Supporting Information

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Synthesis, molecular docking, and in vitro evaluation of 2,4-dichlorobenzylamide derivatives as soluble epoxide hydrolase (sEH) inhibitors

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Supplementary data

Characterization of intermediate compounds 1-6

*4-((3,5-Dimethylisoxazol-4-yl)methoxy)benzoic acid (**1**):* The compound was synthesized using 4-(chloromethyl)-5-methylisoxazole according to the general procedure described above. Yield: 91%; m.p. 221-222 °C. ^{1}H NMR (500 MHz, DMSO-*d*$_6$): δ 12.66 (1H, s), 7.91 (2H, d, *J* = 8.8 Hz, AA' part of AA'BB' system), 7.09 (2H, d, *J* = 8.8 Hz, BB' part of AA'BB' system), 5.00 (2H, s), 2.42 (3H, s), 2.22 (3H, s). HRMS (ESI) *m/z:* $[M+H]^+$ calcd for $C_{13}H_{14}NO_4$, 248.0923; found, 248.0916. CAS: 279250-83-6.

*4-((1-Ethyl-1H-pyrazol-3-yl)methoxy)benzoic acid (**2**)*: The compound was synthesized using 3-(chloromethyl)-1-ethyl-1*H*-pyrazole according to the general procedure described above. Yield: 96%; m.p. 126-127 °C. ^{1}H NMR (500 MHz, DMSO-*d*$_6$): δ 12.63 (1H, s), 7.88 (2H, d, *J* = 8.8 Hz, AA' part of AA'BB' system), 7.72 (1H, d, *J* = 2.3 Hz), 7.10 (2H, d, *J* = 8.8 Hz, BB' part of AA'BB' system), 6.33 (1H, d, *J* = 2.3 Hz), 5.08 (2H, s), 4.13 (2H, q, *J* = 7.2 Hz), 1.37 (3H, t, *J* = 7.2 Hz). HRMS (ESI) *m/z:* $[M+H]^+$ calcd for $C_{13}H_{15}N_2O_3$, 247.1083; found, 247.1080.

*4-((1,3-Dimethyl-1H-pyrazol-4-yl)methoxy)benzoic acid (**3**):* The compound was synthesized using 4-(chloromethyl)-1,3-dimethyl-1*H*-pyrazole according to the general procedure described above. Yield: 92%; m.p. 188-190 °C. ^{1}H NMR (500 MHz, DMSO-*d*$_6$): δ 12.62 (1H, s), 7.88 (2H, d, *J* = 8.8 Hz, AA' part of AA'BB' system), 7.69 (1H, s), 7.06 (2H, d, *J* = 8.8 Hz, BB' part of AA'BB' system), 4.47 (2H, s), 3.74 (3H, s), 2.14 (3H, s). HRMS (ESI) *m/z:* $[M+H]^+$ calcd for $C_{13}H_{15}N_2O_3$, 247.1083; found, 247.1072.

*4-((1,3-Dimethyl-1H-pyrazol-5-yl)methoxy)benzoic acid (**4**):* The compound was synthesized using 5-(chloromethyl)-1,3-dimethyl-1*H*-pyrazole according to the general procedure described above. Yield: 78%; m.p. 223-226 °C. ^{1}H NMR (500 MHz, DMSO-*d*$_6$): δ 12.67 (1H, s), 7.90 (2H, d, *J* = 8.8 Hz, AA' part of AA'BB' system), 7.12 (2H, d, *J* = 8.8 Hz, BB' part of AA'BB' system), 6.17 (1H, s), 5.19 (2H, s), 3.74 (3H, s), 2.12 (3H, s). HRMS (ESI) *m/z:* $[M+H]^+$ calcd for $C_{13}H_{15}N_2O_3$, 247.1083; found, 247.1083. CAS: 1487922-96-2.

*4-(Thiazol-2-ylmethoxy)benzoic acid (**5**):* The compound was synthesized using 2-(chloromethyl)thiazole according to the general procedure described above. Yield: 90%; m.p. 200-202 °C. ^{1}H NMR (500 MHz, DMSO-*d*$_6$): δ 12.71 (1H, s), 7.91 (2H, d, *J* = 8.8 Hz, AA' part of AA'BB' system), 7.86 (1H, d, *J* = 3.2 Hz), 7.80 (1H, d, *J* = 3.2 Hz), 7.15 (2H, d, *J* = 8.8 Hz, BB' part of AA'BB' system), 5.54 (2H, s). HRMS (ESI) *m/z:* $[M+H]^+$ calcd for $C_{11}H_{10}NO_3S$, 236.0381; found, 236.0392. CAS: 1082818-84-5.

*4-((1H-benzo[d][1,2,3]triazol-1-yl)methoxy)benzoic acid (**6**):* The compound was synthesized using 1-(chloromethyl)-1*H*-benzo[d][1,2,3]triazole according to the general procedure described above. Yield: 95%; m.p. 227-229 °C. ^{1}H NMR (500 MHz, DMSO-*d*$_6$): δ 12.79 (1H, s), 8.06-8.12 (1H, m), 7.98 (2H, d, *J* = 8.8 Hz, AA' part of AA'BB' system), 7.62-7.68 (1H, m), 7.43–7.49 (1H, m), 7.26 (2H, d, *J* = 8.8 Hz, BB' part of AA'BB' system), 6.89 (2H, s). HRMS (ESI) *m/z:* $[M+H]^+$ calcd for $C_{14}H_{11}NO_3$, 270.0879; found, 270.0871.

Induced fit docking (IFD) analysis of final compounds

In addition to the docking studies presented in the main manuscript, Induced Fit Docking (IFD) calculations were performed to account for receptor flexibility. The corresponding results, including docking scores (Glide GScores), IFD scores, MM-GBSA ΔG bind, and ligand interaction diagrams, are summarized in Table S1 and Figure S1.

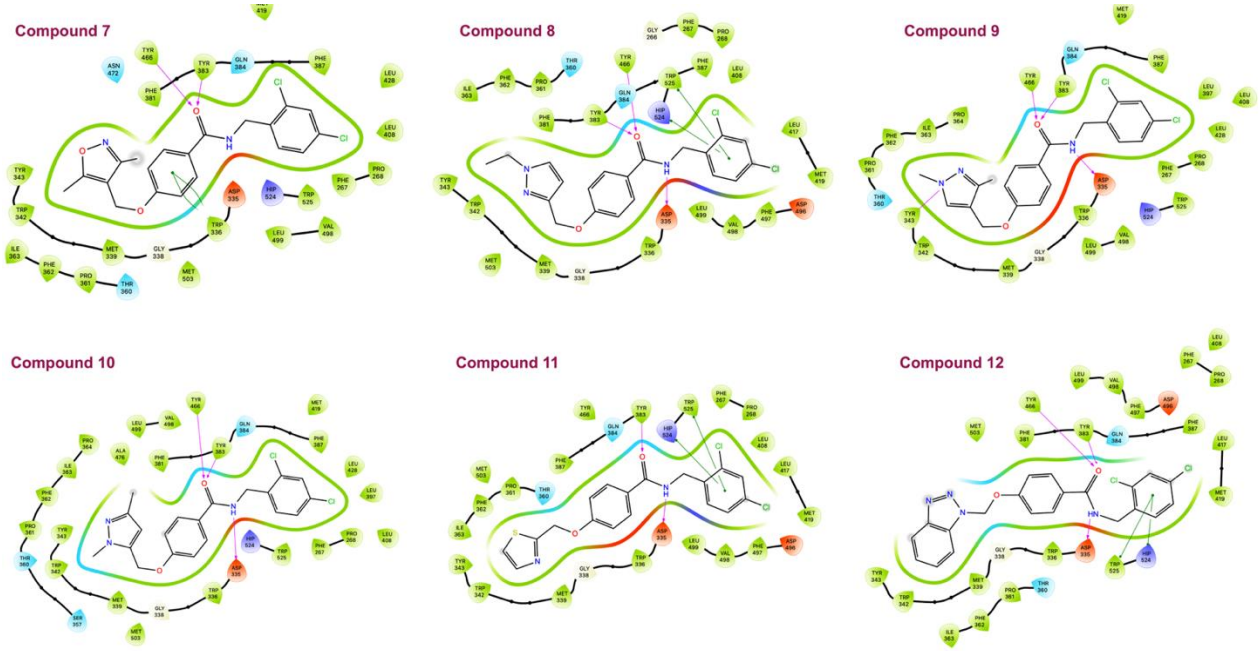
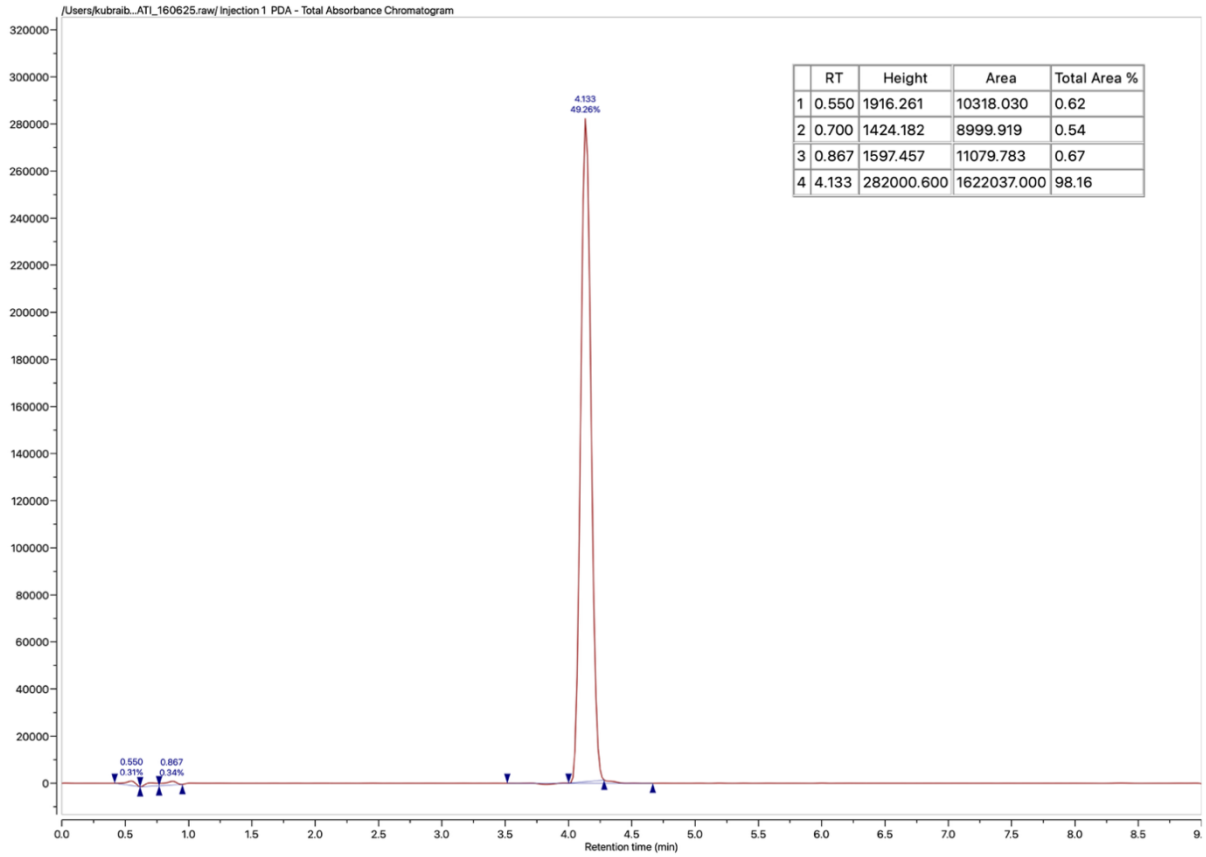


Figure S1. Ligand interaction diagrams showing the best IFD poses of final compounds 7–12 in complex with sEH. The diagrams illustrate hydrogen bonds, hydrophobic contacts, and π–π stacking interactions of the ligands in the IFD-predicted binding site.

Table S1. Docking scores (Glide GScore), Induced Fit Docking (IFD) scores, and MM-GBSA ΔG binding free energies of the final compounds 7-12 against sEH.

Comp.	Glide GScore (kcal/mol)	IFD Score (au, arbitrary unit)	MM-GBSA ΔG binding free energy (kcal/mol)
7	-11.346	-686.36	-114.30
8	-11.810	-684.12	-124.61
9	-12.916	-685.24	-124.59
10	-11.388	-684.03	-110.49
11	-10.491	-679.86	-114.58
12	-11.973	-682.51	-113.02

UPLC, HRMS ,^{1}H, and ^{13}C NMR data of final compounds 7-12



	RT	Height	Area	Total Area %
1	0.550	1916.261	10318.030	0.62
2	0.700	1424.182	8999.919	0.54
3	0.867	1597.457	11079.783	0.67
4	4.133	282000.600	1622037.000	98.16

Figure S2: UPLC spectrum of compound **7**

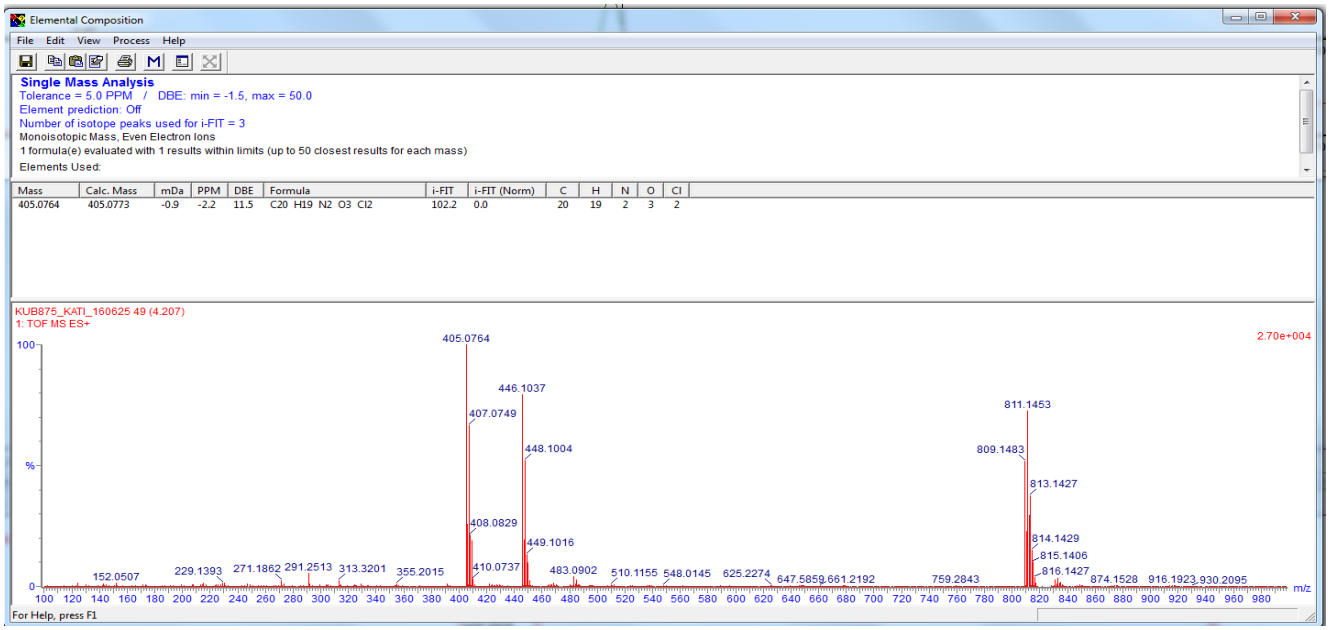


Figure S3: HRMS spectrum of compound **7**

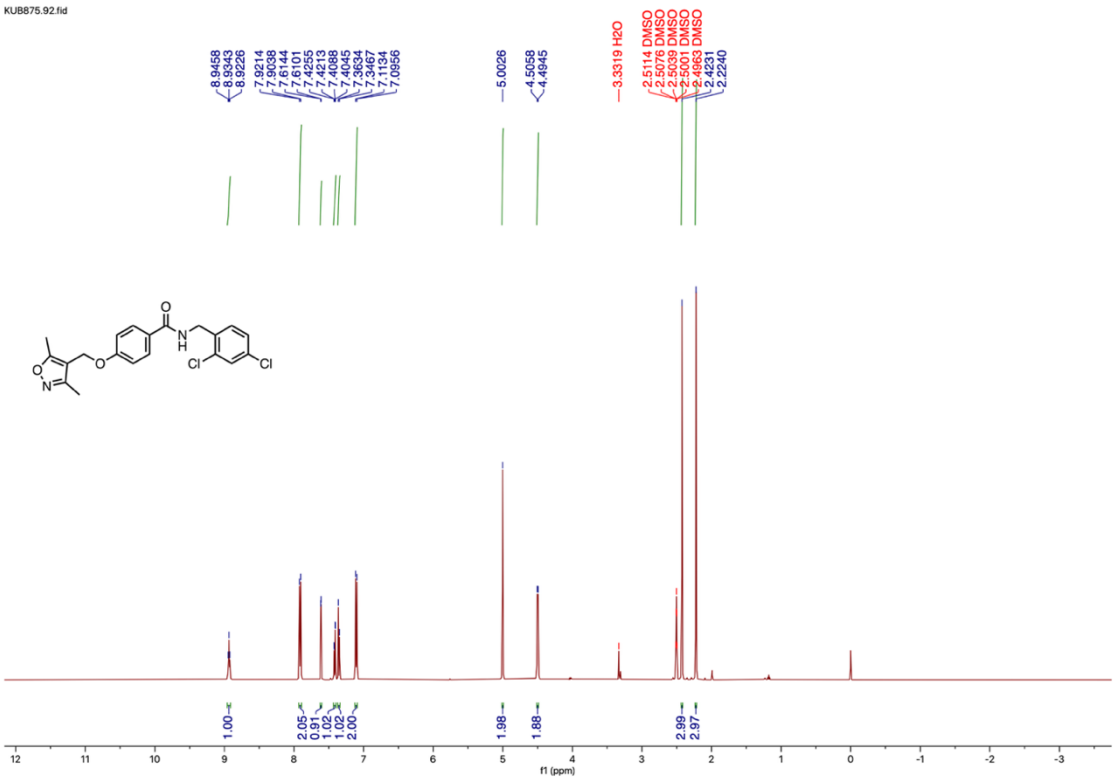


Figure S4a: [1]H-NMR spectrum of compound **7**

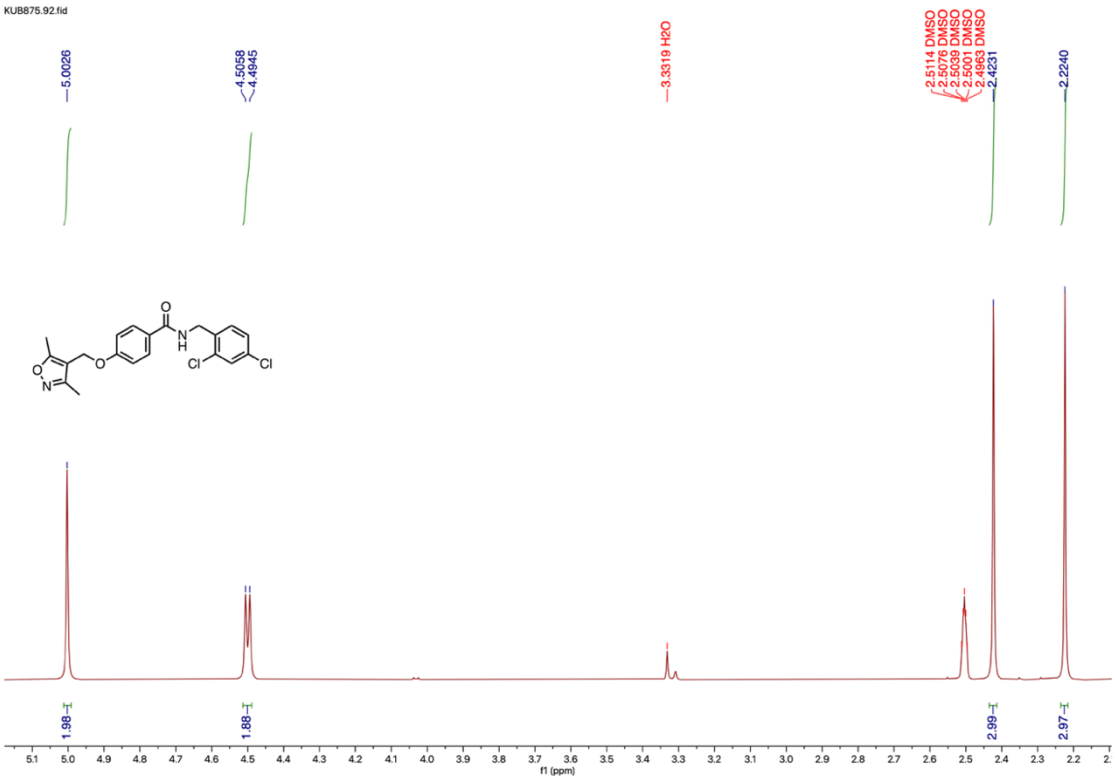


Figure S4b: [1]H-NMR spectrum of compound **7**

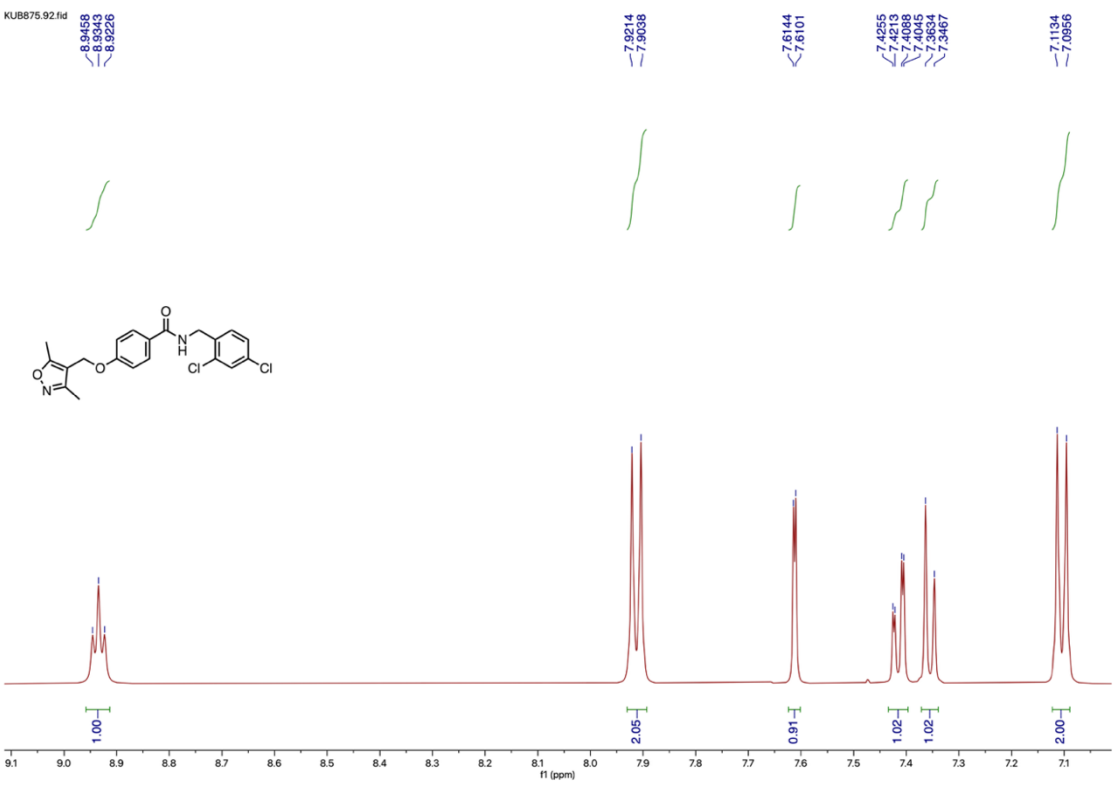


Figure S4c: ^{1}H-NMR spectrum of compound **7**

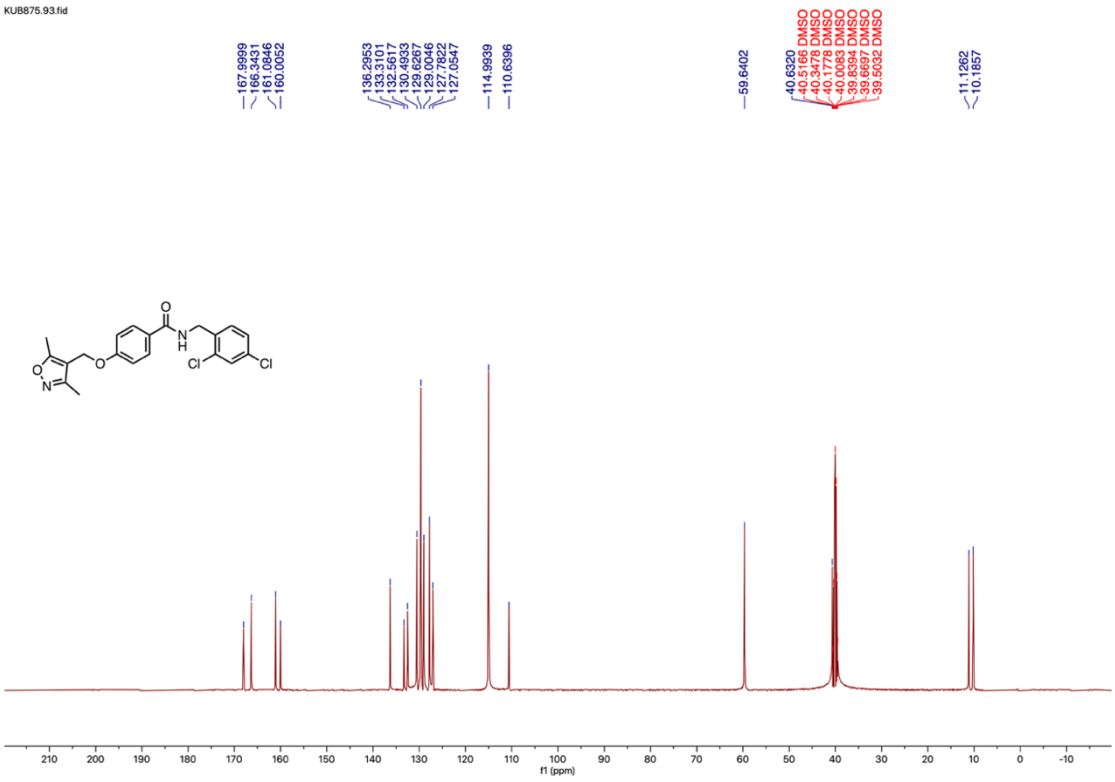


Figure S5a: ^{13}C-NMR spectrum of compound **7**

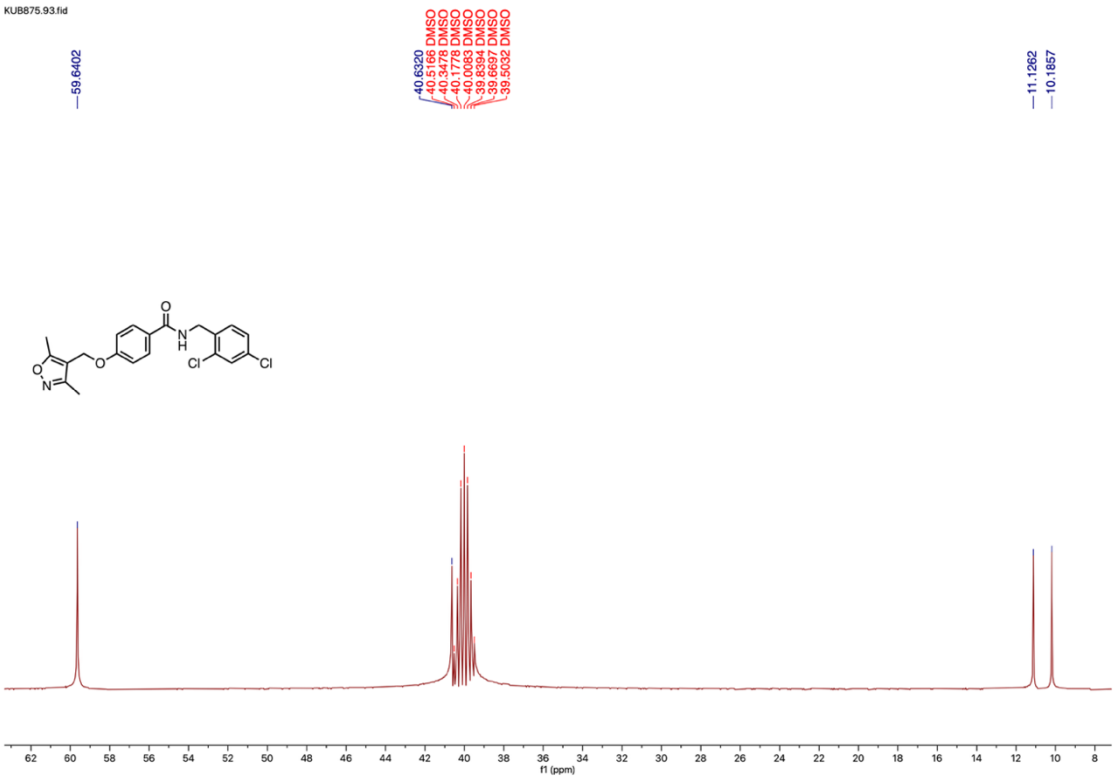


Figure S5b: ^{13}C-NMR spectrum of compound **7**

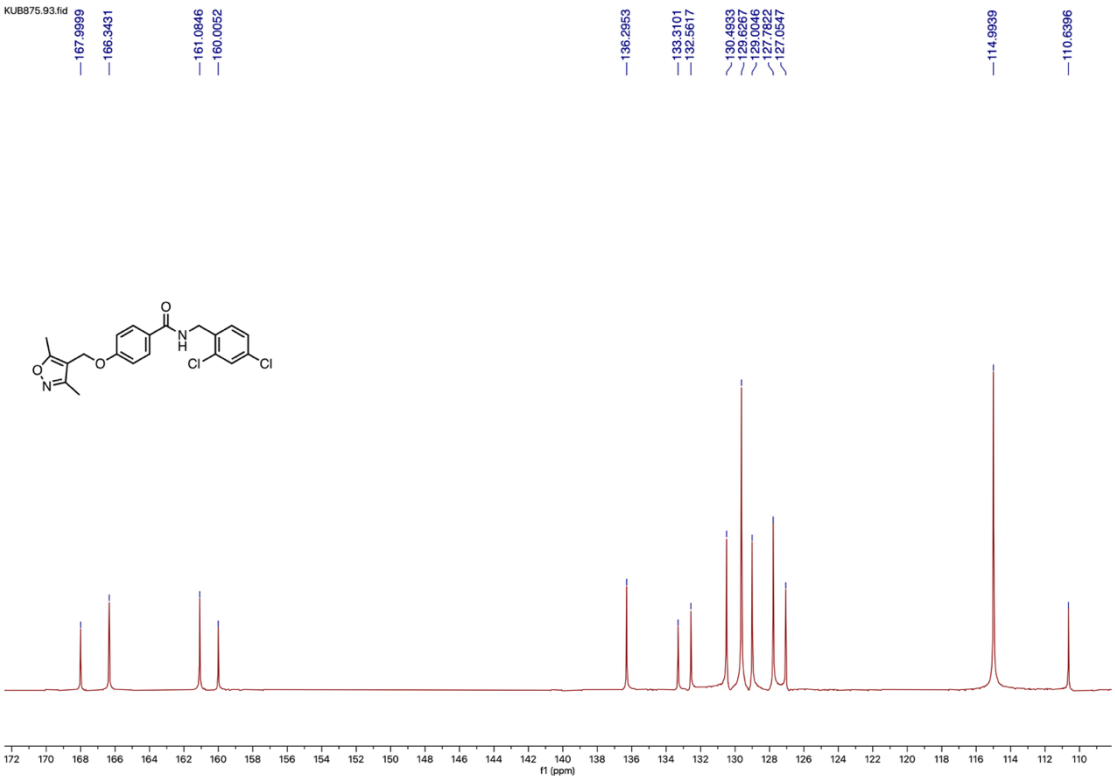


Figure S5c: ^{13}C-NMR spectrum of compound 7

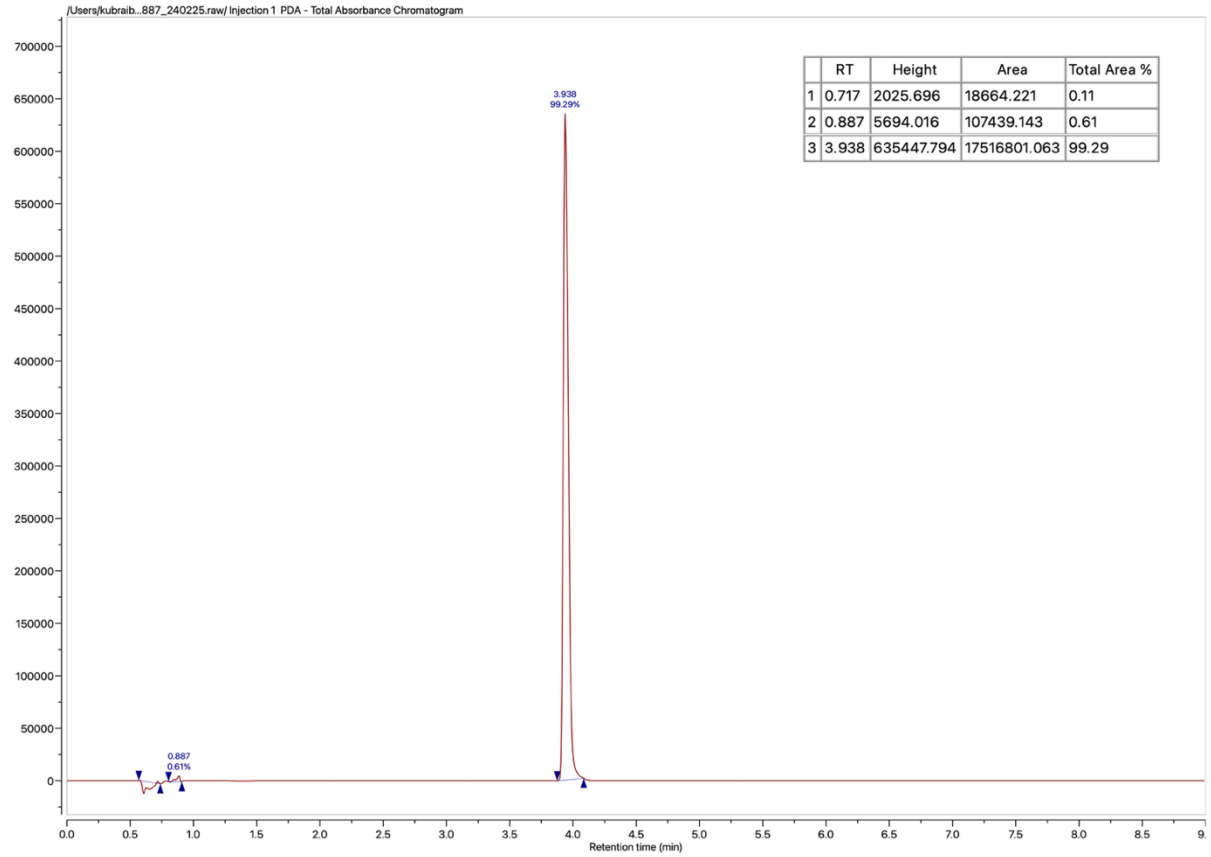


Figure S6: UPLC spectrum of compound **8**

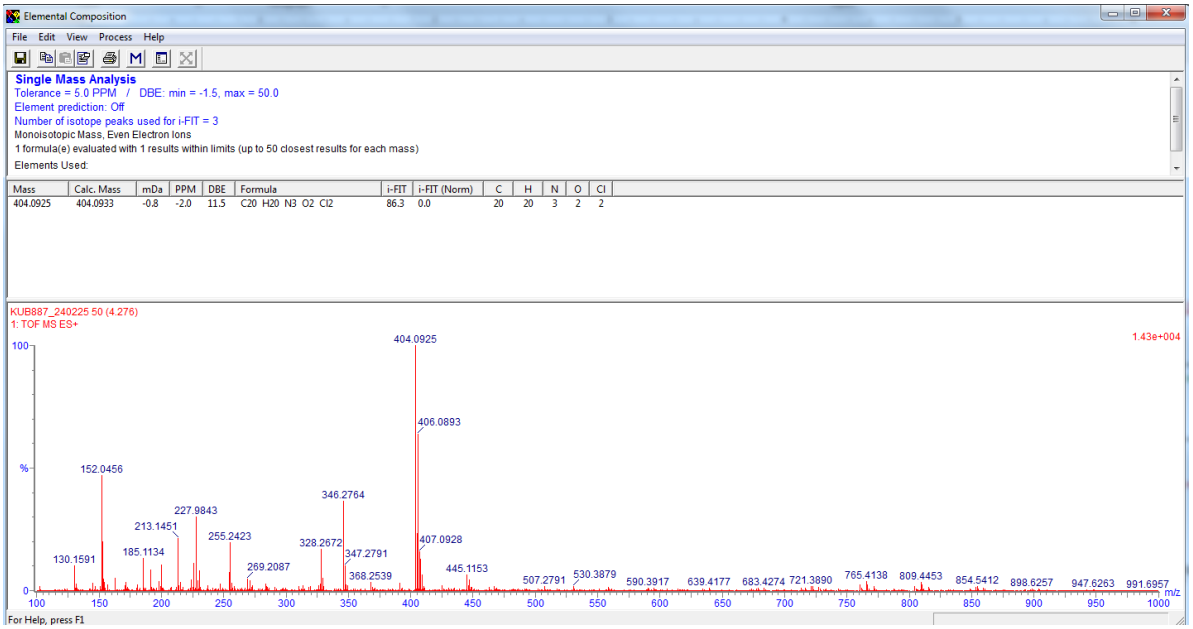


Figure S7: HRMS spectrum of compound **8**

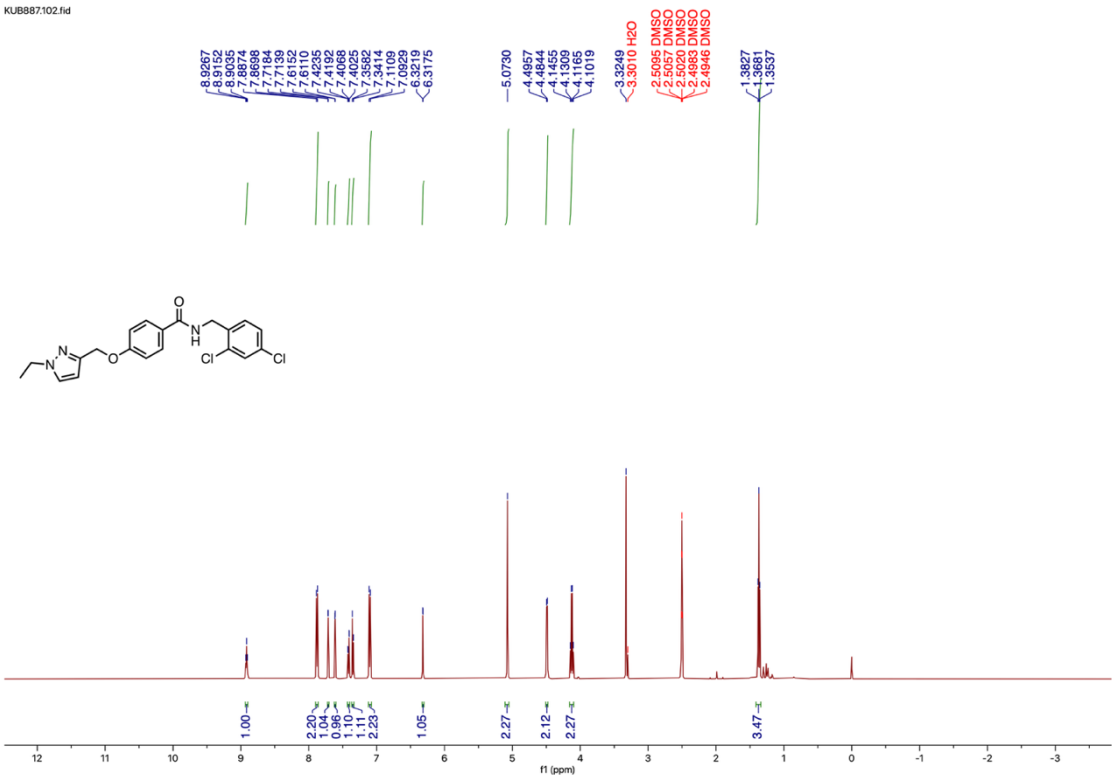


Figure S8a: ^{1}H-NMR spectrum of compound **8**

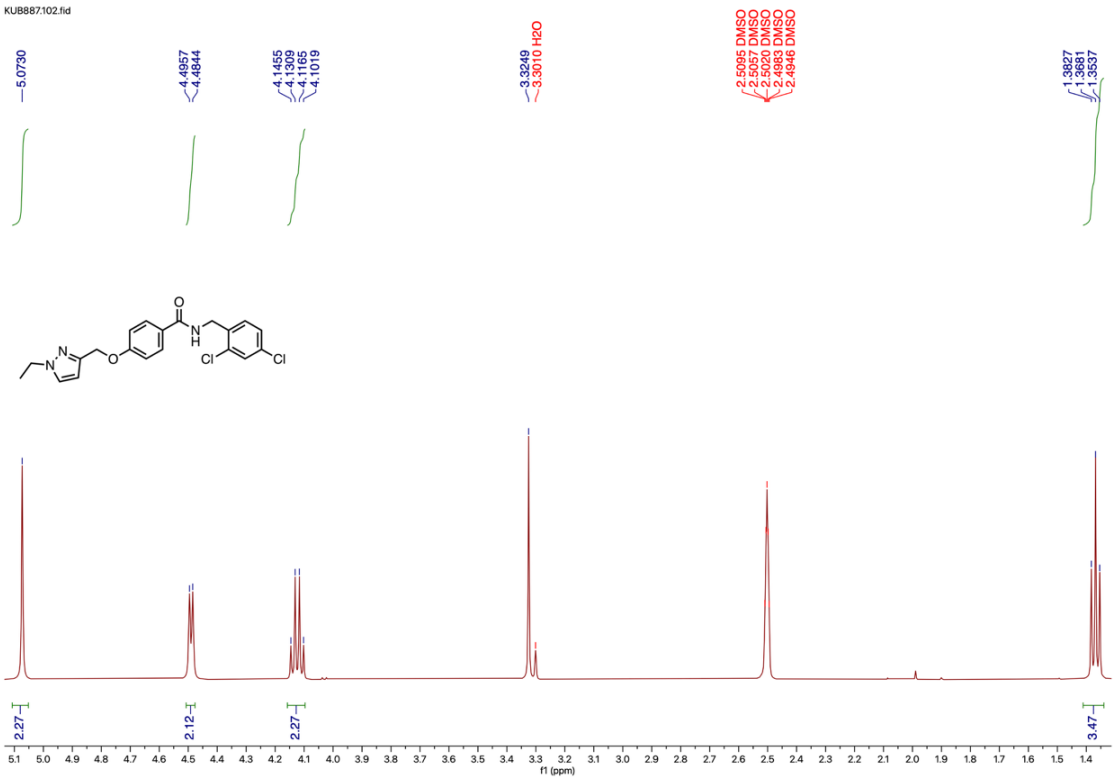


Figure S8b: ^{1}H-NMR spectrum of compound **8**

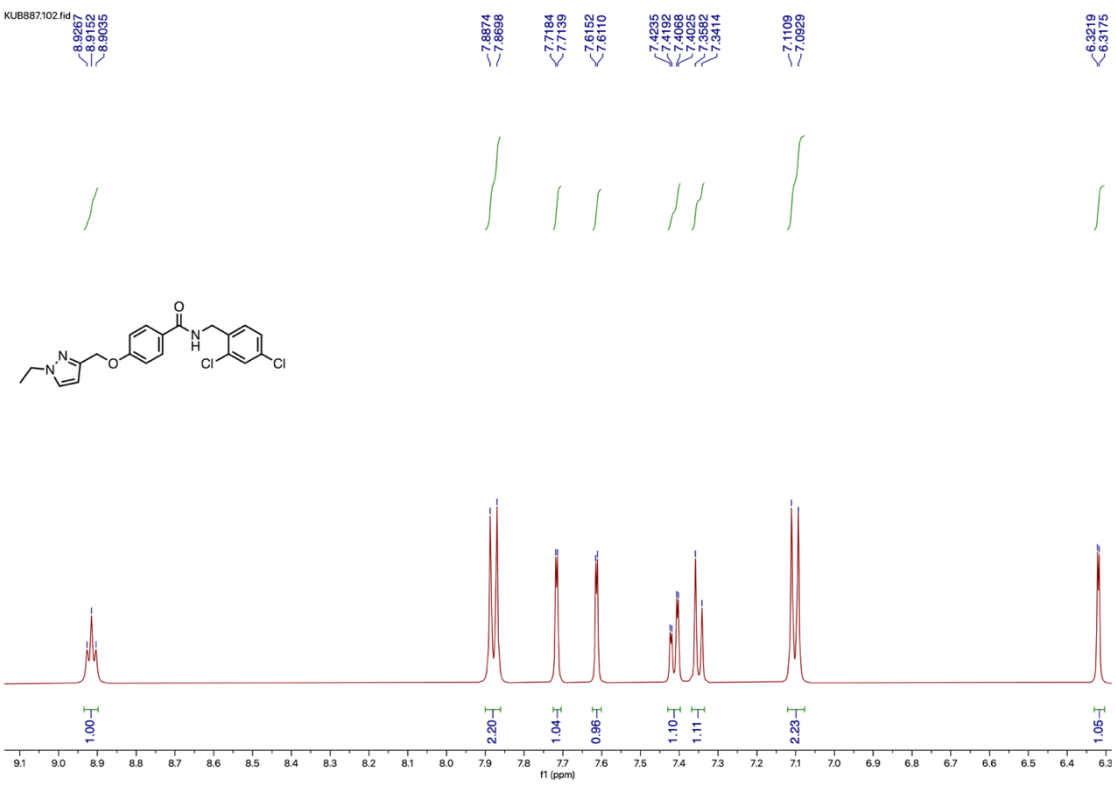


Figure S8c: ^{1}H-NMR spectrum of compound **8**

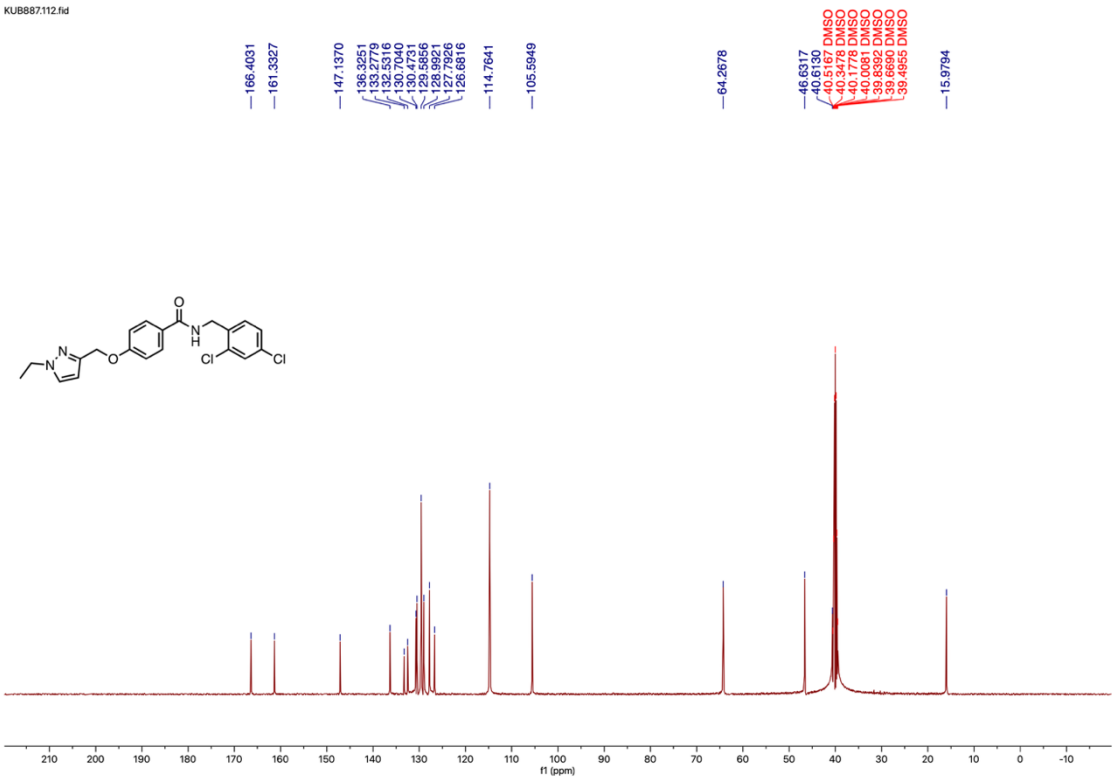


Figure S9a: ^{13}C-NMR spectrum of compound **8**

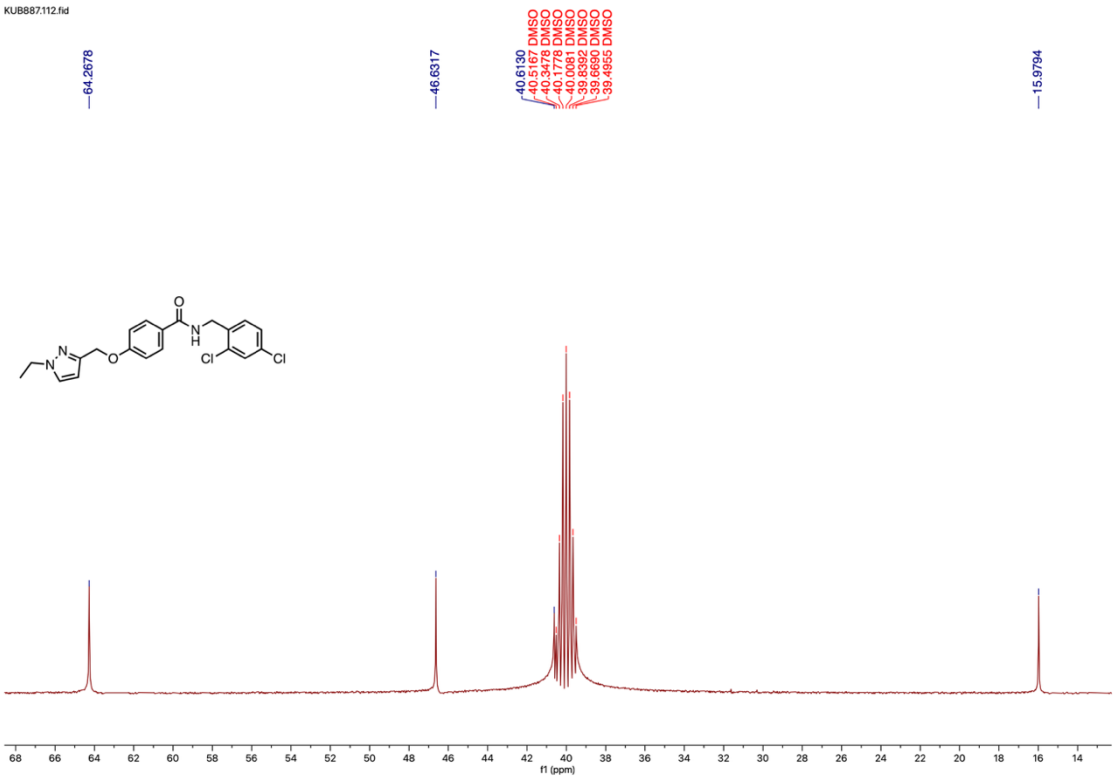


Figure S9b: ^{13}C-NMR spectrum of compound **8**

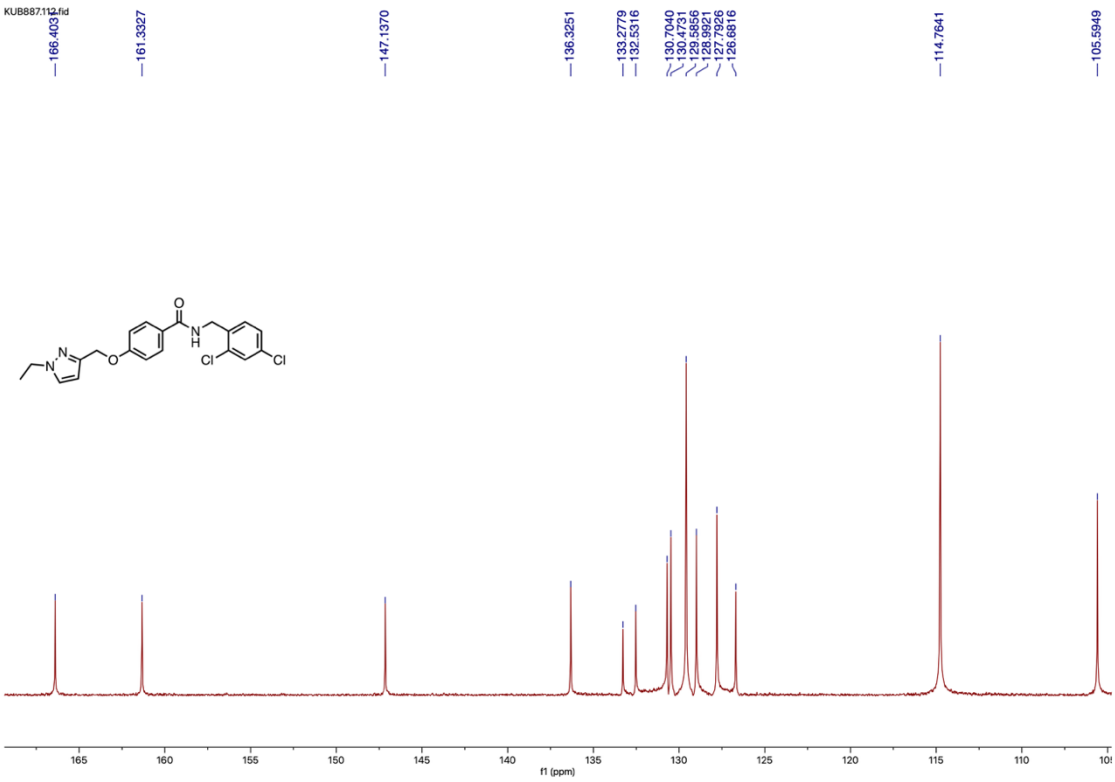
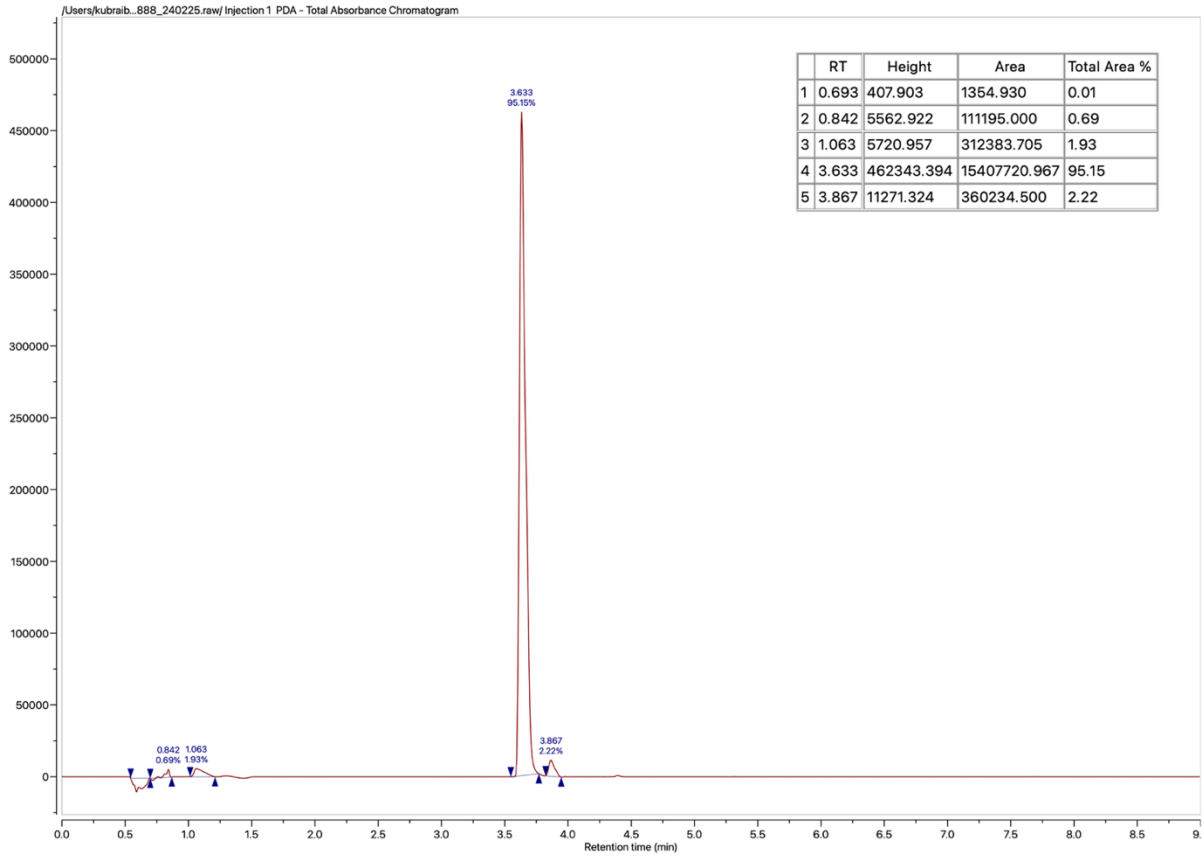


Figure S9c: ^{13}C-NMR spectrum of compound **8**



	RT	Height	Area	Total Area %
1	0.693	407.903	1354.930	0.01
2	0.842	5562.922	111195.000	0.69
3	1.063	5720.957	312383.705	1.93
4	3.633	462343.394	15407720.967	95.15
5	3.867	11271.324	360234.500	2.22

Figure S10: UPLC spectrum of compound **9**

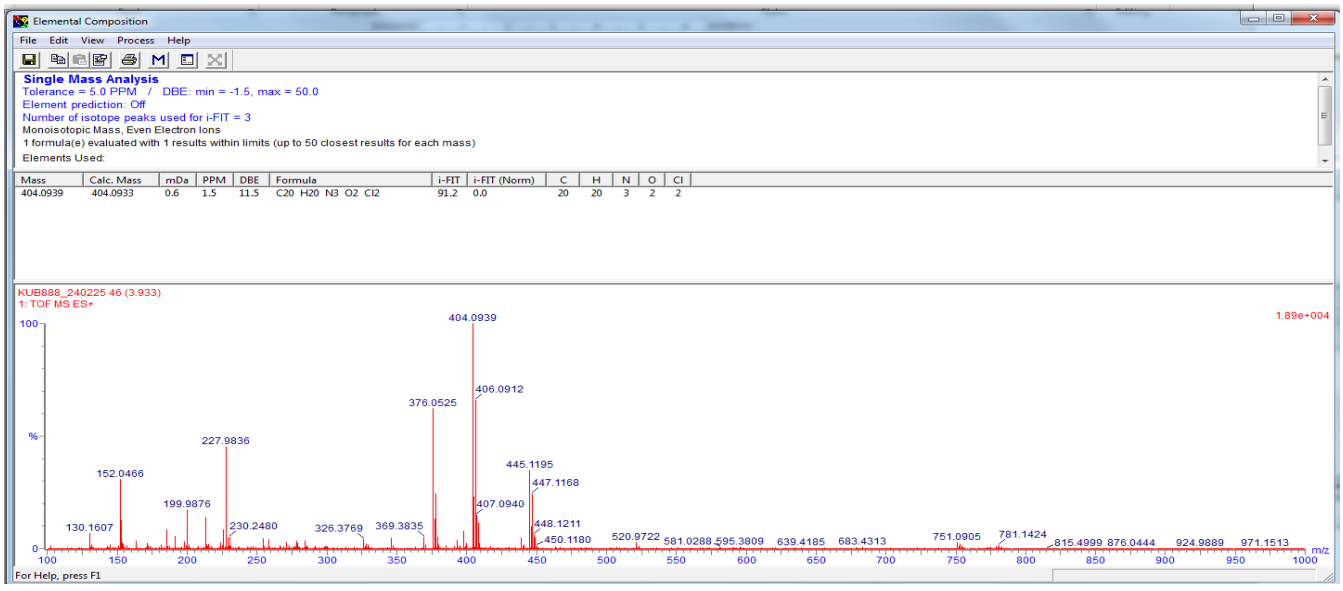


Figure S11: HRMS spectrum of compound **9**

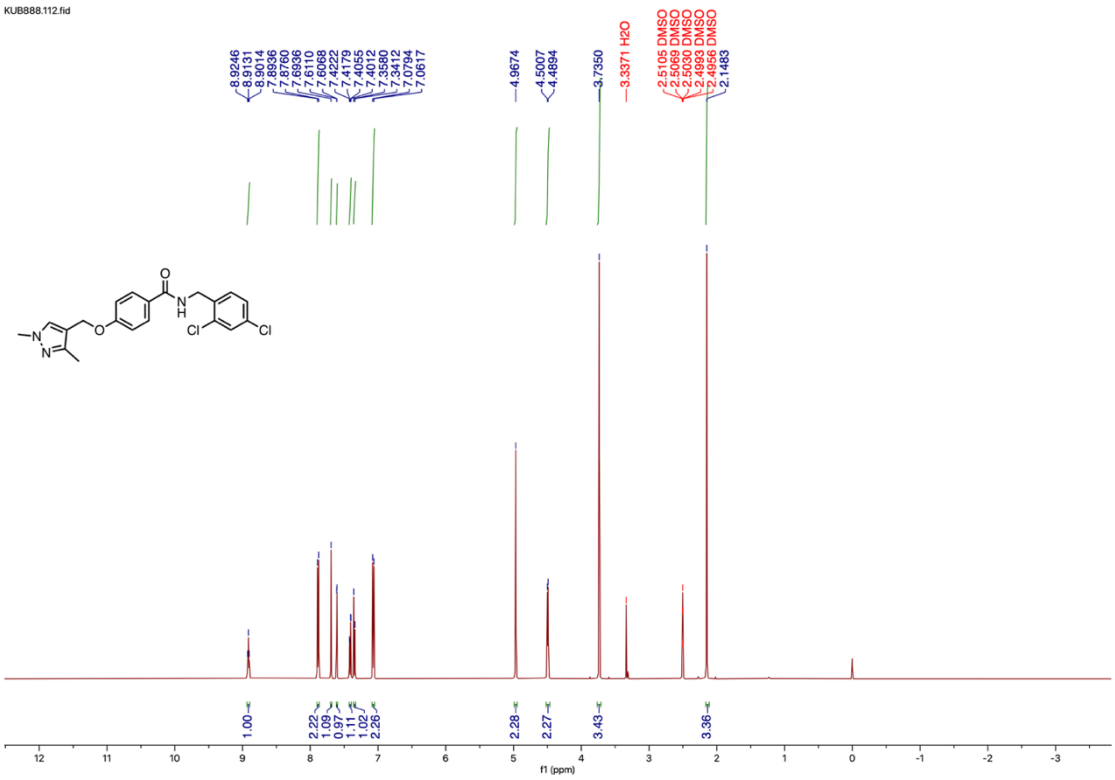


Figure S12a: [1]H-NMR spectrum of compound **9**

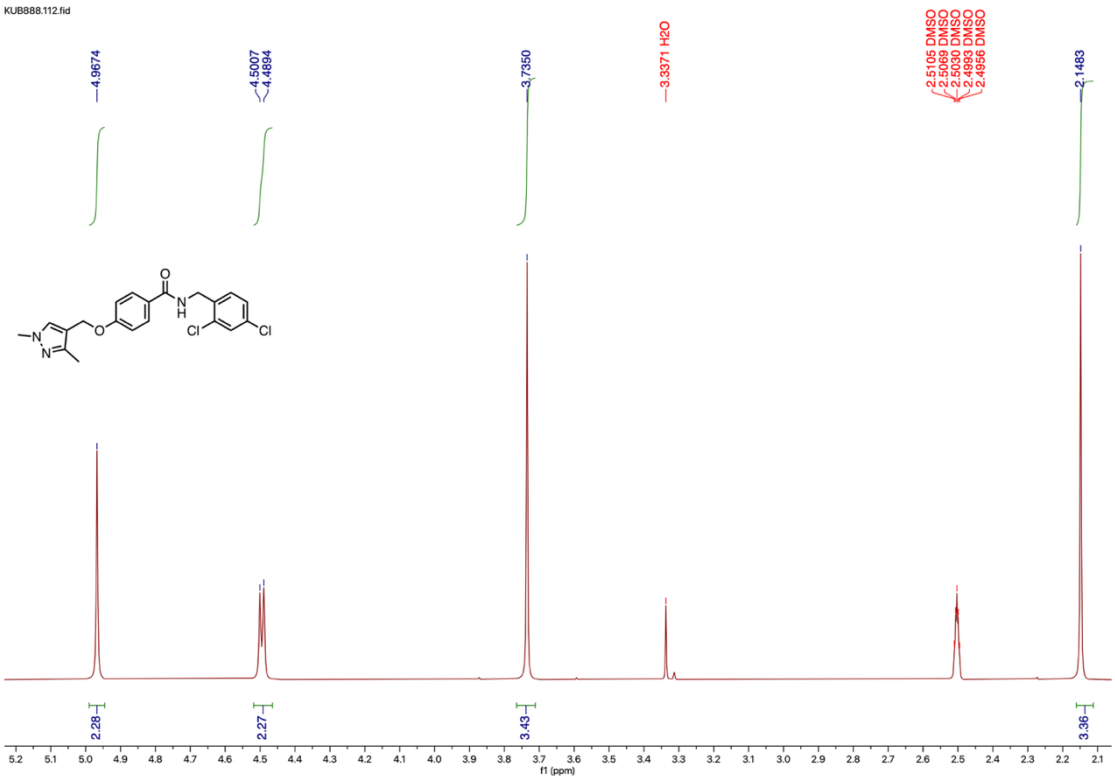


Figure S12b: [1]H-NMR spectrum of compound **9**

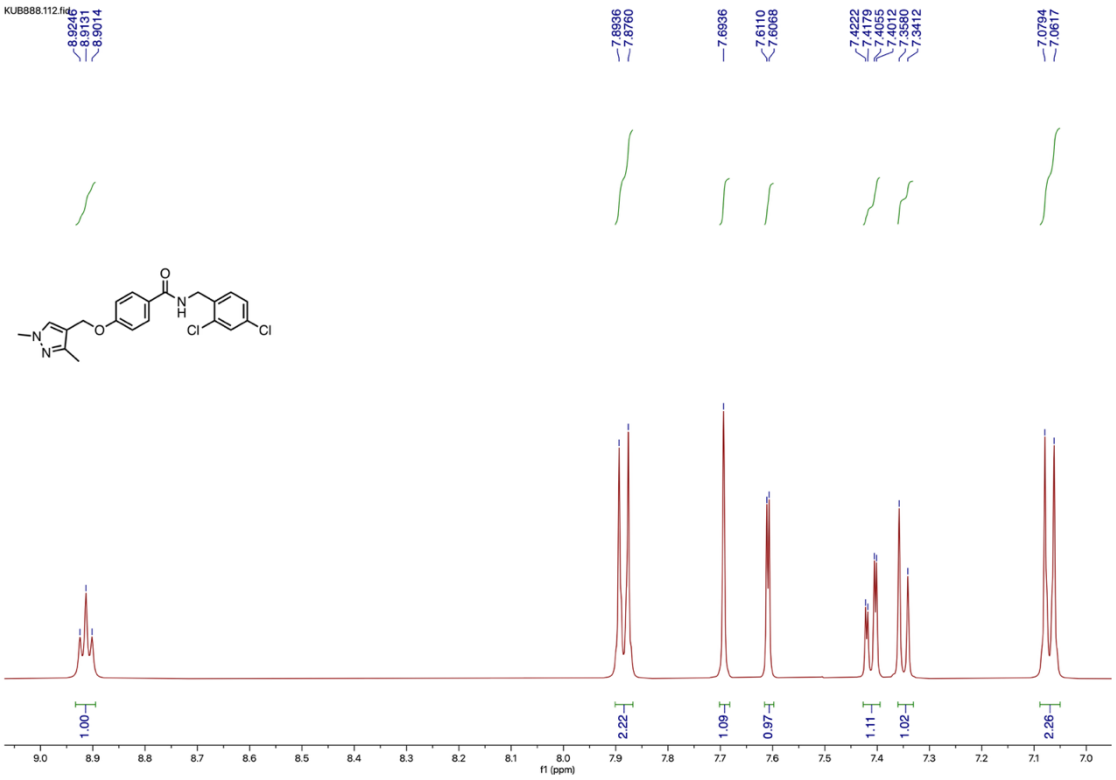


Figure S12c: ^{1}H-NMR spectrum of compound **9**

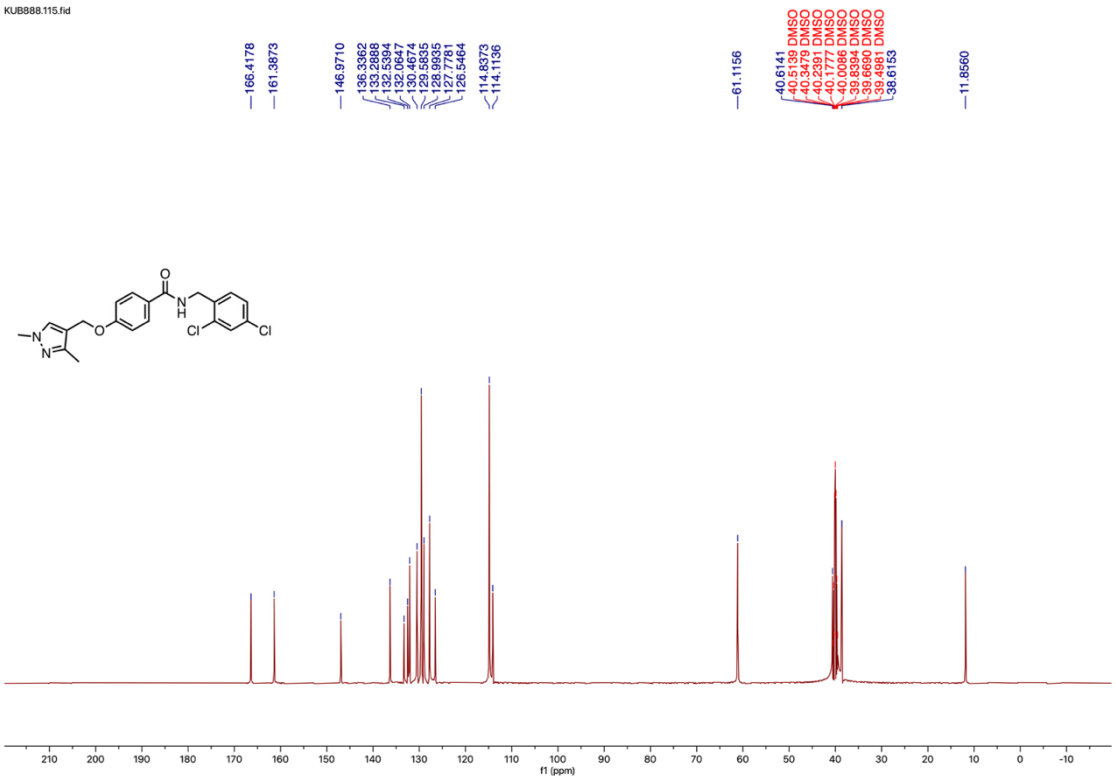


Figure S13a: ^{13}C-NMR spectrum of compound **9**

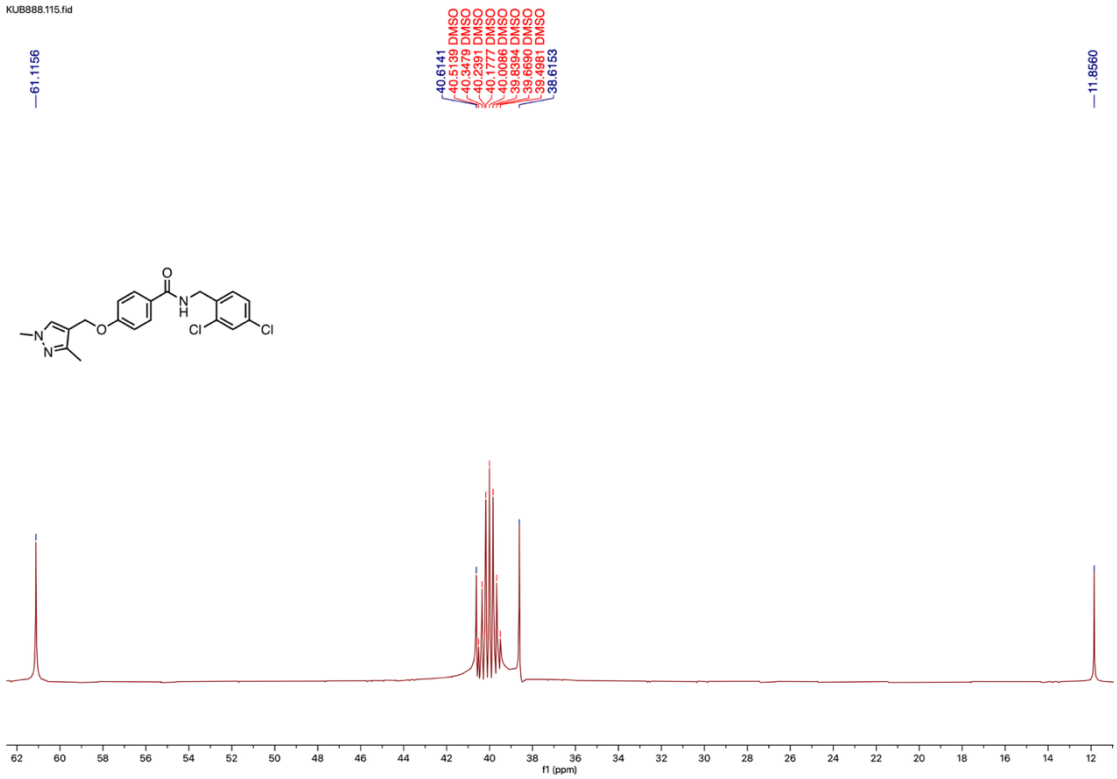


Figure S13b: ^{13}C-NMR spectrum of compound **9**

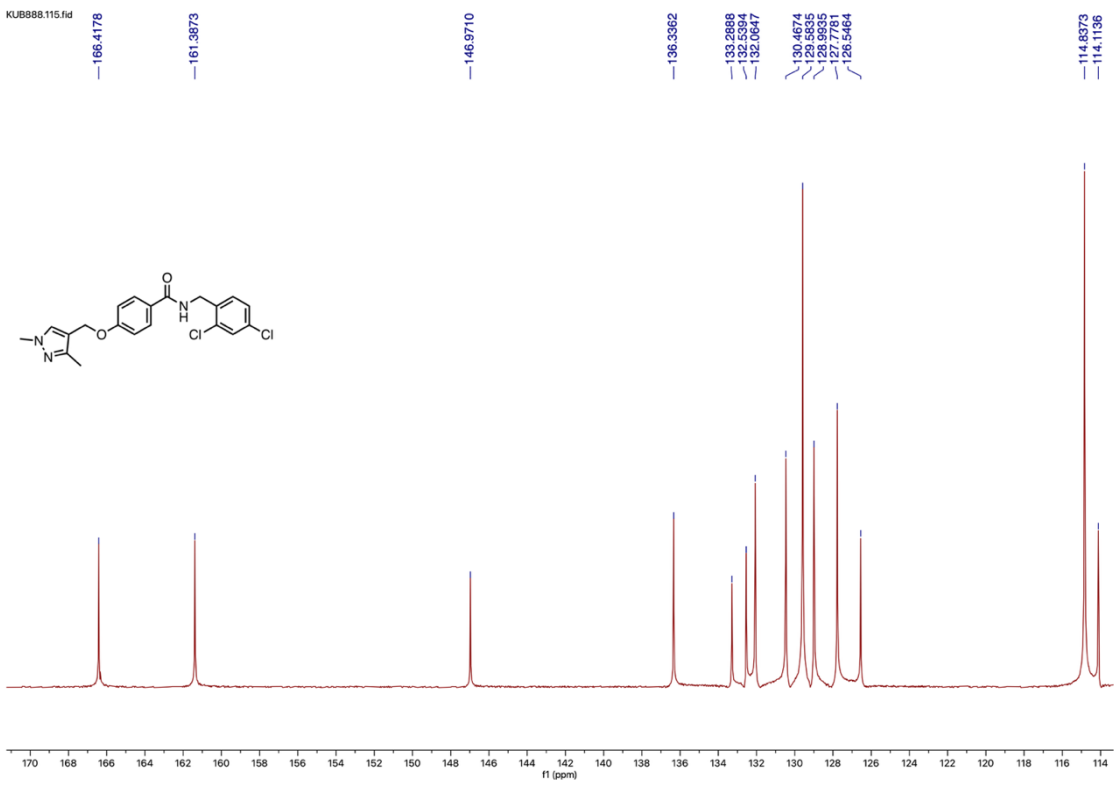
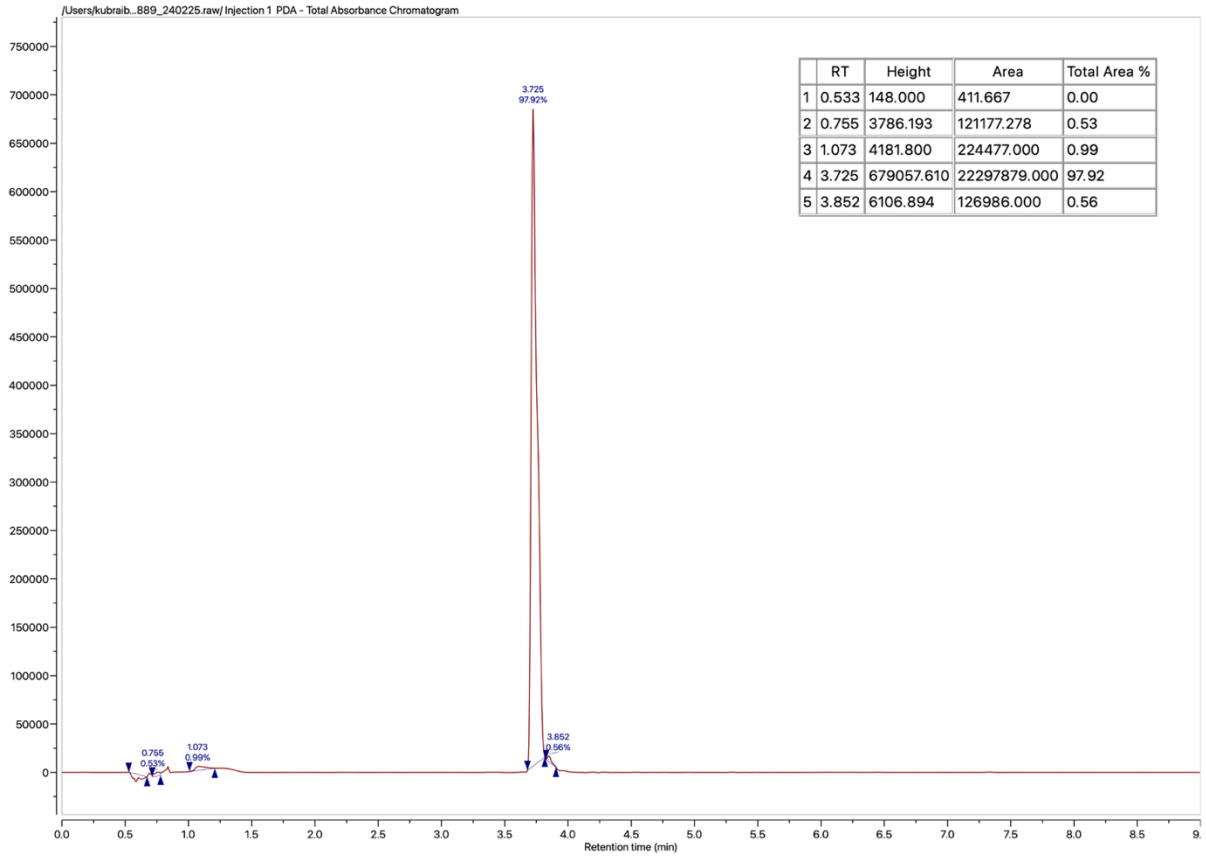


Figure S13c: ^{13}C-NMR spectrum of compound **9**



	RT	Height	Area	Total Area %
1	0.533	148.000	411.667	0.00
2	0.755	3786.193	121177.278	0.53
3	1.073	4181.800	224477.000	0.99
4	3.725	679057.610	22297879.000	97.92
5	3.852	6106.894	126986.000	0.56

Figure S14: UPLC spectrum of compound **10**

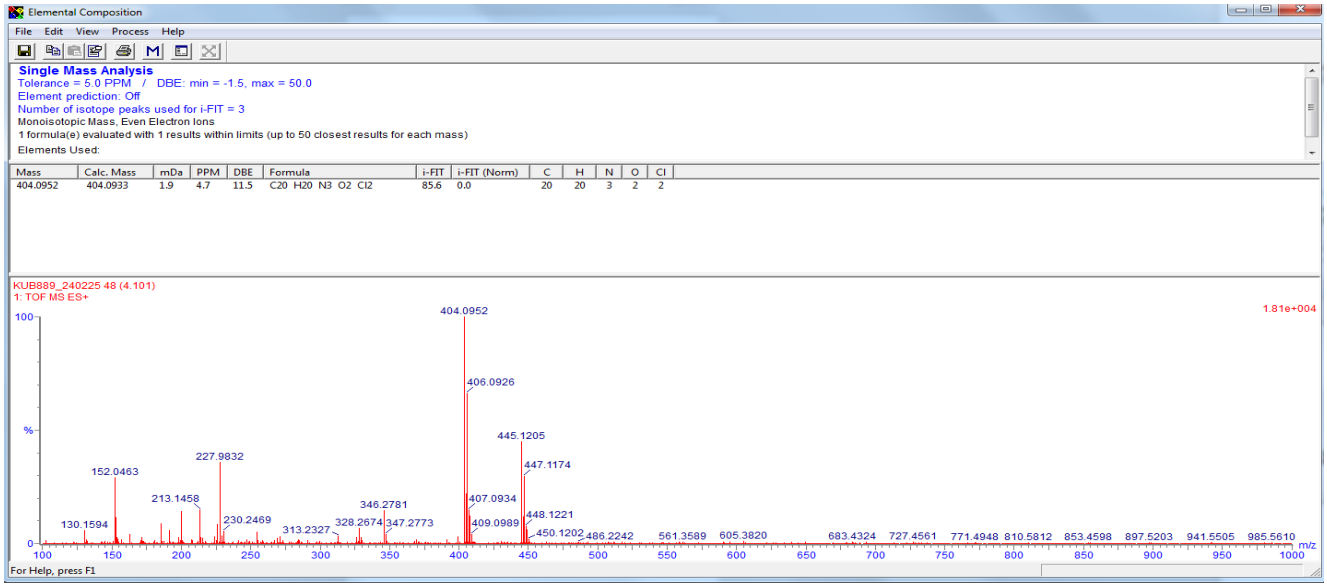


Figure S15: HRMS spectrum of compound **10**

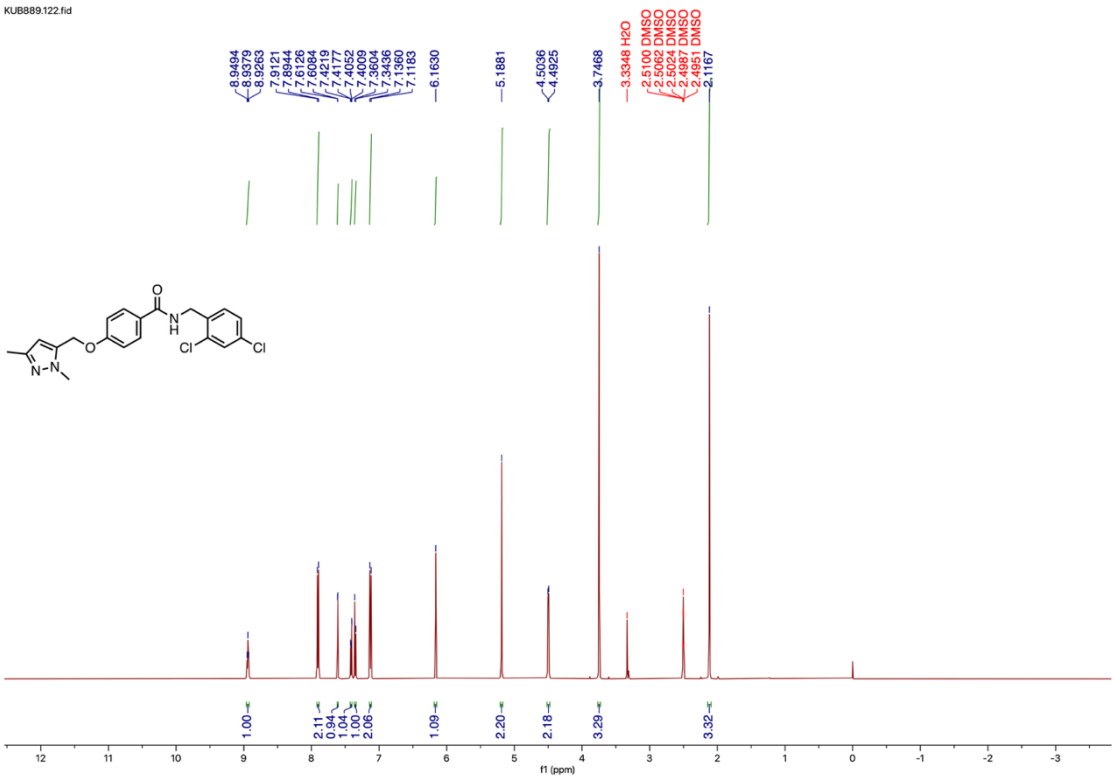


Figure S16a: ^{1}H-NMR spectrum of compound **10**

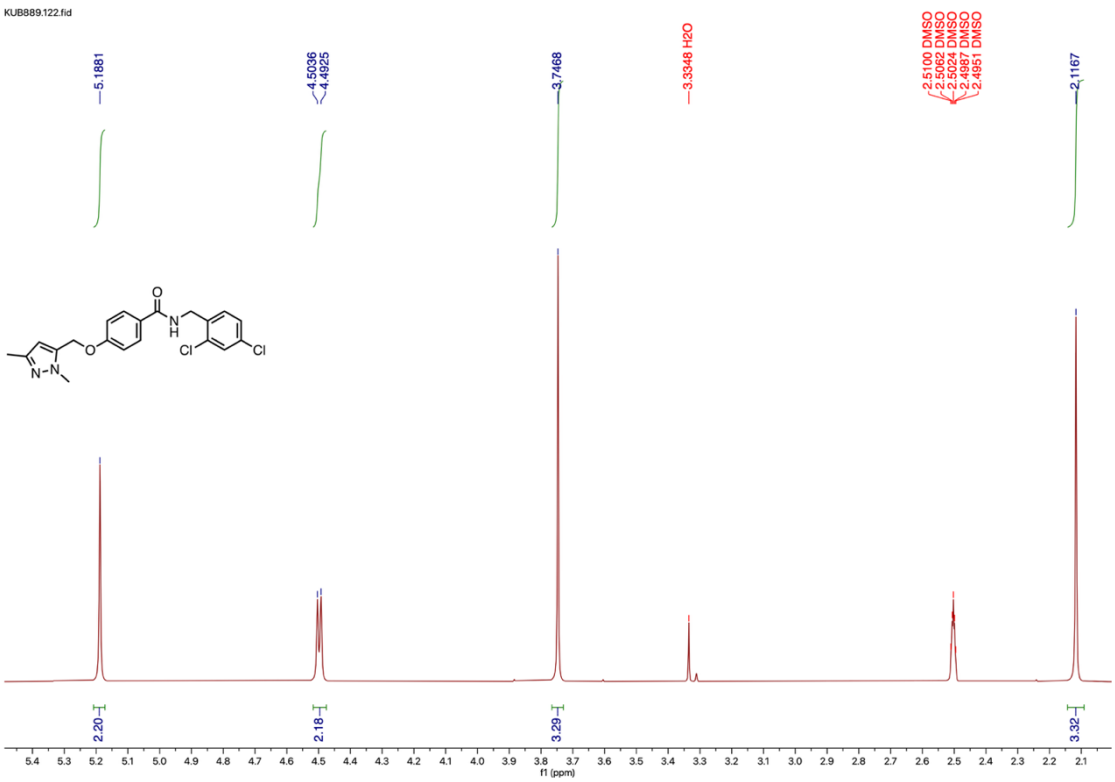


Figure S16b: ^{1}H-NMR spectrum of compound **10**

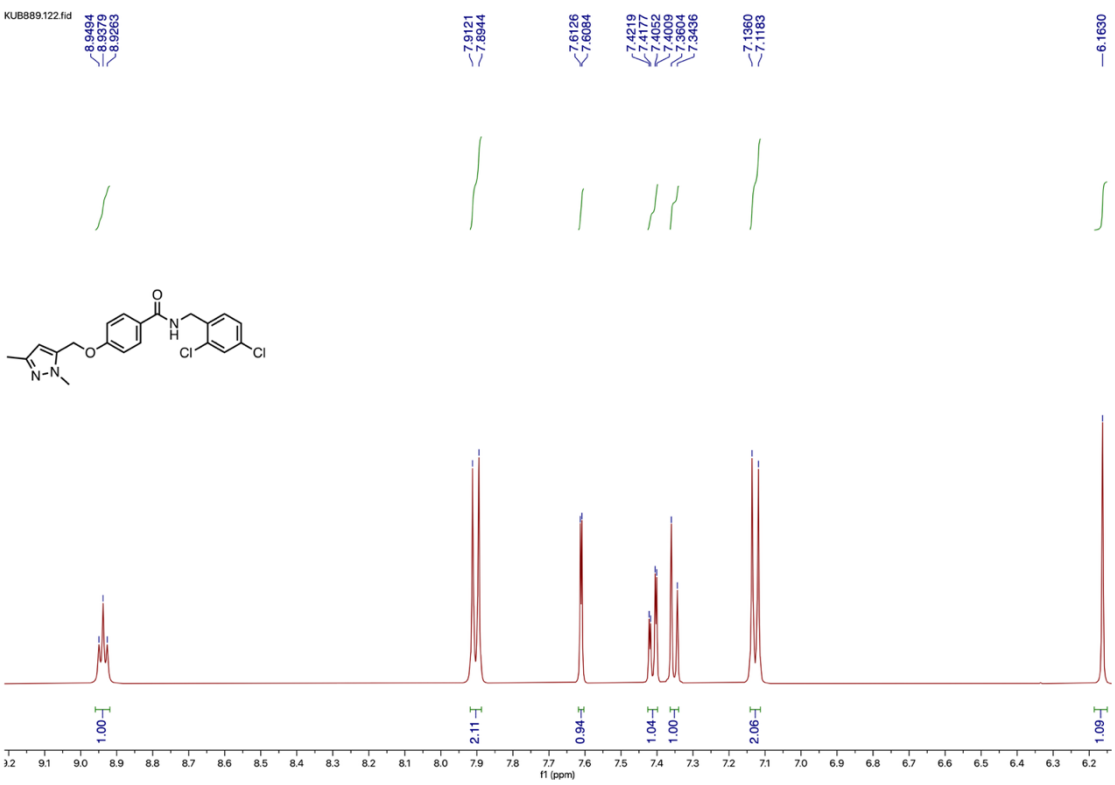


Figure S16c: ^{1}H-NMR spectrum of compound **10**

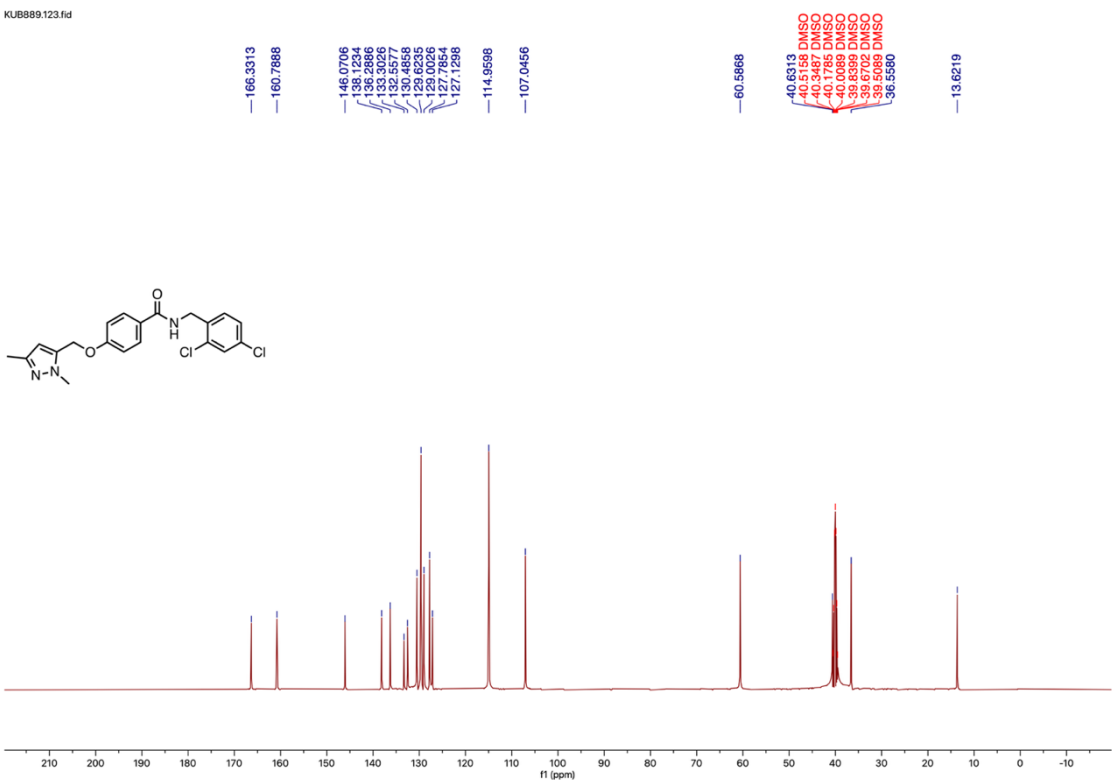


Figure S17a: ^{13}C-NMR spectrum of compound **10**

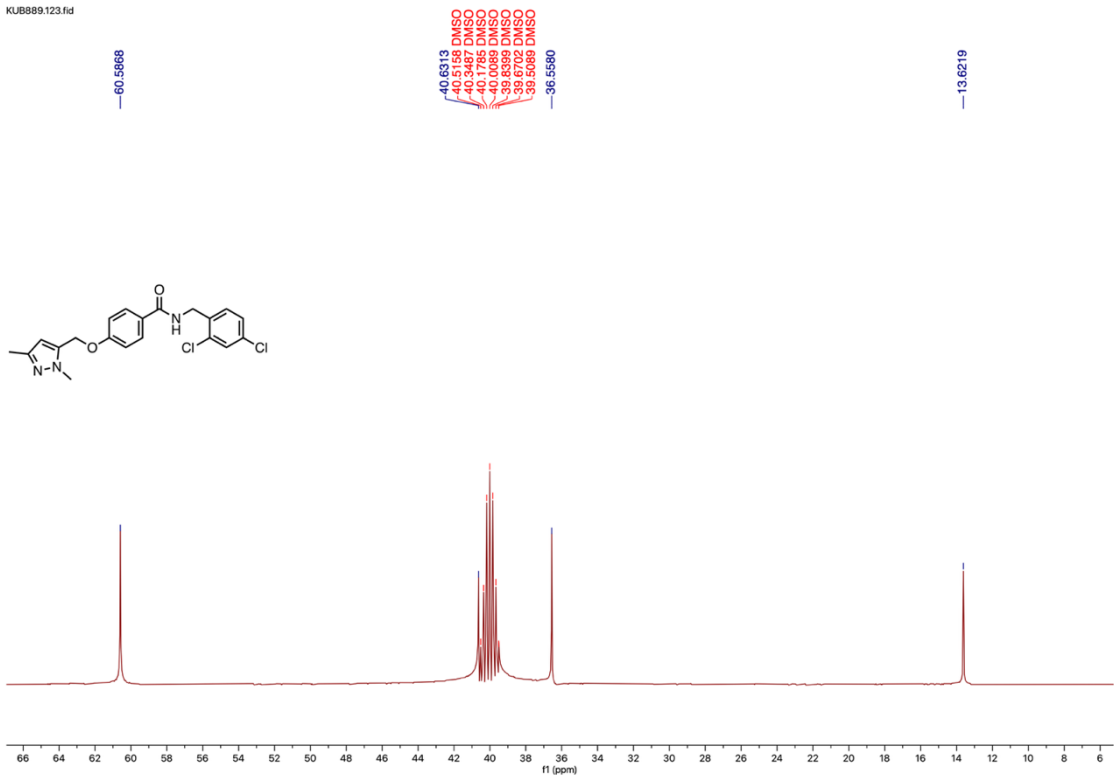


Figure S17b: ^{13}C-NMR spectrum of compound **10**

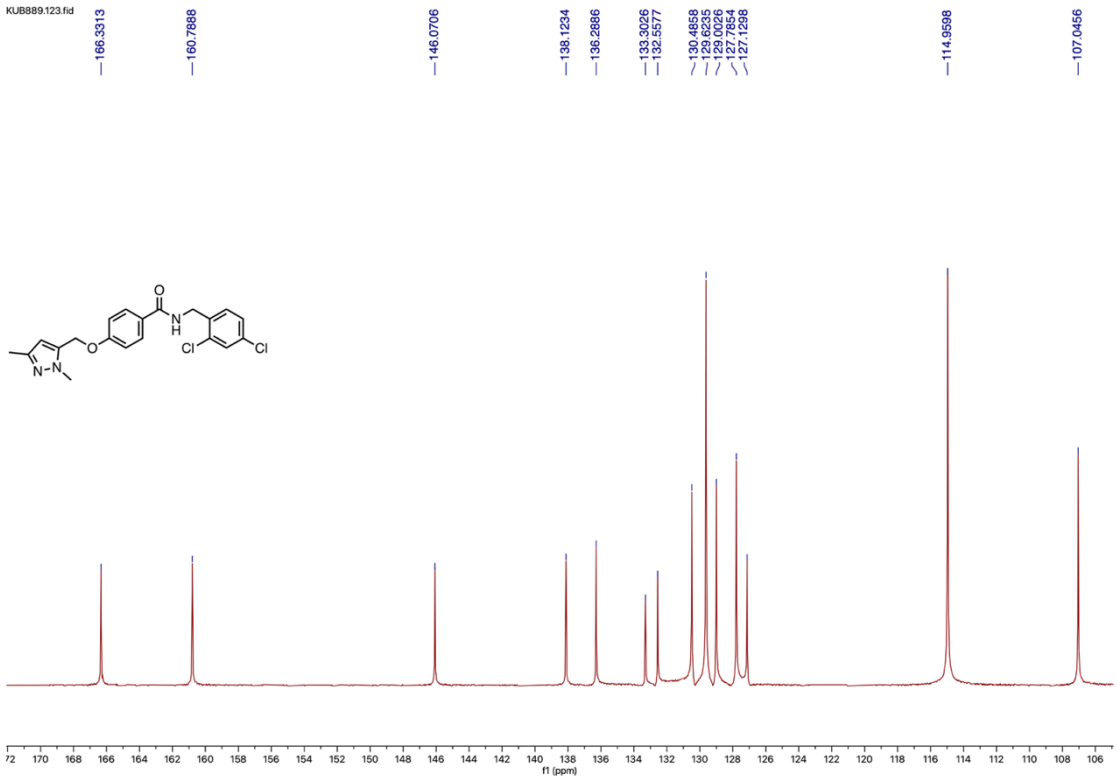


Figure S17c: ^{13}C-NMR spectrum of compound **10**

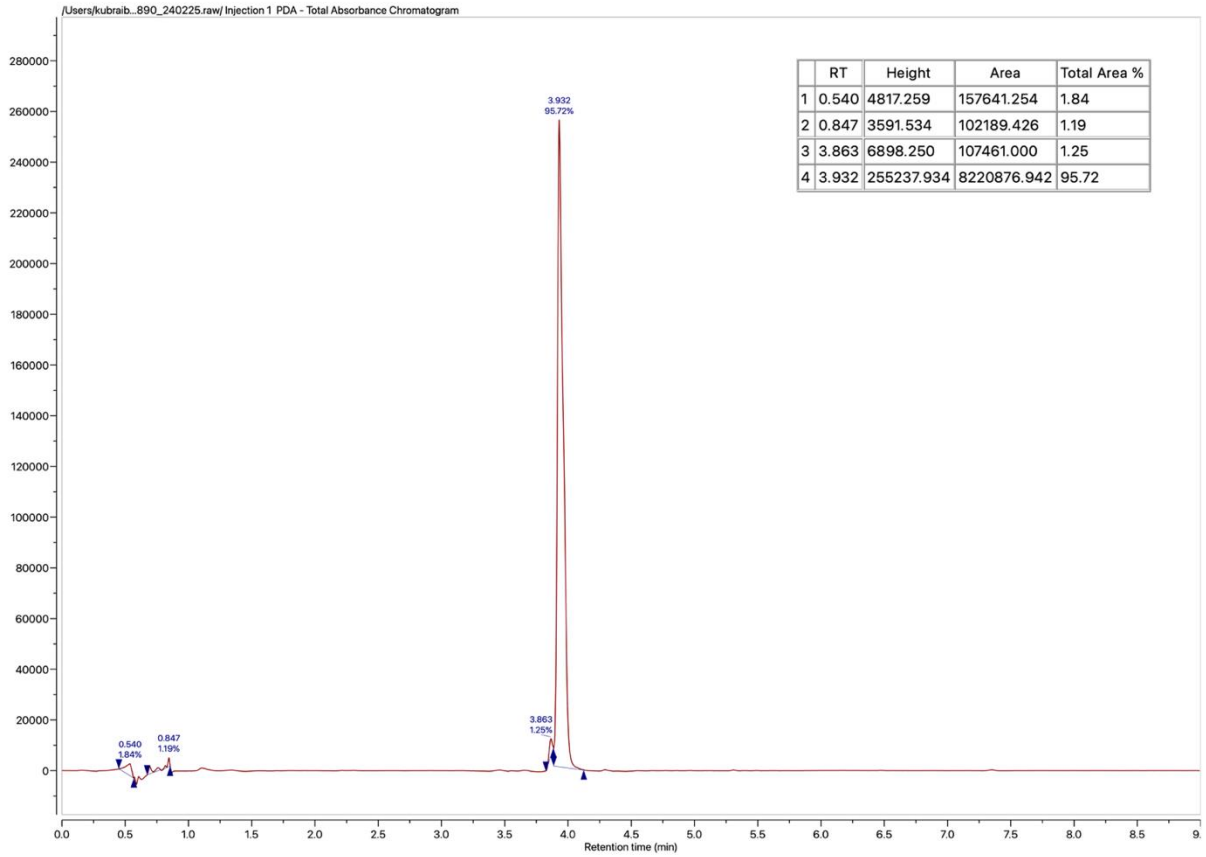


Figure S18: UPLC spectrum of compound **11**

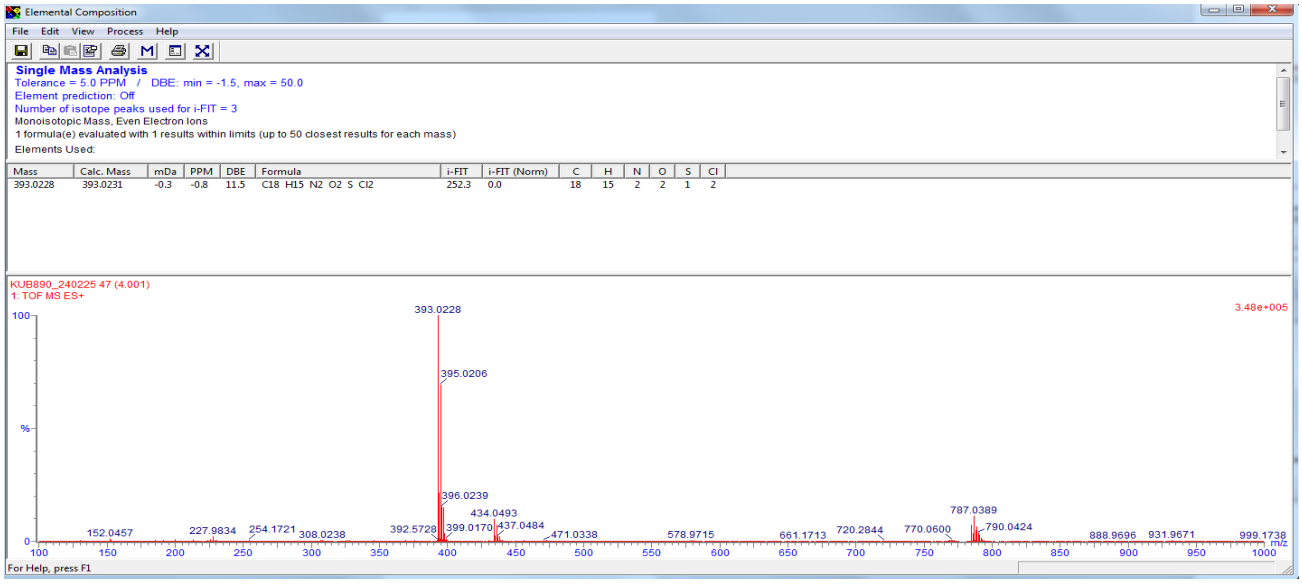


Figure S19: HRMS spectrum of compound **11**

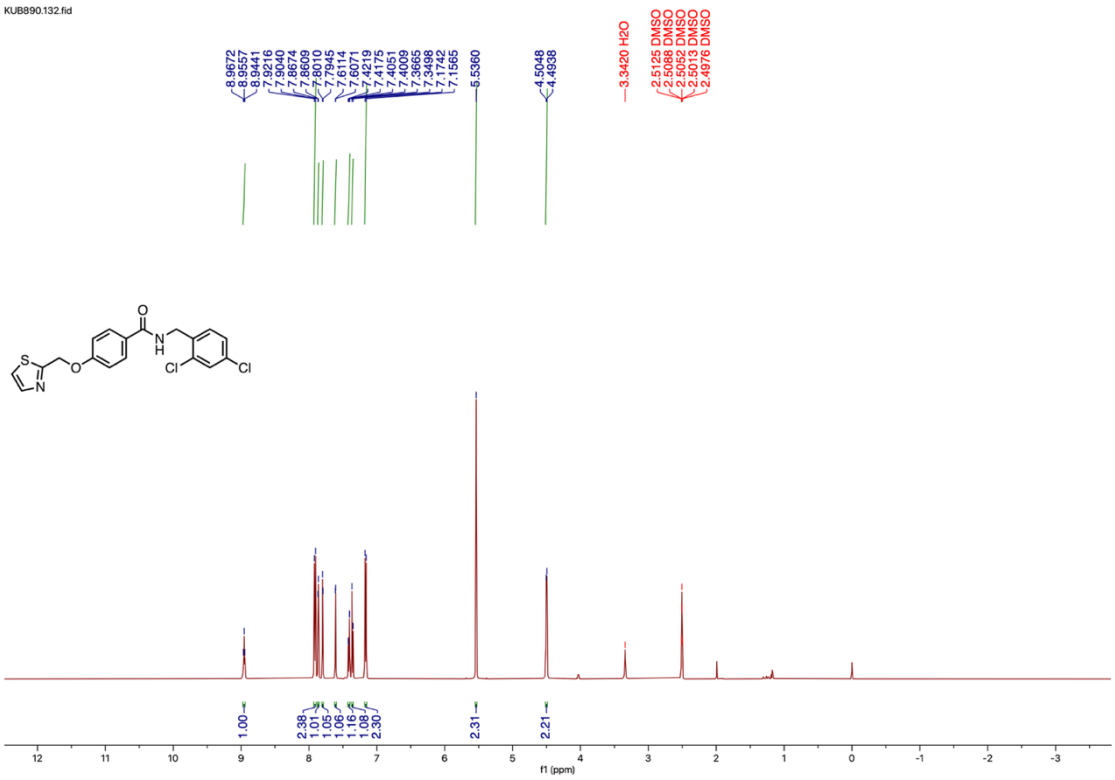


Figure S20a: [1]H-NMR spectrum of compound **11**

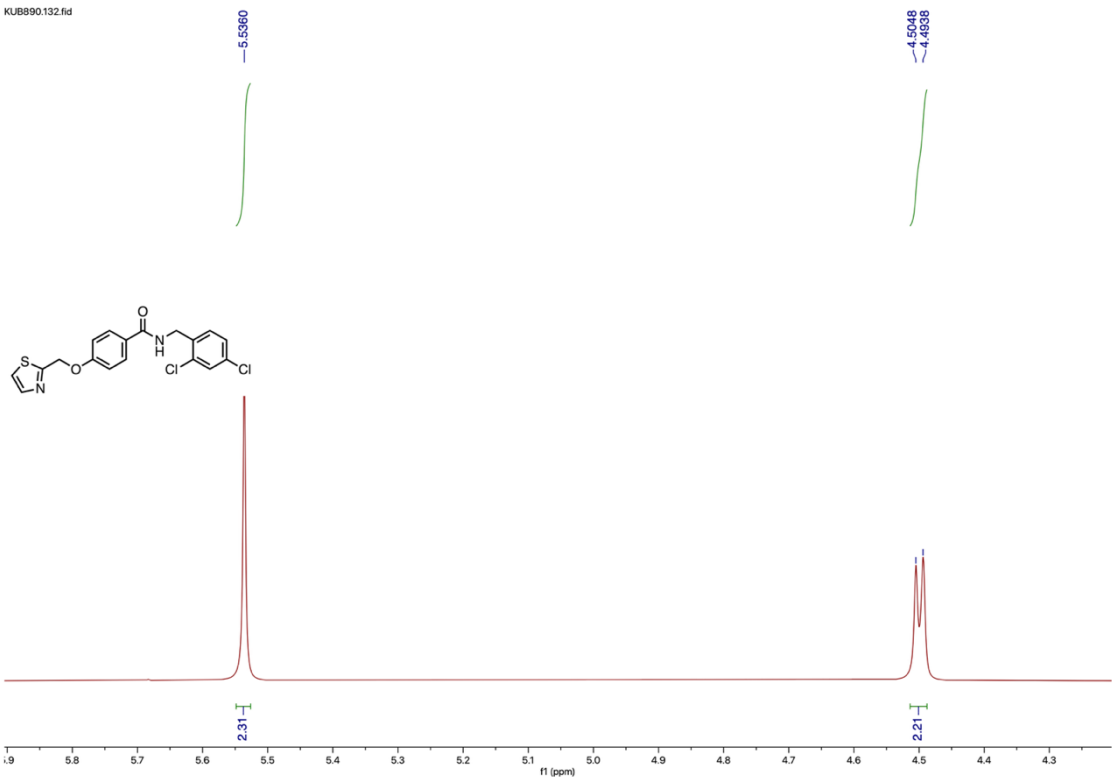


Figure S20b: [1]H-NMR spectrum of compound **11**

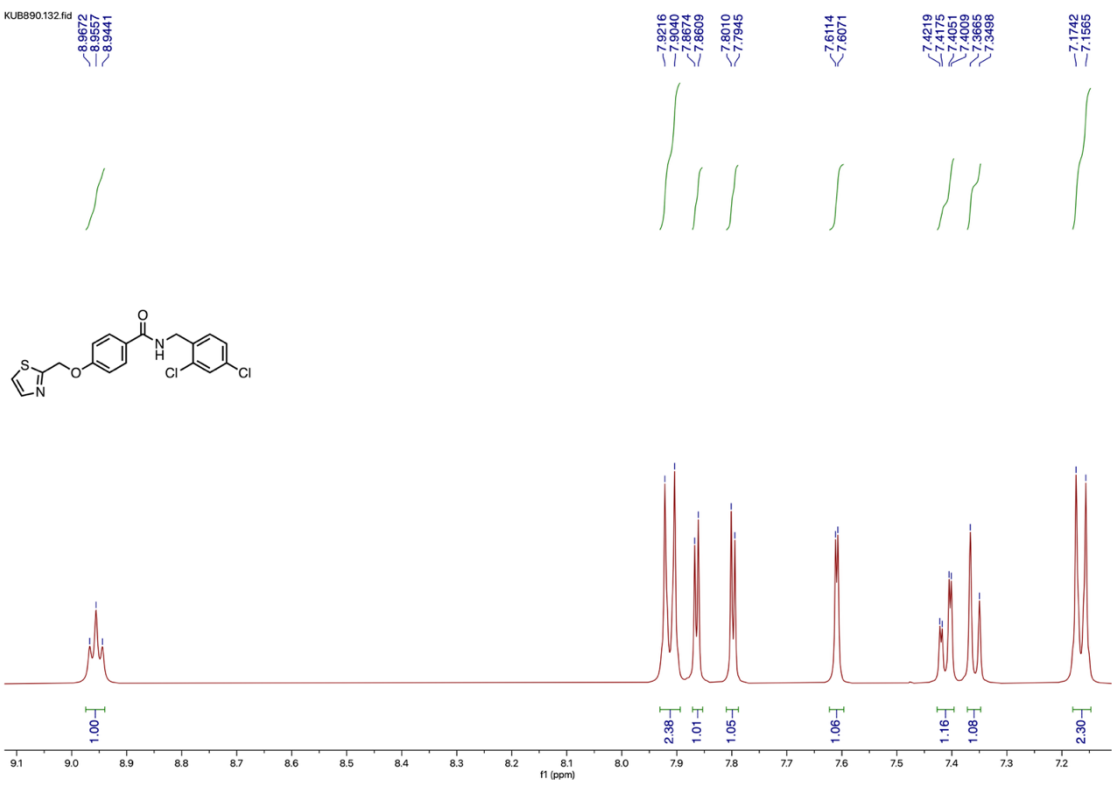


Figure S20c: ^{1}H-NMR spectrum of compound **11**

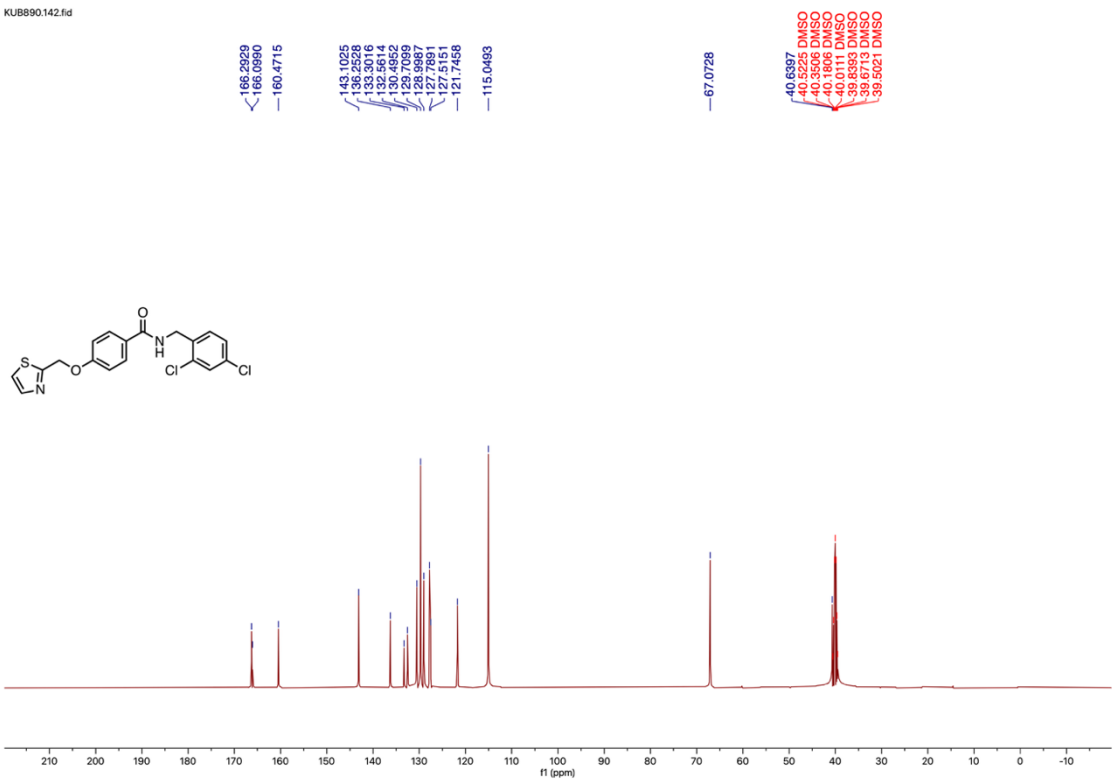


Figure S21a: ^{13}C-NMR spectrum of compound **11**

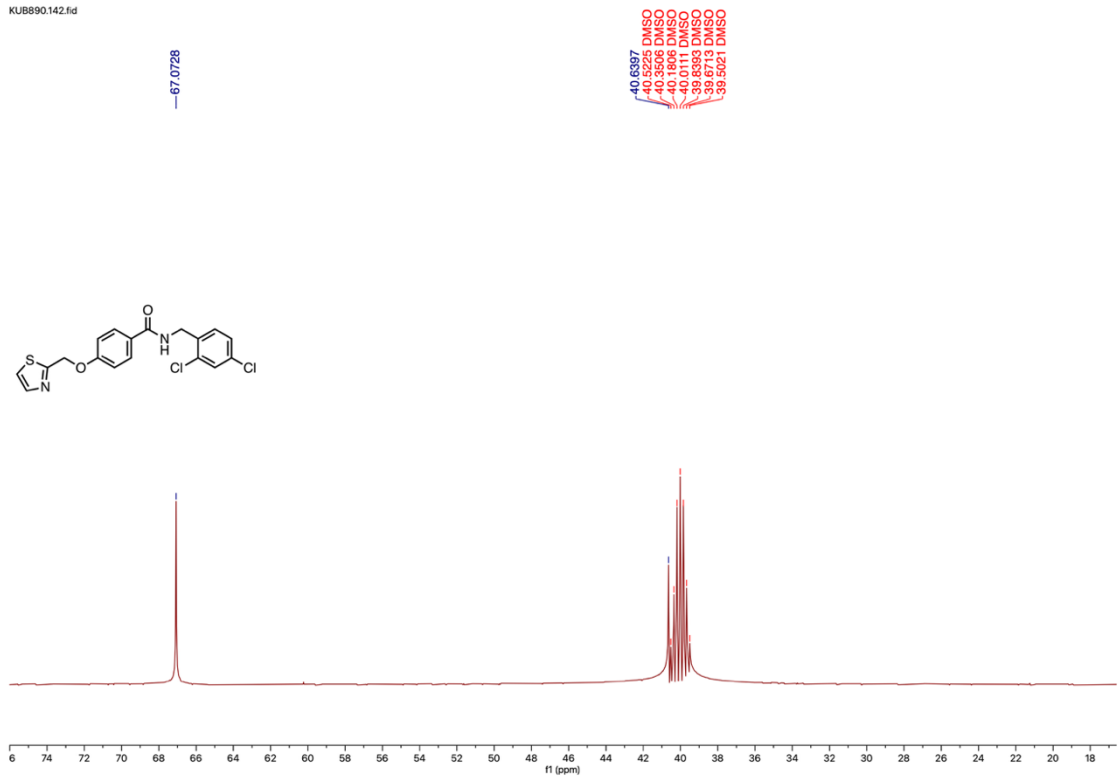


Figure S21b: ^{13}C-NMR spectrum of compound **11**

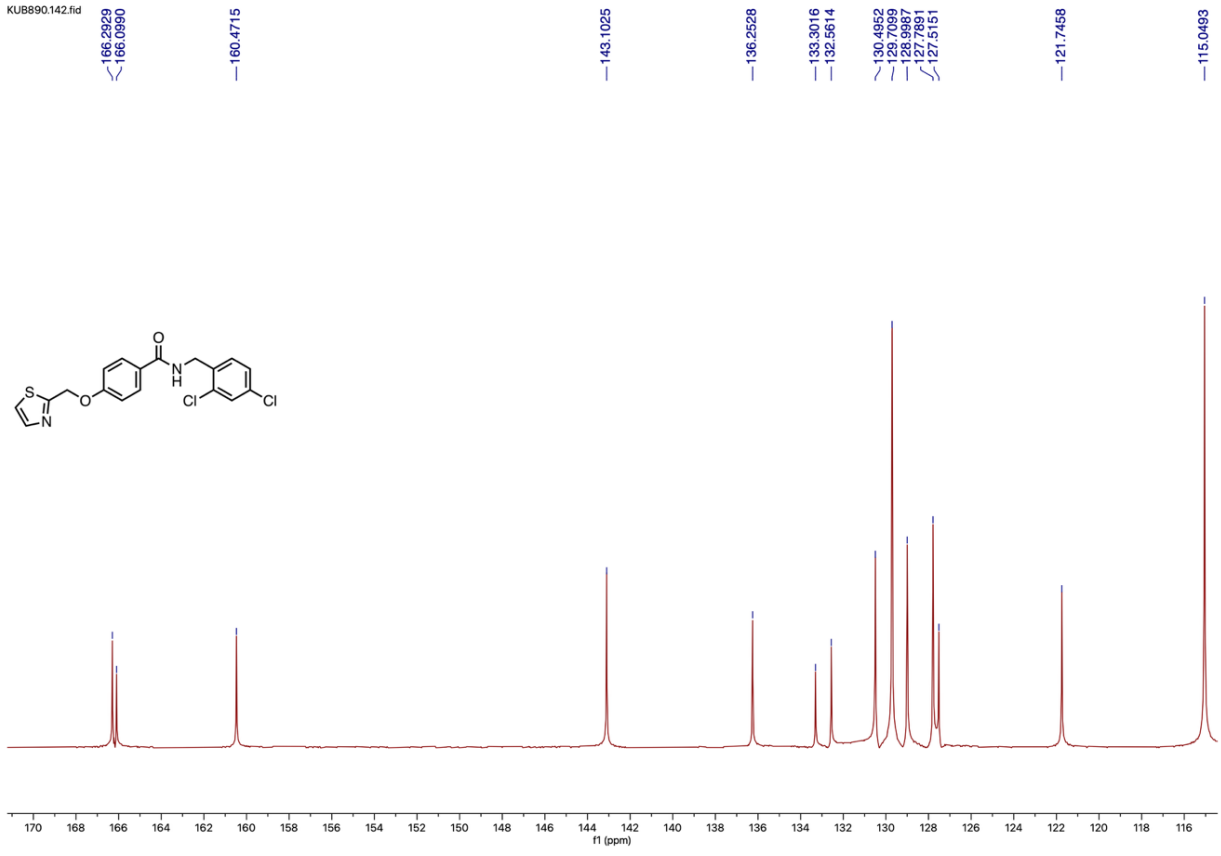
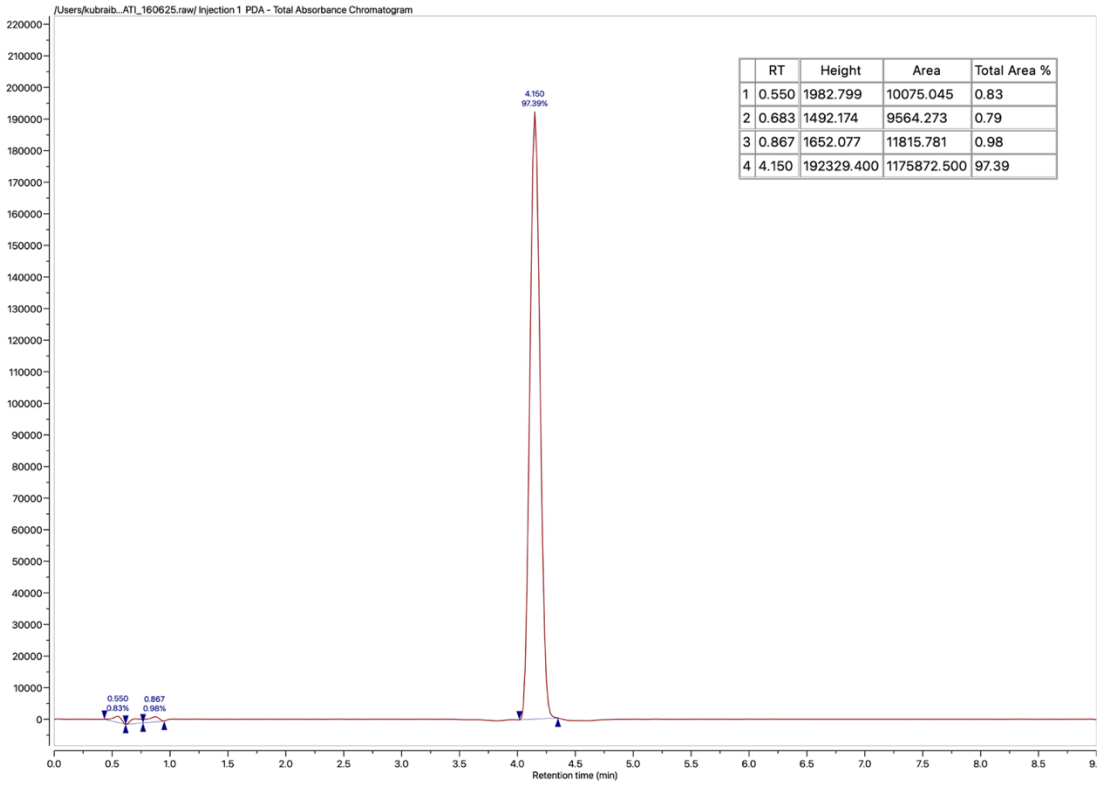


Figure S21c: ^{13}C-NMR spectrum of compound **11**



	RT	Height	Area	Total Area %
1	0.550	1982.799	10075.045	0.83
2	0.683	1492.174	9564.273	0.79
3	0.867	1652.077	11815.781	0.98
4	4.150	192329.400	1175872.500	97.39

Figure S22: UPLC spectrum of compound **12**

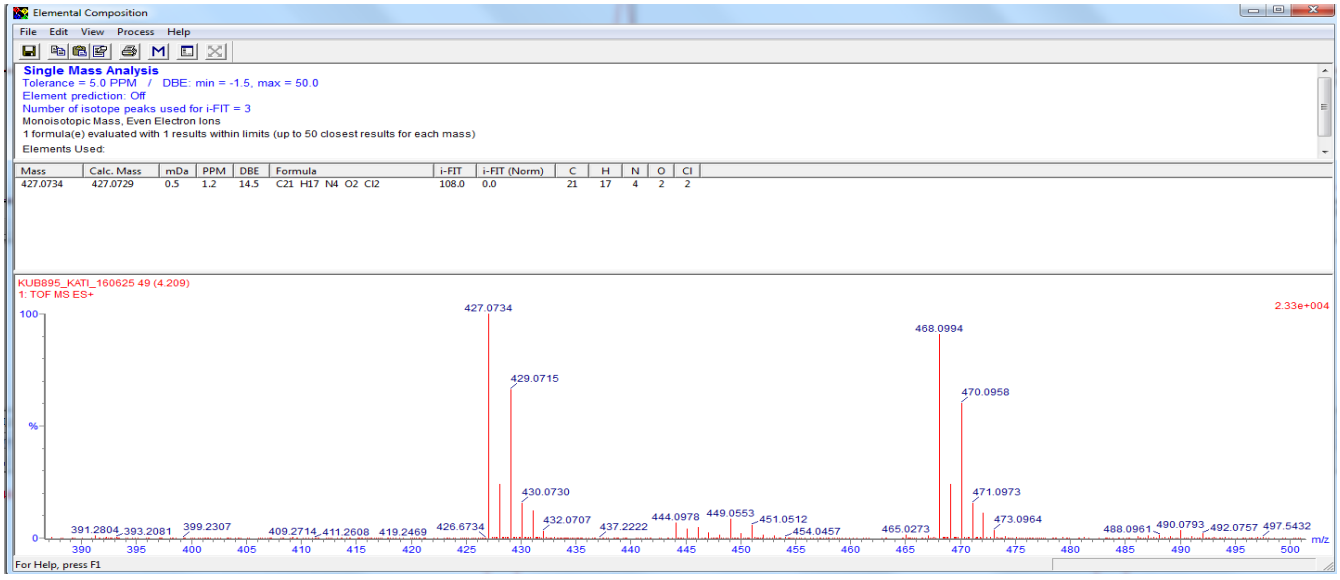


Figure S23: HRMS spectrum of compound **12**

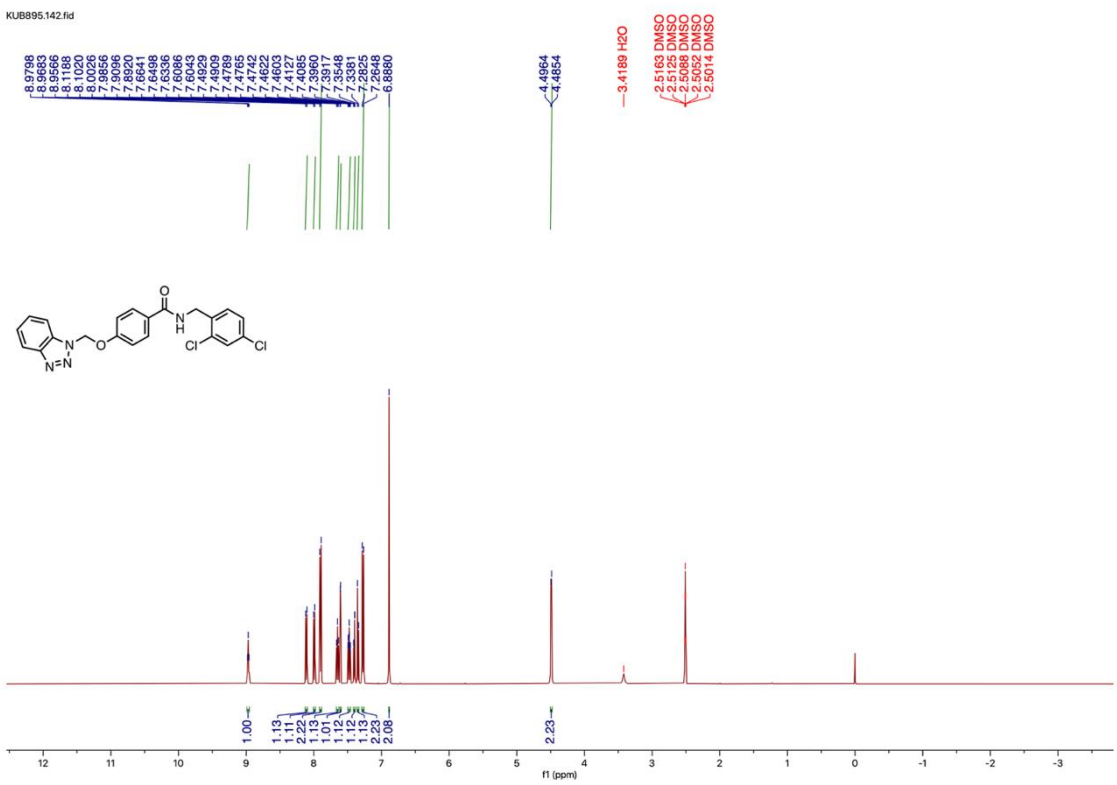


Figure S24a: [1]H-NMR spectrum of compound **12**

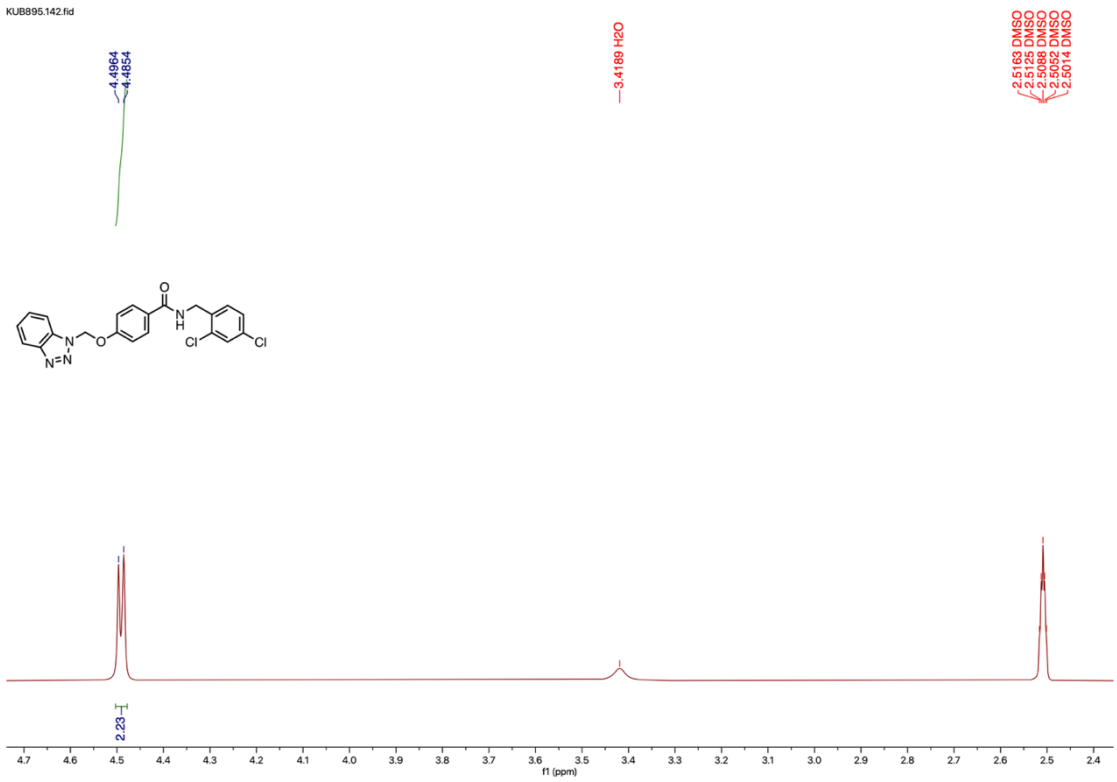


Figure S24b:[1]H-NMR spectrum of compound **12**

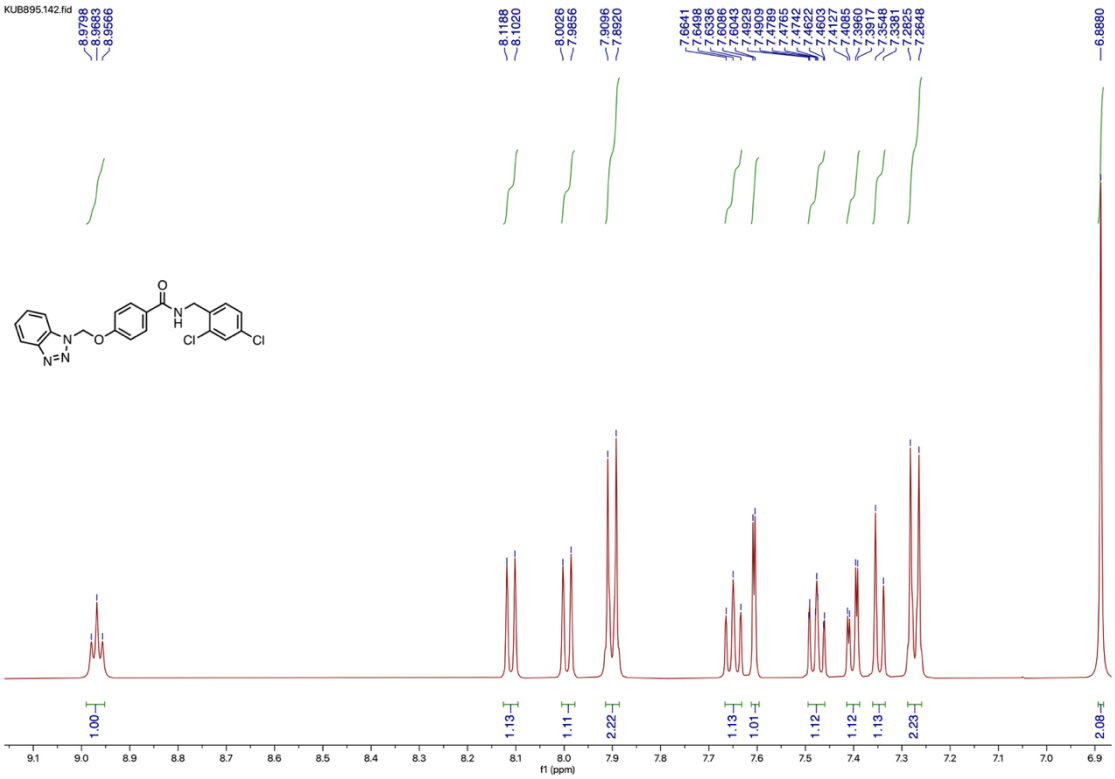


Figure S24c: ^{1}H-NMR spectrum of compound **12**

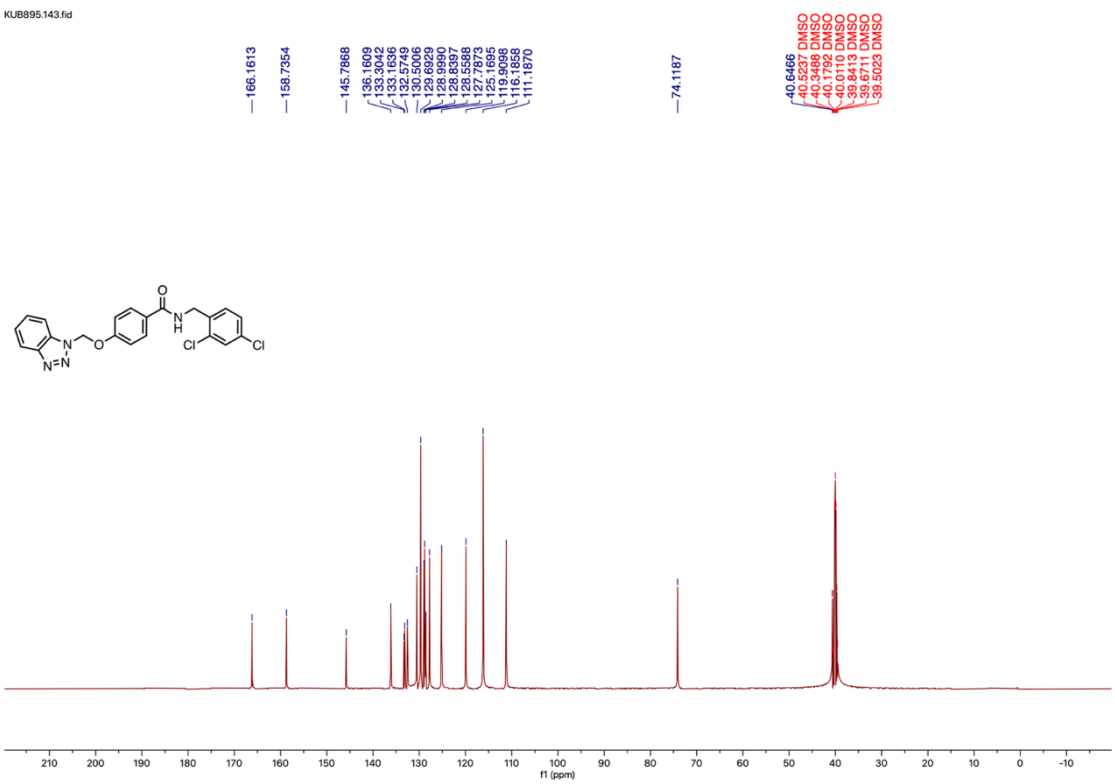


Figure S25a: ^{13}C-NMR spectrum of compound **12**

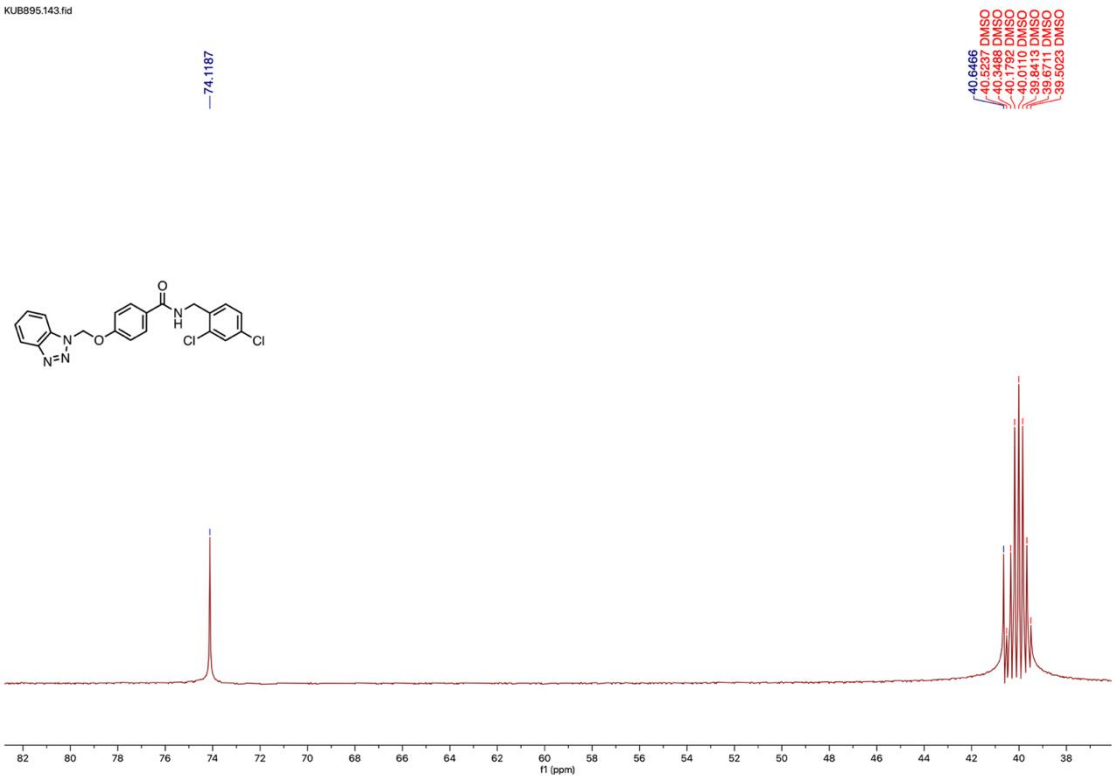


Figure S25b: ^{13}C-NMR spectrum of compound **12**

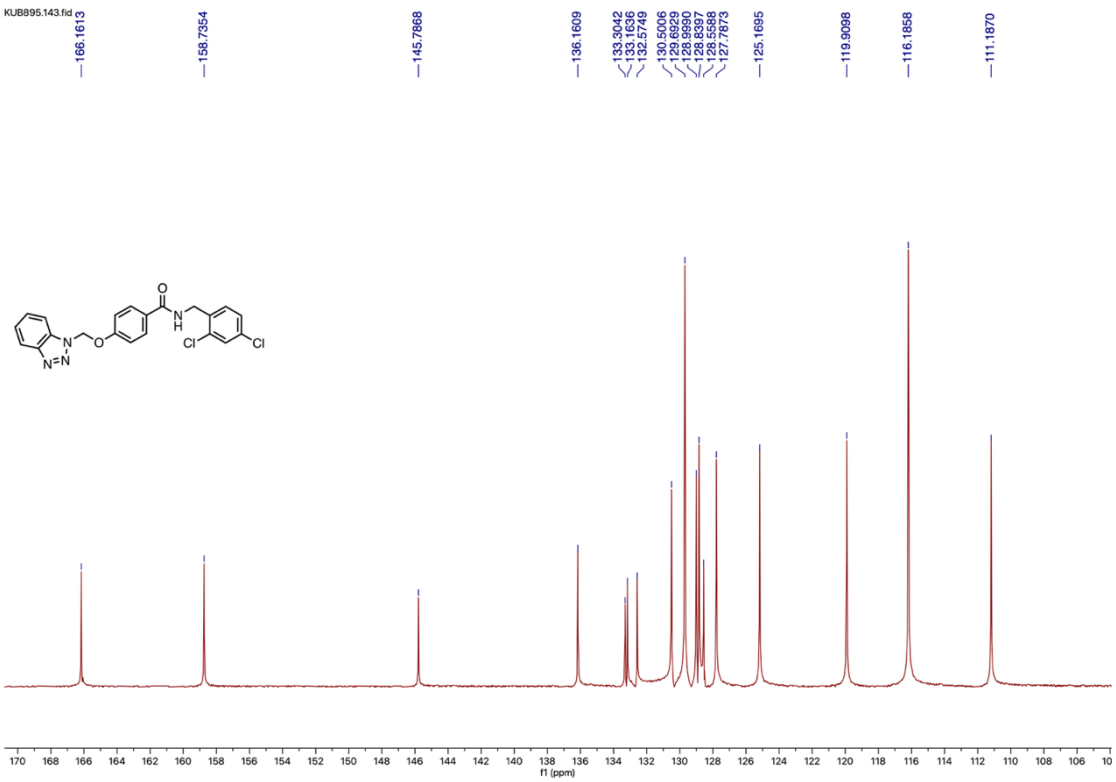


Figure S25c: ^{13}C-NMR spectrum of compound **12**