© 2018 IJSRSET | Volume 4 | Issue 4 | Print ISSN: 2395-1990 | Online ISSN : 2394-4099
Themed Section : Engineering and Technology

A New Pet+Espit Based Nitrobiphenyl Benzothiazole Derived Fluorescent Probe for Sensing of Biothiols

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ABSTRACT

We herewith present a fluorescent probe for the sensing of sulphur containing biomolecules. Sensing function is based on excited state intramolecular proton transfer strategy with large difference in absorption and emission maxima i.e. higher stoke shift. The design of probe is based on biphenyl skeleton exhibiting larger Stokes shift than its monocyclic counterpart. This probe with 2,4- dinitro benzene sulfonate (DNBS) group highly prone to thiolysis was designed based on the amalgamation of photo-induced electron transfer (PET) and excited state intramolecular proton transfer (ESIPT) mechanisms, which initiates a fluorescence turn on response

Keywords: Biothiol, ESPIT, PET, Nitrobiphenyl benzothiazole, DNBS

I. INTRODUCTION

Sulphur bearing amino acid alias biothiols such as cysteine,homocysteine and glutathione, have fascinated scientific community in recent years for their vital role in numerous biochemical phenomenons.[1-5] They contributed in the course of reversible redox reactions to normalize the metabolism and stabilization of the cellular functions. Irregular concentrations of Sulphur containing amino acids associated with various health issues as in, pulmonary embolism[6] cardiovascular diseases[7], neural tube defects[8], and osteoporosis[9], depigmentation of hairs, tiredness, muscle loss, acquired immune deficiency syndrome and dementia[10] .Thus, quantitative estimation of these species is absolutely necessary in biological, therapeutic, and clinical studies. Currently, the detection methods for biothiols consist of electrochemistry [11].High through put liquid chromatography,[12] fluoroscopy,[13] chemi-luminescence.[14] However, costly and sophisticated instrumentation or tedious working procedures are generally linked with these detection methods. In difference to these methods, fluorescence detection has become the leading strategy for monitoring thiols sensing due to its high selectivity, low detection limit, operational ease and little impairment to intact cells.[15-17] Till date, several fluorescent probes for thiols have been developed founded on different strategies including cyclization reactions with aldehydes, [18-19]1,4 additions, deprotection reactions,[20-24].Among these approaches, the 2,4- dinitrobenzene sulfonate group (DNBS) as an efficient sensing group has been utilized to sense thiols with high selectivity against other thiol-free amino acids.[25-30] However, many of them still face some problems, such as low sensitivity, complex synthetic methods and small difference between absorption and emission maxima. Thus, developing new fluorescent probes to monitor the presence of thiols in living cells remain to be a great challenge. 2-(Benzothiazol-2-yl)phenol and its derivatives were good candidates for the design of probes due to their intriguing optical properties such as good photo-sustainability, relatively high

fluorescent quantum yield and good cell membrane permeability.[31-33] Importantly, upon excitation, these derivatives exhibited an excited state intramolecular proton transfer (ESIPT) process from the aromatic OH form to the keto form, resulting in a large Stokes shift .[34] It is known that fluorescent dyes with large Stokes shifts are more desirable for the application because they can improve the sensitivity by reducing the self-quenching and auto-fluorescence resulted from the minimal overlap of excitation and emission spectra.[35-36]

Looking at the perspective of the fluorescence phenomenon we envisioned that the 2,4-dinitrobenzenesulfonate moiety acting as the electron acceptor in probe would block the fluorescent intensity of the fluorophore due to an operative photoinduced electron transfer (PET) process and the inhibition of the ESIPT route. When treated with biothiols, probe would be converted into 4'-hydroxy-3'-(benzothiazol-2-yl)-4- nitrobiphenyl, which emits strong blue fluorescence upon excitation via an ESIPT process

II. MATERIALS AND METHODS:

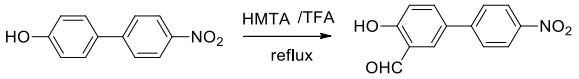
Most of the ingredients used by us for this work were either borrowed or purchased from commercial suppliers and used without further purification.. Solvents needed for this work are being purified and dried by using standard protocols available in literature. Re-distilled water has been used throughout all of our experiments.

For performing all reactions we have made use of oven dried vials with magnetic stirrers and nitrogen to maintain inert atmosphere. Dried solvents and liquid reagents were transferred by sterile syringes cooled to room temperature in a desiccator. Aluminium sheet coated silica plates (TLC) were used for monitoring and to analyse the status of the reactions. TLC plates plate were visualized under UV chamber to locate and analyse the position of spots. Further to confirm, the spots are exposed to KMnO4 solution ,visualized after charring on a hot plate.

Proton NMR spectra and ^{13}C NMR spectra were recorded with Bruker AV instruments in appropriate solvents using Tetramethyl silane as internal standard or the solvent signals are treated as subsidary standards and the chemical shifts are shown in δ scales. All measurements were carried out at room temperature. Measurement of Fluorescence spectra were performed with a Perkin–Elmer UV/Vis Spectrophotometer and a Photon Technology International, QuantaMaster 400 Spectrofluorometer, respectively, in degassed spectral grade.

Synthesis of 3 -formyl-4 -Hydroxy-4-nitro biphenyl.

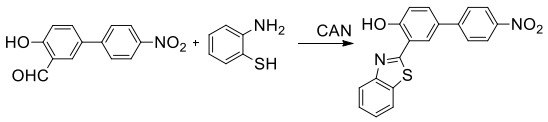
Scheme: 1



4-hydroxy-4-nitrobiphenyl (1mmol),hexa meth- ylene tetramine (HMTA, 5 mmol) dissolved in trifluoro acetic acid were heated to reflux for overnight. The reaction system was cooled to room temperature and adding1.0 M HCl. Resulting mixture was extracted with dichloromethane (3×50 mL). Combined organic phase and washing with water three times and saturated salt water one time, and dried over anhydrous magnesium sulfate. Removing the solvent under reduced pressure to obtain give 3 -formyl-4 -Hydroxy-4-nitro biphenyl. Off-white solid;(70%) 1 H NMR (600 MHz, CDCl3): δ = 11.14 (s, 1 H, -OH), 10.02 (s, 1 H, -CHO), 8.32 (d, 3 J = 8.8 Hz, 2 H, 3,5-H), 7.84 (d, 4 J = 2.2 Hz, 1 H, 2-H), 7.82 (dd, 3 J = 8.6 Hz, 4 J = 2.4 Hz, 1 H, 6-H), 7.72 (d, 3 J = 8.8 Hz, 2 H, 2,6-H), 7.14 ppm (d, 3 J = 8.6 Hz, 1 H, 5-H). 13C NMR (151 MHz, CDCl3): δ = 196.5 (-CHO), 162.3 (-COH), 147.2 (C-4), 145.8 (C-1), 135.8 (C-6), 132.5 (C-2), 130.9 (C-1), 127.3 (C-2,6), 124.5 (C-3,5), 121.0 (C3), 119.0 ppm (C-2). HRMS (EI+): calcd. for C13H9NO4 [M]·+ 243.0532; found 243.0529; Δ = –1.2 ppm. Second fraction (Rf = 0.08):.

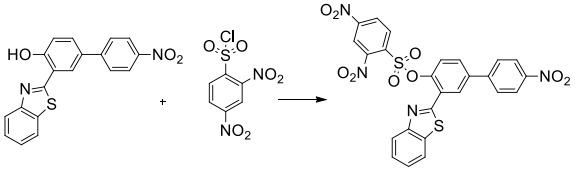
Synthesis of 4'-hydroxy-3'-(benzothiazol-2-yl)-4-nitrobiphenyl:

Scheme:2



General Procedure. A mixture of o-amino thiophenol (1 mmol), 3 -formyl-4 -Hydroxy-4-nitro biphenyl (1 mmol), H_2O_2 (30%, 4 mmol, 0.4 mL), and $NH_4Ce(NO_3)_6$ (0.1 mmol) was heated at 50 °C for 1h. After completion of the reaction, the reaction mixture was dissolved in EtOH (10 mL) and then poured into ice-water. The pure solid product was filtered, washed with ice-water, and subsequently dried. Purified on silica column (6:1 hexane/EA) to yield 4'-hydroxy-3'-(benzothiazol-2-yl)-4-nitrobiphenyl as a light yellow solid (73%). 1H NMR (600-MHz, CDCl3): δ = 11.15 (s, 1 H, -OH), 8.33 (d, 3 J = 8.8 Hz, 2 H, 3,5-H), 7.93(m,1H,12-H),7.85 (d, 4 J = 2.2 Hz, 1 H, 2-H), 7.81 (dd, 3 J = 8.6 Hz, 4 J = 2.4 Hz, 1 H, 6-H), 7.73 (d, 3 J = 8.8 Hz, 2 H, 2,6-H), 7.51(m,1H,13-H),7.41(m,1H,14-H),7.14 ppm (d, 3 J = 8.6 Hz, 1 H, 5-H). 13C NMR (150 MHz, DMSO-d6) 164.38, 156.80, 151.94, 144.40, 135.43, 131.45, 131.29, 129.96, 129.70, 127.50, 125.42, 123.13, 122.80, 120.04, 109.95. HRMS EI) m/z calculated = 348.0730, Found: 348.0742.

Synthesis of probe 1.(Scheme:3)



To a stirred solution of 4'-hydroxy-3'-(benzothiazol-2-yl)-4- nitrobiphenyl (1.0 mmol) and NEt_3 (1.3 mmol) in dry DCM under an nitrogen atmosphere was added 2,4-dinitrobenzenesulfonyl chloride (1.3 mmol) at room temperature. The resulting reaction mixture was allowed to stir at room temperature for 3 h. The reaction was quenched with water and the resulting solution was extracted with twice with portions of CH_2Cl_2. After drying over anhydrous sodium sulphate[4], the organic solvent was filtered and removed under vacuum. The resulting crude product was purified by silica gel column chromatography hexane/ethyl acetate as eluent to yield the desired product.

1H NMR (600 MHz, DMSO-d6) d 8.98 (d, J = 2.2 Hz, 1H), 8.44 (d, J = 2.4 Hz, 1H), 8.36 (dd, J = 8.7, 2.3 Hz, 1H), 8.22 (d, J =8.7 Hz, 1H), 8.16 (d, J = 7.9 Hz, 1H), 8.08-7.98 (m, 6H), 7.59-7.55 (m, 2H), 7.53-7.48 (m, 1H). 13C NMR(150 MHz,DMSO-d6) 161.46, 152.68, 151.26, 148.05, 146.30, 142.63, 139.31, 135.77, 132.13, 132.02, 131.36, 128.49, 128.06, 127.65, 125.12, 122.66, 121.12, 111.53.MS (ESI): found: m/z = 579.1 (M+1)+calculated. for C25H19N3O9S = 578.531.

III. RESULT AND DISCUSSIONS

The desired nitrobiphenyl in three steps as discussed in the material and method section. Formation of all the intermediates and the desired probe is confirmed by NMR,C-13 and MS spectroscopy

Scheme: 1 describes formation of 3 -formyl-4 -Hydroxy-4-nitro biphenyl. 11.10 (1H, s), 9.98 (1H, s) values of chemical shifts indicates OH and aldehydic proton .Scheme: 2 elaborates formation of4'-hydroxy-3'-(benzothiazol-2-yl) - 4- nitro bi phenyl 11.15 (singlet, 1H) indicates free OH along with extra aromatic proton signals from benzothiazole ring ,wherein aldehydic proton signal is absent. Scheme: 3 elaborates synthesis of probe,NMR signal doesn't show OH proton signalin NMR data whereas 8.98 indicates aromatic proton near to NO_2 functionality.

Sensing study of Probe with thiol:

The Probe which has been designed and developed is essentially non-fluorescent due to inhibition of ESPIT process. The sensing ability probe and 4'-hydroxy- 3'-(benzothiazol-2-yl)-4-nitrobiphenyl was determined in MeCN:PBS buffer solution (50.0 mM, v/v = 1:1, pH = 7.4). 4'-hydroxy-3'-(benzothiazol-2-yl)-4-nitro biphe- nyl displayed strong fluoresces (λabs= 300 nm, λem = 482 nm Fig:2) To understand the sensing

process of probe ,the fluorescence spectra changes of probe (10.0 uM) were measured with increasing concentrations of Cys in MeCN: PBS solution (50.0 mM, v/v = 1:1, pH = 7.4). a significant turn on fluorescence response with a maximum at 480 nm was observed upon the addition of Cys , indicating that Cys could rupture the bond liberating 2,4-dinitrobenzenesulfonate moiety thereby initiating ESPIT process.

Time-dependent fluorescence spectra: To obtain an appropriate reaction time, the time-dependent fluorescence experiments were also carried out on probe (10.0 uM) with various concentrations of Cys (100uM) with increasing the reaction time, the increased and reached plateau within 30 min. The higher concentration of Cys resulted in a faster reaction and pronounced fluorescence enhancement. Moreover, probe 1 displayed no observable fluorescence in the absence of Cys during an identical measuring time. All these experiments indicated that probe could serve as an efficient fluorescence turn-on probe for rapid detection of Cys in MeCN-PBS solution (50.0 mM, v/v =1:1,
pH=7.4).

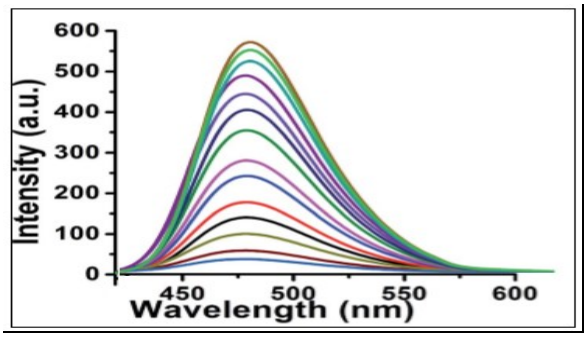


Figure 1

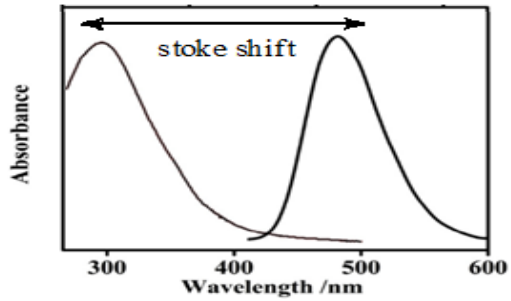


Figure 2

Figure 1:Fluorescence spectra of probe (10.0 mM)) with varying concentration of Cys in PBS -MeCN solution (50.0 mM, v/v = 1:1, pH = 7.4). (λex = 300 nm, λem = 480 nm). Upon treatment with increasing concentrations of cystein, the fluorescence intensity has gradually increased, and reached saturation Fig.2 Absorption and Fluorescence spectra spectra of 4 hydroxy 4 nitrobiphenyl). (λex = 300 nm, λem = 480 nm).

IV. CONCLUSION

In summary, a new PET+ESIPT-based fluorescence probe for biothiols was prepared and reported. ,it was considered that thiol could induced the unanchoring of 2,4-dinitrobenzenesulfonate group (fluorescence quencher) in probe to produce 4 hydroxy 4' nitrobiphenyl (blue fluorescence) After reaction with thiols, inhibition of PET process as well initiation of ESPIT would induced the fluorescence enhancement of probe. The recognition of thiols displayed an excellent selectivity.

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