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A new use for an old molecule: *N*-phenyl-2-(2-hydroxynaphthalen-1-ylmethylene)hydrazinecarbothioamide as a ratiometric 'Off–On' fluorescent probe for iron

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ABSTRACT

N-Phenyl-2-(2-hydroxynaphthalen-1-ylmethylene)hydrazinecarbothioamide has been investigated as a fluorescent sensor for the determination of Fe(III) in aqueous solutions. The probe was prepared by the facile Schiff base condensation of 2-hydroxy-1-napthaldehyde with *N*-phenylhydrazinecarbothioamide. The sensor displayed good selectivity for Fe(III) when tested against a range of biologically and environmentally important cations. A concentration dependent increase in the emission of two fluorescent bands at 425 and 495 nm was observed upon increasing Fe(III) addition resulting in a linear ratiometric response in the 17–37 μM range. The binding stoichiometry was confirmed as 1:1 (host/guest) with the binding constant ($\log\beta$) calculated as 4.56.

Iron is an essential element for normal physiological functioning as it plays an important role in many cellular processes including energy generation, oxygen transport and DNA synthesis.[1] The ability of iron to redox cycle between two stable forms, Fe(II) and Fe(III), enables it to function as both an electron donor and acceptor, a property that makes it suitable as a co-factor in many different enzymes critical for life.[2] However, iron overload can cause deleterious conditions such as β-thalassaemia and Friedreich's ataxia where the presence of excess iron leads to the generation of reactive oxygen species (ROS) via the Fenton reaction.[3] ROS can induce cell death by interacting with many important biomolecules through a series of chemical reactions resulting in DNA oxidation, mitochondrial damage and lipid peroxidation.[4] Iron is also essential for the catalytic activity of ribonucleotide reductase (RR), the enzyme used in the rate determining step of DNA synthesis. As cancer cells proliferate at a faster rate than normal cells, these cells tend to have a greater requirement for iron than healthy cells.[5] Thus iron sequestration has also been a target in certain cancer chemotherapies.[3]

To counter the problems of iron overload, iron chelation therapy utilises ligating drugs that strongly bind to intra- and extracellular iron promoting the excretion and subsequent depletion of toxic iron concentrations in the body.[3] Currently, three drugs, deferiprone, deferasirox and deferoxamine are approved for use in iron chelation therapy.[6] However, in order to administer the optimum dose of these agents it would first be advantageous to determine the level of excess iron present. As a result, there is a need for the development of rapid detection systems that can determine the concentration of iron in aqueous based solutions. In this capacity, fluorescence based methods for analyte detection remain popular due to their high sensitivity, rapid response rate and relative inexpense.[7]

Thiosemicarbazones have been studied extensively as iron chelators in the treatment of cancer. Their high affinity for iron makes them ideal for sequestering iron at the active site of the RR enzyme thus inhibiting its function.[8] A large family of thiosemicarbazone derivatives have been developed and investigated for their chemotherapeutic potential including those derived from 2-hydroxy-1-naphthaldehyde.[9] Given the high affinity these molecules have for Fe(III) and that they possess an inherent fluorophore within their structure, it seems sensible to investigate these compounds as potential fluorescent probes for iron. Surprisingly, to date, the ability of these agents to also function as self indicating fluorescent sensors for iron has been overlooked.

In this Letter, we synthesise the simplest member of this family, *N*-phenyl-2-(2-hydroxynaphthalen-1-ylmethylene)hydrazinecarbothioamide (which in this Letter is designated **NT**)[9] and investigate its ability to function as a fluorescent probe for Fe(III) in aqueous based solution.

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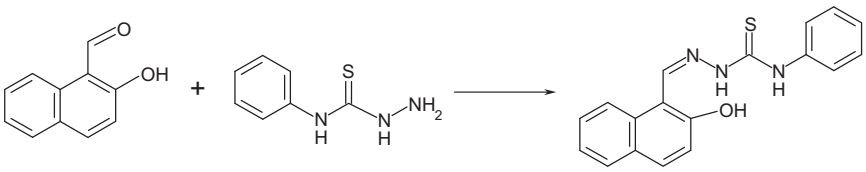

Scheme 1. Synthesis of **NT** from 2-hydroxy-1-naphthaldehyde and 4-phenylthio-semicarbazide.

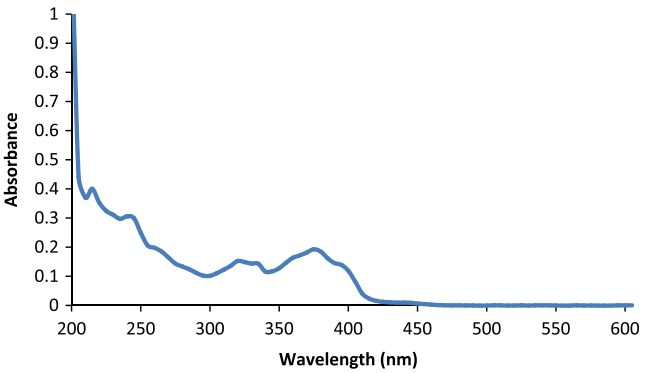

Figure 1. Absorbance spectrum of **NT** recorded in a THF/H_2O (9:1) solvent system buffered at pH 7.4. [**NT**] = 10 μM.

The target compound **NT** was synthesised in one step by the facile Schiff base condensation reaction of 2-hydroxy-1-napthaldehyde with *N*-phenylhydrazinecarbothioamide (4-phenylthiosemicarbazide) (Scheme 1) and was produced in a 65% yield after recrystallisation from ethanol.[10] The UV–Vis spectrum of **NT**, recorded in a THF/H_2O (9:1) HEPES buffered solvent system (pH 7.4) is shown in Figure 1 and is characterised by a broad absorption from 210 to 450 nm. Within this broad absorption profile there is evidence of four main peaks with λ_{max} values of 210, 245, 320 and 370 nm. The 210 and 245 nm peaks are consistent with π–π* transitions of the aromatic rings, the 320 nm peak with a π–π* transition of the C=N group while the 370 nm band reflects an intramolecular charge transfer (CT) band of the entire conjugated molecule.[11] When excited at 370 nm in the same solvent system the fluorescence spectrum exhibited two main bands in the visible region with λ_{max} values of 425 and 485 nm (Fig. 2a). The presence of two bands in the fluorescence spectrum enables the possibility

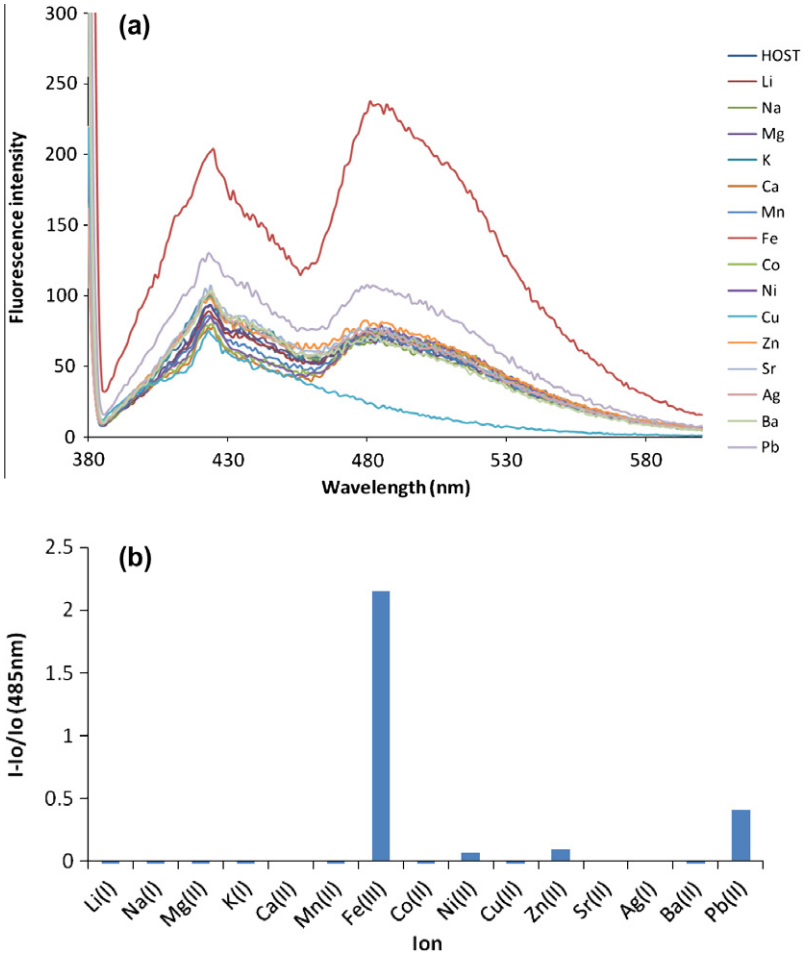

Figure 2. (a) Fluorescence spectra of **NT** (10 μM) in the absence and presence of different metal salts (25 μM) recorded in a THF/H_2O (9:1) solvent system buffered at pH 7.4. Excitation wavelength = 375 nm (b) selectivity plot for **NT** where $I-Io/Io$ is plotted against each metal ion (I is the intensity in the presence of metal ion and Io the intensity in the absence of metal ion).

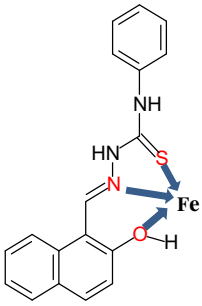

Figure 3. Proposed binding mechanism between **NT** and Fe^{3+}.

of ratiometric analysis by comparing the ratio of the intensities of the two bands as a function of analyte concentration. This is preferred over single wavelength analysis as the method is free from the errors associated with receptor concentration, photobleaching and environmental effects.[12]

The selectivity of **NT** as a fluorescent probe for Fe^{3+} was tested in a THF/H_2O (9:1) solvent system buffered at pH 7.4 by incubating **NT** (10 μM) with a range of environmentally and biologically important metal ions (25 μM) as their chloride salts. The addition of Li^+, Na^+, Mg^{2+}, K^+, Ca^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Sr^{2+}, Ag^+ and Ba^{2+} caused virtually no change to the fluorescence spectrum of **NT**. The addition of Pb^{2+} caused a minor increase in both bands while the addition of Cu^{2+} led to a slight increase of the 425 nm band with a significant quench of the 485 nm band (Fig. 2a). However, the addition of Fe^{3+} resulted in a significant increase of both bands with a 104% increase for the 425 nm band and a 194% increase for the 485 nm band. When a plot of the intensity at 485 nm was performed for each metal ion, excellent selectivity was observed for Fe^{3+} over all the other tested ions (Fig. 2b).

The reason for the large fluorescent enhancement upon the addition of iron can be explained as follows: iron is known to form a tridentate chelate with thiosemicarbazones such as **NT** (Fig. 3), where the oxygen lone pair from the naphthyl hydroxy, the imine nitrogen lone pair and the thiosemicarbazone sulfur lone pair, all bind strongly to the Fe^{3+} ion.[13] This binding event prevents the rapid isomerisation of the C=N group that otherwise happens in the absence of Fe(III) and which leads to non-radiative decay of the excited state.[14] Examples of 'Off–On' fluorescence sensors for Fe(III) are rare given in the redox nature of this ion as it typically quenches the excited state of organic fluorophores by electron transfer processes when brought into close proximity of the fluorophore.[15] In the case of **NT**, the enhancement in fluorescence that arises as a result of the inhibition of the C=N isomerisation process is greater than the quenching ability of Fe^{3+} and an 'Off–On' response was observed.

To determine the sensitivity range over which **NT** can measure Fe(III), a series of solutions were prepared in which the concentration of **NT** was kept constant but the concentration of Fe^{3+} was gradually increased. The fluorescence of each solution was measured and the ratiometric intensity (I_{485}/I_{425}) plotted against concentration (Fig. 4). Excellent linearity was observed in the 17–37 μM range indicating that **NT** can function as a ratiometric 'Off–On' fluorescent sensor in this range. To confirm the binding stoichiometry between **NT** and Fe^{3+} a Job plot was performed.[16] The results of this plot are shown in Figure 5 and indicate the formation of a 1:1 (host/guest) complex. Based on a 1:1 binding stoichiometry the binding constant ($\log\beta$) was calculated to be 4.56 using the equation $\log(F_{max}-F)/(F/F_{min}) = \log[\text{cation}]-\log\beta$, (where F_{max} is the maximum fluorescence intensity, F_{min} the minimum fluorescence intensity and F the measured fluorescence intensity).[17]

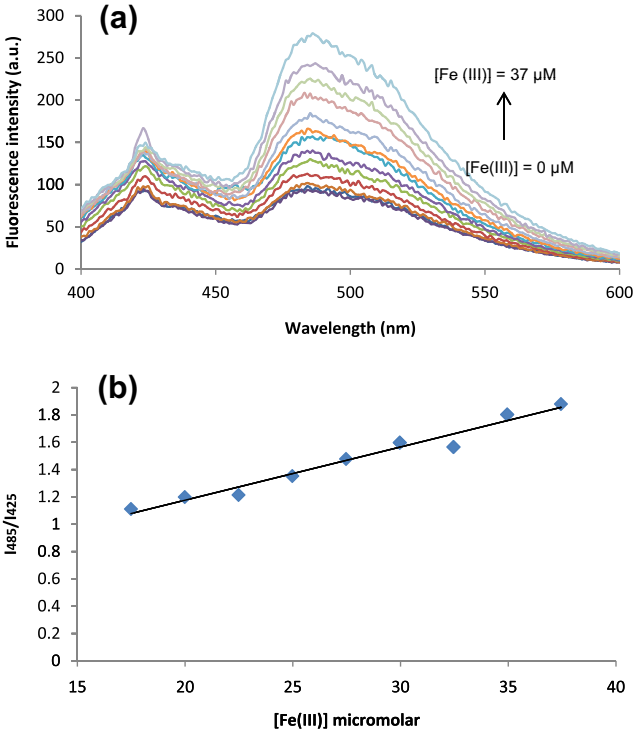

Figure 4. (a) The concentration dependent increase in fluorescence of **NT** upon increasing [Fe^{3+}]. (b) Plot of the ratiometric fluorescence intensity of **NT** (I_{485}/I_{425}) against Fe^{3+} concentration. [**NT**] = 10 μM, solvent: THF/H_2O (9:1) buffered at pH 7.4.

Finally, the effect of solution pH on the fluorescence intensity of **NT** was investigated by recording the fluorescence spectra over a range of different pH values. The emission intensity at 485 nm was plotted as a function of pH and this plot is shown in Figure 6. These results illustrate that the fluorescence intensity remains relatively constant from pH 2–9, but increases dramatically from pH 10–12. This large increase in fluorescence is most likely caused by the deprotonation of the naphthalene hydroxy group of **NT** that otherwise leads to a non-radiative decay from the excited state by vibrationally coupling it to water.[11] As this hydroxy group is also involved in the binding of the Fe^{3+} ion by **NT**, it is also probable that this interaction may also contribute to the fluorescence enhancement observed upon the interaction of **NT** and Fe^{3+}, as this binding event would reduce the ability of the hydroxy group vibrationally coupling to water. The pK_a of this group was calculated to

Figure 5. (a) Job plot for **NT** and Fe^{3+} illustrating the formation of a 1:1 (**NT**/Fe^{3+}) complex.

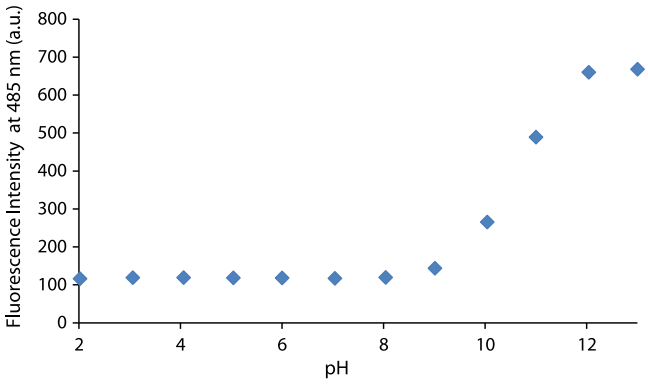

Figure 6. Plot of fluorescence against pH for **NT**. [**NT**] = 10 μM.

be 10.62 using a derivation of the Henderson Hasselbach equation by plotting $-\log(F_{max}-F)/(F-F_{min})$ against pH.[18] Importantly, however, the fluorescence–pH titration showed that the emission intensity of **NT** remained constant in the physiological range.

In summary, we have utilised **NT** as an 'Off–On' ratiometric fluorescence sensor for Fe^{3+} operable in semi aqueous solution. This compound displayed good selectivity for Fe^{3+} when tested against a range of physiological and environmentally important ions and was sensitive in the 17–37 μM range. The fluorescence enhancement upon binding Fe^{3+} was attributed to a cancellation of the C=N isomerisation that otherwise leads to non-radiative decay of the excited state. The binding stoichiometry was confirmed as 1:1 (**NT**/Fe^{3+}) and the binding constant $\log\beta$ was determined as 4.56. Examples of enhancement based ratiometric fluorescence sensors for Fe^{3+} are extremely rare and to the best of our knowledge, **NT** is the first reported example of an enhancement based fluorescence probe for Fe^{3+} that operates via the suppression of C=N isomerisation. In addition, we believe that this provides a good example of how already synthesised molecules can be utilised for a different purpose. We are currently investigating other known iron-chelating compounds for their potential as optical iron sensors.

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10. *UV and Fluorescence Spectroscopy:* Absorbance measurements were recorded on a Varian Cary 50 Spectrometer using 10 mm quartz cuvettes. Fluorescence measurements were recorded on a Cary Eclipse fluorimeter using 10 mm quartz cuvettes. Excitation slit size was 5 nm and emission slit size was 5 nm. *Synthesis of* ***NT***: 2-hydroxy-1-naphthaldehyde (0.5 g, 2.9 mmol) was added to *N*-phenylhydrazinecarbothioamide (0.49 g, 2.9 mmol) in DMF (15 mL) at room temperature. After stirring for 18 h, the solvent was evaporated under reduced pressure and the crude product recrystallised from hot EtOH. The resulting yellow crystals were dried in vacuo to yield 0.61 g of **1** (65% yield). ^{1}H NMR (500 MHz, DMSO-d_6): 6.95 (2H, m, Ar-H), 7.14 (3H, m, Ar-H), 7.32 (3H, m, Ar-H), 7.61 (2H, m, Ar-H), 8.23 (1H, s, Ar-H), 8.90 (1H, s, CH=N), 9.80 (1H, s, NH), 10.34 (1H, s, OH), 11.50 (1H, s, NH). ^{13}C NMR (125 MHz DMSO-d_6): 110.2, 119.0, 124.0, 125.6, 128.6, 129.2, 132.1, 133.1, 139.7, 144.0, 157.1. MS: Calculated for $C_{18}H_{15}N_3SO$ = 321.4, found 321.1.
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