Electronic Supporting Information (ESI)

FRET operated sensor for intracellular pH mapping: Strategically improved efficiency on moving from anthracene to naphthalene derivative

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1. Calculation of Quantum Yield

Fluorescence quantum yields (Φ) were estimated by integrating the area under the fluorescence curves using the equation[1],

Where A was the area under the fluorescence spectral curve, OD was optical density of the compound at the excitation wavelength and η was the refractive indices of the solvent. Anthracene (quantum yield is 0.27 in ethanol)[2] and tris(2,2'-bipyridyl)ruthenium(II) (Φ = 0.042 in water)[3] were used as quantum yield standard for measuring the quantum yields at 365 nm and 450 nm for **ANC** and **AAC** respectively both for their protonated and deprotonated forms.

2. Derivation of the equations which are used to determine the pK_a

In the acidic pH ranges both the –C=N- groups of **AAC** and **ANC** remain protonated.

$$BH + 2H^+ \rightleftharpoons BH_3^{2+}$$

$$K_a = \frac{[BH_3^{2+}]}{[BH][H^+]^2}$$

$$\text{or, } \log K_a = -2\log[H^+] + \log\frac{[BH_3^{2+}]}{[BH]}$$

$$\text{or, } 2pH = -pK_a - \log\frac{[BH_3^{2+}]}{[BH]}$$

$$\text{or, } 2pH = -pK_a - \log\frac{[\text{Protonated form}]}{[\text{Neutral form}]} \quad \text{----------(i)}$$

Upon lowering the pH, the fluorescence intensity may increase or decrease.

If fluorescence intensity increases due to the protonated form, the equation (i) can be written as

$$2\ pH = -pK_a - \log\frac{F_x - F_{min}}{F_{max} - F_x} \quad \text{---------- (ii)}$$

where,

F_{max} = Maximum intensity at the wavelength of the maximum emission due to the protonated form (BH_3^{2+}).

F_{min}= Minimum intensity at same wavelength.

F_x = Intensity at an intermediate pH at the same wavelength.

pK_{a1} of **ANC** has been calculated by using equation (ii). Here F_x, F_{max} and F_{min} were measured at 600 nm, the wavelength at the emission maximum of ANC in the acidic range.

If fluorescence intensity decreases due to protonated form, the working formula becomes

$$2\ pH = -pK_a - \log \frac{F_{max} - F_x}{F_x - F_{min}} - \quad \text{---------- (iii)}$$

where

F_{max} = Maximum intensity at the wavelength of maximum emission due to the neutral form (*BH*).

F_{min}= Minimum intensity at the same wavelength.

F_x= Intensity at an intermediate pH at the same wavelength.

pK_{a1} of **AAC** has been calculated by using equation (iii). Here F_x, F_{max} and F_{min} were measured at 537 nm.

In the basic pH range the –OH of **AAC** and **ANC** is deprotonated

$$BH \rightleftharpoons B^- + H^+$$

$$K_a = \frac{[B^-][H^+]}{[BH]}$$

$$\text{or, } \log K_a = \log[H^+] + \log \frac{[B^-]}{[BH]}$$

$$\text{or, } -\text{pH} = -\text{pK}_a - \log\frac{[B^-]}{[BH]}$$

$$\text{or, } -\text{pH} = -\text{pK}_a - \log\frac{[\text{Deprotonated form}]}{[\text{Neutral form}]}$$

If fluorescence intensity .increases due to deprotonated form

then,

$$\text{pH} = \text{pK}_a + \log\frac{F_x - F_{min}}{F_{max} - F_x}$$

where,

F_{max} = Maximum intensity at the wavelength of maximum emission due to the deprotonated form (B^-).

F_{min}= Minimum intensity at the same wavelength.

F_x = Intensity at an intermediate pH at the same wavelength.

pK_{a2} of **AAC** and **ANC** have been calculated using this equation. Here F_x, F_{max} and F_{min} are measured at 537 nm and 535 nm for **AAC** and **ANC** respectively.

3. Figures

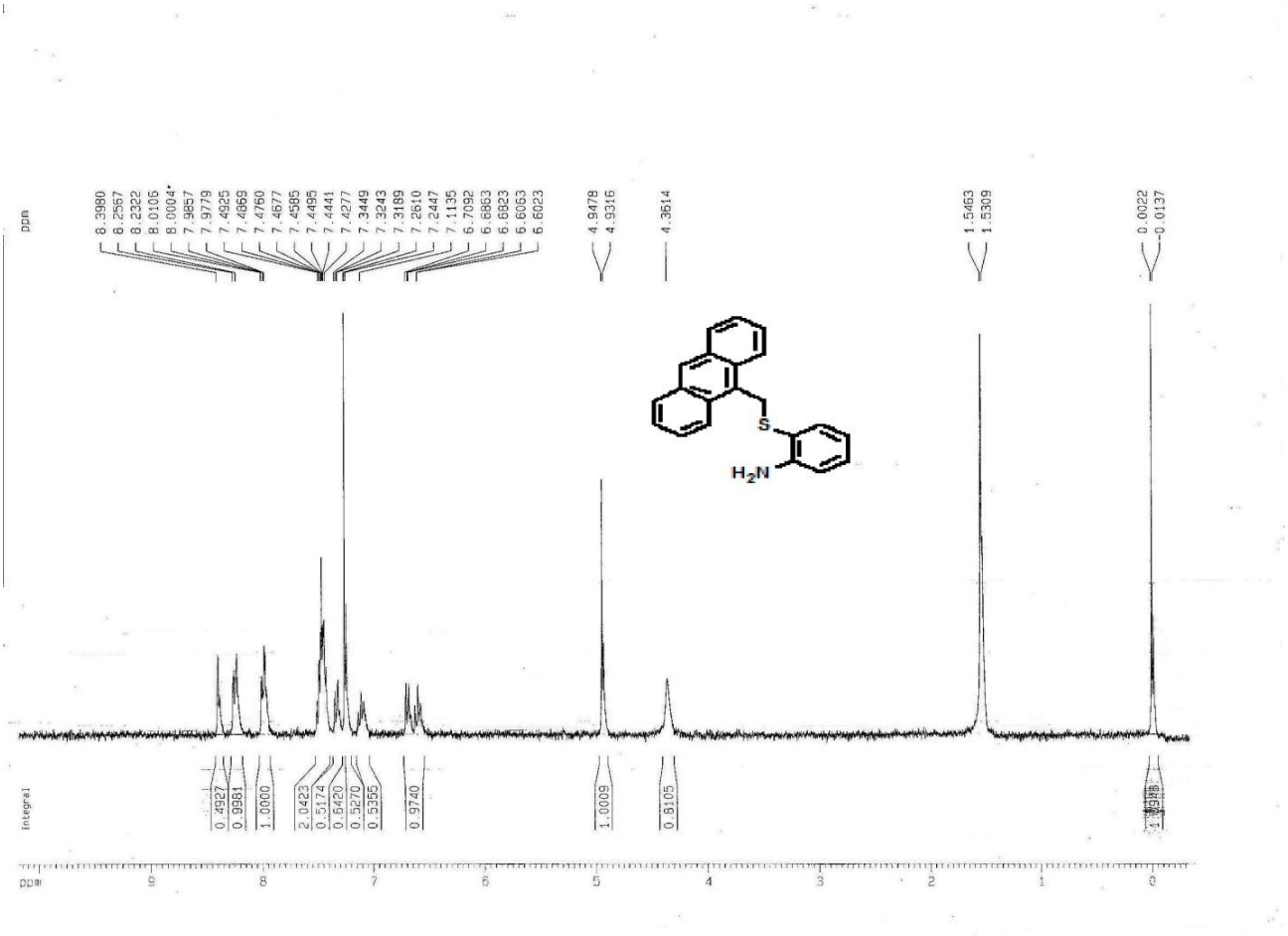

Figure S1. ^{1}H NMR spectrum of **AA** in $CDCl_3$

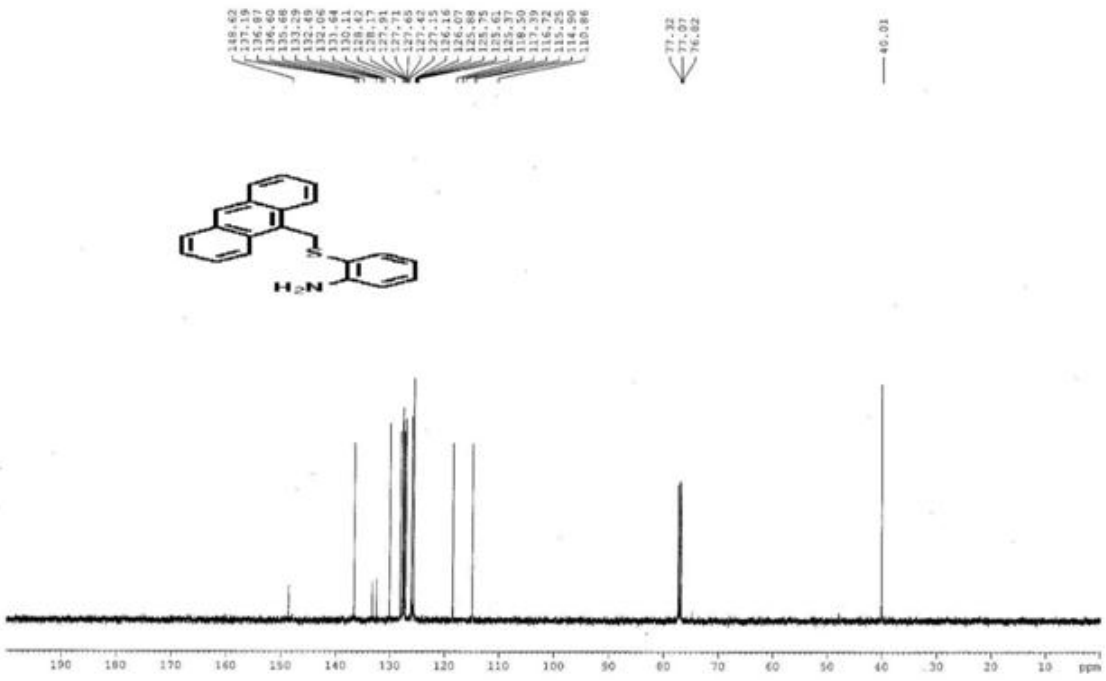

Figure S2**.** ^{13}C NMR spectrum of **AA** in $CDCl_3$

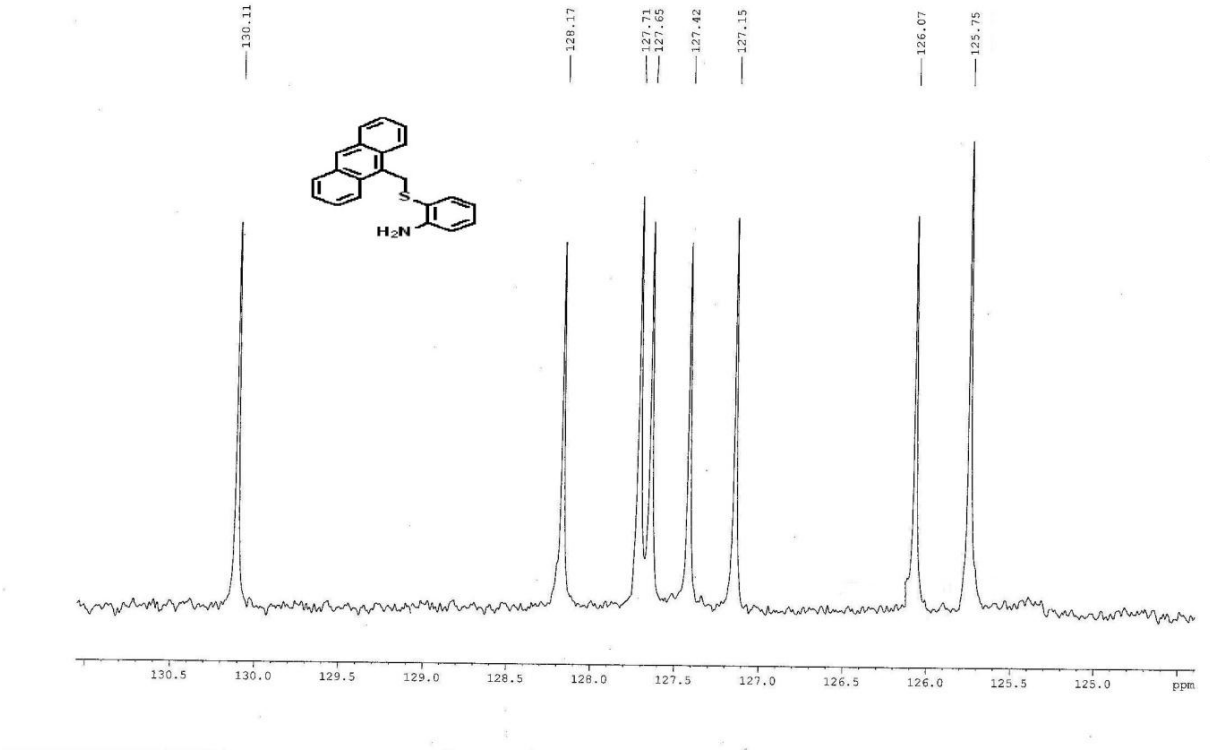

Figure S3**.** Expansion of ^{13}C NMR spectrum of **AA** in $CDCl_3$

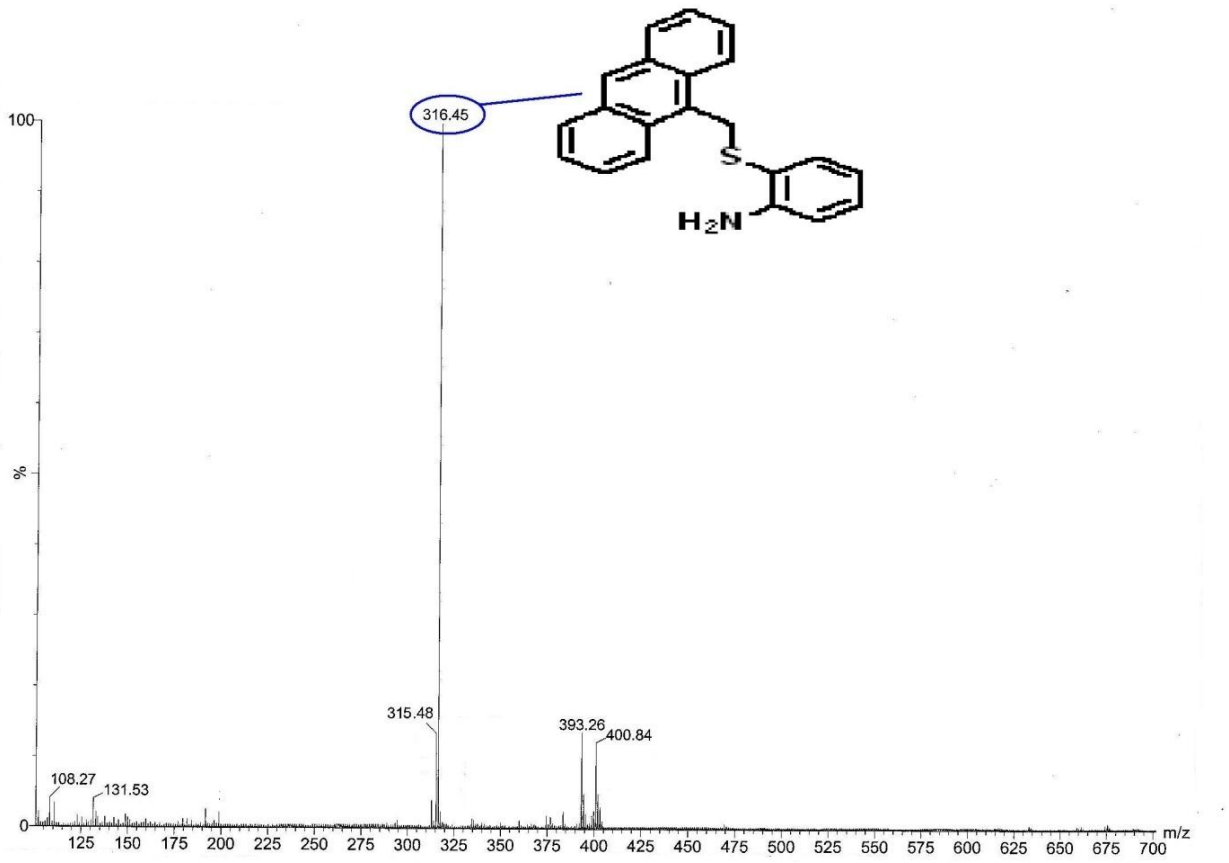

Figure S4**.** QTOF –MS ES^{+} spectrum of **AA**

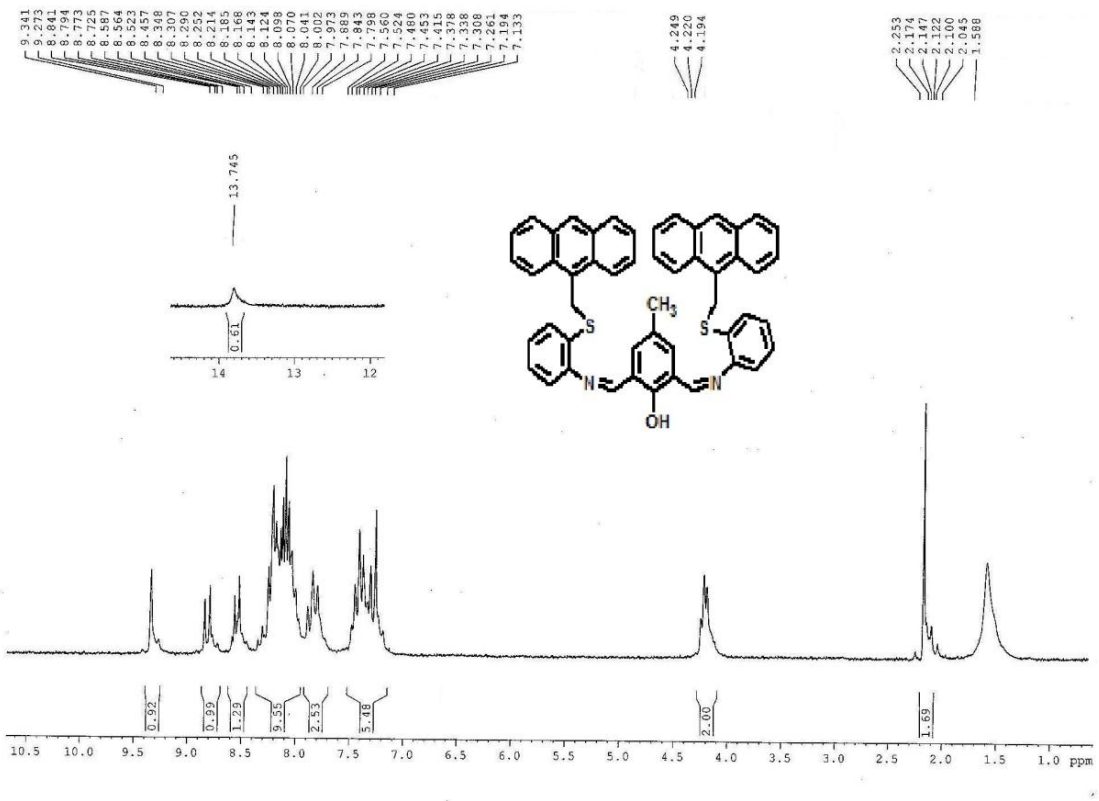

Figure S5. ^{1}H NMR spectrum of **AAC** in $CDCl_3$

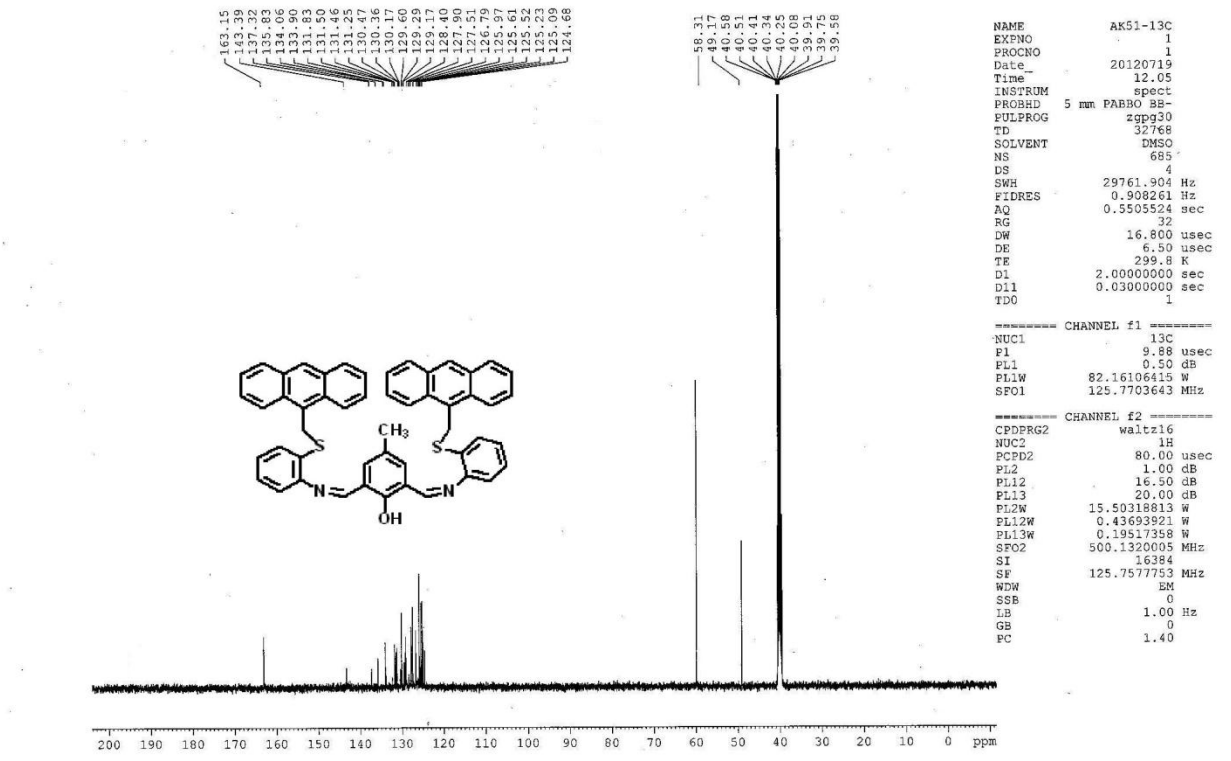

Figure S6. ^{13}C NMR spectrum of **AAC** in DMSO

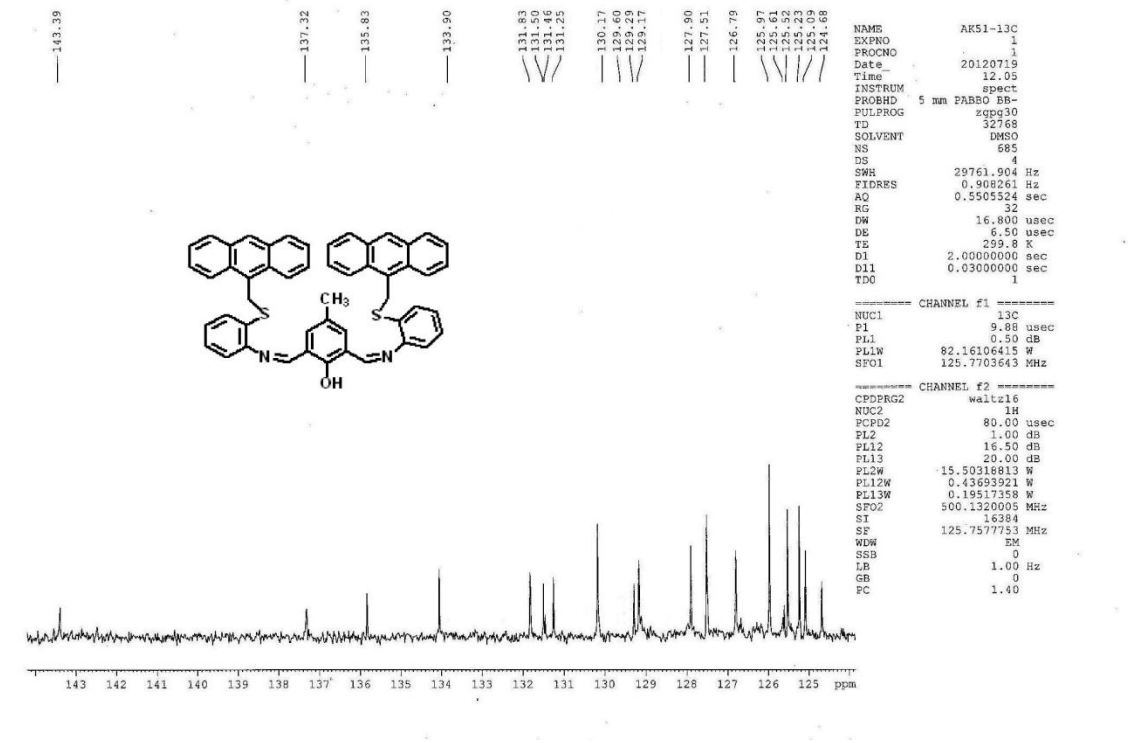

Figure S7**.** Expansion of ^{13}C NMR spectrum of **AAC** in DMSO

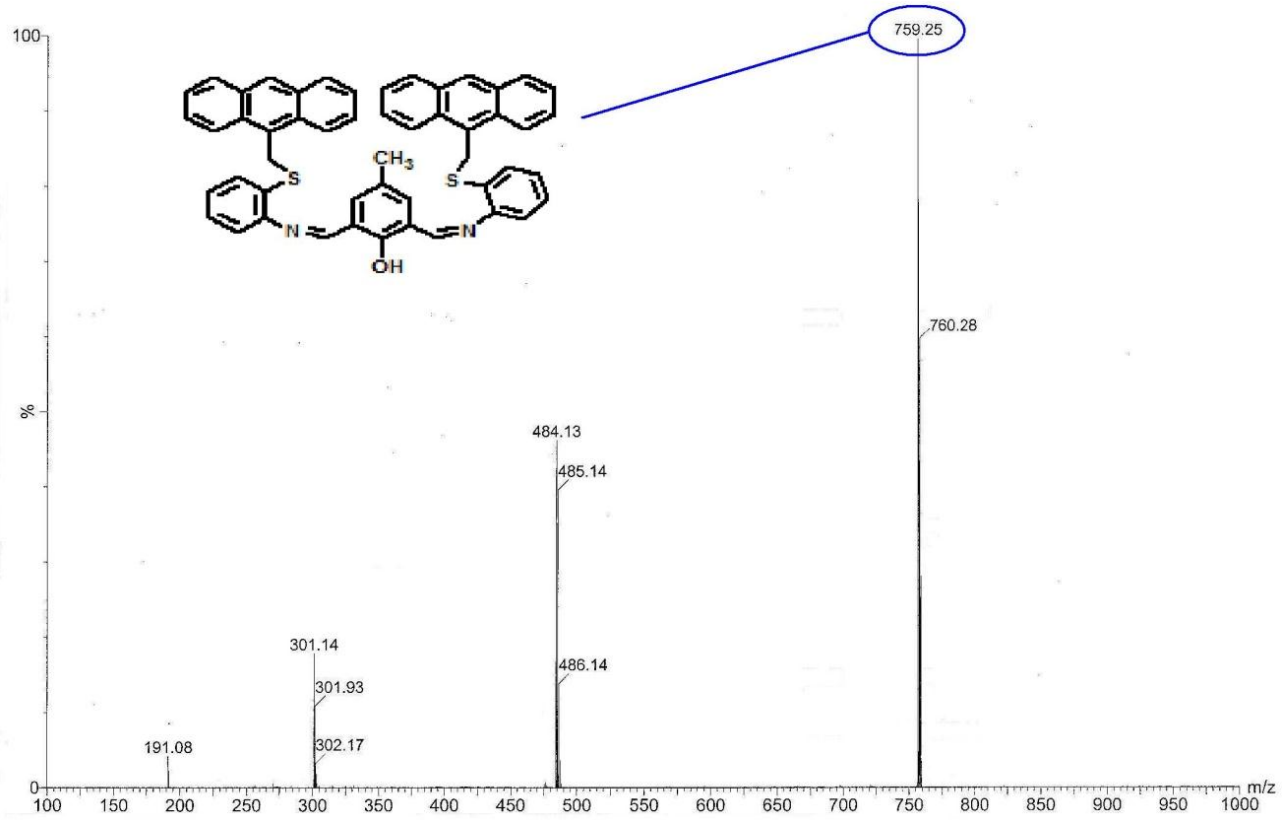

Figure S8. QTOF –MS ES^+ spectrum of **AAC**

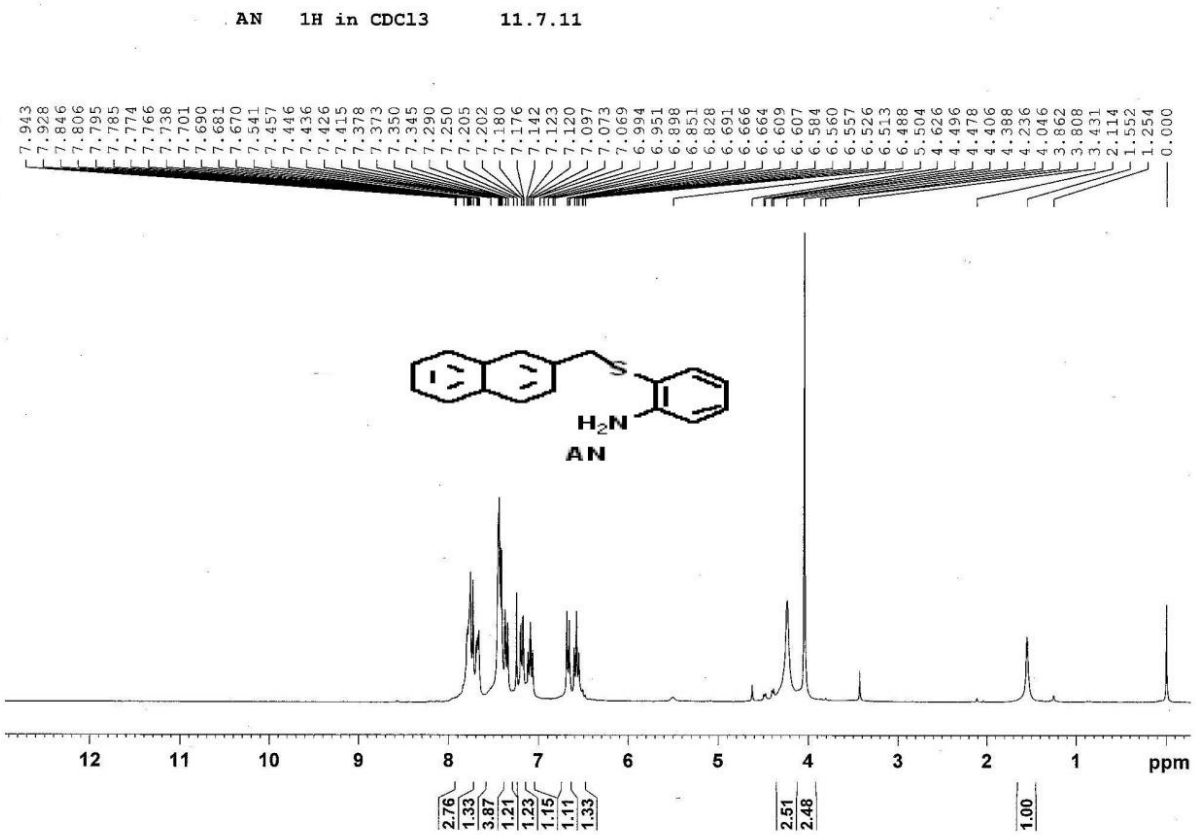

Figure S9. ^{1}H NMR spectrum of **AN** in $CDCl_3$.

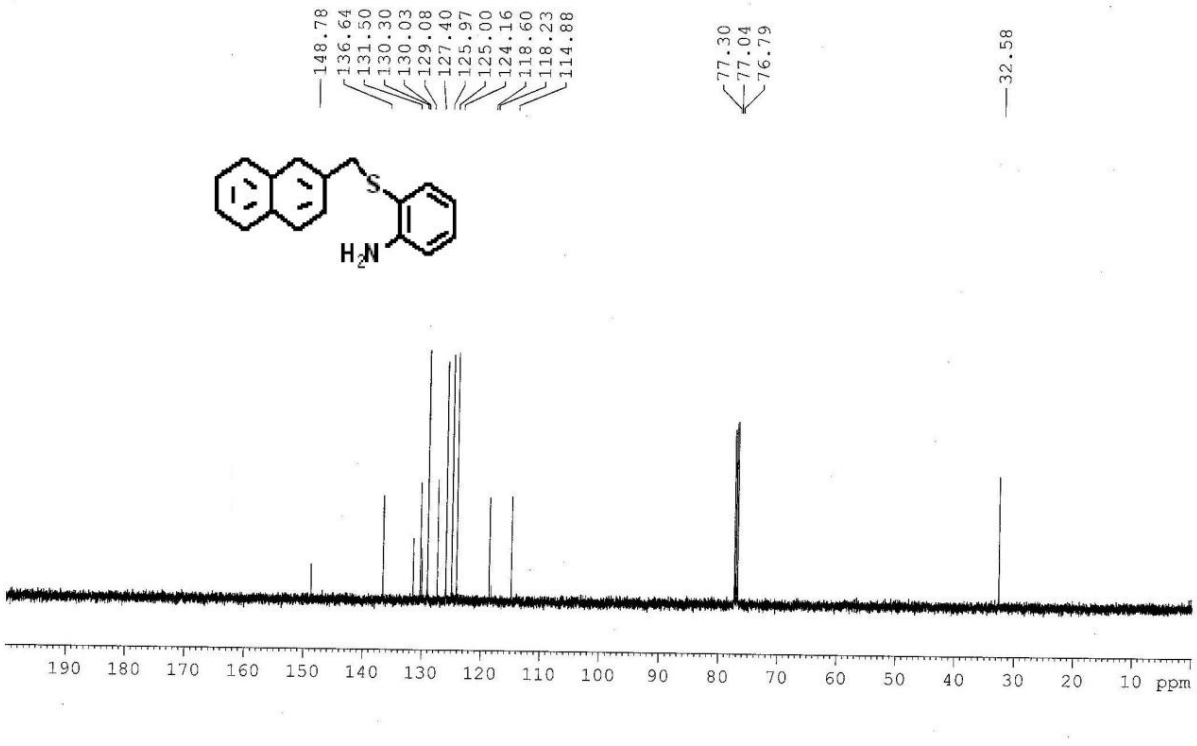

Figure S10. ^{13}C NMR spectrum of **AN** in $CDCl_3$

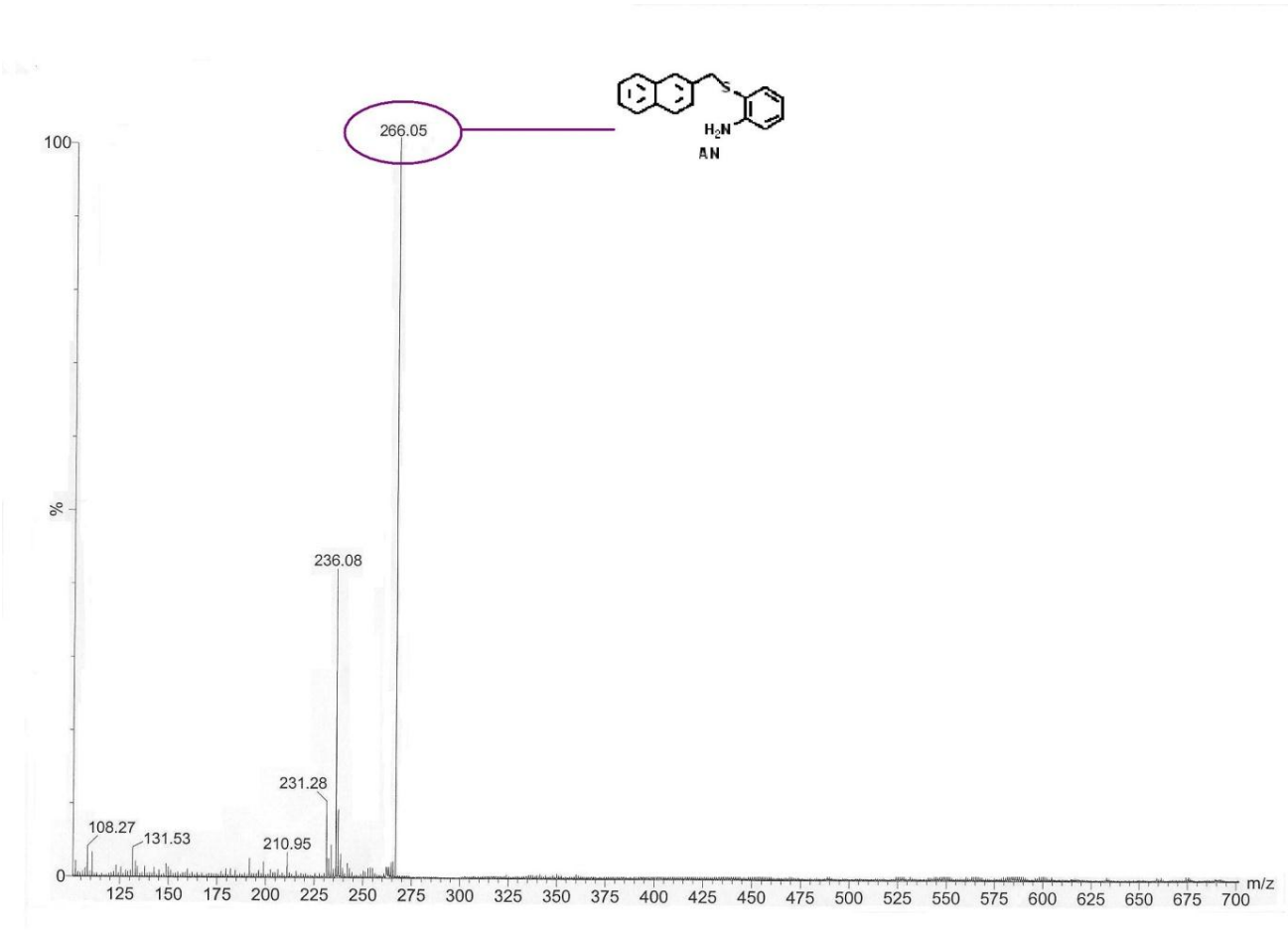

Figure S11. QTOF –MS ES$^+$ spectrum of **AN**

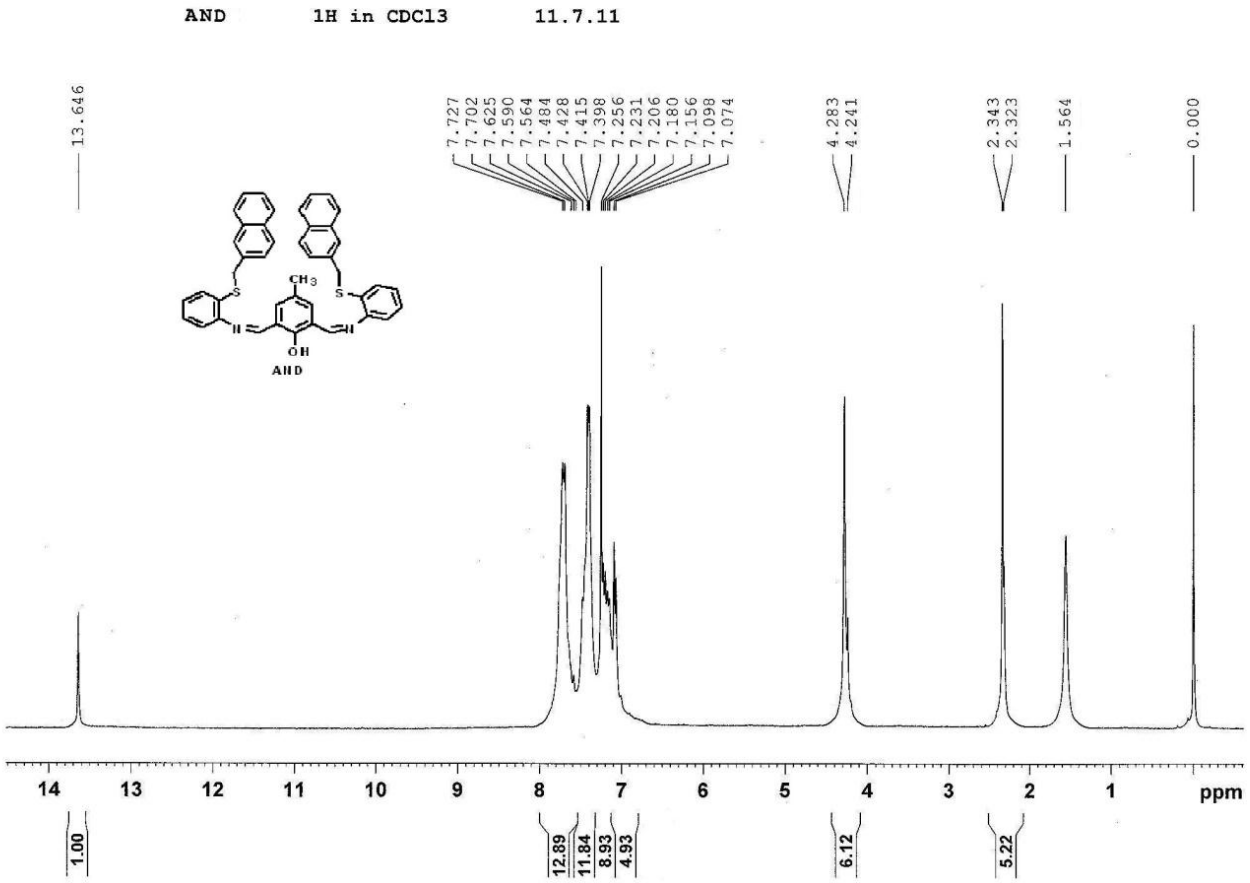

Figure S12**.** ^{1}H NMR spectrum of **ANC** in $CDCl_3$

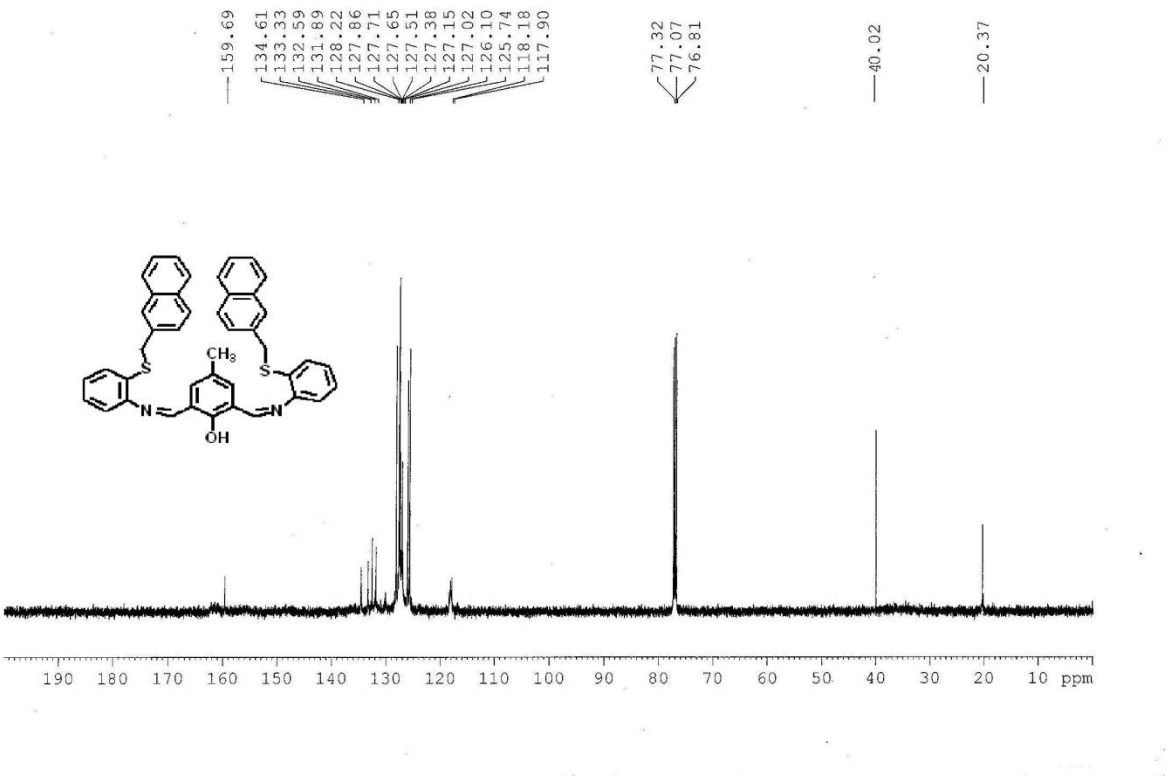

Figure S13**.** ^{13}C NMR spectrum of **ANC** in $CDCl_3$

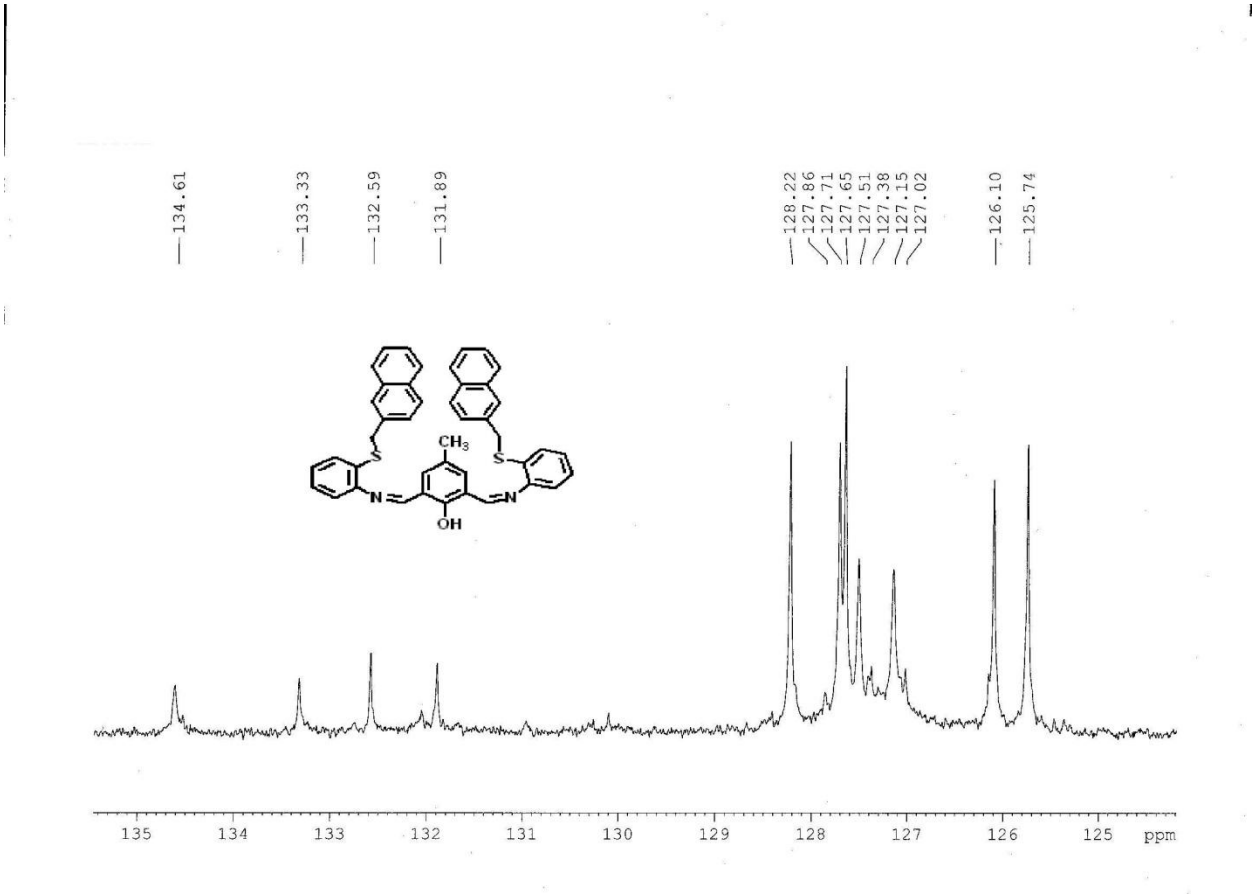

Figure S14**.** Expansion of ^{13}C NMR spectrum of **ANC** in $CDCl_3$

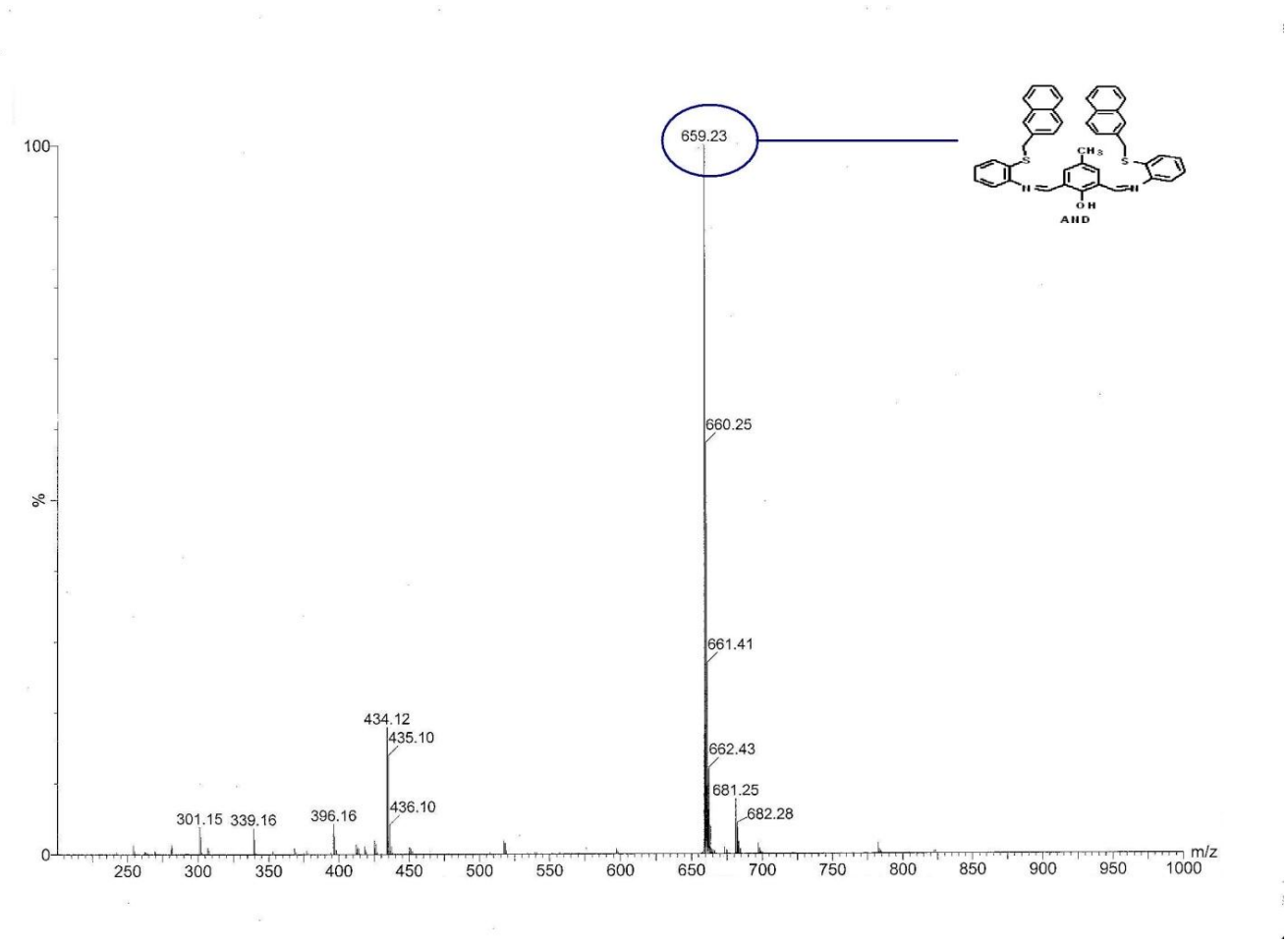

Figure S15.QTOF –MS ES$^+$ spectrum of **ANC**

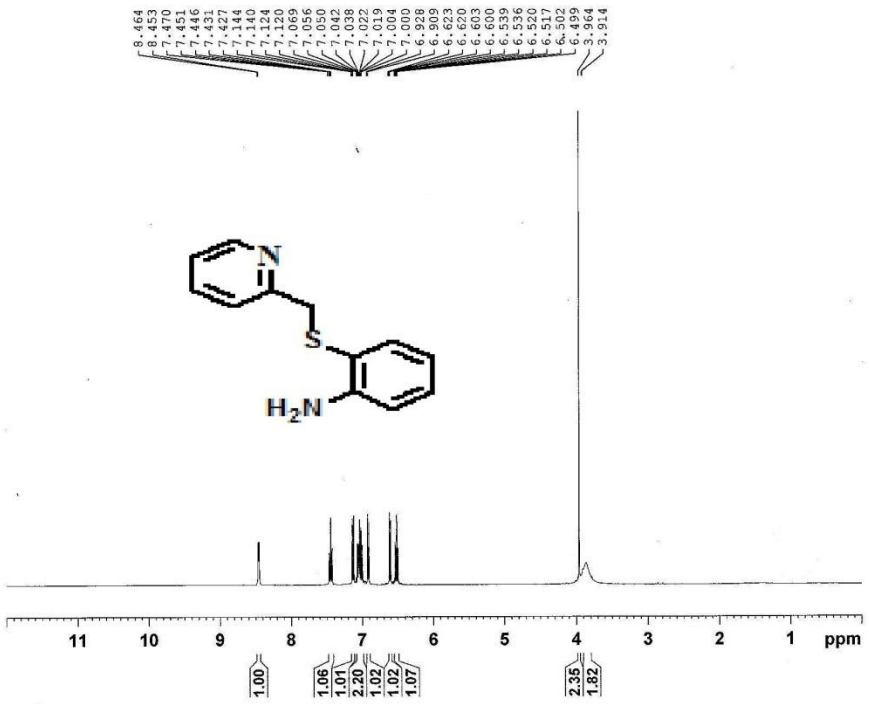

Figure S16. ^{1}H NMR spectrum of **AP** in $CDCl_3$

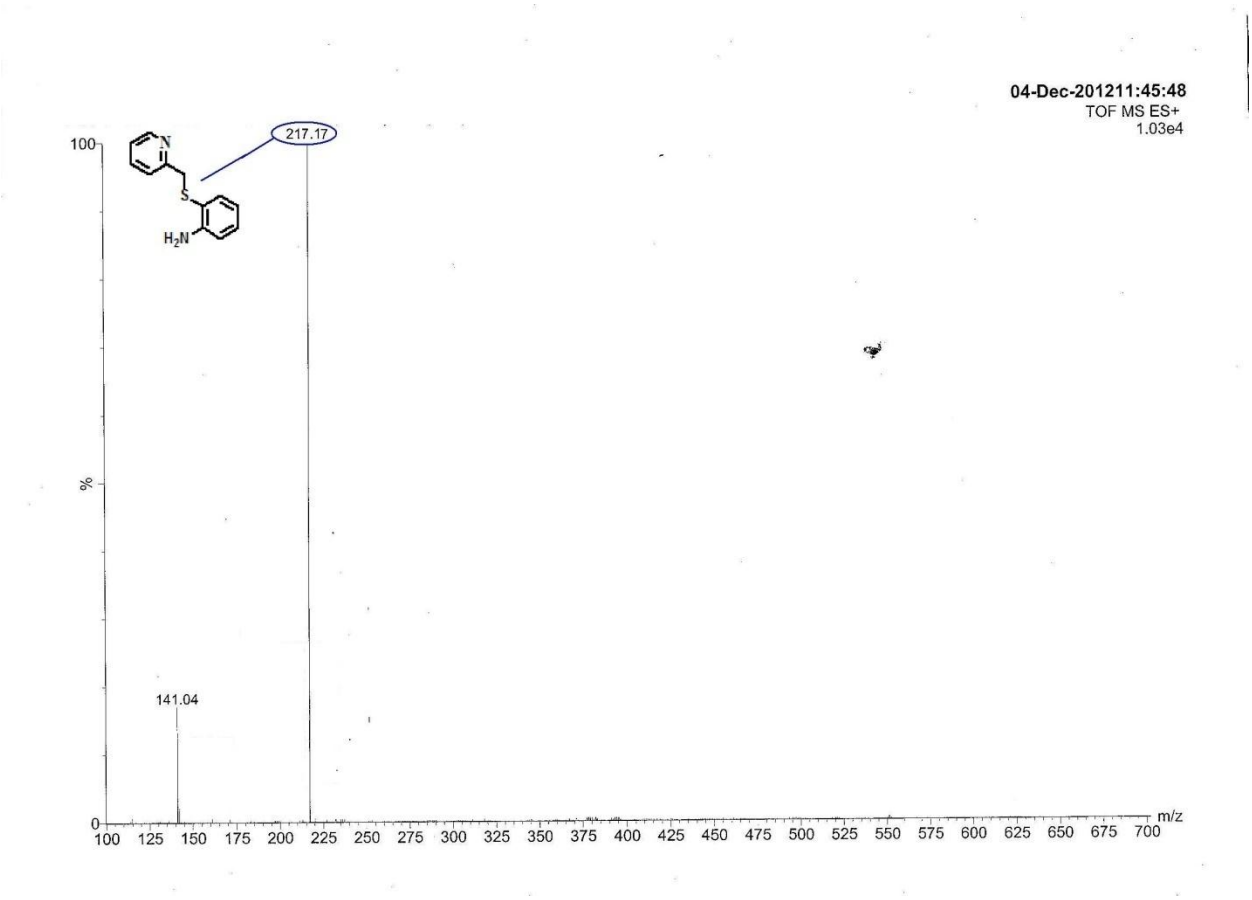

Figure S17.QTOF –MS ES$^+$ spectrum of **AP**

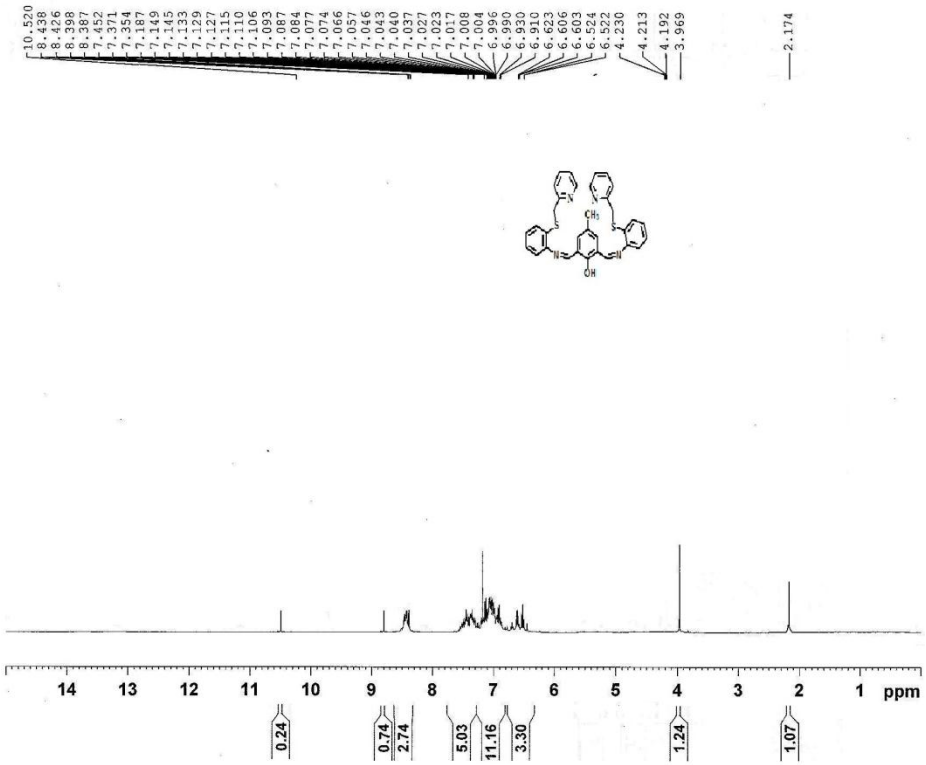

Figure S18. ^{1}H NMR spectrum of **APC** in $CDCl_3$

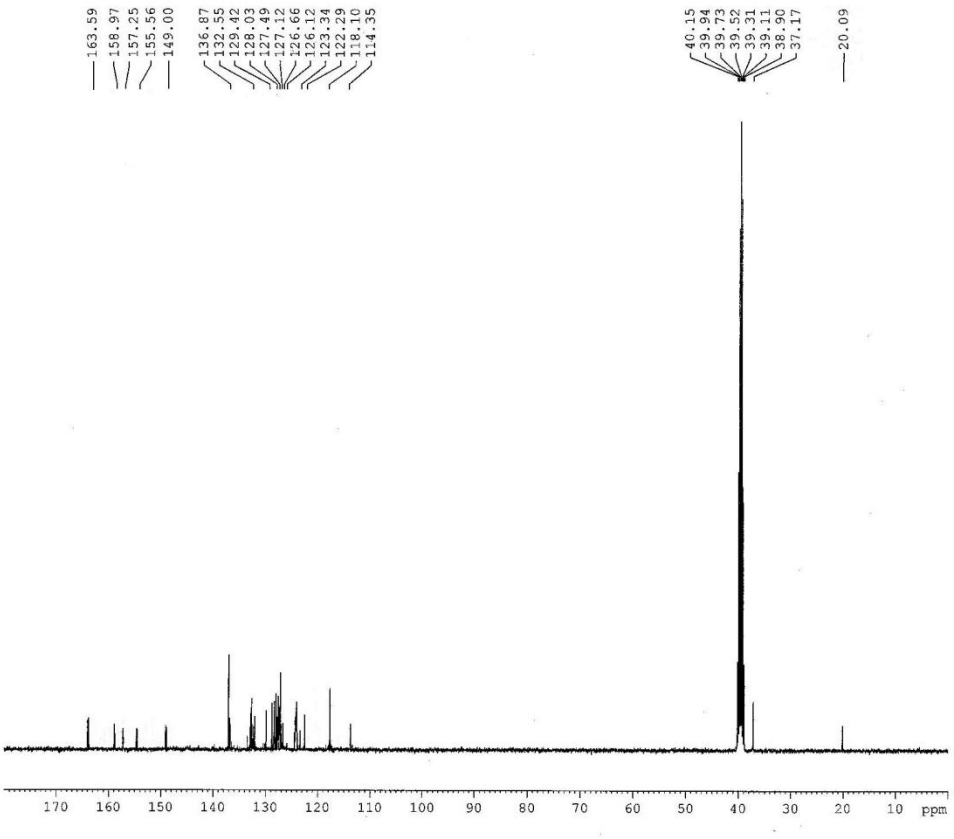

Figure S19**.** ^{13}C NMR spectrum of **APC** in DMSO-d^{6}

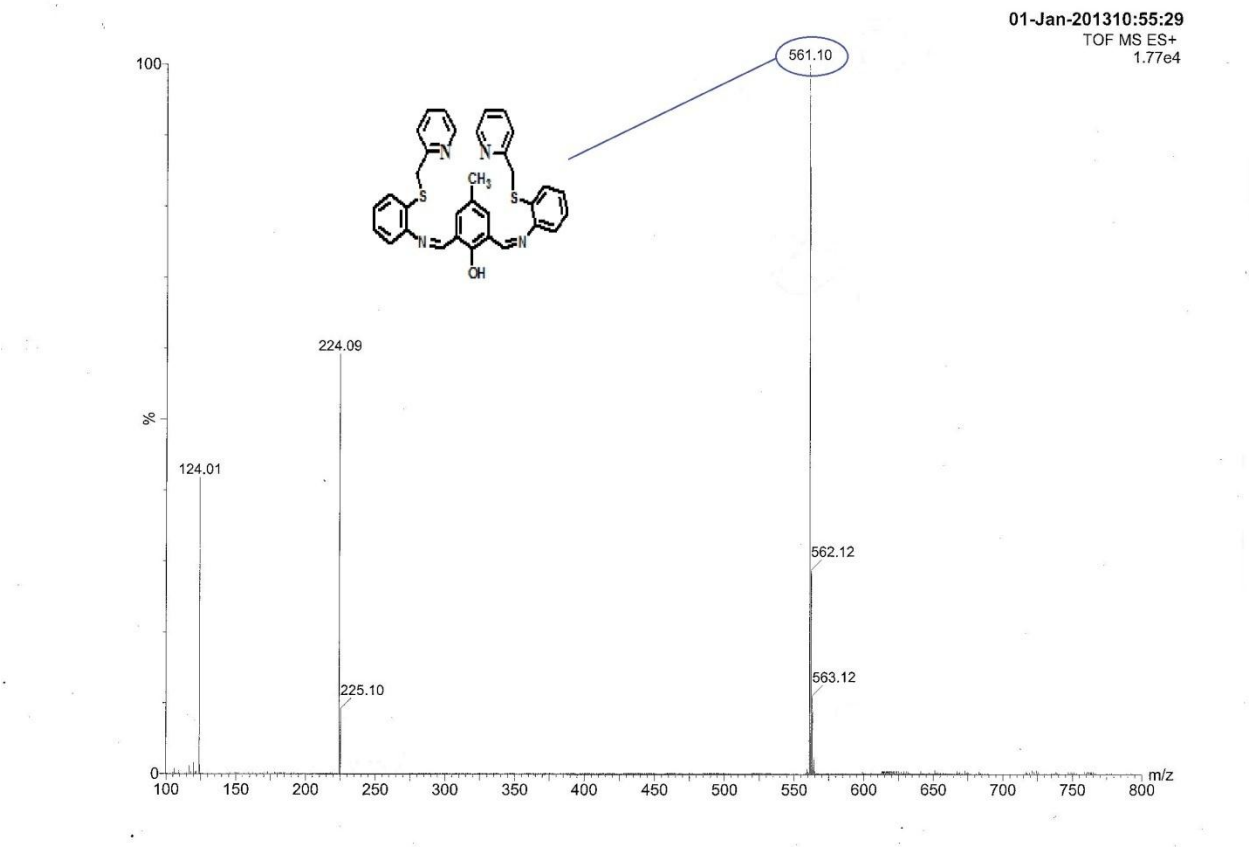

Figure S20.QTOF –MS ES^+ spectrumof **APC**

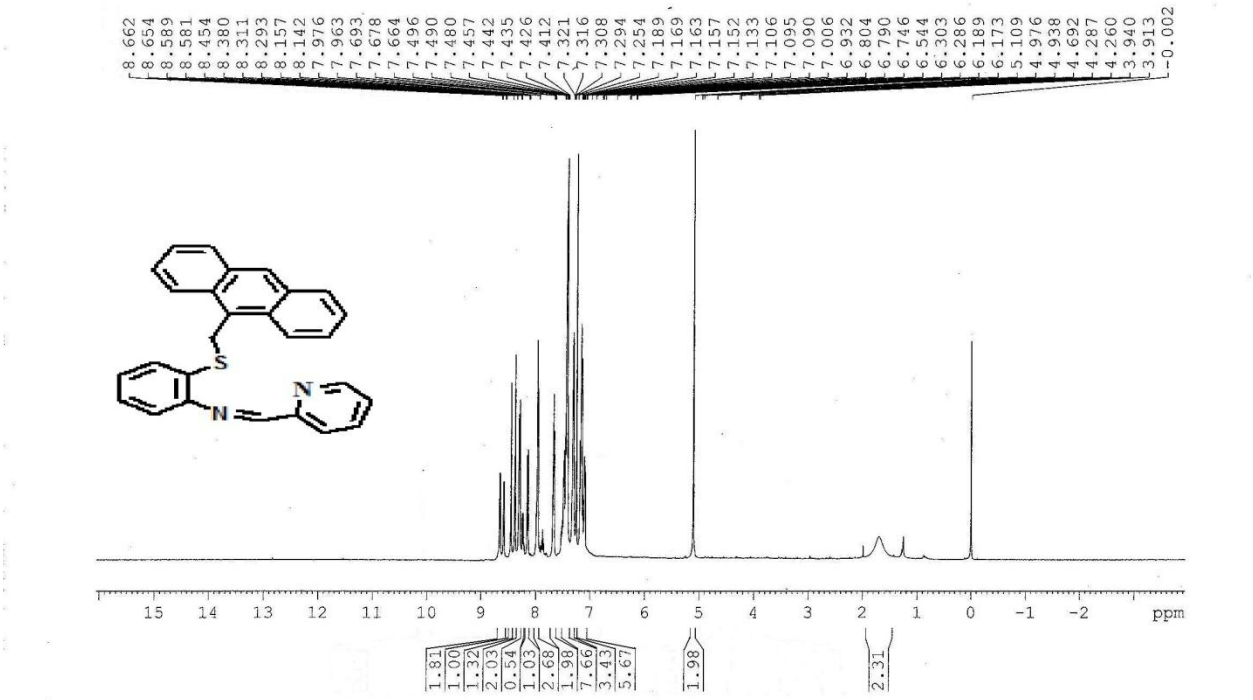

Figure S21. ^{1}H NMR spectrum of **AAP** in $CDCl_3$

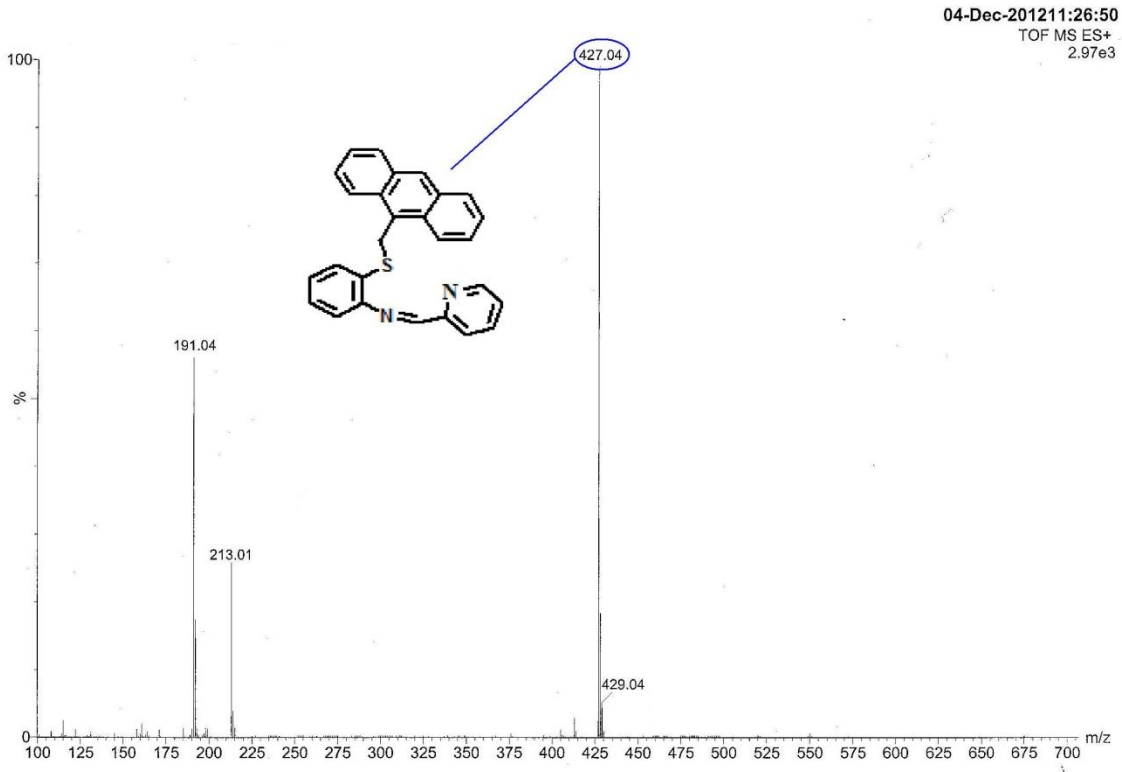

Figure S22. QTOF –MS ES$^+$ spectrum of **AAP**

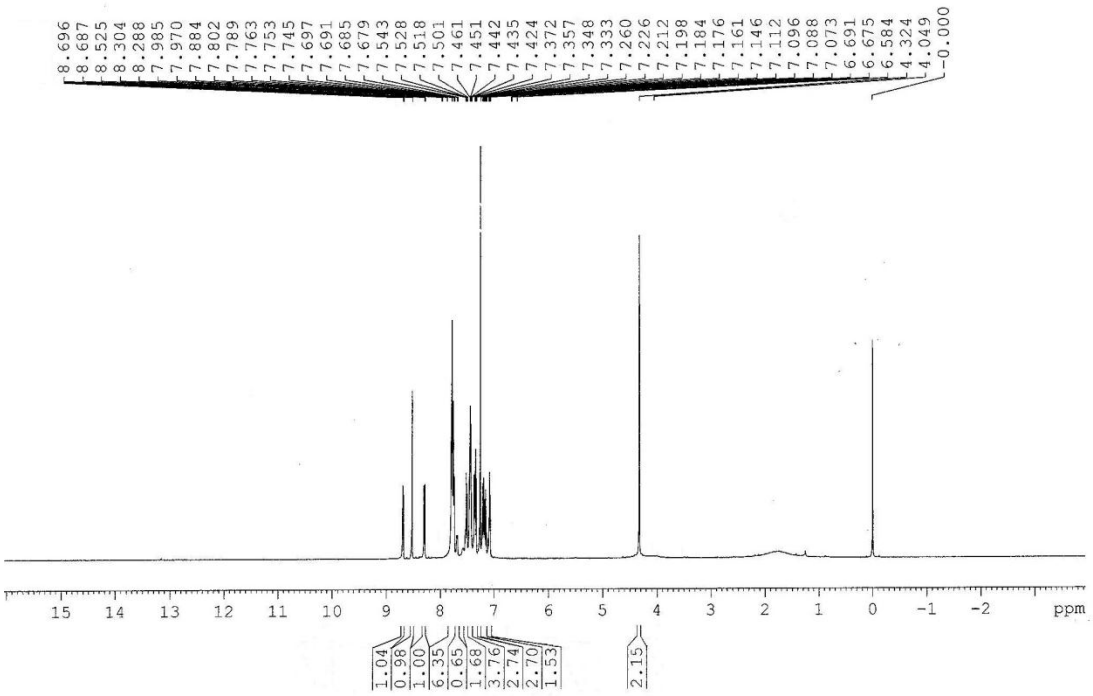

Figure S23. ^{1}H NMR spectrum of **ANP** in $CDCl_3$

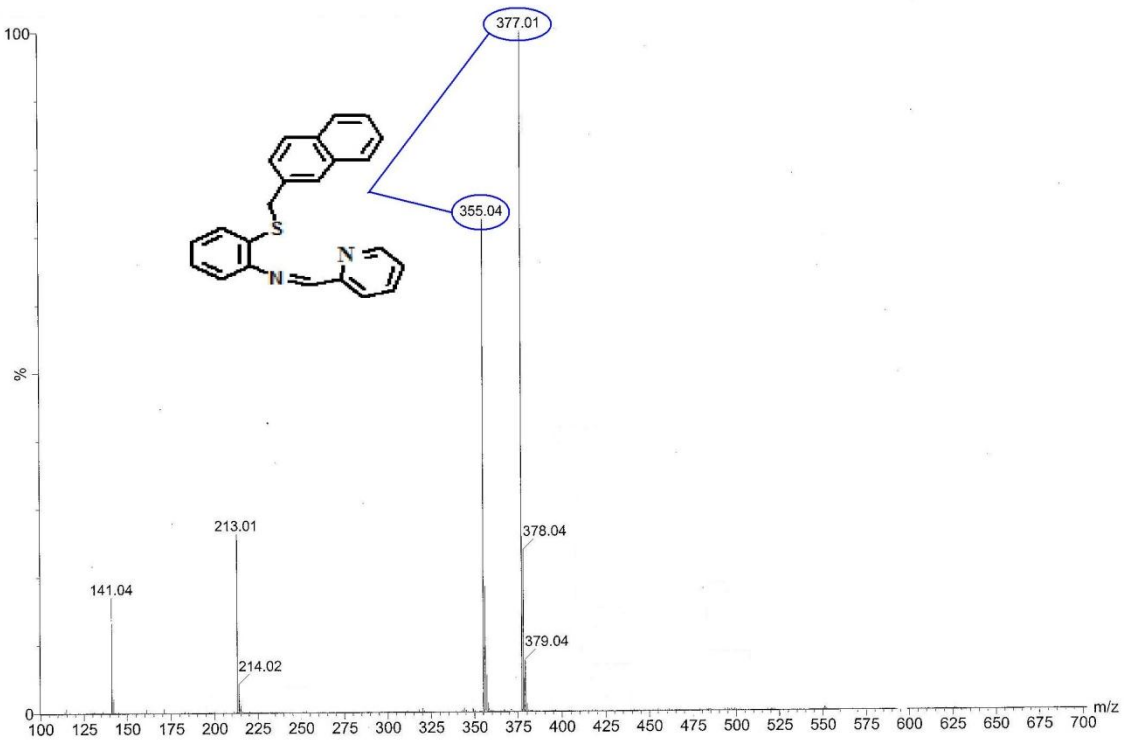

Figure S24.QTOF –MS ES$^+$ spectrum of **ANP**

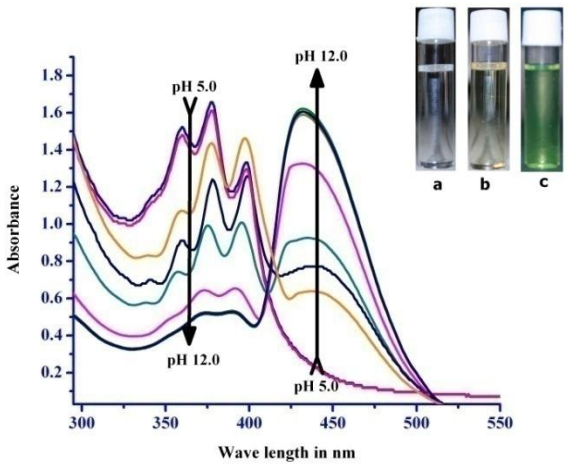

Figure S25. Changes in the absorption spectra of **AAC** (100 µM) as a function of pH (5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0, 11.5, 12.0 respectively). Inset: a, b and c are acidic, neutral and basic pH respectively. Solvent: methanol-water (7:3, v/v).

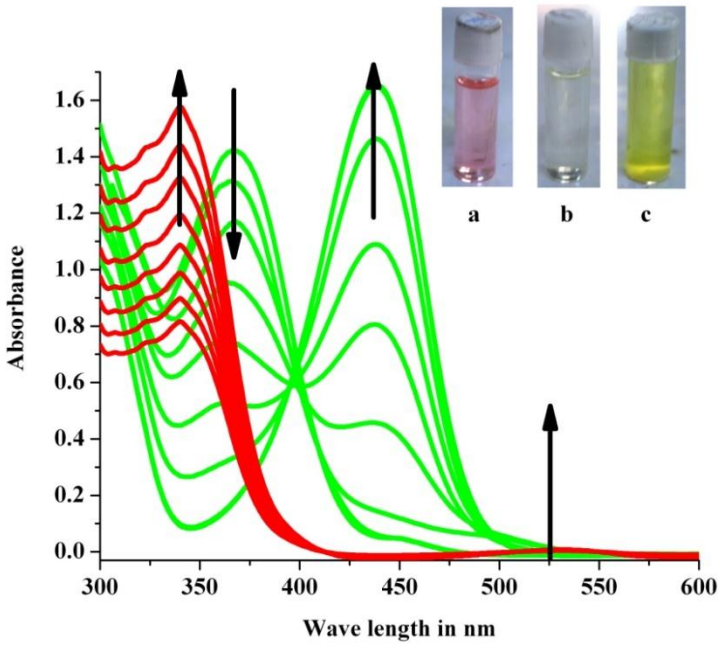

Figure S26. Changes in the absorption spectra of **ANC** (100 µM) as a function of pH. Red lines indicate pH values: 6.5, 6.0, 5.5, 5.0, 4.5, 4.0, 3.5, 3.0 and 2.0; green lines indicate pH values: 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 11.0, and 12.0 (in both the cases, from bottom to top). Inset: a, b and c are acidic, neutral and basic pH respectively. Solvent: methanol-water (7:3, v/v).

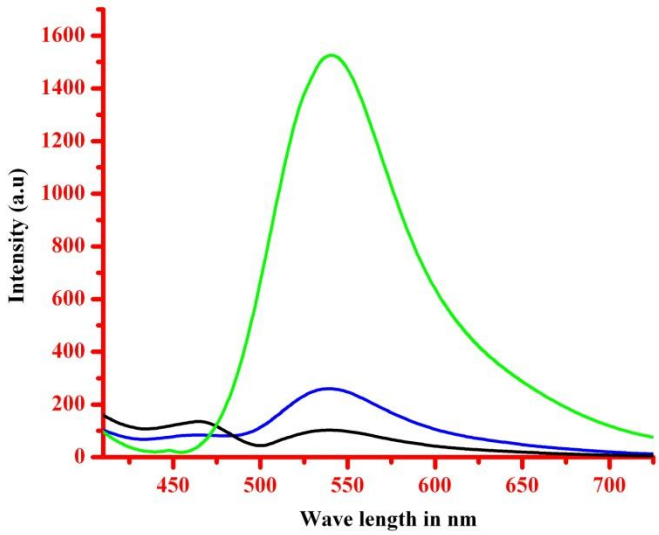

Figure S27. Emission spectra of **AAC** at different pH: pH 11.0 (green), 7.0 (blue), 5.0 (black). λ_{Ex} = 380 nm.

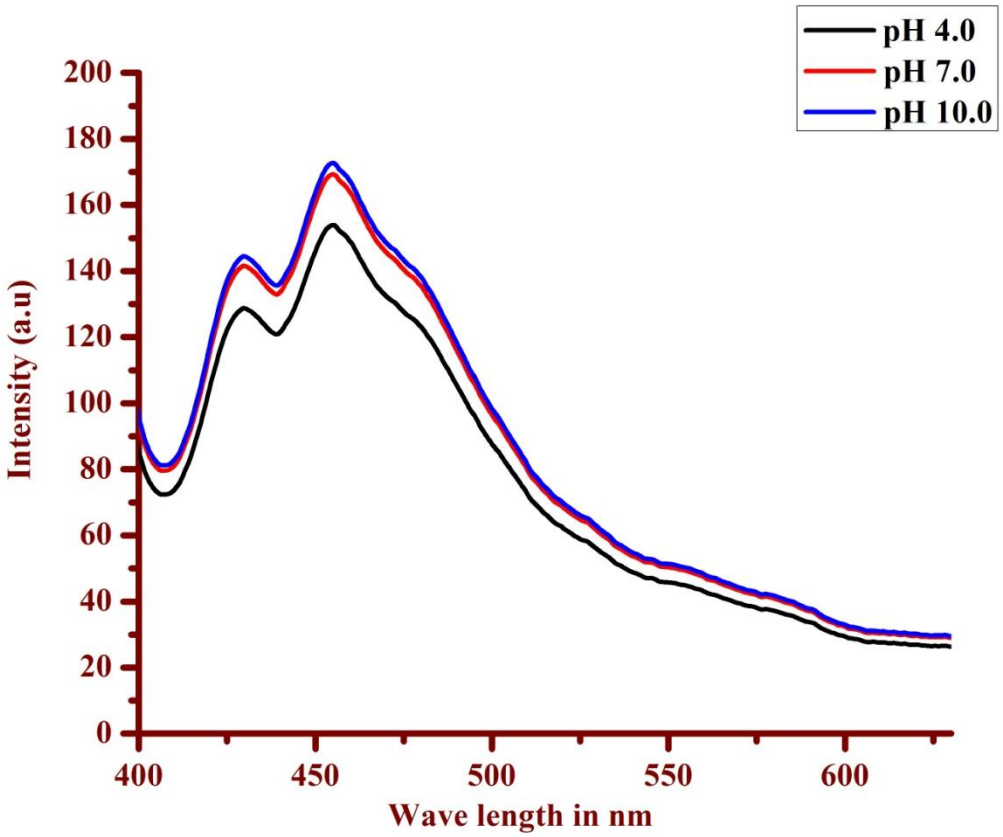

Figure S28. Emission spectra of **AAP** at different pH: pH 10.0 (blue), 7.0 (red), 4.0 (black). λ_{Ex} = 380 nm.

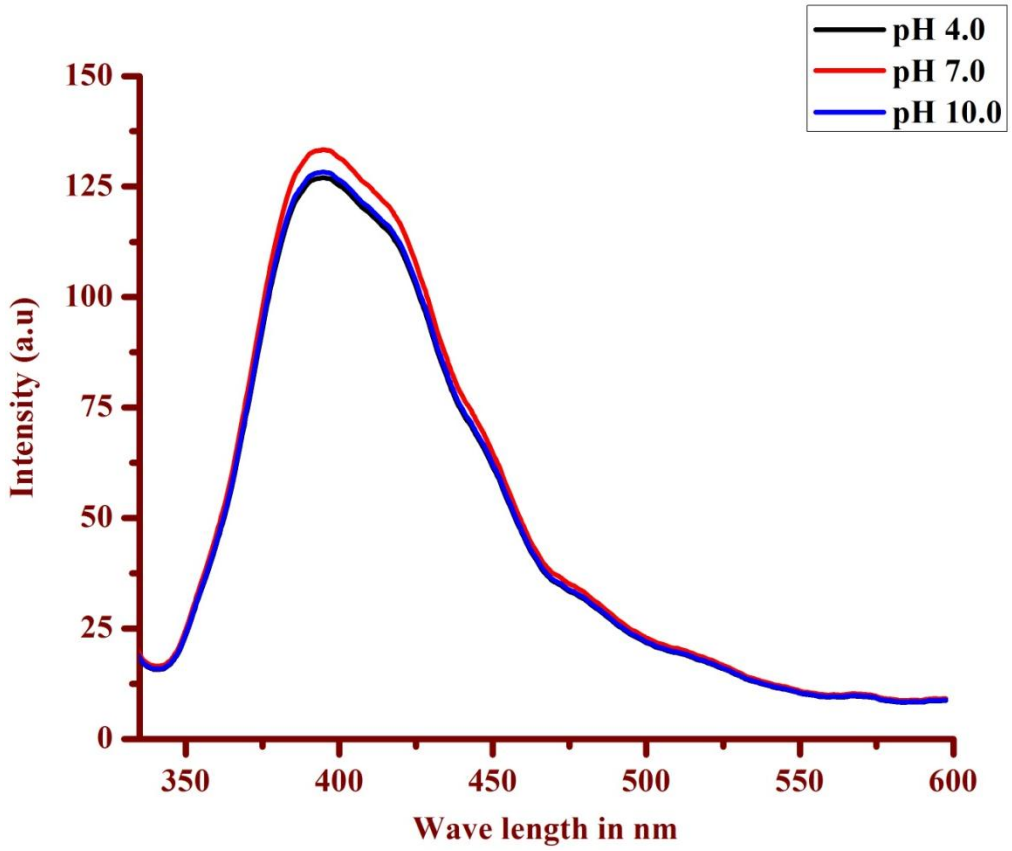

Figure S29. Emission spectra of **ANP** at different pH: pH 10.0 (blue), 7.0 (red), 4.0 (black). λ_{Ex} = 360 nm.

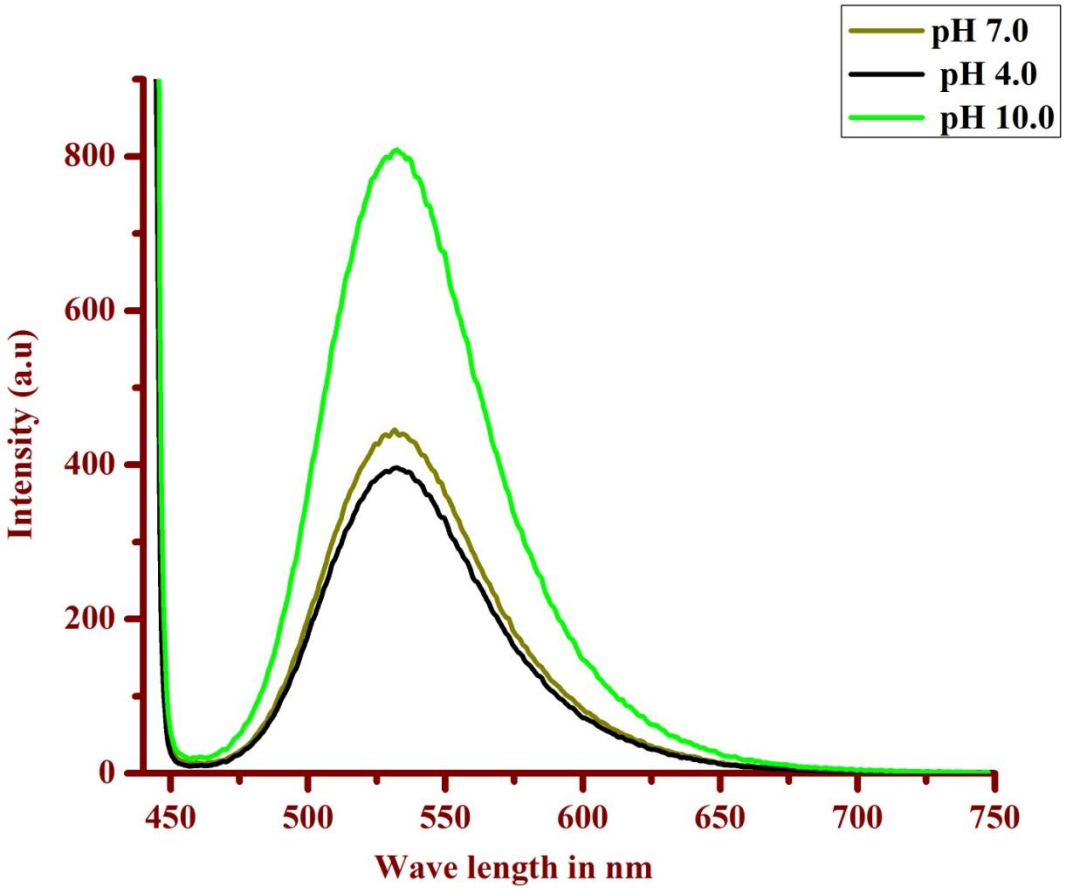

Figure S30. Emission spectra of **APC** at different pH: pH 10.0 (green), 7.0 (deep green), 4.0 (black). λ_{Ex} = 440 nm.

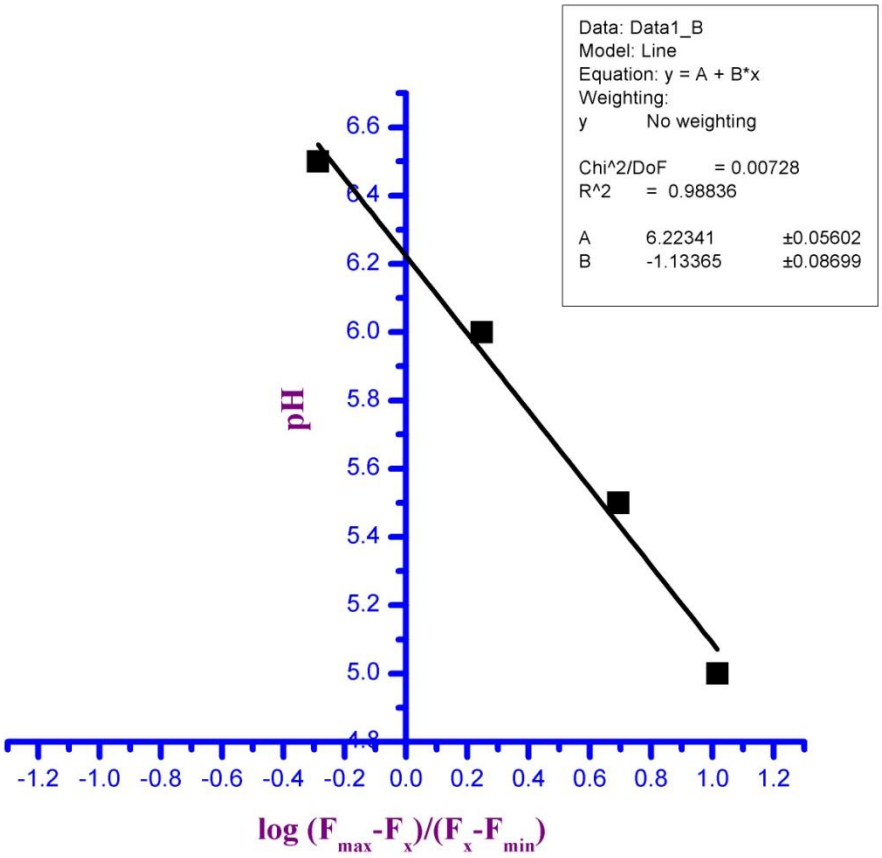

Figure S31. Estimation of $pK_{a\,1}$ of **AAC** from fluorescence experiment using equation (i) (λ_{Em} = 537 nm)

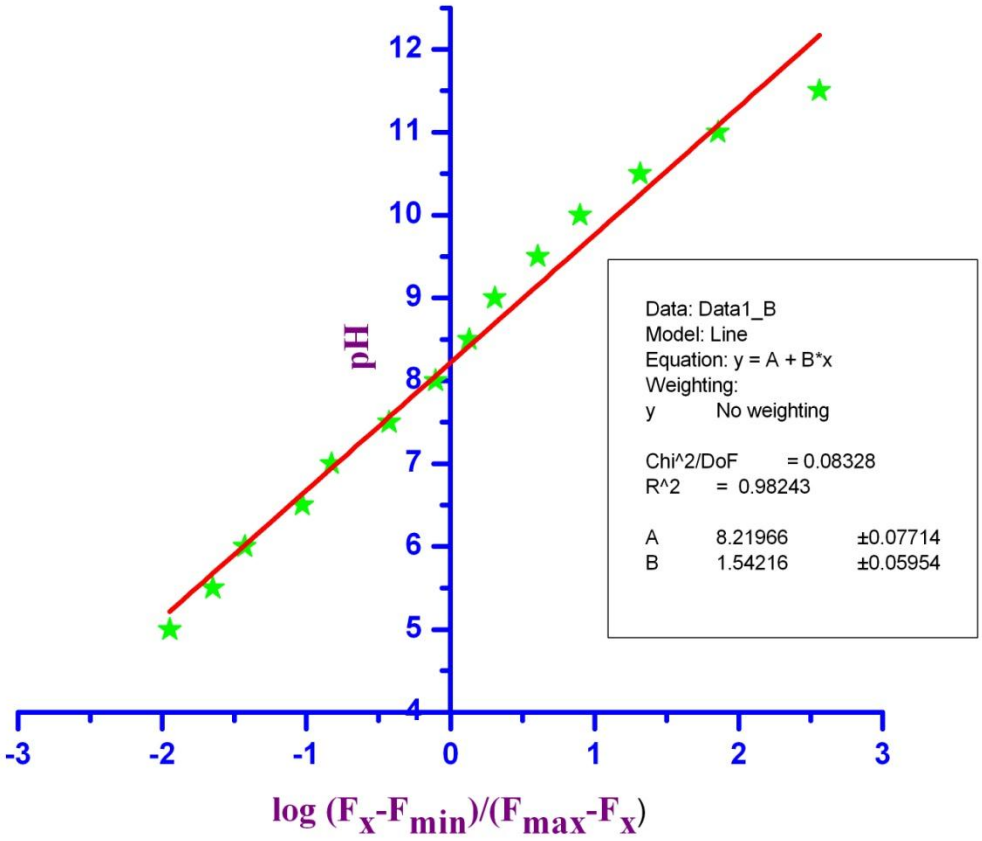

Figure S32. Estimation of pK_{a2} of **AAC** from fluorescence experiment using equation (iii) (λ_{Em} = 537 nm)

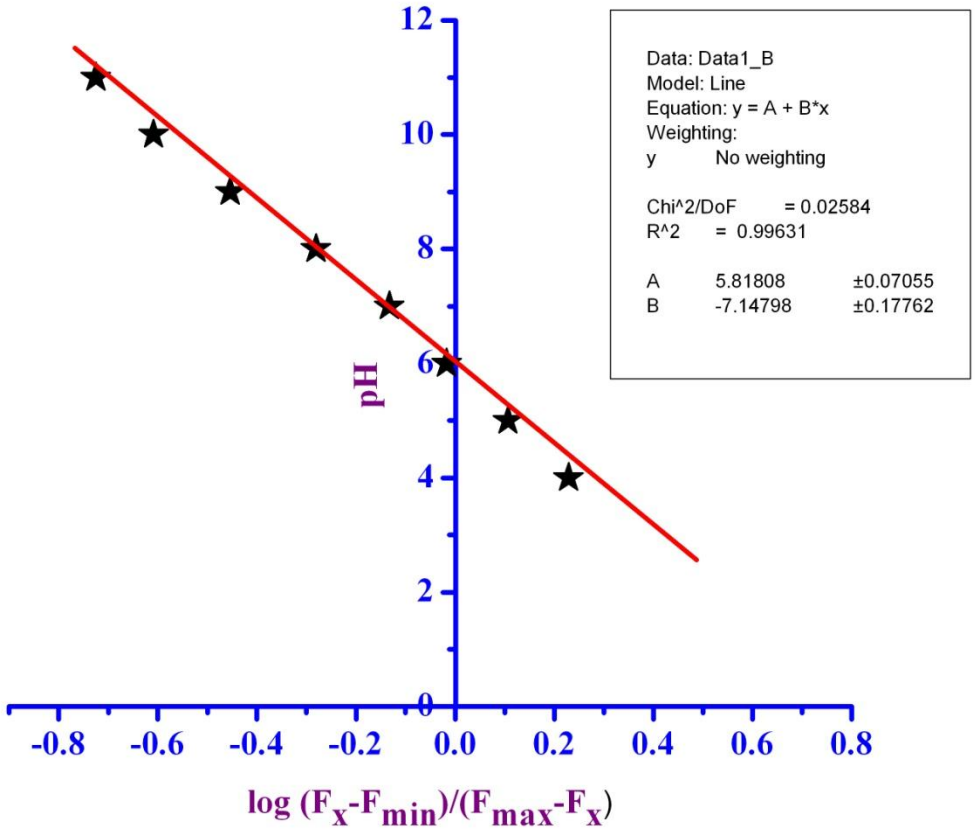

Figure S33. Estimation of pK_{a1} of **ANC** from fluorescence experiment using equation (ii) (λ_{Em} = 600 nm)

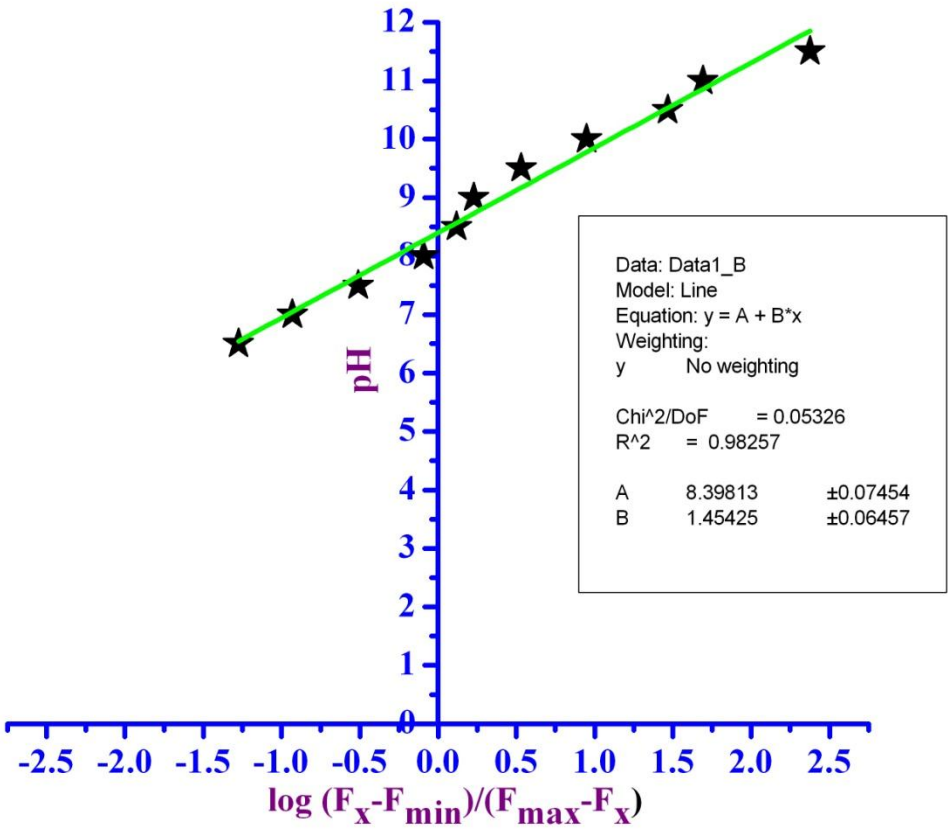

Figure S34. Estimation of pK_{a2} of **ANC** from fluorescence experiment using equation (iii) (λ_{Em} = 535 nm)

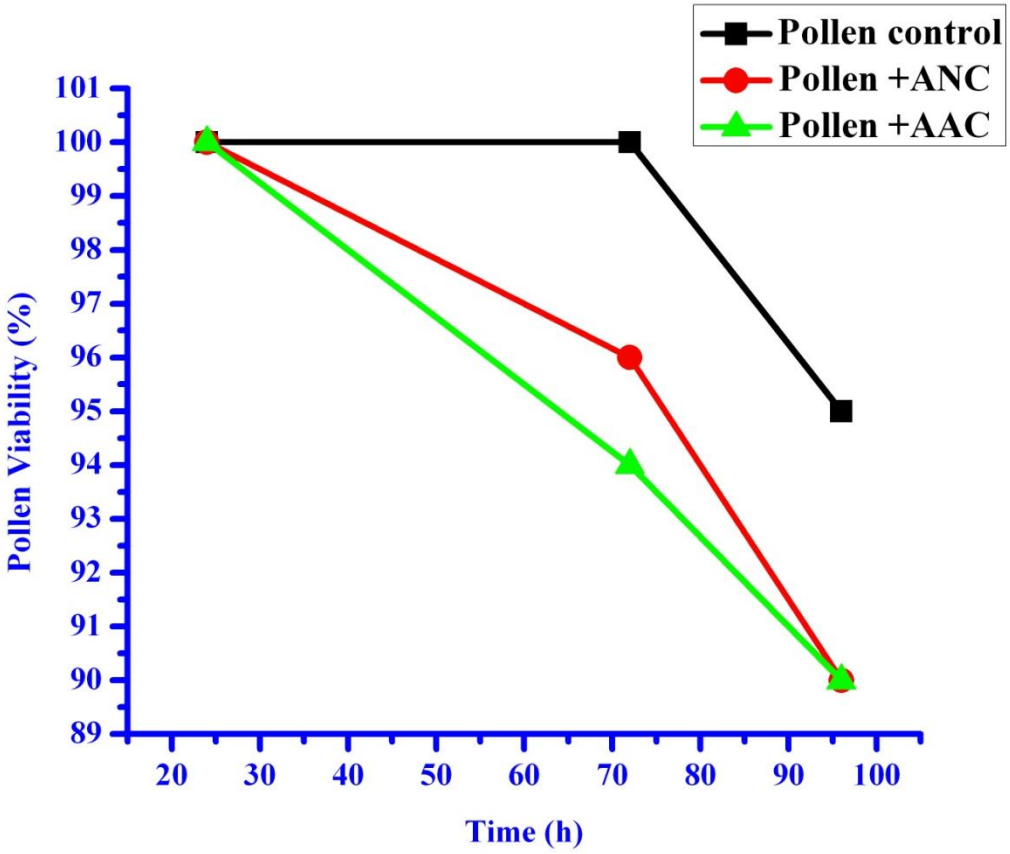

Figure S35. Cell viability graphs: brown line for control, green line for **AAC** and red line for **ANC**

Reference

(1) E. Austin, M. Gouterman, *Bioinorg. Chem.*, 1978, **9**, 281.

(2) W. H. Melhuish, *J. Phys. Chem.*, 1961, **65**, 229.

(3) J. V. Houten, R. J. Watts, *J. Am. Chem. Soc.*, 1976, **98**, 4853.