Supplementary Materials

Pyrazole-Triazole Hybrids as Kinase-Triad Inhibitors: A Triple-Target Strategy for Synergistic Anticancer Therapy

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^{1}HNMR and ^{13}CNMR Spectra

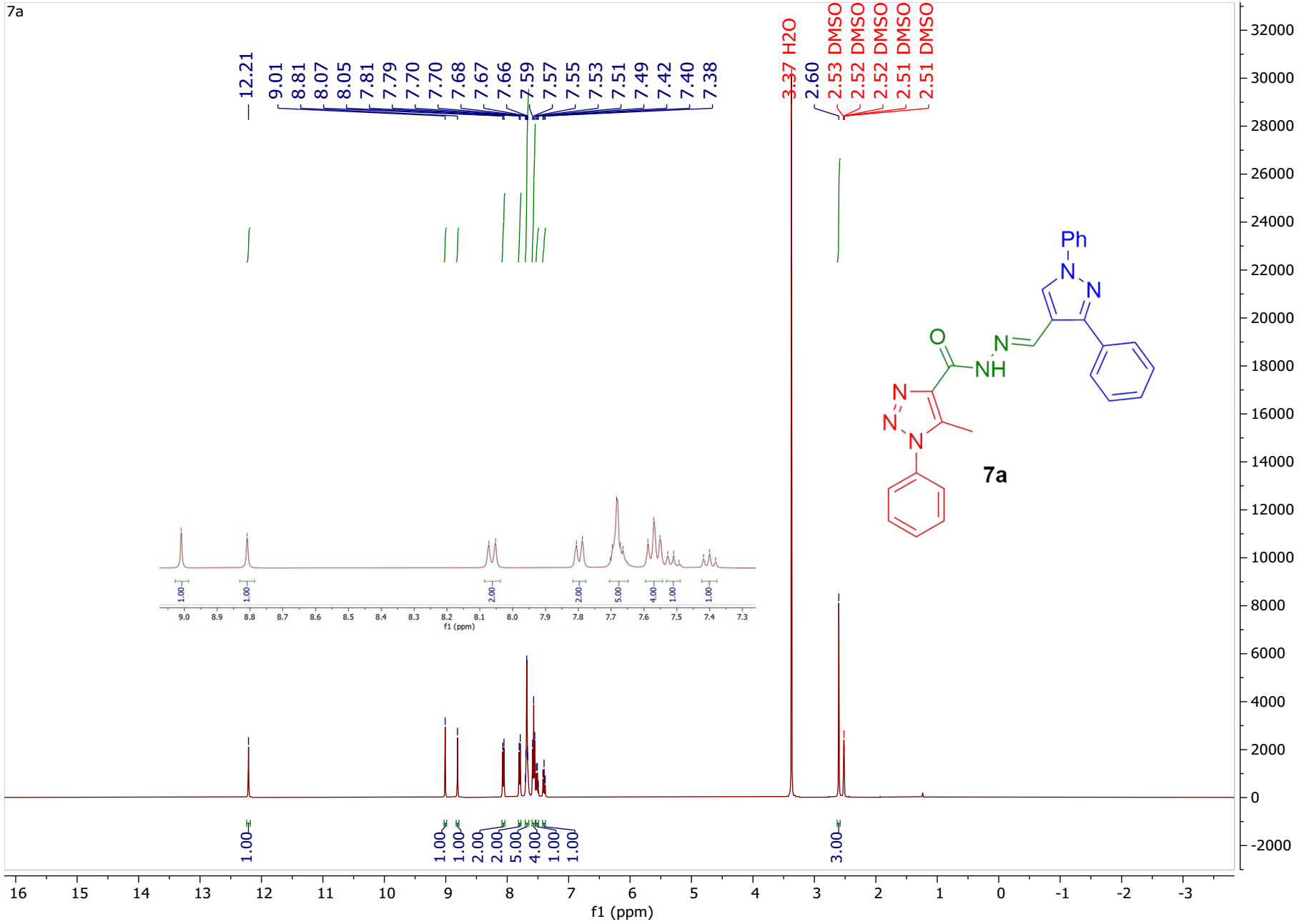

Figure S1. [1]HNMR Spectra of Compound **7a**.

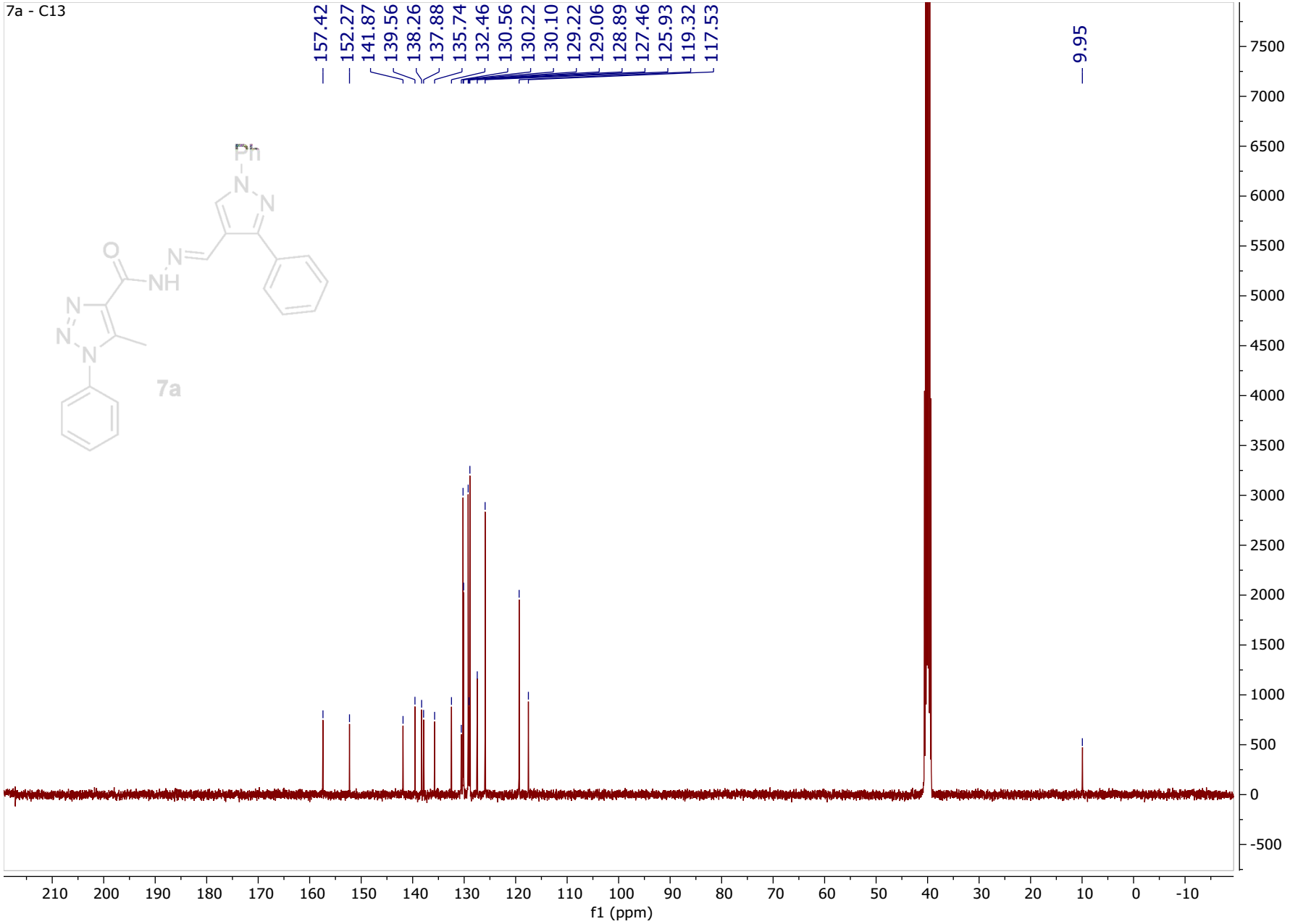

Figure S2. ^{13}CNMR Spectra of Compound **7a**.

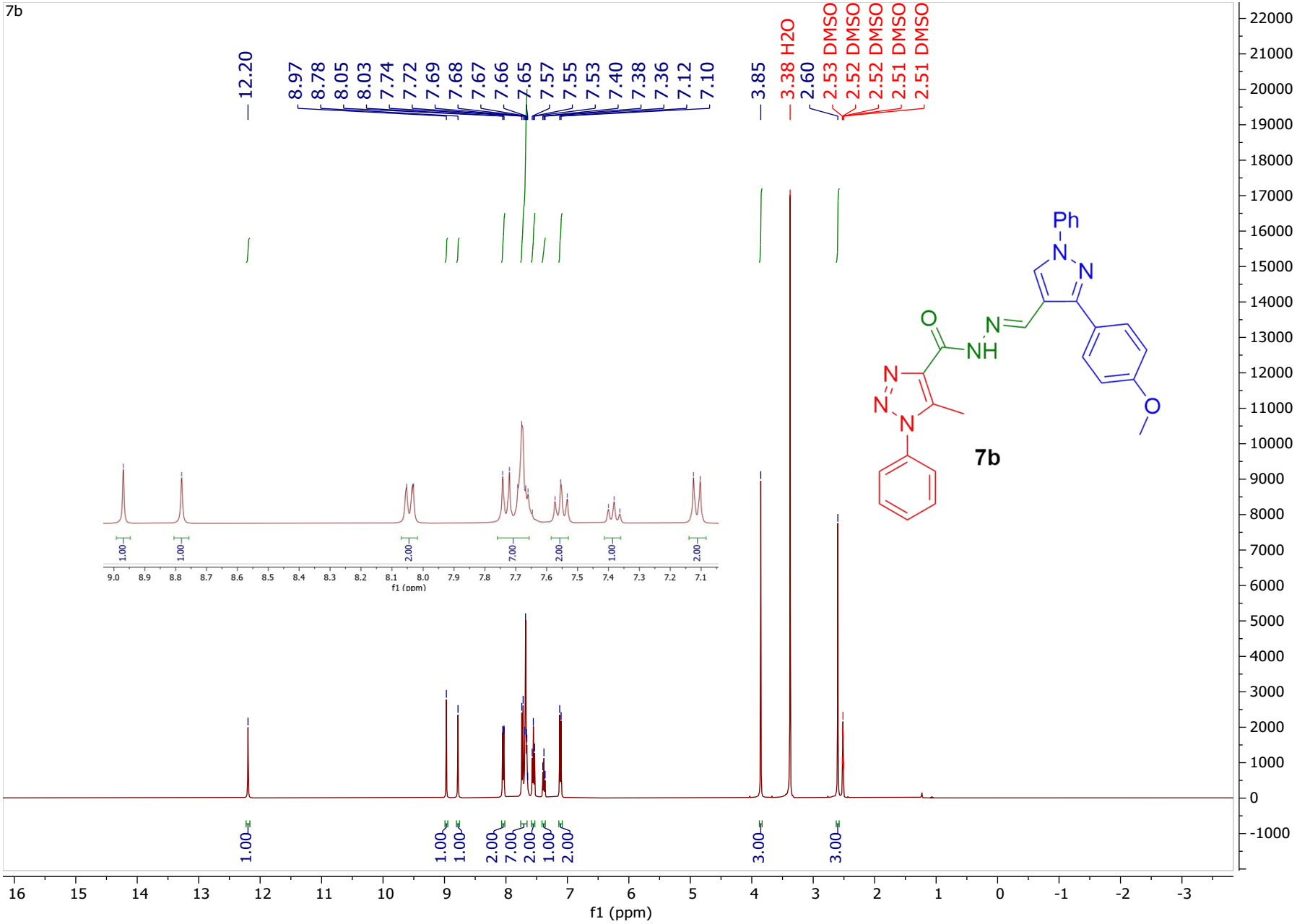

Figure S3. [1]HNMR Spectra of Compound **7b**.

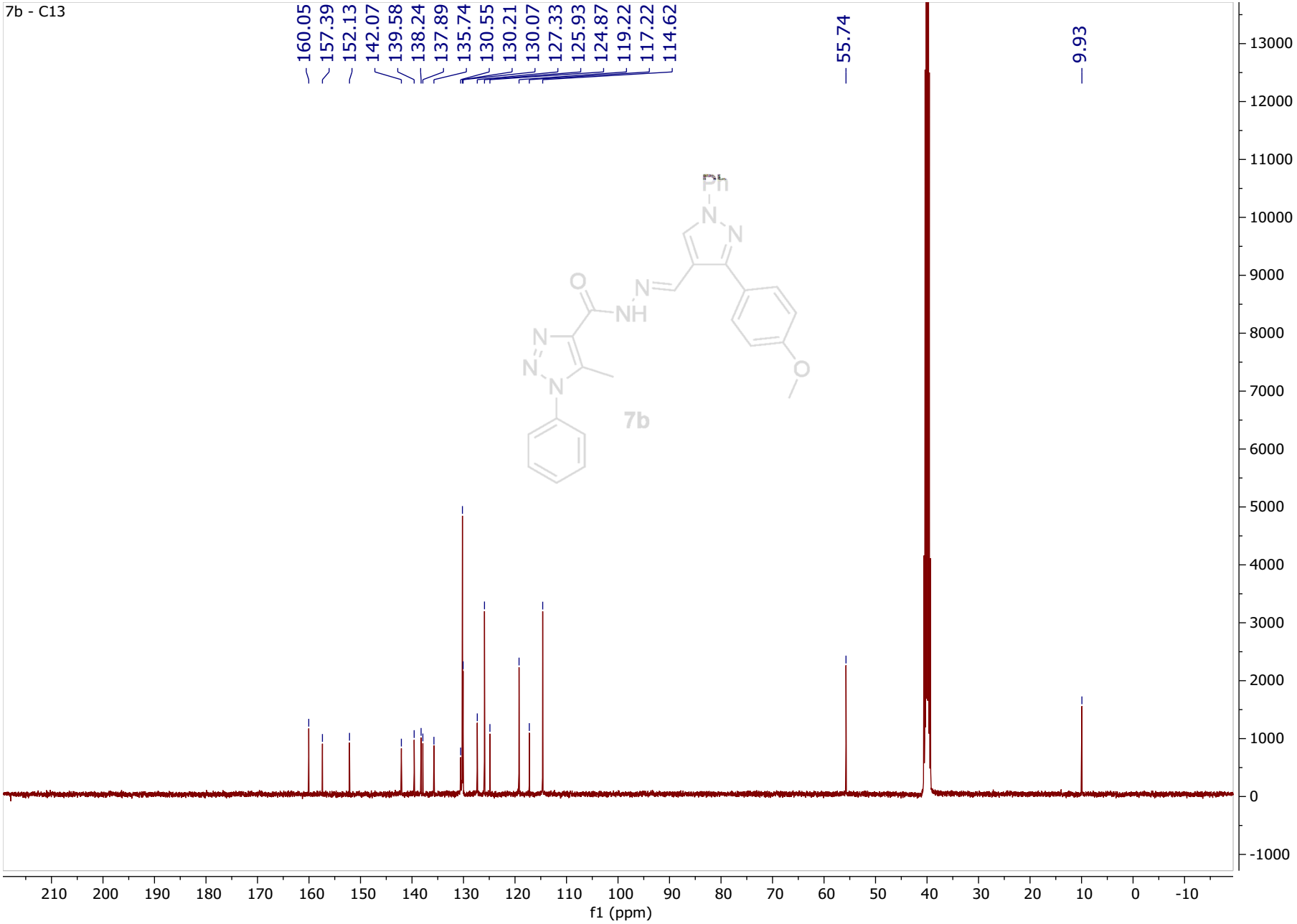

Figure S4. ^{13}CNMR Spectra of Compound **7b**.

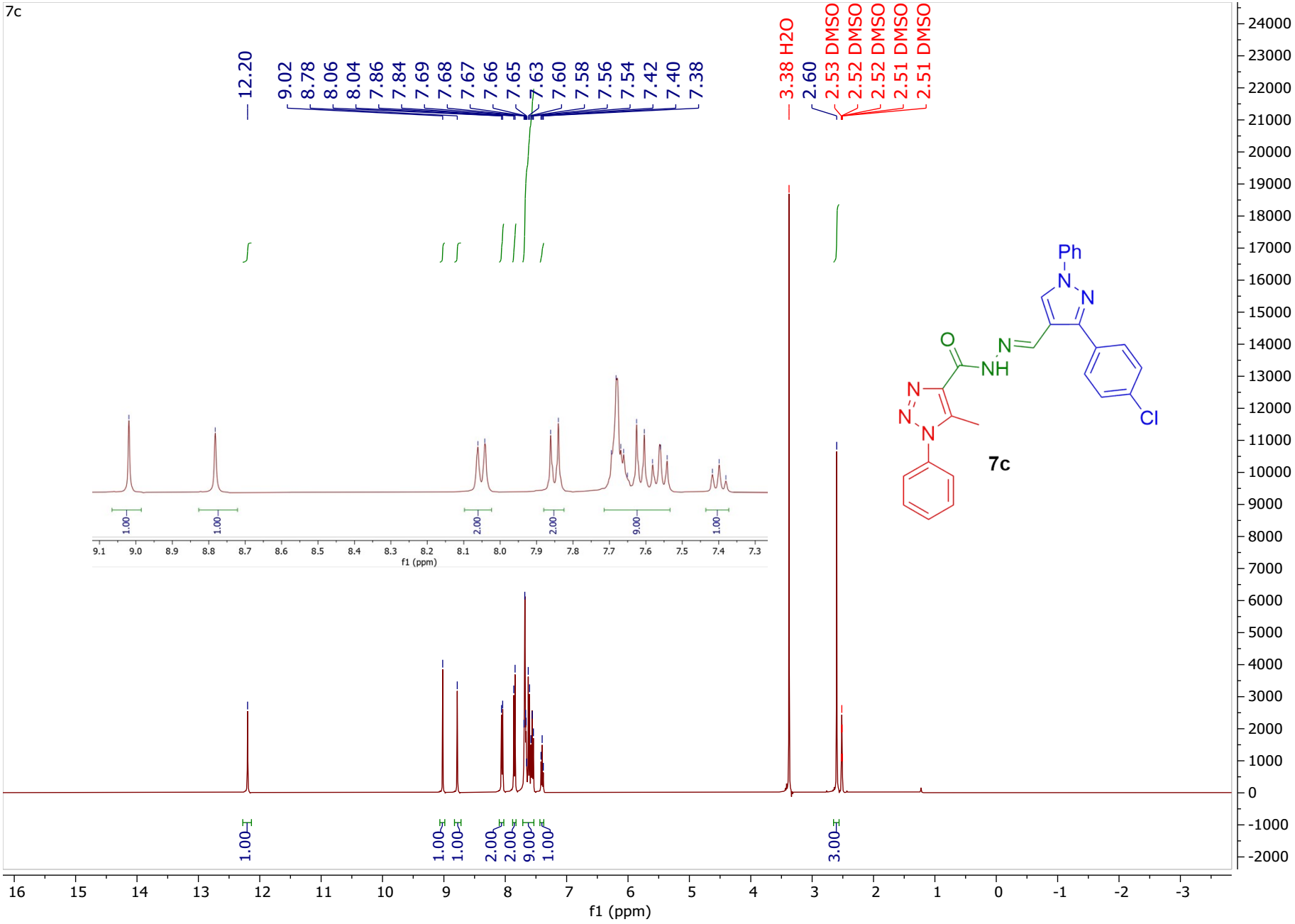

Figure S5. [1]HNMR Spectra of Compound **7c**.

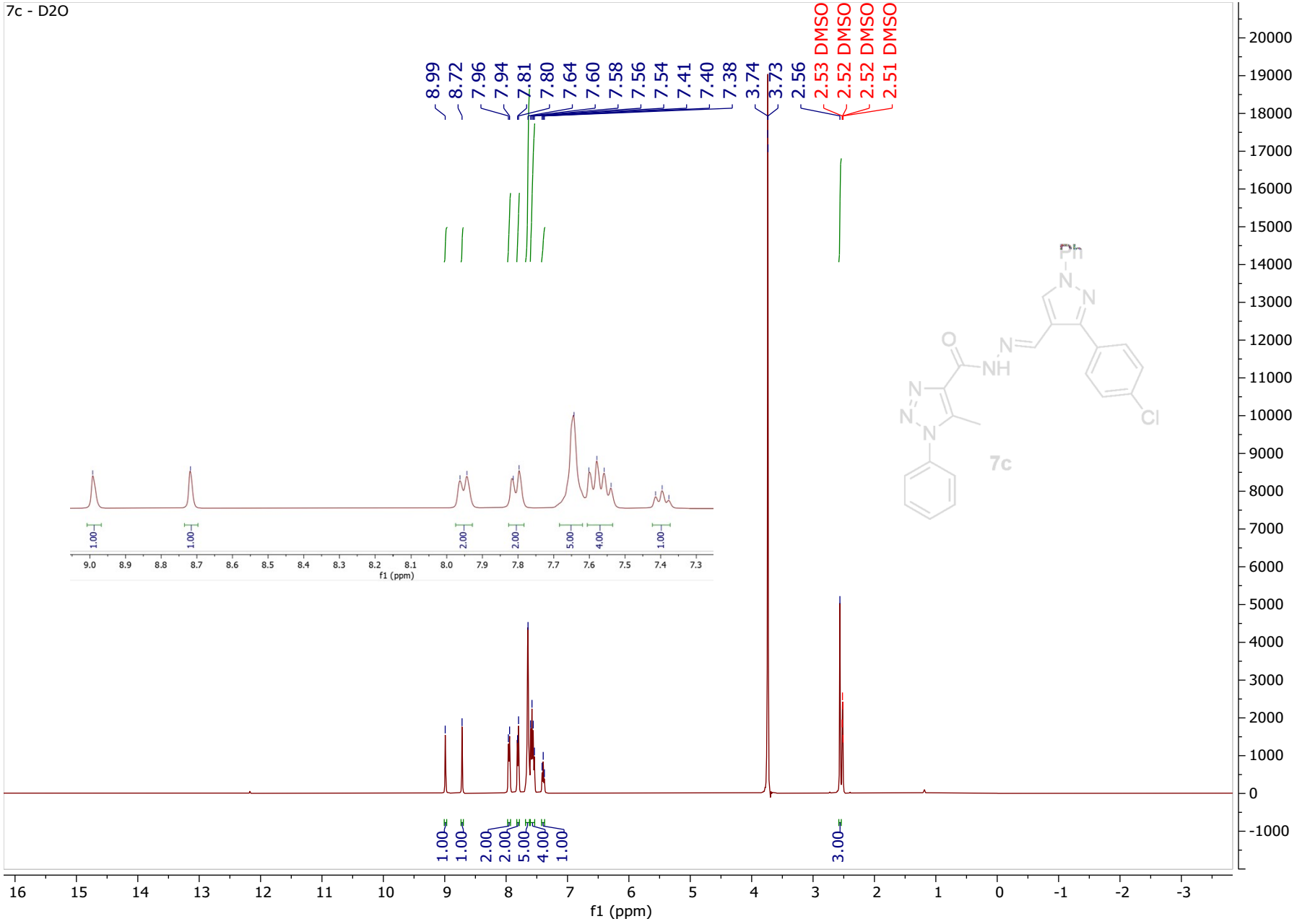

Figure S6. [1]HNMR Spectra – D_2O exchanged of Compound **7c**.

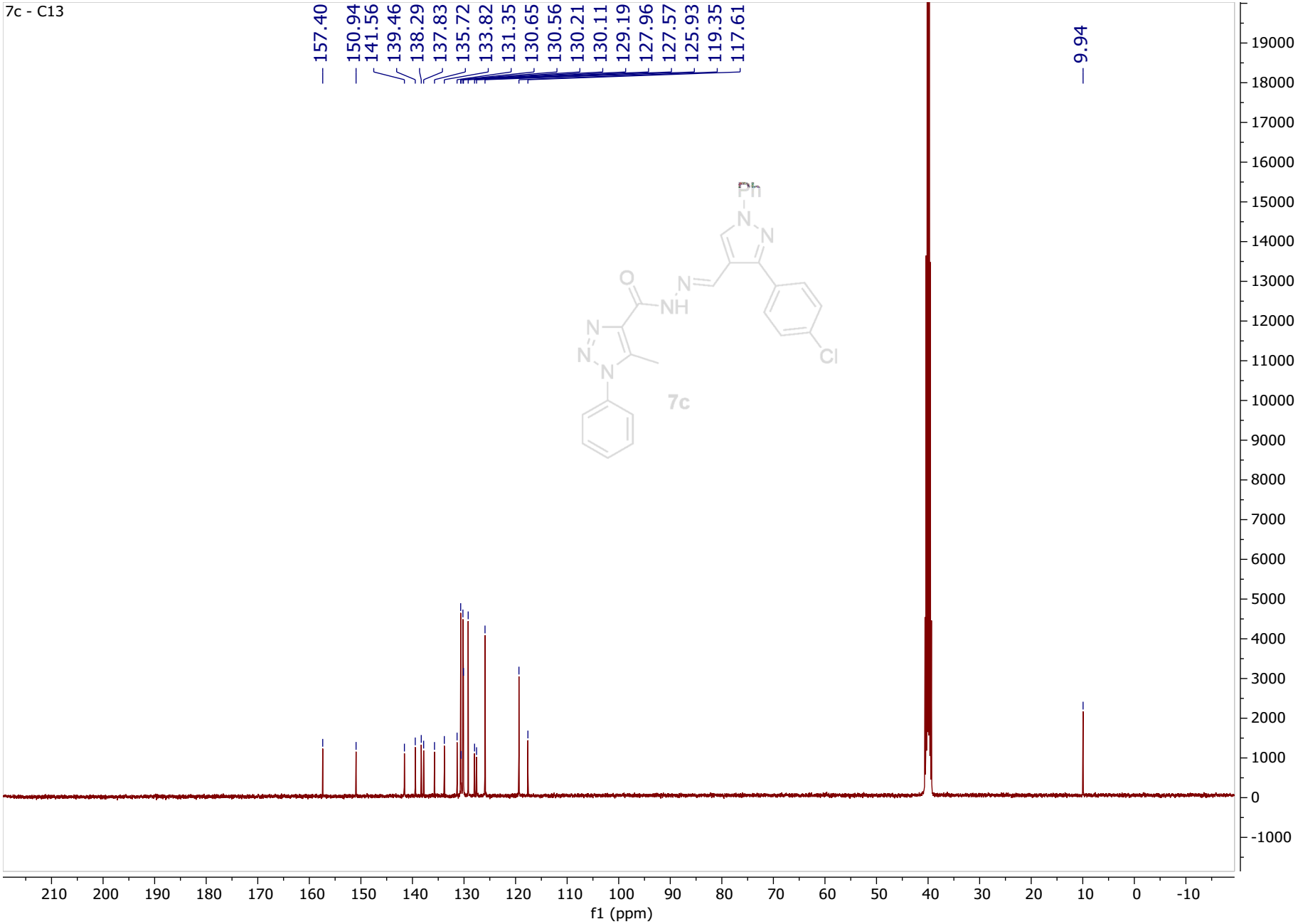

Figure S7. ^{13}CNMR Spectra of Compound **7c**.

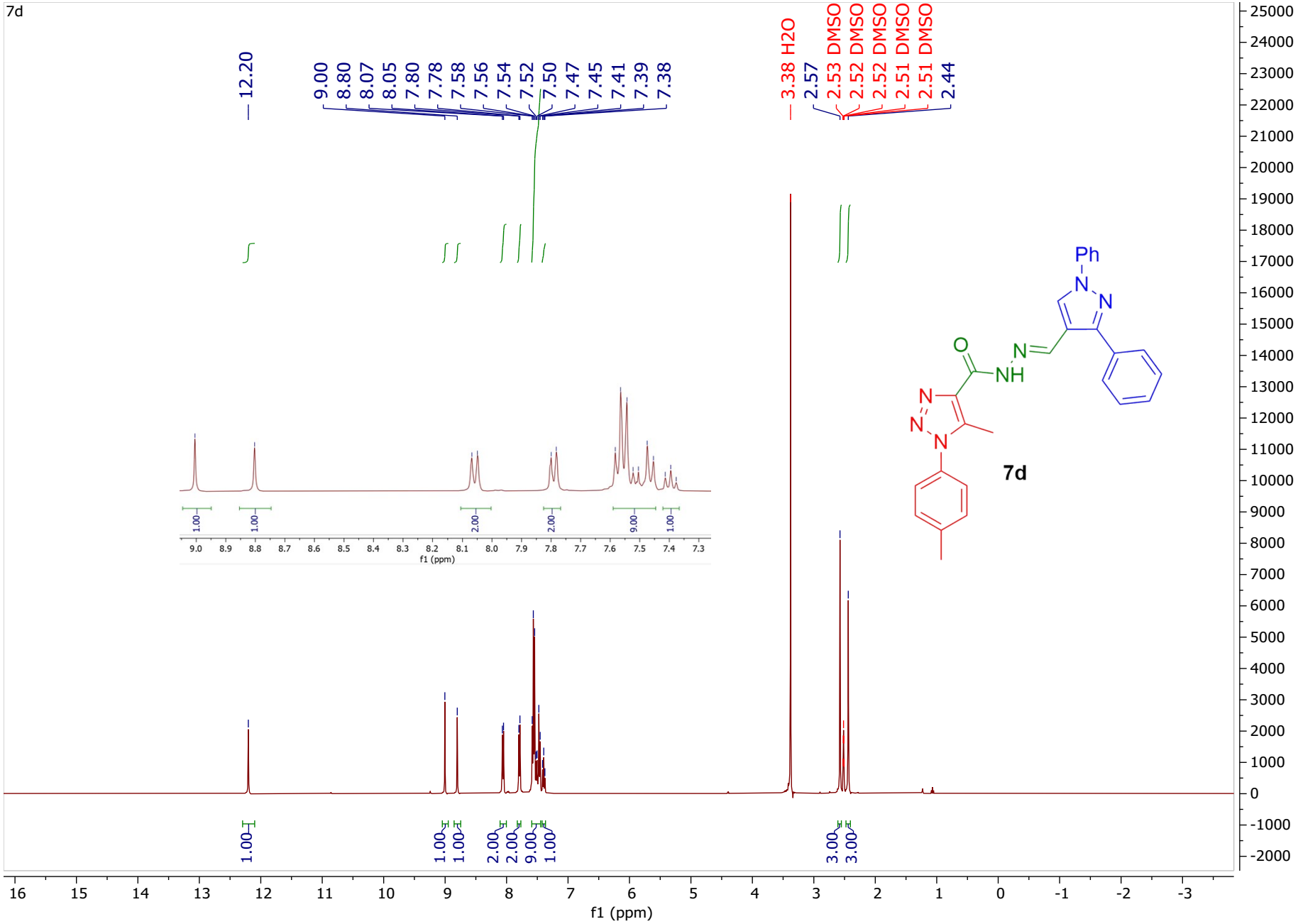

Figure S8. [1]HNMR Spectra of Compound **7d**.

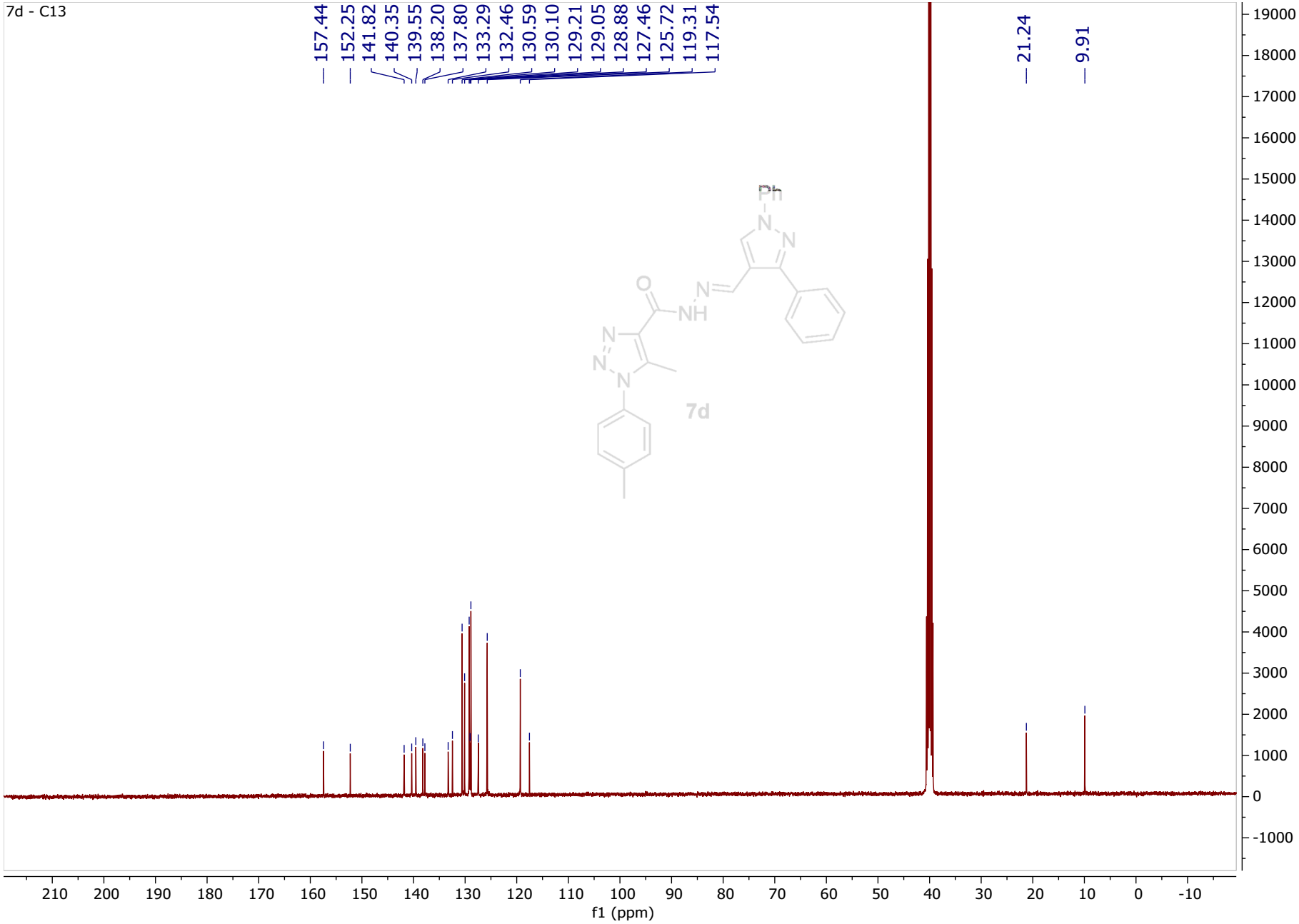

Figure S9. ^{13}CNMR Spectra of Compound **7d**.

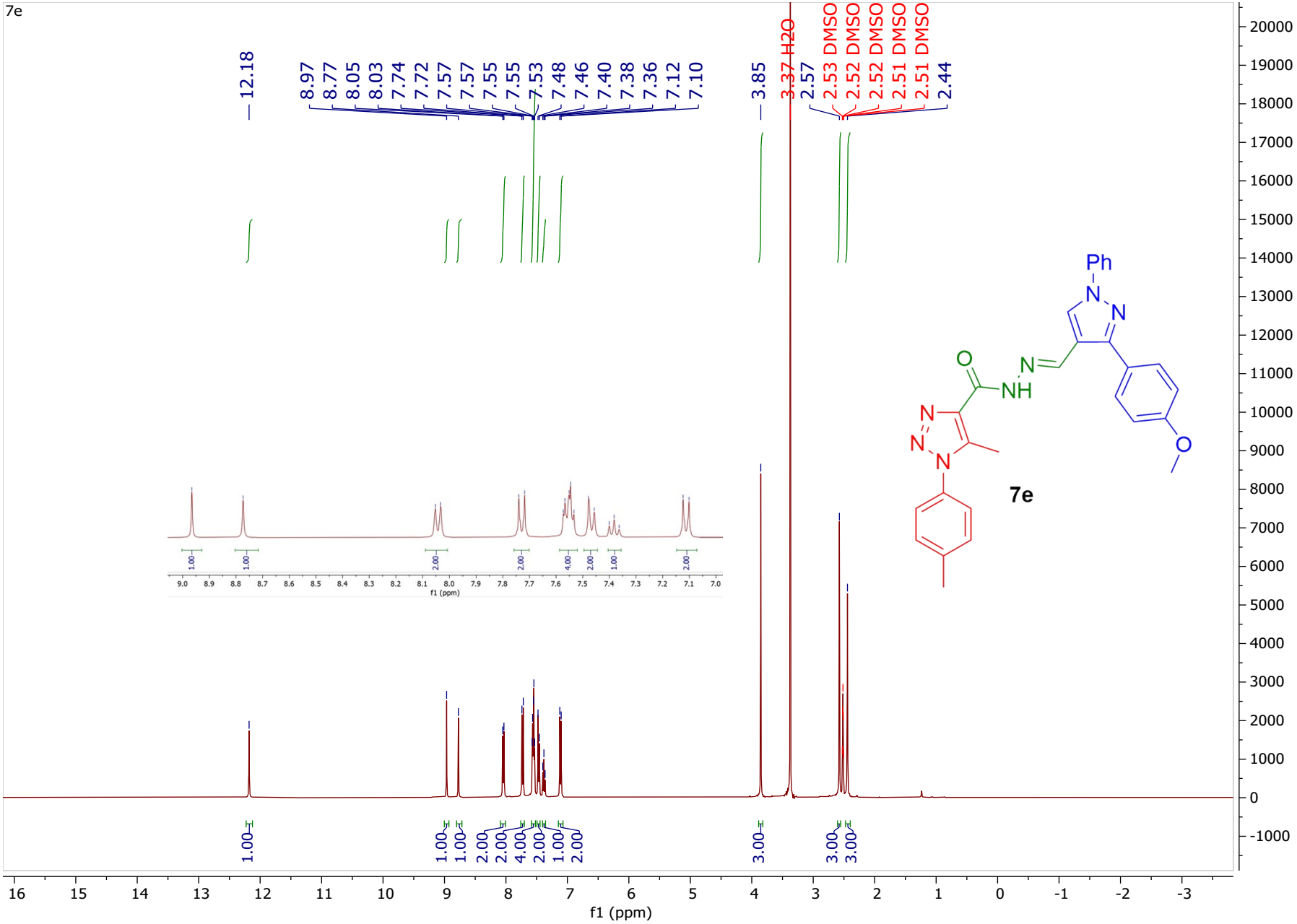

Figure S10. [1]HNMR Spectra of Compound **7e**.

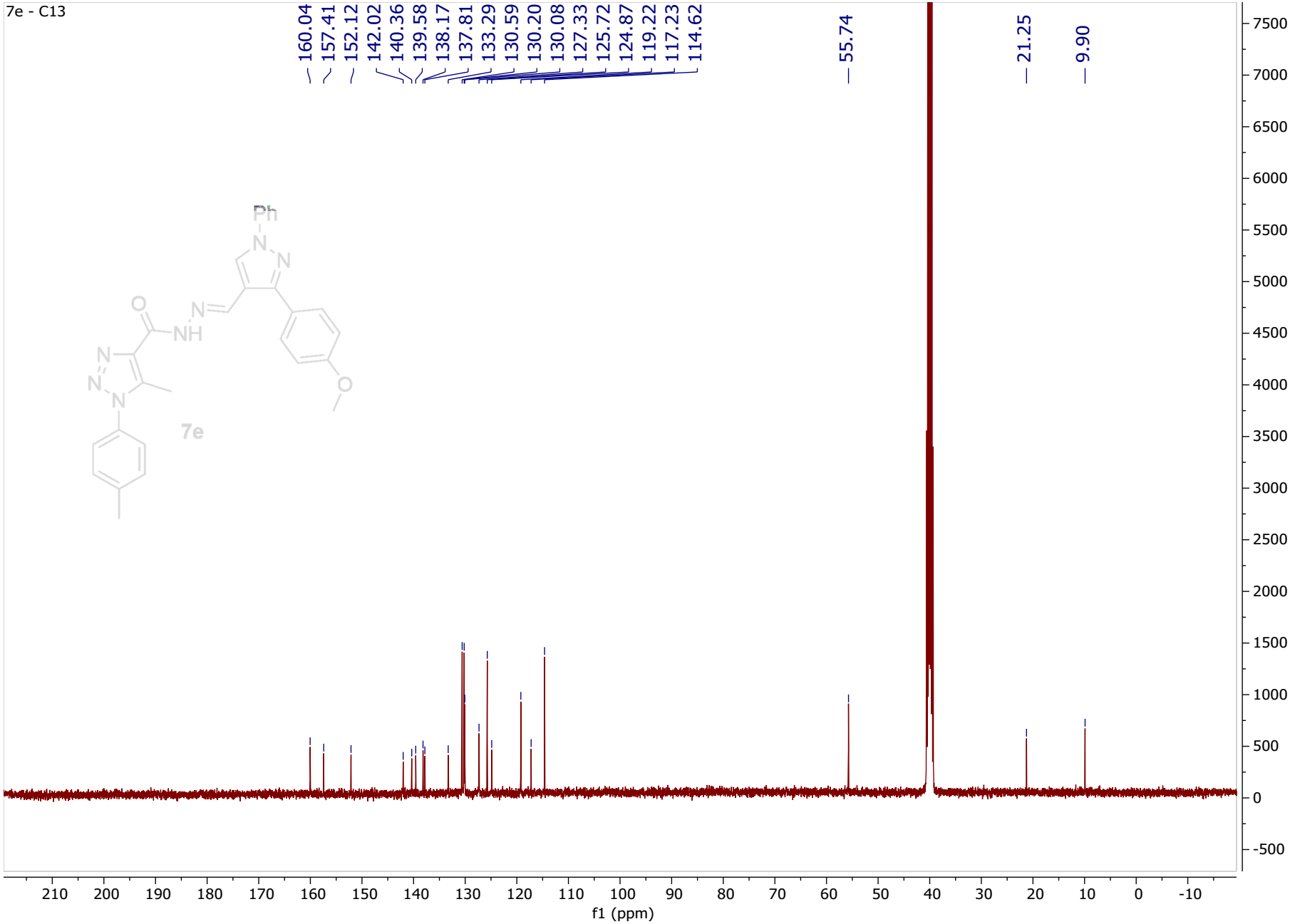

Figure S11. ^{13}CNMR Spectra of Compound **7e**.

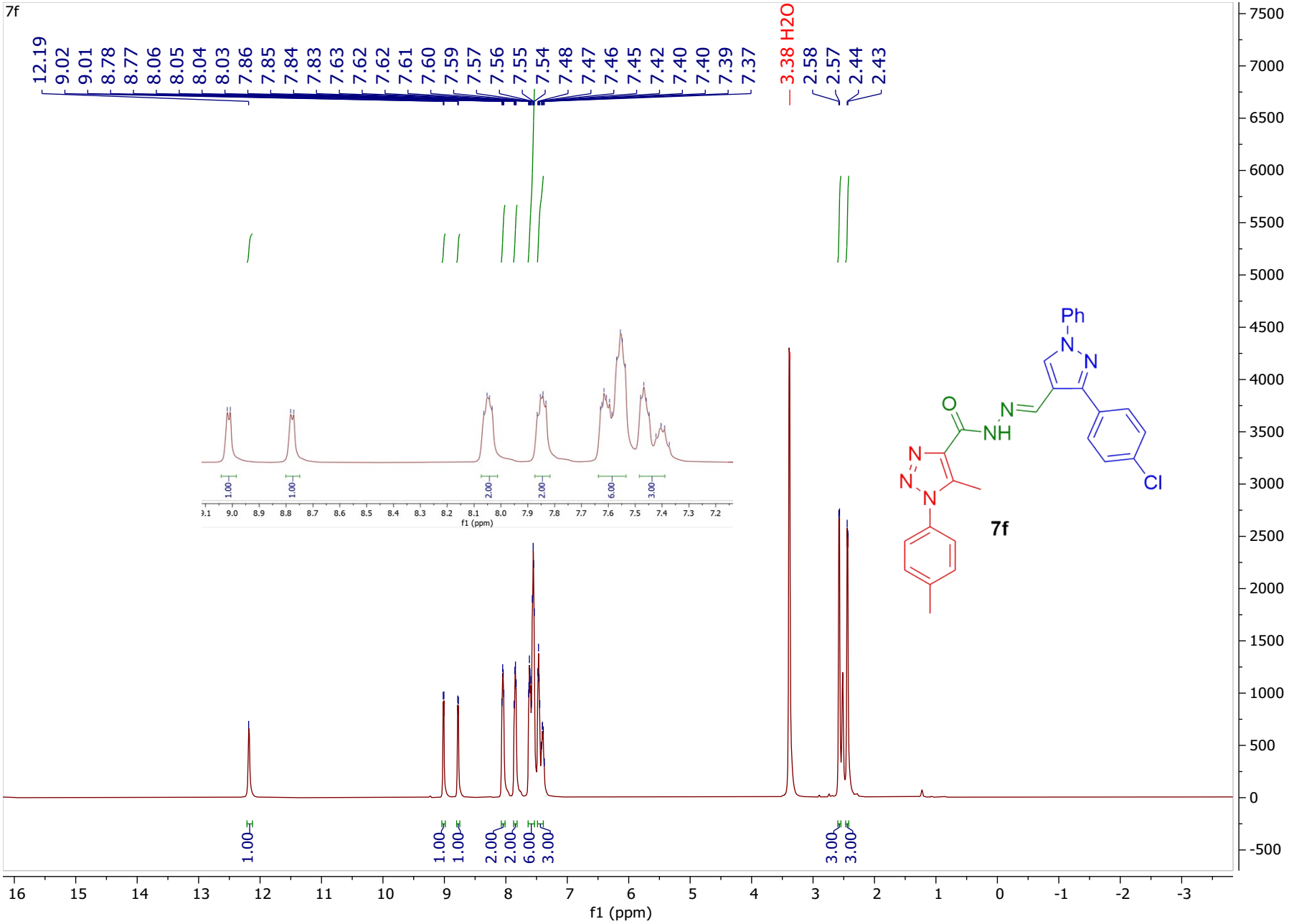

Figure S12. [1]HNMR Spectra of Compound **7f**.

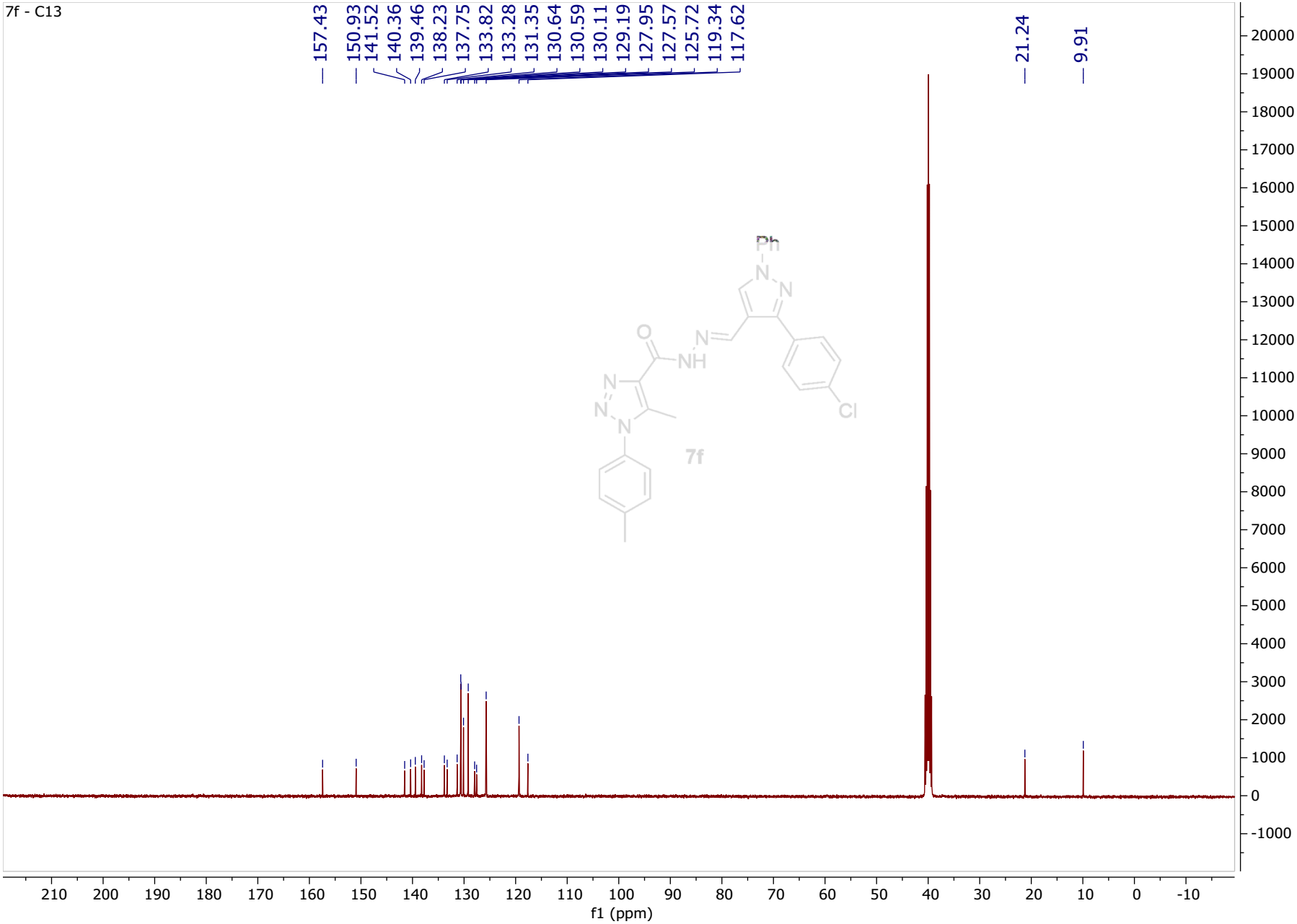

Figure S13. ^{13}CNMR Spectra of Compound **7f**.

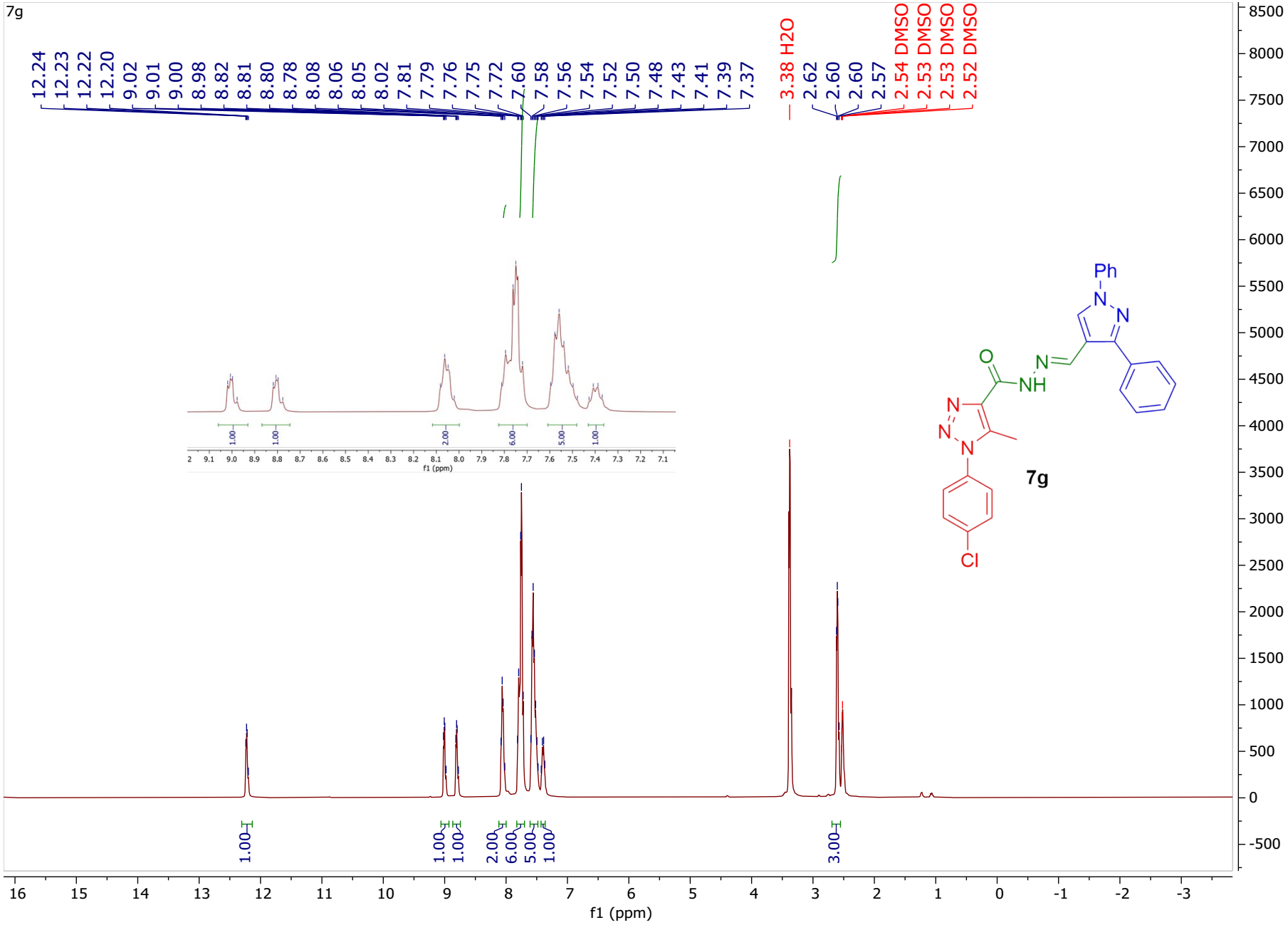

Figure S14. [1]HNMR Spectra of Compound **7g**.

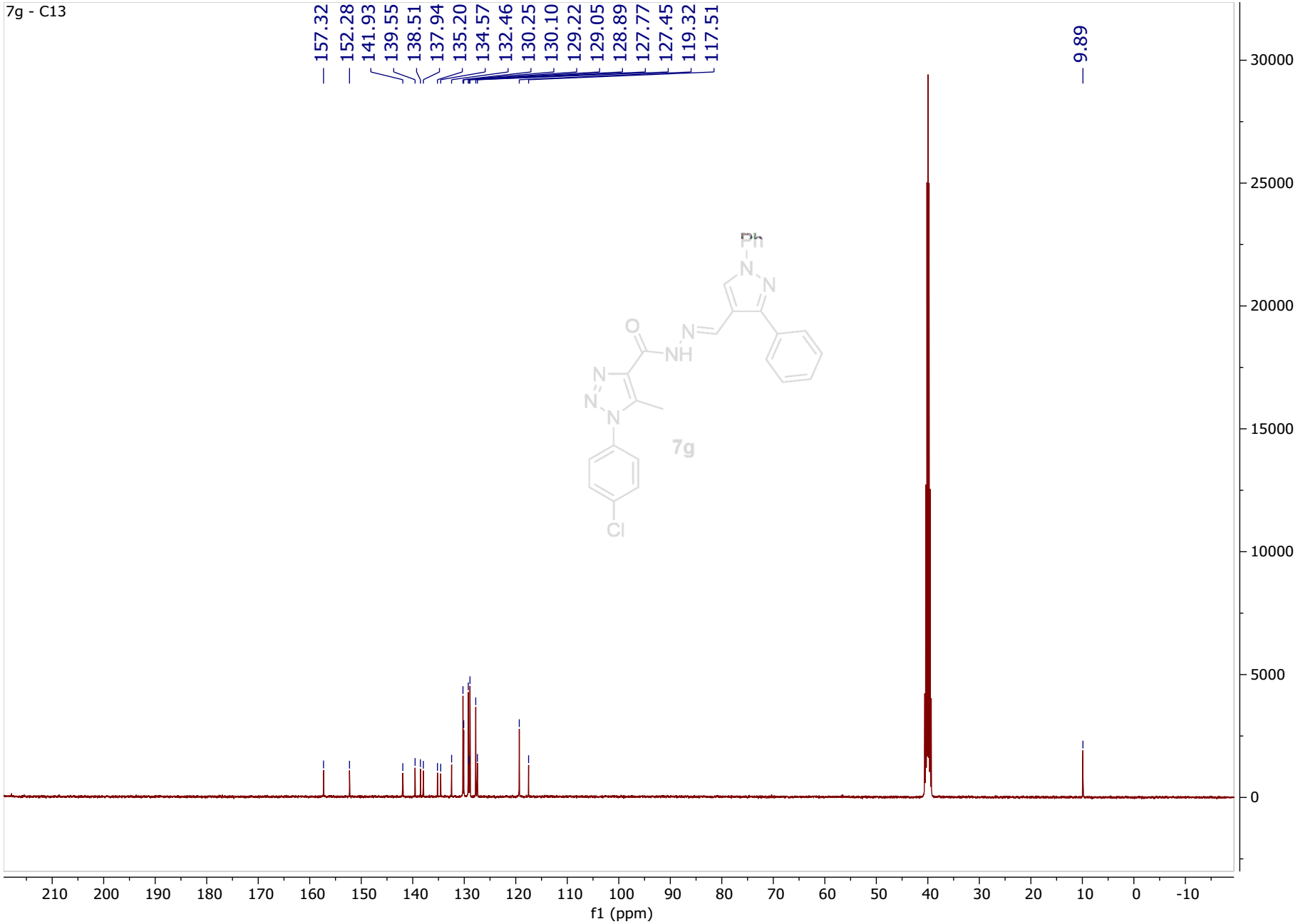

Figure S15. ^{13}CNMR Spectra of Compound **7g**.

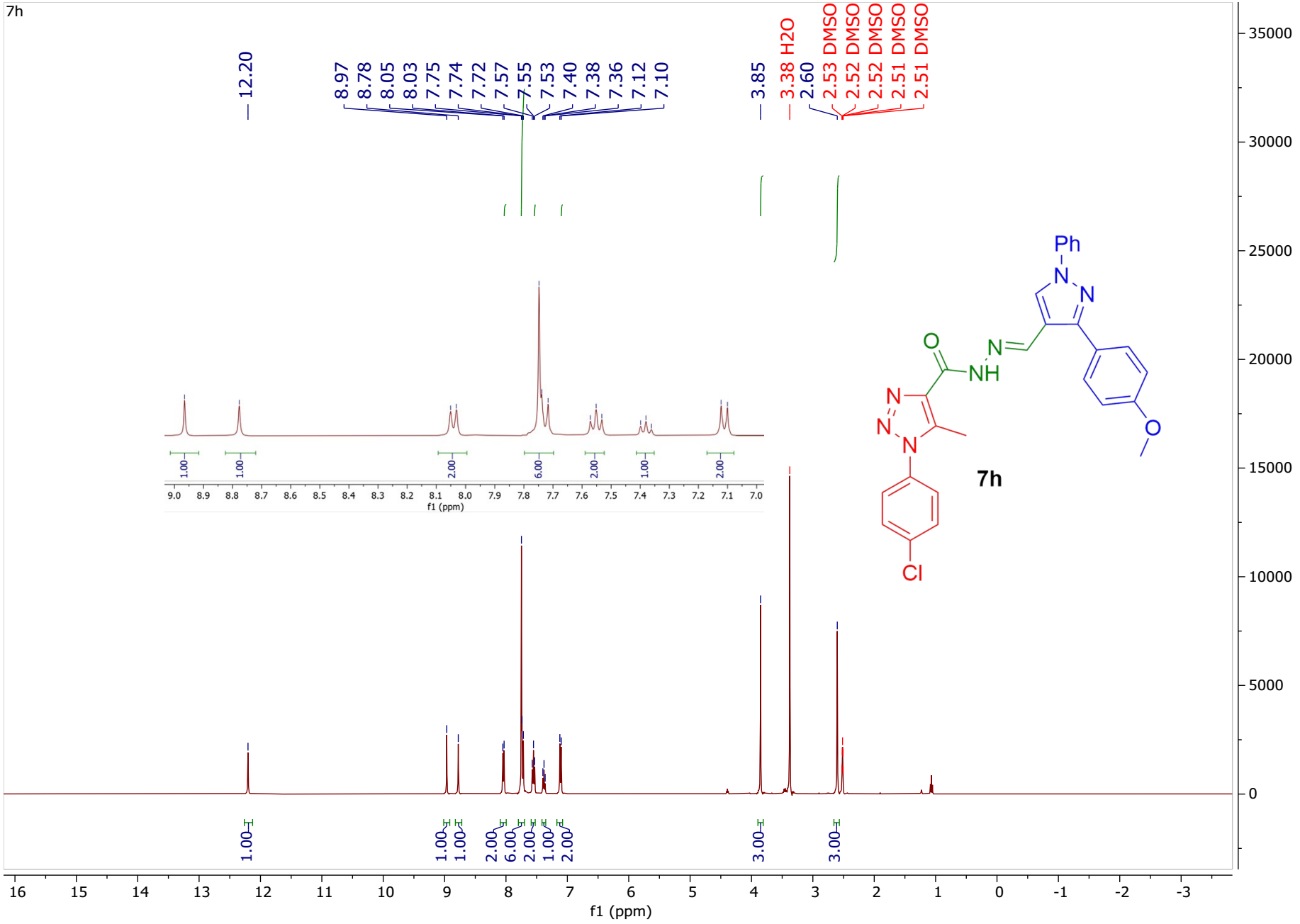

Figure S16. [1]HNMR Spectra of Compound **7h**.

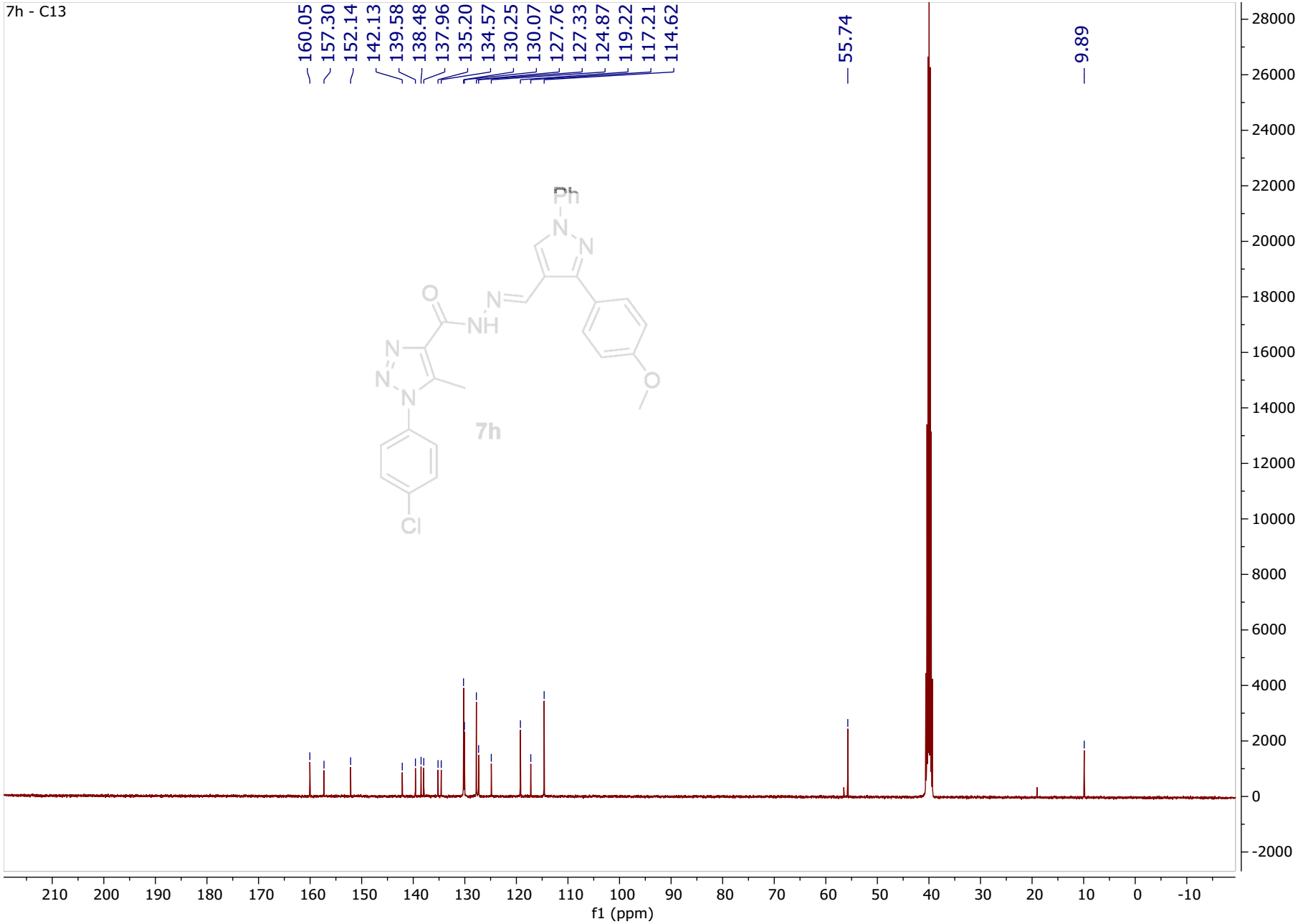

Figure S17. ^{13}CNMR Spectra of Compound **7h**.

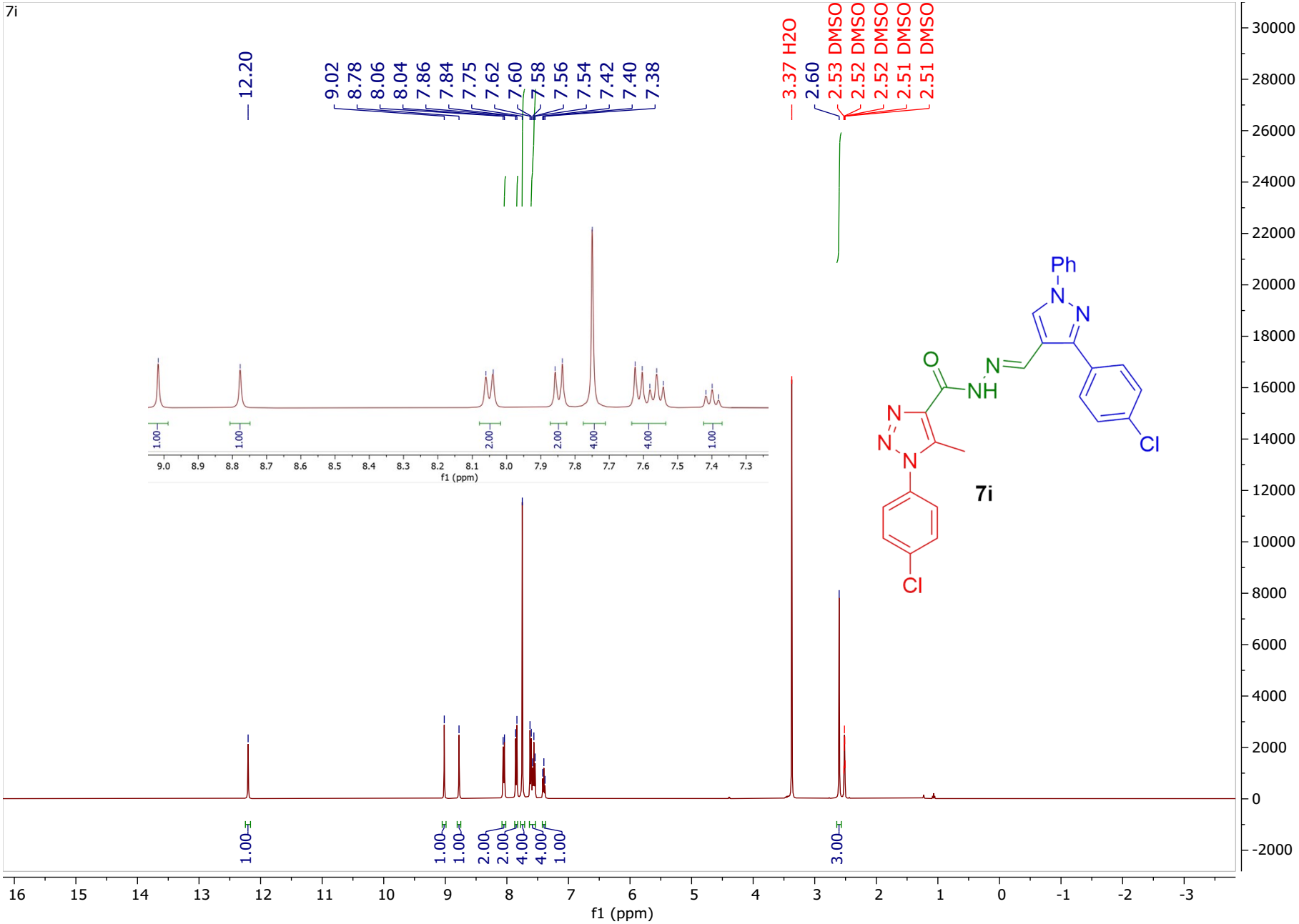

Figure S18. [1]HNMR Spectra of Compound **7i**.

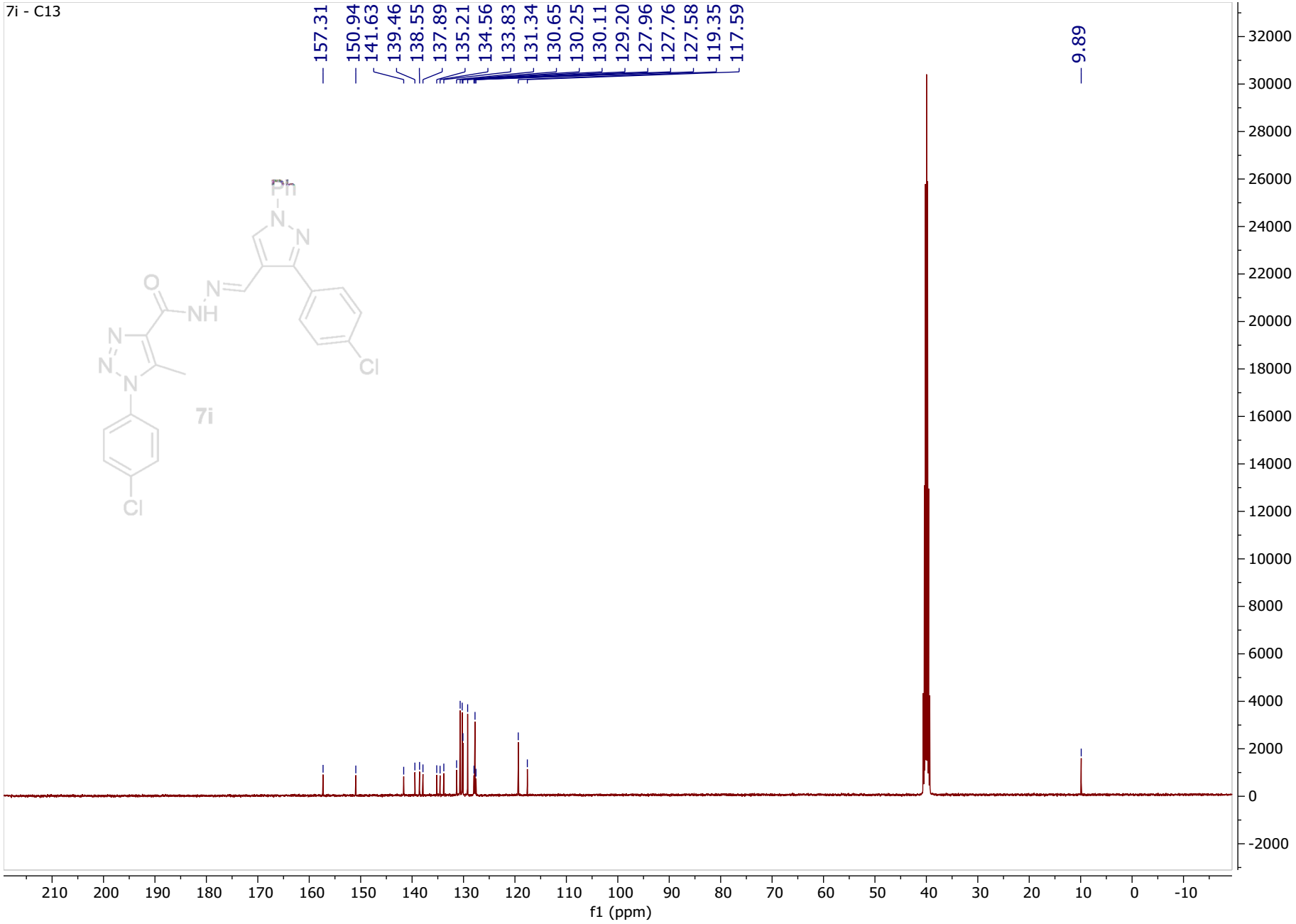

Figure S19. ^{13}CNMR Spectra of Compound **7i**.

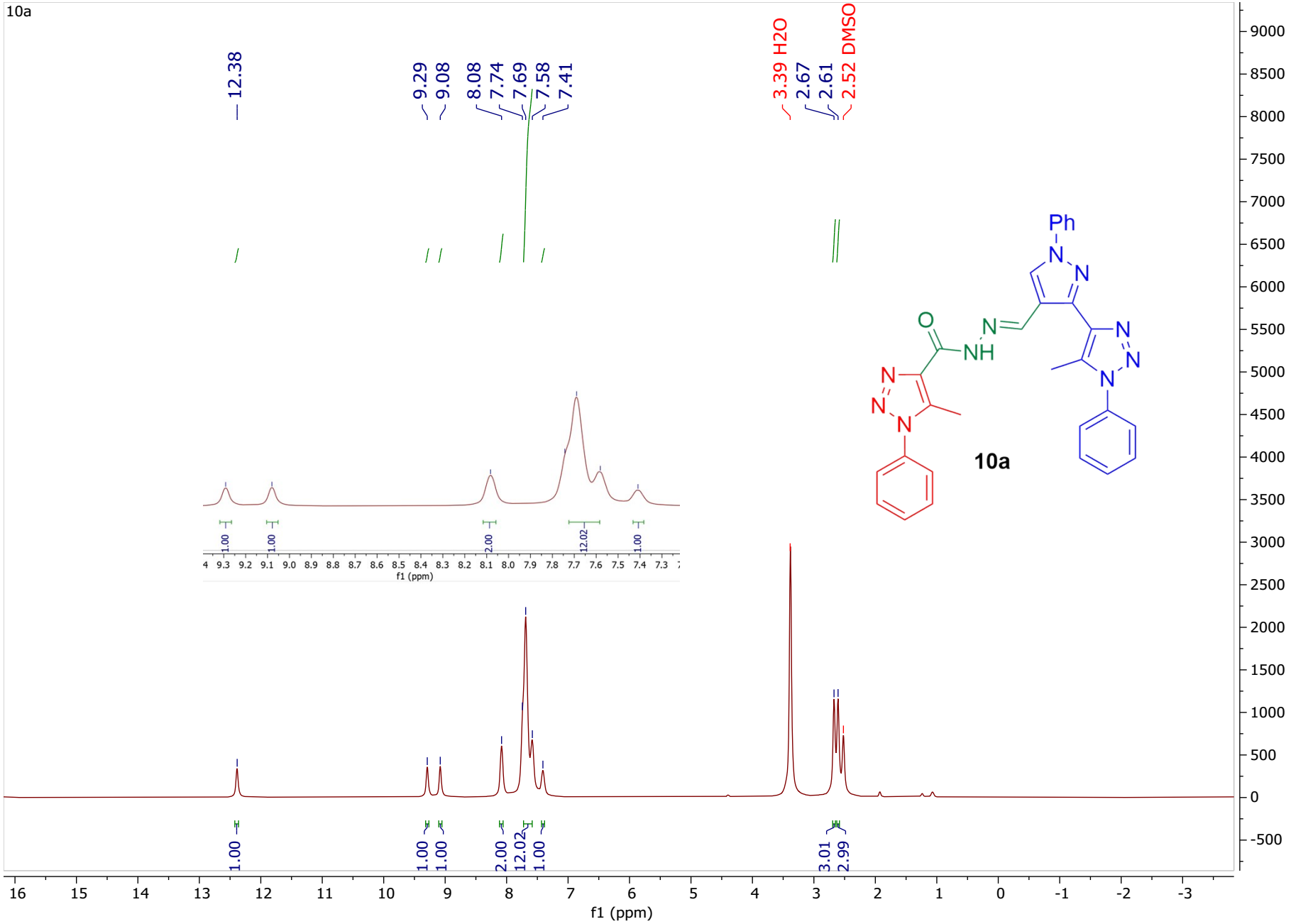

Figure S20. [1]HNMR Spectra of Compound **10a**.

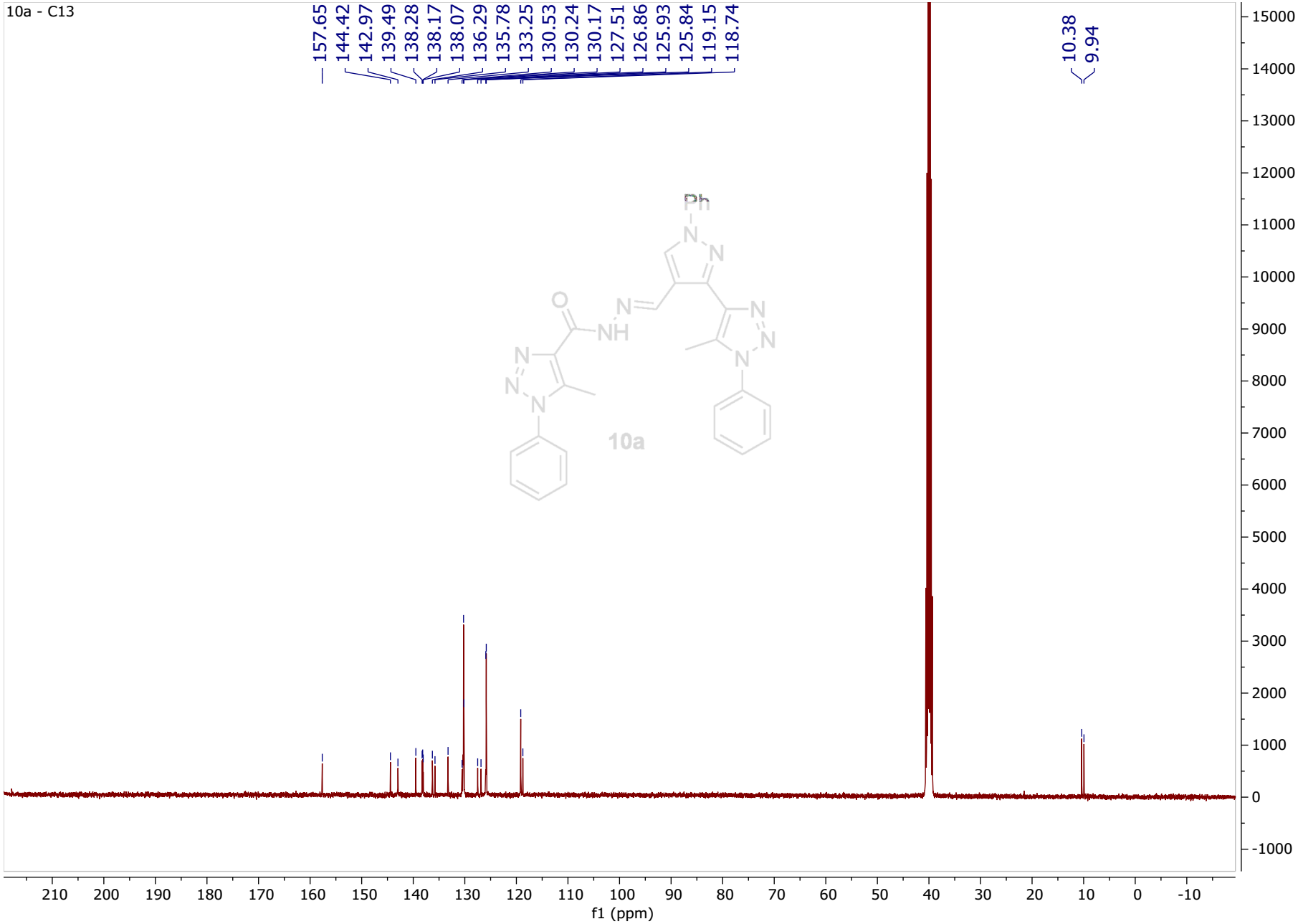

Figure S21. ^{13}CNMR Spectra of Compound **10a**.

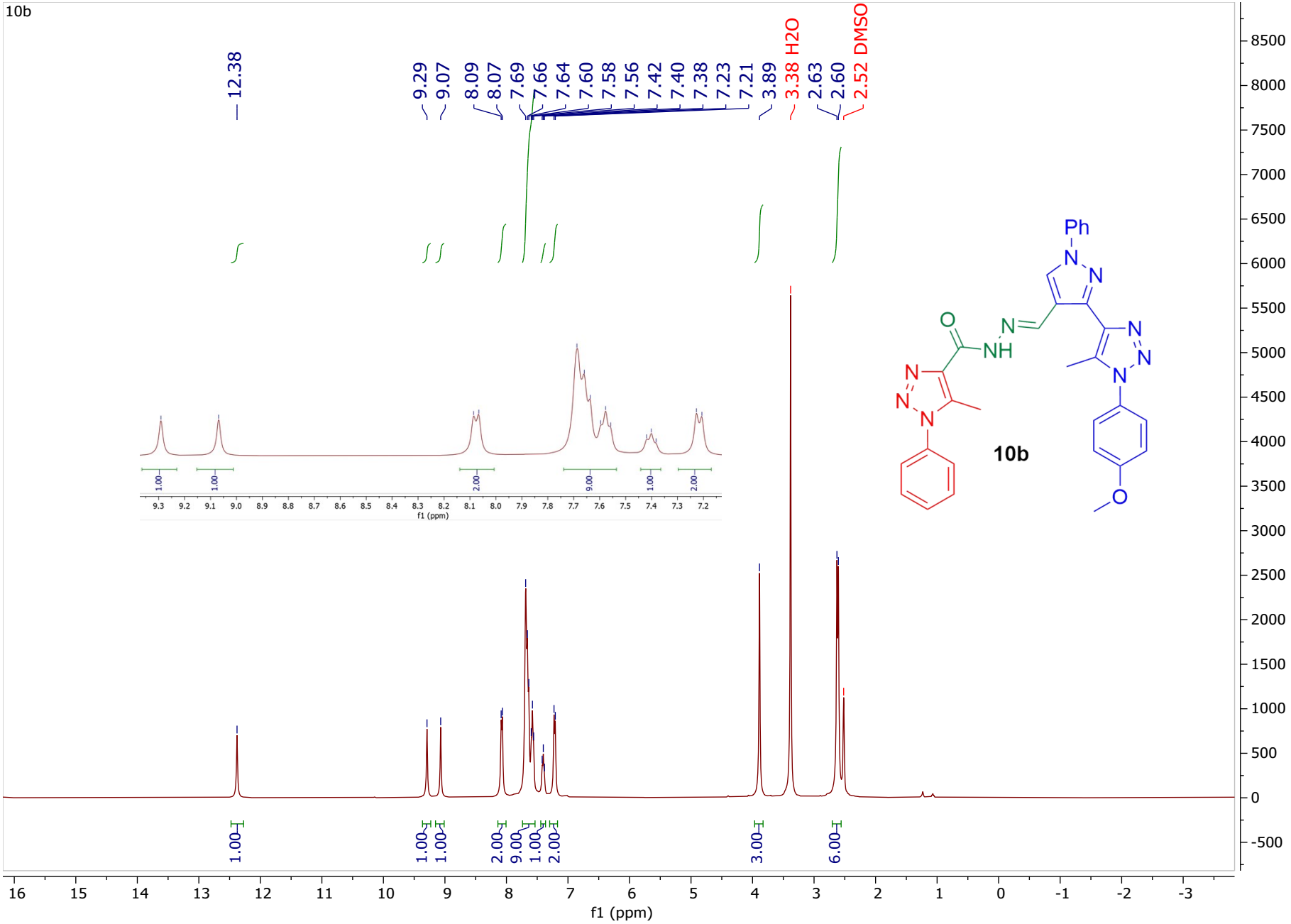

Figure S22. [1]HNMR Spectra of Compound **10b**.

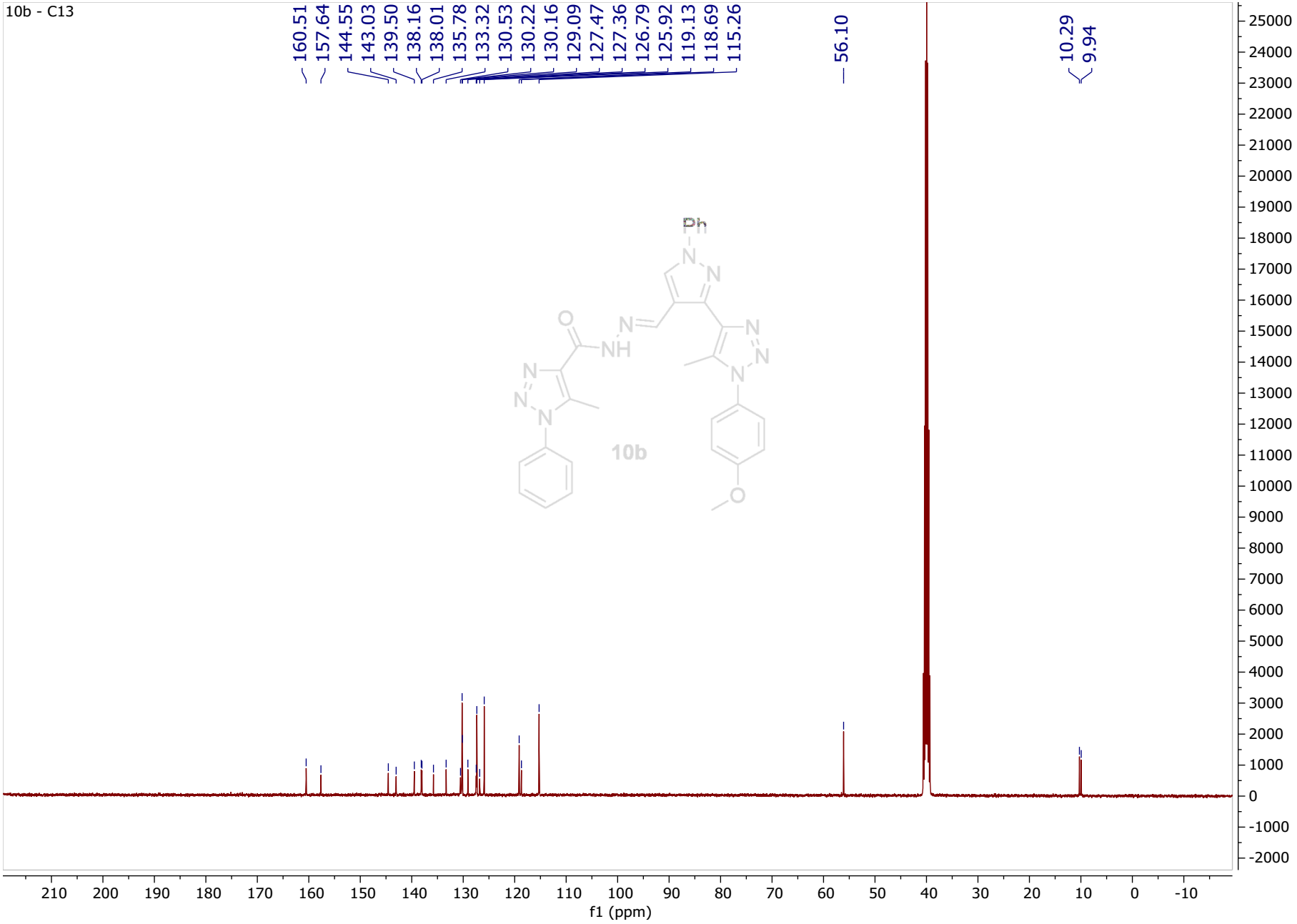

Figure S23. ^{13}CNMR Spectra of Compound **10b**.

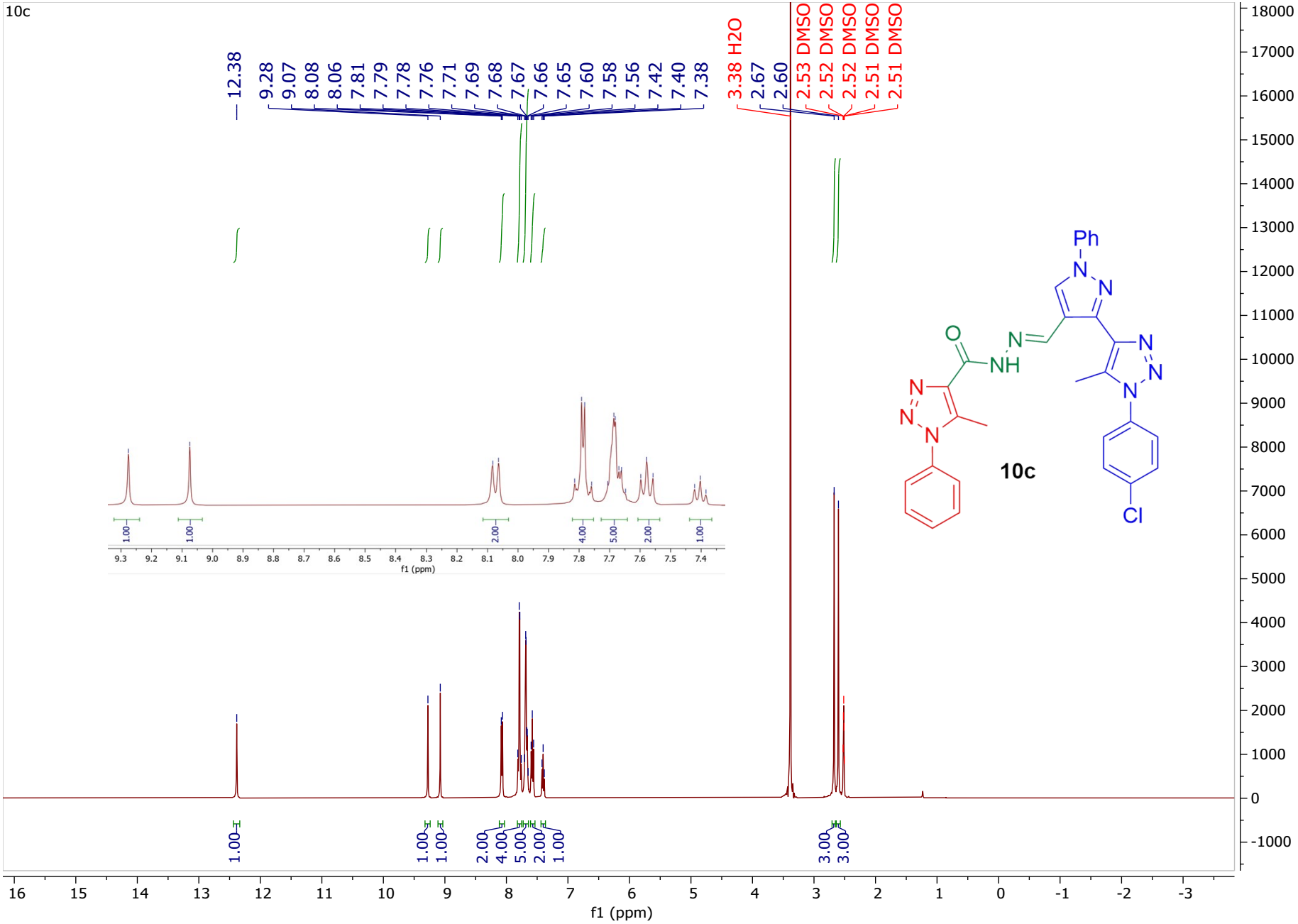

Figure S24. [1]HNMR Spectra of Compound **10c**.

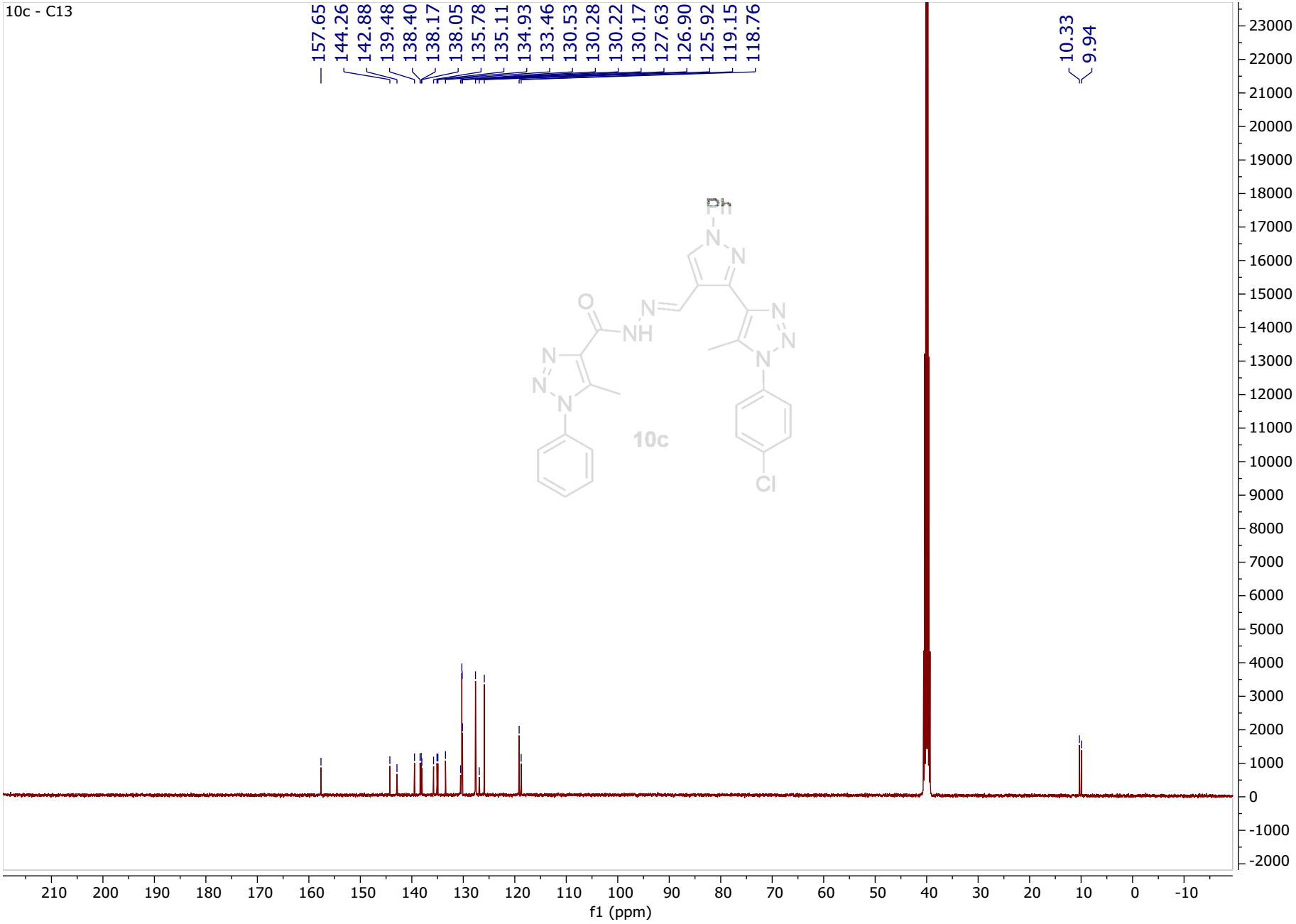

Figure S25. ^{13}CNMR Spectra of Compound **10c**.

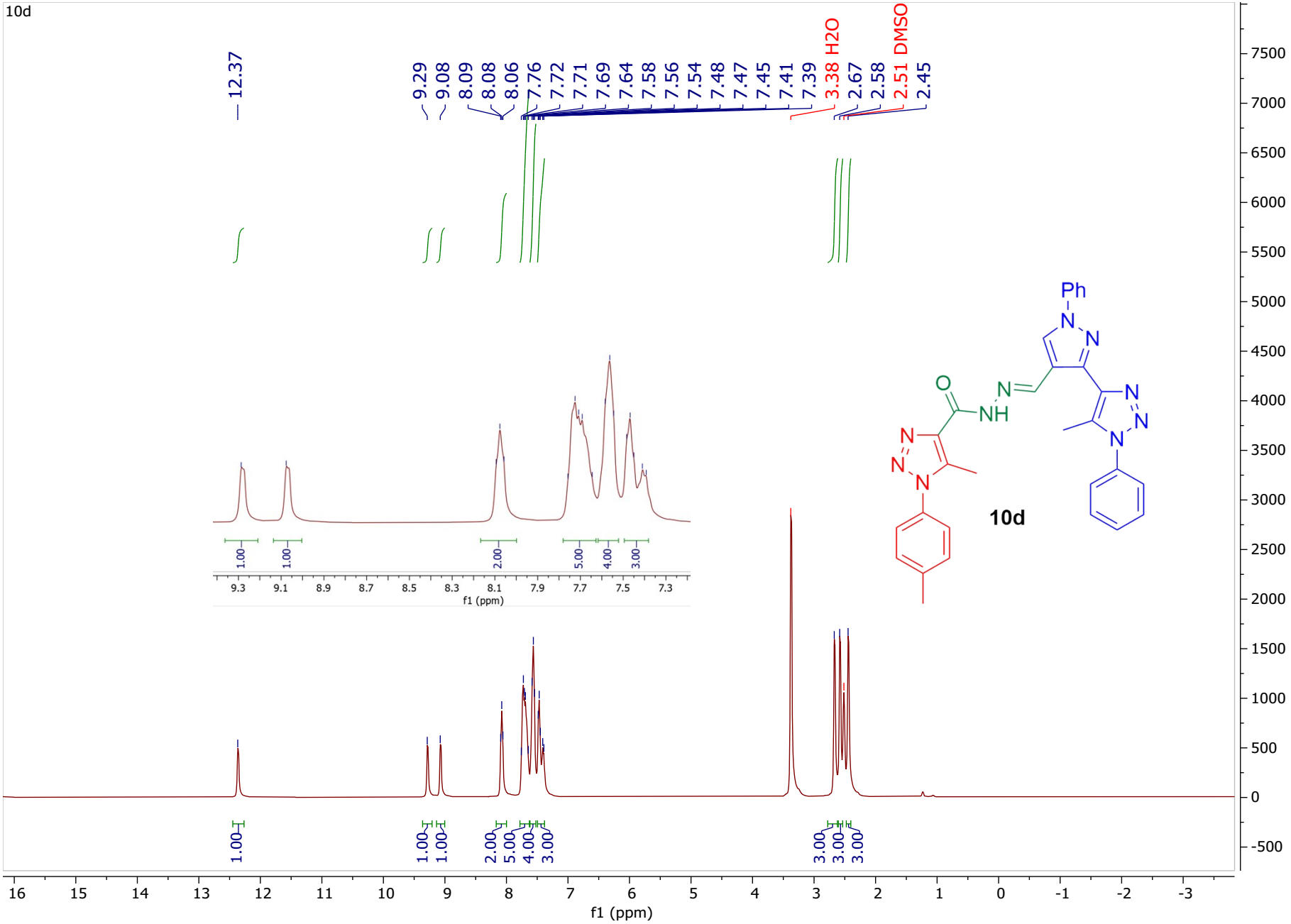

Figure S26. [1]HNMR Spectra of Compound **10d**.

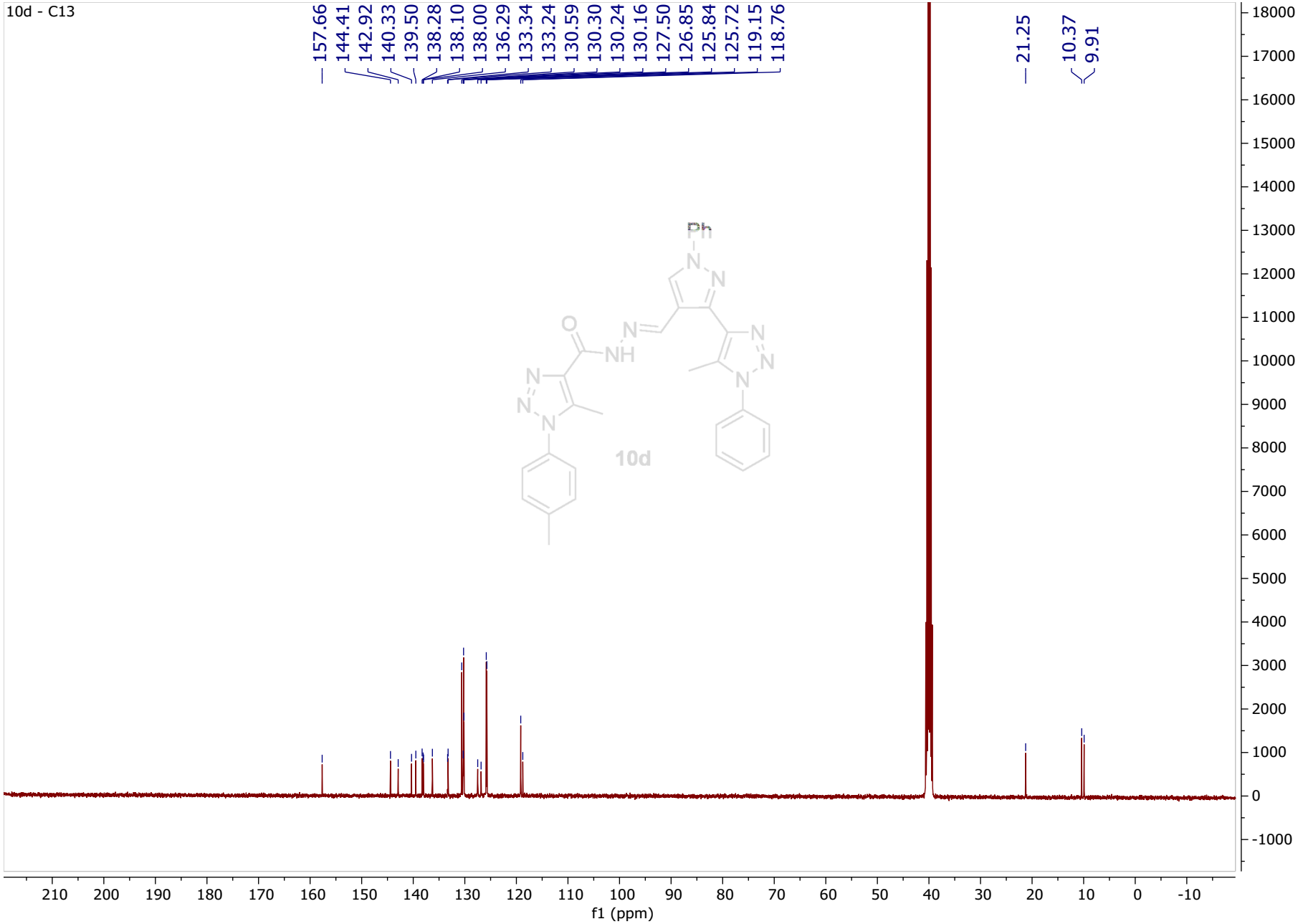

Figure S27. ^{13}CNMR Spectra of Compound **10d**.

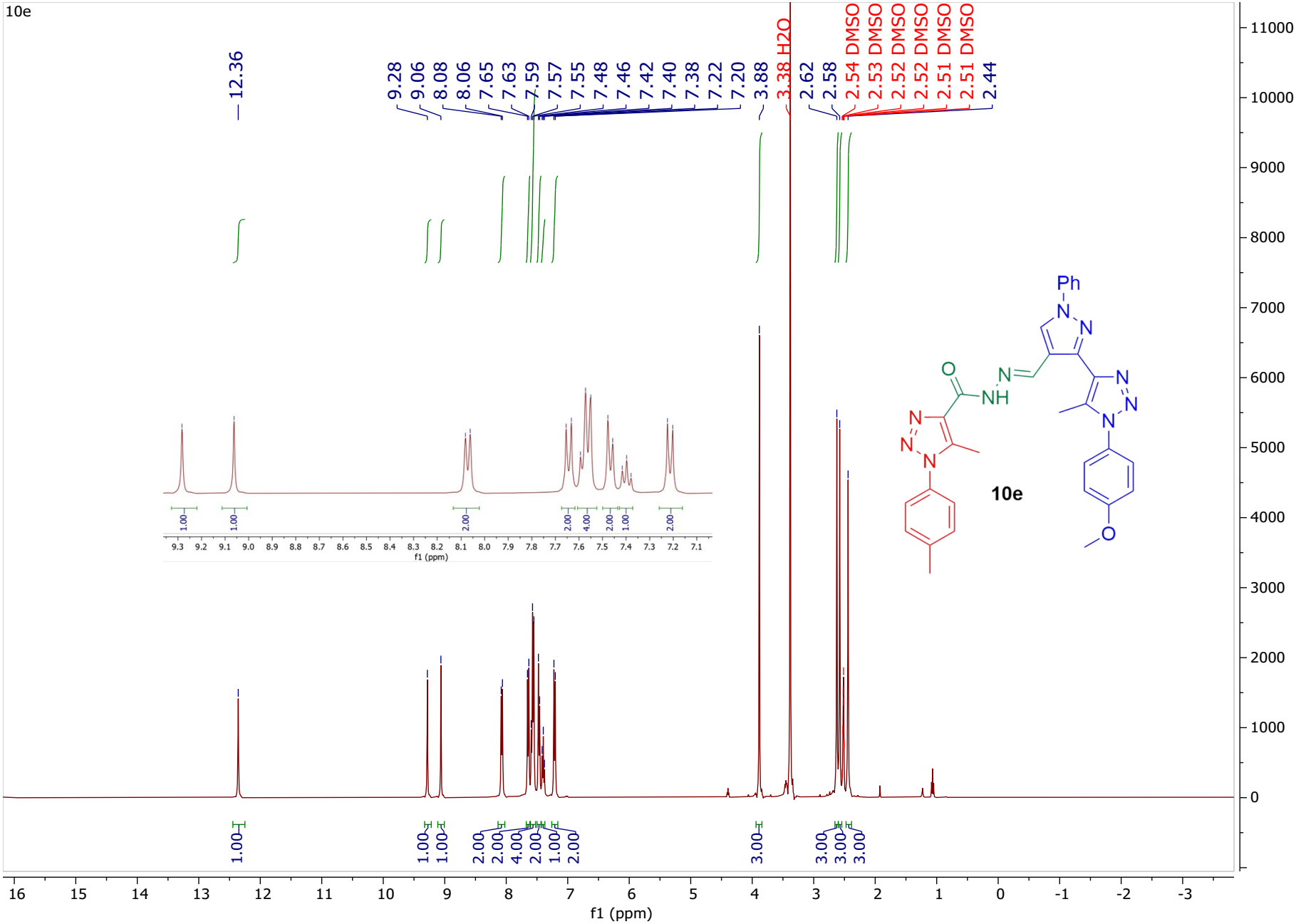

Figure S28. [1]HNMR Spectra of Compound **10e**.

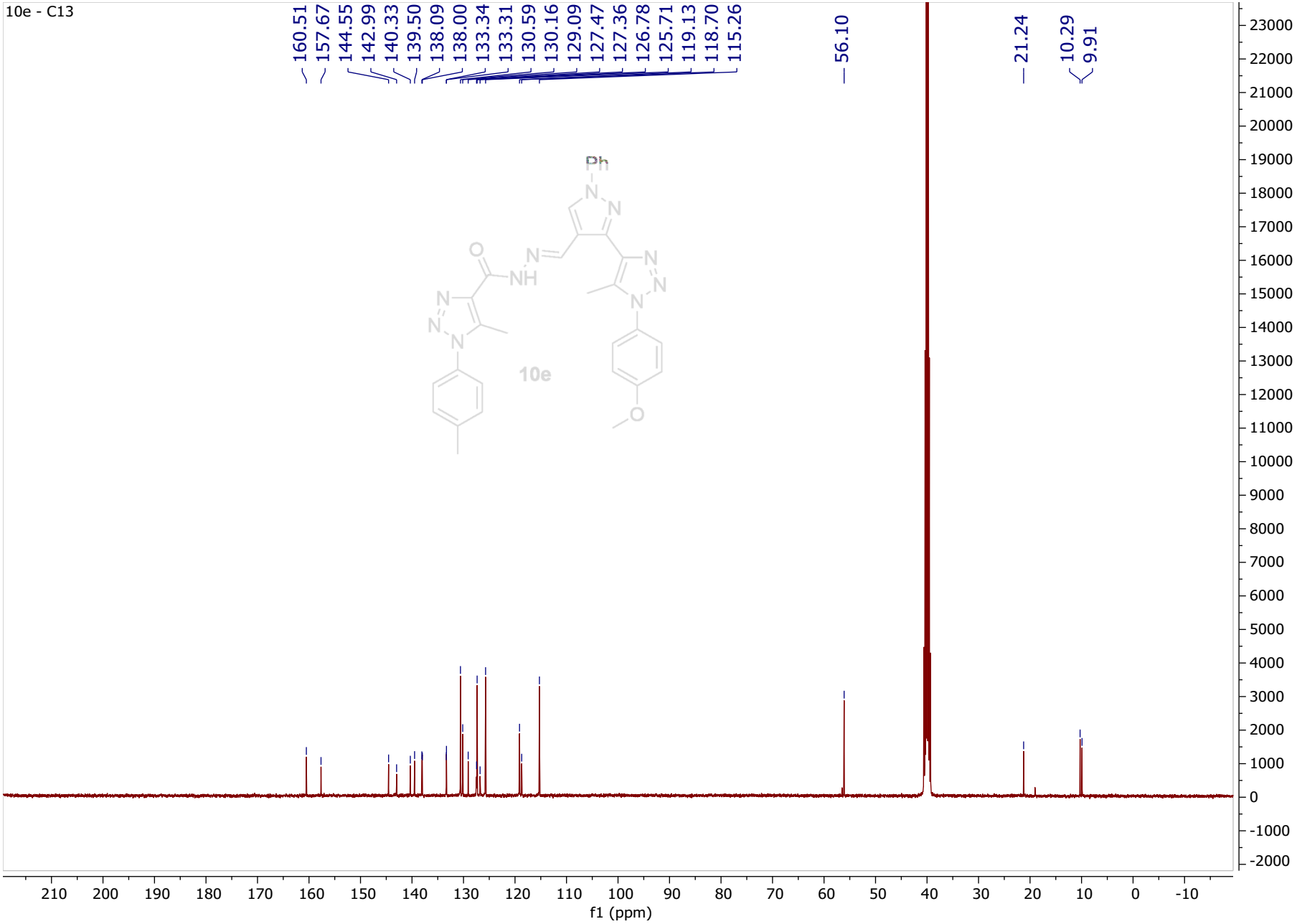

Figure S29. ^{13}CNMR Spectra of Compound **10e**.

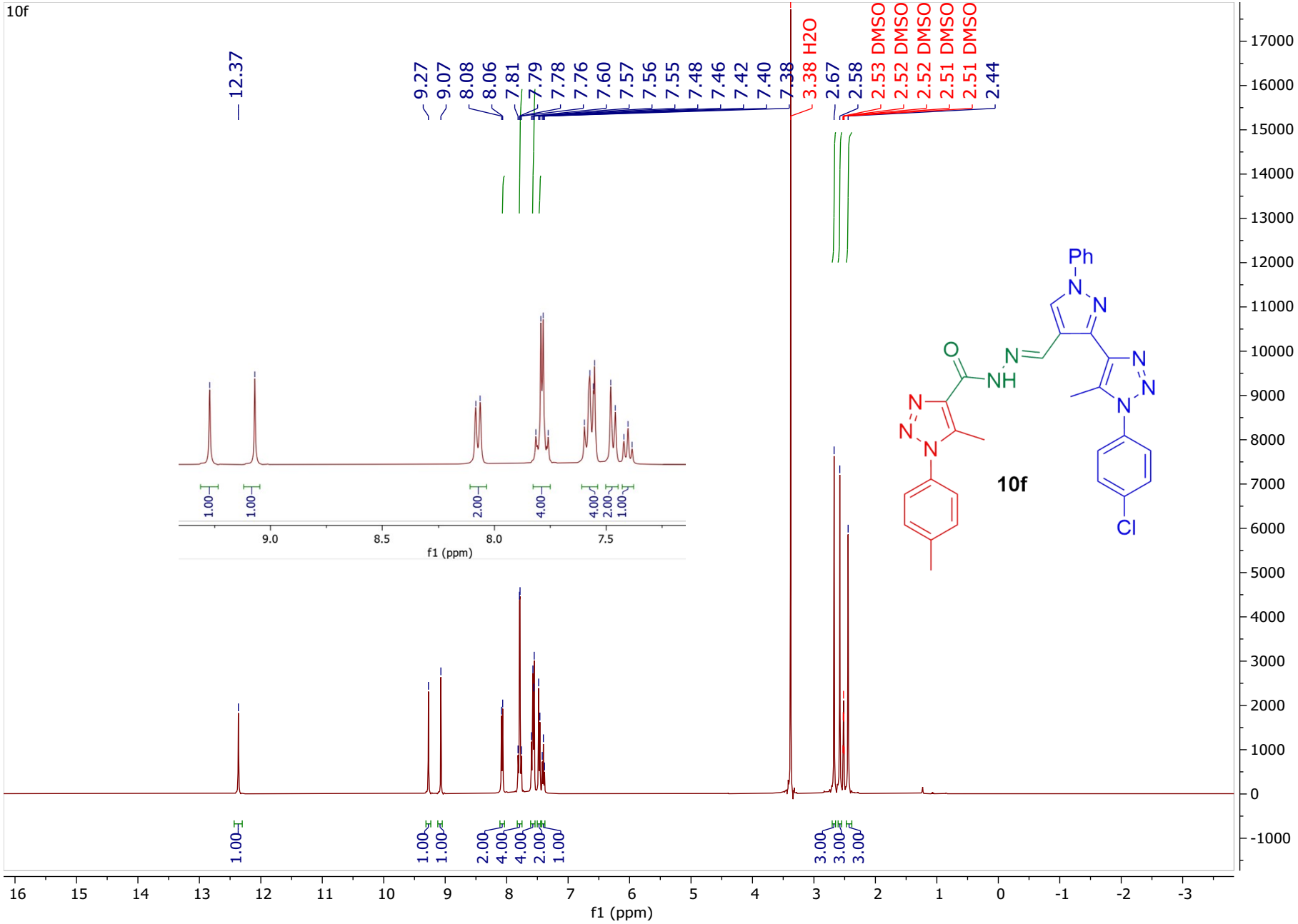

Figure S30. [1]HNMR Spectra of Compound **10f**.

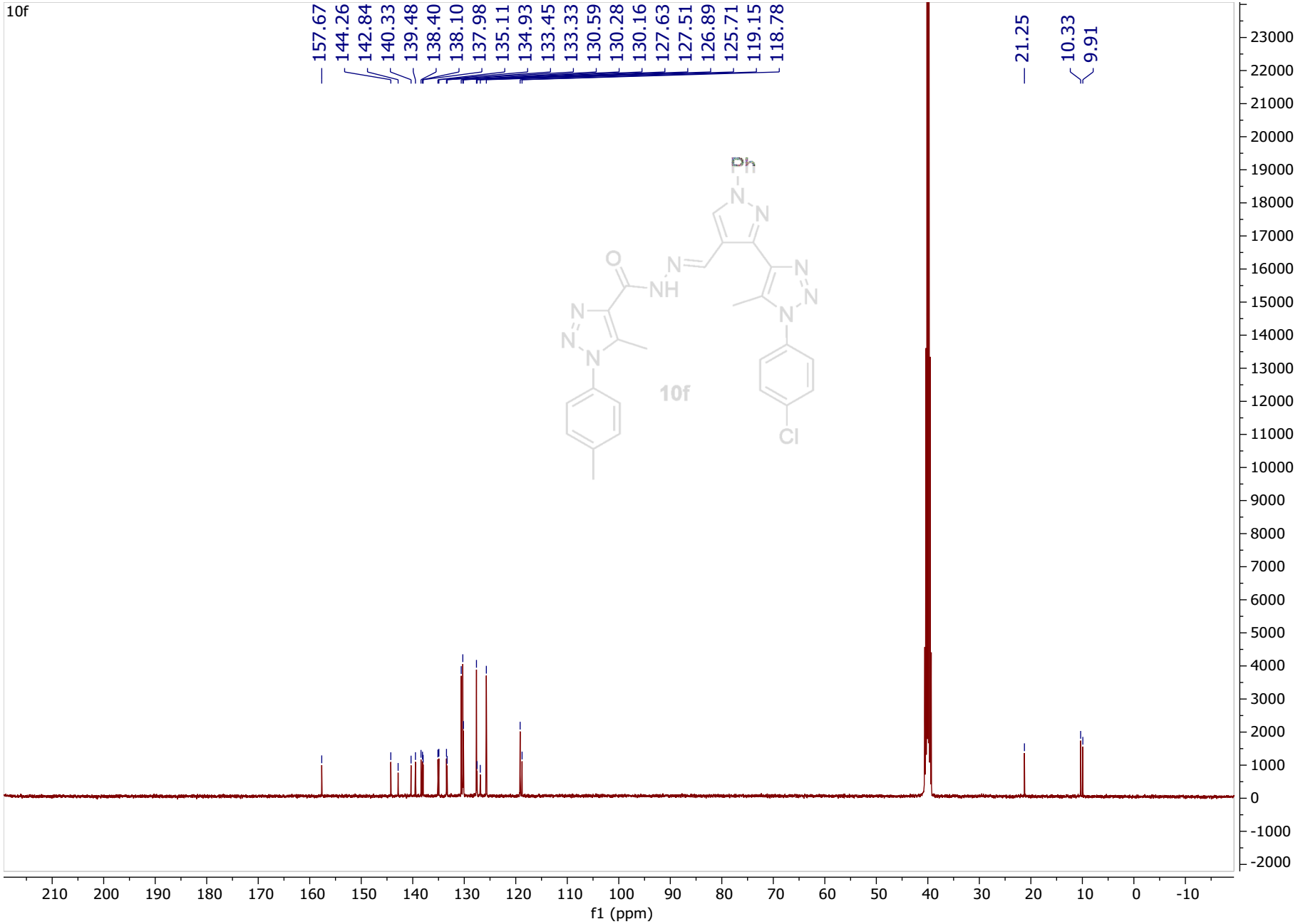

Figure S31. ^{13}CNMR Spectra of Compound **10f**.

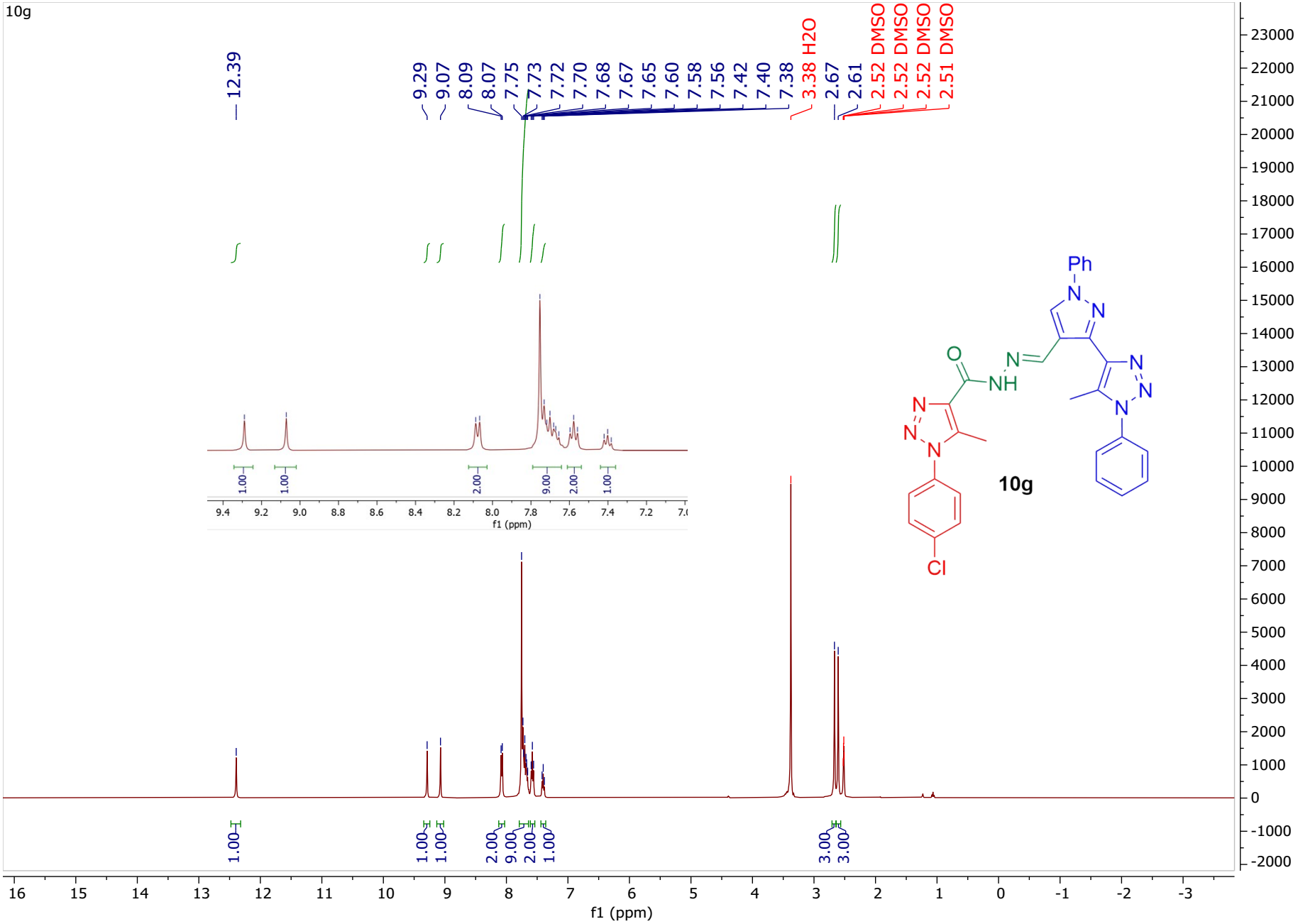

Figure S32. [1]HNMR Spectra of Compound **10g**.

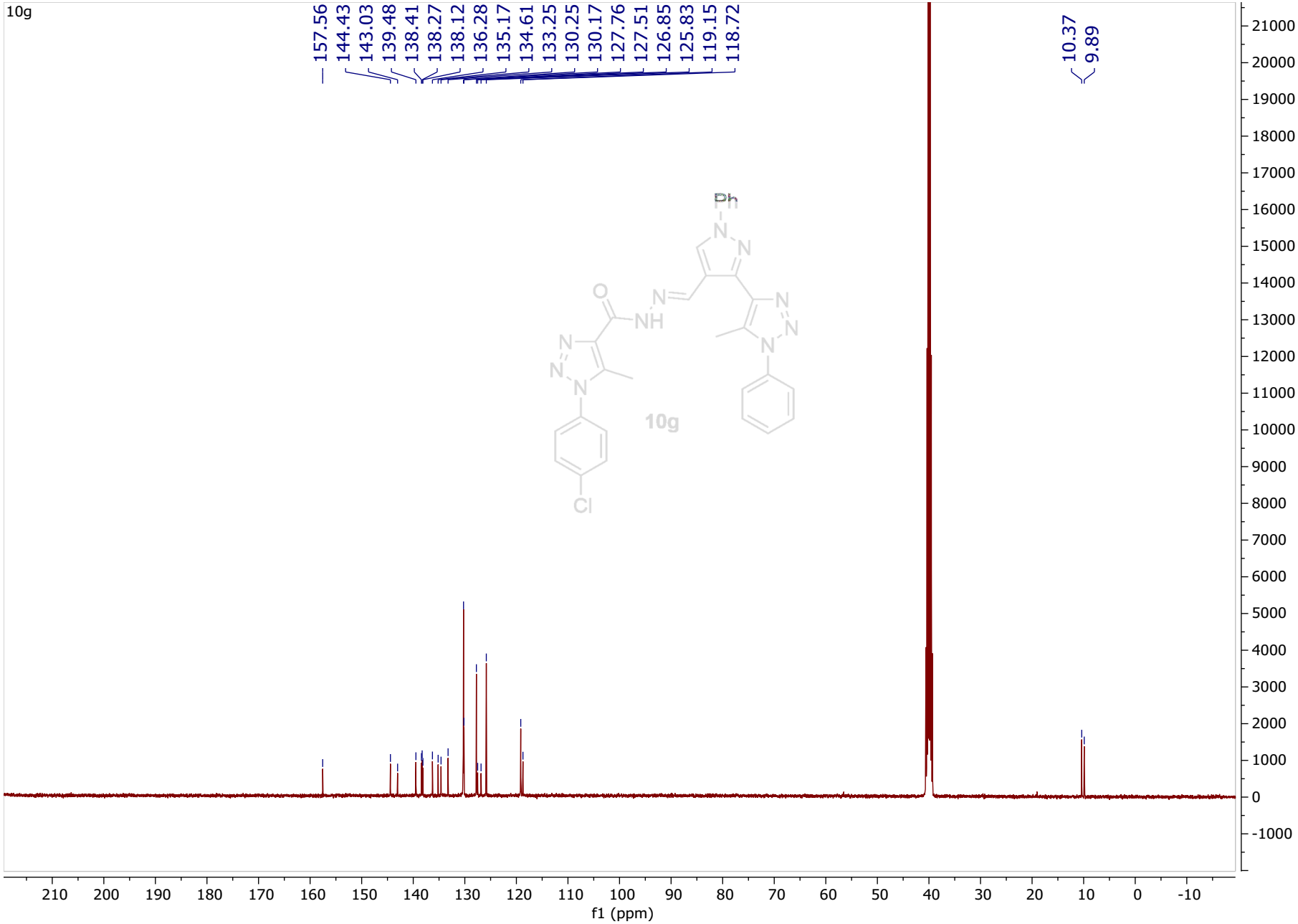

Figure S33. ^{13}CNMR Spectra of Compound **10g**.

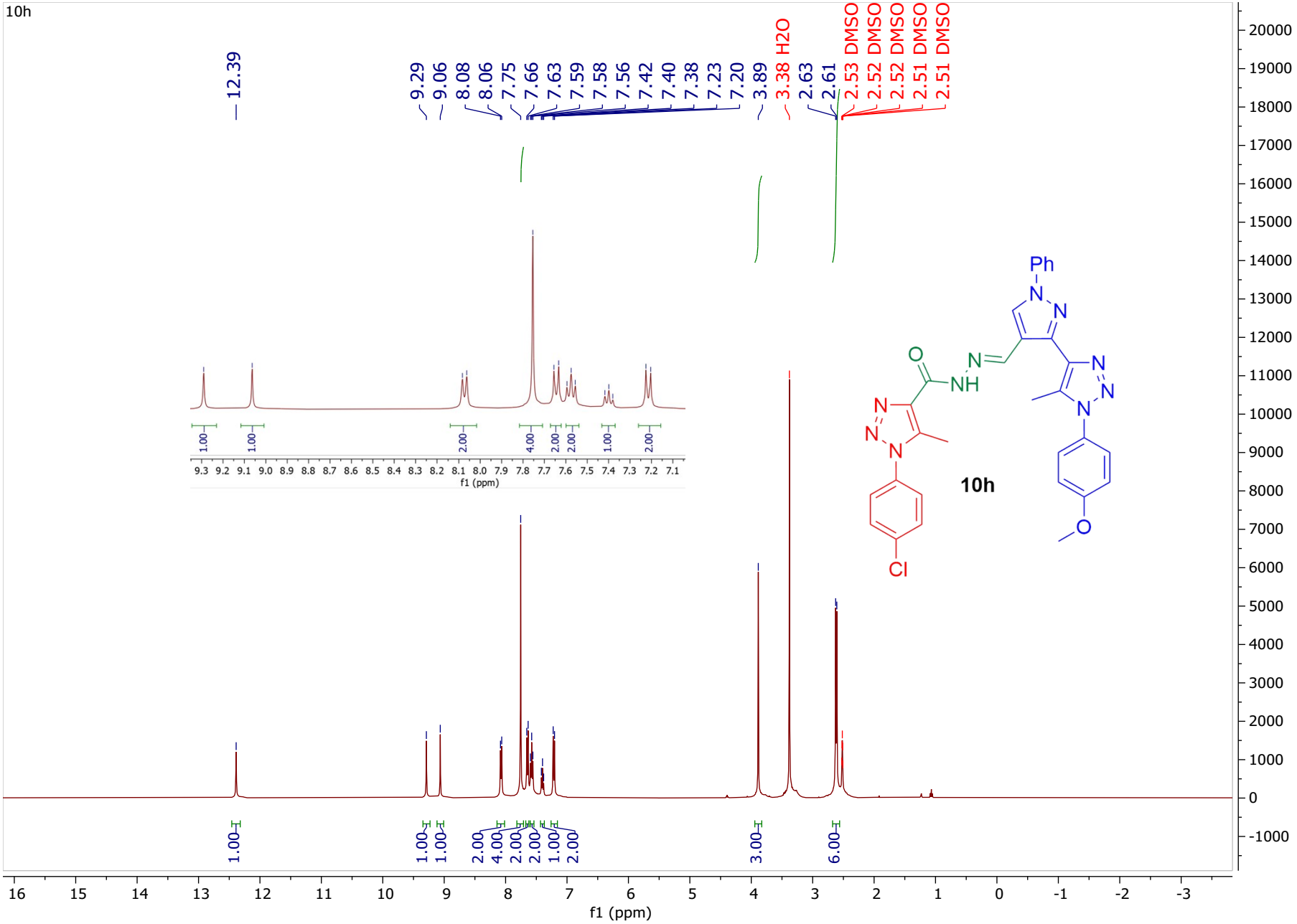

Figure S34. [1]HNMR Spectra of Compound **10h**.

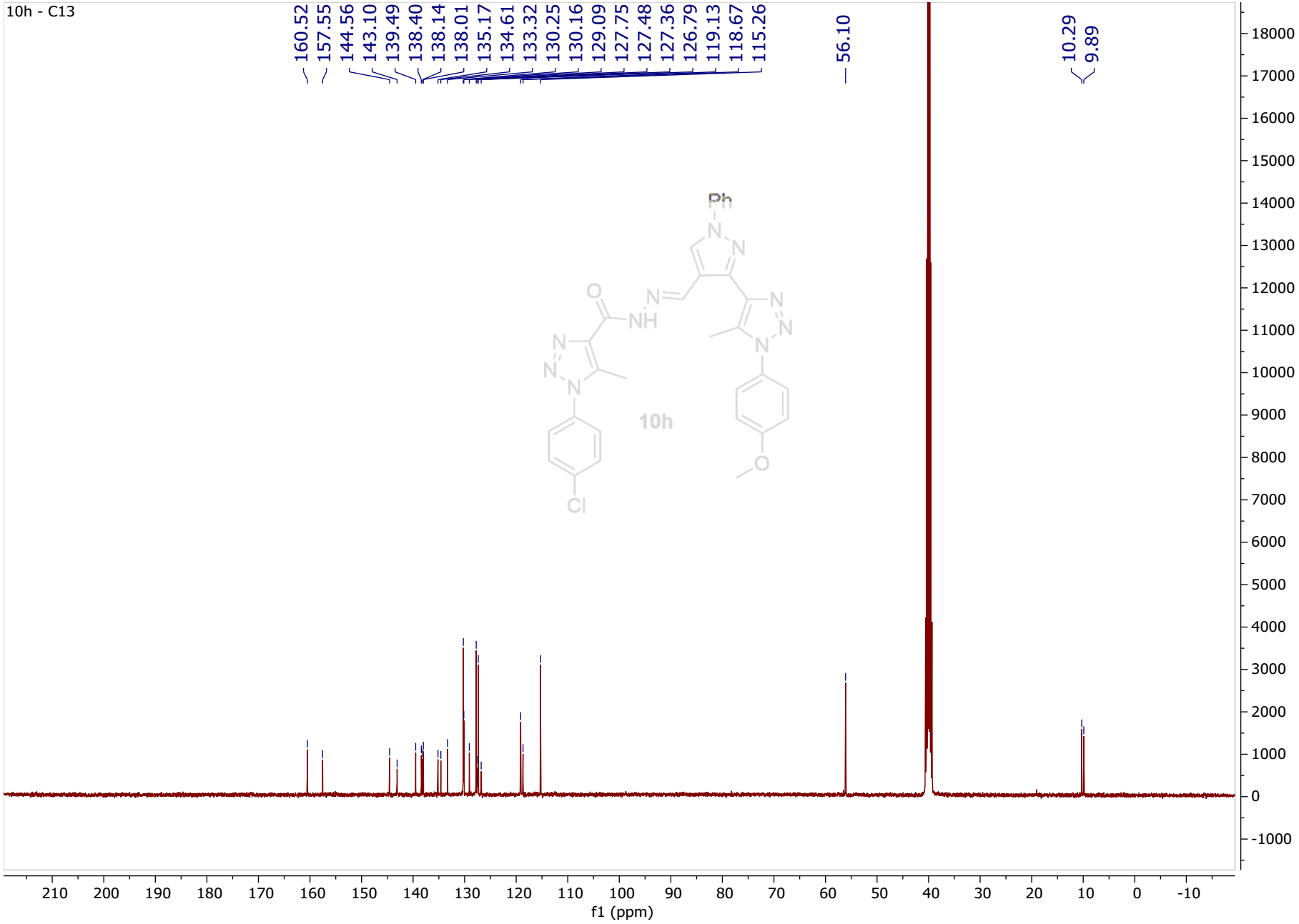

Figure S35. ^{13}CNMR Spectra of Compound **10h**.

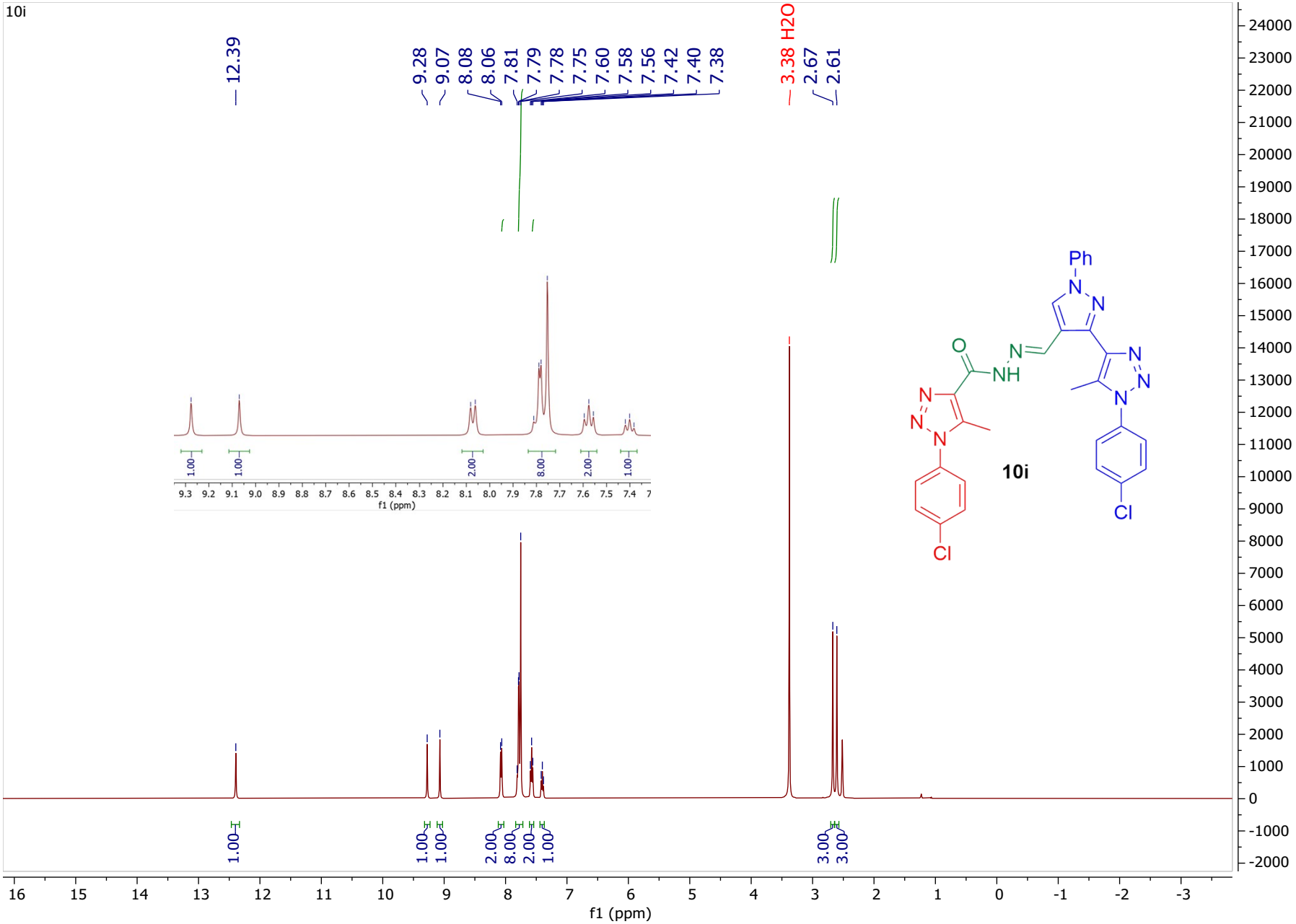

Figure S36. [1]HNMR Spectra of Compound **10i**.

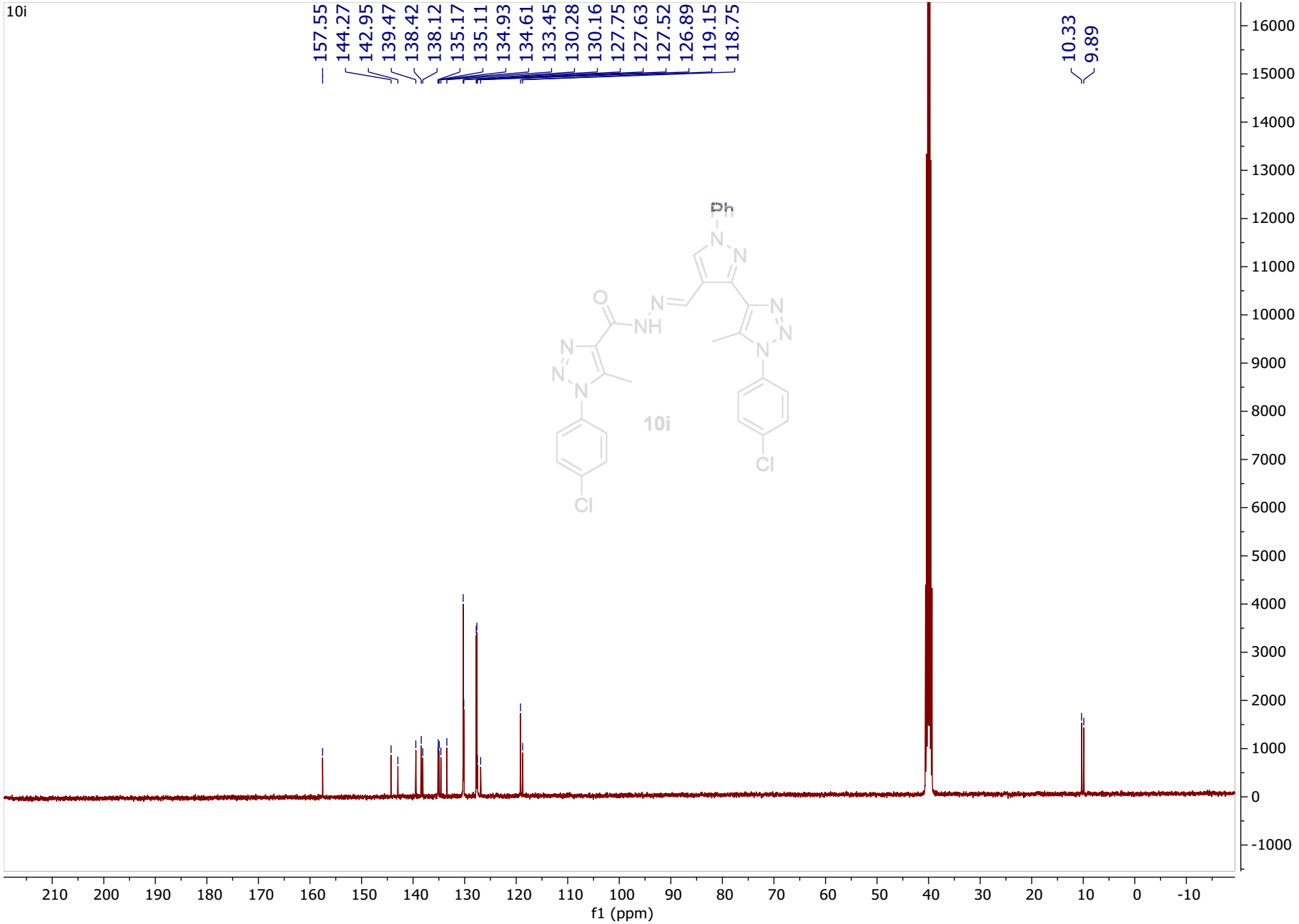

Figure S37. ^{13}CNMR Spectra of Compound **10i**.

ADMETLab 3.0

Table S1. Pharmacokinetic Parameters for Compound **7i**.

ADMETlab 3.0

Cc1c(C(=O)N/N=C/c2cn(-c3ccccc3)nc2-c2ccc(Cl)cc2)nnn1-c1ccc(Cl)cc1

1. Physicochemical Property

Property	Value	Comment
Molecular Weight	515.1	Contain hydrogen atoms. Optimal:100~600
Volume	492.113	Van der Waals volume
Density	1.047	Density = MW / Volume
nHA	8.0	Number of hydrogen bond acceptors. Optimal:0~12
nHD	1.0	Number of hydrogen bond donors. Optimal:0~7
nRot	7.0	Number of rotatable bonds. Optimal:0~11
nRing	5.0	Number of rings. Optimal:0~6
MaxRing	6.0	Number of atoms in the biggest ring. Optimal:0~18
nHet	10.0	Number of heteroatoms. Optimal:1~15
fChar	0.0	Formal charge. Optimal:-4 ~4
nRig	30.0	Number of rigid bonds. Optimal:0~30
Flexibility	0.233	Flexibility = nRot /nRig
Stereo Centers	0.0	Stereo Centers. Optimal: ≤ 2
TPSA	89.99	Topological Polar Surface Area. Optimal:0~140
logS	-7.27	The logarithm of aqueous solubility value.
logP	6.069	The logarithm of the n-octanol/water distribution coefficients at pH=7.4.
logD	4.476	The logarithm of the n-octanol/water distribution coefficient.
pka (Acid)	11.134	Acid-base dissociation constant (pKa) value represents the strength of a drug molecule's acidity or basicity.
pka (Base)	2.12	Acid-base dissociation constant (pKa) value represents the strength of a drug molecule's acidity or basicity.
Melting point	179.805	The predicted melting point of a compound is expressed in degrees Celsius (°C). Melting points below 25°C are classified as liquids, while melting points above 25°C are classified as solids.
Boiling point	442.705	The predicted melting point of a compound is expressed in degrees Celsius (°C). A normal boiling point below 25°C is categorized as a gas.

2. Medicinal Chemistry

Property	Value	Decision	Comment

QED	0.239	🔴	■ A measure of drug-likeness based on the concept of desirability; ■ Attractive: > 0.67; ■ unattractive: 0.49~0.67; ■ too complex: < 0.34
GASA	0.0	🟢	■ ES: Easy to synthesize; HS: Hard to synthesize; ■ The output value represents the probability of being difficult to synthesize, ranging from 0 to 1.
Synth	2.0	🟢	■ Synthetic accessibility score is designed to estimate ease of synthesis of drug-like molecules. ■ SAscore ≥ 6, difficult to synthesize; SAscore <6, easy to synthesize
Fsp3	0.038	🔴	■ The number of sp3 hybridized carbons / total carbon count, correlating with melting point and solubility. ■ Fsp^3 ≥0.42 is considered a suitable value.
MCE-18	27.0	🔴	■ MCE-18 stands for medicinal chemistry evolution. ■ MCE-18≥45 is considered a suitable value.
NPscore	-2.335	-	■ Natural product-likeness score. ■ This score is typically in the range from -5 to 5. ■ The higher the score is, the higher the probability is that the molecule is a NP.
Lipinski Rule	1.0	🔴	■ MW ≤ 500; logP ≤ 5; Hacc ≤ 10; Hdon ≤ 5 ■ If two properties are out of range, a poor absorption or permeability is possible, one is acceptable.
Pfizer Rule	0.0	🟢	■ logP > 3; TPSA < 75 ■ Compounds with a high log P (>3) and low TPSA (<75) are likely to be toxic.
GSK Rule	1.0	🔴	■ MW ≤ 400; logP ≤ 4 ■ Compounds satisfying the GSK rule may have a more favorable ADMET profile
Golden Triangle	1.0	🔴	■ 200 ≤ MW ≤ 500; -2 ≤ logD ≤ 5 ■ Compounds satisfying the Golden Triangle rule may have a more favorable ADMET profile.
PAINS	0 alerts	-	frequent hitters, Alpha-screen artifacts and reactive compound 480 substructures (J Med Chem 201053:2719-40)
ALARM NMR	1 alerts	-	Thiol reactive compounds.
BMS	0 alerts	-	undesirable, reactive compounds 176 substructures (J Chem Inf Model 200646:1060-8)
Chelator Rule	0 alerts	-	Chelating compounds.
Colloidal aggregators	1.0	-	■ Category 0: non-colloidal aggregators; ■ Category 1: colloidal aggregators. ■ The output value is the probability of being colloidal aggregators, within the range of 0 to 1.

FLuc inhibitors	1.0	🔴	■ Category 0: non-fLuc inhibitors; ■ Category 1: fLuc inhibitors. ■ The output value is the probability of being fLuc inhibitors, within the range of 0 to 1.
Blue fluorescence	0.183	🟢	■ Category 0: non-blue fluorescence; ■ Category 1: blue fluorescence. ■ The output value is the probability of being blue fluorescence, within the range of 0 to 1.
Green fluorescence	1.0	🔴	■ Category 0: non-green fluorescence; ■ Category 1: green fluorescence. ■ The output value is the probability of being green fluorescence, within the range of 0 to 1.
Reactive compounds	0.019	🟢	■ Category 0: non-reactive compound; ■ Category 1: reactive compound. ■ The output value is the probability of being reactive compound, within the range of 0 to 1.
Promiscuous compounds	0.0	🟢	■ Category 0: non-promiscuous compound; ■ Category 1: promiscuous compound. ■ The output value is the probability of being promiscuous compound, within the range of 0 to 1.

3. Absorption

Property	Value	Decision	Comment
Caco-2 Permeability	-4.803	🟢	Optimal: higher than -5.15 Log unit
MDCK Permeability	-4.888	🔴	■ low permeability: < 2 × 10-6 cm/s ■ medium permeability: 2-20 × 10^{-6} cm/s ■ high passive permeability: > 20 × 10^{-6} cm/s
PAMPA	0.817	🔴	■ The experimental data for Peff was logarithmically transformed (logPeff). ■ Molecules with log Peff values below 2.0 were classified as low-permeability (Category 0), while those with log Peff values exceeding 2.5 were classified as high-permeability (Category 1).
Pgp-inhibitor	0.001	🟢	■ Category 1: Inhibitor; ■ Category 0: Non-inhibitor; ■ The output value is the probability of being Pgp-inhibitor
Pgp-substrate	0.017	🟢	■ Category 1: substrate; ■ Category 0: Non-substrate; ■ The output value is the probability of being Pgp-substrate
HIA	0.0	🟢	■ Human Intestinal Absorption ■ Category 1: HIA+(HIA < 30%); ■ Category 0: HIA-(HIA >= 30%); ■ The output value is the probability of being HIA+

$F_{20\%}$	0.009	●	■ 20% Bioavailability ■ Category 1: F 20% + (bioavailability < 20%); ■ Category 0: F 20% - (bioavailability ≥ 20%); ■ The output value is the probability of being F 20% +
$F_{30\%}$	0.001	●	■ 30% Bioavailability ■ Category 1: F 30% + (bioavailability < 30%); ■ Category 0: F 30% - (bioavailability ≥ 30%); ■ The output value is the probability of being F 30% +
$F_{50\%}$	0.136	●	■ 50% Bioavailability ■ Category 1: F 50% + (bioavailability < 50%); ■ Category 0: F 50% - (bioavailability ≥ 50%); ■ The output value is the probability of being F 50% +

4. Distribution

Property	Value	Decision	Comment
PPB	99.439	●	■ Plasma Protein Binding Optimal: < 90%. ■ Drugs with high protein-bound may have a low therapeutic index.
VDss	0.154	●	■ Volume Distribution ■ Optimal: 0.04-20L/kg
BBB	0.002	●	■ Blood-Brain Barrier Penetration ■ Category 1: BBB+; Category 0: BBB-; ■ The output value is the probability of being BBB+
Fu	0.335	●	■ The fraction unbound in plasms ■ Low: <5%; Middle: 5~20%; High: > 20%
OATP1B1 inhibitor	0.027	●	■ Category 0: Non-inhibitor; Category 1: inhibitor. ■ The output value is the probability of being inhibitor, within the range of 0 to 1.
OATP1B3 inhibitor	0.879	●	■ Category 0: Non-inhibitor; Category 1: inhibitor. ■ The output value is the probability of being inhibitor, within the range of 0 to 1.
BCRP inhibitor	0.0	●	■ Category 0: Non-inhibitor; Category 1: inhibitor. ■ The output value is the probability of being inhibitor, within the range of 0 to 1.
MRP1 inhibitor	0.019	●	■ Category 0: Non-inhibitor; Category 1: inhibitor. ■ The output value is the probability of being inhibitor, within the range of 0 to 1.

5. Metabolism

Property	Value	Decision	Comment
CYP1A2 inhibitor	1.0	●	■ Category 1: Inhibitor; Category 0: Non-inhibitor; ■ The output value is the probability of being inhibitor.

CYP1A2 substrate	0.0	●	■ Category 1: Substrate; Category 0: Non-substrate; ■ The output value is the probability of being substrate.
CYP2C19 inhibitor	0.998	●	■ Category 1: Inhibitor; Category 0: Non-inhibitor; ■ The output value is the probability of being inhibitor.
CYP2C19 substrate	0.0	●	■ Category 1: Substrate; Category 0: Non-substrate; ■ The output value is the probability of being substrate.
CYP2C9 inhibitor	0.998	●	■ Category 1: Inhibitor; Category 0: Non-inhibitor; ■ The output value is the probability of being inhibitor.
CYP2C9 substrate	0.257	●	■ Category 1: Substrate; Category 0: Non-substrate; ■ The output value is the probability of being substrate.
CYP2D6 inhibitor	0.0	●	■ Category 1: Inhibitor; Category 0: Non-inhibitor; ■ The output value is the probability of being inhibitor.
CYP2D6 substrate	0.112	●	■ Category 1: Substrate; Category 0: Non-substrate; ■ The output value is the probability of being substrate.
CYP3A4 inhibitor	0.208	●	■ Category 1: Inhibitor; Category 0: Non-inhibitor; ■ The output value is the probability of being inhibitor.
CYP3A4 substrate	0.776	●	■ Category 1: Substrate; Category 0: Non-substrate; ■ The output value is the probability of being substrate.
CYP2B6 inhibitor	0.008	●	■ Category 1: Inhibitor; Category 0: Non-inhibitor; ■ The output value is the probability of being inhibitor.
CYP2B6 substrate	0.0	●	■ Category 1: Substrate; Category 0: Non-substrate; ■ The output value is the probability of being substrate.
CYP2C8 inhibitor	1.0	●	■ Category 1: Inhibitor; Category 0: Non-inhibitor; ■ The output value is the probability of being inhibitor.
HLM Stability	0.0	●	■ human liver microsomal (HLM) stability ■ Category 0: stable+ (HLM > 30 min); Category 1: unstable- (HLM ≤ 30 min). The output value is the probability of human liver microsomal instability, where a value closer to 1 indicates a higher likelihood of instability. The range is between 0 and 1.

6. Excretion

Property	Value	Decision	Comment

CL_{plasma}	1.135	🟢	■ The unit of predicted CLplasma penetration is ml/min/kg. >15 ml/min/kg: high clearance; 5-15 ml/min/kg: moderate clearance; < 5 ml/min/kg: low clearance.
$T_{1/2}$	1.56	🟡	■ The unit of predicted T1/2 is hours. ■ ultra-short half-life drugs: 1/2 < 1 hour; short half-life drugs: T1/2 between 1-4 hours; intermediate short half-life drugs: T1/2 between 4-8 hours; long half-life drugs: T1/2 > 8 hours.

7. Toxicity

Property	Value	Decision	Comment
hERG Blockers	0.618	🟠	■ Molecules with IC50 ≤10μM or ≥50% inhibition at 10 μM were classified as hERG+ (Category 1), ■ while molecules with IC50 >10μM or < 50% inhibition at 10μM were classified as hERG - (Category 0). ■ The output value is the probability of being hERG+, within the range of 0 to 1.
hERG Blockers (10um)	0.841	🔴	■ Molecules with IC50 ≤10 μM are classified as hERG+ (Category 1), ■ and molecules with IC50 > 10μM are classified as hERG- (Category 0). ■ The output value is the probability of being hERG+, within the range of 0 to 1.
DILI	0.989	🔴	■ Drug Induced Liver Injury. ■ Category 1: drugs with a high risk of DILI; ■ Category 0: drugs with no risk of DILI. ■ The output value is the probability of being toxic.
AMES Mutagenicity	0.384	🟠	■ AMES Toxicity ■ Category 1: Ames positive(+); ■ Category 0: Ames negative(-); ■ The output value is the probability of being toxic.
Rat Oral Acute Toxicity	0.512	🟠	■ Rat Oral Acute Toxicity. ■ Category 0: low-toxicity, > 500 mg/kg; ■ Category 1: high-toxicity; < 500 mg/kg. ■ The output value is the probability of being toxic, within the range of 0 to 1.
FDAMDD	0.667	🟠	■ FDA Maximum (Recommended) Daily Dose. ■ Category 1: FDAMDD (+); ■ Category 0: FDAMDD (-); The output value is the probability of being positive.
Skin Sensitization	0.549	🟠	■ Category 1: Sensitizer; ■ Category 0: Non-sensitizer. ■ The output value is the probability of being toxic, within the range of 0 to 1.
Carcinogenicity	0.626	🟠	■ Category 1: carcinogens; ■ Category 0: non-carcinogens; ■ The output value is the probability of being toxic.

Eye Corrosion	0.0		■ Eye Corrosion ■ Category 1: corrosives; Category 0: noncorrosives; ■ The output value is the probability of being corrosives.
Eye Irritation	0.063		■ Eye Irritation ■ Category 1: irritants; Category 0: nonirritants; ■ The output value is the probability of being irritants.
Respiratory	0.312		■ Category 1: respiratory toxicants; ■ Category 0: non-respiratory toxicants. ■ The output value is the probability of being toxic, within the range of 0 to 1.
Human Hepatotoxicity	0.767		■ Human Hepatotoxicity ■ Category 1: H-HT positive(+); ■ Category 0: H-HT negative(-); ■ The output value is the probability of being toxic.
Drug-induced Nephrotoxicity	0.941		■ Category 0: non-nephrotoxic (-); ■ Category 1: nephrotoxic (+). ■ The output value is the probability of being nephrotoxic (+), within the range of 0 to 1.
Ototoxicity	0.72		■ Category 0: non-ototoxicity (-); ■ Category 1: ototoxicity (+). ■ The output value is the probability of being ototoxicity (+), within the range of 0 to 1.
Hematotoxicity	0.629		■ Category 0: non-hematotoxicity (-); ■ Category 1: hematotoxicity (+). ■ The output value is the probability of being hematotoxicity (+), within the range of 0 to 1.
Genotoxicity	0.997		■ Category 0: non-Genotoxicity (-); ■ Category 1: Genotoxicity (+). ■ The output value is the probability of being ototoxicity (+), within the range of 0 to 1.
RPMI-8226 Immunitoxicity	0.033		■ Category 0: non-cytotoxicity (-); ■ Category 1: cytotoxicity (+). ■ The output value is the probability of being ototoxicity (+), within the range of 0 to 1.
A549 Cytotoxicity	0.658		■ Category 0: non-cytotoxicity (-); ■ Category 1: cytotoxicity (+). ■ The output value is the probability of being ototoxicity (+), within the range of 0 to 1.
Hek293 Cytotoxicity	0.93		■ Category 0: non-cytotoxicity (-); ■ Category 1: cytotoxicity (+). ■ The output value is the probability of being ototoxicity (+), within the range of 0 to 1.
Drug-induced Neurotoxicity	0.928		■ Category 0: non-neurotoxic (-); ■ Category 1: neurotoxic (+). ■ The output value is the probability of being neurotoxic (+), within the range of 0 to 1.

8. Environmental toxicity

Property	Value	Comment
Bioconcentration Factors	2.078	■ Bioconcentration factors are used for considering secondary poisoning potential and assessing risks to human health via the food chain. ■ The unit is –log10[(mg/L)/(1000*MW)]
IGC_{50}	5.097	■ Tetrahymena pyriformis 50 percent growth inhibition concentration. ■ The unit is –log10[(mg/L)/(1000*MW)]
$LC_{50}FM$	6.747	■ 96-hour fathead minnow 50 percent lethal concentration. ■ The unit is –log10[(mg/L)/(1000*MW)]
$LC_{50}DM$	7.18	■ 48-hour daphnia magna 50 percent lethal concentration. ■ The unit is –log10[(mg/L)/(1000*MW)]

9. Tox21 pathway

Property	Value	Decision	Comment
NR-AhR	0.992	🔴	■ Aryl hydrocarbon receptor ■ Category 1: actives ; ■ Category 0: inactives; ■ The output value is the probability of being active.
NR-AR	0.031	🟢	■ Androgen receptor ■ Category 1: actives ; ■ Category 0: inactives; ■ The output value is the probability of being active.
NR-AR-LBD	0.01	🟢	■ Androgen receptor ligand-binding domain ■ Category 1: actives ; ■ Category 0: inactives; ■ The output value is the probability of being active.
NR-Aromatase	0.467	🟡	■ Category 1: actives ; ■ Category 0: inactives; ■ The output value is the probability of being active.
NR-ER	0.382	🟡	■ Estrogen receptor ■ Category 1: actives ; ■ Category 0: inactives; ■ The output value is the probability of being active.
NR-ER-LBD	0.004	🟢	■ Estrogen receptor ligand-binding domain ■ Category 1: actives ; ■ Category 0: inactives; ■ The output value is the probability of being active.
NR-PPAR-gamma	0.012	🟢	■ Peroxisome proliferator-activated receptor gamma ■ Category 1: actives ; ■ Category 0: inactives; ■ The output value is the probability of being active.
SR-ARE	0.963	🔴	■ Antioxidant response element ■ Category 1: actives ; ■ Category 0: inactives; ■ The output value is the probability of being active.

SR-ATAD5	0.237	●	■ ATPase family AAA domain-containing protein 5 ■ Category 1: actives ; ■ Category 0: inactives; ■ The output value is the probability of being active.
SR-HSE	0.006	●	■ Heat shock factor response element ■ Category 1: actives ; ■ Category 0: inactives; ■ The output value is the probability of being active.
SR-MMP	0.981	●	■ Mitochondrial membrane potential ■ Category 1: actives ; ■ Category 0: inactives; ■ The output value is the probability of being active.
SR-p53	0.145	●	■ p53, a tumor suppressor protein ■ Category 1: actives ; ■ Category 0: inactives; ■ The output value is the probability of being active.

10. Toxicophore Rules

Property	Value	Comment
Acute Toxicity Rule	0	■ 20 substructures; ■ acute toxicity during oral administration
Genotoxic Carcinogenicity Rule	0	■ 117 substructures; ■ carcinogenicity or mutagenicity
NonGenotoxic Carcinogenicity Rule	1 alerts	■ 23 substructures; ■ carcinogenicity through nongenotoxic mechanisms
Skin Sensitization Rule	0	■ 155 substructures; ■ skin irritation
Aquatic Toxicity Rule	1 alerts	■ 99 substructures; ■ toxicity to liquid(water)
NonBiodegradable Rule	1 alerts	■ 19 substructures; ■ non-biodegradable
SureChEMBL Rule	0	■ 164 substructures; ■ MedChem unfriendly status
FAF-Drugs4 Rule	4 alerts	154 toxic substructures from FAF-Drug4

Table S2. Pharmacokinetic Parameters for Compound **10a**.

ADMETlab 3.0

Cc1c(C(=O)N/N=C/c2cn(-c3ccccc3)nc2-c2nnn(-c3ccccc3)c2C)nnn1-c1ccccc1

1. Physicochemical Property

Property	Value	Comment
Molecular Weight	528.21	Contain hydrogen atoms. Optimal:100~600
Volume	532.739	Van der Waals volume
Density	0.991	Density = MW / Volume
nHA	11.0	Number of hydrogen bond acceptors. Optimal:0~12
nHD	1.0	Number of hydrogen bond donors. Optimal:0~7
nRot	8.0	Number of rotatable bonds. Optimal:0~11
nRing	6.0	Number of rings. Optimal:0~6
MaxRing	6.0	Number of atoms in the biggest ring. Optimal:0~18
nHet	11.0	Number of heteroatoms. Optimal:1~15
fChar	0.0	Formal charge. Optimal:-4 ~4
nRig	35.0	Number of rigid bonds. Optimal:0~30
Flexibility	0.229	Flexibility = nRot /nRig
Stereo Centers	0.0	Stereo Centers. Optimal: ≤ 2
TPSA	120.7	Topological Polar Surface Area. Optimal:0~140
logS	-5.016	The logarithm of aqueous solubility value.
logP	3.298	The logarithm of the n-octanol/water distribution coefficients at pH=7.4.
logD	3.05	The logarithm of the n-octanol/water distribution coefficient.
pka (Acid)	7.892	Acid-base dissociation constant (pKa) value represents the strength of a drug molecule's acidity or basicity.
pka (Base)	2.821	Acid-base dissociation constant (pKa) value represents the strength of a drug molecule's acidity or basicity.
Melting point	149.185	The predicted melting point of a compound is expressed in degrees Celsius (°C). Melting points below 25°C are classified as liquids, while melting points above 25°C are classified as solids.
Boiling point	345.54	The predicted melting point of a compound is expressed in degrees Celsius (°C). A normal boiling point below 25°C is categorized as a gas.

2. Medicinal Chemistry

Property	Value	Decision	Comment

QED	0.246	🔴	■ A measure of drug-likeness based on the concept of desirability; ■ Attractive: > 0.67; ■ unattractive: 0.49~0.67; ■ too complex: < 0.34
GASA	0.0	🟢	■ ES: Easy to synthesize; HS: Hard to synthesize; ■ The output value represents the probability of being difficult to synthesize, ranging from 0 to 1.
Synth	2.0	🟢	■ Synthetic accessibility score is designed to estimate ease of synthesis of drug-like molecules. ■ SAscore ≥ 6, difficult to synthesize; SAscore <6, easy to synthesize
Fsp3	0.069	🔴	■ The number of sp3 hybridized carbons / total carbon count, correlating with melting point and solubility. ■ Fsp^3 ≥0.42 is considered a suitable value.
MCE-18	31.0	🔴	■ MCE-18 stands for medicinal chemistry evolution. ■ MCE-18≥45 is considered a suitable value.
NPscore	-1.844	-	■ Natural product-likeness score. ■ This score is typically in the range from -5 to 5. ■ The higher the score is, the higher the probability is that the molecule is a NP.
Lipinski Rule	1.0	🔴	■ MW ≤ 500; logP ≤ 5; Hacc ≤ 10; Hdon ≤ 5 ■ If two properties are out of range, a poor absorption or permeability is possible, one is acceptable.
Pfizer Rule	0.0	🟢	■ logP > 3; TPSA < 75 ■ Compounds with a high log P (>3) and low TPSA (<75) are likely to be toxic.
GSK Rule	1.0	🔴	■ MW ≤ 400; logP ≤ 4 ■ Compounds satisfying the GSK rule may have a more favorable ADMET profile
Golden Triangle	1.0	🔴	■ 200 ≤ MW ≤ 500; -2 ≤ logD ≤ 5 ■ Compounds satisfying the Golden Triangle rule may have a more favorable ADMET profile.
PAINS	0 alerts	-	frequent hitters, Alpha-screen artifacts and reactive compound 480 substructures (J Med Chem 201053:2719-40)
ALARM NMR	1 alerts	-	Thiol reactive compounds.
BMS	0 alerts	-	undesirable, reactive compounds 176 substructures (J Chem Inf Model 200646:1060-8)
Chelator Rule	0 alerts	-	Chelating compounds.
Colloidal aggregators	1.0	-	■ Category 0: non-colloidal aggregators; ■ Category 1: colloidal aggregators. ■ The output value is the probability of being colloidal aggregators, within the range of 0 to 1.

FLuc inhibitors	1.0	🔴	■ Category 0: non-fLuc inhibitors; ■ Category 1: fLuc inhibitors. ■ The output value is the probability of being fLuc inhibitors, within the range of 0 to 1.
Blue fluorescence	0.083	🟢	■ Category 0: non-blue fluorescence; ■ Category 1: blue fluorescence. ■ The output value is the probability of being blue fluorescence, within the range of 0 to 1.
Green fluorescence	0.992	🔴	■ Category 0: non-green fluorescence; ■ Category 1: green fluorescence. ■ The output value is the probability of being green fluorescence, within the range of 0 to 1.
Reactive compounds	0.027	🟢	■ Category 0: non-reactive compound; ■ Category 1: reactive compound. ■ The output value is the probability of being reactive compound, within the range of 0 to 1.
Promiscuous compounds	0.0	🟢	■ Category 0: non-promiscuous compound; ■ Category 1: promiscuous compound. ■ The output value is the probability of being promiscuous compound, within the range of 0 to 1.

3. Absorption

Property	Value	Decision	Comment
Caco-2 Permeability	-4.996	🟢	Optimal: higher than -5.15 Log unit
MDCK Permeability	-4.781	🔴	■ low permeability: < 2 × 10-6 cm/s ■ medium permeability: 2-20 × 10^{-6} cm/s ■ high passive permeability: > 20 × 10^{-6} cm/s
PAMPA	0.899	🔴	■ The experimental data for Peff was logarithmically transformed (logPeff). ■ Molecules with log Peff values below 2.0 were classified as low-permeability (Category 0), while those with log Peff values exceeding 2.5 were classified as high-permeability (Category 1).
Pgp-inhibitor	0.0	🟢	■ Category 1: Inhibitor; ■ Category 0: Non-inhibitor; ■ The output value is the probability of being Pgp-inhibitor
Pgp-substrate	0.002	🟢	■ Category 1: substrate; ■ Category 0: Non-substrate; ■ The output value is the probability of being Pgp-substrate
HIA	0.0	🟢	■ Human Intestinal Absorption ■ Category 1: HIA+(HIA < 30%); ■ Category 0: HIA-(HIA >= 30%); ■ The output value is the probability of being HIA+

$F_{20\%}$	0.035	●	■ 20% Bioavailability ■ Category 1: F 20% + (bioavailability < 20%); ■ Category 0: F 20% - (bioavailability ≥ 20%); ■ The output value is the probability of being F 20% +
$F_{30\%}$	0.042	●	■ 30% Bioavailability ■ Category 1: F 30% + (bioavailability < 30%); ■ Category 0: F 30% - (bioavailability ≥ 30%); ■ The output value is the probability of being F 30% +
$F_{50\%}$	0.408	●	■ 50% Bioavailability ■ Category 1: F 50% + (bioavailability < 50%); ■ Category 0: F 50% - (bioavailability ≥ 50%); ■ The output value is the probability of being F 50% +

4. Distribution

Property	Value	Decision	Comment
PPB	98.669	●	■ Plasma Protein Binding Optimal: < 90%. ■ Drugs with high protein-bound may have a low therapeutic index.
VDss	0.165	●	■ Volume Distribution ■ Optimal: 0.04-20L/kg
BBB	0.002	●	■ Blood-Brain Barrier Penetration ■ Category 1: BBB+; Category 0: BBB-; ■ The output value is the probability of being BBB+
Fu	0.825	●	■ The fraction unbound in plasms ■ Low: <5%; Middle: 5~20%; High: > 20%
OATP1B1 inhibitor	0.007	●	■ Category 0: Non-inhibitor; Category 1: inhibitor. ■ The output value is the probability of being inhibitor, within the range of 0 to 1.
OATP1B3 inhibitor	0.355	●	■ Category 0: Non-inhibitor; Category 1: inhibitor. ■ The output value is the probability of being inhibitor, within the range of 0 to 1.
BCRP inhibitor	0.0	●	■ Category 0: Non-inhibitor; Category 1: inhibitor. ■ The output value is the probability of being inhibitor, within the range of 0 to 1.
MRP1 inhibitor	0.447	●	■ Category 0: Non-inhibitor; Category 1: inhibitor. ■ The output value is the probability of being inhibitor, within the range of 0 to 1.

5. Metabolism

Property	Value	Decision	Comment
CYP1A2 inhibitor	0.914	●	■ Category 1: Inhibitor; Category 0: Non-inhibitor; ■ The output value is the probability of being inhibitor.

CYP1A2 substrate	0.001	🟢	■ Category 1: Substrate; Category 0: Non-substrate; ■ The output value is the probability of being substrate.
CYP2C19 inhibitor	0.994	🔴	■ Category 1: Inhibitor; Category 0: Non-inhibitor; ■ The output value is the probability of being inhibitor.
CYP2C19 substrate	0.0	🟢	■ Category 1: Substrate; Category 0: Non-substrate; ■ The output value is the probability of being substrate.
CYP2C9 inhibitor	0.999	🔴	■ Category 1: Inhibitor; Category 0: Non-inhibitor; ■ The output value is the probability of being inhibitor.
CYP2C9 substrate	0.0	🟢	■ Category 1: Substrate; Category 0: Non-substrate; ■ The output value is the probability of being substrate.
CYP2D6 inhibitor	0.0	🟢	■ Category 1: Inhibitor; Category 0: Non-inhibitor; ■ The output value is the probability of being inhibitor.
CYP2D6 substrate	0.018	🟢	■ Category 1: Substrate; Category 0: Non-substrate; ■ The output value is the probability of being substrate.
CYP3A4 inhibitor	0.162	🟢	■ Category 1: Inhibitor; Category 0: Non-inhibitor; ■ The output value is the probability of being inhibitor.
CYP3A4 substrate	0.945	🔴	■ Category 1: Substrate; Category 0: Non-substrate; ■ The output value is the probability of being substrate.
CYP2B6 inhibitor	0.029	🟢	■ Category 1: Inhibitor; Category 0: Non-inhibitor; ■ The output value is the probability of being inhibitor.
CYP2B6 substrate	0.0	🟢	■ Category 1: Substrate; Category 0: Non-substrate; ■ The output value is the probability of being substrate.
CYP2C8 inhibitor	1.0	🔴	■ Category 1: Inhibitor; Category 0: Non-inhibitor; ■ The output value is the probability of being inhibitor.
HLM Stability	0.008	🟢	■ human liver microsomal (HLM) stability ■ Category 0: stable+ (HLM > 30 min); Category 1: unstable- (HLM ≤ 30 min). The output value is the probability of human liver microsomal instability, where a value closer to 1 indicates a higher likelihood of instability. The range is between 0 and 1.

6. Excretion

Property	Value	Decision	Comment

CL_{plasma}	0.723	●	■ The unit of predicted CLplasma penetration is ml/min/kg. >15 ml/min/kg: high clearance; 5-15 ml/min/kg: moderate clearance; < 5 ml/min/kg: low clearance.
$T_{1/2}$	1.159	●	■ The unit of predicted T1/2 is hours. ■ ultra-short half-life drugs: 1/2 < 1 hour; short half-life drugs: T1/2 between 1-4 hours; intermediate short half-life drugs: T1/2 between 4-8 hours; long half-life drugs: T1/2 > 8 hours.

7. Toxicity

Property	Value	Decision	Comment
hERG Blockers	0.261	●	■ Molecules with IC50 ≤10μM or ≥50% inhibition at 10 μM were classified as hERG+ (Category 1), ■ while molecules with IC50 >10μM or < 50% inhibition at 10μM were classified as hERG - (Category 0). ■ The output value is the probability of being hERG+, within the range of 0 to 1.
hERG Blockers (10um)	0.605	●	■ Molecules with IC50 ≤10 μM are classified as hERG+ (Category 1), ■ and molecules with IC50 > 10μM are classified as hERG- (Category 0). ■ The output value is the probability of being hERG+, within the range of 0 to 1.
DILI	0.95	●	■ Drug Induced Liver Injury. ■ Category 1: drugs with a high risk of DILI; ■ Category 0: drugs with no risk of DILI. ■ The output value is the probability of being toxic.
AMES Mutagenicity	0.729	●	■ AMES Toxicity ■ Category 1: Ames positive(+); ■ Category 0: Ames negative(-); ■ The output value is the probability of being toxic.
Rat Oral Acute Toxicity	0.534	●	■ Rat Oral Acute Toxicity. ■ Category 0: low-toxicity, > 500 mg/kg; ■ Category 1: high-toxicity; < 500 mg/kg. ■ The output value is the probability of being toxic, within the range of 0 to 1.
FDAMDD	0.695	●	■ FDA Maximum (Recommended) Daily Dose. ■ Category 1: FDAMDD (+); ■ Category 0: FDAMDD (-); The output value is the probability of being positive.
Skin Sensitization	0.273	●	■ Category 1: Sensitizer; ■ Category 0: Non-sensitizer. ■ The output value is the probability of being toxic, within the range of 0 to 1.
Carcinogenicity	0.805	●	■ Category 1: carcinogens; ■ Category 0: non-carcinogens; ■ The output value is the probability of being toxic.

Eye Corrosion	0.0		■ Eye Corrosion ■ Category 1: corrosives; Category 0: noncorrosives; ■ The output value is the probability of being corrosives.
Eye Irritation	0.246		■ Eye Irritation ■ Category 1: irritants; Category 0: nonirritants; ■ The output value is the probability of being irritants.
Respiratory	0.516		■ Category 1: respiratory toxicants; ■ Category 0: non-respiratory toxicants. ■ The output value is the probability of being toxic, within the range of 0 to 1.
Human Hepatotoxicity	0.778		■ Human Hepatotoxicity ■ Category 1: H-HT positive(+); ■ Category 0: H-HT negative(-); ■ The output value is the probability of being toxic.
Drug-induced Nephrotoxicity	0.809		■ Category 0: non-nephrotoxic (-); ■ Category 1: nephrotoxic (+). ■ The output value is the probability of being nephrotoxic (+), within the range of 0 to 1.
Ototoxicity	0.586		■ Category 0: non-ototoxicity (-); ■ Category 1: ototoxicity (+). ■ The output value is the probability of being ototoxicity (+), within the range of 0 to 1.
Hematotoxicity	0.517		■ Category 0: non-hematotoxicity (-); ■ Category 1: hematotoxicity (+). ■ The output value is the probability of being hematotoxicity (+), within the range of 0 to 1.
Genotoxicity	0.999		■ Category 0: non-Genotoxicity (-); ■ Category 1: Genotoxicity (+). ■ The output value is the probability of being ototoxicity (+), within the range of 0 to 1.
RPMI-8226 Immunitoxicity	0.043		■ Category 0: non-cytotoxicity (-); ■ Category 1: cytotoxicity (+). ■ The output value is the probability of being ototoxicity (+), within the range of 0 to 1.
A549 Cytotoxicity	0.141		■ Category 0: non-cytotoxicity (-); ■ Category 1: cytotoxicity (+). ■ The output value is the probability of being ototoxicity (+), within the range of 0 to 1.
Hek293 Cytotoxicity	0.642		■ Category 0: non-cytotoxicity (-); ■ Category 1: cytotoxicity (+). ■ The output value is the probability of being ototoxicity (+), within the range of 0 to 1.
Drug-induced Neurotoxicity	0.861		■ Category 0: non-neurotoxic (-); ■ Category 1: neurotoxic (+). ■ The output value is the probability of being neurotoxic (+), within the range of 0 to 1.

8. Environmental toxicity

Property	Value	Comment
Bioconcentration Factors	1.088	■ Bioconcentration factors are used for considering secondary poisoning potential and assessing risks to human health via the food chain. ■ The unit is –log10[(mg/L)/(1000*MW)]
IGC_{50}	4.491	■ Tetrahymena pyriformis 50 percent growth inhibition concentration. ■ The unit is –log10[(mg/L)/(1000*MW)]
$LC_{50}FM$	5.69	■ 96-hour fathead minnow 50 percent lethal concentration. ■ The unit is –log10[(mg/L)/(1000*MW)]
$LC_{50}DM$	5.816	■ 48-hour daphnia magna 50 percent lethal concentration. ■ The unit is –log10[(mg/L)/(1000*MW)]

9. Tox21 pathway

Property	Value	Decision	Comment
NR-AhR	0.951	🔴	■ Aryl hydrocarbon receptor ■ Category 1: actives ; ■ Category 0: inactives; ■ The output value is the probability of being active.
NR-AR	0.002	🟢	■ Androgen receptor ■ Category 1: actives ; ■ Category 0: inactives; ■ The output value is the probability of being active.
NR-AR-LBD	0.0	🟢	■ Androgen receptor ligand-binding domain ■ Category 1: actives ; ■ Category 0: inactives; ■ The output value is the probability of being active.
NR-Aromatase	0.026	🟢	■ Category 1: actives ; ■ Category 0: inactives; ■ The output value is the probability of being active.
NR-ER	0.493	🟡	■ Estrogen receptor ■ Category 1: actives ; ■ Category 0: inactives; ■ The output value is the probability of being active.
NR-ER-LBD	0.0	🟢	■ Estrogen receptor ligand-binding domain ■ Category 1: actives ; ■ Category 0: inactives; ■ The output value is the probability of being active.
NR-PPAR-gamma	0.011	🟢	■ Peroxisome proliferator-activated receptor gamma ■ Category 1: actives ; ■ Category 0: inactives; ■ The output value is the probability of being active.
SR-ARE	0.556	🟡	■ Antioxidant response element ■ Category 1: actives ; ■ Category 0: inactives; ■ The output value is the probability of being active.

Experimental Procedures

Chemistry Instruments:

Melting points were determined using an uncorrected Stuart melting point apparatus. The FT-IR 8400S spectrophotometer was used to record infrared spectra. The Bruker spectrometer was used to capture the ^{1}H NMR spectra at 400 MHz. ^{13}C NMR spectra were obtained in deuterated dimethylsulfoxide (DMSO-d_6) at 100 MHz. Chemical shifts (H) are reported concerning TMS, the internal standard. The values of all coupling constants (*J*) are given in hertz. As internal standards, chemical shifts (C) are reported to DMSO-d_6. The abbreviations are s, singlet; bs, broad singlet; d, doublet; dd, doublet of doublet; t, triplet; m, multiplet. Thin layer chromatography (TLC) on silica gel precoated F_{254} Merck plates was used to monitor reaction courses and product mixtures regularly. Elemental analyses were conducted at Al-Azhar University's Regional Center for Microbiology and Biotechnology in Cairo, Egypt. Unless otherwise specified, all solvents and reagents were commercially available and used without further purification.

MTT Assay:

The anti-proliferative effects of the synthesized pyrazolyl-triazole hybrids and pyrazolyl-di(triazole) hybrids were evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay against MCF-7 human breast adenocarcinoma cells (ATCC HTB-22) derived from metastatic pleural effusion and A549 human lung carcinoma cells (ATCC CCL-185). Both cell lines were cultured as adherent epithelial cells and maintained under standard conditions. The cytotoxicity assessment was performed by seeding cells at 1×105 cells/ml (100 µl/well) in 96-well tissue culture plates and incubating for 24 hours at 37°C to establish confluent monolayers. After washing with maintenance medium, cells were treated with serial two-fold dilutions of test compounds in RPMI medium containing 2% serum, with three wells serving as untreated controls. Following compound exposure, MTT solution (5 mg/ml in PBS) was added (20 µl/well), and plates were incubated at 37°C with 5% CO2 for 4 hours to allow metabolic conversion of the tetrazolium salt to purple formazan crystals by viable cells. The formazan product was subsequently solubilized in 200 µl DMSO with gentle shaking, and optical density was measured at 560 nm with background correction at 620 nm using a microplate reader. Cell viability was calculated as a percentage relative to untreated controls, with the assay principle based on the direct proportionality between formazan formation and the number of metabolically active cells.

Flow Cytometric Analysis in A549 Cells:

Cell cycle analysis of A549 cells was performed using the COULTER DNA PREP Reagents Kit (PN 6607055) purchased from Beckman Coulter, Inc. (Brea, CA, USA). A549 cells were harvested and prepared for flow cytometric analysis following the manufacturer's protocol, where cells were treated with DNA PREP LPR (lysing/permeabilizing reagent containing <0.1% potassium cyanide, <0.1% NaN3, nonionic detergents, saline and stabilizers) to permeabilize the cell membrane, followed by staining with DNA PREP Stain solution containing 50 µg/mL propidium iodide and RNAse (Type III-A, Bovine Pancreas, 4 KU/mL) to ensure specific DNA binding by removing interfering RNA. The reagents were brought to room temperature (20-25°C) before use, and cells were incubated with the staining solution while minimizing light exposure to prevent photobleaching of the propidium iodide fluorophore. Flow cytometric analysis was performed using appropriate excitation at 488 nm and emission detection at 560-680 nm, with the fluorescence intensity directly proportional to cellular DNA content, allowing for quantitative assessment of cell cycle distribution phases (G0/G1, S, and G2/M) and identification of any sub-G1 population indicative of apoptotic cells. Data acquisition and analysis were conducted following standard flow cytometry protocols with proper instrument calibration using fluorospheres to ensure a half-peak coefficient of variation (CV) less than 2% for red fluorescence.

Clonogenic Assay:

A clonogenic assay was performed to evaluate the antiproliferative effects of test compounds on A549 cells. Cells were seeded in 6-well tissue culture plates at appropriate densities and allowed to attach. After 24 hours, cells were treated with **compounds 7i**, **10a**, or **Doxorubicin**, followed by incubation for colony formation over 10–14 days. Colonies consisting of at least 50 cells were fixed with 10% formalin and stained using 0.5% crystal violet solution. Plates were air-dried, and colonies were manually counted under a stereomicroscope. The plating efficiency (PE) and surviving fraction (SF) were calculated, with PE representing the percentage of seeded untreated cells that formed colonies, and SF derived from treated groups relative to the PE of the control. All assays were conducted in quadruplicate, and data are presented as mean ± standard error.

Biochemical Markers:

Human Ki-67 PicoKine™ ELISA Kit (Catalog No. MBS1751629) was used to quantify Ki-67 expression in A549 cell lysates. After cell lysis and clarification, 100 µL of each sample or standard was added to precoated wells and incubated for 2 h at 37 °C. Wells were washed, then 100 µL biotinylated anti-Ki-67 antibody (1:100) was added for 90 min, followed by 100 µL ABC-HRP for 30 min. After washing, 200 µL TMB substrate was developed for 25 min, stopped with acid, and absorbance read at 450 nm.

Human PCNA ELISA Kit (Catalog No. MBS762155) measured PCNA levels in A549 lysates. Samples (100 µL) were incubated in anti-PCNA-coated plates for 90 min at 37 °C, washed, then 100 µL biotinylated detection antibody (1:100) was added for 60 min, followed by 100 µL HRP-streptavidin for 30 min. After washing, 90 µL TMB was added for 15 min, stopped with acid, and O.D. read at 450 nm.

Human p21 ELISA Kit (Cat. No. MBS2605968) quantified p21 in A549 cell lysates. Standards and 100 µL of each sample were incubated in anti-p21-coated wells for 90 min at 37 °C. After washing, 100 µL biotinylated anti-p21 antibody (1:100) was added for 60 min, washed, then 100 µL enzyme conjugate for 30 min. Following five washes, 90 µL TMB was added for up to 30 min, stopped, and read at 450 nm.

Cloud-Clone Corp. Bax ELISA Kit (SEB343Hu; 96T) assessed Bax in A549 lysates. Samples (100 µL) were incubated in precoated wells for 1 h at 37 °C. After three washes, 100 µL Detection Reagent A was added for 1 h, washed, then 100 µL Detection Reagent B for 30 min. Following five washes, 90 µL TMB was added for 10–20 min, stopped, and absorbance measured at 450 nm.

Human Bcl-2 ELISA Kit (SEA778Hu; Cloud-Clone Corp.) measured Bcl-2 in A549 lysates. 100 µL of standards or samples were incubated for 1 h at 37 °C in precoated wells. After washing, 100 µL Detection Reagent A was added for 1 h, washed, then 100 µL Detection Reagent B for 30 min. Following five washes, 90 µL TMB was added for 10–20 min, stopped, and read at 450 nm.

Caspase-3 ELISA Kit (SEA626Hu; Cloud-Clone Corp.) quantified Caspase-3 in A549 lysates. 100 µL of standards or samples were incubated for 1 h at 37 °C in precoated wells. After

washing, 100 µL Detection Reagent A was added for 1 h, washed, then 100 µL Detection Reagent B for 30 min. Following five washes, 90 µL TMB was added for 10–20 min, stopped, and absorbance read at 450 nm.

VEGFR2/KDR ELISA Kit (MBS765022; MyBioSource, Inc.) measured VEGFR2 in A549 lysates. Samples (100 µL) were incubated in precoated wells for 90 min at 37 °C, washed twice, then 100 µL biotinylated detection antibody (1:100) for 60 min. After three washes, 100 µL SABC (1:100) was incubated for 30 min, washed five times, and 90 µL TMB added for 15–30 min. The reaction was stopped with 50 µL acid, and O.D. read at 450 nm.

P-gp ELISA Kit (MBS2506188; MyBioSource, Inc.) quantified P-glycoprotein in A549 lysates. After sample preparation, 100 µL of standards or samples were incubated for 90 min at 37 °C in precoated wells. 100 µL biotinylated detection antibody (1:100) was added for 60 min, washed thrice, then 100 µL HRP conjugate (1:100) for 30 min. Following five washes, 90 µL substrate was added for 15 min, stopped, and absorbance measured at 450 nm.

E-Cadherin ELISA Kit (Kit No. KIT10204; Sino Biological, Inc., Beijing, China) assessed E-cadherin in A549 lysates. After preparing 100 µL of standards or samples, plates were washed three times, then incubated for 2 h at room temperature. Wells were washed, then 100 µL HRP-conjugated detection antibody (0.5 µg/mL) was added for 1 h, washed, and 200 µL TMB added for 20 min. The reaction was stopped with 50 µL 2 N H_2SO_4, and O.D. read at 450 nm.

EGFR, VEGFR-2, and AURKA Inhibitory Activity:

The inhibitory potency of compounds (7i) and (10a) against EGFR kinase was assessed using the EGFR Kinase Assay Kit (Catalog No. 40321; BPS Bioscience, 6042 Cornerstone Court W, Ste B, San Diego, CA 92121, USA). Recombinant EGFR enzyme (1 ng/µL) and PTK substrate (Poly(Glu:Tyr) 4:1) were incubated in kinase buffer at 30 °C for 40 min in the presence of serial dilutions of each compound (0.1 nM to 10 µM) in duplicate. Reactions were terminated with Kinase-Glo® MAX reagent, and luminescence was measured on a microplate reader.

Compound potency against VEGFR-2 kinase was measured with the VEGFR-2 (KDR) Kinase Assay Kit (Catalog No. 40325; BPS Bioscience, San Diego, CA, USA). Purified VEGFR-2 (1 ng/µL) and PTK substrate were combined in 1× kinase buffer and incubated at 30 °C for 45 min

with serial dilutions of 7i and 10a (0.1 nM–10 µM) in white 96-well plates. Following incubation, Kinase-Glo® MAX reagent was added, and luminescence was recorded.

Aurora Kinase A inhibition by (7i) and (10a) was evaluated using the Chemi-Verse™ Aurora Kinase A Assay Kit (Cat. No. 82095; BPS Bioscience, San Diego, CA, USA), employing ADP-Glo™ detection. Reactions containing 5 ng/µL Aurora A, Kemptide substrate, and ATP (500 µM) were assembled in assay buffer and treated with compound dilutions (0.1 nM–10 µM) for 45 min at 30 °C. After reaction quenching and ADP-to-ATP conversion, luminescence was measured.

***In-silico* Studies:**

Molecular docking studies were carried out using **AutoDock Vina v1.2.5** to predict the binding modes of **compounds (7i)** and **(10a)** within EGFR **(PDB ID: 4HJO)**, VEGFR-2 **(PDB ID: 3VHE)**, and AURKA **(PDB ID: 4UYN)**. Protein structures were prepared by removing all water molecules and co-crystallized ligands, adding polar hydrogens, and assigning Gasteiger charges using AutoDockTools. Grid boxes were defined as follows: EGFR (center_x = 24.214, center_y = 8.727, center_z = 0.296; size_x = 30 Å, size_y = 28 Å, size_z = 30 Å), VEGFR-2 (center_x = –24.709, center_y = –1.299, center_z = –10.738; size_x = 32 Å, size_y = 32 Å, size_z = 30 Å), and AURKA (center_x = 26.770, center_y = –5.043, center_z = 14.907; size_x = 26 Å, size_y = 24 Å, size_z = 32 Å). Ligand structures were energy-minimized using the MMFF94 force field in OpenBabel and then converted to PDBQT format. For each docking run, exhaustiveness was set to 32 and the number of binding modes to 10. The top-ranked poses, according to Vina score, were visualized and analyzed for intermolecular interactions using **Discovery Studio v21.1.0.20298** and **UCSF ChimeraX v1.7rc202312020245**.

Pharmacokinetic and drug-likeness profiles of compounds were predicted using **SwissADME** (http://www.swissadme.ch), where SMILES inputs generated physicochemical descriptors, and **ADMETLab 3.0** (https://admetlab3.scbdd.com) with results downloadable as interactive tables and CSV files.

HPLC

Spectra

Area Percent Report

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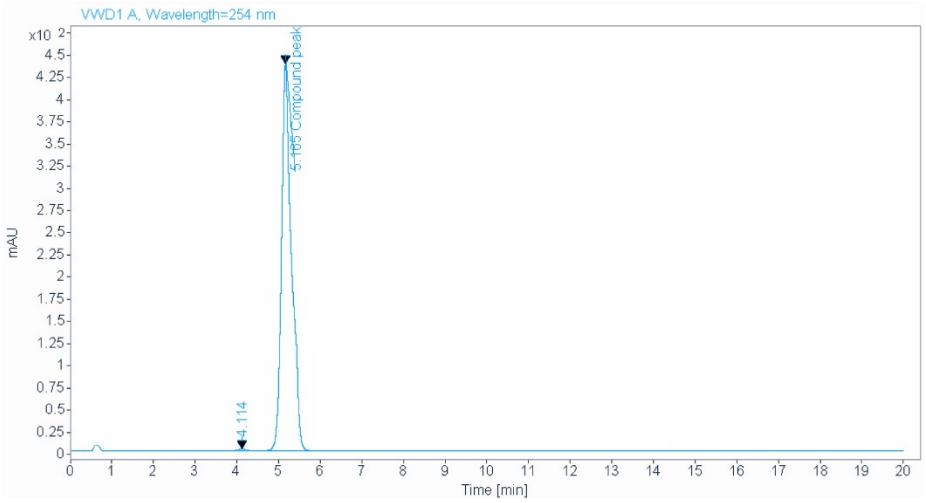
Data file:	C:\CHEM32\1\DATA\HAYTHAM\7I-003.D		
Sample name:	7i		
Description:	Solvent: DMSO + ACN Mobile phase: 60% ACN + 40% Phosphate buffer		
Sample amount:	5.000	**Sample type:**	Sample
Instrument:	HPLC	**Location:**	Vial 1
Injection date:	2/10/2026 4:19:04 AM	**Injection:**	1 of 1
Acq. method:	HAYTHAM.M	**Injection volume:**	5.000
Analysis method:	HAYTHAM.M	**Acq. operator:**	SYSTEM
Last changed:	2/10/2026 4:30:16 AM (modified after loading)		



Signal: VWD1 A, Wavelength=254 nm

RT [min]	Type	Width [min]	Area	Height	Area%	Name
4.114	BB	0.2801	41.1634	2.1610	0.4871	
5.165	BV	0.2607	8410.2714	435.2385	99.5129	Compound peak
		Sum	8451.4348			

Figure S38. HPLC Spectra for compound **7i**.

Area Percent Report

Agilent Technologies

Data file:	C:\CHEM32\1\DATA\HAYTHAM\10A-001.D 10a		
Sample name:	Solvent: DMSO + ACN, Flow rate: 1.5 mL/min		
Description:	Mobile phase: 60% ACN + 40% Phosphate buffer		
Sample amount:	2.500	**Sample type:**	Sample
Instrument:	HPLC	**Location:**	Vial 2
Injection date:	2/10/2026 4:26:32 AM	**Injection:**	1 of 1
Acq. method:	HAYTHAM.M	**Injection volume:**	5.000
Analysis method:	HAYTHAM.M	**Acq. operator:**	SYSTEM
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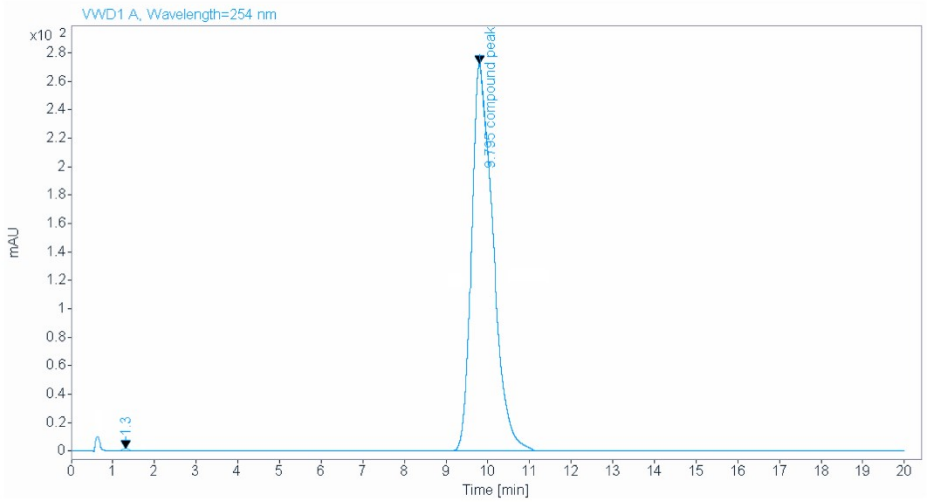

Signal: VWD1 A, Wavelength=254 nm

RT [min]	Type	Width [min]	Area	Height	Area%	Name
1.300	BV	0.2665	13.3155	3.5147	0.1341	
9.795	VV	0.5309	9921.1729	268.8050	99.8659	compound peak
		Sum	9934.4884			

Figure S39. HPLC Spectra for compound **10a**.

IC_{50} Curves

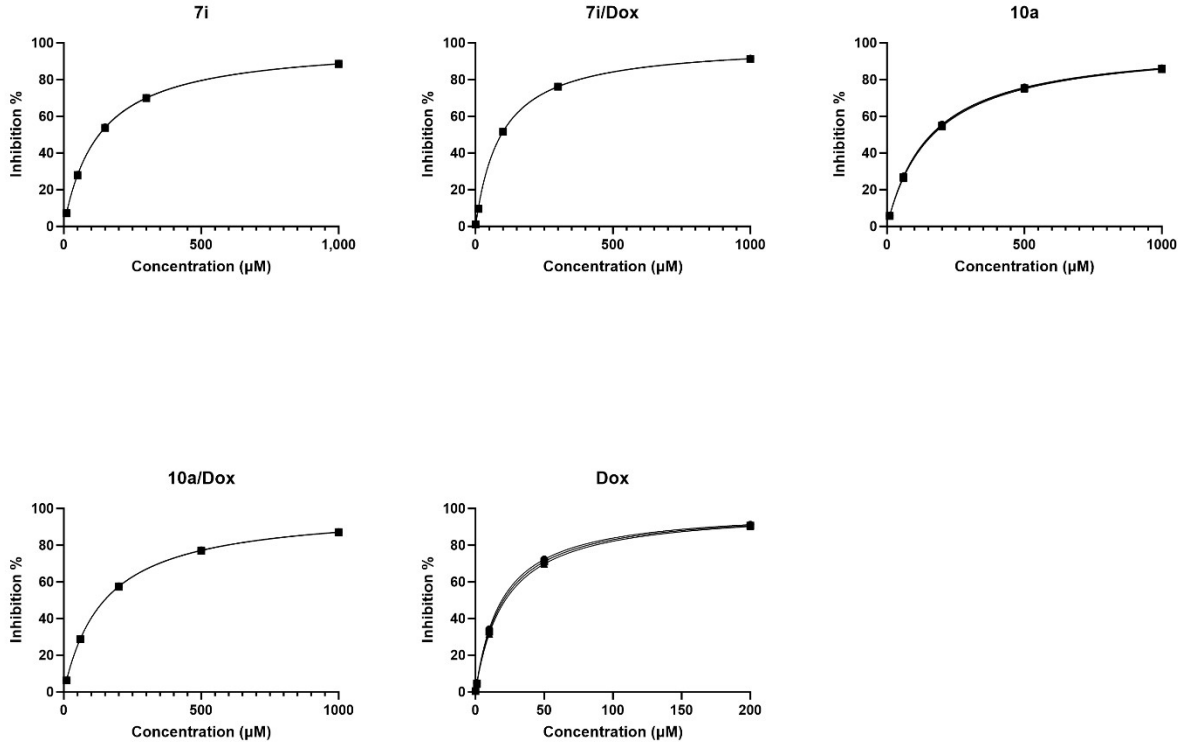

Figure S40. IC_{50} curves of compounds **7i** and **10a**, administered alone and in combination with doxorubicin, against the normal human lung fibroblast cell line WI-38, as determined by the MTT assay.

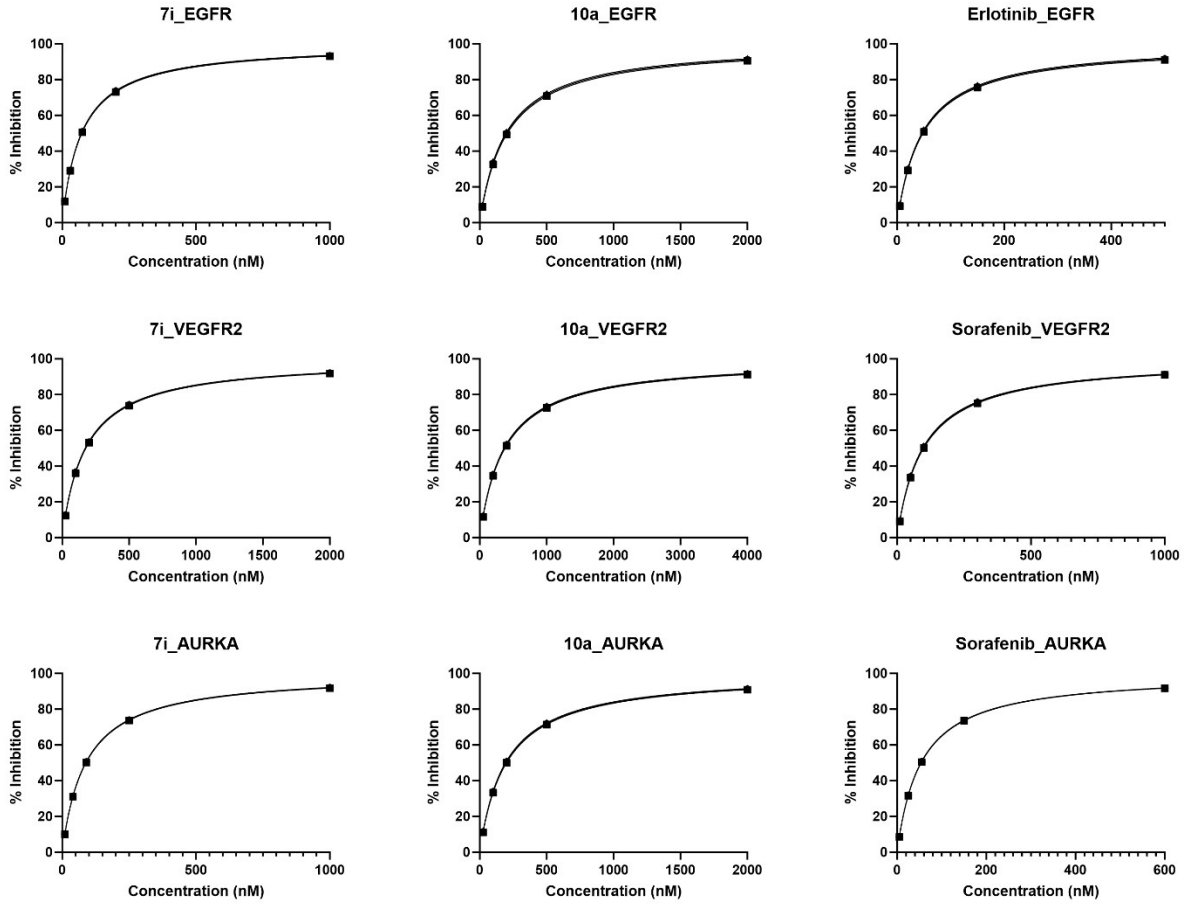

Figure S41. Concentration-response curves showing the inhibitory activity of compounds **7i** and **10a** against EGFR, VEGFR-2, and AURKA kinases. IC_{50} values were calculated from nonlinear regression fitting of the percentage inhibition versus concentration.