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Calix[4]arene based molecular probe for sensing copper ions

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A B S T R A C T

A novel calix[4]arene based molecular probe for metal ions has been designed, synthesized and evaluated. Studies on its binding with different metal ions reveal a noticeable naked eye color change, bathochromic shift in absorption spectrum and remarkable enhancement in fluorescence emission in the presence of Cu^{2+} only. The role of calix[4]arene scaffold for selective recognition of Cu^{2+} has been demonstrated by repeat evaluation and analysis of an appropriate reference molecule. A rational explanation for fluorescence enhancement in **3** on interaction with copper has been suggested.

The development of chemosensors for recognition of copper ions has attracted immense interest because of its critical role in chemical and biological systems.[1,2] Disproportionate amounts of copper ions may lead to neurodegenerative diseases and amyotrophic lateral sclerosis.[2c] A large volume of research investigations have been documented which describe different types of sensors for copper ions which utilize UV, near infra red and emission spectroscopies and sensing mechanisms that include photoinduced electron transfer, fluorescence resonance energy transfer, internal charge transfer, nanotechnology and other photo-physical properties compatible with analysis of Cu^{2+} in biological systems.[3] Diverse nature of functions of copper ions, both beneficial and otherwise, and its analysis requirements justify strong academic interest in Cu^{2+} selective molecular probes especially because it is a typical ion that leads to fluorescence quenching inherent to its paramagnetic nature.[4] There is a significant design challenge for obtaining molecular receptors for copper that lead to enhancement of intensity of the emission spectrum.

Fluorescence[5] and colorimetric[6] sensing modes have several advantages over other method of evaluating ionic recognition due to their ease of operation, high sensitivity and straight forward application modes. Reported chemosensors for copper ions mostly involve the attachment of fluorophore units to the macrocyclic or chelating scaffolds. Among well known macrocyclic frameworks, calix[4]arenes are considered to be extremely useful building blocks for achieving ionic and molecular recognition[7] plausibly due to their preorganized cavity and facile modifiability. Coumarin and its derivatives have been extensively employed as fluorophores because of their tunable photophysical properties in the visible region.[8] Schiff bases (imines) are also known to act as good chelating sites for metal ions.[9] Thus a molecular probe possessing a calixarene scaffold, fluorescence moiety and a Schiff base substructure should be worth examining for recognition of metal ions.[10] Herein we report the synthesis of a new calix[4]arene derivative (**3**) with different functional units cited above for recognition of copper ions.

The distinct advantage expected from the designed molecular probe is that it emits in the visible region to promote least interference from background emissions.[11]

The designed molecular receptor **3** was synthesized from *p-tert*-butyl calix[4]arene via the known intermediates[10] while **2** was synthesized by following the synthetic protocol depicted in Scheme S1 (Supplementary data). Reaction of calix[4]arene derivative (**1**) with (**2**) in the presence of glacial acetic acid in ethanol yielded the product which when washed with methanol gave **3** in satisfactory yield (71%) (Scheme 1).[12–14]

The synthesized compounds were characterized by ^1H NMR, ^{13}C NMR, IR, and HRMS (ESI, Fig. S2). For example, **3** showed a >C=N– signal at 1605 cm^{-1} in IR and a sharp pair of doublets at δ 3.32 and δ 4.23 for axial and equatorial protons respectively in the ^1H NMR spectrum and a distinct signal at δ 31.18 for the methylene carbons in its ^{13}C NMR spectrum indicated its symmetric cone conformation for the calix[4]arene scaffold. It was further confirmed by observing a D_2O exchangeable singlet at δ 7.76 due to –OH protons in its ^1H NMR spectrum. Non deuterable singlets at δ 8.60 and 8.55 for the azo-methine proton (–N=CH) confirmed the structure for **3** as depicted in Scheme 1.

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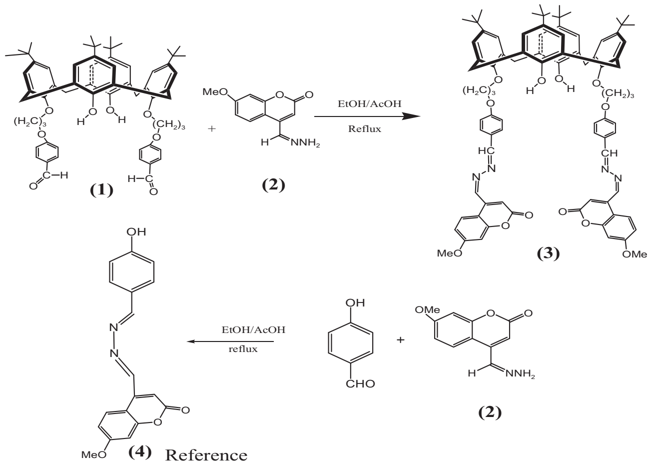

Scheme 1. Synthesis of novel calixarene derivative.

A reference compound (**4**) with similar binding sites and the signalling unit was also synthesized (Scheme 1) to evaluate the role of calix[4]arene platform in the ion recognition process.

The sensing ability of **3** was investigated by monitoring the absorption spectra on gradual addition of several metal cations such as Na^+, Li^+, K^+, Cs^+, Ag^+, Ca^{2+}, Mn^{2+}, Zn^{2+}, Co^{2+}, Cd^{2+}, Pb^{2+}, Hg^{2+}, Fe^{2+}, Ni^{2+} and Cu^{2+} as their perchlorate salts in acetonitrile. The absorption spectrum of receptor **3** in the absence of any metal ion showed a band centered around 339 nm which is characteristic of the coumarin moiety. This absorption band remained unchanged (only slight decrease in the absorbance value due to dilution was observed) on addition of 5.5 equiv of Na^+, Li^+, K^+, Cs^+, Ag^+, Ca^{2+}, Mn^{2+}, Zn^{2+}, Co^{2+}, Cd^{2+}, Pb^{2+}, Hg^{2+}, Fe^{2+}, Ni^{2+} with no color change in the solution (ESI, Fig. S3). The 339 nm band red shifted to 346 nm with decreased intensity on addition of increasing concentration of Cu^{2+} ion which was also accompanied by a color change of the solution from colorless to light yellow (Fig. 1). Thus **3** showed a specific selectivity for Cu^{2+} ion to allow its facile naked eye detection. Job's plot was deduced to determine the stoichiometry of the interaction between the Cu^{2+} ion and receptor **3**. Analysis of the data revealed that the stoichiometry of molecular complex was 1:1 (Fig. 1 inset) which was further confirmed by straight line obtained from Benesi–Hildebrand plot (ESI, Fig. S4).

Fluorescence spectra of **3** were also recorded after addition of Cu^{2+} ions to its acetonitrile solution. When excited at 340 nm, the emission spectrum of **3** showed a weak fluorescence band at 420 nm.

Fluorescence emission spectra of **3** in the presence of Cu^{2+} at various concentrations are shown in Figure 2. It was determined that addition of Cu^{2+} ions (0–130 μM) resulted in enhancement of the fluorescence signal with a 90 nm red shift in the position of fluorescence peak indicating that it can be used as turn-on fluorescence sensor for Cu^{2+}.

The enhancement in intensity of fluorescence of **3** may be rationalized by considering photoinduced electron transfer (PET) in the designed molecular probe. Such an electron transfer is facile in the Schiff base moiety. The presence of a lone pair of electrons on nitrogen results in an effective quenching of the fluorescence intensity of **3** in its off state. When Schiff base substructure interacts with Cu^{2+} ions, this photoinduced electron transfer seems to get blocked which results in enhancement of fluorescence intensity. That this indeed is the case was substantiated by the observation that fluorescence intensity also got markedly enhanced when measurements were made at low pHs plausibly due to protonation of the nitrogen lone pair of electrons. These findings are in consonance with recent literature precedents.[15] Enhancement in fluorescence intensity can also be attributed to the suppression of C=N isomerization[15a,16] in the excited state when complexation occurs with Cu^{2+} (as shown in Figure 3).

Compound **3** showed a remarkably high selectivity for Cu^{2+} which was confirmed by observing an insignificant increase in the fluorescence intensity upon addition of other metal ions (ESI, Fig. S5). Fluorescence spectra of solution of **3** on addition of different metal ions are given in Figure 4.

Selectivity coefficients were calculated for all the studied metal ions using the relationship $K_{M^{n+}, Cu^{2+}} = \Delta F_{M^{n+}}/\Delta F_{Cu^{2+}}$. It was determined that ions other than copper have a very small value for $K_{M^{n+}}/_{Cu^{2+}}$ indicating an insignificant change in the selectivity of **3** for copper.

The practical application of synthesized novel receptor **3** as fluorescence turn on probe for Cu^{2+} was also examined by recording its fluorescence response to Cu^{2+} in the presence of other competing ions. As shown in Figure 5, most of the competing ions such as Co^{2+}, Hg^{2+}, Zn^{2+}, Cd^{2+}, Mn^{2+}, Fe^{2+} and Ag^+ exhibited no conflict in the detection of Cu^{2+} in the presence of other metal ions. A little interference from nickel ions was however noticed. Thus **3** can be used for selective detection of Cu^{2+} ions even in the presence of mentioned competing ions.

Fluorescence properties of the reference compound **4** in the presence of Cu^{2+} were investigated with the aim to assess the

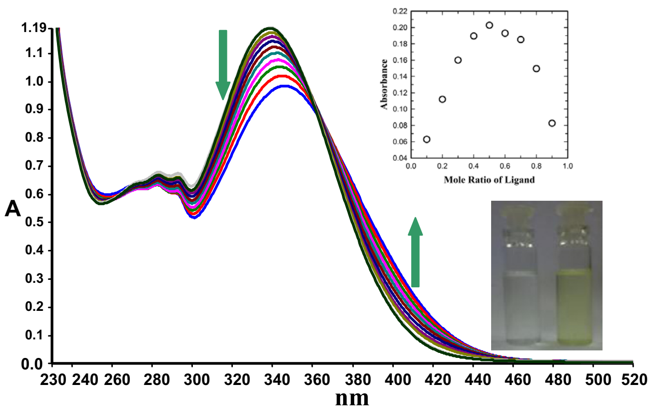

Figure 1. Change in the UV–vis spectra of Ligand (15 μM) upon addition of Cu^{2+} (0–70 μM) in ACN. Inset: Job's plot of Cu^{2+} complex formation. {[**3**]/[**3**]+[Cu^{2+}] is the mole fraction of ligand **3**}and color change after addition of Cu^{2+}.

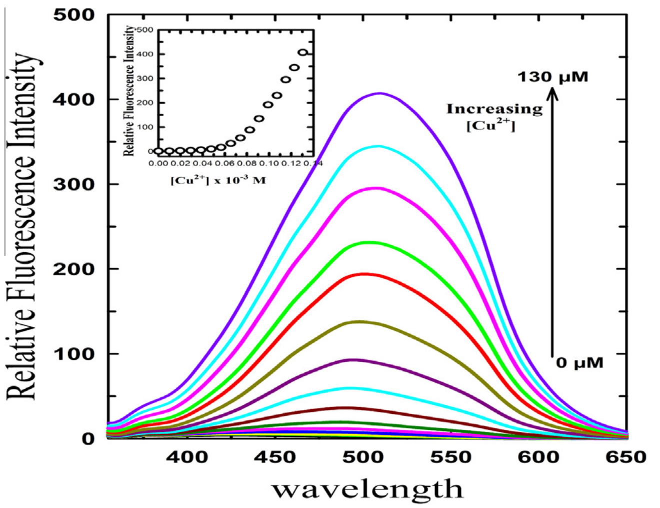

Figure 2. Enhancement in the fluorescence intensity of **3** (15 μM) in ACN in presence of Cu^{2+} (0–130 μM); Inset shows change in the fluorescence intensity of the ligand with varying concentration of Cu^{2+} (λ_{ex} = 340 nm).

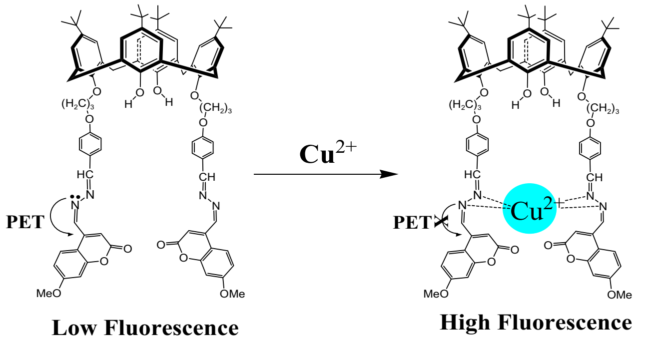

Figure 3. Proposed model for rationalizing the enhancement in fluorescence intensity upon addition of Cu^{2+} to the solution of receptor **3** (15 μM) in ACN.

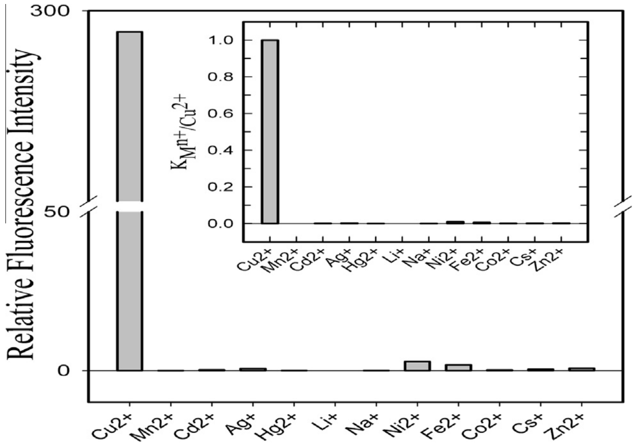

Figure 4. Change in the fluorescence intensity of the ligand **3** upon addition of 10 equiv of various metal ions to ligand in ACN (λ_{ex} = 340 nm). Inset shows selectivity coefficients, $K_{M^{n+}}/_{Cu^{2+}} = \Delta F_{M^{n+}}/\Delta F_{Cu^{2+}}$.

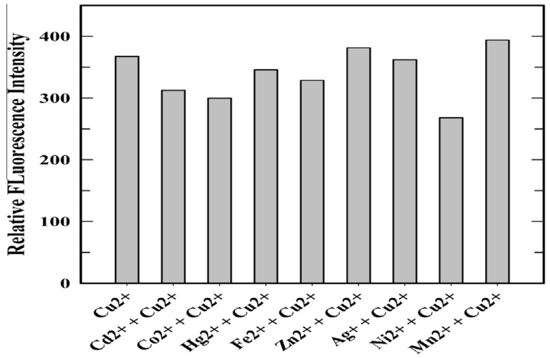

Figure 5. Comparative study of molecular receptor **3** in ACN in the presence of 10 equiv of various metal ions + 10 equiv of Cu^{2+} by exciting at 340 nm.

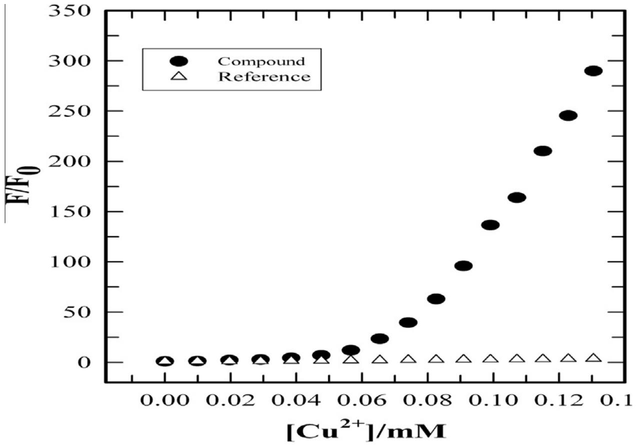

Figure 6. Comparative study of molecular receptor **3** in ACN with its reference compound **4** by exciting at 340 nm.

importance of calix[4]arene scaffold for recognition ability of ligand. Figure 6 represents a relative enhancement in the fluorescence intensity of **3** and the reference compound **4**, respectively, in the presence of Cu^{2+}. It was quite evident that the prominent fluorescence enhancement observed for ligand **3** was absent in the *reference* compound **4** thereby indicating that calixarene framework has an important control on the recognition of copper ions plausibly by providing an appropriate rigidity in the supramolecular complex.

The binding constant of **3** for Cu^{2+} was calculated to be 1.15×10^4 M^{-1} (ESI, Fig. S6). Limit of detection (LOD) for Cu^{2+} by **3** could be calculated by using calibration sensitivity (m) of relative fluorescence intensity versus $[Cu^{2+}]$ in the aforementioned range.[17] Minimum change in the fluorescence intensity due to the presence of Cu^{2+} was taken to be $3 \times s_o$ where s_o represents the standard deviation of Fo for ten replicate measurements.[18] These measurements gave the limit of detection (LOD) of copper as 0.6 μM which is fair within the recommended US EPA[19] limits of ~20 μM.

The ^1H NMR titrations revealed the presence of broadened signals for 3-Cu^{2+} complex as expected due to paramagnetic nature of Cu^{2+}.[20]

In conclusion, we have reported the synthesis and evaluation of a novel calix[4]arene derivative with appended coumarin moiety for selective recognition of Cu^{2+} ions. **3** can function as a 'turn-on' fluorescence and a chromogenic molecular probe.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2014.02.021.

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13. *Procedure for the synthesis of **3**:* To a solution of calix[4]arene derivative (**1**) in ethanol was added **2** and a catalytic amount of acetic acid. The reaction mixture was refluxed for 24 h. After completion of the reaction (TLC), the precipitate was filtered and washed with water. The structure of **4** was confirmed by ^{1}H NMR and ^{13}C NMR spectra as well as MALDI-TOF MS analysis.
14. *Analytical data for **3**:* light yellow solid; melting point: 340 °C (decomposed); UV (λ_{max}, ACN): 339 nm. IR (KBr pellet, cm^{-1}): 3445, 1682, 1605, 756; ^{1}H NMR (300 MHz, $CDCl_3$ δ in ppm): 8.60 (s, 2H, $\mathrm{C}H = \mathrm{N}_{cou}$), 8.55 (s, 2H, $\mathrm{N} = \mathrm{C}H_{calix}$), 8.46 (d, 2H, $\mathrm{Ar}H_{cou}$), 7.76 (s, 2H, D_2O exchangeable, $\mathrm{ArO}H$), 7.71 (d, 4H, $\mathrm{Ar}H$), 7.06 (s, 4H, $\mathrm{Ar}H_{calix}$), 6.99 (d, 4*H*, $\mathrm{Ar}H$), 6.87 (d + s, 6H, $\mathrm{Ar}H_{cou} + \mathrm{Ar}H_{calix}$), 6.79 (s, 2H, $\mathrm{Ar}H_{cou}$), 6.54 (s, 2H, $\mathrm{Ar}H_{cou}$), 4.42 (t, 4H, $–\mathrm{OC}H_2–CH_2–CH_2–$), 4.23 (d, 4H, $\mathrm{ArC}H_2\mathrm{Ar}$), 4.12 (t, 4H, $–OCH_2–CH_2–\mathrm{C}H_2–$), 3.87 (s, 6H, $\mathrm{OC}H_3$), 3.32 (d, 4H, $\mathrm{ArC}H_2\mathrm{Ar}$), 2.38 (qu, 4H, $–OCH_2–\mathrm{C}H_2–CH_2$), 1.28 (s, 18H, $–\mathrm{C}(\mathrm{C}H_3)_3$), 1.00 (s, 18H, $–\mathrm{C}(\mathrm{C}H_3)_3$); ^{13}C NMR (75 MHz, DMSO, δ in ppm): 164.30, 157.37, 150.09, 148.82, 141.26, 132.22, 130.55, 127.16, 125.16, 124.68, 114.66, 114.51, 112.08, 55.22, 33.53, 31.18, 30.58; HRMS (ESI-MS) m/z: calcd 1395.6604, found 1395.6506 (M+Na^{+}).
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