

**Mitochondria-Targeting Symmetric Diiminoguanidines: Potent and Selective Anticancer Agents
Against Pancreatic Tumors**

Sigrid Lacaille^a and Andreea R. Schmitzer^{*a}

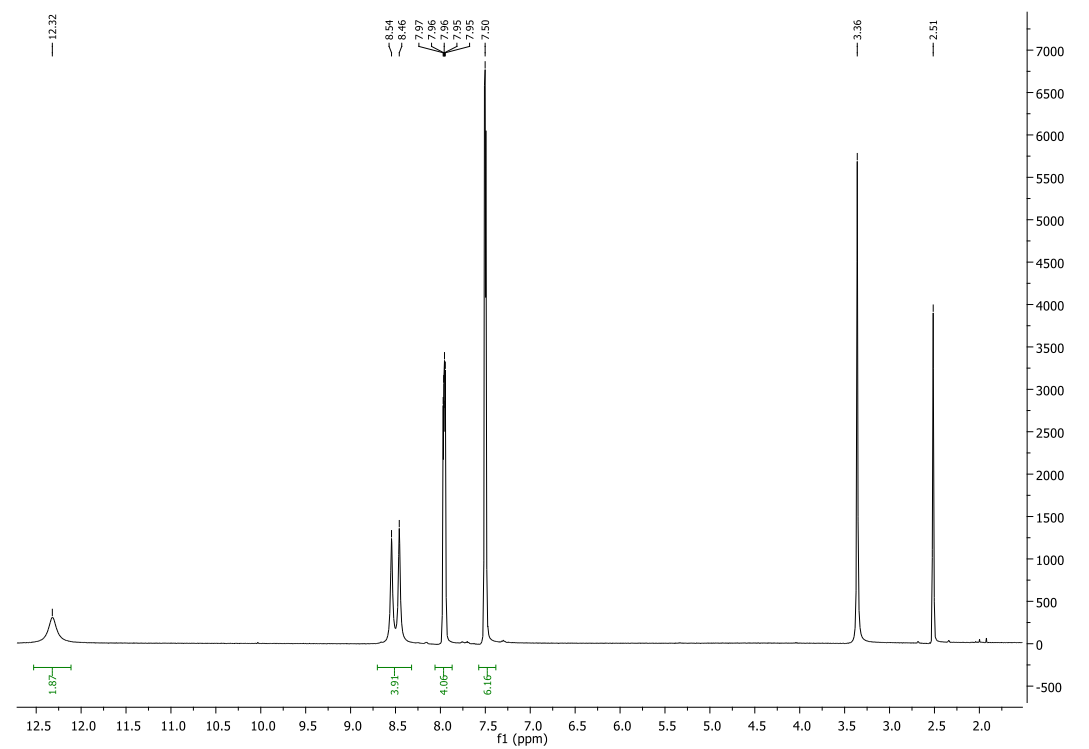
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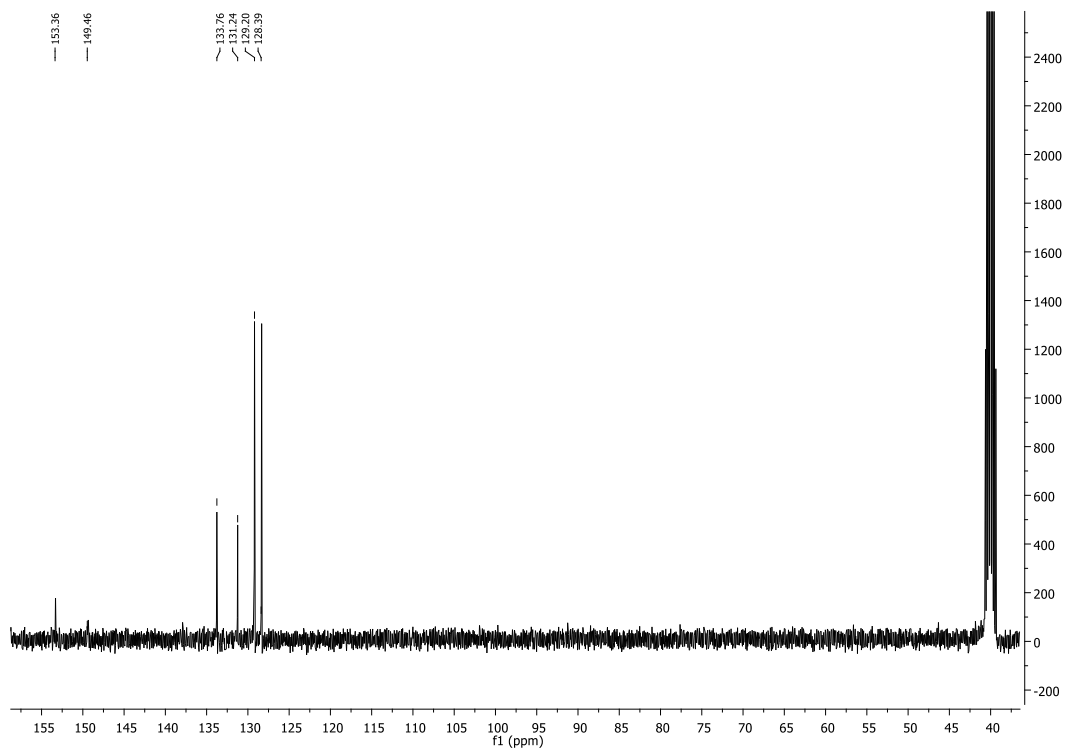
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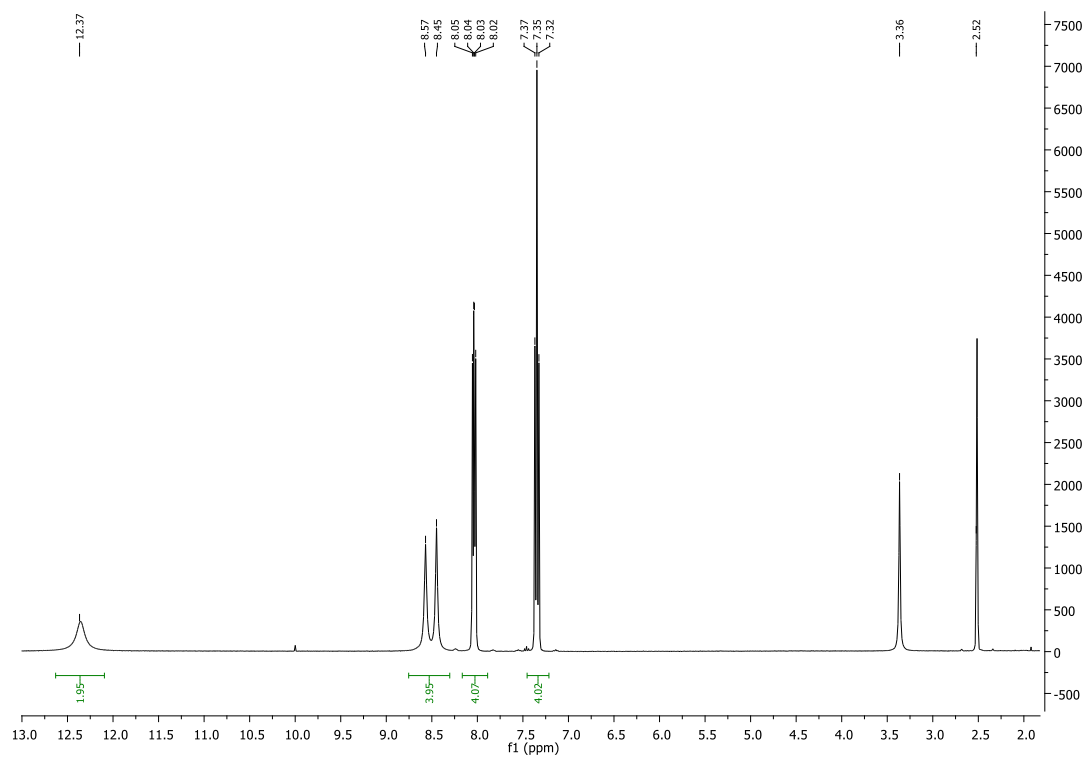
¹H and ¹³C NMR Spectra



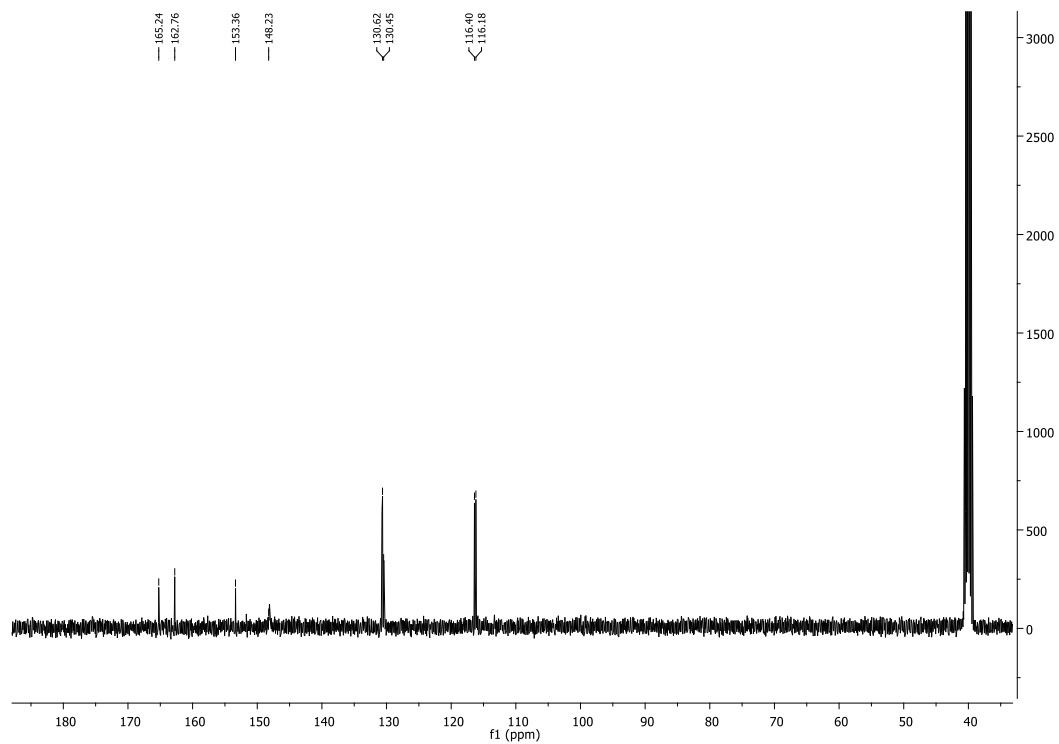
¹H NMR (400 MHz, DMSO-*d*₆) of **1a**



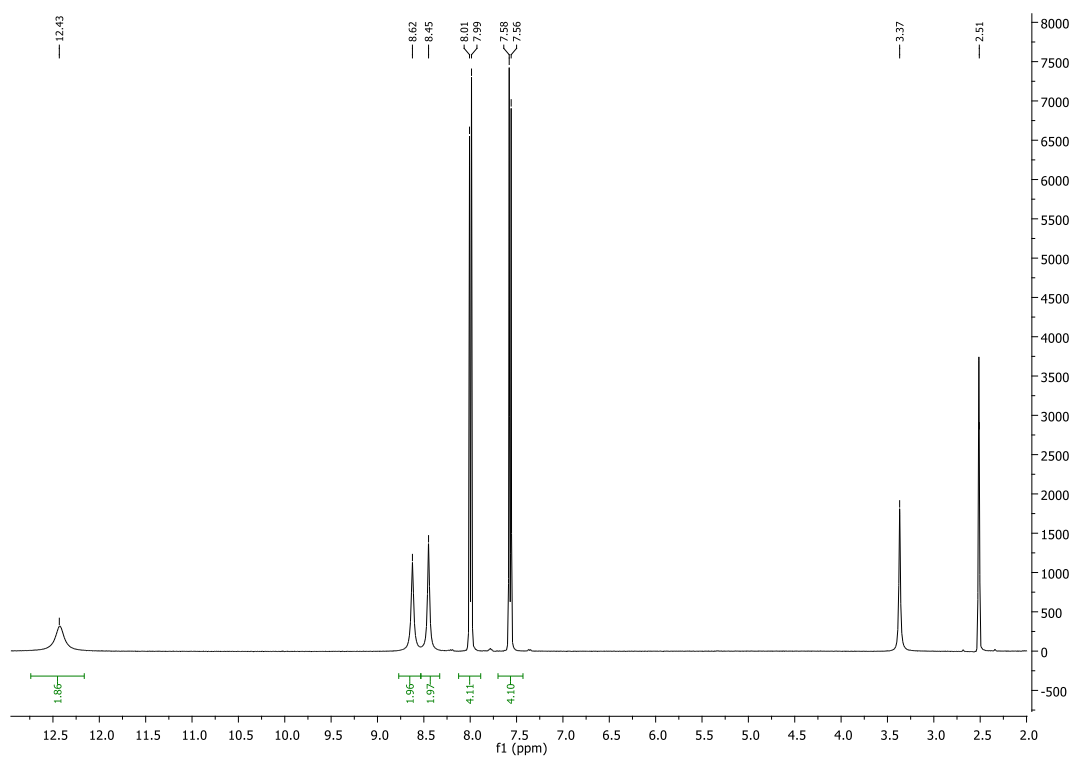
^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) of **1a**



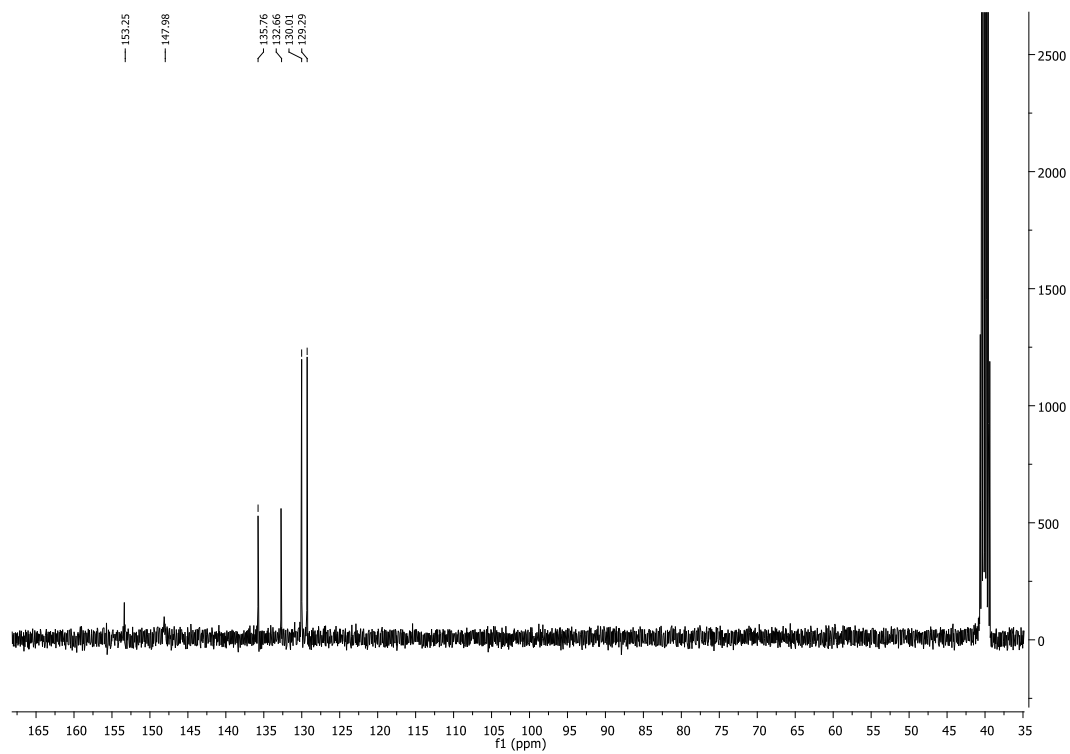
^1H NMR (400 MHz, $\text{DMSO}-d_6$) of **1b**



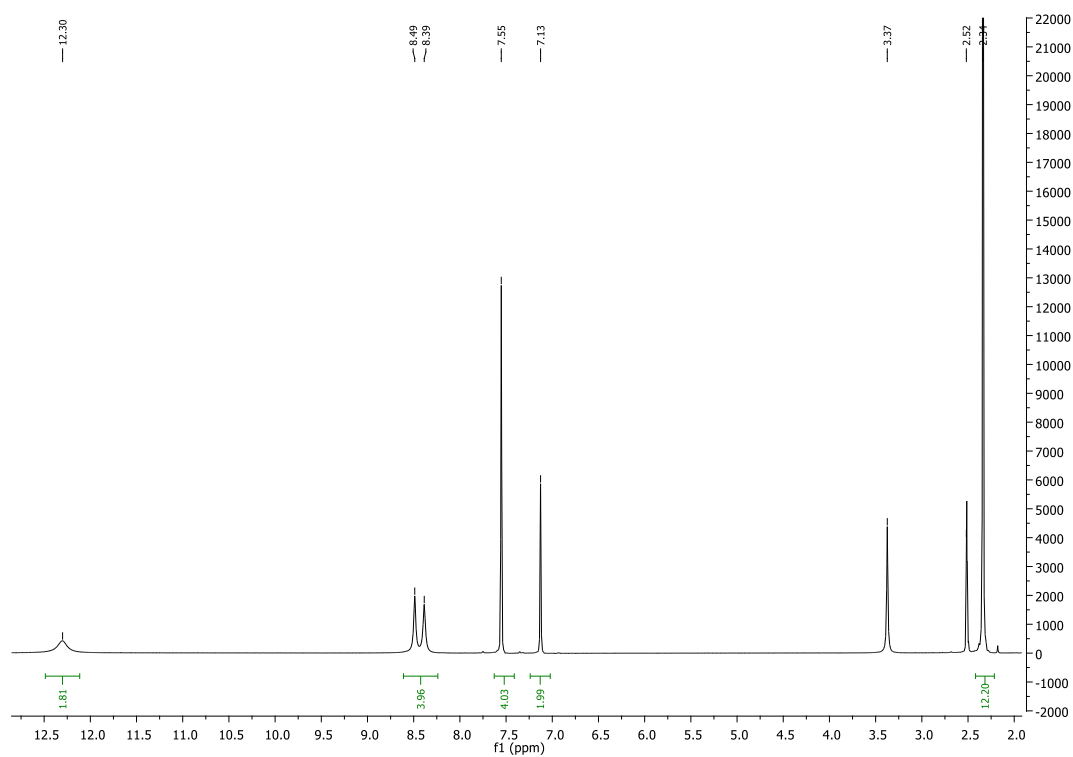
^{13}C NMR (101 MHz, DMSO- d_6) of **1b**



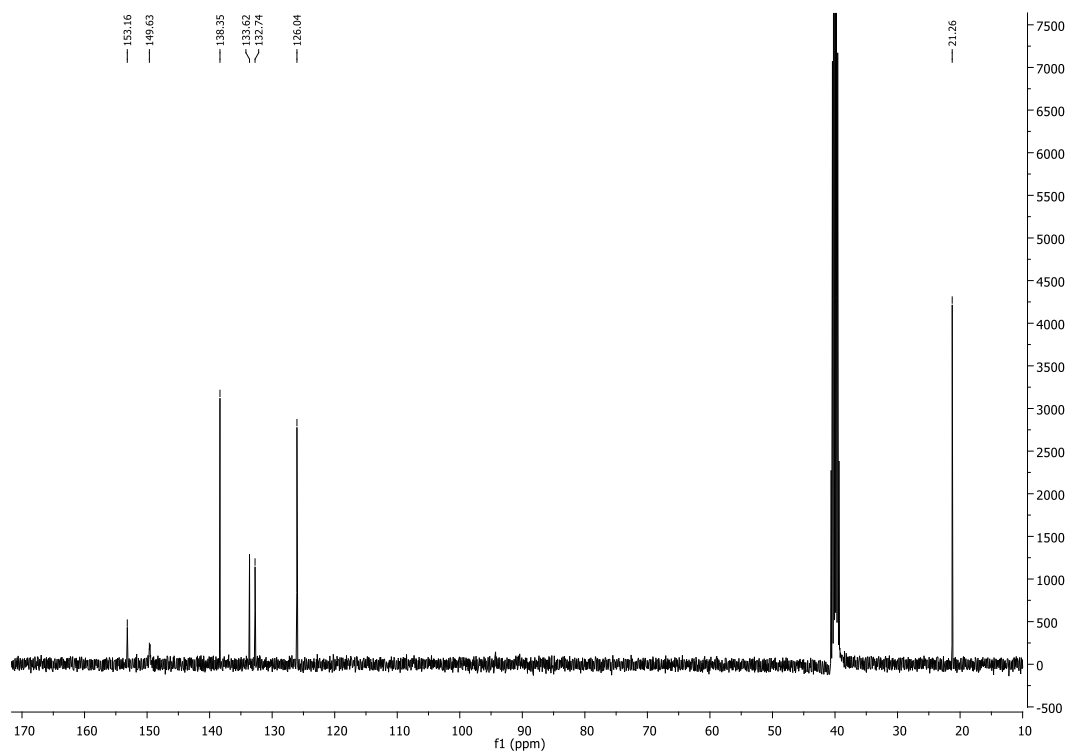
^1H NMR (400 MHz, DMSO- d_6) of **1c**



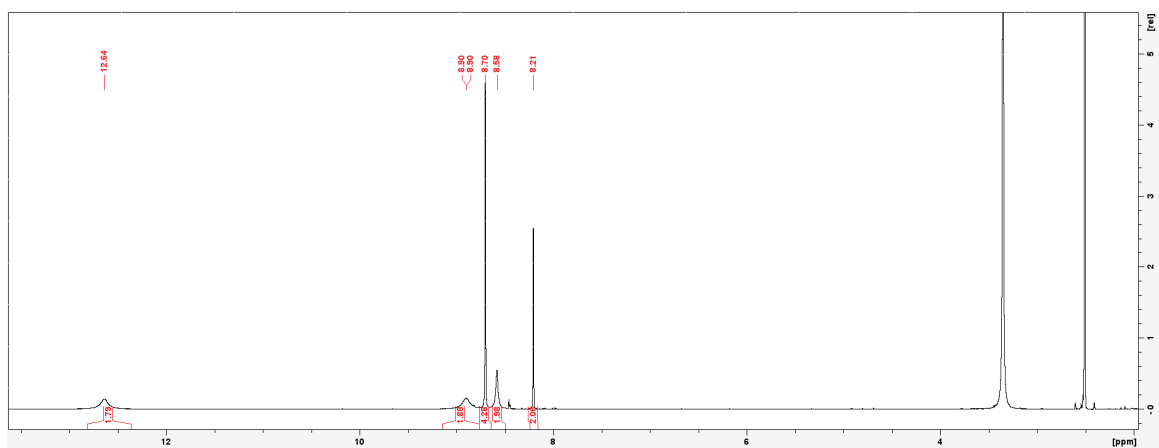
^{13}C NMR (101 MHz, DMSO- d_6) of **1c**



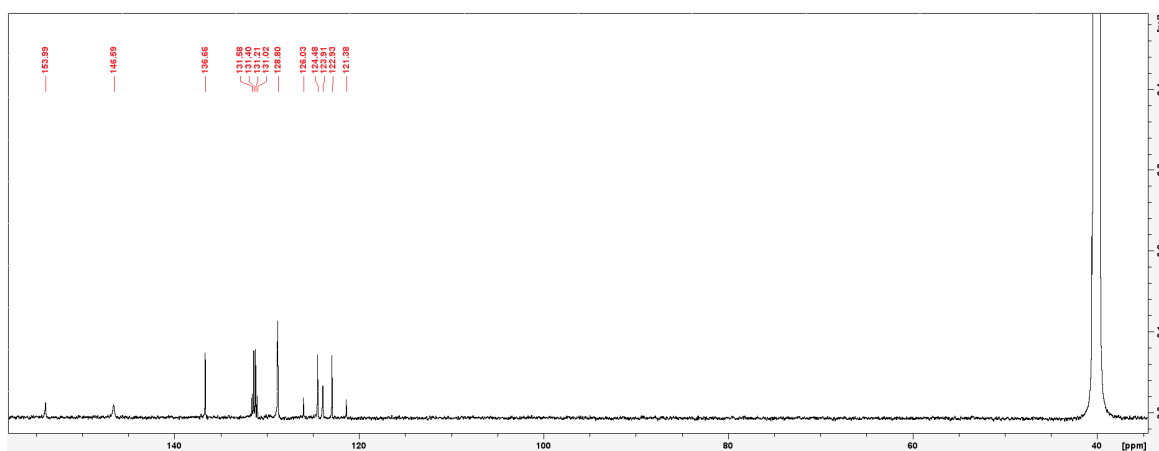
^1H NMR (400 MHz, DMSO- d_6) of **1d**



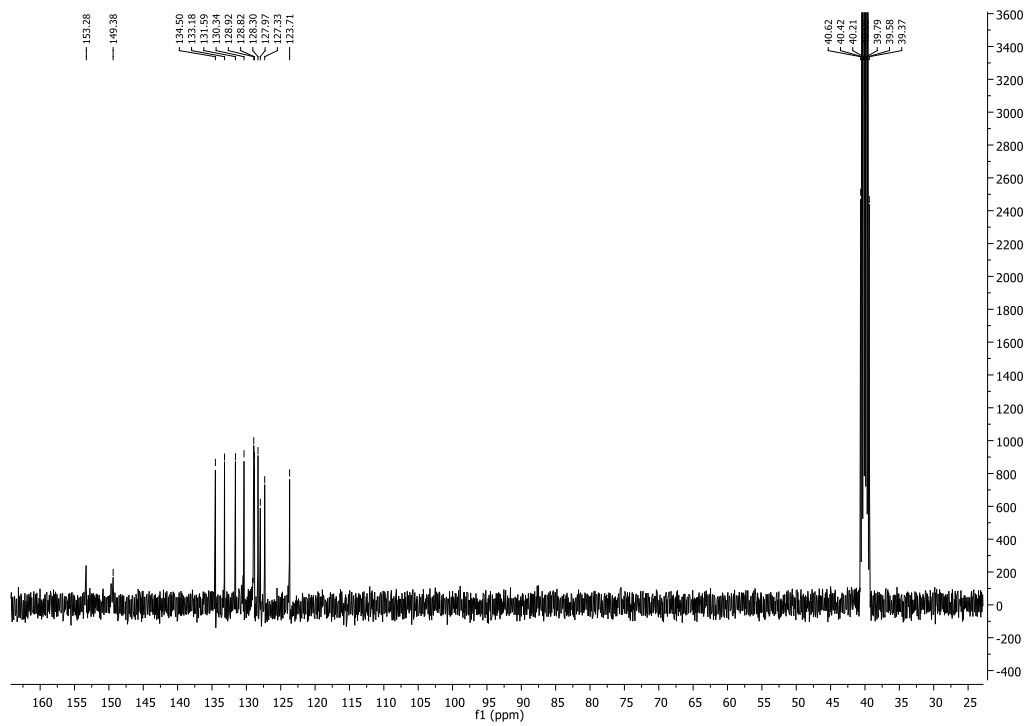
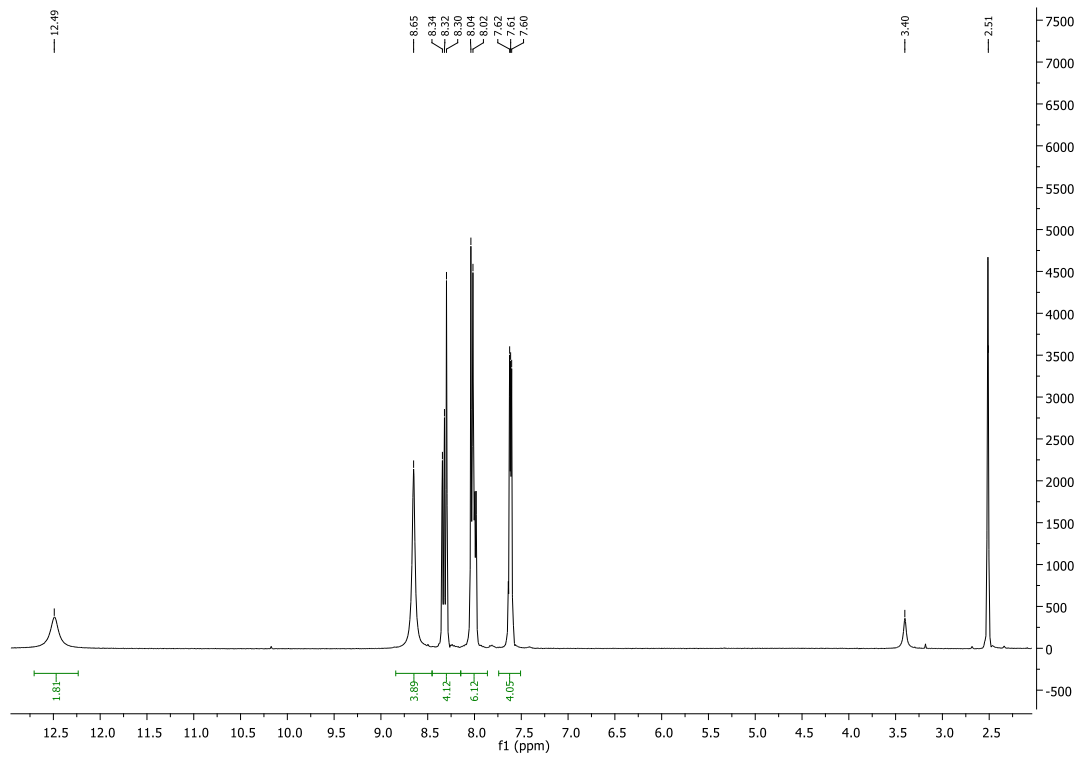
^{13}C NMR (101 MHz, DMSO-*d*₆) of **1d**

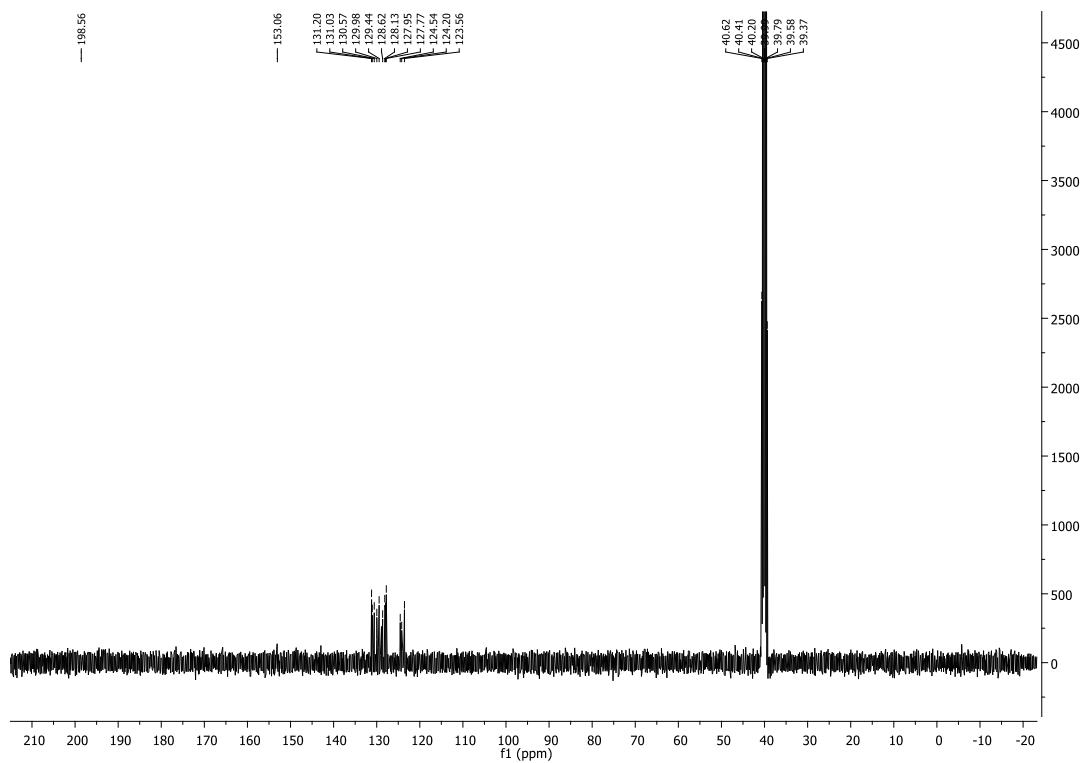
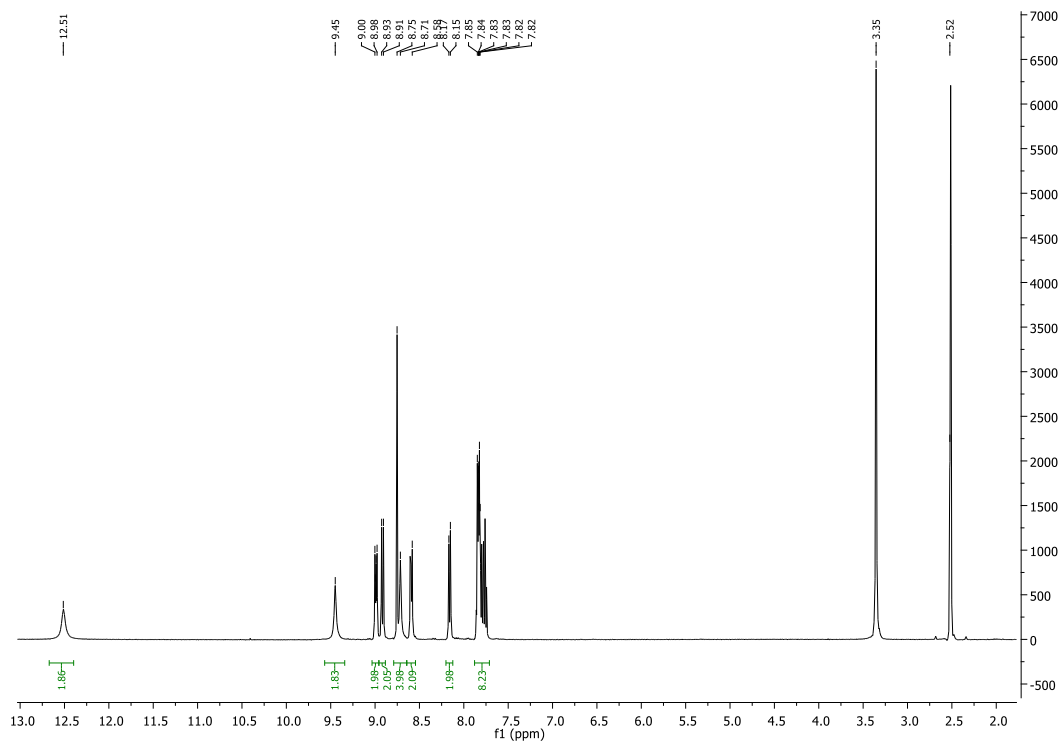


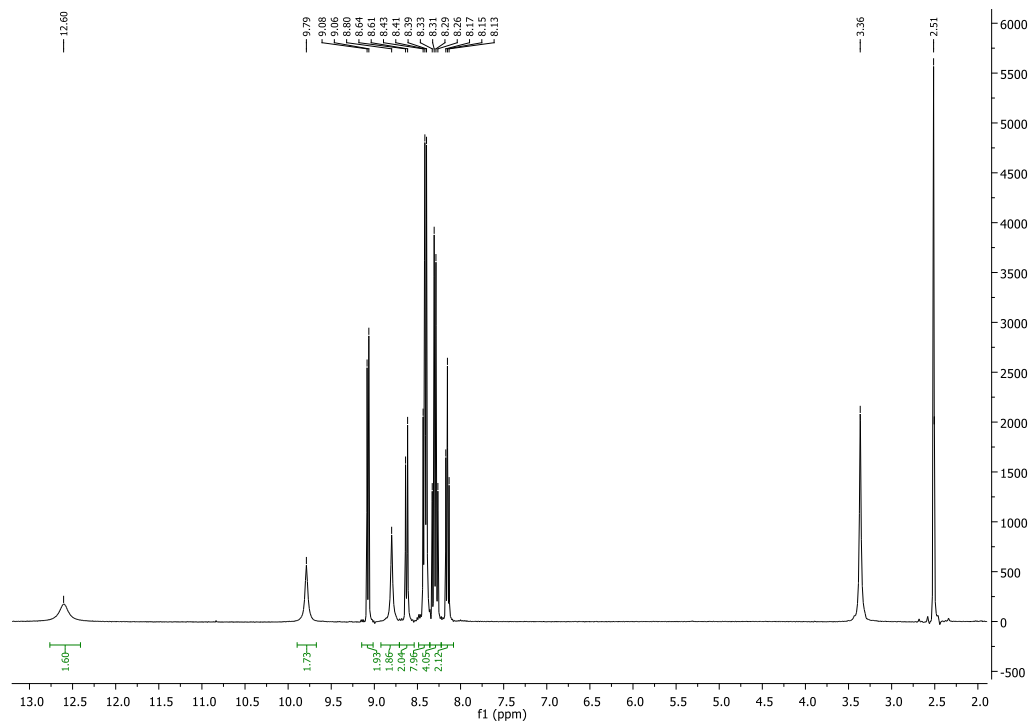
^1H NMR (700 MHz, DMSO-*d*₆) of **1e**



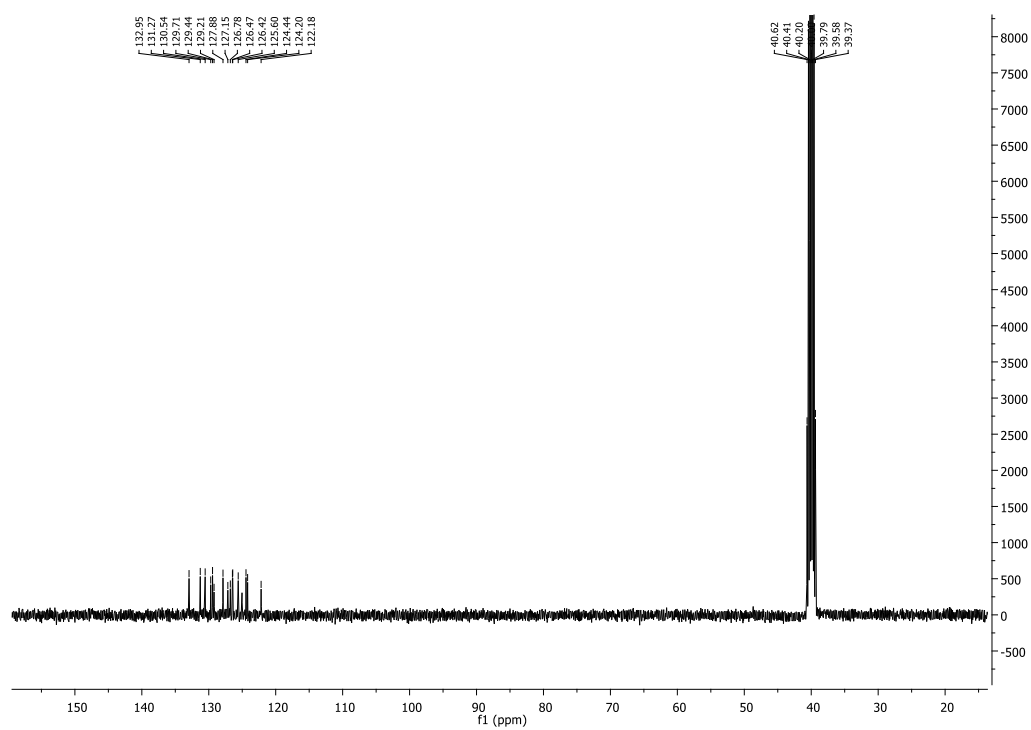
^{13}C NMR (176 MHz, DMSO-*d*₆) of **1e**



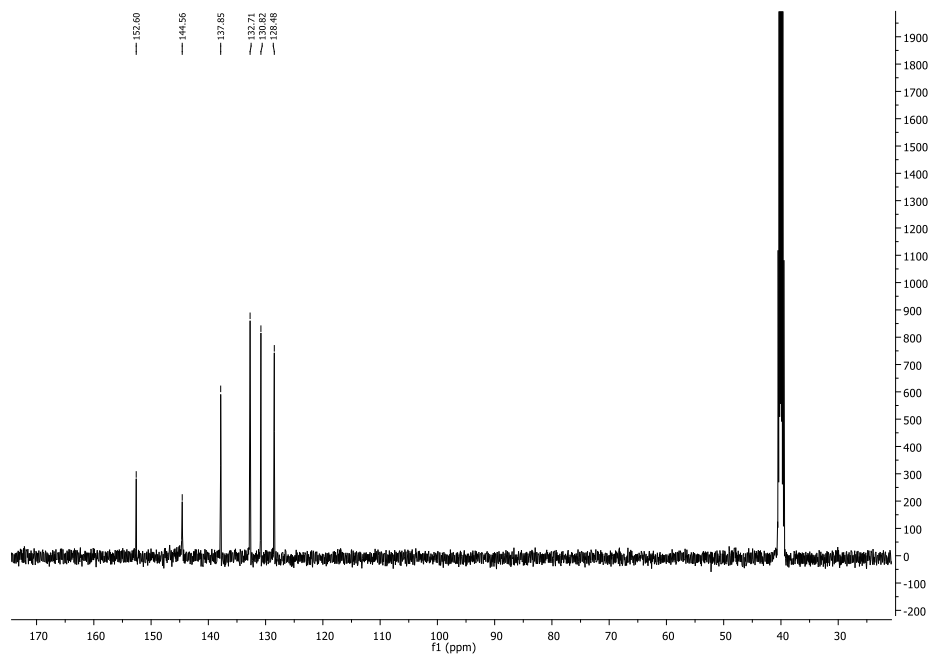
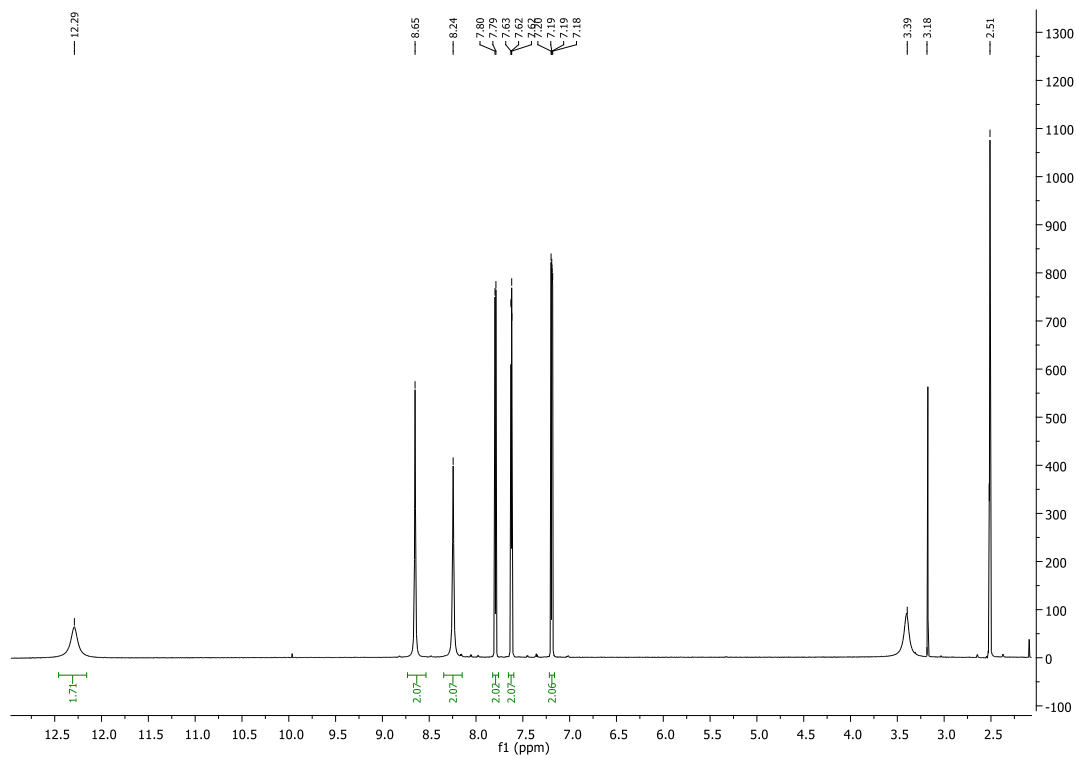


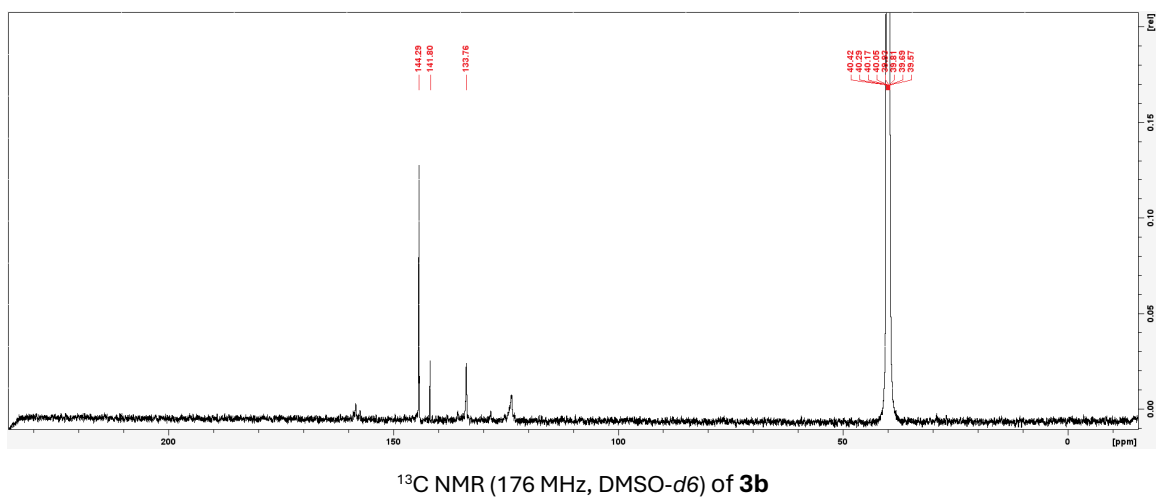
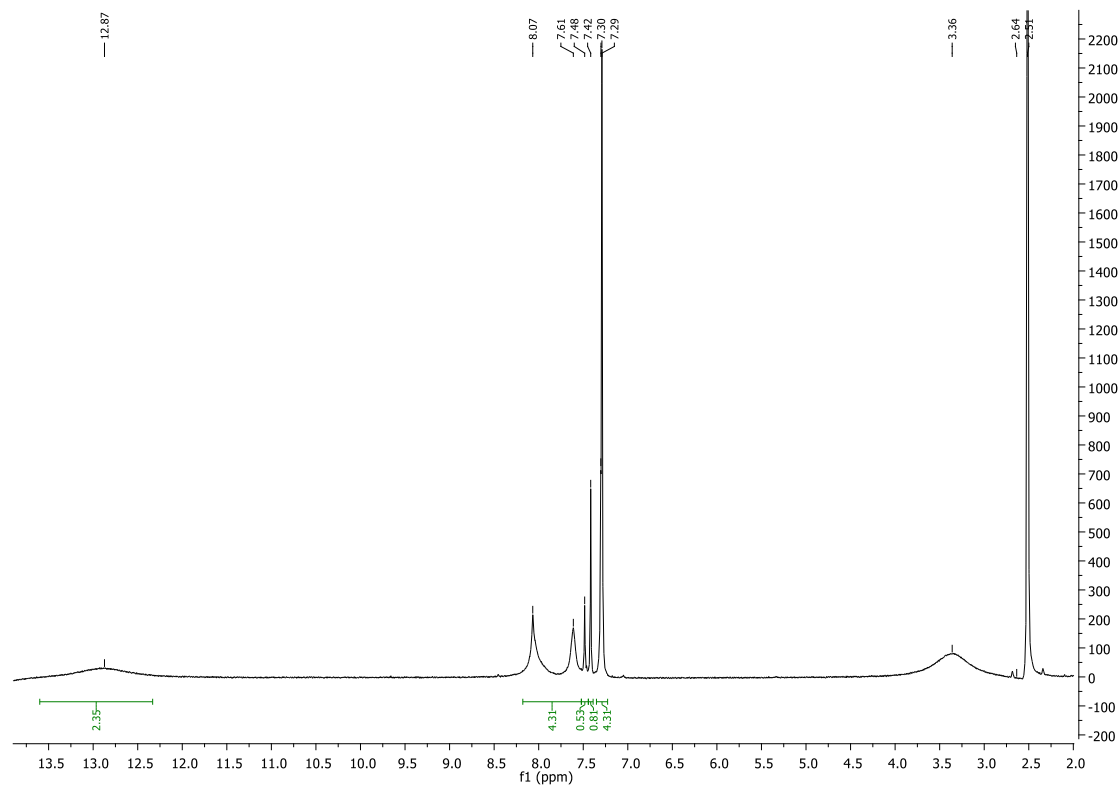


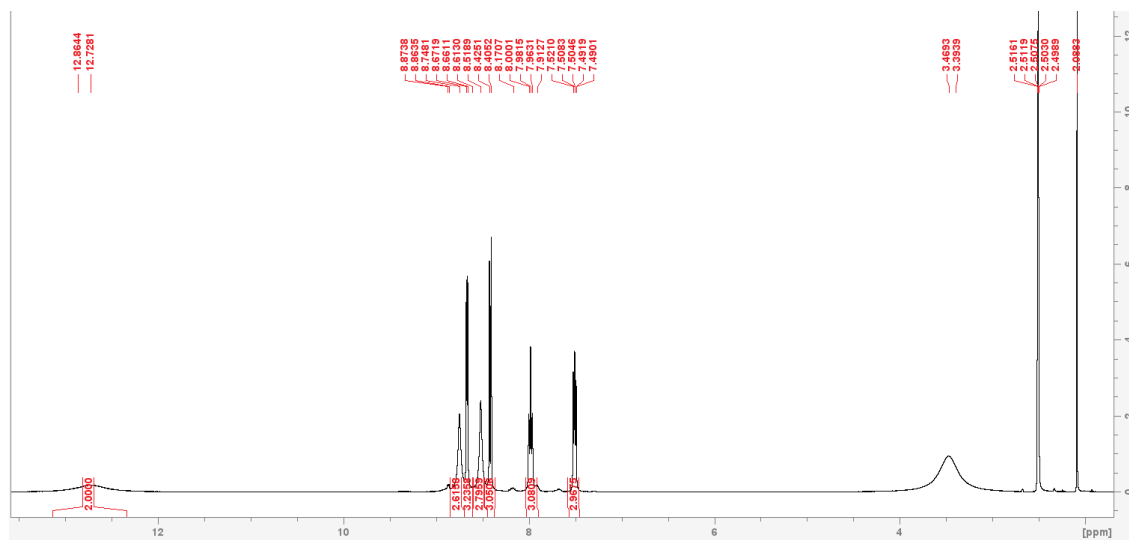
¹H NMR (400 MHz, DMSO-*d*₆) of **2c**



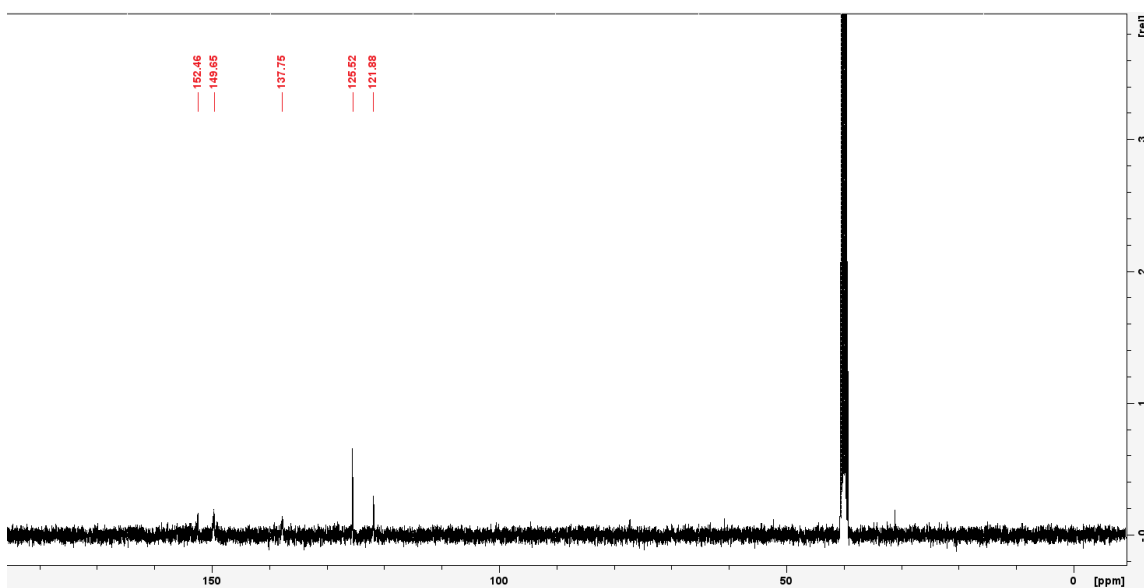
¹³C NMR (101 MHz, DMSO-*d*₆) of **2c**



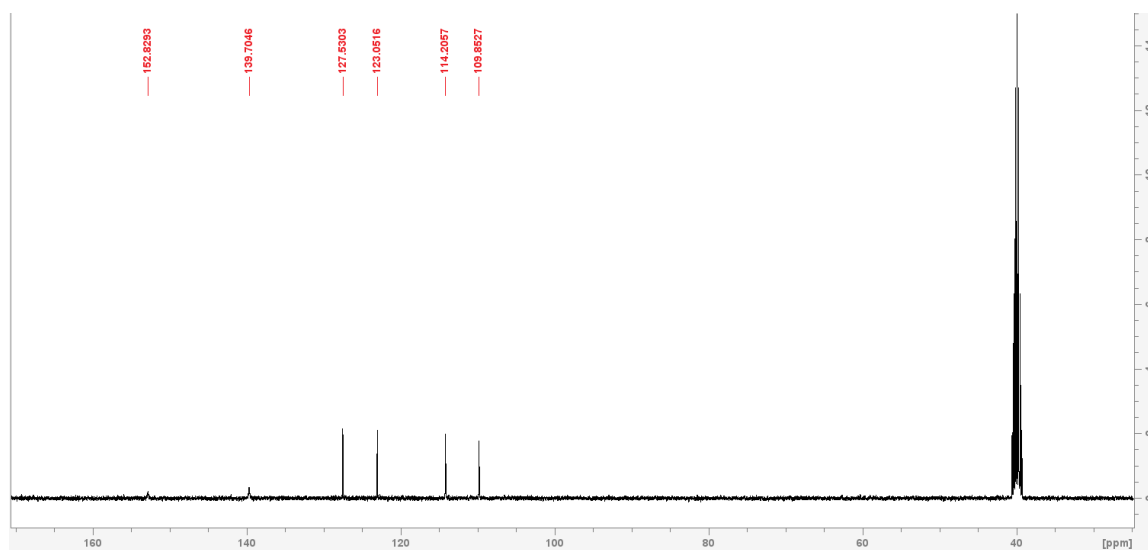
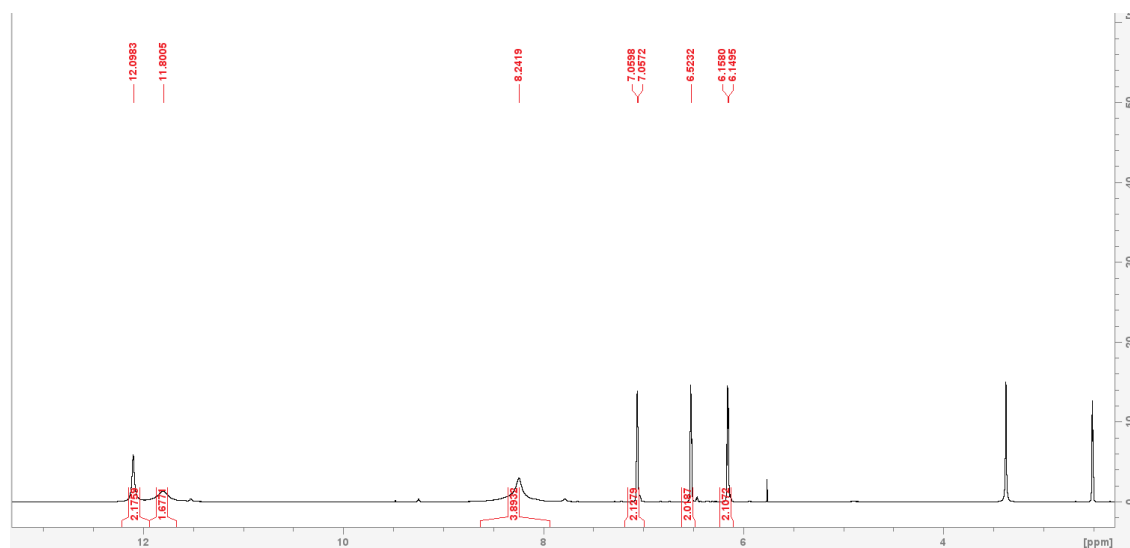


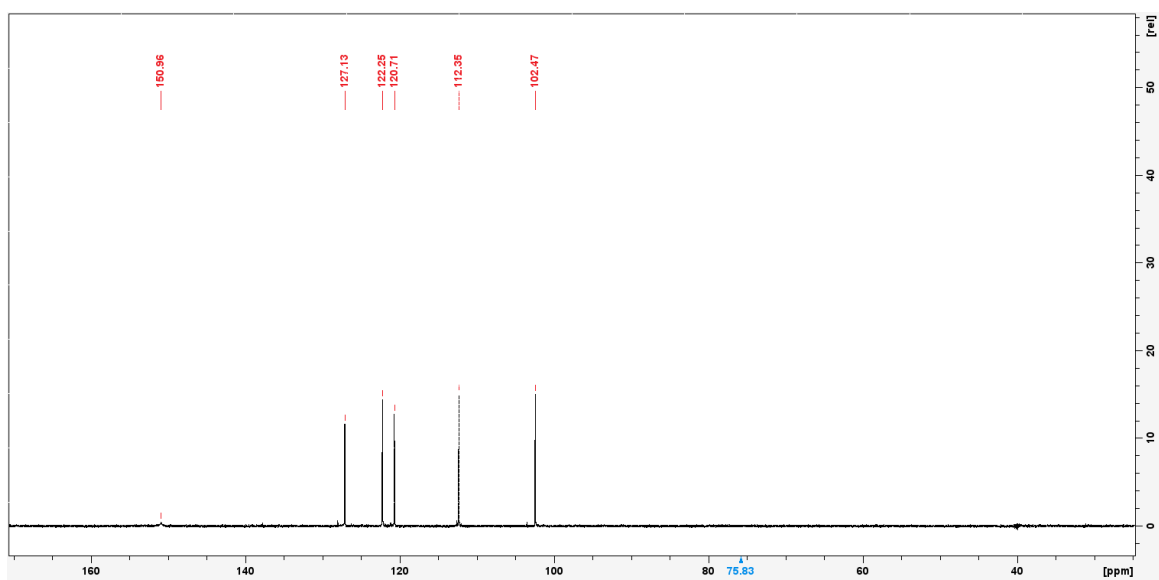
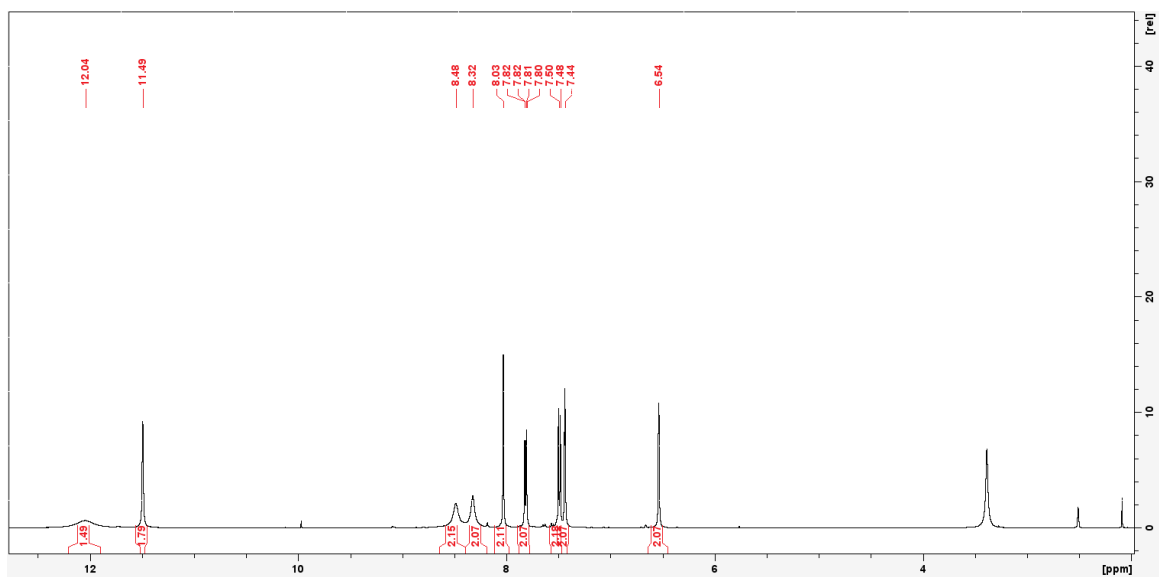


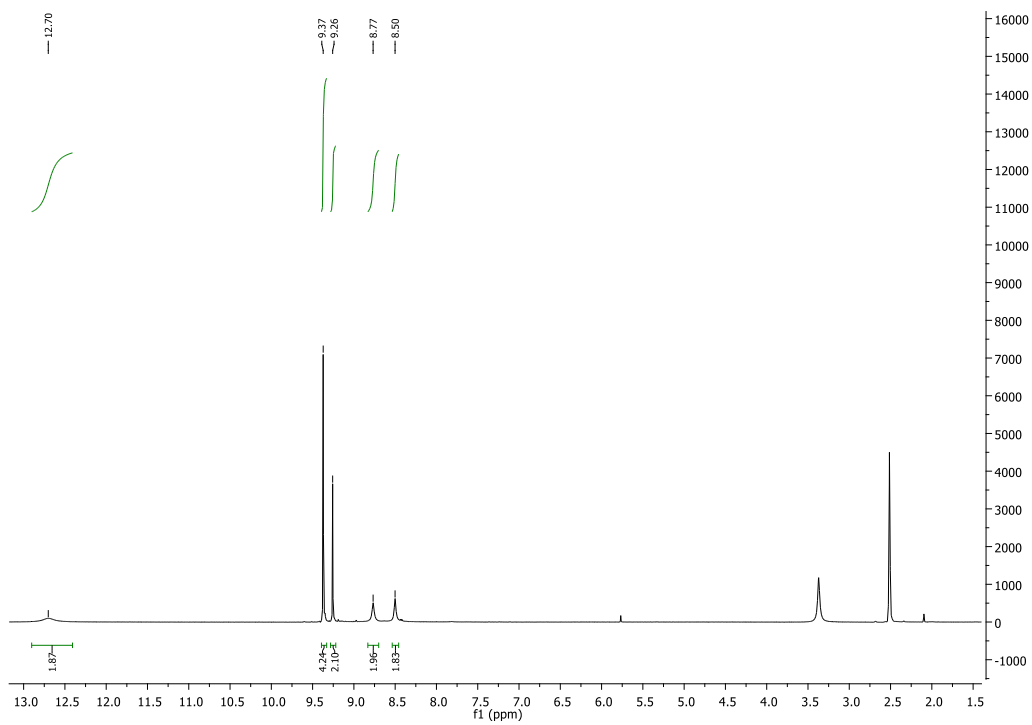
¹H NMR (400 MHz, DMSO-*d*₆) of **3c**



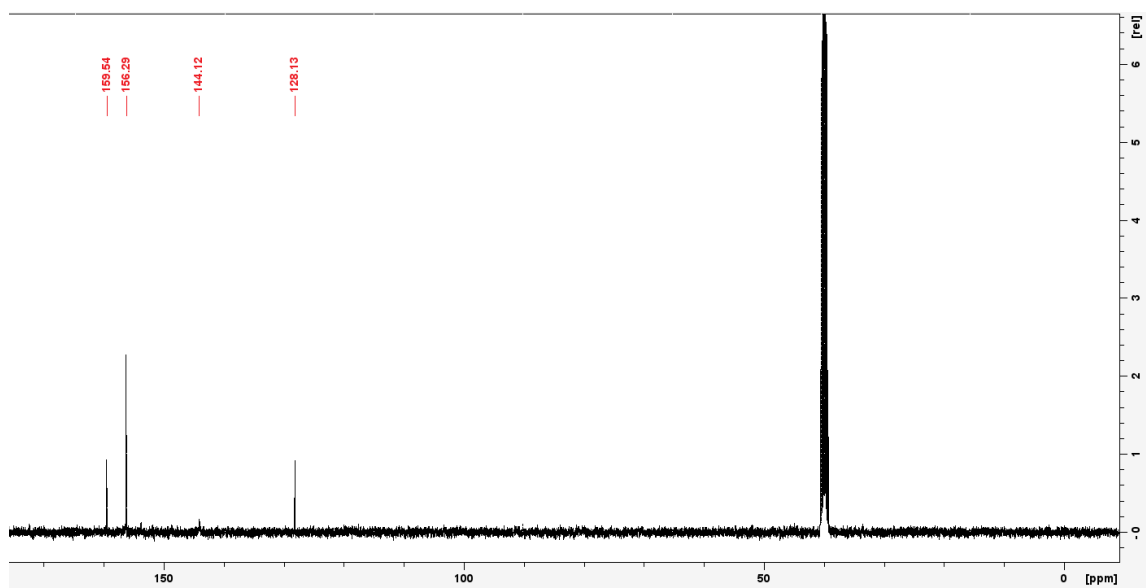
¹³C NMR (126 MHz, DMSO-*d*₆) of **3c**





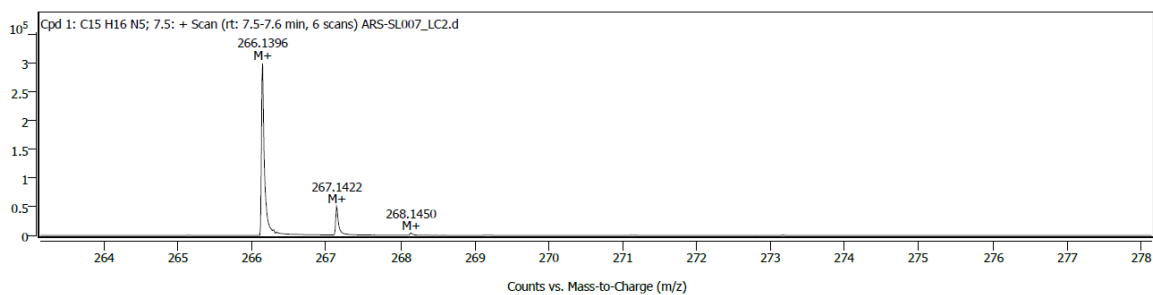


¹H NMR (400 MHz, DMSO-*d*₆) of **3f**

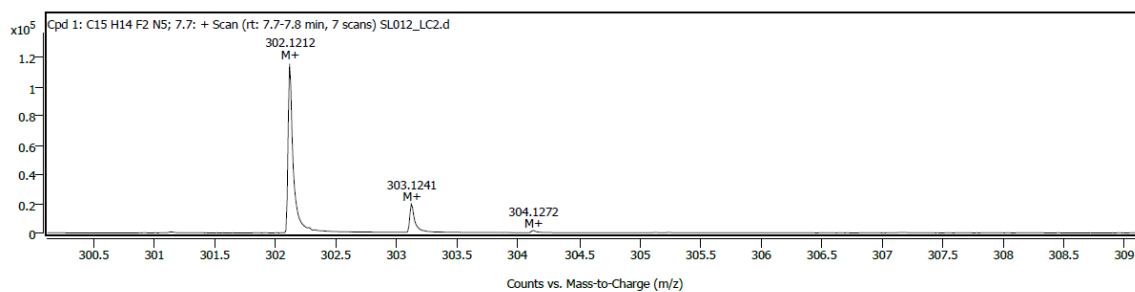


¹³C NMR (126 MHz, DMSO-*d*₆) of **3f**

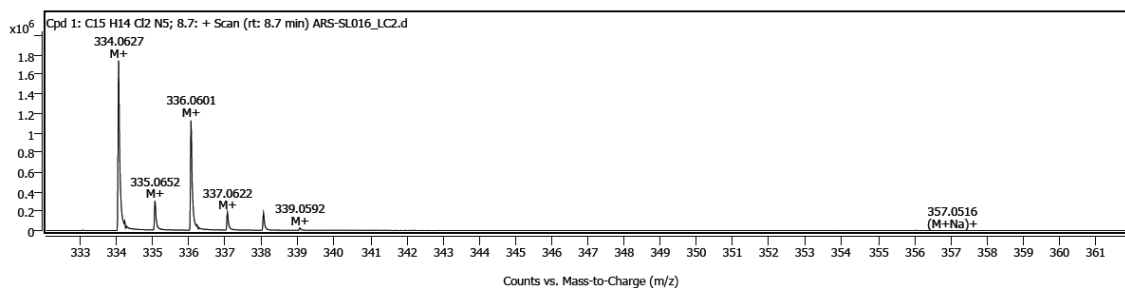
Exact Mass analysis



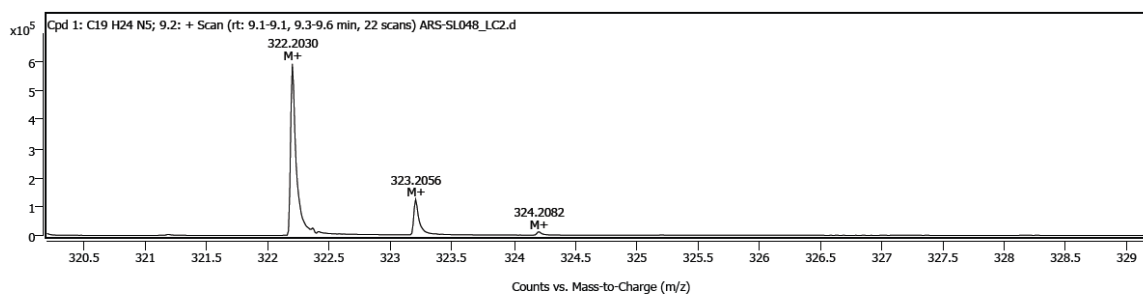
Exact mass spectrum of **1a**



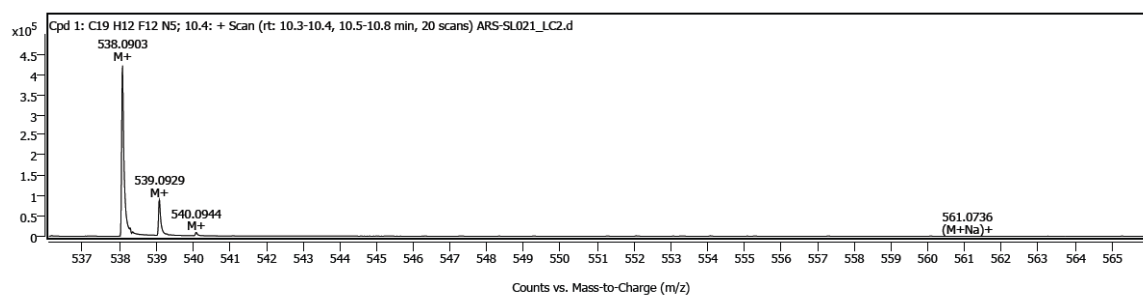
Exact mass spectrum of **1b**



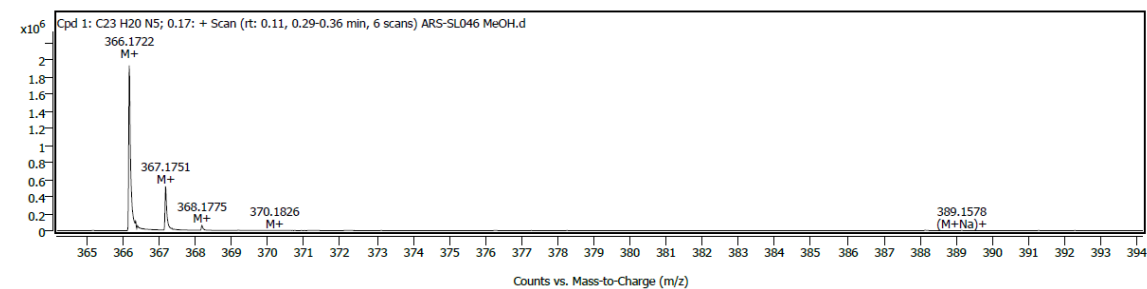
Exact mass spectrum of **1c**



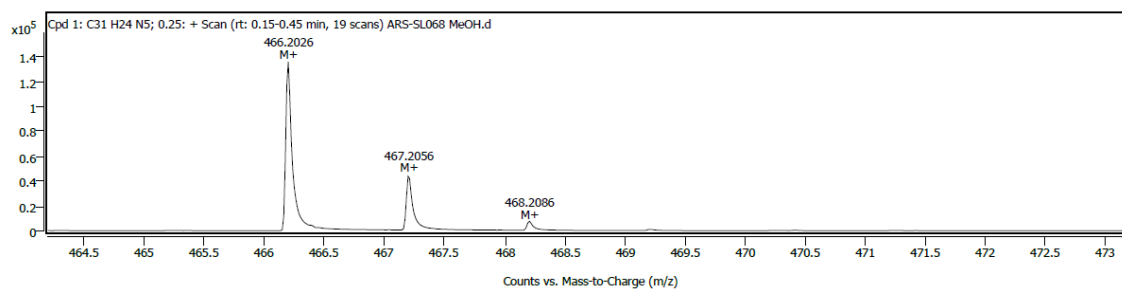
Exact mass spectrum of **1d**



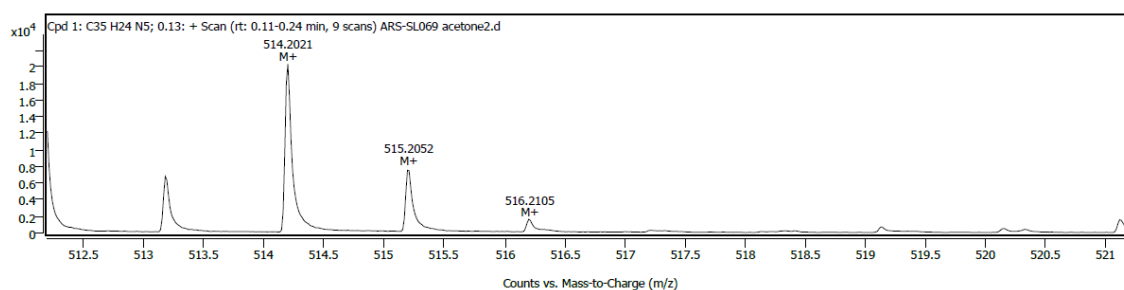
Exact mass spectrum of **1e**



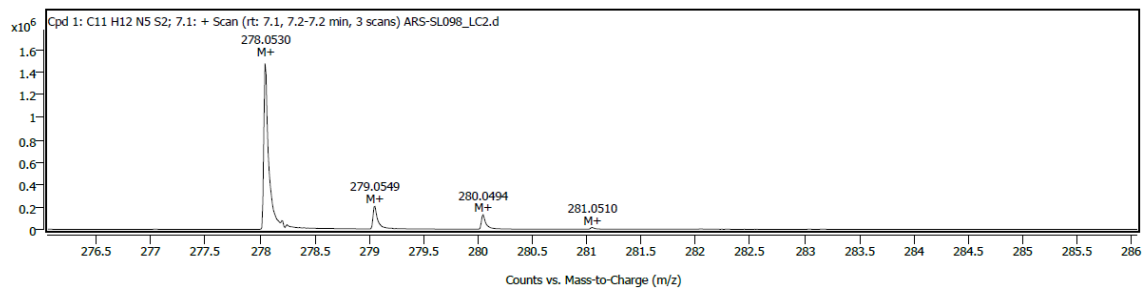
Exact mass spectrum of **2a**



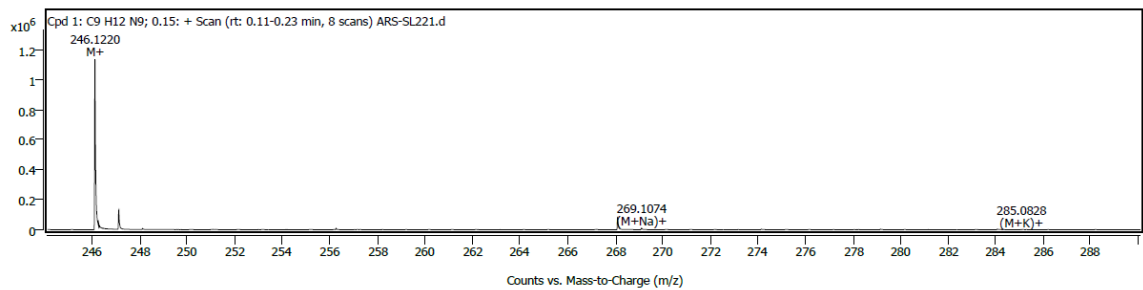
Exact mass spectrum of **2b**



Exact mass spectrum of **2c**



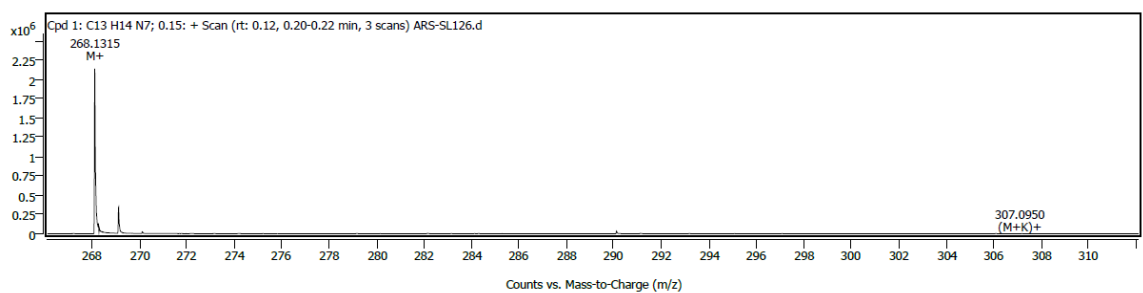
Exact mass spectrum of **3a**



Spectrum Peaks (Max. 1)

Ion Species	Formula	Abund	m/z	m/z (Calc)	Diff (ppm)	Diff (mDa)
M+	C ₉ H ₁₂ N ₉	1137443	246.12196	246.12102	3.85	0.95

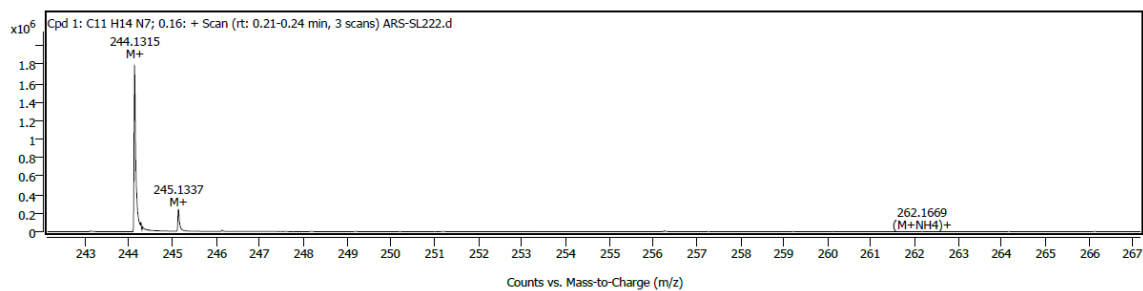
Exact mass spectrum of **3b**



Spectrum Peaks (Max. 1)

Ion Species	Formula	Abund	m/z	m/z (Calc)	Diff (ppm)	Diff (mDa)
M+	C ₁₃ H ₁₄ N ₇	2215892	268.13152	268.13052	3.73	1.00

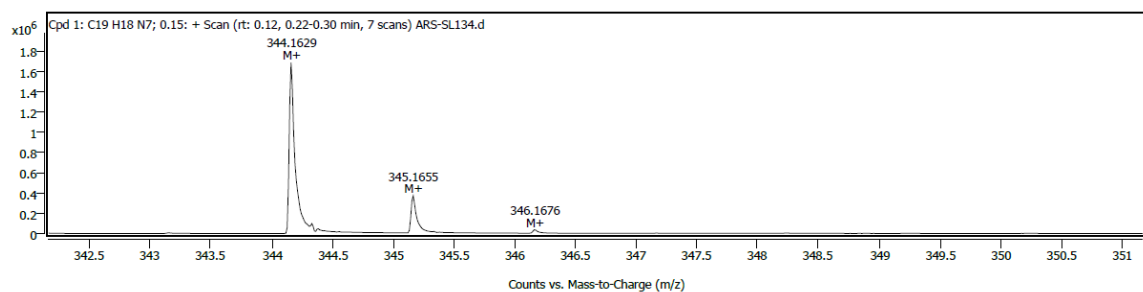
Exact mass spectrum of **3c**



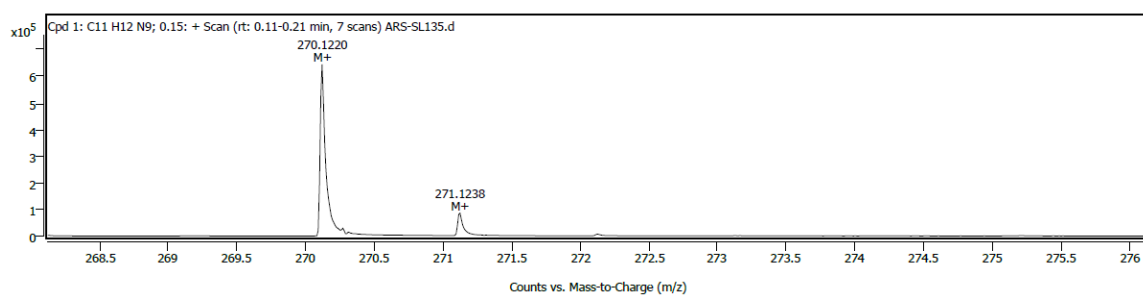
Spectrum Peaks (Max. 1)

Ion Species	Formula	Abund	m/z	m/z (Calc)	Diff (ppm)	Diff (mDa)
M+	C ₁₁ H ₁₄ N ₇	1844175	244.13147	244.13052	3.89	0.95

Exact mass spectrum of **3d**

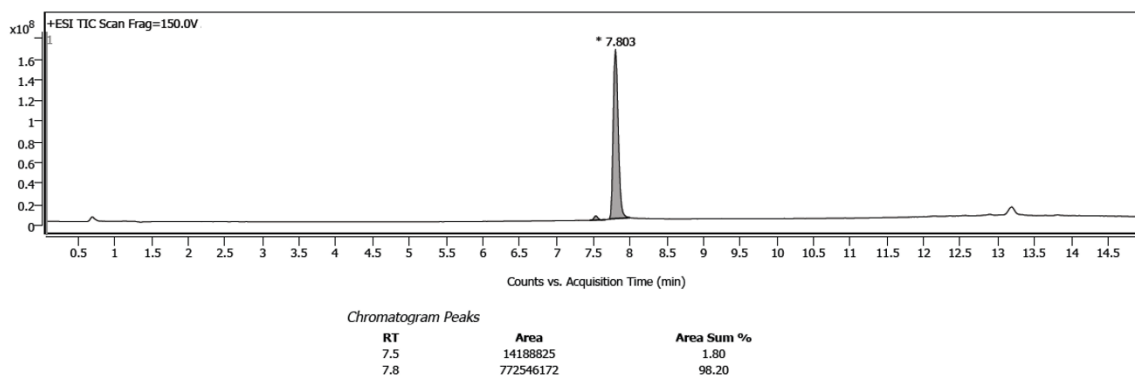


Exact mass spectrum of **3e**

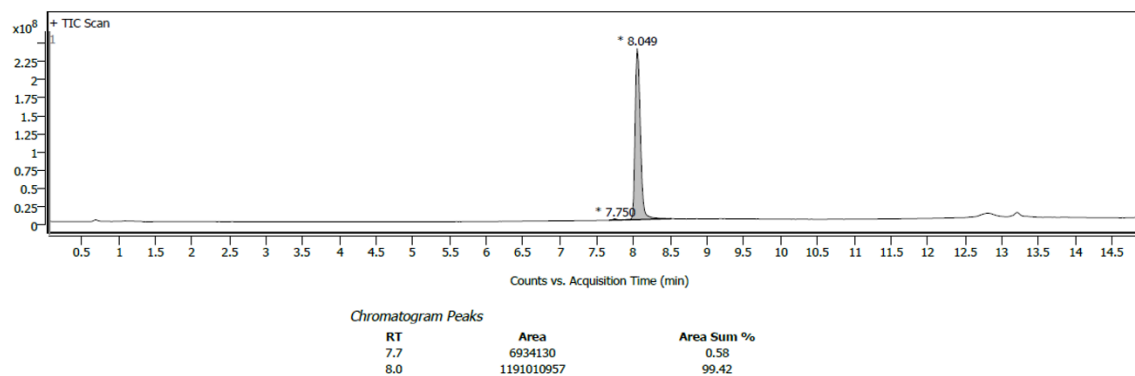


Exact mass spectrum of **3f**

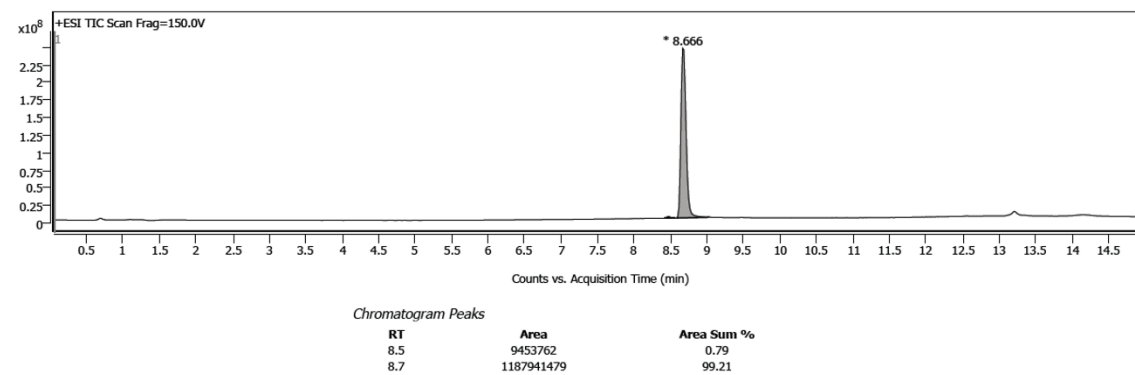
Determination of the purity of diiminoguanidines



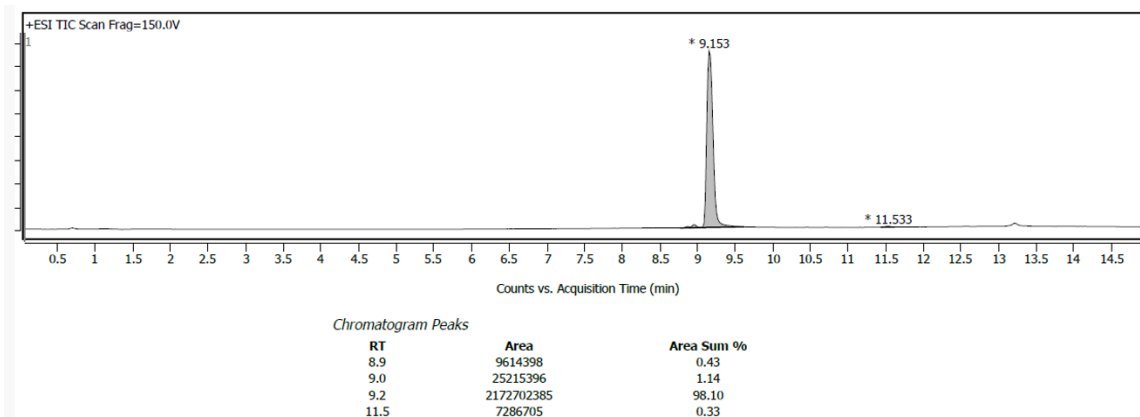
LCMS chromatogram of **1a**



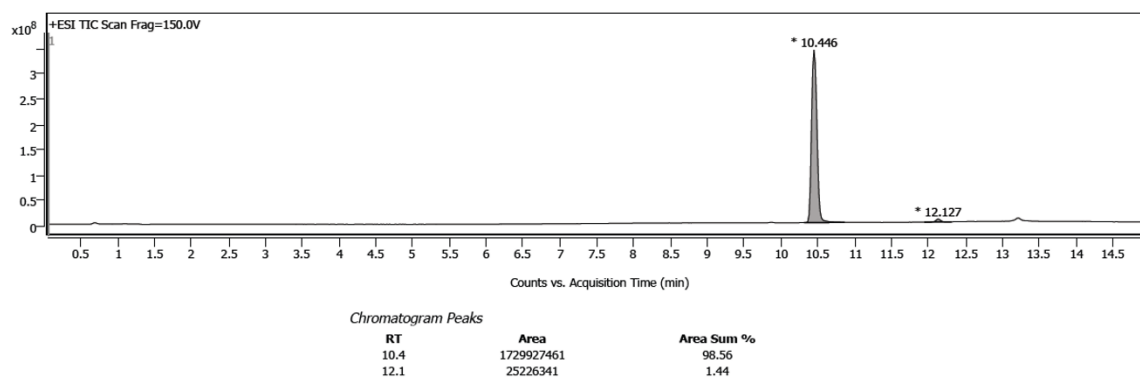
LCMS chromatogram of **1b**



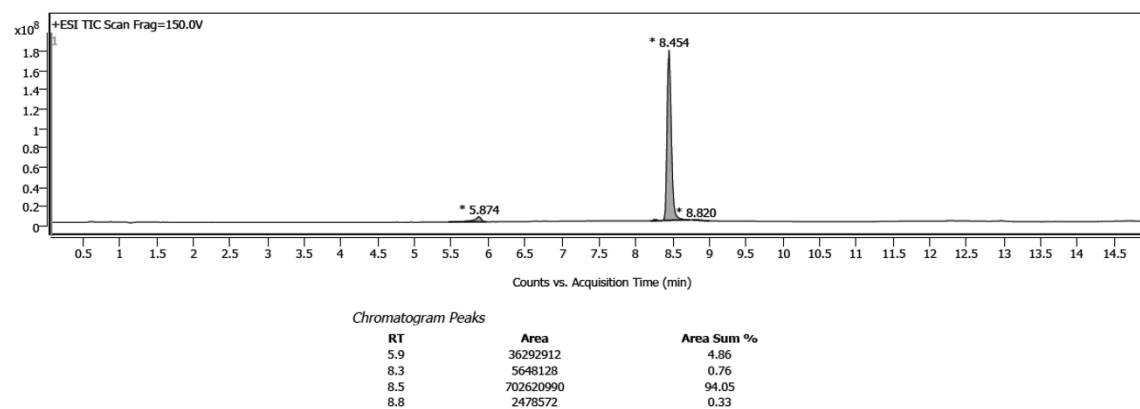
LCMS chromatogram of **1c**



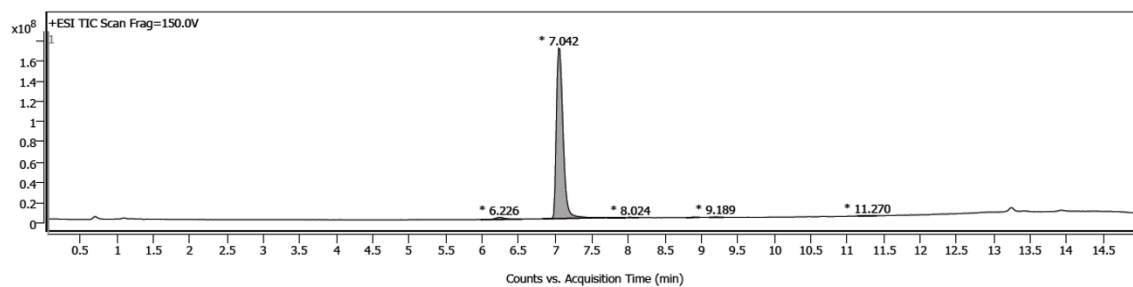
LCMS chromatogram of **1d**



LCMS Spectrum of **1e**



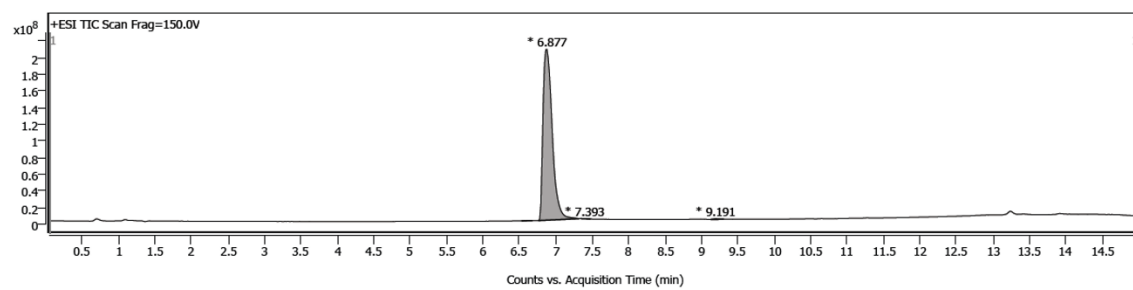
LCMS chromatogram of **2a**



Chromatogram Peaks

RT	Area	Area Sum %
6.2	16677840	1.50
7.0	1083382547	97.71
7.8	2699901	0.24
8.0	1699885	0.15
8.9	1241436	0.11
9.2	1161037	0.10
11.3	1873800	0.17

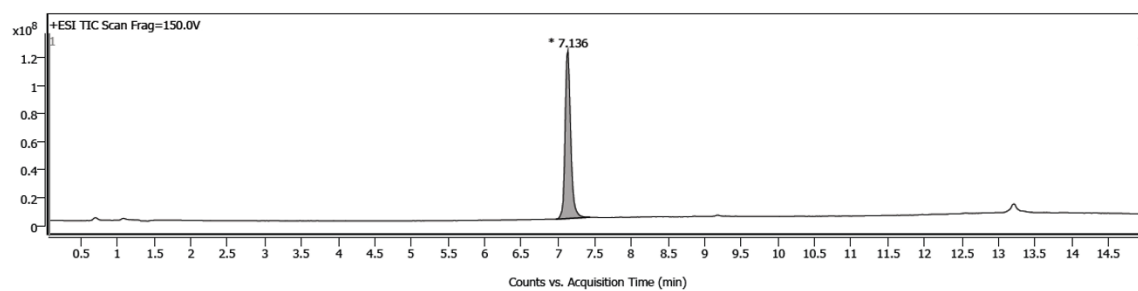
LCMS chromatogram of **2b**



Chromatogram Peaks

RT	Area	Area Sum %
6.7	734585	0.04
6.9	1814971100	99.74
7.4	1056864	0.06
9.2	2907458	0.16

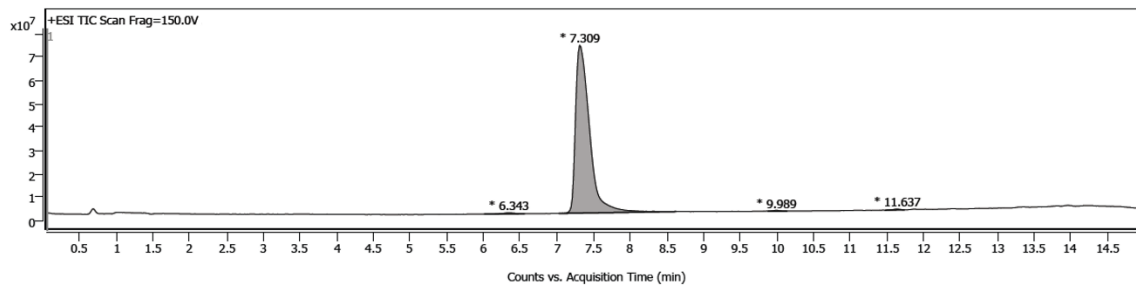
LCMS chromatogram of **2c**



Chromatogram Peaks

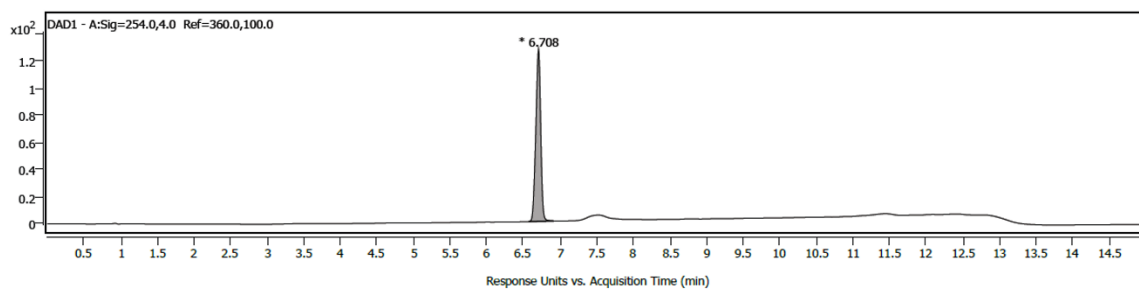
RT	Area	Area Sum %
7.1	640496362	100.00

LCMS chromatogram of **3a**



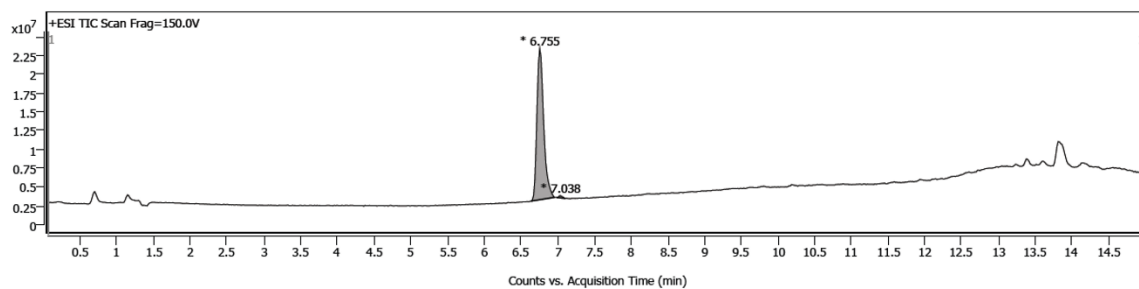
Chromatogram Peaks		
RT	Area	Area Sum %
6.3	4899370	0.51
7.3	953688948	98.98
10.0	2288037	0.24
11.6	2660829	0.28

LCMS chromatogram of **3b**



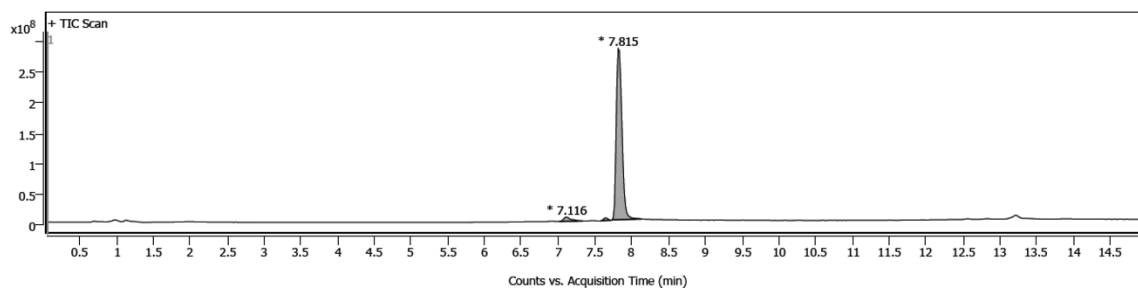
Chromatogram Peaks		
RT	Area	Area Sum %
6.7	589	100.00

LCMS chromatogram of **3c**



Chromatogram Peaks		
RT	Area	Area Sum %
6.8	129033833	99.28
7.0	940597	0.72

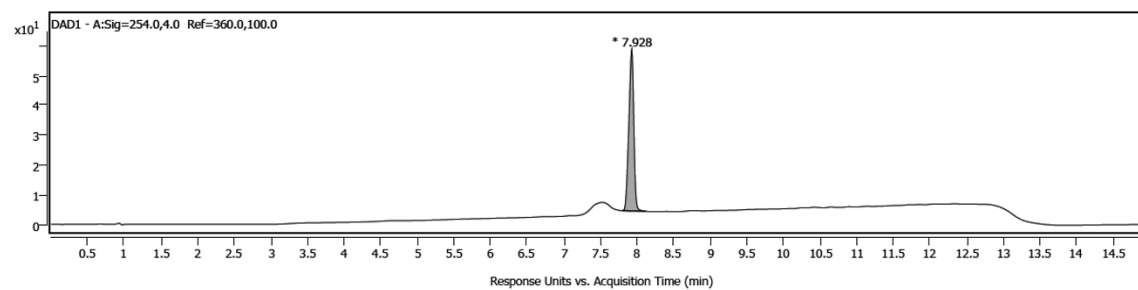
LCMS chromatogram of **3d**



Chromatogram Peaks

RT	Area	Area Sum %
7.1	48823007	3.08
7.6	17323486	1.09
7.8	1518685050	95.83

LCMS chromatogram of **3e**

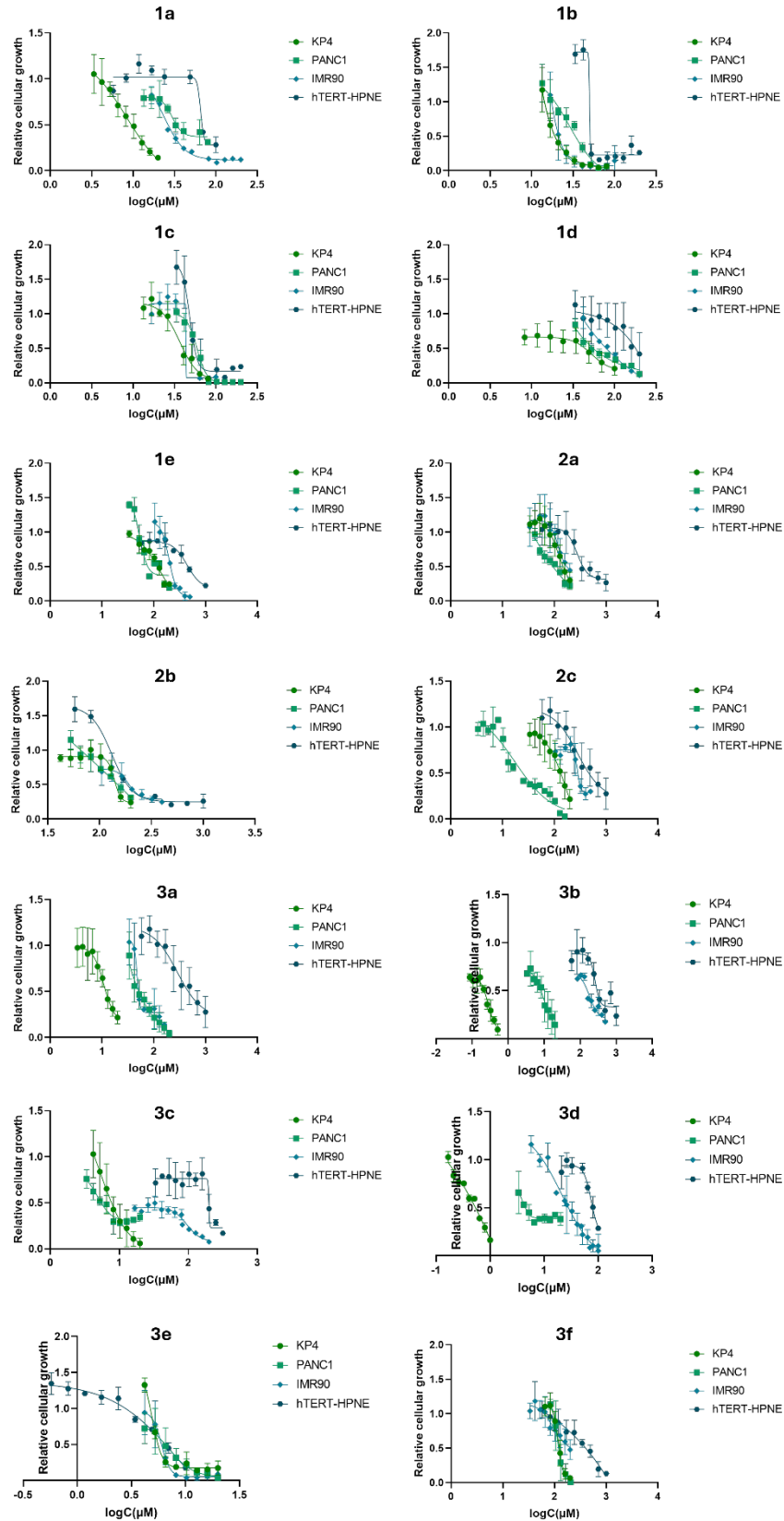


Chromatogram Peaks

RT	Area	Area Sum %
7.9	255	100.00

LCMS chromatogram of **3f**

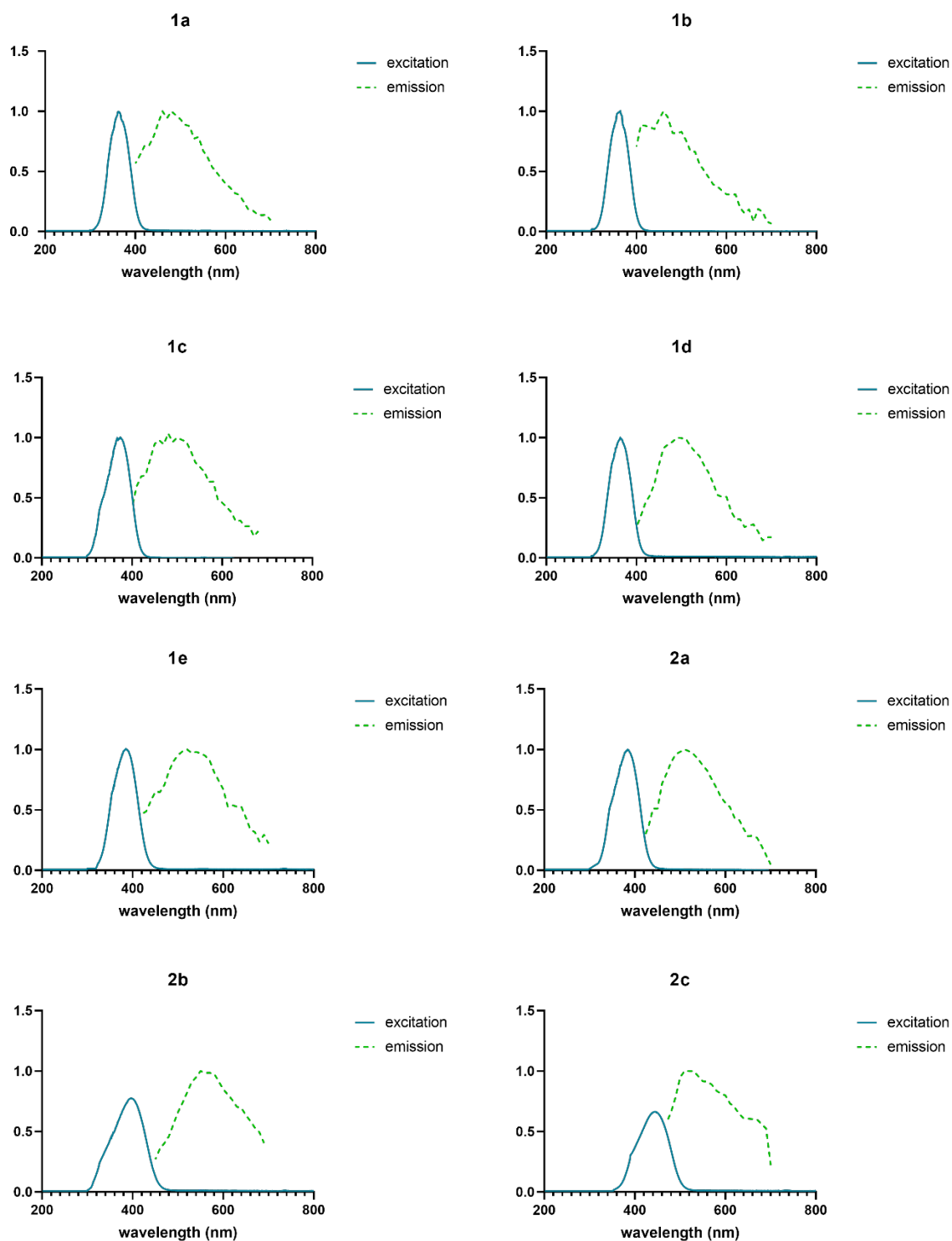
Biological tests and IC₅₀ determination

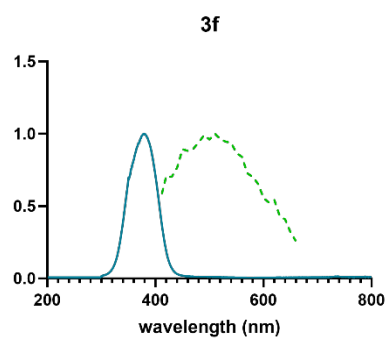
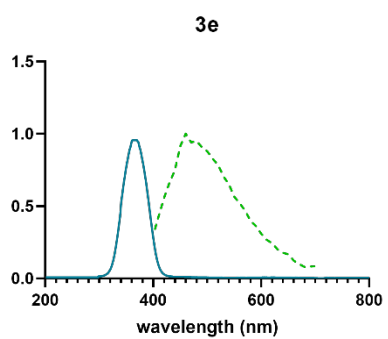
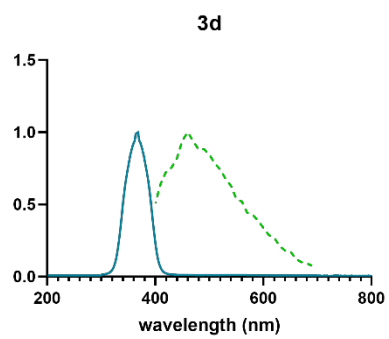
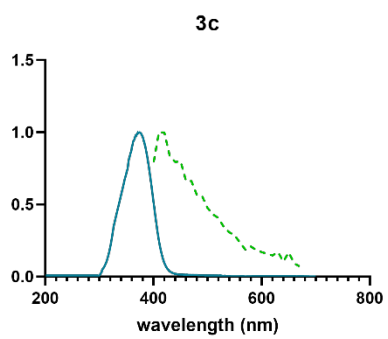
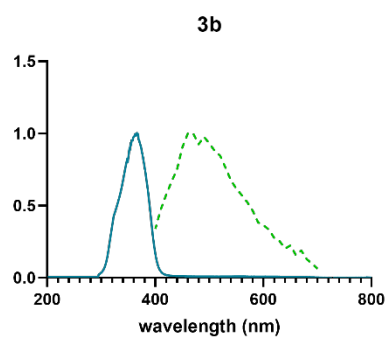
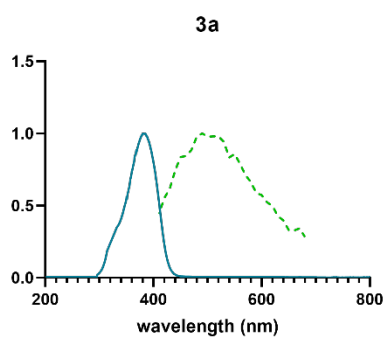


Selectivity indexes on PANC1 cancer cells

Compound	Substituent R	IC ₅₀ cancer cells (μM)		IC ₅₀ healthy cells (μM)		SI IMR90/PANC1	SI hTERT-HPNE/PANC1	clogP*
		KP4	PANC1	IMR90	hTERT-HPNE			
1a	phenyl	9.3 ± 1.4	29.6 ± 2.9	23.6 ± 0.9	62.9 ± 7.5	0.8	2.1	2.24
1b	p-fluorophenyl	9.4 ± 1.3	28.6 ± 4.3	16.3 ± 2.9	50.0 ± 5.4	0.6	1.7	2.83
1c	p-chlorophenyl	36.6 ± 5.5	59.1 ± 4.1	43.5 ± 5.2	48.5 ± 9.7	0.7	0.8	3.29
1d	3,5-dimethylphenyl	29.1 ± 3.5	56.2 ± 3.4	89.1 ± 5.3	140.7 ± 21.1	3.4	6.4	3.57
1e	3,5-bis(trifluoro)methylphenyl	134.0 ± 10.7	58.2 ± 8.4	197.0 ± 27.6	374.9 ± 30.0	1.6	2.5	6.4
2a	2-naphthyl	122.2 ± 21.9	104.7 ± 7.3	119.2 ± 27.4	276.0 ± 55.2	1.1	2.6	4.05
2b	9-phenanthryl	138.6 ± 16.6	151.4 ± 13.6	190.5 ± 24.7	130.6 ± 10.4	1.3	0.9	5.83
2c	1-pyrenyl	163.2 ± 24.5	240.3 ± 28.3	280.9 ± 25.3	284.3 ± 54	1.2	1.2	6.76
3a	2-thiophenyl	10.8 ± 1.7	50.1 ± 6.0	47.9 ± 7.7	120.8 ± 20.5	1	2.4	2.26
3b	2-imidazole	0.28 ± 0.02	9.7 ± 1.3	180.1 ± 10.8	257.9 ± 33.5	18.6	26.6	-0.63
3c	2-pyridinyl	5.4 ± 1.0	4.8 ± 0.3	95.0 ± 4.6	198.8 ± 23.8	19.8	41.4	0.78
3d	2-pyrole	0.52 ± 0.03	4.1 ± 0.3	21.6 ± 2.6	74.3 ± 7.4	5.3	18.1	0.62
3e	2-indole	4.9 ± 0.4	7.01 ± 1.2	5.9 ± 1.0	8.8 ± 0.8	0.8	1.3	2.47
3f	5-pyrimidine	118.9 ± 14.3	113.5 ± 22.7	95.6 ± 17.2	398.1 ± 55.7	0.8	3.5	-0.42

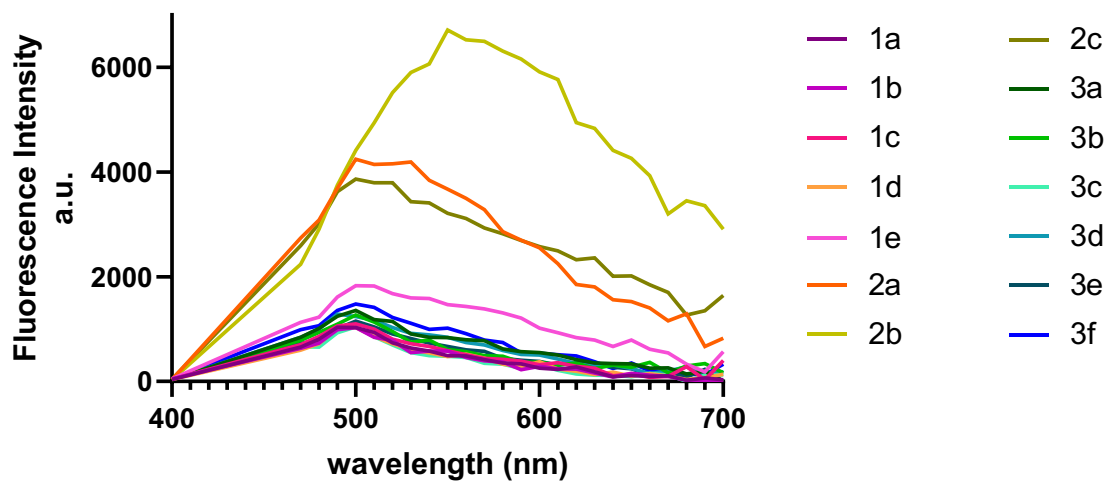
Excitation and emission spectra of diiminoguanidines





Compound	λ excitation (nm)	λ emission (nm)
1a	360	460
1b	360	460
1c	375	480
1d	365	490
1e	385	520
2a	385	510
2b	395	550
2c	445	530
3a	380	490
3b	365	475
3c	375	415
3d	365	460
3e	365	460
3f	380	510

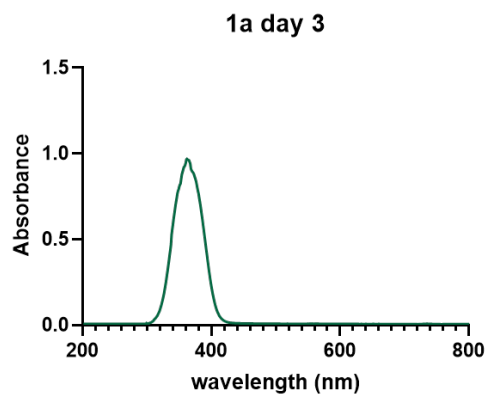
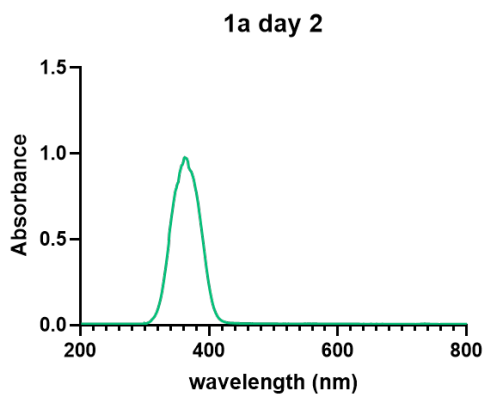
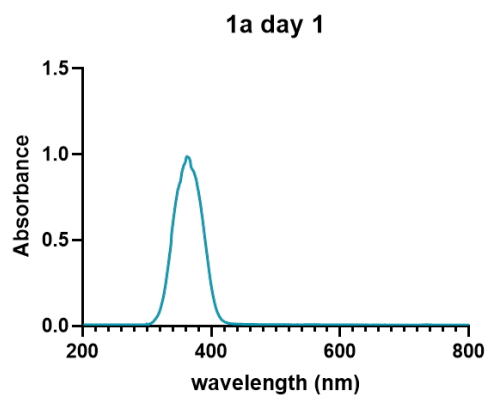
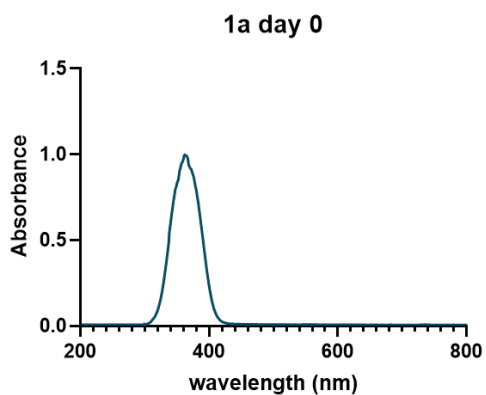
To ensure that the fluorescence diiminoguanidines can be visualised with a fluorescence microscopy GFP filter ($\lambda_{\text{exc}} = 350 \text{ nm}$, $\lambda_{\text{em}} = 500 \text{ nm}$), their fluorescence spectra were recorded with $\lambda_{\text{exc}} = 350 \text{ nm}$. All diiminoguanidines had a fluorescence maximum of intensity in the range of 500 – 550 nm.



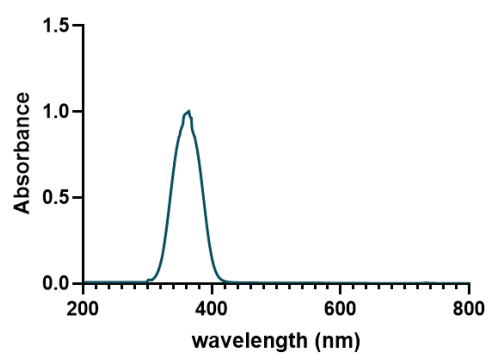
Fluorescence spectra of diiminoguanidines 1a-3f ($\lambda_{\text{exc}} = 450 \text{ nm}$)

Stability assays

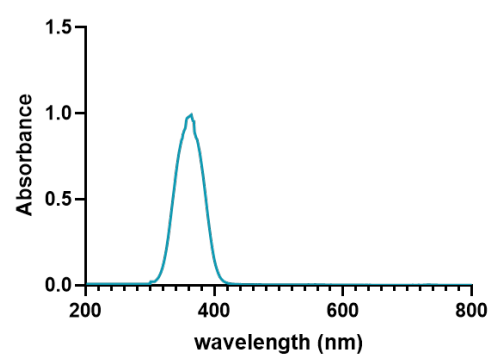
Stability was confirmed by UV-Vis. Compounds were stirred in PBS + 4% DMSO at 37°C for 3 days, and spectra were recorded everyday.



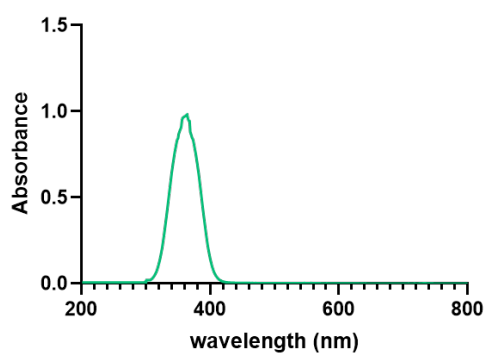
1b day 0



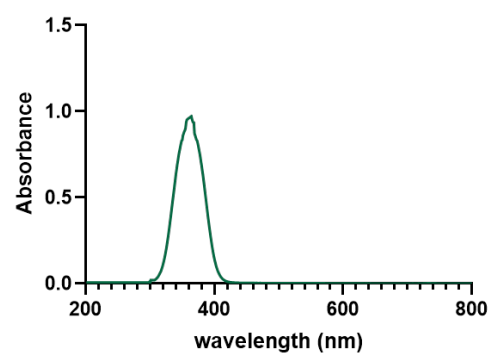
1b day 1

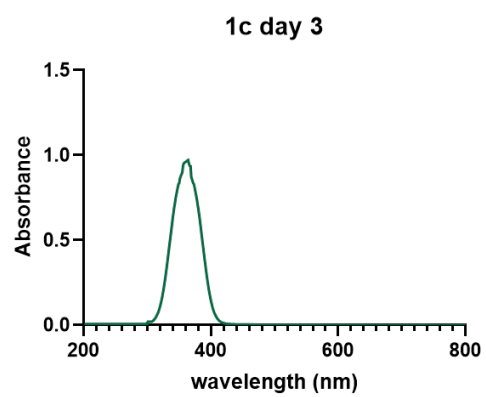
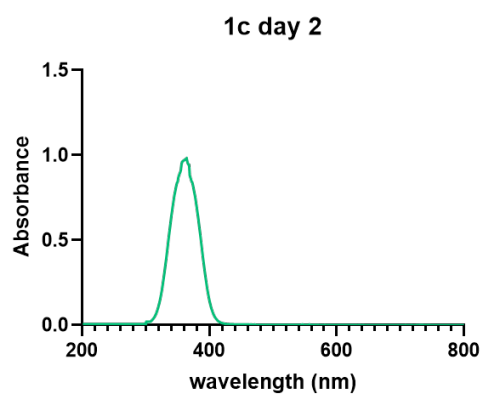
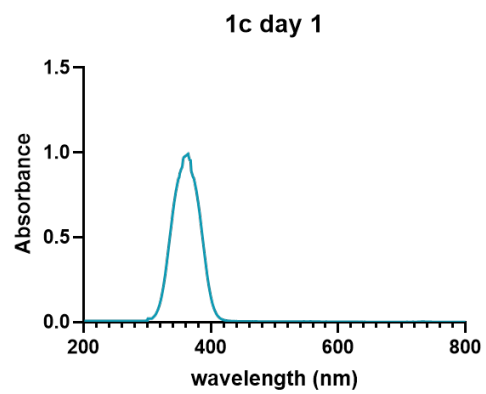
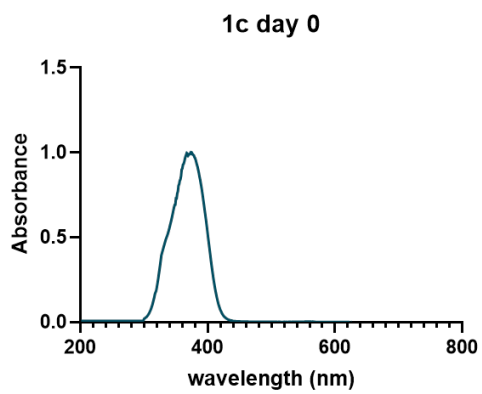


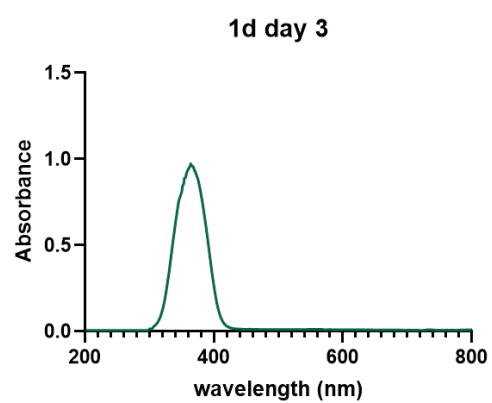
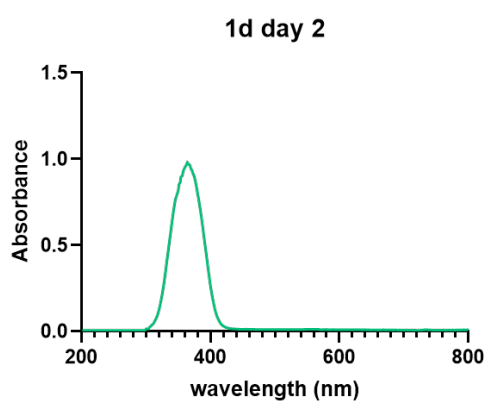
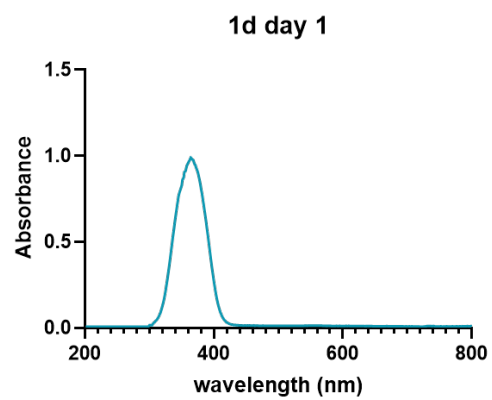
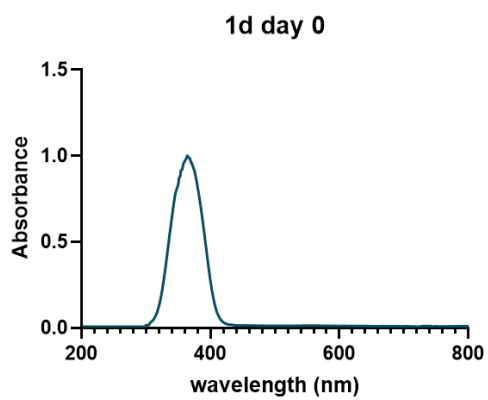
1b day 2

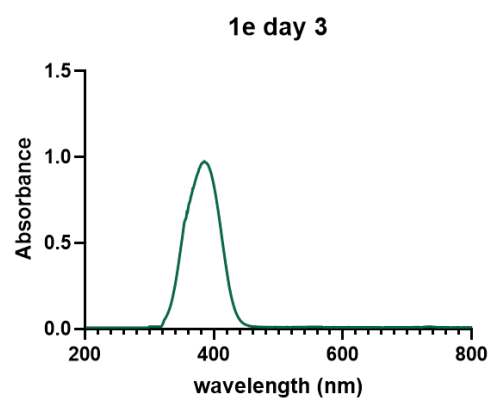
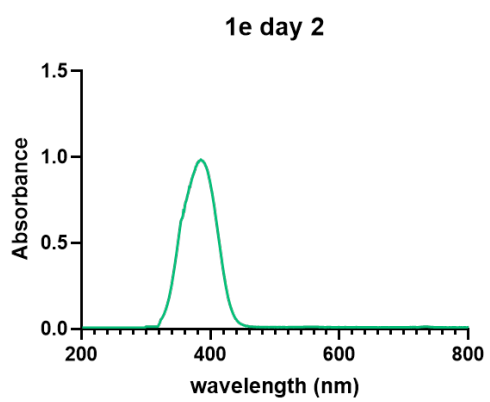
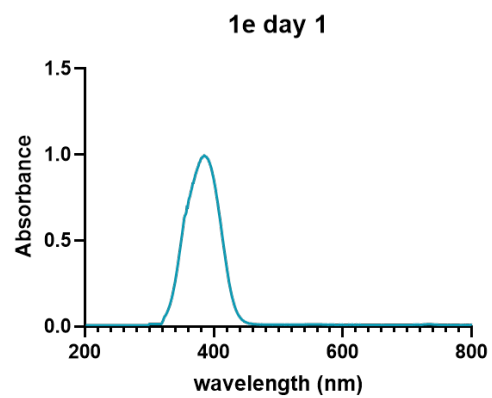
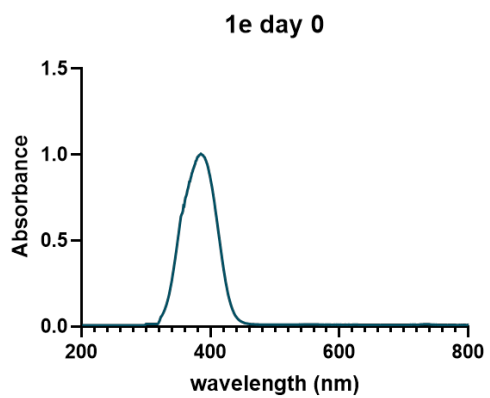


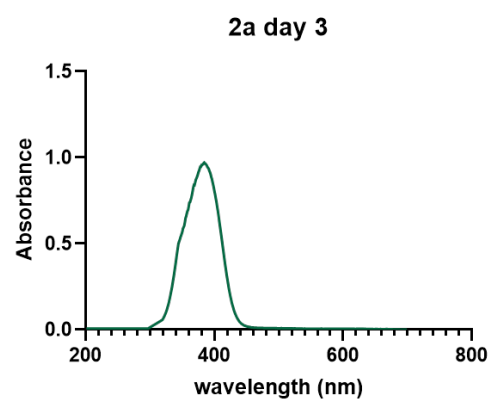
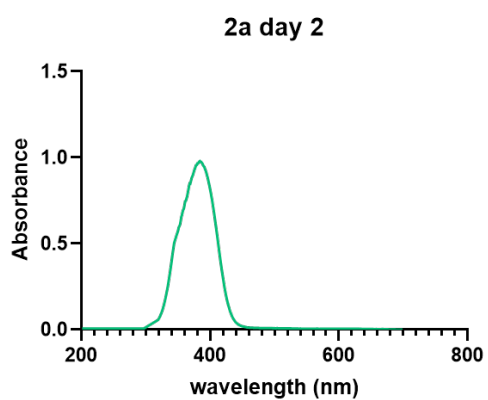
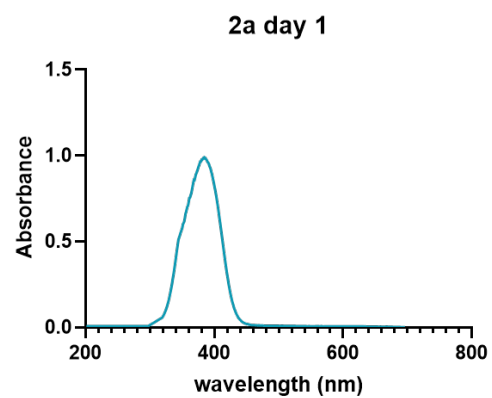
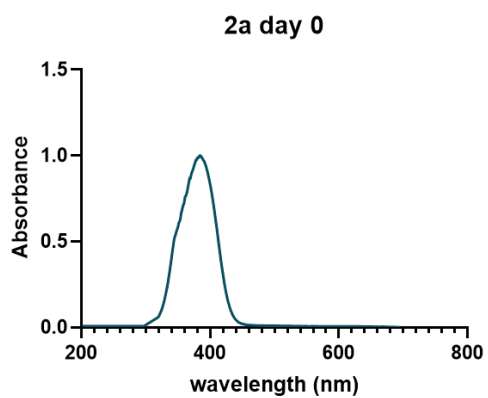
1b day 3

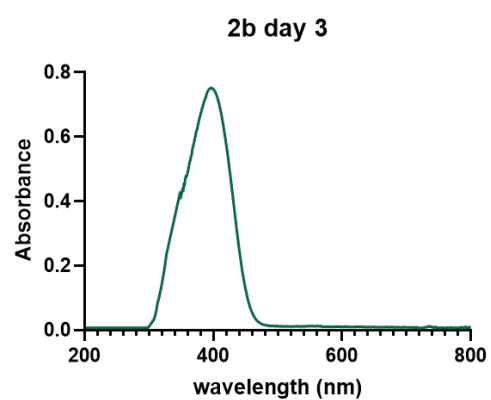
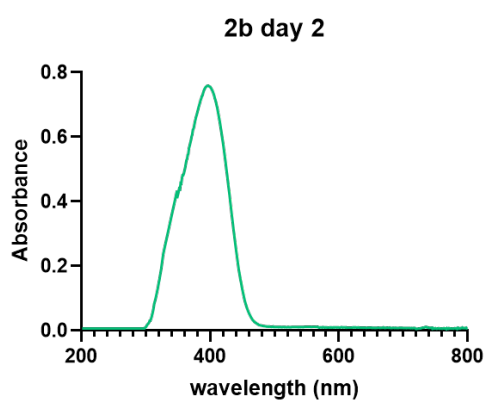
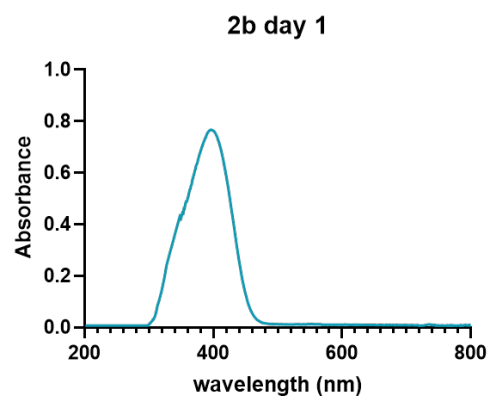
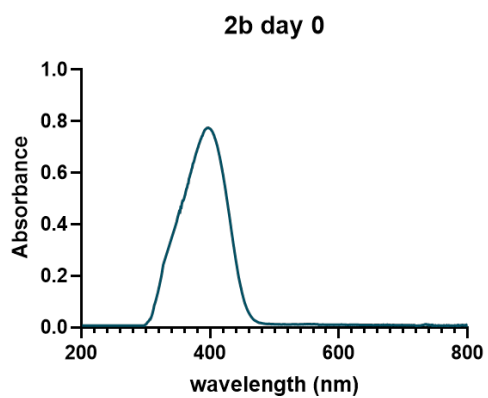


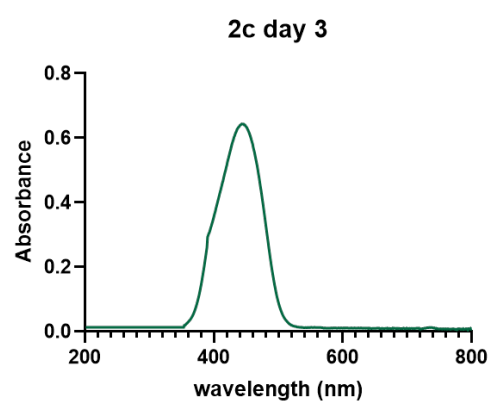
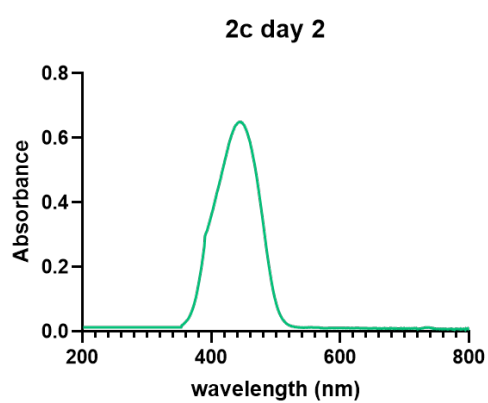
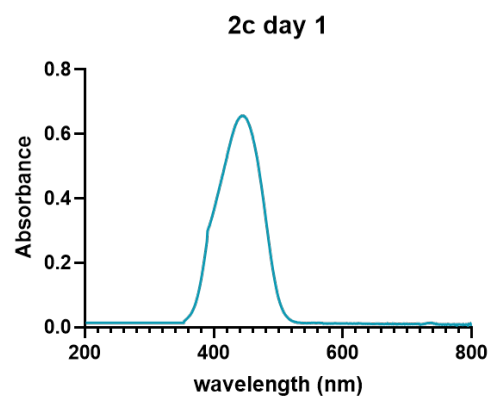
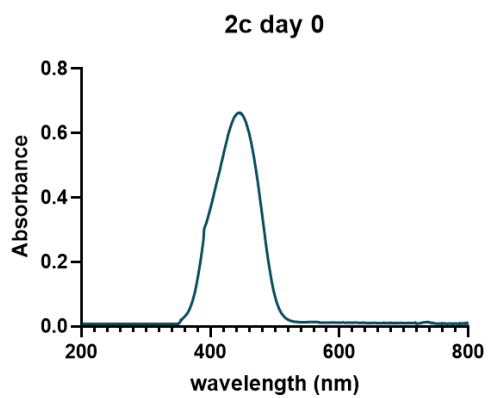


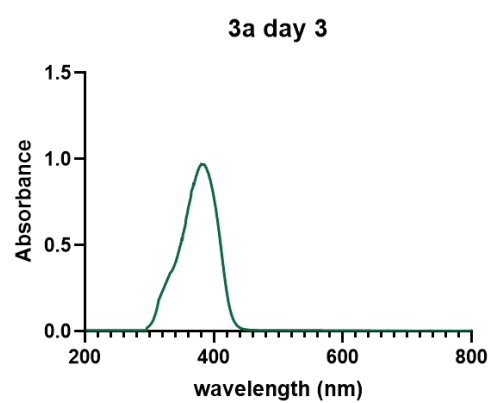
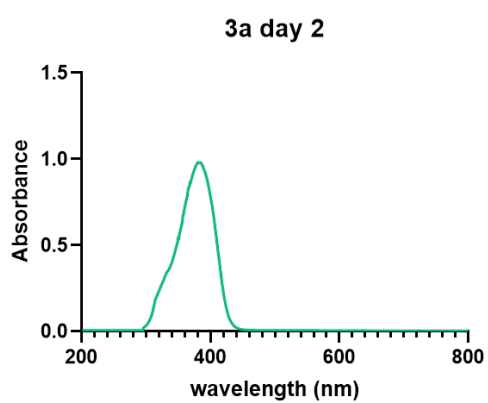
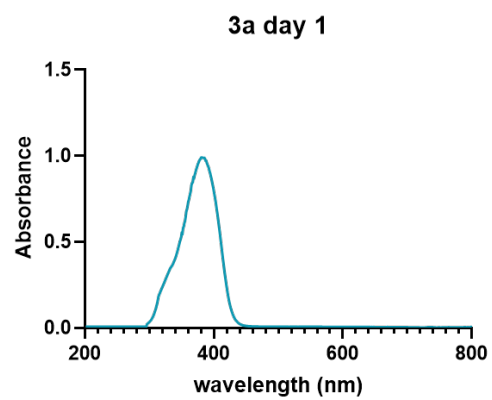
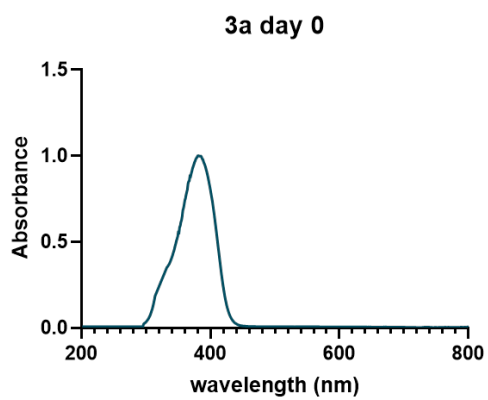


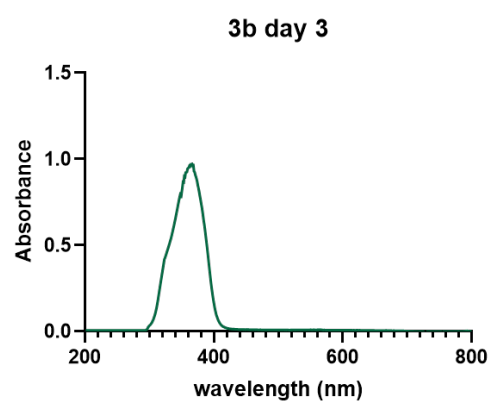
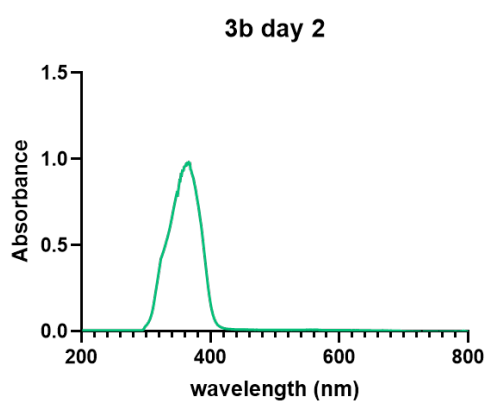
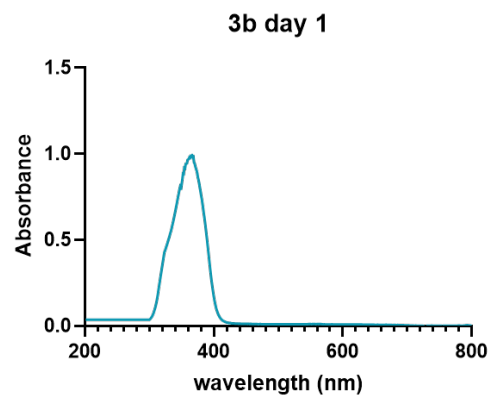
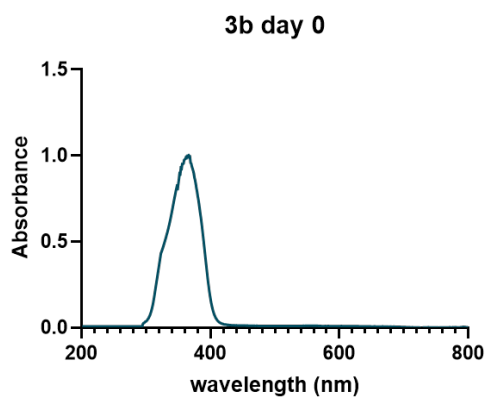


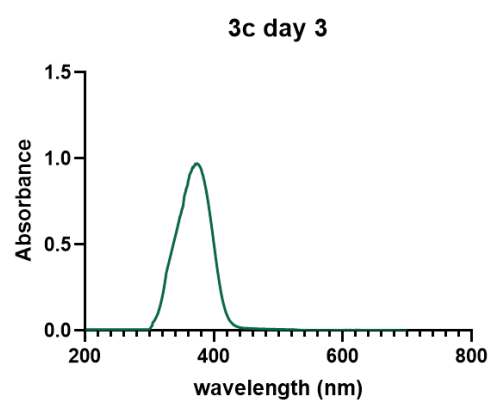
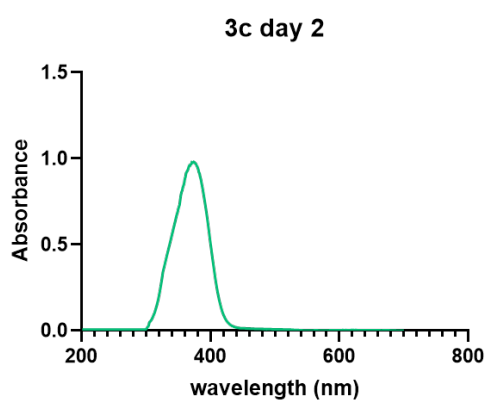
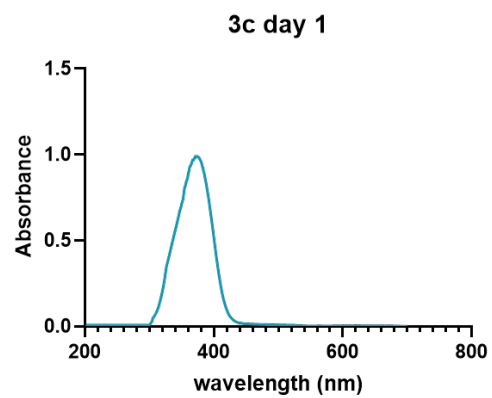
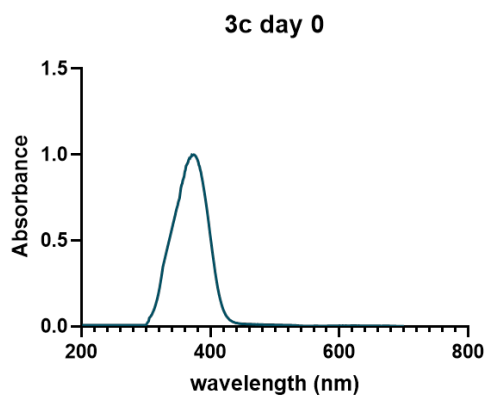


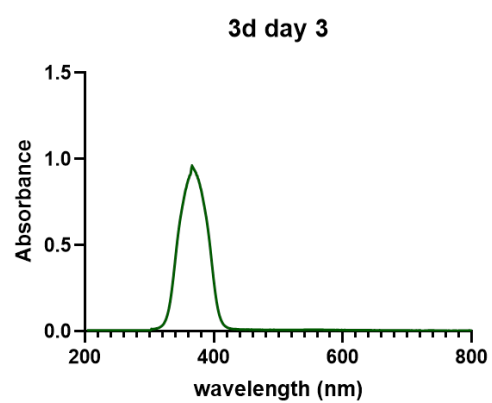
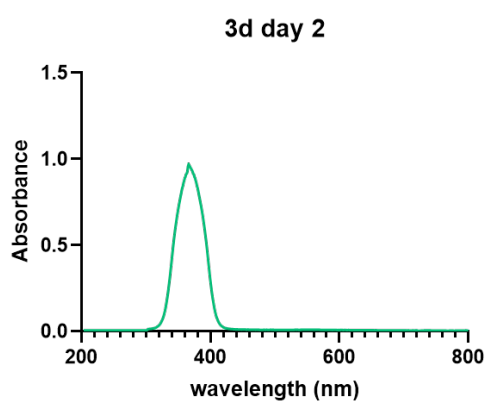
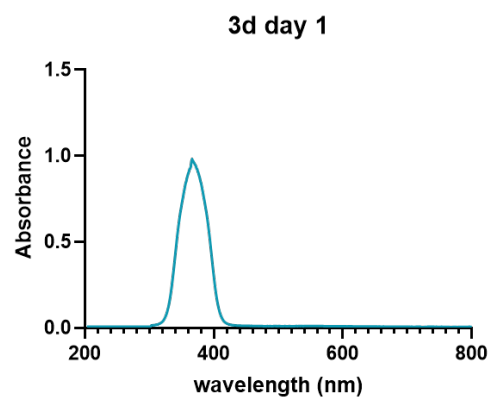
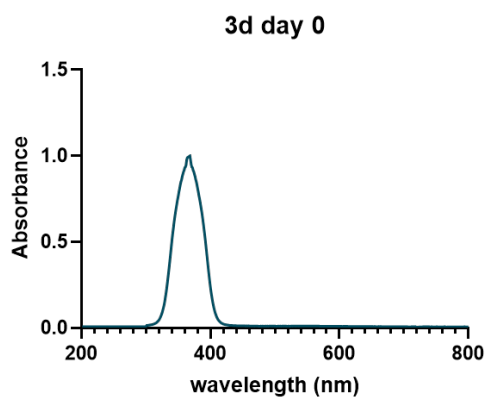


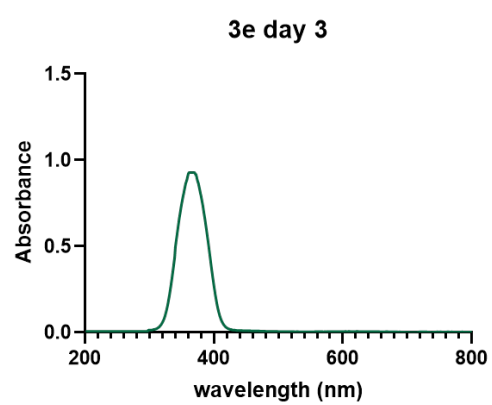
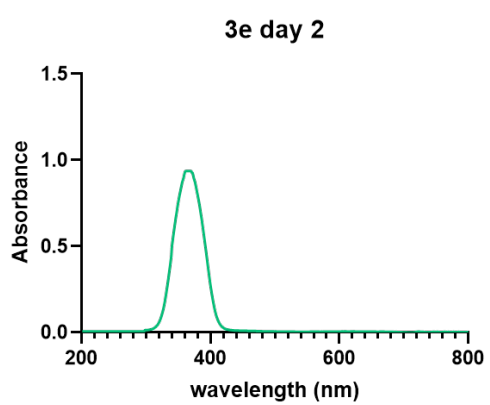
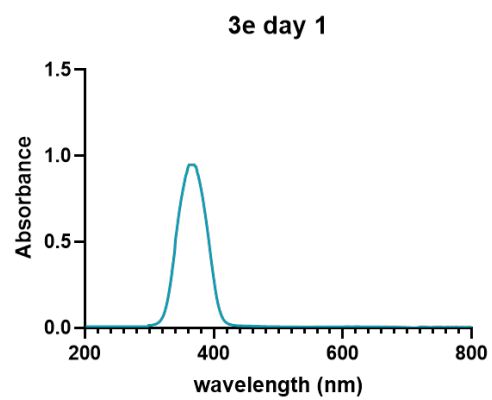
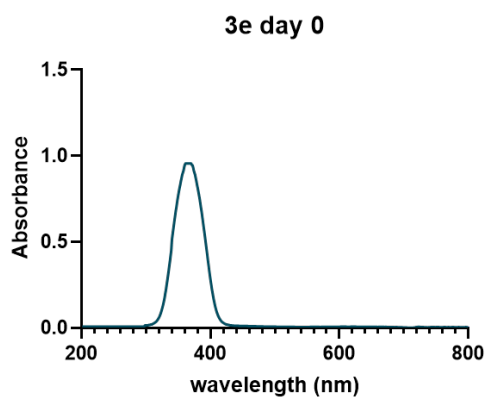


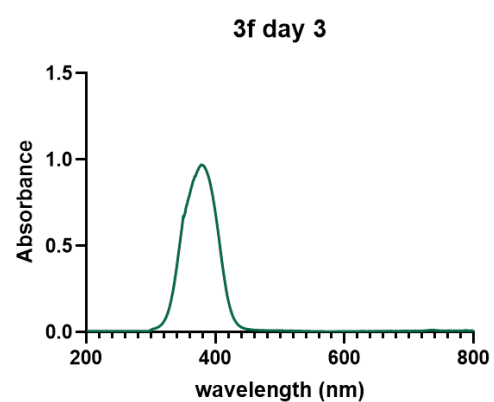
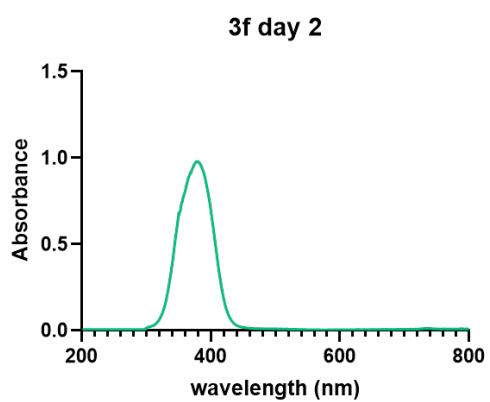
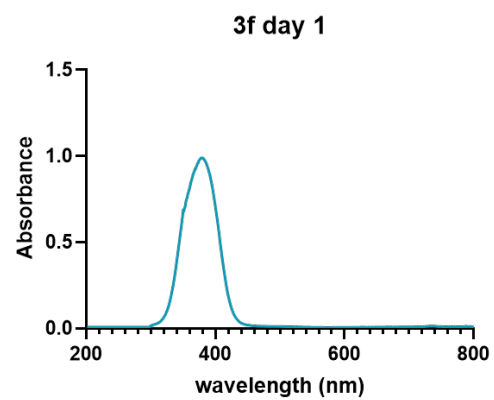
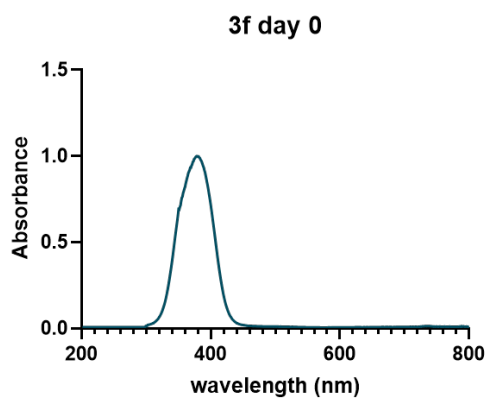




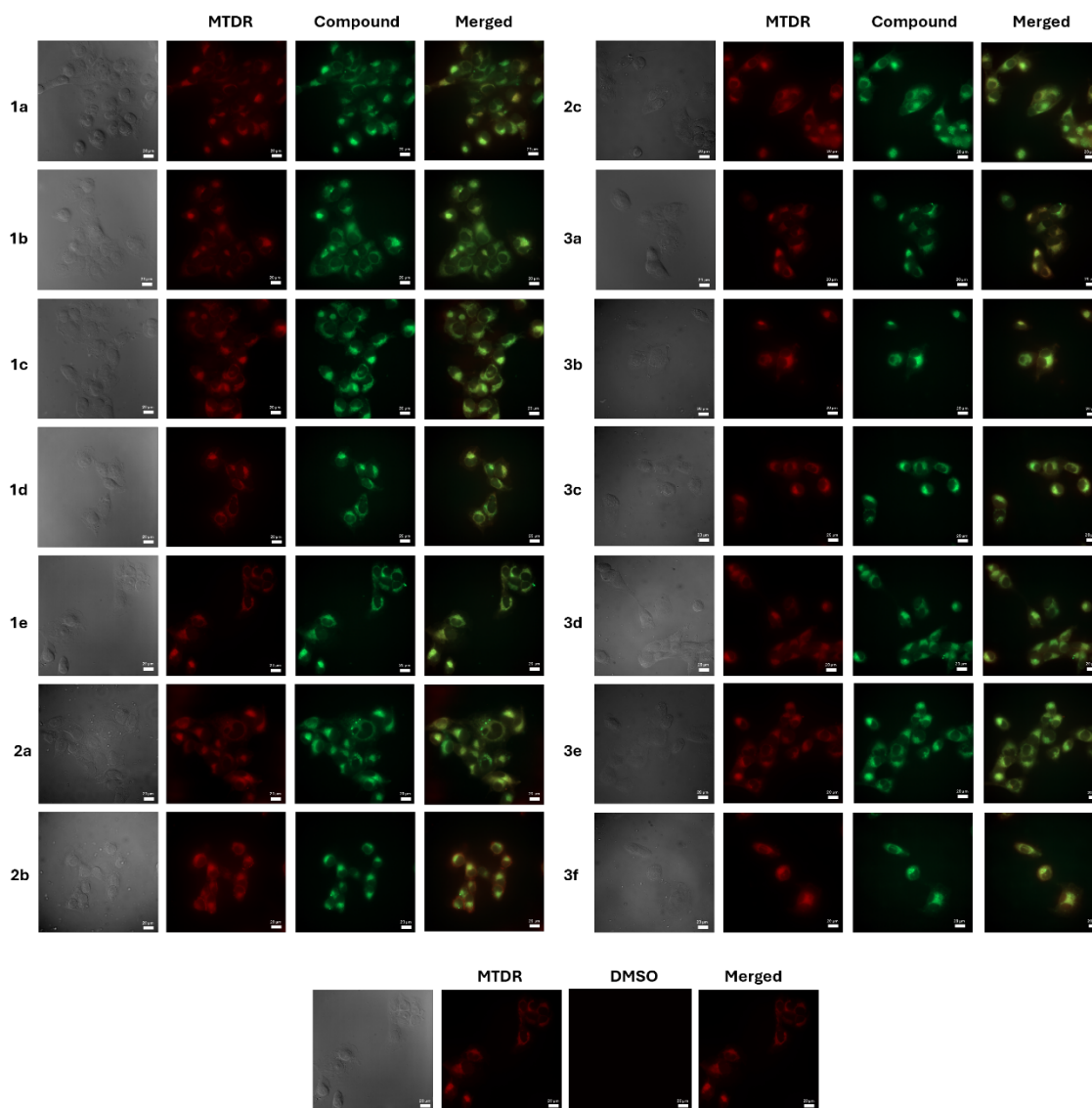








Confocal microscopy images



1a $r_P = 0.92$; **1b** $r_P = 0.97$; **1c** $r_P = 0.94$; **1d** $r_P = 0.92$; **1e** $r_P = 0.95$; **2a** $r_P = 0.89$; **2b** $r_P = 0.89$; **2c** $r_P = 0.94$; **3a** $r_P = 0.93$; **3b** $r_P = 0.93$; **3c** $r_P = 0.92$; **3d** $r_P = 0.92$; **3e** $r_P = 0.96$; **3f** $r_P = 0.90$). Scale: 0.2cm=20 μ m.