Supplementary Information

Design, Synthesis, and Biological Evaluation of Novel Chalcone Derivatives: Integrating Molecular Docking with *In Vivo* Anti-Inflammatory and Antinociceptive Studies

Abida Shamim[1], Humaira Nadeem[2], Syed Muzzammil Masaud[2], Tajamul Hussain[3, 4, *], Kashif Bashir[1], Salman Alrokayan[4], Ihsan-ul-Haq[1], Muhamamd Latif[5,6,7], Muhammad Imran[8], Nadeem Irshad[1*]

[1]Department of Pharmacy, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad 45320, Pakistan; abidashamim@bs.qau.edu.pk (A.S.), kbashir@qau.edu.pk (K.B.), ihaq@qau.edu.pk (I.H.), nirshad@qau.edu.pk (N.I.)
[2]Riphah Institute of Pharmaceutical Sciences, Riphah International University, Islamabad, Pakistan; humaira.nadeem@riphah.edu.pk (H.N.), muzzammil.masaud@riphah.edu.pk (S.M.M.)
[3]Center of Excellence in Biotechnology Research, King Saud University, Riyadh 11451, Saudi Arabia; thussain@ksu.edu.sa (T.H.)

[4]Research Chair for Biomedical Application of Nanomaterials, Biochemistry Department, College of Science, King Saud University, Riyadh 11451, Saudi Arabia; salrokayan@ksu.edu.sa (S.A.R.)

[5]Centre for Genetics and Inherited Diseases (CGID), Taibah University, Madinah 42353, Saudi Arabia; latifmayo@gmail.com (M.L.)
[6]Department of Basic Medical Sciences, College of Medicine, Taibah University, Madinah 42353, Saudi Arabia; latifmayo@gmail.com (M.L.)

[7]Department of Chemistry, Rawalpindi Women University, Rawalpindi-46300 Pakistan; latifmayo@gmail.com (M.L.)

[8]State Key Laboratory of Microbial Metabolism, Joint International Research Laboratory of Metabolic and Developmental Sciences, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai, P. R. China.

Correspondence: nirshad@qau.edu.pk (N.I.); Ph: 925190644099, thussain@ksu.edu.sa (T.H.), Ph: 9660114661867

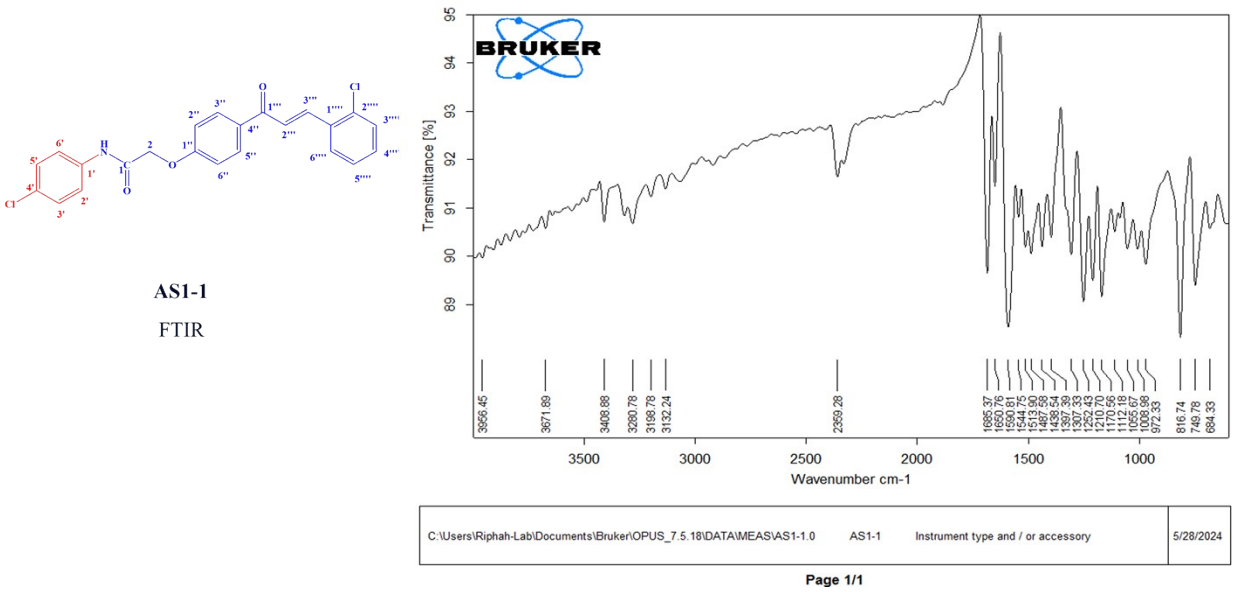
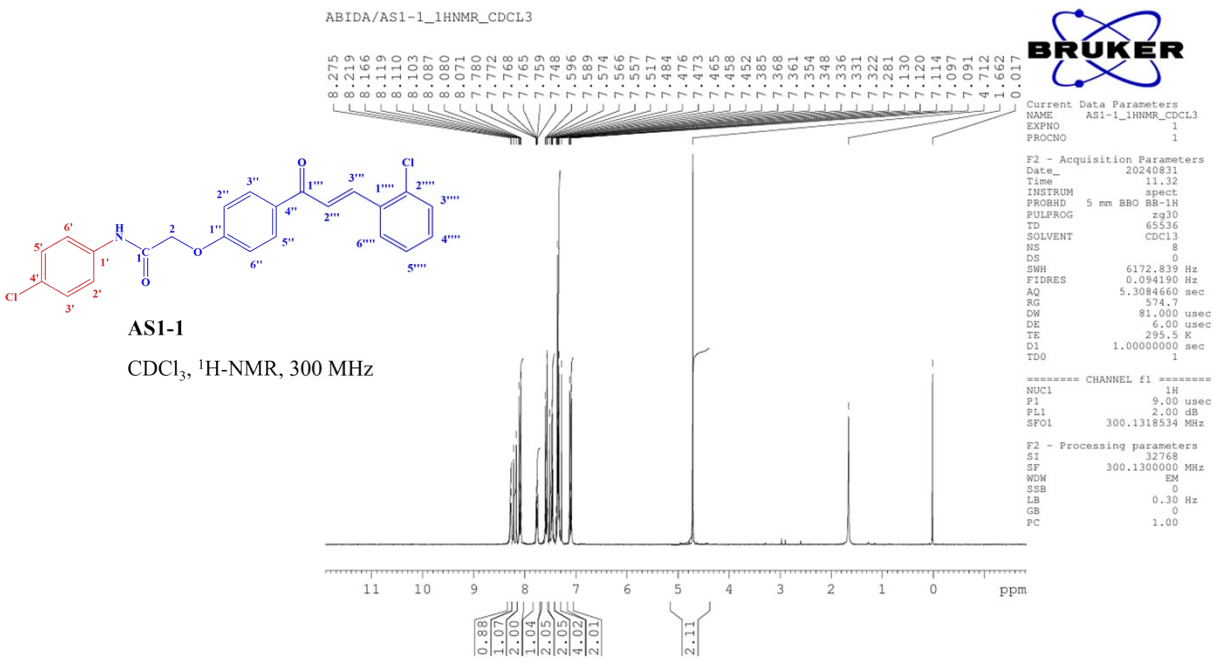


Figure S1. FTIR Spectrum of AS1-1.



AS1-1

$CDCl_3$, ^{1}H-NMR, 300 MHz

Figure S2. ^{1}H-NMR Spectrum of AS1-1.

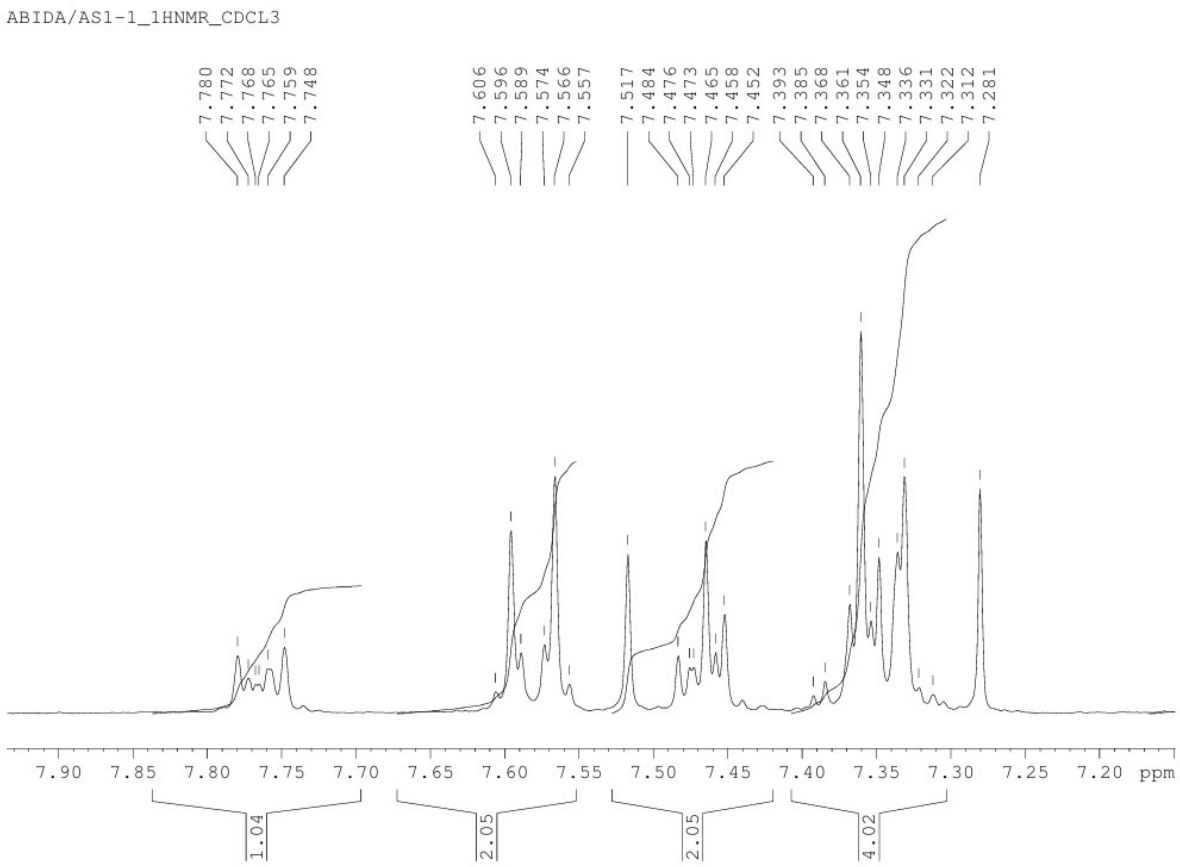


Figure S3. ^{1}H-NMR expansion of AS1-1

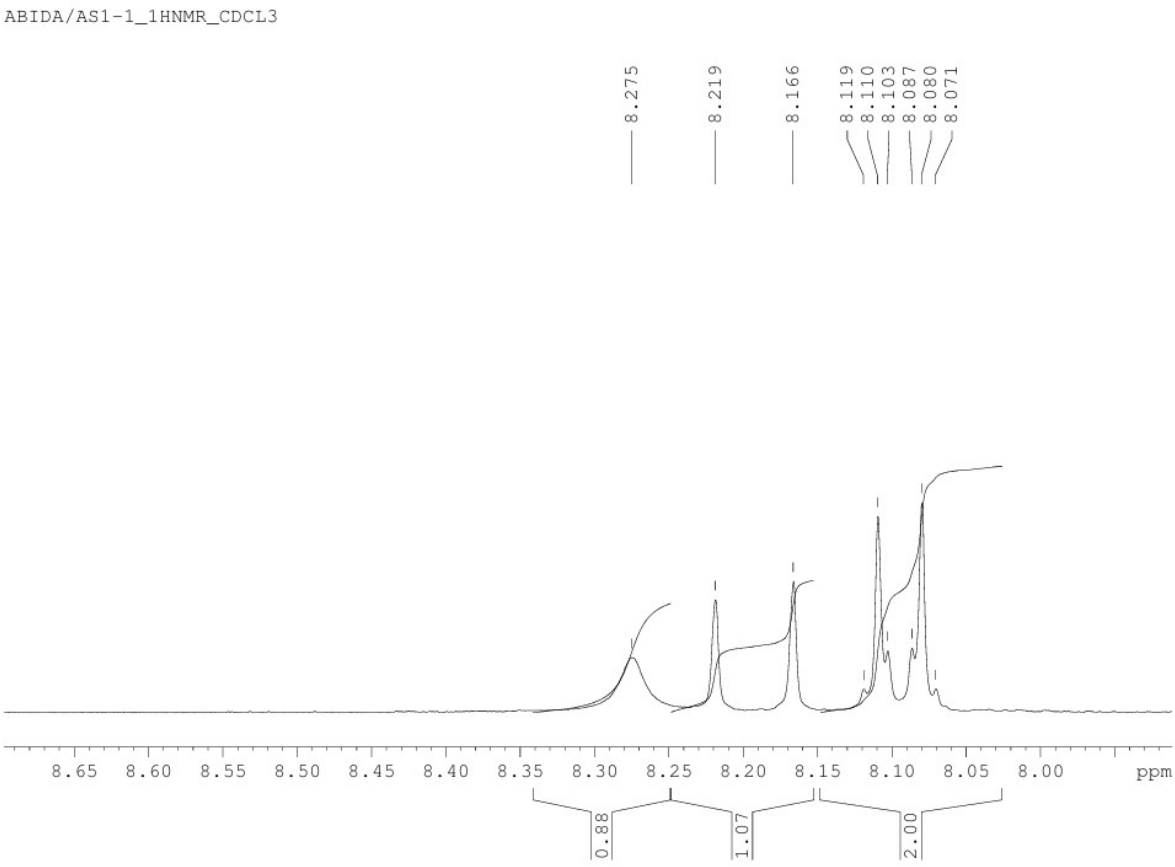


Figure S4. ^{1}H-NMR expansion of AS1-1

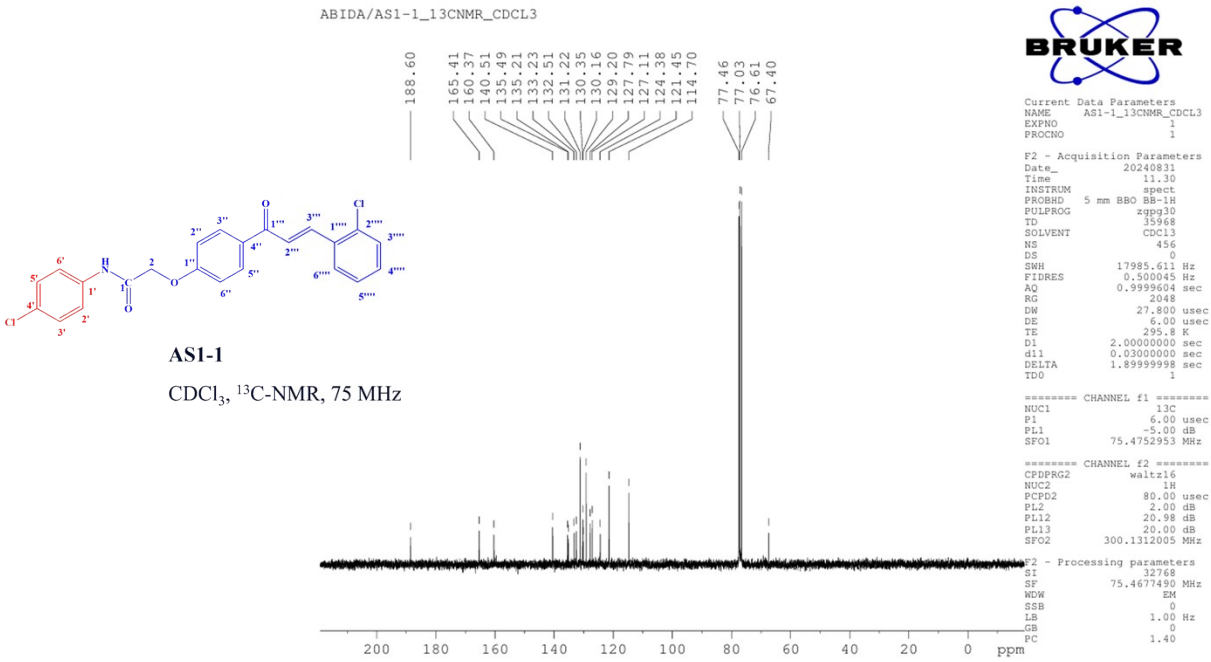


Figure S5. ^{13}C-NMR Spectrum of AS1-1.

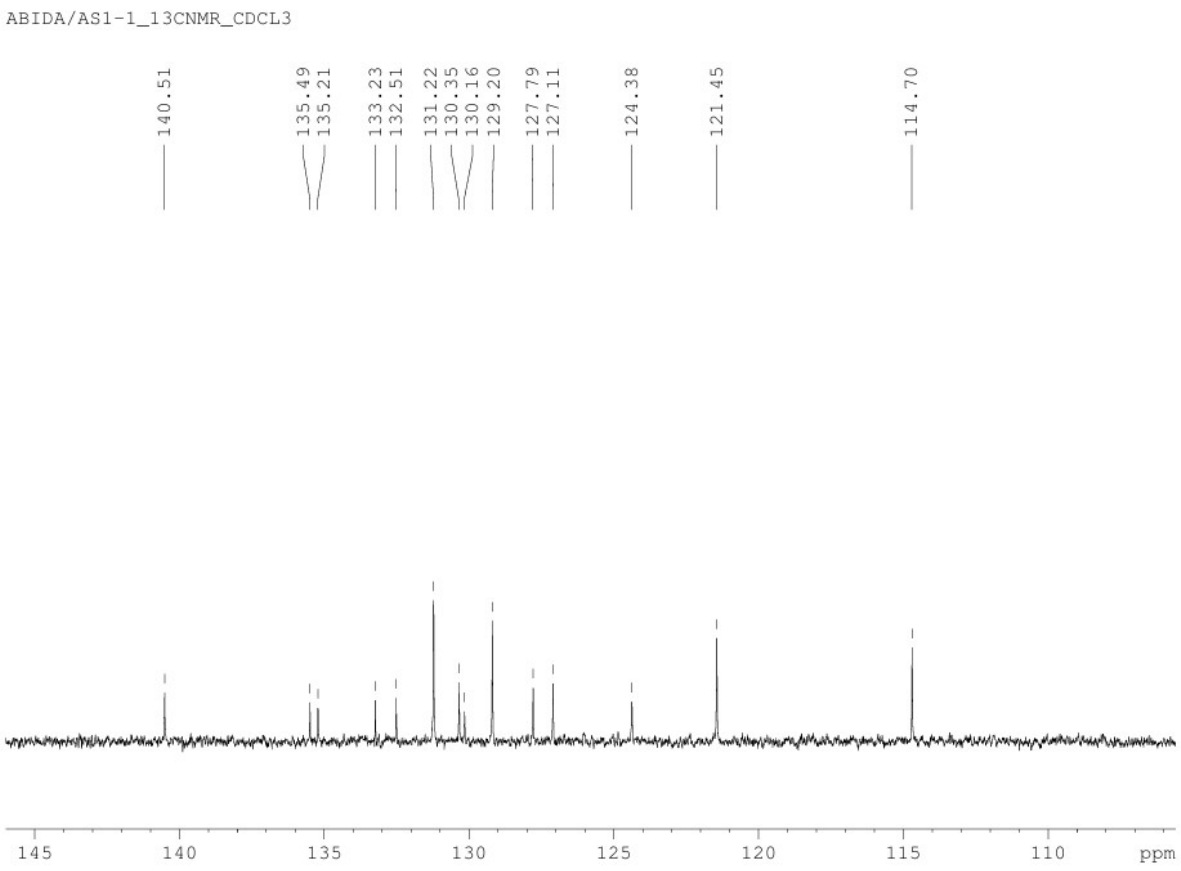


Figure S6. ^{13}C-NMR expansion of AS1-1.

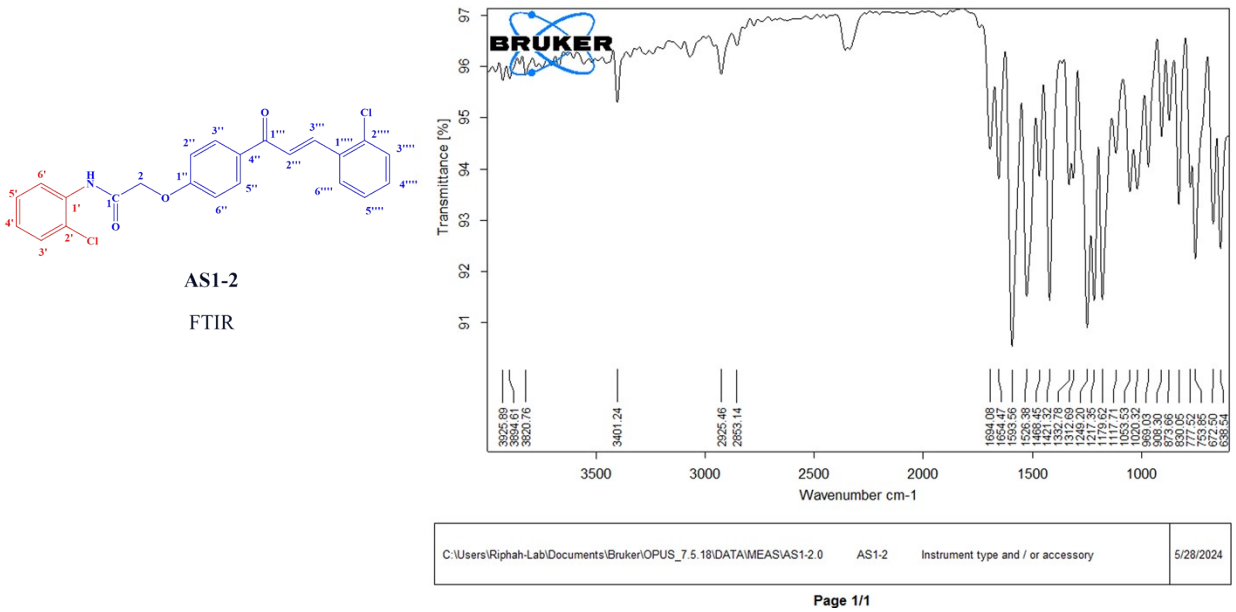


Figure S7. FTIR Spectrum of AS1-2.

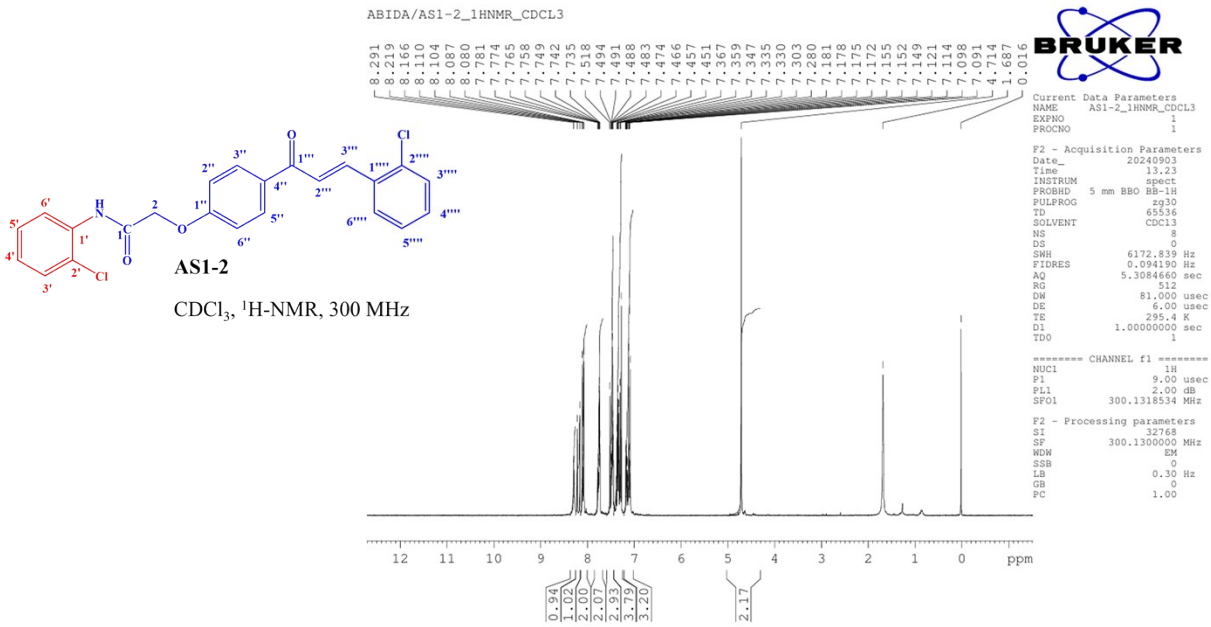


Figure S8. ^{1}H-NMR Spectrum of AS1-2.

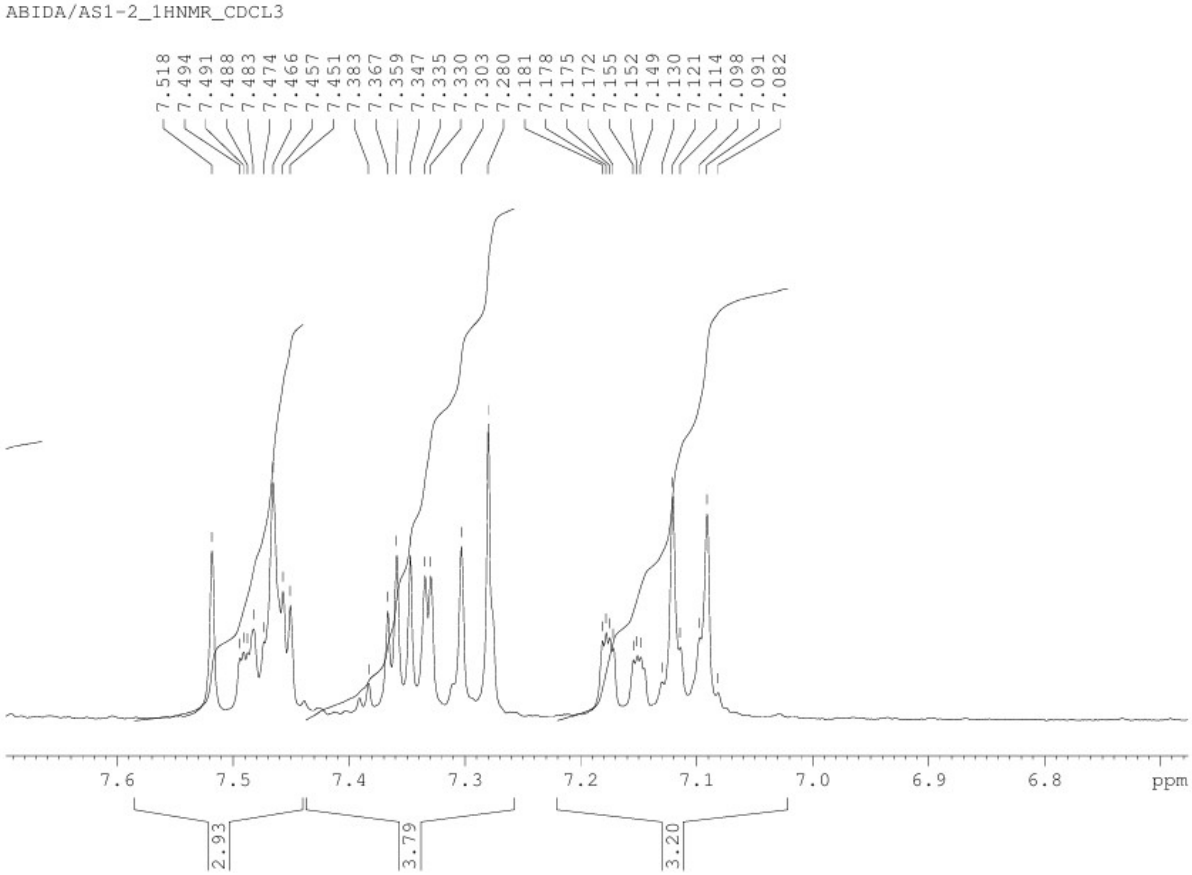


Figure S9. [1]H-NMR expansion of AS1-2

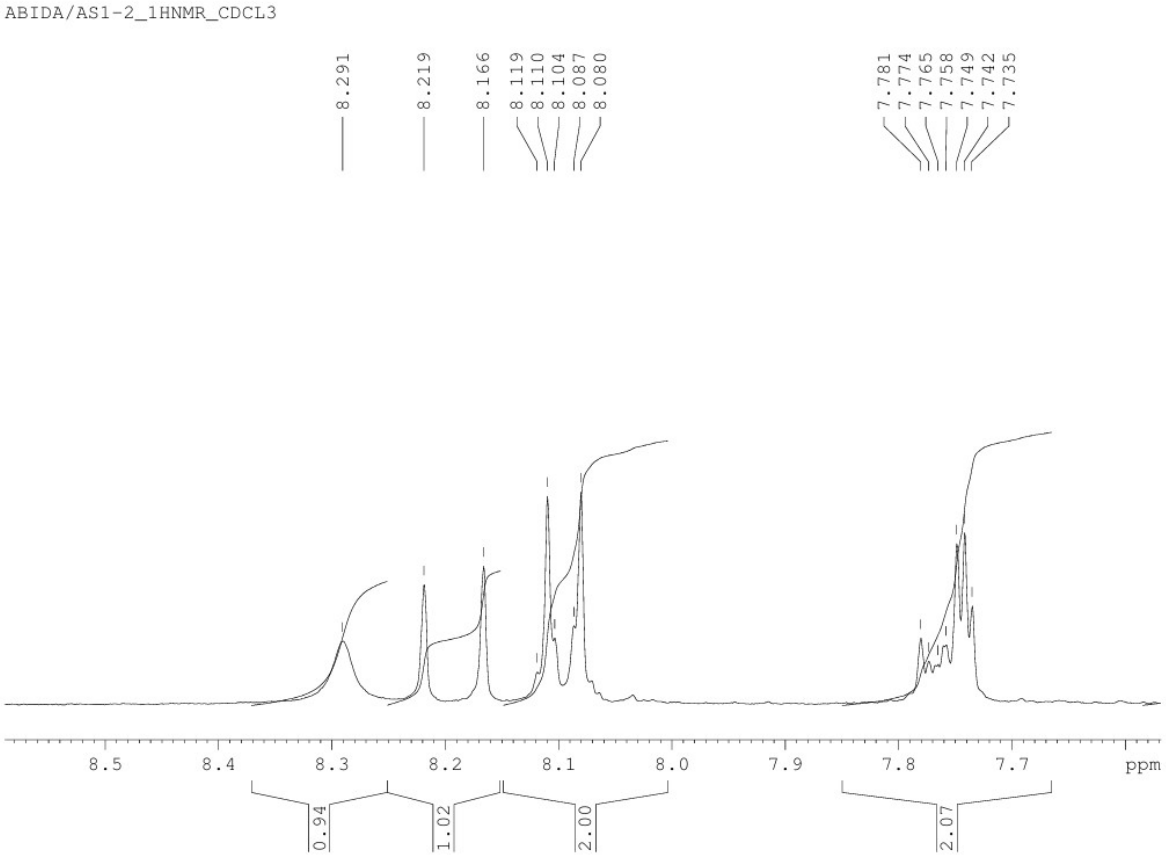


Figure S10. ^{1}H-NMR expansion of AS1-2

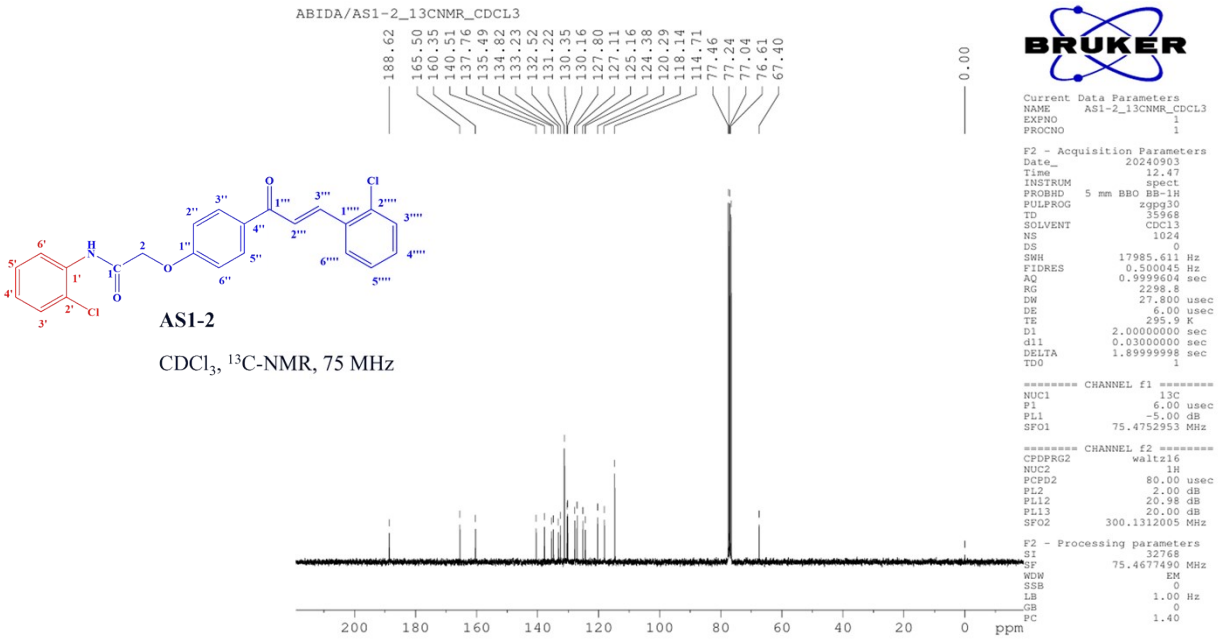


Figure S11. ^{13}C-NMR Spectrum of AS1-2.

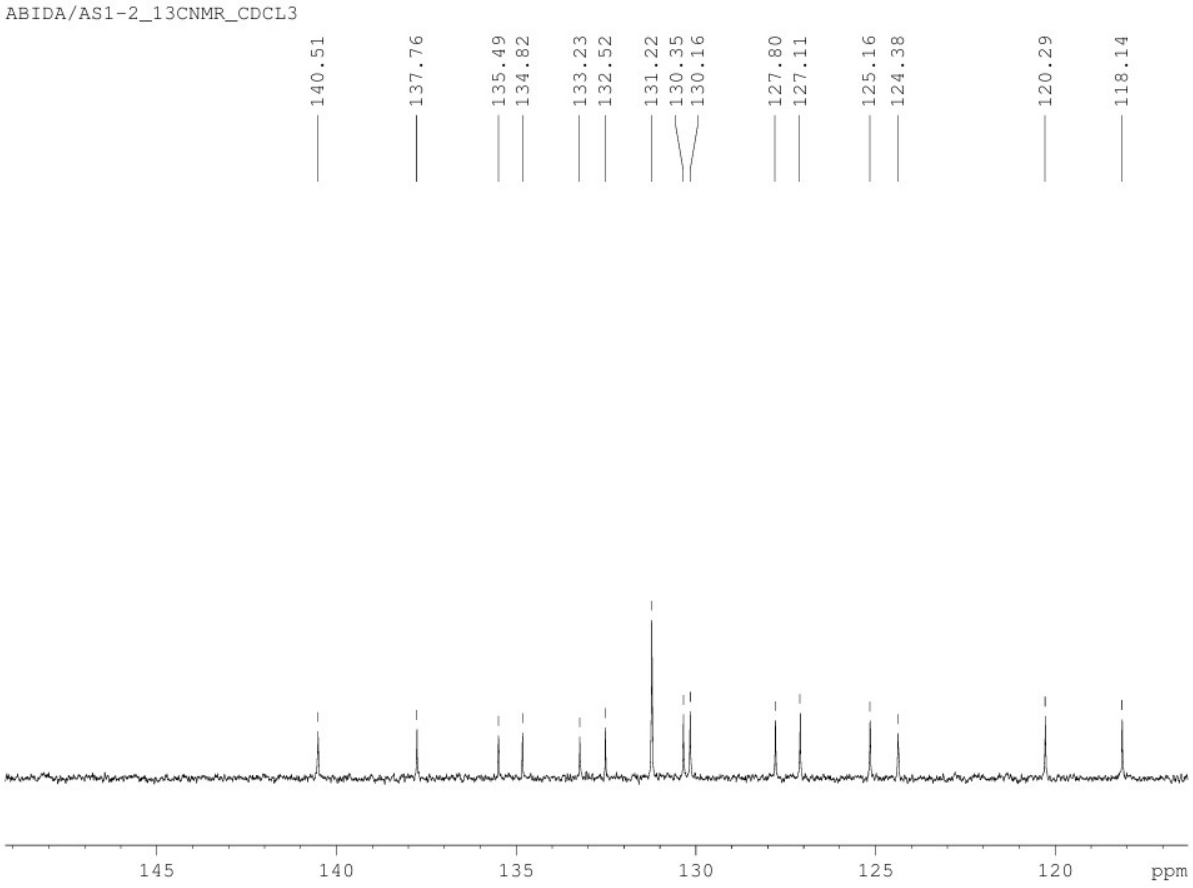


Figure S12. ^{13}C-NMR expansion of AS1-2

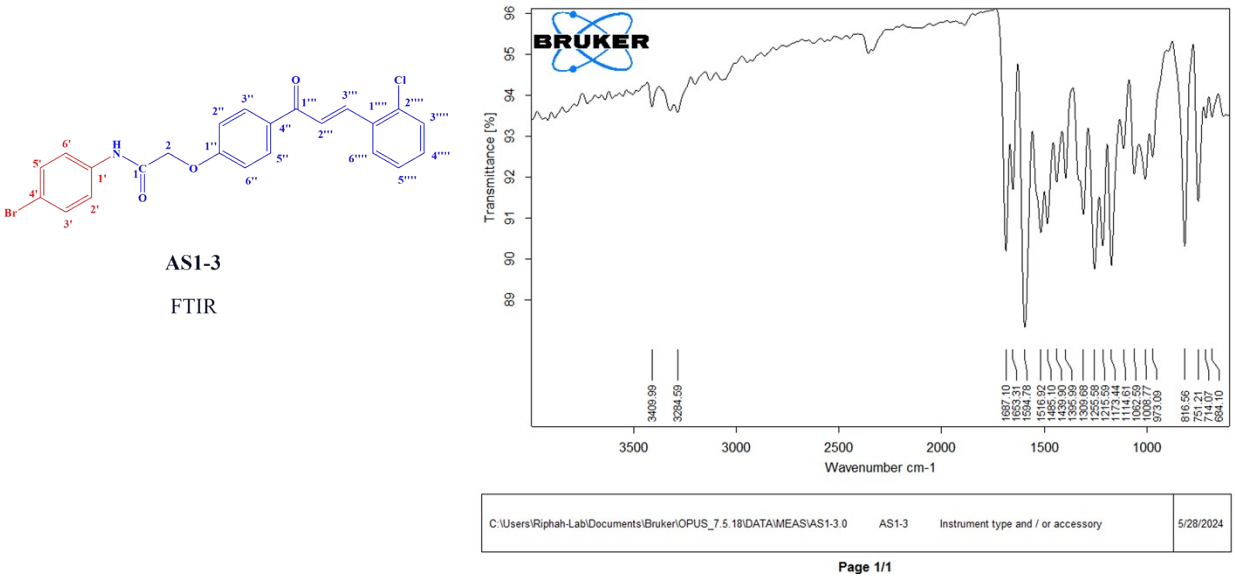


Figure S13. FTIR Spectrum of AS1-3.

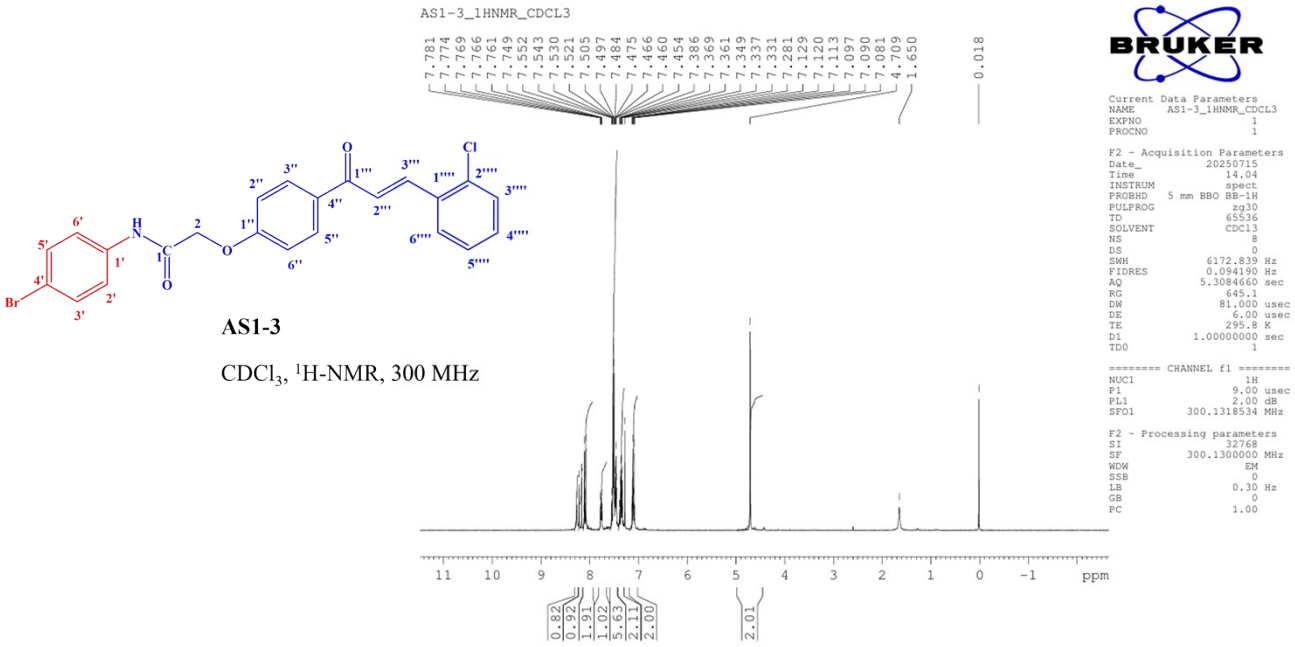


Figure S14. ^{1}H-NMR Spectrum of AS1-3.

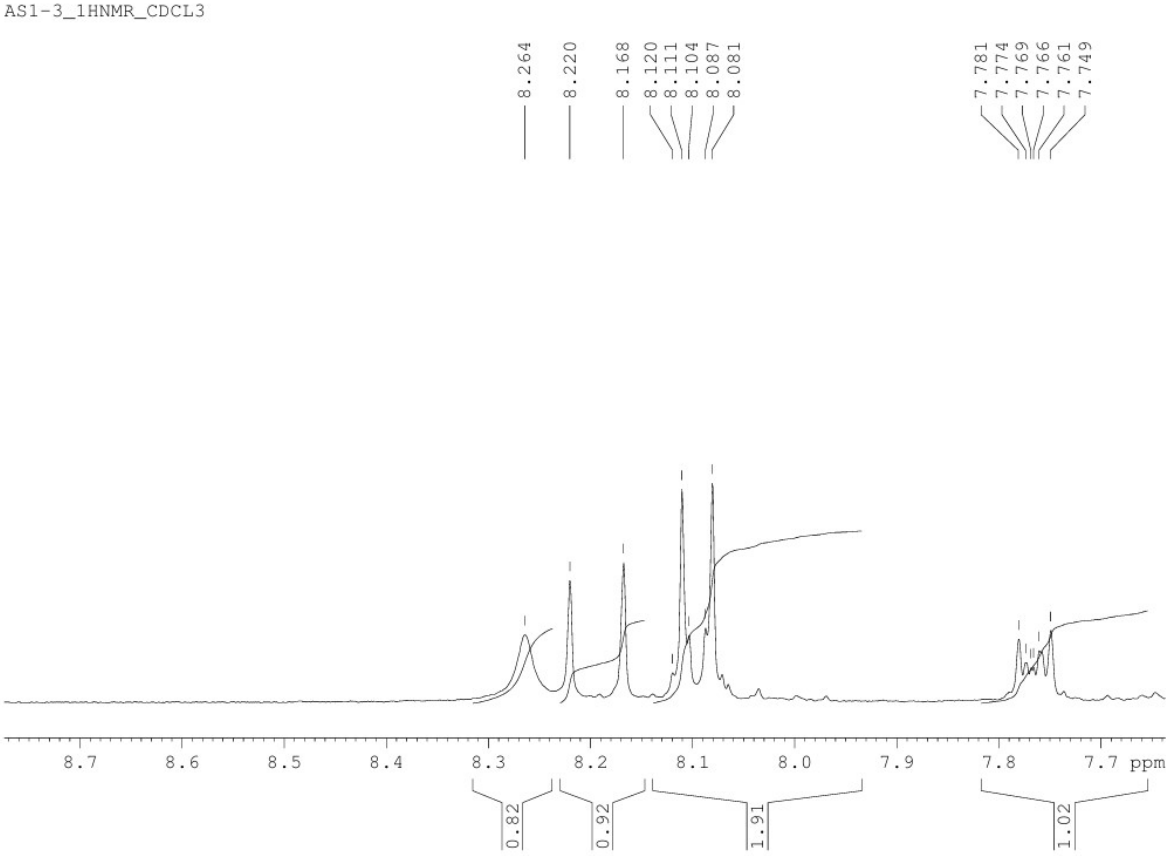


Figure S15. ^{1}H-NMR expansion of AS1-3.

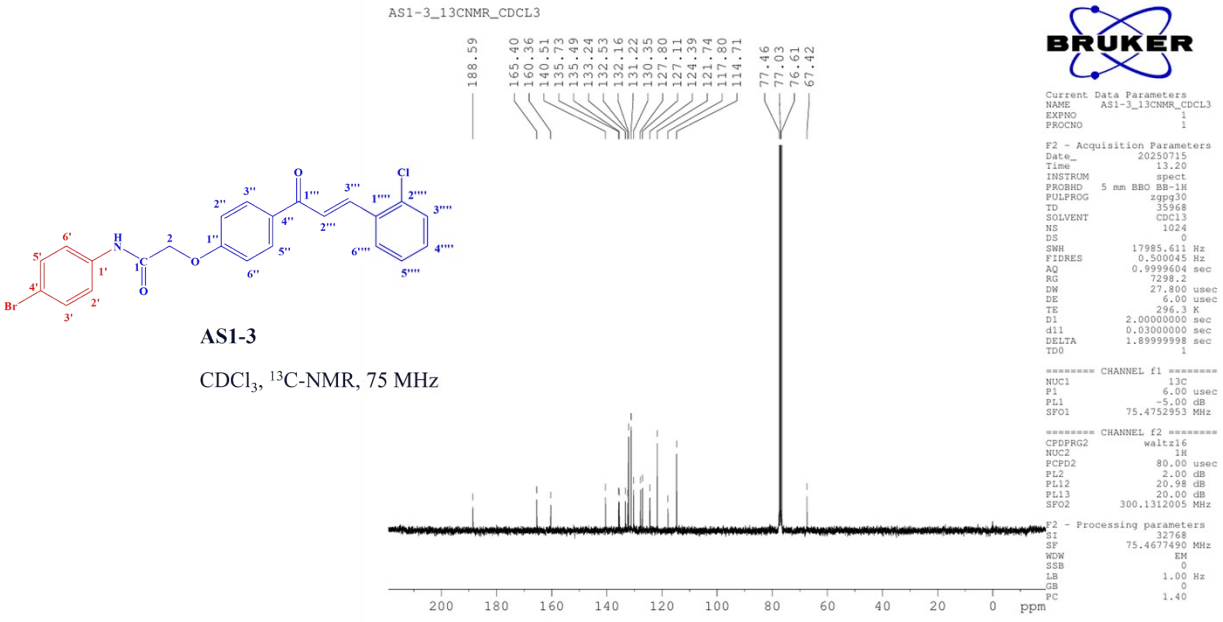


Figure S16. ^{13}C-NMR Spectrum of AS1-3.

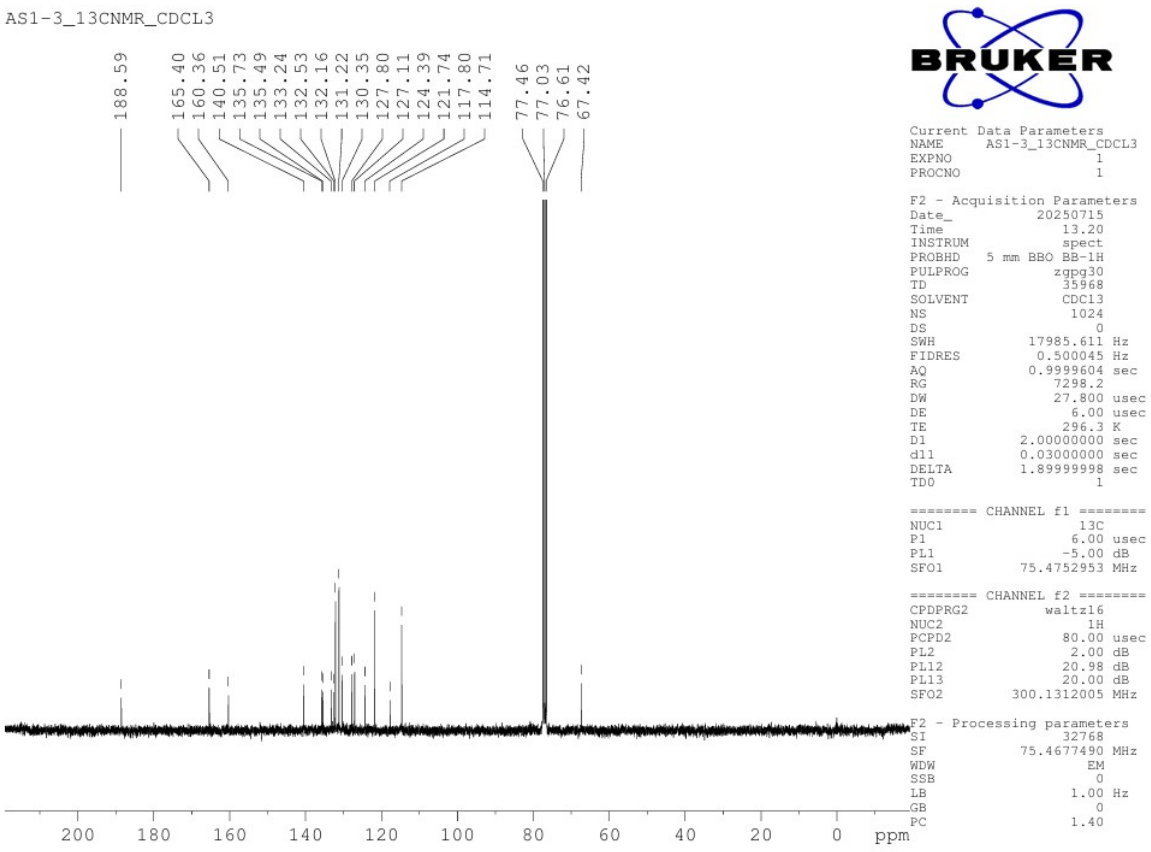


Figure S17. ^{13}C-NMR Spectrum of AS1-3.

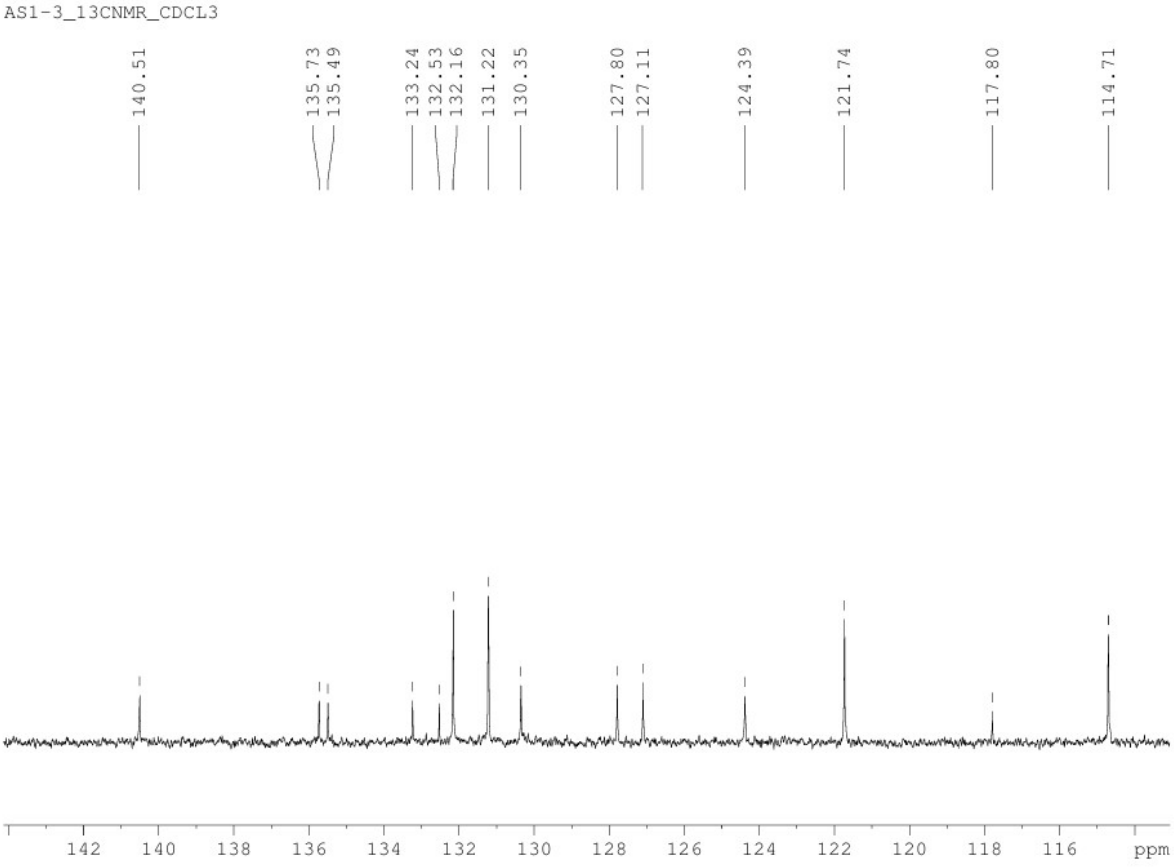


Figure S18. ^{13}C-NMR expansion of AS1-3.

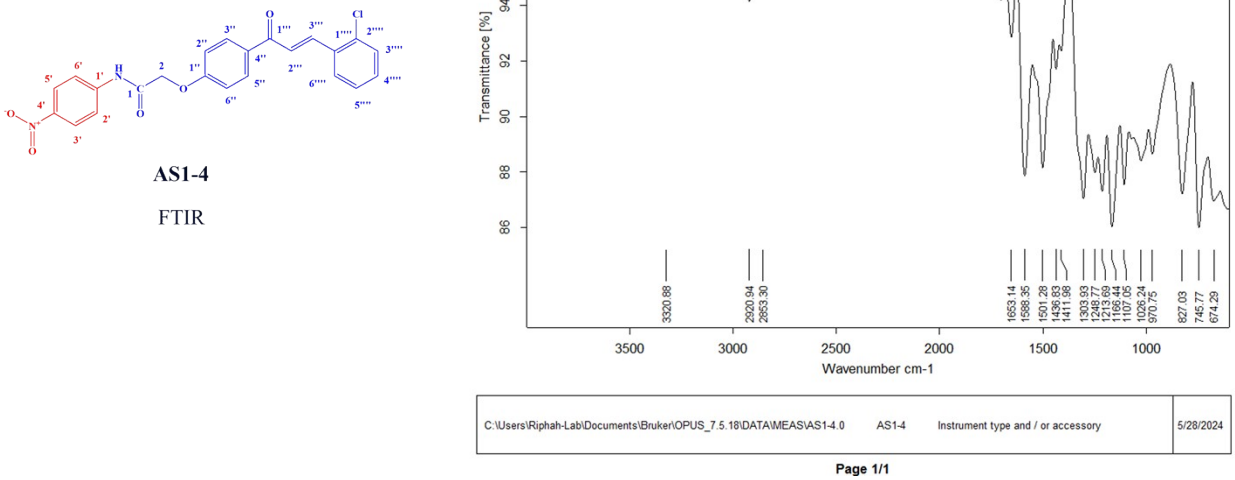


Figure S19. FTIR Spectrum of AS1-4.

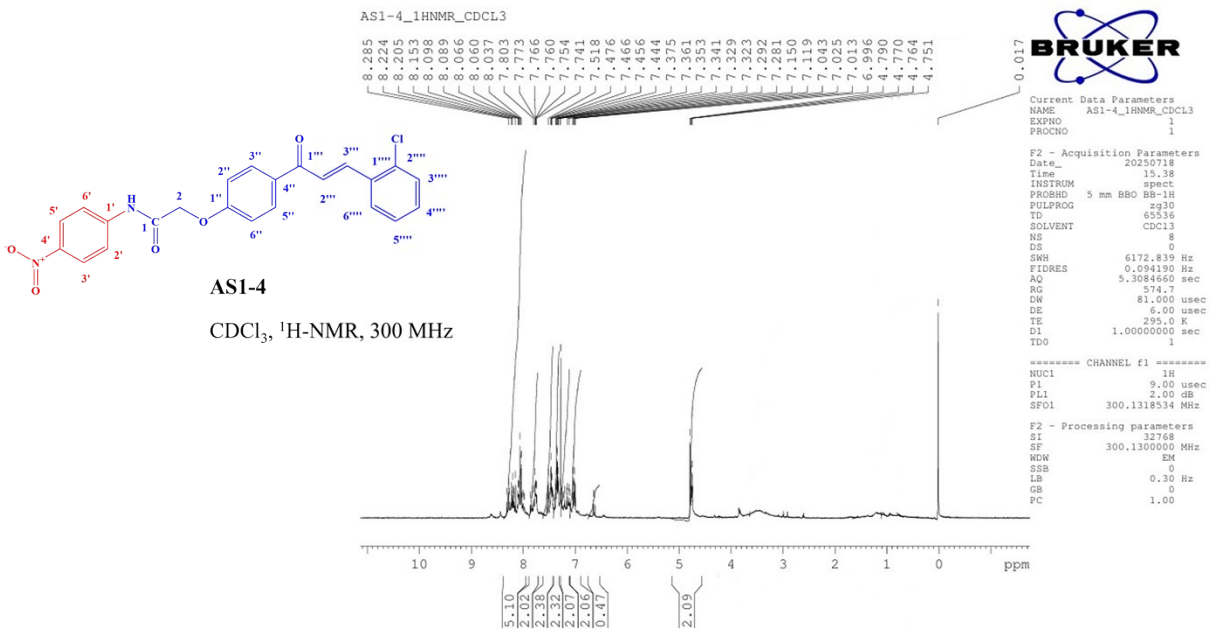


Figure S20. ^{1}H-NMR Spectrum of AS1-4.

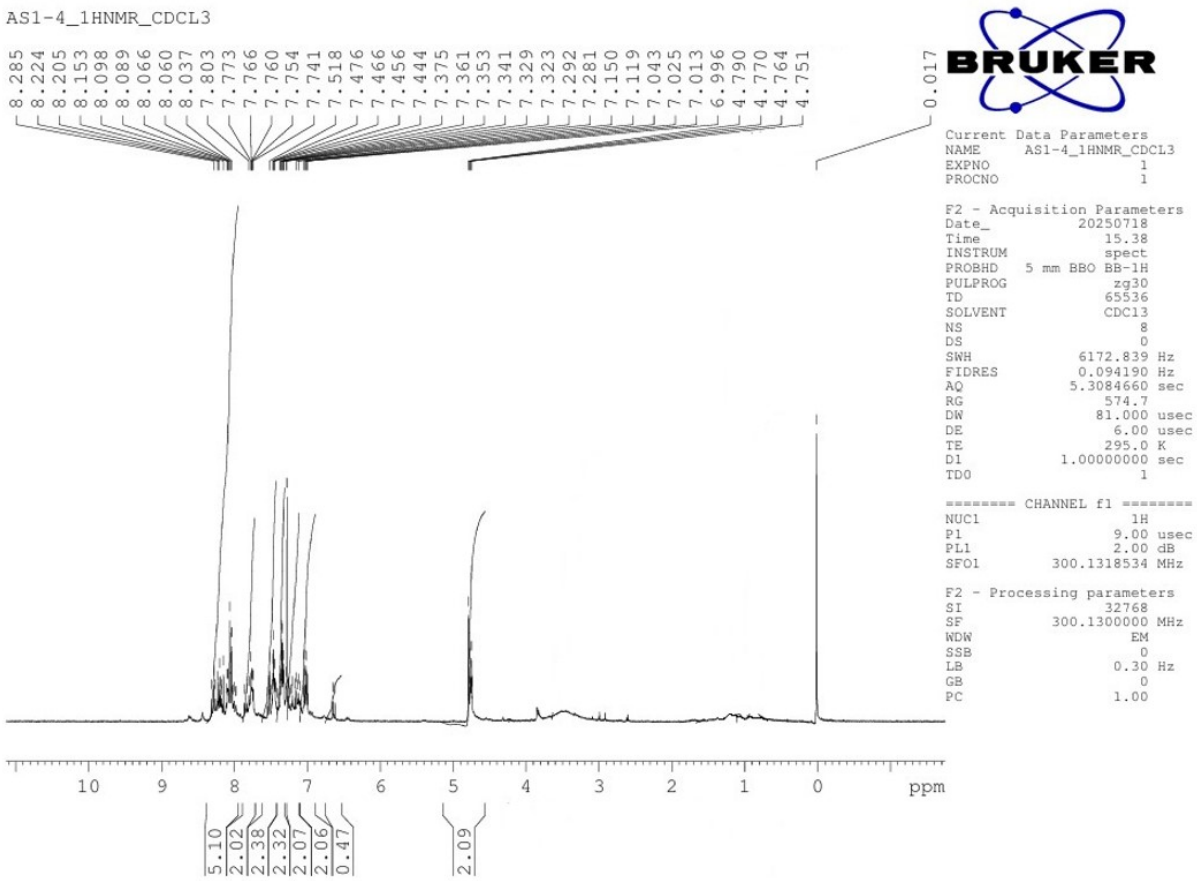


Figure S21. ^{1}H-NMR Spectrum of AS1-4.

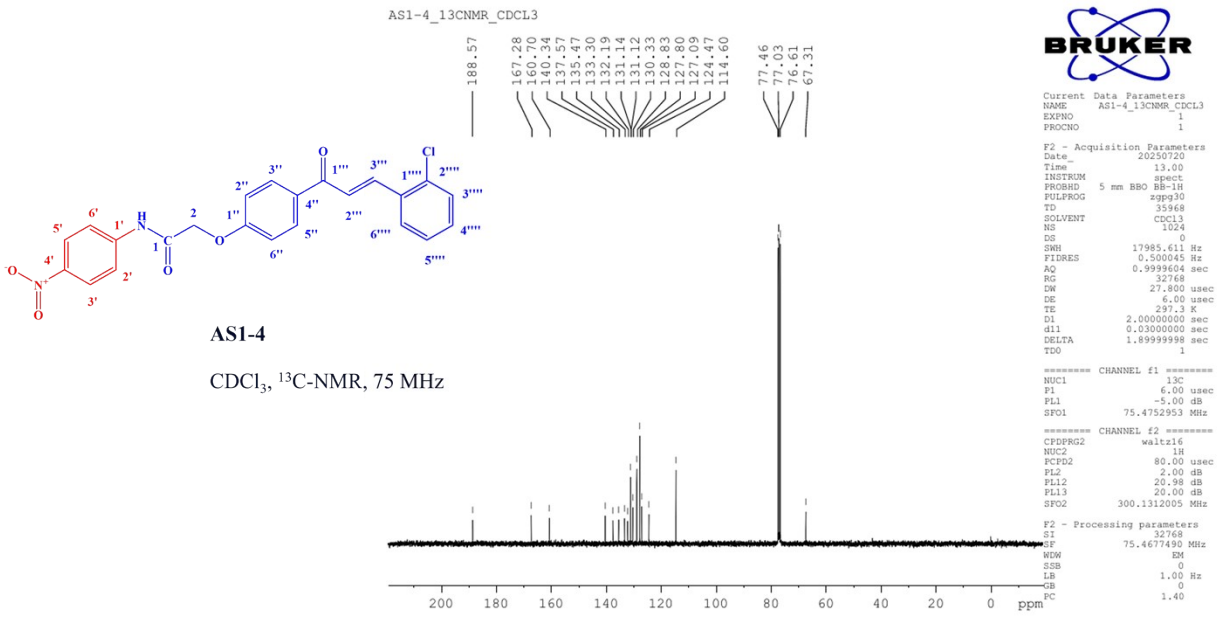


Figure S22. ^{13}C-NMR Spectrum of AS1-4.

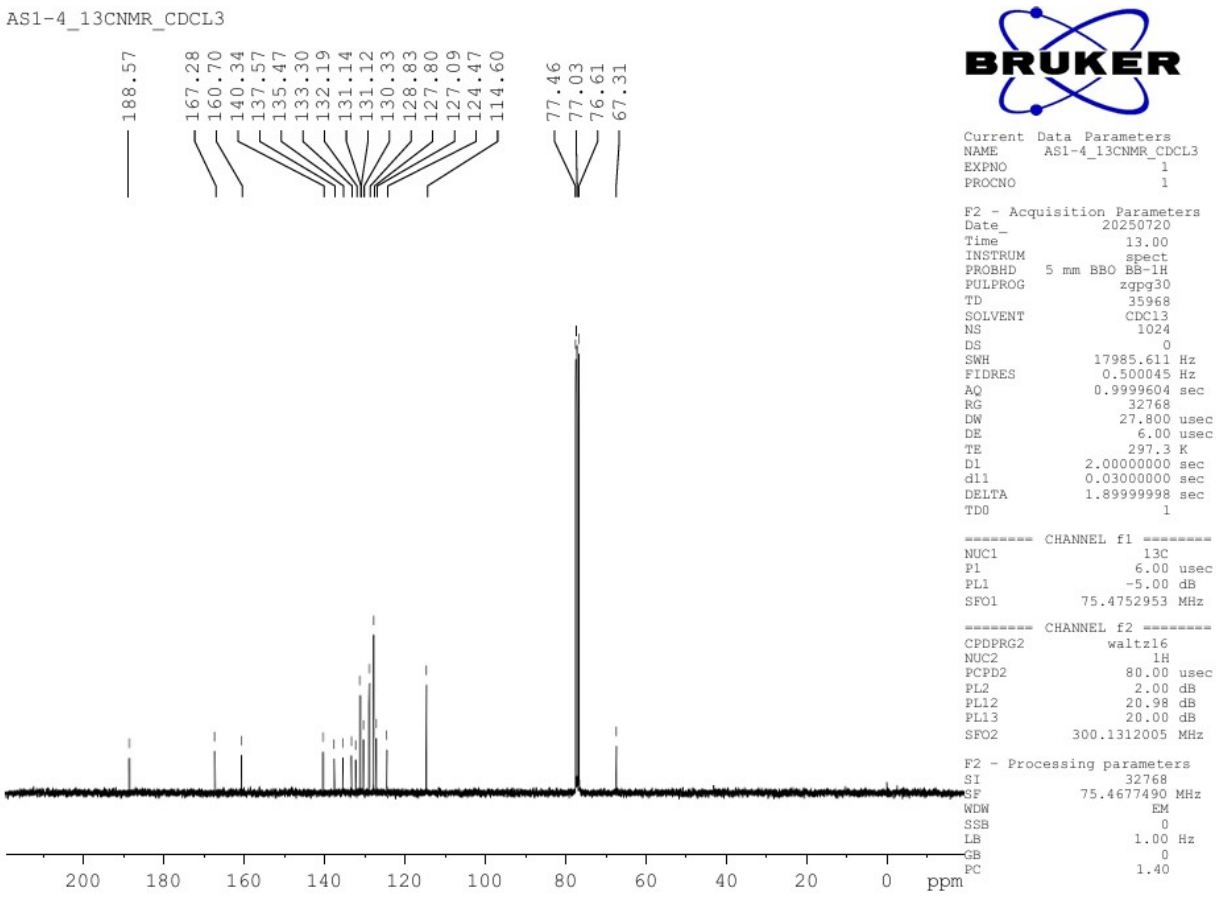
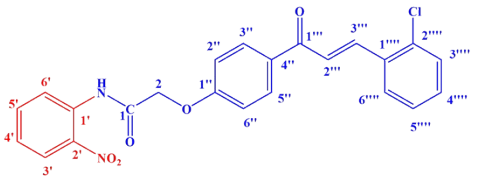


Figure S23. ^{13}C-NMR Spectrum of AS1-4.



AS1-5

FTIR

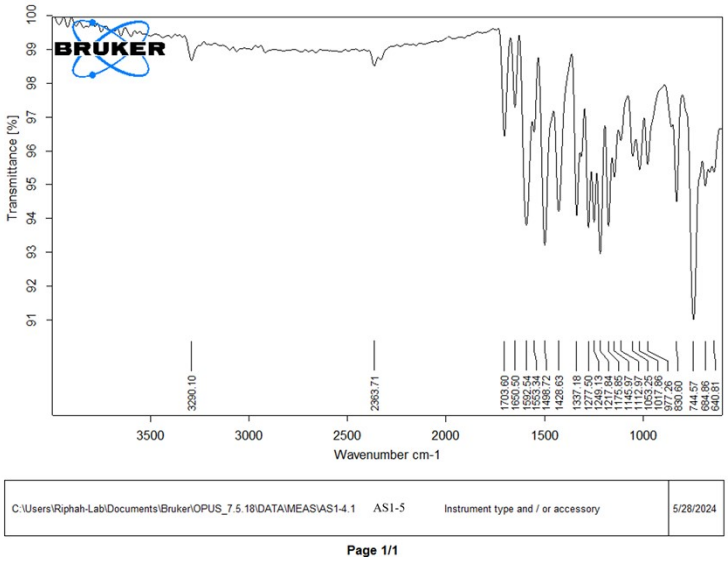


Figure S24. FTIR Spectrum of AS1-5.

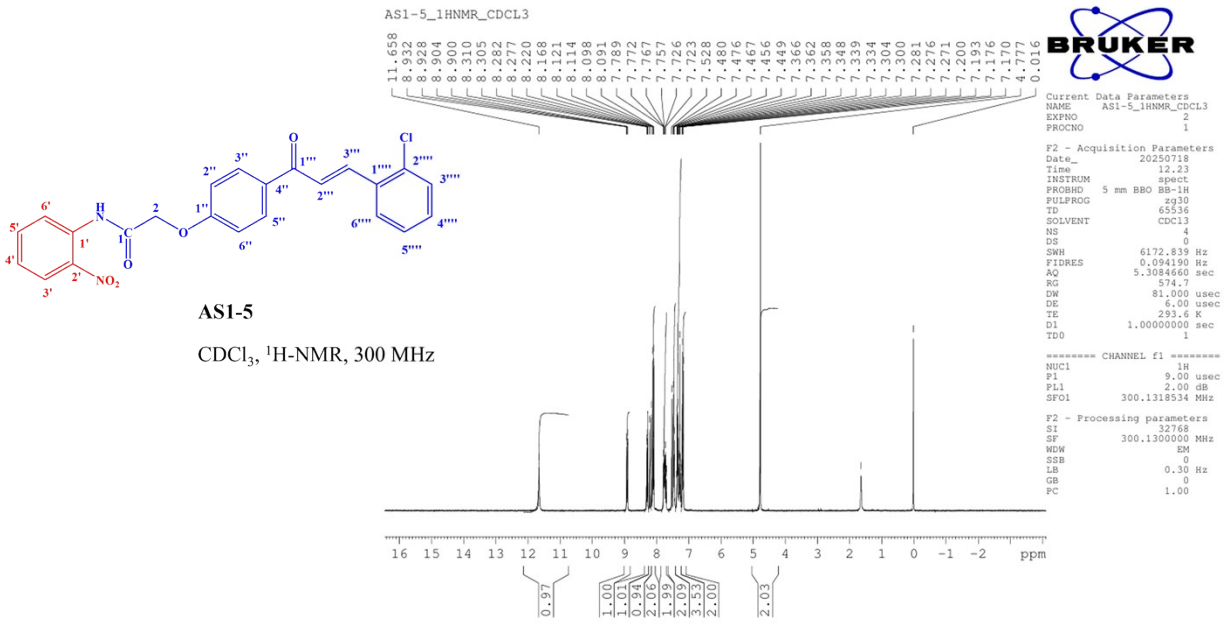


Figure S25. ^{1}H-NMR Spectrum of AS1-5.

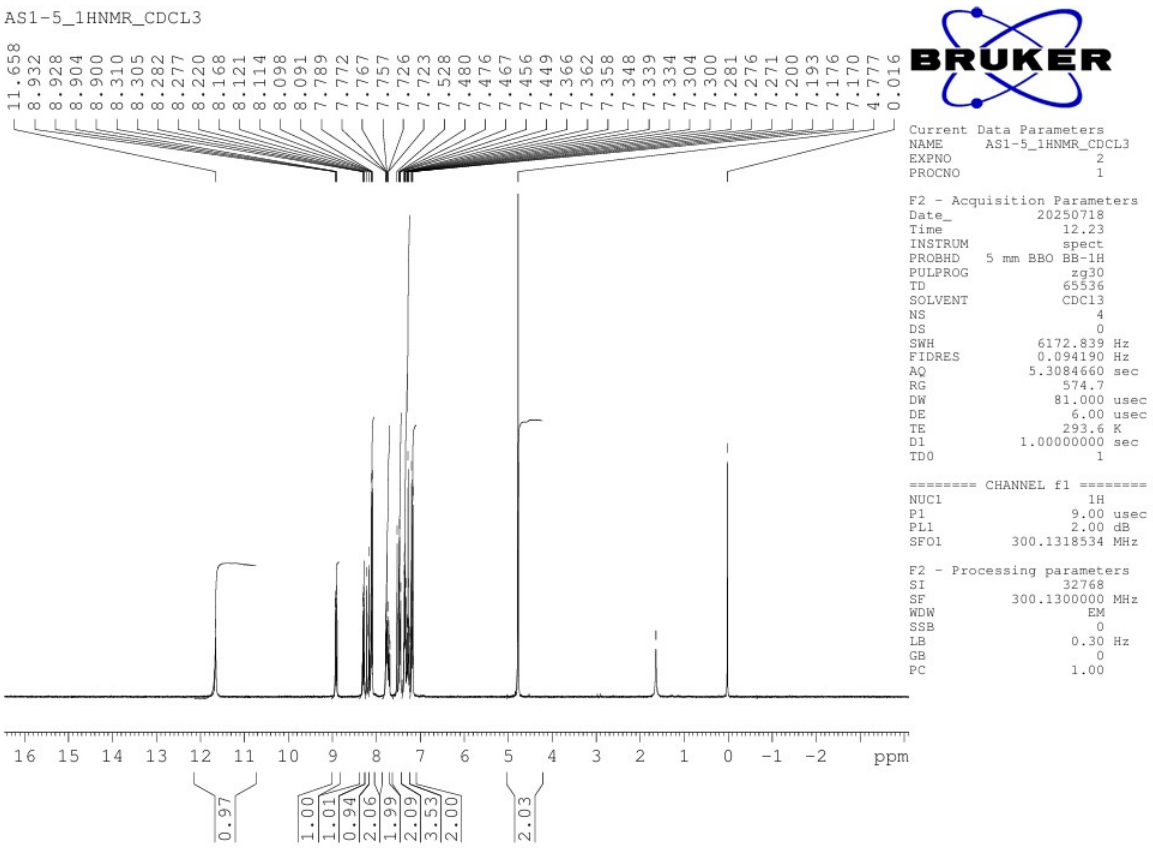


Figure S26. ^{1}H-NMR Spectrum of AS1-5.

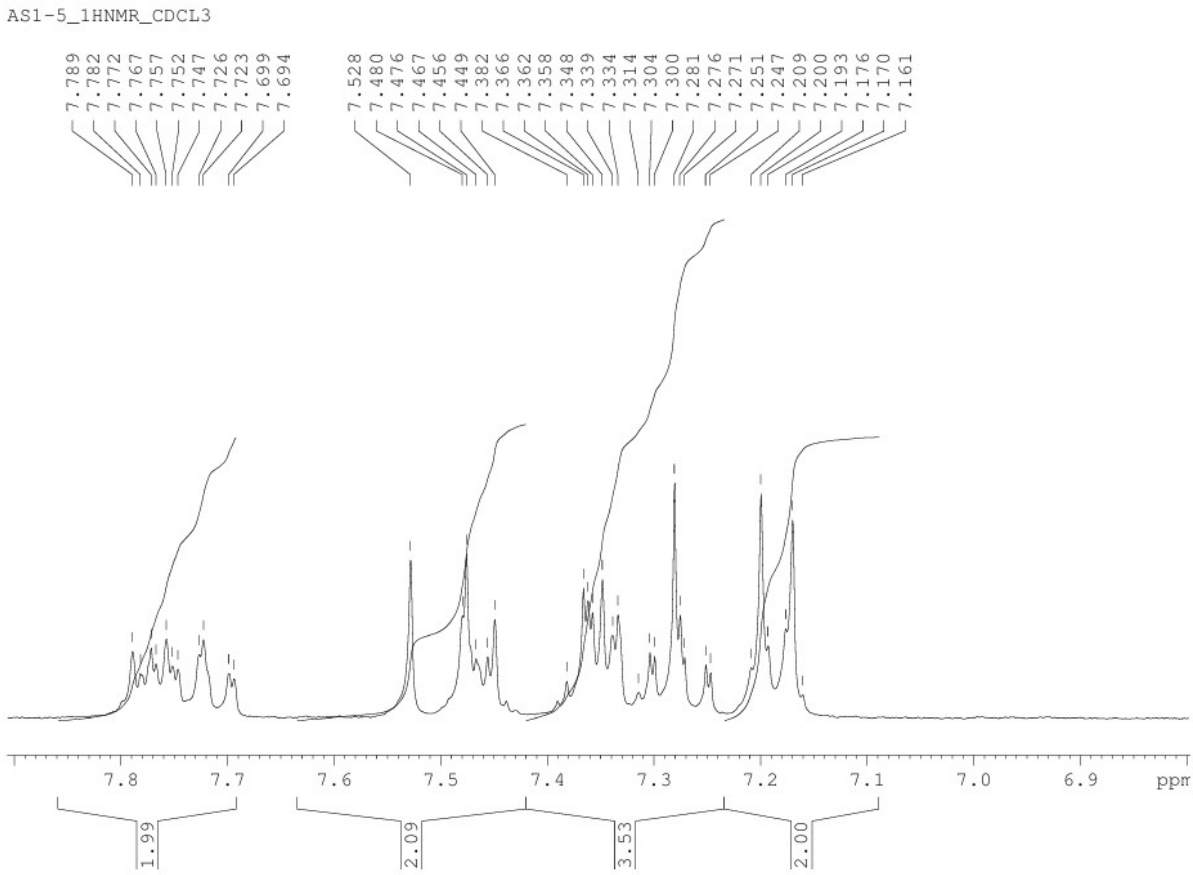


Figure S27. ^{1}H-NMR expansion of AS1-5.

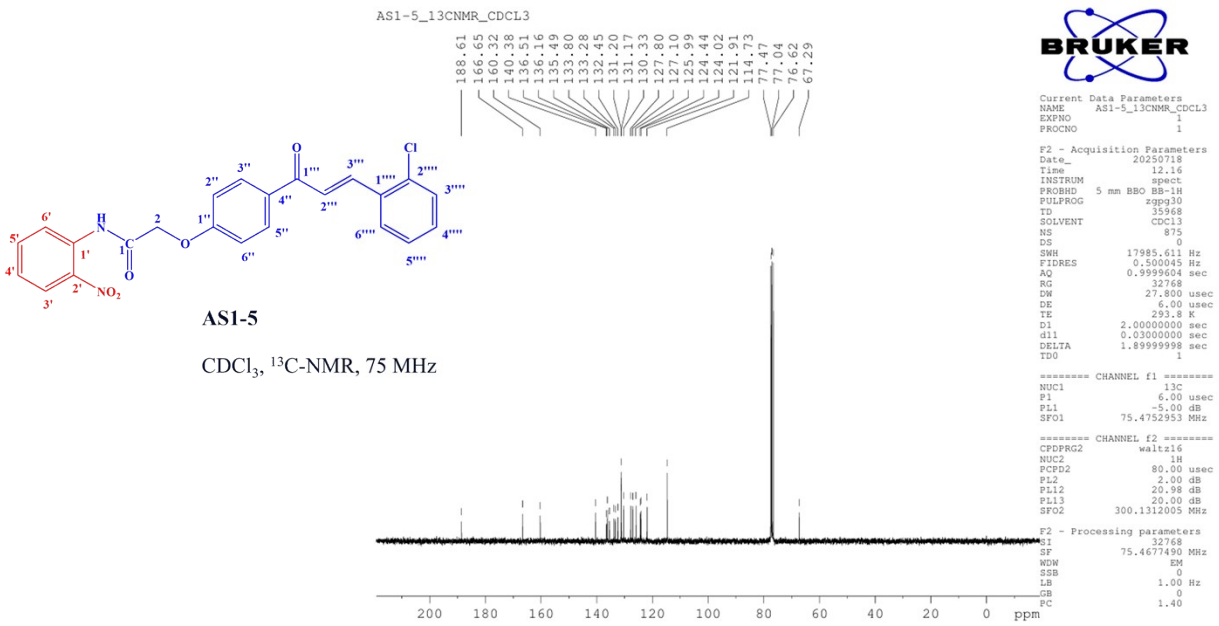


Figure S28. ^{13}C-NMR Spectrum of AS1-5.

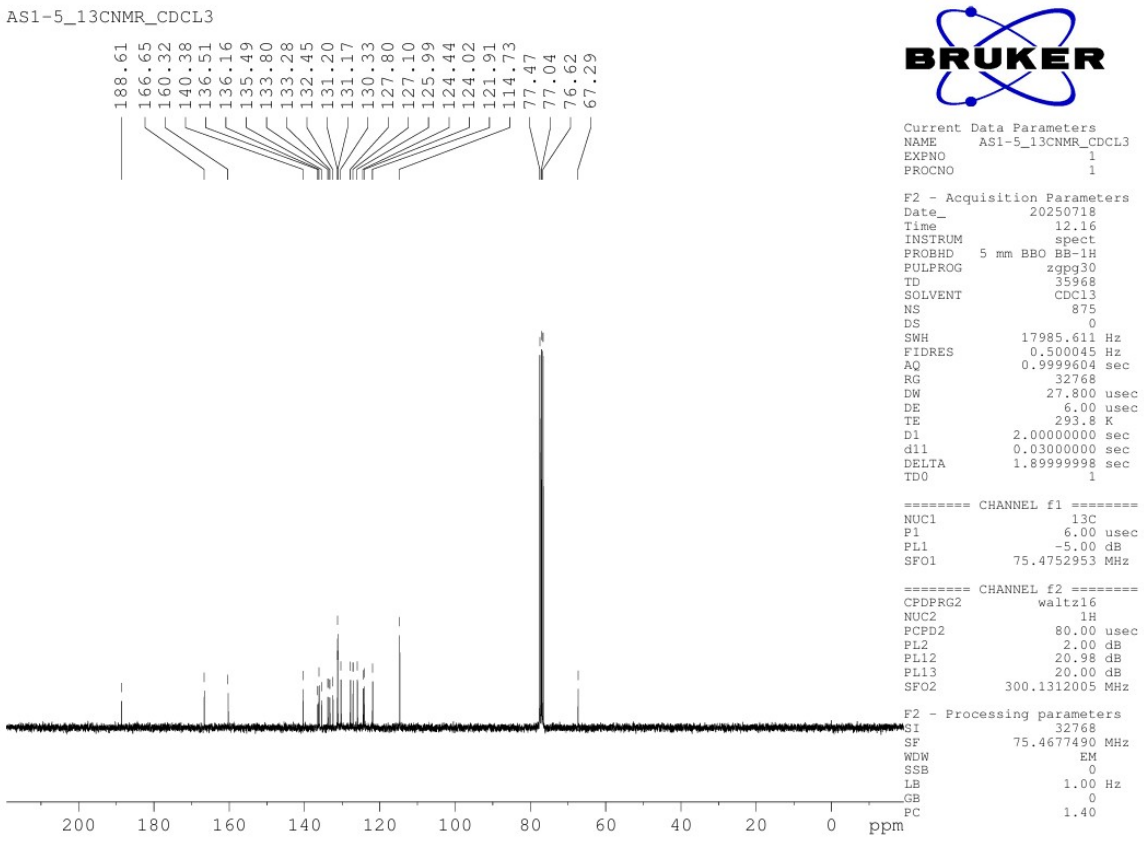


Figure S29. ^{13}C-NMR Spectrum of AS1-5.

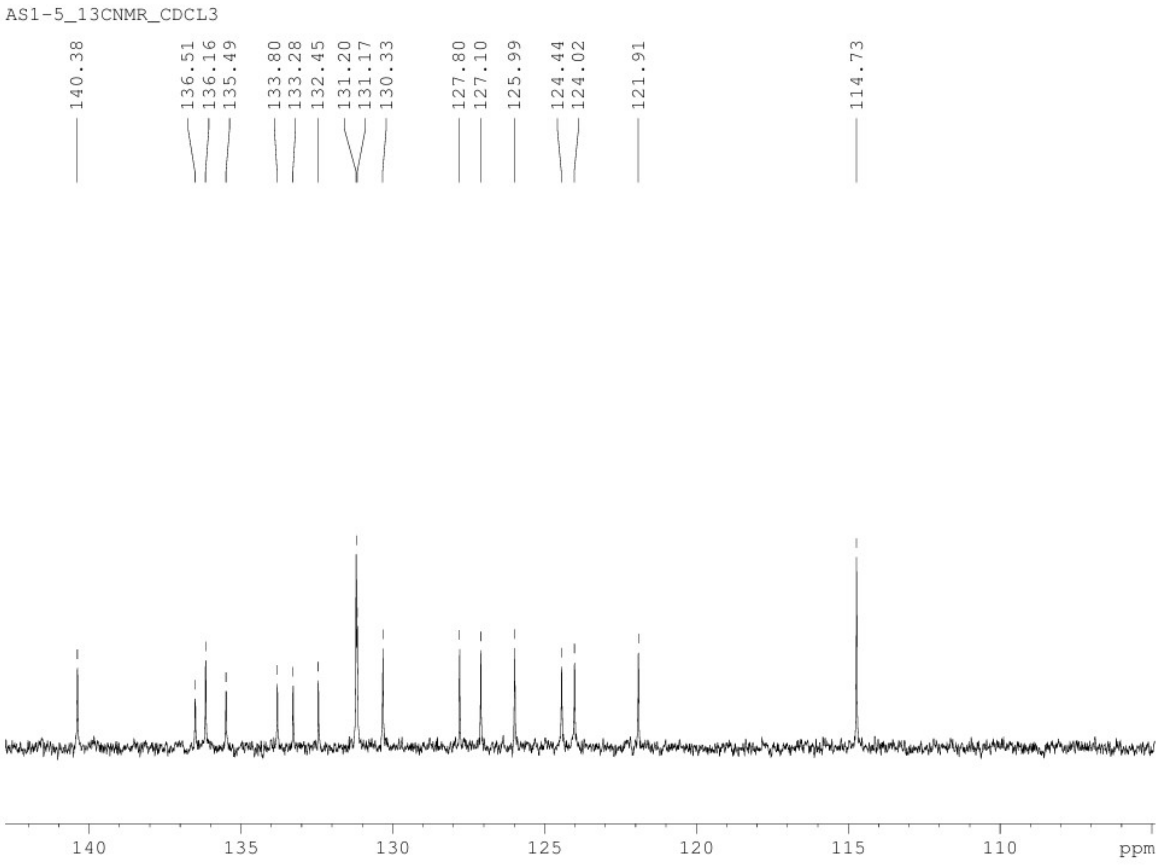


Figure S30. ^{13}C-NMR expansion of AS1-5.

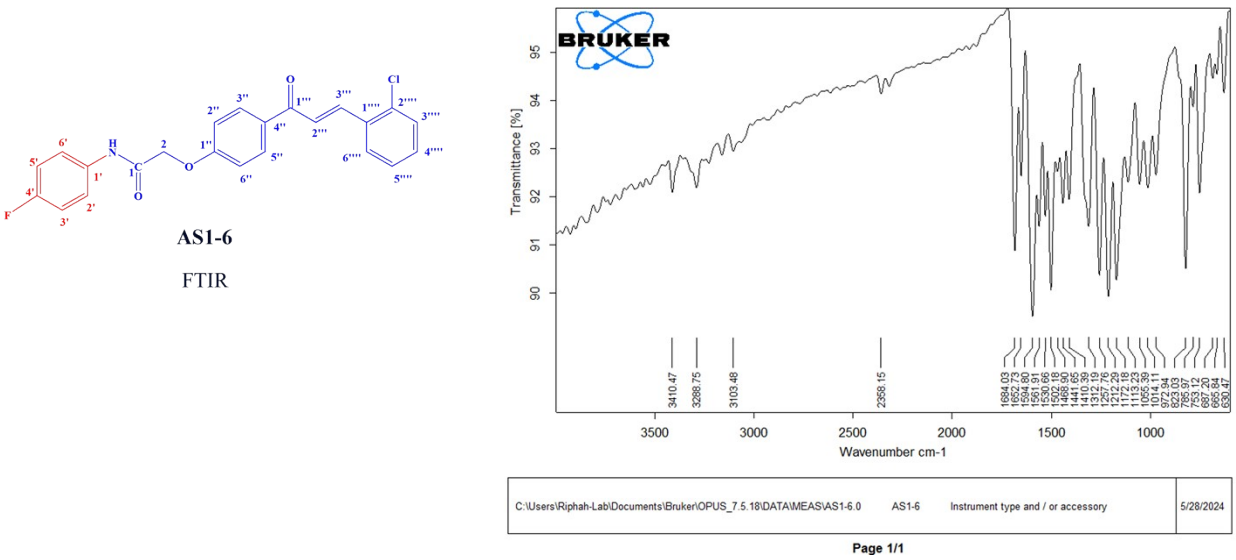


Figure S31. FTIR Spectrum of AS1-6.

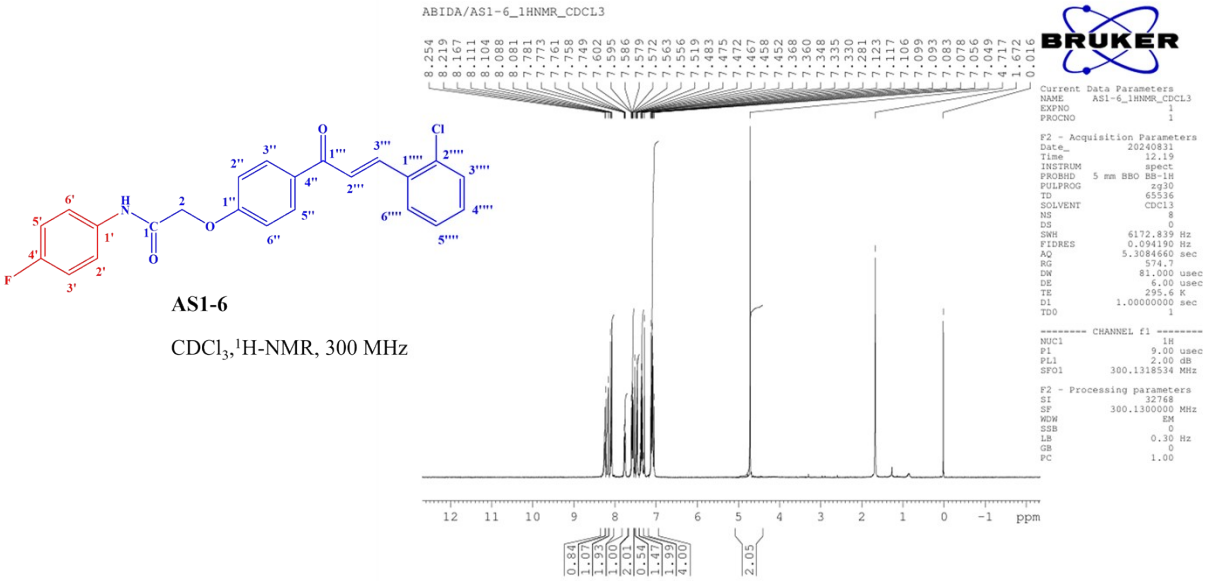


Figure S32. ^{1}H-NMR Spectrum of AS1-6.

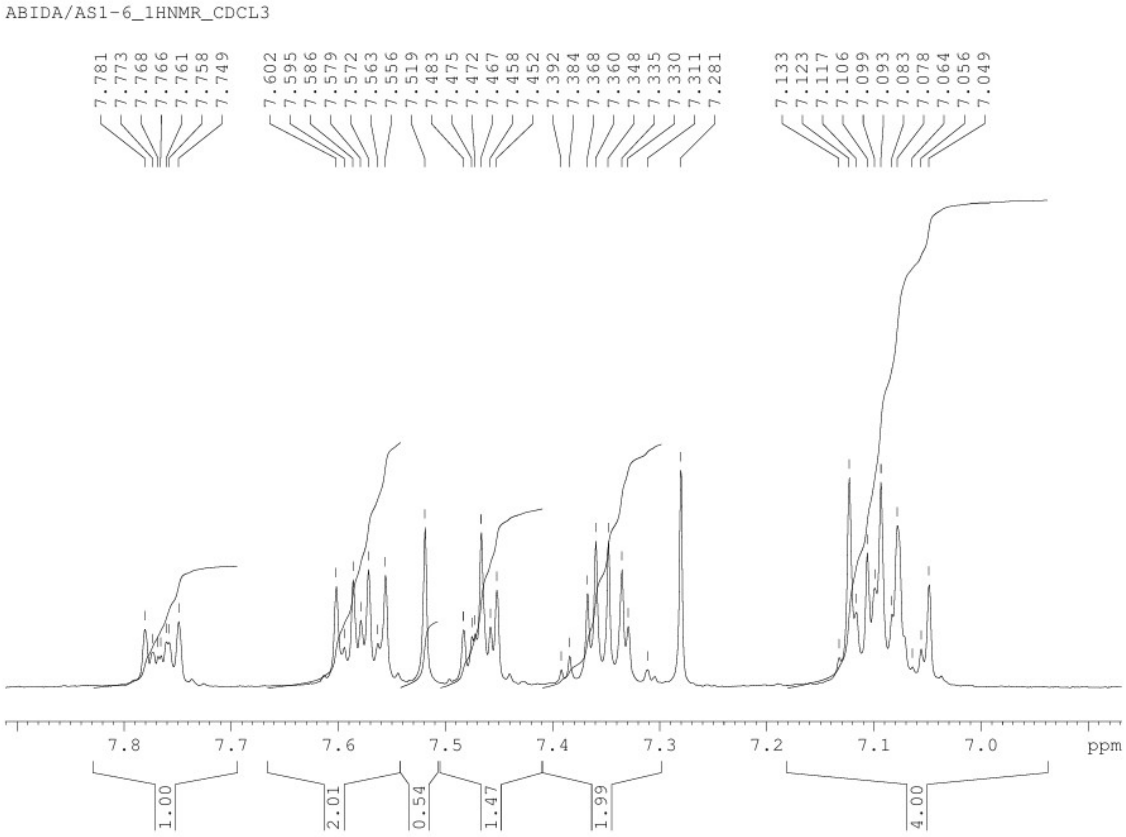


Figure S33. ^{1}H-NMR expansion of AS1-6.

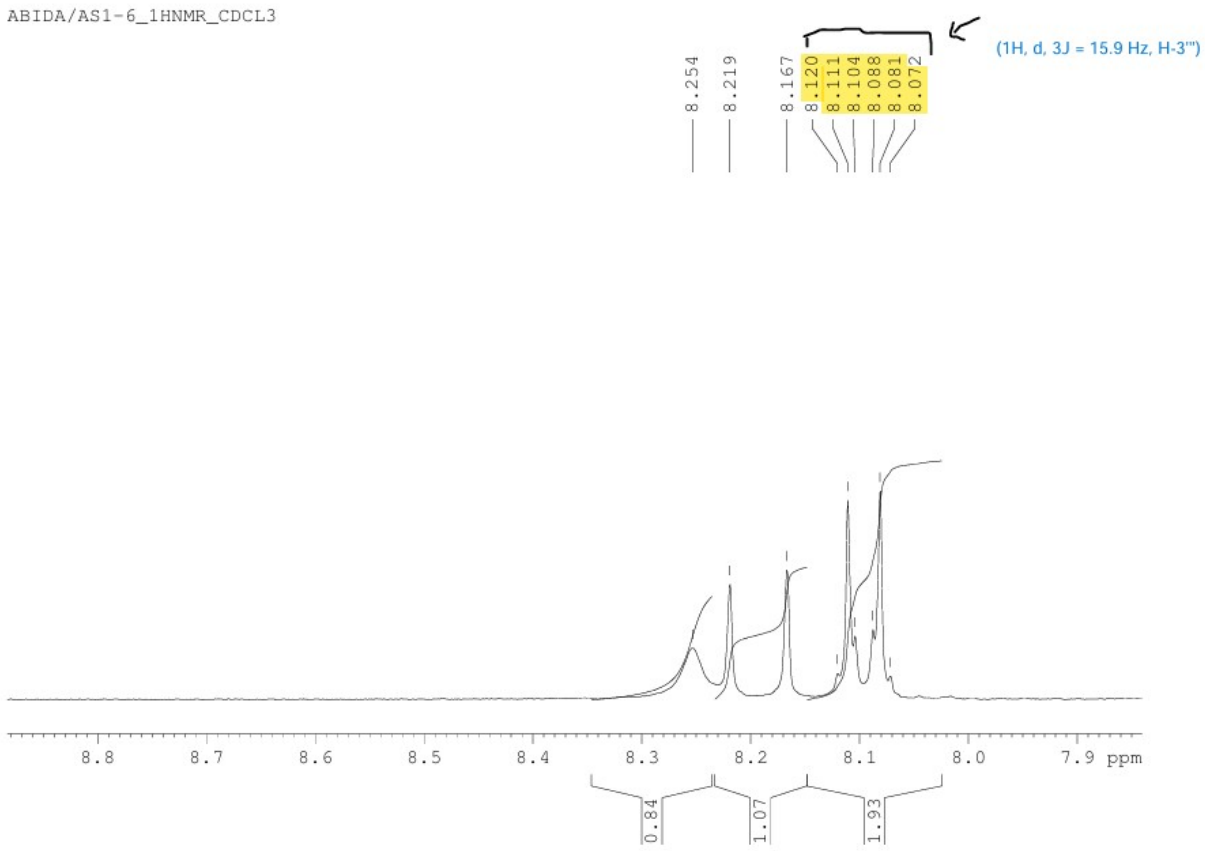


Figure S34. ^{1}H-NMR expansion of AS1-6.

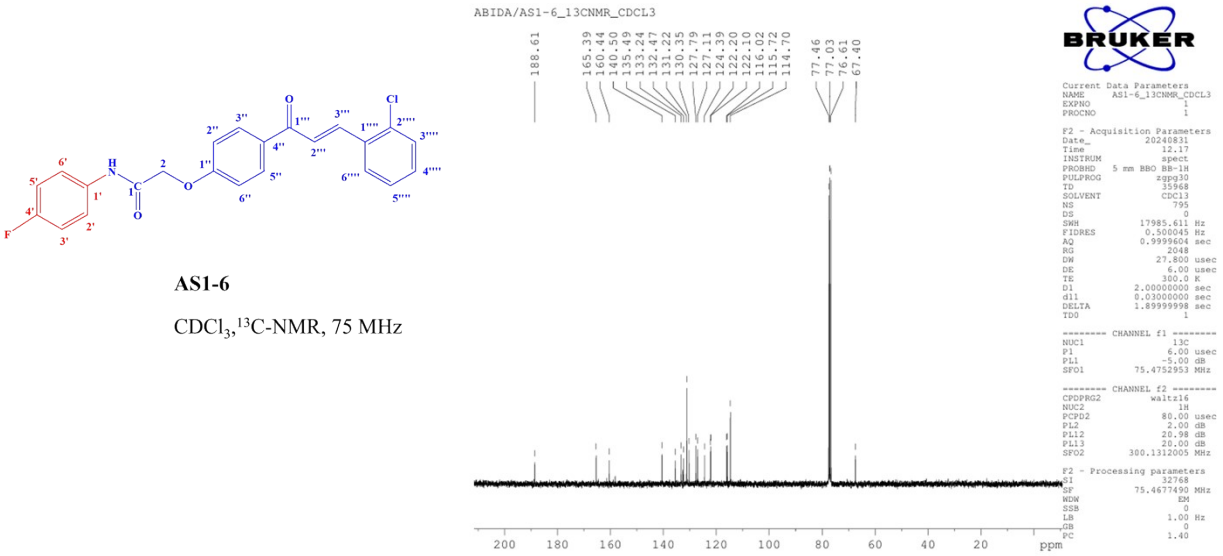


Figure S35. ^{13}C-NMR Spectrum of AS1-6.

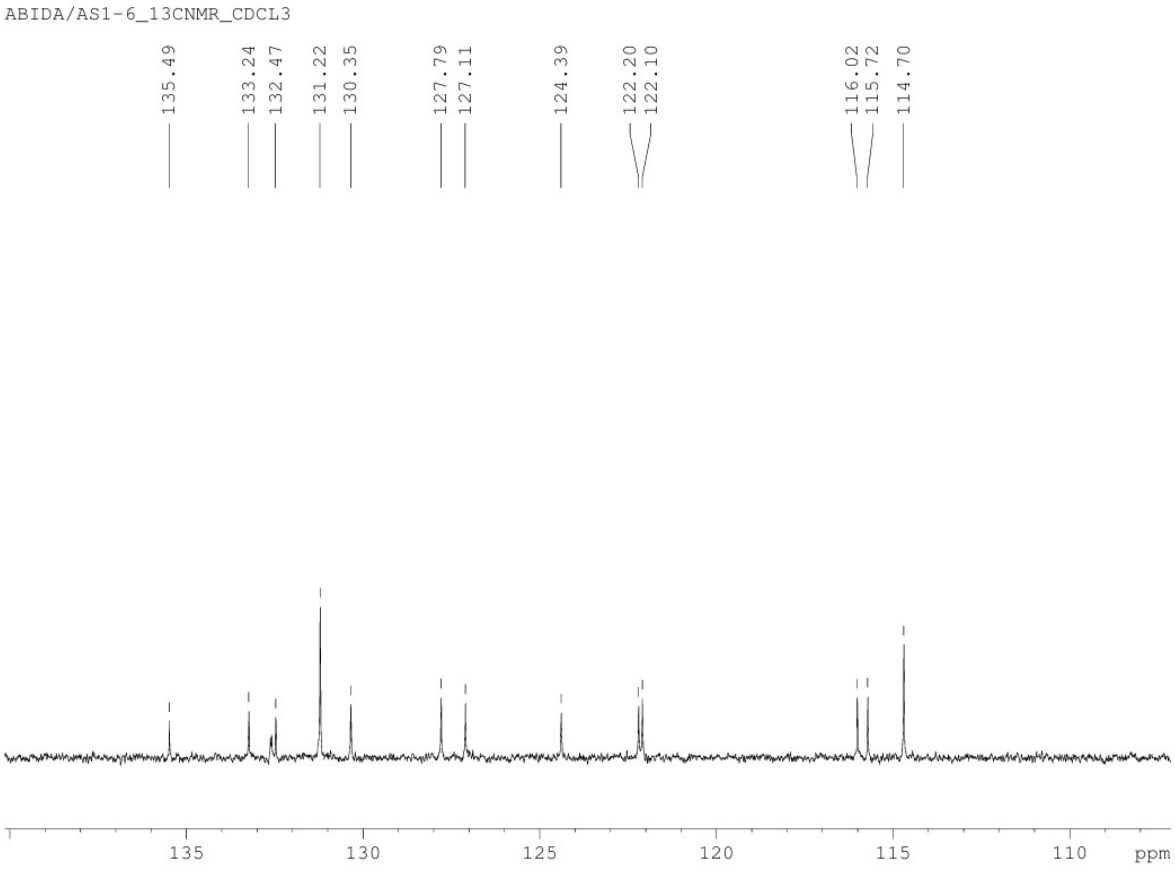


Figure S36. ^{13}C-NMR expansion of AS1-6.

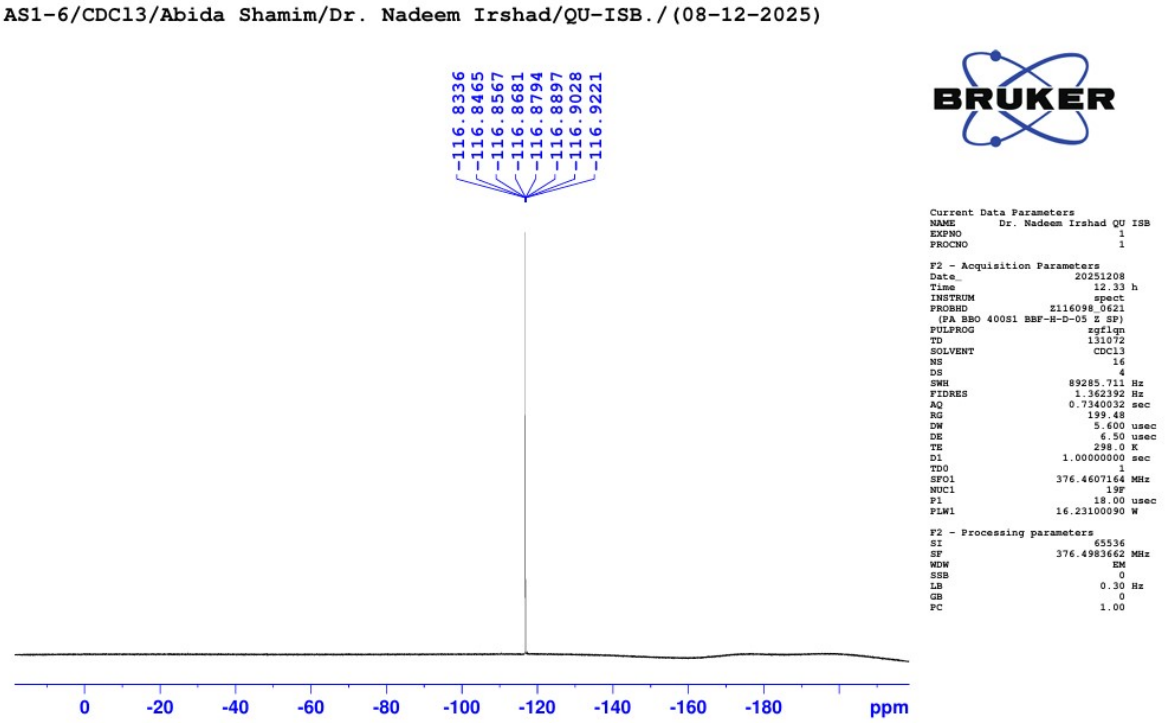


Figure S37. ^{19}F-NMR Spectrum of AS1-6.

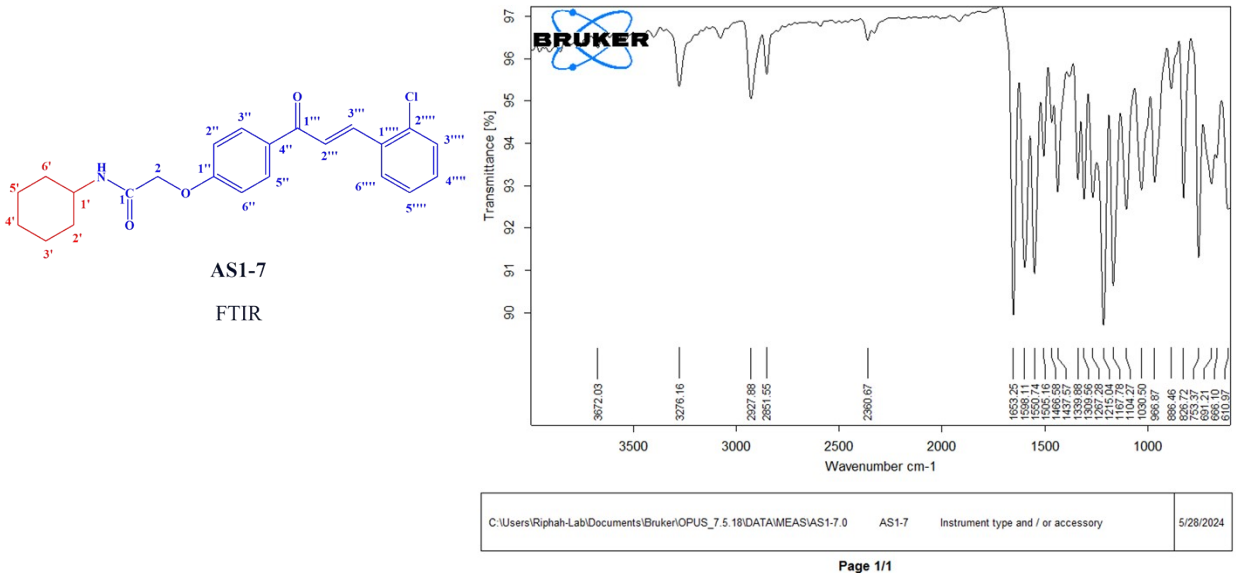


Figure S38. FTIR Spectrum of AS1-7.

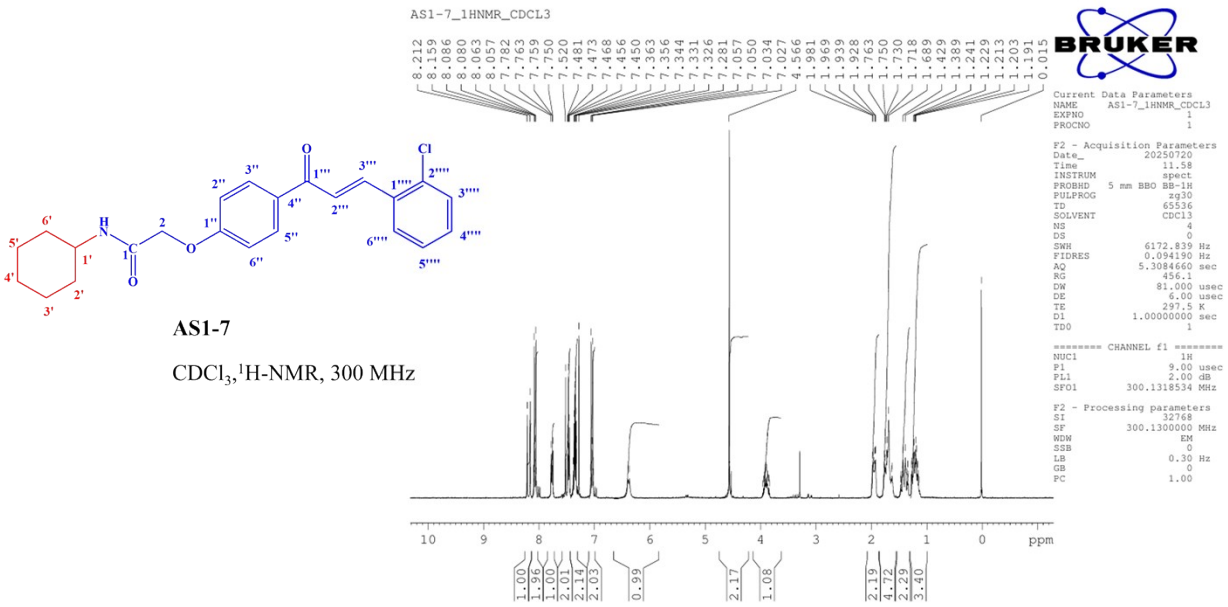


Figure S39. ^{1}H-NMR Spectrum of AS1-7.

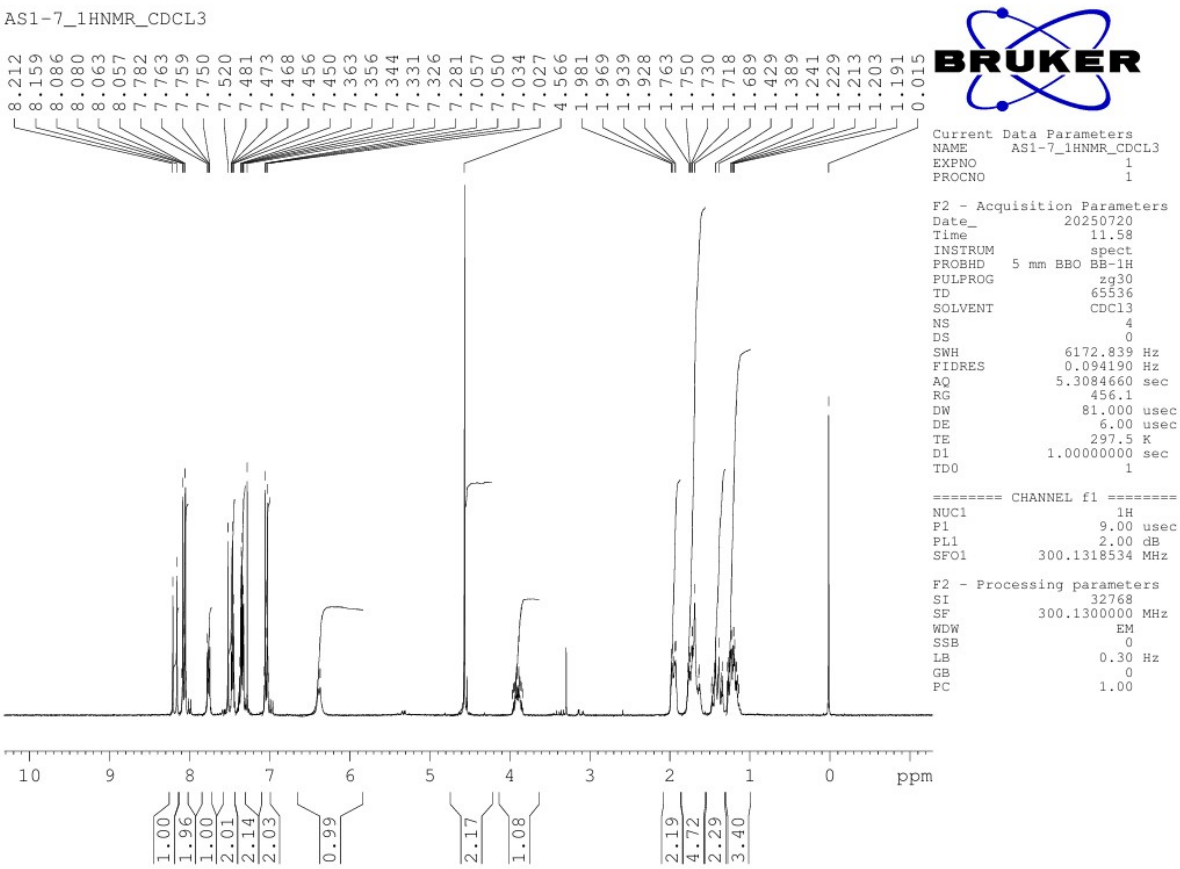


Figure S40. ^{1}H-NMR Spectrum of AS1-7.

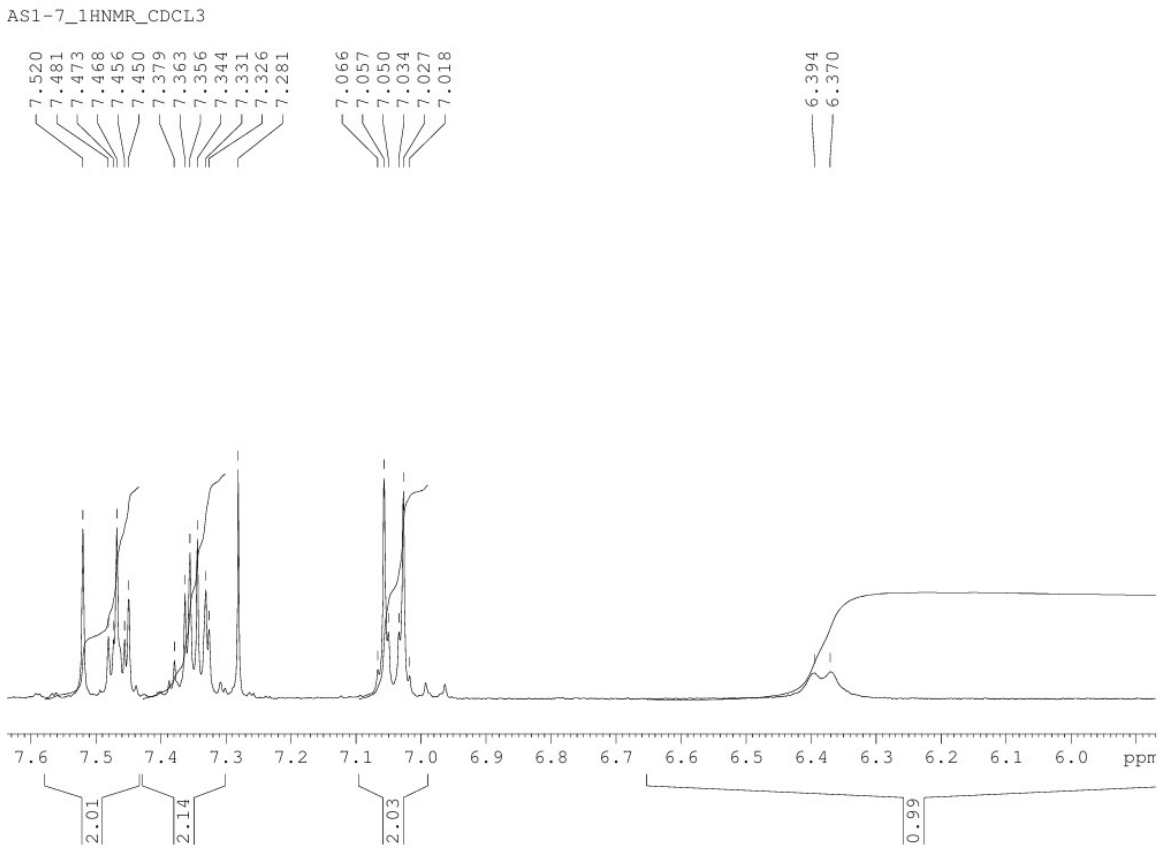


Figure S41. ^{1}H-NMR expansion of AS1-7.

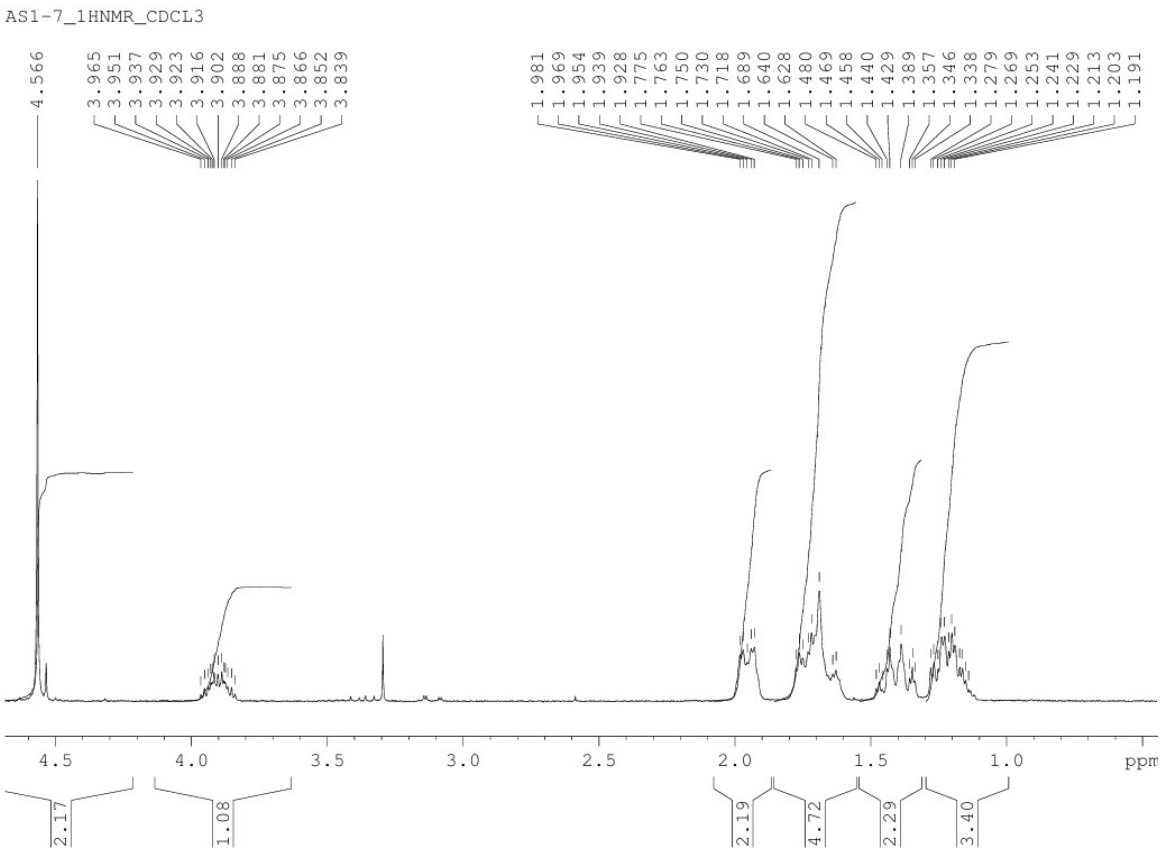


Figure S42. ^{1}H-NMR expansion of AS1-7.

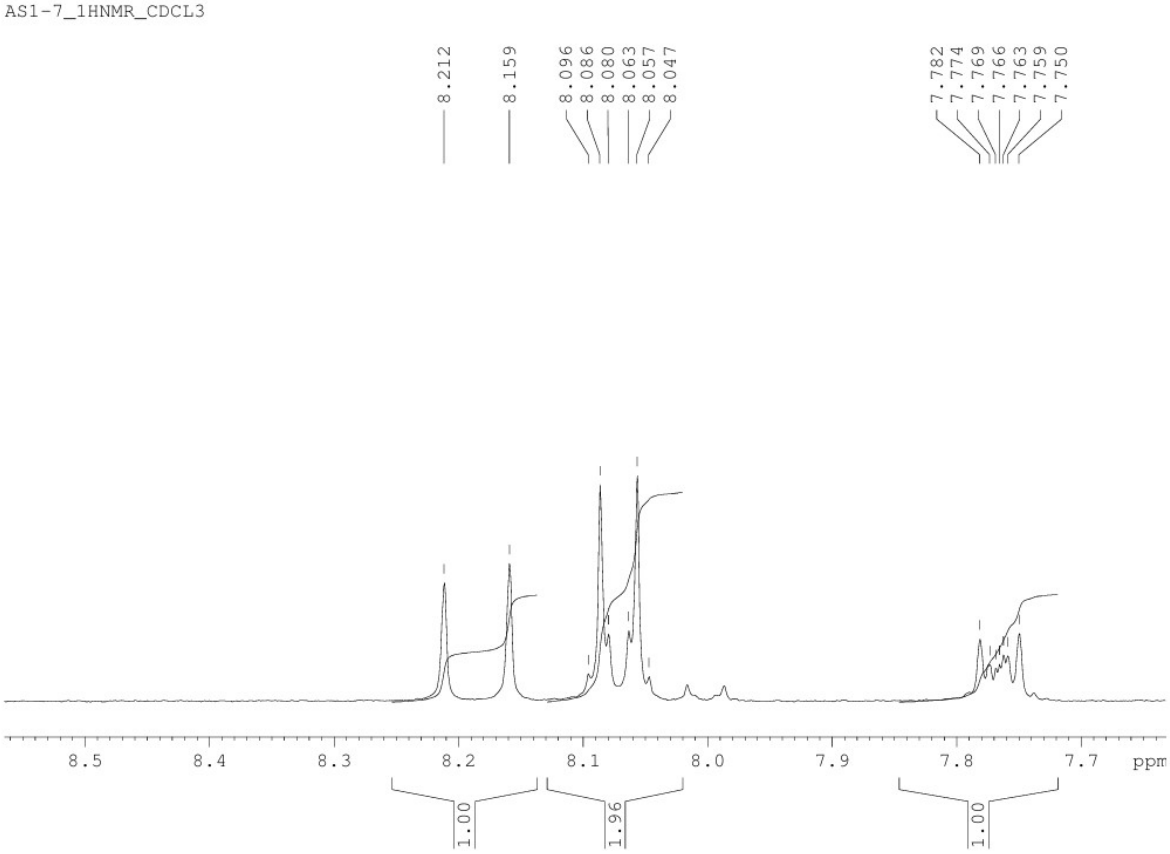
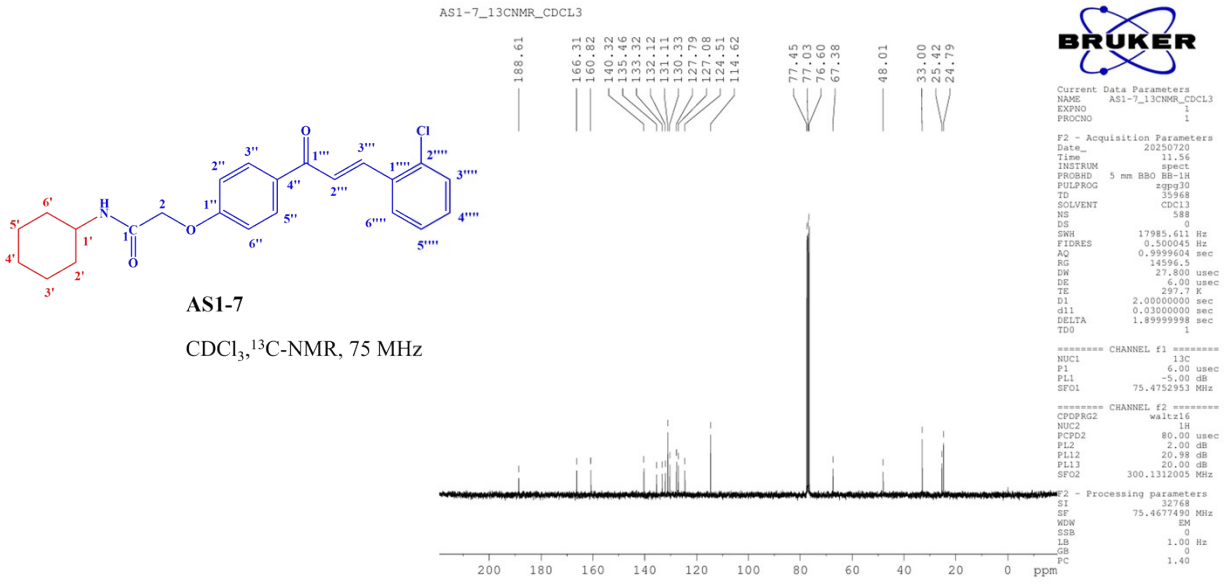


Figure S43. ^{1}H-NMR expansion of AS1-7.



AS1-7

$CDCl_3$,^{13}C-NMR, 75 MHz

Figure S44. ^{13}C-NMR Spectrum of AS1-7.

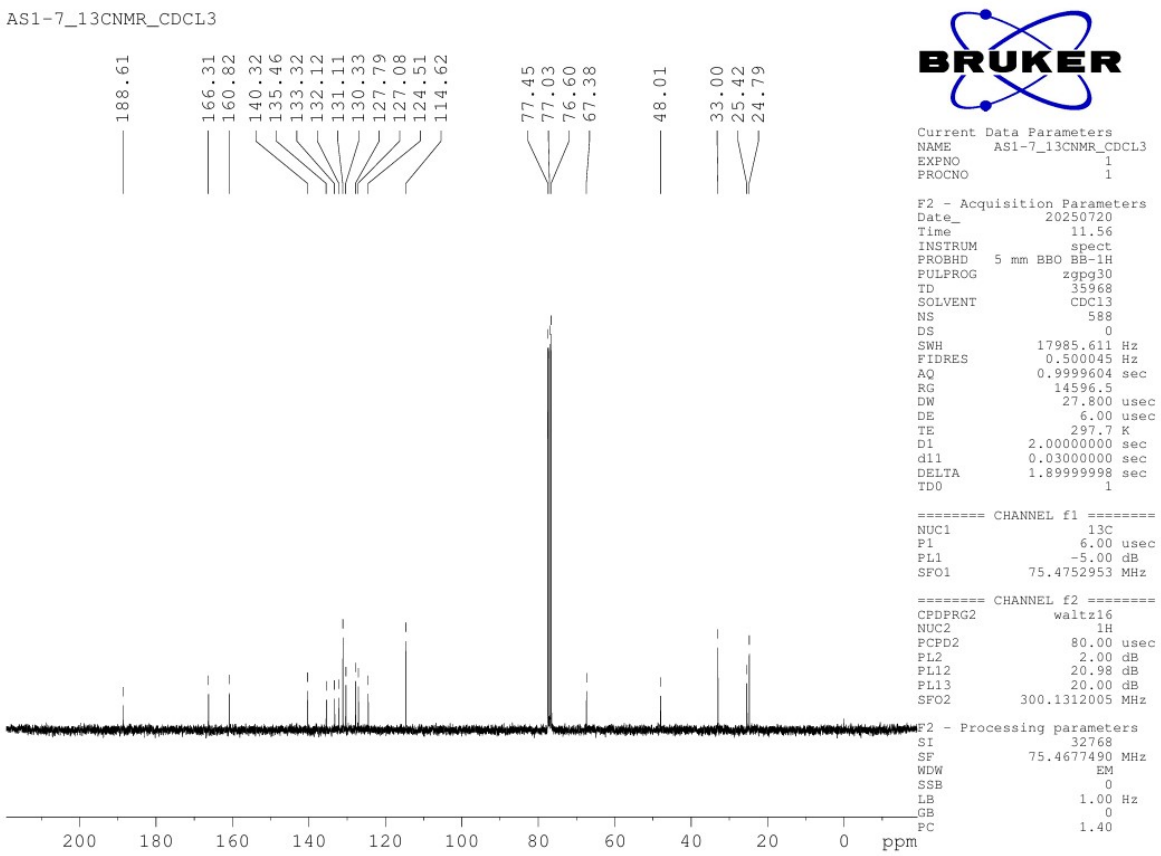


Figure S45. ^{13}C-NMR Spectrum of AS1-7.

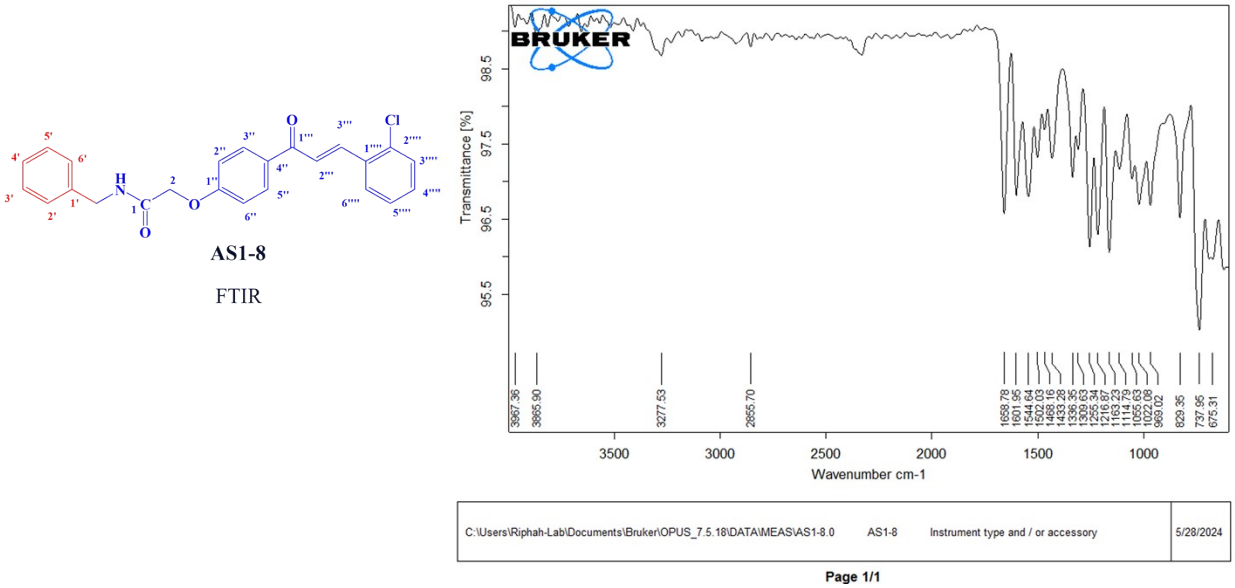
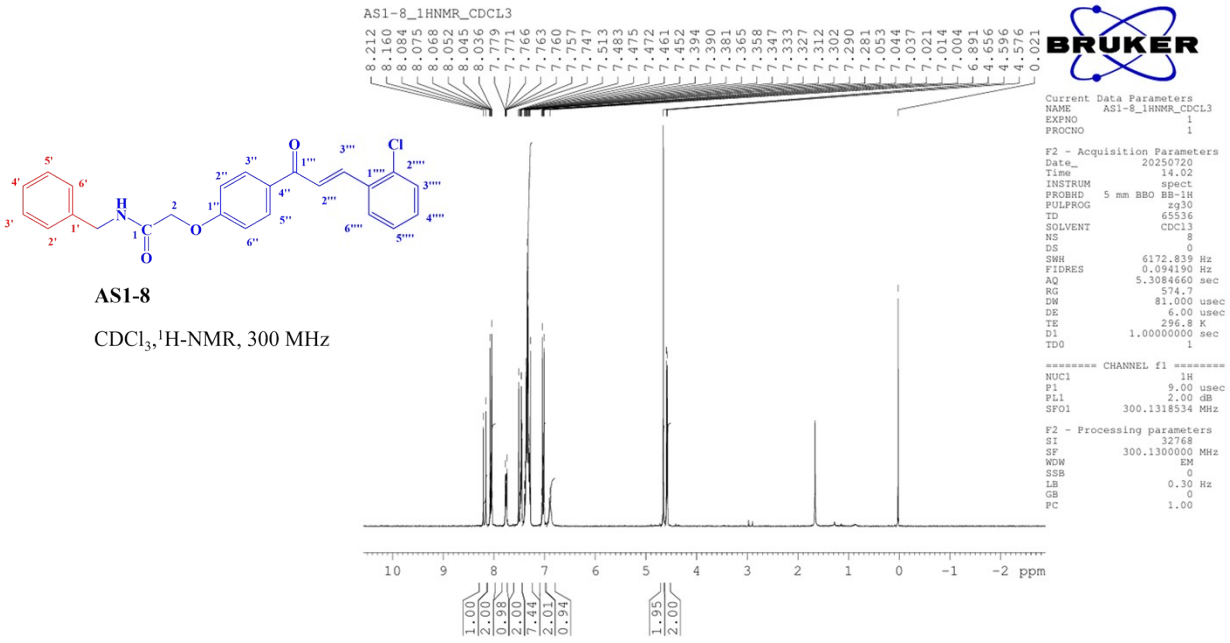


Figure S46. FTIR Spectrum of AS1-8.



AS1-8

$CDCl_3$,^{1}H-NMR, 300 MHz

Figure S47. ^{1}H-NMR Spectrum of AS1-8.

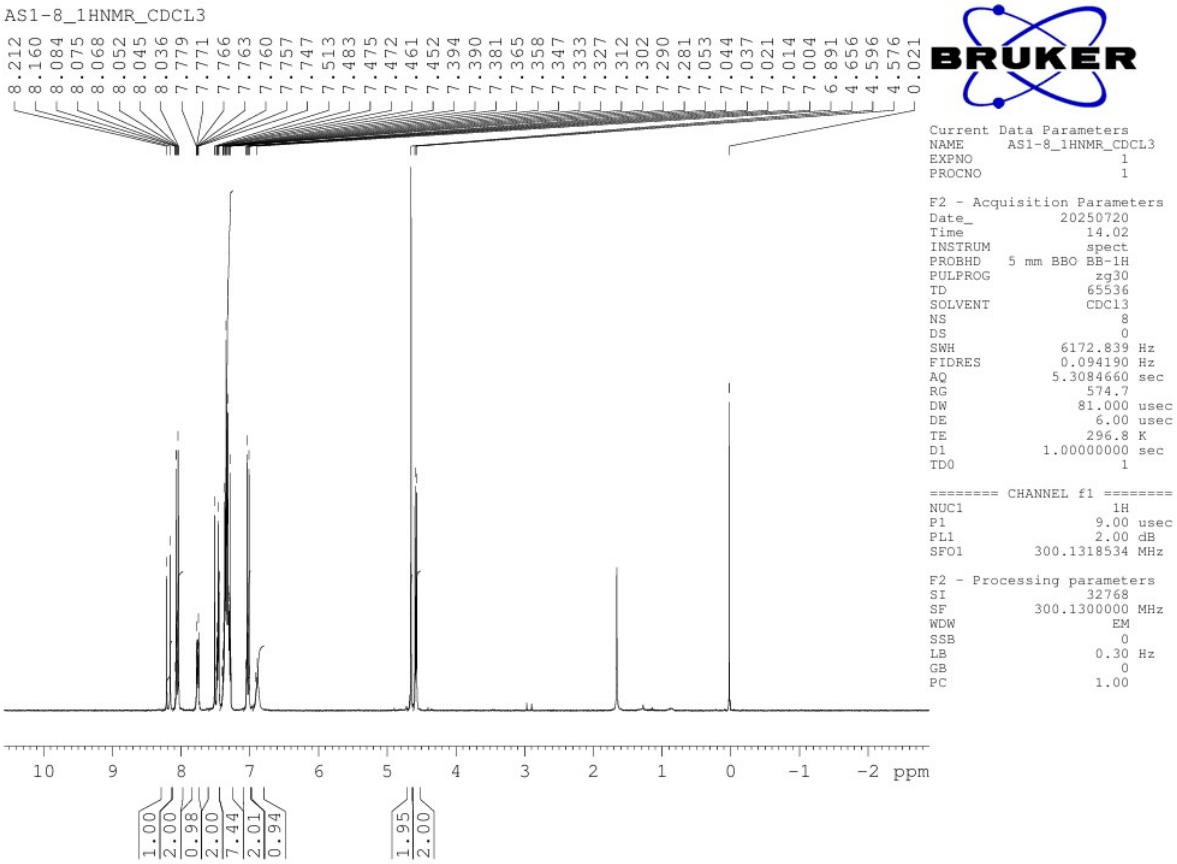


Figure S48. ^{1}H-NMR Spectrum of AS1-8.

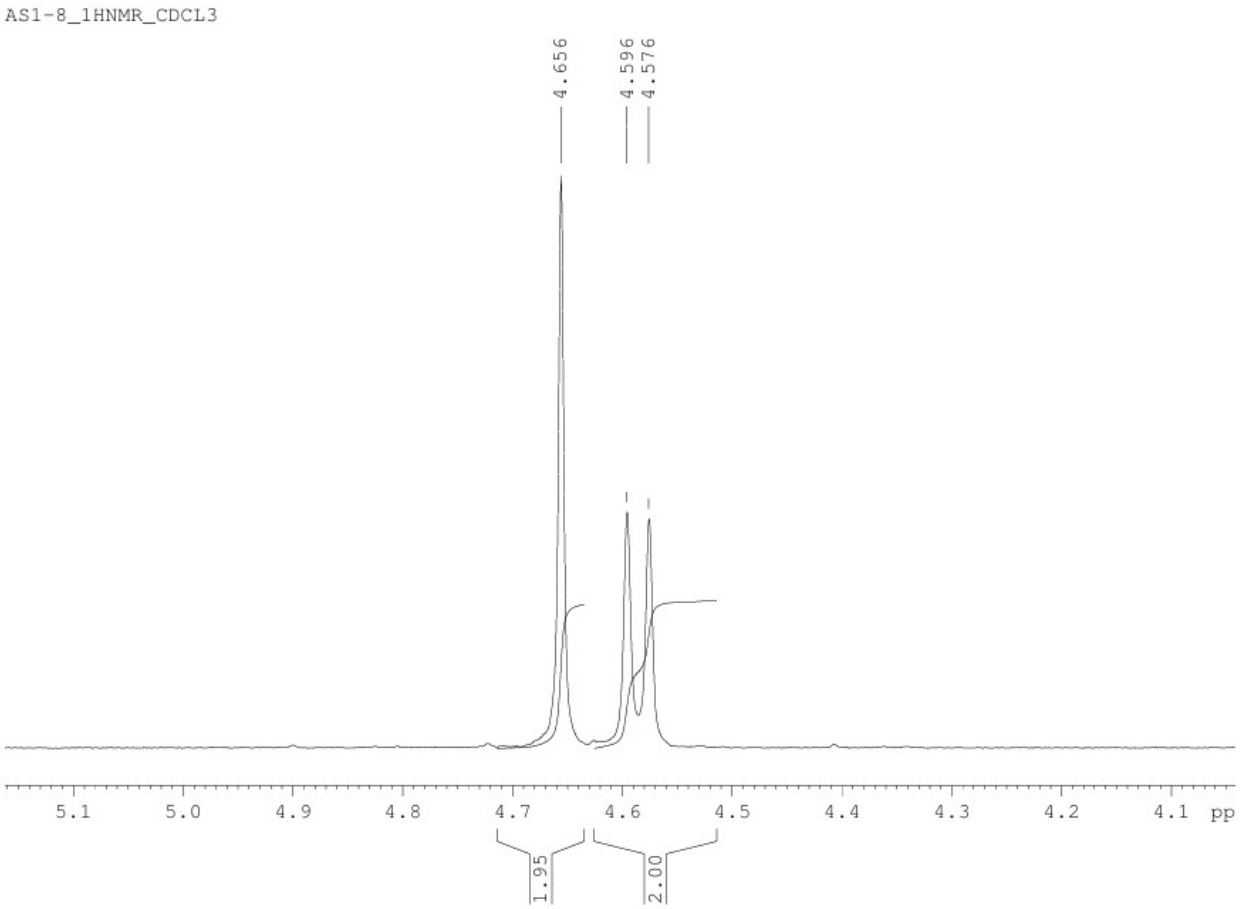


Figure S49. ^{1}H-NMR expansion of AS1-8.

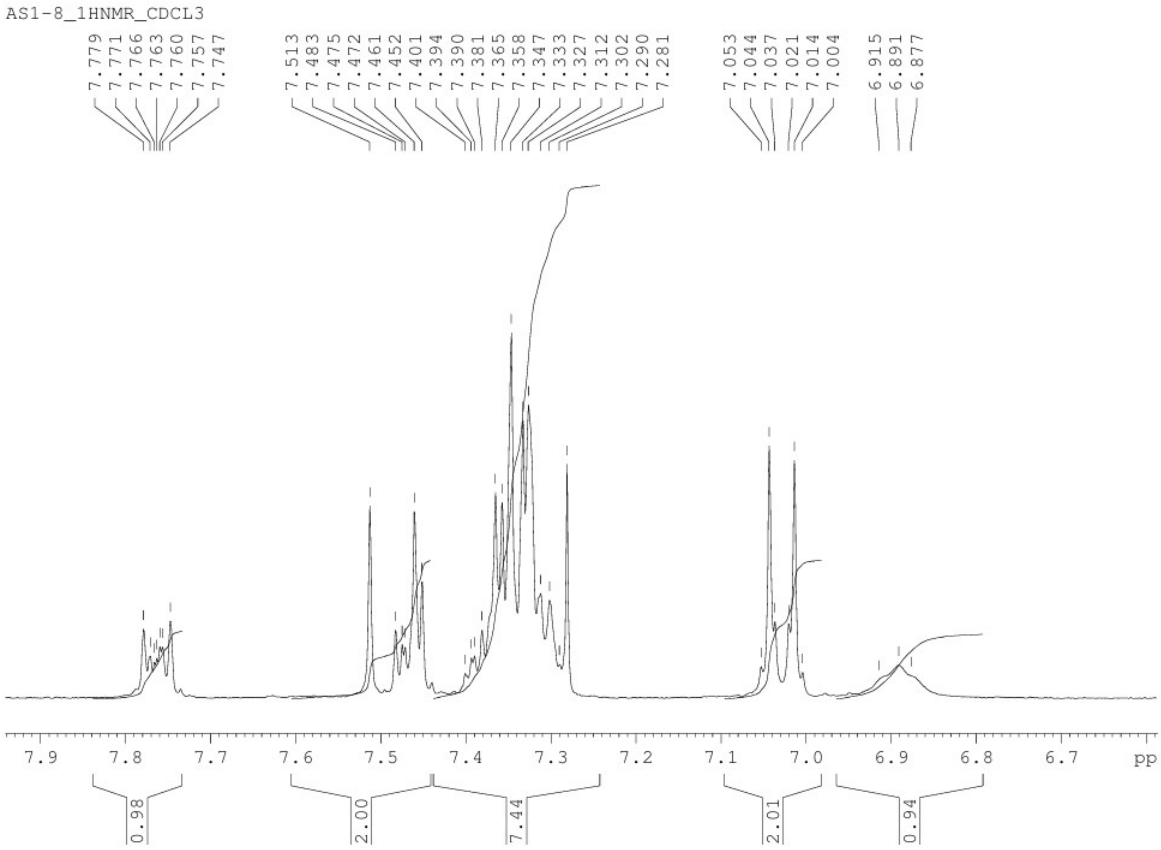


Figure S50. ^{1}H-NMR expansion of AS1-8.

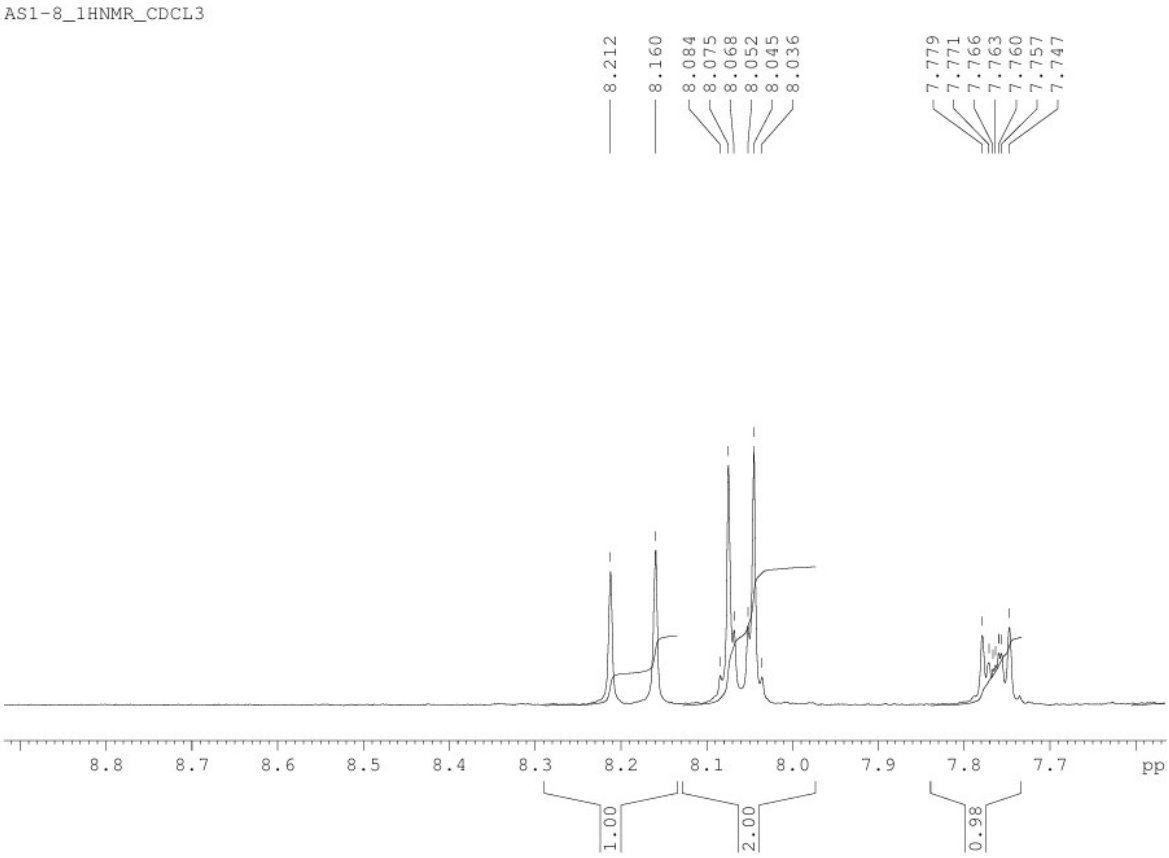
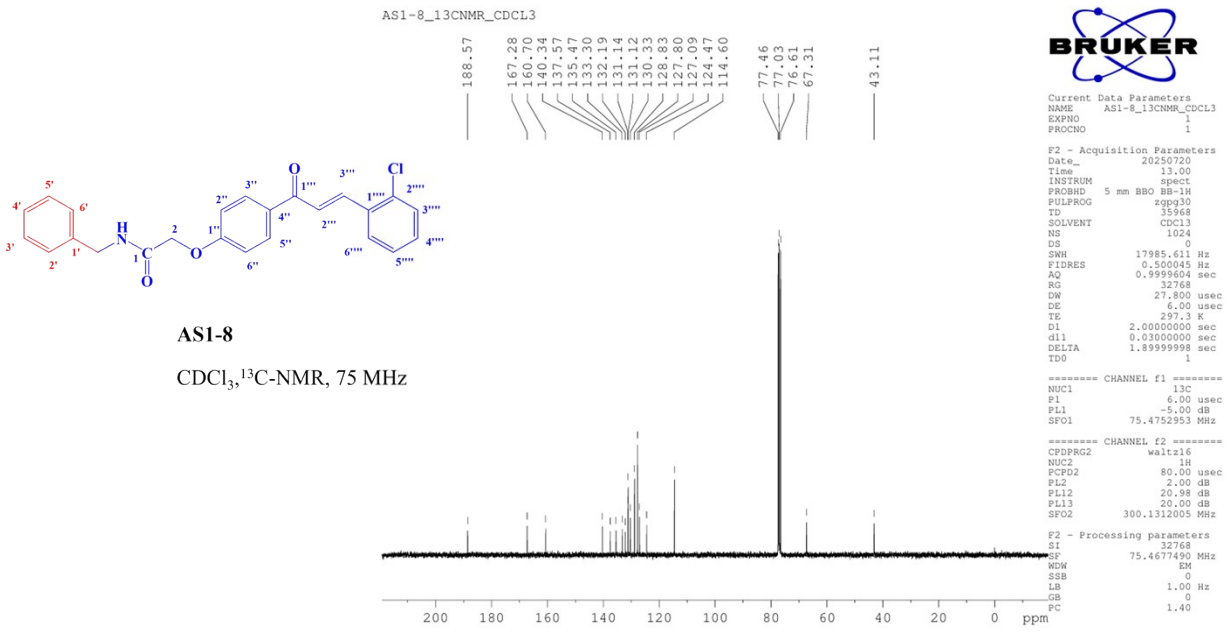


Figure S51. ^{1}H-NMR expansion of AS1-8.



AS1-8

$CDCl_3$, ^{13}C-NMR, 75 MHz

Figure S52. ^{13}C-NMR Spectrum of AS1-8.

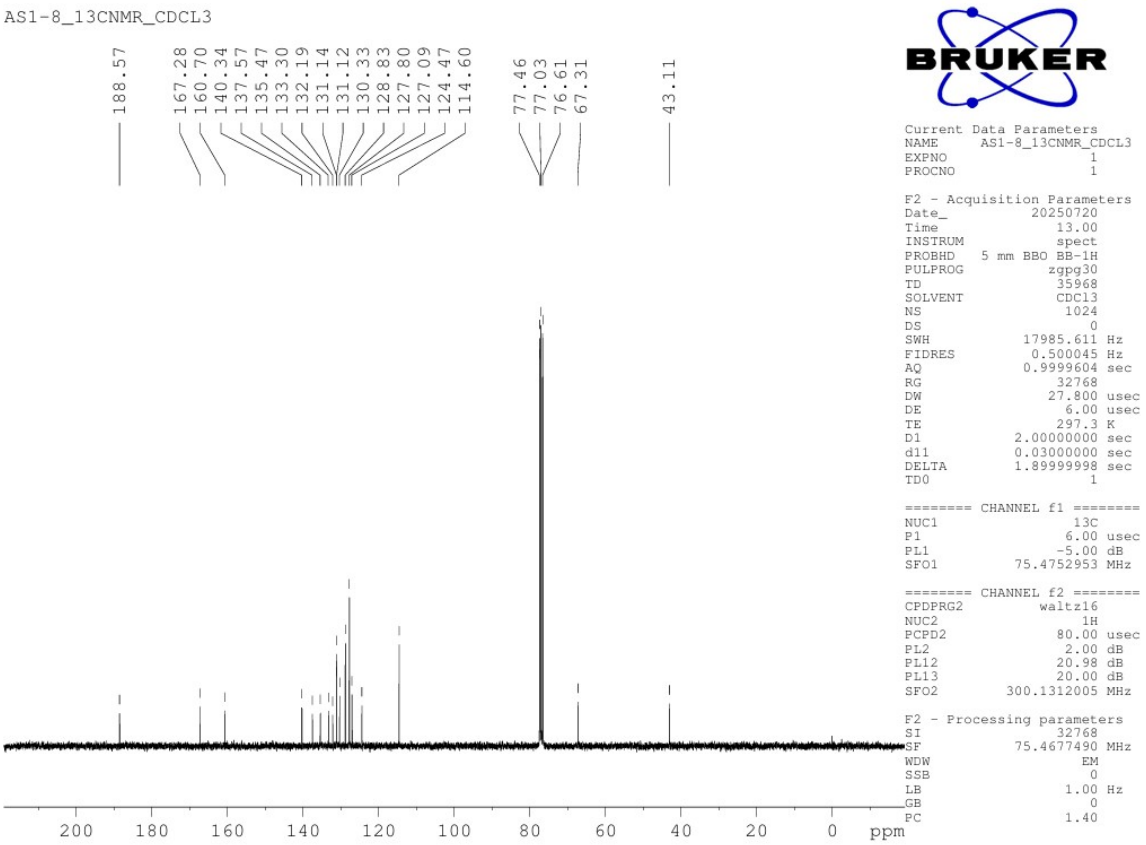


Figure S53. ^{13}C-NMR Spectrum of AS1-8.

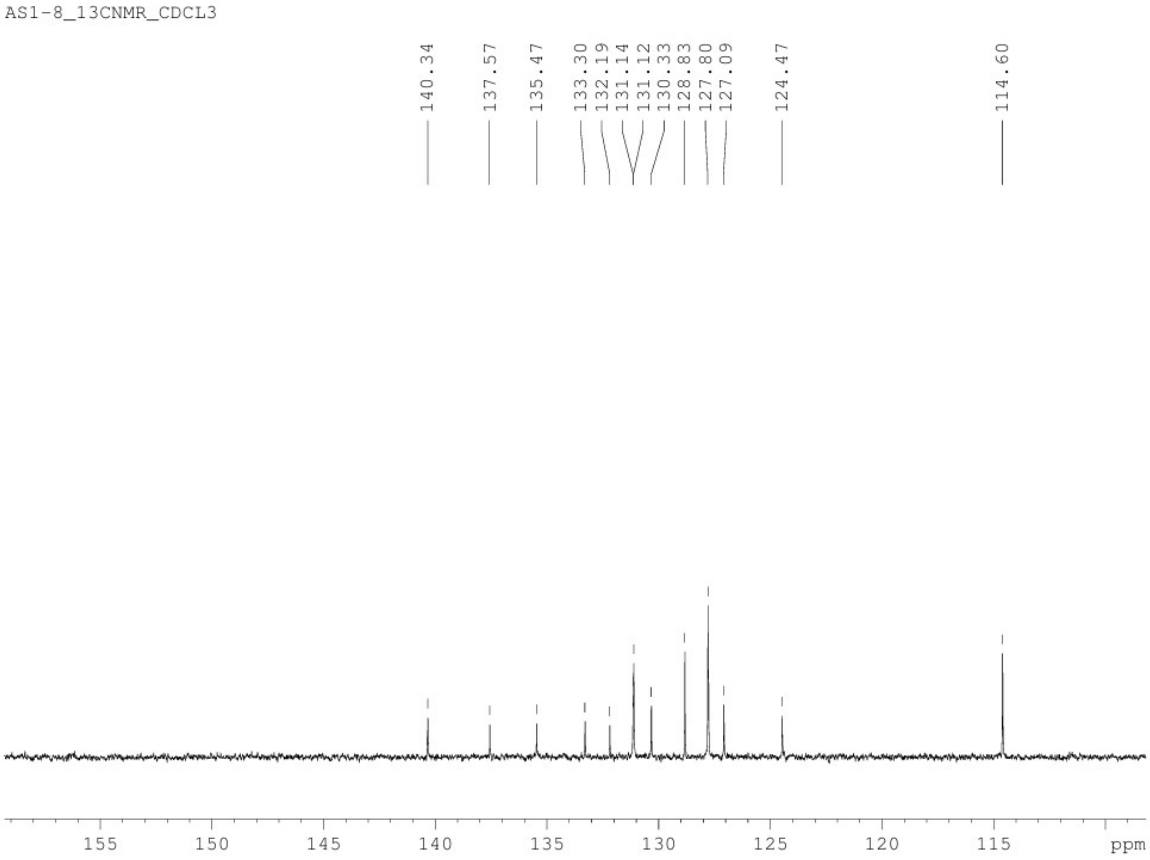


Figure S54. ^{13}C-NMR expansion of AS1-8.

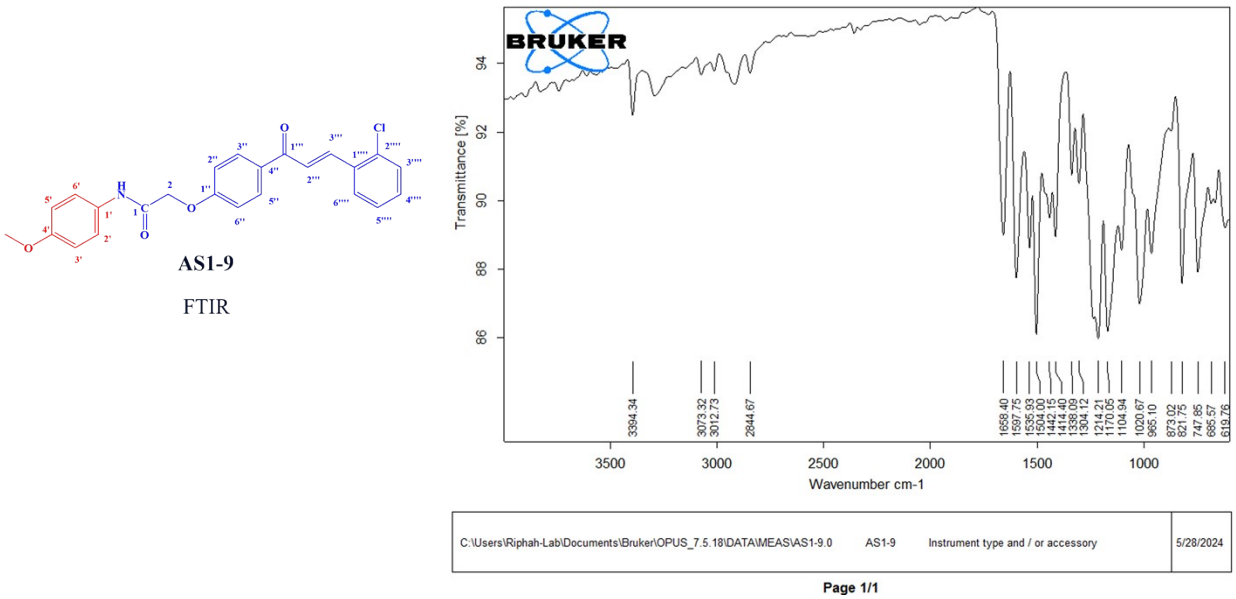


Figure S55. FTIR Spectrum of AS1-9.

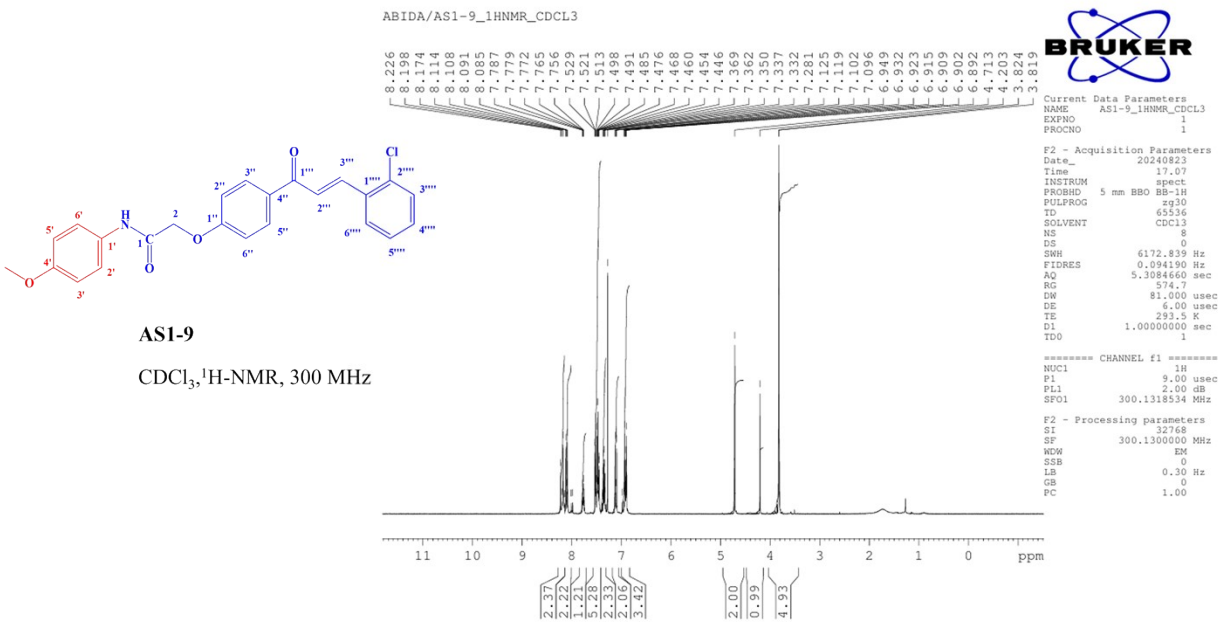


Figure S56. ^{1}H-NMR Spectrum of AS1-9.

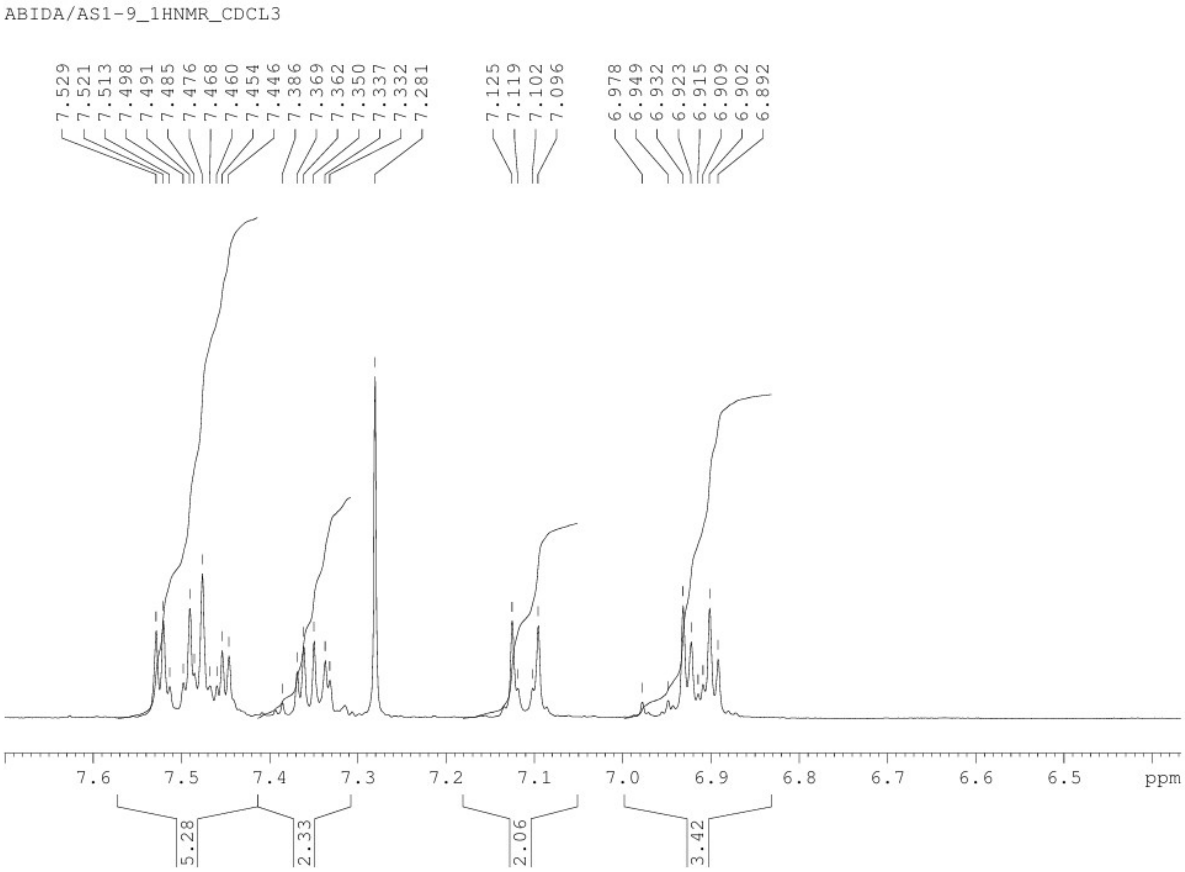


Figure S57. ^{1}H-NMR expansion of AS1-9.

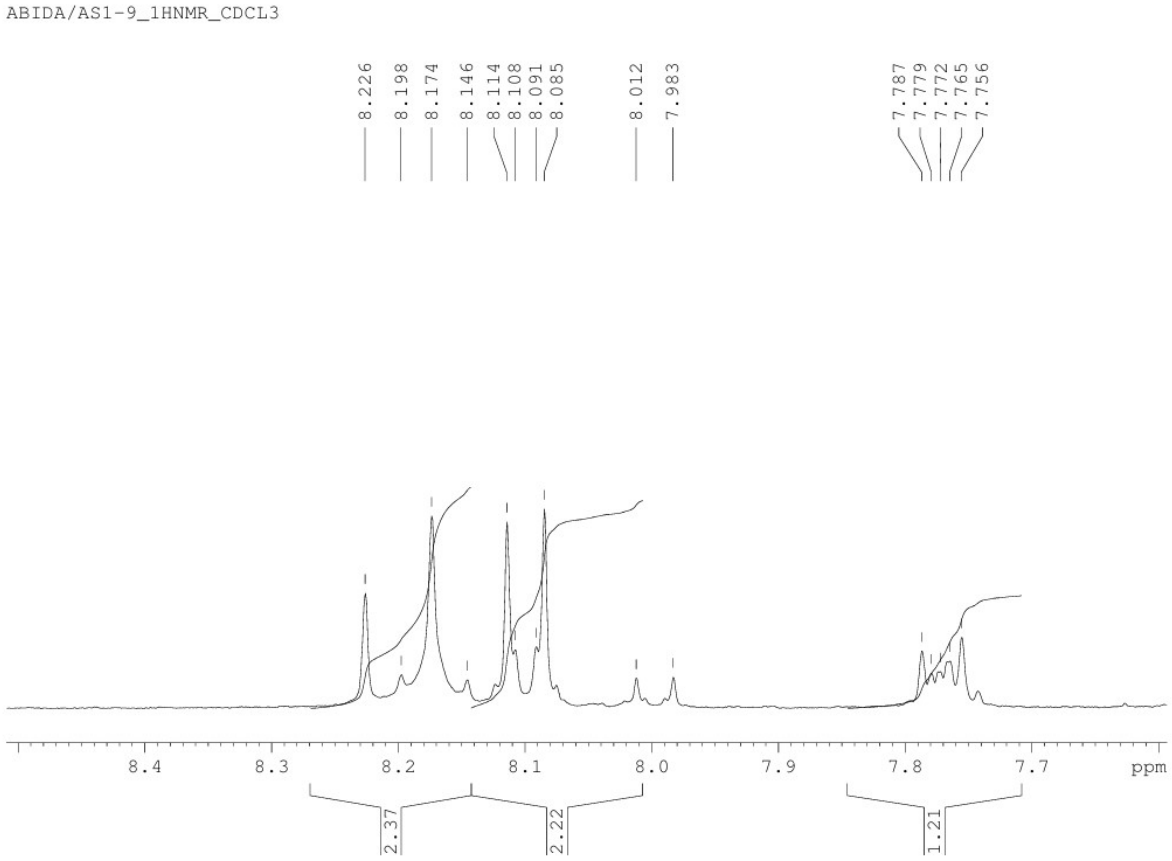
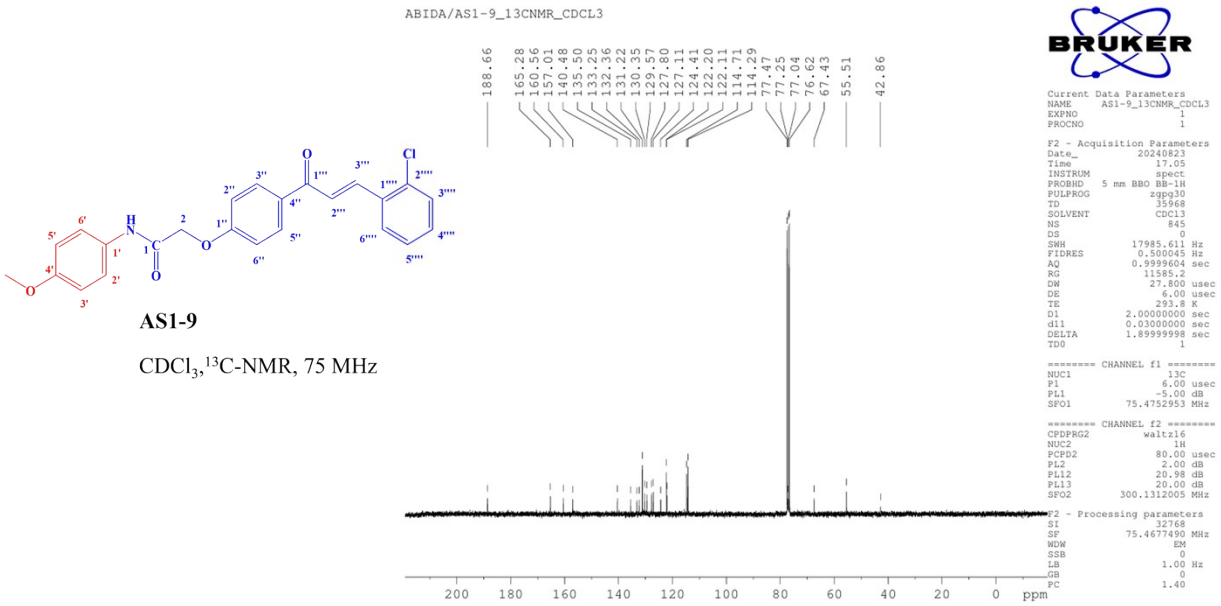


Figure S58 ^{1}H-NMR expansion of AS1-9.



AS1-9

$CDCl_3$,^{13}C-NMR, 75 MHz

Figure S59. ^{13}C-NMR Spectrum of AS1-9.

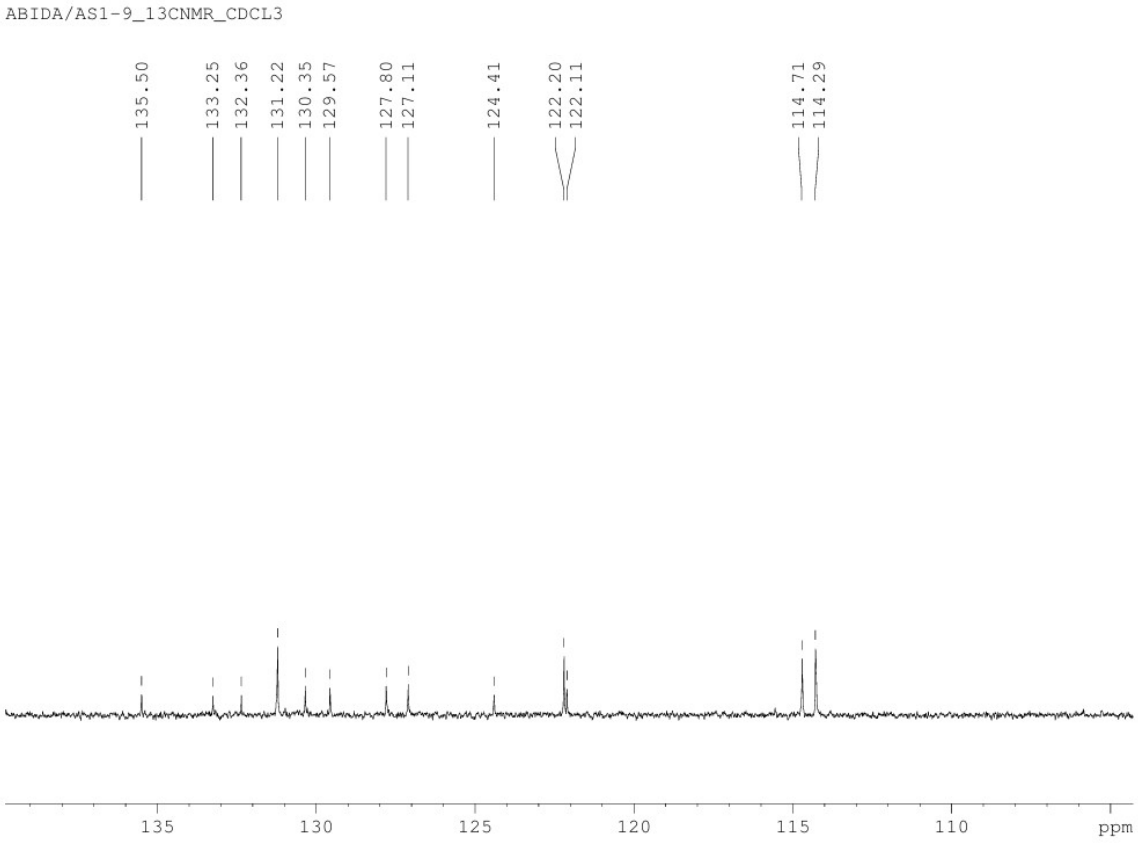
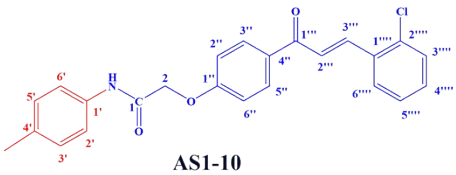


Figure S60. ^{13}C-NMR expansion of AS1-9.



AS1-10

FTIR

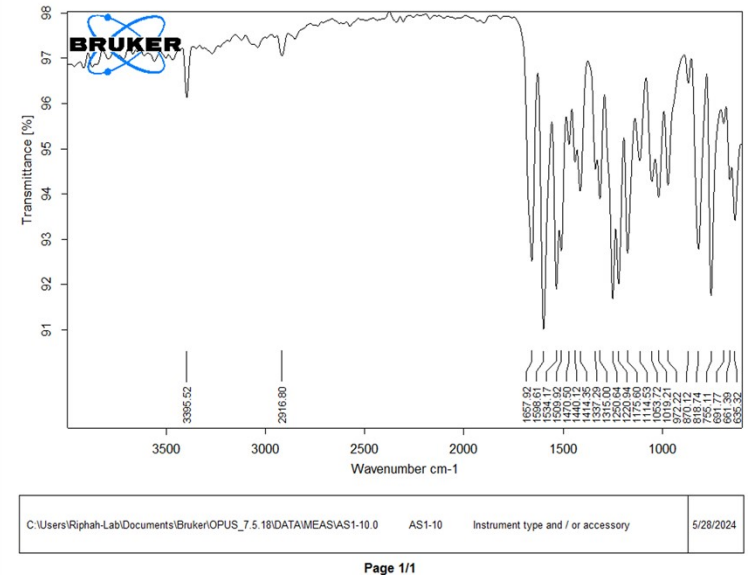


Figure S61. FTIR Spectrum of AS1-10.

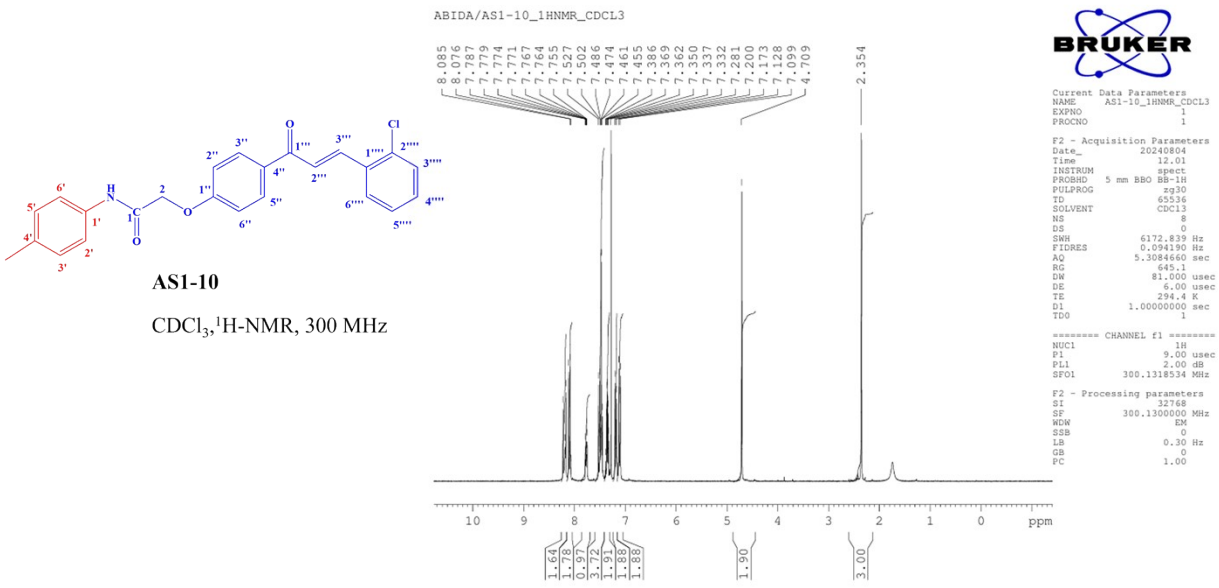


Figure S62. ^{1}H-NMR Spectrum of AS1-10.

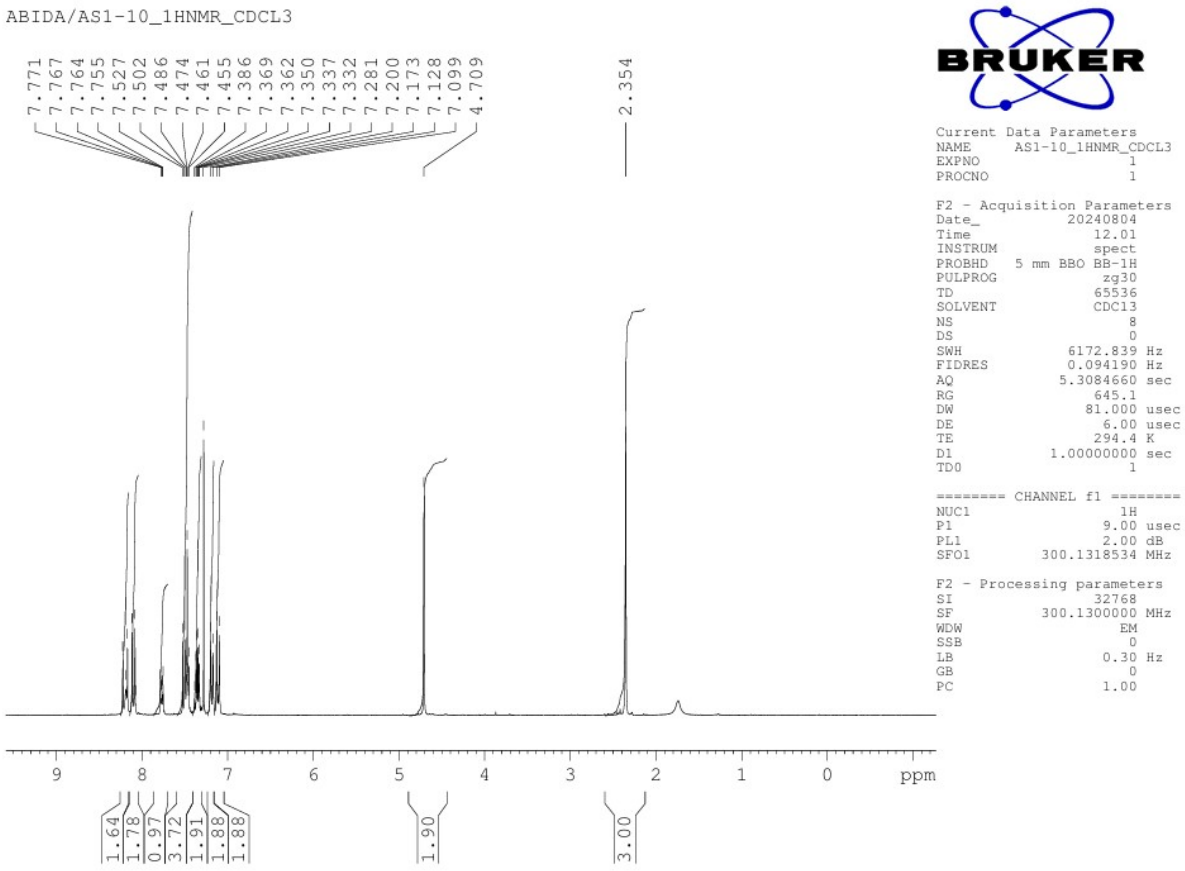


Figure S63. ^{1}H-NMR expansion of AS1-10.

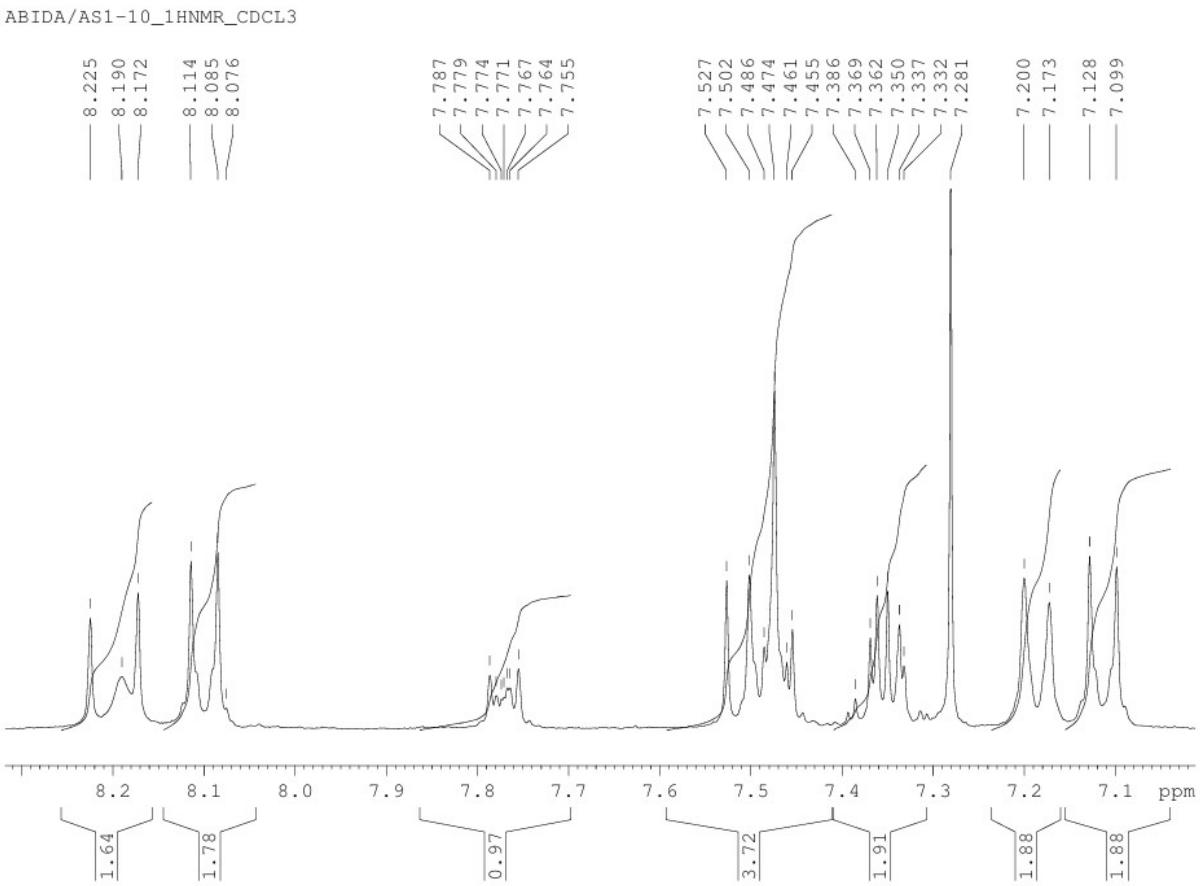


Figure S64. ^{1}H-NMR expansion of AS1-10.

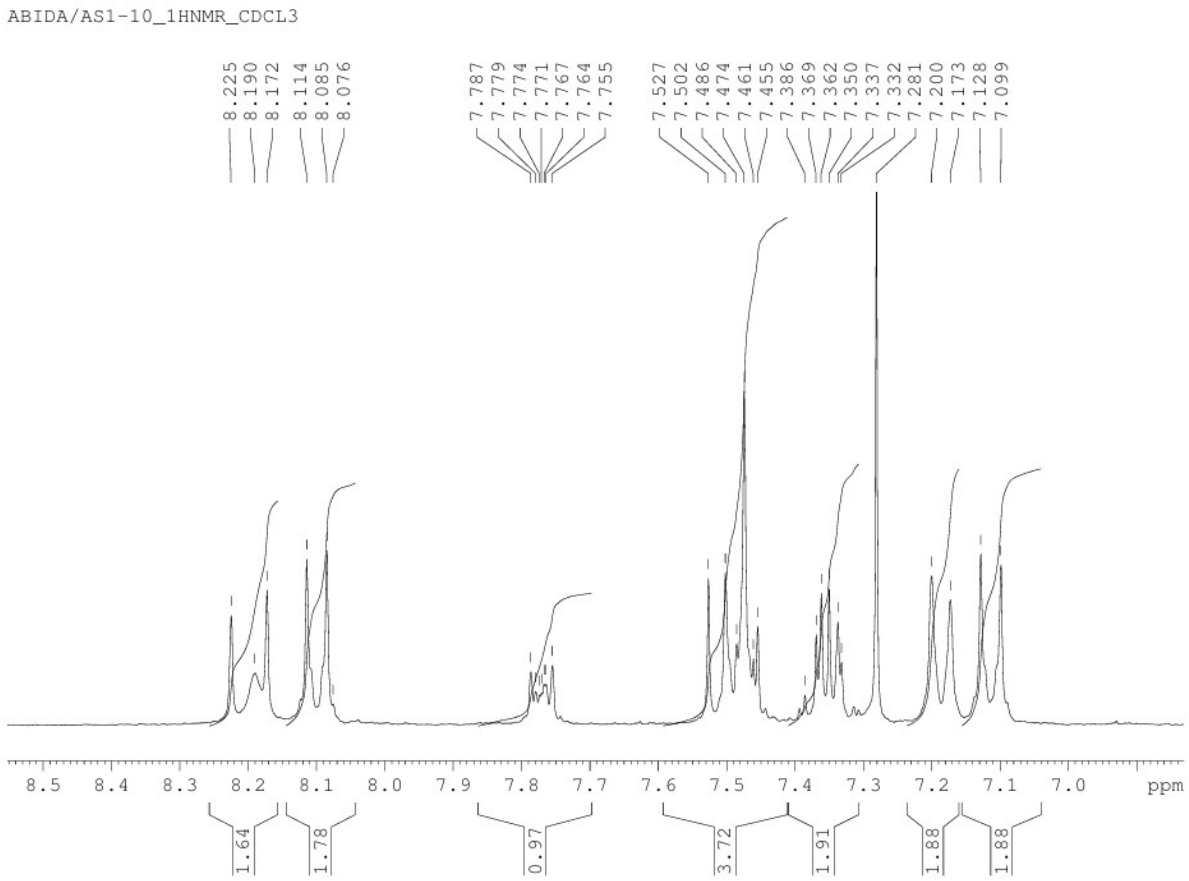
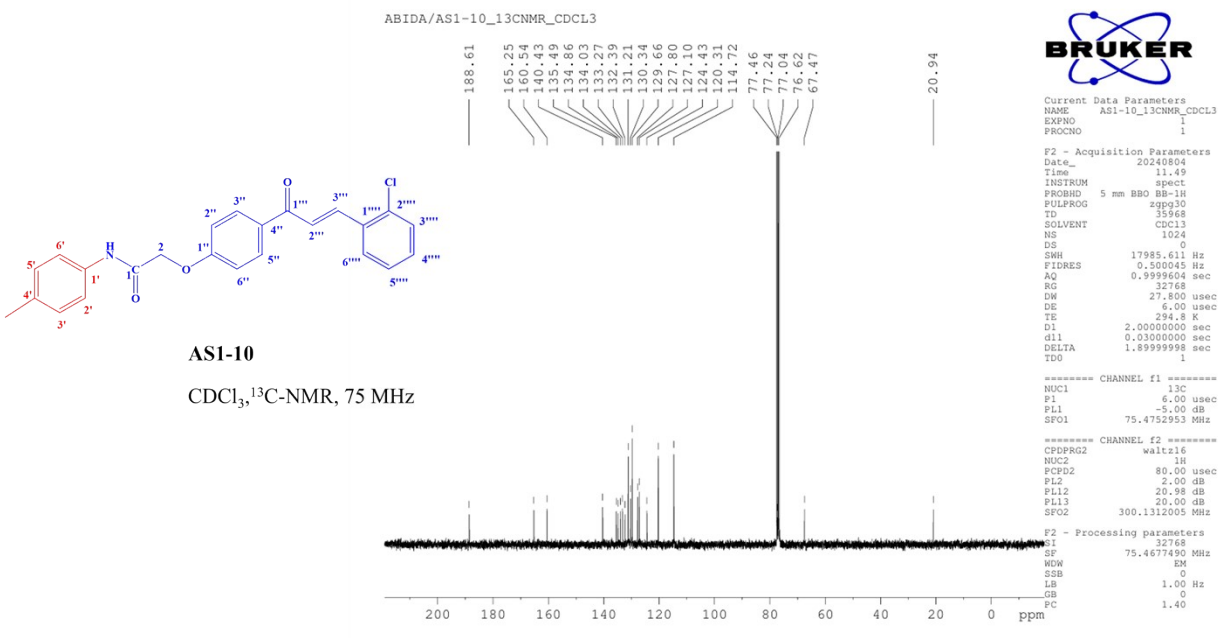


Figure S65. ^{1}H-NMR expansion of AS1-10.



AS1-10

$CDCl_3$,^{13}C-NMR, 75 MHz

Figure S66. ^{13}C-NMR Spectrum of AS1-10.

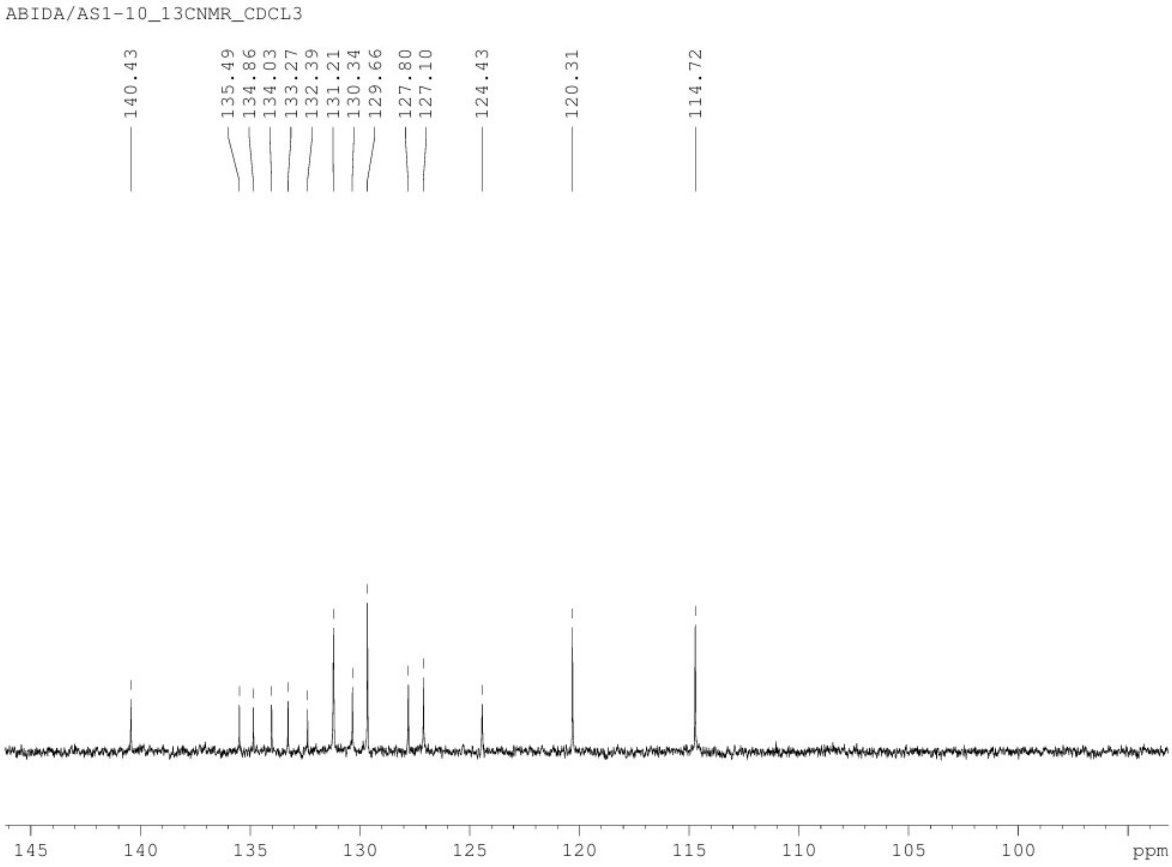


Figure S67. ^{13}C-NMR expansion of AS1-10.

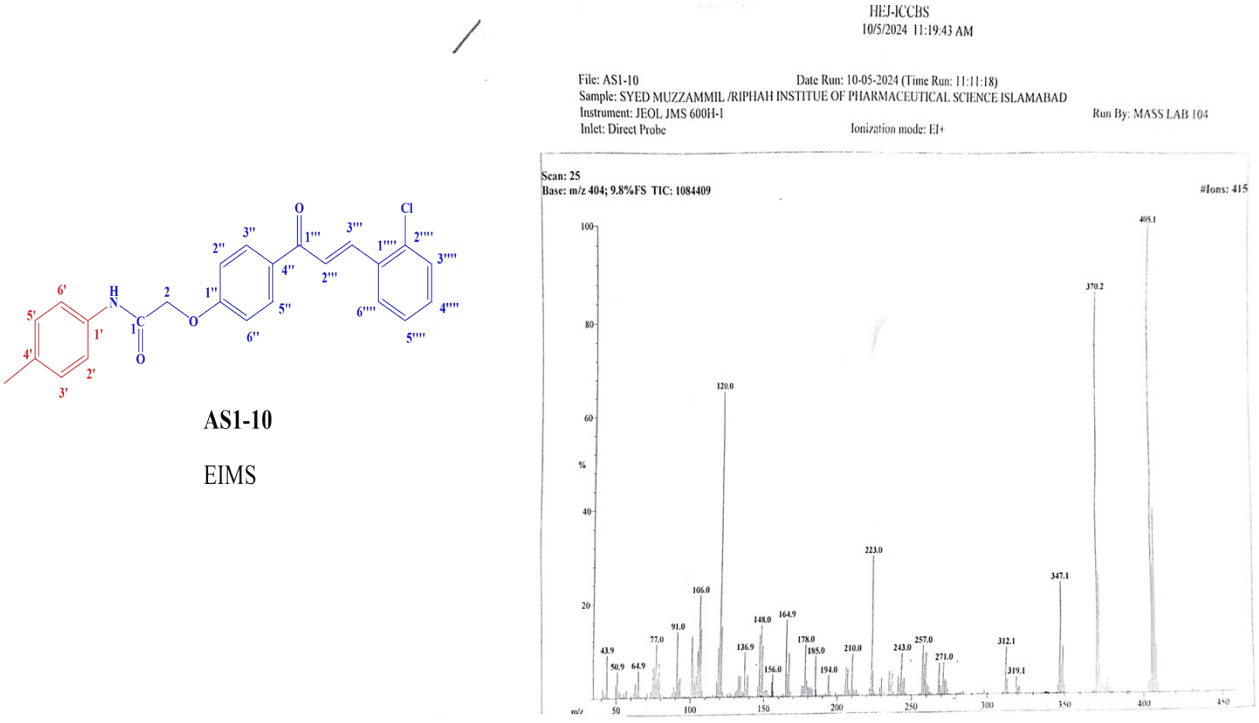


Figure S68. EIMS Spectrum of AS1-10.

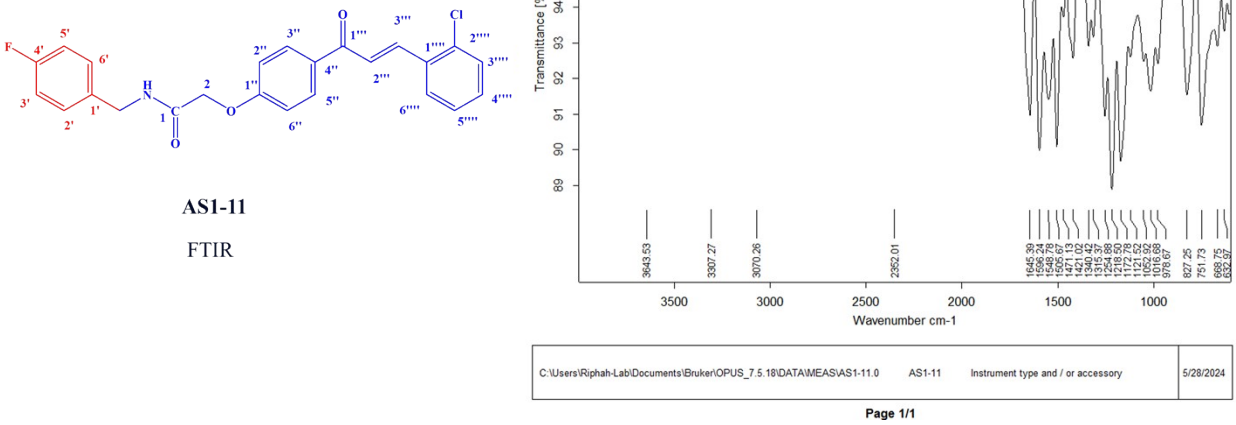


Figure S69. FTIR Spectrum of AS1-11.

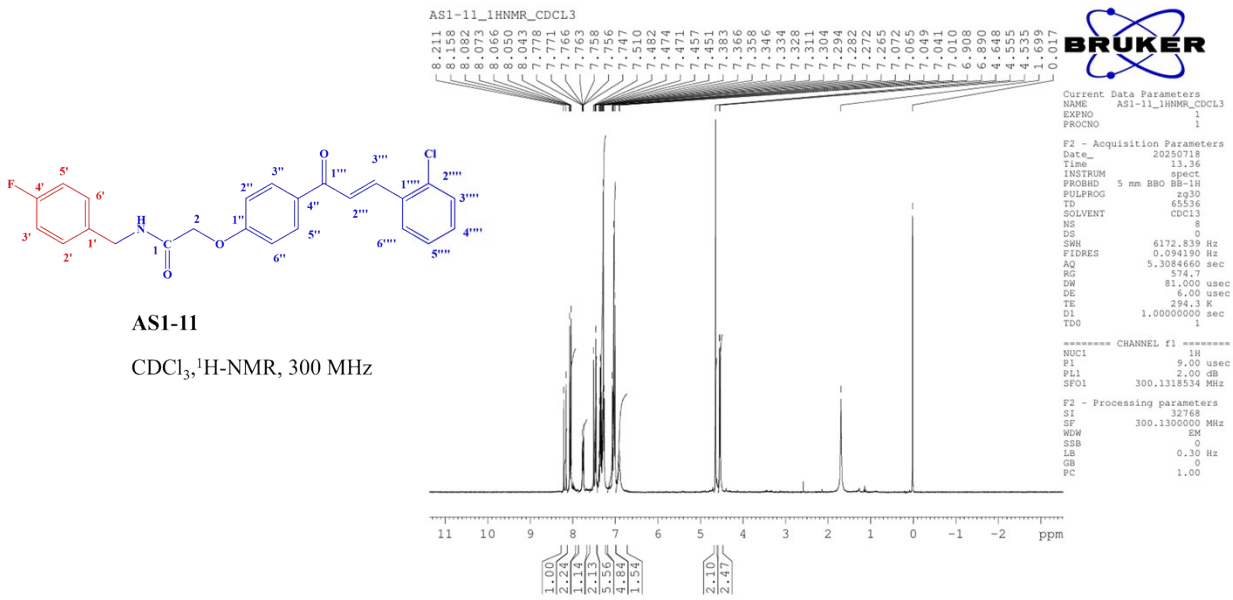


Figure S70. ^{1}H-NMR Spectrum of AS1-11.

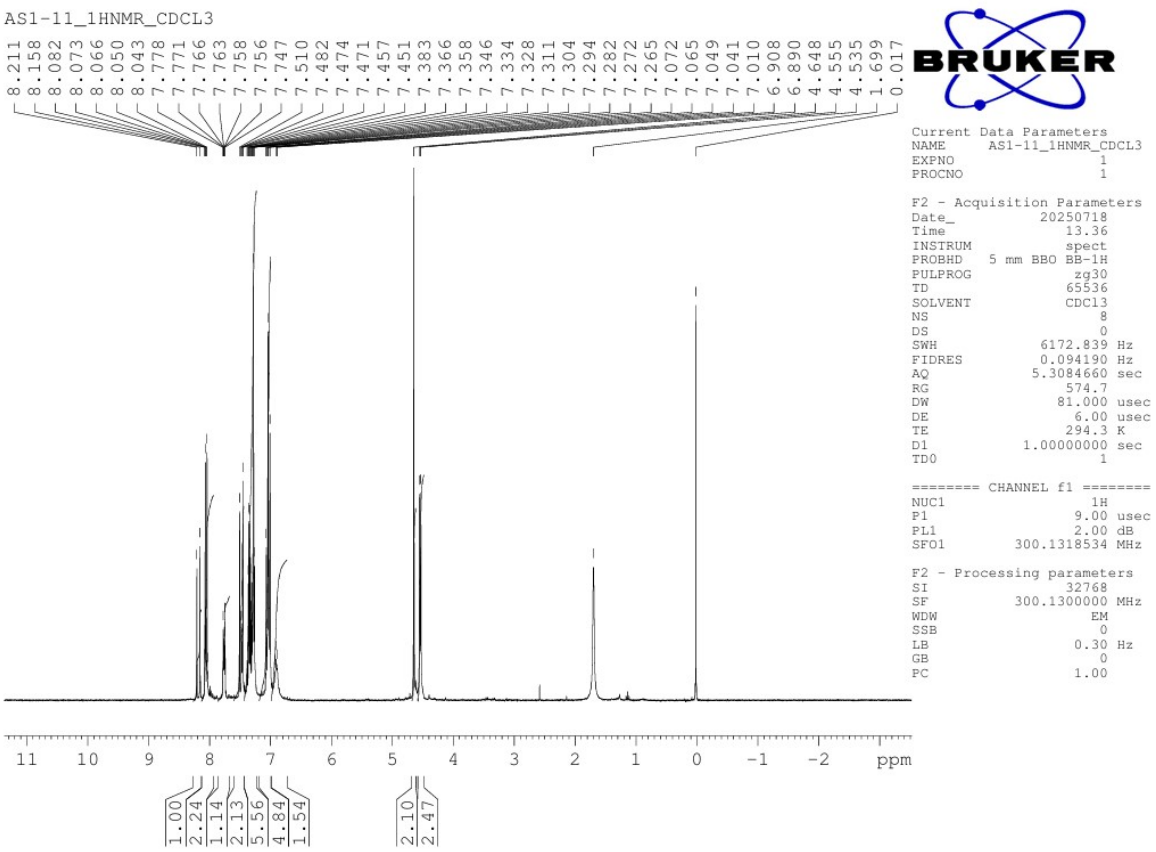


Figure S71. ^{1}H-NMR Spectrum of AS1-11.

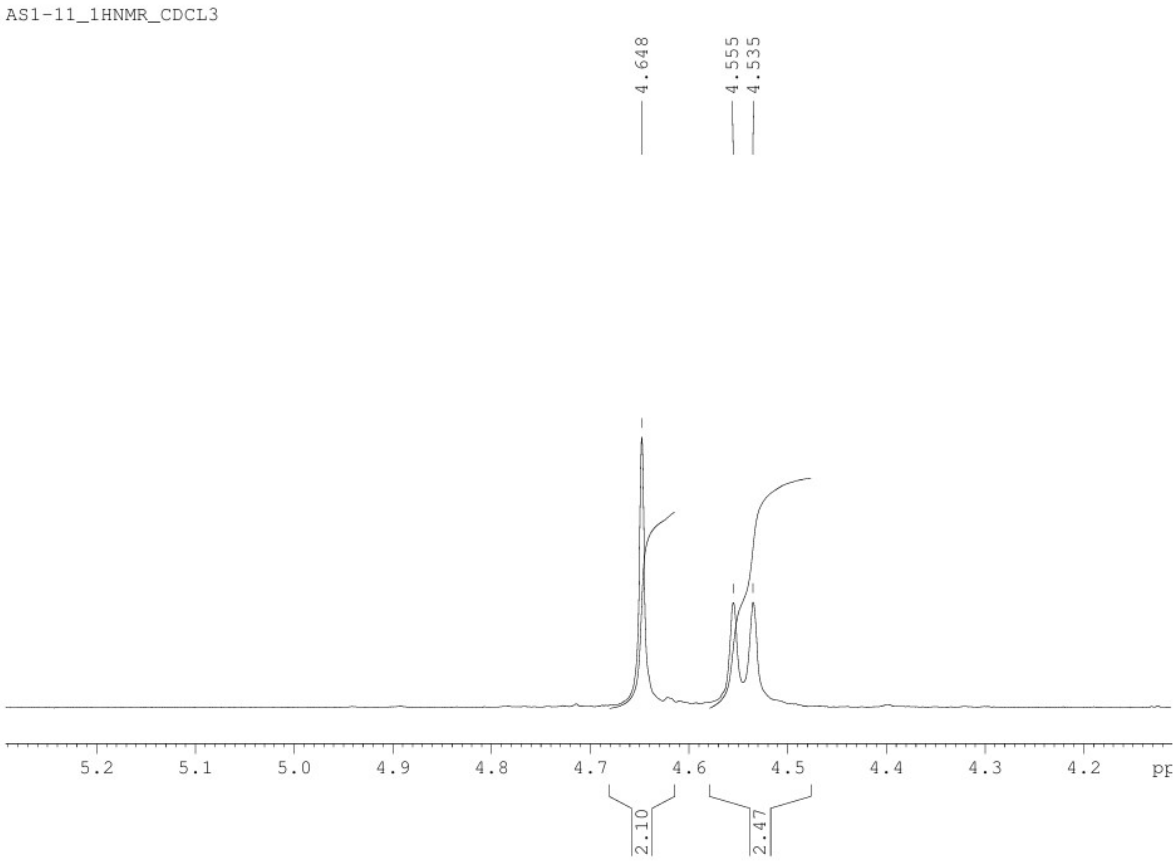


Figure S72. ^{1}H-NMR expansion of AS1-11.

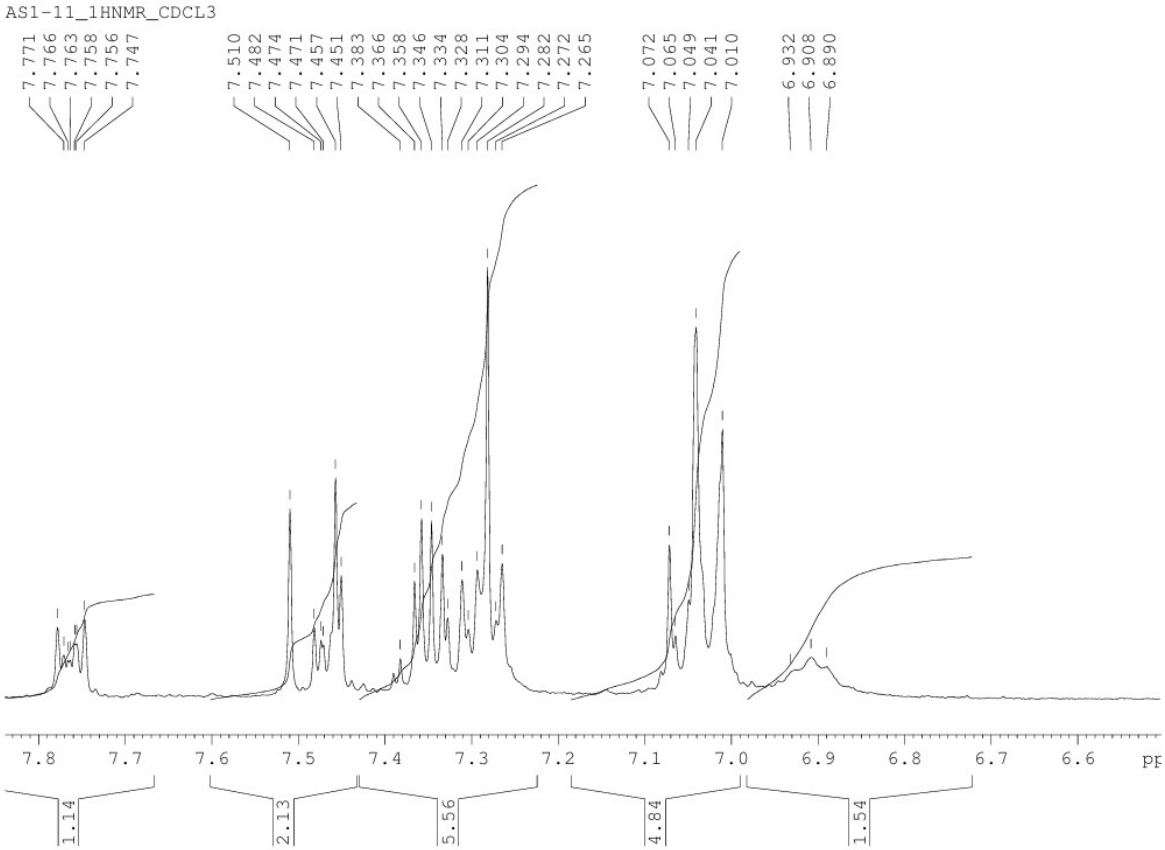


Figure S73. ^{1}H-NMR expansion of AS1-11.

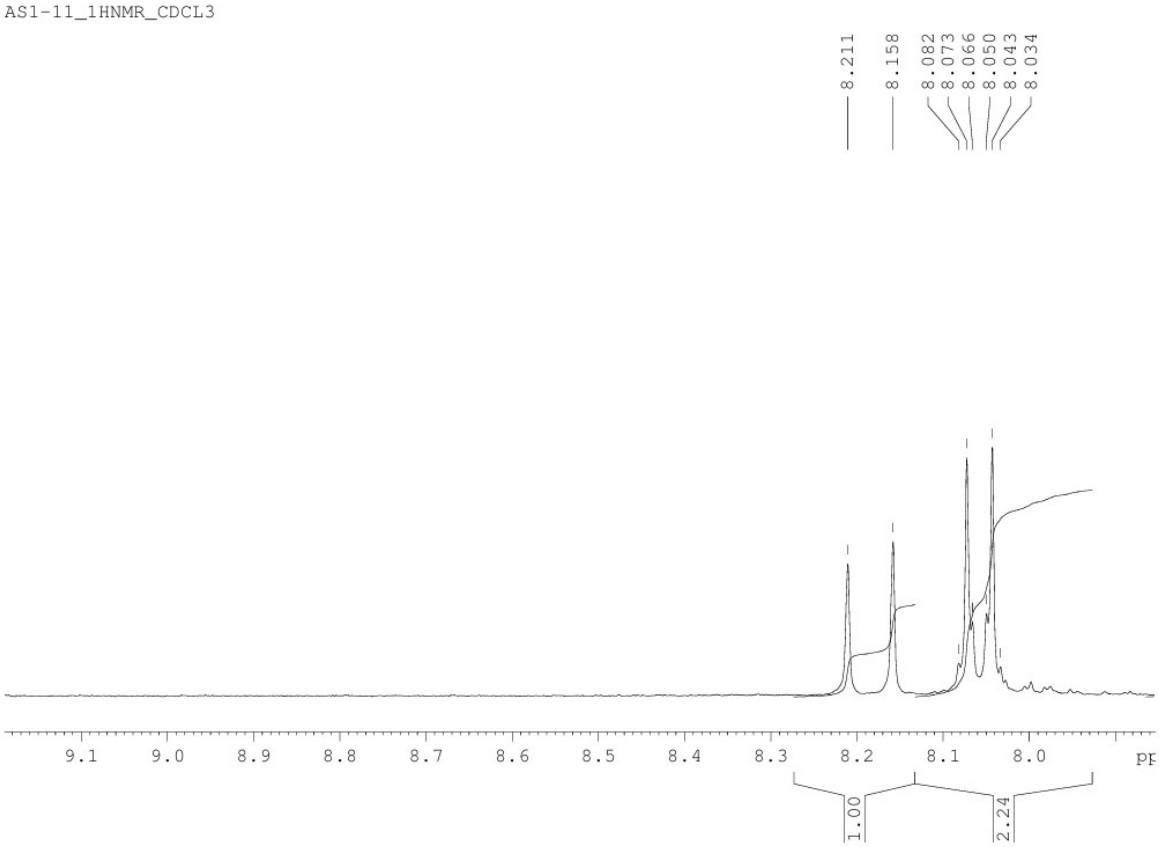


Figure S74. [1]H-NMR expansion of AS1-11.

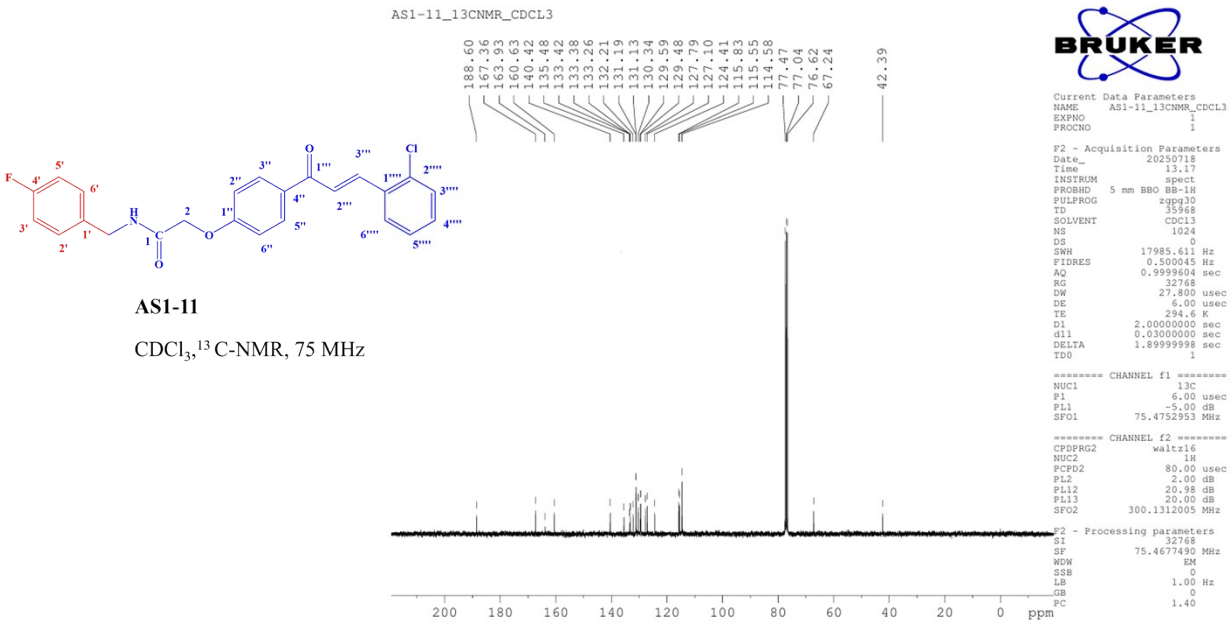


Figure S75. ^{13}C-NMR Spectrum of AS1-11.

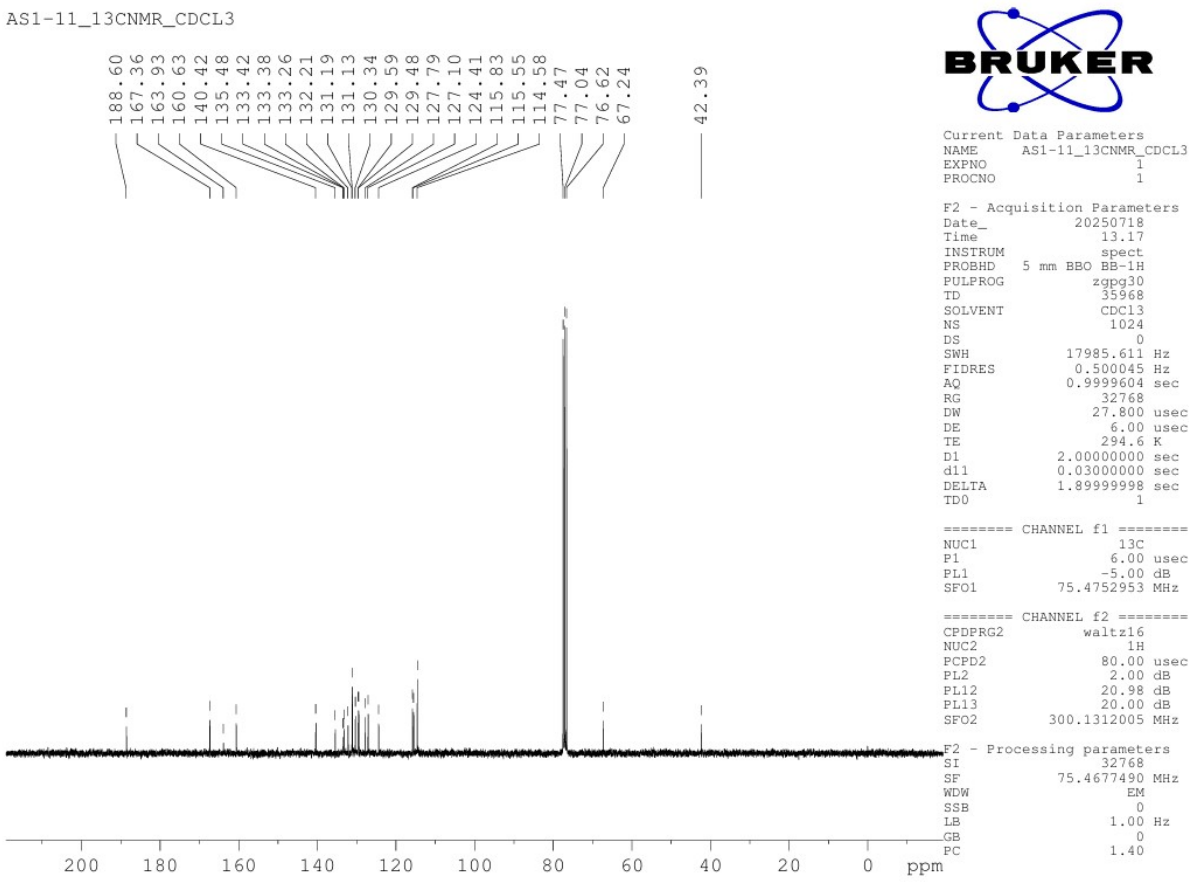


Figure S76. ^{13}C-NMR Spectrum of AS1-11.

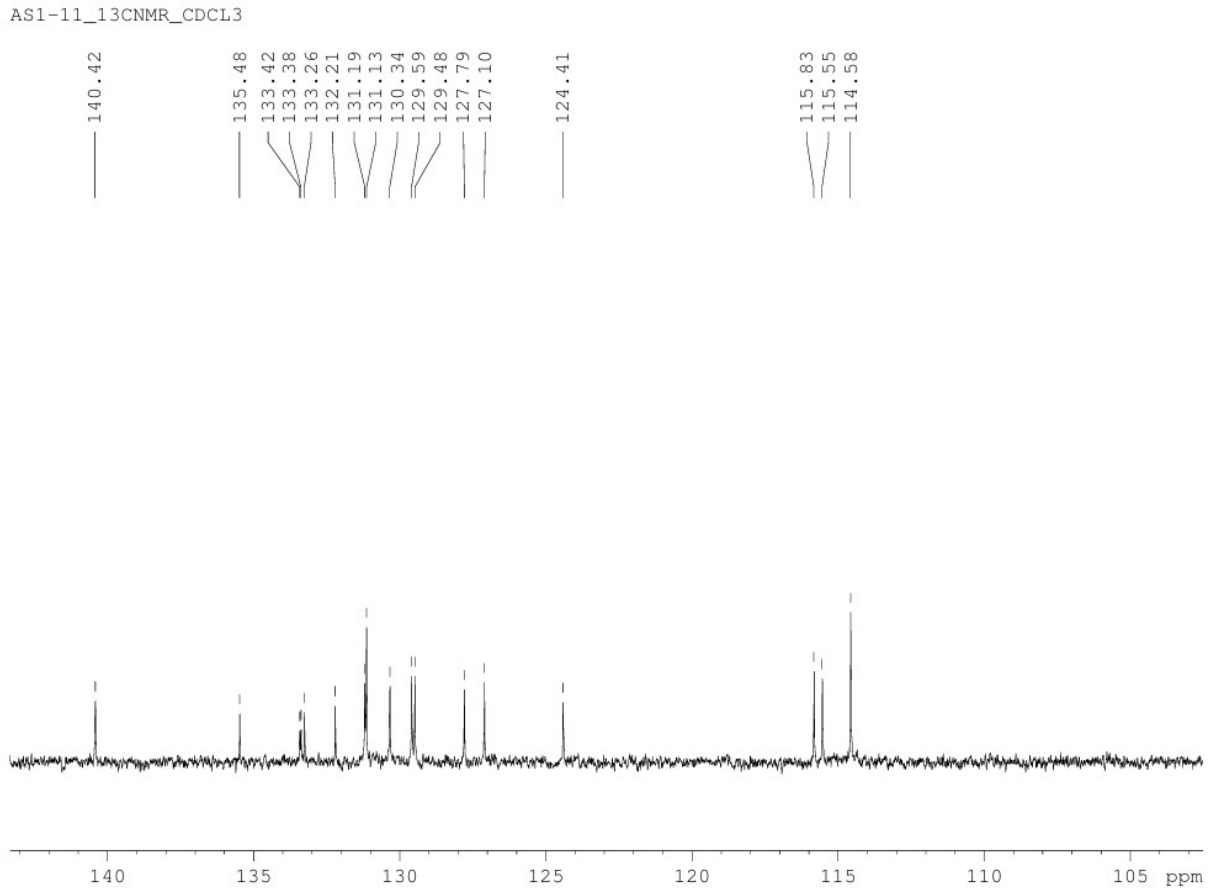


Figure S77. ^{13}C-NMR expansion of AS1-11.

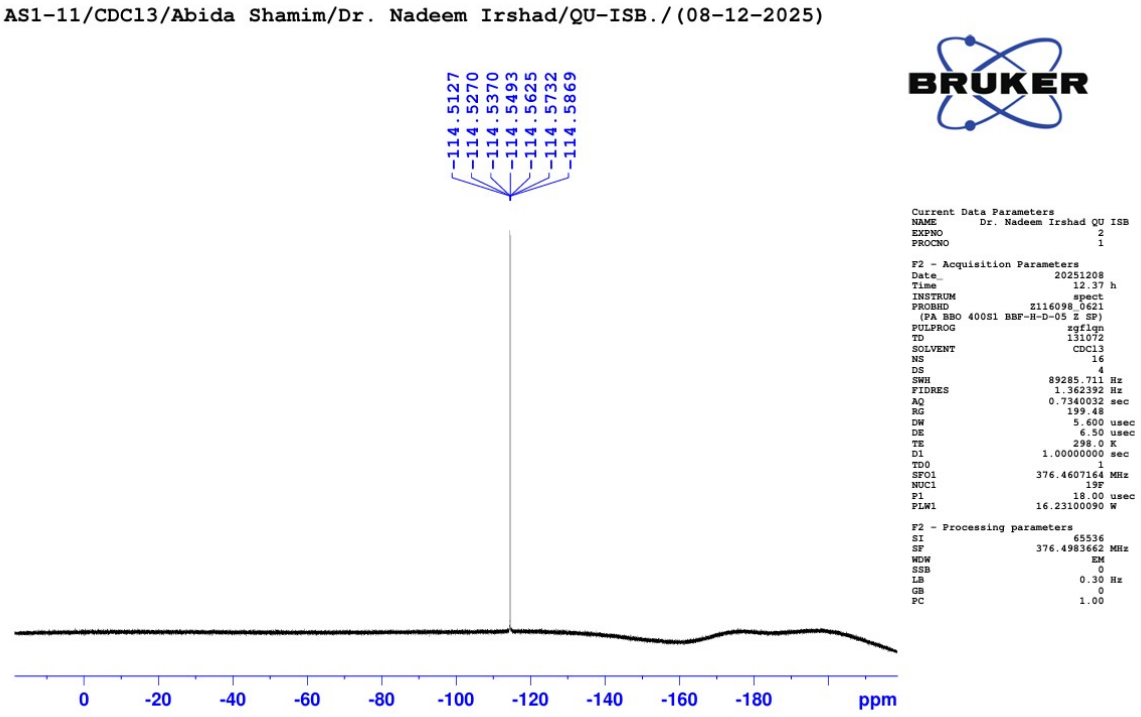


Figure S78. ^{19}F-NMR Spectrum of AS1-11.

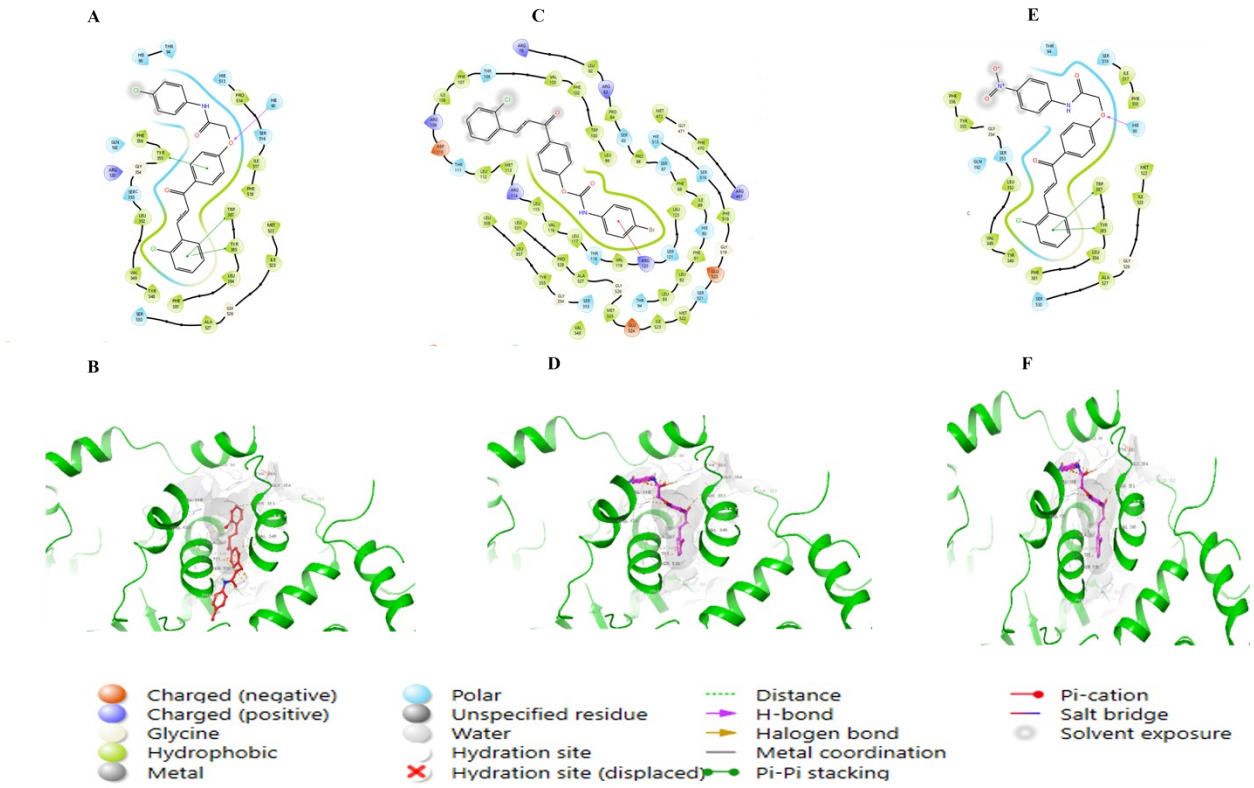


Figure S79. 2D and 3D molecular docking interactions of compound AS1-1 (A and B), AS1-3 (C and D) and AS1- 4 (E and F) against COX-1 active site.

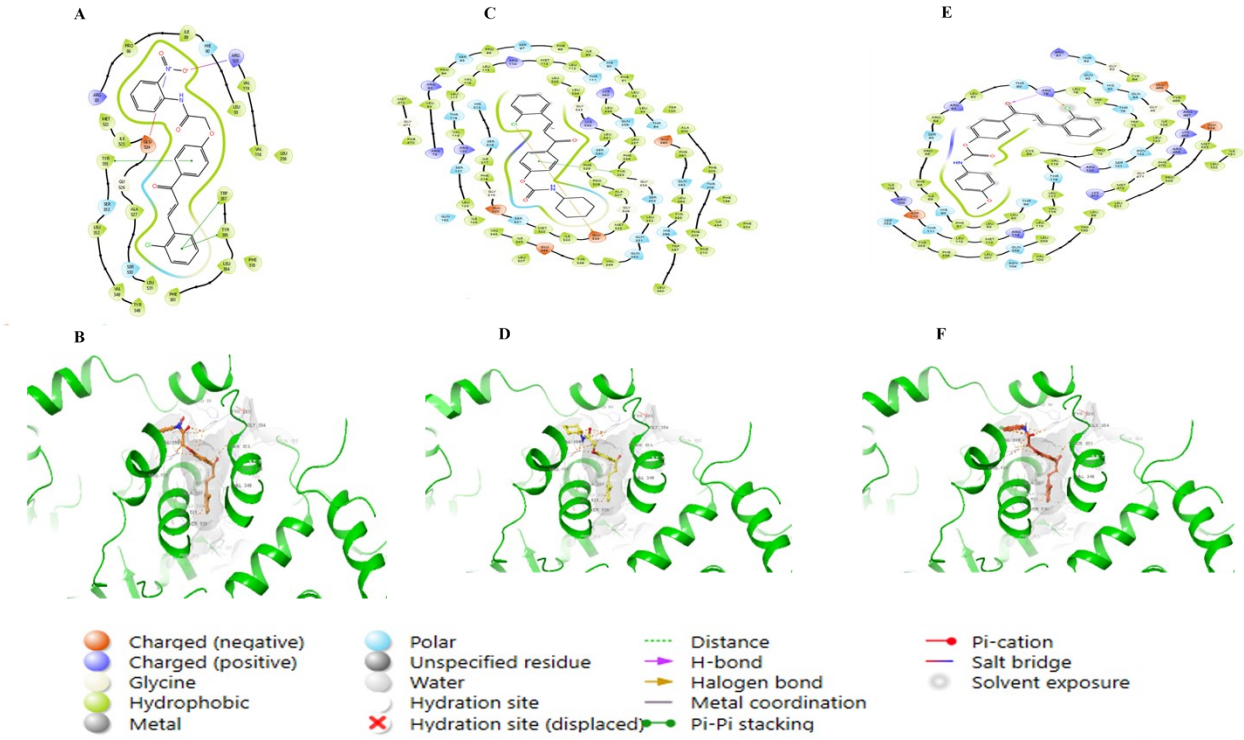


Figure S80. 2D and 3D molecular docking interactions of compound AS1-5 (A and B), AS1-7 (C and D) and AS1- 9 (E and F) against COX-1 active site.

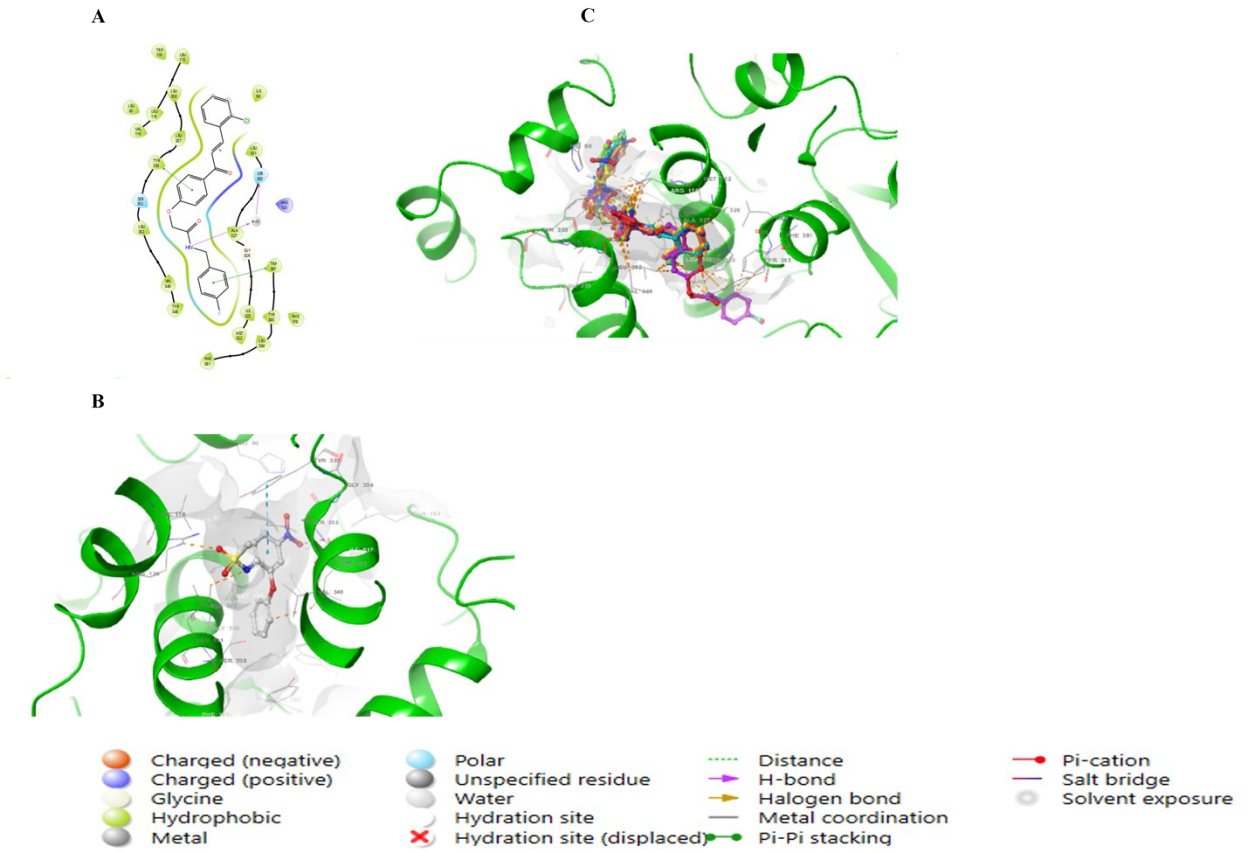


Figure S81. 2D and 3D molecular docking interactions of compound AS1-11 (A and B) and 3D superimpose interaction (C)of all the newly synthesized chalcone derivatives against COX-1 active site.

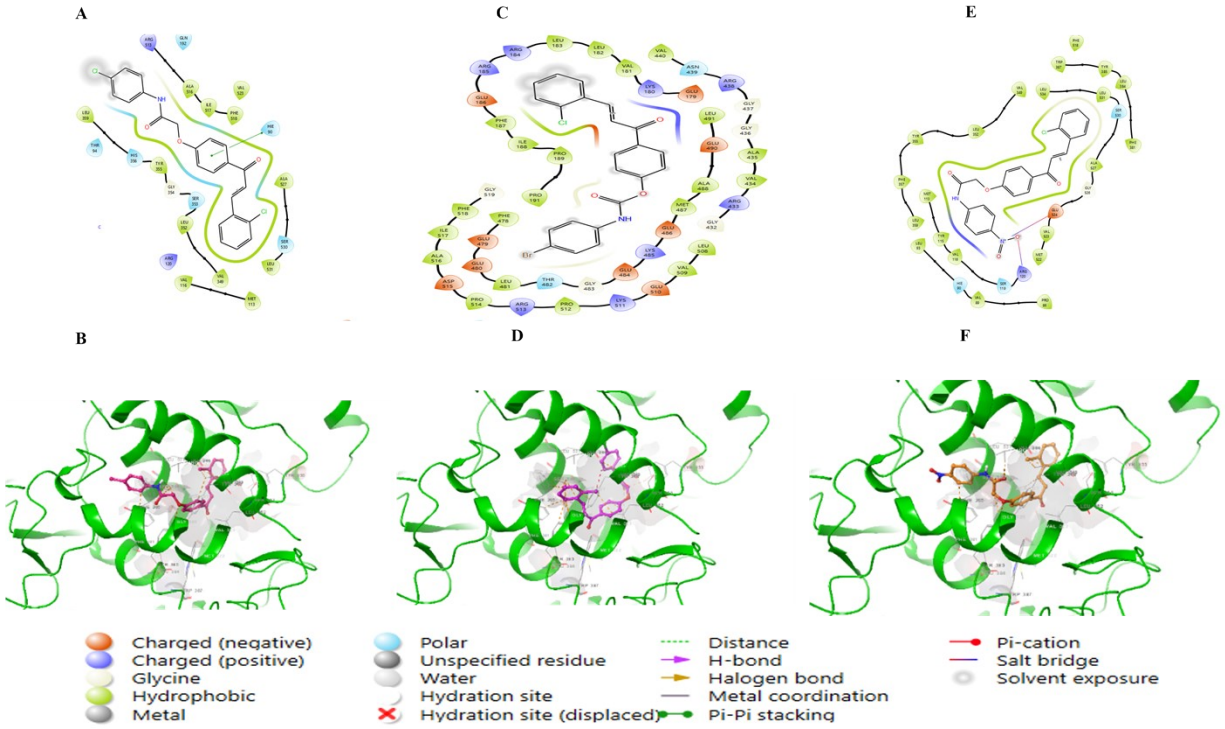


Figure S82. 2D and 3D molecular docking interactions of compound AS1-1 (A and B), AS1-3 (C and D) and AS1- 4 (E and F) against COX-2 active site.

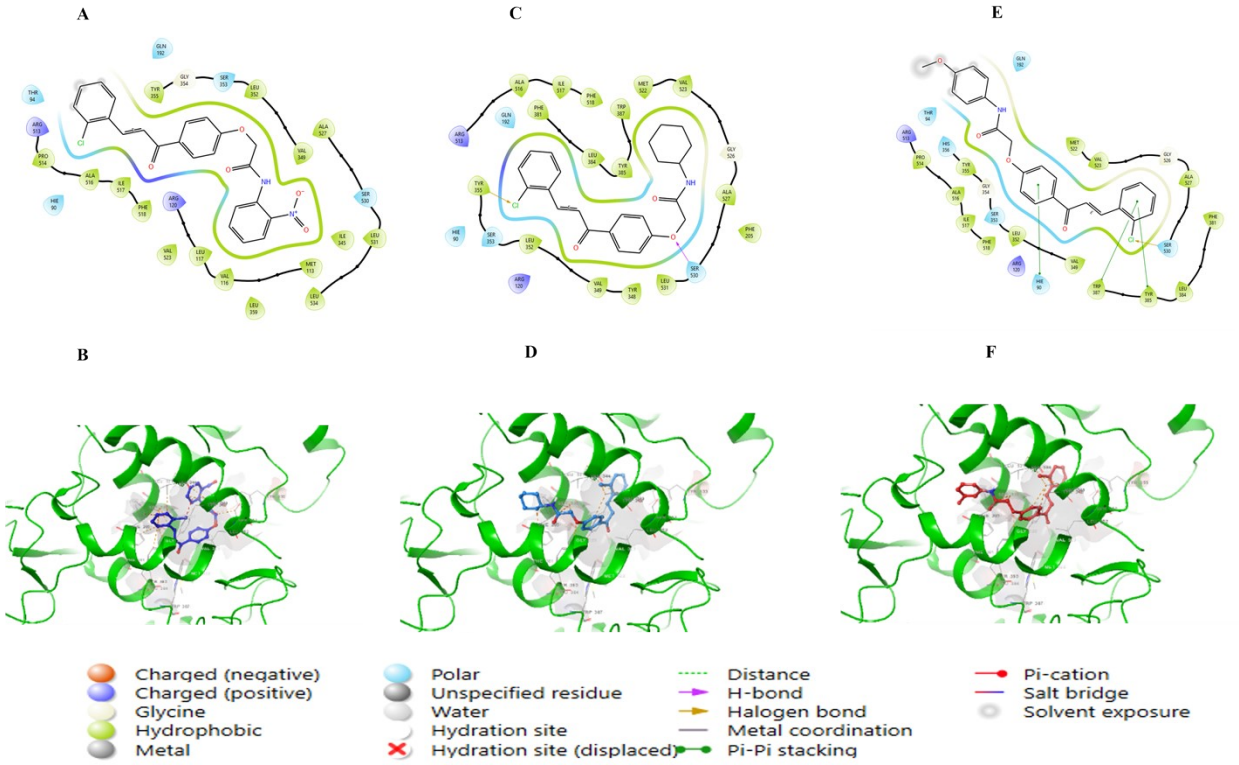


Figure S83. 2D and 3D molecular docking interactions of compound AS1-5 (A and B), AS1-7 (C and D) and AS1- 9 (E and F) against COX-2 active site.

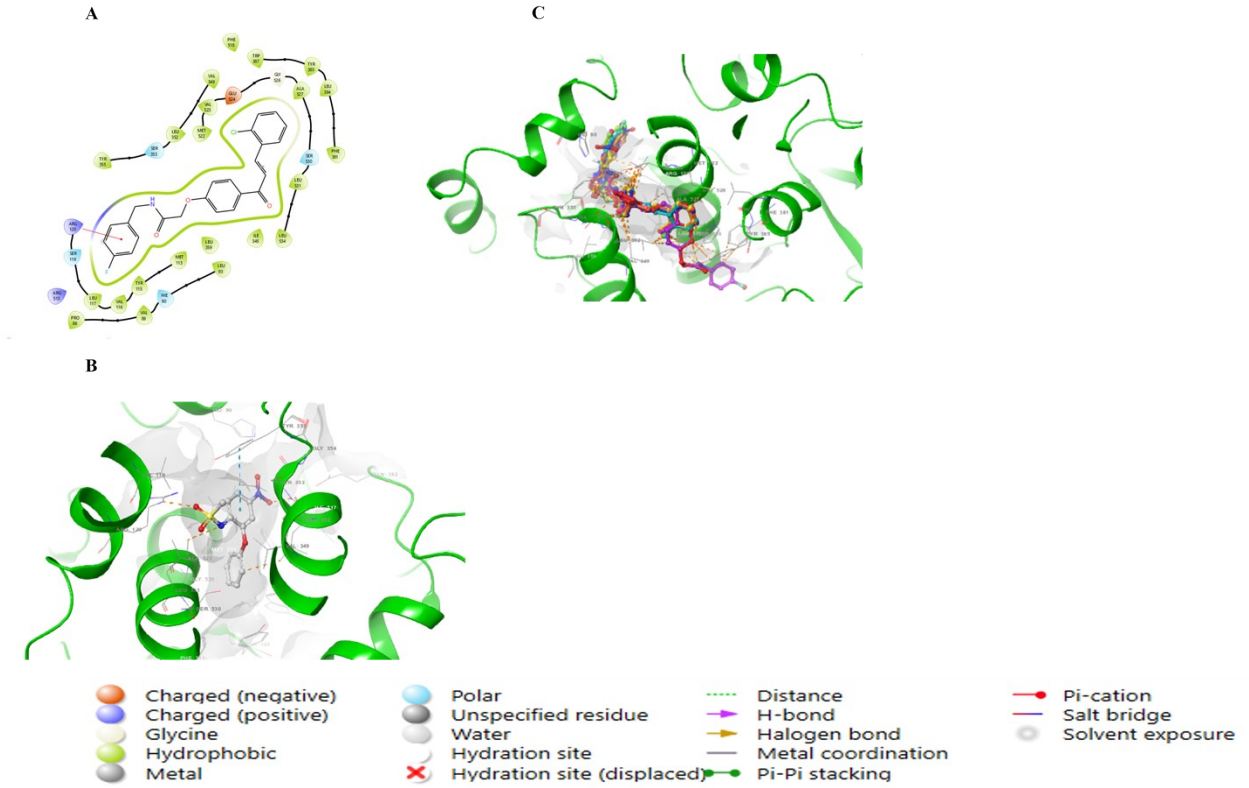
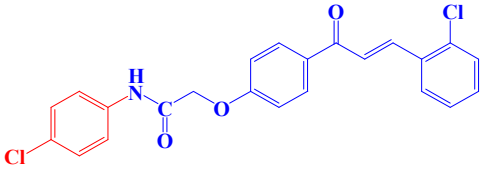
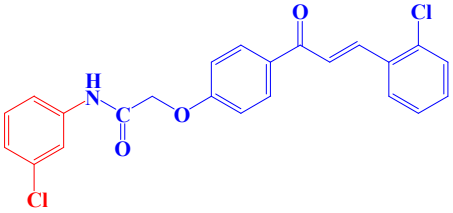
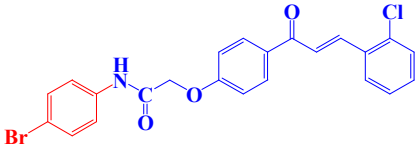
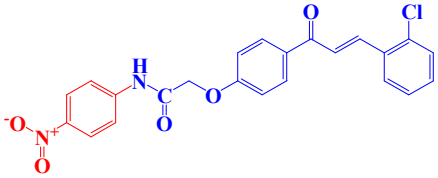
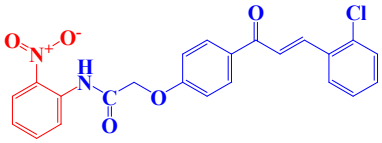
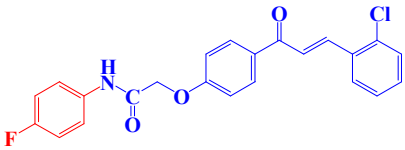
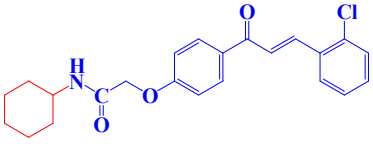
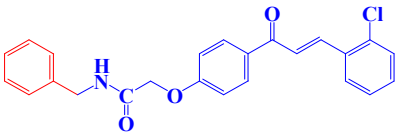


Figure S84. 2D and 3D molecular docking interactions of compound AS1-11 (A and B) and 3D superimpose interaction (C) of all the newly synthesized chalcone derivatives against COX-1 active site

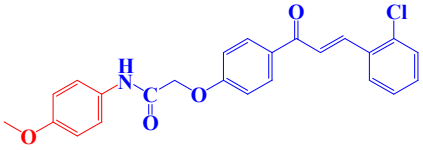
Table S1. Structures of the newly synthesized chalcone derivatives (AS1-1 – AS1-11).

Compounds	Structures
AS1-1	
AS1-2	
AS1-3	
AS1-4	
AS1-5	
AS1-6	
AS1-7	

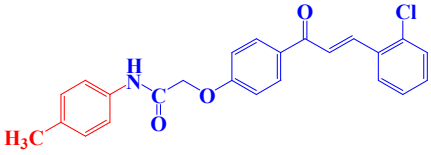
AS1-8



AS1-9



AS1-10



AS1-11

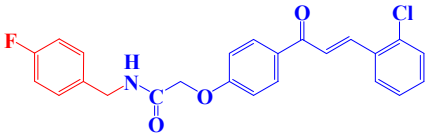


Table S2. Physical data of newly synthesized chalcone derivatives (AS1-1 – AS1-11).

Code	R	Molecular Formula	Mol. Wt.	Colour	R_f*	m.p. (°C)	Yield (%)
AS1-1	4-Cl-Ph	$C_{24}H_{19}Cl_2NO_2$	424.32	Light yellow	0.72	182-184	87
AS1-2	3-Cl-Ph	$C_{23}H_{17}Cl_2NO_3$	426.29	Yellow	0.68	176-178	84
AS1-3	4-Br-Ph	$C_{23}H_{17}BrClNO_3$	470.74	Yellow	0.68	166-168	82
AS1-4	4-NO_2-Ph	$C_{23}H_{17}ClN_2O_5$	436.84	Dark brown	0.65	155-157	67
AS1-5	2-NO_2-Ph	$C_{23}H_{17}ClN_2O_5$	436.84	Off white	0.78	185-187	71
AS1-6	4-F-Ph	$C_{23}H_{17}ClFNO_3$	409.84	Light brown	0.73	172-174	83
AS1-7	Cyclohexyl	$C_{23}H_{24}ClNO_3$	397.89	Off white	0.65	146-148	74
AS1-8	Benzyl	$C_{24}H_{20}ClNO_3$	405.87	Off white	0.61	168-170	79
AS1-9	4-OCH_3-Ph	$C_{24}H_{20}ClNO_4$	421.87	Light brown	0.71	135-137	81
AS1-10	4-CH_3-Ph	$C_{24}H_{20}ClNO_3$	405.87	Light yellow	0.68	167-169	90
AS1-11	4-F-Benzyl	$C_{24}H_{19}ClFNO_3$	423.86	Brown	0.67	136-138	80

R_f* (chloroform:acetone; 9:1)

Table S3. Predicted physicochemical properties of newly synthesized chalcone derivatives (AS1-1 – AS1-11) using SwissADME

Compounds code	*M.wt	Num. heavy atoms	Num. arom. heavy atoms	Fraction Csp3	Num. rotatable bonds	*NHAs	*NHDs	Molar Refractivity	TPSA
AS1-1	424.32	29	18	0.08	8	2	1	119.88	46.17
AS1-2	426.29	29	18	0.04	8	3	1	116.59	55.4
AS1-3	470.74	29	18	0.04	8	3	1	119.28	55.4
AS1-4	436.84	31	18	0.04	9	5	1	120.41	101.22
AS1-5	436.84	31	18	0.04	9	5	1	120.41	101.22
AS1-6	409.84	29	18	0.04	8	4	1	111.54	55.4
AS1-7	397.89	28	12	0.3	8	3	1	112.29	55.4
AS1-8	405.87	29	18	0.08	9	3	1	114.85	55.4
AS1-9	421.87	30	18	0.08	9	4	1	118.08	64.63
AS1-10	405.87	29	18	0.08	8	3	1	116.55	55.4
AS1-11	423.86	30	18	0.08	9	4	1	114.81	55.4

M.wt: Molecular weight; NHAs: Number of Hydrogen bond acceptors; NHDs: Number Hydrogen bond donors; TPSA: Topological Polar Surface Area;

Table S4. Lipophilicity of the newly synthesized chalcone derivatives (AS1-1 – AS1-11) using SwissADME

Compounds	Log Po/w (iLOGP)	Log Po/w (XLOGP3)	Log Po/w (WLOGP)	Log Po/w (MLOGP)	Log Po/w (SILICOS-IT)	Consensus Log Po/w
AS1-1	3.73	6.01	6.16	5.02	6.78	5.54
AS1-2	3.41	5.82	5.61	4.23	5.92	5
AS1-3	3.58	5.88	5.72	4.33	5.96	5.09
AS1-4	2.8	5.02	4.86	2.8	3.13	3.72
AS1-5	3.21	5.57	4.86	2.8	3.13	3.91
AS1-6	3.26	5.29	5.51	4.13	5.7	4.78
AS1-7	3.63	5.44	4.95	3.31	5.38	4.54
AS1-8	3.5	5.13	4.67	3.69	5.67	4.53
AS1-9	3.41	5.16	4.96	3.4	5.35	4.46
AS1-10	3.26	5.56	5.26	3.96	5.8	4.77
AS1-11	3.43	5.23	5.23	4.07	6.1	4.81

Table S5. Water solubility prediction values, based on three alternative models of newly synthesized chalcone derivatives (AS1-1 – AS1-11) using SwissADME

Code	Log S (ESOL)	Solubility	Class		Log S (Ali)	Solubility	Class		Log S (SILICOSIT)	Solubility	Class	
	ESOL Log S	ESOL Solubility (mg/ml)	ESOL Solubility (mol/l)	ESOL Class	Ali Log S	Ali Solubility (mg/ml)	Ali Solubility (mol/l)	Ali Class	Silicos-IT LogSw	Silicos-IT Solubility (mg/ml)	Silicos-IT Solubility (mol/l)	Silicos-IT class
AS1-1	-6.19	2.75E-04	6.48E-07	Poorly soluble	-6.76	7.42E-05	1.75E-07	Poorly soluble	-9.41	1.64E-07	3.87E-10	Poorly soluble
AS1-2	-6.08	3.54E-04	8.30E-07	Poorly soluble	-6.75	7.51E-05	1.76E-07	Poorly soluble	-8.75	7.65E-07	1.79E-09	Poorly soluble
AS1-3	-6.39	1.90E-04	4.03E-07	Poorly soluble	-6.82	7.19E-05	1.53E-07	Poorly soluble	-8.94	5.44E-07	1.16E-09	Poorly soluble
AS1-4	-5.55	1.24E-03	2.84E-06	Moderately soluble	-6.89	5.68E-05	1.30E-07	Poorly soluble	-7.5	1.38E-05	3.17E-08	Poorly soluble
AS1-5	-5.89	5.59E-04	1.28E-06	Moderately soluble	-7.46	1.53E-05	3.49E-08	Poorly soluble	-7.5	1.38E-05	3.17E-08	Poorly soluble
AS1-6	-5.65	9.28E-04	2.26E-06	Moderately soluble	-6.2	2.56E-04	6.25E-07	Poorly soluble	-8.42	1.54E-06	3.77E-09	Poorly soluble
AS1-7	-5.52	1.19E-03	3.00E-06	Moderately soluble	-6.36	1.74E-04	4.37E-07	Poorly soluble	-7.08	3.35E-05	8.41E-08	Poorly soluble
AS1-8	-5.45	1.43E-03	3.52E-06	Moderately soluble	-6.04	3.72E-04	9.16E-07	Poorly soluble	-8.55	1.14E-06	2.80E-09	Poorly soluble
AS1-9	-5.56	1.17E-03	2.78E-06	Moderately soluble	-6.26	2.30E-04	5.46E-07	Poorly soluble	-8.26	2.31E-06	5.47E-09	Poorly soluble
AS1-10	-5.79	6.57E-04	1.62E-06	Moderately	-6.48	1.33E-04	3.28E-07	Poorly soluble	-8.54	1.18E-06	2.92E-09	Poorly soluble

				soluble								
AS1-11	-5.61	1.03E-03	2.44E-06	Moderately soluble	-6.14	3.06E-04	7.21E-07	Poorly soluble	-8.82	6.47E-07	1.53E-09	Poorly soluble

Table S6. Calculated pharmacokinetic parameters of newly synthesized chalcone derivatives (AS1-1 – AS1-11) using SwissADME

Compounds	GI absorption	BBB permeant	P-gp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	Log Kp (skin permeation)
AS1-1	High	No	Yes	Yes	Yes	Yes	No	Yes	-4.62
AS1-2	High	Yes	Yes	Yes	Yes	Yes	No	Yes	-4.77
AS1-3	High	No	Yes	Yes	Yes	Yes	No	Yes	-5
AS1-4	High	No	No	No	Yes	Yes	No	Yes	-5.4
AS1-5	High	No	No	No	Yes	Yes	No	Yes	-5.01
AS1-6	High	Yes	No	Yes	Yes	Yes	No	Yes	-5.04
AS1-7	High	Yes	No	No	Yes	Yes	Yes	Yes	-4.86
AS1-8	High	Yes	Yes	Yes	Yes	Yes	No	Yes	-5.13
AS1-9	High	Yes	No	Yes	Yes	Yes	No	Yes	-5.21
AS1-10	High	Yes	Yes	Yes	Yes	Yes	No	Yes	-4.83
AS1-11	High	Yes	Yes	Yes	Yes	Yes	No	Yes	-5.17

Table S7. Drug likeness, medicinal chemistry and lead-likeness parameters of the newly synthesized chalcone derivatives (AS1-1 – AS1-11) using SwissADME

Compounds	Lipinski	Ghose	Veber	Egan	Muegge	Bioavailability score

AS1-1	Yes; 1 violation: MLOGP>4.15	No; 1 violation: WLOGP>5.6	Yes	No; 1 violation: WLOGP>5.88	No; 1 violation: XLOGP3>5	0.55
AS1-2	Yes; 1 violation: MLOGP>4.15	No; 1 violation: WLOGP>5.6	Yes	Yes	No; 1 violation: XLOGP3>5	0.55
AS1-3	Yes; 1 violation: MLOGP>4.15	No; 1 violation: WLOGP>5.6	Yes	Yes	No; 1 violation: XLOGP3>5	0.55
AS1-4	Yes; 0 violation	Yes	Yes	Yes	No; 1 violation: XLOGP3>5	0.55
AS1-5	Yes; 0 violation	Yes	Yes	Yes	No; 1 violation: XLOGP3>5	0.55
AS1-6	Yes; 0 violation	Yes	Yes	Yes	No; 1 violation: XLOGP3>5	0.55
AS1-7	Yes; 0 violation	Yes	Yes	Yes	No; 1 violation: XLOGP3>5	0.55
AS1-8	Yes; 0 violation	Yes	Yes	Yes	No; 1 violation: XLOGP3>5	0.55
AS1-9	Yes; 0 violation	Yes	Yes	Yes	No; 1 violation: XLOGP3>5	0.55
AS1-10	Yes; 0 violation	Yes	Yes	Yes	No; 1 violation: XLOGP3>5	0.55
AS1-11	Yes; 0 violation	Yes	Yes	Yes	No; 1 violation: XLOGP3>5	0.55

Table S8. Predicted toxicological data of the newly synthesized chalcone derivatives (AS1-1 – AS1-11) using pkCSM

Compounds	AMES toxicity	Max. tolerated dose (human) log(mg/kg/day)	hERG I inhibitor	hERG II inhibitr	Oral Rat acute Toxicity (LD50) mol/kg	Oral Rat Chronic Toxicity (LOAEL) log (mg/kg_bw/day)	Hepatotoxicity	Skin sensitization	*T. Pyriformis* toxicity log(ug/L)	Minnow toxicity (log mM)
AS1-1	No	0.526	No	Yes	2.357	0.828	Yes	No	0.455	-1.026
AS1-2	Yes	0.571	No	Yes	2.35	1.22	Yes	No	0.561	-1.593
AS1-3	No	0.536	No	Yes	2.361	1.223	Yes	No	0.548	-1.607
AS1-4	Yes	0.089	No	Yes	2.56	1.71	Yes	No	0.383	-1.378
AS1-5	Yes	0.313	No	Yes	2.502	1.563	Yes	No	0.343	-1.593
AS1-6	Yes	0.558	No	Yes	2.331	0.863	Yes	No	0.461	-1.408
AS1-7	No	0.153	No	Yes	2.695	1.31	No	No	1.669	0.225
AS1-8	Yes	0.531	No	Yes	2.309	1.55	Yes	No	0.593	-1.305
AS1-9	Yes	0.494	No	Yes	2.373	0.914	Yes	No	0.461	-1.269
AS1-10	Yes	0.529	No	Yes	2.314	1.377	Yes	No	0.555	-1.243
AS1-11	Yes	0.526	No	Yes	2.381	0.941	Yes	No	0.495	-1.388