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Naked Eye Detection of Thiols by using Pyrene based Chemosensor in Aqueous Medium

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ABSTRACT

Thiols are well known biological species plays a major physiological role. Sensitive and quantitative sensing thiols is therefore of great significance to public health investigation A new Pyrene based turn-on fluorescent probe based on the Anchoring and Unanchoring strategy for the selective detection of thiol was synthesized. The pyrene potrntially used as the fluorophore and an DNBS group was combined as both a fluorescence quencher and a recognition unit .which can react with thiol and releases the hydroxypyrene which has strong fluorescence, The fluorogenic reaction is selectively initiated by the presence of thiols. The results indicate high application potential in analytical chemistry and diagnostics.

Keywords : Thiols, Anchoring- Unanchoring, hydroxypyrene, DNBS, CTAB, Chemosensor.

I. INTRODUCTION

In last few decades there has been specific attention on the chemosensing of mercaptans, as they play a key role in extensive array of physiological processes and biological functions[1-5] ,biothiols such as cysteine ,homocysteine, and glutathione are a component of many amino acids and peptides, Preservation of appropriate concentrations of these low molecular-weight thiols is necessary for numerous cellular functions, conversely, the abnormal levels of thiols can give rise to various health complications. For instance, the inadequacy of Cys contribute to slowed growth in children, hair depigmentation, edema, lethargy, liver damage, loss of muscle and fat, skin lesions, and weakness,an elevated level of Hcy in human plasma is a risk factor for Alzheimer's, cardiovascular diseases, neural tube defect, and coronary heart disease[6-14] . Therefore, a rapid, sensitive, and selective detection of thiols in biological samples is of significance.

Typical instrumental detection methods for biothiols include liquid chromatography, capillary electrophoresis, voltammetry and flow injection[15-20]. However, those techniques require relatively complex sample preparation protocols and sophisticated instrumentation. Among various methods for detection of biothiols, the fluorescence method[21-41] based on fluorescence probe is expedient due to its desirable features such as high sensitivity, simplicity, and potential for in vivo imaging . Noteworthy efforts have been devoted to construction of fluorescent probes for biothiols . Many fluorescent probes, involved in different detecting mechanisms, have been developed for thiols detecting, such as Michael addition reaction[42], cyclization reaction based on aminothiols and aldehyde , cleavage reaction induced by thiols ,and ligand displacement of metal complexes by thiols.[21-41]

The thiol mediated nucleophilic displacement strategy[43] provides a superior performance of chemosensors for recognition of thiols. However, it is

still a great challenge to develop thiol chemosensors with prompt response, high sensitivity, and large spectral shift other than only "turn-on/off" features. The interest in thiol sensing urges us to develop effective sensors with distinct spectral shift that can provide colorimetric and ratiometric fluorescence sensing with self calibration enabling quantitative analysis, easy observation, and practical applications.
Herein we reported a sensor for colorimetric determination of thiol. The sensor molecule consists of a pyrene derivative, 8-hyroxylpyrene-1 carboxaldehyde, as the chromophore, and is readily prepared by capping with a DNBS unit to the hydroxyl group. The sensing proceeds through a removal[44] of sulphonyl group mediated by thiol and a consequently changes colour change of the pyrene derivative.

II. MATERIALS AND METHODS

(GENERAL INFORMATION)

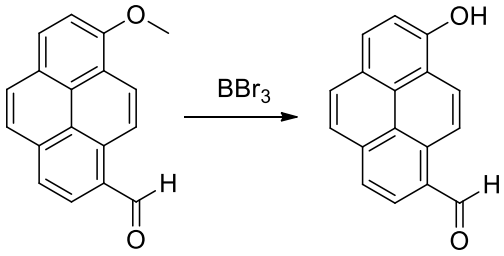
All reactions were carried out in oven dried vials with magnetic stirring under nitrogen atmosphere. Dried solvents and liquid reagents were transferred by oven-dried syringes or hypodermic syringe. All experiments were monitored by analytical thin layer chromatography (TLC). TLC was performed on pre-coated silica gel plates. After elution, plate was visualized under UV chamber. Further visualization was achieved by staining $KMnO_4$ and charring on a hot plate.

Melting points were determined on a Thomas Hoover capillary melting point apparatus with a digital thermometer. IR spectra were determined on JASCO FTIR 4000 spectrophotometers. ^1H NMR spectra and ^{13}C NMR spectra were recorded with Bruker AV, 200/400/500 MHz spectrometers in appropriate solvents using TMS as internal standard or the solvent signals as secondary standards and the chemical shifts are shown in δ scales. Fluorescence spectra measurement were performed with a Perkin–Elmer LAMBDA 950 UV/Vis Spectrophotometer .

DESIGN AND SYNTHESIS OF PROBE:

Step 1. Synthesis of 8-hydroxypyrene-1carbaldehyde
Step 2. Synthesis of Probe from 8-hydroxypyrene-1carbaldehyde

Step 1.



General Procedure: OH-PC (1mmol) and dry methylene chloride .And then, BBr3 (3 mmol) was added slowly to the solution at 0°C , and the stirring was continued for the next 12 hours at room temperature. Finally the reaction solution was poured into ice water, the crude extracted with dichloromethane, and the separated organic phase was dried over Na_2SO4. The solvent was evaporated and the residue was purified by column chromatography using dichloromethane: Hexane as eluent to obtain yellow powder. Yield: (85%)

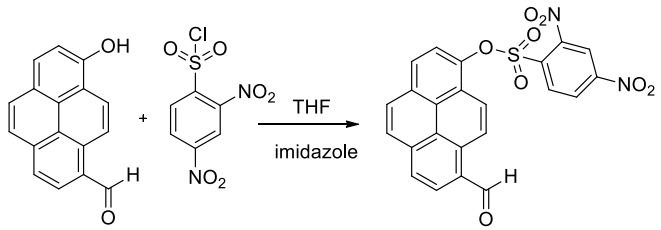
Spectral Data:

1H NMR (400 MHz, DMSO) δ = 11.12 (s, 1H), 10.72 (s, 1H), 9.31 (d, J = 9.5 Hz, 1H), 8.62 (d, J = 9.5 Hz, 1H), 8.52 – 8.43 (m, 1H), 8.34 – 8.21 (m, 3H), 8.00 (d, J = 8.8 Hz, 1H), 7.68 (d, J = 8.3 Hz, 1H).

13C NMR (101 MHz, DMSO) δ =193.8, 154.5, 136.4, 131.9, 131.7, 131.3, 129.4, 126.3, 125.4, 125.3, 124.7, 124.1, 124.0, 123.8, 121.2, 118.2, 114.3.

HRMS (ESI) m/z [M□H]+calc. 245.05971 found: 245.06026,

Step 2. Synthesis of Probe (DNBS-OPC)



OHPC DNBS DNBS-OPC

General Procedure: A solution of 2,4 dinitrobenzene sulphonyl chloride (1.0 mmol,) in dry THF (5 mL) was added to a THF solution (10 mL) of imidazole (1.0 mmol,) and 8-hydroxylpyrene-1-carbaldehyde (1 mmol,) at 0 °C, and the mixture was stirred for 0.5 h at 0 °C and then 1 h at ambient temperature. The solids were filtered off and the filtrate was evaporated under vacuum. Subsequently, CH2Cl2 (10 mL) was added into the residue. The solution was washed with deionized water for three times, and dried over Na2SO4. Finally, the solvent was evaporated under vacuum and the residue was purified by silica-gel column chromatography

Spectral Data:

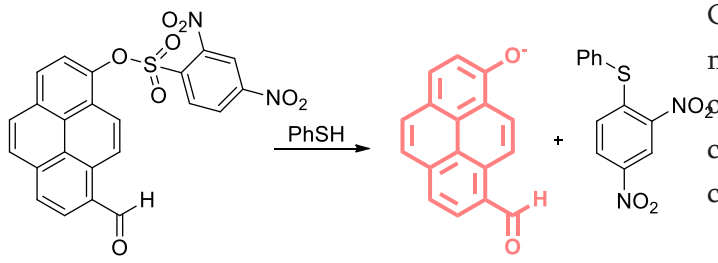
1H NMR (400 MHz, DMSO) δ 10.79 (s, 1H), 9.85 (s ,1H),9.65(d, J = 8.2 Hz, 1H),9.45(d, J = 8.2 Hz, 1H),9.49 (d, J = 9.6 Hz, 1H),,8.96 (d, J = 9.6 Hz, 1H),,8.42 (d, J = 8.0 Hz, 1H),,8.18 (d, J = 8.0 Hz, 1H), 8.02 (d, J = 8.8 Hz,1H), 7.91 (d, J = 8.8 Hz, 1H), 7.43–7.46 (m, 2H),

13C NMR (100 MHz, CDCl3): δ 193.1, 153.63,151.73, 147.23,144.23,136.37, 135.56, 132.31, 131.95, 131.87, 130.75, 130.36, 128.17, 127.81, 126.82, 125.71, 125.44, 125.37, 125.13, 124.98, 124.03, 122.34, 121.92, 117.33,

IR (KBr) ν (cm–1): 1050 (S=O), 1688 (O=C), 2720 (C(O)-H).

MS m/z [M+H]+ calc. 477.79, found: 476.76.

SENSING EXPERIMENT:



OH-PC bears an EWG- aldehyde unit and an EDG-hydroxyl group, giving it a typical ICT feature. Anchoring of OH-PC with DNBS will quench the fluorescence ,deprotection of it will induce spectral shifts, which can be used for the colorimetric sensing. Water-solubility and interaction with biomolecules are always the major problems with the organic dyes used as intracellular probes, and OHPC is no exception. A cationic surfactant, CTAB was introduced to solubilize the DNBS-OPC in water, which can also significantly improve the performance of sensors in aqueous environment by attracting SH ions and facilitating the reaction between sensor molecule and thiol. The stability of the sensor candidates toward water was evaluated by monitoring the variation of absorption and emission spectra of their CTAB aqueous solutions in the absence of fluoride ion. No change was observed for DNBS-OPC after standing for more than one hour, demonstrating their good stability against water Upon addition thiol

Optical Response of Probe to thiol:

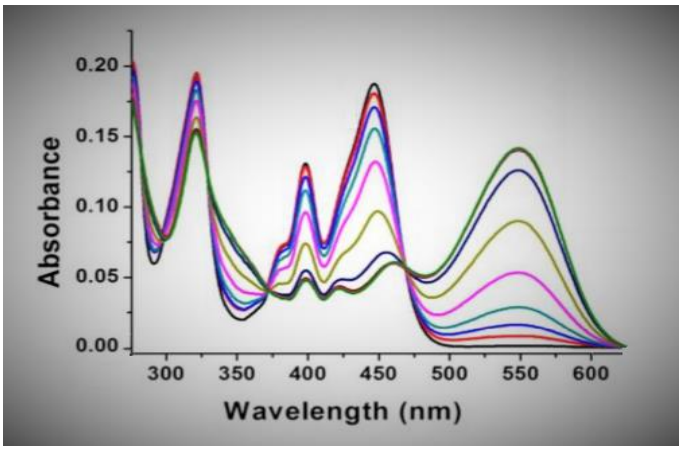
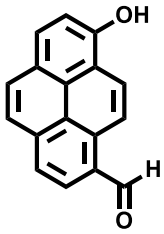
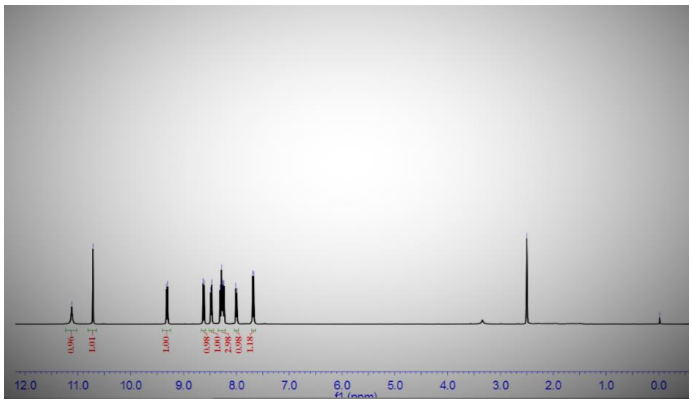


Figure 1. Absorption spectra of Probe (10 μM) with 2 mM surfactant after addition of various concentrations of PhSH (0–100 μM) at room temperature Three distinct absorbance peaks are observed 320 , 390, 445 nm after exposing Probe + CTAB with different conc of thiol will give rise to new absorbace peak at 550 appears due to formation of anionic chromophore. The color of the solution changes from colourless to pale red, indicating colorimetric detection of thiols

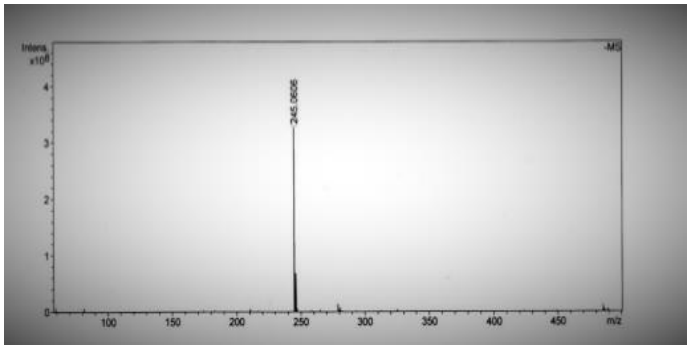


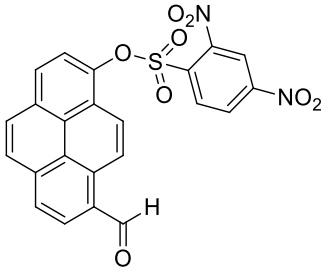
NMR Spectrum of OH-PC:

Shows characteristic peak of OH and aldehydic proton



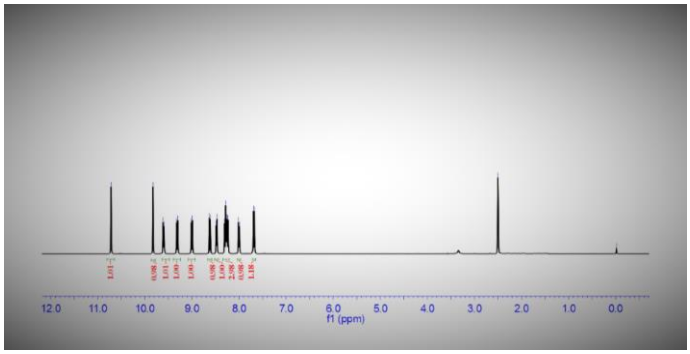
Mass Spectrum of OH-PC:



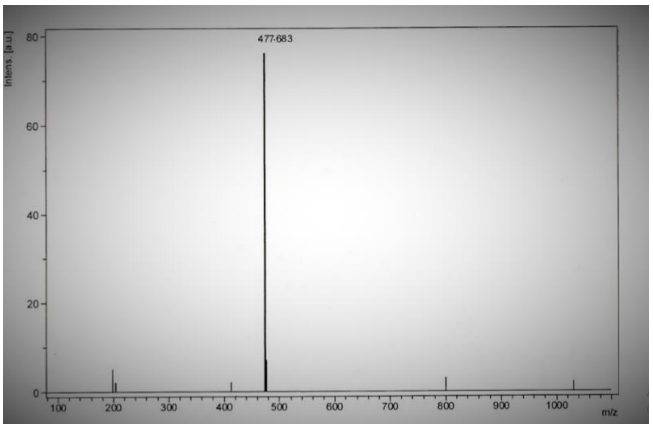


NMR spectrum of DNBS-OPC

Characterastic peak of C-H aldehyde, No OH peak as in case of OH-PC



Mass spectrum: DNBS-OPC



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