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New water soluble Hg^{2+} selective fluorescent calix[4]arenes: Synthesis and application in living cells imaging

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ABSTRACT

The present study demonstrates the synthesis of water-soluble fluorescent calix[4]arenes (**6** and **7**) and its application in living cell imaging for Hg^{2+} detection at a low level. The synthesized fluorescent ligands **6** and **7** were characterized by ^1H NMR technique. The fluorescent study showed both water soluble ligands were Hg^{2+} selective and follow photo-induced electron transfer (PET) process. From the fluorimeter titration experiment detection limit was calculated as 1.14×10^{-5} and 3.42×10^{-5} for ligand **6** and 7, respectively. From the Benesi-Hildebrand plot binding constant values were evaluated as 666.7 and 733.3 M^{-1} for **6** and **7**, respectively. The interactions between ligands **6** and 7 and Hg^{2+} were also demonstrated in living cells, SW-620, using Fluorescent Cell Imager. While ligands **6** and **7** alone show fluorescent properties, they loss their action with the presence of Hg^{2+} in SW-620 cells.

1. Introduction

For the rapid, distant and valid analysis, fluorescence chemosensors have taken the lead in all techniques including atomic absorption/emission spectroscopy, inductively coupled plasma-mass spectroscopy (ICPMS), inductively coupled plasma-atomic emission spectrometry (ICP-AES) and voltammetry due to simplicity, reduced instrumental drift, real-time detection and miniaturization capabilities which are reliable and low cost [1–4]. Particularly in bioanalytical science, the combination of fluorescent sensing and bio-imaging technology can be exploited as a powerful approach to investigate biomolecules of interest with high temporal and spatial resolution in a noninvasive manner. From last few decades, the development of fluorescent chemosensors for the detection of metal ions and anions have been an important topic with much attention has been focused on new methods to monitor Hg^{2+} in biological and environmental samples [5–10]. Because mercury is one of the most toxic heavy metal and is in the list of the Agency for Toxic Substances and Disease Registry (ATSDR) of the U.S. Department of Health and Human Services with a standard maximum allowable level in dietary and environmental sources to be 2 ppb (10 nM) [11]. Due to the high affinity of Hg^{2+} for thiol groups in proteins, bioaccumulation leads to the malfunction of cells and consequently causes much damage to the brain, kidney, and central nervous system [12].

Many fluorescent chemosensors have been designed consisting of two components, an ionophore that selectively binds the guest and a fluorophore that provides a response to binding host depending on the receptor-reporter mechanism, these include photo-induced electron/energy transfer (PET), metal-ligand charge transfer (MLCT), intramolecular charge transfer (ICT), excimer/exciplex formation, imine isomerization, chelation- enhanced fluorescence (CHEF), and fluorescence resonance energy transfer (FRET) [13–16]. A number of Hg^{2+} selective fluorescent chemosensors have been designed and applied in cell imaging. Wang et.al synthesized pyrene derivatives (MS1, MS2, and MS3) containing two triazole units as fluorescent probes for detecting Hg^{2+} in living cells through fluorescence microscopy experiments [9]. In 2009, Fan et al. reported a fluorescent chemosensor, B2, for Hg^{2+} containing a BODIPY fluorophore and carboxylmetylthiol metal bonding moieties that show the concentration of ppb range with a detection limit of 77 nM. The B2 was hydrolyzed by membrane-permeable ethyl ester *in vivo*, and successfully applied to image intracellular Hg^{2+} in living cell [17]. New FRET fluorophore bearing rhodamine B and naphthalimide was developed by Song et.al for Hg^{2+} in aqueous solution. They demonstrated its value of practical applications in biological systems by fluorescence imaging experiments of Hg^{2+} in living EC 109 cells [6].

Besides, many calixarene-based fluorescence chemosensor for Hg^{2+} have been reported with specific binding sites in its rigid conformations

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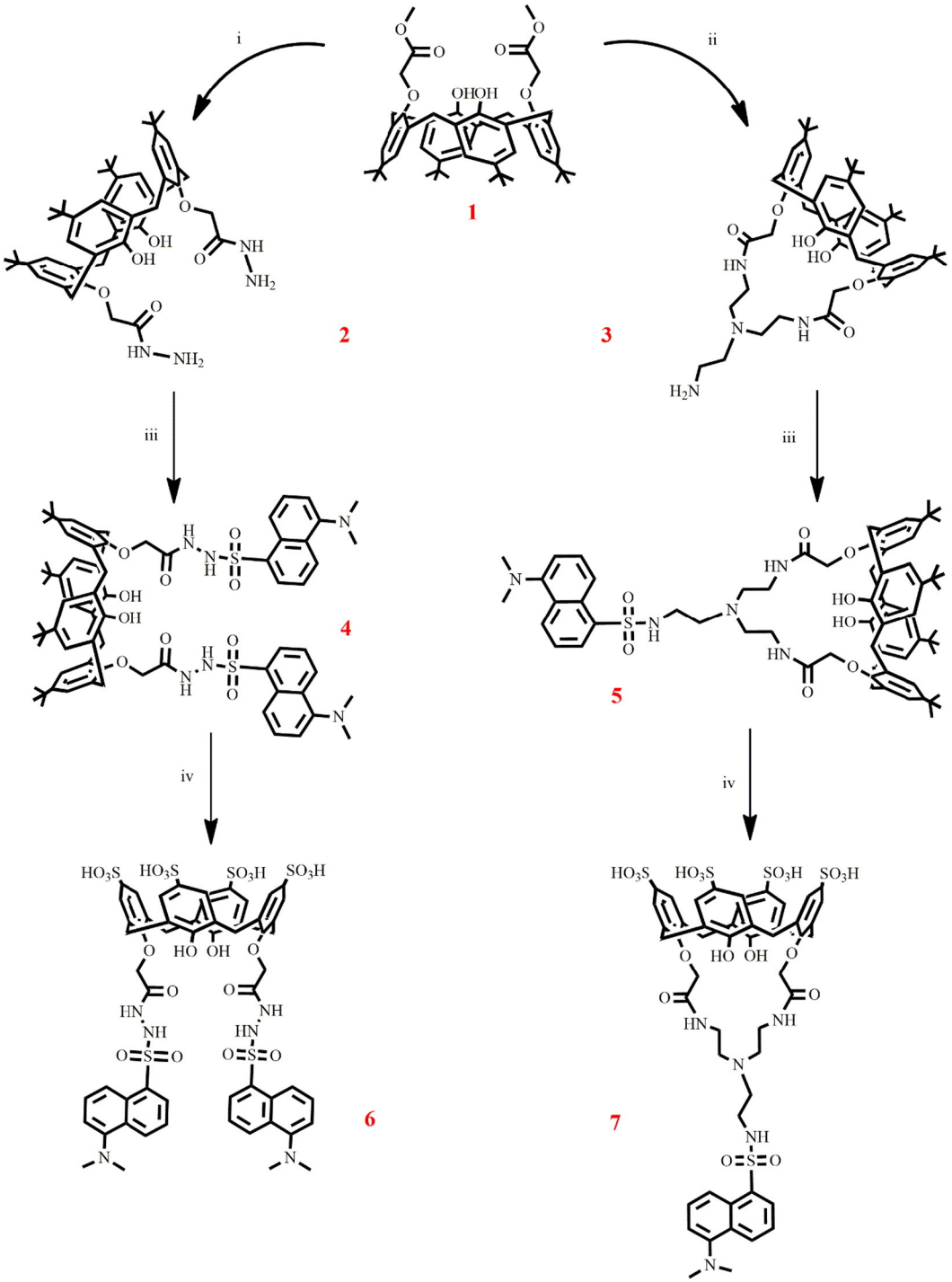
but they are not water soluble and have limited applications in biological assays [18–23]. The different types of water-soluble calix[4]arenes with hydrophilic substituents such as SO_3^-, COO^-, PO_3^-, $SO_2N(CH_2CH_2\_OH)2$, Me_3N^+, or polyethyleneoxy chains can be utilized for bioassay [24–26]. Keeping in view different possible applications of calixarenes and its properties, in this article, we report the synthesis of selective water-soluble calix[4]arene containing dansyl groups as fluorescence probes for facile detection and fluorescent imaging of biological cells containing Hg^{2+}. The synthesized fluorescent derivatives were characterized by ^1H NMR and fluorescent properties were evaluated by the fluorimeter.

2. Experimental

2.1. General section

All the reagents and solvents were commercial and used without further purification. Melting points were determined on an Electrothermal IA9100 digital melting point apparatus in a sealed capillary tube and are uncorrected. ^1H NMR spectra were referenced to tetramethylsilane (TMS) at 0.00 ppm as the internal standard solution and recorded on a Varian 400 MHz spectrometer at room temperature (25 ± 1 °C). Analytical TLC was performed on pre-coated silica gel plates



Scheme 1. The schematic route for the synthesis of ligand **6** and **7**. (i) Ethylenediamine (ii) Tris(2-aminoethyl)amine.(iii) Dansyl chloride. (iv) H_2SO_4.