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A bodipy based hydroxylamine sensor

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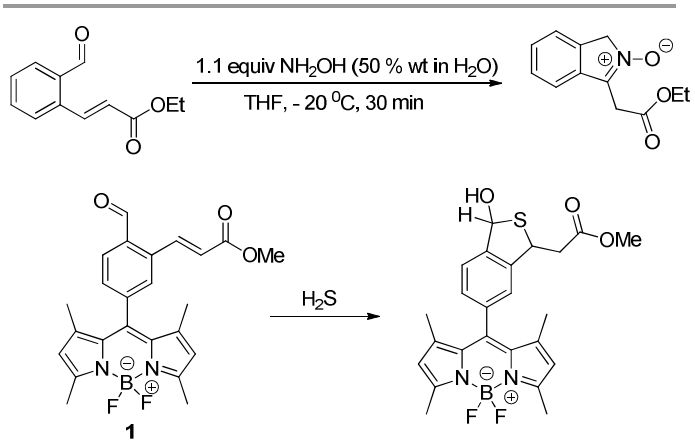
With this research we have developed a bodipy based system as the first "turn-on" fluorescence system for the detection hydroxylamine.

Hydroxylamine, (HA) NH_2OH, is an oxygenated form of ammonia, which is widely used in industrial and pharmaceutical processes[1]. HA is widely known as a nitric oxide (NO) donor and participates in a wide range of biological processes.[2-4] In cellular metabolism, HA is an intermediate in the conversion L-arginine to nitric oxide (NO).[5, 6] This process involves the hydrolysis of oxime arginine to L-citrulline and HA. HA is then converted by catalase to NO and superoxide ($^{-}O_2$) in the presence of hydrogen peroxide.[7] It is also believed that HA can be converted to NO by the non-enzymatic attack by $^{-}O_2$. Therefore, in inflammatory cells a reaction between HA and $^{-}O_2$ could take place to generate NO.[3, 8] For example, HA has shown to be a vasodilator in a dose-dependent manner in the blood vessels of rats' kidneys.[9]. HA has also shown to be an inhibitor for the release of insulin and HA has been shown to activate K^+ channels.[3, 10]

Despite HA being a product of metabolism and a NO donor, it is moderately toxic to humans, animals and plants. HA toxicity occurs at concentrations that are substantially greater than the normal physiological concentrations. Exposure to HA has been shown to be hemotoxic, mutagenic whilst also being an enzyme and virus inhibitor.[11]

Given these important biological aspects of HA we set out to develop a fluorescence probe for the detection of HA. To our knowledge, there are currently no HA fluorescence probes. HA is currently detected using: HPLC, GC, potentiometric, polarographic, biamperometric and electrochemical methods.[12-19]

Previously we have developed a synthetic route to nitrones by reacting an aromatic aldehyde containing an *ortho* α,β-unsaturated ester with NH_2OH (50 % wt in H_2O) in THF at – 20 ^{O}C to produce isoindole nitrones with high yields,[20] Scheme 1. Therefore, we set out to integrate this reaction with a fluorophore for the detection of HA. Luckily, an appropriate system probe **1** had previously been prepared for the detection of hydrogen sulphide (H_2S).[21, 22] Scheme 1.



Scheme 1. Nitrone synthesis and sensor for hydrogen sulphide.

The synthesis of probe **1**, is given in the electronic supplementary information (ESI)

We also confirmed that probe **1** reacts as predicted with hydroxyl amine as shown by the ^{1}HNMR, ^{13}CNMR and MS. (ESI). More importantly when HA is added to a 0.5 μM solution of probe **1** in a 1% DMSO PBS Buffer solution at pH 7.4 the fluorescence is significantly enhanced as the concentration of HA is increased. (Fig 1)

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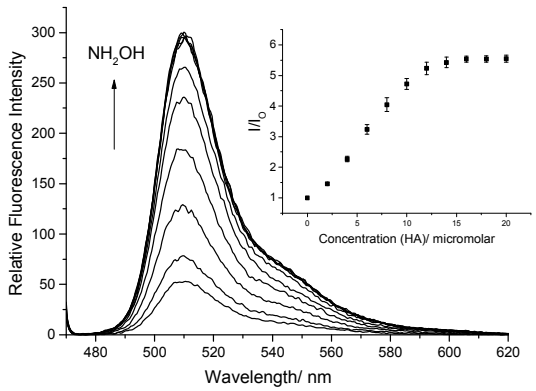

Fig 1. Fluorescence changes observed for probe **1** (0.5 μM) during the addition of HA (0 – 20 μM), in 1% DMSO PBS Buffer at pH 7.4. λex = 465 nm, with a 5 min delay between additions of HA. *Insert:* Relative fluorescence changes (I/I_0) as a function of HA concentration (0 – 20 μM)

Having shown that probe **1** works with HA we set out to investigate the selectivity amongst several hydroxyl amines: *N*-(Benzyl)hydroxylamine, *N*-(Propargyl)Hydroxylamine, *N*-(tert-Butyl)Hydroxylamine, *O*-(Benzyl)Hydroxylamine, and *N*-(Phenyl)Hydroxylamine. (Fig 2)

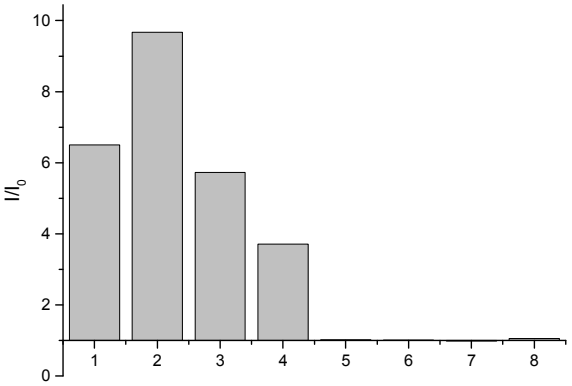

Fig 2. Selectivity of probe **1** (0.5 μM) towards various hydroxylamines (1) Hydroxylamine, (2) N-(methyl)hydroxylamine, (3) N-(Benzyl)hydroxylamine, (4) N-(Propargyl)Hydroxylamine, (5) N-(*tert*-Butyl)Hydroxylamine, (6) O-(Benzyl)Hydroxylamine, (7) N-(Phenyl)Hydroxylamine, (8) Blank. 15 min incubation with each analyte (50 μM) in PBS Buffer, 1% DMSO pH = 7.4. slit width ex = 5 nm, em = 2.5 nm. λ_{exc} = 465 nm, λ_{em} = 510 nm.

From these results it is clear that the system works for simple primary hydroxyl amines (1) Hydroxylamine, (2) N-(methyl)hydroxylamine (3) *N*-(Benzyl)hydroxylamine, (4) *N*-(Propargyl)Hydroxylamine,) However, larger alkyl groups or aromatic hydroxyl amines such as (5) *N*-(tert-Butyl)Hydroxylamine and (7) *N*-(Phenyl)Hydroxylamine do not work. It is evident that the system also requires the hydroxyl of the hydroxyl amine (6) *O*-(Benzyl)Hydroxylamine. These observations are consistent with the proposed mechanism of the reaction (Fig 3). Where, a nucleophillic nitrogen[23] and exchangeable OH are required to facilitate condensation of HA with probe **1**.

We have carried out a more extensive screen amongst relavant biological species (ESI), and as expected only hydrogen sulphide (50 μM) produced a fluorescence response. We then attempted to detect hydroxylamine in a cellular environment using probe **1.** HeLa cells were incubated HA (0 - 150 μM) for 30 min and washed with DPBS then 1 μM of probe **1** was added and left for 30 minutes, fluorescence images were then acquired by confocal microscopy. From these fluorescence images it is clear that probe 1 was fluorescent in HeLa cells even without added HA (ESI Fig 3). The strong background fluorescence is attributed to cellular thiols including endogenous H_2S present in the HeLa cells.

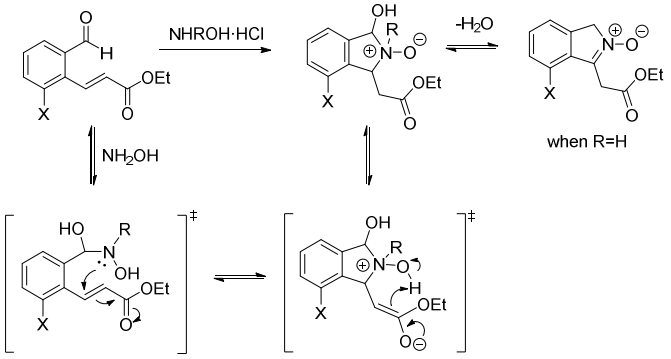

Fig 3. Mechanism for the reaction of probe **1** with HA (adapted from reference 20).

In conclusion we have developed a fluorescence turn-on probe for the detection of hydroxylamine. The system works well *in vitro* but with the *in vivo* cellular experiments endogenous thiols react to turn on probe **1**. We are currently working to develop a system with enhanced selectivity for hydroxyl amines over thiols. However, it is interesting to note that the system using *N*-(Propargyl) Hydroxylamine could be used as fluorescent "click" tag.

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Notes and references

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All data created during this research are openly available from the University of Bath data archive at http://doi.org/XXXXXXX

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