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A simple chalcone based ratiometric chemosensor for sensitive and selective detection of Nickel ion and its imaging in live cells

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ABSTRACT

A new highly selective fluorescent chemosensor for Nickel ion was developed based on pyrene-conjugated pyridine. The ratiometric sensing of Nickel ions by the chemosensor in solution is by the formation of a monomer-excimer emissions. It's a reversible sensor and has a detection limit up to micro molar level. All the findings were supported by the DFT studies. In addition, ratiometric fluorescent changes upon the addition of nickel ions, is also applied in cell imaging.

1. Introduction

Metal ion recognition with highest sensitivity and selectivity gains immense interest as it offers cost effective, simple, quantitative and qualitative solution for probing environmentally toxic metal ions as well as to understand their role in the functions of biological systems. Such sensors should have exclusive sensitivity over the detection of targeted metal ions in the pool of different metal ions preferably in aqueous or mixture of aqueous and organic mediums [1–6]. Among the various transition metal ions, Ni^{2+} is an essential nutrient for living organisms and have been involved in the biological processes such as respiration, metabolism and biosynthesis [7–10]. While the deficiency of nickel, affects the prokaryotic and eukaryotic organisms, excess of it creates adverse effects on blood and kidneys and leads to asthma, lung cancer, sinus and other disorders in central nervous systems [11–13]. Extensive usage of Nickel in industrial applications like rechargeable batteries, electrochromic devices, supercapacitors, electroless nickel plating (ENP) technology and precursor for catalysts in chemical reaction [14–16] leads to wide exposure of nickel which eventually affect the respiratory system [17–19]. Thus, developing a method for selective sensing of Ni^{2+} in environmental, industrial, food and biological samples is highly desirable.

Various methodologies have been reported for the quantification of Ni^{2+} ions for industrial and biological samples. However, detection of Ni^{2+} ion at low concentrations are quite expensive and requires sophisticated analytical facility with time consuming, tedious sample preparation methods [20,21]. Though, these methods are accurate but not suitable for onsite analysis. On the other hand, fluorescence based methods offer cost effective, highly selective and sensitive detection of specific analytes with simple sample preparation methods [22–24]. Thus, many researchers focused on fluorescence chemosensors for Ni^{2+} due to the d^8 system of paramagnetic Ni^{2+}-ions which involves the spin forbidden process in the ground and excited states and totally affects the spectral response, which leads to severe interferences from other paramagnetic metal ions [25–30]. Nevertheless, only a minimum number of reports were available for detection of Ni^{2+} than

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other metal ions. Nearly most of the reported chemosensors for Ni^{2+} have some drawbacks such as poor water solubility, lack of selectivity, low sensitivity and quenching nature. Therefore, development of new fluorescent chemosensors for Ni^{2+}, by overcoming the above mentioned problems and also with turn-on fluorescence and ratiometric responses would be a desirable feature. Ratiometric fluorescence sensors become important as when a quantitative estimation of analytes with greater accuracies are needed [31–34]. To the best of our knowledge, no reports are available for the detection of Ni^{2+} *via* ratiometrically. Therefore, it is more important and highly challenging to synthesize ratiometric sensors for the detection of Ni^{2+}-ion in aqueous solution.

Herein, we designed and synthesized a simple chalcone based fluorescent probe **1**, which is anticipated to provide a ratiometric sensor for Ni^{2+} *via* π-π stacking between two closely bounded pyrene rings upon coordination with Ni^{2+}-ion. Therefore, pyrene was chosen as a reporter unit due to ideal monomer and excimer emission and pyridine behaves as a good coordinating ability for metal ion binding sites [35,36], are covalently linked with π-conjugated C=C bridge. DFT theoretical calculations were employed to understand the behaviour of the receptor towards the Ni^{2+} ions. The ratiometric and reversible fluorescence sensor **1** was further applied for the detection of Ni^{2+} ions in living cells.

2. Experimental

2.1. Materials and measurements

Double distilled water was used throughout all experiments. Reagents were purchased from Sigma–Aldrich and solvents were of analytical grade (Merck), used without further purification. ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker 250 and 63 MHz NMR spectrometer, with chemical shifts reported in ppm (in $CDCl_3$, TMS as internal standard). ESI-HRMS were determined on a Agilent 6520 (Q-TOF). UV–vis absorption spectra were measured using Shimadzu UV-240 spectrophotometer. Fluorescence measurements were performed using Jasco FP-8200 spectrofluorometer. All emission spectra were recorded at 24 ±1 °C. Stock solutions for analysis were prepared (2×10^{-3} M for compound **1** in MeOH-H_2O, 1:1 v/v, HEPES = 50 mM, pH = 7.0) immediately before the experiments. The solutions of metal ions Na^+, K^+, Al^{3+}, Cu^{2+}, Cd^{2+}, Hg^{2+}, La^{3+}, Pb^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+}, Ca^{2+}, Mn^{2+}, Cr^{3+}, Ba^{2+}, Ce^{3+}, Mg^{2+}, Fe^{2+}, Fe^{3+} and Ag^+ were prepared from their respective nitrate salt.

2.2. Synthesis of receptor 1 ((E)-1-(pyren-1-yl)-3-(pyridin-2-yl)prop-2-en-1-one)

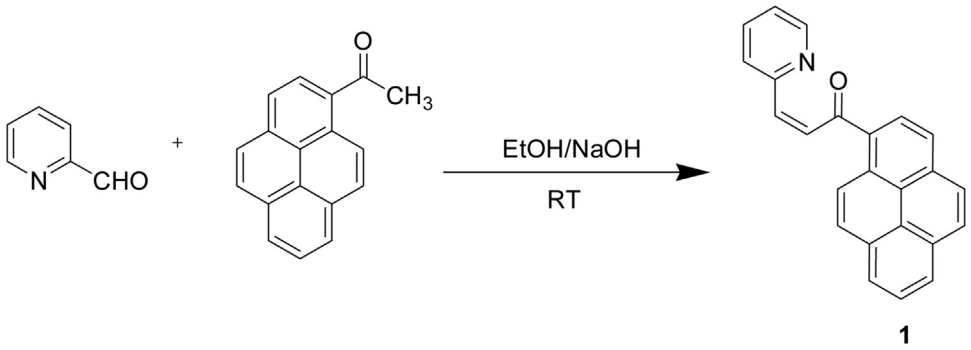
The receptor **1** was synthesized in quantitative yields (85%) as given in the Scheme 1. Briefly, aqueous sodium hydroxide (4 mL, 10%) was added to a mixture of Pyridine-2-carbaldehyde 0.2 g (1.86 mmol), 1-acetylpyrene 0.45 g (1.86 mmol) and 25 mL of ethanol. The reaction mixture was continuously stirred overnight at room temperature. The resulting precipitate was filtered, washed with excess of ethanol and recrystallized from methanol. Yield 85%. M.p. 240–242 °C, ^{1}H NMR (250 MHz, $CDCl_3$): δ: 8.65–8.74 (m, 2H), 8.33 (d, *J* = 5 Hz, 1H), 7.97–8.23 (m, 8H), 7.46–7.69 (m, 2H), 7.26 (d, *J* = 5 Hz, 1H), 7.16 (d, *J* = 2.5 Hz, 1H) ppm. ^{13}C NMR (62.5 MHz, $CDCl_3$): δ = 195.4, 153.2, 150.2, 143.8, 136.7, 133.5, 132.2, 131.1, 130.6, 130.5, 129.6, 129.3, 129.2, 127.1, 126.6, 126.3, 126.1, 126.0, 125.0, 124.9, 124.7, 124.3, 124.0 ppm. ESI-HRMS: *m*/*z* = 334.12 [M^++H].

2.3. Biocompatibility and fluorescence property of 1

The cytotoxicity of as synthesized compound **1** was evaluated using MTT assay. For this study we used HaCaT (Immortalized Human keratinocytes) as an *in vitro* model. Briefly the cell culture flask having 80% confluency of HaCaT cells were harvested by trypsinizatiion using 0.25% trypsin EDTA and approximately 15–20 thousands cells were seeded on to 24 well microtiter plate. The plate was kept for 24 h in a 5% CO_2 and 95% air humidified incubator. On the following day the cells were examined and confirmed the cells had taken morphology and attained confluency. Then these cells were treated with various concentration of the compound **1** such as 0, 25, 50, 100, 150, 175, 200 μM *etc.* and were tested. The experiment was carried out in triplicates. After addition of the complex the plates were kept in a CO_2 incubator for 24 h. After completion of 24 h incubation, the cells were viewed under fluorescence microscopy (Leica system) and documented using Qwin software. After documenting and evaluating the fluorescence the medium containing various concentrations of complex was removed and treated with 0.5 mg/ml of MTT (3-4,5dimethylthiozolyl-2)2,5-diphenyltetrazoliumbromide)salt in 1X PBS (250 μL/well) and incubated it for 4 h in a CO_2 incubator. After 4 h incubation the MTT solution was removed and added 200 μL DMSO to solubilise the blue formazan crystal. The intensity of blue coloured formazan product was measured at 570 nm using BioRad elisa plate reader.

2.4. In vitro *fluorescence study*

In order to investigate the ability of **1** as a chemosensor for Ni^{2+} ions under biological environment, *in vitro* cell based fluorescent study was performed. The experiment was designed to evaluate the fluorescence property of **1** in presence and absence of Ni^{2+} ions. Briefly, 15–20 thousands HaCaT cells/well were seeded on to 24 well mirotitre plate. The plate was kept for 24 h in a 5% CO_2 and 95% air humidified incubator. The experiment was carried out by treating the cells with 50 μM of **1** and kept in a CO_2 incubator for 24 h. After 24 h incubation the **1** containing medium was removed and exposed with different concentrations of Ni^{2+} likewise 0.1, 0.5, 1, 5, 10 and 50 μM and incubate it for one hour. After one hour



Scheme 1. Synthetic route of the receptor **1.**