Supporting Information

Halogenated Coumarin–Chalcones as Multifunctional Monoamine oxidase-B and Butyrylcholinesterase Inhibitors

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Supplementary Figures and Tables

CC2: 3-[(2*E*)-3-(2-chlorophenyl)prop-2-enoyl]-2*H*-1-benzopyran-2-one

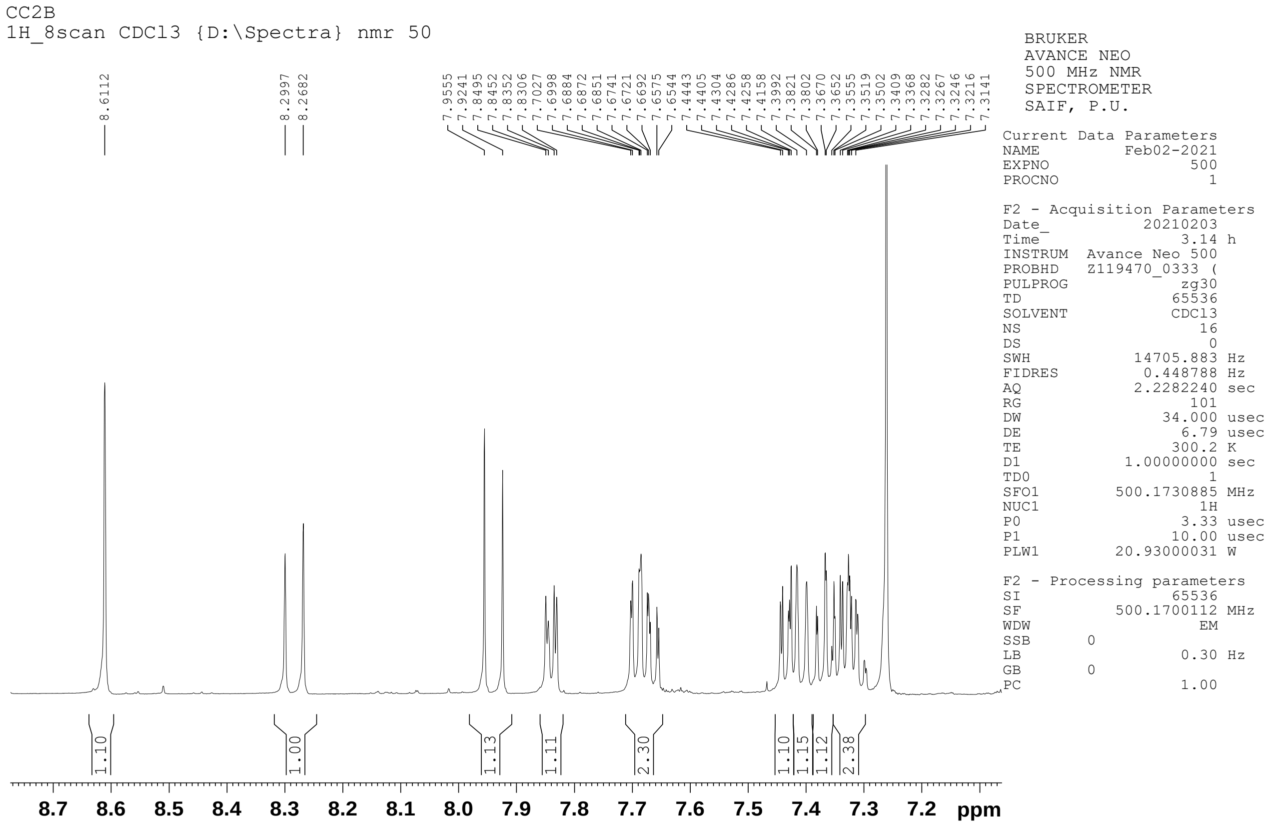


Figure S1. ^{1}**H-NMR of CC2**

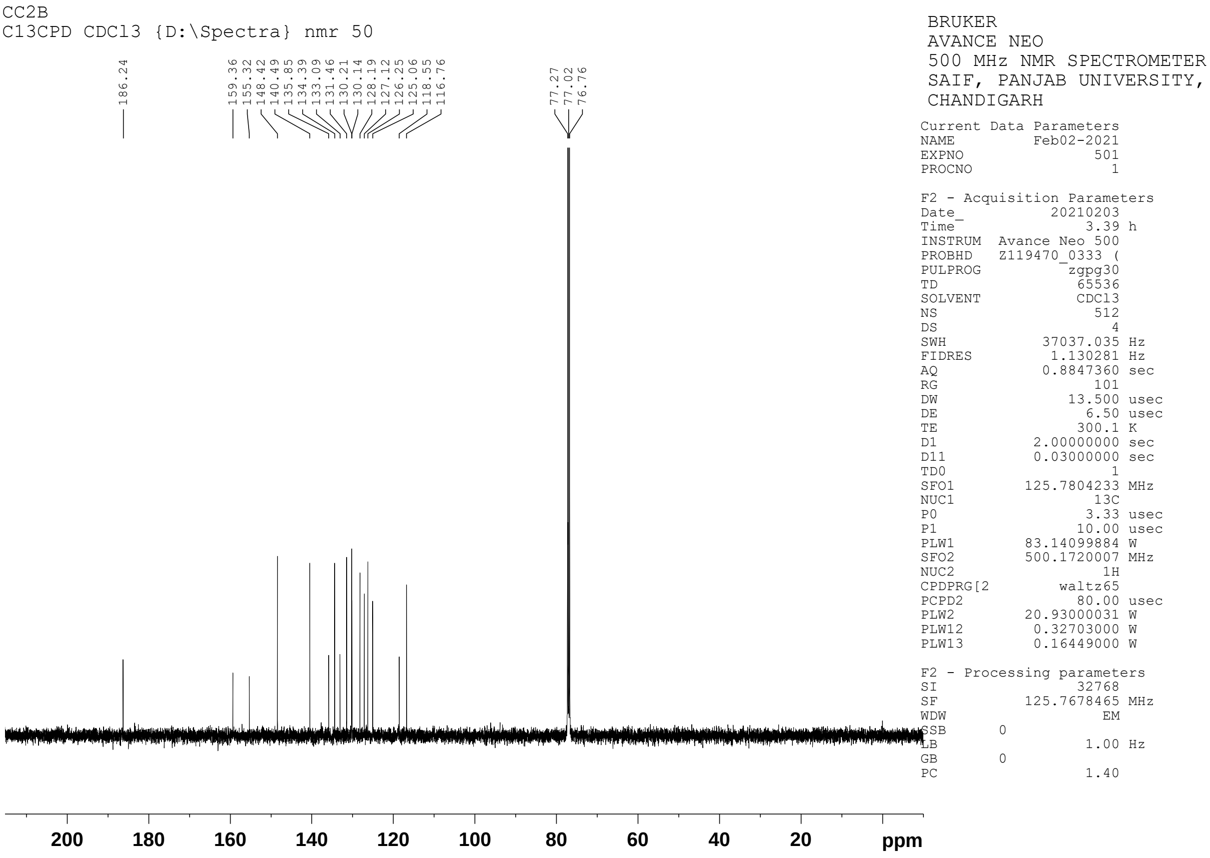


Figure S2. ^{13}C-NMR of CC2

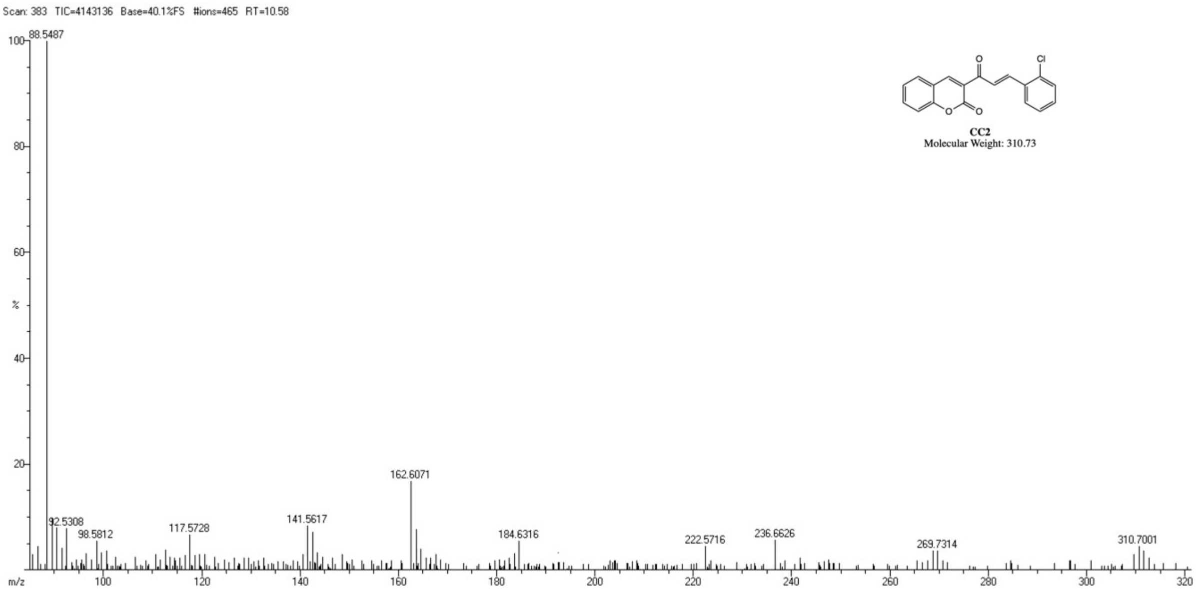
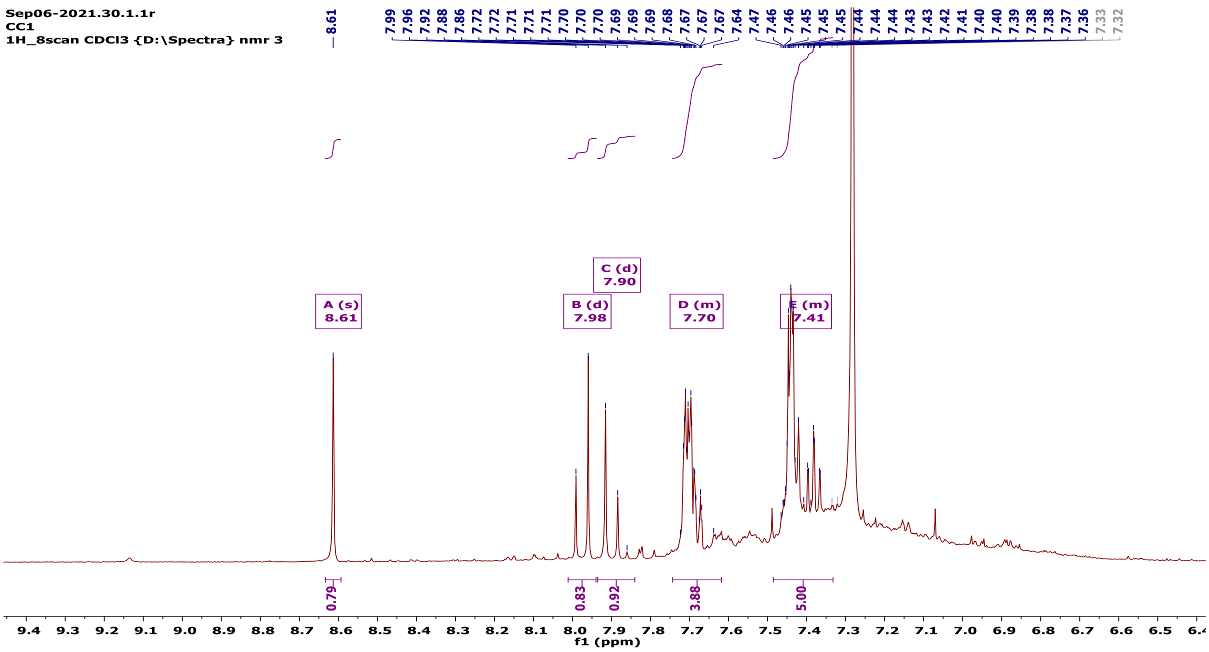


Figure S3. HRMS of CC2



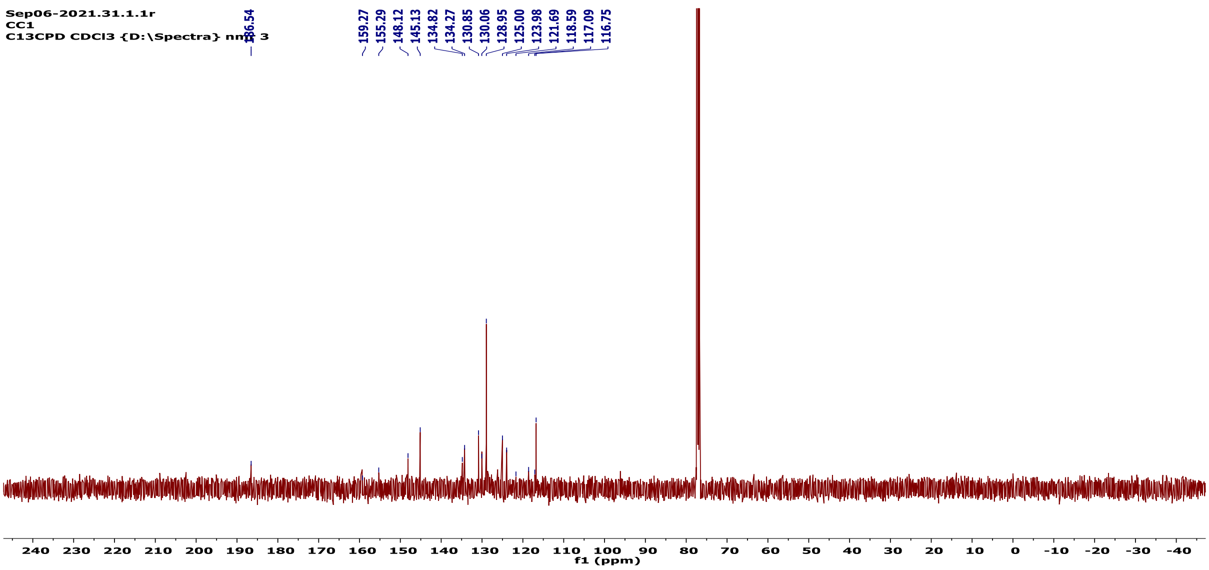


Figure S4. [1]H- and [13]C-NMR of CC1

^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.61 (s, 1H), 7.98 (d, J = 15.7 Hz, 1H), 7.90 (d, J = 15.7 Hz, 1H), 7.74 – 7.62 (m, 4H), 7.49 – 7.33 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 186.54, 159.27, 155.29, 148.12, 145.13, 134.82, 134.27, 130.85, 130.06, 128.95, 125.00, 123.98, 121.69, 118.59, 117.09, 116.75.

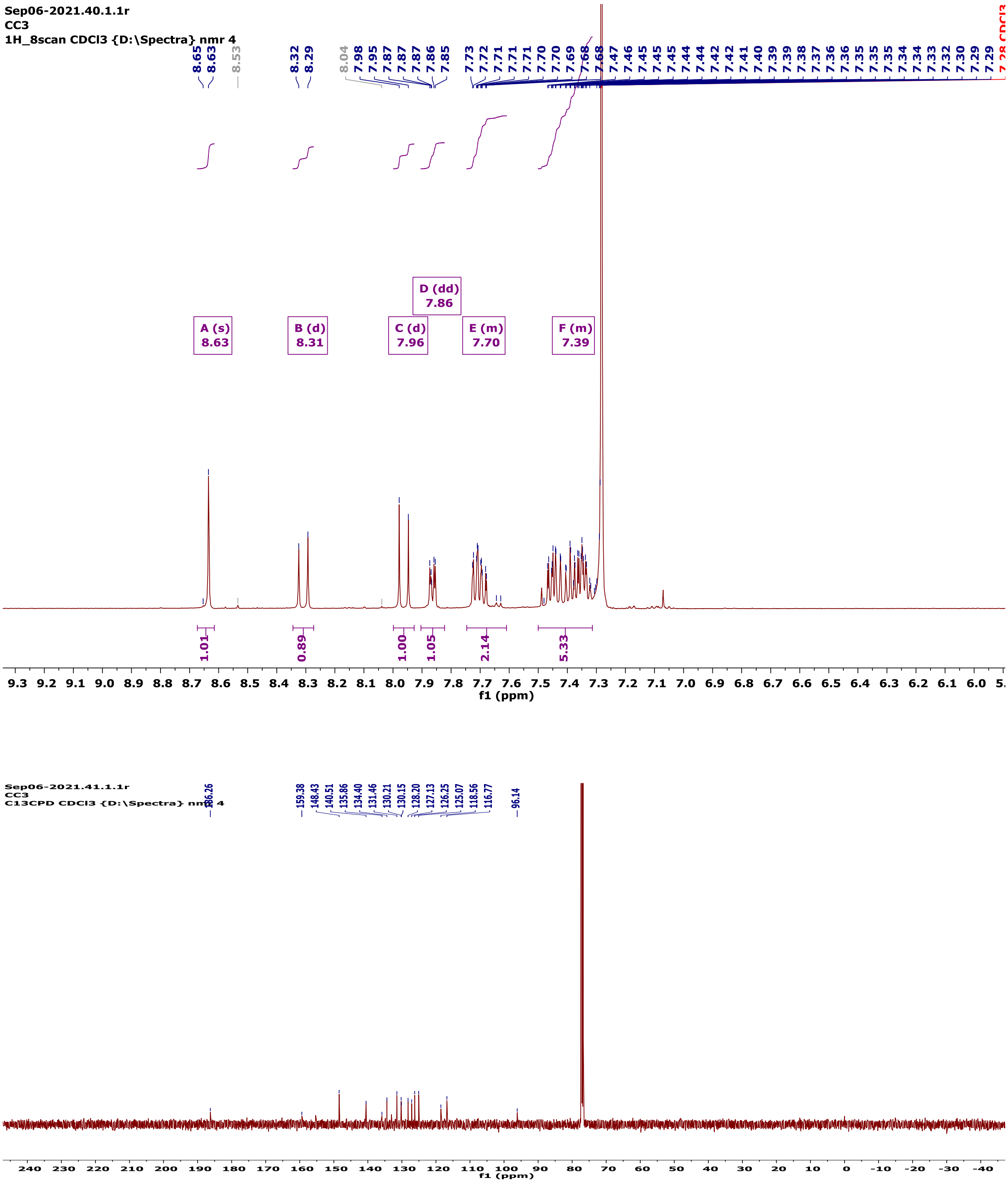
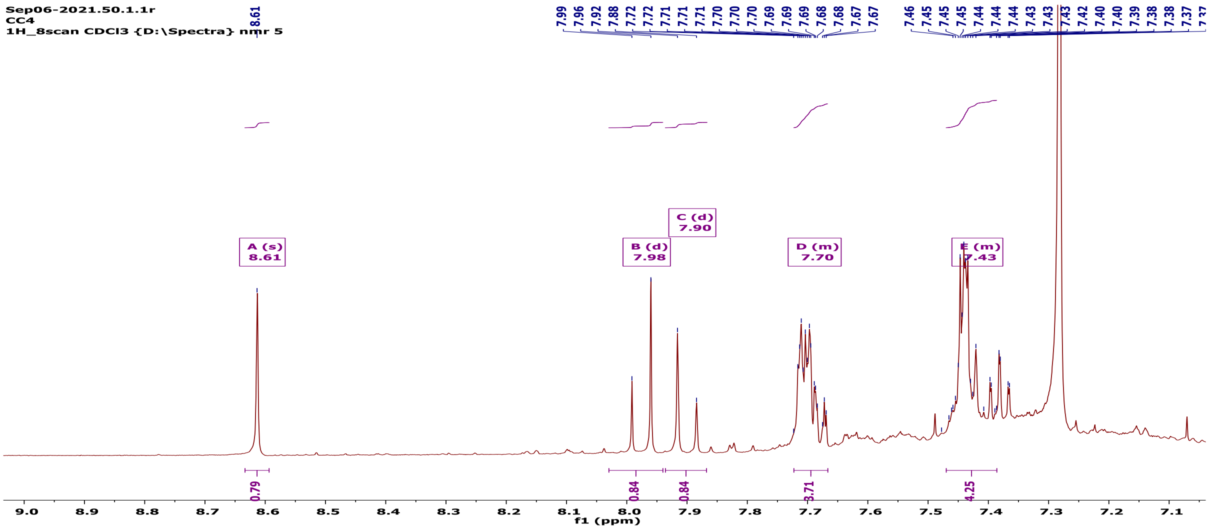


Figure S5. ^{1}H- and ^{13}C-NMR of CC3

^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.63 (s, 1H), 8.31 (d, J = 15.7 Hz, 1H), 7.96 (d, J = 15.8 Hz, 1H), 7.86 (dd, J = 7.2, 2.2 Hz, 1H), 7.75 – 7.61 (m, 2H), 7.50 – 7.31 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 186.26, 159.38, 148.43, 140.51, 135.86, 134.40, 131.46, 130.21, 130.15, 128.20, 127.13, 126.25, 125.07, 118.56, 116.77, 96.14.



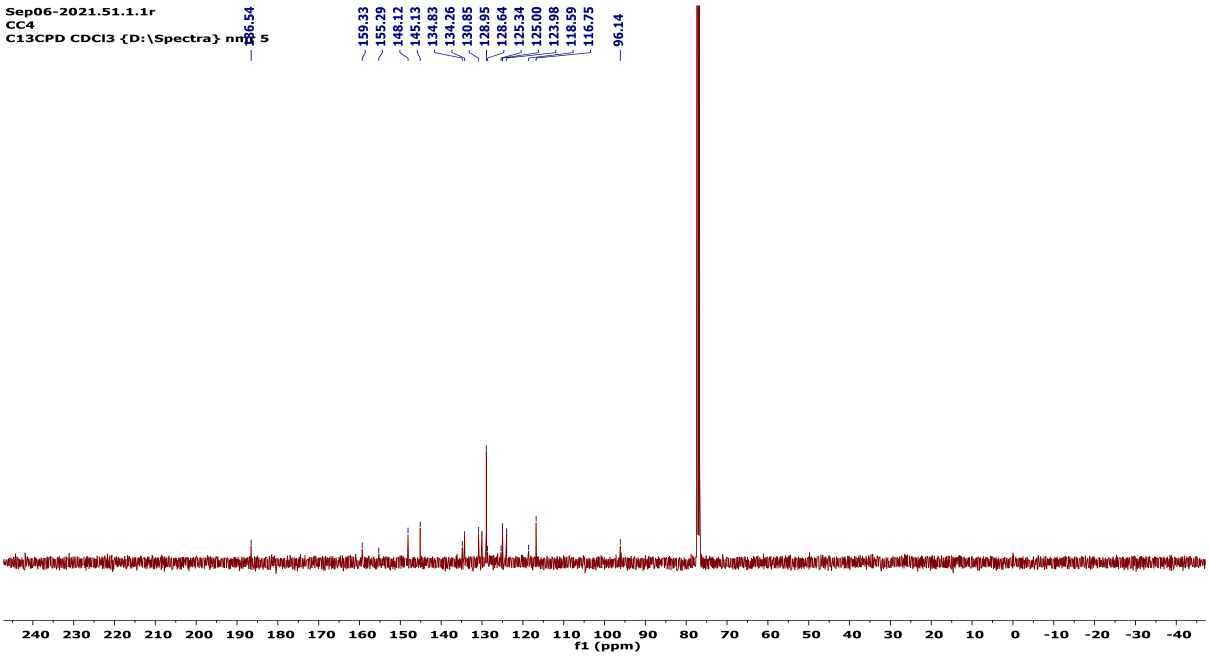
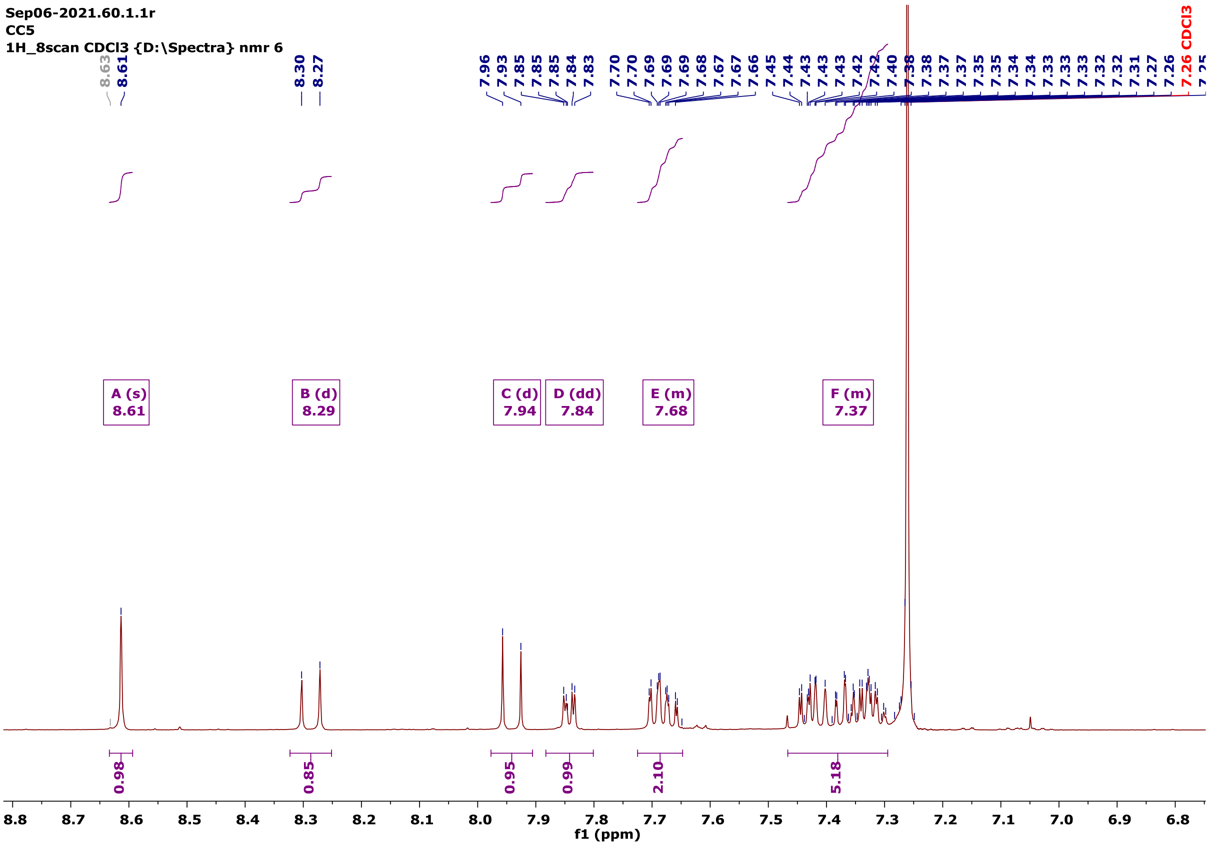


Figure S6. ^{1}H- and ^{13}C-NMR of CC4

^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.61 (s, 1H), 7.98 (d, J = 15.7 Hz, 1H), 7.90 (d, J = 15.8 Hz, 1H), 7.72 – 7.67 (m, 4H), 7.47 – 7.39 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 186.54, 159.33, 155.29, 148.12, 145.13, 134.83, 134.26, 130.85, 128.95, 128.64, 125.34, 125.00, 123.98, 118.59, 116.75, 96.14.



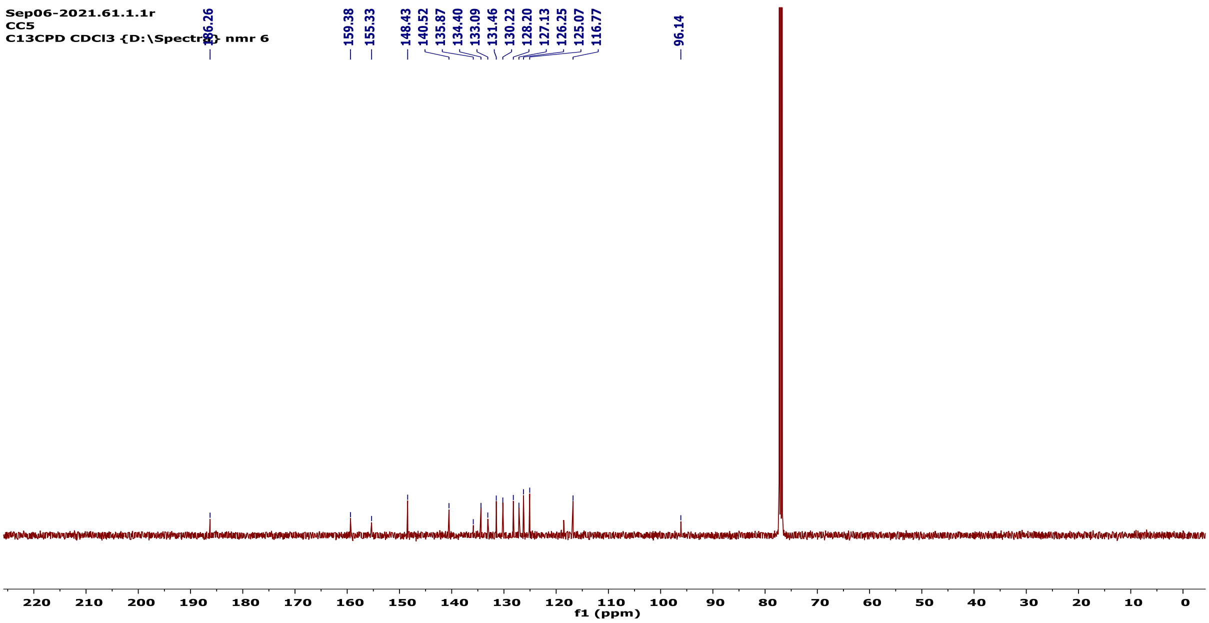
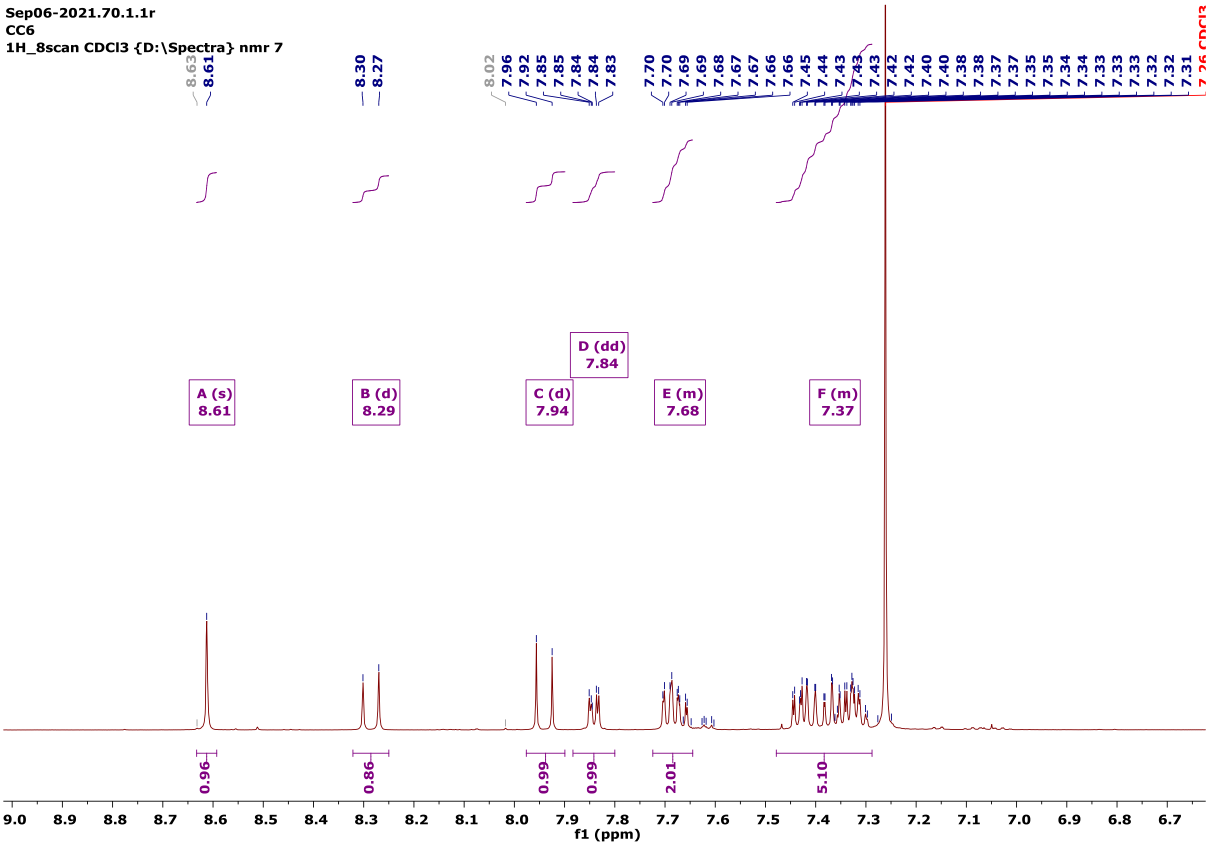


Figure S7. ^{1}H- and ^{13}C-NMR of CC5

^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.61 (s, 1H), 8.29 (d, *J* = 15.8 Hz, 1H), 7.94 (d, *J* = 15.7 Hz, 1H), 7.84 (dd, *J* = 7.2, 2.3 Hz, 1H), 7.72 – 7.65 (m, 2H), 7.47 – 7.29 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 186.26, 159.38, 155.33, 148.43, 140.52, 135.87, 134.40, 133.09, 131.46, 130.22, 128.20, 127.13, 126.25, 125.07, 116.77, 96.14.



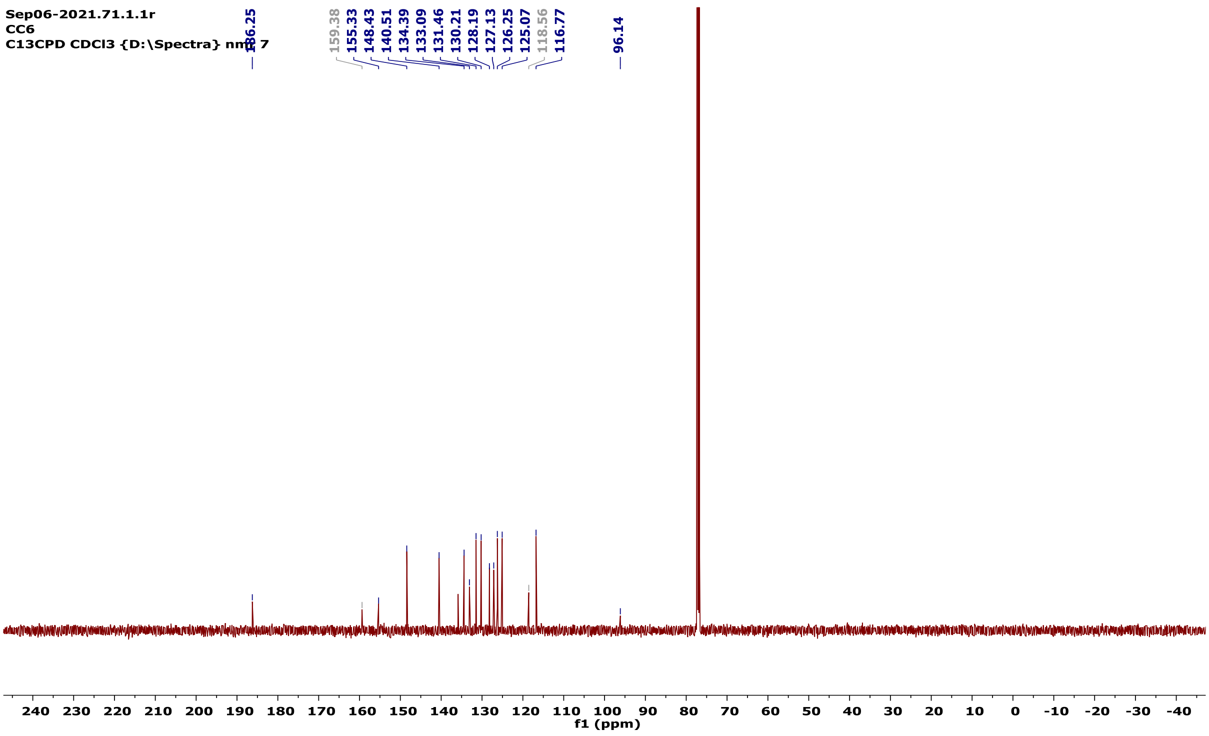
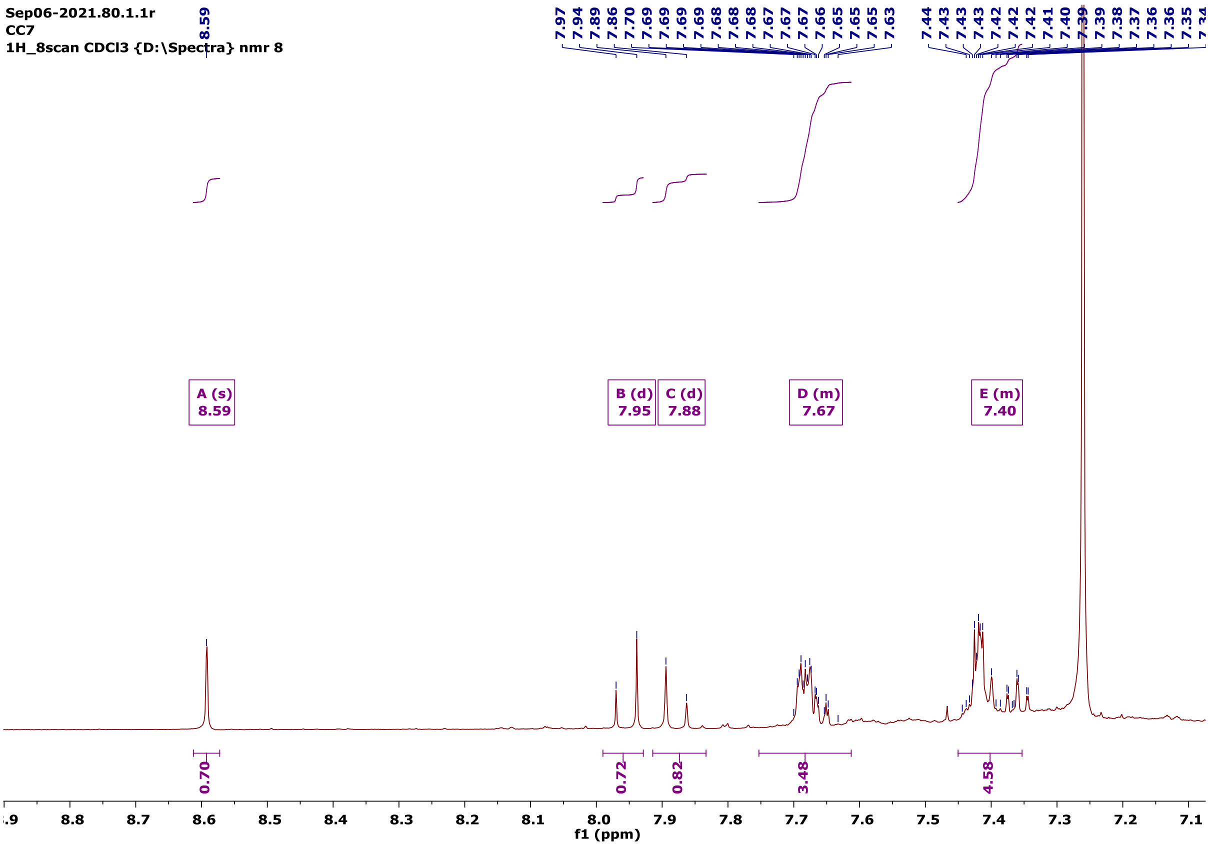


Figure S8. ^{1}H- and ^{13}C-NMR of CC6

^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.61 (s, 1H), 8.29 (d, *J* = 15.8 Hz, 1H), 7.94 (d, *J* = 15.7 Hz, 1H), 7.84 (dd , *J* = 7.3, 2.2 Hz, 1H), 7.72 – 7.64 (m, 2H), 7.48 – 7.29 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 186.25, 159.38, 155.33, 148.43, 140.51, 134.39, 133.09, 131.46, 130.21, 128.19, 127.13, 126.25, 125.07, 118.56, 116.77, 96.14.



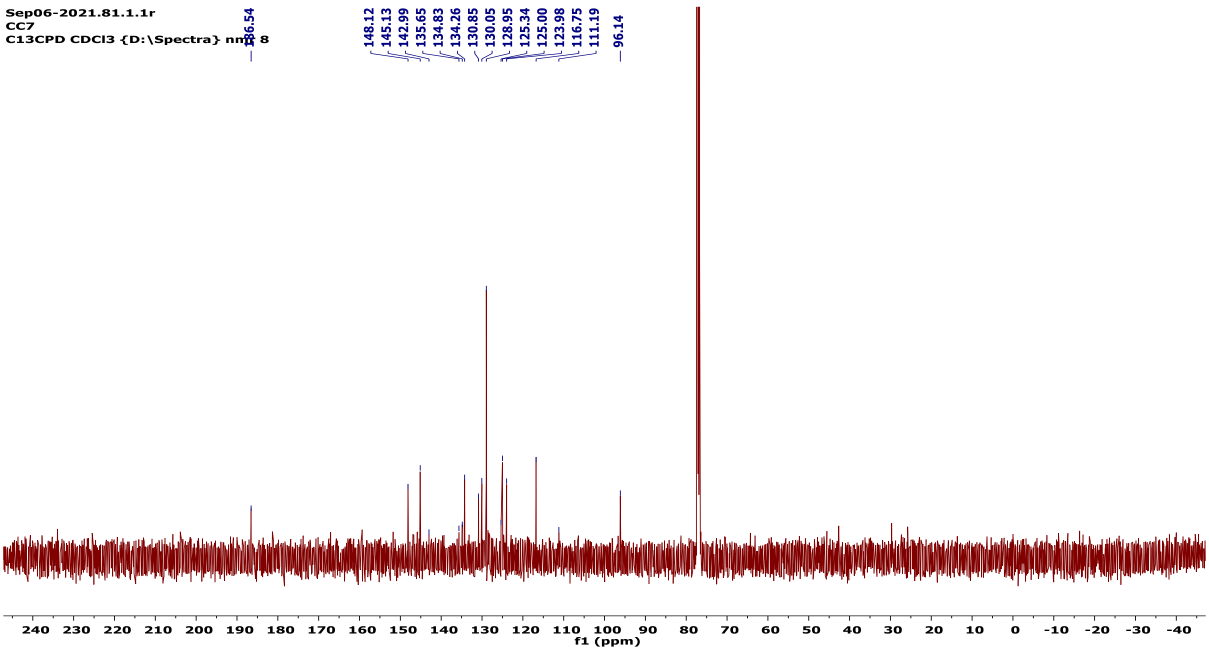
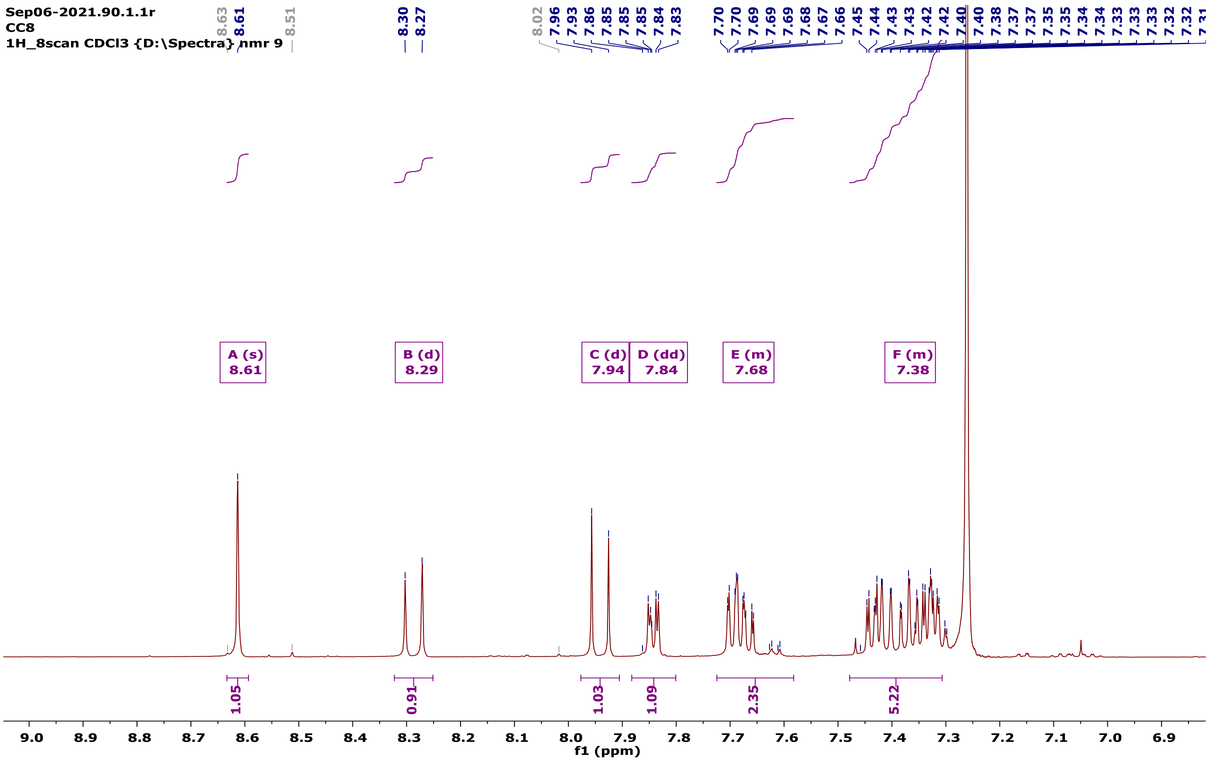


Figure S9. ^{1}H- and ^{13}C-NMR of CC7

^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.59 (s, 1H), 7.95 (d, *J* = 15.7 Hz, 1H), 7.88 (d, *J* = 15.7 Hz, 1H), 7.75 – 7 .61 (m, 3H), 7.45 – 7.35 (m, 5H);^{13}C NMR (125 MHz, $CDCl_3$) δ 186.54, 148.12, 145.13, 142.99, 135.65, 134.83, 134.26, 130.85, 130.05, 128.95, 125.34, 125.00, 123.98, 116.75, 111.19, 96.14.



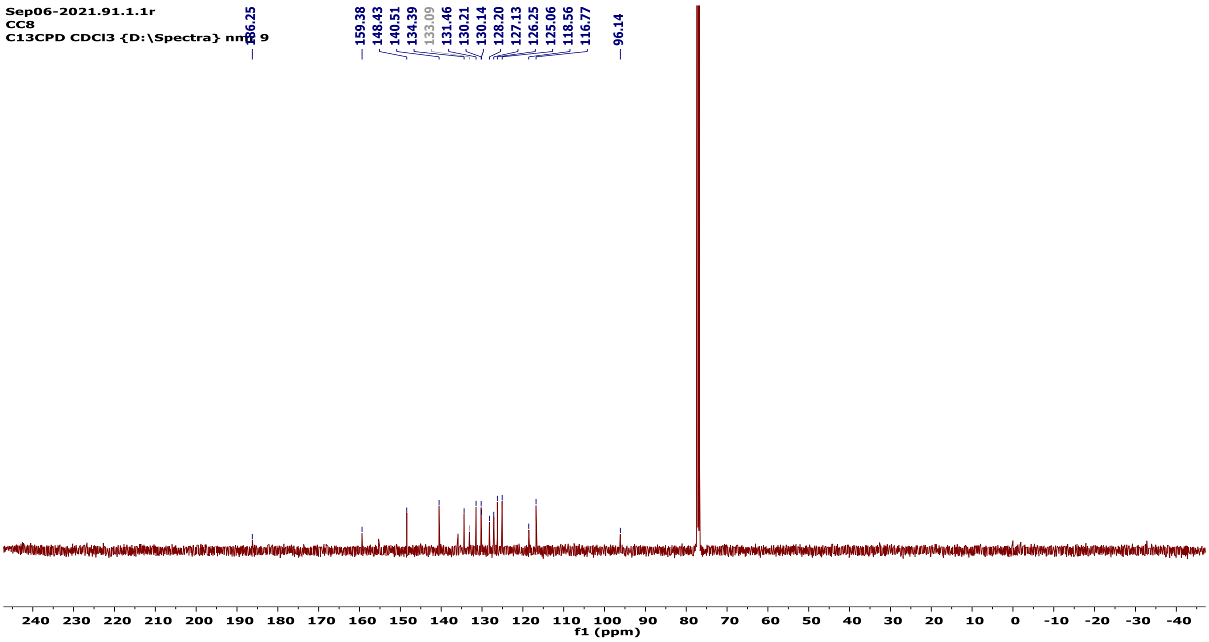


Figure S10. ^{1}H- and ^{13}C-NMR of CC8

^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.61 (s, 1H), 8.29 (d, *J* = 15.8 Hz, 1H), 7.94 (d, *J* = 15.7 Hz, 1H), 7.84 (dd, *J* = 7.2, 2.2 Hz, 1H), 7.72 – 7.58 (m, 2H), 7.48 – 7.31 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 186.25, 159.38, 148.43, 140.51, 134.39, 133.09, 131.46, 130.21, 130.14, 128.20, 127.13, 126.25, 125.06, 118.56, 116.77, 96.14.

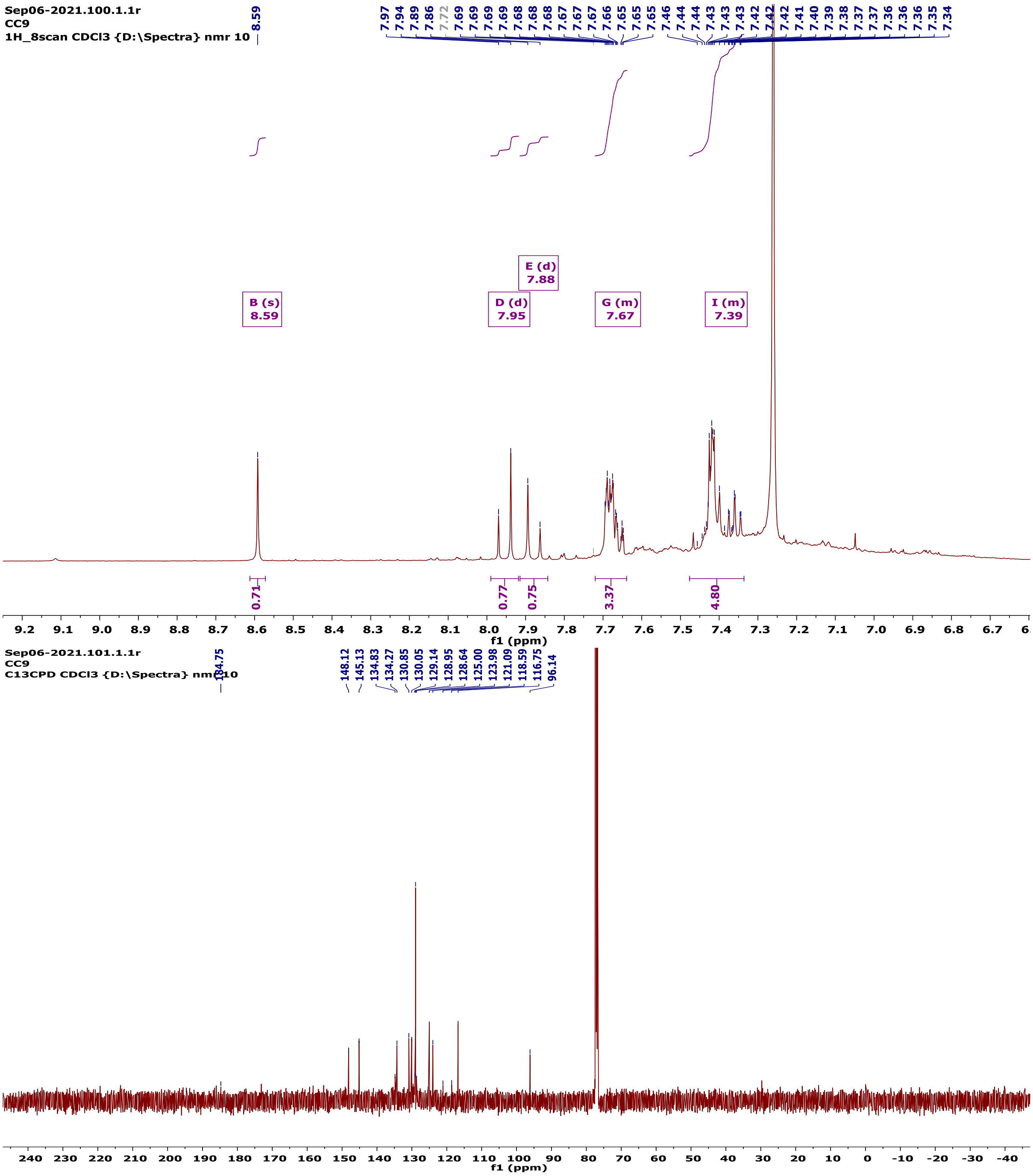
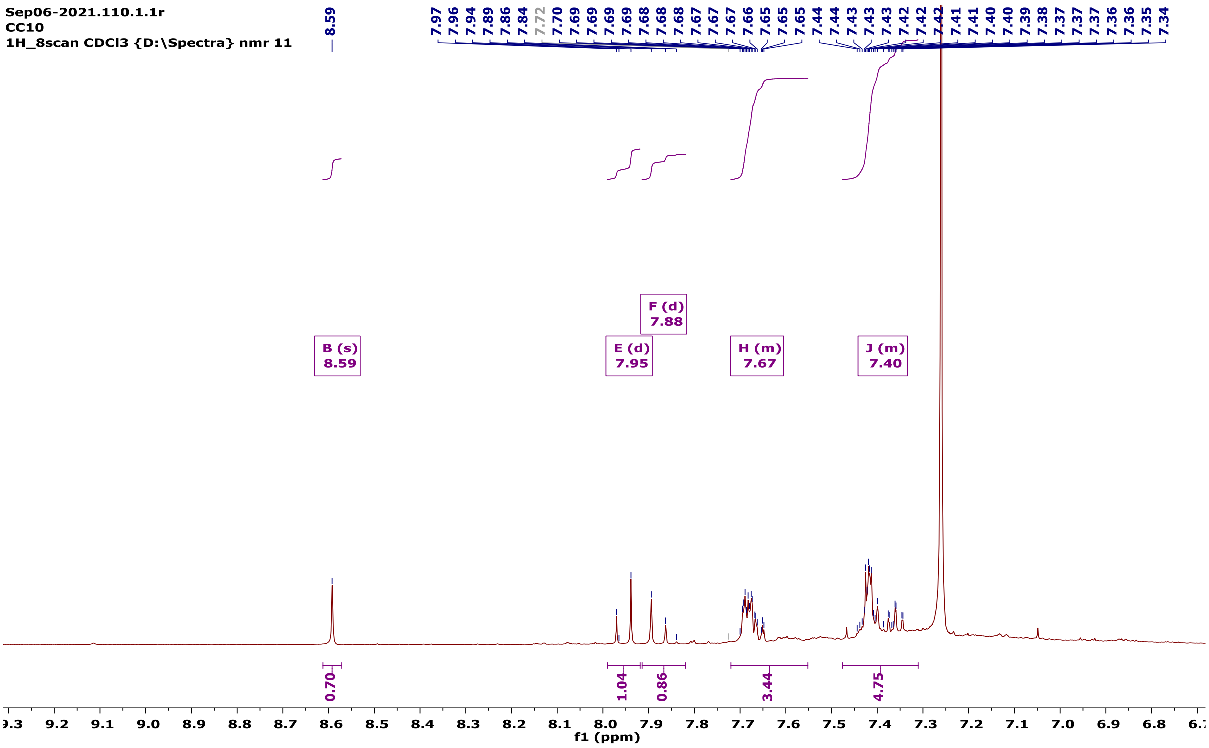


Figure S11. ^{1}H- and ^{13}C-NMR of CC9

^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.59 (s, 1H), 7.95 (d, J = 15.7 Hz, 1H), 7.88 (d, J = 15.8 Hz, 1H), 7.72 – 7.64 (m, 3H), 7.48 – 7.34 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 184.75, 148.12, 145.13, 134.83, 134.27, 130.85, 130.05, 129.14, 128.95, 128.64, 125.00, 123.98, 121.09, 118.59, 116.75, 96.14.



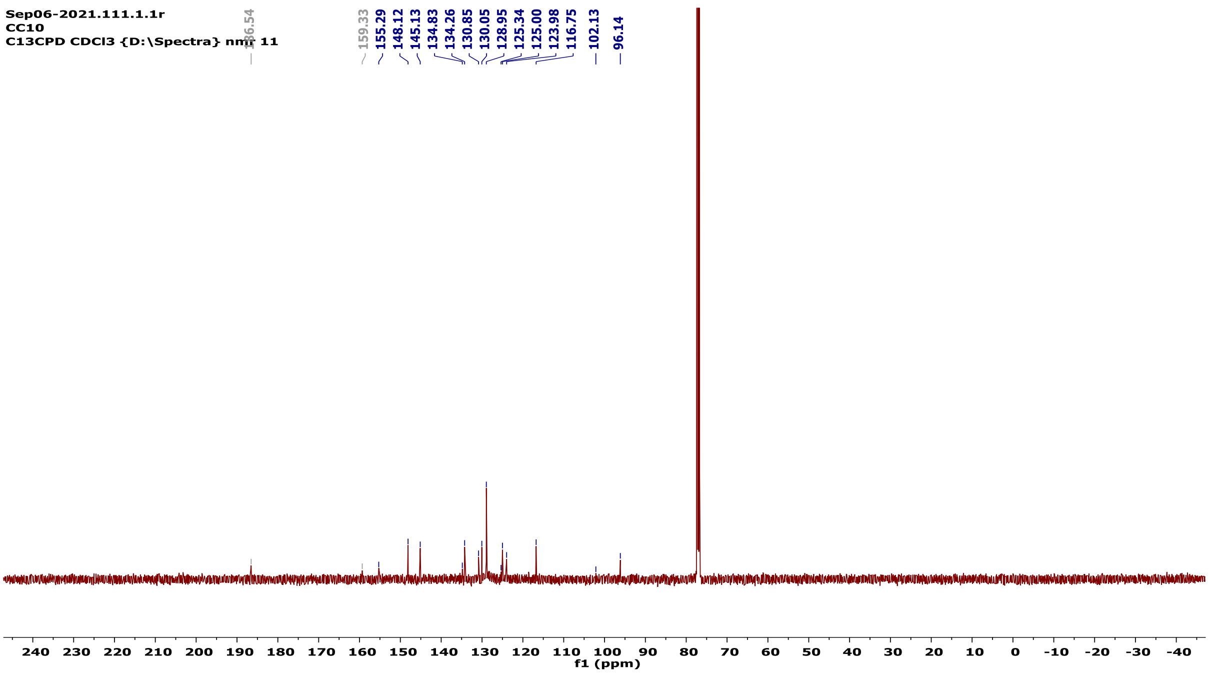


Figure S12. ^{1}**H- and** ^{13}**C-NMR of CC10**

^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.59 (s, 1H), 7.95 (d, *J* = 15.7 Hz, 1H), 7.88 (d, *J* = 15.7 Hz, 1H), 7.72 – 7.55 (m, 3H), 7.48 – 7.31 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 186.54, 159.33, 155.29, 148.12, 145.13, 134.83, 134.26, 130.85, 130.05, 128.95, 125.34, 125.00, 123.98, 116.75, 102.13, 96.14

CC1

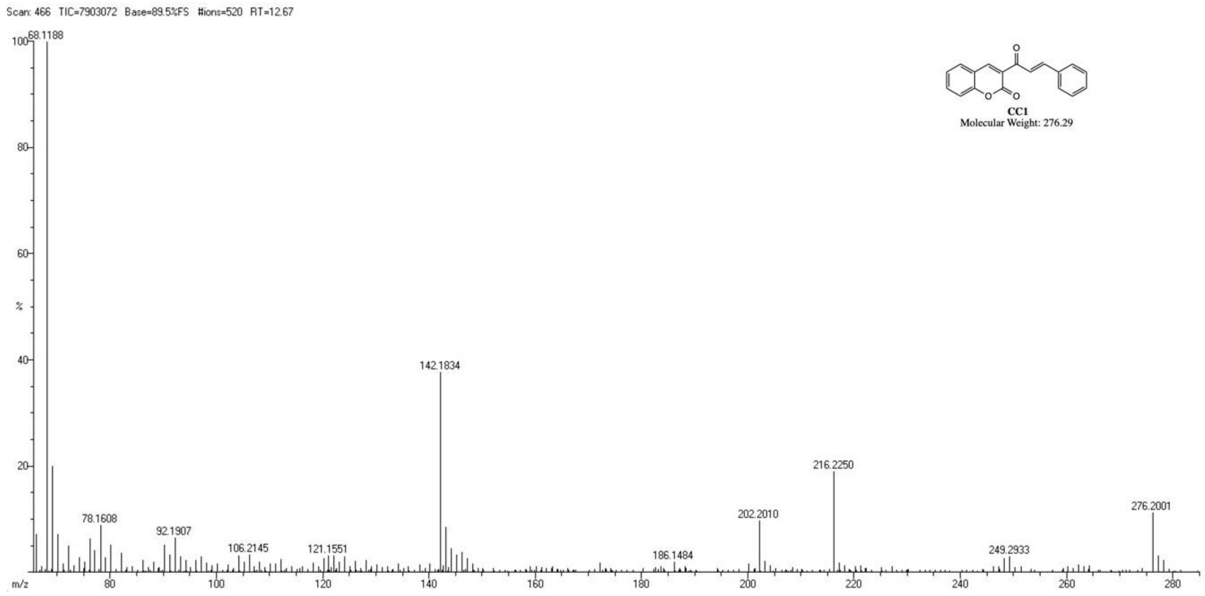


Figure S13. MS of CC1

CC3

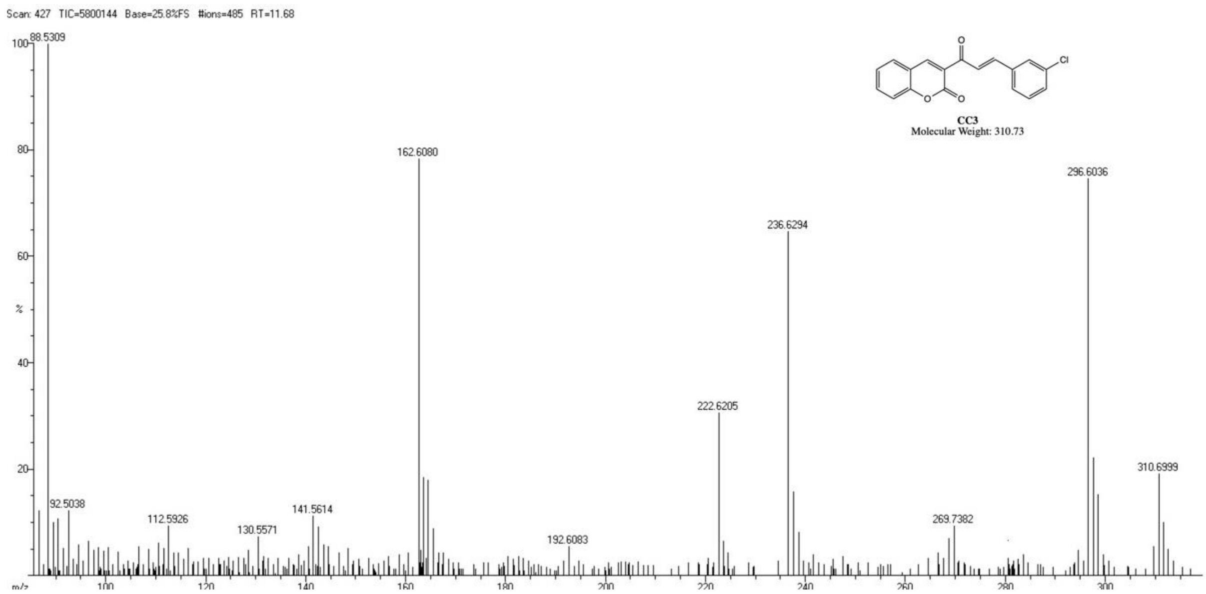


Figure S14. MS of CC3

CC4

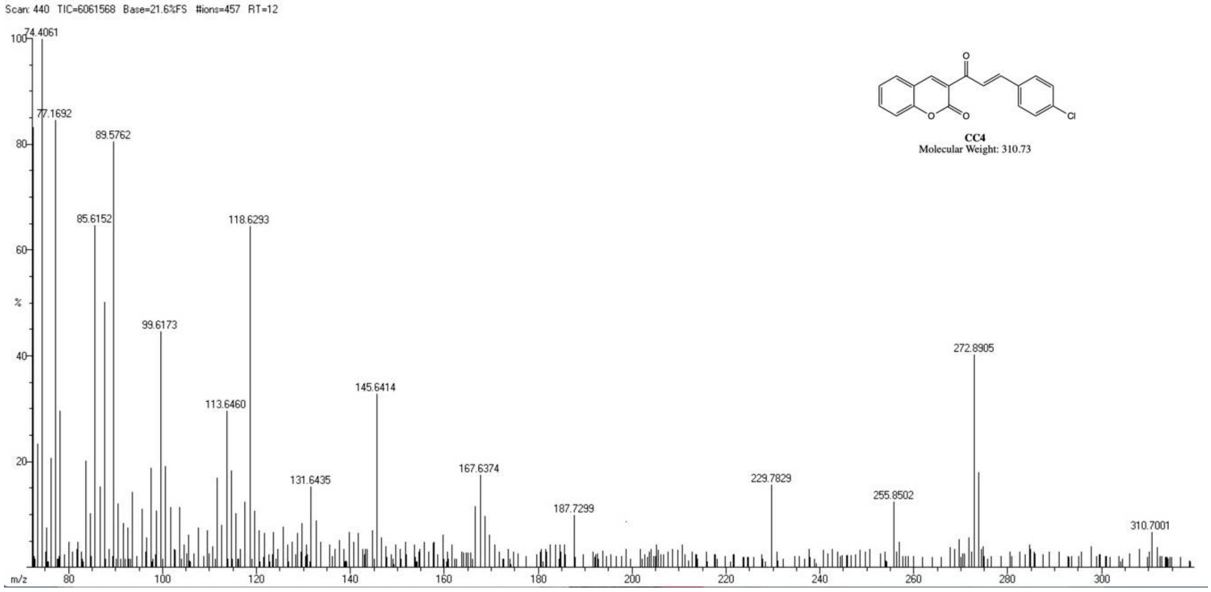


Figure S15. MS of CC4

CC5

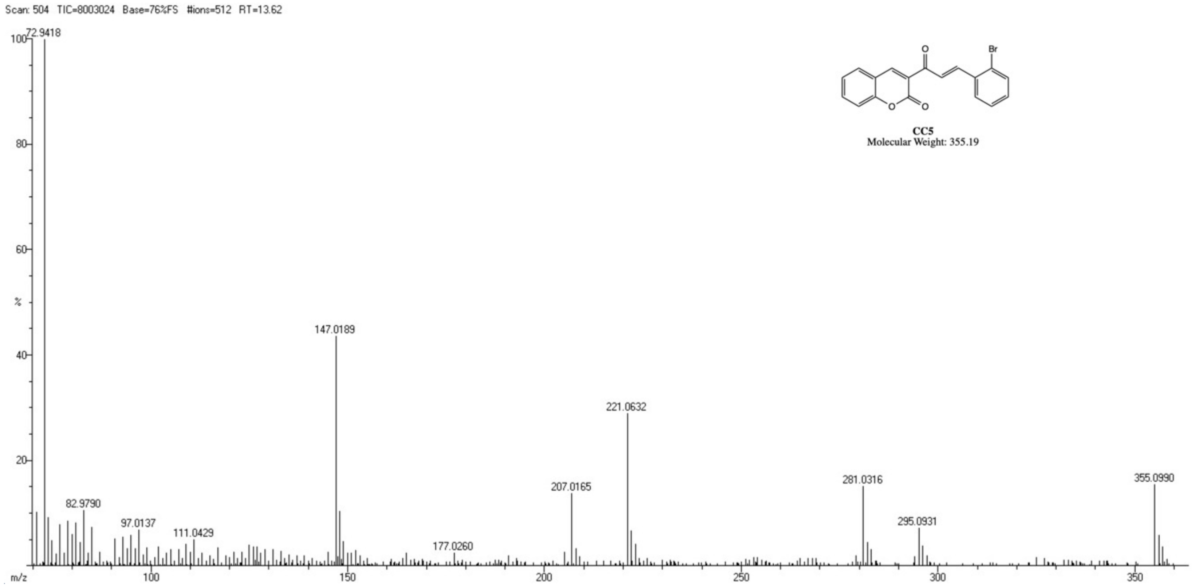


Figure S16. MS of CC5

CC6

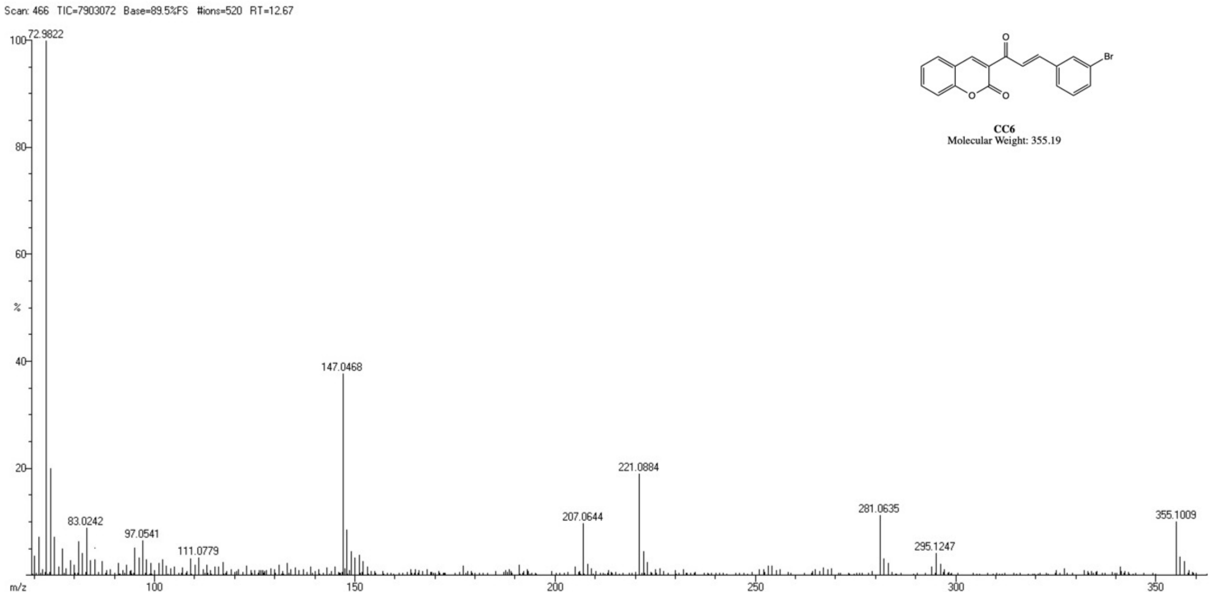


Figure S17. MS of CC6

CC7

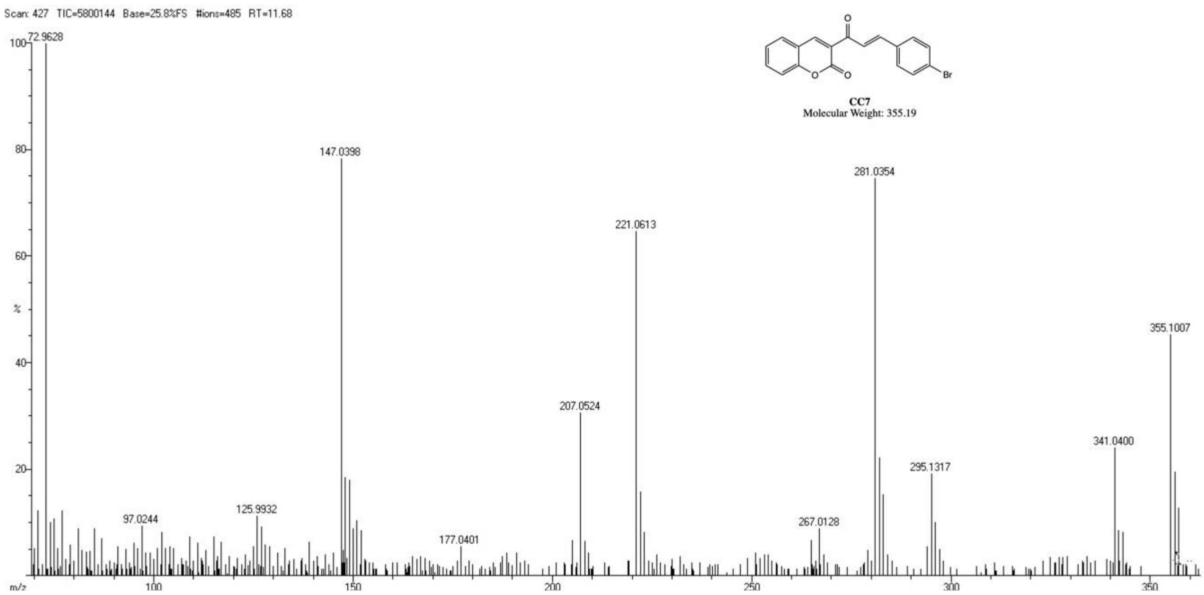


Figure S18. MS of CC7

CC8

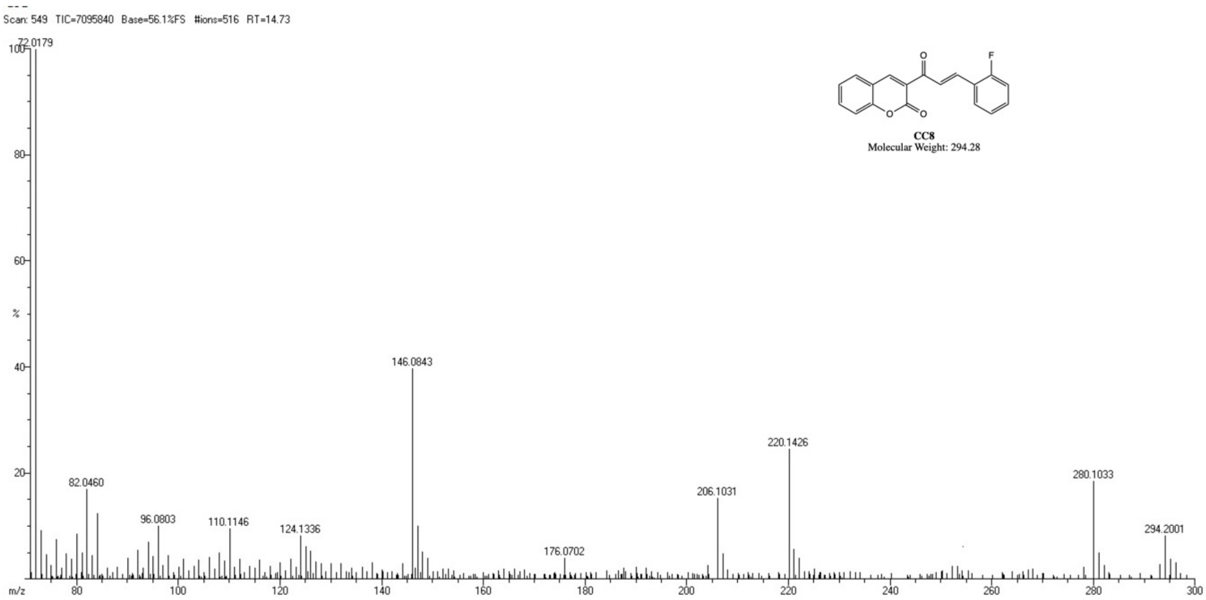


Figure S19. MS of CC8

CC9

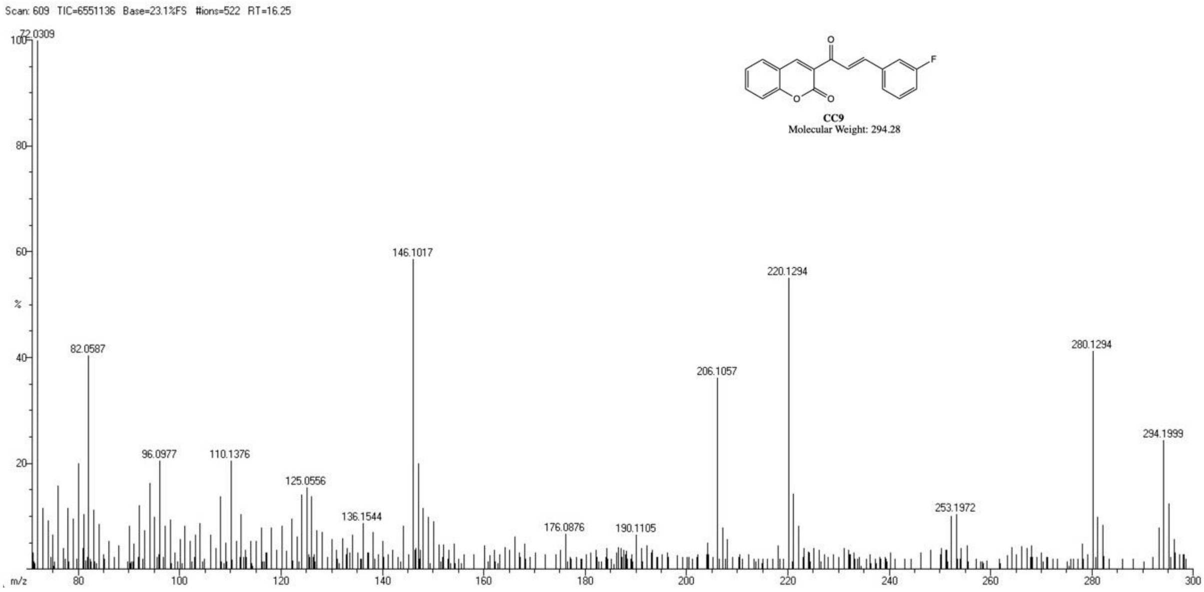


Figure S20. MS of CC9

CC10

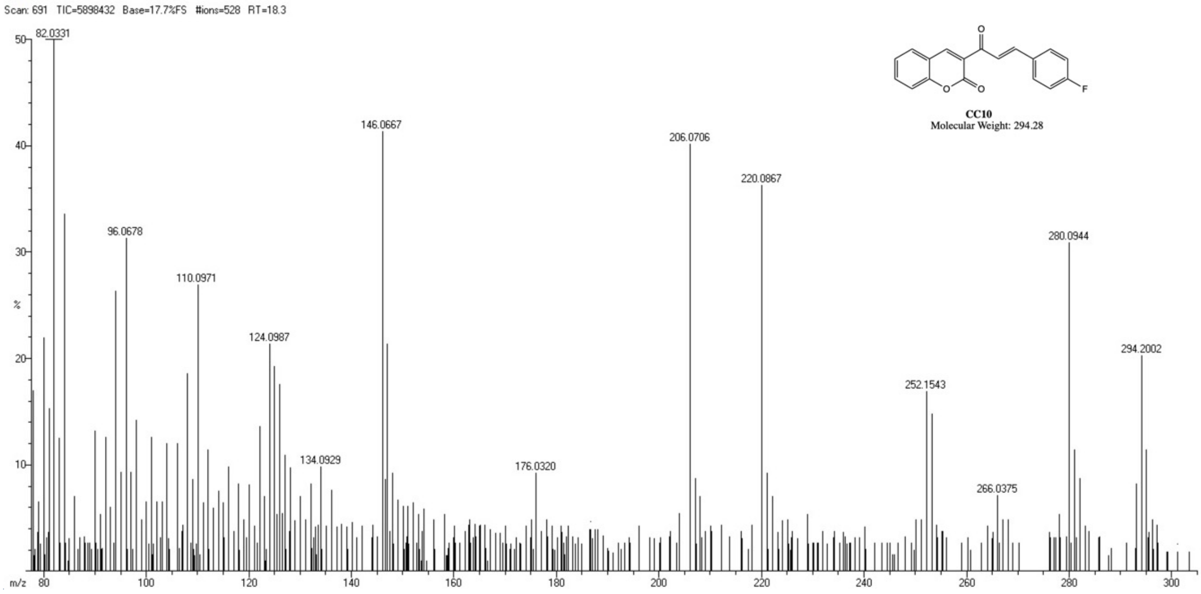


Figure S21. MS of CC10

Experimental Notes

1. Cytotoxicity

The cell line of Vero (African green monkey kidney cells) was procured from NCCS, Pune and were grown in liquid medium (DMEM) containing, 100 ug/ml penicillin, 100 µg/ml streptomycin, and 10% Fetal Bovine Serum (FBS) and preserved under an atmosphere of 5% CO_2 at 37ºC. The **CC1** and **CC2** sample was assayed for *in vitro* cytotoxicity by MTT assay using the cultured Vero cells. Briefly, the cultured Vero cells were produced by cell dissociation with trypsin (trypsinization), collectively in a 15 ml tube. At a density of 1×10^5 cells/ml cells/well (200 µL) the cells were plated for 24-48 hour at 37°C into 96-well tissue culture plate in DMEM medium containing 10 % FBS and 1% antibiotic solution. The wells were washed with sterile Phosphate buffered saline and further allowed to react with varying concentrations of the **CC1** and **CC2** sample in a serum free DMEM medium. The samples were triplicated and the cells were kept for incubation at 37°C for 24 h in a humidified 5% CO2 incubator. MTT (20 µL of 5 mg/ml) was added into each well after the incubation and the cells were incubated for another 2-4 h. The end point was determined by the purple precipitation which was clearly seen under an inverted microscope. At the end, the medium along with MTT (220 µL) were used for the aspiration of the wells and later washed with 1X PBS (200 µl). In order, to dissolve formazan crystals, DMSO (100 µL) was added to the plate with shaking for 5 min. The absorbance at 570 nm was measured using a micro plate reader (Thermo Fisher Scientific, USA). The IC_{50} value and the percentage cell viability was calculated using GraphPad Prism 8.0 software (USA).

2. ROS assay

The **CC1 and CC2** sample was tested for ROS using Vero cells. In Brief, the cultured Vero cells were grown by cell dissociation with trypsin (trypsinization), in a 15 ml tube. Then, the cells were plated at a density of 1×10^6 cells/ml into 24-well tissue culture plate in DMEM medium containing 10 % FBS and 1% antibiotic solution for 24 hour at 37°C. The wells were washed and pretreated with 126.4 µg/ml of **CC1** and **CC2** sample in serum free DMEM medium and incubated at 37°C for 24 hrs. 24 hrs later, 1 ml of ROS assay buffer was added followed by 100 µl of 1X ROS assay staining solution was added to the wells and mixed gently. Then the plate was incubated for 60 minutes in a 37ºC incubator with 5% CO_2. After the incubation period, the cells were treated with 100 µM/ml of 30% H2O2 and the production of ROS was evaluated immediately by fluorescence imaging system (ZOE, BIO-RAD).

Table S1: Docking score and MMGBSA values of MAO-A, MAO-B, AChE, and BChE cognate ligands.

Compounds	**RMSD** (Å)	**Docking Score** (kcal/mol)	**MMGBSA** (kcal/mol)
HRM (MAO-A cognate ligand)	0.762	-9.34	-73.24
SAG (MAO-B cognate ligand)	0.390	-10.76	-80.21
Donepezil (AChE cognate ligand)	0.190	-13.43	-94.84
L3H (BChE cognate ligand)	0.349	-10.00	-70.95

MAO-A (PDB code 2Z5X); MAO-B (PDB code 2V5Z); AChE (PDB code 4EY7[1]); and BChE (PDB code 6SAM[2])

Table S2: Docking score and MMGBSA values of **CC1** and **CC2** towards AChE and BChE.

	AChE		BChE	
	Docking score (kcal/mol)	MM-GBSA (kcal/mol)	Docking Score (kcal/mol)	MM-GBSA (kcal/mol)
CC1	-8.89	-60.71	-7.72	-51.39
CC2	-9.10	-65.02	-7.47	-54.37

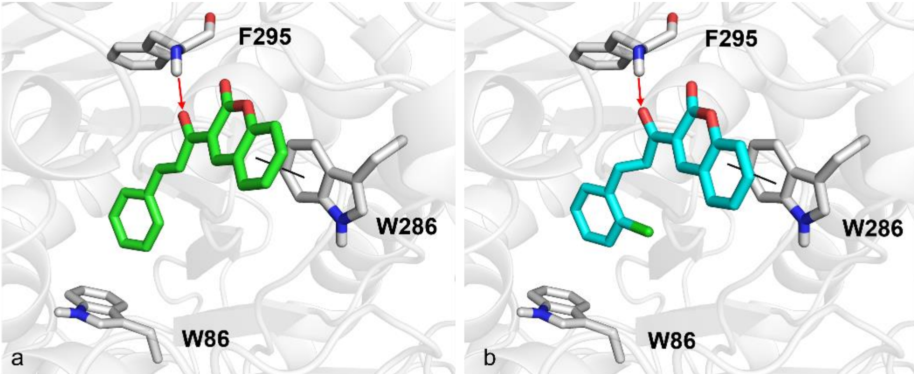


Figure S22: Zoomed in view of an AChE binding pocket. Panels (a) and (b) report the best pose returned from docking analysis for **CC1** (green sticks) and **CC2** (cyan sticks), respectively. Black lines and red arrows indicate π–π contacts and hydrogen bonds, respectively.

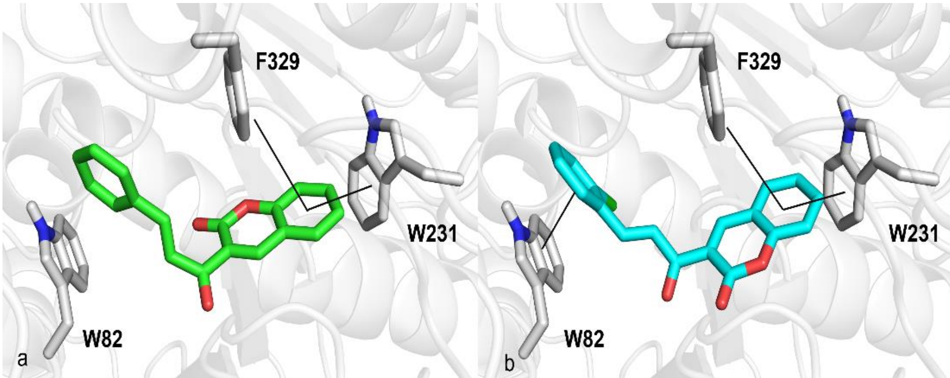


Figure S23: Zoomed in view of a BChE binding pocket. Panels (a) and (b) reported the best pose returned from docking analysis for **CC1** (green sticks) and **CC2** (cyan sticks), respectively. Black lines indicate π–π contacts.

References

(1) Cheung, J.; Rudolph, M. J.; Burshteyn, F.; Cassidy, M. S.; Gary, E. N.; Love, J.; Franklin, M. C.; Height, J. J. Structures of Human Acetylcholinesterase in Complex with Pharmacologically Important Ligands. *J. Med. Chem.* **2012**, *55* (22), 10282–10286.

(2) Košak, U.; Strašek, N.; Knez, D.; Jukič, M.; Žakelj, S.; Zahirović, A.; Pišlar, A.; Brazzolotto, X.; Nachon, F.; Kos, J.; Gobec, S. N-Alkylpiperidine Carbamates as Potential Anti-Alzheimer's Agents. *Eur. J. Med. Chem.* **2020**, *197*, 112282.