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A triphenylene based zinc ensemble as an oxidation inhibitor†

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The zinc complex of a new triphenylene based receptor is evaluated for its anti-oxidant activity which is better in comparison to that of commercially available anti-oxidants.

Zinc, an essential nutrient, plays an important role as an anti-oxidant in the human body.[1] Deficiency of zinc increases the production of reactive oxygen species (ROS),[2] which are a class of radical or non-radical oxygen-containing molecules that show high reactivity to bio-molecules.[3] Excessive ROS generation is involved in the pathogenesis of many diseases, including cardiovascular disease, cancer, and neurological disorders.[4] Thus, regulation of generation of these ROS is highly significant.

Our research involves the design, synthesis and evaluation of artificial receptors for metal ions and anions.[5] In continuation of this work, in the present manuscript, we have synthesized a new triphenylene based receptor **3** (*vide infra*) (Scheme 1) which selectively senses Zn^{2+} ions.[6] Further, the zinc ensemble of **3** (**Zn-3**) has been evaluated for its anti-oxidizing property and it was observed that the anti-oxidant activity of **Zn-3** is better than five commercially available anti-oxidants. Earlier, a number of artificial receptors for Zn^{2+} ions with bio-mimetic applications have been reported in the literature[7] which includes complexes for understanding the intrinsic properties of substrate or inhibitor recognition by Zn^{2+} ions at the active centers of enzymes.[8] Recently, Reinaud *et al.* reported the tripodal bio-mimetic macrocyclic Zn^{2+} complexes.[9] However, the development of bio-mimetic synthetic zinc complexes showing anti-oxidant activity has not been explored. Thus, to the best of our knowledge, this is the first report where a triphenylene based receptor for Zn^{2+} ions shows anti-oxidant activity.

Suzuki–Miyaura coupling of 2,3,6,7,10,11-hexabromotriphenylene **1a** with boronic ester **1b**[10] yielded hexamine **1c** in 70% yield (Scheme 1) (for synthetic details see S3, S9–S11 of ESI†). Condensation of hexamine **1c** with quinoline-2-carboxaldehyde **2** in THF at room temperature furnished compound **3** in 75% yield (Scheme 1). The structure of compound **3** was confirmed from its spectroscopic data (for synthetic details see S4, S12–S14 of ESI†) and its purity was determined from HPLC (see ESI†, S26).

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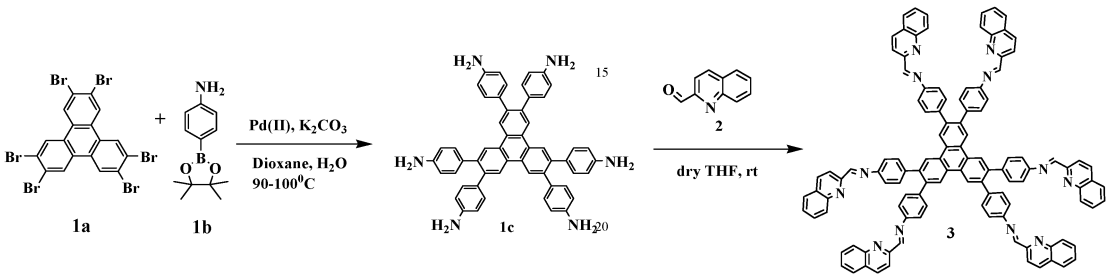
The binding behaviour of compound **3** toward different metal ions was evaluated with the help of UV-vis and fluorescence spectroscopy. The absorption spectrum of compound **3** (10 μM) shows three absorption bands at 246, 296 and 355 nm in THF : H_2O (95 : 5). Among all the metal ions tested (Zn^{2+}, Fe^{3+}, Fe^{2+}, Cu^{2+}, Hg^{2+}, Ni^{2+}, Cd^{2+}, Pb^{2+}, Mn^{2+}, Co^{2+}, Li^{+}, Na^{+}, Mg^{2+}, K^{+}, Ca^{2+}, Ba^{2+}) two new bands are formed at 290 and 325 nm upon addition of only Zn^{2+} ions (200 equiv.) with two isosbestic points at 285 and 335 nm (see ESI†, S6). These changes in the UV-vis spectrum of **3** upon addition of Zn^{2+} ions are ascribed to the formation of a complex between **3** and Zn^{2+} ions.

Compound **3** showed no fluorescence emission in THF : H_2O (95 : 5) (Fig. 1) when excited at 355 nm, which may be attributed to photo-induced electron transfer (PET)[11] from imino nitrogen to the photo-excited quinoline moiety. Upon addition of only Zn^{2+} ions (20 equiv.), an enhancement in the fluorescence spectrum was observed at 438 nm. The fluorescence enhancement of receptor **3** in the presence Zn^{2+} ions is attributed to the co-ordination of imino and quinolinyl nitrogens of **3** with Zn^{2+} leading to the formation of a supramolecular complex, as a result of which the PET from imino nitrogen to the quinoline moiety is suppressed. Under the same conditions, the fluorescence behaviour of **3** was tested with other metal ions (Fe^{3+}, Fe^{2+}, Cu^{2+}, Hg^{2+}, Ni^{2+}, Cd^{2+}, Pb^{2+}, Mn^{2+}, Co^{2+}, Li^{+}, Na^{+}, Mg^{2+}, K^{+}, Ca^{2+}, Ba^{2+}), however no change in emission spectrum was observed with any other metal ion (see ESI†, S7). The fluorescence spectra of **3** (5 μM) at various concentrations of Zn^{2+} ions are shown in Fig. 1. Fitting the changes in the fluorescence spectra of compound **3** with Zn^{2+} ions, using the nonlinear regression analysis program SPECFIT,[12] gave a good fit and demonstrated that a 1 : 3 stoichiometry (host : guest) was the most stable species in the solution with a binding constant $\log \beta_{1,3} = 13.23$ (see ESI†, S15 and S16). The complex formation was further confirmed by mass and IR spectroscopy (for mass spectrum see ESI†, S25). The IR absorption band at 1616 cm^{-1} due to the imine moiety of **3** shifted to a lower frequency at 1590 cm^{-1} ($n = 26$ cm^{-1}) upon complexation with zinc, which confirms the coordination of imino nitrogen atoms with Zn^{2+} ions (see ESI†, S23 and S24). To test the practical applicability of compound **3** as a Zn^{2+} selective fluorescence sensor, competitive experiments were carried out in the presence of Zn^{2+} ions at 20 equiv. mixed with other cations (Fe^{3+}, Fe^{2+}, Cu^{2+}, Hg^{2+}, Ni^{2+}, Cd^{2+}, Pb^{2+}, Mn^{2+}, Co^{2+}, Li^{2+}, Na^{+}, Mg^{2+}, K^{+}, Ca^{2+}, Ba^{2+}) at 200 equiv., no significant variation was found by comparison with and without the other metal ions



Scheme 1

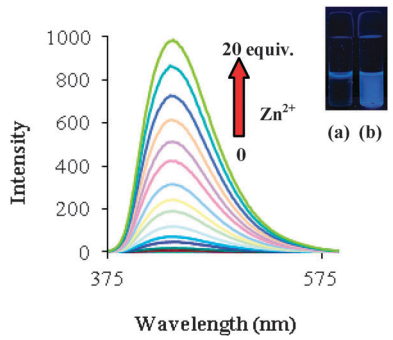


Fig. 1 Fluorescence emission spectrum of **3** (5 μM) upon addition of Zn^{2+} (20 equiv.) ions in THF : H_2O (95 : 5). Inset shows the fluorescence intensity changes upon the addition of Zn^{2+} ions from (a) 0 to (b) 20 equiv.

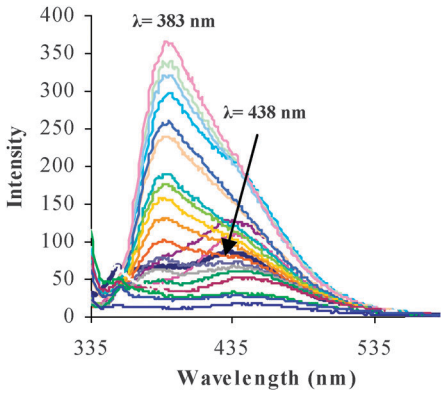
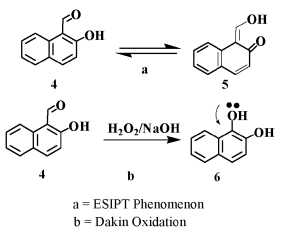


Fig. 2 Fluorescence emission spectra of **4** (3 μM) upon addition of H_2O_2 (750 μl) in THF : H_2O (95 : 5). Inset shows the (a) ESIPT phenomenon in β-OH napthaldehyde and (b) its Dakin's oxidation.



Scheme 2

(see ESI†, S7). This means that compound **3** has a high affinity for Zn^{2+} ions. The detection limit of **3** as a fluorescent sensor for the analysis of Zn^{2+} was found to be 1×10^{-6} mol l^{-1}, which is sufficiently low for the detection of submillimolar concentrations of Zn^{2+} ions found in many chemical systems.[13] The fluorescence quantum yield[14] (ϕ_f) of compound **3** in the free and **3**·Zn^{2+} bound state was found to be 0.001 and 0.65, respectively (for calculations see ESI†, S4). This substantial increase in the quantum yield of compound **3** in the presence of Zn^{2+} ions showed its credibility as a good Zn^{2+} sensor.

Further, we evaluated the zinc ensemble of **3** (**Zn-3**) for its anti-oxidant activity using fluorescence spectroscopy and cyclic voltammetric studies (*vide infra*). For this, we utilized β-hydroxy naphthaldehyde[15] as a template, carried out its Dakin oxidation[16] and observed its fluorescence behaviour in the presence and absence of **Zn-3** as fluorescent probes for detection of ROS, which has been reported recently.[17] However, such an evaluation of anti-oxidant activity of a metal coordinated ligand using an organic template is unprecedented.

β-Hydroxy naphthaldehyde **4** (3 μM) shows an emission at 438 nm in THF : H_2O (95 : 5) when excited at 318 nm (Fig. 2). The fluorescence emission of compound **4** is attributed to fast keto–enol tautomerism (representing the enol form **4** and keto form **5**, Scheme 2a) involving the phenomenon of excited state intramolecular proton transfer (ESIPT).[18] To carry out Dakin oxidation we added alkaline H_2O_2/NaOH (pH = 7.4) to the solution of **4** (3 μM) at 25 °C. Upon addition of H_2O_2 (225 μl) the emission band at 438 nm is blue shifted to 383 nm. We propose that this fluorescence response of compound **4** is due to the modulation of the existing ESIPT state (**5**) by the interaction of HOO^- ions with the hydroxyl group of **4**. Upon further addition of H_2O_2 (525 μl) quenching of the fluorescence emission was observed. This quenching is ascribed to the oxidative conversion of **4** to species **6** (Scheme 2b). In species **6**, the inhibition of ESIPT makes photoinduced electron transfer (PET) operational (Scheme 2b) and as a result fluorescence emission gets quenched. Further, to observe the anti-oxidation effect of **Zn-3**, we performed the fluorescence titration with H_2O_2 under similar conditions in the presence of 250 μl of 100 μM of **Zn-3**. We observed that in the presence of **Zn-3** the rate of oxidation of **4** decreases and the amount of H_2O_2 required to quench the fluorescence is larger (1300 μl) (see ESI†, S17) than in the absence of **Zn-3**. This may be ascribed to the fact that hydroperoxide anions (HOO^-) generated during the reaction[16] (which are responsible for the oxidation) show preferential binding to the charged zinc centre of **Zn-3**. The rate of oxidation slows down, indicating that **Zn-3** has an anti-oxidant effect.

We also studied the fluorescence behaviour of **Zn-3** toward other anions (F^-, CN^-, OAc^-, Cl^-, Br^-, I^-, $EDTA^{-4}$, HSO^{4-}, NO_3^- $H_2PO_4^-$, ClO_4^-) and observed that no change in fluorescence emission of **Zn-3** was observed with any other anions (see ESI†, S8). Thus, **Zn-3** shows a selective fluorescence response towards hydroperoxide anions.

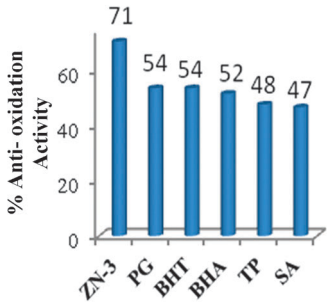


Fig. 3 Comparison of percentage anti-oxidation activity of **Zn-3** with other commercial anti-oxidants.

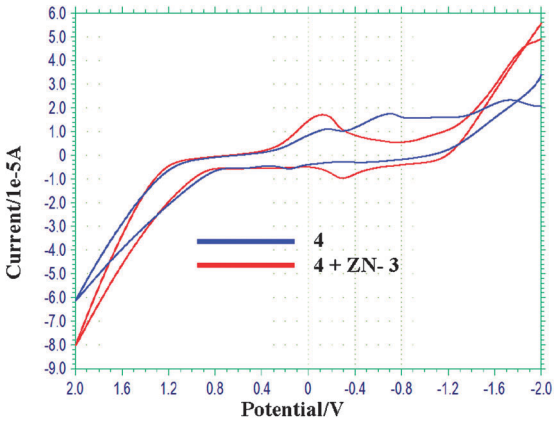


Fig. 4 Cyclic voltammogram of (a) **4** and (b) **4** + **Zn-3**.

We also compared the anti-oxidant activity of **Zn-3** with five different commercially available anti-oxidants *viz*. propyl gallate (PG), butylated hydroxyl toluene (BHT), butylated hydroxyl anisole (BHA), α-tocopherol (TP) and sodium ascorbate (SA).[19] It was observed that in the presence of 250 μl of 100 μM PG (see ESI†, S18), BHT (see ESI†, S19), BHA (see ESI†, S20), TP (see ESI†, S21) and SA (see ESI†, S22) 1025 μl, 1000 μl, 1000 μl, 975 μl and 950 μl of H_2O_2 were used, respectively, to quench the fluorescence of β-hydroxy naphthaldehyde **4**. The lower amount of hydrogen peroxide used in the presence of all these commercially available anti-oxidants in comparison to **Zn-3** shows that the **Zn-3** has a better anti-oxidant property. The percentage oxidation inhibition calculated for **Zn-3**, PG, BHT, BHA, TP, SA is 71%, 54%, 54%, 52%, 48% and 47% respectively (Fig. 3) (see ESI†, S5).

The anti-oxidative nature of **Zn-3** observed from the above spectroscopic studies was further confirmed from electrochemical studies. The cyclic voltammogram of **4** [THF, $c = 1 \times 10^{-3}$, $[(n\text{Bu})_4\text{N}]\text{ClO}_4$ as supporting electrolyte, using a glassy carbon working electrode, a (Ag/Ag^+) reference electrode, and a Pt wire counter electrode] exhibits two electrochemical oxidation waves at $E_{1/2} = -0.175$ and $E_{1/2} = -0.70$ (Fig. 4). The cyclic voltammogram of **4** + **Zn-3** under the same conditions showed one oxidation wave at $E_{1/2} = -0.13$ and a reduction wave at $E_{1/2} = -0.30$ (Fig. 4b). Such a dramatic shift in the oxidation waves of **4** in the presence of **Zn-3** is attributed to the slow oxidation of **4** in the presence of **Zn-3**. Thus, the electrochemical studies firmly support the spectroscopic studies and prove the anti-oxidant behaviour of **Zn-3**.

To conclude, we synthesized a new triphenylene based receptor **3** incorporating quinoline moieties which behaves as a Zn^{2+} selective chemosensor. The Zn^{2+} ensemble of **3** shows anti-oxidant properties and may be used in the real world by diluting with THF prior to a hydroperoxide assay. This is proved by different spectral and electrochemical studies using β-hydroxy naphthaldehyde as an organic template and performing its Dakin oxidation in the absence and presence of **Zn-3**.

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