Electronic Supplementary Information

Naphthyridine Based Fluorescent Receptors for Recognition of Uric Acid

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1. Complexation studies of R1 by UV-vis and fluorescence method

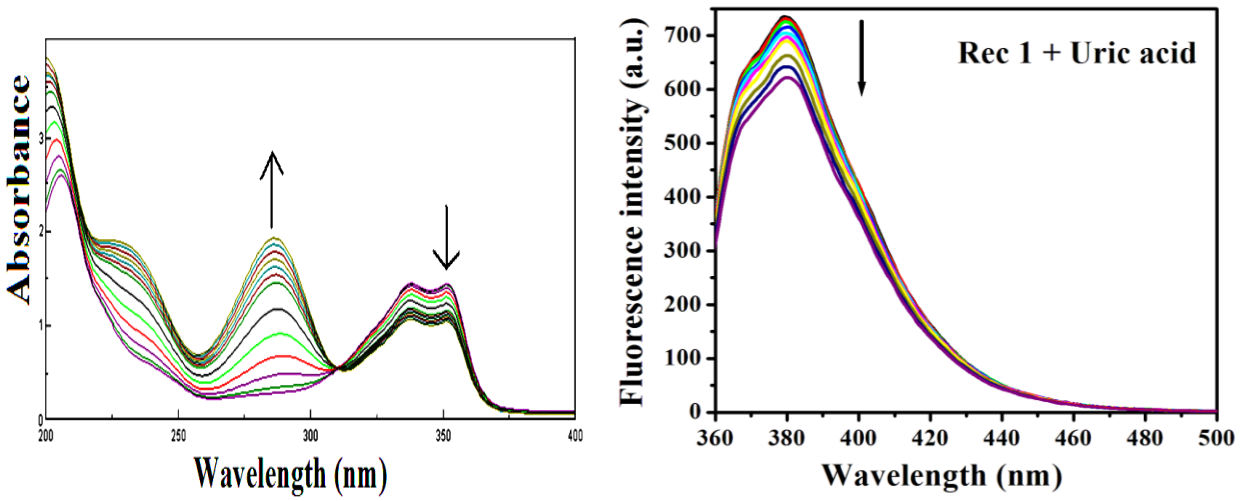


Figure 1: (i) UV-Vis absorption spectra of **R1** (λ_{max}= 351 nm) with uric acid; (ii) Fluorescence emission spectra of **R1** (λ_{max}= 380 nm) on complexation with uric acid (after excitation at 351 nm).

2. Complexation studies of R2 by UV-vis and fluorescence method

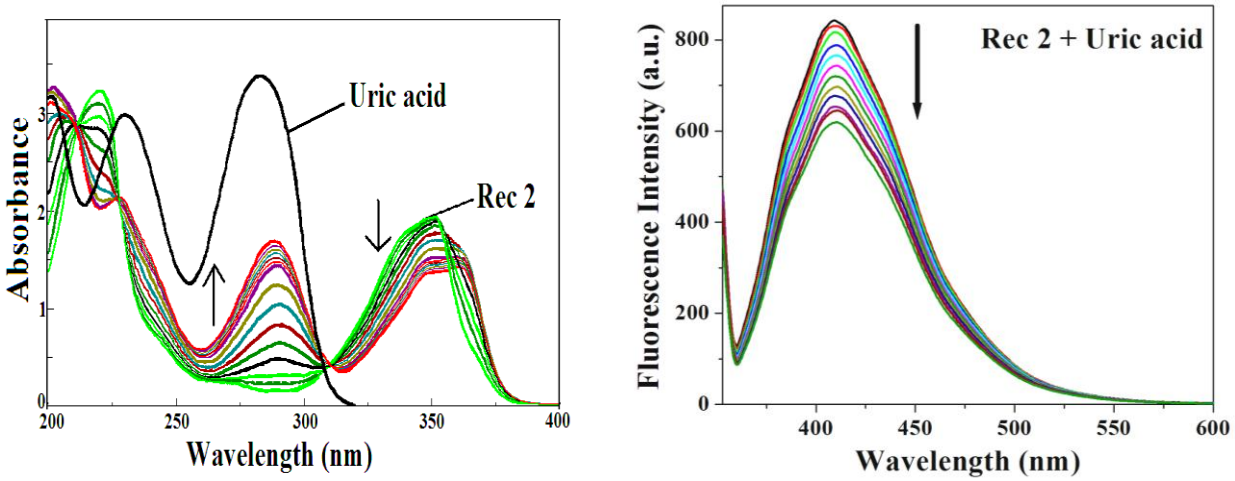


Figure 2: (i) UV-Vis absorption spectra of **R2** (λ_{max}= 351 nm) with uric acid; (ii) Fluorescence emission spectra of **R2** (λ_{max}= 409 nm) on complexation with uric acid (after excitation at 351 nm).

3. Complexation studies of R3 by UV-vis and fluorescence method

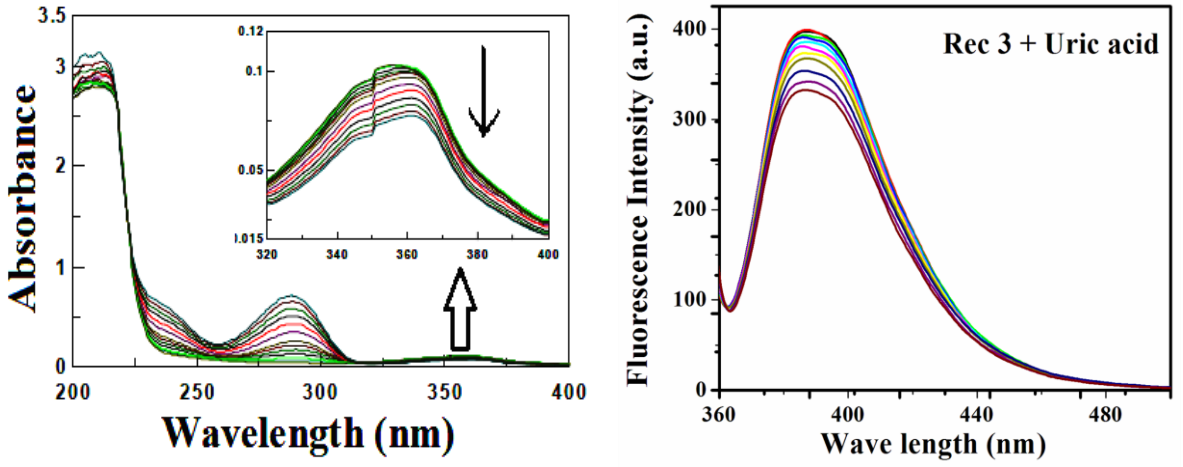


Figure 3: (i) UV-Vis absorption spectra of **R3** (λ_{max}= 355 nm) with uric acid; (ii) Fluorescence emission spectra of **R3** (λ_{max}= 390 nm) on complexation with uric acid (after excitation at 355 nm).

4. Complexation studies of R4 by UV-vis and fluorescence method:

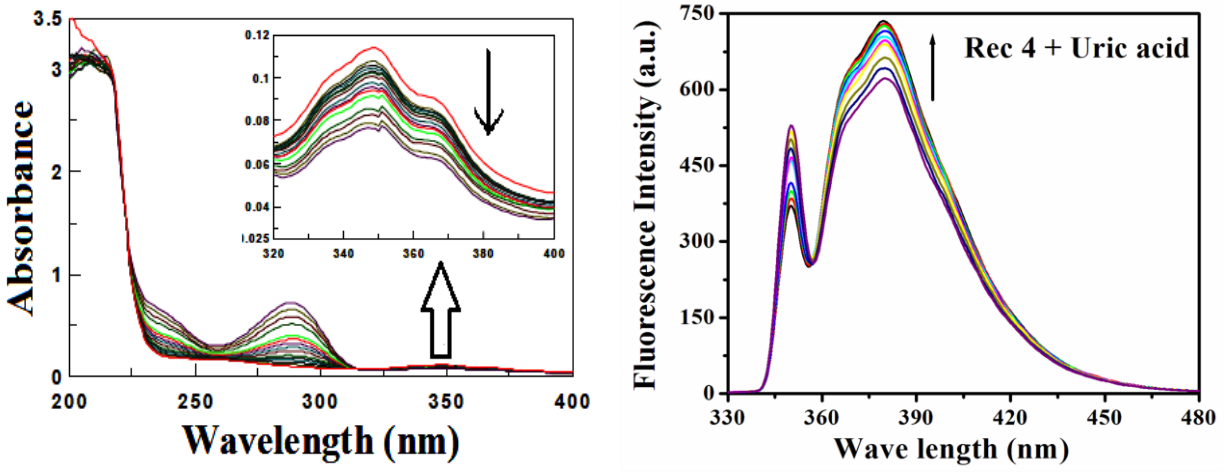


Figure 4: (i) UV-Vis absorption spectra of **R4** (λ_{max}= 348 nm) with uric acid; (ii) Fluorescence emission spectra of **R4** (λ_{max}= 380 nm) on complexation with uric acid (after excitation at 348 nm).

5. Experimental

General

^{1}H NMR spectra were recorded either on a Bruker AM 300L MHz or a Bruker 500 MHz spectrometer. For NMR spectra, $CDCl_3$ was used as solvent unless otherwise mentioned using TMS as internal standard. UV-VIS spectra were recorded on JASCO V-530. Mass and fluorescence spectra, JEOL JMS600 and Perkin Elmer Instruments, LS 55, were used.

7-Amino-1H-[1,8]naphthyridin-2-one [R1]:

2,6-diaminopyridine (2.2 g, 0.02 mmol) and malic acid (3.0 g, 0.02 mmol) were thoroughly mixed and taken in a round bottomed (RB) flask and to this conc. H_2SO_4 (10 mL) was added dropwise in continuous cooling. The mixture was warmed until no more gases formed (3.5 hrs). Then it was poured onto ice and made basic with ammonium hydroxide. The yellow precipitate was filtered and washed with water and finally it was dried to yield the desired product [5.2g, Mp. 222^{o}C-223^{o}C].

^{1}H-NMR, d_6-DMSO; 300MHz): δ 11.55 (bs, 1H), 7.62 (d, 2H, *J* = 9.0 Hz), 6.75 (bs, 2H), 6.31 (d, 1H, *J* = 8.4 Hz), 6.08 (d, 1H, *J* = 9.3 Hz).

MS (EI) (m/z, %): 178.8 (20), 161.0 (M^+, 100), 147.0 (5).

FT-IR (KBr, cm^{-1}): 3468 (NH str.), 2974, 1650 (C=O str.), 1563 (Ar. C=C str.), 1465 (Ar. C=N str.), 1431.

1,8-Naphthyridine-2,7-diamine [R2]:

R2 was prepared by the procedure, which was previously reported in our laboratory.[5c]

^{1}H-NMR (d_6-DMSO; 300MHz) of R2: δ 7.58 (d, 2H, *J* = 8.4 Hz), 6.37 (d, 2H, *J* = 8.4 Hz), 6.24 (bs, 4H).

MS (EI) (m/z, %): 160.1 (M^+, 100), 121.2 (25), 93.2 (10).

FT-IR (KBr, cm^{-1}): 3478 (NH str.), 2932, 1630, 1571 (Ar. C=C str.), 1454 (Ar. C=N str.).

^{1}H-NMR (d_6-DMSO; 300MHz) of complex of R2 with uric acid: δ10.57 (bs, 4H), 7.76 (d, 2H, *J* = 8.5 Hz), 6.93 (bs, 4H), 6.50 (d, 2H, *J* = 8.6 Hz).

2-{[(7-{[(E)-1-(2-hydroxyphenyl)methylidine]amino}[1,8-]naphthyridin-2-yl)imino]methyl}phenol (R3):

2,7-diaminonaphthyridine (0.5 g, 3.12 mmol) and salicylaldehyde (1.0 g, 8.19 mmol) were taken in a dry RB,. To this RB, dry methanol (5.0 mL) was added to it. The RB was put on a oil bath and refluxed for 12 hrs. A solid residue was found, adhered to the wall of RB. The residue was filtered and washed several times with methanol. Finally an orange residue was obtained (Mp > 250^0C; 0.4 g, yield 35%).

^{1}H-NMR (d_6-DMSO; 500MHz): δ 10.19 (bs, 2H), 8.27 (s, 2H), 7.59 (d, 2H, *J* = 8.1 Hz), 7.45 (bs, 2H), 6.92 (bs, 2H), 6.37 (d, 2H, *J* = 8.1 Hz), 6.26 (bs, 4H).

MS (EI) (m/z, %): Mass spectra not found.

FT-IR (KBr, cm^{-1}): 3420 (OH str.), 2964, 1759, 1608 (Ar. C=C str.), 1509, 1456 (Ar. C=N str.).

^{1}H-NMR (d_6-DMSO; 500MHz) of R3 with uric acid: δ 10.66 (bs, 2H), 10.51 (bs, 2H), 8.27(s, 2H), 7.84 (d, 2H, *J* = 8.7 Hz), 7.64 (d, 2H, *J* = 9.3 Hz), 7.50 (t, 2H, *J* = 8.5 Hz), 7.28(bs, 2H), 6.98-6.92 (m, 2H), 6.54 (d, 2H, *J* = 8.7 Hz).

N1-{7-[(2-hydroxybenzoyl)amino][1,8]naphthyridin-2-yl}-2-hydroxybenzamide (R4):

Salicylic acid (0.5 g, 3.6 mmol) was taken in dry benzene (8.0 mL). Then excess thionyl chloride (2.5 mL) was added to it and then refluxed for 4 hrs. After refluxation completed, excess thionyl

chloride and benzene were distilled out. CH_2Cl_2 was poured into it and the whole mixture was transferred into a two-neck rb.

On the other side, naphthyridine diamine (1.15 g, 7.24 mmol), dry Et_3N (0.4 mL) and CH_2Cl_2 (5.0 mL) were mixed together. The whole system was kept under nitrogen atmosphere. This solution was added dropwise to the acid chloride during one hour. After 12 hours with continuous stirring, tlc was checked. The desired compound was purified through preparative tlc using 8% methanol-chloroform solvent (yield 25%, Mp. > 250^0C).

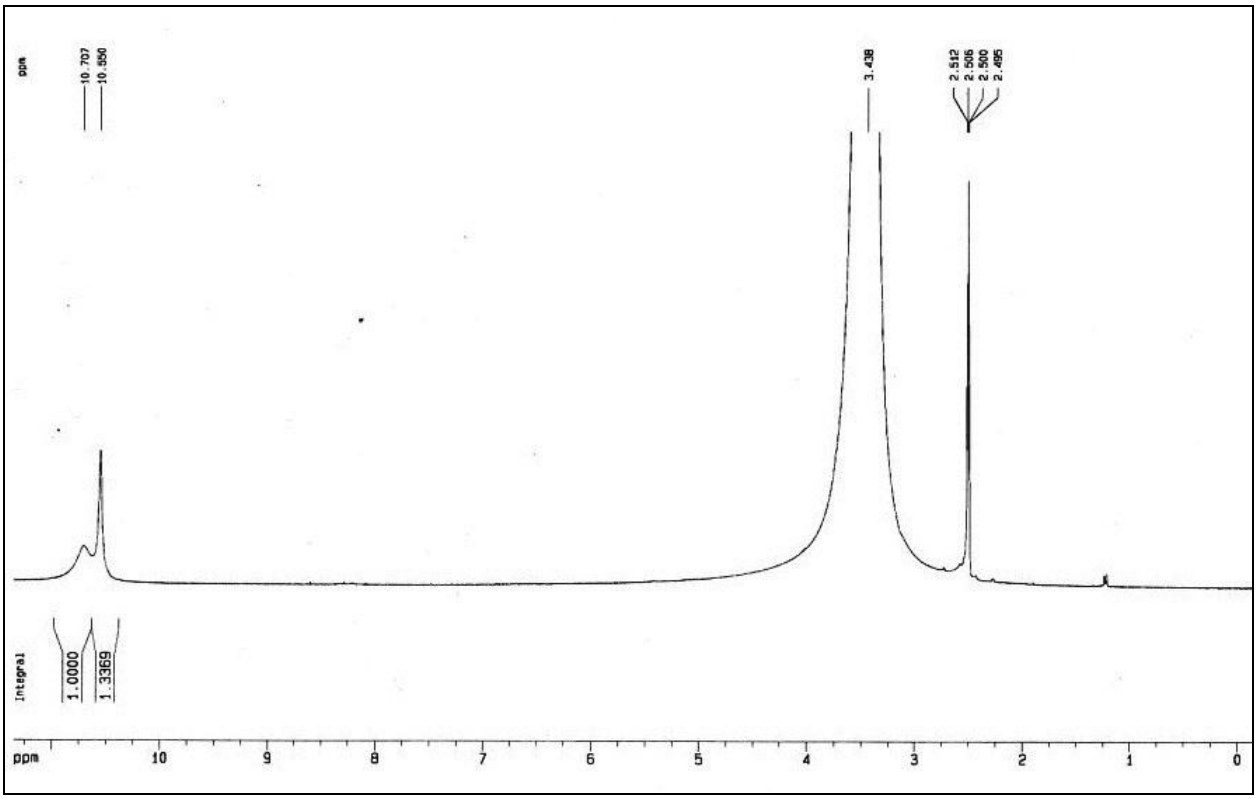
^{1}H-NMR (d_6-DMSO; 500MHz): δ 11.32 (bs, 2H), 9.69 (bs, 2H), 8.47-8.41 (m, 4H), 8.05 (d, 2H, *J* = 6.3 Hz), 7.47 (t, 2H, *J* = 8.4 Hz), 7.07 (d, 2H, *J* = 8.1 Hz), 7.00 (t, 2H, *J* = 7.4 Hz).

MS (EI) (m/z, %): 400 (M^+, 69.5), 280 (34), 160 (92), 121 (21), 28 (100).

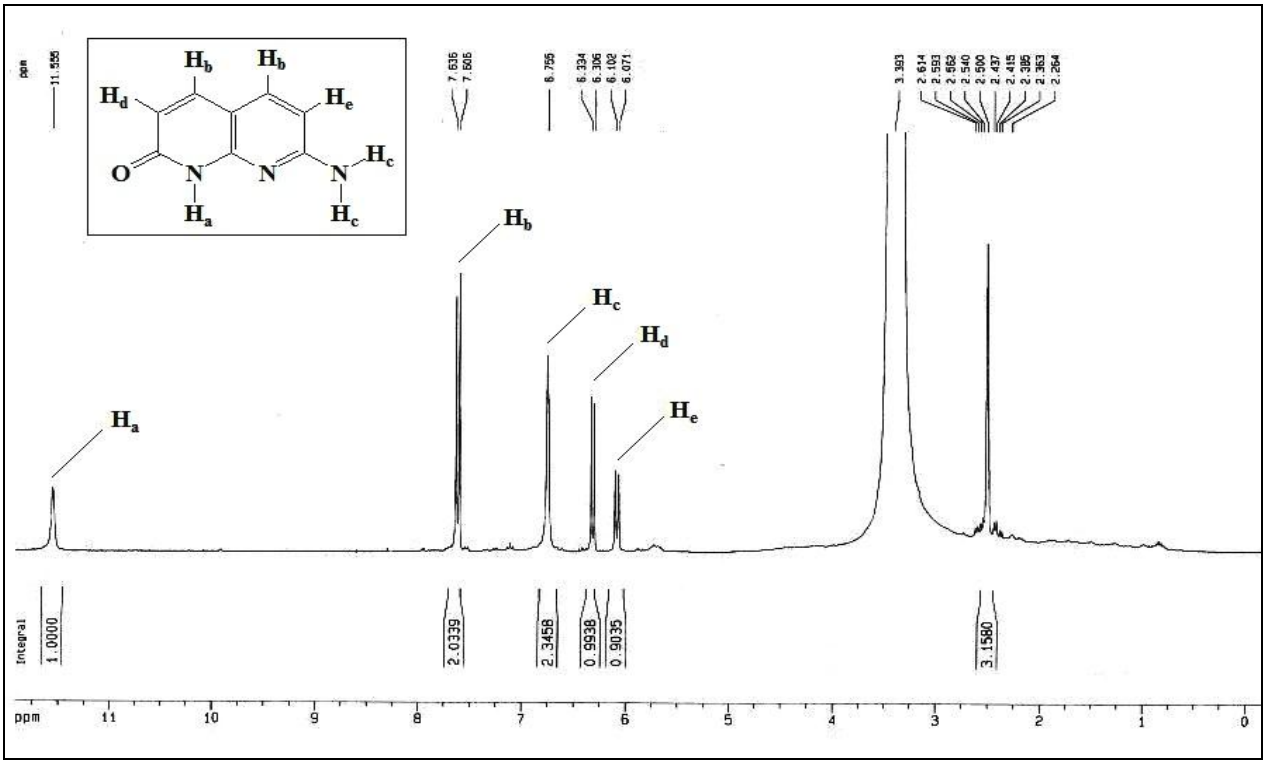
FT-IR (KBr, cm^{-1}): 3407 (OH str.), 1606 (C=O str.), 1502 (Ar. C=N str.), 1111.

^{1}H-NMR (d_6-DMSO; 500MHz) of R4 with uric acid: δ11.08 (bs, 2H), 10.50 (bs, 2H), 10.34 (bs, 2H), 9.40 (bs, 2H), 8.28-8.23 (m, 4H), 7.87(d, 2H, *J* = 9.3 Hz), 7.29 (t, 2H, *J* = 8.4 Hz), 6.89 (d, 2H, *J* = 8.1 Hz), 6.82 (t, 2H, *J* = 7.4 Hz).

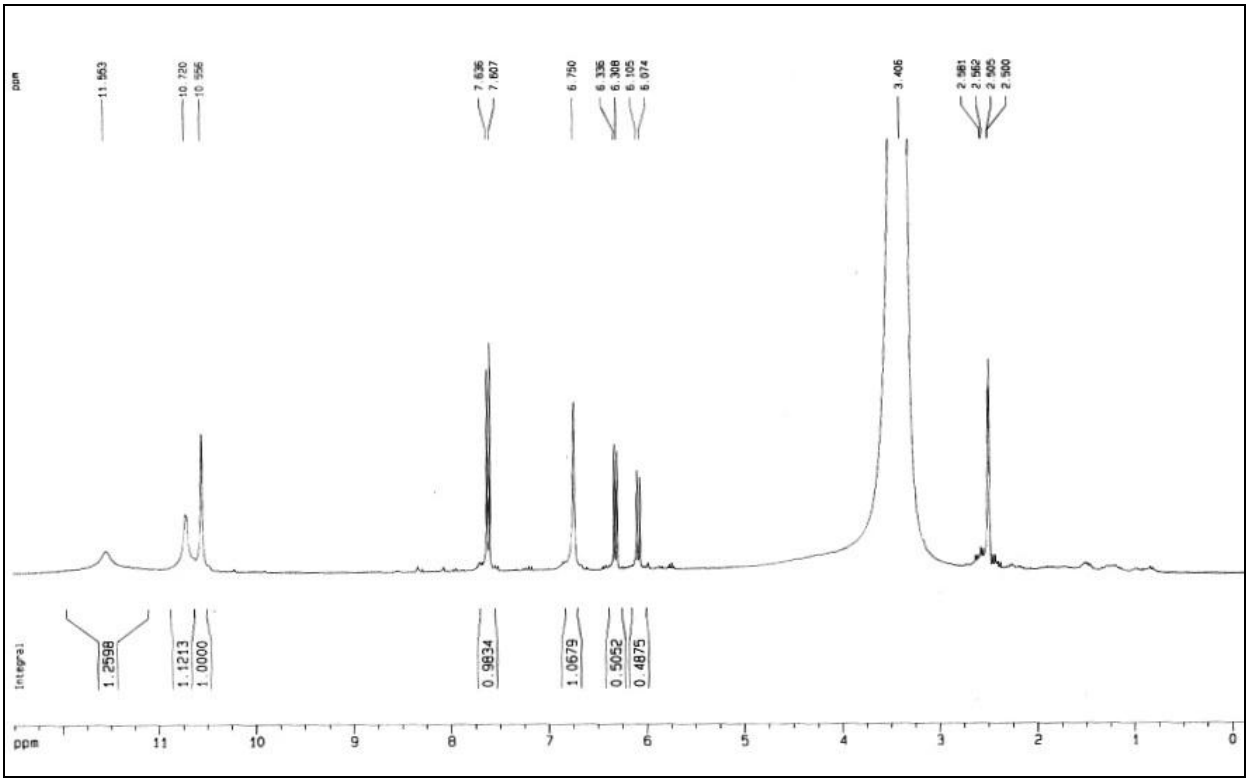
^{1}H-NMR (500 MHz) spectra of Uric acid (**UA**) in d_6-DMSO



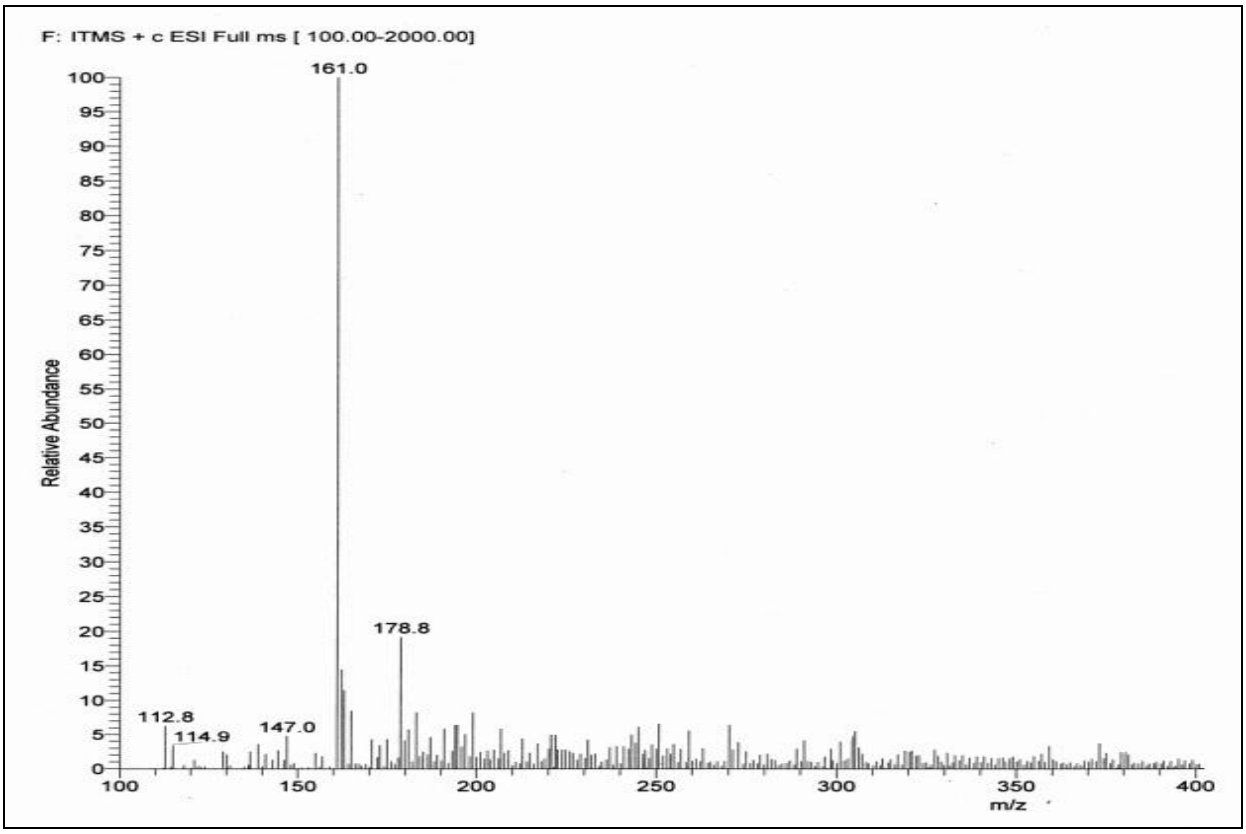
^{1}H-NMR (300 MHz) spectra of **R1** in d_6-DMSO



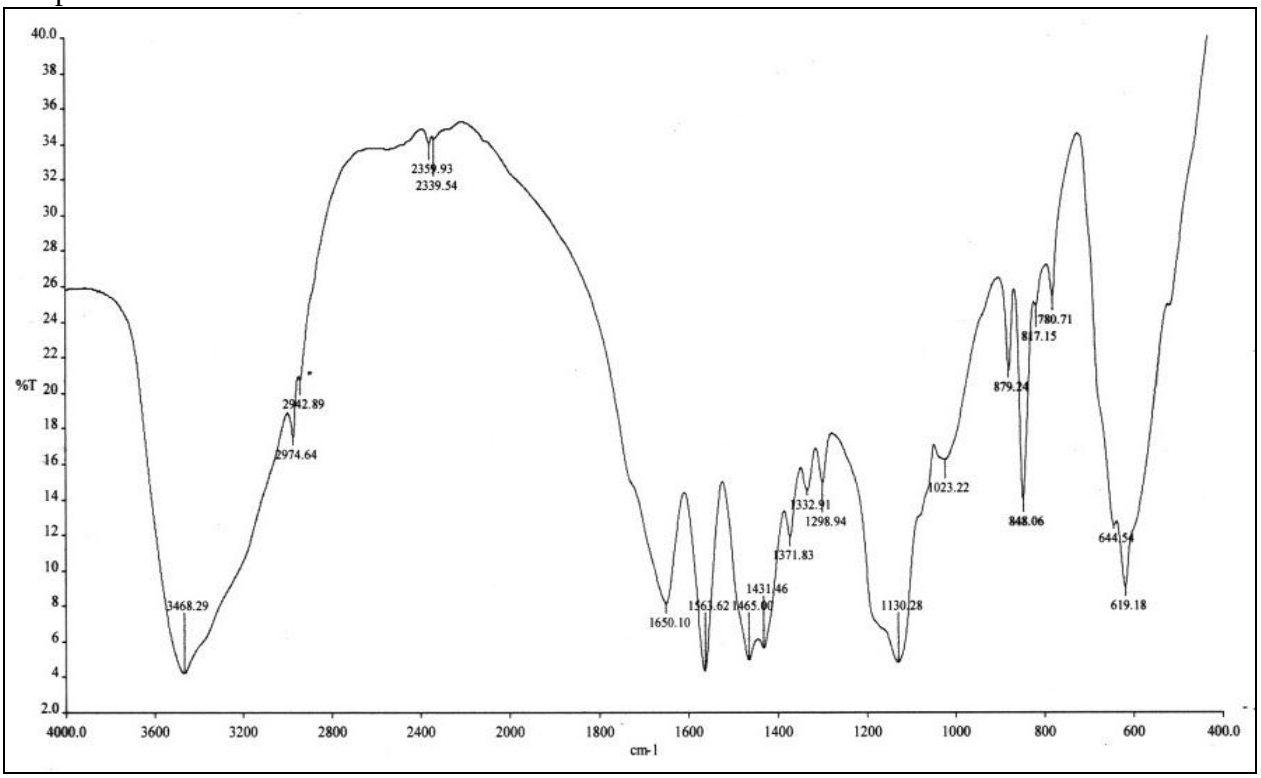
^{1}H-NMR (300 MHz) spectra of **R1**with uric acid in d_6-DMSO



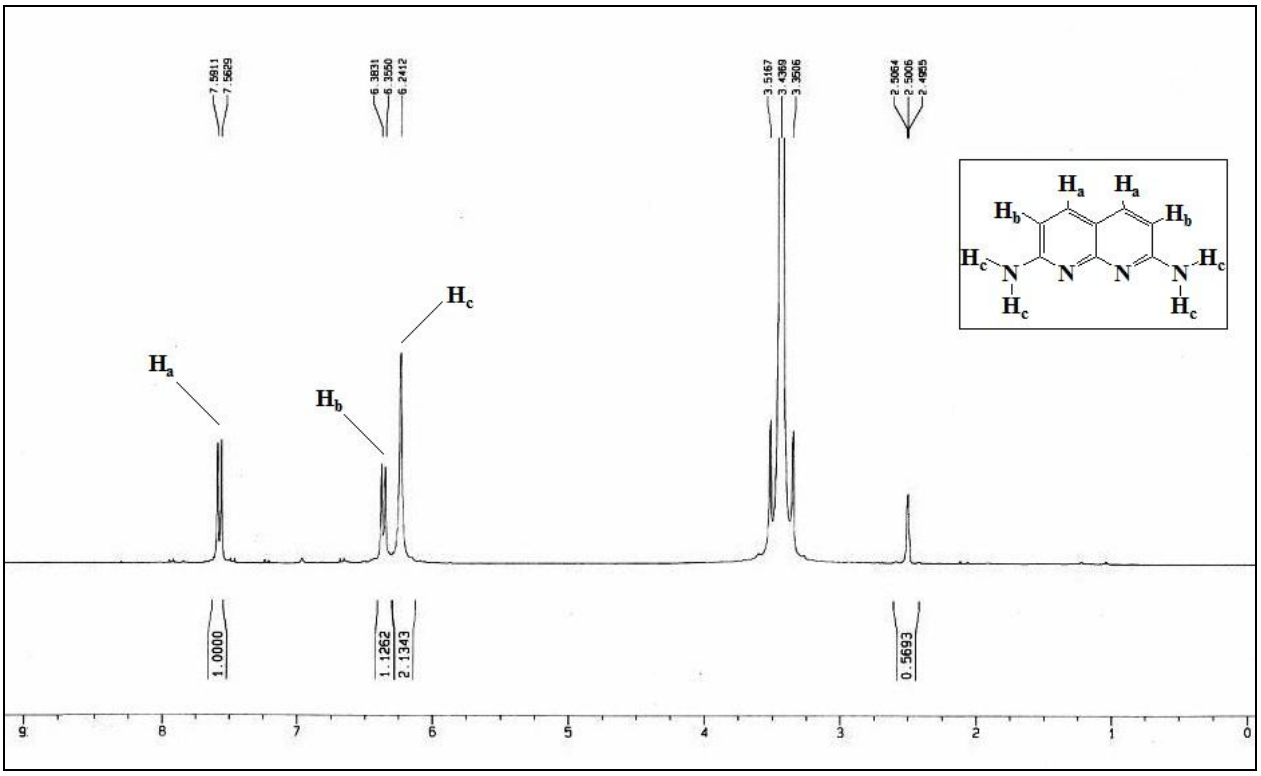
Mass Spectra of **R1**



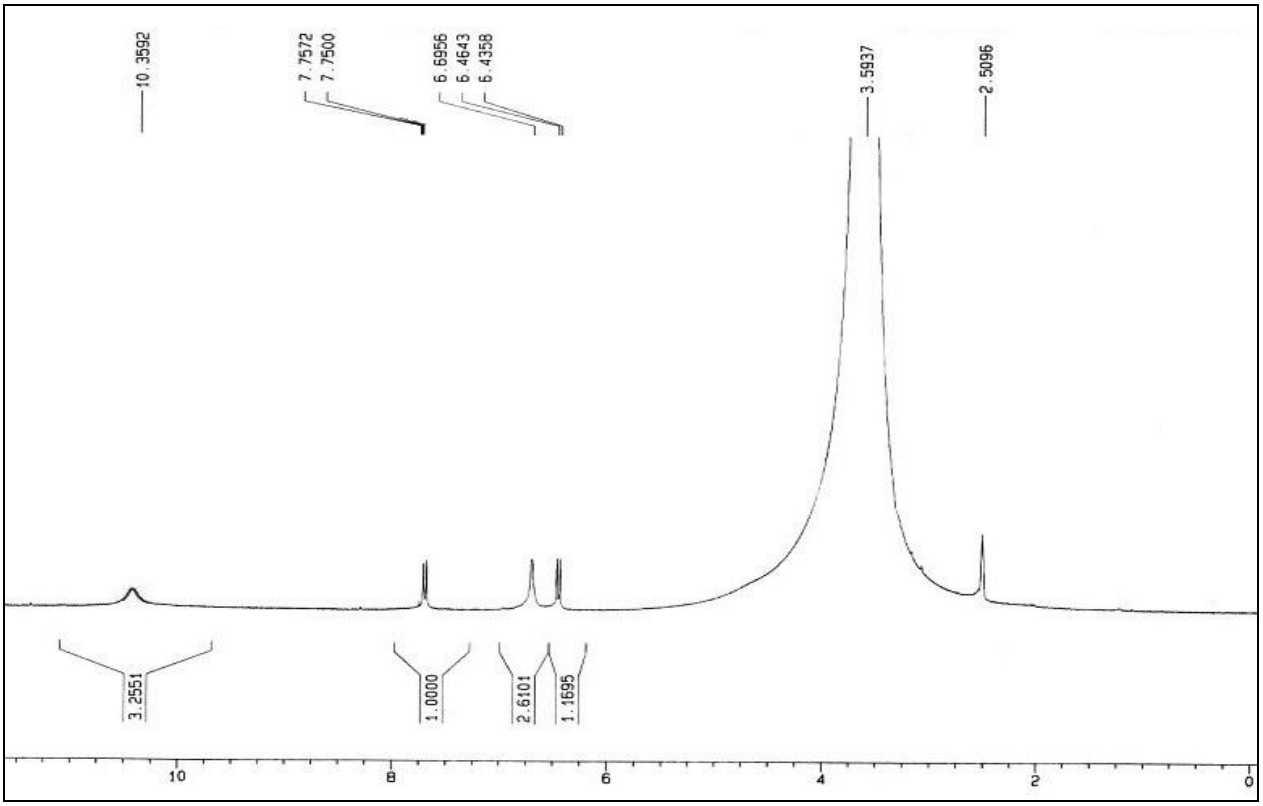
IR spectra of **R1**



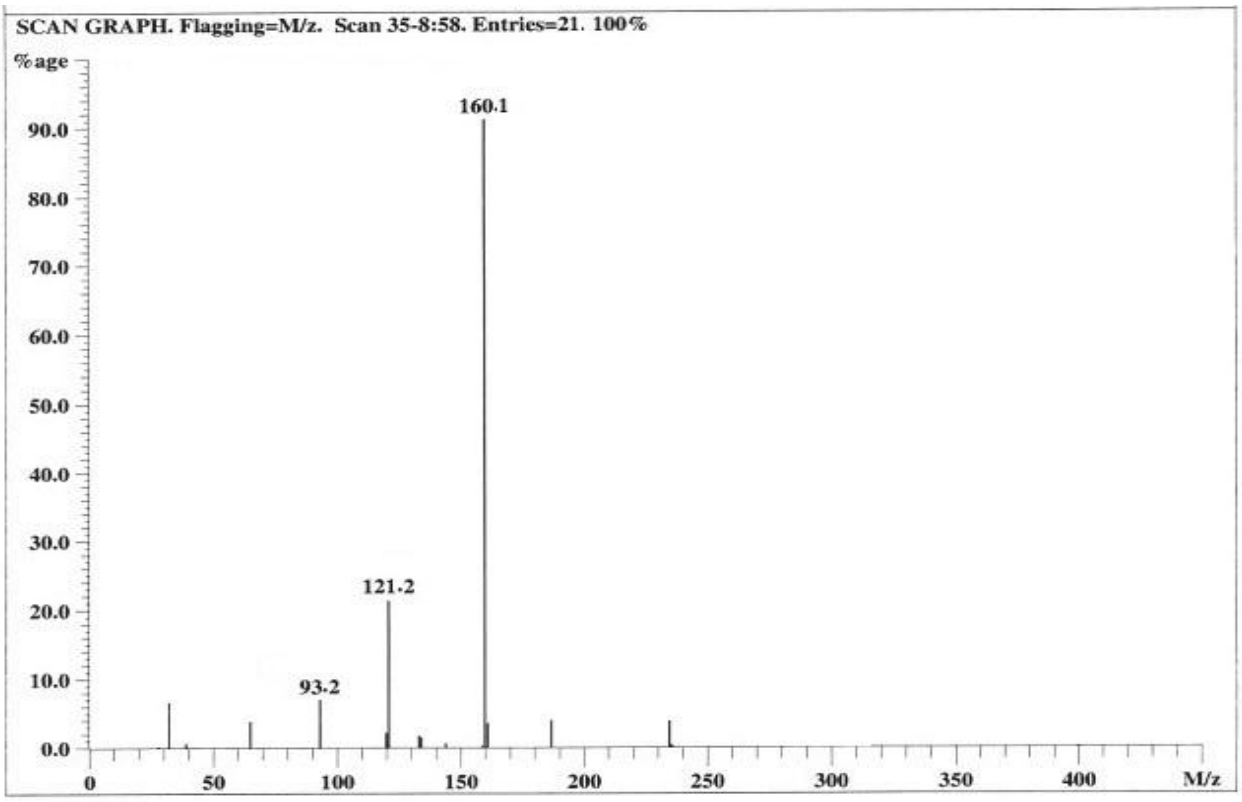
^{1}H-NMR (300 MHz) spectra of **R2** in d_6-DMSO



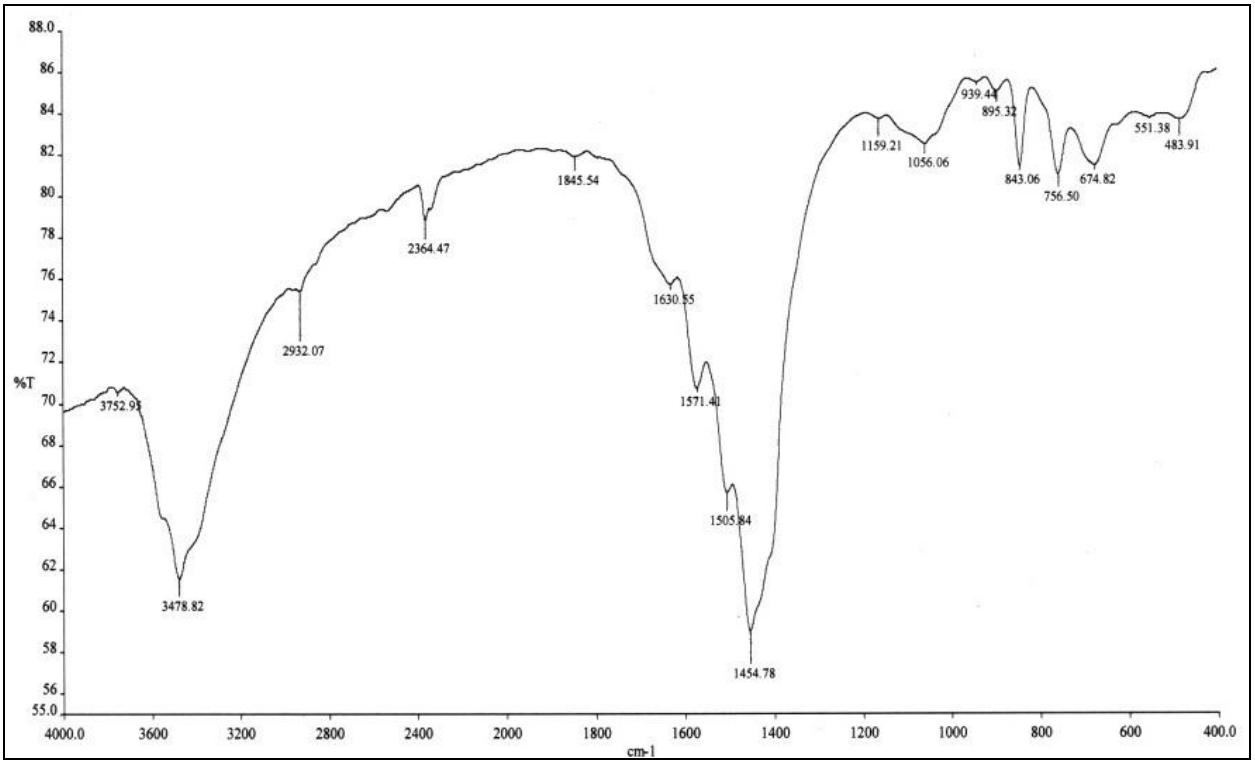
^{1}H-NMR (300 MHz) spectra of **R2** with uric acid in d_6-DMSO



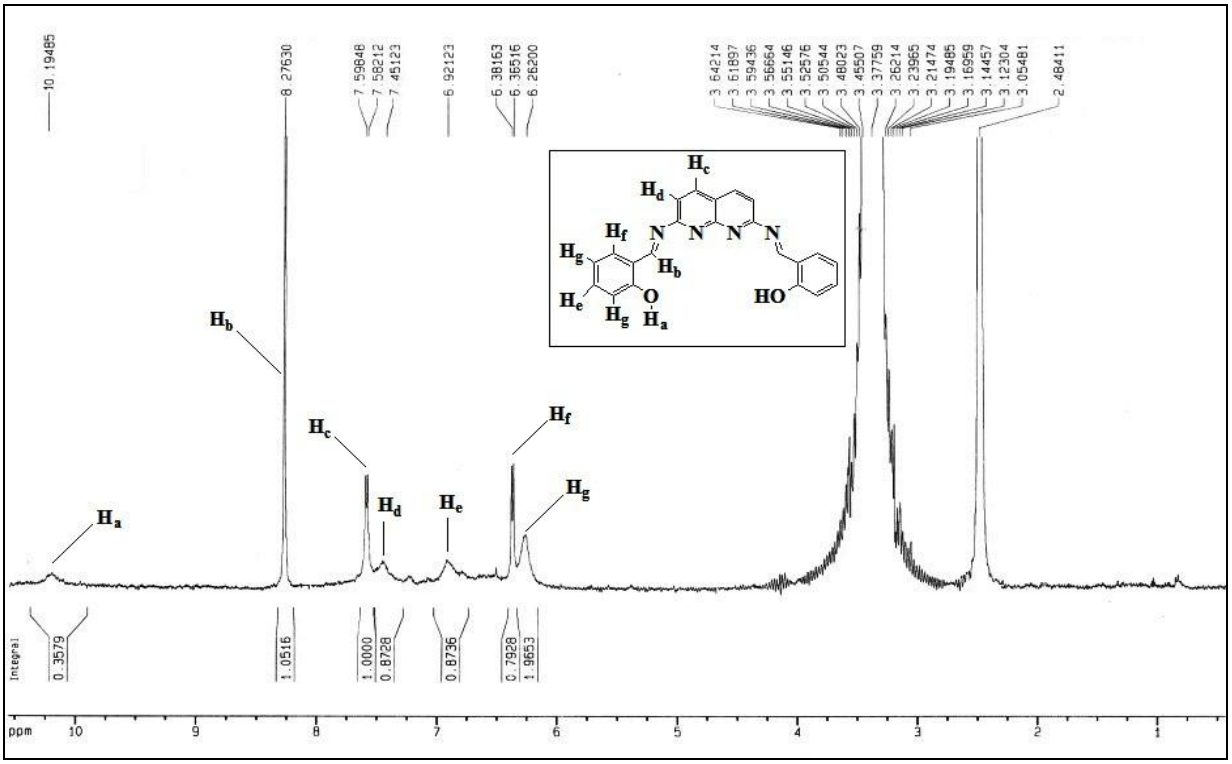
Mass Spectra of **R2**



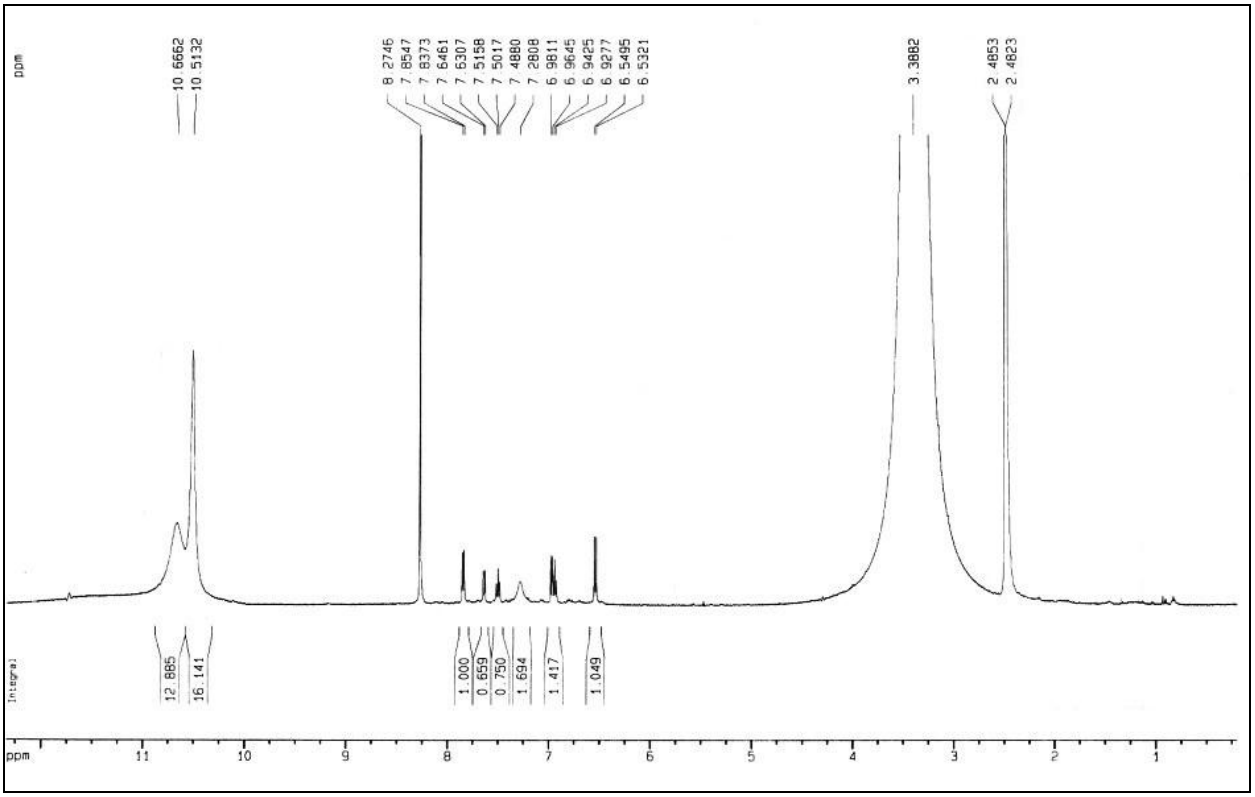
IR spectra of **R2**



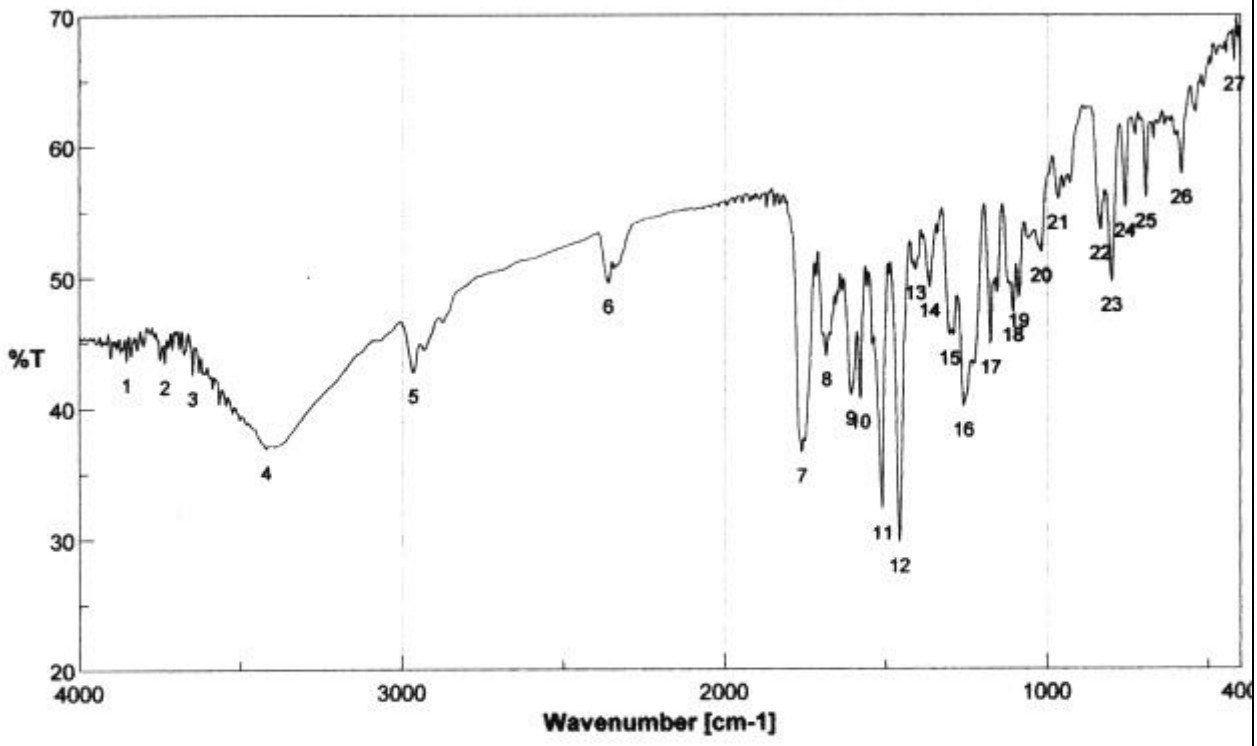
^{1}H-NMR (500 MHz) spectra of **R3** in d_6-DMSO



^{1}H-NMR (500 MHz) spectra of **R3** with Uric acid in d_6-DMSO

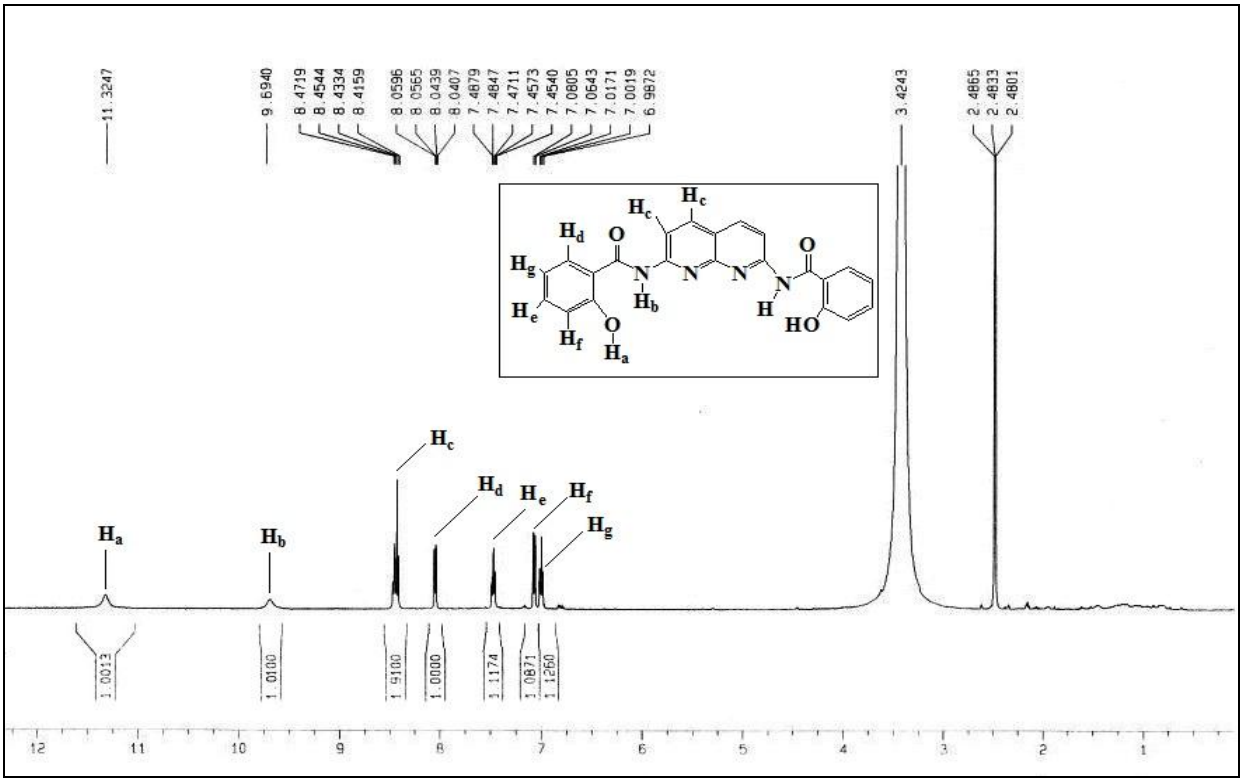


IR spectra of **R3**

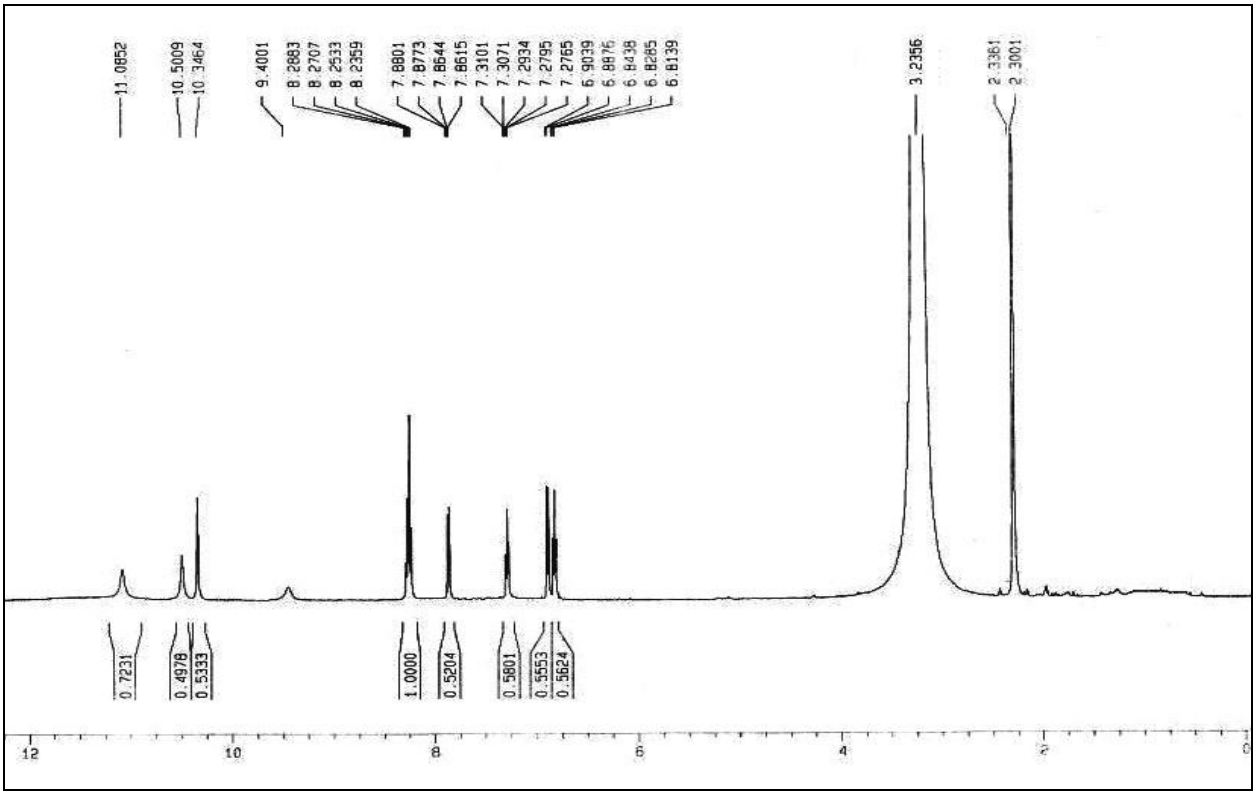


No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T
1	3853.08	43.6344	2	3734.48	43.4708	3	3648.66	42.5873
4	3420.14	36.9255	5	2964.05	42.7168	6	2360.44	49.5816
7	1759.73	36.5682	8	1683.55	43.9465	9	1608.34	40.9493
10	1579.41	40.6733	11	1509.03	32.2011	12	1456.96	29.7012
13	1406.82	50.5843	14	1363.43	49.23	15	1301.72	45.5779
16	1258.32	40.0715	17	1174.44	44.8501	18	1105.01	47.3473
19	1086.69	48.4529	20	1018.23	51.9405	21	965.198	55.9684
22	834.062	53.6449	23	799.35	49.684	24	756.923	55.358
25	693.284	56.0926	26	583.361	57.8852	27	419.442	66.5205

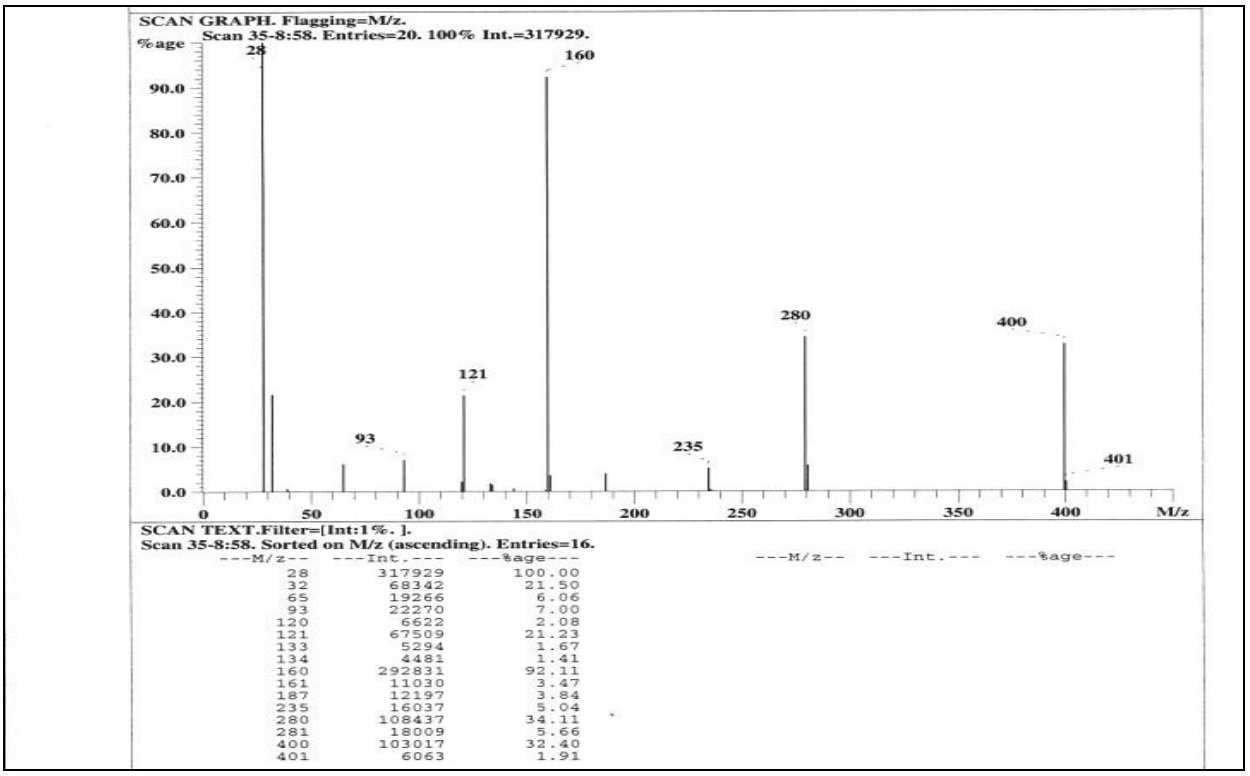
^{1}H-NMR (500 MHz) spectra of **R4** in d_6-DMSO



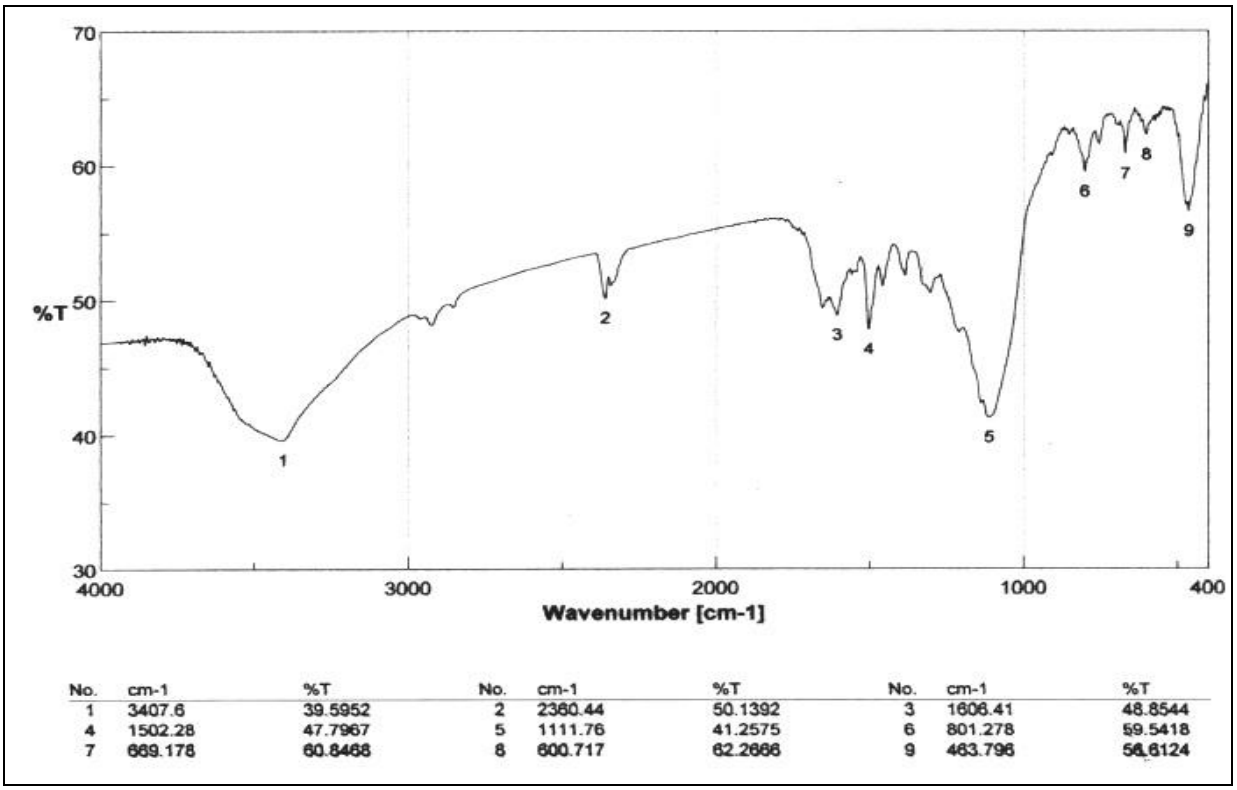
^{1}H-NMR (500 MHz) spectra of **R4** with uric acid in d_6-DMSO



Mass Spectra of **R4**



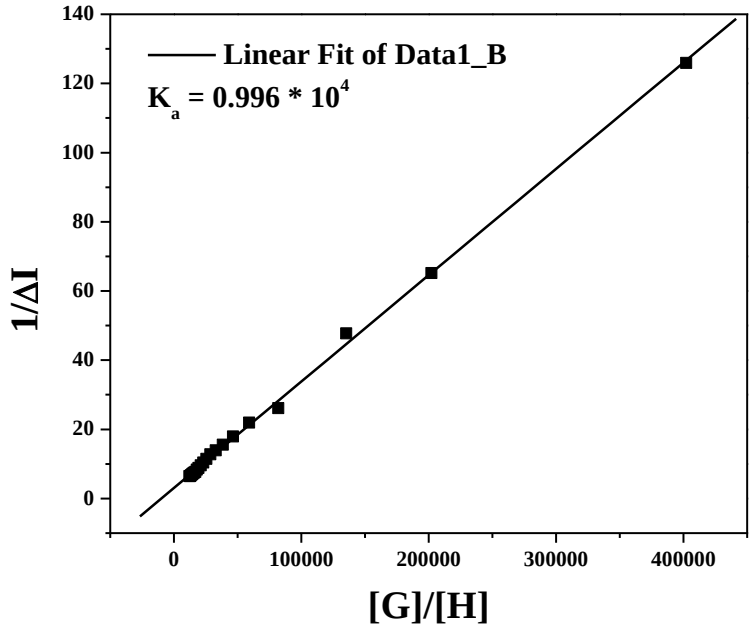
IR spectra of **R4**



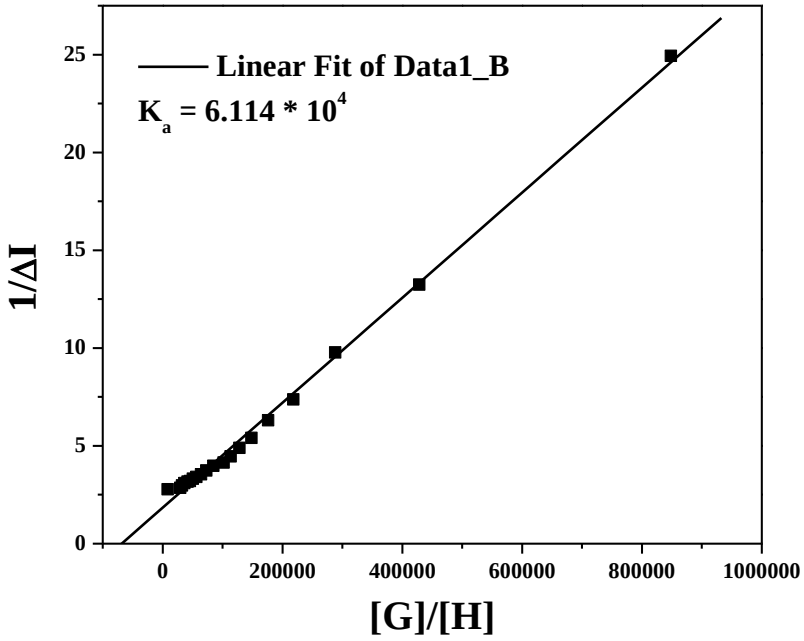
No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T
1	3407.6	39.5952	2	2360.44	50.1392	3	1606.41	48.8544
4	1502.28	47.7967	5	1111.76	41.2575	6	801.278	59.5418
7	669.178	60.8468	8	600.717	62.2666	9	463.796	56.6124

Linear regression analysis (1/[G] vs 1/ΔI) for the calculation of association constant values by UV-Vis titration method:

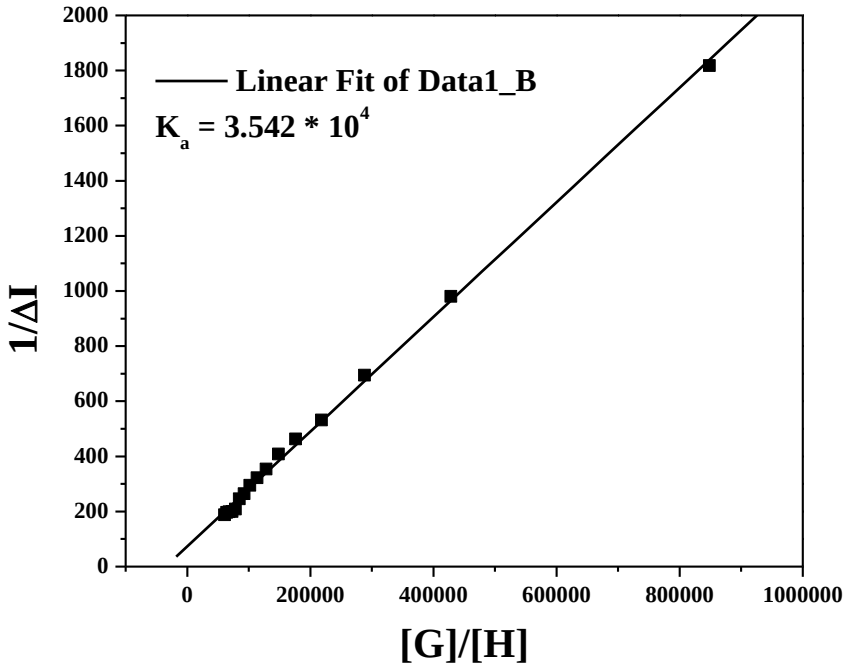
(i) R1 with Uric acid:



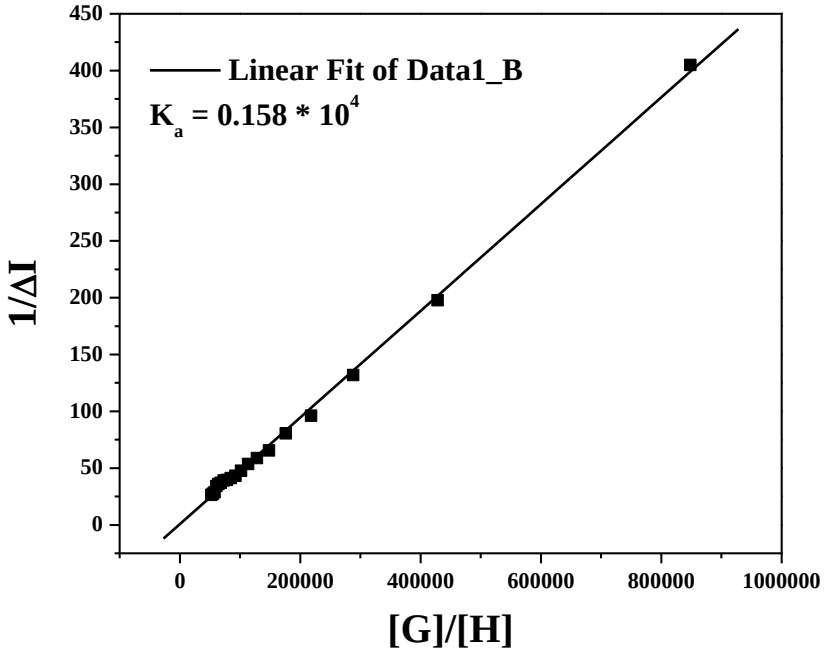
(ii) R2 with Uric acid:



(iii) R3 with Uric acid:

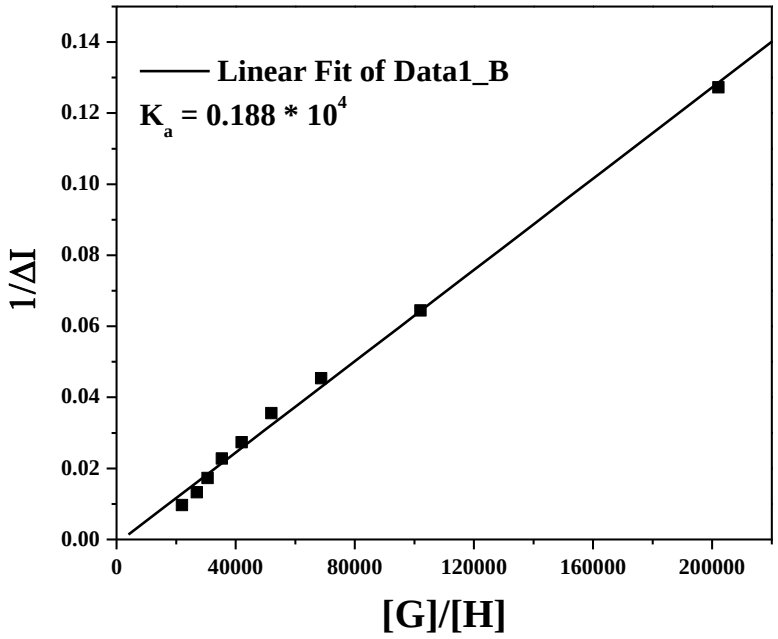


(iv) R4 with Uric acid:

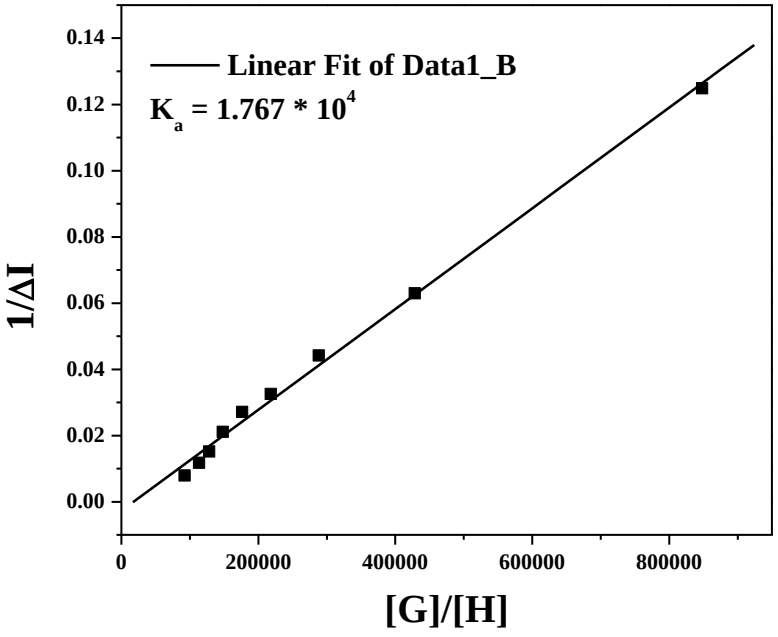


Linear regression analysis (1/[G] vs 1/ΔI) for the calculation of association constant values by Fluorescence titration method:

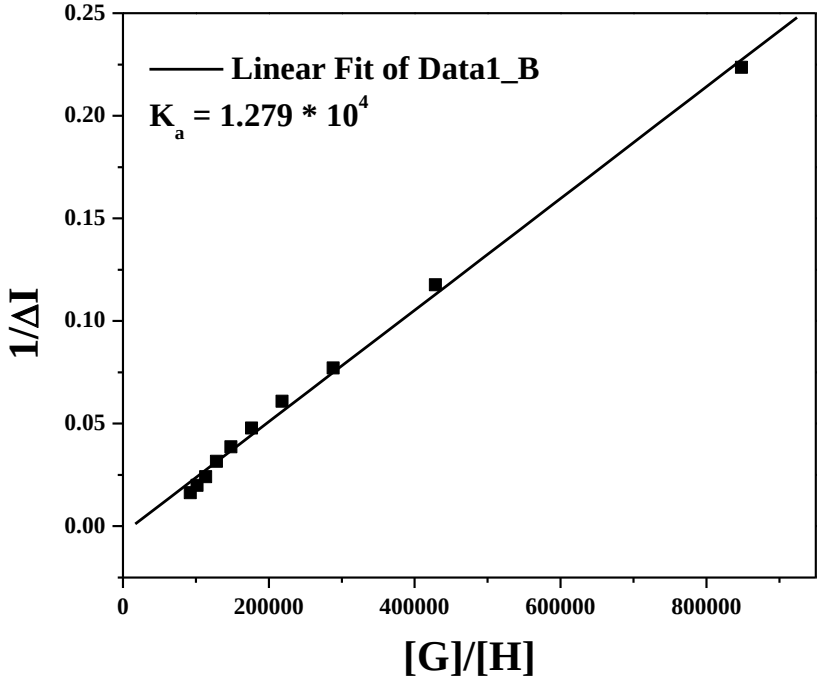
(i) R1 with Uric acid:



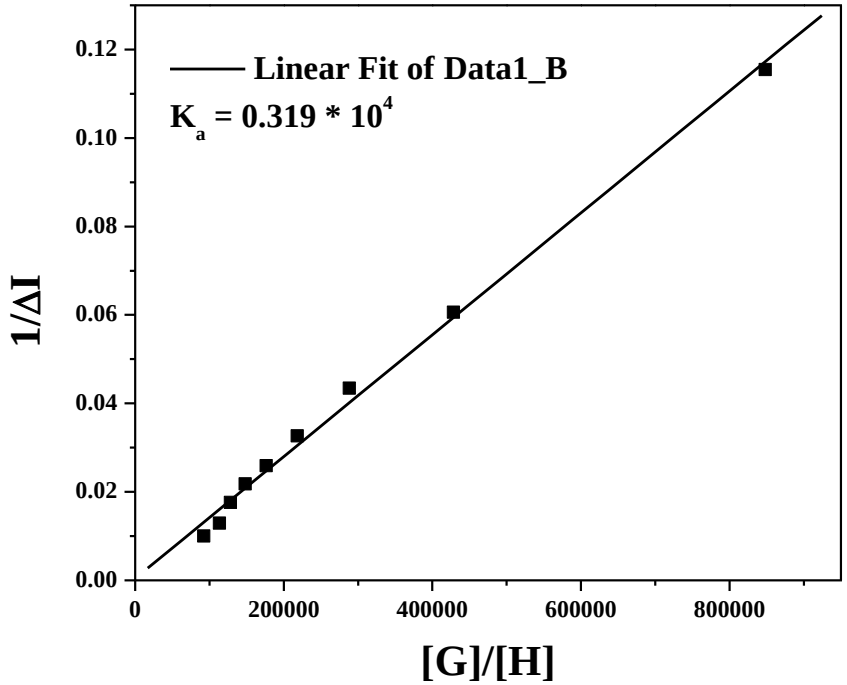
(ii) R2 with Uric acid:



(iii) R3 with Uric acid:



(iv) R4 with Uric acid:



Calculation of limit of detection (LOD):

The detection limit of the receptors for uric acid was calculated on the basis of fluorescence titration. To determine the standard deviation for the fluorescence intensity, the emission intensity of four individual receptors without uric acid was measured by 10 times and the standard deviation of blank measurements was calculated.

The limit of detection (LOD) of four receptors for sensing uric acid was determined from the following equation[1]:

$LOD = K \times SD/S$

Where K = 2 or 3 (we take 3 in this case); SD is the standard deviation of the blank receptor solution; S is the slope of the calibration curve.

For R1 with uric acid:

From the linear fit graph we get slope = -7.88496×10^6, and SD value is 0.51284

Thus using the above formula we get the Limit of Detection = 19.512×10^{-8} M i.e. **R1** can detect uric acid up to this very lower concentration by fluorescence techniques.

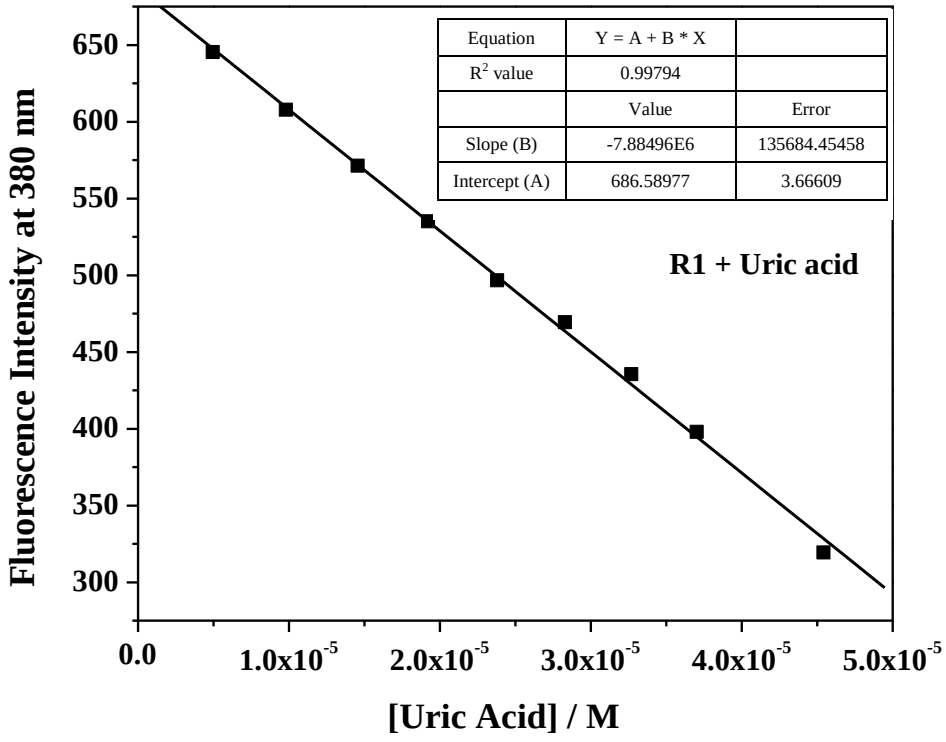


Figure: Linear fit curve of **R1** at 380 nm with respect to uric acid concentration.

For R2 with uric acid:

From the linear fit graph we get slope = -1.62726×10^{7}, and SD value is 0.49148

Thus using the above formula we get the Limit of Detection = 9.0609×10^{-8} M i.e. **R2** can detect uric acid up to this minimum concentration by fluorescence techniques.

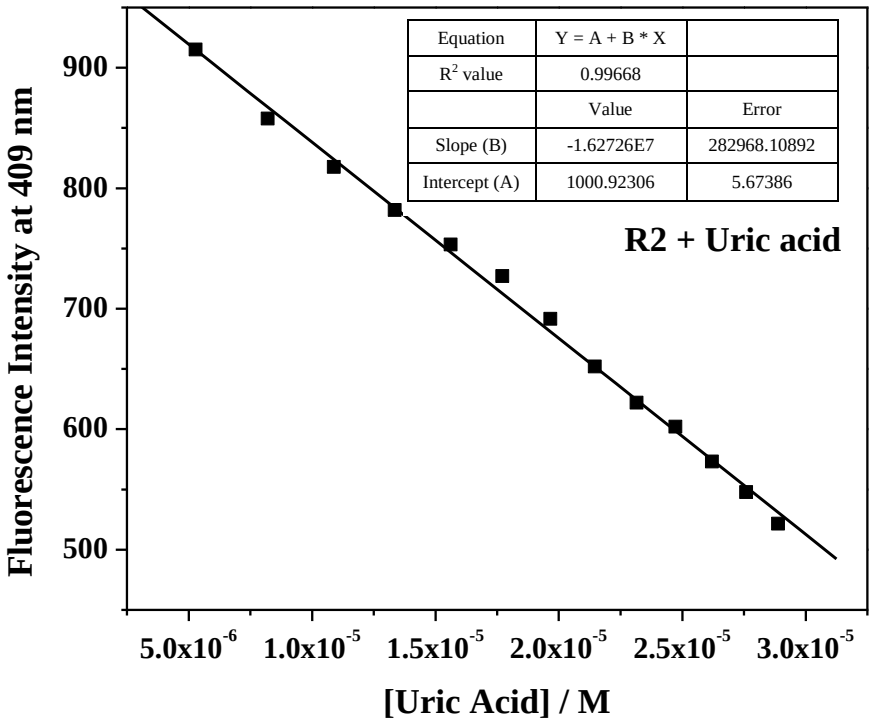


Figure: Linear fit curve of **R2** at 409 nm with respect to uric acid concentration.

For R3 with uric acid:

From the linear fit graph we get slope = -1.52969×10^{7}, and SD value is 0.49559

Thus using the above formula we get the Limit of Detection = 9.7194×10^{-8} M i.e. **R3** can detect uric acid up to this minimum concentration by fluorescence techniques.

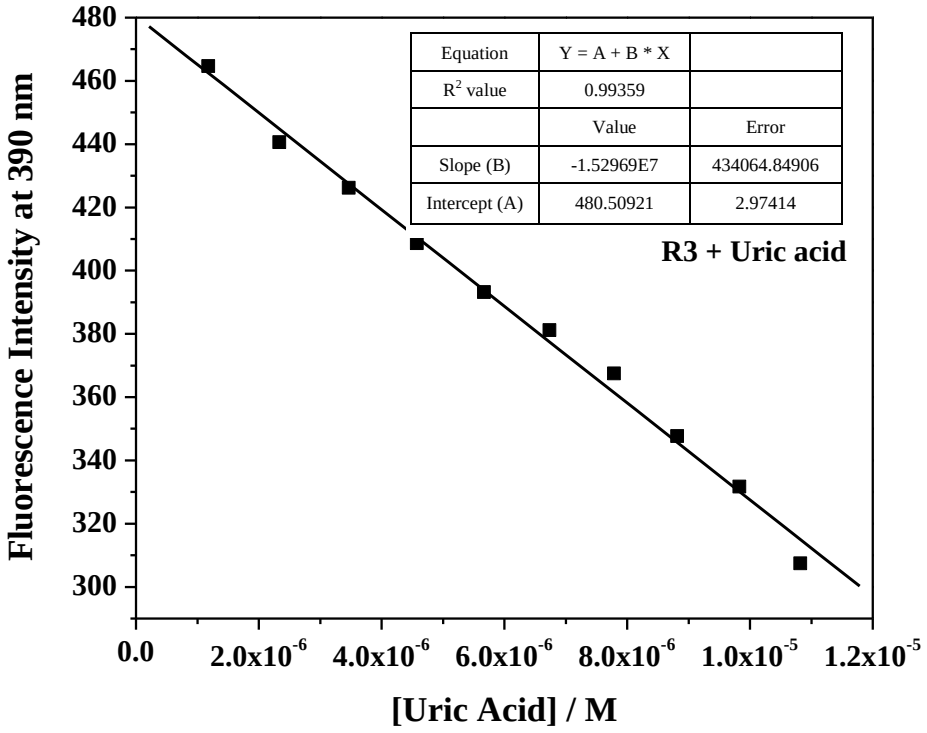


Figure: Linear fit curve of **R3** at 390 nm with respect to uric acid concentration.

For R4 with uric acid:

From the linear fit graph we get slope = -1.50734×10^{7}, and SD value is 0.50085

Thus using the above formula we get the Limit of Detection = 9.9682×10^{-8} M i.e. **R4** can detect uric acid up to this minimum concentration by fluorescence technique.

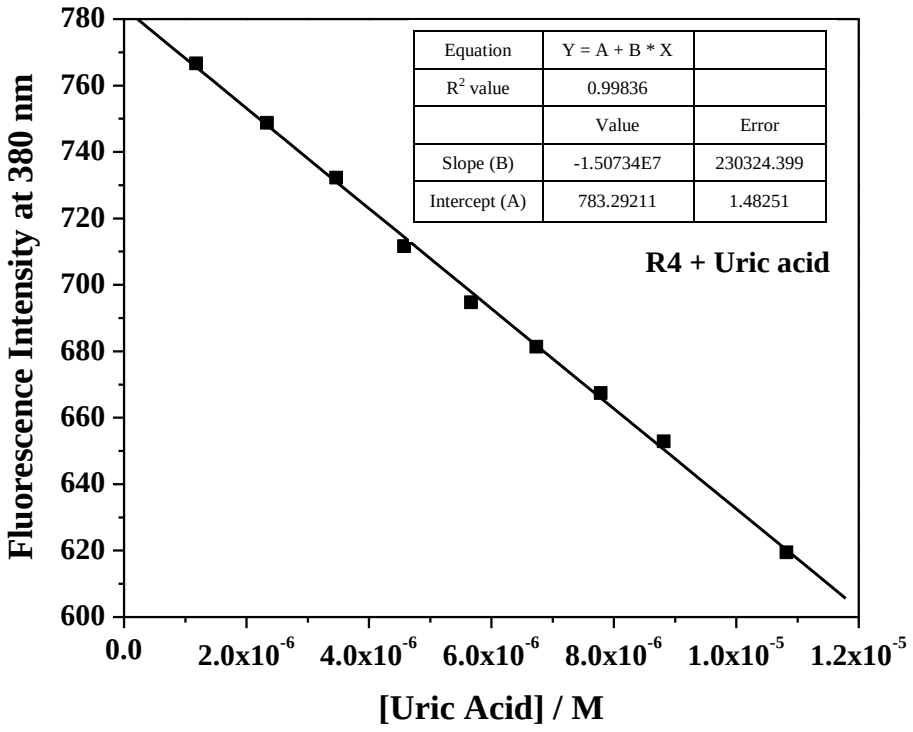


Figure: Linear fit curve of **R4** at 380 nm with respect to uric acid concentration.

References:

1. (a) M. Shortreed, R. Kopelman, M. Kuhn and B. Hoyland *Anal. Chem.* 1996, **68**, 1414; (b) Y. Yang, T. Cheng, W. Zhu, Y. Xu and X. Qian *Org. Lett.* 2011, **13**, 264; (c) W. Lin, L. Yuan, Z. Cao, Y. Feng and L. Long *Chem. Eur. J.* 2009, **15**, 5096.