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Electronic Supplementary Information

New Visible Light Excitable Donor-Acceptor 7-Hydroxy-coumarins as Blue Fluorescent Probes for Selective Staining of Vacuoles in Yeasts and *L. donovani*

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Photophysical properties of Synthesized dyes 4a-d, 5-8 .

Absorption, Excitation and Emission spectra were recorded with UV-vis and fluorescence spectrophotometer. Samples were prepared in HEPES buffer (pH=7.2 at 25℃) final concentration of 2.5×10^{-6} M.

Table S1: Photophysical data of 4a-d, in HEPES (pH=7.2)

Entry	Excitation Wavelength λ_{max} (nm)[a]	Emission Wavelength λ_{max} (nm)[b]	Quantum Yield Φ (%)[c]	HOMO[d] (eV)	LUMO[d] (eV)	Energy gap E_g[d] (eV)
4a	419	483	37	-6.915	-3.048	3.868
4b	425	487	54	-6.931	-3.467	3.464
4c	431	506	29	-6.992	-3.231	3.761
4d	426	488	20	-6.710	-2.668	4.042

[a]Excitation maxima in HEPES Buffer at pH 7.2 [b]Fluorescence maxima in HEPES Buffer at pH 7.2. [c]Fluorescence quantum yield in HEPES Buffer at pH 7.2 relative to harmine in 0.1M H_2SO_4 as a standard (Φ = 45%). [d]The HOMO and LUMO and E_g of **CHMC**s using the TDDFT B3LYP/6-311G(d,p) method calculations.

TDDFT study of probe CHMCs 4a-d

To study the electronic behaviour of **CHMC**s **4a-d** time-dependent density functional theory (TDDFT) calculations were performed with a Gaussian 09 package.[1] The geometries were optimized at DFT/B3LYP level using a 6-31G(d,p) basis set. TDDFT calculations were performed using a B3LYP/6-311++G(d,p) method.

Table S2. Computed molecular orbital energy diagrams and isodensity surface plots of **CHMC**s **4a-d** as obtained from TDDFT calculation and computed values of vertical excitations, oscillator strength (*f*), assignment, HOMO, and LUMO values.

Compound	λ_{max} (nm)	*f*	Assignment
4a	351.56 228.90	0.5329 0.1059	HOMO → LUMO (96.5%) HOMO → LUMO +2 (61.1%)
4b	384.41 352.35	0.3649 0.2164	HOMO-1 → LUMO (63.2%) HOMO-2→ LUMO (85.1%)
4c	368.94 341.68	0.3104 0.1356	HOMO → LUMO (89.4%) HOMO-2 → LUMO (88.3%)
4d	342.45 332.00	0.3979 0.1104	HOMO → LUMO (77.6%) HOMO-1 → LUMO (73.6%)

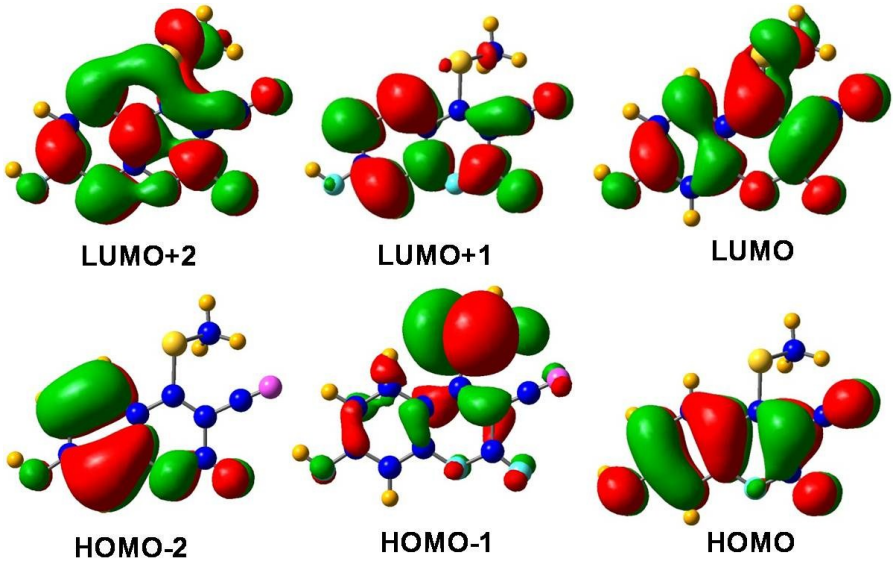


Figure S1. Computed molecular orbital energy diagrams and isodensity surface plots of compound **4a.**

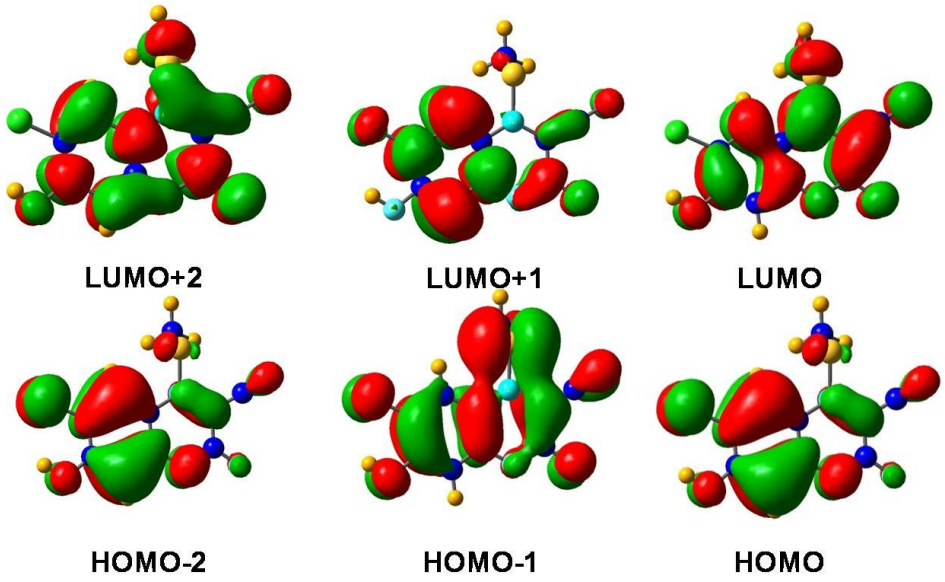


Figure S2. Computed molecular orbital energy diagrams and isodensity surface plots of compound **4b.**

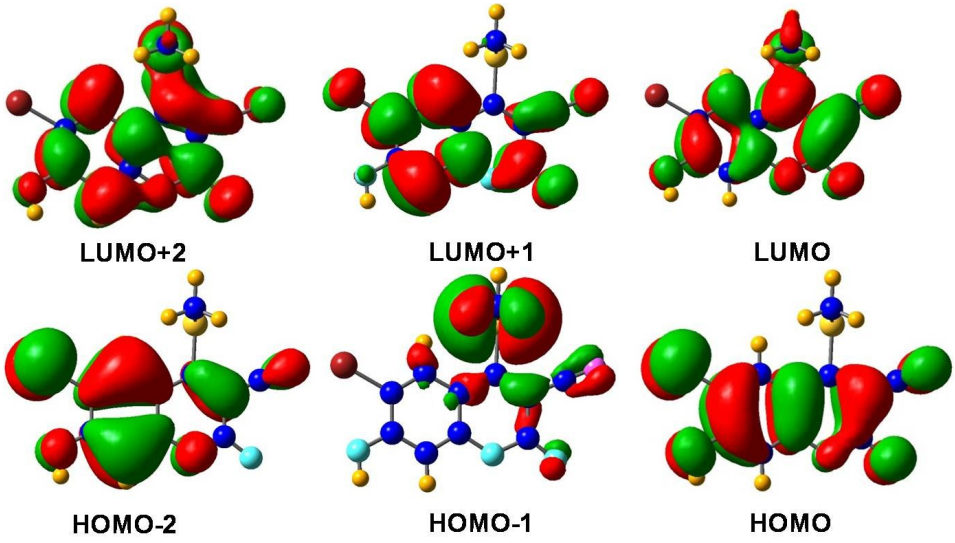


Figure S3. Computed molecular orbital energy diagrams and isodensity surface plots of compound **4c.**

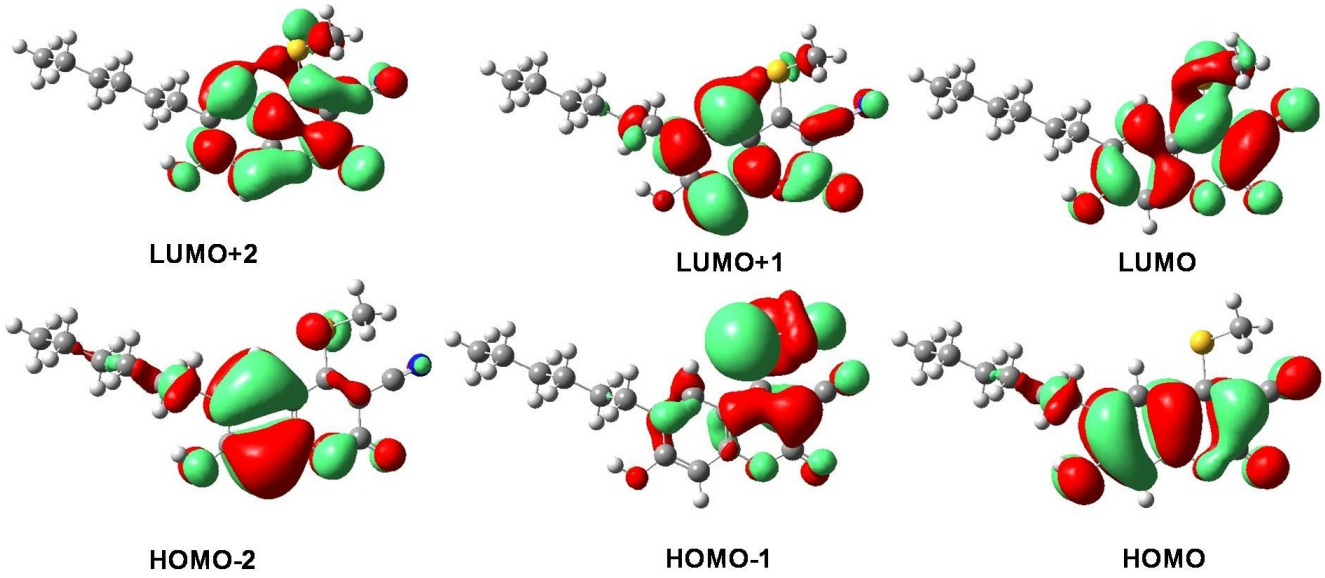


Figure S4. Computed molecular orbital energy diagrams and isodensity surface plots of compound **4d.**

Cytotoxicity study in *Leishmania donovani* at 24 h

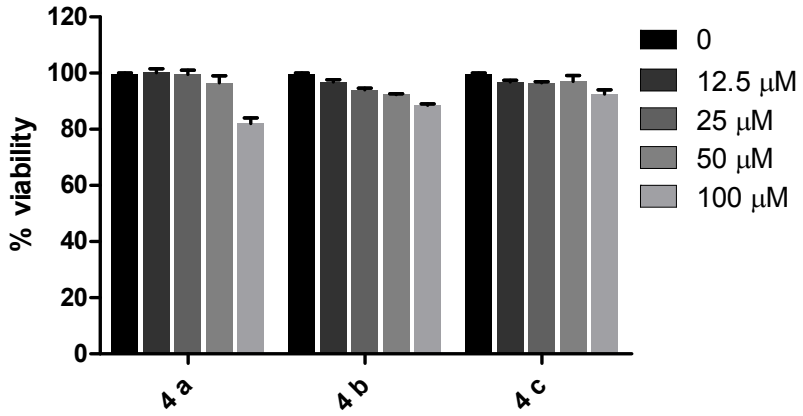


Figure S5. The cell viability assay of compounds 4a-c (100 μM to 12.5 μM) in *Leishmania donovani* promastigotes cells for 24 h at 26°C.

MDY-64 staining in *S. cerevisiae* and *S. pombe.*

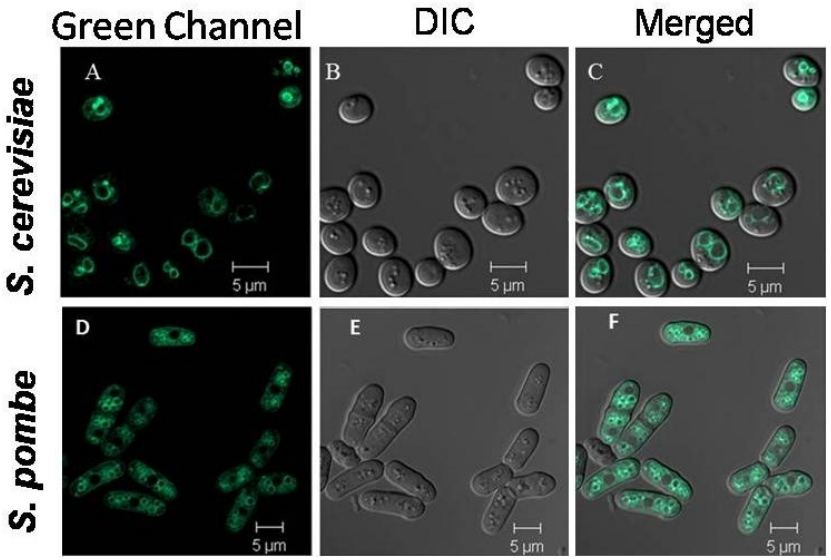


Figure S6. Confocal microscopy images showing **MDY-64** to stain the vacuolar membranes of *S. cerevisiae* and *S. Pombe.* **MDY-64:** $\lambda_{ex,\ max}$= 458 nm, $\lambda_{em,\ max}$= 491 nm; Scale bar: 5 µm.

Cytotoxicity study in yeast strains

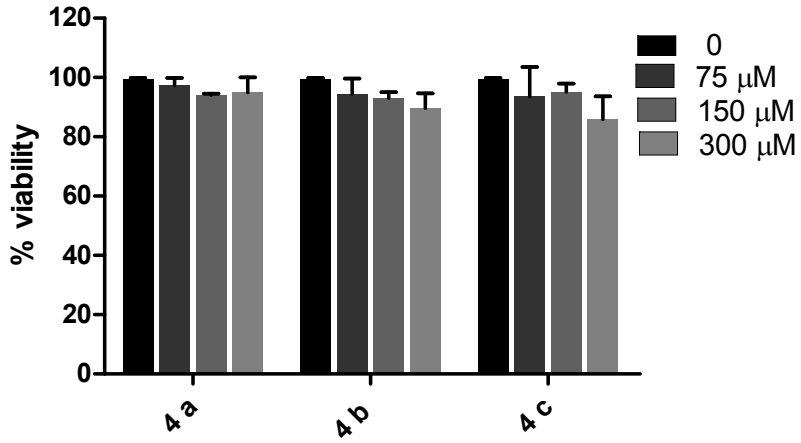


Figure S7. The cell viability assay of compounds **4a-c** (300 µM to 75 µM) in *S. cerevisiae* yeast cells for 5 h at 26°C.

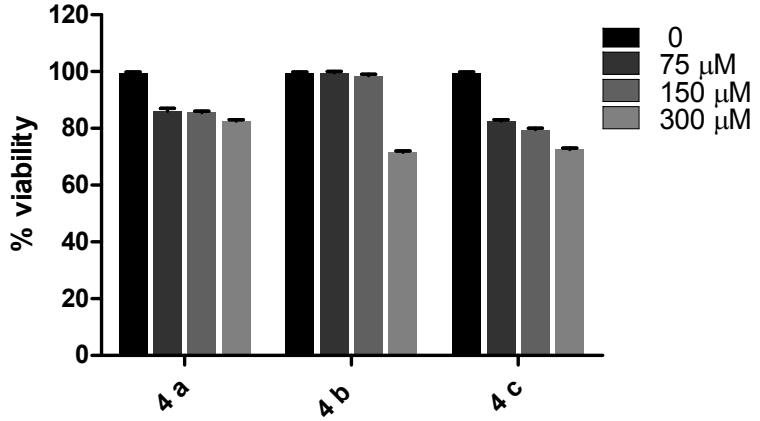


Figure S8. The cell viability assay of compounds **4a-c** (300 μM to 75 μM) in *S. pombe* yeast cells for 5 h at 26°C.

Evaluation of photo-stability of the CHMC derivative (4b)

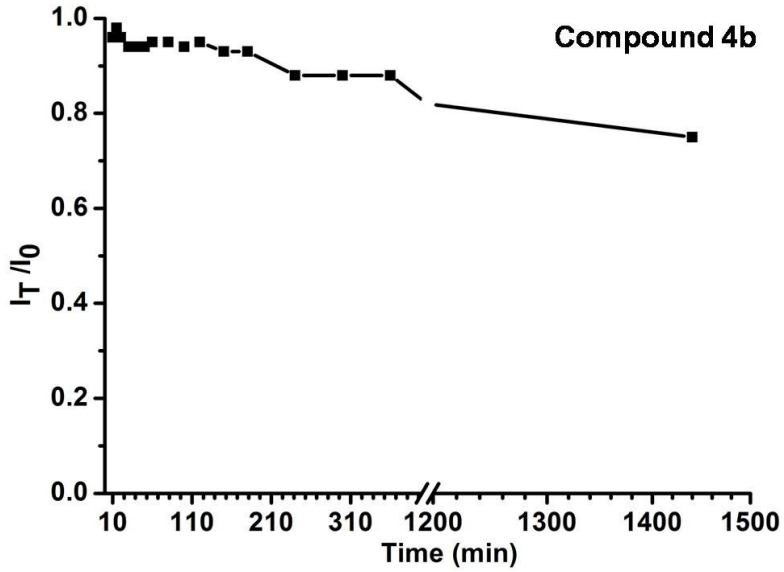


Figure S9. Photo-stability of compound **4b** (in HEPES buffer, 2.5×10^{-6} M) under continuous UV irradiation for 65 hours. (I_T= Intensity at time t min, I_0= Initial Intensity). λ_{ex}= 420 nm .

pH Stability experiments:

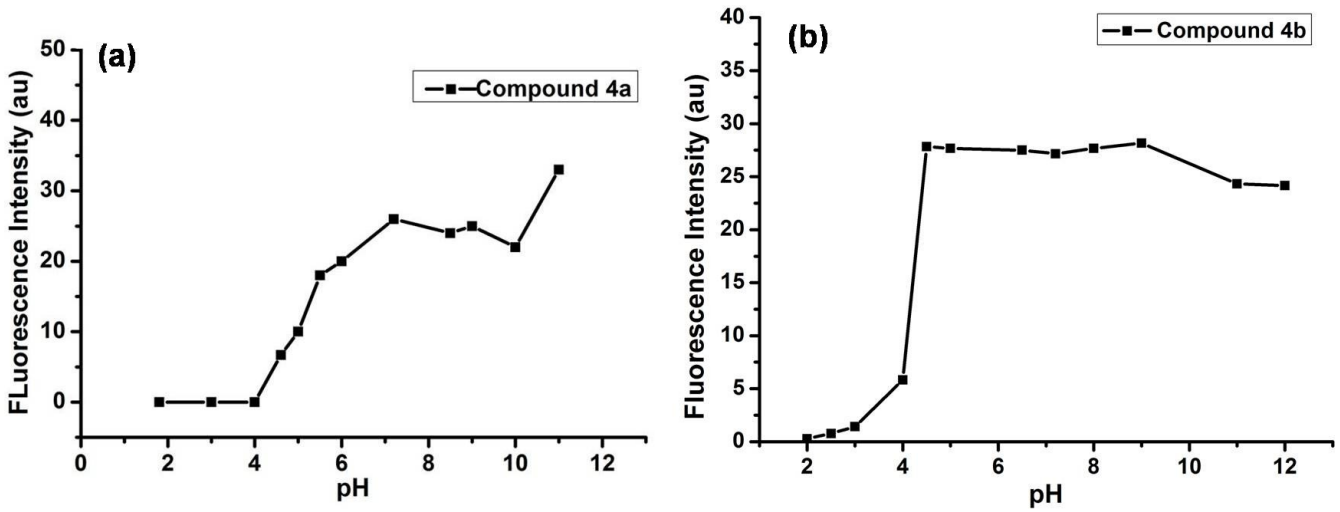


Figure S10. pH study of compound **4a, b** (2.5×10^{-6} M) in solutions of different pH (TDW). λ_{ex}= 420 nm.

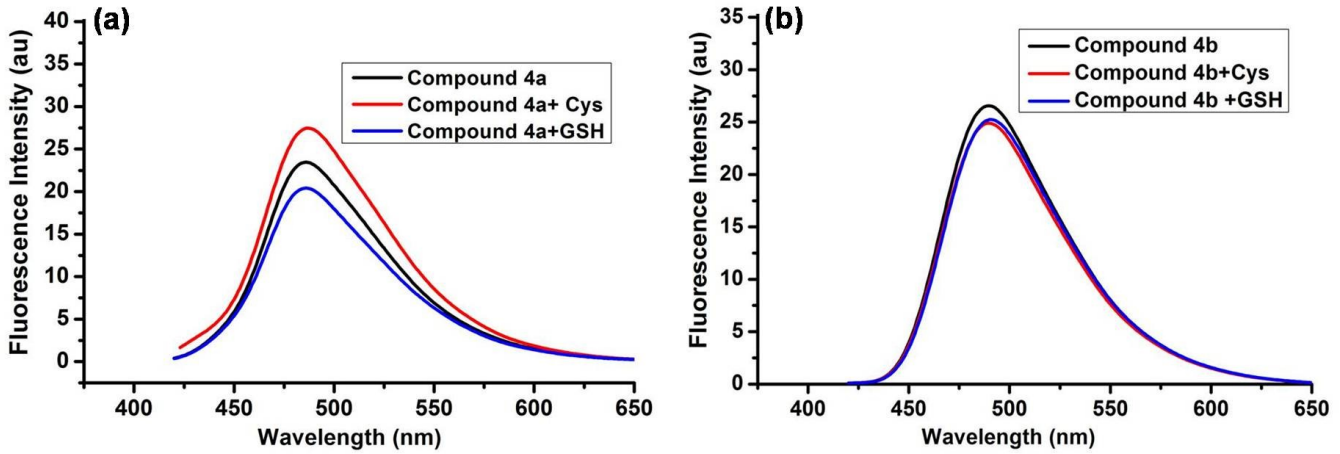
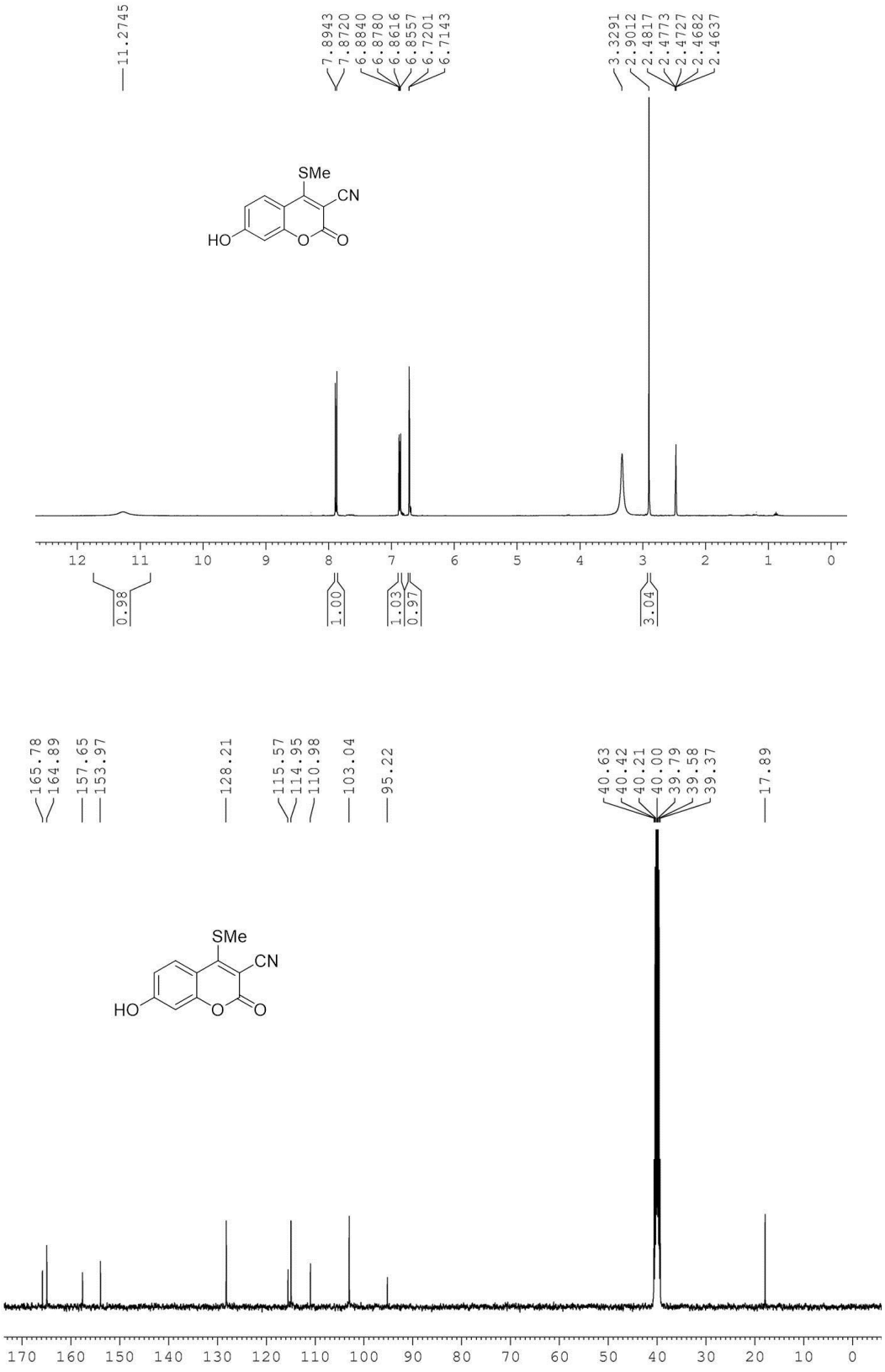


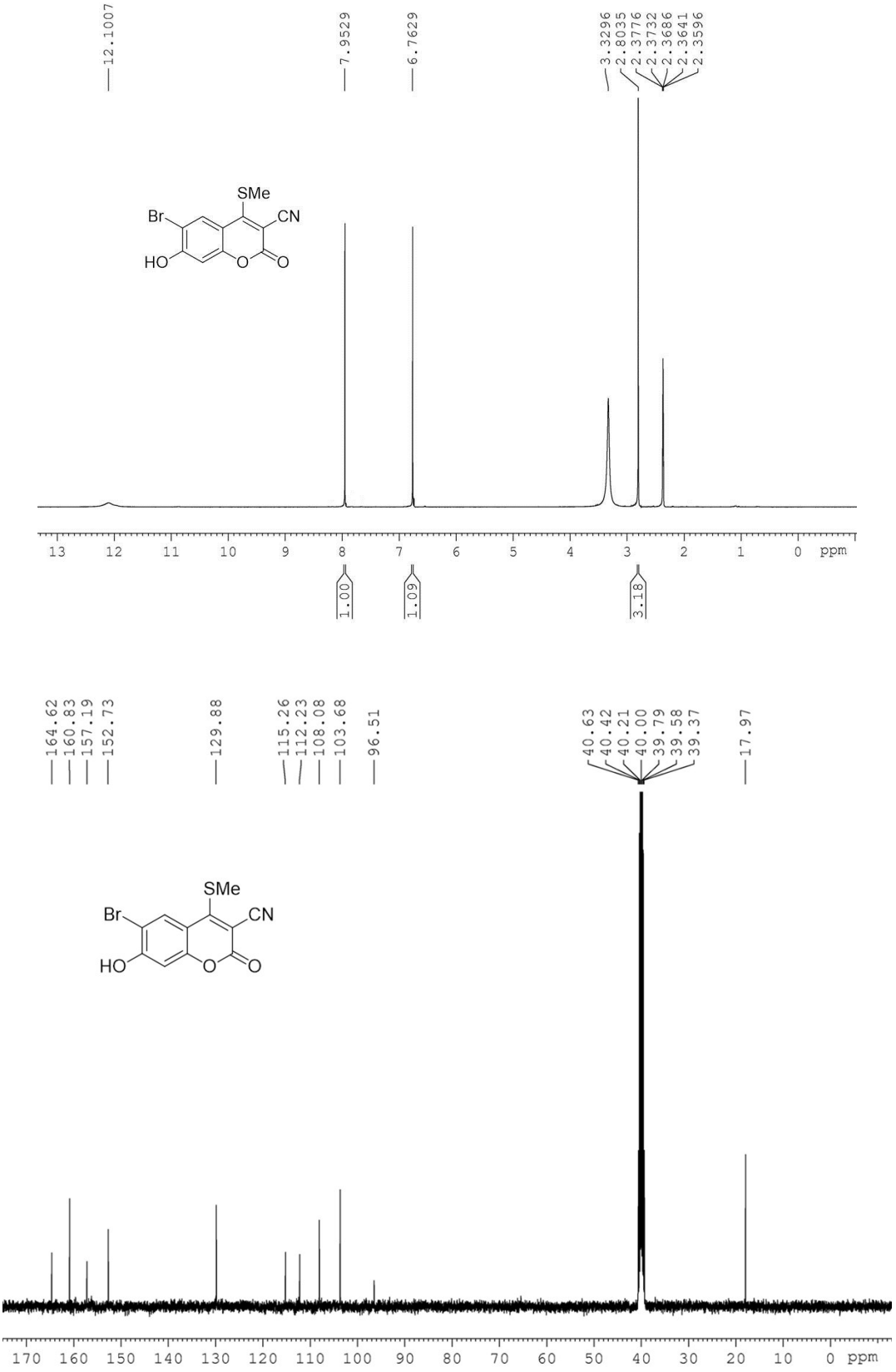
Figure S11. Competitive experiment between compound **4a,b**,Cysteine (Cys) and Glutathione (GSH). Variation in the fluorescence intensity of **4a,b** (2.5×10^{-6} M) in presence of Cys (10mM), and GSH (10 mM). λ_{ex}= 420 nm.

^{1}H and ^{13}C NMR of 4a in DMSO-d_6

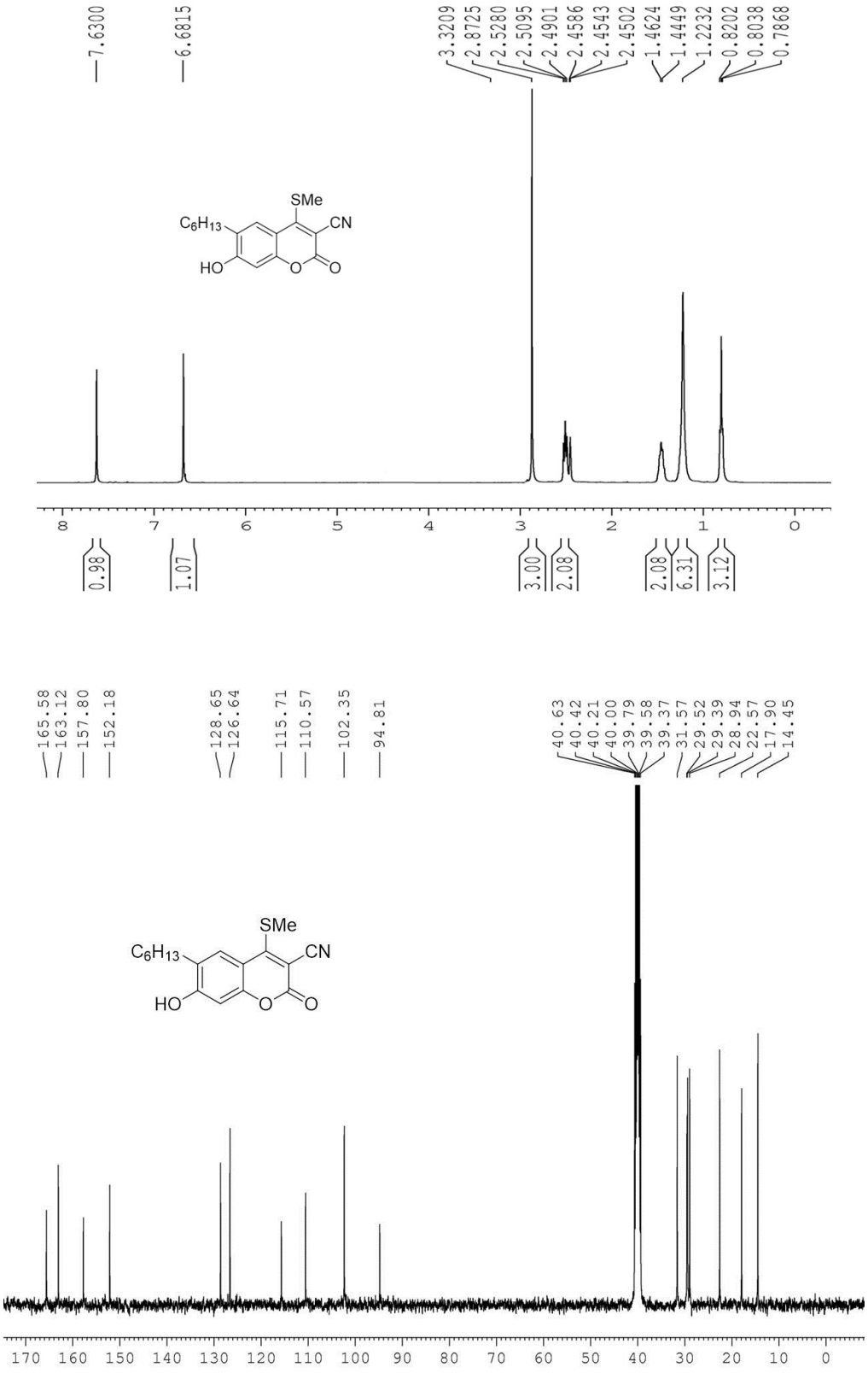


^{1}H and ^{13}C NMR of 4b in DMSO-d_6

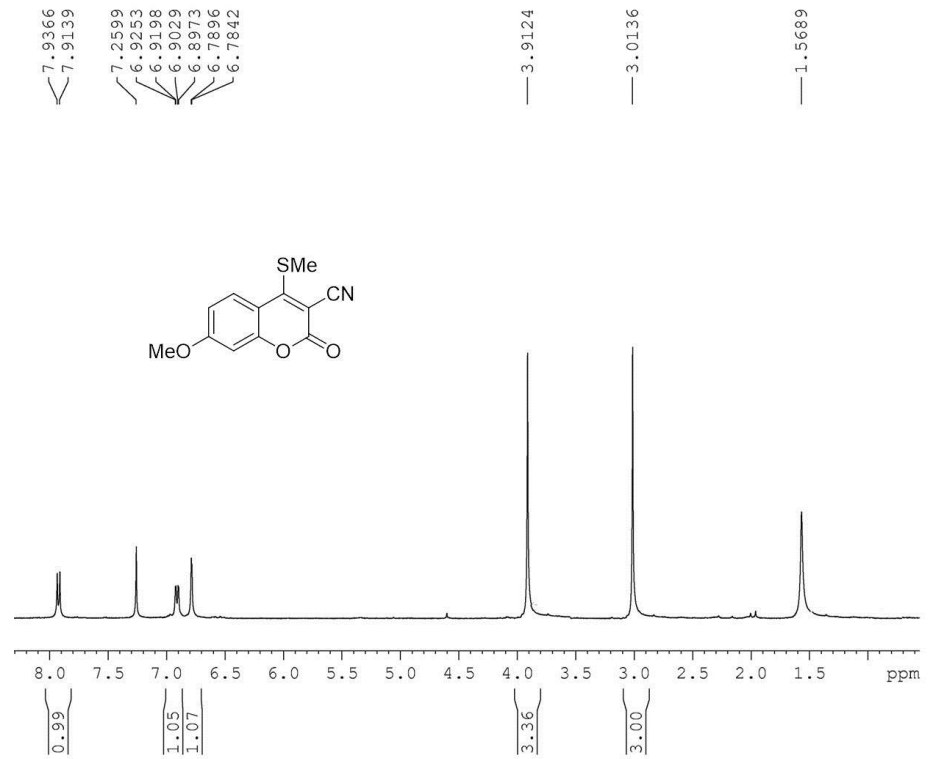
^{1}H and ^{13}C NMR of 4c in DMSO-d_6

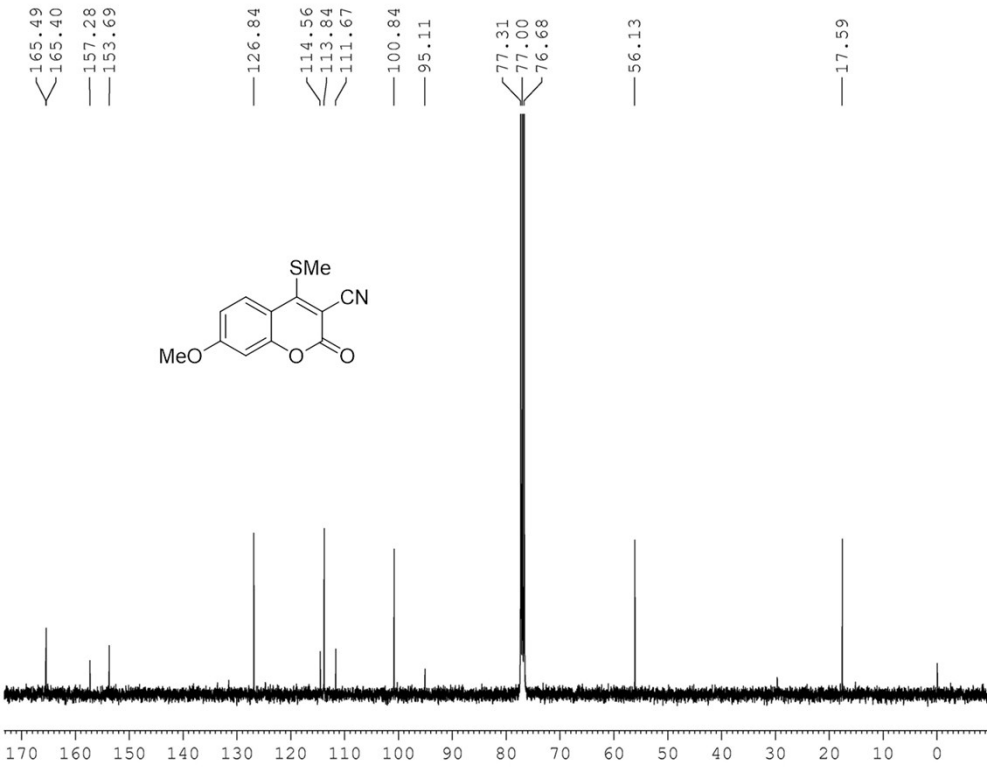


^{1}H and ^{13}C NMR of 4d in DMSO-d_6

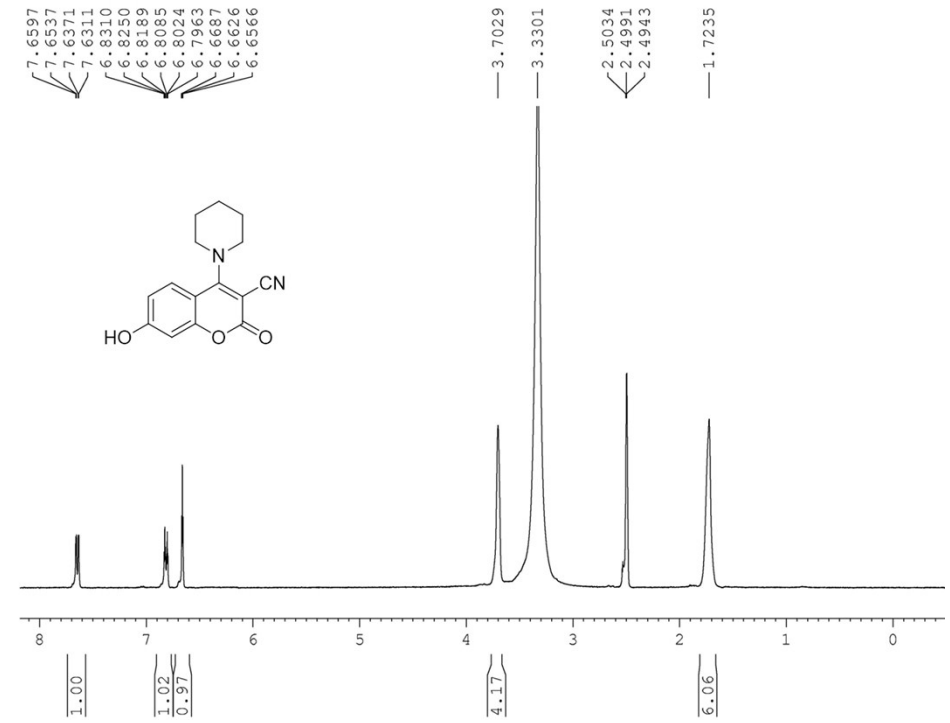


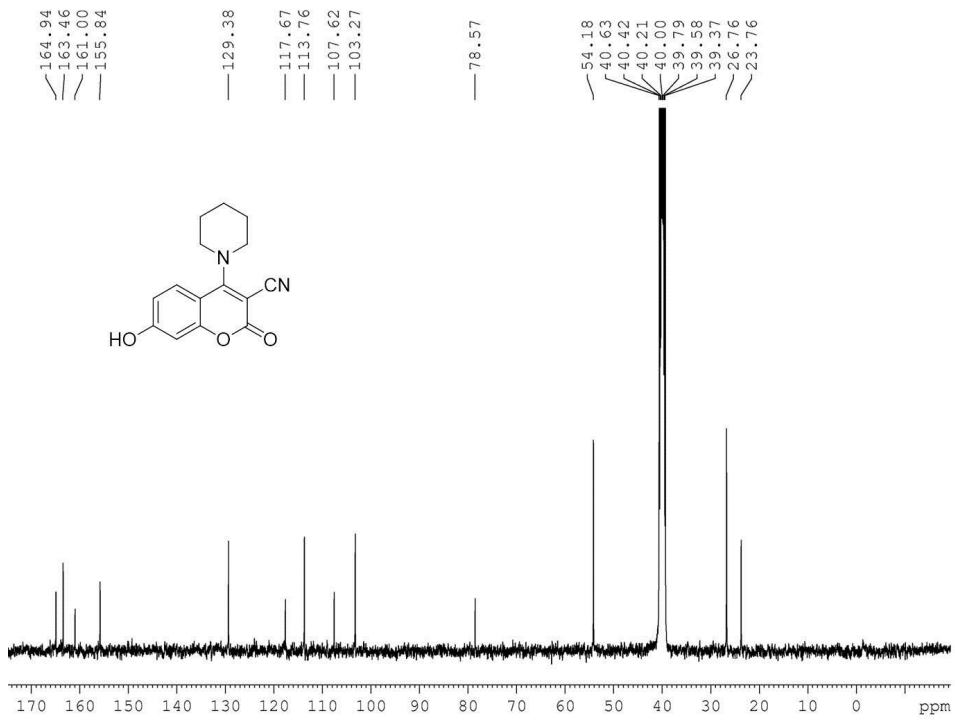
^{1}H and ^{13}C NMR of 5 in $CDCl_3$



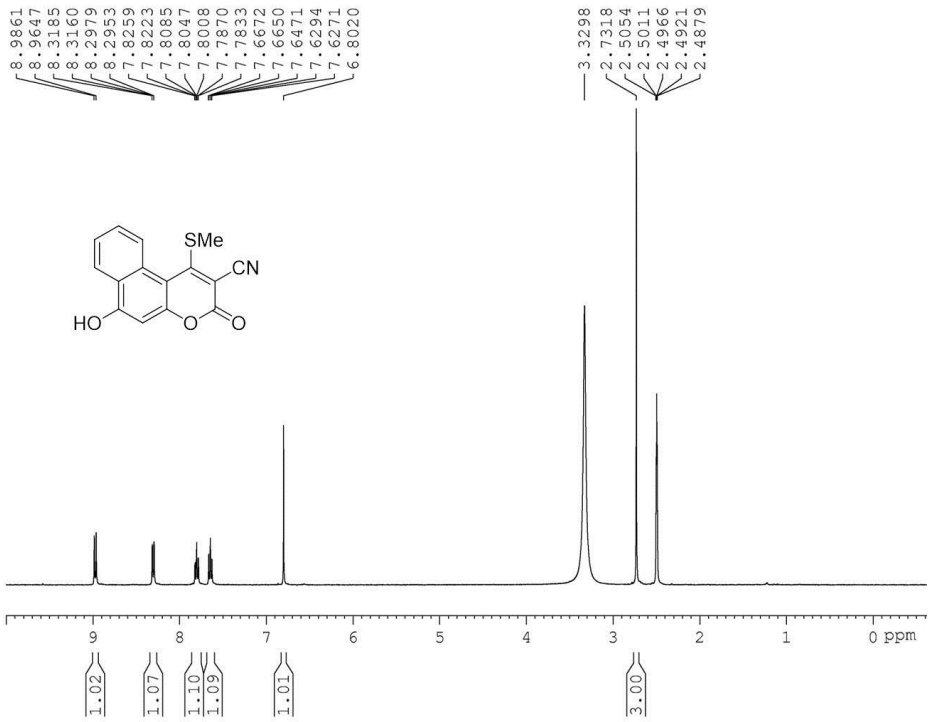


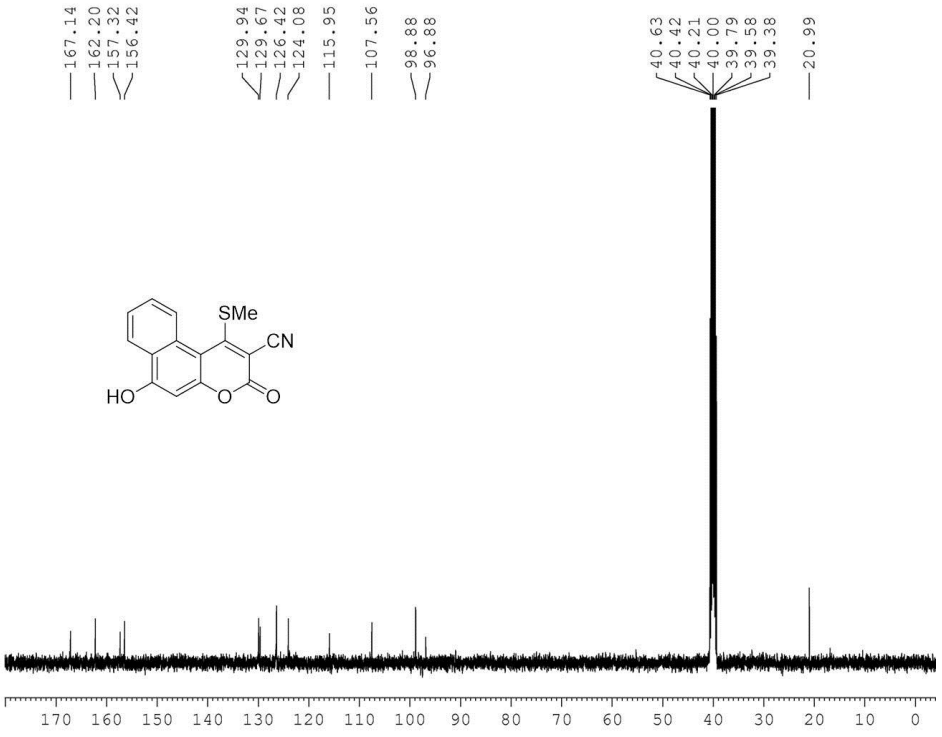
^{1}H and ^{13}C NMR of 6 in DMSO-d_6





^{1}H and ^{13}C NMR of 8 in DMSO-d_6





Cartesian coordinates (Å) of CHMCs optimized at the B3LYP/6-31G(d,p)

Compound **4a**

Atom	X	Y	Z
C	-3.43786980	-0.15088969	0.03228947
C	-2.63417030	0.98921804	0.10617144
C	-1.25730419	0.86683111	0.03776495
C	-0.63144987	-0.39552619	-0.10790799
C	-1.46359475	-1.53664123	-0.16464758
C	-2.83608135	-1.42091409	-0.10014190
H	-3.08624310	1.96300412	0.21880627
C	0.79755966	-0.42927786	-0.18615188
H	-1.00269744	-2.51054632	-0.25409852
H	-3.45480155	-2.30876377	-0.14483414
C	1.52947868	0.73171945	-0.11568871
C	0.89854605	2.03888444	0.06745892
O	-4.78828920	0.03864028	0.10247261
H	-5.28436103	-0.82039256	0.04414106
S	1.62903697	-2.05141559	-0.44626972
O	1.46462765	3.11918263	0.15512082
O	-0.51322095	2.02705988	0.12891149
C	2.93648241	0.76923279	-0.24761016
N	4.09768470	0.81628310	-0.35203018
C	2.75439686	-2.14901764	1.10471599
H	2.14084424	-1.97168941	1.98563239
H	3.13158018	-3.17163265	1.09203931
H	3.56639378	-1.43208873	1.01328580

Compound **4b**

Atom	X	Y	Z
C	2.76213675	-1.04087654	0.06787253
C	1.64861757	-1.88423210	0.12532752
C	0.37359728	-1.35844713	0.06192453
C	0.13602750	0.03466505	-0.05361957
C	1.27085644	0.87186741	-0.11079673
C	2.54741770	0.33803059	-0.05472328
H	1.78924481	-2.95349697	0.21767214
C	-1.22411881	0.45833711	-0.10158341
H	1.16713779	1.94526522	-0.19993794
C	-2.28934306	-0.50133091	-0.07977646
C	-2.00258341	-1.90012714	0.04095591
O	3.99216869	-1.60822506	0.13605619
H	4.67487955	-0.92386688	0.08429339
S	-1.62147828	2.11877495	-0.50878718
O	-2.81267401	-2.81026484	0.09342204
O	-0.66242931	-2.26456102	0.11594132

C	-3.64209677	-0.13147598	-0.15981616
N	-4.75185566	0.21266944	-0.23009542
C	-1.88419581	2.93001005	1.08227932
H	-0.99092992	2.79576994	1.69587631
H	-2.08996529	3.98050801	0.88706416
H	-2.73197238	2.44297478	1.57166492
Cl	3.94551955	1.40527327	-0.12805834

Compound **4c**

Atom	X	Y	Z
C	2.02131928	0.15099985	-0.04777192
C	2.02981954	1.55050106	0.13881855
C	0.82092291	2.24390753	0.20585095
C	-0.36677413	1.53924417	0.09184167
C	-0.40658790	0.13902770	-0.08131176
C	0.83338184	-0.53653209	-0.15863689
O	-1.54174606	2.28007084	0.14469144
C	-2.81545519	1.69939654	0.01100357
C	-2.84564605	0.24756736	-0.14837361
C	-1.68948831	-0.50041214	-0.18054460
S	-1.80900183	-2.33264138	-0.41835803
O	-3.79107467	2.44965174	0.05302553
C	-1.44657061	-2.91245820	1.36765072
Br	3.70444428	-0.80332676	-0.15900699
O	3.24411927	2.17431344	0.23786370
C	-4.14467668	-0.32768365	-0.24954398
N	-5.22762562	-0.75692742	-0.32792280
H	0.79447557	3.31519204	0.33692554
H	0.84134146	-1.60164062	-0.32319837
H	-1.48812185	-3.99552870	1.31600738
H	-0.45948150	-2.58442597	1.67143152
H	-2.21666992	-2.52938322	2.02618514
H	3.17845845	3.13934036	0.34854037

Compound **4d**

Atom	X	Y	Z
C	-0.93329871	0.72319649	-0.78341736
C	-0.67628781	2.08411786	-0.53511173
C	0.57401416	2.51838723	-0.14173736
C	1.58548554	1.59566537	0.02260051
C	1.38482217	0.23643869	-0.20193207
C	0.10670594	-0.16118663	-0.61697932

O	2.78459238	2.08296405	0.39416210
C	3.87031522	1.31507611	0.60442046
C	3.68612349	-0.13612156	0.42715984
C	2.51047619	-0.66341397	0.01416281
O	4.88580448	1.82672038	0.92135762
O	-1.61548215	3.02934211	-0.67321292
S	2.21820919	-2.39963395	-0.21308995
C	3.65344341	-3.00847423	-1.15114531
C	4.82451139	-0.92973421	0.79798306
N	5.71368186	-1.56707985	1.10414309
C	-8.03360069	-1.14783814	1.39507302
C	-7.05124636	-1.00417546	0.23390746
C	-5.66959118	-0.52002263	0.67634433
C	-4.68095881	-0.37484678	-0.48145590
C	-3.30031708	0.10852947	-0.03552886
C	-2.30667954	0.23642656	-1.20112869
H	0.75855805	3.56032941	0.03167545
H	-0.06923970	-1.19957472	-0.81967739
H	-2.43403999	2.67611310	-0.97727699
H	3.30921955	-3.92616514	-1.60921054
H	3.92081746	-2.30569870	-1.92705682
H	4.49787725	-3.20937262	-0.51472267
H	-9.00284613	-1.49249163	1.04865799
H	-7.67145056	-1.86211411	2.12923732
H	-8.18204962	-0.19907192	1.90321875
H	-7.45809979	-0.31032137	-0.49935240
H	-6.95112222	-1.96109254	-0.27476212
H	-5.26377615	-1.21427927	1.41057297
H	-5.77081379	0.43700601	1.18635141
H	-5.08781625	0.31804183	-1.21737848
H	-4.57874984	-1.33205576	-0.99053333
H	-2.88805456	-0.58089343	0.69696333
H	-3.39765329	1.06402131	0.47743962
H	-2.72248109	0.88753709	-1.97052479
H	-2.19822113	-0.73057282	-1.68092769