Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information for

Thiosemicarbazone based chemo and fluorogenic sensor for Zn^{2+} with CHEF and ESIPT behaviours: computational studies and cell imaging application

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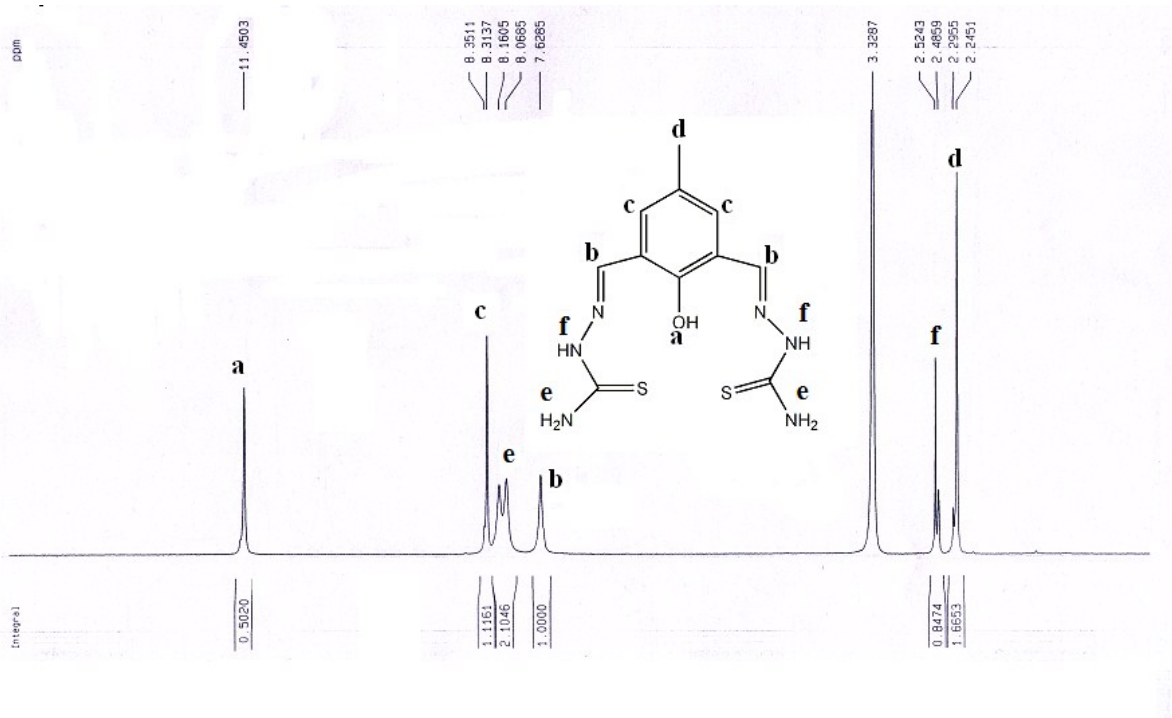


Figure S1. ^{1}H NMR spectrum of **DFC-TS**,in DMSO-d_6 in Bruker 300 MHz instrument.

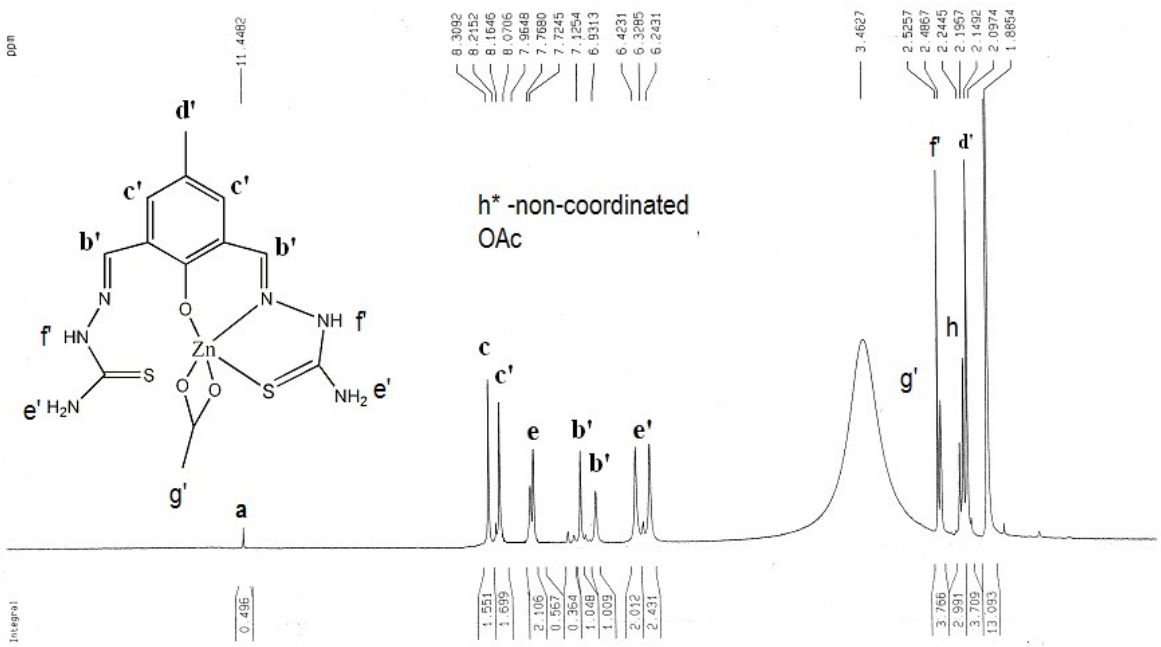


Figure S2. ^{1}H NMR spectrum of **DFCTS+ Zn^{2+}(1:1),**in DMSO-$d_6$in Bruker 300 MHz instrument.

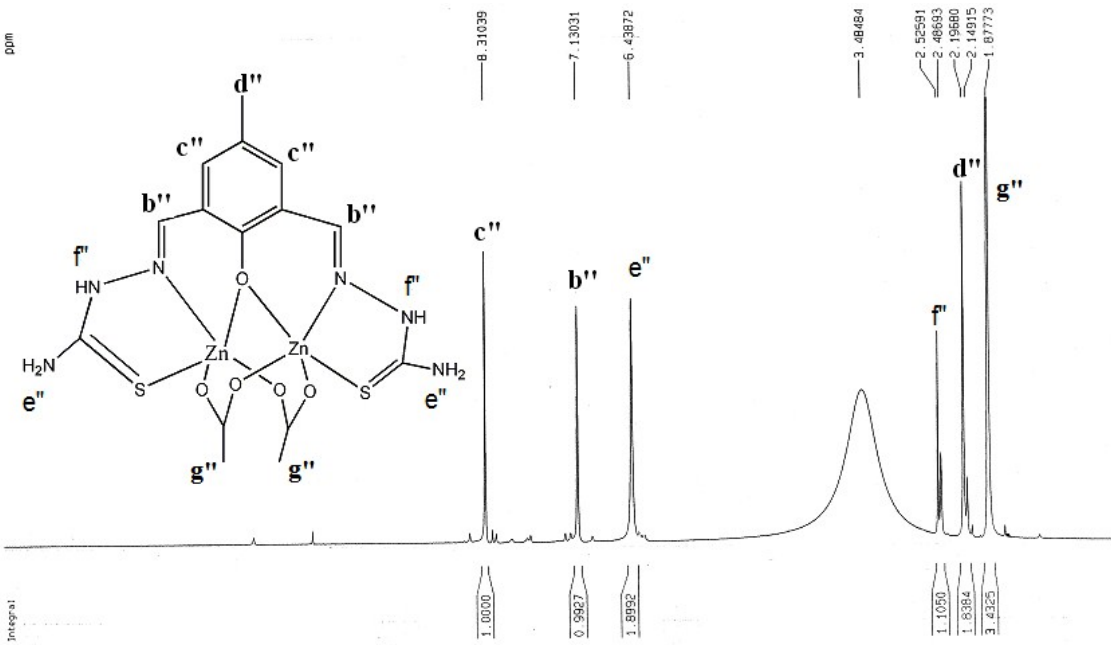
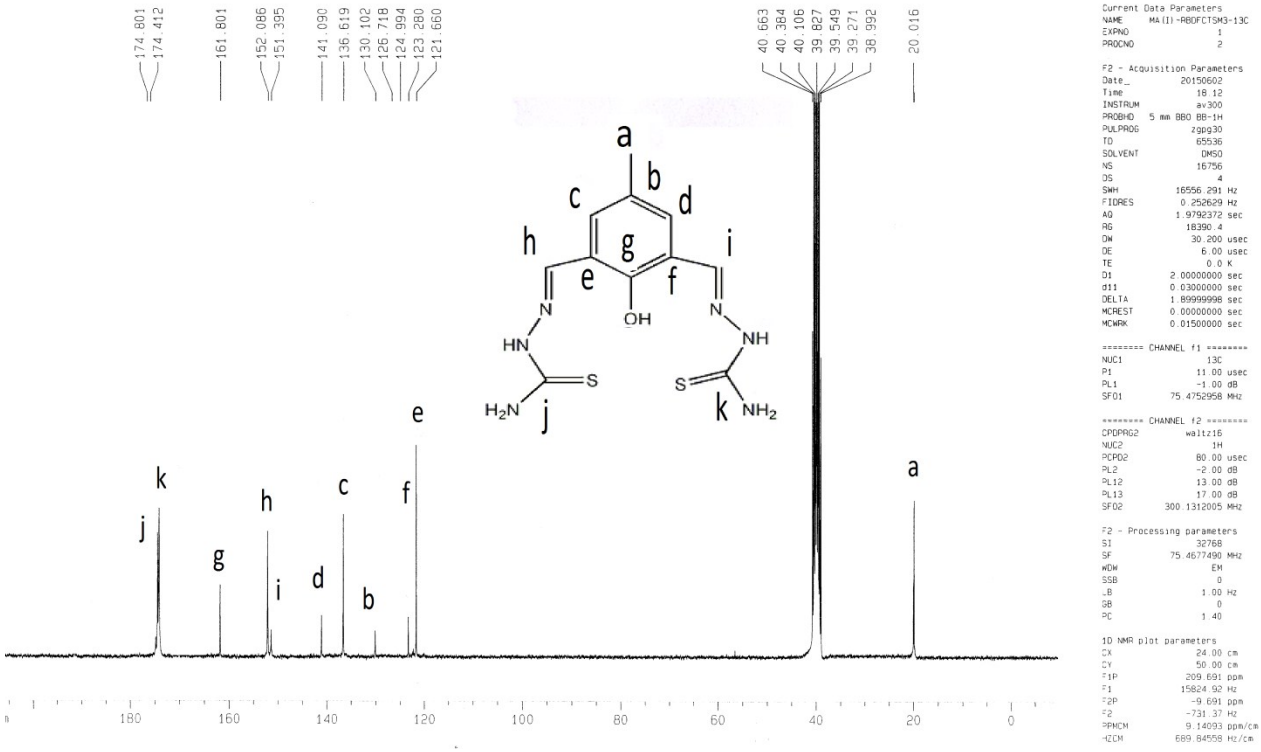


Figure S3. ^{1}H NMR spectrum of**DFC-TS +** Zn^{2+}(1:2),in DMSO-d_6 in Bruker 300 MHz instrument.



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PROCNO 2

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1D NMR plot parameters
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F1 15824.92 Hz
F2P -9.691 ppm
F2 -731.37 Hz
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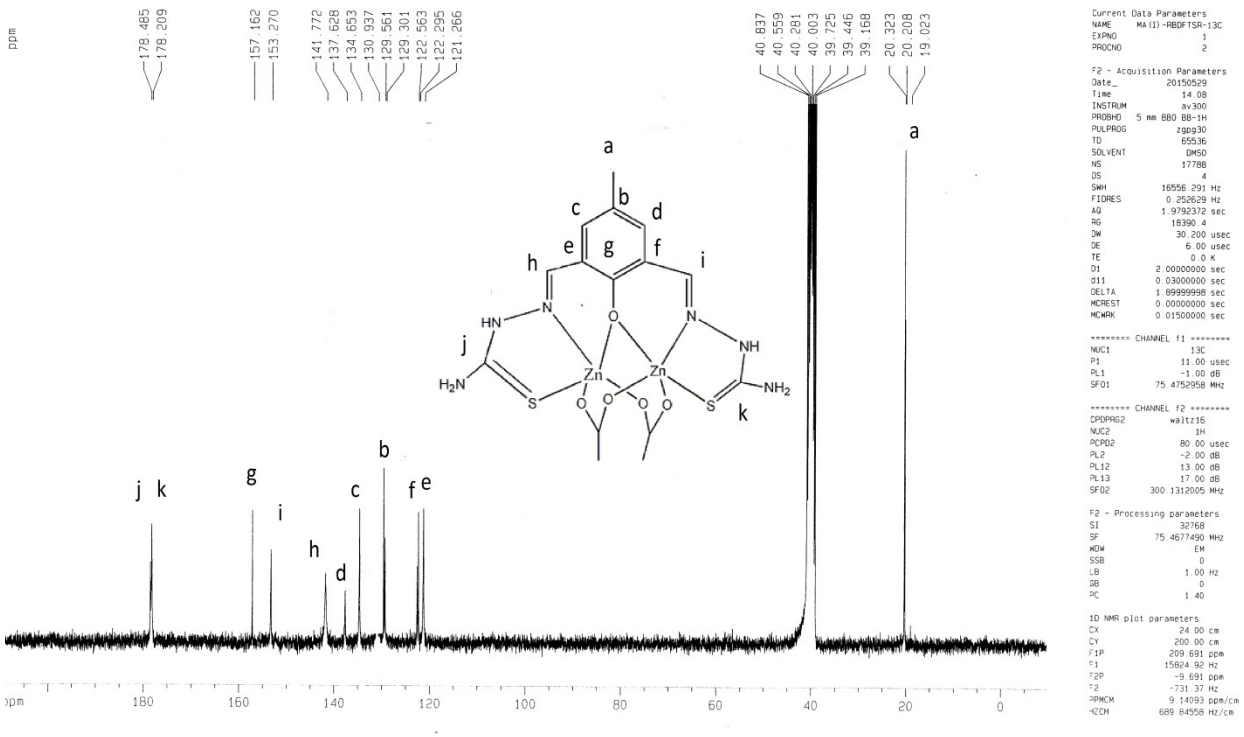
HZCM 689.84558 Hz/cm

Figure S4. ^{13}C NMR spectrum of **DFC-TS**,in DMSO-d_6 in Bruker 300 MHz instrument.



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F2 - Processing parameters
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WDW EM
SSB 0
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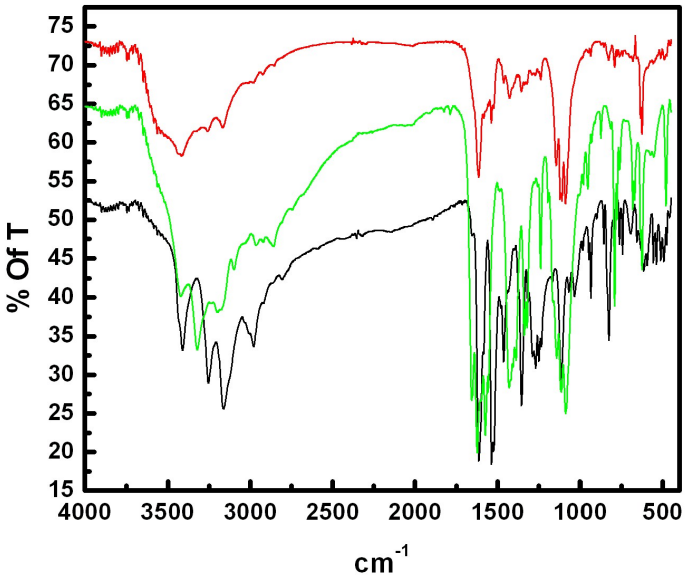
1D NMR plot parameters
CX 24.00 cm
CY 200.00 cm
F1P 209.691 ppm
F1 15824.92 Hz
F2P -9.691 ppm
F2 -731.37 Hz
PPMCM 9.14093 ppm/cm
HZCM 689.84558 Hz/cm

Figure S5. ^{13}C NMR spectrum of **DFC-TS + Zn^{2+}(1:2)**,in DMSO-d_6 in Bruker 300 MHz instrument.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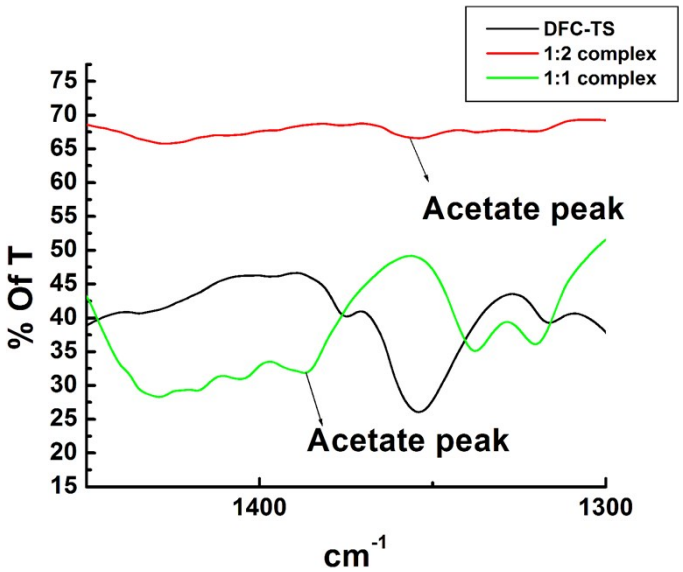
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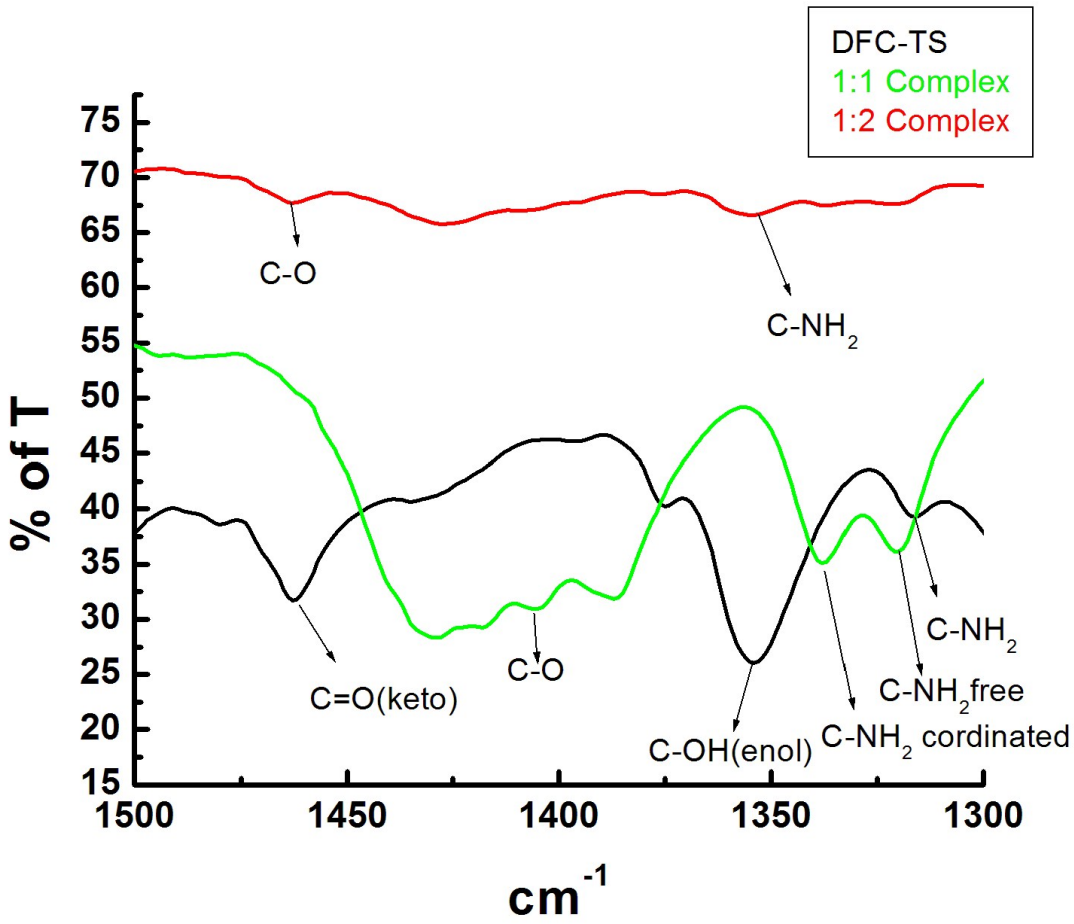
1:1 complex
Acetate peak
Acetate peak
% Of T
cm-1
1400
1300



FigureS6. IR Spectrum of the ligand **DFC-TS** (shown in black), **Zn-complex** (1:1) (shown in green),**Zn-complex**(1:2) (shown in red) in solid state.

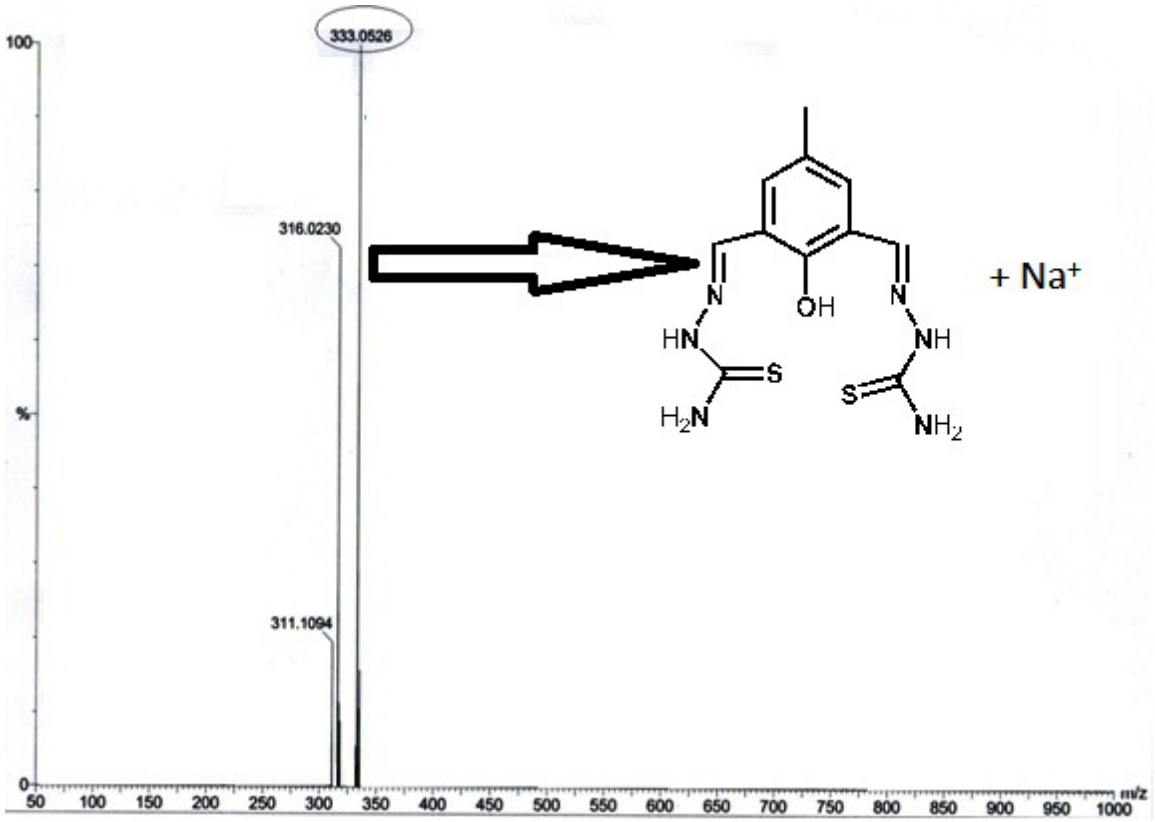
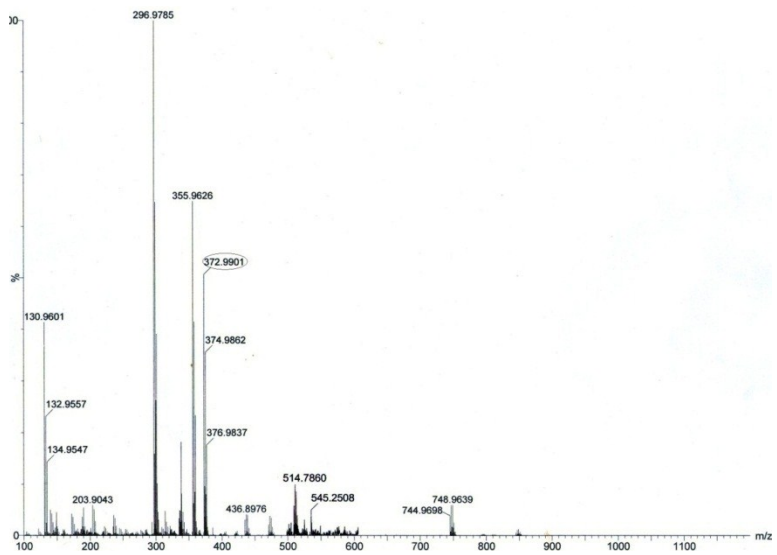
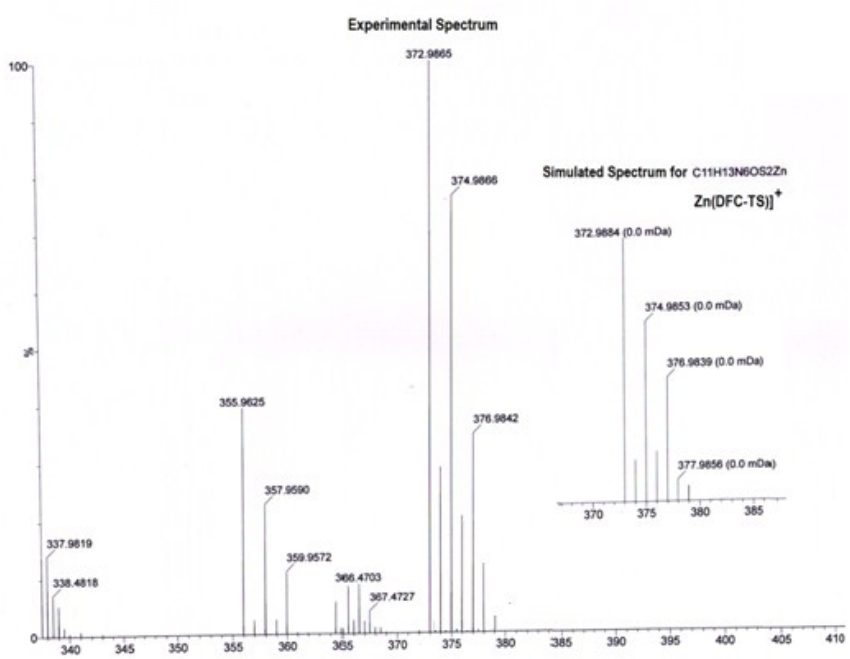


Figure S7. Mass spectrum of**DFC-TS**inMeCN.

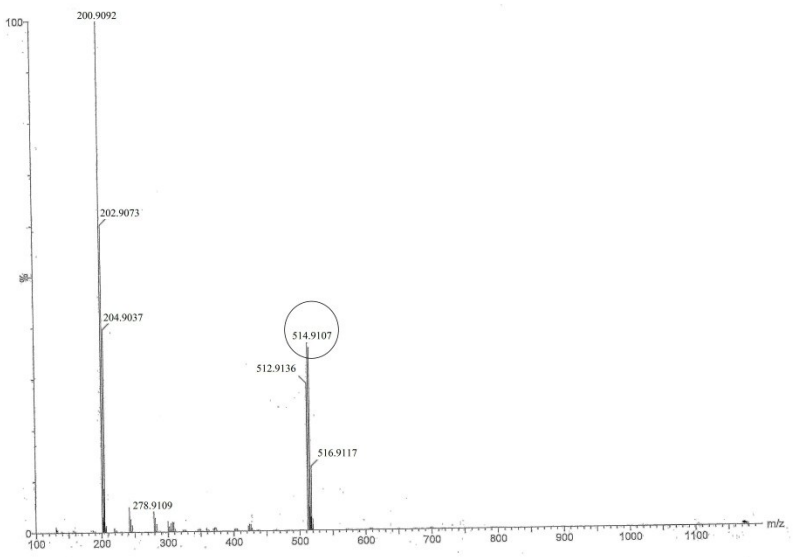


(A)

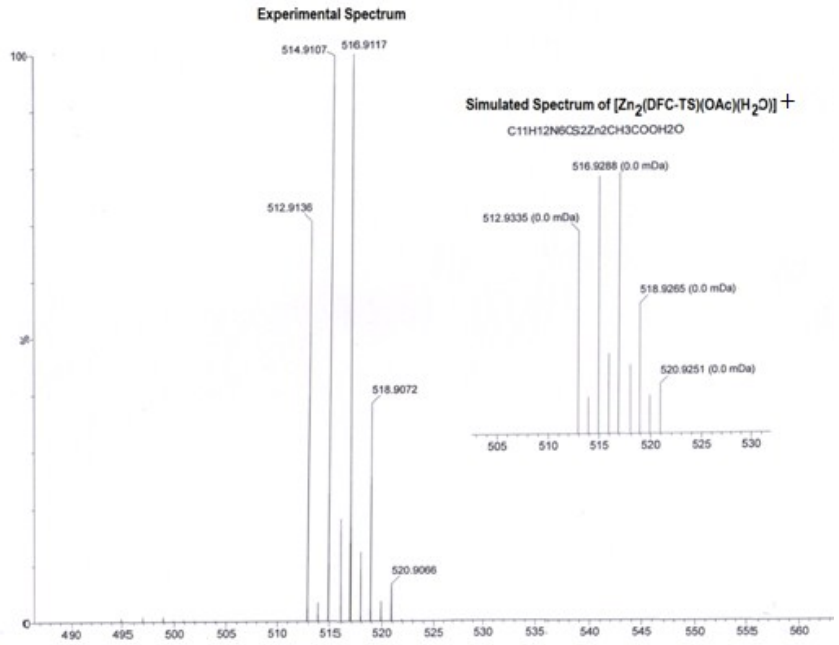


(B)

Figure S8. Mass spectrum of**DFC-TS+Zn2**$^{+}$ (1:1)in MeCN: (A) Full range spectrum and (B) simulated spectrum.



(A)



(B)

Figure S9. Mass spectrum of**DFC-TS+Zn^{2+}**(1:2)in MeCN: (A) Full range spectrum and (B) simulated spectrum.

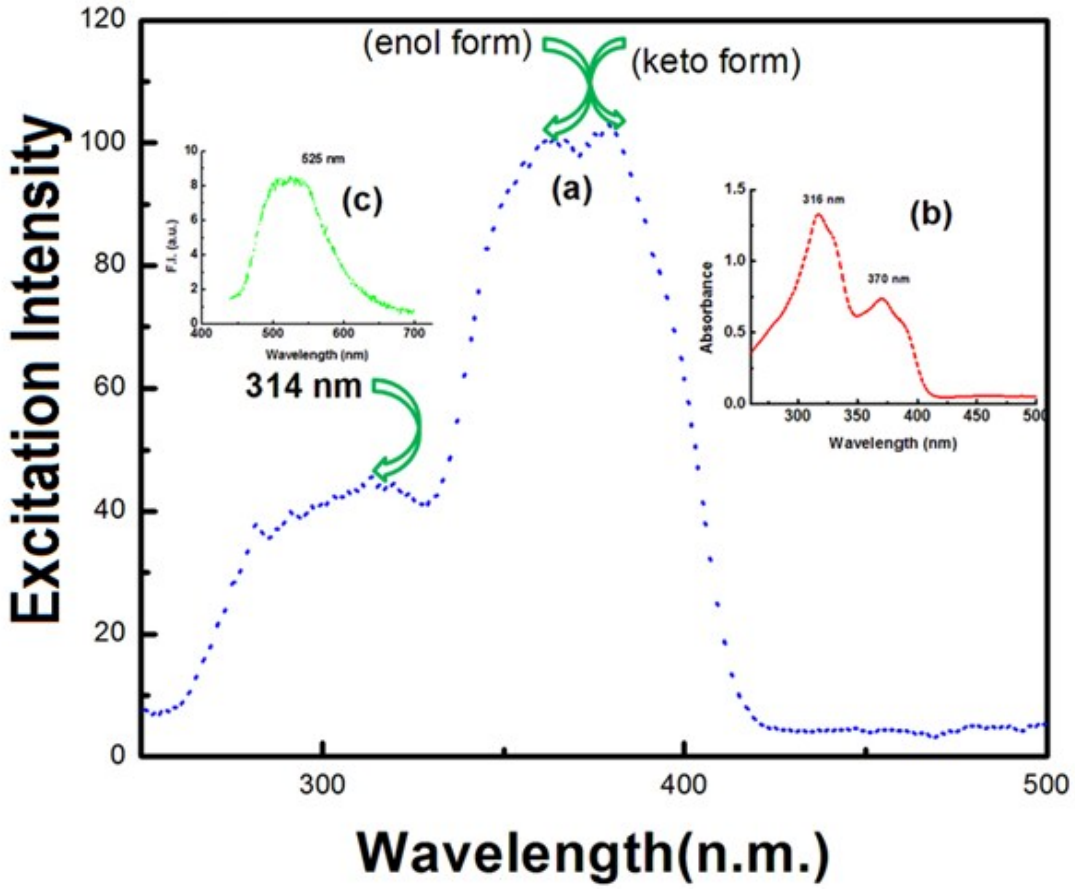


Figure S10. UV-vis, emission and excitation spectra of **DFC-TS**inDMSO-water(9:1 v/v) .

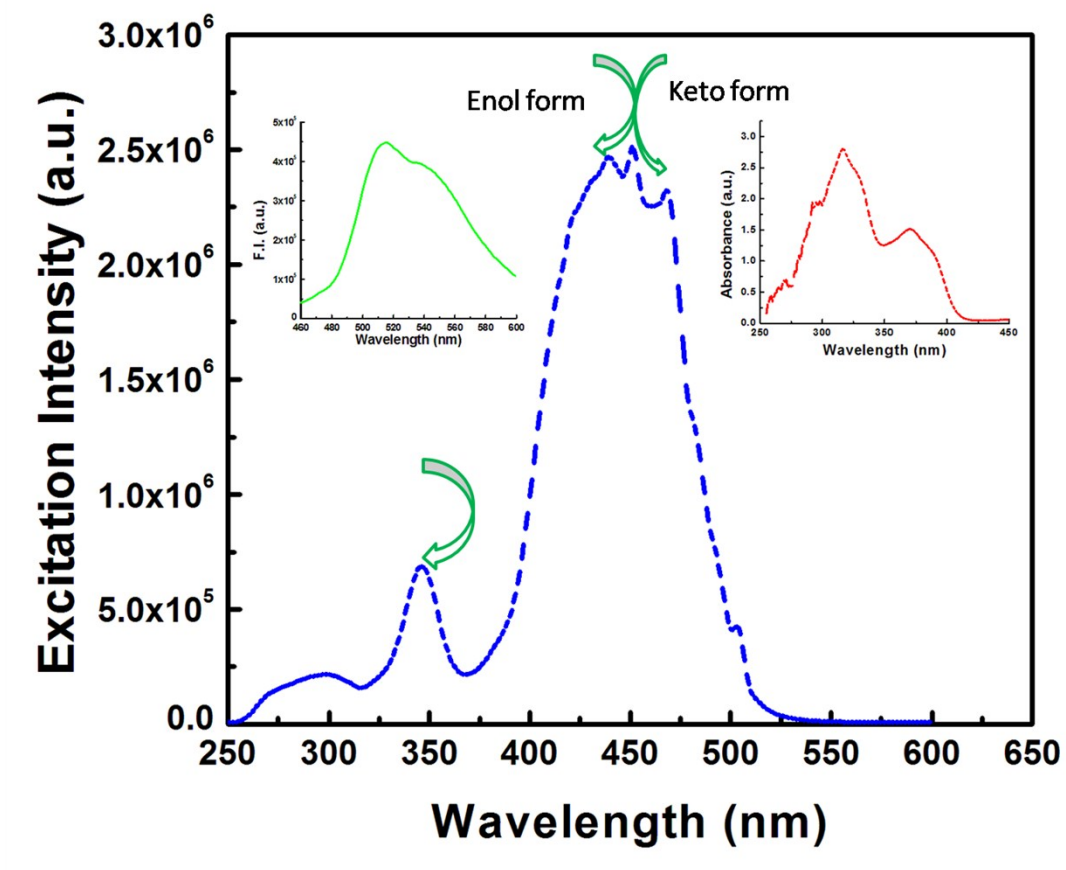


Figure S11.UV, Emission and Excitation plot of DFC-TS in non proton solution (DMSO)**.**

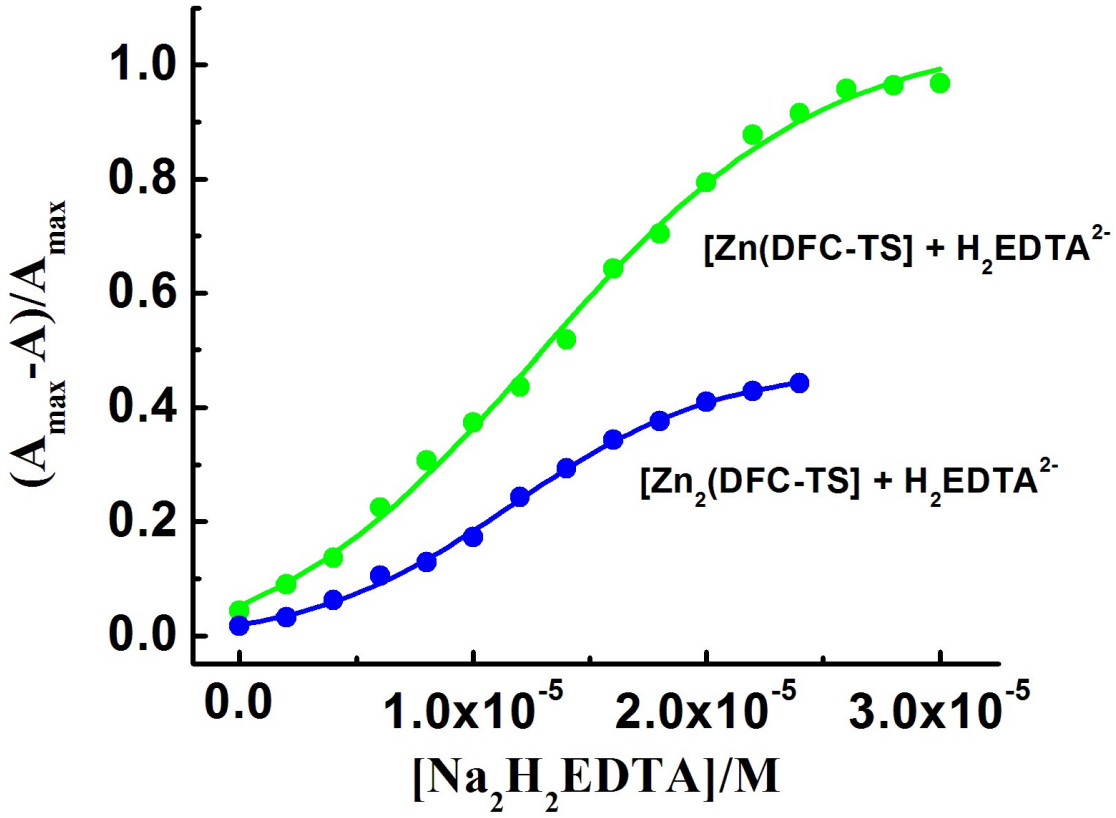


Figure S12. Absorbance plot for competition method.

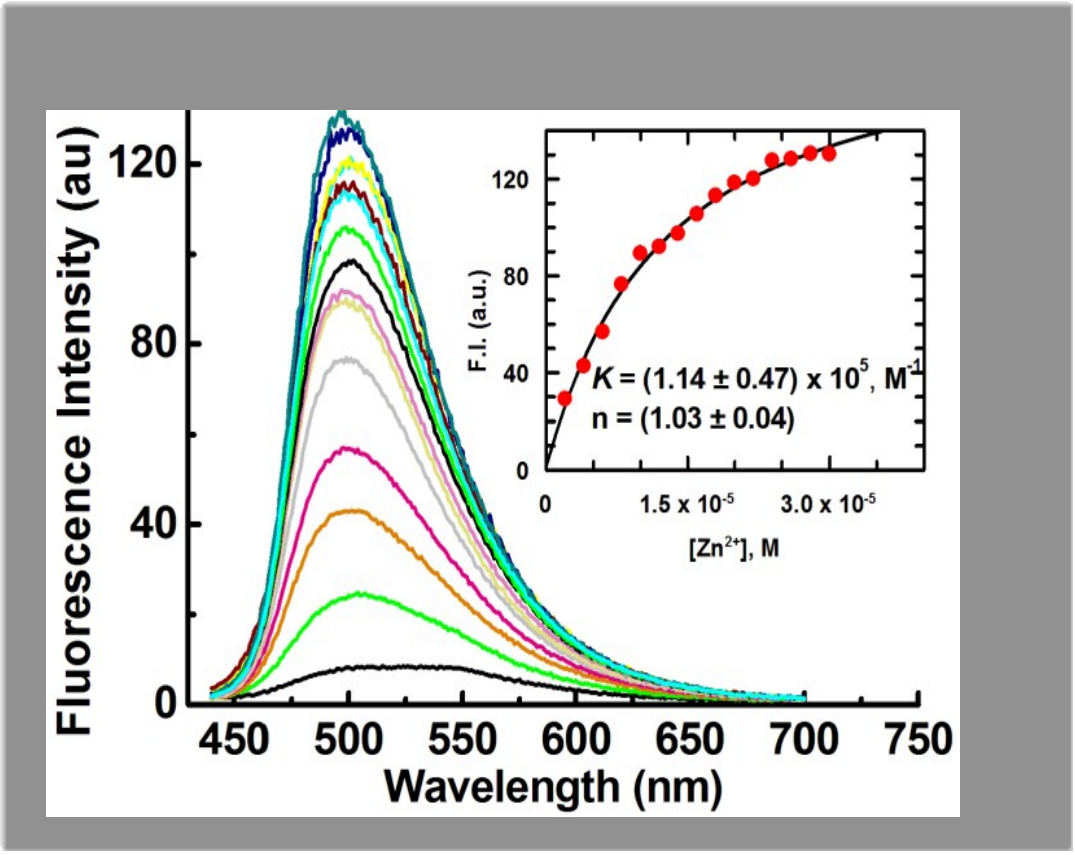


Figure S13a. Fluorescence titration of **DFC-TS**(20µM) with Zn^{2+}(0-48µM) in DMSO-H_2O (9:1, v/v, pH 7.20, 10mM HEPES buffer). Inset shows the non-linear least squares curve-fit of fluorescence titration data for the formation of the complex. λ_{ex} = 430 nm, R^2= 0.99.

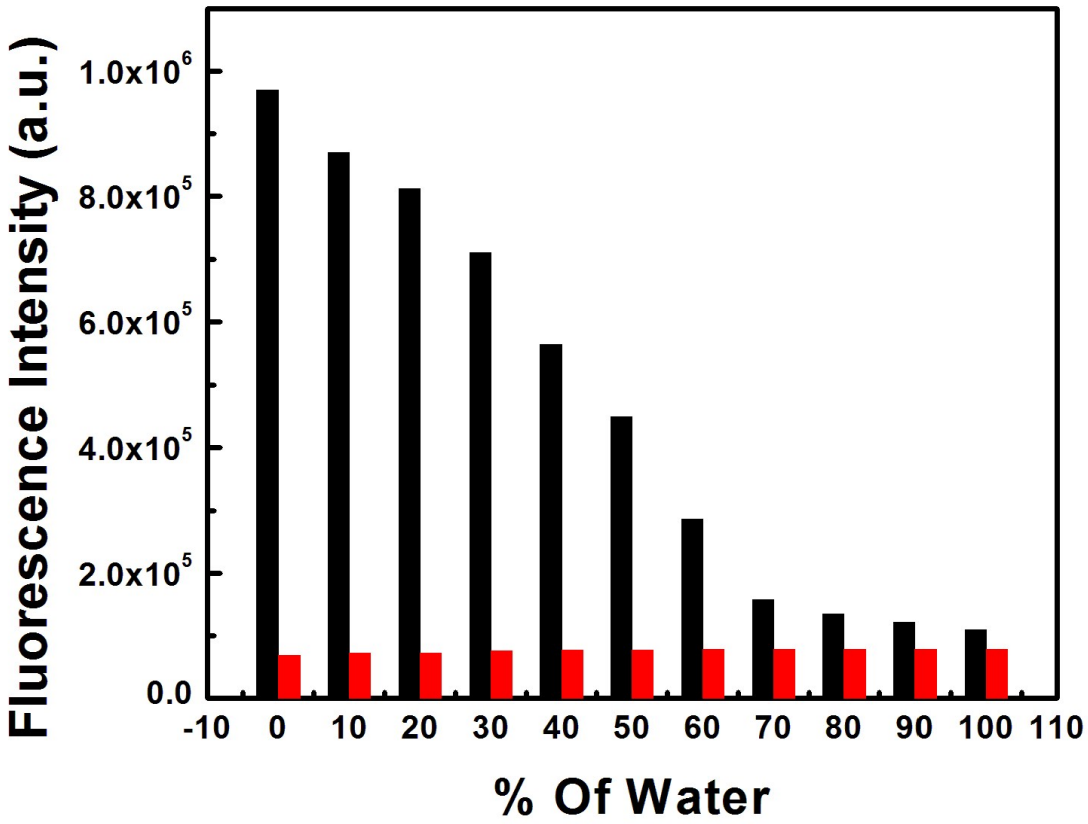


Figure S13b Fluorescence emission bar diagram of varying water, λ_{ex} = 390 nm, red= DFC-TS and black= DFC-TS+ Zn^{2+}.

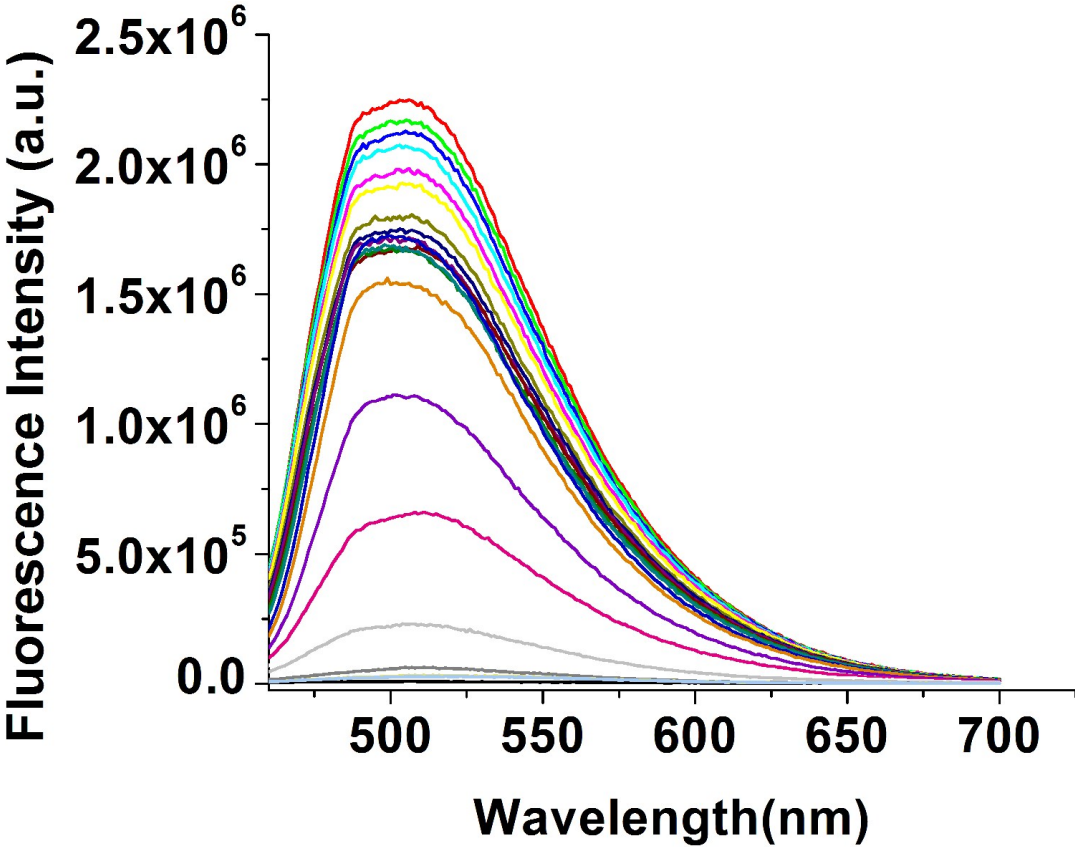


Figure S14. Competitivefluorescence titration plot for the displacement of Zn^{2+} from Zn_2(DFC-TS) ensemble (20 µM with respect to DFC-TS) with Na_2H_2EDTA (0-50 µM) in DMSO-H_2O (9:1, v/v) at pH 7.2 (HEPES buffer).

Quantum Yield Calculation:

Fluorescence quantum yields (**Φ**) were estimated by integrating the area under the fluorescence curves with the equation: $\Phi_{sample} = \frac{OD_{std}}{OD_{sample}} \times \frac{A_{sample}}{A_{std}} \times \Phi_{std}$

where, A is the area under the fluorescence spectral curve and OD is optical density of the compound at the excitation wavelength. The standard used for the measurement of fluorescence quantum yield was coumarine153 Φ_{std} =0.56 in DMSO).

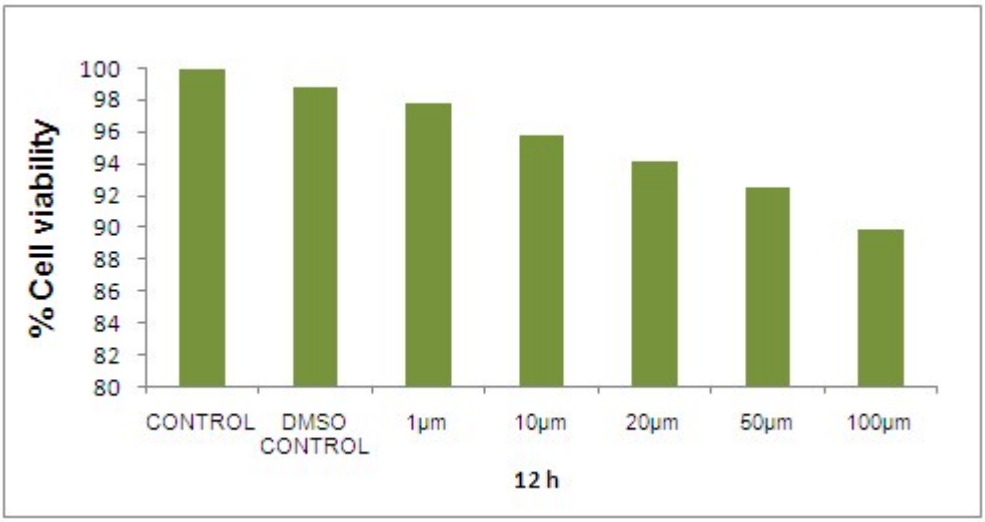


Figure S15. % cell viability of HepG2 cells treated with different concentrations (1 µM-100 µM) of **DFC-TS** for 12 hour determined by MTT assay. Results were expressed as mean of three independent experiments.

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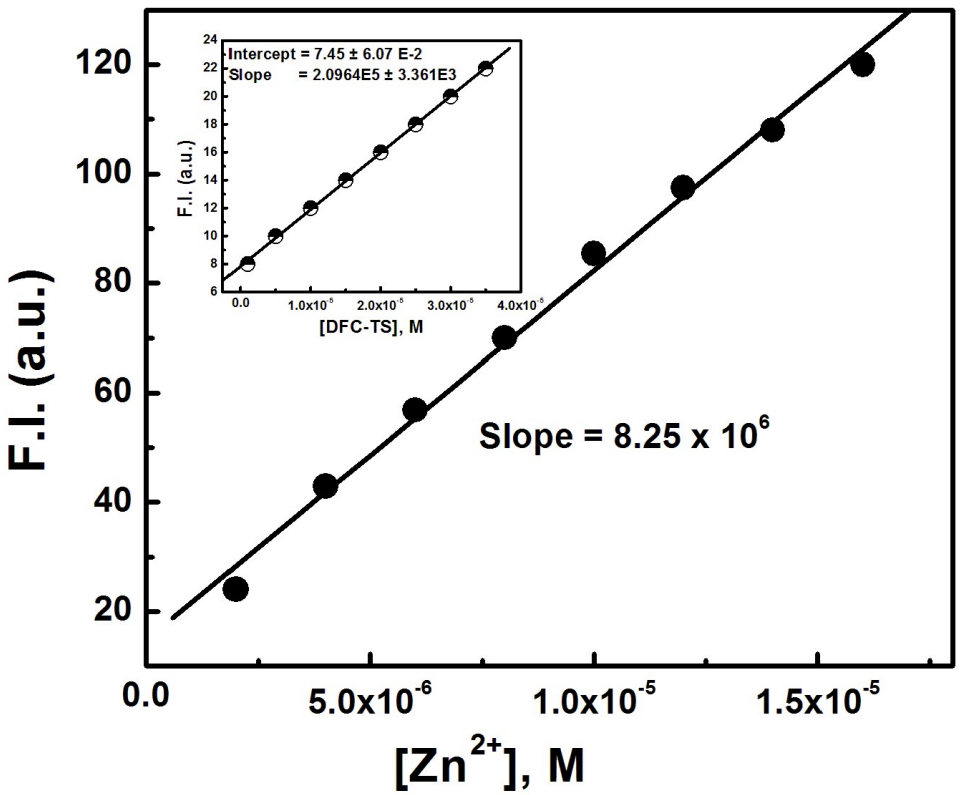

Figure S16. Calculation of Limit of detection(LOD): LOD=3X Standard deviation/ Slope = $3x6.07x10^{-2}/8.25X10^{6}=2.20X10^{-8}$= 22 nM.

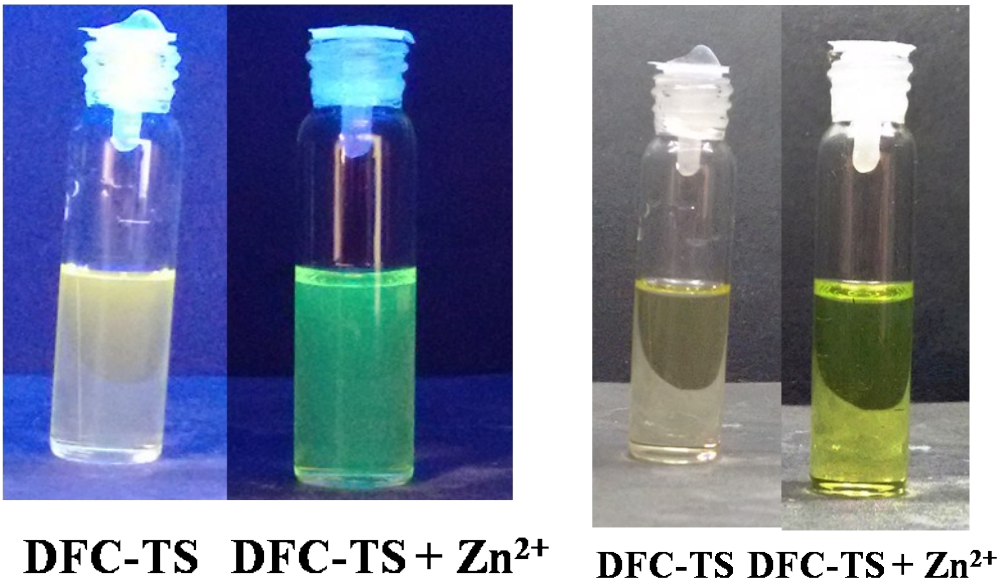

Figure S17. Photograph of DFC-TS and DFC-TS+Zn^{2+} in UV exposed and visually exposed light.

Table S1.Selected bond distances of the optimized DFC-TS and complexes 1 and 2 in the ground state calculated at B3LYP Levels.

Bond Length	DFC-TS (enol)	DFC-TS (keto)	$[Zn_1DFCTS(OAc)]^0$	$[Zn_1DFCTS(OAc)]^-$	$[Zn_2(DFC\text{-}TS)(OAc)_2]$
C6-O26	1.34	1.26	1.283	1.283	1.3054(C7-017)
O26-H34	0.988	1.73			
C9-N13	1.290	1.330	1.307	1.299	1.3007(C28-N10)
N13-N14	1.356	1.38	1.3798	1.376	1.381(N10-N11)
N14-C15	1.378	1.39	1.353	1.314	1.360(N11-C30)
C15-N16	1.374	1.36	1.358	1.387	1.36598(C30-N42)
C15-S19	1.663	1.65	1.7	1.756	1.67823(C30-S40)
C11-N20	1.288	1.288	1.290	1.290	1.298(C19-N21)
N20-N21	1.352	1.352	1.352	1.362	1.365(N21-N8)
N21-N22	1.358	1.37	1.376	1.366	1.327(N8-C22)
C22-N23	1.356	1.34	1.344(C22-S40)	1.342(C22-S39)	1.369(C22-N45)
C22-S32	1.680	1.680	1.685	1.699	1.737(C22-S41)
Zn32-O26			1.980	2.009(Zn31-O26)	2.135(Zn1-O17)
Zn32-N13			2.203	2.178(Zn31-N13)	2.171(Zn1-N10)
Zn32-S19			2.543	2.442(Zn31-S19)	2.667(Zn1-S40)
Zn32-O33			2.115	2.15(Zn31-O32)	2.001(Zn1-O3)
Zn32-O34			2.130	2.239(Zn31-O33)	2.008(Zn1-O6)
Zn2-O17					2.287
Zn2-N21					2.143
Zn2-S41					2.441
Zn2-O4					2.054
Zn2-O5					2.053

Table S2. Selected bond angles of the optimized complexes 1 and 2 in the ground state calculated at B3LYP Levels.

Bond Angle	Zn1DFCTS(NEUTRAL)	Zn1DFCTS(ANION)	ZN2DFCTS
N13-Zn32-O26	82.67	84.904(N13-Zn31-O26)	84.965(N10-Zn1-O17)
N13-Zn32-S19	78.12	80.445(N13-Zn31-S19)	77.086(N10-Zn1-S40)
O26-Zn32-O34	99.03	91.1313(O26-Zn31-O33)	100.095(O17-Zn1-O6)
O33-Zn32-O34	62.60	60.642(O32-Zn31-O33)	123.968(O3-Zn1-O6)
N13-Zn32-O34	176.45	174.04(N13-Zn31-O33)	105.689(N10-Zn1-O6)
S19-Zn32-O26	139.90	139.9(S19-Zn31-O26)	160.55(S40-Zn1-O17)
			84.221(N21-Zn2-O17)
			82.402(N21-Zn2-S40)
			88.191(O17-Zn2-O5)
			113.071(O4-Zn2-O5)
			133.332(N21-Zn2-O5)
			165.547(S40-Zn2-O17)

Table S3. Vertical excitation energies (E_{cal}), oscillator strengths (f_{cal}), and type of excitations of the lowest few excited singlets obtained from TDDFT calculations of $[Zn(DFC\text{-}TS)(OAc)]^-$ and $[Zn_2(DFC\text{-}TS)(OAc)_2]$ in MeCN.

compound	States	E_{cal}/nm	f_{cal}	excitation
DFC-TS (enol)	S_1	369	0.372	HOMO→LUMO (65%)
	S_3	326	0.4195	HOMO-1→LUMO (48%) HOMO-1→LUMO+1(34%) HOMO → LUMO+1(27%)
	S_5	320	0.7823	HOMO-1→LUMO (34%) HOMO→ LUMO+1(53%)
	S_{10}	275	0.1343	HOMO−5→LUMO (59%) HOMO−4→LUMO +1(28%)
	S_{18}	239	0.1785	HOMO-1→LUMO+2 (59%) HOMO→LUMO+4(28%)
DFC-TS(keto)	S1	439	0.3915	HOMO→LUMO(65%)
	S5	340	0.1444	HOMO-3→LUMO(34%) HOMO-1→LUMO(43%) HOMO→LUMO+1(39%)
	S6	327	0.3778	HOMO-1→LUMO(32%) HOMO→LUMO+1(52%)
	S10	293	0.1685	HOMO-6→LUMO(65%)
	S13	279	0.3612	HOMO-1→LUMO+1(66%)
	S20	245	0.1449	HOMO-7→LUMO(42%) HOMO-6→LUMO+1(26%) HOMO→LUMO+4(33%)
ZnDFCTS (NEUTRAL)	S1	424	0.3782	HOMO→LUMO(66%)
	S2	340	0.2001	HOMO-1→LUMO(32%) HOMO→LUMO+1(58%)
	S6	303	0.3557	HOMO-3→LUMO(32%) HOMO-1→LUMO+1(58%)
	S8	290	0.2314	HOMO-3→LUMO(51%) HOMO-1→LUMO+1(30%)
	S15	253	0.1391	HOMO-7→LUMO(52%) HOMO→LUMO+4(34%)
Zn_2DFCTS	S1	431	0.2832	HOMO→ LUMO(66%)
	S2	362	0.4158	HOMO-1→LUMO(31%) HOMO→LUMO+1(58%)
	S3	348	0.3591	HOMO-1→LUMO(57%) HOMO→LUMO+1(28%)
	S10	281	0.179	HOMO-2→LUMO+1 (54%) HOMO-4→LUMO+1(26%)
	S14	265	0.2195	HOMO-6→LUMO (23%) HOMO-7→LUMO(56%)

Table S4. Theoretical and experimental IR intensities.ǂ

IR	DFC-TS (enol)cm^{-1}	DFC-TS (keto) cm^{-1}	$[Zn_1DFCTS(OAc)]^0$ cm^{-1}	$[Zn_1DFCTS(OAc)]^-$ cm^{-1}	$[Zn_2(DFC\text{-}TS)(OAc)_2]$ cm^{-1}
C=S	1281(1110)	1280	1265	1268	1340 (1110)
C-S	-	-	1325	1295(1090)	-
C-O	1492(1353)	-	1500	1506(1405)	1495(1463)
C=O	-	1597(1462)	-	-	-
C-NH_2	1305(1315)	1452	1470	1470(1319)	1383(1352)

ǂParenthetical values are experimentally observed values.