Dyes and Pigments 106 (2014) 25–31

Contents lists available at ScienceDirect

Dyes and Pigments

journal homepage: www.elsevier.com/locate/dyepig

Iminocoumarin based fluorophores: Indispensable scaffolds for rapid, selective and sensitive detection of thiophenol

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ARTICLE INFO

Article history:
Received 23 October 2013
Received in revised form
29 January 2014
Accepted 2 February 2014
Available online 28 February 2014

Keywords:
Iminocoumarin
Thiophenol sensing
Off-on probe
Fast responsive
Selectivity
Benzothiazole

ABSTRACT

Off-on fluorescence probes for the detection of thiophenol were designed based on the reaction mechanism to ensure rapid sensing process. 2,4-Dinitrophenylsulfonyl based probes with iminocoumarin fluorophores were validated by theoretical calculations for their *off*-fluorescence states. Reaction of these probes with thiophenol released strong fluorescent iminocoumarin species with good response times. These results, upon comparing with reported probes, confirmed that higher pK_{aH} values of the imino nitrogen of iminocoumarins are important for rapid sensing process. Reactivity of these probes was limited to only thiophenol and no reaction was observed with aliphatic thiols. The benzothiazole substituted probe displayed up to 260-fold fluorescence enhancement upon reaction with thiophenol. Sensing of the analyte by the probe was characterized by change in color from red to yellow and with appearance of the green fluorescence. A detection limit up to 6.9×10^{-9} M was also determined for this probe.

1. Introduction

Thiophenols, widely used in the production of polymers, pesticides and pharmaceuticals [1–3], are highly toxic and polluting chemicals. These aromatic thiols target central nervous system, kidney and liver [4–6] resulting in burning sensation, wheezing, nausea, vomiting, etc. [4,7] Presence of thiophenols in water and soil are also reported to cause damage to natural habitats [8]. Since, the first report of 2,4-dinitrophenylsulfonyl (DNs) based fluorescent thiophenol probe **1** by Jiang et al.,[9] considerable number of DNs-based [10–13] and other thiophenol probes [14] have been developed [15]. DNs-based thiophenol probes undergo thiolate mediated SNAr reaction on the DNs-ring to release the fluorophore (Fig. 1(a)). Either an intramolecular charge transfer (ICT) or photoinduced electron transfer (PET) pathway from the fluorophore to the DNs moiety is responsible for the *off*-fluorescence state of these probes. Selectivity of these probes toward aromatic thiols by discriminating aliphatic ones, has paved their significance in sensing applications *e.g.* detection of thiophenol in water, living cells. However, slow reactivity of these probes with PhSH has resulted in relatively long response times (t_Rs). For example, with a 1:10 molar ratio of probe versus PhSH, probe **1** displayed a $t_R = 17$ min (Fig. 1(b)). For probe **2**, an increased $t_R = 20$ min was determined, when studied under comparable conditions. The fastest responsive DNs-based probe **3** which provided a $t_R = 12$ min was reported by our group [13]. To date the best $t_R = 2$ min was reported by Lin et al. for a probe in which a coumarin fluorophore was linked to the 2,4-dinitrophenol quencher. However, a molar ratio of 1:20 was used during the sensing experiment and a decrease in molar ratio to 1:5 resulted in an increase in t_R value to 7 min [14]. High reactivity of the analyte towards various Michael acceptors is expected to result in fluctuations of actual concentration of the species. Hence, it is essential to develop fast responsive *off-on* thiophenol probes for quantitative detection of the species particularly applicable in biological and natural systems.

A rational approach for controlling the rates of decomposition of DNs-amides was reported recently by Malwal et al [16]. A structure activity relationship (SAR) study provided crucial insight into the reaction mechanism of cysteine-activated SO_2 (a gaseous anti-mycobacterial agent) release from these pro-drugs. During the thiol mediated decomposition of DNs-linked arylamines **4**–**6** to free aniline derivatives **4a**–**6a**, a reactivity order **6 > 5 > 4** was observed based on half-lives ($t_{1/2}$) of these DNs-derivatives (Fig. 2). A correlation of pK_{aH} values of **6a**, **5a** and **4a** with determined $t_{1/2}$ values confirmed a linear relation between these parameters *i.e.* a faster cleavage of DNs group was achieved with higher pK_{aH} value of the released aniline derivative. This structure activity relationship (SAR) study suggests that the protonation of aniline *N*-center is the

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http://dx.doi.org/10.1016/j.dyepig.2014.02.001
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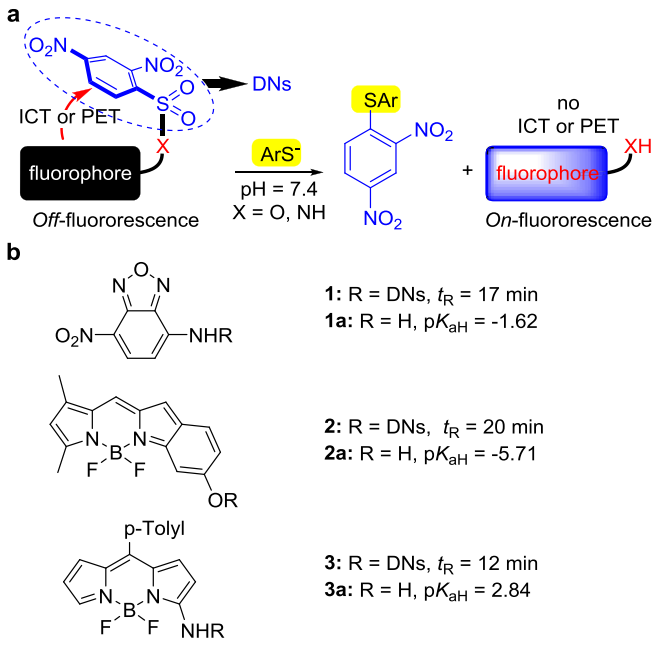


Fig. 1. The *off-on* mechanism for DNs appended fluorescent probes (a), Structures, response times of reported DNs-based thiophenol probes **1-3** and pK_{aH} values released fluorophores **1a-3a** (b).

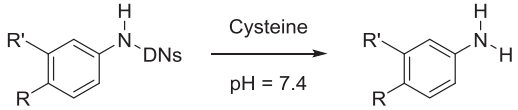
rate determining step (*rds*) and this step occurs after a thiol mediated *ipso* attack (for −SH group of Cys, pK_a = 8.0) on the DNs ring. This study has provided a simple and effective methodology to alter onset times of SO_2 releasing prodrugs by adjusting the basicity (pK_{aH}) of the released amine. We realized the importance of this work in the design of new thiophenol probes to ensure faster response times compared to reported probes **1-3**.

Herein, we report two DNs-derivatized iminocoumarin probes **7** and **8** for rapid, selective and sensitive detection of thiophenol (Fig. 3). We speculate that an intramolecular charge transfer (ICT) pathway from iminocoumarin to DNs moiety is responsible for the fluorescence *off*-state in these probes. Free iminocoumarins **7a** and **8a** can be released as strongly fluorescent species from these probes via the thiolate (PhS^-) mediated cleavage of DNs moiety. Particularly in **8a**, an electron donating diethylamino group at the 7-position and electron withdrawing benzothiazole group at 3-position favor strong fluorescence properties at longer wavelength compared to **7a** via the intramolecular electron transfer process [17].

2. Materials and methods

2.1. General methods

All reactions were conducted under the nitrogen atmosphere. All the chemicals were purchased from commercial sources



4: R = H, R' = F, $t_{1/2}$ = 63 min
5: R, R' = H, $t_{1/2}$ = 25 min
6: R = OMe, R' = H, $t_{1/2}$ = 12 min
4a: R = H, R' = F, pK_{aH} = 3.38
5a: R, R' = H, pK_{aH} = 4.64
6a: R = OMe, R' = H, pK_{aH} = 5.29

Fig. 2. Correlation of calculated half lives ($t_{1/2}$) of prodrugs **4-6** with and pK_{aH} values of released amines **4a-6a**.

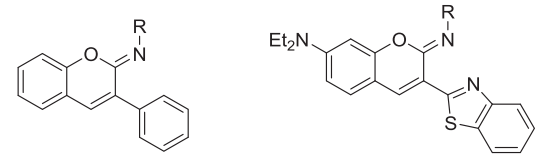


Fig. 3. Structures of iminocoumarin based thiophenol probes **7-8** and active fluorophores **7a** and **8a**.

(Aldrich, Spectrochem, and Sdfine Chemical Limited) and used as received unless stated otherwise. Solvents: petroleum ether and ethyl acetate (EtOAc) were distilled prior to thin layer and column chromatography. Column chromatography was performed on Merck silica gel (100–200 mesh). TLC was carried out with E. Merck silica gel 60-F-$_{254}$ plates.

2.2. Physical measurements

^1H and ^{13}C spectra were recorded on 400 MHz Jeol ECS-400 (or 100 MHz for ^{13}C) spectrometers using either residual solvent signals as an internal reference or from internal tetramethylsilane on the δ scale ($CDCl_3$ δ H, 7.24 ppm, δ C 77.0 ppm and for DMSO-d_6 δ H, 2.52 ppm, δ C 41.23 ppm). The chemical shifts (δ) are reported in ppm and coupling constants (J) in Hz. The following abbreviations are used: m (multiplet), s (singlet), br s (broad singlet), d (doublet), *t* (triplet) dd (doublet of doublet). High-resolution mass spectra were obtained from Micro Mass ESI-TOF MS spectrometer. Absorption spectra were recorded on a Thermo Scientific, Evolution 300 UV-VIS spectrophotometer. Steady State fluorescence experiments were carried out in a micro fluorescence cuvette (Hellma, path length 1.0 cm) on a FluoroMax-4 (Horiba JobinYvon). FT-IR spectra were obtained using NICOLET 6700 FT-IR spectrophotometer as KBr disc and reported in cm^{-1}. Melting points were measured using a VEEGO Melting point apparatus. All melting points were measured in open glass capillary and values are uncorrected. Crystal structures were recorded on a Bruker single crystal X-Ray diffractometer.

2.3. Theoretical calculations

Geometry optimization of **7** and **8** were carried out in the gas phase using density functional theory (DFT) at the B3LYP/6-311G (d,p) level using Gaussian 09 software [18,19]. Time-dependent DFT (TDDFT) calculations were carried out on the geometry optimized structures of **7** and **8** at the B3LYP/6-311G(d,p) level to predict excitation and emission properties.

2.4. Synthesis

2.4.1. 2,4-Dinitro-N-(3-phenyl-2H-chromen-2-ylidene) benzene sulfonamide ($C_{21}H_{13}N_3O_7S$) 7

To a solution of 3-phenyl-2*H*-chromen-2-imine **7a** (200 mg, 0.9 mmol) in pyridine (10 mL) placed in a 100 mL two-neck round bottomed flask were added 2,4-dinitrobenzene-1-sulfonyl chloride (362 mg, 1.35 mmol) at 0 °C slowly with stirring. The reaction mixture was placed under nitrogen atmosphere and stirred at room temperature for 1 h. After completion of the reaction, the solvent was removed under reduced pressure. The residue was poured into H_2O (10 mL) and extracted with CH_2Cl_2 (15 mL × 3). The combined organic layer was washed with water (10 mL × 3), brine (10 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure to obtain a yellow residue which was purified by column