Supporting Information

Novel Etodolac Derivatives as Eukaryotic Elongation Factor 2 Kinase (eEF2K) Inhibitors for Targeted Cancer Therapy

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Short title: **Etodolac Derivatives as Eukaryotic Elongation Factor 2 Kinase (eEF2K) Inhibitors**

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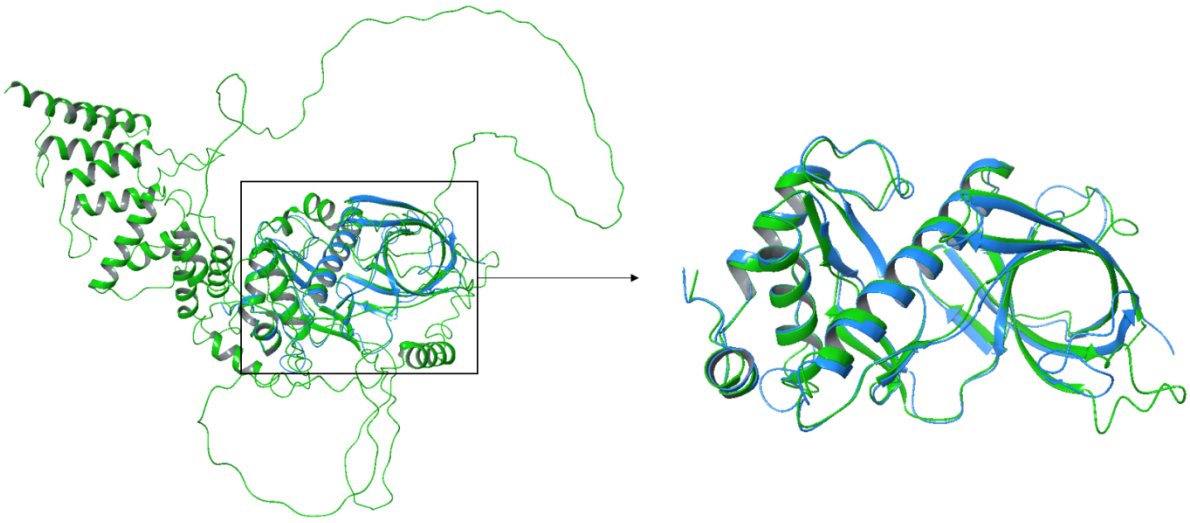


Figure S1. Alignment of 3D structures of alphafold (green) and homology model (blue) structure used in this study (RMSD: 1.5 Å).

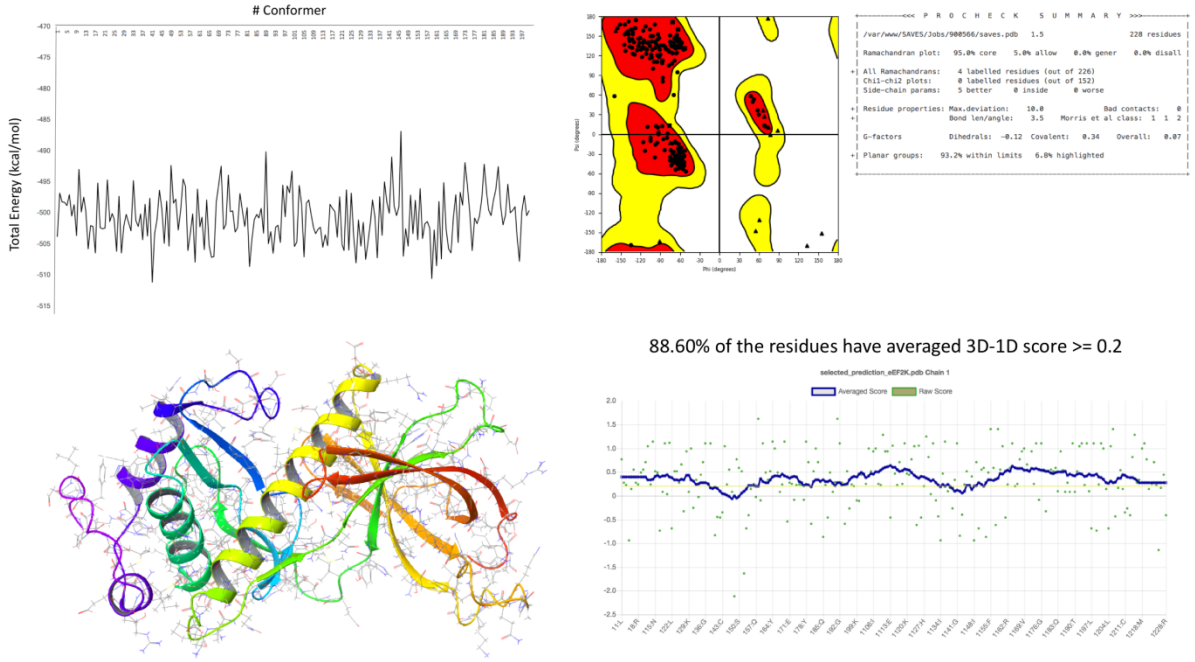


Figure S2. Developed model by combined Alphafold and Rosetta approaches. The model that has the lowest total energy (model no #41) was used. Ramachandran's plot showed that all the residues at the allowed region. Verify3D and PROCHECK protein validation tools validated the used model.

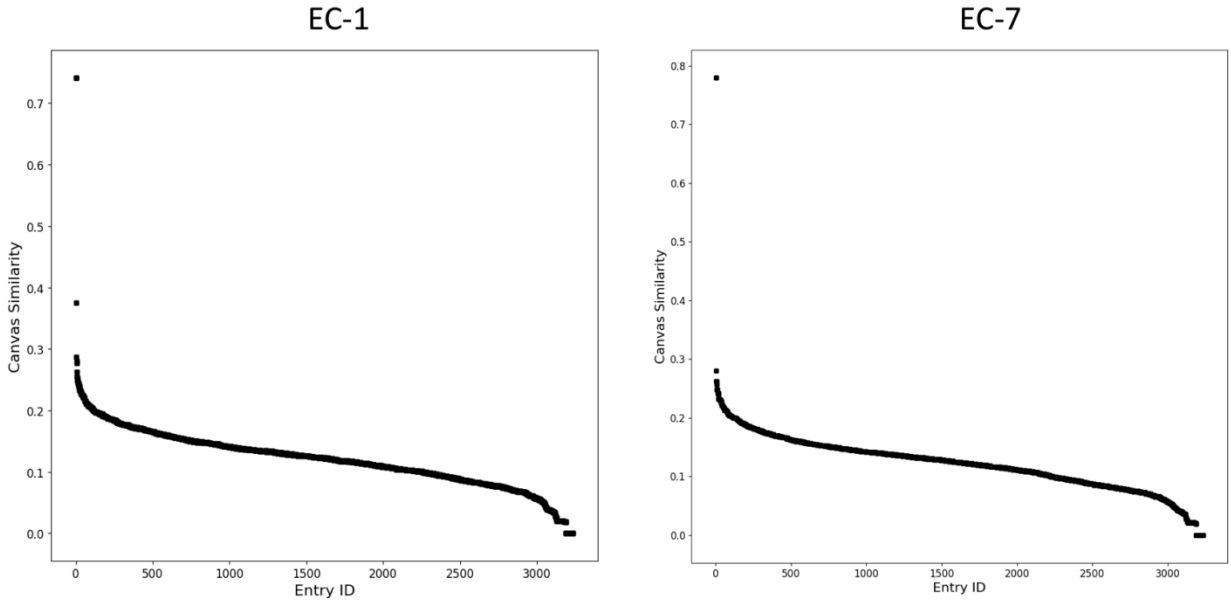


Figure S3. Structural similarity comparison of EC1 (left) and EC7 (right) with FDA approved compounds.

Table S1. Toxicity prediction analysis of the synthesized compounds.

Name	EC1	EC2	EC3	EC4	EC5	EC6	EC7
AMES (TP) [1]	0.6	0.46	0.57	0.51	0.4	0.5	0.38
Anemia (TP) [2]	0.31	0.19	0.22	0.3	0.22	0.34	0.21
Carcinogenicity (TP) [3]	0.06	0.08	0.06	0.04	0.07	0.04	0.05
Carcinogenicity Mouse Female (TP) [4]	0.12	0.18	0.12	0.04	0.1	0.04	0.12
Carcinogenicity Mouse Male (TP) [5]	0.1	0.12	0.1	0.05	0.11	0.05	0.14
Carcinogenicity Rat Female (TP) [6]	0.06	0.13	0.06	0.07	0.16	0.07	0.19
Carcinogenicity Rat Male (TP) [7]	0.07	0.19	0.07	0.05	0.16	0.05	0.16
Cardiotoxicity (TP) [8]	0.33	0.18	0.33	0.31	0.16	0.31	0.3
Cytotoxicity model, -log GI50 (M) (TP) [9]	5.9	5.98	5.9	5.64	5.61	5.6	5.44
Epididymis toxicity (TP) [10]	0.23	0.23	0.23	0.15	0.15	0.15	0.19
Genotoxicity (TP) [11]	0.31	0.28	0.31	0.4	0.48	0.46	0.38
Hepatotoxicity (TP) [12]	0.14	0.19	0.13	0.16	0.19	0.15	0.15
Kidney Necrosis (TP) [13]	0.04	0.11	0.04	0.04	0.1	0.04	0.07
Kidney Weight Gain (TP) [14]	0.04	0.04	0.04	0.04	0.06	0.04	0.06

Liver Cholestasis (TP) [15]	0.15	0.2	0.15	0.5	0.52	0.5	0.43
Liver Lipid Accumulation (TP) [16]	0.15	0.16	0.15	0.13	0.13	0.13	0.13
Liver Necrosis (TP) [17]	0.18	0.16	0.18	0.15	0.16	0.15	0.16
Liver Weight Gain (TP) [18]	0.18	0.18	0.16	0.2	0.22	0.2	0.18
MRTD (TP) [19]	0.06	0.16	0.06	0.0	0.08	0.08	0.05
Nasal pathology (TP) [20]	0.19	0.23	0.19	0.18	0.31	0.18	0.35
Nephron Injury (TP) [21]	0.17	0.1	0.17	0.21	0.21	0.21	0.23
Nephrotoxicity (TP) [22]	0.07	0.08	0.07	0.1	0.08	0.1	0.06
Neurotoxicity (TP) [23]	0.23	0.38	0.27	0.3	0.39	0.3	0.35
Pulmonary toxicity (TP) [24]	0.07	0.08	0.07	0.07	0.08	0.07	0.14
SkinSens, EC3 (TP) [25]	62.11	66.84	52.7	45.62	44.24	45.98	44.71
Testicular toxicity (TP) [26]	0.11	0.09	0.11	0.13	0.13	0.13	0.17

1. Potential to be mutagenic (AMES positive), range from 0 to 1. A value of 1 is AMES positive (mutagenic), and a value of 0 is AMES negative (non-mutagenic). Cutoff is 0.5. Values close to zero are preferable. The AMES assay is based upon the reversion of mutations in the histidine operon in the bacterium Salmonella enterica sv Typhimurium.

2. Potential for causing anemia. Cutoff is 0.5. Values higher than 0.5 indicate potentially toxic compounds. Training set consists of chemicals and drugs causing anemia in vivo. Model organisms: human. Model description: Training set N=324, Test set N=51, Sensitivity= 0.82, Specificity=0.90, Accuracy=0.86, MCC=0.72.

3. Potential for inducing carcinogenicity in rats and mice. Cutoff is 0.5. Values higher than 0.5 indicate potentially toxic compounds. Training set consists of chemicals and drugs causing carcinogenicity in vivo. Model organisms: mouse, rat. Model description: Training set N=1210, Test set N=185, Sensitivity= 0.96, Specificity=0.90, Accuracy=0.93, MCC=0.86.

4. Potential for inducing carcinogenicity in female mice. Cutoff is 0.5. Values higher than 0.5 indicate potentially toxic compounds. Training set consists of chemicals and drugs causing carcinogenicity in vivo. Model organisms: female mice. Model description: Training set N=640, Test set N=94, Sensitivity= 0.90, Specificity=0.93, Accuracy=0.92, MCC=0.83.

5. Potential for inducing carcinogenicity in male mice. Cutoff is 0.5. Values higher than 0.5 indicate potentially toxic compounds. Training set consists of chemicals and drugs causing carcinogenicity in vivo. Model organisms: mouse male. Model description: Training set N=584, Test set N=93, Sensitivity= 0.91, Specificity=0.88, Accuracy=0.89, MCC=0.78.

6. Potential for inducing carcinogenicity in female rats. Cutoff is 0.5. Values higher than 0.5 indicate potentially toxic compounds. Training set consists of chemicals and drugs causing carcinogenicity in vivo. Model organisms: female rat. Model description: Training set N=667, Test set N=120, Sensitivity= 0.90, Specificity=0.96, Accuracy=0.93, MCC=0.86.

7. Potential for inducing carcinogenicity in male rats. Cutoff is 0.5. Values higher than 0.5 indicate potentially toxic compounds. Training set consists of chemicals and drugs causing carcinogenicity in vivo. Model organisms: male rat. Model description: Training set N=715, Test set N=117, Sensitivity= 0.92, Specificity=0.88, Accuracy=0.90, MCC=0.79.

8. Potential for inducing cardiotoxicity. Cutoff is 0.5. Values higher than 0.5 indicate potentially toxic compounds. Training set consists of chemicals and drugs causing cardiotoxicity in vivo. Model organisms: mouse, rat, human. Model description: Training set N=143, Test set N=30, Sensitivity= 0.80, Specificity=1.00, Accuracy=0.90, MCC=0.82.

9. Growth inhibition of MCF7 cell line (human caucasian breast adenocarcinoma), pGI50. Cutoff is 6. Values from 6 to 8 correspond to a toxic metabolite, values less than 6 are preferable, values less than 3 are more preferable and less toxic. Model description: N=1474, R2=0.9, RMSE=0.05.

10. Potential for inducing epididymis toxicity. Training set consists of chemicals and drugs causing epididymis toxicity in vivo. Model organisms: mouse, rat, human. Cutoff is 0.5. Values higher than 0.5 indicate potentially toxic compounds. Model description: Training set N=252, Test set N=42, Sensitivity= 0.90, Specificity=0.86, Accuracy=0.88, MCC=0.76.

11. Potential for inducing genotoxicity. Cutoff is 0.5. Values higher than 0.5 indicate potentially toxic compounds. Training set consists of chemicals and drugs causing genotoxicity in vivo. Model organisms: mouse, rat. Model description: Training set N=372, Test set N=86, Sensitivity= 0.75, Specificity=0.84, Accuracy=0.79, MCC=0.59.

12. Potential for inducing hepatotoxicity. Cutoff is 0.5. Values higher than 0.5 indicate potentially toxic compounds. Training set consists of chemicals and drugs causing hepatotoxicity in vivo. Model organisms: mouse, rat, human. Model description: Training set N=1380, Test set N=231, Sensitivity= 0.73, Specificity=0.88, Accuracy=0.81, MCC=0.62.

13. Potential for inducing kidney necrosis. Cutoff is 0.5. Values higher than 0.5 indicate potentially toxic compounds. Training set consists of chemicals and drugs causing renal necrosis in vivo. Model organisms: mouse, rat, human. Model description: Training set N=221, Test set N=42, Sensitivity= 0.96, Specificity=1.00, Accuracy=0.98, MCC=0.95.

14. Potential for inducing kidney weight gain. Cutoff is 0.5. The values higher than 0.5 indicate potentially toxic compounds. Training set consists of chemicals and drugs causing kidney weight gain in vivo. Model organisms: mouse, rat. Model description: Training set N=240, Test set N=49, Sensitivity= 0.95, Specificity=1.00, Accuracy=0.98, MCC=0.96.

15. Potential for inducing liver cholestasis. Cutoff is 0.5. Values higher than 0.5 indicate potentially toxic compounds. Training set consists of chemicals and drugs causing cholestasis in vivo. Model organisms: mouse, rat, human. Model description: Training set N=218, Test set N=35, Sensitivity= 0.79, Specificity=0.67, Accuracy=0.74, MCC=0.46.

16. Potential for inducing liver lipid accumulation. Cutoff is 0.5. Values higher than 0.5 indicate potentially toxic compounds. Training set consists of chemicals and drugs causing lipid accumulation in vivo. Model organisms: mouse, rat, human. Model description: Training set N=172, Test set N=28, Sensitivity= 0.80, Specificity=0.85, Accuracy=0.82, MCC=0.64.

17. Potential for inducing liver necrosis. Cutoff is 0.5. Values higher than 0.5 indicate potentially toxic compounds. Training set consists of chemicals and drugs causing hepatic necrosis in vivo. Model organisms: mouse, rat, human. Model description: Training set N=300, Test set N=57, Sensitivity= 0.91, Specificity=0.91, Accuracy=0.91, MCC=0.82.

18. Potential for inducing liver weight gain. Cutoff is 0.5. Values higher than 0.5 indicate potential liver weight-changing compounds. Training set consists of chemicals and drugs causing liver weight gain in vivo. Model organisms: mouse, rat. Model description: Training set N=292, Test set N=52, Sensitivity= 1.00, Specificity=1.00, Accuracy=1.00, MCC=1.00.

19. Maximum Recommended Therapeutic Dose, log mg/kg-bm/day, range is from -5 to 3. Cutoff is 0.5. Chemicals with high log MRTDs can be classified as mildly toxic compounds, chemicals with low log MRTDs as highly toxic compounds. Model description: N=1209, R2= 0.86, RMSE=0.42.

20. Potential for causing nasal pathology. Training set consists of chemicals and drugs causing nasal pathology in vivo. Model organisms: mouse, rat, human. Cutoff is 0.5. Values higher than 0.5 indicate potentially toxic compounds. Model description: Training set N=246, Test set N=47, Sensitivity= 1.00, Specificity=0.93, Accuracy=0.96, MCC=0.92.

21. Potential for inducing nephron injury. Cutoff is 0.5. Values higher than 0.5 indicate potentially toxic compounds. Training set consists of chemicals and drugs causing nephron injury in vivo. Model organisms: mouse, rat, human. Model description: Training set N=598, Test set N=109, Sensitivity= 0.91, Specificity=1.00, Accuracy=0.96, MCC=0.93.

22. Potential for inducing nephrotoxicity. Cutoff is 0.5. Values higher than 0.5 indicate potentially toxic compounds. Training set consists of chemicals and drugs causing nephrotoxicity in vivo. Model organisms: mouse, rat, human. Model description: Training set N=847, Test set N=154, Sensitivity= 0.90, Specificity=0.84, Accuracy=0.87, MCC=0.74.

23. Potential for inducing neurotoxicity. Training set consists of chemicals and drugs causing neurotoxicity in vivo. Model organisms: mouse, rat, human. Cutoff is 0.5. Values higher than 0.5 indicate potentially toxic compounds. Model description: Training set N=175, Test set N=34, Sensitivity= 0.94, Specificity=0.94, Accuracy=0.94, MCC=0.88.

24. Potential for inducing pulmonary toxicity. Training set consists of chemicals and drugs causing pulmonary toxicity in vivo. Model organisms: mouse, rat, human. Cutoff is 0.5. Values higher than 0.5 indicate potentially toxic compounds. Model description: Training set N=482, Test set N=87, Sensitivity= 0.89, Specificity=0.88, Accuracy=0.89, MCC=0.77.

25. Skin sensitization potential expressed as effective concentration 3, EC3 %. Values higher than 10 indicate weak and moderate sensitizers. Model description: N=89, R2=0.67, RMSE=22.56.

26. It consists of chemicals and drugs causing testicular toxicity in vivo. Model organisms: mouse, rat, human. Cutoff is 0.5. Values higher than 0.5 indicate potentially toxic compounds. Model description: Training set N=439, Test set N=88, Sensitivity= 0.81, Specificity=0.85, Accuracy=0.83, MCC=0.66. a. Potential activity against cancer. Cutoff is 0.5. Values higher than 0.5 indicate potentially active compounds. Training set consists of approved drugs. Model description: Training set N=886, Test set N=167, Sensitivity= 0.89, Specificity=0.83, Accuracy=0.86, MCC=0.72.

Spectroscopic analysis

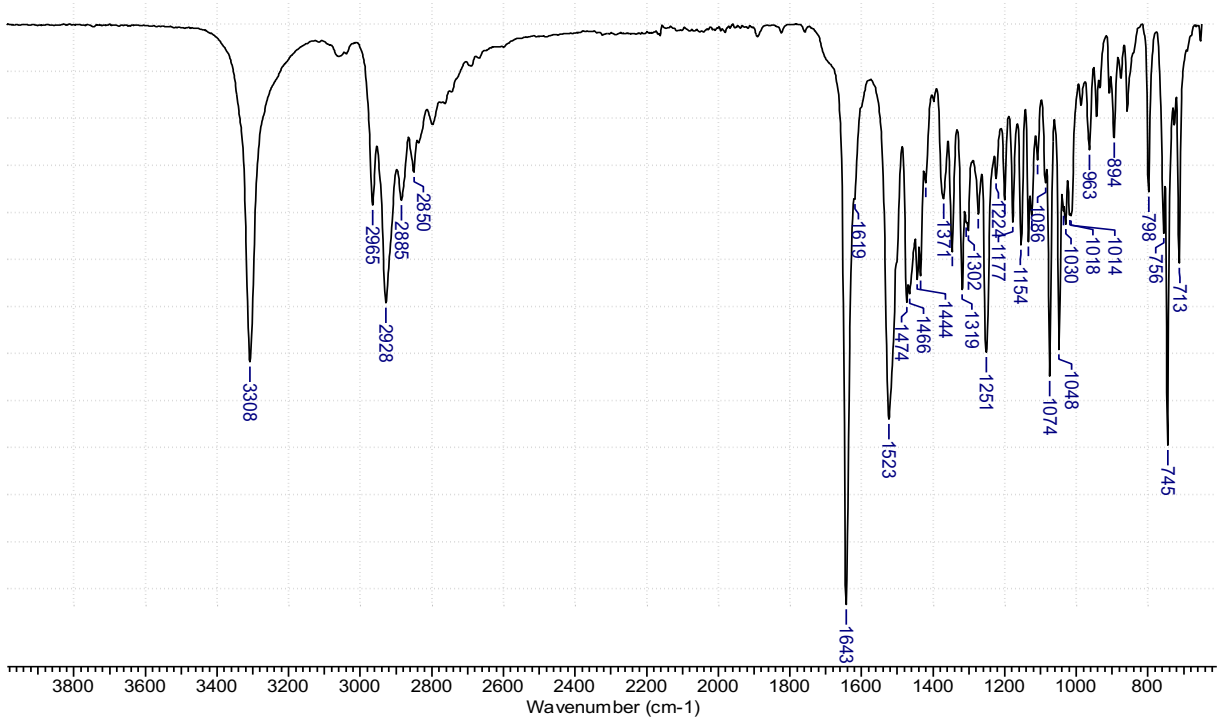


Figure S4. FT-IR spectra of compound **EC1**

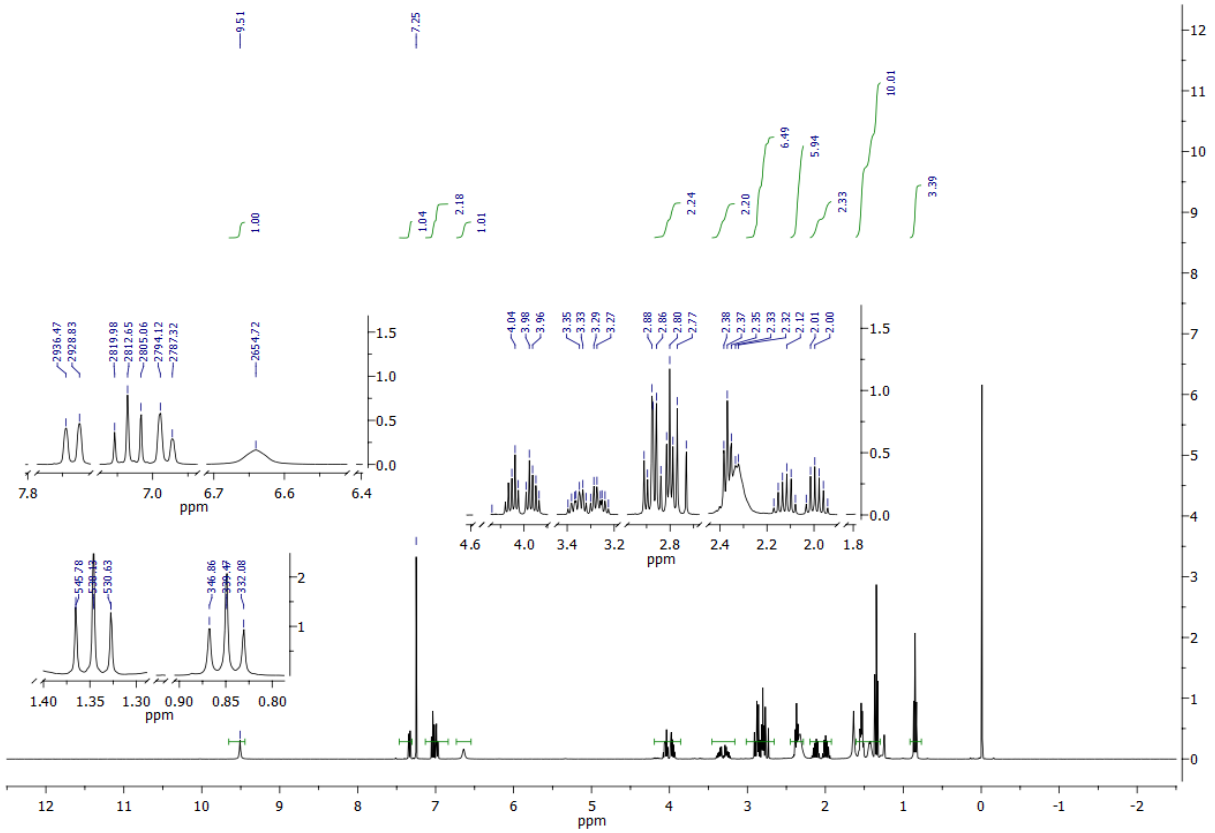


Figure S5. [1]H NMR spectra of compound **EC1**

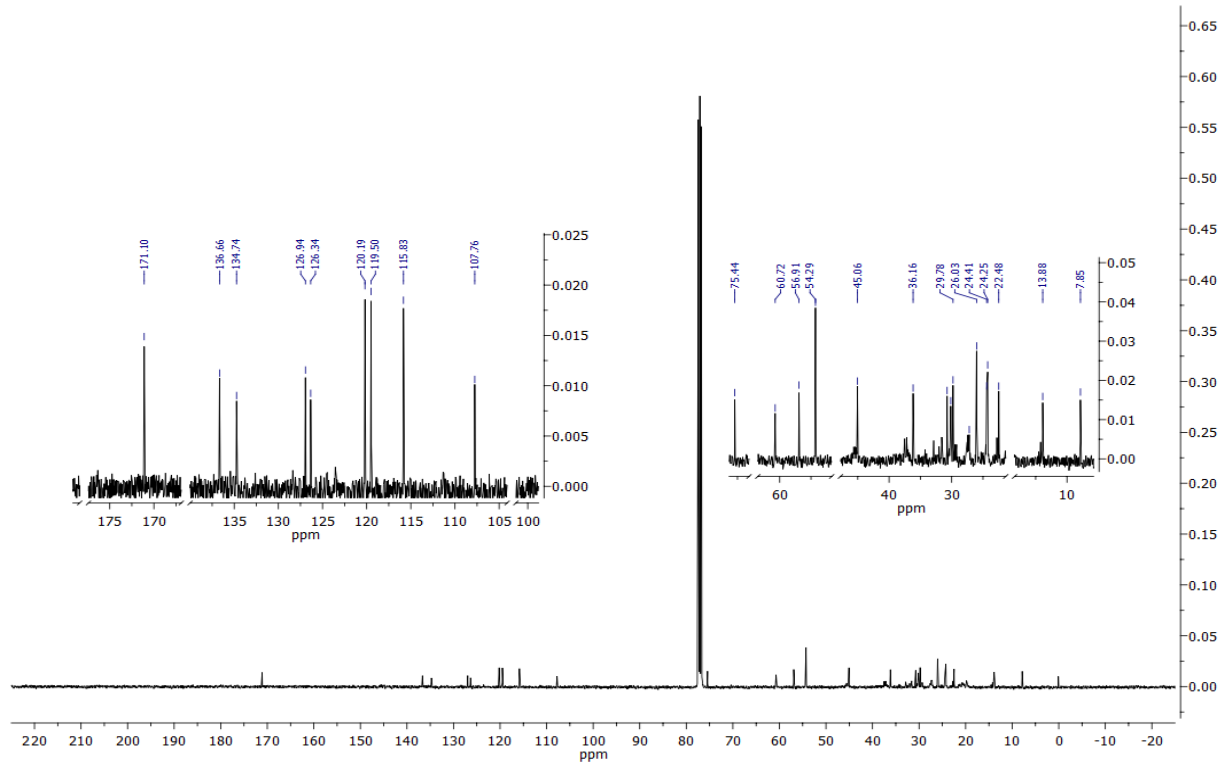


Figure S6. ^{13}C NMR spectra of compound **EC1**

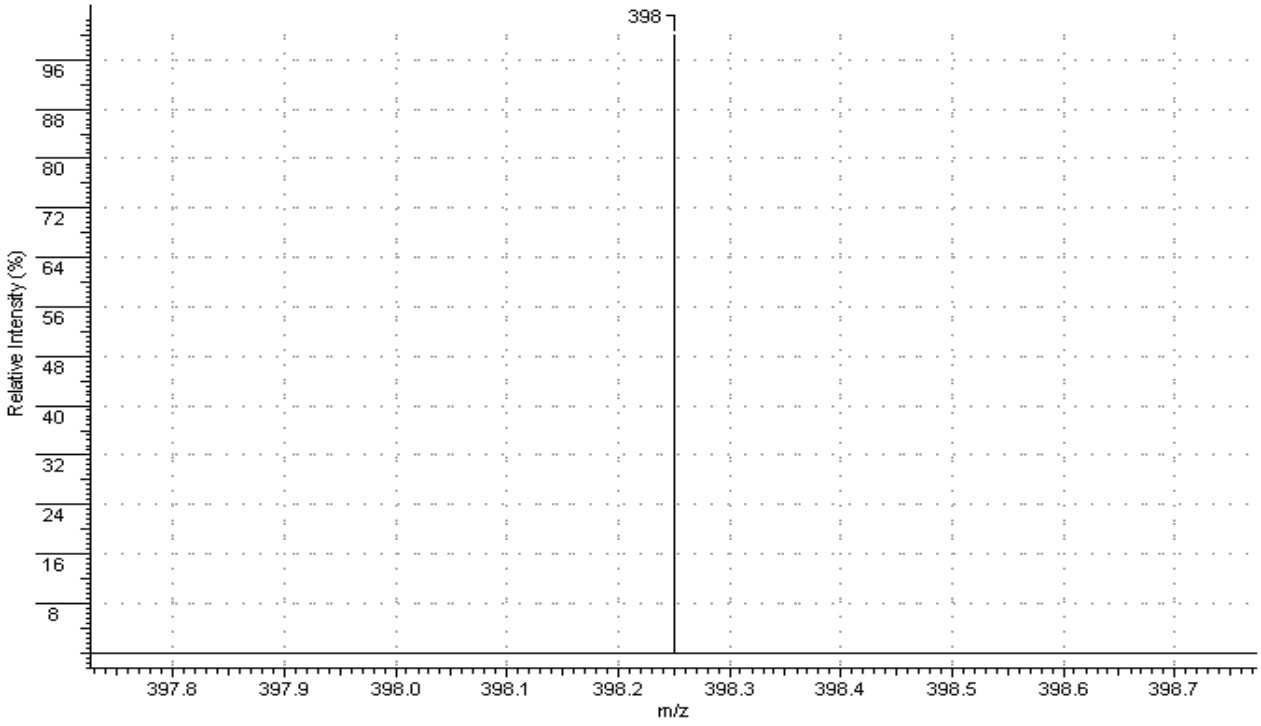


Figure S7. LC-MS/MS spectra of compound **EC1**

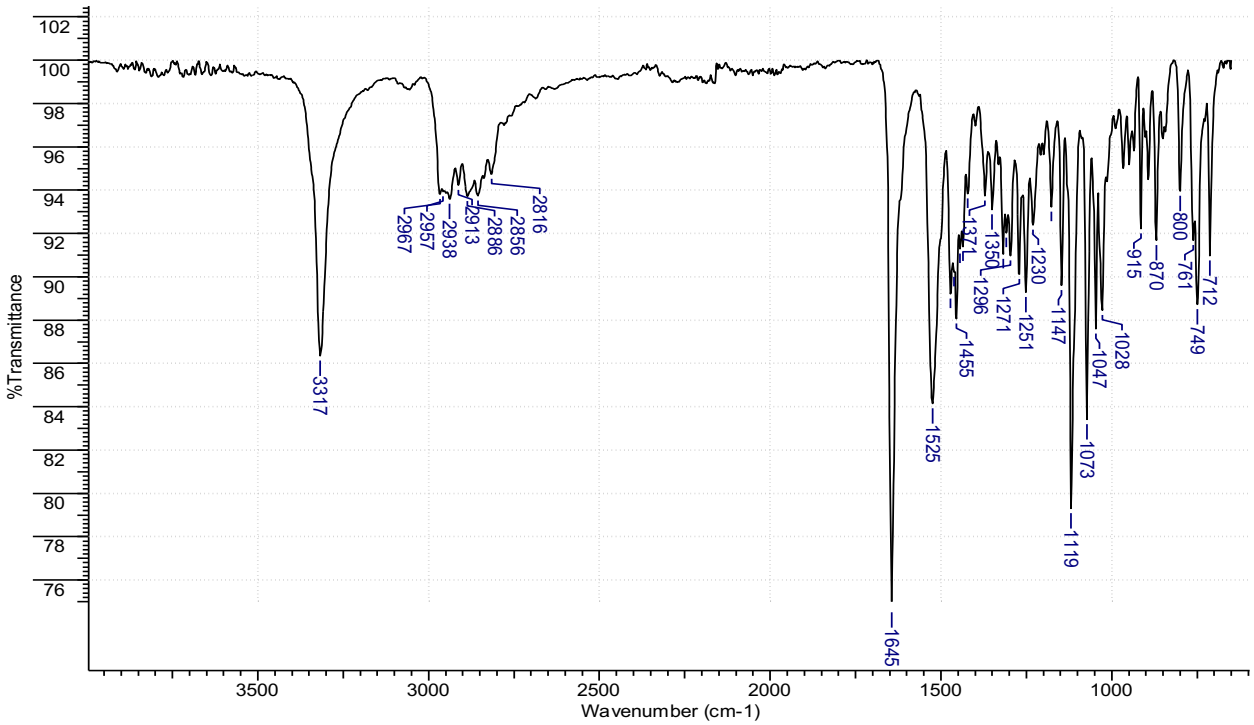


Figure S8. FT-IR spectra of compound **EC2**

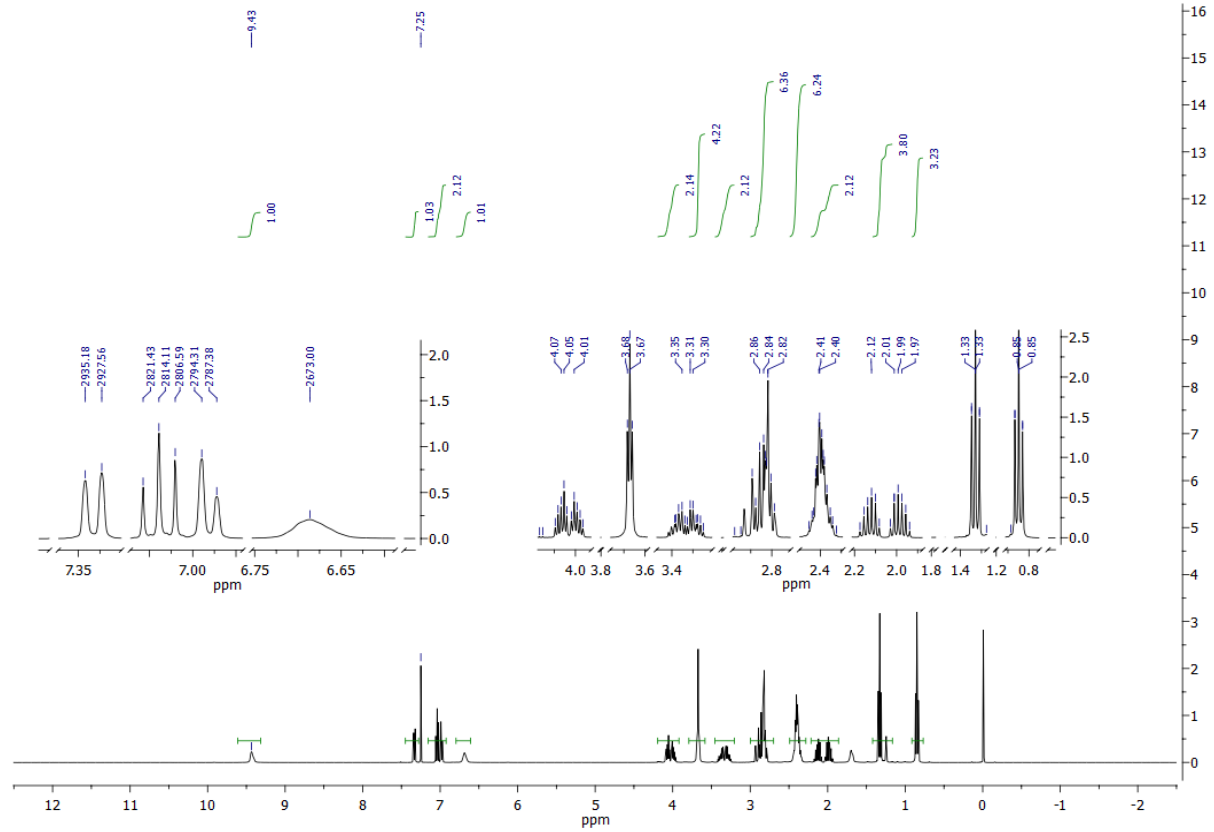


Figure S9. ^{1}H NMR spectra of compound **EC2**

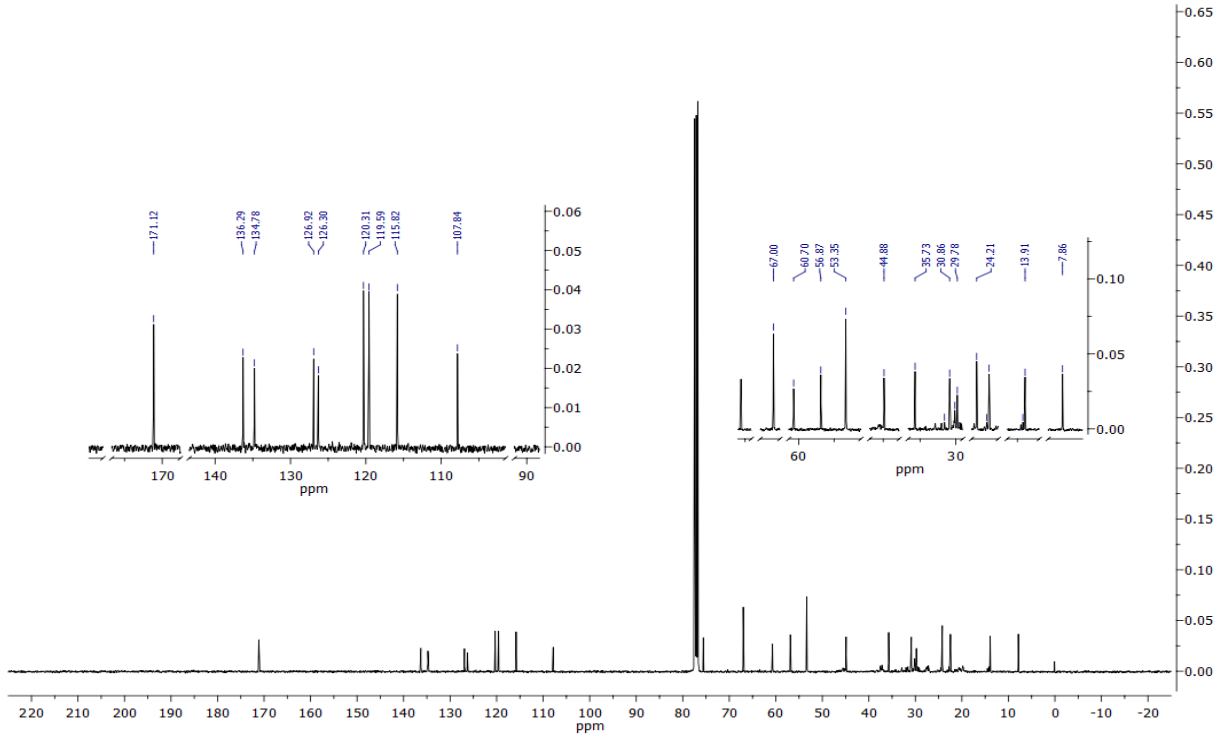


Figure S10. ^{13}C NMR spectra of compound **EC2**

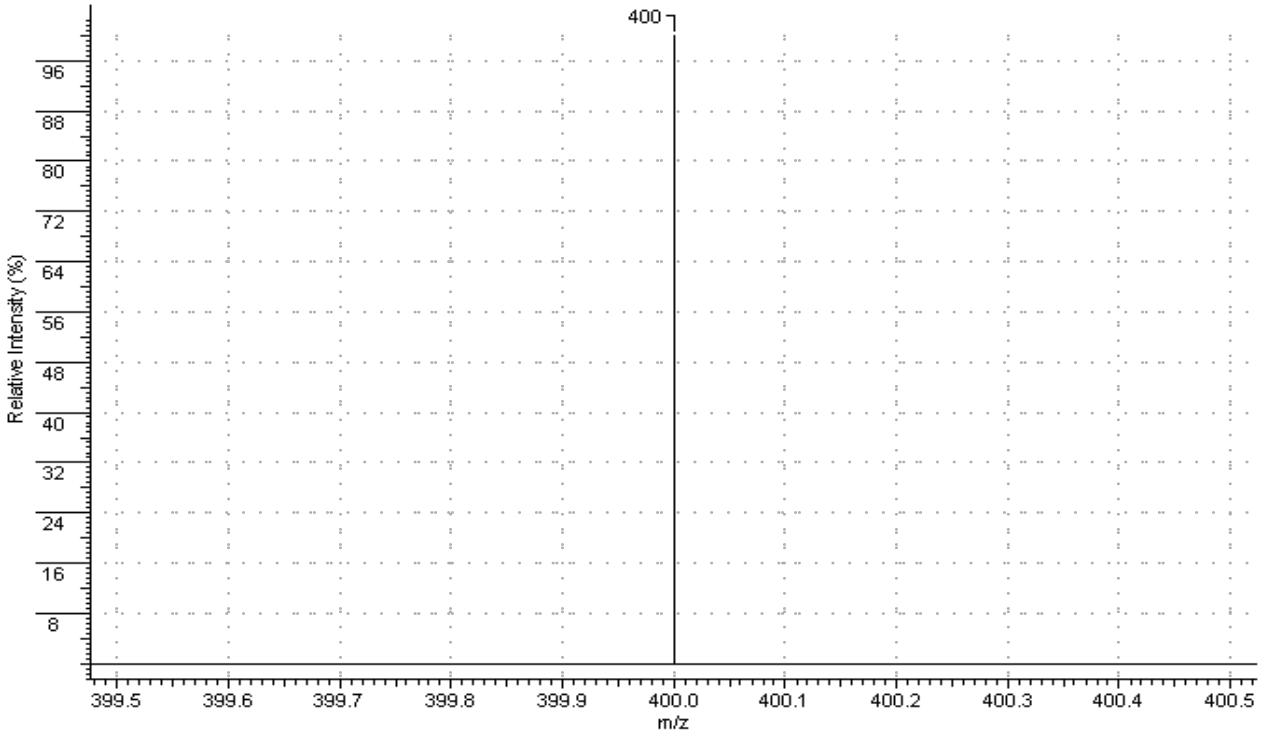


Figure S11. LC-MS/MS spectra of compound **EC2**

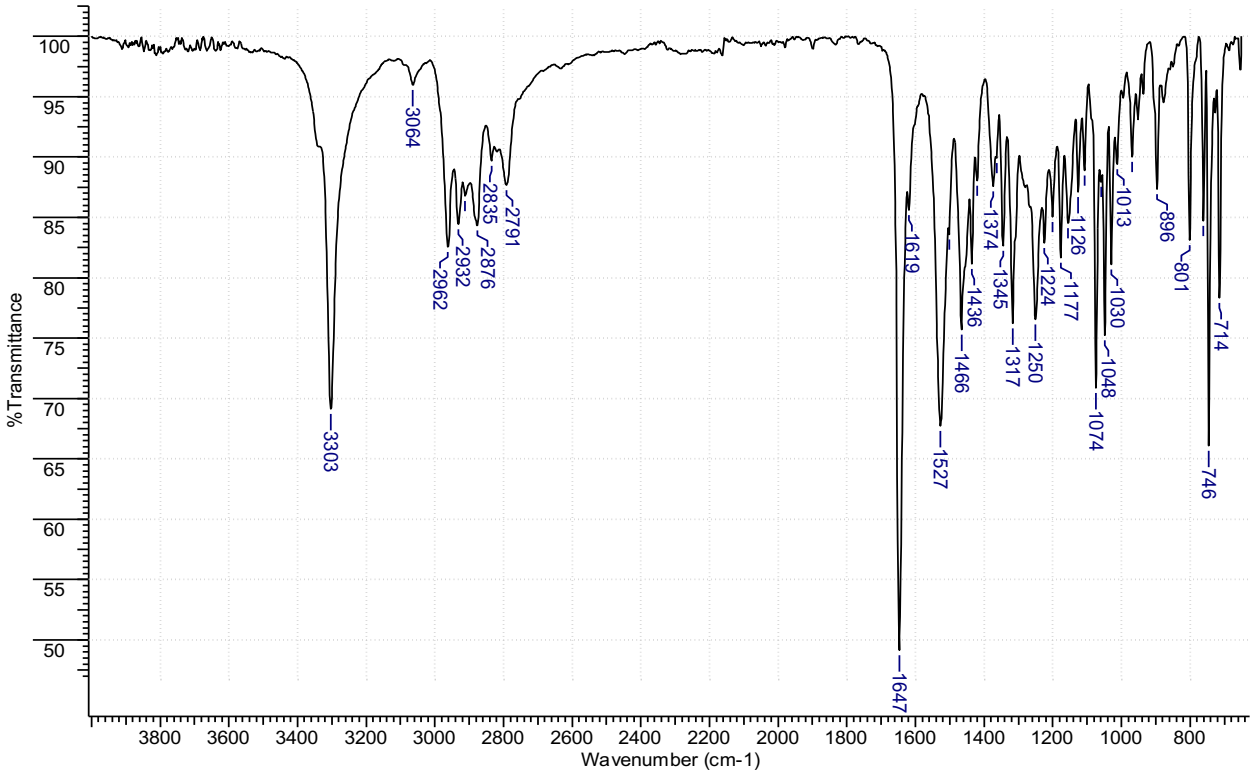


Figure S12. FT-IR spectra of compound **EC3**

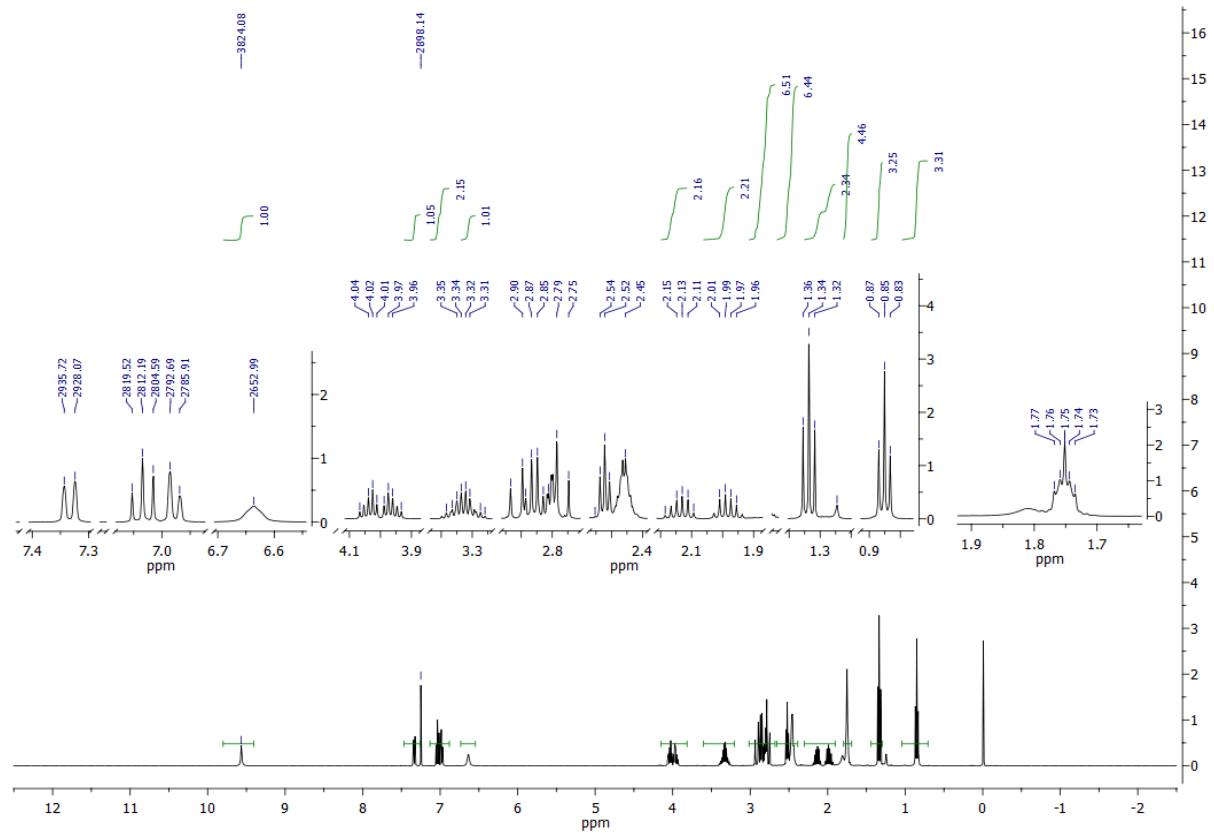


Figure S13. ^{1}H NMR spectra of compound **EC3**

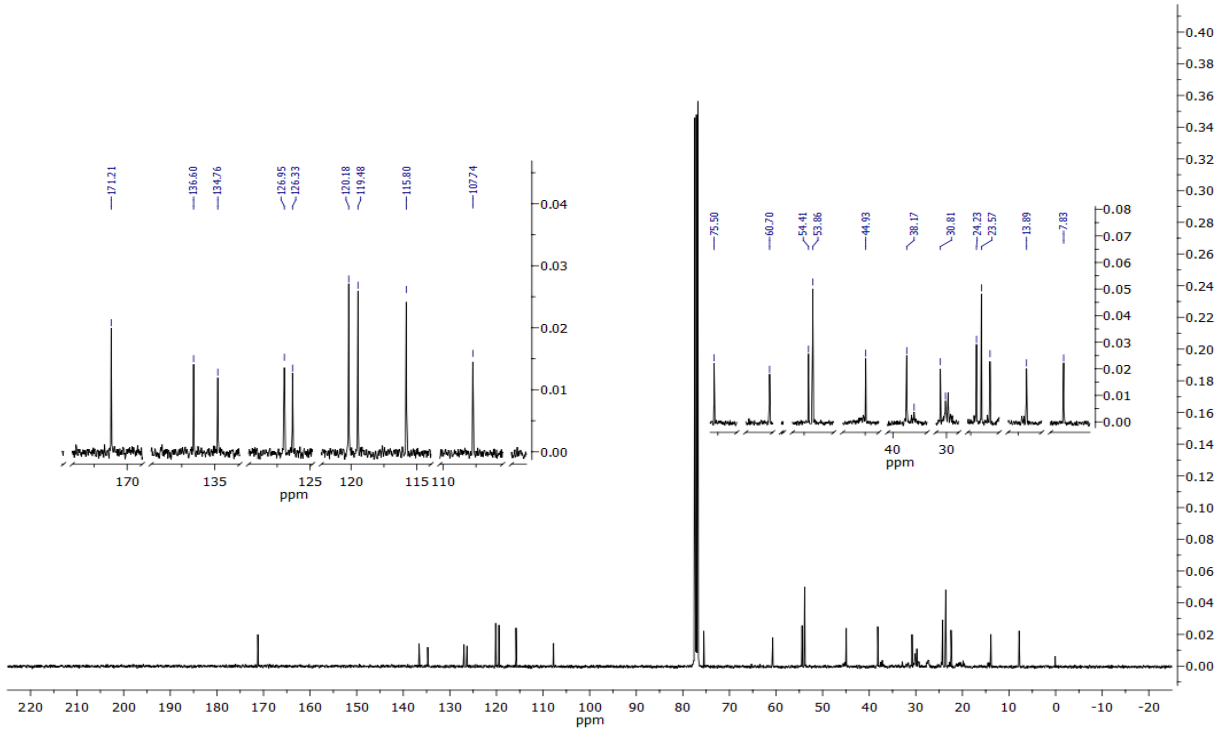


Figure S14. ^{13}C NMR spectra of compound **EC3**

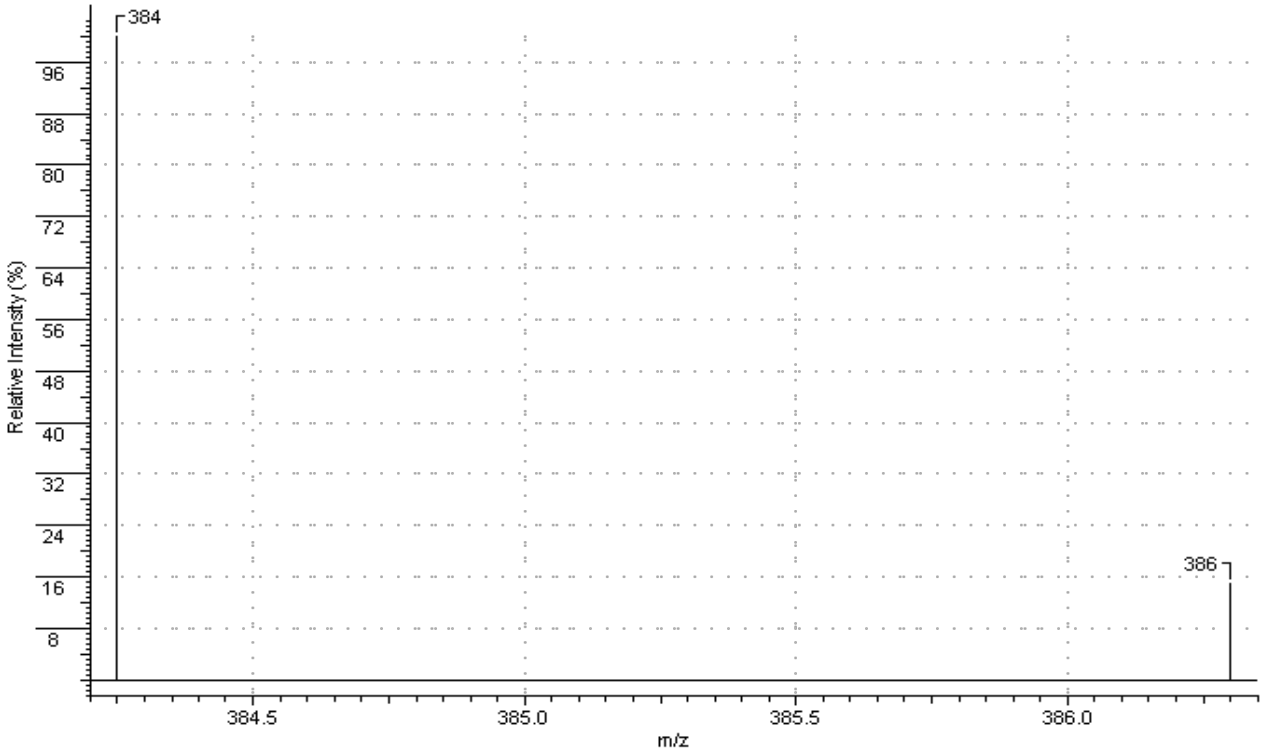


Figure S15. LC-MS/MS spectra of compound **EC3**

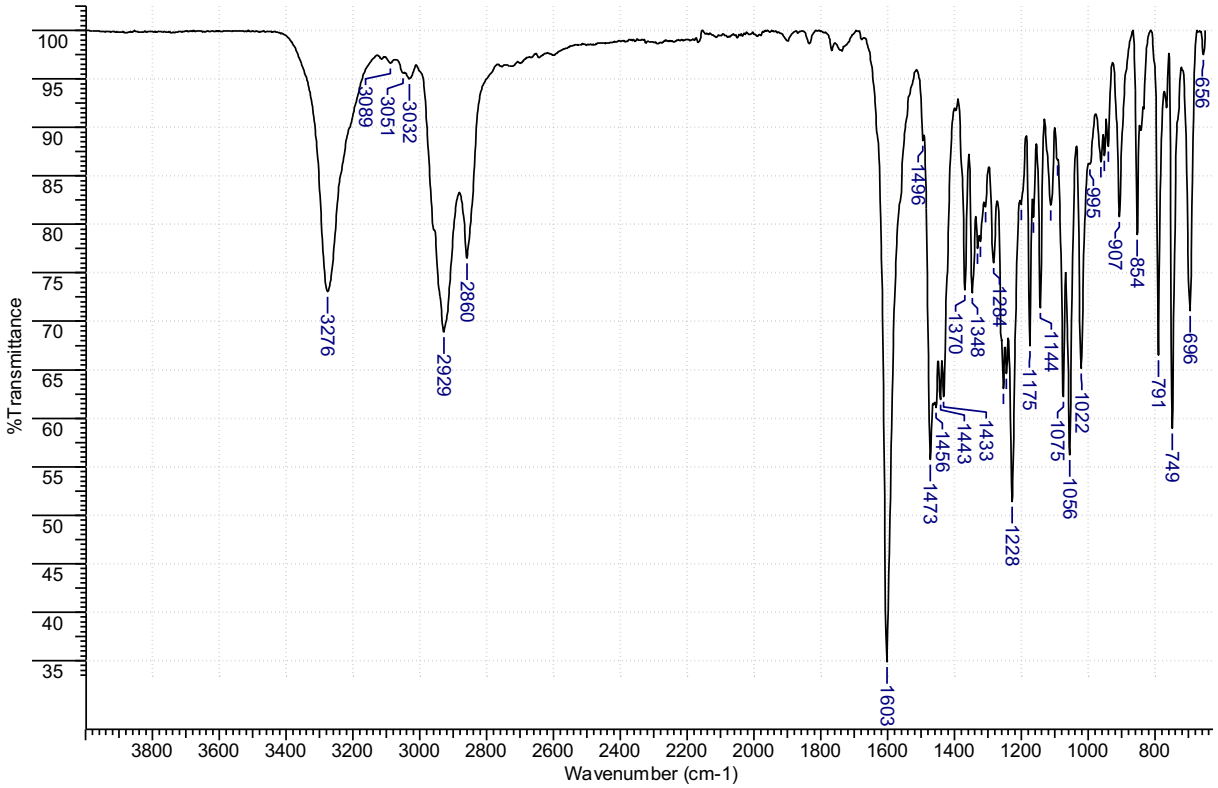


Figure S16. FT-IR spectra of compound **EC4**

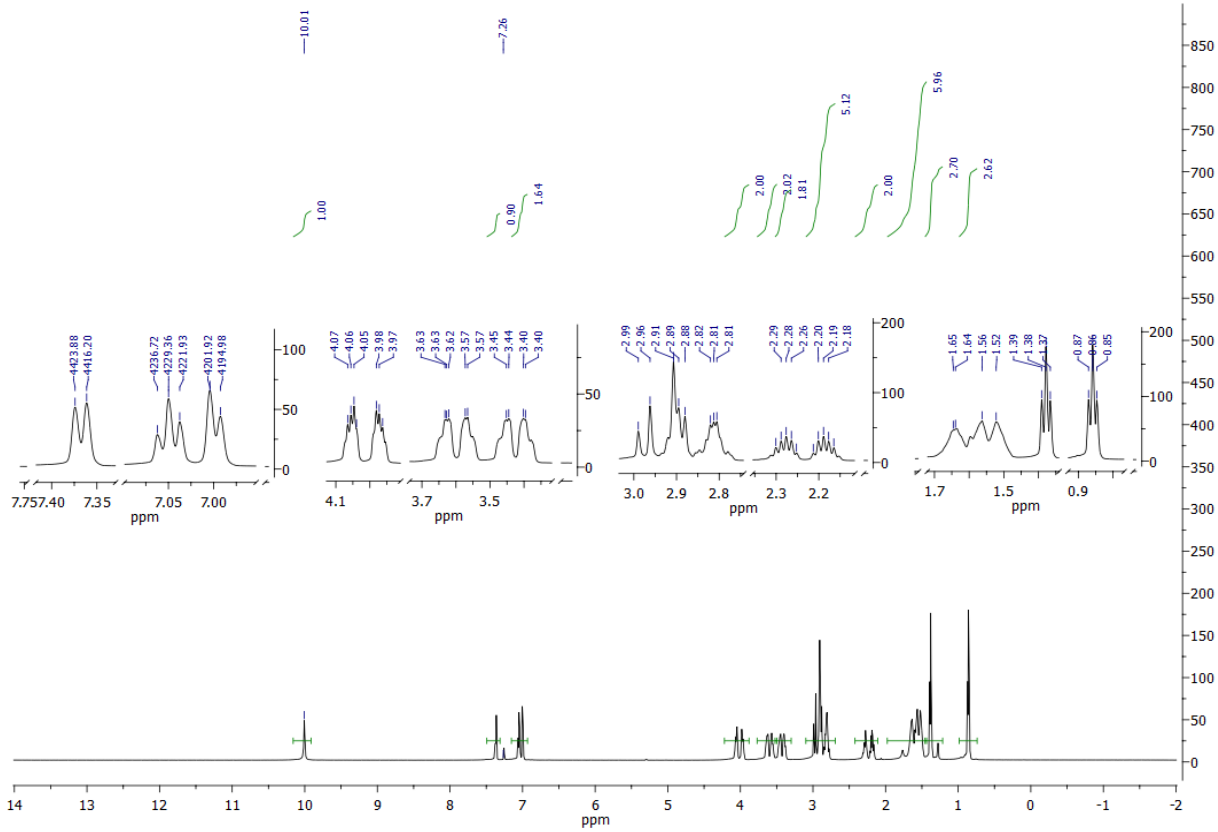


Figure S17. [1]H NMR spectra of compound **EC4**

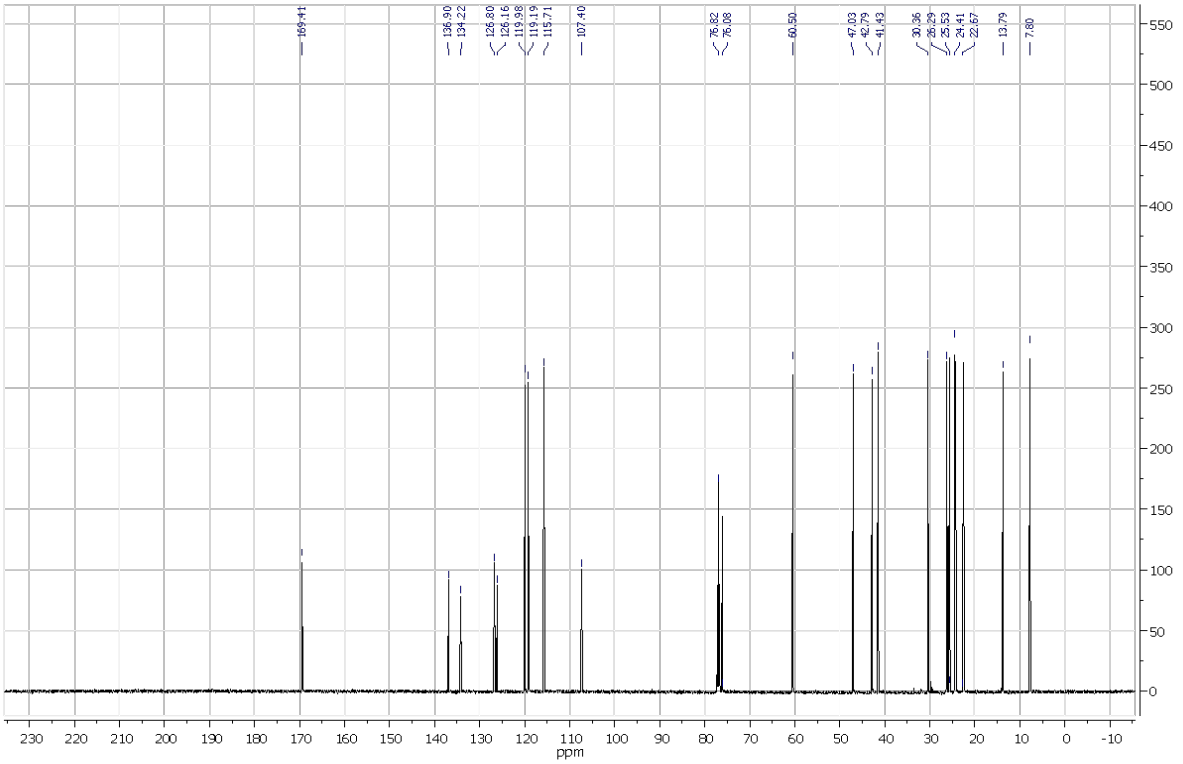


Figure S18. ^{13}C NMR spectra of compound **EC4**

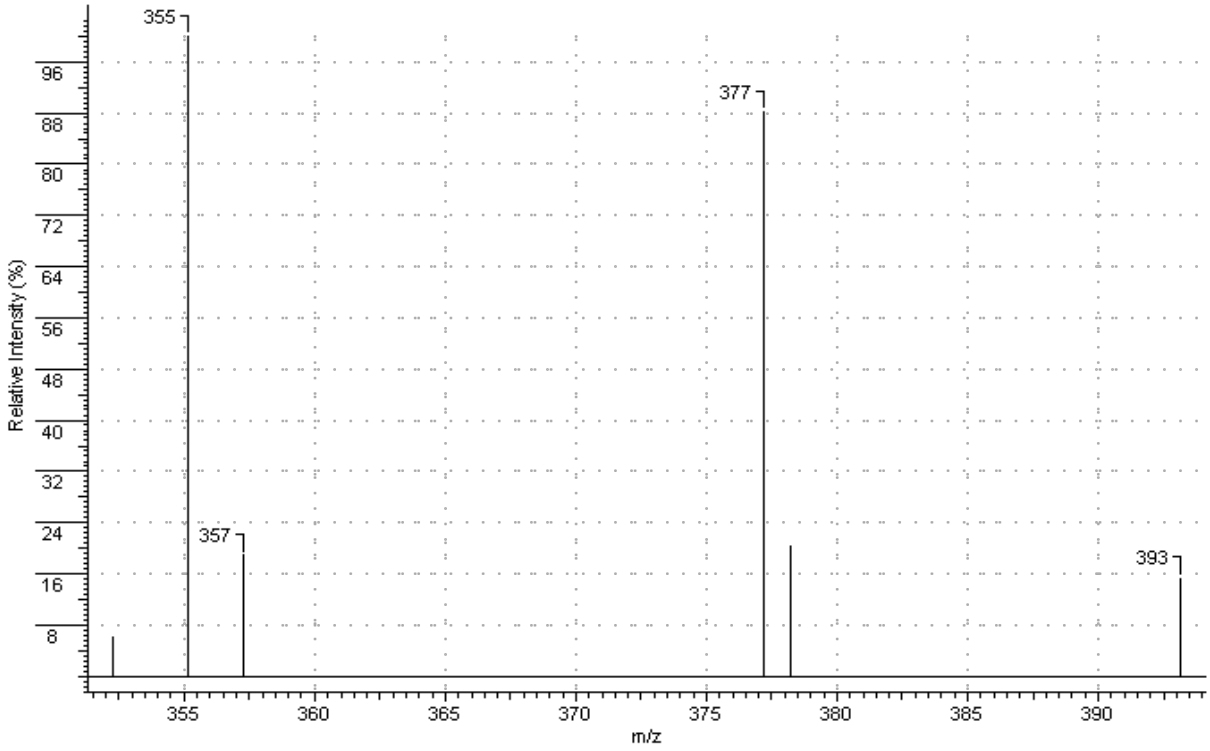


Figure S19. LC-MS/MS spectra of compound **EC4**

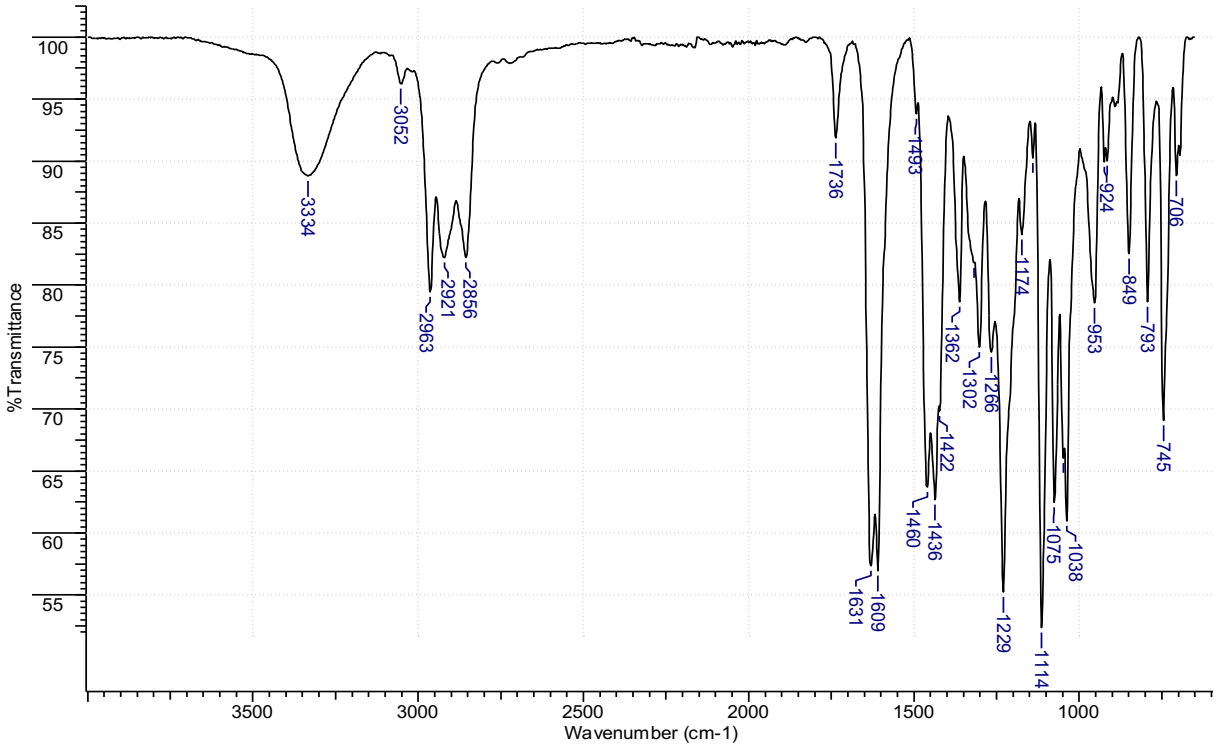


Figure S20. FT-IR spectra of compound **EC5**

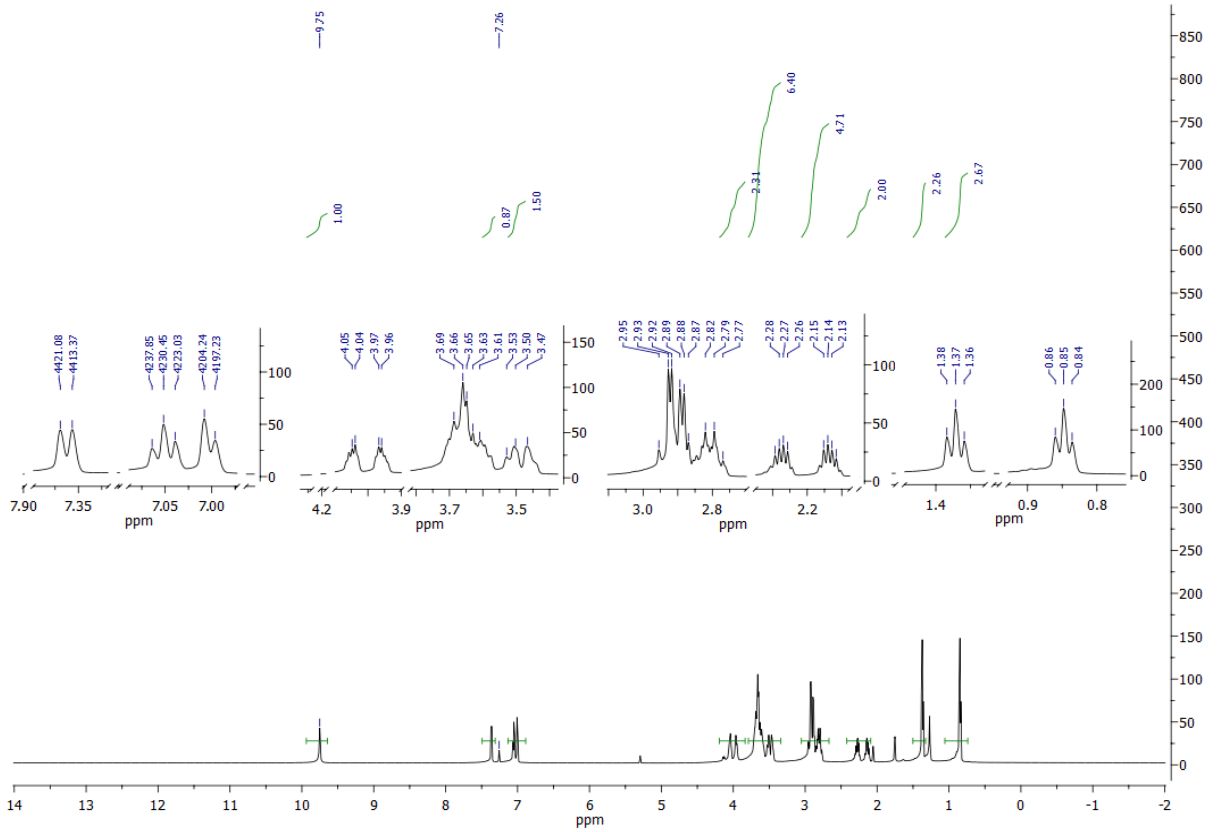


Figure S21. [1]H NMR spectra of compound **EC5**

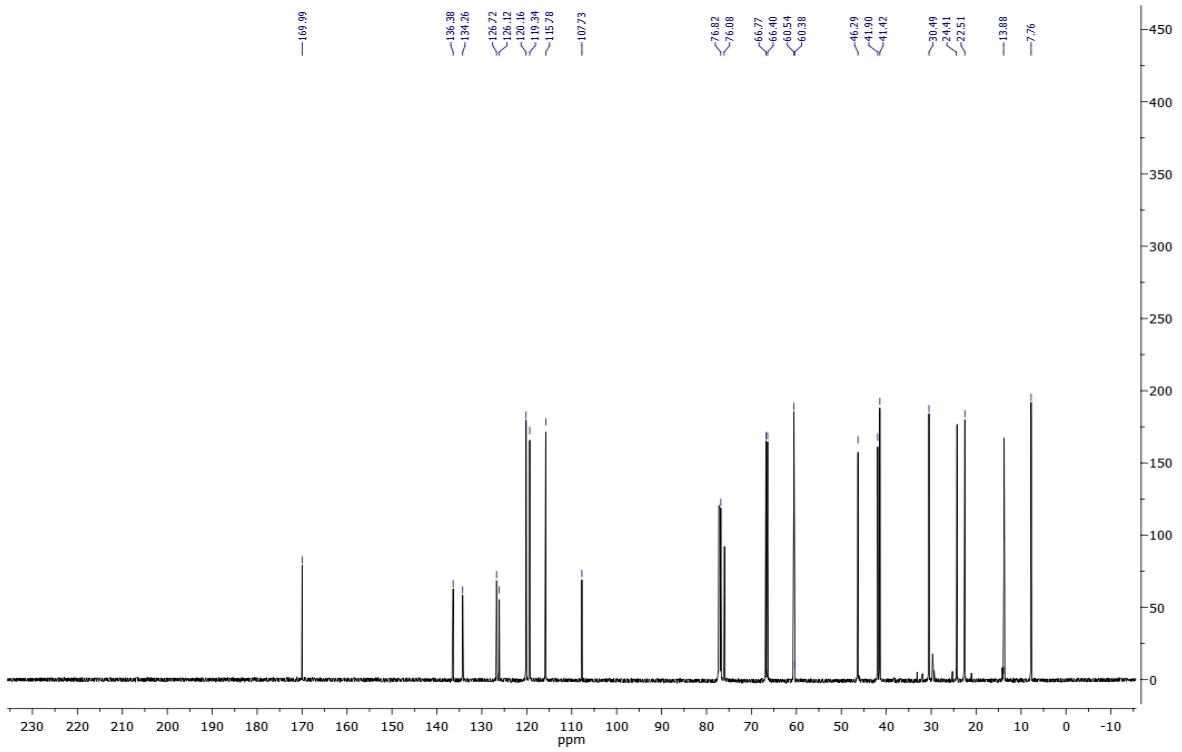


Figure S22. ^{13}C NMR spectra of compound **EC5**

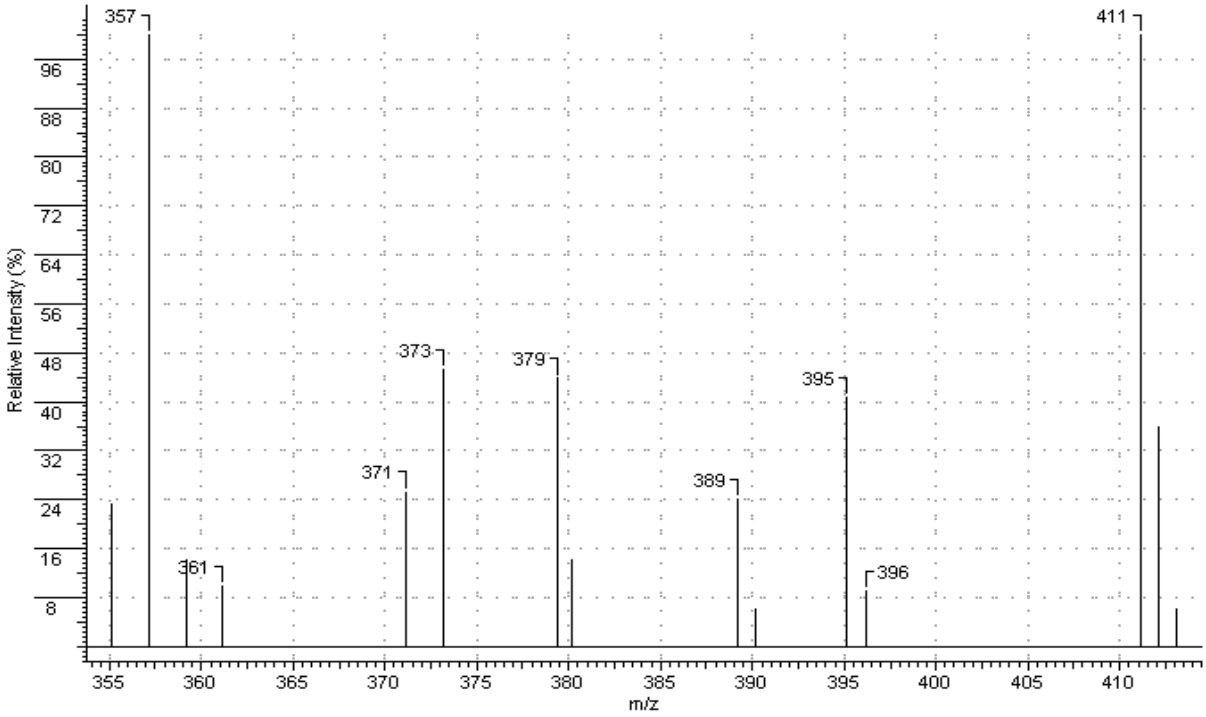


Figure S23. LC-MS/MS spectra of compound **EC5**

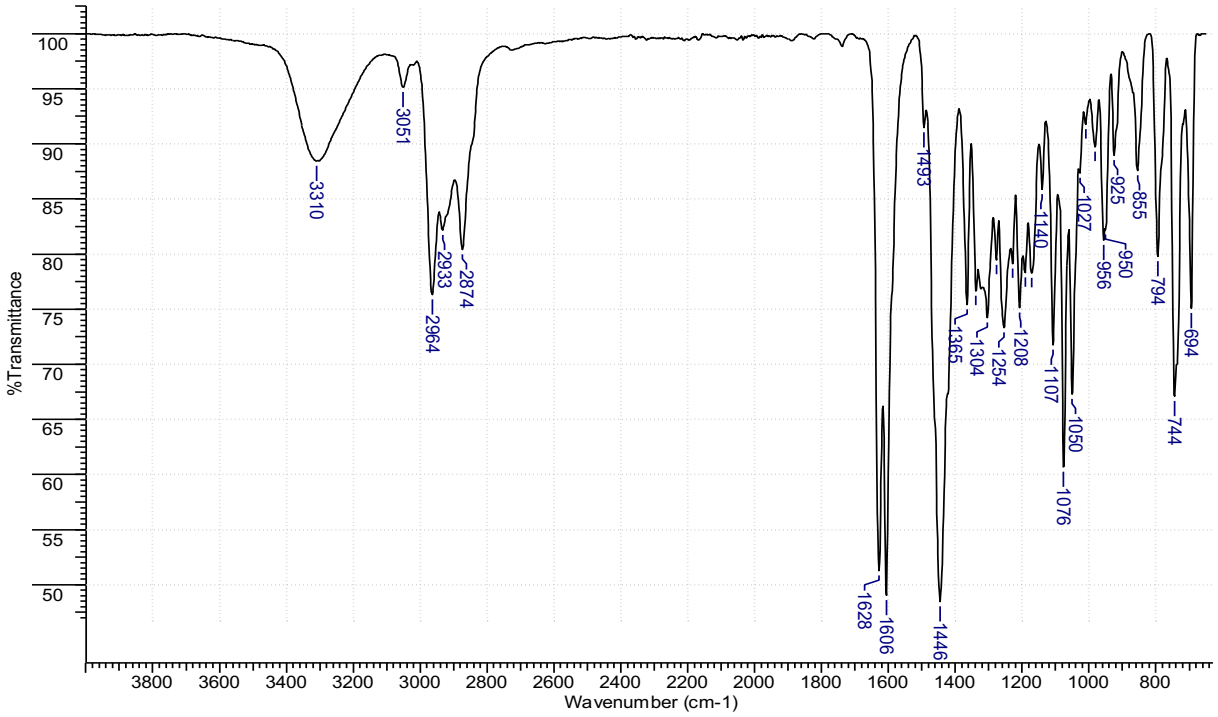


Figure S24. FT-IR spectra of compound **EC6**

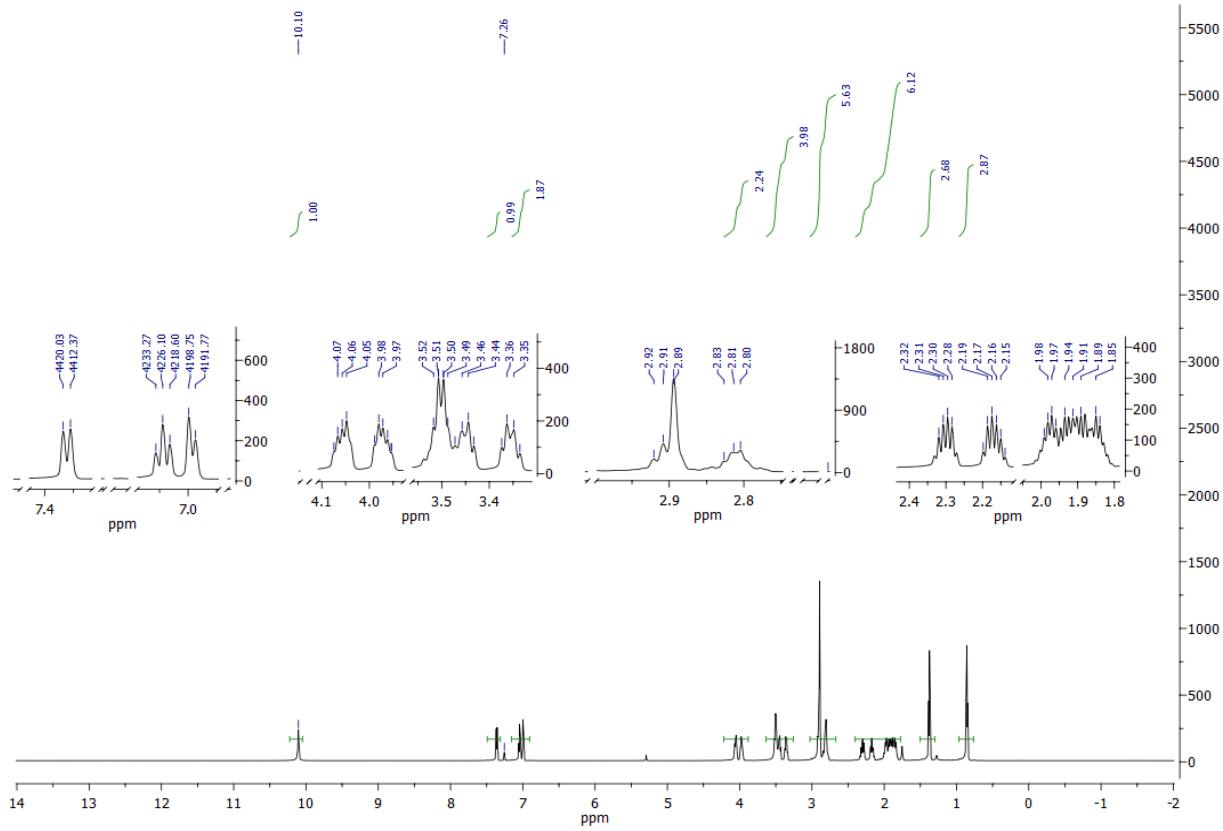


Figure S25. ^{1}H NMR spectra of compound **EC6**

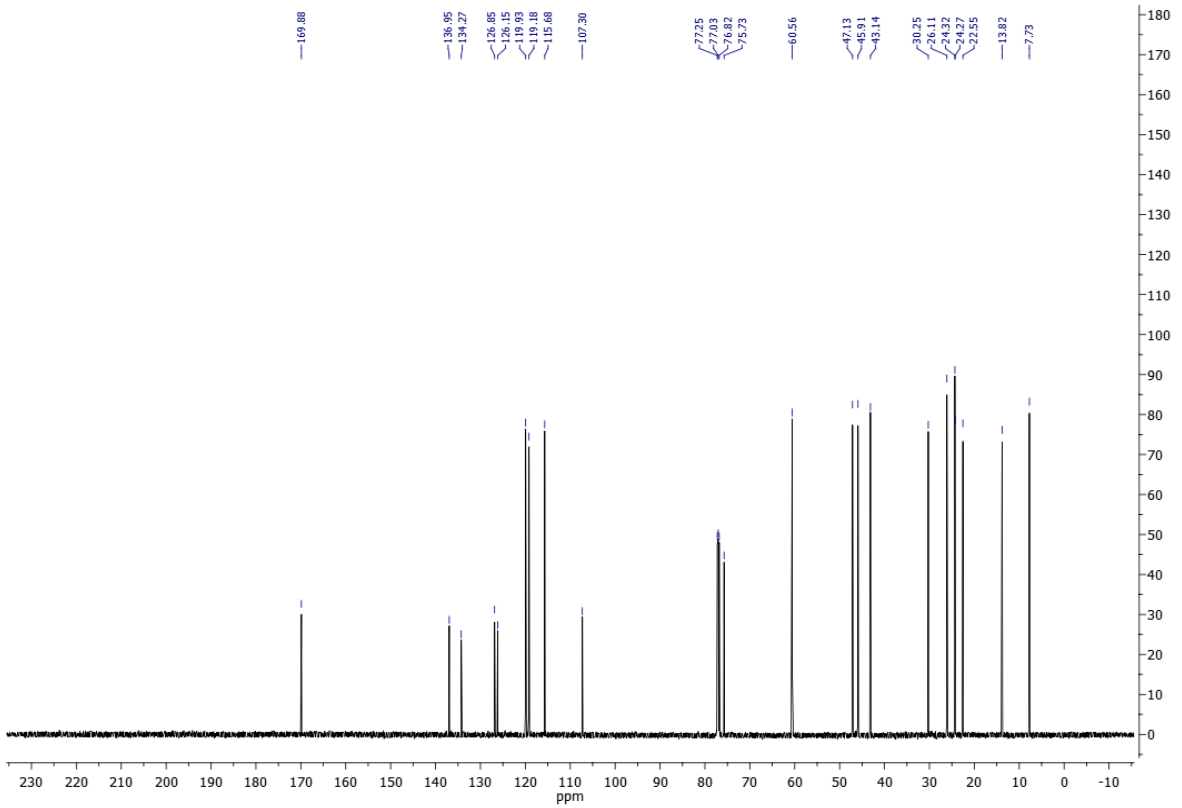


Figure S26. ^{13}C NMR spectra of compound **EC6**

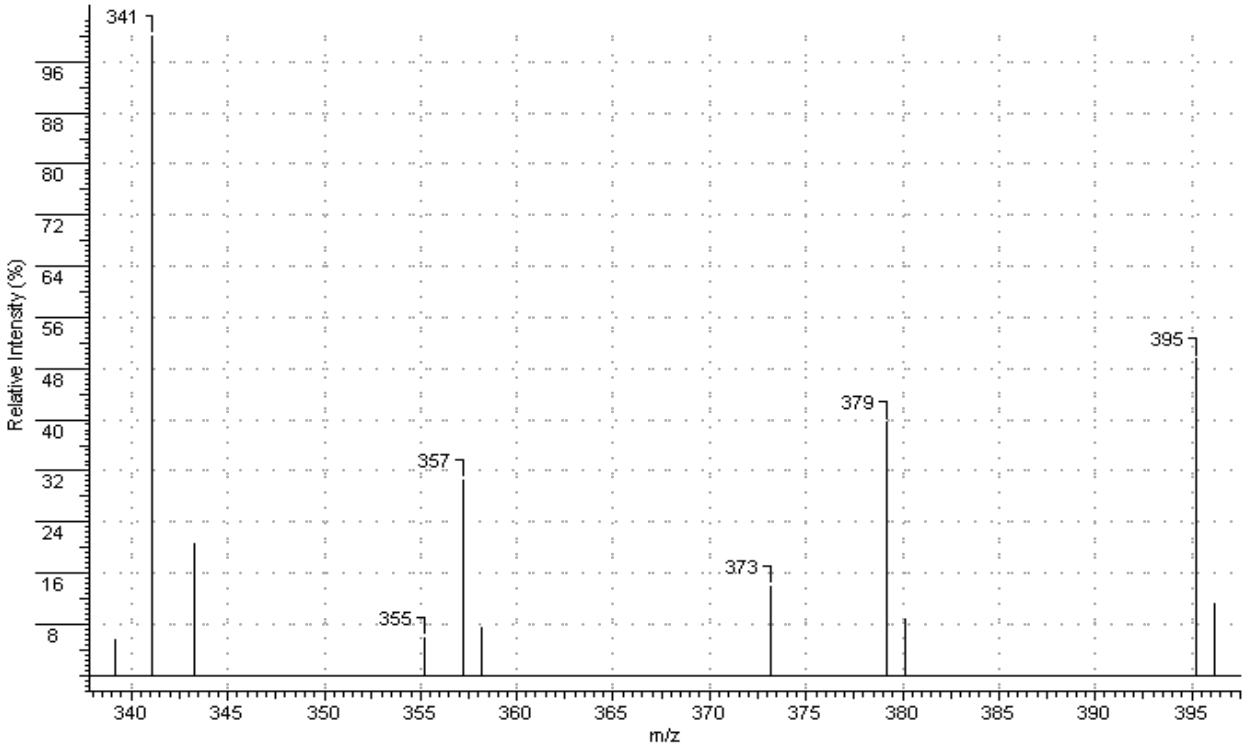


Figure S27. LC-MS/MS spectra of compound **EC6**

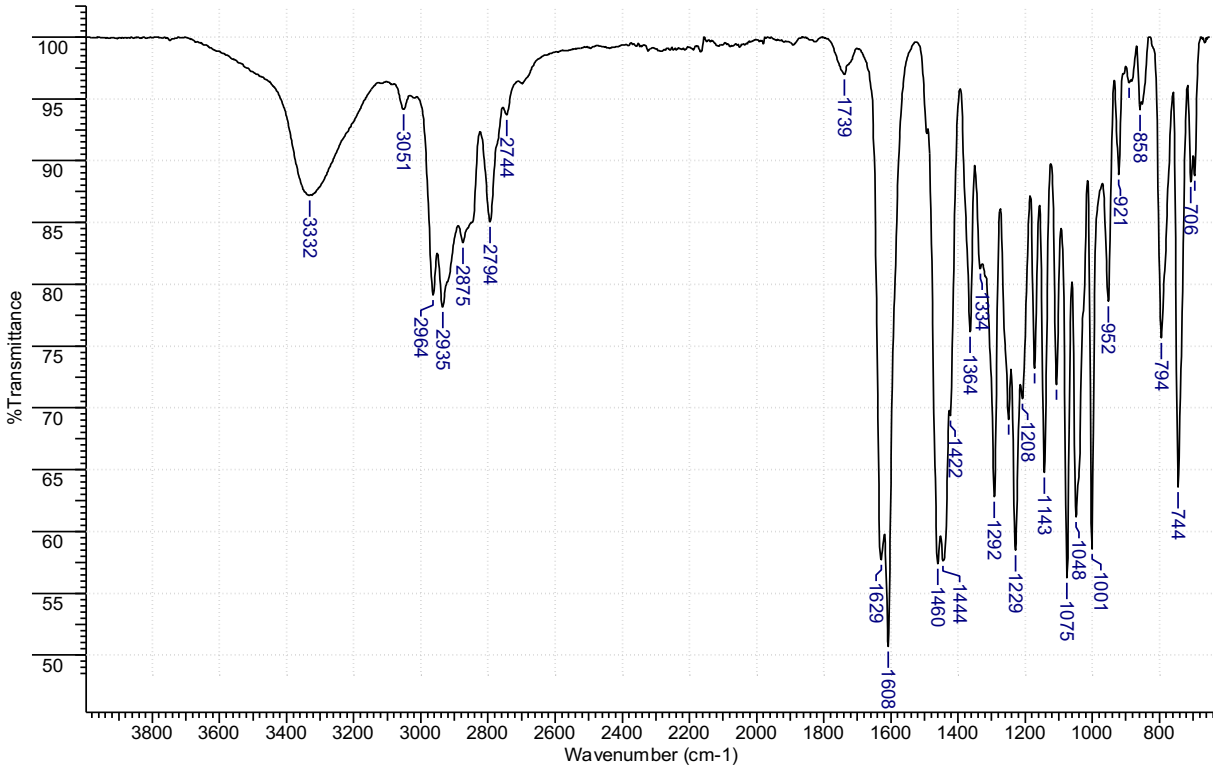


Figure S28. LC-MS/MS spectra of compound **EC7**

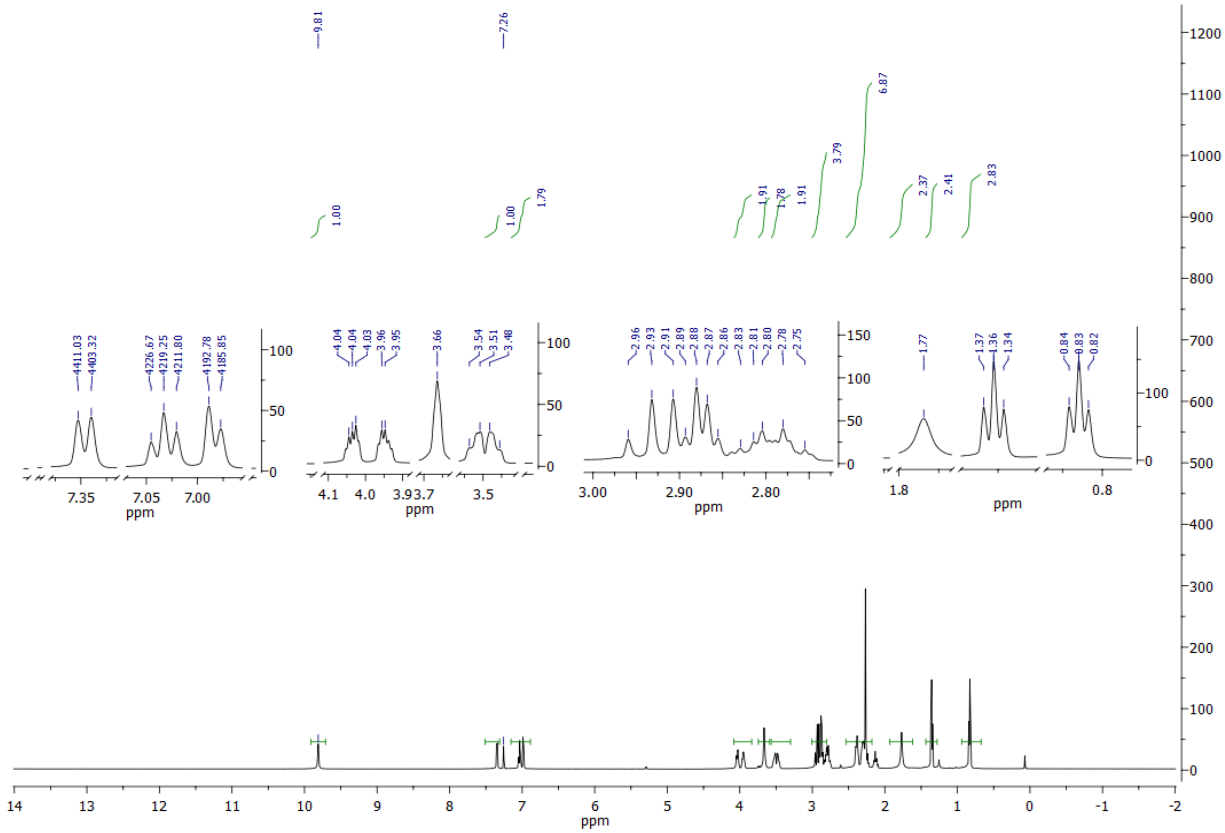


Figure S29. ^{1}H NMR spectra of compound **EC7**

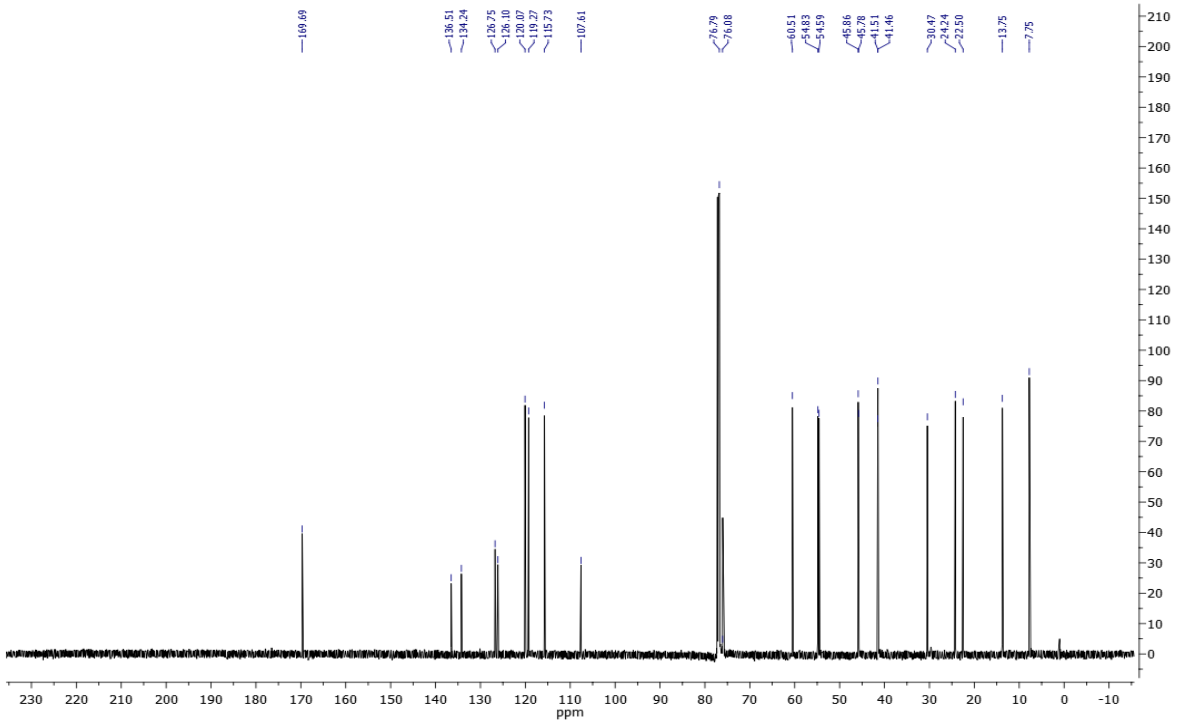


Figure S30. ^{13}C NMR spectra of compound **EC7**

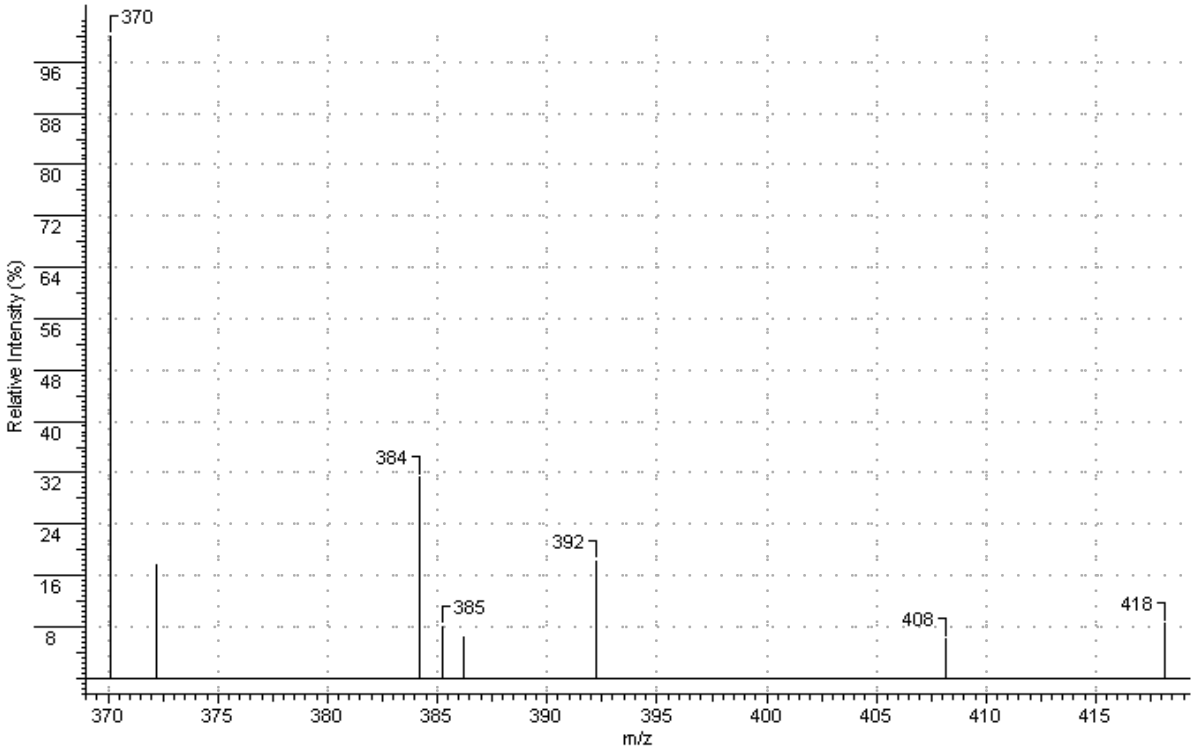
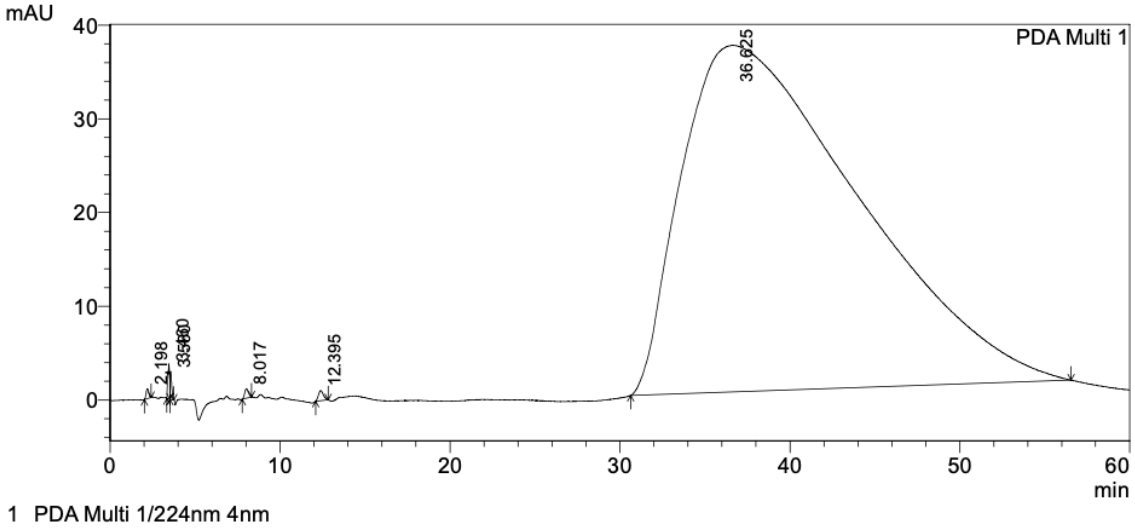


Figure S31. LC-MS/MS spectra of compound **EC7**

HPLC Chromotogram

HPLC Chromatogram of **EC1**



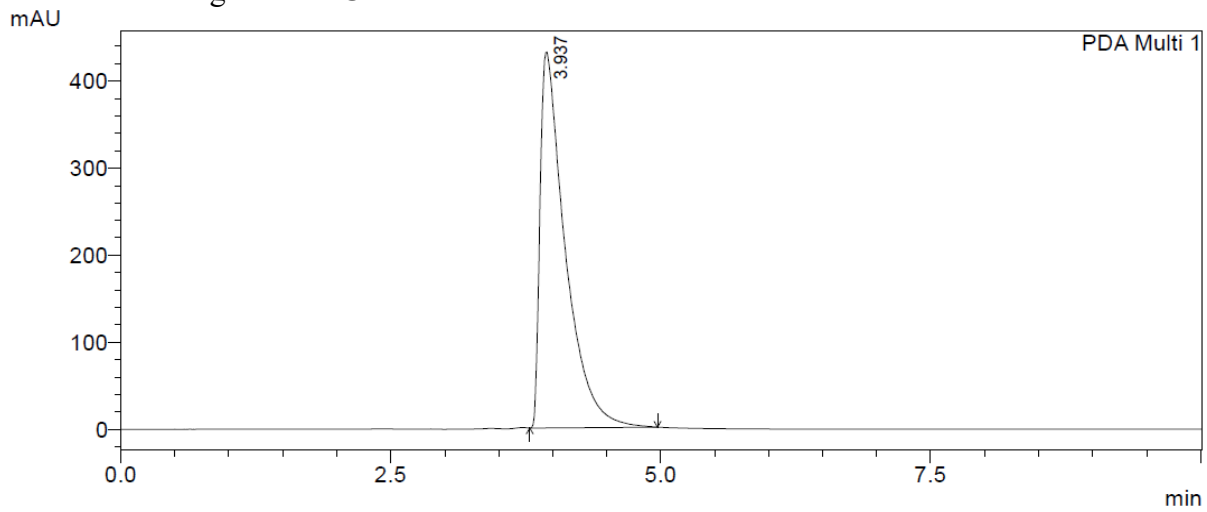
1 PDA Multi 1/224nm 4nm

PeakTable

PDA Ch1 224nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.198	11535	1047	0.043	2.234
2	3.460	27364	3754	0.102	8.008
3	3.560	19328	2992	0.072	6.381
4	8.017	15645	1016	0.058	2.167
5	12.395	22310	1093	0.083	2.332
6	36.625	26701159	36978	99.641	78.878
Total		26797340	46880	100.000	100.000

HPLC Chromatogram of **EC2**



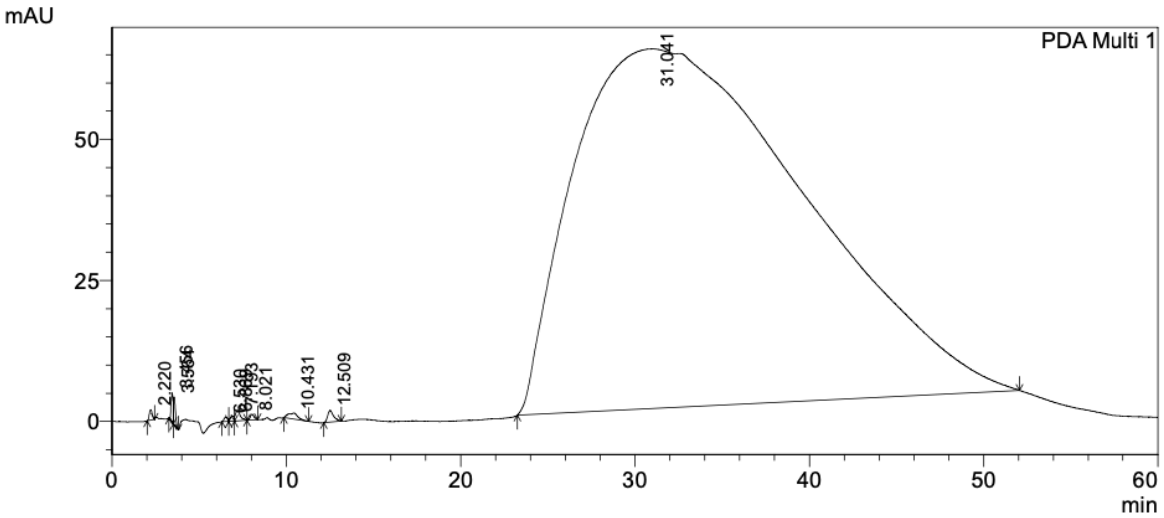
1 PDA Multi 1/254nm 4nm

PeakTable

PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.937	6980793	431544	100.000	100.000
Total		6980793	431544	100.000	100.000

HPLC Chromatogram of **EC3**



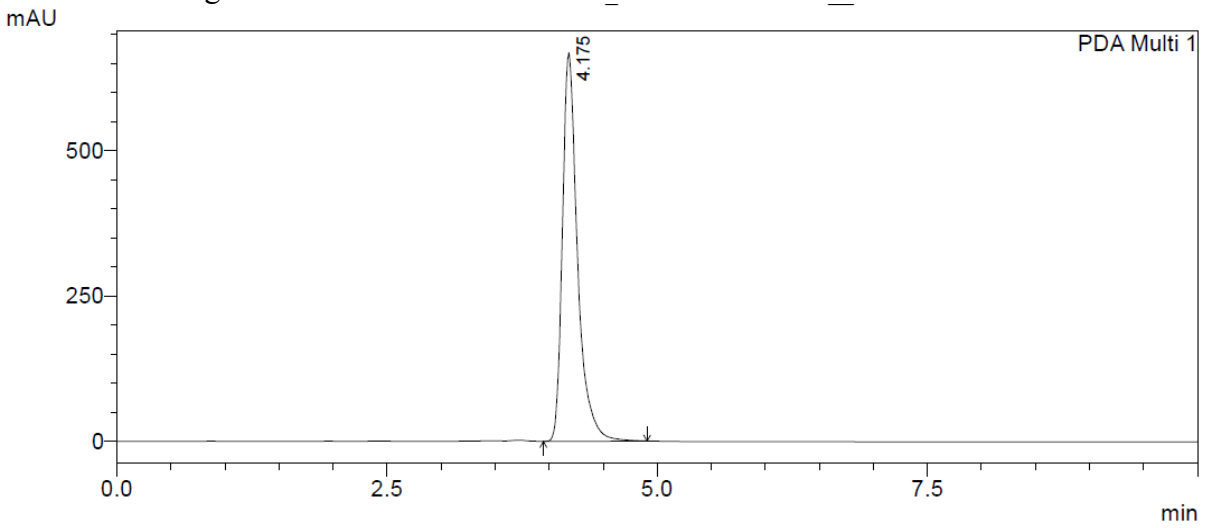
1 PDA Multi 1/224nm 4nm

PeakTable

PDA Ch1 224nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.220	20857	1860	0.035	2.230
2	3.456	40665	5207	0.069	6.241
3	3.564	39393	4915	0.067	5.891
4	6.530	11896	783	0.020	0.938
5	6.869	14145	871	0.024	1.044
6	7.193	35743	1864	0.061	2.235
7	8.021	16274	1036	0.028	1.242
8	10.431	40238	1089	0.068	1.306
9	12.509	49262	2071	0.084	2.482
10	31.041	58585534	63724	99.544	76.390
Total		58854007	83419	100.000	100.000

HPLC Chromatogram of **EC4**



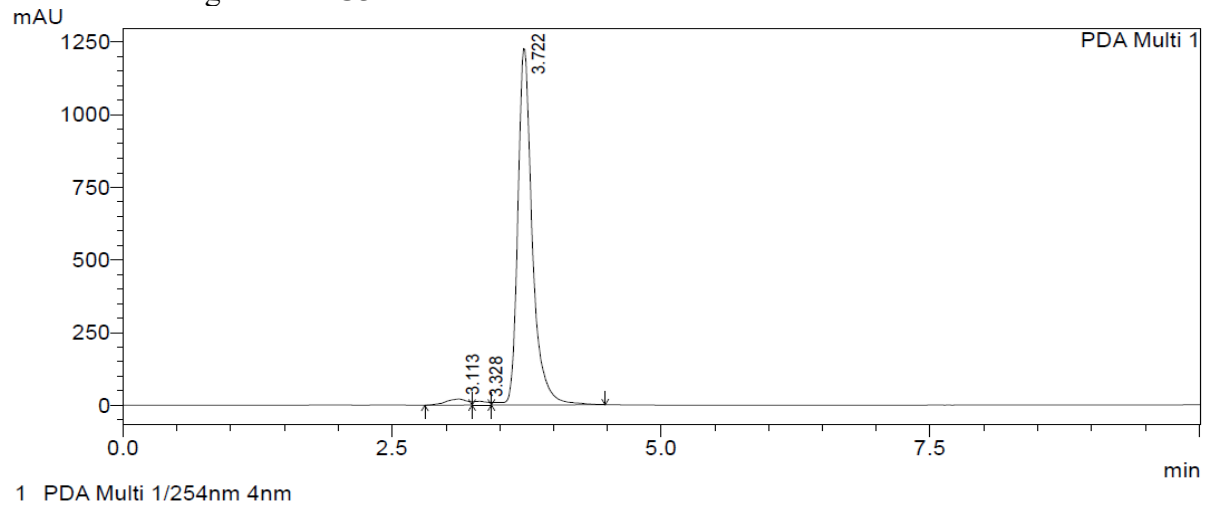
1 PDA Multi 1/254nm 4nm

PeakTable

PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.175	6508082	668564	100.000	100.000
Total		6508082	668564	100.000	100.000

HPLC Chromatogram of **EC5**



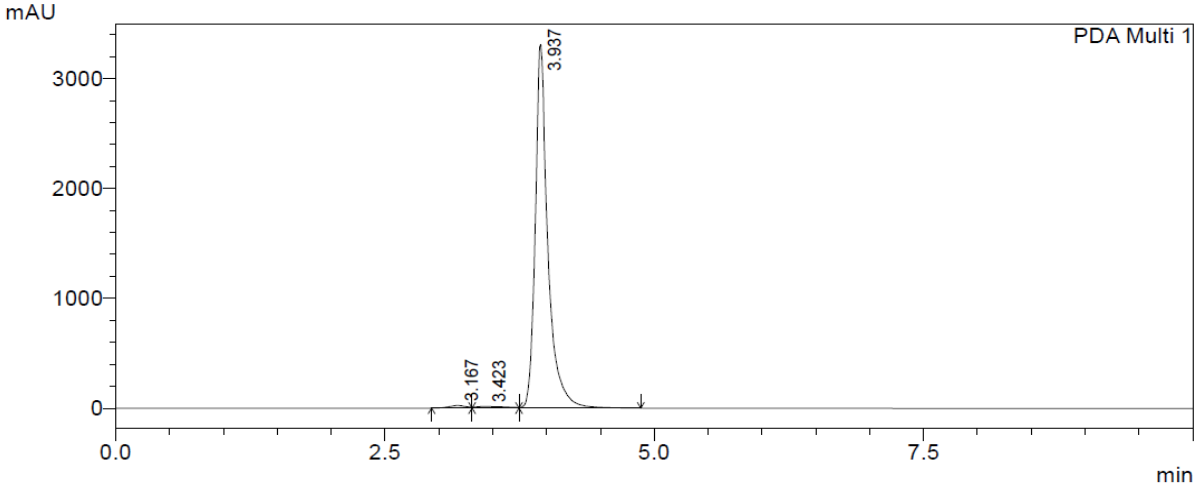
1 PDA Multi 1/254nm 4nm

PeakTable

PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.113	291743	20914	2.448	1.660
2	3.328	119529	12604	1.003	1.001
3	3.722	11505630	1226156	96.549	97.339
Total		11916902	1259674	100.000	100.000

HPLC Chromatogram of **EC6**



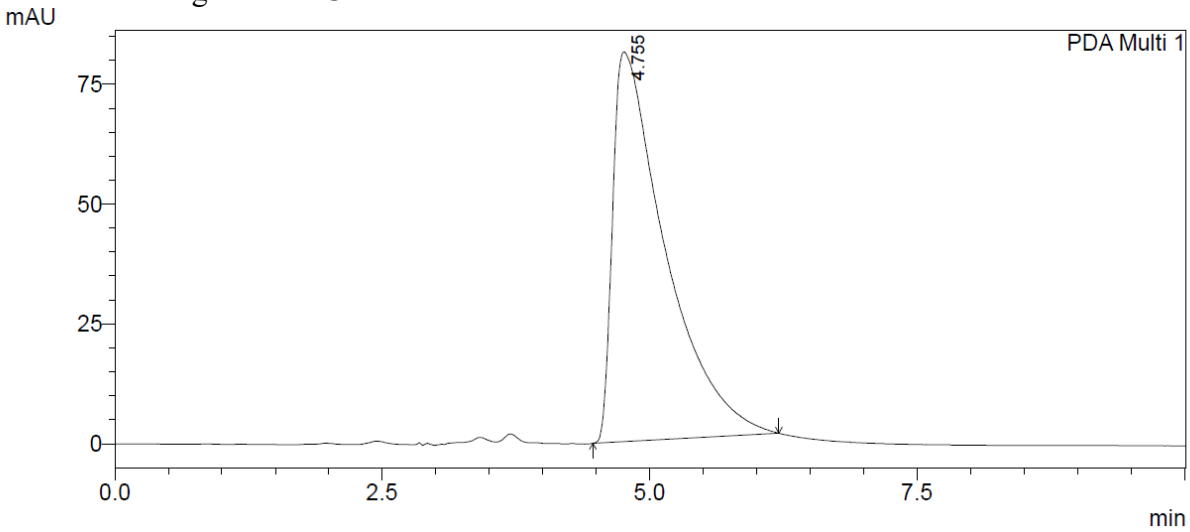
1 PDA Multi 1/254nm 4nm

PeakTable

PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.167	275342	24492	0.997	0.731
2	3.423	329188	17529	1.192	0.523
3	3.937	27004201	3306659	97.810	98.745
Total		27608732	3348680	100.000	100.000

HPLC Chromatogram of **EC7**



1 PDA Multi 1/254nm 4nm

PeakTable

PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.755	2781252	81307	100.000	100.000
Total		2781252	81307	100.000	100.000