

# Supporting Information

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

Nisha Abdul Rehuman<sup>1,#</sup>, Jong Min Oh<sup>2,#</sup>, Lekshmi R Nath<sup>3</sup>, Ahmed Khames<sup>4</sup>, Mohamed A Abdelgawad<sup>5</sup>, Nicola Gambacorta<sup>6</sup>, Orazio Nicolotti<sup>6</sup>, Rakesh Kumar Jat<sup>7</sup>, Hoon Kim<sup>2,\*</sup>, Bijo Mathew<sup>8,#,\*</sup>

<sup>1</sup> Department of Pharmaceutical Chemistry, Dr. Joseph Mar Thoma Institute of Pharmaceutical Sciences & Research, Kerala 690503, India.

<sup>2</sup> Department of Pharmacy, and Research Institute of Life Pharmaceutical Sciences, Sunchon National University, Suncheon 57922, Republic of Korea.

<sup>3</sup> Department of Pharmacognosy, Amrita School of Pharmacy, Amrita Vishwa Vidyapeetham, AIMS Health Sciences Campus, Kochi-682, India.

<sup>4</sup> Department of Pharmaceutics and Industrial Pharmacy, College of Pharmacy, Taif University, P.O. Box 11099, Taif-21944, Saudi Arabia

<sup>5</sup> Department of Pharmaceutical Chemistry, College of Pharmacy, Jouf University, Sakaka, Al Jouf 72341, 14 Saudi Arabia.

<sup>6</sup> Dipartimento di Farmacia—Scienze del Farmaco, Università degli Studi di Bari “Aldo Moro”, Via E. Orabona, 4, I-70125 Bari, Italy.

<sup>7</sup> Department of Pharmaceutical Chemistry, JJTU University, Jhunjhunu 333001, India.

<sup>8</sup> Department of Pharmaceutical Chemistry, Amrita School of Pharmacy, Amrita Vishwa Vidyapeetham, AIMS Health Sciences Campus, Kochi-682 041, India.

#Authors contributed equally.

\*Correspondence: Bijo Mathew (B. Mathew) ([bijomathew@aims.amrita.edu](mailto:bijomathew@aims.amrita.edu); [bijovilaventgu@gmail.com](mailto:bijovilaventgu@gmail.com)); Hoon Kim (H. Kim) ([hoon@sunchon.ac.kr](mailto:hoon@sunchon.ac.kr))

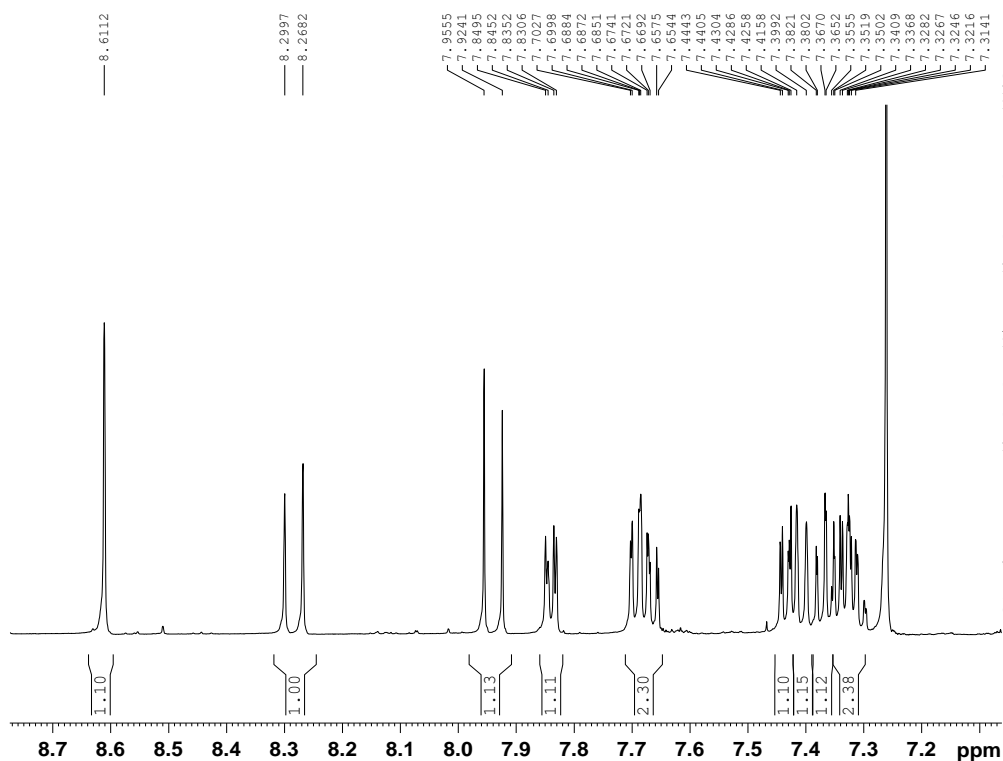
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CC2: 3-[(2*E*)-3-(2-chlorophenyl)prop-2-enoyl]-2*H*-1-benzopyran-2-one

CC2B  
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BRUKER  
 AVANCE NEO  
 500 MHz NMR  
 SPECTROMETER  
 SAIF, P.U.



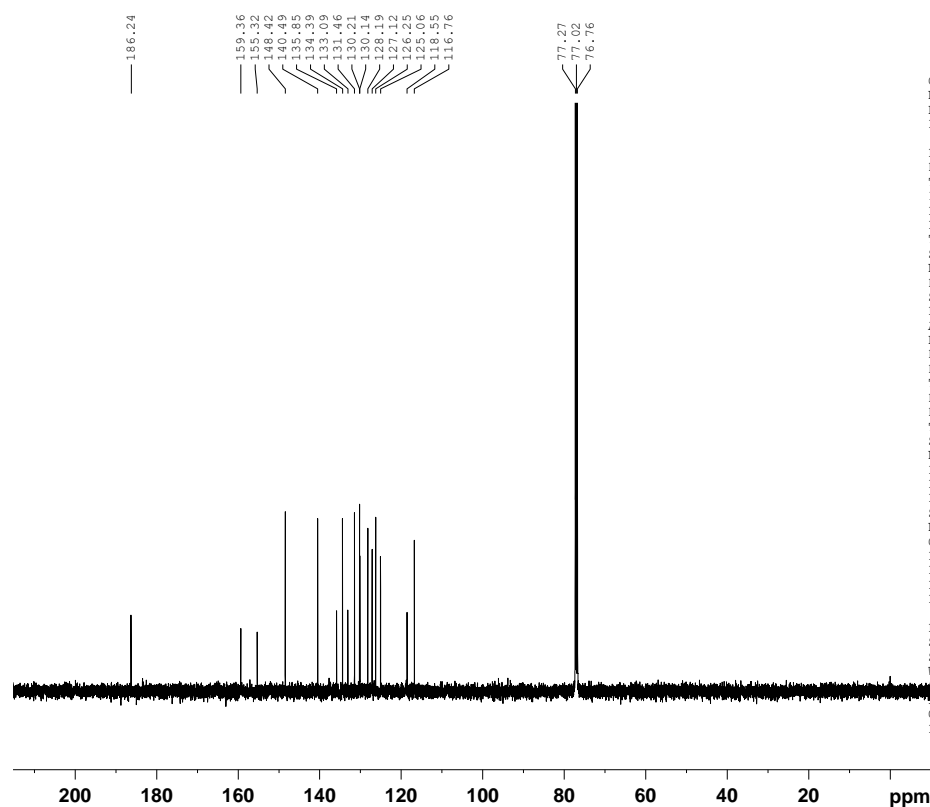
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 FIDRES 0.448788 Hz  
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 DW 34.000 usec  
 DE 6.79 usec  
 TE 300.2 K  
 D1 1.00000000 sec  
 TD0 1  
 SFO1 500.1730885 MHz  
 NUC1 1H  
 P0 3.33 usec  
 P1 10.00 usec  
 PLW1 20.93000031 W

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 SSB 0  
 LB 0.30 Hz  
 GB 0  
 PC 1.00

Figure S1. <sup>1</sup>H-NMR of CC2

CC2B  
 C13CPD CDC13 {D:\Spectra} nmr 50



BRUKER  
 AVANCE NEO  
 500 MHz NMR SPECTROMETER  
 SAIF, PANJAB UNIVERSITY,  
 CHANDIGARH

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 DS 4  
 SWH 37037.035 Hz  
 FIDRES 1.130281 Hz  
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 DW 13.500 usec  
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 TE 300.1 K  
 D1 2.00000000 sec  
 D11 0.03000000 sec  
 TD0 1  
 SFO1 125.7804233 MHz  
 NUC1 13C  
 P0 3.33 usec  
 P1 10.00 usec  
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 NUC2 1H  
 CPDPRG2 waltz65  
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 PLW13 0.16449000 W

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Figure S2. <sup>13</sup>C-NMR of CC2

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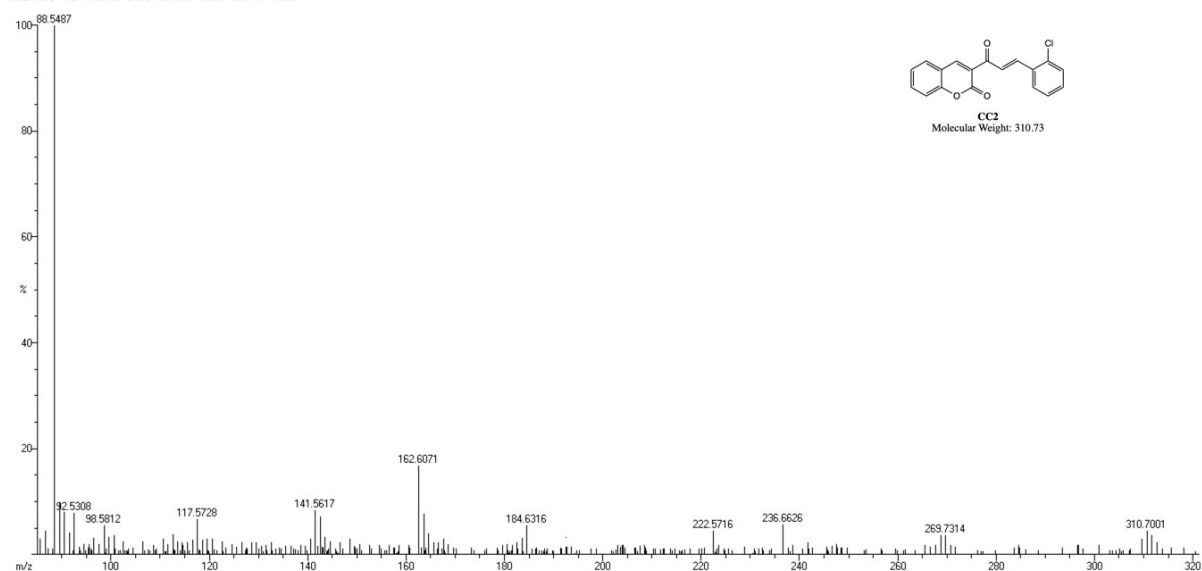


Figure S3. HRMS of CC2

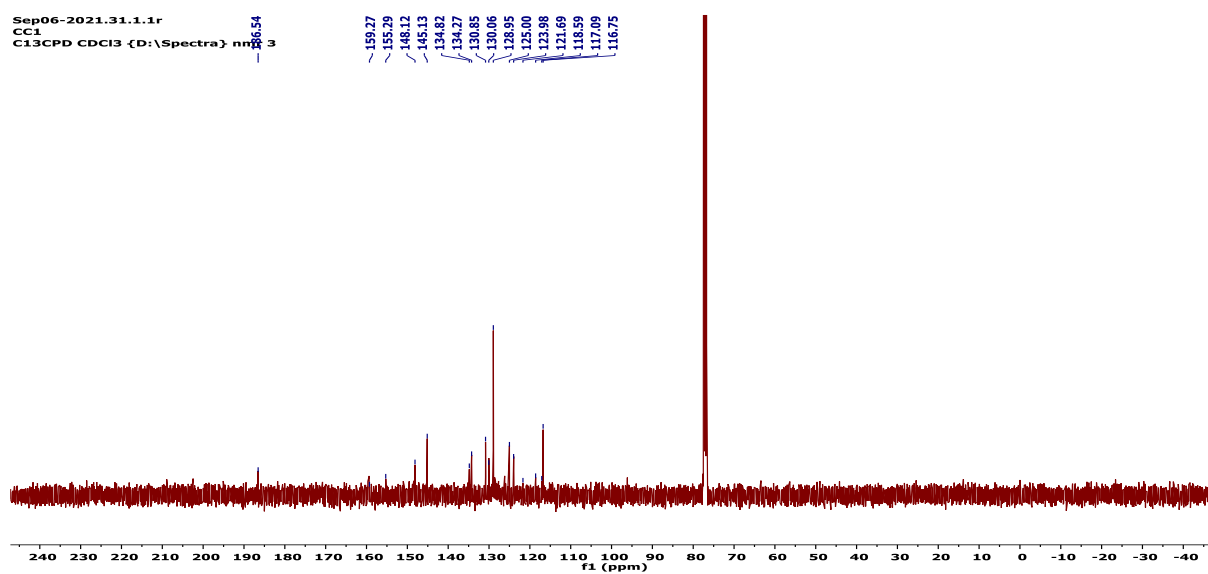
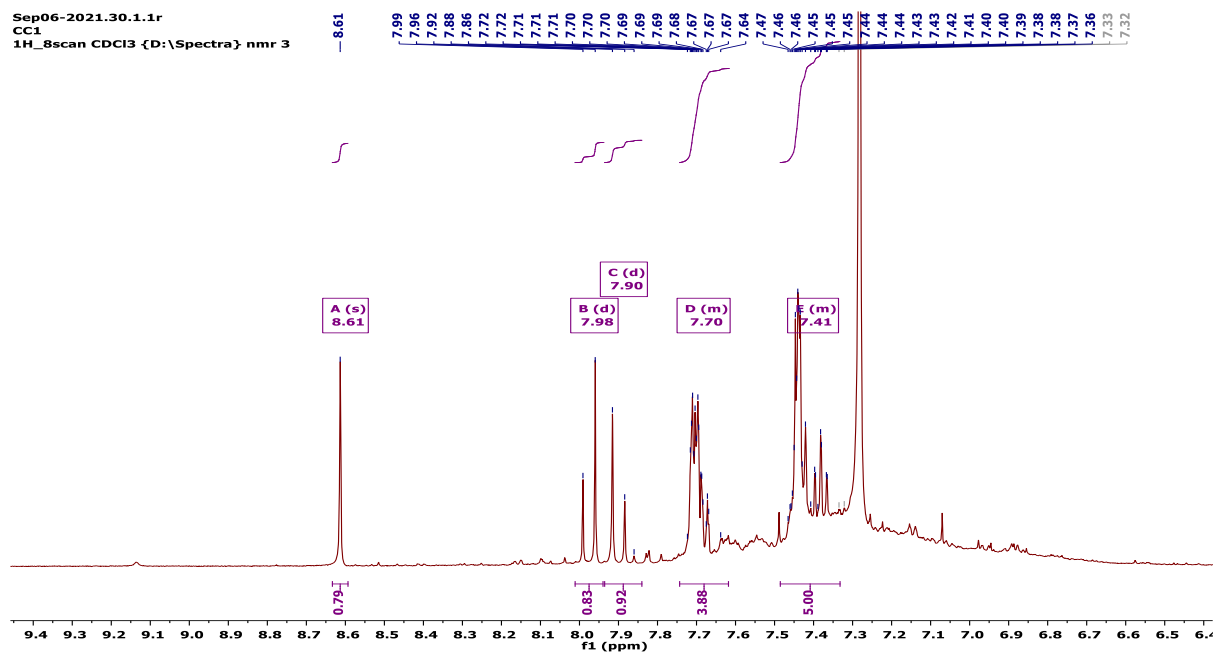


Figure S4.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR of CC1

$^1\text{H}$  NMR (500 MHz, Chloroform-*d*)  $\delta$  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);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  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.

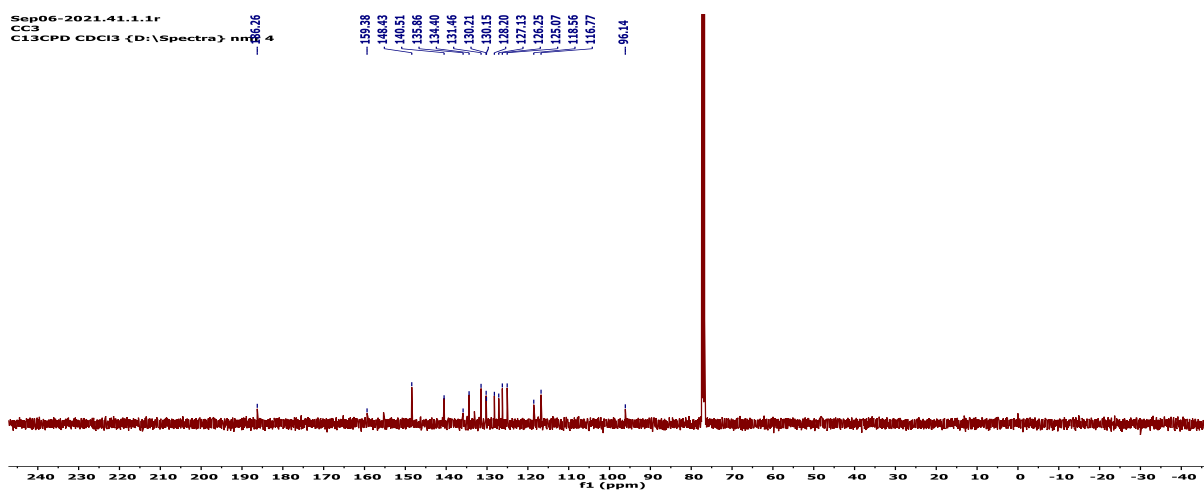
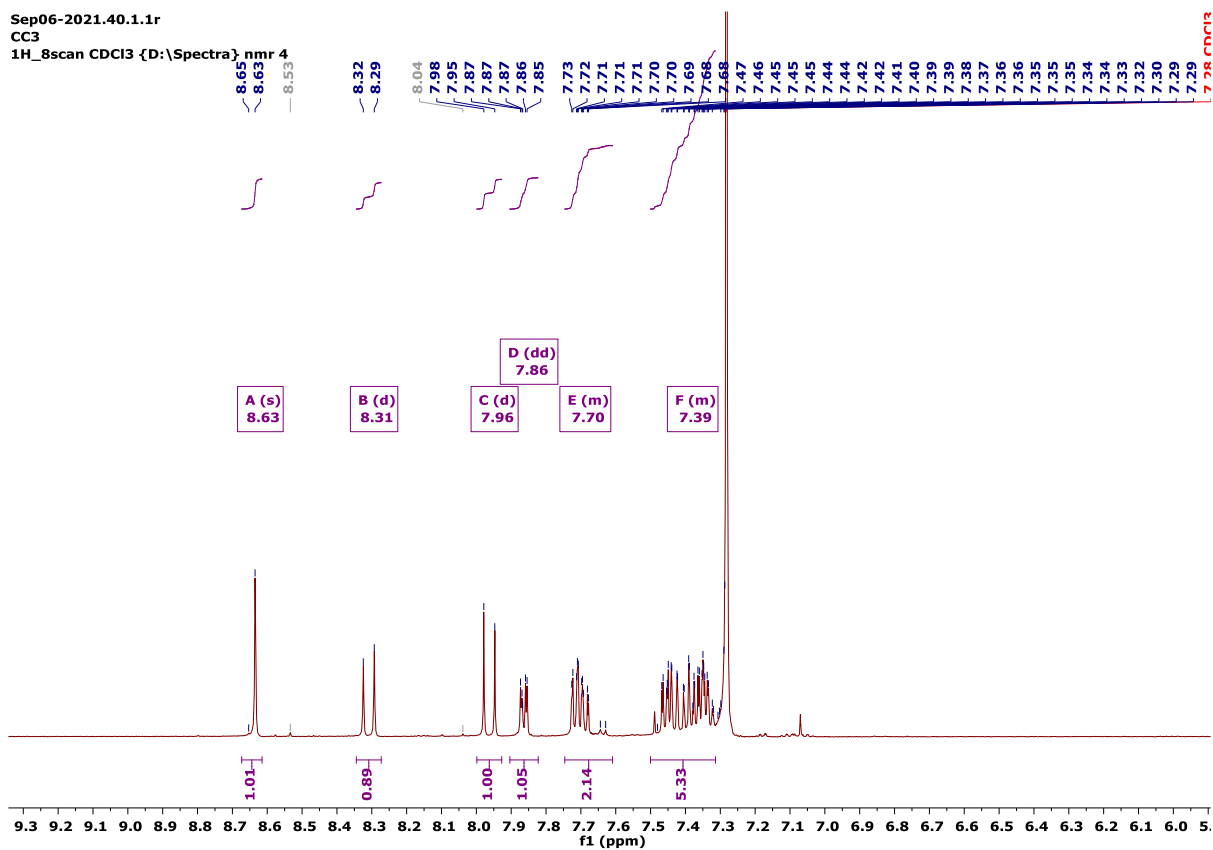


Figure S5.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR of CC3

$^1\text{H}$  NMR (500 MHz, Chloroform-*d*)  $\delta$  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);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  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.

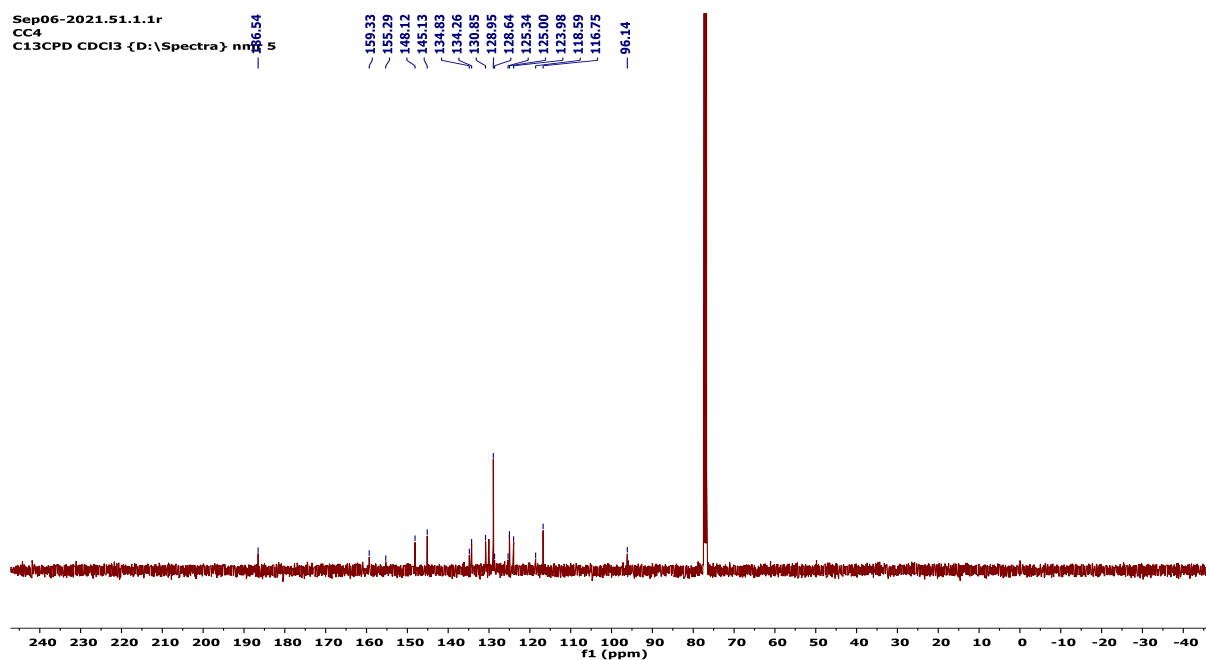
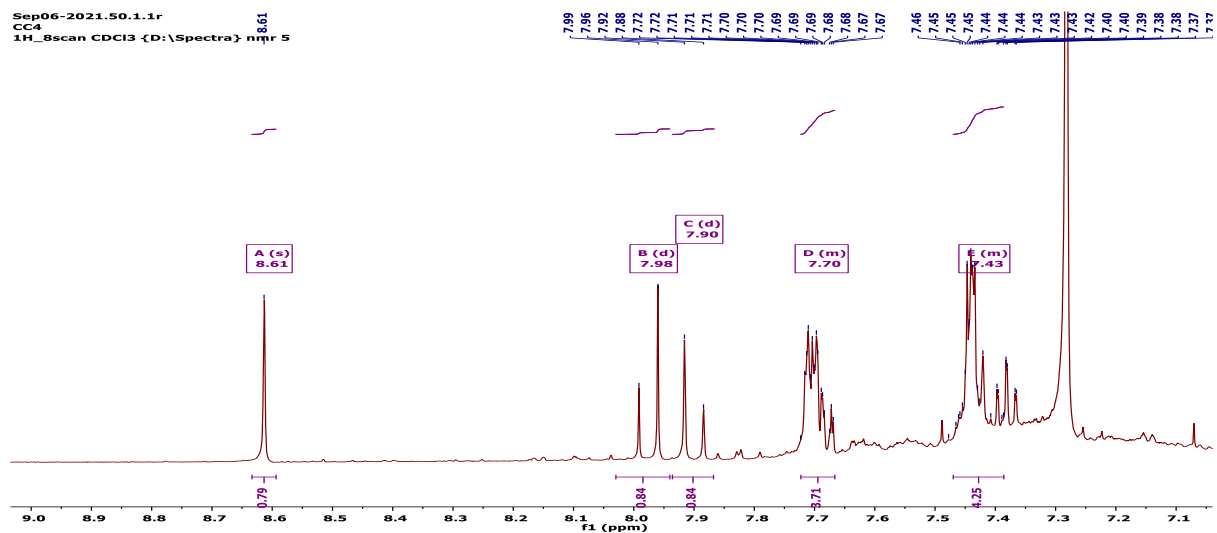


Figure S6.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR of CC4

$^1\text{H}$  NMR (500 MHz, Chloroform-*d*)  $\delta$  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);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  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.





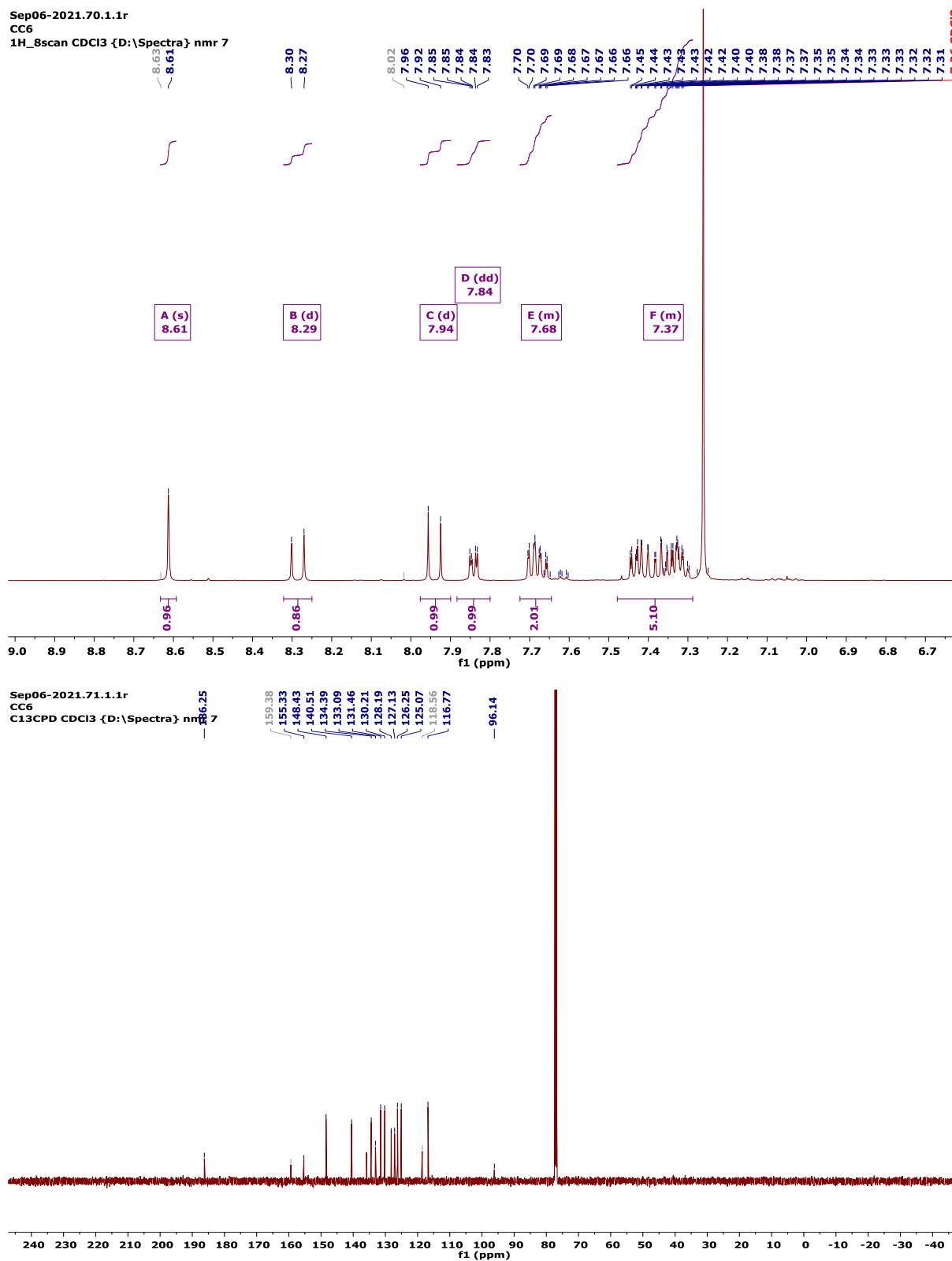


Figure S8.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR of CC6

$^1\text{H}$  NMR (500 MHz, Chloroform-*d*)  $\delta$  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);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  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.

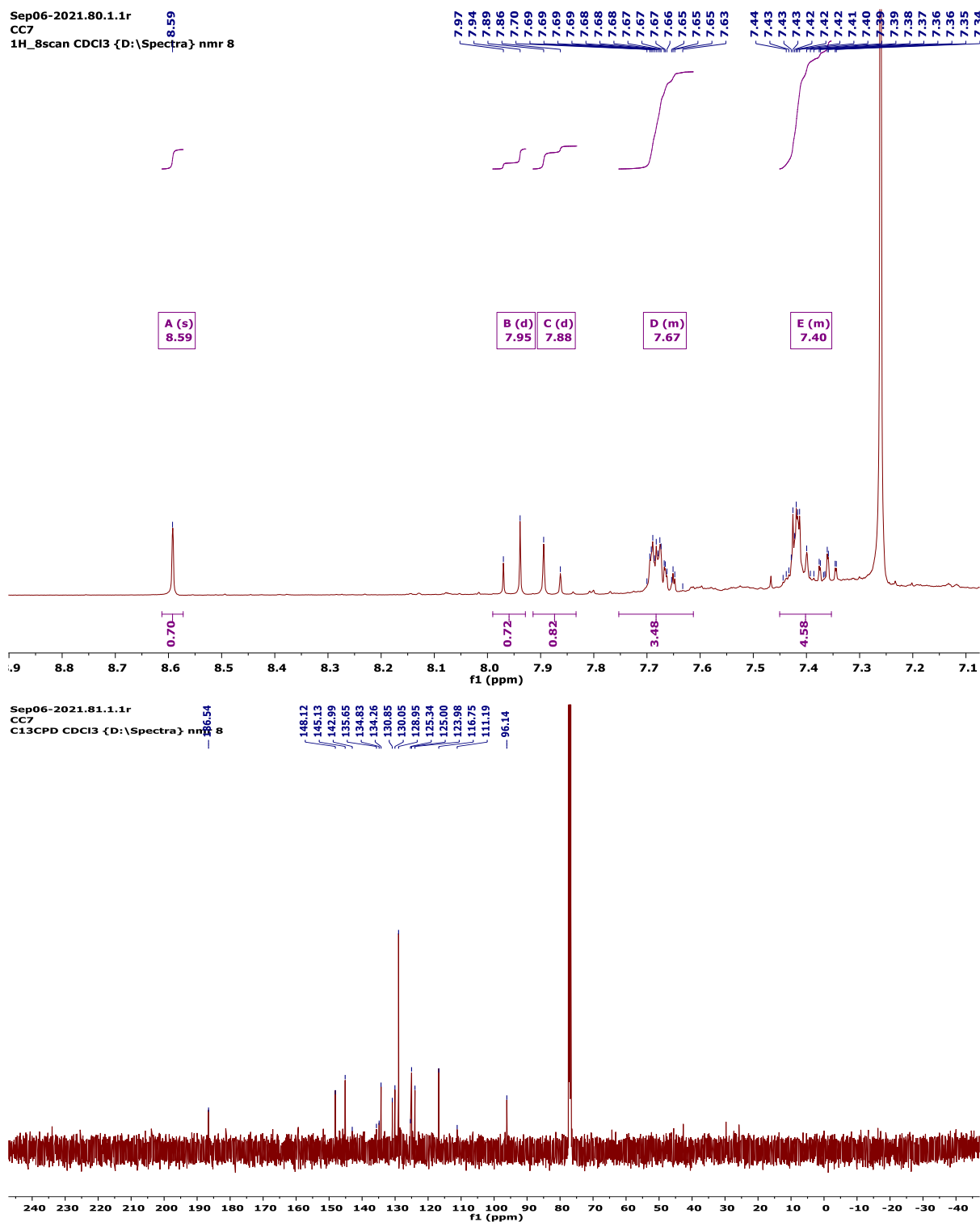


Figure S9.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR of CC7

$^1\text{H}$  NMR (500 MHz, Chloroform-*d*)  $\delta$  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);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  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.

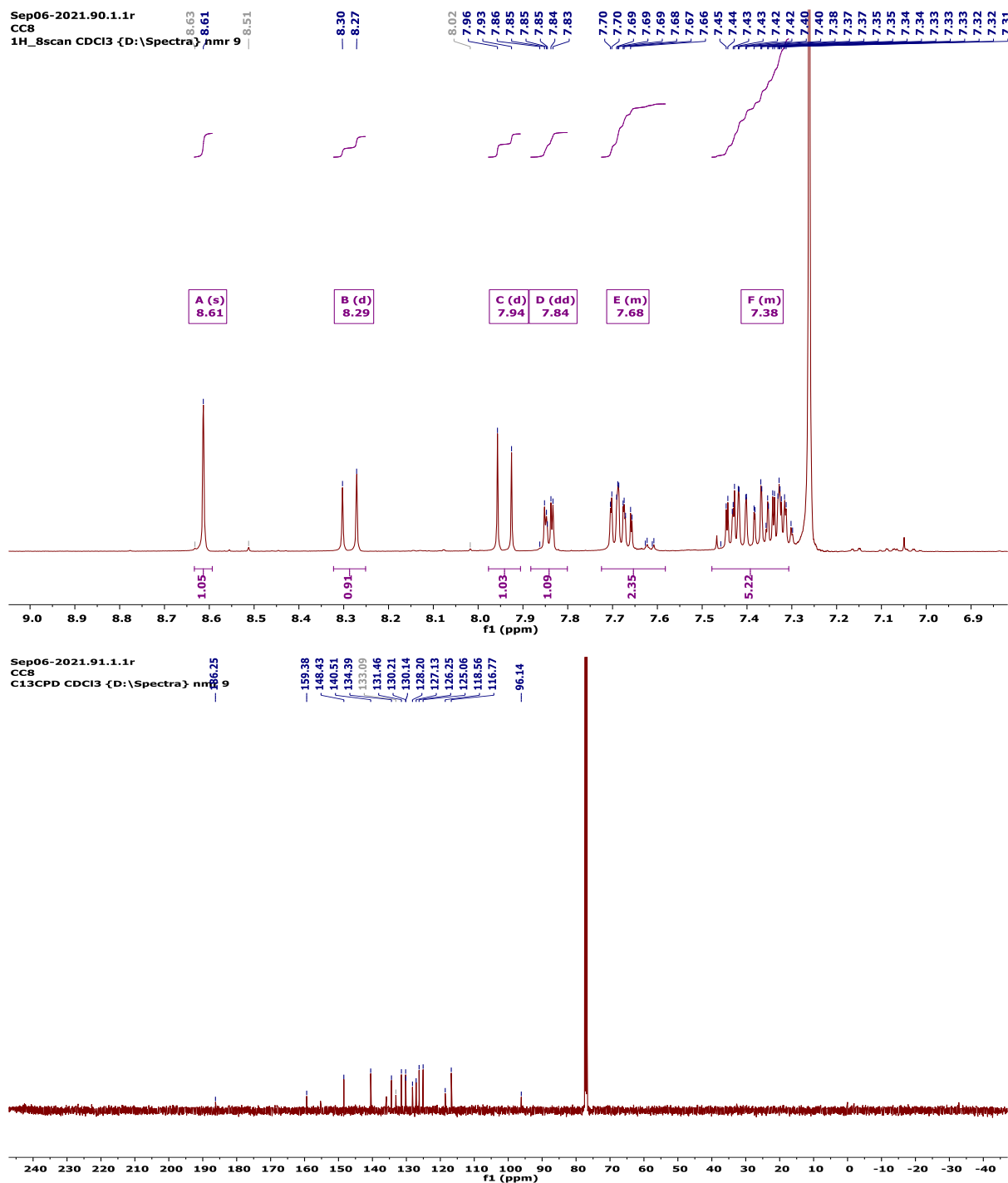


Figure S10. <sup>1</sup>H- and <sup>13</sup>C-NMR of CC8

<sup>1</sup>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); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) 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.

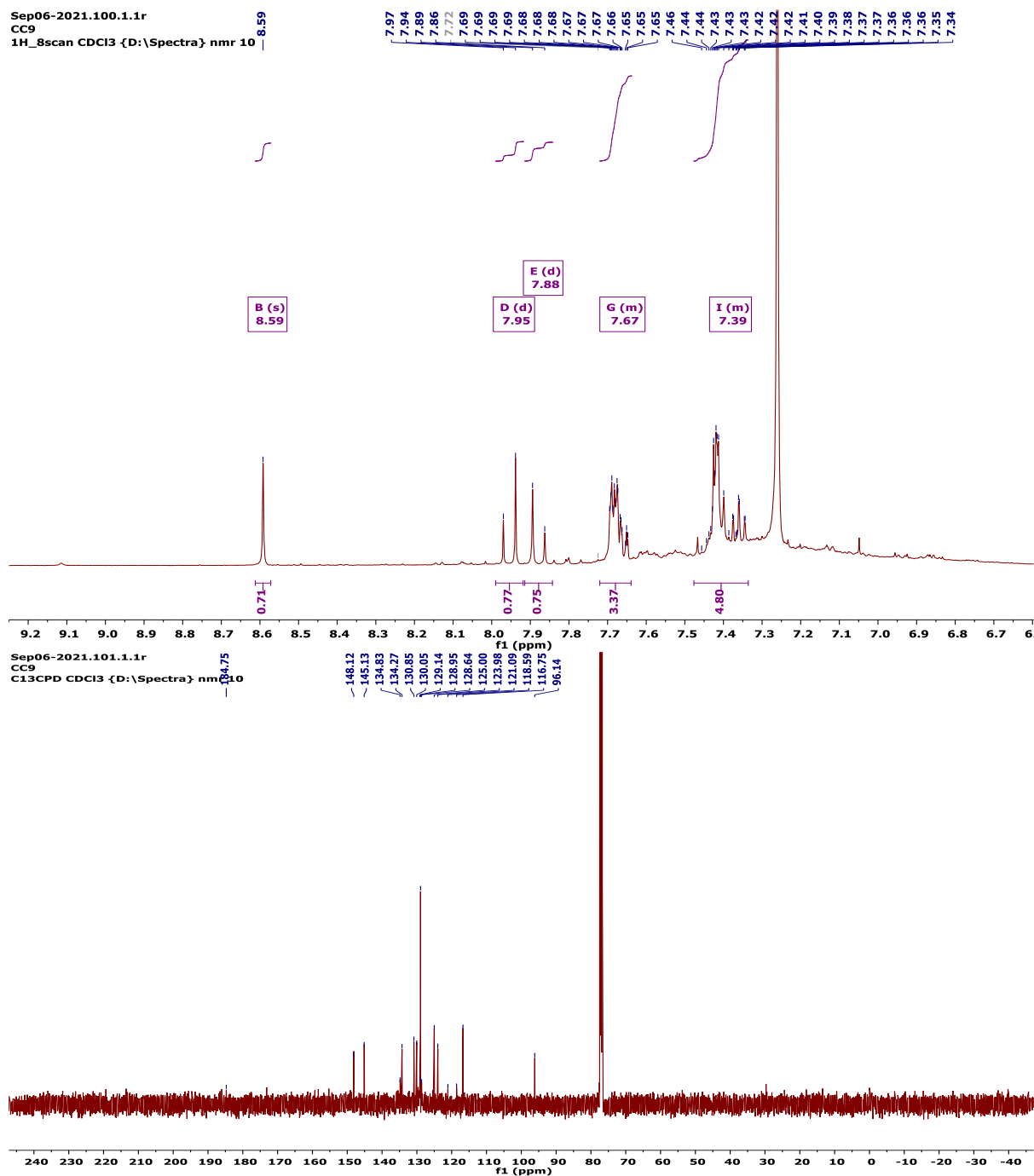


Figure S11.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR of CC9

$^1\text{H}$  NMR (500 MHz, Chloroform-*d*)  $\delta$  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);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  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.

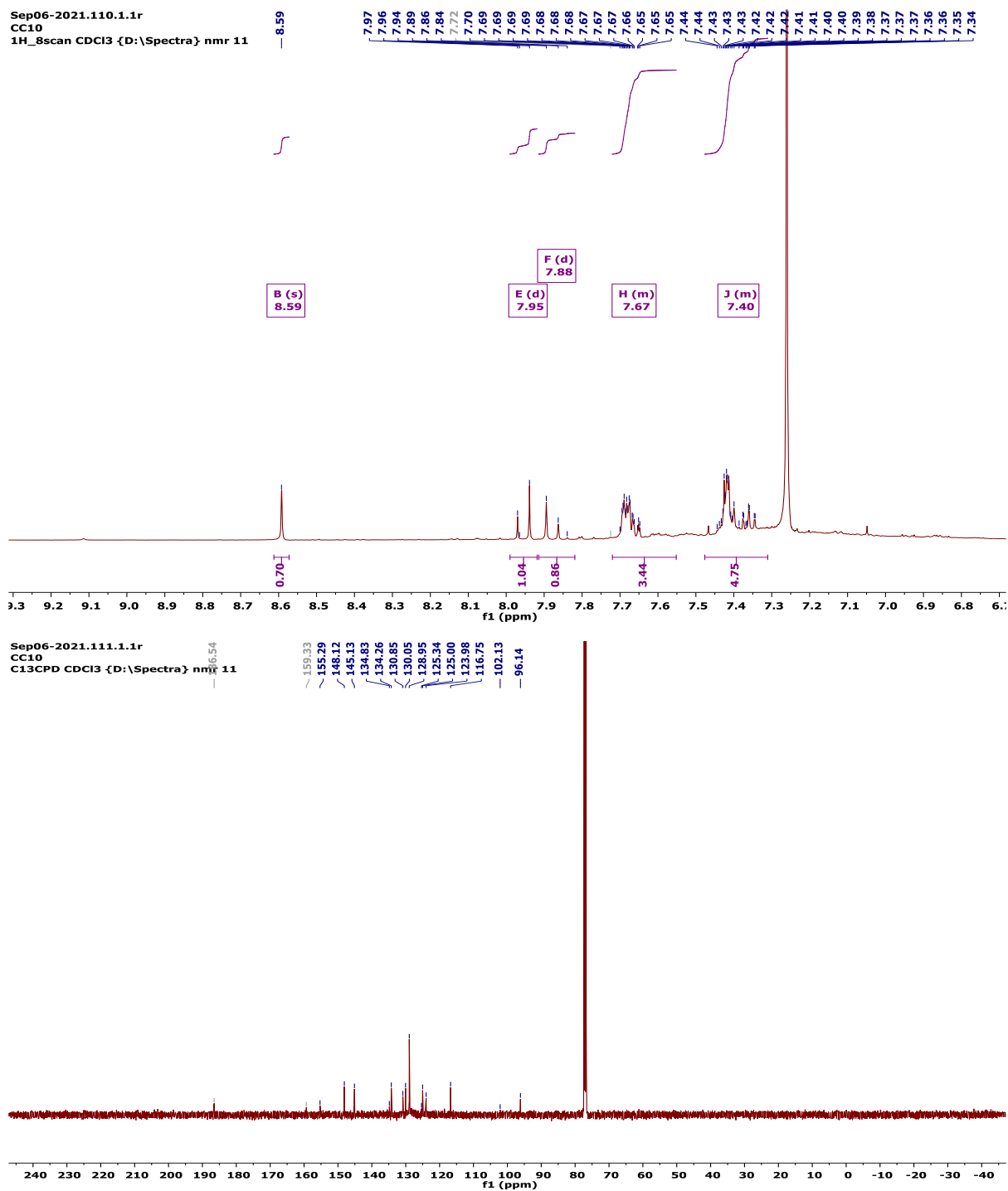


Figure S12. <sup>1</sup>H- and <sup>13</sup>C-NMR of CC10

<sup>1</sup>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); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) 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

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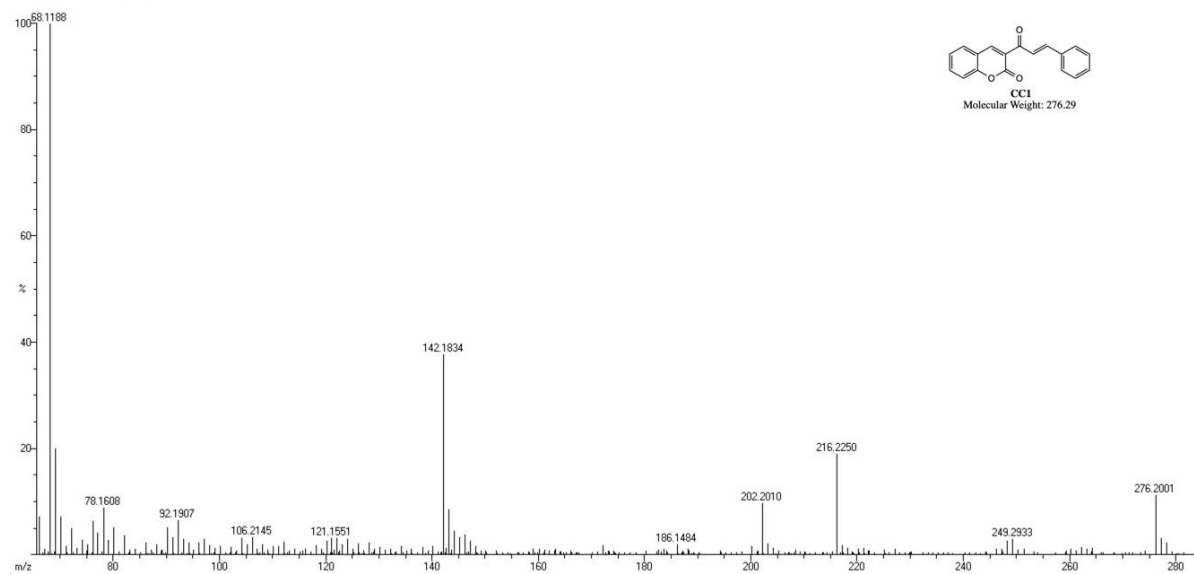


Figure S13. MS of CC1

# CC3

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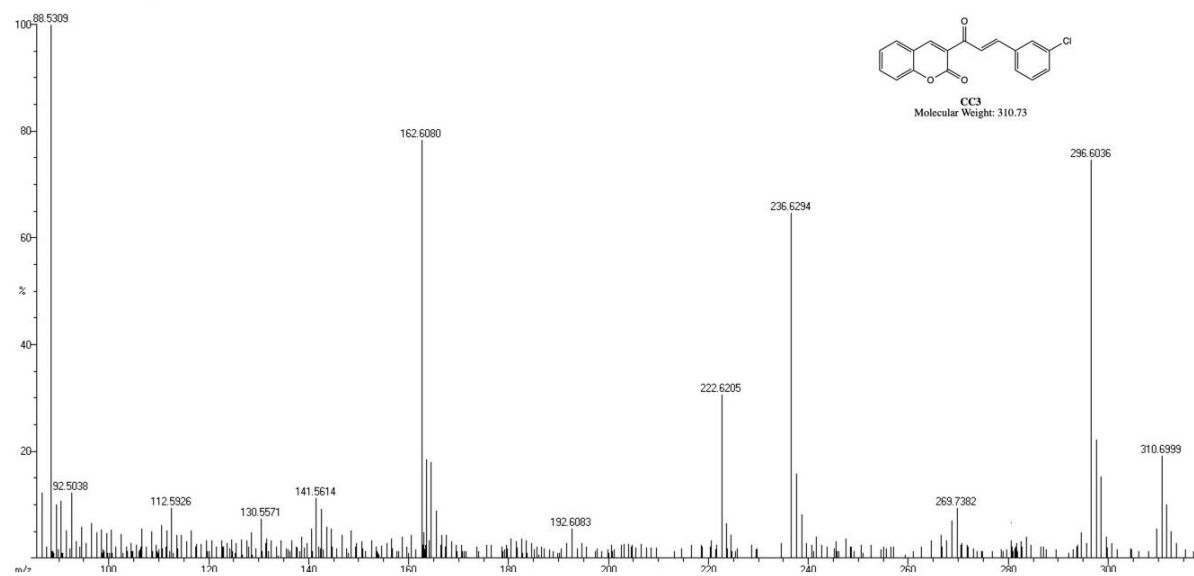


Figure S14. MS of CC3

## CC4

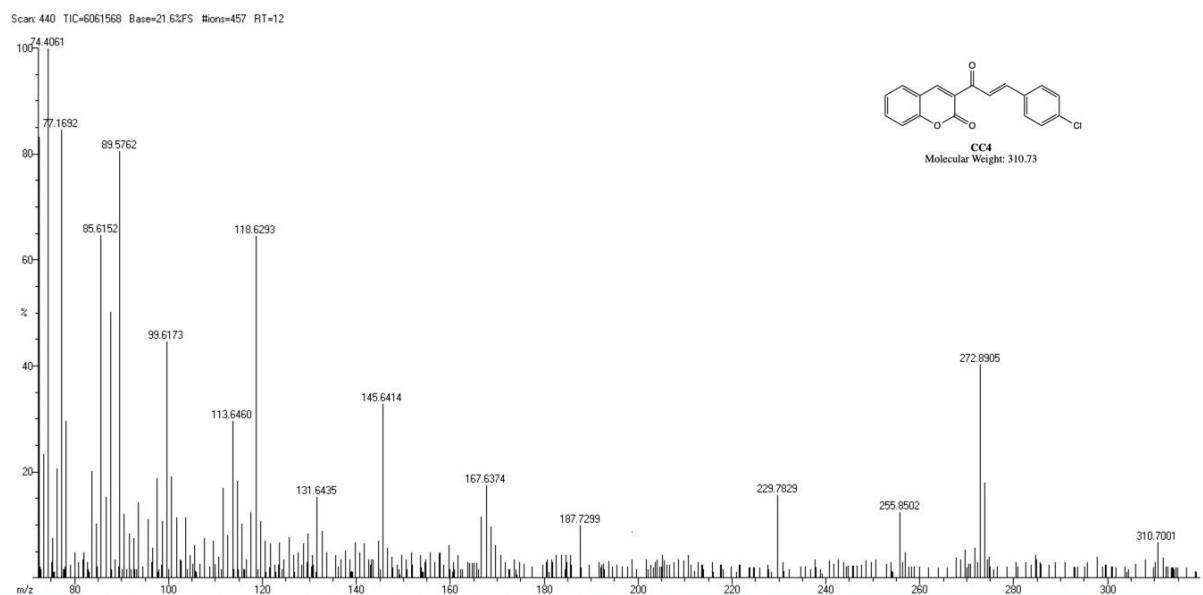


Figure S15. MS of CC4

## CC5

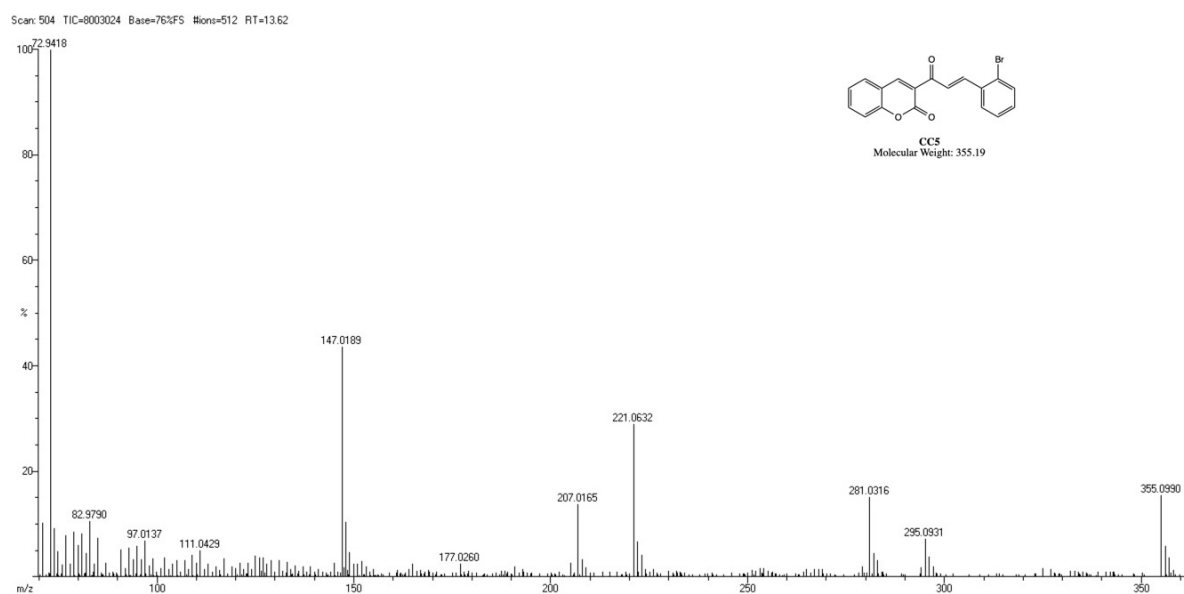


Figure S16. MS of CC5

## CC6

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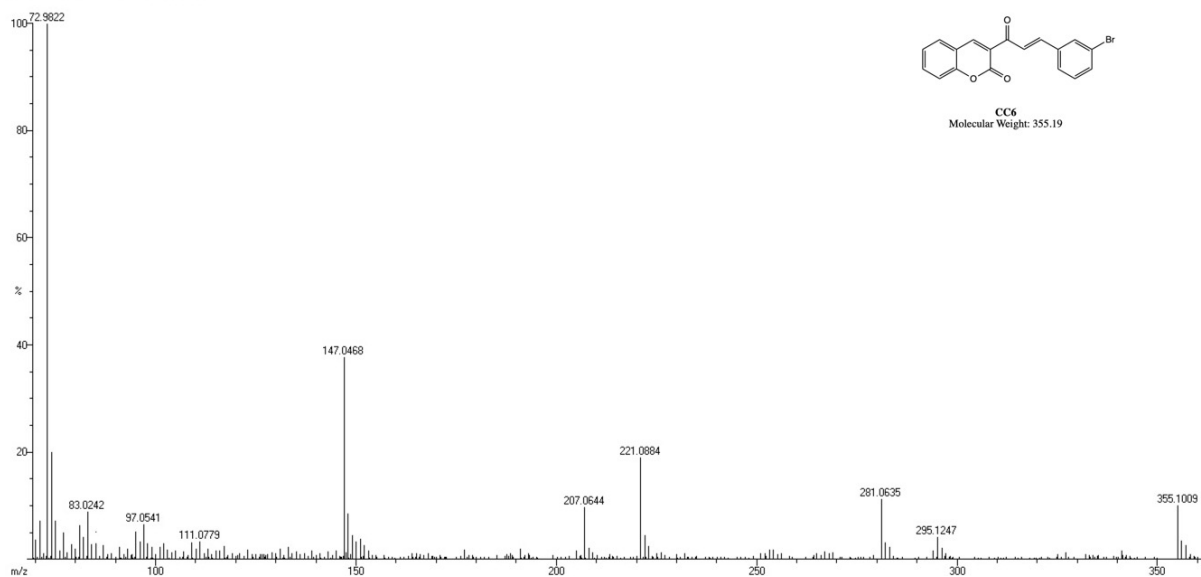


Figure S17. MS of CC6

## CC7

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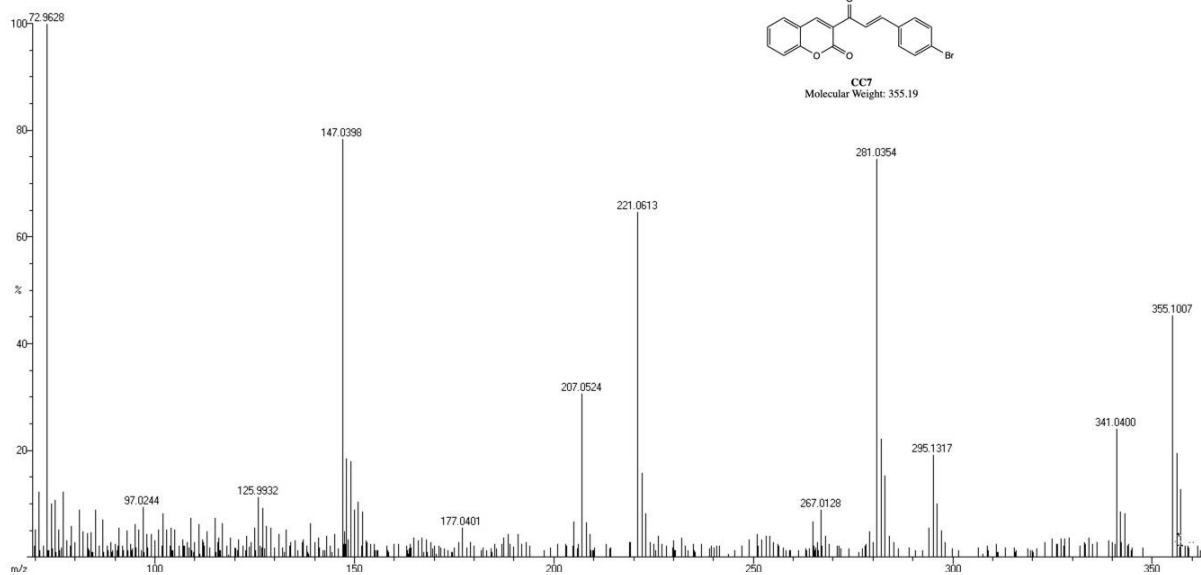


Figure S18. MS of CC7



## CC8

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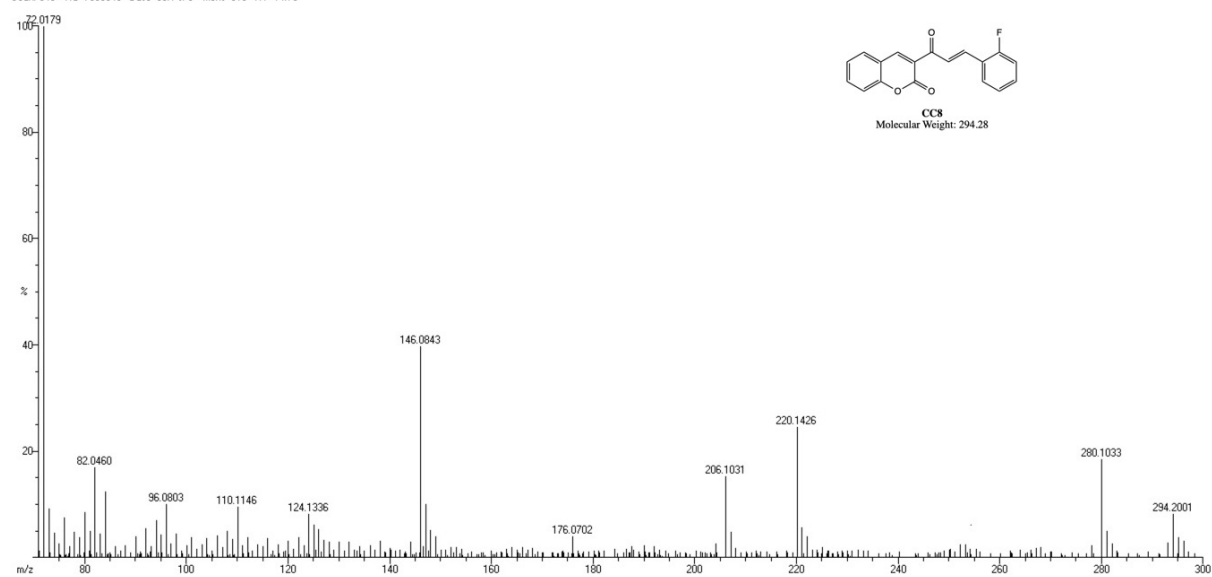


Figure S19. MS of CC8

## CC9

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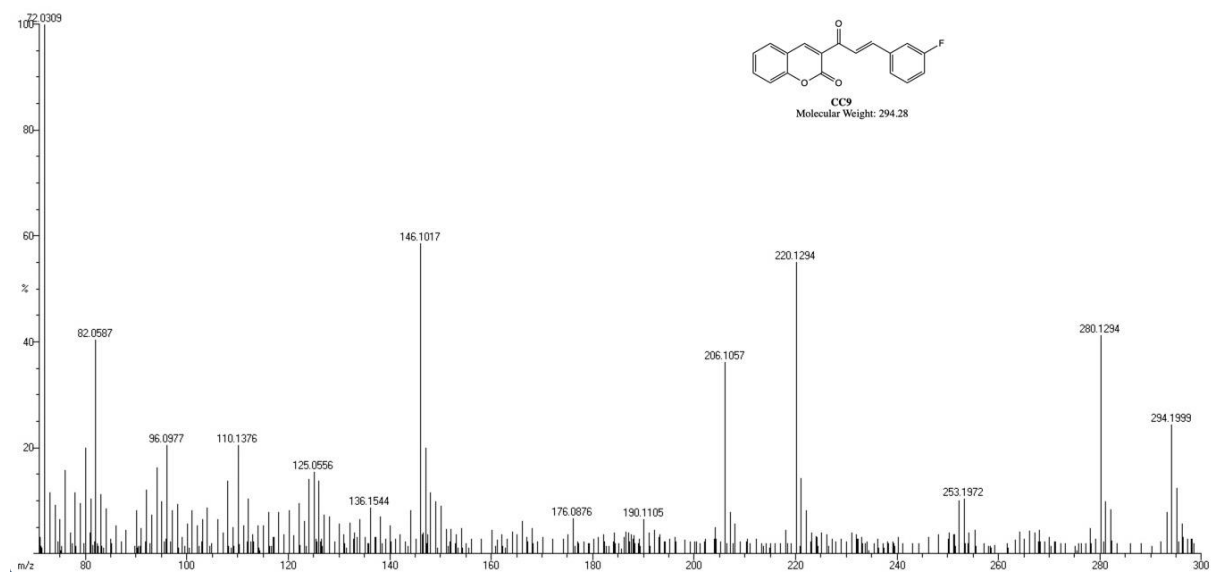


Figure S20. MS of CC9

# CC10

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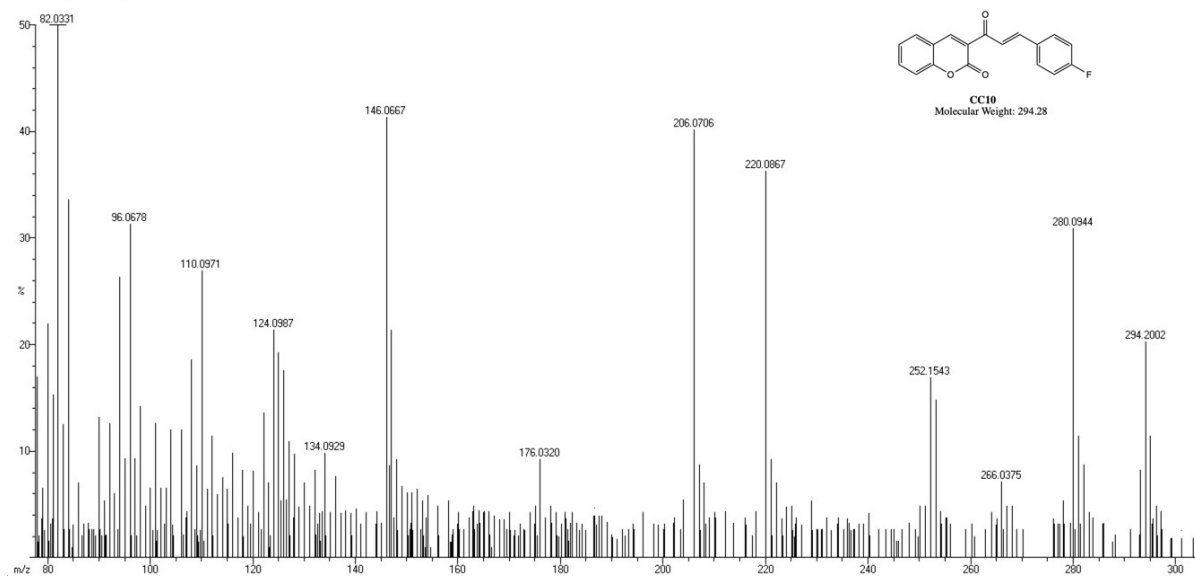


Figure S21. MS of CC10

## 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<sub>2</sub> at 37°C. 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<sup>5</sup> 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% CO<sub>2</sub> 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<sub>50</sub> 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<sup>6</sup> 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 37°C incubator with 5% CO<sub>2</sub>. After the incubation period, the cells were treated with 100 µM/ml of 30% H<sub>2</sub>O<sub>2</sub> and the production of ROS was evaluated immediately by fluorescence imaging system (ZOE, BIO-RAD).

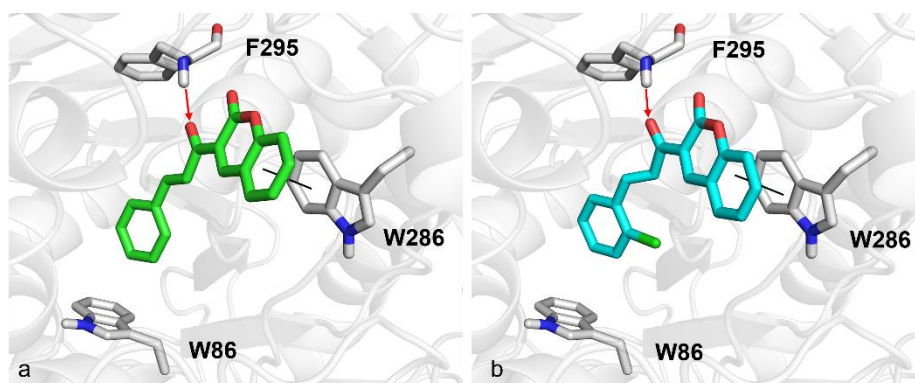
**Table S1:** Docking score and MMGBSA values of MAO-A, MAO-B, AChE, and BChE cognate ligands.

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

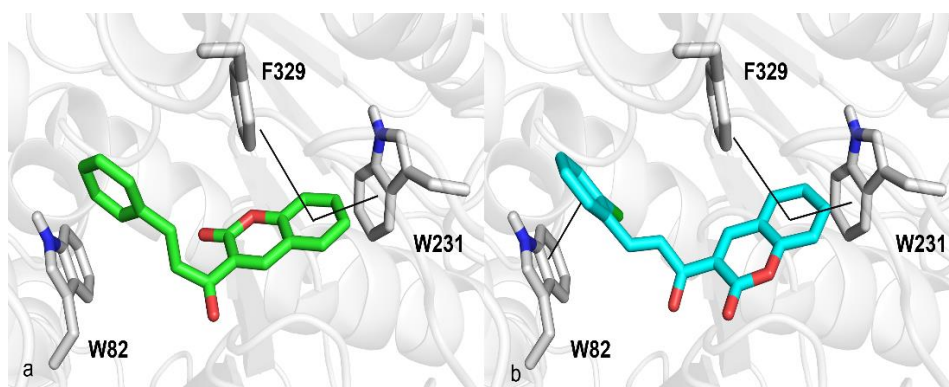
MAO-A (PDB code 2Z5X); MAO-B (PDB code 2V5Z); AChE (PDB code 4EY7<sup>1</sup>); and BChE (PDB code 6SAM<sup>2</sup>)

**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)
<b>CC1</b>	-8.89	-60.71	-7.72	-51.39
<b>CC2</b>	-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  $\pi$ - $\pi$  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  $\pi$ - $\pi$  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.