

Supporting Information

Org. Commun. 18:3 (2025) 166-176

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

Kübra Çalışkan^{1*}, Esra Sadak¹, Paul M. Jordan² and Oliver Werz²

¹Gazi University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 06330, Ankara, Türkiye

²Department of Pharmaceutical/Medicinal Chemistry, Institute of Pharmacy, Friedrich Schiller University Jena, Philosophenweg 14, D-07743, Jena, Germany

Table of Contents	Page
Characterization of intermediate compounds 1-6	3
Induced fit docking (IFD) analysis of final compounds 7-12	4
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.	4
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.	4
UPLC, HRMS, ¹ H, and ¹³ C NMR data of final compounds 7-12	5
Figure S2: UPLC spectrum of compound 7	5
Figure S3: HRMS spectrum of compound 7	5
Figure S4a: ¹ H-NMR spectrum of compound 7	6
Figure S4b: ¹ H-NMR spectrum of compound 7	6
Figure S4c: ¹ H-NMR spectrum of compound 7	7
Figure S5a: ¹³ C-NMR spectrum of compound 7	7
Figure S5b: ¹³ C-NMR spectrum of compound 7	8
Figure S5c: ¹³ C-NMR spectrum of compound 7	8
Figure S6: UPLC spectrum of compound 8	9
Figure S7: HRMS spectrum of compound 8	9
Figure S8a: ¹ H-NMR spectrum of compound 8	10
Figure S8b: ¹ H-NMR spectrum of compound 8	10
Figure S8c: ¹ H-NMR spectrum of compound 8	11
Figure S9a: ¹³ C-NMR spectrum of compound 8	11
Figure S9b: ¹³ C-NMR spectrum of compound 8	12
Figure S9c: ¹³ C-NMR spectrum of compound 8	12
Figure S10: UPLC spectrum of compound 9	13
Figure S11: HRMS spectrum of compound 9	13
Figure S12a: ¹ H-NMR spectrum of compound 9	14
Figure S12b: ¹ H-NMR spectrum of compound 9	14
Figure S12c: ¹ H-NMR spectrum of compound 9	15
Figure S13a: ¹³ C-NMR spectrum of compound 9	15

© 2025 ACG Publications. All rights reserved.

Figure S13b: ^{13}C -NMR spectrum of compound 9	16
Figure S13c: ^{13}C -NMR spectrum of compound 9	16
Figure S14: UPLC spectrum of compound 10	17
Figure S15: HRMS spectrum of compound 10	17
Figure S16a: ^1H -NMR spectrum of compound 10	18
Figure S16b: ^1H -NMR spectrum of compound 10	18
Figure S16c: ^1H -NMR spectrum of compound 10	19
Figure S17a: ^{13}C -NMR spectrum of compound 10	19
Figure S17b: ^{13}C -NMR spectrum of compound 10	20
Figure S17c: ^{13}C -NMR spectrum of compound 10	20
Figure S18: UPLC spectrum of compound 11	21
Figure S19: HRMS spectrum of compound 11	21
Figure S20a: ^1H -NMR spectrum of compound 11	22
Figure S20b: ^1H -NMR spectrum of compound 11	22
Figure S20c: ^1H -NMR spectrum of compound 11	23
Figure S21a: ^{13}C -NMR spectrum of compound 11	23
Figure S21b: ^{13}C -NMR spectrum of compound 11	24
Figure S21c: ^{13}C -NMR spectrum of compound 11	24
Figure S22: UPLC spectrum of compound 12	25
Figure S23: HRMS spectrum of compound 12	25
Figure S24a: ^1H -NMR spectrum of compound 12	26
Figure S24b: ^1H -NMR spectrum of compound 12	26
Figure S24c: ^1H -NMR spectrum of compound 12	27
Figure S25a: ^{13}C -NMR spectrum of compound 12	27
Figure S25b: ^{13}C -NMR spectrum of compound 12	28
Figure S25c: ^{13}C -NMR spectrum of compound 12	28

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. ¹H 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 C₁₃H₁₄NO₄, 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-1H-pyrazole according to the general procedure described above. Yield: 96%; m.p. 126-127 °C. ¹H 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 C₁₃H₁₅N₂O₃, 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-1H-pyrazole according to the general procedure described above. Yield: 92%; m.p. 188-190 °C. ¹H 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 C₁₃H₁₅N₂O₃, 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-1H-pyrazole according to the general procedure described above. Yield: 78%; m.p. 223-226 °C. ¹H 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 C₁₃H₁₅N₂O₃, 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. ¹H 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 C₁₁H₁₀NO₃S, 236.0381; found, 236.0392. CAS: 1082818-84-5.

4-((1H-benzotriazol-1-yl)methoxy)benzoic acid (6): The compound was synthesized using 1-(chloromethyl)-1H-benzotriazole according to the general procedure described above. Yield: 95%; m.p. 227-229 °C. ¹H 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 C₁₄H₁₁NO₃, 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.

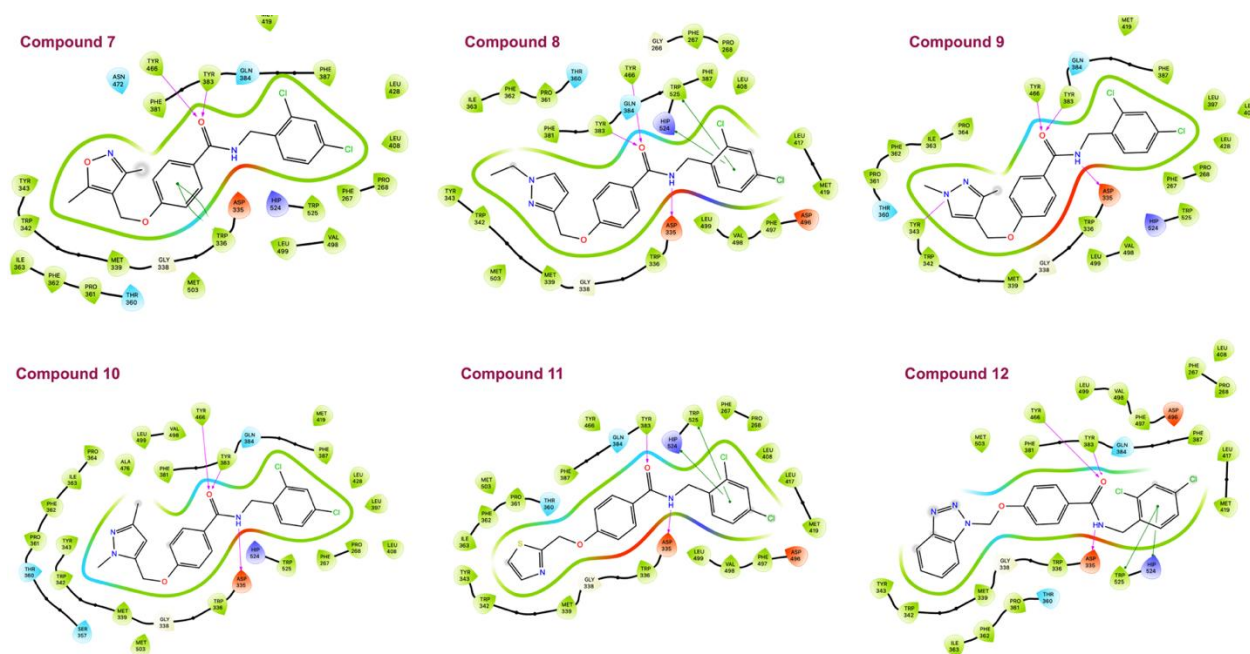


Figure S1. Ligand interaction diagrams showing the best IFD poses of final compounds 7–12 in complex with sEH. The diagrams illustrate hydrogen bonds, hydrophobic contacts, and π – π stacking interactions of the ligands in the IFD-predicted binding site.

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

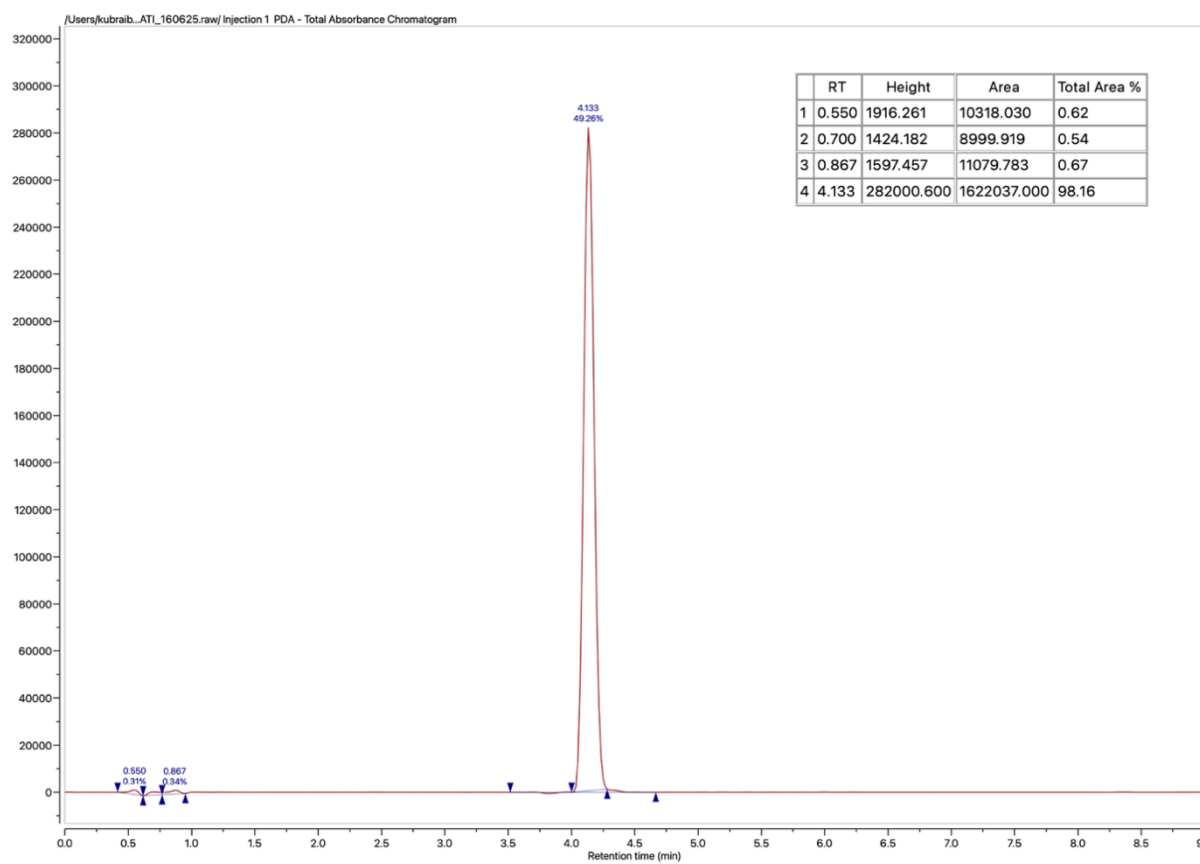


Figure S2: UPLC spectrum of compound 7

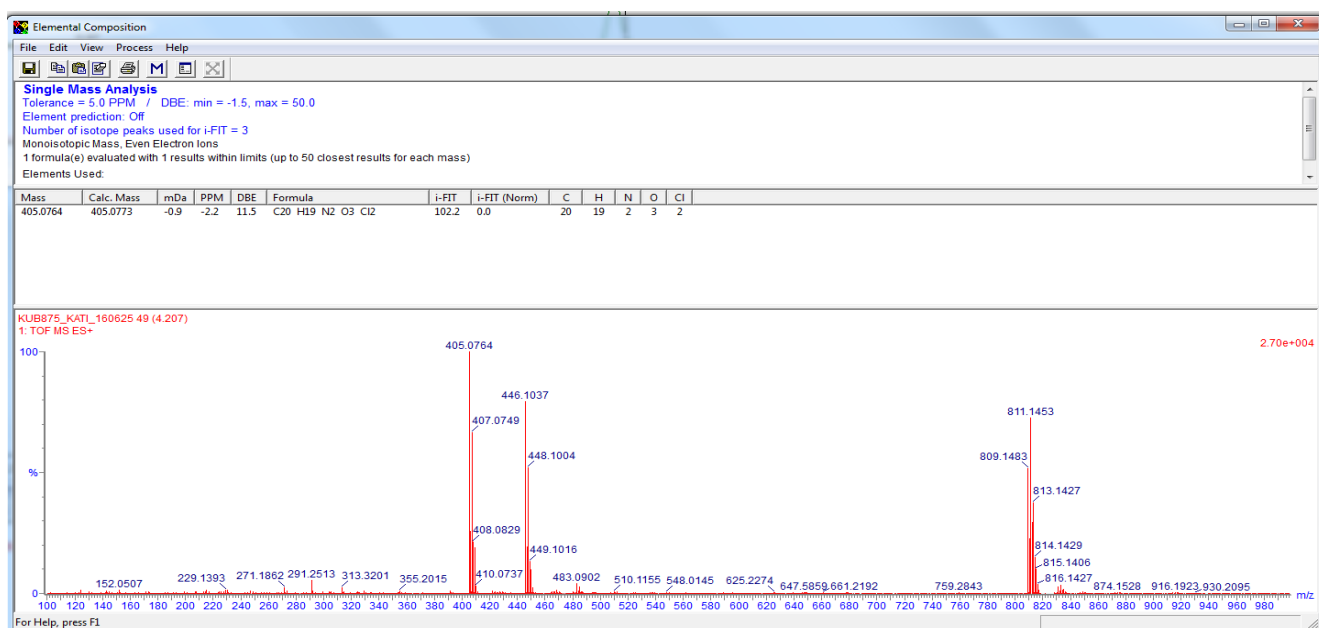


Figure S3: HRMS spectrum of compound 7

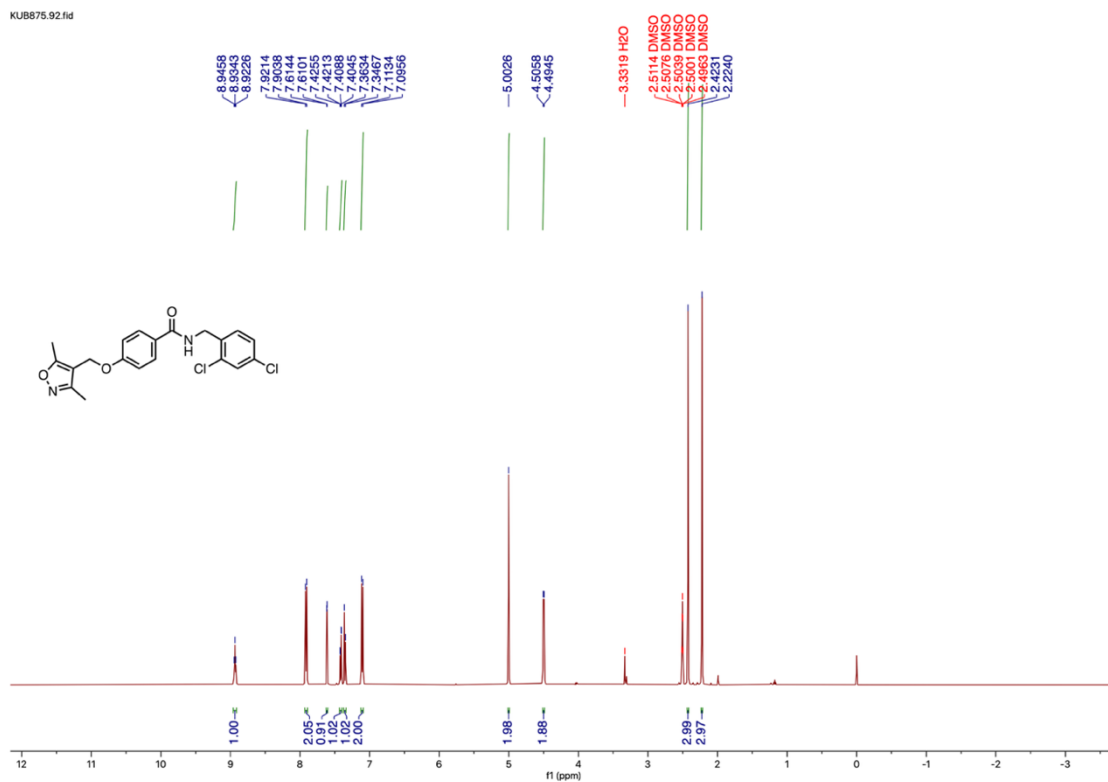


Figure S4a: ¹H-NMR spectrum of compound 7

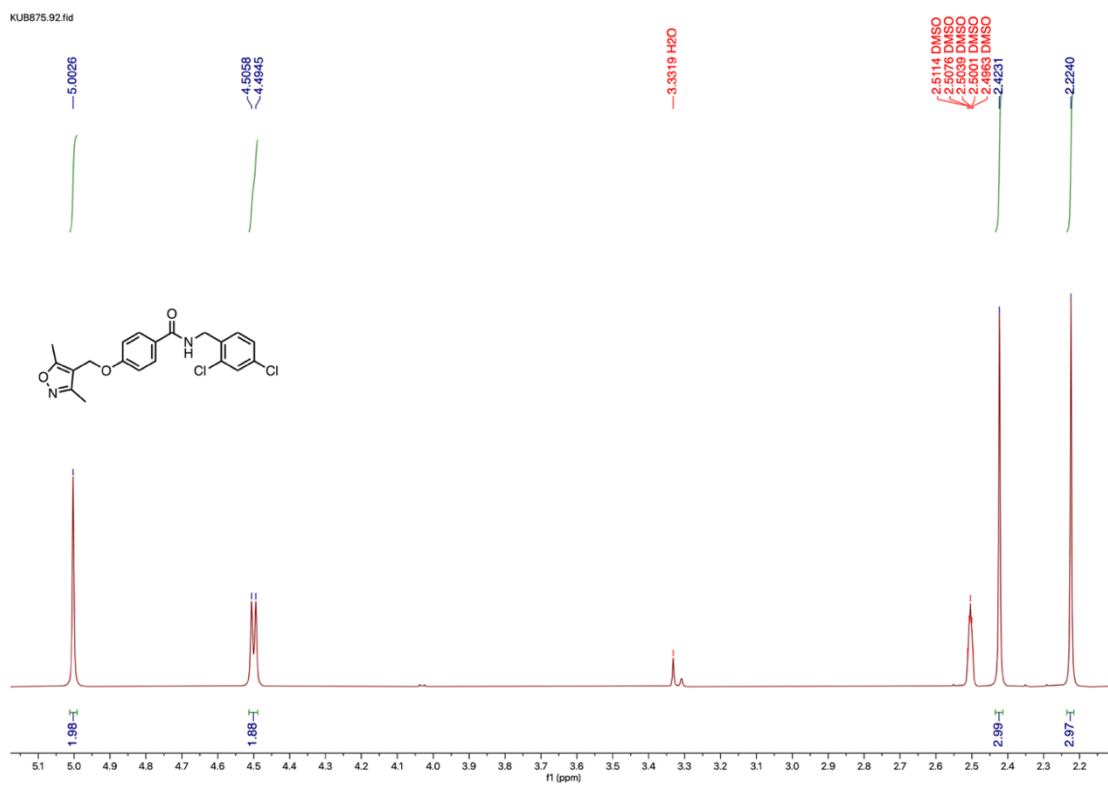


Figure S4b: ¹H-NMR spectrum of compound 7

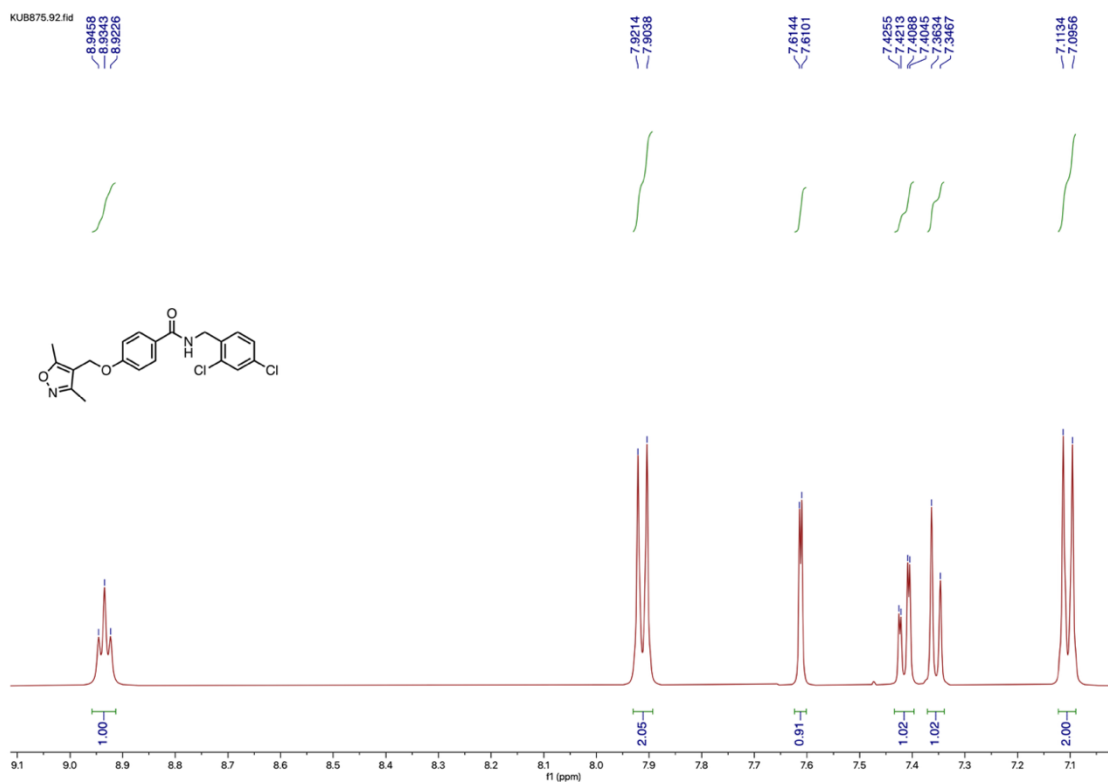


Figure S4c: ^1H -NMR spectrum of compound **7**

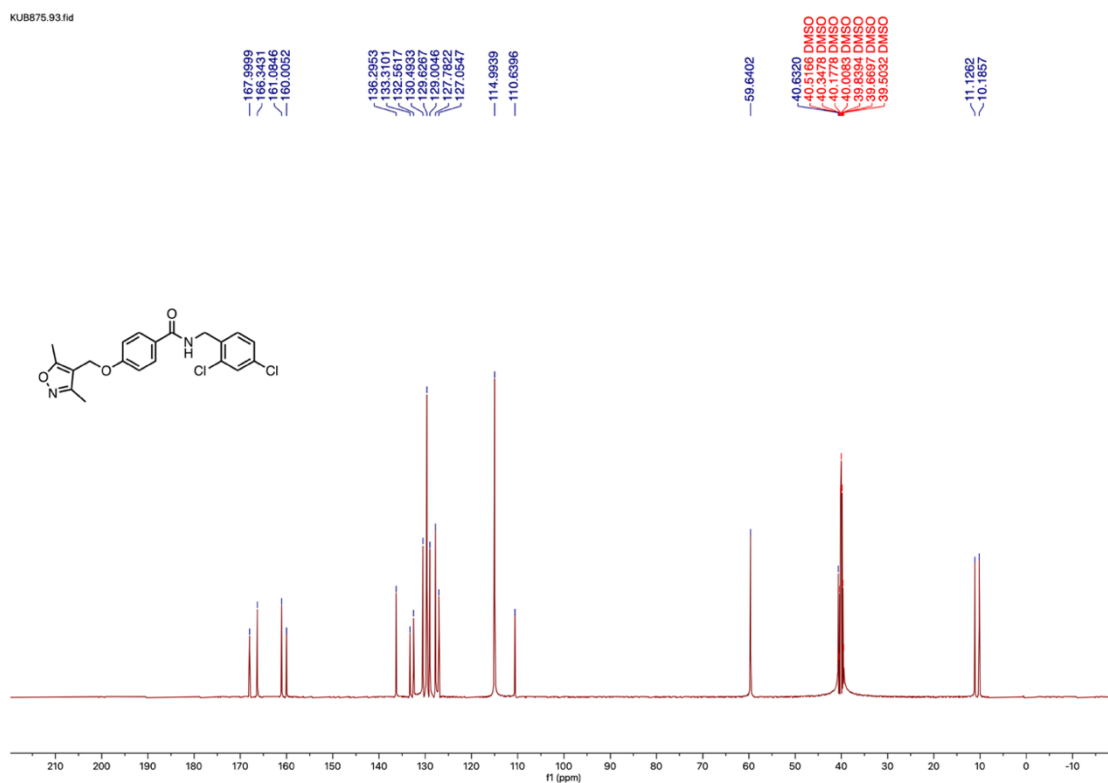


Figure S5a: ^{13}C -NMR spectrum of compound **7**

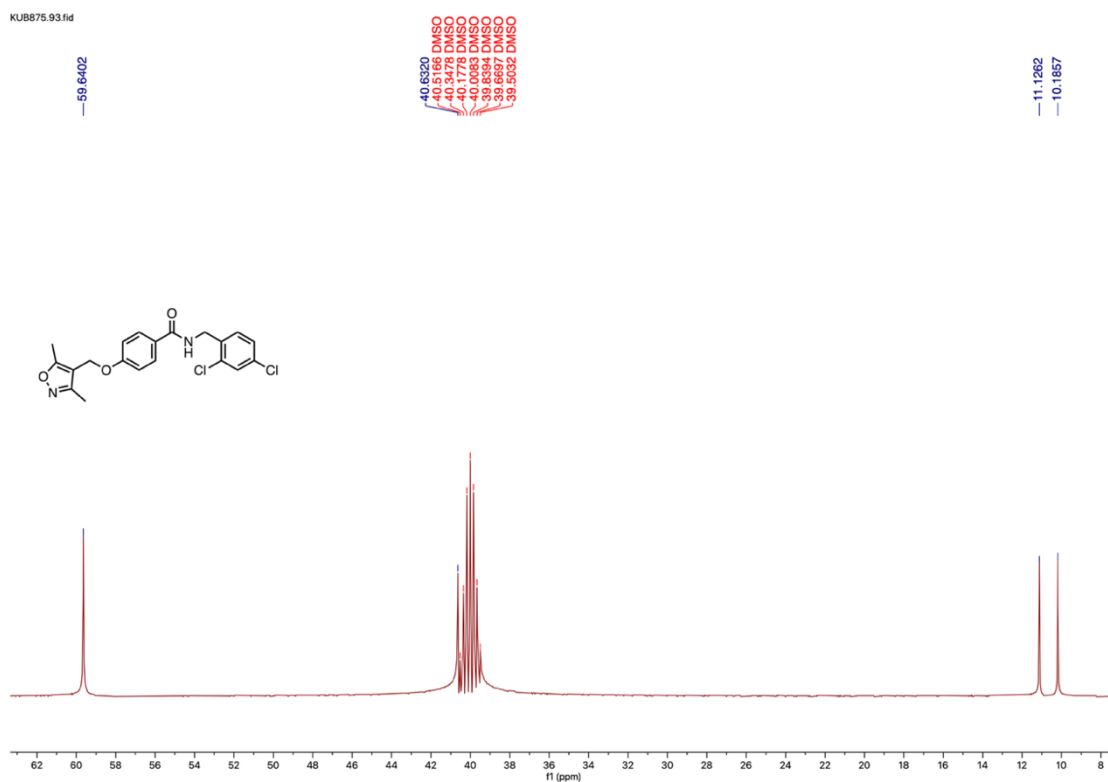


Figure S5b: ^{13}C -NMR spectrum of compound 7

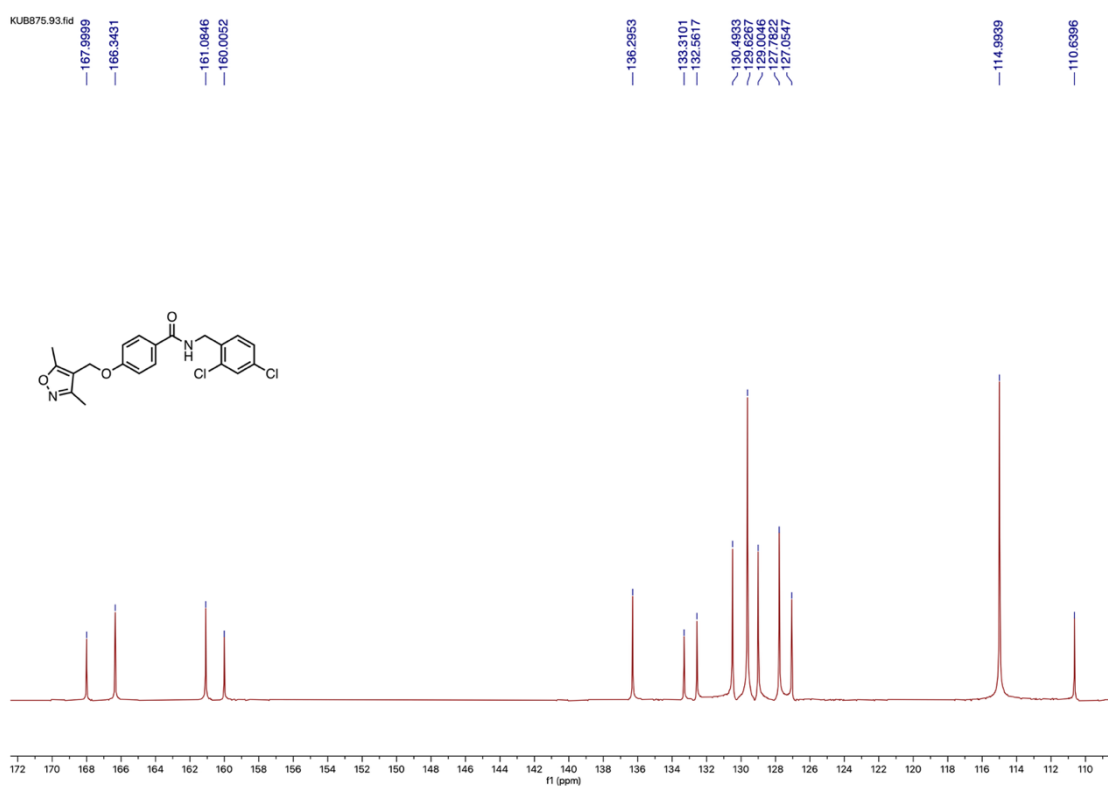


Figure S5c: ^{13}C -NMR spectrum of compound 7

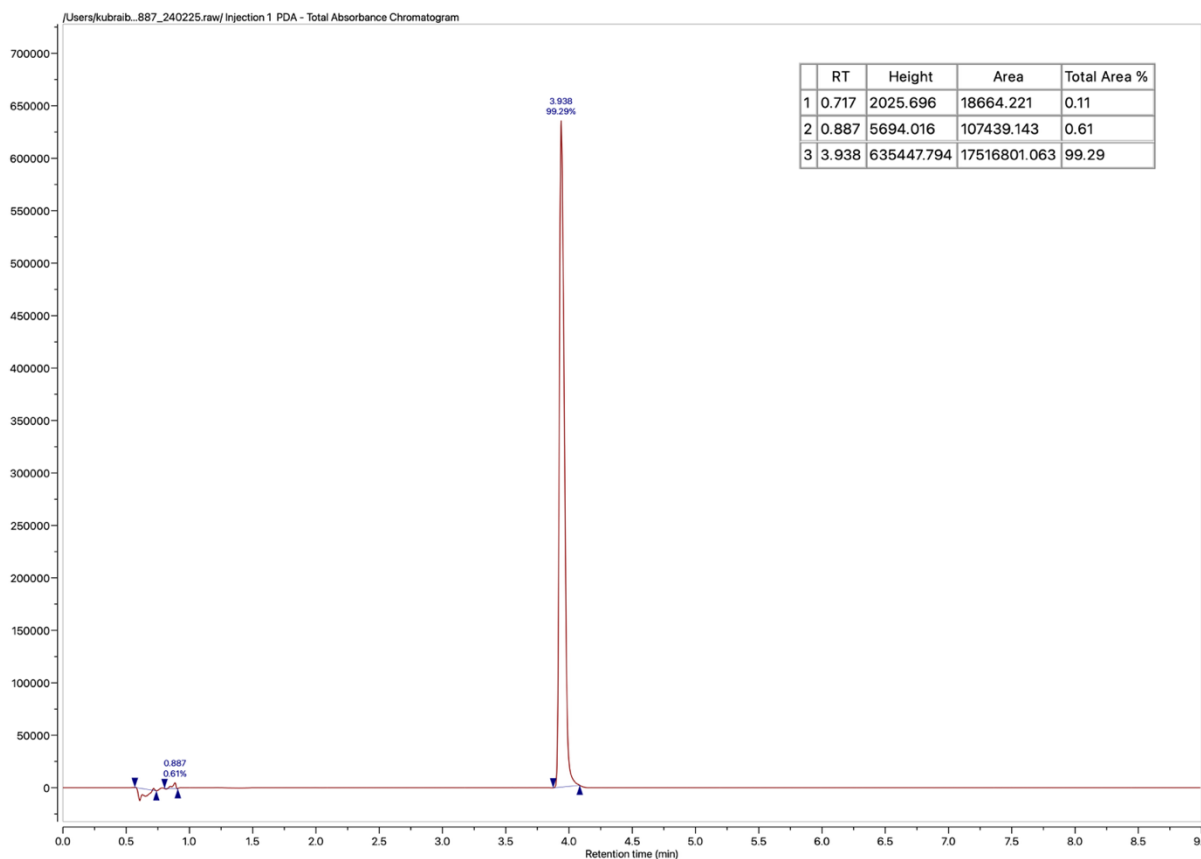


Figure S6: UPLC spectrum of compound 8

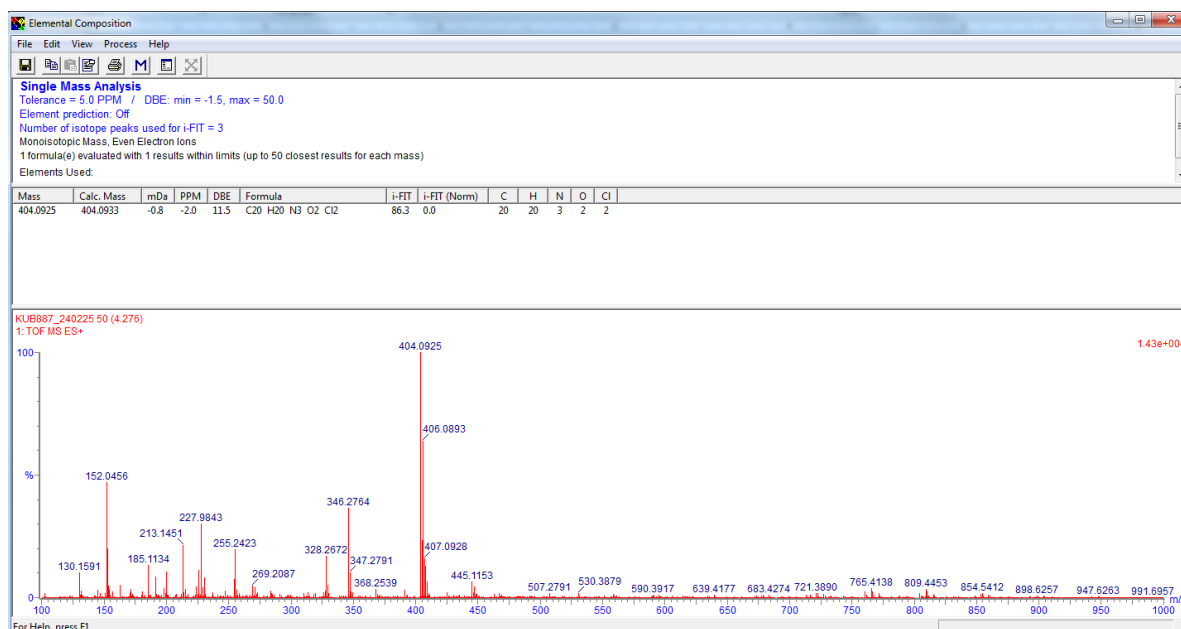


Figure S7: HRMS spectrum of compound 8

KU8887102.fid

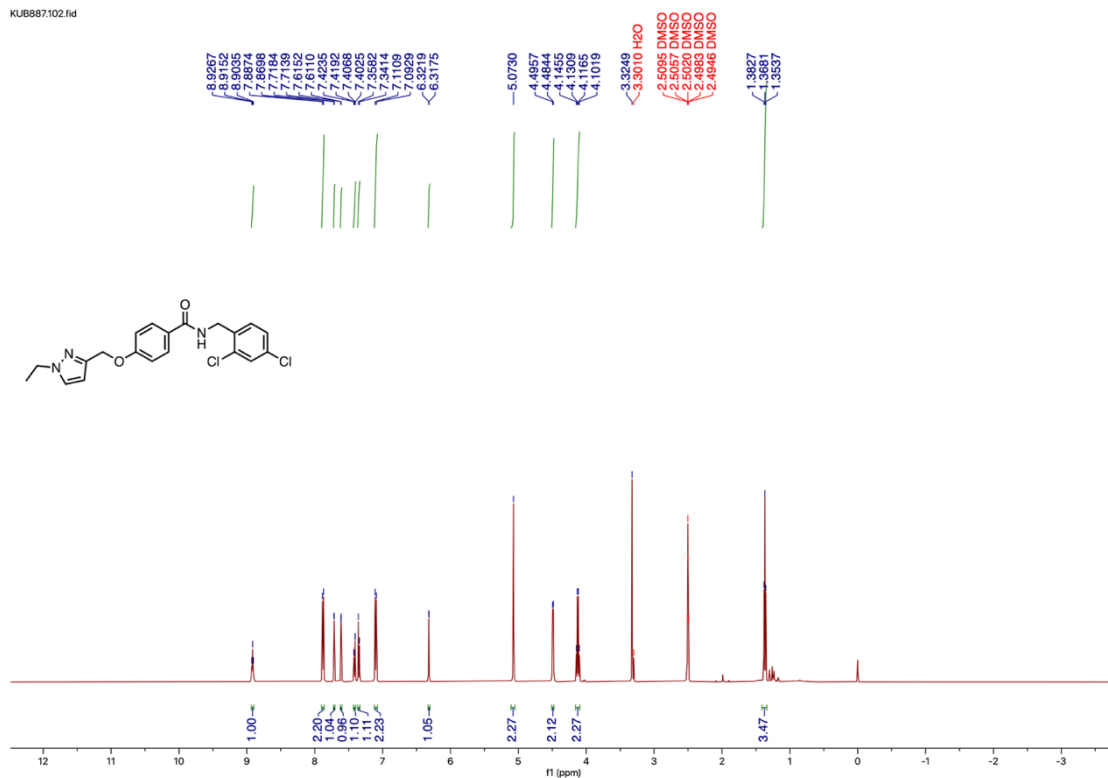


Figure S8a: ¹H-NMR spectrum of compound 8

KU8887102.fid

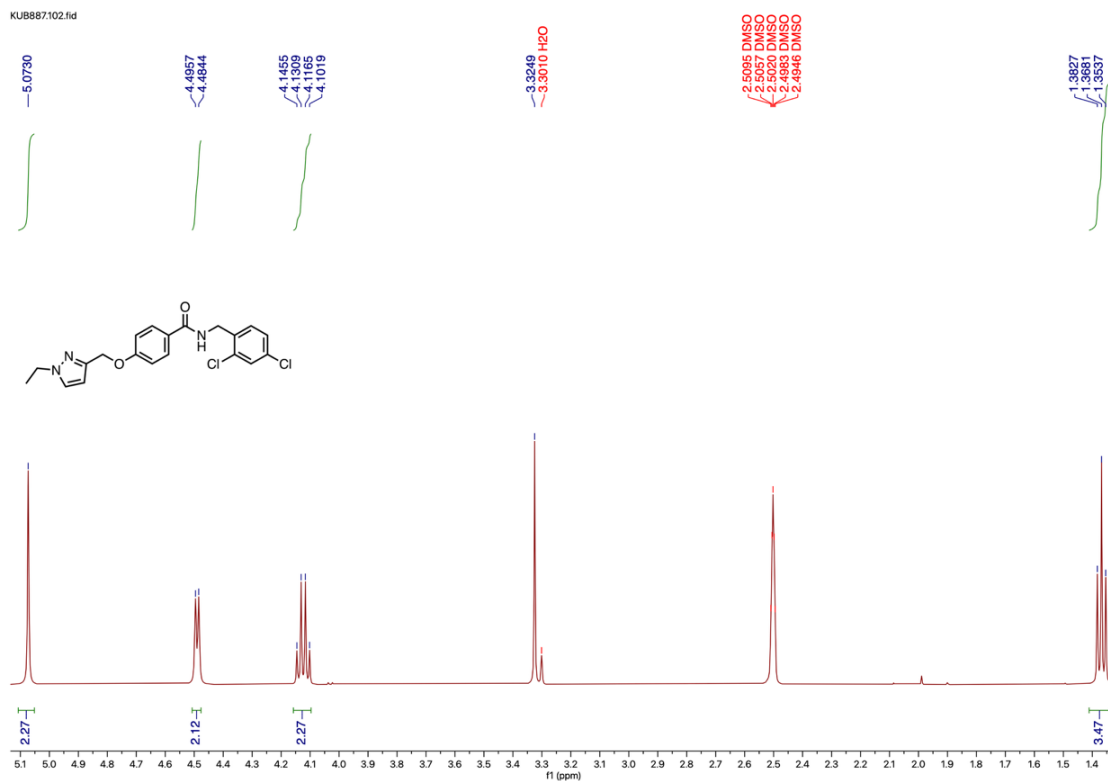


Figure S8b: ¹H-NMR spectrum of compound 8

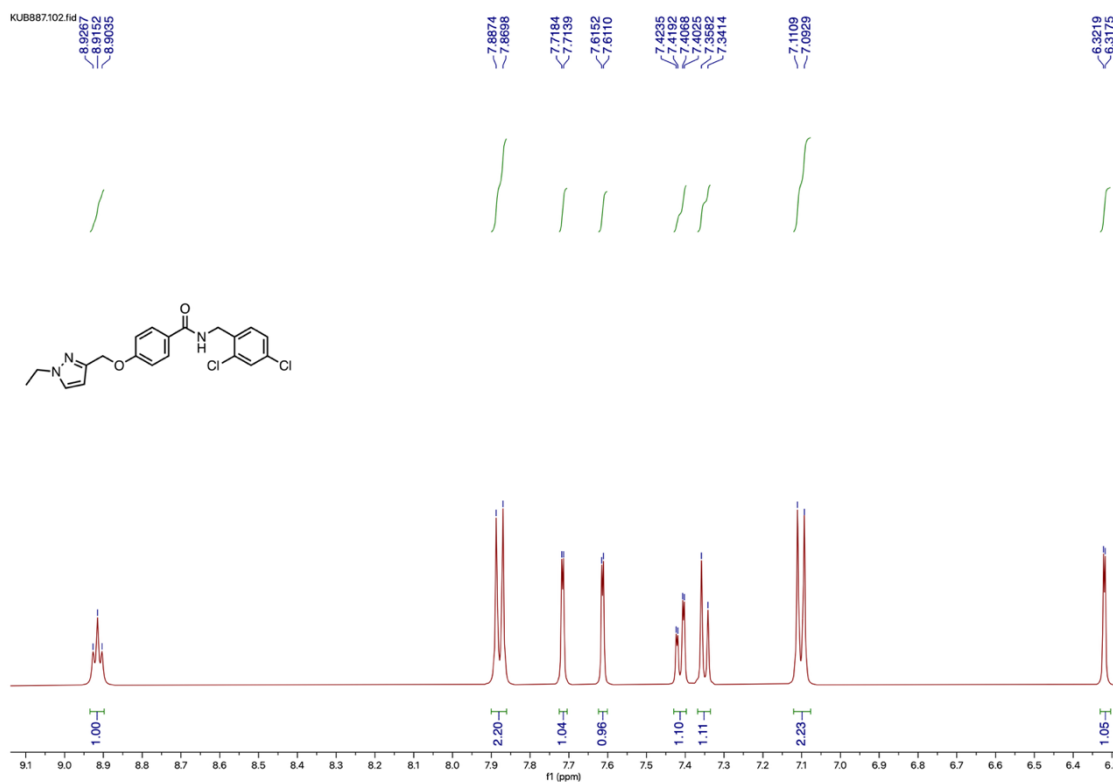


Figure S8c: ^1H -NMR spectrum of compound **8**

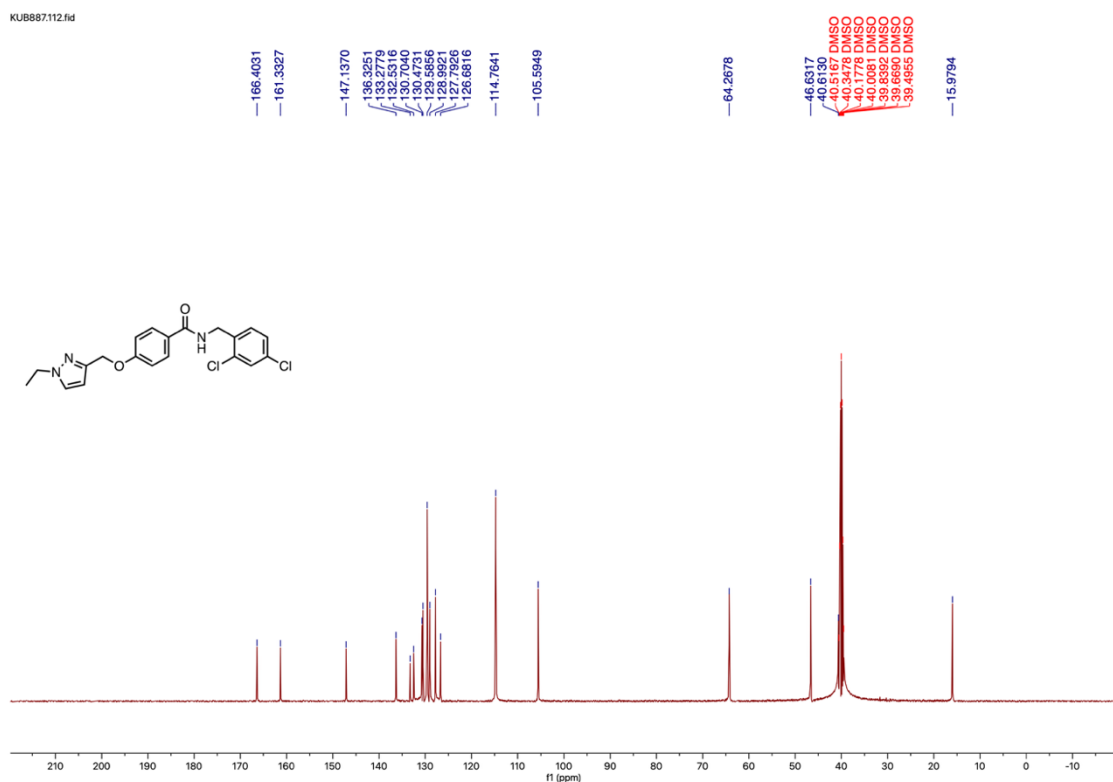


Figure S9a: ^{13}C -NMR spectrum of compound **8**

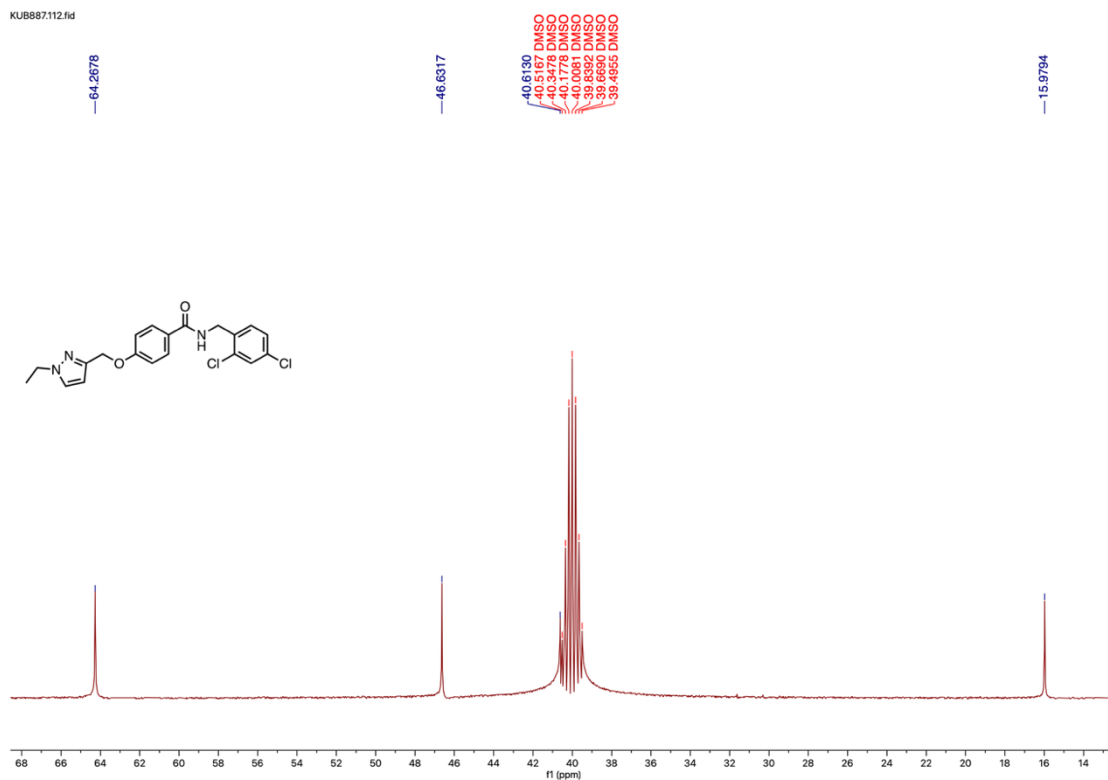


Figure S9b: ^{13}C -NMR spectrum of compound **8**

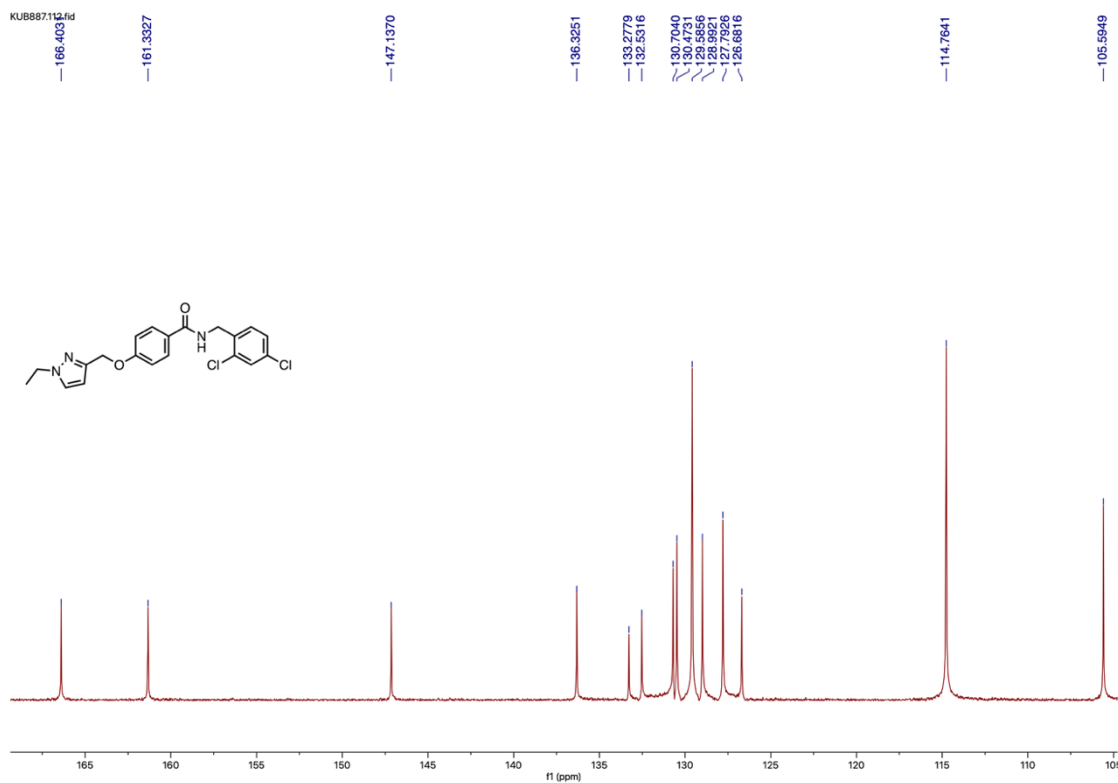


Figure S9c: ^{13}C -NMR spectrum of compound **8**

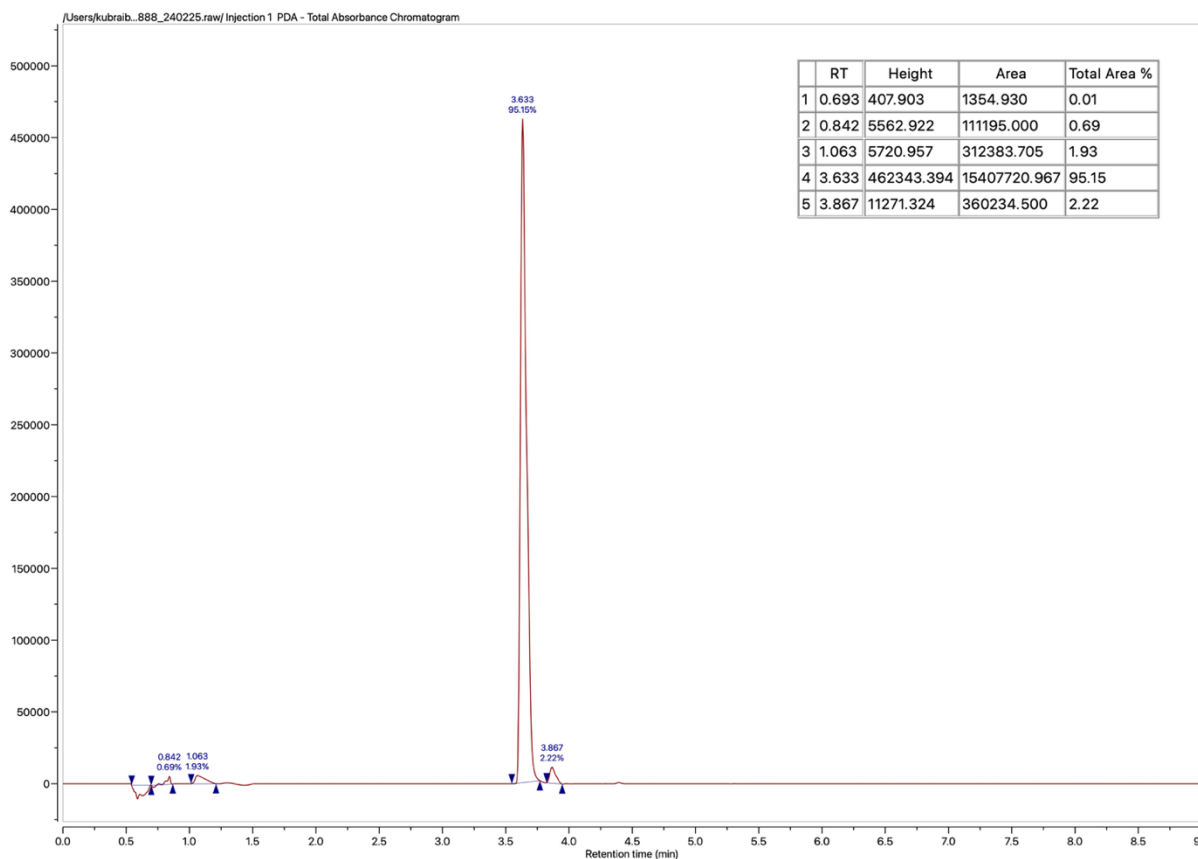


Figure S10: UPLC spectrum of compound 9

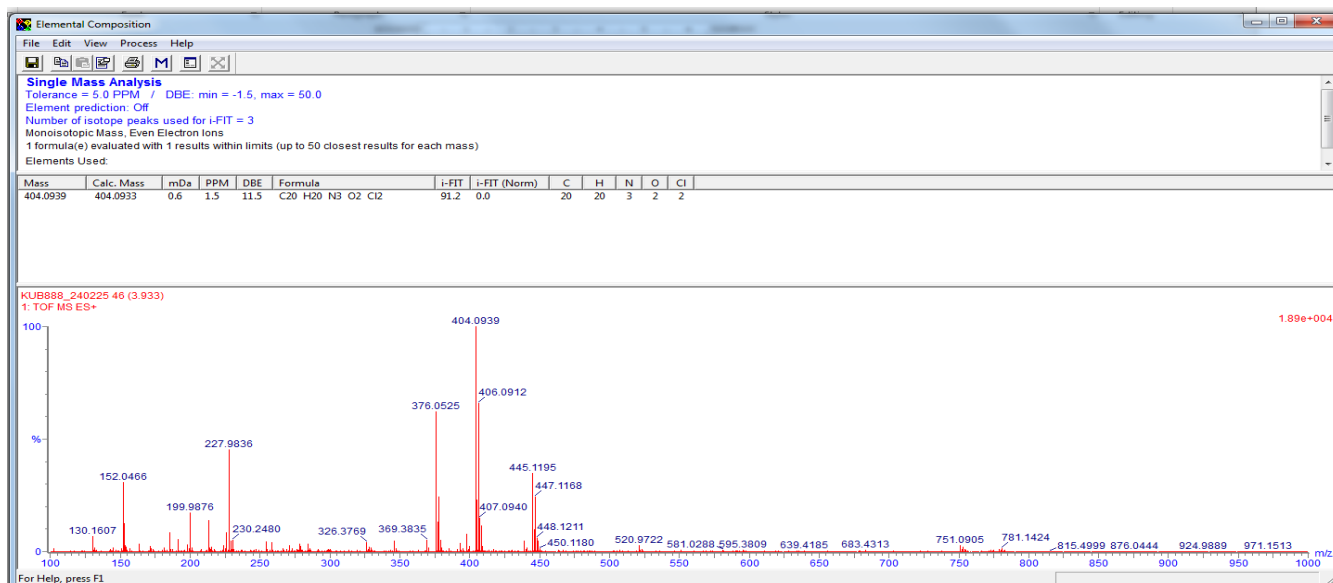


Figure S11: HRMS spectrum of compound 9

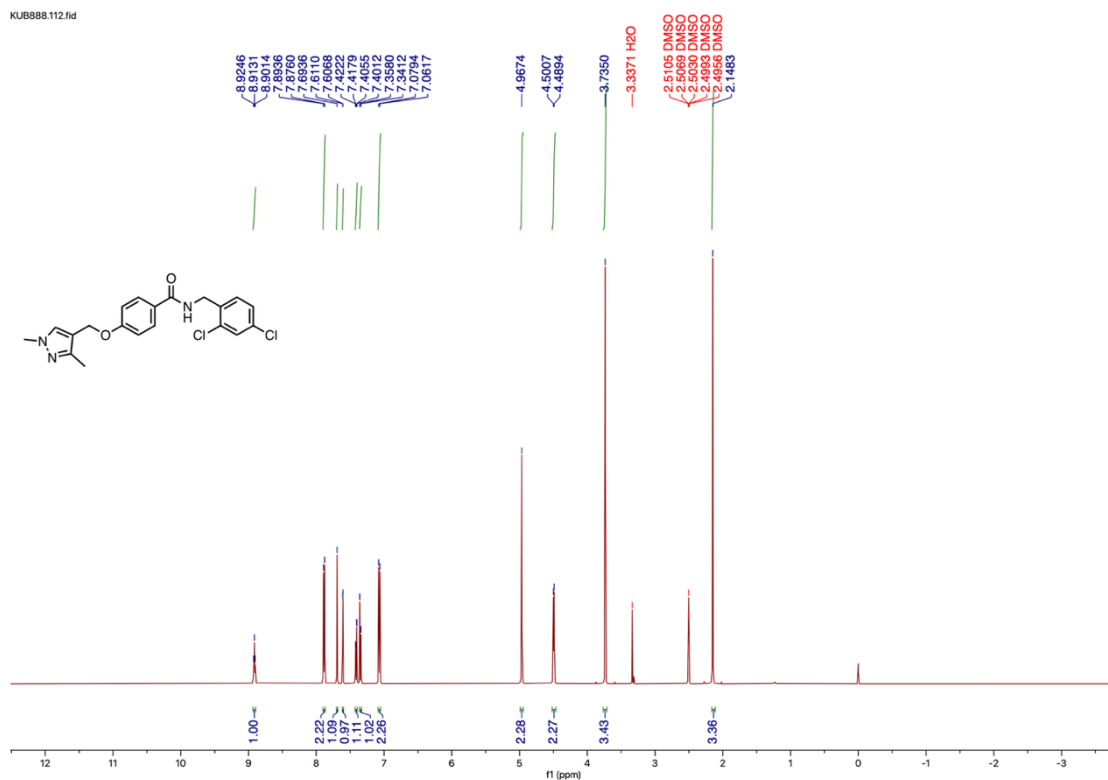


Figure S12a: ¹H-NMR spectrum of compound 9

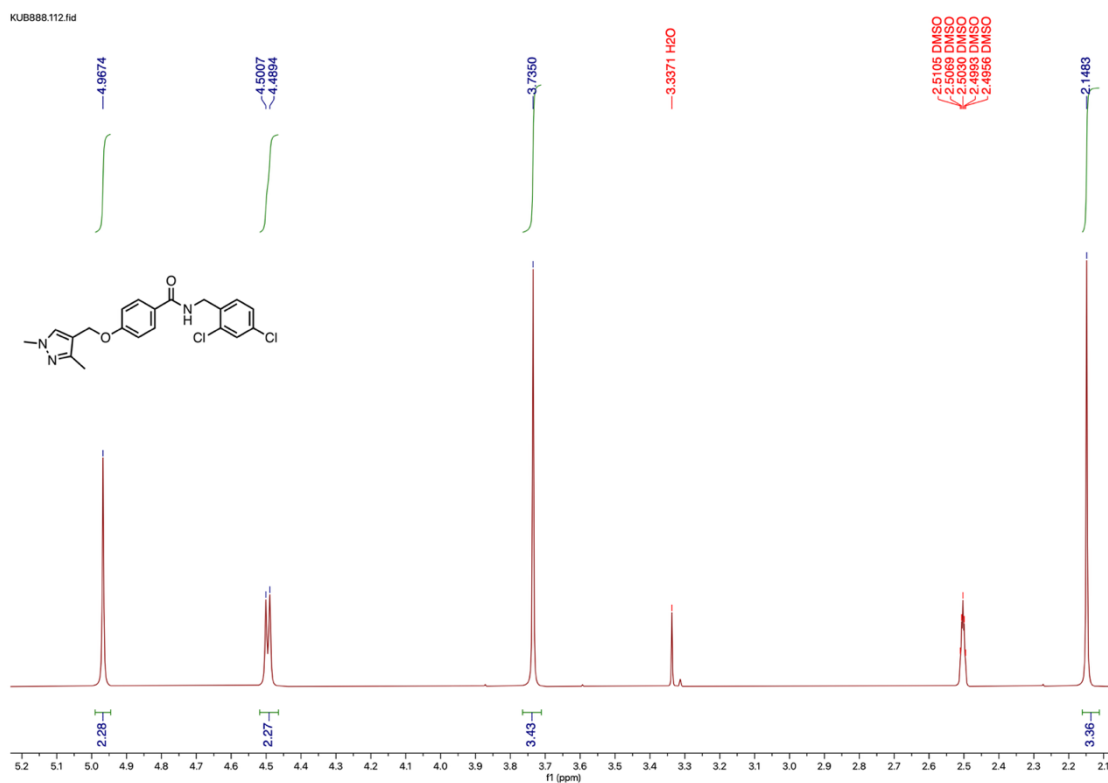


Figure S12b: ¹H-NMR spectrum of compound 9

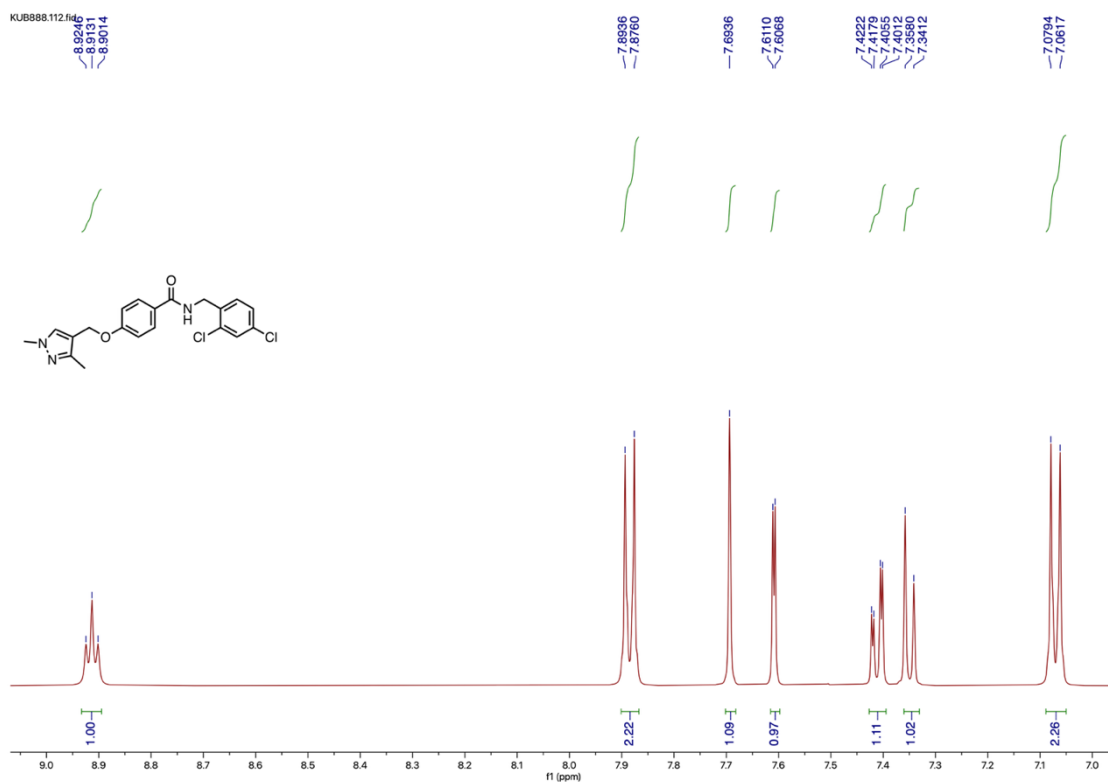


Figure S12c: ^1H -NMR spectrum of compound **9**

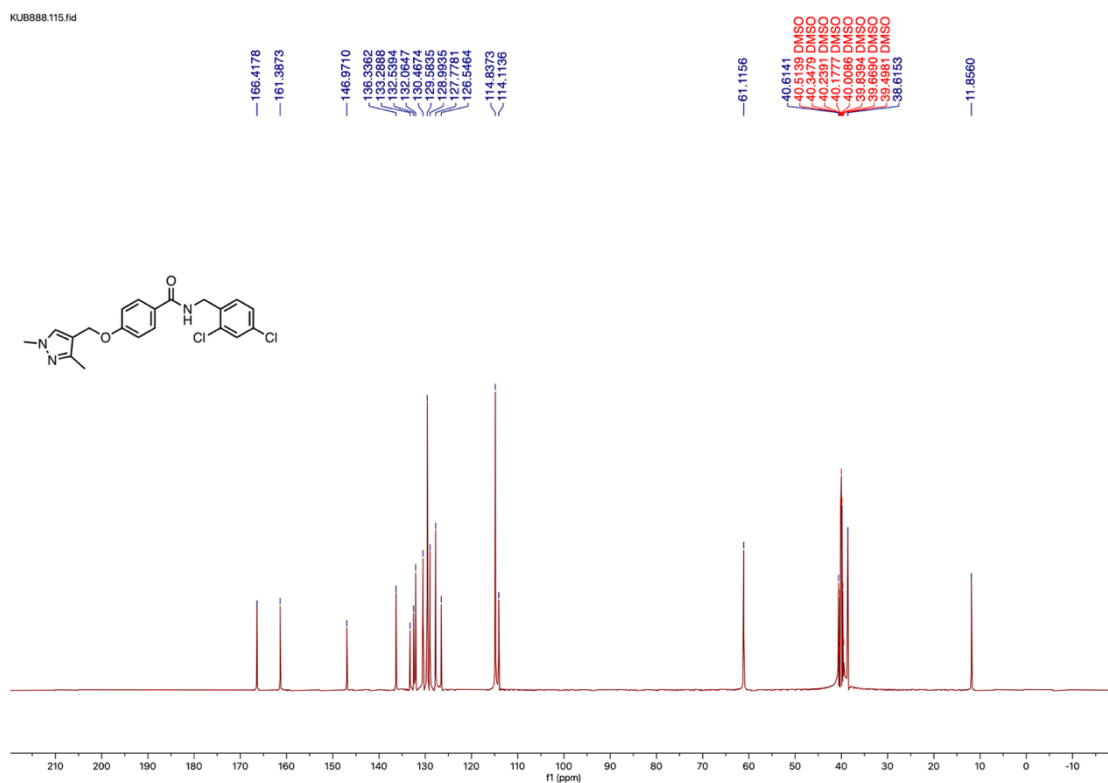


Figure S13a: ^{13}C -NMR spectrum of compound **9**

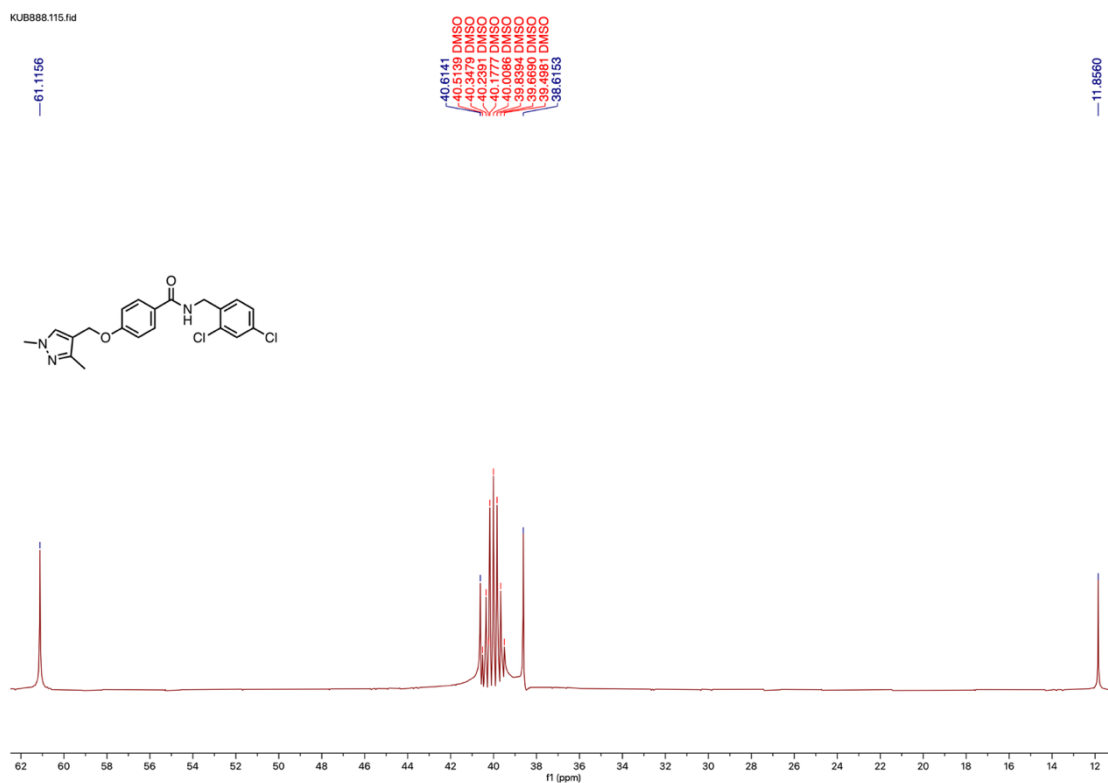


Figure S13b: ^{13}C -NMR spectrum of compound 9

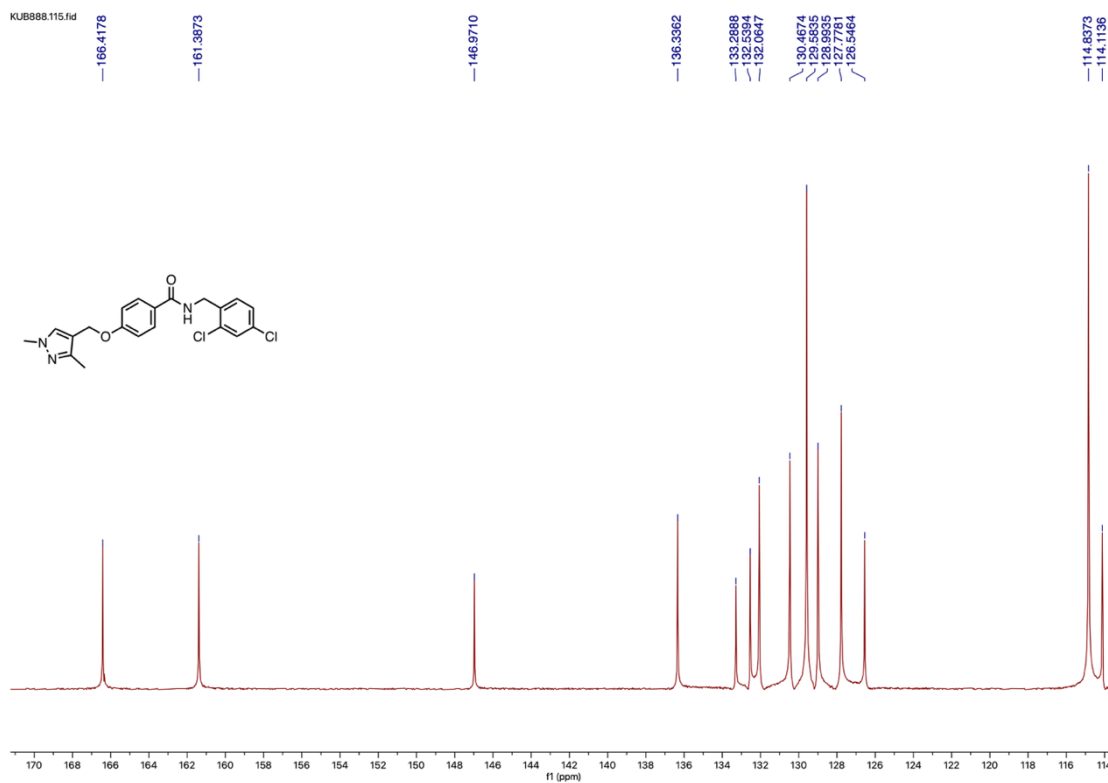


Figure S13c: ^{13}C -NMR spectrum of compound 9

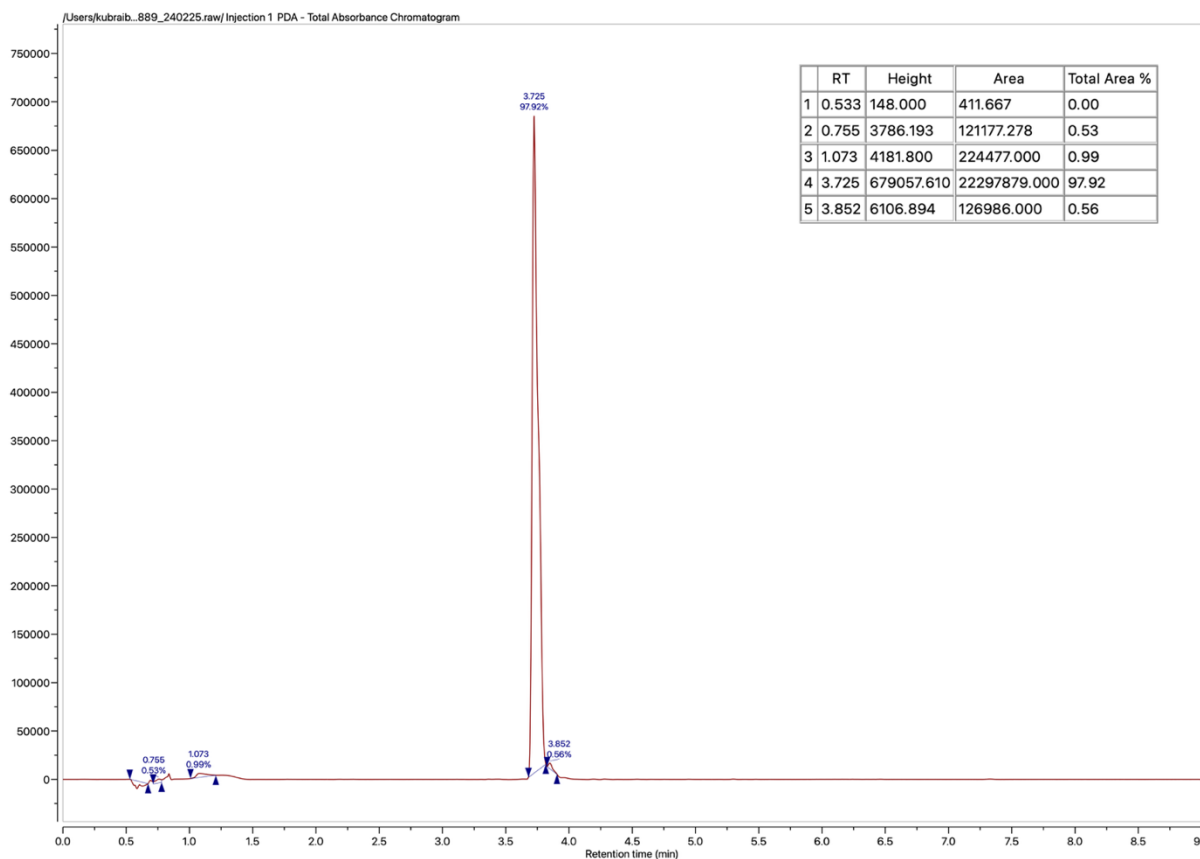


Figure S14: UPLC spectrum of compound **10**

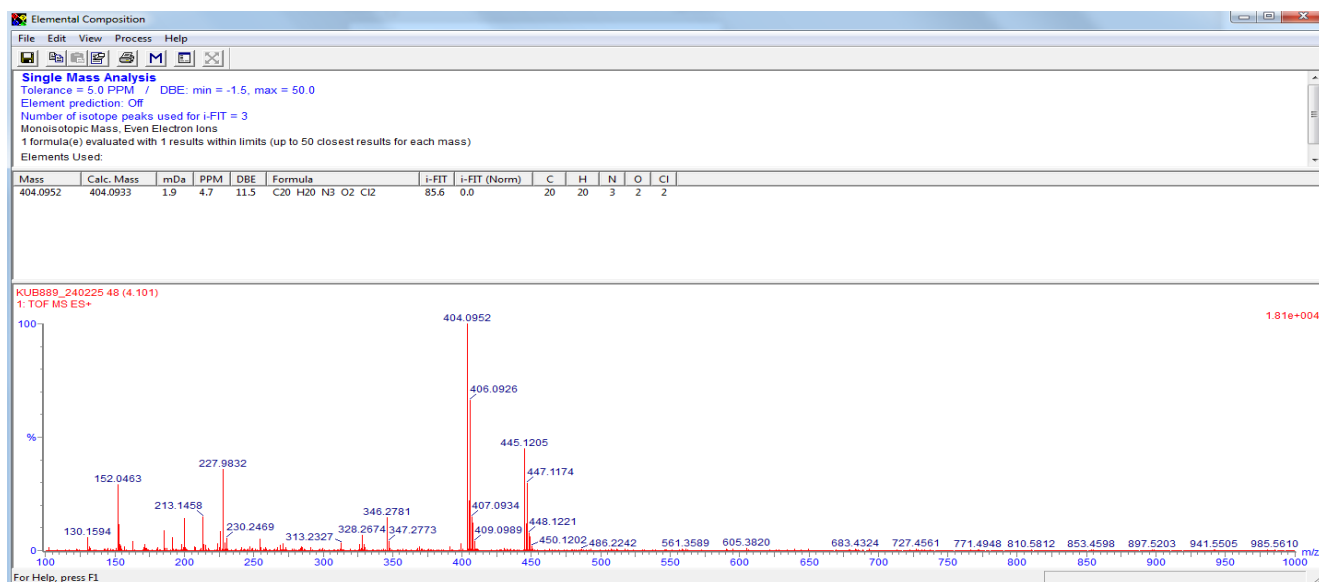


Figure S15: HRMS spectrum of compound **10**

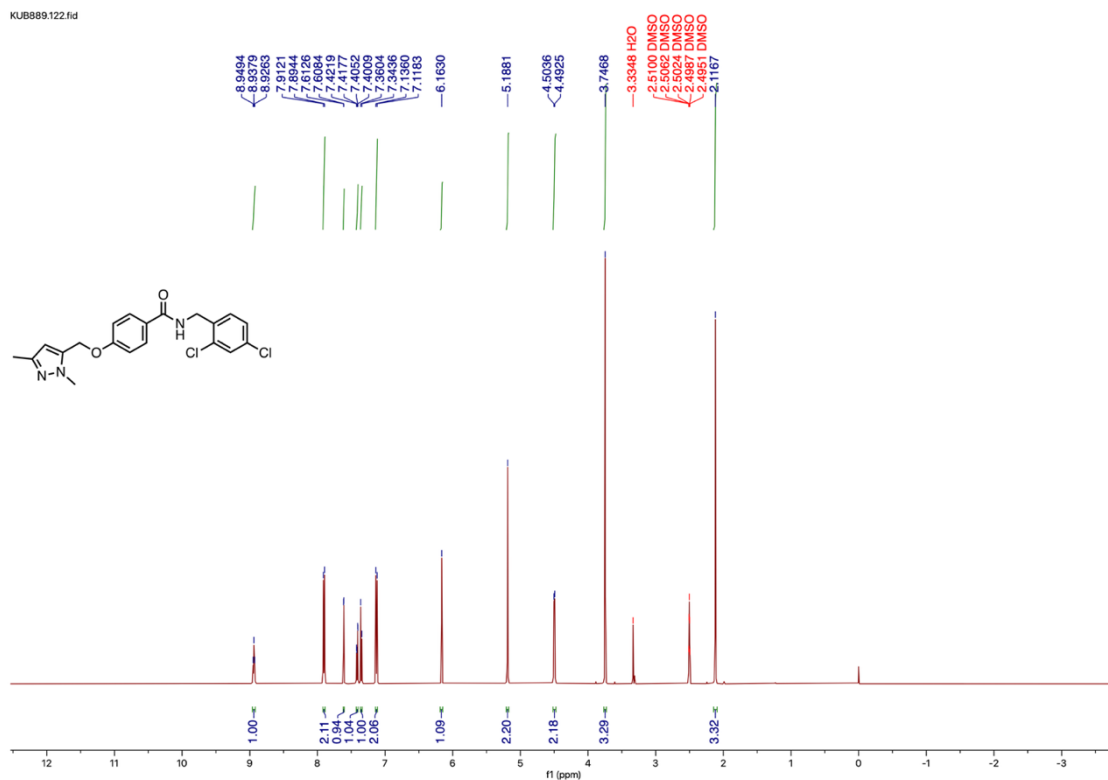


Figure S16a: ^1H -NMR spectrum of compound 10

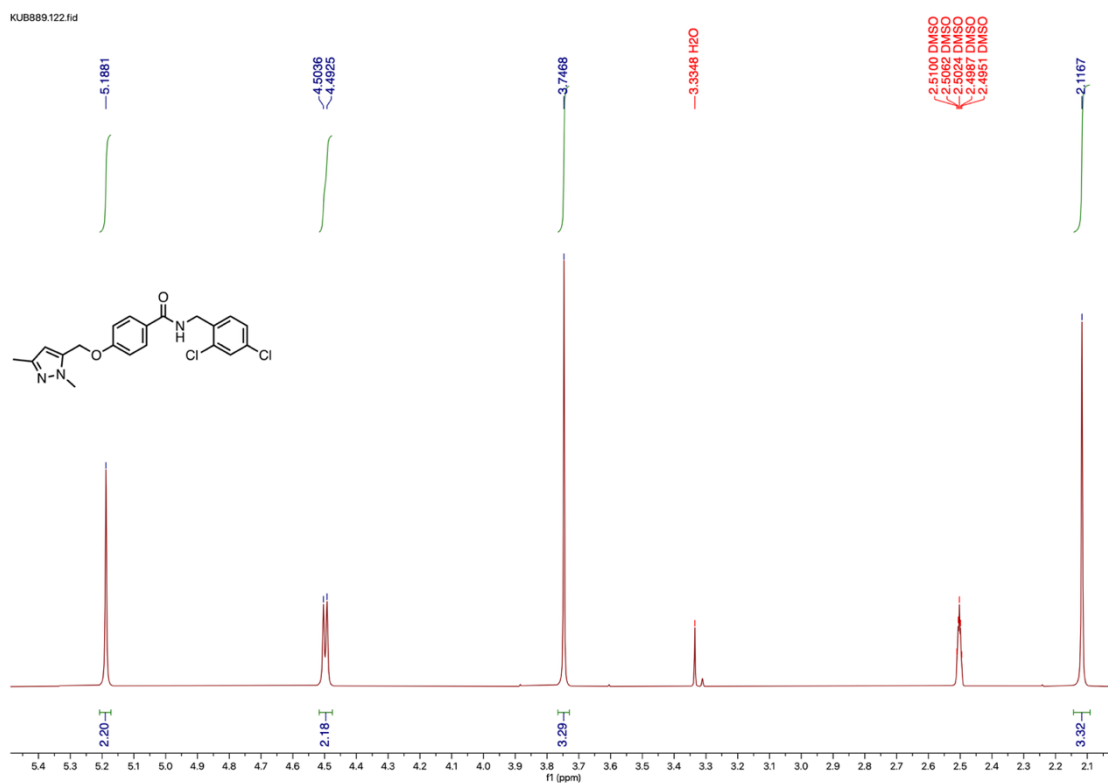


Figure S16b: ^1H -NMR spectrum of compound 10

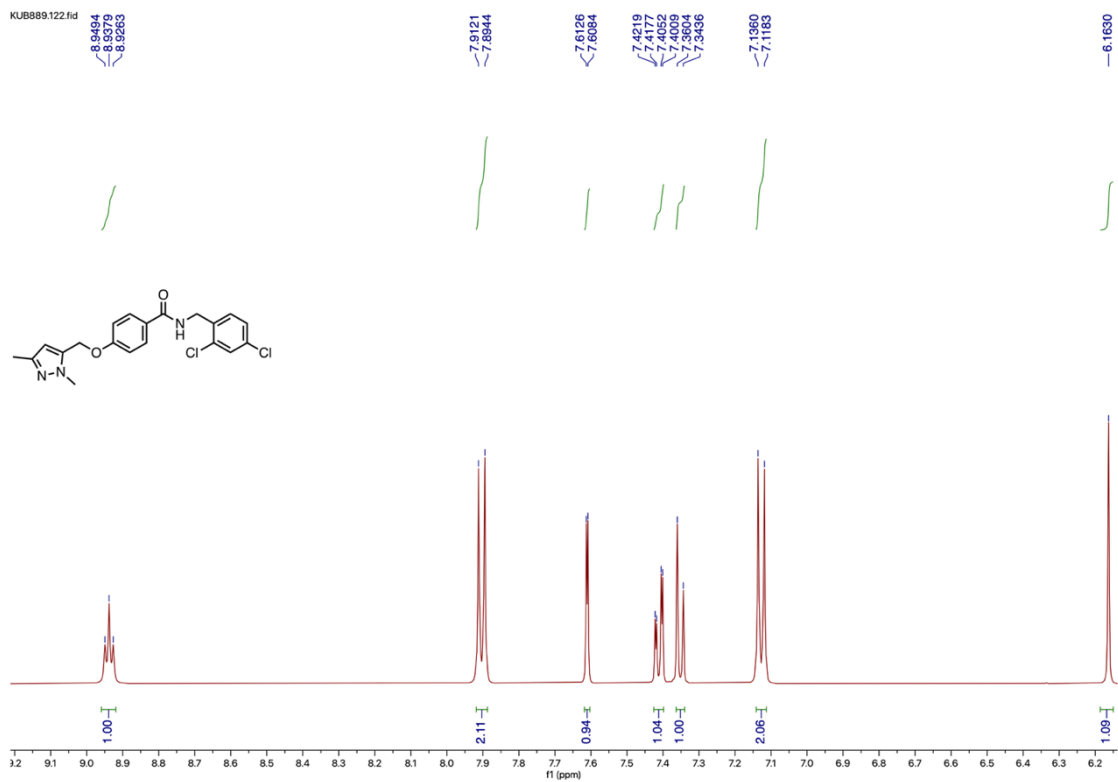


Figure S16c: ¹H-NMR spectrum of compound **10**

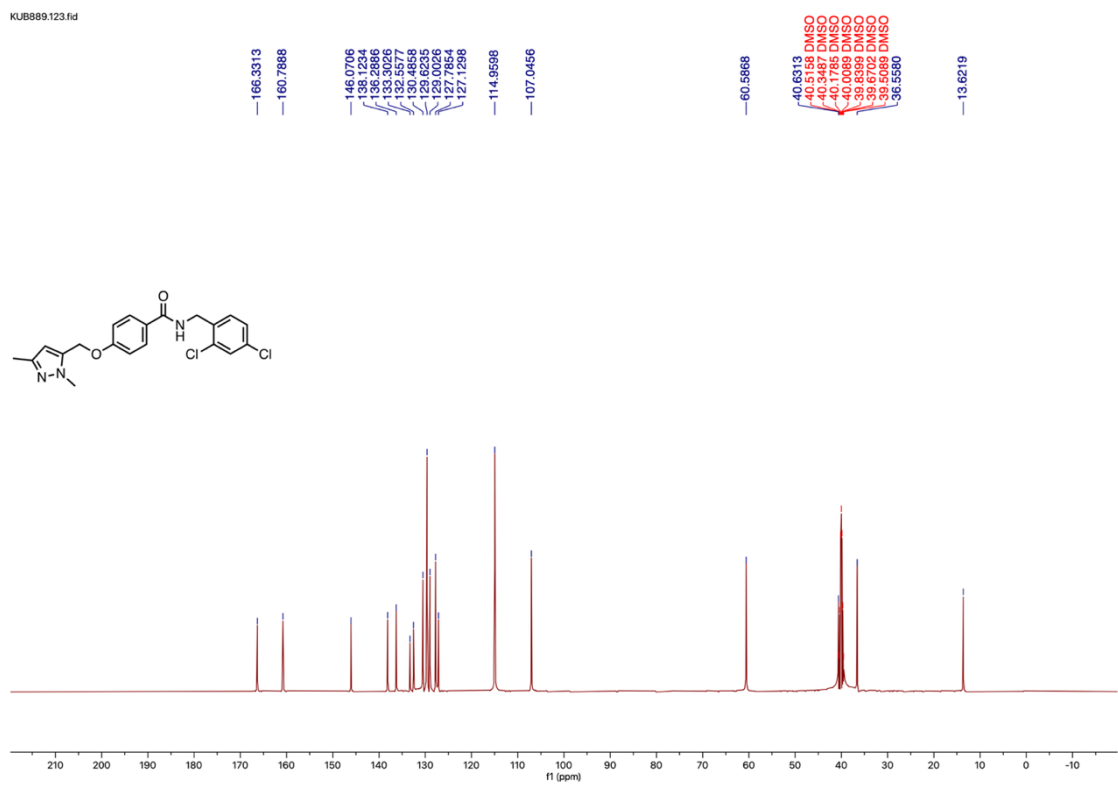


Figure S17a: ¹³C-NMR spectrum of compound **10**

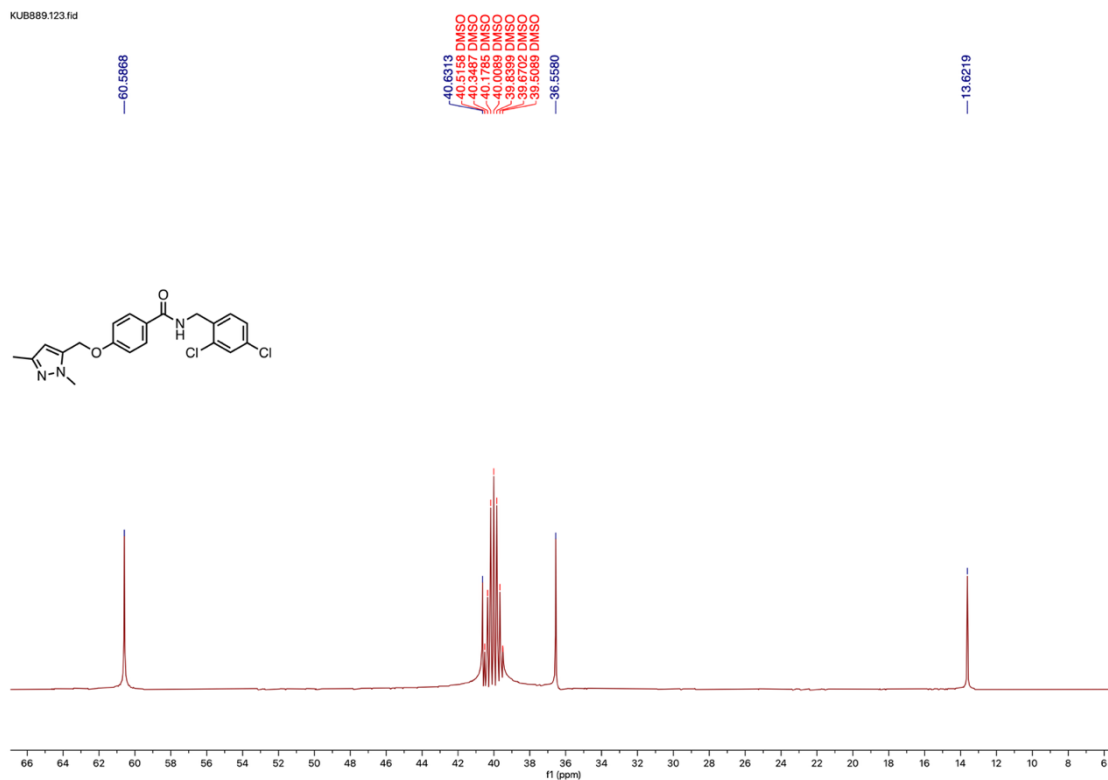


Figure S17b: ^{13}C -NMR spectrum of compound **10**

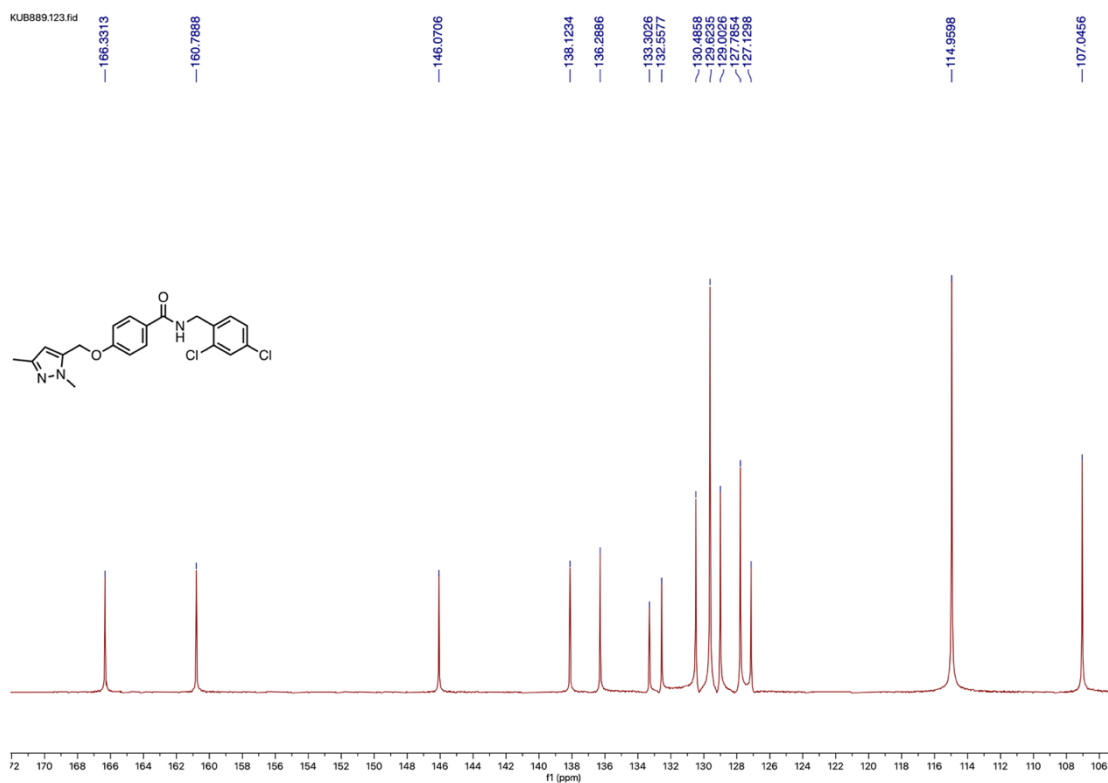


Figure S17c: ^{13}C -NMR spectrum of compound **10**

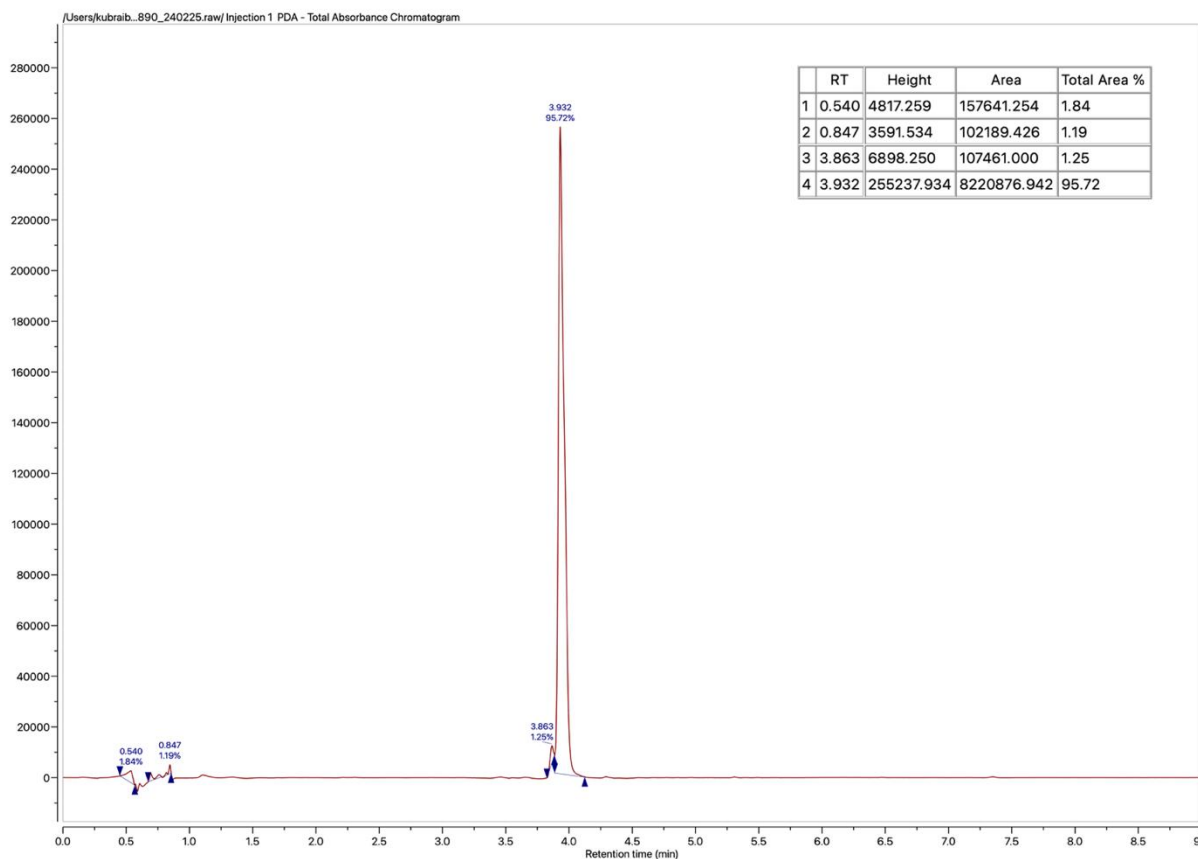


Figure S18: UPLC spectrum of compound 11

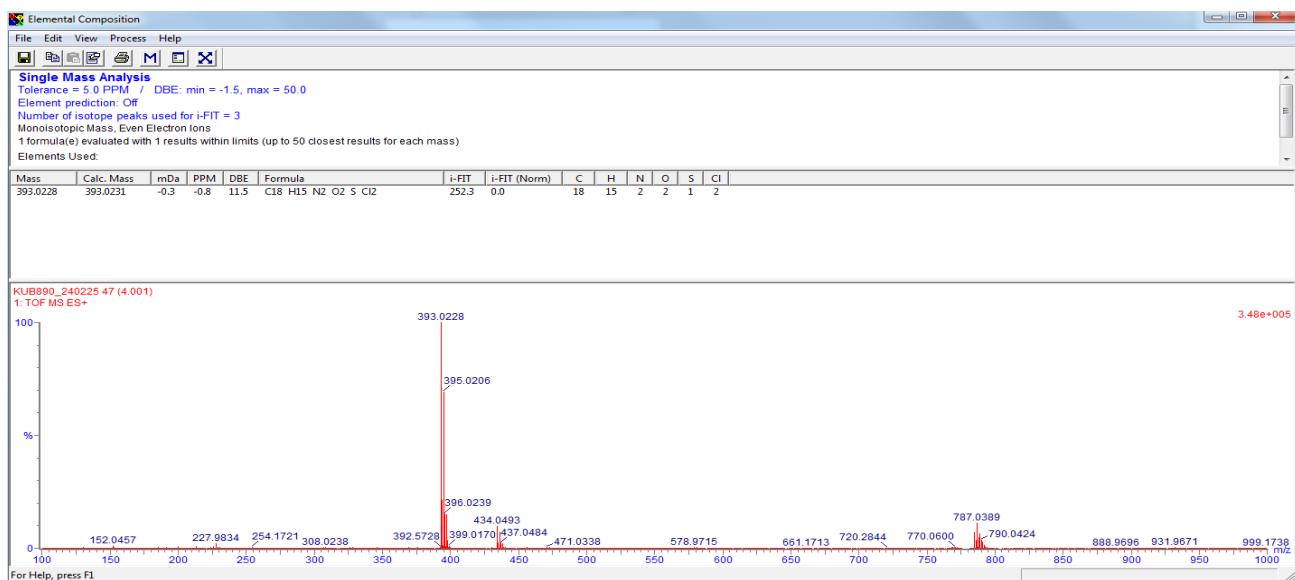
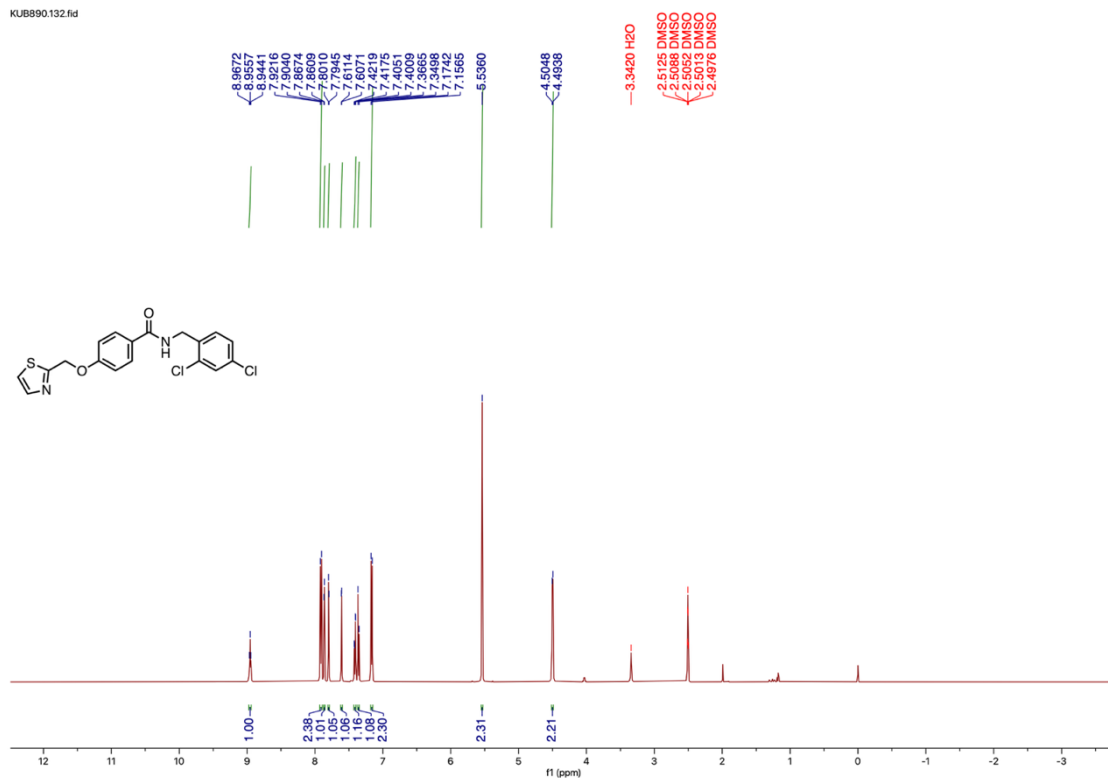
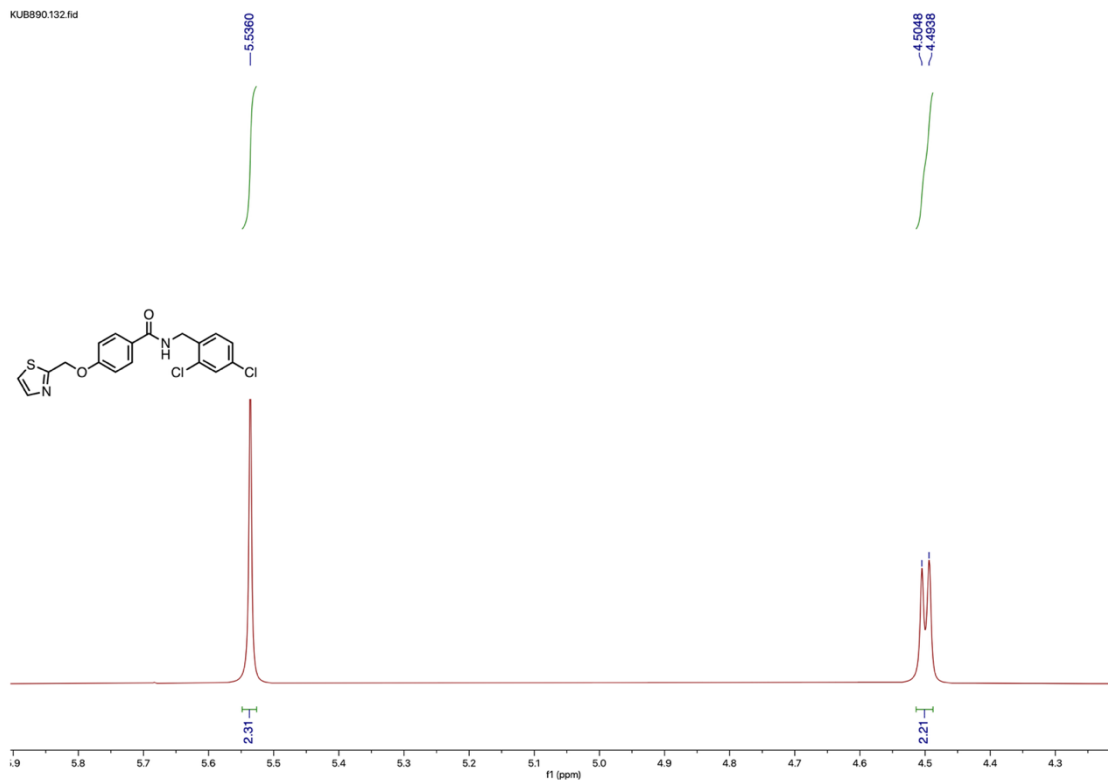


Figure S19: HRMS spectrum of compound 11

KUB890.132.fid

Figure S20a: ^1H -NMR spectrum of compound 11

KUB890.132.fid

Figure S20b: ^1H -NMR spectrum of compound 11

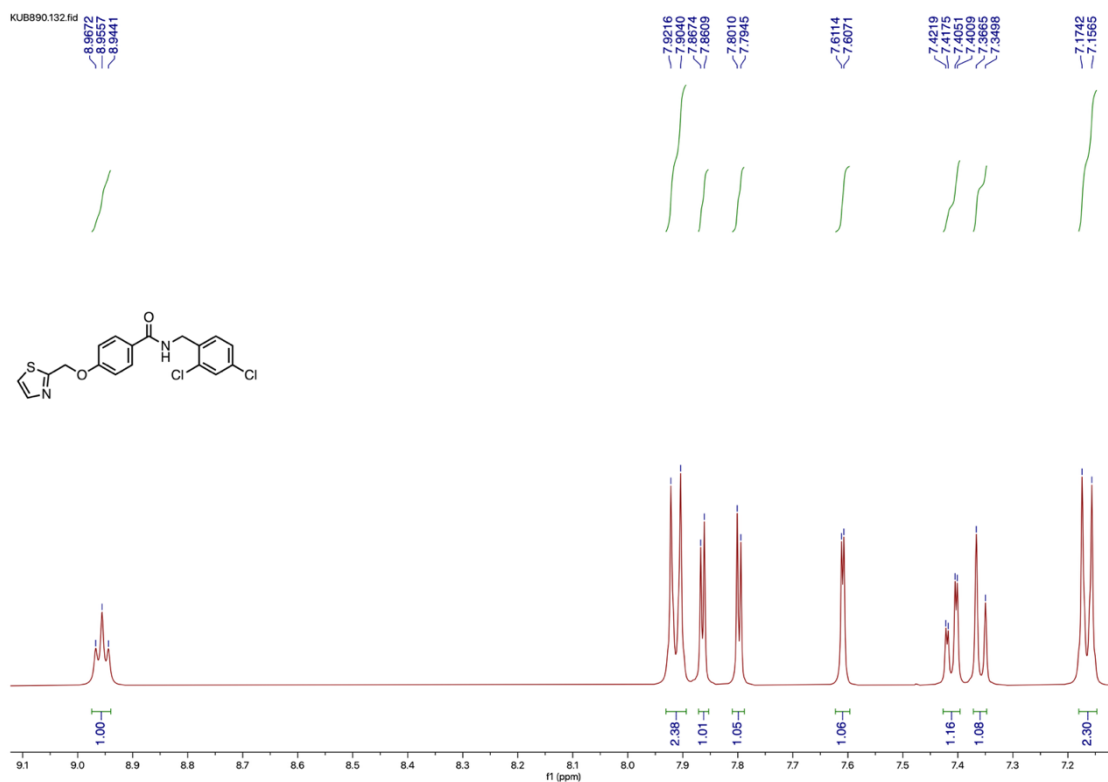


Figure S20c: ¹H-NMR spectrum of compound 11

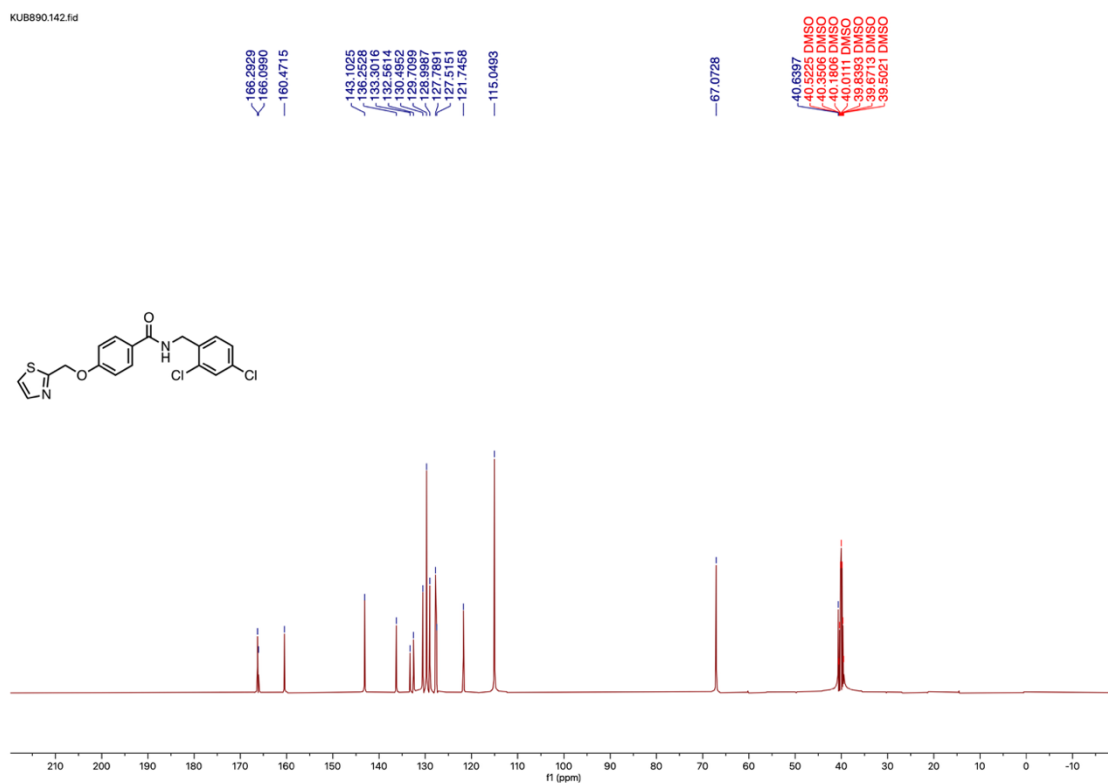
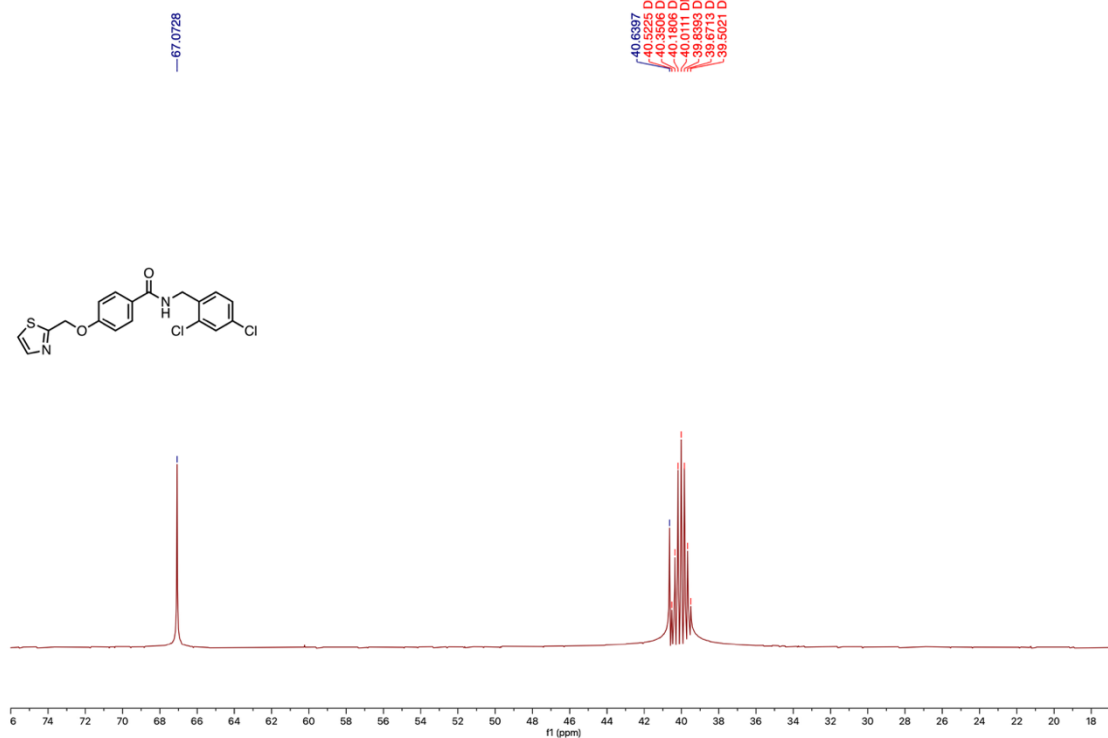
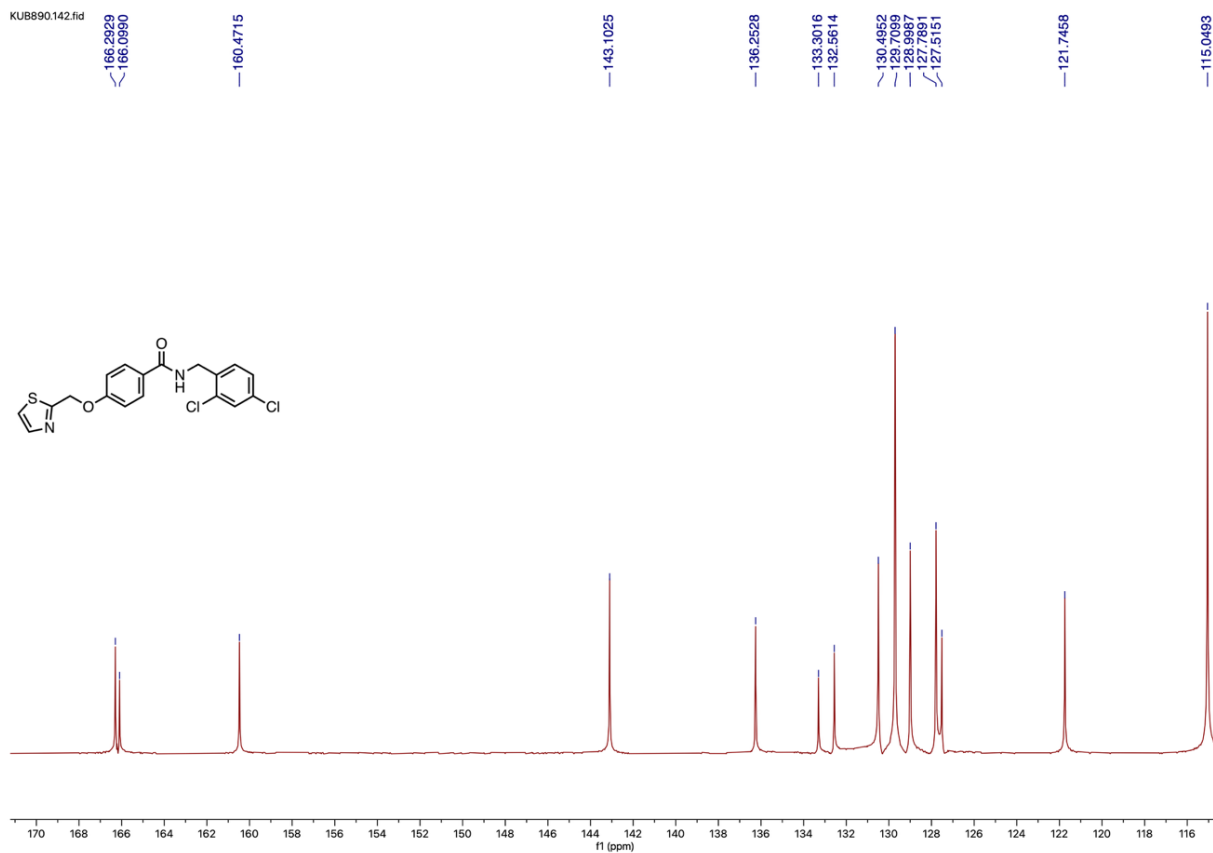


Figure S21a: ¹³C-NMR spectrum of compound 11

KUB890.142.fid

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

KUB890.142.fid

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

© 2025 ACG Publications. All rights reserved.

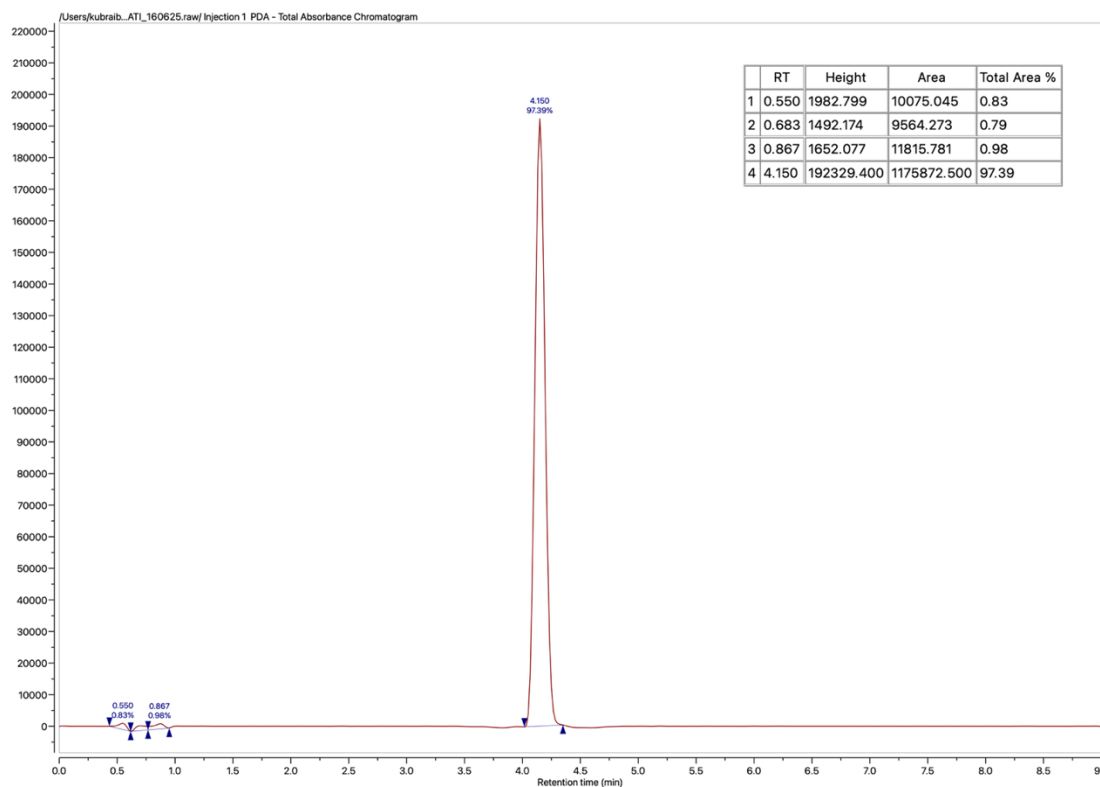


Figure S22: UPLC spectrum of compound **12**

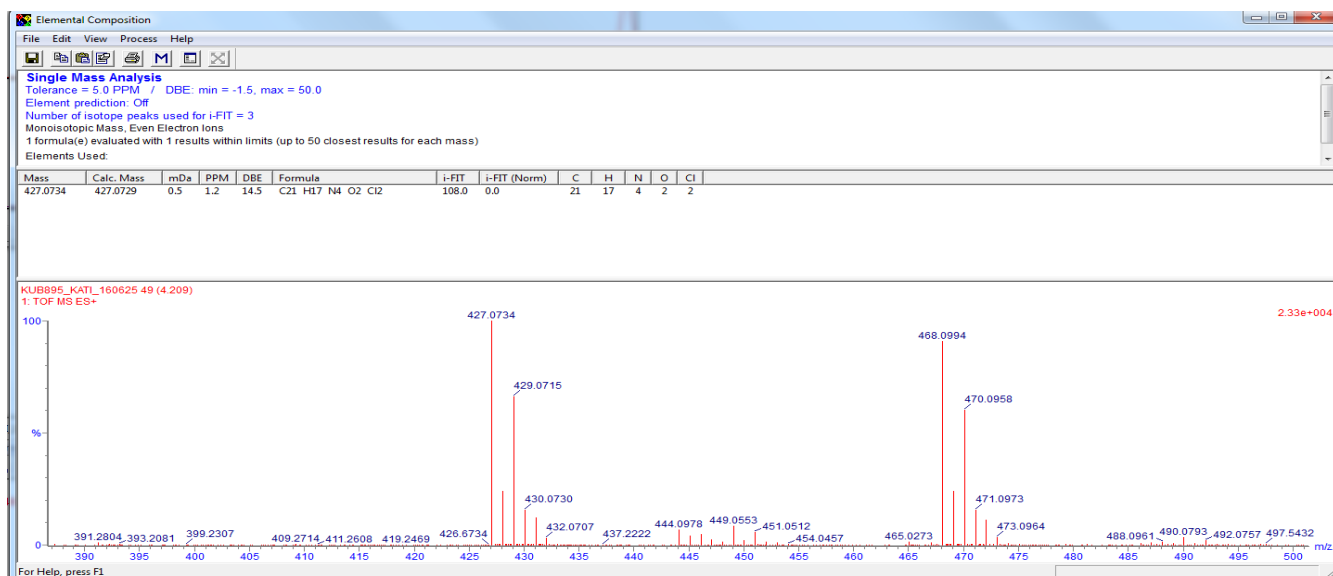


Figure S23: HRMS spectrum of compound **12**

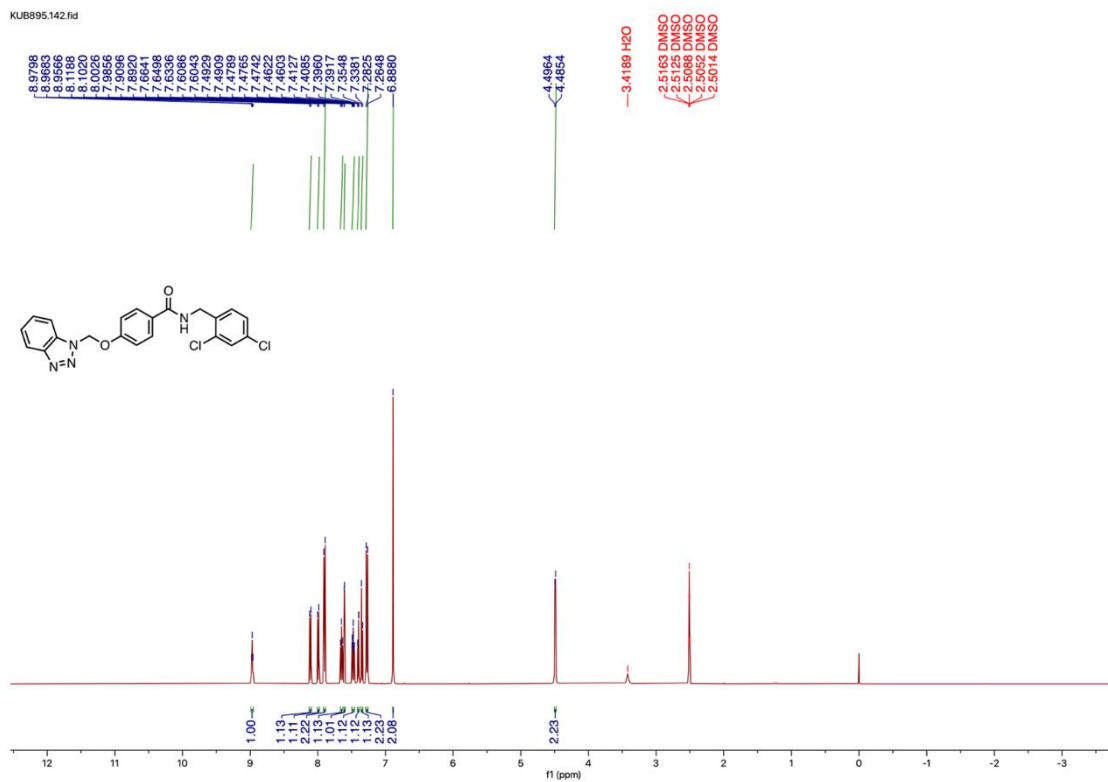


Figure S24a: ^1H -NMR spectrum of compound **12**

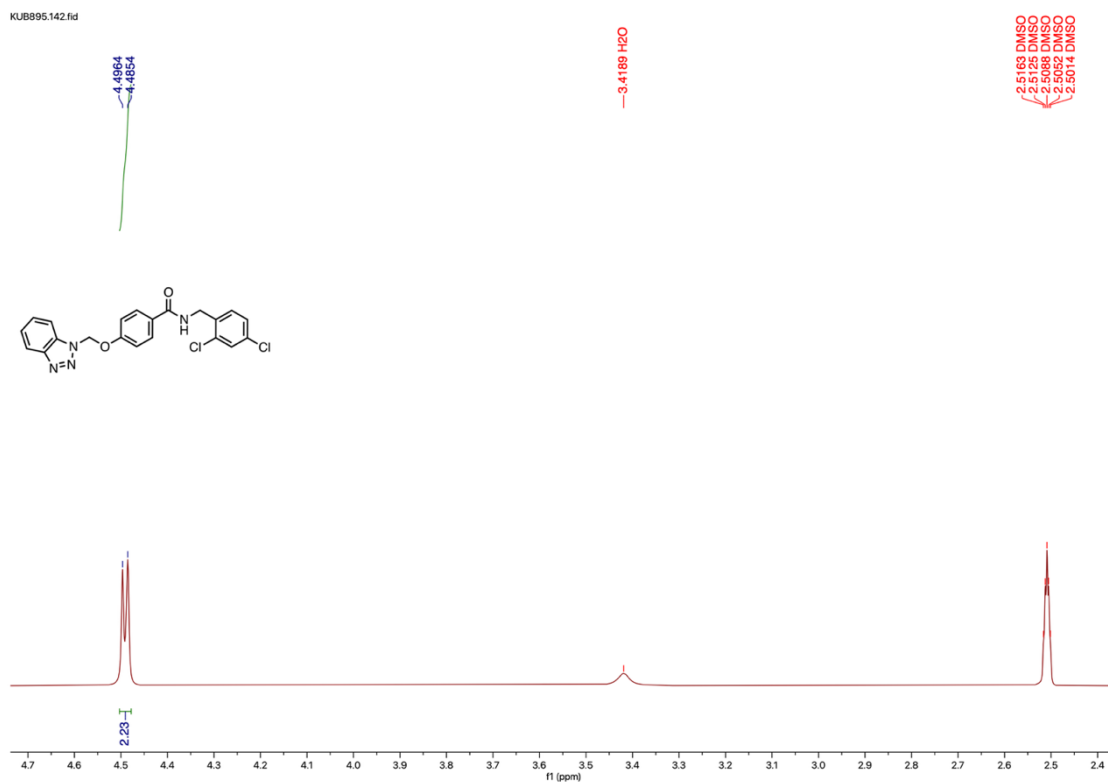


Figure S24b: ^1H -NMR spectrum of compound **12**

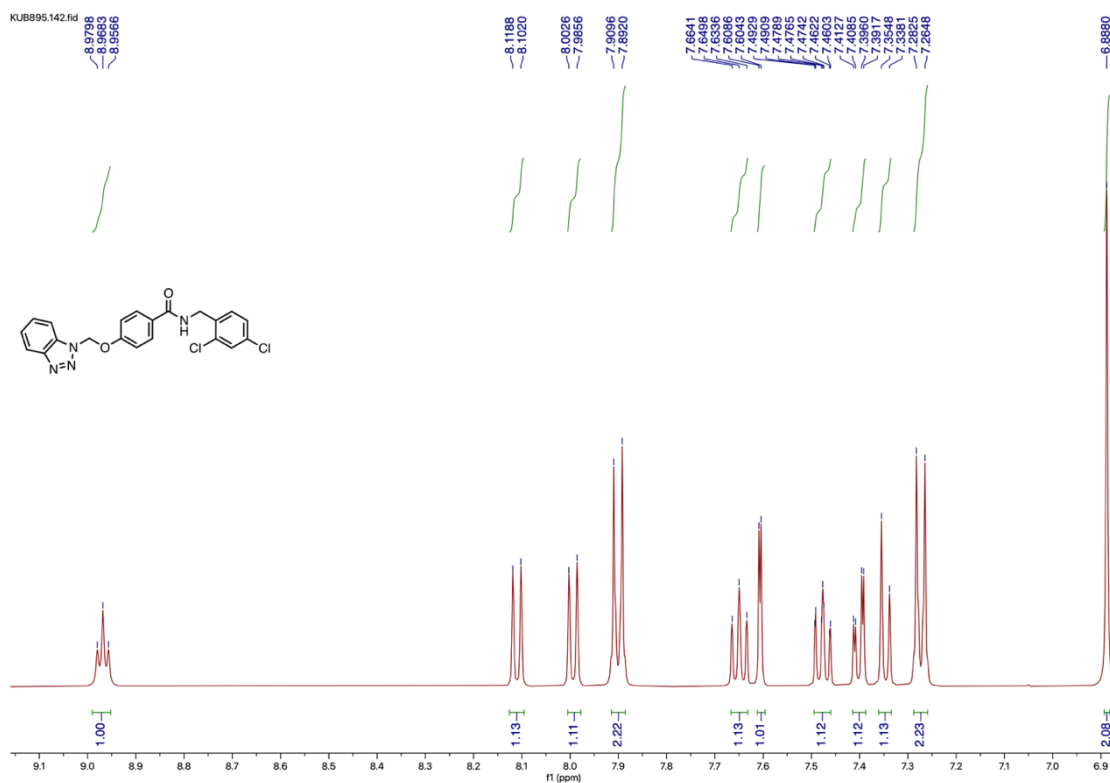


Figure S24c: ^1H -NMR spectrum of compound **12**

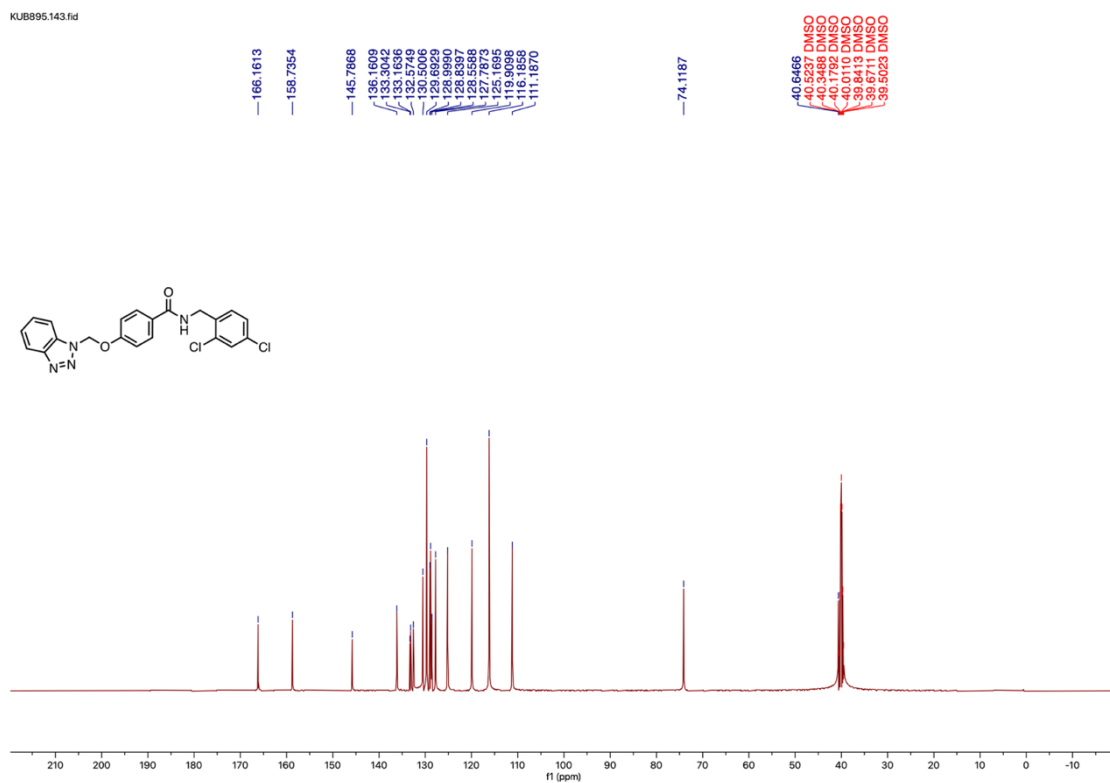


Figure S25a: ^{13}C -NMR spectrum of compound **12**

