Novel structurally tuned DAMN receptor for “in-situ” diagnosis of bicarbonate in environmental waters

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General information

All solvents were purchased from commercial sources and used without further purification. Starting materials, 4-nitrocinamaldehyde, 98%, (predominantly trans) and 1, 2-diaminomalenonitrile were purchased from Sigma-Aldrich. Mass spectra were recorded on a Bruker HR-MS spectrometer using CH$_3$CN as solvent. $^{13}$CMR and $^1$H nmr spectra were recorded using a Bruker instrument operating at 500MHz. Single-Crystal XRD data was collected on a Bruker APEX II diffractometer (Mo KR, $\lambda = 0.71069$ Å). Absorption measurements were carried out using an Agilent spectrophotometer (Product no: G9821A, Serial no: MY1321007, Cary Win Uv software. For absorption titration experiments, 3ml volumes of receptor in DMSO were used in a quartz cuvette (Hellma) at 25 ± 3 °C, followed by addition of stock solutions of the appropriate anions in the form of Sodium or potassium salts dissolved in Milli-Q water (resistivity=18.2 MΩcm at 25 °C). Graph plotting and liner-curve fitting was done in Origin Pro 8 and Excel 2007. The synthesis of receptor molecule was carried under ambient conditions (298 K).

Single-crystals were obtained through solvent evaporation method. Receptor was dissolved in minimal amount of DMSO and sample vial was left under ambient conditions via perforated cap. After 3-4 weeks, needle shaped crystals obtained were suitable for X-ray diffraction, were collected by filtration. Single-crystal analysis revealed that receptor molecule crystallizes in the P21/c space group.
**Synthesis and characterization of receptor**

Receptor was synthesised by the below mentioned procedure:

It involves dropwise addition of equimolar methanolic solution of diaminomalonitrile (50 mg, 0.46 mM) to a stirring solution of 4-nitrocinamaldehyde (81.94 mg) in H₂O, containing 1-2 drops of concentrated HCl. A yellow-colored solid got precipitated immediately. Later is filtered, washed several times with absolute EtOH and ultimately dried under vacuum and characterised by standard spectroscopic techniques, like NMR and HR-MS and Single-crystal XRD.

![Synthetic Scheme of receptor](image)

**Fig. 1** Synthetic Scheme of receptor.

**Characterisation of receptor (NMR and HR-MS):**

(Yellow colour, 95% yield); (Ratio of *trans* and *cis* isomers: 6:1); \(^1\)H NMR- (500MHz; \(d_6\)-DMSO): \(\delta\) 8.26, 8.22 (two diastereomers, d, \(J = 9, 9\) Hz, 2H), \(\delta\) 8.12, 8.06 (two diastereomers, d, \(J = 9, 4\) Hz, 1H), 7.98 (s, 2H), \(\delta\) 7.85, 7.93 (two diastereomers, d, \(J = 3.5, 9\) Hz, 2H), \(\delta\) 7.93, 7.57 (two diastereomers, d, \(J = 16, 16\) Hz, 1H), \(\delta\) 7.29, 7.20 (two diastereomers, dd, \(J = 8.8, 16\) Hz, dd, \(J = 8.8, 16\) Hz, 1H); \(^13\)C NMR- (500 MHz; \(d_6\)-DMSO): \(\delta\) 156.40, 147.79, 142.44, 141.27, 131.59, 128.93, 127.68, 124.64, 114.73, 114.07, 103.73. MS (HR-MS, negative mode) found 267.064 for C\(_{13}\)H\(_9\)N\(_3\)O\(_2\).
Calcd. 267.067.

**Fig. 2** $^1$H nmr spectra of receptor.
**Selectivity of receptor and Naked-eye changes**

**Fig. 5** Diagram representing exclusive recognition response of receptor 3 (16 µM) with various anions. Observations were made in presence of 10 equivalents of HCO$_3^-$, while rest have been added with more than 200 equivalents.

**Reversal of receptor carbonate interaction**
Fig. 6 Recyclable nature of receptor (3), driven by H\(^+\) sources. Observations were made with 25uM of 3 with 60uL of 10 mM HCO\(_3^\)\(^-\). The reversibility was obtained upon addition of 20 µl of 1 mM H\(_2\)SO\(_4\).

Jobs Plot

![Jobs Plot](image)

Fig. 7 Jobs plot for determination of stoichiometry of receptor-bicarbonate interaction (R = receptor 3).

Interaction of various receptor derivatives with bicarbonate

![Interaction of various receptor derivatives with bicarbonate](image)
**Fig. 8** Absorption changes of -OCH$_3$ (1) receptor with bicarbonate anion.

**Fig. 9** Absorption changes of –H (2) receptor with bicarbonate anion.

**Binding constant calculations**

For the 1:1 interaction, binding constants were obtained with the help of Benesi-Hilderbrand Plots below. Here variation of reciprocal of change in emerging absorption signal after bicarbonate interaction ($I/AA$) was fitted with respect to reciprocal of HCO$_3^-$ concentration in µM ($1/ C$). Binding constant (K) can be calculated by the below relation:

\[ K = \left(\frac{\text{Intercept}}{\text{Slope}}\right) \times 1000000 \text{ M}^{-1} \]
Fig. 10 Inverse of absorption changes of -OCH₃ (1, 28µM) receptor with inverse of HCO₃⁻ concentrations.

Fig. 11 Inverse of absorption changes of –H (2, 20 µM) receptor with inverse of HCO₃⁻ concentrations.
Fig. 12 Inverse of absorption changes of \(-\text{NO}_2\) receptor (3, 30 \(\mu\text{M}\)) with inverse of \(\text{HCO}_3^-\) concentrations.

**Sensitivity of various receptors**

Sensitivity of receptor is presented by the slope of below curve \((\theta)\), presenting variation of absorbance of emerging signal with bicarbonate anion concentrations.

Fig.13 Variation of absorbance at 470nm of \(-\text{OCH}_3\) receptor (1, 28 \(\mu\text{M}\)) with bicarbonate.
**Fig 14** Variation of absorbance at 450 nm of –H receptor (2, 20 µM) with bicarbonate anion concentrations.

**Fig. 15** Variation of absorbance at 555 nm of –NO$_2$ receptor (3, 30 µM) with bicarbonate anion concentrations.
Interaction of Hydroxide anion with receptor

Fig. 16 Interaction of hydroxide anion (OH⁻) with –NO₂ receptor (3).

Limit of Detection

Fig. 17 Calibration curve for determination of bicarbonate anion in water samples. Here receptor concentration used is 170 µM. All the experiments were carried out at 298 K.

Limit of detection (LOD) = \(3\sigma/m\)

Here “\(\sigma\)” refers to the standard deviation of blank measurements (\(n=3\)).

“\(m\)” refers to the slope of the calibration curve in the above figure.
Hence LOD = (3*0.000834/0.00006147) = 40.71µM

**Response time of receptor**

![Graph showing sensor response](image)

**Fig. 18** Sensor response of receptor (3) in presence of HCO$_3^-$ . The studies were conducted at room temperature, with receptor concentration of (20 µM) with 80-100 µM of HCO$_3^-$. 