

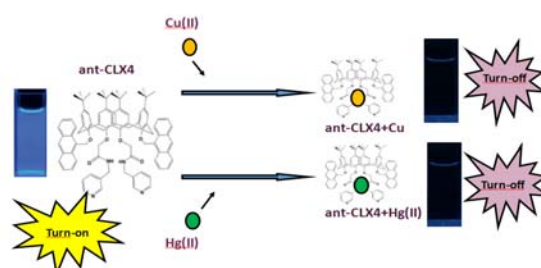
HIGHLY SENSITIVE CHEMOSENSOR FOR Cu^{2+} AND Hg^{2+} BASED ON ANTHRACENE ANCHORED CALIX[4]ARENE PYRIDINE AMIDE RECEPTOR

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In this study, anthracene anchored calix[4]arene pyridine amide (ant-CLX4) receptor was synthesized for the detection of Hg^{2+} and Cu^{2+} metal ions and fully characterized by spectroscopic methods. The ion binding properties of ant-CLX4 towards some selected metal ions such as Cu^{2+} , Hg^{2+} , Cr^{3+} , Co^{2+} , Ag^+ , Tb^{3+} , Zn^{2+} , Cd^{2+} , Ni^{2+} , Ga^{3+} , Mn^{2+} , Yb^{3+} and Gd^{3+} were studied by absorption and emission spectra. The ant-CLX4 demonstrated excellent fluorescence response with quenching mechanism (turn-off) in the presence of trace amount of Hg^{2+} and Cu^{2+} ions. This quenching response of ant-CLX4 receptor showing the possible binding interaction between ant-CLX4 receptor and metal ions was also observed by a fluorescence color change from bright blue to colorless in presence of metal ions as Hg^{2+} and Cu^{2+} . Furthermore, the binding stoichiometry of ant-CLX4 with metal ions was confirmed a 1:1 (ant-CLX4- Hg^{2+} and ant-CLX4- Cu^{2+}) binding model by Job's Plot method. The detection limits of copper and mercury ions were calculated to be 3.8×10^{-7} M and 3.3×10^{-7} M with a satisfying level for the detection of such ions in the micromolar scale, respectively. This work showed that anthracene anchored calix[4]arene pyridine amide (ant-CLX4) receptor could be used as a member of family of highly sensitive synthetic chemosensor towards toxic ions as Hg^{2+} and Cu^{2+} .



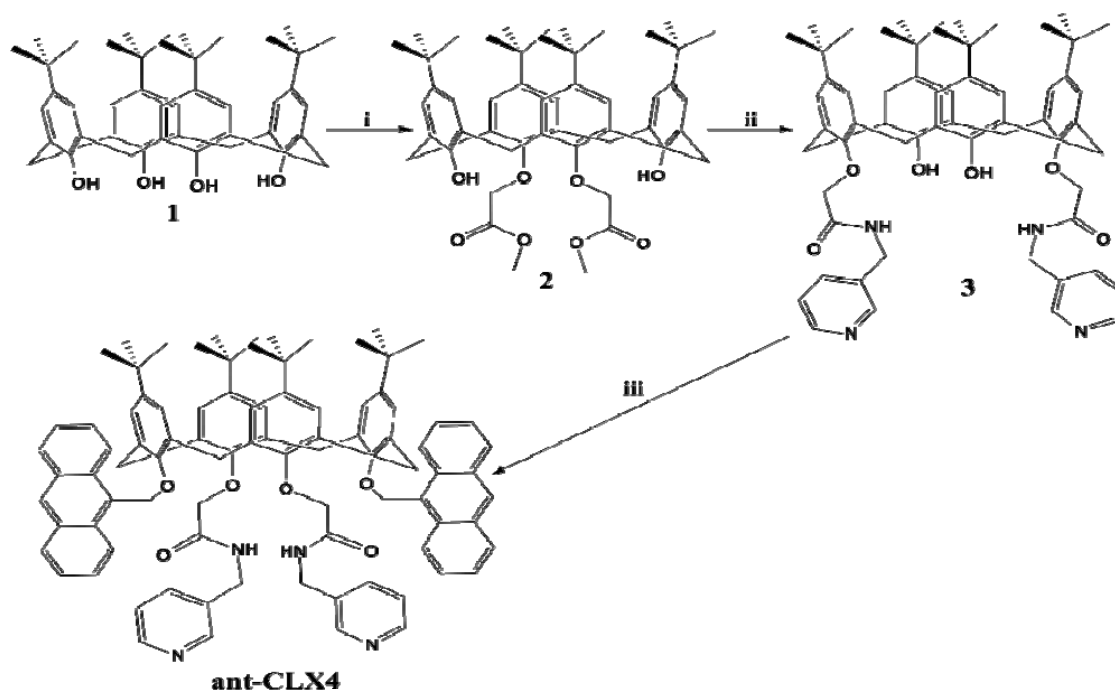
INTRODUCTION

The design and synthesis of fluorescent chemosensors for detection and removal of low-level contamination environmentally and biologically relevant ionic species, particularly for heavy metal ions, is currently of great interest.^{1,2} Therefore, fluorescent sensors for the detection and measurement of toxic metal ions are widely investigated.³ As is well-known, mercury and copper can cause serious human health problems even at lowest concentration. These metal ions can reason a number of toxicological problems such as cancer, asthma and other respiratory diseases, reproductive disorders, neurological and immune system impairments, and skin disease, as well as cardiovascular, renal, hepatic, and psychological disorders.^{4,5} Toxic metal ion is a complex case of chemical constituents encompassing

diverse chemical structures, it appears significantly threaten human health.⁶ Toxic metal contamination is commonly correlated with surface soils, waters in evaporation areas, and land water contaminated with leachate from the different sources. Recently, restoration of metal ion-contaminated sites to their original condition is a worldwide challenge.⁷ In this regard, supramolecular chemistry has improved new synthetic methods directed to research areas such as host-guest chemistry, ionic and molecular recognition, aggregation, signal transfer, fluorescent sensor, etc. Much attention has been paid to the derivatization for supramolecular that can serve as building blocks for the development of fluorescence sensor particularly for ionic guest species.^{8,9}

Fluorescent sensors offer several distinct advantages such as resolution, high sensitivity and selectivity.^{10,11} Different type of chemical sensors

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Scheme 1 – Synthesis of ant-CLX4. (i) Dry acetone, K_2CO_3 , Bromomethyl acetate, 48h, reflux under N_2 ; (ii) 3-picolinamine, toluene-methanol, 36 h, reflux under N_2 ; (iii) 9-(Chloromethyl)anthracene, dry acetone, Na_2CO_3 , NaI, 72 h, reflux under N_2 .

based supramolecular are quite abundant.^{12,13} However, sensing studies related to design and synthesis of chemical sensors including calixarene molecules that are one of the most important compounds of supramolecular chemistry are very limited.¹⁴ Calixarene molecules are macrocyclic compounds having hydrophobic cavity with upper and lower rims that can be easily functionalized by different groups.¹⁵ Since these compounds can be synthesized and derivatized more easily than other macrocyclic compounds, they are recognized as the most popular compounds in supramolecular chemistry.^{16,17} In addition, calixarene frameworks are used extensively as supramolecular platform in synthesis and various applications such as sensor, catalyst, chromatograph, resolution agent.^{18,19} These macromolecules have a large number of conformational isomers because of their different size and spacing structure. Calixarenes are usually found in the conformation of four main conformations such as cone, partial cone, 1,2-alternative and 1,3-alternative.²⁰ The cone conformation is the strongest structure for constructing more useful and stable calixarene structures than the other forms, and this conformation has less polarity than the other conformational points.^{20,21}

The design and synthesis of the calixarene chemosensor having cone conformation was still rare and very limited studies based on fluorescence quenching (turn off) response towards Cu^{2+} and

Hg^{2+} by calixarene chemosensors were presented till now. For this reason, the development of a new cone calixarene receptor decorated with anthracene and pyridine amide units for Cu^{2+} and Hg^{2+} detection is center of this presented study. From this perspective, the present work aimed to report the design, synthesis and sensor application of anthracene anchored calix[4]arene pyridine amide.

RESULTS AND DISCUSSION

Synthesis and characterization

The synthesis of ant-CLX4 molecule was given in the Scheme 1. As seen in Scheme 1, the ester derivative of p-tert-butyl-calix[4]arene (2) firstly reacted with 3-aminomethyl pyridine to obtain bis-pyridine amide functionalized calix[4]arene (3).²² And then, compound (3) was reacted with 9-(chloromethyl) anthracene to gain the finally product ant-CLX4 in suitable reaction condition. 1H and ^{13}C NMR measurements were carried out for the clarification of structure of ant-CLX4. From the NMR data, it was observed that calixarene framework was fully modified with both pyridine and anthracene units due to singlet assigned of the phenolic hydroxyl groups. In addition, one singlet around 5.7 ppm attributable to the OCH_2 protons of anthracene fragments was also observed in

^1H NMR data. Furthermore, in the ^{13}C NMR spectra of compound ant-CLX4, it was obviously seen that ant-CLX4 was symmetrical and therefore the number of signals observed in the ^{13}C NMR was lesser than the number of C atoms in the related compound ant-CLX4.

Spectroscopic studies

The chemosensor behavior of ant-CLX4 were studied with Cr^{3+} , Co^{2+} , Ag^+ , Tb^{3+} , Zn^{2+} , Cd^{2+} , Ni^{2+} , Ga^{3+} , Mn^{2+} , Cu^{2+} , Hg^{2+} , Yb^{3+} and Gd^{3+} by fluorescence and UV-vis absorption spectroscopy. Absorbance spectra of ant-CLX4 with and without metal ions was given in Fig. 1(a). As seen in Fig. 1(a), the absorption spectra of ant-CLX4 exhibited three characteristic absorption bands at around 360 nm, 375 nm and 390 nm attributable the anthracene units, respectively. These related characterization bands at around 360-390 nm may be attributable to π - π transition of the aromatic units of anthracene groups²³. The chemosensor behavior of ant-CLX4 in the presence of the above-mentioned set of metal ions indicated that only Cu^{2+} and Hg^{2+} ions showed a notable response with the increasing of absorption bands of ant-CLX4 at around 360-390 nm, while the considerable response was not observed for the other tested metal ions. When compound ant-CLX4 was complexed with Cu^{2+} and/or Hg^{2+} , the original peak at around 280 nm increased, and a

slightly bathochromic shift of absorption band of anthracene conjugated system around 360-390 nm was also observed in presence of Cu^{2+} and/or Hg^{2+} ions²⁴. In order to support changes in the absorption spectra, emission spectra of the compound ant-CLX4 with same metal ions were also studied. Therefore, the fluorescence behavior of ant-CLX4 was investigated upon addition of same metal ions in methanol/chloroform mixture at room temperature. The fluorescence spectra of ant-CLX4 were measured in the presence of each metal ions Cu^{2+} , Hg^{2+} , Cr^{3+} , Co^{2+} , Ag^+ , Tb^{3+} , Zn^{2+} , Cd^{2+} , Ni^{2+} , Ga^{3+} , Mn^{2+} , Yb^{3+} and Gd^{3+} and presented in Fig.1(b). The ant-CLX4 indicated potent emission spectra at around 490 nm when the excitation wavelength was at 330 nm. Upon addition of metal cations to solutions of ant-CLX4 except Cu^{2+} and Hg^{2+} , a considerable increase or decrease and/or shift of the maximum emission of receptor ant-CLX4 at around 430 and 490 nm was not observed for Cr^{3+} , Co^{2+} , Ag^+ , Tb^{3+} , Zn^{2+} , Cd^{2+} , Ni^{2+} , Ga^{3+} , Mn^{2+} , Yb^{3+} and Gd^{3+} excited at 330 nm (Fig.1(b)). However, an excellent quenching response was seen for ant-CLX4 in presence of Cu^{2+} , and Hg^{2+} . This fluorescence quenching response indicated that a possible strong complex formation between ant-CLX4 with Cu^{2+} or Hg^{2+} ions was occurred. Furthermore, this increase in emission spectra of ant-CLX4 showed a selective turn off fluorescence response to Cu^{2+} and Hg^{2+} among tested metal ions in organic solutions.

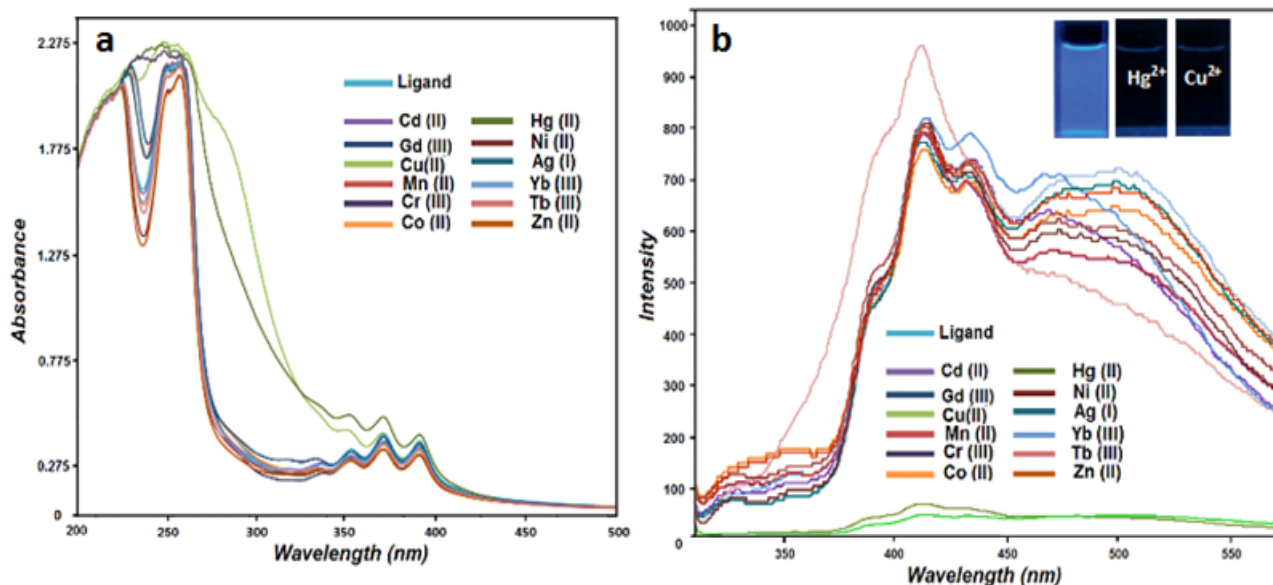


Fig. 1 – UV-vis absorption (a) and fluorescence emission (b) spectra of ant-CLX4 (1 μM) in $\text{MeOH}/\text{CHCl}_3$ upon respectively addition of metal ions (10 μM).

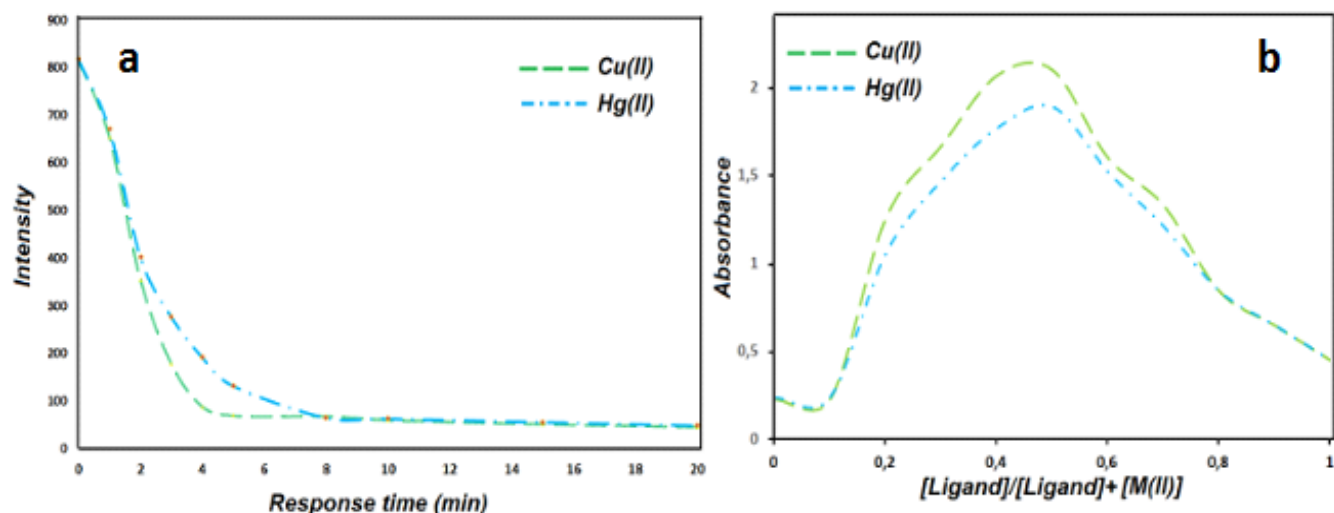


Fig. 2 – Kinetics of fluorescence spectra (a), and Job's plot (b) for ant-CLX4.

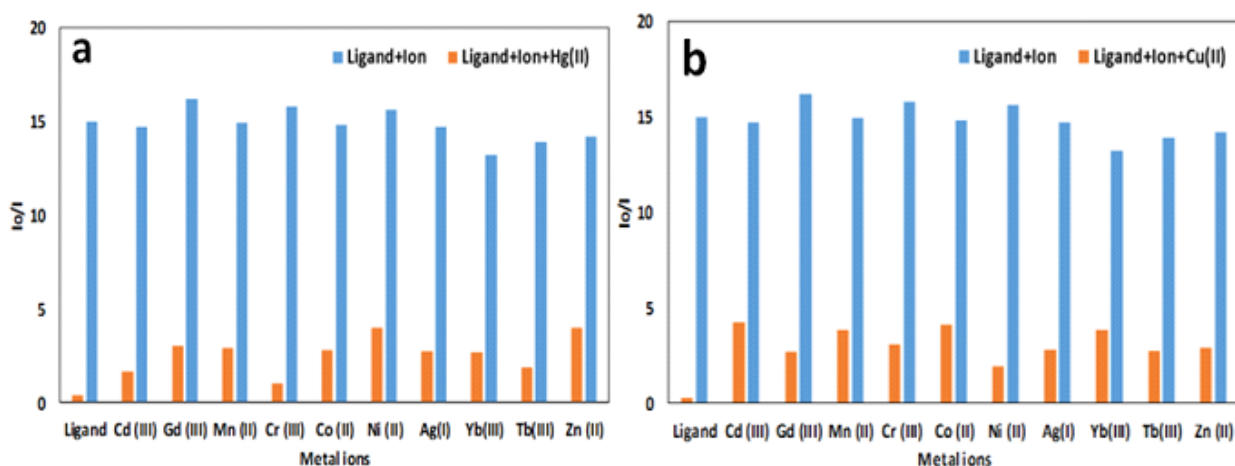


Fig. 3 – Relative fluorescence intensities of ant-CLX4 upon addition of (a) Hg^{2+} (b) Cu^{2+} ions in presence of competing metal ions.

The effect of response time on the fluorescence intensity of ant-CLX4 was investigated at 490 nm. The change in the fluorescence intensity of ant-CLX4 was specified for different selected time intervals in the range 0 – 20 min. As it can be seen in Fig. 2(a), the fluorescence intensity gradually decreased until the 4th min for Cu^{2+} and 7th min for Hg^{2+} , and then remains almost unchanged upon further increments in response time from 4 or 7 to 20 min. Moreover, no obvious emission intensity variation for receptor ant-CLX4 was observed even for prolonged response times up to 30 minutes. Thus, it was concluded that complex formation between ant-CLX4 and Cu^{2+} or Hg^{2+} , reached an equilibration state within the first seven minutes. Since long response time periods are not preferable for many fluorescent sensors, which record for the receptor ant-CLX4 is evaluated as reasonably good. The Job's plot analysis was performed to understand the binding stoichiometry of the

complex formation between ant-CLX4 and Cu^{2+} or Hg^{2+} . The binding stoichiometry of ant-CLX4 with Cu^{2+} and/or Hg^{2+} was found by keeping the total of the concentration of the metal ions and ant-CLX4 constant and varying the molar component of Cu^{2+} and Hg^{2+} from 1 to 9. In Fig. 2(b), the absorbance data was plotted against the molar fraction of Cu^{2+} and Hg^{2+} . Maximum absorbance spectra were measured for a molar fraction of 0.5, indicating a 1:1 complex formation between ant-CLX4 and Cu^{2+} and Hg^{2+} .

To verify the practical application of ant-CLX4 as an Cu^{2+} or Hg^{2+} selective and sensitive fluorescent sensor, competing experiments were also studied by adding of Cu^{2+} or Hg^{2+} into ant-CLX4 solution mixed with other coexisting metal ions such as Cr^{3+} , Co^{2+} , Ag^+ , Tb^{3+} , Zn^{2+} , Cd^{2+} , Ni^{2+} , Ga^{3+} , Mn^{2+} , Yb^{3+} and Gd^{3+} . As depicted in Fig. 3(a-b), relatively low interference was seen for the detection of Hg^{2+} or Cu^{2+} in the presence of other

competing metal ions. Although, the slightly increasing in emission intensity of ant-CLX4 at around 490 nm was observed in the presence of Zn^{2+} , and Mg^{2+} , fluorescent response was relatively detectable. However, upon addition of other competing metal ions under same conditions, it was seen that the fluorescence emission intensity at around 490 nm did not change considerably and ant-CLX4 still have an efficient “turn-off” rate for the detection of Cu^{2+} or Hg^{2+} . Consequently, it was concluded that ant-CLX4 could be a promising selective and sensitive fluorescent sensor for the detection of Cu^{2+} or Hg^{2+} in the presence of competing metal ions. Furthermore, the detection limits of Cu^{2+} and/or Hg^{2+} were estimated based on the fluorescence technique.²⁵ The detection limits of ant-CLX4 in recognizing Cu^{2+} and/or Hg^{2+} were found to be 3.8×10^{-7} M and 3.3×10^{-7} M. The successful detection of Cu^{2+} and/or Hg^{2+} in solutions in the concentration range of $10 \text{ nM} - 50 \text{ }\mu\text{M}$ indicated the applicability of the developed sensor for effective monitoring and controlling the level of Cu^{2+} and Hg^{2+} in biological and environmental systems.²⁶

EXPERIMENTAL

Synthesis of ant-CLX4

Calixarene compounds 5,11,17,23-Tetra-tert-butyl-25,26,27,28-tetrahydroxycalix[4]arene, 5,11,17,23-Tetra-tert-butyl-25,27-di-(methoxycarbonylmethoxy)-26,28-dihydroxycalix[4]arene and 5,11,17,23-Tetra-tert-butyl-25,27-bis-(2-aminomethyl-pyridinecarbonylmethoxy)-26,28-dihydroxycalix[4]arene were synthesized by following literature procedure.²² Anthracene anchored-based receptor ant-CLX4 was obtained for the first time as mentioned below (Scheme 1). To a stirred suspension of compound **3** (1 mmol) and Na_2CO_3 (10 mmol) in 200 mL dry acetone was added 9-(Chloromethyl) anthracene (2.2 mmol) in the presence of NaI (10 mmol); the reaction mixture was stirred under reflux for 72 h under nitrogen atmosphere. The solvent was removed under reduced pressure and the residue was treated with CH_2Cl_2 (40 mL) and water (30 mL). The organic phase was washed twice with water (2x25 mL), dried over MgSO_4 , and the solvent was evaporated. Column chromatography on silica gel eluting with hexane and ethyl acetate gave the pale yellow solid product ant-CLX4 in 77% yield. ¹H NMR (400 MHz CDCl_3): δ 0.67 (s, 18H, tBu), 1.21 (s, 18H, tBu), 2.3 (d, $J = 13.1$ Hz, 4H, ArCH_2Ar), 4.1 (d, 4H, $J = 13.2$ Hz, ArCH_2Ar), 4.71 (s, 4H, OCH_2CO), 5.2 (d, 4H, ArCH_2NH), 5.81 (s, 4H, $\text{OCH}_2\text{-Antr}$), 6.0 (s, 4H, ArH), 6.7 (s, 4H, ArH), 7.1 (m, 2H, PyH), 7.3 (m, 8H, Anth-H), 7.5 (d, 2H, $J = 7.8$ Hz, PyH), 7.7 (m, 2H, PyH), 7.8 (d, 4H, $J = 8.4$ Hz, Anth-H), 7.9 (d, 4H, $J = 7.2$ Hz, Anth-H), 8.4 (s, 2H, Anth-H), 8.5 (d, 2H, $J = 5.1$ Hz, PyH), 9.2 (m, 2H, NH). ¹³C NMR (CDCl_3): 173.3, 155.5, 153.7, 150.7, 149.8, 144.7, 144.2, 135.5, 134.1, 133.2, 131.4, 130.8, 127.8, 126.6, 124.9, 123.1, 122.9, 122.2, 124.4, 123.4, 122.5, 121.1, 76.9, 67.8, 47.1, 34.4, 33.1, 31.3, 31.2, 30.8. Anal. calcd. For $\text{C}_{90}\text{H}_{94}\text{O}_6\text{N}_4$: C, 81.41; H, 7.14; N, 4.22. Found: C, 81.31; H, 7.13; N, 4.14%.

Spectroscopic Studies

1 mM stock solution of ant-CLX4 was prepared in absolute $\text{MeOH}/\text{CHCl}_3$ (1:3). The stock solutions of metal ions (2 mM) Cu^{2+} , Hg^{2+} , Cr^{3+} , Co^{2+} , Ag^+ , Tb^{3+} , Zn^{2+} , Cd^{2+} , Ni^{2+} , Ga^{3+} , Mn^{2+} , Yb^{3+} and Gd^{3+} were prepared in $\text{MeOH}/\text{CHCl}_3$. The test solutions were obtained by placing 1 mL stock solution of ant-CLX4 into a test tube, added with 1 mL metal ions. The solution was mixed and measured using spectrophotometric methods (Shimadzu UV-1800 and Hitachi F-7100) at room temperature.²⁵

CONCLUSION

In summary, the synthesis and metal binding ability of a new anthracene anchored-based receptor (ant-CLX4) for Cu^{2+} and Hg^{2+} was studied. The metal binding capability of ant-CLX4 molecule towards some selected metal ions was performed by spectrophotometric measurement as absorption and emission spectra. The results demonstrated that Cu^{2+} and Hg^{2+} metal ions interacting with ant-CLX4 given rise to the fluorescence quenching (turn off) of ant-CLX4. However, no interaction with other metal ions was observed. Furthermore, selectivity experiments showed a novel approach for the selective identification of the two transition metal ions (Hg^{2+} and Cu^{2+}) among the various metal ions. All spectrophotometric experiments suggested that ant-CLX4 could be successfully applied as a fluorescence sensor with high accuracy and detection limit for the determination of Hg^{2+} and Cu^{2+} in different environments.

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