

Electronic Supporting Information

Discovery of N-Substituted-2-Oxoindolin Benzoylhydrazines as c-MET/SMO Modulators in EGFRi-Resistant Non-Small Cell Lung Cancer

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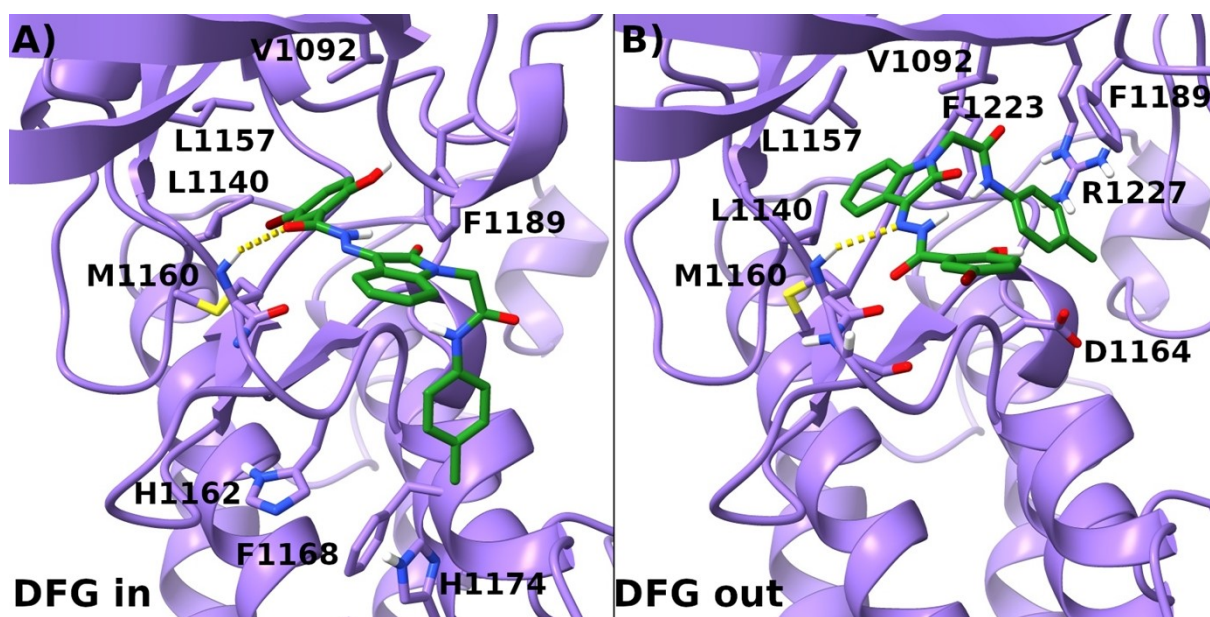


Figure S1. Predicted binding mode of **3** in complex with DFG-in (A) and DFG-out (B) conformation of c-MET, respectively. The ligand and receptor are in green sticks and purple sticks and ribbons, respectively. H-bonds are represented as dashed yellow lines.

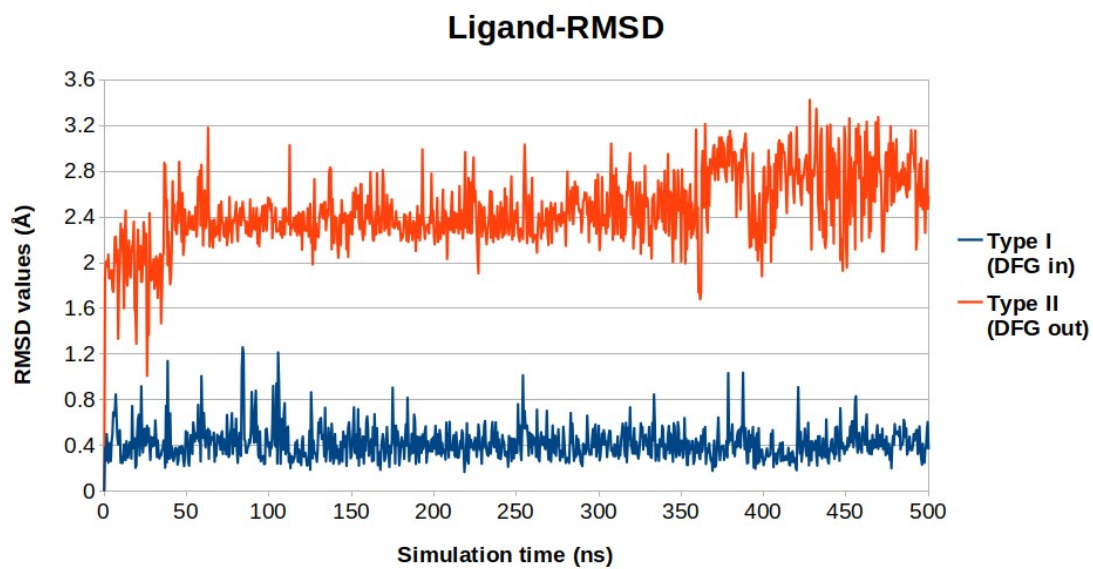


Figure S2. RMSD (Å) plot over simulation time (ns) of **3** in complex with DFG in (blue line) and DFG out (orange line) conformation of c-MET.

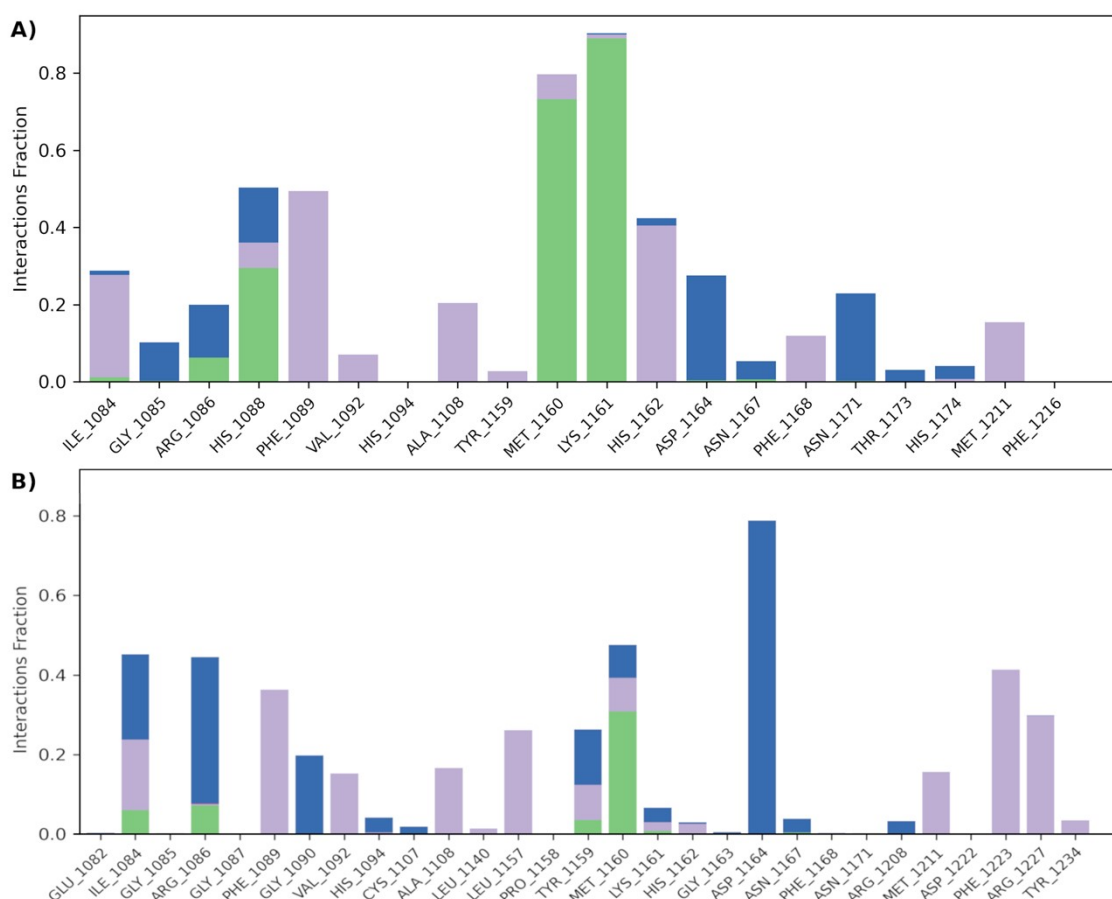


Figure S3. Histogram of protein-ligand interactions fraction of the 500 ns long MD simulation for **3** in complex with DFG in (A) and DFG out (B) conformations of c-MET, respectively. H-bonds are in green, hydrophobic contacts are in purple, and water-mediated interactions are in blue.

Table S1. Description of the employed 20 kinases.

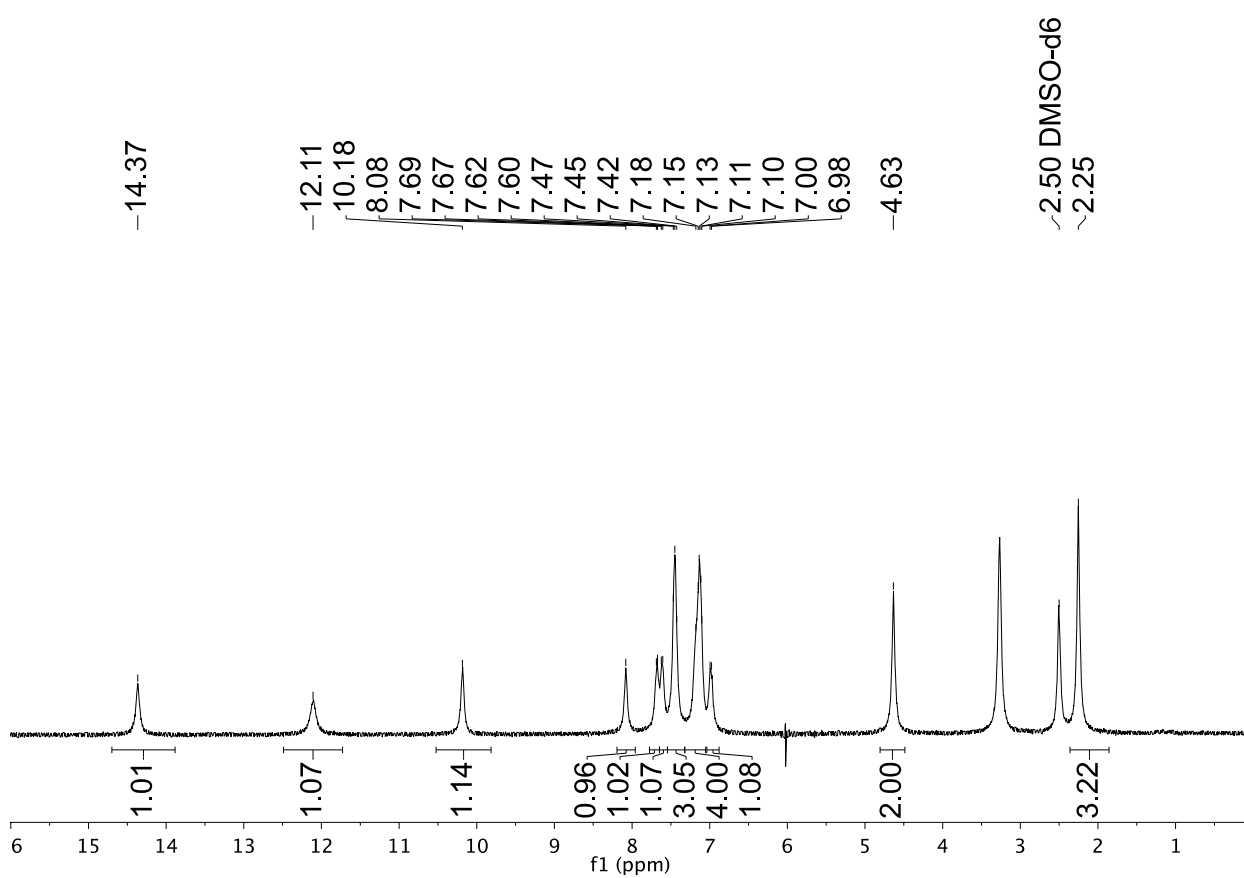
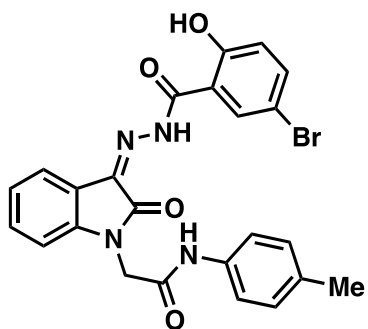
TK	Description
ABL(ABL1)	Full-length human ABL [2-1130(end) amino acids of accession number NP_005148.2] was expressed as N-terminal His-tagged protein (126 kDa) using baculovirus expression system. His-tagged ABL was purified by using Ni-NTA affinity chromatography.
CSK	Full-length human CSK [1-450(end) amino acids of accession number NP_004374.1] was expressed as N-terminal GST-fusion protein (78 kDa) using baculovirus expression system. GST-CSK was purified by using glutathione sepharose chromatography.
EGFR	Human EGFR, cytoplasmic domain [669-1210(end) amino acids of accession number NP_005219.2] was expressed as N-terminal GST-fusion protein (89 kDa) using baculovirus expression system. GST-EGFR was purified by using glutathione sepharose chromatography.
EPHA2	Human EPHB4, cytoplasmic domain [577-987(end) amino acids of accession number NP_004435.3] was expressed as N-terminal GST-protein (73 kDa) using baculovirus expression system. GST-EPHB4 was purified by using glutathione sepharose chromatography.
FGFR1	Human FGFR1, cytoplasmic domain [398-822(end) amino acids of accession number NP_075598.2] was expressed as N-terminal GST-fusion protein (75 kDa) using baculovirus expression system. GST-FGFR1 was

	purified by using glutathione sepharose chromatography.
FLT3	Human FLT3, cytoplasmic domain [564-993(end) amino acids of accession number NP_004110.2] was expressed as N-terminal GST-fusion protein (77 kDa) using baculovirus expression system. GST-FLT3 was purified by using glutathione sepharose chromatography.
IGF1R	Human IGF1R, cytoplasmic domain [959-1367(end) amino acids of accession number NP_000866.1] was expressed as N-terminal GST-fusion protein (73 kDa) using baculovirus expression system. GST-IGF1R was purified by using glutathione sepharose chromatography.
ITK	Full-length human ITK [2-620(end) amino acids of accession number NP_005537.3] was expressed as N-terminal GST-fusion protein (99 kDa) using baculovirus expression system. GST-ITK was purified by using glutathione sepharose chromatography.
JAK3	Human JAK3, catalytic domain [795-1124(end) amino acids of accession number NP_000206.2] was expressed as N-terminal His-tagged protein (41 kDa) using baculovirus expression system. His-tagged JAK3 was purified by using Ni-NTA affinity chromatography and gel filtration chromatography.
KDR	Human KDR, cytoplasmic domain [790-1356(end) amino acids of accession number NP_002244.1] was expressed as N-terminal GST-fusion protein (90 kDa) using baculovirus expression system. GST-KDR was purified by using glutathione sepharose chromatography.
LCK	Full-length human LCK [1-509(end) amino acids of accession number NP_005347.2] was expressed as N-terminal GST-fusion protein (85 kDa) using baculovirus expression system. GST-LCK was purified by using glutathione sepharose chromatography.
c-MET	Human MET, cytoplasmic domain [956-1390(end) amino acids of accession number NP_000236.2] was expressed as N-terminal GST-fusion protein (76 kDa) using baculovirus expression system. GST-MET was purified by using glutathione sepharose chromatography.
PDGFR α (PDGFRA)	Human PDGFR α , cytoplasmic domain [550-1089(end) amino acids of accession number NP_006197.1] was expressed as N-terminal GST-fusion protein (89 kDa) using baculovirus expression system. GST-PDGFR α was purified by using glutathione sepharose chromatography.
PYK2(PTK2B)	Full-length human PYK2 [1-967(end) amino acids of accession number NP_775267.1] was expressed as N-terminal GST-fusion protein (138 kDa) using baculovirus expression system. GST-PYK2 was purified by using glutathione sepharose chromatography.
SRC	Full-length human SRC [1-536(end) amino acids of accession number NP_005408.1] was expressed as N-terminal GST-fusion protein (87 kDa) using baculovirus expression system. GST-SRC was purified by using glutathione sepharose chromatography.
SYK	Full-length human SYK [1-635(end) amino acids of accession number NP_003168.2] was expressed as N-terminal GST-fusion protein (99 kDa) using baculovirus expression system. GST-SYK was purified by using glutathione sepharose chromatography.
TIE2(TEK)	Human TIE2, cytoplasmic domain [771-1124(end) amino acids of accession number NP_000450.1] was expressed as N-terminal GST-fusion protein (68 kDa) using baculovirus expression system. GST-TIE2 was purified by using glutathione sepharose chromatography.
TRKA(NTRK1)	Human TRKA, cytoplasmic domain [436-790(end) amino acids of

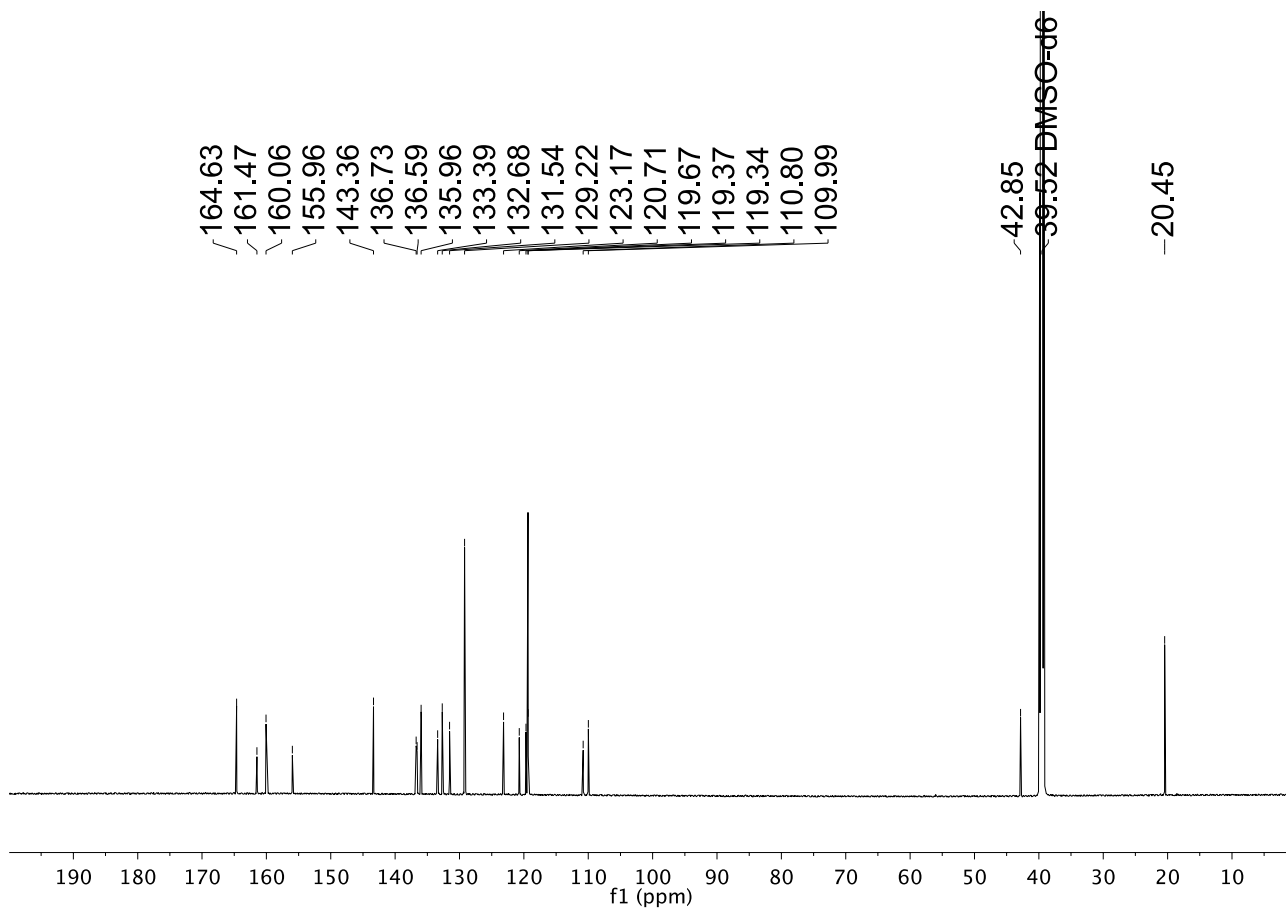
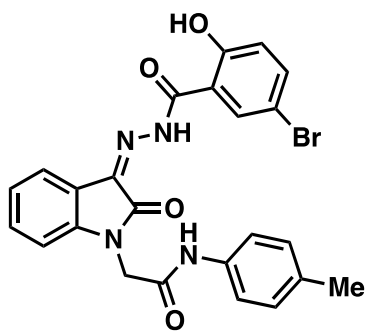
	accession numberNP_001012331.1] was expressed as N- terminal GST-fusion protein (67 kDa) usingbaculovirus expression system. GST-TRKA was purified by using glutathione sepharosechromatography
TYRO3	Human TYRO3, cytoplasmic domain of [453-890(end) amino acids of accession numberNP_006284.2] was expressed as N-terminal GST fusion protein (76 kDa) usingbaculovirus expression system. GST-TYRO3 was purified by using glutathione sepharosechromatography

NMR spectra.

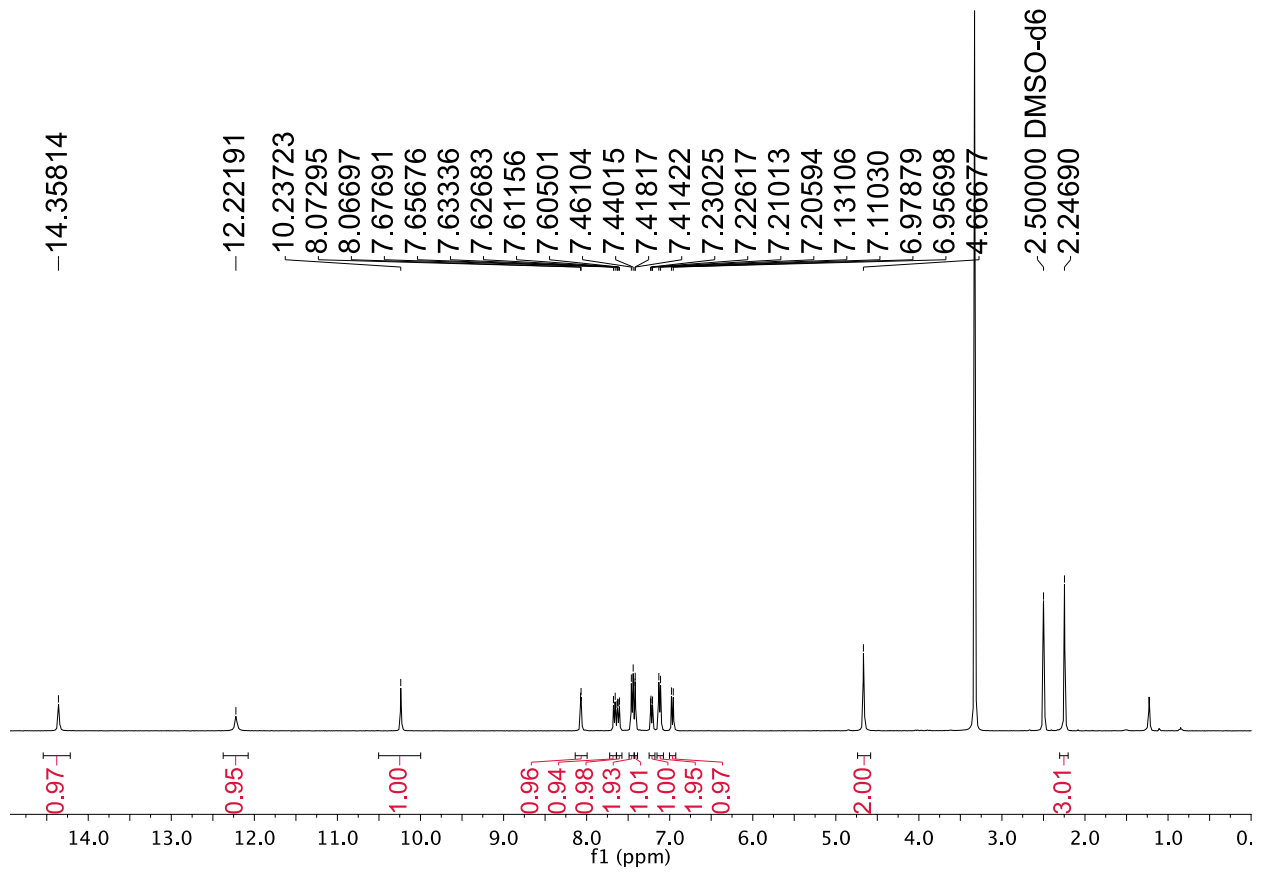
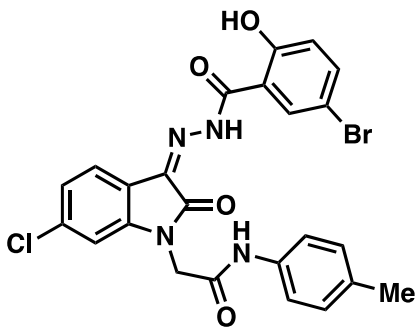
Compound (3) ^1H NMR (400 MHz, $\text{DMSO-}d_6$)



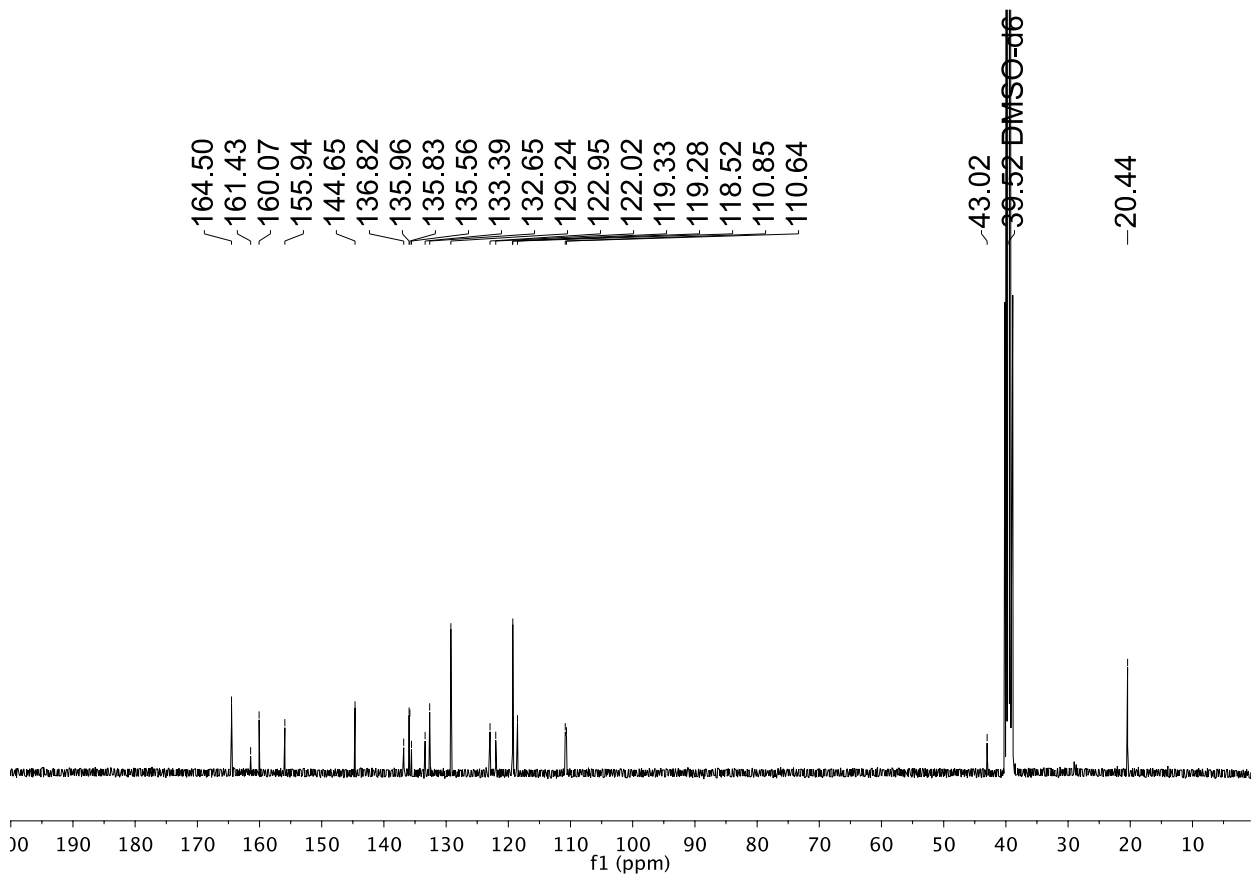
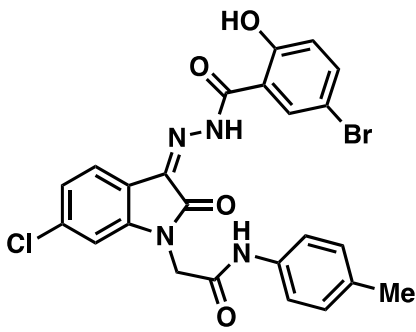
Compound (3) ¹³C NMR (101 MHz, DMSO-d₆)



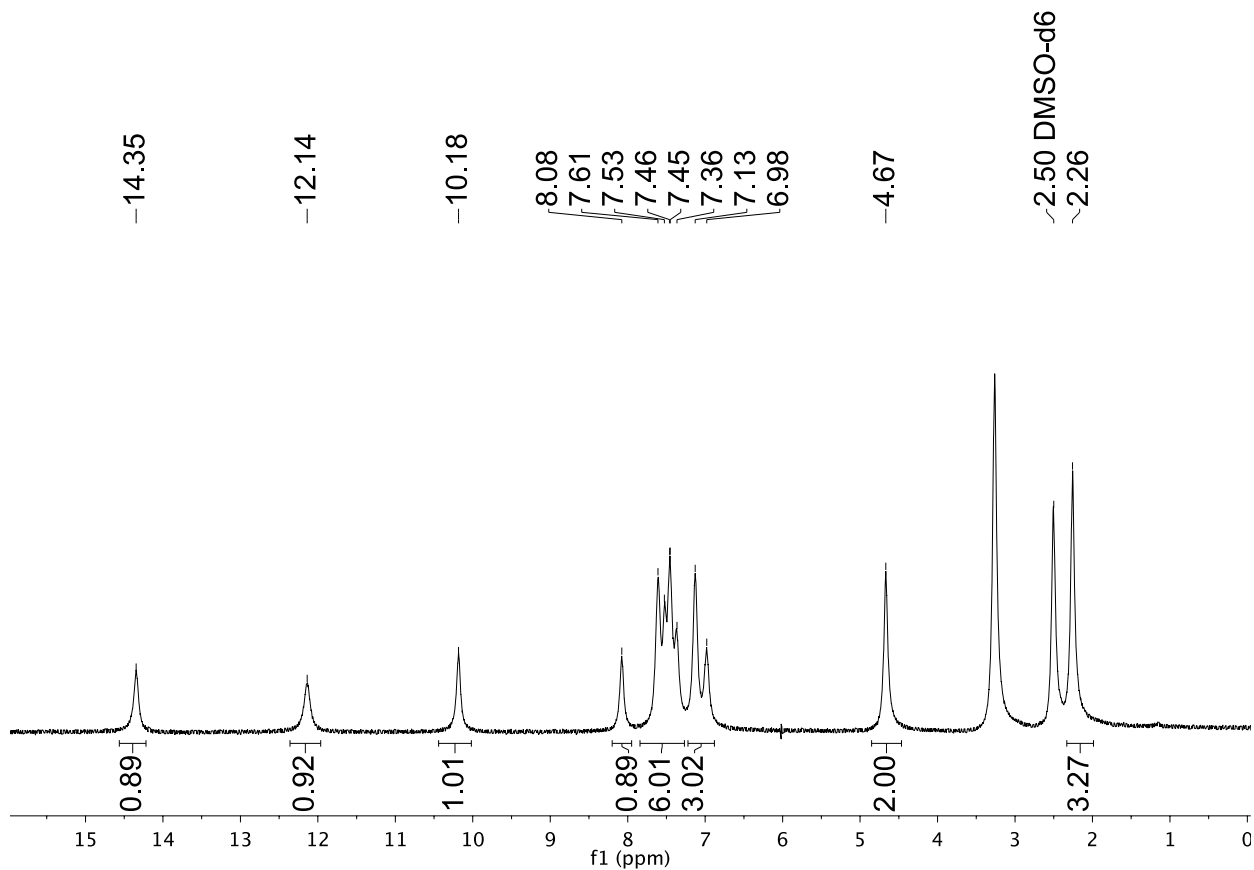
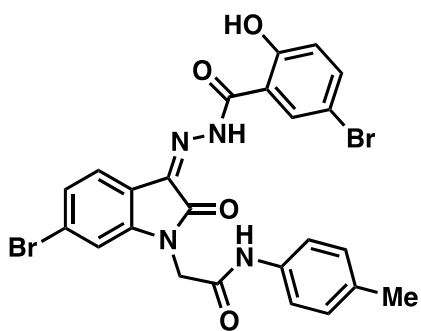
Compound (6) ¹H NMR (400 MHz, DMSO-d₆)



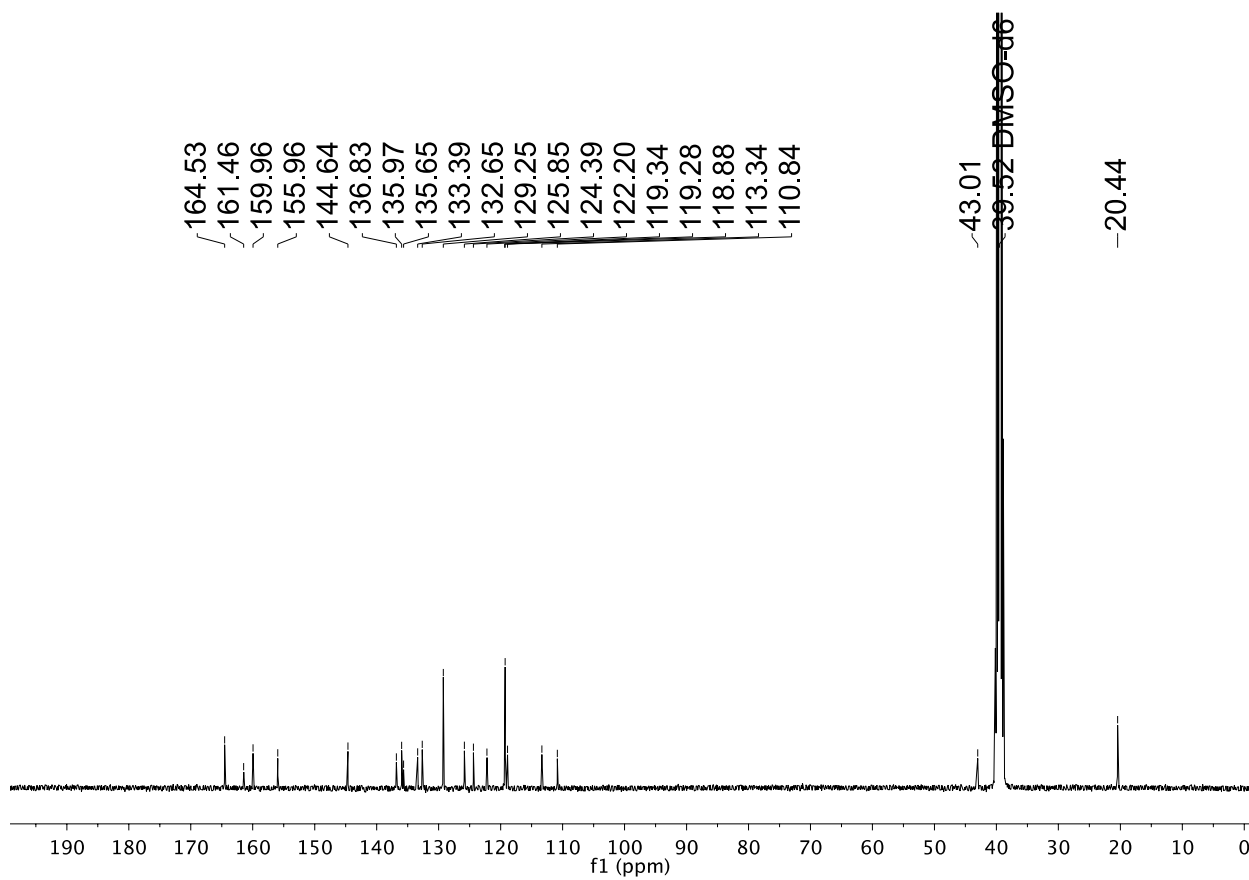
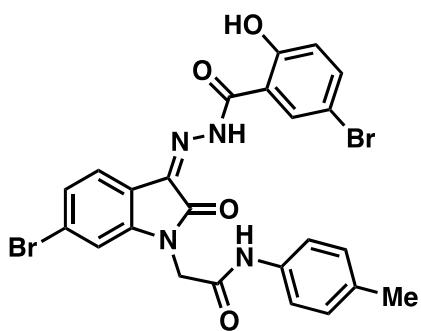
Compound (6) ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$)



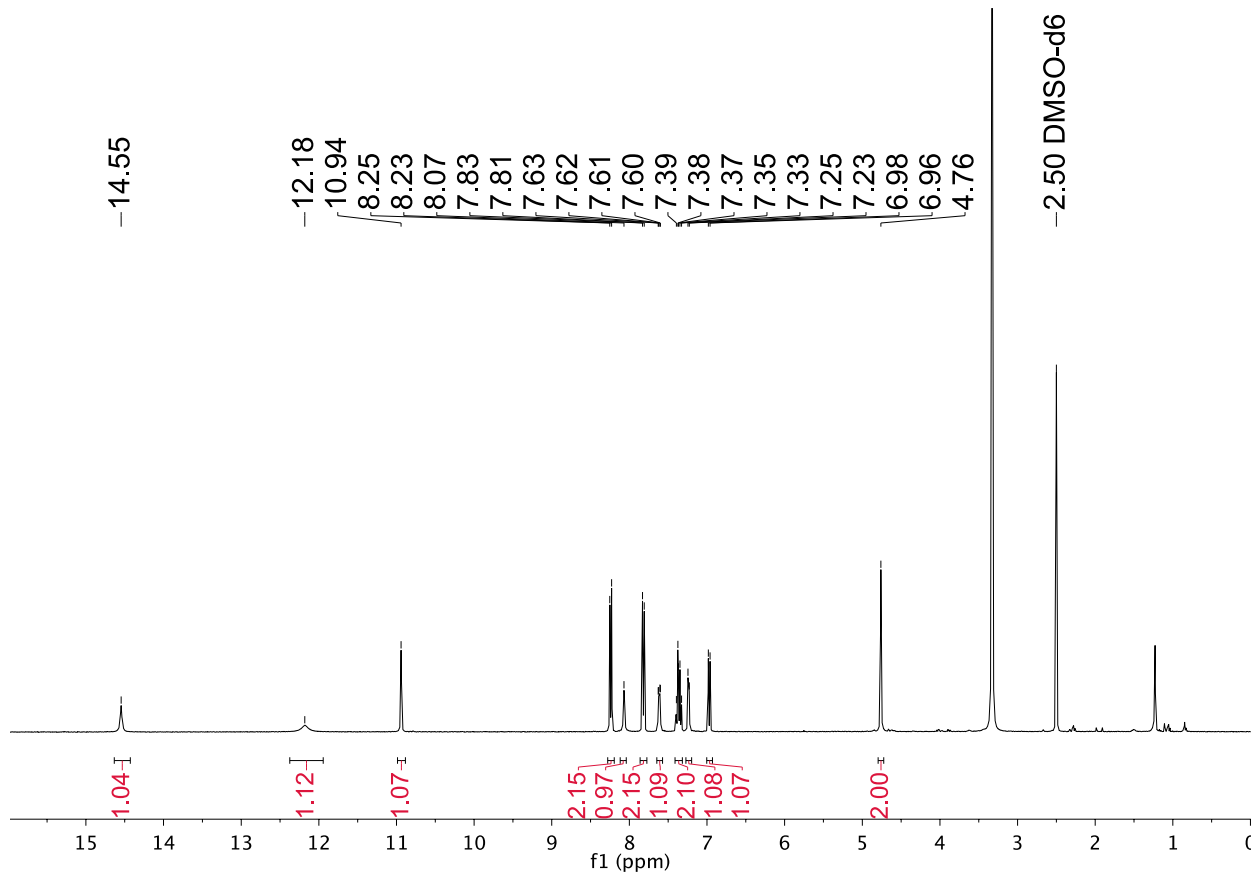
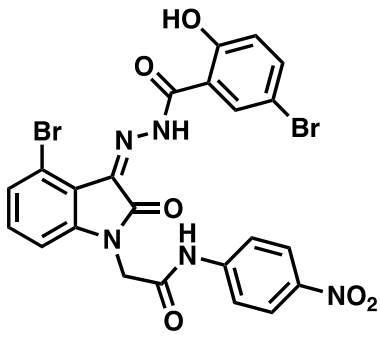
Compound (10) ^1H NMR (400 MHz, $\text{DMSO-}d_6$)



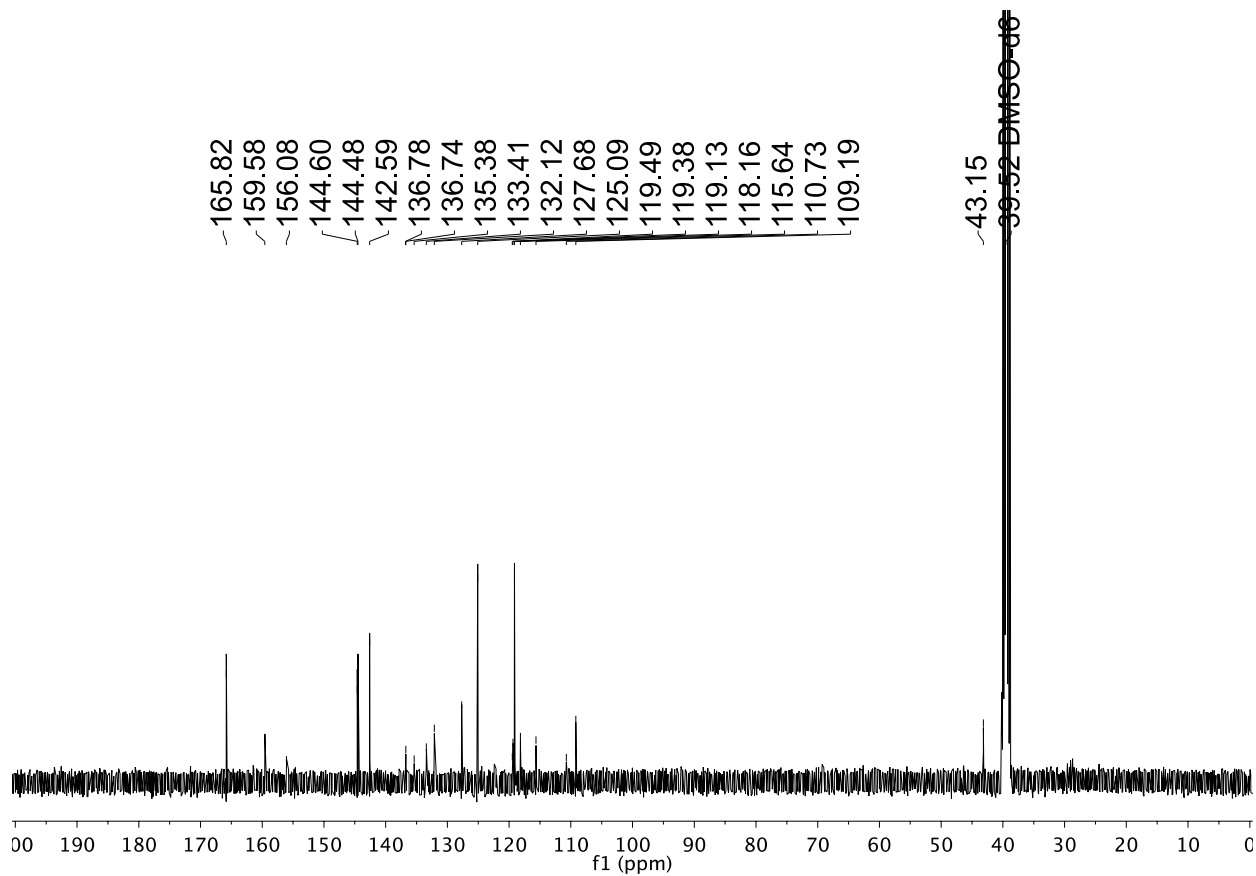
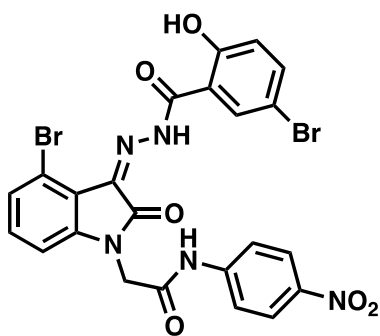
Compound (10) ¹³C NMR (101 MHz, DMSO-d₆)



Compound (25) ¹H NMR (400 MHz, DMSO-d₆)



Compound (25) ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$)



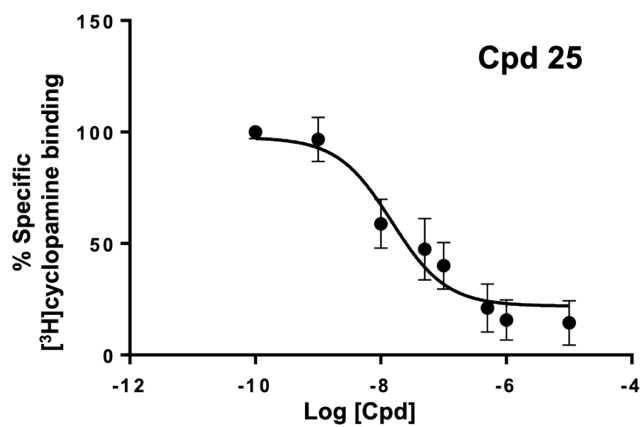
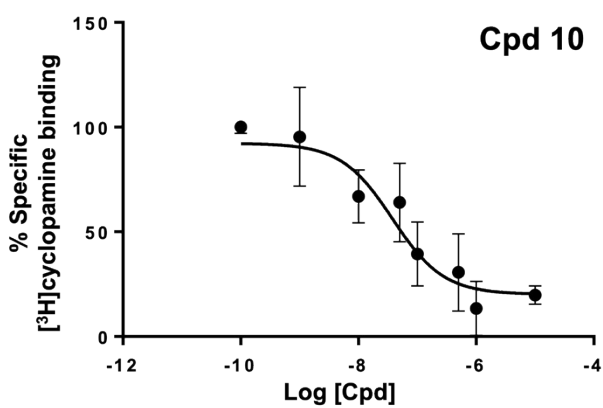
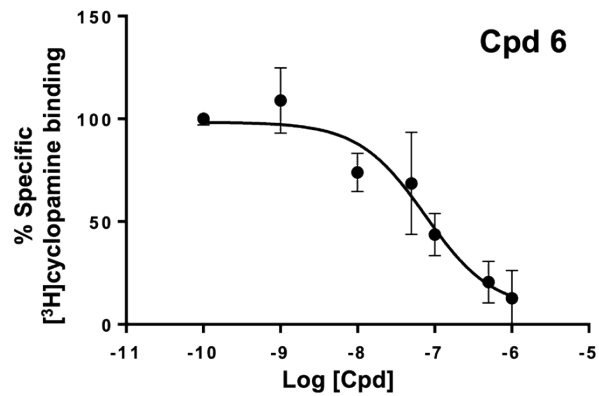
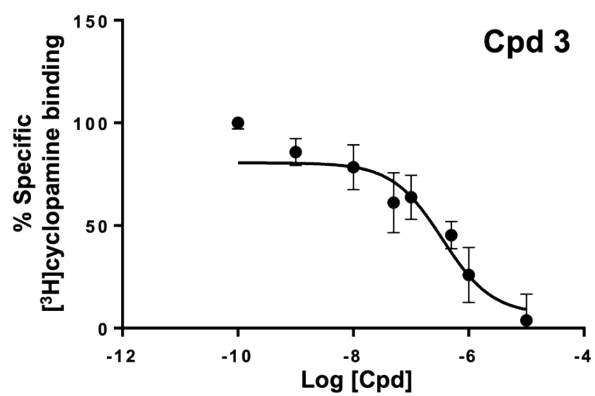
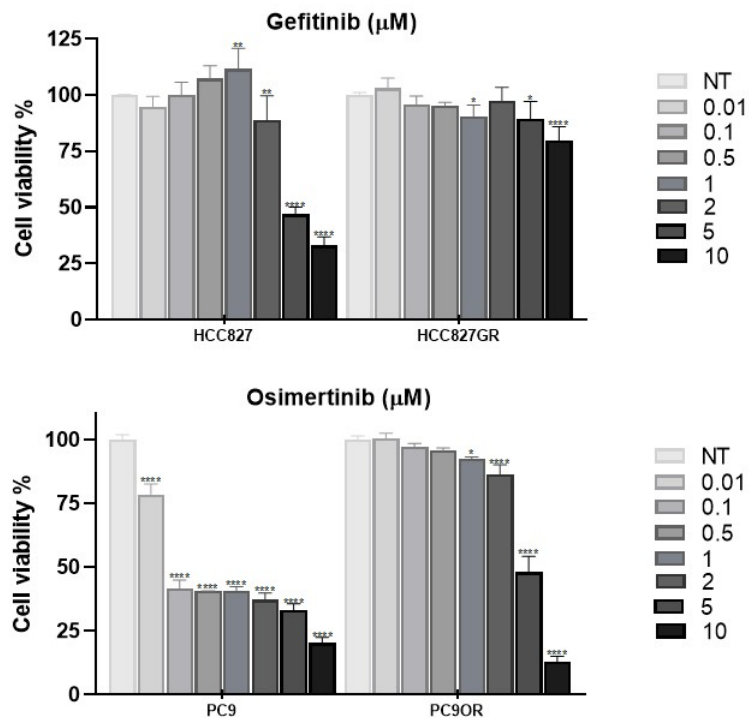
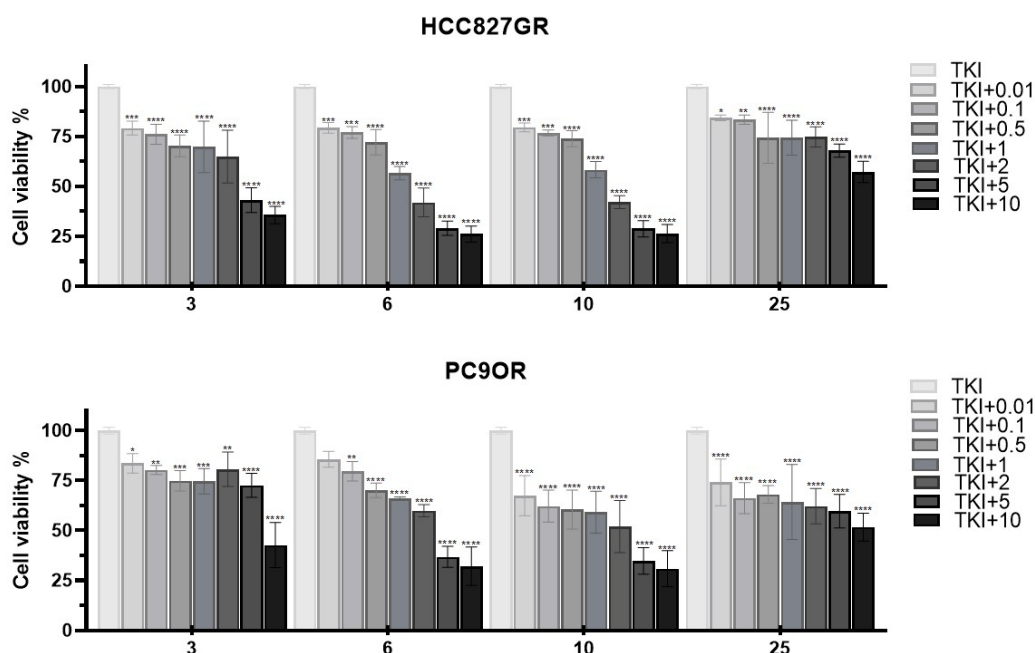


Figure S4. SMO binding curves of compounds 3, 6, 10, and 25.



IC_{50} (μM) \pm SD	HCC827	HCC827GR	PC9	PC9OR
Gefitinib	5.98 \pm 0.11	83.94 \pm 6.21	-	-
Osimertinib	-	-	0.18 \pm 0.06	4.81 \pm 0.51

Figure S5. MTT Cell viability Assay with increasing concentrations of respective TKI (gefitinib for HCC827-GR and osimertinib for PC9-OR). Table shows the IC_{50} values for parental versus resistant NSCLC cell lines. Data are presented as mean of three biological replicates and four technical replicates \pm SD. Statistical significance ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$.



IC ₅₀ (μM) ± SD	TKI + 3	TKI + 6	TKI + 10	TKI + 25
HCC827-GR	5.61 ± 0.61	1.89 ± 0.53	2.10 ± 0.34	22.24 ± 1.76
PC9-OR	10.80 ± 1.51	3.57 ± 0.52	1.03 ± 0.62	11.74 ± 1.11

Figure S6. MTT Cell viability Assay with increasing concentrations of compounds (**3**, **6**, **10**, **25**) in combination with IC₅₀ value dose for respective TKI (gefitinib for HCC827-GR and osimertinib for PC9-OR). Table shows the IC₅₀ values of the compound in combination with gefitinib or osimertinib for HCC827-GR and PC9-OR cell lines, respectively. Data are presented as mean of three biological replicates and four technical replicates ± SD. Statistical significance**p<0.01, ***p< 0.001 and ****p<0.0001.

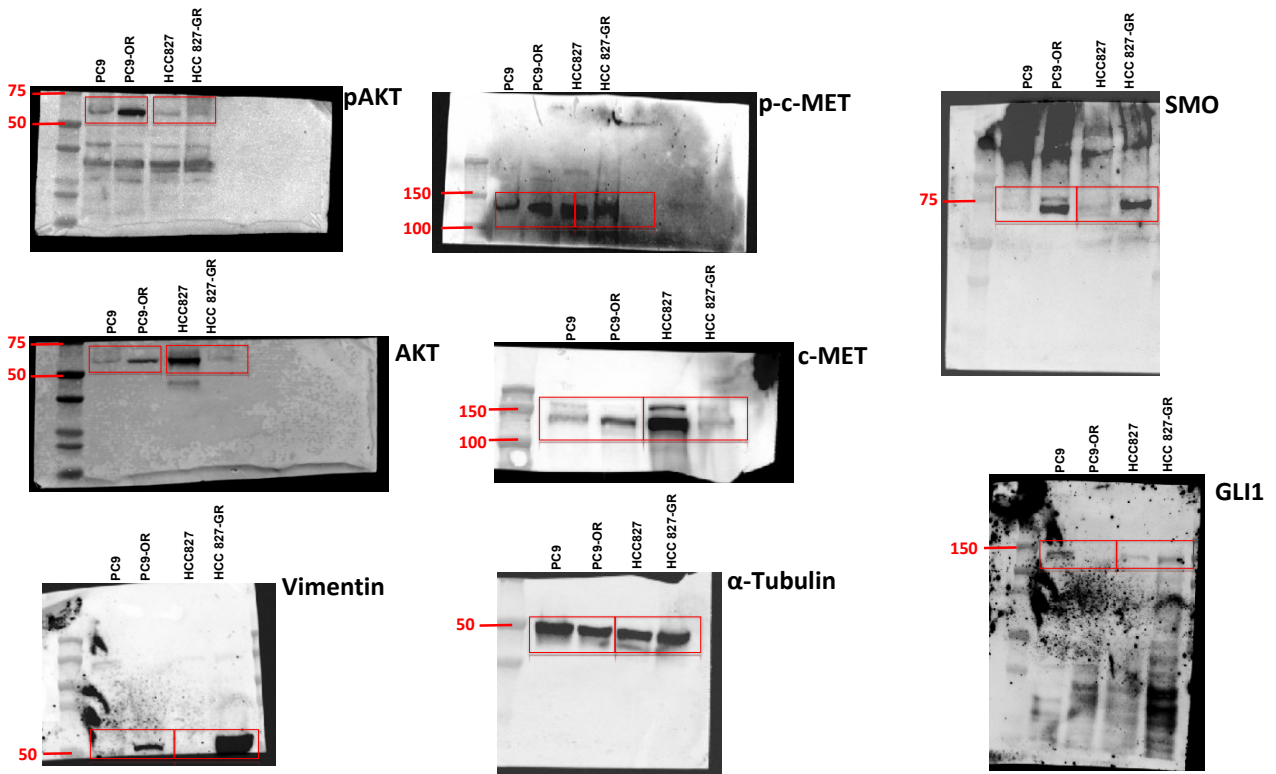


Figure S7. Western Blot raw gels for Figure 4 in the main text.

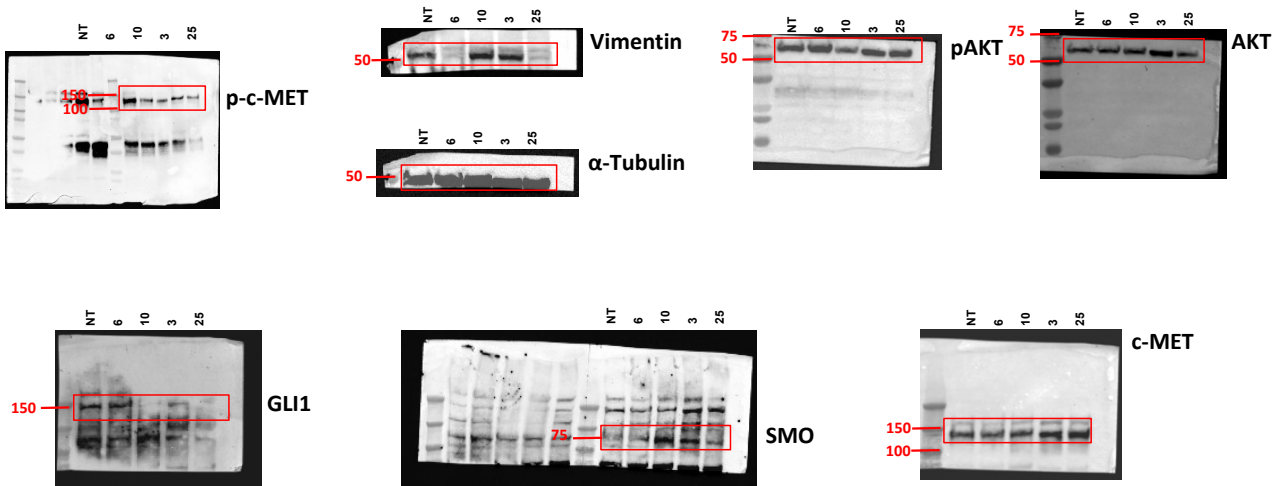


Figure S8. Western Blot raw gels using HCC827-GR cell lines for Figure 6 in the main text.

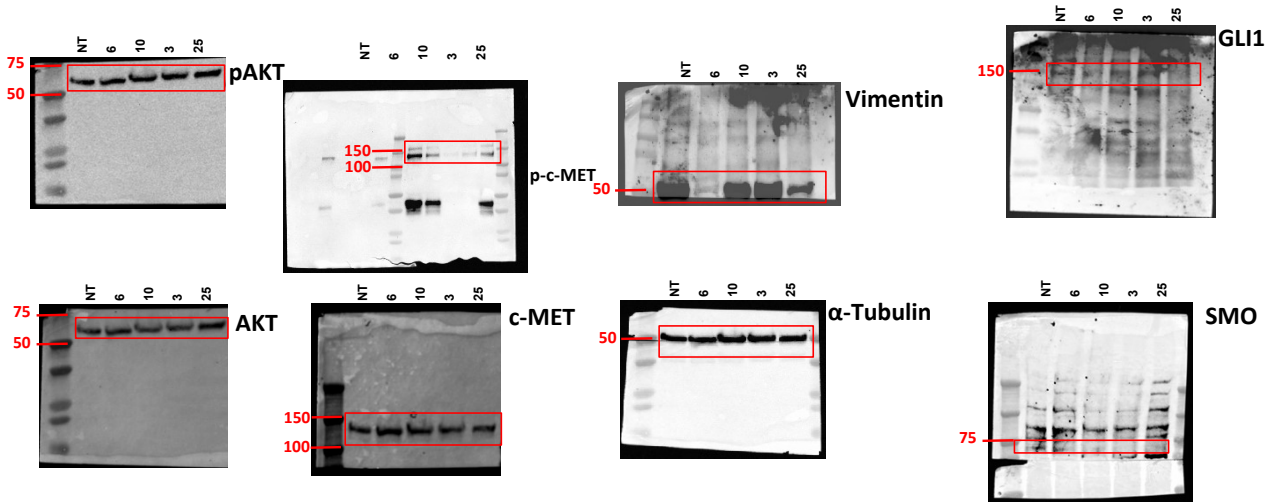


Figure S9. Western Blot raw gels using PC9-OR cell lines for Figure 6 in the main text.

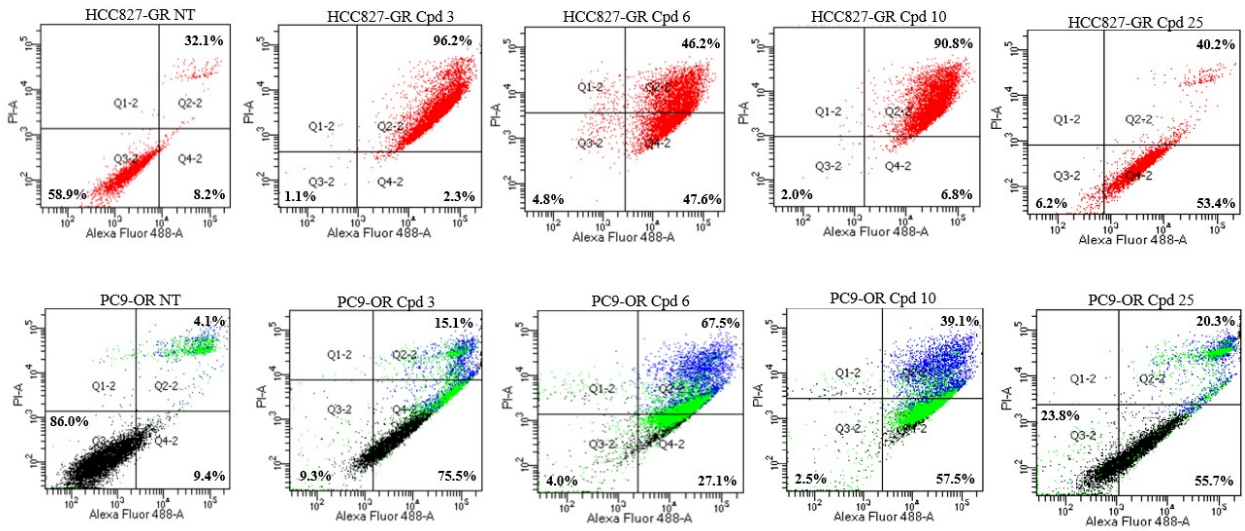


Figure S10. Annexin V/Propidium Iodide Apoptosis assay of Figure 7 in the main text.

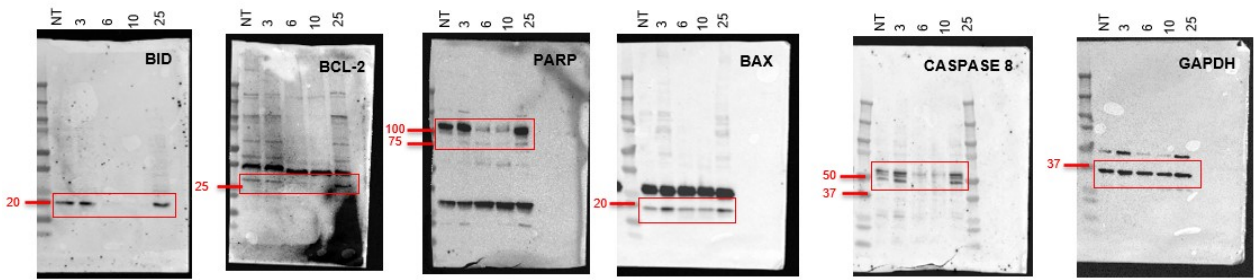


Figure S11. Western Blot raw gels using PC9-OR cell lines for Figure 7 in the main text.

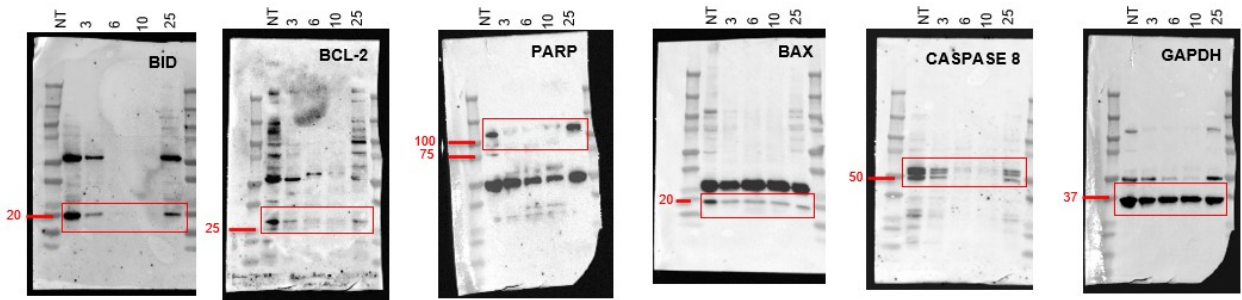


Figure S12. Western Blot raw gels using HCC827-GR cell lines for Figure 7 in the main text.