

Supporting Information

Synthesis and evaluation of biological activities of Bis(spiropyrazolone)cyclopropanes: a potential application against leishmaniasis.

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- † These authors contributed equally to this work.

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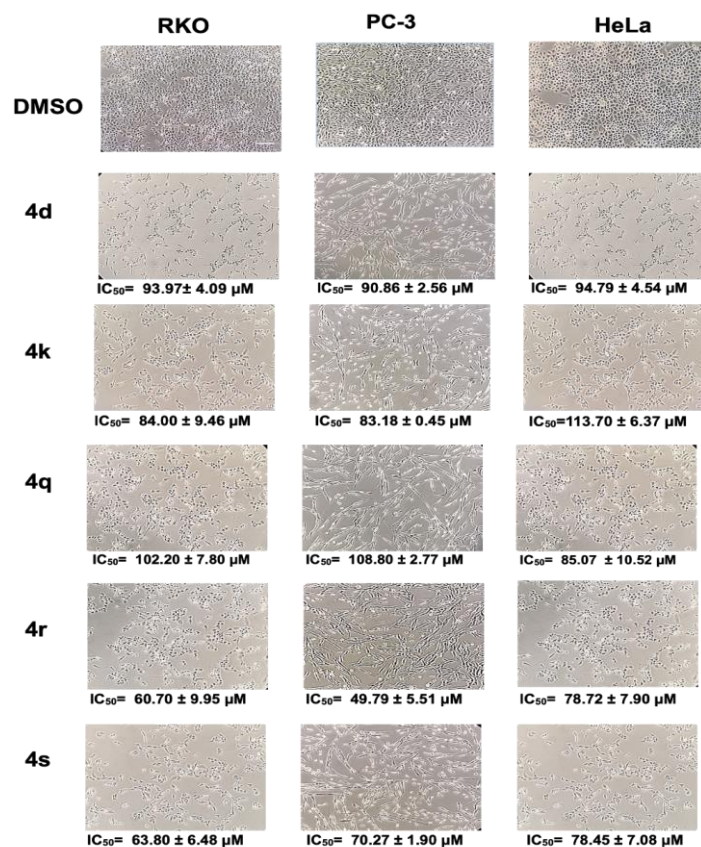


Figure S1. Cell morphology of RKO, PC-3 and HeLa cell lines exposed to the Inhibitory concentration (IC₅₀) of the most active bis (spiropyrazolone) cyclopropanes for 48 hours. Data are given as mean and standard error (SEM) of at least three independent experiments. Horizontal bar in the figure below = 50 μm.

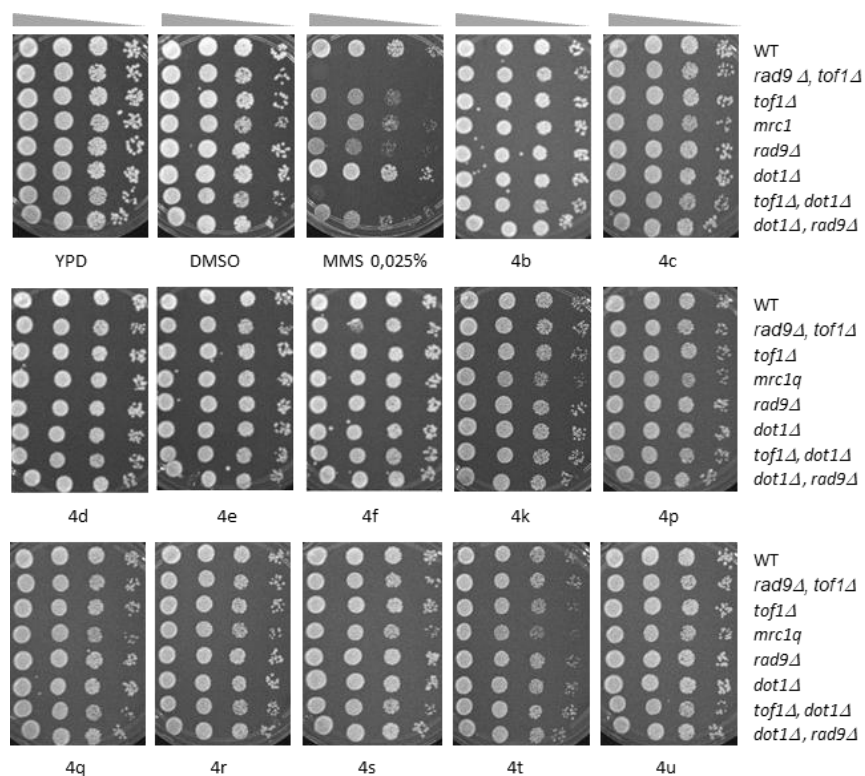
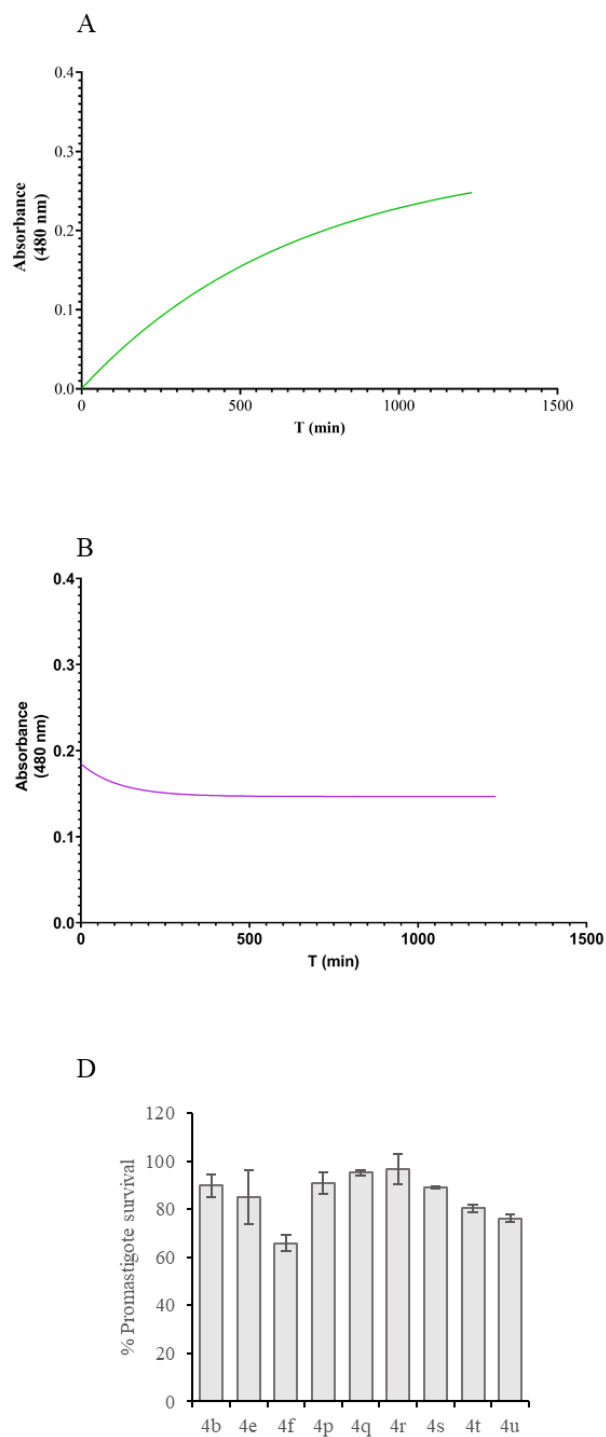


Figure S2. Drop test for *S. cerevisiae* strains in presence of bis(spiropyrazolone)cyclopropanes 4. Each line of serial-diluted cells dots corresponds with the strain indicated at the left of the panel. The compound evaluated in each panel is indicated below.



4b in DMSO	
One phase exponential decay	
Best-fit values	
Span	-0.2963
K	0.001472
Plateau	0.2965
HalfLife	471.0
Tau	679.5
95% CI (profile likelihood)	
Span	-0.3016 to -0.2914
K	0.001406 to 0.001538
Plateau	0.2906 to 0.3027
HalfLife	450.8 to 493.0
Tau	650.3 to 711.3
Goodness of Fit	
Degrees of Freedom	39
R squared	0.9991
Sum of Squares	0.0001981
Sy.x	0.002254
Constraints	
K	K > 0
Number of points	

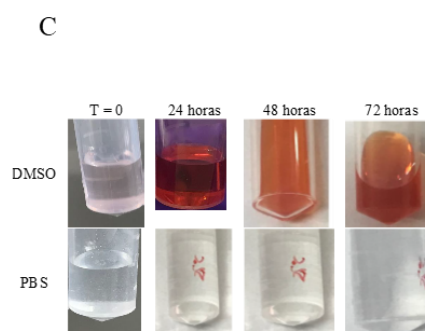


Figure S3. Stability of bis(spiropyrazolone)cyclopropanes **4**. (A) Absorbance spectra at 480 nm of **4b** (100 μ M) in DMSO at different time of incubation at 25 $^{\circ}$ C. (B) Absorbance spectra at 480 nm of **4b** (100 μ M) in PBS 1X pH 7.4 at different time of incubation at 25 $^{\circ}$ C. (C) Stability of **4b** (2300 μ M) in DMSO and PBS 1X pH 7.4 at different time of incubation at 25 $^{\circ}$ C. (D) Inhibitory activity of products of thermal isomerization of bis(spiropyrazolone)cyclopropanes **4** against *L. mexicana* promastigotes at 10 μ M.

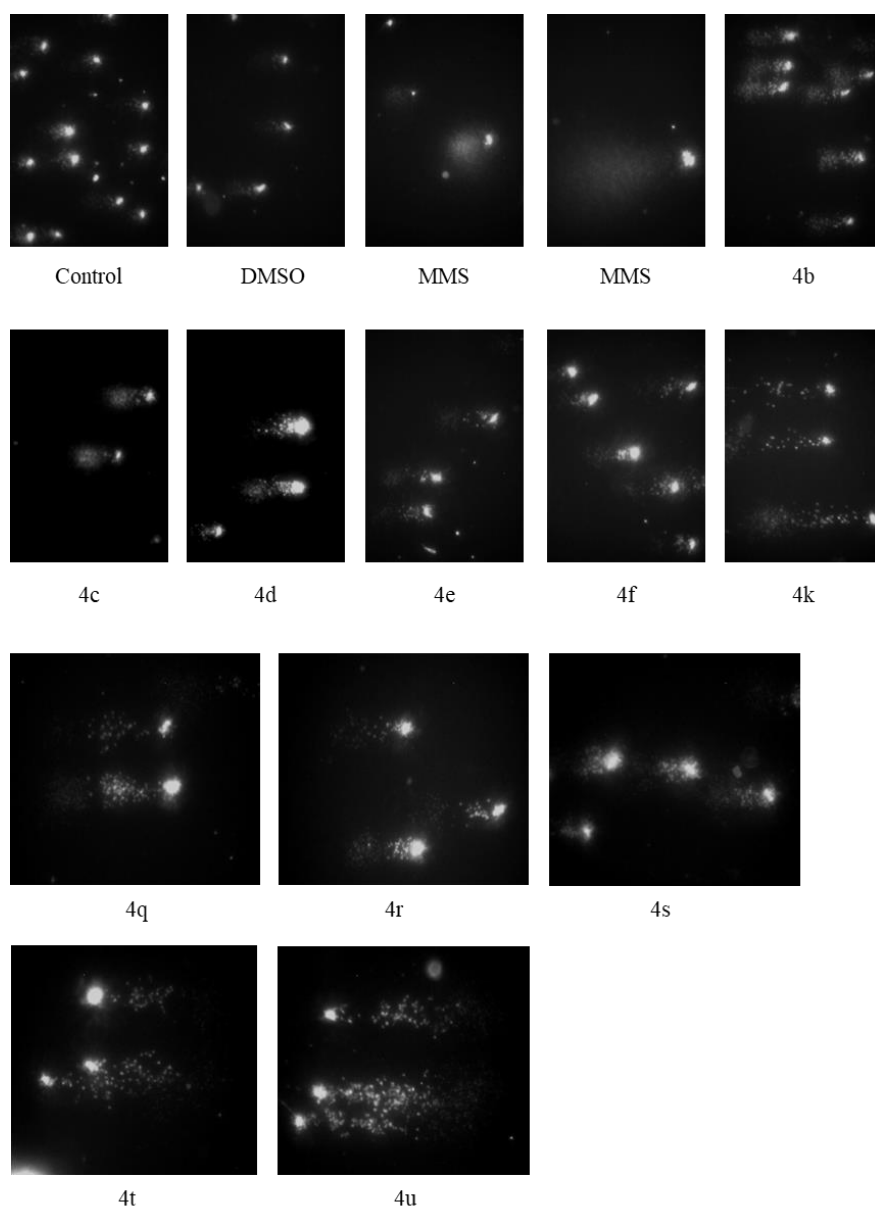
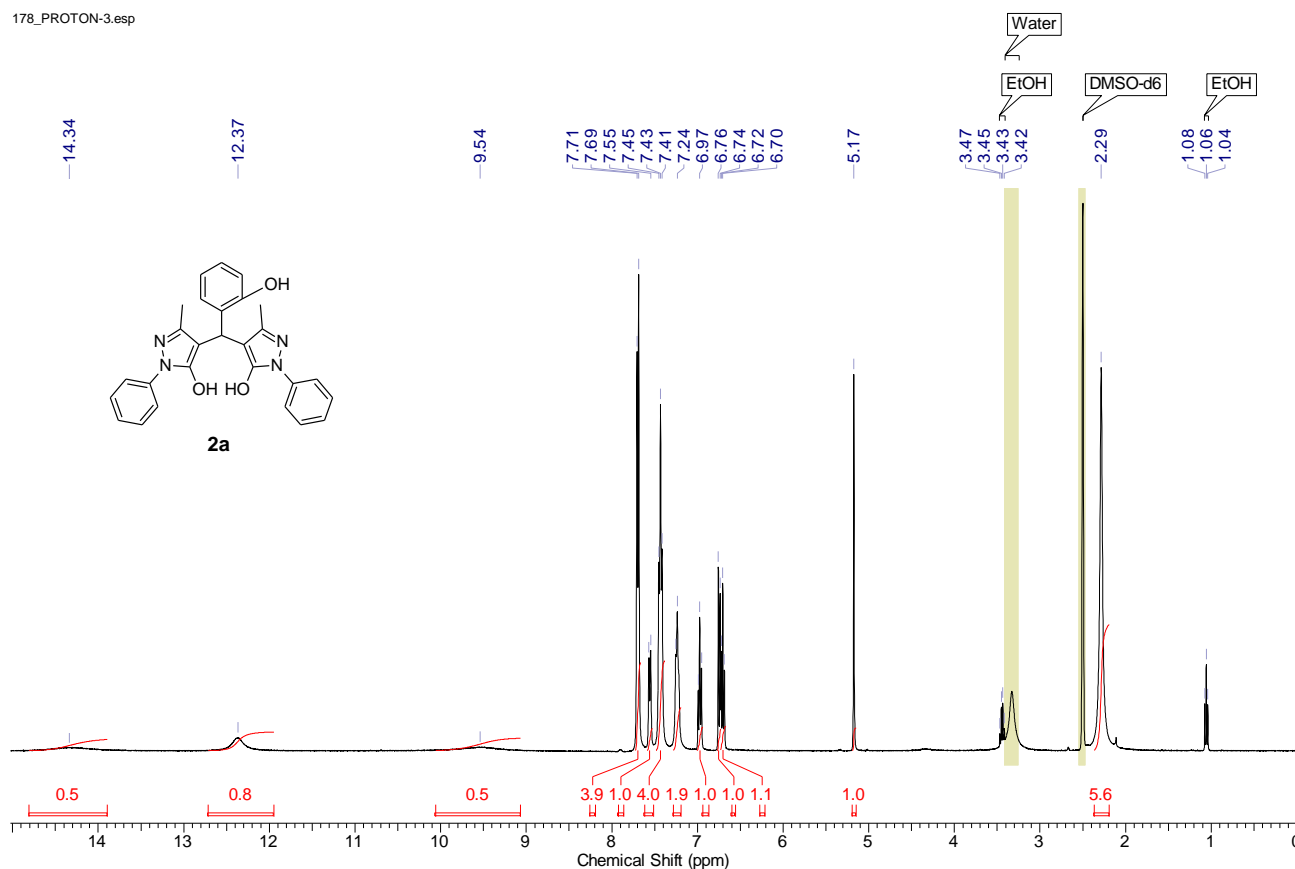
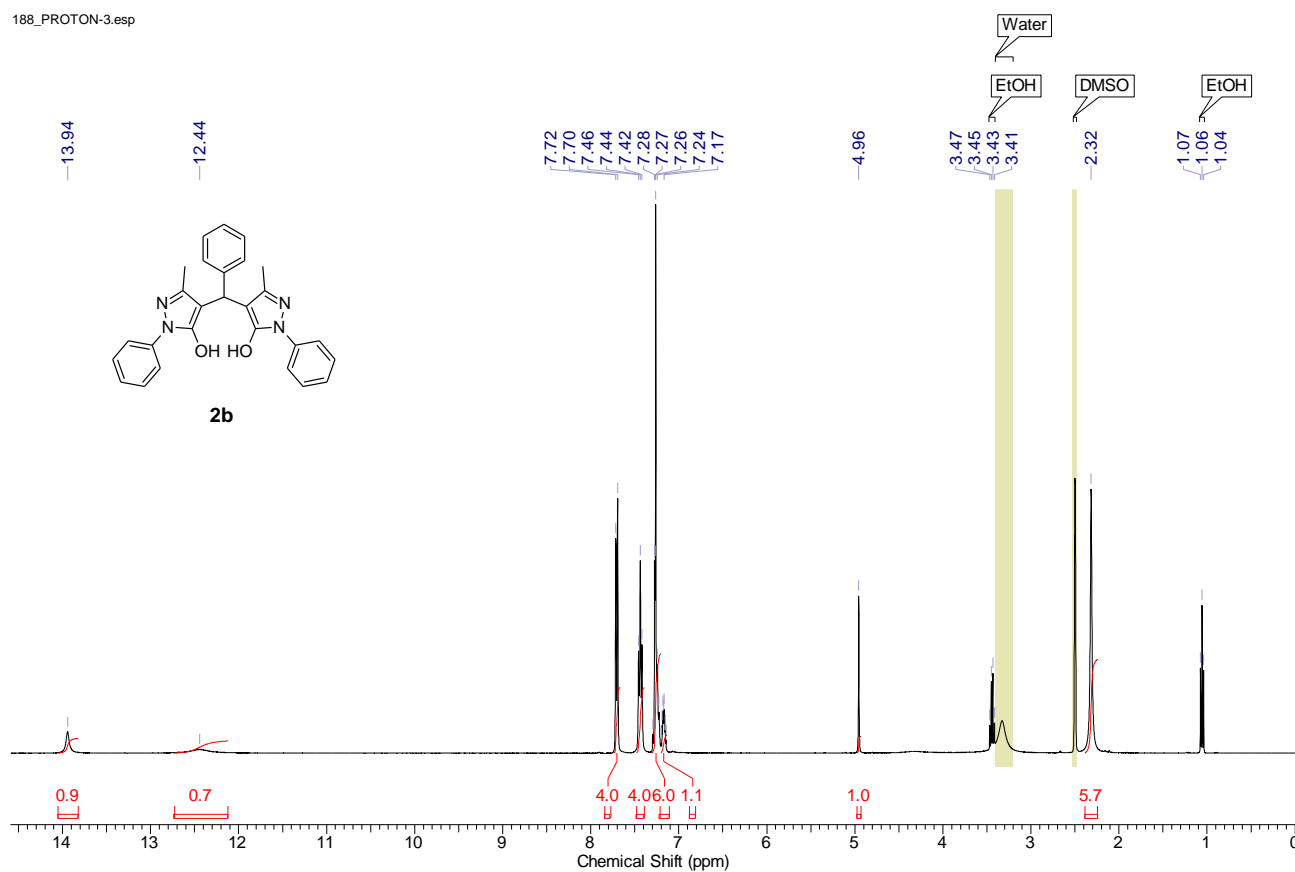
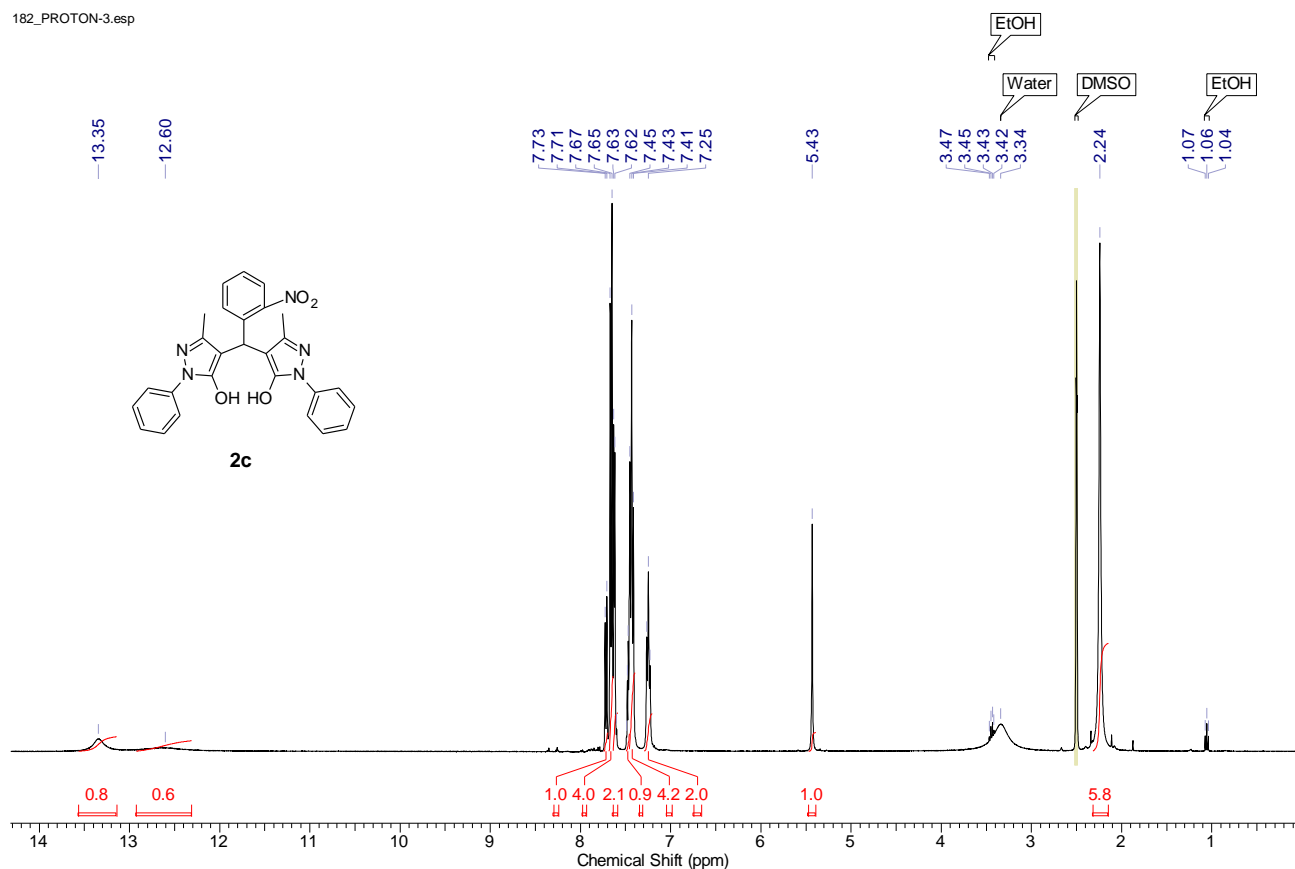
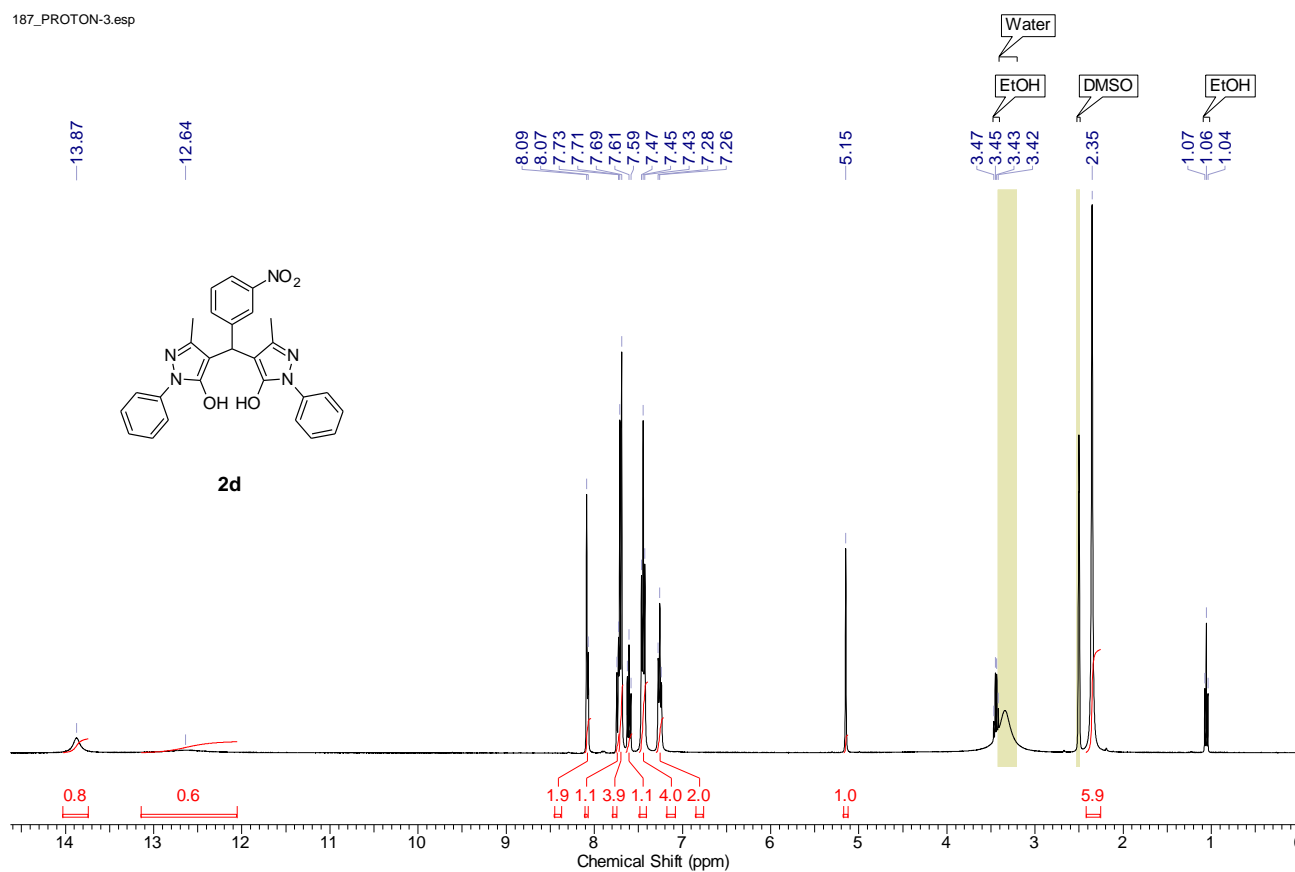


Figure S4. Representative examples of *Leishmania mexicana* cells exposed to bis(spiropyrazolone)cyclopropanes **4** and subjected to comet assay. C, control unexposed cells; DMSO, dimethyl sulfoxide; MMS, methyl methanesulfonate.

Figure S5. ¹H NMR spectrum of compound 2aFigure S6. ¹H NMR spectrum of compound 2b

Figure S7. ¹H NMR spectrum of compound **2c**Figure S8. ¹H NMR spectrum of compound **2d**

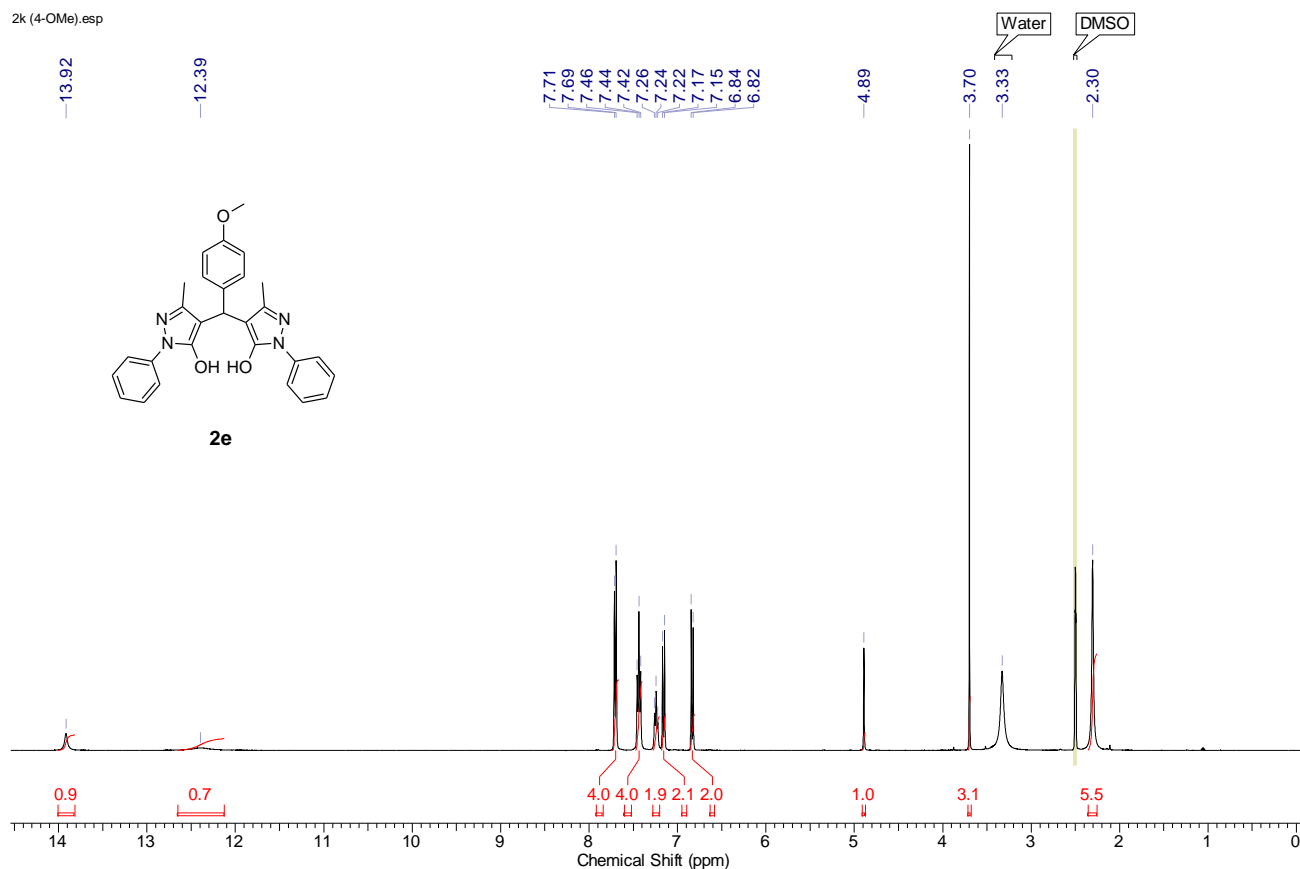


Figure S9. ^1H NMR spectrum of compound **2e**

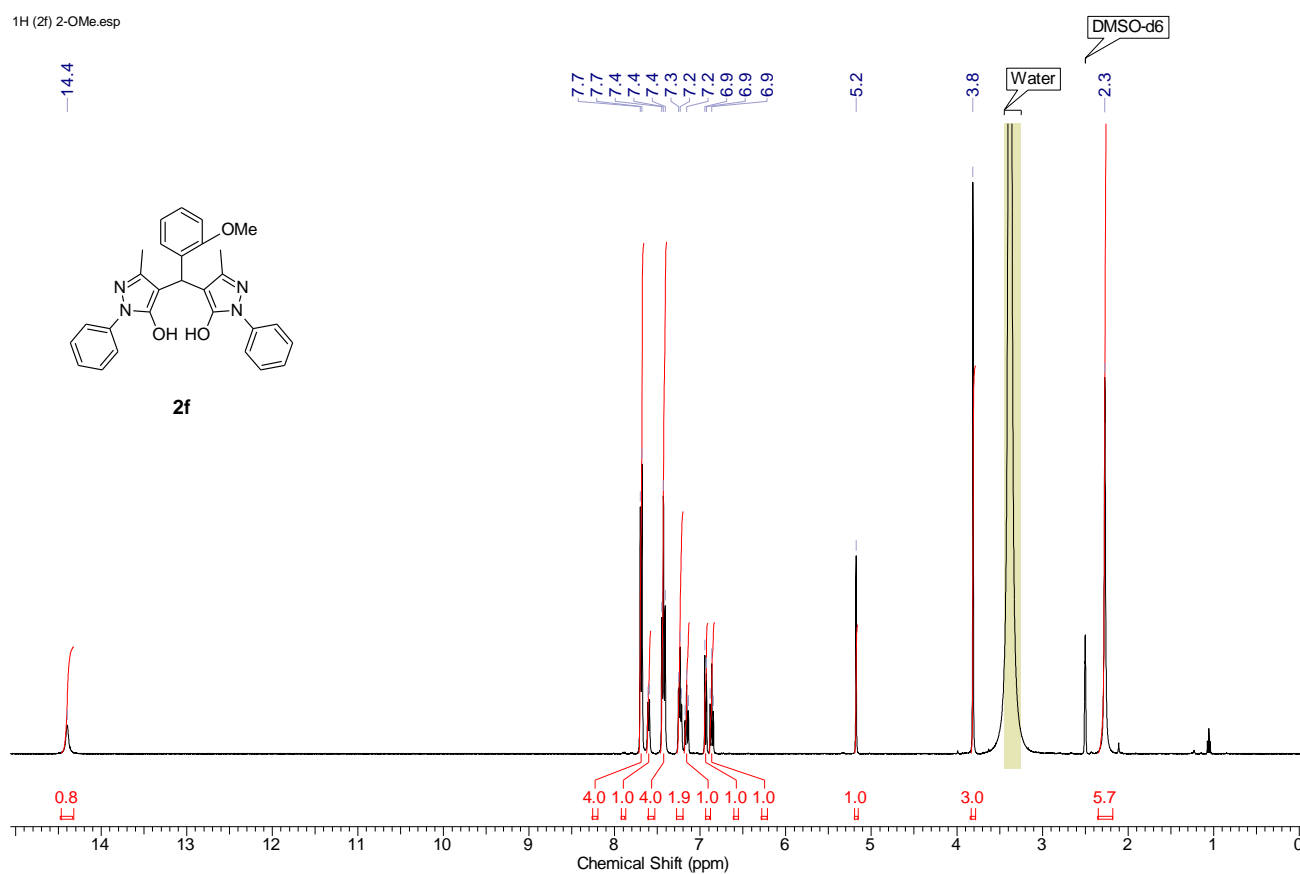


Figure S10. ^1H NMR spectrum of compound **2f**

181_PROTON-3.esp

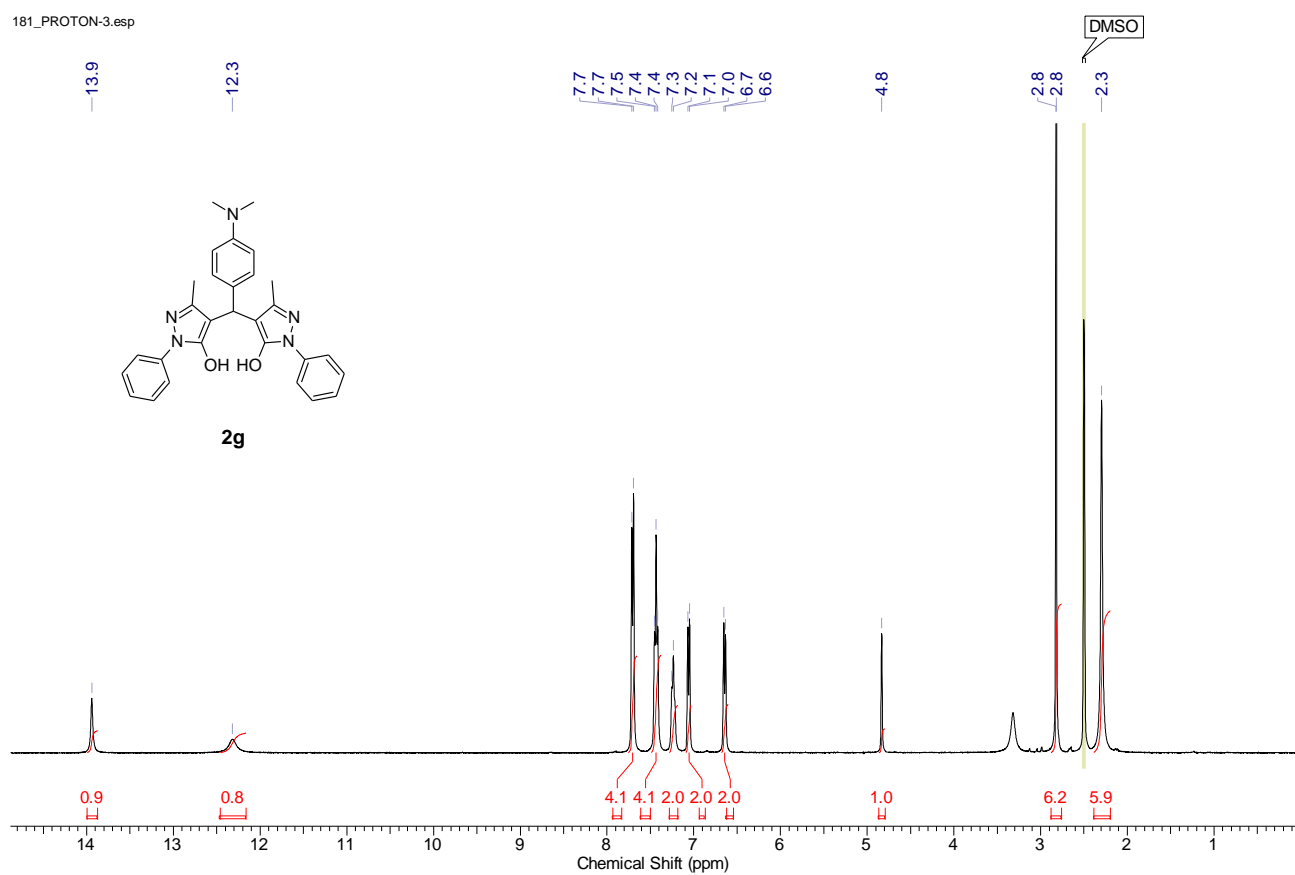


Figure S11. ¹H NMR spectrum of compound **2g**

JH-203-33 (1H).esp

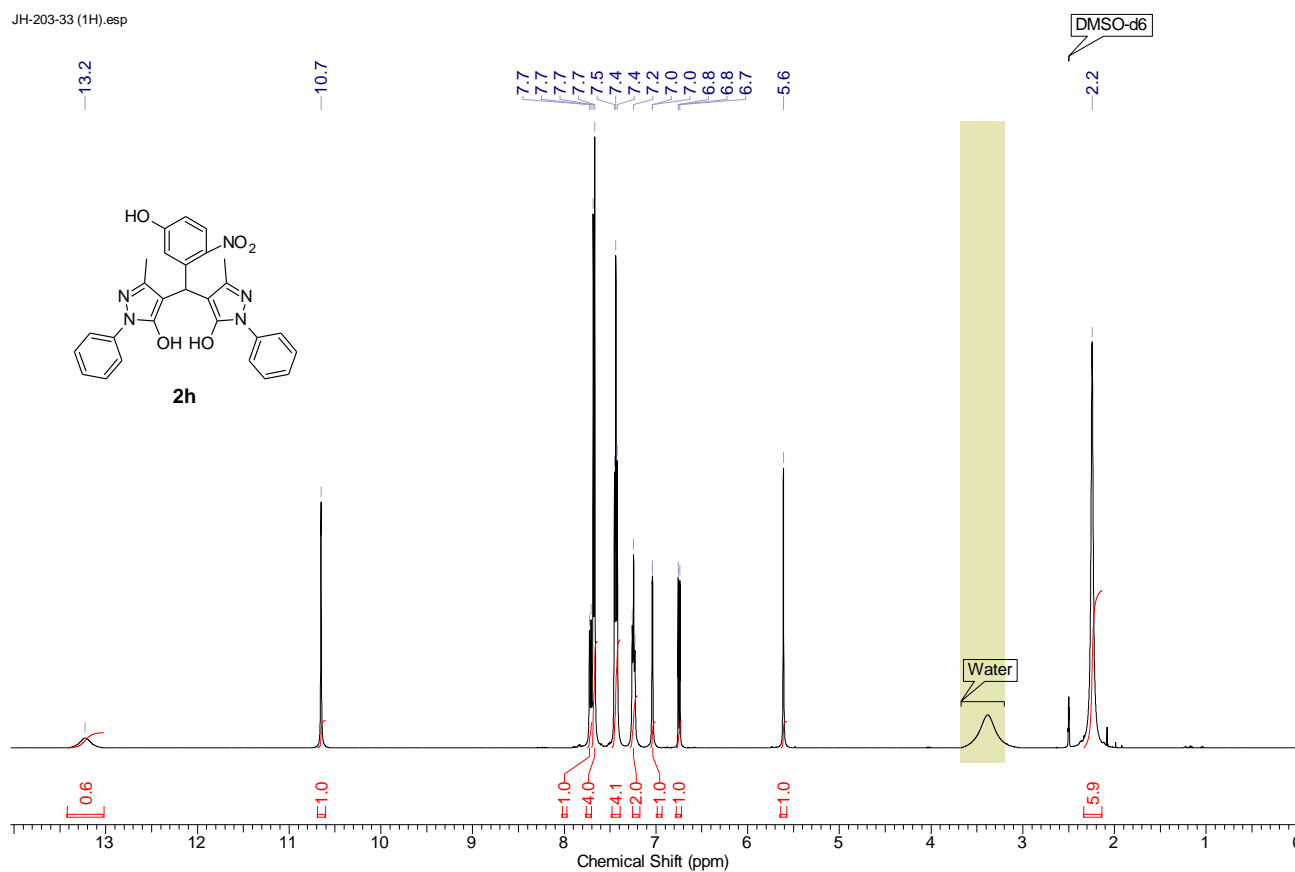


Figure S12. ¹H NMR spectrum of compound **2h**

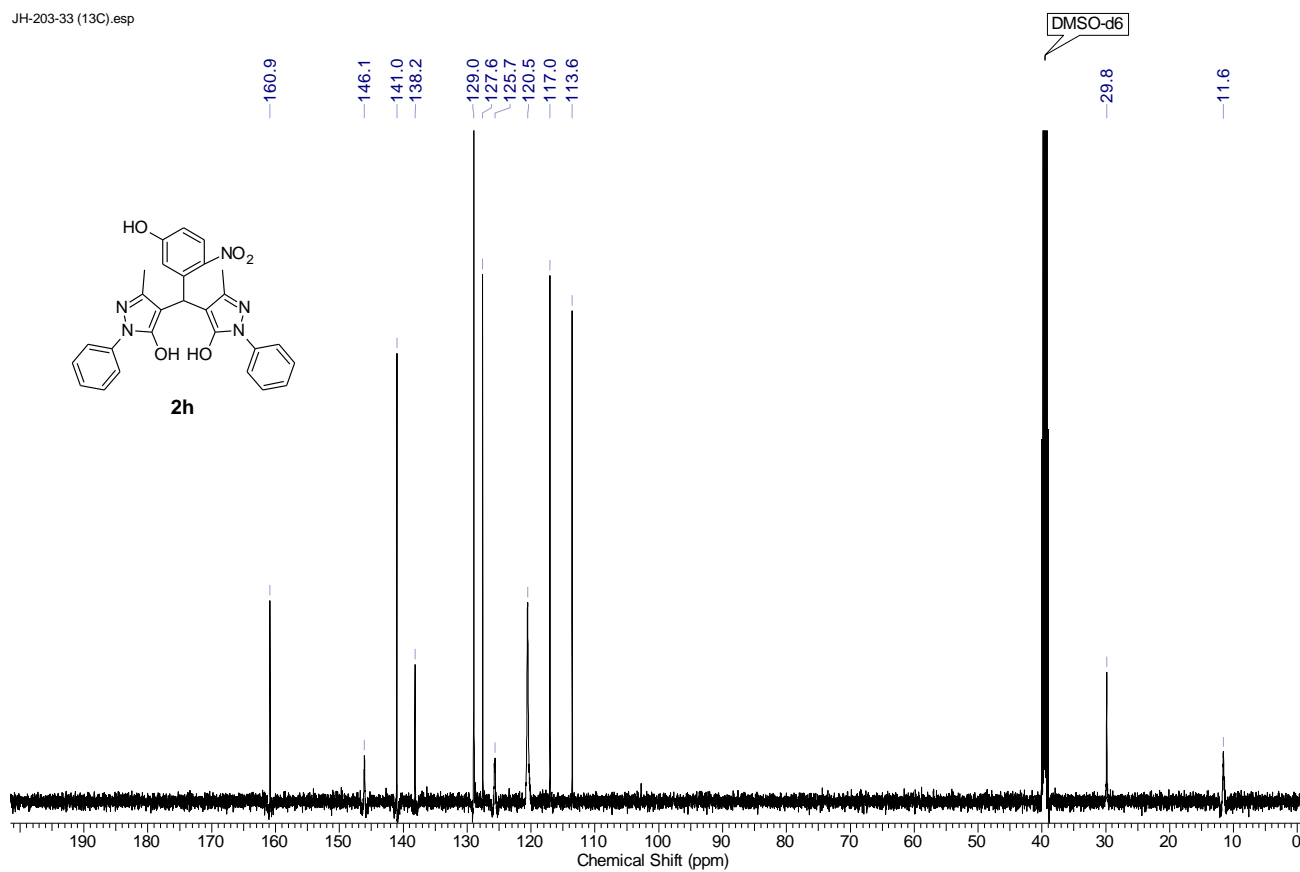
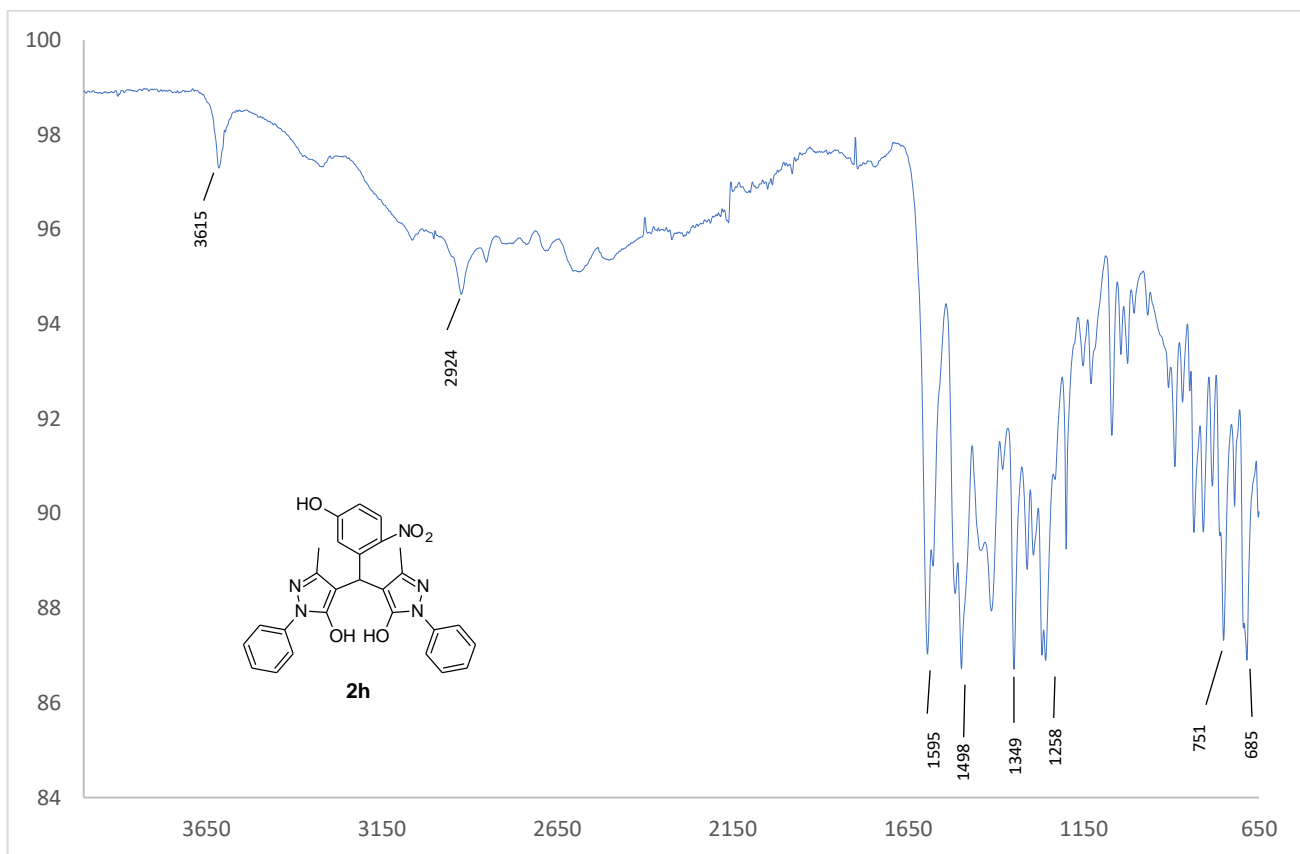
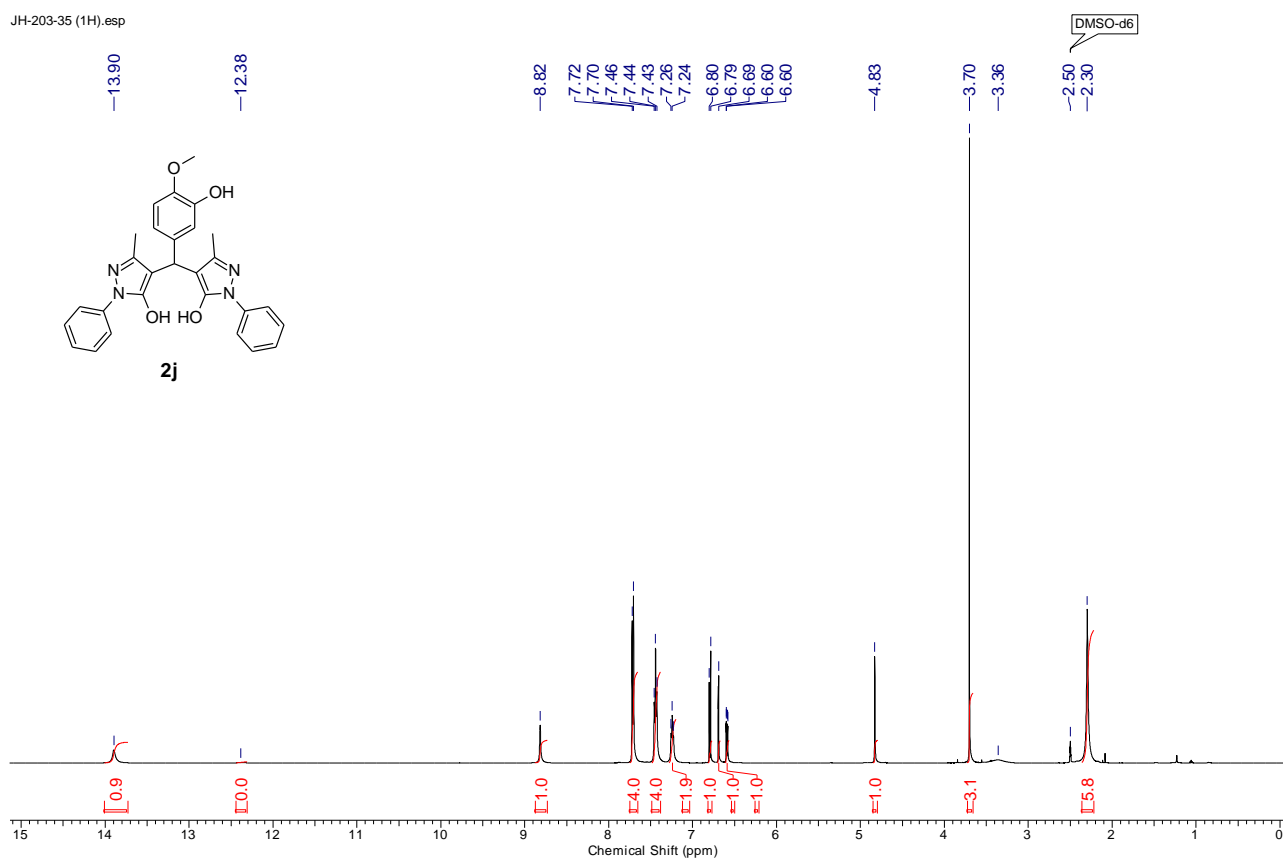
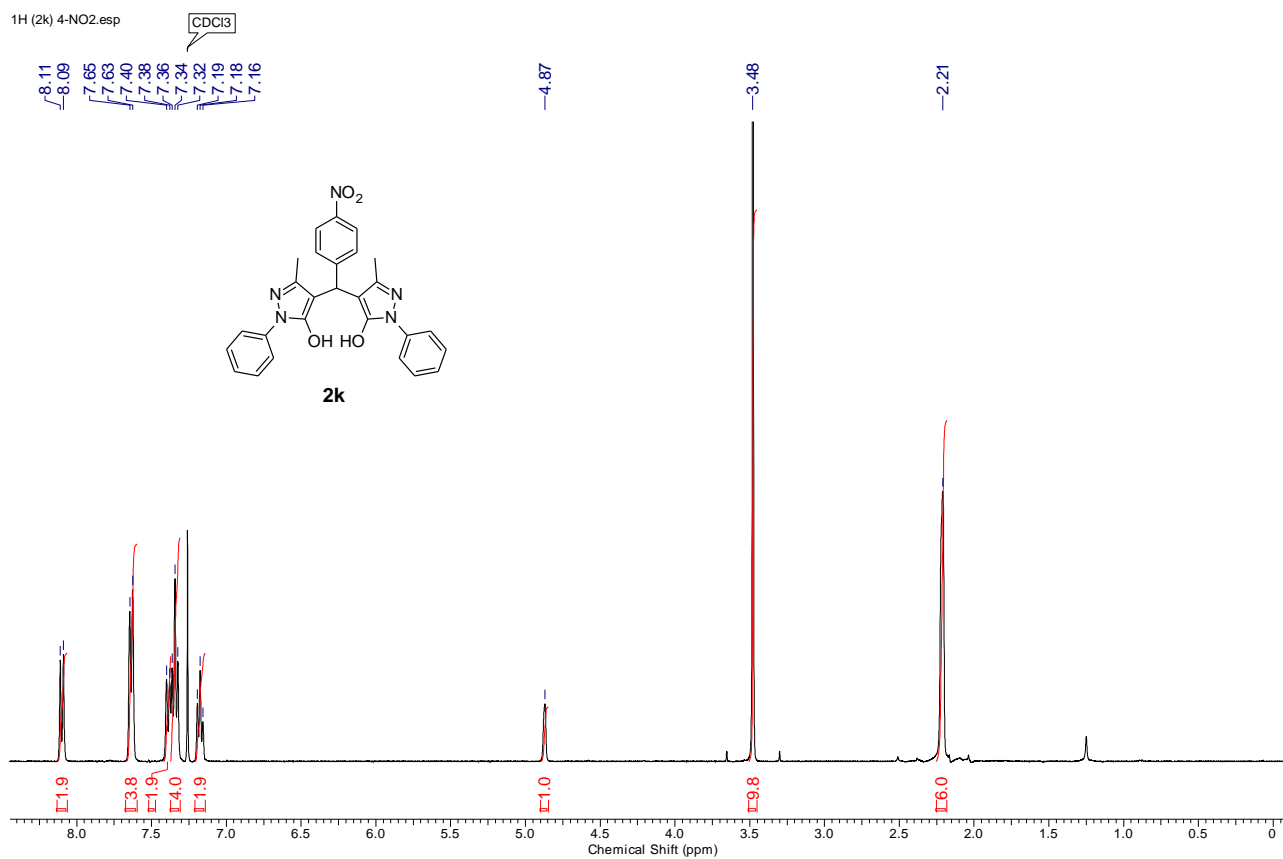
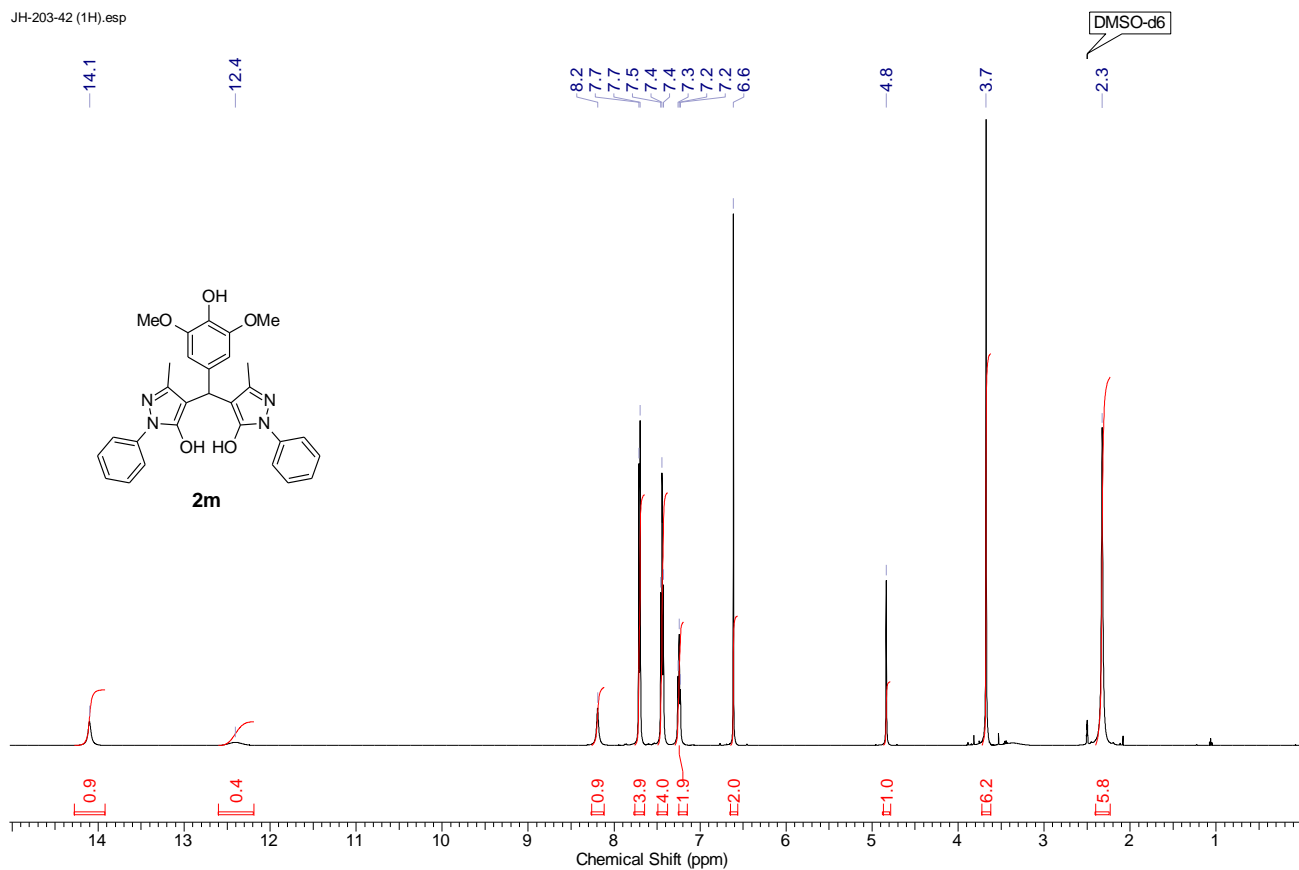
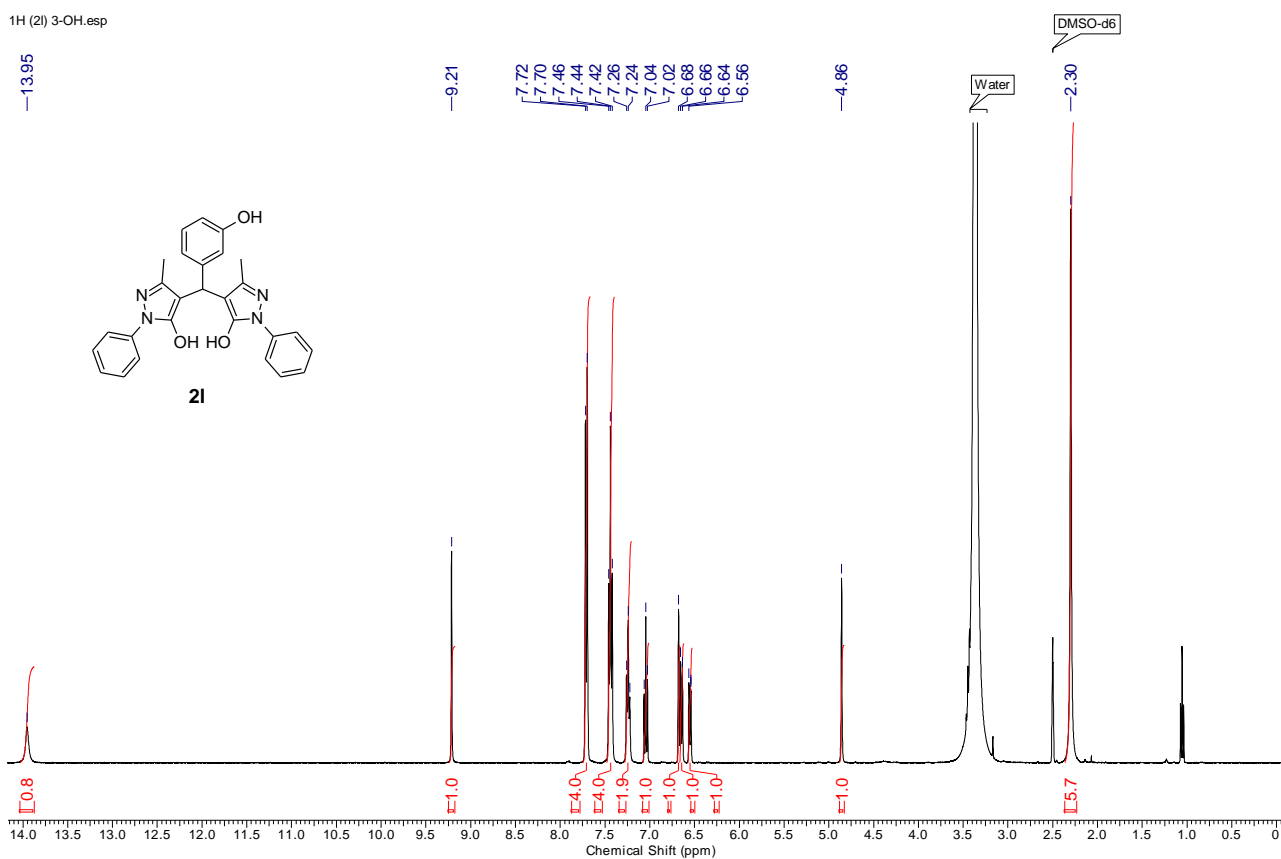
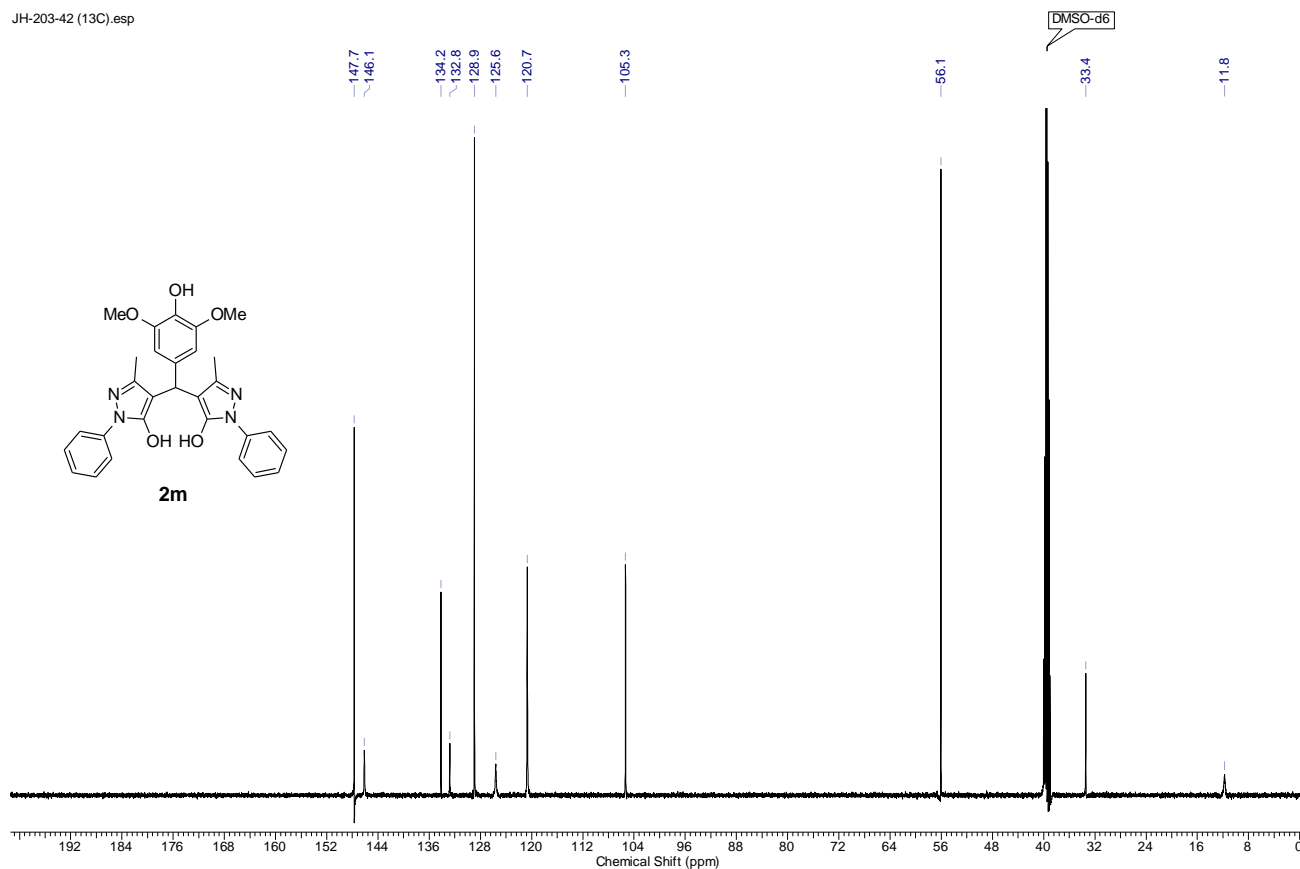
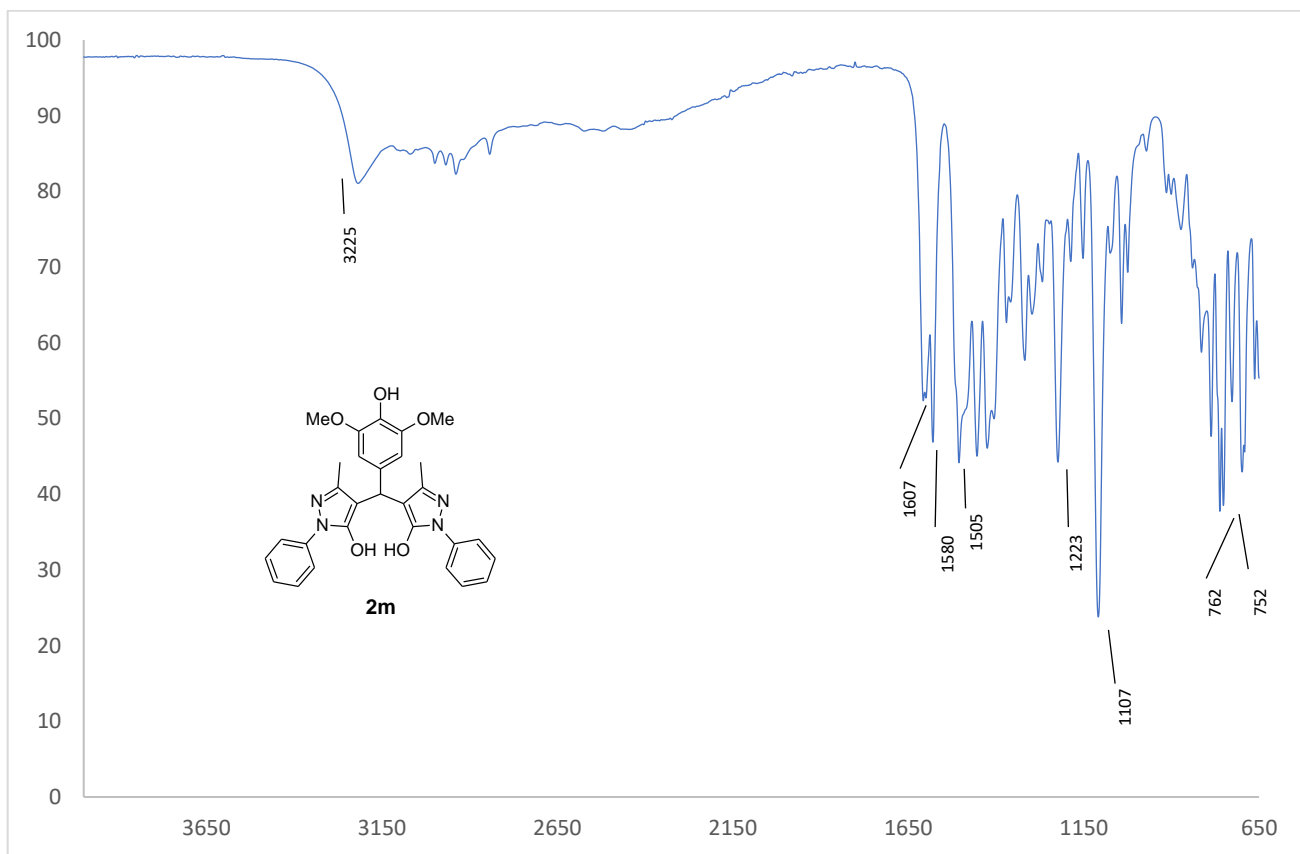
Figure S13. ¹³C NMR spectrum of compound 2h

Figure S14. FTIR spectrum of compound 2h

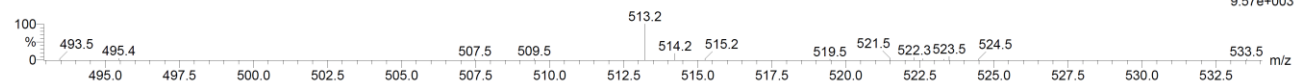
Figure S17. ^1H NMR spectrum of compound **2j**.Figure S18. ^1H NMR spectrum of compound **2k**.



Figure S21. ^{13}C NMR spectrum of compound **2m**Figure S22. FTIR spectrum of compound **2m**

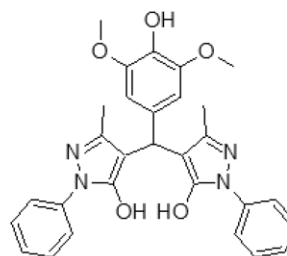
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 118 formula(e) evaluated with 4 results within limits (up to 19 closest results for each mass)
 Elements Used:
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 24-Jun-2016
 jhm-24jun16-203-42 167 (3.089) Cn (Cen,7, 50.00, Ar); Sm (SG, 3x5.00); Sb (12,5.00)

TOF MS ES+
 9.57e+003



Minimum: -2.0
 Maximum: 1000.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
513.2136	513.2138	-0.2	-0.4	17.5	539.1	C29 H29 N4 O5
	513.2079	5.7	11.1	26.5	942.9	C36 H25 N4
	513.2197	-6.1	-11.9	8.5	222.9	C22 H33 N4 O10
	513.2044	9.2	17.9	4.5	125.0	C18 H33 N4 O13



Molecular Formula = C₂₉H₂₈N₄O₅
 [M+H]⁺ = 513.213246 Da

Figure S23. HRMS spectrum of compound 2m

¹H (2n) 3,4-diOH.esp

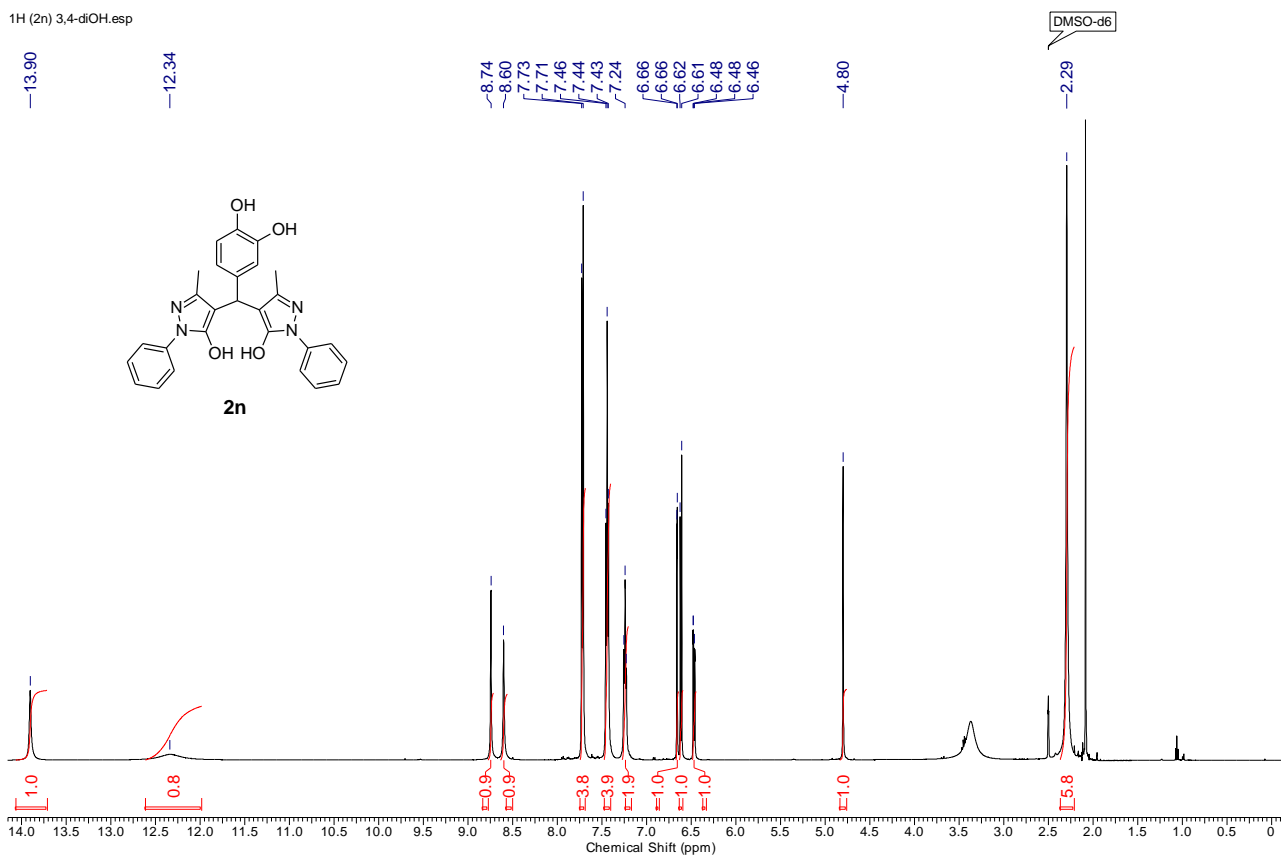


Figure S24. ¹H NMR spectrum of compound 2n.

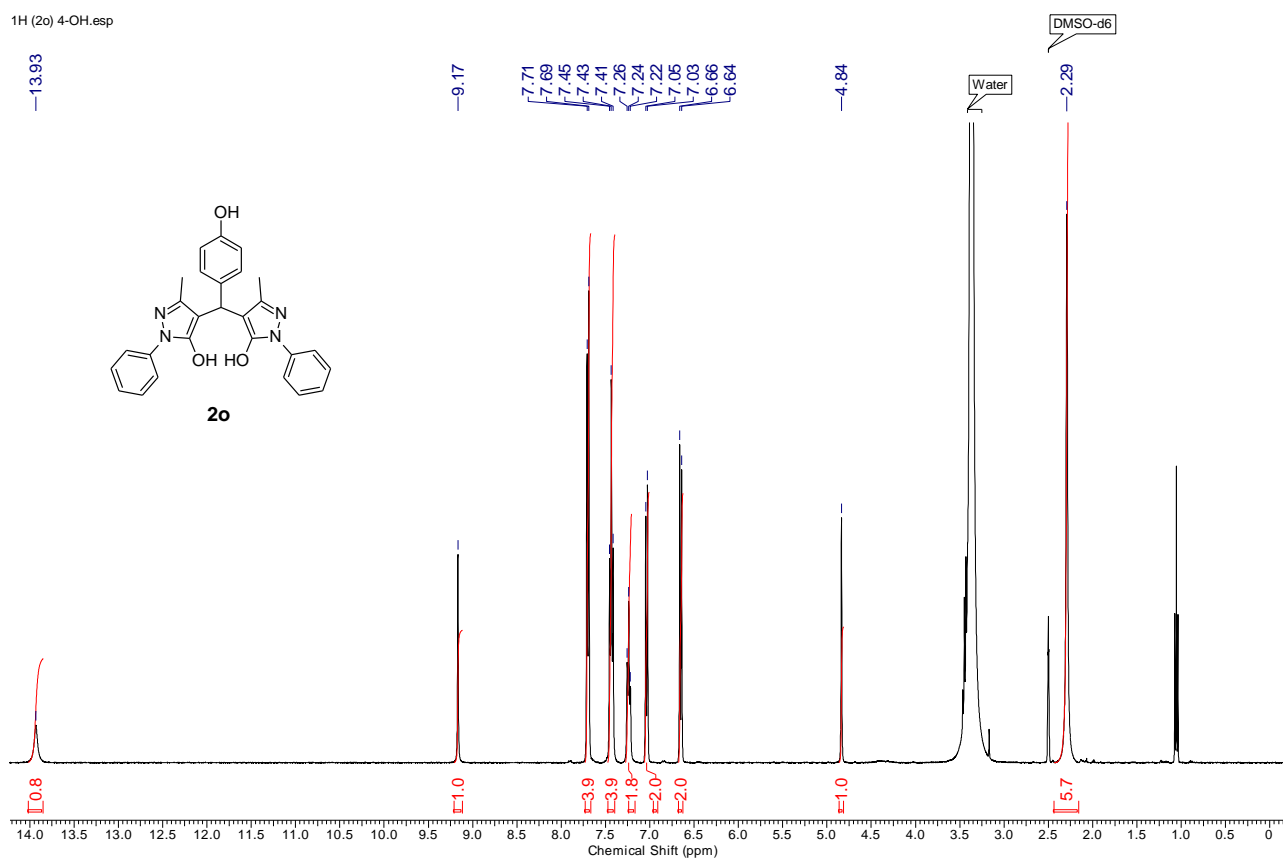


Figure S25. ¹H NMR spectrum of compound **2o**.

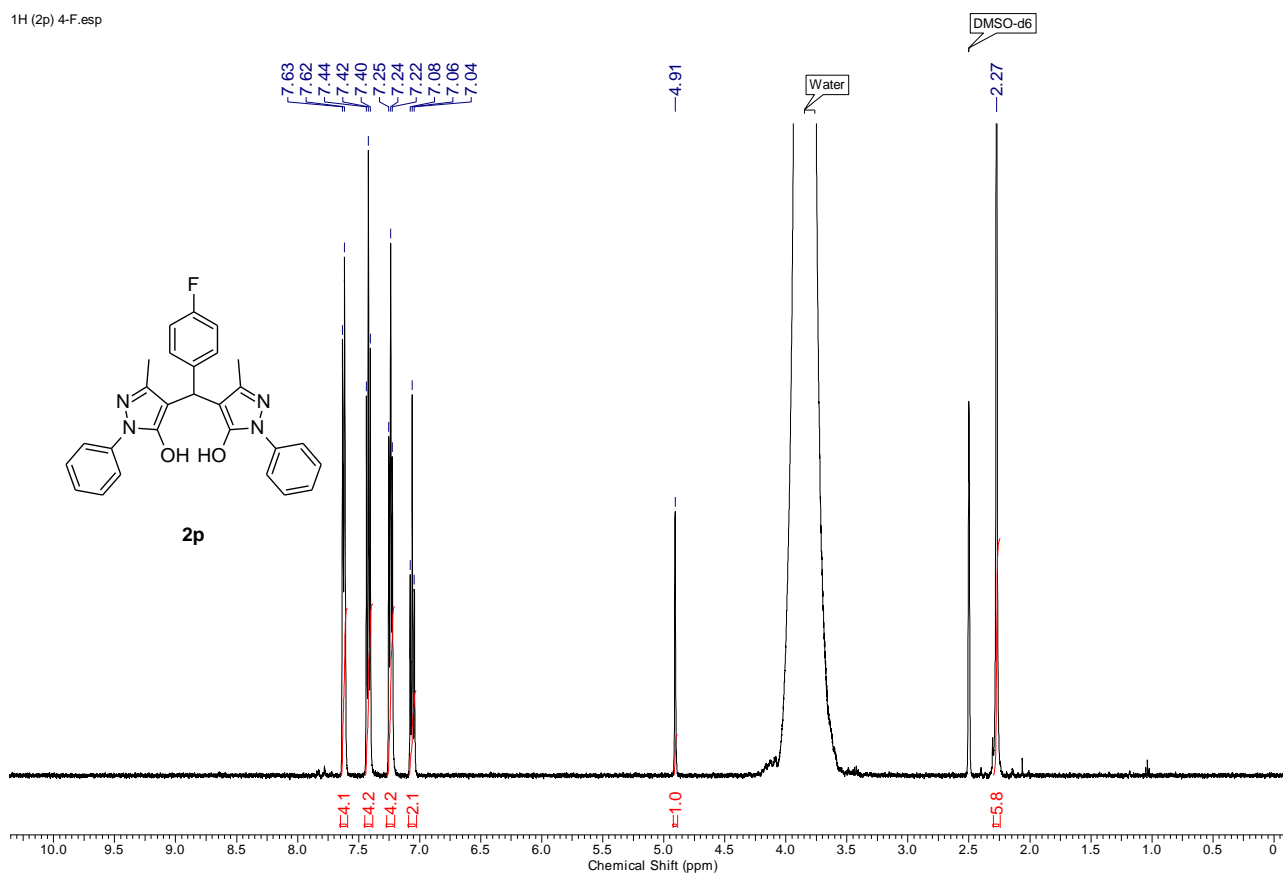


Figure S26. ¹H NMR spectrum of compound **2p**.

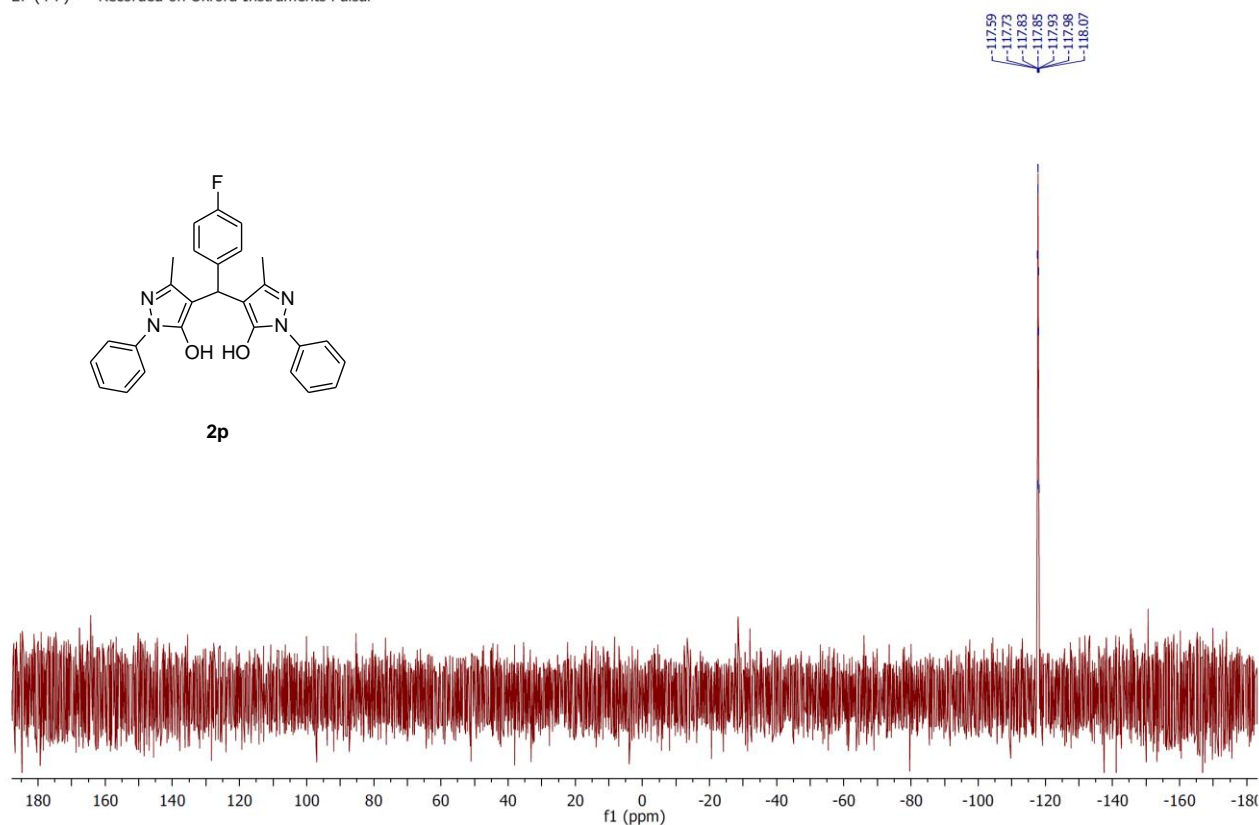


Figure S27. ^{19}F NMR spectrum of compound **2p**.

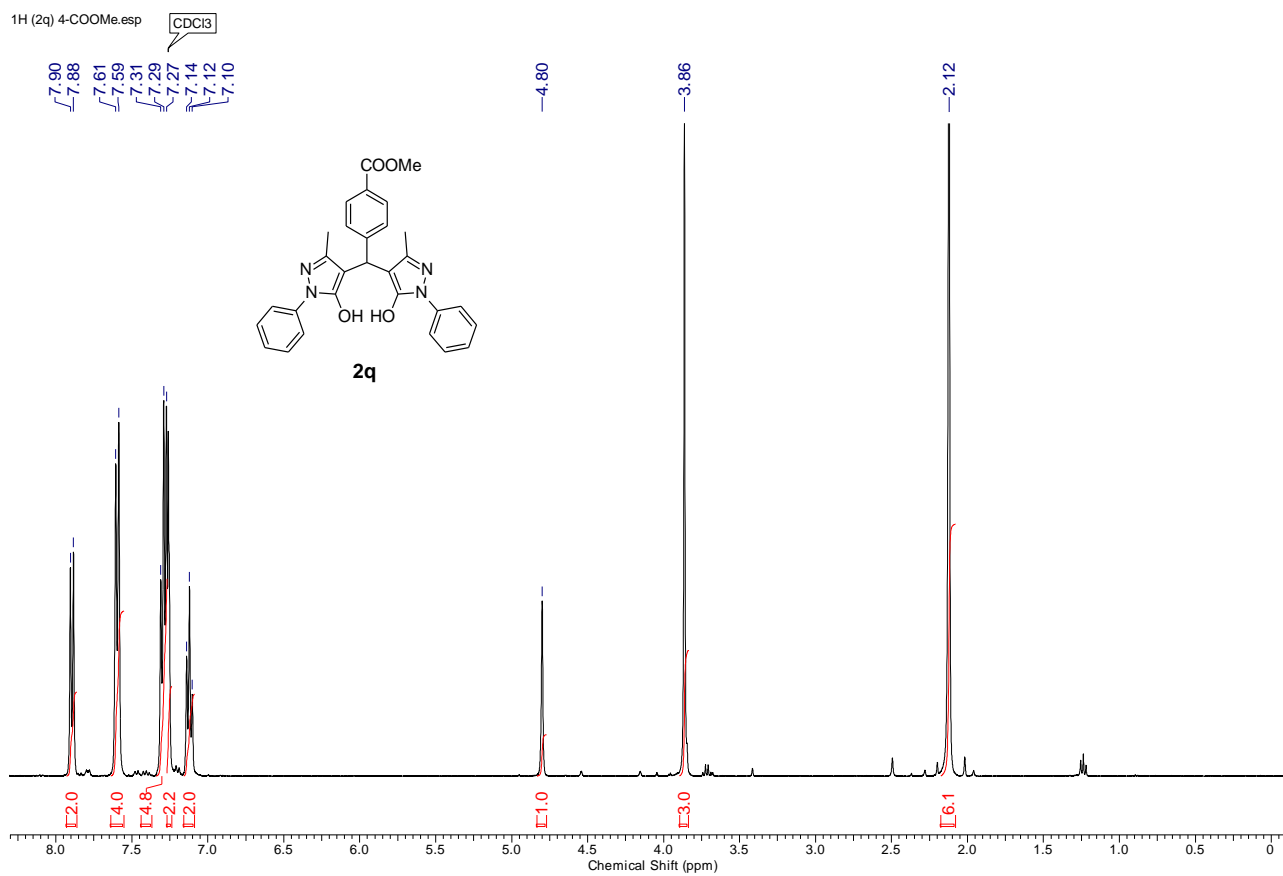


Figure S28. ^1H NMR spectrum of compound **2q**.

1H (2r) 4-CF3.esp

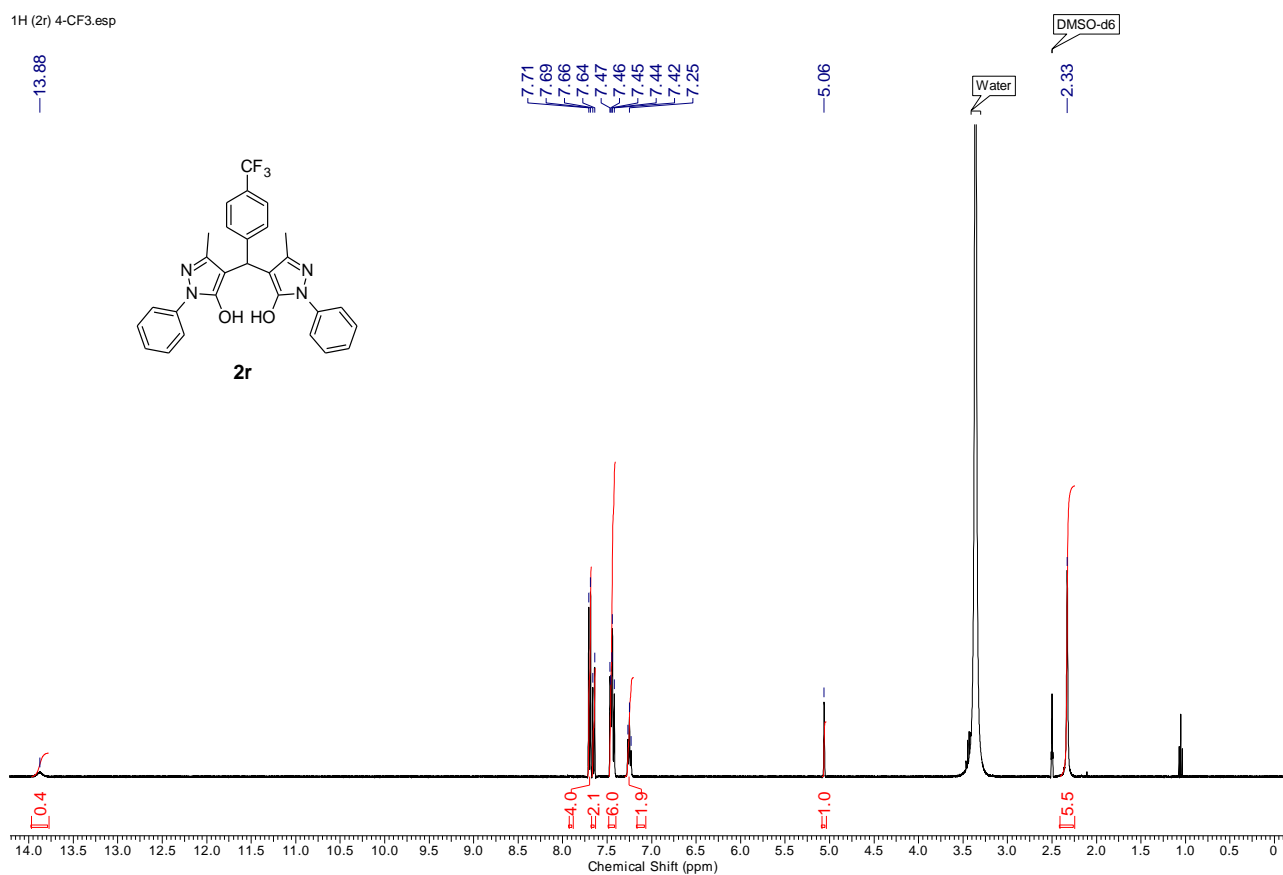


Figure S29. ¹H NMR spectrum of compound **2r**.

1H (2s) 500 MHz.esp

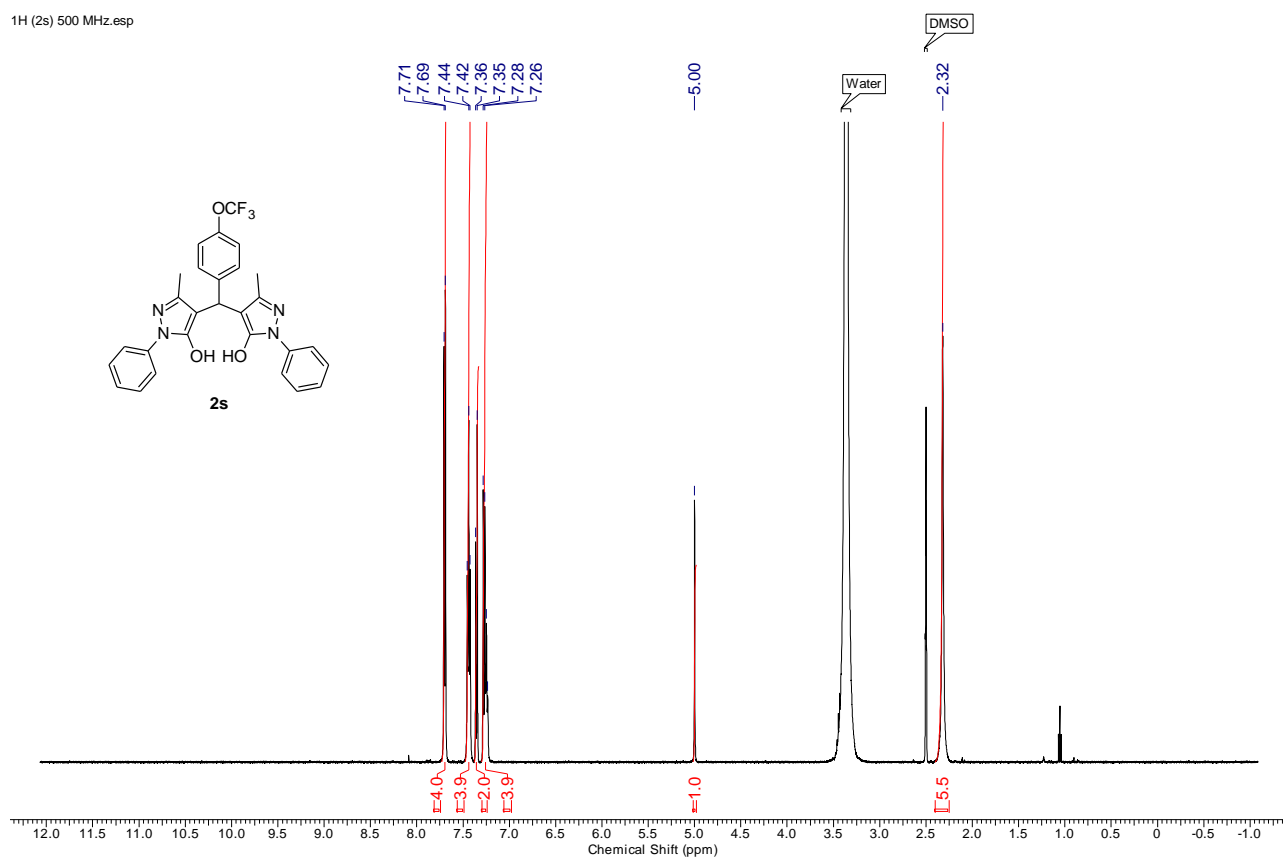


Figure S30. ¹H NMR spectrum of compound **2s**.

1H (2t) 3-F.esp

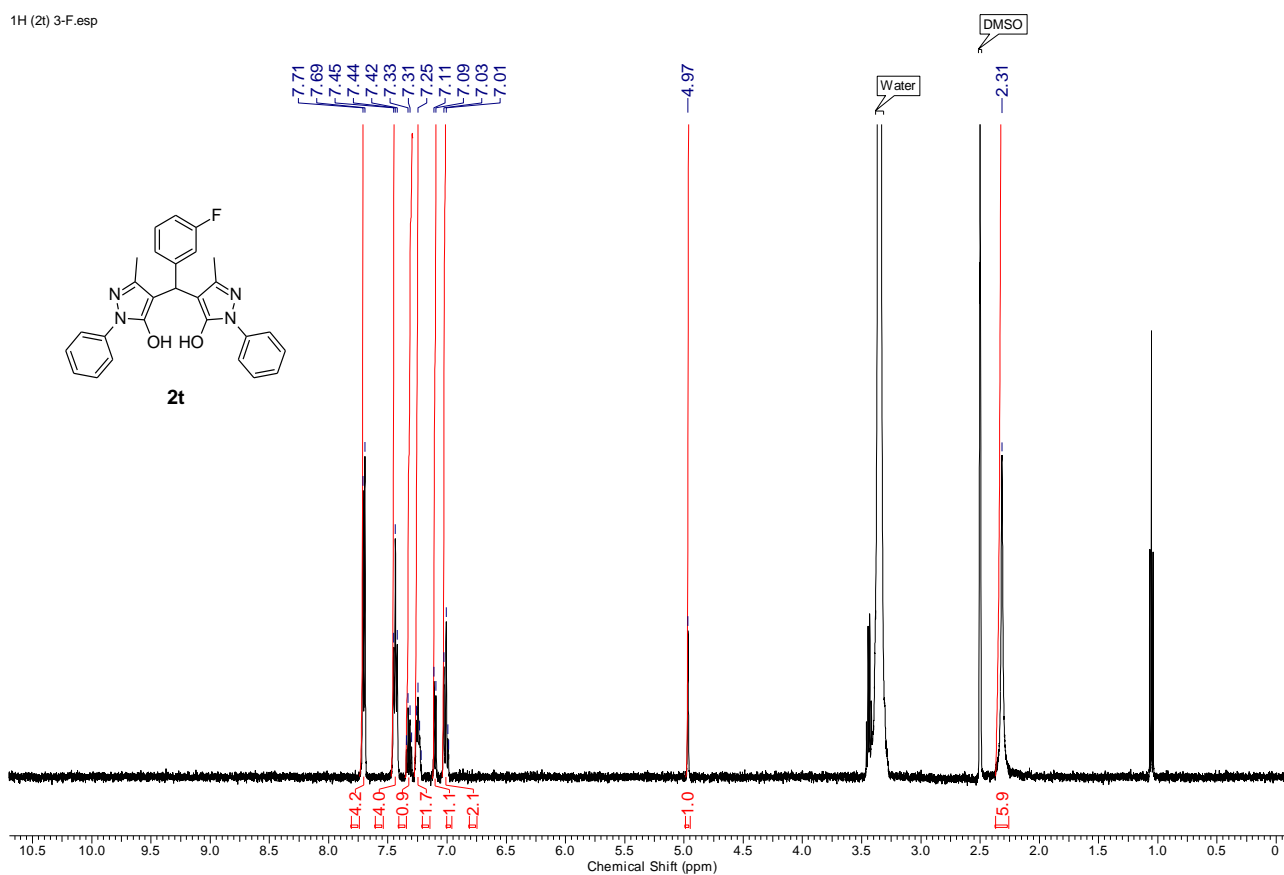


Figure S31. ¹H NMR spectrum of compound **2t**.

1H (2u) 4-SMe.esp

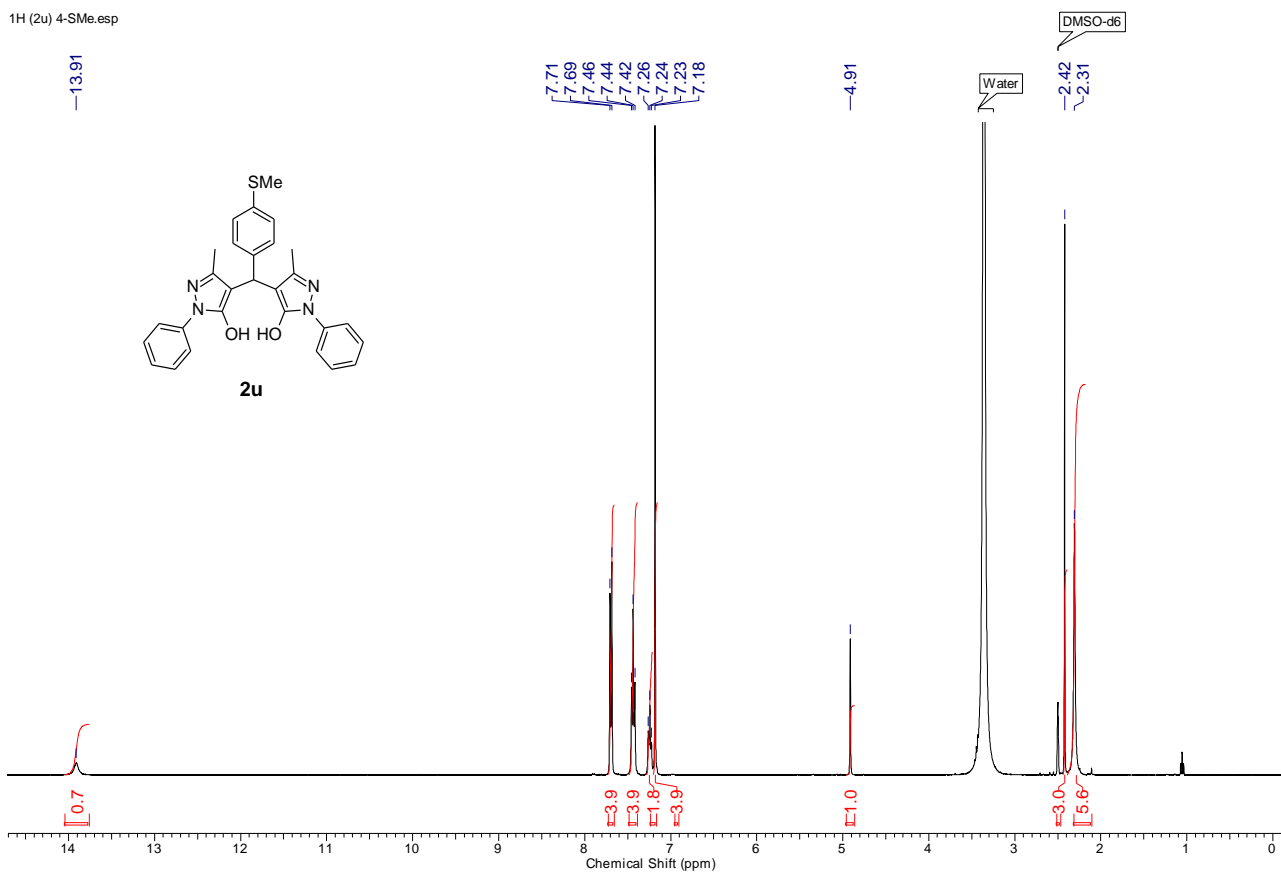


Figure S32. ¹H NMR spectrum of compound **2u**.

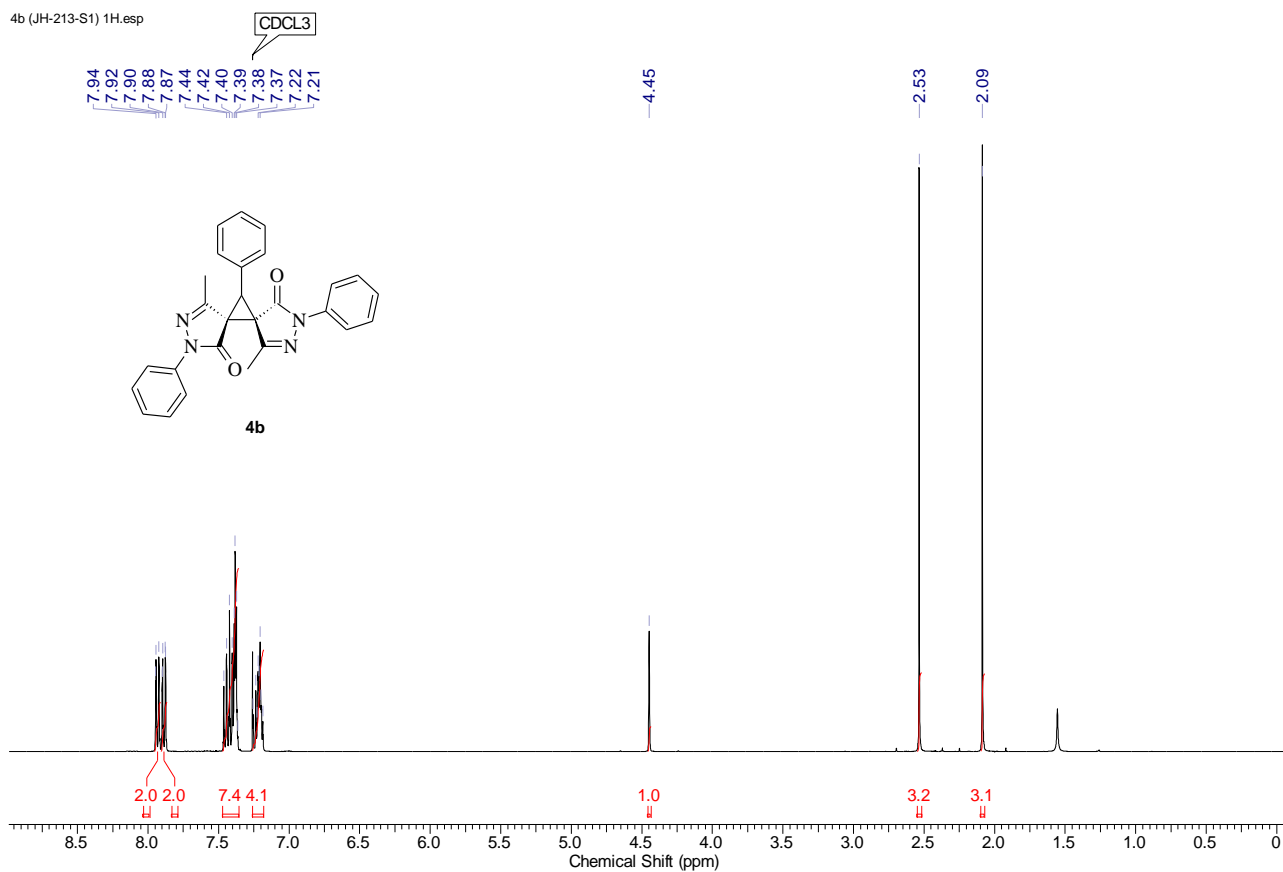


Figure S33. ¹H NMR spectrum of compound 4b

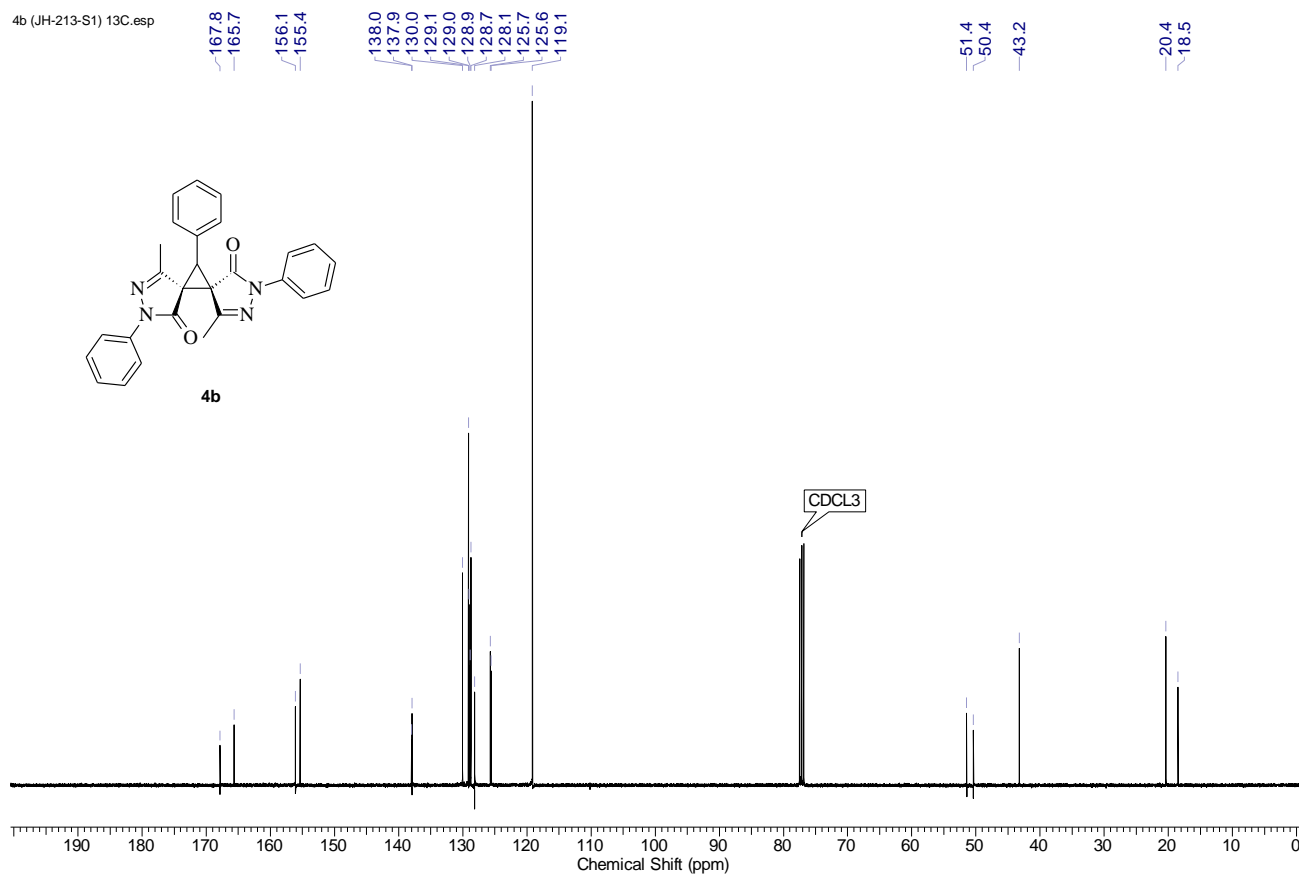


Figure S34. ¹³C NMR spectrum of compound 4b

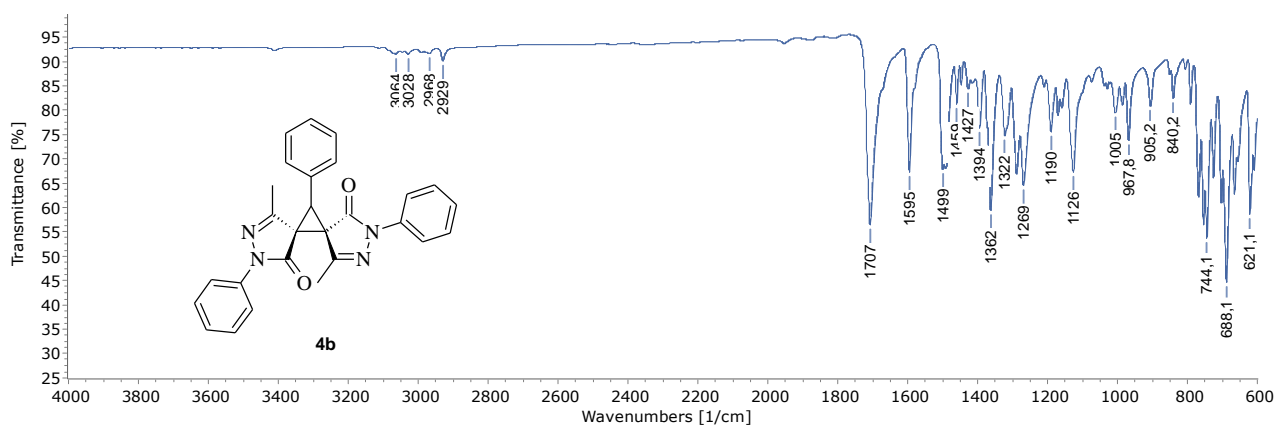
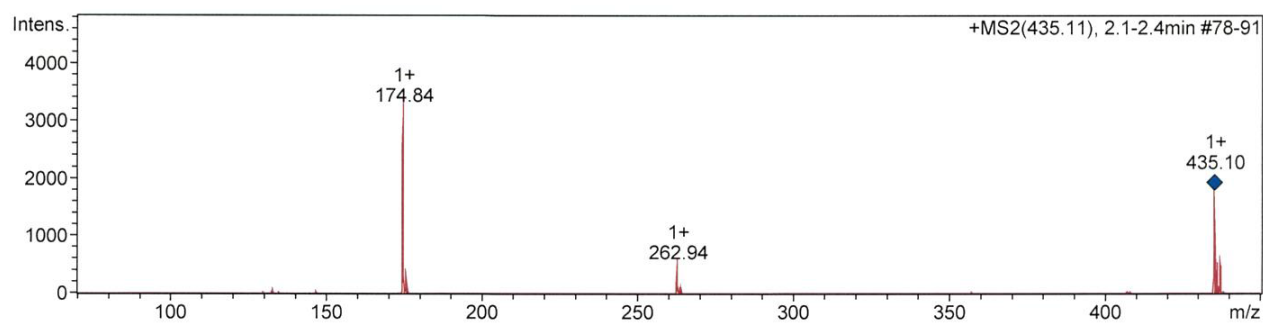


Figure S35. FTIR spectrum of compound **4b**

Acquisition Parameter

Ion Source Type	ESI	Ion Polarity	Positive	Alternating Ion Polarity	off
Mass Range Mode	UltraScan	Scan Begin	70 m/z	Scan End	450 m/z
Accumulation Time	200000 μ s	RF Level	63 %	Trap Drive	54.1
SPS Target Mass	400 m/z	Averages	5 Spectra	n/a	n/a



Component	Molecular Mass	Molecule	Absolute Abundance	Relative Abundance	
A	174.84	174.93	[M + H] ⁺	3230	100.00
B	435.11	435.30	[M + H] ⁺	1784	55.22
C	262.94	263.10	[M + H] ⁺	521	16.14

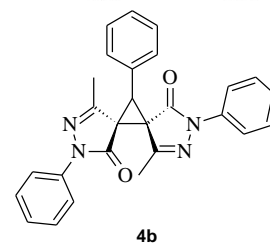


Figure S36. ESI-MS spectrum of compound **4b**

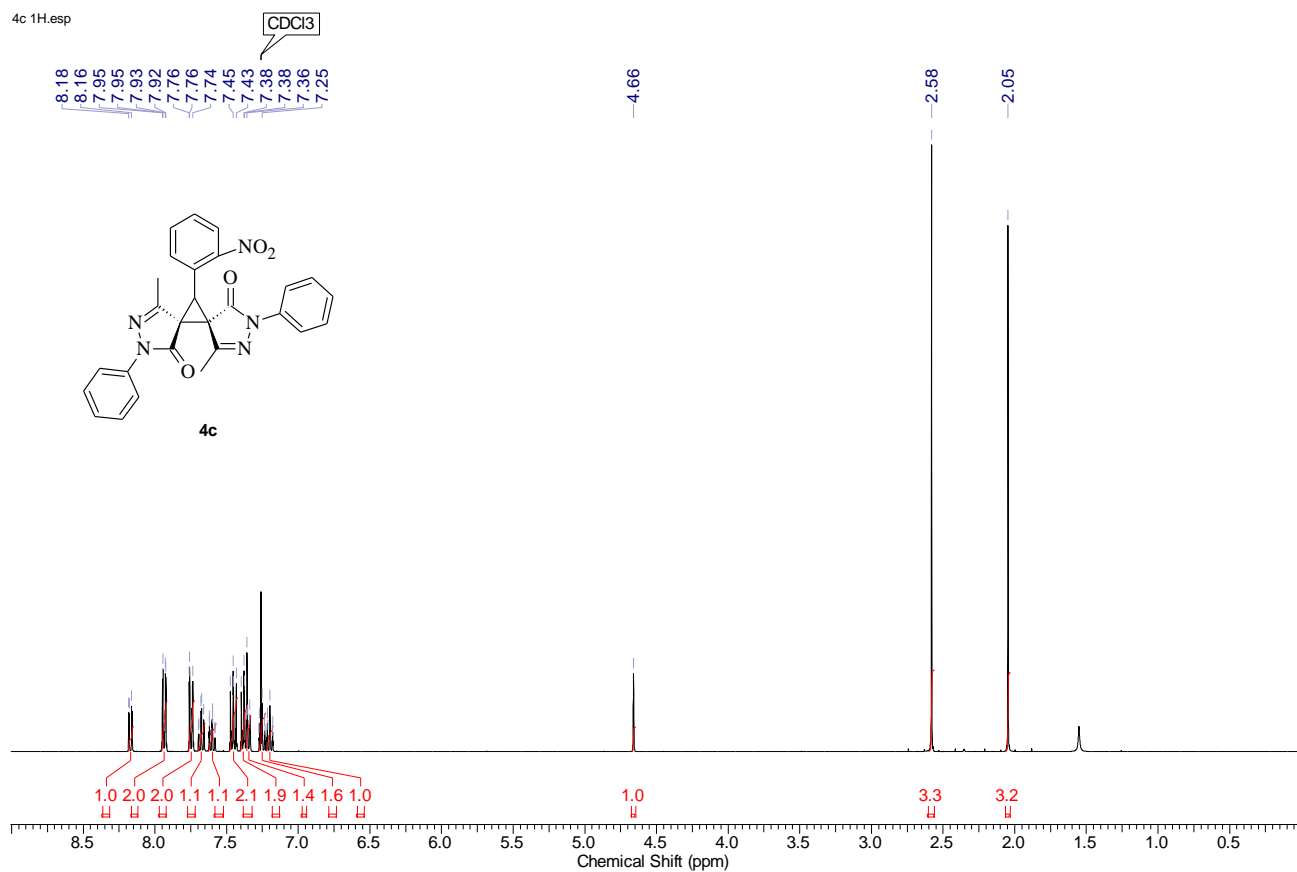


Figure S37. ¹H NMR spectrum of compound 4c

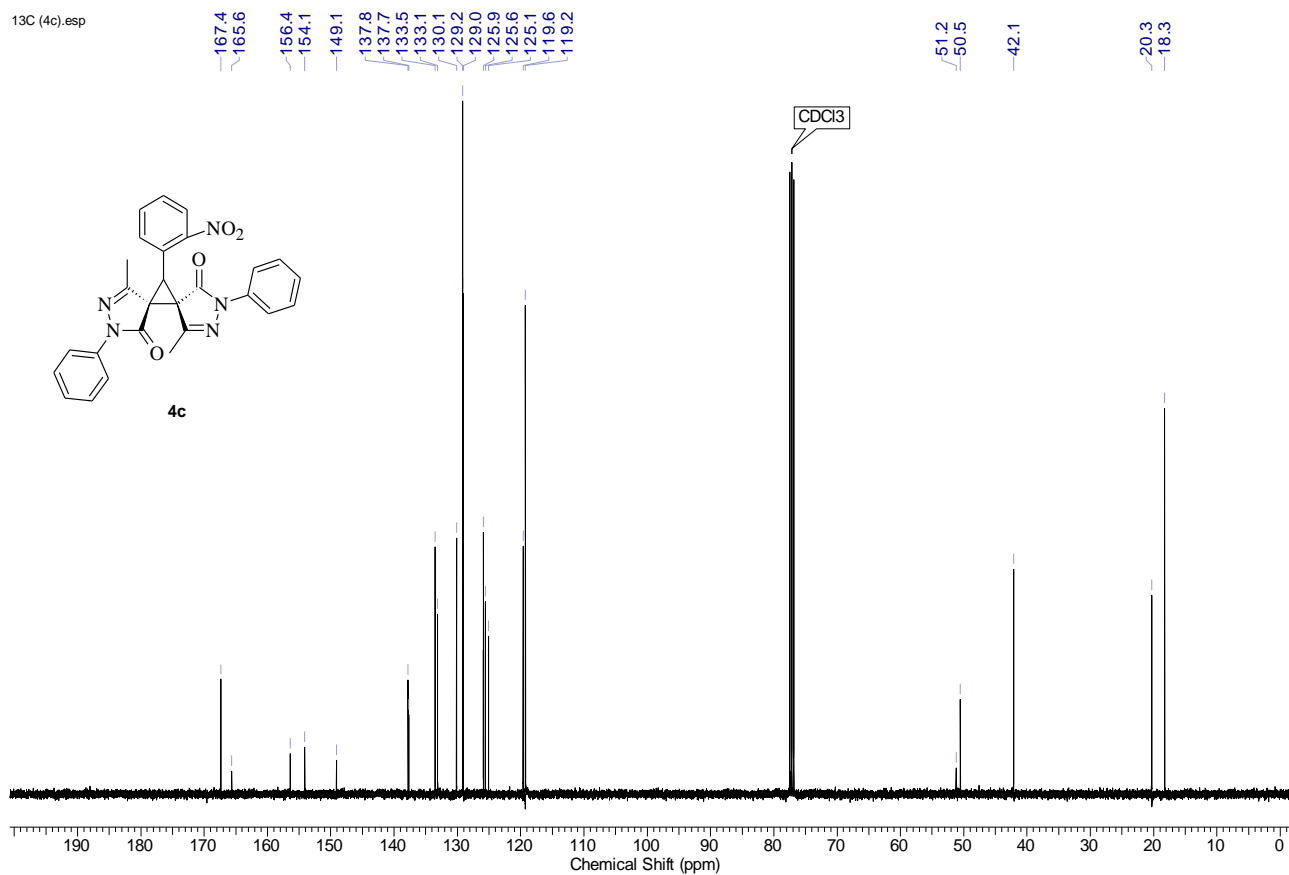


Figure S38. ¹³C NMR spectrum of compound 4c

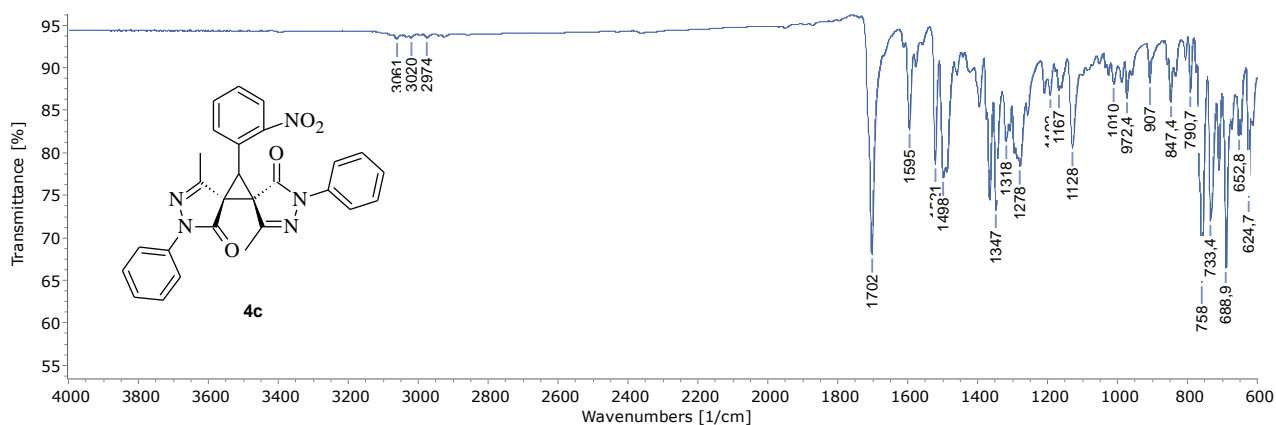
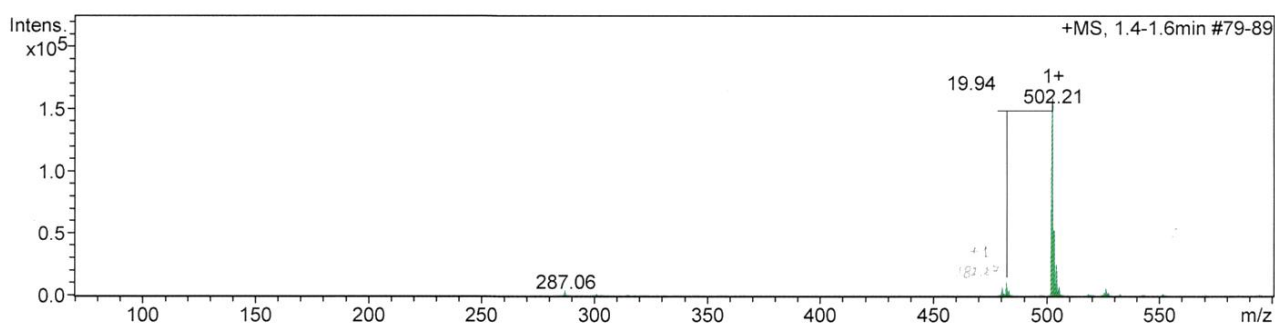


Figure S39. FTIR spectrum of compound **4c**

Acquisition Parameter

Ion Source Type	ESI	Ion Polarity	Positive	Alternating Ion Polarity	off
Mass Range Mode	UltraScan	Scan Begin	70 m/z	Scan End	600 m/z
Accumulation Time	200000 μ s	RF Level	63 %	Trap Drive	54.1
SPS Target Mass	400 m/z	Averages	5 Spectra	n/a	n/a



Component	Molecular Mass	Molecule	Absolute Abundance	Relative Abundance
A	502.18	502.66	[M + H] ⁺	150245
B	482.27	482.67	[M + H] ⁺	8078

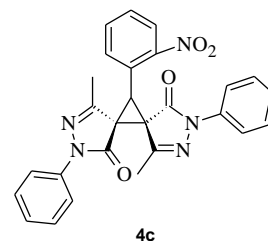


Figure S40. ESI-MS spectrum of compound **4c**

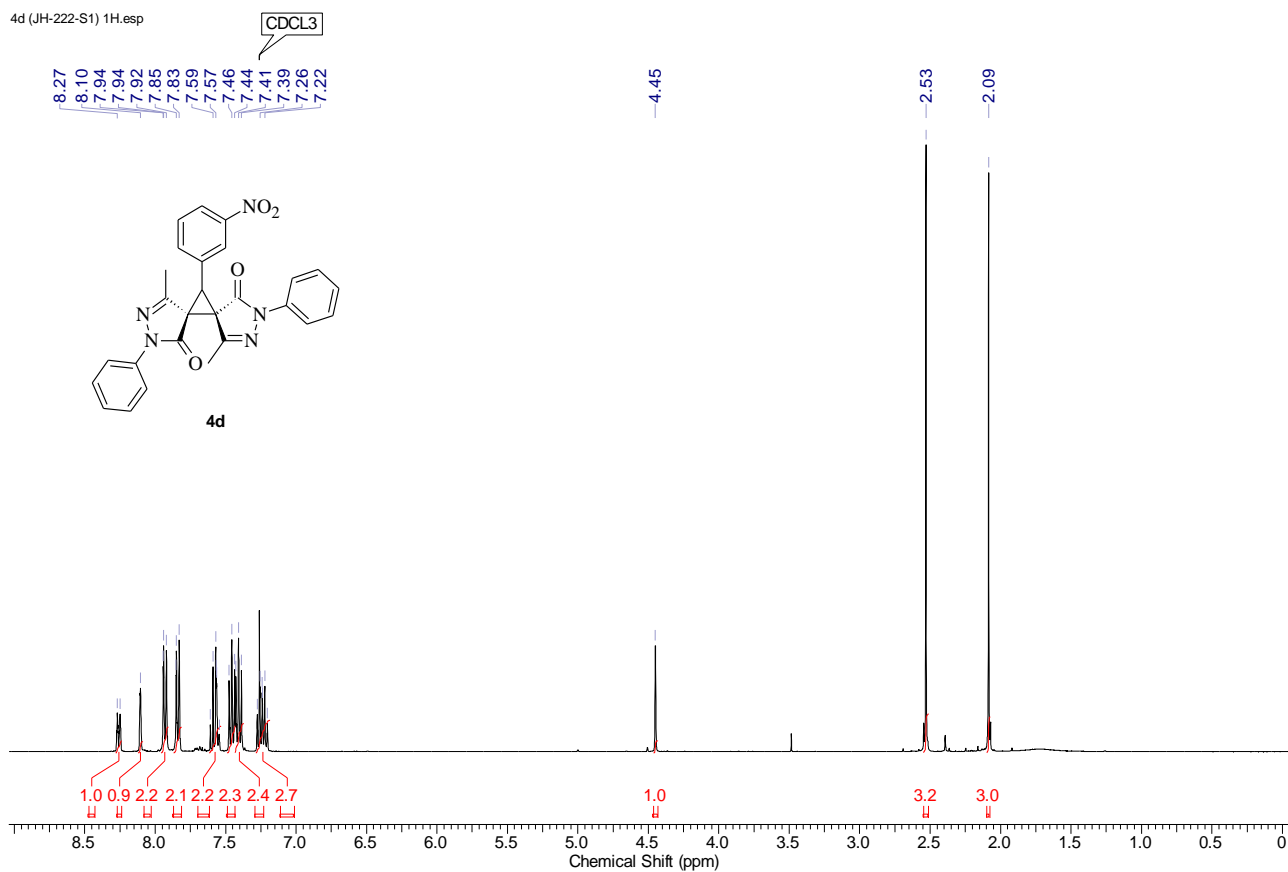


Figure S41. ¹H NMR spectrum of compound **4d**

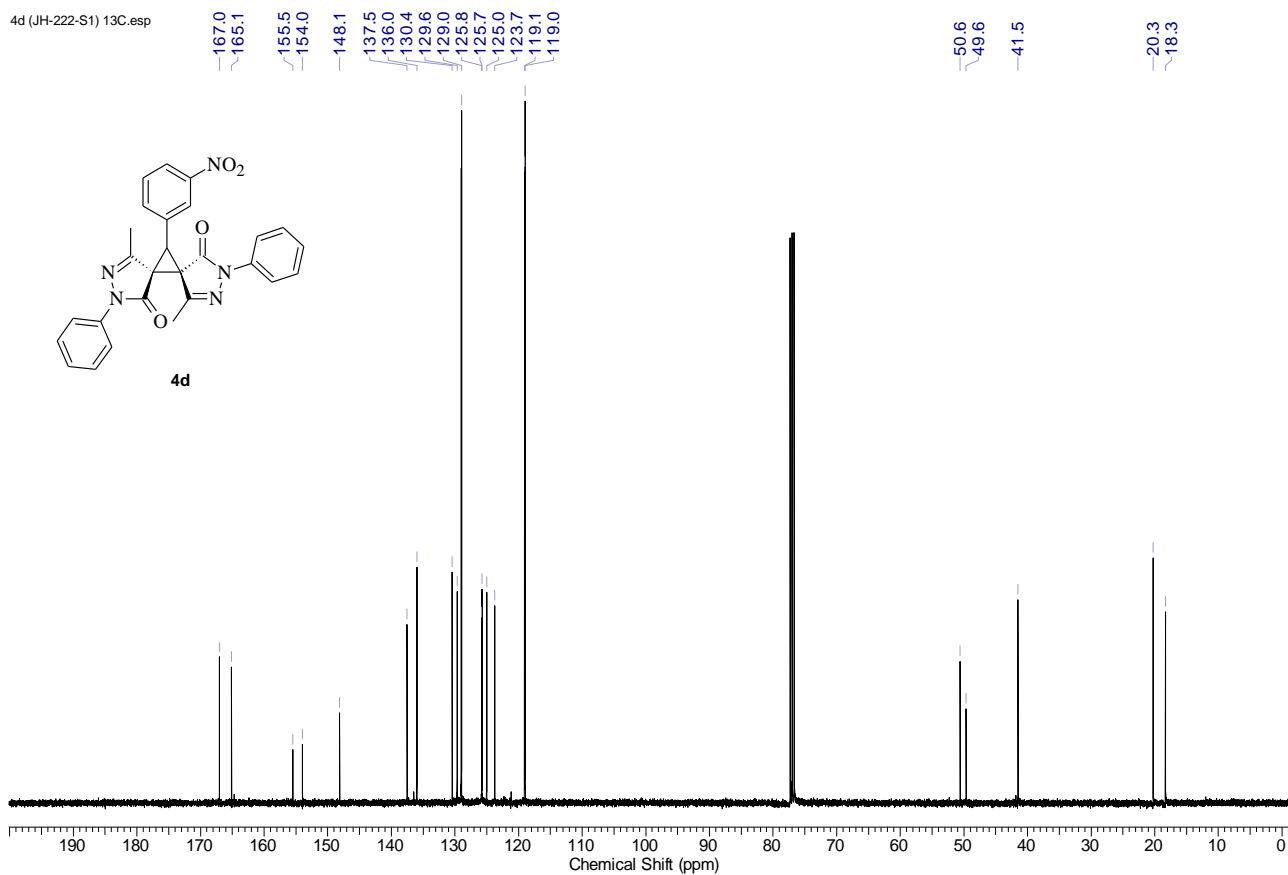


Figure S42. ¹³C NMR spectrum of compound **4d**

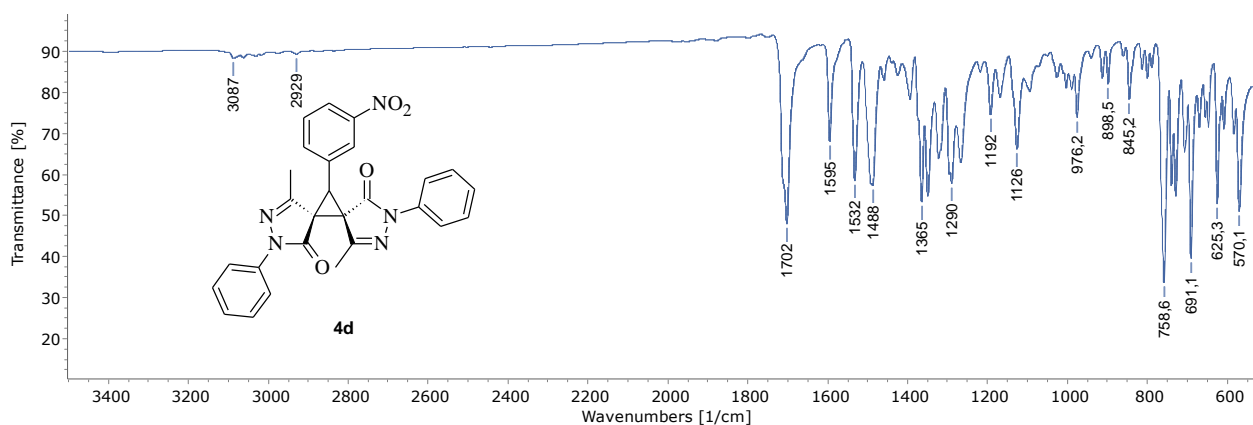
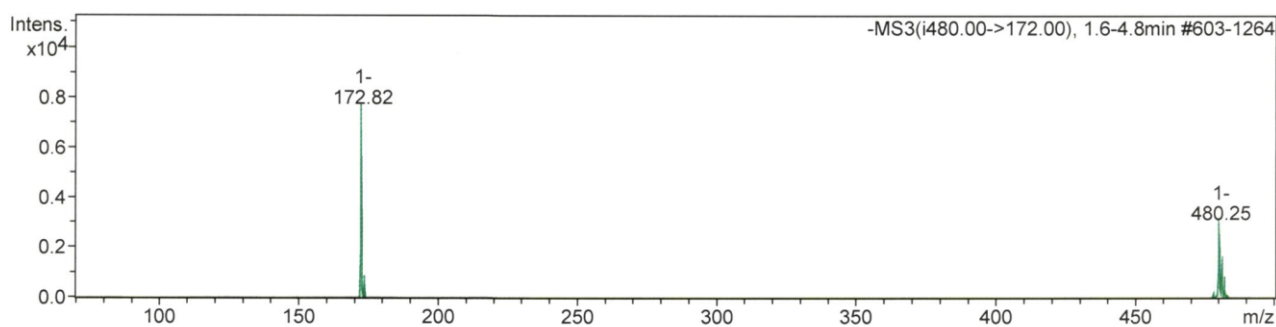


Figure S43. FTIR spectrum of compound **4d**

Acquisition Parameter

Ion Source Type	ESI	Ion Polarity	Positive	Alternating Ion Polarity	off
Mass Range Mode	XtremeScan	Scan Begin	70 m/z	Scan End	400 m/z
Accumulation Time	20000 μ s	RF Level	63 %	Trap Drive	54.1
SPS Target Mass	400 m/z	Averages	3 Spectra	n/a	n/a



Component	Molecular Mass	Molecule	Absolute Abundance	Relative Abundance	
A	172.82	172.90	[M - H] -	7518	100.00
B	480.23	480.77	[M - H] -	2933	39.01

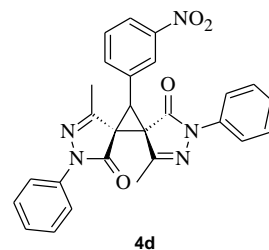


Figure S44. ESI-MS spectrum of compound **4d**

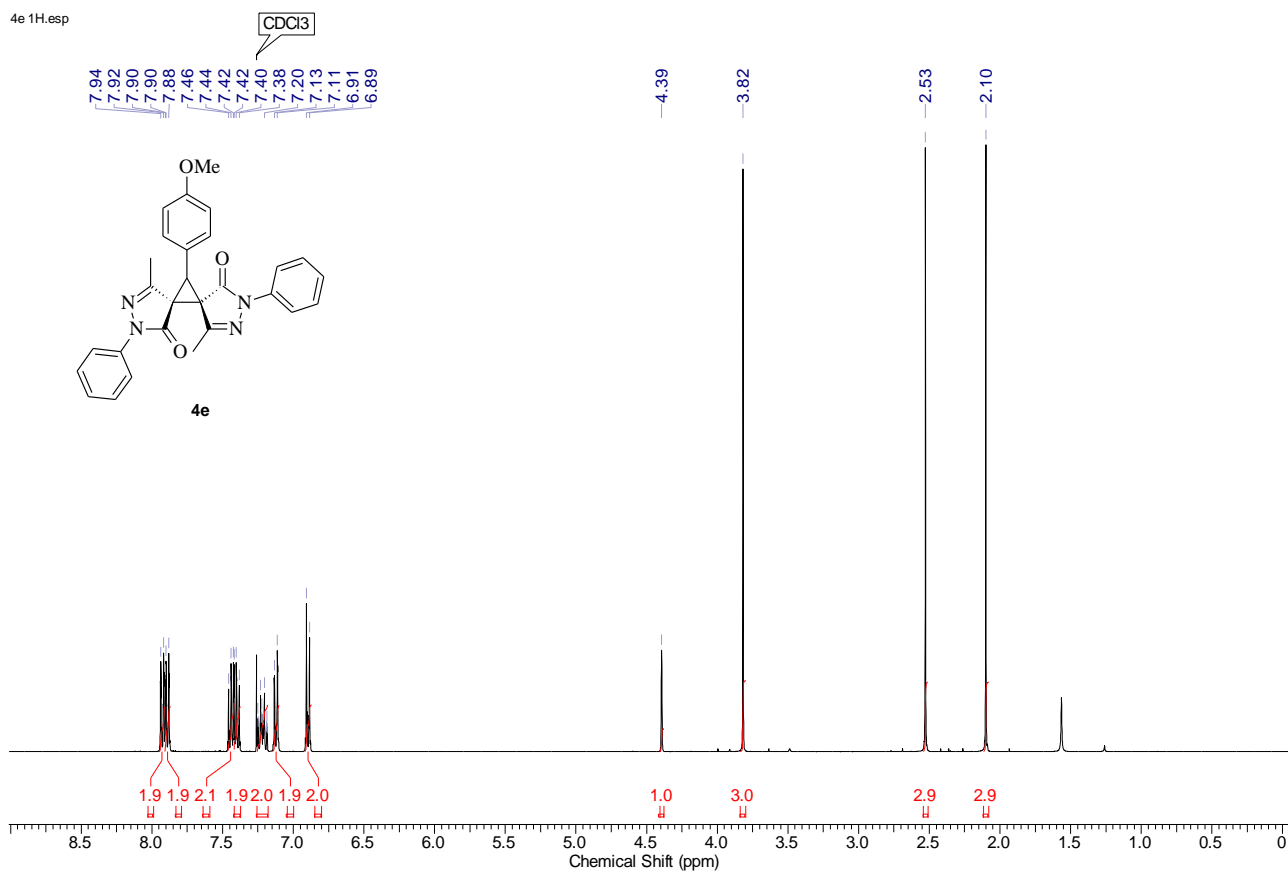


Figure S45. ¹H NMR spectrum of compound **4e**

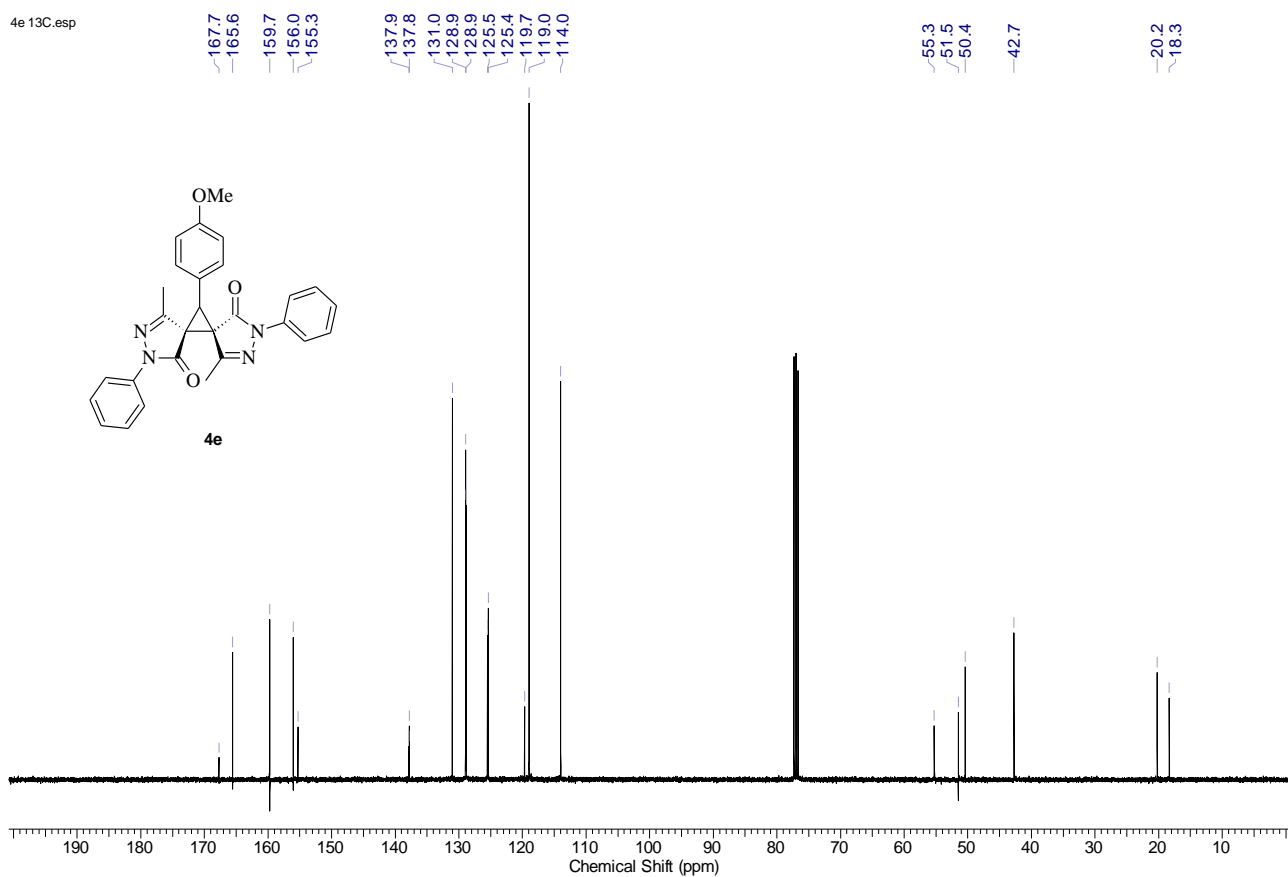


Figure S46. ¹³C NMR spectrum of compound **4e**

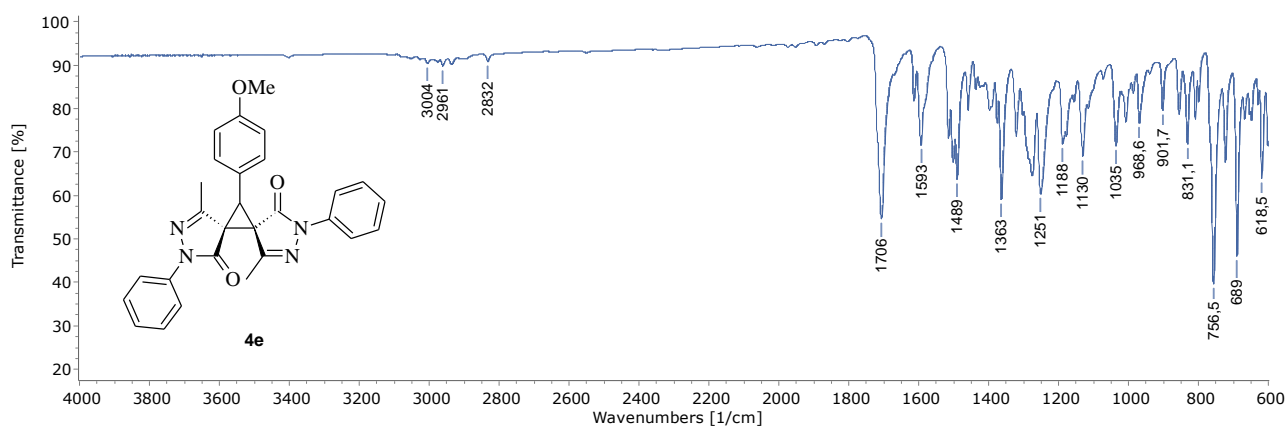
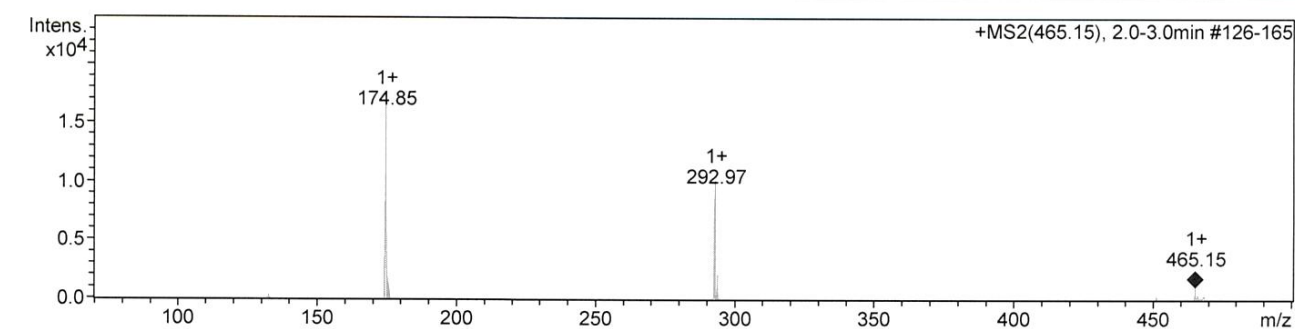


Figure S47. FTIR spectrum of compound **4e**

Acquisition Parameter

Ion Source Type	ESI	Ion Polarity	Positive	Alternating Ion Polarity	off
Mass Range Mode	UltraScan	Scan Begin	70 m/z	Scan End	500 m/z
Accumulation Time	199196 μ s	RF Level	63 %	Trap Drive	54.1
SPS Target Mass	400 m/z	Averages	5 Spectra	n/a	n/a



Component	Molecular Mass	Molecule	Absolute Abundance	Relative Abundance
A	174.85	174.92	[M + H] ⁺	100.00
B	292.97	293.12	[M + H] ⁺	59.57
C	465.15	465.53	[M + H] ⁺	6.66

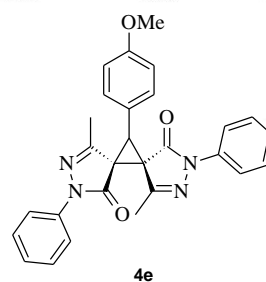


Figure S48. ESI-MS spectrum of compound **4e**

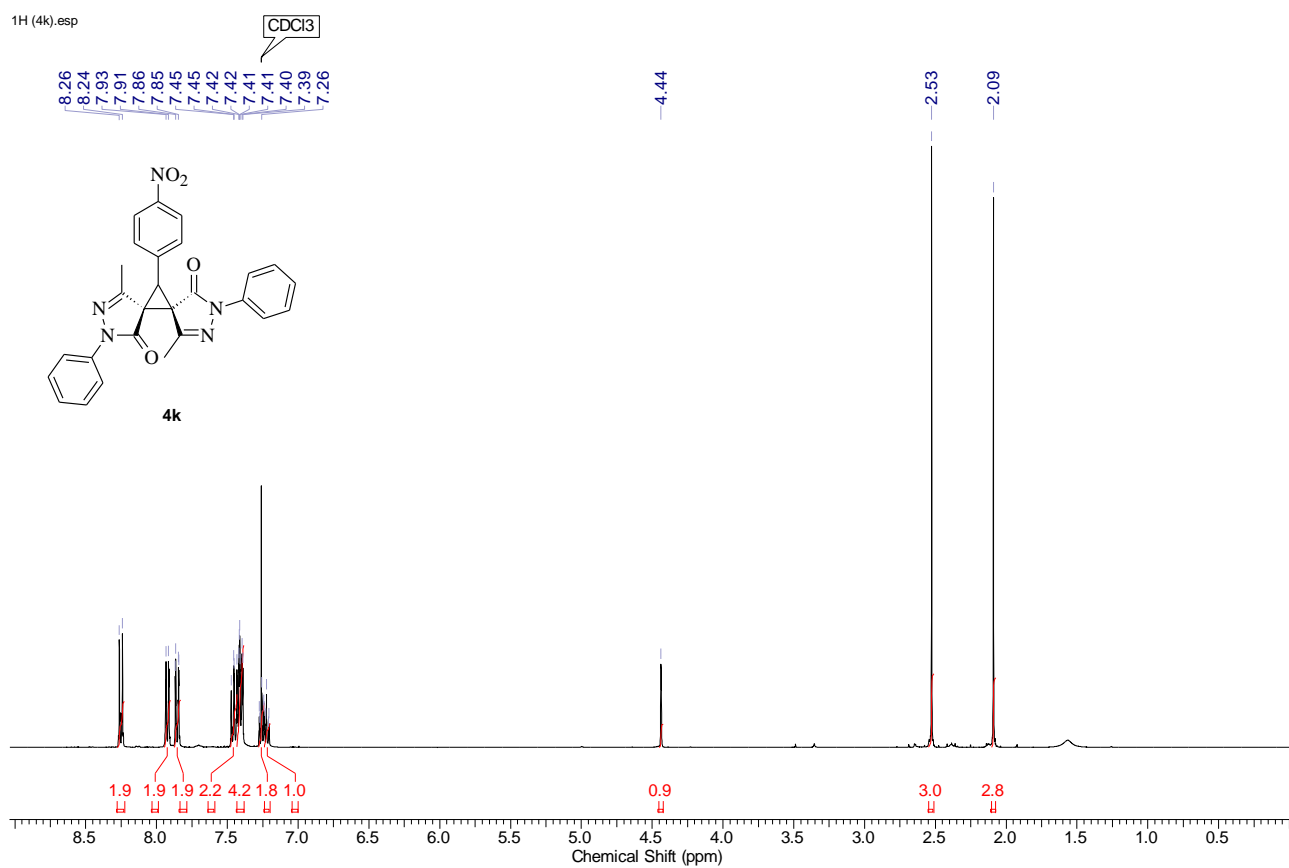


Figure S51. ¹H NMR spectrum of compound **4k**

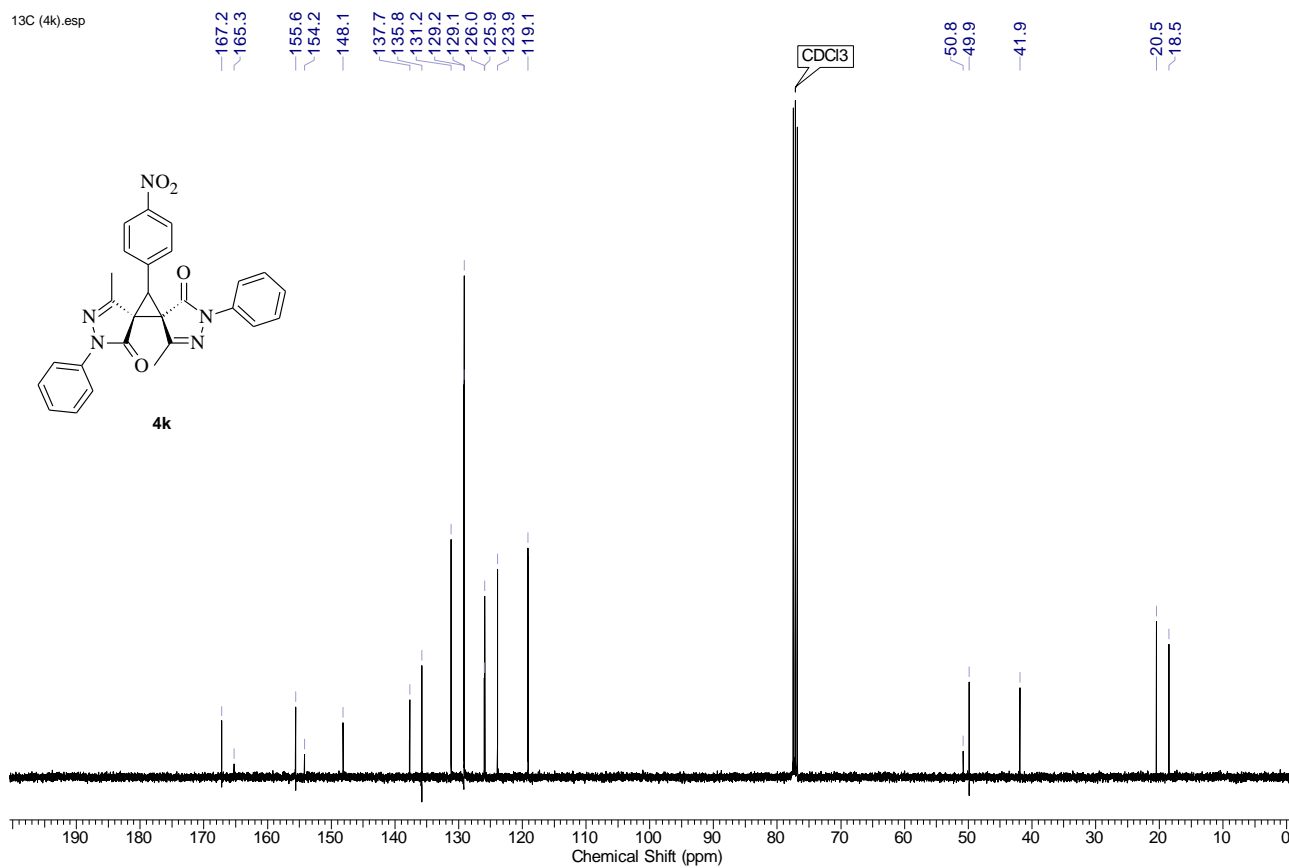


Figure S52. ¹³C NMR spectrum of compound **4k**

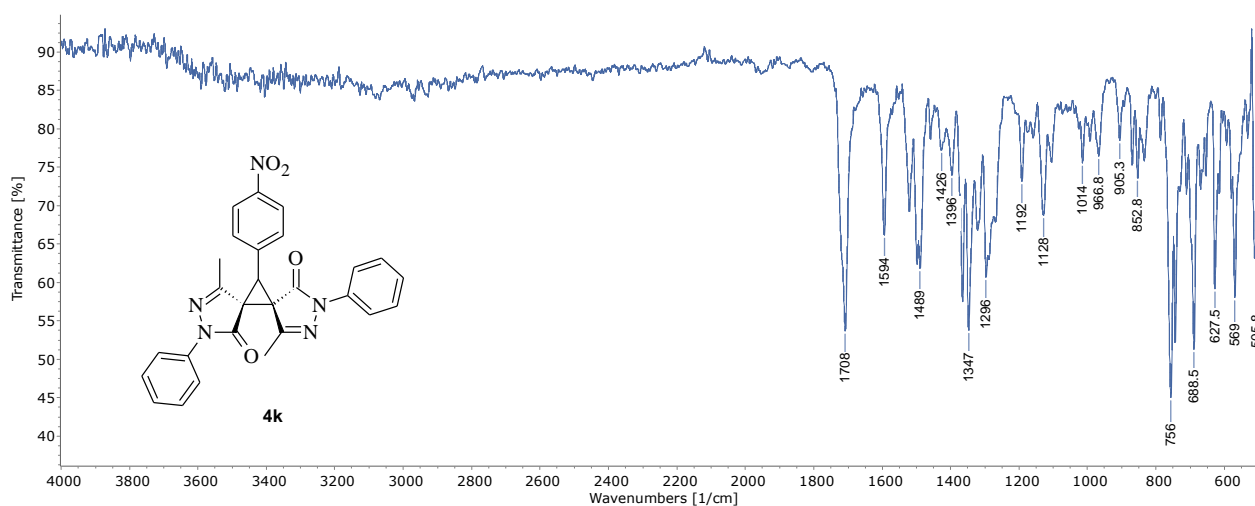
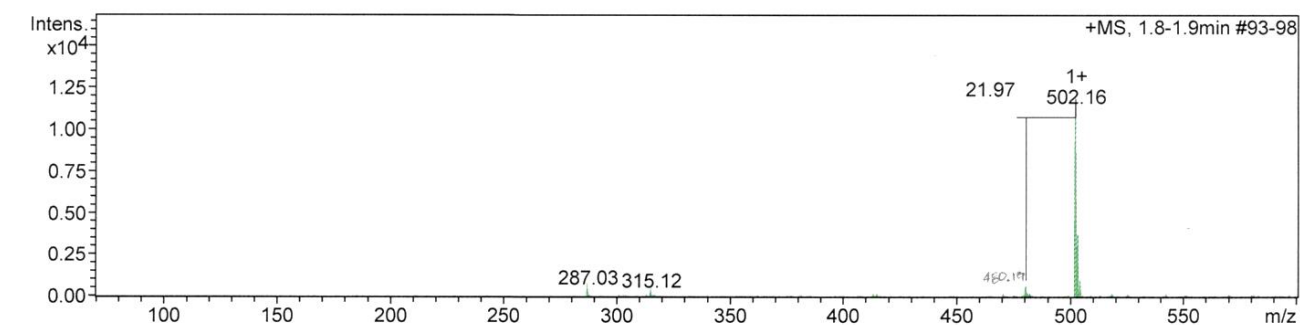


Figure S53. FTIR spectrum of compound **4k**

Acquisition Parameter

Ion Source Type	ESI	Ion Polarity	Positive	Alternating Ion Polarity	off
Mass Range Mode	UltraScan	Scan Begin	70 m/z	Scan End	600 m/z
Accumulation Time	200000 μ s	RF Level	63 %	Trap Drive	54.1
SPS Target Mass	400 m/z	Averages	5 Spectra	n/a	n/a



Component	Molecular Mass	Molecule	Absolute Abundance	Relative Abundance	
A	502.16	502.49	[M + H] ⁺	11262	100.00

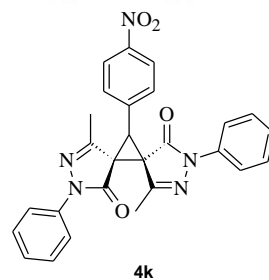


Figure S54. ESI-MS spectrum of compound **4k**

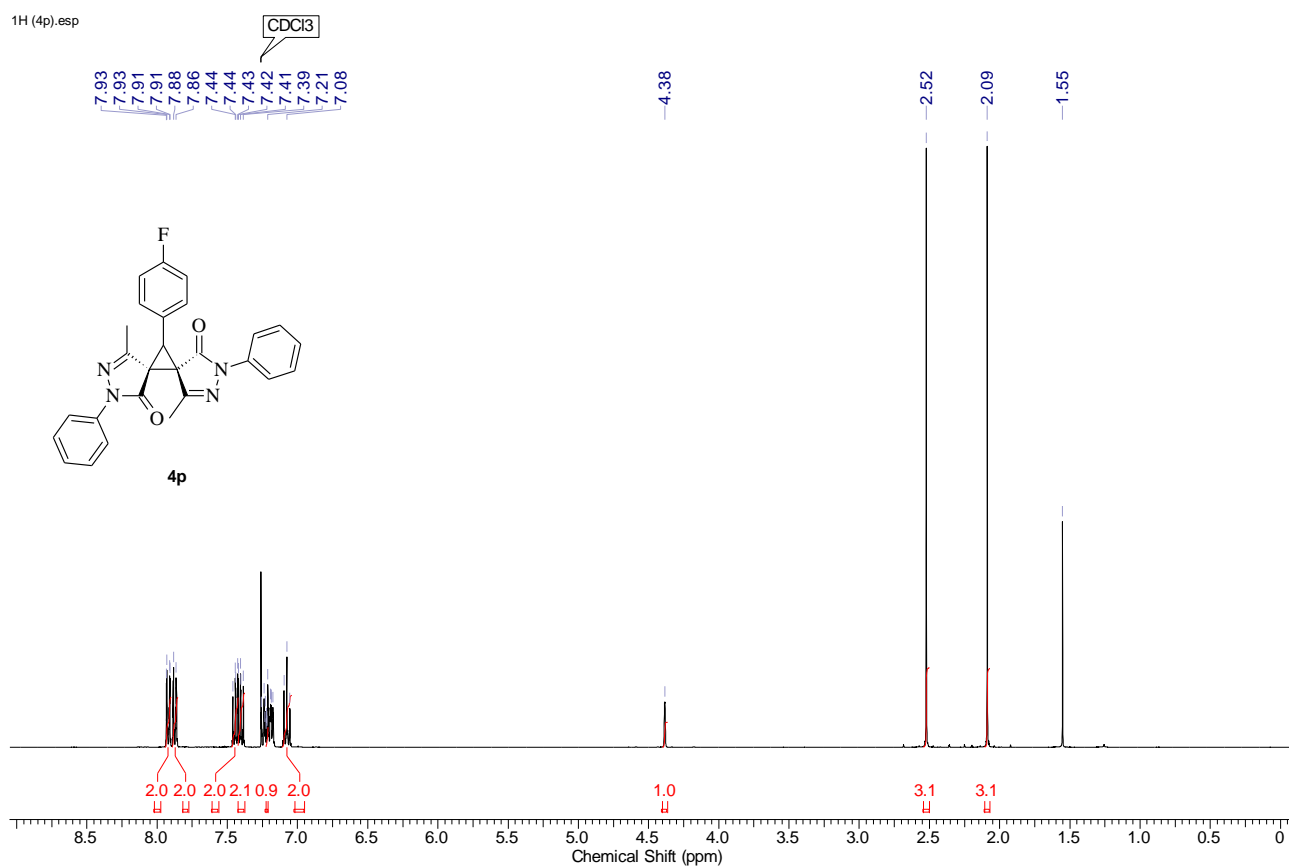


Figure S55. ^1H NMR spectrum of compound **4p**

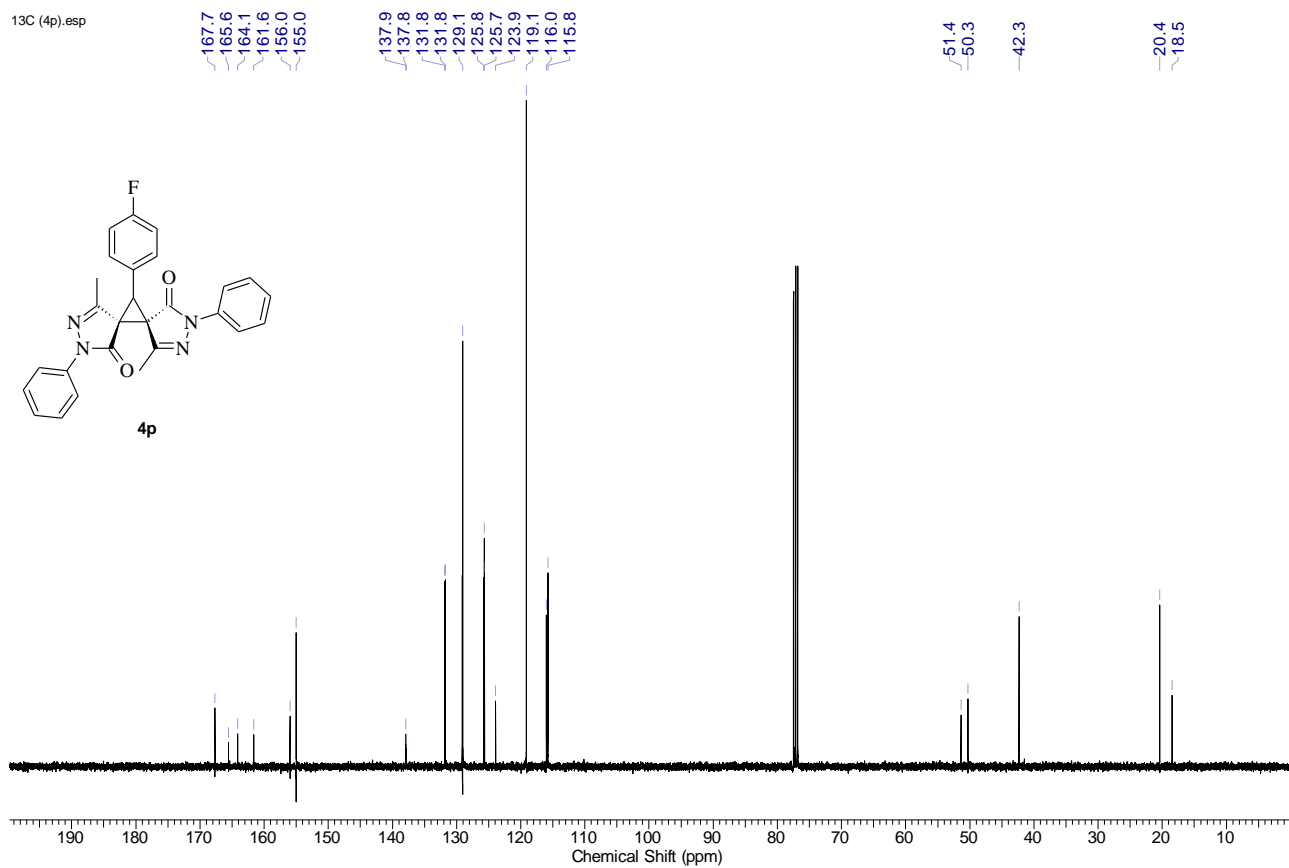


Figure S56. ^{13}C NMR spectrum of compound **4p**

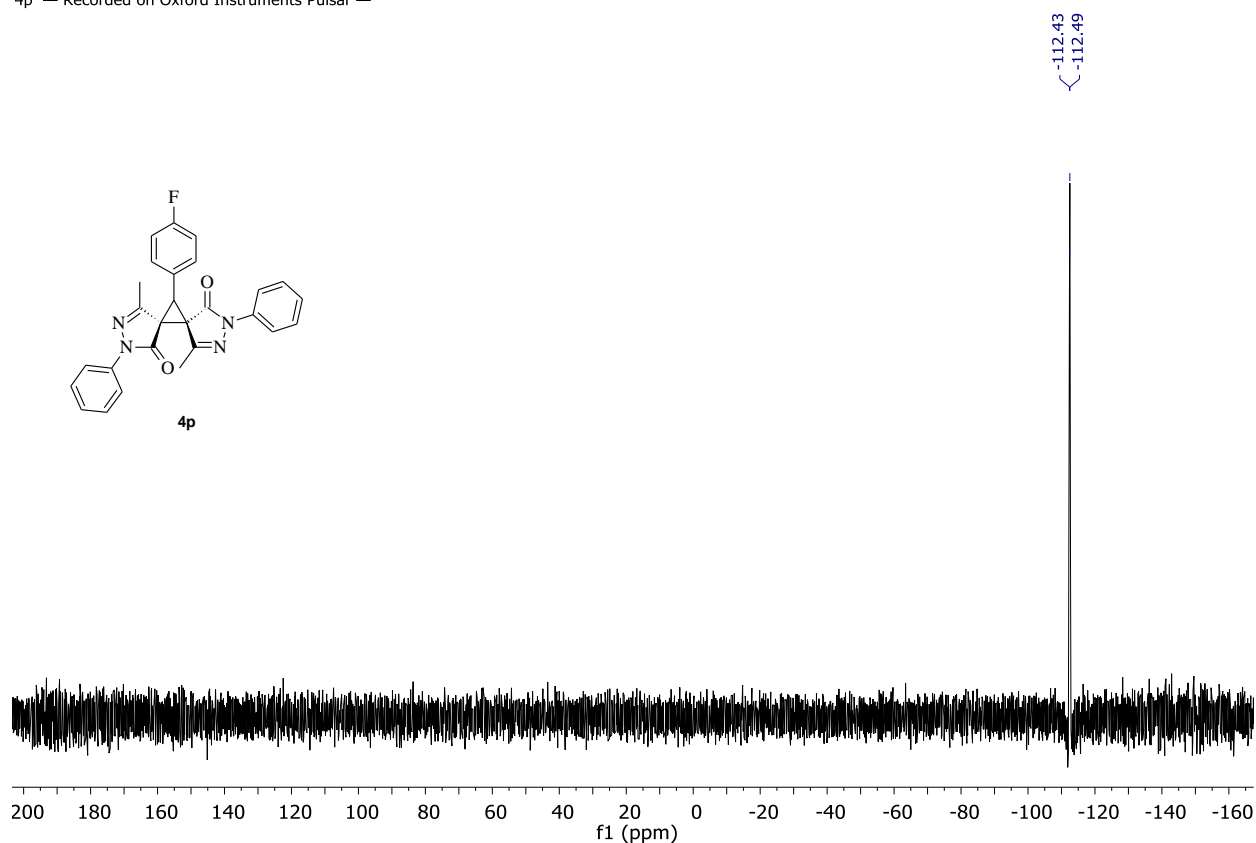


Figure S57. ^{19}F NMR spectrum of compound **4p**

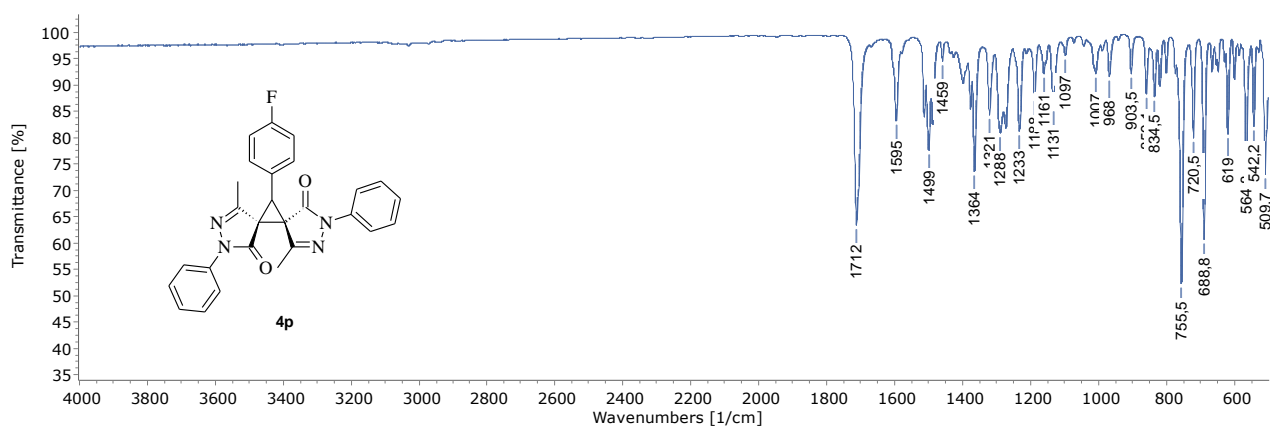


Figure S58. FTIR spectrum of compound **4p**

Acquisition Parameter

Ion Source Type	ESI	Ion Polarity	Positive	Alternating Ion Polarity	off
Mass Range Mode	UltraScan	Scan Begin	70 m/z	Scan End	600 m/z
Accumulation Time	9580 μ s	RF Level	63 %	Trap Drive	54.1
SPS Target Mass	400 m/z	Averages	5 Spectra	n/a	n/a

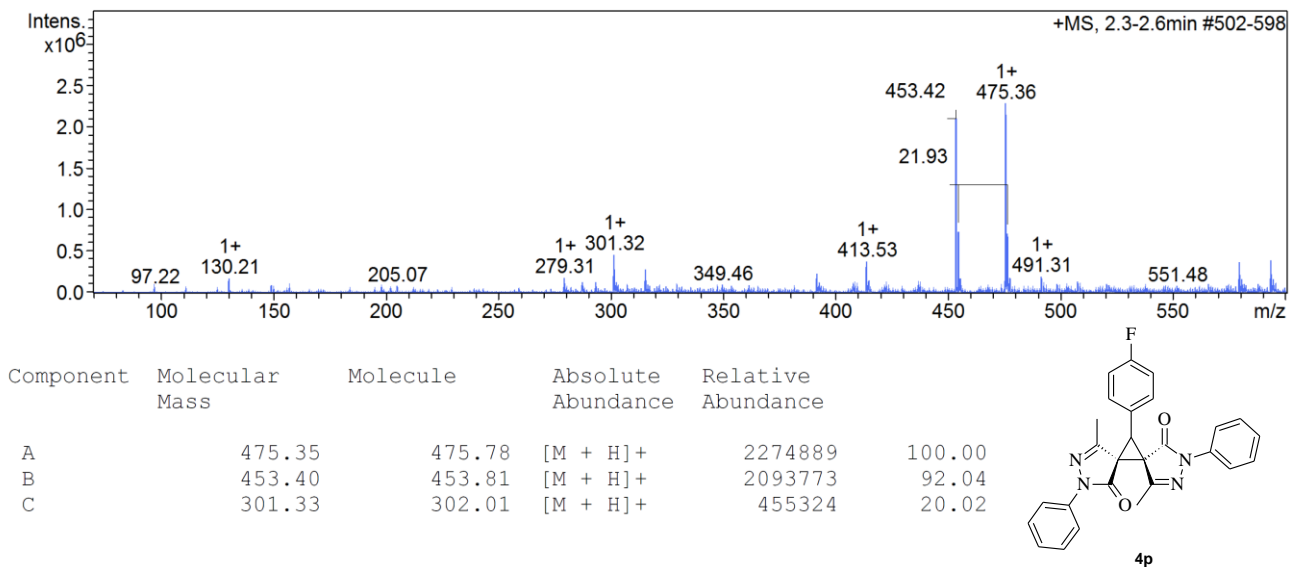


Figure S59. ESI-MS spectrum of compound 4p

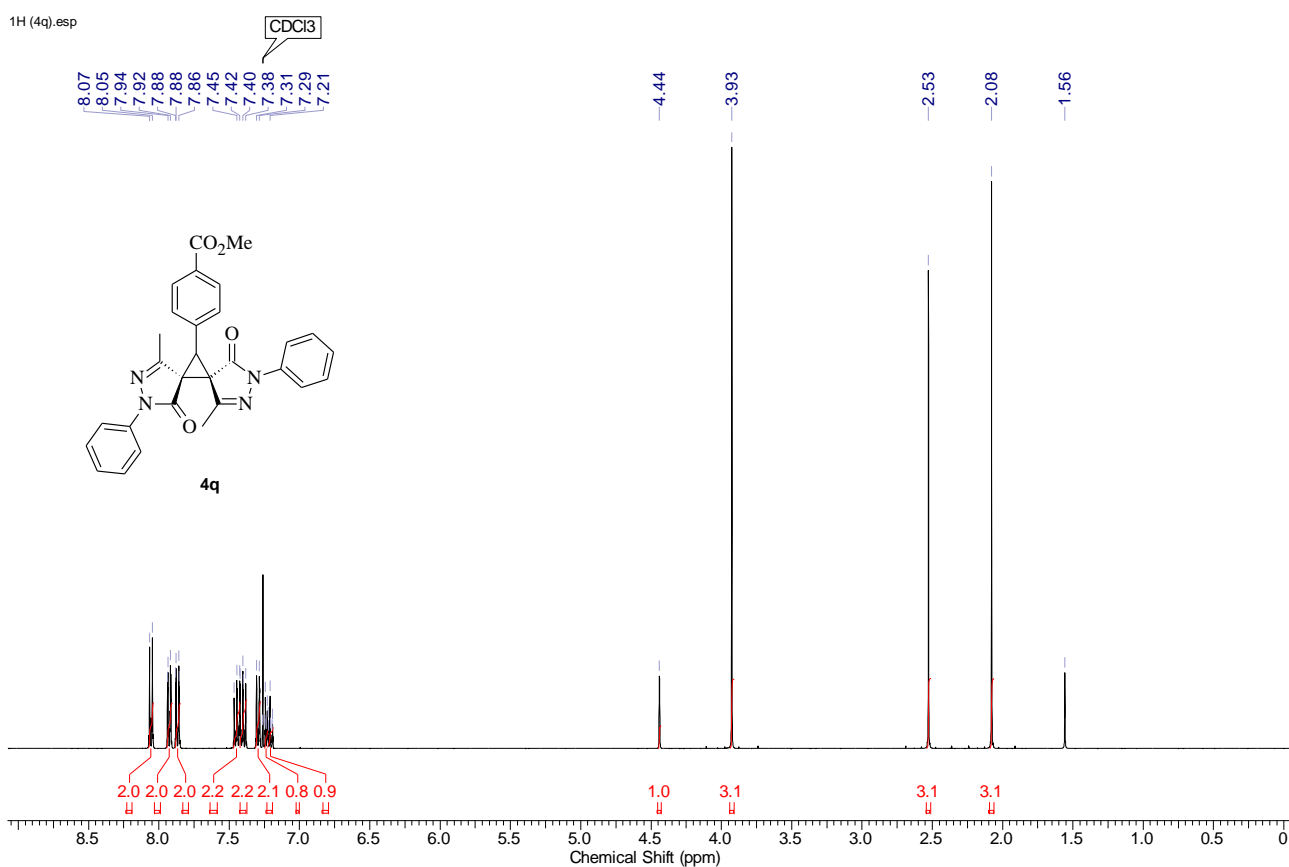


Figure S60. ¹H NMR spectrum of compound 4q

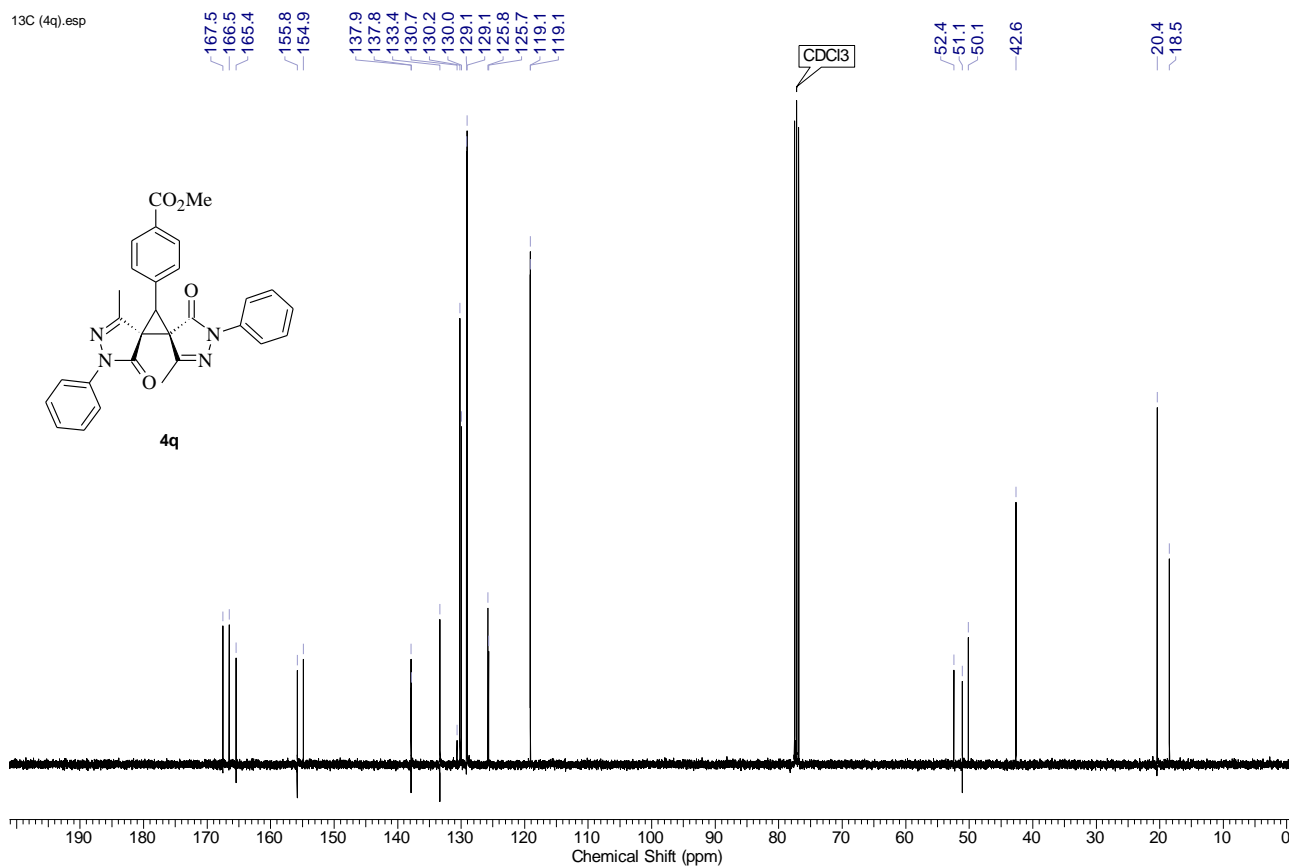


Figure S61. ¹³C NMR spectrum of compound **4q**

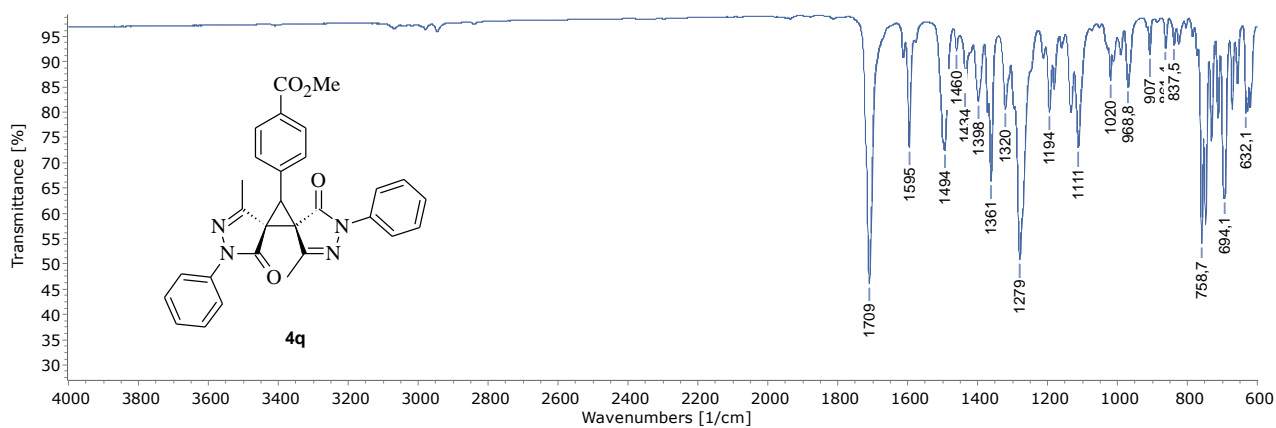


Figure S62. FTIR spectrum of compound **4q**

Acquisition Parameter

Ion Source Type	ESI	Ion Polarity	Positive	Alternating Ion Polarity	off
Mass Range Mode	UltraScan	Scan Begin	70 m/z	Scan End	600 m/z
Accumulation Time	6959 μ s	RF Level	63 %	Trap Drive	54.1
SPS Target Mass	400 m/z	Averages	5 Spectra	n/a	n/a

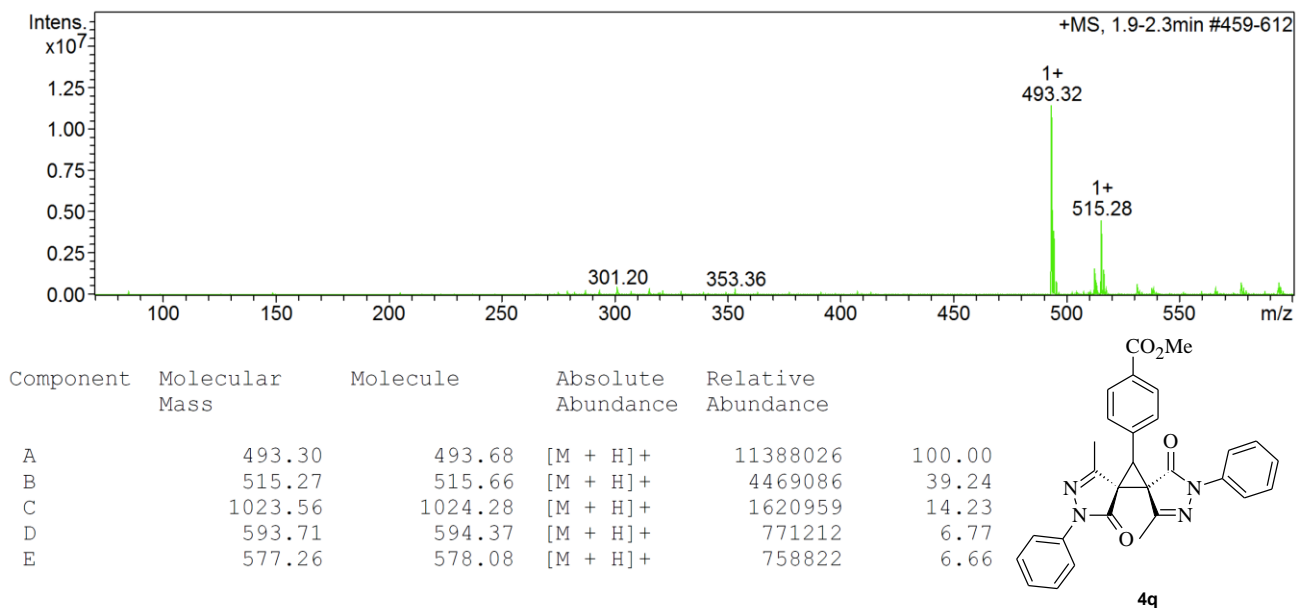


Figure S63. ESI-MS spectrum of compound **4q**

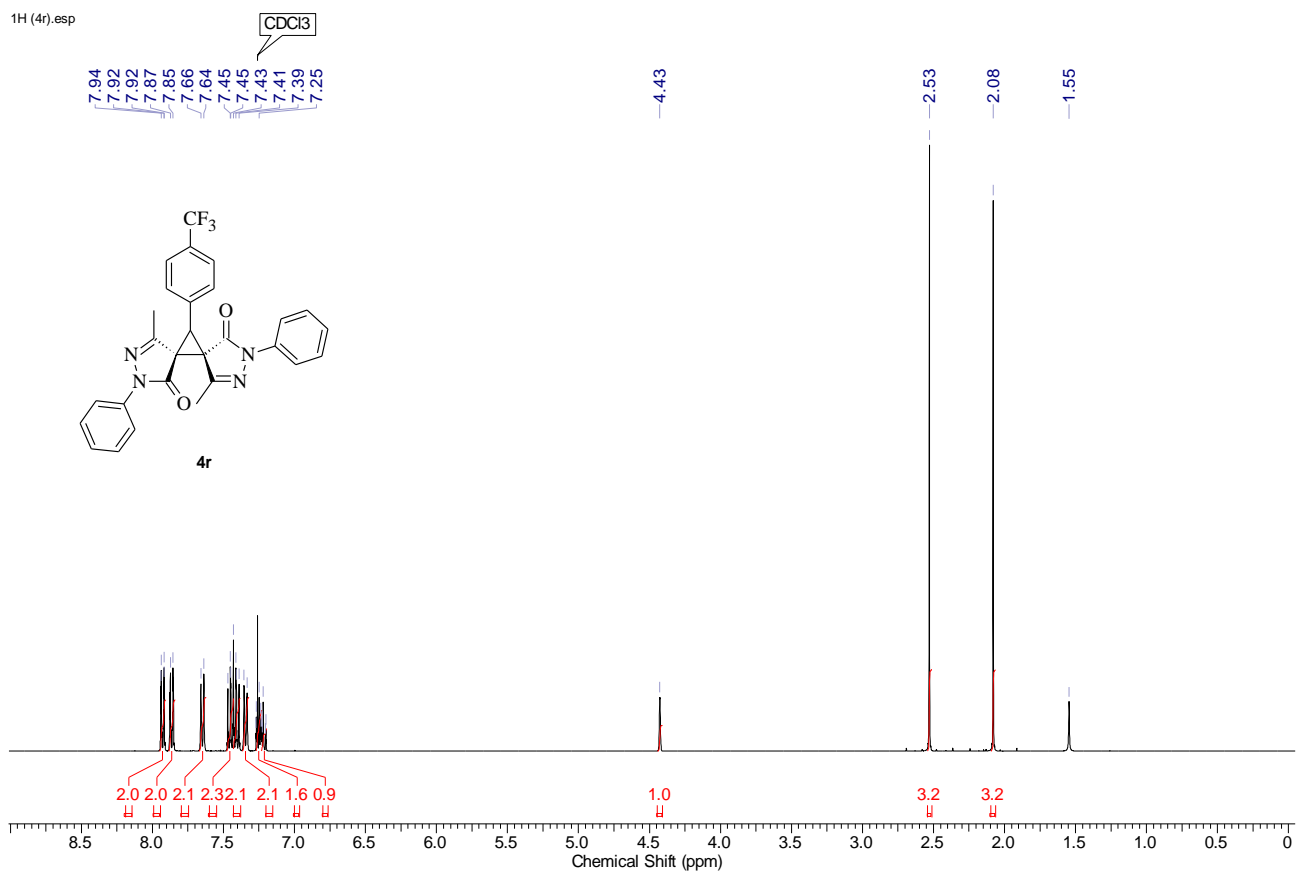


Figure S64. ¹H NMR spectrum of compound **4r**

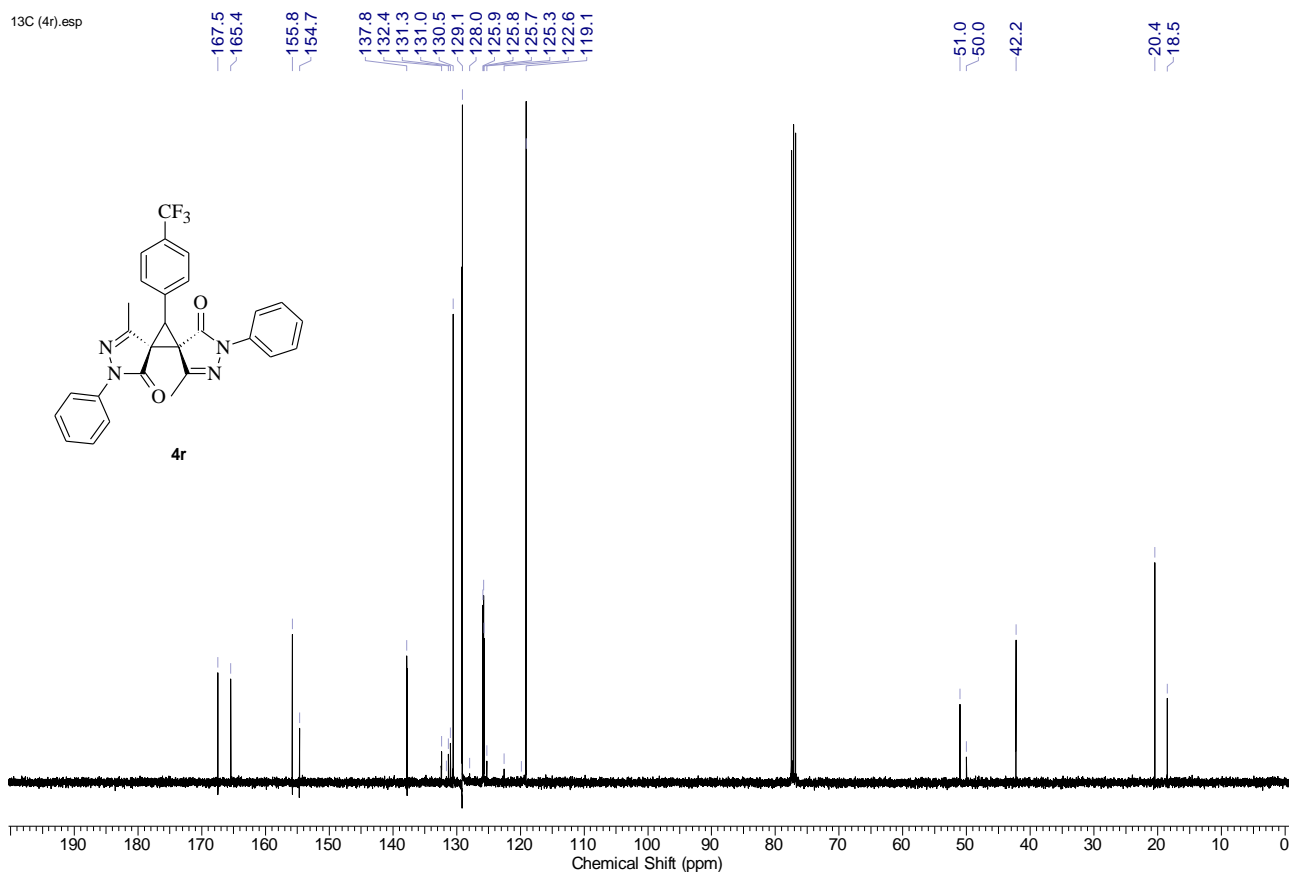


Figure S65. ^{13}C NMR spectrum of compound **4r**

4r — Recorded on Oxford Instruments Pulsar —

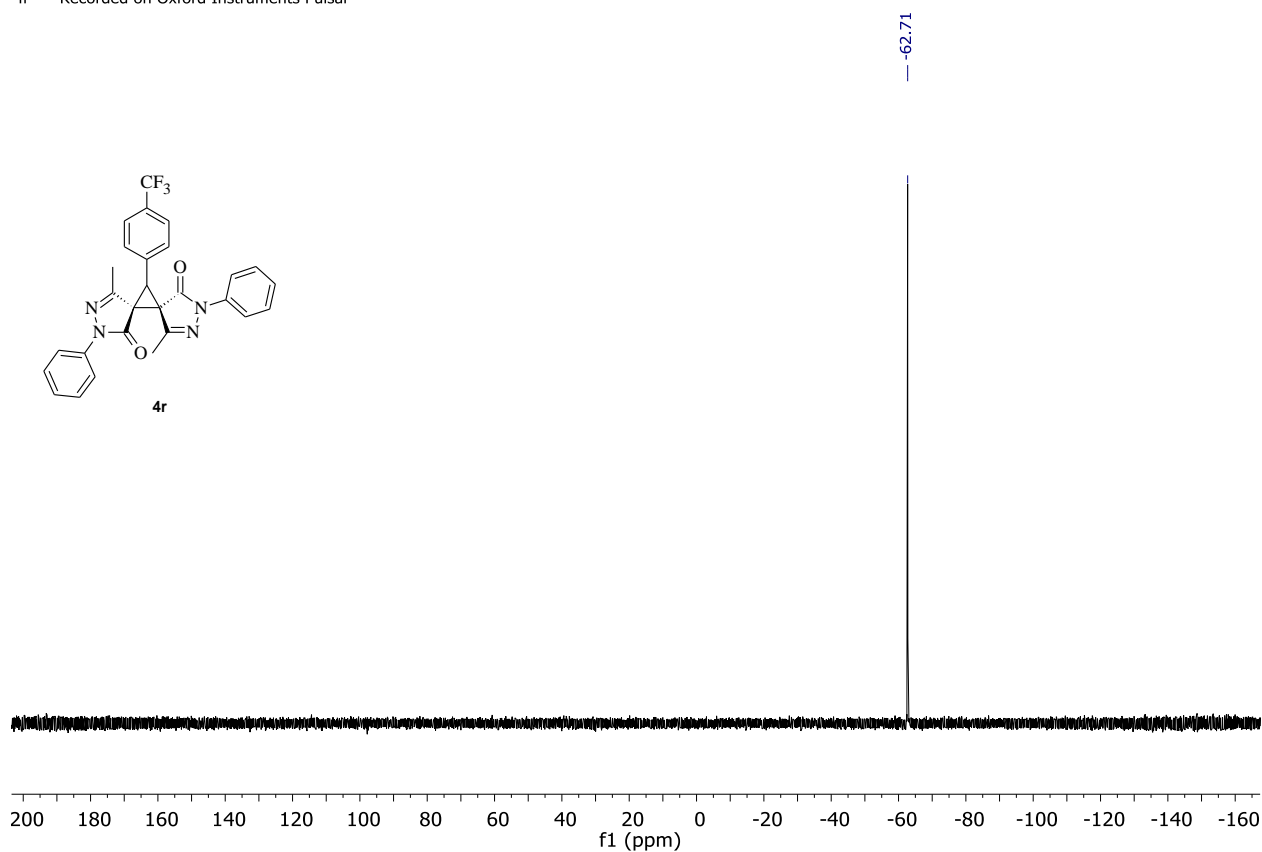


Figure S66. ^{19}F NMR spectrum of compound **4r**

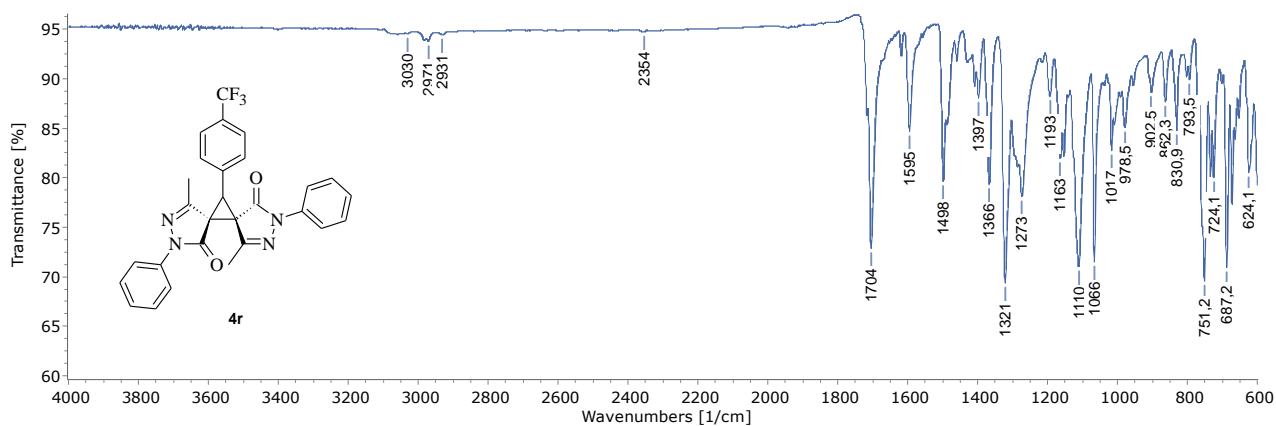
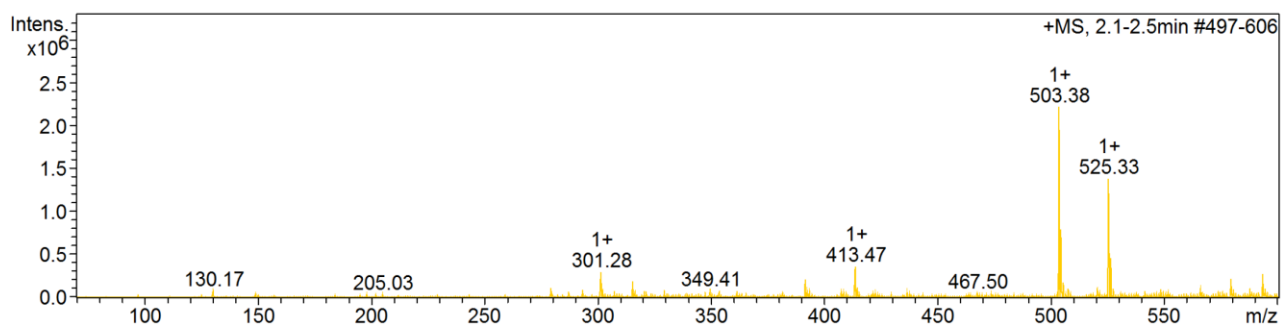


Figure S67. FTIR spectrum of compound **4r**

Acquisition Parameter

Ion Source Type	ESI	Ion Polarity	Positive	Alternating Ion Polarity	off
Mass Range Mode	UltraScan	Scan Begin	70 m/z	Scan End	600 m/z
Accumulation Time	13368 μ s	RF Level	63 %	Trap Drive	54.1
SPS Target Mass	400 m/z	Averages	5 Spectra	n/a	n/a



Component	Molecular Mass	Molecule	Absolute Abundance	Relative Abundance	
A	503.36	503.75	[M + H] ⁺	2206733	100.00
B	525.32	525.72	[M + H] ⁺	1378402	62.46
C	413.47	413.97	[M + H] ⁺	356961	16.18
D	301.28	301.90	[M + H] ⁺	294262	13.33

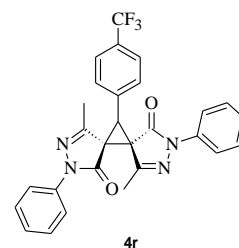


Figure S68. ESI-MS spectrum of compound **4r**

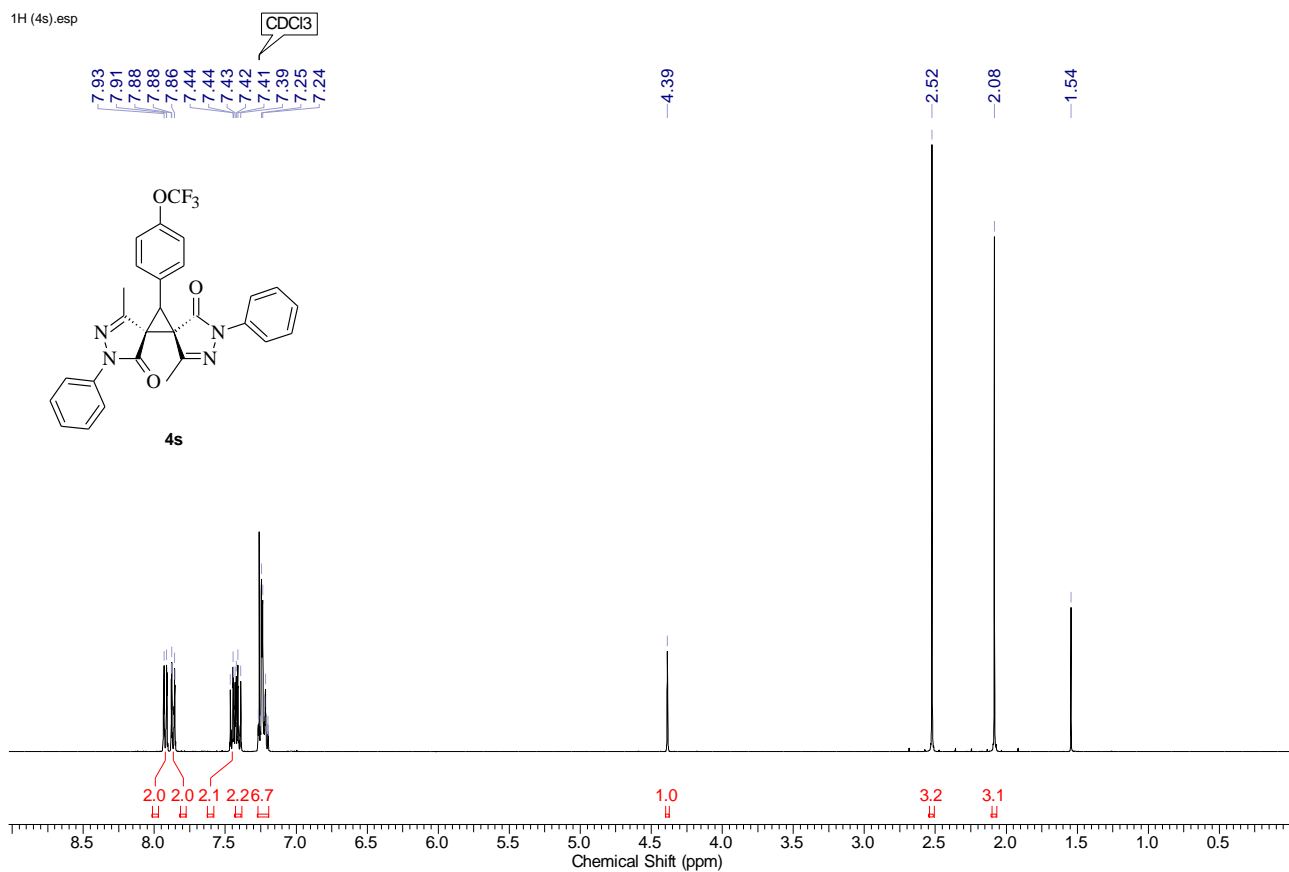


Figure S69. ¹H NMR spectrum of compound 4s

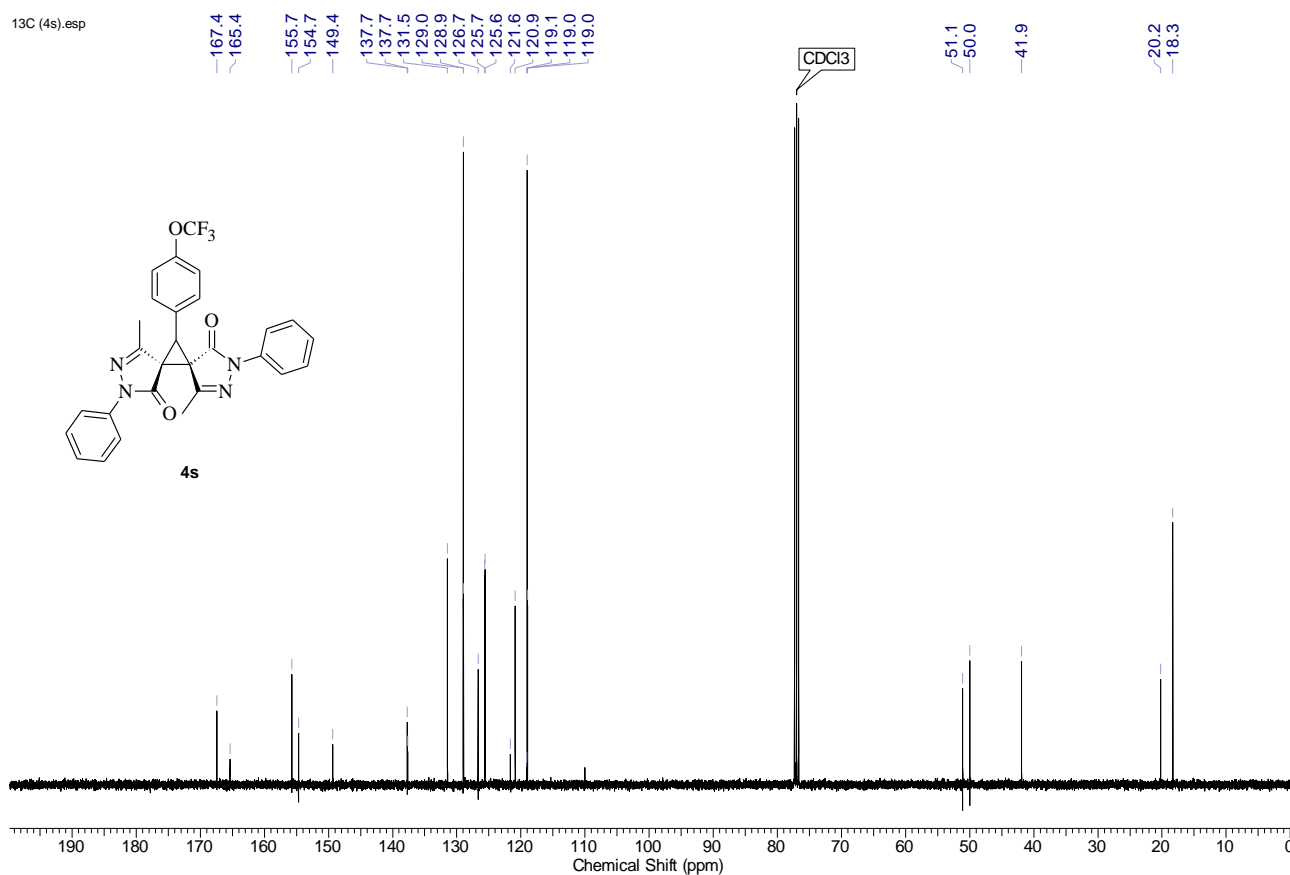


Figure S70. ¹³C NMR spectrum of compound 4s

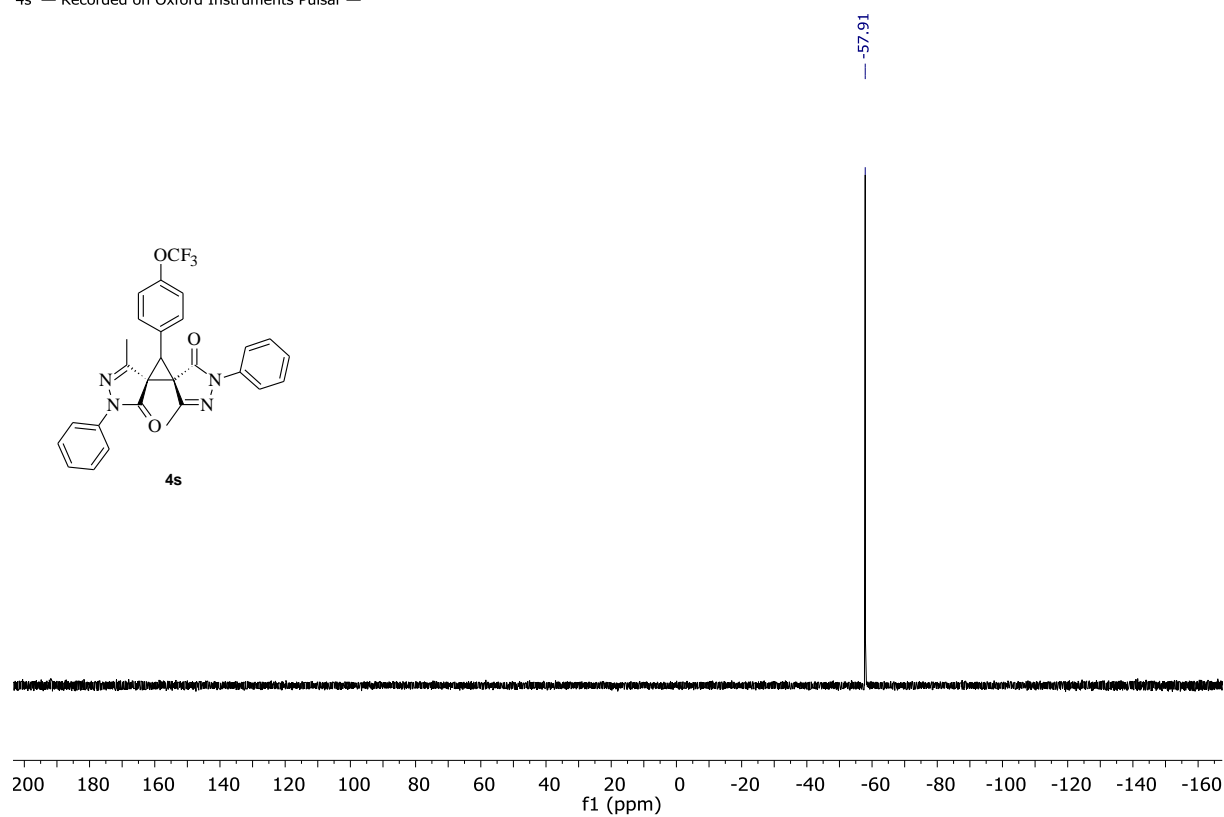


Figure S71. ¹⁹F NMR spectrum of compound 4s

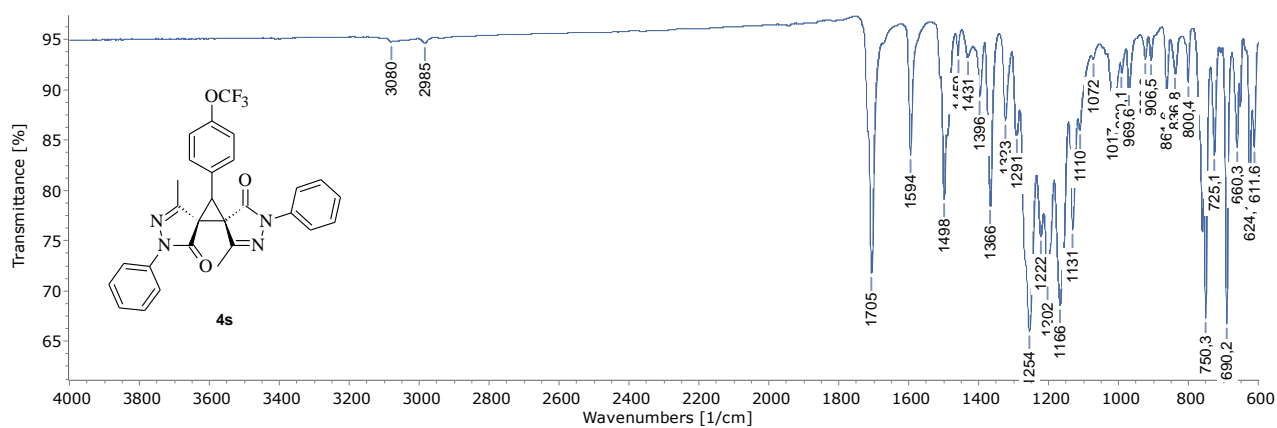


Figure S72. FTIR spectrum of compound 4s

Acquisition Parameter

Ion Source Type	ESI	Ion Polarity	Positive	Alternating Ion Polarity	off
Mass Range Mode	UltraScan	Scan Begin	70 m/z	Scan End	600 m/z
Accumulation Time	9765 μ s	RF Level	63 %	Trap Drive	54.1
SPS Target Mass	400 m/z	Averages	5 Spectra	n/a	n/a

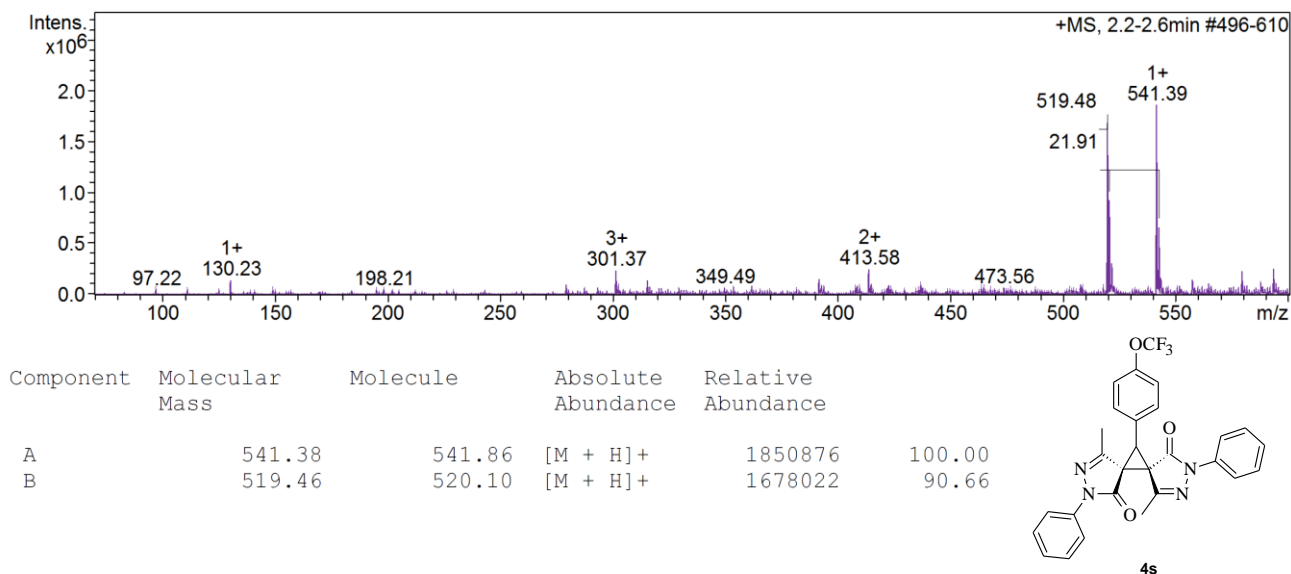


Figure S73. ESI-MS spectrum of compound 4s

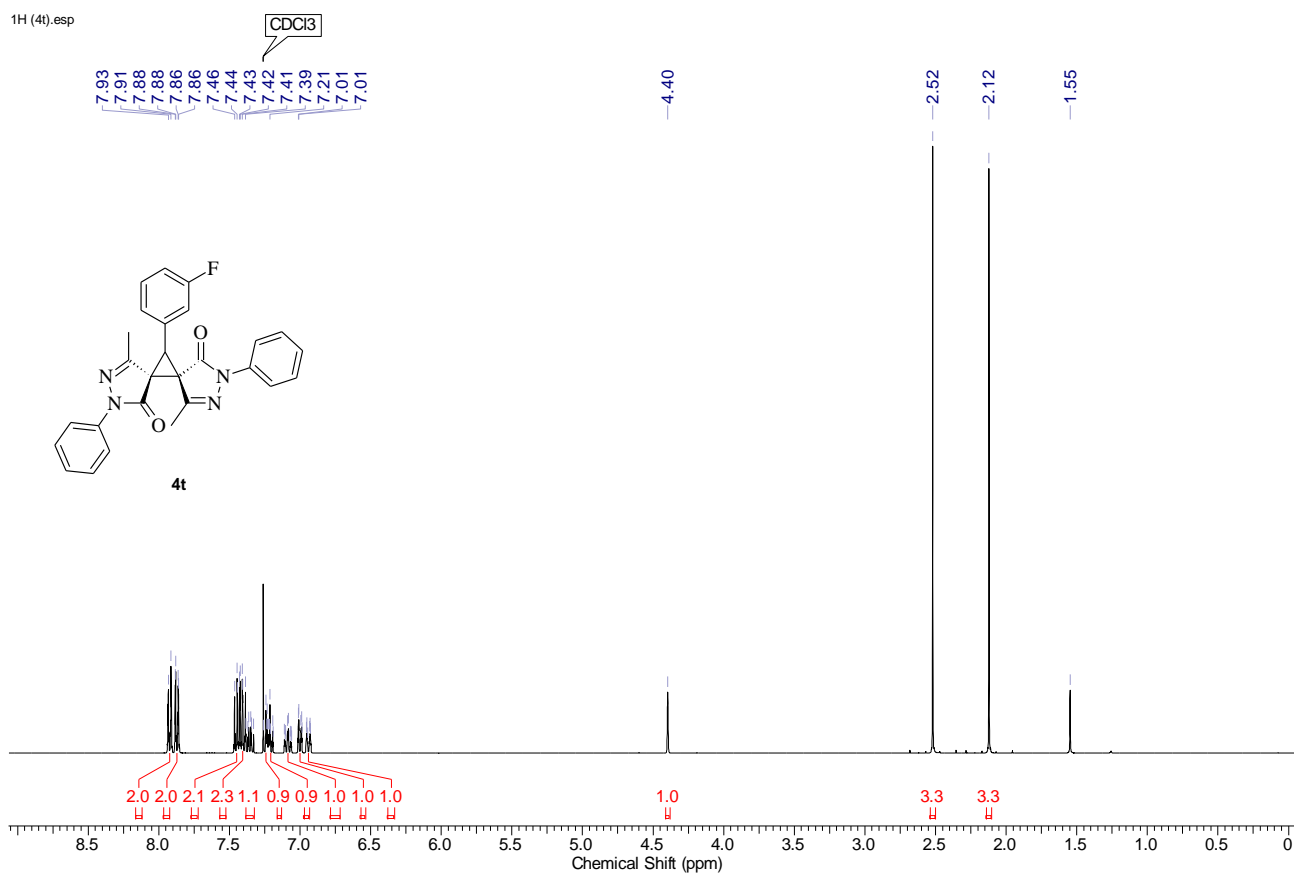


Figure S74. ¹H NMR spectrum of compound 4t

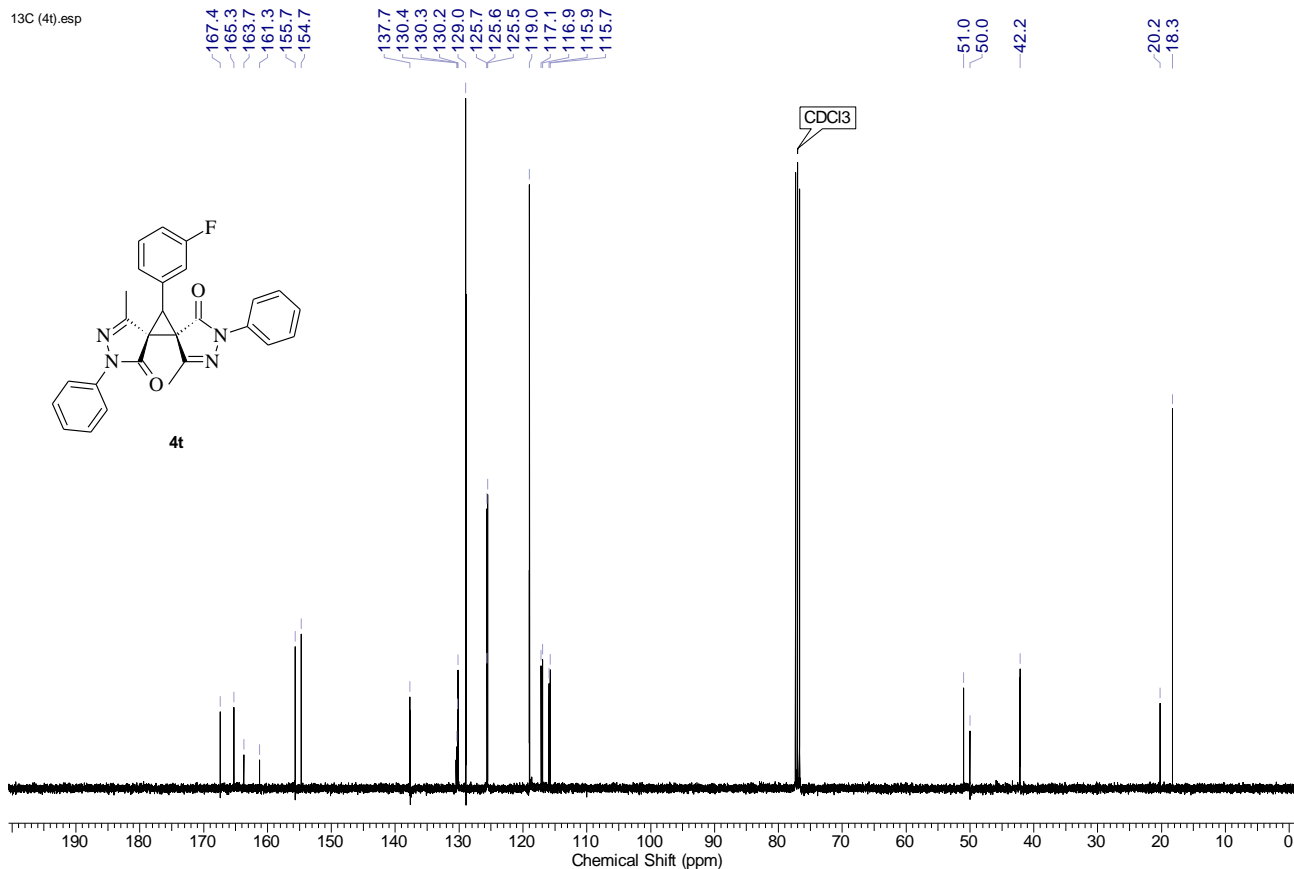


Figure S75. ^{13}C NMR spectrum of compound **4t**

4t — Recorded on Oxford Instruments Pulsar —

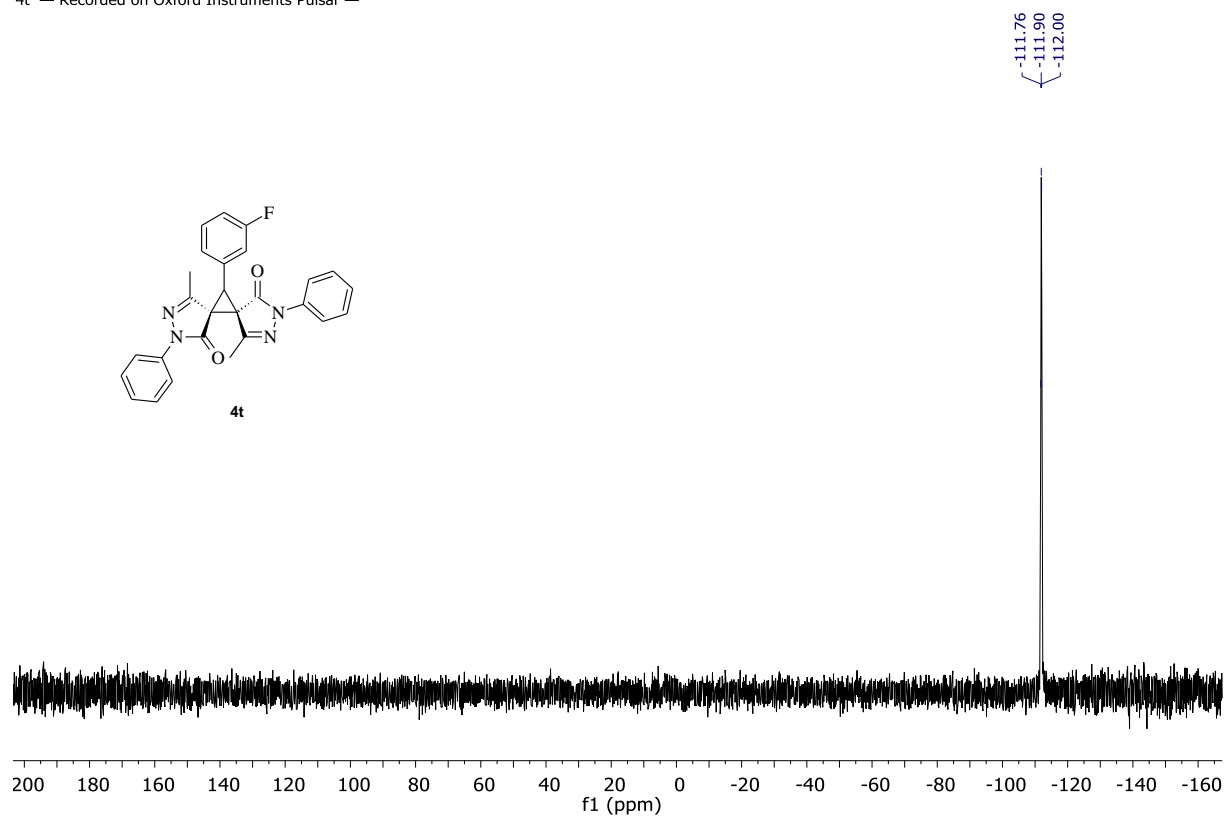


Figure S76. ^{19}F NMR spectrum of compound **4t**

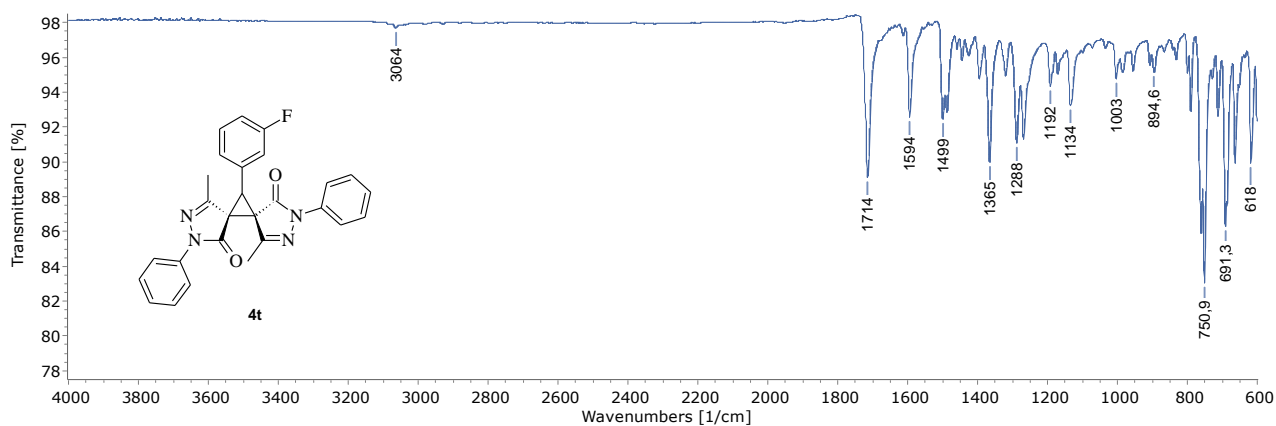
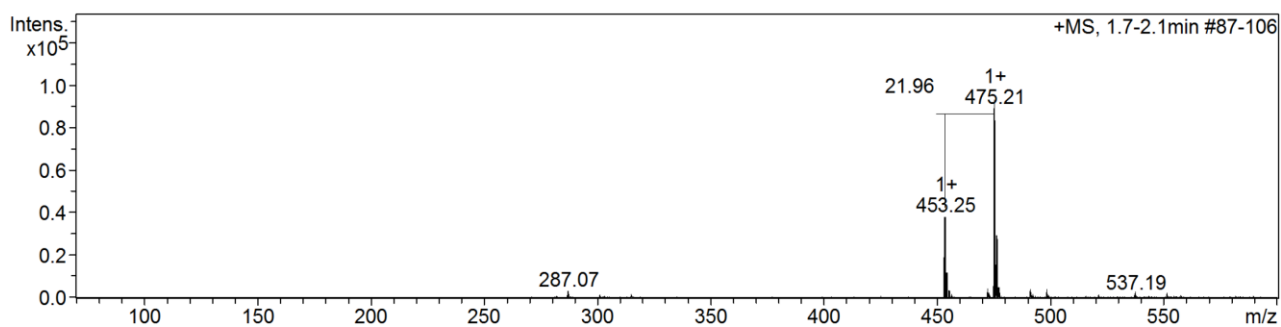


Figure S77. FTIR spectrum of compound **4t**

Acquisition Parameter

Ion Source Type	ESI	Ion Polarity	Positive	Alternating Ion Polarity	off
Mass Range Mode	UltraScan	Scan Begin	70 m/z	Scan End	600 m/z
Accumulation Time	200000 μ s	RF Level	63 %	Trap Drive	54.1
SPS Target Mass	400 m/z	Averages	5 Spectra	n/a	n/a



Component	Molecular Mass	Molecule	Absolute Abundance	Relative Abundance	
A	475.19	475.53	[M + H] ⁺	88901	100.00
B	453.24	453.65	[M + H] ⁺	37933	42.67

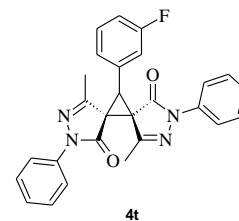


Figure S78. ESI-MS spectrum of compound **4t**

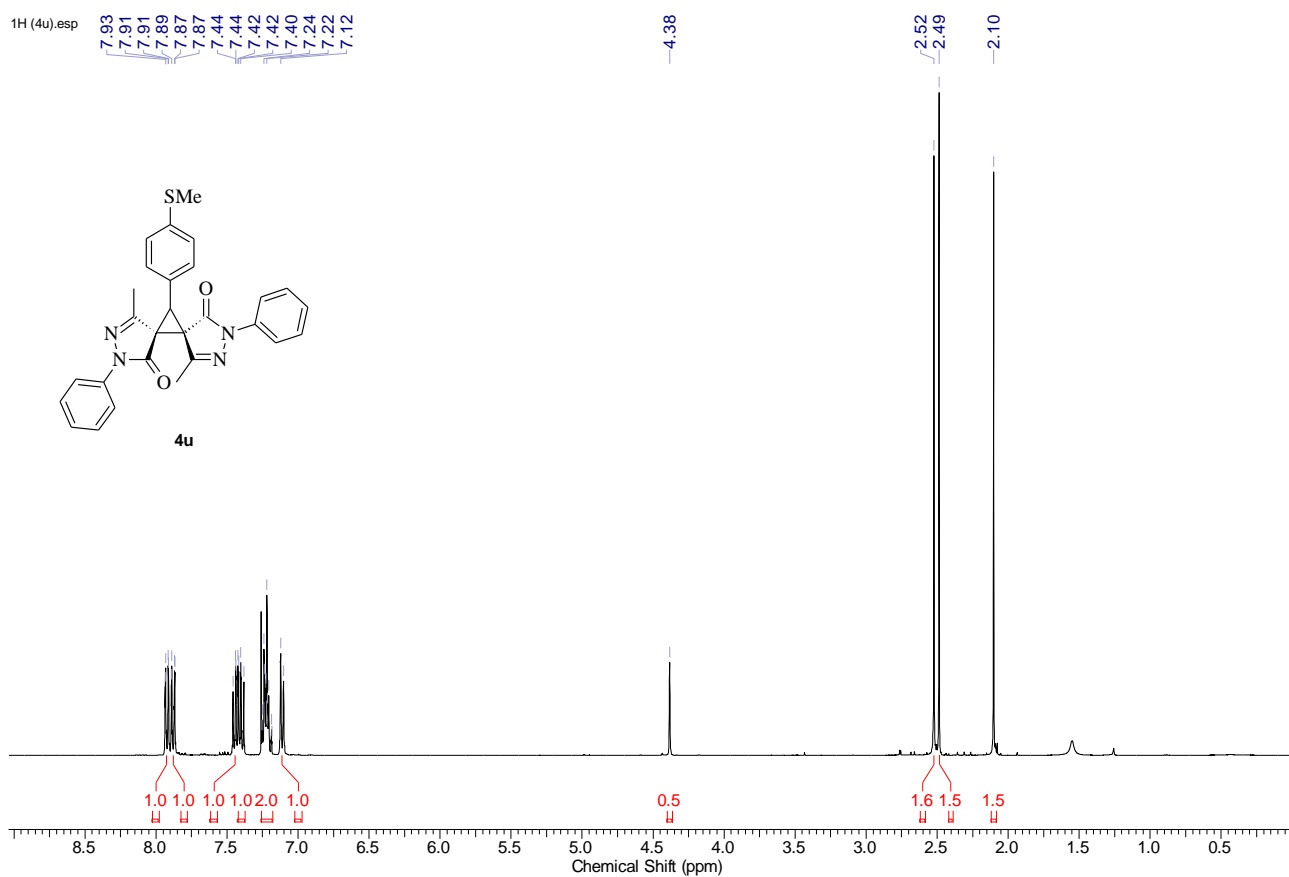


Figure S79. ¹H NMR spectrum of compound **4u**

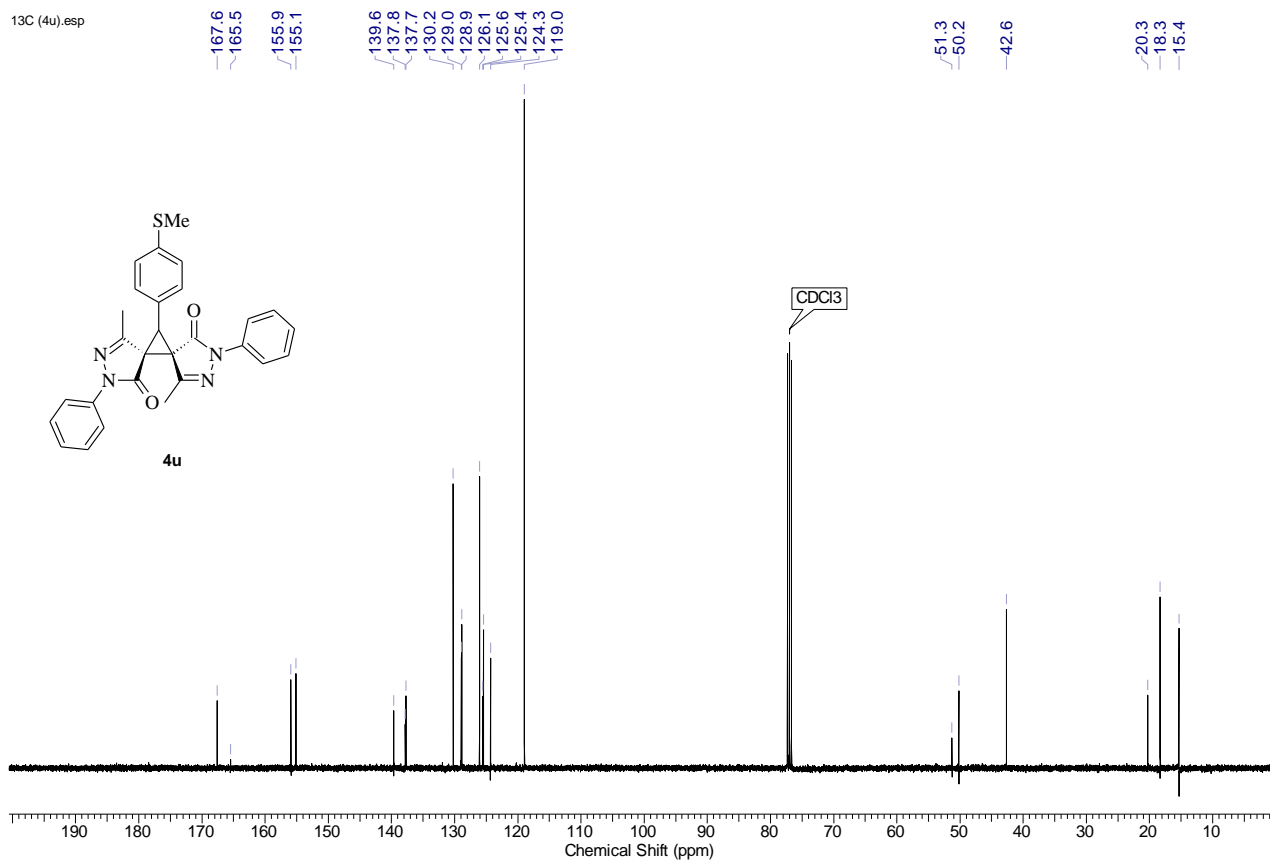


Figure S80. ¹³C NMR spectrum of compound **4u**

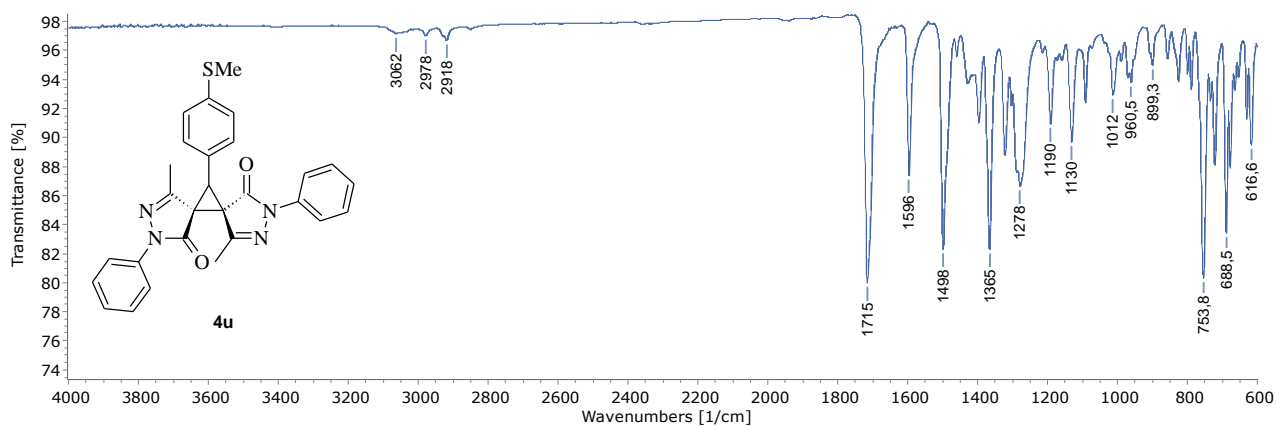


Figure S81. FTIR spectrum of compound **4u**

Acquisition Parameter

Ion Source Type	ESI	Ion Polarity	Positive	Alternating Ion Polarity	off
Mass Range Mode	UltraScan	Scan Begin	70 m/z	Scan End	600 m/z
Accumulation Time	200000 μ s	RF Level	63 %	Trap Drive	54.1
SPS Target Mass	400 m/z	Averages	5 Spectra	n/a	n/a

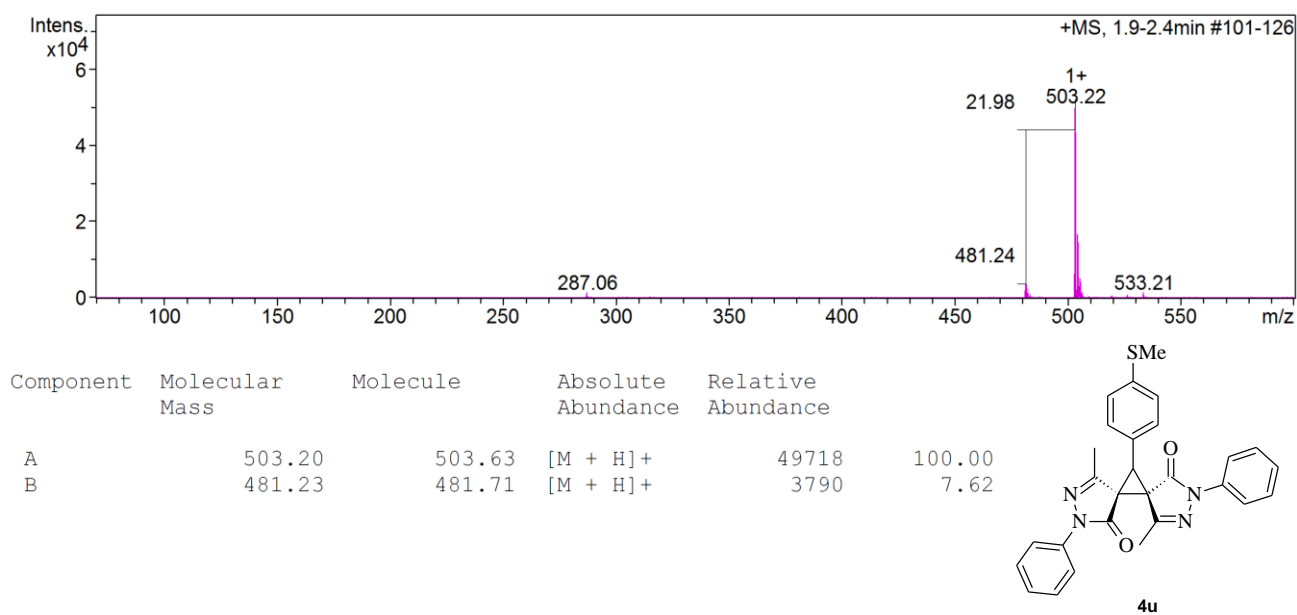


Figure S82. ESI-MS spectrum of compound **4u**

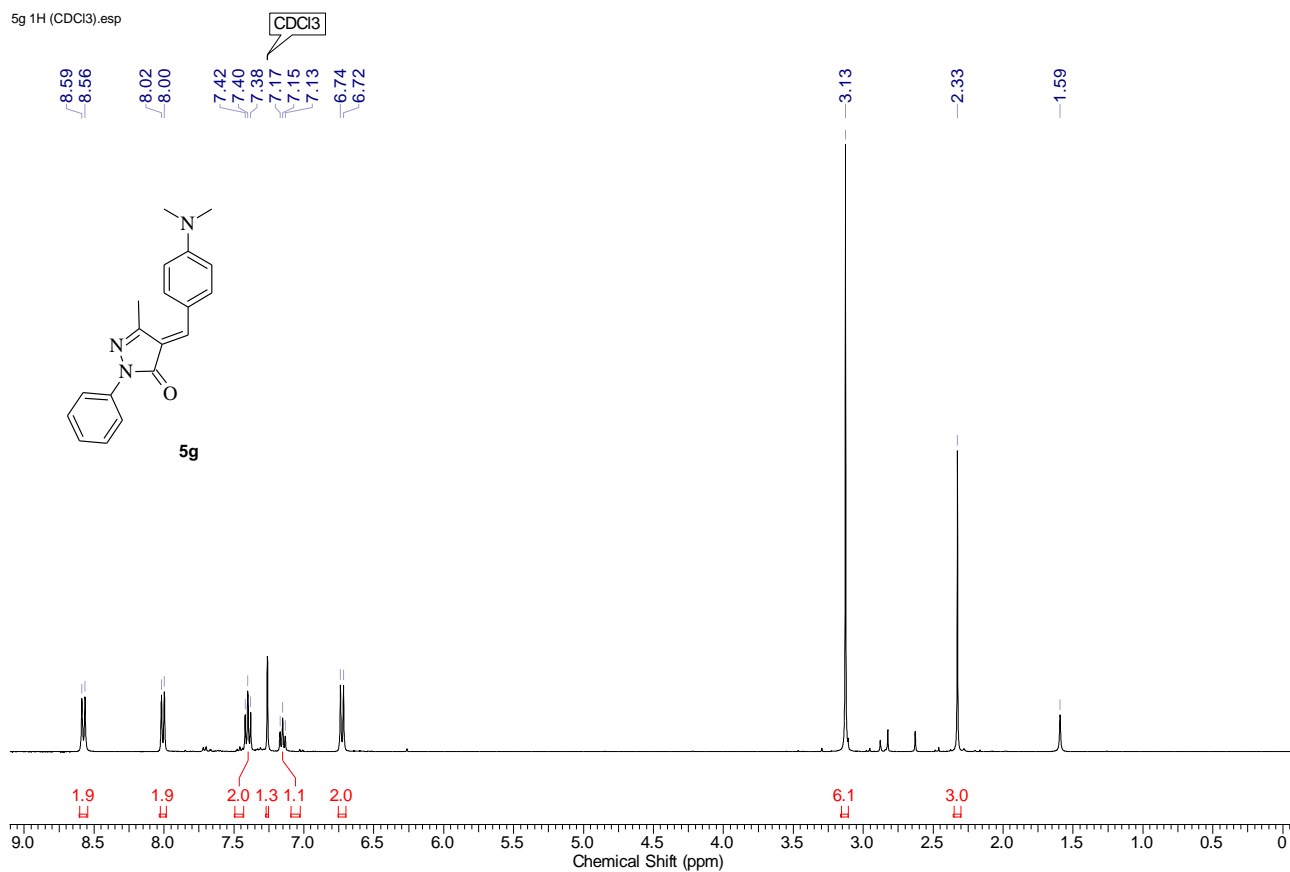


Figure S83. ¹H NMR spectrum of compound 5g

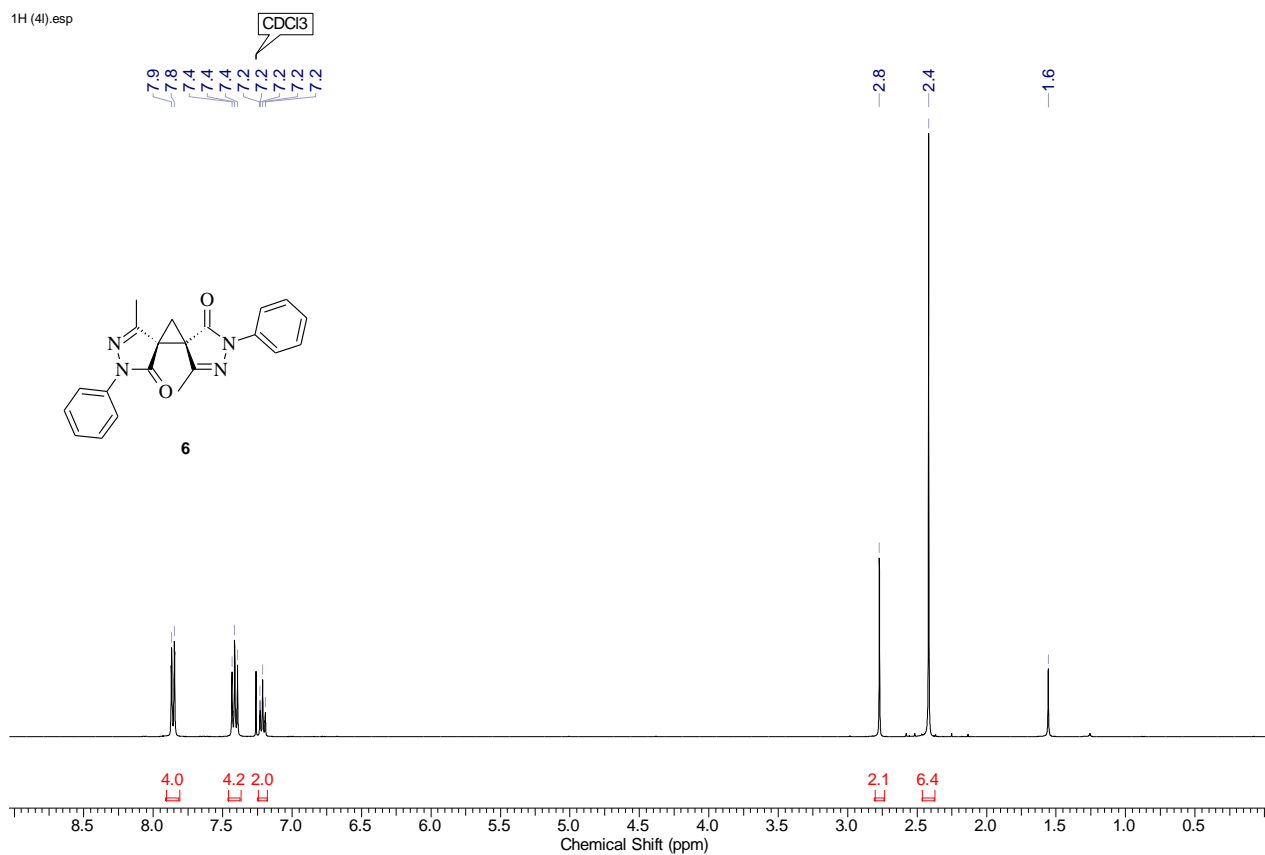


Figure S84. ¹H NMR spectrum of compound 6

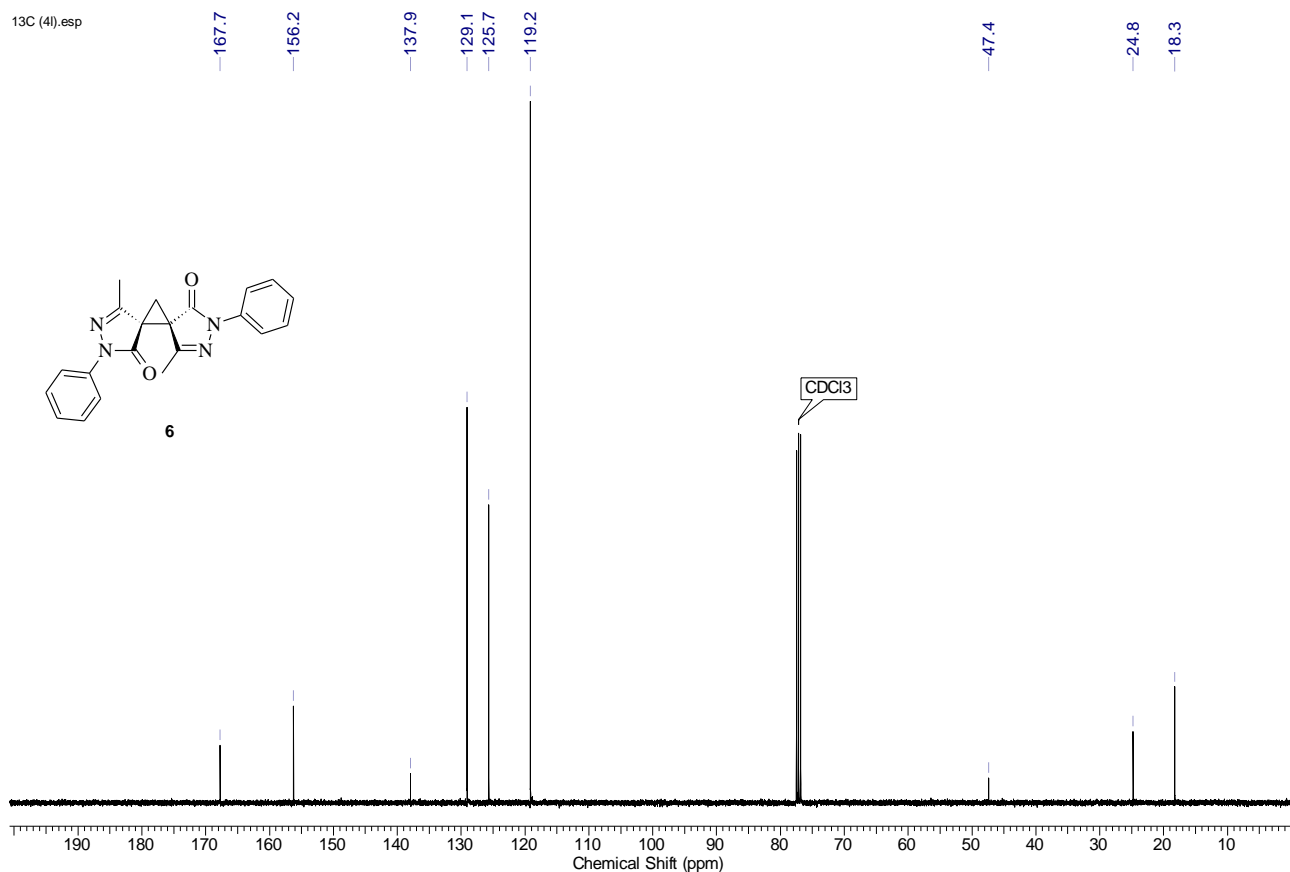


Figure S85. ¹³C NMR spectrum of compound 6

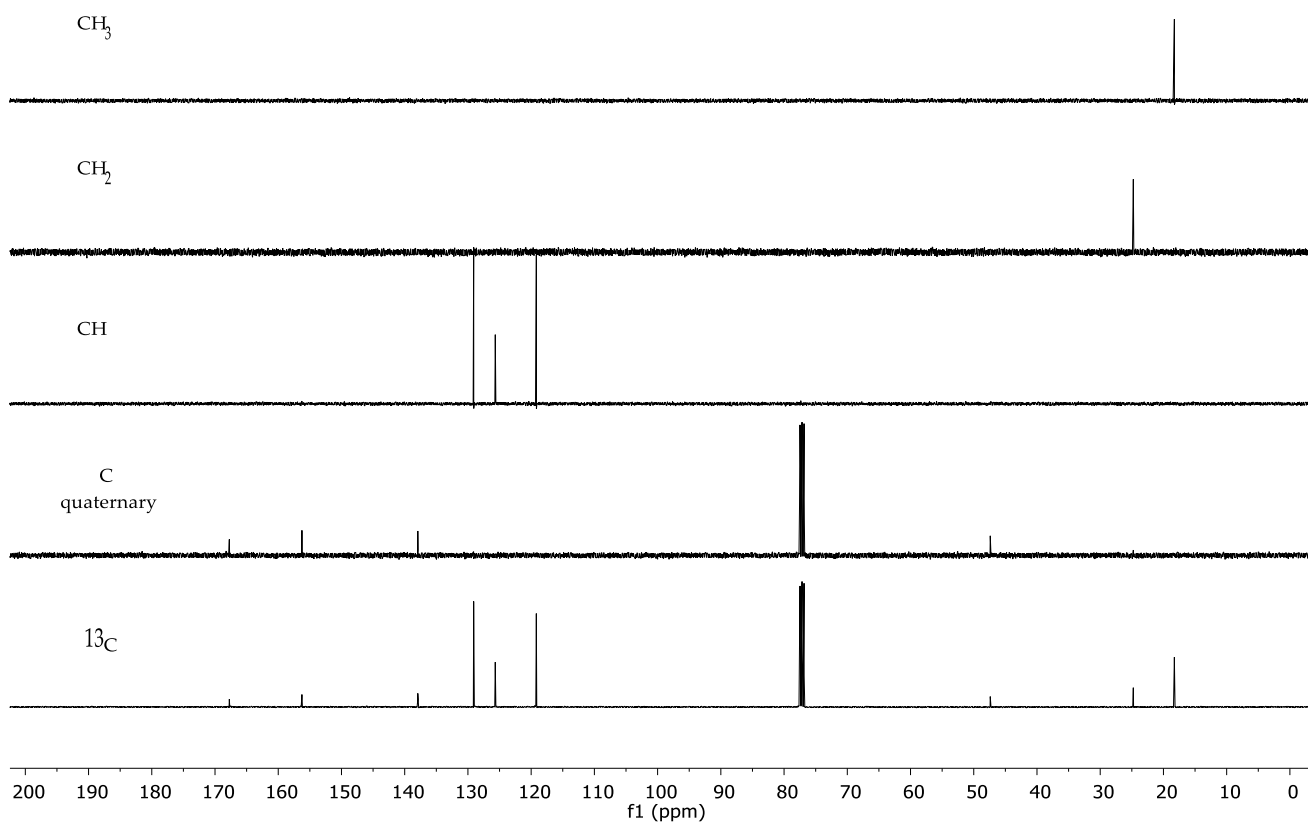


Figure S86. DEPT spectrum of compound 6

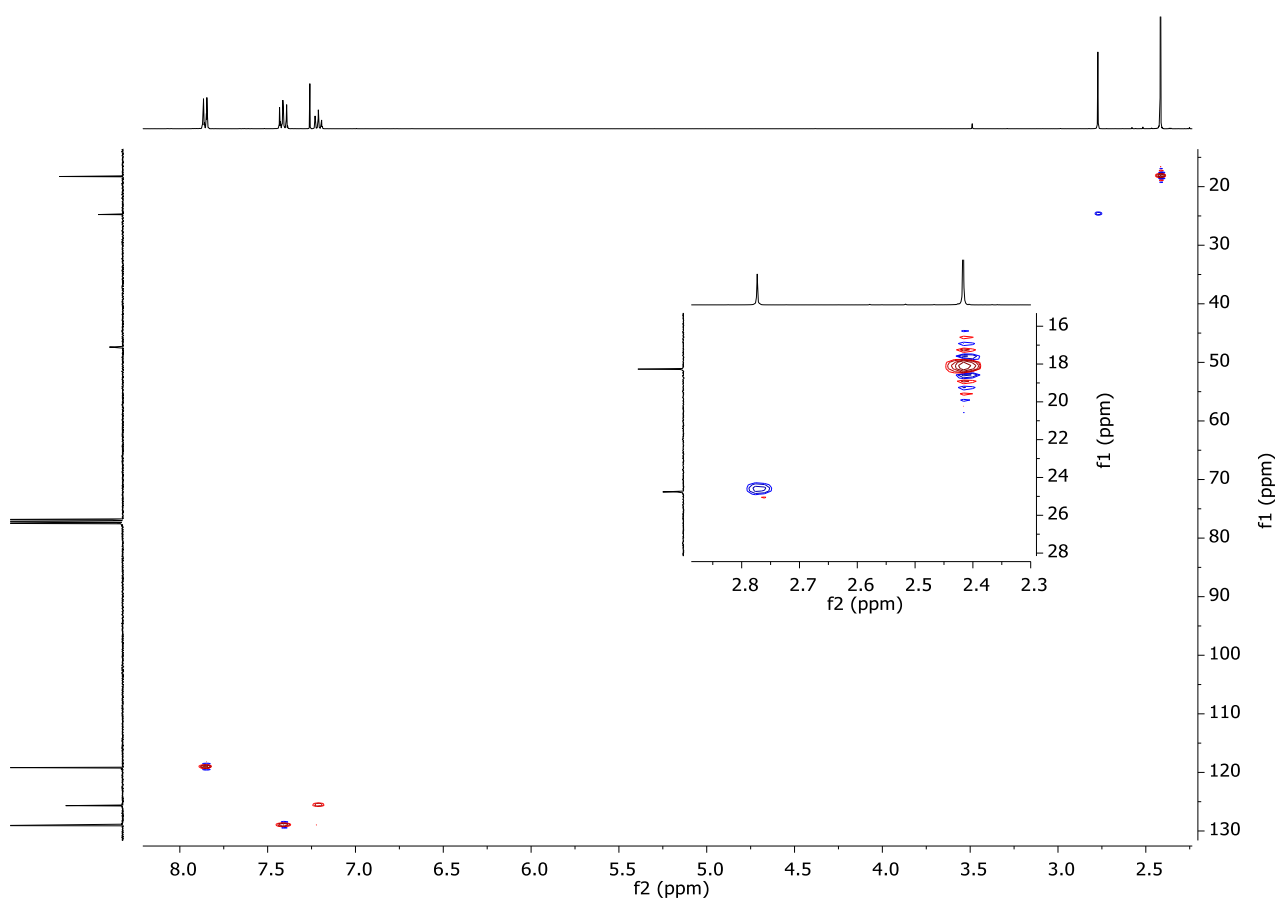


Figure S87. HSQC spectrum of compound 6

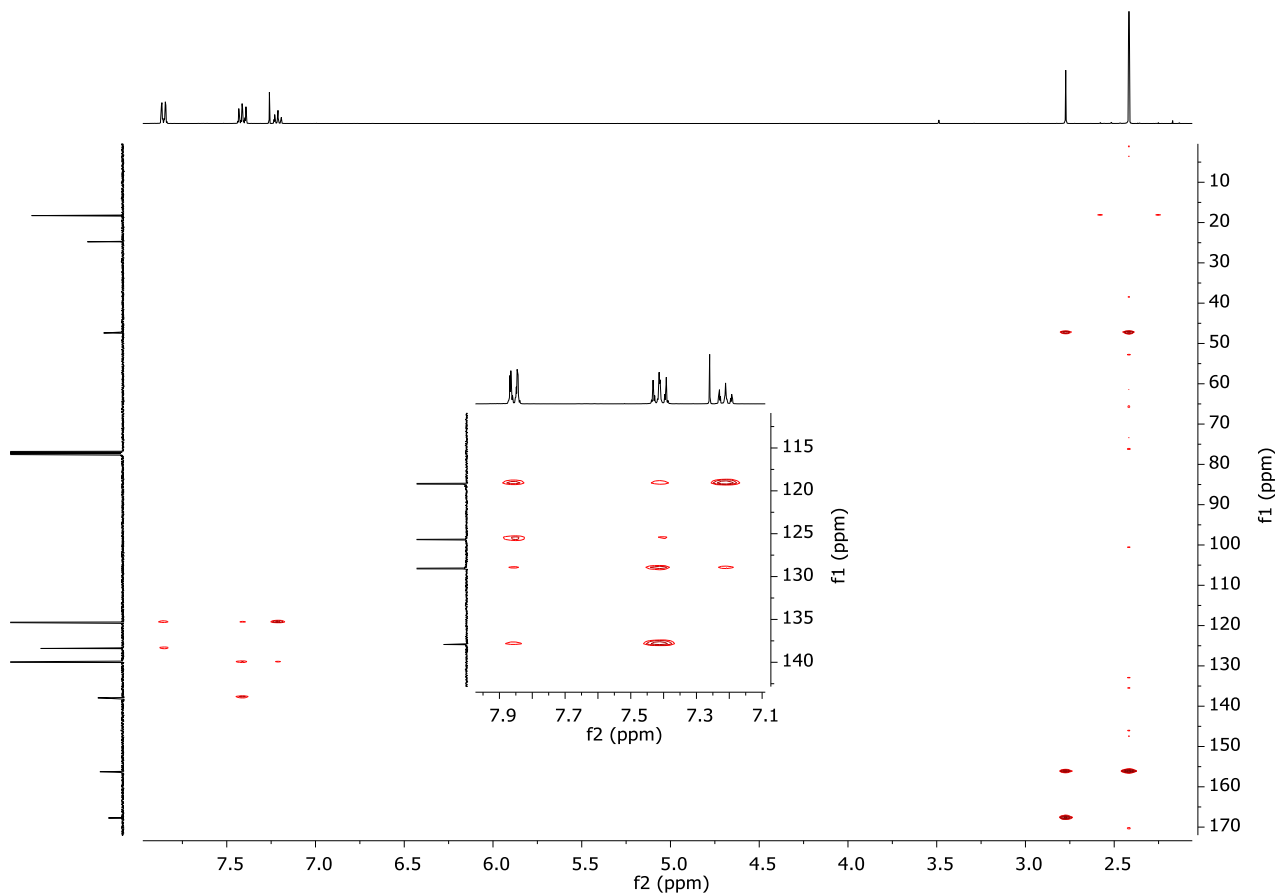


Figure S88. HMBC spectrum of compound 6

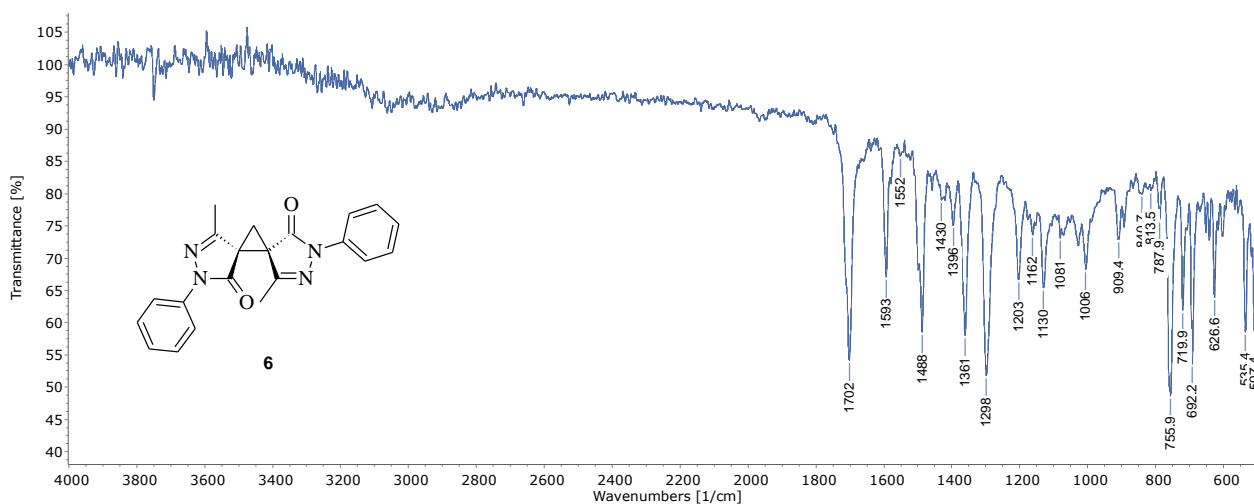
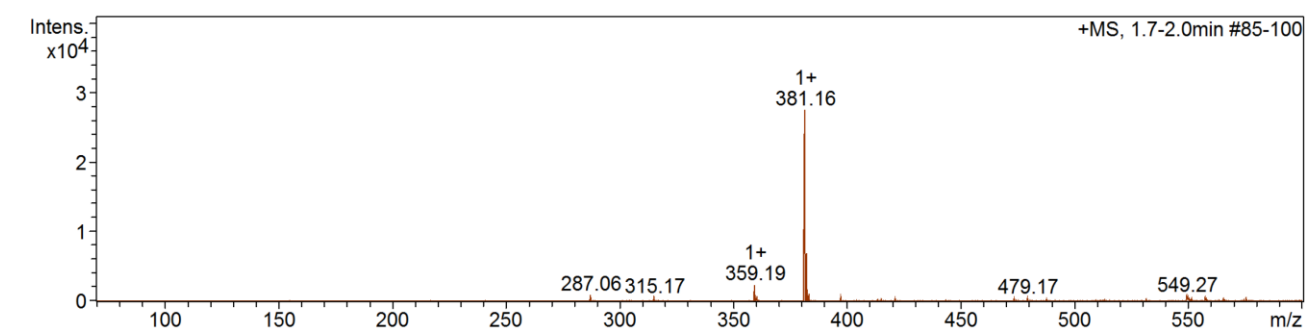


Figure S89. FTIR spectrum of compound 6

Acquisition Parameter

Ion Source Type	ESI	Ion Polarity	Positive	Alternating Ion Polarity	off
Mass Range Mode	UltraScan	Scan Begin	70 m/z	Scan End	600 m/z
Accumulation Time	200000 μ s	RF Level	63 %	Trap Drive	54.1
SPS Target Mass	400 m/z	Averages	5 Spectra	n/a	n/a



Component	Molecular Mass	Molecule	Absolute Abundance	Relative Abundance	
A	381.15	381.42	[M + H] ⁺	27417	100.00
B	359.19	359.55	[M + H] ⁺	2350	8.57

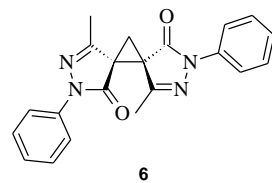


Figure S90. ESI-MS spectrum of compound 6

Table S1. Percentage (%) of inhibition of five human cancer cell lines, and immortalized cell CHO K-1 exposed 48 h to 100 μ M of each bis(spiropyrazolone)cyclopropanes **4**.

	RKO	PC-3	A-549	MCF-7	HeLa	CHO-K1
4b	21.74 \pm 2.37	17.56 \pm 2.83	NA	7.87 \pm 2.92	NA	5.76 \pm 3.08
4c	37.29 \pm 1.22	11.03 \pm 3.50	6.10 \pm 0.86	16.69 \pm 5.36	24.80 \pm 1.74	2.62 \pm 2.20
4d	68.88 \pm 1.72	46.08 \pm 1.91	13.64 \pm 0.74	39.41 \pm 2.29	48.56 \pm 4.91	4.40 \pm 0.72
4e	36.77 \pm 1.55	17.93 \pm 3.40	7.97 \pm 1.46	4.47 \pm 3.48	47.84 \pm 2.45	NA
4f	28.74 \pm 0.29	28.32 \pm 2.56	10.36 \pm 3.28	NA	NA	2.79 \pm 1.49
4k	67.59 \pm 0.77	46.72 \pm 3.78	26.71 \pm 3.03	43.03 \pm 3.46	47.22 \pm 1.36	NA
4p	18.59 \pm 1.37	5.25 \pm 2.60	9.08 \pm 1.14	13.64 \pm 2.55	5.98 \pm 2.19	1.95 \pm 1.42
4q	62.87 \pm 1.53	50.51 \pm 2.38	13.00 \pm 1.99	32.48 \pm 2.33	31.72 \pm 1.52	2.29 \pm 2.60
4r	64.71 \pm 0.52	54.66 \pm 1.95	28.65 \pm 3.71	45.75 \pm 2.11	50.16 \pm 0.52	4.74 \pm 3.04
4s	70.23 \pm 1.19	49.74 \pm 2.11	24.42 \pm 1.36	9.91 \pm 3.37	43.56 \pm 1.75	NA
4t	47.57 \pm 0.95	13.81 \pm 2.87	3.43 \pm 2.42	31.41 \pm 3.65	5.06 \pm 2.43	5.08 \pm 2.84
4u	40.96 \pm 1.49	38.68 \pm 3.77	3.64 \pm 1.81	19.51 \pm 2.96	34.73 \pm 2.29	5.76 \pm 3.65
Dox	85.63 \pm 1.06	87.98 \pm 1.65	9.16 \pm 2.16	46.23 \pm 7.77	9.18 \pm 9.55	2.62 \pm 1.42

Data are given as mean and standard error (SEM) of at least three independent experiments; NA: No activity; Dox: Doxorubicin (1 μ M).

Table S2. Inhibitory activity of bis(spiropyrazolone)cyclopropanes **4** against *S. cerevisiae* (W303 background).

	<i>S. cerevisiae</i>
	W303
4b	1377.67 \pm 303.94
4c	NA
4d	NA
4e	NA
4f	NA
4k	NA
4p	NA
4q	1087.00 \pm 38.50
4r	1270.00 \pm 118.82
4s	789.03 \pm 37.29
4t	NA
4u	NA

Table S3. DPPH scavenging activity of bis(spiropyrazolone)cyclopropanes **4**.

DPPH scavenging activity	
	IC ₅₀ μ M
4b	> 300
4c	> 300
4d	> 300
4e	> 300
4f	> 300
4k	233.8
4p	> 300
4q	> 300
4r	> 300
4s	> 300
4t	> 300
4u	> 300
Ascorbic acid	14.0
Ampho B	ND

Statistical Analyses S1. Test for statistical significance were based on one-way ANOVA test applied to leishmanicidal and cytotoxicity activities of bis(spiropyrazolone)cyclopropanes **4** (Table 3).

```
>bartlett.test(log(IC50+1)~Drug)
```

Bartlett test of homogeneity of variances

data: log(IC50 + 1) by Drug

Bartlett's K-squared = 18.182, df = 12, p-value = 0.1103

```
> ic50m=lm(log(IC50+1)~Drug)
```

```
> anova(ic50m)
```

Analysis of Variance Table

Response: log(IC50 + 1)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Drug	12	3.01683	0.251403	46.477	6.327e-12 ***
Residuals	20	0.10818	0.005409		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Study: ic50m ~ "Drug"

HSD Test for log(IC50 + 1)

Mean Square Error: 0.005409134

Drug, means

	log ₁₀ IC ₅₀ ...	std	r	Min	Max
4b	0.1463159	0.003543739	3	0.1431475	0.1501427
4c	0.1397771	0.034249039	3	0.1045402	0.1729444
4d	0.1377132	0.013678017	2	0.1280413	0.1473850
4e	0.9040759	0.103044742	3	0.7943491	0.9987920
4f	1.1732861	0.022310818	2	1.1575099	1.1890622
4k	0.2568298	0.074736324	3	0.1709235	0.3068963
4p	0.4487139	0.146863896	2	0.3448655	0.5525624
4q	0.2442968	0.033416047	3	0.2147887	0.2805819
4r	0.1716096	0.051448633	2	0.1352299	0.2079893
4s	0.1719498	0.057499706	2	0.1312914	0.2126082
4t	0.1948935	0.041308595	2	0.1656839	0.2241031
4u	0.3409402	0.174190218	2	0.2177691	0.4641113
Amphotericina	0.1573406	0.060319051	4	0.1024663	0.2312704

Alpha: 0.05 ; DF Error: 20

Critical Value of Studentized Range: 5.281538

Groups according to probability of means differences and alpha level(0.05)

Treatments with the same letter are not significantly different.

	log(IC ₅₀ + 1)	groups
4f	1.1732861	a
4e	0.9040759	b
4p	0.4487139	c
4u	0.3409402	cd
4k	0.2568298	cd
4q	0.2442968	cd
4t	0.1948935	cd
4s	0.1719498	d
4r	0.1716096	d
Amphotericina	0.1573406	d
4b	0.1463159	d
4c	0.1397771	d
4d	0.1377132	d

Original values:

	Group.1	x.1 (Mean)	x.2 (SD)
1	4b	0.157566667	0.004104063
2	4c	0.150466667	0.039335650
3	4d	0.147700000	0.015697771
4	4e	1.478333333	0.252224767

5	4f	2.233000000	0.072124892
6	4k	0.295200000	0.094711351
7	4p	0.574750000	0.230446100
8	4q	0.277200000	0.042880415
9	4r	0.188000000	0.061094026
10	4s	0.188600000	0.068306515
11	4t	0.215700000	0.050204581
12	4u	0.416950000	0.245578185
13	Amphotericina	0.172000000	0.071210158

```
> bartlett.test(log(CC50)~Drug)
```

Bartlett test of homogeneity of variances

data: log(CC50) by Drug

Bartlett's K-squared = 8.5922, df = 11, p-value = 0.6595

Analysis of Variance Table

```
Response:      log(CC50)
             Df Sum Sq Mean Sq F value Pr(>F)
Drug          11  10.3815  0.94377  11.409 8.51e-08 ***
Residuals     29   2.3989  0.08272
```

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```
> HSD.test(CC50,"Drug",console=T)
```

Study: CC50m~ "Drug"

HSD Test for log(CC50)

Mean Square Error: 0.08271965

Drug, means

	log(CC50.	std	r	Min	Max
4b	1.1719100	0.43666050	3	0.7323679	1.605631
4c	0.8128903	0.06772344	2	0.7650026	0.860778
4d	1.3342413	0.29842066	4	0.9783261	1.625705
4e	1.9649729	0.28245877	2	1.7652444	2.164701
4f	2.5482028	0.09670494	3	2.4379897	2.618855
4k	1.1645144	0.32332882	3	0.7911809	1.354029
4p	1.2466569	0.23752428	3	1.0466170	1.509175
4q	1.3739465	0.18446436	5	1.1568812	1.658228
4r	1.8612149	0.14882827	4	1.6420987	1.957698

4s	2.1056371	0.39517555	3	1.6787774	2.458734
4t	0.9820859	0.27456625	5	0.5300398	1.229641
4u	2.1711028	0.41188059	4	1.6152212	2.511224

Alpha: 0.05 ; DF Error: 29

Critical Value of Studentized Range: 5.014273

Groups according to probability of means differences and alpha level(0.05)

Treatments with the same letter are not significantly different.

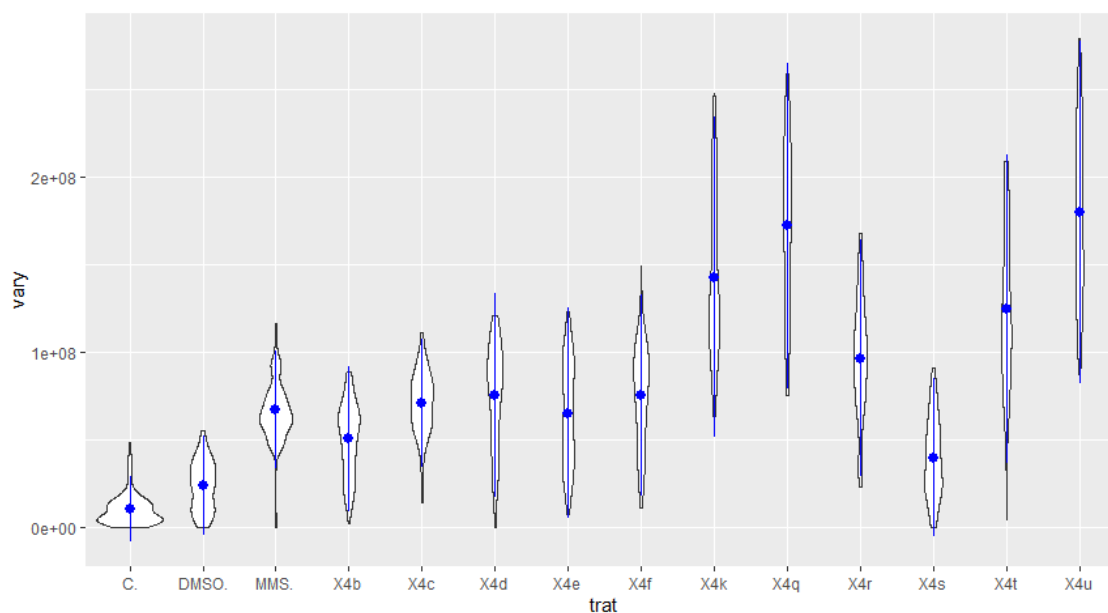
log(CC50) groups

4f	2.5482028	a
4u	2.1711028	a
4s	2.1056371	ab
4e	1.9649729	abc
4r	1.8612149	abc
4q	1.3739465	bcd
4d	1.3342413	bcd
4p	1.2466569	cd
4b	1.1719100	cd
4k	1.1645144	cd
4t	0.9820859	d
4c	0.8128903	d

Original values:

	Group.1	x.1 (Mean)	x.2(sd)
1	4b	3.4360000	1.4597058
2	4c	2.2570000	0.1527351
3	4d	3.9225000	1.1214757
4	4e	7.2775000	2.0286894
5	4f	12.8233333	1.2077389
6	4k	3.3100000	0.9561553
7	4p	3.5463333	0.8714978
8	4q	4.0066000	0.7724440
9	4r	6.4827500	0.8992754
10	4s	8.6300000	3.1707698
11	4t	2.7450000	0.6646112
12	4u	9.2952500	3.3663162

Statistical Analyses S1. Kruskal Wallis test's applied to comet assay of *Leishmania mexicana* cells exposed to bis(spiropyrazolone)cyclopropanes **4** (Figure 2).



Study: Trat
Kruskal-Wallis test's
Ties or no Ties

Critical Value: 1004.543
Degrees of freedom: 13
Pvalue Chisq : 0

trat, means of the ranks

trat	mean	r
C.	97.280	100
DMSO.	207.115	100
MMS.	606.840	100
X4b	449.315	100
X4c	648.065	100
X4d	694.660	100
X4e	595.490	100
X4f	691.135	100
X4k	1111.660	100
X4q	1208.625	80
X4r	861.180	100
X4s	352.245	100
X4t	1016.210	100
X4u	1230.805	100

Post Hoc Analysis

P value adjustment method: bonferroni

t-Student: 3.463614

Alpha : 0.05

Groups according to probability of treatment differences and alpha level.

Treatments with the same letter are not significantly different.

```

vary groups
X4u 1230.805  a
X4q 1208.625  ab
X4k 1111.660  bc
X4t 1016.210  c
X4r  861.180  d
X4d  694.660  e
X4f  691.135  e
X4c  648.065  e
MMS. 606.840  e
X4e  595.490  e
X4b  449.315  f
X4s  352.245  f
DMSO. 207.115  g
C.    97.280   h

```

\$statistics

```

Chisq Df p.chisq
1004.543 13    0

```

\$parameters

```

test p.adjusted name.t ntr alpha
Kruskal-Wallis bonferroni  trat 14 0.05

```

\$means

```

vary rank std r Min Max Q25 Q50 Q75
C. 10290000 97.280 9224796 100 0.0e+00 4.80e+07 4000000 8000000 14000000
DMSO. 23610000 207.115 14071890 100 0.0e+00 5.50e+07 11000000 25000000 35000000
MMS. 67050000 606.840 16924894 100 0.0e+00 1.16e+08 58000000 65000000 74250000
X4b 50400000 449.315 20682301 100 2.0e+06 8.90e+07 32750000 55000000 64000000
X4c 70710000 648.065 18193847 100 1.4e+07 1.11e+08 58750000 72500000 83250000
X4d 75120000 694.660 29056295 100 0.0e+00 1.21e+08 52000000 83500000 97250000
X4e 65120000 595.490 29979549 100 7.0e+06 1.23e+08 39750000 64500000 90250000
X4f 75100000 691.135 28784641 100 1.1e+07 1.49e+08 55000000 80500000 99000000
X4k 142780000 1111.660 45723716 100 6.3e+07 2.48e+08 110000000 128000000 182500000
X4q 172025000 1208.625 46560965 80 7.5e+07 2.59e+08 129750000 177500000 205750000
X4r 96310000 861.180 33823277 100 2.3e+07 1.68e+08 76500000 98500000 117500000
X4s 39720000 352.245 22688528 100 0.0e+00 9.10e+07 23000000 39500000 55250000
X4t 124410000 1016.210 44002317 100 4.0e+06 2.09e+08 95250000 116000000 157000000
X4u 179560000 1230.805 48875087 100 8.7e+07 2.79e+08 142000000 177500000 223000000

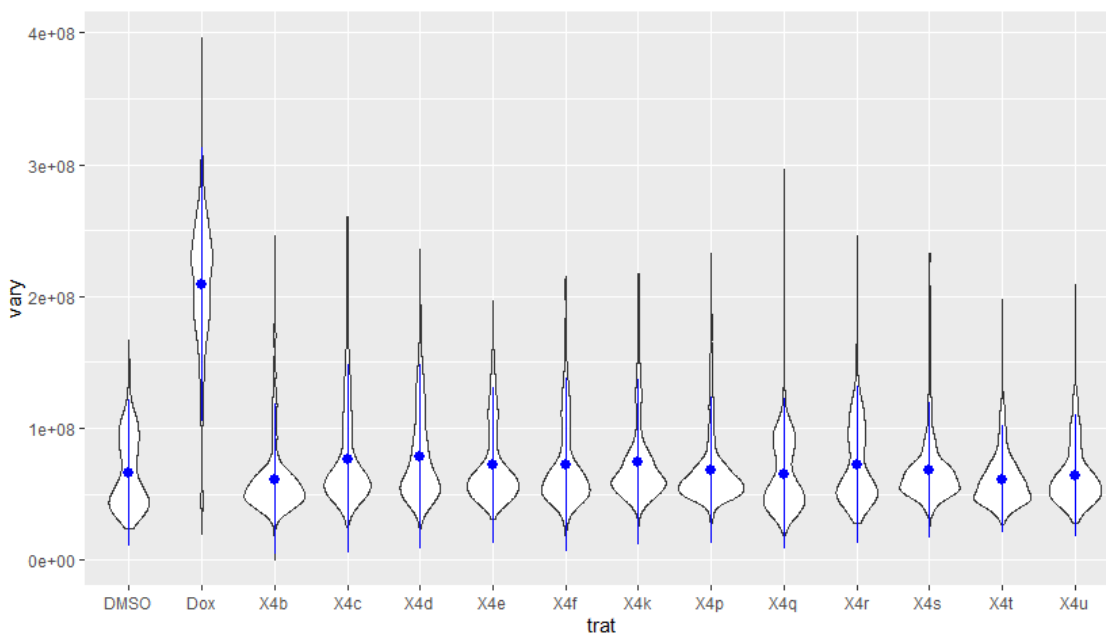
```


\$comparison

	Difference	pvalue	Signif.	LCL	UCL
C. - DMSO.	-109.835	0.0187	*	-212.036162	-7.633838
C. - MMS.	-509.560	0.0000	***	-611.761162	-407.358838
C. - X4b	-352.035	0.0000	***	-454.236162	-249.833838
C. - X4c	-550.785	0.0000	***	-652.986162	-448.583838
C. - X4d	-597.380	0.0000	***	-699.581162	-495.178838
C. - X4e	-498.210	0.0000	***	-600.411162	-396.008838
C. - X4f	-593.855	0.0000	***	-696.056162	-491.653838
C. - X4k	-1014.380	0.0000	***	-1116.581162	-912.178838
C. - X4q	-1111.345	0.0000	***	-1219.745702	-1002.944298
C. - X4r	-763.900	0.0000	***	-866.101162	-661.698838
C. - X4s	-254.965	0.0000	***	-357.166162	-152.763838
C. - X4t	-918.930	0.0000	***	-1021.131162	-816.728838
C. - X4u	-1133.525	0.0000	***	-1235.726162	-1031.323838
DMSO. - MMS.	-399.725	0.0000	***	-501.926162	-297.523838
DMSO. - X4b	-242.200	0.0000	***	-344.401162	-139.998838
DMSO. - X4c	-440.950	0.0000	***	-543.151162	-338.748838
DMSO. - X4d	-487.545	0.0000	***	-589.746162	-385.343838
DMSO. - X4e	-388.375	0.0000	***	-490.576162	-286.173838
DMSO. - X4f	-484.020	0.0000	***	-586.221162	-381.818838
DMSO. - X4k	-904.545	0.0000	***	-1006.746162	-802.343838
DMSO. - X4q	-1001.510	0.0000	***	-1109.910702	-893.109298
DMSO. - X4r	-654.065	0.0000	***	-756.266162	-551.863838
DMSO. - X4s	-145.130	0.0001	***	-247.331162	-42.928838
DMSO. - X4t	-809.095	0.0000	***	-911.296162	-706.893838
DMSO. - X4u	-1023.690	0.0000	***	-1125.891162	-921.488838
MMS. - X4b	157.525	0.0000	***	55.323838	259.726162
MMS. - X4c	-41.225	1.0000		-143.426162	60.976162
MMS. - X4d	-87.820	0.2702		-190.021162	14.381162
MMS. - X4e	11.350	1.0000		-90.851162	113.551162
MMS. - X4f	-84.295	0.3954		-186.496162	17.906162
MMS. - X4k	-504.820	0.0000	***	-607.021162	-402.618838
MMS. - X4q	-601.785	0.0000	***	-710.185702	-493.384298
MMS. - X4r	-254.340	0.0000	***	-356.541162	-152.138838
MMS. - X4s	254.595	0.0000	***	152.393838	356.796162
MMS. - X4t	-409.370	0.0000	***	-511.571162	-307.168838
MMS. - X4u	-623.965	0.0000	***	-726.166162	-521.763838
X4b - X4c	-198.750	0.0000	***	-300.951162	-96.548838
X4b - X4d	-245.345	0.0000	***	-347.546162	-143.143838
X4b - X4e	-146.175	0.0001	***	-248.376162	-43.973838
X4b - X4f	-241.820	0.0000	***	-344.021162	-139.618838
X4b - X4k	-662.345	0.0000	***	-764.546162	-560.143838
X4b - X4q	-759.310	0.0000	***	-867.710702	-650.909298
X4b - X4r	-411.865	0.0000	***	-514.066162	-309.663838
X4b - X4s	97.070	0.0936	.	-5.131162	199.271162
X4b - X4t	-566.895	0.0000	***	-669.096162	-464.693838
X4b - X4u	-781.490	0.0000	***	-883.691162	-679.288838
X4c - X4d	-46.595	1.0000		-148.796162	55.606162
X4c - X4e	52.575	1.0000		-49.626162	154.776162
X4c - X4f	-43.070	1.0000		-145.271162	59.131162
X4c - X4k	-463.595	0.0000	***	-565.796162	-361.393838

X4c - X4q	-560.560 0.0000	***	-668.960702	-452.159298
X4c - X4r	-213.115 0.0000	***	-315.316162	-110.913838
X4c - X4s	295.820 0.0000	***	193.618838	398.021162
X4c - X4t	-368.145 0.0000	***	-470.346162	-265.943838
X4c - X4u	-582.740 0.0000	***	-684.941162	-480.538838
X4d - X4e	99.170 0.0727	.	-3.031162	201.371162
X4d - X4f	3.525 1.0000		-98.676162	105.726162
X4d - X4k	-417.000 0.0000	***	-519.201162	-314.798838
X4d - X4q	-513.965 0.0000	***	-622.365702	-405.564298
X4d - X4r	-166.520 0.0000	***	-268.721162	-64.318838
X4d - X4s	342.415 0.0000	***	240.213838	444.616162
X4d - X4t	-321.550 0.0000	***	-423.751162	-219.348838
X4d - X4u	-536.145 0.0000	***	-638.346162	-433.943838
X4e - X4f	-95.645 0.1108		-197.846162	6.556162
X4e - X4k	-516.170 0.0000	***	-618.371162	-413.968838
X4e - X4q	-613.135 0.0000	***	-721.535702	-504.734298
X4e - X4r	-265.690 0.0000	***	-367.891162	-163.488838
X4e - X4s	243.245 0.0000	***	141.043838	345.446162
X4e - X4t	-420.720 0.0000	***	-522.921162	-318.518838
X4e - X4u	-635.315 0.0000	***	-737.516162	-533.113838
X4f - X4k	-420.525 0.0000	***	-522.726162	-318.323838
X4f - X4q	-517.490 0.0000	***	-625.890702	-409.089298
X4f - X4r	-170.045 0.0000	***	-272.246162	-67.843838
X4f - X4s	338.890 0.0000	***	236.688838	441.091162
X4f - X4t	-325.075 0.0000	***	-427.276162	-222.873838
X4f - X4u	-539.670 0.0000	***	-641.871162	-437.468838
X4k - X4q	-96.965 0.1808		-205.365702	11.435702
X4k - X4r	250.480 0.0000	***	148.278838	352.681162
X4k - X4s	759.415 0.0000	***	657.213838	861.616162
X4k - X4t	95.450 0.1134		-6.751162	197.651162
X4k - X4u	-119.145 0.0052	**	-221.346162	-16.943838
X4q - X4r	347.445 0.0000	***	239.044298	455.845702
X4q - X4s	856.380 0.0000	***	747.979298	964.780702
X4q - X4t	192.415 0.0000	***	84.014298	300.815702
X4q - X4u	-22.180 1.0000		-130.580702	86.220702
X4r - X4s	508.935 0.0000	***	406.733838	611.136162
X4r - X4t	-155.030 0.0000	***	-257.231162	-52.828838
X4r - X4u	-369.625 0.0000	***	-471.826162	-267.423838
X4s - X4t	-663.965 0.0000	***	-766.166162	-561.763838
X4s - X4u	-878.560 0.0000	***	-980.761162	-776.358838
X4t - X4u	-214.595 0.0000	***	-316.796162	-112.393838

Statistical Analyses S2. Kruskal Wallis test's applied to comet assay of CHO cells exposed to bis(spiropyrazolone)cyclopropanes **4** (Figure 3).



\$statistics

Chisq	Df	p.chisq	t.value	MSD
1708.307	13	0	1.960247	245.1376

\$parameters

test	p.adjusted	name	t	ntr	alpha
Kruskal-Wallis	none	trat	14	0.05	

\$means

	vary	rank	std	r	Min	Max	Q25	Q50	Q75
DMSO	66045840	3568.301	27896533	600	23429540.0	166553500	42784380	57045840	89134120
Dox	209447368	7865.864	51979579	600	19864180.0	396264900	183361600	217996600	240916800
X4b	61401527	3115.134	28639223	600	509337.8	246519500	45331070	53989810	65195240
X4c	76526316	4376.037	35814130	600	24957560.0	260781000	52843805	62648560	92954160
X4d	78343803	4521.320	34892342	600	23429540.0	235314100	52461800	65704580	95755520
X4e	71876061	4164.706	29613974	600	31069610.0	197113800	51443120	61120540	82640065
X4f	72036501	4055.789	32998506	600	18845500.0	215449900	49915110	61629880	83022070
X4k	74173175	4427.186	31543285	600	25976230.0	217487300	55008490	63667230	80093375
X4p	68013582	3946.407	27613069	600	27504240.0	232767400	51825125	60101870	71816640
X4q	65551784	3537.702	28621584	600	18336160.0	296944000	43293720	56791170	86714765
X4r	72132428	4106.395	29902529	600	27504240.0	246010200	48896430	61629880	94736840
X4s	68389643	4118.727	25773413	600	25976230.0	232258100	52971140	62648560	73344650
X4t	61194397	3386.662	20212790	600	26485570.0	198132400	46859090	57045840	69779290
X4u	64116299	3616.770	23301875	600	28013580.0	209337900	47877760	58573850	72325970

\$comparison

NULL

\$groups

	vary	groups
Dox	7865.864	a
X4d	4521.320	b

X4k	4427.186	b
X4c	4376.037	bc
X4e	4164.706	cd
X4s	4118.727	d
X4r	4106.395	d
X4f	4055.789	d
X4p	3946.407	d
X4u	3616.770	e
DMSO	3568.301	e
X4q	3537.702	e
X4t	3386.662	e
X4b	3115.134	f

\$statistics

Chisq	Df	p.chisq	t.value	MSD
1708.307	13	0	3.456746	432.2815

\$parameters

test	p.adjusted	name.t	ntr	alpha
Kruskal-Wallis	bonferroni	trat	14	0.05

\$means

	vary	rank	std	r	Min	Max	Q25	Q50	Q75
DMSO	66045840	3568.301	27896533	600	23429540.0	166553500	42784380	57045840	89134120
Dox	209447368	7865.864	51979579	600	19864180.0	396264900	183361600	217996600	240916800
X4b	61401527	3115.134	28639223	600	509337.8	246519500	45331070	53989810	65195240
X4c	76526316	4376.037	35814130	600	24957560.0	260781000	52843805	62648560	92954160
X4d	78343803	4521.320	34892342	600	23429540.0	235314100	52461800	65704580	95755520
X4e	71876061	4164.706	29613974	600	31069610.0	197113800	51443120	61120540	82640065
X4f	72036501	4055.789	32998506	600	18845500.0	215449900	49915110	61629880	83022070
X4k	74173175	4427.186	31543285	600	25976230.0	217487300	55008490	63667230	80093375
X4p	68013582	3946.407	27613069	600	27504240.0	232767400	51825125	60101870	71816640
X4q	65551784	3537.702	28621584	600	18336160.0	296944000	43293720	56791170	86714765
X4r	72132428	4106.395	29902529	600	27504240.0	246010200	48896430	61629880	94736840
X4s	68389643	4118.727	25773413	600	25976230.0	232258100	52971140	62648560	73344650
X4t	61194397	3386.662	20212790	600	26485570.0	198132400	46859090	57045840	69779290
X4u	64116299	3616.770	23301875	600	28013580.0	209337900	47877760	58573850	72325970

\$comparison

	Difference	pvalue	Signif.	LCL	UCL
DMSO - Dox	-4297.56333	0.0000	***	-4729.844865	-3865.281801
DMSO - X4b	453.16667	0.0266	*	20.885135	885.448199
DMSO - X4c	-807.73583	0.0000	***	-1240.017365	-375.454301
DMSO - X4d	-953.01917	0.0000	***	-1385.300699	-520.737635
DMSO - X4e	-596.40500	0.0002	***	-1028.686532	-164.123468
DMSO - X4f	-487.48833	0.0089	**	-919.769865	-55.206801
DMSO - X4k	-858.88500	0.0000	***	-1291.166532	-426.603468
DMSO - X4p	-378.10583	0.2280		-810.387365	54.175699
DMSO - X4q	30.59917	1.0000		-401.682365	462.880699
DMSO - X4r	-538.09417	0.0016	**	-970.375699	-105.812635
DMSO - X4s	-550.42667	0.0010	***	-982.708199	-118.145135
DMSO - X4t	181.63833	1.0000		-250.643199	613.919865

DMSO - X4u	-48.46917 1.0000		-480.750699 383.812365
Dox - X4b	4750.73000 0.0000	***	4318.448468 5183.011532
Dox - X4c	3489.82750 0.0000	***	3057.545968 3922.109032
Dox - X4d	3344.54417 0.0000	***	2912.262635 3776.825699
Dox - X4e	3701.15833 0.0000	***	3268.876801 4133.439865
Dox - X4f	3810.07500 0.0000	***	3377.793468 4242.356532
Dox - X4k	3438.67833 0.0000	***	3006.396801 3870.959865
Dox - X4p	3919.45750 0.0000	***	3487.175968 4351.739032
Dox - X4q	4328.16250 0.0000	***	3895.880968 4760.444032
Dox - X4r	3759.46917 0.0000	***	3327.187635 4191.750699
Dox - X4s	3747.13667 0.0000	***	3314.855135 4179.418199
Dox - X4t	4479.20167 0.0000	***	4046.920135 4911.483199
Dox - X4u	4249.09417 0.0000	***	3816.812635 4681.375699
X4b - X4c	-1260.90250 0.0000	***	-1693.184032 -828.620968
X4b - X4d	-1406.18583 0.0000	***	-1838.467365 -973.904301
X4b - X4e	-1049.57167 0.0000	***	-1481.853199 -617.290135
X4b - X4f	-940.65500 0.0000	***	-1372.936532 -508.373468
X4b - X4k	-1312.05167 0.0000	***	-1744.333199 -879.770135
X4b - X4p	-831.27250 0.0000	***	-1263.554032 -398.990968
X4b - X4q	-422.56750 0.0665	.	-854.849032 9.714032
X4b - X4r	-991.26083 0.0000	***	-1423.542365 -558.979301
X4b - X4s	-1003.59333 0.0000	***	-1435.874865 -571.311801
X4b - X4t	-271.52833 1.0000		-703.809865 160.753199
X4b - X4u	-501.63583 0.0055	**	-933.917365 -69.354301
X4c - X4d	-145.28333 1.0000		-577.564865 286.998199
X4c - X4e	211.33083 1.0000		-220.950699 643.612365
X4c - X4f	320.24750 0.9517		-112.034032 752.529032
X4c - X4k	-51.14917 1.0000		-483.430699 381.132365
X4c - X4p	429.63000 0.0541	.	-2.651532 861.911532
X4c - X4q	838.33500 0.0000	***	406.053468 1270.616532
X4c - X4r	269.64167 1.0000		-162.639865 701.923199
X4c - X4s	257.30917 1.0000		-174.972365 689.590699
X4c - X4t	989.37417 0.0000	***	557.092635 1421.655699
X4c - X4u	759.26667 0.0000	***	326.985135 1191.548199
X4d - X4e	356.61417 0.3967		-75.667365 788.895699
X4d - X4f	465.53083 0.0181	*	33.249301 897.812365
X4d - X4k	94.13417 1.0000		-338.147365 526.415699
X4d - X4p	574.91333 0.0004	***	142.631801 1007.194865
X4d - X4q	983.61833 0.0000	***	551.336801 1415.899865
X4d - X4r	414.92500 0.0829	.	-17.356532 847.206532
X4d - X4s	402.59250 0.1174		-29.689032 834.874032
X4d - X4t	1134.65750 0.0000	***	702.375968 1566.939032
X4d - X4u	904.55000 0.0000	***	472.268468 1336.831532
X4e - X4f	108.91667 1.0000		-323.364865 541.198199
X4e - X4k	-262.48000 1.0000		-694.761532 169.801532
X4e - X4p	218.29917 1.0000		-213.982365 650.580699
X4e - X4q	627.00417 0.0000	***	194.722635 1059.285699
X4e - X4r	58.31083 1.0000		-373.970699 490.592365
X4e - X4s	45.97833 1.0000		-386.303199 478.259865
X4e - X4t	778.04333 0.0000	***	345.761801 1210.324865
X4e - X4u	547.93583 0.0011	**	115.654301 980.217365
X4f - X4k	-371.39667 0.2719		-803.678199 60.884865
X4f - X4p	109.38250 1.0000		-322.899032 541.664032

X4f - X4q	518.08750 0.0032	**	85.805968	950.369032
X4f - X4r	-50.60583 1.0000		-482.887365	381.675699
X4f - X4s	-62.93833 1.0000		-495.219865	369.343199
X4f - X4t	669.12667 0.0000	***	236.845135	1101.408199
X4f - X4u	439.01917 0.0409	*	6.737635	871.300699
X4k - X4p	480.77917 0.0111	*	48.497635	913.060699
X4k - X4q	889.48417 0.0000	***	457.202635	1321.765699
X4k - X4r	320.79083 0.9399		-111.490699	753.072365
X4k - X4s	308.45833 1.0000		-123.823199	740.739865
X4k - X4t	1040.52333 0.0000	***	608.241801	1472.804865
X4k - X4u	810.41583 0.0000	***	378.134301	1242.697365
X4p - X4q	408.70500 0.0989	.	-23.576532	840.986532
X4p - X4r	-159.98833 1.0000		-592.269865	272.293199
X4p - X4s	-172.32083 1.0000		-604.602365	259.960699
X4p - X4t	559.74417 0.0007	***	127.462635	992.025699
X4p - X4u	329.63667 0.7649		-102.644865	761.918199
X4q - X4r	-568.69333 0.0005	***	-1000.974865	-136.411801
X4q - X4s	-581.02583 0.0003	***	-1013.307365	-148.744301
X4q - X4t	151.03917 1.0000		-281.242365	583.320699
X4q - X4u	-79.06833 1.0000		-511.349865	353.213199
X4r - X4s	-12.33250 1.0000		-444.614032	419.949032
X4r - X4t	719.73250 0.0000	***	287.450968	1152.014032
X4r - X4u	489.62500 0.0083	**	57.343468	921.906532
X4s - X4t	732.06500 0.0000	***	299.783468	1164.346532
X4s - X4u	501.95750 0.0055	**	69.675968	934.239032
X4t - X4u	-230.10750 1.0000		-662.389032	202.174032

\$groups
NULL