Supporting information

A New Receptor with a FRET Based Fluorescence Response for Selective Recognition of Fumaric and Maleic Acid in Aqueous Medium

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Experimental Section

Materials:

Dansyl chloride, Hydrazine Monohydrate (98%), 1-Isothiocyanato-naphthalene, Tosyl chloride, Phthalic anhydride were obtained from Sigma Aldrich and were used as received. 2-2'-Diaminodiethylamine was obtained from Koch-Light laboratory in India. Various AR grade mono- and di-carboxylic acids, such as Oxalic acid, Maleic acid, Fumaric acid, Suberic acid, Sebasic acid, Malonic acid, Acetic acid, Glutaric acid, Adipicacid, Citric acid, Malic acid were purchased from SD Fine Chemicals in India.Tetrabutylammonium (TBA) salts of fumaric and maleic acids were prepared following standard procedure and used for further studies. Compound **1, 2** and **A** was synthesized by following the method reported in the literature. Further the reference compound (R) was synthesized following a previously reported methodology. Solvents used for synthesis of various intermediates and final compounds were of AR grade (S.D. Fine Chemicals) and were used as received without further purification. Solvents used for various spectroscopic, and HPLC studies were of HPLC grade (S.D. Fine Chemicals). Solvents were dried, as and when necessary, following standard procedures.

Analytical Methods

^{1}H NMR spectra were recorded on a Bruker 200 MHz FT NMR (Model: Avance-DPX 200) or on a Bruker 500 MHz FT NMR (Model: Avance-DPX 500) using CD_3CN, CD_2Cl_2 and CD_3OD as the solvent and tetra methyl silane (TMS) as an internal standard. ESI-Ms measurements were carried out on a Waters QTof-Micro instrument. Electronic spectra were recorded with a Shimadzu UV-3101 PC spectrophotometer; while fluorescence spectra were recorded using an Edinburgh instrument Xe 900 spectrofluorometer.

General experimental procedure for UV-Vis and Fluorescence studies

1.0×10^{-4}M, 1.0×10^{-3} M solution of the **A** and1.0×10^{-4} M solution of **B** in aq. HEPES buffer acetonitrile (1:1 (v/v); pH 7.4 and 6) and acetonitrile medium was prepared and stored in dark. This solution was used for all spectroscopic studies after appropriate dilution. 1.0×10^{-2} M of diacid solutions was prepared in 10 mM HEPES buffer (pH 6.0): acetonitrile (1: 1, v/v). Solution of the compound A was further diluted for spectroscopic titrations, and the effective final concentration of the solution of compound **A** used for the fluorescence study was 2.0×10^{-5} M, while the final analyte concentration during emission spectral scanning was 4.0×10^{-3} M. For all luminescence measurements, $\lambda_{Ext} = 290$ nm with an emission slit width of 2 nm. The relative fluorescence quantum yields (ϕ_f) were estimated using equation 1 by using the integrated emission intensity of dansyl amide ($\phi_f = 0.37$)for **A** and naphthalene($\phi_f = 0.23$) for **B** as a reference.

$$\phi_f = \phi_f{'} (I_{sample}/I_{std})(A_{std}/A_{sample})(\eta^2_{sample}/\eta^2_{std}) \qquad \text{Eq. 1}$$

Where, $\phi_f{'}$ is the absolute quantum yield for the dansylamide and naphthalene used as references; I_{sample} and I_{std} are the integrated emission intensities; A_{sample} and A_{std} are the absorbances at the excitation wavelength, and η_{sample} and η_{std} are the refractive indices.

Energy transfer efficienc (Φ_{ET}) was evaluated using the expression shown in Eq. 2,

$$\Phi_{ET} = 1-(F{'}_D/F_D) \qquad \text{Eq. 2}$$

where $F{'}_D$ and F_D denote the donor fluorescence intensity with and without an acceptor, respectively.

General experimental procedure for ^{1}H NMR

2.83 x 10^{-3} M solution of the reagent **A** in CD_3CN (500 μL) was taken in an NMR tube. Complexation studies with maleate and fumarate, both as their tetrabutylammonium (TBA) salts, were studied by ^{1}H NMR technique. Concentration for the stock solutions of both fumarate (in CD_3CN: DMSO; 99:1 (v/v)) and maleate (in CD_3CN) ions were maintained at 150 x 10^{-3} M. ^{1}H NMR spectra of the guest molecule (**A**) were recorded with increasing concentration of the host ions (fumarate and maleate ions). Such addition was continued until no further change in the chemical shifts was observed.

HPLC Instrument & Methodology

Apple juice was analysed by HPLC (Waters Alliance model with Waters 2996 Photo diode array Detector (PDA)) using the Supelco Gel 610-H Column (Length: 30 cm, ID: 7.8 mm), in conjugation with a column heating device at 30ºC. Solutions with known [Fumaric acid] was analysed by HPLC method under the identical conditions. Elution was carried out with Water (100%) – contain 0.1 % of Phosphoric acid at a flow rate of 0.5 ml/min for 30 min. The mobile phase was filtered through 0.2 μM PVDF membrane filter and degassed before use. Detection was performed with Photo diode array Detector (PDA). Calibration standard solution of fumaric acid was freshly prepared at concentrations of 0.78, 1.56, 3.125, 6.25, 12.5, 25 and 50ppm in distilled water. The elution was monitored using an UV-detector at 210 nm and for each standard fumaric acid solution injection volume was 20 μL to get the standard plot for obtaining the calibration plot, while that for commercial apple juice was 40 μL,. The standard curve for fumaric acid passed virtually through the origin and a good linear fit (R^2 = 0.999) was obtained.

Synthesis:
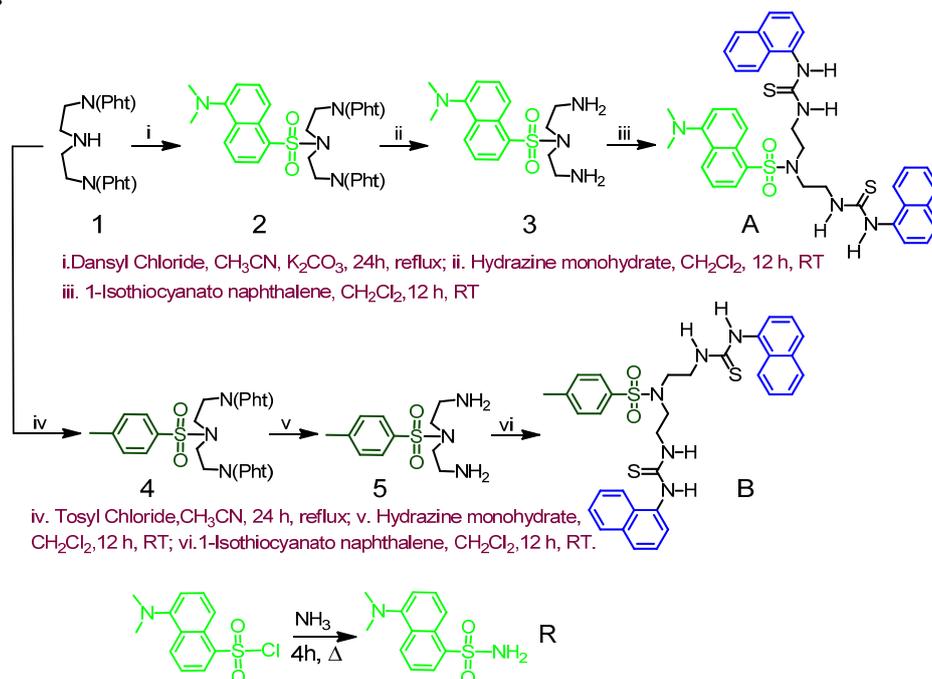
Scheme 1 Methodologies that were adopted for synthesis of **A**, **B** and **R**.

Synthesis of 2: Compound **1** (700 mg, 1.92 mmol) and dansyl chloride (647 mg, 2.40 mmol) were dissolved in 50 ml dry acetonitrile. To this solution, Na_2CO_3 (407 mg, 3.84 mmol) was added and allowed to reflux for 24 h. Then the reaction mixture was filtered to separate out Na_2CO_3. Filtrate was completely evaporated and redissolved in minimum volume of dichloromethane. To this solution, 20 mL of ethanol was added to precipitate the desired compound. This was collected through filtration and was washed further with ethanol. This isolated light yellow compound was further dried over P_2O_5 in a dissector. Yield: 550 mg, 48%. ESI-Ms (m/z) calculated for $C_{32}H_{28}N_4O_6S$: 596, observed: 597 [M + H$^+$]. ^{1}H NMR [500 MHz, CDCl$_3$: δ (ppm)]: 8.16 (1H, d, J = 8.5 Hz, ArH); 8.12 (1H, d, J = 7.5 Hz, ArH); 7.76 (1H, d, J = 8.5 Hz, ArH); 7.65-7.60(8H, m, ArH); 7.38 (1H, t, J = 8.0 Hz, ArH); 7.22 (1H, t, J = 8.0 Hz, ArH); 6.18 (1H, d, J = 7.5 Hz, ArH); 3.94 (4H, t, J = 5.0 Hz, CH$_2$); 3.88(4H, t, J = 5.0 Hz, CH$_2$); 2.70(6H, s, CH$_3$).

Synthesis of 3: To a dichloromethane solution of compound **2** (500mg, 0.84mmol), hydrazine monohydrate (16.8 mmol) was added. Reaction mixture was stirred for 12 h at

room temperature, while a white precipitate was formed. Precipitate was removed through filtration using a G_4 sinter crucible. Filtrate was collected and evaporated under the vacuum. Solid residue was redissolved in dichloromethane and undesired impurities were subsequently extracted is aqueous layer. The organic layer was collected and dried over anhydrous Na_2SO_4 and evaporated in vacuum. A sticky pale green compound was obtained. Yield: 250 mg; 89 %.ESI-Ms (m/z) calculated for $C_{16}H_{24}N_4 O_2S$: 336, observed: 337 [M + H^+].^{1}H NMR [500 MHz, $CDCl_3$: δ (ppm)]. 8.53 (1H, d, J = 8.5 Hz, ArH); 8.30(1H, d, J = 8.5 Hz, ArH); 8.15 (1H, d, J = 7.5 Hz, ArH); 7.56-7.49 (2H, m, ArH); 7.17 (1H, d, J = 8 Hz, ArH); 3.38(4H, t, J = 6 Hz, CH_2); 2.91 (4H, t, J = 6Hz, CH_2); 2.87 (6H, s, CH_3).

Synthesis of A: Compound **3** (130 mg, 0.39 mmol) and 1-isothiocyanato naphthalene (144 mg, 0.78 mmol) were dissolved in 10 ml dichloromethane and stirred at room temperature for 24 h. Progress of the reaction was monitored by checking the TLC and stopped when no further change was observed. Volume of the reaction mixture was reduced to 5 ml under reduced pressure. To this, diethyl ether was added to precipitate the desired compound. White solid compound was isolated through filtration and was further washed with cold dichloromethane. Yield: 180mg, 66 %. ESI-Ms(m/z) calculated for $C_{38}H_{38}N_6 O_2S_3$: 706, observed: 707 [M + H^+]. ^{1}H NMR [500 MHz, $CDCl_3$: δ (ppm)]: 8.47 (1H, d, J = 8.0 Hz, ArH); 7.98 (1H, d, J = 8.5 Hz, ArH); 7.98 (2H, d, J = 5.0 Hz, ArH); 7.88-7.86 (4H, m, ArH); 7.82 (1H, d, J = 7.0 Hz, ArH); 7.76 (2H, s, NH); 7.54-7.53 (4H, m, ArH); 7.48 (2H, t, J = 7.5 Hz, ArH); 7.42-7.35 (4H, m, ArH); 7.08 (1H, d, J = 7.0 Hz, ArH); 6.28(2H, s, NH); 3.65 (4H, t, J = 8.0 Hz,CH_2); 3.39 (4H, t, J = 8.0 Hz, CH_2); 2.84 (6H,s,CH_3).^{13}C NMR (500 MHz, CD3CN+ 1% DMS0-d_6: δ (ppm)]: 183.72, 152.64, 135.71, 135.34, 134.96, 131.18, 130.70, 130.03, 129.14, 128.31, 127.45, 126.62, 124.57, 123.91, 120.17, 116.23, 47.90, 45.74, 43.81. Elemental analysis (in %): Calculated for $C_{37}H_{40}N_6O_2S_3$: C, 63.76; H, 5.79; N, 12.06; S, 13.80 and Observed: C, 63.55; H, 5.75; N, 12.0; S, 13.63

Synthesis of 4: Synthetic procedure that was adopted for synthesis of **4** was similar to that of **2**, except 4-toluene sulphonyl chloride (104 mg, 0.55 mmol) was used for the reaction instead of dansylchloride. Similar workup procedure was also followed for isolating the desired white solid compound **5**. Yield: 140 mg, 50 %. ESI-Ms (m/z) calculated for $C_{27}H_{23}N_3O_6S$: 517, observed: 556 [M + K$^+$]. ^{1}H NMR [200 MHz, CDCl$_3$: δ (ppm)]:9.71 (8H, m, ArH); 9.38 (2H, d, J = 7.6Hz, ArH); 8.86 (2H, d, J = 7.8 Hz, ArH); 5.83 (4H, t, J = 5.6 Hz, CH$_2$); 5.63 (4H, t,J = 5.6 Hz, CH$_2$);4.06 (3H, s,CH$_3$).

Synthesis of 5: Synthetic procedure that was adopted for synthesis of **5** was similar to that of **3**, except **4** (150 mg, 0.3 mmol) was used for the reaction instead of the compound **2**. After following the similar work-up procedure a white sticky compound was isolated. Yield: 40 mg, 58 %. ESI-Ms (m/z) calculated for $C_{11}H_{19}N_3O_2S$: 257, observed: 258[M + H$^+$]. ^{1}H NMR [200 MHz, CDCl$_3$: δ (ppm)]:9.67 (2H, d, J = 7.8 Hz, ArH); 9.28 (2H, d, J = 7.4 Hz, ArH); 5.08 (4H, t, J = 12 Hz, CH$_2$); 4.82 (4H, t, J = 12 Hz, CH$_2$); 4.39 (3H, s, CH$_3$).

Synthesis of B: Synthetic procedure that was adopted for synthesis of **B** was similar to that of **A**, except **5** (40 mg, 0.15 mmol) and accordingly 57 mg (0.31 mmol) of 1-isothiocyanato naphthalene were used for the reaction. After following the similar work-up procedure, as it was adopted for **4**, desired compound **B** was isolated as a white solid compound. Yield: 17 mg, 18 %. ESI-Ms (m/z) calculated for $C_{33}H_{33}N_5O_2S_3$: 627, observed: 628 [M + H$^+$]. ^{1}H NMR [500 MHz, CD$_2$Cl$_2$: δ (ppm)]. 7.95(2H, d, J = 7.5 Hz, ArH); 7.92-7.87(4H, m, ArH); 7.83 (2H, s, NH); 7.58-7.53(4H, m, ArH);7.51-7.47 (4H, m, ArH); 7.42(2H, d, J = 10 Hz);6.33 (2H, s, NH); 3.66 (4H, t,J = 10 Hz, CH$_2$); 3.19 (4H, t, J = 10 Hz, CH$_2$); 2.37(3H, s, CH$_3$).^{13}C NMR (500 MHz, CDCl3, δ (ppm)]: 182.04, 143.81, 134.97, 134.64, 131.36, 129.88, 129.03, 128.55, 127.38, 127.03, 126.91, 125.89, 122.36, 48.67, 44.43, 29.70, 21.50. Elemental analysis (in %): Calculated for $C_{33}H_{33}N_5O_2S_3$: C, 63.13; H, 5.30; N, 11.15; S, 15.32 and Observed: C, 63.1; H, 5.28; N, 11.08; S, 15.25.
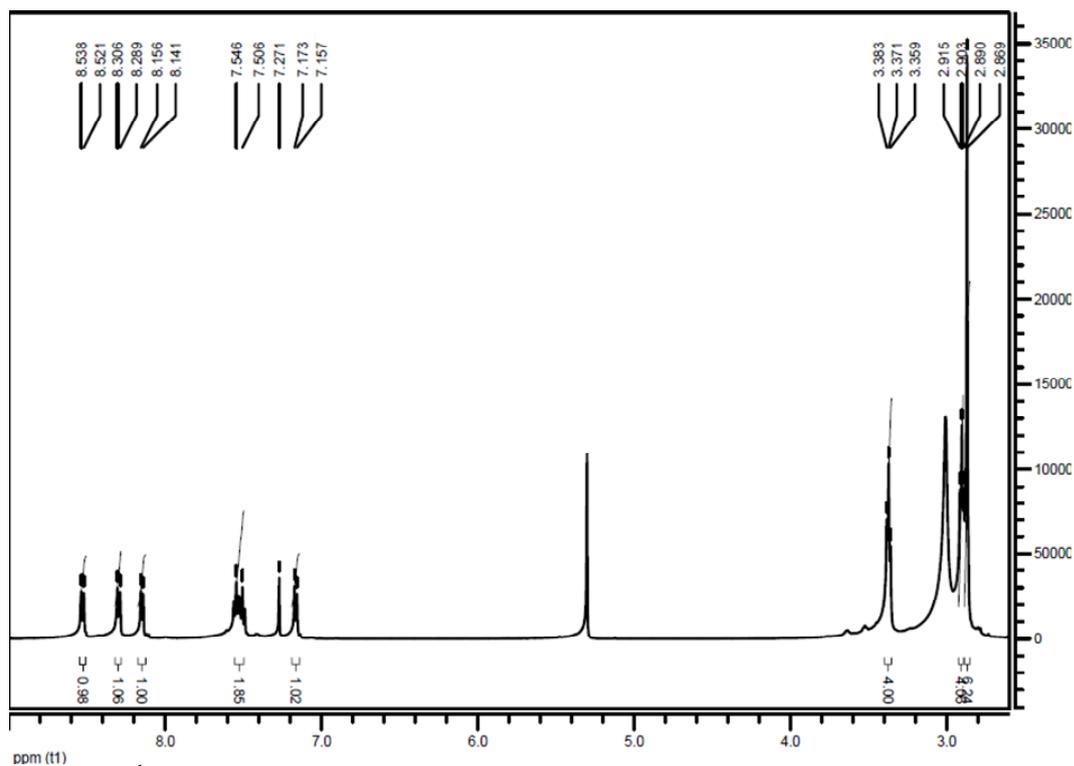
8
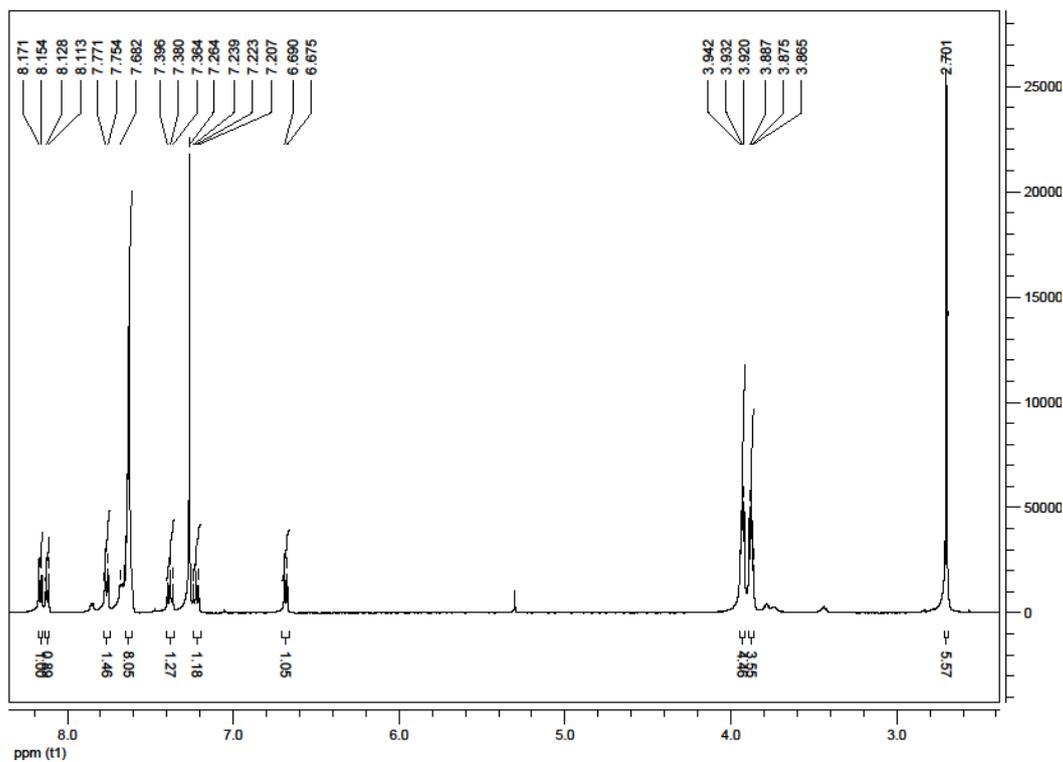
^{1}H NMR spectra of 2

SI Figure 1: ^{1}H NMR spectra of **2** in CDCl$_3$ medium.
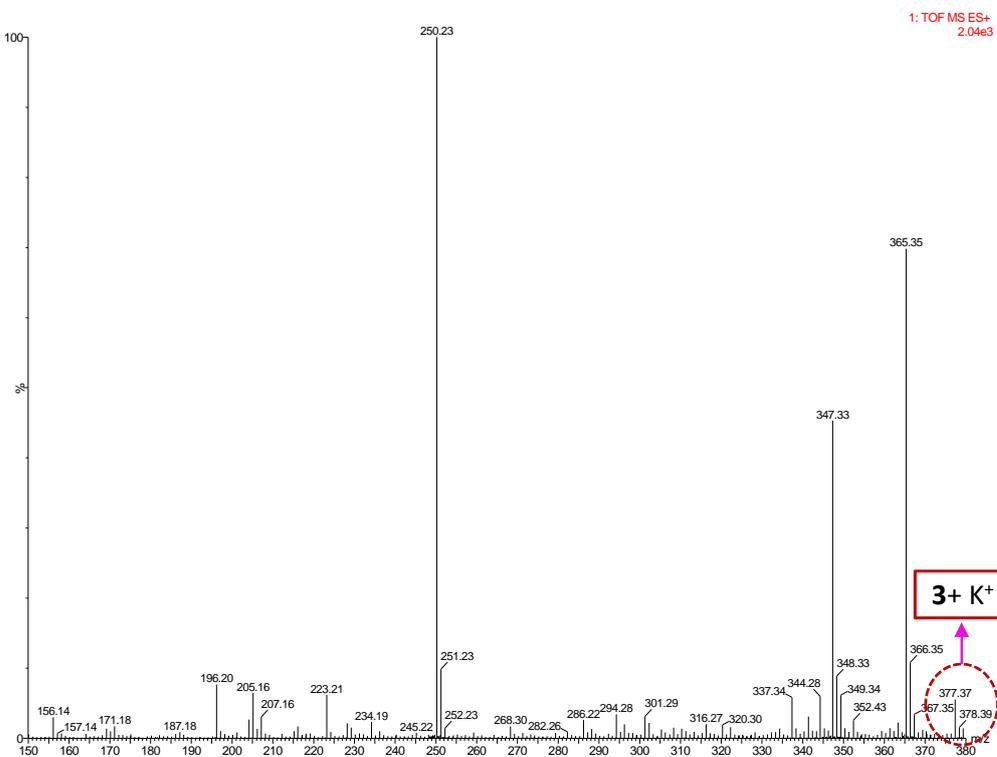
Mass spectra of 2
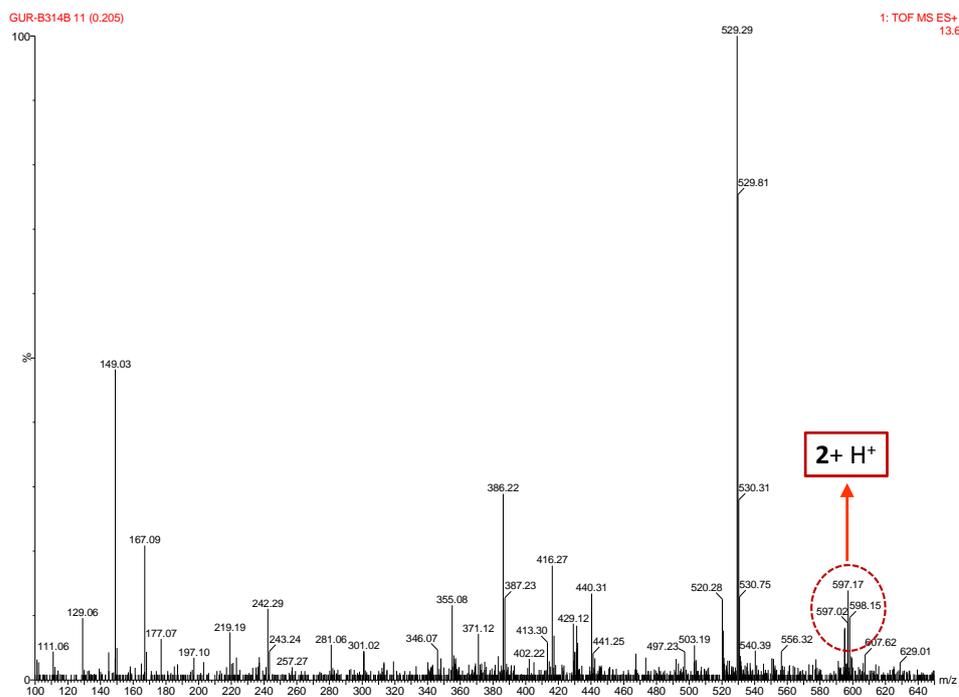
SI Figure 2: ESI- mass spectra of **2**.

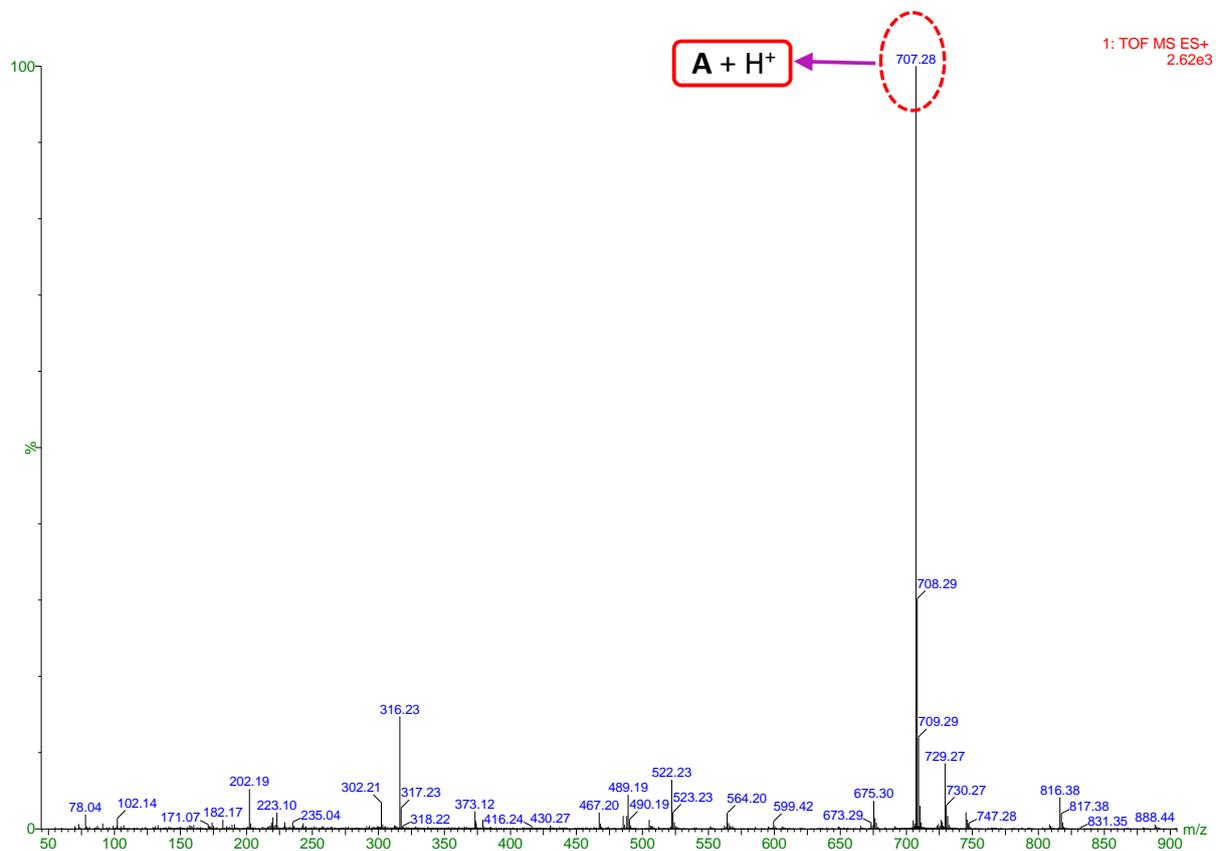## ^{1}H NMR spectra of 3

SI Figure 3: ^{1}H NMR spectra of **3** in CDCl$_3$ medium.
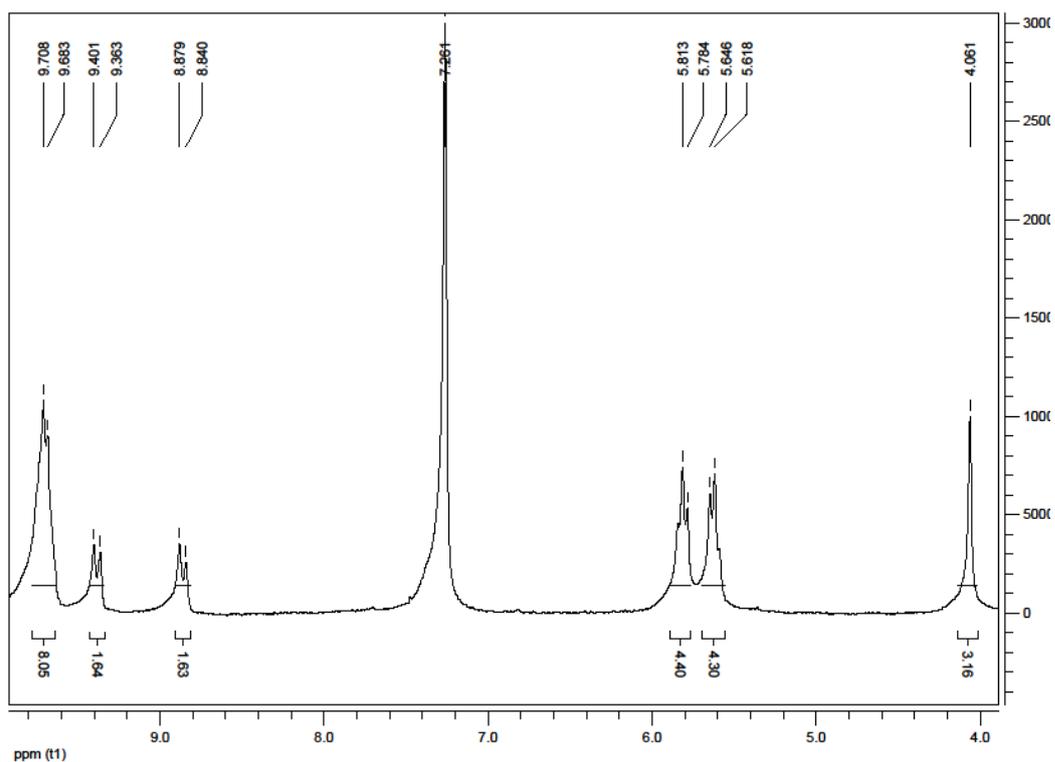
Mass spectra of 3

SI Figure 4: ESI- mass spectra of **3**.

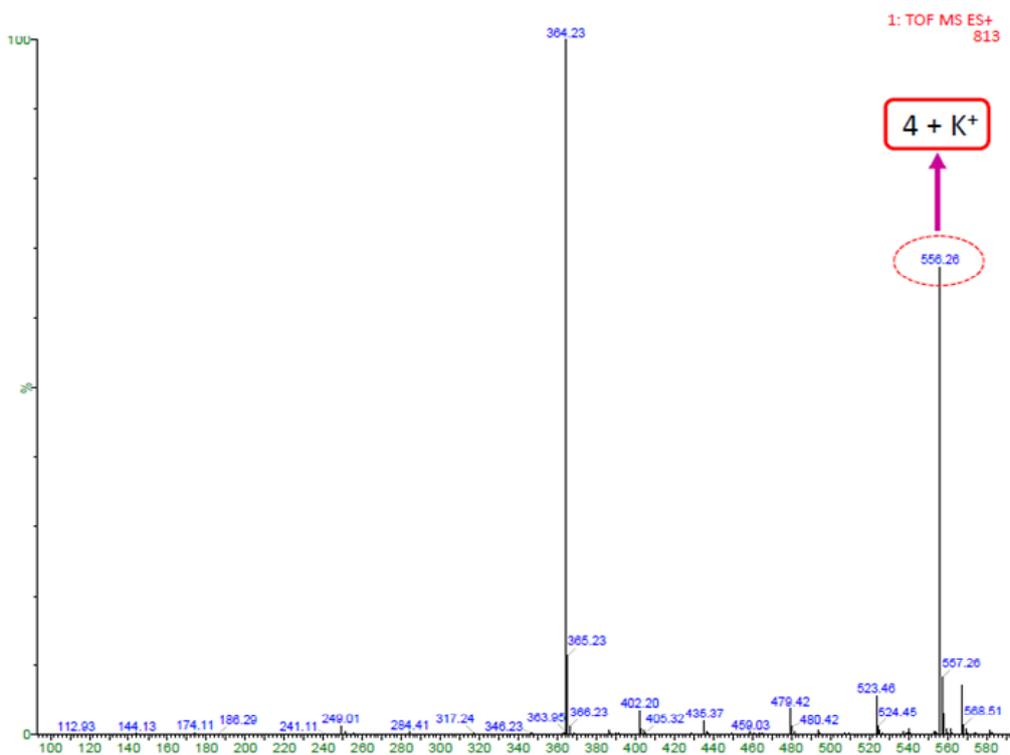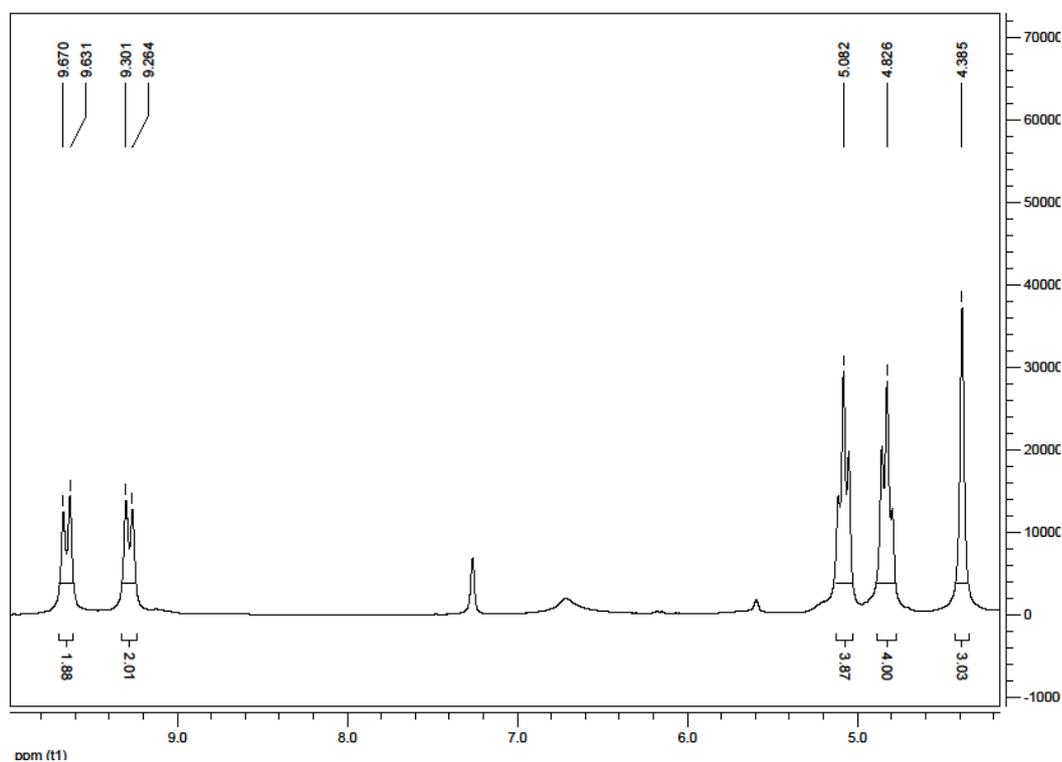## ^{1}H NMR spectra of A

SI Figure 5: ^{1}H NMR spectra of **A** in CDCl$_3$ medium.

^{13}C NMR spectra of A

SI Figure 6: ^{13}C NMR spectra of **4** in CD$_3$CN having 1% DMSO-d$_6$.

Mass spectra of A

SI Figure 7: ESI- mass spectra of **A**.

^{1}H NMR spectra of 4

SI Figure 8: ^{1}H NMR spectra of **4** in CDCl$_3$ medium.
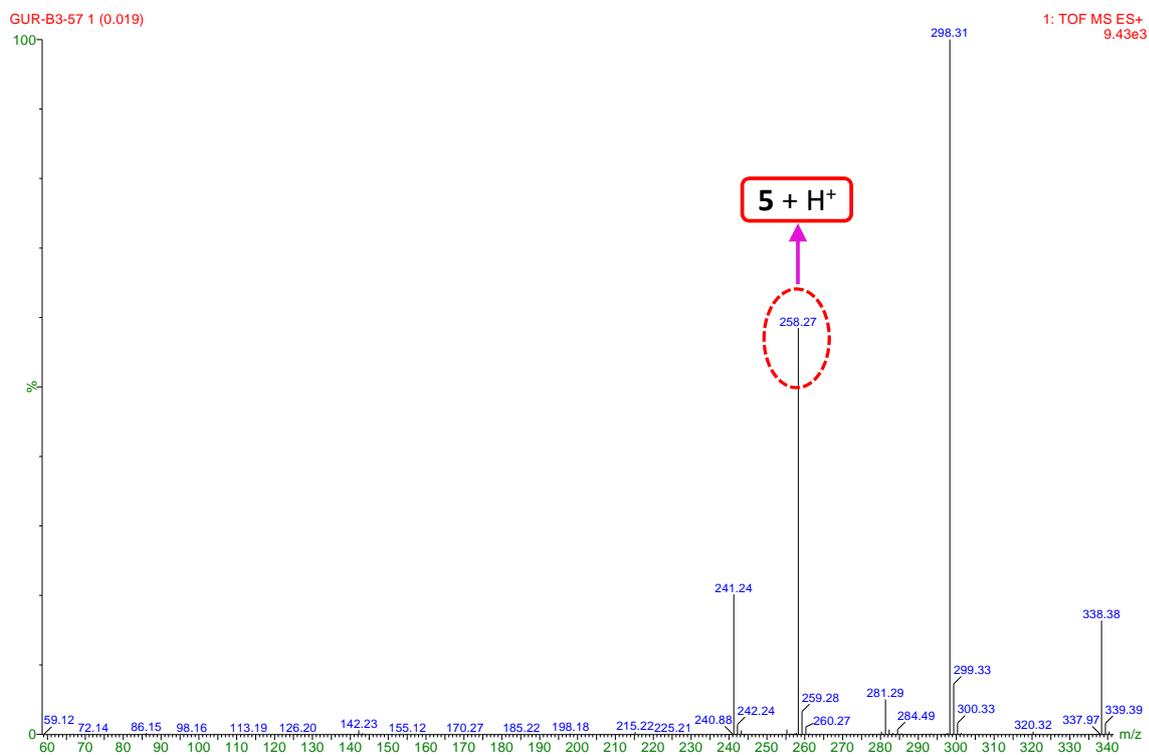
Mass spectra of 4

SI Figure 9: ESI- mass spectra of **4.**
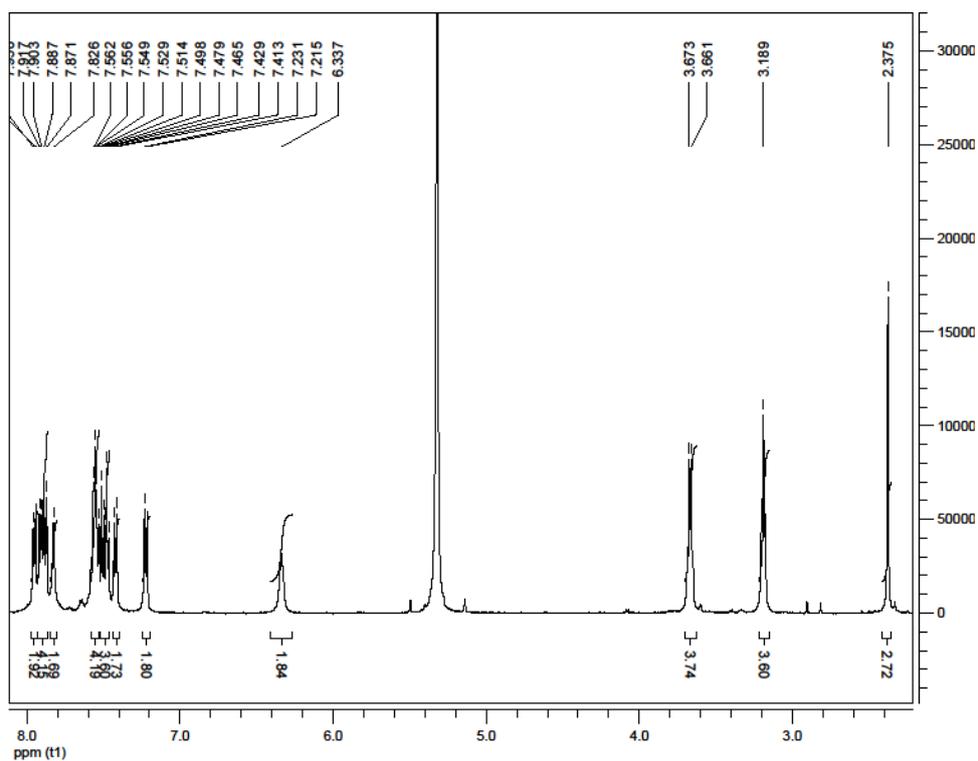
^{1}H NMR spectra of 5

SI Figure 10: ^{1}H NMR spectra of **5** in CDCl$_3$ medium.
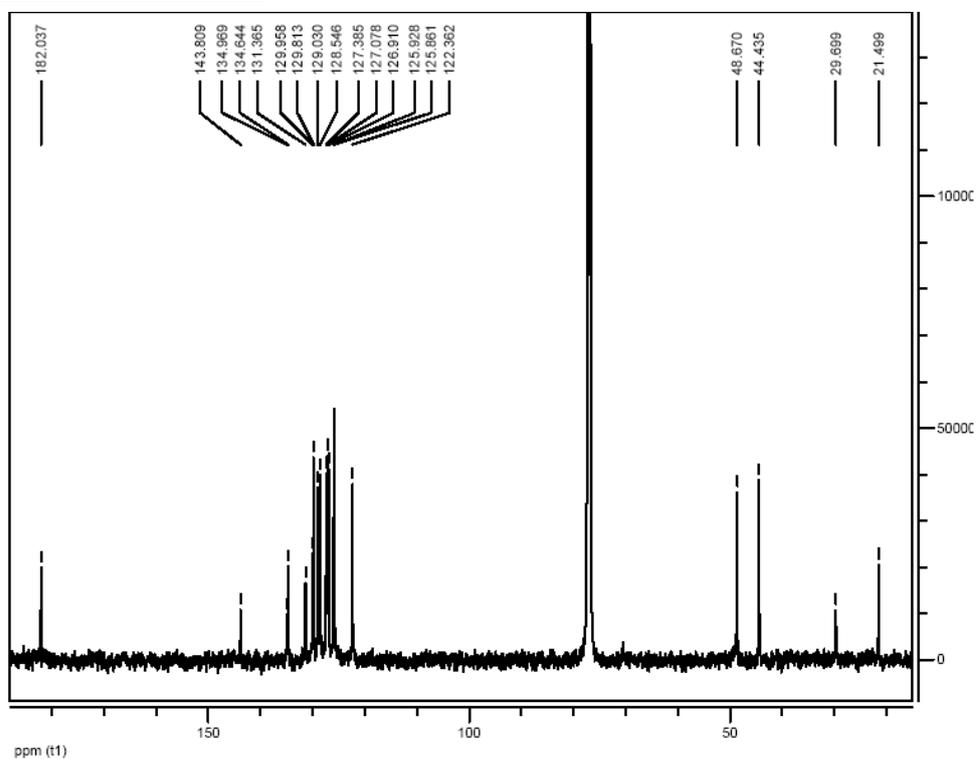
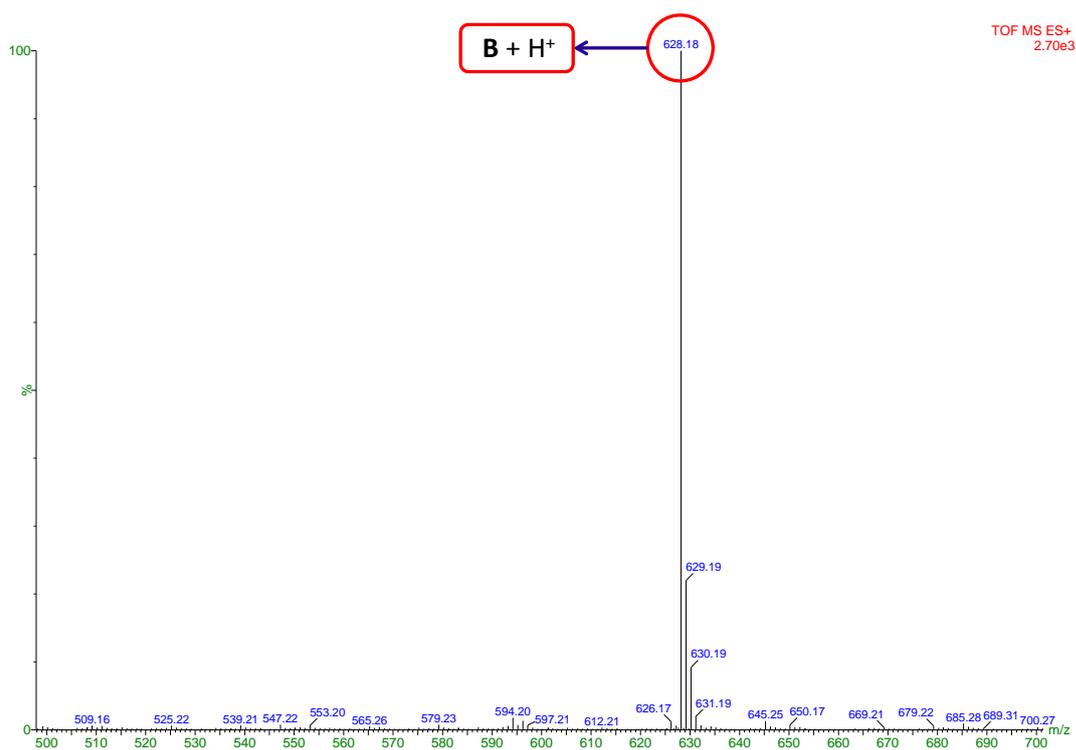**Mass spectra of 5**

SI Figure 11: ESI- mass spectra of **5.**

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^{1}H NMR spectra of B

SI Figure 12: ^{1}H NMR spectra of **B** in CD_2Cl_2 medium.
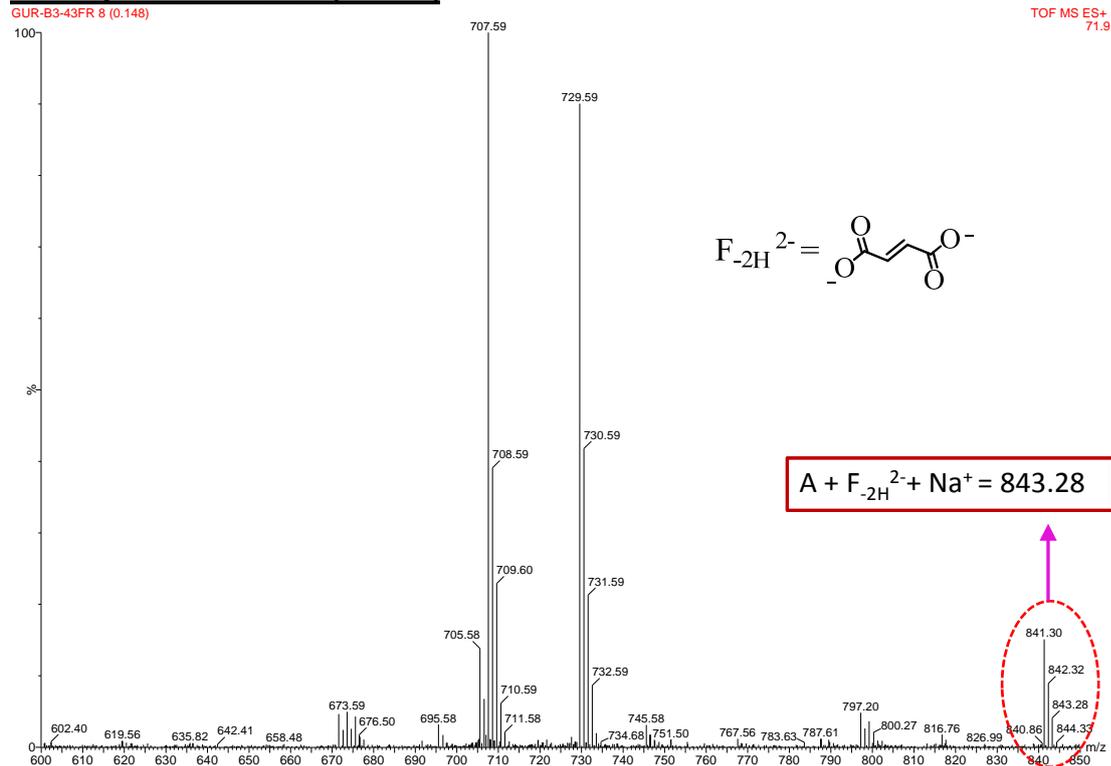
^{13}C NMR spectra of B

SI Figure 13: ^{13}C NMR spectra of **B** in $CDCl_3$ medium.

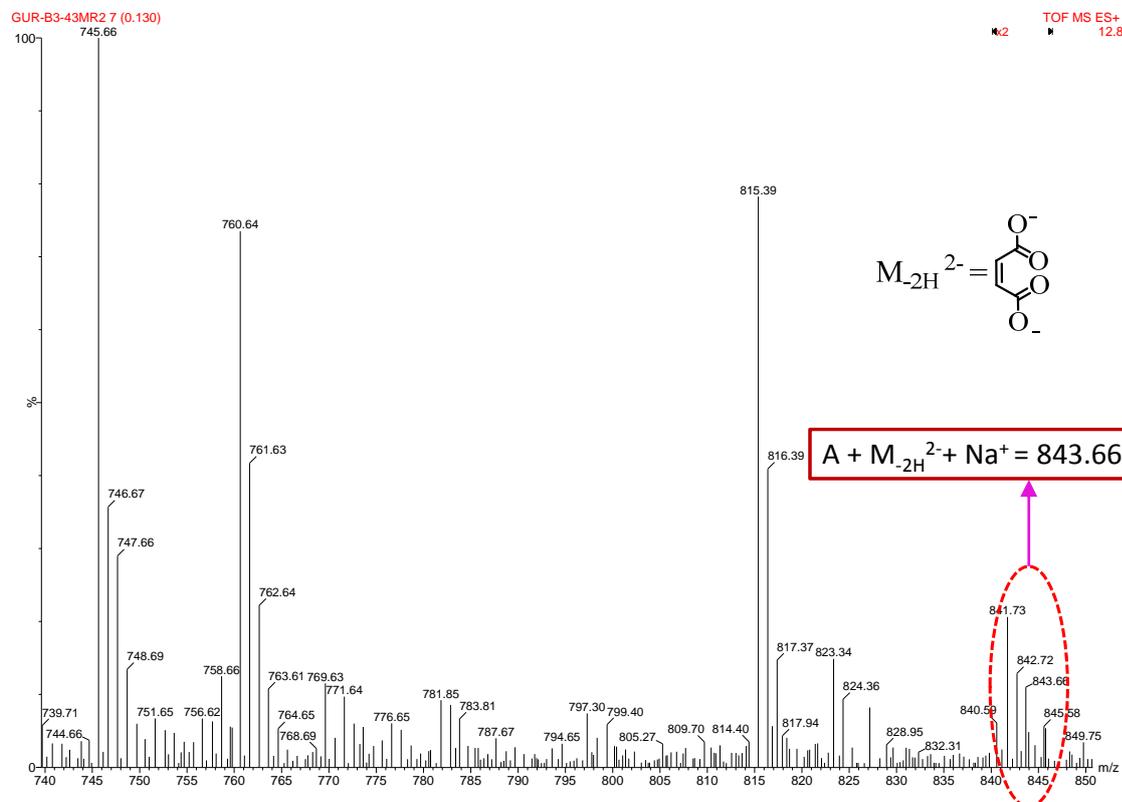**Mass spectra of B**

SI Figure 14: ESI-Ms spectra of **B.**
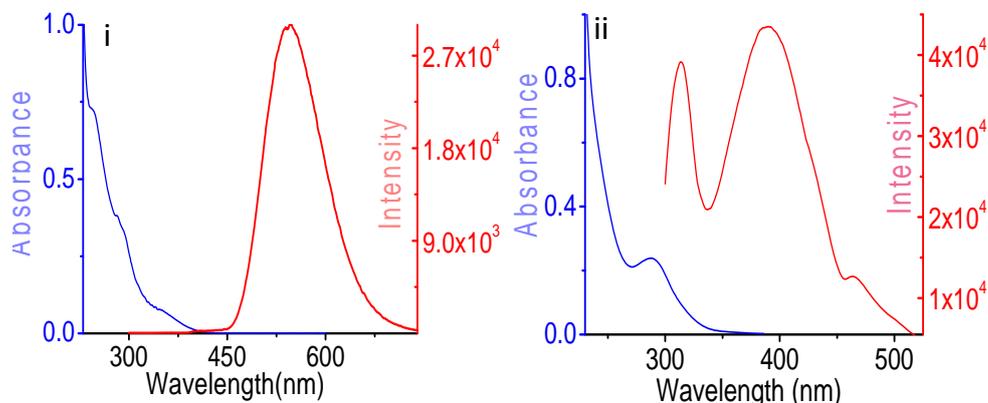
Mass spectra of A with [TBAF]

SI Figure 15: ESI- mass spectra of **A** with [TBAF].

Mass spectra of A with [TBAM]

SI Figure 16: ESI- mass spectra of **A** with [TBAM].

Electronic and Emission spectra of A and B

SI Figure 17: Electronic and luminescence (λ_{Ext} = 290 nm) spectra recorded for 2.0 x 10^{-5} M solution of (i) **A** (slit width: 2/2 nm) and (ii) **B** (slit width: 3/3 nm); All spectra were recorded in 10 mM aq. HEPES buffer: acetonitrile (1: 1 (v/v); pH 7.4) medium.

Emission spectra of R and Overlap spectra of B and R
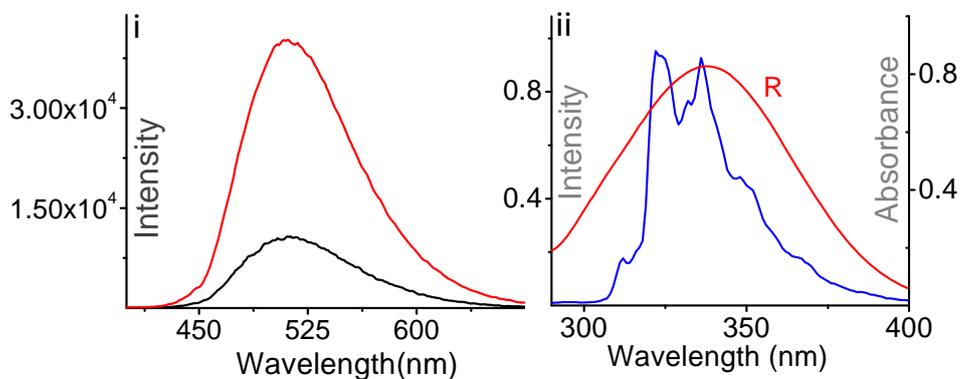
SI Figure 18: (i) Emission spectra for R following excitation at (red) 355and (black) 290 nm. (ii) Spectra showing the strong overlap between the emission spectra of naphthalene and Uv-vis spectra of R; all spectra were recorded in 10 mM aq. HEPES buffer: acetonitrile (1: 1 (v/v); pH 7.4) medium.

Emission response of B with Fumaric and Maleic acid
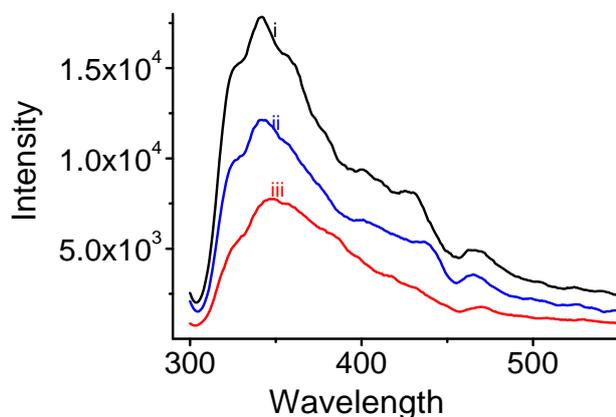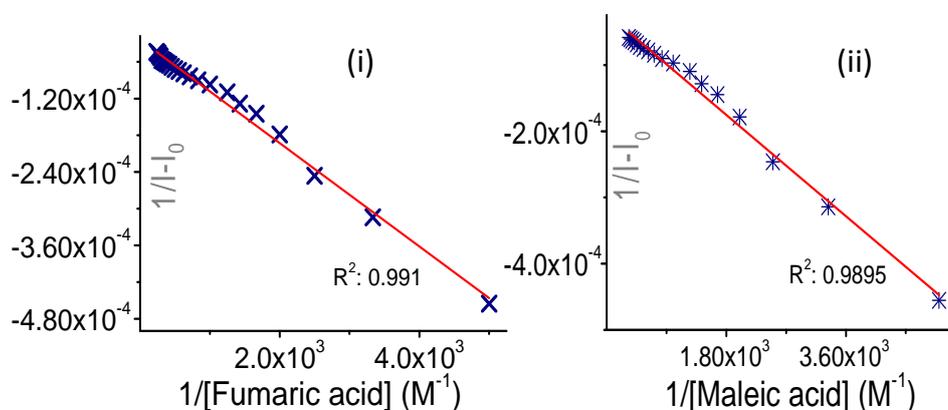

SI Figure 19: Luminescence response of (i) B, (ii) B with Maleic acid (iii) B with Fumaric acid was recorded in 10 mM aq. HEPES buffer: acetonitrile (1: 1, v/v; pH 6.0):) medium. λ_{Ext} = 290 nm.

Benesi-Hildebrand plot for binding studies of Fumaric and Maleic acids towards A

SI Figure 20: Figures i and ii show the respective Benesi-Hildebrand plot for fumaric and maleic acids. For both B-H plots, good linear fit confirms the 1:1 binding stoichiometry. For all studies λ_{Ext}= 290 nm, λ_{Mon}= 542 nm with slit width: 2 nm was used. All measurements were performed in 10 mM aq. HEPES buffer: acetonitrile (1:1, v/v; pH 6.0) medium.

Partial ^{1}H NMR spectra of A with [TBAM]
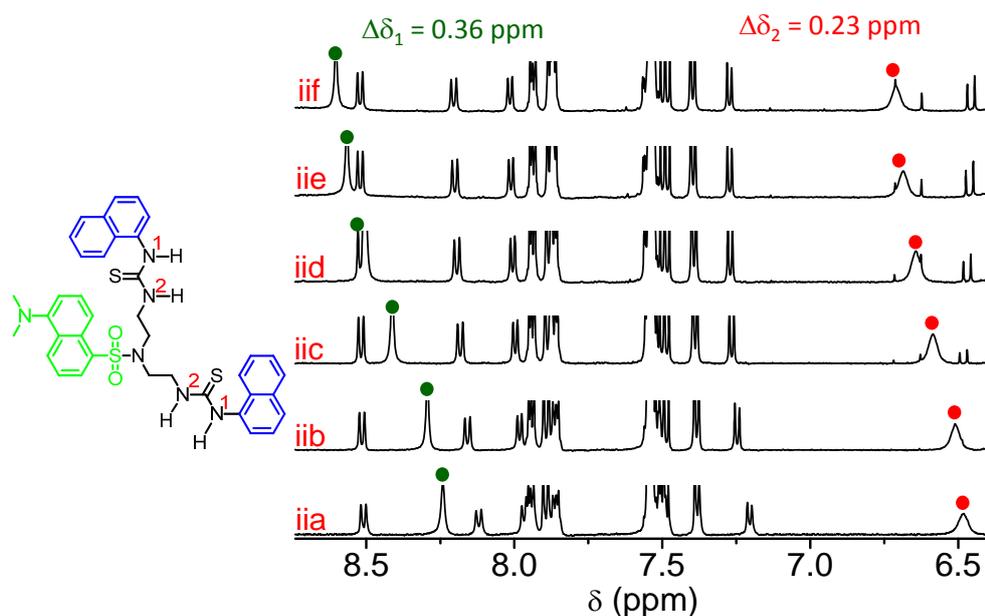
SI Figure 21: Partial ^{1}H NMR spectra of **A** (2.83 mM) in absence and presence of varying [TBAM] in CD$_3$CN medium.

^{1}HNMR titration of probe A with [TBAF] and [TBAM]
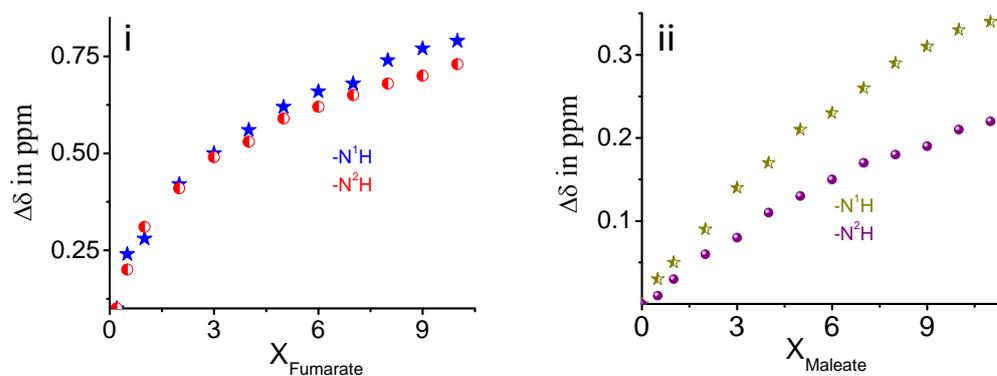
SI Figure 22: Changes in chemical shift values of thiourea protons in probe molecule **A** with varying no. of equivalents of (i) TBAF and (ii) TBAM.

Computational methods: Conformational searches for probe **A** was performed using the Monte Carlo algorithm for the random variation of all of the rotatable bonds combined with Semi empirical 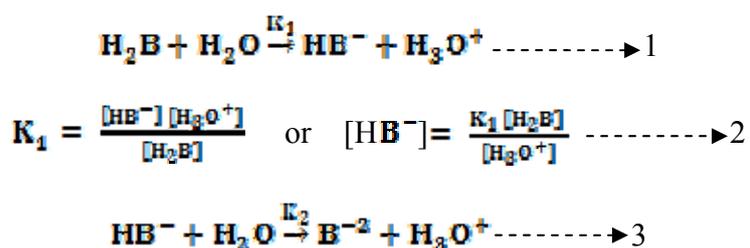PM3 in Spartan 06.[1] For each calculation, 5000 Monte Carlo steps were carried out. Lowest energy conformers were taken for the energetic comparisons with semi empirical calculations. All the geometries of probe **A** were fully optimized at the PM3 level[2] of theory using the Gaussian 03 program.[3] The lowest energy conformer is taken for the complexation study. We have also optimized fumaric and maleic acids (in neutral, mono and bis deprotonated form) at PM3 level of theory in gas phase. The optimized geometries were checked with imaginary frequencies to identify the ground-state minimum. Next, we have taken probe **A** for complexation with diastereomeric diacids (fumaric and maleic acid) in neutral, mono and bisdeprotonated form optimised at PM3 level of theory in gas phase. Single-point calculations were performed for these complexes at the B3LYP/6-31G* level[4] using the PM3 level optimized complex geometries for more accurate estimation of the energies. The binding energies were computed using the equation, $\Delta E = \Delta E_{complex} - (\Delta E_{probeA} + \Delta E^{diacid/mono/bisdeprotonated})$.

Determination of the composition of Fumaric and Maleic acid as a function of pH

In order to find the effect of pH of the solvent on the respective acids we could evaluate the fractional distribution curves, which were obtained from experimental pK_a values.[5] The curves explain that the formation of α_1 and α_2 of fumaric and maleic acid at different pH range. The detailed derivation for composition calculations were given below.

Fumaric and maleic acids are the diprotic acids and shows the following equilibrium in aqueous solution.

$$H_2B + H_2O \overset{K_1}{\rightleftharpoons} HB^- + H_3O^+ \quad \text{-------}\rightarrow 1$$

$$K_1 = \frac{[HB^-][H_3O^+]}{[H_2B]} \quad \text{or} \quad [HB^-] = \frac{K_1[H_2B]}{[H_3O^+]} \quad \text{-------}\rightarrow 2$$

$$HB^- + H_2O \overset{K_2}{\rightleftharpoons} B^{-2} + H_3O^+ \quad \text{-------}\rightarrow 3$$

$$K_2 = \frac{[B^{-2}][H_3O^+]}{[HB^-]} \quad \text{or} \quad [B^{-2}] = \frac{K_2[HB^-]}{[H_3O^+]} \quad \text{--------} \rightarrow 4$$

Let the compositions of di acid $= \alpha_0 = \dfrac{[H_2B]}{[H_3O^+]}$

And for the 1st conjugate base $= \alpha_1 = \dfrac{[HB^-]}{[H_3O^+]}$

For the 2nd conjugate base $= \alpha_2 = \dfrac{[B^{2-}]}{[H_3O^+]}$

So the total concentration of the parent acid is $CH_2B = [H_2B] + [HB^-] + [B^{2-}]$

Substituting the value of $[HB^-]$ and $[B^{2-}]$ from equation 2 and 4 we get,

$$CH_2B = [H_2B] + \frac{K_1[H_2B]}{[H_3O^+]} + \frac{K_2[HB^-]}{[H_3O^+]}$$

Substituting value of HB^- in terms of H_2B and Multiplying with $[H_3O^+]^2$ on both sides we get

$$CH_2B[H_3O^+]^2 = [H_2B][H_3O^+]^2 + K_1[H_2B][H_3O^+] + K_1K_2[H_2B]$$

$$\text{or,} \quad \frac{[H_2B]}{CH_2B} = \frac{[H_3O^+]^2}{[H_3O^+]^2 + K_1[H_3O^+] + K_1K_2} = \alpha_0$$

$$\text{or,} \quad \frac{[HB^-]}{CH_2B} = \frac{K_1[H_3O^+]}{[H_3O^+]^2 + K_1[H_3O^+] + K_1K_2} = \alpha_1$$

$$\text{or,} \quad \frac{[B^{2-}]}{CH_2B} = \frac{K_1K_2}{[H_3O^+]^2 + K_1[H_3O^+] + K_1K_2} = \alpha_2$$

The sum of α_0, α_1 and α_2 will be equal to 1.

Respective values for K_1 & K_2 for fumaric and maleic acids are 9.55×10^{-5} & 3.63×10^{-5}

and 1.2×10^{-2} & 8.5×10^{-9} as derived from the respective pK_a from the literature.[6]

References

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Fractional distribution curve for Fumaric and Maleic acids
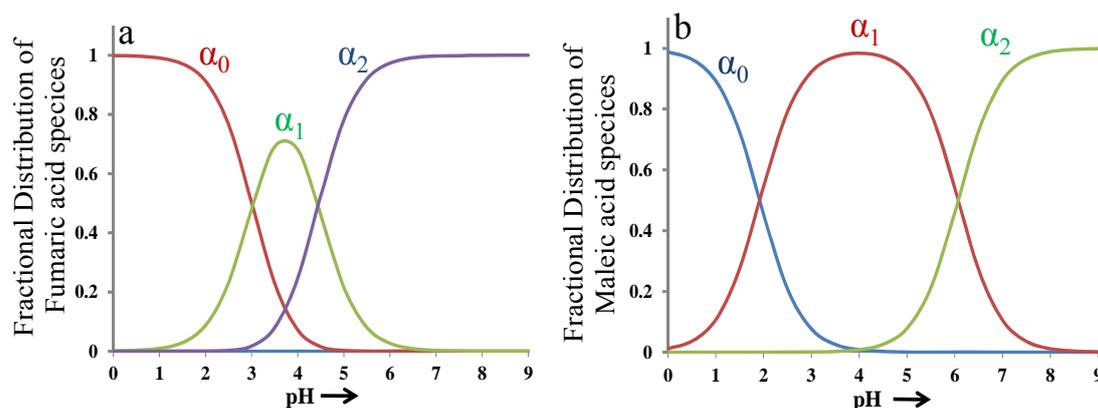

SI Figure 23: Fractional distributions of the (a) fumaric acid (b) maleic acid calculated from the pKa values are plotted as a function of pH value. Where α_0, α_1, α_2 are the zero, first and second dissociation constants of fumaric and maleic acids.

Energy optimized structure for the receptor A
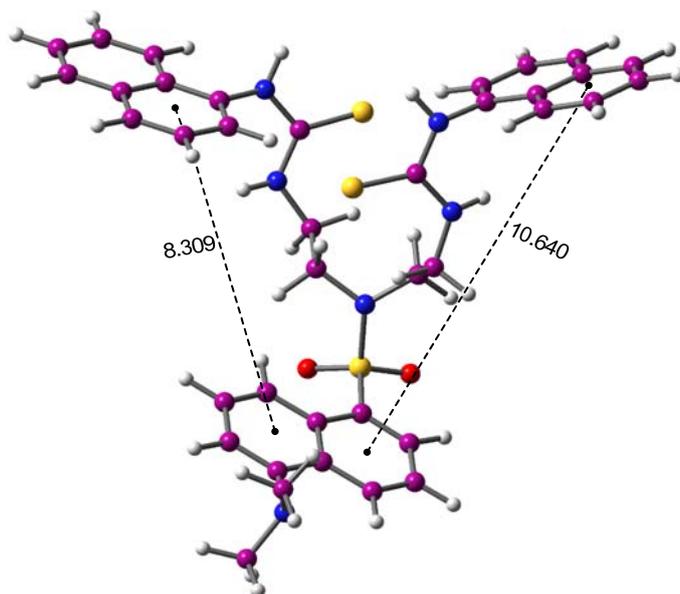
SI Figure 24: RHF/PM3 optimized geometry of probe A. Distances are given in Å. (Atom colour code: red = oxygen; blue = nitrogen; white = hydrogen; yellow = sulfur; dark magenta = carbon).

HPLC analysis
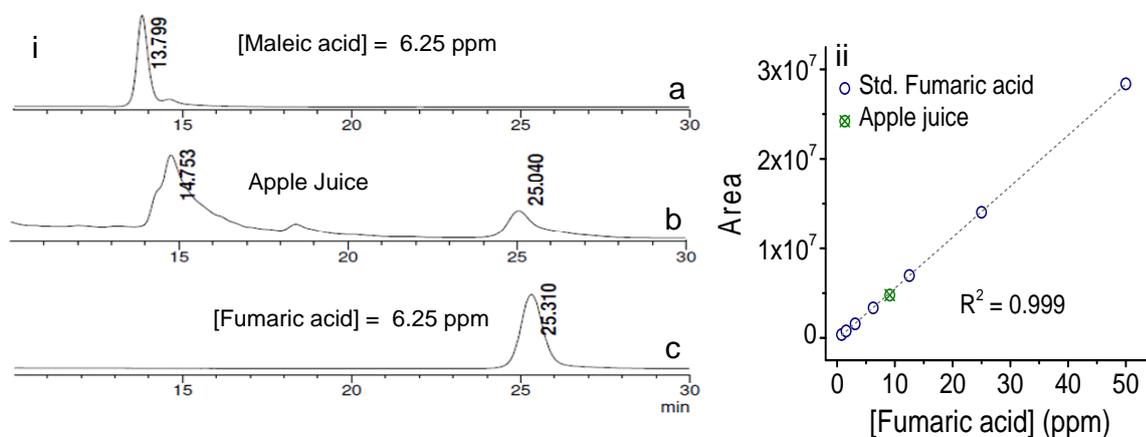
SI Figure 24:(i) HPLC chromatogram of (a) standard Maleic acid (6.25 ppm) solution with retention time 13.799 min. (b) commercial apple juice and (c) standard fumaric acid (6.25 ppm) with retention time 25.221 min; (ii) Standard calibration curve obtained from the HPLC data for different standard fumaric acid (0.78, 1.56, 3.125, 6.25, 12.5, 25 and 50 ppm) solutions and the use of that calibration plot for evaluation of [Fumaric acid] in the commercial sample of apple juice.