Electronic Supplementary Informations

A radical approach for fluorescent turn 'on' detection, differentiation and bioimaging of methanol

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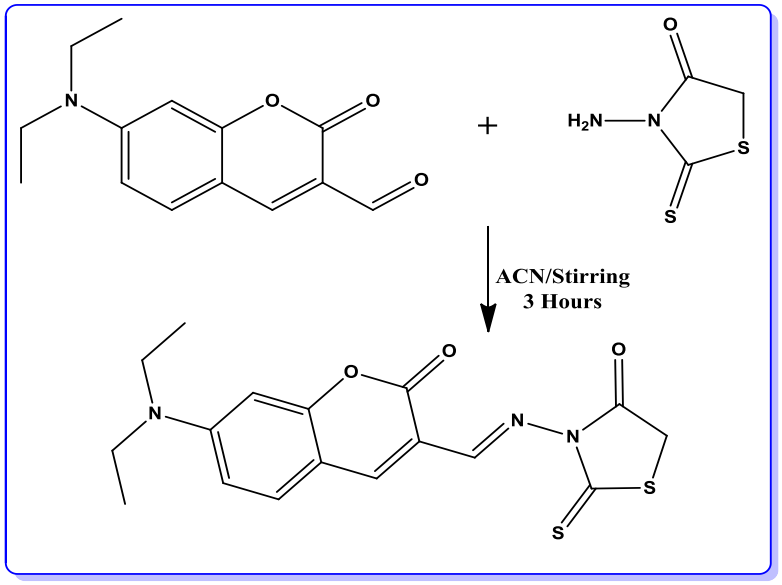
Experimental section

1.1. **Synthesis of RC: RC** was synthesized by adding 2.0 mM acetonitrile solution of 7-(diethylamino)-2-oxo-2H-chromene-3-carbaldehyde to the equimolar acetonitrile solution of 3-aminorhodanine followed by constant stirring for three hours at room temperature (**Scheme 1**). A brick red solid was precipitated which was filtered and washed with diethyl ether and finally dried under vacuum over anhydrous $CaCl_2$. **RC** was characterized through various spectroscopic techniques like IR, ^{1}H & ^{13}C NMR spectral studies along with mass determination through ESI-MS **(ESI; Fig. S1-S4)**.



Scheme 1: Synthesis of **RC**

Spectroscopic characterization data: **Yield:** 86%, **IR/cm^{-1}:** 2971, 2929, 1741, 1709, 1620, 1598, 1563, 1513, 1483, 1428, 1379, 1350, 1311, 1295, 1259, 1233, 1188, 1133, 1078, 1029, 955, 904, 875, 835, 796, 761, 688, 637; **^{1}H NMR (300 MHz, $CDCl_3$, 298K, TMS):** δ = 1.233-1.279 (t, 6H, CH_3), 3.433-3.504 (q, 4H, CH_2), 4.077 (s, 2H, CH_2), 6.490 (s, 1H, Ar-H), 6.613-6.635**,** (d, 1H, Ar-H), 7.369-7.399 (d, 1H, Ar-H), 8.587**,** (s, 1H, Ar-H), 8.740 (s, 1H, –CH=N) δ ppm; **^{13}C NMR (75**

MHz, $CDCl_3$, 298K, TMS): δ=194.83, 169.03, 161.07, 158.34, 152.90, 143.23, 131.79, 131.41, 110.23, 109.81, 108.46, 97.30, 96.92, 45.17, 33.73, 12.63, 12.27 δ ppm; **MS m/z (ESI) = 376.00** Calc. for, $C_{17}H_{17}N_3O_3S_2$= **375.47.**

1.2. **Apparatus:** IR Spectra were recorded with a Perkin-Elmer spectrometer using KBr pellets. The corresponding ^{1}H NMR and ^{13}C NMR spectra were recorded in **$CDCl_3$** with a JEOL AL 300 FT NMR Spectrometer instrument using tetramethylsilane ($Si(CH_3)_4$) as an internal standard. **^{1}H** and **^{13}C** chemical shifts are reported in parts per million (ppm) relative to the residual proton signal of the deuterated solvents. Mass spectrometric analysis was carried out on a MDS Sciex API 2000 LCMS spectrometer while HRMS of **RO** was recorded at Water-Q-Tof Premier-HAB213. The electronic spectra and UV-visible titrations were carried out room temperature (298 K) on a UV-1700/1800 Pharmaspec spectrophotometer with quartz cuvette (path length=1 cm). The emission spectra were recorded at JY HORIBA Fluorescence spectrophotometer.

1.3. **Materials:** All the reagents and solvents for synthesis were purchased from Sigma-Aldrich and were used without further purification. All reactions were carried out using commercial-grade solvents.

1.4. **Theoretical Calculations:** The geometric and energy optimizations were performed with the Gaussian 03 program based on the density functional theory (DFT) method.[S1] Becke's three parameter hybrid functional with the Lee-Yang-Parr correlation functional (B3LYP) was employed for all the calculations. The 3-21G** basis set was used to treat all atoms.

1.5. **X-ray diffraction studies**: *S*ingle crystal X-ray diffraction measurements were carried out on an Oxford Diffraction Xcalibur system with a Ruby CCD detector using graphite-monochromated MoKa radiation (k = 0.71073 Å). All the determinations of unit cell and intensity data were performed with graphite-mono-chromated Mo-Kα radiation (λ= 0.71073 A°). Data for the ligand and metal complexes were collected at room temperature/liquid nitrogen temperature. The structures were solved by direct methods, using Fourier techniques, and refined by full-matrix least-squares on F^2 using the SHELXTL-97 program package.[S2]

1.6. **Cell Imaging Studies:** *E. coli* strains (DH5-α) were grown in LB media at 37° C overnight in shaker incubator. The cells were collected in sterile water and vortexed to make the suspension homogeneous. These cell cultures were incubated with **RC**

(10μM) from 1.0 mM stock in 50mM phosphate buffer (pH 7.54) for 1 hour. The treated cells were examined by the excitation range from 450-490 nm and emission range from 500-560 nm on a fluorescence microscope (Nikon-E800, Japan).

References:

S1. M. J. Frisch, et al., GAUSSIAN 03, (Revision D.01), Gaussian, Inc., Wallingford, CT, 2004.

S2. (a) G. M. Sheldrick, SHELXL-97, Program for X-ray Crystal Structure Refinement, Göttingen University, Göttingen, Germany, 1997; **(b)** G. M. Sheldrick, SHELXS-97, Program for X-ray Crystal Structure Solution, Göttingen University, Göttingen, Germany, 1997.

Figure S1: ^{1}H NMR spectrum of **RC** in $CDCl_3$**:**

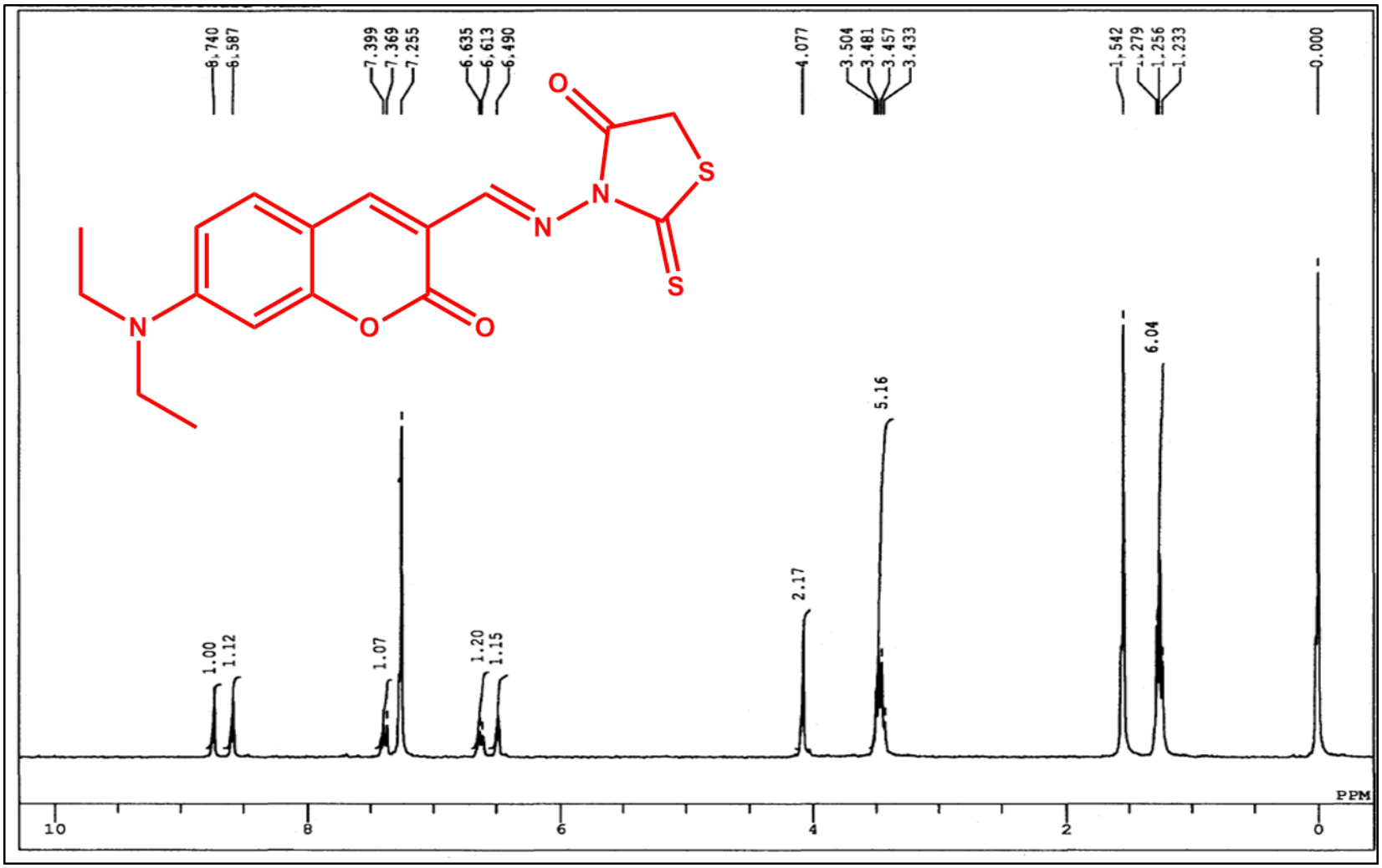


Figure S2: ^{13}C NMR spectrum of **RC:**

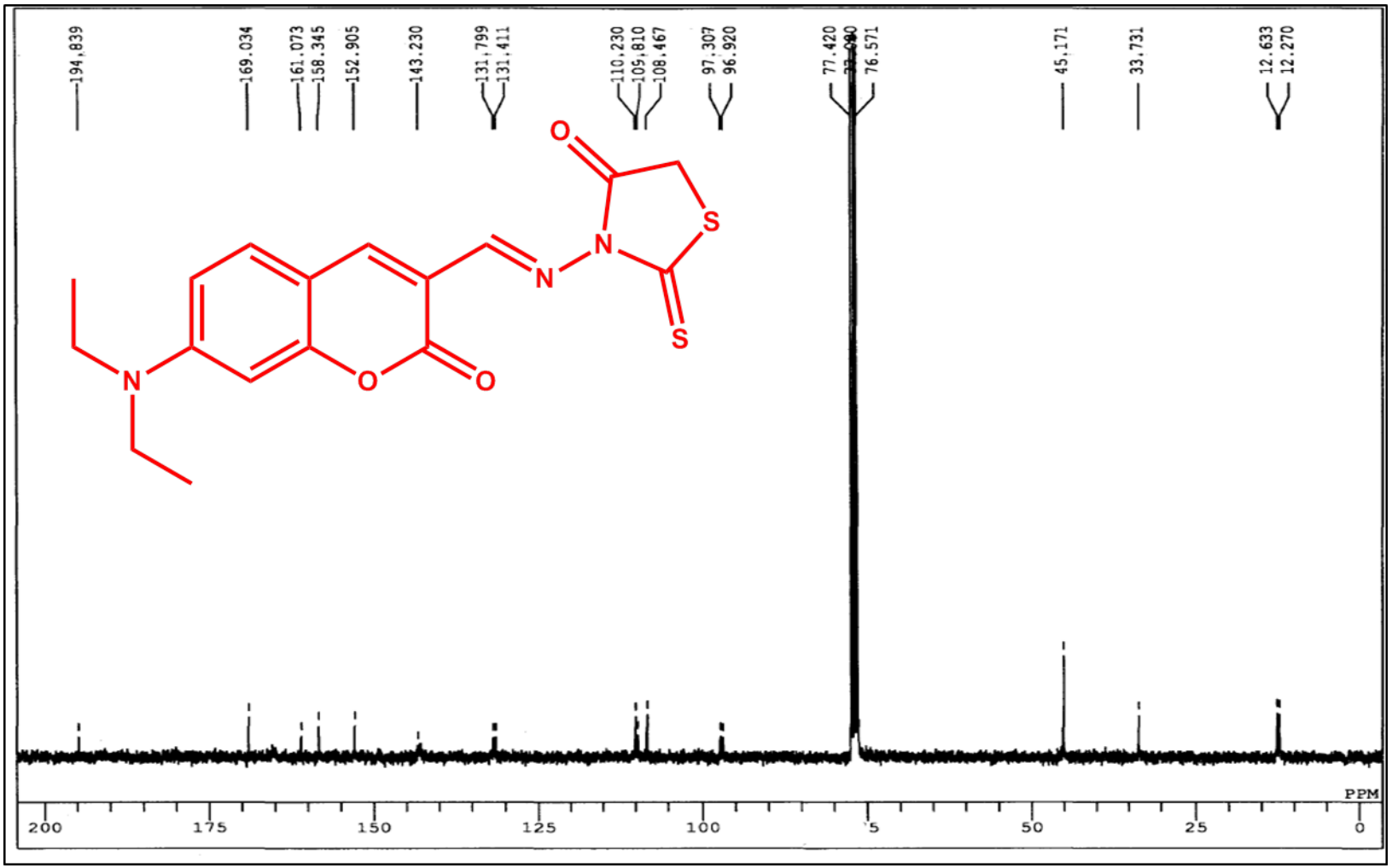


Figure S3: IR spectrum of **RC:**

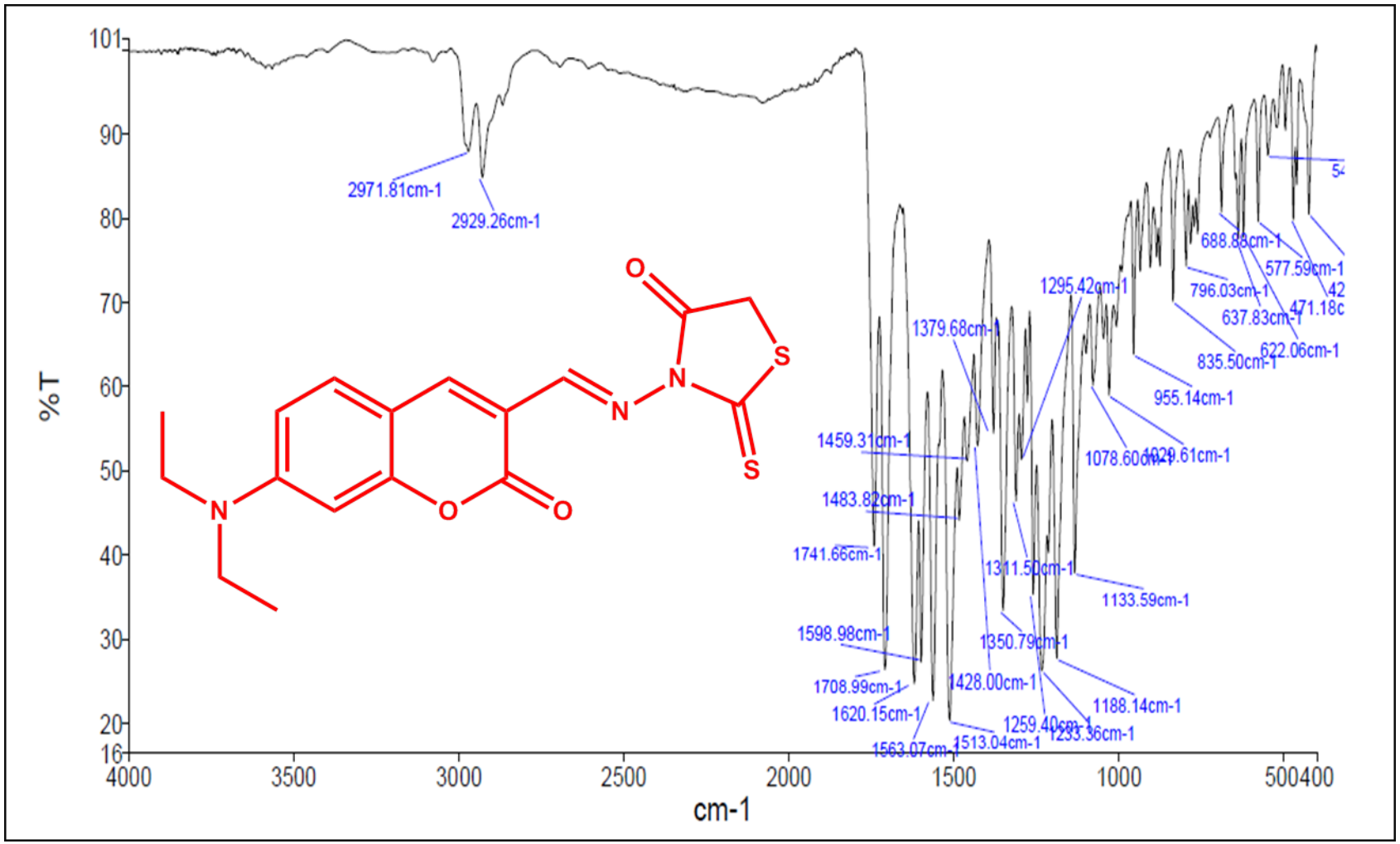


Figure S4: Mass spectrum of **RC:**

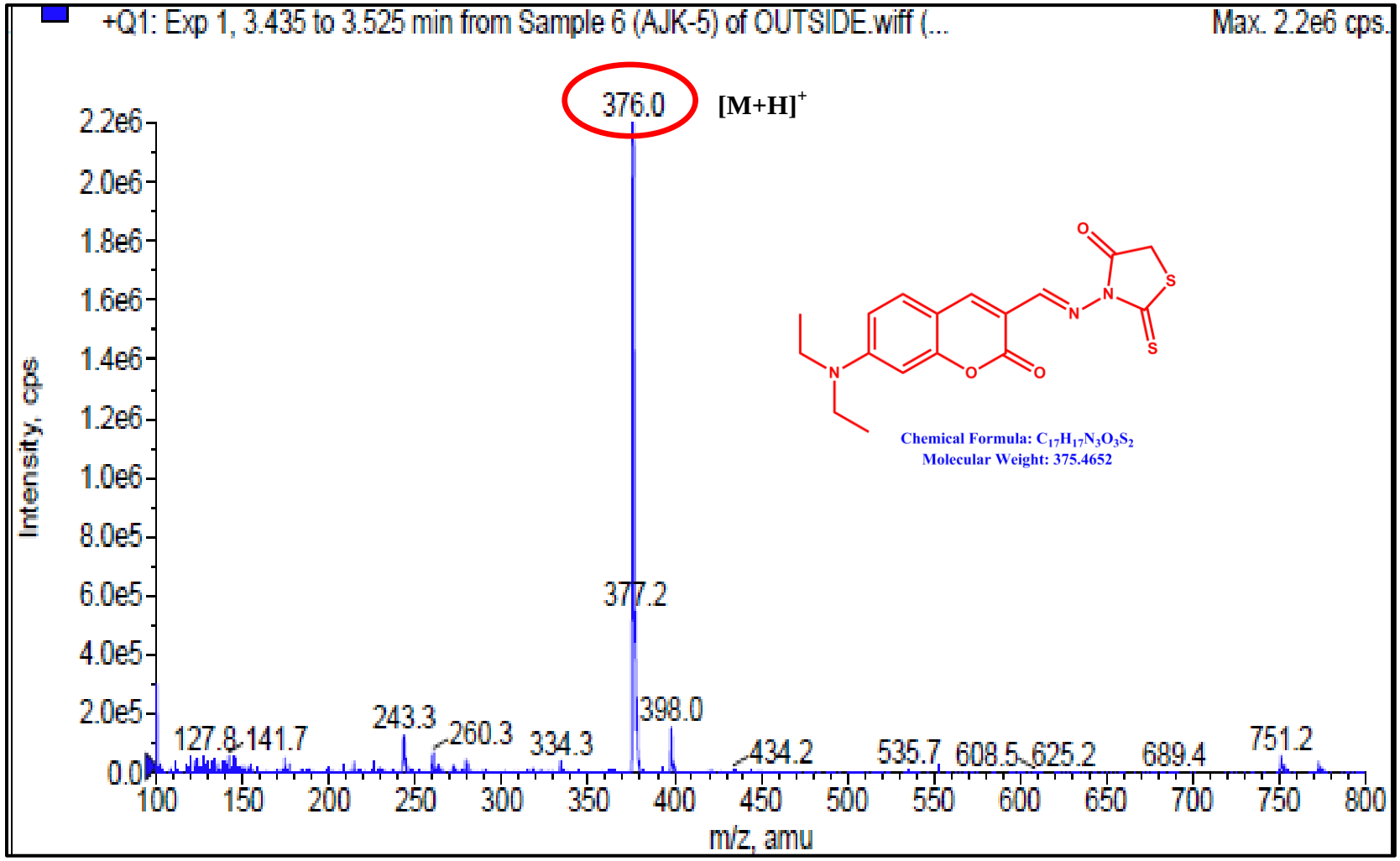


Figure S5: UV-visible absorbance spectrum of **RC** in different solvent:

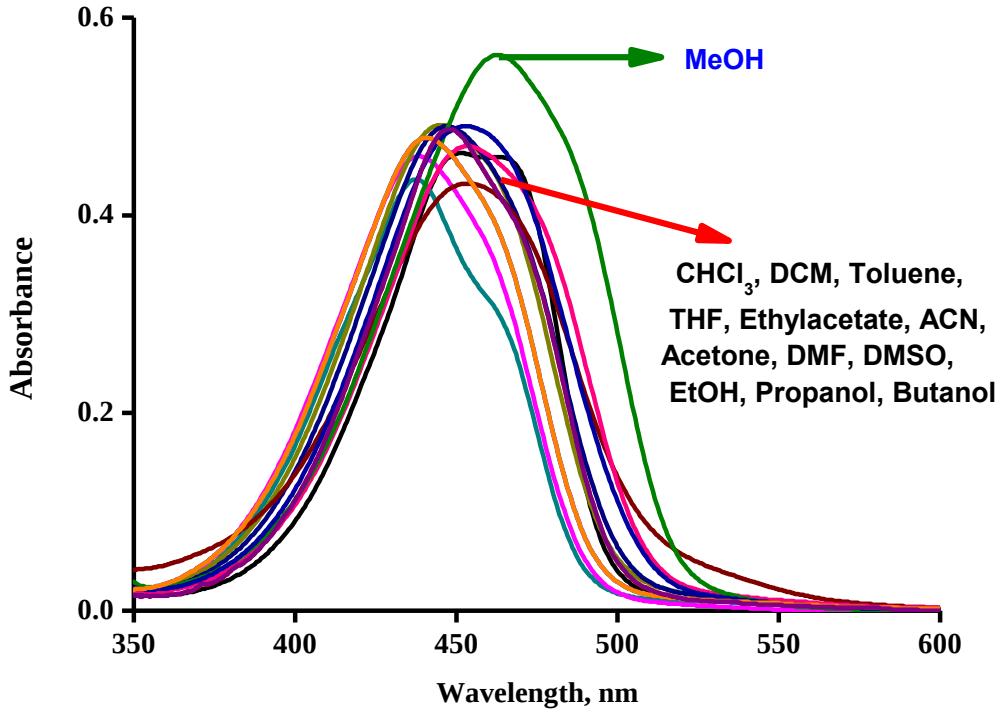


Figure S6a: Selective visible color changes of **RC** in various solvents; from left to right: $CHCl_3$, DCM, Toluene, THF, Ethylacetate, ACN, Acetone, MeOH, EtOH, Propanol, Butanol, DMF, and DMSO

Figure S6b: Selective fluorescence color changes (Under UV light) of **RC** in various solvents; from left to right: **RC**, MeOH, EtOH, Propanol, Butanol, Acetone, Ethyl acetate, Toluene, DCM, $CHCl_3$, THF, ACN, DMF, DMSO and Water.

Figure S7: ^{1}H NMR spectrum of **RO** in $CDCl_3$**:**

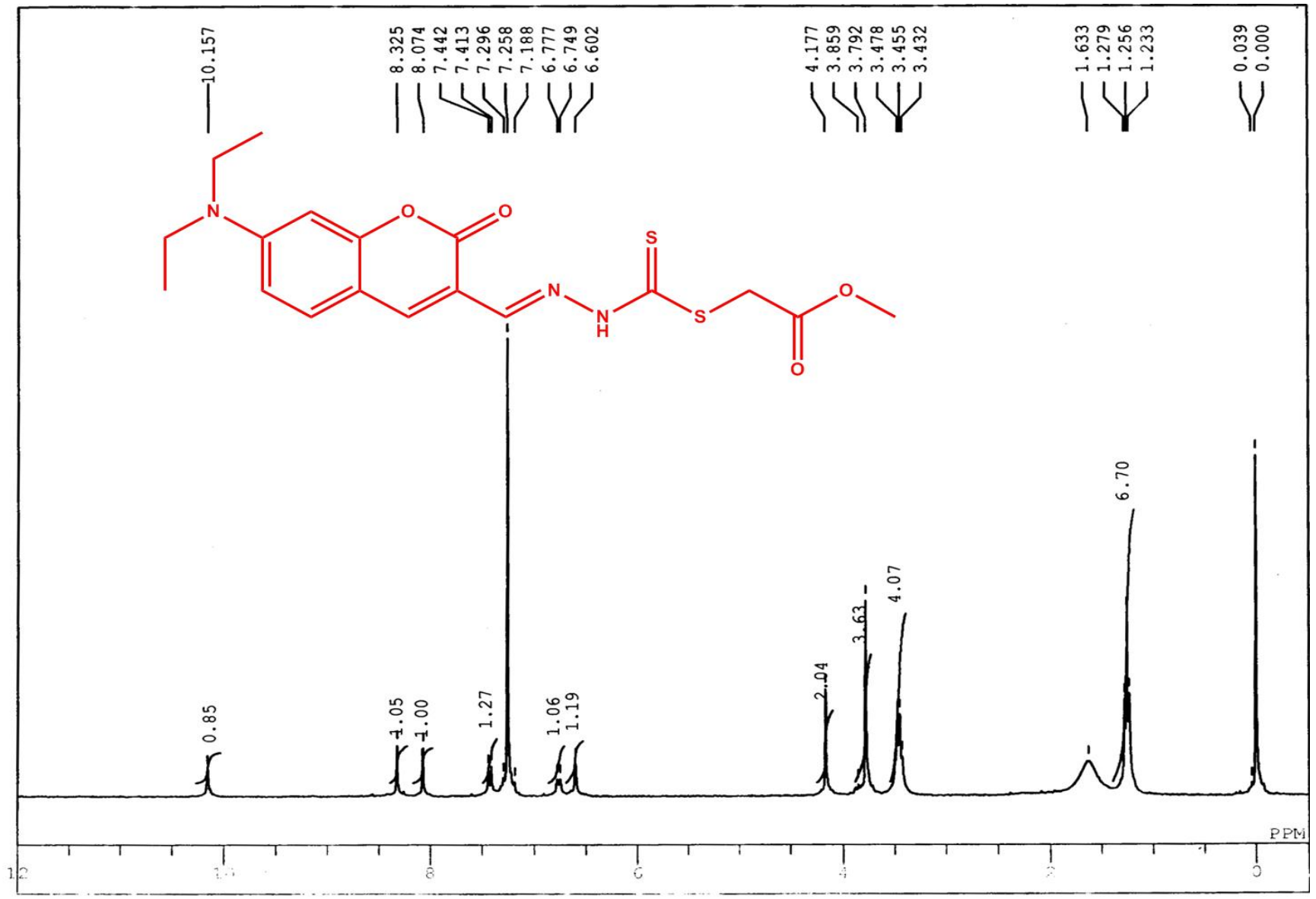


Figure S8: Proposed mechanism of nucleophile attack of methanol over **RC:**

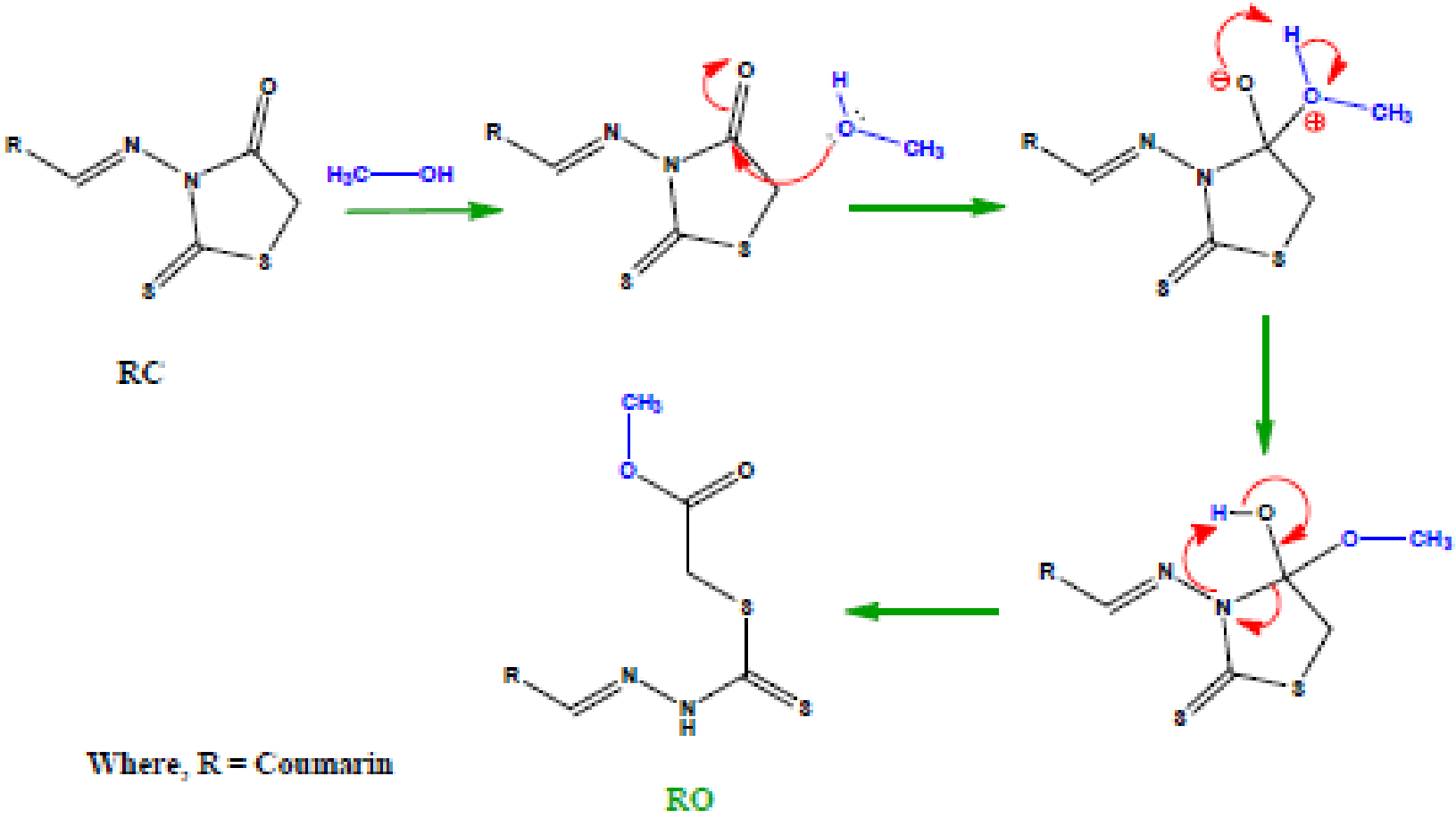


Figure S9: IR spectrum of **RO:**

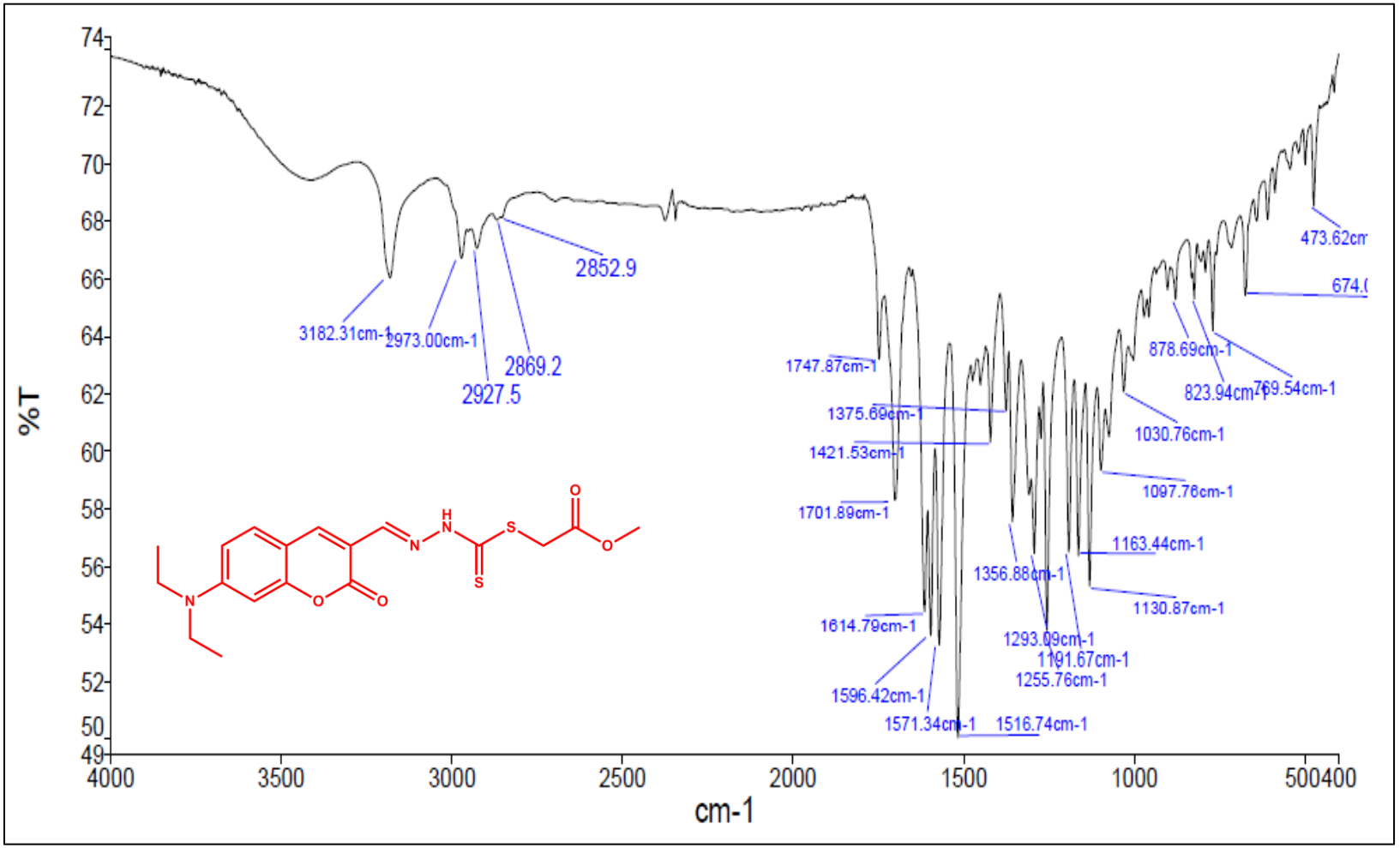


Figure S10: Overlay IR spectrum of **RC** and **RO:**

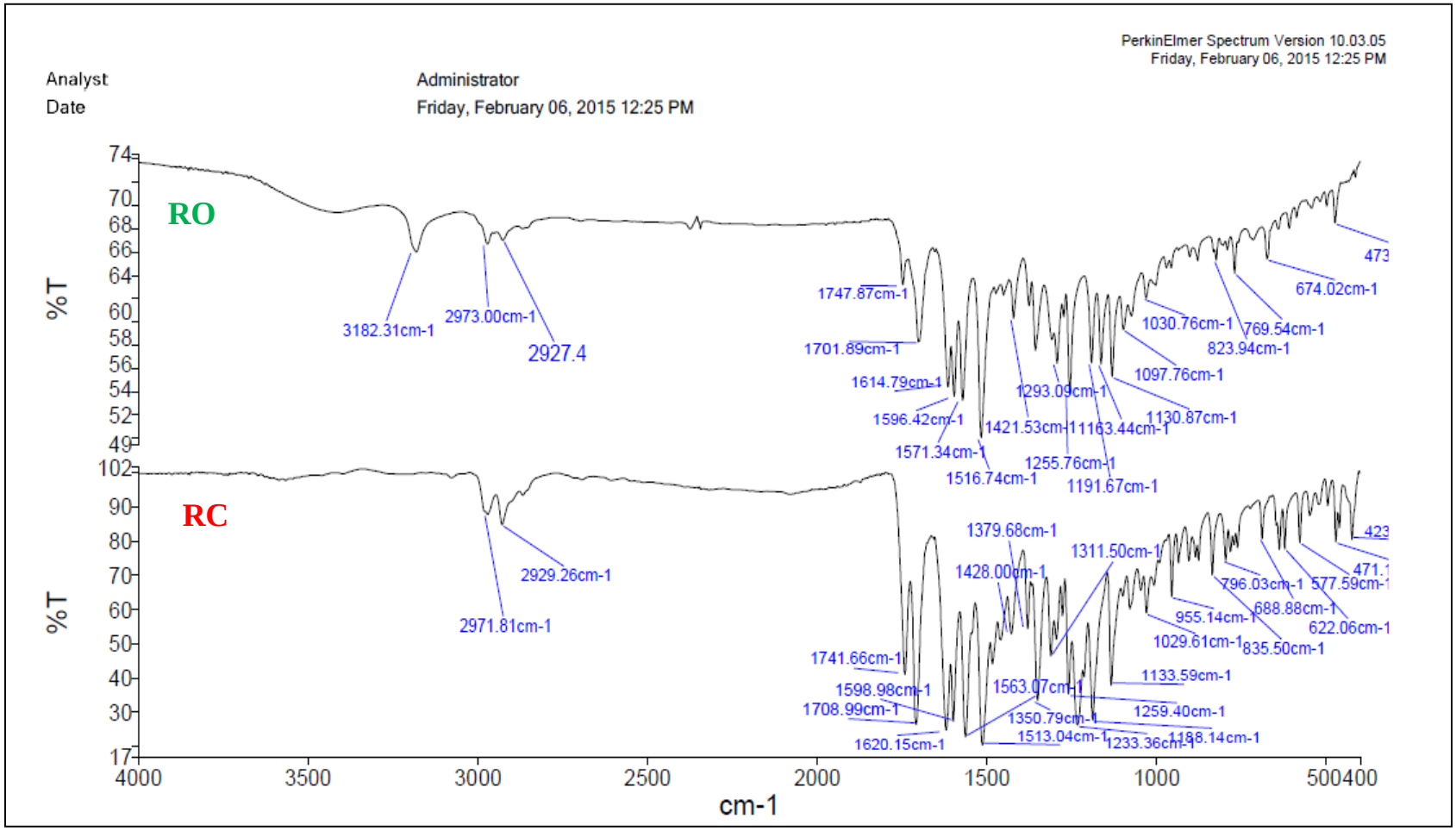


Figure S11: Mass spectrum of **RO:**

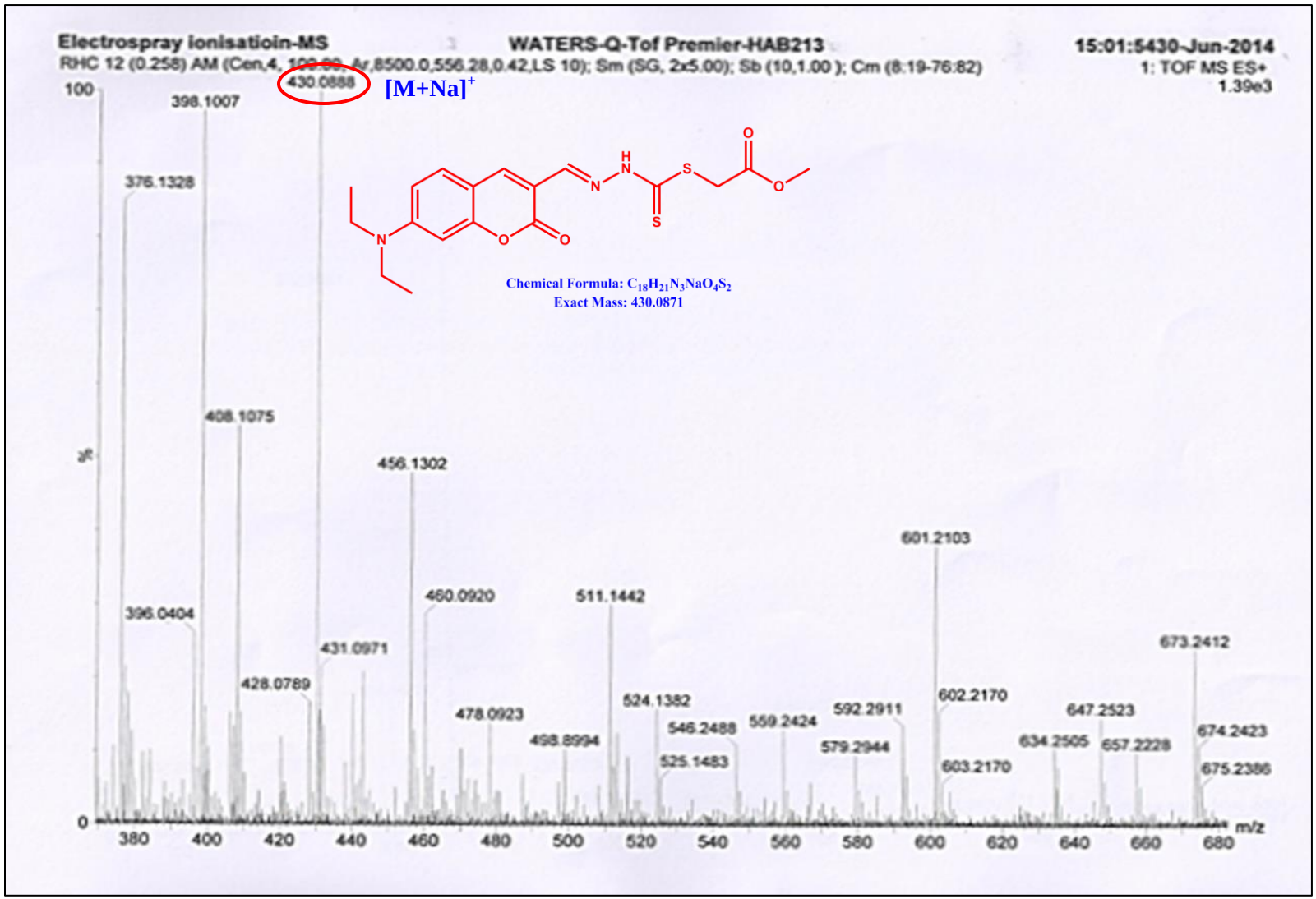


Figure S12: HOMO-LUMO orbitals of **RC** and **RO** their calculated energy and energy gaps are shown:

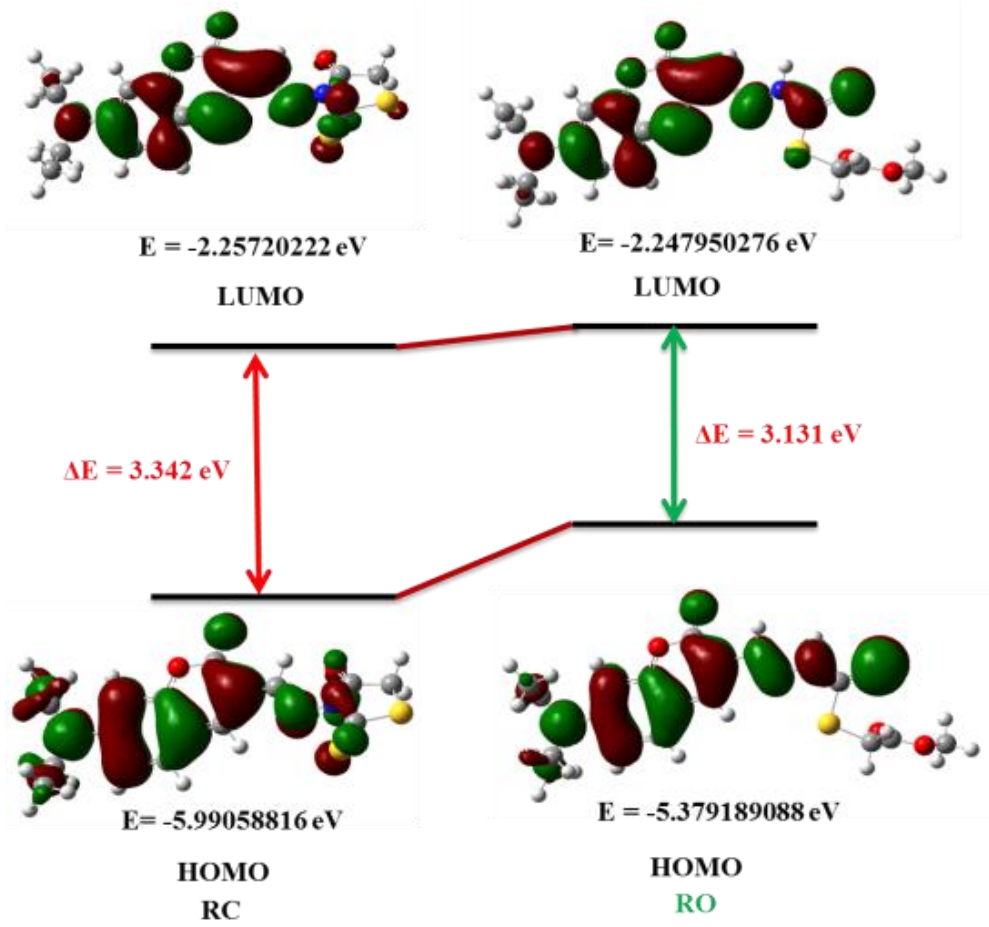


Figure S13: Fluorescence reaction time profile of receptor **RC** at 0.5μM in MeOH:

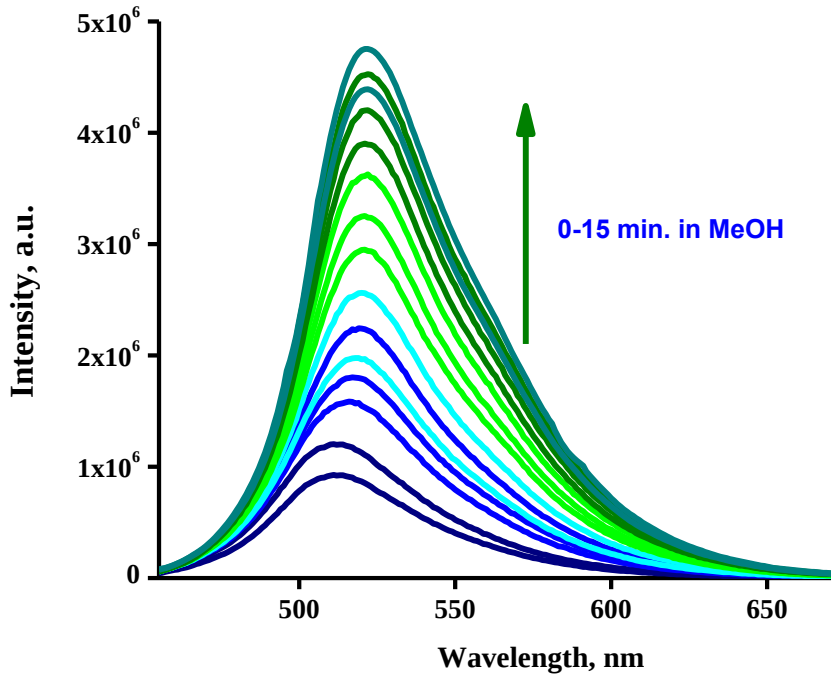


Figure S14: Calibration curve of **RC** in water with increasing MeOH%:

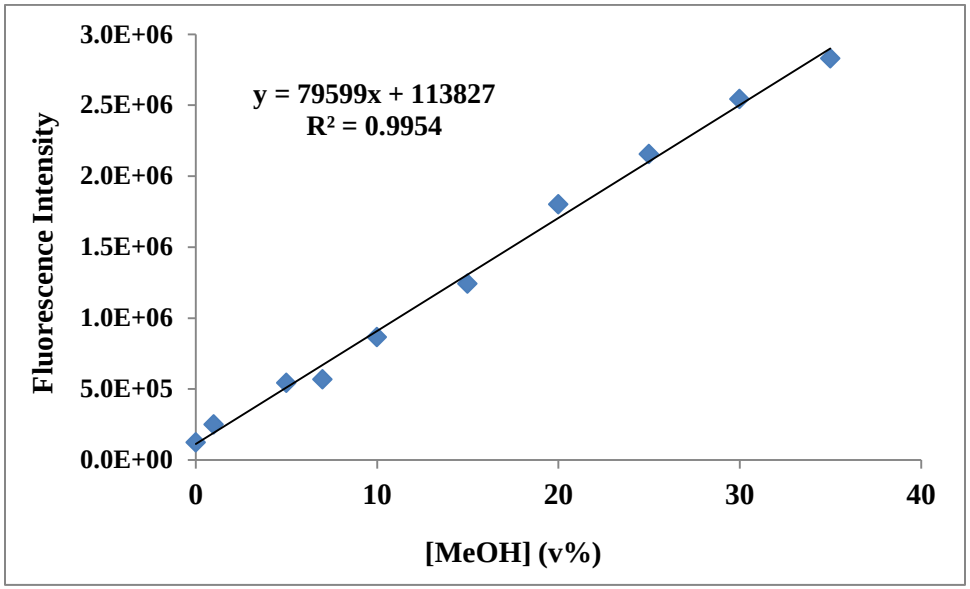
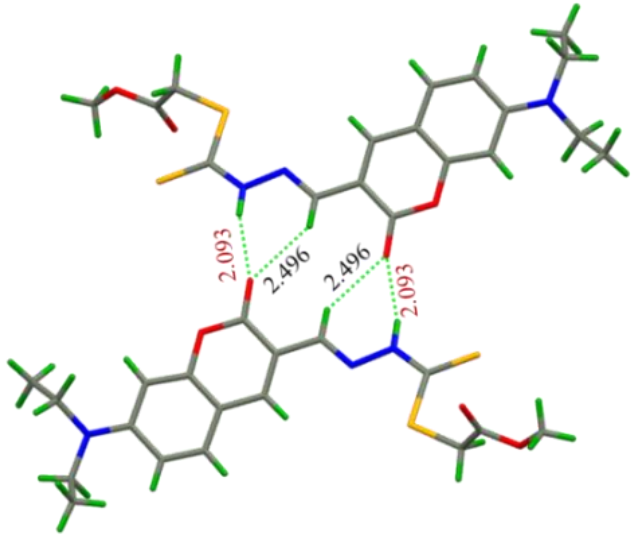


Table S1: Important bond length and bond angle of **RO:**

Atom	Bond Length(Å)	Atom	Bond angle
S(2)- C(16)	1.786(3)	C(16)-S(2)-C(15)	100.(1)
S(2)-C(15)	1.752(2)	C(9)-O(1)-C(13)	122.7(2)
S(1)-C(15)	1.643(2)	N(3)-N(2)-C(14)	116.1(2)
O(1)- C(9)	1.377(3)	C(17)-O(4)-C(18)	115.1(2)
O(1)-C(13)	1.381(3)	N(2)-N(3)-C(15)	119.2(2)
N(2)-N(3)	1.383(2)		
N(2)-C(14)	1.271(4)		
O(4)-C(17)	1.343(4)		
O(4)-C(18)	1.445(3)		
O(2)-C(13)	1.202(3)		
N(3)-C(15)	1.339(4)		

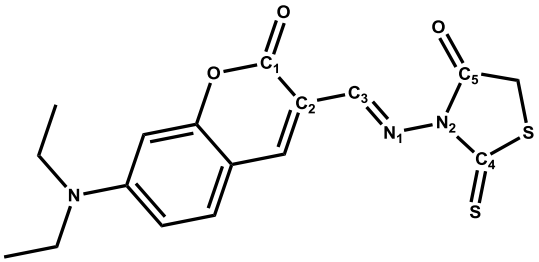


Showing intermolecular hydrogen bonding in **RO**

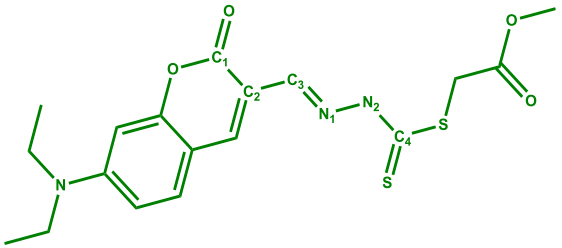
Table S2: Crystal data and structure refinement for **RO:**

Identification code	**RO**
CCDC No.	**980304**
Empirical formula	C18 H21 N3 O4 S2
Formula weight	407
Temperature	293(2) K
Wavelength	0.71073Å
Crystal system, space group	Triclinic, P -1
Unit cell dimensions	a = 6.9629(13) Å alpha = 102.726(18) deg. b = 10.231(3) Å beta = 97.404(16) deg. c = 14.781(2) Å gamma = 94.658(18) deg.
Volume	1012.0(3) Å3
Z, Calculated density	2, 1.334 Mg/m3
Absorption coefficient	0.291 mm-1
F(000)	428.0
Crystal size	0.34 x 0.28 x 0.22 mm
Theta range for data collection	2.97 to 28.99 deg.
Limiting indices	-8<=h<=9, -9<=k<=13, -19<=l<=19
Reflections collected / unique	6963 / 4036 [R(int) = 0.0397]
Completeness to theta = 25.00	99.0 %
Max. and min. transmission	1.00000 and 0.94362
Refinement method	Full-matrix least-squares on F2
Data / restraints / parameters	4036 / 0 / 248
Goodness-of-fit on F2	0.960
Final R indices [I>2sigma(I)]	R1 = 0.0574, wR2 = 0.0556
R indices (all data)	R1 = 0.1294, wR2 = 0.0712
Largest diff. peak and hole	0.213 and -0.161 e.Å-3

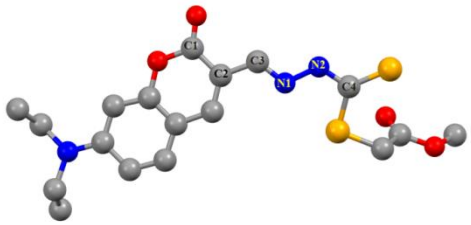
Table S3: The selected experimental and calculated dihedral angles in **RC** and **RO:**



RC



RO



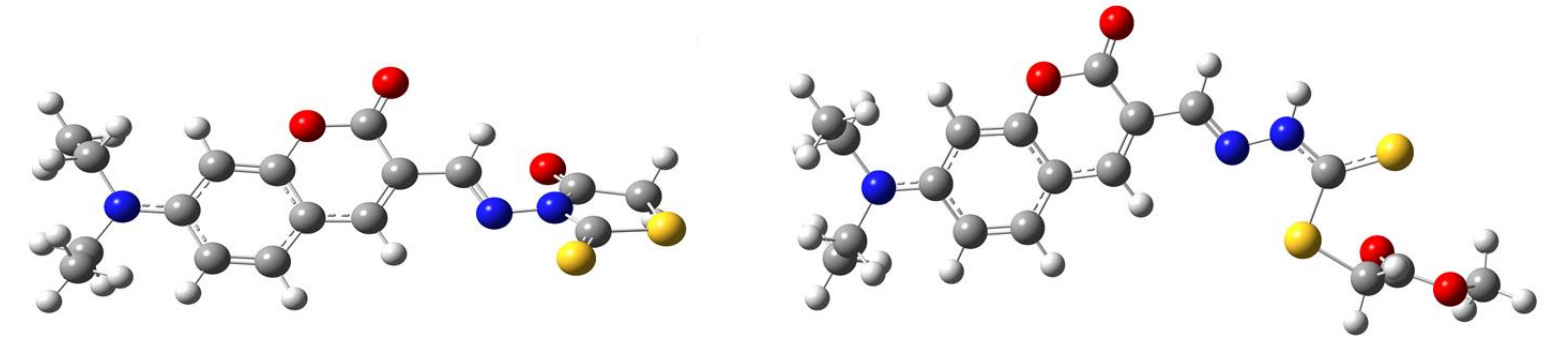
Crystal structure RO

Dihedral angles of RC and RO

DA1 = C1-C2-C3=N1
DA2= C2-C3=N1-N2
DA3 = C3-N1-N2-C4
DA4 = N1-N2-C4-S1

DFT calculated structure								**Single Crystal Structure**			
RC				**RO**				**RO**			
DA1	**DA2**	**DA3**	**DA4**	**DA1**	**DA2**	**DA3**	**DA4**	**DA1**	**DA2**	**DA3**	**DA4**
177.19	177.62	139.15	-11.13	178.96	179.64	-179.97	179.73	166.62	177.61	-171.71	179.80

Table S4: Theoretical calculation of absorption maxima of **RC** and **RO** in MeOH using TD-DFT study:



RC **RO**

Entry	Major transitions	Wavelength	Oscillator strength	Energy	Contributions of Excitation %
RO	107 →108 0.63996	449.17 nm	f=1.3047	2.7603 eV	HOMO → LUMO = 81.9
RC	98 → 99 0.63028	428.38 nm	f=0.9064	2.8943 eV	HOMO → LUMO = 79.45