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 ($\lambda_{\text{max}}$ = 351 nm) with uric acid; (ii) Fluorescence emission spectra of R1 ($\lambda_{\text{max}}$ = 380 nm) on complexation with uric acid (after excitation at 351 nm).](image)
2. Complexation studies of R2 by UV-vis and fluorescence method

Figure 2: (i) UV-Vis absorption spectra of R2 ($\lambda_{\text{max}}$= 351 nm) with uric acid; (ii) Fluorescence emission spectra of R2 ($\lambda_{\text{max}}$= 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 ($\lambda_{\text{max}}$= 355 nm) with uric acid; (ii) Fluorescence emission spectra of R3 ($\lambda_{\text{max}}$= 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 ($\lambda_{\text{max}}$ = 348 nm) with uric acid; (ii) Fluorescence emission spectra of R4 ($\lambda_{\text{max}}$ = 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-$^1$H-$[1,8]$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$_2$SO$_4$ (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 [5.2 g, Mp. 222°C-223°C].

$^1$H-NMR, d$_6$-DMSO; 300MHz): $\delta$ 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

¹H-NMR (d₆-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.), 2964, 1759, 1608 (Ar, C=C str.), 1509, 1456 (Ar, C=N str.).

¹H-NMR (d₆-DMSO; 300MHz) of complex of R2 with uric acid: δ 10.57 (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)methyldine][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 > 250⁰C; 0.4 g, yield 35%).

¹H-NMR (d₆-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.).

¹H-NMR (d₆-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$_2$Cl$_2$ 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$_3$N (0.4 mL) and CH$_2$Cl$_2$ (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$^\circ$C).

$^1$H-NMR (d$_6$-DMSO; 500MHz): $\delta$ 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: $\delta$11.08 (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

![Mass Spectra of R1](image)

IR spectra of R1

![IR Spectra of R1](image)
$^1$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
$^1$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
Linear regression analysis ($1/[G]$ vs $1/\Delta I$) for the calculation of association constant values by UV-Vis titration method:

(i) R1 with Uric acid:

![Graph showing linear fit with $K_a = 0.996 \times 10^4$](image1)

(ii) R2 with Uric acid:

![Graph showing linear fit with $K_a = 6.114 \times 10^4$](image2)
(iii) R3 with Uric acid:

\[
\text{Linear Fit of Data1_B} \\
K_a = 3.542 \times 10^4
\]

(iv) R4 with Uric acid:

\[
\text{Linear Fit of Data1_B} \\
K_a = 0.158 \times 10^4
\]
Linear regression analysis (1/[G] vs 1/ΔI) for the calculation of association constant values by Fluorescence titration method:

(i) R1 with Uric acid:

![Graph showing linear fit with Ka = 0.188 * 10^4](image)

(ii) R2 with Uric acid:

![Graph showing linear fit with Ka = 1.767 * 10^4](image)
(iii) R3 with Uric acid:

$$K_a = 1.279 \times 10^4$$

(iv) R4 with Uric acid:

$$K_a = 0.319 \times 10^4$$
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:\(^1\):\[
\text{LOD} = K \times \text{SD}/\text{S}
\]
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 = \(-7.88496 \times 10^6\), and SD value is 0.51284
Thus using the above formula we get the Limit of Detection = \(19.512 \times 10^{-8}\) 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 = \(-1.62726 \times 10^7\), and SD value is 0.49148

Thus using the above formula we get the Limit of Detection = \(9.0609 \times 10^{-8}\) 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 = $-1.52969 \times 10^7$, and SD value is 0.49559.

Thus using the above formula we get the Limit of Detection = $9.7194 \times 10^{-8}$ 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 = $-1.50734 \times 10^7$, and SD value is 0.50085
Thus using the above formula we get the Limit of Detection = $9.9682 \times 10^{-8}$ M i.e. R4 can detect uric acid up to this minimum concentration by fluorescence technique.

![Linear fit curve of R4 at 380 nm with respect to uric acid concentration.](image)

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

**References:**