ORGANIC LETTERS

2012
Vol. 14, No. 23
6008–6011

Reactive Probes for Ratiometric Detection of Co^{2+} and Cu^{+} Based on Excited-State Intramolecular Proton Transfer Mechanism

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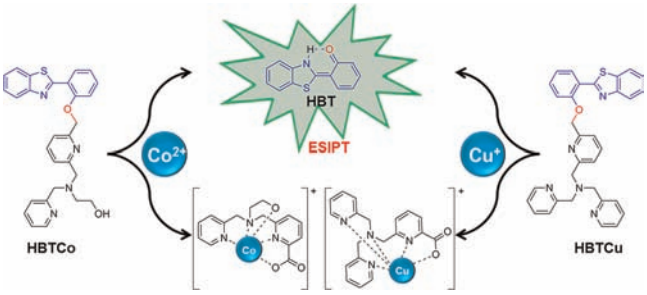
Received October 22, 2012

ABSTRACT



An excited-state intramolecular proton transfer (ESIPT) mechanism based two reactive probes HBTCo and HBTCu is reported for the selective detection of Co^{2+} and Cu^{+} respectively in a reducing aqueous environment. Co^{2+} and Cu^{+} mediated oxidative benzylic ether (C−O) bond cleavage offers ratiometric detection of these metal ions.

A number of chemosensors have been developed for the optical detection of various analytes based on conventional signaling mechanisms such as photoinduced electron transfer (PET), intramolecular charge transfer (ICT), metal−ligand charge transfer (MLCT), twisted intramolecular charge transfer (TICT), fluorescence resonance energy transfer (FRET), and excimer/exciplex formation. In recent times, a new sensing mechanism known as excited-state intramolecular proton transfer (ESIPT) is emerging as a suitable optical response for designing a molecular probe to sense biologically important species and their functions in vitro and in vivo.[1] The ESIPT chromophores exhibit interesting photophysical properties including a large Stokes shift, a desired optical response from any fluorescent probe as it minimizes artifacts such as self-absorption and an inner-filter effect.[2] Dual channel emissive ESIPT chromophores are particularly useful for developing ratiometric probes as they become strategically more advantageous over normal probes by minimizing the error arising from physical or chemical fluctuations in the sample and experimental conditions.[3] While several anion selective probes have been developed, metal cation detection based on modulation of ESIPT has not been much explored.[4]

Cobalt is an important element for all multicellular organisms present in minute amounts as part of cobalamin and a few metalloproteins.[5] Although cobalt is mildly toxic, unregulated exposure may cause detrimental effects

(1) (a) Henary, M. M.; Fahrni, C. J. *J. Phys. Chem. A* **2002**, *106*, 5210. (b) Kim, T. H.; Choi, M. S.; Sohn, B. H.; Park, S. Y.; Lyoo, W. S.; Lee, T. S. *Chem. Commun.* **2008**, 2364. (c) Wu, J.; Liu, W.; Ge, J.; Zhang, H.; Wang, P. *Chem. Soc. Rev.* **2011**, *40*, 3483.

(2) (a) Taki, M.; Wolford, J. L.; O'Halloran, T. V. *J. Am. Chem. Soc.* **2004**, *126*, 712. (b) Seo, J.; Kim, S.; Park, S. Y. *J. Am. Chem. Soc.* **2004**, *126*, 11154. (c) Zhao, J.; Ji, S.; Chen, Y.; Guo, H.; Yang, P. *Phys. Chem. Chem. Phys.* **2012**, *14*, 8803.

(3) (a) Xu, Z.; Yoon, J.; Spring, D. R. *Chem. Commun.* **2010**, *46*, 2563. (b) Zhang, J. F.; Zhou, Y.; Yoon, J.; Kim, Y.; Kim, S. J.; Kim, J. S. *Org. Lett.* **2010**, *12*, 3852. (c) Maity, D.; Govindaraju, T. *Chem. Commun.* **2010**, *46*, 4499. (d) Liu, Z.; Zhang, C.; Wang, X.; He, W.; Guo, Z. *Org. Lett.* **2012**, *14*, 4378. (e) Maity, D.; Govindaraju, T. *Chem. Commun.* **2012**, *48*, 1039. (f) Maity D.; Karthigeyan D.; Kundu T. K.; Govindaraju T. *Sens. Actuators, B* **2013**, *162*, 831.

(4) (a) Chu, Q. H.; Medvetz, D. A.; Pang, Y. *Chem. Mater.* **2007**, *19*, 6421. (b) Singh, N.; Kaur, N.; Mulrooney, R. C.; Callan, J. F. *Tetrahedron Lett.* **2008**, *49*, 6690. (c) Kim, T.; Kang, H. J.; Han, G.; Chung, S. J.; Kim, Y. *Chem.Commun.* **2009**, 5895. (d) Jung, H. S.; Kim, H. J.; Vicens, J.; Kim, J. S. *Tetrahedron Lett.* **2009**, *50*, 983. (e) Xu, Y. Q.; Pang, Y. *Chem. Commun.* **2010**, *46*, 4070. (f) Hu, R.; Feng, J.; Hu, D. H.; Wang, S. Q.; Li, S. Y.; Li, Y.; Yang, G. Q. *Angew. Chem., Int. Ed.* **2010**, *49*, 4915. (g) Xu, Z.; Xu, L.; Zhou, J.; Xu, Y.; Zhu, W.; Qian, X. *Chem. Commun.* **2012**, *48*, 10871.

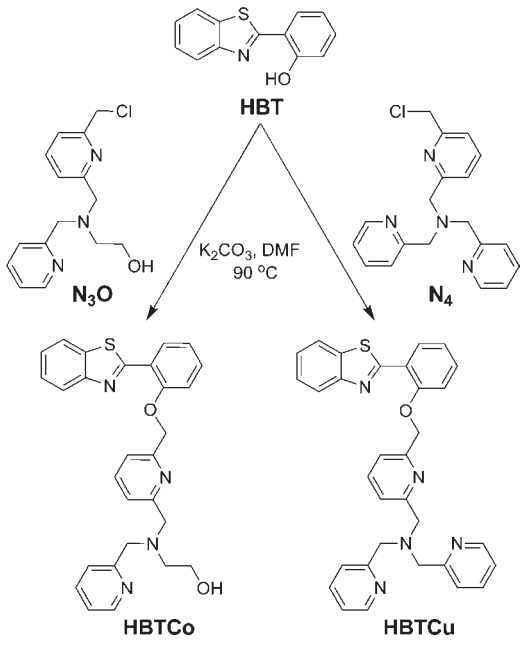
(5) Okamoto, S.; Eltis, L. D. *Metallomics* **2011**, *3*, 963.

10.1021/ol302904c
Published on Web 11/29/2012

including decreased cardiac output, cardiac and thyroid enlargements, heart disease, and elevated red blood cells accompanied by increased cells in bone marrow, increased blood volume and vasodilation, and flushing.[6] Soft transition metal copper is the third most abundant essential trace element in the human body.[7] The alterations in the cellular homeostasis of copper are connected to serious neurodegenerative diseases, including Menkes and Wilson, Alzheimer's, and prion diseases.[7] Therefore, it is necessary to develop a novel method for the detection of these transition metals in biological and environmental samples in bioavailable forms. In recent years chemists are more fascinated about developing metal-responsive fluorescent probes for their selectivity, sensitivity, and real-time monitoring of exchangeable metal ions in living cells.[8] Designing fluorescent probes for Co and Cu is challenging due to the fluorescence quenching nature of paramagnetic character associated with these metal ions. We and others have developed colorimetric and fluorometric probes for Co^{2+}.[9] Although there are several probes reported for Cu^{2+},[10] probes to monitor intracellular copper (Cu^{+}) are rare. Cu^{+} is the dominant oxidation state in a cytosolic reducing environment. Researchers have developed a few 'turn-on' probes for Cu^{+}.[11] These facts emphasize the need for the development of novel ratiometric fluorescence probes for Co^{2+} and Cu^{+} based on a common molecular platform. Furthermore such a design principle can be easily adopted to develop a ratiometric fluorescent probe library for other cations as well.

Our design strategy is based on the 2-(2′-hydroxyphenyl) benzothiazole (**HBT**) molecular platform as this ESIPT chromophore shows a large Stokes shift and corresponding efficient ratiometric fluorescence response. We designed two probes **HBTCo** and **HBTCu** with ESIPT chromophore **HBT** linked to tetradentate ligands $\mathbf{N_3O}$[9h] and $\mathbf{N_4}$[11d] respectively (Scheme 1). The tetradentate ligands $\mathbf{N_3O}$ and $\mathbf{N_4}$ linked chromophores were developed as nonfluorescent probes for reaction-based turn-on sensing of Co^{2+} and Cu^{+} by Chang et al.[9h] and Taki et al.[11d] respectively. The metal ion mediated cleaving of the benzylic ether bond in the nonfluorescent probes generates a fluorescent dye under physiologically reducing conditions. In our probes Co^{2+} and Cu^{+} assisted benzyl ether bond (C−O) cleavage releases an ESIPT active **HBT** chromophore. The released **HBT** chromophore undergoes enol to keto tautomeric transformation as a result of ESIPT, and thus a large Stokes shift in the fluorescence emission was achieved.

Scheme 1. Synthesis of **HBTCo** and **HBTCu**



In this letter, we report the synthesis and fluorometric properties of two ESIPT probes for the detection of Co^{2+} and Cu^{+} under physiologically reducing conditions. **HBTCo** and **HBTCu** were synthesized by linking **HBT** with tetradentate $\mathbf{N_3O}$ and $\mathbf{N_4}$ respectively under basic conditions in excellent yields (Scheme 1). The metal ion selective $\mathbf{N_3O}$ and $\mathbf{N_4}$ ligands were synthesized following the literature procedure.[9h,12] We studied the fluorescence properties of **HBTCo** and **HBTCu** in aqueous buffer solution (50 mM HEPES, pH 7.2) in the presence of 2 mM glutathione (GSH) for mimicking the intracellular

(6) (a) Barceloux, D. G. *Clin. Toxicol.* **1999**, *37*, 201. (b) Seldén, A. I.; Norberg, C.; Karlson-Stiber, C.; Hellström-Lindberg, E. *Environ. Toxicol. Pharmacol.* **2007**, *23*, 129.

(7) (a) Kim, B. E.; Nevitt, T.; Thiele, D. J. *Nat. Chem. Biol.* **2008**, *4*, 176. (b) Domaille, D. W.; Que, E. L.; Chang, C. J. *Nat. Chem. Biol.* **2008**, *4*, 168. (c) Maity, D.; Govindaraju, T. *Chem.—Eur. J.* **2011**, *17*, 1410. (d) Nagarjun, N.; Govindaraju, T. *Sens. Actuators, B* **2012**, *161*, 304.

(8) (a) Hargrove, A. E.; Nieto, S.; Zhang, T.; Sessler, J. L.; Anslyn, E. V. *Chem. Rev.* **2011**, *111*, 6603. (b) Maity, D.; Govindaraju, T. *Eur. J. Inorg. Chem.* **2011**, 5479. (c) Zhou, Y.; Yoon, J. *Chem. Soc. Rev.* **2012**, *41*, 52.

(9) (a) Monteil-Rivera, F.; Dumonceau *J. Anal. Bioanal. Chem.* **2002**, *374*, 1105. (b) Montalti, M.; Prodi, L.; Zaccheroni, N. *J. Mater. Chem.* **2005**, *15*, 2810. (c) Lin, W.; Yuan, L.; Long, L.; Guo, C.; Feng, J. *Adv. Funct. Mater.* **2008**, *18*, 2366. (d) Yao, Y.; Tian, D.; Li, H. *ACS Appl. Mater. Interfaces* **2010**, *2*, 684. (e) Zhen, S. J.; Guo, F. L.; Chen, L. Q.; Li, Y. F.; Zhang, Q.; Huang, C. Z. *Chem. Commun.* **2011**, *47*, 2562. (f) Grabchev, I.; Staneva, D.; Dumas, S.; Chovelon, J.-M. *J. Mol. Struct.* **2011**, *999*, 16. (g) Maity, D.; Govindaraju, T. *Inorg. Chem.* **2011**, *50*, 11282. (h) Au-Yeung, H. Y.; New, E. J.; Chang, C. J. *Chem. Commun.* **2012**, *48*, 5268.

(10) (a) Jung, H. S.; Park, M.; Han, D. Y.; Kim, E.; Lee, C.; Ham, S.; Kim, J. S. *Org. Lett.* **2009**, *11*, 3378. (b) Ko, K. C.; Wu, J.-S.; Kim, H. J.; Kwon, P. S.; Kim, J. W.; Bartsch, R. A.; Lee, J. Y.; Kim, J. S. *Chem. Commun.* **2011**, *47*, 3165. (c) Maity, D.; Manna, A. K.; Karthigeyan, D.; Kundu, T. K.; Pati, S. K.; Govindaraju, T. *Chem.—Eur. J.* **2011**, *17*, 11152. (d) Jo, J.; Lee, H. Y.; Liu, W.; Olasz, A.; Chen, C.-H.; Lee, D. *J. Am. Chem. Soc.* **2012**, *134*, 16000.

(11) (a) Zeng, L.; Miller, E. W.; Pralle, A.; Isacoff, E. Y.; Chang, C. J. *J. Am. Chem. Soc.* **2006**, *128*, 10. (b) Viguier, R. F. H.; Hulme, A. N. *J. Am. Chem. Soc.* **2006**, *128*, 11370. (c) Verma, M.; Chaudhry, A. F.; Morgan, M. T.; Fahrni, C. J. *Org. Biomol. Chem.* **2010**, *8*, 363. (d) Taki, M.; Iyoshi, S.; Ojida, A.; Hamachi, I.; Yamamoto, Y. *J. Am. Chem. Soc.* **2010**, *132*, 5938. (e) Chaudhry, A. F.; Verma, M.; Morgan, M. T.; Henary, M. M.; Siegel, N.; Hales, J. M.; Perry, J. W.; Fahrni, C. J. *J. Am. Chem. Soc.* **2010**, *132*, 737. (f) Dodani, S. C.; Leary, S. C.; Cobine, P. A.; Winge, D. R.; Chang, C. J. *J. Am. Chem. Soc.* **2011**, *133*, 8606. (g) Morgan, M. T.; Bagchi, P.; Fahrni, C. J. *J. Am. Chem. Soc.* **2011**, *133*, 15906. (h) Chaudhry, A. F.; Mandal, S.; Hardcastle, K. I.; Fahrni, C. *J. Chem. Sci.* **2011**, *2*, 1016. (i) Hirayama, T.; Van de Bittner, G. C.; Gray, L. W.; Lutsenko, S.; Chang, C. J. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 2228. (j) Cao, X.; Lin, W.; Wan, W. *Chem. Commun.* **2012**, *48*, 6247.

(12) Lucchese, B.; Humphreys, K. J.; Lee, D.; Incarvito, C. D.; Sommer, R. D.; Rheingold, A. L.; Karlin, K. D. *Inorg. Chem.* **2004**, *43*, 5987.

environment (Figure 1). Upon 350 nm excitation probes **HBTCo** and **HBTCu** emit blue fluorescence (E_{max} = 380 nm) that correspond to **HBT** 'enol-form' emission. The fluorometric behavior of 20.0 μM probes was investigated in the presence of several metal ions such as Li^+, Na^+, K^+, Ba^{2+}, Mg^{2+}, Ca^{2+}, Al^{3+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^+, Cu^{2+}, Zn^{2+}, Cd^{2+}, Ag^+, Hg^{2+}, and Pb^{2+} after 2 h of mixing. The examined millimolar alkali and alkaline earth metals showed no or negligible change in the fluorescence of **HBTCo** and **HBTCu**. While the addition of Co^{2+} to the **HBTCo** solution led to quenching of the blue emission (E_{max} = 380 nm) and subsequent appearance of a new intense emission band in the green region (E_{max} = 510 nm) (Figure 1a). Other metal ions tested quenched the blue fluorescence of **HBTCo** to different extents, but there was no new emission band in the green region. The observed large red shift (130 nm) in emission indicates the Co^{2+} catalyzed oxidative cleavage of benzylic ether (C−O) linkage in the presence of O_2 releasing ESIPT active phenolic **HBT**. The decrease in blue emission and corresponding increase in the green emission band was also observed during sequential addition of Co^{2+} in a titration experiment. A minimum of 20.0 μM Co^{2+} can be easily detected by this ratiometric study employing **HBTCo** (Figure S1, Supporting Information). A time dependent study showed that the addition of Co^{2+} quenches **HBTCo** blue fluorescence (E_{max} = 380 nm) rapidly with the appearance of a new peak around 460 nm (Figure 2a). The rapid fluorescence quenching of the probe is due to binding of paramagnetic Co^{2+}. Subsequent Co^{2+} catalyzed oxidative cleavage of the benzyl ether bond releases **HBT**. The weak emission band at 460 nm is due to an enolic rotamer,[2c] as the Co^{2+} coordination with the phenolic (−OH) moiety seems to initially stabilize the enol rotamer. Thus a weak emission band at 460 nm may be viewed as evidence for the existence of an enolic intermediate. Then, the enol form slowly converts to a keto tautomer upon excitation to emit bright green fluorescence at a longer wavelength (E_{max} = 510 nm).

Similarly, quenching of blue fluorescence (E_{max} = 390 nm) accompanied by a new intense green emission band at E_{max} = 515 nm was observed when **HBTCu** was treated with copper ions (Figure 1b). This significant transformation in fluorecence behavior of **HBTCu** is indistinguishable for both oxidative states of copper, as GSH in the medium rapidly reduces Cu^{2+} to Cu^+. Other control metal ions did not show blue fluorescence quenching or a new emission band in the green region. The ESIPT based ratiometric emission study helped to improve the detection limit of Cu^+ down to a submicromolar concentration (Figure S2). A time dependent study showed that the blue emission band around 390 nm decreases with the increase in intensity of the green emission band around 515 nm upon addition of just 1.0 μM Cu^+ after a period of 1 h (Figure 2b). In contrast to **HBTCo**, no emission band around 460 nm is observed with **HBTCu**; instead a clear isoemissive point is found at 460 nm. As expected Cu^+ catalyzed oxidative cleavage of the benzylic ether (C−O) bond in **HBTCu** releases the ESIPT active **HBT** fluorophore in the presence of O_2. The phenolic **HBT** rapidly

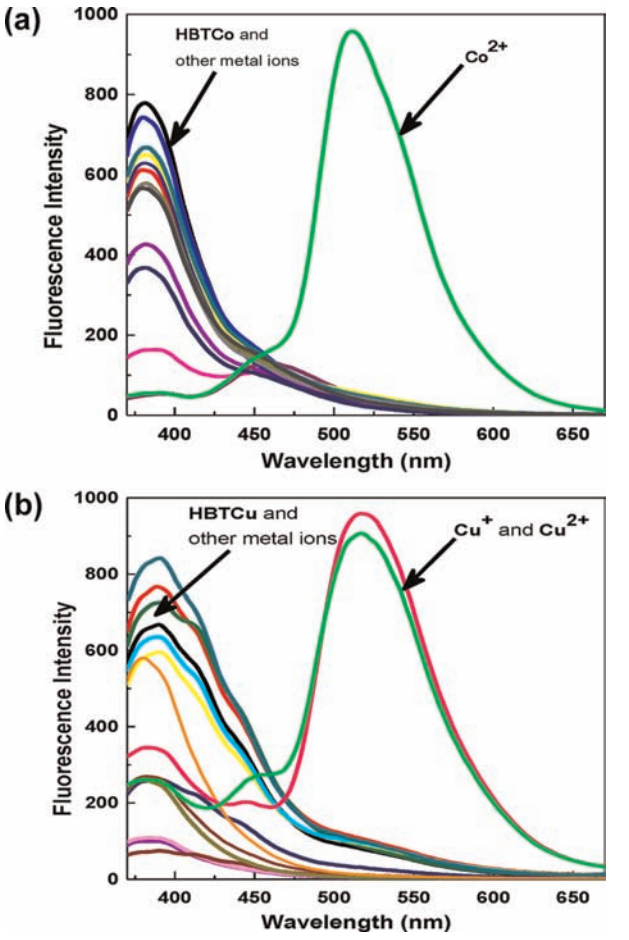


Figure 1. Fluorescence responses of (a) **HBTCo** (20 μM) and (b) **HBTCu** (20 μM) and upon addition 1 mM of Li^+, Na^+, K^+, Ba^{2+}, Mg^{2+}, Ca^{2+}, and Al^{3+} and 20 μM of Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^+, Cu^{2+}, Zn^{2+}, Cd^{2+}, Ag^+, Hg^{2+}, and Pb^{2+} after 2 h in aqueous solution (50 mM HEPES, pH 7.2, 2 mM GSH) (λ_{ex} = 350 nm).

transformed to the keto-form upon excitation, which is responsible for green fluorescence emission (ESIPT) at a longer wavelength (515 nm, Stokes shift = 125 nm).

The effect of pH on the Co^{2+} and Cu^+ mediated oxidative cleavage of the benzylic ether bond was studied to understand the efficiency of the process (Figures S3 and S4). **HBTCo** and **HBTCu** reacted efficiently with Co^{2+} and Cu^+ respectively in the biologically relevant pH range of 6.5−8.5 to release the ESIPT active **HBT** fluorophore. Hence, these probes are very convenient for the ratiometric detection of Co^{2+} and Cu^+ without interference from the pH-dependent effects. The mass peak at m/z 346.16 corresponds to the carboxylated **N_3O**-Co complex (calcd 346.06 for $C_{15}H_{15}CoN_3O_3$) and that at m/z 396.26 corresponds to the carboxylated **N_4**-Cu complex (calcd 396.06 for $C_{19}H_{17}CuN_4O_2$), in agreement with the literature data (Figures S5 and S6).[9h,11d] The ESI-MS data confirmed the Co^{2+} and Cu^+ mediated oxidative benzylic ether (C−O) bond cleavage reaction in **HBTCo**/**HBTCu** and

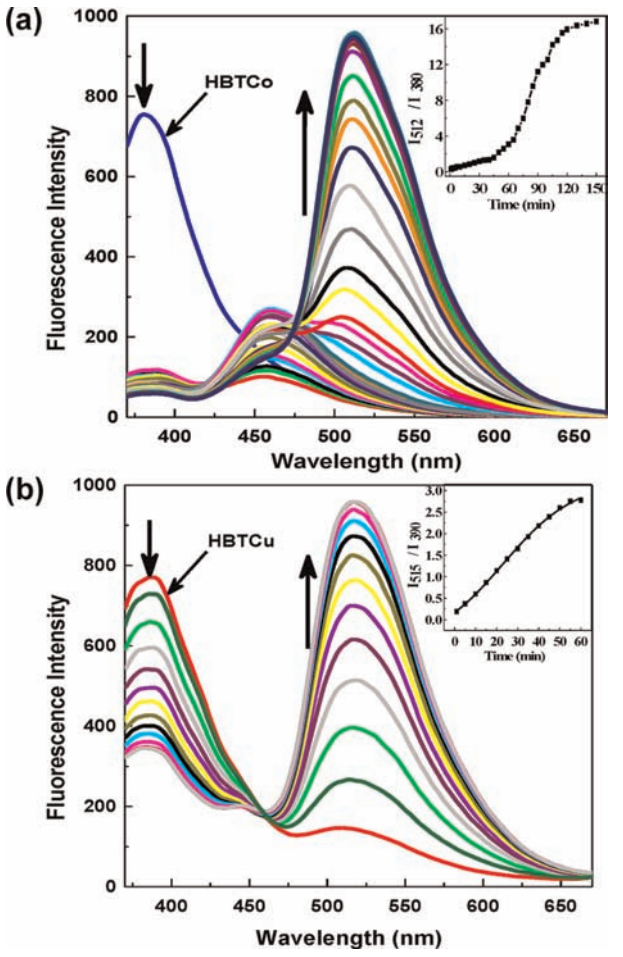
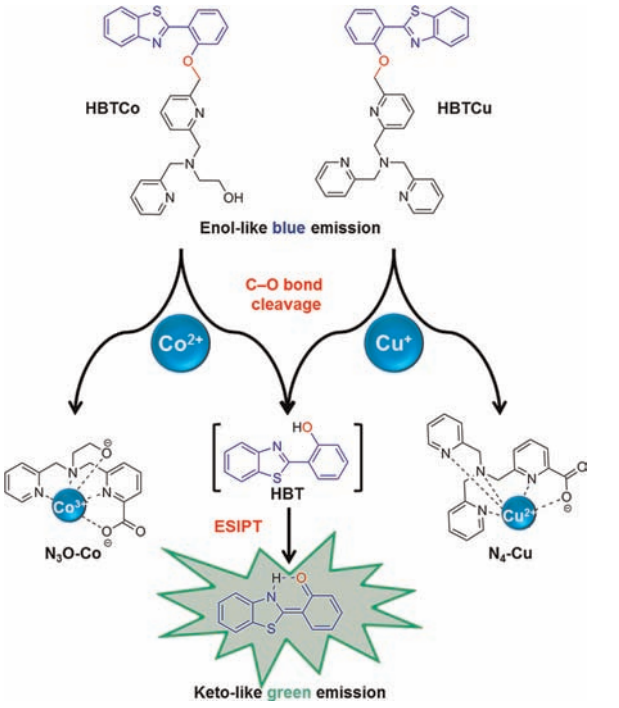


Figure 2. Time dependent fluorescence responses of (a) **HBTCo** (20 μM) and (b) **HBTCu** (20 μM) after addition of 100 μM of Co^{2+} and 1 μM of Cu^{+} respectively in aqueous solution (50 mM HEPES, pH 7.2, 2 mM GSH) (λ_{ex} = 350 nm).

formation of Co/Cu complexes with carboxylated pentadentate ligands $\mathbf{N_3O}$ and $\mathbf{N_4}$ respectively (Scheme 2). We propose a tentative meachanism for the metal ion (Co^{2+}/Cu^{+}) catalyzed oxidative clavage of the benzylic ether (C−O) bond between the **HBT** and $\mathbf{N_3O/N_4}$ ligand based on the C−N bond cleavage reported by C. J. McKenzie et al.[13] The benzylic carbon of the ligand ($\mathbf{N_3O/N_4}$) oxidized to a carboxylate via benzylic radical formation in the presence of activated oxygen (Figures S9 and S10). A subsequent transformation involves the formation of an oxonium ion which hydrolyzed to aldehyde liberating ESIPT active **HBT**. The aldehyde further oxidized to a carboxylate by the hydroperoxide of the metal complex through a Bayer−Villiger type reaction to form the final $\mathbf{N_3O}$-**Co** and $\mathbf{N_4}$-**Cu** complexes (Scheme 2).[14] Structurally, **HBTCo** and **HBTCu** only differ in the single cation coordination (hydroxyl/pyridyl) site. This minor

(13) Vad, M. S.; Nielsen, A.; Lennartson, A.; Bond, A. D.; McGrady, J. E.; McKenzie, C. J. *Dalton Trans.* **2011**, *40*, 10698.
(14) Bayer, A.; Villiger, V. *Ber.* **1899**, *32*, 3625.

Scheme 2. Co^{2+}/Cu^{+} Mediated Release of ESIPT Fluorophore **HBT** from **HBTCo** and **HBTCu** Respectively



change in the coordination site played a major role by rendering differential metal ion selectivity to the probes, as C−O bond cleavage proceeded through the formation of the Co/Cu complex with pentadentate moieties of $\mathbf{N_3O/N_4}$ (Scheme 2). The reducing environment created by GSH is very crucial as confirmed by control exeriments in the absence of GSH, which did not lead to benzylic ether bond cleavage (Figures S7 and S8).

In conclusion, we developed a novel ESIPT-based molecular platform (**HBTCo** and **HBTCu**) for ratiometric fluoroscence detection of Co^{2+} and Cu^{+} under physiologically reducing conditions. The metal ion catalyzed oxidative benzylic ether bond cleavage was effectively used in the switch-on ESIPT in **HBT** for fluoremetric detection of paramagnetic metal ions overcoming their inherently associated fluorescence quenching property. These ratiometric probes can be efficiently used for monitoring Co^{2+} or Cu^{+} in physiological and environmental samples. Generalizing this design strategy for the development of ESIPT-based ratiometric probes for other analytes is in progress.

Acknowledgment. We thank Prof. C. N. R. Rao, FRS for encouragement, JNCASR and the DST, India for a research grant, and CSIR for awarding SRF to D.M.

Supporting Information Available. Experimental and synthetic and meachanistic details, characterization, and spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.