

Novel Benzimidazole Hybrids: Design, Synthesis, Mechanistic Antifungal Potential and Molecular Dynamics

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1- Chemistry data

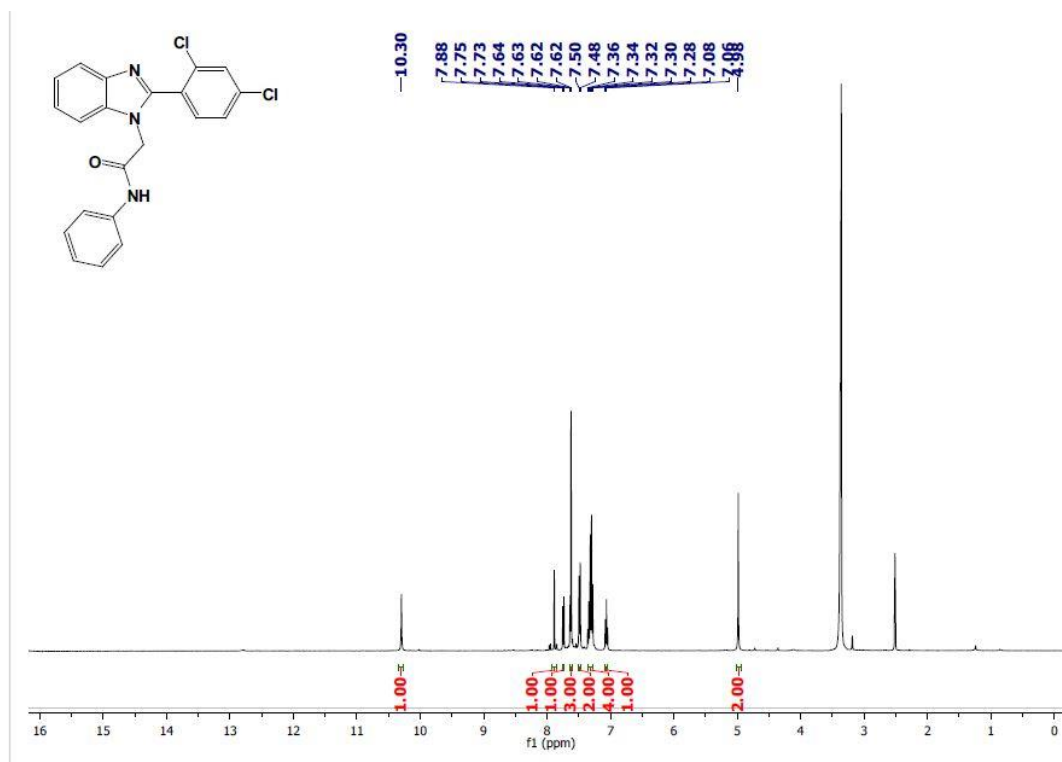


Figure S 1. ¹H-NMR Chart of Compound 3a

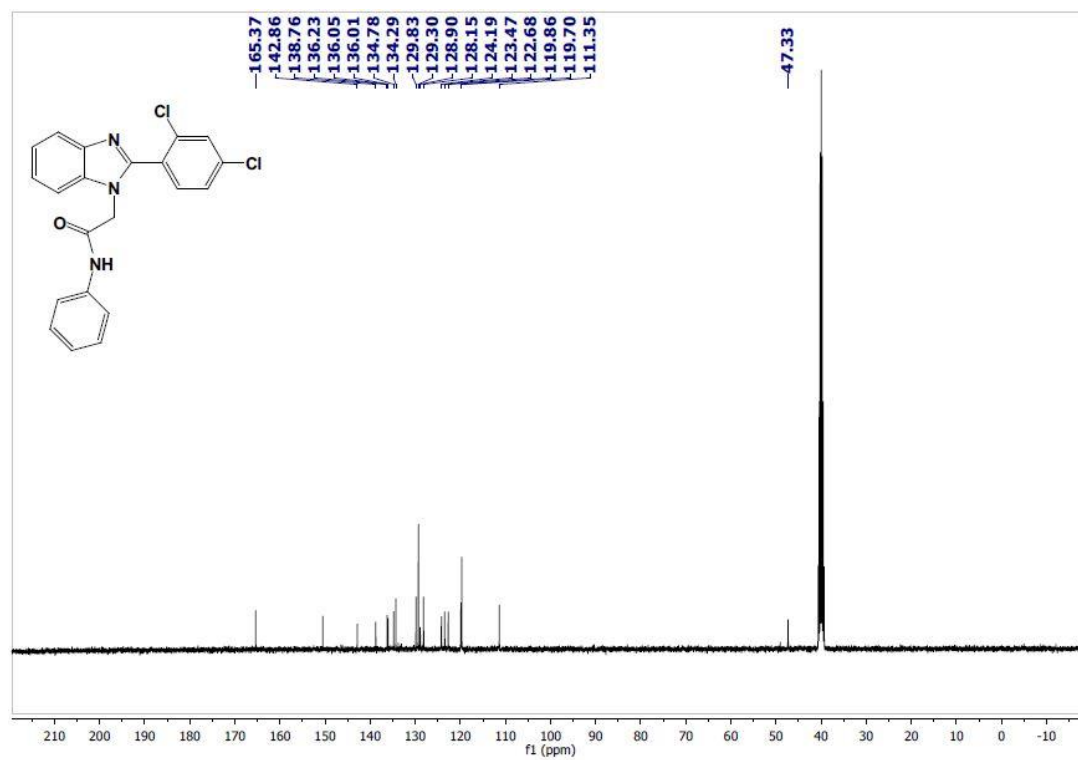


Figure S 2. ^{13}C -NMR Chart of Compound 3a

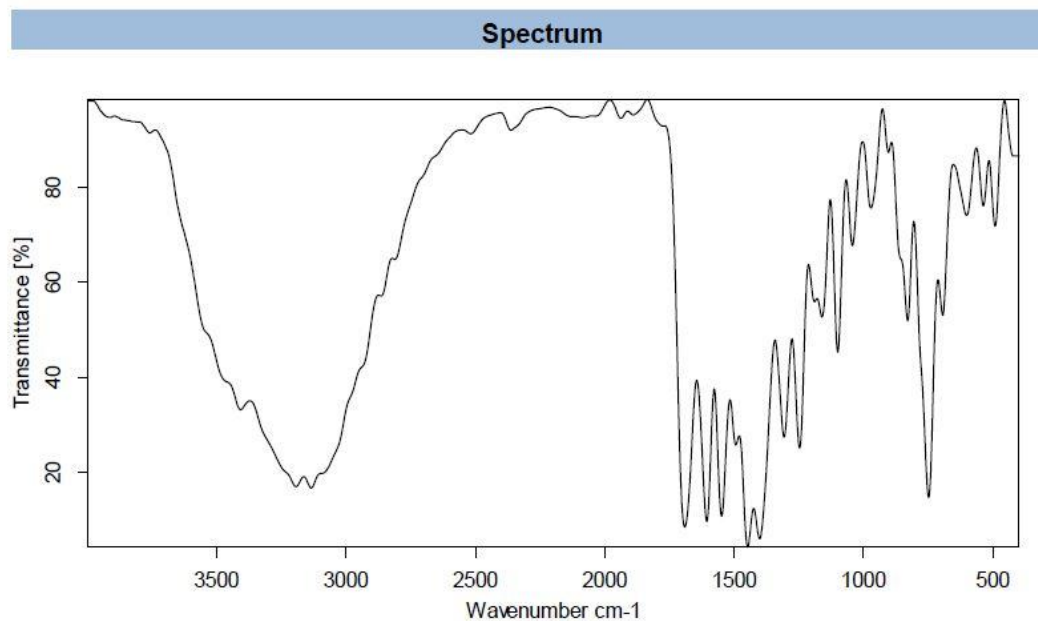


Figure S 3. IR Chart of Compound 3a

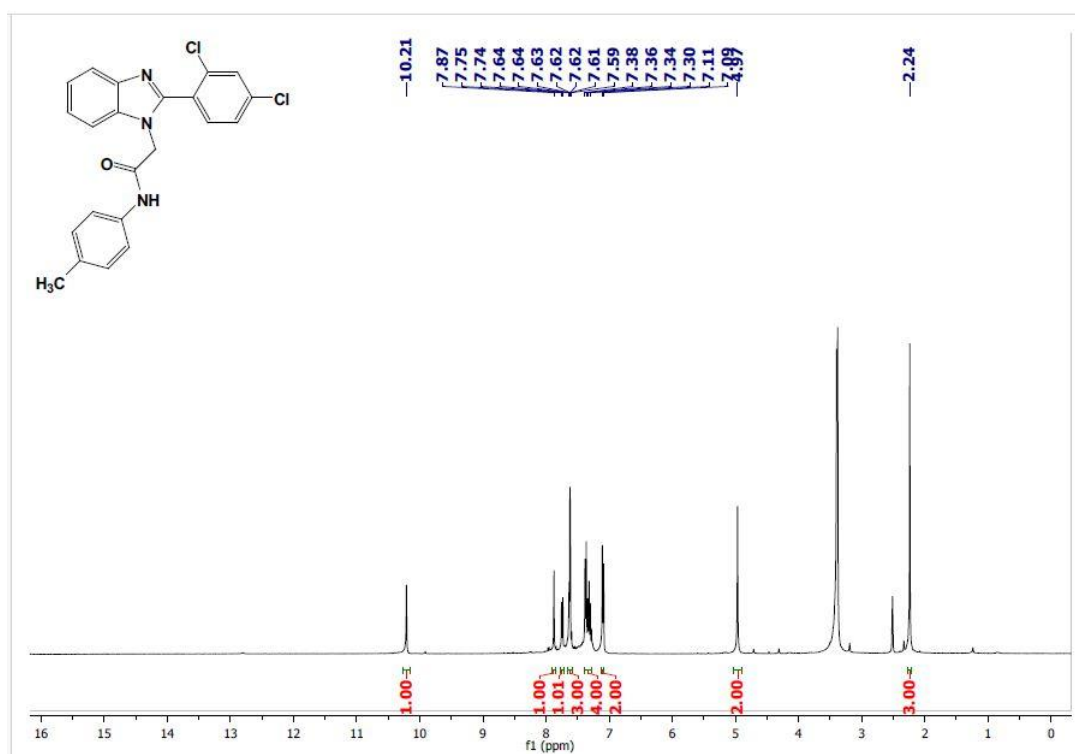


Figure S 4. ^1H -NMR Chart of Compound 3b

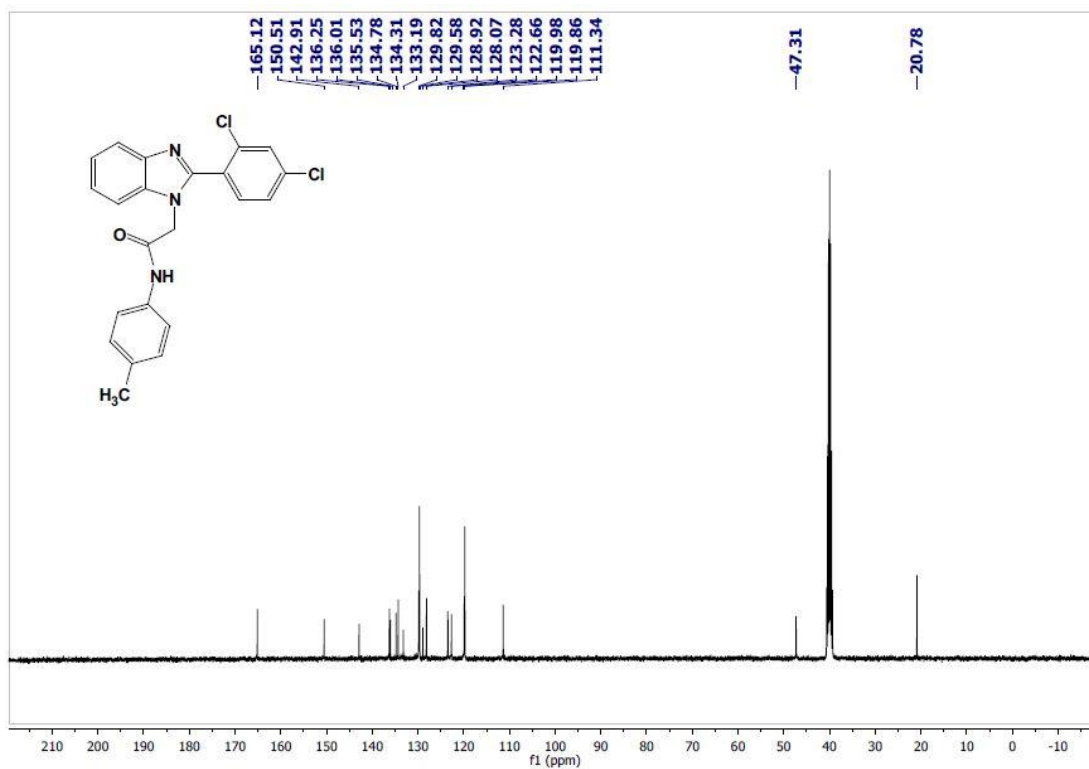


Figure S 5. ¹³C-NMR Chart of Compound 3b

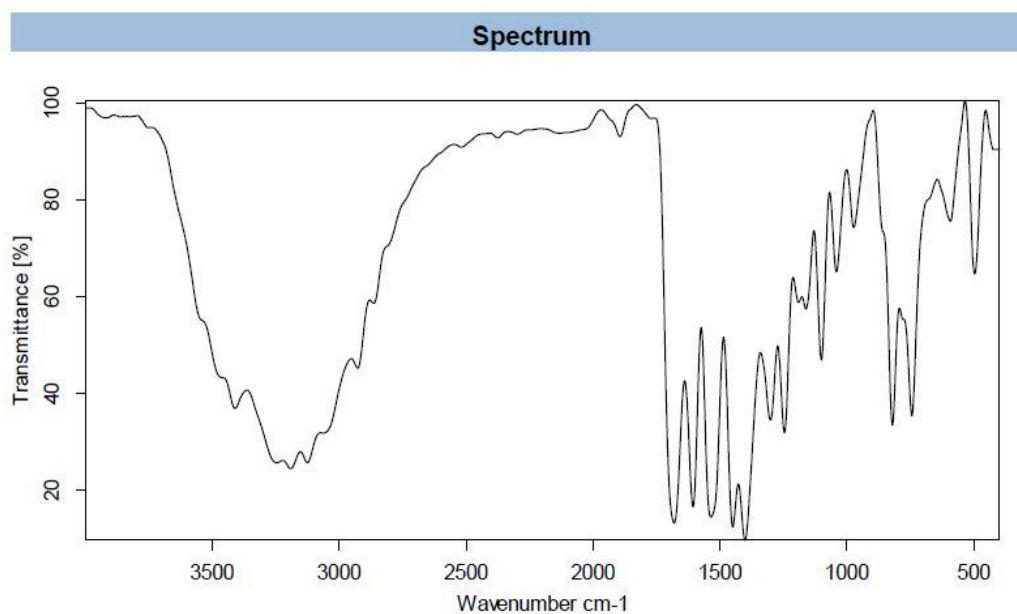


Figure S 6. IR Chart of Compound 3b

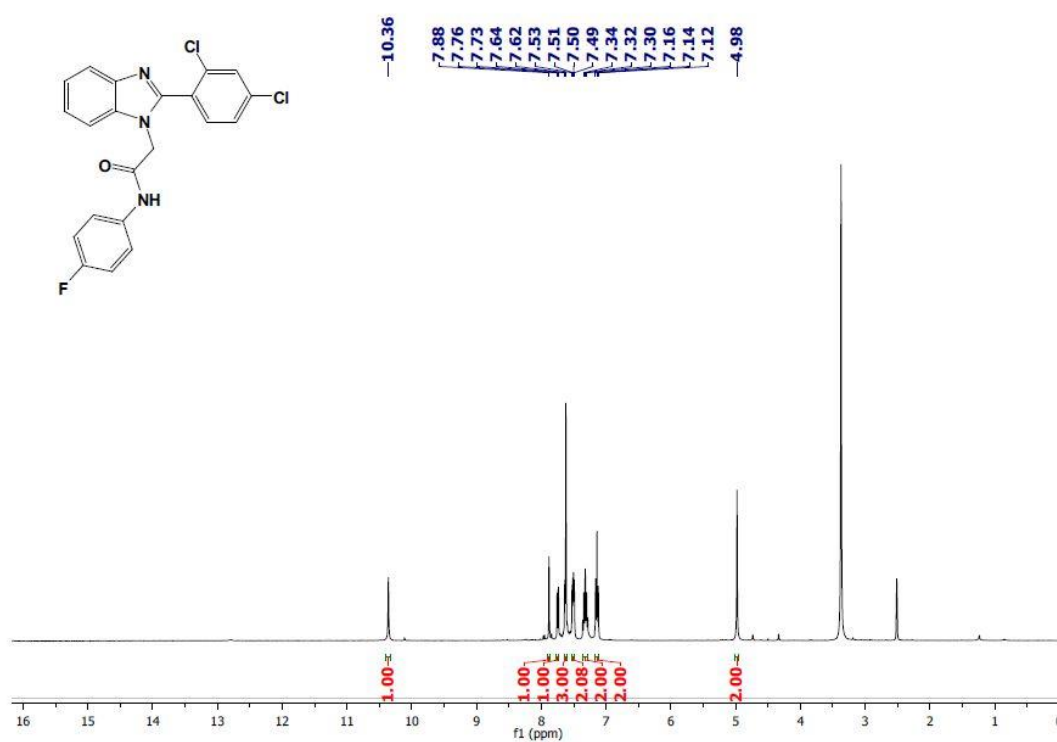


Figure S 7. ¹H-NMR Chart of Compound 3c

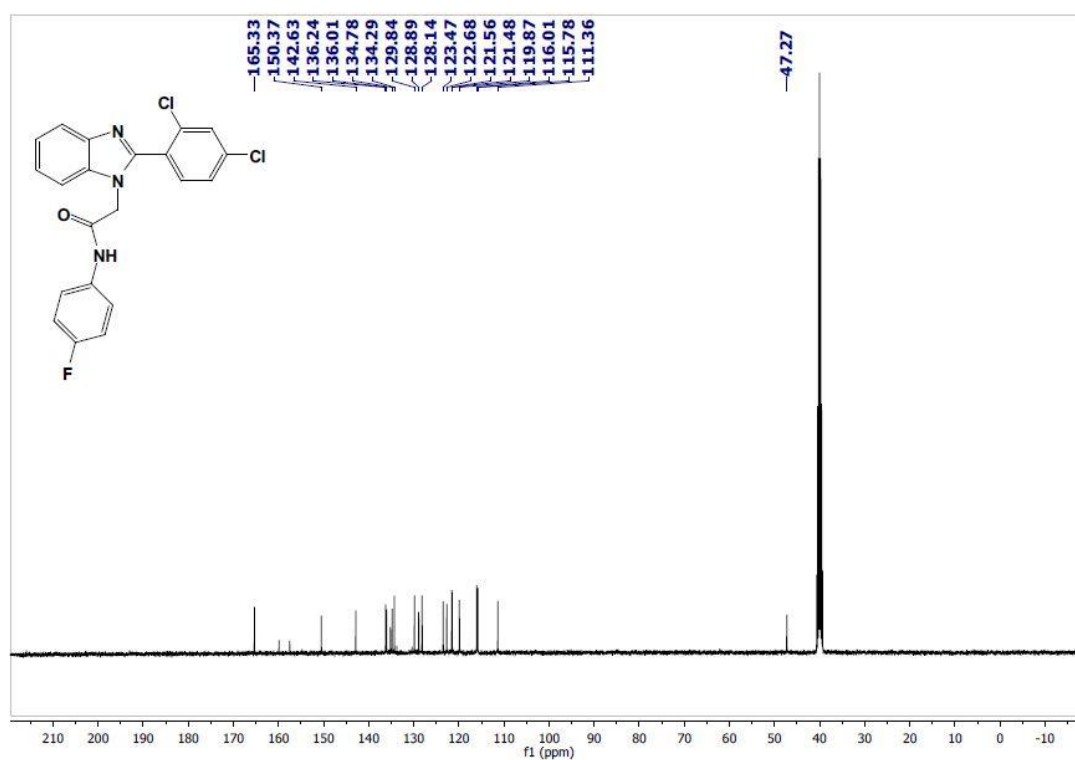


Figure S 8. ¹³C-NMR Chart of Compound 3c

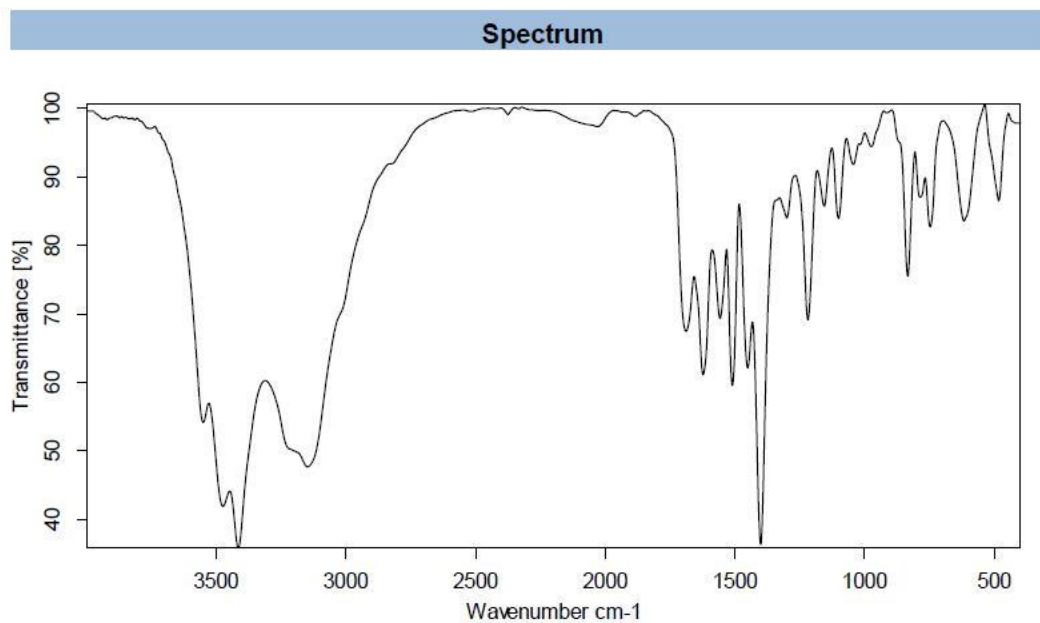


Figure S 9. IR Chart of Compound 3c

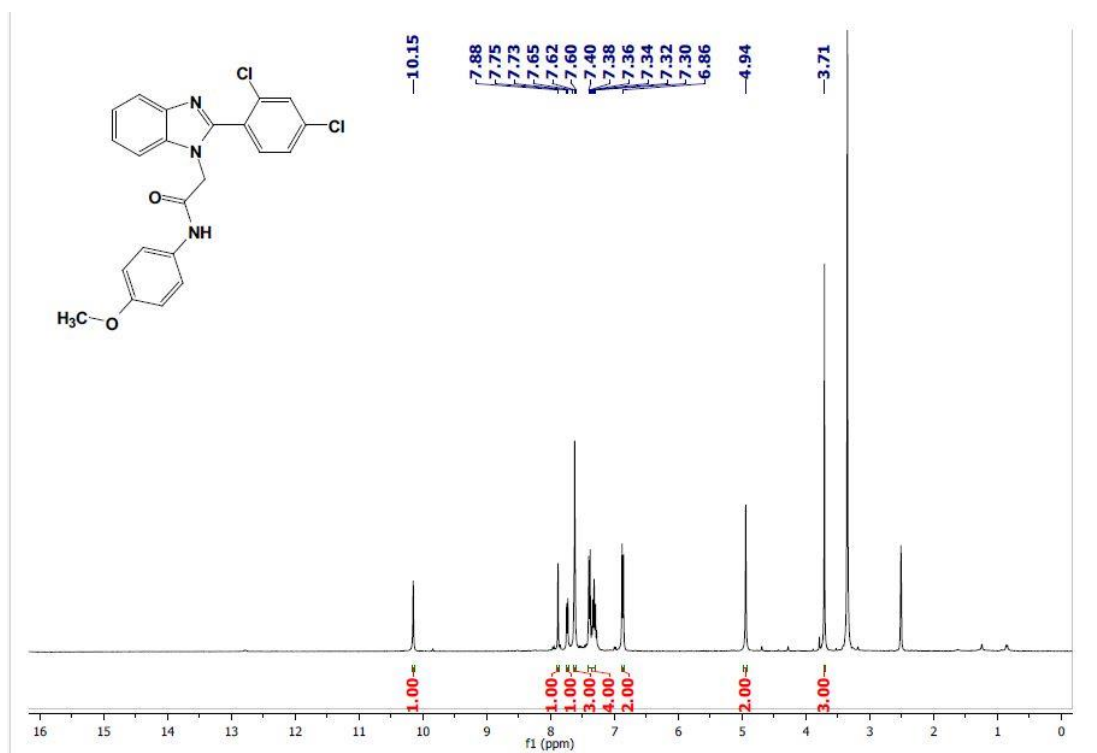


Figure S 10. ¹H-NMR Chart of Compound 3d

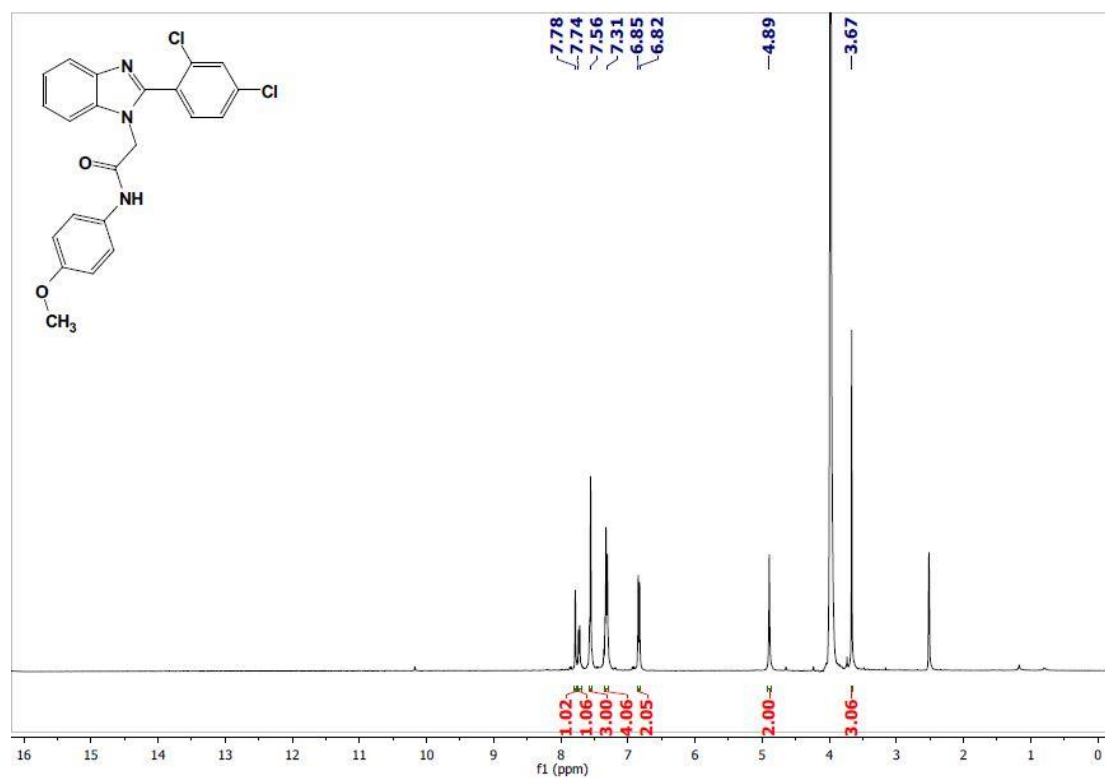


Figure S 11. ¹H-NMR D₂O Chart of Compound 3d

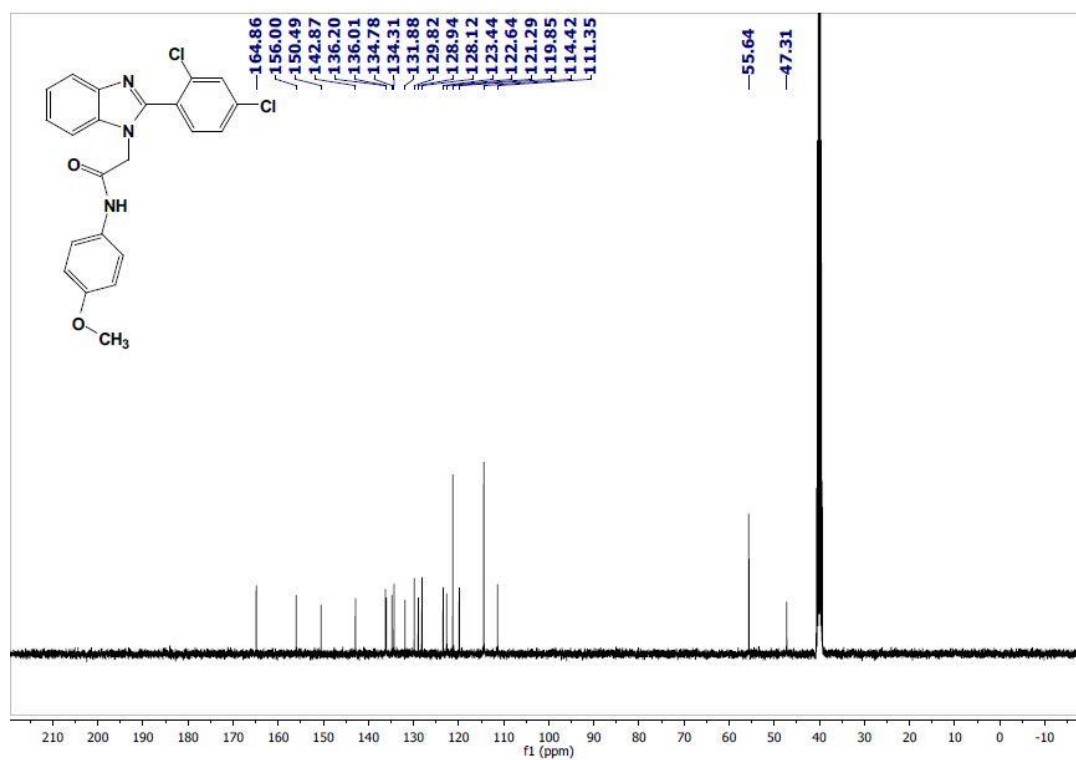


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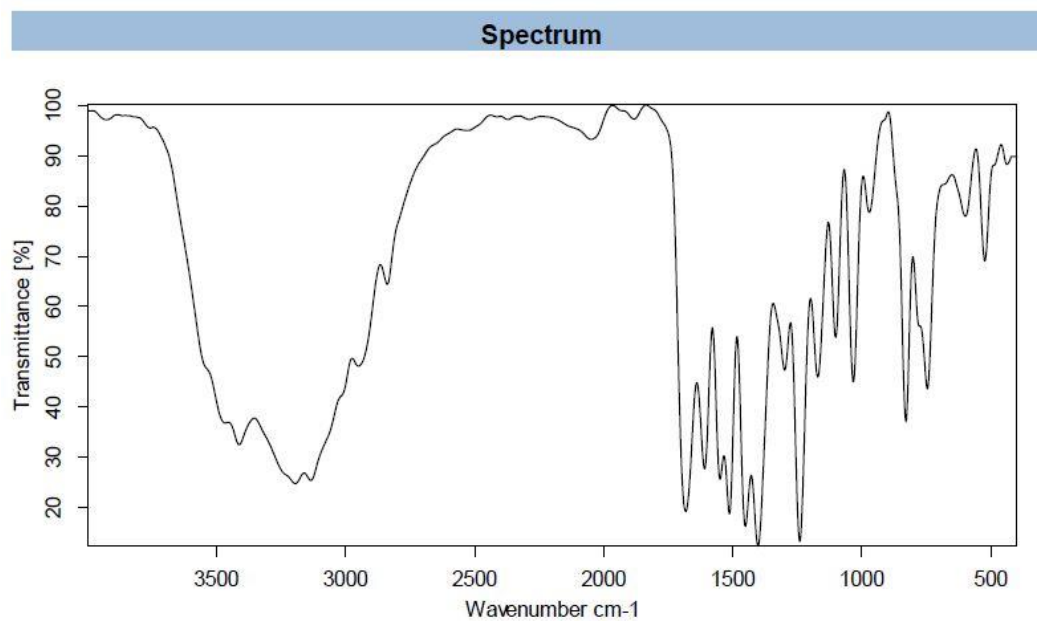


Figure S 13. IR Chart of Compound 3d

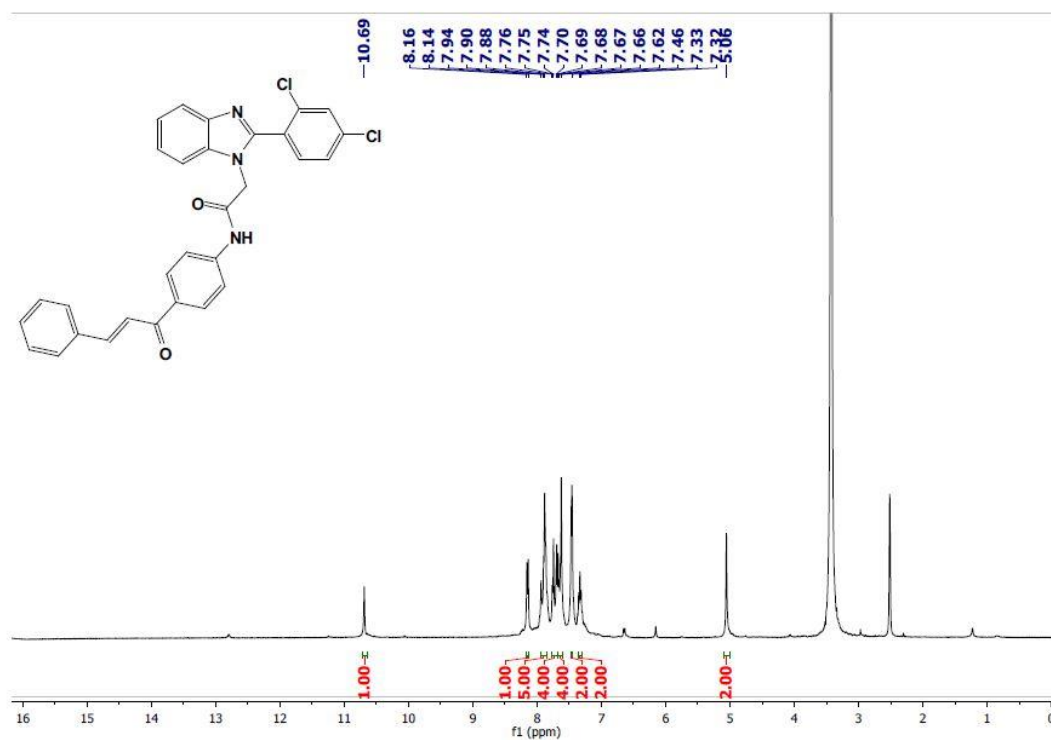


Figure S 14. ^1H -NMR Chart of Compound 5a

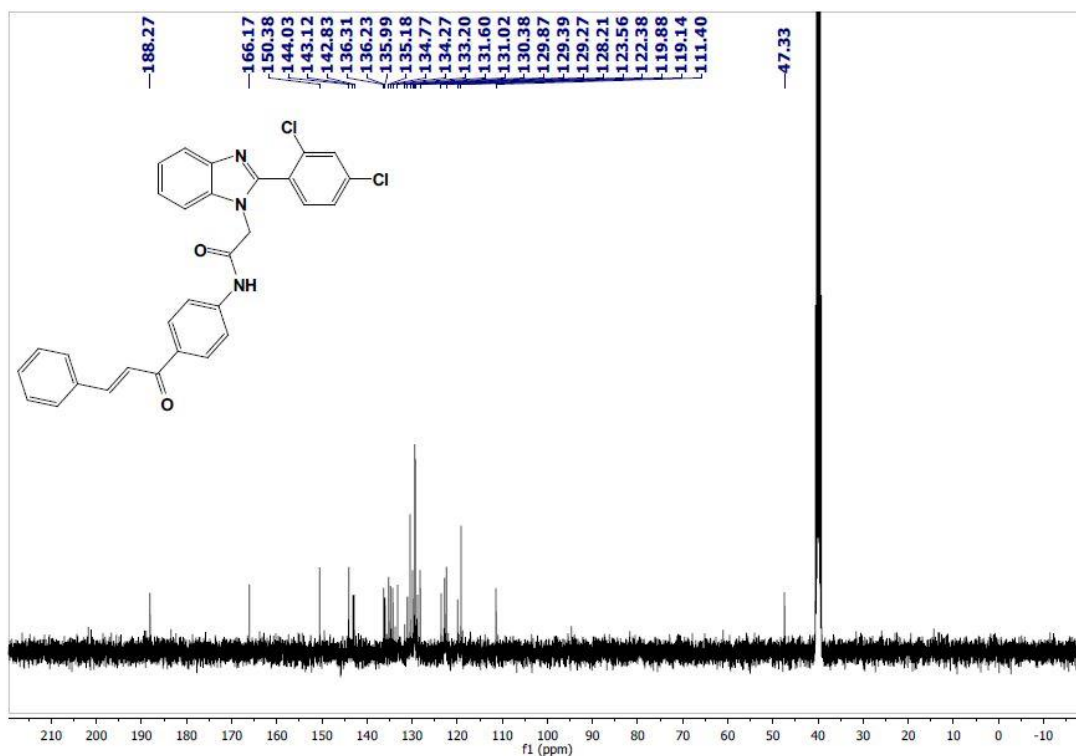


Figure S 15. ¹³C-NMR Chart of Compound 5a

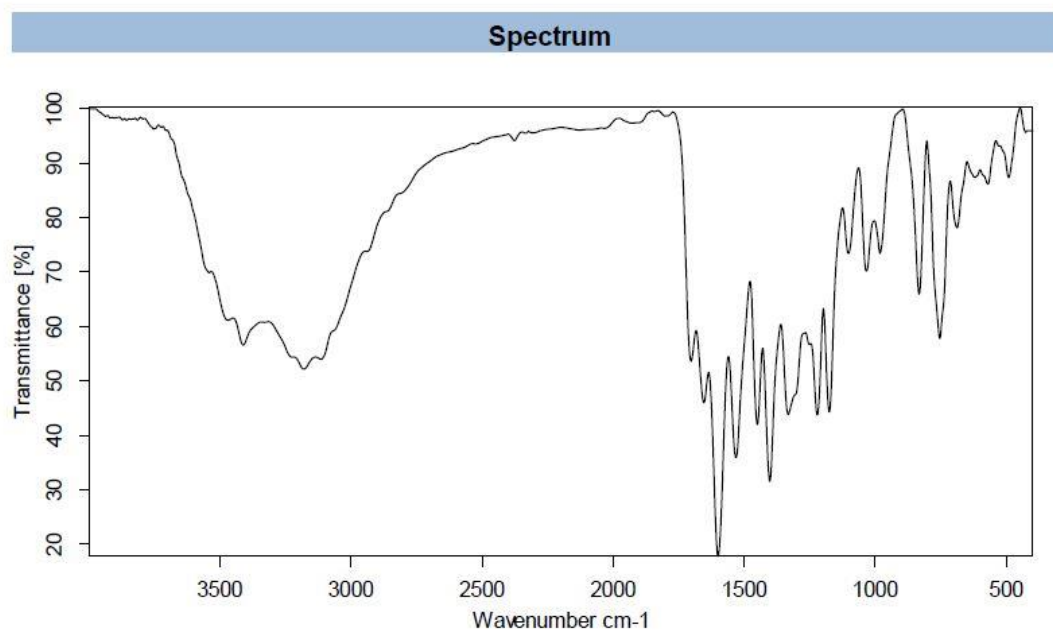


Figure S 16. IR Chart of Compound 5a

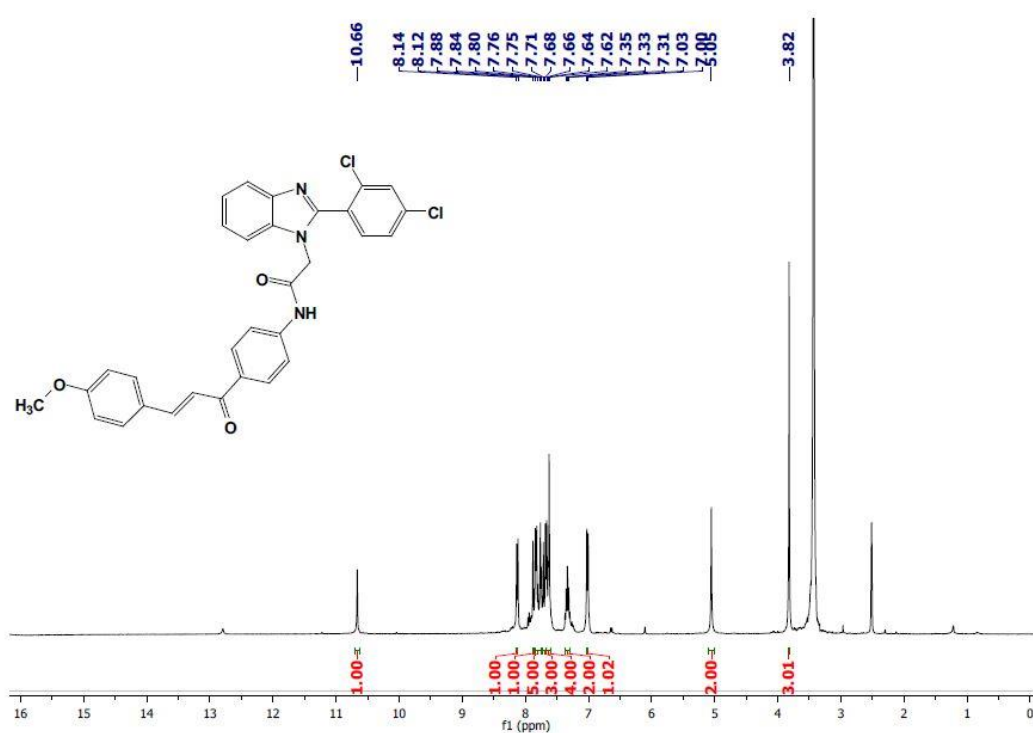


Figure S 17. ¹H-NMR Chart of Compound 5b

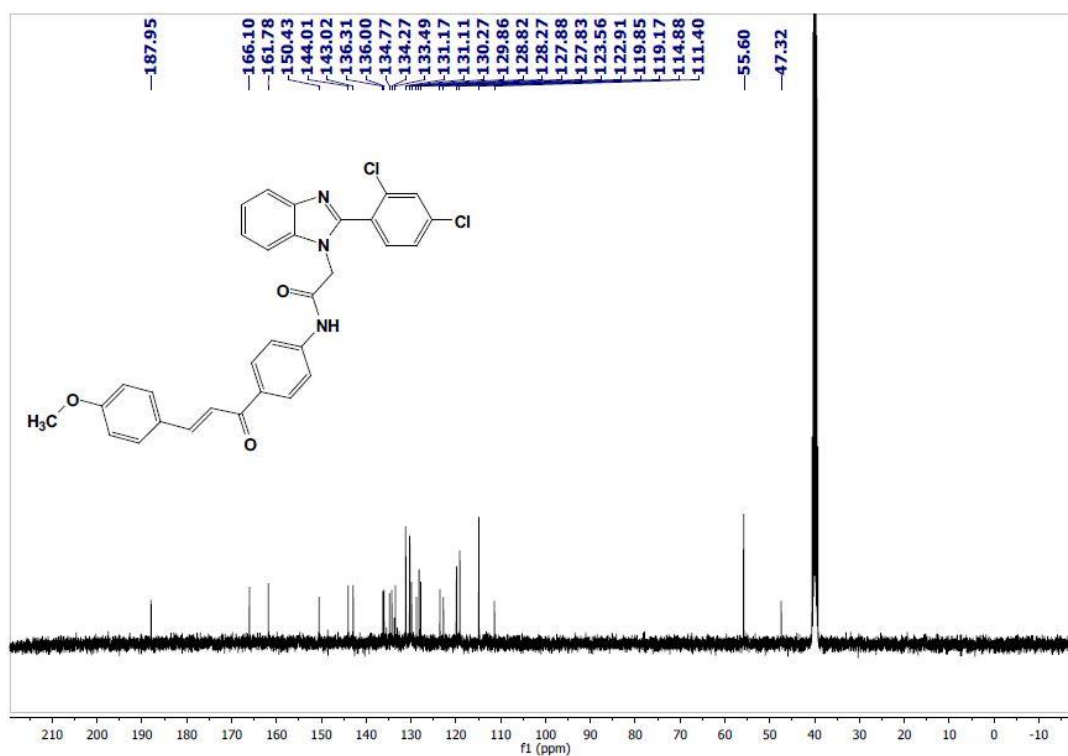


Figure S 18. ¹³C-NMR Chart of Compound 5b

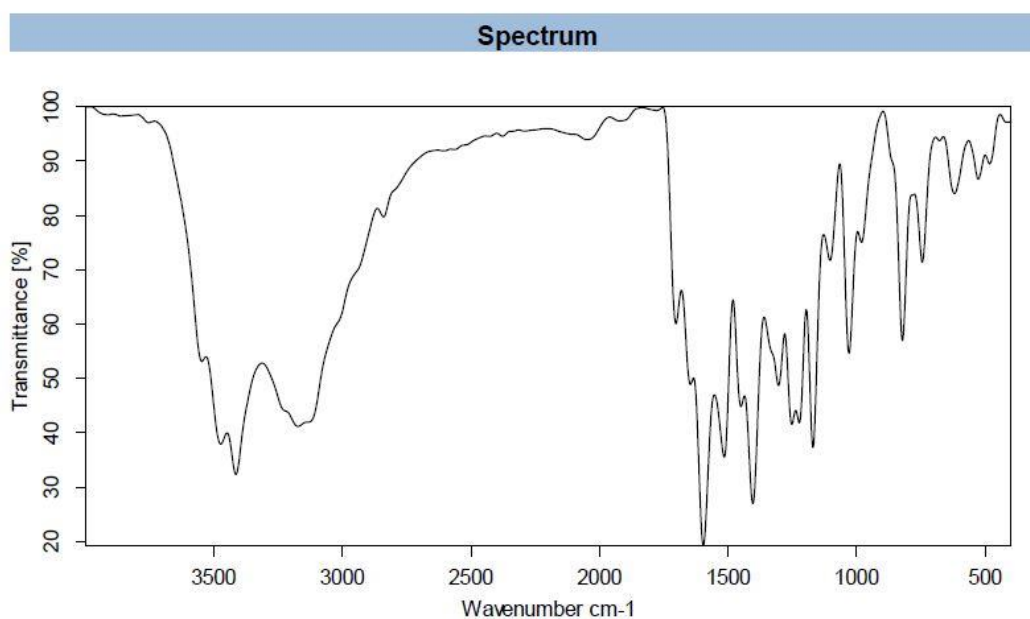


Figure S 19. IR Chart of Compound 5b

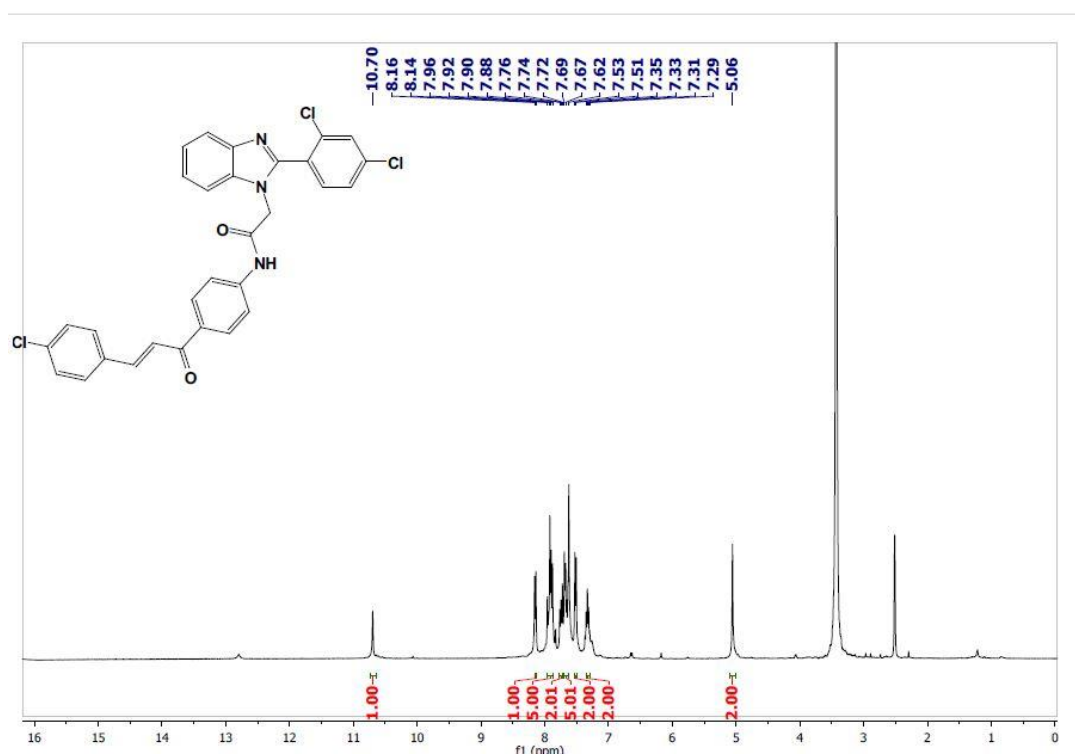


Figure S 20. ¹H-NMR Chart of Compound 5c

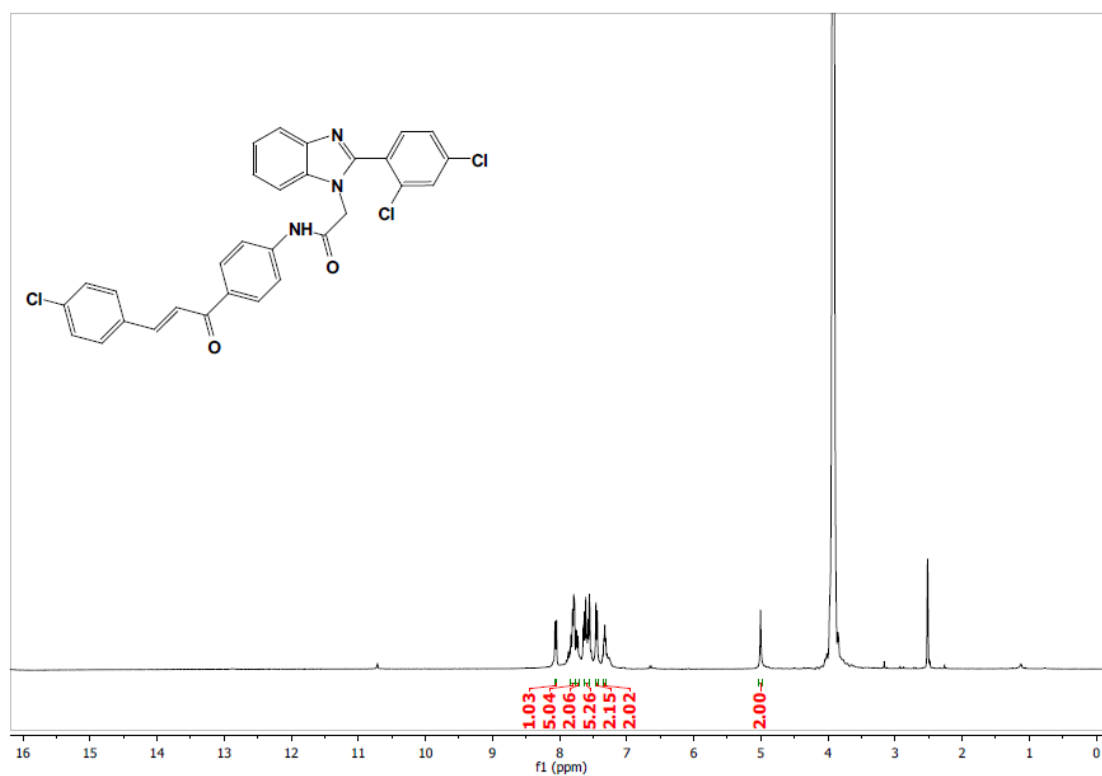


Figure S 21. ¹H-NMR D₂O Chart of Compound 5c

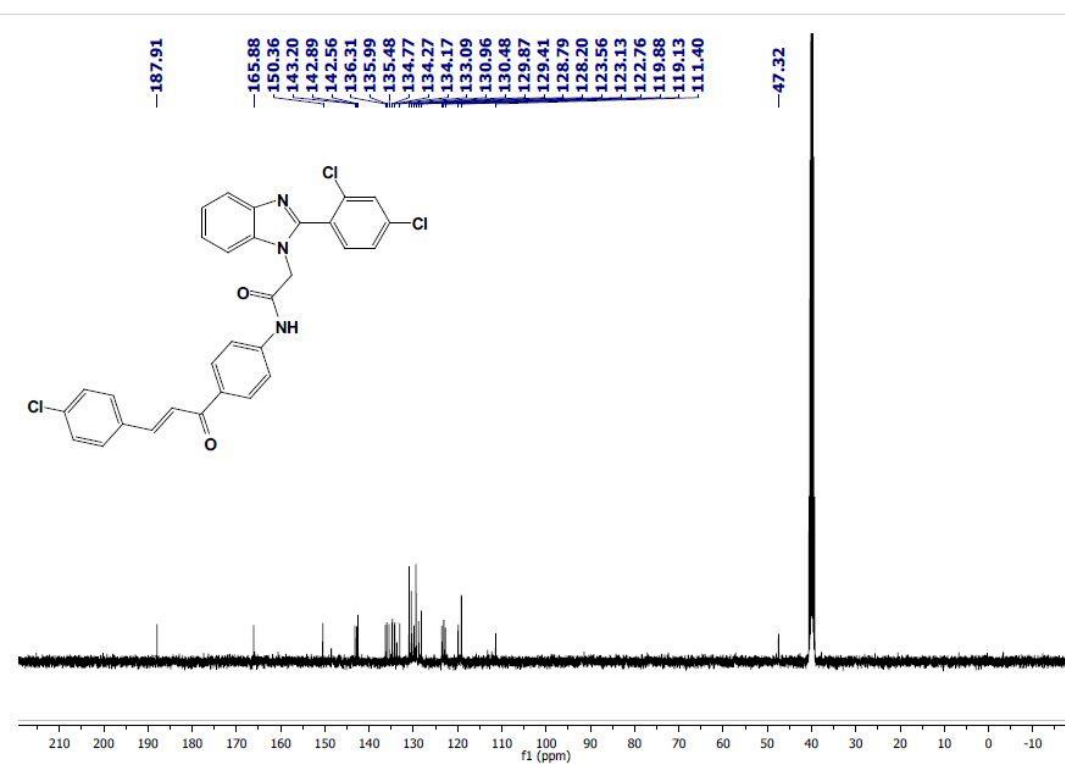


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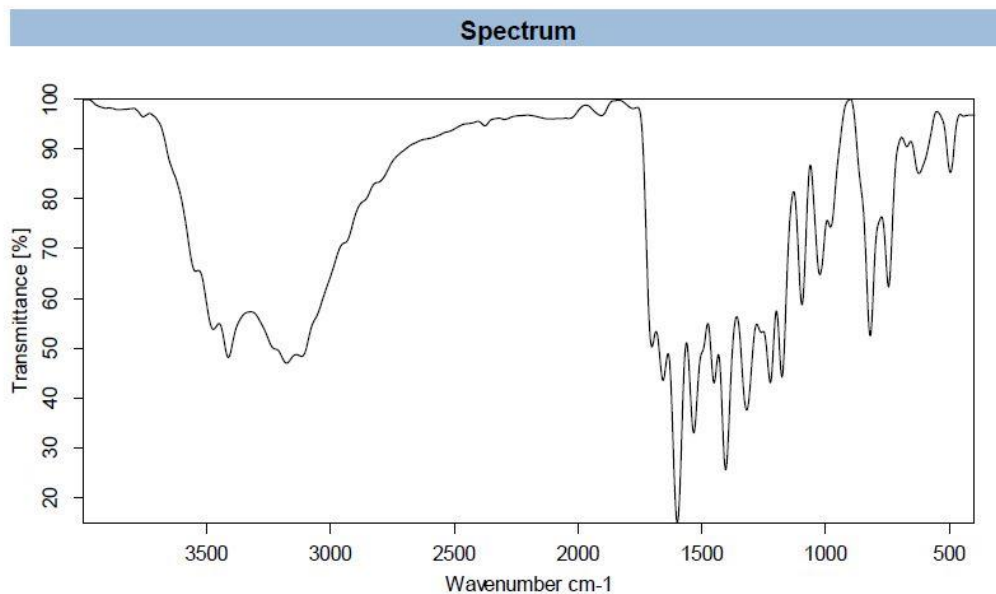


Figure S 23. IR Chart of Compound 5c

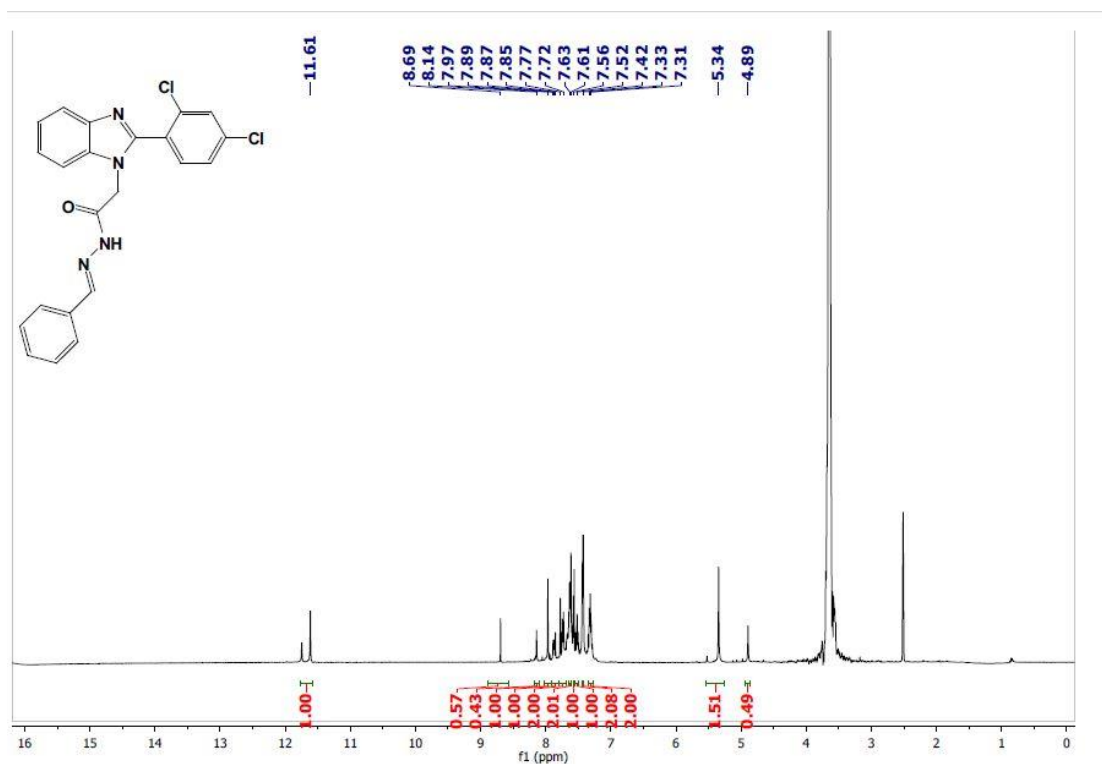


Figure S 24. ^1H -NMR Chart of Compound 8a

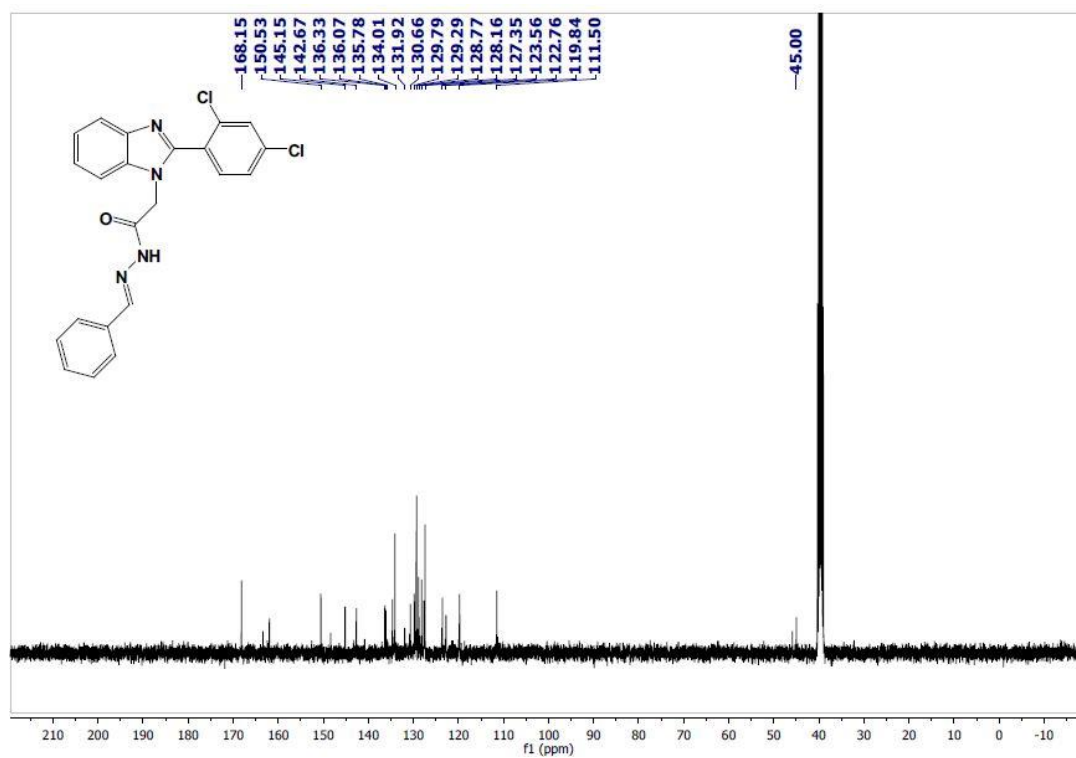


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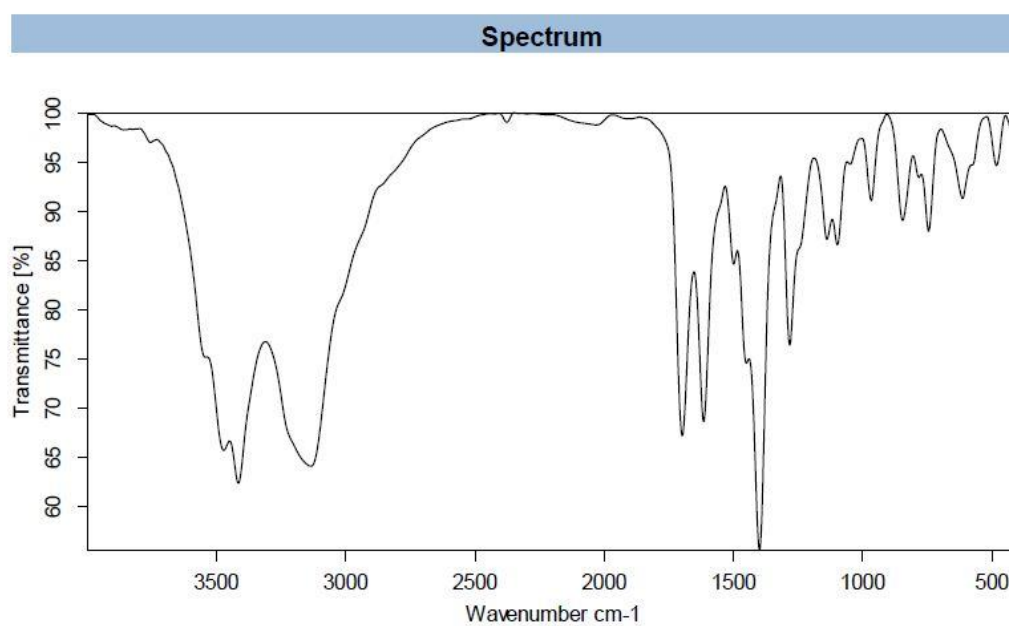


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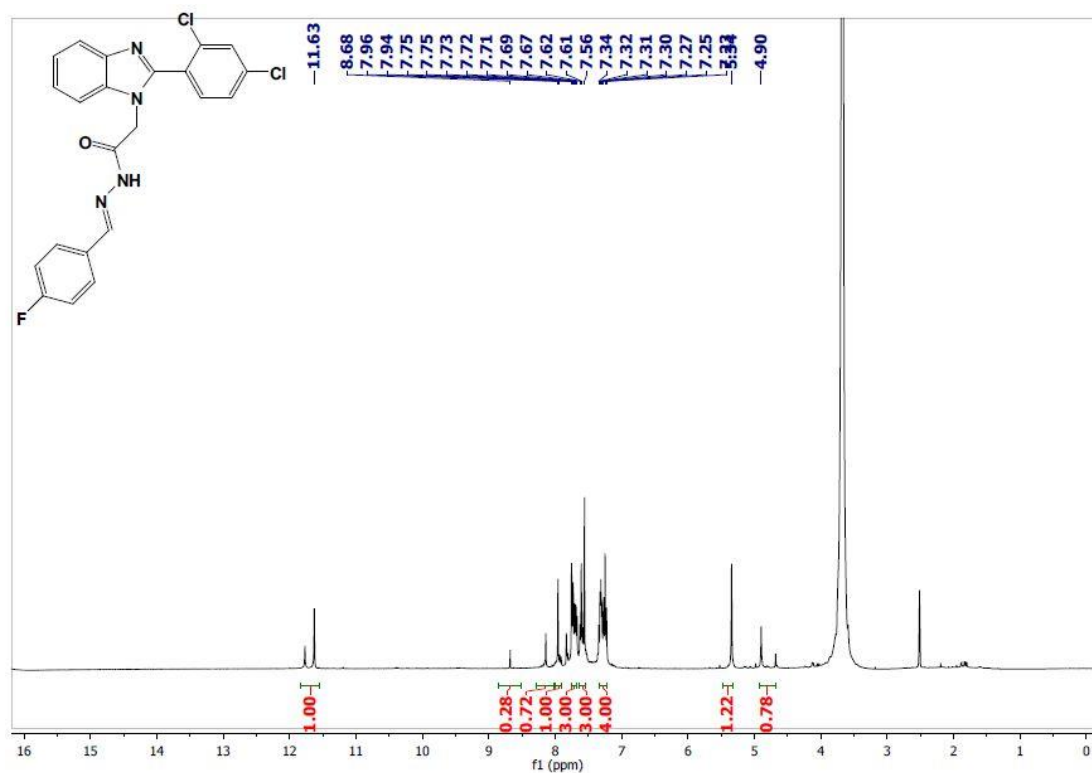


Figure S 27. ¹H-NMR Chart of Compound 8b

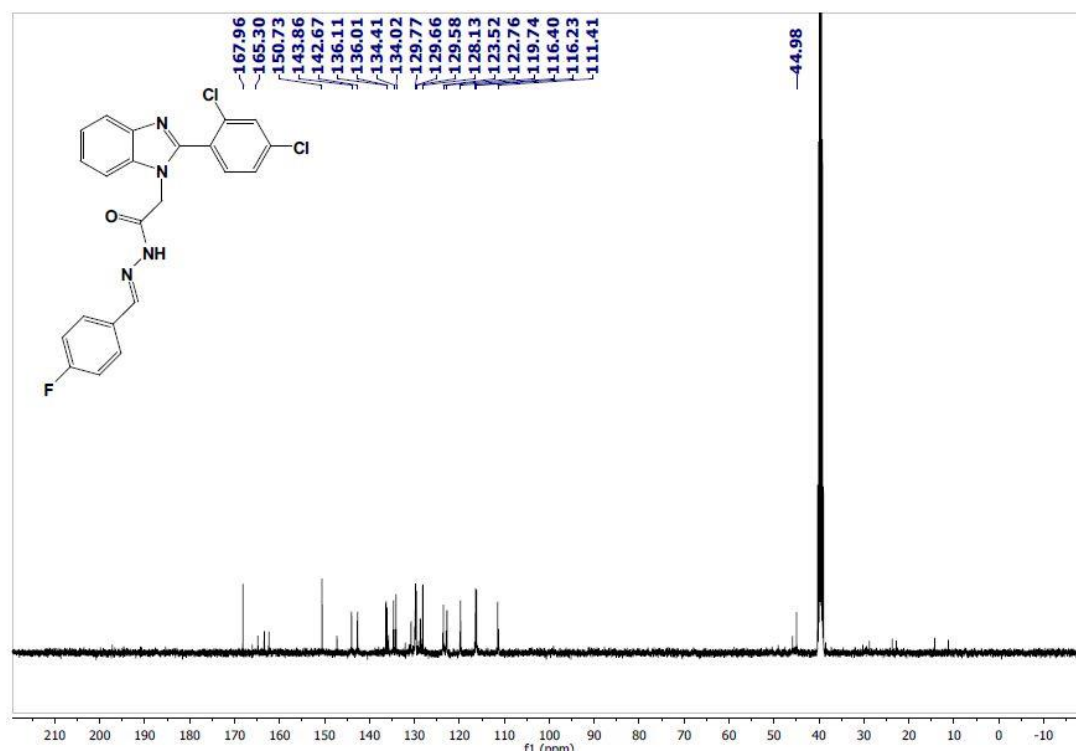


Figure S 28. ¹³C-NMR Chart of Compound 8b

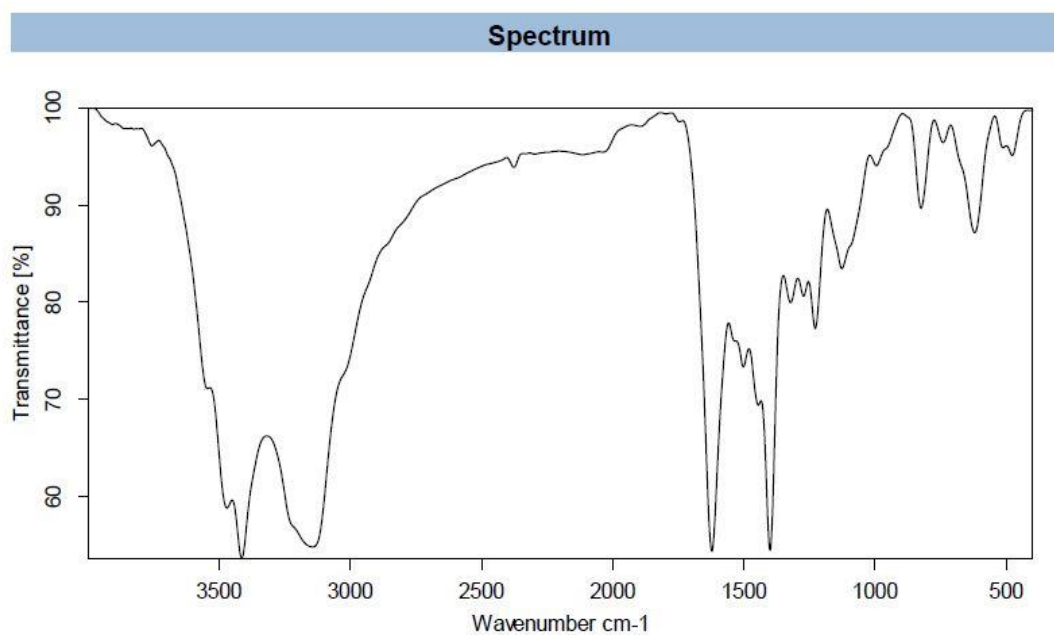


Figure S 29. IR Chart of Compound 8b

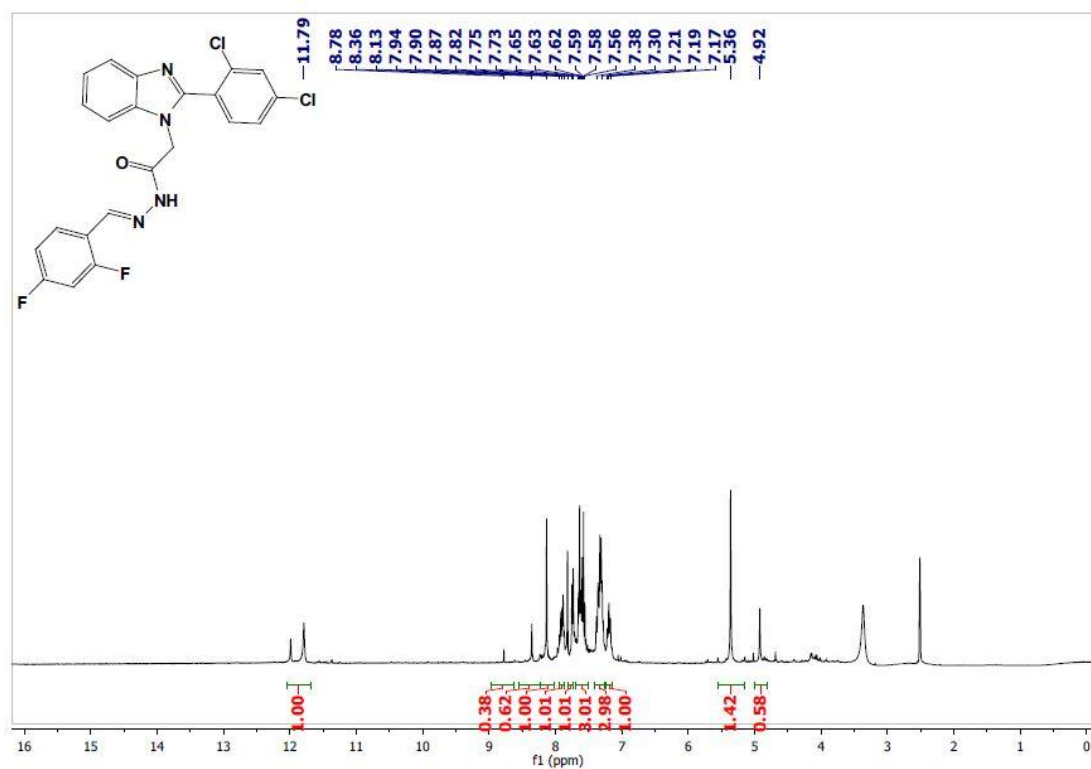


Figure S 30. ¹H-NMR Chart of Compound 8c

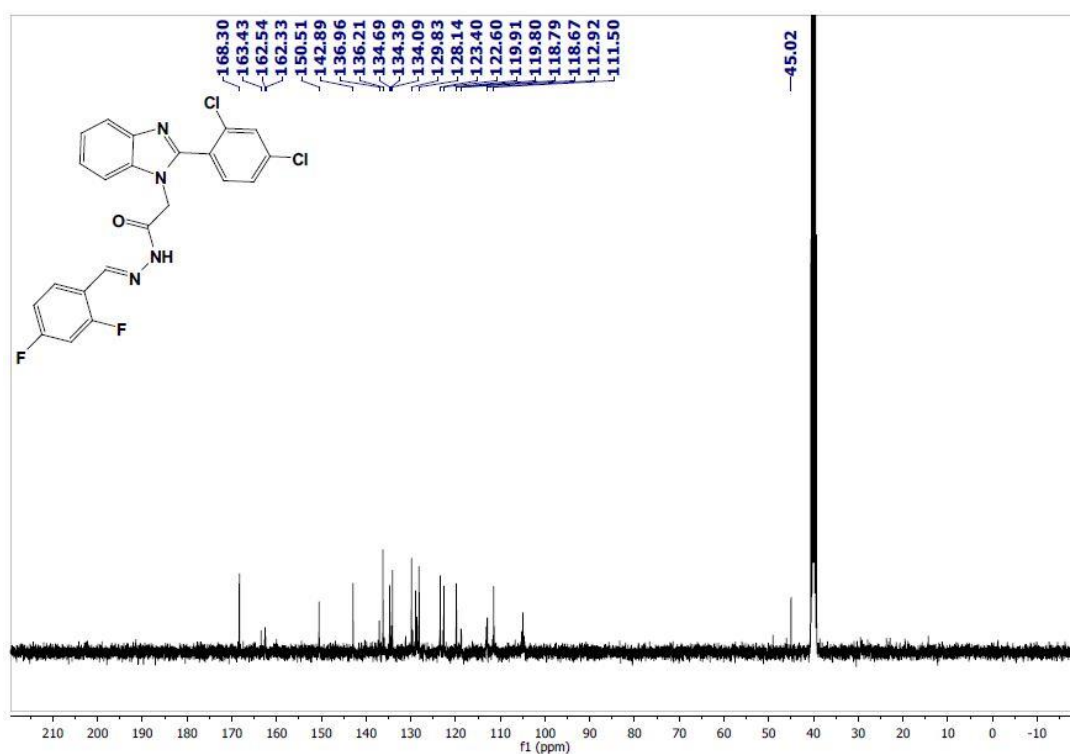


Figure S 31. ¹³C-NMR Chart of Compound 8c

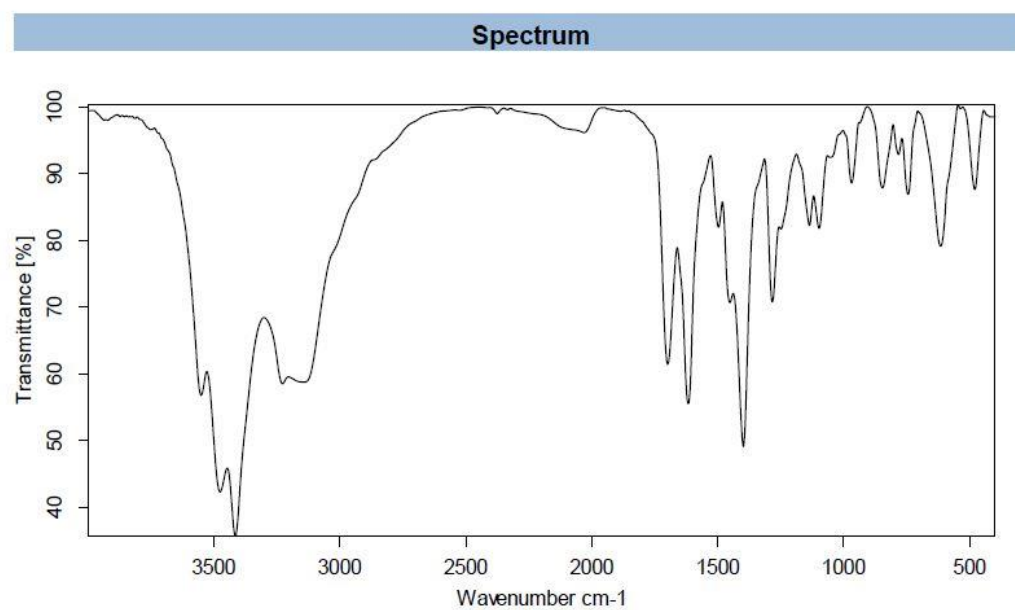


Figure S 32. IR Chart of Compound 8c

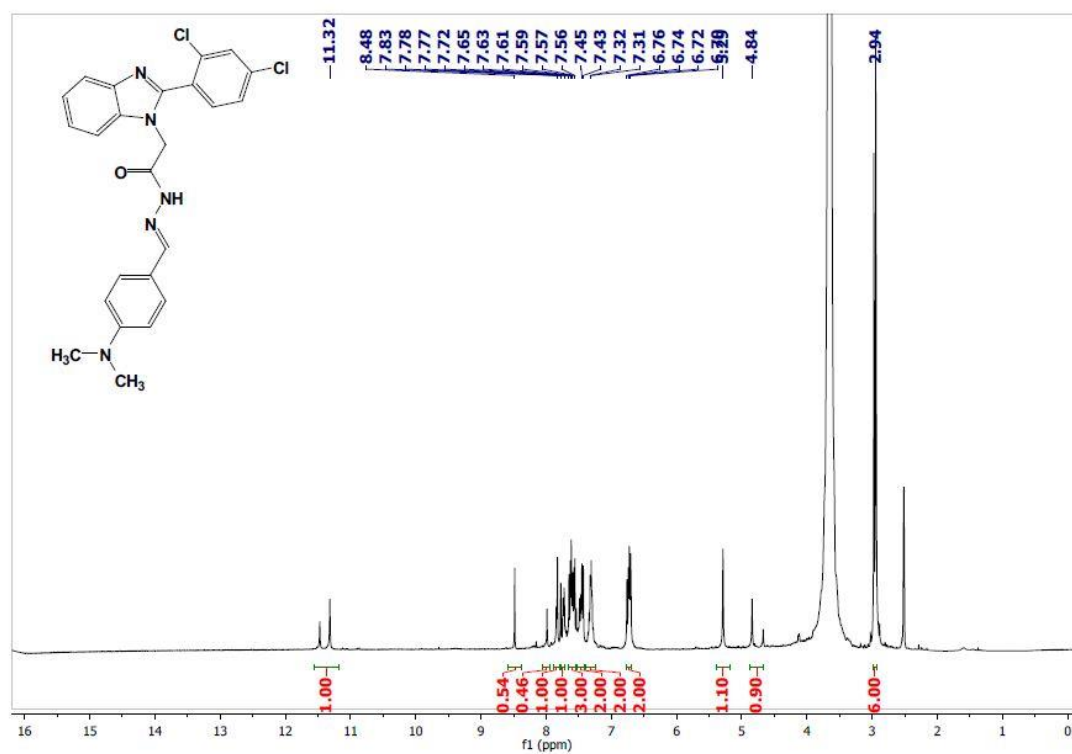


Figure S 33. ¹H-NMR Chart of Compound 8d

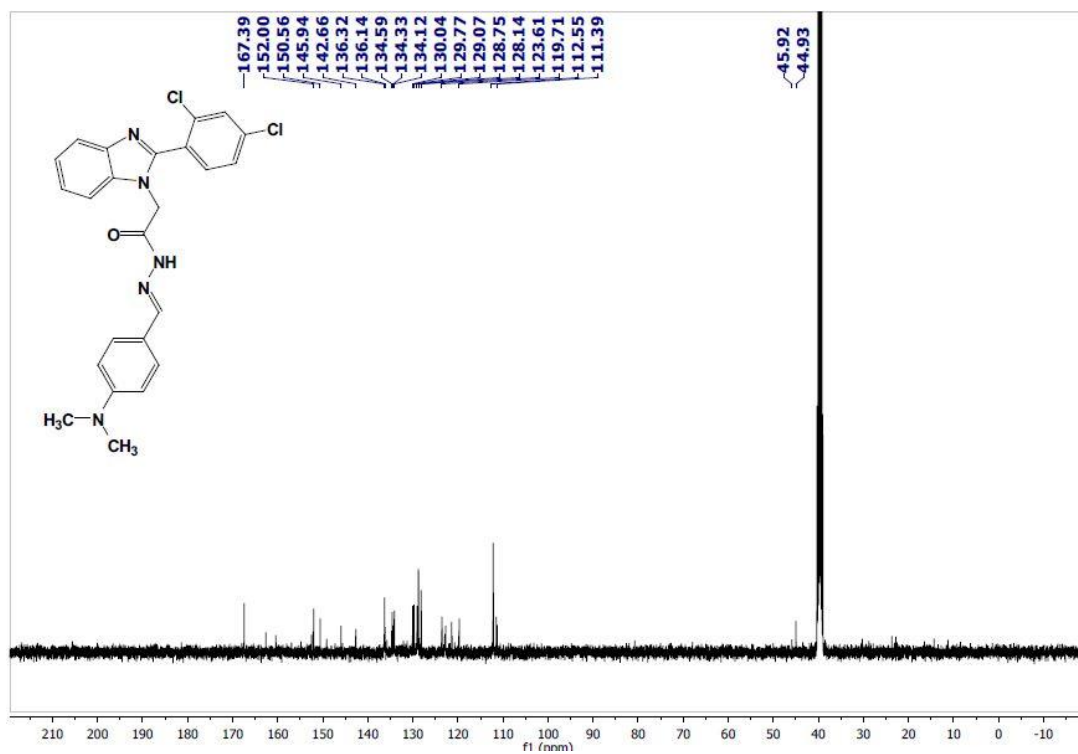


Figure S 34. ¹³C-NMR Chart of Compound 8d

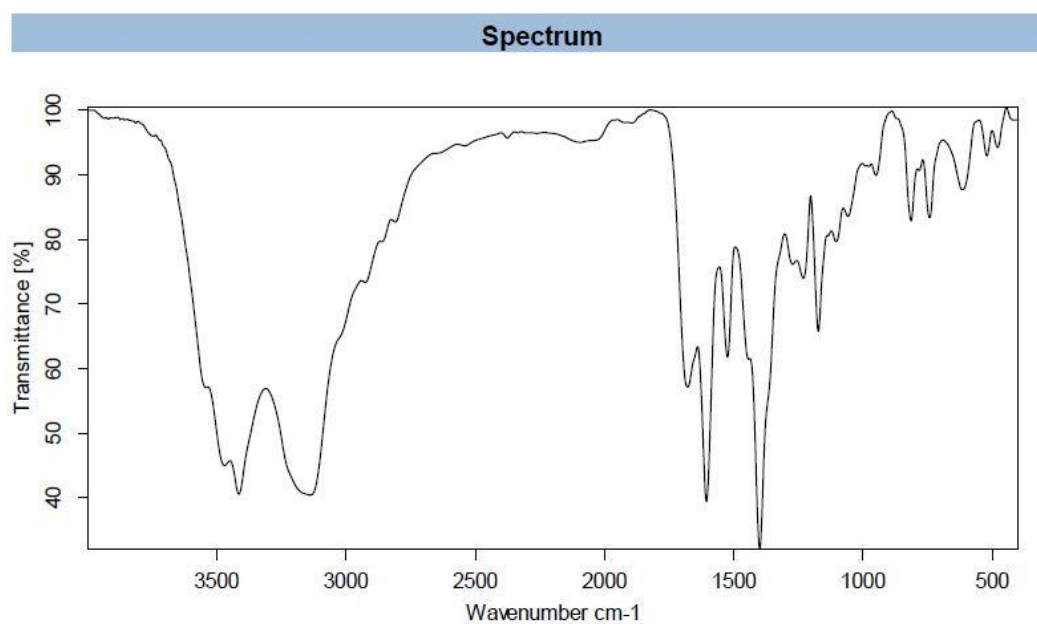


Figure S 35. IR Chart of Compound 8d

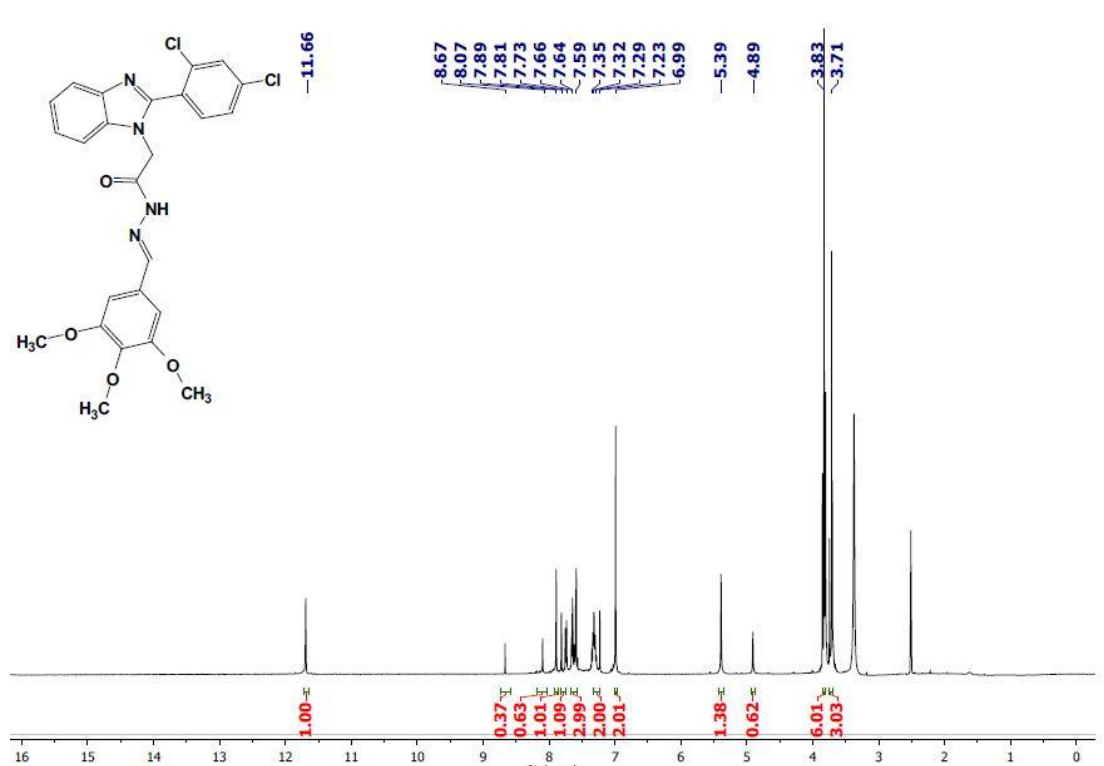


Figure S 36. ¹H-NMR Chart of Compound 8e

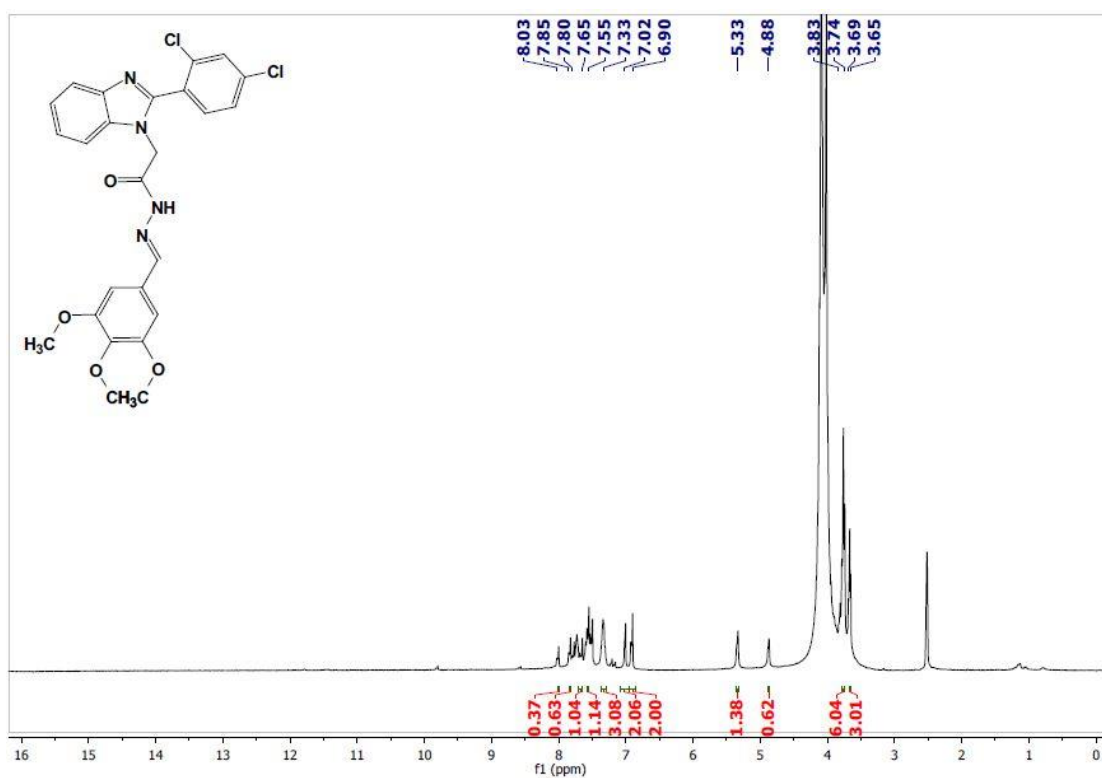


Figure S 37. ¹H-NMR D2O Chart of Compound 8e

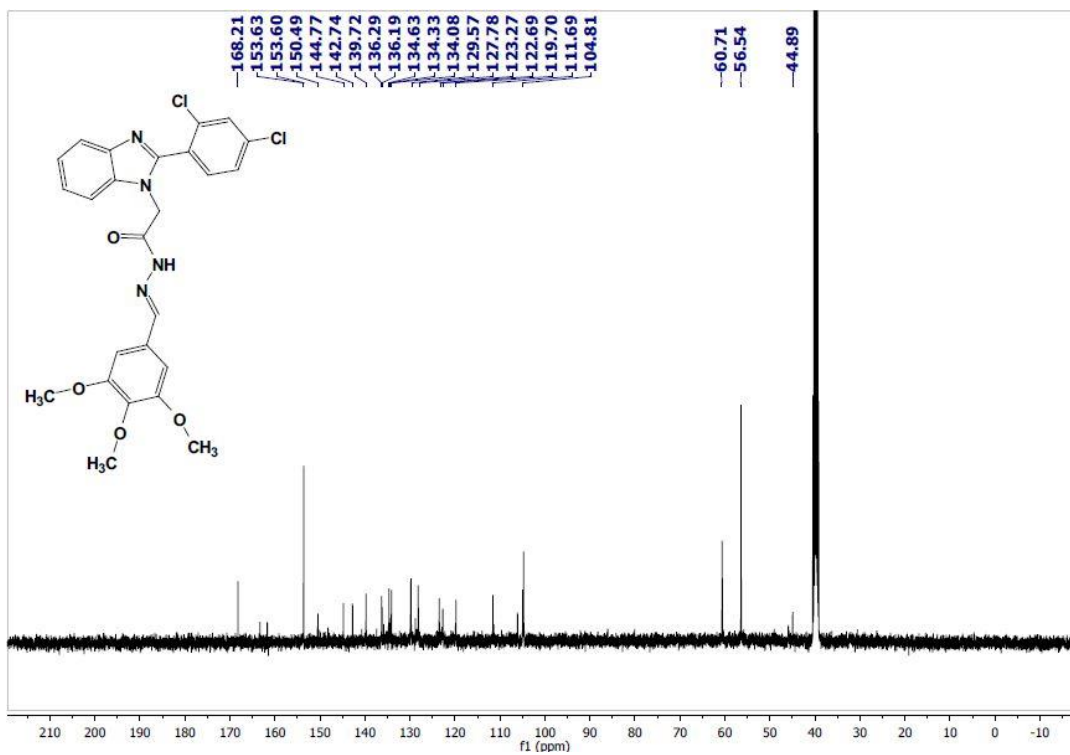


Figure S 38. ¹³C-NMR Chart of Compound 8e

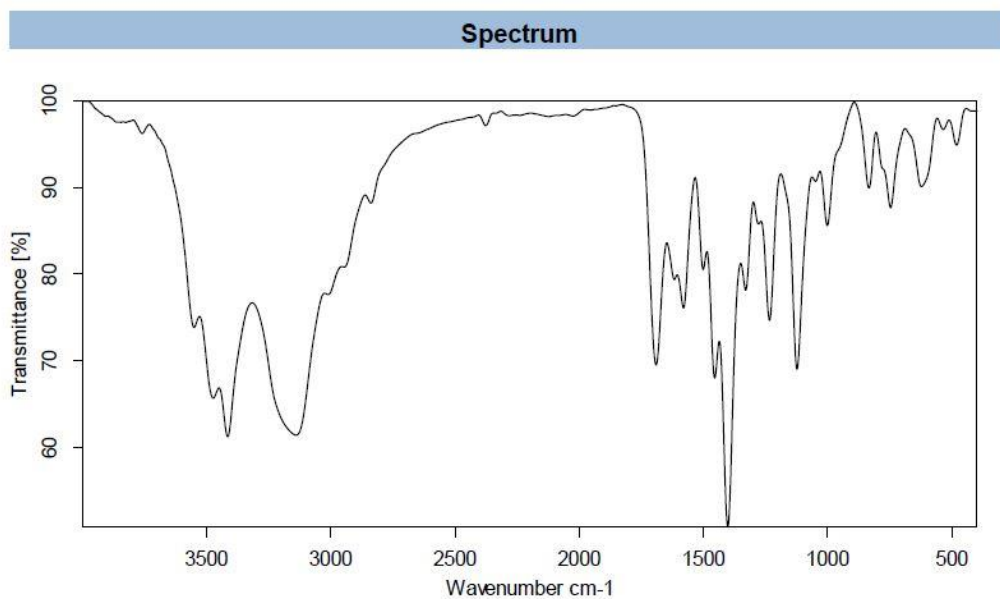


Figure S 39. IR Chart of Compound 8e

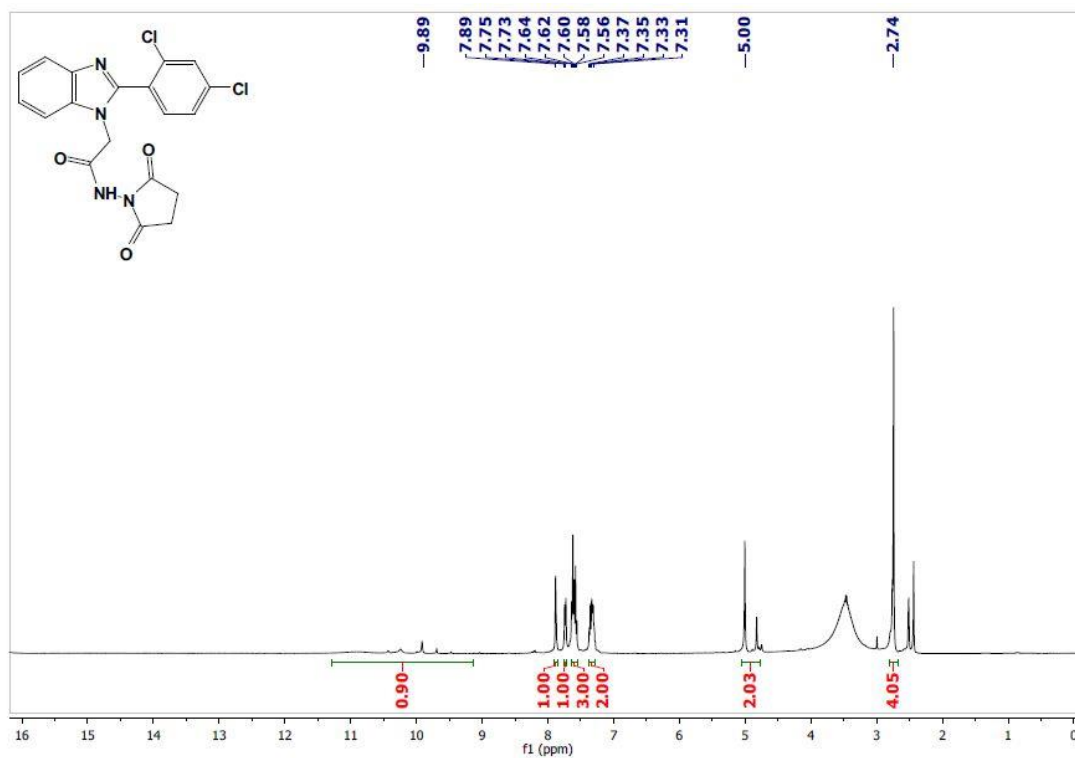


Figure S 40. ¹H-NMR Chart of Compound 9a

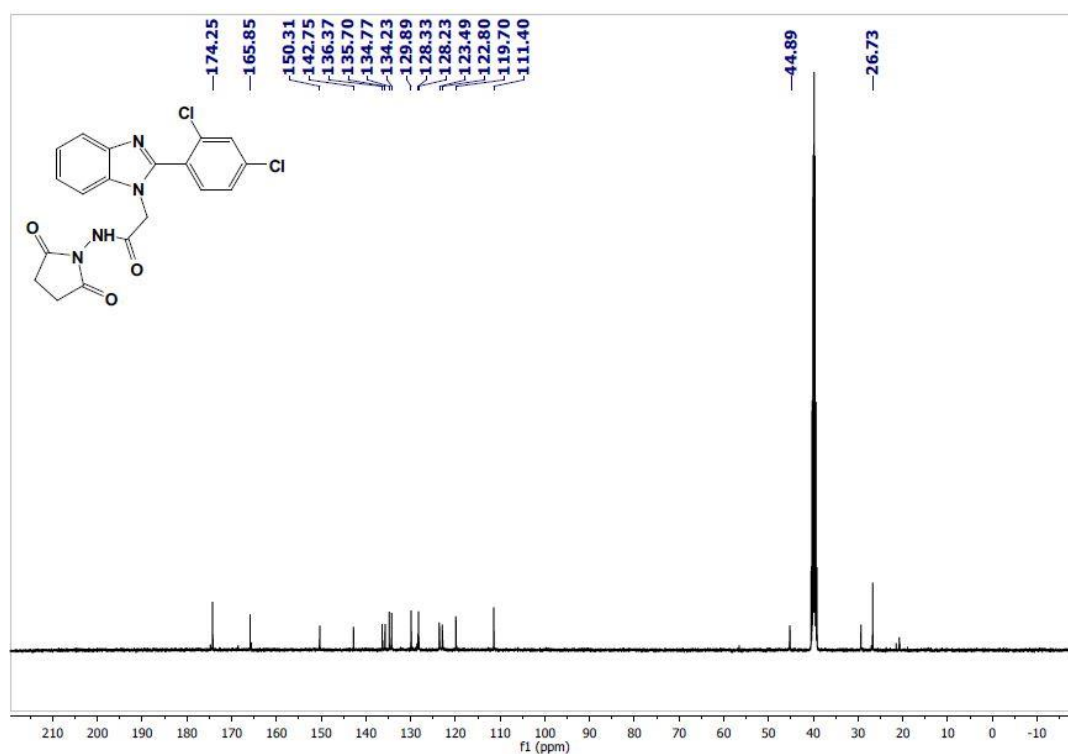


Figure S 41. ¹³C-NMR Chart of Compound 9a

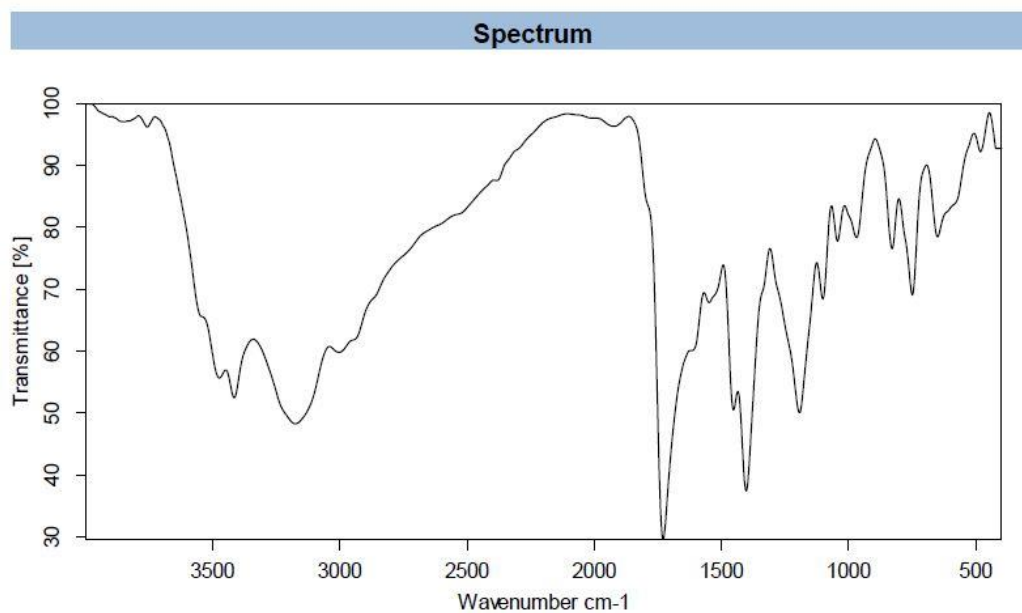


Figure S 42. IR Chart of Compound 9a

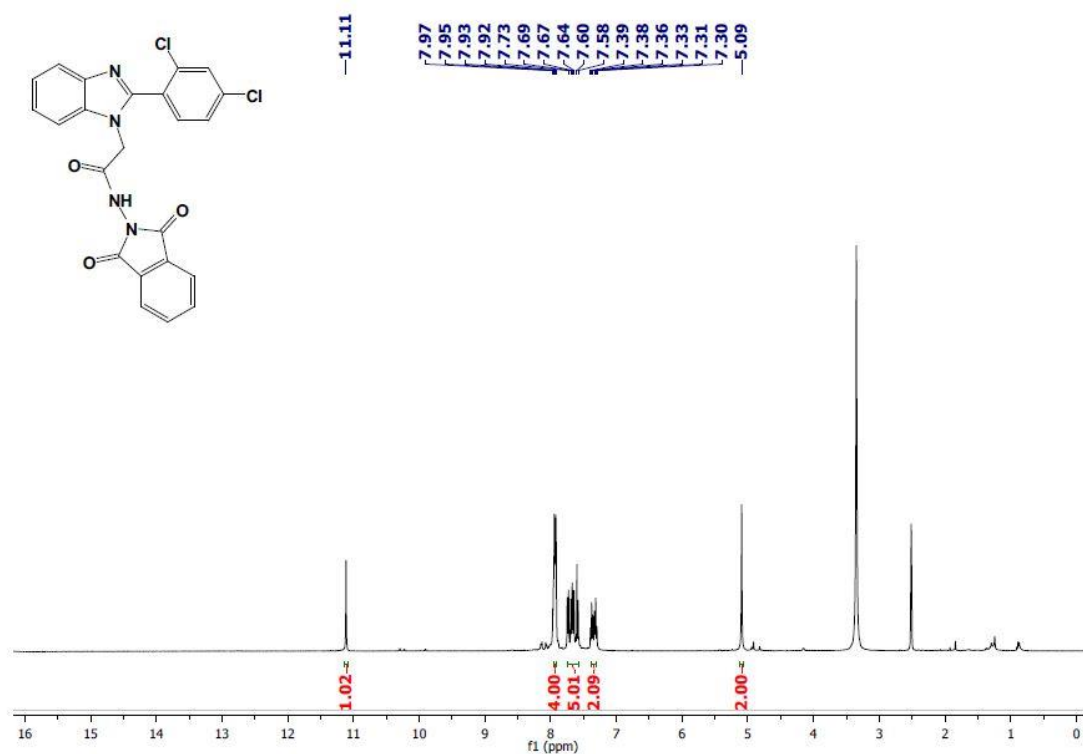


Figure S 43. ¹H-NMR Chart of Compound 9b

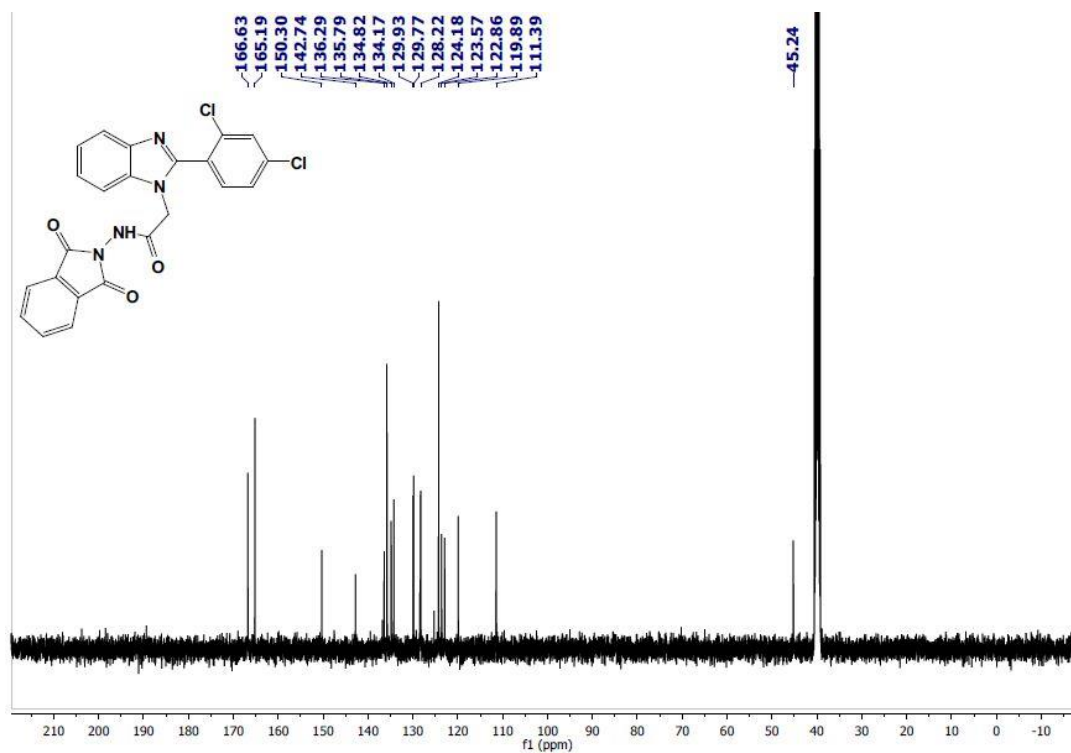


Figure S 44. ¹³C-NMR Chart of Compound 9b

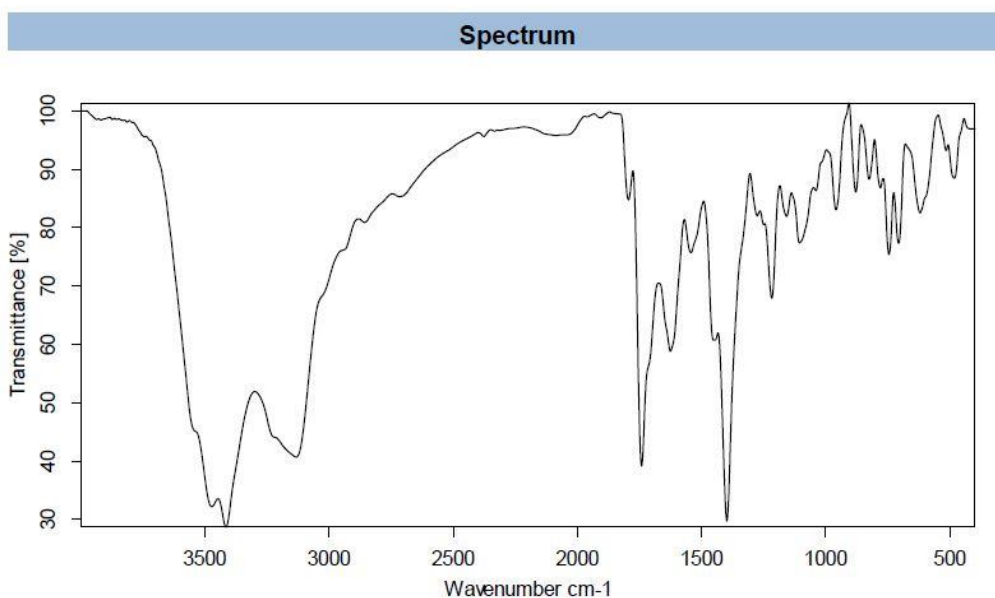


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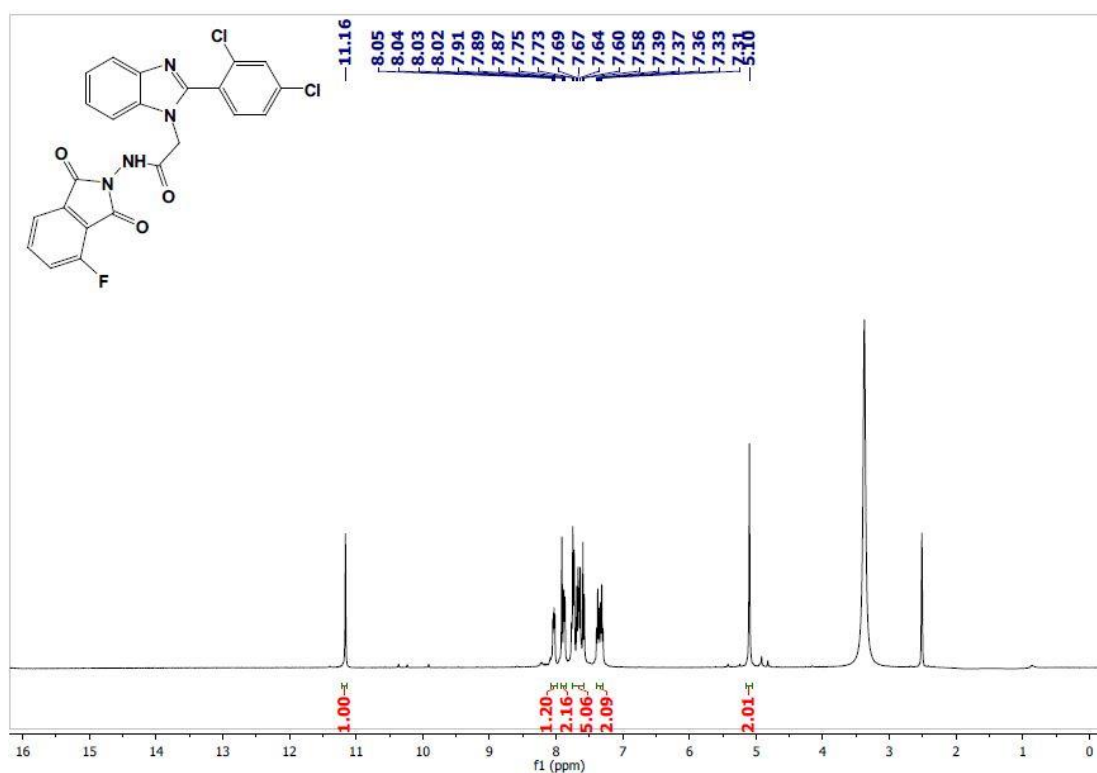


Figure S 46. ¹H-NMR Chart of Compound 9c

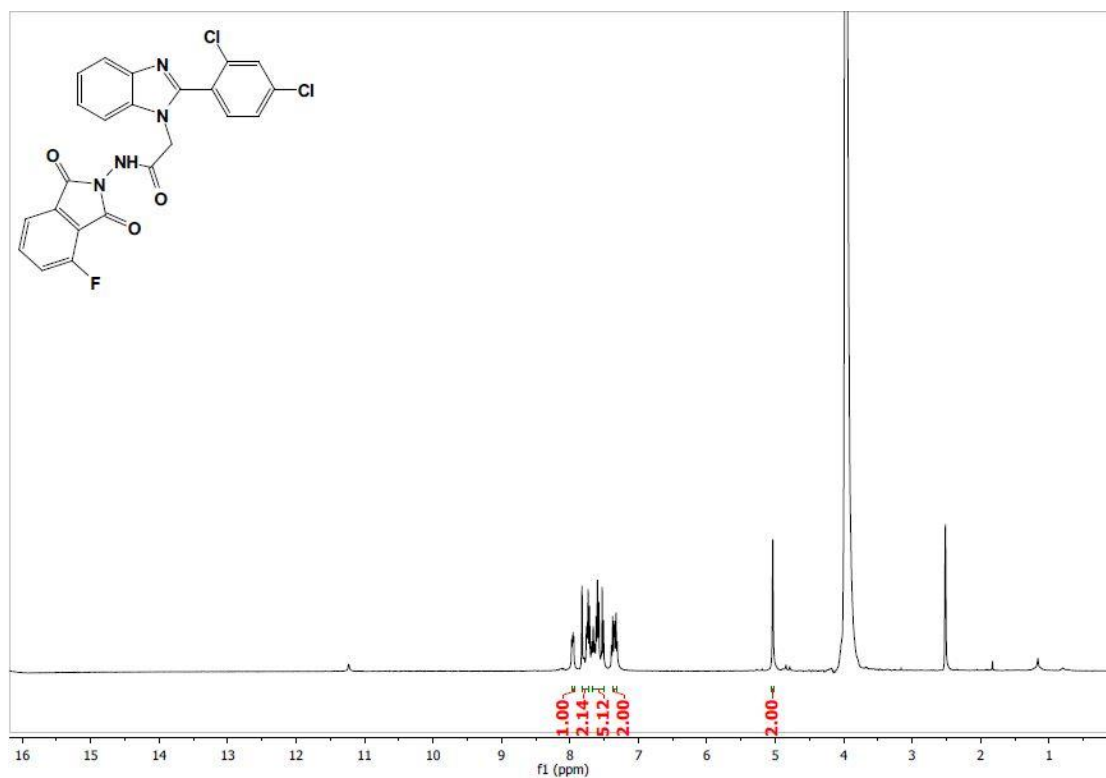


Figure S 47. ¹H-NMR D₂O Chart of Compound 9c

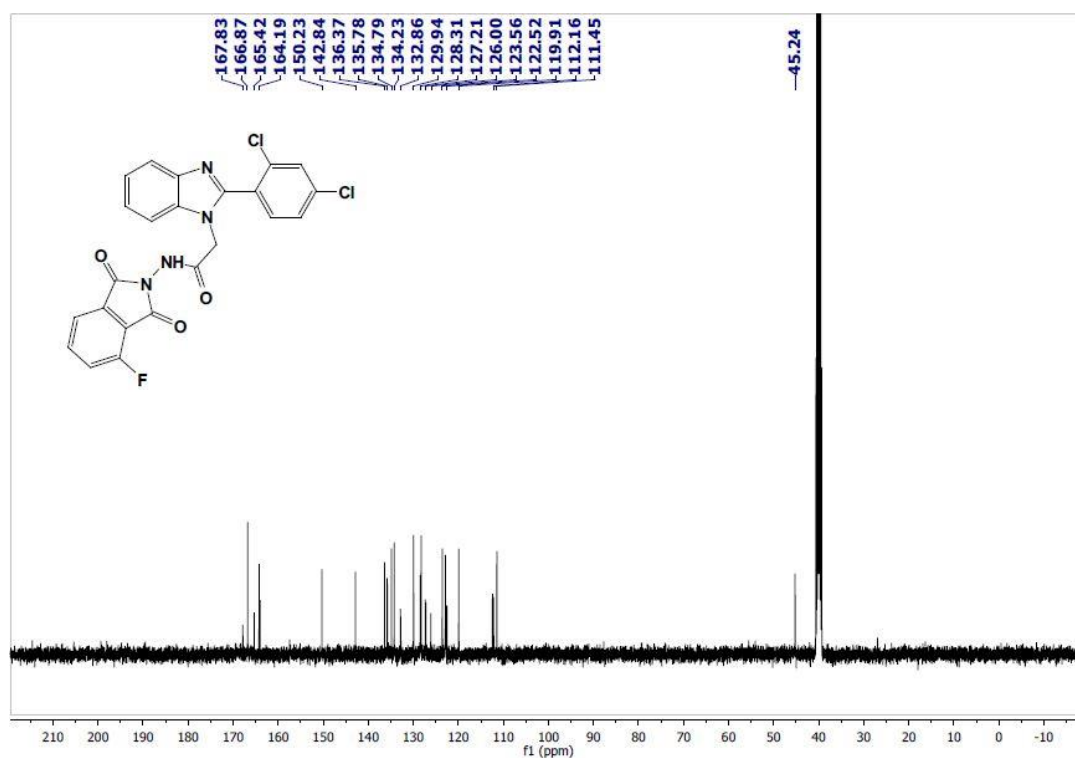


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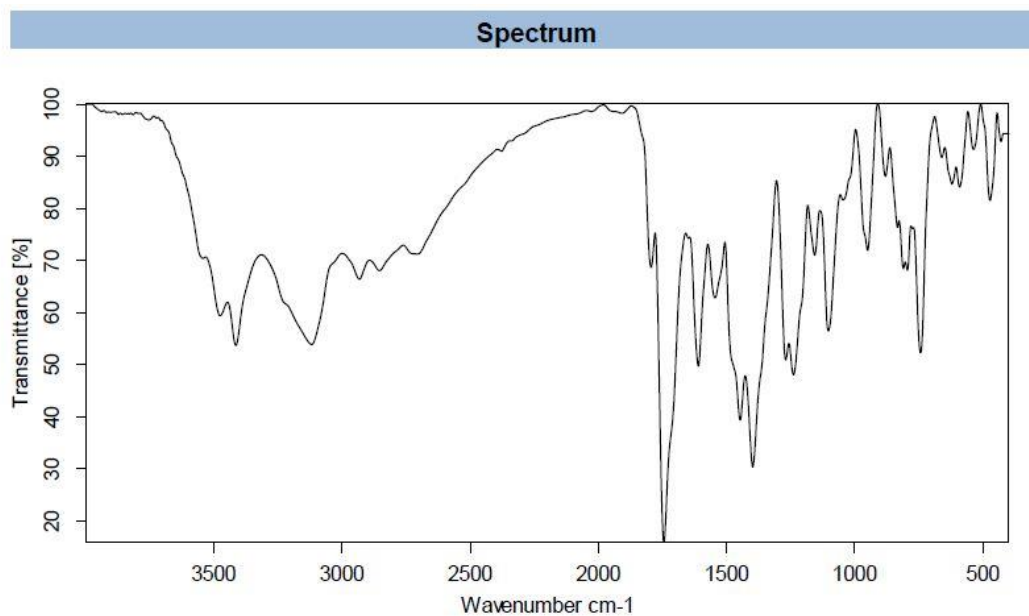


Figure S 49. IR Chart of Compound 9c

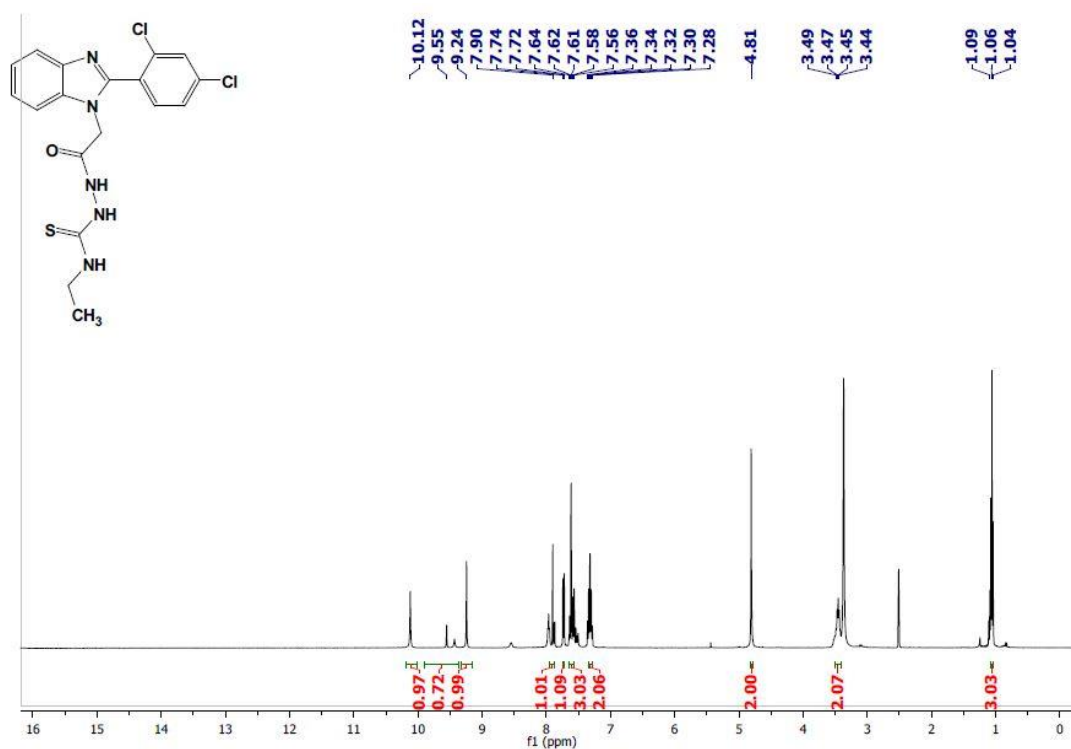


Figure S 50. ¹H-NMR Chart of Compound 10a

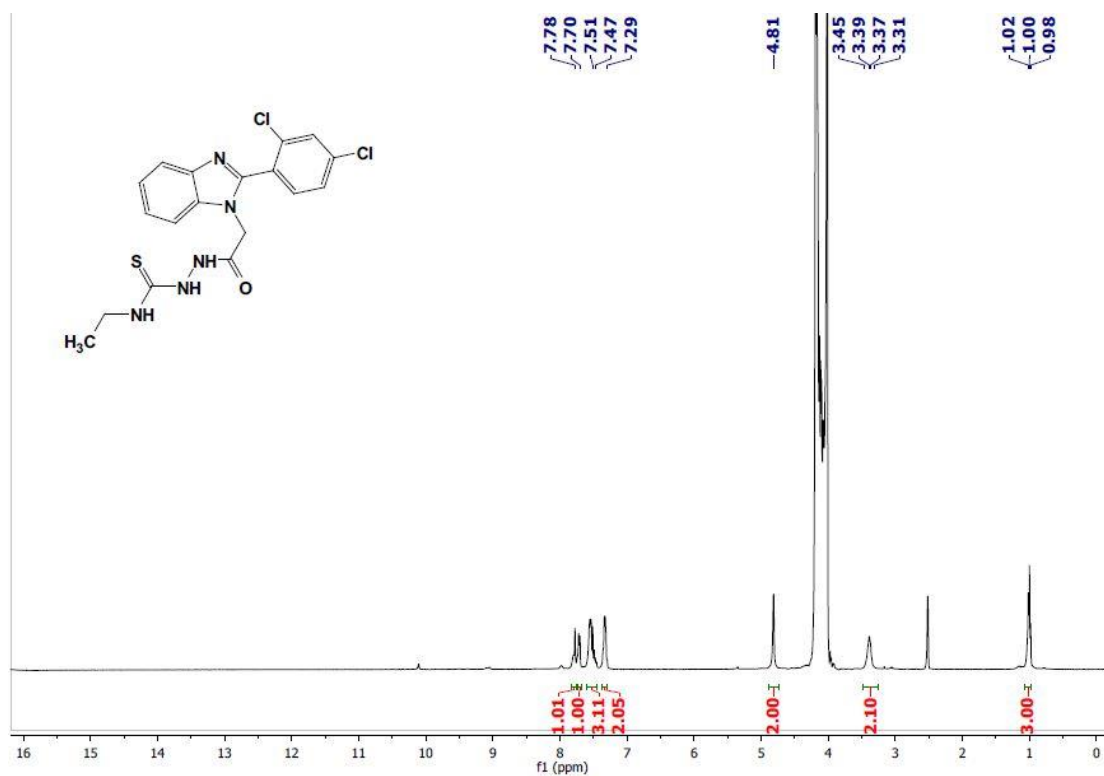


Figure S 51. ¹H-NMR D₂O Chart of Compound 10a

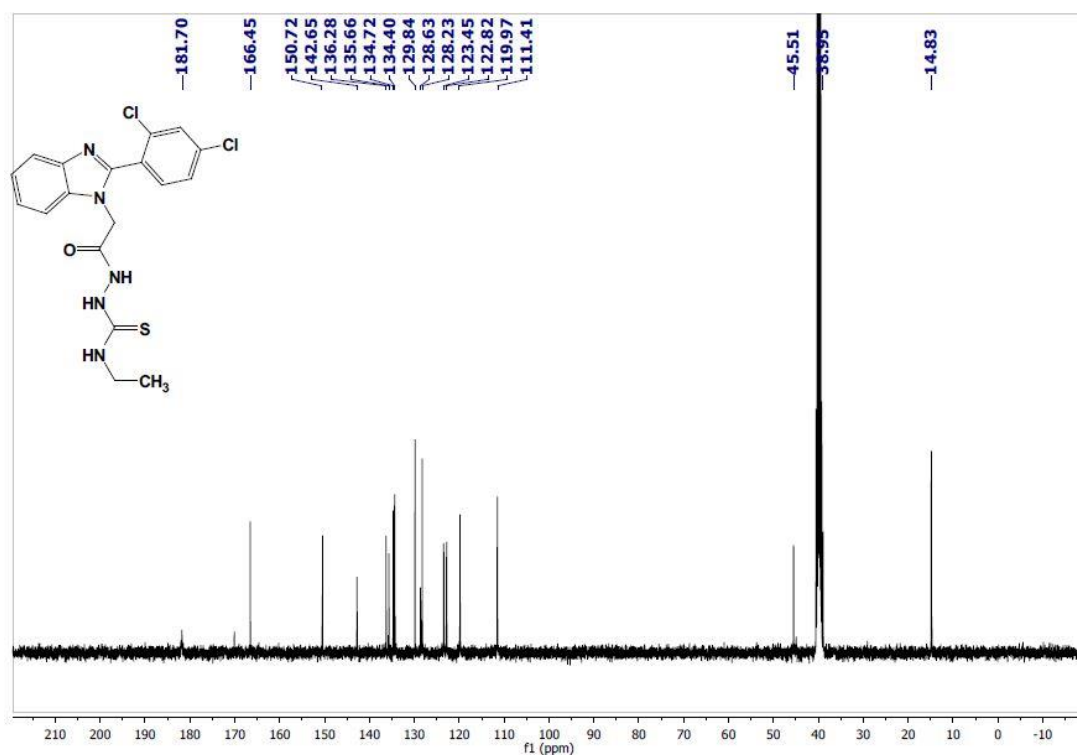


Figure S 52. ¹³C-NMR Chart of Compound 10a

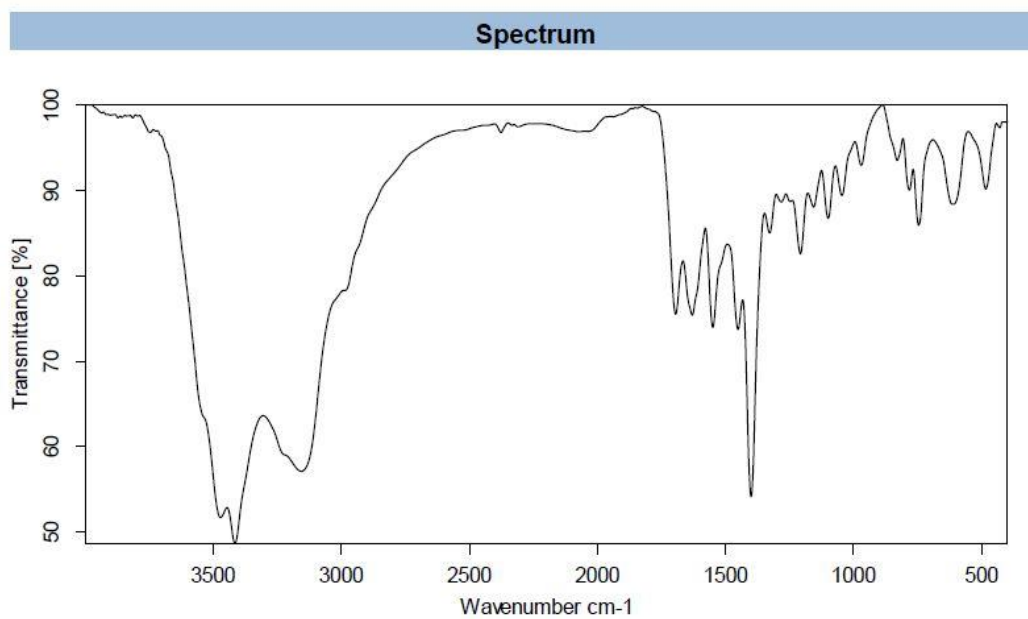


Figure S 53. IR Chart of Compound 10a

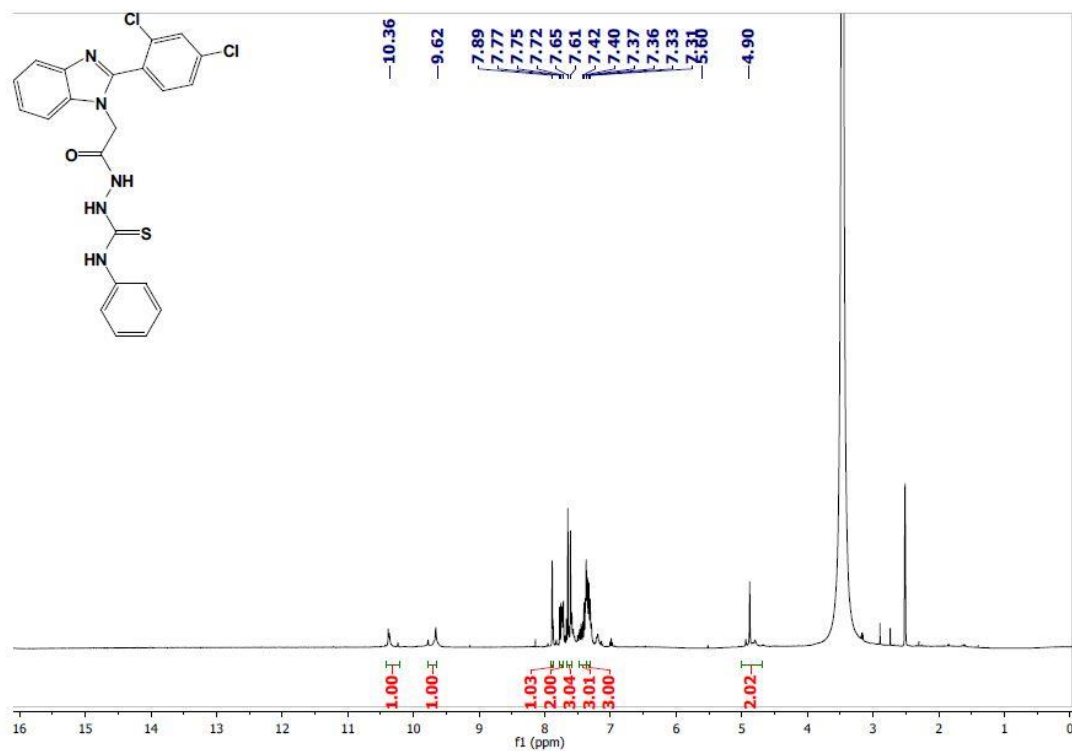


Figure S 54. ¹H-NMR Chart of Compound 10b

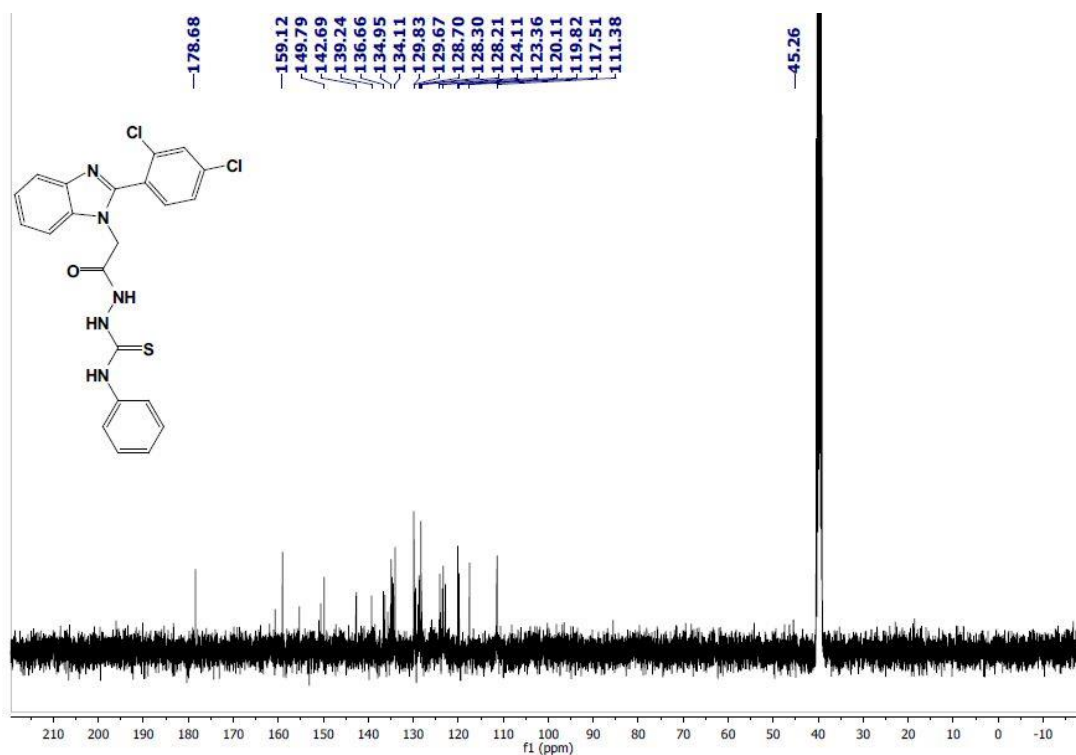


Figure S 55. ¹³C-NMR Chart of Compound 10b

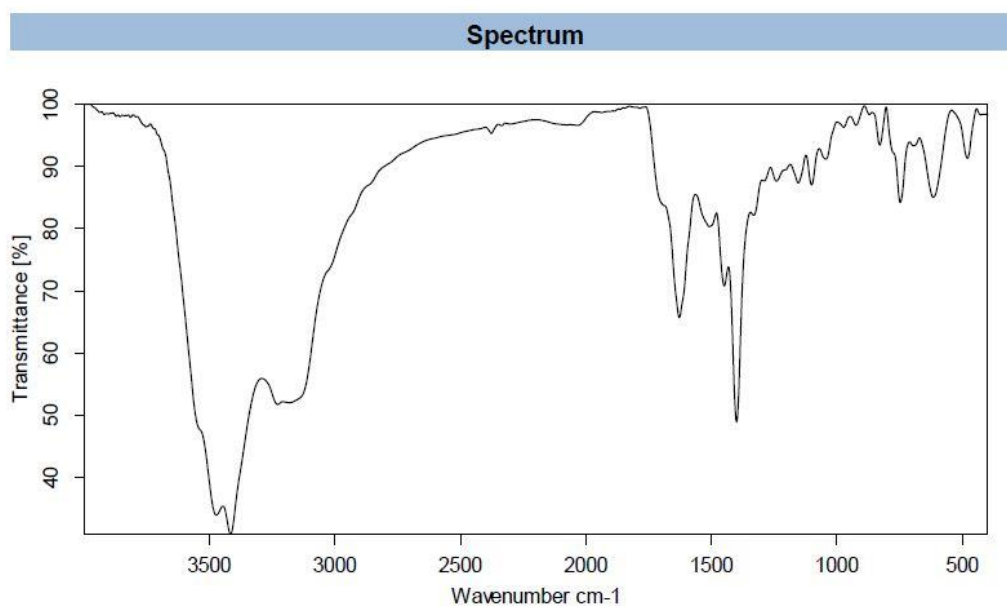


Figure S 56. IR Chart of Compound 10b

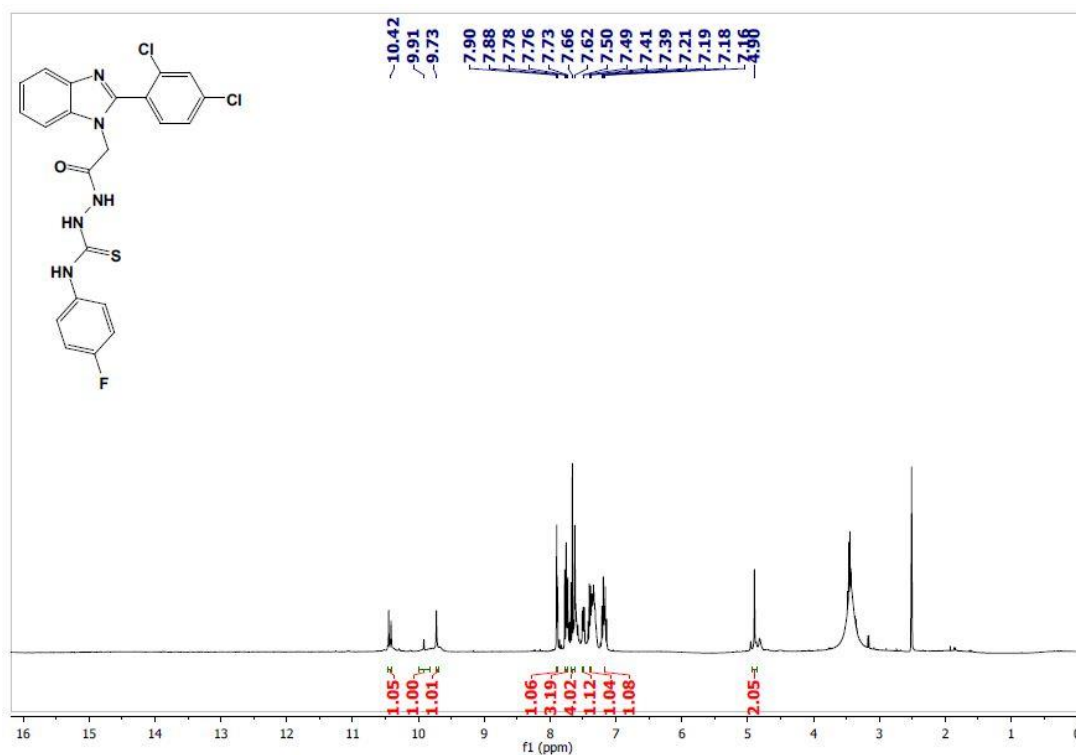


Figure S 57. ¹H-NMR Chart of Compound 10c

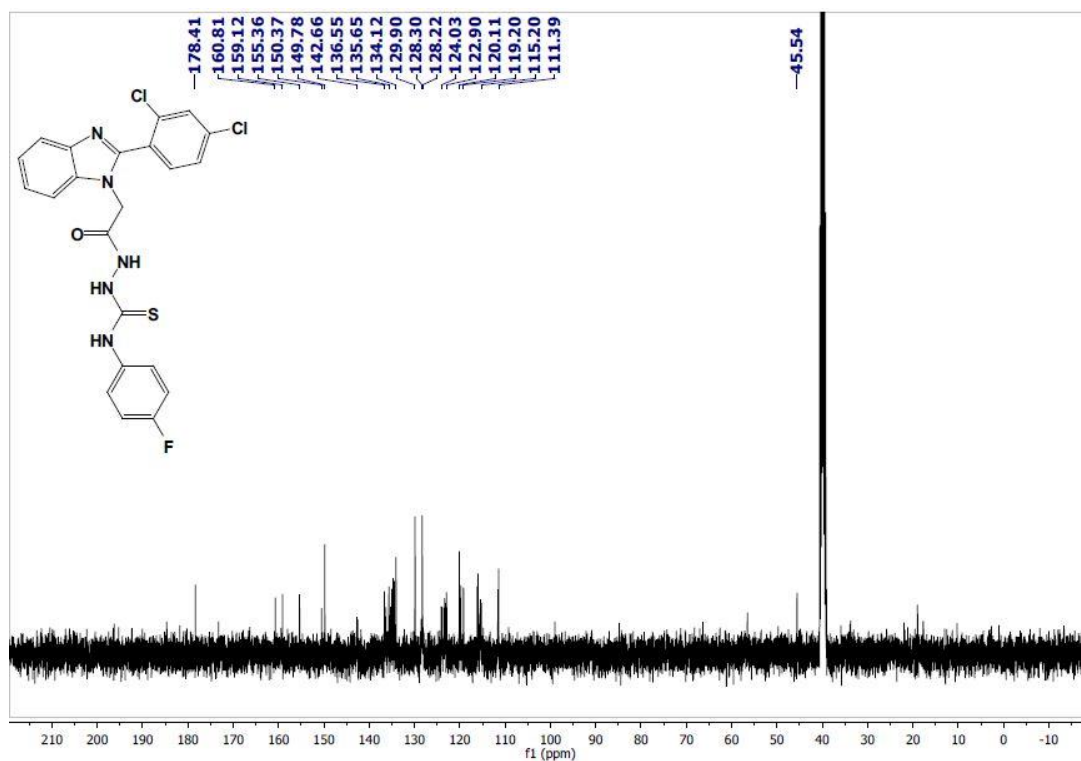


Figure S 58. ¹³C-NMR Chart of Compound 10c

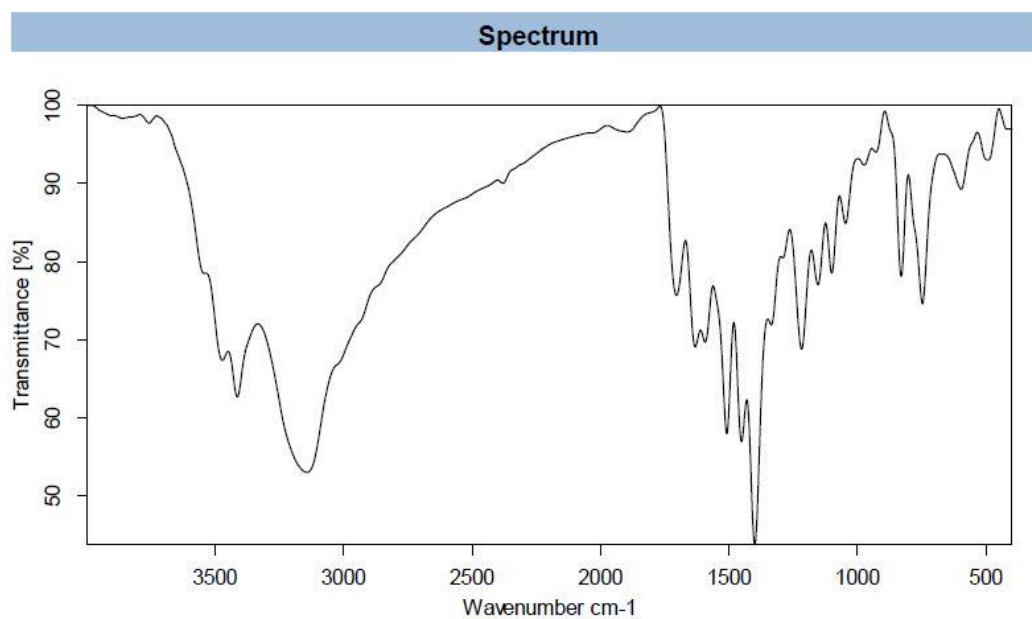


Figure S 59. IR Chart of Compound 10c

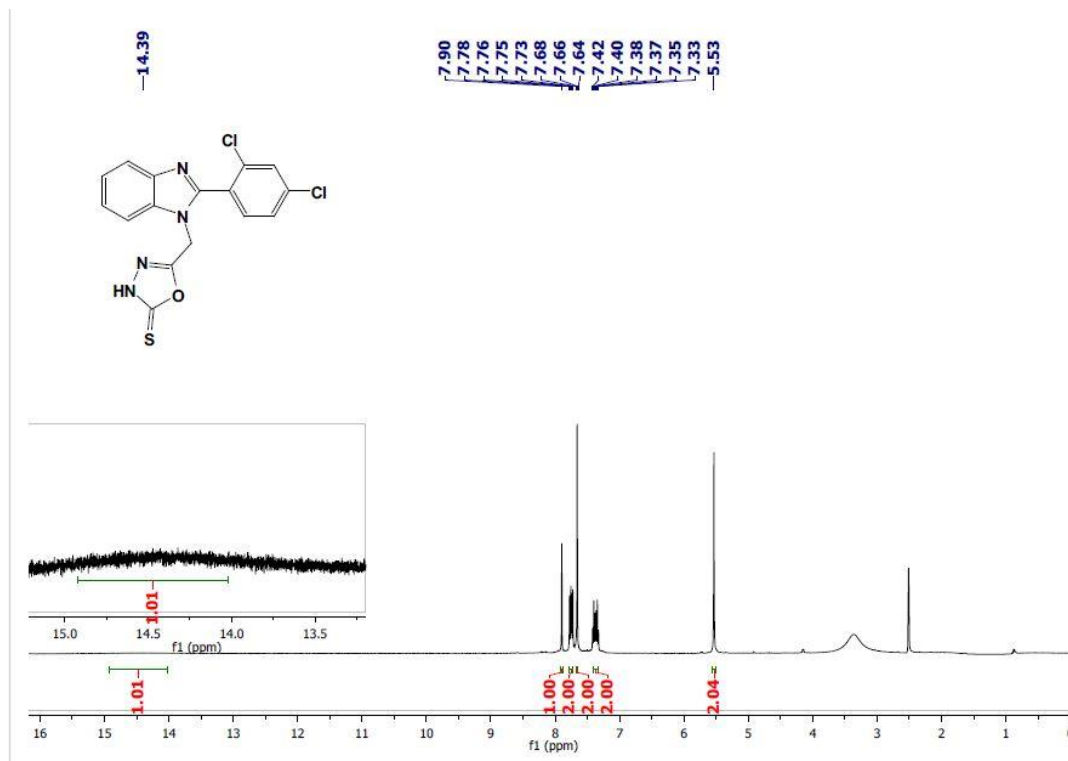


Figure S 60. ¹H-NMR Chart of Compound 11

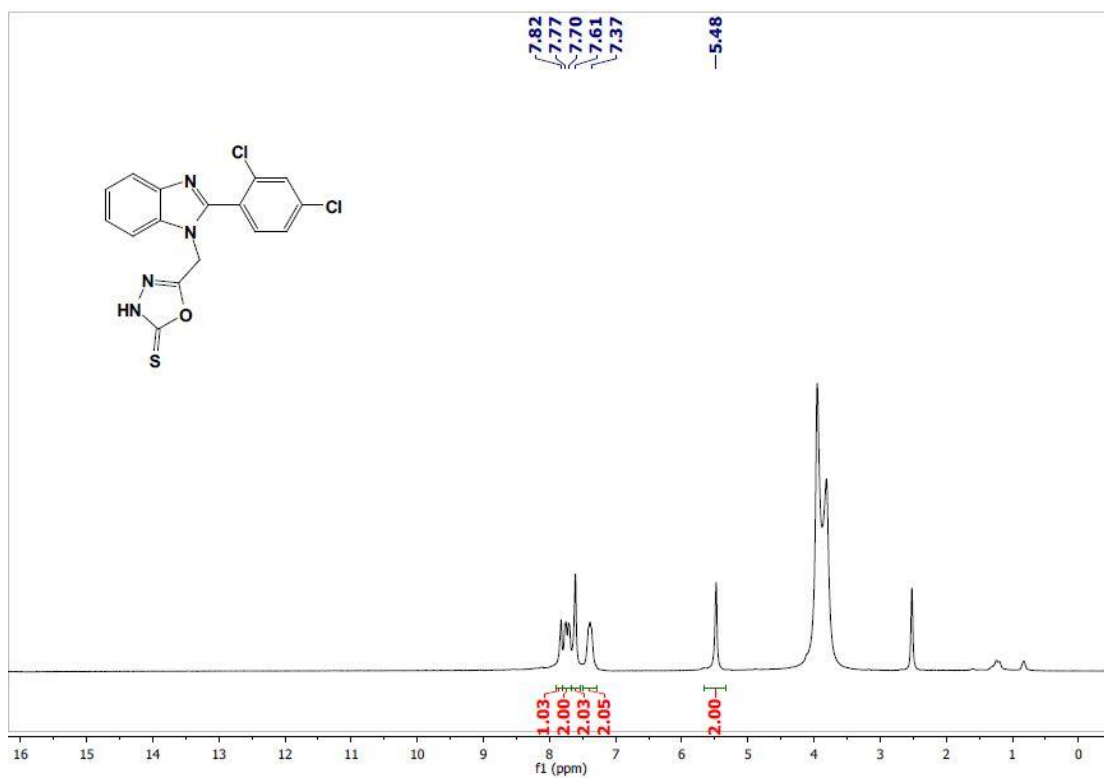


Figure S 61. ¹H-NMR D₂O Chart of Compound 11

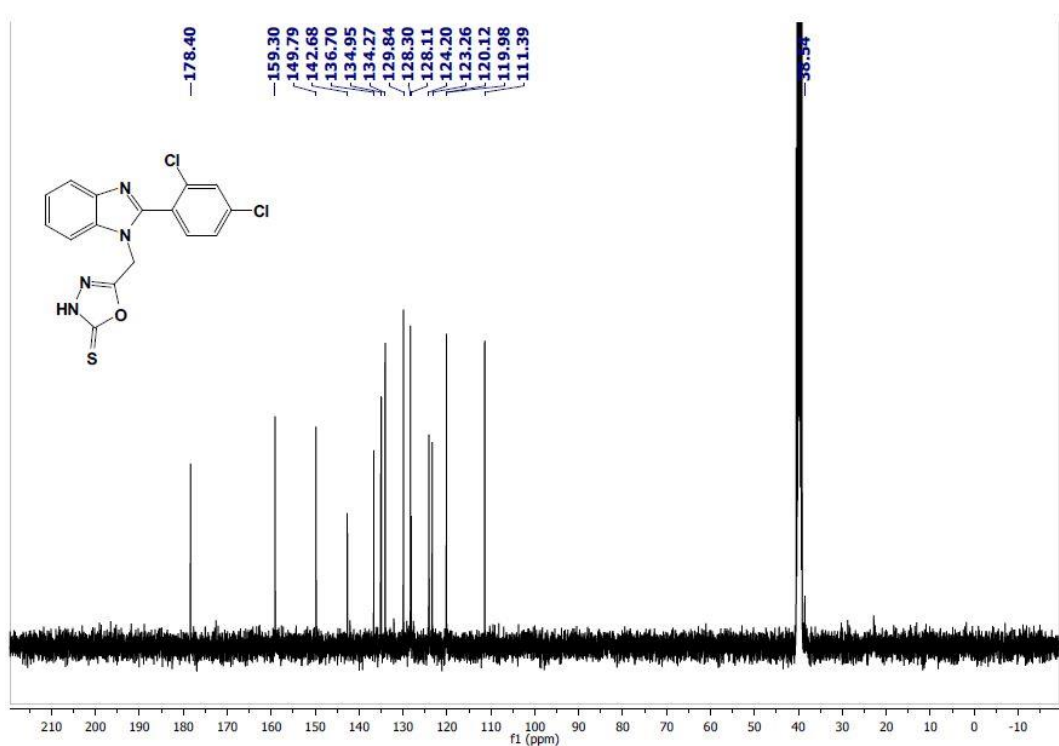


Figure S 62. ¹³C-NMR Chart of Compound 11

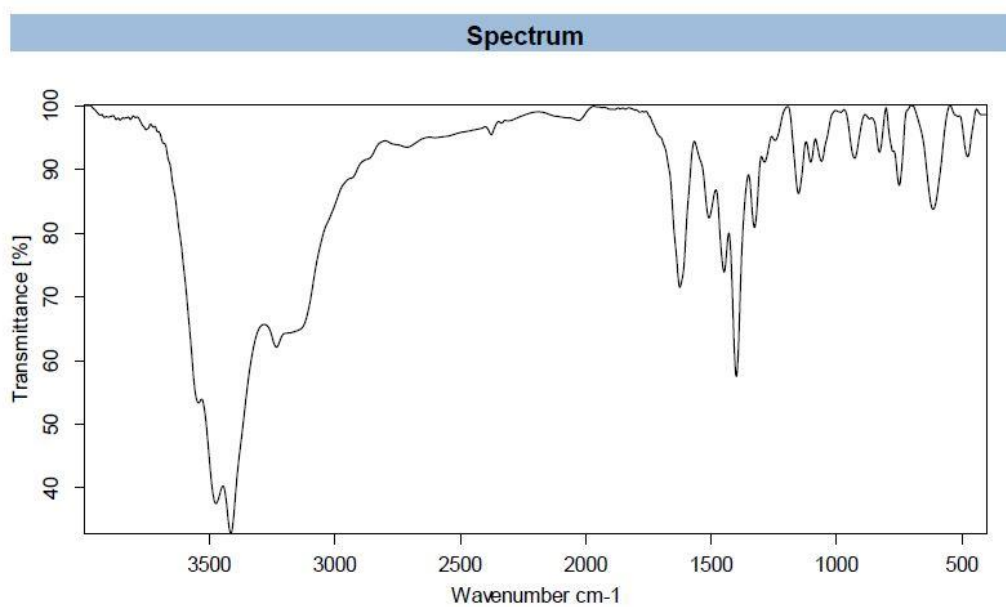


Figure S 63 . IR Chart of Compound 11

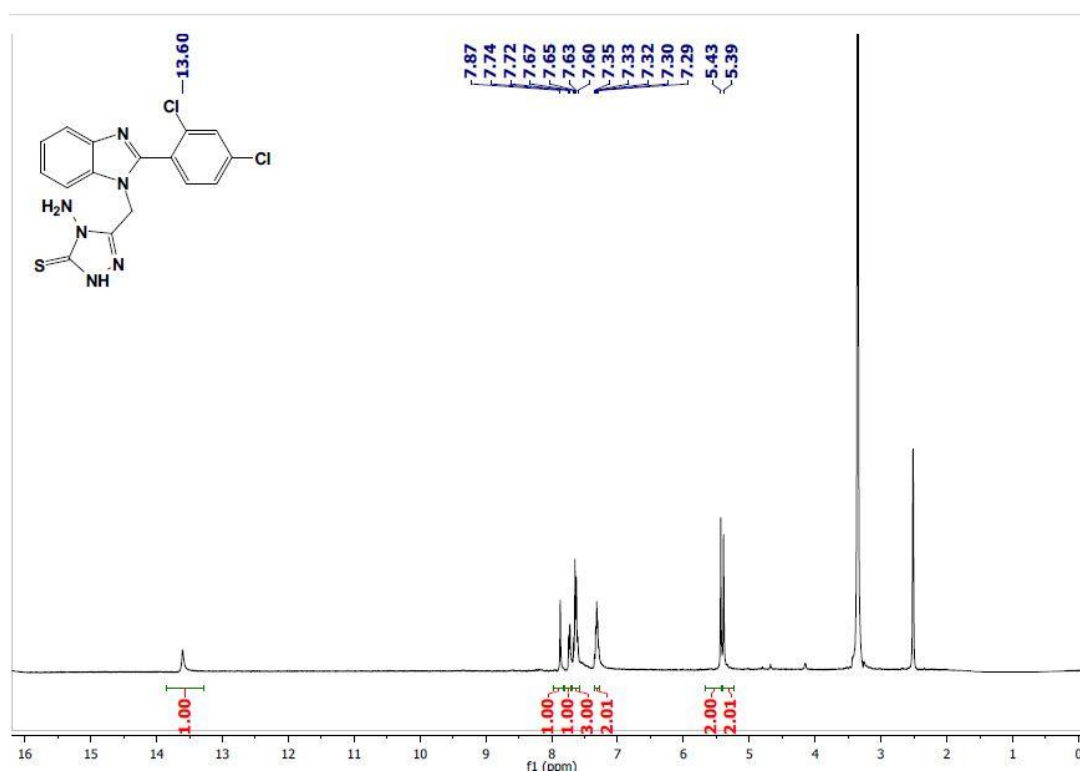


Figure S 64. ^1H -NMR Chart of Compound 12

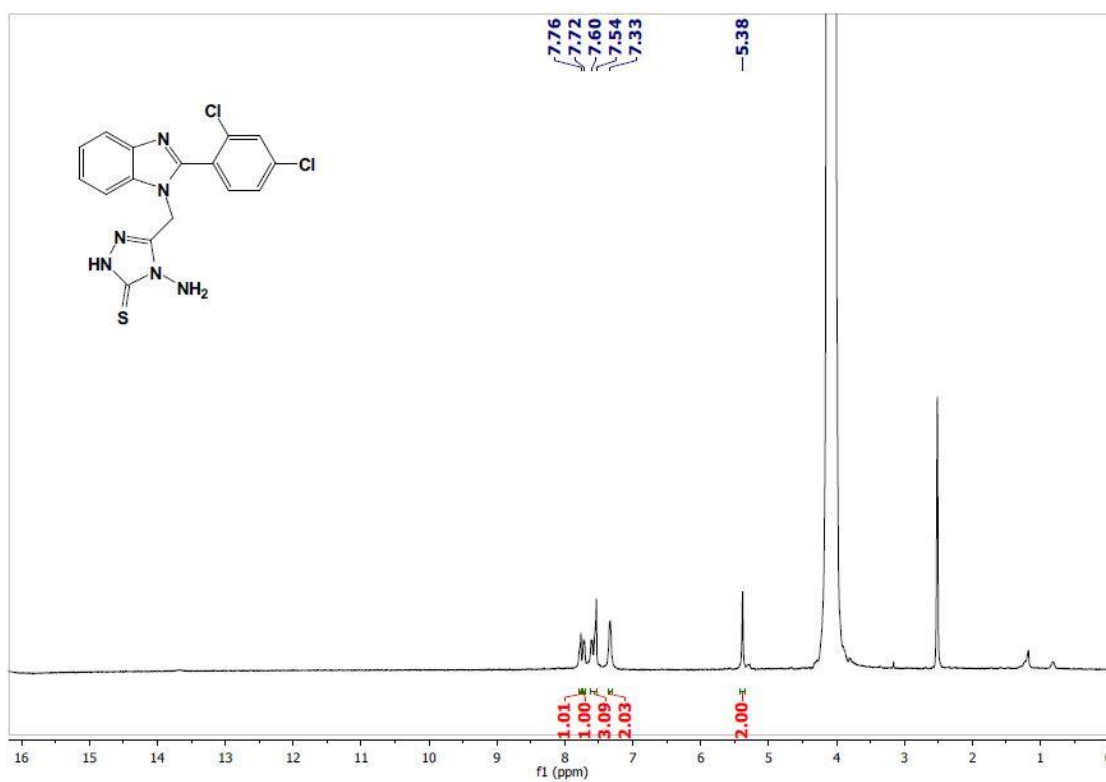


Figure S 65. ¹H-NMR D₂O Chart of Compound 12

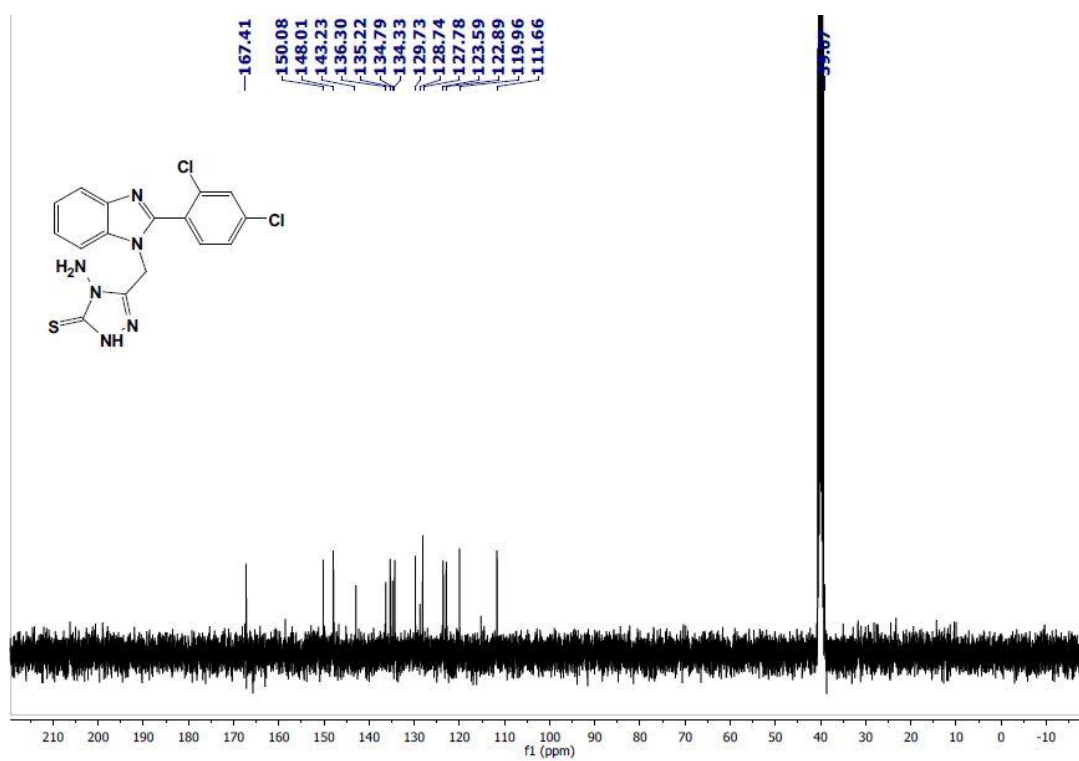


Figure S 66. ¹³C-NMR Chart of Compound 12

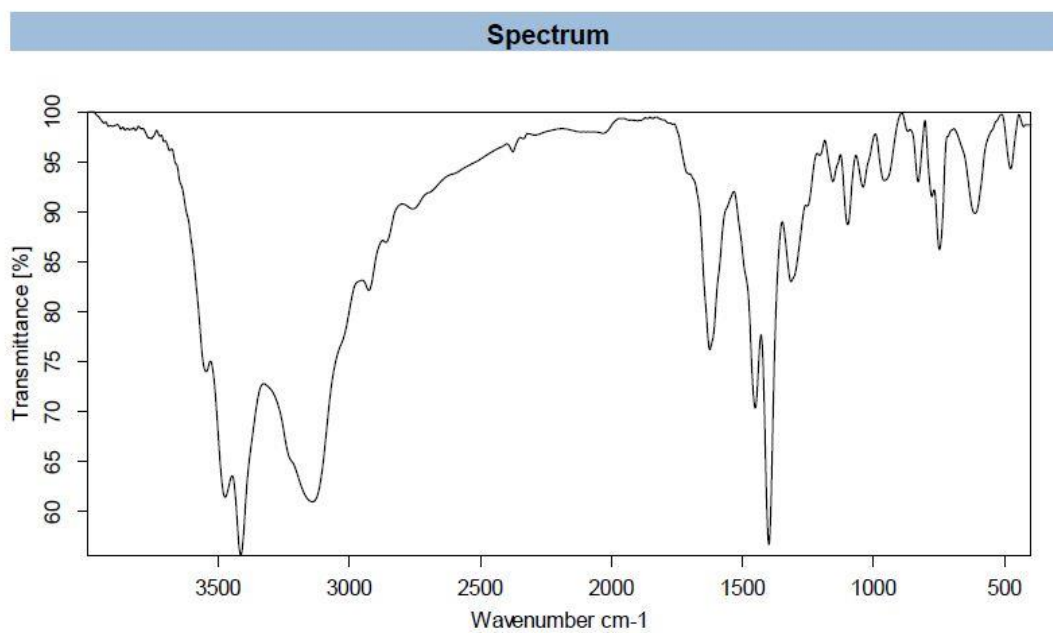


Figure S 67. IR Chart of Compound 12

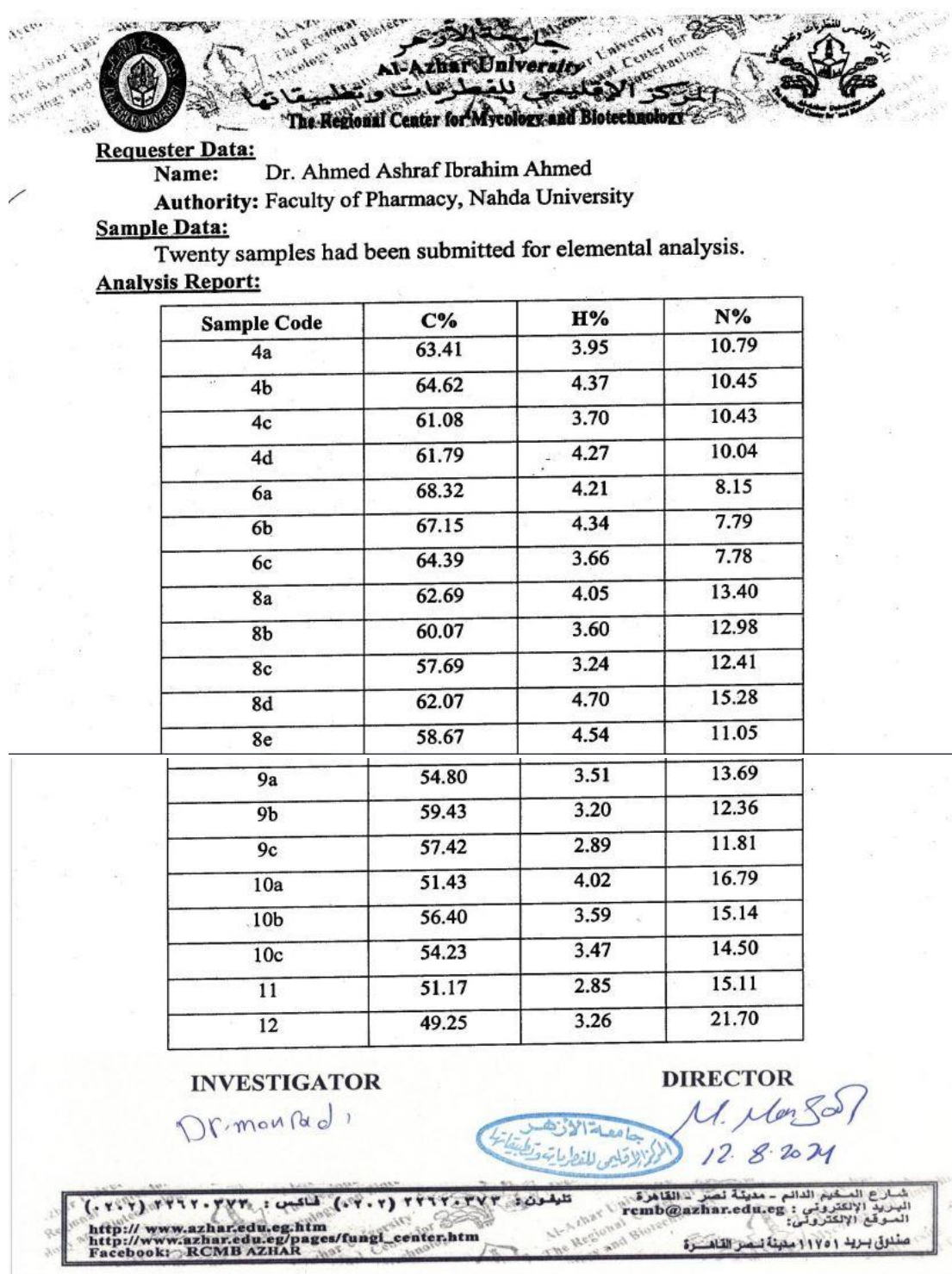


Figure S 68. elemental analysis of synthesized compounds

2- Biological evaluation data

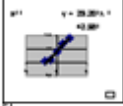
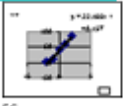
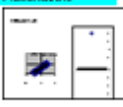
Lab report

ser	compound			Candida 14 α -demethylase CYP51	SD
	code	MW	conc	IC50 ug/ml	
1	s11	---	---	2.13 $\pm$ 0.09	
2	s12	---	---	2.781 $\pm$ 0.12	
3	Fluconazole	---	---	0.739 $\pm$ 0.03	

Figure S 69. Final results of IC50 of active compounds 11 and 12

Detailed Results:

CYP51a

code	IC50	conc. μ M	log conc	%inh	T2	T1	Δ T	RFU2	RFU1	Δ RFU	slope	K _d activity
s11		100	2	89.9	10	0	30	6185	0	6185	6105.7	4.051951
		10	1	68.8	10	0	30	19034	0	19034	6105.7	12.46966
		1	0	39.6	10	0	30	36886	0	36886	6105.7	24.16496
		0.1	-1	10.7	10	0	30	54531	0	54531	6105.7	35.72465
		0.01	-2	2.93	10	0	30	59267	0	59267	6105.7	38.82733
		EC		0	10	0	30	61057	0	61057	6105.7	40
s12		100	2	86.3	10	0	30	8337	0	8337	6105.7	5.461782
		10	1	61.5	10	0	30	23513	0	23513	6105.7	15.40397
		1	0	41.4	10	0	30	35752	0	35752	6105.7	23.42205
		0.1	-1	10.5	10	0	30	54663	0	54663	6105.7	35.81113
		0.01	-2	1.03	10	0	30	60427	0	60427	6105.7	39.58727
		EC		0	10	0	30	61057	0	61057	6105.7	40
Fluconazole		100	2	93.9	10	0	30	3755	0	3755	6105.7	2.459996
		10	1	78.1	10	0	30	13382	0	13382	6105.7	8.76689
		1	0	52.9	10	0	30	28775	0	28775	6105.7	18.85124
		0.1	-1	30.1	10	0	30	42687	0	42687	6105.7	27.96534
		0.01	-2	9.36	10	0	30	55342	0	55342	6105.7	36.25596
		EC		0	10	0	30	61057	0	61057	6105.7	40

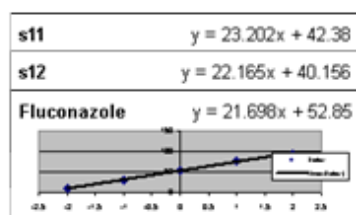


Figure S 70. raw data results of IC50 of active compounds 11and 12

Lab Report

s	compound		Antimicrobial		SD
			MIC ug/ml		
	code	mw	c.albicans	c.neoformans	
1	s11	---	3	1.5	
2	s12	---	12	6	
***	Fluconazole	---	0.75	3	

Figure S 71. MIC results of active compounds

Lab Report

ser	compound			Biofilm inhibition			
				Cryptococcus Neoformans			
	code	M.W g/mol	Treatment conc μ M	XCR	BSA	BMV	
1	s11	--	--	3.546	19.578	8.045	
2	Fluconazole	--	--	2.469	18.749	7.662	
3	Mix	--	--	1.206	1.498	0.597	

Figure S 72. MIC results of biofilm biomass for compound 11

		1	2	3	4	5	6	7	8	9	10
	conc ug/ml	0	1000	500	250	125	62.5	31.2	15.6	7.8	3.9
s11	A	C	s11	s11	s11	s11	s11	s11	s11	s11	s11
Flu	B	C	Flu	Flu	Flu	Flu	Flu	Flu	Flu	Flu	Flu
Mix	C	C	Mix	Mix	Mix	Mix	Mix	Mix	Mix	Mix	Mix

		XXT									
		0	1000	500	250	125	62.5	31.2	15.6	7.8	3.9
s11	A	0.025	0.043	0.067	1.311	1.386	1.555	1.584	1.661	1.739	1.765
Flu	B	0.019	0.055	0.046	0.048	0.049	1.287	1.431	1.543	1.696	1.811
Mix	C	0.022	0.038	0.051	0.042	0.037	1.303	1.539	1.584	1.765	1.802
	Δ										

		crystal violet CV									
		0	1000	500	250	125	62.5	31.2	15.6	7.8	3.9
s11	A	0.057	0.061	0.084	1.272	1.565	1.941	2.218	2.376	2.551	2.584
Flu	B	0.062	0.059	0.051	0.061	0.067	0.081	2.044	2.303	2.547	2.588
Mix	C	0.059	0.048	0.066	0.058	0.064	0.077	1.849	2.297	2.492	2.705
	Δ										

	s11	Flu	Mix
XTTN = (XTT - ContXTT)/ContXTT	1.68	1.578947368	0.6818182
CVN = (CV - ContCV)/ContCV	0.473684	0.080645161	0.0847458

XCR = XTTN/CVN

BSA = (XTTN/CVN) - [(CVN + XTTN)/2]

BMV = XTTN - CVN

	s11	Flu	Mix
XCR=	3.546667	19.57894737	8.0454545
BSA=	2.469825	18.7491511	7.6621726
BMV =	1.206316	1.498302207	0.5970724



Figure S 73. Raw data results of biofilm biomass for compound 11

Lab Report

Assay: Time dependant killing

Organism: Cryptococcus Neoformans

$CFU/ml = \frac{(No. of colonies * Total dilution factor)}{Vol. of culture plated in ml}$
 Start = 5600000 CFU/ml

	CFU/ml				
	1h	3h	6h	12h	24h
s11	6600000	17000000	21000000	2400000	0
Fluconazole	6200000	11000000	800000	0	0
cont	8100000	46000000	171000000	225000000	338000000

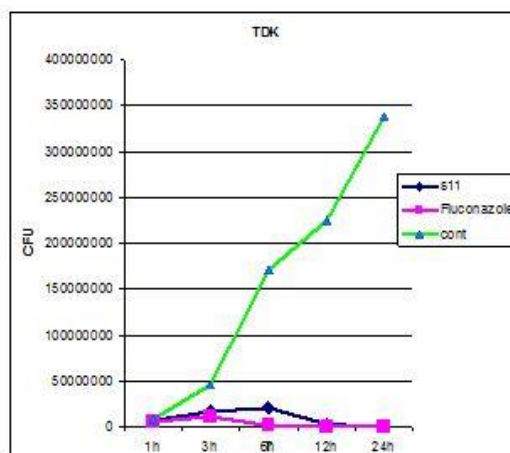


Figure S 74. Time dependent kill assay for compound 11

UPLC results of the synthesized compounds

Compound **3a** purity (99.7%)

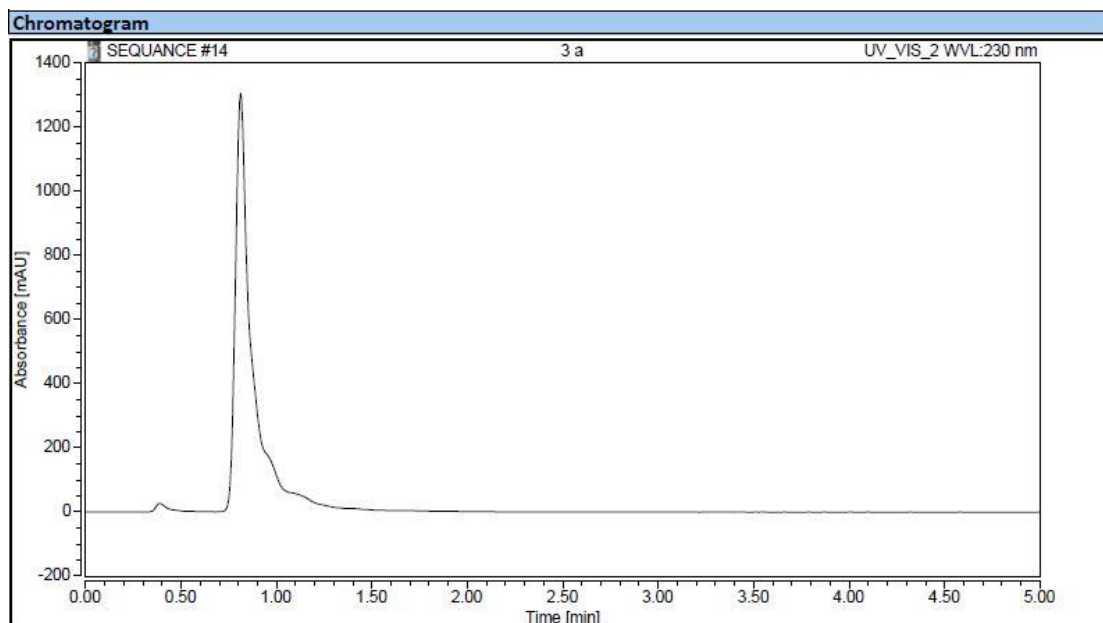


Figure S 75. Compound 3a purity

Compound **3b** purity (98.4%)

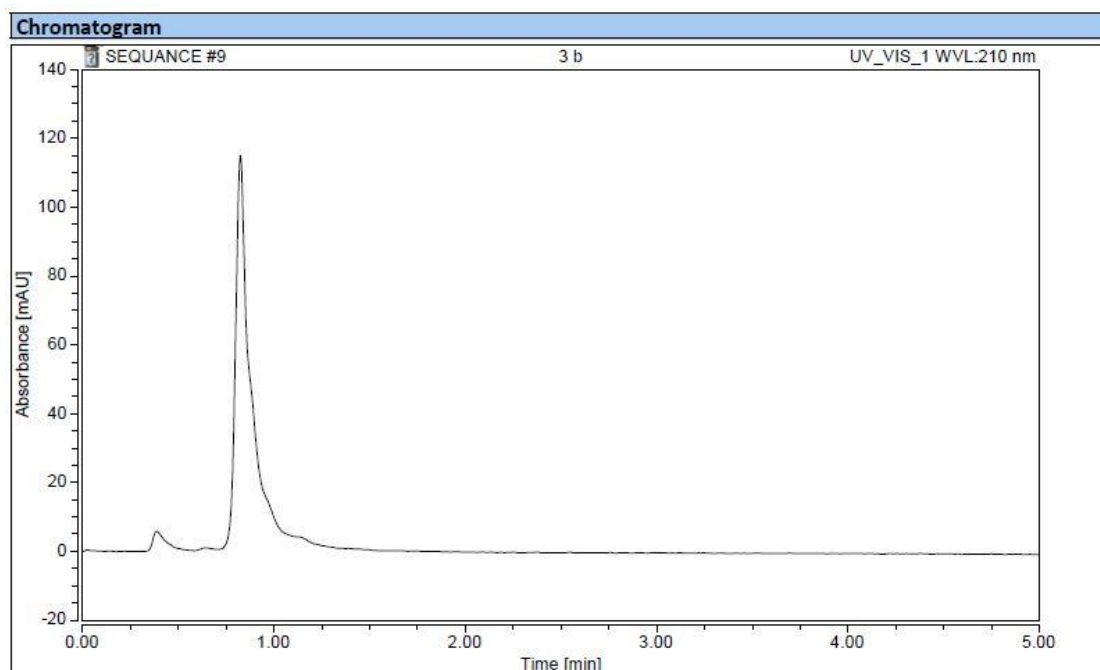


Figure S 76. Compound 3b purity

Compound **3c** purity (99.2%)

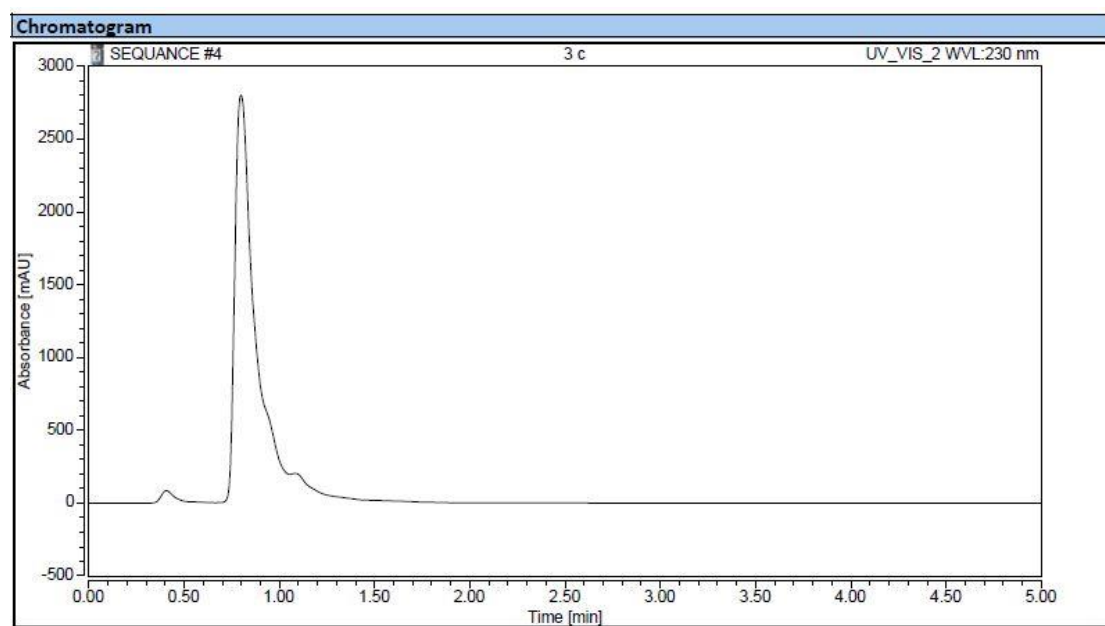


Figure S 77. Compound 3c purity

Compound **3d** purity (99.3%)

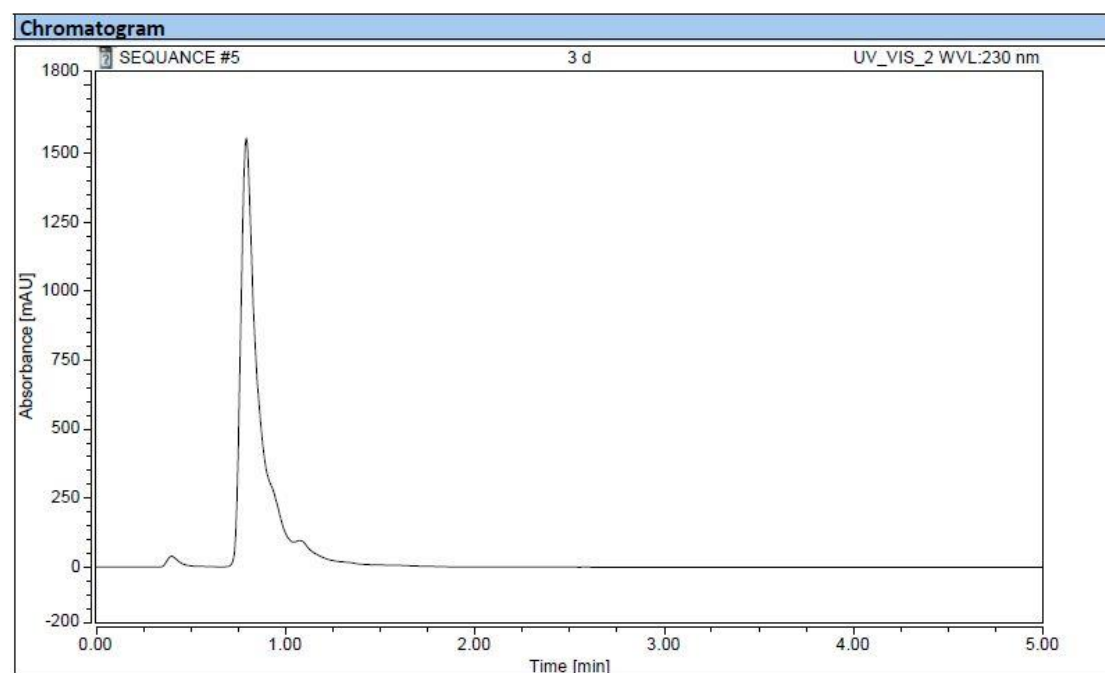


Figure S 78. Compound 3d purity

Compound **5a** purity (97.1%)

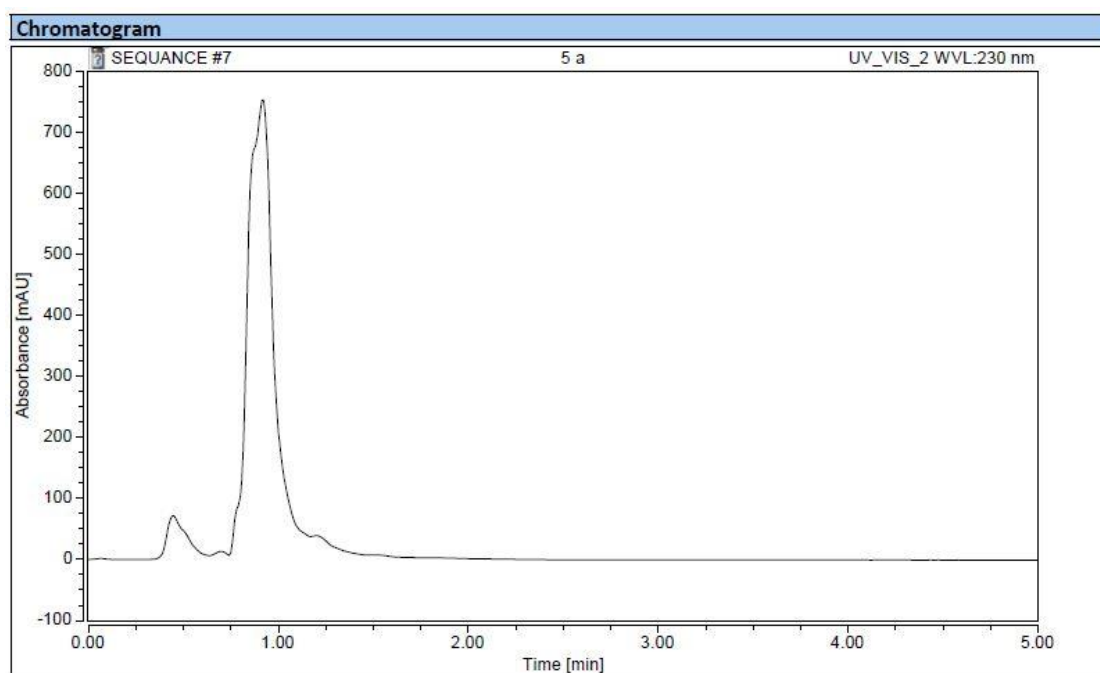


Figure S 79. Compound 5a purity

Compound **5b** purity (97.5%)

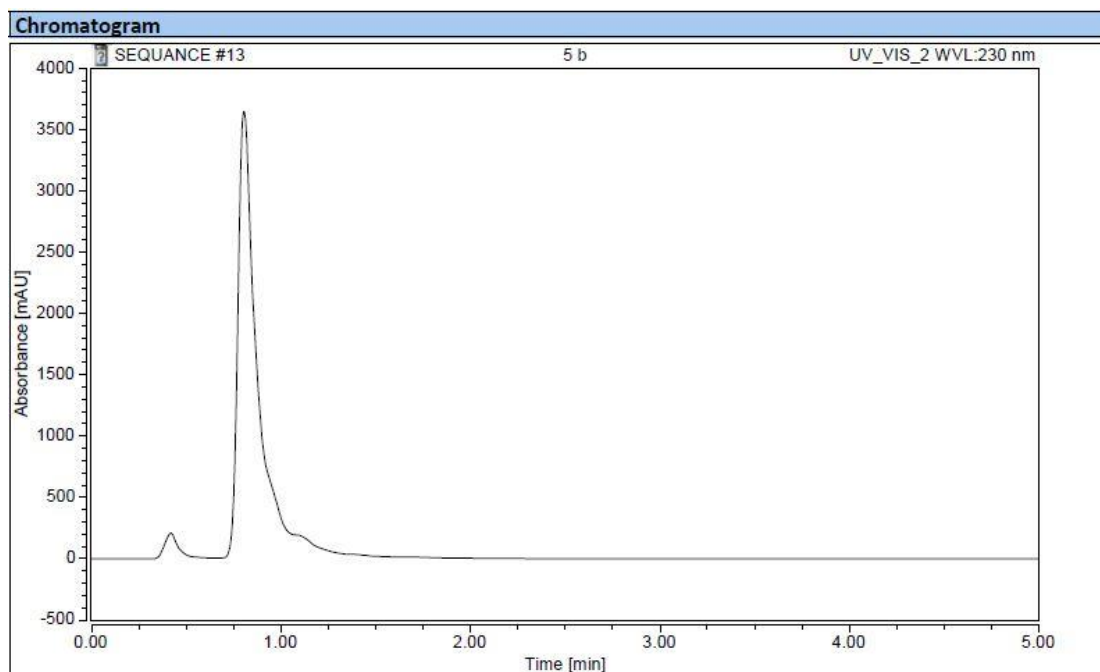


Figure S 80. Compound 5b purity

Compound **5c** purity (97.8%)

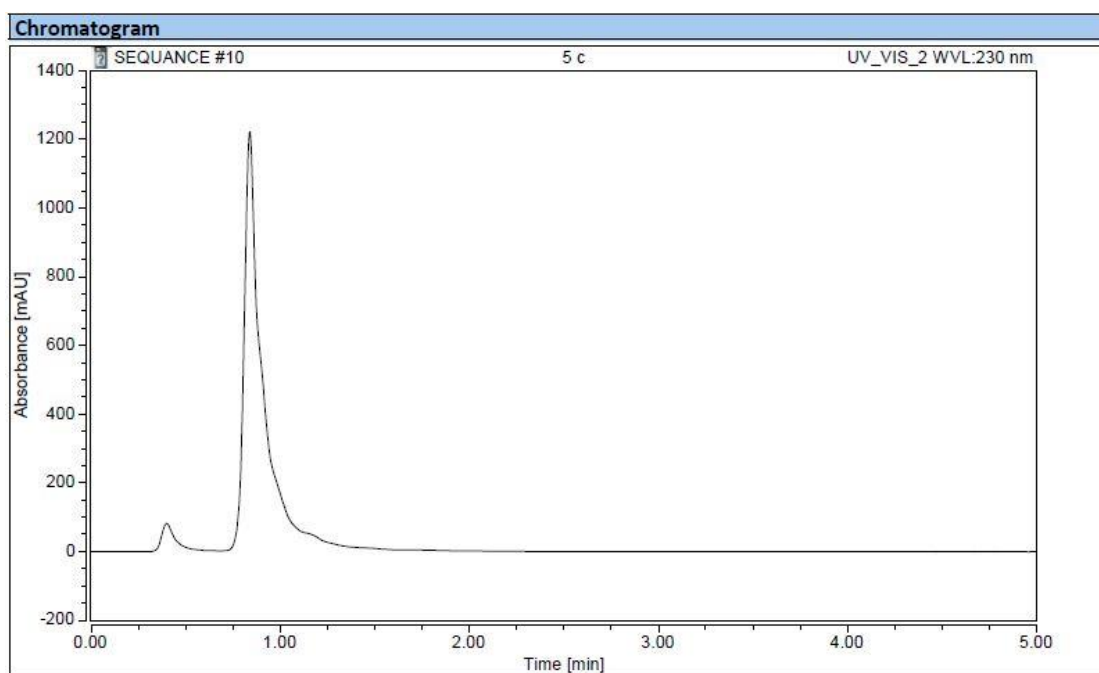


Figure S 81. Compound 5c purity

Compound **8a** purity (99.1%)

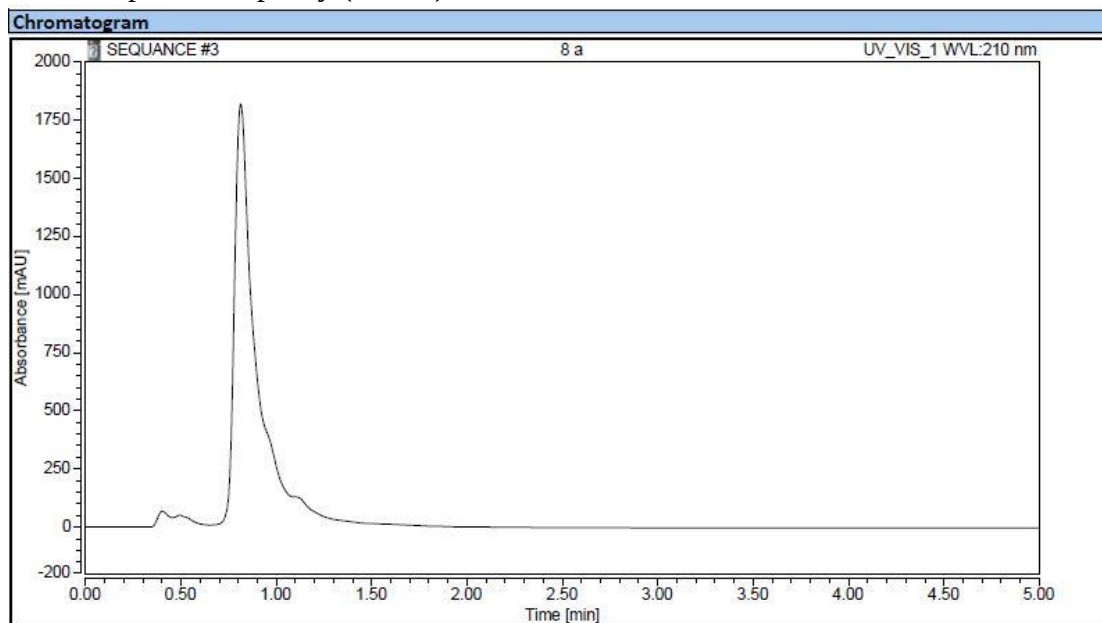


Figure S 82. Compound 8a purity

Compound **8b** purity (99.6%)

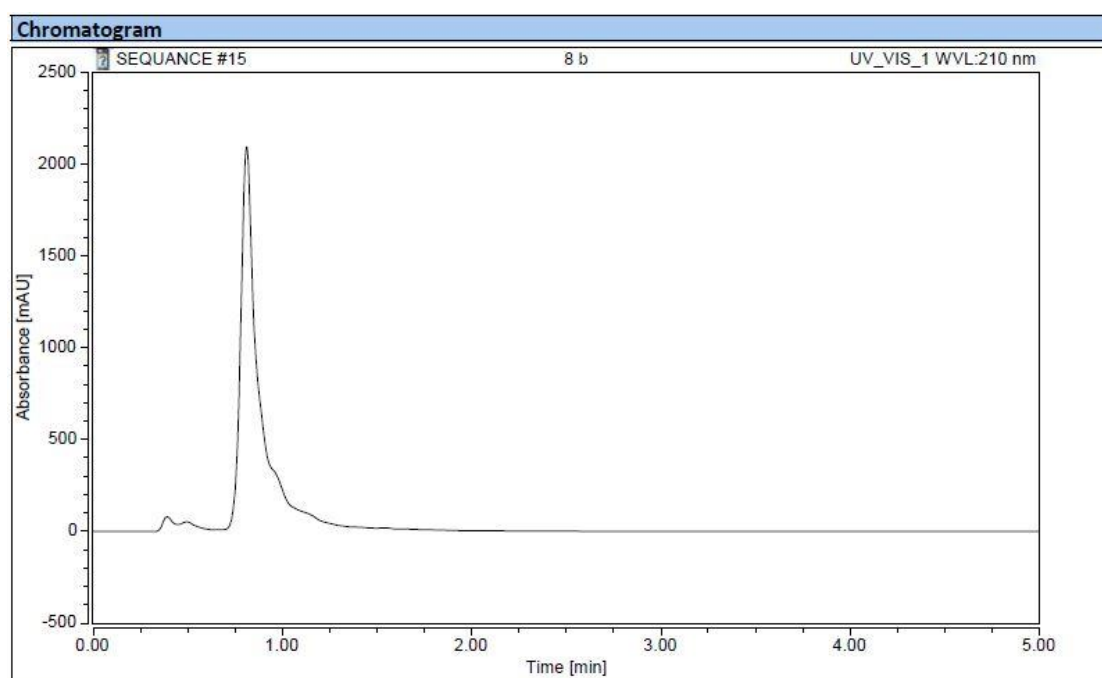


Figure S 83. Compound 8b purity

Compound **8c** purity (99.8%)

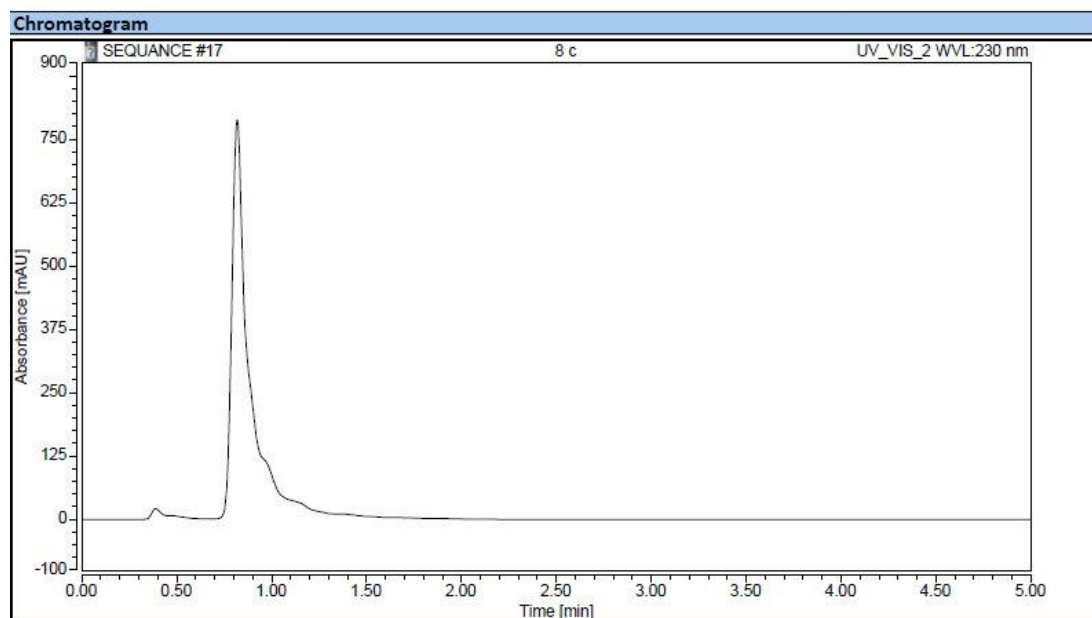


Figure S 84. Compound 8c purity

Compound **8d** purity (99.7%)

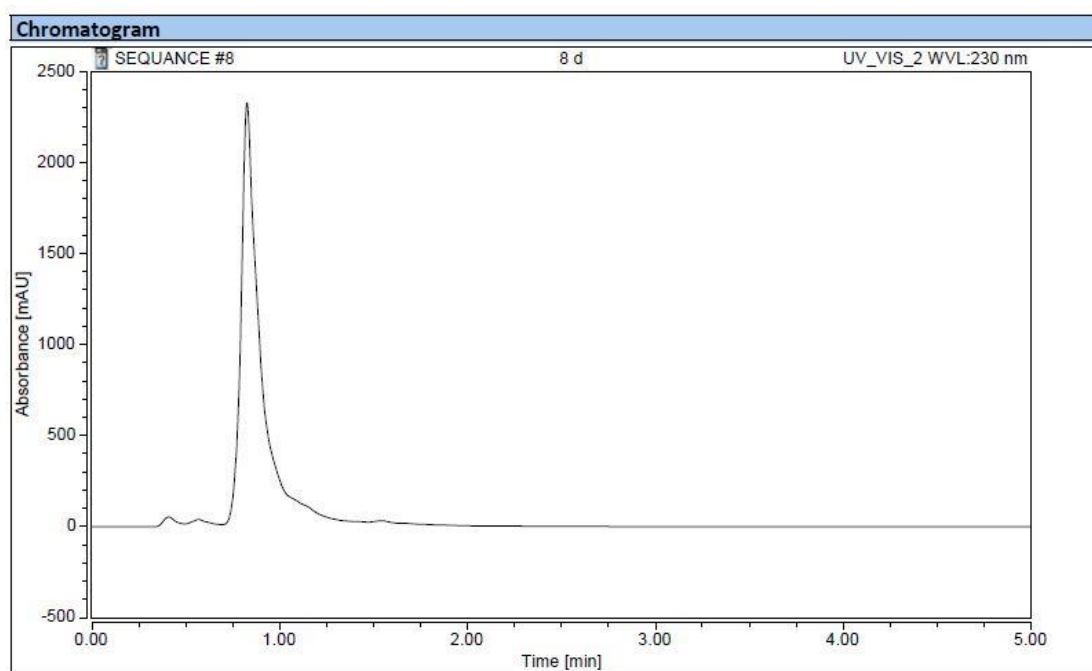


Figure S 85. Compound 8d purity

Compound **8e** purity (99.5%)

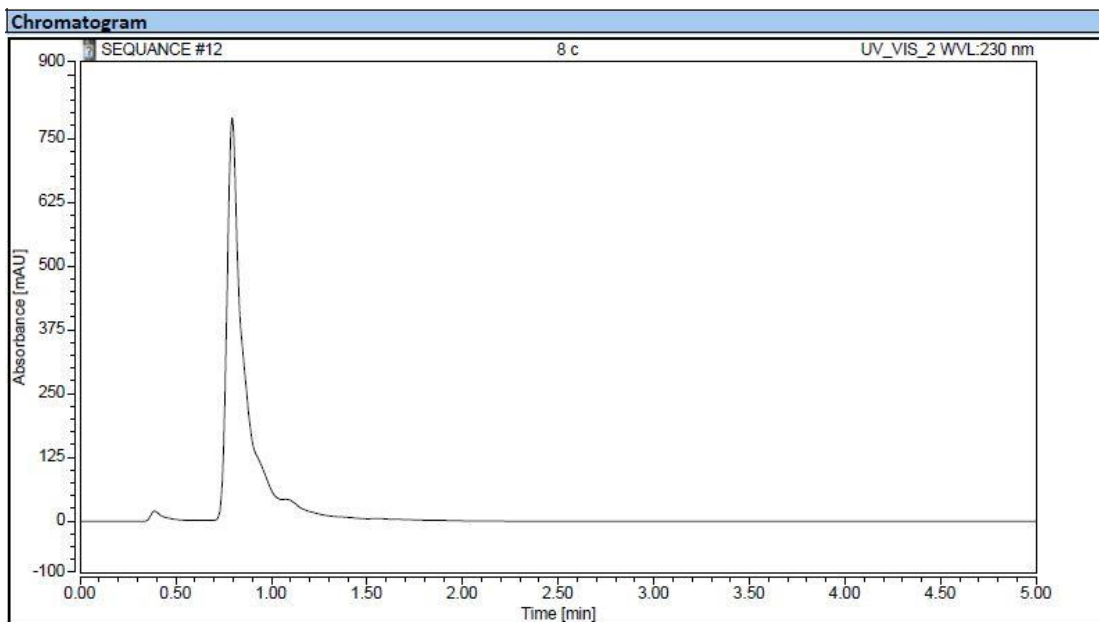


Figure S 86. Compound 8e purity

Compound **9a** purity (99.7%)

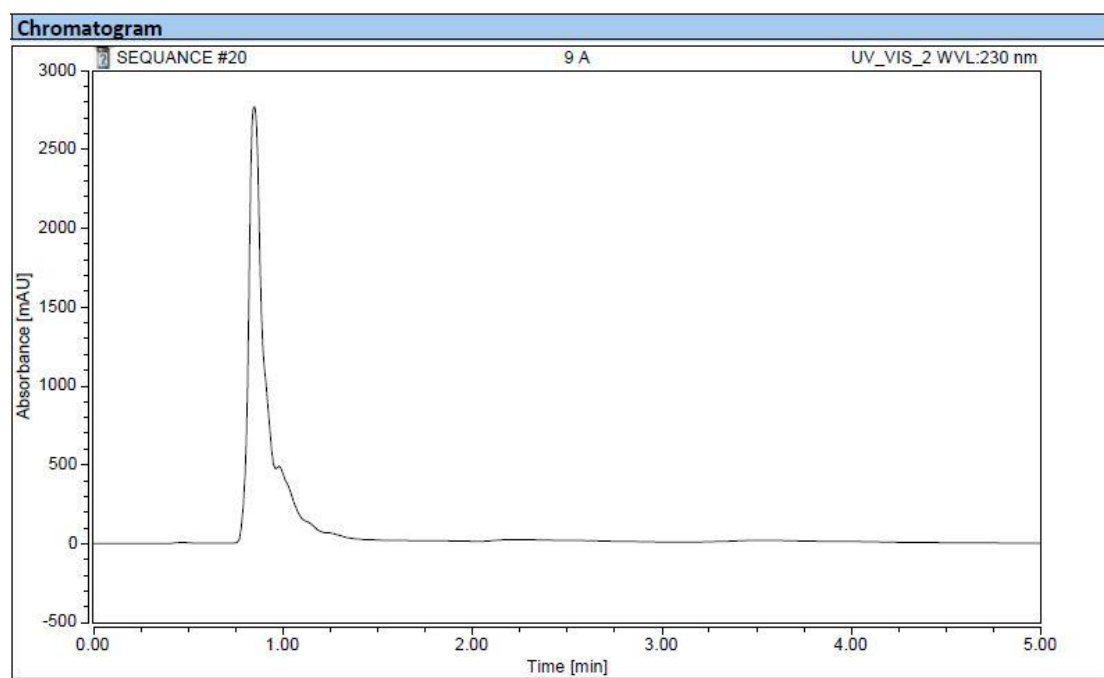


Figure S 87. Compound 9a purity

Compound **9b** purity (98.8%)

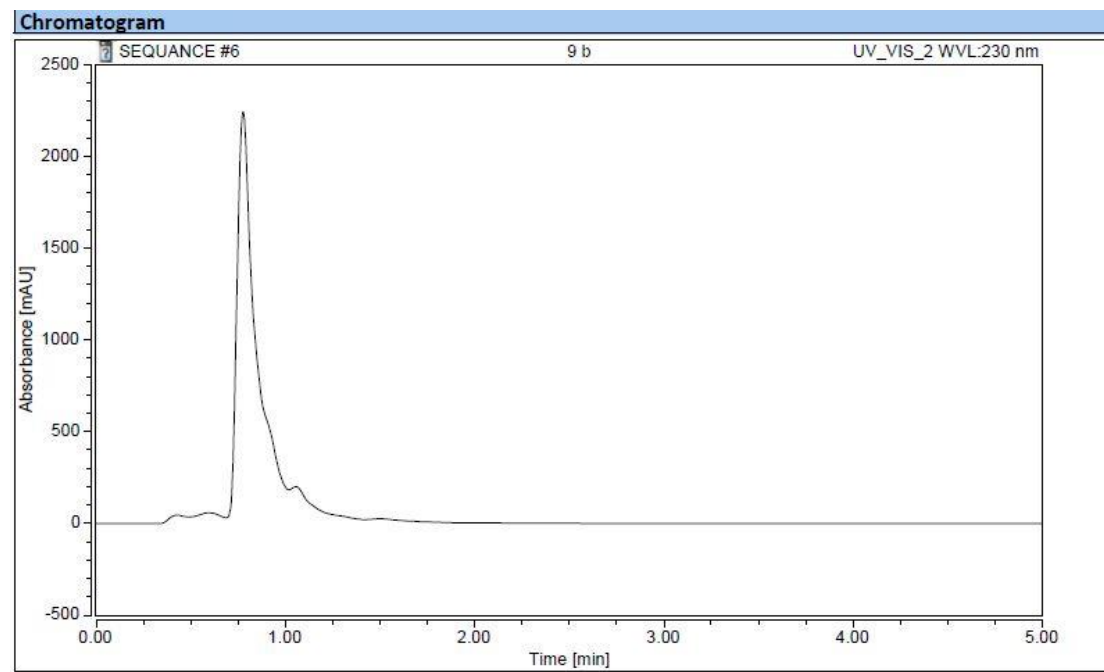


Figure S 88. Compound 9b purity

Compound **9c** purity (98.9%)

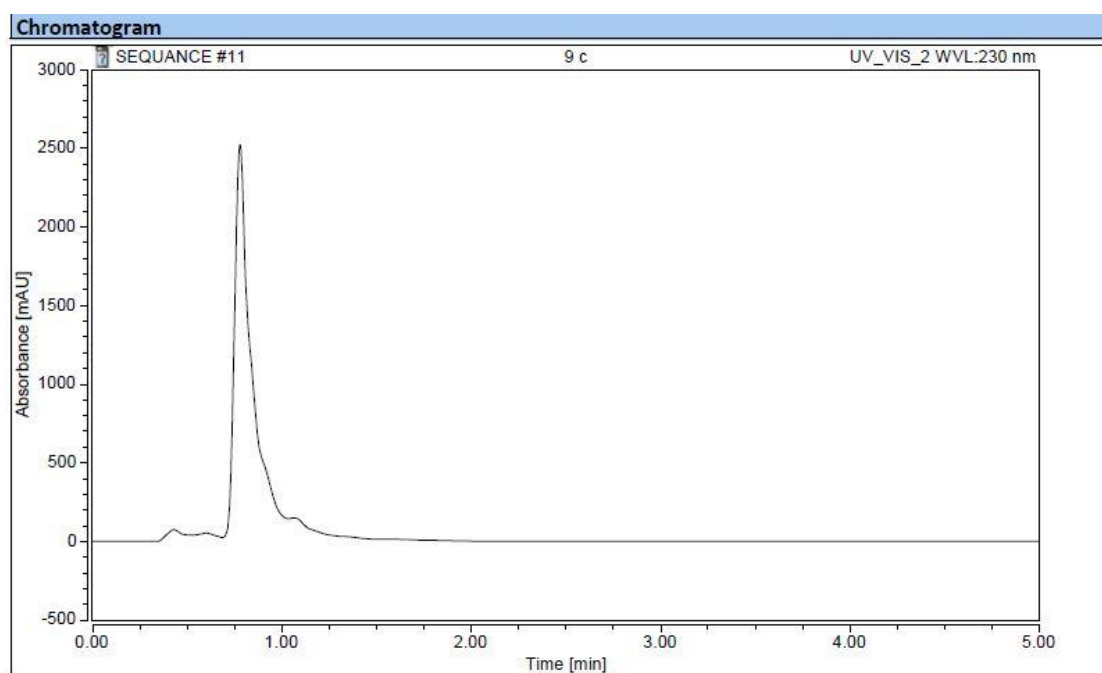


Figure S 89. Compound 9c purity

Compound **10a** purity (99.6%)

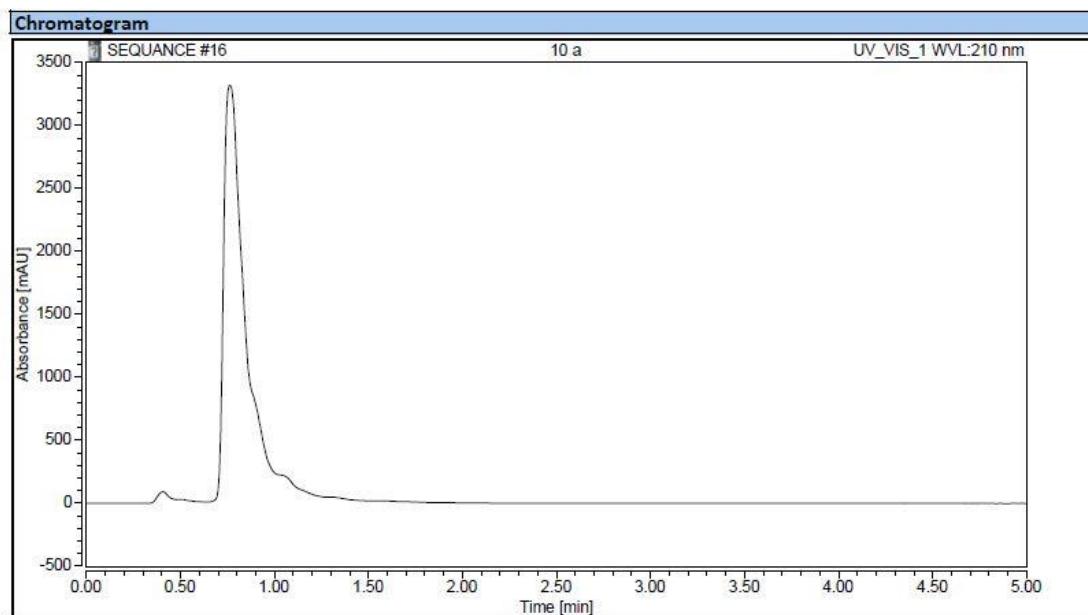


Figure S 90. Compound 10a purity

Compound **10b** purity (99.9%)

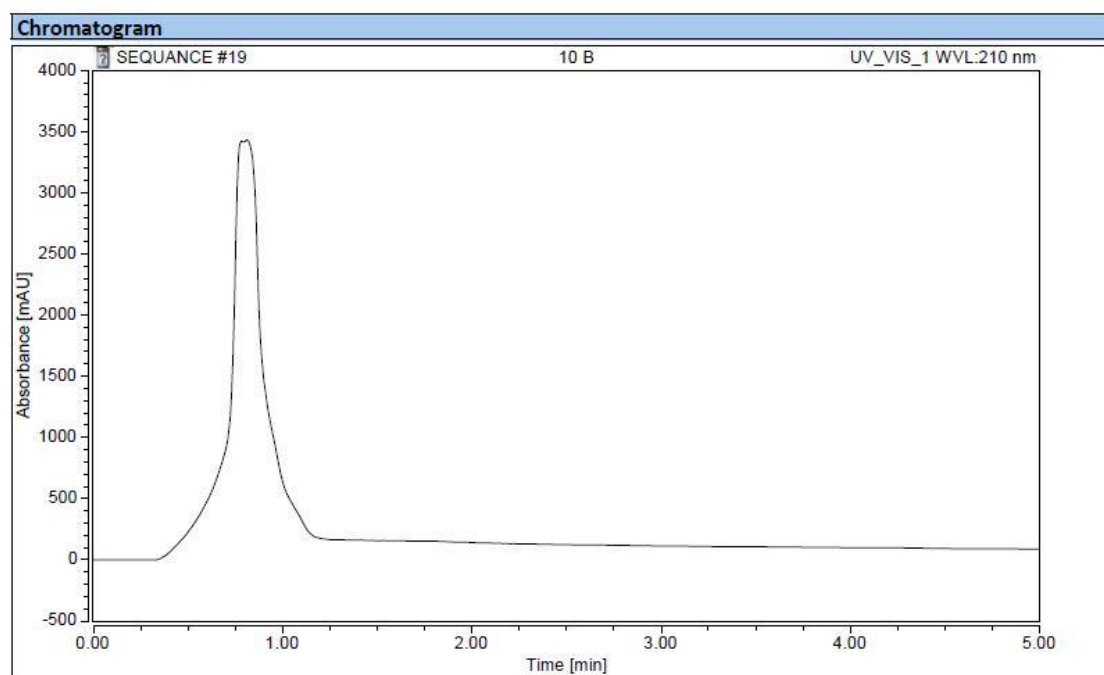


Figure S 91. Compound 10b purity

Compound **10c** purity (99.8%)

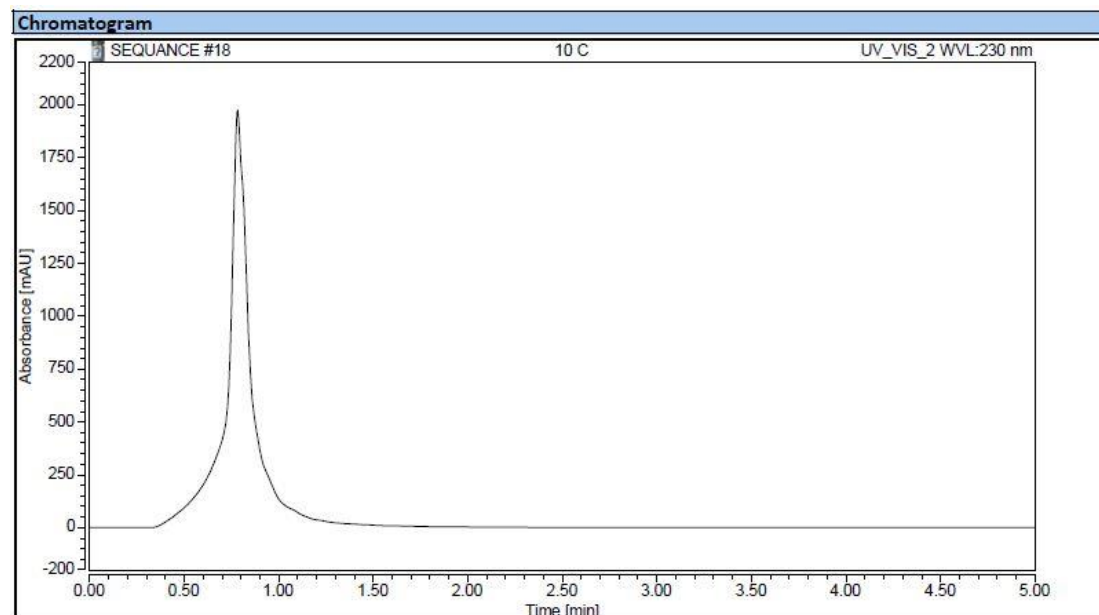


Figure S 92. Compound 10c purity

Compound **11** purity (99.7%)

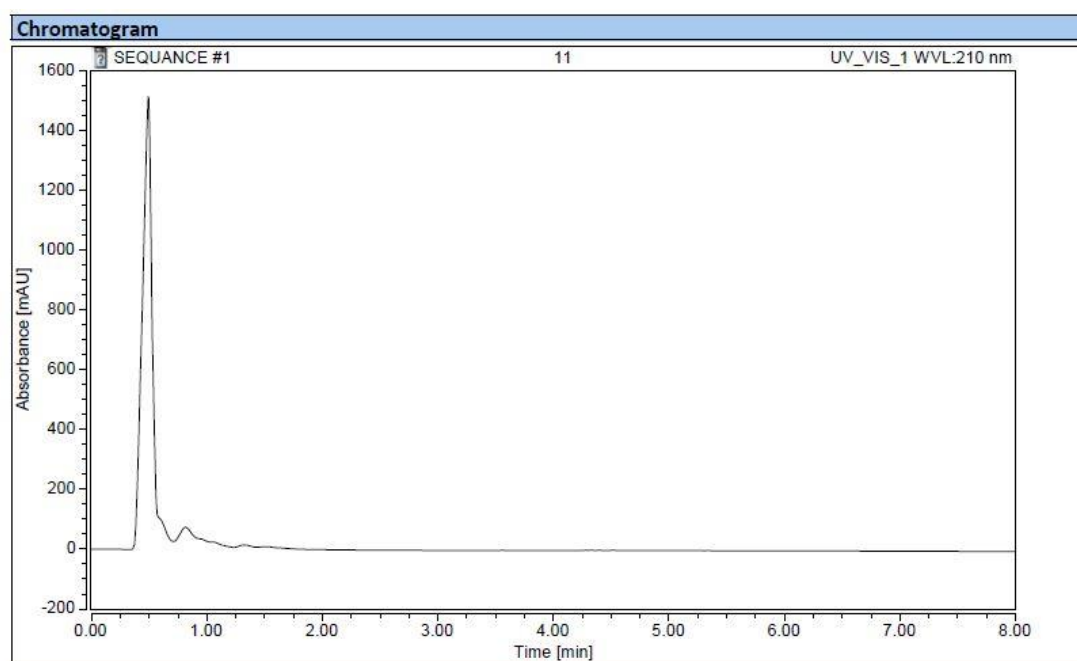


Figure S 93. Compound 11 purity

Compound **12** purity (99.6%)

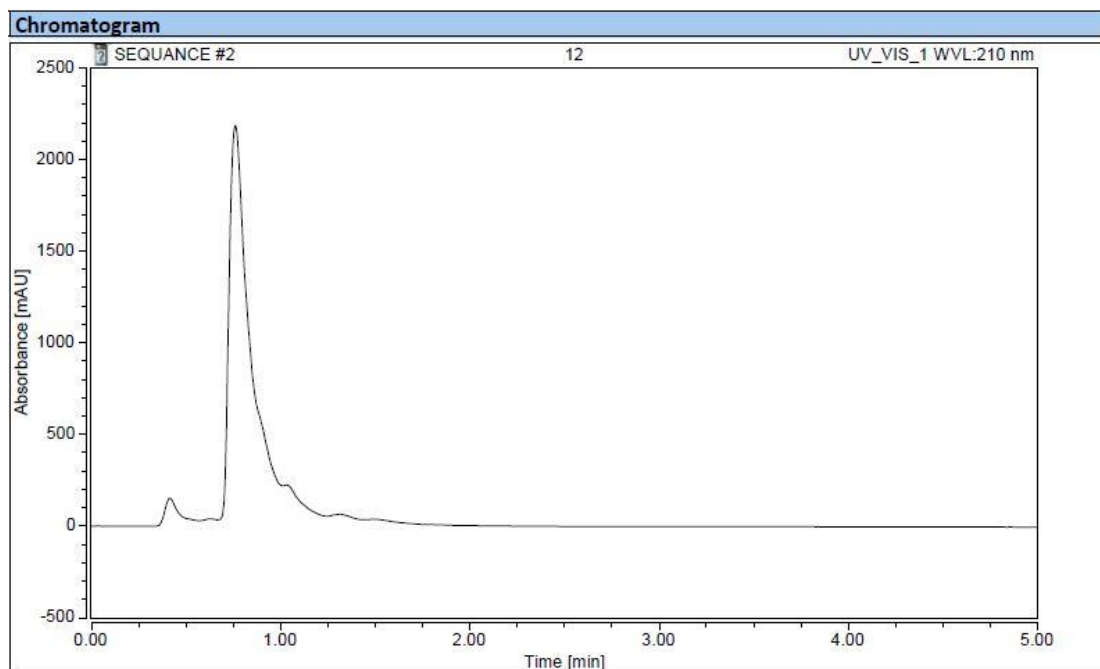


Figure S 94. Compound 12 purity