Supplementary Information (SI) for RSC Advances.
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Discovery of Thieno[3,2-c]pyran-Based Leads for Liver, Lung and Glioma Cancer: Synthesis, Docking-Guided Optimization and Apoptotic Profiling

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Supporting information

1. Experimental Section:

1.1 General remarks: Reagents and solvents used as they are available commercially means no further purification was performed. ^{1}H and ^{13}C NMR spectra were recorded on 400 MHz NMR spectrometer, respectively. $CDCl_3$ and DMSO-d_6 were used as solvent for NMR. Chemical shift is reported in ppm considering ($CDCl_3$) δ 7.26 ppm for ^{1}H NMR and δ 77.00 ppm for ^{13}C NMR as an internal standard. Signal patterns are indicated as s, singlet; d, doublet; dd, double doublet; t, triplet; m, multiplet; bs, broad singlet. Coupling constants (*J*) are given in hertz (Hz). Infrared (IR) spectra were recorded on AX-1 spectrophotometer and reported as wave number (cm^{-1}). HRMS was recorded on Brucker-Daltonics, Micro-TOF-Q II mass spectrometer. Elemental analysis of compounds was done by CHNS techniques.

1.2 Synthesis of substrate 4-(methylthio)-2-oxo-6-aryl-*2H*-pyran-3-carbonitriles/ carboxylate: The pyran substrates were prepared from the reaction of 2-(bis(methylthio)methylene)malonate or 2-cyano-3,3-bis(methylthio)acrylate with aryl methyl ketone in presence of KOH in DMSO.[1] Whereas 2-(bis(methylthio)methylene)malonate and 2-cyano-3,3-bis(methylthio)acrylate were prepared from malonate ester and methyl 2-cyanoacetate, respectively.[2]

2. Characterisation Data of Compound 5a-o

5a. Methyl 3-amino-4-oxo-6-phenyl-*4H*-thieno[3,2-*c*]pyran-2-carboxylate: Yield: 90%; R_f = 0.32 (50% hexane in DCM); yellow solid; mp: 204–206 ^{o}C; IR (KBr): 3457, 3351, 1707, 1675, 1573 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.87-785 (m, 2H, ArH), 7.48 (dd, *J* = 3.9, 2.5 Hz, 3H, ArH), 7.03 (s, 1H, ArH), 6.77 (bs, 2H, -NH_2), 3.87 (s, 3H, -OMe); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 164.3, 158.4, 157.6, 153.0, 152.2, 131.9, 130.9, 129.0, 126.4, 125.6, 112.1, 98.5, 51.5; HRMS (*m/z*): calculated for ($M+Na^+$) of $C_{15}H_{11}NO_4S$: 324.0301; found: 324.0301.

5b. Methyl 3-amino-4-oxo-6-(p-tolyl)-*4H*-thieno[3,2-*c*]pyran-2-carboxylate: Yield: 92%; R_f = 0.35 (50% hexane in DCM); yellow solid; mp: 193–195 ^{o}C; IR (KBr): 3459, 3349, 1705, 1672, 1576 cm^{-1} ; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.73 (d, *J* = 8.2 Hz, 2H, ArH), 7.25 (d, J = 8.2 Hz, 2H, ArH), 6.97 (s, 1H, ArH), 6.75 (bs, 2H, -NH_2), 3.84 (s, 3H, -OMe), 2.39 (s, 3H, -Me), ^{13}C-NMR (101 MHz, $CDCl_3$) δ 164.3, 158.5, 157.8, 153.3, 152.5, 141.5, 129.7, 128.1, 127.2, 125.6, 111.8, 97.8, 51.4, 21.4; HRMS (*m/z*): calculated for ($M+H^+$) of $C_{16}H_{13}NO_4S$: 316.0638; found: 316.0635.

5c. Methyl 3-amino-6-(4-fluorophenyl)-4-oxo-*4H*-thieno[3,2-*c*]pyran-2-carboxylate: Yield: 90%; R_f = 0.22 (50% hexane in DCM); yellow solid; mp: 196–198 °C; IR (KBr): 3470, 3360, 1700, 1680, 1660, 1586 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.34 (dd, J = 7.0, 1.8 Hz, 2H, ArH), 8.05-8.03 (m, 2H, ArH), 7.19 (s, 1H, ArH), 6.78 (bs, 2H, -NH_2), 3.89 (s, 3H, -OMe); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 164.2 (d, *J* = 258.4 Hz), 164.2, 158.3, 156.7, 152.9, 128.7, 127.8 (d, *J* = 8.6 Hz), 127.2 (d, *J* = 2.9 Hz), 124.9, 116.2 (d, *J* = 21.9 Hz), 112.0, 98.3, 51.5; HRMS (*m/z*): calculated for ($M+H^+$) of $C_{15}H_{10}FNO_4S$: 320. 0387; found: 320.0383.

5d. Methyl 3-amino-6-(4-chlorophenyl)-4-oxo-*4H*-thieno[3,2-*c*]pyran-2-carboxylate: Yield: 88%; R_f = 0.25 (50% hexane in DCM); yellow solid; mp: 208–210 °C; IR (KBr): 3490, 3380, 1710, 1680, 1662 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.80 (d, J = 8.7 Hz, 2H, ArH), 7.46 (d, J = 9.2 Hz, 2H, ArH), 7.02 (s, 1H, ArH), 6.77 (bs, 2H, -NH_2), 3.87 (s, 3H, -OMe); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 164.2, 160.5, 158.2, 156.5, 152.7, 137.1, 129.4, 129.3, 126.9, 114.1, 112.2, 98.7, 51.6; HRMS (*m/z*): calculated for ($M+Na^+$) of $C_{15}H_{10}ClNO_4S$: 336.0092; found: 336.0102.

5e. Methyl 3-amino-6-(4-bromophenyl)-4-oxo-*4H*-thieno[3,2-*c*]pyran-2-carboxylate: Yield: 89 %; R_f = 0.25 (50% hexane in DCM); yellow solid; mp: 230–232 °C; IR (KBr): 3478, 3362, 1708, 1675, 1604 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.73 (dd, *J* = 8.9, 2.1 Hz, 2H, ArH), 7.61 (dd, *J* = 8.9, 2.1 Hz, 2H, ArH), 7.03 (s, 1H, ArH), 6.76 (bs, 2H, -NH_2), 3.87 (s, 3H, -OMe); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 164.3, 158.5, 157.7, 153.1, 148.2, 131.0, 129.3, 129.0, 126.7, 125.7, 112.1, 98.6, 51.5; HRMS (*m/z*): calculated for ($M+H^+$) of $C_{15}H_{10}$ $BrNO_4S$: 379.9587; found: 379.9585.

5f. Methyl 3-amino-4-oxo-6-(thiophen-2-yl)-*4H*-thieno[3,2-*c*]pyran-2-carboxylate: yield: 83%; R_f = 0.35 (50% hexane in DCM); yellow solid; mp: 212-214 °C, IR (KBr): 3468, 3340, 1725, 1676, 1582 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ δ 7.65 (d, *J* = 2.9 Hz, 1H, ArH), 7.48 (d, *J* = 5.1 Hz, 1H, ArH), 7.13 (t, *J* = 4.4 Hz, 1H, ArH), 6.86 (s, 1H, ArH), 6.76 (bs, 2H, -NH_2), 3.87 (s, 3H, -OMe); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 164.3, 158.5, 157.9, 153.3, 152.4, 141.5, 129.7, 128.1, 125.6, 111.8, 97.8, 51.5; HRMS (*m/z*): calculated for ($M+Na^+$) of $C_{13}H_9NO_4S_2$: 329.9865; found: 329.9866.

5g. Methyl 3-amino-6-(furan-2-yl)-4-oxo-*4H*-thieno[3,2-*c*]pyran-2-carboxylate: yield: 88%; R_f = 0.27 (50% hexane in DCM); yellow solid; mp: 204-206 °C, IR (KBr): 3475, 3347, 1725, 1677, 1604 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.54 (s, 1H, ArH), 7.05 (d, *J* = 3.2 Hz, 1H, ArH), 6.95 (s, 1H, ArH), 6.76 (bs, 2H, -NH_2), 6.57 (q, *J* = 1.7 Hz, 1H, ArH), 3.85 (s, 3H, -OMe); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 164.3, 157.8, 152.8, 149.6, 146.1, 145.0, 112.6, 112.3, 111.5, 96.9, 51.5; HRMS (*m/z*): calculated for (M+Na^+) of $C_{13}H_9NO_5S$: 314.0094; found: 314.0096.

5h. Methyl 3-amino-6-(naphthalen-1-yl)-4-oxo-*4H*-thieno[3,2-*c*]pyran-2-carboxylate: yield: 76%; R_f = 0.30 (50% hexane in DCM); yellow solid; mp: 222-224 °C, IR (KBr): 3472, 3323, 1730, 1677, 1585 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.20 (d, *J* = 8.9 Hz, 1H, ArH), 7.99-7.91 (m, 2H, ArH), 7.74 (d, *J* = 7.1 Hz, 1H, ArH), 7.61-7.52 (m, 3H, ArH), 6.91 (s, 1H, ArH), 6.82 (bs, 2H, -NH_2), 3.89 (s, 3H, -OMe); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 164.4, 159.0, 158.9, 152.8, 152.4, 133.8, 131.3, 130.7, 130.4, 129.9, 128.7, 127.9, 127.5, 126.5, 125.0, 124.8, 112.3, 103.7, 51.5; HRMS (*m/z*): calculated for (M+H^+) of $C_{19}H_{13}NO_4S$: 352.0638; found: 352.0639.

5i. methyl 3-amino-6-(4-nitrophenyl)-4-oxo-*4H*-thieno[3,2-*c*]pyran-2-carboxylate: Yield: ~8%; R_f = 0.30 (50% hexane in DCM); brown solid; mp: 198–200 °C; IR (KBr): 3490, 3380, 1710, 1680, 1662, 1548, 1332 cm^{-1}; ^1H-NMR (400 MHz, $CDCl_3$) δ 8.34 (d, J = 9.5 Hz, 2H, ArH), 8.04 (d, J = 8.8 Hz, 2H, ArH), 7.19 (s, 1H, ArH), 6.78 (bs, 2H, -NH_2), 3.89 (s, 3H, -OMe); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 165.8, 160.8, 156.4, 153.4, 148.8, 136.7, 134.0, 126.4, 124.3, 123.8, 120.9, 101.2, 51.7; HRMS (*m/z*): calculated for (M+H^+) of $C_{15}H_{10}N_2O_6S$: 347.0332; found: 347.0332.

5j. Methyl 3-amino-6-(2-methoxyphenyl)-4-oxo-*4H*-thieno[3,2-*c*]pyran-2-carboxylate: Yield: 75%; R_f = 0.28 (50% hexane in DCM); yellow solid; mp: 189–191 °C; IR (KBr): 3495, 3383, 1722, 1681, 1666 cm^{-1}; ^1H-NMR (400 MHz, $CDCl_3$) δ 7.88 (dd, J = 6.7, 1.6 Hz, 1H, ArH), 7.35-7.30 (m, 1H, ArH), 7.01-6.95 (m, 2H, ArH), 6.91 (s, 1H, ArH), 6.66 (bs, 2H, -NH_2), 3.89 (s, 3H, -OMe), 3.77 (s, 3H, -OMe); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 164.2, 158.2, 156.5, 154.6, 152.7, 134.1, 132.3, 129.9, 127.0, 125.5, 119.1, 112.2, 112.1, 98.8, 53.4, 51.6; HRMS (*m/z*): calculated for (M+H^+) of $C_{16}H_{13}NO_5S$: 332.0587; found: 332.0578.

5k. Methyl 3-amino-4-oxo-6,7-diphenyl-*4H*-thieno[3,2-*c*]pyran-2-carboxylate: yield: 93%; R_f = 0.28 (50% hexane in DCM); yellow solid; mp: 236-238 °C; IR(KBr): 3467, 3345, 1726, 1678 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.41-7.35 (m, 5H, ArH), 7.31-7.28 (m, 3H, ArH), 7.22 (t, *J*

= 7.3 Hz, 2H, ArH), 6.77 (bs, 2H, -NH_2), 3.81 (s, 3H, -OMe); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 164.3, 158.4, 157.1, 154.0, 152.7, 133.4, 131.5, 129.9, 129.7, 129.3, 129.3, 129.0, 128.1, 115.5, 112.2, 98.3, 51.4; HRMS (*m/z*): calculated for (M+H^+) of $C_{21}H_{15}NO_4S$: 378.0795 found: 378.0786.

5l. Methyl 3-amino-6,7-bis(4-methoxyphenyl)-4-oxo-*4H*-thieno[3,2-*c*]pyran-2-carboxylate: yield: 90%; R_f = 0.23 (50% hexane in DCM); yellow solid; mp: 252-254 °C; IR(KBr): 3465, 3343, 1724, 1677 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.33 (d, *J* = 8.8 Hz, 2H, ArH), 7.22 (d, *J* = 8.8 Hz, 2H, ArH), 6.94 (d, *J* = 8.8 Hz, 2H, ArH), 6.76-6.74 (m, 4H, ArH and -NH_2), 3.85 (s, 3H, -OMe), 3.81 (s, 3H, -OMe), 3.78 (s, 3H, -OMe); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 164.4, 160.7, 159.9, 158.6, 158.1, 153.9, 130.9, 130.8, 125.8, 124.0, 114.8, 113.9, 113.6, 111.6, 100.9, 55.3, 51.4; HRMS (*m/z*): calculated for (M+H^+) of $C_{23}H_{19}NO_6S$: 438.1006; found: 438.1003.

5m. Methyl 3-amino-7-methyl-4-oxo-6-phenyl-*4H*-thieno[3,2-*c*]pyran-2-carboxylate: yield: 91%; R_f = 0.31 (50% hexane in DCM); yellow solid; mp: 213-215 °C, IR (KBr): 3488, 3368, 1718, 1670, 1578 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.60 (t, *J* = 4.0 Hz, 2H, ArH), 7.48 (s, 3H, ArH), 6.77 (bs, 2H, -NH_2), 3.87 (s, 3H, -OMe), 2.27 (s, 3H, -Me); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 164.3, 158.7, 156.9, 154.6, 152.9, 131.8, 130.0, 129.1, 128.4, 122.1, 112.1, 108.9, 51.5, 14.4; HRMS (*m/z*): calculated for (M+H^+) of $C_{16}H_{13}NO_4S$: 316.0638; found: 316.0638.

5n. Methyl 1-amino-11-oxo-5,11-dihydro-4H-benzo[h]thieno[3,2-c]chromene-2-carboxylate: yield: 95%; R_f = 0.32 (50% hexane in DCM); yellow solid; mp: 232–234 °C; IR(KBr): 3463, 3342, 1708, 1589, 1294 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (q, J = 2.9 Hz, 1H, Ar-H), 7.35-7.31 (m, 2H, Ar-H), 7.23 (t, J = 4.4 Hz, 1H, Ar-H), 6.79 (s, 2H, -NH_2), 3.86 (s, 3H, -OMe), 3.02 (t, *J* = 7.7 Hz, 2H, -CH_2), 2.78 (dd, J = 8.4, 7.0 Hz, 2H, -CH_2); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 164.3, 158.5, 154.5, 153.0, 151.5, 136.9, 130.3, 128.0, 127.5, 127.3, 123.8, 123.6, 112.0, 109.2, 51.5, 26.9, 22.5; HRMS (m/z): calculated for (M+H^+) of $C_{17}H_{13}NO_4S$: 328.0638; found: 328.0638.

5o. Methyl 1-amino-7-methoxy-11-oxo-5,11-dihydro-4H-benzo[h]thieno[3,2-c]chromene-2-carboxylate: yield: 84%; R_f = 0.26 (50% hexane in DCM); yellow solid; mp: 240–242 °C; IR(KBr): 3469, 3336, 1721, 1578, 1299 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, J = 8.7 Hz, 1H, Ar-H), 6.83 (dd, J = 8.7, 2.3 Hz, 2H, Ar-H), 6.77 (s, 2H, -NH_2), 3.86 (s, 3H, -OMe), 3.85 (s, 3H, -OMe), 2.99 (t, *J* = 7.8 Hz, 2H, -CH_2), 2.76 (t, *J* = 8.0 Hz, 2H, -CH_2); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 164.4, 161.4, 158.6, 154.9, 152.0, 139.2, 125.5, 120.4, 114.2, 113.1, 112.2, 111.2, 107.2,

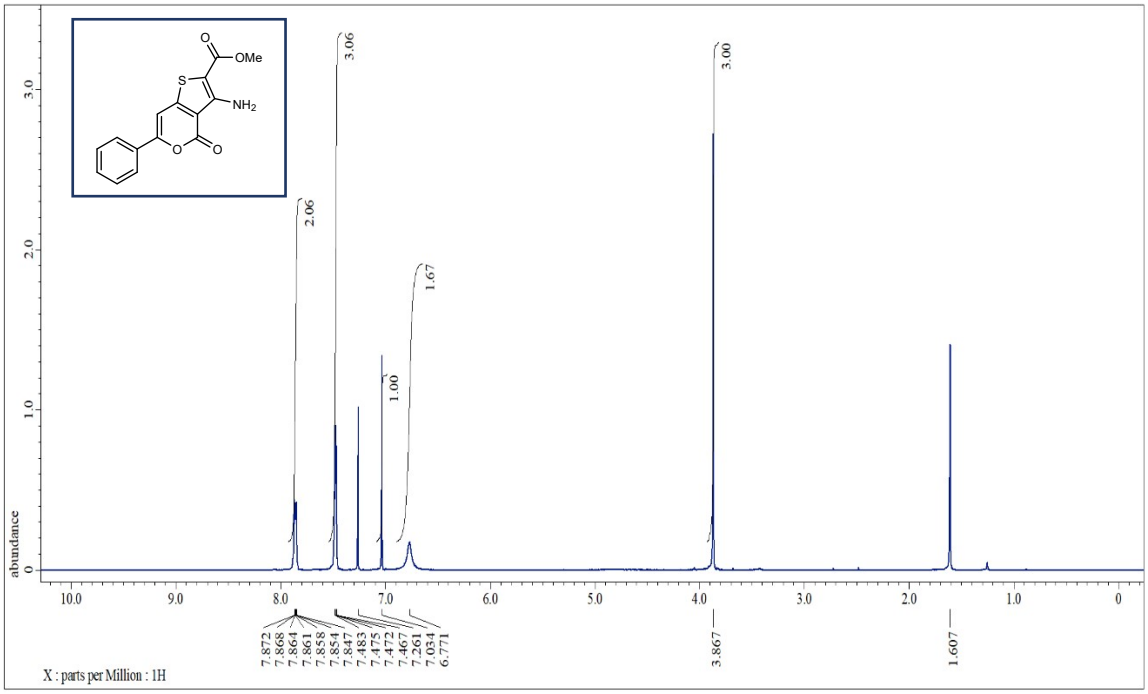
55.4, 51.4, 27.4, 22.6; HRMS (m/z): calculated for ($M+H^+$) of $C_{18}H_{14}O_6S$: 359.0584; found: 359.0582.

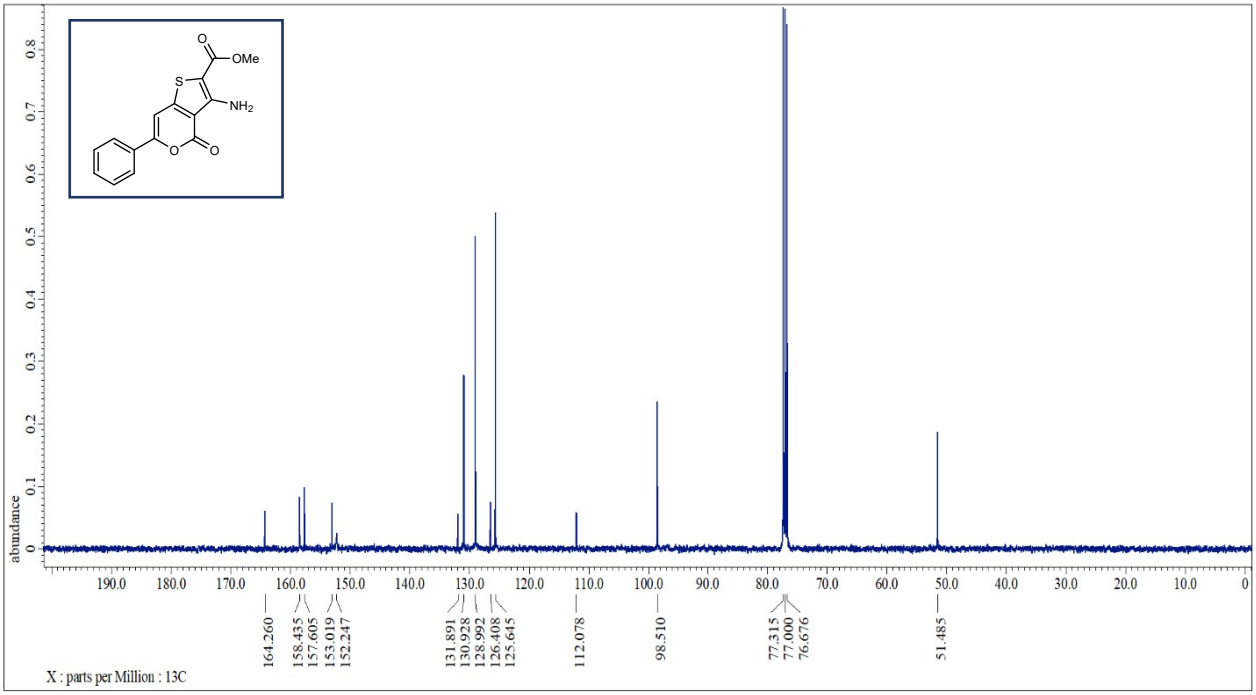
3. References

1. Yadav, P., Shaw, R., Panwar, R., Sahu, S.N., Kumar, A. and Pratap, R., A Base-Mediated 6-exo-trig versus 6-exo-dig Carbocyclization Strategy for the Synthesis of Functionalized Biaryl Compounds. *Asian Journal of Organic Chemistry*, 2017, 6(10), 1394-1397.
2. Shaw, R. and Pratap, R., A Green and Base-Free Arylation of Thiomethylated 2-pyranones and Ketene Dithioacetals via Liebeskind-Srogl Coupling in Water. *Asian Journal of Organic Chemistry*, 2022, 11(6), e202200078.

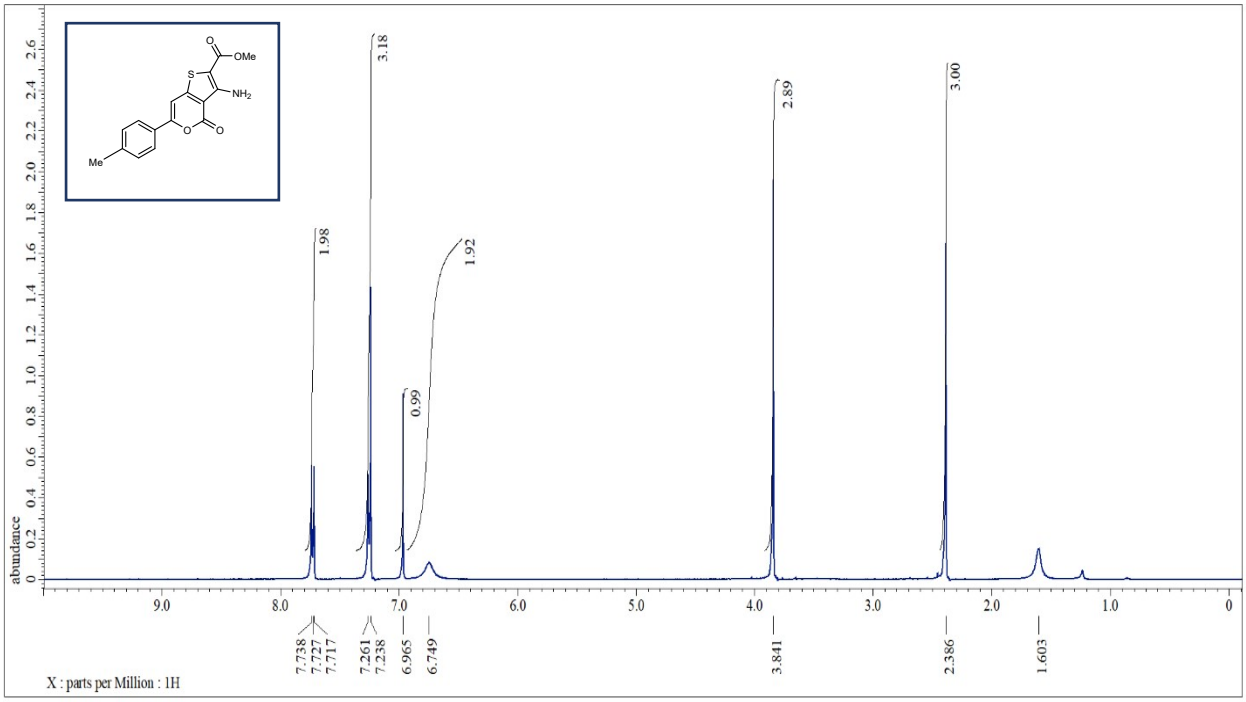
^{1}H and ^{13}C NMR Spectra

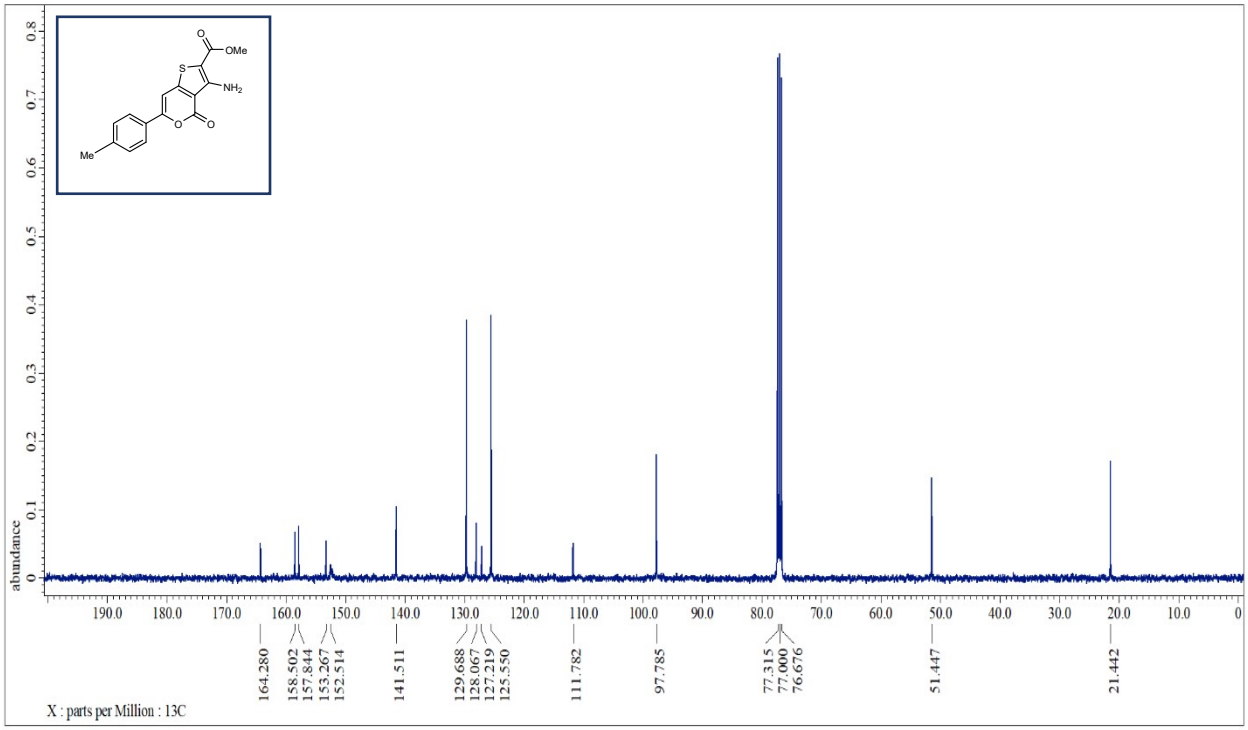
^{1}H and ^{13}C NMR spectra of 5a





^{1}H and ^{13}C NMR spectra of 5b





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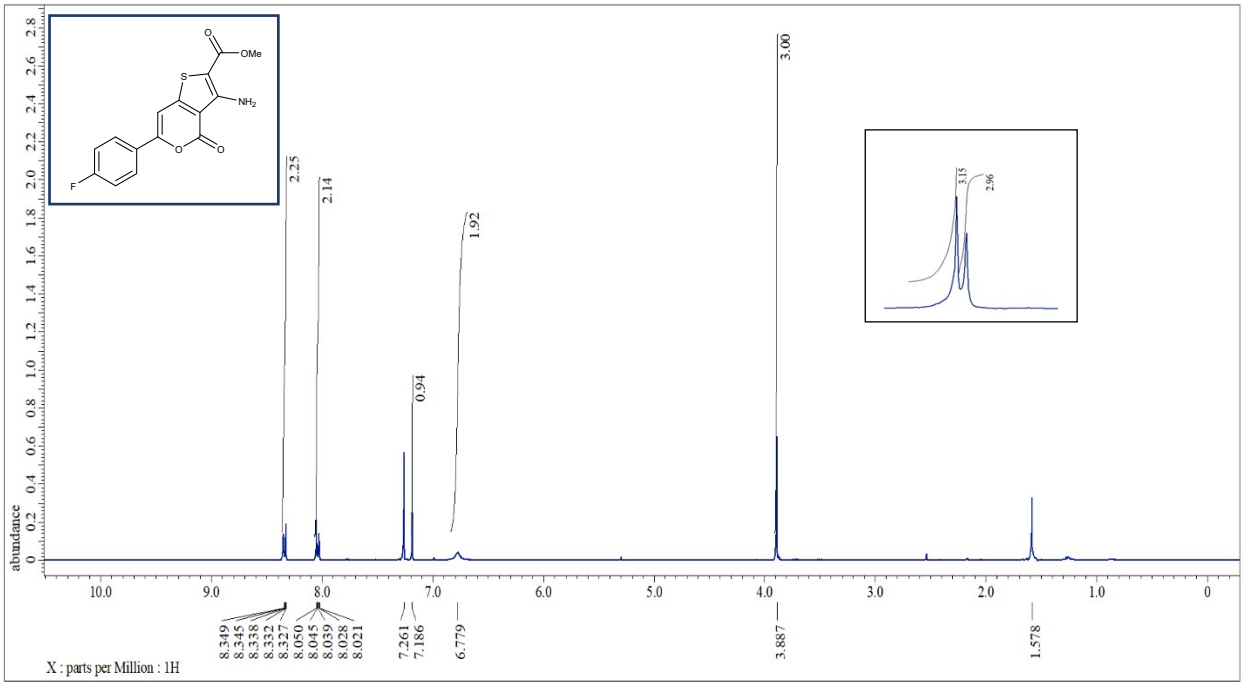
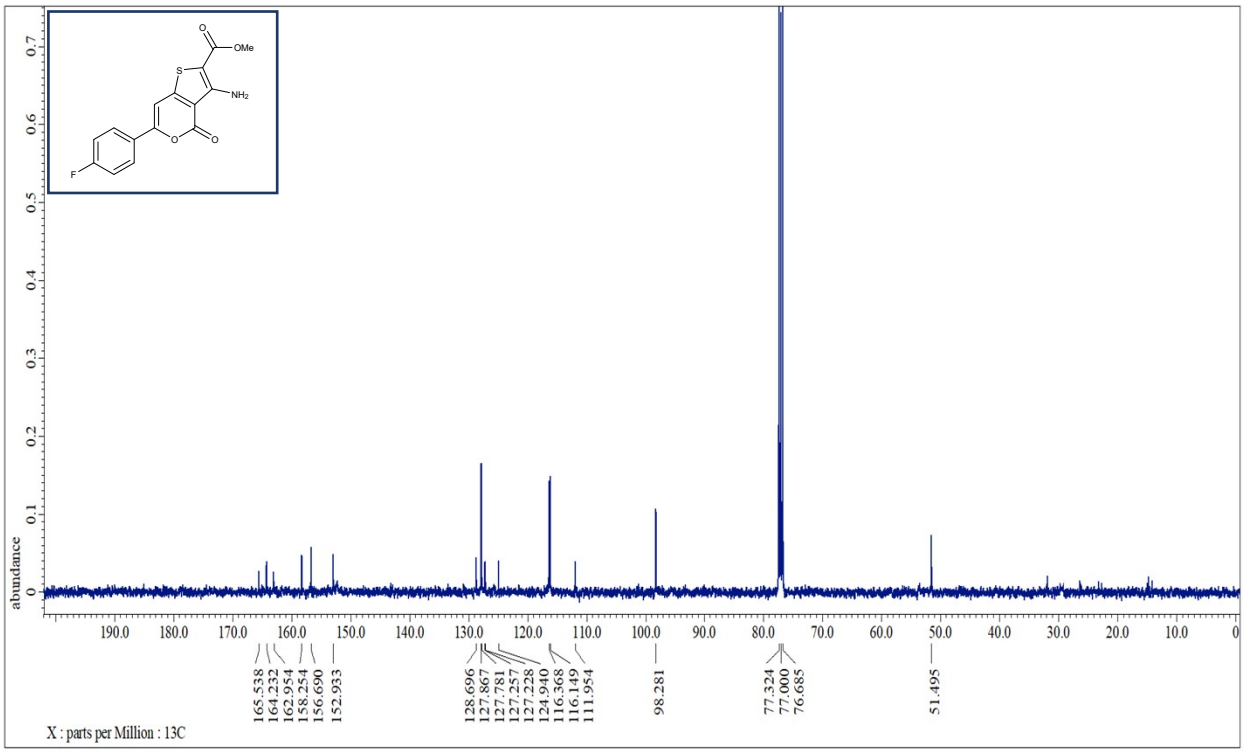
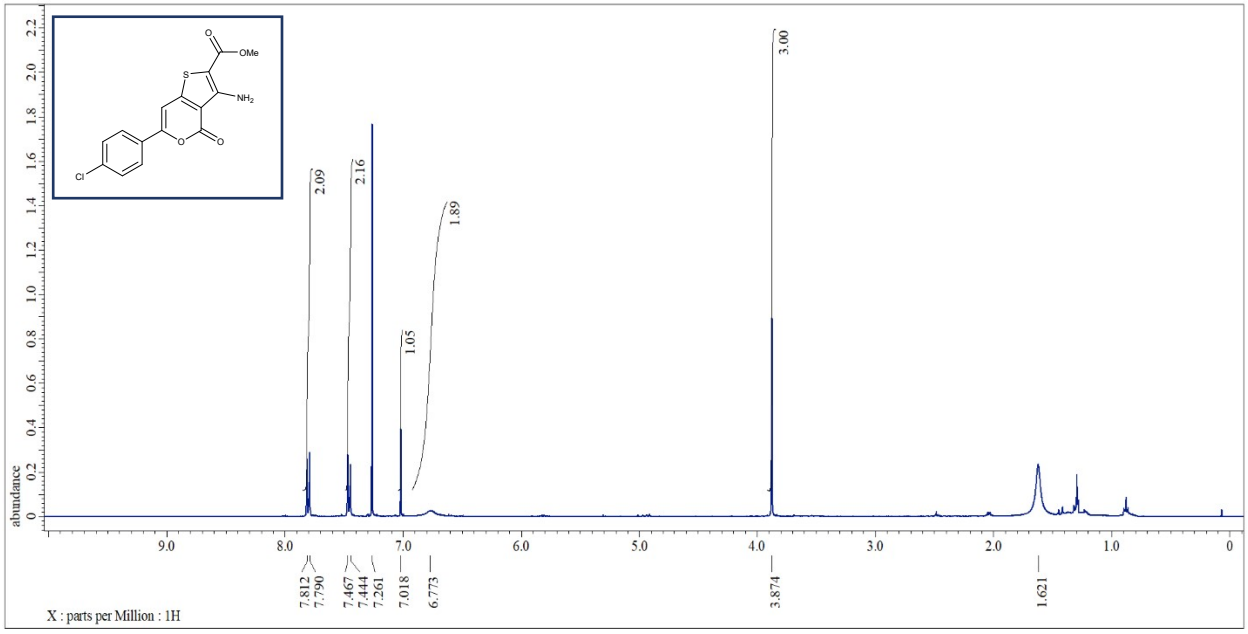
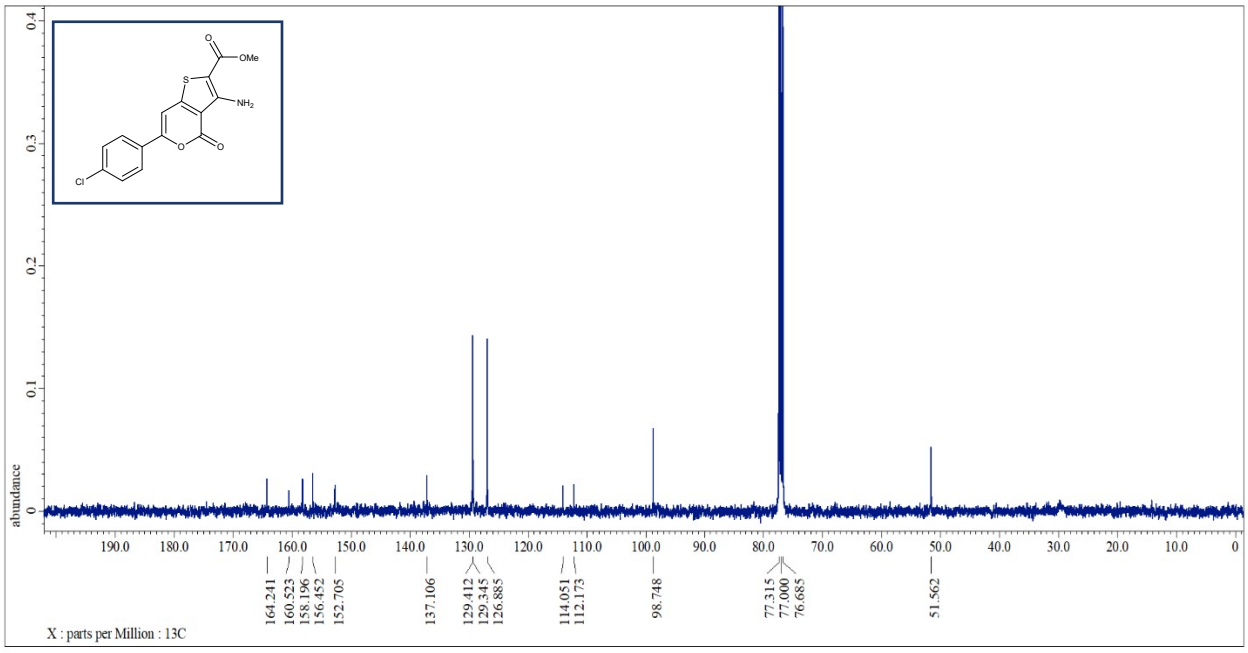




^{1}H and ^{13}C NMR spectra of 5d





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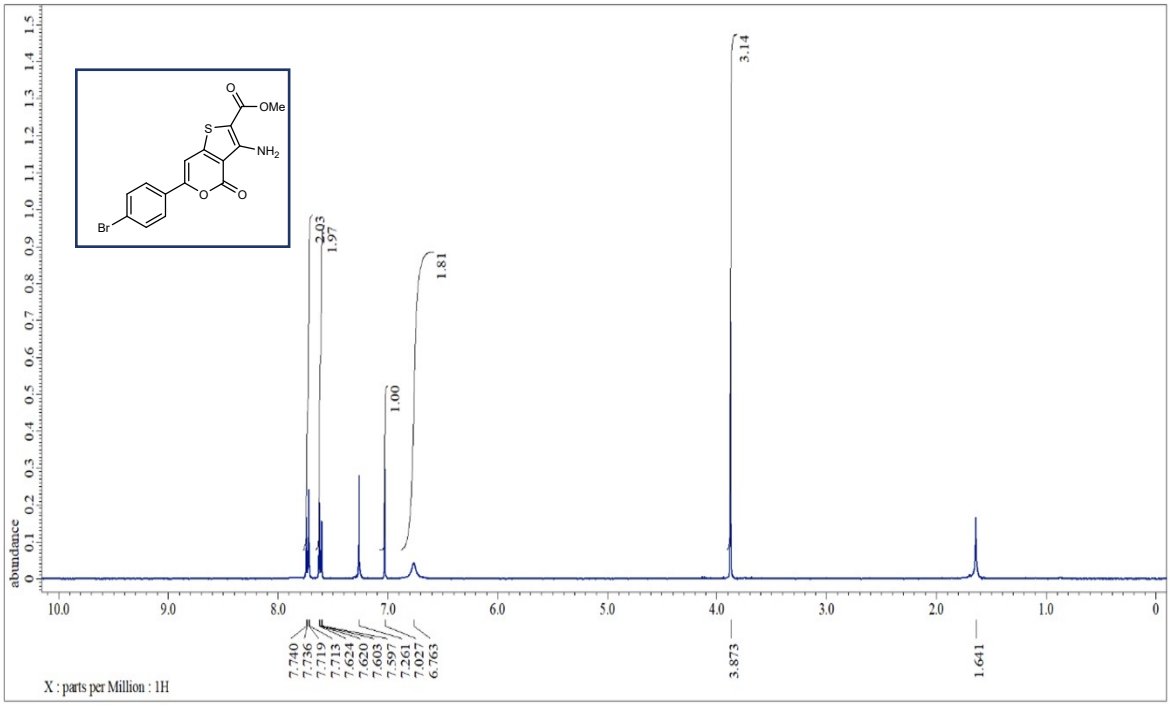

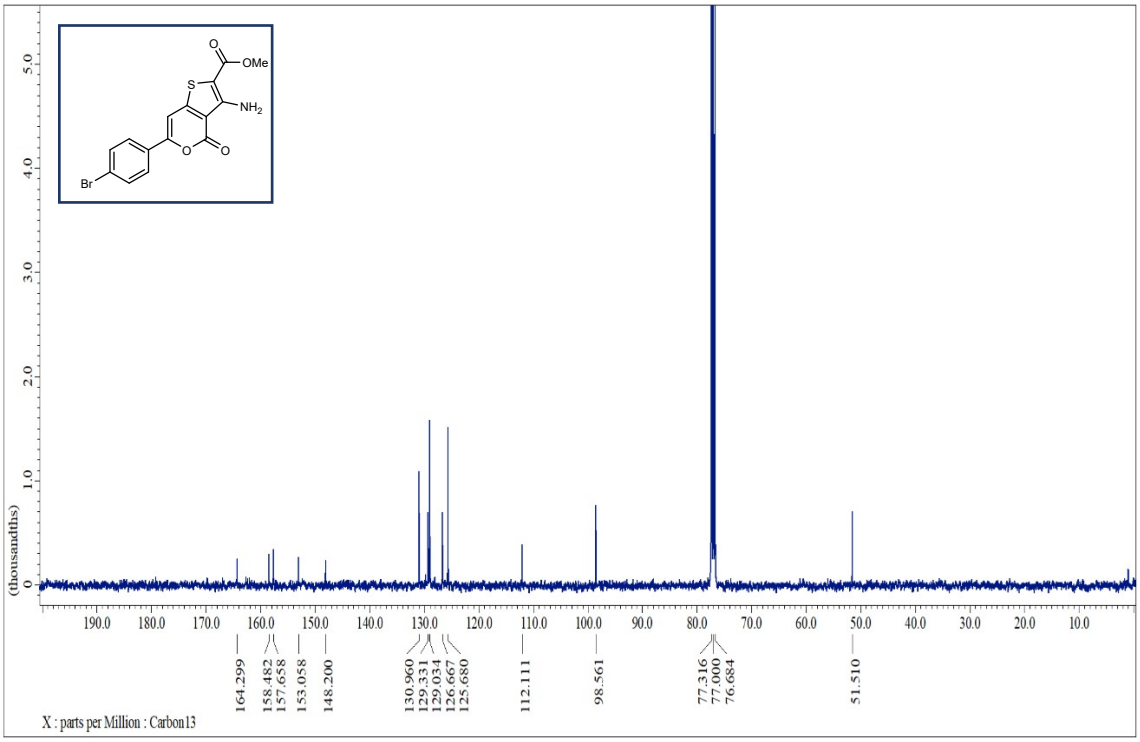

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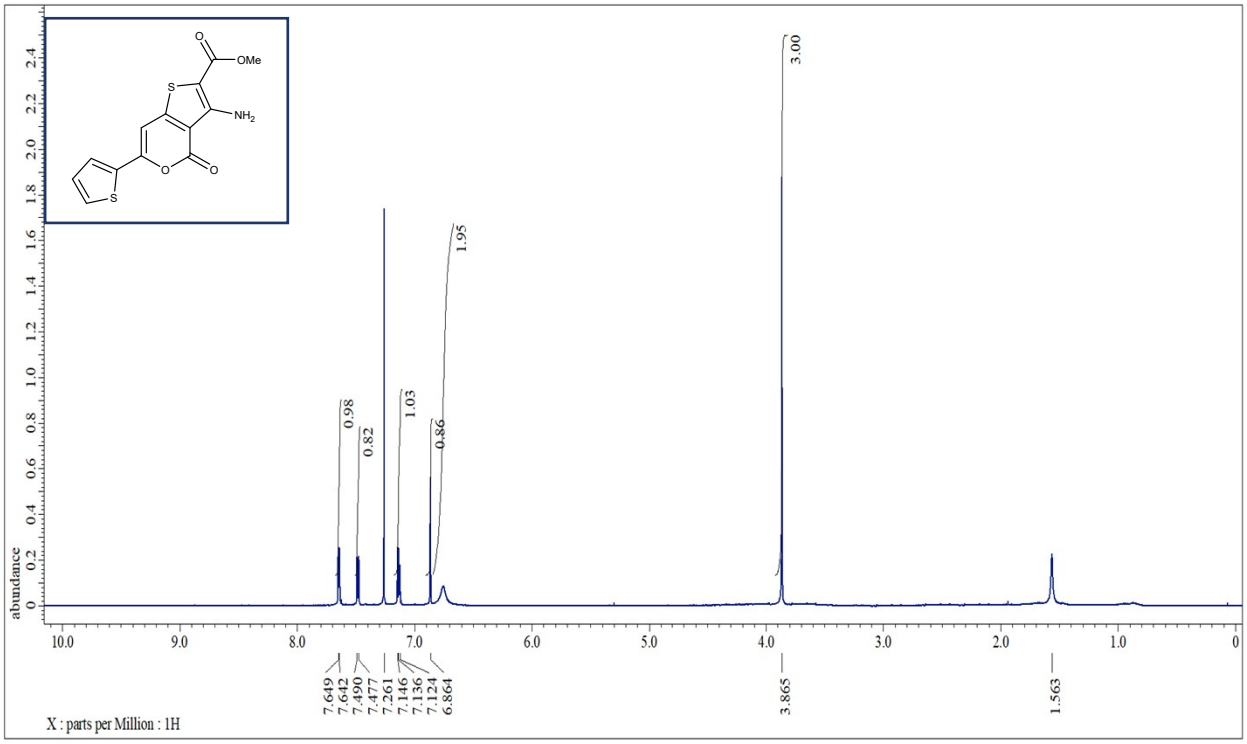

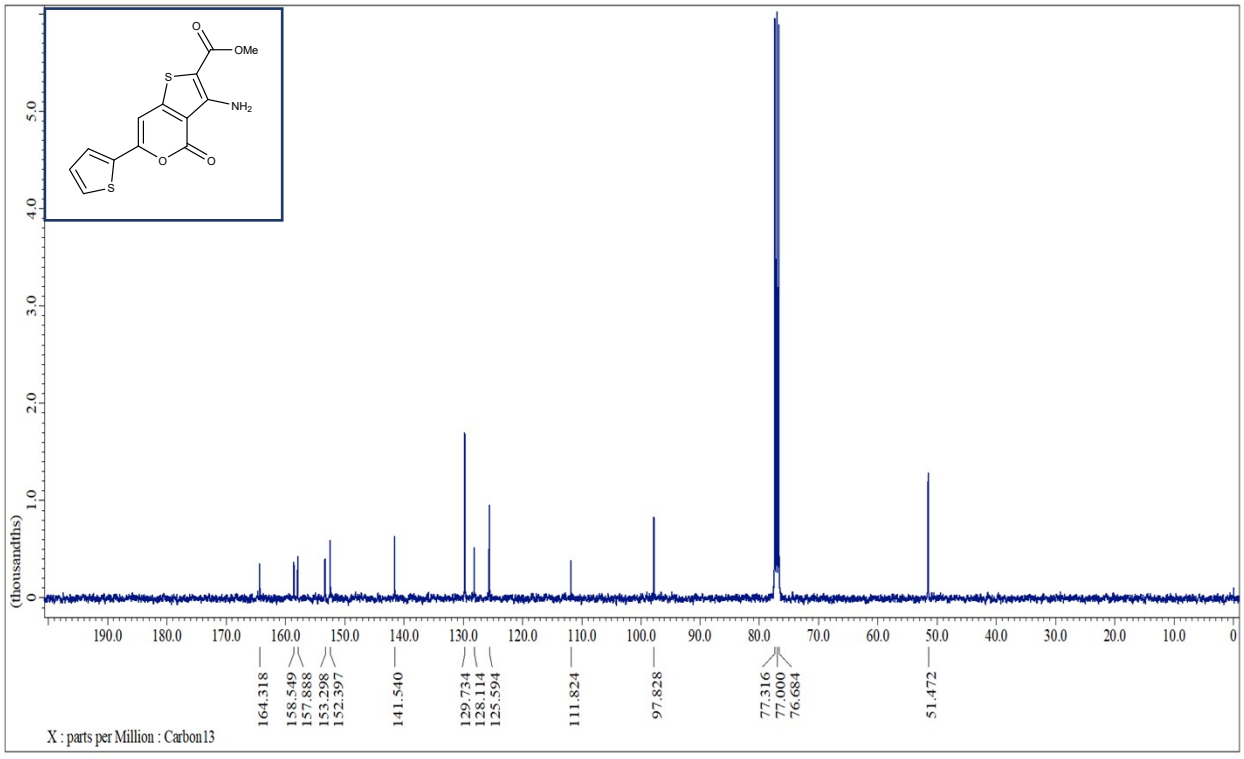

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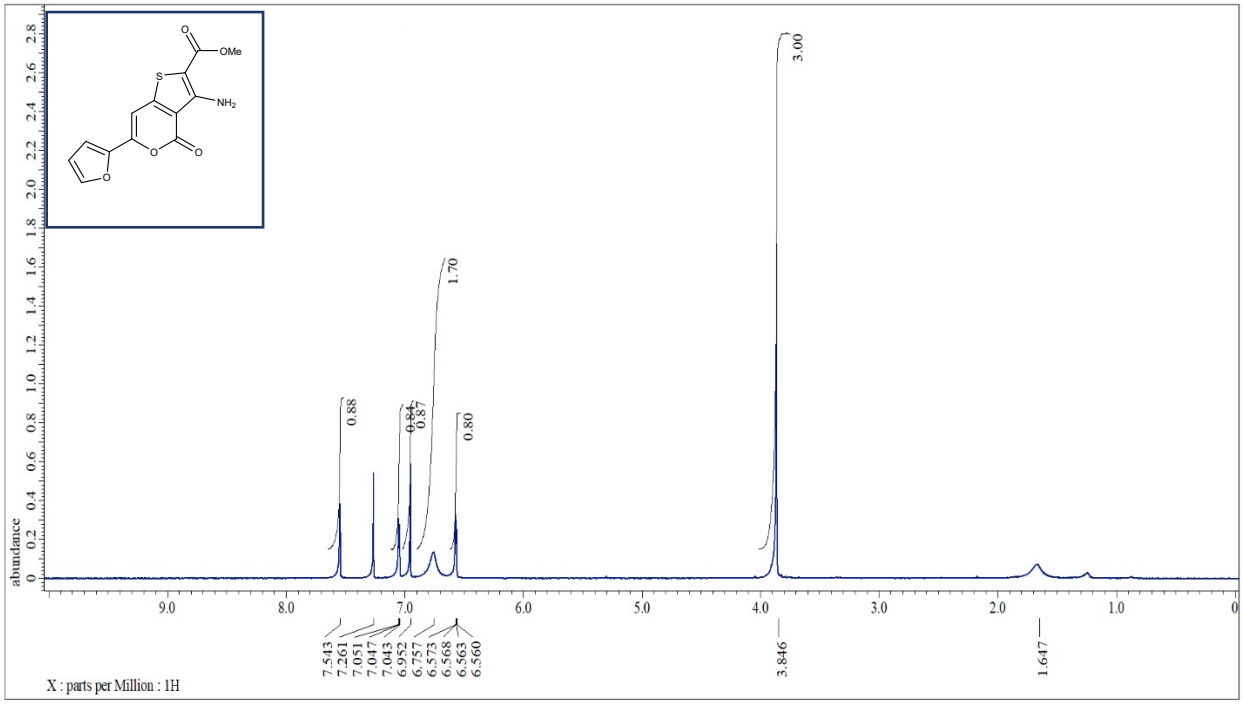

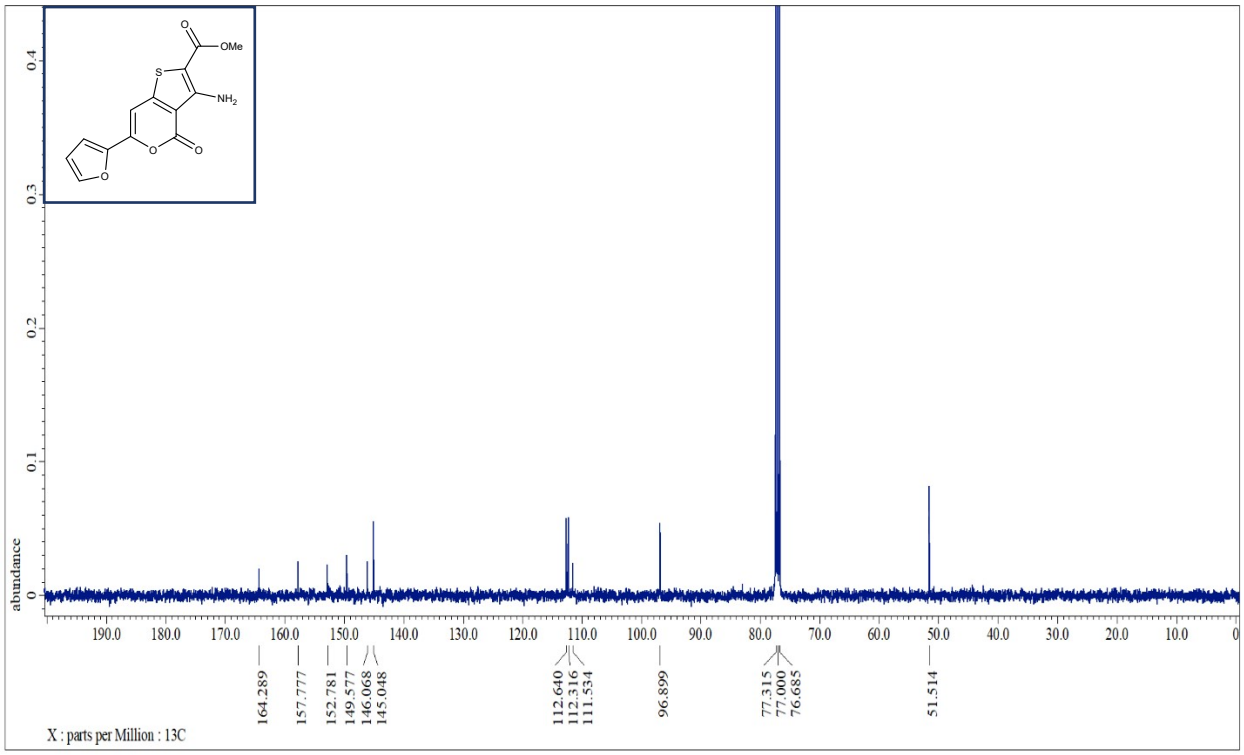

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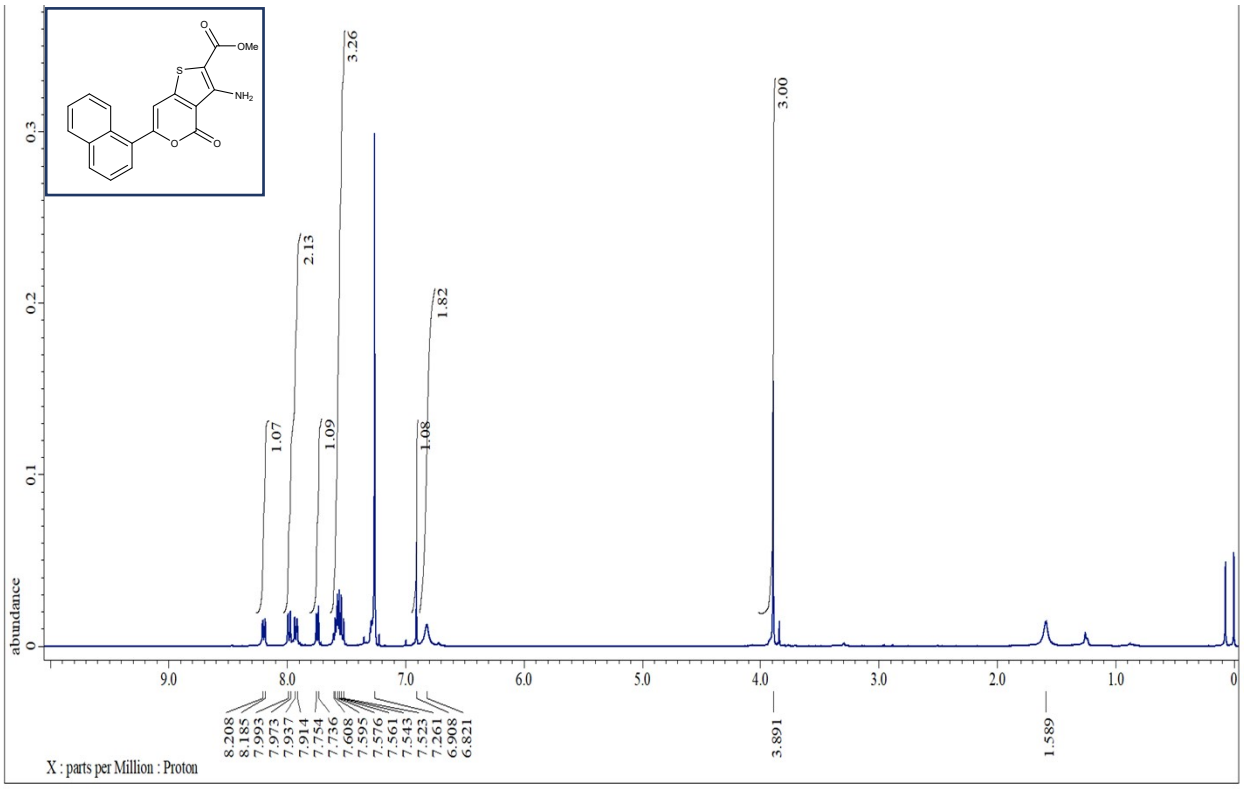
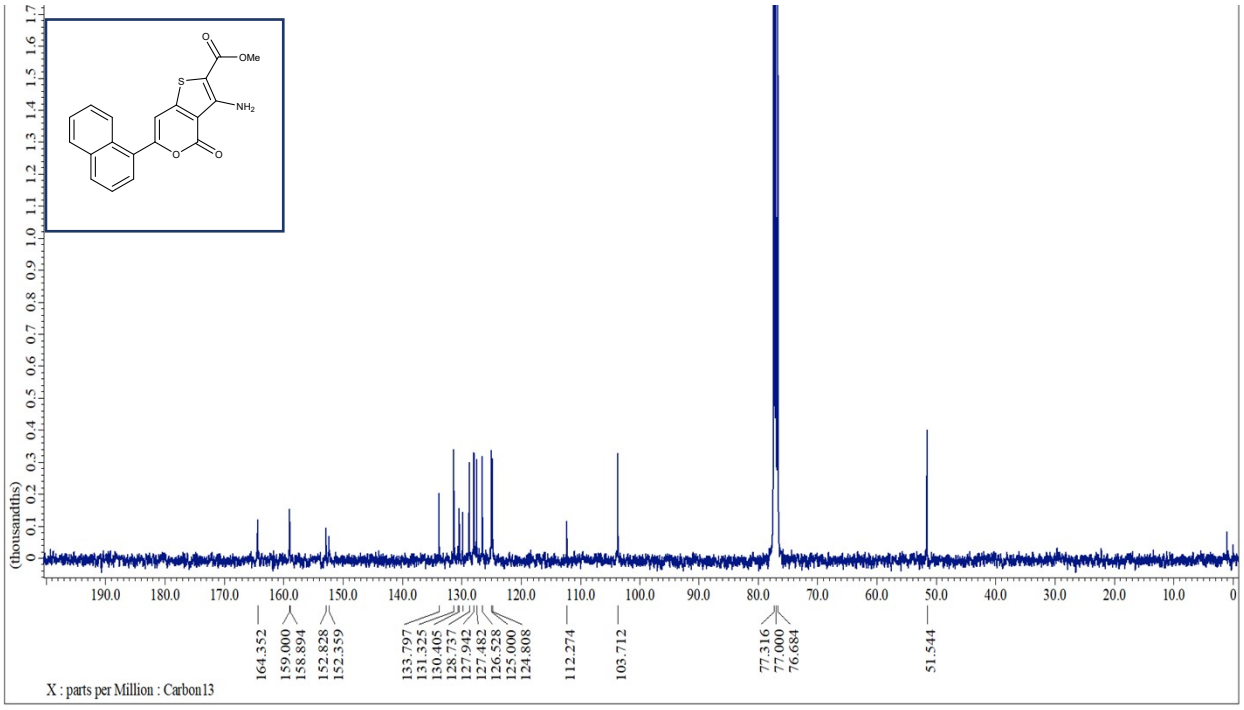
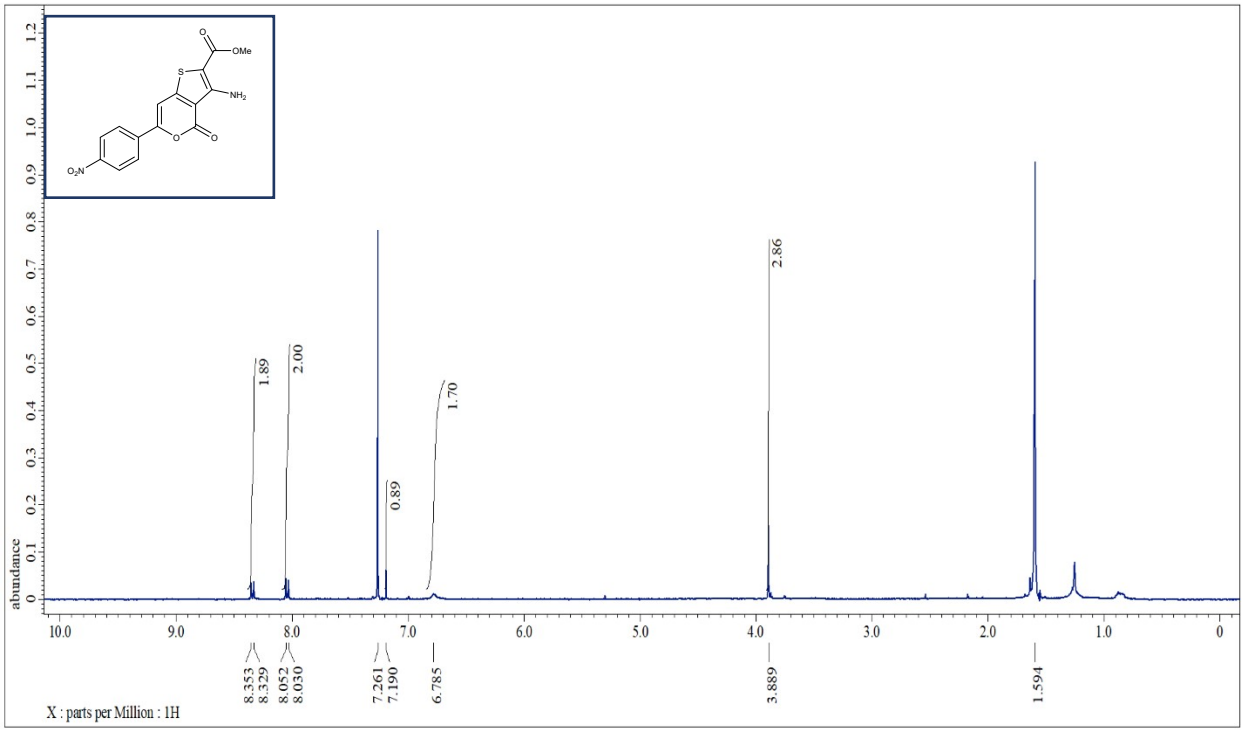
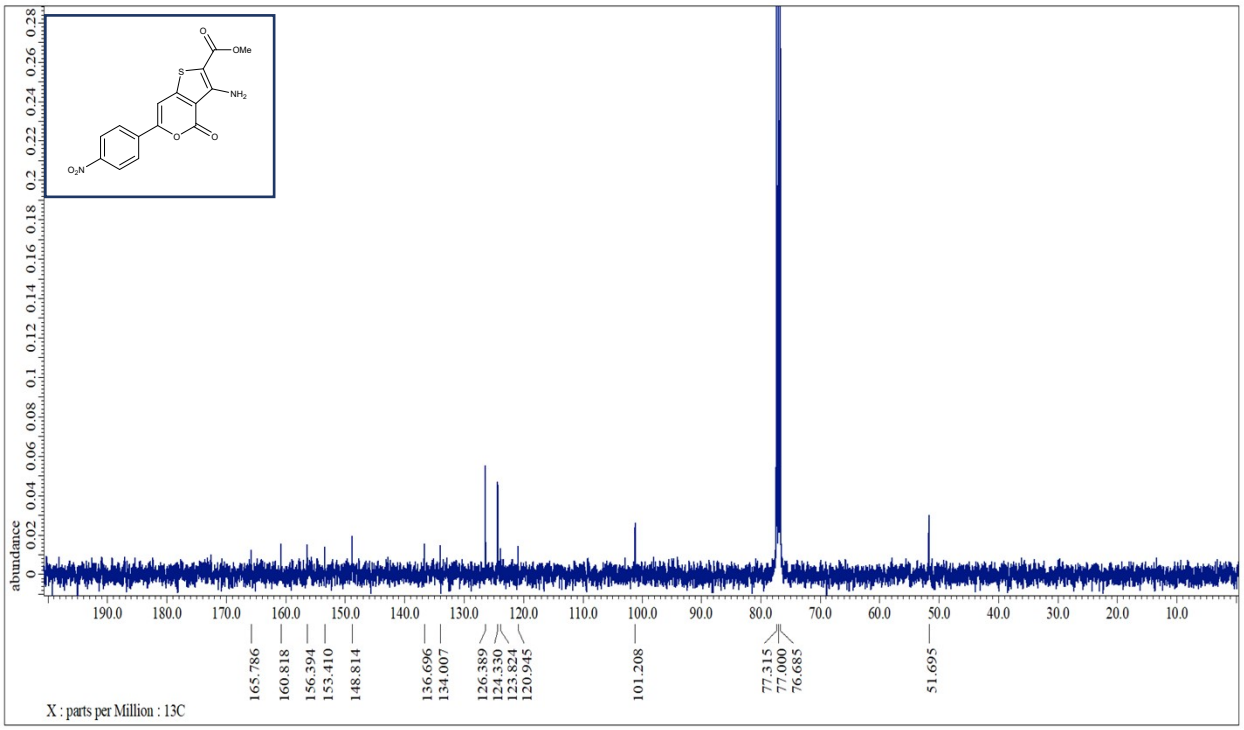


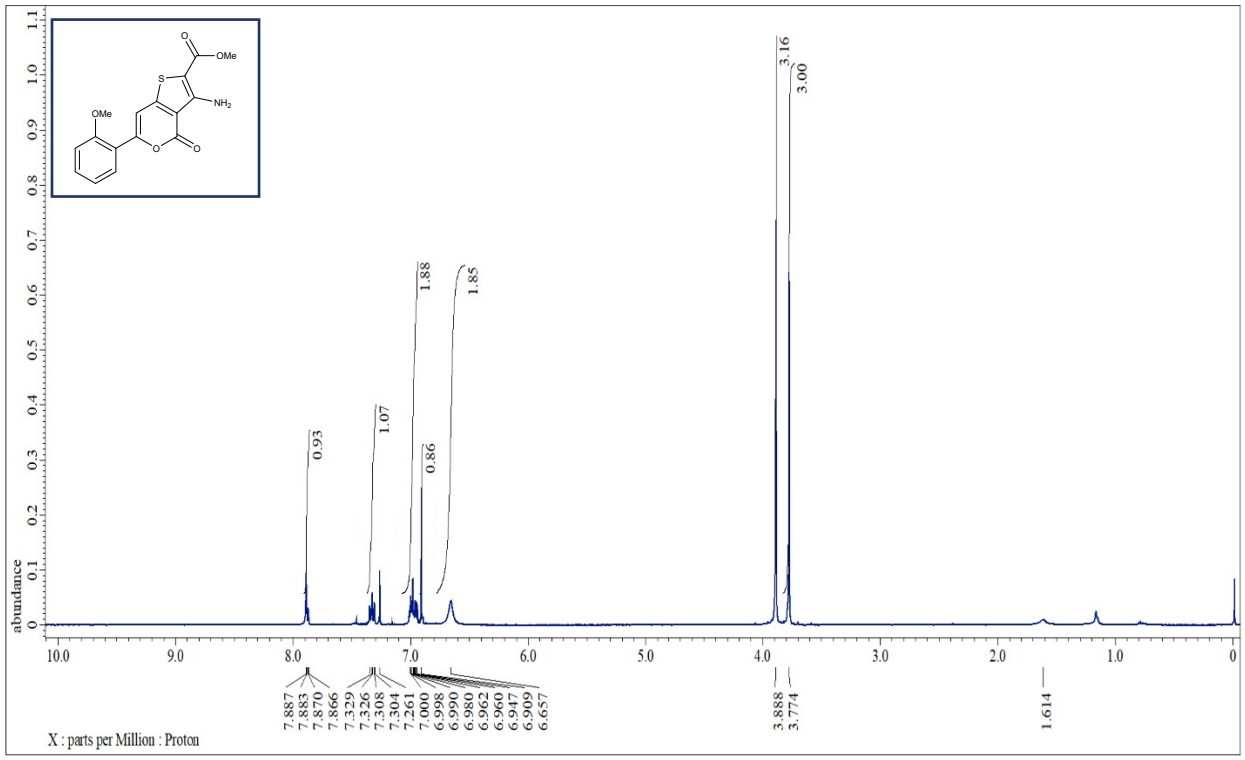


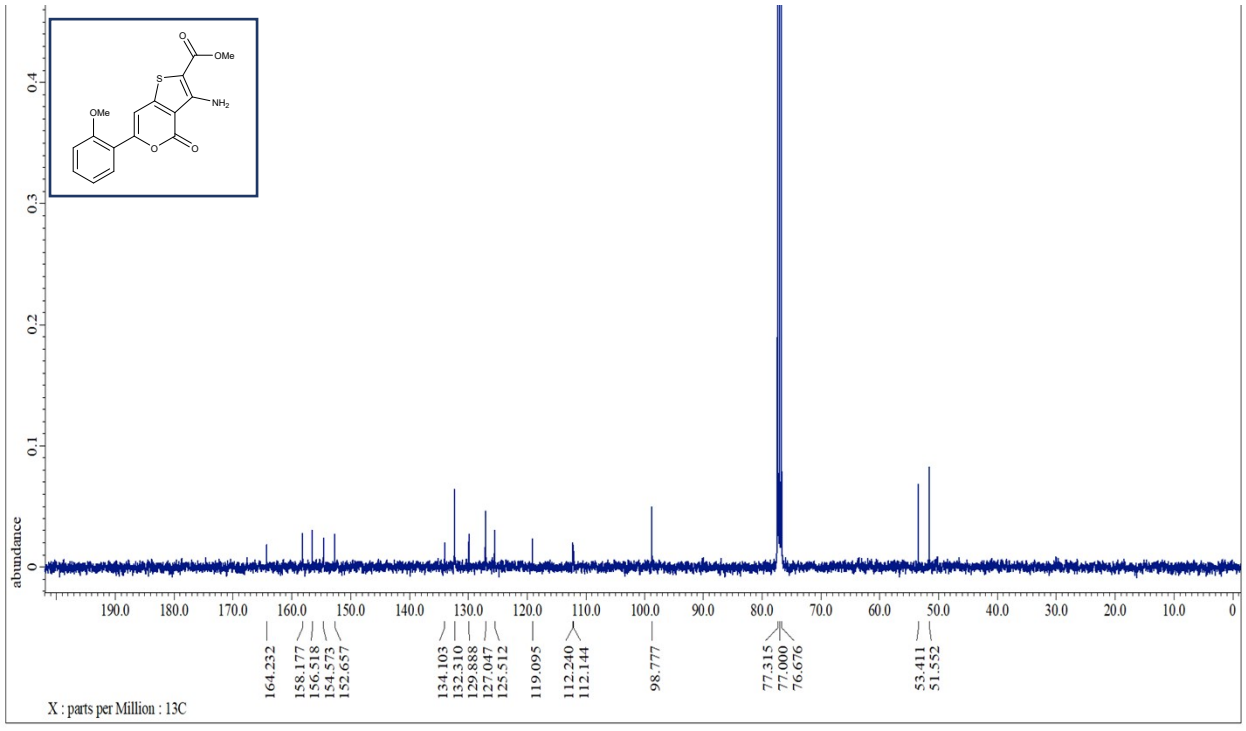
^{1}H and ^{13}C NMR spectra of 5i



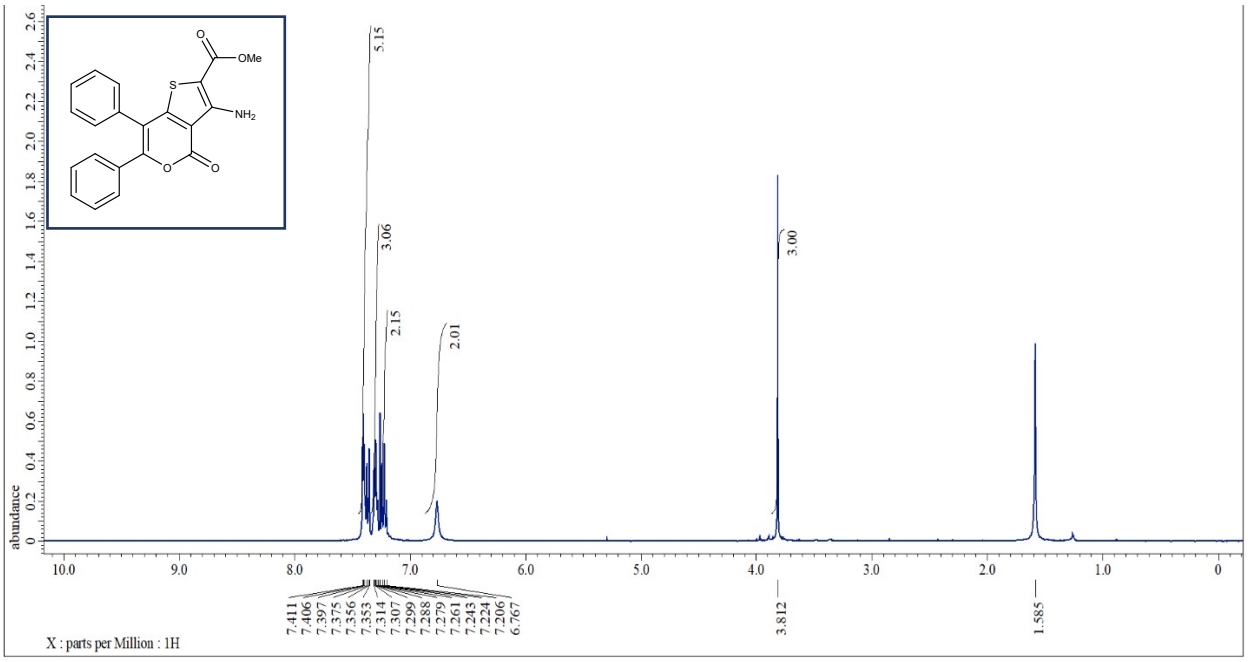


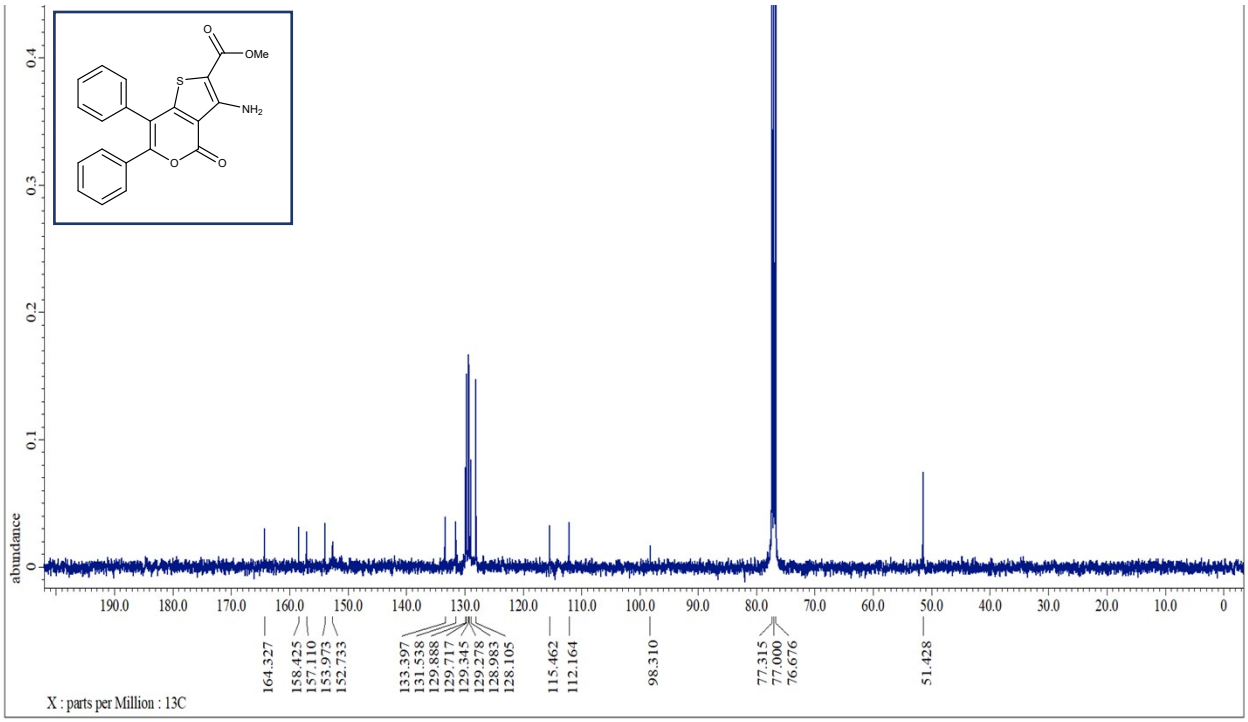
^{1}H and ^{13}C NMR spectra of 5j



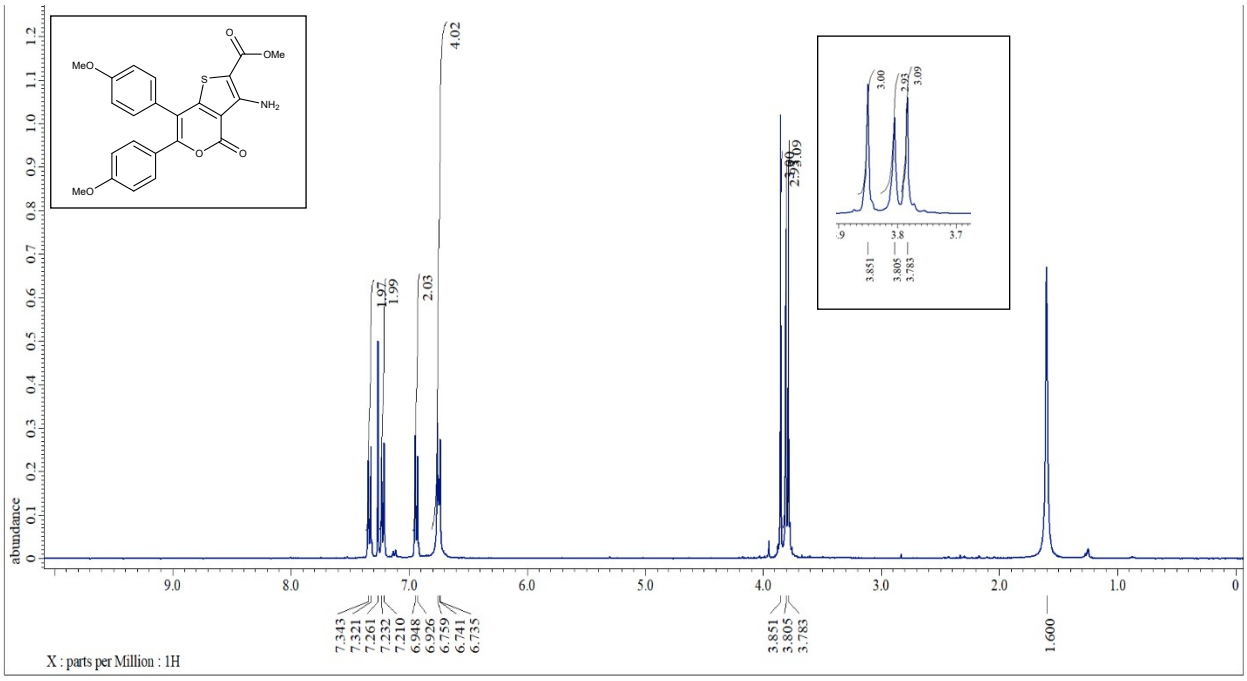


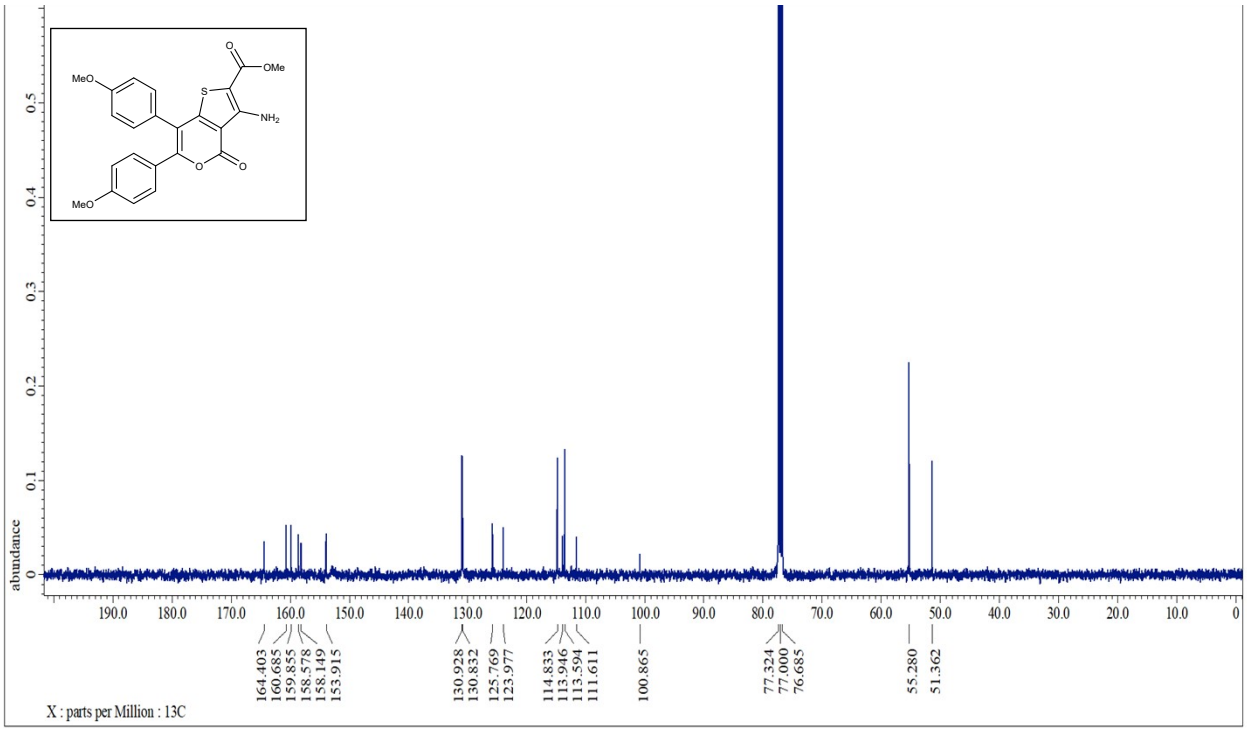
^{1}H and ^{13}C NMR spectra of 5k



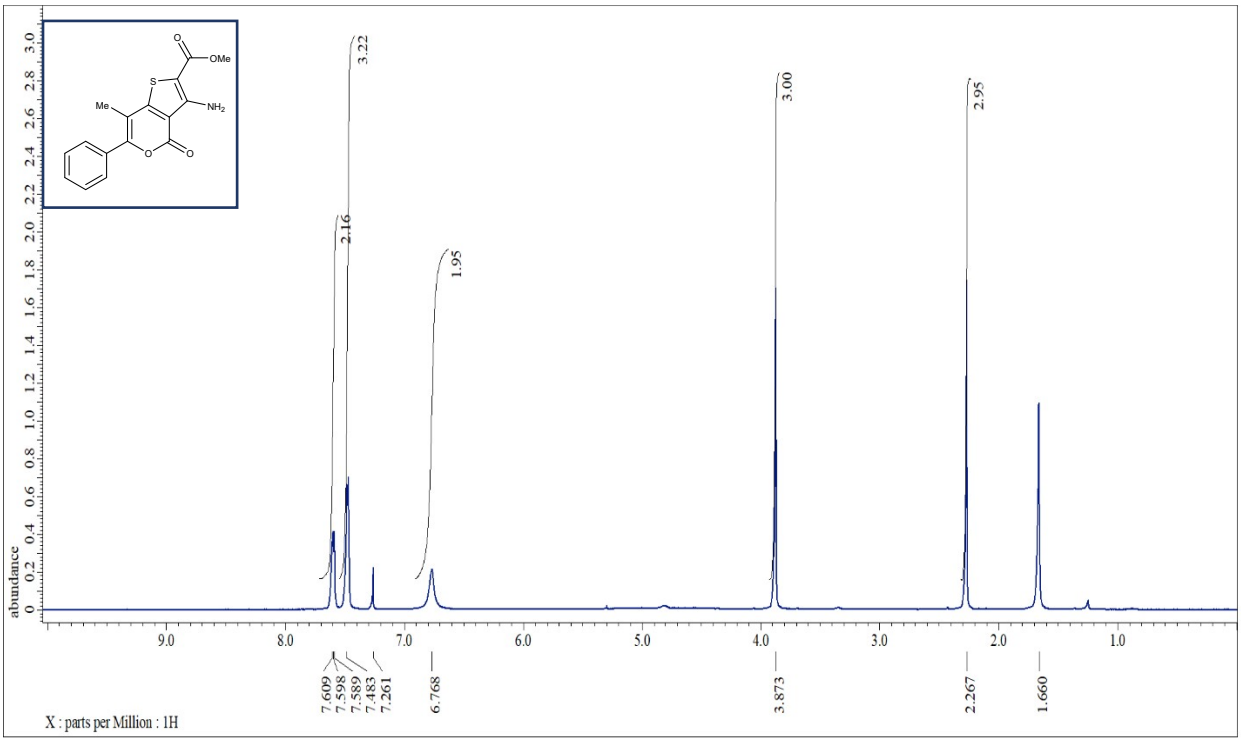


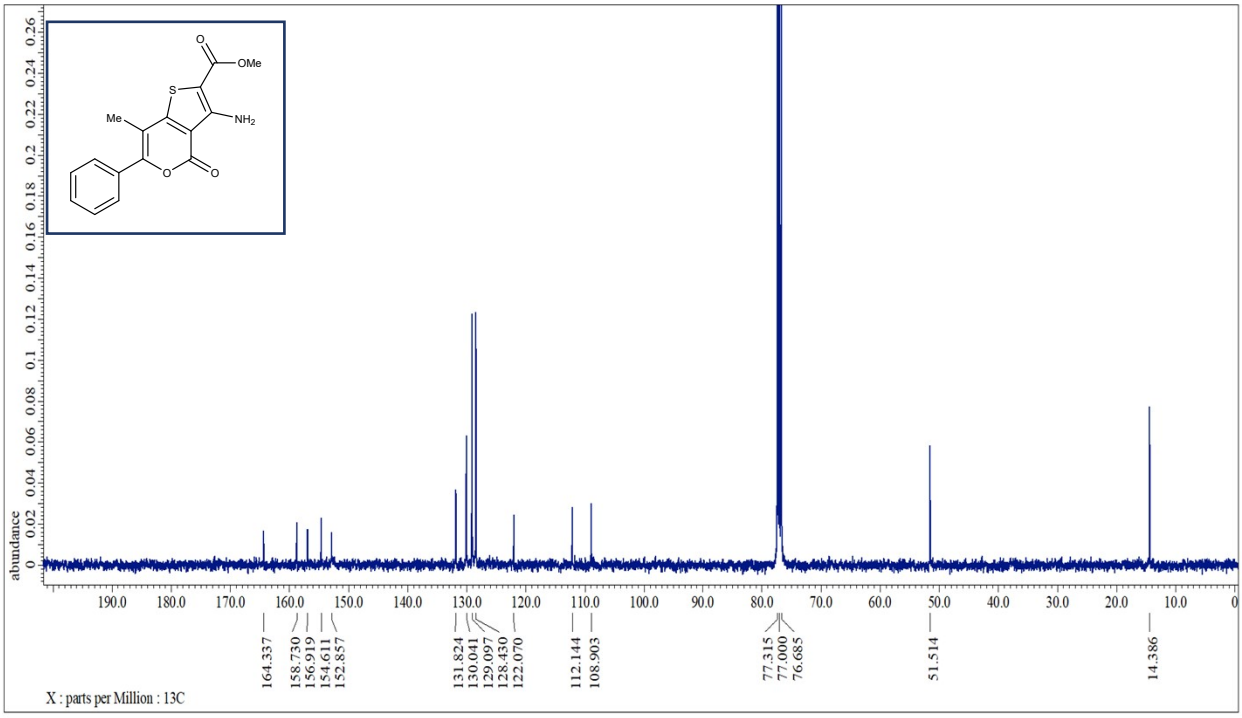
^{1}H and ^{13}C NMR spectra of 5l



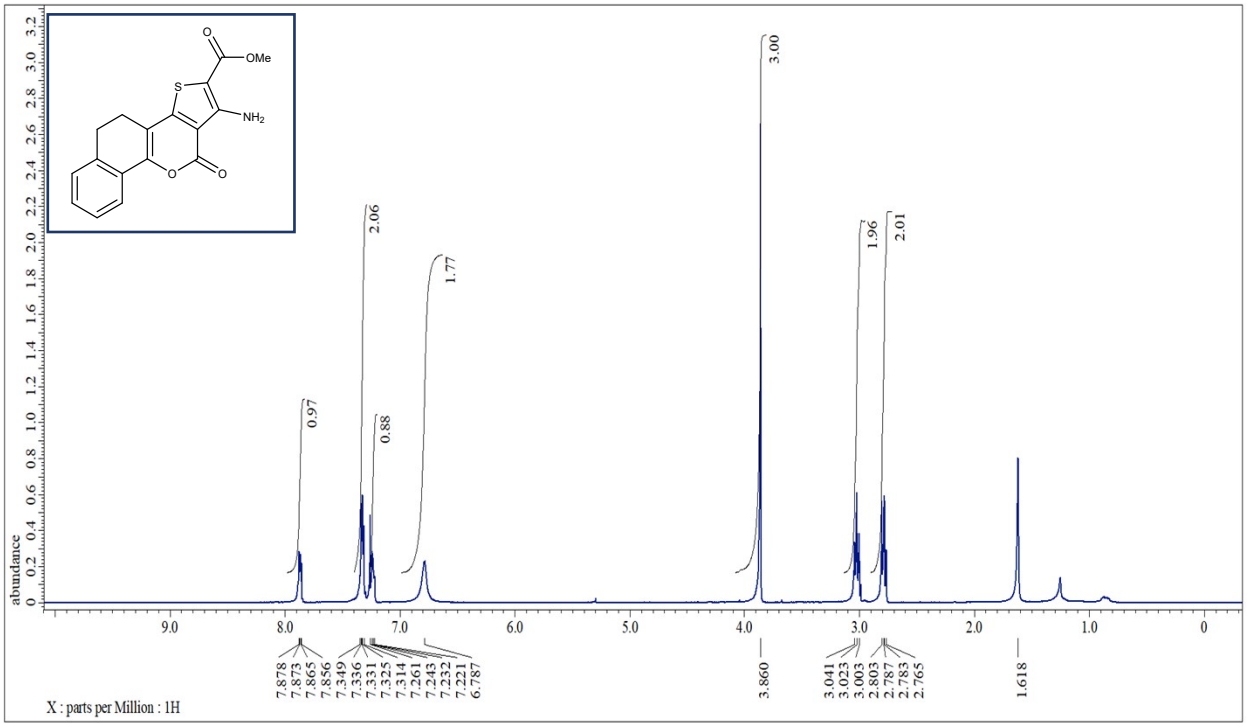


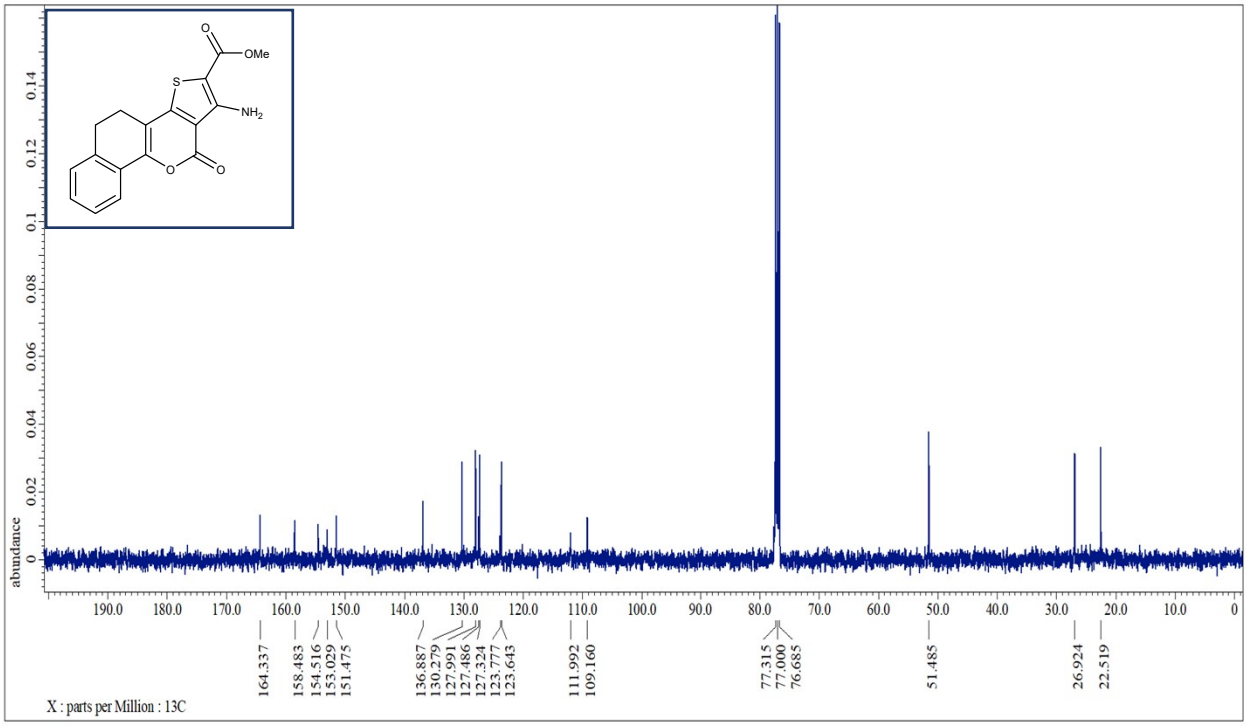
^{1}H and ^{13}C NMR spectra of 5m



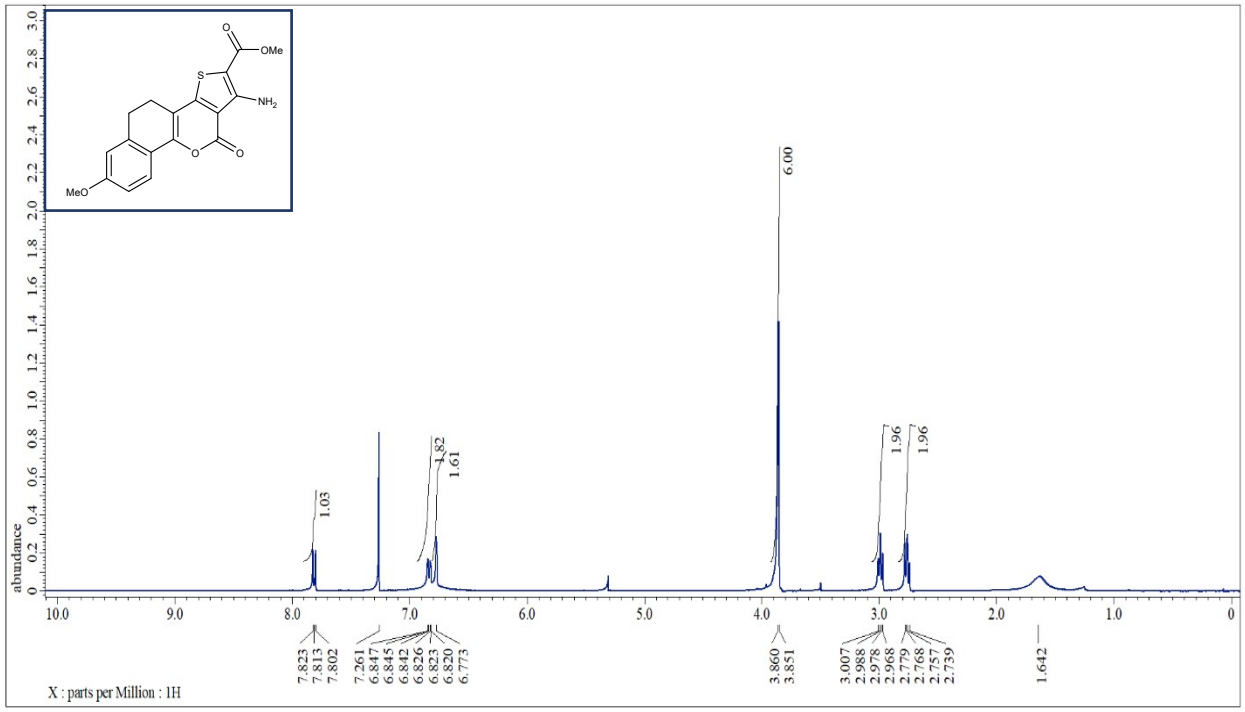


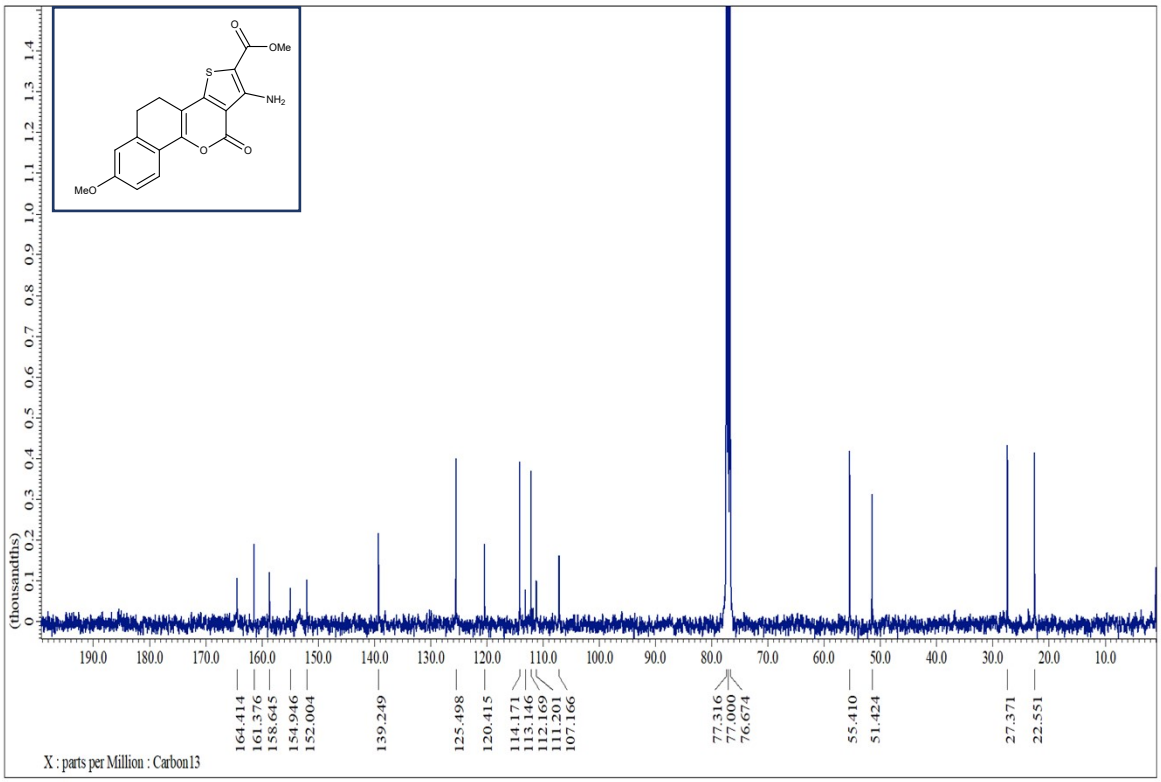
^{1}H and ^{13}C NMR spectra of 5n





^{1}H and ^{13}C NMR spectra of 5o





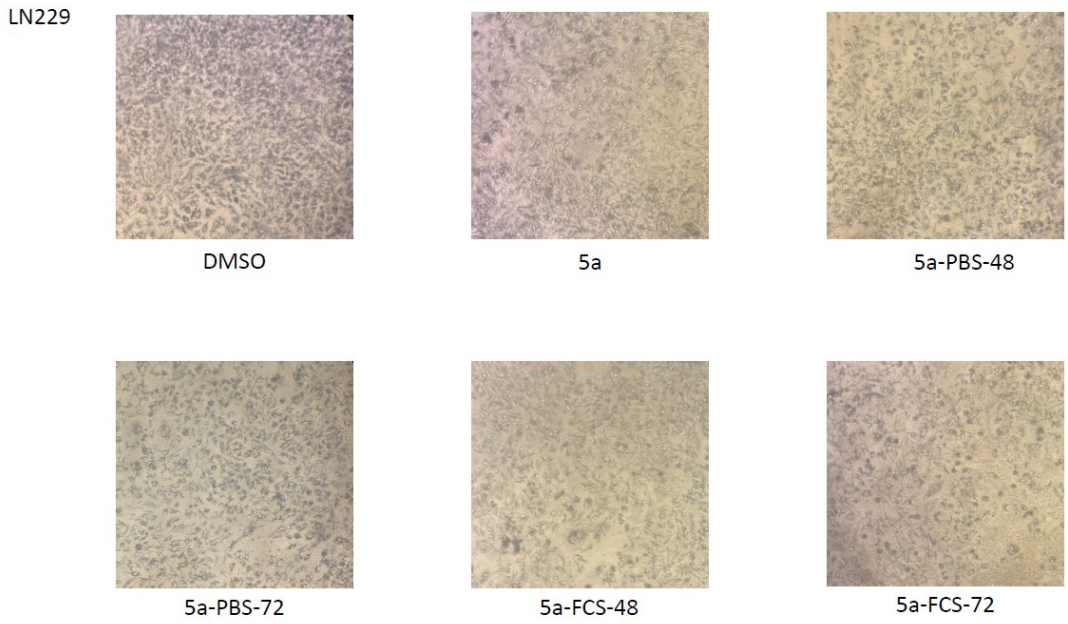


Figure S1: LN229 Cell images taken after incubation with MTT, cell number is same but the intensity of crystal formation is lower in cells treated with 5a**.**

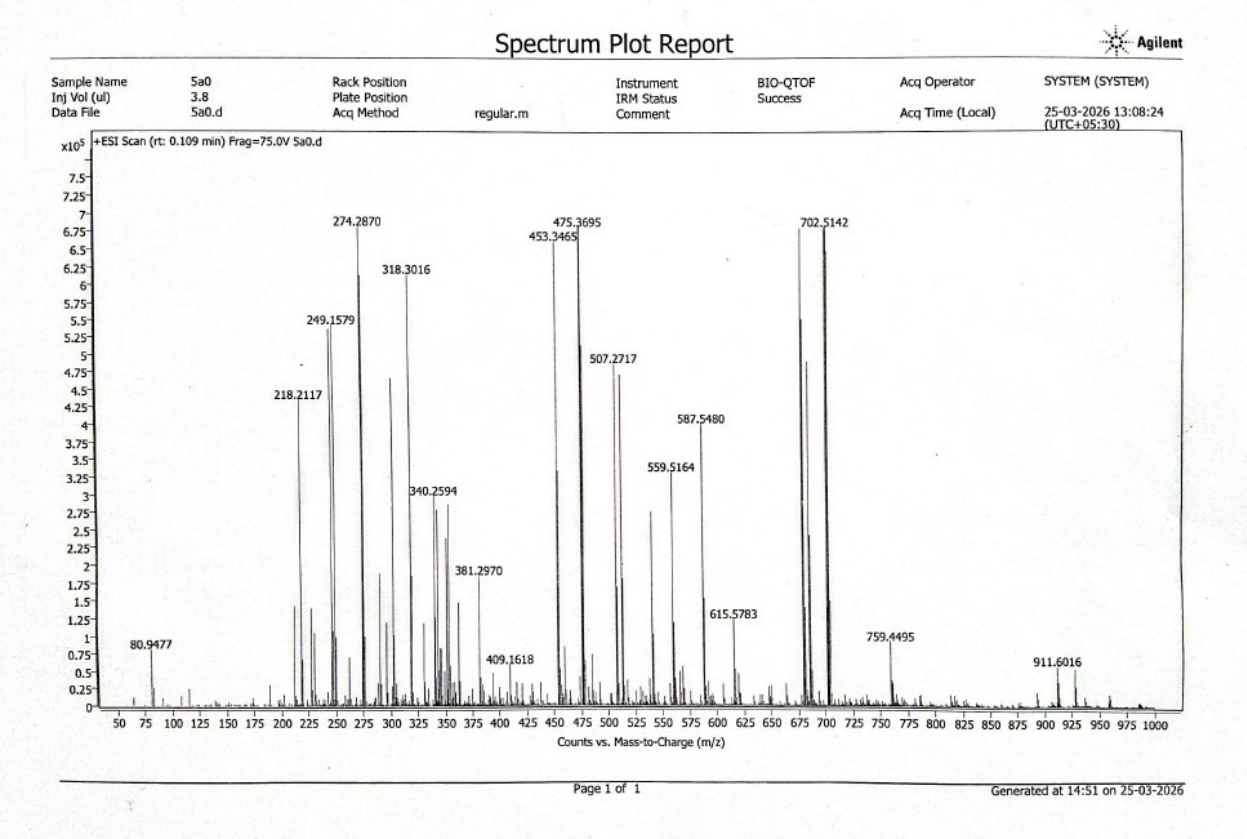
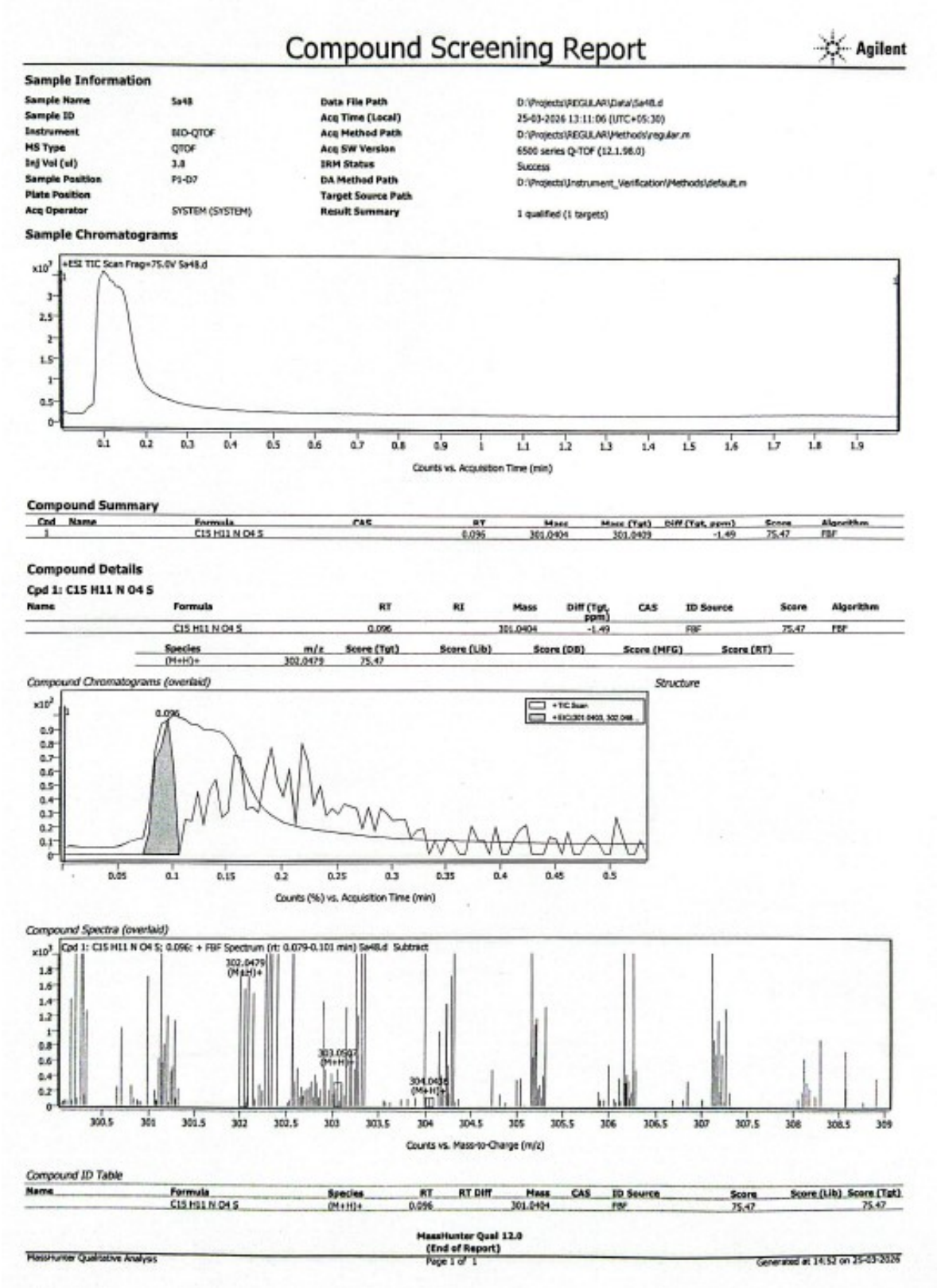


Figure S2: HRMS data of PBS without compound



Compound Screening Report

Agilent

Sample Information

Sample Name	Sa48	**Data File Path**	D:\Projects\REGULAR\Data\Sa48.d
Sample ID		**Acq Time (Local)**	25-03-2026 13:11:06 (UTC+05:30)
Instrument	BIO-QTOF	**Acq Method Path**	D:\Projects\REGULAR\Methods\regular.m
MS Type	QTOF	**Acq SW Version**	6500 series Q-TOF (12.1.98.0)
Inj Vol (ul)	3.0	**IRM Status**	Success
Sample Position	P1-D7	**DA Method Path**	D:\Projects\Instrument_Verification\Methods\default.m
Plate Position		**Target Source Path**	
Acq Operator	SYSTEM (SYSTEM)	**Result Summary**	1 qualified (1 targets)

Sample Chromatograms

+ESI TIC Scan Frag=75.0V Sa48.d

Counts vs. Acquisition Time (min)

Compound Summary

Cpd	Name	Formula	CAS	RT	Mass	Mass (Tgt)	Diff (Tgt, ppm)	Score	Algorithm
1		C15 H11 N O4 S		0.096	301.0404	301.0429	-1.49	75.47	FBF

Compound Details

Cpd 1: C15 H11 N O4 S

Name	Formula	RT	RI	Mass	Diff (Tgt, ppm)	CAS	ID Source	Score	Algorithm
	C15 H11 N O4 S	0.096		301.0404	-1.49		FBF	75.47	FBF

Species	m/z	Score (Tgt)	Score (Lib)	Score (DB)	Score (MFG)	Score (RT)
(M+H)+	302.0479	75.47				

Compound Chromatograms (overlaid)

Structure

Counts (%) vs. Acquisition Time (min)

Compound Spectra (overlaid)

Cpd 1: C15 H11 N O4 S: 0.096: + FBF Spectrum (rt: 0.079-0.101 min) Sa48.d Subtract

302.0479 (M+H)+

303.0507 (M+H)+

304.0436 (M+H)+

Counts vs. Mass-to-Charge (m/z)

Compound ID Table

Name	Formula	Species	RT	RT Diff	Mass	CAS	ID Source	Score	Score (Lib)	Score (Tgt)
	C15 H11 N O4 S	(M+H)+	0.096		301.0404		FBF	75.47		75.47

MassHunter Qual 12.0
(End of Report)

MassHunter Qualitative Analysis

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Generated at 14:52 on 25-03-2026

Figure S3: HRMS data of 5a in PBS after 48h

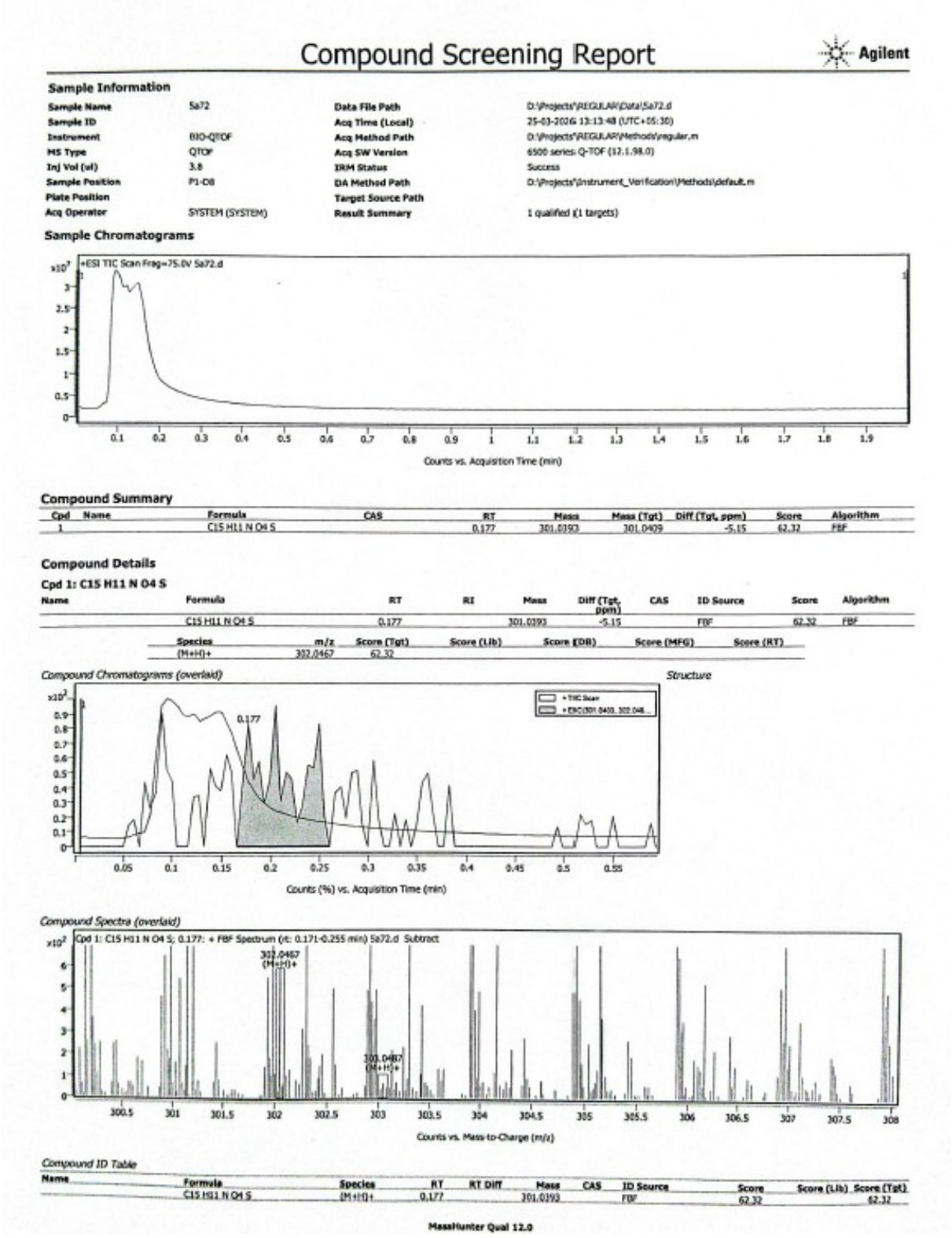

Figure S4: HRMS data of 5a in PBS after 72h

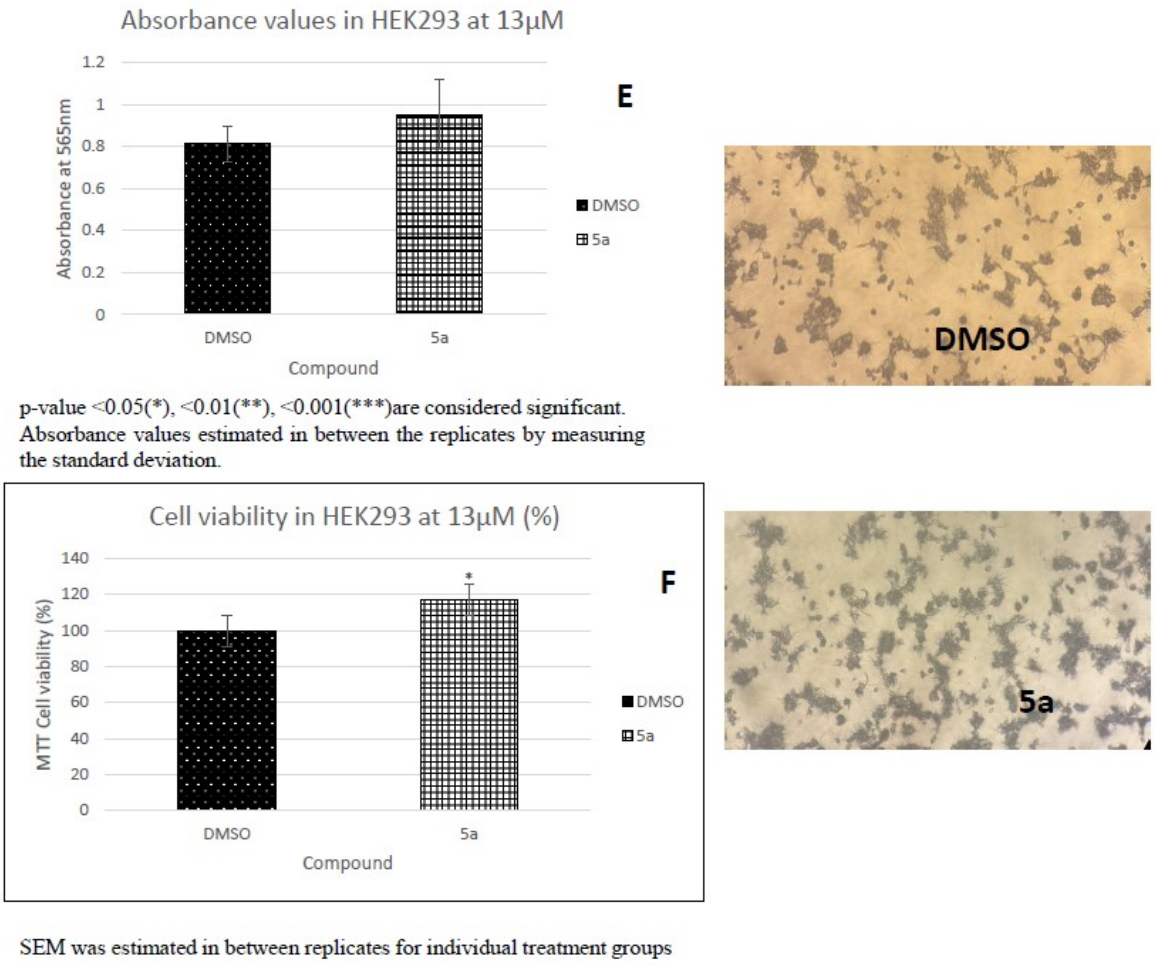

Figure S5: HEK293 Cell images taken after incubation with MTT reagent, cell morphology not affected, but the intensity of crystal formation is higher in cells treated with 5a [**E- Absorbance values at 565 nm**; **F- Cell viability (%)**]

Solubility study in solvents and media: We have tested the solubility of compounds. Compounds are completely soluble in DMSO but insoluble in water. Further, the solubility of compounds was assessed after suspending in PBS at 26°C, and dissolved properly (Figure S6-J). Further, the PBS containing compound was incubated for 72 HRS at 26°C and later was stored at 4°C for cell cytotoxicity assessment. Mild precipitation was seen at the bottom when compounds were incubated at 4°C (Figure S6 I). However, compounds again dissolved in PBS, when warmed at 26°C.

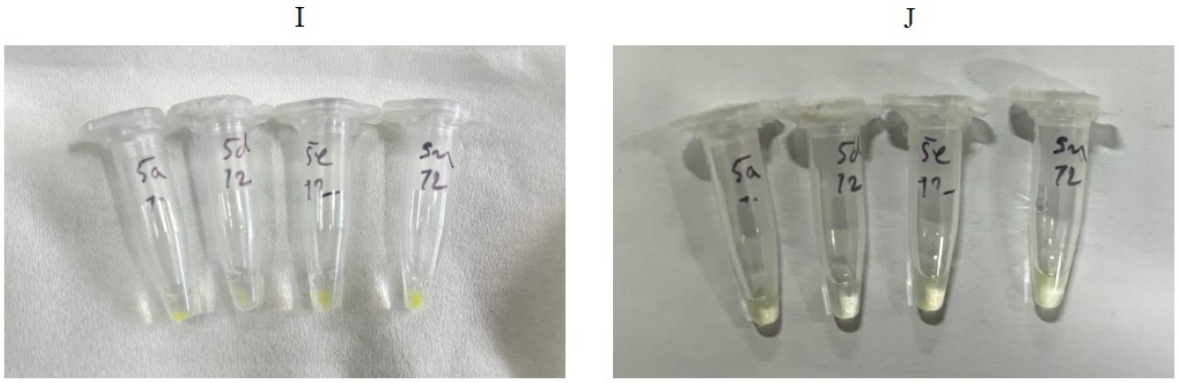

Figure S6- (I) Compounds were slightly precipitated in PBS at 4°C (During storage); Figure (J)- Compounds dissolved in PBS at 26°C (good solubility).