SUPPORTING INFORMATION

Design, synthesis, and evaluation of benzhydrylpiperazine-based novel dual COX-2/5-LOX inhibitors with anti-inflammatory and anti-cancer activity

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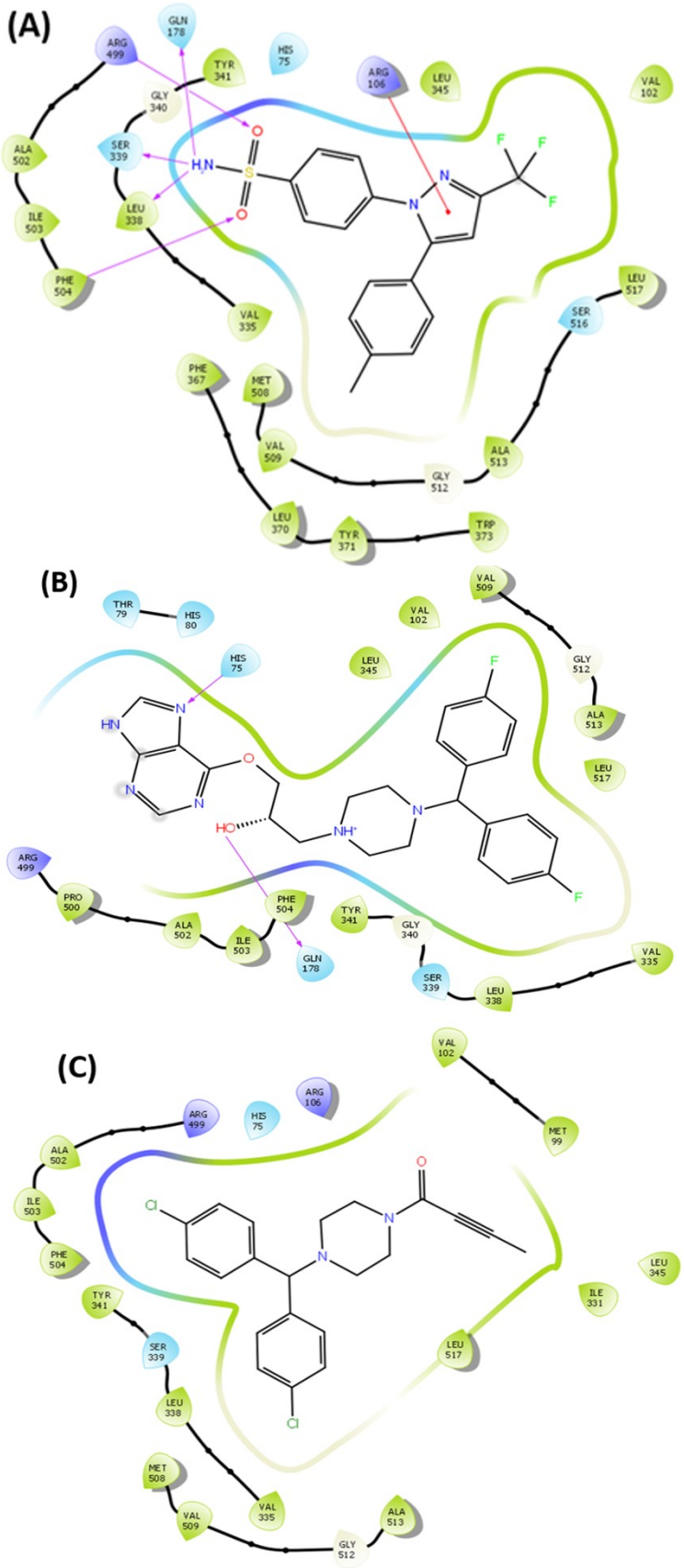

Figure S1. 2-D docking poses PDB Id- 3LN1: (A) Celecoxib (B) Hit 1 ChEMBL342253 (C) Hit 2 ChEMBL4794855

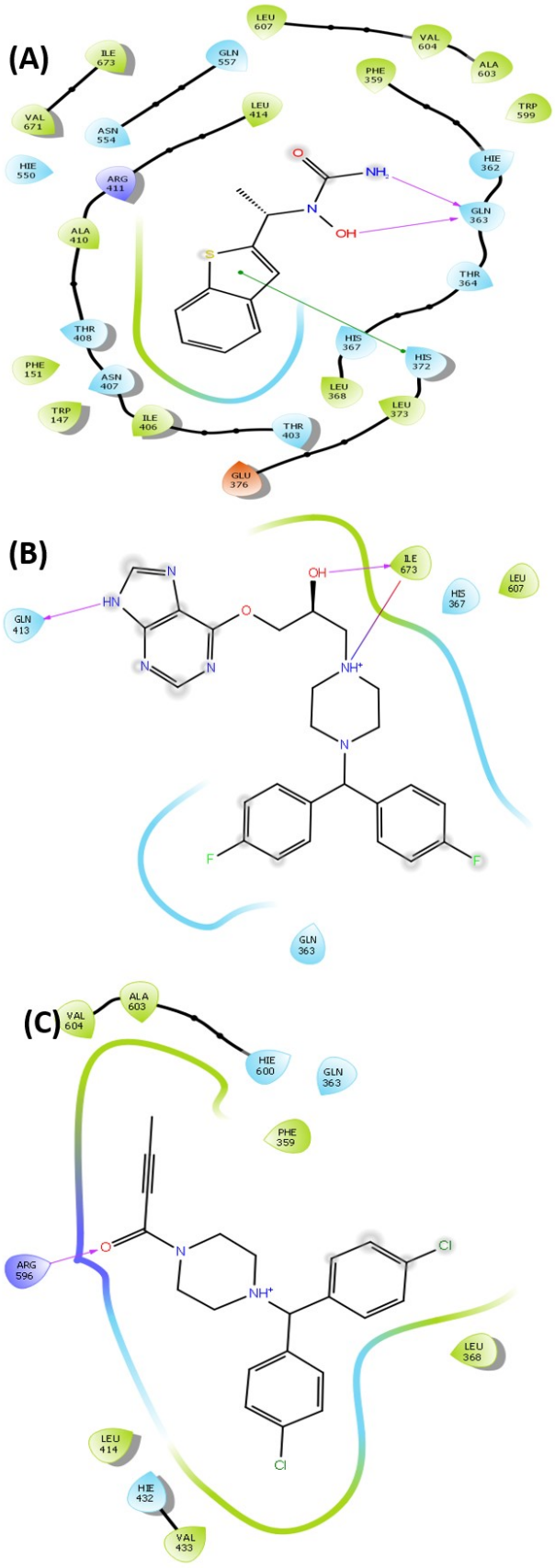

Figure S2. 2-D docking poses PDB Id- 6N2W: (A) Zileuton (B) Hit 1 ChEMBL342253 (C) Hit 2 ChEMBL4794855

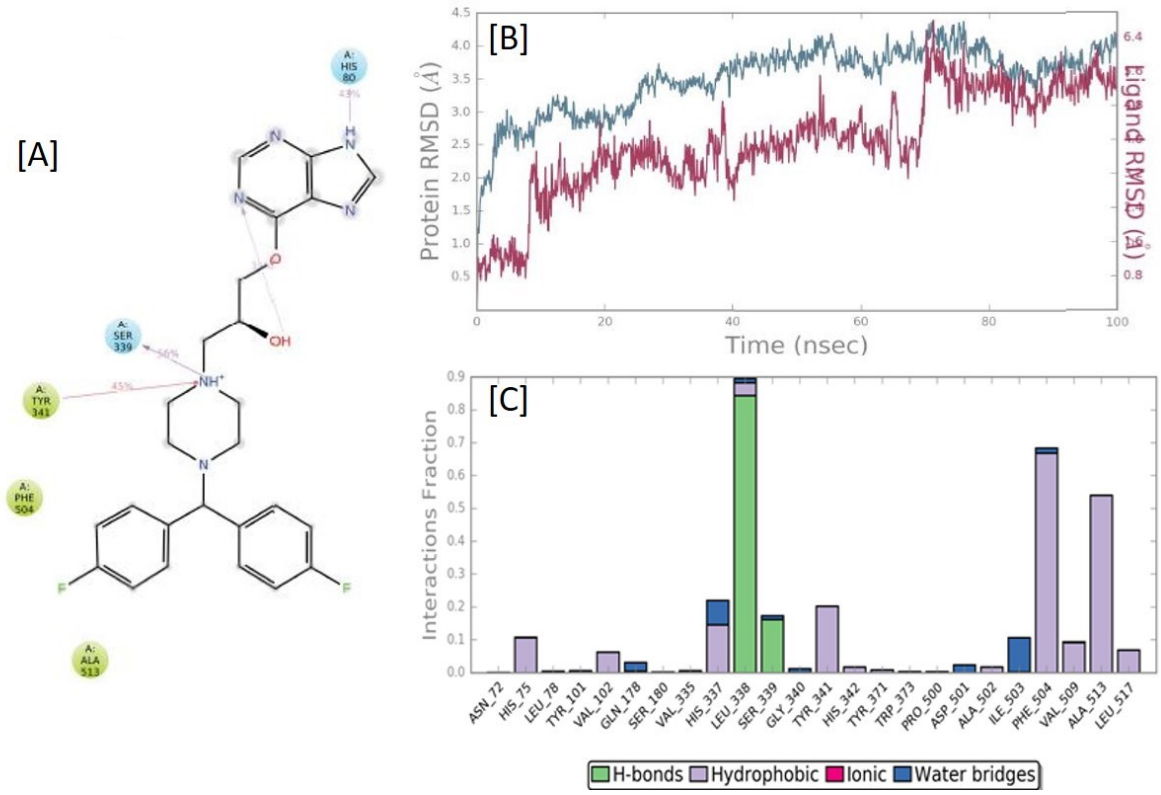

Figure S3. MD Simulation studies of screened hit 1 compound ChEMBL342253-COX-2 **(**3LN1) docked complex: [A] 2D-representation showing percent interaction with active site amino acid residues; [B] RMSD graph of ChEMBL342253 for 100ns run; [C] Histogram depicting interaction between ChEMBL342253 and protein.

The RMSD graph showed that the hit obtained from the CHEMBL database did not exhibit RMSD within the acceptable range of 1-3 Å. Moreover, the hit did not show favourable interactions with some important amino acid residues which are essential for the activity. Thus, the hit was further modified to develop novel molecules to enhance the stability of the docked molecules in molecular dynamic simulation run. Interaction with the active site amino acid residues were minimum in the hit ChEMBL342253, while the designed molecule displayed favourable interactions which are important for overall stability of the inhibitors in the active site of COX-2 enzyme.

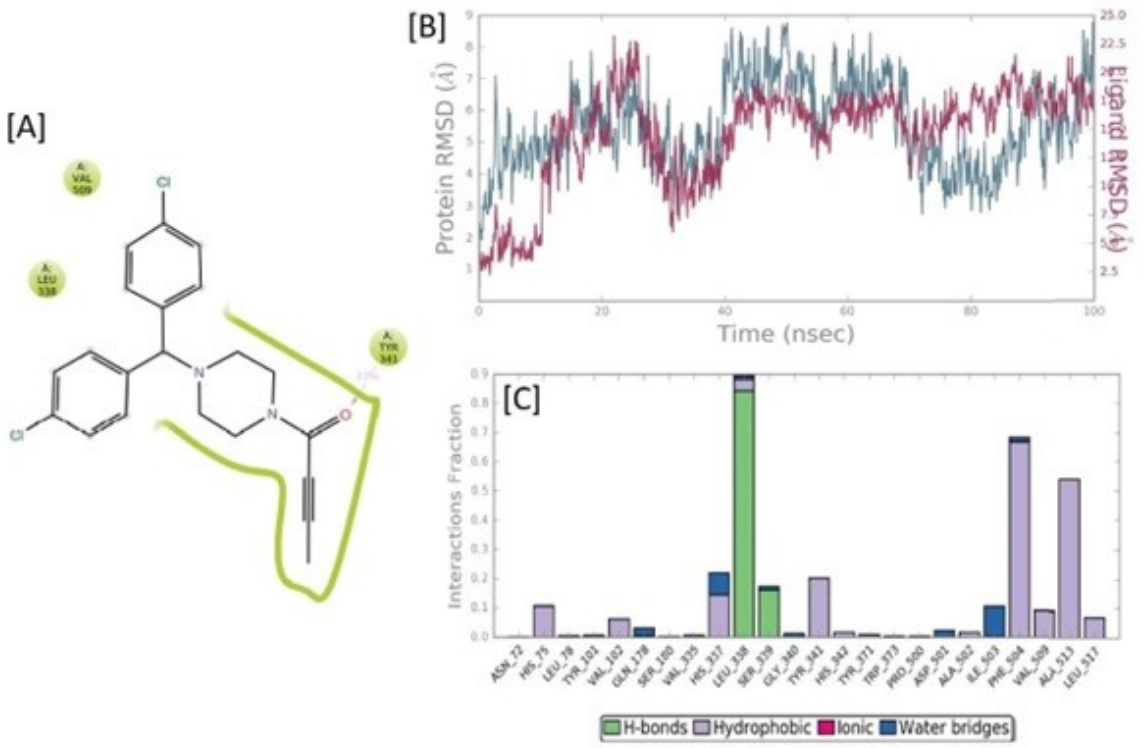

Figure S4. MD Simulation studies of screened hit 2 ChEMBL4794855-COX-2 **(**3LN1) docked complex: [A] 2D-representation showing percent interaction with active site amino acid residues; [B] RMSD graph of ChEMBL4794855 for 100ns run; [C] Histogram depicting interaction between ChEMBL342253 and protein.

The second hit obtained from the CHEMBL database (ChEMBL4794855) was also checked for its molecular stability, but the results were unsatisfactory. RMSD was outside the acceptable range of 1-3Å and the molecule did not show favorable interactions with the crucial amino acid residues. While, in contrast to this, the designed molecule displayed molecular stability and interactions with the important amino acid residues required for the activity of the inhibitor molecules.

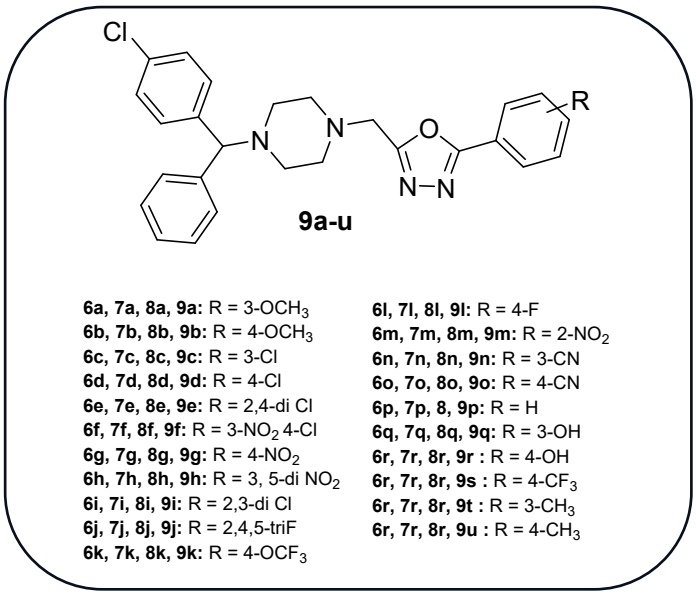

Figure S5. General structure of the designed compounds **9a-u**

Table S1. Docking scores of hits ChEMBL database, compounds, and standard celecoxib for COX-2 and zileuton for 5-LOX enzymes

	Compounds	**Docking Scores on**	
S.No.		**COX-2 (PDB:3LN1)**	**5-LOX (PDB: 6N2W)**
1.	**Hit 1 ChEMBL342253**	-10.711	3.347
2.	**Hit 2 ChEMBL4794855**	-10.521	3.142
3.	**9d**	-10.830	-5.859
4.	**9g**	-8.440	-4.372
5.	**Celecoxib**	-12.636	NA
6.	**Zileuton**	NA	-5.287

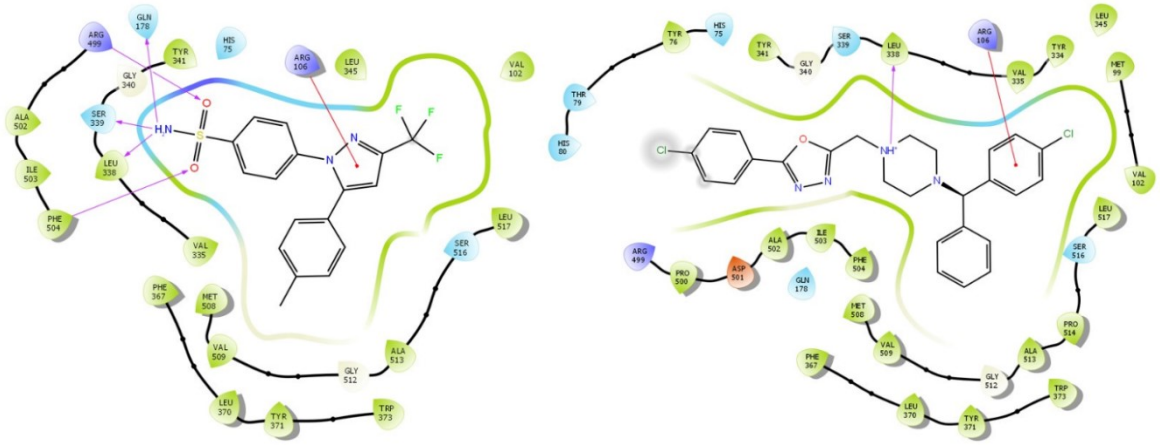

Figure S6. 2-D docking poses PDB Id- 3LN1: (A) Celecoxib (B) Compound **9d**

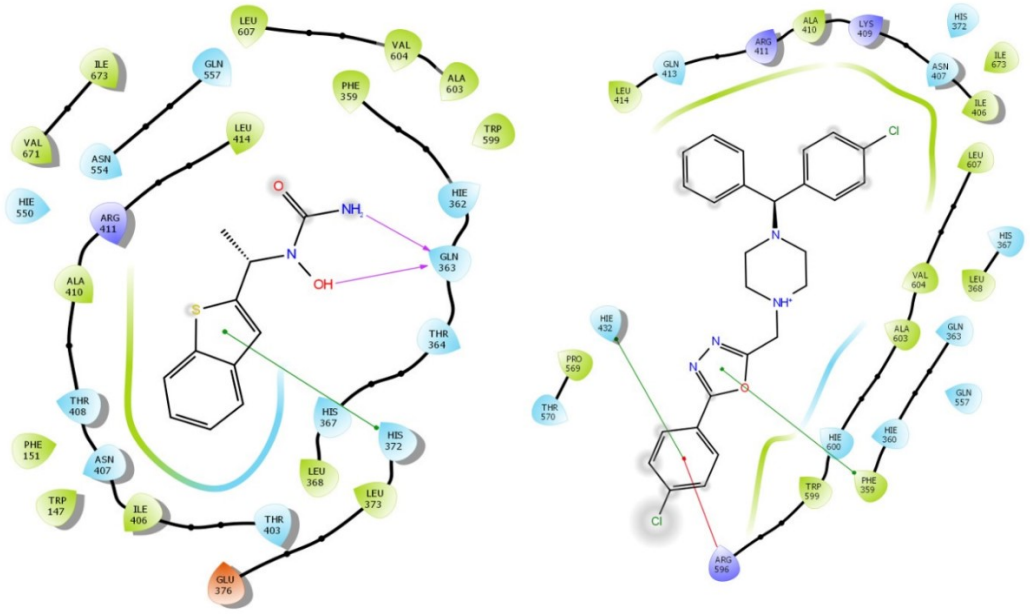

Figure S7. 2-D docking poses PDB Id- 6N2W: (A) Zileuton (B) Compound **9d**

Table S2. MM-GBSA analysis of compound **9d** with COX-2 and 5-LOX enzymes

Enzyme targets	Compounds	ΔG binding free energy
COX-2	Celecoxib	-41.416
	9d	-36.628
5-LOX	Zileuton	-44.286
	9d	-48.907

Table S3. In silico calculations of molecular characteristics

Compd	Mol wt.	Donor HB	Acceptor HB	QPlogPo/w	$n_{violations}$	QPlogPC16	QPlogPoct
Rule	**< 500**	**≤ 5**	**≤ 10**	**≤ 5**	**≤ 1**	**4-18**	**8-43**
9d	479.4	0.0	6.5	4.9	0	16.4	21.8
Celecoxib	383.8	2.0	5.5	3.3	0	10.8	20.2
Zileuton	231.2	3.0	3.7	0.9	0	8.5	14.6

Drug-likeliness determination by Qikprop module

The drug-likeliness for the most promising compound **9d** and the standard drugs were determined using the Qikprop Software module of Schrödinger Maestro 2018.1 as shown in Table S3. The prediction ensured that the compound abided by Lipinski's rule along with prediction of other important parameters including Log P estimation. The experimental Log P values in the range of ≤5 and other molecular characteristics determine if a novel compound with specific pharmacological potential would be considered an orally active medication, crucial in obtaining good bioavailability in humans.

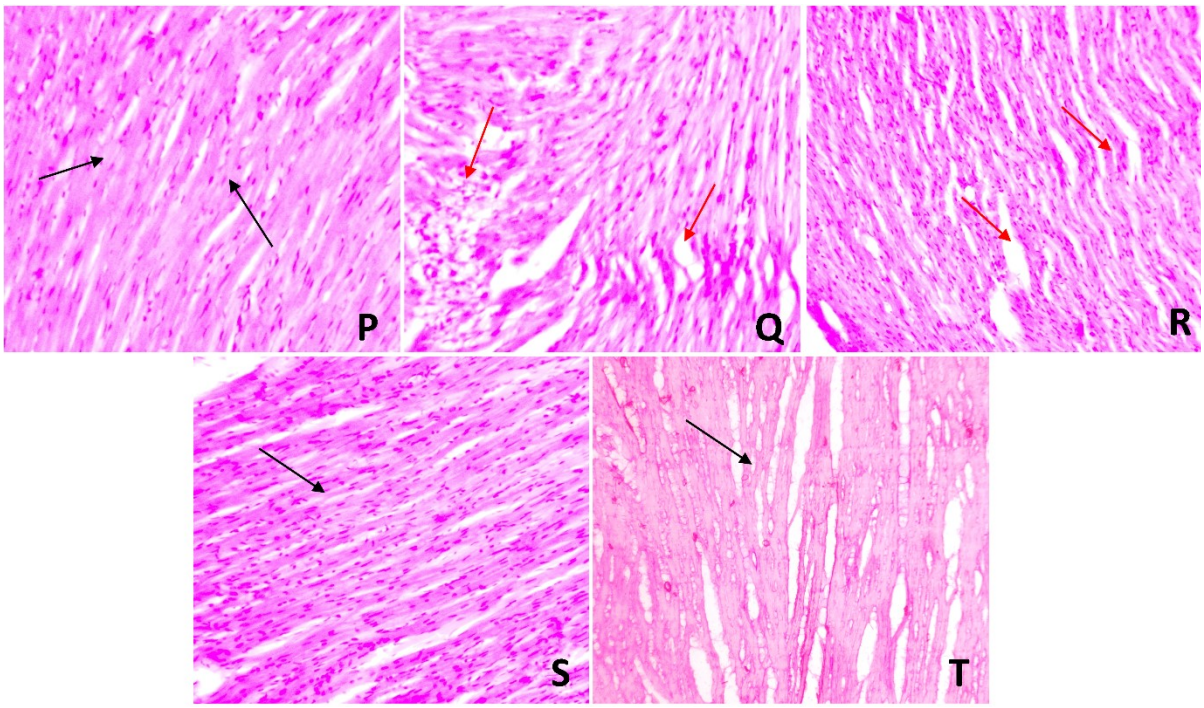

Figure S8. Microscopic evaluation of rat heart tissues (H & E staining): **P,** display control group with well-organized tissues and nuclei; **Q,** physiology of heart of rats treated with isoproterenol (red arrows) showing portion of damaged tissues and dislocated nuclei; **R,** displaying the celecoxib group with minor damage to the heart tissues; **S** & **T,** showed the promising derivative **9d** and **9g** with normal tissue framework and well-organized nuclei.

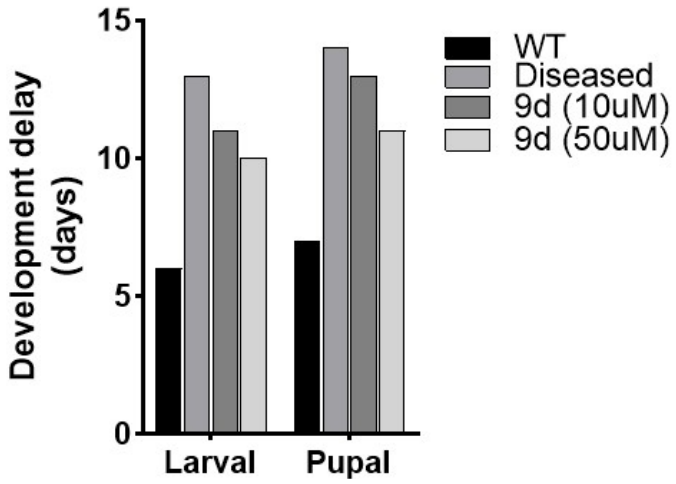

Figure S9. Histogram represents percent of fly eclosed in untreated control group and 9d treated with 10 μM, and 50 μM concentrations.

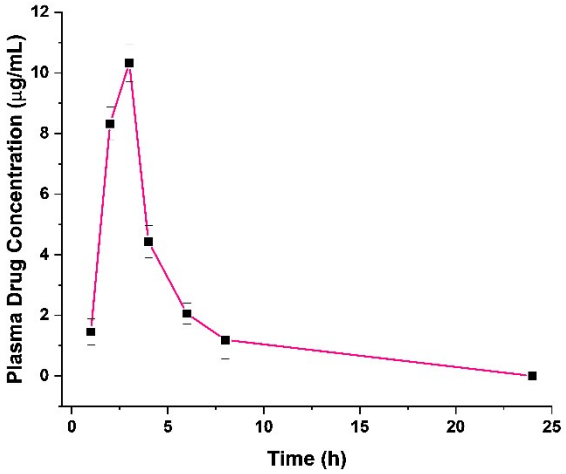

Figure S10. The drug release profile of orally administered drug **9d** in the rat model.

^{1}H NMR, ^{13}C NMR spectra of the representative intermediate compounds

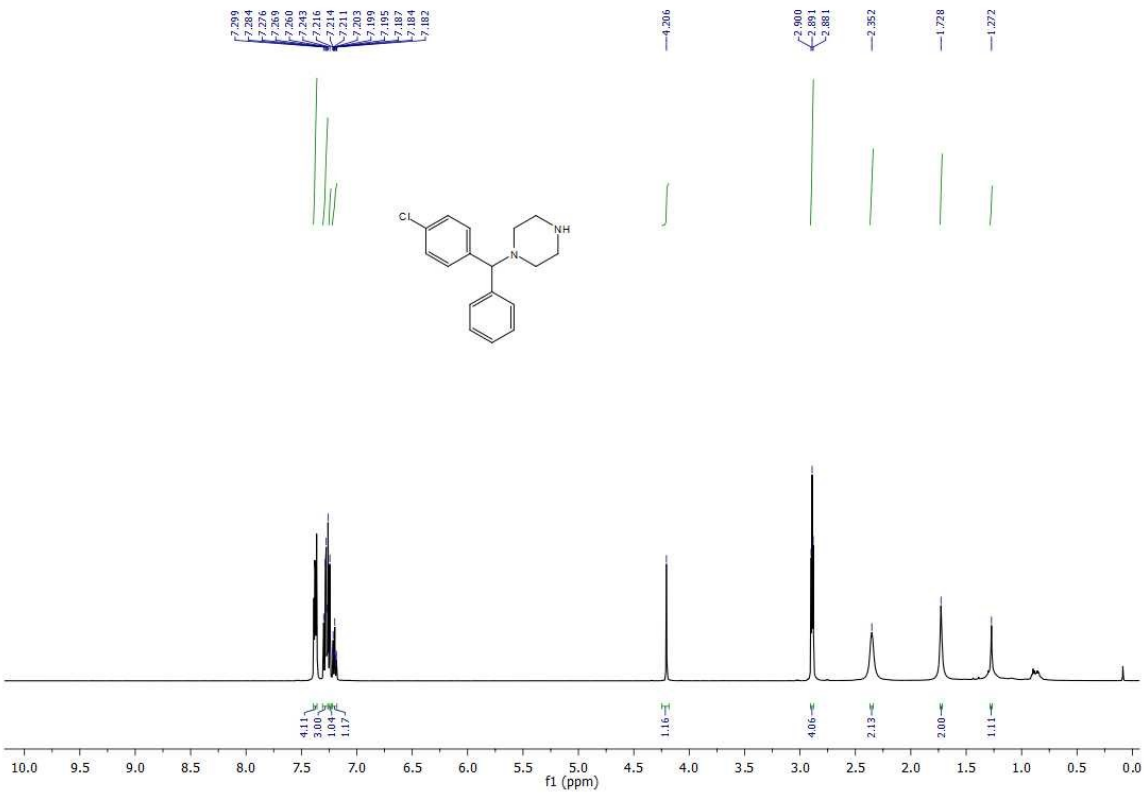

Figure S11. ^{1}H NMR spectra of target compound **5.**

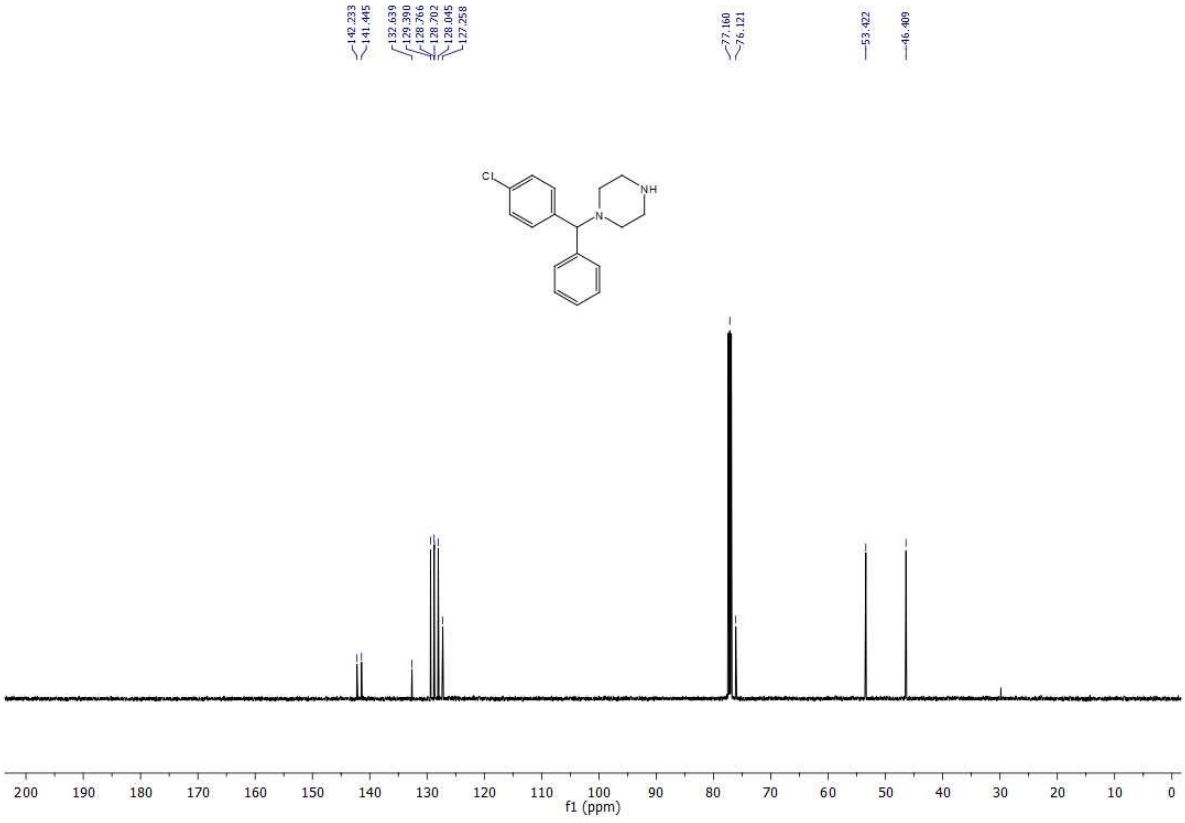

Figure S12. ^{13}C NMR spectra of target compound **5.**

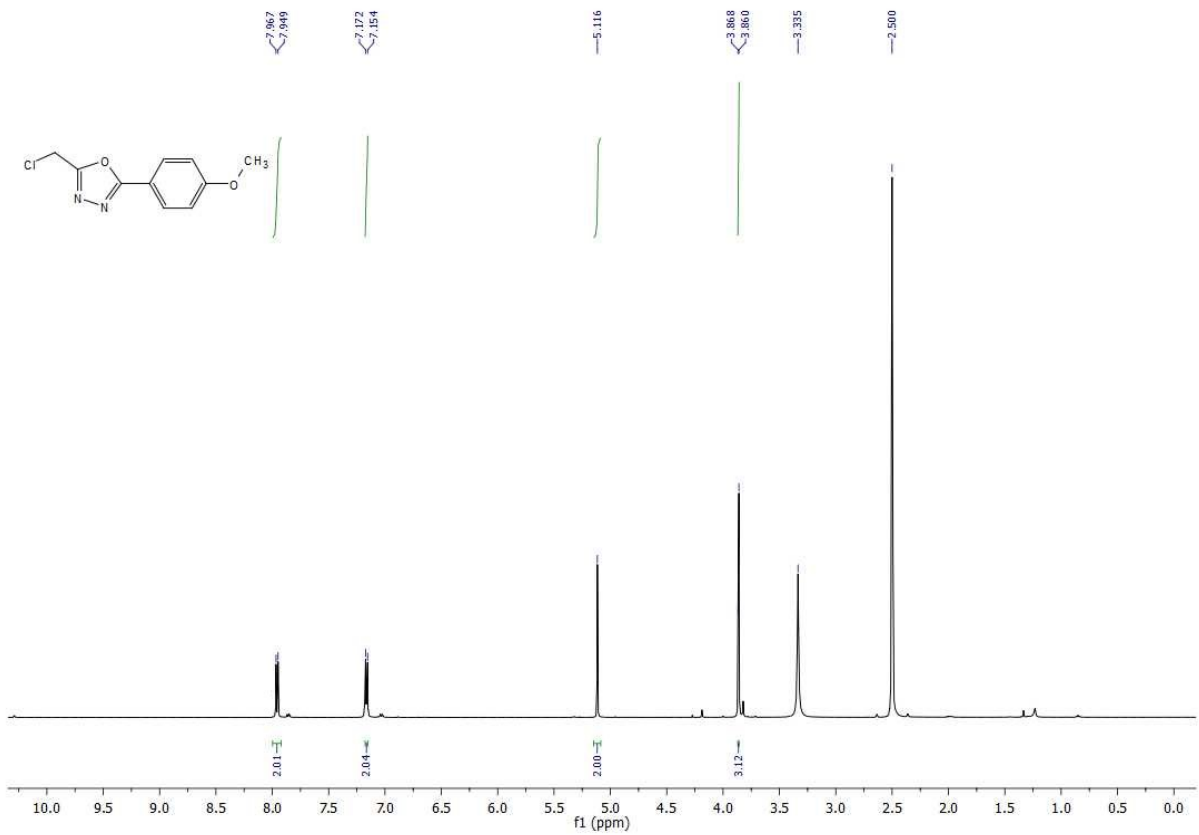

Figure S13. [1]H NMR spectra of target compound **8b**

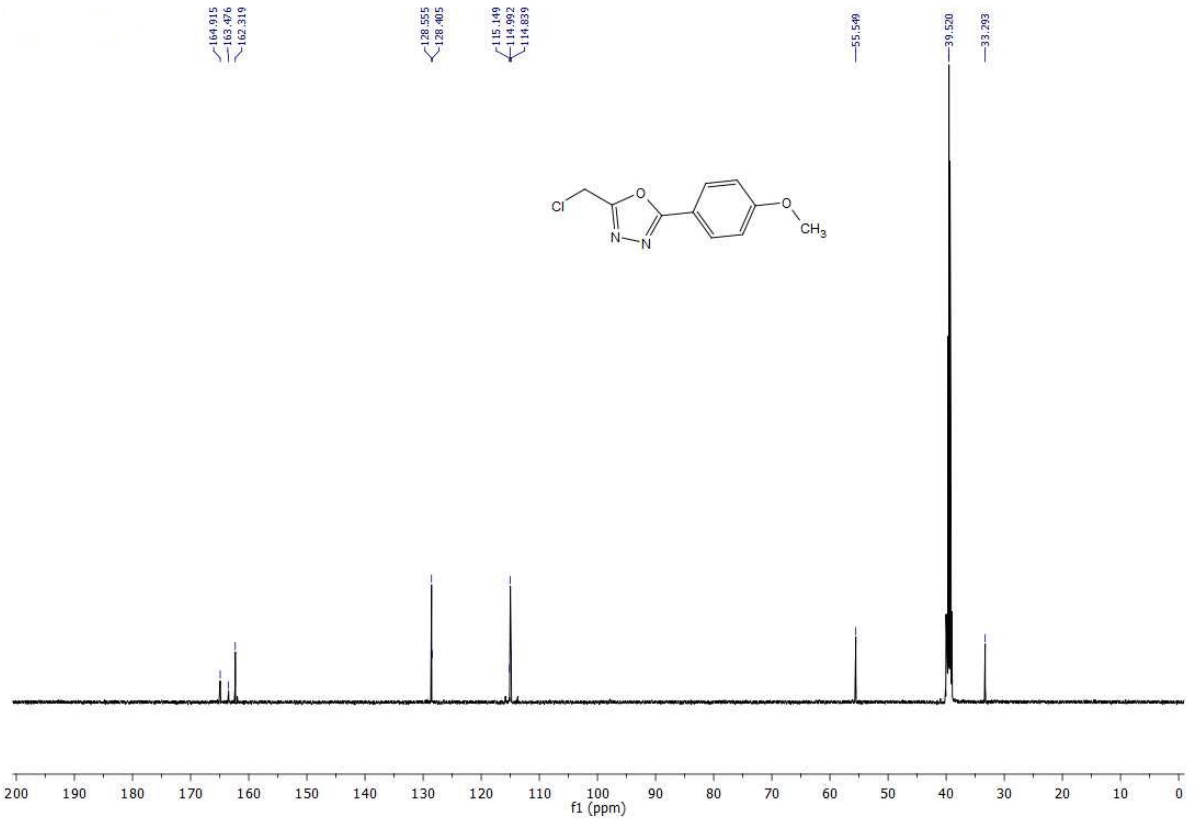

Figure S14. [13]C NMR spectra of target compound **8b**

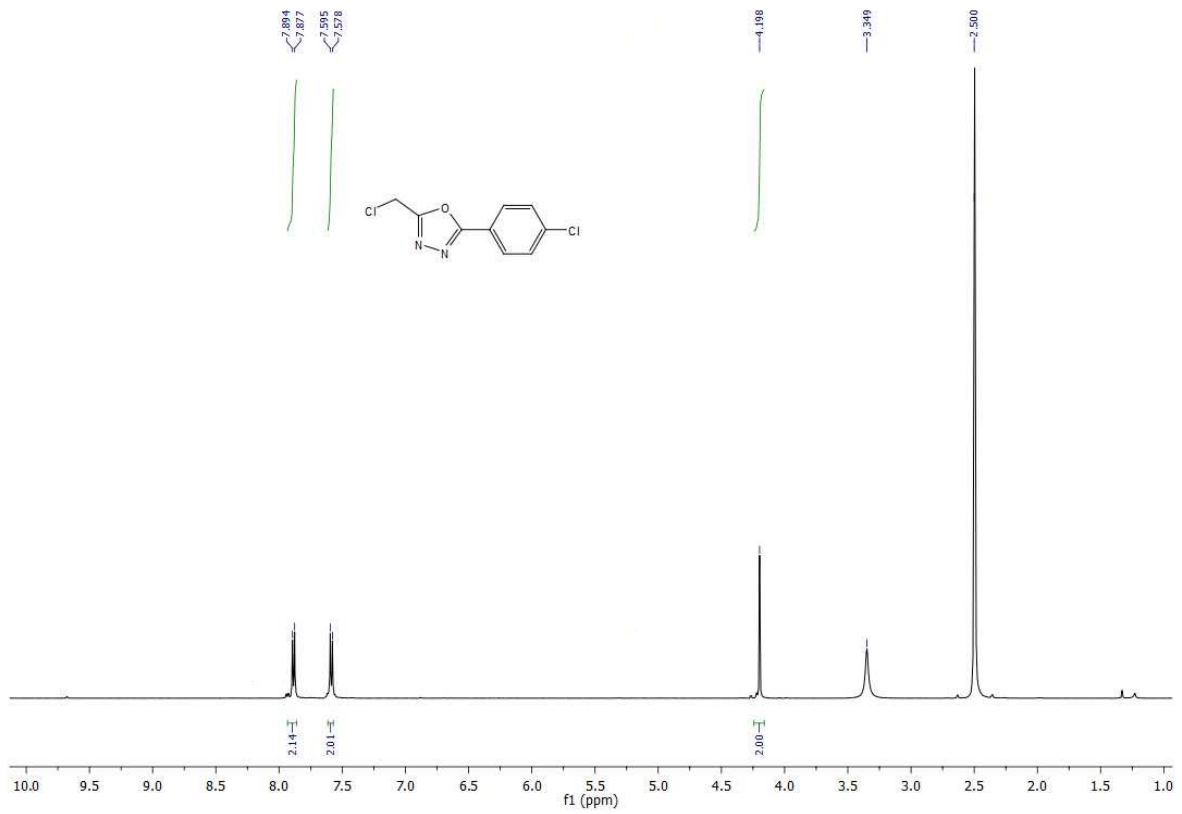

Figure S15. ^{1}H NMR spectra of target compound **8d.**

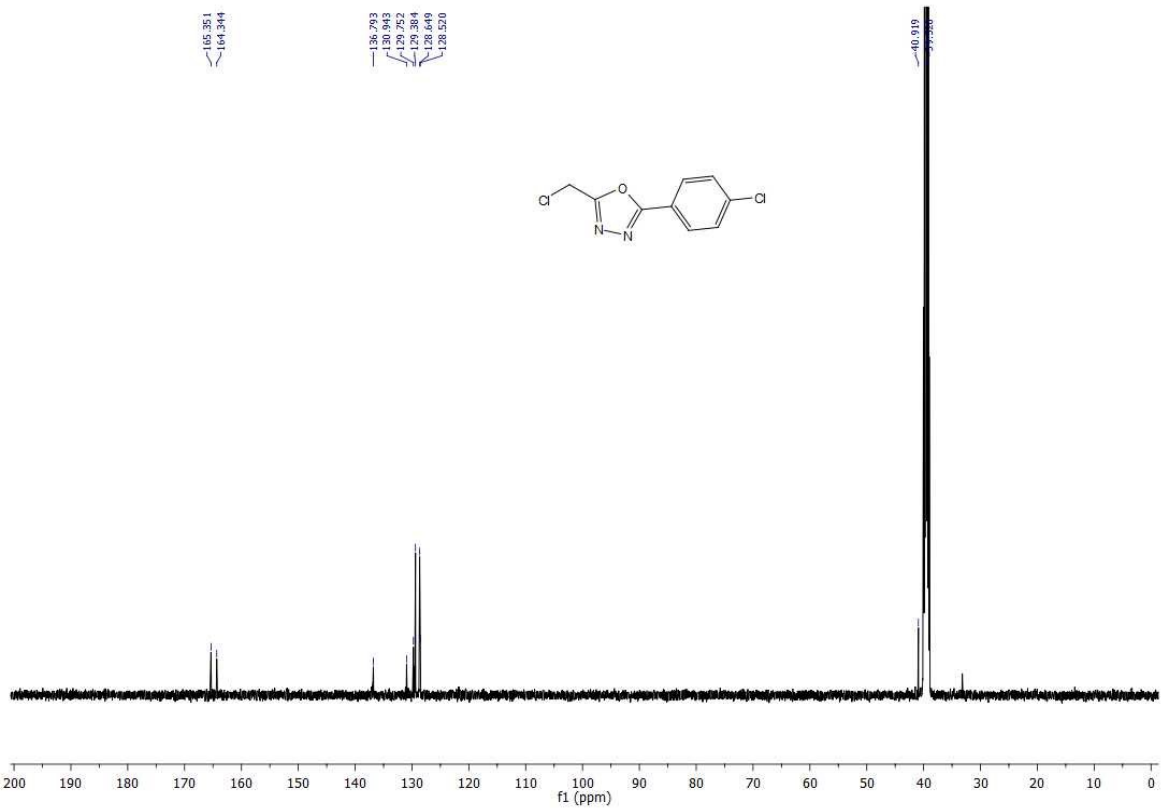

Figure S16. ^{13}C NMR spectra of target compound **8d.**

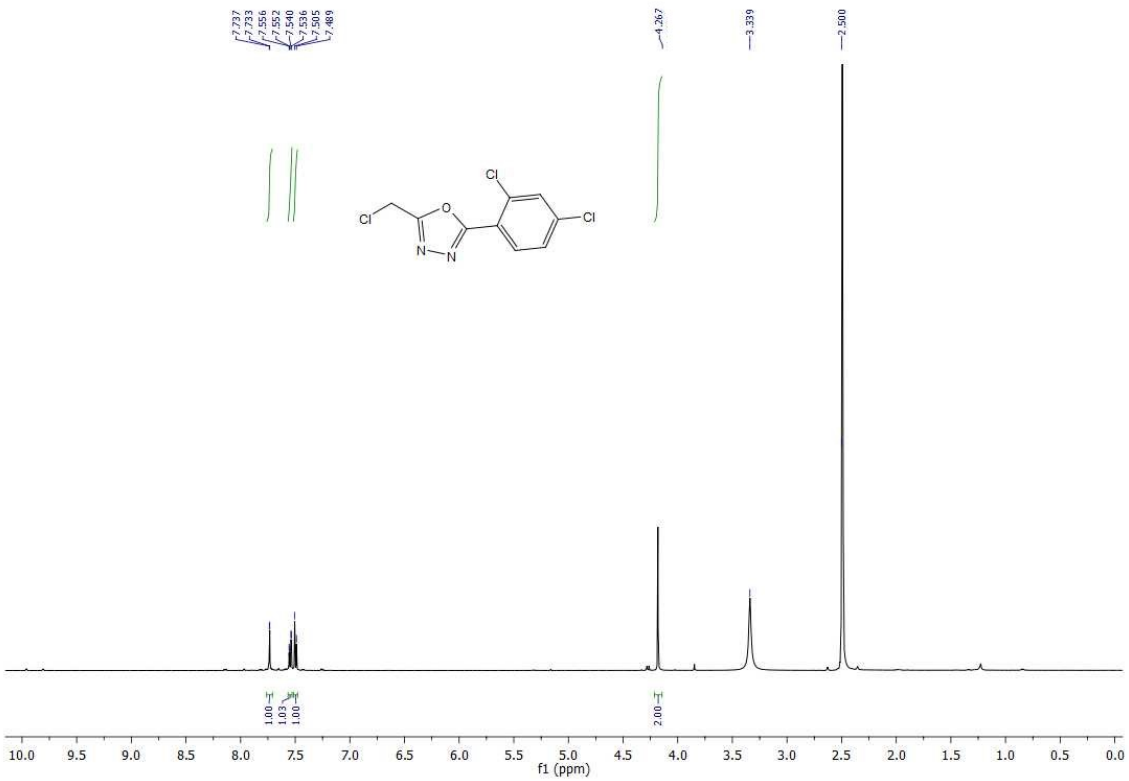

Figure S17. ^{1}H NMR spectra of target compound **8e.**

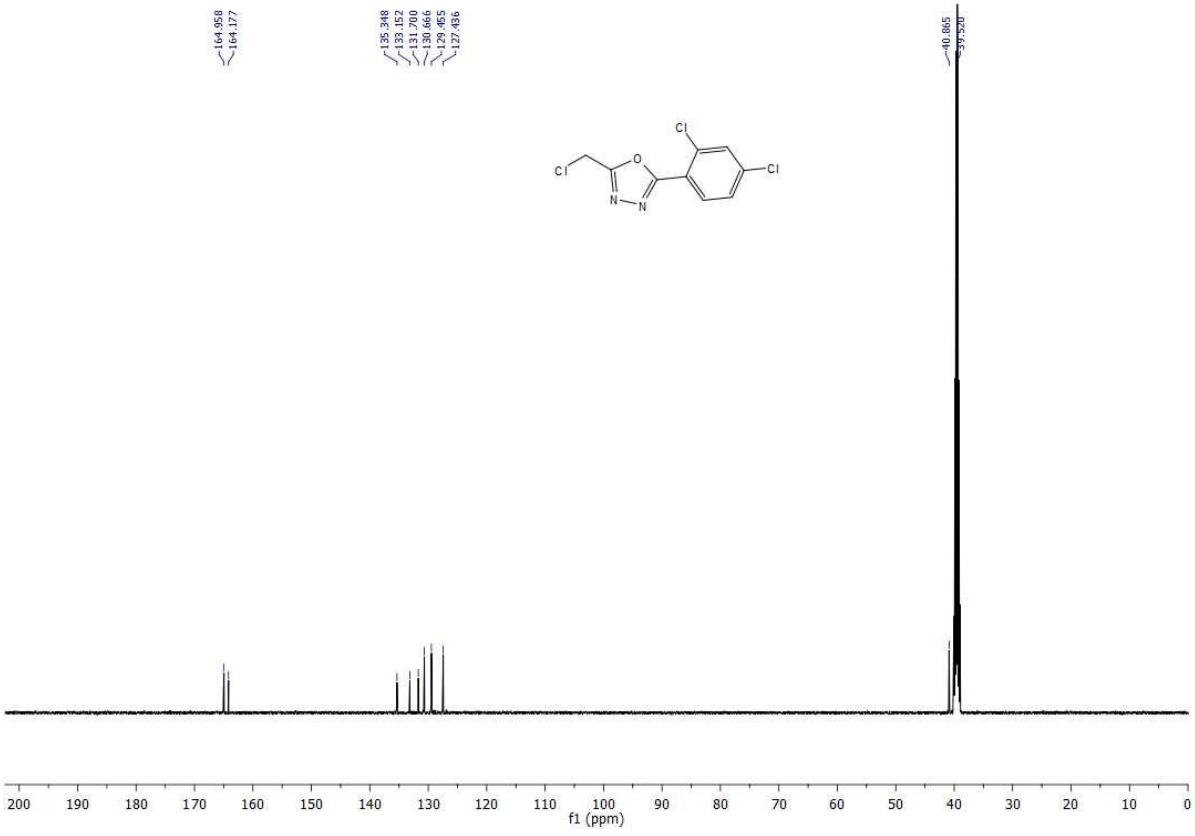

Figure S18. ^{13}C NMR spectra of target compound **8e.**

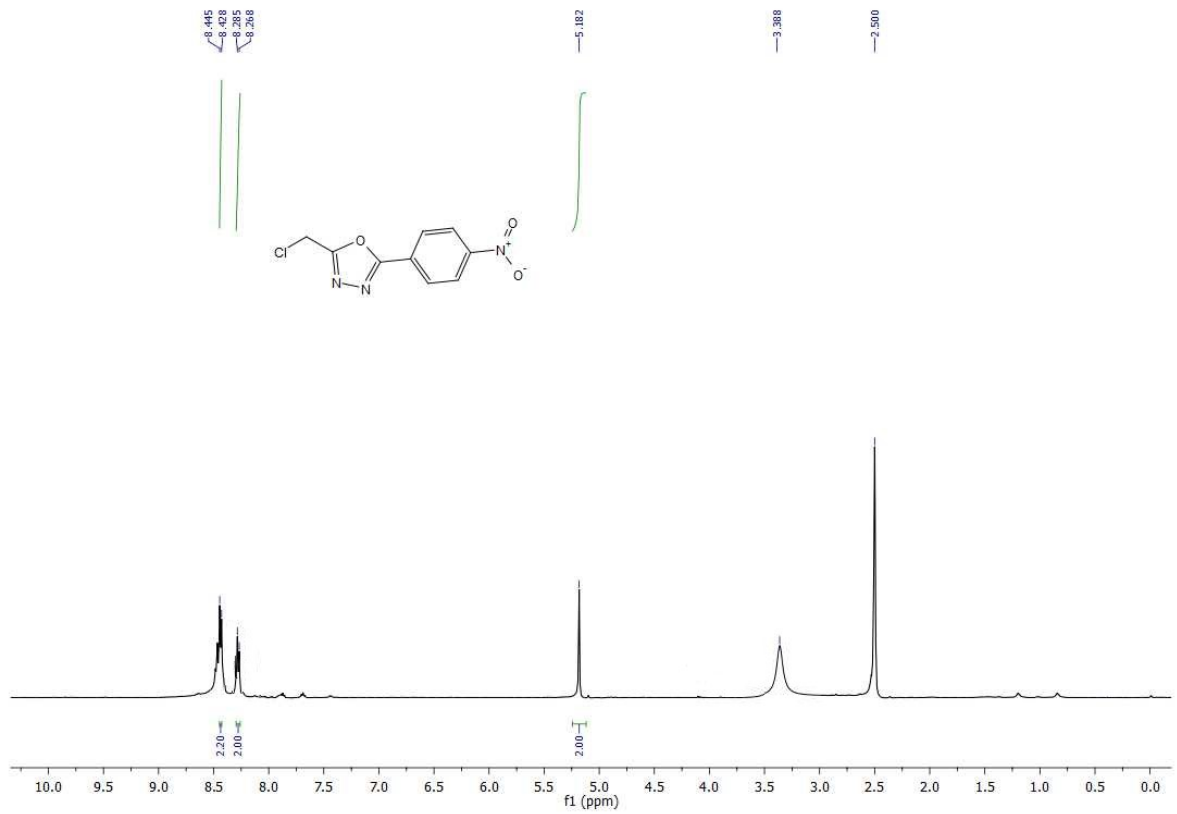

Figure S19. ^{1}H NMR spectra of target compound **8g.**

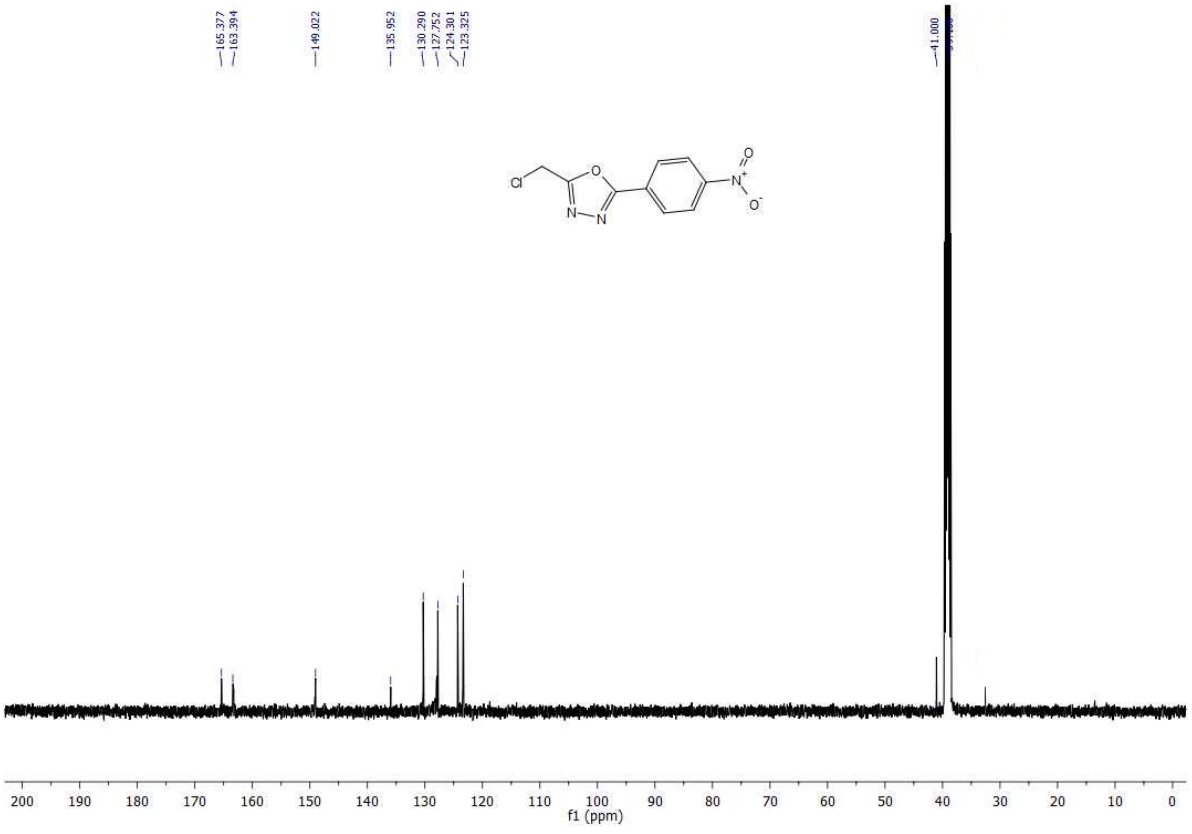

Figure S20. ^{13}C NMR spectra of target compound **8g.**

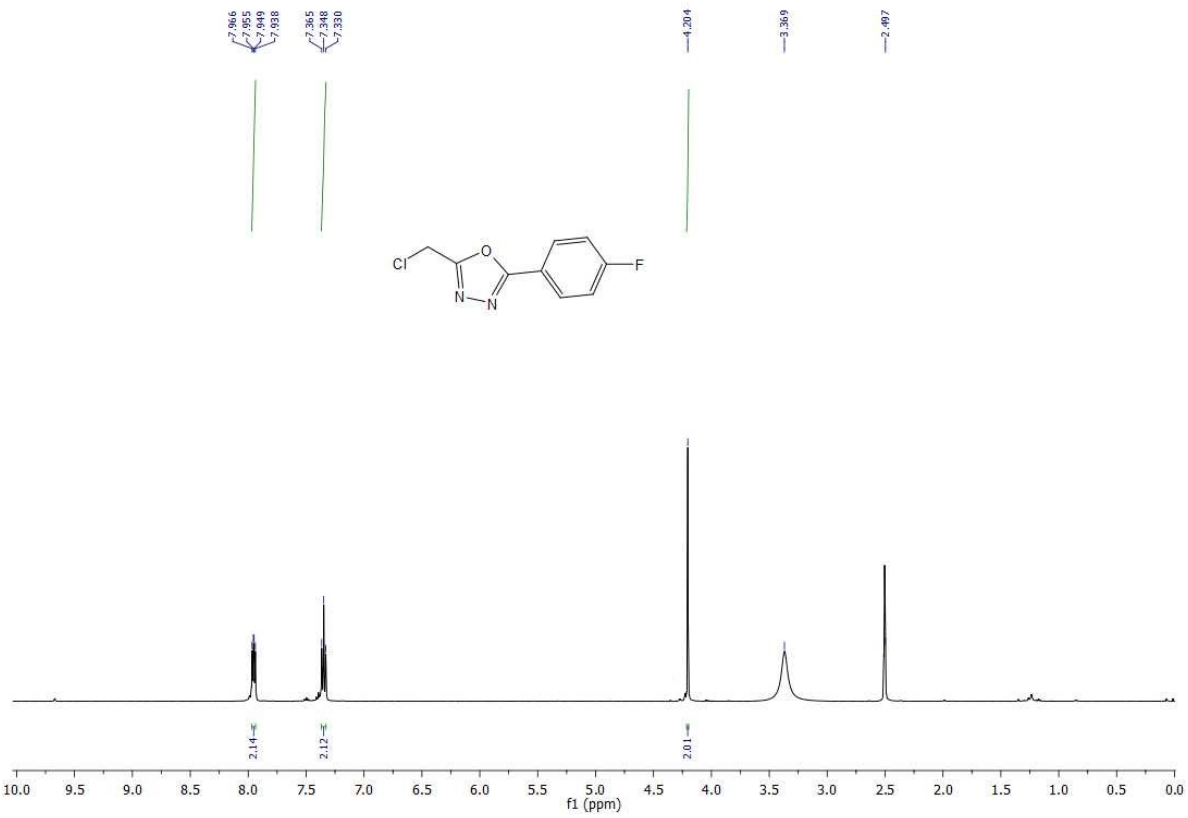

Figure S21. [1]H NMR spectra of target compound **8l.**

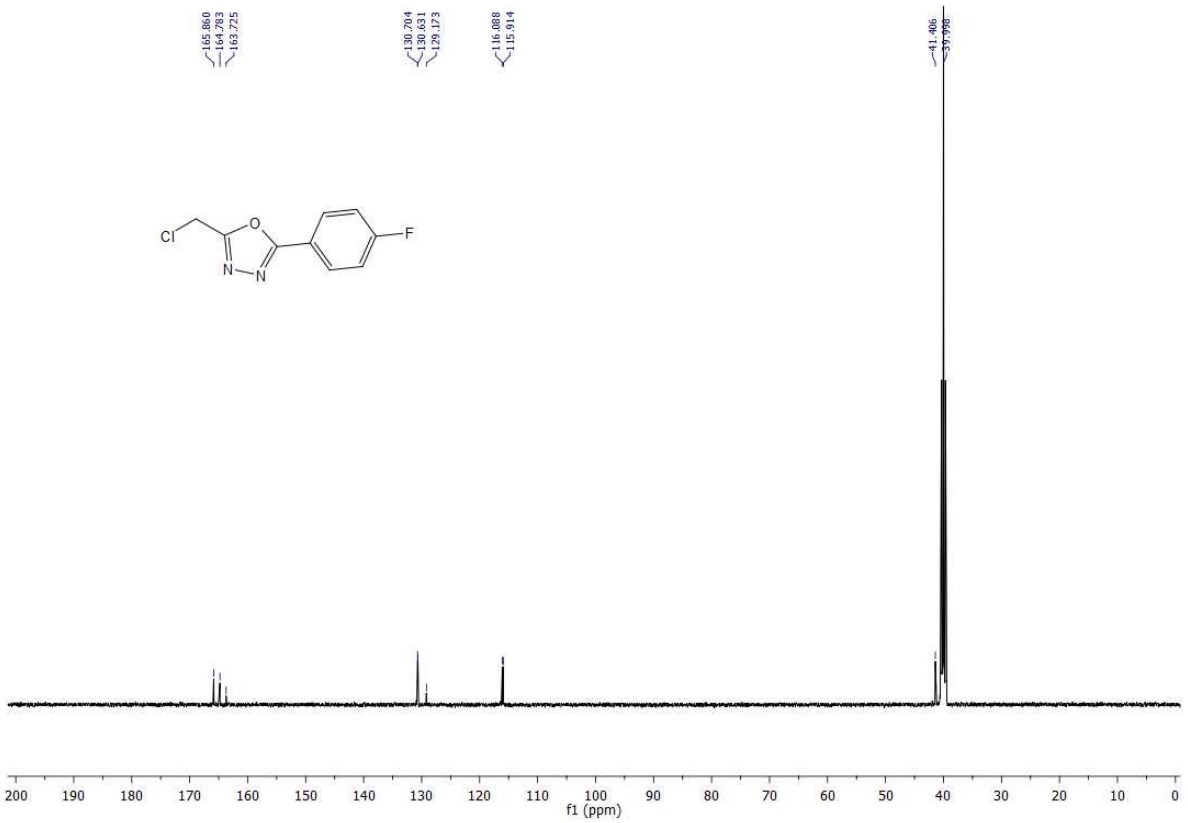

Figure S22. [13]C NMR spectra of target compound **8l.**

^{1}H NMR, ^{13}C NMR, HRMS, and HPLC spectra of the representative final compounds

(9b, 9d, 9e, 9g, 9l)

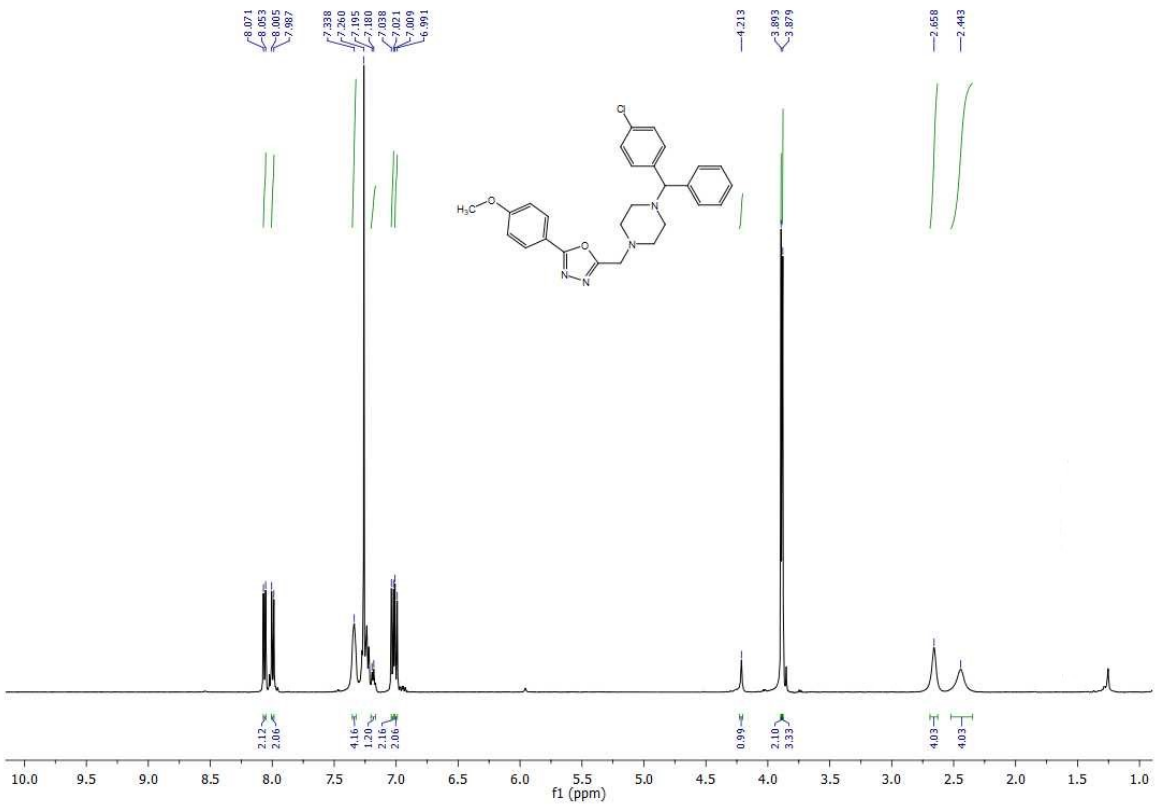

Figure S23.[1]H NMR spectra of target compound **9b.**

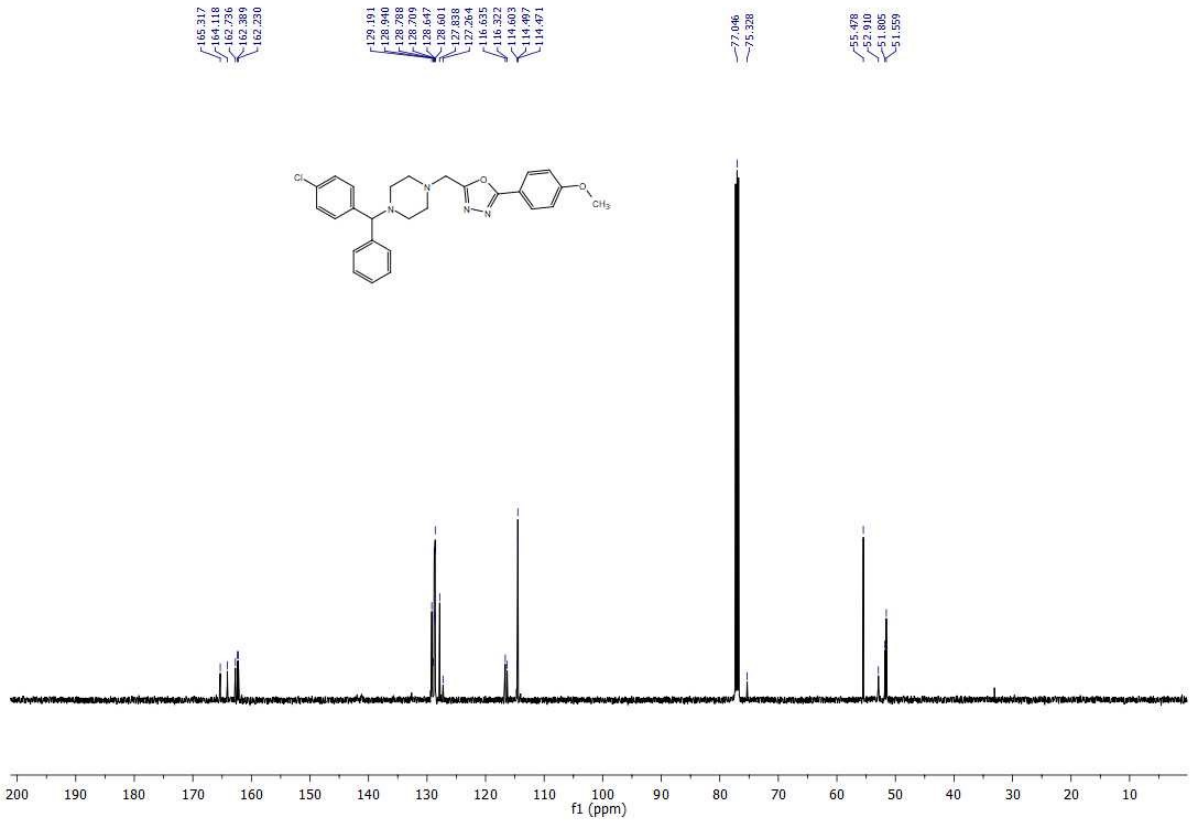

Figure S24. [13]C NMR spectra of target compound **9b.**

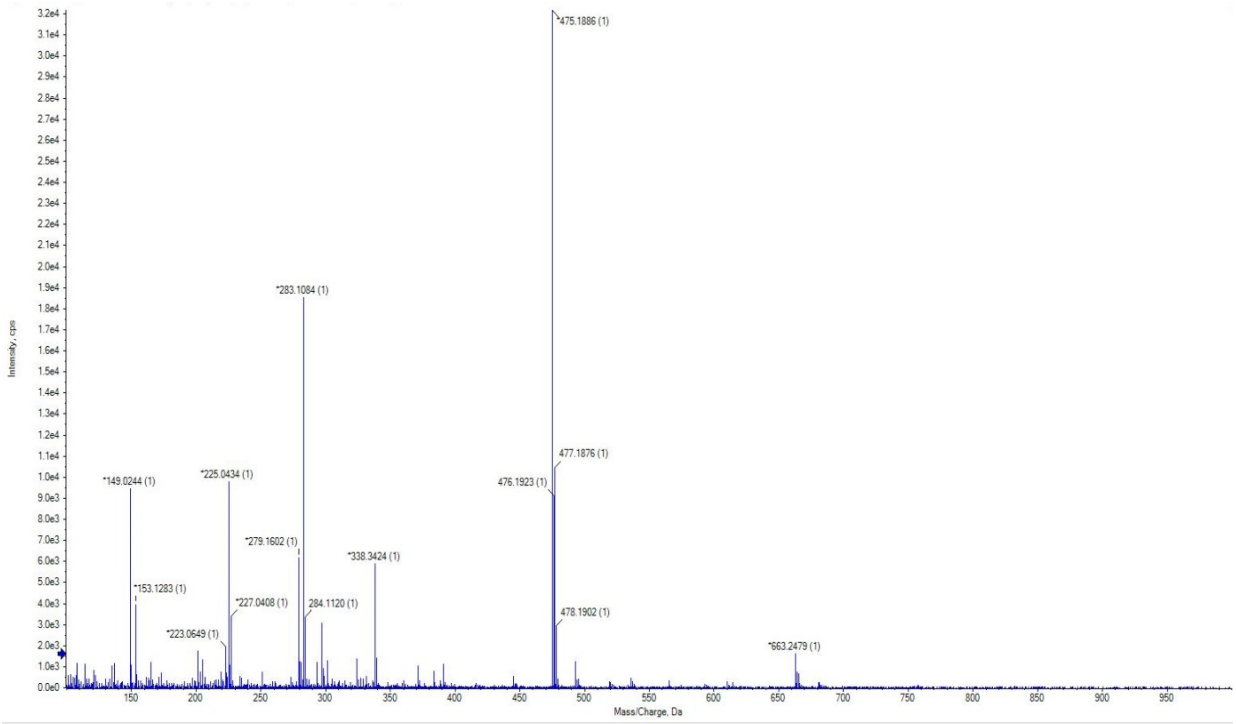

Figure S25. HRMS $[M + H]^+$spectra of target compound **9b.**

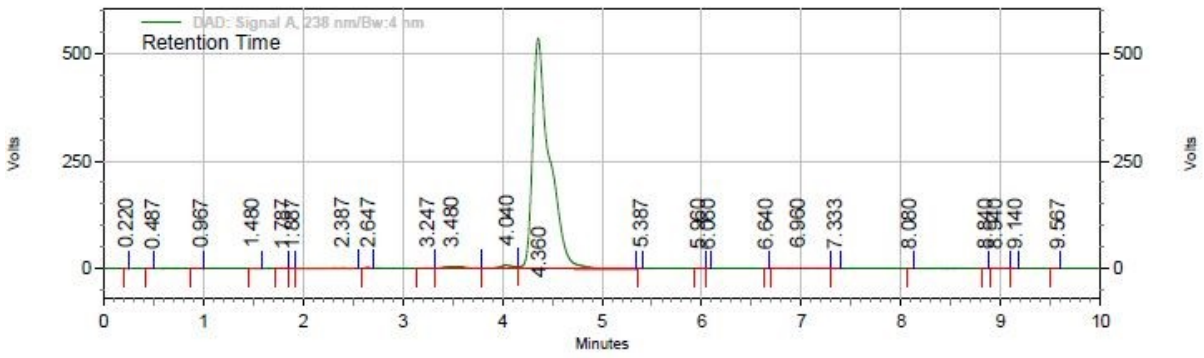

Figure S26. HPLC chromatogram of target compound **9b**

Percentage purity of compound 9b:

- ✓Determined using the Agilent 1200 Infinity high-performance liquid chromatography (HPLC) system, USA.
- ✓ Column: Quasar C_{18} 250 × 4.6mm, 5µm Cat. No. N9308801
- ✓ Mobile phase: Methanol (90): Water (10)
- ✓ Flow rate: 1ml/min.
- ✓ Sample volume: 20 µl
- ✓ Detection range: λmax = 254

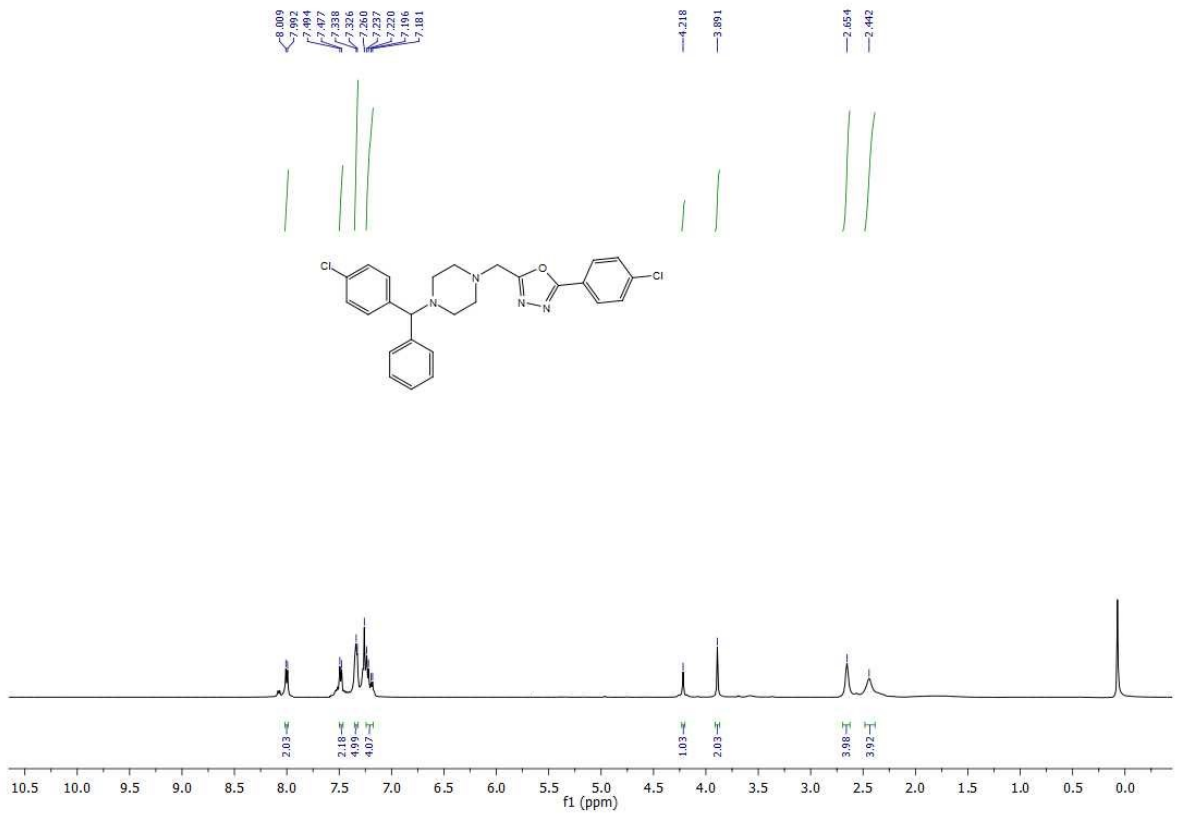

Figure S27. ^{1}H spectra of target compound **9d**

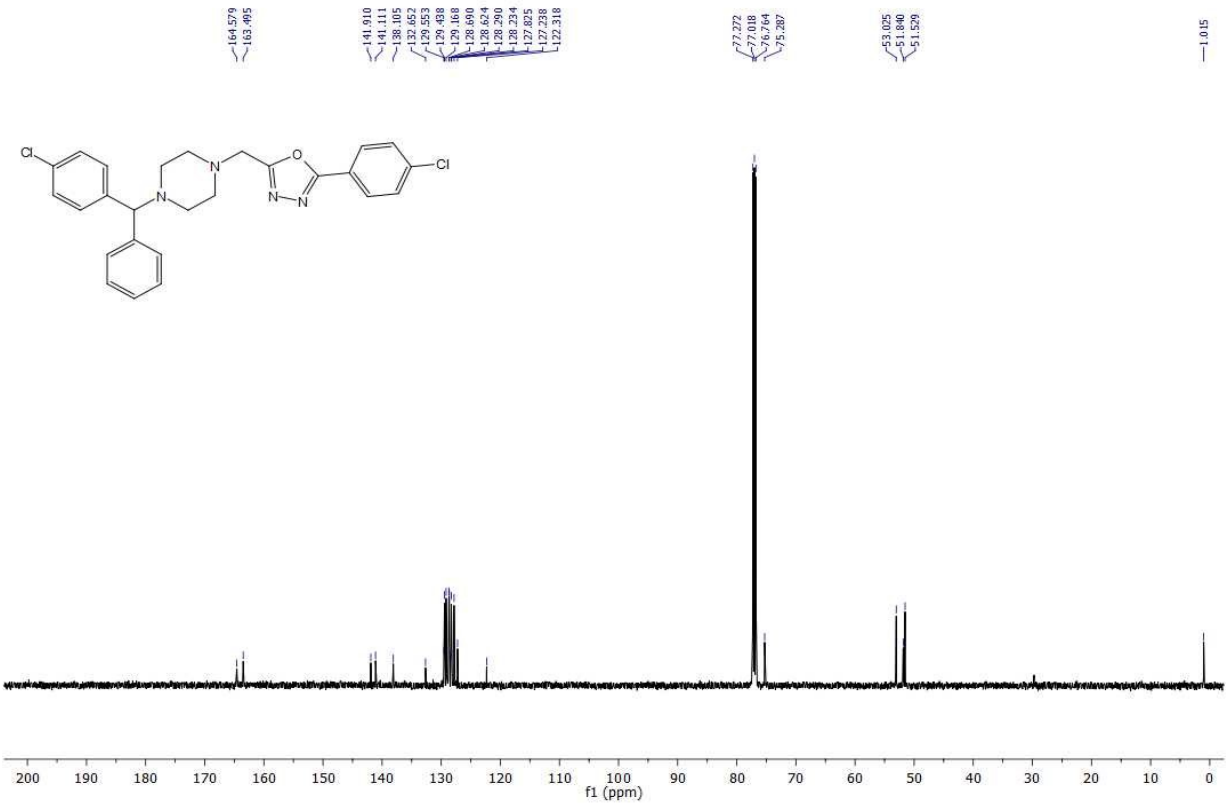

Figure S28. ^{13}C spectra of target compound **9d**

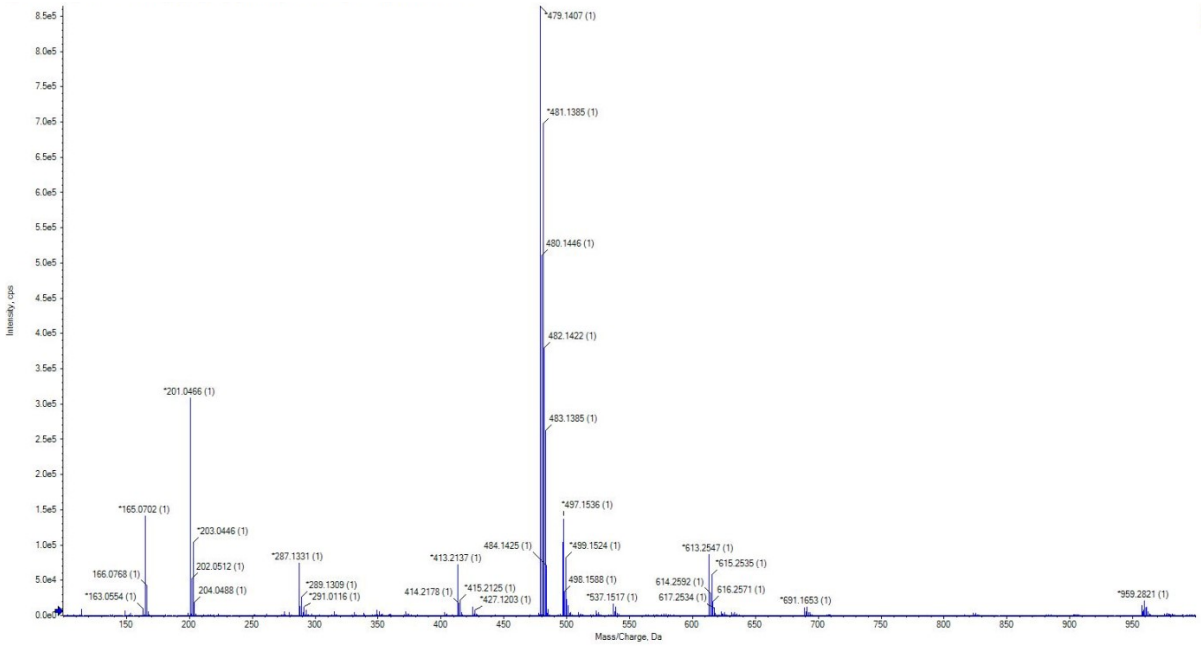

Figure S29. HRMS $[M + H]^+$ spectra of target compound **9d.**

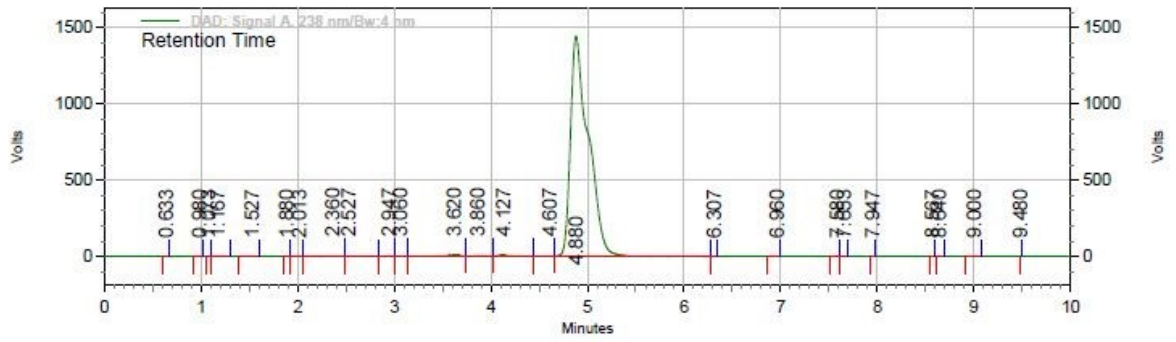

Figure S30. HPLC chromatogram of target compound **9d**

Percentage purity of compound 9d:

- ✓Determined using the Agilent 1200 Infinity high-performance liquid chromatography (HPLC) system, USA.
- ✓ Column: Quasar C_{18} 250 × 4.6mm, 5µm Cat. No. N9308801
- ✓ Mobile phase: Methanol (90): Water (10)
- ✓ Flow rate: 1ml/min.
- ✓ Sample volume: 20 µl
- ✓ Detection range: λmax = 254

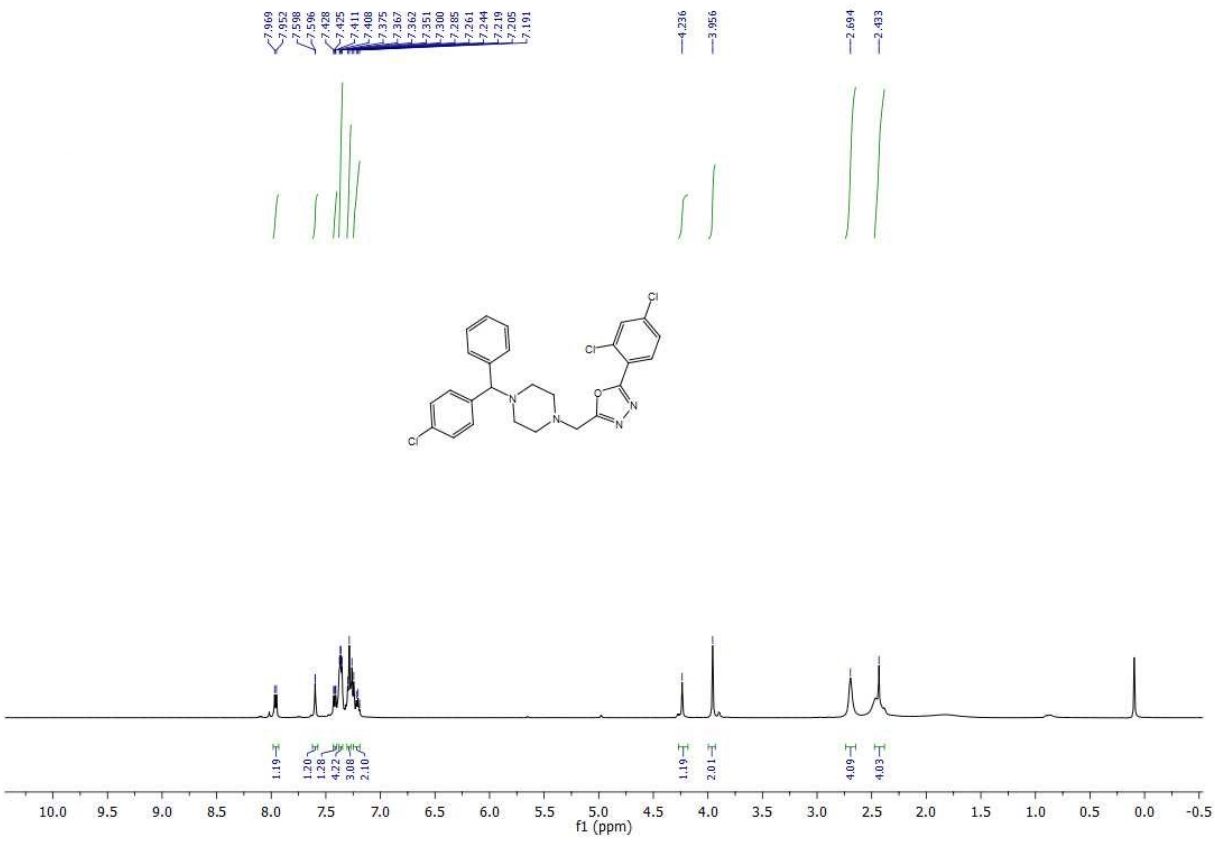

Figure S31. [1]H spectra of target compound **9e**

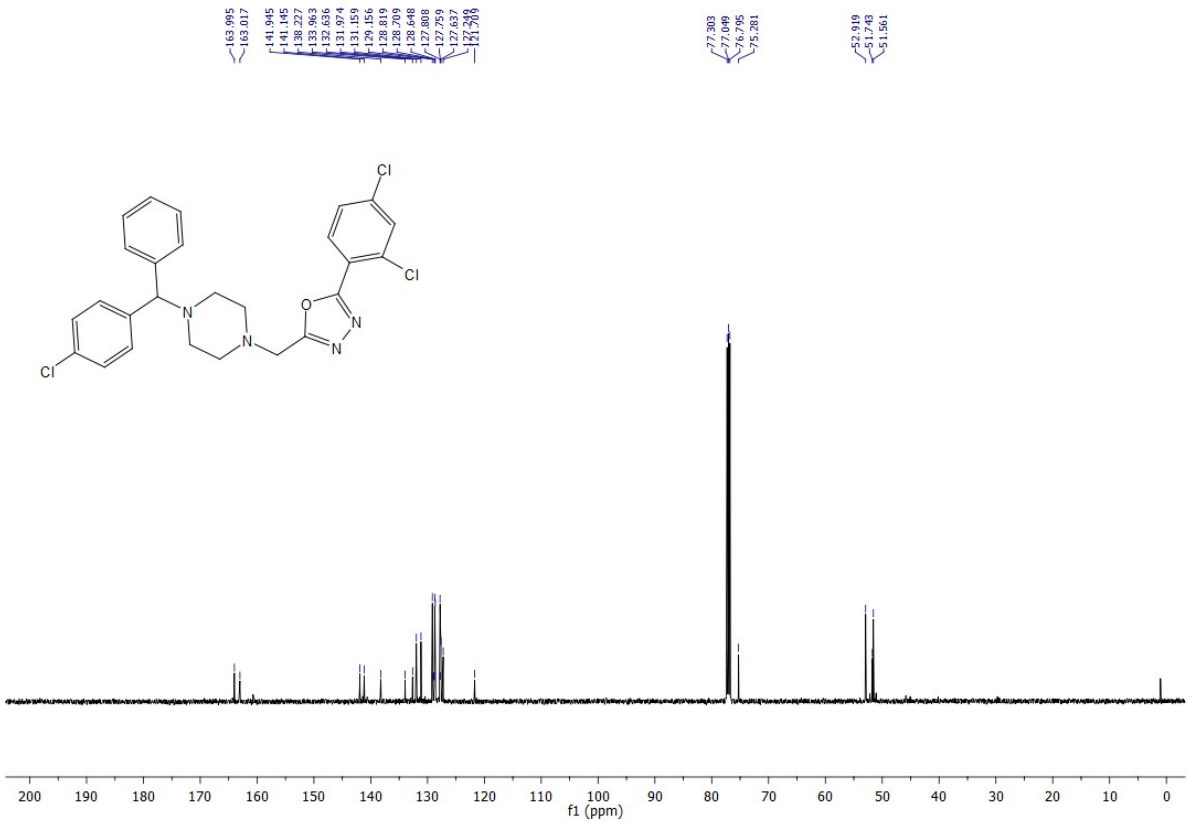

Figure S32. [13] C spectra of target compound **9e**

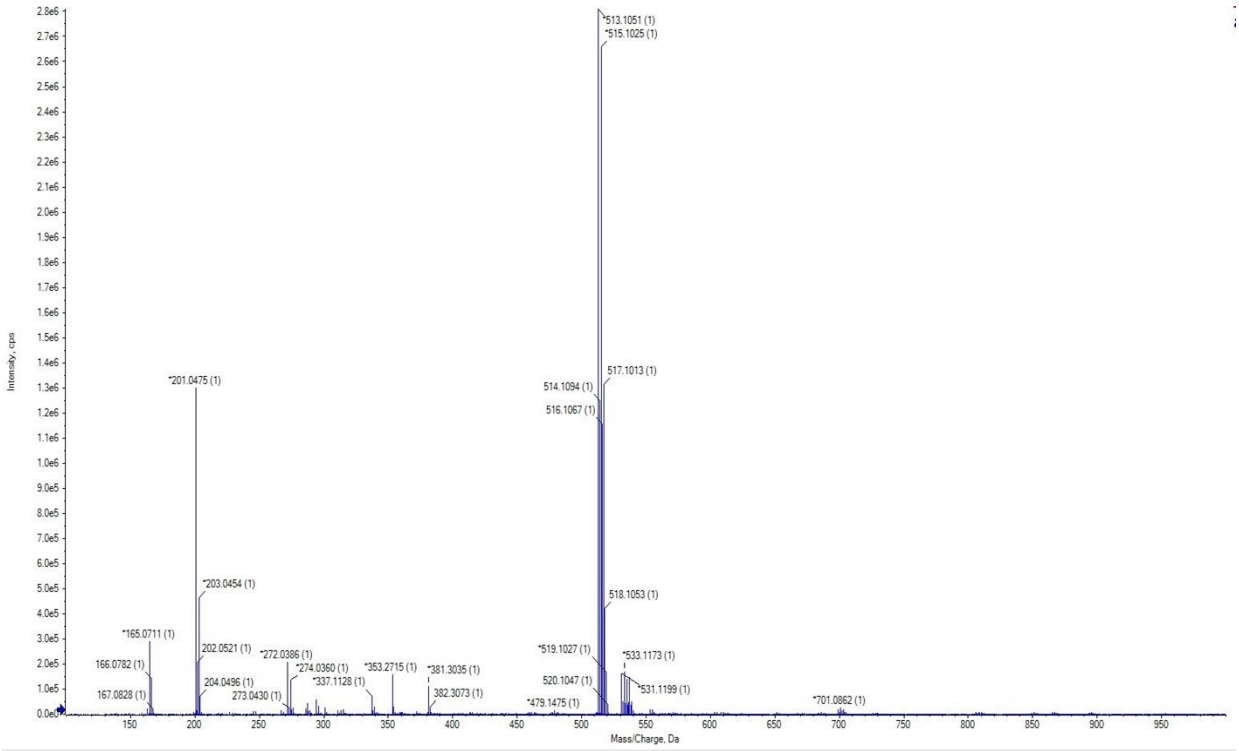

Figure S33. HRMS spectra of target compound **9e**

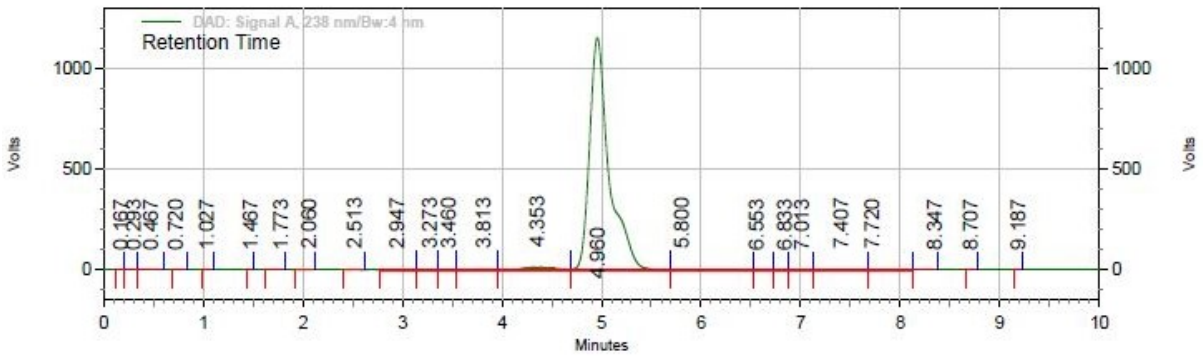

Figure S34. HPLC chromatogram of target compound **9e**

Percentage purity of compound 9e:

- ✓Determined using the Agilent 1200 Infinity high-performance liquid chromatography (HPLC) system, USA.
- ✓ Column: Quasar C_{18} 250 × 4.6mm, 5µm Cat. No. N9308801
- ✓ Mobile phase: Methanol (90): Water (10)
- ✓ Flow rate: 1ml/min.
- ✓ Sample volume: 20 µl
- ✓ Detection range: λmax = 254

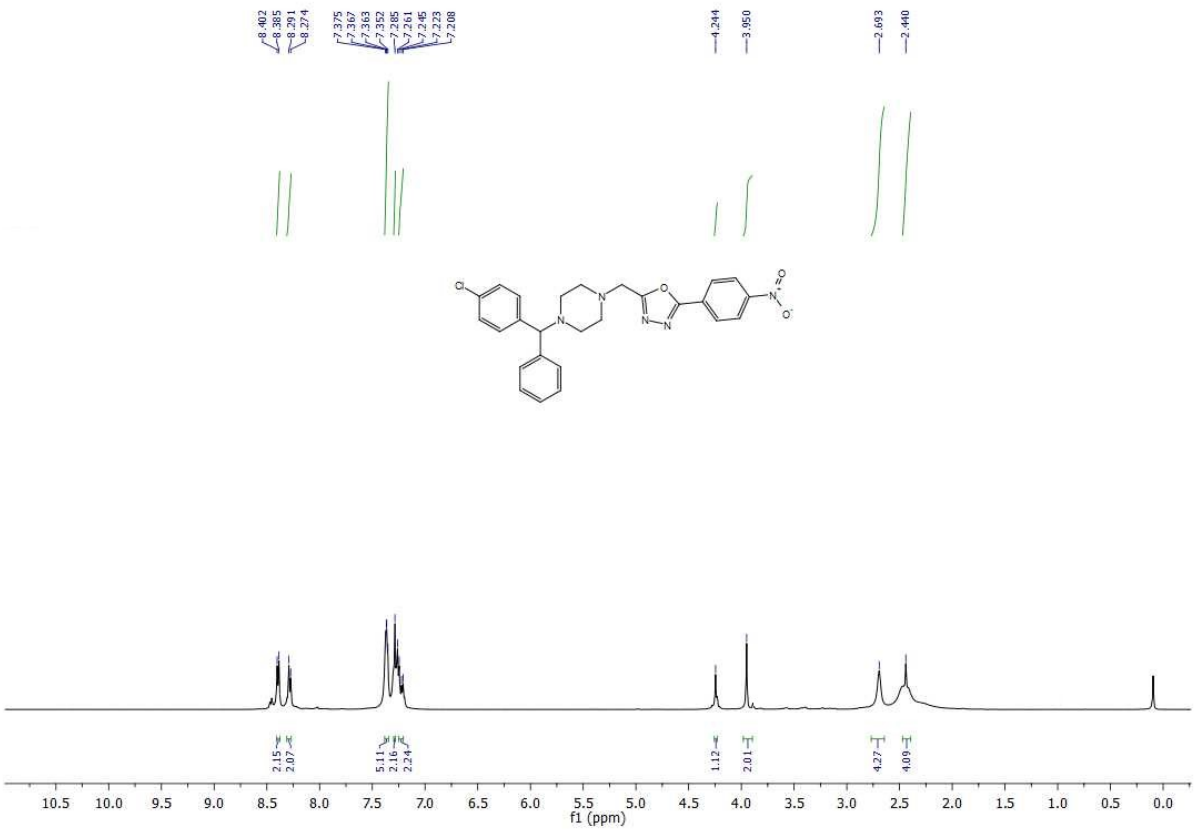

Figure S35. [1]H spectra of target compound **9g**

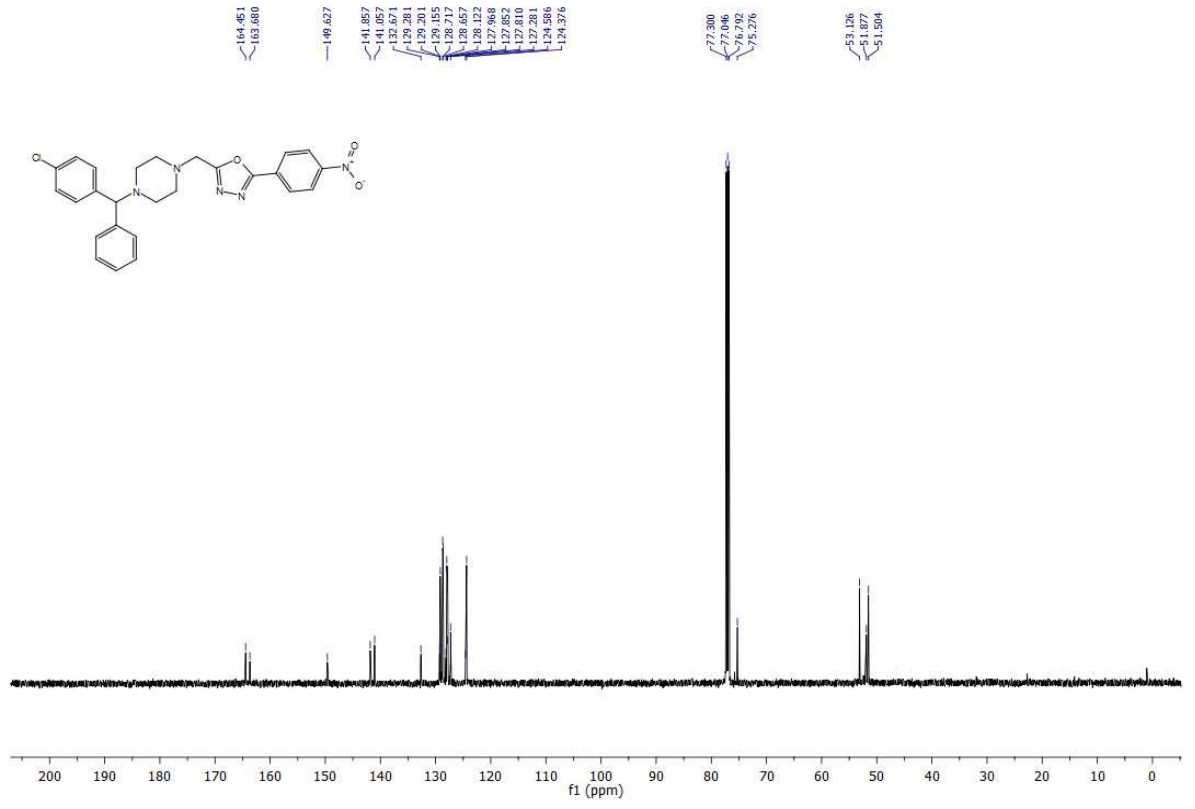

Figure S36. [13] C spectra of target compound **9g**

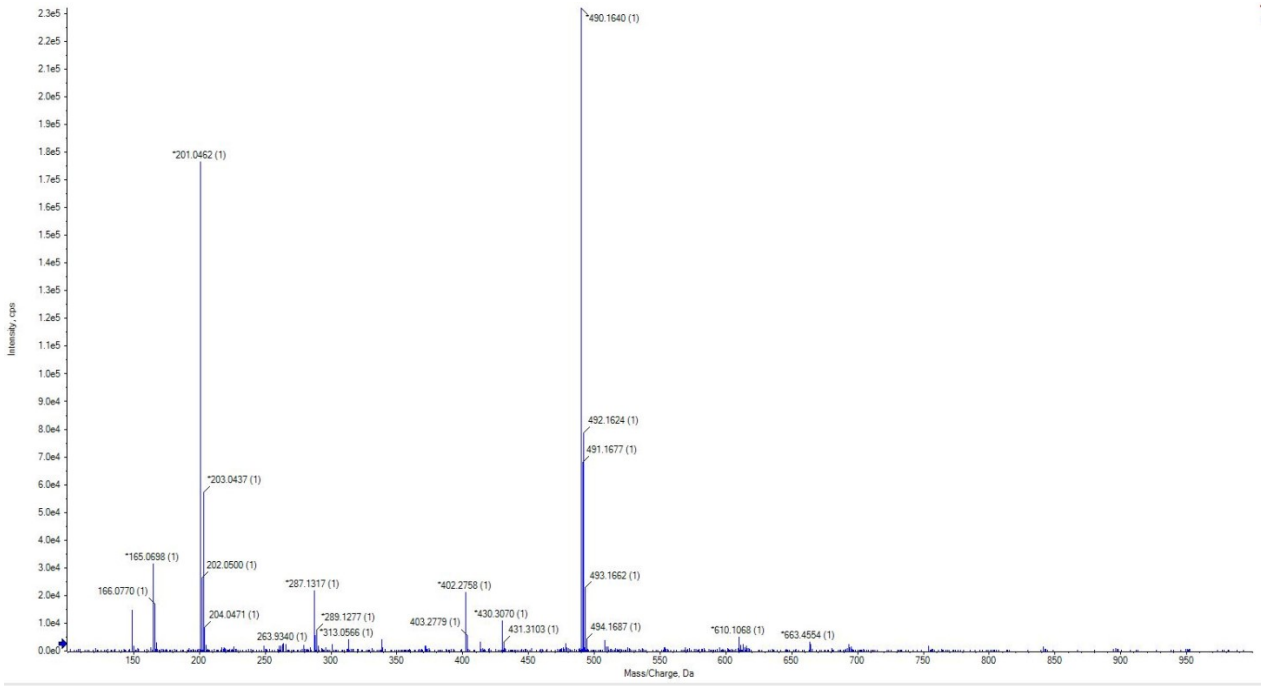

Figure S37. HRMS spectra of target compound **9g**

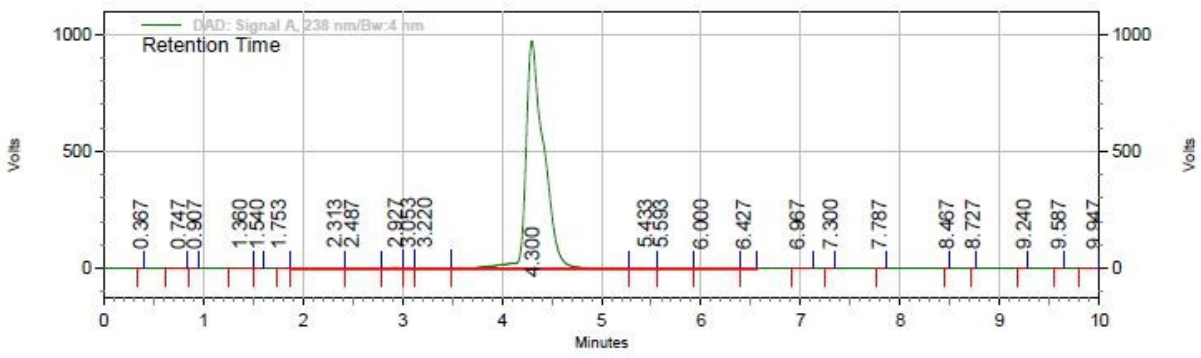

Figure S38. HPLC chromatogram of target compound **9g**

Percentage purity of compound 9g:

- ✓Determined using the Agilent 1200 Infinity high-performance liquid chromatography (HPLC) system, USA.
- ✓ Column: Quasar C_{18} 250 × 4.6mm, 5µm Cat. No. N9308801
- ✓ Mobile phase: Methanol (90): Water (10)
- ✓ Flow rate: 1ml/min.
- ✓ Sample volume: 20 µl
- ✓ Detection range: λmax = 254

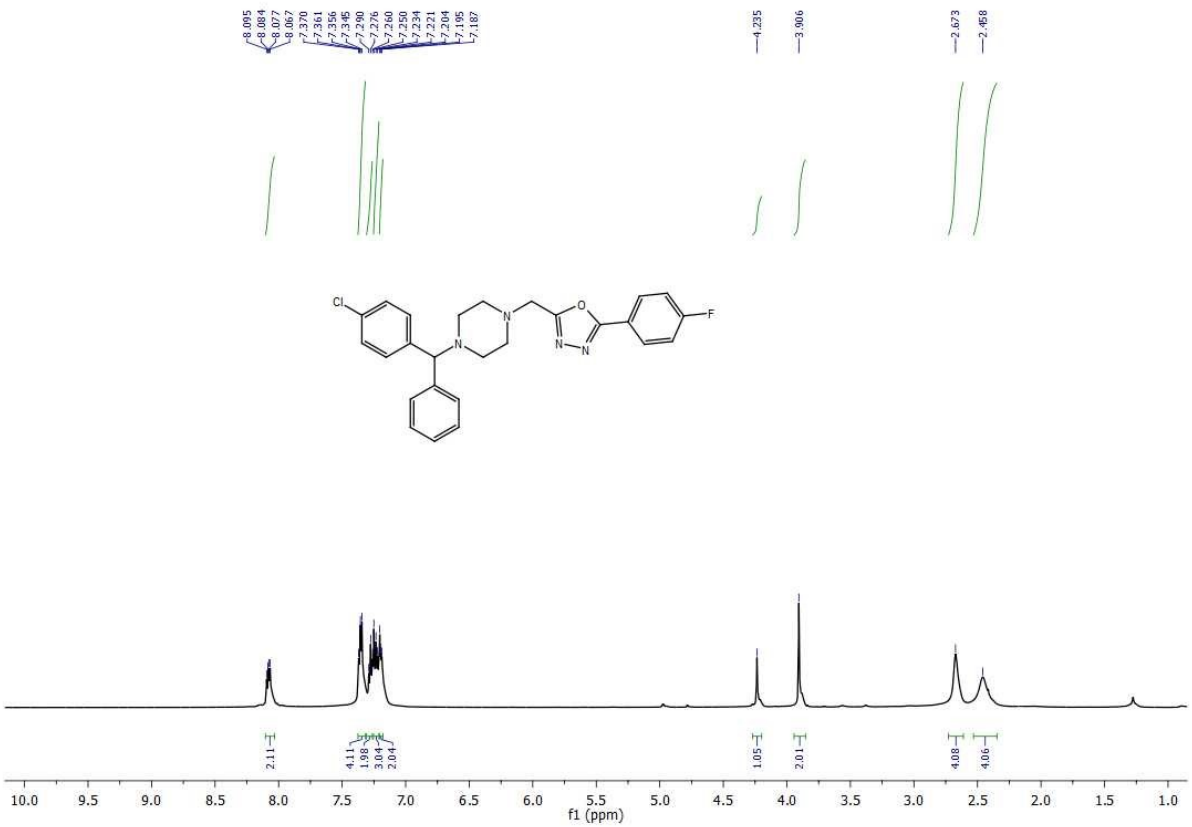

Figure S39 [1] H spectra of compound **9l**

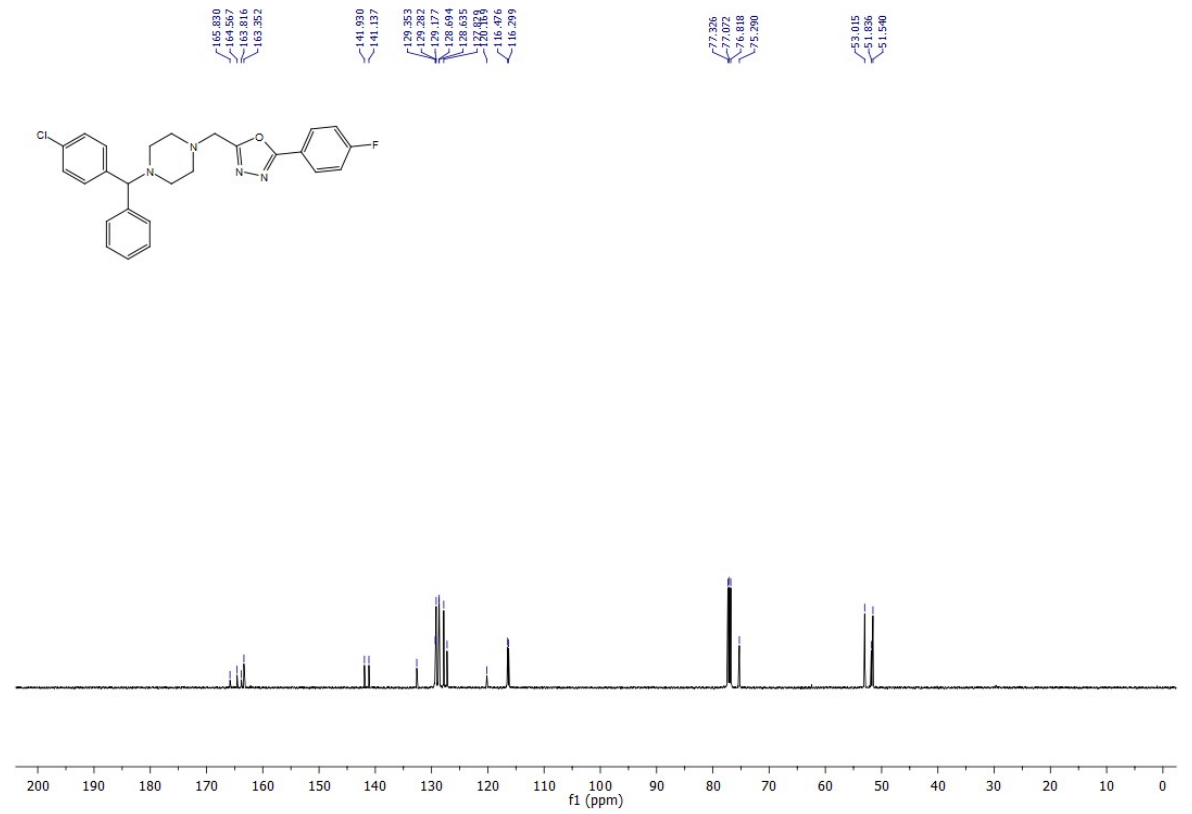

Figure S40. [13] C spectra of compound **9l**

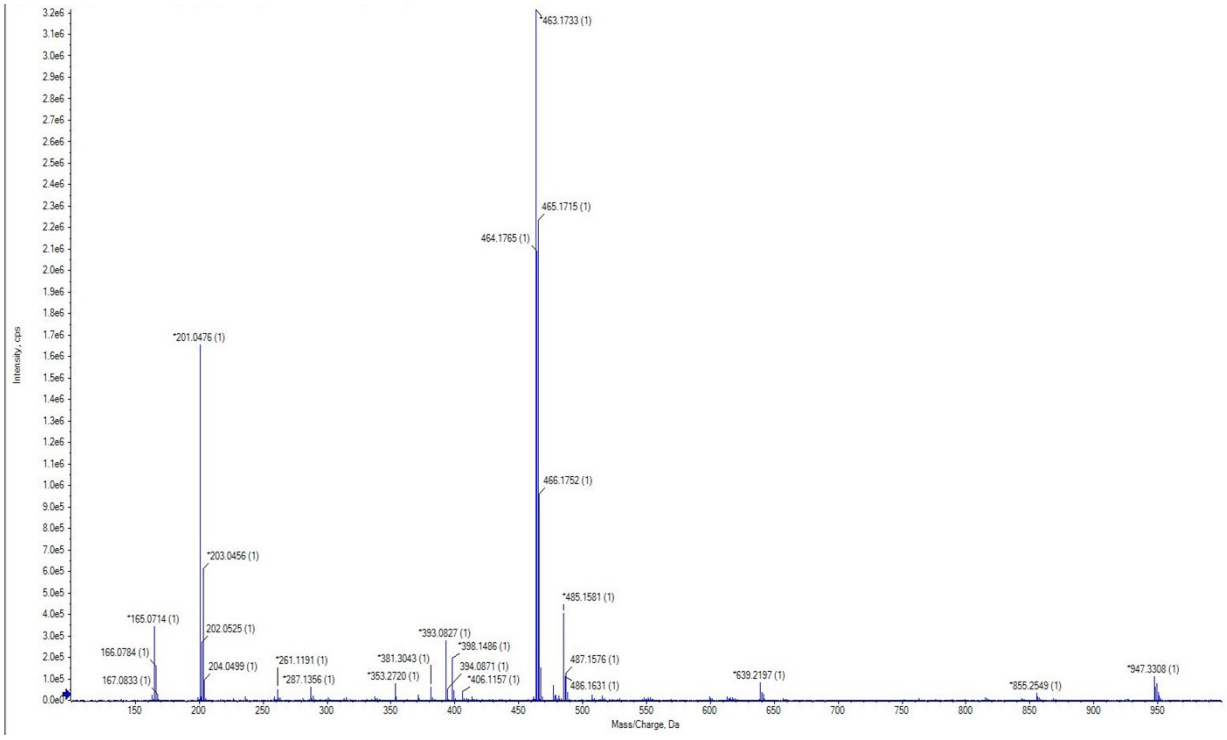

Figure S41. HRMS spectra of target compound **9l**

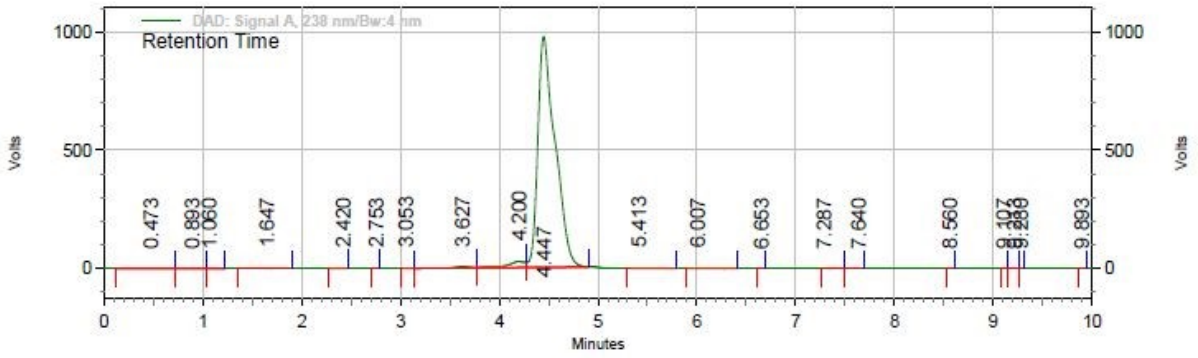

Figure S42. HPLC chromatogram of target compound **9l**

Percentage purity of compound 9i:

- ✓Determined using the Agilent 1200 Infinity high-performance liquid chromatography (HPLC) system, USA.
- ✓ Column: Quasar C_{18} 250 × 4.6mm, 5µm Cat. No. N9308801
- ✓ Mobile phase: Methanol (90): Water (10)
- ✓ Flow rate: 1ml/min.
- ✓ Sample volume: 20 µl
- ✓ Detection range: λmax = 254

^{1}H NMR spectra of each of the corresponding final compounds

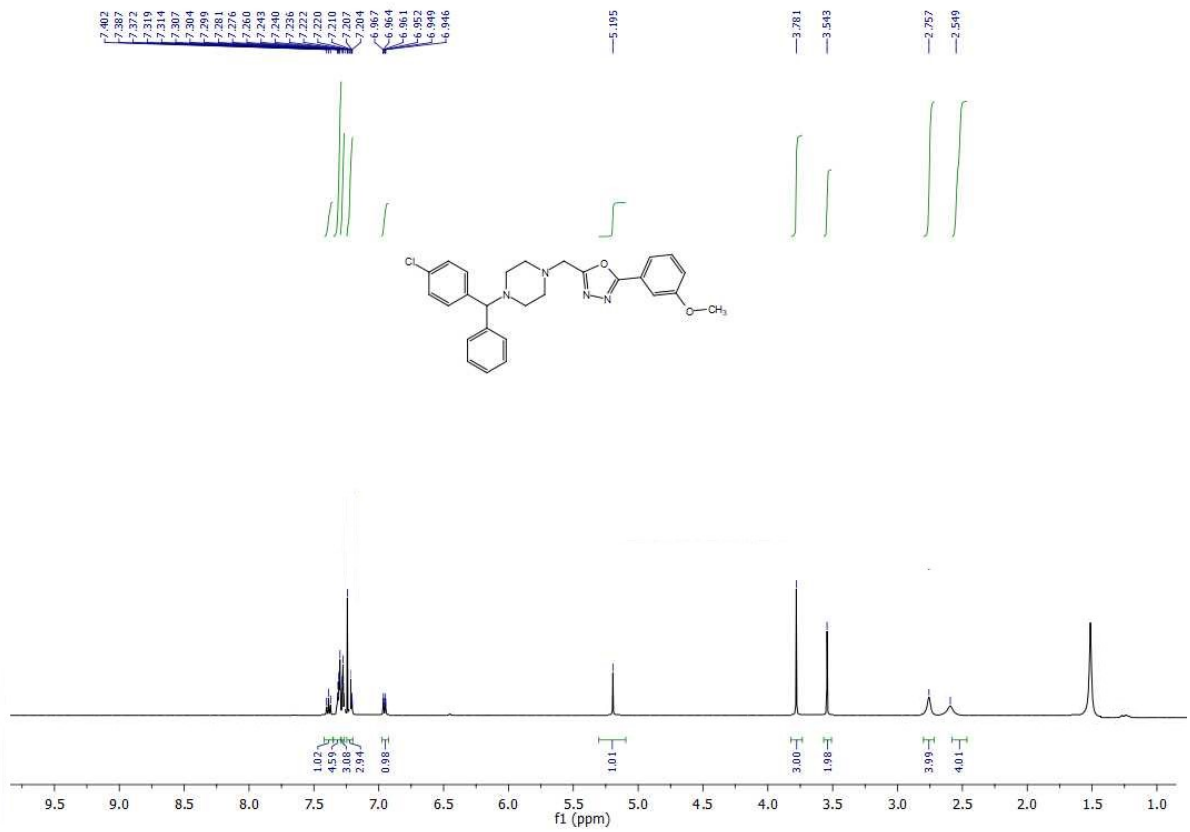

Figure S43. [1]H spectra of compound **9a**

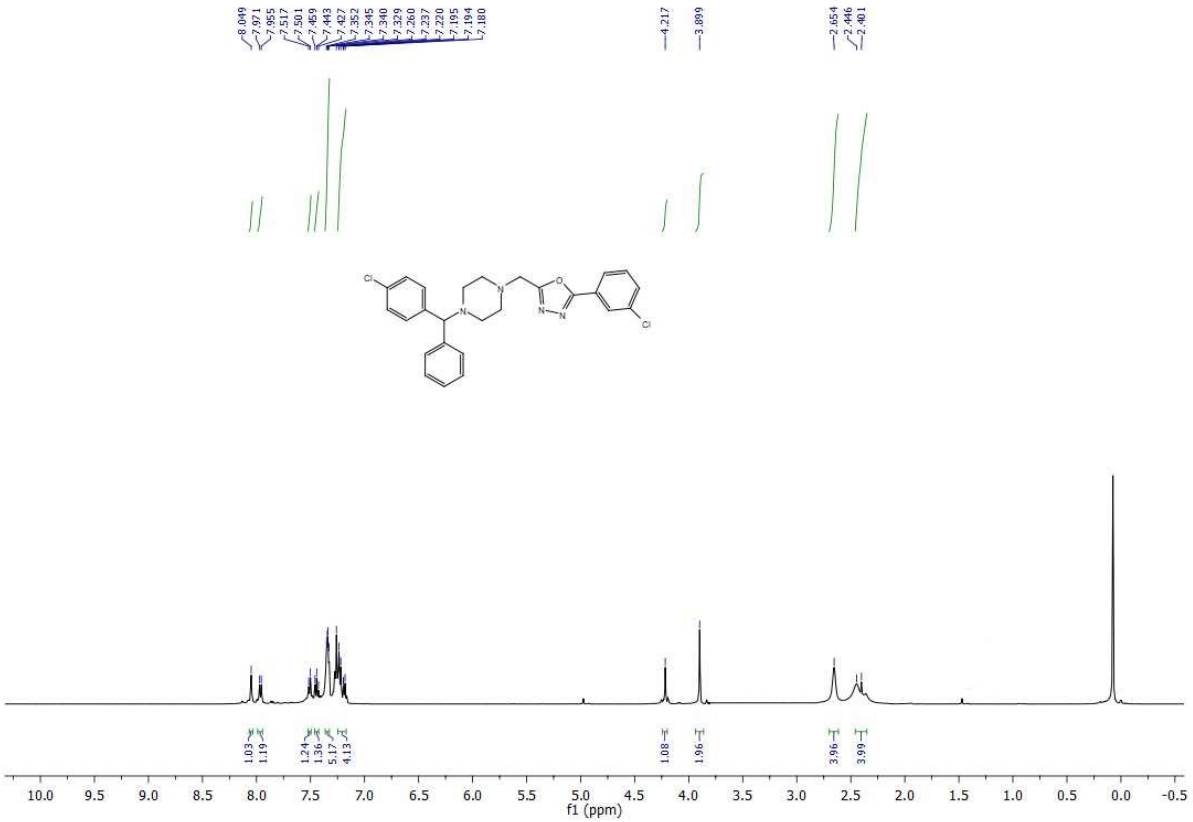

Figure S44. [1]H spectra of compound **9c**

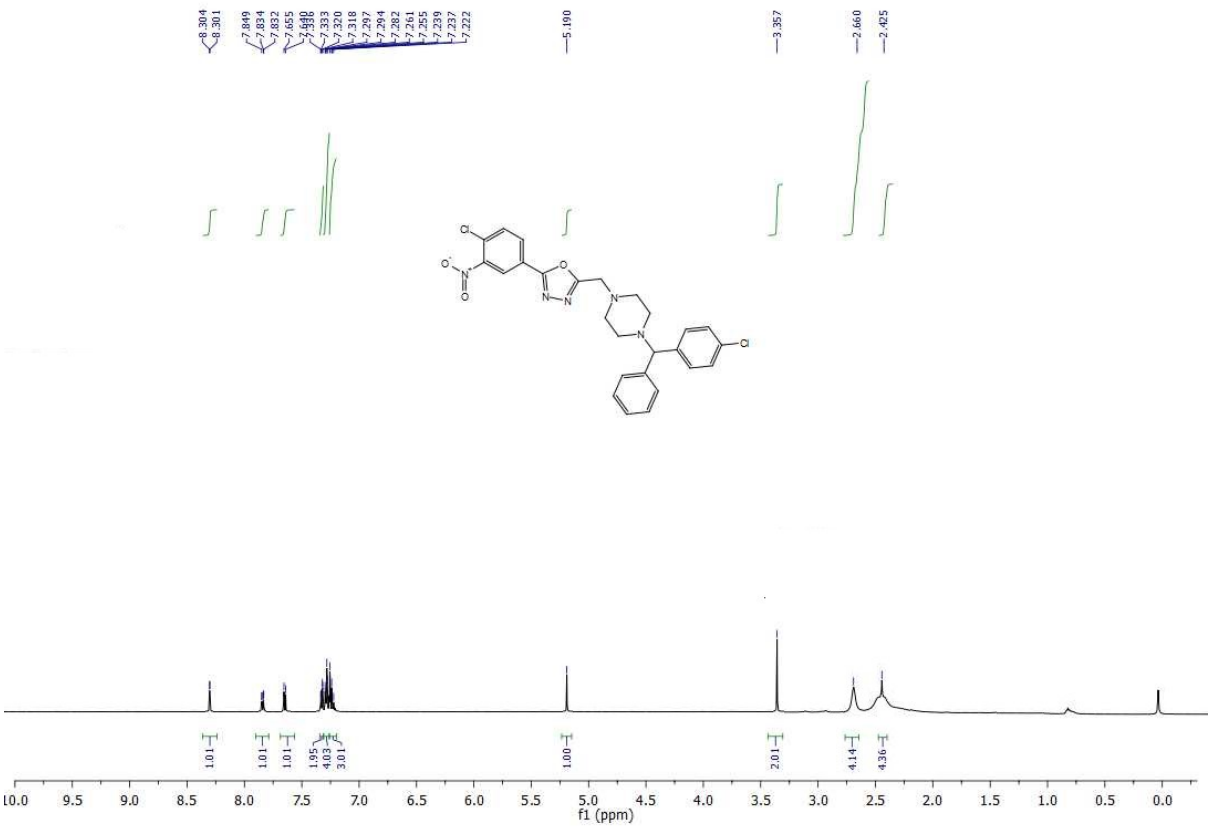

Figure S45. ^{1}H spectra of compound **9f**

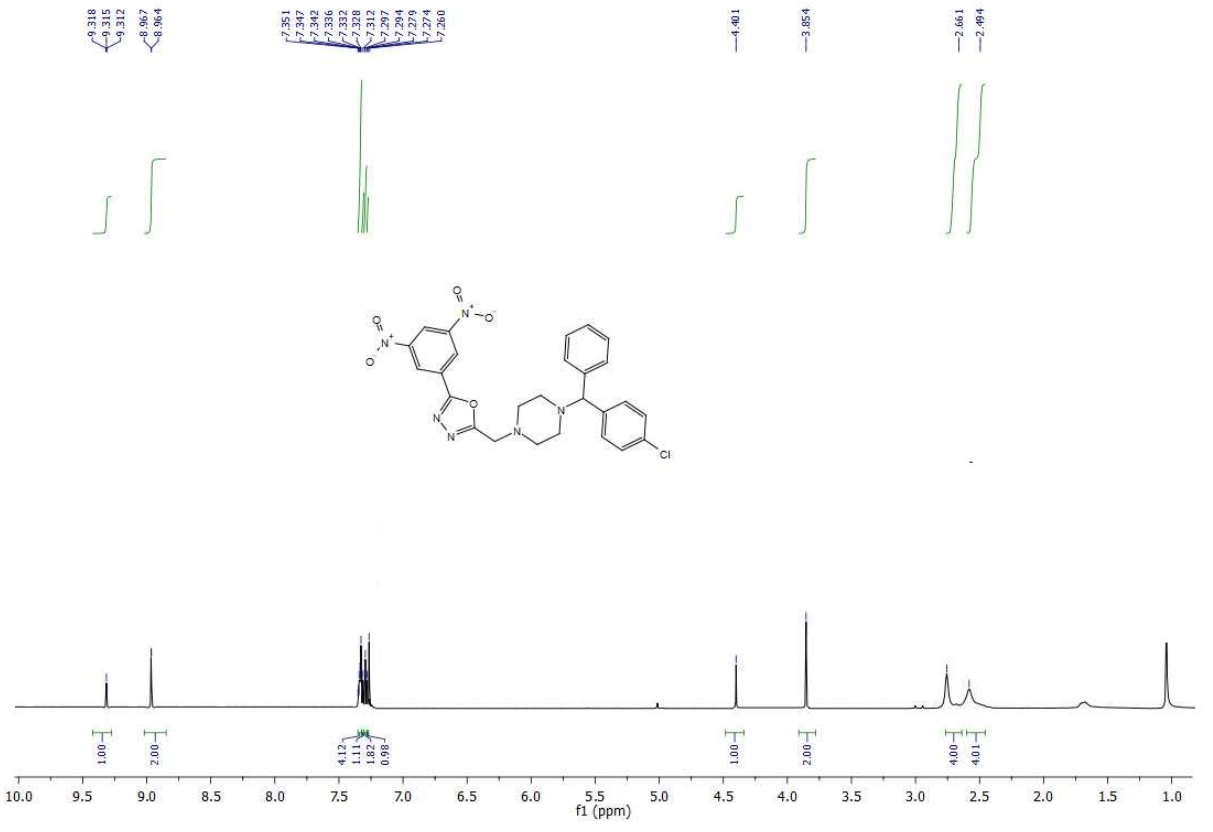

Figure S46. ^{1}H spectra of compound **9h**

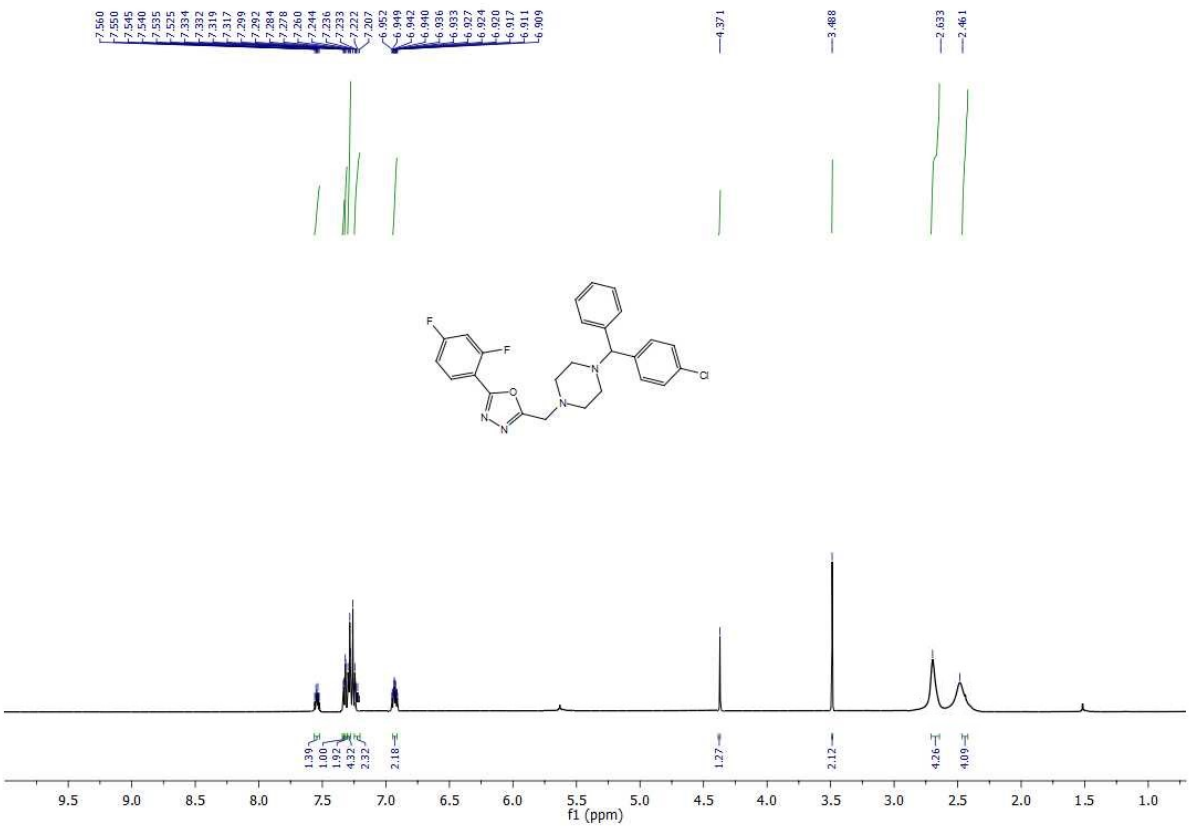

Figure S47. ^{1}H spectra of compound **9i**

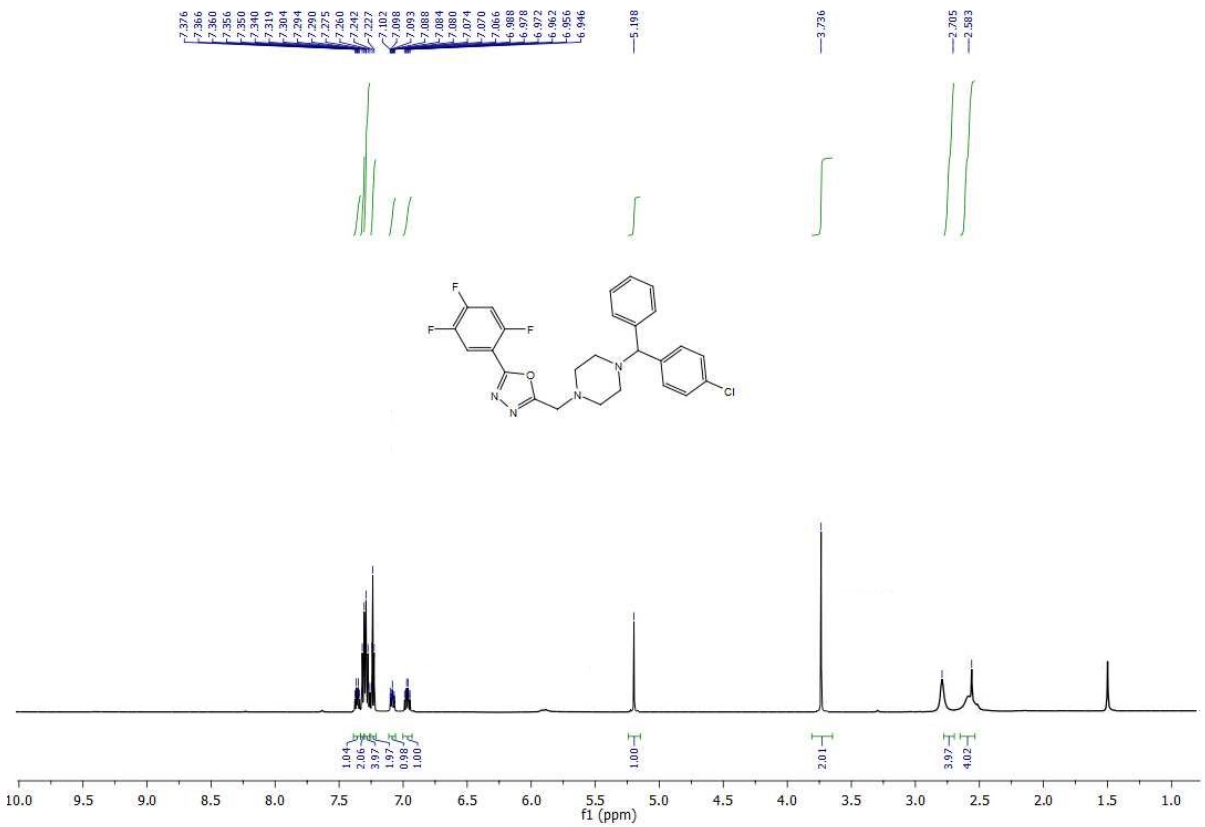

Figure S48. ^{1}H spectra of compound **9j**

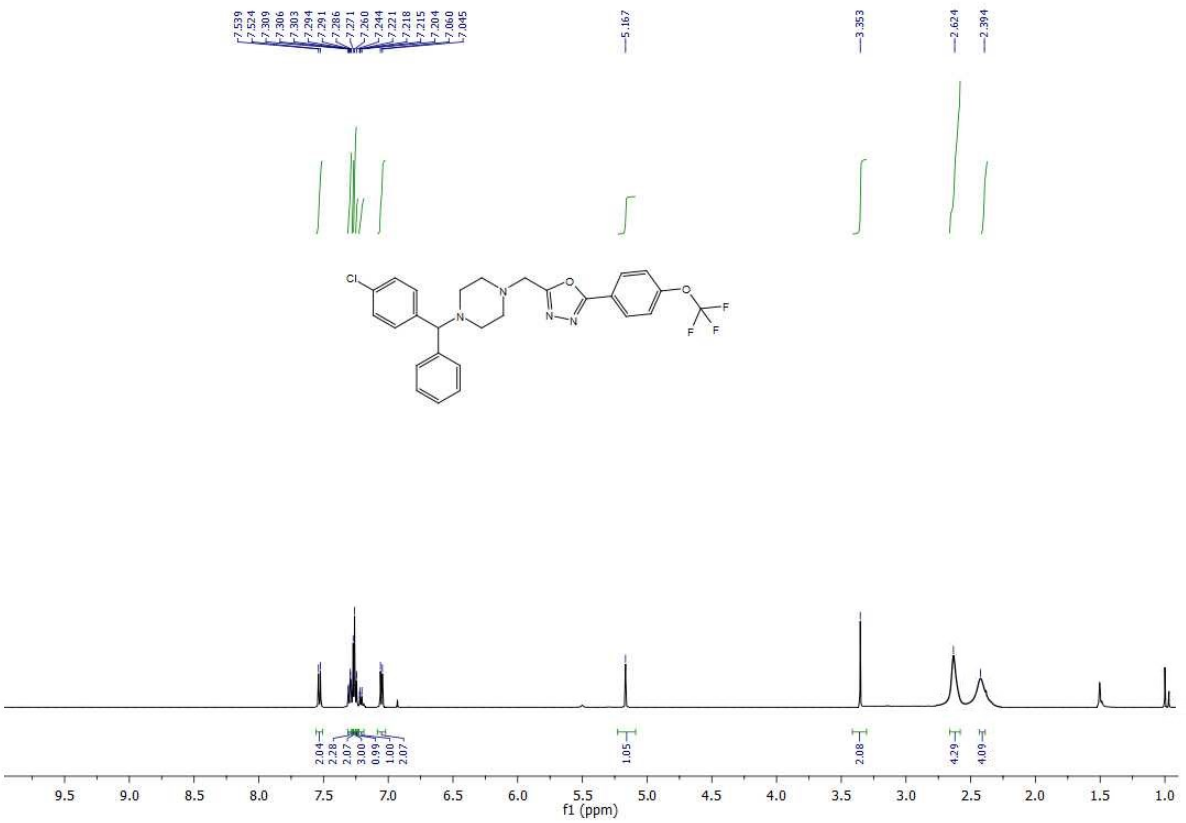

Figure S49. ^{1}H spectra of compound **9k**

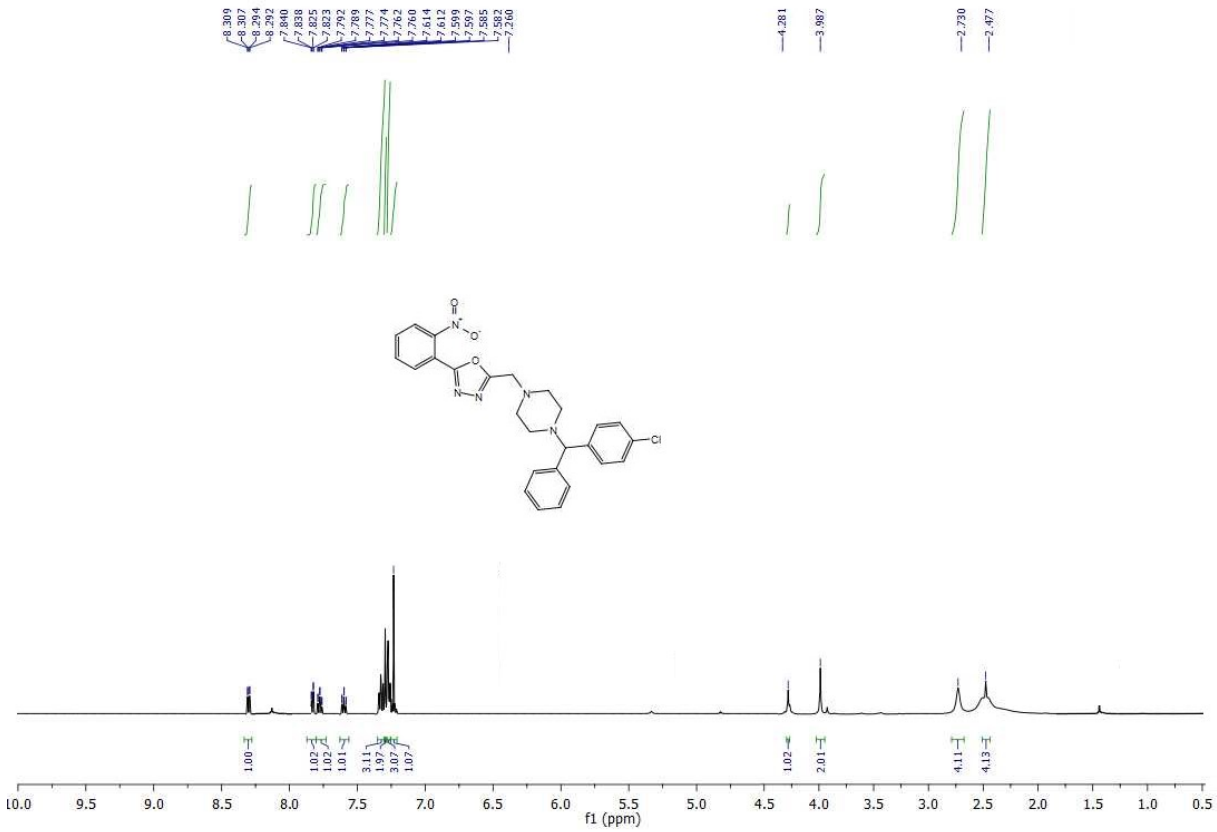

Figure S50. ^{1}H spectra of compound **9m**

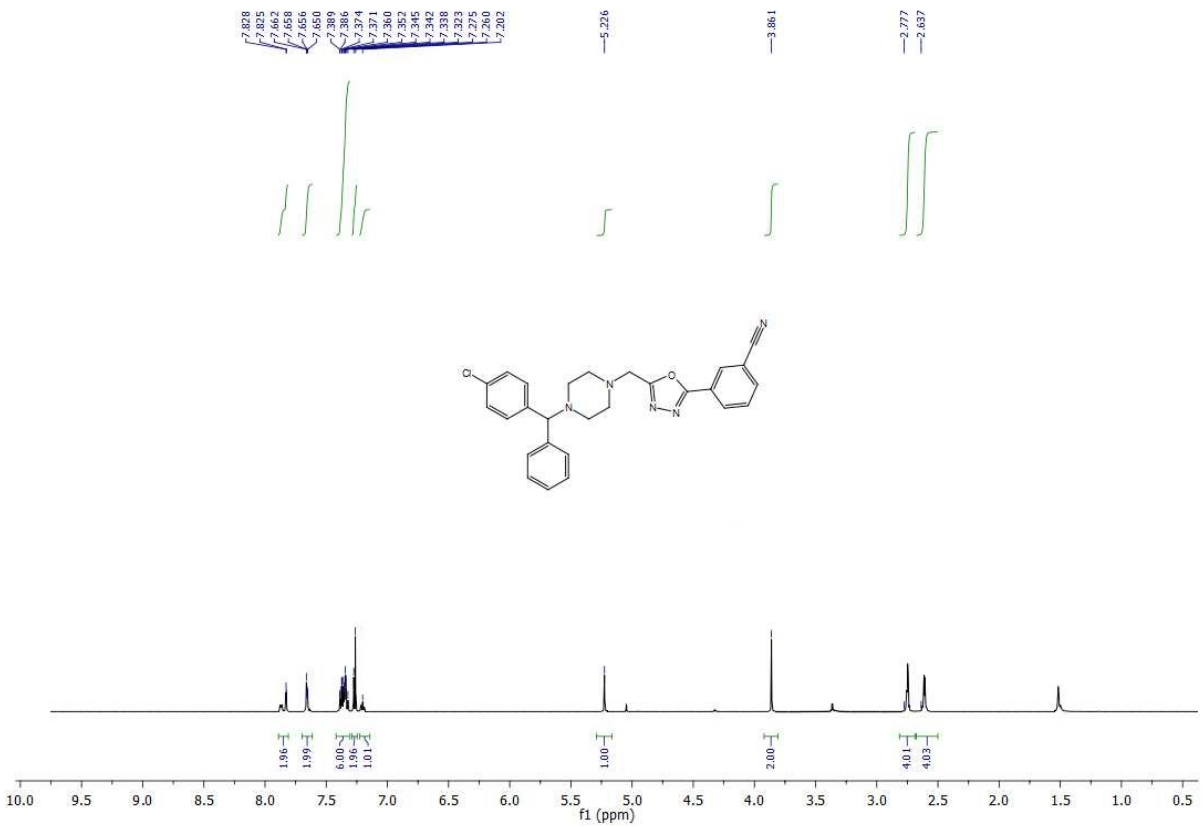

Figure S51. ^{1}H spectra of compound **9n**

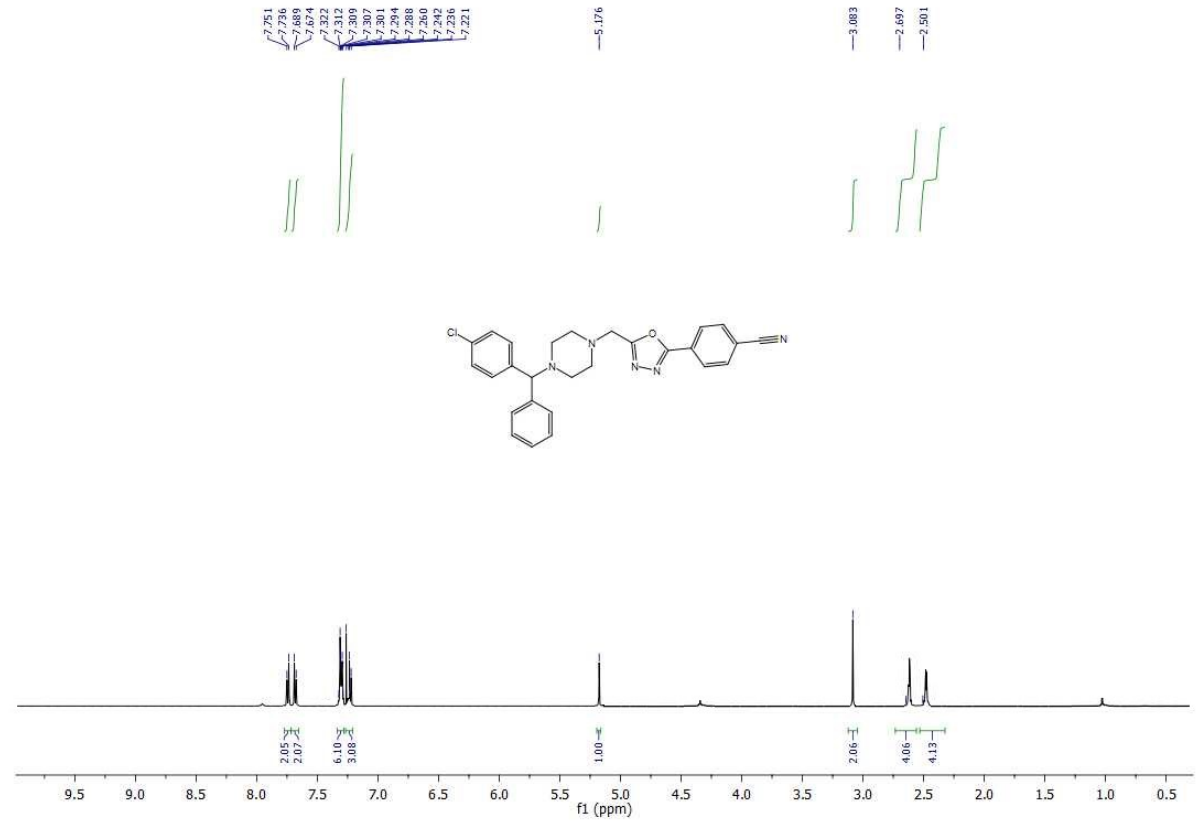

Figure S52. ^{1}H spectra of compound **9o**

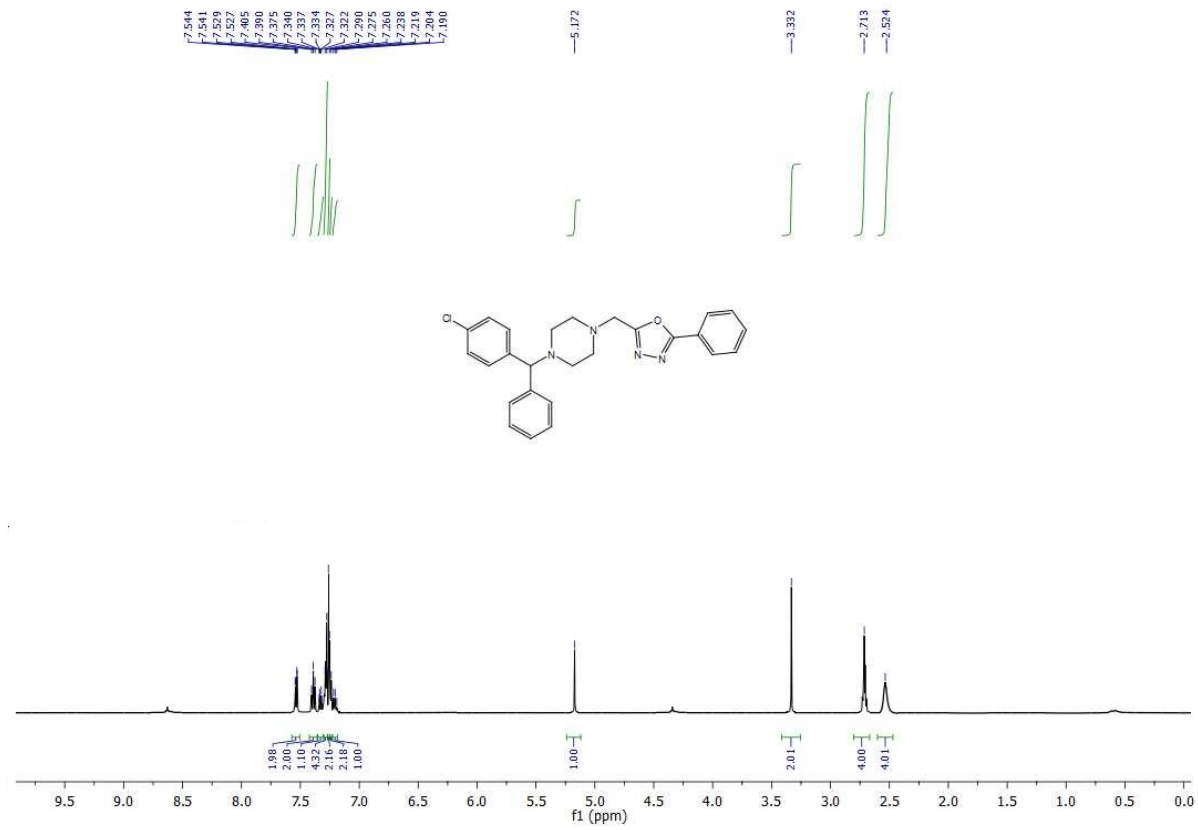

Figure S53. [1]H spectra of compound **9p**

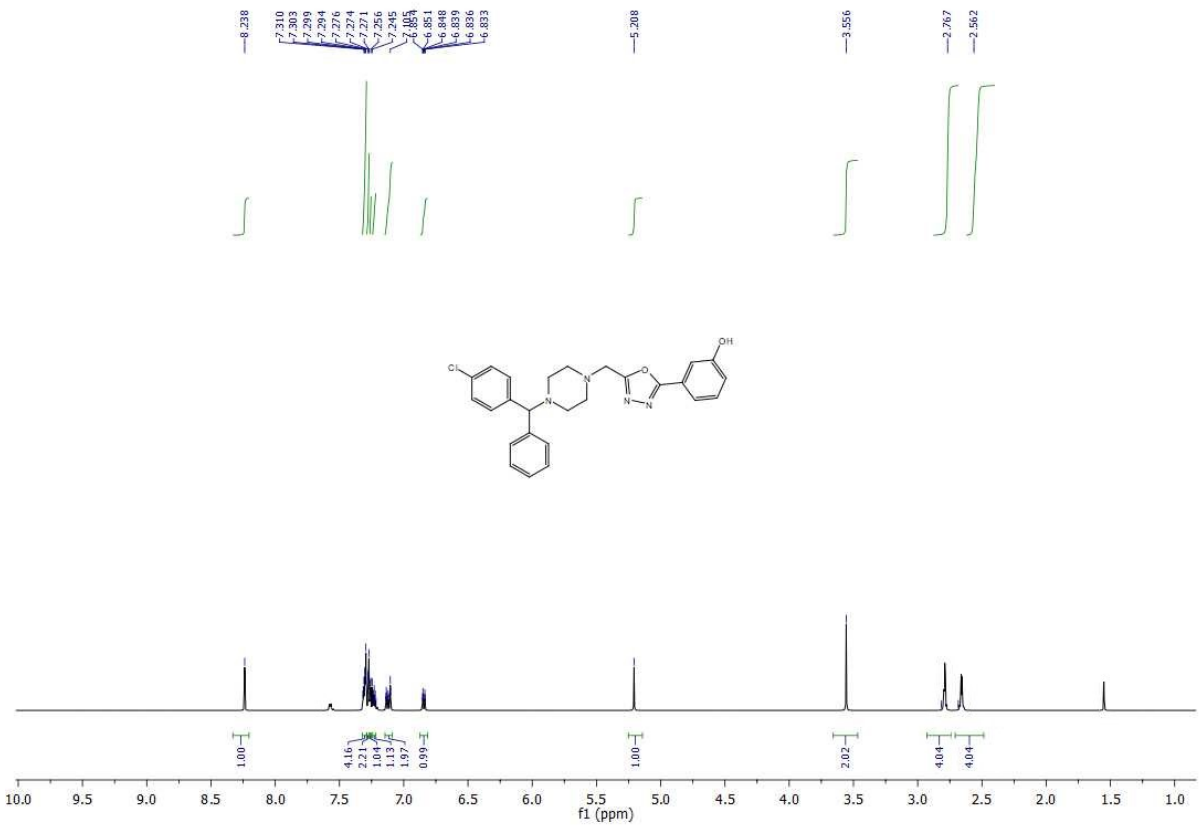

Figure S54. [1]H spectra of compound **9q**

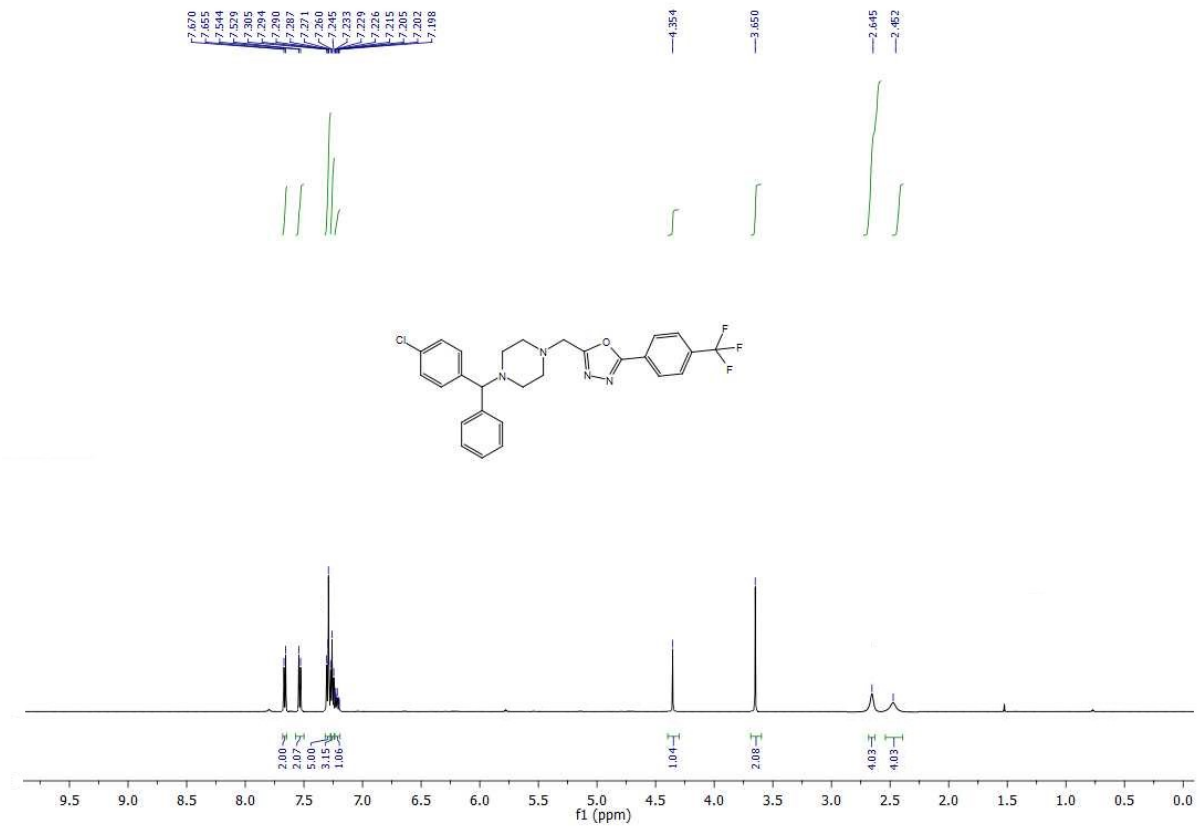

Figure S55. ^{1}H spectra of compound **9s**

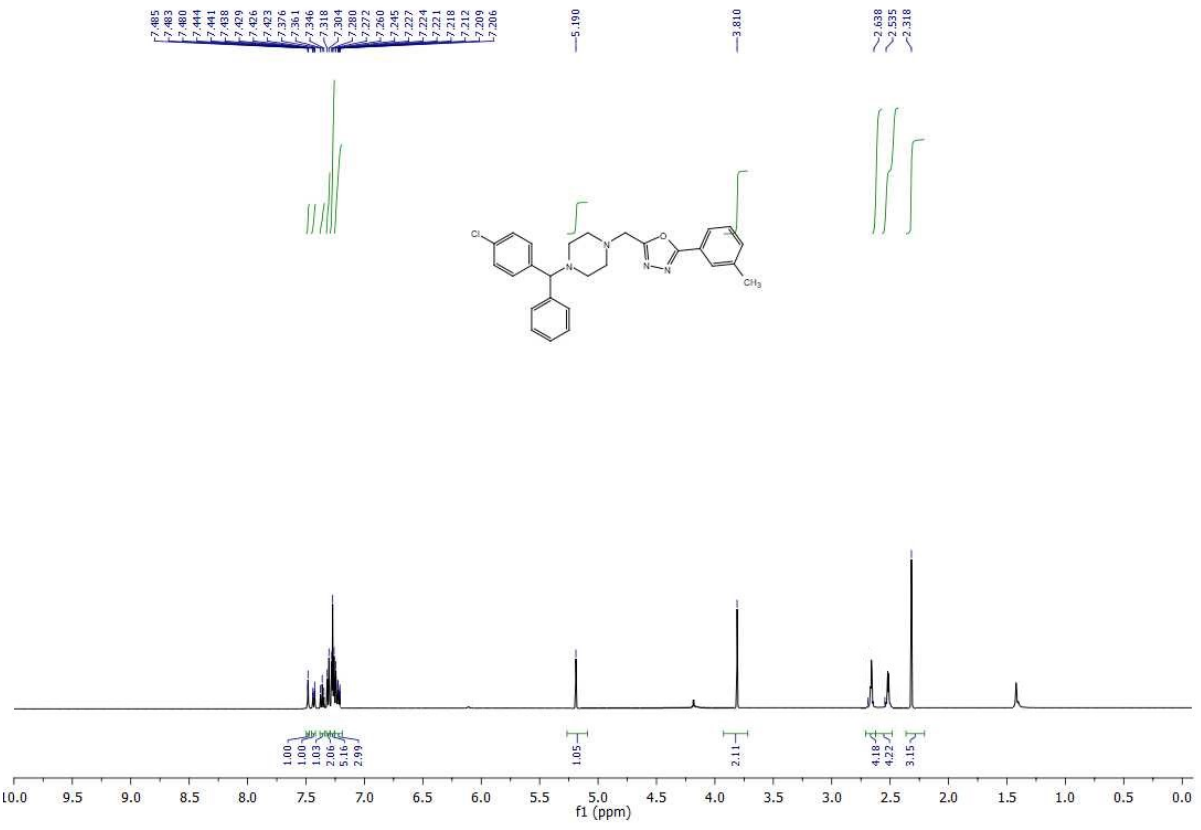

Figure S56. ^{1}H spectra of compound **9t**

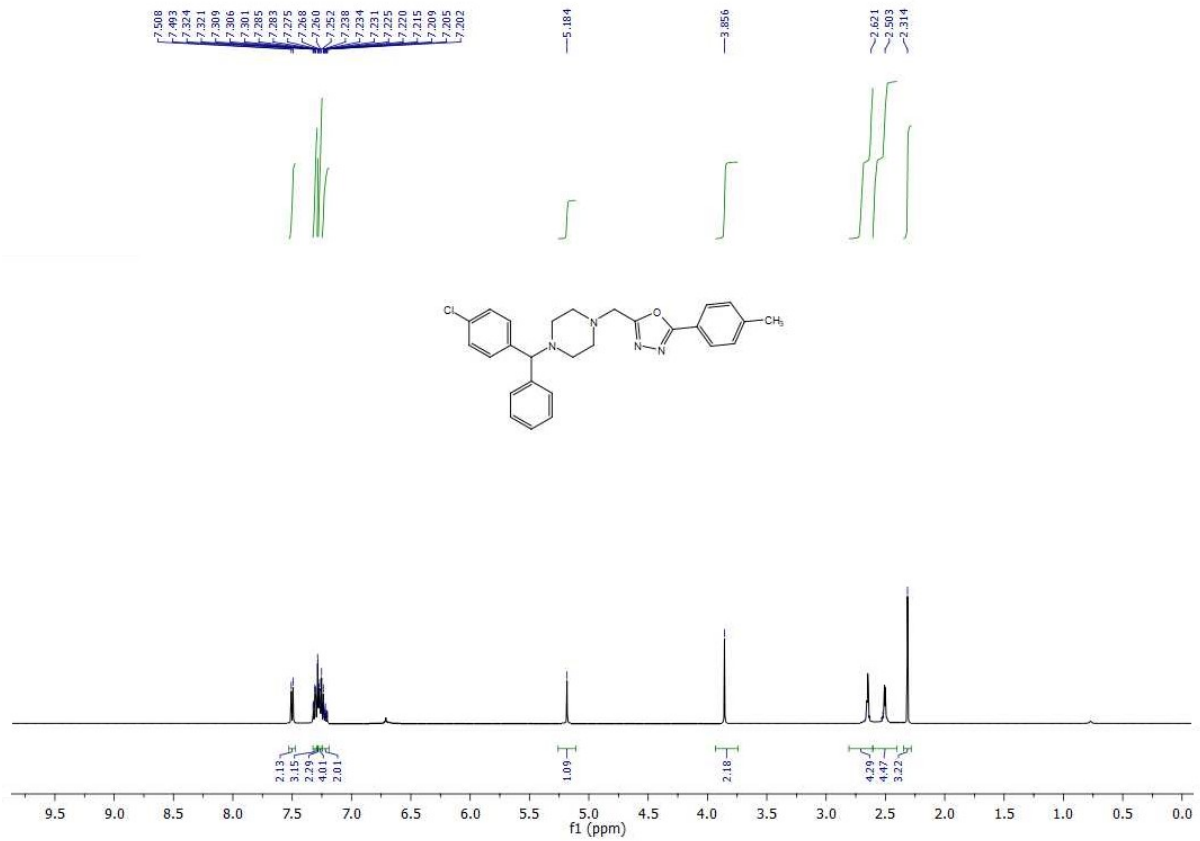

Figure S57. ^{1}H spectra of compound **9u**