Supporting Information

for

TfOH-triggered denitrogenative union of diazo carbonyl compounds and indoline-2-thiones toward functionalized indole derivatives and their initiatory anticancer assessment

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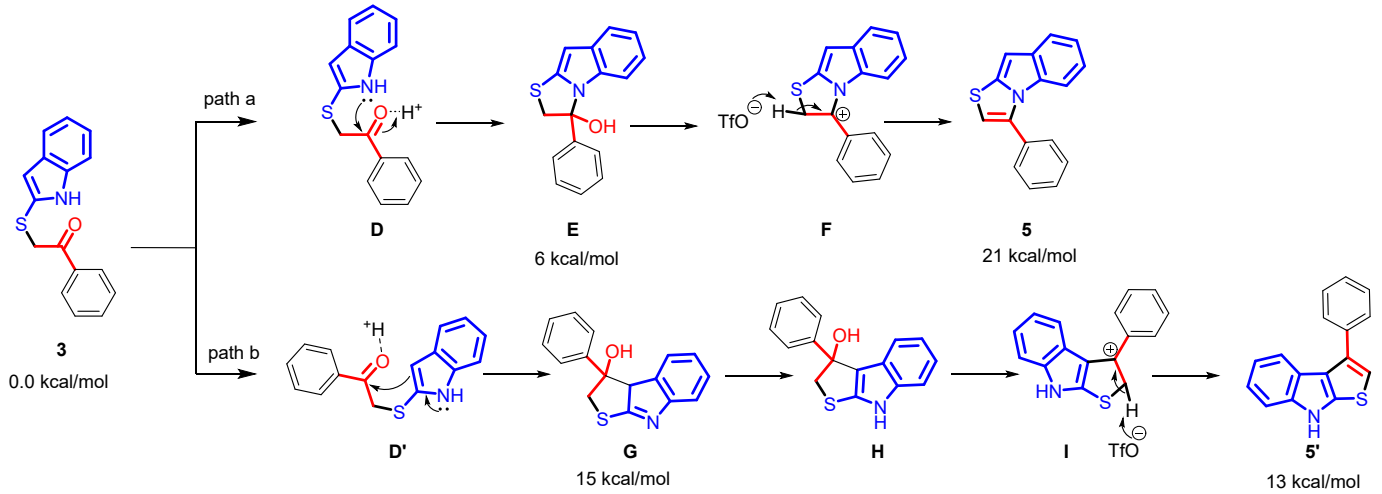
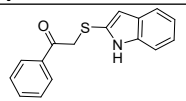
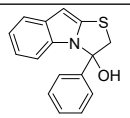
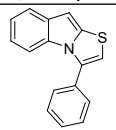
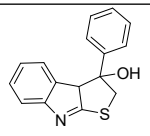
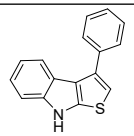
Table of contents

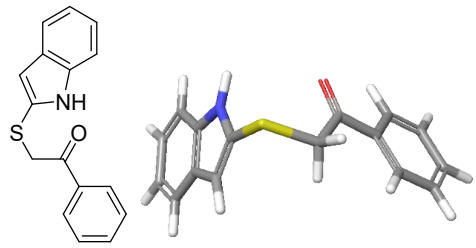
Density functional theory (DFT) computations



All molecular simulations were performed using the Schrödinger 2021-4 program. Geometry optimizations were carried out in the gas phase using the B3LYP functional, with an effective core potential (ECP) for Sc and the split-valence Pople-type 6-31G(d,p) basis set for all other atoms. The optimized geometries of the ground states were obtained using the Jaguar "Optimization" module at the B3LYP/6-31G(d,p) level. Vibrational frequency calculations were performed at the same level of theory to confirm the nature of the stationary points as minima or first-order saddle points and to obtain thermal energy corrections at 298 K and 1 atm. Thermal corrections derived from the optimized geometries were added to the electronic energies obtained from single-point calculations at the same level of theory.

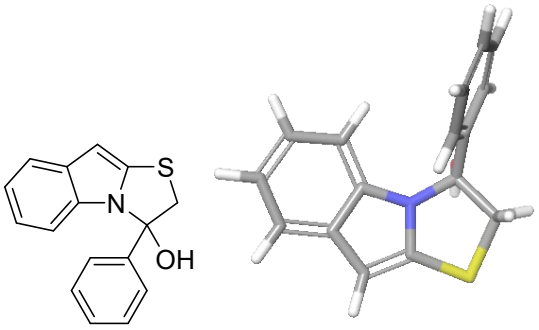
Basis set: **B3LYP/6-31G (d,p)**			in (kcal/mol)		
Structure	 **3**	 **E**	 **5**	 **G**	 **5'**
Gas phase E	-718953.0289945	-718947.0776933	-670979.7406203	-718937.40024017	-670987.45334089
Solution Phase E	-718958.026481	-718952.4052499	-670981.7693589	-718945.6250086	-670993.0526091
Total Enthalpy (ΔH)	-718790.4550602	-718784.398965	-670832.1240051	-718777.8929454	-670843.0621251
Total Free Energy (ΔG)	-718827.0012198	-718819.3418375	-670865.5263415	-718812.8552707	-670876.5403902
Total internal E	-718791.0474293	-718784.9913341	-670832.7163742	-718778.4853145	-670843.6544941

Cartesian Coordinates for optimized geometry



Compound 3

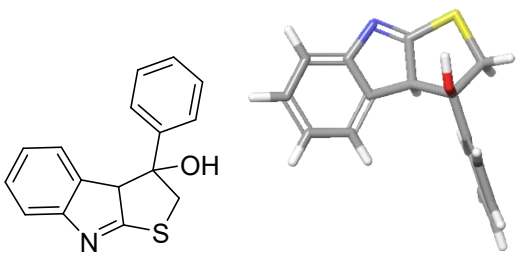
C	1.18546	8.56988	0.89878
C	-0.11305	8.58806	0.33934
C	-0.74612	7.41847	-0.06415
C	-0.04968	6.21206	0.10622
C	1.26431	6.17418	0.66241
C	1.87889	7.37886	1.06208
C	1.66533	4.79969	0.68511
C	0.61479	4.06250	0.18071
N	-0.42044	4.91589	-0.17546
S	0.50253	2.31080	-0.03915
C	0.13702	1.73463	1.66448
C	-1.24127	2.14241	2.19085
C	-1.48323	2.01385	3.65884
C	-0.58829	1.35884	4.52352
C	-0.86700	1.27200	5.88667
C	-2.03277	1.84627	6.40050
C	-2.92787	2.50212	5.54671
C	-2.65748	2.58019	4.18485
O	-2.11254	2.56977	1.44190
H	1.64354	9.50587	1.20642
H	-0.62714	9.53871	0.22618
H	-1.74470	7.43618	-0.49045
H	2.87739	7.37053	1.49560
H	2.60503	4.39569	1.03804
H	-1.29962	4.62015	-0.58379
H	0.20247	0.63659	1.62724
H	0.93003	2.08279	2.33279
H	0.32049	0.90446	4.14111
H	-0.17347	0.75882	6.54785
H	-2.24360	1.78439	7.46459
H	-3.83271	2.95269	5.94530
H	-3.34194	3.08577	3.51040



Compound E

C	-1.40977	4.62396	0.03080
C	-1.58897	4.88564	1.38556
C	-1.17294	3.96333	2.36470
C	-0.57698	2.75359	2.01386
C	-0.40293	2.48905	0.65147
C	-0.80321	3.41588	-0.35680
C	-0.46642	2.84685	-1.63963
C	0.10789	1.63349	-1.37273
N	0.12853	1.38311	-0.00789
C	0.83426	0.18407	0.45250
C	0.83426	-0.74483	-0.79957

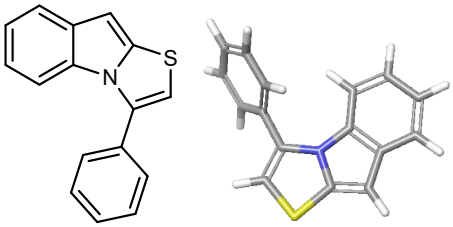
S	0.89821	0.34964	-2.28979
O	2.14936	0.49886	0.87341
C	0.09741	-0.49862	1.60554
C	-1.29929	-0.62100	1.55357
C	-1.99118	-1.27227	2.57267
C	-1.29486	-1.81435	3.65639
C	0.09472	-1.70280	3.70839
C	0.79090	-1.04966	2.68661
H	-1.72925	5.34325	-0.71890
H	-2.05372	5.81761	1.69655
H	-1.31809	4.19787	3.41575
H	-0.25825	2.04912	2.77356
H	-0.63347	3.28604	-2.61357
H	1.69852	-1.41200	-0.78877
H	-0.08237	-1.33505	-0.84425
H	2.65298	0.83980	0.11184
H	-1.85024	-0.19398	0.72025
H	-3.07397	-1.35127	2.52279
H	-1.83406	-2.31973	4.45327
H	0.64343	-2.12467	4.54593
H	1.87064	-0.96666	2.73133



Compound G

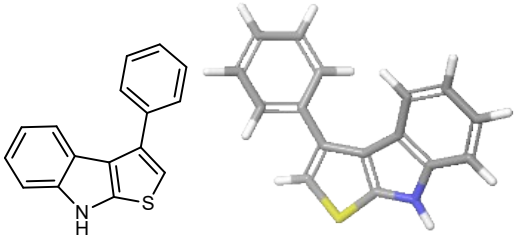
C	-2.55945	-0.34417	4.15564
C	-1.56488	-0.99679	4.89642
C	-0.37996	-1.42803	4.29202
C	-0.15761	-1.22619	2.92126
C	-1.13176	-0.56785	2.18331
C	-2.32712	-0.13313	2.79970
N	-3.19876	0.54581	1.88785
C	-2.59724	0.48744	0.74646
C	-1.29503	-0.27727	0.71445
C	-0.33856	0.57394	-0.16486
C	-1.22469	0.92309	-1.40277
S	-2.91859	1.35454	-0.74778
C	0.91919	-0.18313	-0.59345
C	2.18951	0.37591	-0.41392
C	3.33484	-0.32143	-0.80766
C	3.22793	-1.58479	-1.38902
C	1.96344	-2.14828	-1.57781
C	0.81988	-1.45440	-1.18458
O	0.06308	1.76148	0.50950
H	-3.48317	-0.01209	4.62041
H	-1.71728	-1.17230	5.95819
H	0.37675	-1.92918	4.88984
H	0.75953	-1.57726	2.45670
H	-1.48629	-1.22123	0.18288
H	-0.83046	1.78290	-1.95024
H	-1.32176	0.07278	-2.08020
H	2.28247	1.35641	0.03806

H	4.31283	0.12820	-0.65699
H	4.11916	-2.12742	-1.69309
H	1.86511	-3.13152	-2.03039
H	-0.14964	-1.91994	-1.34694
H	-0.70263	2.34915	0.62980



Compound 5

C	-0.35604	5.42070	-0.03573
C	0.86391	4.71981	0.05941
C	0.90070	3.32781	0.06938
C	-0.31779	2.64287	-0.02408
C	-1.56750	3.33430	-0.09909
C	-1.56673	4.74330	-0.11120
C	-2.62691	2.36305	-0.12484
C	-2.00915	1.13836	-0.07209
N	-0.61607	1.27688	-0.02924
C	0.08568	0.05859	0.04536
C	-0.75152	-1.00543	0.09062
S	-2.45969	-0.55631	0.01258
C	1.56193	-0.03775	0.01260
C	2.29976	0.51753	-1.04757
C	3.68496	0.36612	-1.09505
C	4.34962	-0.34554	-0.09126
C	3.62219	-0.90749	0.96026
C	2.23589	-0.75434	1.01375
H	-0.34655	6.50752	-0.04244
H	1.79589	5.27353	0.13073
H	1.84344	2.80213	0.15390
H	-2.50530	5.28850	-0.17219
H	-3.69094	2.55466	-0.15842
H	-0.46355	-2.04808	0.10867
H	1.78644	1.05957	-1.83708
H	4.24499	0.79870	-1.91961
H	5.42937	-0.46075	-0.13008
H	4.13318	-1.45878	1.74491
H	1.67017	-1.17851	1.83862



Compound 5'

C	-1.30506	5.18996	-1.20375
C	0.00481	4.69029	-1.33445
C	0.34652	3.43351	-0.84447
C	-0.63483	2.65268	-0.20774
C	-1.96283	3.17551	-0.09922
C	-2.30439	4.44035	-0.58732
N	-2.77198	2.23292	0.53114
C	-1.98168	1.14811	0.81654

C	-0.66622	1.34023	0.40158
C	0.15900	0.17820	0.66048
C	-0.57065	-0.82878	1.24247
S	-2.26592	-0.41831	1.51081
C	1.61029	0.04127	0.38398
C	2.13287	-1.16458	-0.11678
C	3.50225	-1.31505	-0.33752
C	4.37906	-0.26163	-0.06753
C	3.87398	0.94278	0.42863
C	2.50468	1.09238	0.65328
H	-1.54246	6.17577	-1.59432
H	0.76101	5.29573	-1.82751
H	1.35886	3.05970	-0.96006
H	-3.31717	4.82140	-0.49166
H	-3.76528	2.33221	0.72384
H	-0.21247	-1.78886	1.59239
H	1.45630	-1.98206	-0.35080
H	3.88434	-2.25413	-0.72994
H	5.44536	-0.37757	-0.24375
H	4.54827	1.76639	0.64922
H	2.12832	2.02440	1.06386

Table S1. IC_{50} (in μM) values of compounds on 4T-1, MDA-MB-231, DU-145 and BEAS-2B cell lines

Compounds	4T-1 (μM)	MDA-MB-231 (μM)	DU-145 (μM)	BEAS-2B (μM)
3a	>20	>20	>20	>20
3b	>20	>20	>20	>20
3c	19.01±2.25	16.69±3.6	>20	>20
3d	11.04±0.77	15.17±1.23	11.34±1.39	13.56±1.07
3e	>20	>20	>20	>20
3f	3.98±0.19	9.45±0.40	5.34±1.74	15.61±1.54
3g	>20	>20	>20	>20
3h	>20	>20	>20	>20
3i	>20	>20	>20	>20
3j	18.92±4.31	>20	6.35±0.64	9.32±3.27
3k	>20	>20	12.12±1.22	>20
5a	>20	>20	>20	>20
5b	>20	>20	>20	>20
5c	>20	>20	>20	>20
5d	8.91±0.98	16.83±2.13	6.62±0.77	8.23±0.93
5e	>20	>20	>20	>20
5f	>20	>20	>20	>20
5g	>20	>20	>20	>20
5i	>20	>20	>20	>20
5j	>20	>20	>20	>20
Vinblastine	0.13±0.04	0.01±0.02	0.59±0.16	--

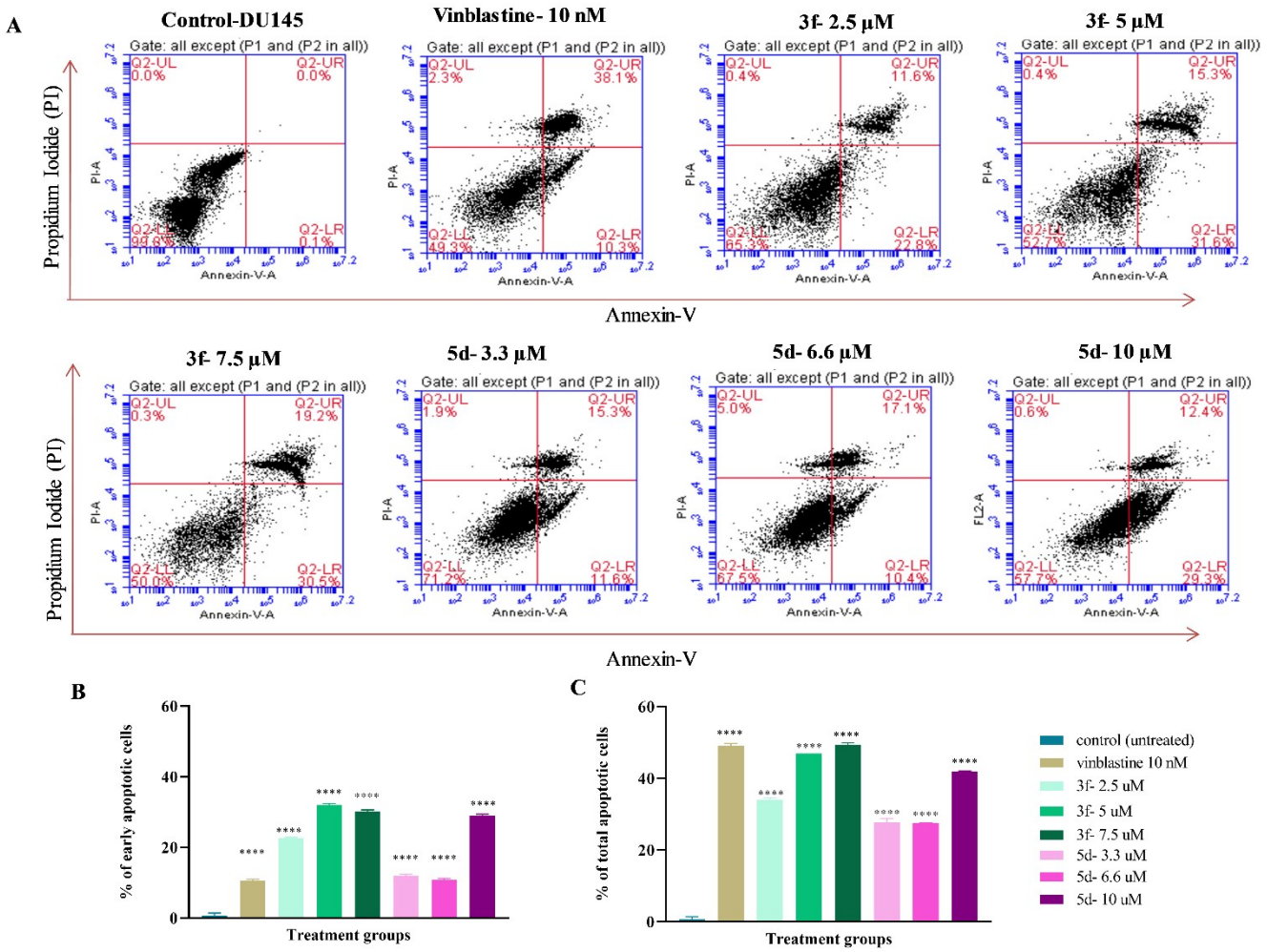


Figure S1: Apoptosis assay: DU-145 cells were treated with various concentrations of **3f** and **5d** for 24 hrs and subjected to apoptosis analysis using Annexin-V assay, and data analyzed using BD-Accuri flow-cytometer. A: FACS plots and B: Quantification of early apoptosis. C: Quantification of total apoptosis.

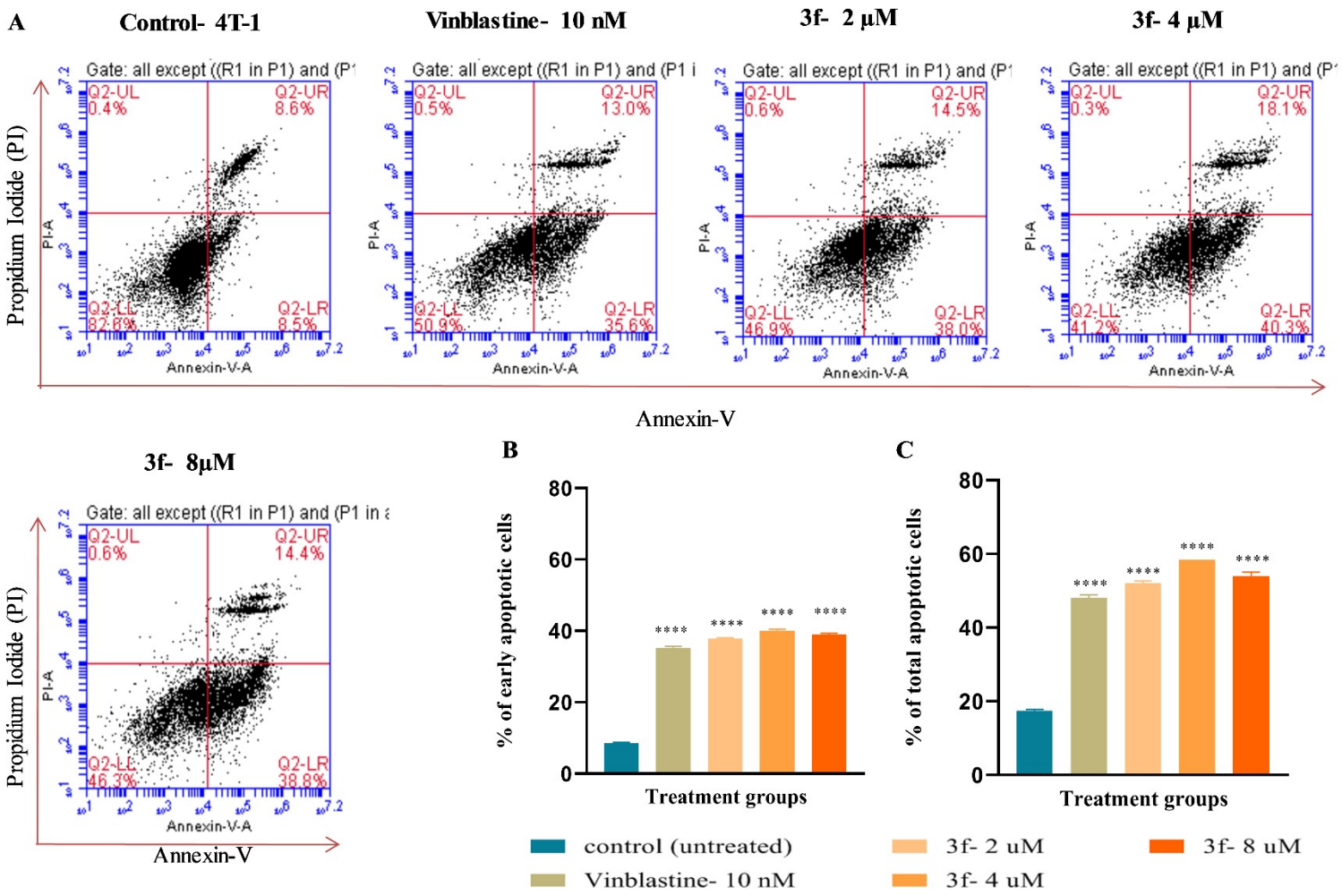


Figure S2: Apoptosis assay: 4T-1 cells were treated with various concentrations of **3f** for 24 hrs and subjected to apoptosis analysis using Annexin-V assay, and data analyzed using BD-Accuri flow-cytometer. A: FACS plots and B: Quantification of early apoptosis. C: Quantification of total apoptosis.

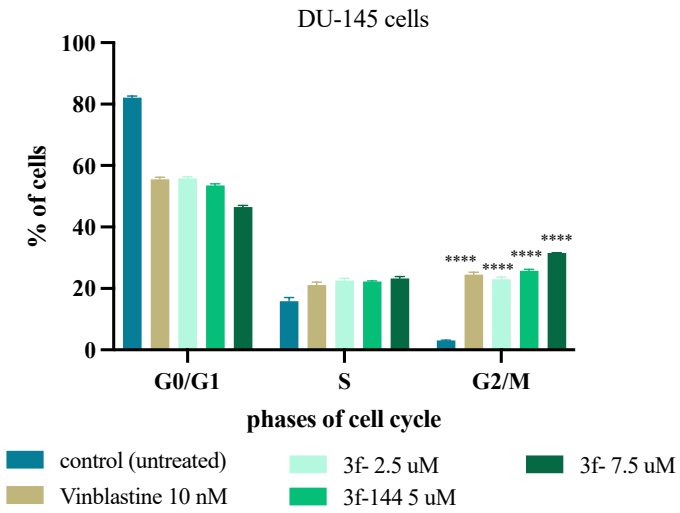


Figure S3: Cell cycle analysis of 3f in DU-145 cell line: DU-145 cells were treated with various concentrations of **3f** for 48 hrs and subjected to cell cycle analysis using BD-Accuri flow-cytometer.

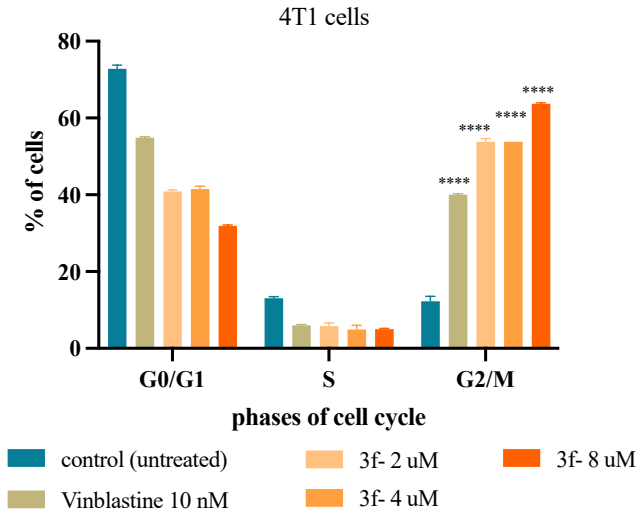


Figure S4: Cell cycle analysis of 3f in 4T-1 cell line: 4T-1 cells were treated with various concentrations of 3f for 48 hrs and subjected to cell cycle analysis using BD-Accuri flow-cytometer.

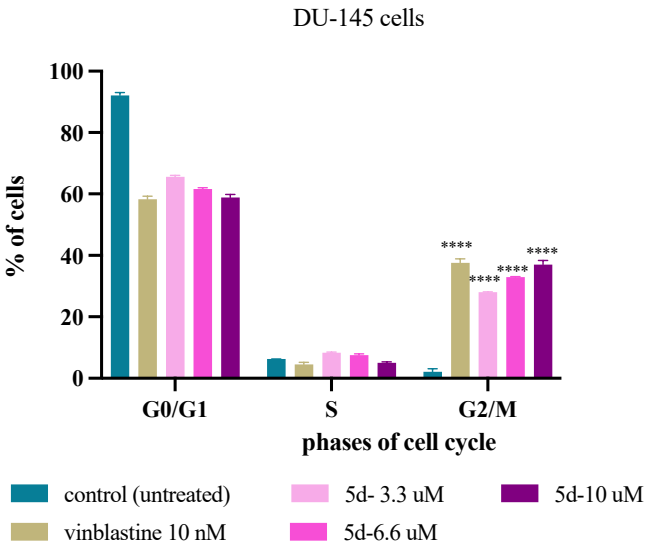
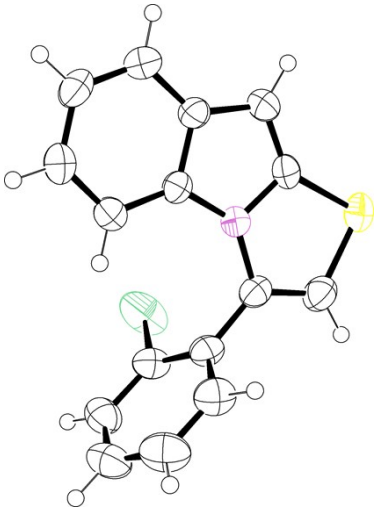


Figure S5: Cell cycle analysis of 5d in DU-145 cell line: DU-145 cells were treated with various concentrations of **5d** for 48 hrs and subjected to cell cycle analysis using BD-Accuri flow-cytometer.

Figure S6. ORTEP view and crystallographic data for **5c** (CCDC: 2450009)



Compound	**5c**
Emp form.	$C_{16}H_{10}ClNS$
Form wt.	283.78
Sp. Grp.	*P 21 21 21*
T (K)	296
a (Å)	7.6596(4)
b (Å)	10.8313(5)
c (Å)	16.1412(7)
α (º)	90
β (º)	90
γ (º)	90
Z	4
V (Å^3)	1339.13(11)
ρcalcd (g/cm3)	1.408
Rflns. collect	3206
Unique rflns.	2571
Obsd. Rflns.	3201
R_1	0.0375
wR_2	0.0942
Diffractometer	Bruker APEX-II CCD

EXPERIMENTAL SECTION

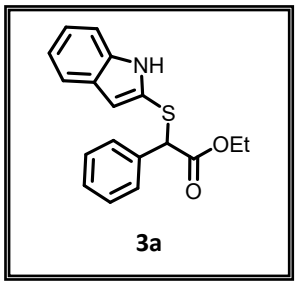
General Information. All reagents and chemicals were used as obtained from commercial sources, unless specified. Dulbecco's Modified Eagle medium (DMEM), heat-inactivated Fetal bovine serum (FBS) and antibiotic solution (pen strep) were acquired from Gibco. Acetic acid, sulforhodamine B (SRB), and Tris base were obtained from Sigma-Aldrich. Trichloroacetic acid (TCA) was obtained from TCI. Pvt. Ltd. Annexin V (FITC labelled) Apoptosis Detection Kit with PI procured from Biolegend. PI/RNAase staining solution purchased from Invitrogen by Thermo Scientific. Air and moisture-sensitive reactions were performed under positive N_2 or a blanket in flame- or oven-dried glassware. Melting points were determined on a capillary melting point apparatus and uncorrected. IR spectra were recorded neat on a Bruker Alpha FT-IR spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded (300/400/500 and 101/126/151 MHz, respectively) on a Bruker 300/400/500/600 MHz using $CDCl_3$. Chemical shifts for proton and carbon resonances are reported for the major isomer in parts per million (δ) relative to tetramethylsilane (δ 0.00) and chloroform (δ 77.7), respectively. Multiplicities are indicated by singlet (*s*), doublet (*d*), triplet (*t*), quartet (*q*), multiplet (*m*), broad singlet (br s). Coupling constants (*J*) were reported in hertz (*Hz*). High resolution mass analyses were performed using ESI-TOF technique. Single-crystal X-ray data were collected on a Bruker APEX-II CCD and XtaLAB Synergy diffractometer, using Mo Kα radiation at 298 K. Structure solution and refinements were performed in Olex2 using the SHELX program. The crystal for single crystal X-ray diffraction analysis were obtained by slow evaporation in pentane at 25 °C. Thin layer chromatography (TLC) was performed using pre-coated silica gel 60 F_{254} (Merck) and visualized through UV light, iodine, and *p*-anisaldehyde stain. Column chromatography was performed using silica gel (100-200 mesh). Diazo carbonyl compounds **1a-k** & **4a-h** were prepared using a reported literature procedure.[1] Indolin-2-thiones **2a-d** were synthesized by adopting known procedures.[2]

General procedure for synthesis of 3

To a stirred solution of diazo carbonyl compound **1** (1 equiv) and indolin-2-thione **2** (1 equiv) in 2 mL dry CH_2Cl_2, TfOH (1 equiv) was added at -10 °C, and the reaction was continued stirring at the same temperature for another 30 minutes. After that, the reaction mixture was quenched with a saturated aq. $NaHCO_3$ solution and extracted with DCM. The organic layer was washed with water, brine solution, and dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue was purified by column chromatography (SiO_2 and EtOAc: Hexane) to get the desired thioether-substituted indoles **3a-3k**.

Ethyl 2-((1*H*-indol-2-yl)thio)-2-phenylacetate (3a)

Starting materials: **1a** and **2a**



3a

Yield: 140 mg (86%)

Description: White Solid

Rf: 0.5 (10% EtOAc/Hexane)

Melting point: 113-115 °C; lit.[18] 113-114 °C

FTIR(ATR): 3368, 1731, 1267 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48 (s, 1H), 7.52 (d, *J* = 7.9 Hz, 1H), 7.34 – 7.28 (m, 6H), 7.21 – 7.17 (m, 1H), 7.09 – 7.05 (m, 1H), 6.61 – 6.60 (m, 1H), 4.82 (s, 1H), 4.21 – 4.12 (m, 2H), 1.17 (t, *J* = 7.1 Hz, 3H) ppm.

^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 171.3, 137.5, 135.4, 128.8, 128.4, 128.3, 128.1, 125.5, 123.2, 120.7, 120.1, 111.7, 110.9, 62.2, 57.3, 14.0 ppm.

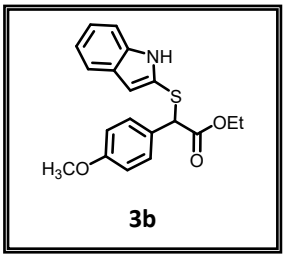
HRMS: *m/z* $[M + H]^+$ calcd for $C_{18}H_{18}NO_2S$ 312.1058; found 312.1062

Ethyl 2-((1*H*-indol-2-yl)thio)-2-(4-methoxyphenyl)acetate (3b)

Starting materials: **1b** and **2a**

Yield: 115 mg (75%)

Description: White Solid



3b

Rf (TLC): 0.39 (20% EtOAc/Hexane)

Melting point: 111-114 °C

FTIR(ATR): 3388, 1722, 1149 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49 (s, 1H), 7.53 (s, 1H), 7.29 – 7.24 (m, 3H), 7.20 – 7.16 (m, 1H), 7.09 – 7.05 (m, 1H), 6.81 (d, *J* = 8.8 Hz, 1H), 6.61 (dd, *J* = 2.0, 0.8 Hz, 1H), 4.80 (s, 1H), 4.21 – 4.09 (m, 2H), 3.76 (s, 3H), 1.14 (d, *J* = 7.1 Hz, 3H) ppm.

$^{13}C\{^1H\}$ NMR (126 MHz, $CDCl_3$) δ 171.5, 159.6, 137.5, 129.6, 128.6, 127.4, 125.7, 123.1, 120.7, 120.0, 114.2, 111.5, 110.8, 62.1, 56.7, 55.3, 14.0 ppm.

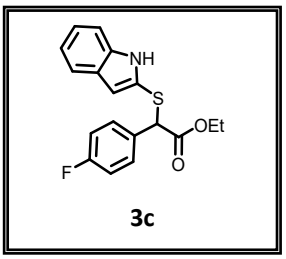
HRMS: *m/z* $[M + H]^+$ calcd for $C_{19}H_{20}NO_3S$ 342.1164; found 342.1163

Ethyl 2-((1*H*-indol-2-yl)thio)-2-(4-fluorophenyl)acetate (3c)

Starting materials: **1c** and **2a**

Yield: 154 mg (97%)

Description: Greenish white solid



3c

Rf: 0.65 (10% EtOAc/Hexane)

m.p: 110-115 °C

FTIR(ATR): 3388, 1724, 1283, 1224 cm^{-1}

^1H NMR (400 MHz, $CDCl_3$) δ 8.49 (s, 1H), 7.53 (d, *J* = 7.9 Hz, 1H), 7.29 (tt, *J* = 8.4, 2.4 Hz, 3H), 7.25 – 7.18 (m, 1H), 7.10 – 7.06 (m, 1H), 6.99 – 6.94 (m, 2H), 6.59 (d, *J* = 1.3 Hz, 1H), 4.80 (s, 1H), 4.21 – 4.12 (m, 2H), 1.17 (t, *J* = 7.1 Hz, 3H) ppm.

^{13}C NMR (101 MHz, $CDCl_3$) δ 171.2, 162.6 (d, *J* = 247.9 Hz), 137.5, 131.2 (d, *J* = 3.0 Hz), 130.1 (d, *J* = 8.3 Hz), 128.1, 125.0, 123.3, 120.7, 120.6, 115.7 (d, *J* = 21.7 Hz), 111.9, 110.9, 62.3, 57.0, 14.0 ppm.

^{19}F NMR (376 MHz, $CDCl_3$) δ -113.16 ppm.

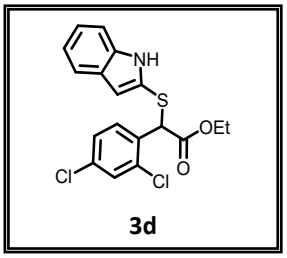
HRMS: *m/z* $[M + H]^+$ calcd for $C_{18}H_{17}FNO_2S$ 330.0964; found 330.0968

Ethyl 2-((1*H*-indol-2-yl)thio)-2-(2,4-dichlorophenyl)acetate (3d)

Starting materials: **1d** and **2a**

Yield: 126 mg (86%)

Description: Yellow Solid



3d

Rf: 0.6 (10% EtOAc/Hexane)

m.p: 84-86 °C

FTIR(ATR): 3392, 1725, 1267 cm^{-1}

^1H NMR (300 MHz, $CDCl_3$) δ 8.68 (s, 1H), 7.52 (dd, *J* = 7.9, 0.6 Hz, 1H), 7.40 (d, *J* = 2.1 Hz, 1H), 7.34 (dd, *J* = 8.2, 0.8 Hz, 1H), 7.24 – 7.18 (m, 2H), 7.11 – 7.06 (m, 2H), 6.57 (dd, *J* = 2.0, 0.8 Hz, 1H), 5.24 (s, 1H), 4.25 – 4.14 (m, 2H), 1.19 (t, *J* = 7.1 Hz, 3H) ppm.

^{13}C NMR (101 MHz, $CDCl_3$) δ 170.8, 137.6, 134.7, 134.4, 131.9, 130.3, 129.6, 128.0, 127.3, 124.3, 123.5, 120.8, 120.2, 112.5, 110.9, 62.6, 52.7, 14.0 ppm.

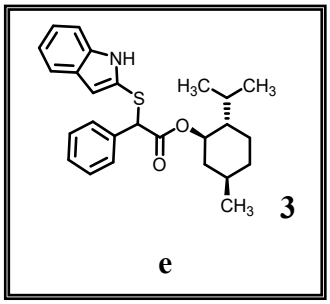
HRMS: *m/z* $[M + H]^+$ calcd for $C_{18}H_{16}Cl_2NO_2S$ 380.0279; found 380.0288

2-Isopropyl-5-methylcyclohexyl 2-((1*H*-indol-2-yl)thio-2-phenylacetate (3e)

Starting materials: **1e** and **2a**

Yield: 123 mg (88%)

Description: White solid



Rf: 0.5 (10% EtOAc/Hexane)

m.p: 133-137 °C

FTIR(ATR): 3365, 1720, 1280 cm^{-1}

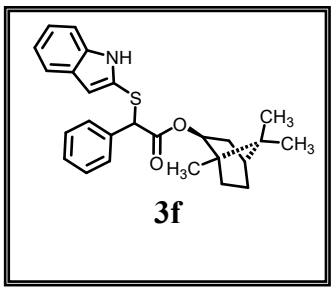
dr ratio: 8:2 (based on NMR)

^1H NMR (400 MHz, $CDCl_3$) δ 8.49 (s, 1H), 7.52 (d, *J* = 7.9 Hz, 1H), 7.33 – 7.27 (m, 6H), 7.18 (t, *J* = 7.6 Hz, 1H), 7.07 (t, *J* = 7.5 Hz, 1H), 6.60 (d, *J* = 1.6 Hz, 1H), 4.83 (d, *J* = 15.6 Hz, 1H), 4.69 (ddd, *J* = 15.3, 11.3, 4.4 Hz, 1H), 1.89 (d, *J* = 12.1 Hz, 1H), 1.65 – 1.61 (m, 2H), 1.43 (ddd, *J* = 13.8, 6.9, 2.8 Hz, 2H), 1.30 – 1.23 (m, 1H), 0.97 (ddd, *J* = 16.0, 11.2, 3.9 Hz, 1H), 0.85 (t, *J* = 5.0 Hz, 5H), 0.68 (d, *J* = 7.0 Hz, 3H), 0.57 (dd, *J* = 16.6, 6.9 Hz, 3H) ppm.

^{13}C NMR (101 MHz, $CDCl_3$) δ 171.0, 137.4, 135.6, 128.74, 128.68, 128.3, 128.2, 128.1, 125.7, 123.1, 120.6, 120.0, 111.3, 110.8, 76.4, 76.2, 57.7, 57.6, 47.0, 46.9, 40.6, 40.3, 34.1, 31.4, 26.0, 25.8, 23.3, 22.0, 20.5, 16.0 ppm.

HRMS: *m/z* $[M + H]^+$ calcd for $C_{26}H_{32}NO_2S$ 422.2154; found 422.2170

(4*R*)-1,7,7-Trimethylbicyclo[2.2.1]heptan-2-yl 2-((1*H*-indol-2-yl)thio)-2-phenylacetate (3f)



3f

Starting materials: **1f** and **2a**

Yield: 80 mg (62%)

Description: White solid

Rf: 0.5 (10% EtOAc/Hexane)

m.p: 110-113 °C

FTIR(ATR): 3378, 1720, 1278 cm^{-1}

dr ratio: 1:1 (based on NMR)

^1H NMR (300 MHz, $CDCl_3$) δ 8.41 (s, 1H), 7.46 (s, 1H), 7.29 – 7.18 (m, 6H), 7.10 (dd, *J* = 11.2, 4.0 Hz, 1H), 7.00 (t, *J* = 7.4 Hz, 1H), 6.54 (d, *J* = 0.9 Hz, 1H), 4.86 (dd, *J* = 11.0, 4.2 Hz, 1H), 4.78 (s, 1H), 2.30 – 2.17 (m, 1H), 1.67 – 1.53 (m, 4H), 1.18 – 1.03 (m, 1H), 0.94 (ddd, *J* = 13.4, 8.8, 3.3 Hz, 1H), 0.80 (s, 3H), 0.74 (d, *J* = 3.1 Hz, 3H), 0.62 (d, *J* = 7.9 Hz, 3H) ppm.

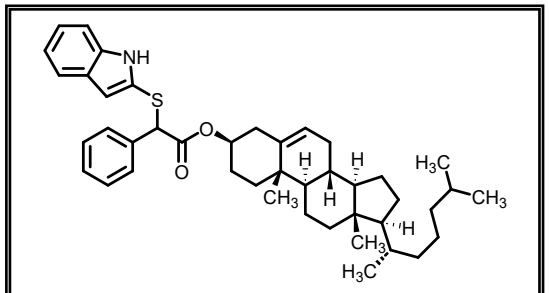
^{13}C NMR (101 MHz, $CDCl_3$) δ 171.6, 171.5, 137.4, 135.8, 135.7, 128.7, 128.3, 128.1, 125.7, 123.1, 120.6, 120.0, 111.3, 111.2, 110.8, 82.2, 81.9, 57.6, 57.5, 49.1, 48.9, 47.9, 44.81, 44.75, 36.5, 36.4, 27.9, 27.8, 27.0, 26.9, 19.6, 18.8, 13.41, 13.37 ppm.

HRMS: *m/z* $[M + H]^+$ calcd for $C_{26}H_{30}NO_2S$ 420.1997; found 420.2009

(10*S*,13*S*)-10,13-Dimethyl-17-((*S*)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[a]phenanthren-3-yl 2-((1*H*-indol-2-yl)thio)-2-phenylacetate (3g)

Starting materials: **1g** and **2a**

Yield: 100 mg (85%)



3g

Description: White solid

Rf: 0.67 (10% EtOAc/Hexane)

m.p: 171-174 ℃

FTIR(ATR): 3371, 1726, 1280 cm^{-1}

dr ratio: 1:1 (based on NMR)

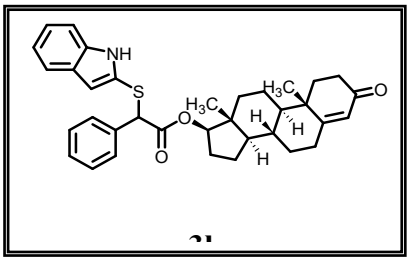
^1H NMR (400 MHz, $CDCl_3$) δ 8.42 (s, 1H), 7.45 (d, *J* = 7.7 Hz, 1H), 7.22 (td, *J* = 10.7, 5.6 Hz, 6H), 7.12 (t, *J* = 7.5 Hz, 1H), 7.00 (t, *J* = 7.4 Hz, 1H), 6.53 (s, 1H), 5.26 – 5.23 (m, 1H), 4.73 (s, 1H), 4.60 – 4.54 (m, 1H), 2.14 (dd, *J* = 16.7, 8.4 Hz, 2H), 1.90 (t, *J* = 15.6 Hz, 2H), 1.73 (d, *J* = 14.3 Hz, 3H), 1.49 – 1.19 (m, 12H) 1.04 – 1.01 (m, 9H), 0.87 (s, 3H), 0.84 (d, *J* = 6.6 Hz, 3H),0.79 (dd, *J* = 6.5, 1.6 Hz, 6H), 0.59 (s, 3H) ppm.

^{13}C NMR (75 MHz, $CDCl_3$) δ 170.8, 139.3, 137.5, 135.50, 135.46, 128.7, 128.3, 128.1, 125.5, 123.2, 123.0, 120.6, 120.0, 111.6, 110.8, 76.03, 75.98, 57.3, 56.7, 56.2, 50.0, 42.3, 39.7, 39.6, 37.74, 37.71, 36.92, 36.88, 36.7, 36.2, 35.8, 31.92, 31.85, 28.3, 28.1, 27.5, 24.3, 23.9, 22.9, 22.6, 21.1, 19.3, 18.8, 11.9 ppm.

HRMS: *m/z* $[M + H]^+$ calcd for $C_{43}H_{58}NO_2S$ 652.4188, found 652.4206

(10*S*,13*R*)-10,13-Dimethyl-3-oxo-2,3,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[a]phenanthren-17-yl 2-((1*H*-indol-2-yl)thio)-2-phenylacetate (3h)

Starting materials: **1h** and **2a**



3h

Yield: 85 mg (62%)

Description: Orange gummy solid

Rf: 0.26 (20% EtOAc/Hexane)

FTIR(ATR): 3284, 1726, 1273 cm^{-1}

dr ratio: 1:1 (based on NMR)

^1H NMR (500 MHz, $CDCl_3$) δ 8.60 (s, 1H), 7.44 (d, *J* = 7.9 Hz, 1H), 7.27 (dd, *J* = 13.6, 5.2 Hz, 2H), 7.22 – 7.18 (m, 4H), 7.10 (t, *J* = 7.5 Hz, 1H), 6.99 (t, *J* = 7.4 Hz, 1H), 6.55 – 6.52 (m,

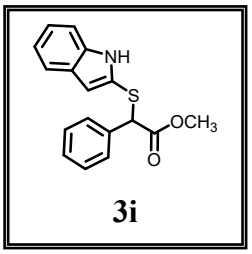
1H), 5.65 (s, 1H), 4.78 (dd, *J* = 15.9, 3.6 Hz, 1H), 4.55 (dt, *J* = 16.7, 8.7 Hz, 1H), 2.33 – 2.23 (m, 3H), 2.16 (d, *J* = 13.2 Hz, 1H), 2.02 (dd, *J* = 16.4, 10.1 Hz, 1H), 1.91 – 1.88 (m, 1H), 1.71 (d, *J* = 12.0 Hz, 1H), 1.60 – 1.47 (m, 4H), 1.41 (d, *J* = 11.4 Hz, 2H), 1.34 – 1.32 (m, 1H), 1.21 – 1.18 (m, 1H), 1.15 (d, *J* = 6.0 Hz, 1H), 1.05 (s, 3H), 0.98 – 0.85 (m, 3H), 0.51 (d, *J* = 18.2 Hz, 3H) ppm.

^{13}C NMR (101 MHz, $CDCl_3$) δ 199.6, 171.2, 171.13, 171.06, 171.0, 137.5, 135.9, 135.4, 128.8, 128.5, 128.4, 128.3, 128.9, 128.0, 126.6, 125.71, 125.66, 124.0, 123.1, 120.6, 120.0, 111.2, 110.9, 84.2, 84.0, 57.6, 57.41, 53.6, 50.1, 42.9, 42.7, 38.6, 36.5, 36.4, 35.7, 35.3, 34.0, 32.7, 31.4, 27.2, 27.1, 23.4, 20.5, 20.4, 17.4, 11.8, 11.7 ppm.

HRMS: *m/z* $[M + H]^+$ calcd for $C_{35}H_{40}NO_3S$ 554.2729; found 554.2723

Methyl 2-((1*H*-indol-2-yl)thio)-2-phenylacetate (3i)

Starting materials: **1i** and **2a**



3i

Yield: 50 mg (50%)

Description: White gummy solid

Rf: 0.37 (10% EtOAc/Hexane)

FTIR(ATR): 3365, 1720, 1280 cm^{-1}

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.46 (s, 1H), 7.53 (s, 1H), 7.30 (t, *J* = 3.0 Hz, 6H), 7.22 – 7.17 (m, 1H), 7.08 (d, *J* = 7.8 Hz, 1H), 6.61 (d, *J* = 1.1 Hz, 1H), 4.84 (s, 1H), 3.71 (s, 3H) ppm.

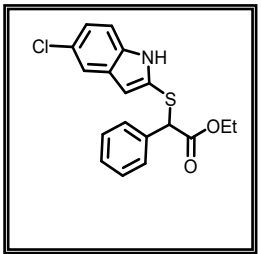
^{13}C NMR (75 MHz, $CDCl_3$) δ 171.8, 137.5, 135.3, 128.8, 128.5, 128.3, 128.1, 125.4, 123.2, 120.7, 120.1, 111.7, 110.9, 57.2, 53.1 ppm.

HRMS: *m/z* $[M + H]^+$ calcd for $C_{17}H_{16}NO_2S$ 298.0902; found 298.0912

Ethyl 2-((5-chloro-1*H*-indol-2-yl)thio)-2-phenylacetate (3j)

Starting materials: **1a** and **2b**

Yield: 128 mg (72%)



Description: White solid

Rf: 0.4 (10% EtOAc/Hexane)

m.p: 92-95 ℃

FTIR(ATR): 3359, 1721, 1299, 698 cm^{-1}

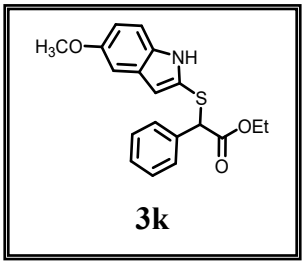
^1H NMR (400 MHz, $CDCl_3$) δ 8.55 (s, 1H), 7.41 (d, *J* = 8.5 Hz, 1H), 7.28 (s, 6H), 7.05 – 7.03 (m, 1H), 6.55 – 6.54 (m, 1H), 4.82 (s, 1H), 4.23 – 4.14 (m, 2H), 1.19 (t, *J* = 7.1 Hz, 3H) ppm.

^{13}C NMR (101 MHz, $CDCl_3$) δ 171.7, 137.7, 135.2, 129.1, 128.8, 128.4, 128.2, 126.6, 126.3, 121.5, 120.9, 111.7, 110.7, 62.3, 57.0, 14.0 ppm.

HRMS: *m/z* $[M + H]^+$ calcd for $C_{18}H_{17}ClNO_2S$ 346.0669; found 346.0673

Ethyl 2-((5-methoxy-1*H*-indole-2yl)thio)-2-phenylacetate (3k)

Starting materials: **1a** and **2c**



3k

Yield: 135mg (76%)

Description: White solid

Rf: 0.17 (10% EtOAc/Hexane)

m.p: 113-115 ℃

FTIR(ATR): 3368, 2836, 1724, 1218 cm^{-1}

^1H NMR (400 MHz, $CDCl_3$) δ 8.37 (s, 1H), 7.34 – 7.28 (m, 5H), 7.18 (d, *J* = 8.8 Hz, 1H), 6.96 (d, *J* = 2.4 Hz, 1H), 6.86 (dd, *J* = 8.8, 2.5 Hz, 1H), 6.53 (dd, *J* = 2.0, 0.8 Hz, 1H), 4.81 (s, 1H), 4.17 (m, *J* = 14.2, 9.0, 5.4 Hz, 2H), 3.82 (s, 3H), 1.19 – 1.15 (m, 3H) ppm.

^{13}C NMR (126 MHz, $CDCl_3$) δ 171.3, 154.2, 135.5, 132.7, 128.8, 128.7, 128.4, 128.3, 125.8, 113.9, 111.7, 111.3, 101.8, 62.1, 57.3, 55.8, 14.0 ppm.

HRMS: *m/z* $[M + H]^+$ calcd for $C_{19}H_{20}NO_3S$ 342.1164; found 342.1176

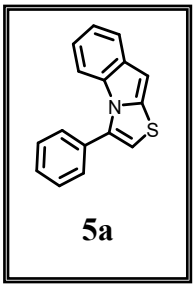
General procedure for the synthesis of 5

To a stirred solution of diazo carbonyl compound **4** (1 equiv) and indolin-2-thione **2** (1 equiv) in 2 mL dry CH_2Cl_2, TfOH (1 equiv) was added at -10 °C, and the reaction was continued by stirring at the same temperature for another 30 minutes. After that, the reaction mixture was shifted to 0 °C for another 1h and continued stirring at room temperature for another 1.5h and starting material consumption monitored by TLC. After that the reaction mixture was quenched with a saturated aq. $NaHCO_3$ solution and extracted with DCM. The organic layer was washed with water, brine solution, and dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue was purified by column chromatography (SiO_2 and EtOAc: Hexane) to get the desired product **5**.

Note: The thiazolo[3,2-a]indoles 5 obtained in these studies were found to be unstable at room temperature (~25 °C) and also showed limited stability in $CDCl_3$ solution. However, the solid compounds were stable when stored at ≤ 0 °C, retaining their integrity for at least 6 months under these conditions.

3-Phenylthiazolo[3,2-*a*]indole (**5a**)

Starting Material: **4a** and **2a**



5a

Yield: 112 mg (66%)

Description: Yellowish green gummy solid

Rf: 0.5 (10% EtOAc/Hexane)

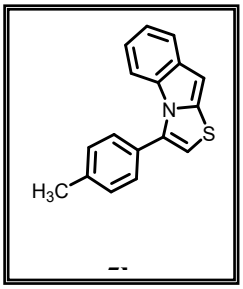
FTIR(ATR): 1170, 1457 cm^{-1}

^1H NMR (500 MHz, $CDCl_3$) δ 7.63 (ddd, J = 11.0, 6.2, 4.4 Hz, 3H), 7.56 – 7.52 (m, 3H), 7.17 – 7.13 (m, 1H), 7.02 – 7.00 (m, 1H), 6.89 (ddd, J = 8.3, 7.1, 1.1 Hz, 1H), 6.60 (s, 1H), 6.37 (s, 1H) ppm.

^{13}C NMR (101 MHz, $CDCl_3$) δ 138.7, 135.4, 133.3, 130.7, 130.1, 129.7, 129.4, 128.8, 121.1, 119.6, 118.6, 111.8, 106.8, 91.5 ppm.

HRMS: *m/z* $[M + H]^+$ calcd for $C_{16}H_{12}NS$ 250.0689; found 250.0690

3-(*p*-Tolyl)thiazolo[3,2-*a*]indole (5b)



Starting Material: **4b** and **2a**

Yield: 96 mg (59%)

Description: White solid

Rf: 0.6 (10% EtOAc/Hexane)

m.p: 93-95 °C

FTIR(ATR): 1602, 1306 cm^{-1}

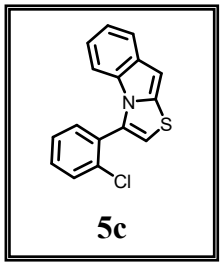
^1H NMR (400 MHz, $CDCl_3$) δ 7.61 – 7.59 (m, 1H), 7.51 – 7.49 (m, 2H), 7.32 (d, *J* = 7.8 Hz, 2H), 7.16– 7.12 (m, 1H), 7.05 (dd, *J* = 8.4, 0.7 Hz, 1H), 6.90 – 6.86 (m, 1H), 6.58 (d, *J* = 0.7 Hz, 1H), 6.31 (s, 1H), 2.47 (s, 3H) ppm.

^{13}C NMR (101 MHz, $CDCl_3$) δ 139.8, 138.7, 135.5, 133.3, 130.1, 129.5, 129.3, 127.7, 121.0, 119.6, 118.6, 111.9, 106.4, 91.5, 21.6 ppm.

HRMS: *m/z* $[M + H]^+$ calcd for $C_{17}H_{14}NS$ 264.0847; found 264.0853

3-(2-Chlorophenyl)thiazolo[3,2-*a*]indole (5c)

Starting Material: **4c** and **2a**



5c

Yield :79 mg (51%)

Description: White gummy solid

Rf: 0.52 (10% EtOAc/Hexane)

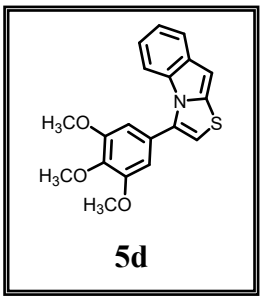
FTIR(ATR): 1451, 1308, 746 cm^{-1}

^1H NMR (300 MHz, $CDCl_3$) δ 7.62 (d, *J* = 3.1 Hz, 1H), 7.60 (d, *J* = 1.3 Hz, 1H), 7.56 (t, *J* = 1.7 Hz, 1H), 7.54 – 7.51 (m, 1H), 7.46 – 7.41 (m, 1H), 7.17 – 7.12 (m, 1H), 6.91 – 6.85 (m, 1H), 6.67 (dd, *J* = 8.4, 0.7 Hz, 1H), 6.60 (d, *J* = 0.6 Hz, 1H), 6.47 (s, 1H) ppm.

^{13}C NMR (101 MHz, $CDCl_3$) δ 137.9, 135.4, 133.0, 132.5, 131.7, 131.4, 130.1, 129.94, 129.92 127.2, 121.1, 119.6, 119.0, 110.8, 108.1, 91.4 ppm.

HRMS: *m/z* $[M + H]^+$ calcd for $C_{16}H_{11}ClNS$ 284.0301; found 284.0296

3-(3,4,5-Trimethoxyphenyl)thiazolo[3,2-*a*]indole (5d)



5d

Starting Material: **4d** and **2a**

Yield: 85 mg (59%)

Description: White solid

Rf: 0.6 (10% EtOAc/Hexane)

m.p: 85-87 ℃

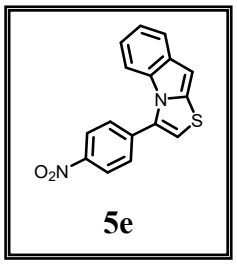
FTIR (ATR): 1734, 1661, 1333, 1227 cm^{-1}

^1H NMR (500 MHz, $CDCl_3$) δ 7.62 (s, 1H), 7.19 – 7.12 (m, 2H), 6.95 (ddd, *J* = 8.3, 7.1, 1.1 Hz, 1H), 6.84 (s, 2H), 6.61 (d, *J* = 0.4 Hz, 1H), 6.39 (s, 1H), 3.98 (s, 3H), 3.87 (s, 6H) ppm.

^{13}C NMR (101 MHz, $CDCl_3$) δ 153.5, 139.2, 138.5, 135.2, 133.3, 130.0, 125.8, 121.2, 119.7, 118.7, 112.0, 106.6, 91.6, 61.2, 56.3 ppm.

HRMS: *m/z* $[M + H]^+$ calcd for $C_{19}H_{18}NO_3S$ 340.1027; found 340.1006

3-(4-Nitrophenyl)thiazolo[3,2-a]indole (5e)



5e

Starting Material: **4e** and **2a**

Yield :105 mg (68%)

Description: Reddish orange solid

Rf: 0.5 (2% EtOAc/Hexane)

m.p: 180-182 ℃

FTIR(ATR): 2824, 1516, 1345 cm^{-1}

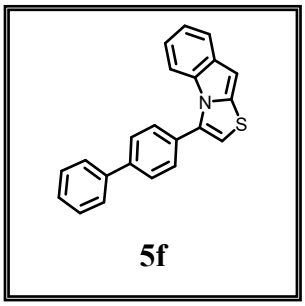
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 (d, J = 8.8 Hz, 2H), 7.85 (dd, J = 9.0, 2.1 Hz, 2H), 7.65 (d, J = 8.0 Hz, 1H), 7.26 (s, 1H), 7.20 (ddd, J = 8.0, 7.0, 1.0 Hz, 1H), 7.03 (d, J = 8.4 Hz, 1H), 6.96 (ddd, J = 8.3, 6.9, 1.1 Hz, 1H), 6.55 (s, 1H) ppm.

^{13}C NMR (126 MHz, $CDCl_3$) δ 148.5, 138.5, 136.9, 133.5, 133.2, 129.9, 124.1, 121.5, 120.2, 119.2, 111.5, 109.9, 92.4 ppm.

HRMS: *m/z* $[M + H]^+$ calcd for $C_{16}H_{11}N_2O_2S$ 295.0541; found 295.0529

3-([1,1-Biphenyl]-4-yl)thiazole[3,2-*a*]indole (5f)

Starting Material: **4f** and **2a**



5f

Yield: 80 mg (55%)

Description: White solid

Rf: 0.6 (10% EtOAc/Hexane)

m.p: 129-131 °C

FTIR(ATR): 1661, 1303 cm^{-1}

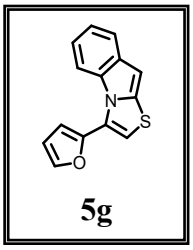
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (d, J = 8.1 Hz, 2H), 7.70 (d, J = 6.1 Hz, 4H), 7.63 (d, J = 7.9 Hz, 1H), 7.50 (t, J = 7.6 Hz, 2H), 7.41 (t, J = 7.4 Hz, 1H), 7.16 (t, J = 8.5 Hz, 2H), 6.92 (t, J = 7.6 Hz, 1H), 6.62 (s, 1H), 6.41 (s, 1H) ppm.

^{13}C NMR (101 MHz, $CDCl_3$) δ 142.5, 140.2, 138.7, 135.2, 133.4, 130.1, 129.8, 129.5, 129.0, 127.9, 127.4, 127.2, 121.1, 119.7, 118.7, 111.9, 107.0, 91.6 ppm.

HRMS: *m/z* $[M + H]^+$ calcd for $C_{22}H_{16}NS$ 326.1003; found 326.1000.

3-(Furan-2-yl)thiazolo[3,2-a]indole (5g)

Starting Material: **4g** and **2a**



5g

Yield: 76 mg (40%)

Description: Off-white gummy solid

Rf: 0.6 (10% EtOAc/Hexane)

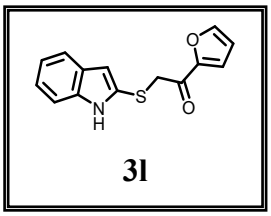
FTIR (ATR): 1605, 1314 cm^{-1}

^1H NMR (500 MHz, $CDCl_3$) δ 7.67 (dd, *J* = 1.9, 0.8 Hz, 1H), 7.64 – 7.60 (m, 1H), 7.27 – 7.24 (m, 1H), 7.19 (ddd, *J* = 8.0, 7.1, 1.0 Hz, 1H), 7.04 (ddd, *J* = 8.3, 7.1, 1.2 Hz, 1H), 6.76 (dd, *J* = 3.3, 0.8 Hz, 1H), 6.64 (s, 1H), 6.61 – 6.58 (m, 2H) ppm.

^{13}C NMR (101 MHz, $CDCl_3$) δ 143.6, 143.2, 137.8, 133.1, 129.9, 125.5, 121.3, 119.7, 119.3, 112.3, 111.8, 111.6, 110.2, 92.0 ppm.

HRMS: *m/z* $[M + H]^+$ calcd for $C_{14}H_{10}NOS$ 240.0483; found 240.0478.

2-((1*H*-Indol-2-yl)thio)-1-(furan-2-yl)ethan-1-one (3l)



3l

Starting materials: **4h** and **2a**

Yield: 132 mg (70%)

Description: White solid

Rf: 0.17 (10% EtOAc/Hexane)

m.p: 121-122 ℃

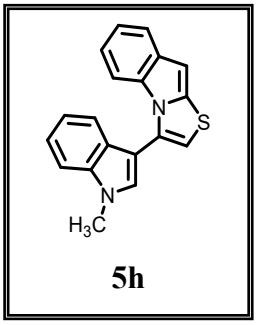
FTIR(ATR): 3368, 2836, 1724, 1218 cm^{-1}

^1H NMR (500 MHz, $CDCl_3$) *δ* 9.04 (s, 1H), 7.60 (d, *J* = 0.7 Hz, 1H), 7.53 (d, *J* = 7.9 Hz, 1H), 7.33 (d, *J* = 8.2 Hz, 1H), 7.28 – 7.24 (m, 1H), 7.21 – 7.14 (m, 1H), 7.07 (t, *J* = 7.5 Hz, 1H), 6.69 – 6.65 (m, 1H), 6.55 (dd, *J* = 3.5, 1.5 Hz, 1H), 4.04 (s, 2H) ppm.

^{13}C NMR (101 MHz, $CDCl_3$) *δ* 185.1, 151.5, 147.5, 137.5, 128.1, 127.2, 122.9, 120.4, 120.1, 119.0, 112.9, 111.0, 109.2, 42.0 ppm.

HRMS: *m/z* $[M + H]^+$ calcd for $C_{14}H_{12}NO_2S$ 258.0589; found 258.0580

3-(1-Methyl-1H-indol-3-yl)thiazolo[3,2-a]indole (5h)



5h

Starting Material: **4h** and **2a**

Yield: trace

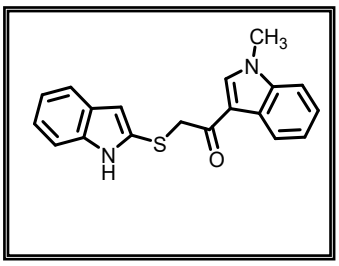
Description: White gummy solid

Rf: 0.5 (10% EtOAc/Hexane)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, J = 8.0 Hz, 1H), 7.46 (dd, J = 8.2, 4.4 Hz, 2H), 7.36 – 7.30 (m, 2H), 7.12 (ddd, J = 10.4, 8.8, 4.1 Hz, 2H), 7.04 (d, J = 8.2 Hz, 1H), 6.80 – 6.71 (m, 1H), 6.61 (d, J = 2.6 Hz, 1H), 6.40 (s, 1H), 3.93 (s, 3H) ppm.

HRMS: *m/z* $[M + H]^+$ calcd for $C_{19}H_{15}N_2S$ 303.0956; found 303.0945.

2-((1*H*-Indol-2-yl)thio)-1-(1-methyl-1H-indol-3-yl)ethan-1-one (**3m**)



Starting Material: **4h** and **2a**

Yield: 100 mg (62%)

Description: White gummy solid

Rf: 0.2 (10% EtOAc/Hexane)

FTIR(ATR): 3351, 2904, 1717, 1204 cm^{-1}

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.38 (s, 1H), 8.42 – 8.24 (m, 1H), 7.44 (d, J = 7.8 Hz, 1H), 7.31 (s, 1H), 7.28 – 7.16 (m, 4H), 7.11 – 7.04 (m, 1H), 7.02 – 6.95 (m, 1H), 6.57 (d, J = 1.1 Hz, 1H), 3.90 (s, 2H), 3.53 (s, 3H) ppm.

^{13}C NMR (101 MHz, $CDCl_3$) δ 190.9, 137.6, 137.4, 136.3, 128.5, 128.3, 126.4, 123.9, 123.2, 122.6, 120.2, 120.0, 115.2, 111.0, 109.9, 108.2, 43.0, 33.5 ppm.

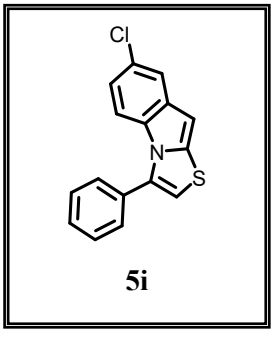
HRMS: *m/z* $[M + H]^+$ calcd for $C_{19}H_{17}N_2OS$ 321.1062; found 321.1058.

7-Chloro-3-phenylthiazolo[3,2-*a*]indole (**5i**)

Starting Material: **4a** and **2b**

Yield: 109 mg (57%)

Description: White solid



5i

Rf: 0.6 (10% EtOAc/Hexane)

m.p: 128-131 °C

FTIR(ATR): 1499, 1215, 746 cm^{-1}

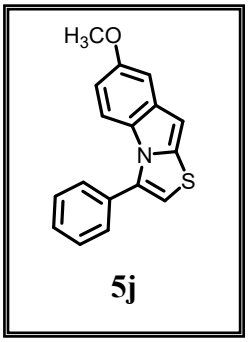
^1H NMR (300 MHz, $CDCl_3$) δ 7.61 – 7.53 (m, 5H), 7.50 (d, *J* = 8.6 Hz, 1H), 7.11 (dd, *J* = 8.6, 1.9 Hz, 1H), 6.99 (d, *J* = 1.7 Hz, 1H), 6.56 (d, *J* = 0.6 Hz, 1H), 6.38 (s, 1H) ppm.

^{13}C NMR (151 MHz, $CDCl_3$) δ 139.3, 135.1, 131.7, 130.0, 129.9, 129.2, 129.0, 124.3, 121.7, 120.3, 111.8, 107.5, 91.6 ppm.

HRMS: *m/z* $[M + H]^+$ calcd for $C_{16}H_{11}ClNS$ 284.0301, found 284.0295.

7-Methoxy-3-phenylthiazolo[3,2-*a*]indole (5j)

Starting Material: **4a** and **2c**



5j

Yield: 112 mg (59%)

Description: White gummy solid

Rf: 0.63 (10% EtOAc/Hexane)

FTIR(ATR): 3109, 2854, 1609, 1033 cm^{-1}

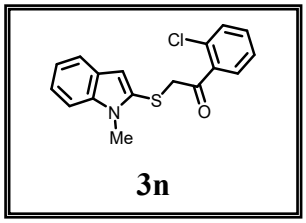
^1H NMR (300 MHz, $CDCl_3$) δ 7.61 – 7.57 (m, 2H), 7.51 (dd, *J* = 5.2, 1.8 Hz, 3H), 7.05 (d, *J* = 2.5 Hz, 1H), 6.90 (s, 1H), 6.55 – 6.50 (m, 2H), 6.30 (s, 1H), 3.81 (s, 3H) ppm.

^{13}C NMR (126 MHz, $CDCl_3$) δ 154.9, 139.3, 135.4, 134.1, 130.6, 129.7, 129.4, 128.8, 125.4, 112.5, 108.7, 106.2, 101.2, 91.3, 55.7 ppm.

HRMS: *m/z* $[M + H]^+$ calcd for $C_{17}H_{14}NOS$ 280.0796; found 280.0801

1-(2-Chlorophenyl)-2-((1-methyl-1H-indol-2-yl)thio)ethan-1-one (3n)

Starting materials: **4c** and **2e**



3n

Yield: 136 mg (82%)

Description: Yellowish liquid

Rf: 0.17 (10% EtOAc/Hexane)

FTIR(ATR): 3368, 2836, 1724, 1218 cm^{-1}

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.53 (d, *J* = 7.9 Hz, 1H), 7.41 (d, *J* = 7.8 Hz, 1H), 7.39 – 7.36 (m, 2H), 7.29 – 7.19 (m, 3H), 7.09 (t, *J* = 7.3 Hz, 1H), 6.61 (s, 1H), 4.20 (s, 2H), 3.75 (s, 3H) ppm.

^{13}C NMR (75 MHz, $CDCl_3$) δ 196.5, 138.4, 137.3, 132.4, 131.4, 130.5, 130.3, 129.1, 127.3, 127.0, 122.7, 120.6, 119.9, 109.7, 109.6, 46.5, 29.9 ppm.

HRMS: *m/z* $[M + H]^+$ calcd for $C_{17}H_{15}ClNOS$ 316.0563; found 316.0552

Biological Experimental:

Cell lines and culture conditions:

For evaluating anti-cancer activity, triple-negative breast cancer (TNBC) cell lines-mouse-derived 4T1 and human MDA-MB-231 along with human prostate cancer (DU-145) and normal human bronchial epithelial cells (BEAS-2B) were procured from ATCC (USA). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic solution, and maintained under standard conditions in a humidified 5% CO_2 incubator at 37 °C.

Experiments:

Cell cytotoxicity assay

The Sulforhodamine B (SRB) assay is a widely used colorimetric method to evaluate cell cytotoxicity and the efficacy of test compounds by quantifying total cellular protein content. In this study, cancer cell lines were seeded in 96-well plates at the following densities: 4T1 (3×10^3 cells/well), MDA-MB-231 and DU-145 (5×10^3 cells/well), and BEAS-2B (7×10^3 cells/well) in DMEM supplemented with 10% FBS. After 24 hours of incubation, cells were treated with test compounds at concentrations of 20 μM, 10 μM, 5 μM, and 2.5 μM for 48 hours. Following treatment, 100 μL of cold 10% trichloroacetic acid (TCA) was added to each well to fix the cells at 4 °C for 1 hour, followed by four washes with running tap water. Dried plates were stained with 100 μL of 0.057% SRB solution and incubated at room temperature for 30 minutes in the dark. Unbound dye was removed by washing with 1% acetic acid, repeated at least three times. After drying, 200 μL of 10 mM Tris base was added, and plates were shaken for 5 minutes. Absorbance was measured at 510 nm using an ELx800 microplate reader (Biotek, USA). The half-maximal inhibitory concentration (IC_{50}) values were determined using the curve-fitting method in GraphPad Prism 5.

Annexin V/PI assay:[3]

Cancer cells (DU-145 or 4T-1) were seeded in 12-well plates (5×10^4 cells/well) and incubated overnight. Based on IC_{50} values, test compounds **3f** and **5d** were treated at three different concentrations for 48 hrs (Table. I). Cells were collected by the initial step of trypsinization and then washed with 1x PBS for two times. Then, cells were resuspended in Annexin-V-FITC and propidium iodide using the Annexin-V Apoptosis Detection Kit. Cells were analysed by flow cytometry (using Becton Dickinson FACS accuri) after 20 min of incubation.

Cell cycle analysis:[4]

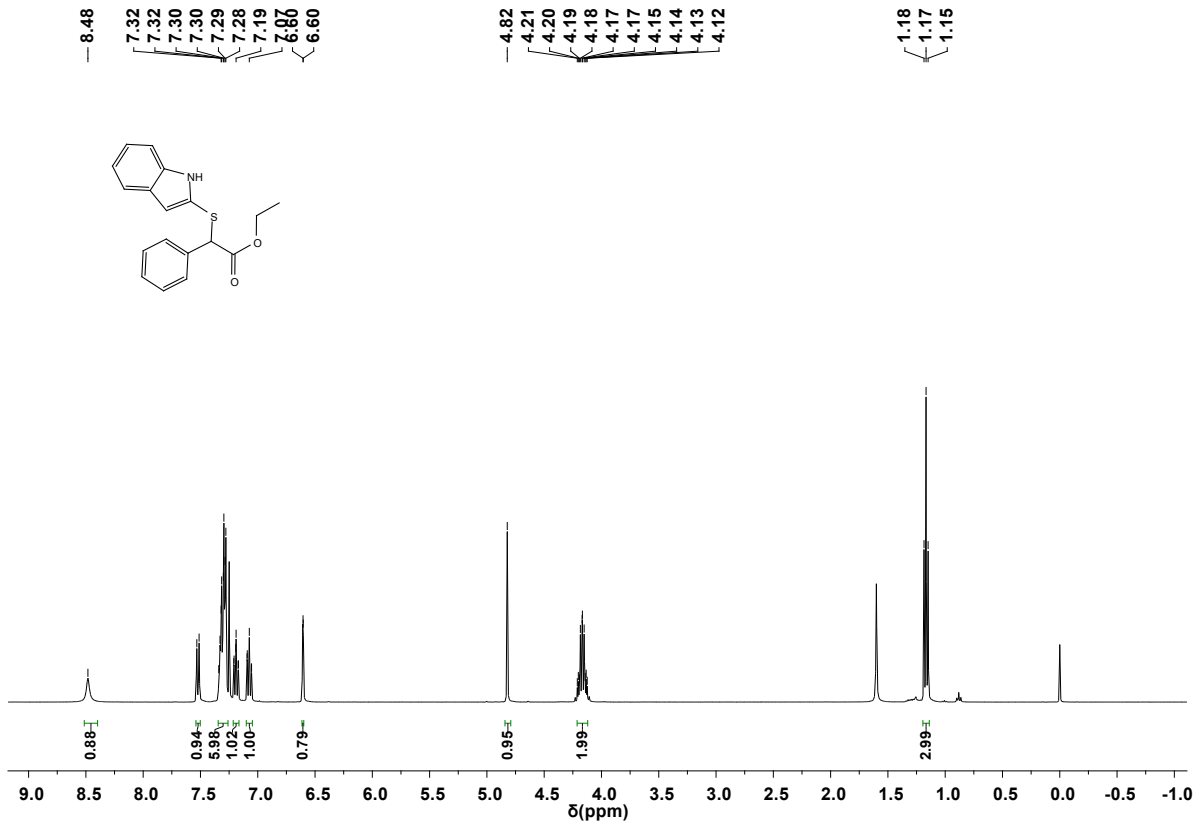
For cell cycle analysis, DU-145 and 4T-1 cells were treated with **3f** and **5d**. Cells were seeded in 12-well plates at a density of 5×10^4 cells/well and incubated for 24 h. Subsequently, cells were exposed to the test compounds at three different concentrations (Table I) for 48 h. Following treatment, cells were trypsinized, washed twice with 1× PBS, and fixed in 70% ethanol at 4 °C for 1 h. The fixed cells were then centrifuged, and the resulting pellet was resuspended in PI/RNase staining solution and incubated for 1 h. Cell cycle distribution was analyzed using flow cytometry (Becton Dickinson FACS Accuri).

Table.I selected compounds, cell lines and concentrations used for cell cycle and apoptosis analysis.

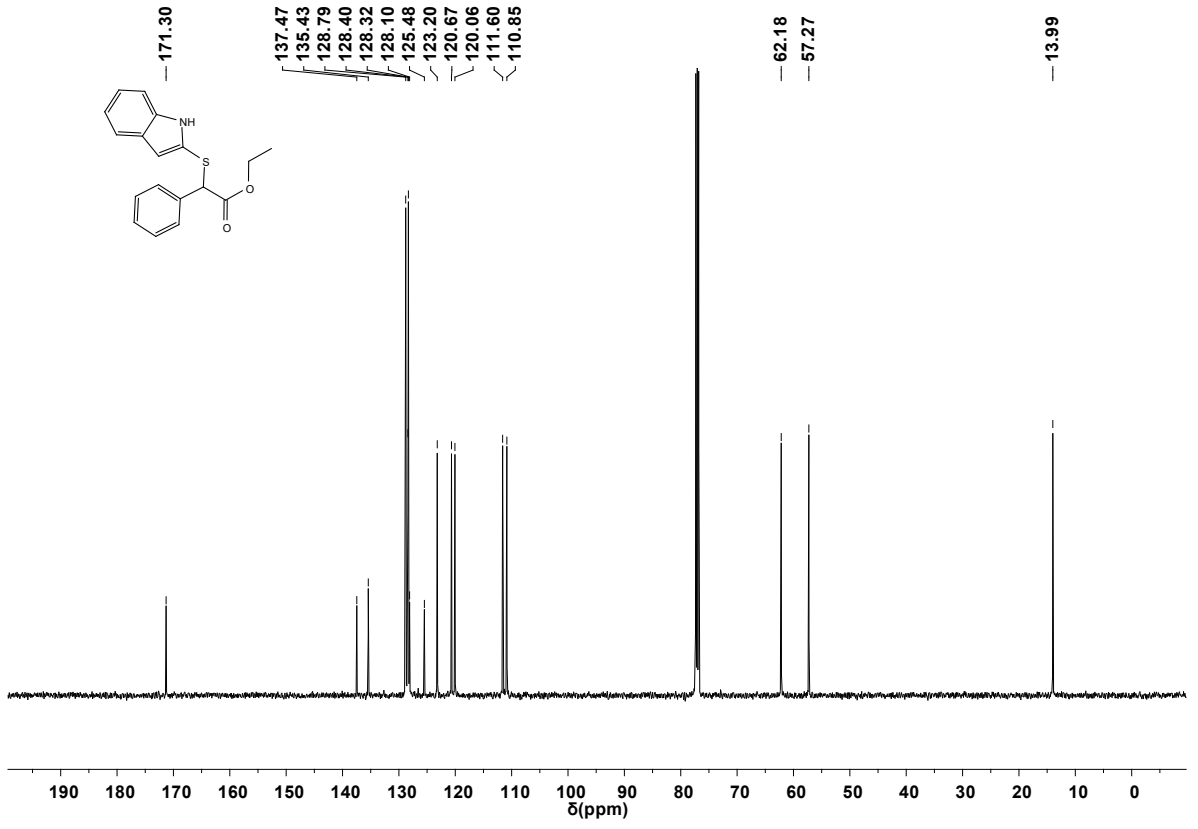
Compounds	Cell lines	Concentrations treated
3f	4T-1	2 µM, 4 µM, and 8 µM
3f	DU-145	2.5 µM, 5 µM, and 7.5 µM
5d	DU-145	3.3 µM, 6.6 µM, and 10 µM

References

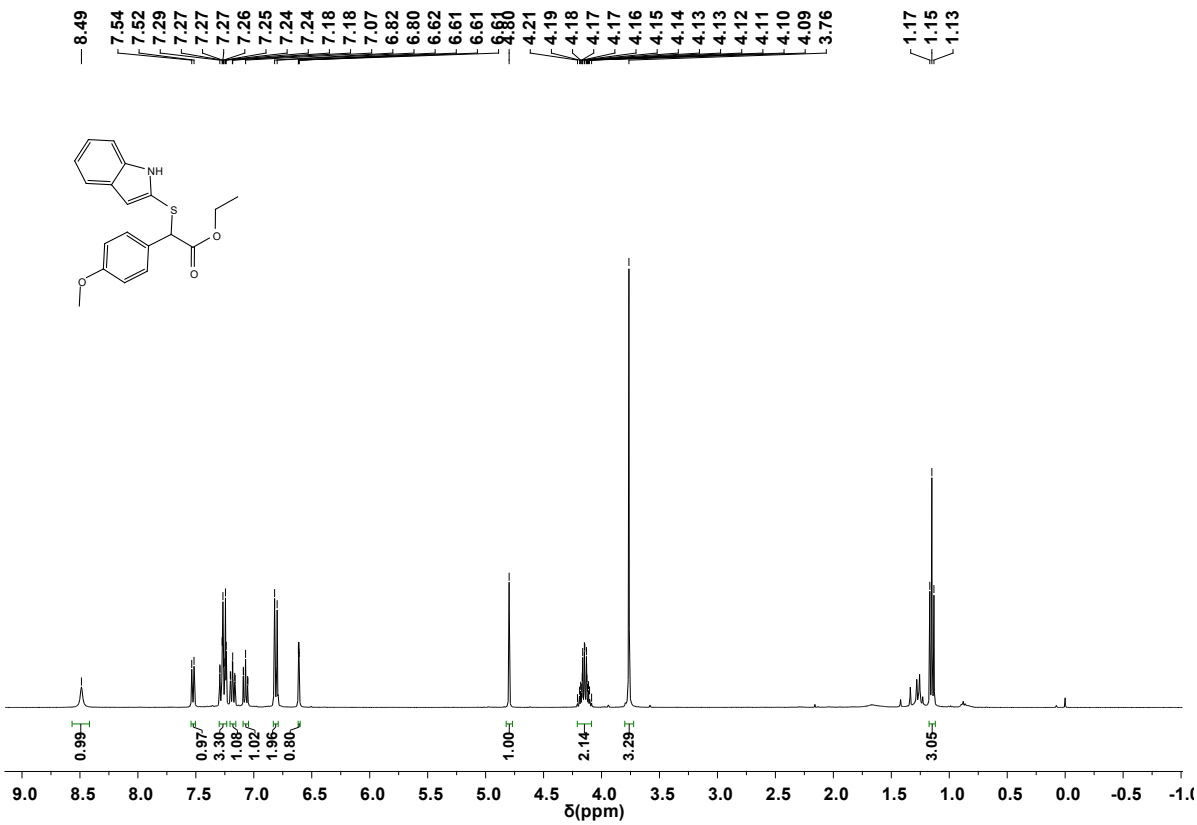
1. H. Keipour, A. Jalba, L. Delage-Laurin and T. Ollevier, *J. Org. Chem.* 2017, **82**, 3000-3010.
2. M. S. C. Pedras and M. Jha, *J. Org. Chem.* 2005, **70**, 1828-1834.
3. S. A. Balaji, N. Udupa, M. R. Chamallamudi, V. Gupta and A. Rangarajan, *PLoS One*. 2016**, 11**, e0155013.
4. S. Vaidya, A. Mohod, A. C. Eedara, S. B. Andugulapati and S. Pabbaraja, *ChemMedChem*, 2023, **18**, e202300097



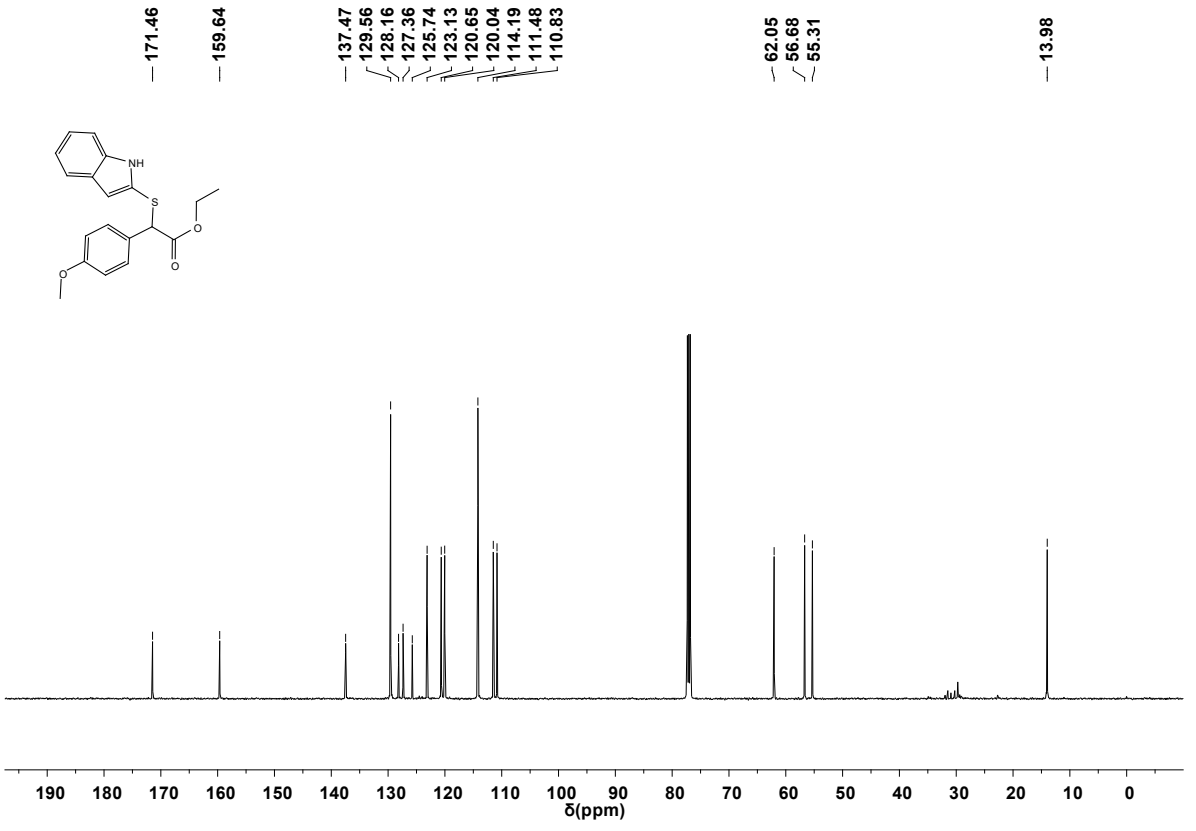
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **3a**



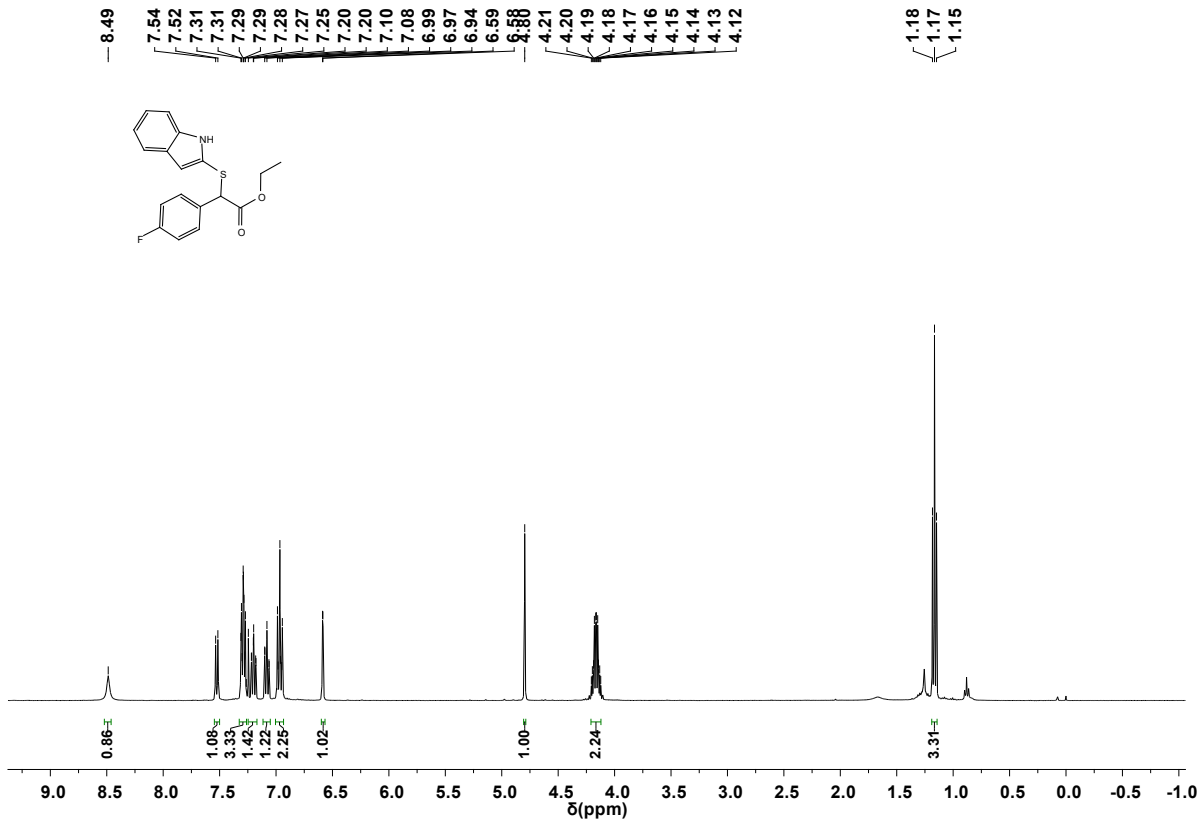
^{13}C NMR (126 MHz, $CDCl_3$) spectrum of **3a**



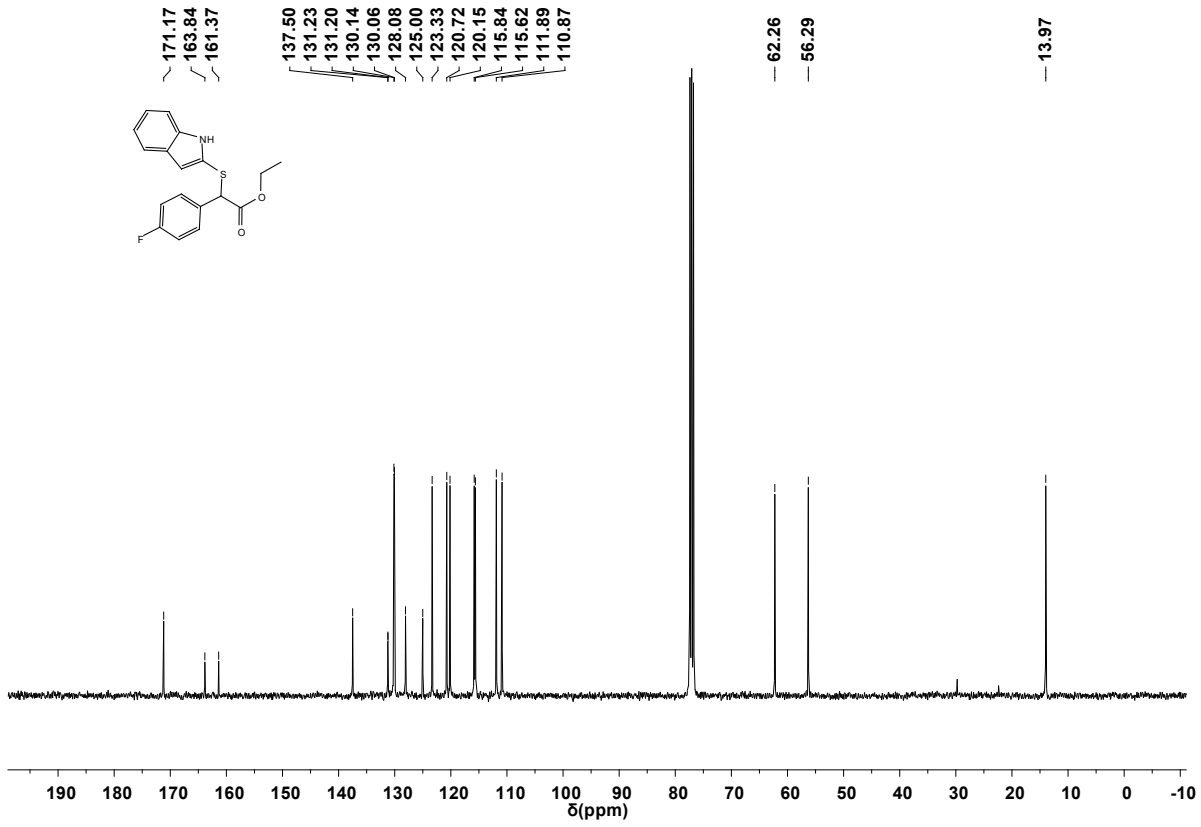
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **3b**



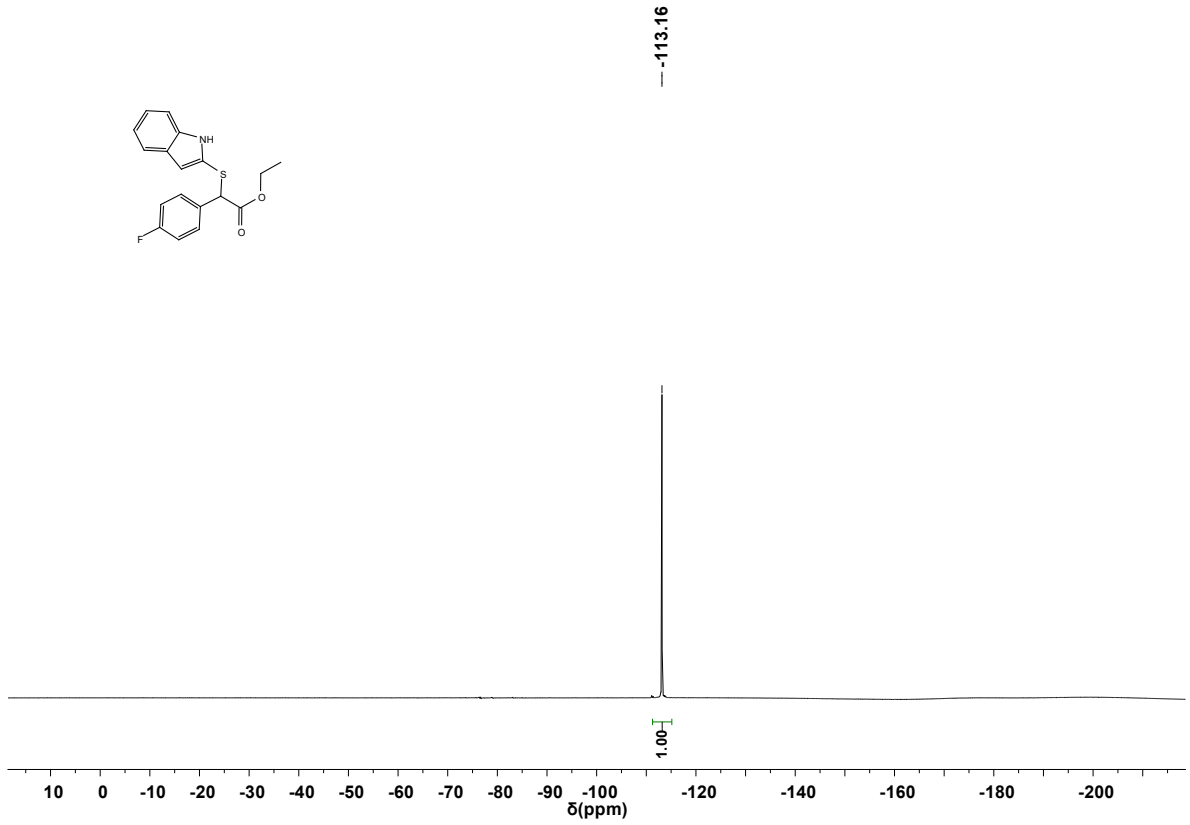
^{13}C NMR (126 MHz, $CDCl_3$) spectrum of **3b**



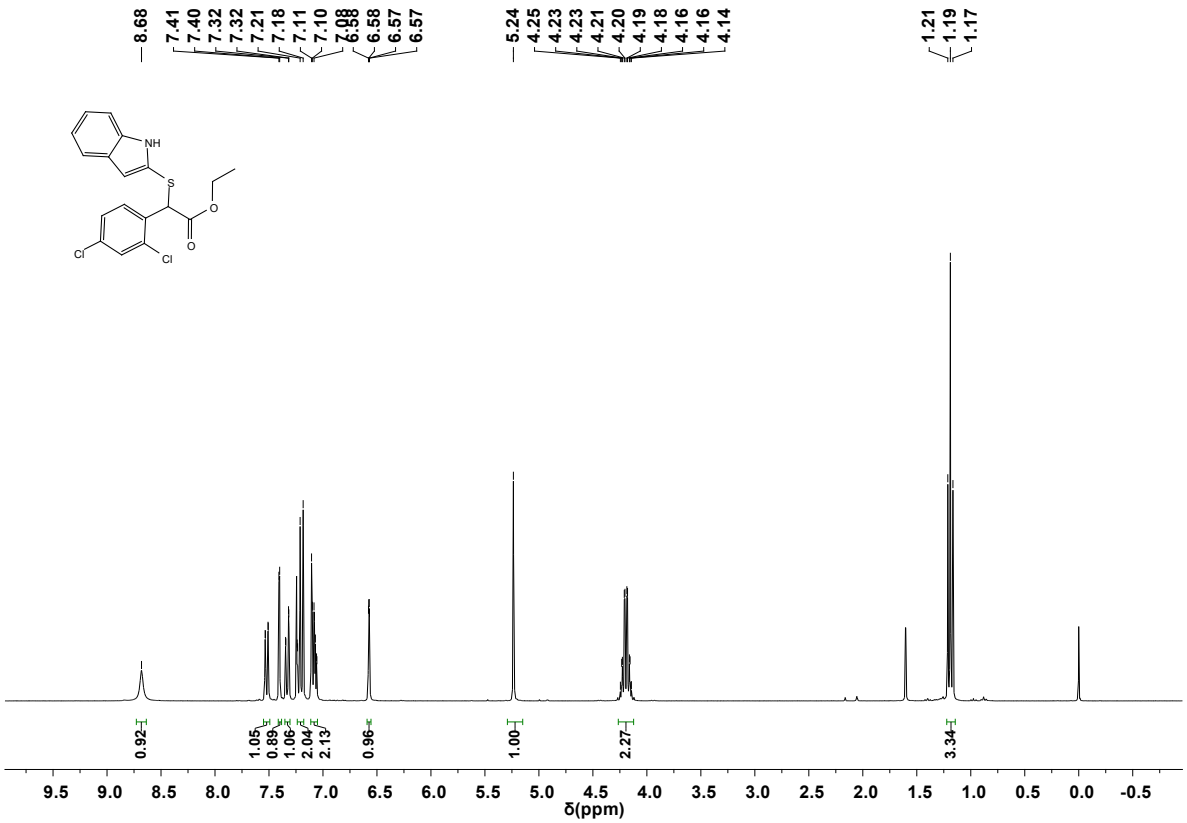
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **3c**



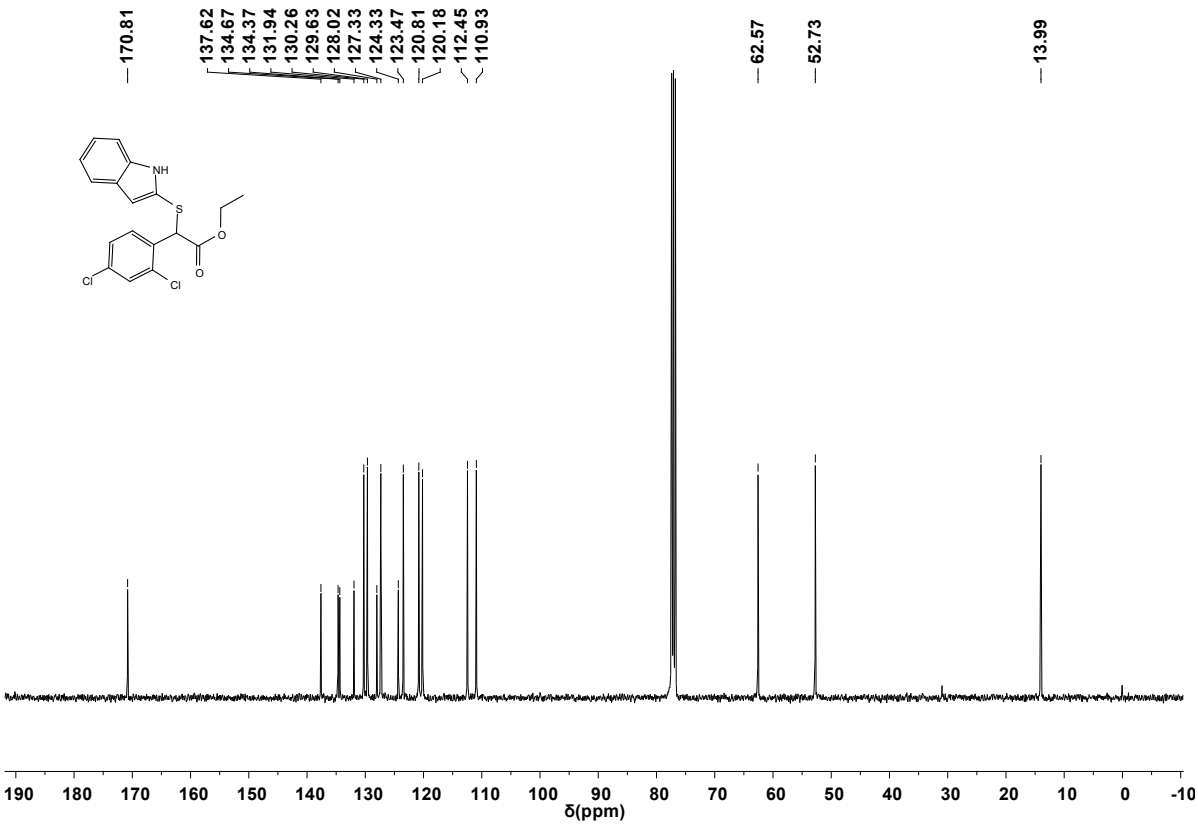
^{13}C NMR (101 MHz, $CDCl_3$) spectrum of **3c**



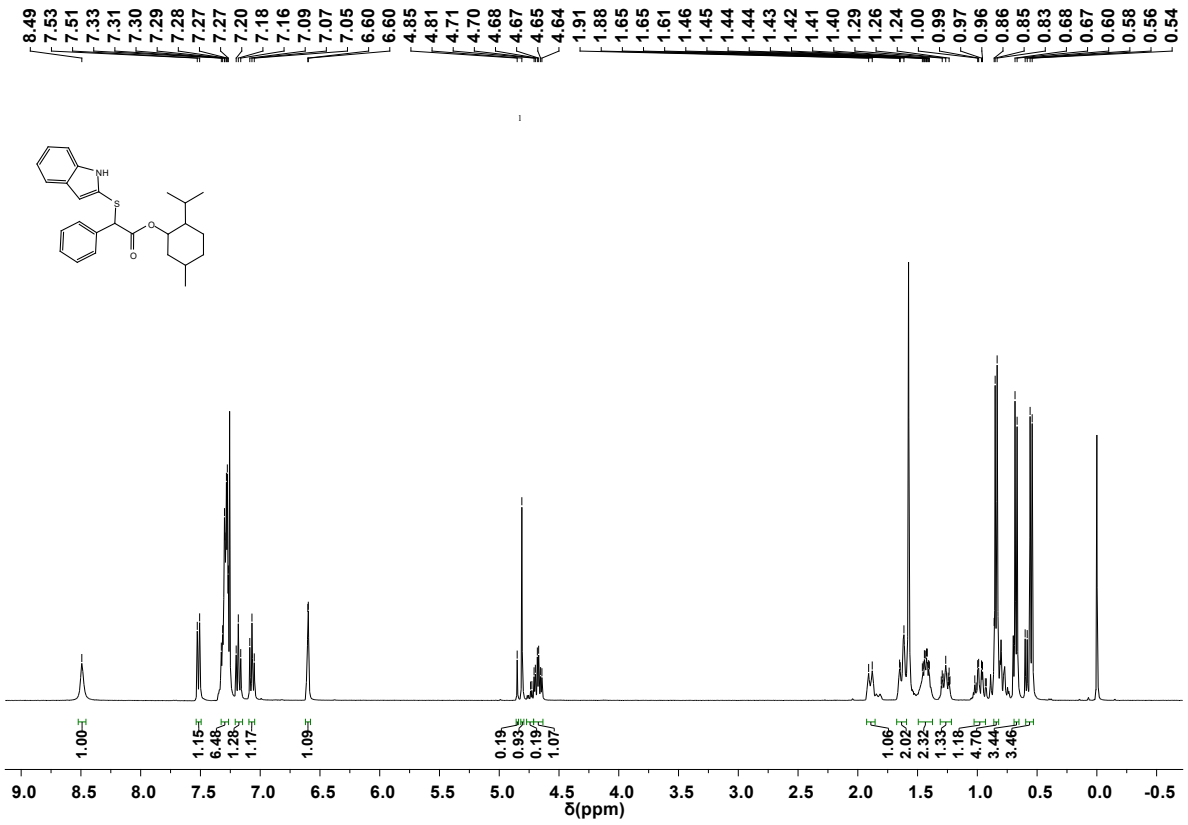
^{19}F NMR (376 MHz, $CDCl_3$) spectrum of **3c**



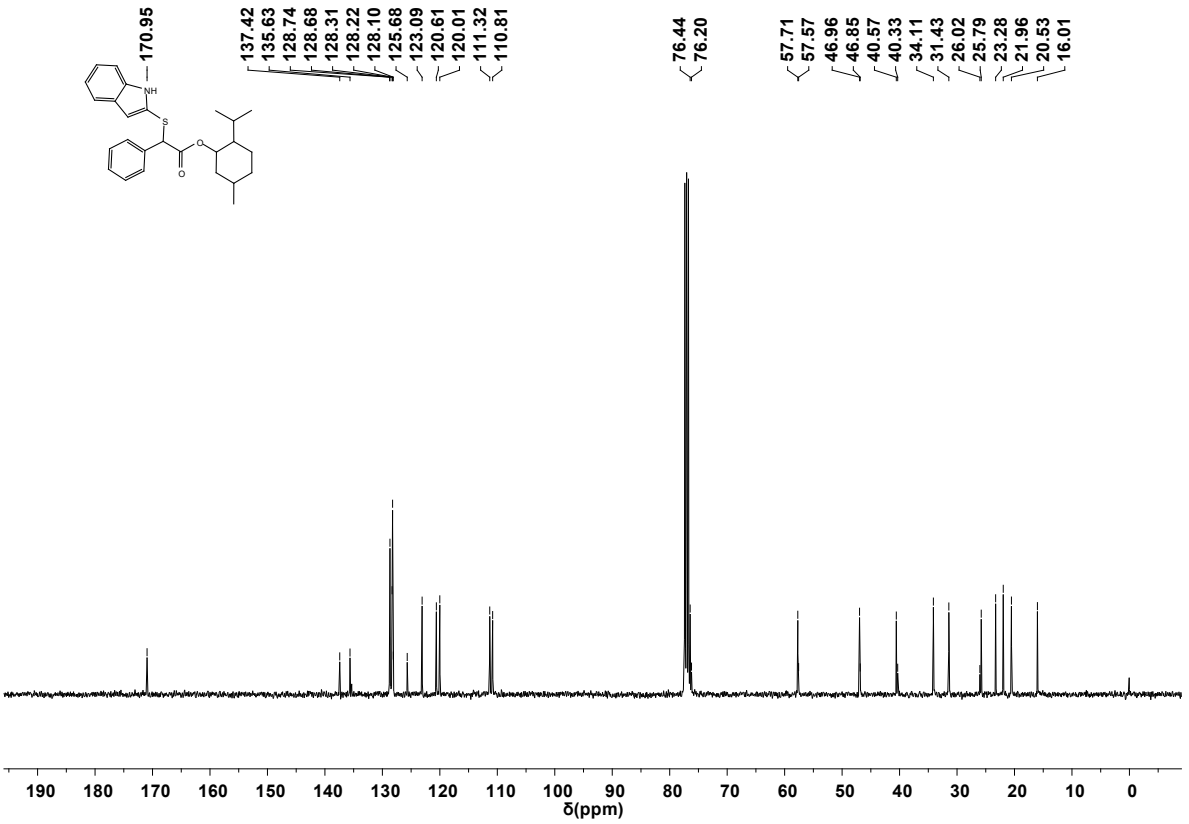
^{1}H NMR (300 MHz, $CDCl_3$) spectrum of **3d**



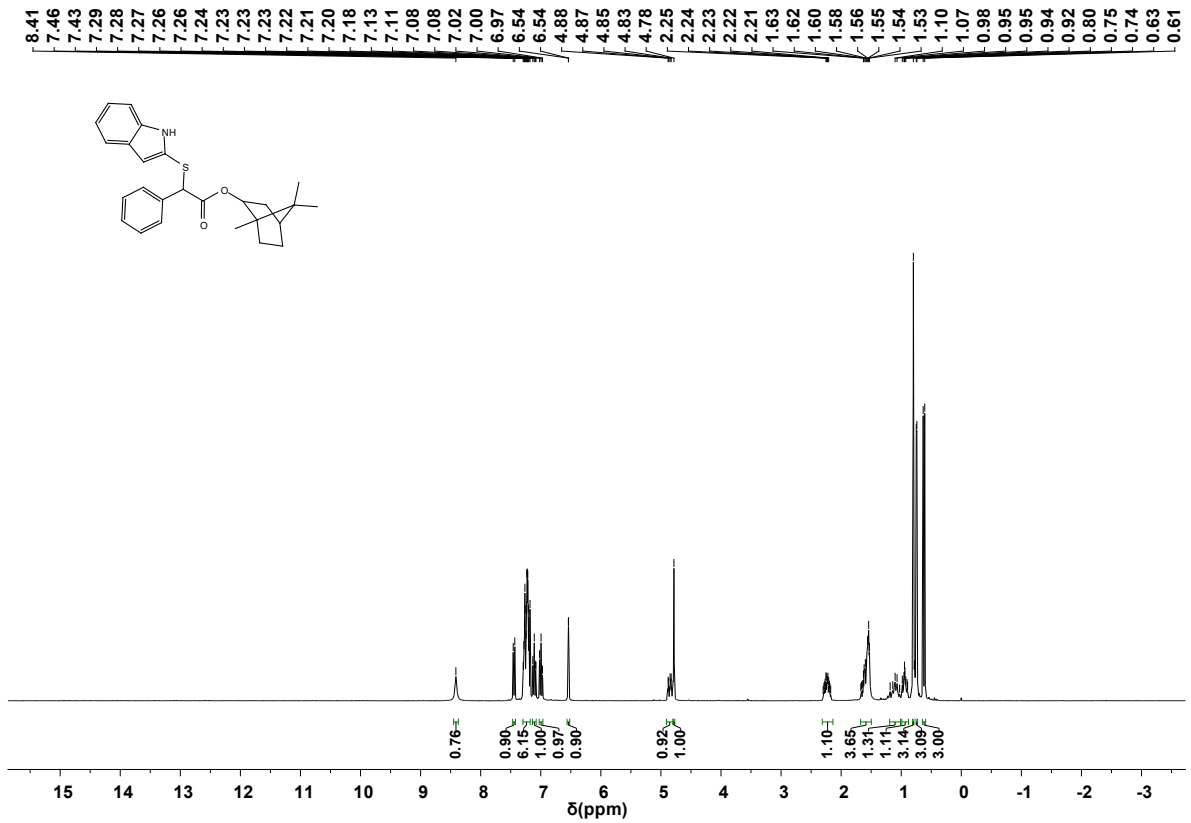
^{13}C NMR (101 MHz, $CDCl_3$) spectrum of **3d**



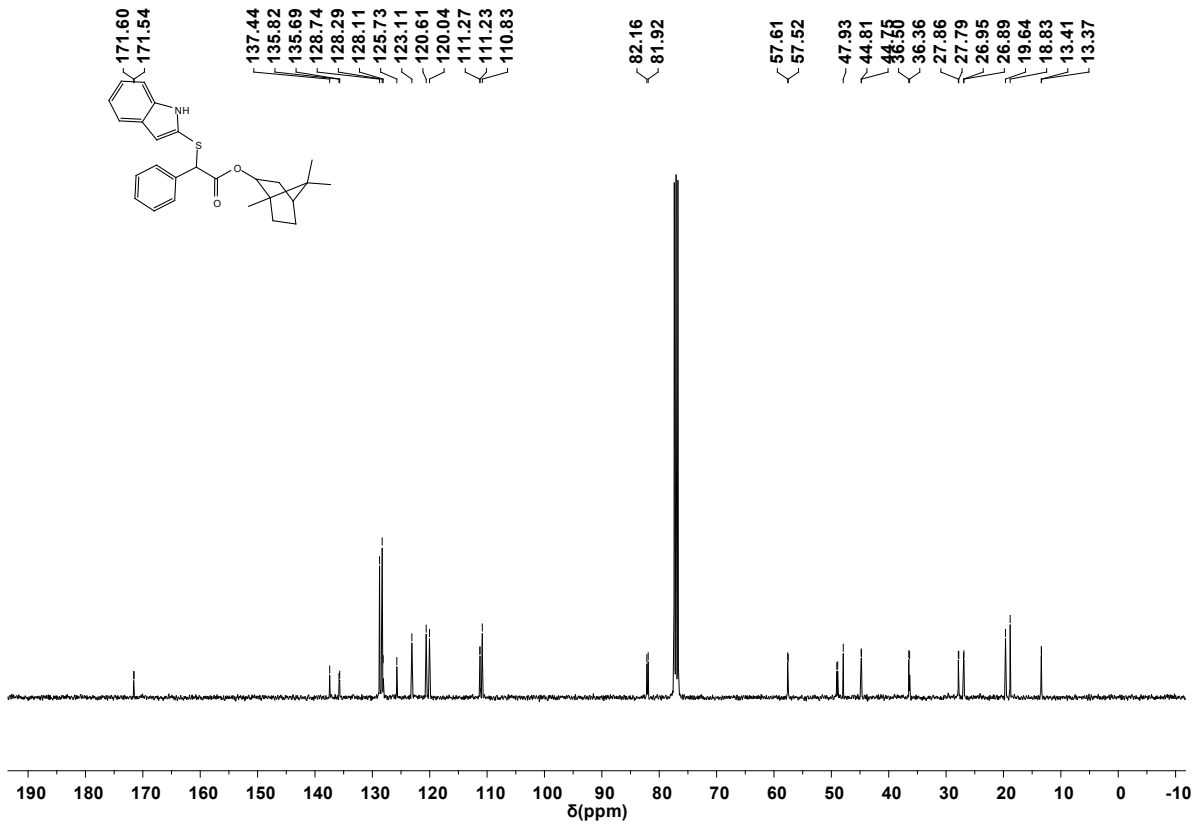
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **3e**



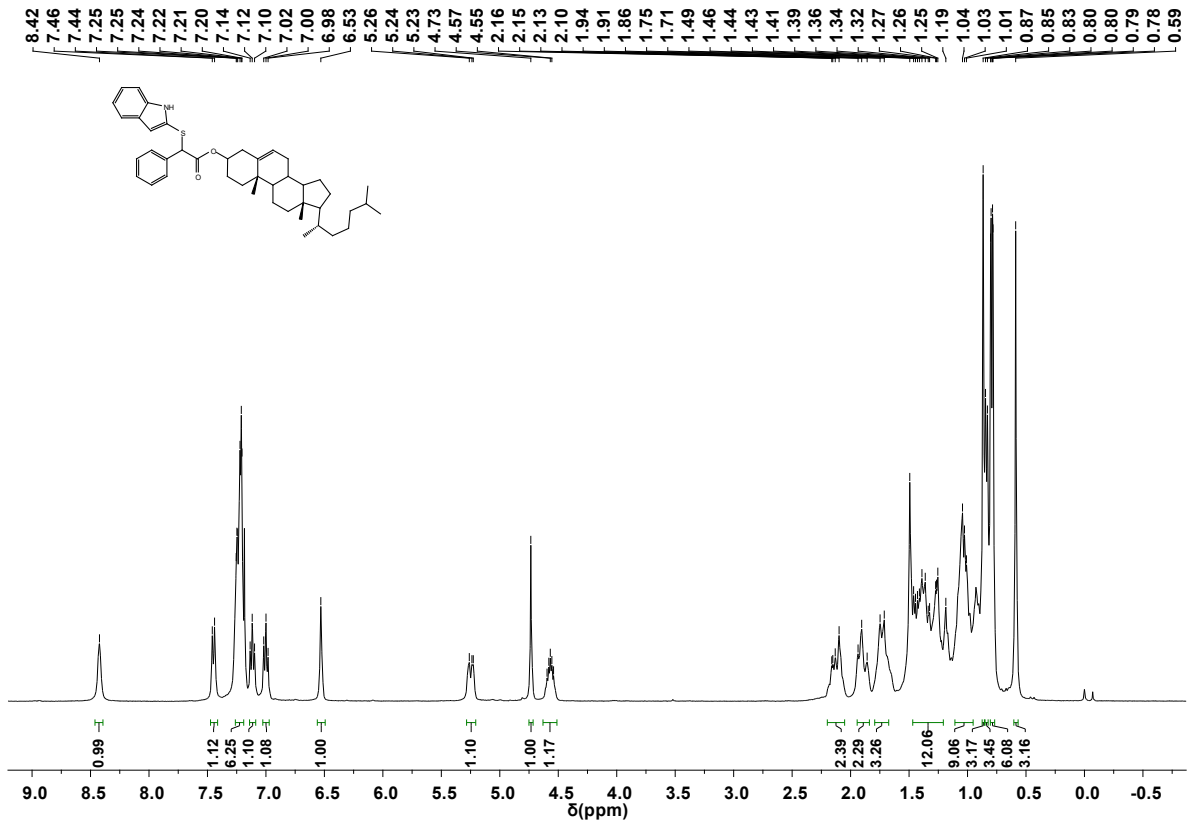
^{13}C NMR (101 MHz, $CDCl_3$) spectrum of **3e**



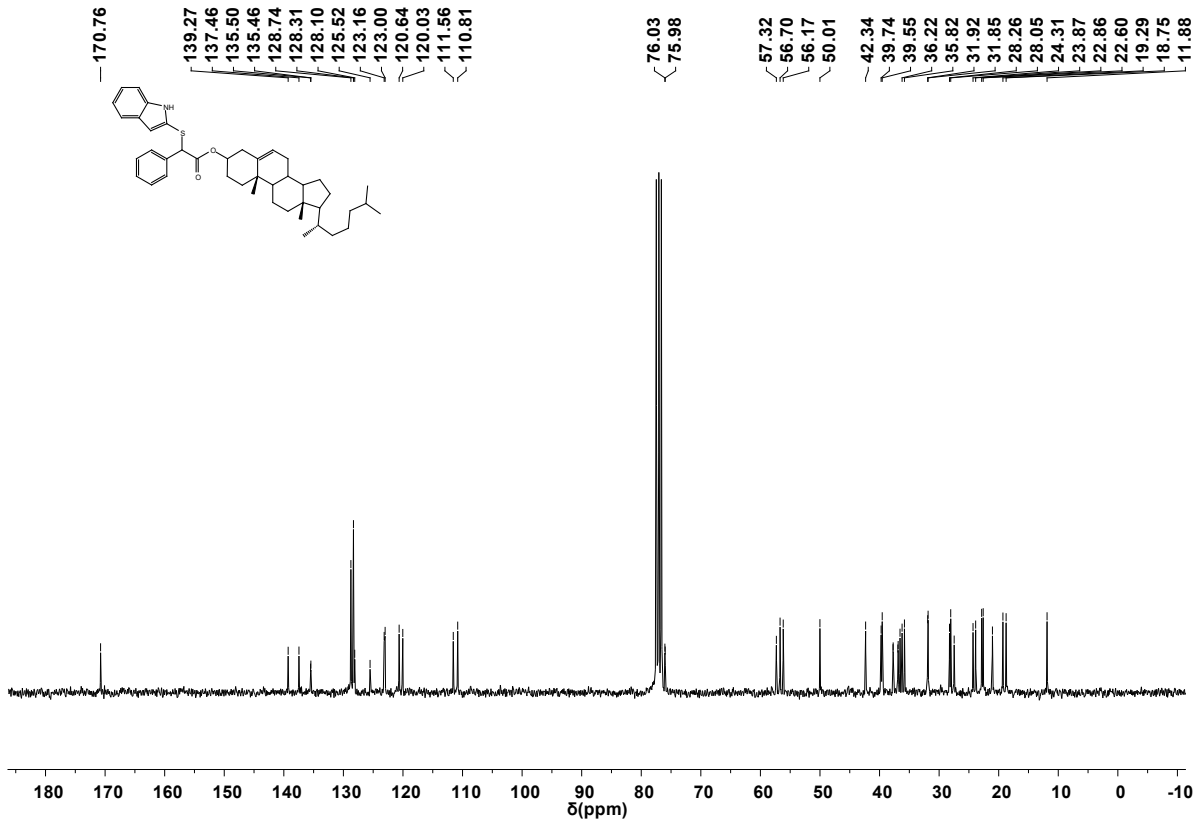
^{1}H NMR (300 MHz, $CDCl_3$) spectrum of **3f**



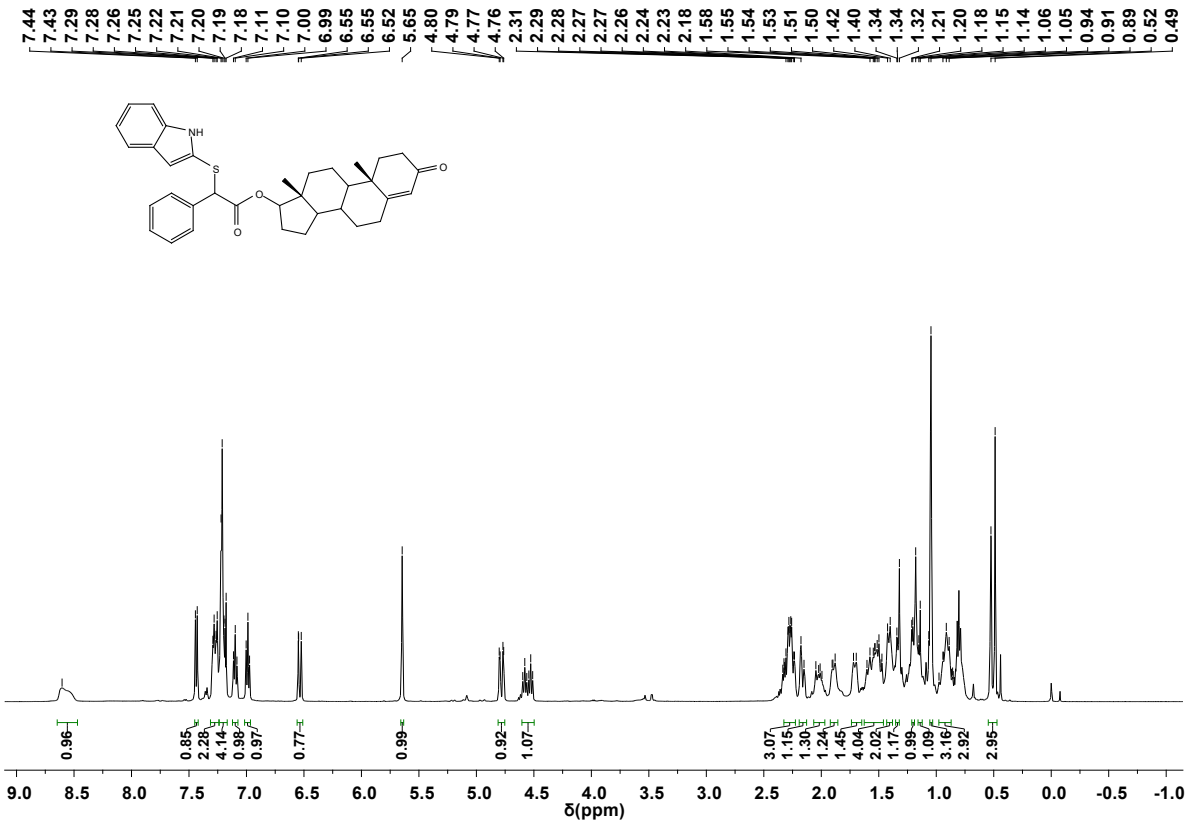
^{13}C NMR (101 MHz, $CDCl_3$) spectrum of **3f**



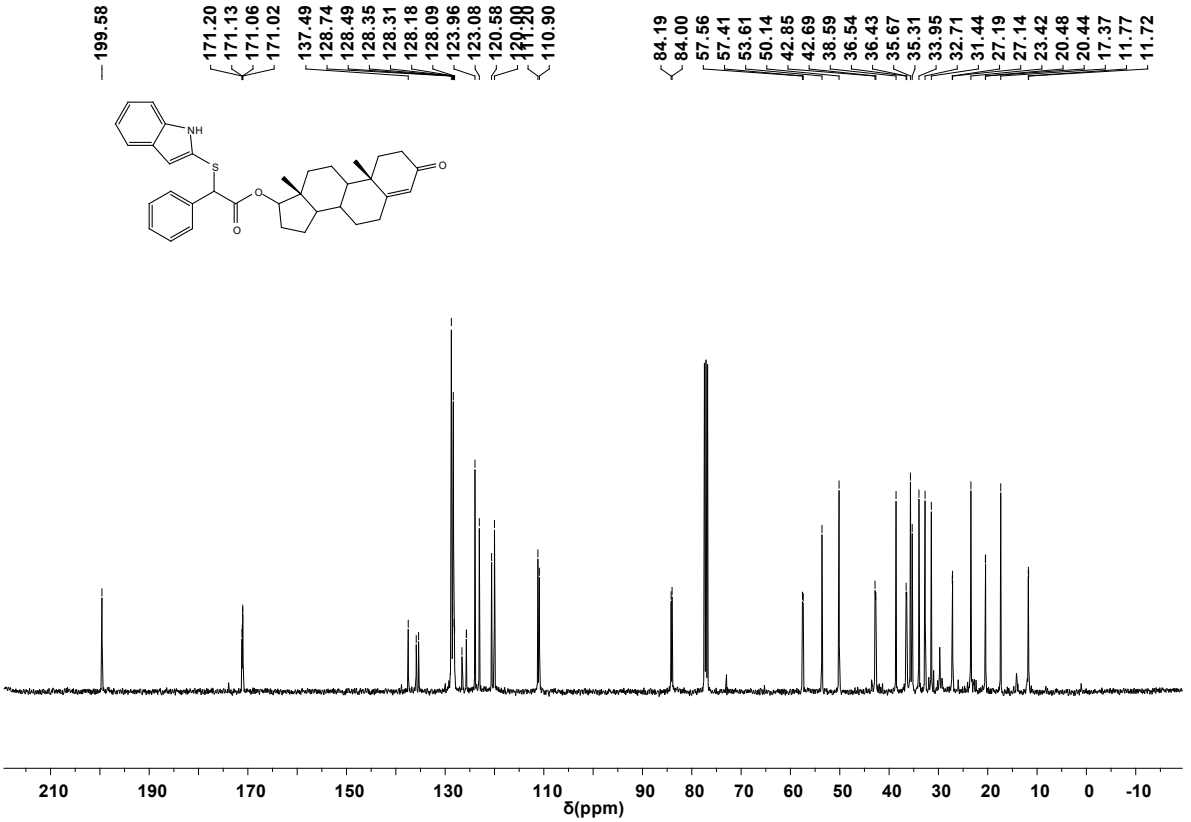
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **3g**



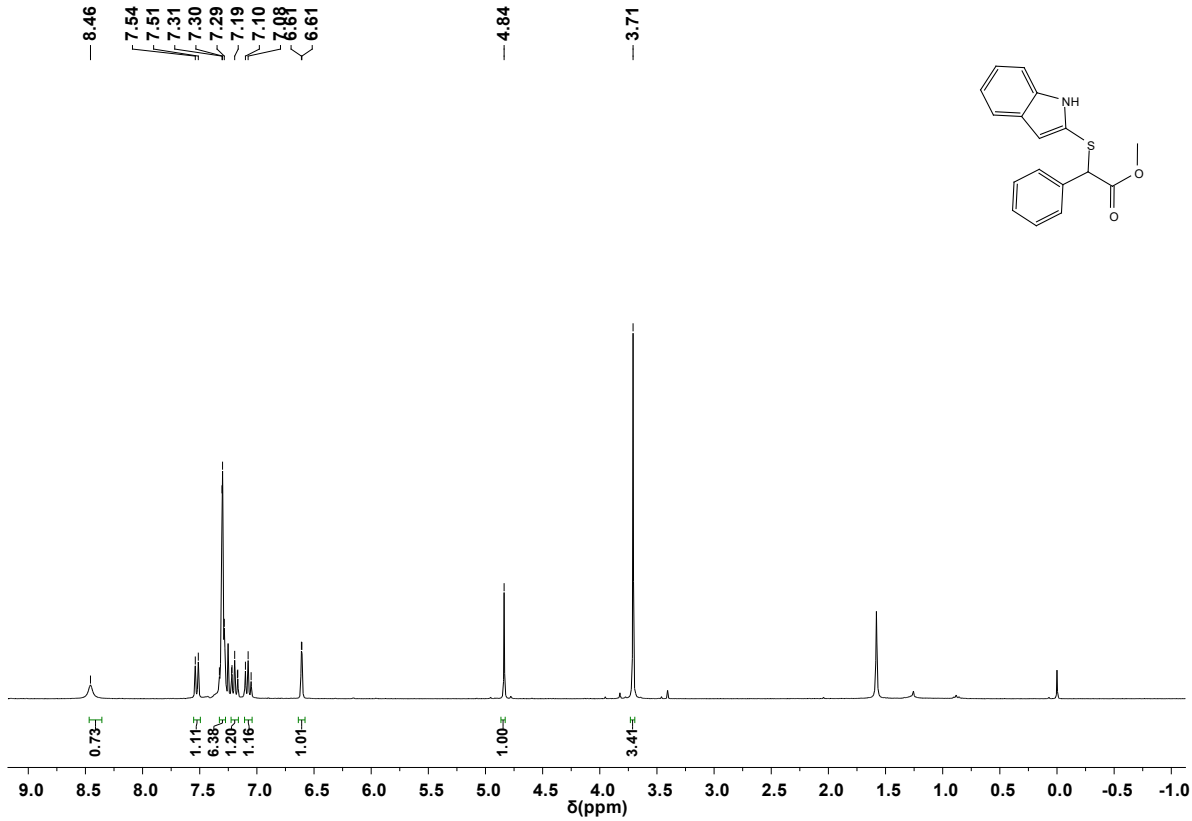
^{13}C NMR (75 MHz, $CDCl_3$) spectrum of **3g**



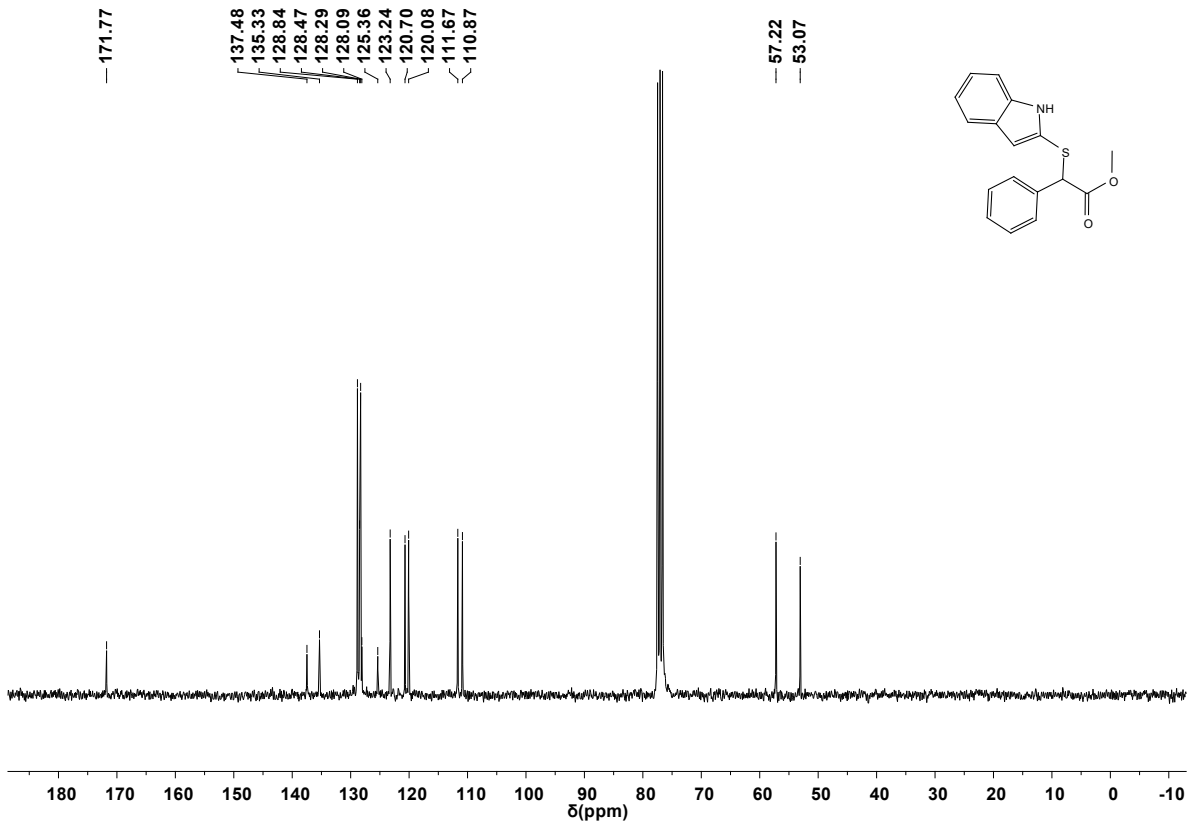
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **3h**



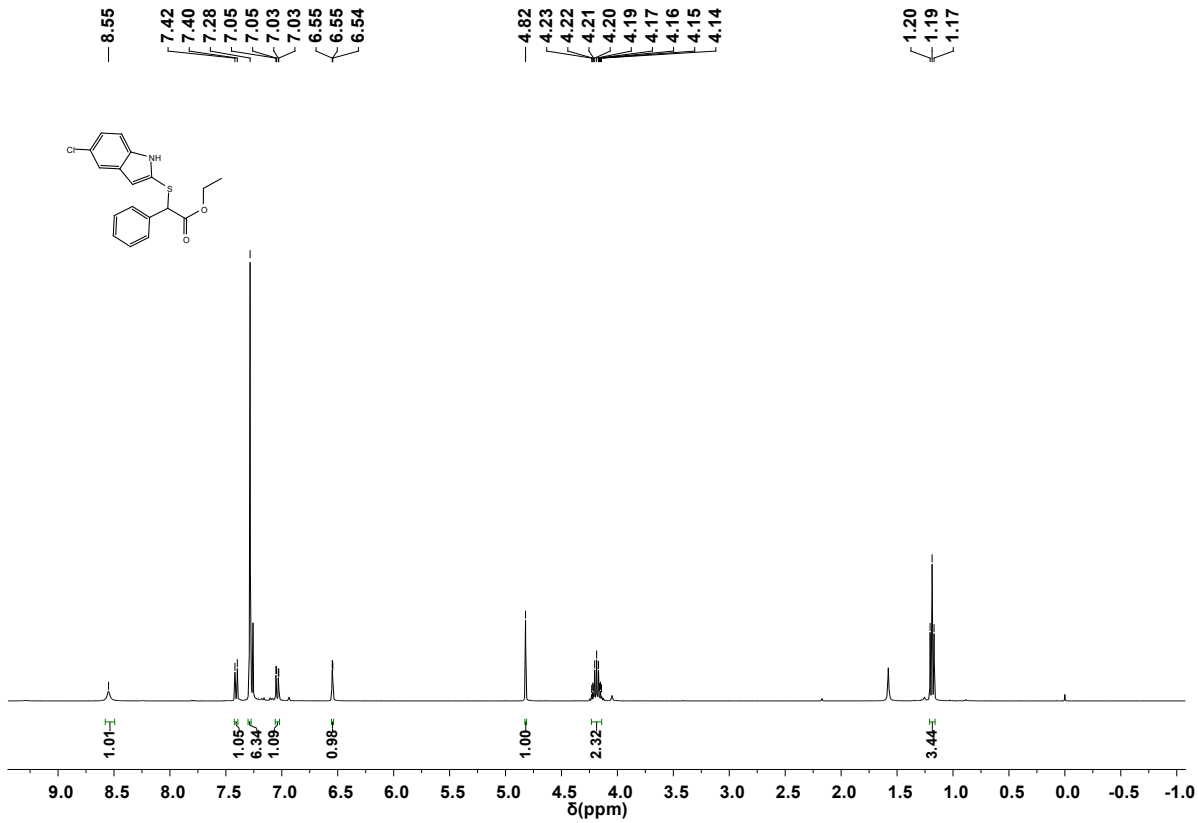
^{13}C NMR (75 MHz, $CDCl_3$) spectrum of **3h**



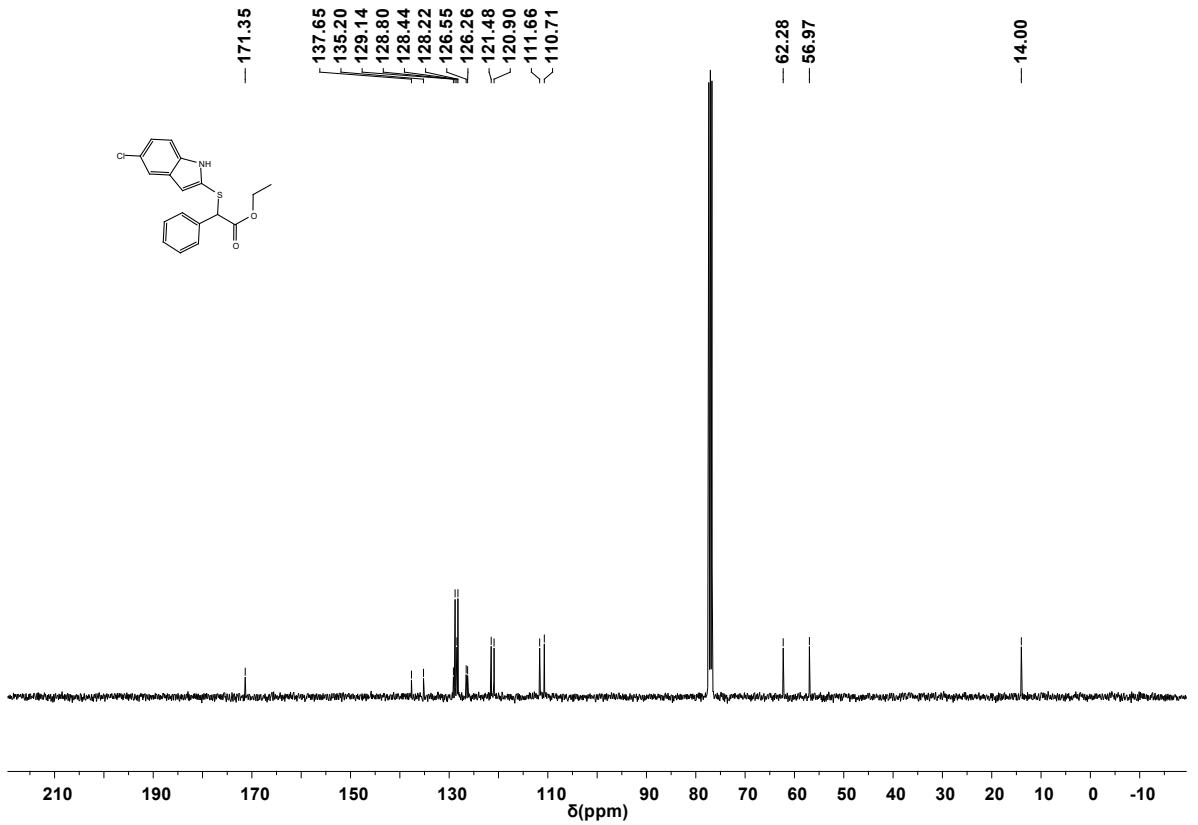
^{1}H NMR (300 MHz, $CDCl_3$) spectrum of **3i**



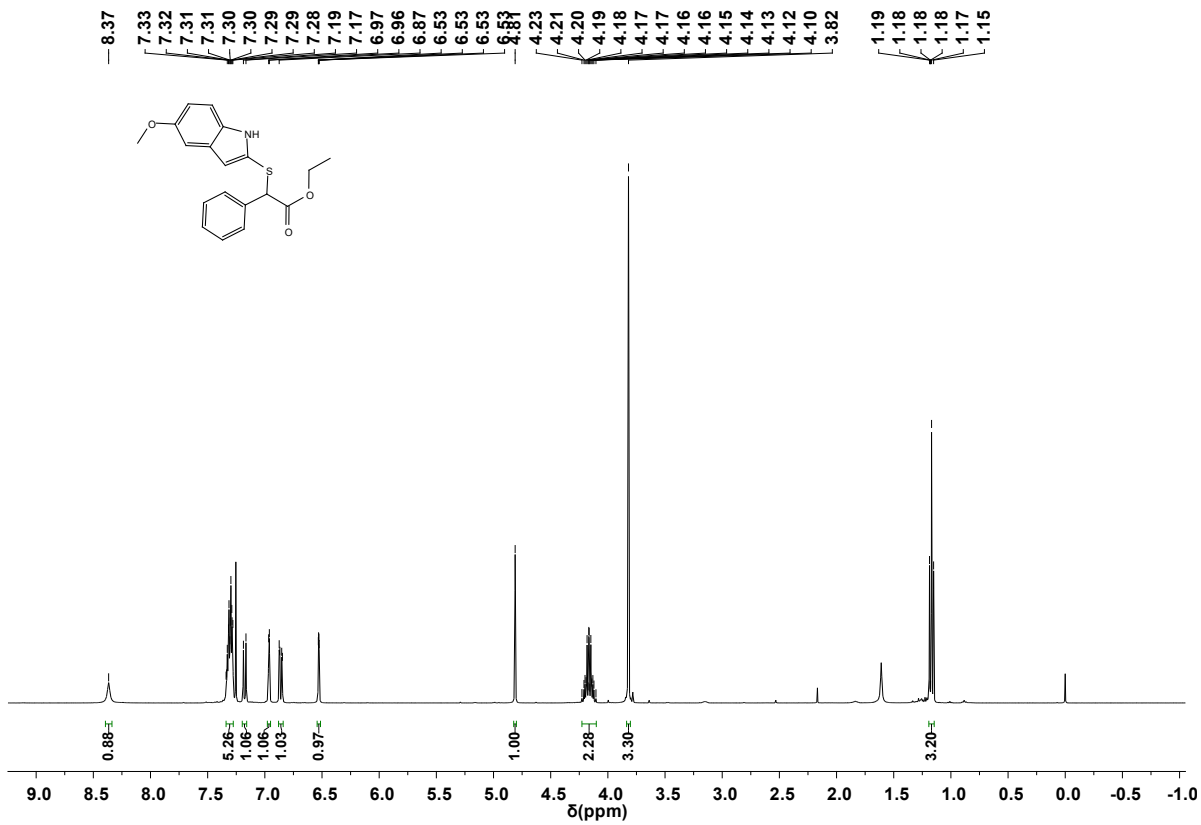
^{13}C NMR (75 MHz, $CDCl_3$) spectrum of **3i**



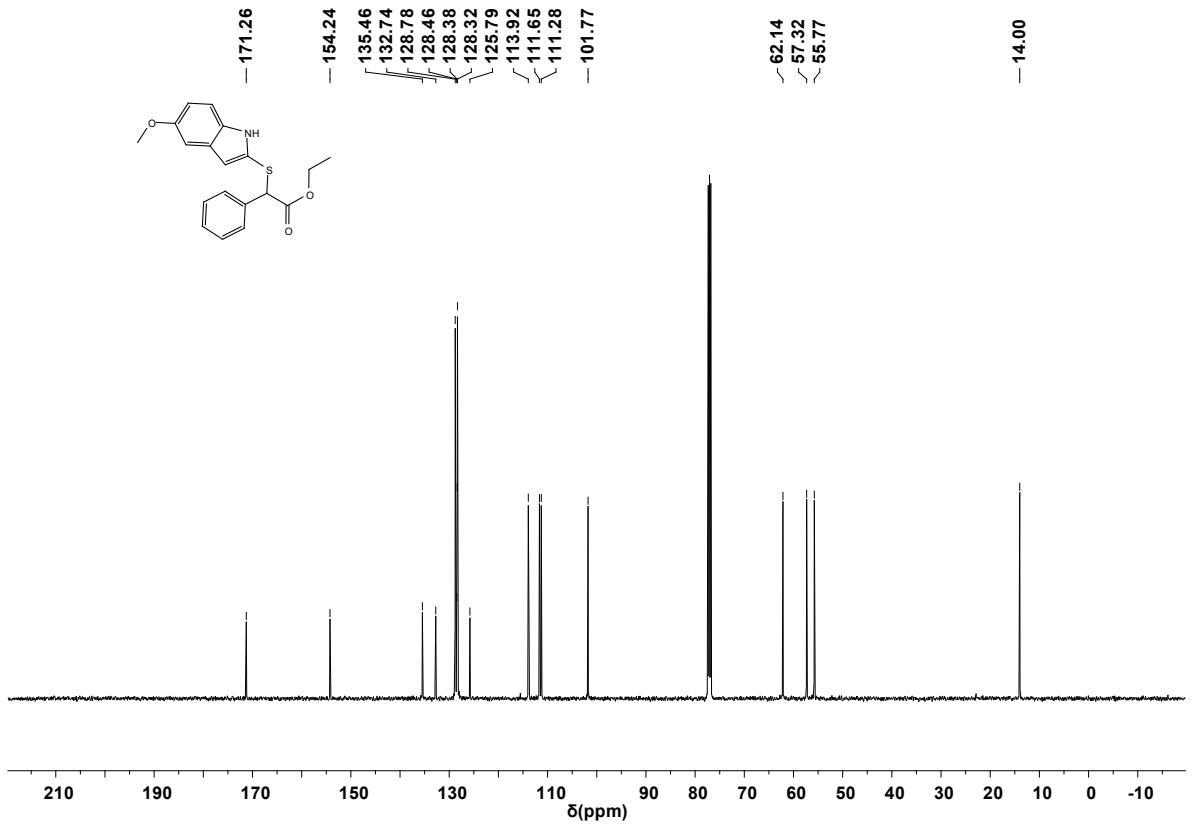
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **3j**



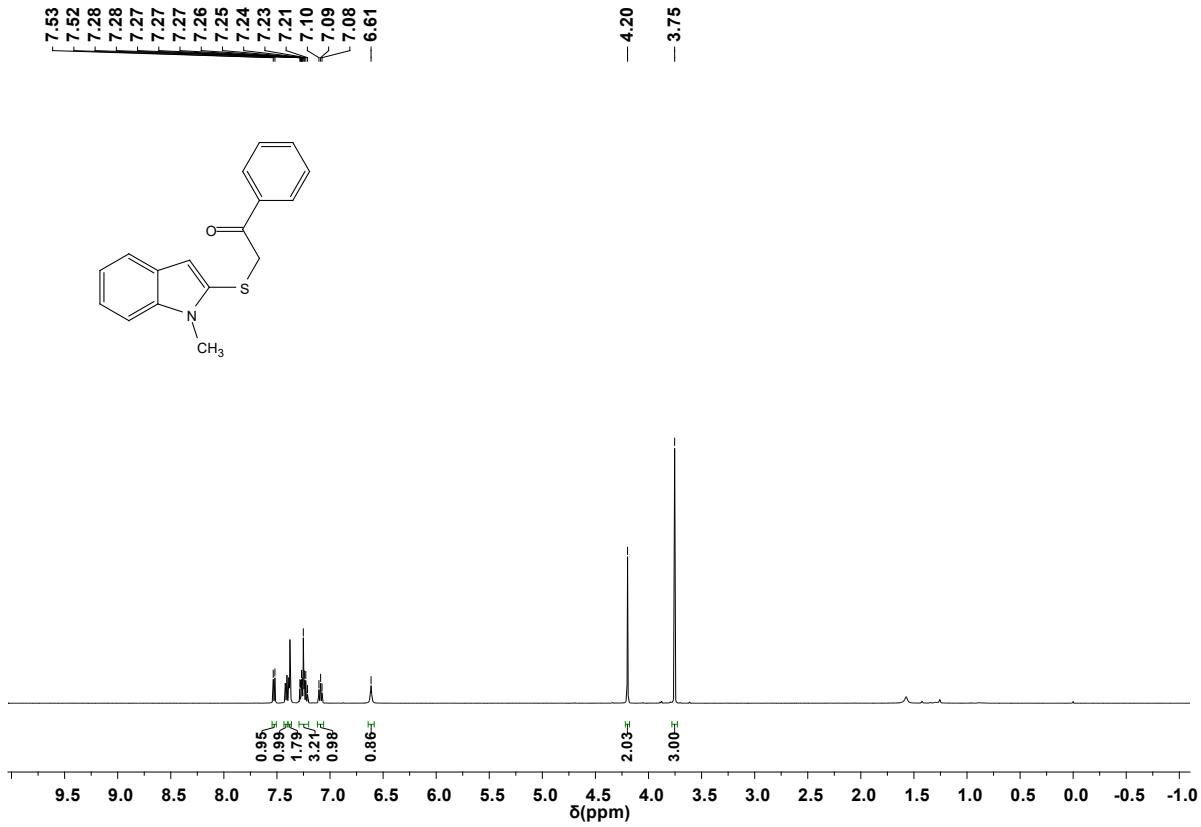
^{13}C NMR (101 MHz, $CDCl_3$) spectrum of **3j**



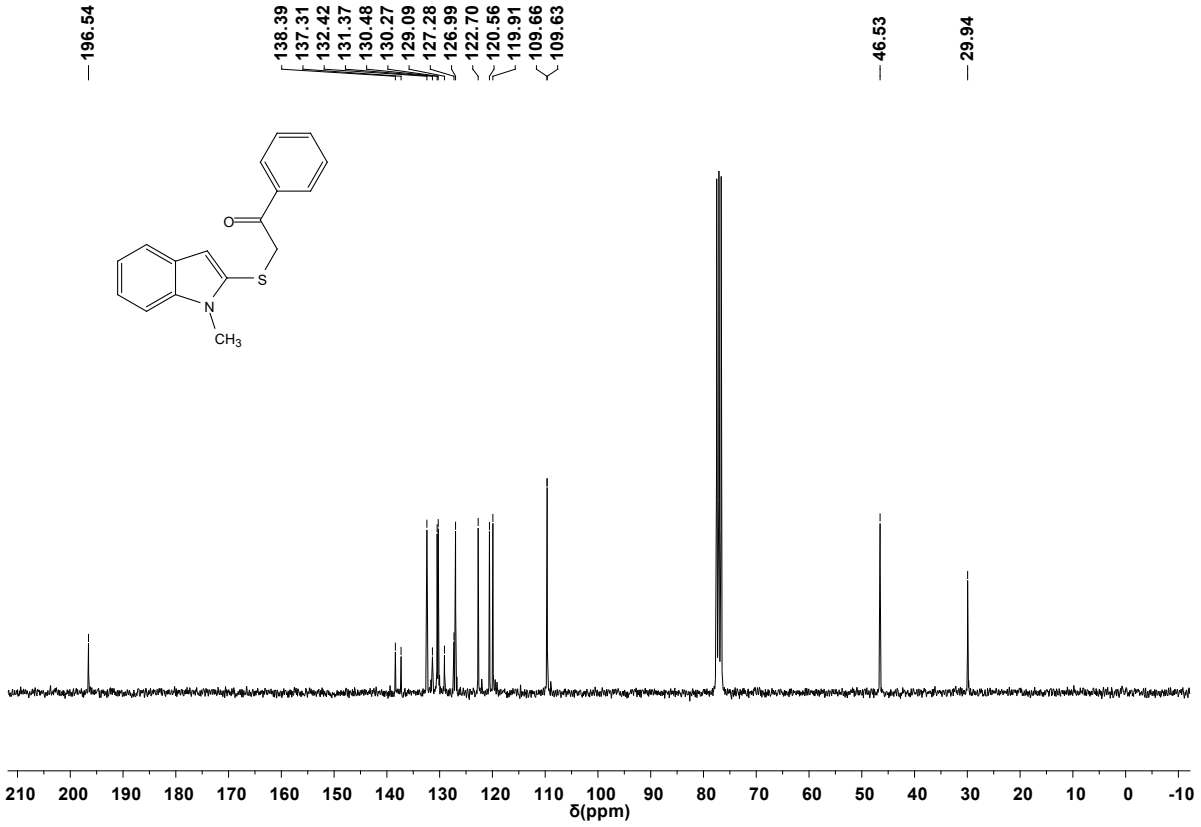
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **3k**



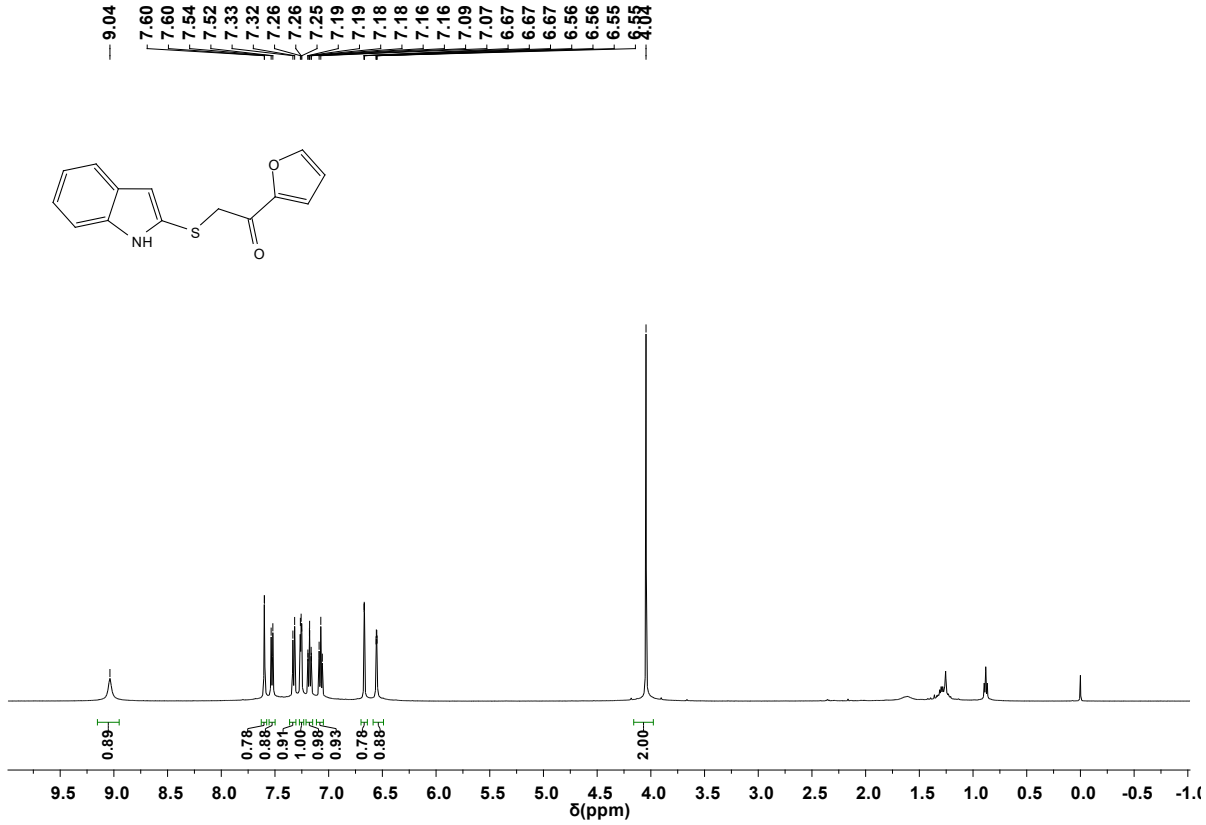
^{13}C NMR (126 MHz, $CDCl_3$) spectrum of **3k**



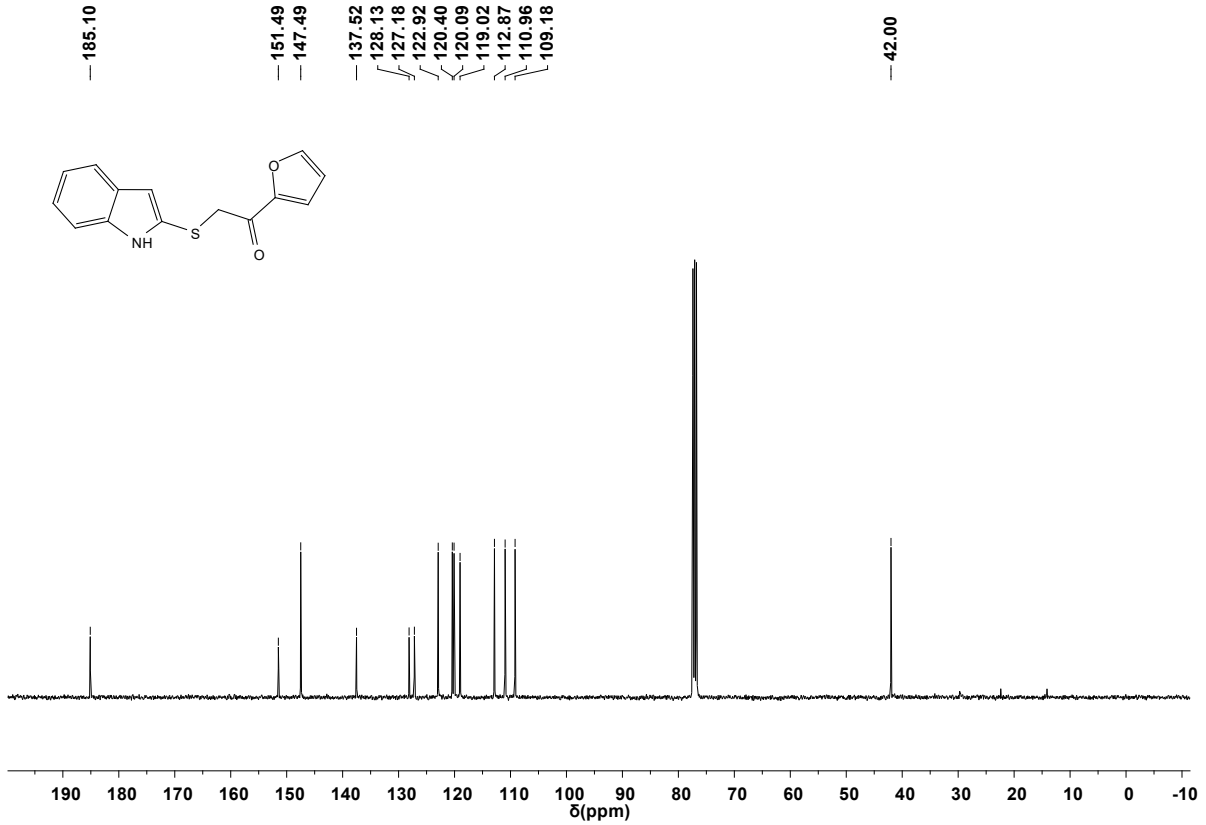
^{1}H NMR (500 MHz, $CDCl_3$) spectrum of **3n**



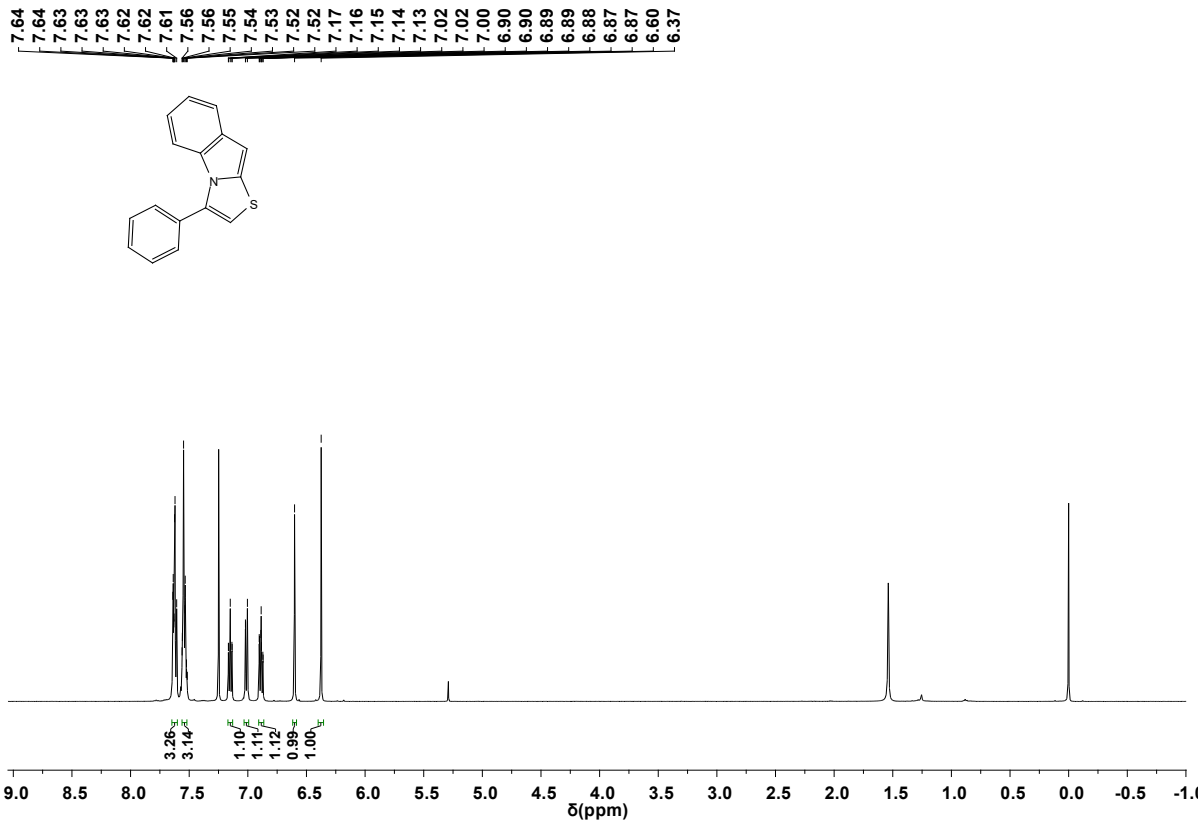
^{13}C NMR (75 MHz, $CDCl_3$) spectrum of **3n**



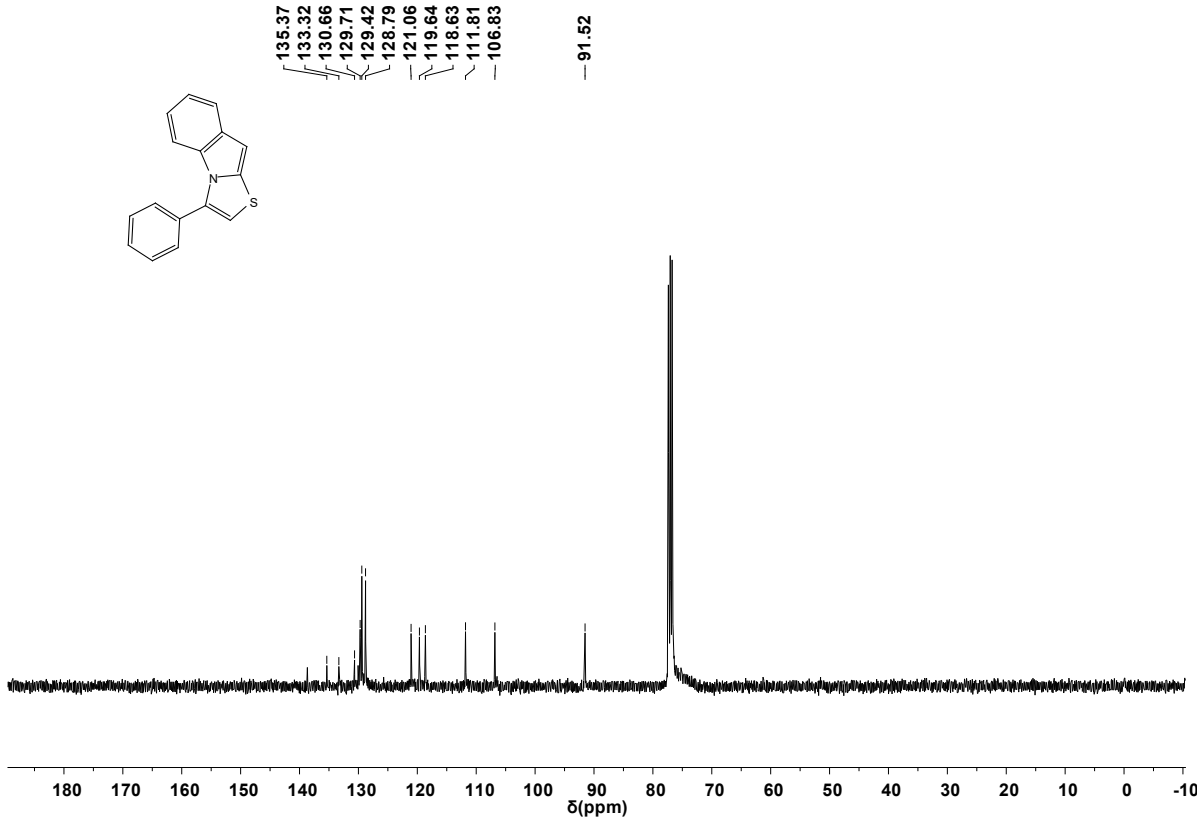
^{1}H NMR (500 MHz, $CDCl_3$) spectrum of **3l**



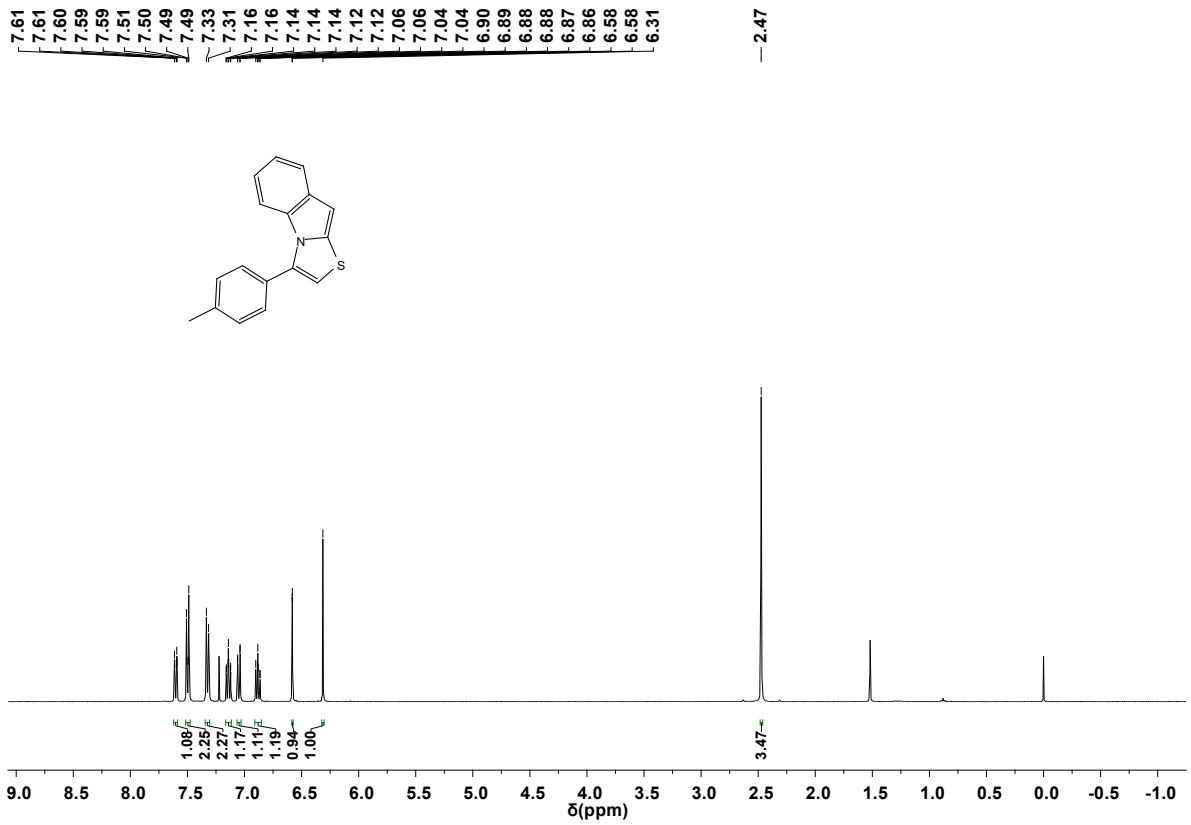
^{13}C NMR (101 MHz, $CDCl_3$) spectrum of **3l**



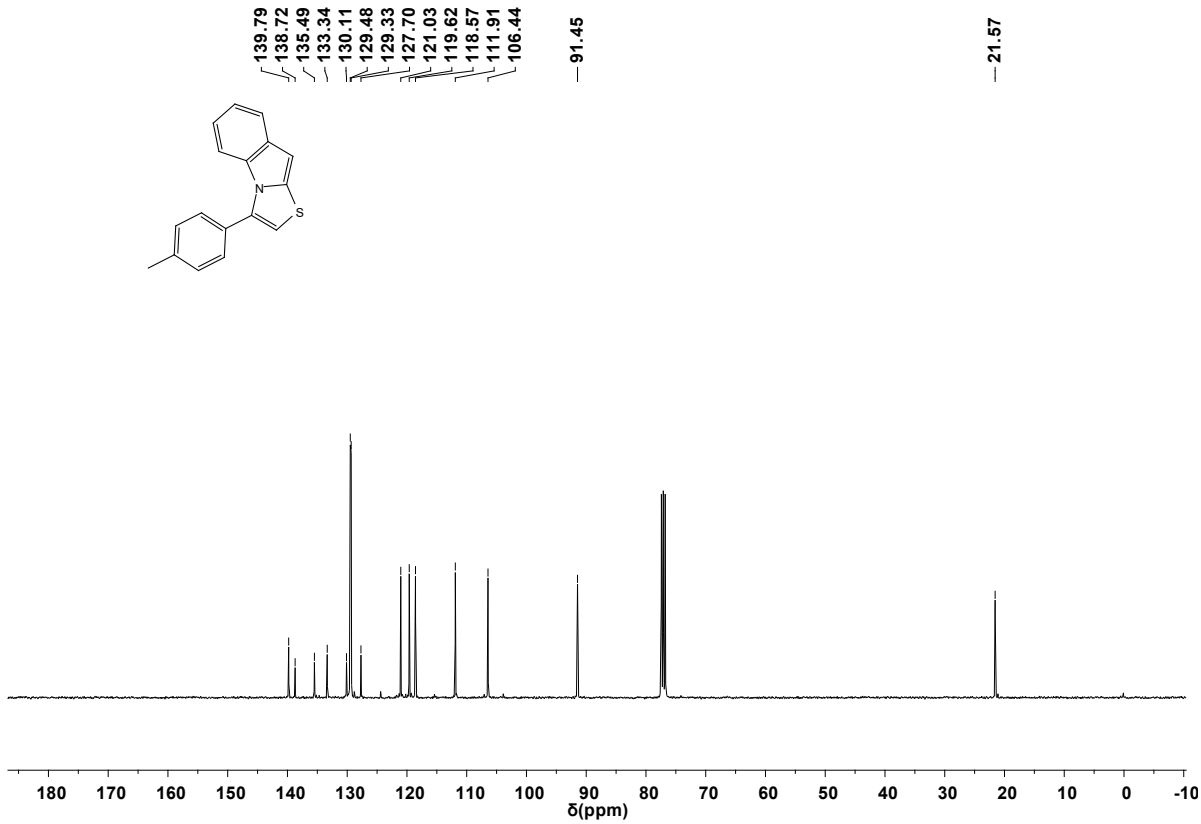
^{1}H NMR (500 MHz, $CDCl_3$) spectrum of **5a**



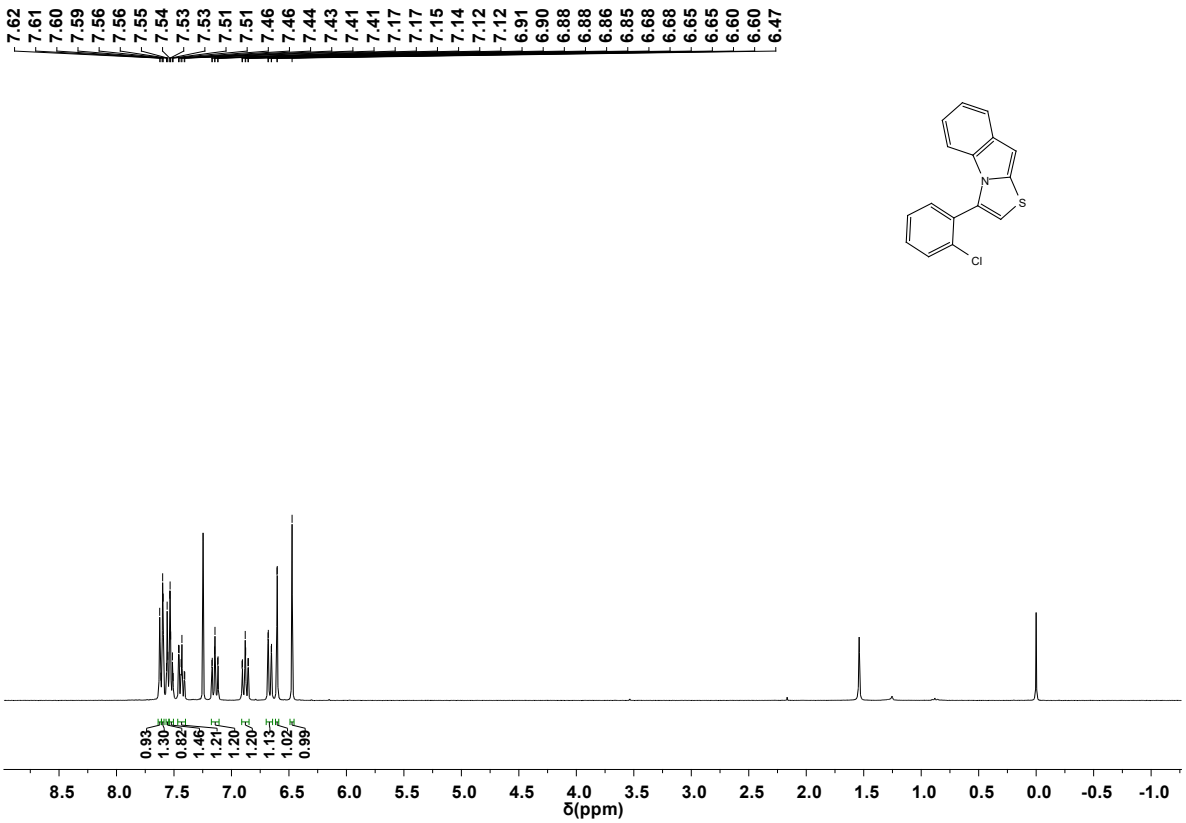
^{13}C NMR (101 MHz, $CDCl_3$) spectrum of **5a**



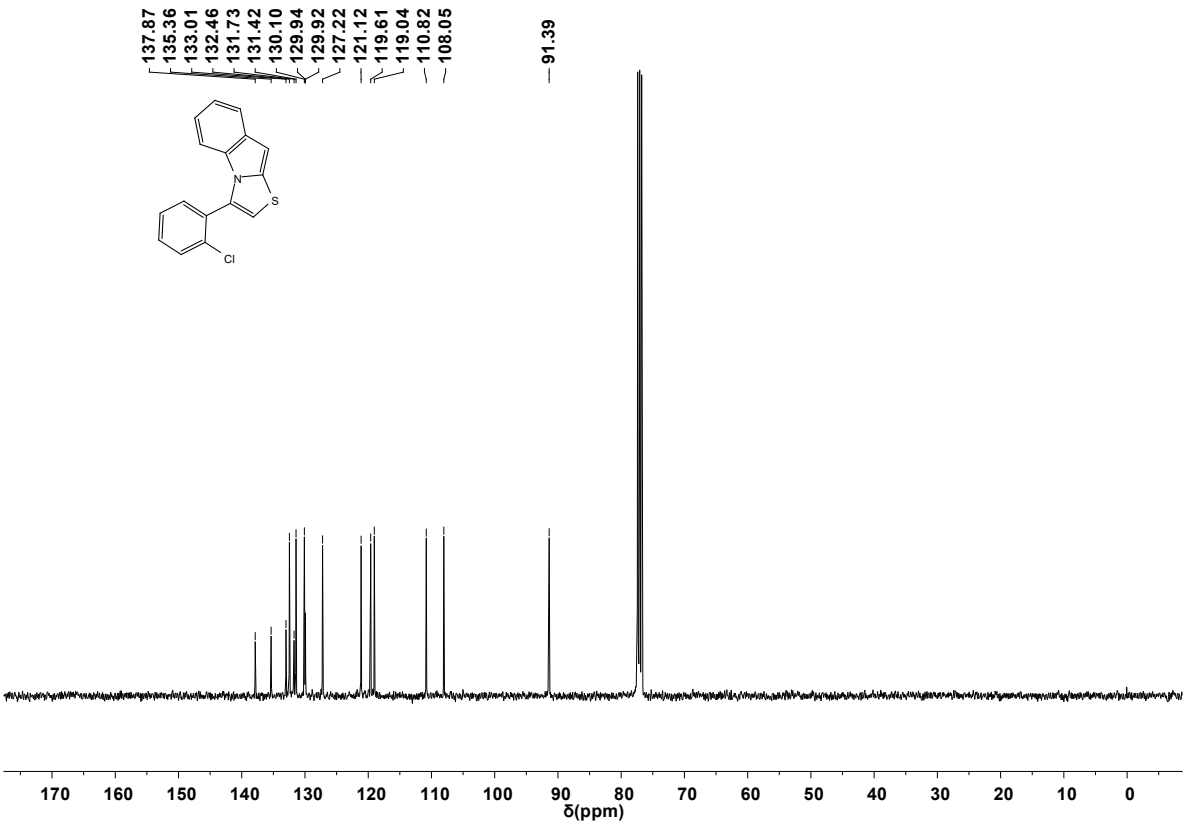
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **5b**



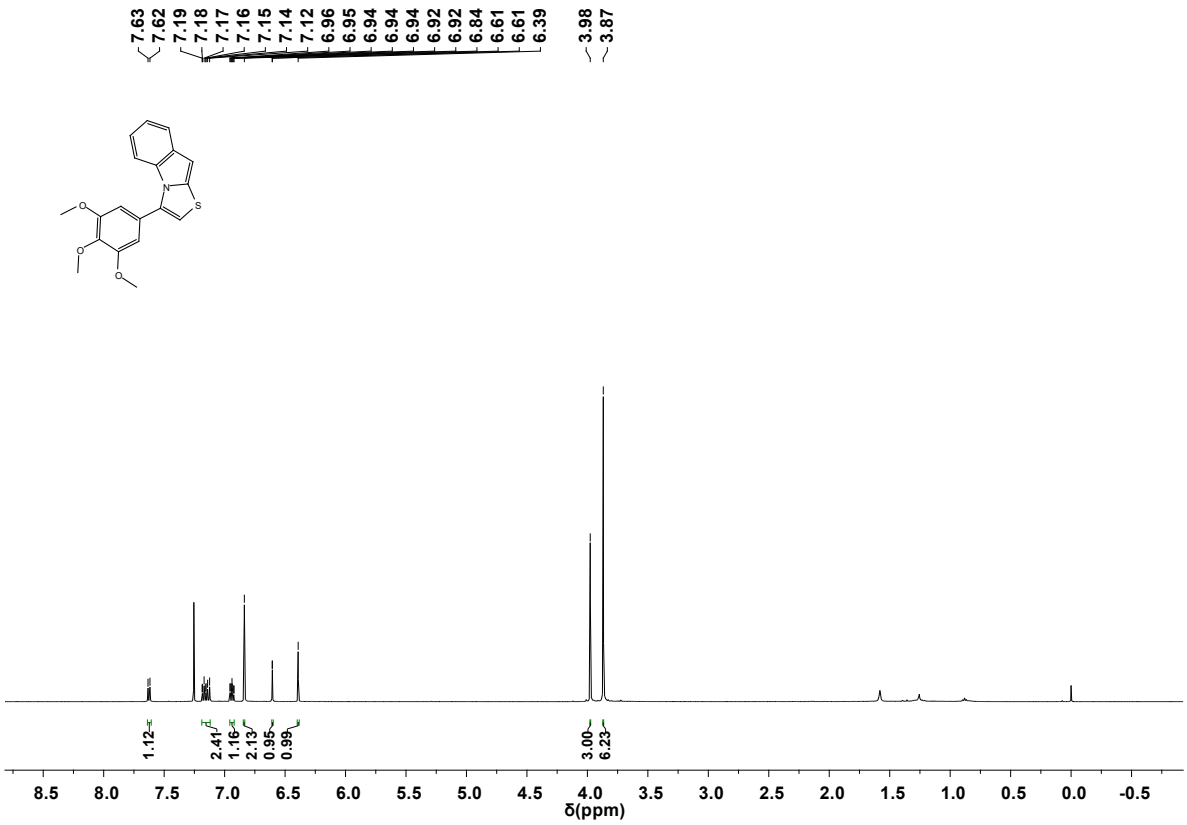
^{13}C NMR (101 MHz, $CDCl_3$) spectrum of **5b**



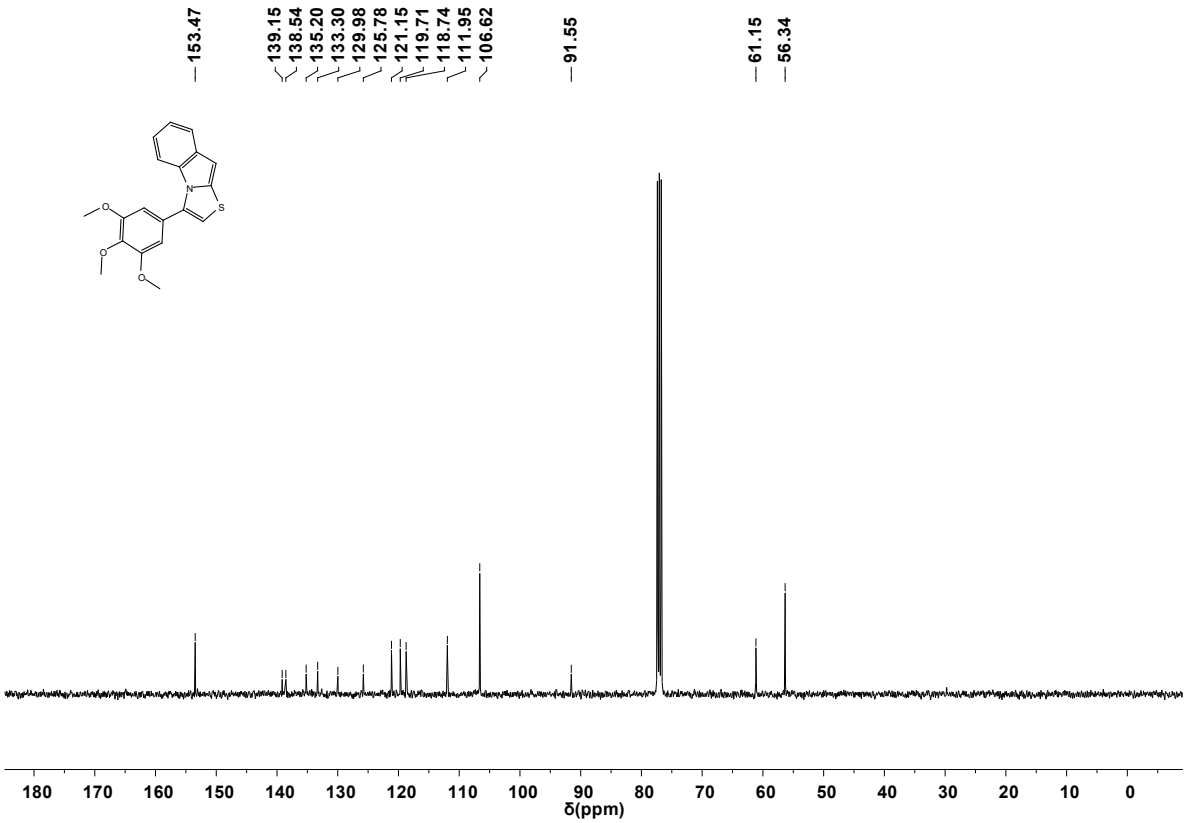
^{1}H NMR (300 MHz, $CDCl_3$) spectrum of **5c**



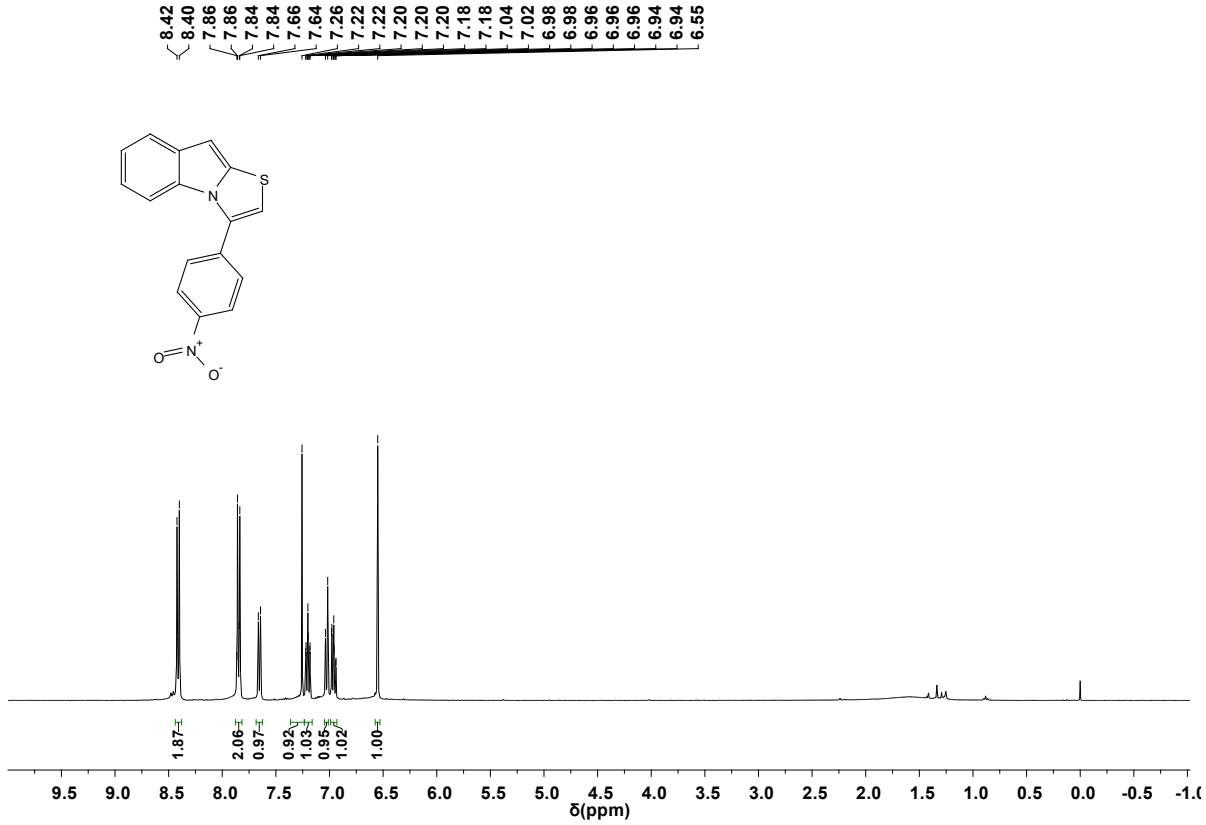
^{13}C NMR (101 MHz, $CDCl_3$) spectrum of **5c**



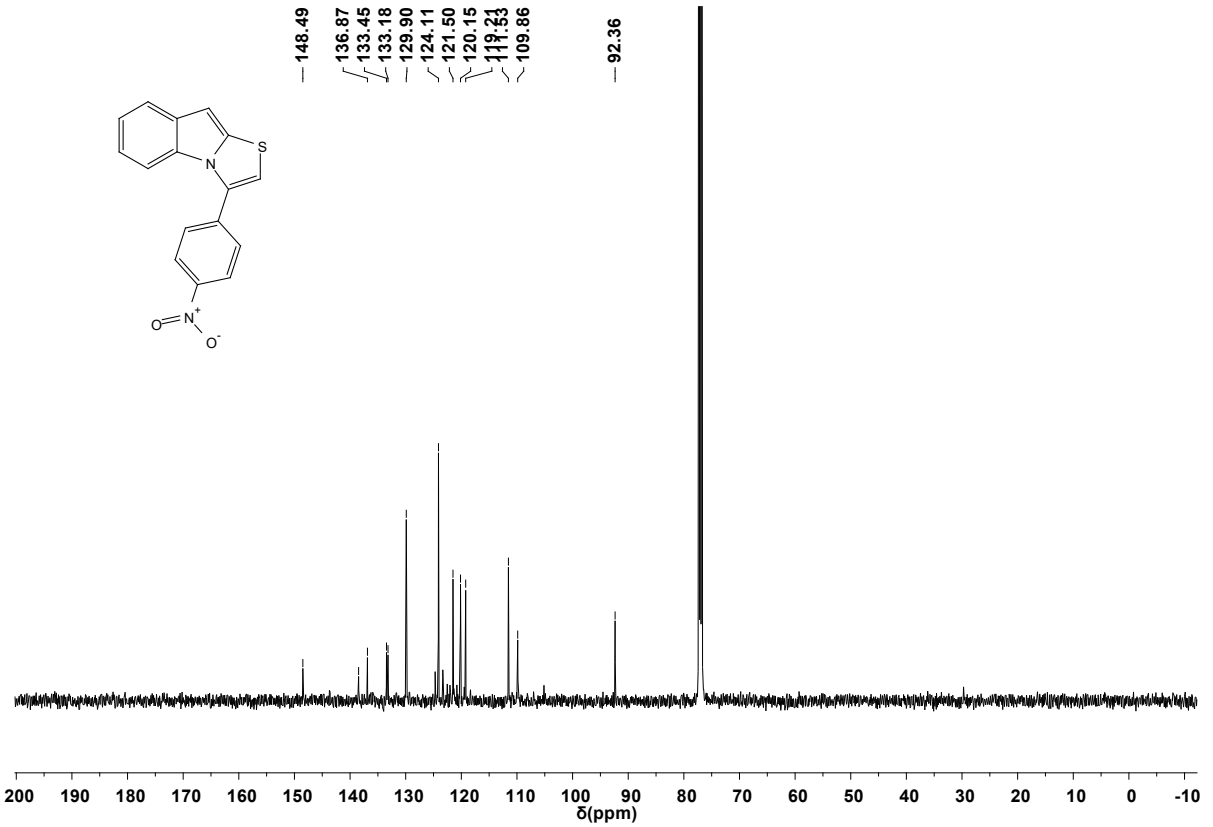
^{1}H NMR (500 MHz, $CDCl_3$) spectrum of **5d**



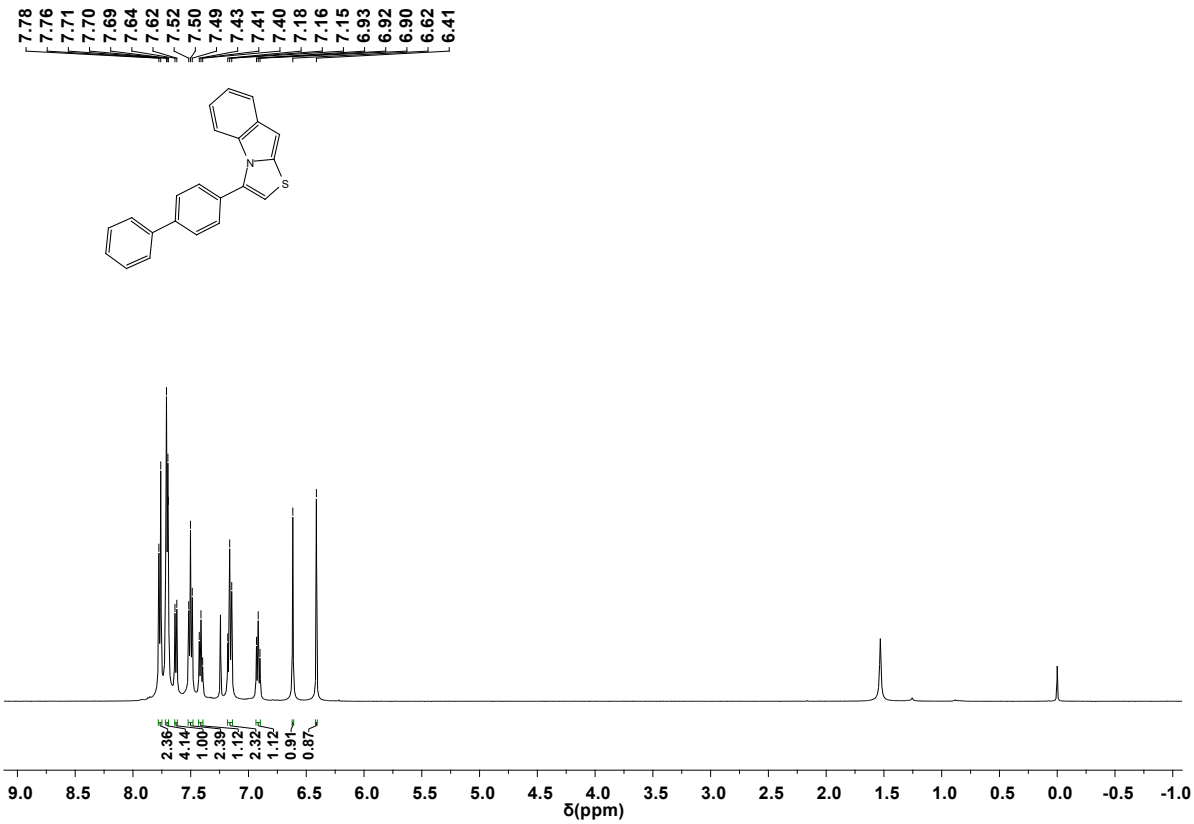
^{13}C NMR (101 MHz, $CDCl_3$) spectrum of **5d**



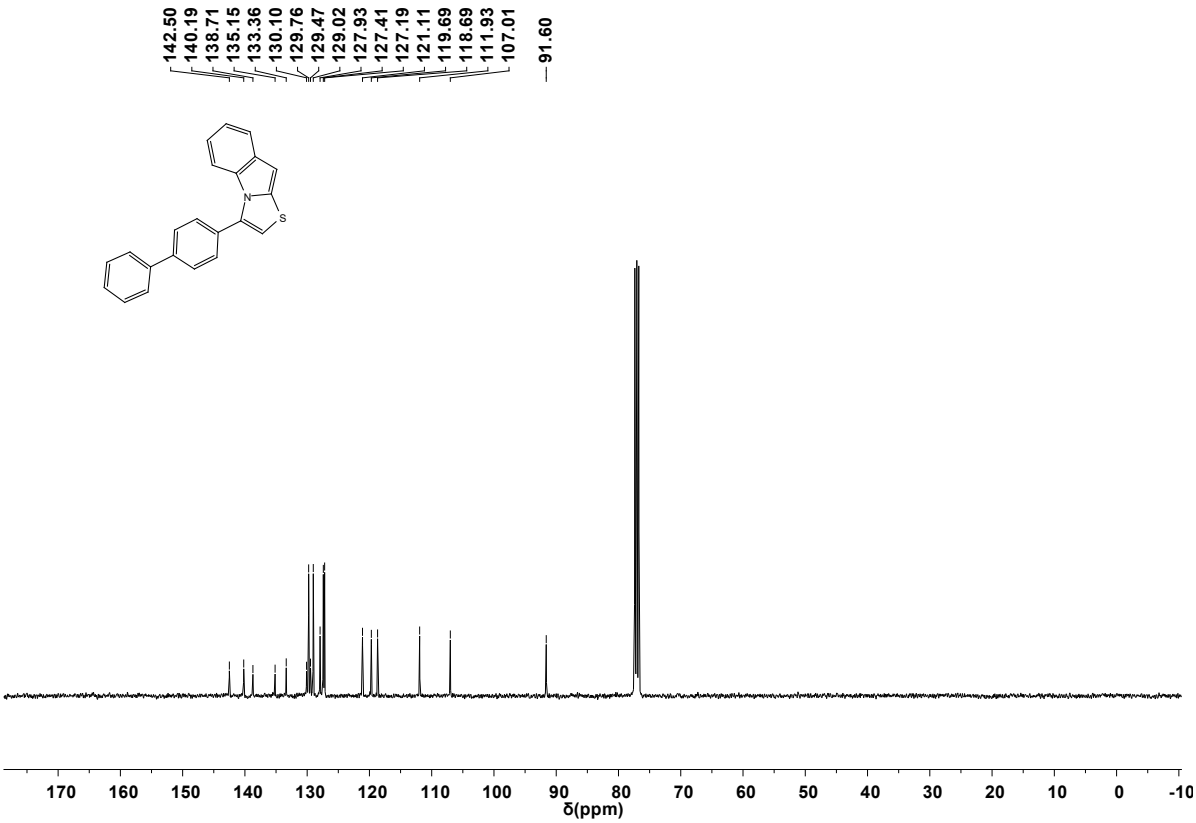
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **5e**



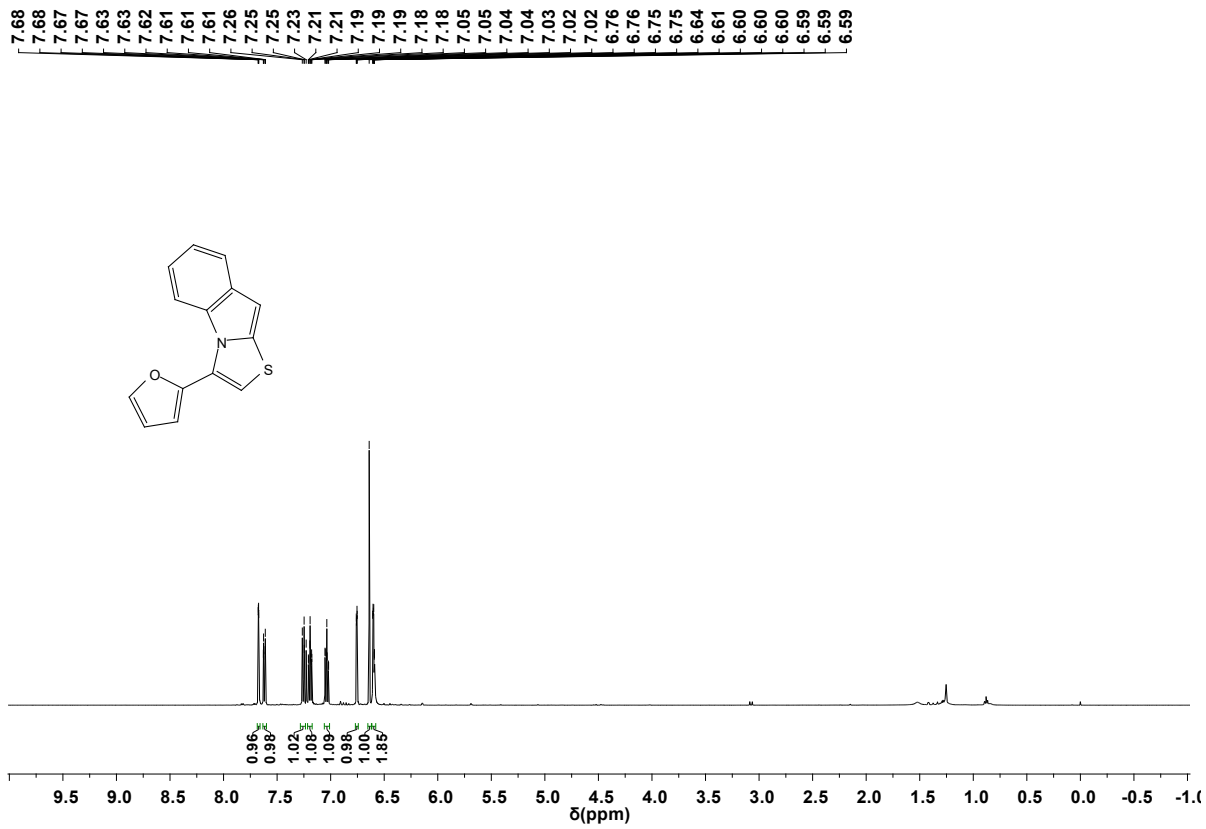
^{13}C NMR (126 MHz, $CDCl_3$) spectrum of **5e**



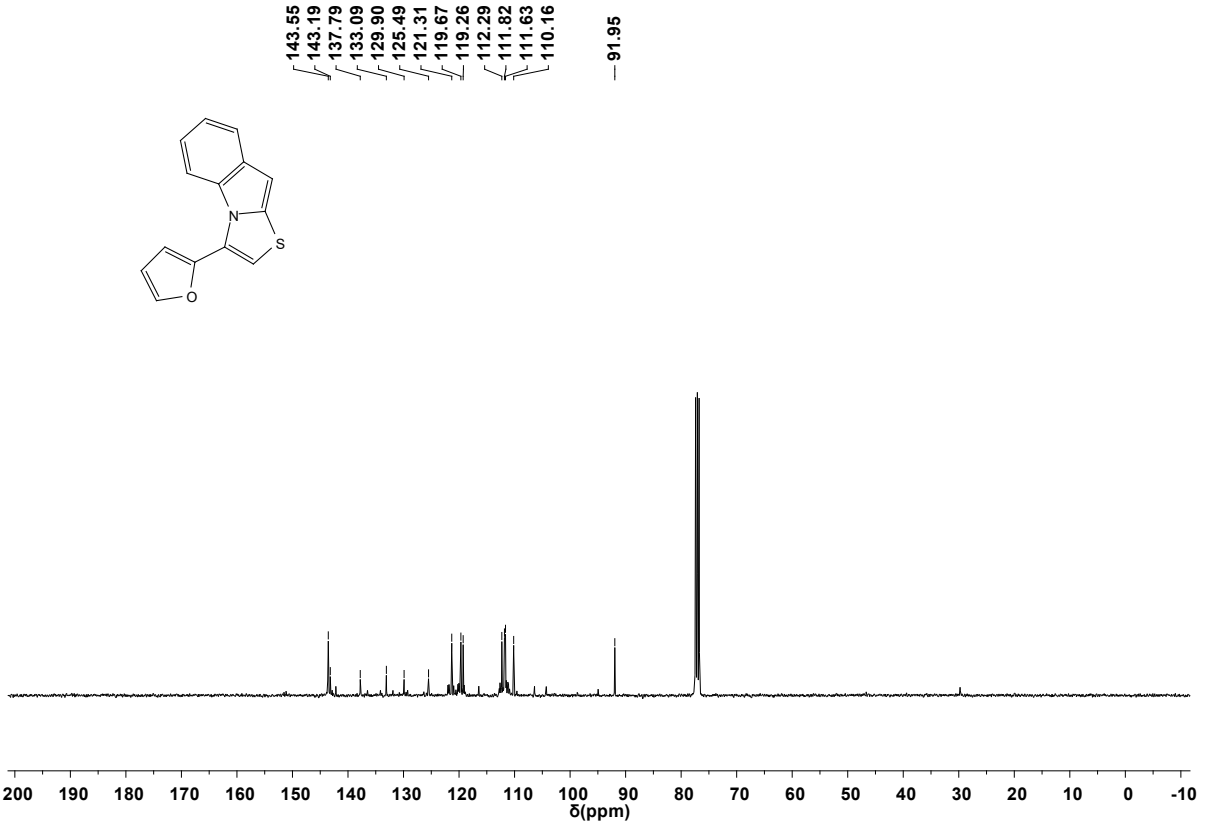
^{1}H NMR (500 MHz, $CDCl_3$) spectrum of **5f**



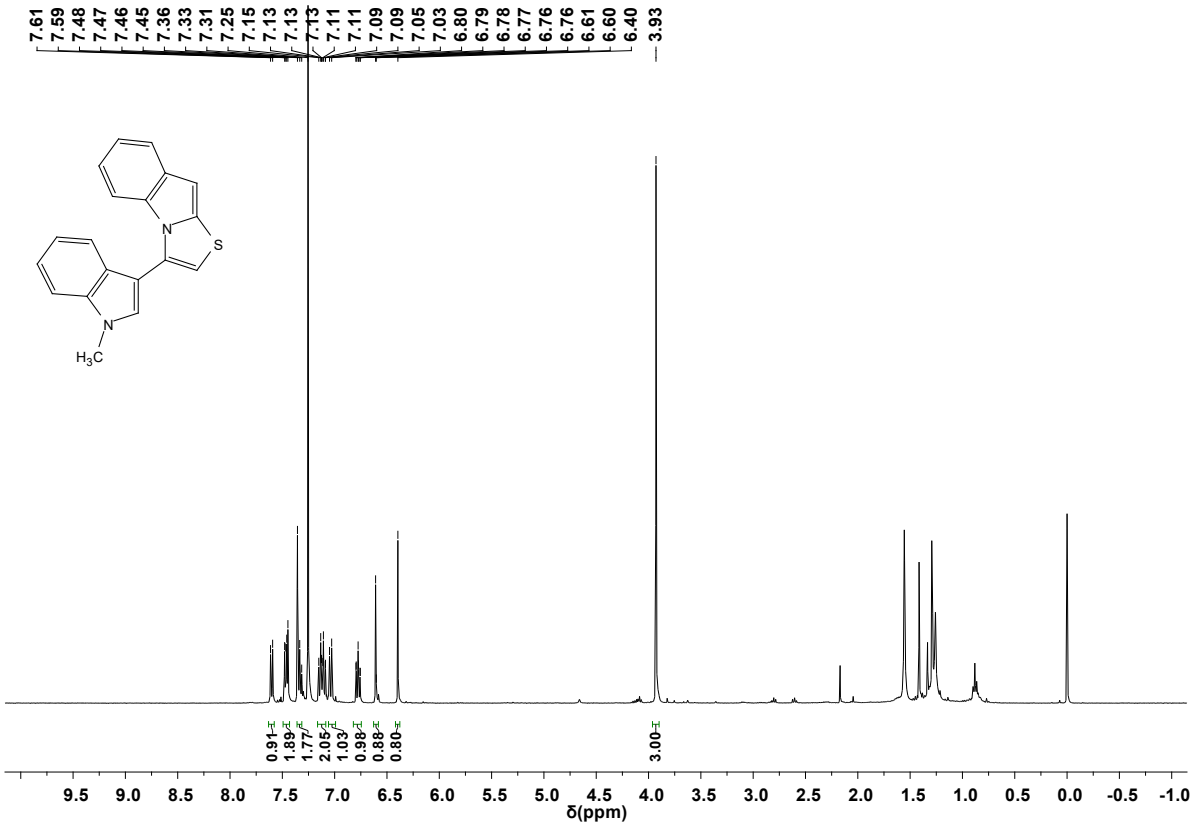
^{13}C NMR (101 MHz, $CDCl_3$) spectrum of **5f**



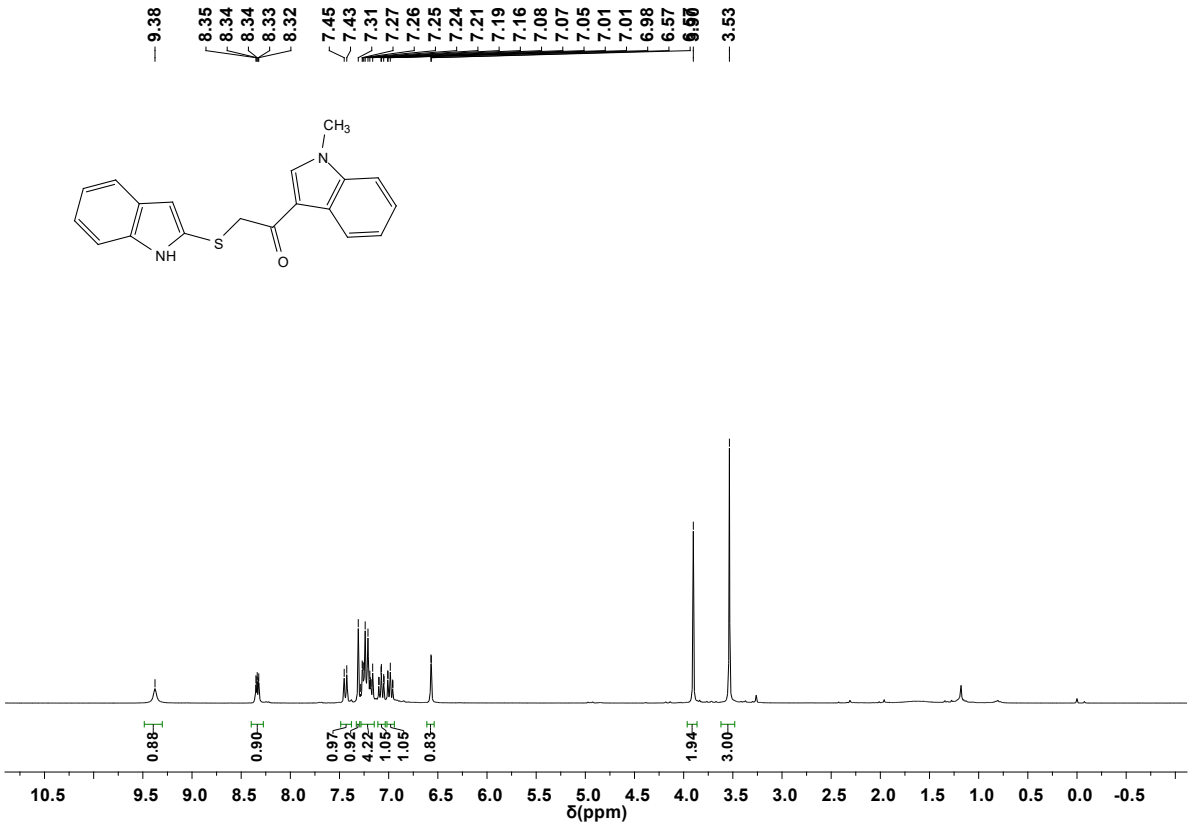
^{1}H NMR (500 MHz, $CDCl_3$) spectrum of **5g**



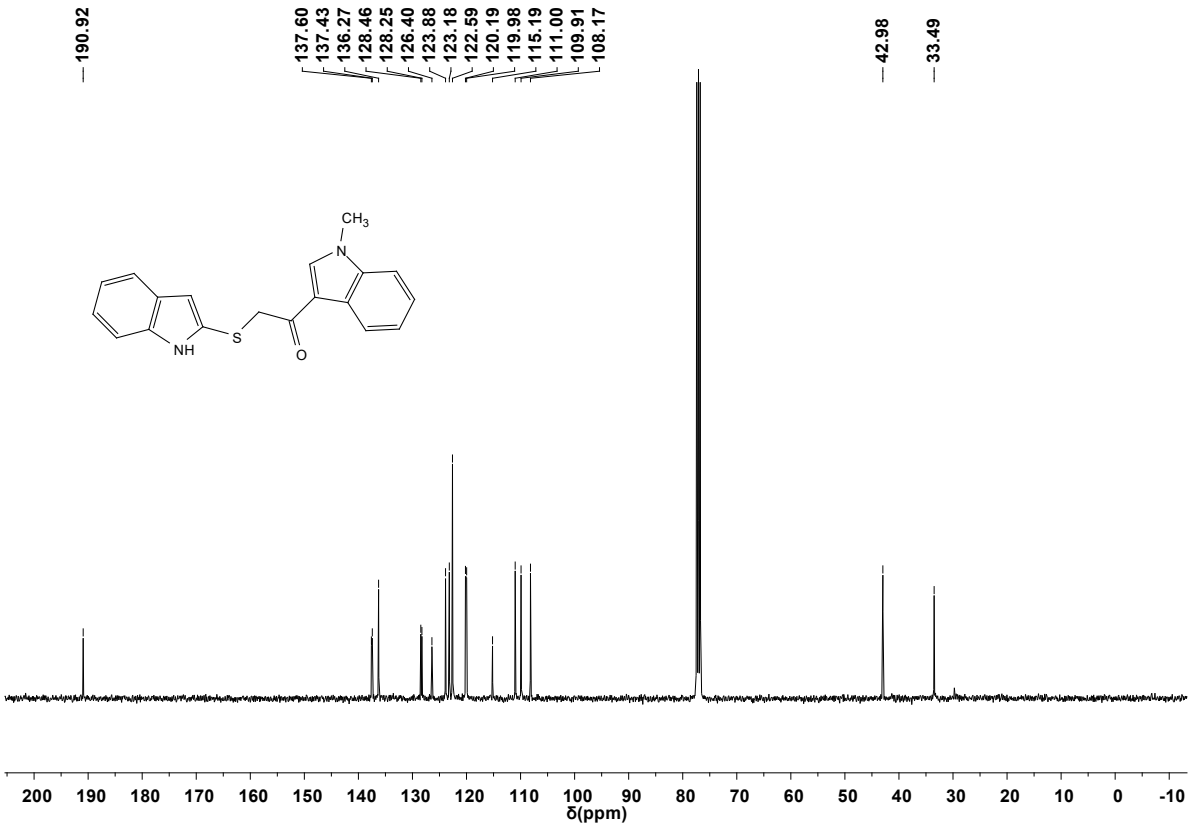
^{13}C NMR (126 MHz, $CDCl_3$) spectrum of **5g**



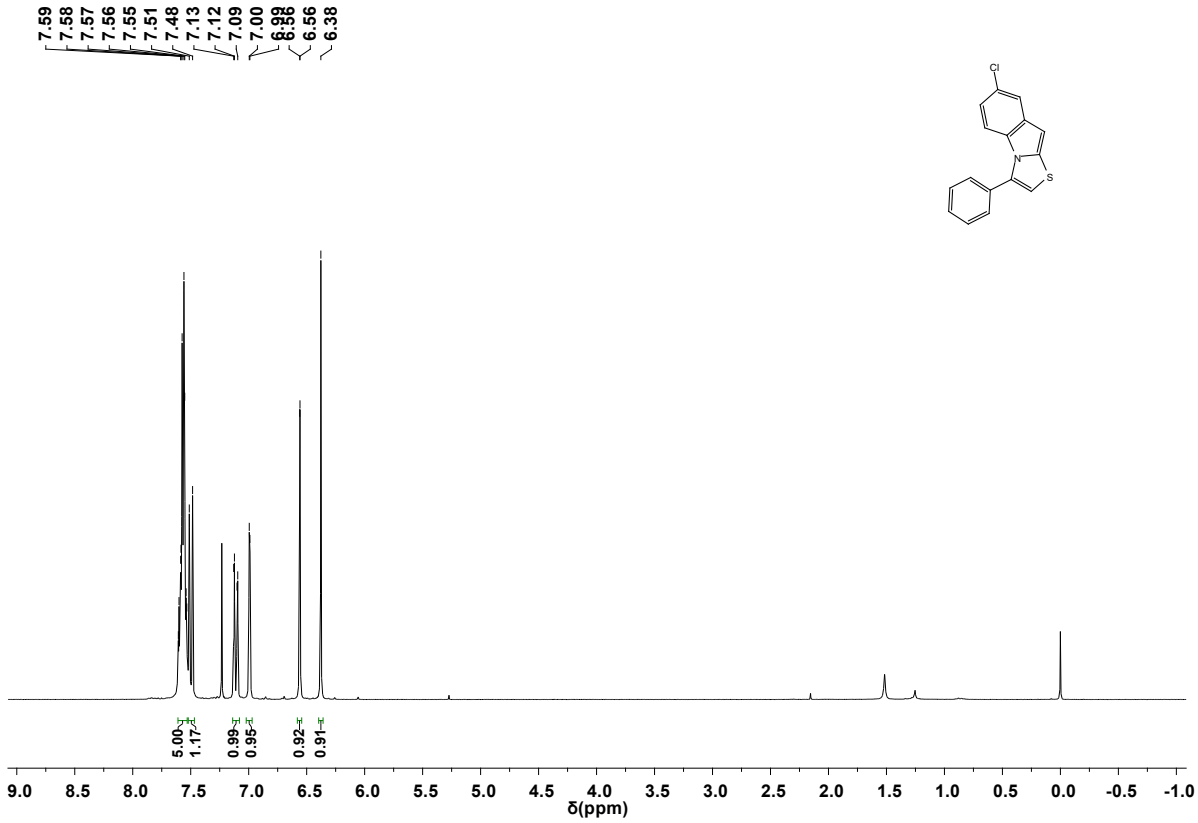
^{1}H NMR (500 MHz, $CDCl_3$) spectrum of **5h**



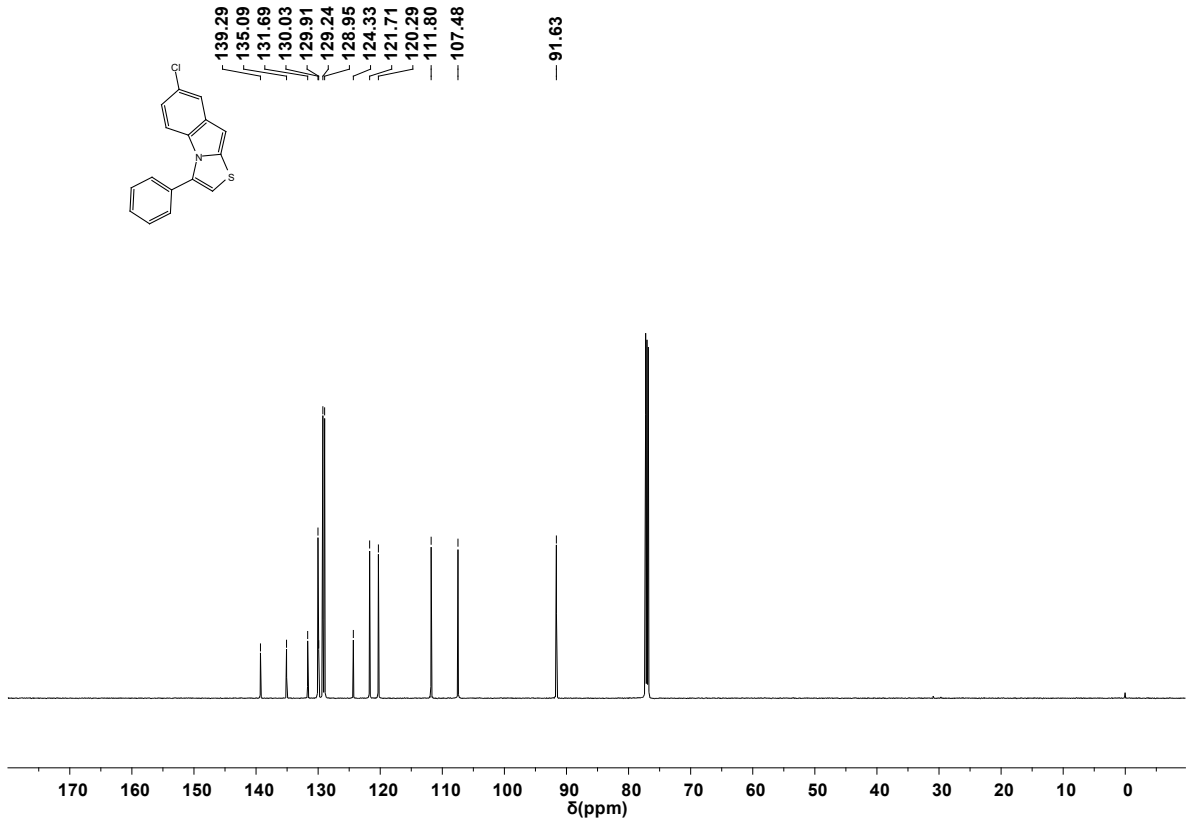
^{1}H NMR (300 MHz, $CDCl_3$) spectrum of **3m**



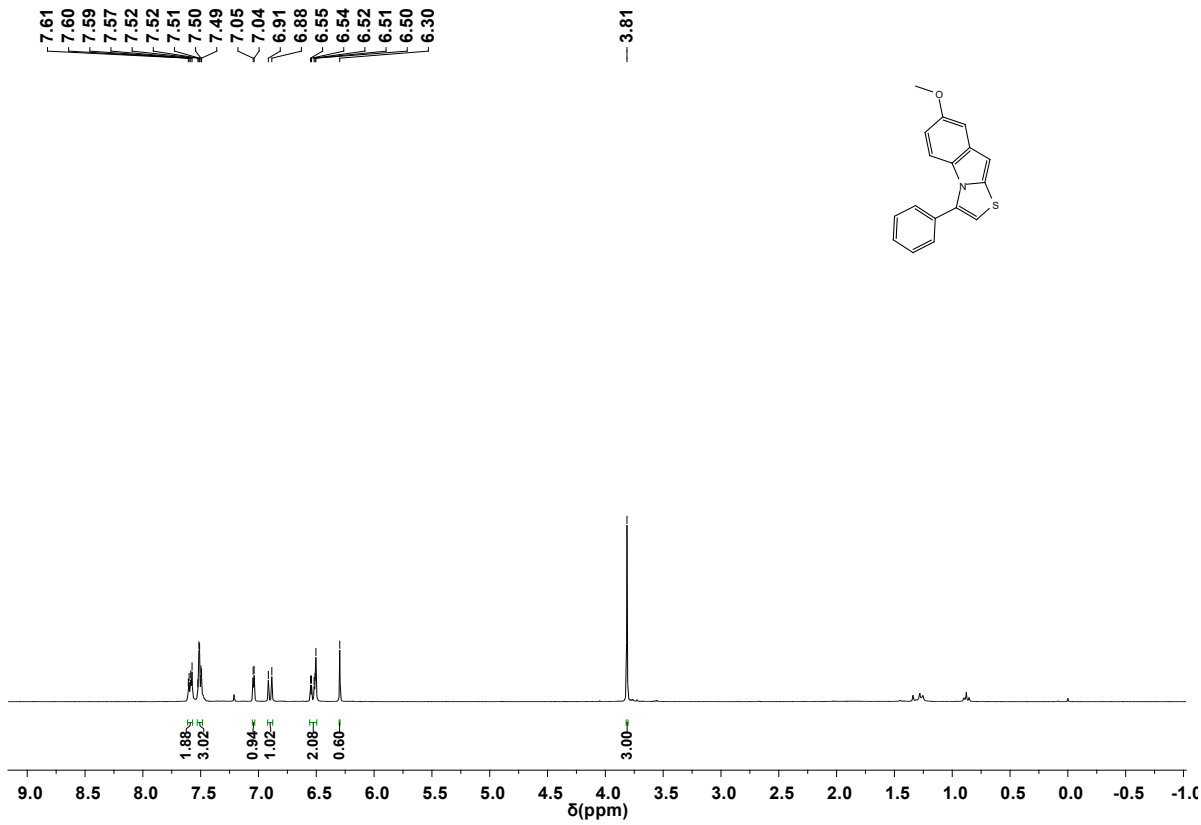
^{13}C NMR (101 MHz, $CDCl_3$) spectrum of **3m**



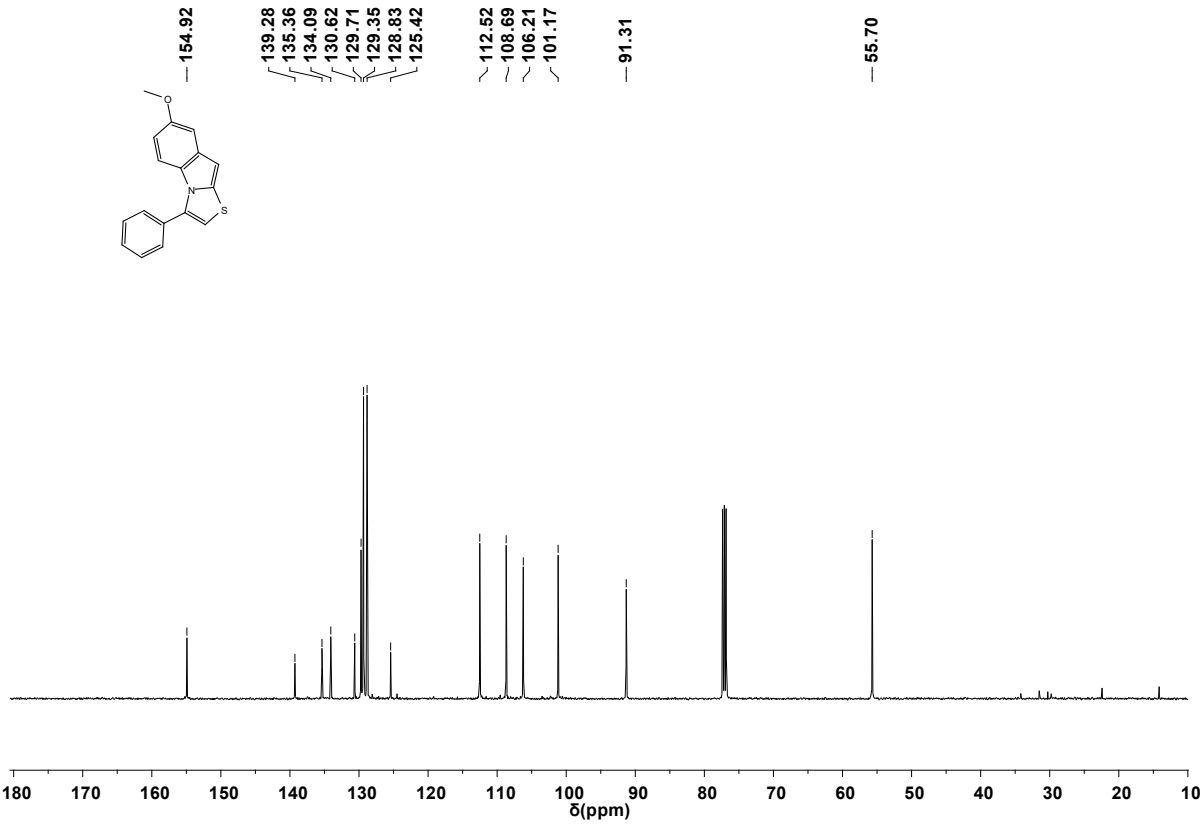
^{1}H NMR (300 MHz, $CDCl_3$) spectrum of **5i**



^{13}C NMR (151 MHz, $CDCl_3$) spectrum of **5i**



^{1}H NMR (300 MHz, $CDCl_3$) spectrum of **5j**



^{13}C NMR (126 MHz, $CDCl_3$) spectrum of **5j**

HPLC Traces

Column: Phenyl Hexyl (4.6 × 100 mm, 3.5 μm)

Column Temperature: 30 °C

Mobile Phase: A = 0.1% Formic acid in water and B = 0.1% Formic acid in MeOH

Wavelength: 220 nm

Gradient elution program:
0–3 min: 95% A / 5% B
3–15 min: linear gradient to 5% A / 95% B
15–20 min: hold at 5% A / 95% B
20–22 min: linear return to 95% A / 5% B
22–30 min: hold at 95% A / 5% B for re-equilibration

Figure S7. HPLC chromatogram of blank

```
================================================================================
Acq. Operator   : admin                          Seq. Line :   3
Sample Operator : admin
Acq. Instrument : IICT-ANA-FA-EQ-19_DAD          Location :   D1F-A1
Injection Date  : 09-10-2025 13:29:38                 Inj :   1
                                                 Inj Volume : 10.000 µl
Method          : E:\Chemstation Data\1\Data\OCT_2025\PSH_SS_A_S1_09OCT2025 2025-10-09 12-25-
                  49\Purity method_29AUG2025.M (Sequence Method)
Last changed    : 16-09-2025 16:04:46 by admin
Additional Info : Peak(s) manually integrated
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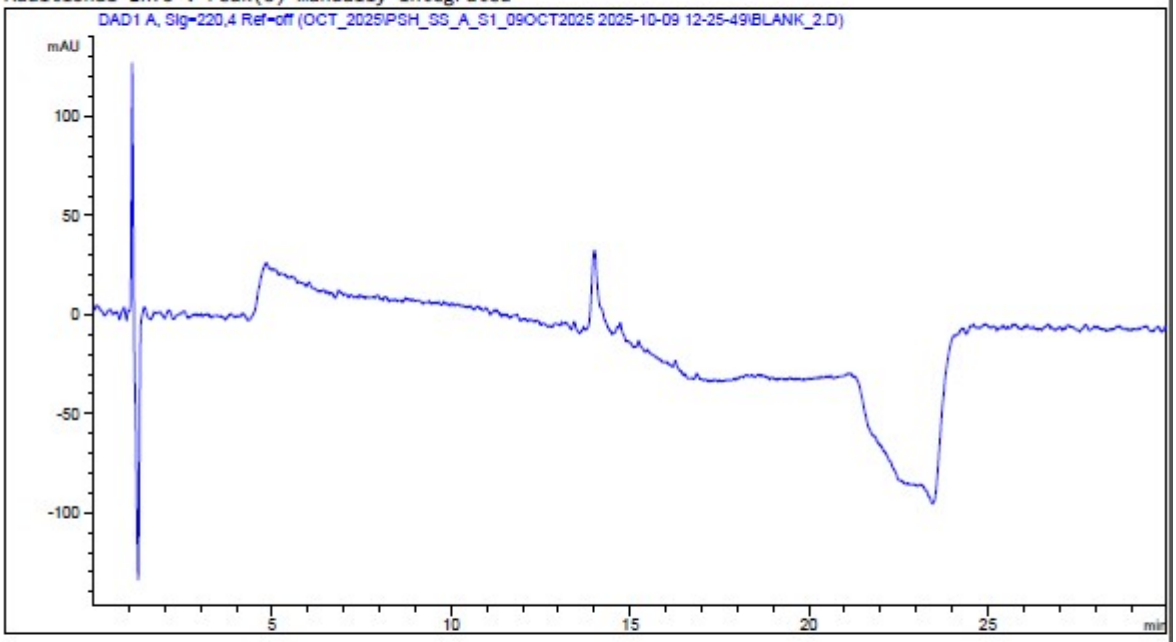


```
================================================================================
                          Area Percent Report
================================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs

No peaks found

================================================================================
                           *** End of Report ***
```

Figure S8. HPLC chromatogram of **3f**

```
Acq. Operator   : admin                          Seq. Line :   4
Sample Operator : admin
Acq. Instrument : IICT-ANA-FA-EQ-19_DAD          Location :   D1F-A3
Injection Date  : 09-10-2025 14:00:56                Inj :   1
                                                Inj Volume : 10.000 µl
Method          : E:\Chemstation Data\1\Data\OCT_2025\PSH_SS_A_S1_09OCT2025 2025-10-09 12-25-
                  49\Purity method_29AUG2025.M (Sequence Method)
Last changed    : 16-09-2025 16:04:46 by admin
Additional Info : Peak(s) manually integrated
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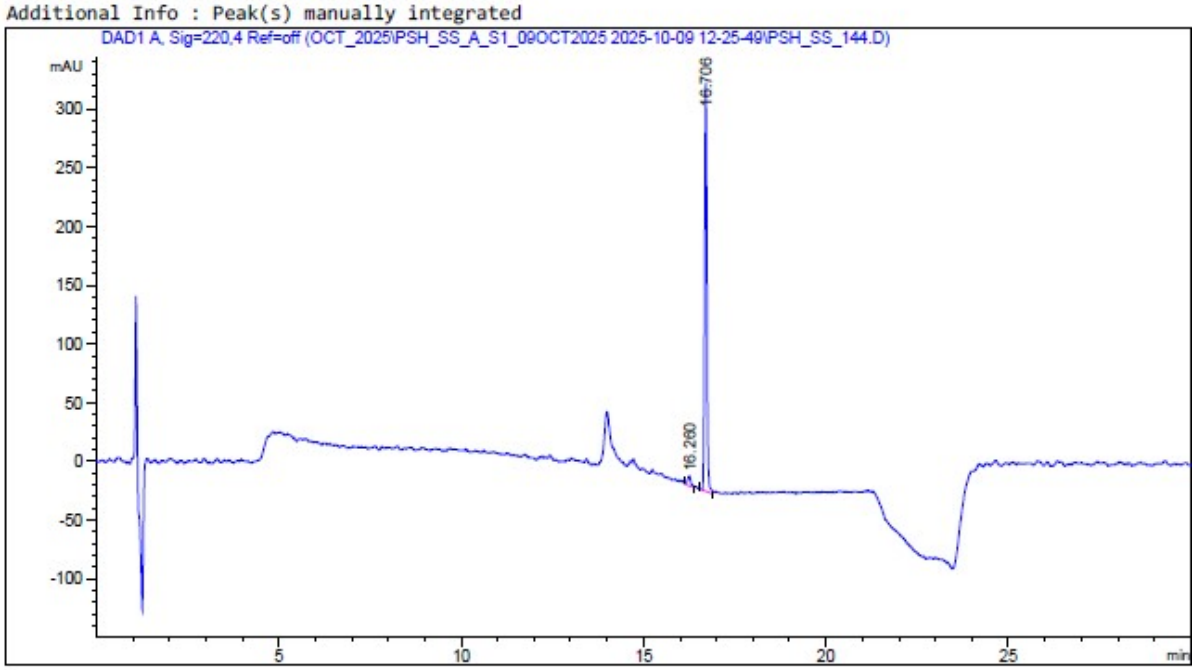


```
                        Area Percent Report

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=220,4 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]       [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  16.260 MM R  0.1097   55.36644    8.41293   3.3095
   2  16.706 MM R  0.0773 1617.59167  348.78268  96.6905

Totals :                  1672.95811  357.19561


                        *** End of Report ***
```

Figure S9. HPLC chromatogram of **5d**

```
Acq. Operator   : admin                          Seq. Line :   2
Sample Operator : admin
Acq. Instrument : IICT-ANA-FA-EQ-19_DAD          Location :   D1F-A2
Injection Date  : 09-10-2025 12:58:15                 Inj :   1
                                                Inj Volume : 10.000 µl
Method          : E:\Chemstation Data\1\Data\OCT_2025\PSH_SS_A_S1_09OCT2025 2025-10-09 12-25-
                  49\Purity method_29AUG2025.M (Sequence Method)
Last changed    : 16-09-2025 16:04:46 by admin
Additional Info : Peak(s) manually integrated
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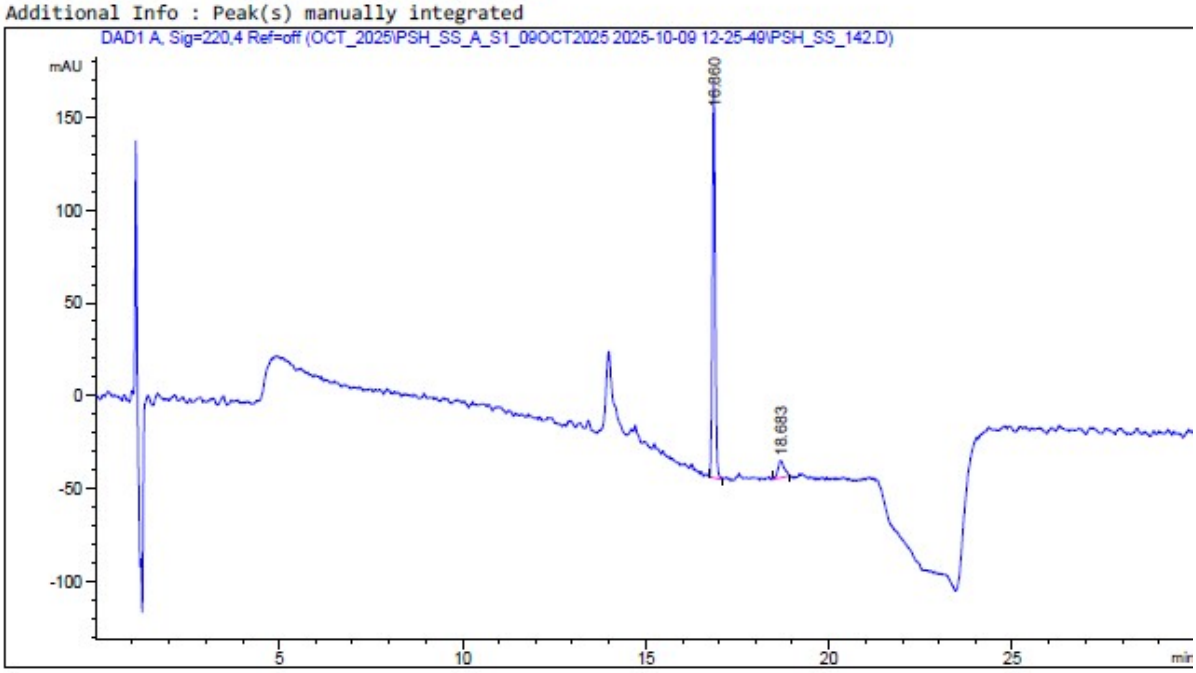


```
                        Area Percent Report

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=220,4 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  16.860 MM R  0.0889 1137.92212  213.33295  91.2443
   2  18.683 MM R  0.2004  109.19339    9.08108   8.7557

Totals :                  1247.11551  222.41403


                        *** End of Report ***
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