, 156.11, 157.05, 168.02. HRMS calculated for $C_{30}H_{38}N_5O_6$ $(M + H)^+$, 564.2817; found, 564.2769. Analytical RP-HPLC R_t = 16.66 min.

4-[(E)-2-{4-[({5-[(2-{2-[2-(2-{[1-(1H-1,3-Benzodiazol-2-yl)isoquinolin-6-yl]oxy}acetamido)ethoxy]ethoxy}ethyl)carbamoyl]pentyl}carbamoyl)methoxy]phenyl}ethenyl]-2,2-difluoro-12-(thiophen-2-yl)-1 lambda5,3-diaza-2 boratricyclo[7.3.0.0^{3,7}]dodeca-1(12),4,6,8,10-pentaen-1-ylium-2-uid (31)



To a solution of **26** (7 mg, 0.01 mmol) in DCM (2.0 mL) at 0 °C was added TFA (0.5 mL). The reaction mixture was warmed to rt and stirred for 2 h, volatiles were removed under reduced pressure to provide *N*-{2-[2-(2-aminoethoxy)ethoxy]ethyl}-2-{[1-(1H-1,3-benzodiazol-2-yl)isoquinolin-6-yl]oxy}acetamide trifluoroacetate in quantitative yield. To a solution of this semiprep RP-HPLC purified trifluoroacetate salt (5.2 mg, 9.22 μmol) in DMF (200 μL), was added a solution of DIPEA (6.42 μL, 36.90 μmol, 4 equiv) in DMF (100 μL), followed by addition of solution of BOPIPY630/650-SE (1.7 mg, 2.52 μmol, 1 equiv) in DMF (700 μL). The reaction was stirred for 12 h with the exclusion of light then concentrated under reduced pressure and purified by RP-HPLC, freeze-dried, neutralised with Amberlyst A21 ion exchange resin to give **31** (2.1 mg, 2.11 μmol, 84% yield) as a blue solid. HRMS calculated for $C_{53}H_{53}BF_2N_8NaO_7S$ $(M + Na)^+$, 1017.3720; found, 1017.3773. Analytical RP-HPLC R_t = 21.44 min.

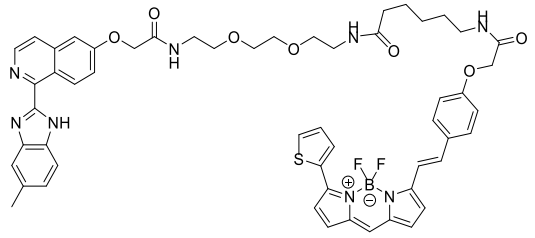
2- Fluoraniumyl-2-fluorescent-4-[(E)-2-(4-{[(5-{[8-(2-{[1-(5-methyl-1H-1,3-benzodiazol-2-yl)isoquinolin-6-yl]oxy}33acetamido)octyl]carbamoyl}pentyl)carbamoyl]methoxy}-phenyl)ethenyl]-12-(thiophen-2-yl)-1lambda4,3-diaza-2-boratricyclo[7.3.0.0^{3,7}]dodeca-1(12),4,6,8,10-pentaen-2-uide (32)



According to the procedure for **31**, **27** (6 mg, 0.01 mmol) was treated with TFA to give N-(8-aminooctyl)-2-{[1-(5-methyl-1H-1,3-benzodiazol-2-yl)isoquinolin-6-yl]oxy}acetamide trifluoroacetate salt in quantitative yield as yellowish solid. This trifluoroacetate salt (3.9 mg, 6.79 μmol) on reaction with BOPIPY630/650-SE

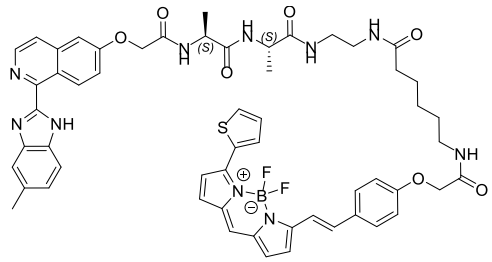
(1.2 mg, 1.9 μmol) provided **32** (0.8 mg, 0.83 μmol, 44% yield) as dark blue solid. HRMS calculated for $C_{56}H_{59}BF_2N_8NaO_5S$ $(M + Na)^+$, 1027.4292; found, 1027.4322. Analytical RP-HPLC R_t = 22.85 min.

[12-(2-{4-[({5-[(2-{2-[2-(2-{[1-(5-Methyl-1H-1,3-benzodiazol-2-yl)isoquinolin-6-yl]oxy}acetamido)ethoxy]ethoxy}ethyl)carbamoyl]pentyl}carbamoyl)methoxy]phenyl}ethenyl)-4-(thiophen-2-yl)-2-(λ²-fluoranidyl)-1λ⁴ -aza-3λ⁴ -aza-2λ¹-boratricyclo[7.3.0.0³,⁷]dodeca-3,5,7,9,11-pentaene-2,2,2-triium-1-id-2-yl]-λ²-fluoranide (33)



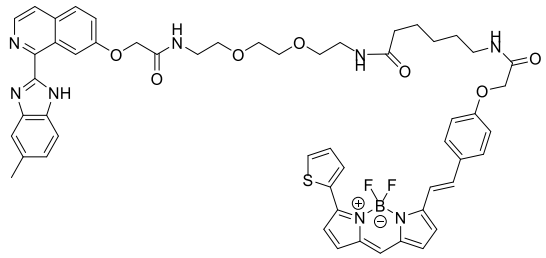
According to the procedure for **31**, **28** (10 mg, 0.02 mmol) was treated with TFA to give *N*-{2-[2-(2-aminoethoxy)ethoxy]ethyl}-2-{[1-(5-methyl-1H-1,3-benzodiazol-2-yl)isoquinolin-6-yl]oxy}acetamide trifluoroacetate salt in quantitative yield as yellowish solid. This trifluoroacetate salt (5.1 mg, 8.83 μmol) on reaction with BOPIPY630/650-SE (1.2 mg, 1.9 μmol) provided **33** (1.0 mg, 0.99 μmol, 54% yield) as dark blue solid. HRMS calculated for $C_{54}H_{55}BF_2N_8NaO_7S$ $(M + Na)^+$, 1031.3877; found, 1031.3959. Analytical RP-HPLC R_t = 21.63 min.

(12-{2-[4-({[5-({2-[(2S)-2-[(2S)-2-(2-{[1-(5-Methyl-1H-1,3-benzodiazol-2-yl)isoquinolin-6-yl]oxy}acetamido)propanamido]propanamido]ethyl}carbamoyl)pentyl]carbamoyl}methoxy)phenyl]ethenyl}-4-(thiophen-2-yl)-2-(λ²-fluoranidyl)-1λ⁴ -aza-3λ⁴ -aza-2λ¹-boratricyclo[7.3.0.0³,⁷]dodeca-3,5,7,9,11-pentaene-2,2,2-triium-1-id-2-yl)-λ²-fluoranide (34)



According to the procedure for **31, 29** (15 mg, 0.02 mmol) was treated with TFA to give (2*S*)-*N*-[(1*S*)-1-[(2-aminoethyl)carbamoyl]ethyl]-2-(2-{[1-(5-methyl-1H-1,3-benzodiazol-2-yl)isoquinolin-6-yl]oxy}acetamido)propanamide trifluoroacetate salt in quantitative yield as yellowish solid. Tthis trifluoroacetate salt (5.5 mg, 8.7 μmol) on reaction with BOPIPY630/650-SE (1.2 mg, 1.9 μmol) provided **34** (0.63 mg, 0.59 μmol, 33% yield) as dark blue solid. HRMS calculated for $C_{56}H_{57}BF_2N_{10}NaO_7S$ $(M + Na)^+$, 1085.4095; found, 1085.4162. Analytical RP-HPLC R_t = 21.05 min.

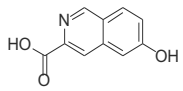
[12-(2-{4-[({5-[(2-{2-[2-(2-{[1-(5-Methyl-1H-1,3-benzodiazol-2-yl)isoquinolin-7-yl]oxy}acetamido)ethoxy]ethoxy}ethyl)carbamoyl]pentyl}carbamoyl)methoxy]phenyl}ethenyl)-4-(thiophen-2-yl)-2-(λ²-fluoranidyl)-1λ⁴ -aza-3λ⁴ -aza-2λ¹-boratricyclo[7.3.0.0³,⁷]dodeca-3,5,7,9,11-pentaene-2,2,2-triium-1-id-2-yl]-λ²-fluoranide (35)



According to the procedure for **31**, **30** (7 mg, 0.01 mmol) was treated with TFA to give N-{2-[2-(2-aminoethoxy)ethoxy]ethyl}-2-{[1-(5-methyl-1H-1,3-benzodiazol-2-yl)isoquinolin-7-yl]oxy}trifluoroacetate salt in quantitative yield as yellowish solid. This trifluoroacetate salt **2.32** (5.5 mg, 9.52 µmol) on reaction with BOPIPY630/650-SE (1.2 mg, 1.9 µmol) provided **2.38** (0.9 mg, 0.89 µmol, 49% yield) as dark blue solid. HRMS calculated for $C_{54}H_{55}BF_2N_8NaO_7S$ $(M + Na)^+$, 1031.3877; found, 1031.3969. Analytical RP-HPLC R_t = 21.68 min.

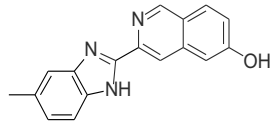
1.3 Experimental details for scheme 3

6-Hydroxyisoquinoline-3-carboxylic acid (36)



Following the procedure described for **15**, a mixture of **11** (0.6 g, 2.95 mmol) and LiOH (0.212 g, 8.86 mmol) gave **36** (0.5 g, 2.64 mmol, 90% yield) as yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.55 – 7.63 (m, 2H, ArH), 8.39 (d, 1H, J = 8.7 Hz, ArH), 8.66 (s, 1H, ArH), 9.46 (s, 1H, ArH), 11.79 (br s, 1H, OH). ^{13}C NMR (101 MHz, DMSO-d_6) δ 109.88, 122.88, 123.65, 123.92, 132.49, 135.02, 139.40, 148.32, 163.39, 163.77. HRMS calculated for $C_{10}H_8NO_3$ $(M + H)^+$, 190.0499; found, 190.0512.

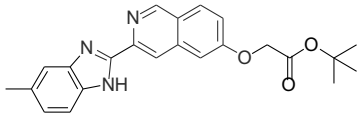
3-(6-Methyl-1H-1,3-benzodiazol-2-yl)isoquinolin-6-ol (37)



Following the procedure described for **17**, a mixture of **36** (0.2 g, 1.05 mmol) and 3,4-diaminotoluene (0.13 g, 1.05 mmol) gave **37** (75 mg, 0.27 mmol, 26% yield) as off-white solid. ^{1}H NMR (400 MHz, MeOD-d_4) δ 2.49 (s, 3H, C**H**$_3$), 7.12 (d, 1H, J = 8.4 Hz, ArH isoquinoline or ArH benzimidazole), 7.20 – 7.30 (m, 2H, ArH isoquinoline and ArH benzimidazole), 7.44 (s, 1H, ArH benzimidazole), 7.54 (s, 1H, ArH benzimidazole), 7.98 (d, 1H, J = 8.8 Hz, ArH isoquinoline), 8.43 (s, 1H, ArH isoquinoline), 9.15 (s, 1H, ArH isoquinoline). ^{13}C NMR (101 MHz, MeOD-d_4) δ 21.78, 109.11, 118.02, 122.20, 125.57, 131.01, 139.78,

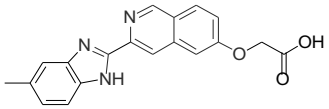
143.17, 152.99, 161.65. HRMS calculated for $C_{17}H_{14}N_3O$ $(M + H)^+$, 276.1131; found, 276.1114. Analytical RP-HPLC R_t = 13.83 min.

***tert*-Butyl 2-{[3-(5-methyl-1H-1,3-benzodiazol-2-yl)isoquinolin-6-yl]oxy}acetate (38)**



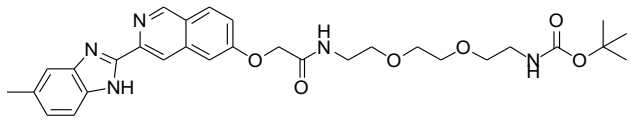
Following the procedure described for **20**, a mixture of **37** (18 mg, 0.06 mmol), K_2CO_3 (25 mg, 0.18 mmol) and *tert*-butyl bromoacetate (20 µL, 0.13 mmol) gave **38** (24 mg, 0.06 mmol, 94% yield) as an off-white solid.. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.53 (s, 9H, C(C**H**$_3$)$_3$), 2.50 (s, 3H, CH_3), 4.68 (s, 2H, CH_2), 7.07 (d, 1H, *J* = 2.5 Hz, ArH isoquinoline or ArH benzimidazole), 7.09 – 7.16 (m, 1H, ArH isoquinoline or ArH benzimidazole), 7.34 (dd, 1H, *J* = 2.5, 8.9 Hz, ArH isoquinoline), 7.40 – 7.82 (s, 2H, ArH benzimidazole), 7.91 (d, 1H, *J* = 8.9 Hz, ArH isoquinoline), 8.68 (s, 1H, ArH isoquinoline), 9.10 (s, 1H, ArH isoquinoline), 10.74 (br s, 1H, NH). ^{13}C NMR (126 MHz, $CDCl_3$) δ 21.92, 28.25, 65.75, 83.14, 106.05, 107.48, 117.69, 121.32, 125.23, 129.84, 138.22, 142.54, 151.42, 159.93, 167.31. HRMS calculated for $C_{29}H_{34}N_3O_5$ $(M + H)^+$, 504.2493; found, 504.2455.

2-{[3-(5-Methyl-1H-1,3-benzodiazol-2-yl)isoquinolin-6-yl]oxy}acetic acid (39)



Following the procedure described for **23**, a mixture of **38** (40 mg, 0.10 mmol) and TFA gave **39** in quantitative yield as a yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.43 (s, 3H, CH_3), 4.91 (s, 2H, CH_2), 7.06 (d, 1H, *J* = 8.2 Hz, ArH isoquinoline and/or ArH benzimidazole), 7.32 – 7.45 (m, 2H, ArH isoquinoline and/or ArH benzimidazole), 7.46 – 7.55 (m, 2H, ArH isoquinoline and/or ArH benzimidazole), 8.15 (d, 1H, *J* = 8.9 Hz, ArH isoquinoline), 8.66 (s, 1H, ArH isoquinoline), 9.33 (s, 1H, ArH isoquinoline). HRMS calculated for $C_{19}H_{14}N_3O_3$ $(M + H)^+$, 332.1041; found, 332.1064.

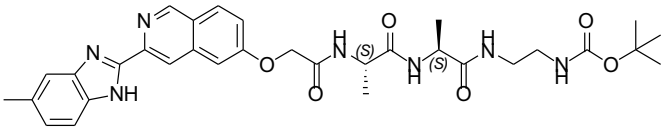
***tert*-Butyl N-(2-{2-[2-(2-{[3-(5-methyl-1H-1,3-benzodiazol-2-yl)isoquinolin-6-yl]oxy}acetamido)-ethoxy]ethoxy}ethyl)carbamate (40)**



According to the procedure described for **26**, a mixture of **39** (17 mg, 0.05 mmol), *tert*-butyl {2-[2-(2-aminoethoxy)ethoxy] ethyl}carbamate (38 mg, 0.15 mmol), HATU (20 mg, 0.05 mmol) and DIPEA (20 µL, 0.11 mmol) gave a residue that was purified by silica gel flash column chromatography (50% EtOAc/hexane to 10% MeOH/EtOAc) to give **40** (11 mg, 0.02 mmol, 39% yield) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 (s, 9H, C(C**H**$_3$)$_3$), 2.50 (s, 3H, CH_3), 3.23 – 3.36 (m, 2H, CONHC**H**$_2$ or C**H**$_2$NHBoc), 3.47 – 3.65 (m, 10H, CH_2 peg), 4.66 (s, 2H, OCH_2), 5.04 (br s, 1H, NH), 7.05 (s, 1H, NH), 7.10 – 7.15 (m, 1H, ArH benzimidazole or ArH isoquinoline), 7.17 (d, 1H, *J* = 2.4, 2.5 Hz, ArH benzimidazole or ArH isoquinoline),

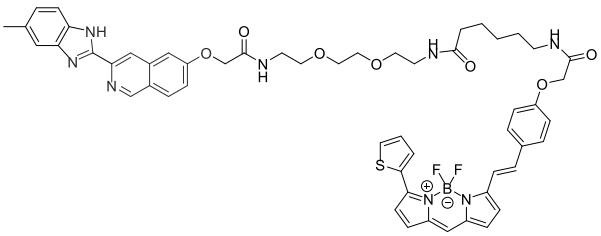
7.31 (dd, 1H, J = 2.4, 8.9 Hz, ArH isoquinoline), 7.37 – 7.49 (s, 1H, ArH benzimidazole), 7.50 – 7.70 (s, 1H, ArH benzimidazole), 7.94 (d, 1H, J = 9.0 Hz, ArH isoquinoline), 8.69 (s, 1H, ArH isoquinoline), 9.12 (s, 1H, ArH isoquinoline). ^{13}C NMR (101 MHz, $CDCl_3$) δ 21.91, 28.55, 39.09, 40.46, 67.44, 69.83, 70.35, 70.41, 79.51, 107.07, 118.46, 118.48, 120.82, 125.28, 125.48, 130.05, 133.97, 138.00, 150.33, 151.61, 156.14, 159.10, 167.43. HRMS calculated for $C_{30}H_{38}N_5O_6$ $(M + H)^+$, 564.2817; found, 564.2784. Analytical RP-HPLC R_t = 16.81 min.

***tert*-Butyl N-{2-[(2S)-2-[(2S)-2-(2-{[3-(5-methyl-1H-1,3-benzodiazol-2-yl)isoquinolin-6-yl]oxy}acetamido)propanamido]propanamido]ethyl}carbamate (41)**



According to the procedure described for **26**, a mixture of **39** (20 mg, 0.06 mmol), Ala-Ala-CH_2-CH_2-NH-Boc (54 mg, 0.18 mmol), HATU (24 mg, 0.06 mmol) and DIPEA (20 μL, 0.12 mmol) gave a residue that was purified by silica gel flash column chromatography (50% EtOAc/hexane to 12% MeOH/EtOAc) to give **41** (16 mg, 0.02 mmol, 37% yield) as an off-white solid ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.19 (d, 3H, J = 7.1 Hz, CH_3 Ala), 1.29 (d, 3H, J = 7.1 Hz, CH_3 Ala), 1.35 (s, 9H, C(C**H**$_3$)$_3$), 2.44 (s, 3H, C**H**$_3$, benzimidazole), 2.91 – 2.99 (m, 2H, CH_2), 3.00 – 3.13 (m, 2H, CH_2), 4.18 – 4.26 (m, 1H, CH), 4.36 – 4.44 (m, 1H, CH), 4.72 – 4.82 (m, 2H, OCH_2), 6.74 (t, 1H, J = 5.8 Hz, NH), 7.05 – 7.09 (m, 1H, ArH benzimidazole), 7.42 (s, 1H, ArH benzimidazole), 7.45 (dd, 1H, J = 2.4, 8.9 Hz, ArH isoquinoline), 7.49 – 7.55 (m, 2H, ArH benzimidazole or ArH isoquinoline), 7.86 (t, 1H, J = 5.7 Hz, NH), 8.11 (d, 1H, J = 7.5 Hz, NH), 8.17 (d, 1H, J = 9.0 Hz, ArH isoquinoline), 8.36 (d, 1H, J = 7.4 Hz, NH), 8.67 (s, 1H, ArH isoquinoline), 9.34 (s, 1H, ArH isoquinoline). ^{13}C NMR (126 MHz, DMSO-d_6) δ 18.19, 18.21, 21.37, 28.21, 38.73, 39.52*, 48.09, 48.32, 66.85, 77.65, 106.67, 117.29, 120.94, 124.07, 124.49, 129.79, 137.57, 142.44, 150.73, 151.60, 155.58, 159.53, 166.84, 171.52, 172.11 (*underneath DMSO-d_6 peaks, identified through HSQC experiment). HRMS calculated for $C_{32}H_{40}N_7O_6$ $(M + H)^+$, 618.3035; found, 618.3073.

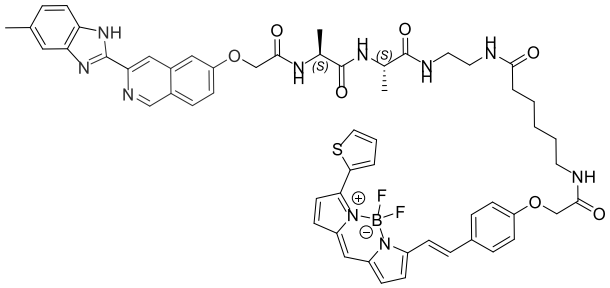
[12-(2-{4-[({5-[(2-{2-[2-(2-{[3-(5-Methyl-1H-1,3-Benzodiazol-2-yl)isoquinolin-6-yl]oxy}acetamido)ethoxy]ethoxy}ethyl)carbamoyl]pentyl}carbamoyl)methoxy]phenyl}ethenyl)-4-(thiophen-2-yl)-2-(λ²-fluoranidyl)-1λ⁴ -aza-3λ⁴ -aza-2λ¹-boratricyclo[7.3.0.0³,⁷]dodeca-3,5,7,9,11-pentaene-2,2,2-triium-1-id-2-yl]-λ²-fluoranide (42)



According to the procedure for **31**, **40** (5.0 mg, 0.01 mmol) was treated with TFA to give *N*- {2- [2- (2-aminoethoxy)ethoxy]ethyl}- 2- {[3- (5- methyl- 1H- 1,3- benzodiazol- 2- yl)isoquinolin- 6-

yl]oxy}acetamide trifluoroacetate salt in quantitative yield as yellowish solid, this trifluoroacetate salt (5.3 mg, 0.01 mmol) on reaction with BOPIPY630/650-SE (1.7 mg, 2.52 μmol) provided **42** (2.2 mg, 2.18 μmol, 86% yield) as dark blue solid. HRMS calculated for $C_{54}H_{55}BF_2N_8NaO_7S$ $(M + Na)^+$, 1031.3877; found, 1031.3849. Analytical RP-HPLC R_t = 20.96 min.

(12-{2-[4-({[5-({2-[(2S)-2-[(2S)-2-(2-{[3-(5-methyl-1H-1,3-Benzodiazol-2-yl)isoquinolin-6-yl]oxy}acetamido)propanamido]propanamido]ethyl}carbamoyl)pentyl]carbamoyl}methoxy)phenyl]ethenyl}-4-(thiophen-2-yl)-2-(λ²-fluoranidyl)-1λ⁴ -aza-3λ⁴ -aza-2λ¹-boratricyclo[7.3.0.0³,⁷]dodeca-3,5,7,9,11-pentaene-2,2,2-triium-1-id-2-yl)-λ²-fluoranide (43)



According to the procedure for **31**, **41** (9.0 mg, 0.01 mmol) was treated with TFA to give (2*S*)- *N*- [(1*S*)- 1-[(2- aminoethyl)carbamoyl]ethyl]- 2- (2- {[3- (5- methyl- 1H- 1,3- benzodiazol- 2- yl)isoquinolin- 6-yl]oxy}acetamido)propanamide trifluoroacetate salt, this trifluoroacetate salt (9.2 mg, 0.01 mmol) on reaction with BOPIPY630/650-SE (1.7 mg, 2.52 μmol) provided **43** (1.9 mg, 1.83 μmol, 73% yield) as dark blue solid. HRMS calculated for $C_{56}H_{57}BF_2N_{10}NaO_7S$ $(M + Na)^+$, 1085.4095; found, 1085.4166. Analytical RP-HPLC R_t = 20.51 min.

1.4 Excitation and emission spectra of fluorescent compounds

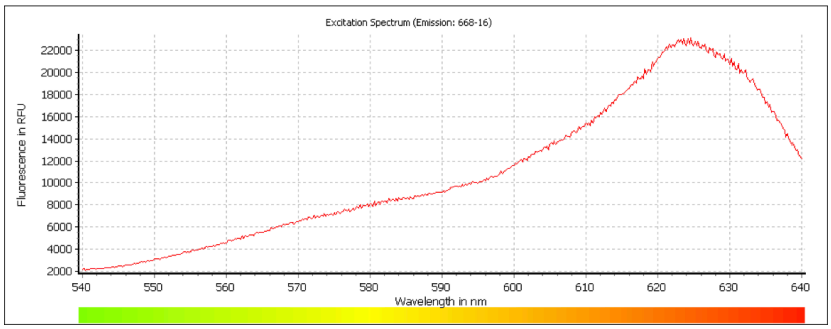
The excitation and emission spectra for each fluorescent compound (10 μM in methanol) were measured in Griener Bio-One white 96 well flat bottom plates using a BMG LabTech CLARIOstar read type 'top optic' (software version 5.20 RS; firmware version 1.20) and analysed using BMG MARS (software version 3.10 RS). Alexa Flour 633 presets were used. Excitation wavelength 540 – 640 nm (1.0 stepwidth), excitation bandwidth 10 nm, emission wavelength 668 nm, emission bandwidth 16 nm, gain 1500, measured 0.2 sec. And excitation wavelength 592 nm excitation bandwidth 16 nm, emission wavelength 620 – 700 nm (0.2 stepwidth), emission bandwidth 10 nm, gain 1500, measured 0.2 sec.

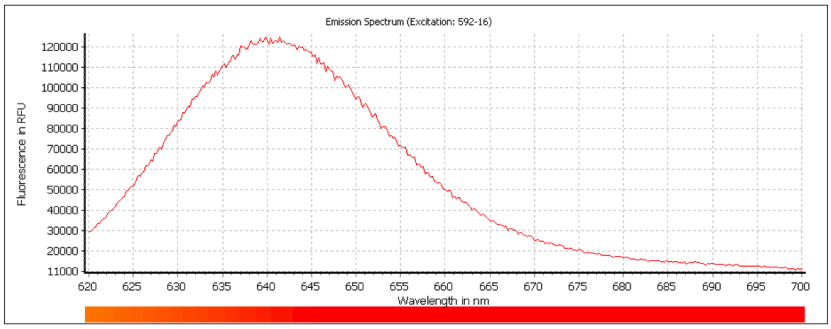
Summary of results:

Fluorescent compound	Excitation (max) (nm)	Emission (max) (nm)
31	624	641
33	624	641
32	624	641
34	624	641
35	624	642
42	624	642
43	624	641

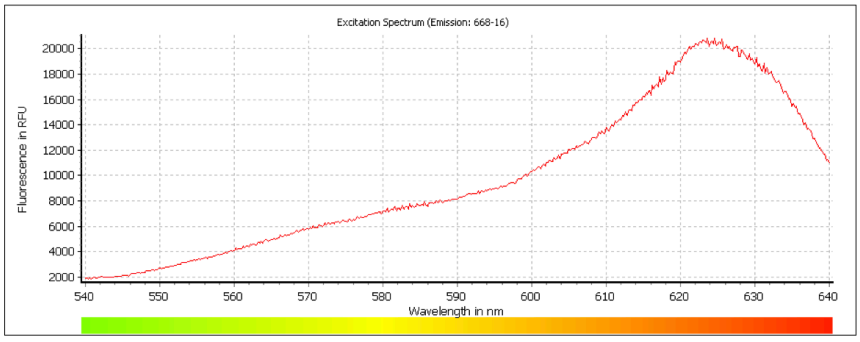
Representative spectra: Fluorescent ligands 31 and 33, from Figure 2

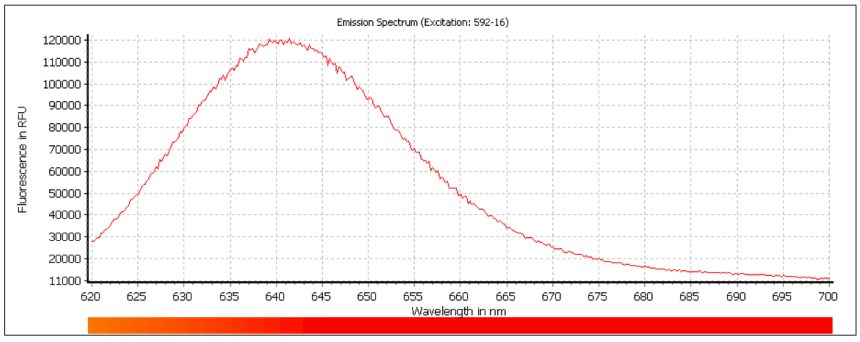
Compound 31 -0.2nm resolution





Compound 33 – 0.2nm resolution





2. Conformers of 18

Benzimidazoles are well known for exhibiting tautomerism,[15-20] however the (benzimidazolyl)isoquinolinol scaffold has not been investigated as such. Since multiple sets of peaks were observed in initial NMR spectra of **17-19**, we sought to investigate this using **18** as a case study. Tautomers of **18** would constitute the chemical exchange of protons between N9 and N11 (Figure S1.A), usually with the participation of solvent molecules. Rotamer conformers could also be present, due to hindered rotation around a single bond; for **18** this would be around the C1-C10 bond connecting the benzimidazole and isoquinoline heterocycles (Figure S1.B). If it is conformers rather than discrete isomers that are present, then peak appearance and multiplicity in NMR and HPLC spectra should be sensitive to factors such as solvent (hydrogen bond acceptor or donor ability), temperature and concentration of the compound because the rate of conformer interconversion is likely affected by these factors. Also the atoms nearest to the point of difference between potential conformers should show the most non-equivalent NMR signals between conformers. Therefore, NMR and HPLC studies were carried out using **18** as a representative example from the compounds **17-19** to confirm the presence of interconvertible conformers versus discrete isomers/regioisomers.

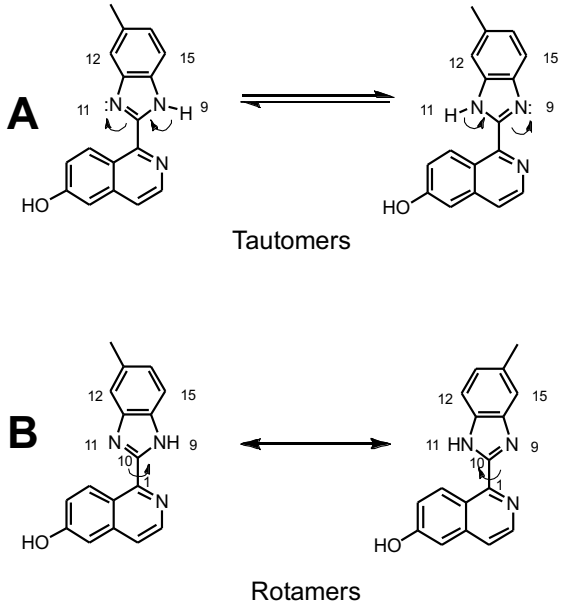


Figure S1: Interconversion of possible (A) tautomers or (B) rotamers of **18**.

2.1 NMR studies

The ^{1}H NMR spectrum of **18** in MeOD-d_4 showed the protons belonging to the isoquinoline ring as sharp peaks while protons of the benzimidazole were broadened peaks (Figure S2.A). The ^{13}C NMR spectrum of **18** in MeOD-d_4 showed carbons of the isoquinoline ring as sharp peaks compared to a low intensity or absence of benzimidazole carbon signals (Figure S2.B) (refer to Experimental section in paper for peak lists in MeOD-d_4). ^{1}H and ^{13}C NMR spectra signals were assigned using gCOSY (Figure S3), HSQC (Figure S4) and gHMBC (Figure S5) spectra. However, more proton signals were seen in the ^{1}H NMR spectrum of **18** in DMSO-d_6 than observed in MeOD-d_4 (Figure S6.A) and correspondingly in the ^{13}C NMR spectrum (Figure S6.B).

^{1}H NMR (400 MHz, DMSO-d_6) δ 2.45 (s, 2.84H, 16-H), 7.07 (d, 0.62H, J = 7.6 Hz, 14-H), 7.12 (d, 0.41H, J = 8.0 Hz, 14-H), 7.21 (d, 1H, J = 2.4 Hz, 5-H), 7.33 – 7.40 (m, 1.53H, 7-H and 12-H), 7.47 (d, 0.41H, J = 8.3 Hz, 15-H), 7.60 (s, 0.41H, 12-H), 7.68 (d, 0.60H, J = 8.3 Hz, 15-H), 7.74 (d, 0.96H, J = 5.6 Hz, 4-H), 8.49 (d, 1.0H, J = 5.6 Hz, 3-H), 9.90 – 9.99 (m, 1.02H, 8-H), 10.53 (s, 1.53H, 17-H), 12.98 (s, 0.63H, 9-H), 12.99 (s, 0.40H, 9-H). (Additional ^{1}H NMR signals, than expected by magnetic equivalence for one chemical structure of **18**, are due to presence of another conformer)
^{13}C NMR (101 MHz, DMSO-d_6) δ 21.33 (16-C), 21.48 (16-C), 107.63 (5-C), 111.52 (15-C), 111.59 (12-C), 119.18 (12-C and 15-C), 120.78 (4-C), 121.07 (7-C), 123.54 (14-C), 125.02 (14-C), 130.12 (8-C), 130.78 (13-C), 132.01 (11a-C or 15a-C), 132.86 (13-C), 134.21 (11a-C or 15a-C), 139.16 (8a-C or 4a-C), 141.68 (3-C), 142.24 (11a-C or 15a-C), 144.40 (11a-C or 15a-C), 146.19 (8a-C or 4a-C), 150.97 (10-C), 151.31 (1-C), 158.93 (6-C). (Additional ^{13}C NMR signals, than expected by magnetic equivalence for one chemical structure of **18**, are due to presence of another conformer)

The ^{13}C NMR spectrum of **18** in DMSO-d_6 revealed 23 aromatic carbon signals, however only 17 carbons are present in **18**. The HSCQ spectrum (Figure S8) of **18** showed 12 C-H correlations, however **18** has only nine carbons attached to at least one hydrogen. These HSQC correlations with data from gCOSY (Figure S7) and gHMBC spectra (Figure S9) revealed that additional three C-H correlations in HSQC spectrum and additional carbon signals in ^{13}C NMR spectrum were from atoms belonging to the benzimidazole moiety. Assignment of atoms to the benzimidazole moiety was guided by HSQC and gHMBC correlations to clearly identifiable H-16 protons.

Variable temperature ^{1}H NMR spectra

The rate of interconversion of conformers is dependent on temperature. Therefore, a complex NMR spectrum of two conformers simplifies at higher temperature as the rate of conversion of conformers increases and reaches beyond that observable on NMR timescale. ^{1}H NMR spectra carried out on **18** in DMSO-d_6 at three different temperatures (Figure S10) showed coalescence of proton signals as temperature increased (Figure S10), supporting the presence of conformers rather than discrete non-interchangeable isomers.

Addition of D_2O

One reason for the difference in complexity of the NMR spectra signals for **18** in MeOD-d_4 versus DMSO-d_6 could be the strong hydrogen bond accepting property of DMSO-d_6 compared to MeOD-d_4. Thus, if conformers are present, addition of D_2O to a solution of **18** dissolved in DMSO-d_6 should weaken a hydrogen bond between an NH of benzimidazole and DMSO-d_6 and thus increase rate of interconversion of tautomers. In agreement with this, less complex signals were observed in both ^{1}H and ^{13}C NMR spectra of **18** in DMSO-d_6 spiked with D_2O (Figure S11, Figure S12).

Tautomer or rotamer?

NMR spectra indicated it is highly likely tautomers are present in **18** because it is the benzimidazole atoms most affected by the change in solvent and temperature. If it was predominantly rotamers then the atoms belonging to isoquinoline would also be affected (FigureS1(B)). In MeOD-d_4, exchange of hydrogen between N9 and N11 exposes neighbouring atoms: 11a, 12, 13, 14, 15 and 15a to a fluctuating local chemical environment resulting in either broadening or absence of $^{1}H/^{13}C$ NMR signals of their corresponding atoms (FigureS1(A)). Compound **18** in DMSO-d_6 presumably makes a strong hydrogen bond with the NH benzimidazole, thus slowing down the rate of exchange of hydrogen between N9 and N11 and consequently rate of interconversion of tautomers, thus leading to observation of both tautomers in NMR spectra time scale (FigureS1(A)). The multiplicity of 8-H in DMSO-d_6 is presumably due to its closeness to benzimidazole moiety exhibiting tautomerism.

2.2 RP-HPLC studies

Chromatograms from analytical HPLC of **17-19** revealed that peak shape was concentration dependent. Again using **18** as the model compound, at higher concentrations an elongated, square shoulder peak was observed (Figure S13). We hypothesised that the peculiar peak shape was either due to the presence of conformers, peak tailing, or the presence of chemical impurities/non-interconvertible isomers. To rule out that the shoulder peak is not due to chemical impurities/non-interconvertible isomers, further RP-HPLC experiments were done. Semi-preparative RP-HPLC purification of **18** was performed whereby the peak (Figure S14) was fractionated into 10 separate fractions. An analytical HPLC of each fraction was carried out (Figure S15 A), which showed broad peaks for fractions with high concentration and sharp peaks for samples with dilute concentration. An aliquot from each fraction was combined and analysed by analytical RP-HPLC at a dilute concentration, which showed a single peak (figure S15(B)), thus making the presence of non-interconvertible or discrete chemical impurities unlikely. To investigate the effect of temperature on the more concentrated analytical HPLC chromatogram of **18** (Figure S13), HPLC experiments were carried out at 25-40 °C using a column oven. However no significant change in chromatogram peak shape was observed in this temperature range (results not shown). A much higher temperature is likely needed to see any significant change in chromatogram of **18**, in alignment with ^{1}H NMR spectra results that showed little change from 25 °C to 45 °C but a much larger change at 60 °C. Higher temperature HPLC experiments were not carried out because of solvent and column incompatibility with higher temperatures.

2.3 Summary and Conclusions

NMR spectra and HPLC experiments indicated **18** is a mixture of interconvertible tautomers. The synthetic route to **18** made the presence of regioisomers very unlikely, and this was reinforced by the high temperature experiments since discrete regioisomers would not be interconvertible and the ratio would be the same at low and high temperature (which it was not). As the HRMS spectrum of **18** matched the expected molecular formula mass and no other parent peaks were observed the possibility of an impurity with different molecular formula was also ruled out.

2.4 NMR Spectra and HPLC chromatograms

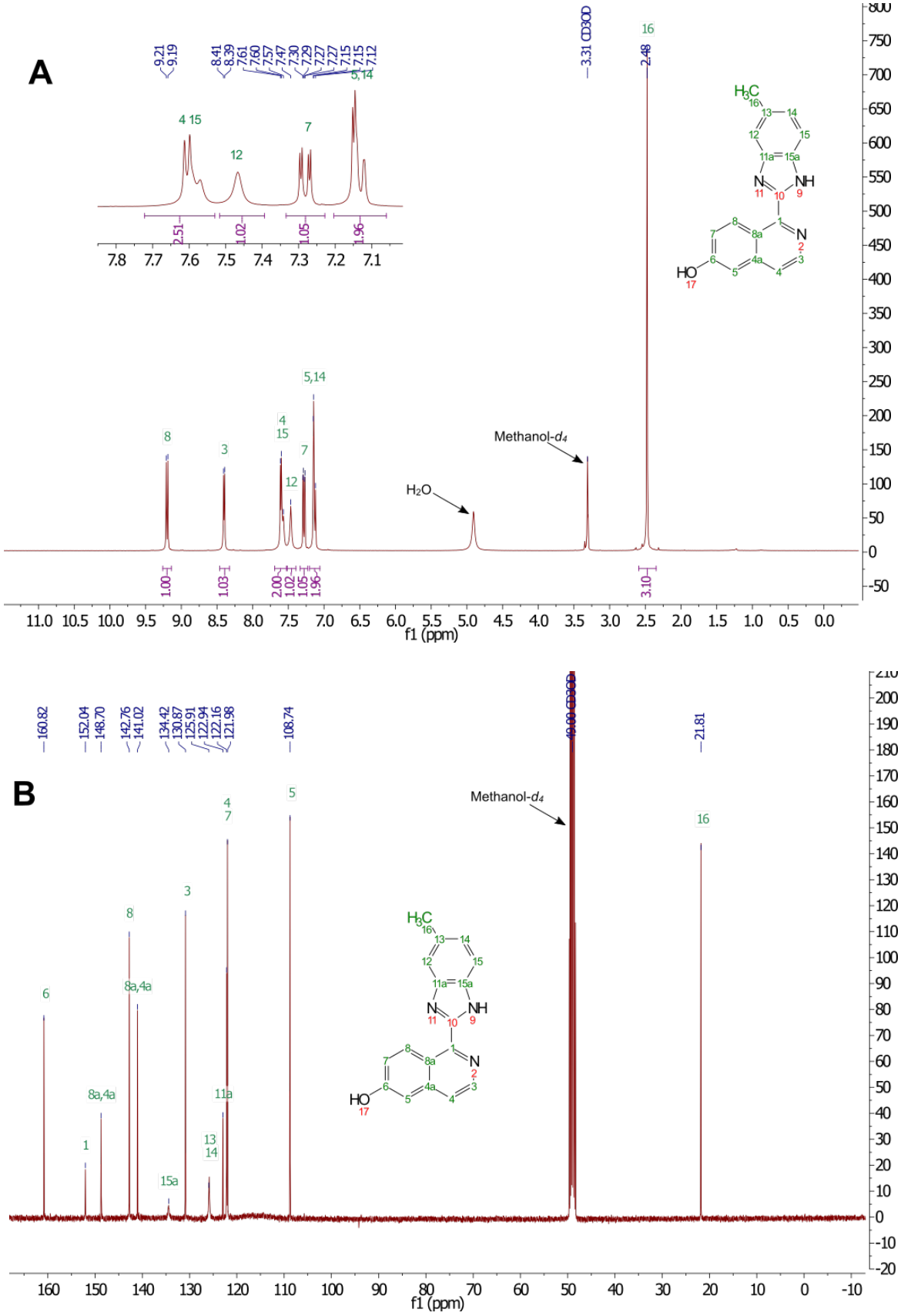


Figure S2. NMR spectra of **18** in MeOD-*d*4 at 25 °C: **(A)** ^{1}H NMR (inset shows aromatic region) and **(B)** ^{13}C NMR spectrum.

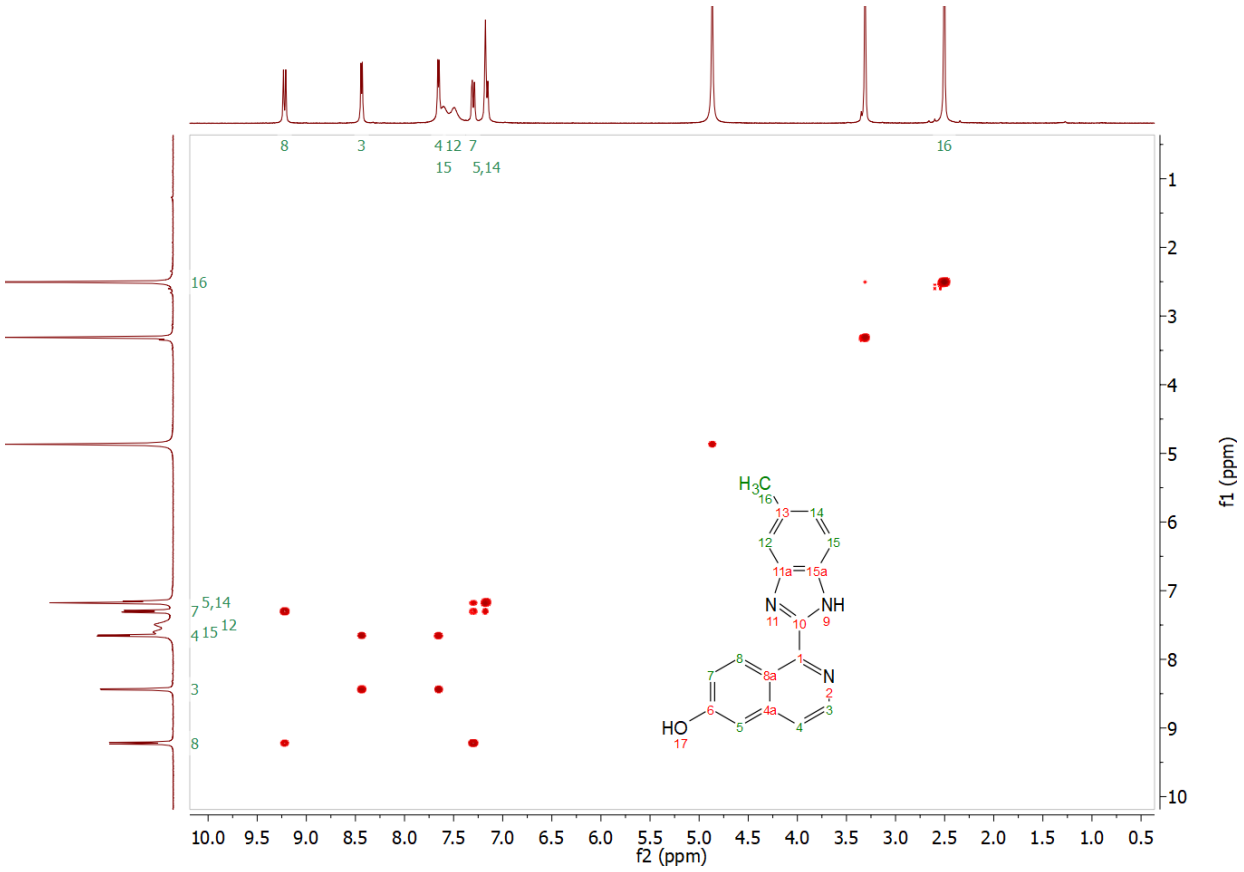


Figure S3. gCOSY spectrum of **18** in MeOD-d_4 at 25 °C

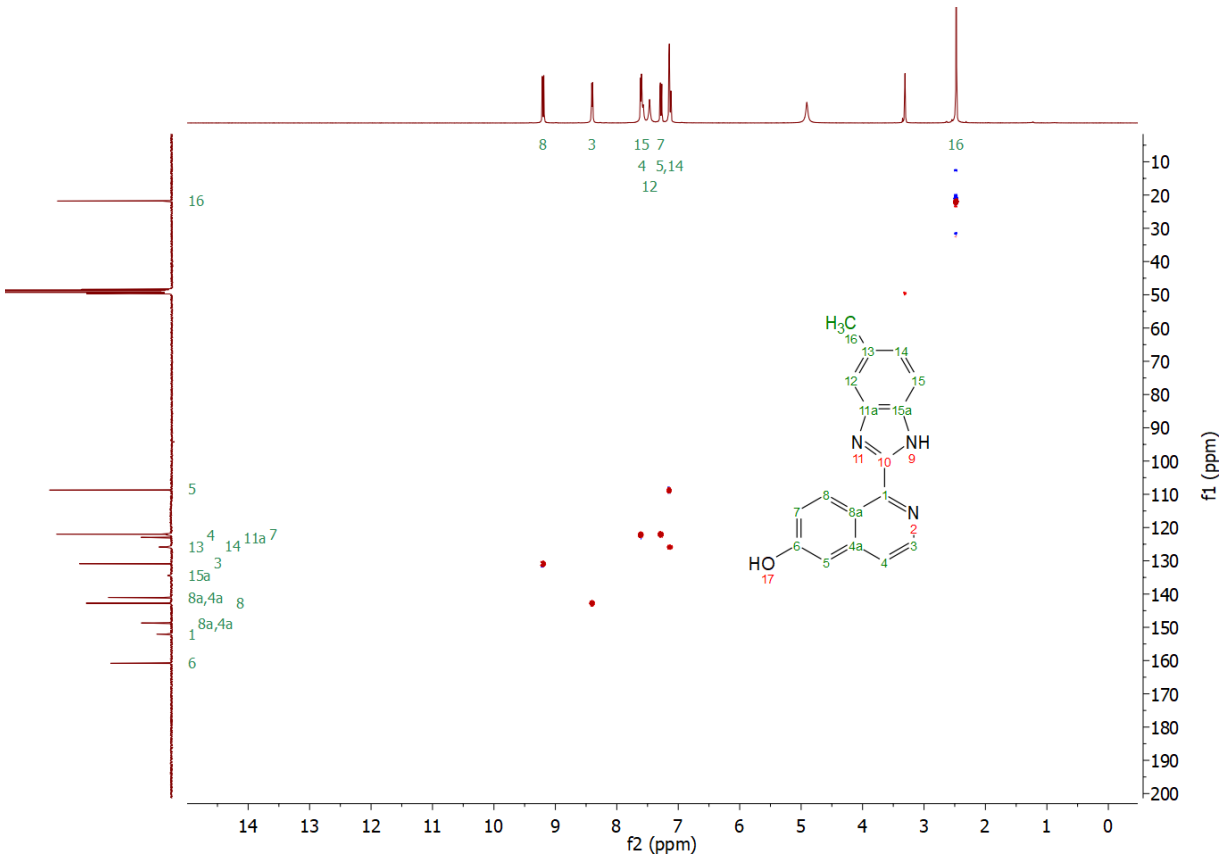


Figure S4. gHSQC spectrum of **18** in MeOD-d_4 at 25 °C

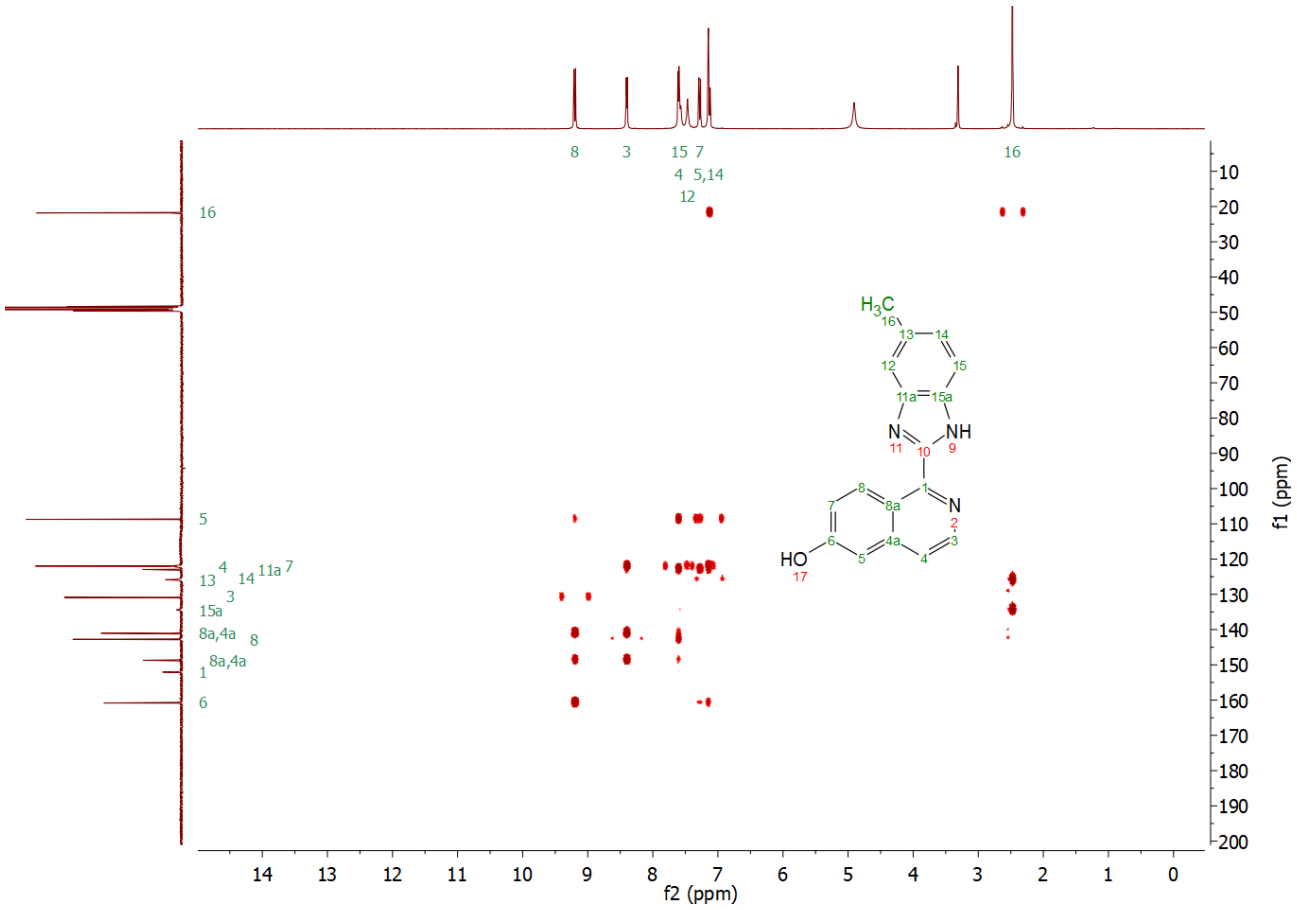


Figure S5. gHMBC spectrum of **18** in MeOD-d_4 at 25 °C

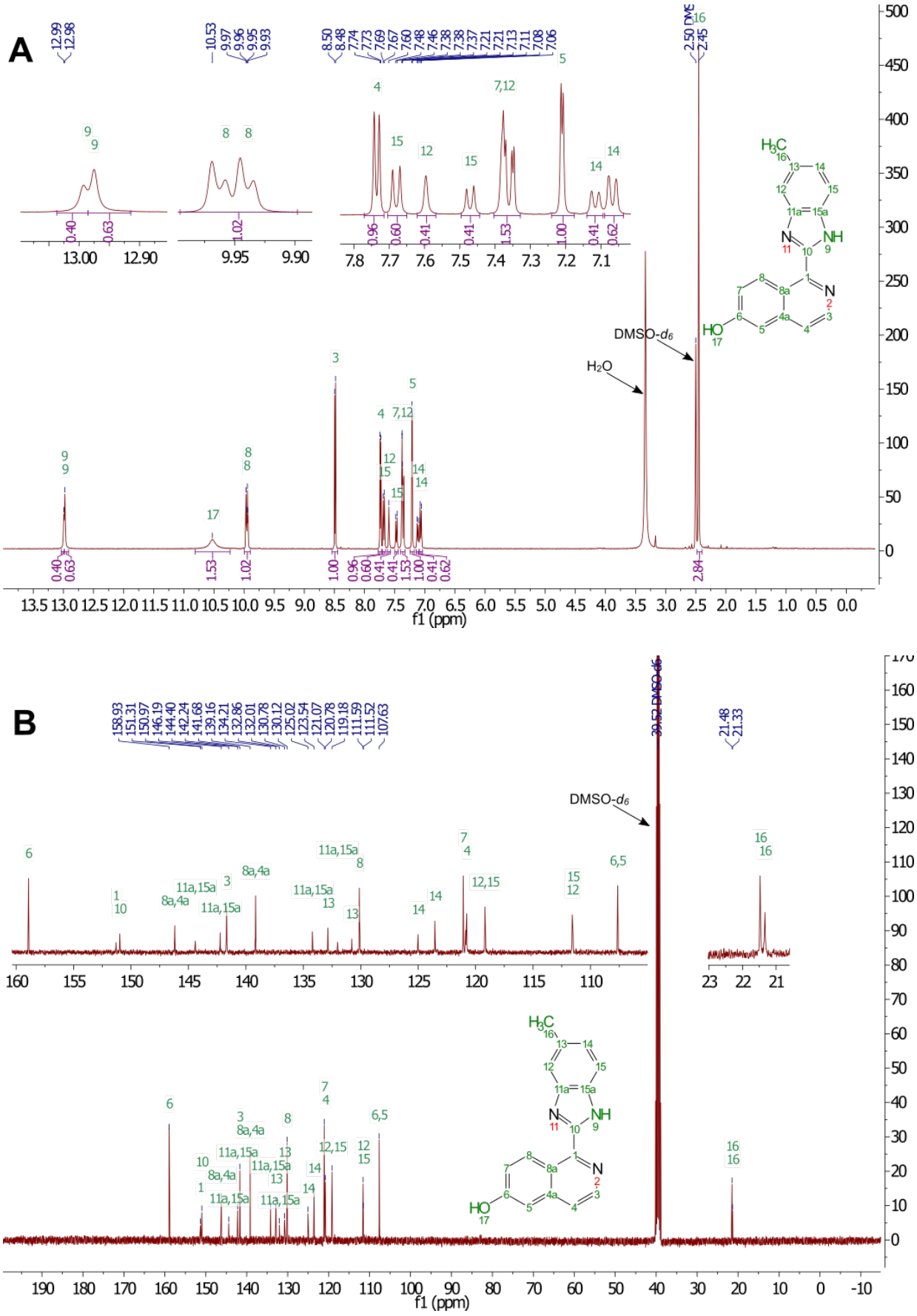


Figure S6. NMR spectra of **18** in DMSO-d_6 at 25 °C: **(A)** ^{1}H NMR (inset shows aromatic region) and **(B)** ^{13}C spectrum (inset shows expanded view of ^{13}C spectrum).

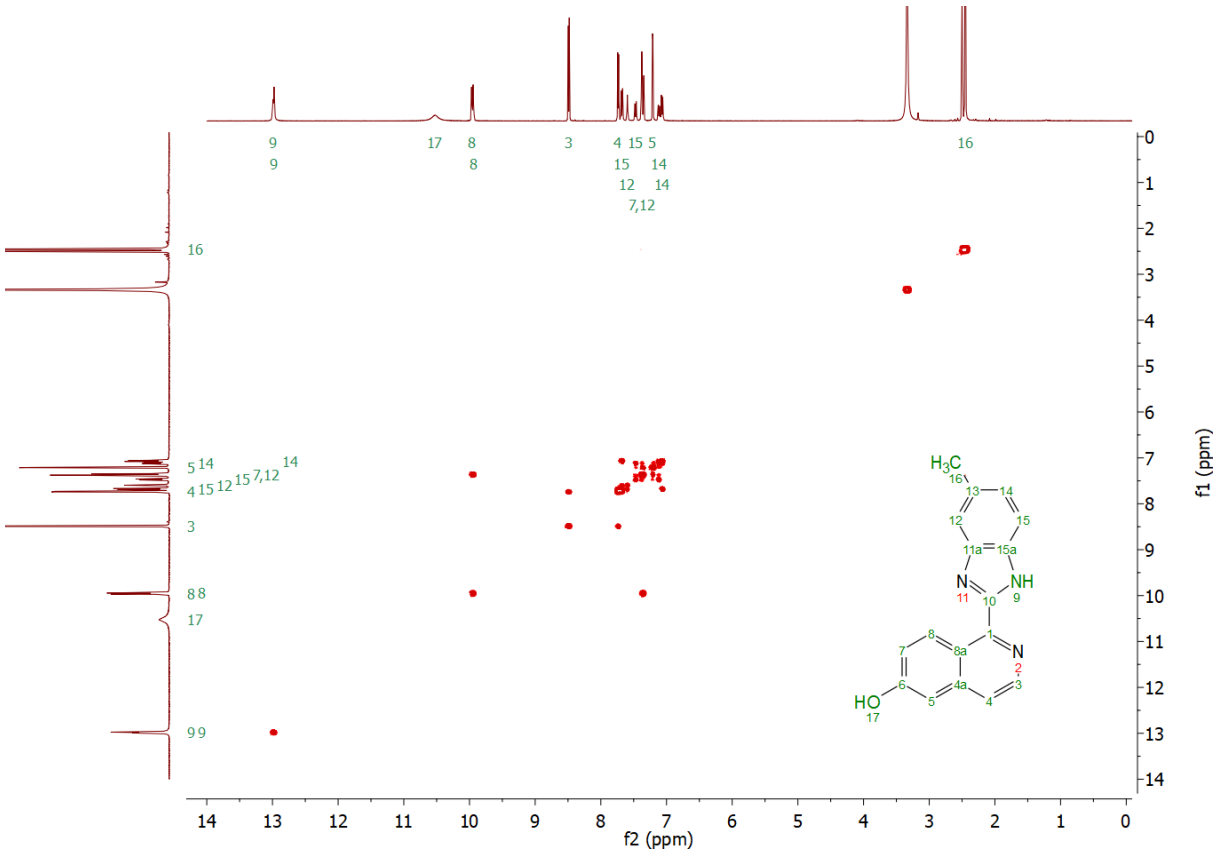


Figure S7. gCOSY spectrum of **18** in DMSO-d_6 at 25 °C

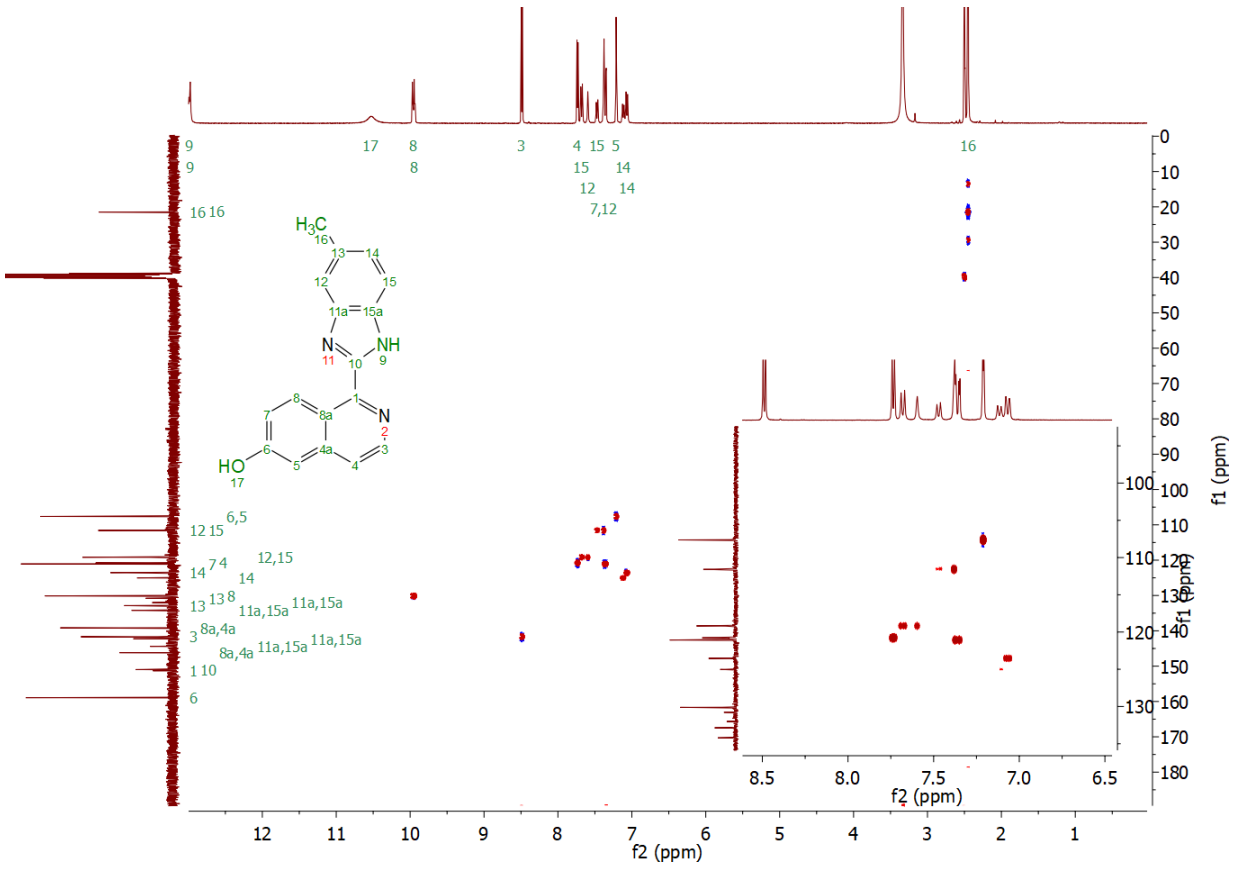


Figure S8. HSQC spectrum (inset shows aromatic region) of **18** in DMSO-d_6 at 25 °C

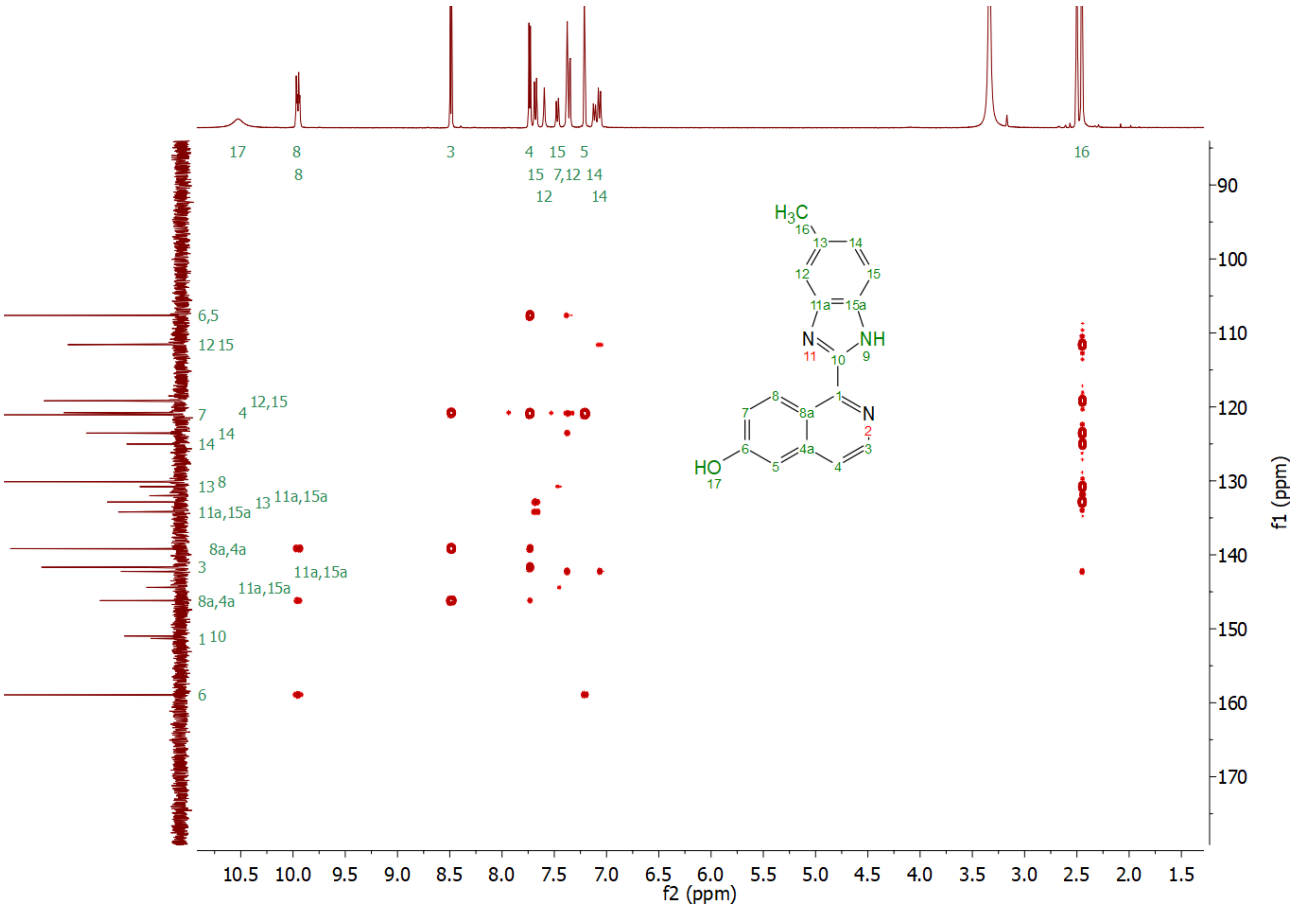


Figure S9. gHMBC spectrum of **18** in DMSO-d_6 at 25 °C

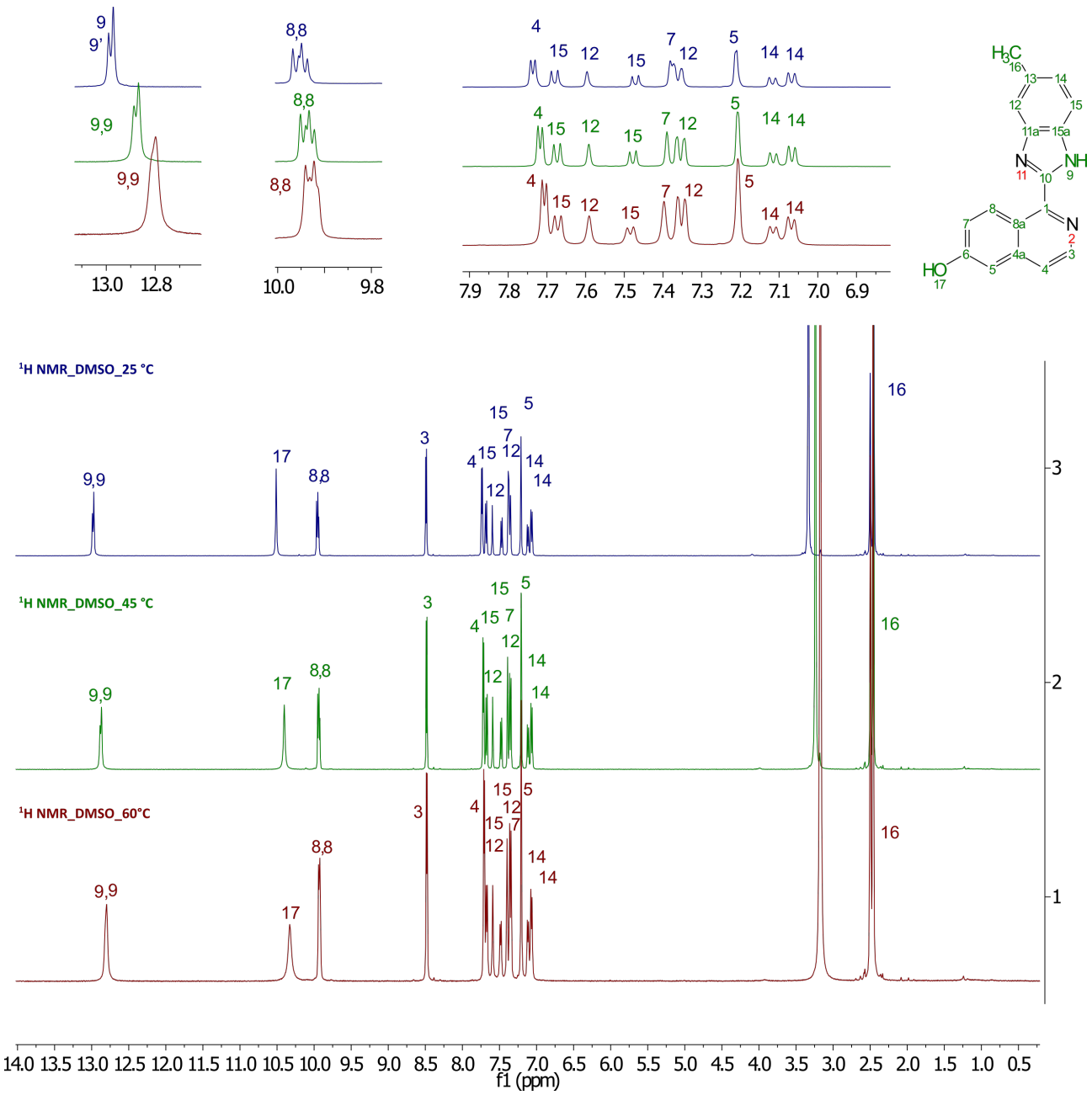


Figure S10: ^{1}H NMR experiments of **18** in DMSO-d_6 were carried at three different temperatures: Top (25 °C in blue), middle (45 °C in green), bottom (60 °C in red), inset shows aromatic region.

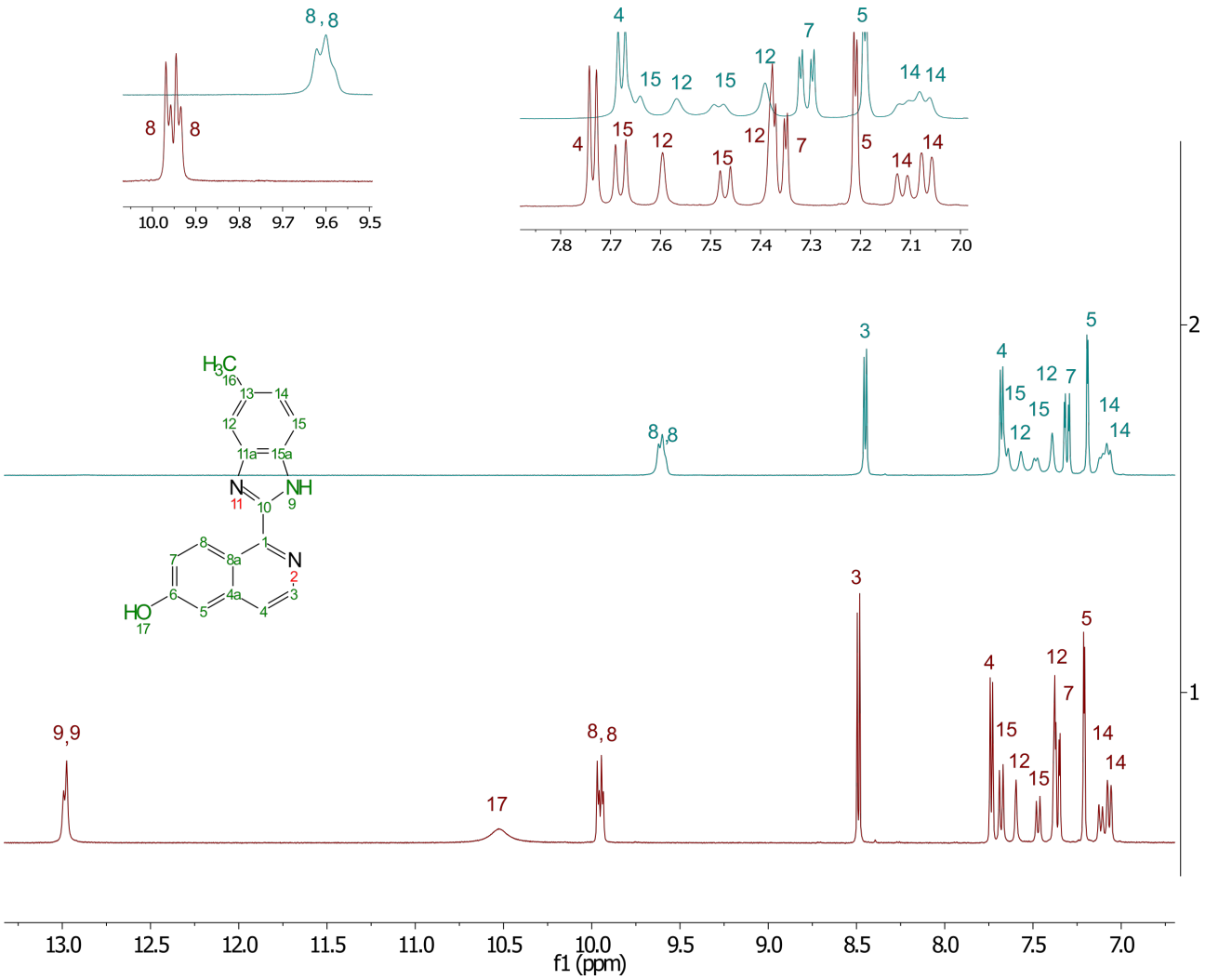


Figure S11: ^{1}H NMR spectrum of **18** in DMSO-d_6 (0.7 mL) at 25 °C (bottom), and after addition of 0.1 mL D_2O (Top).

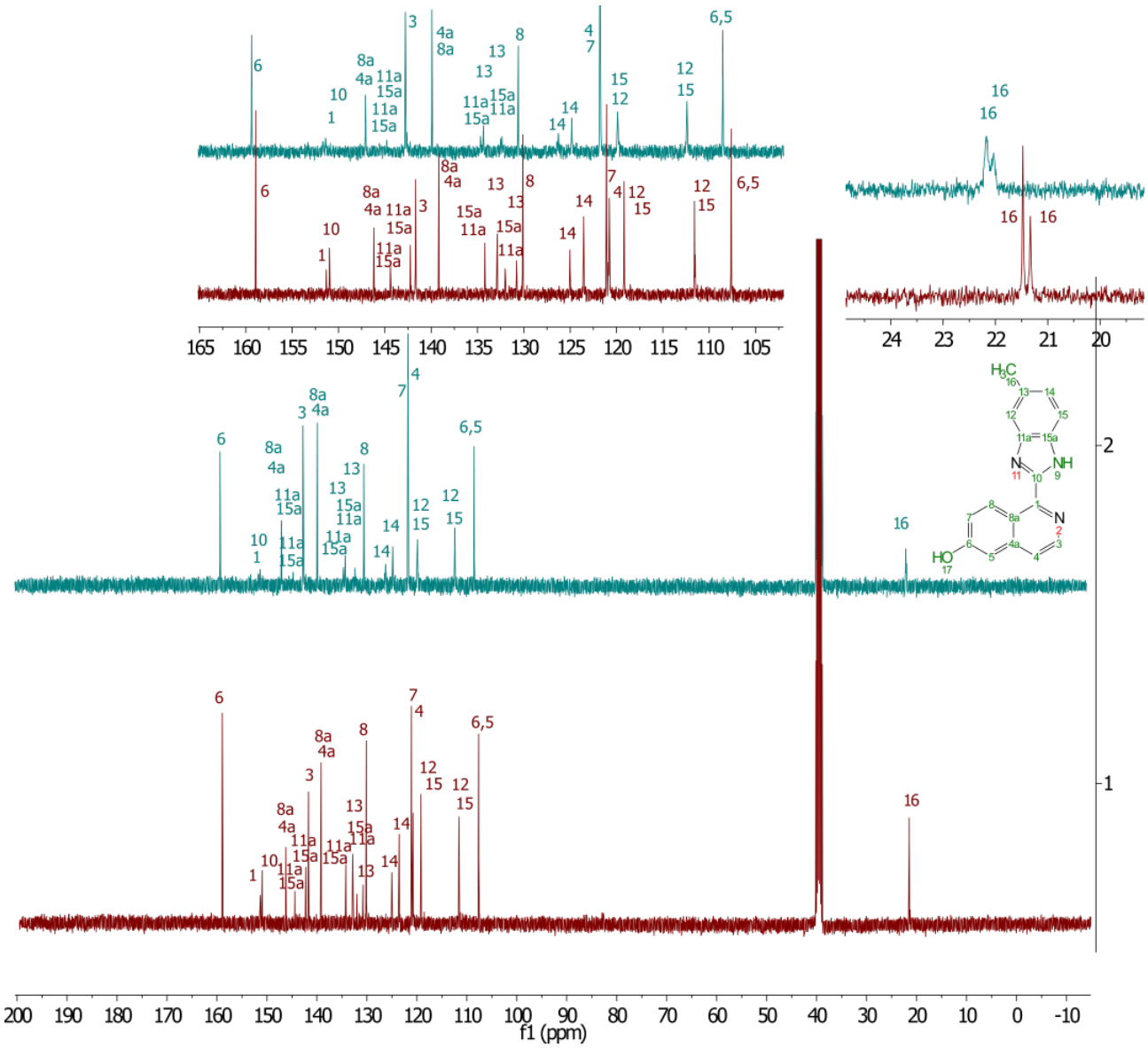


Figure S12: ^{13}C NMR spectrum of **18** in DMSO-d_6 (0.7 mL) at 25 °C (bottom), ^{13}C NMR spectrum after addition of 0.1 mL D_2O (Top).

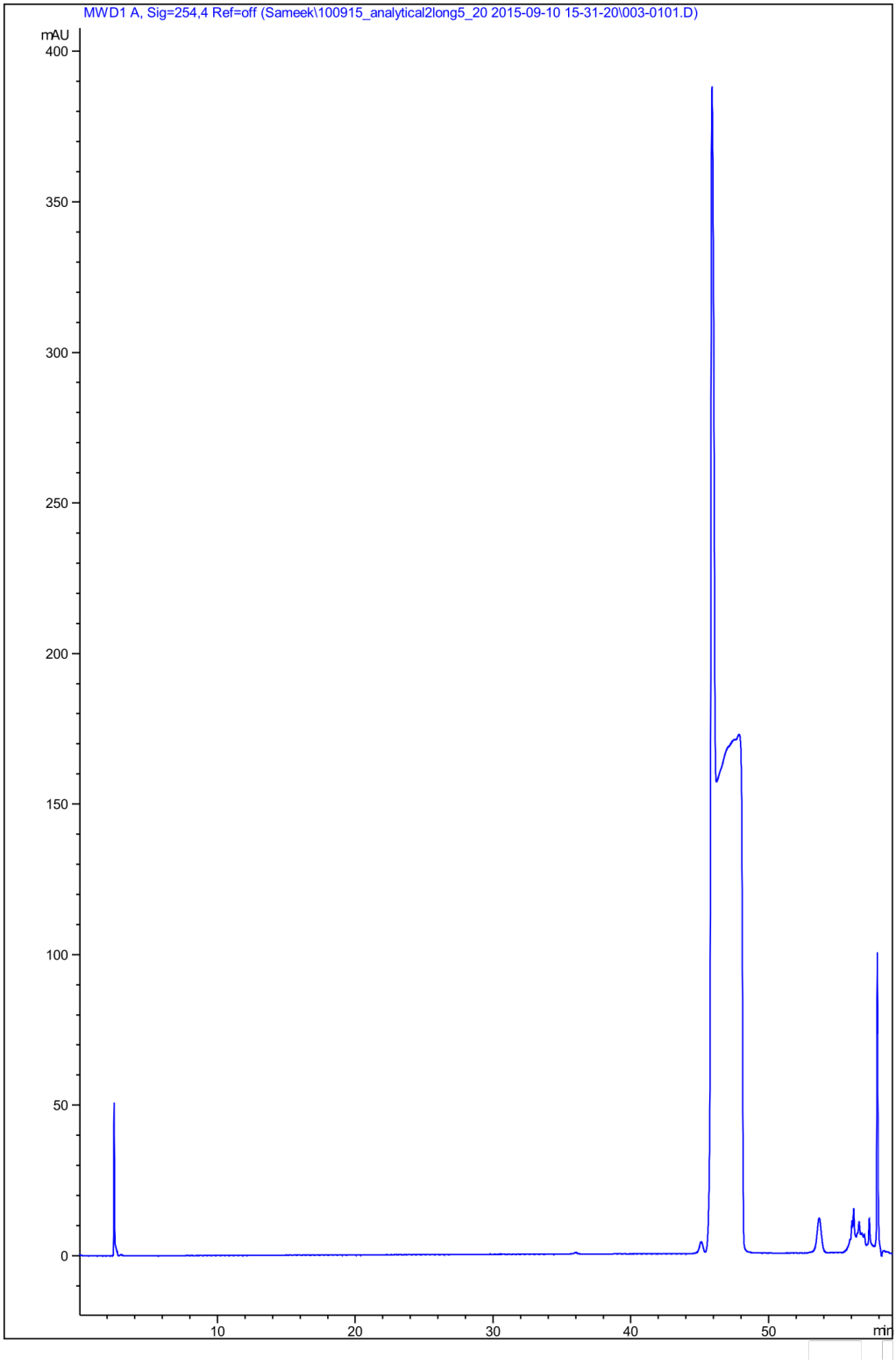


Figure S13: Analytical HPLC chromatogram of **18** at high concentration showed a shoulder peak.

The following Analytical RP-HPLC method was used for obtaining analytical HPLC chromatograms reported in Figure S13, Figure S15(A) and (B) - 5% solvent B 1 min, gradient of 5-20% solvent B 1-51 mins, 95% solvent B 52-53 mins, gradient of 95-5% solvent B 53-55 mins, 5% solvent B 55-59 mins.

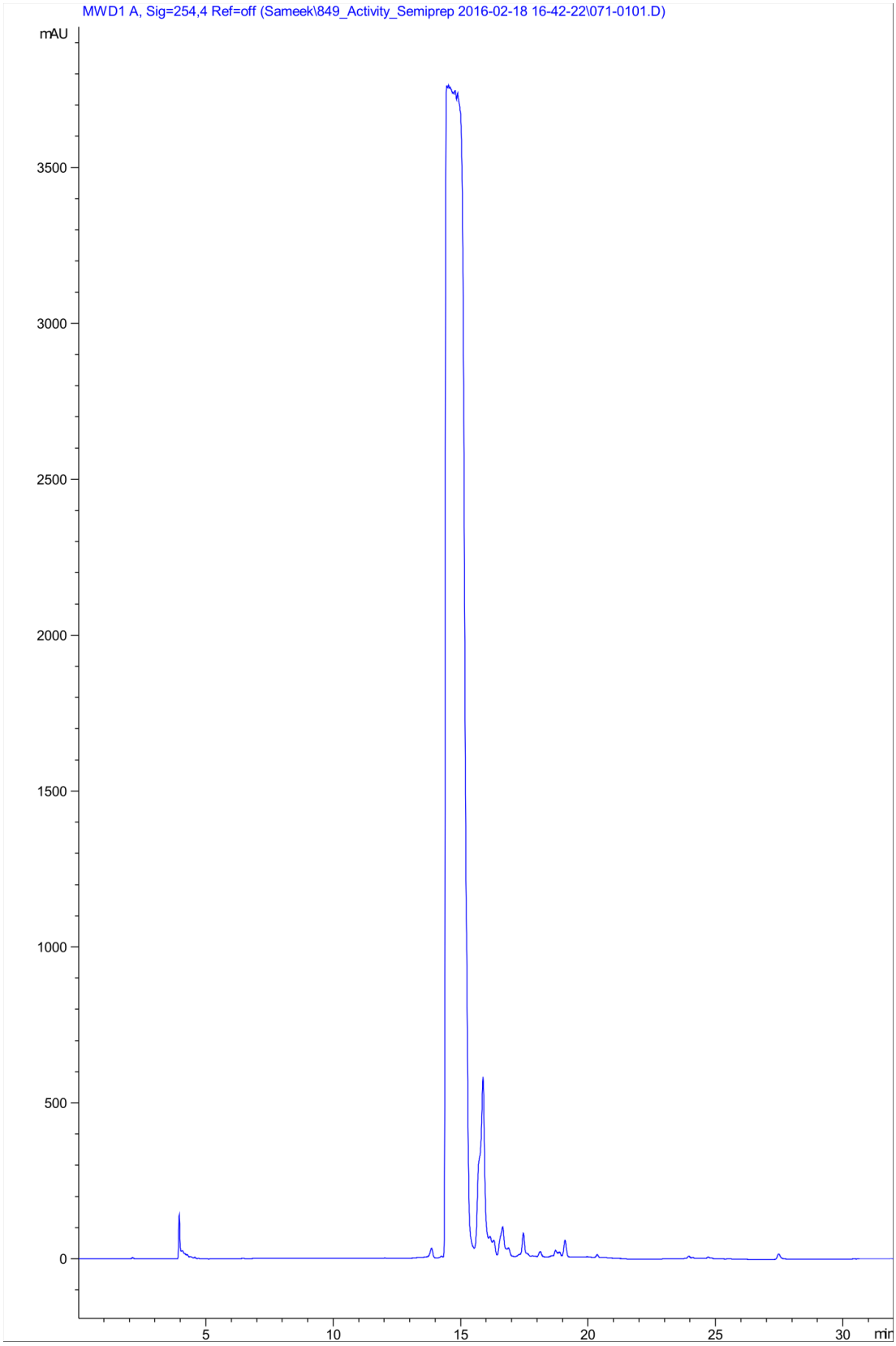
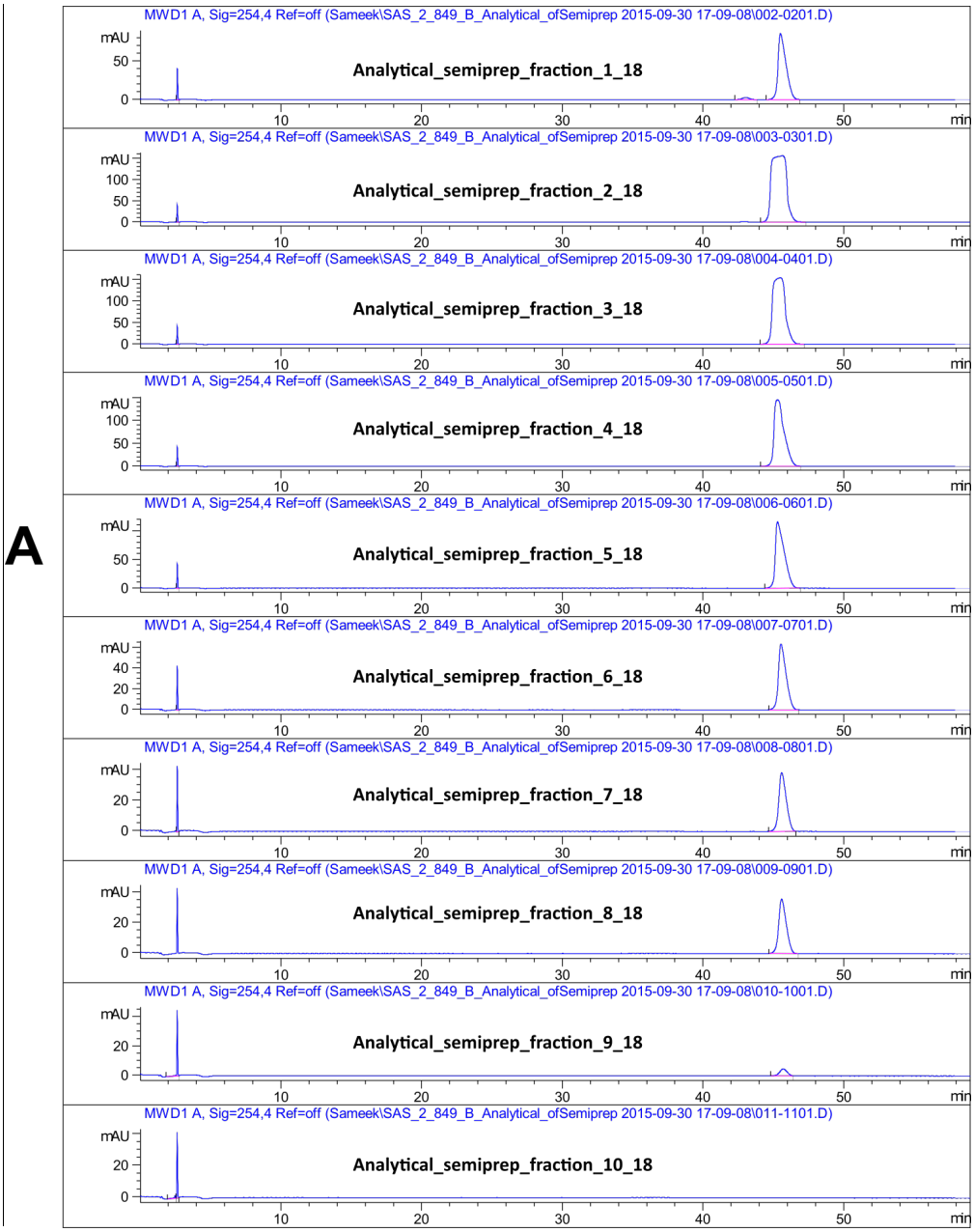


Figure S14: Semi-preparative HPLC chromatogram of **18**.



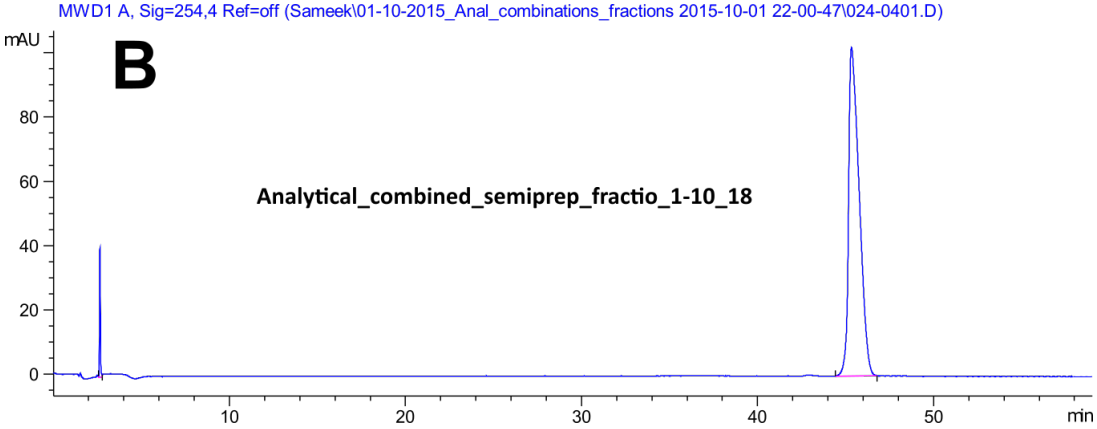


Figure S15: (A) Analytical HPLC chromatogram of 10 separate fractions collected from peak corresponding to **18** (14.5 to 15.5 min) in semi-preparative RP-HPLC chromatogram (Figure S14). **(B)** Analytical HPLC chromatogram of combined fractions.

3. Pharmacology

3.1 Material and Methods

NLuc-A_1AR and NLuc-A_3AR receptor constructs, stable cell line generation and cell culture were carried out as described by Stoddart *et al*.[21] Briefly, the full length NLuc luciferase sequence (pNL1.1 vector; Promega Corporation) was amplified and fused in frame within pcDNA3.1 vector along with a short membrane signal sequence derived from the 5HT3A receptor. Full length human adenosine A_1 or A_3 receptor subtypes (initiation methionine removed) were then fused to the 3' end of signal sequence-NLuc in pcDNA3.1. The resulting constructs were termed NLuc A_1AR and NLuc A_3AR respectively. Untagged A_1AR was obtained from cDNA.org.

HEK293 cells (NLuc A_1AR; American Type Culture Collection (ATCC)) or HEK293G cells (Glosensor™ cAMP HEK293 for NLuc A_3AR; Promega Corporation) were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum at 37°C, 5% CO_2. Mixed population cell lines were generated following transfection with Lipofectamine (Life Technologies) and geneticin (G418; Thermo-Fisher Scientific) selection pressure. The NLuc A_3AR cell line was then dilution cloned to generate cell lines derived from a single cell

Bioluminescence resonance energy transfer (BRET) assays

The BRET A_1AR and A_3AR receptor-ligand binding assays were undertaken following the protocol described in Stoddart *et al*.[21] In brief, saturation assays were performed on stably transfected cells that were seeded 24 h before experimentation in poly-$_D$-lysine coated (2mg/ml; Sigma Aldrich) white Thermo Scientific Matrix 96-well microplates. The medium was removed from each well and replaced with HEPES Buffered Saline Solution (HBSS: 145 mmol/L NaCL, 5 mmol/L KCl, 1.7 mmol/L $CaCl_2$, 1 mmol/L $MgSO_4$, 10 mmol/L HEPES, 2 mmol/L sodium pyruvate, 1.5 mmol/L $NaHCO_3$, 10 mmol/L D-glucose, pH 7.2–7.45) with the required concentration of fluorescent ligand and competing ligand if present (1h at 37 °C). Non-specific binding was assessed in the absence and presence of 1μM DPCPX (NLuc-A_1AR) or 1μM MRS1220 (NLuc-A_3AR). For competition experiments, NLuc A_1AR cells were incubated with 25mM CA200645 in the presence or absence of increasing concentrations of unlabelled ligand (1h at 37 °C). For both saturation and competition assays, following ligand stimulation the NLuc substrate furimazine (Promega Corporation, USA) was added to give a final concentration of 10μM. Plates were left for 5min and then luminescence and fluorescence emissions were measured using a PHERAstar FS plate reader (BMG Labtech) at room temperature. Filtered light emissions were sequentially measured at 460 nm (80-nm bandpass; NLuc emission) and >610 nm (longpass; fluorescence emission) and the raw BRET ratio calculated by dividing the >610-nm emission by the 460-nm emission. Data was pooled from independent experiments (n=4-7) and for saturation experiments, data was baseline corrected (minus vehicle + furimazine BRET ratios) and expressed as fold increase in BRET ratios over basal. For saturation experiemnts, NLuc A_1AR data were fit using GraphPad Prism_v6 using a one site saturation binding fit (total and non specific binding) with shared

values for non specific binding. Specific binding for A_3AR was calculated by subtracting corrected BRET values obtained in the presence of competing unlabelled MRS1220 from those obtained for compound alone. Data were fit using Graph Prism_v6 using one site saturation binding. K_d values were estimated from each individual experiment, which were then averaged and expressed as mean ± standard error of the mean. For displacement experiments, data were pooled from 5 independent experiments. When possible, binding affinities (K_i) of unlabeled ligands were calculated using the Cheng-Prusoff equation, using K_d values derived from CA200645 saturation binding and are expressed as mean ± standard error of the mean.

High content fluorescence imaging

HEK293 cells were seeded 48 h before experimentation in poly-$_D$-lysine coated (2mg/ml; Sigma Aldrich) white Thermo Scientific Matrix 96-well microplates and were transiently transfected with the A_1AR (untagged) construct 24 h before experimentation using Fugene (1:3 DNA:reagent ratio; 100ng cDNA/well). Additionally, HEK293 cells stably transfected with NanoLuc N-terminal, tagged A_1AR were seeded 24 h before experimentation in poly-$_D$-lysine coated (2mg/ml; Sigma Aldrich) white Thermo Scientific Matrix 96-well microplates. On the day of experimentation, medium was removed from each well and replaced with HBSS. Cells were then incubated with increasing concentrations of the fluorescent ligand, CA200645 and incubated for 1hr at 37°C. Non-specific binding was defined using 1μM DPCPX. Plates were then washed with phosphate-buffered saline (PBS) and fixed using 3% paraformaldehyde in PBS. Plates were imaged at 4 sites per well using a IX Ultra Confocal Plate Reader (Molecular Devices, CA, USA) fitted with a 40x ELWD objective and using a long pass 650nm filter (60% laser power, 600 gain).[22] Fluorescence intensity was quantified per well using a multi wavelength cell sorting algorithm (MetaXpress, Molecular Devices). Data were fit using Graph Prism_v6 using one site saturation binding. K_d values were estimated from each individual experiment (n=3/4), averaged and expressed as mean ± standard error of the mean.

3.2 Supplementary data

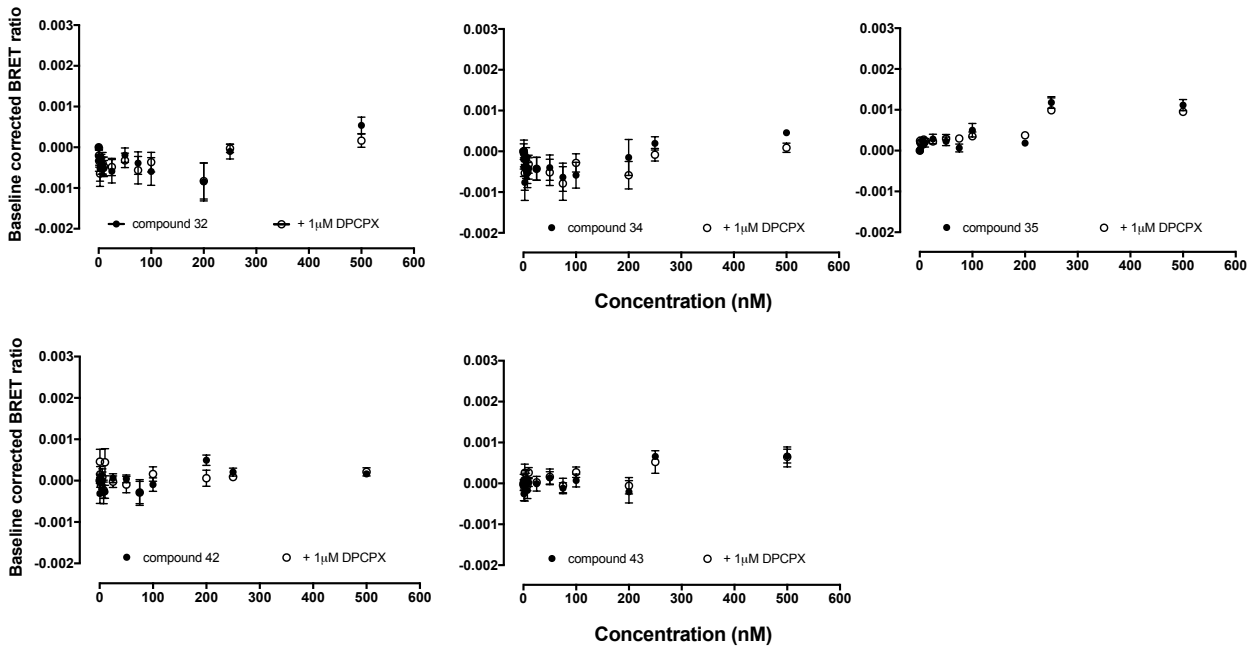


Figure S16. HEK293 cells stably transfected with *N*-terminally NLuc-labelled A_1AR were treated with increasing concentrations of fluorescent ligand and the BRET ratio measured after direct addition of the NLuc substrate furimazine (10μM).[21] Non-specific binding was assessed in the absence and presence of 1μM DPCPX. Pooled raw BRET ratio's were baseline corrected (minus vehicle + furimazine BRET ratios) with data expressed as fold increase in BRET ratios over basal. Data represents five - seven independent experiments (in triplicate).

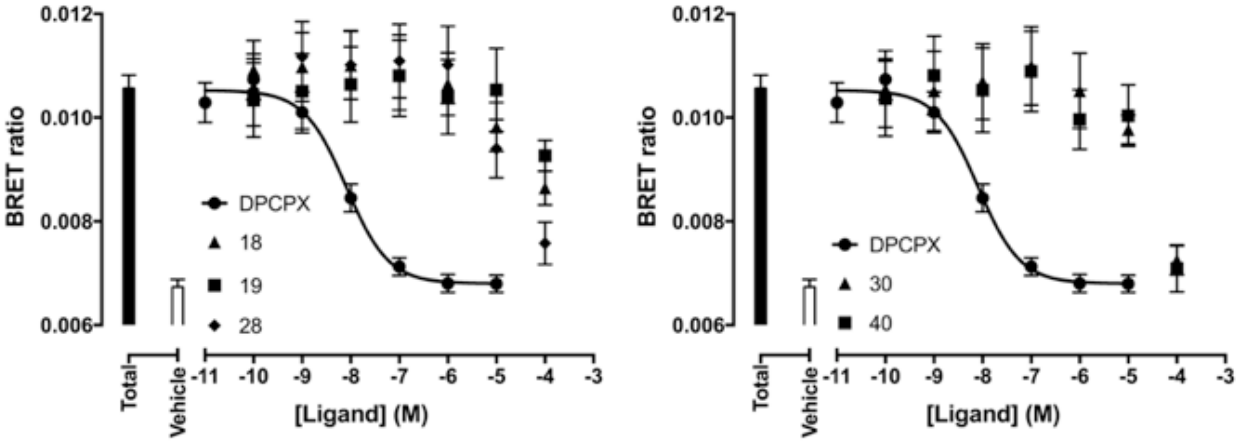


Figure S17. HEK293 cells stably expressing *N*-terminal NLuc tagged A1 were co-incubated with a fixed concentration of CA200645 (25nM) and increasing concentrations of unlabelled ligand (1hr at 37°C). The A_1AR selective antagonist DPCPX was included as a positive control. Total CA200645 binding and vehicle are shown by the black and white bars respectively. Data were pooled from five independent experiments and are expressed as mean ± S.E.M.

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