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Development of a cell permeable red-shifted CHEF-based chemosensor for Al^{3+} ion by controlling PET

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ABSTRACT

A structurally modified quinazoline derivative (**L**) acts as highly selective chemosensor for Al^{3+} ions in DMSO–H_2O (1:9, v/v) over the other competitive metal ions. **L** shows a red shifted fluorescence after the addition of Al^{3+} ions and later the further fluorescence enhancement is due to chelation enhanced fluorescence (CHEF) through inhibition of photoinduced electron transfer (PET). This probe (**L**) detects Al^{3+} ions as low as 9 nM in DMSO–H_2O (1:9, v/v) at biological pH. The non-cytotoxic probe (**L**) can efficiently detect the intercellular distribution of Al^{3+} ions in living cells under a fluorescence microscope to exhibit its sensible applications in the biological systems.

1. Introduction

The development of selective chemosensors for the detection of various metal ions has received significant attention because of their important roles in medicine, living systems and the environment [1]. Among them, aluminium is the third most abundant element in the Earth after oxygen and silicon and there is extensive exposure of humans to aluminium due to its use in food additives, aluminium-based pharmaceuticals and storage/cooking utensils [2–5]. The World Health Organization (WHO) prescribed the average human intake of aluminium as around 3–10 mg day^{-1} and limited its drinking water concentration to 7.41 μM, the tolerable weekly dietary intake as 7 mg kg^{-1} body weight [6]. The excess intake of aluminium influences the absorption of calcium in the bowel, causing softening of the bone, atrophy and even aberrance, and also affects the absorption of iron in the blood, causing anaemia. In addition, the toxicity of aluminium causes damage to the central nervous system, which is suspected to be involved in neurodegenerative diseases such as Alzheimer's and Parkinson's diseases, gastrointestinal problems, decreased liver and kidney function, and intoxication in hemodialysis patients [7].

In recent years, many efforts have been devoted to design various chemosensors including specific for Al^{3+} ion detection [8]. However, many of them have suffered from cost of synthesis, the number of synthesis steps, selectivity, binding constant, detection limit, use of harmful organic solvents, and poor water solubility; in addition, the controlling factor for the change of fluorescence in support of the proposed mechanistic path is scarce [9]. Modification of molecular structures is the most common approach to govern the fluorescence properties of the probe. Controlling of fluorescence intensity occurred *via* different photophysical processes like PET, ICT, CHEF, FRET, *etc.* [1,8,10–11] can be possible by various factors. Of which photo-induced electron transfer (PET) acts in such a system where long range electronic interaction is possible to quench the emission in the signalling event. Thus, fluorescence intensity can be enhanced after inhibiting PET partially or fully to develop a fluorescent sensor.

Pursuing our continuous interest in exploring the controlling factor on various photophysical processes and in addition of the importance of Al^{3+} ion detection, herein we report a chemosensor, *6-[2-(4-nitrophenyl)-vinyl]-5,6-dihydrobenzo-[4,5]imidazo[1,2-c]quinazoline* (**L**), which is a positional isomer of our previous *ortho* substituted product [12] for the selective sensing of Al^{3+} ions in DMSO–H_2O (1:9, v/v) solution based on chelation-enhanced fluorescence mechanism. This simple and easy to synthesize chemosensor (**L**) has high emission yield, excellent photostability and significant fluorescent behaviour in the visible region. Furthermore, the presence of an excess of the other competitive metal ions including all alkali and alkaline earth metal ions does not affect this fluorescence enhancement behaviour of **L** due to the selective formation of **L′–Al** species.

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2. Experimental section

The spectroscopic studies of **L** were recorded in DMSO:water (1:9, v/v) HEPES buffer. The 100 mM HEPES buffer solution was used for pH study by adjusting the pH with HCl or NaOH and the *in vitro* study was carried out at biological pH ~7.4 using 50 mM PBS buffer solution. For the selectivity study of **L**, the stock solutions (~10^{-2} M) of different metal ions were prepared taking nitrate salts of Na^+, K^+, Cu^{2+}, Cr^{3+}, Pb^{2+}, Cd^{2+} and Ag^+; acetate salt of Mn^{2+} and Zn^{2+}; chloride salts of Co^{2+}, Ni^{2+}, Ca^{2+}, Hg^{2+}, Mg^{2+}, Fe^{3+}, and Fe^{2+} sulphate; in DMSO–H_2O (1:9, v/v) HEPES buffer. In this selectivity study, the amount of the metal ions was hundred times greater than that of the probe used. Fluorescence titration with aluminium nitrate was performed in DMSO–H_2O (1:9, v/v) varying the metal concentration from 0 to 100 μM and the concentration of **L** was fixed at 10 μM.

2.1. Preparation of 6-[2-(4-nitro-phenyl)vinyl]-5,6-dihydro-benzo[4,5]imidazo[1,2-c]quinazo-line (*L*)

To the ethanolic solution of *2-(2-aminophenyl)benzimidazole* (0.209 g, 1.0 mmol) *4-nitro cinnamaldehyde* (0.177 g, 1.0 mmol) was added dropwise at room temperature with continuous stirring. Then the resulting reaction mixture was refluxed for 6.0 h. After reducing the solvent on a rotary evaporator a slight yellow coloured precipitate was collected through filtration followed by washing with hot ethanol to remove the unreacted reactants. The precipitate was recrystallised from methanol for purification and was used throughout the experiment and characterisation.

$C_{22}H_{16}N_4O_2$: Anal. Found: C, 71.85; H, 4.32; N, 15.51; Calc.: C, 71.73; H, 4.38; N, 15.21. ESI-MS: $[M + H]^+$, m/z, 369.1239 (100%) (calcd.: m/z, 369.1273. IR (KBr, cm^{-1}): $\nu_{NH,}$ 3076.44, $\nu_{CH=N}$, 1628.72. ^1H NMR (400 Hz DMSO-d_6): δ, 8.07 (d, 2 H, J = 8); 7.88 (d, 1H, J = 7.6); 7.66 (t, 2H, J = 8.4); 7.62–7.55 (m, 2H); 7.24 (t, 1H, J = 7.04); 7.18–7.15 (m, 3H); 6.95(d, 1H, J = 16); 6.87 (d, 1H, J = 8); 6.80 (t, 1H, J = 8); 6.70–6.64 (m, 2H). ^{13}C NMR (400 Hz DMSO-d_6): δ, 147, 146, 144, 142.83, 142.03, 133, 131.9, 130.99, 129.90, 128.09, 124.87, 123.99, 122.52, 122.42, 118.75, 118.53, 115.25, 112, 110, 66.95. Yield: 90%.

2.2. Preparation of aluminium (III) complex (*L′-Al*)

To the methanolic solution of **L** (368.0 mg, 1.0 mmol) aluminium(III) nitrate nonahydrate (375 mg, 1.0 mmol) was added very slowly with vigorous stirring and then the reaction mixture was stirred at ambient temperature for another 5.0 h. The resulting solution thus obtained was then kept aside for slow evaporation at room temperature. After a few days, a deep yellow coloured complex was precipitated out duly washed with cold methanol and water thoroughly and then dried *in vacuo* for characterisation purpose.

$C_{22}H_{18}AlN_7O_{12}$: Anal. Found: C, 44.12; H, 3.14; N, 16.77; Calc.: C, 44.08; H, 3.03; N, 16.36. IR (cm^{-1}): ν_{NO3}, 1338. ESI-MS in methanol: $[M + Na]^+$, m/z, 622.1821 (obsd. with 8% abundance) (calcd.: m/z, 622.0727) where M = $[Al(L')(ONO_2)_3(H_2O)]$. ^1H NMR (δ, ppm in DMSO-d_6): 8.26 (s, 1H); 8.10 (d, 2H, 8); 7.94 (d, 1H, J = 8); 7.69–7.59(m, 4 H); 7.28 (t, 1H, J = 8.1); 7.22–7.20 (d, 1H, J = 15.28); 7.00 (d, 1H, J = 7.6); 6.84 (d, 1H, J = 7.6); 6.73–6.66 (m, 2H). Yield: 75%.

2.3. Preparation of cell and in vitro cellular imaging with *L*

AGS cell line used in this study was maintained in Dulbecco's Modified Eagle's Medium (DMEM) and supplemented with 10% foetal bovine serum (FBS), 100 $\mu g \cdot ml^{-1}$ of penicillin, 100 $\mu g \cdot ml^{-1}$ of streptomycin and 2 mM Glutamax at 37 °C in a humidified incubator at 5% CO_2. The adherent cultures were grown as monolayer and passed once in 4–5 days by trypsinizing with 0.25% Trypsin–EDTA. AGS cells (4×10^4 cells/mm^2), plated on cover slips, were incubated with **L** (2, 5 and 10 μM, 1% DMSO) for 30 min. After washing with 50 mM phosphate buffer of pH 7.4 containing 150 mM NaCl (PBS), required volumes of aluminium nitrate stock solution in DMSO were added such that the final concentration of aluminium nitrate adjusted to 2.0 μM, 5.0 μM and 10.0 μM (DMSO will be 1%) and incubated for 30 min. The cells were fixed with 4% paraformaldehyde for 10 min at room temperature (RT). After washing with PBS, mounted in 90% glycerol solution containing Mowiol, an anti-fade reagent, and sealed. Images were acquired using an Apotome fluorescence microscope (Carl Zeiss, Germany) using an oil immersion lens at 63× magnification. The images were analysed using the AxioVision Rel 4.8.2 (Carl Zeiss, Germany) software [13].

2.4. Cell cytotoxicity assay

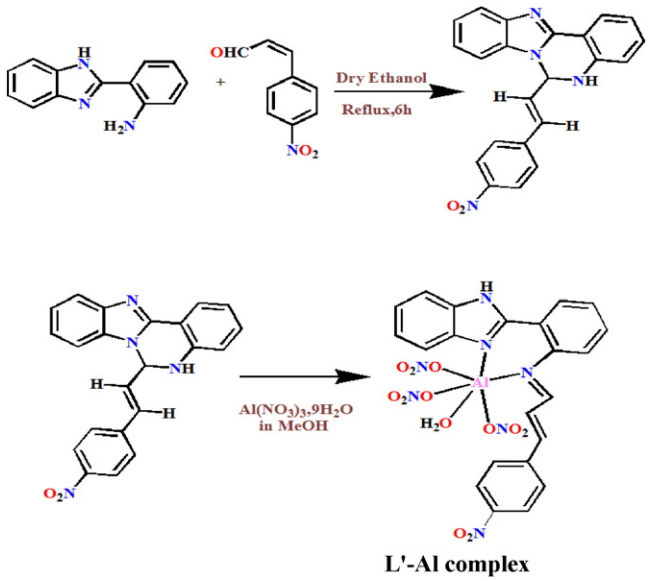
To test the cytotoxicity of **L**, MTT [*3-(4,5-dimethyl-thiazol-2-yl)-2,S-diphenyl tetrazolium bromide*] assay was performed [14].After treatments of the probe (5, 10, 25, 50, and 100 μM), 10 μl of MTT solution (10 mg/ml PBS) was added in each well of a 96-well culture plate and incubated continuously at 37 °C for 8 h. All mediums were removed from wells and replaced with 100 μl of acidic isopropanol. The intracellular formazan crystals (blue-violet) formed were solubilized with 0.04 N acidic isopropanol and the absorbance of the solution was measured at 595 nm wavelength with a microplate reader. Values are means ± S.D. of three independent experiments.

3. Results and discussion

3.1. Synthesis and characterisation

The probe (**L**) was synthesized by condensing *2-(2-aminophenyl)benzimidazole* with *4-nitro cinnamaldehyde* in ethanol medium (Scheme 1). **L** is characterised by different physico-chemical and spectroscopic tools which support the formulation of **L** (Figs. S1–S3). **L** is soluble in common polar organic solvents and sparingly soluble in water.

IR spectrum of **L** shows the characteristic stretching of ν_{N-H} and $\nu_{C=N}$ bonds at 3076 and 1628 respectively. The ESI mass spectrum of the probe (**L**) in methanol shows a peak at m/z 369.1239 with 100% abundance assignable to $[M + H]^+$ (calculated value at m/z, 369.1273) where M = molecular weight of **L** (Fig. S1) conforming the formation of the probe. Also the ^1H NMR spectrum and ^{13}C NMR of **L**



Scheme 1. Synthetic strategy of the probe **L** and its Al(III) complex (**L′-Al**).