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Electronic Supplementary Information (ESI)

Visible light excitable ON fluorescence and naked eye detection of Cu^{2+} *via* hydrolysis of rhodamine- thiophene conjugate: Human breast cancer cell (MCF7) imaging studies

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1. General method of UV-Vis and fluorescence titration

Path length of the cells used for absorption and emission studies is 1 cm. 20 μM stock solution of **RDHDTCA** is prepared in CH_3CN/ water (9/1, v/v). Working solutions of **RDHDTCA** and Cu^{2+} are prepared from their respective stock solutions. Fluorescence measurements are performed using 5 nm x 5 nm slit width. All the fluorescence and absorbance spectra are recorded after 40 minutes of mixing of Cu^{2+} and **RDHDTCA**.

2. Job's plot from fluorescence experiments

A series of solutions containing **RDHDTCA** and $Cu(NO_3)_2.3H_2O$ are prepared such that the total concentration of Cu^{2+} and **RDHDTCA** remain constant (20 μM) in all the sets. The mole fraction (X) of **RDHDTCA** is varied from 0.1 to 0.8. The fluorescence intensity at 581 nm is plotted against the mole fraction of **RDHDTCA**.

3. Calculation of quantum yield

Fluorescence quantum yields (Φ) are estimated by integrating the area under the fluorescence curves using the equation,

$$\phi_{sample} = \phi_{ref} \times \frac{OD_{ref} \times A_{sample} \times \eta^2_{sample}}{OD_{sample} \times A_{ref} \times \eta^2_{ref}}$$

where A is the area under the fluorescence spectra and OD is optical density of the compound at the excitation wavelength. Rhodamine B is used as reference with a known φ_{ref} value of 0.65 in basic EtOH[1]. The area of the emission spectrum is integrated using the software available in the instrument. φ_{sample} and φ_{ref} are the fluorescence quantum yields of the sample and reference respectively. A_{sample} and A_{ref} are the area under the fluorescence spectra of the sample and the reference, respectively. OD_{sample} and OD_{ref} are the corresponding optical densities of the sample and the reference solution at the wavelength of excitation. η_{sample} and η_{ref} are the refractive indeces of the sample and reference, respectively. Using the above formula, the quantum yields of **RDHDTCA** and its Cu^{2+} complex are 1.19×10^{-2} and 10.54×10^{-2} respectively.

4. Estimation of detection limit

The detection limit is calculated from the fluorescence titration data based on a reported and widely accepted method.[2] Emission intensities (at 581 nm) obtained from fluorescence titration experiment are normalized between the minimum intensity (0.0 equiv. free Cu^{2+}) and the maximum intensity as displayed in Fig.4. A linear regression curve is then fitted to these normalized fluorescent intensity data, and the point at which the line crossed the ordinate axis is considered as the detection limit.

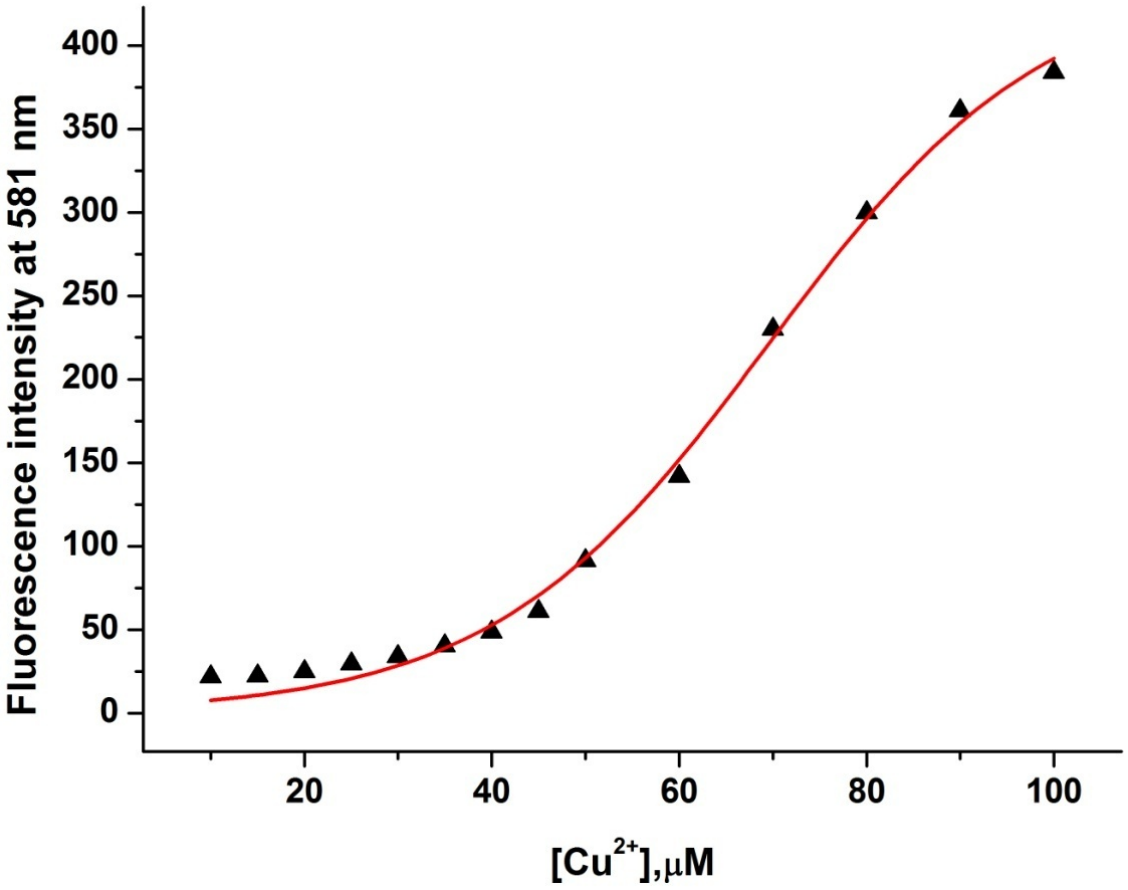

Fig. S1 Plot of fluorescence intensities of **RDHDTCA** (20 μM, λ_{ex} = 500 nm, λ_{em} = 581 nm) as a function of externally added Cu^{2+} (5-100 μM).

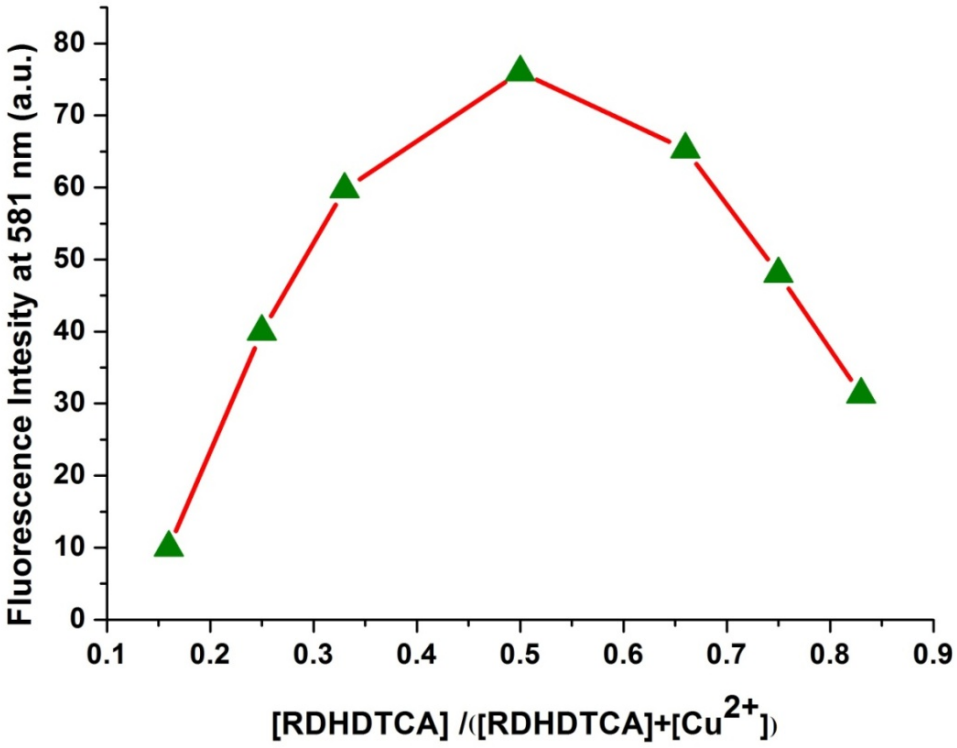

Fig. S2 Job's plot for determination of stoichiometry of the [**RDHDTCA-**Cu^{2+}] complex in CH_3CN: water (9:1, v/v, λ_{ex} = 500 nm, λ_{em} = 581 nm)

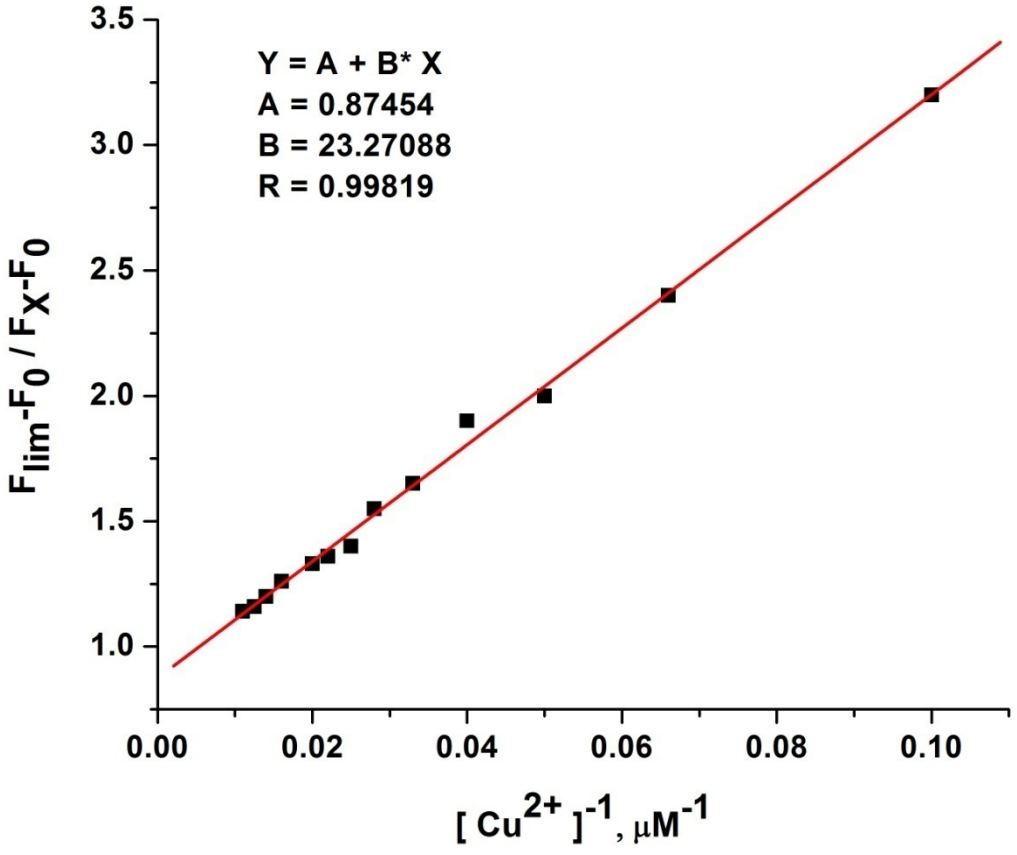

Fig. S3 Determination of association constant of **RDHDTCA** for Cu^{2+} in CH_3CN: water (9:1, v/v, λ_{ex} = 500 nm, λ_{em} = 581 nm) using fluorescence technique.

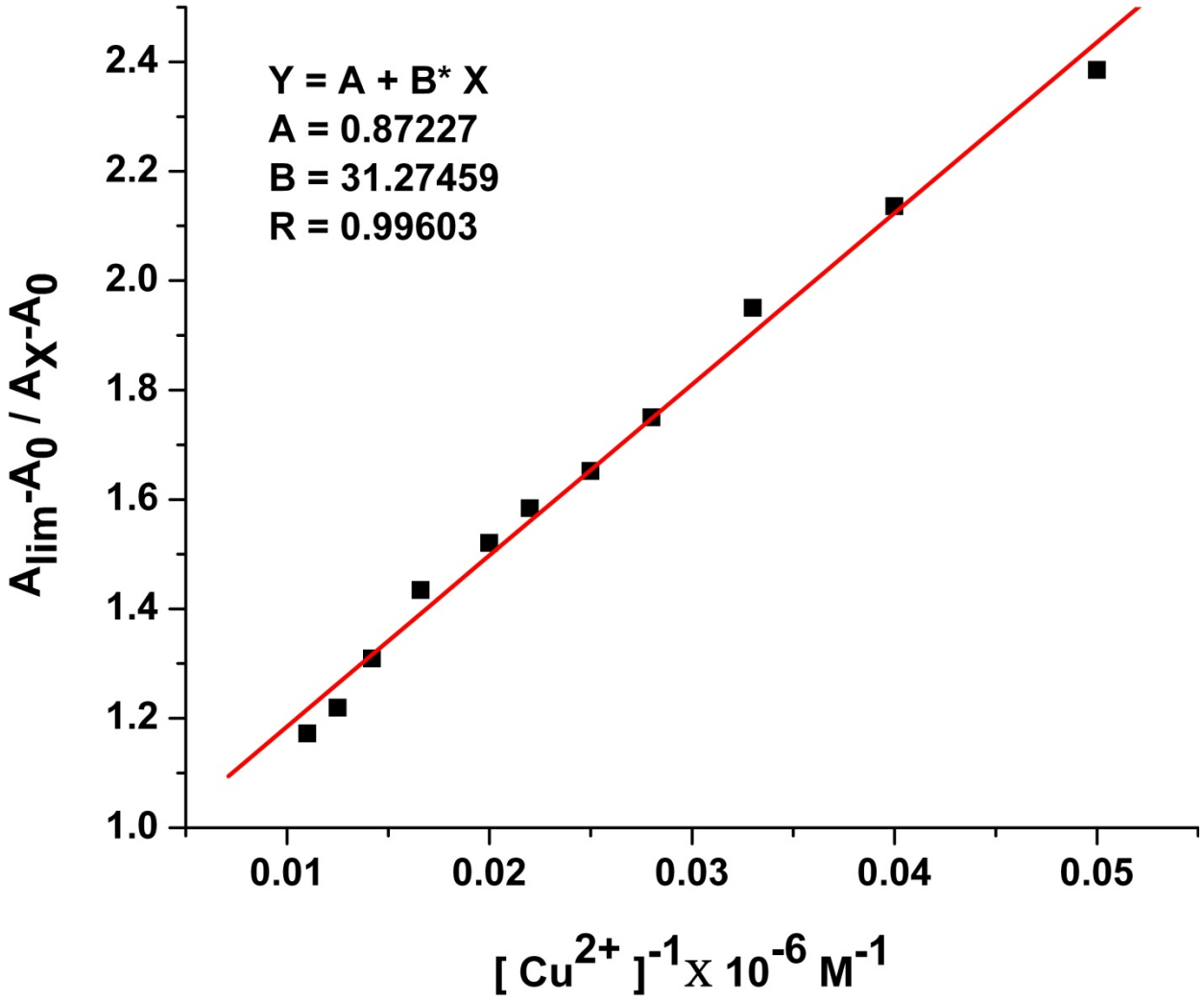

Fig. S4 Determination of association constant of **RDHDTCA** for Cu^{2+} in CH_3CN: water (9:1, v/v) using UV-Vis technique.

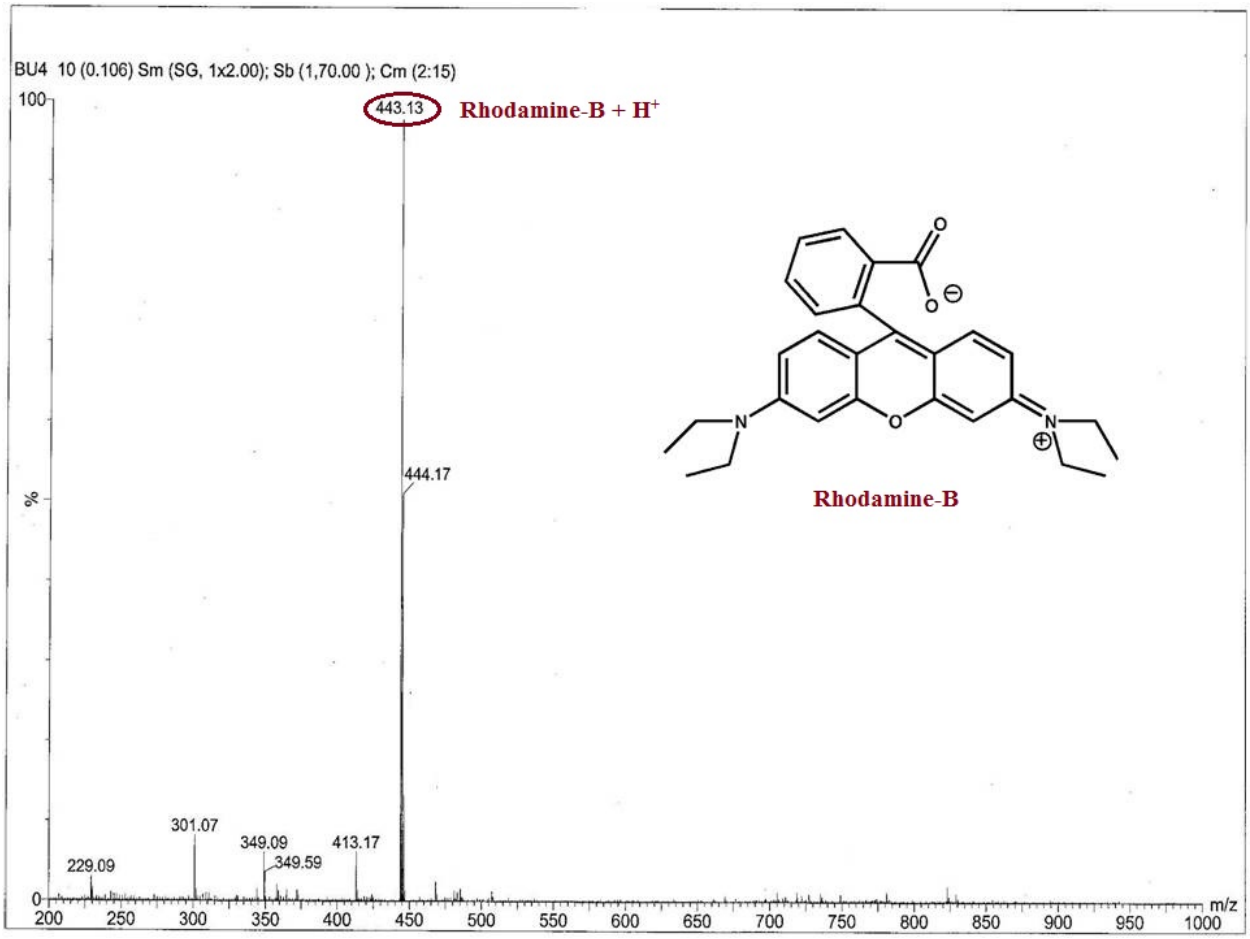

Fig. S5. QTOF-MS spectrum of the product formed after Cu^{2+} assisted hydrolysis of **RDHDTCA**.

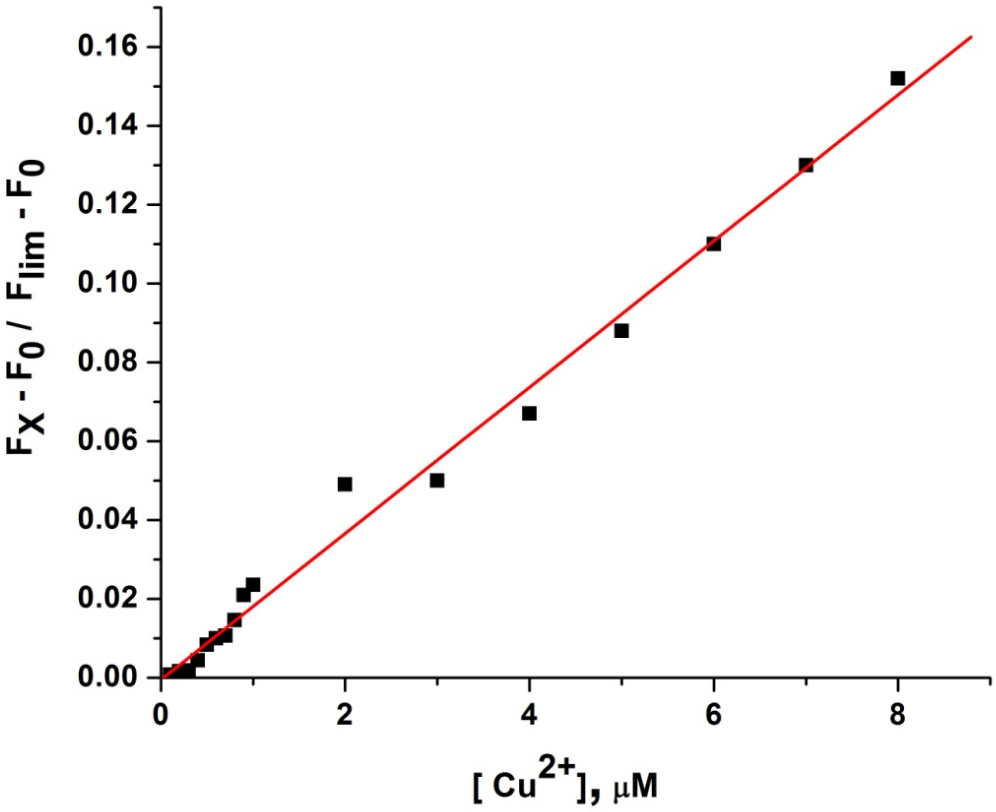

Fig.S6 Emission intensity of **RDHDTCA** (20 μM) as a function of externally added Cu^{2+} (0.1-100 μM) in CH_3CN: water (9:1, v/v, λ_{ex} = 500 nm, λ_{em} = 581 nm)

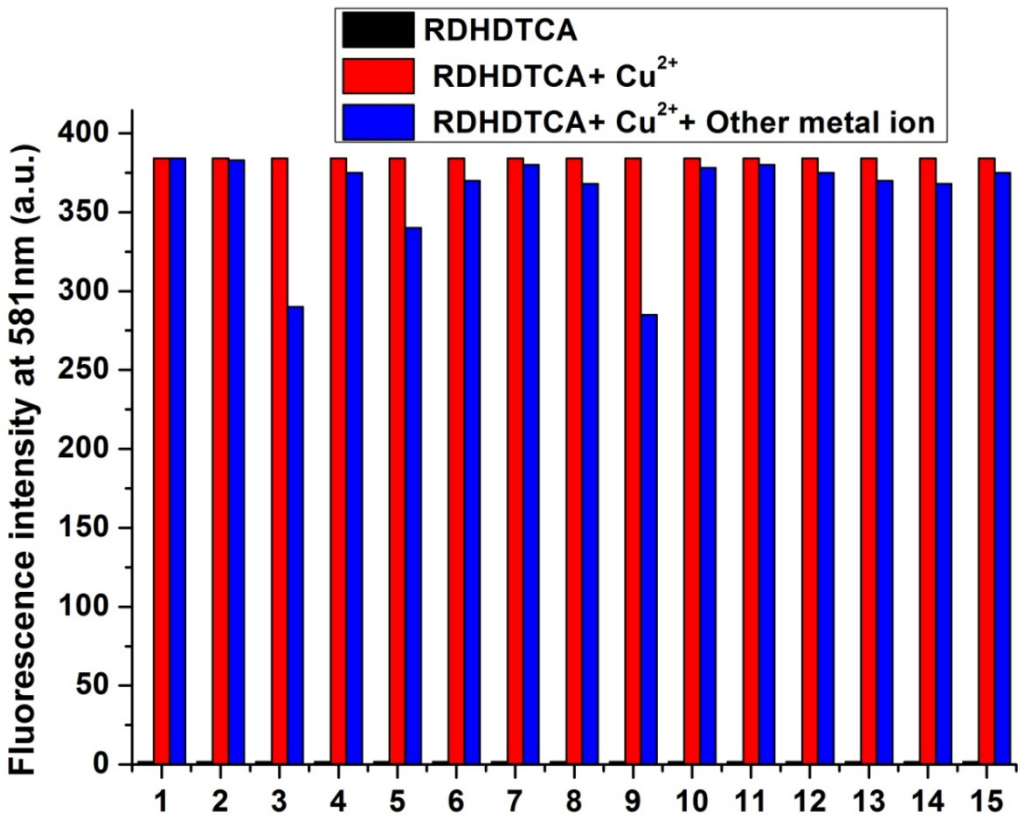

Fig. S7 Emission intensities of**[RDHDTCA-Cu^{2+}]** systemin presence of different cations (100 μM): (**1**) Cu^{2+},(**2**) Na^{+}, (**3**) Fe^{2+}, (**4**)Co^{2+}, (**5**)Al^{3+}, (**6**) Zn^{2+}, (**7**)Mg^{2+}, (**8**)Hg^{2+}, (**9**) Fe^{3+}, (**10**) Mn^{2+}, (**11**) Ni^{2+}, (**12**)Cr^{3+}, (**13**)Pb^{2+}, (**14**)Cd^{2+}, (**15**)Sn^{2+} in CH_3CN: water (9:1, v/v,λ_{ex}= 500 nm, λ_{em}= 581 nm).

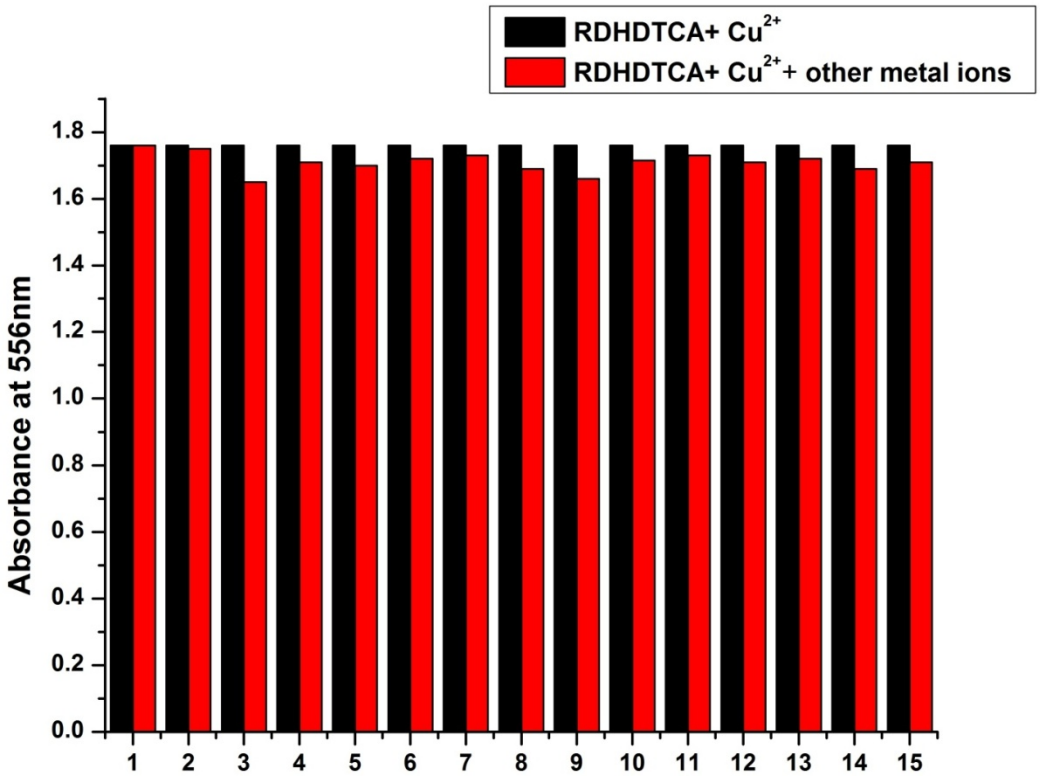

Fig. S8 Absorbance of **[RDHDTCA-Cu^{2+}]** system (at 556 nm) in presence of different cations (50 μM): (**1**) Cu^{2+}, (**2**) Na^{+}, (**3**) Fe^{2+}, (**4**) Co^{2+}, (**5**) Al^{3+}, (**6**) Zn^{2+}, (**7**) Mg^{2+}, (**8**) Hg^{2+}, (**9**) Fe^{3+}, (**10**) Mn^{2+}, (**11**) Ni^{2+}, (**12**) Cr^{3+}, (**13**) Pb^{2+}, (**14**) Cd^{2+} and (**15**) Sn^{2+} in CH_3CN: water (9:1, v/v)

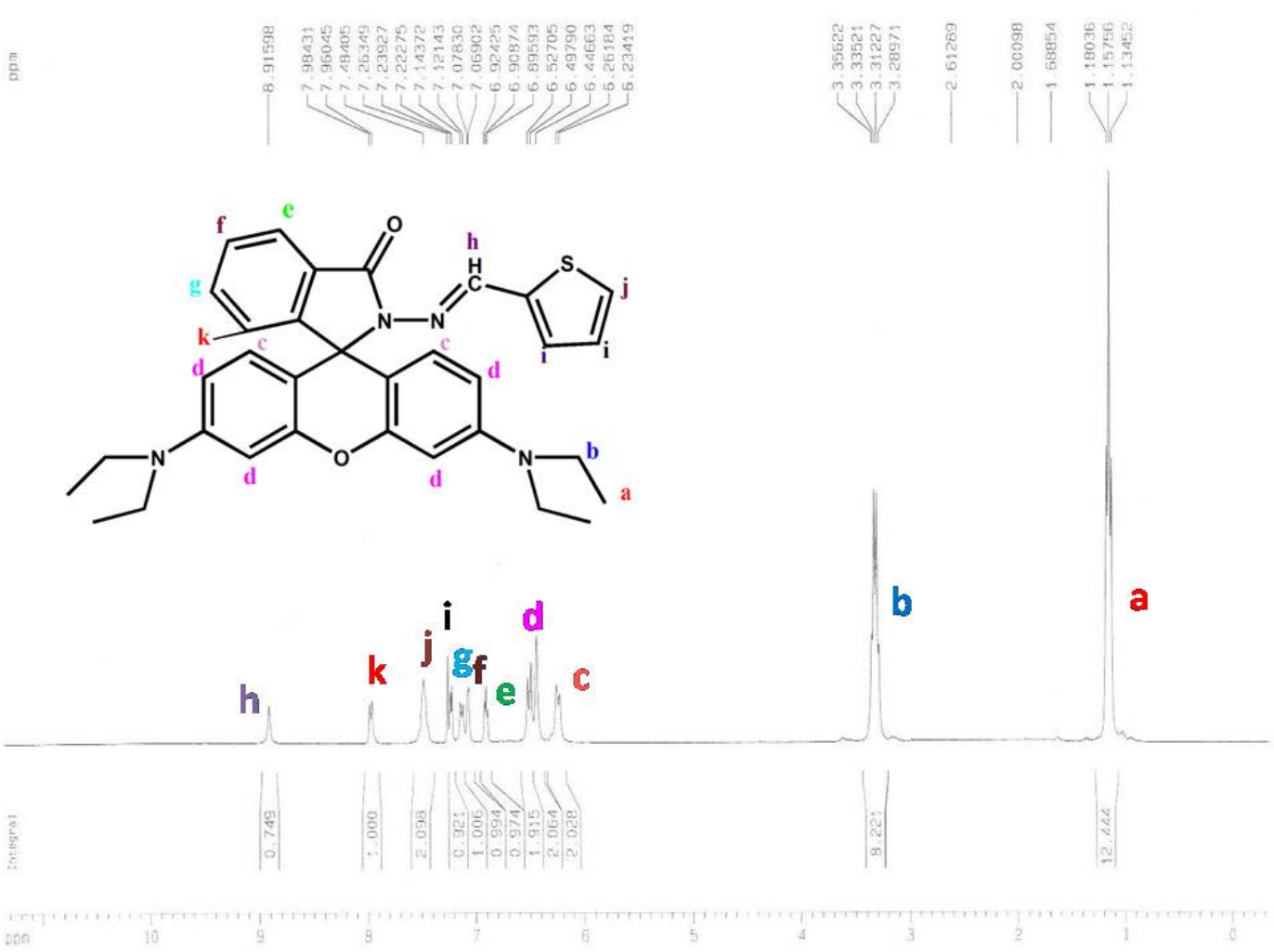

Fig. S9 ^{1}H NMR spectrum of **RDHDTCA** in $CDCl_3$

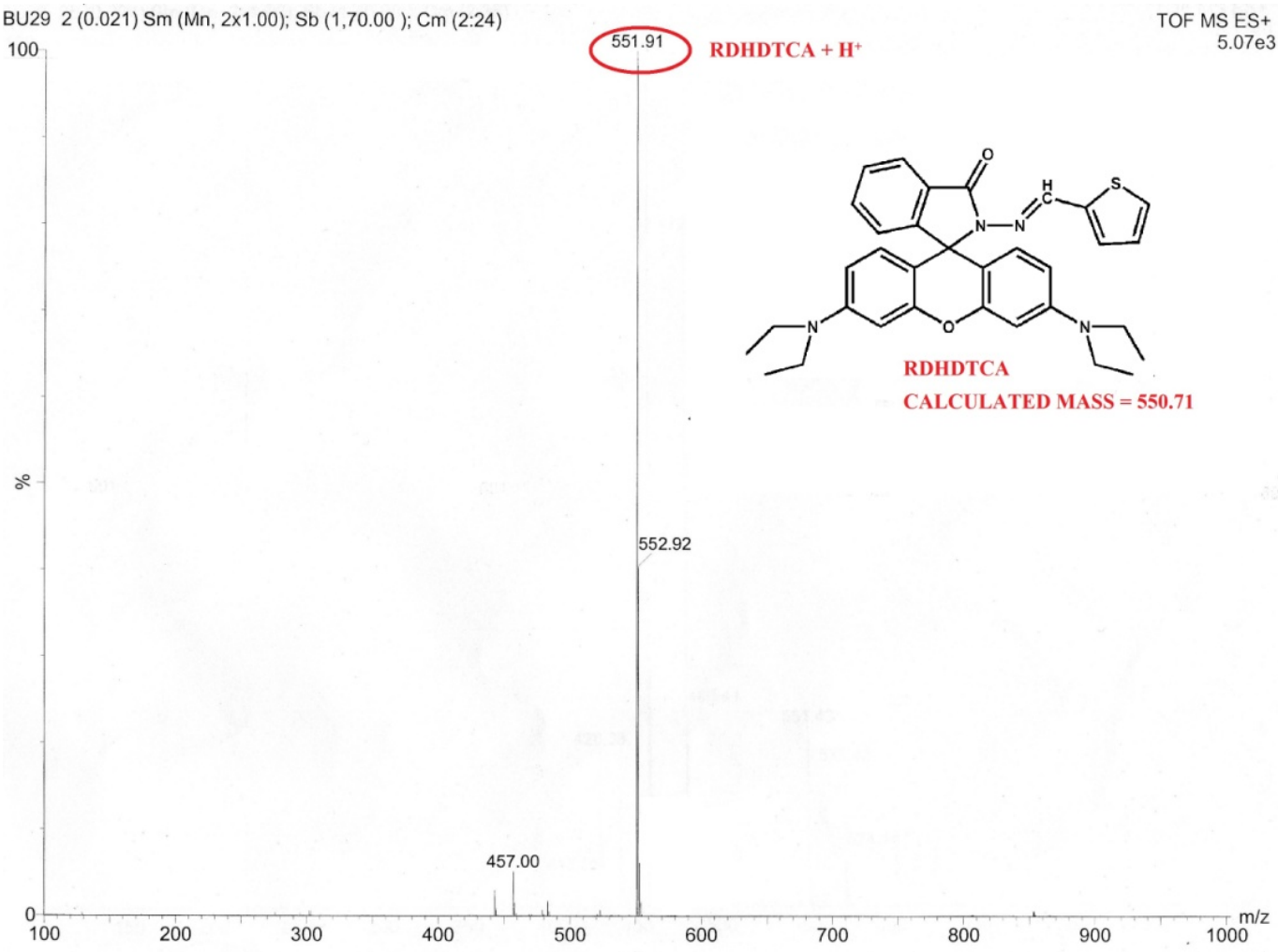

Fig. S10. QTOF-MS spectrum of **RDHDTCA**

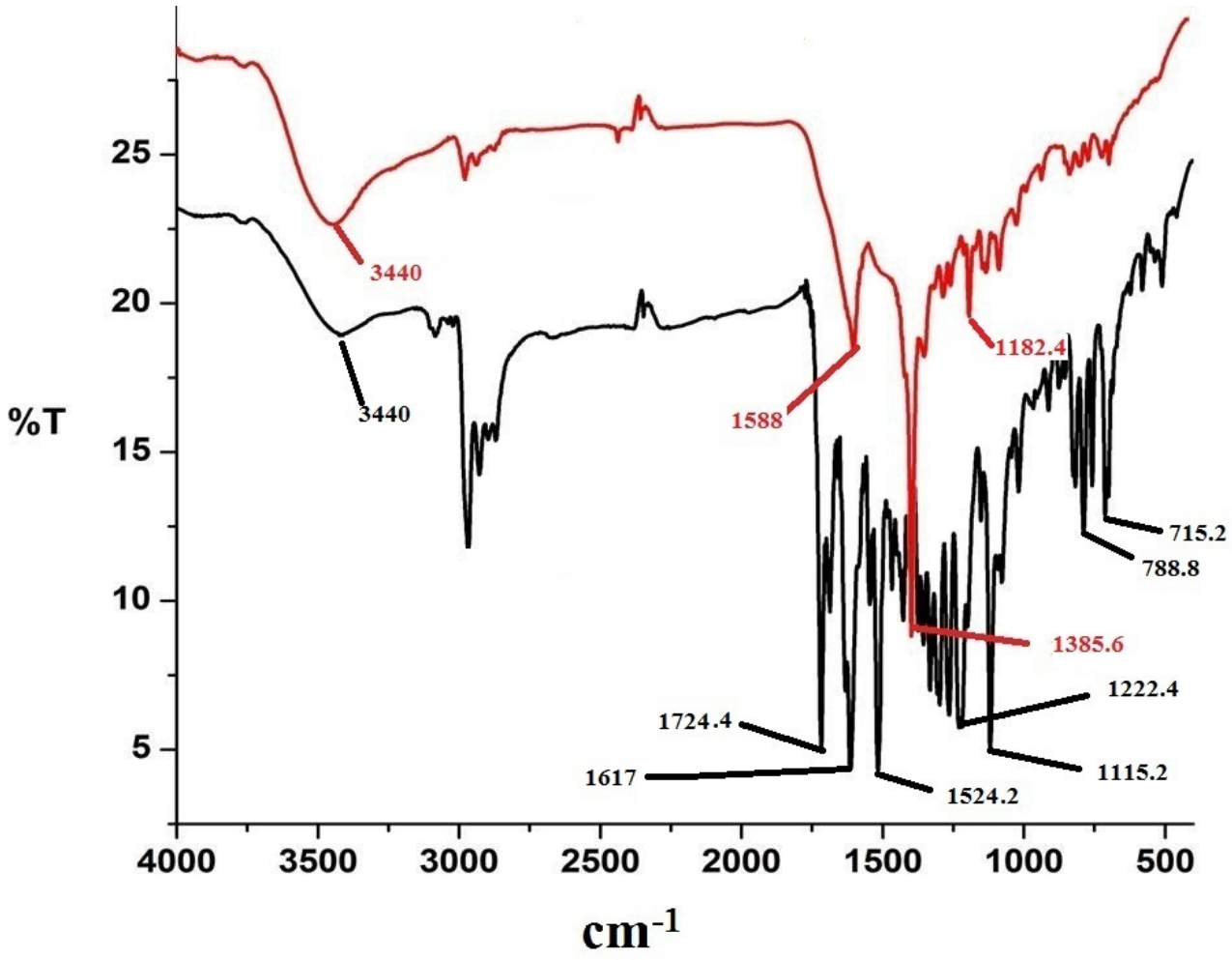

Fig. S11 FTIR spectra of **RDHDTCA** (black) and [**RDHDTCA-Cu^{2+}**] complex (red)

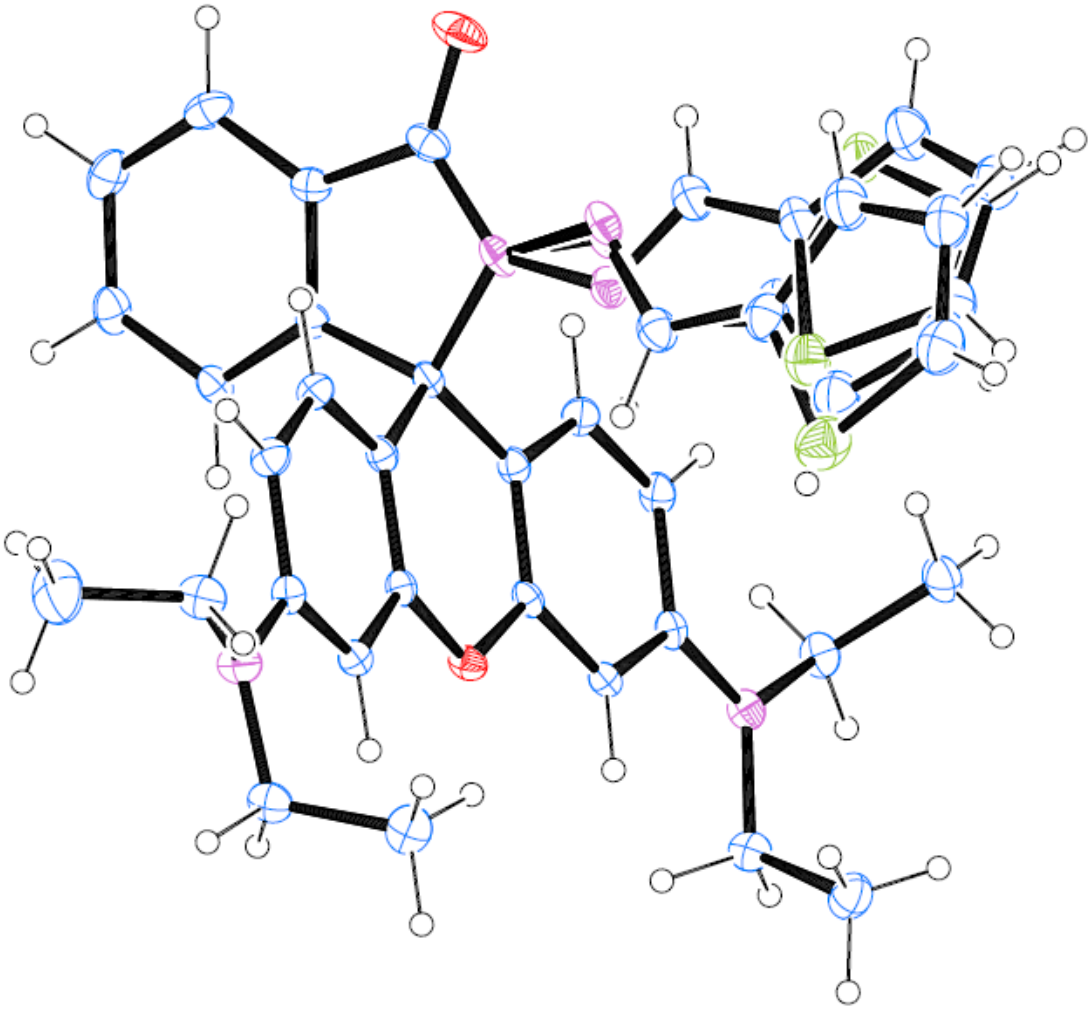

Fig. S12. Molecular structure of **RDHDTCA** showing the disorder ataldehyde residue

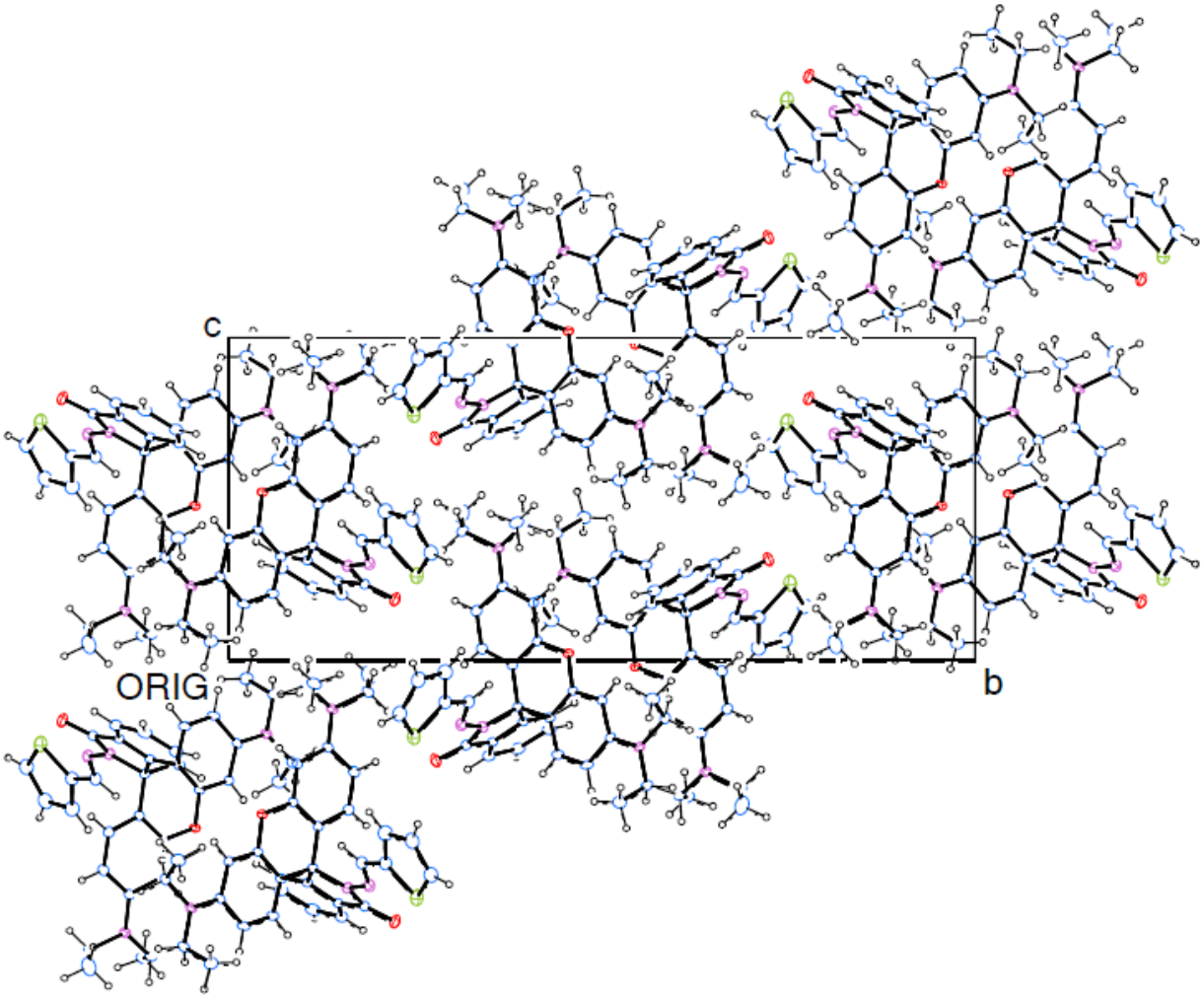

Fig. S13 Crystal packing of **RDHDTCA**

Table S1. Selected bond lengths [Å] for **RDHDTCA**

O(1)-C(31)		N(37)-C(38)	
O(1)-C(11)		C(38)-C(39)	
C(11)-C(16)		C(40)-C(41)	
C(11)-C(12)		C(50)-N(51)	
C(12)-C(13)		C(50)-C(59)	
C(13)-N(17)		N(51)-N(60)	
C(13)-C(14)		N(51)-C(52)	
C(14)-C(15)		C(52)-O(53)	
C(15)-C(16)		C(52)-C(54)	
C(16)-C(50)		C(54)-C(55)	
N(17)-C(18)		C(54)-C(59)	
N(17)-C(20)		C(55)-C(56)	
C(18)-C(19)		C(56)-C(57)	
C(20)-C(21)		C(57)-C(58)	
C(31)-C(32)		C(58)-C(59)	
C(31)-C(36)		N(60)-C(61)	
C(32)-C(33)		C(61)-C(62)	
C(33)-N(37)		C(62)-C(66)	
C(33)-C(34)		C(62)-S(63)	
C(34)-C(35)		S(63)-C(64)	
C(35)-C(36)		C(64)-C(65)	
C(36)-C(50)		C(65)-C(66)	
N(37)-C(40)			

Table S2. Selected bond angles [°] for **RDHDTCA**

C(31)-O(1)-C(11)		N(37)-C(38)-C(39)	
C(16)-C(11)-O(1)		N(37)-C(40)-C(41)	
C(16)-C(11)-C(12)		N(51)-C(50)-C(36)	
O(1)-C(11)-C(12)		N(51)-C(50)-C(16)	
C(11)-C(12)-C(13)		C(36)-C(50)-C(16)	
N(17)-C(13)-C(12)		N(51)-C(50)-C(59)	
N(17)-C(13)-C(14)		C(36)-C(50)-C(59)	
C(12)-C(13)-C(14)		C(16)-C(50)-C(59)	
C(15)-C(14)-C(13)		N(60)-N(51)-C(52)	
C(14)-C(15)-C(16)		N(60)-N(51)-C(50)	
C(11)-C(16)-C(15)		C(52)-N(51)-C(50)	
C(11)-C(16)-C(50)		O(53)-C(52)-N(51)	
C(15)-C(16)-C(50)		O(53)-C(52)-C(54)	
C(13)-N(17)-C(18)		N(51)-C(52)-C(54)	
C(13)-N(17)-C(20)		C(55)-C(54)-C(59)	
C(18)-N(17)-C(20)		C(55)-C(54)-C(52)	
N(17)-C(18)-C(19)		C(59)-C(54)-C(52)	
N(17)-C(20)-C(21)		C(56)-C(55)-C(54)	
O(1)-C(31)-C(32)		C(55)-C(56)-C(57)	
O(1)-C(31)-C(36)		C(58)-C(57)-C(56)	
C(32)-C(31)-C(36)		C(59)-C(58)-C(57)	
C(31)-C(32)-C(33)		C(58)-C(59)-C(54)	
N(37)-C(33)-C(32)		C(58)-C(59)-C(50)	
N(37)-C(33)-C(34)		C(54)-C(59)-C(50)	
C(32)-C(33)-C(34)		C(61)-N(60)-N(51)	
C(35)-C(34)-C(33)		N(60)-C(61)-C(62)	
C(34)-C(35)-C(36)		C(61)-C(62)-C(66)	
C(31)-C(36)-C(35)		C(61)-C(62)-S(63)	
C(31)-C(36)-C(50)		C(66)-C(62)-S(63)	
C(35)-C(36)-C(50)		C(62)-S(63)-C(64)	
C(33)-N(37)-C(40)		C(65)-C(64)-S(63)	
C(33)-N(37)-C(38)		C(66)-C(65)-C(64)	
C(40)-N(37)-C(38)		C(65)-C(66)-C(62)	

Table S3. Single crystal X-ray structural parameters of **RDHDTCA**.

Empirical formula	$C_{33}H_{34}N_4O_2S$
Formula weight	550.70
Temperature	100(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	$P2_1/c$
Unit cell dimensions	a = 9.1327(12) Å
	b = 26.729(4) Å
	c = 11.9313(16) Å
	□= 90°
	□= 104.556(2)°
	□ = 90°
Volume	2819.0(6) $Å^3$
Z	4
Density (calculated)	1.298 Mg/m^3
Absorption coefficient	0.153 mm^{-1}
F(000)	1168
Crystal size	0.220 x 0.220 x 0.160 mm^3
Theta range for data collection	1.524 to 26.749°
Index ranges	-11<=h<=11, -33<=k<=33, -15<=l<=15
Reflections collected	24897
Independent reflections	5984 [R(int) = 0.0532]
Completeness to theta = 25.242°	100.0 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.977 and 0.794
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	5984 / 1080 / 494
Goodness-of-fit on F^2	1.053
Final R indices [I>2sigma(I)]	R1 = 0.0587, wR2 = 0.1462
R indices (all data)	R1 = 0.0886, wR2 = 0.1619
Largest diff. peak and hole	0.586 and -0.416 $e.Å^{-3}$

References

[1] R. Kubin, *J. Lumin.*, 1983, **27**, 455.

[2] M. Shortreed, R. Kopelman, M.Kuhn, B.Hoyland; *Anal.Chem*. 1996, **68**, 1414. (b) A.Caballero, R.Martinez, V.Lloveras, I.Ratera, J.Vidal-Gancedo, K.Wurst, A.Tárraga, P.Molina, J.Veciana; *J. Am. Chem. Soc*. 2005, **127**, 15666.