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A new fluorogenic sensing platform for salicylic acid derivatives based on π-π and NH-π interactions between electron-deficient and electron-rich aromatics

Anup Pandith, Giridhari Hazra, Hong-Seok Kim *

Department of Applied Chemistry, School of Applied Chemical Engineering, Kyungpook National University, Daegu 41566, Republic of Korea

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ABSTRACT

A novel simple fluorescent probe was designed for the recognition of electron-rich salicylic acid derivatives (SAs). The imidazole-appended aminomethyl perylene probe **1** selectively differentiated between electron-rich amino-SAs and electron-deficient nitro-SAs in EtOH, exhibiting the highest selectivity and sensitivity toward 5-aminosalicylic acid (5-ASA) and showing strong 1:1 binding ($K_a = 1.37 \times 10^7\ M^{-1}$). This high selectivity and sensitivity resulted from the synergistic multiple hydrogen bonding interactions of secondary amine and imidazole units and π-π interactions between electron-rich and electron-deficient rings, along with the unusual NH-π interactions between 5-ASA and the perylene moiety of **1**. The limit of detection (LOD) for 5-ASA in EtOH was 0.012 ppb.

1. Introduction

Among the various salicylic acid derivatives (SAs), the electron-rich aminosalicylic acids; 5-aminosalicylic acid (5-ASA or mesalazine), 4-aminosalicylic acid (4-ASA), and 3-aminosalicylic acid (3-ASA) are used as drugs owing to their varied bioactivities [1–4]. Unlike aminobenzoic acid derivatives, aminosalicylic acid counterparts are more biochemically potent due to the presence of an additional *ortho*-hydroxyl group [5–7]. 5-ASA exhibits enzyme inhibition, immune transduction, analgesic, and anti-inflammatory properties [8,9], and is used as a potential drug to treat ulcerative colitis and Crohn's disease [10–12]. Apart from this, 4-ASA and other amino-substituted SAs are also used as anti-mycobacterial agents [13,14] and antioxidants [15], with the structurally distinct and biologically potent behavior of these amino aromatic acids making them vital bioactive natural products. Such structure-related pharmacological activities may cause predominant actions, thereby exhibiting few systematic side effects (such as fever, nausea, joint pain, unusual skin rash, and tiredness) when these compounds are present at non-optimal levels [16]. In nature, due to their complex biosynthetic pathways (as vital secondary metabolites), the SAs are usually found in complex biological fluids as mixtures with their isomers or homologues. Therefore, the selective differentiation between electron-rich and electron-deficient salicylic acid derivatives using highly efficient and rapid response techniques is highly sought in supramolecular chemistry. Fluorimetry is an economical and simple technique with a very fast response [17–25]. Recently, we reported *turn-on* and *turn-off* probes for electron-deficient SAs [26,27]. To further explore the sensing strategy, we have now extended our work toward more biologically potent electron-rich SAs. Fluorescent probes for electron-rich ASA derivatives and their differentiation from electron-deficient counterparts have not been reported yet.

Perylene is a strong UV-light absorber that exhibits high molar absorptivity. High quantum yield and low extent of photo-bleaching account for its highly unusual and distinct photophysical properties arising from its unsymmetrical electron density distribution. Therefore, even with its small Stokes shift, perylene derivatives have become an attractive choice for fluorescent probes. So far, few examples of perylene-based (other than perylene-diimides) probes have been reported for the detection of ions, neutral molecules and bioimaging studies along with structural based photophysics [28–30]. Typical rational design of fluorescent molecular probes for the detection of neutral molecules usually relies on protonation, coulombic interactions, hydrogen bonding and π-π interactions that can commonly induce fluorescence via electron density redistribution. However, along with the mentioned synergistic interactions, NH-π-, CH-π-, OH-π-, and halogen-π-interaction-oriented *turn-on* molecular probes are rarely reported in literature. Exploring such unusual interactions greatly helps better understand the complex biochemical reactions and design novel receptors for bioactive molecules. Owing to such importance of non-covalent interactions, we herein report a new strategy for the selective sensing of SAs at up to sub-nM levels based on a *turn-on* response.

* Corresponding author.
E-mail address: kimhs@knu.ac.kr (H.-S. Kim).

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2. Experimental

2.1. Materials and Methods

Analytical-grade ethanol was purchased from Merck. 1-(3-Aminopropyl)imidazole and other compounds used for synthesis were purchased from Aldrich Chemical Co. and were used as received. 3-Perylenecarboxaldehyde was prepared by the procedure reported in the literature [31]. Fluorescence quantum yields were determined by integration of the corrected fluorescence spectra, using a solution of quinine hemisulfate in 0.5 M H_2SO_4 as a standard ($\Phi = 0.54$). The fluorescence spectra were recorded immediately after sample preparation. Analytical-grade ethanol and deionized water were used for each measurement. Stock solutions (10×10^{-6} M) of SAs were freshly prepared during the analysis. Stock solutions (1.0 or 0.02×10^{-6} M) of the yellowish solid probes **1**/**2**/**3** were prepared in respective solvents (EtOH or EtOH/H_2O (9:1, v/v)).

2.2. Instrumentation

Melting points were determined using the Thomas-Hoover capillary melting-point apparatus and were uncorrected. The ^1H and ^{13}C NMR spectra were recorded on a Bruker AM-400 spectrometer. The high-resolution fast atom bombardment (HR-FAB) mass spectra were recorded on a JEOL 700 high resolution mass spectrometer at the KBSI Daegu center. UV–vis absorption spectra were recorded on a Shimadzu UV-1650PC spectrophotometer. Fluorescence spectra were measured on a Shimadzu RF-5301 fluorescence spectrometer equipped with a xenon discharge lamp, using 1-cm quartz cells with excitation and emission slit widths of 3/5. All measurements were performed at 298 K.

2.3. Calculation of Association Constants and LODs

The association constants of probes **1**/**2**/**3** were calculated using Origin 8.0 and Gnuplot ver. 5.1 software to obtain an accurate estimate of the binding constants and to minimize the error bound. Origin 8.0 was used to fit the fluorescence titration data employing the reduced chi-square method (error bound within ±10.0%) using the $Y = Y_0 + A_1 \times \exp((x - x_0)K_a)$ equation, where x and x_0 are the emission intensities of probes **1**/**2**/**3** in the presence and absence of SAs, respectively, A_1 is the concentration of probes **1**/**2**/**3**, and Y and Y_0 are the total concentrations of probes **1**/**2**/**3** in presence and absence of SAs, respectively. The fitted data points were used as an input for the curve-fitting method (error bound ±10.0%) of Gnuplot ver. 5.1 that used nonlinear regression analysis and an adjusted r-square method [32]. Solutions ($1.00/0.02 \times 10^{-6}$ M) of probes **1**/**2**/**3** were prepared in 50 mL volumetric flasks (±0.025 mL), and the detection limit was calculated based on the results of fluorescence titration. To determine the signal-to-noise (S/N) ratio, the emission intensity of probe **1** was measured five times, and the standard deviation of blank measurements were determined. LODs were calculated from the linear relationship between fluorescence intensity and analyte concentration after appropriate calibration.

2.4. Theoretical Calculations

Theoretical calculations were performed according to literature [33].

2.5. Synthesis of Probes

2.5.1. Probe *1*

A mixture of 3-perylenecarboxaldehyde (50 mg, 0.18 mmol), 1-(3-aminopropyl)imidazole (36 mg, 0.29 mmol), and $TiCl(O^iPr)_3$ (0.37 mL, 0.29 mmol) in CH_2Cl_2 (8 mL) was stirred at room temperature for 15 h under Ar, followed by the addition of $NaBH(OAc)_3$ (61 mg, 0.29 mmol). The resulting mixture was further stirred for 2 h. After the reaction was complete, the solvent was removed, and the residue was neutralized with $NaHCO_3$ solution and extracted with ethyl acetate. The organic layer was dried over anhydrous Na_2SO_4 and was concentrated to dryness. The crude product was purified by silica gel (neutralized with 8% triethylamine in hexane) chromatography (elution with Hex:DCM:MeOH:NH_4OH: 5:4:0.5:0.5, $R_f = 0.5$) to give **1** as a brownish-orange solid in 86% yield (60 mg). m.p. = 114–115 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 1.98 (m, 2H, H_e), 2.73 (t, $J = 5.1$ Hz, 2H, H_f), 3.95 (t, $J = 8.5$ Hz, 2H, H_d), 4.95 (bs, 1H, −NH), 6.79 (s, 1H, H_c), 6.95 (s, 1H, H_b), 7.42 (s, 1H, H_a), 7.96–8.00 (m, 5H, Py), 8.07 (m, 5H, Py), 8.07–8.17 (m, 5H, Py), 8.28 (d, $J = 7.5$ Hz, 1H, Py); ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.2, 44.4, 45.4, 50.7, 118.8, 122.8, 124.6, 124.7, 124.8, 125.2, 125.4, 126.0, 127.3, 127.5, 127.6, 128.0, 129.1, 129.2, 130.5, 130.6, 131.1, 131.2, 137.1; HR Mass $C_{27}H_{23}N_3$ $[M + H]^+$: 389.1892, Found: m/z 389.1895.

2.5.2. Probe *2*

A mixture of 3-perylenecarboxaldehyde (50 mg, 0.18 mmol), *n*-butylamine (21 mg, 0.29 mmol), and $TiCl(O^iPr)_3$ (0.37 mL, 0.29 mmol) in CH_2Cl_2 (8 mL) was stirred at room temperature for 12 h under Ar, followed by the addition of $NaBH(OAc)_3$ (61 mg, 0.29 mmol). The reaction mixture was further stirred at room temperature for 2 h. After the reaction was complete, work-up was performed as described for probe **1**. The residue was purified by silica gel (neutralized with 8% triethylamine in hexane) column chromatography (elution with Hex:DCM:MeOH:NH_4OH: 6:3:0.4:0.6, $R_f = 0.7$) to give **2** as an orange amorphous solid in 83% yield (50 mg). m.p. = 140–141 °C; ^1H NMR (400 MHz, DMSO-d_6) δ 0.88 (t, $J = 7.2$ Hz, 3H, $NHCH_2CH_2CH_2C\underline{H_3}$), 1.35 (m, 2H, $NHCH_2CH_2C\underline{H_2}$), 1.49 (m, 2H, $NHCH_2C\underline{H_2}$), 2.66 (t, $J = 6.8$ Hz, 2H, $NHC\underline{H_2}$), 4.12 (s, 2H, $C\underline{H_2}NH$), 7.52–7.60 (m, 4H, Py), 7.79 (m, 2H, Py), 8.05 (d, $J = 8.4$ Hz, 1H, Py), 8.32–8.40 (m, 4H, Py); ^{13}C NMR (100 MHz, DMSO-d_6) δ 14.0, 20.1, 31.7, 49.0, 50.9, 120.3, 120.3, 120.6, 120.6, 124.3, 126.6, 126.9, 127.6, 127.7, 127.8, 128.2, 129.3, 130.6, 130.7, 132.7, 134.3, 136.8; HR Mass $C_{25}H_{23}N$ $[M + H]^+$: 337.1830, Found: m/z 337.1831.

2.5.3. Probe *3*

A mixture of 3-perylenecarboxaldehyde (50 mg, 0.18 mmol), *n*-methylbutylamine (25 mg, 0.29 mmol), and $TiCl(O^iPr)_3$ (0.37 mL, 0.29 mmol) in CH_2Cl_2 (8 mL) was stirred at room temperature for 18 h under Ar, followed by the addition of $NaBH(OAc)_3$ (61 mg, 0.29 mmol). The reaction mixture was further stirred at room temperature for 2 h. After the reaction was complete, work-up was performed as described for probe **1**. The residue was purified by silica gel (neutralized with 8% triethylamine in hexane) column chromatography (elution with Hex:DCM:MeOH:NH_4OH: 6:3:0.3:0.7, $R_f = 0.7$) to give **3** as an orange amorphous solid in 87% yield (55 mg). m.p. = 145–146 °C; ^1H NMR (400 MHz, DMSO-d_6) δ 0.81 (t, $J = 8.0$ Hz, 3H, $N(CH_3)CH_2CH_2CH_2C\underline{H_3}$), 1.25 (m, 2H, $N(CH_3)CH_2CH_2C\underline{H_2}$), 1.46 (m, 2H, $NHCH_2C\underline{H_2}$), 2.14 (s, 3H, $N(C\underline{H_3})$), 2.40 (t, $J = 7.2$ Hz, 2H, $N(CH_3)C\underline{H_2}$), 3.77 (s, 2H, $C\underline{H_2}N(CH_3)$), 7.45 (d, $J = 7.6$ Hz, 1H, Py), 7.51–7.57 (m, 3H, Py), 7.77 (d, $J = 8.0$ Hz, 2H, Py), 8.13 (d, $J = 8.4$ Hz, 1H, Py), 8.28 (d, $J = 7.6$ Hz, 1H, Py), 8.32–8.37 (m, 3H, Py); ^{13}C NMR (100 MHz, DMSO-d_6) δ 14.3, 20.5, 29.3, 57.3, 60.9, 120.3, 120.4, 120.6, 120.7, 124.3, 126.6, 129.9, 127.6, 127.7, 127.9, 128.2, 129.4, 130.7, 130.8, 132.7, 134.3, 136.8; HR Mass $C_{26}H_{25}N$ $[M + H]^+$: 351.1986, Found: m/z 351.1987.

3. Results and Discussion

3.1. Synthesis and Sensing Properties of Probes

Unlike naphthalene, anthracene, and pyrene fluorophores, perylene exhibits a different electron density distribution. The former fluorophores feature electron-rich aromatic systems, oriented either axially (naphthalene, anthracene) or radially (pyrene). However, the anti-aromatic nature (20 π-electrons, according to Hückel's rule) of perylene,