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A red fluorescence '*off–on*' molecular switch for selective detection of Al^{3+}, Fe^{3+} and Cr^{3+}: experimental and theoretical studies along with living cell imaging†

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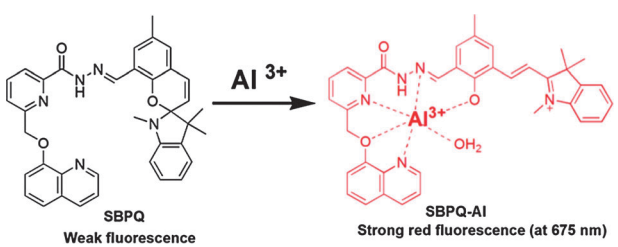
A spirobenzopyran-quinoline (SBPQ) based sensor was synthesized which selectively detects trivalent ions *viz.* Al^{3+}, Fe^{3+} and Cr^{3+} through a fluorescence turn on signal in the red region (~675 nm) with the detection limit in the order of 10^{-8} M. The potentiality of the probe was confirmed by employing it for fluorescence bio-imaging with Al^{3+} in three different types of live-cells.

Trivalent ions such as Al^{3+}, Fe^{3+} and Cr^{3+} play many important roles in living organisms. Aluminium is the most abundant metallic element in the earth's crust and is extensively used in our daily life, such as aluminium-based pharmaceuticals and storage/cooking utensils which results in a moderate increase in the Al^{3+} concentration in food. Alzheimer's disease, osteoporosis, colic, rickets, gastrointestinal problems, anaemia, headache, memory loss and aching muscles can all be caused by aluminium toxicity.[1] Iron in its trivalent form is indispensable for most organisms, as it is involved in both electron transfer and oxygen transport. High levels of Fe^{3+} within the body have been associated with increasing incidence of certain cancers and dysfunction of certain organs, such as the heart, pancreas, and liver.[2] Trivalent chromium, Cr^{3+}, is one of the most important heavy metal elements. Cr^{3+} adversely affects cellular structures and plays an important role in the metabolism of carbohydrates, lipids, proteins and nucleic acids.[3] In the past few years, many sensors have been reported for selective detection of Al^{3+}, Fe^{3+} or Cr^{3+} (ref. 4–6) while a single sensor which can detect all these trivalent ions selectively over other divalent and monovalent ions has been scarcely reported. Recently, Barba-Bon *et al.*,[7a] Chen *et al.*[7b] and we[7c] reported three sensors which can selectively detect these trivalent ions. However, the successful design of a sensor which can give a signal in the red region after binding with a target metal ion is still a challenging task and has a great demand in the viewpoint of biological applications. The benefits of using red emitting sensors in biological applications are minimal photo damage to biological samples and minimum interference from background auto-fluorescence in living systems.[8] Therefore, it is necessary to develop a fluorescence probe that can be used for the detection of trivalent ions under physiological conditions, preferably with 'turn on' emission located in the red region.

Spirobenzopyrans are well known due to their photochromic behaviour.[9] These spiropyran moieties have some advantages to be used as sensors, such as rapid response, reversibility, good quantum yield and optical behaviour. Taking advantage of these spiropyran skeletons, during recent decades, a number of receptors have been reported for the optical and/or fluorescence sensing of metal ions.[10] To the best of our knowledge, this is the first report of sensing of Al^{3+}, Fe^{3+} or Cr^{3+} ions based on the spiropyran platform, signalling in the red region. On the basis of these considerations and the continuation of our effort to the development of fluorescence 'turn on' sensors,[11] we report here the design, synthesis and the cation sensing properties of a new spirobenzopyran-quinoline (SBPQ) conjugated dyad.

The detailed synthetic strategy of the sensor (SBPQ) is shown in Scheme 1 (ESI†). A spirobenzopyran moiety (R1), the signalling unit, was synthesised in analogy to the literature procedure[10e] and R2, the chelating unit, was also synthesised according to the procedure previously reported by us.[11a] Finally, the sensor was achieved by treatment of R1 with R2 in methanol solution at room temperature with 67% yield. ^{1}H NMR, ^{13}C NMR, ESI-MS and elemental analyses were used for the structural elucidation of the sensor molecule (ESI,† Fig. S22–S29).



Scheme 1 Probable binding mode of SBPQ with Al^{3+}.

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† Electronic supplementary information (ESI) available: Detailed synthetic procedure, NMR, MS, DFT data, *etc.*. See DOI: 10.1039/c3cc46860g

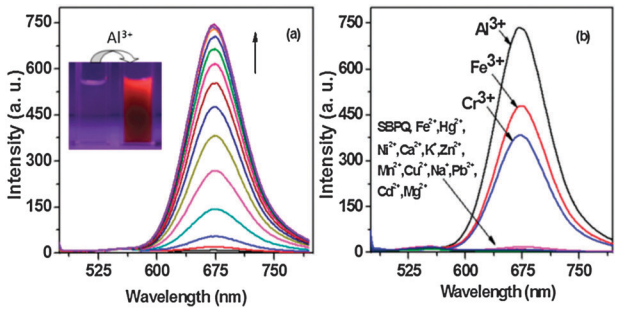

Fig. 1 (a) Change in fluorescence spectra of SBPQ (20 μM) upon gradual addition of Al^{3+} (0 to 2 equiv.). Inset: visible emission observed from SBPQ in the absence and presence of 2 equivalents of Al^{3+} after irradiation under UV light. (b) Changes in emission spectra of SBPQ (20 μM) upon addition of 2 equivalents of stated metal ions. λ_{ex} = 460 nm.

In order to investigate the sensing mechanism, fluorescence spectral changes of SBPQ (20 μM) were obtained at different concentrations of trivalent ions (Al^{3+}, Fe^{3+} and Cr^{3+}) in CH_3CN–HEPES buffer solution (1/1, v/v, pH = 7.4). We used here Al^{3+} as a representative of other trivalent ions (Fe^{3+} and Cr^{3+}) and performed the fluorescence titration of SBPQ at different concentrations of Al^{3+}. Upon gradual addition of Al^{3+} to the solution of SBPQ (20 μM), excited at 460 nm, the relatively weak emission band at 552 nm decreased with simultaneous formation of a red shifted peak at 675 nm (Fig. 1). The intensity of the new peak (at 675 nm) increased regularly as the concentration of Al^{3+} was increased. A significant enhancement (88 fold) of emission intensity at 675 nm was observed upon addition of 1 equivalent of Al^{3+}. A further increase in concentration of Al^{3+} (2 equiv.) did not lead to more enhancement of fluorescence intensity at 675 nm. Al^{3+} triggers the opening of the spiropyran ring of SBPQ to the formation of metastable merocyanine form, which was accompanied by the intramolecular charge transfer (ICT), and it was responsible for the observed bathochromic shift (~120 nm) and the large enhancement of fluorescence intensity after binding with Al^{3+}. It may also be accounted that Al^{3+} mediated opening of the spiropyran ring followed by formation of a tight complex could enhance the rigidity of the system (ESI,† Fig. S30), which may also be a cause of the dramatic enhancement of fluorescence intensity.

The emission intensity of the receptor at 675 nm increased linearly with the amount of Al^{3+} in the range of 0 to 16 μM (R^2 = 0.9959) (ESI,† Fig. S2a). The detection limit of the sensor for Al^{3+} was determined from the emission spectral change upon addition of Al^{3+} to be 3.24×10^{-8} M (ESI,† Fig. S10). In order to quantify the stoichiometry of the complex of SBPQ and Al^{3+}, Job's plot analysis was carried out, in which the maxima appear at a mole fraction of 0.5, which correspond to the 1 : 1 complex formation of SBPQ and Al^{3+} (ESI,† Fig. S7). The ESI-MS spectrum of SBPQ shows a peak at *m*/*z* 596.2659 possibly for $[SBPQ + H]^+$ whereas the Al^{3+} complex shows a peak at *m*/*z* 640.2118 possibly for $[SBPQ + Al^{3+} + H_2O]^+$, which also proves the mononuclear complex of SBPQ with Al^{3+} (ESI,† Fig. S30).

The acid–base titration experiment revealed that the sensor does not undergo any significant enhancement of emission intensity at 675 nm within the pH range 6–10, which suggests that the molecule prefers the spirocyclic form in this pH range. But under strong acidic conditions (pH < 4), protonation causes the yellow coloration along with a red fluorescence due to opening of the spiro ring (ESI,† Fig. S1). Thus SBPQ can be employed for the detection of trivalent ions in the near-neutral pH range (pH = 7.4). We also monitored the fluorescence titration experiment of SBPQ with Cr^{3+} and Fe^{3+} (ESI,† Fig. S2b and c). The results were similar to that of Al^{3+}. In the case of Cr^{3+} and Fe^{3+} (1 equiv.) the fluorescence intensity of SBPQ at 675 nm was increased by 34 and 40 fold respectively. The comparison of spectra between these three trivalent metal ions indicates that Al^{3+} can form a tighter complex with SBPQ may be due to its smaller size than Fe^{3+} and Cr^{3+}. The fluorescence quantum yield of the sensor was increased from 0.01 to 0.42, 0.18 and 0.25, respectively, in the presence of 1 equivalent of Al^{3+}, Cr^{3+} and Fe^{3+} (ESI†). The difference in quantum yield is high enough to discriminate between these cations from each other.

In the same way we have investigated the emission studies of metal ion binding properties of SBPQ toward other metal ions (2 equiv.) *viz.* Na^+, K^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Cu^{2+}, Cd^{2+}, Pb^{2+}, Ni^{2+}, Fe^{2+}, Hg^{2+} and Zn^{2+} (as their chloride salts). A comparative view of emission intensity of the receptor, after adding 2.0 equivalents of each guest cation is shown in Fig. 1. To utilize SBPQ as a selective sensor for these trivalent ions, a competing experiment was also performed by adding Al^{3+} (1.0 equiv.) in the presence of 2.0 equivalents of different cations. The studies revealed that Al^{3+} can be detected in the presence of almost all of the alkali and transition metal ions (ESI,† Fig. S13). The competing experiments were also performed using Cr^{3+} and Fe^{3+} and the results were similar to that of Al^{3+} (ESI,† Fig. S14 and S15). This indicates that the sensor can be used potentially to quantitatively detect Al^{3+} as well as trivalent ions (Cr^{3+} or Fe^{3+}) at low concentration with high selectivity.

The cation binding properties of the sensor (SBPQ) was also investigated in the ground state by the UV-vis spectroscopic method. We investigated the absorption spectral changes of SBPQ upon a gradual increase in concentration of Al^{3+}. The UV-vis spectrum of the metal-free form of SBPQ (20 μM) in CH_3CN–H_2O (1/1, v/v, pH = 7.4, 1 mM HEPES buffer solution) exhibits two λ_{max} peaks at 306 and 362 nm (ESI,† Fig. S3a) corresponding to the spiropyran form. Upon addition of increasing concentrations of Al^{3+} to the colourless solution of SBPQ, the band at 306 nm decreases slightly whereas the band at 362 nm increases along with formation of a new peak at around 440 nm. The intensity of the new peak increases (~14 fold) regularly upon increasing the concentration of Al^{3+} and turns the colorless solution of SBPQ to deep yellow (ESI,† Fig. S4). This may be due to the formation of a tight complex between SBPQ and Al^{3+} which is accompanied by the opening of the spiro ring to formation of open merocyanine form. An excellent linear correlation exists between the added Al^{3+} (0–20 μM) concentration and the absorbance intensity with a good R^2 value of 0.9921 (ESI,† Fig. S3a, inset).

Similar changes in UV-vis spectra, to that of Al^{3+}, were obtained in the case of Cr^{3+} and Fe^{3+} (ESI,† Fig. S4). To establish the selectivity of SBPQ to trivalent ions we also recorded the absorption spectral changes of SBPQ upon addition of different metal ions under the same experimental conditions (ESI†). Interestingly, the other common ions showed inertness (except Cu^{2+}) towards the absorption spectrum of SBPQ (ESI,† Fig. S3b). The detailed fluorescence and UV-vis titration spectra of SBPQ with different metal ions are shown in the ESI.†

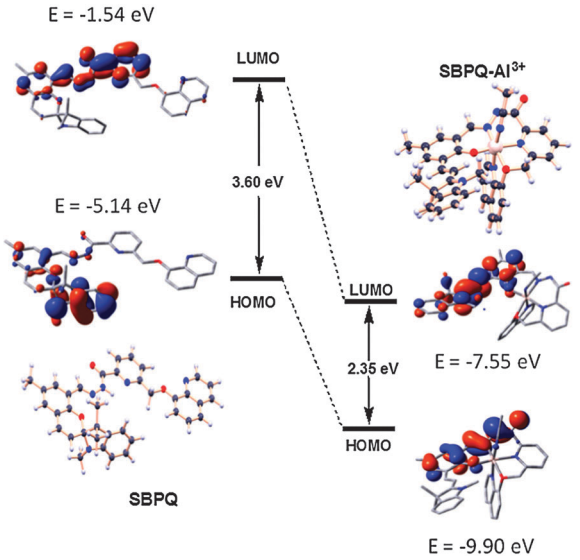

Fig. 2 Energy diagrams of HOMO and LUMO orbitals of SBPQ and the SBPQ–Al^{3+} complex calculated at the DFT level using a B3LYP/6-31+G(d,p) basis set.

To further understand the relationship between the structural changes of SBPQ and its complex with Al^{3+} and the optical response of SBPQ to Al^{3+}, we carried out density functional theory (DFT) and time dependent density functional theory (TDDFT) calculations with the B3LYP/6-31+G(d,p) method basis set using the Gaussian 03 program. The optimized geometry and the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) of SBPQ and its Al^{3+} complex are presented in Fig. 2. UV-vis spectra of SBPQ and its Al complex were calculated using the TDDFT method in acetonitrile medium. Calculated absorption peaks agree well with the experimentally observed peaks (ESI,† Fig. S20 and S21). In the case of SBPQ, the transition from HOMO to LUMO, HOMO − 1 to LUMO, HOMO − 3 to LUMO and HOMO − 4 to LUMO + 2 contributed mainly to the excitation at 397 nm, 369 nm, 325 nm and 268 nm respectively (ESI,† Table S1). For the Al^{3+} complex, main absorption peaks in the long wavelength region were at 553 nm, 453 nm, 428 nm, 371 nm and 359 nm generated from the transition of HOMO to LUMO, HOMO to LUMO + 2, HOMO − 1 to LUMO, HOMO to LUMO + 3 and HOMO − 2 to LUMO respectively (ESI,† Table S3).

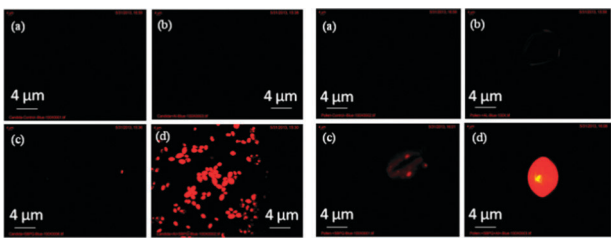

Fig. 3 Fluorescence microscopic photographs of (left) *Candida albicans* cells and (right) *Tecoma stans* pollen grains, (a) cells without any treatment, (b) cells treated with 5 μM aluminium nitrate, (c) cells treated with 5 μM SBPQ, (d) cells treated with 5 μM aluminium nitrate, washed and then treated with 5 μM SBPQ.

Considerations of the practical application led to further examination of the ability of the probe (SBPQ) to sense Al^{3+} in living cells. We use here three types of cells for the bioimaging application of SBPQ. *Candida albicans* cells, *Tecoma stans* pollen grains (Fig. 3) and *Bacillus thuringiensis* cells (ESI,† Fig. S5) were incubated with 5 μM aluminium nitrate and 5 μM SBPQ separately for 30 min (37 °C), and the cells showed no or weak red fluorescence. Once the cells treated with aluminium nitrate were incubated with SBPQ (5 μM) for 30 min (37 °C) in the culture medium, a significant deep red fluorescence was observed in the intracellular area. These results suggest that the probe, SBPQ, was cell membrane permeable and could be used as a chemosensor to detect Al^{3+} in living cells.

In summary, we reported here the synthesis of a quinoline appended spirobenzopyran derivative which showed the selective 'turn on' fluorescence response in the red region upon complexation with trivalent (Al^{3+}, Fe^{3+} and Cr^{3+}) ions. The phenomenon is further supported by DFT and TDDFT calculations. The probe was applied to detect intracellular Al^{3+} in live-cells.

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Notes and references

1 (*a*) G. D. Fasman, *Coord. Chem. Rev.*, 1996, **149**, 125; (*b*) P. Nayak, *Environ. Res.*, 2002, **89**, 101; (*c*) G. Berthon, *Coord. Chem. Rev.*, 2002, **228**, 319.
2 (*a*) D. Touati, *Arch. Biochem. Biophys.*, 2000, **373**, 1; (*b*) D. Galaris, V. Skiada and A. Barbouti, *Cancer Lett.*, 2008, **266**, 21.
3 (*a*) Committee on Animal Nutrition and National Research Council, *The Role of Chromium in Animal Nutrition*, National Academy Press, Washington, DC, 2007; (*b*) A. K. Singh, V. K. Gupta and B. Gupta, *Anal. Chim. Acta*, 2007, **585**, 171.
4 (*a*) D. Maity and T. Gobindoraju, *Chem. Commun.*, 2012, **48**, 1039; (*b*) X. Sun, Y.-W. Wang and Y. Peng, *Org. Lett.*, 2012, **14**, 3420; (*c*) S. H. Kim, H. S. Choi, J. Kim, S. J. Lee, D. T. Quang and J. S. Kim, *Org. Lett.*, 2010, **12**, 560; (*d*) T. Han, X. Feng, B. Tong, J. Shi, L. Chen, J. Zhi and Y. Dong, *Chem. Commun.*, 2012, **48**, 416–418.
5 (*a*) R. K. Pathak, J. Dessingou, V. K. Hinge, A. G. Thawari, S. K. Basu and C. P. Rao, *Anal. Chem.*, 2013, **85**, 3707; (*b*) N. R. Chereddy, S. Thennarasu and A. B. Mandal, *Analyst*, 2013, **138**, 1334; (*c*) S. K. Sahoo, D. Sharma, R. K. Bera, G. Crisponi and J. F. Callan, *Chem. Soc. Rev.*, 2012, **41**, 7195.
6 (*a*) Z. Zhou, M. Yu, H. Yang, K. Huang, F. Li, T. Yi and C. Huang, *Chem. Commun.*, 2008, 3387; (*b*) Z. Li, W. Zhao, Y. Zhang, L. Zhang, M. Yu, J. Liu and H. Zhang, *Tetrahedron*, 2011, **67**, 7096.
7 (*a*) A. Barba-Bon, A. M. Costero, S. Gil, M. Parra, J. Soto, R. Martínez-Máñez and F. Sancenon, *Chem. Commun.*, 2012, **48**, 3000; (*b*) X. Chen, X. Y. Shen, E. Guan, Y. Liu, A. Qin, J. Z. Sun and B. Z. Tang, *Chem. Commun.*, 2013, **49**, 1503; (*c*) S. Goswami, K. Aich, A. K. Das, A. Manna and S. Das, *RSC Adv.*, 2013, **3**, 2412.
8 (*a*) R. Weissleder, *Nat. Biotechnol.*, 2001, **19**, 316; (*b*) B. Tang, H. Huang, K. Xu, L. Tong, G. Yang, X. Liu and L. An, *Chem. Commun.*, 2006, 3609; (*c*) L. Yuan, W. Lin, K. Zheng, L. He and W. Huang, *Chem. Soc. Rev.*, 2013, **42**, 622.
9 (*a*) E. Fischer and Y. Hirshberg, *J. Chem. Soc.*, 1952, 4522; (*b*) S. Giordani and F. M. Raymo, *Org. Lett.*, 2003, **5**, 3559; (*c*) V. I. Minkin, *Chem. Rev.*, 2004, **104**, 2751.
10 (*a*) Z.-Q. Guo, W.-Q. Chen and X.-M. Duan, *Org. Lett.*, 2010, **12**, 2202; (*b*) N. Shao, J. Y. Jin, H. Wang, Y. Zhang, R. H. Yang and W. H. Chan, *Anal. Chem.*, 2008, **80**, 3466; (*c*) T. Sakata, D. K. Jackson, S. Mao and G. Marriott, *J. Org. Chem.*, 2008, **73**, 227; (*d*) J.-F. Zhu, H. Yuan, W.-H. Chan and A. W. M. Lee, *Org. Biomol. Chem.*, 2010, **8**, 3957; (*e*) J.-F. Zhu, W.-H. Chan and A. W. M. Lee, *Tetrahedron Lett.*, 2012, **53**, 2001.
11 (*a*) S. Goswami, K. Aich, S. Das, A. K. Das, A. Manna and S. Halder, *Analyst*, 2013, **138**, 1903; (*b*) S. Goswami, D. Sen, N. K. Das, H.-K. Fun and C. K. Quah, *Chem. Commun.*, 2011, **47**, 9101.