A Fluorescence Polarization Assay for Binding to Macrophage Migration Inhibitory Factor and Crystal Structures for Complexes of Two Potent Inhibitors

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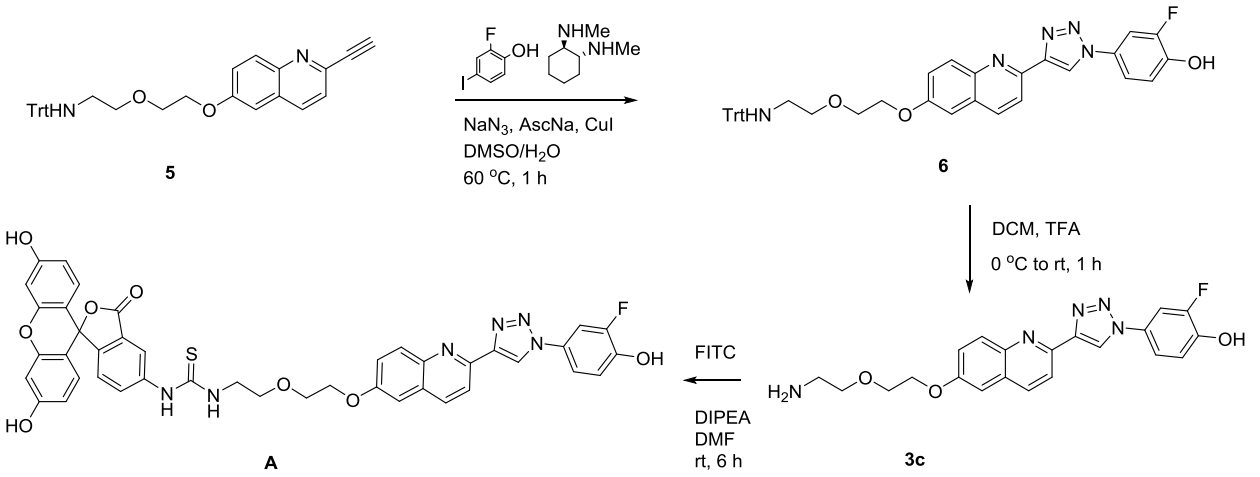
Supporting Information

Table of Contents

1. General Information

NMR spectra were recorded on Agilent DD2 600 (600 MHz), DD2 500 (500 MHz) and DD2 400 (400 MHz) instruments. Column chromatography was carried out using CombiFlash over redisep column cartridges employing Merck silica gel (Kieselgel 60, 63-200 μm) and Grace C18 reversed-phase (40 μm). Pre-coated silica gel plates F-254 were used for thin-layer analytical chromatography. Mass determinations were performed using electrospray ionization on water Micromass ZQ (LC-MS) and on an Agilent Technologies 6890N (GC-MS). HRMS (ESI-TOF) analyses were performed on Waters Xevo QTOF equipped with Z-spray electrospray ionization source. The purity (≥ 95%) of all final synthesized compounds was determined by reverse phase HPLC, using a Waters 2487 dual λ absorbance detector with a Waters 1525 binary pump and a Phenomenex Luna 5μ C18(2) 250 x 4.6 mm column. Samples were run at 1 mL/min using gradient mixtures of 5-100% of water with 0.1% trifluoroacetic acid (TFA) (A) and 10:1 acetonitrile:water with 0.1% TFA (B) for 22 min followed by 3 min at 100% B.

2. Synthesis of ligands A and B



Scheme S1. Synthesis of ligand **A**

2-(2-((2-ethynylquinolin-6-yl)oxy)ethoxy)-N-tritylethan-1-amine (**5**). Synthesized as previously reported.[1]

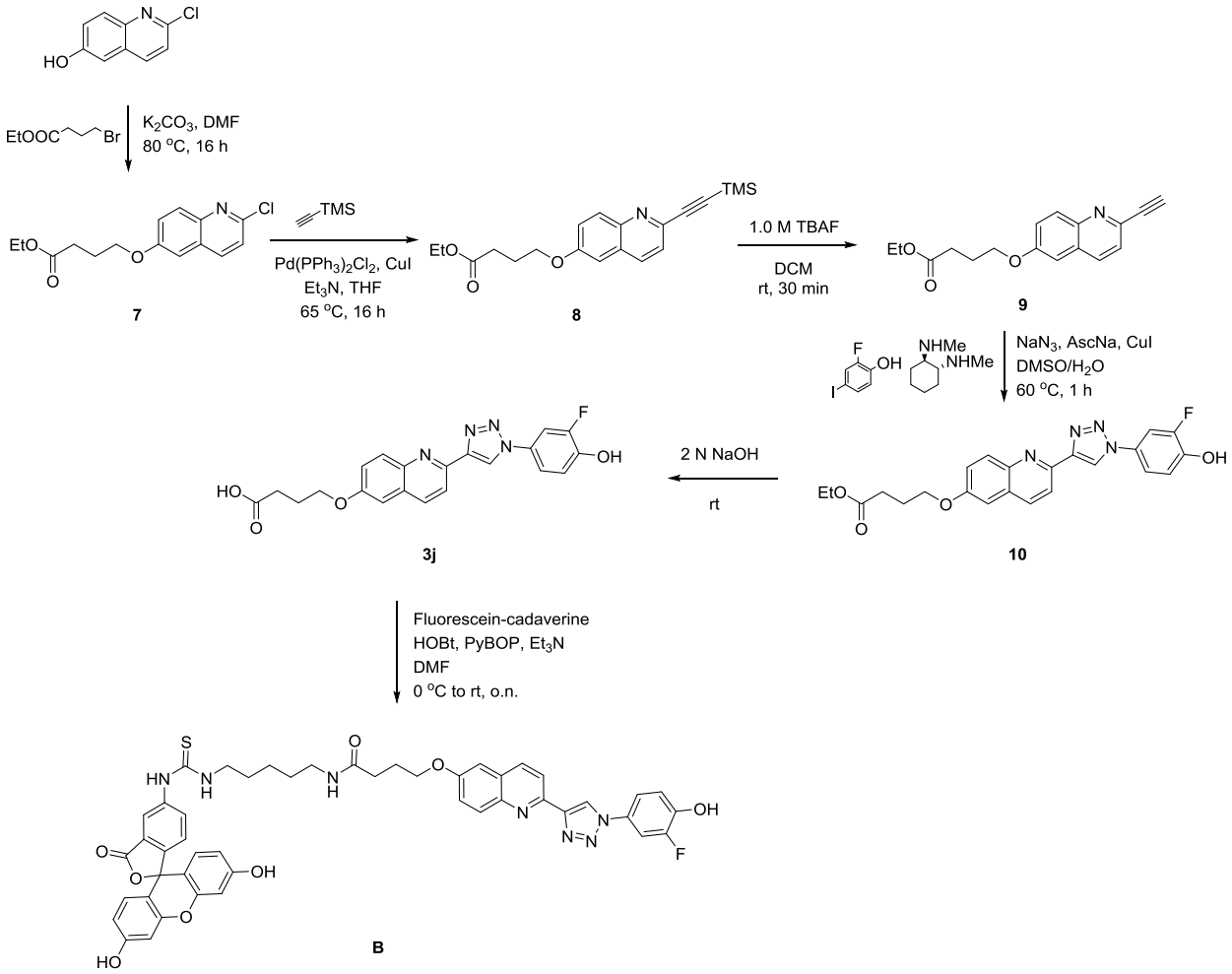
2-fluoro-4-(4-(6-(2-(2-(tritylamino)ethoxy)ethoxy)quinolin-2-yl)-1H-1,2,3-triazol-1-yl)phenol (**6**). 2-fluoro-4-iodopehnol (0.23 mmol) followed by trans-*N*,*N*'-dimethylcyclohexane-1,2-diamine (0.034 mmol), sodium ascorbate (0.023 mmol), copper iodide (0.023 mmol) and sodium azide (0.23 mmol) were added to DMSO (1.0 mL). The mixture was stirred at 70 °C for 2 h and then **5** (0.23 mmol) followed by H_2O (1.0 mL) were added to the reaction which was stirred overnight. The solution was then diluted with EtOAc and extracted with H_2O (x1) and brine (x1). The aqueous phase was washed with EtOAc, the organic phases were combined and dried with anh Na_2SO_4 and solvent evaporated. The crude product was purified by flash chromatography (hexanes/EtOAc). (27%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 (s, 1H), 8.32 (d, *J* = 8.6 Hz, 1H), 8.11 (d, *J* = 8.6 Hz, 1H), 7.92 (d, *J* = 9.2 Hz, 1H), 7.66 (dd, *J* = 10.8, 2.5 Hz, 1H), 7.53 - 7.39 (m, 7H), 7.30 (dd, *J* = 9.2, 2.7 Hz, 1H), 7.26 - 7.23 (m, 6H), 7.20 - 7.13 (m, 4H), 7.08 (d, *J* = 2.8 Hz,

1H), 4.22 (t, *J* = 4.7 Hz, 2H), 3.80 (t, *J* = 4.7 Hz, 2H), 3.71 (t, *J* = 5.3 Hz, 2H), 2.39 (t, *J* = 5.3 Hz, 2H).

4-(4-(6-(2-(2-aminoethoxy)ethoxy)quinolin-2-yl)-1H-1,2,3-triazol-1-yl)-2-fluorophenol (**3c**). **6** (42 mg, 0.06 mmol) was dissolved in anh DCM (0.24 mL) and cooled to 0 °C. Trifluoroacetic acid (0.16 mL) was added dropwise, the reaction warmed to rt and stirred for 1 h. Saturated $NaHCO_3$ solution was added to neutralize the reaction and extracted with DCM. Combined organic layers were dried over anh Na_2SO_4 and the final compound purified by flash chromatography (DCM/MeOH). (78%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.56 (s, 1H), 9.31 (s, 1H), 8.39 (d, *J* = 8.6 Hz, 1H), 8.24 (d, *J* = 8.6 Hz, 1H), 8.02 - 7.79 (m, 4H), 7.76 - 7.69 (m, 1H), 7.52 - 7.41 (m, 2H), 7.17 (t, *J* = 9.0 Hz, 1H), 4.36 - 4.25 (m, 2H), 3.93 - 3.86 (m, 2H), 3.71 (t, *J* = 5.2 Hz, 2H), 3.04 (q, *J* = 5.3 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 156.4, 150.7 (d, *J* = 242.8 Hz), 148.2, 147.5, 145.6 (d, *J* = 11.9 Hz), 143.5, 136.1, 130.0, 128.5, 128.3 (d, *J* = 8.8 Hz), 122.7, 121.4, 118.7, 118.2 (d, *J* = 3.5 Hz), 116.9 (d, *J* = 3.1 Hz), 109.3 (d, *J* = 23.2 Hz), 106.7, 68.83, 67.4, 66.8, 38.6. HRMS (ESI): calcd. for $[M+H]^+$ ($C_{21}H_{20}FN_5O_3$) 410.1623, found 410.1625.

5-(3-(2-(2-((2-(1-(3-fluoro-4-hydroxyphenyl)-1H-1,2,3-triazol-4-yl)quinolin-6-yl)oxy)ethoxy)ethyl)thioureido)-2-(6-hydroxy-3-oxo-3H-xanthen-9-yl)benzoic acid (**A**). To a solution of **3c** (30 mg) in 1 mL DMF under N_2, 0.7 mL DIPEA were added followed by the addition of 40 mg FITC and the reaction stirred for 6 h at rt protected from light. After solvent evaporation, final compound was purified by preparative HPLC. (80 %). ^{1}H NMR (400 MHz, CD_3OD) δ 9.14 (s, 1H), 8.51 - 8.43 (m, 1H), 8.24 - 8.14 (m, 1H), 8.11 - 7.98 (m, 2H), 7.76 - 7.67 (m, 2H), 7.63 - 7.50 (m, 2H), 7.44 (s, 1H), 7.15 (t, *J* = 8.8 Hz, 1H), 7.09 - 6.98 (m, 1H), 6.83 - 6.75 (m, 2H), 6.73 - 6.62 (m, 2H), 6.54 (d, *J* = 9.1 Hz, 2H), 4.37 (s, 2H), 3.97 (s, 3H), 3.85 (s,

4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 168.6, 159.5, 158.1, 156.5, 151.9, 151.9, 150.7 (d, J = 242.8 Hz), 148.2, 147.5, 145.6 (d, J = 11.4 Hz), 143.4, 141.5, 136.1, 132.9, 130.0, 129.3, 129.0, 128.5, 128.3 (d, J = 9.2 Hz), 122.8, 121.4, 118.7, 118.2 (d, J = 3.6 Hz), 116.9 (d, J = 3.0 Hz), 116.4, 116.2, 112.6, 109.7, 109.4 (d, J = 22.9 Hz), 106.8, 102.2, 83.0, 68.7, 68.6, 67.6, 41.5. HRMS (ESI): calcd. for $[M+H]^+$($C_{42}H_{31}FN_6O_8S$) 799.1981, found 798.1992.



Scheme S2. Synthesis of ligand **B**

Ethyl 4-((2-chloroquinolin-6-yl)oxy)butanoate (7). 2-chloroquinolin-6-ol (200 mg, 1.1 mmol) was added to a pressure vial. Next, DMF (5 mL), ethyl 4-bromobutanoate (0.24 mL, 1.68 mmol) and K_2CO_3 (306 mg, 2.2 mmol) were added and the solution was stirred at 80 °C for 16 h. The

reaction mixture was cooled to rt, diluted with EtOAc and washed with H_2O (x1) and brine (x3). The organic phase was dried over anh Na_2SO_4 and concentrated under vacuum. Intermediate **7** was used in the next step without further purification. (84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, *J* = 8.6 Hz, 1H), 7.90 (d, *J* = 9.2 Hz, 1H), 7.39 - 7.30 (m, 2H), 7.06 (d, *J* = 2.7 Hz, 1H), 4.18 - 4.09 (m, 4H), 2.55 (t, *J* = 7.2 Hz, 2H), 2.22 - 2.13 (m, 2H), 1.25 (t, *J* = 7.1 Hz, 3H).

Ethyl 4-((2-((trimethylsilyl)ethynyl)quinolin-6-yl)oxy)butanoate (8). Dry THF (4 mL), **7** (270 mg, 0.92 mmol), ethynyltrimethylsilane (1.4 mmol), $Pd(PPh_3)_2Cl_2$ (0.046 mmol,), CuI (0.046 mmol) and dry Et_3N (3.7 mmol) were added to a pressure vial. The reaction mixture was stirred at 60 °C for 16 h. The crude reaction mixture was filtered through celite pad and washed with EtOAc. After solvent evaporation, desired compound was purified by flash chromatography (hexanes/EtOAc). (76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (t, *J* = 8.4 Hz, 2H), 7.48 (d, *J* = 8.4 Hz, 1H), 7.34 (dd, *J* = 9.3, 2.7 Hz, 1H), 7.02 (d, *J* = 2.7 Hz, 1H), 4.19 - 4.09 (m, 4H), 2.55 (t, *J* = 7.2 Hz, 2H), 2.22 - 2.13 (m, 2H), 1.25 (t, *J* = 7.1 Hz, 3H), 0.29 (s, 9H).

Ethyl 4-((2-ethynylquinolin-6-yl)oxy)butanoate (9). **8** (250 mg, 0.7 mmol) was dissolved in anh DCM (7 mL). Next, 1.0 M TBAF in hexanes (0.84 mL) was added dropwise under N_2 and the reaction mixture was stirred at rt for 1 min. Upon completion 10 mL of 10% citric acid were added and the mixture stirred for 30 min. After washing with H_2O, the organic phase was dried over anh Na_2SO_4, concentrated under vacuum and the product used in the next step without further purification. (92%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (d, *J* = 2.3 Hz, 1H), 7.97 (d, *J* = 3.2 Hz, 1H), 7.49 (d, *J* = 8.5 Hz, 1H), 7.35 (dd, *J* = 9.3, 2.7 Hz, 1H), 7.03 (d, *J* = 2.7 Hz, 1H), 4.21 - 4.07 (m, 4H), 3.19 (s, 1H), 2.55 (t, *J* = 7.2 Hz, 2H), 2.25 - 2.12 (m, 2H), 1.26 (t, *J* = 7.1 Hz, 4H).

Ethyl 4-((2-(1-(3-fluoro-4-hydroxyphenyl)-1H-1,2,3-triazol-4-yl)quinolin-6-yl)oxy)butanoate (10). Following the synthesis of **6**. Purified by flash chromatography (DCM/EtOAc) (57%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (s, 1H), 8.33 (d, *J* = 8.6 Hz, 1H), 8.15 (d, *J* = 8.6 Hz, 1H), 7.97 (d, *J* = 9.2 Hz, 1H), 7.67 (dd, *J* = 10.7, 2.5 Hz, 1H), 7.53 - 7.47 (m, 1H), 7.37 (dd, *J* = 9.2, 2.7 Hz, 1H), 7.18 (t, *J* = 8.9 Hz, 1H), 7.10 (d, *J* = 2.8 Hz, 1H), 4.20 - 4.08 (m, 4H), 2.58 (t, *J* = 7.2 Hz, 2H), 2.24 - 2.17 (m, 3H), 1.27 (t, *J* = 7.1 Hz , 3H).

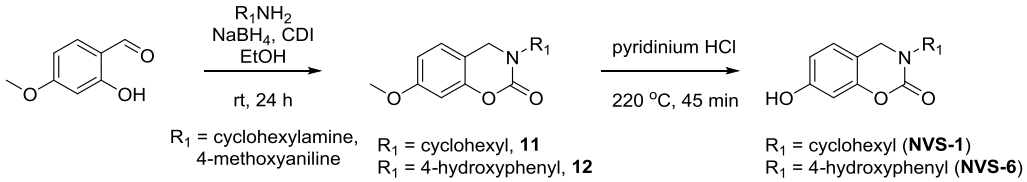
4-((2-(1-(3-fluoro-4-hydroxyphenyl)-1H-1,2,3-triazol-4-yl)quinolin-6-yl)oxy)butanoic acid (3j). Ethyl ester **10** (25 mg, 0.06 mmol) was dissolved in dioxane (3.5 mL) and 2 N NaOH solution (1.6 mL) was added to the solution which was then stirred at rt. Upon completion, solvent was evaporated, the mixture diluted in H_2O and the pH was adjusted to around 3-4 with 1 N HCl solution. After cooling at 4 °C the crude was filtered and dried to give the carboxylic acid **11** without further purification. (85%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.26 (s, 1H), 8.35 (d, *J* = 8.6 Hz, 1H), 8.19 (d, *J* = 8.6 Hz, 1H), 7.99 - 7.84 (m, 2H), 7.67 (ddd, *J* = 9.1, 2.6, 1.3 Hz, 1H), 7.41 (d, *J* = 7.8 Hz, 2H), 7.16 (t, *J* = 9.0 Hz, 1H), 4.13 (t, *J* = 6.5 Hz, 2H), 2.39 (t, *J* = 7.3 Hz, 2H), 2.01 (quint, *J* = 6.9 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.4, 156.6, 151.8 (d, *J* = 262.5 Hz), 148.2, 147.4, 146.5 (d, *J* = 14.0 Hz), 143.4, 136.0, 130.0, 128.6, 128.3 (d, *J* = 8.5 Hz), 122.8, 121.3, 118.6, 118.3 (d, *J* = 3.9 Hz), 116.8 (d, *J* = 2.4 Hz), 109.3 (d, *J* = 20.0 Hz), 106.7, 67.3, 30.7, 24.4. HRMS (ESI): calc for $[M+H]^+$ ($C_{21}H_{17}FN_4O_4$) 409.1307. found 409.1466.

5-(3-(5-(4-((2-(1-(3-fluoro-4-hydroxyphenyl)-1H-1,2,3-triazol-4-yl)quinolin-6-yl)oxy)butanamido)pentyl)thioureido)-2-(6-hydroxy-3-oxo-3H-xanthen-9-yl)benzoic acid (B). To an ice-cooled solution of **11** (50 mg, 0.12 mmol) in anh DMF (2 mL), Et_3N (20 µL), HOBt (19 mg, 0.15 mmol) and PyBOP (76 mg, 0.15 mmol) were added. The mixture was stirred

at 0 °C for 20 min followed by the addition of 72 mg (0.15 mmol) of fluorescein-cadaverine. The reaction was warmed to rt and stirred overnight. Upon completion of the reaction, the mixture was diluted in EtOAc and washed with saturated NH_4Cl solution. The organic phase is dried over anh Na_2SO_4, solvent evaporated and the desired compound purified by C18 reverse phase chromatography (H_2O/ACN). (48%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.77 (s, 1H), 10.57 (s, 1H), 10.19 (s, 1H), 9.29 (s, 1H), 8.63 (s, 1H), 8.43 (s, 1H), 8.38 (d, *J* = 8.6 Hz, 1H), 8.21 (d, *J* = 8.5 Hz, 1H), 7.97 - 7.89 (m, 3H), 7.87 - 7.82 (m, 1H), 7.73 - 7.70 (m, 1H), 7.45 - 7.40 (m, 2H), 7.21 (t, *J* = 9.0 Hz, 1H), 7.15 (d, *J* = 8.3 Hz, 1H), 6.70 (d, *J* = 2.2 Hz, 2H), 6.62 - 6.51 (m, 4H), 4.13 (t, *J* = 6.4 Hz, 2H), 3.50 - 3.44 (m, 2H), 3.08 (q, *J* = 6.4 Hz, 2H), 2.31 (t, *J* = 7.3 Hz, 2H), 2.06 - 2.00 (m, 2H), 1.59 - 1.53 (m, 2H), 1.47 - 1.41 (m, 2H), 1.39 - 1.32 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.3, 168.6, 166.2, 159.5, 156.6, 151.9, 150.7 (d, *J* = 242.7 Hz), 149.7, 148.2, 147.4, 146.6, 145.6 (d, *J* = 11.9 Hz), 143.4, 141.8, 136.0, 130.0, 129.0, 129.0, 128.6, 128.3 (d, *J* = 9.1 Hz), 126.4, 123.9, 122.8, 121.4, 118.6, 118.2 (d, *J* = 3.4 Hz), 116.8 (d, *J* = 3.3 Hz), 112.6, 109.7, 109.3 (d, *J* = 23.2 Hz). 106.7, 102.2, 83.0, 67.5, 43.5, 38.4, 31.7, 28.8, 28.0, 24.9, 23.9. HRMS (ESI): calcd. for $[M+H]^+$($C_{47}H_{40}FN_7O_8S$) 882.2716, found 882.2645.

3. Synthesis of NVS compounds

Final compounds NVS-1, and NVS-6 were obtained following the synthetic route previously reported (Scheme S3).[2]



Scheme S3. Synthesis of NVS-1 and NVS-6

General method for synthesis of 3,4-dihydro-2H-benzo[e][1,3]oxazin-2-one (11, 12). Cyclohexylamine or 4-methoxyaniline (3.96 mmol, 1 eq) were added to a solution of 2-hydroxy-4-methoxybenzaldehyde (3.96 mmol) in 19 mL of absolute EtOH at rt. The mixture obtained was stirred for 1.5 h. After cooled to 0 °C, $NaBH_4$ (11.88 mmol, 3 eq) was added in portions. The mixture was stirred for 3 h at rt, poured into H_2O (30 mL) and extracted with DCM (50 mL). The organic layer was dried over anh Na_2SO_4. After filtration carbonyl-diimidazole (5.94 mmol, 1.5 eq) was added and the mixture stirred for 16 h at rt. Additional carbonyl-diimidazole (3.09 mmol, 0.78 eq) was added and the reaction stirred for 3 h at rt. Then, the mixture was washed with 1 N HCl solution (50 mL), a saturated $NaHCO_3$ solution (50 mL) and brine (50 mL), dried over anh Na_2SO_4 and concentrated under vacuum. Desired intermediates were used in the next step without further purification.

3-cyclohexyl-7-methoxy-3,4-dihydro-2H-benzo[e][1,3]oxazin-2-one (11). (84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.99 (d, *J* = 8.4 Hz, 1H), 6.66 (dd, *J* = 8.4, 2.5 Hz, 1H), 6.57 (d, *J* = 2.4 Hz, 1H), 4.34 (s, 2H), 4.24 (tt, *J* = 11.9, 3.5 Hz, 1H), 3.78 (s, 3H), 1.89 - 1.78 (m, 4H), 1.74 - 1.66 (m, 1H), 1.59 - 1.36 (m, 4H), 1.19 - 1.05 (m, 1H).

7-methoxy-3-(4-methoxyphenyl)-3,4-dihydro-2H-benzo[e][1,3]oxazin-2-one (12). (27%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (d, *J* = 8.8 Hz, 2H), 7.00 (d, *J* = 8.4 Hz, 1H), 6.78 (d, *J* = 8.8 Hz, 2H), 6.70 (dd, *J* = 8.4, 2.5 Hz, 1H), 6.54 (d, *J* = 2.5 Hz, 1H), 4.74 (s, 2H), 3.81 (s, 3H), 3.82 (s, 3H).

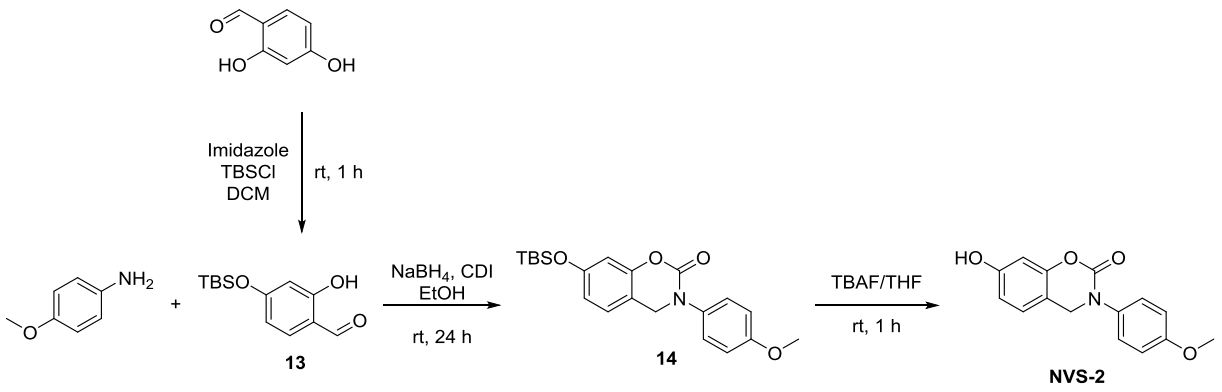
General method for synthesis of NVS-1 and NVS-6. A mixture of 0.85 g (3.25 mmol) of **11** or **12** and 2.63 g (22.28 mmol) of pyridinium HCI were heated without solvent on a metal bath to

220 °C for 45 min while stirring. After the mixture was cooled to rt, the melt obtained was dissolved in H_2O (75 mL) and EtOAc (50 mL). The aqueous phase was extracted with EtOAc (25 mL). Organic layer was washed with 1N HCl solution (50 mL), dried over anh Na_2SO_4 and concentrated under vacuum. The residue was filtered over silica gel and the filtration residue concentrated without further purification.

3-cyclohexyl-7-hydroxy-3,4-dihydro-2H-benzo[e][1,3]oxazin-2-one, NVS-1. (85%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.02 (d, *J* = 8.3 Hz, 1H), 6.57 (dd, *J* = 8.3, 2.3 Hz, 1H), 6.40 (d, *J* = 2.2 Hz, 1H), 4.34 (s, 2H), 4.15 - 4.05 (m, 1H), 1.93 - 1.58 (m, 7H), 1.48 - 1.36 (m, 2H), 1.26 - 1.17 (m, 1H). ^{13}C NMR (101 MHz, CD_3OD) δ 159.3, 153.1, 151.3, 127.6, 112.9, 110.0, 103.2, 57.8, 42.5, 30.2, 26.8, 26.5. HRMS (ESI): calcd. for $[M+H]^+$($C_{14}H_{18}NO_3$) 248.1281, found 248.1296.

7-hydroxy-3-(4-hydroxyphenyl)-3,4-dihydro-2H-benzo[e][1,3]oxazin-2-one, NVS-6. (21%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.22 (d, *J* = 8.8 Hz, 2H), 7.02 (d, *J* = 8.4 Hz, 1H), 6.84 (d, *J* = 8.8 Hz, 2H), 6.61 (dd, *J* = 8.3, 2.3 Hz, 1H), 6.48 (d, *J* = 2.2 Hz, 1H), 4.71 (s, 2H). ^{13}C NMR (101 MHz, CD_3OD) δ 159.4, 158.1, 153.1, 151.6, 135.0, 128.3, 127.5, 116.9, 113.1, 110.1, 103.5, 52.0. HRMS (ESI): calcd. for $[M+H]^+$($C_{14}H_{12}NO_4$) 258.0761, found 258.0774.

NVS-2 was synthesized following the synthetic route described in the Scheme S4. In order to avoid deprotection of both methoxy groups this compound has been obtained through intermediate **13**.



Scheme S4. Synthesis of NVS-2

4-((tert-butyldimethylsilyl)oxy)-2-hydroxybenzaldehyde (13). To a stirred solution of 2,4-dihydroxybenzaldhyde (2.01 g, 14.25 mmol) and imidazole (1.08 g, 15.68 mmol) in DCM (33mL), tert-butylchlorodimethylsilane (2.21 g, 14.25 mmol) was added slowly and the reaction stirred at rt for 1 h. The mixture was diluted with DCM (25 mL), washed with H_2O (3 x 50 mL) and brine (50 mL) and dried over anh Na_2SO_4. Concentration under vacuum yielded **13** as a clear oil. The product was used the next step without further purification. (99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.33 (s, 1H), 9.72 (s, 1H), 7.40 (d, *J* = 8.5 Hz, 1H), 6.47 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.39 (d, *J* = 2.2 Hz, 1H), 0.98 (s, 9H), 0.26 (s, 6H).

7-((tert-butyldimethylsilyl)oxy)-3-(4-methoxyphenyl)-3,4-dihydro-2H-benzo[e][1,3]oxazin-2-one (14). Following the synthesis of **11** and **12**. (25%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (d, *J* = 8.8 Hz, 2H), 6.97 - 6.94 (m, 3H), 6.67 - 6.59 (m, 2H), 4.73 (s, 2H), 3.82 (s, 3H), 0.99 (s, 9H), 0.22 (s, 6H).

7-hydroxy-3-(4-methoxyphenyl)-3,4-dihydro-2H-benzo[e][1,3]oxazin-2-one, NVS-2. A 1.0 M solution of TBAF in THF (0.78 mL, 0.78 mmol) was slowly added to a cold (0 °C) solution of **14** (300 mg, 0.78 mmol) in THF (20 mL) and stirred at 0 °C for 1 h. The mixture was diluted with EtOAc (20 mL) and washed with saturated NH_4Cl solution (20 mL) and H_2O (20 mL). The

organic phase was dried over anh Na_2SO_4 and solvent evaporated under vacuum to yield the desired final compound without further purification. (99%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.33 (d, *J* = 8.9 Hz, 2H), 7.04 - 6.98 (m, 3H), 6.62 (dd, *J* = 8.3, 2.2 Hz, 1H), 6.49 (d, *J* = 2.1 Hz, 1H), 4.73 (s, 2H), 3.82 (s, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 160.3, 159.5, 153.1, 151.6, 136.1, 128.2, 127.5, 115.6, 113.1, 110.1, 103.5, 56.0, 51.9. HRMS (ESI): calcd. for $[M+H]^+$($C_{15}H_{14}NO_4$) 272.0917, found 272.0925.

4. Synthesis of compounds 5.

(*E*)-1-((1,2-dichlorovinyl)oxy)-4-methoxybenzene (15). To a solution of 500 mg of 4-metthoxyphenol (4 mmol) in 4 mL DMSO, 160 mg of powder NaOH were added and stirred for 2h at rt. 1,1,2-trichloroethene (0.36 mL, 4 mmol) was added dropwise and the resulting solution stirred at rt for 2h. After completion of the reaction, the reaction was quenched with H_2O and extracted with DCM (x2). The organic phases were combined and dried over Na_2SO_4. After solvent evaporation, the resulting product was used in the next step without further purification. (55%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.99 – 6.89 (m, 2H), 6.87 – 6.77 (m, 2H), 5.83 (s, 1H), 3.74 (s, 3H).

1-(ethynyloxy)-4-methoxybenzene (16). To a solution of 480 mg of 15 (2.2 mmol) in 22 mL of anhydrous Et_2O, 8.8 mL of 2.2 M *n*-BuLi in cyclohexane were added dropwise at -78 °C. The mixture was stirred 1h at -78 °C, and 2h at -40 °C. The reaction was quenched with H_2O and extracted with Et_2O (x2). The organic phases were combined and dried over Na_2SO_4. After solvent evaporation, the resulting product was used in the next step without further purification. (54%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23 – 7.18 (m, 2H), 6.91 – 6.84 (m, 2H), 3.79 (s, 3H), 2.03 (s, 1H).

2-fluoro-4-(4-(4-methoxyphenoxy)-1H-1,2,3-triazol-1-yl)phenol (5a). Following the synthesis of **6**. Purification by flash chromatography (hexanes/EtOAc). (10%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.06 (s, 1H), 7.62 (dd, *J* = 11.5, 2.6 Hz, 1H), 7.46 (ddd, *J* = 8.8, 2.7, 1.4 Hz, 1H), 7.14 – 7.03 (m, 3H), 6.97 – 6.91 (m, 2H), 3.79 (s, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 161.0, 157.9, 152.7 (d, *J* = 242.9 Hz), 151.9, 147.3 (d, *J* = 12.7 Hz), 130.7 (*J* = 8.3 Hz), 120.1, 119.3 (*J* = 3.7 Hz), 117.7 (*J* = 3.4 Hz), 115.9, 110.1, 110.0 (*J* = 23.5 Hz), 56.1. HRMS (ESI): calcd. for $[M+H]^+$($C_{15}H_{12}FN_3O_3$) 302.0935, found 302.0898.

4-(4,5-dihydrooxazol-2-yl)phenol (17). 1.2 g (8 mmol) of methyl 4-hydroxybenzoate were dissolved in 0.96 mL (16 mmol) of ethanolamine and heated at 180 °C for 1h. Solvent was evaporated and the residue resuspended in 20 mL DCM. The mixture was cooled to 0 °C followed by the addition of $SOCl_2$ (0.58 mL, 8 mmol). The reaction was stirred at rt for 16h. The solid reaction was filtered and washed with DCM. The solid was dissolved in 0.5 M $NaHCO_3$ and extracted with Et_2O. Combined organic layers were dried over anh Na_2SO_4 and concentrated under vacuum. The desired intermediate was used in the next step without further purification. (38%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.03 (s, 1H), 7.71 – 7.66 (m, 2H), 6.83 – 6.78 (m, 2H), 4.33 (t, *J* = 9.4 Hz, 2H), 3.88 (t, *J* = 9.4 Hz, 2H).

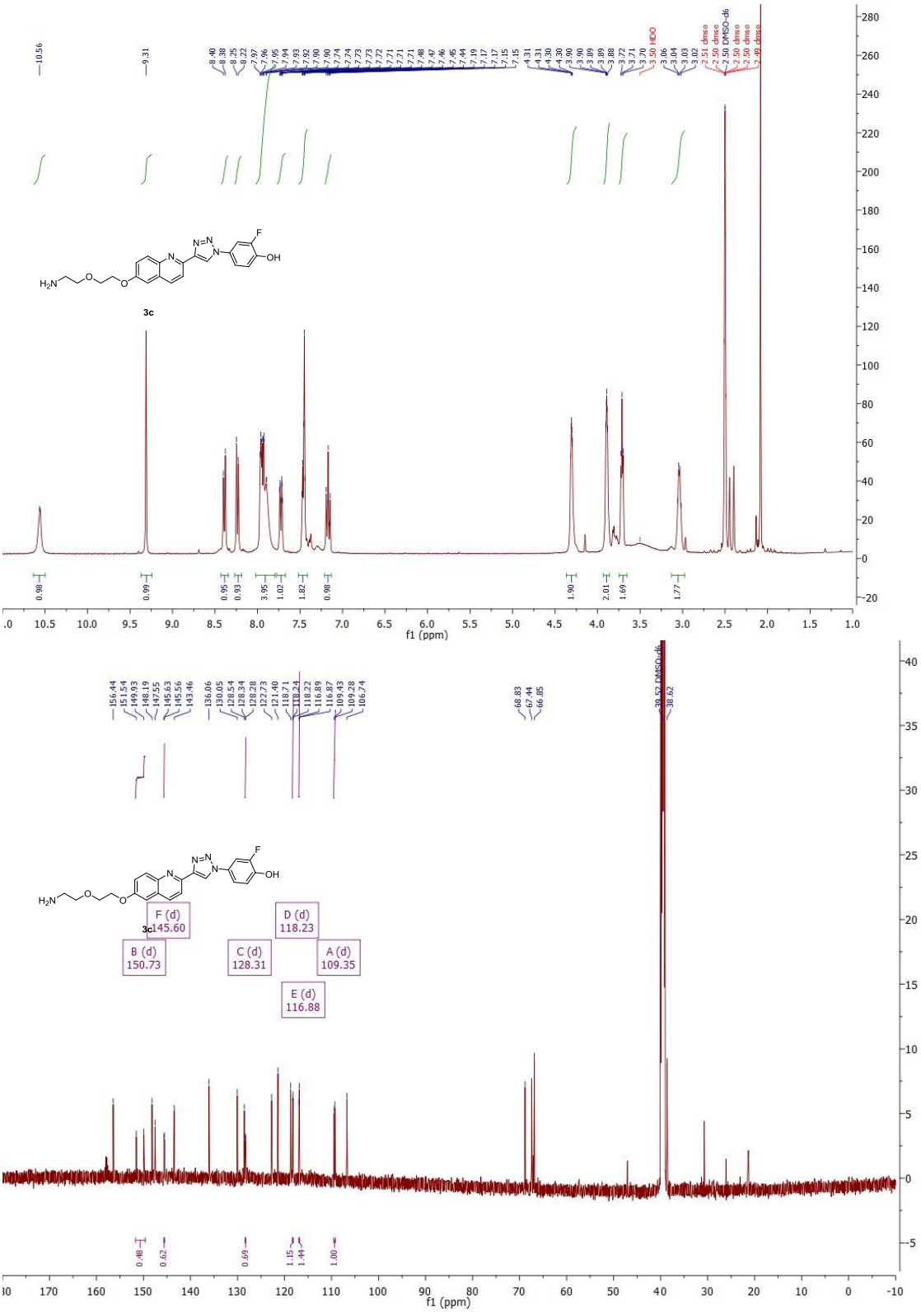
(*E*)-2-(4-((1,2-dichlorovinyl)oxy)phenyl)-4,5-dihydrooxazole (18). According to the synthesis of **15**. (60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 – 7.94 (m, 2H), 7.12 – 7.05 (m, 2H), 6.01 (s, 1H), 4.43 (t, *J* = 9.5 Hz, 2H), 4.05 (t, *J* = 9.5 Hz, 2H).

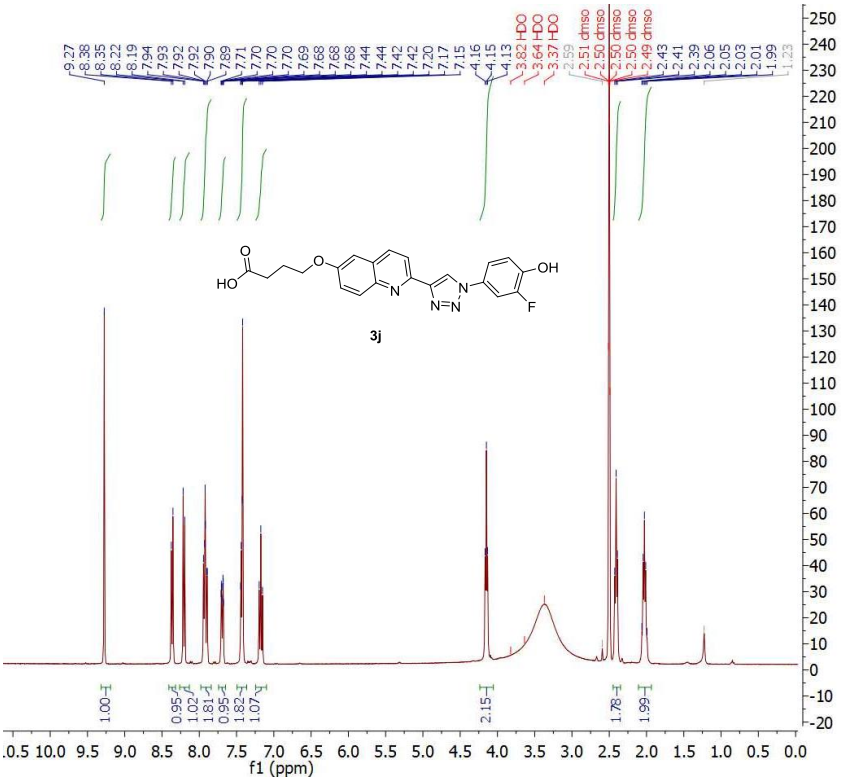
2-(4-(ethynyloxy)phenyl)-4,5-dihydrooxazole (19). Following the synthesis of **16**. Purification by flash chromatography (hexanes/EtOAc). (10%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 – 7.93 (m, 2H), 7.35 – 7.30 (m, 2H), 4.44 (t, *J* = 9.5, 2H), 4.06 (t, *J* = 9.5, 2H), 2.16 (s, 1H).

4-(4-(4-(4,5-dihydrooxazol-2-yl)phenoxy)-1H-1,2,3-triazol-1-yl)-2-fluorophenol (20). According to synthesis of **6**. Purification through flash chromatography (DCM/EtOAc). (16%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (d, *J* = 8.3 Hz, 2H), 7.62 (s, 1H), 7.52 (dd, *J* = 10.8, 2.5 Hz, 1H), 7.35 – 7.29 (m, 1H), 7.11 – 7.08 (m, 1H), 6.81 (d, *J* = 8.3 Hz, 2H), 4.47 (t, *J* = 9.5 Hz, 2H), 4.06 (t, *J* = 9.5 Hz, 2H).

4-((1-(3-fluoro-4-hydroxyphenyl)-1H-1,2,3-triazol-4-yl)oxy)benzoic acid (5b). 20 mg of **20** (0.06 mmol) were dissolved in 1.5 mL of 4N HCl and the mixture heated at reflux for 14h. After cooling to 4 °C, the solution was extracted with EtOAc (x2), the organic phases combined and dried over Na_2SO_4. (37%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.78 (bs, 1H), 10.46 (s, 1H), 8.74 (s, 1H), 7.97 (d, *J* = 8.3 Hz, 2H), 7.83 – 7.75 (m, 2H), 7.23 (d, *J* = 8.3 Hz, 2H), 7.15 (t, *J* = 8.8 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 166.6, 160.1, 155.9, 150.8 (d, *J* = 243.0 Hz), 145.6 (d, *J* = 11.7 Hz), 131.6, 128.6 (d, *J* = 9.0 Hz), 126.0, 118.2 (d, *J* = 3.5 Hz), 116.6 (d, *J* = 3.3 Hz), 116.4, 111.8, 109.1 (d, *J* = 23.2 Hz). HRMS (ESI): calcd. for $[M+H]^+$($C_{15}H_{10}FN_3O_4$) 316.0728, found 316.0878.

5. ^{1}H and ^{13}C spectra

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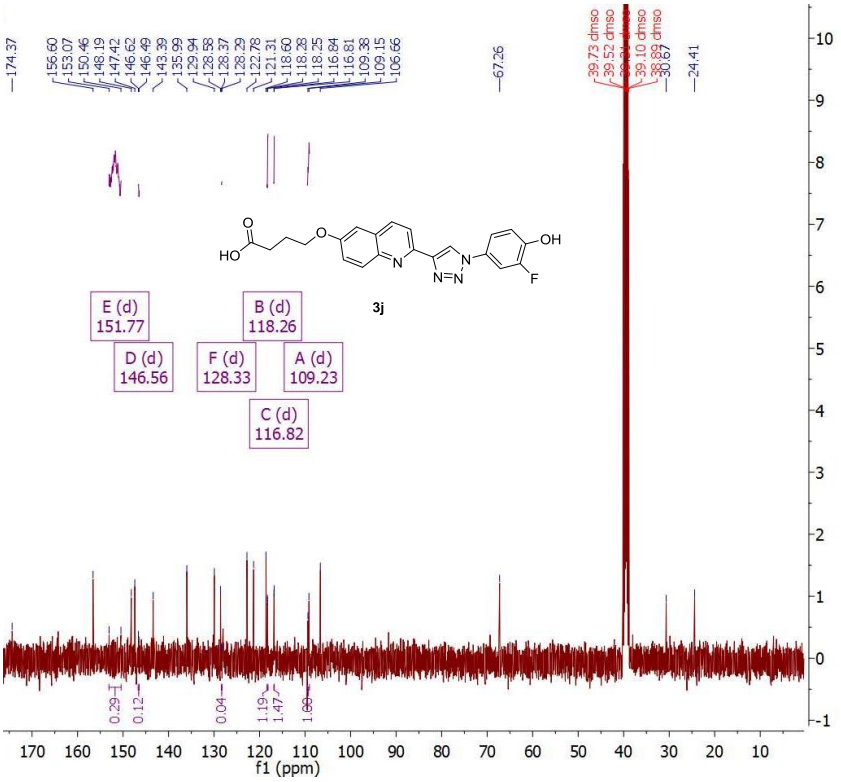
f1 (ppm)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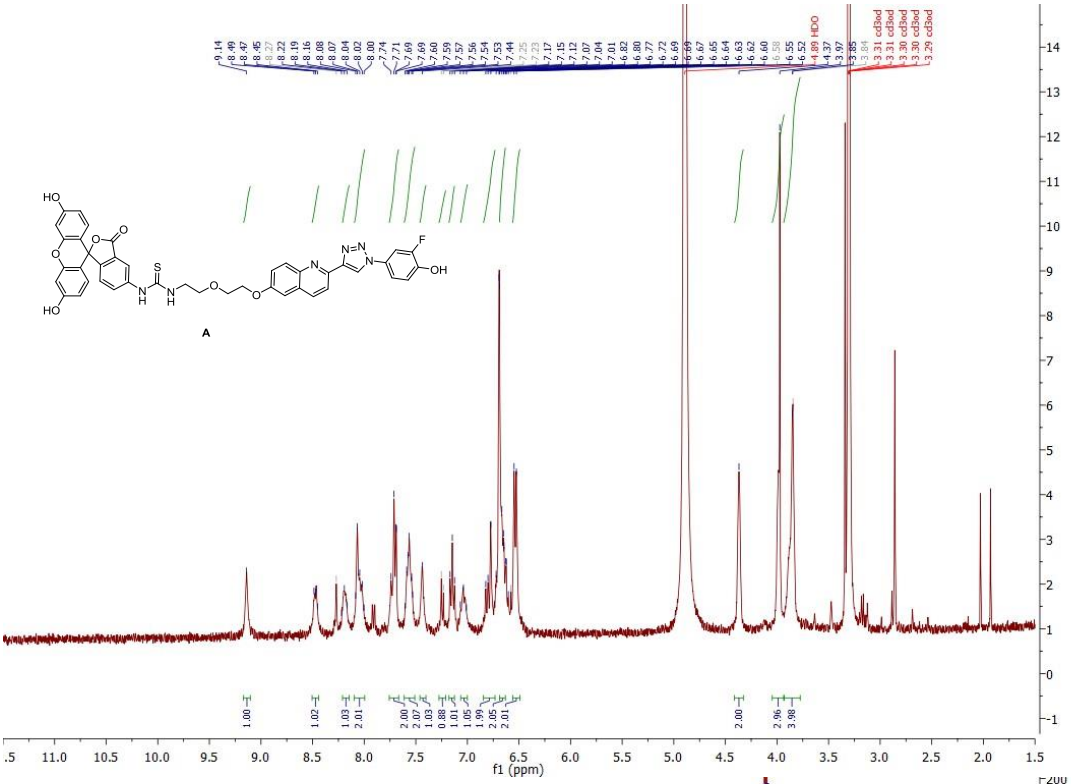
f1 (ppm)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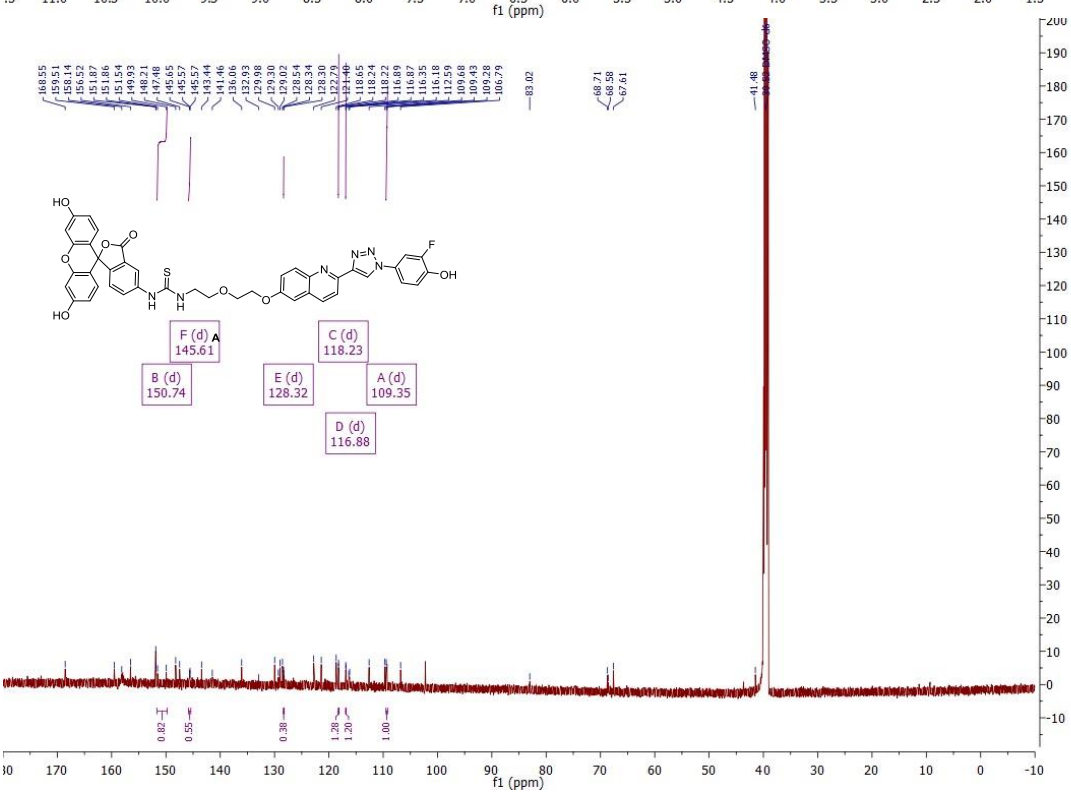
O
O
S
N
H
N
H
O
O
N
N
N
F
OH
A
f1 (ppm)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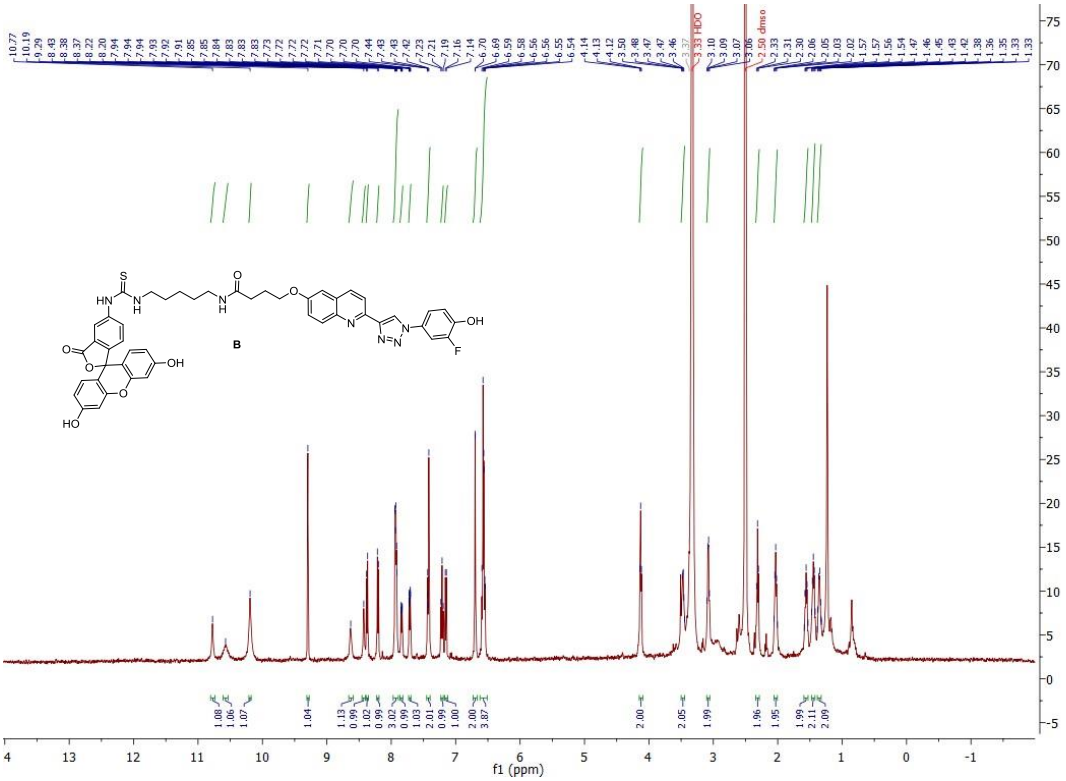
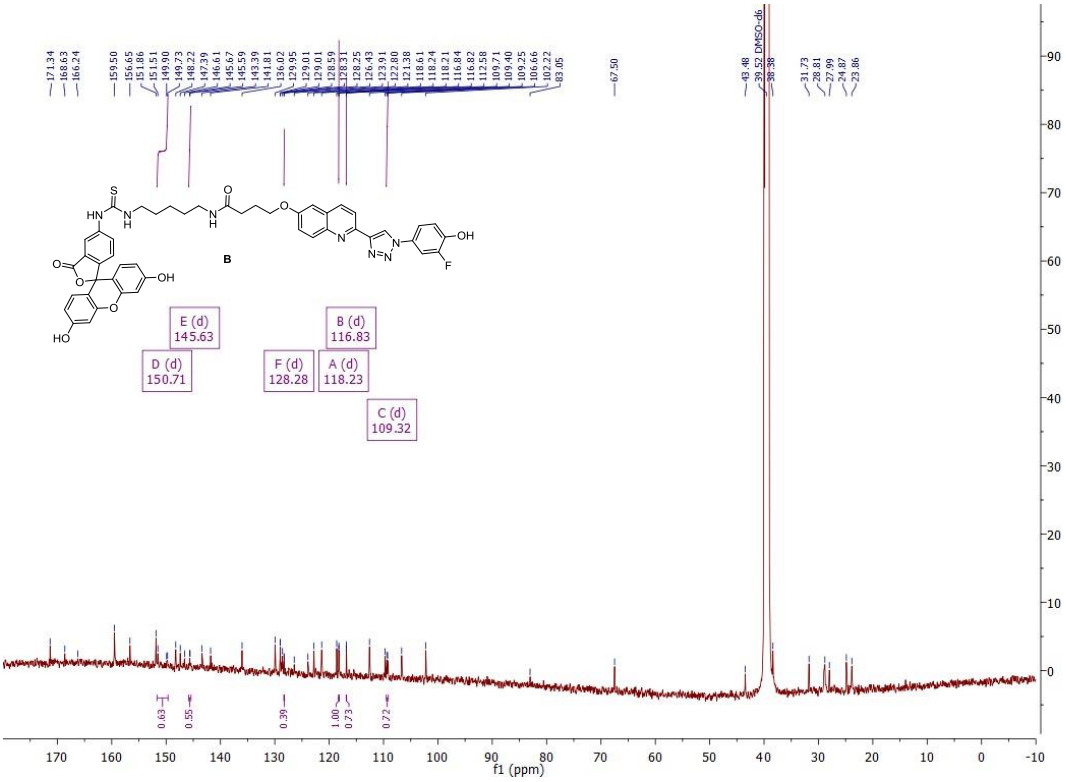
HO
O
O
O
S
N
H
N
H
O
O
N
N
N
F
OH
F (d) A
145.61
B (d)
150.74
E (d)
128.32
C (d)
118.23
A (d)
109.35
D (d)
116.88
f1 (ppm)


B
f1 (ppm)


B
E (d)
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D (d)
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B (d)
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F (d)
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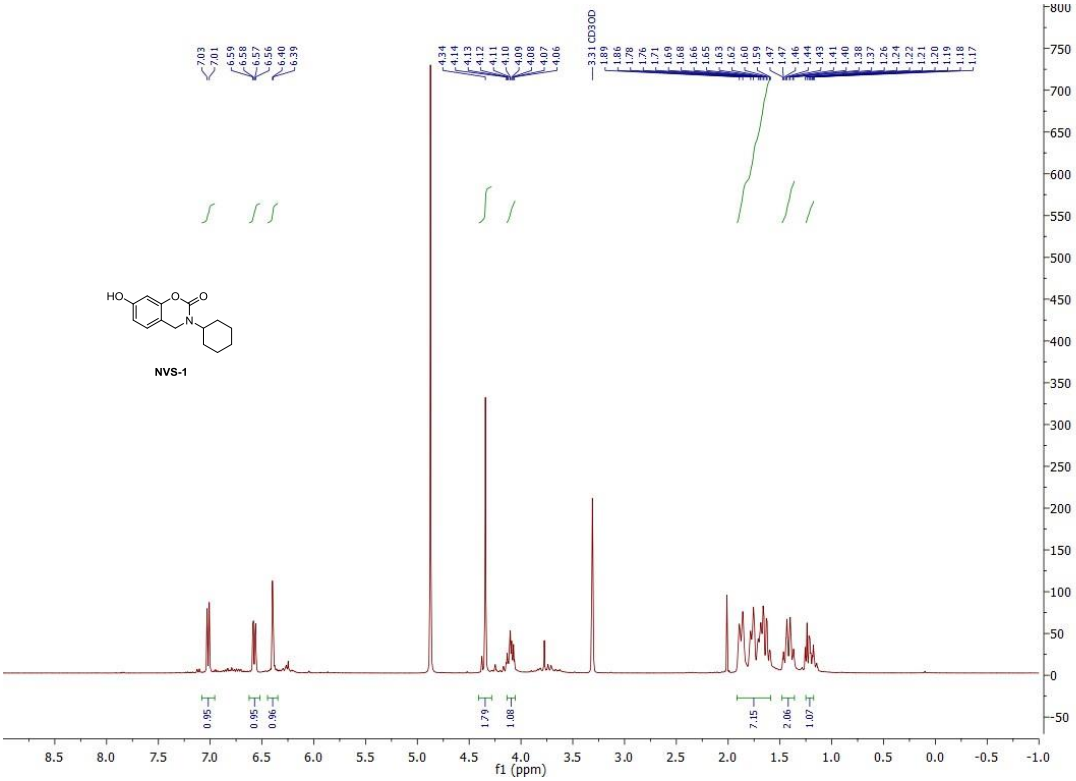
A (d)
118.23
C (d)
109.32
f1 (ppm)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6.56
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6.39
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4.14
4.13
4.12
4.11
4.10
4.09
4.08
4.07
4.06
3.31 CD3OD
1.89
1.86
1.78
1.76
1.71
1.69
1.68
1.66
1.65
1.63
1.62
1.60
1.59
1.47
1.47
1.46
1.44
1.43
1.41
1.40
1.38
1.37
1.26
1.24
1.22
1.21
1.20
1.19
1.18
1.17
HO
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O
N
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0.95
0.96
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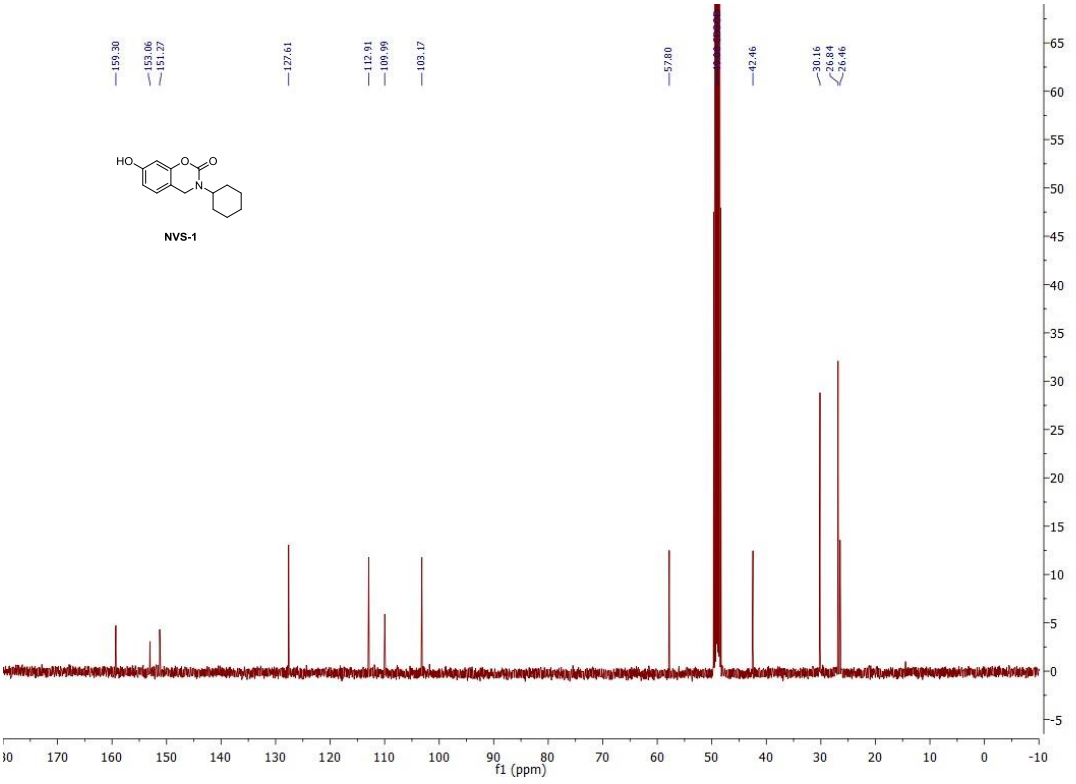
1.08
7.15
2.06
1.07
f1 (ppm)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30.16
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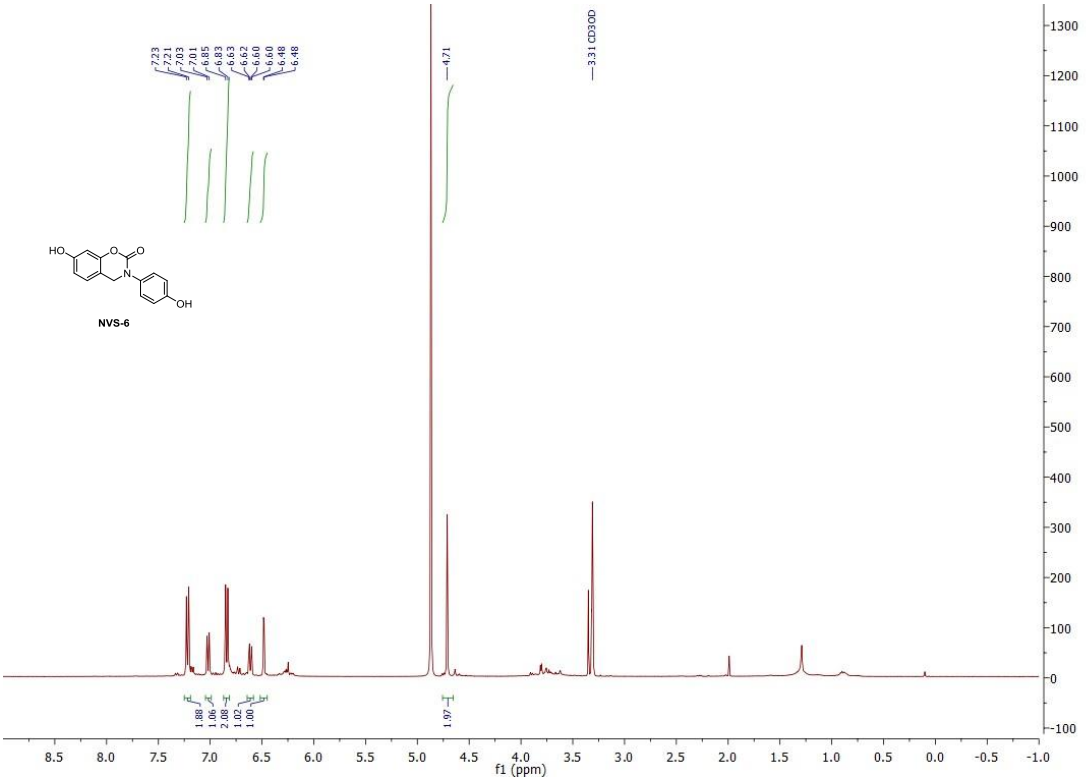
26.46
HO
O
O
N
NVS-1
f1 (ppm)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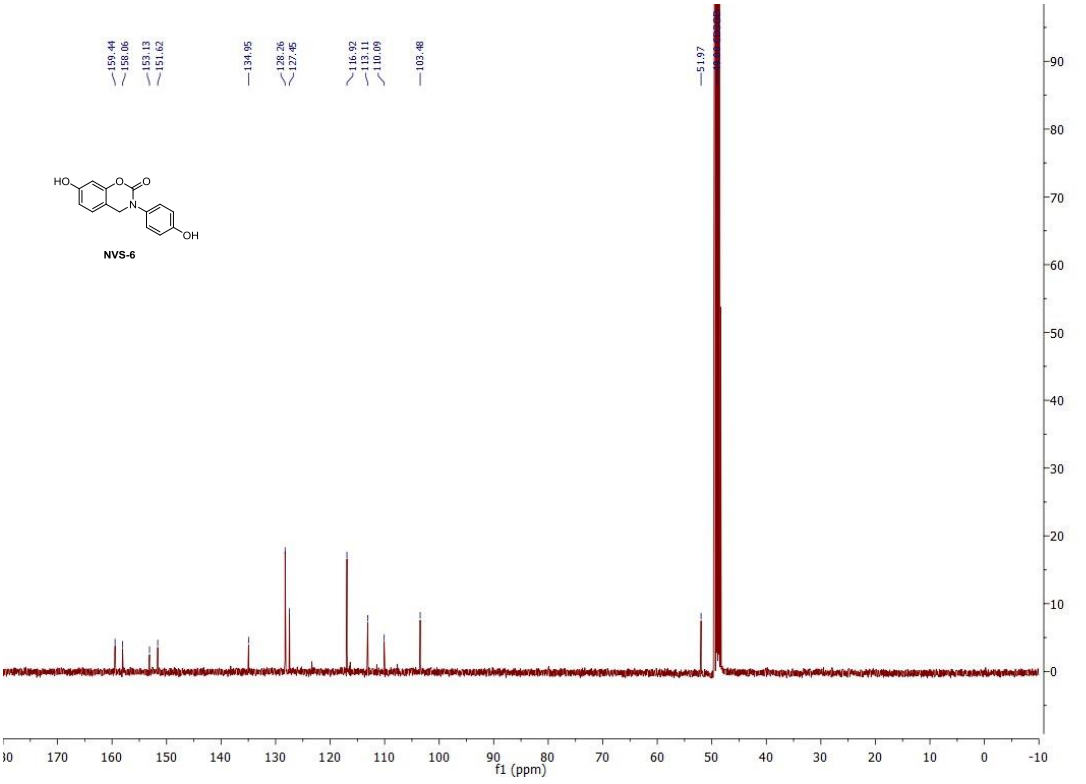
6.63
6.62
6.60
6.60
6.48
6.48
4.71
3.31 CD3OD
HO
O
O
N
OH
NVS-6
1.88
1.06
2.08
1.02
1.00
1.97
f1 (ppm)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110.09
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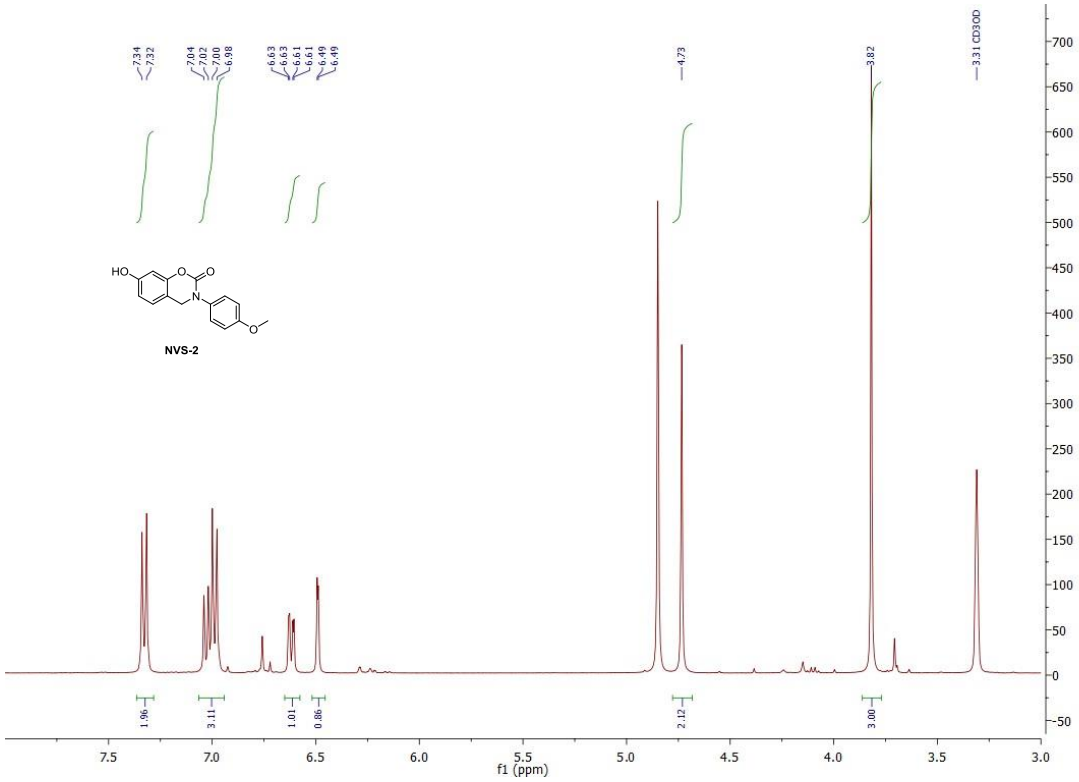
O
O
N
OH
NVS-6
f1 (ppm)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6.63
6.61
6.61
6.49
6.49
4.73
3.82
3.31 CD3OD
HO
O
O
N
O
NVS-2
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1.01
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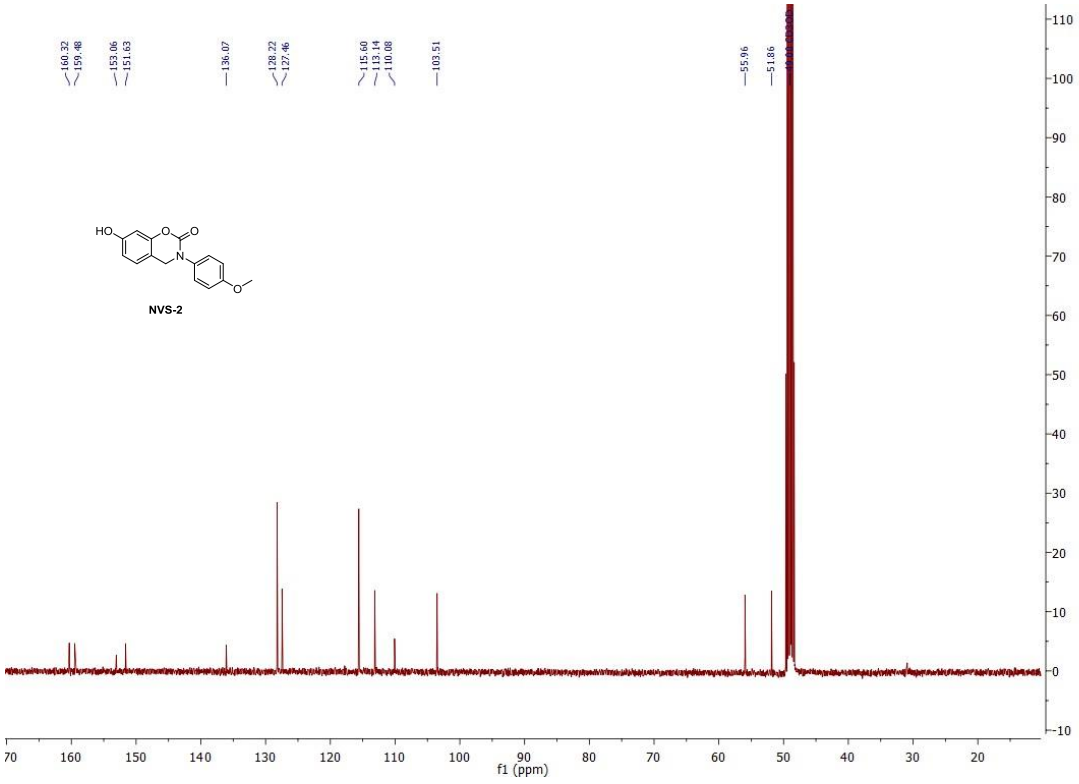
2.12
3.00
f1 (ppm)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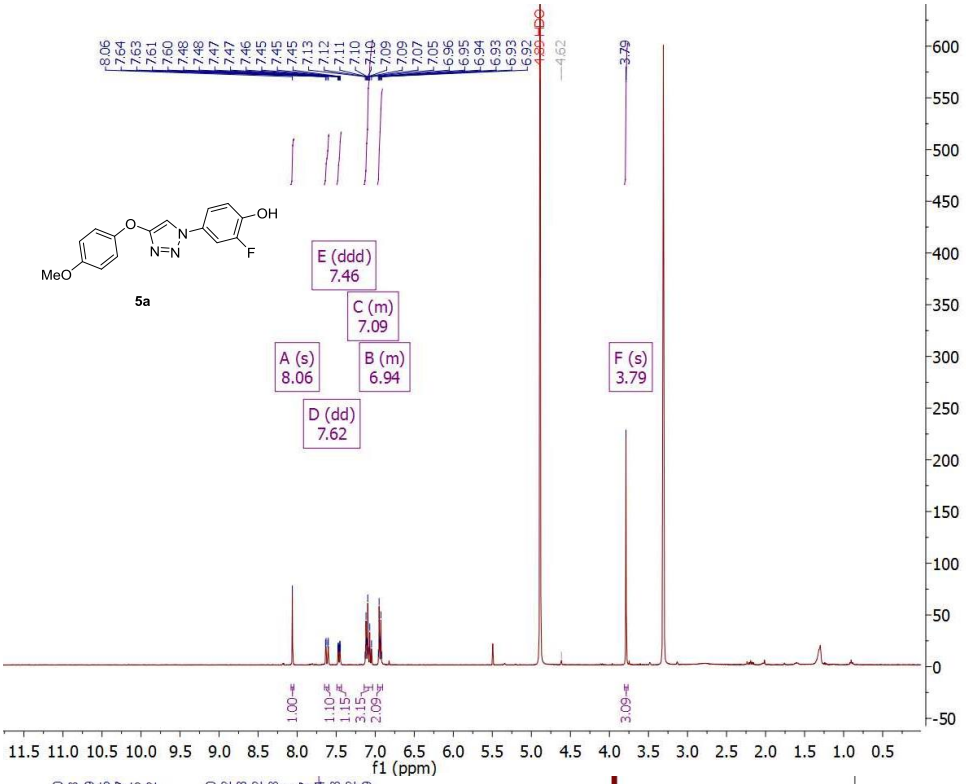
HO
O
O
N
O
NVS-2
f1 (ppm)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C (m)
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D (dd)
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F (s)
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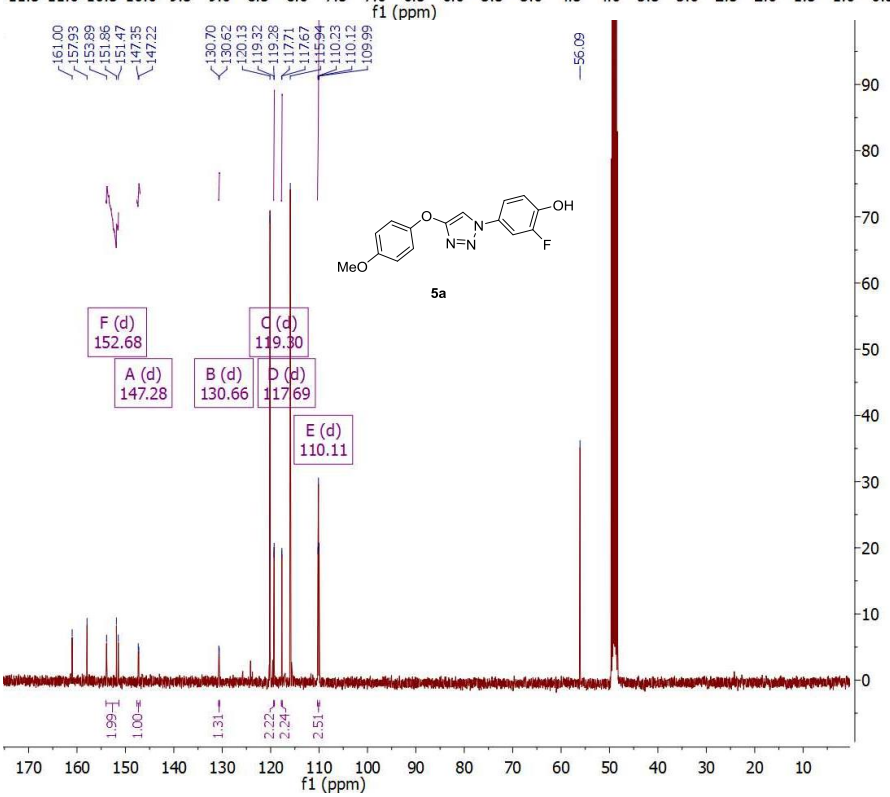
5a
f1 (ppm)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D (d)
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C (d)
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E (d)
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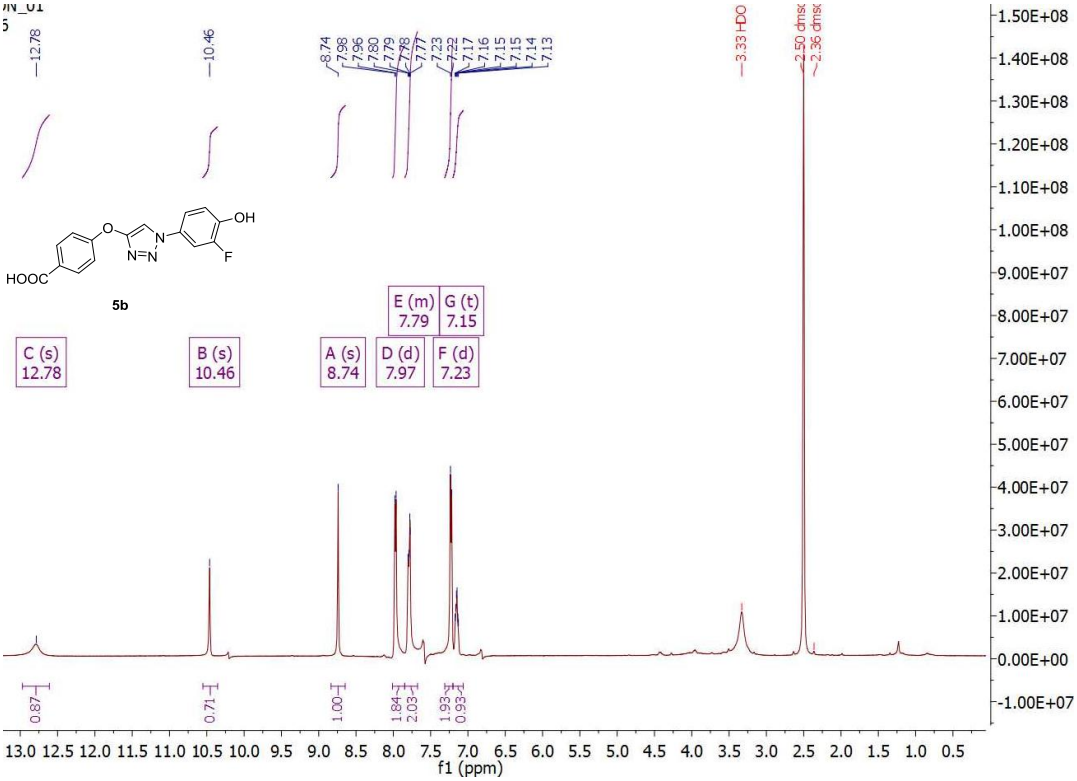
5a
f1 (ppm)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7.78
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7.23
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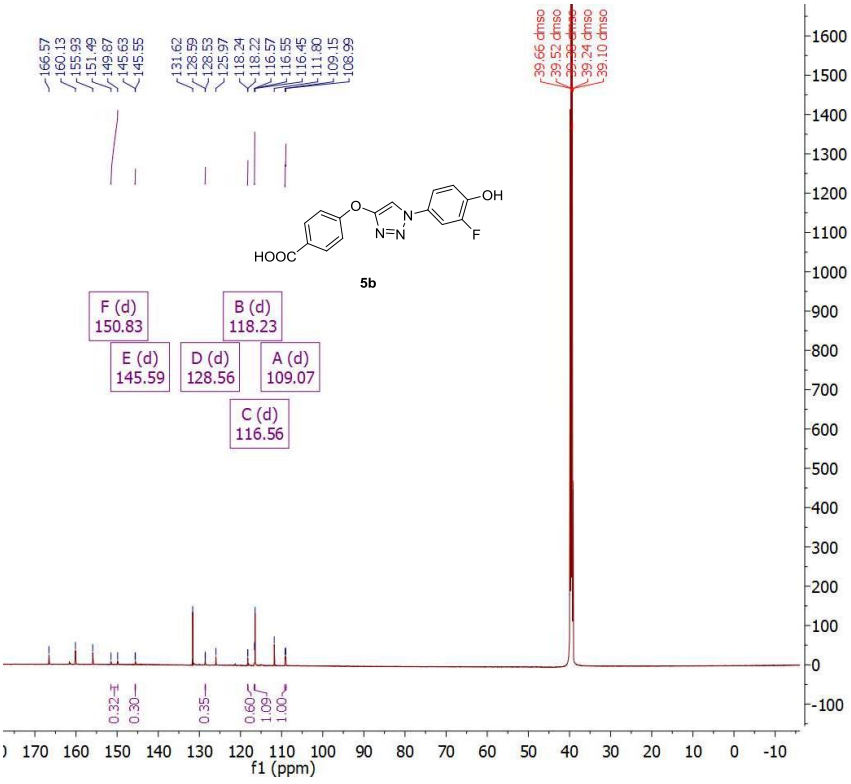
7.17
7.16
7.15
7.15
7.14
7.13
3.33 HDO
2.50 dmso
2.36 dmso
HOOC
OH
F
5b
C (s) 12.78
B (s) 10.46
A (s) 8.74
D (d) 7.97
E (m) 7.79
F (d) 7.23
G (t) 7.15
0.87
0.71
1.00
1.84
2.03
1.93
0.93
f1 (ppm)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128.53
125.97
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116.57
116.55
116.45
111.80
109.15
108.99
39.66 dmso
39.52 dmso
39.38 dmso
39.24 dmso
39.10 dmso
HOOC
OH
F
5b
F (d) 150.83
B (d) 118.23
E (d) 145.59
D (d) 128.56
A (d) 109.07
C (d) 116.56
0.32
0.30
0.35
0.60
1.09
1.00
f1 (ppm)

6. HPP and FP assays

Tautomerase assay. Inhibition of the tautomerase activity of MIF was measured using 4-hydroxyphenyl pyruvic acid (HPP) as substrate, largely following previously reported protocols.[1] HPP was dissolved in 0.5 M acetate buffer, pH 6.0 to a final concentration of 10 mM and incubated overnight at rt to allow equilibration of the keto and enol forms. MIF (6 µL) was premixed in 500 mM boric acid, pH 6.2 (142 µL) and transferred to a transparent U bottom 96-well plate (Falcon) to a final concentration of 50 nM MIF. For K_i determination, compounds were placed into wells (2 µL) at 6 different concentrations and incubated for 20 min until the assay was started by addition of HPP (50 µL) at two concentrations (1.0 and 2.5 mM). The negative control was MIF incubated with DMSO vehicle. MIF activity was monitored at 305 nm for formation of the borate-enol complex using an Infinite F500 plate reader (TECAN, Morrisville, NC) for 175 seconds. Calculation of initial velocities and the nonlinear regression analyses for the enzyme kinetics were repeated three times with the program Prism 6 (GraphPad, La Jolla, CA). Samples of Orita-13 and (*R*)-ISO-1 were purchased both from Alfa Aesar and Santa Cruz Biotechnology.

Determination of the affinities for A and B by FP assay. Determination of K_d values for the tracers followed previously reported protocols for other proteins.[3] Experiments are carried out by quadruplicates in three independent experiments. In a flat black bottom 96 well plate (Corning), add to the third column 300 µL of 1.8 µM MIF in FP buffer (20 mM HEPES, 150 mM NaCl, 0.01% Tween-20, pH 7.4). From this column, using a multichannel pipette make serial dilutions (1:2) into the following wells to a total volume of 150 µL. First column contains 200 µL buffer and is used as a blank, while second column contains 150 µL buffer. 2 µL of DMSO are added to

each well to keep 1% of DMSO, followed by the addition of 16.7 nM ligand **A** or **B** (48 µL) except first column (blank). Fluorescence polarization is measured at λ_{exc} = 485 ± 20 nm, λ_{em} = 535 ± 25 nm using an Infinite F500 plate reader until no FP variation was observed (typically 1 h). From the lowest and highest FP values (tracer free and tracer fully bound to MIF) we calculated the fraction of ligand bound to the protein to ligand total (L_b/L_t) for each concentration of MIF (Figure 1). We plotted these data to provide a typical saturation binding curve and using Prism 6 fit the results to the Hill equation.

In order to determine the existence of non-specific binding, if any, the same experiment with the same conditions is carried out by adding 2 µL of 1 mM NVS-2. No increase in FP values is detected in the presence of the inhibitor, therefore we assume the lack of non-specific binding.

Competitive FP assay. In order to calculate the K_d values of the unlabeled compounds, competitive assays were carried out in quadruplicates in three independent experiments. In a flat black bottom 96 well plate (Corning) 140 µL of FP buffer are added to columns 3-12. First column contains 200 µL buffer (blank), while second column contains 150 µL FP buffer. 10 µL of 1.1 µM MIF are added to columns 3-12 followed by the addition of 2 µL of inhibitor in DMSO at 9 different concentrations. 2 µL of DMSO are added to columns 1-3. After 20 min of incubation at rt 48 µL of 16.7 nM ligand **B** are added to columns 2-12 and fluorescence polarization was measured at λ_{exc} = 485 ± 20 nm, λ_{em} = 535 ± 25 nm for 1 h. Data are analyzed by a least-squares non-linear fit, generated using Prism 6 in order to determine the compound's IC_{50}. K_d values for each inhibitor are calculated using the following equation based on the IC_{50}, K_d of the tracer (K_d^t), total (L_t) and bound (L_b) tracer, as well as total MIF concentration (P_t).[3]

Samples of 4-IPP and Pontamine Sky Blue were purchased both from Tocris and Santa Cruz Biotechnology.

$$K_d^I = \frac{L_b IC_{50} K_d^t}{P_t L_t + L_b(P_t - L_t + L_b - K_d^t)}$$

7. Protein crystallography

Expression and Purification of MIF. Expression and purification of MIF followed previously reported procedures.[1]

NVS-2 and 3i Crystallography. In the case of NVS-2, apo MIF was crystallized by the hanging drop method with a reservoir solution containing 2.0 M Ammonium Sulfate, 3% isopropanol and 0.1 M Tris pH 7.0. For **3i**, a reservoir solution containing 2.0 M Ammonium Sulfate, 3% isopropanol, 0.1M Tris pH 8.0 was used to grow crystals of apo MIF. A 100 mM solution of NVS-2 or **3i** in DMSO was diluted with the corresponding reservoir solution to a 10 mM suspension. 0.25 µM of the suspension was added to the respective 2 µL drops containing apo MIF crystals. In both cases, after several weeks, the initial protein crystals cracked and dissolved, and new crystals formed (Figure SI-1). Crystals were cryoprotected with 25% glycerol, 2.4 M ammonium sulfate, 3% isopropanol, and 0.1 M Tris at pH 7.0. Data collection was performed on a Rigaku 007HF+ x-ray source with a Saturn 944+ CCD detector. Data processing, phasing, model building, and refinement were performed as described previously (Figures SI-2 and SI-3).[1] Crystals of NVS-2 were found to occupy the $P3_121$ space group, while those of **3i** were *I*222.

Table S1. Crystallography Statistics for NVS-2

Complex	NVS-2
PDB Code	5HVT
Data Collection	
X-Ray Source	Rigaku 007 HF+
Wavelength, Å	1.5418
Resolution Å (last shell)	50-1.75 (1.80-1.75)
Space group	$P3_121$
Unit cell, a,b,c in Å (α,β,γ, in °)	a=96.023 b=96.023 c=104.039 α=β=90,γ=120
No. of Reflections	396624
No. Unique Reflections	56042
Redundancy (last shell)	7.1 (4.0)
Avg. I/σ (last shell)	34.7 (2.2)
R-merge	0.09
Refinement	
Resolution (last shell)	50.00-1.75 (1.80-1.75)
Completeness, % (last shell)	99.72 (99.51)
Working Set (last shell)	50398 (3629)
Test Set (last shell)	5644 (426)
R_{cryst} (last shell)	0.177 (0.280)
R_{free} (last shell	0.203 (0.307)
RMS deviation bond lengths (Å)	0.011
RMS deviation bond angles (°)	1.407
Total Number of Atoms	3096
Protein	2692
Inhibitor	60
Solvent	314
Ions	30
Avg. B-factor	21.57
Protein	20.23
Inhibitor	23.48
Solvent	30.66
Ions	42.31
Ramachandran Favored, Allowed, Outliers (%) {MolProbity}	97.99, 2.01, 0

Table S2. Crystallography Statistics for 3i

Complex	3i
PDB Code	5HVS
Data Collection	
X-Ray Source	Rigaku 007 HF+
Wavelength, Å	1.5418
Resolution Å (last shell)	50-1.75 (1.80-1.75)
Space group	*I*222
Unit cell, a,b,c in Å (α,β,γ, in °)	a=68.531 b=69.744 c=133.793 α=β=γ=90
No. of Reflections	114815
No. Unique Reflections	32486
Redundancy (last shell)	3.5 (2.0)
Avg. I/σ (last shell)	35.5 (6.4)
R-merge	0.054
Refinement	
Resolution (last shell)	50.00-1.75 (1.79-1.75)
Completeness, % (last shell)	99.58 (96.43)
Working Set (last shell)	29385 (2077)
Test Set (last shell)	3281 (222)
R_{cryst} (last shell)	0.170 (0.210)
R_{free} (last shell	0.206 (0.257)
RMS deviation bond lengths (Å)	0.008
RMS deviation bond angles (°)	1.497
Total Number of Atoms	3035
Protein	2721
Inhibitor	99
Solvent	185
Ions	30
Avg. B-factor	19.18
Protein	18.43
Inhibitor	23.84
Solvent	24.89
Ions	35.90
Ramachandran Favored, Allowed, Outliers (%) {MolProbity}	98.31, 1.69, 0

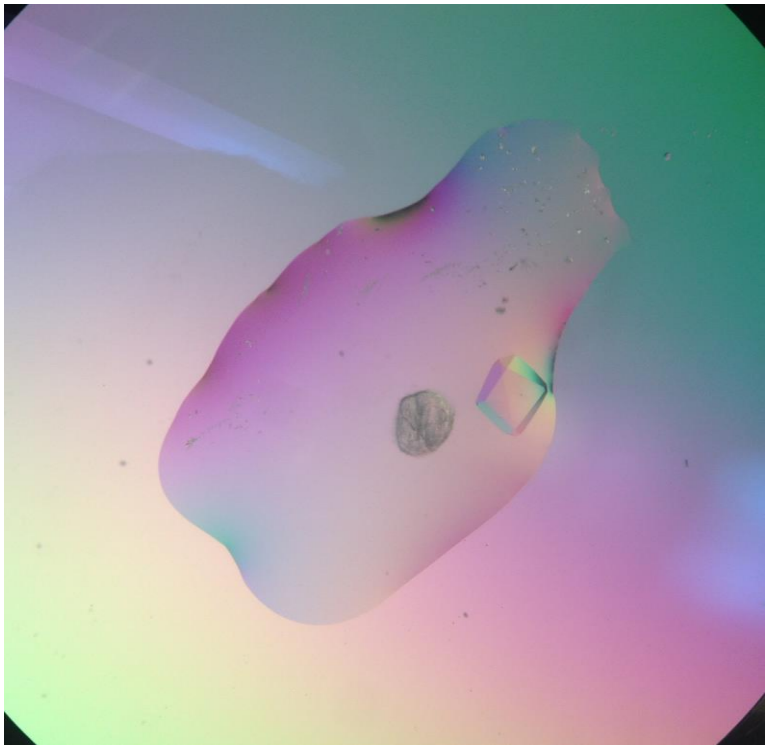

Figure S1. Photograph of an extensively cracked apo-MIF crystal adjacent to a newly formed co-crystal of NVS-2 with MIF.

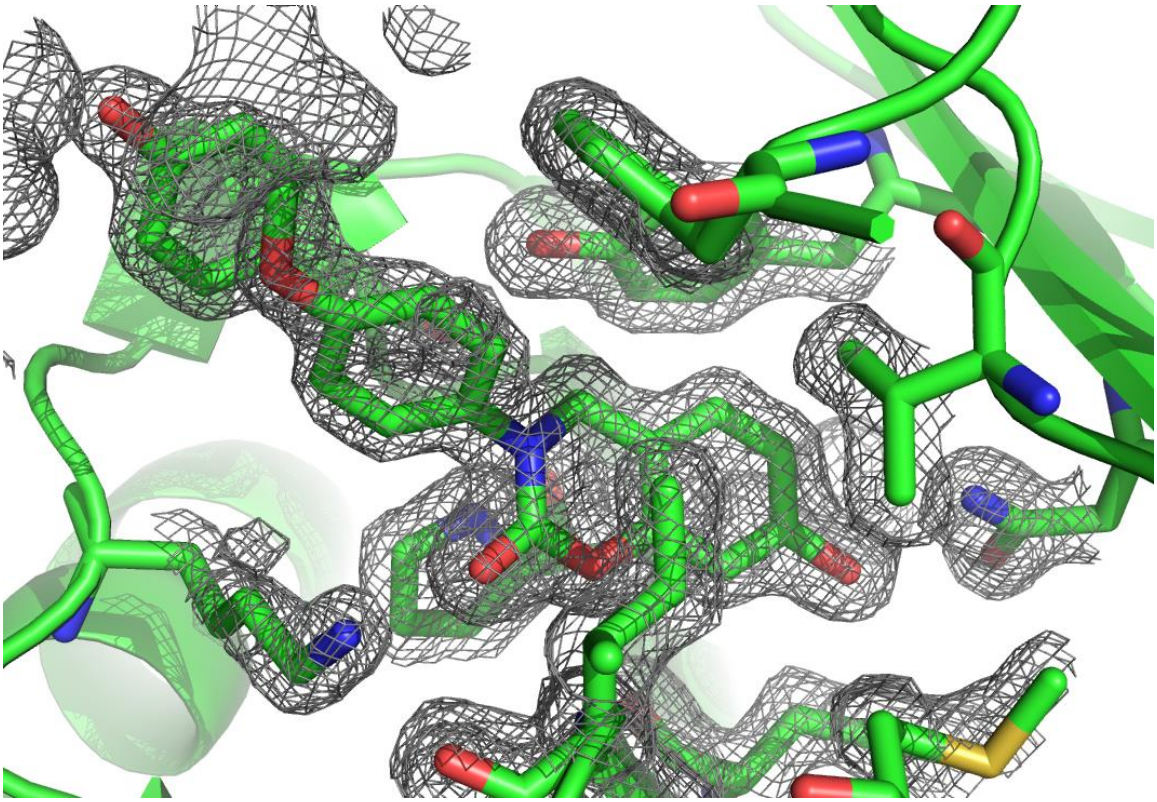

Figure S2. 2Fo-Fc map of NVS-2 within 5 Å of the ligand, contoured at σ=1.5. Some residues in front of the ligand have been removed for clarity.

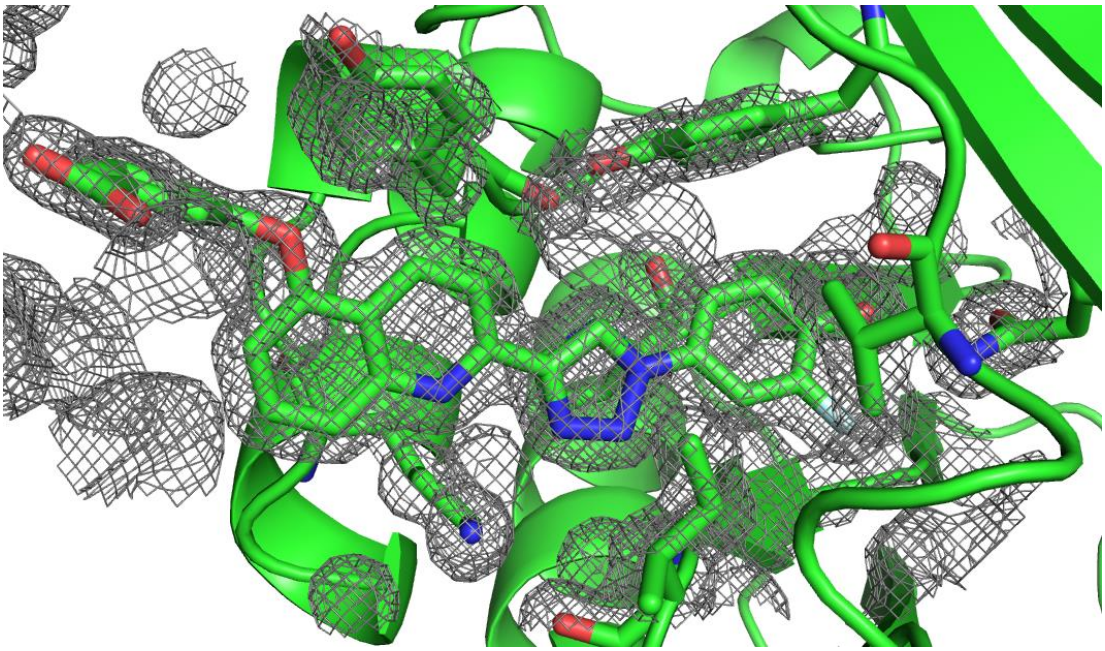

Figure S3. 2Fo-Fc map of **3i** within 4.5 Å of the ligand contoured at σ=1.0. Some residues in front of the ligand have been removed for clarity.

8. References

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