Journal of Photochemistry & Photobiology A: Chemistry 364 (2018) 758–763

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology A: Chemistry

journal homepage: www.elsevier.com/locate/jphotochem

Monoterpenoid derivative based ratiometric fluorescent chemosensor for bioimaging and intracellular detection of Zn^{2+} and Mg^{2+} ions

Manohar Patil[a], Karunesh Keshav[b], Mukesh Kumar Kumawat[c], Shilpa Bothra[d], Suban K. Sahoo[d,*], Rohit Srivastava[c], Jamatsing Rajput[a], Ratnamala Bendre[a,*], Anil Kuwar[a,*]

[a] School of Chemical Science, North Maharashtra University, Jalgaon-425001, India
[b] Department of Chemistry, Indian Institute of Technology Bombay, Powai, Mumbai-400076, India
[c] Department of Biosciences and Bioengineering, Indian Institute of Technology Bombay, Powai, Mumbai-400076, India
[d] Department of Applied Chemistry, SV National Institute of Technology, Surat-395007, India

ARTICLE INFO

Keywords:
Fluorescent sensor
Zn^{2+}
Mg^{2+}
Monoterpenoid
Nanomolar detection
Cellular imaging

ABSTRACT

A new monoterpenoid based fluorescent receptor (E)-2-(5-allyl-2-hydroxy-3-methoxybenzylidene)-*N*-phenylhydrazinecarbothioamide (**1**) was synthesized and applied as a fluorescent chemosensor for the selective detection of bioactive Zn^{2+} and Mg^{2+} ions over other tested cations and anions. The selective complexation with the receptor **1** provides a fluorescence enhancement that is highly specific for the determination of Zn^{2+} and Mg^{2+} ions. Under optimal conditions, the limit of detection was estimated down to 59.4 nM and 89.1 nM for Zn^{2+} and Mg^{2+} ions, respectively. Furthermore, the receptor **1** showed good cell permeability and was successfully applied for the monitoring of Zn^{2+} and Mg^{2+} ions in live cells.

1. Introduction

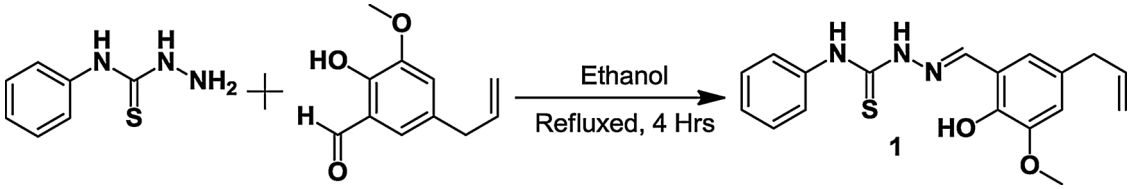
In recent years, the artificial organic receptors have significantly used for the development of chemosensors [1–5]. The chemosensor imparts through a change in magnetic, electronic or optical properties when it binds to an analyte. Out of the different chemosensing approaches, the development of fluorescence-based chemosensors gained a burgeoning interest because of simplicity, high sensitivity [6–8] and its real application in biological systems without any need of pretreatment procedure [9–12]. The chemosensor possesses a unique binding site and a light-emitting group which upon binding of the target analyte shows a selective change in the fluorescence profile [13–16]. Due to the importance of cations in the industrial, biological and environmental processes, the researchers got attracted towards the determination of metal ions [17,18].

Among the various metal ions, Zn^{2+} is the second most abundant and essential trace element after iron in the body [19,20] and known to involve in various biological processes like gene transcription, cell apoptosis, and DNA binding or recognition. It also serves as an essential ingredient for the enzyme. On the other hand, excess accumulation of Zn^{2+} ions in the body marks the symptoms of some diseases like Parkinson disease, Alzheimer's disease, Wilson's disease, prostate cancer and diabetes [21,22]. Likewise, the Mg^{2+} ions are also equally important and play vital roles in biological and environmental processes. Magnesium is a most copious intracellular metal ion essential for many processes such as ion channel regulation, DNA and protein synthesis, membrane stabilization and cytoskeletal activity. Also, the allocation of Mg^{2+} in the cytosol and subcellular areas emerges to be important in the control and regulation of the cell cycle and cell differentiation [23–26]. Therefore, there is expedite growth in the development of novel fluorescent sensors for the qualitative and quantitative monitoring of Zn^{2+} and Mg^{2+} ions. However, the magnetically silent properties and the similar coordination properties with other metal ions create challenges for the designing of selective fluorescent sensors for Zn^{2+} and Mg^{2+} ions [27–32].

In this communication, we develop a new monoterpenoid based fluorogenic receptor (E)-2-(5-allyl-2-hydroxy-3-methoxybenzylidene)-N-phenylhydrazinecarbothioamide (**1**) for the fluorescence turn-on detection of Zn^{2+} and Mg^{2+} ions with a nanomolar detection limit even in the presence of several other metal ions (Scheme 1). In this receptor **1**, the aldehyde used *i.e.* 5-allyl-2-hydroxy-3-methoxybenzaldehyde is structurally similar to eugenol found in essential oils of many plants that classified under naturally occurring phenolic monoterpenoids [33] whereas the analyte binding part N-phenylhydrazine-carbothioamide is well know to form complex with various metal ions.

* Corresponding authors.
E-mail addresses: suban_sahoo@rediffmail.com (S.K. Sahoo), bendrers@rediffmail.com (R. Bendre), kuwaras@gmail.com (A. Kuwar).

https://doi.org/10.1016/j.jphotochem.2018.07.015
Received 17 March 2018; Received in revised form 21 June 2018; Accepted 5 July 2018



Scheme 1. Synthesis of the receptor **1**.

2. Experimental

2.1. Materials and methods

All reactions were carried out by using oven-dried glassware under a slight positive pressure of nitrogen unless otherwise specified. All necessary solvents were purified before use by following the standard procedure. All chemicals were purchased from Sigma Aldrich, India. All reactions were magnetically stirred and monitored by thin-layer chromatography (TLC). The ^{1}HNMR (400 MHz) and ^{13}CNMR (100 MHz) spectra were recorded on a Bruker AVANCE II 400 spectrometer. Chemical shifts for NMR are reported in parts per million (ppm), calibrated to the solvent peak set. Fluorescence measurements were made with a HORIBA JOBIN YVON, Fluoromax-4 Spectrofluorometer equipped with a xenon lamp. UV–Vis absorption spectra were recorded on a Shimadzu UV-2450 spectrophotometer. The receptor **1** was synthesized by following the reported method and characterized by various spectral data [34].

2.2. Spectral analysis

All stocks and working solutions were prepared in ultrapure water and spectroscopic grade acetonitrile. The stock solutions of receptor **1** ($c = 1 \times 10^{-5}$M) were prepared in acetonitrile whereas the cations and anions ($c = 1 \times 10^{-4}$M) solutions were prepared in water. The UV–vis absorption and fluorescence experiments were carried out at room temperature (298 K) with the aim of determining the selectivity of the receptor **1** towards different cations such as Cr^{3+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+}, Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}, Al^{3+}, Cs^{2+} and Ag^{+}. The spectral titrations with the selective metal ions (*i.e.* Zn^{2+} and Mg^{2+}) showed the satisfactory linear relationship between the added concentrations and the fluorescence intensity. These titrations were accomplished through a stepwise addition of metal salt solutions (0.01 ml, 1×10^{-4} M) in water to a solution of receptor **1** (2 ml, 1×10^{-5} M) in acetonitrile. The fluorescence spectra were recorded at an excitation wavelength of 323 nm after each aliquot addition of the metal ion. The excitation and emission slits were both set to 5.0 nm. The collected titration data were processed by using the BindFit v0.5 program available freely at supramolecular.org website to calculate the association constant (log K_a) of the appropriate cation complexes [35]. This program allowed to fit the experimental titration data with various possible complexation modes.

2.3. Cellular imaging study

To investigate the sensing of Mg^{2+} and Zn^{2+} ions in a biological sample, ~20,000 L929 mouse fibroblasts cells were seeded over acid-etched sterilized glass coverslips immersed in each well of the 12-well plates containing complete media. The cells were allowed to grow for 24 h under humidified incubation condition with 5% CO_2 and 37 °C temperature. After incubation, the cells of one well were kept untreated considering them as control cells, and the cells from other wells were treated with the receptor **1** retaining the final concentration 50 μg/mL in the complete media. After 30 min, the treated cells were washed gently with PBS thrice to ensure the removal of traces of receptor **1** from the extracellular environment. A well with the receptor **1**-treated cells is set as a reaction control and remaining probe-treated cells from different wells are treated with the salt solutions of Mg^{2+} and Zn^{2+} ions for 10 min. The final salt concentrations in each well are set as 500 μg/mL. After treatment, the cells were gently washed thrice to remove the traces of ions and culture media. The cells were fixed using 3.7% formaldehyde solution (pH = 7.0). The cells were imaged under the confocal microscope in different filters.

3. Results and discussion

Receptor **1** was synthesized by Schiff base condensation of one mole of N-phenylhydrazine-carbothioamide with one mole of 5-allyl-2-hydroxy-3-methoxy benzaldehyde in ethanol (Scheme 1) [34]. The molecular structure of the receptor **1** was characterised by various spectral data and then applied for the sensing of cations.

The interactions of receptor **1** (1×10^{-5} M) with cations were

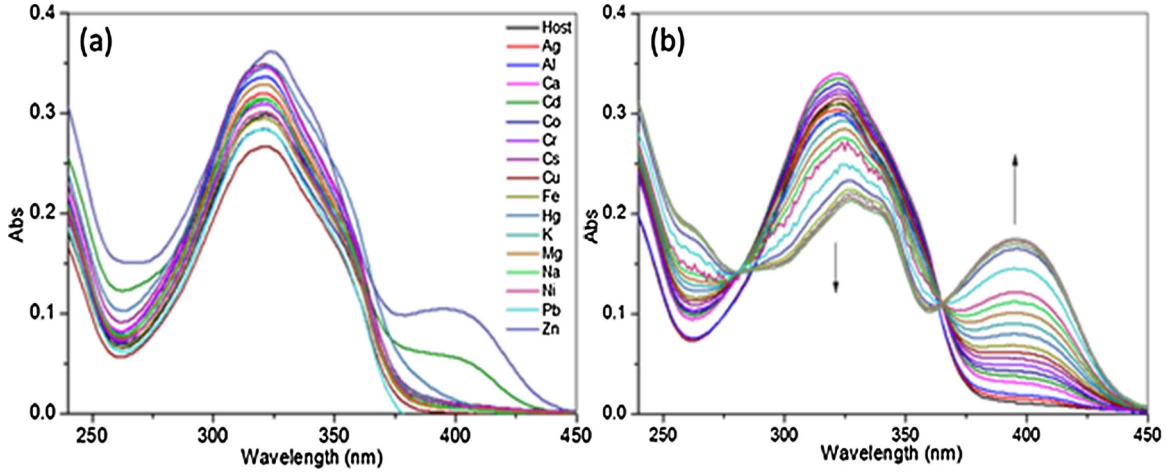


Fig. 1. The UV–vis absorption spectral changes of **1** (1×10^{-5} M) in the presence of equivalent amount of different metal ions (a), and the titration experiment with incremental addition of Zn^{2+} ions (0.01 ml, 1×10^{-4} M) (b).