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Anticancer activity of new 5-(2-oxodihydrobenzimidazolyl)pyrrole-3-carbonitriles, pharmacophore analogues of opnurasib

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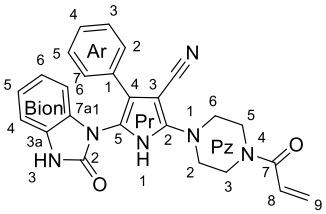
Experimental section

General Methods. The melting points were determined on a Boetius hot-stage apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Bruker Vector-22 spectrometer. The pH of the reaction mixtures was measured using universal indicator paper pH 0-12 (Lachema o.p. Brno, plant Neratovice) and using the HANNA instruments pH 211 Laboratory pH meter. (State Register of SI No. 20738-00). Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker spectrometer Avance III-500.1 (500.1 MHz for ^{1}H, 125.8 MHz for ^{13}C) in DMSO-d_6 at 303 K. The spectrometer was equipped with a pulsed gradient unit capable of producing magnetic field pulse gradients in the z-direction of 53.5 G cm^{-1}. All the spectra were obtained in a 5-mm gradient broadband probehead. Chemical shifts are reported on the δ (ppm) scale and referred to the solvent DMSO-d_6 (2.50 ppm for ^{1}H and 39.5 ppm for ^{13}C NMR). The ^{1}H and ^{13}C NMR spectra of synthesized compounds were completely assigned by using a combination of 2D NMR experiments, which included ^{1}H-^{1}H COSY, HSQC and HMBC sequences. The high-resolution mass spectra (HRMS) with electrospray ionization (ESI) were obtained on ESI-QTOF Impact II mass spectrometer (Bruker Daltonik GmbH, Germany). The relative error in determining the exact mass values was no more than 3 ppm.

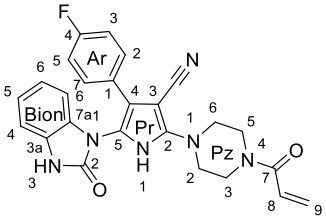
General Procedure for the Synthesis of 1:

Starting α-(piperazino)pyrroles **3a,b** were prepared as described [V. A. Mamedov, E. A. Khafizova, N. E. Algaeva, Sh. K. Latypov and O. G. Sinyashin, *J. Org. Chem.*, 2020, **85**, 9887; https://doi.org/10.1021/acs.joc.0c01180].

To a solution of **3** (1.14 mmol, 1.0 equiv.) in freshly distilled dichloromethane (15 ml) cooled to 0 °C was added Et_3N (3.46 mmol, 0.48 ml, 3.0 equiv.) with stirring, followed by the dropwise addition of acryloyl chloride (2.32 mmol, 0.19 ml, 2.0 equiv.). The reaction mixture was stirred for 4 h at the same temperature under a nitrogen atmosphere and then left overnight (12 h) at room temperature. After evaporation of the reaction mixture to dryness, a brown solid was obtained, which was purified by column chromatography.



2-(4-Acryloylpiperazin-1-yl)-5-(2-oxo-2,3-dihydro-1H-benzimidazol-1-yl)-4-phenyl-1H-pyrrole-3-carbonitrile **1a**: the compound was obtained as above from 5-(2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-1-yl)-4-phenyl-2-(piperazin-1-yl)-1*H*-pyrrole-3-carbonitrile **(3a)** (0.44 g, 1.14 mmol, 1.0 equiv) and acryloyl chloride (0.21 g, 2.32 mmol, 0.19 ml, 2.0 equiv) and triethylamine (0.35 g, 3.46 mmol, 0.48 ml, 3.0 equiv) in dichloromethane (15 ml). Yield 0.48 g, 96%; brown solid; mp 282−283 °C, R_f = 0.5 ($CHCl_3$/MeOH:9/1). IR (KBr, cm^{-1}): ν_{max} 3423, 3201, 2203, 1707, 1601, 1441, 1243, 754, 736, 701. NMR data for **1a**:^{1}H NMR (500.1 MHz, DMSO-d_6): δ 3.39 (m, 4H, H3/H5-Pz), 3.70 and 3.75 (m, 4H, H2/H6-Pz), 5.72 (dd, *J* = 10.4 Hz, *J* = 2.3 Hz, 1H, H9-Pz (cis to H8-Pz)), 6.16 (dd, *J* = 16.6 Hz, *J* = 2.3 Hz, 1H, H9-Pz (trans to H8-Pz)), 6.65 (d, *J* = 7.9 Hz, 1H, H7-Bion), 6.83 (dd, *J* = 16.6 Hz, *J* = 10.4 Hz, 1H, H6-Pz), 6.92 (m, 1H, H6-Bion), 7.00 (m, 1H, H5-Bion), 7.01 (m, 1H, H4-Bion), 7.21 (m, 1H, H4-Ar), 7.29 (m, 1H, H3/H5-Ar), 7.29 (m, 1H, H2/H6-Ar), 11.09 (s, 1H, H3(NH)-Bion), 11.725 (s, 1H, H1(NH)-Pr). ^{13}C{^{1}H} NMR (125.8 MHz, DMSO-d_6): δ 164.4 (C7-Pz), 154.2 (C2-Bion), 147.1 (C2-Pr), 131.4 (C7a-Bion), 131.3 (C1-Ar), 128.5 (C3/C5-Ar), 128.3 (C3a-Bion), 128.0 (C8-Pz), 127.7 (C9-Pz), 127.3 (C2/C6-Ar), 127.1 (C4-Ar), 122.0 (C5-Bion), 121.5 (C4-Pr), 121.1 (C6-Bion), 117.8 (C5-Pr), 111.9 (C3a-Pr), 109.1 (C4-Bion), 108.3 (C7-Bion), 73.7 (C3-Pr), 48.7 and 48.1 (C3/C5-Pz), 44.4 and 40.4 (C2/C6-Pz). HRMS (ESI), *m/z*: $[M+H]^+$calcd for $C_{25}H_{22}N_6O_2$ 439.1877, found 439.1876.



2-(4-Acryloylpiperazin-1-yl)-4-(4-fluorophenyl)-5-(2-oxo-2,3-dihydro-1H-benzimidazol-1-yl)-1H-pyrrole-3-carbonitrile **1b**: the compound was obtained as above from 4-(4-fluorophenyl)-5-(2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-1-yl)-2-(piperazin-1-yl)-1*H*-pyrrole-3-carbonitrile **(3b)** (0.46 g, 1.14 mmol, 1.0 equiv) and acryloyl chloride (2.32 mmol, 0.19 ml, 2.0 equiv) and triethylamine (3.46 mmol, 0.48 ml, 3.0 equiv) in dichloromethane (15 ml) following the general procedure. Yield 0.47 g, 90%; brown solid; mp 294−295 °C, R_f = 0.49 ($CHCl_3$/MeOH:9/1). IR (KBr, cm^{-1}): ν_{max} 3432, 2924, 2200, 1705, 1640, 1608, 1443, 1242, 842, 753, 735, 702. NMR data for **6b**:^{1}H NMR (500.1 MHz, DMSO-d_6): δ 3.40 (m, 4H, H3/H5-Pz), 3.70 and 3.75 (m, 4H, H2/H6-Pz), 5.72 (dd, *J* = 10.5 Hz, *J* = 2.3 Hz, 1H, H9-Pz (cis to H8-Pz)), 6.16 (dd, *J* = 16.7 Hz, *J* = 2.3 Hz, 1H, H9-Pz (trans to H8-Pz)), 6.65 (d, *J* = 7.8 Hz, 1H, H7-Bion), 6.86 (dd, *J* = 16.7 Hz, *J* = 10.5 Hz, 1H, H6-Pz), 6.93 (m, 1H, H6-Bion), 7.02 (m, 1H, H5-Bion), 7.02 (m, 1H, H4-Bion), 7.15 (dd, *J* = 8.8 Hz, *J(HF)* = 9.1 Hz, 2H, H3/H5-Ar), 7.32 (dd, *J* = 8.8 Hz, *J(HF)* = 5.5 Hz, 2H, H2/H6-Ar), 11.11 (s, 1H, H3(NH)-Bion), 11.74 (s, 1H, H1(NH)-Pr). ^{13}C{^{1}H} NMR (125.8 MHz, DMSO-d_6): δ 164.4 (C7-Pz), 161.2 (d, *J(CF)* = 243.2 Hz, C4-Ar), 154.1 (C2-Bion), 147.1 (C2-Pr), 131.2 (C7a-Bion), 129.4 (d, *J(CF)* = 8.5 Hz, C2/C6-Ar), 128.3 (C3a-Bion), 128.0 (C8-Pz), 127.8 (d, *J(CF)* = 2.2 Hz, C1-Ar), 127.7 (C9-Pz), 122.1 (C5-Bion), 121.1 (C6-Bion), 120.5 (C4-Pr), 117.7 (C5-Pr), 115.5 (d, *J(CF)* = 21.6 Hz, C3/C5-Ar), 111.9 (C3a-Pr), 109.2 (C4-Bion), 108.3 (C7-Bion), 73.6 (C3-Pr), 48.7 and 48.1 (C3/C5-Pz), 44.4 and 40.8 (C2/C6-Pz). HRMS (ESI), *m/z*: $[M+H]^+$calcd for $C_{25}H_{21}FN_6O_2$ 457.1783, found 457.1780.

Cell Culture

The human cancer cell lines NCI-H358 and AsPC-1 were obtained from the American Type Culture Collection (ATCC). Cells were cultured in RPMI-1640 medium (Gibco, UK) supplemented with 10% fetal bovine serum (FBS, Gibco, UK), penicillin (100 U ml^{-1}), streptomycin (100 μg ml^{-1}), and GlutaMAX (2.0 mM, Gibco, UK). All cell lines were maintained at 37 °C in a humidified atmosphere containing 5% CO_2 and 95% air. Subconfluent monolayers in the logarithmic growth phase were harvested by brief treatment with TrypLE Express solution (Gibco, UK) in phosphate-buffered saline (PBS, Capricorn Scientific, Germany), followed by three washes with serum-free PBS. Cell viability was determined using the trypan blue exclusion assay.

Antiproliferative assay

The effects of the synthesized compounds on cell viability were evaluated using the MTT colorimetric assay. Cells were diluted in growth medium to a density of 10.0×10^4 cells ml^{-1}, and aliquots (2×10^3 cells in 200 μl) were seeded into individual wells of 96-well plates (Eppendorf, Germany) and incubated for 24 h. Subsequently, the cells were treated with the synthesized compounds and incubated for 72 h at 37 °C in a 5% CO_2 atmosphere. After incubation, 40 μL of MTT solution (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, 5 mg ml^{-1} in PBS) was added to each well, and the plates were incubated for 4 h. The medium was then removed, and 150 μL of DMSO was added to dissolve the formazan crystals. The plates were shaken for 10 min, and the absorbance was measured at 560 nm using a GloMax® Multi+ microplate reader (Promega, USA). Each compound was tested for cytotoxicity in three independent experiments.

Molecular Modeling

Molecular modeling was performed using the Schrodinger Maestro software package. The CovDock covalent docking algorithm was used to connect the test compounds and control ligands. Crystallographic data for the KRAS G12C protein in complex with 6-chloro-7-(2-fluoro-6-hydroxyphenyl)-4-(4-propanoylpiperazin-1-yl)-1-[2-(propan-2-yl)phenyl]quinazolin-2(1*H*)-one (PDB ID: 6PGP) were used. A receptor network for the test compounds was constructed based on the centroid of this control ligand. Cys12 was selected as the reactive residue for the Michael addition reaction with the specified SMARTS formula of the reactive group [6](=[8])-[6]=[6]. No constraints were imposed. Virtual screening was chosen as the mode for covalent docking.

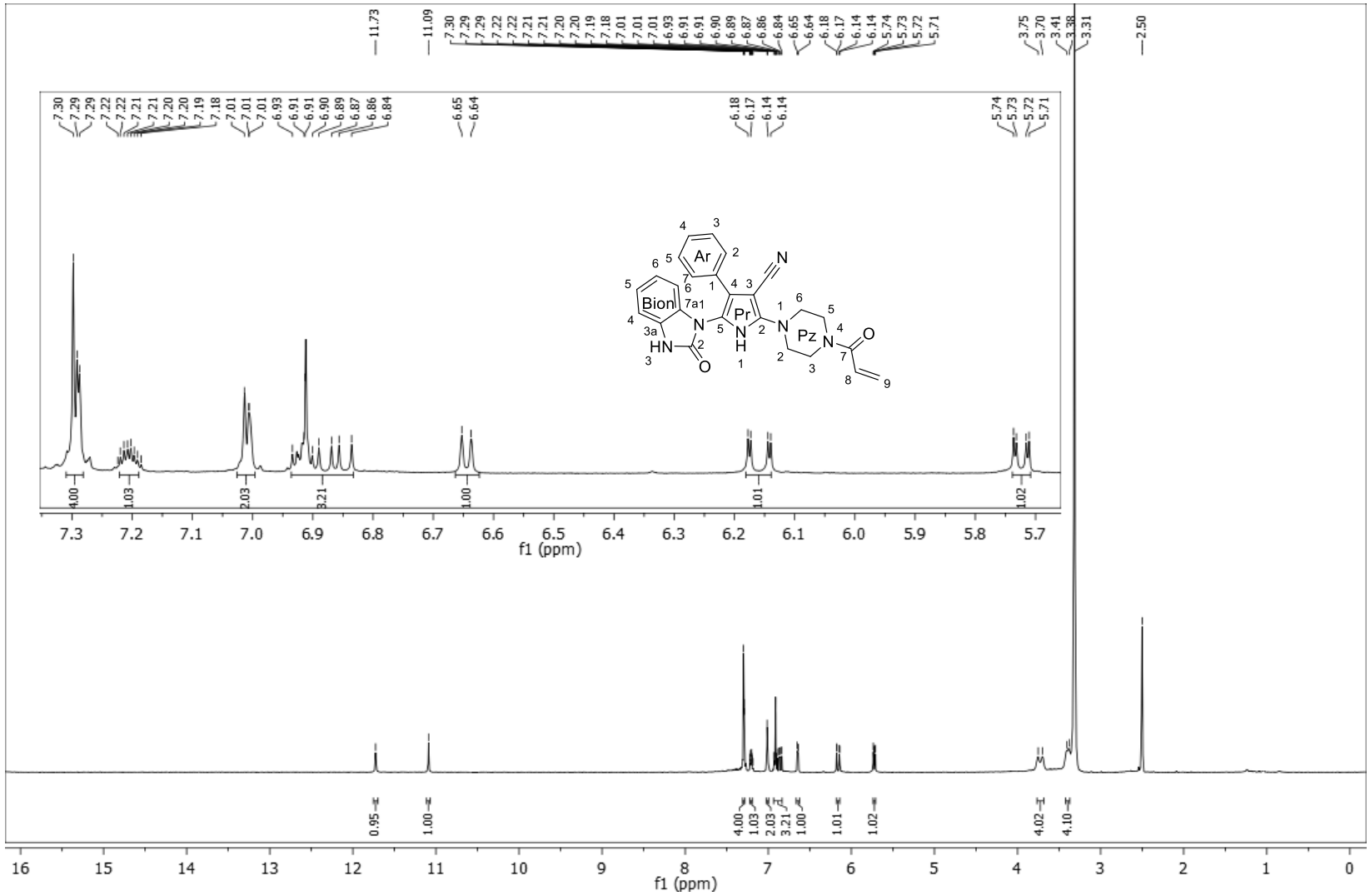


Figure S1. 1D ^{1}H NMR spectrum of **1a** in DMSO-d_6 at T = 303 K. Chemical shifts are given in ppm (Bruker spectrometer at 500.1 MHz).

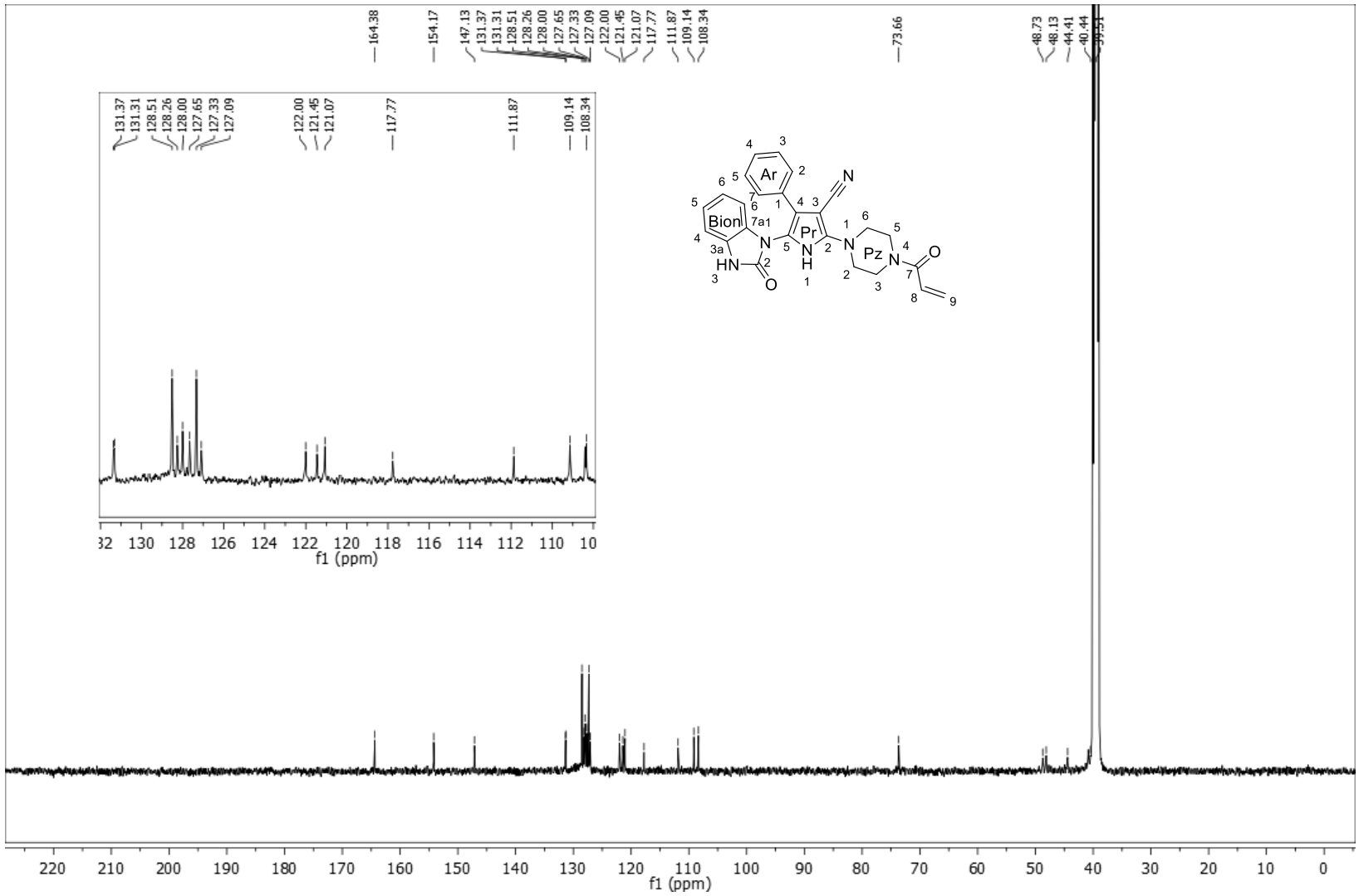


Figure S2. $^{13}C\{^{1}H\}$ NMR spectrum of **1a** in DMSO-d_6 at T = 303 K (Bruker spectrometer at 125.7 MHz).

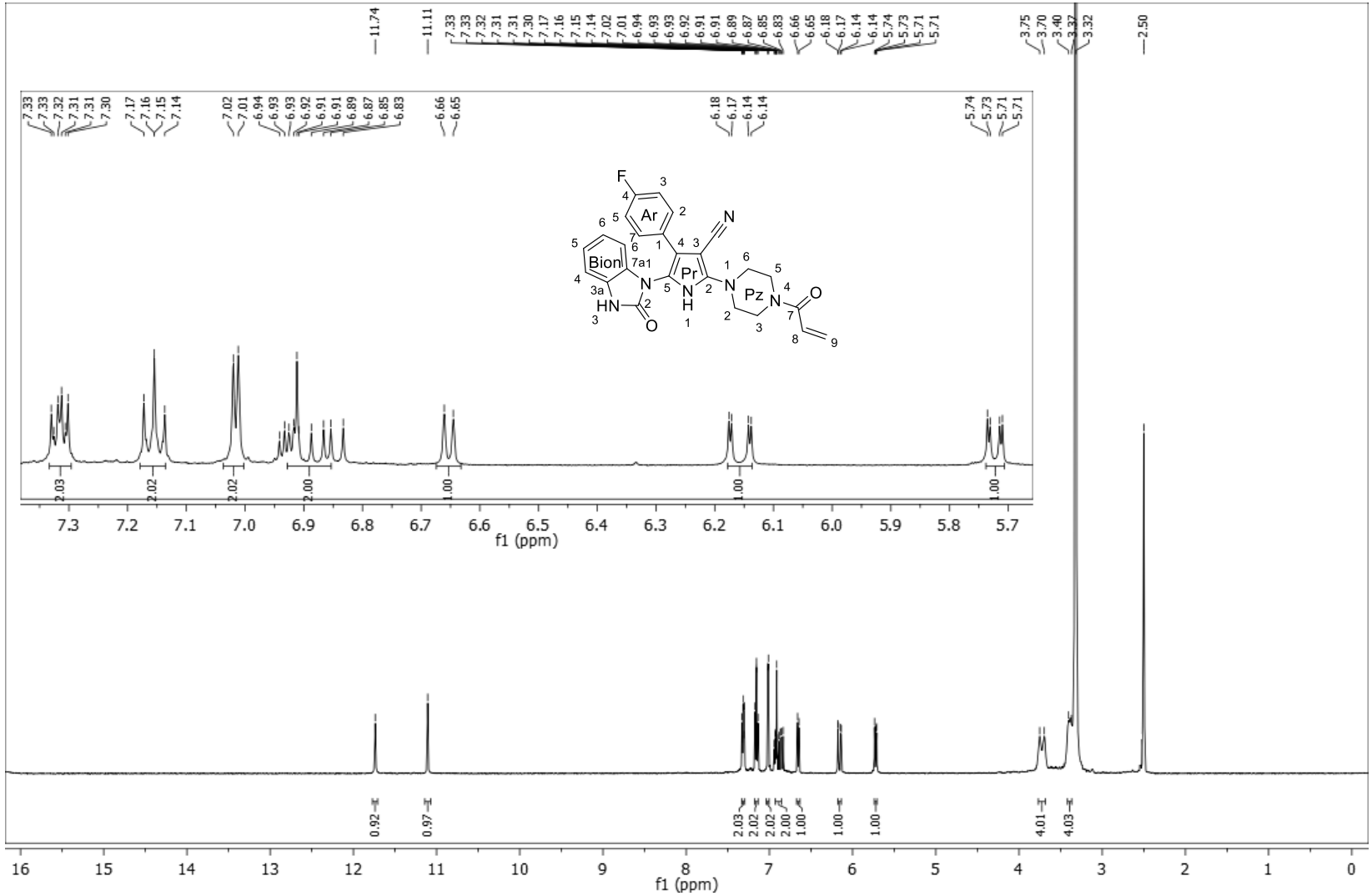


Figure S3. 1D ^{1}H NMR spectrum of **1b** in DMSO-d_6 at T = 303 K. Chemical shifts are given in ppm (Bruker spectrometer at 500.1 MHz).

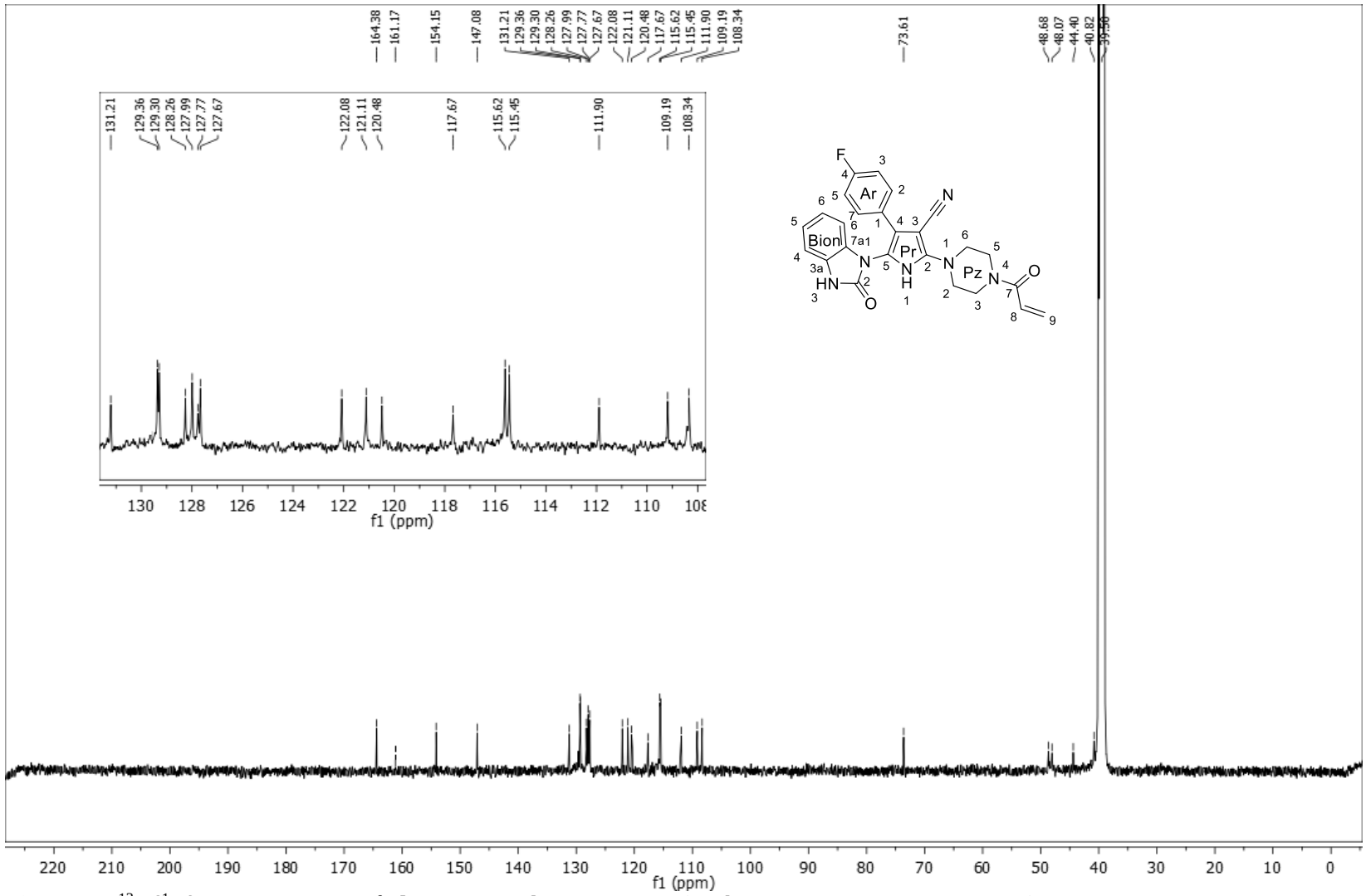


Figure S4. $^{13}C\{^{1}H\}$ NMR spectrum of **1b** in DMSO-d_6 at T = 303 K (Bruker spectrometer at 125.7 MHz).

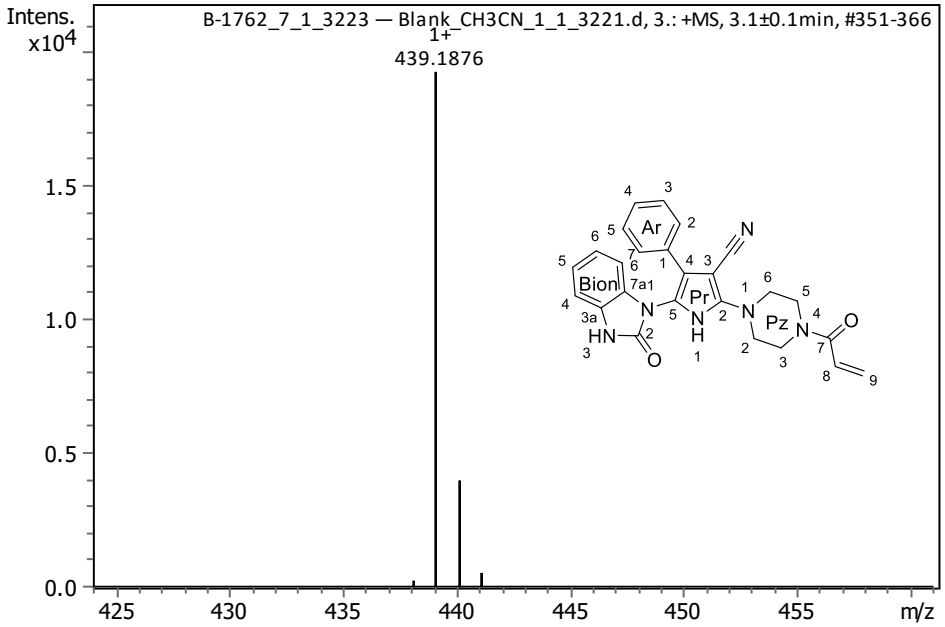


Figure S5. HRMS (ESI) of **1a**, *m/z*: $[M+H]^+$calcd for $C_{25}H_{23}N_6O_2$ 439.1877, found 439.1876.

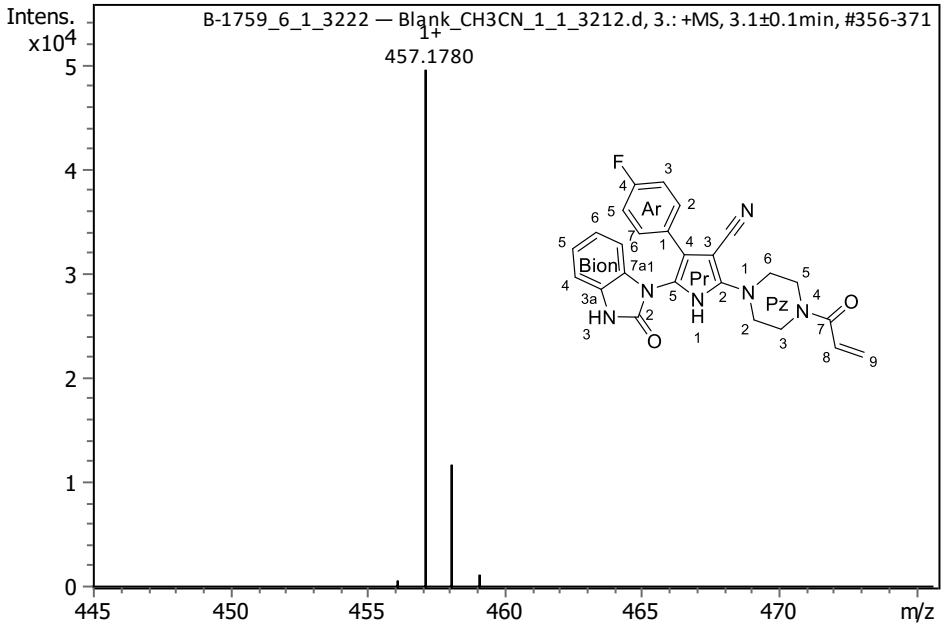


Figure S6. HRMS (ESI) of **1b**, *m/z*: $[M+H]^+$calcd for $C_{25}H_{22}FN_6O_2$ 457.1783, found 457.1780.