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Unusual large Stokes shift and solvatochromic fluorophore: Synthesis, spectra, and solvent effect of 6-substituted 2,3-naphthalimide

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ABSTRACT

In this article, four new series of 6-substituted 2,3-naphthalimides (**1a–d**, **2a–d**, **3a–d**, **4a–d** and **4 e–h**) have been designed and synthesized through the formation of key cyclic anhydride intermediate, which was the precursor of the well known environment-sensitive fluorophore [6-*N*,*N*-dimethylaminonaphthalimides (6-DMN)] and other 6-substituted 2,3-naphthalimide series (**2**, **3**, **4**). Based on 6-amino-2,3-naphthalimide (6-ANP) compound, a new type of fluorophore was found to exhibit moderate to unusual large Stokes shift (297–303 nm). 6-ANP derivatives display relatively low fluorescence quantum yields in high polar protic solvents such as water ($\Phi_F \sim 0.004$, 571–576 nm) and a significant unusual red shift due to (1) hydrogen bonding interaction of the excited state of the molecule with the solvents, which presumably enhance the intersystem crossing process in the system to quench fluorescence, (2) this large Stokes shift was assumed to be the consequence of a substantial change of the geometric structure from the ground state (S_0) to the first excited state (S_1). Compared with the other compounds studied, the fluorescence of the nitro- and halo-derivatives was rather weak, probably due to the efficient intersystem crossing leading to a non-reactive triplet state.

1. Introduction

Environment-sensitive fluorophores are a special class of molecules that have spectroscopic properties depending on the physicochemical properties of their immediate surroundings. Typical examples of these fluorophores include donor–acceptor systems like 4-dimethylamino phthalimide (4-DMAP) [1], 2-propionyl-6-dimethylaminonaphthalene (PRODAN) [2], 4-amino-1,8-naphthalimide (4-DMN) [3] 6-*N*,*N*-dimethylamino-2,3-naphthalimide (6-DMN) [4] and 6-chloro-2,3-naphthalimide derivative (6-ClNP) [5] (Fig. 1). The applications of these new solvatochromic fluorophores have greatly expanded the scope of potential as unique chemical tools [6–8]. On one hand, peptides containing the 4-DMAP and 6-DMN residues have been developed recently as probes for the phosphotyrosine binding SH2 and for the phosphoserine and phospothreonine binding [14-3-3] domains [9], and on the other hand, PRODAN has been used to measure RNase assembly [10], δ-opioid antagonist binding, and electrostatics within protein G [11].

Naphthalimide (NP) belongs to a class of chromophores for which the excited-state properties can be drastically changed by the nature of a substituent presented on the aromatic ring [12–14]. The studies of the photophysical behavior of naphthalimides have contributed to the development of new fluorescent probes [15] and optical sensors [16], and the dual fluorescence was observed for several substituted phenyl naphthalimides [17–20]. From previous reports the fluorescence of 4-aminonaphthalimide and its derivatives were characterized by the high quantum yield in all the examined solvents, but, their Stokes shifts were not very large, which would hinder their applications in solar fluorescence material and sensor as the presence of intersecting self-absorption between their emissions and excitations. Ones hope has fluorescent sensor with large Stokes shift in aqueous solution or water, as most of sensors will get application in environmental or biological condition. Additionally fluorescent materials with large Stokes shifts have advantages that they could be more easily detected in the presence of other fluorescent materials, for example, immunoassays are typically carried out in body, which contain many endogenous fluorescent molecules, such as bilins, flavins and drugs. Since the vast majority of interfering fluorescent materials have relatively short Stokes shifts, the use of a fluorescent label that emits at a wavelength far greater than its excitation wavelength makes the label easier to distinguish from background fluorescence. Fluorescent materials with a large Stokes shift can be used in combination with those with a smaller Stokes shift where both materials excite at the same wavelength, but emit at different wavelengths, giving multiple signals that can be resolved using optical filters or monochromators. Recently, Imperiali et al. have synthesized and studied the fluorescence properties of the new environmentally sensitive fluorophore, 6-*N*,*N*-dimethylamino-2,3-

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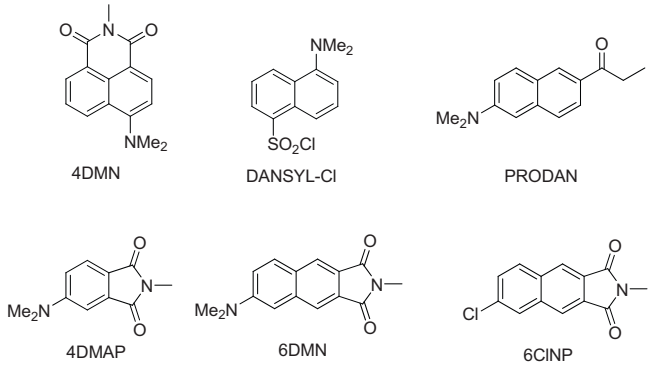


Fig. 1. Structures of some common environment-sensitive fluorophores.

naphthalimide (6-DMN) [4] and Katritzky et al. synthesized a novel environmentally sensitive chlorine substituted naphthalimide-based fluorophore (6-ClNP) which can be utilized for the labelling of amino acids [5]. The fluorescence quantum yield of 6-DMN chromophore changes from $\Phi_F = 0.225$ in chloroform to $\Phi_F = 0.002$ in water and the fluorescence quantum yield of 6-ClN chromophore changes from $\Phi_F = 0.53$ in chloroform to $\Phi_F = 0.15$ in water. The Stokes shift of 6-DMN was reported around 204 nm and that of 6-ClN was around 141 nm in aqueous solutions. Therefore, we here concentrated to design and synthesize some naphthalimides with unusual large Stokes shift, especially in aqueous or water solution.

In addition, it was observed from the literature [4,5,21] that the synthesis of 6-substituted naphthalimides required multiple synthetic steps and special purification methods. Synthesis of the 6-N,N-dimethylamino-2,3-naphthalene anhydride required 6 steps and resulted the mixture of isomers at positions **2** and **4** in a 1:1 ratio in the second step, the yield of the desired product 2-(2-phenylsufonylmethyl-5-nitrophenyl)-1,3-dioxolane is only 35%, which reflects the overall yield of the synthesis. On the other hand, chlorine substituted 2,3-naphthalimide required 5 steps.

Thus in this work we also reported the short and efficient synthesis of three new groups of 6-nitro, 6-amino and 6-iodo-substituted derivatives **2**, **3**, **4** and their spectral characterization. Finally, we also reported the detailed investigation of the photophysical behavior in solvents of diverse polarity including polar to non-polar and protic to aprotic solvents. We examined the effects of substituents on 2nd position of 2,3-naphthalimide ring substituted by phenyl, alkyl and on 6th position of naphthalene ring substituted from electron donating (6-NH_2-NP and 6-Me_2N-NP) to electron-withdrawing (6-nitro-1H-benzo[f]isoindole-1,3(2H)-dione, O_2N–NP), and halogenated groups (6-Cl-NP, 6-I-NP).

Then greater attention was paid to 6-amino-2,3-naphthalimide (6-ANP) and the unusual large Stokes shift of 6-ANP (highest value 297–303 nm) was systemically studied in aqueous solution. The results showed that: (1) amino group acts as electron donor in all the solvents except water. In water it behaves as a proton acceptor since proton-donating capability of water was reported to be more (hence a blue shift relatively $\lambda_{abs} = 274$ nm), respectively; (2) water can interact at two sites with the two lone pairs of amino and imino groups. So, the delocalization of two lone pairs is restricted. Hence, these interactions cause a significant blue shift in absorption spectra.

2. Experimental

2.1. General

All reagents and solvents were obtained from commercial suppliers and used without further purification. ^{1}H NMR spectra were collected in $CDCl_3$/DMSO at 25 °C in NMR spectrometer (400 MHz). Absorption measurements were performed with a Varian Cary 500 spectrophotometer (1 cm quartz cell) and fluorescence spectra were recorded on a Varian Cary Eclipse fluorescence spectrophotometer (1 cm quartz cell). The experimental solutions were prepared just before taking measurements. Mass spectra (MS) were recorded on an MA1212 instrument using standard conditions (ESI, 70 eV). All the experiments were performed at 25.0 ± 0.1 °C. Melting points were determined by an X-6 micro-melting point apparatus and uncorrected.

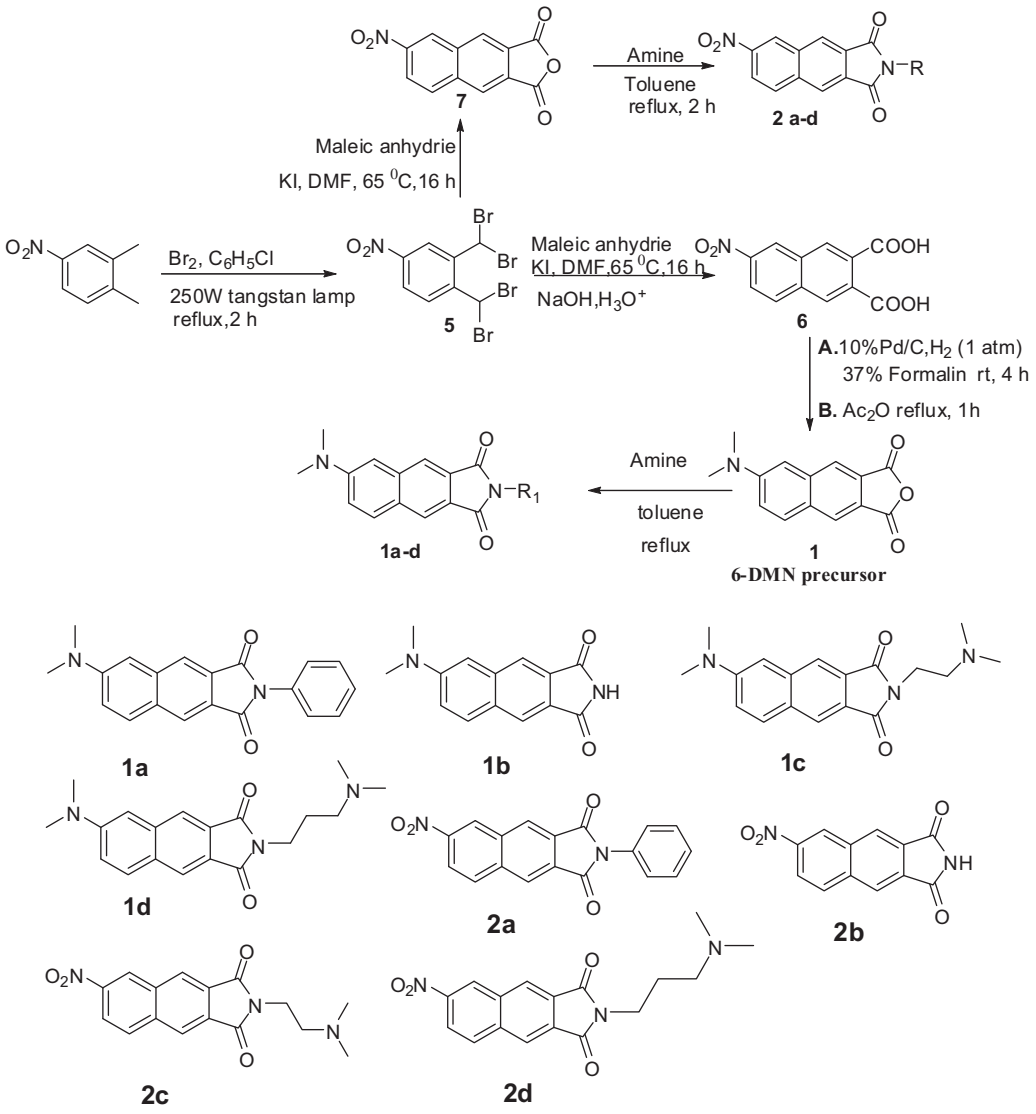
2.2. Synthesis

The designed target compounds of 6-sub-2,3-naphthalimides (**1a–d**, **2a–d**, **3a–d**, **4a–d** and **4e–4h**) were synthesized from commercially available starting materials which are illustrated in Schemes 1 and 2.

*Preparation of 1, 2-bis (dibromomethyl)-4-nitrobenzene (**5**)*: 4-Nitro-o-xylene (15.1 g, 100 mmol) in 100 mL of chlorobenzene was placed in two-necked flask equipped with dropping funnel, and a reflux condenser attached to a sodium hydroxide trap for evolved hydrogen bromide (HBr) gas. Liquid bromide (67.2 g, 420 mmol) in 50 mL chlorobenzene was added drop wise while the solution was irradiated with 250 W tungsten lamp. When the addition was completed, the reaction mixture was then cooled, washed successively with aqueous $NaHSO_3$ and distilled water, dried over magnesium sulfate, filtered, and concentrated with a rotary evaporator. The resulting yellowish precipitate which was recrystallized from CCl_4 and dried 100 °C to afford the desired compound as a pale yellow powder (82%). m.p. 125–127 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ): 8.57 (s, 1H), 8.29 (dd, $J_1 = 8.4$ Hz, $J_2 = 8.8$ Hz, 1H), 7.96 (d, $J = 7.2$ Hz, 1H), 7.15 (s, 1H), 7.10 (s, 1H). HRMS-EI (m/z): [M−Br] calcd for $C_8H_5NO_2Br_3$: 385.7850. Found: 385.7847.

*6-Nitro-2,3-naphthalenedicarboxylic acid (**6**)*: The procedure of Carlson [28] was employed with minor modifications. A mixture of tetrabromide **5** (20.0 g, 42.82 mmol), maleic anhydride (12.59 g, 128.46 mmol), potassium iodide (46.2 g, 0.278 mol), and dry dimethylformamide (150 mL) was stirred at 65 °C for 16 h under N_2. The cooled reaction mixture was poured into ice water (1000 mL), and the brown color due to iodine was discharged by the gradual addition of aqueous sodium hydrogen sulfite. The yellow precipitate was dissolved in dilute sodium hydroxide, the solution decolorized by charcoal, filtered and the filtrate acidified with conc. sulfuric acid under cool condition. The yellow precipitate of diacid was washed with cold water and dried, which was recrystallized from dichloromethane to give compound **6** as light yellow crystals (9.72 g, 87.0%). m.p. 179–181 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 13.9 (br, 2H, –COOH), 9.16 (s, 1H), 8.72 (s, 1H), 8.51 (s, 1H), 8.37 (dd, $J = 2.2$ Hz, 1H), 8.34 (s, 1H). ^{13}C NMR (DMSO-d_6): 168.6, 168.3, 147.0, 135.9, 134.0, 132.2, 132.1, 132.0, 131.0, 129.67, 125.5, 121.8. HRMS-ESI (m/z): [M−H$^+$] calcd for $C_{12}H_6NO_6$: 260.0195. Found: 260.0208.

*6-(Dimethylamino)-2,3-naphthalenedicarboxylic anhydride (**1**) 6-DMN precursor*: Compound **6** (4 g) was dissolved in MeOH (400 mL). Formalin (40 mL) and Pd/C 10% (1 g) were added to the solution. The resulting mixture was stirred under hydrogen for 2.5–3 h until TLC showed that the starting material has been consumed. The reaction mixture was then filtered through celite and concentrated under reduced pressure to get the crude precipitate which was washed thoroughly with water to eliminate formalin fraction to give the desired pure diacid product (3.40 g, 85%, $R_f = 0.5$ 20% CH_3OH/DCM). m.p. 248–250 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.15 (s, 1H), 7.87 (s, 2H), 7.32 (dd, $J_1 = 9.2$ Hz, $J_2 = 2.6$ Hz, 1H), 7.05 (s, 1H), 3.05 (s, 6H); ^{13}C NMR (400 MHz, DMSO-d_6, δ): 169.6, 168.2, 150.0, 135.1, 131.3, 129.7, 129.5, 126.1, 124.7, 123.6, 117.6, 105.3, 48.8(2). For diacid intermediate: HRMS-ESI (m/z): [M−H$^+$] Calcd for $C_{14}H_{12}NO_4$: 258.0766. Found: 258.0764. 3 g of



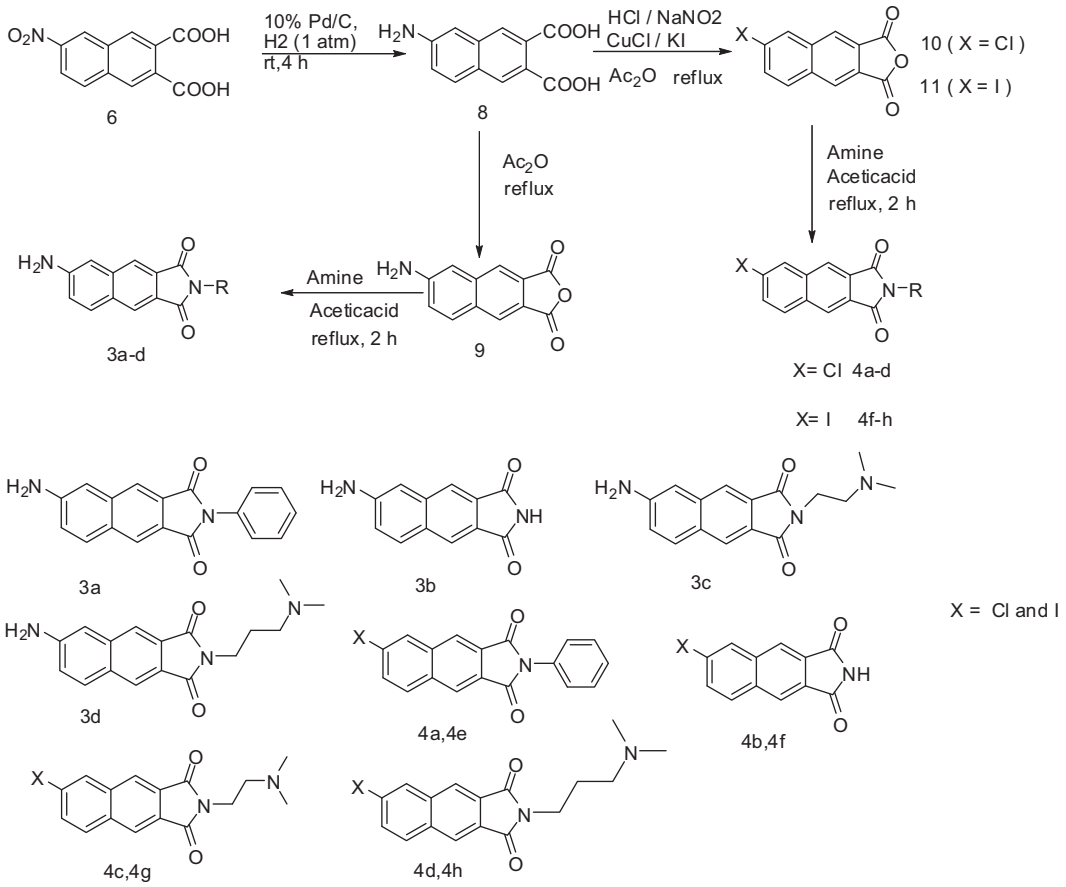
Scheme 1. Preparation of **1a–d** and **2a–d**.

the diacid was placed in a 250 mL flask containing 15 mL acetic anhydride and reflux for 1 h, remove the solvent on a rotary evaporator at 25 °C. Dissolve the solid residue in a 100 mL of toluene and remove the solvent on a rotary evaporator two times. The crude product was purified by column chromatography (EtOAc/hexanes: 30/70) to furnish 2.7 g (90%) of desired anhydride **1** (R_f = 0.64, 30% EtOAc/hexanes). m.p. 245–247 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ): 8.34 (s, 1H), 8.26 (s, 1H), 7.98 (d, J = 9.2 Hz, 1H), 7.44 (dd, J_1 = 9.2 Hz, J_2 = 2.6 Hz, 1H), 7.21 (d, J = 2.4 Hz, 1H), 3.22 (s, 6H); HRMS-EI (m/z): calcd for $C_{14}H_{11}NO_3$: 241.0739. Found: 241.0738.

*6-Amino-2,3-naphthalenedicarboxylic acid (**8**)*: Compound **6** (15 g) was dissolved in MeOH (1500 mL) and Pd/C 10% (1.5 g) was added to the solution. The resulting mixture was stirred under hydrogen for 3–6 h until TLC showed that the starting material has been consumed. The reaction mixture was then filtered through celite and concentrated under reduced pressure to get the crude precipitate diacid product (12.50 g, 85%, R_f = 0.3, 20% CH_3OH/DCM). m.p. 127–129 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 14.0 (b, 2H), 8.20 (s, 1H), 7.85 (s, 1H), 7.74 (d, J = 8.8 Hz, 1H), 7.04 (dd, J_1 = 1.2 Hz, J_2 = 1.6 Hz, 1H), 6.88 (s, 1H), 6.02 (Br, 2 NH,); ^{13}C NMR (DMSO-d_6): 170.0, 168.8, 149.6, 135.9, 132.1, 131.3, 130.1, 126.9, 125.7, 124.2, 120.5, 106.0; HRMS-ESI ($M-H^+$): Calcd for $C_{12}H_8NO_4$: 230.0532. Found: 230.0475.

*6-Amino-2,3-naphthalenedicarboxylic anhydride (**9**)*: A solution of diacid **8** (12 g, 0.05 mol) in acetic anhydride (50 mL) was refluxed for 15 min. The solvent was removed under reduced pressure. Benzene (150 mL) was added to it, and the solvent was again evaporated to remove traces of acetic anhydride, affording (93%) of cyclic anhydride as yellow solid. m.p. 212–214 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ): 8.44 (s, 1H), 8.29 (s, 1H), 7.98 (d, J = 2.4 Hz, 1H), 7.24 (dd, J_1 = 8.8 Hz, J_2 = 9.2 Hz, 1H), 7.04 (s, 1H), 2.83 (s, 2H); ^{13}C NMR (DMSO-d_6): 168.6, 168.5, 134.1, 133.2, 133.1, 132.5, 131.7, 131.6, 131.1, 130.7, 130.6, 129.0, 127.5; EIMS m/z 213.0.

*6-Chloro-2,3-naphthalenedicarboxylic anhydride (**10**)*: A heterogeneous mixture of 6-amino-2,3-naphthalenedicarboxylic acid (4.20 g, 20 mmol) in 10 mL of concentrated HCl was stirred at 0 °C as a solution of $NaNO_2$ (1.66 g, 24 mmol) in 10 mL of ice water was added. The resultant homogeneous orange solution was stirred at 0 °C for 1 h, and then added slowly with stirring to a solution of $CuCl_2$ (6.8 g, 50 mmol) in 30 mL of water at 0 °C. The reaction mixture was slowly warmed to room temperature over 2 h. The aqueous solution was extracted with EtOAc, and the organic layer was washed with water, and brine, dried, and concentrated to give 4.39 g (89%) of the diacid. m.p. 245–247 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 15.5 (b, 2H), 8.56 (s, 1H), 8.50 (s, 1H), 8.23 (s, 1H), 8.14 (d, J = 8.8 Hz, 1H), 7.66 (d, J = 8.4 Hz, 1H); ^{13}C NMR



Scheme 2. Preparation of **3a–d** and **4a–d** and **4e–h**.

(DMSO-d_6): 168.6, 168.5, 134.1, 133.2, 132.5, 131.7, 131.6, 131.1, 130.7, 130.6, 129.0, 127.5; HRMS-EI (m/z): Calcd for $C_{12}H_7ClO_4$: 250.0033. Found: 250.0040. Follow the same procedure mentioned for 6-amino-2,3-naphthalenedicarboxylic anhydride except starting material afford anhydride of chloro compound (10) as colorless solid. m.p. 218–220 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ): 8.56 (s, 1H), 8.48 (s, 1H), 8.15 (d, J = 2 Hz, 1H), 8.12 (d, J = 9.6 Hz, 1H), 7.78 (dd, J = 2, 2 Hz, 1H); ^{13}C NMR ($CDCl_3$, δ): 162.5(2), 136.9, 136.7, 134.3, 131.8, 131.5, 129.1, 127.7, 127.1, 126.7, 126.2; HRMS-EI (m/z): Calcd for $C_{12}H_5ClO_3$: 231.9927. Found: 231.9928.

*6-Iodo-2,3-naphthalenedicarboxylic anhydride (**11**)*: A heterogeneous mixture of 6-amino-2,3-naphthalenedicarboxylic acid (4.20 g, 20 mmol) in 10 mL of concentrated HCl was stirred at 0 °C as a solution of $NaNO_2$ (1.66 g, 24 mmol) in 10 mL of ice water was added. The resultant homogeneous orange solution was stirred at 0 °C for 1 h, and then added slowly with stirring to a solution of KI (8.3 g, 50 mmol) in 30 mL of water at 0 °C. The reaction mixture was slowly warmed to room temperature over 2 h. The aqueous solution was extracted with EtOAc, and the organic layer was washed with water, aqueous saturated sodium metabisulfite, and brine, dried, and concentrated to give 5.7 g (85%) of diacid as a brown solid. m.p. 180–182 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 14.3 (b, 2H), 8.59 (s, 1H), 8.31 (s, 1H), 8.24 (s, 1H), 7.94 (dd, J_1 = 1.2 Hz, J_2 = 1.6 Hz, 1H), 7.90 (s, 1H); ^{13}C NMR (DMSO-d_6, δ): 168.9, 168.8, 137.2, 137.0, 134.8, 131.9, 131.2, 130.7, 130.6, 129.6, 128.3, 95.9; HRMS-EI (m/z) calcd for $C_{12}H_7IO_4$: 341.9389. Found: 341.9399. Follow the same procedure as mentioned for compound **9** except starting material given Iodo anhydride as light red color solid. m.p. 196–198 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ): 8.57 (s, 1H), 8.53 (s, 1H), 8.44 (s, 1H), 8.07 (dd, J = 1.6 Hz, 1H), 7.88 (d, J = 8.4 Hz, 1H); ^{13}C NMR ($CDCl_3$, δ): 162.6, 162.5, 139.2, 139.1, 137.1, 134.8, 131.4, 127.8, 126.8, 126.4, 126.3, 97.58; HRMS-EI (m/z) calcd for $C_{12}H_5IO_3$: 323.9283. Found: 323.9279.

2.2.1. General procedure for the synthesis of N-phenyl-naphthalimides (*1a–4a* and *4e*) [22]

Compounds **1a–4a** and **4e** have been synthesized by mixing equimolar quantities of anhydride (1 mmol) and aniline (1 mmol) in glacial acetic acid were stirred under reflux for 1 h and the solvent was evaporated under reduced pressure to yield the crude product, which was recrystallized from acetone or acetic acid.

*6-(Dimethylamino)-2-phenyl-1H-benzo[f]isoindole-1,3(2H)-dione (**1a**)*: Green yellow solid. m.p. 248–249 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.33 (s 1H), 8.22 (s, 1H), 8.04 (d, J = 7.2 Hz, 1H), 7.51 (d, J = 4.8 Hz, 2H), 7.49 (d, J = 6.0 Hz, 4H), 7.27 (s, 1H), 3.09 (s, 6H); ^{13}C NMR (DMSO-d_6, δ) 167.4, 167.3, 150.9, 137.9, 132.7, 131.6, 129.2, 128.4, 128.3, 127.7, 127.4, 125.27, 125.23, 124.9, 122.8, 122.4, 118.5, 107.9, 40.2 (2); HRMS-EI calcd for $C_{20}H_{16}N_2O_2$: 316.1212. Found: 316.1208.

*6-Nitro-2-phenyl-1H-benzo[f]isoindole-1,3(2H)-dione (**2a**)*: Colorless solid. m.p. 296–299 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 9.30 (s, 1H), 8.93 (s, 1H), 8.78 (s, 1H), 8.49 (qt, J = 22.4, 22 Hz, 2H), 7.47–7.58 (m, 5H); ^{13}C NMR (DMSO-d_6, δ): 166.6 (2), 147.3, 138.3, 134.8, 132.6, 132.3, 131.2, 129.6, 129.3, 129.0, 128.7, 127.8, 127.0, 126.7, 124.9, 122.7, 119.4; HRMS-EI calcd for $C_{18}H_{10}N_2O_4$: 318.0641. Found: 318.0642.

*6-Amino-2-phenyl-1H-benzo[f]isoindole-1,3(2H)-dione (**3a**)*: Green solid. m.p. 278–279 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.27 (s 1H), 8.11 (s, 1H), 7.93 (d, J = 9.2 Hz, 1H), 7.43-7.53 (m, 5H), 7.15 (dd, J = 2.4, 2.4 Hz, 1H), 7.11 (s, 1H), 6.13 (s, 2H-NH_2); ^{13}C NMR

(DMSO-d_6, δ) 167.5, 167.4, 150.7, 138.3, 132.8, 131.9, 129.2, 128.3, 128.2, 127.8, 127.7, 125.4, 124.9, 122.1, 121.9, 121.4, 121.0, 108.9; HRMS-EI calcd for $C_{18}H_{12}N_2O_2$: 288.0899. Found: 288.0900.

*6-Chloro-2-phenyl-1H-benzo[f]isoindole-1,3(2H)-dione (**4a**)*: colorless solid. m.p. > 300 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.66 (s, 1H), 8.60 (s, 1H), 8.44 (s, 1H), 8.34 (d, J = 8.4 Hz, 2H), 7.83 (d, J = 8.0 Hz, 1H), 7.46–7.56 (m, 5H); ^{13}C NMR (DMSO-d_6, δ): 166.8, 166.7, 136.4, 136.2, 134.4, 134.2, 134.1, 132.7, 132.4, 130.1, 129.33, 129.30, 129.0, 128.6, 128.3, 127.8, 125.2, 124.4; HRMS-EI calcd for $C_{18}H_{10}ClNO_2$: 307.0400 Found: 307.0398.

*6-Iodo-2-phenyl-1H-benzo[f]isoindole-1,3(2H)-dione (**4e**)*: Bright color solid. m.p. 276–278 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.75 (d, J = 1.2 Hz, 1H), 8.58 (s, 1H), 8.52 (d, J = 1.2 Hz, 1H), 8.04 (s, 2H), 7.49–7.58 (m, 5H); ^{13}C NMR (DMSO-d_6, δ): 166.9, 166.8, 138.9, 137.9, 137.0, 134.5, 134.4, 132.4, 132.2, 132.1, 129.2, 128.65, 128.63, 128.2, 127.8, 125.3, 124.1, 97.3; HRMS-EI calcd for $C_{18}H_{10}INO_2$: 398.9756. Found: 398.9753.

2.2.2. General procedure for the synthesis of N-(H)imides (**1b**–**4b** and **4f**)

A mixture of anhydride (1.1 mmol) and urea (2.2 mmol) was heated at 170 °C for 2 h. The mixture was cooled and triturated with water (30 mL) and the resulting solid collected by filtration and dried to give desired imide (95%). The following compounds were prepared by this general method.

*6-(Dimethylamino)-1H-benzo[f]isoindole-1,3(2H)-dione (**1b**)*: Dark yellow solid. m.p. 290–292 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 11.19 (s, 1H NH), 8.15 (s, 1H), 8.04 (s, 1H), 7.97 (d 1H, J = 8.4 Hz), 7.74 (d 1H, J = 7.6 Hz), 7.19 (s, 1H), 3.07 (s, 6H); ^{13}C NMR (DMSO-d_6, δ): 169.68, 169.58, 150.68, 137.69, 131.4, 129.7, 127.31, 124.48, 123.97, 122.17, 118.31, 107.92, 40.2 (2); EIMS m/z 240.1.

*6-Nitro-1H-benzo[f]isoindole-1,3(2H)-dione (**2b**)*: Colorless solid. m.p. > 300 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 11.8 (br 1-NH), 9.21 (s, 1H), 8.72 (s, 1H), 8.56 (s, 1H), 8.41 (dd, 2H, J_1 = 18.8 Hz, J_2 = 18.4 Hz); ^{13}C NMR (DMSO-d_6, δ): 168.67 (2C), 150.37, 147.18, 138.19, 134.68, 132.47, 130.77, 126.65, 126.50, 124.37, 122.31; EIMS m/z 242.1.

*6-Amino-1H-benzo[f]isoindole-1,3(2H)-dione (**3b**)*: Dark green solid. m.p. > 300 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 11.12 (s, 1H, NH), 8.12 (s, 1H), 7.9 (s, 1H), 7.89 (d 1H, J = 8.8 Hz), 7.11 (dd, 1H, J_1 = 1.6 Hz, J_2 = 1.6 Hz), 7.06 (s, 1H), 6.06 (s, 2H); ^{13}C NMR (DMSO-d_6, δ): 169.77, 169.69, 151.70, 150.68, 150.46, 138.14, 131.83, 129.71, 127.65, 123.46, 121.43, 120.87, 108.38; EIMS m/z 212.0.

*6-Chloro-1H-benzo[f]isoindole-1,3(2H)-dione (**4b**)*: Dark color solid. m.p. 280–284 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 11.57 (s, 1H, –NH), 8.49 (s, 1H), 8.43 (s, 1H), 8.38 (d, 1H, J = 2.0 Hz), 8.28 (d 1H, J = 8.8 Hz), 7.78 (dd, 1H, J_1 = 2.0 Hz, J_2 = 2.0 Hz); ^{13}C NMR (DMSO-d_6, δ): 169.05 (2C), 136.36, 134.17, 133.97, 132.61, 130.28, 129.88, 129.57, 129.26, 124.61, 123.83; EIMS m/z 231.0. DMSO-d_6.

*6-Iodo-1H-benzo[f]isoindole-1,3(2H)-dione (**4f**)*: Dark red solid. m.p. 215–218 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 11.58 (s, 1H, NH), 8.73 (s, 1H), 8.42 (d, 2H, J = 21.2 Hz), 8.02 (s, 2H); ^{13}C NMR (DMSO-d_6, δ): 169.11, 169.04, 138.90, 137.67, 136.95, 134.31, 132.14, 129.93, 129.52, 124.72, 123.56, 96.91; EIMS m/z 323.0.

2.2.3. General procedure for the synthesis of N-(aminoalky1) imides (**1c**,**d**–**4c**,**d** and **4g**,**h**)

Unless otherwise stated, all imides reported were prepared by the following general procedure. To a stirred solution of 0.01 mol of the proper anhydride in 100 mL of toluene was added drop wise 0.015 mol of the appropriate N,N-dialky1amino alkylamine in 10 mL of toluene within 5 min. The mixture was stirred at room temperature for 60 min and then heated under reflux while connected to a Dean-Stark trap for 2 h. After the theoretical amount of water was removed, the reaction mixture was cooled, washed successively with H_2O (2 × 50 mL), 5% $NaHCO_3$ (2 × 50 mL), and H_2O (2 × 50 mL), and then dried (Na_2SO_4). Purified by chromatography and yields of these imides was generally high 75–85%.

*6-(Dimethylamino)-2-(2-(dimethylamino) ethyl)-1H-benzo[f]isoindole-1,3(2H)-dione (**1c**)*: Green yellow solid. m.p. 182–185 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ): 8.11 (s, 1H), 8.05 (s, 1H), 7.83 (d, J = 9.2 Hz, 1H), 7.22 (d, J = 8.8 Hz, 1H), 6.99 (s, 1H), 3.85 (t, J_1 = 6.4 Hz, J_2 = 6.6 Hz, 2H), 3.28 (s, 6H), 2.65 (t, J = 2H, 6.4 Hz, 2H), 2.33 (s, 6H); ^{13}C NMR (DMSO-d_6, δ): 168.65, 168.57, 150.36, 137.55, 131.18, 128.69, 127.30, 124.53, 123.14, 122.49, 117.60, 107.82, 57.11, 45.46, 40.32, 35.84. HRMS-ESI (M+H$^+$): calcd for $C_{18}H_{21}N_3O_2$: 312.1712. Found: 312.1699.

*6-(Dimethylamino)-2-(3-(dimethylamino)propyl)-1H-benzo[f]isoindole-1,3(2H)-dione (**1d**)*: Green yellow solid. m.p. 155–158 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ): 8.12 (s, 1H), 8.06 (s, 1H), 7.85 (d, J = 9.2 Hz, 1H), 7.23 (dd, J = 2.2 Hz, 1H), 7.01 (s, 1H), 3.77 (t, J_1 = 7.2 Hz, J_2 = 6.8 Hz, 2H), 3.14 (s, 6H), 2.41 (t, J_1 = 7.2 Hz, J_2 = 7.6 Hz, 2H), 2.26 (s, 6H), 1.70–1.90 (m, 2H); ^{13}C NMR (DMSO-d_6, δ): 168.66, 168.59, 150.39, 137.56, 131.18, 128.68, 127.31, 124.47, 123.14, 122.43, 117.62, 107.83, 57.05, 45.29, 40.31, 36.22, 26.60; HRMS-ESI (M+H$^+$): calcd for $C_{19}H_{23}N_3O_2$: 326.1869. Found: 326.1864.

*2-(2-(Dimethylamino) ethyl)-6-nitro-1H-benzo[f]isoindole-1,3(2H)-dione hydrochloride (**2c**)*: Colorless solid. m.p. > 300 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 9.32 (d, J = 1.6 Hz, 1H), 8.90 (s, 1H), 8.72 (s, 1H), 8.51 (dd, J_1 = 9.2 Hz, J_2 = 2.0 Hz, 1H), 8.49 (d, J = 2 Hz, 1H), 3.79 (t, J = 6.4 Hz, 2H), 2.85 (s,, 6H), 2.3 (t, J = 8.4 Hz, 1H); ^{13}C NMR (DMSO-d_6, δ): 167.67, 167.59, 147.37, 138.26, 134.69, 132.58, 131.44, 129.83, 126.74, 126.59, 124.55, 122.68, 54.63, 54.511, 42.48, 35.61, 23.61; HRMS-ESI (M+H$^+$): calcd for $C_{16}H_{15}N_3O_4$: 314.1141. Found: 314.1135.

*2-(3-(Dimethylamino) propyl)-6-nitro-1H-benzo[f]isoindole-1,3(2H)-dione hydrochloride (**2d**)*: Light solid. m.p. > 300 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 9.31 (d,, J = 1.6 Hz, 1H), 8.88 (s, 1H), 8.72 (s, 1H), 8.50 (dd, J_1 = 9.2 Hz, J_2 = 2.0 Hz, 1H), 8.47 (d, J = 2.0 Hz, 1H), 3.73 (t, J = 6.4 Hz, 2H), 3.14 (qt, J_1 = 5.2 Hz, J_2 = 4.4 Hz, 2H), 2.72 (s, 6H), 2.30 (t, J = 8.4 Hz, 1H); ^{13}C NMR (DMSO-d_6, δ): 167.67, 167.59, 147.37, 138.26, 134.69, 132.58, 131.44, 129.83, 126.74, 126.59, 124.55, 122.68, 54.63, 54.511, 42.48, 35.61, 23.61; HRMS-ESI (M+H$^+$): calcd for $C_{17}H_{17}N_3O_4$: 328.1297. Found: 328.1292.

*6-Amino-2-(2-(dimethylamino) ethyl)-1H-benzo[f]isoindole-1,3(2H)-dione (**3c**)*: Green solid. m.p. 198–201 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.16 (s, 1H), 8.00 (s, 1H), 7.89 (d, J = 8.8 Hz, 1H), 7.11 (dd, J = 2.0 Hz, 1H), 7.07 (s, 1H), 6.10 (s, 2H), 3.70 (t, J = 6.4 Hz, 2H), 2.57 (t, J = 5.6 Hz, 2H), 2.23 (s, 6H); ^{13}C NMR (DMSO-d_6, δ): 168.36, 168.27, 150.65, 138.08, 131.93, 128.48, 127.47, 124.89, 122.12, 121.71, 120.88, 108.54, 56.80, 45.30, 35.70; HRMS-ESI (M+H$^+$): calcd for $C_{16}H_{17}N_3O_2$: 284.1399. Found: 284.1394.

*6-Amino-2-(3-(dimethylamino)propyl)-1H-benzo[f]isoindole-1,3(2H)-dione (**3d**)*: Green solid. m.p. 130–133 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.15 (s, 1H), 7.99 (s, 1H), 7.88 (d, J = 8.4 Hz, 1H), 7.10 (dd, J = 2.0 Hz, 1H), 7.06 (s, 1H), 6.08 (s, 2H), 3.60 (t, J = 7.2 Hz, 2H), 2.24 (t, J_1 = 7.2 Hz, J_2 = 6.8 Hz, 2H), 2.18 (s, 6H), 1.67-1.74 (m, 2H); ^{13}C NMR (DMSO-d_6, δ): 168.43, 168.36, 150.59, 138.07, 131.89, 128.54, 127.46, 124.80, 122.20, 121.63, 120.82, 108.53, 56.93, 45.47, 36.20, 26.45; HRMS-ESI (M+H$^+$): calcd for $C_{17}H_{19}N_3O_2$: 298.1556. Found: 298.1541.

*6-Chloro-2-(2-(dimethylamino)ethyl)-1H-benzo[f]isoindole-1,3(2H)-dione (**4c**)*: Colorless solid. m.p. 280–283 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.57 (s, 1H), 8.51 (s, 1H), 8.43 (s, 2H), 8.32 (d, J = 8.8 Hz, 2H), 7.82 (d, J = 8.8 Hz, 2H), 4.02 (t, J_1 = 6.4 Hz, J_2 = 6.0 Hz, 2H), 3.41 (t, J = 5.2 Hz, 2H), 2.83 (s, 6H); ^{13}C NMR (DMSO-d_6, δ): 167.84, 167.79, 136.30, 134.43, 133.90, 132.71, 130.15, 129.40, 129.37, 128.68, 124.82, 124.03, 54.59, 54.53, 42.65, 33.64; HRMS-ESI (M+H$^+$): calcd for $C_{16}H_{15}N_3O_4$: 303.0900. Found: 303.0889.

6-Chloro-2-(3-(dimethylamino)propyl)-1H-benzo[f]isoindole-1,3(2H)-dione (***4d***): Colorless solid. m.p. 290–292 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.55 (s, 1H), 8.50 (s, 1H), 8.41 (s, 2H), 8.31 (d, J = 8.8 Hz, 2H), 7.81 (d, J = 8.8 Hz, 2H), 3.78 (t, J_1 = 6.4 Hz, J_2 = 6.0 Hz, 2H), 3.13-3.21 (m, 2H), 2.75 (s, 6H), 2.03 (t, J_1 = 7.2 Hz, J_2 = 8.0 Hz, 2H); ^{13}C NMR (DMSO-d_6, δ): 168.52, 168.35, 136.34, 134.43, 133.36, 132.01, 130.69, 129.48, 129.29, 127.71, 124.85, 120.33, 54.59, 54.55, 42.69, 33.66, 23.62; HRMS-ESI (M+H$^+$): calcd for $C_{17}H_{18}ClN_2O_2$: 317.1057 Found: 317.1049.

2-(2-(Dimethylamino)ethyl)-6-iodo-1H-benzo[f]isoindole-1,3(2H)-dione hydrochloride (***4g***): Brown solid. m.p. 285–288 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.76 (s, 1H), 8.49 (d, J = 22.4 Hz, 2H), 8.05 (s, 2H), 4.01 (t, J_1 = 5.6 Hz, J_2 = 6.0 Hz, 2H), 3.41 (t, J = 5.2 Hz, 2H), 2.82 (s, 6H); ^{13}C NMR (DMSO-d_6, δ): 167.89, 167.81, 138.95, 137.91, 136.85, 134.23, 132.21, 129.06, 124.90, 123.72, 97.26, 54.48, 54.44, 42.55, 33.61; HRMS-ESI (M+H$^+$): calcd for $C_{16}H_{15}IN_2O_2$: 3950256. Found: 395.0243.

2-(3-(Dimethylamino)propyl)-6-iodo-1H-benzo[f]isoindole-1,3(2H)-dione hydrochloride (***4h***): Brown solid. m.p. 292–295 °C; ^{1}H NMR (400 MHz, DMSO-d_6): 8.75 (s, 1H), 8.51 (s, 1H), 8.48 (s, 1H), 8.04 (s, 2H), 3.70 (t, J_1 = 6.4 Hz, J_2 = 6.0 Hz, 2H), 3.09–3.15 (m, 2H), 2.71 (s, 6H), 2.03 (t, J_1 = 7.2 Hz, J_2 = 8.0 Hz, 2H); ^{13}C NMR (DMSO-d_6, δ): 167.89, 167.81, 138.93, 136.86, 134.25, 132.19, 128.86, 128.44, 124.86, 123.69, 97.24, 54.60, 54.55, 42.41, 35.47, 23.65; HRMS-ESI (M+H$^+$): calcd for $C_{17}H_{17}IN_2O_2$: 409.0413. Found: 409.0435.

3. Results and discussions

3.1. Synthesis

There were few reports available on the synthesis of 6-substituted 2,3-naphthalimide derivatives [4,5, and 21]. Here we have attempted to synthesize various 6-substituted naphthalimides as described.

Synthesis of 6-sub-2,3-naphthaleneanhydrides and its imide analogs were derived successfully from key intermediate (**6**) in the above efficient route (Schemes 1 and 2), with different groups like strong (e.g. NO_2) and poor electron-withdrawing groups (e.g. Cl, I) and strong electron-donating groups (NH_2). To synthesize the intermediate **6**, started from 1,2-dimethyl 4-nitrobenzene, which was subjected to photobromination with bromine in chlorobenzene to get 1,2-bis(dibromomethyl)-4-nitrobenzene (**5**). The resultant tetrabromide (**5**) was treated with maleic anhydride and KI followed by base hydrolysis to obtain the Diels–Alder adduct of nitro diacid **6**, we found nitro anhydride (**7**) instead of compound **6** with good yield and high purity, under similar reaction conditions except base hydrolysis (Scheme 1). The above Diels–Alder adducts were recrystallized from dichloromethane. The reductive amination of intermediate (**6**) with 37% formalin solution and hydrogen in the presence of catalytic amount of Pd/C at room temperature and 30 psi pressure afforded the desired 6-N,N-dimenthylamino-2,3-naphthalene dicarboxylic acid in excellent yield with high purity after several times washing with water, after dehydration on acetic anhydride under reflux obtained the desired anhydride **1** (6-DMN Precursor) (Scheme 1).

For the preparation of derivatives **3a**–**d** and **4a**–**h**, we have tried direct reductive amination of compound **7**, but were unsuccessful due to the instability of the ring. Then we selected the alternative route as shown in Scheme 2. Compound **6** was subjected to catalytic hydrogenation to get 6-amino dicarboxylic acid (**8**), which was further diazotized, followed by Sand Mayer reaction to afford 6-halo dicarboxylic acid. We are the first one to characterize these diacids. The crude 6-substituted diacid was directly converted to anhydrides by treating with acetic anhydride under reflux. Then condensation with appropriate amine in acetic acid as solvent affords 6-substitued 2,3-naphthalimides.

Table 1
Photophysical properties of **1a**–**4a** and **4e** in DMSO.

Compound	Absorption maximum and excitation (nm)	Emission maximum (nm)	Quantum yield (Φ)
1a	385 (390)	566	0.18
2a	364 (375)	465	0.13
3a	372 (375)	537	0.21
4a (chloro)	289 (300)	386	0.16
4e (iodo)	271 (300)	403	0.09

3.2. Absorption and emission spectra

The absorption maximum for unsubstituted 2,3-NP appears at 340–350 nm, while its fluorescence emerges at 380–400 nm. All the UV spectra of the studied compounds (6-sub 2,3-NP) were ranged in the 280–390 nm and some of them are shown in Fig. 2. The maxima of the absorption, excitation and emission wavelength were given in Table 1. As is typical of a charge-transfer transition, an increase in the polarity of the medium leads to a Stokes shift of the absorption maximum. A change of the solvent from toluene to ethanol typically results in 10–20 nm bathochromic shift of the absorption maximum of the systems. On one hand, substitution of the hydrogen in N–H by a alkyl amine group, or by a phenyl group, leads to a slight red shift of the absorption maximum: 363 nm for N–H compared with 369 nm for N-$(CH_2)_2$N$(Me)_2$ and 372 nm for N–Ph (average). On the other hand, a minor bathochromic shift was observed if the 2,3-naphthaline ring was substituted with an electron-donating group, such as dimethyl-amino [6-DMN, **1a**–**d**] (average of 380 nm).

The absorption spectra of **3a** in different solvents are shown in Fig. 3, respectively. Surprisingly, in aqueous medium, it showed significant difference in the spectral behavior, the absorption around the 360 nm became negligible. In polar solvents the large Stokes shift was due to major stabilization of S_1 state with contribution of an ICT effect and also it was well known that compounds containing lone pair of electrons undergo a blue shift (to higher energy) in their n–π^* electronic transition when solvation occurs. The reason for this blue shift was a lowering of the energy of the lone-pair orbital caused by the molecule's strong interaction with protic solvents, such as amino group with water through hydrogen-bond formation.

In the case of **3a**, we studied the effect of polarity and hydrogen bonding interaction of the solvents on the spectroscopic properties, and the results were reported in Table 2. Increase of the polarity of aprotic solvents leads to a red shift of the emission wavelength, from 496 nm in toluene to 515 nm in acetonitrile. The effect was less important on the absorption maximum than on the emission maximum, which suggests an excited state more polar than the ground state. The change in charge distribution following the excitation may be quantified by determination of the dipole moment variation between the ground state and the excited state using the Lippert–Mataga equation [23]. The charge transfer occurs from the amino group to the carbonyl and the phenyl group was more electronic donating than the hydrogen atom; therefore a lower value of the dipole moment of **3a** was expected. Almost no change of the fluorescence quantum yield with the polarity was observed in aprotic solvents (average of Φ_F = 0.20) as in the case of **3a**. In protic solvents such as ethanol and water, an important red shift (Table 2) of the emission wavelength was noticed compared to polar aprotic solvents (ACN, DMSO). For instance, $\lambda_{fluo\ max}$ = 559 nm in ethanol and $\lambda_{fluo\ max}$ = 515 nm in acetonitrile, although acetonitrile and ethanol have almost the same polarity, but hydrogen-bonding capacity of the ethanol leads to n–π transition. On the other hand, the unexpected large Stokes shift observed in water (nearly 297 nm) was not attributed to the increase of the solvent polarity, but to the hydrogen bonding interaction between the carbonyl groups of the

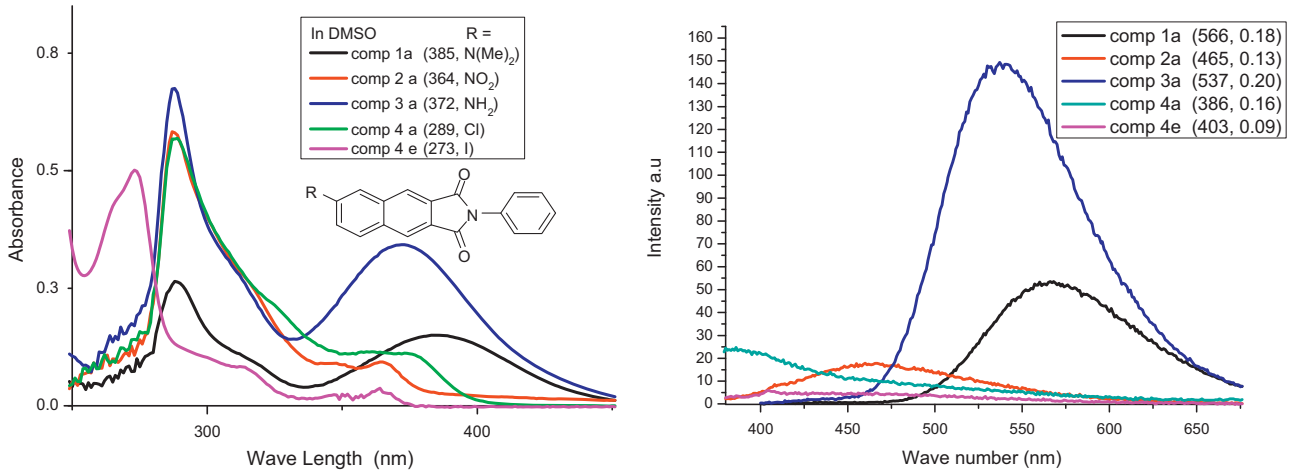


Fig. 2. Absorption and fluorescence spectra of 6-sub 2,3-naphthalimides (**1a**–**4a** and **4e**) in DMSO.

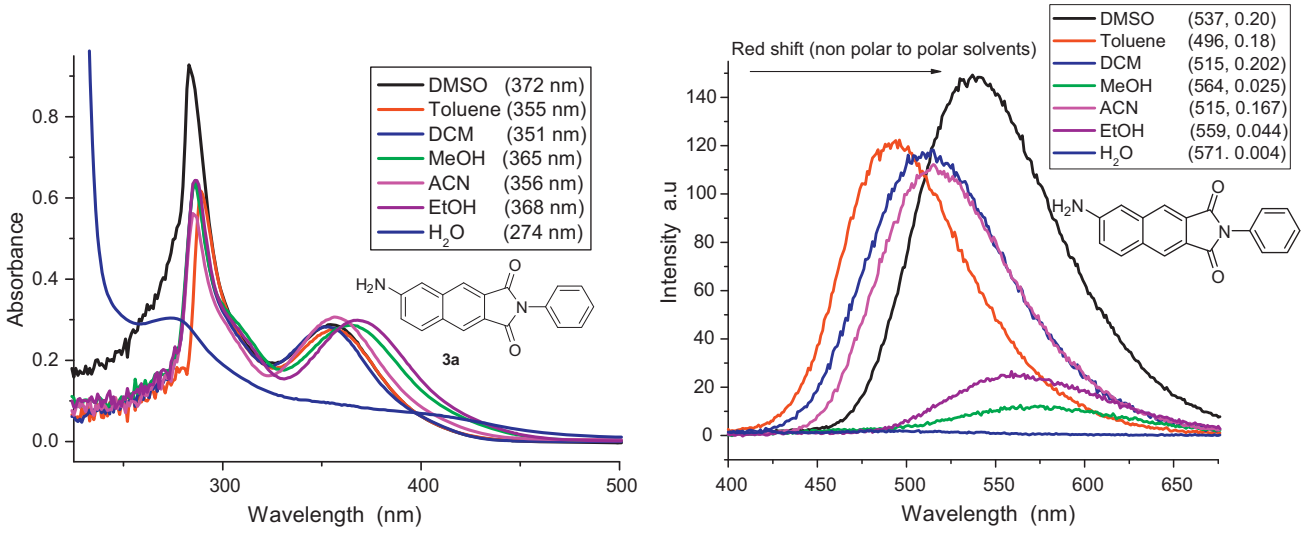


Fig. 3. Absorption (A) and fluorescence (F) spectra of 6-amino-2,3-naphthalimide (**3a**) in water, methanol, ethanol, acetonitrile, DMSO, dichloromethane and toluene. Excitation wavelength: 375 nm and 300 nm (H_2O).

naphthalimide (which acts as hydrogen bonding acceptor) and the hydroxylated solvents (which acts as hydrogen bond donor). This interaction was also responsible for the decrease of the fluorescence quantum yield. In water, Φ_F was almost 50 times weaker than in the aprotic solvents, almost 10–20 times weaker than in polar and protic solvents, e.g. methanol, ethanol, which also means that the hydrogen bonding interaction between **3a** and water was much stronger than those between **3a** and methanol or ethanol, as water has more hydrogen bonding sites than methanol and ethanol. Non-radiative deactivation of the excited state through hydrogen bonding [24] has been suggested to explain this specific behavior in protic solvents. The other three compounds of 6-AMN family (**3b**, **3c**, **3d**) exhibit similar behaviors in terms of their absorption and emission properties compared with **3a**. Substitution of the hydrogen in N-H (**3b**) by the alkyl amine groups (**3c**, **3d**), leads to a slight red shift of the absorption maxima and emission spectra.

The absorption and emission spectra of **3b** in different solvents are shown in Fig. 4, respectively. From Fig. 4 the absorption maxima of studied compound **3b** in all solvents were at 273–368 nm and emission maxima were at 478–576 nm. The absorption peak

Table 2
Photophysical properties of **3a** in different solvents.[a]

Solvent	Absorption maximum (nm)	Emission maximum (nm)[b]	Quantum yield (Φ)	Solvent type
Water	274	571	0.0042	
Methanol	365	564	0.025	Protic
Ethanol	368	559	0.044	
ACN	356	515	0.167	Polar aprotic
DMSO	372	537	0.209	
DCM	351	515	0.202	Apolar aprotic
Toluene	355	496	0.182	

[a] With quinine sulfate in 0.1 mol/L sulfuric acid as quantum yield standard (Φ = 0.58) [27].
[b] Excitation wavelength: 375 nm and 300 nm (H_2O).

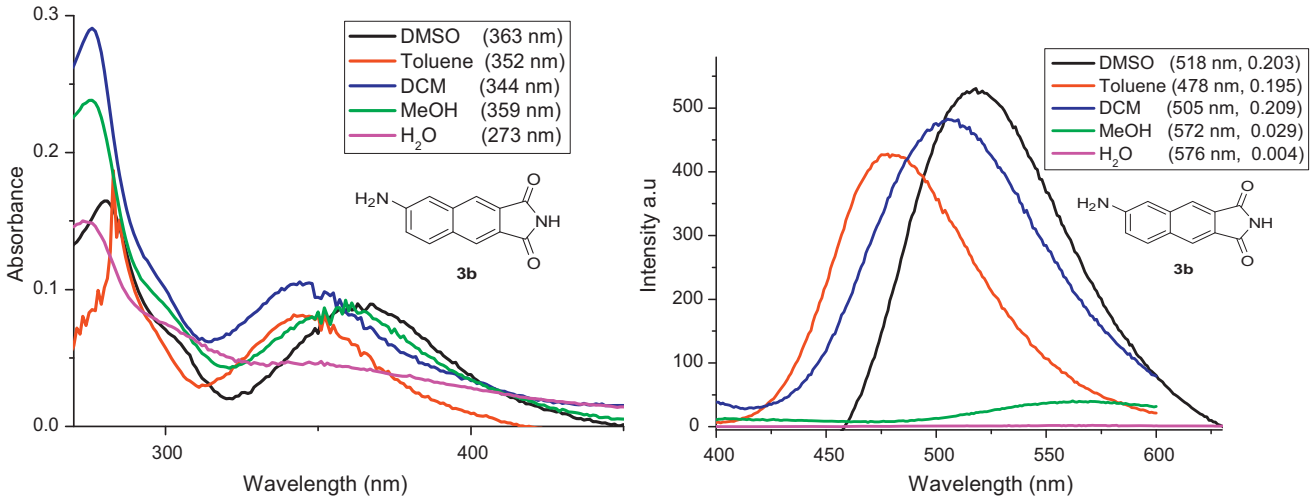


Fig. 4. Absorption (A) and fluorescence (F) spectra of **3b** in water, methanol, DMSO, dichloromethane and toluene. Excitation wavelength: 375 nm and 300 nm (H_2O).

position for **3b** was unusually blue-shifted (273 nm) in aqueous media relatively to that of other solvents same as **3a** and a minor short wavelength occurs due to imide hydrogen (–N–H).

The absorption and emission spectra of **3c** in different solvents are shown in Fig. 5, respectively. From Fig. 5 the absorption maxima of studied compound **3c** in all solvents were at 276–369 nm and emission maxima were at 482–576 nm. Substitution of the hydrogen in N–H by a N,N-dimethylethylenediamine and dimethylaminopropylamine, leads to a slight red shift of the absorption and emission maximum.

6-ANP compounds displayed a marked positive solvatochromic effect in the emission spectra, accompanied by a reduction of the fluorescence quantum yield (Φ_F) with increasing solvent's polarity. The three main reasons given for this effect were namely: (1) an increase in dipole moment of S_1 relative to S_0; (2) the formation of much stronger hydrogen bond in the S_1-ICT state than in the S_0 state. Recently Wang et al. reported the results of the theoretical study on the excited-state hydrogen-bonding dynamics of 4-AP chromophore (4-aminophthalimide) in hydrogen-donating water solvent using the TDDFT method [25]. On the other hand, Topp and co-workers studied the properties of 4-AP and the hydrogen-bonded 4-AP-$(H_2O)_{1,2}$ complexes under jet-cooled conditions and showed the results of infrared double-resonance experiments on these complexes. The structures of the hydrogen-bonded 4AP-$(H_2O)_{1,2}$ clusters were given [26]. 4-AP and 6-ANP belong to the same class of family. Then we like to adopt same principle to our 6-amino 2,3-naphthalimides. The fluorescence maximum was located at 490 nm for ANP in aprotic solvents, while it was at 570 nm for hydrogenated solvents. The large red shift was due to the alternative formation of intermolecular hydrogen bond C=O···H–O and N–H···O–H (Fig. 6).

Compounds **1a** and **1b** have similar properties with 6-DMN described by Loving and Imperiali [3]. The maximum excitation wavelength of 6-DMN was at 370 nm (water), and contrary to it, fluorescence of 6-DMN was strongly influenced by polarity of the medium. The fluorescence in polar methanol or water was strongly bathochromically shifted from around 482 nm in toluene up to 585 nm in methanol, and the fluorescence quantum yield decreased from dichloromethane ($\Phi_F = 0.212$) to water ($\Phi_F = 0.002$) (Fig. 7).

Substitution by $-NO_2$, leads to some changes in the absorption and emission spectra compared with unsubstituted 2,3-naphthalimide (free naphthalene ring) (2,3-NP). A red shift about 44 nm was observed relative to the spectra of 2,3-NP (generally emission maxima ~ 379 nm) [29], and the absorption and emission bands become broad and structureless, probably due to an efficient intersystem crossing (S_1–T_1) leading to a non-reactive triplet state. These results were consistent with the presence of the CT excited states. In the case of O_2N–NP, the charge transfer occurs in the opposite direction: from aromatic ring toward the nitro substituent (Fig. 8).

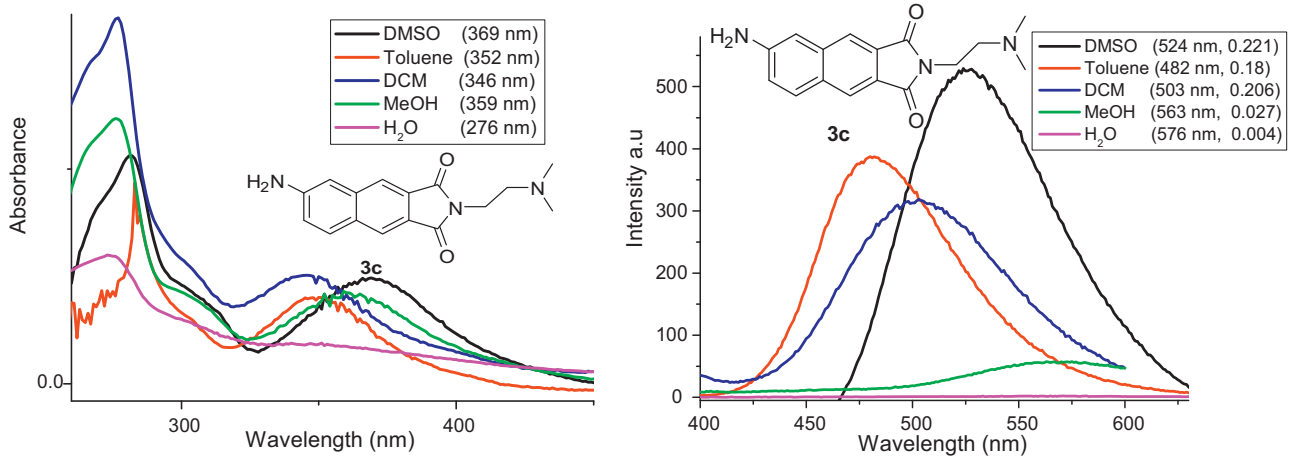


Fig. 5. Absorption (A) and fluorescence (F) spectra of **3c** in water, methanol, DMSO, dichloromethane and toluene. Excitation wavelength: 375 nm and 300 nm (H_2O).

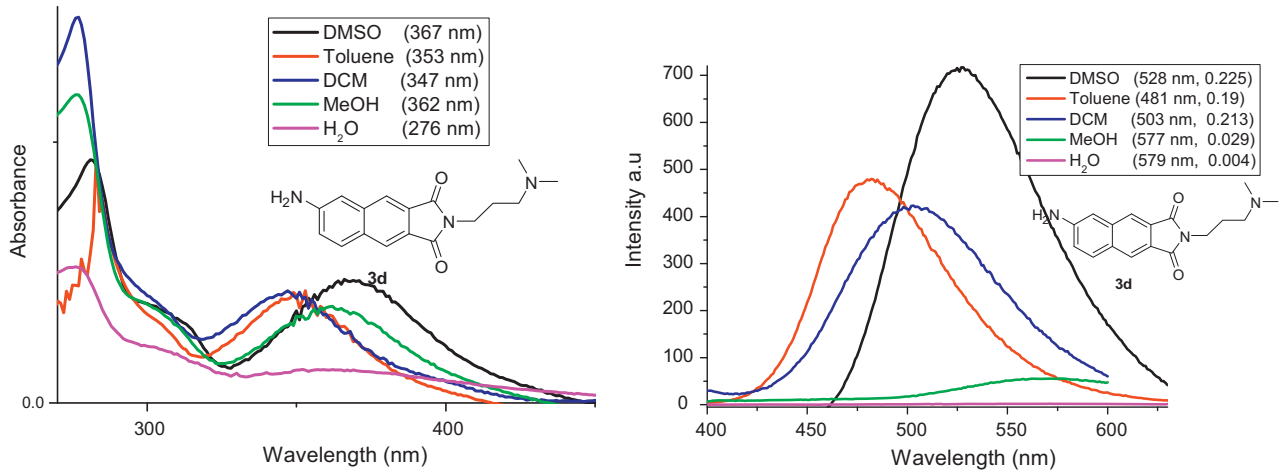


Fig. 6. Absorption (A) and fluorescence (F) spectra of **3d** in water, methanol, DMSO, dichloromethane and toluene. Excitation wavelength: 375 nm and 300 nm (H_2O).

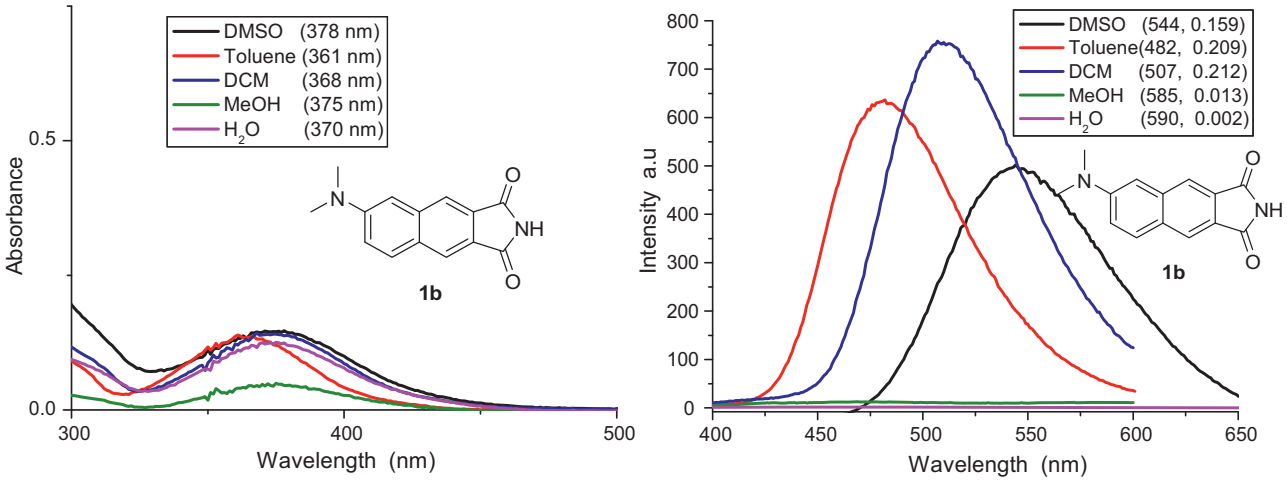


Fig. 7. Absorption (A) and fluorescence (F) spectra of 6-dimethyl 2,3-naphthalimide (6-DMN) in DMSO, methanol, dichloromethane, toluene and water. Excitation wavelength: 380 nm.

Substitution by –iodo group leads to minor changes in the absorption and emission spectra. A small red shift was observed in the spectra of 6-INP relative to 2,3-NP. The nature of the iodo substituent was unusual: it was an electron-donating substituent due to its lone pair of electrons, while it behaves as an electron withdrawing substituent due to the relative electron negativity of iodine atom. Thus, the overall effect of iodo substitution was minor, and the character of the excited state was very similar to the parent 2,3-NP. The absorption maxima (271 nm) and the fluorescence emission maxima (403 nm) for compound **4e** were summarized in

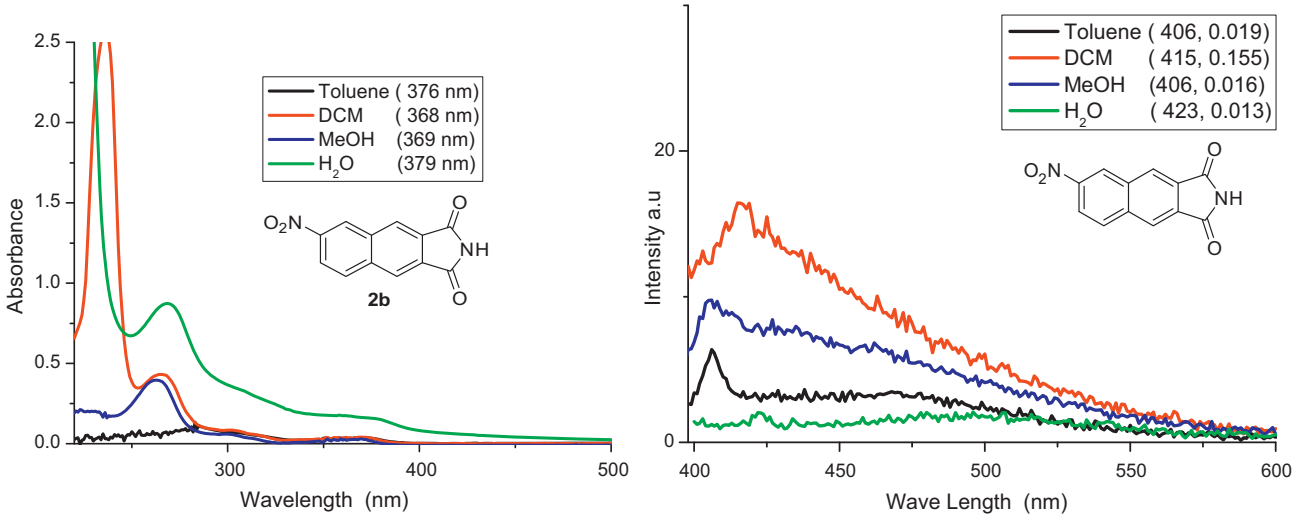


Fig. 8. Absorption (A) and fluorescence (F) spectra of 6-nitro 2,3-naphthalimide (**2b**) in methanol, dichloromethane, toluene and water. Excitation wavelength: 380 nm.

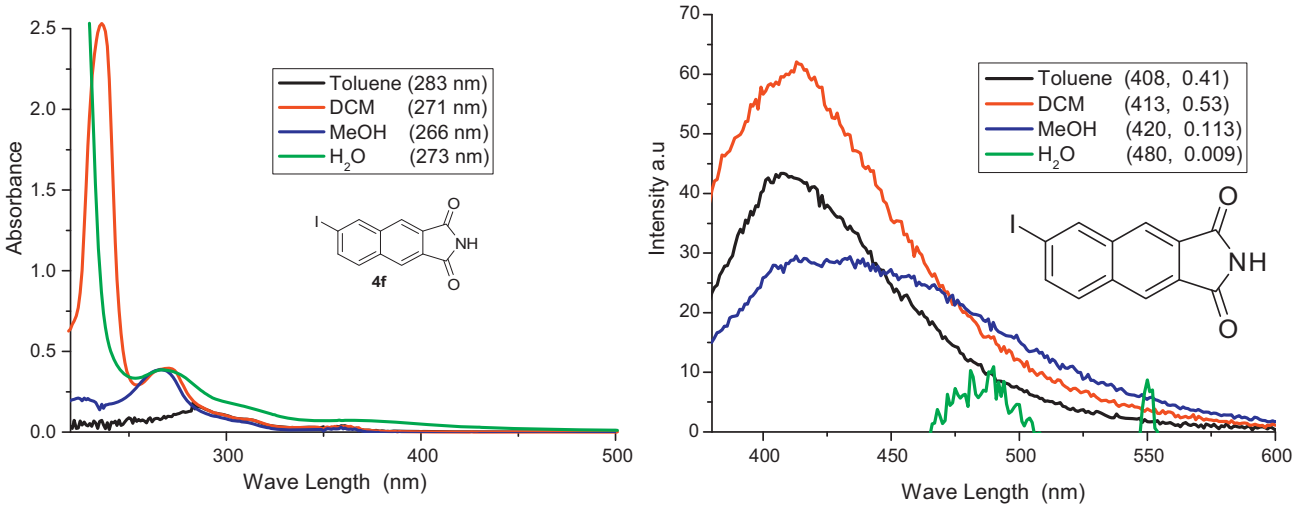


Fig. 9. Absorption (A) and fluorescence (F) spectra of 6-iodo 2,3-naphthalimide (**4f**) in methanol, dichloromethane, toluene and water. Excitation wavelength: 300 nm.

Table 1. From Fig. 9 the absorption maxima of studied compound **4f** in all three solvents were at 266–288 nm and emission maxima were at 400–480 nm.

4. Conclusion

In this paper we presented the results on the synthesis, spectral (absorption and emission) and photophysical studies of 6-amino-2,3-naphthalimides and other 6-sub-2,3-naphthlimides in a range of solvents. A new environment-sensitive fluorophore, 6-ANP displays excellent fluorescent properties: long excitation (375 nm) and emission wavelengths (480–590 nm) and also an acceptable quantum yield ($\Phi_F = 0.2$) to ($\Phi_F = 0.004$), and unusual large Stokes shift. The unexpected large Stokes shift observed in water (about 300 nm) was not only attributed to the increase of the solvent polarity, but also to the hydrogen bonding interaction between the carbonyl groups of the naphthalimide (which act as hydrogen bonding acceptor) and the hydroxylated solvents (which act as hydrogen bond donor). This interaction was also responsible for the decrease of the fluorescence quantum yield. We also reported photophysical properties of some other 6-sub-2,3-naphthalimides (NO_2, I).

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References

[1] G. Saroja, T. Soujanya, B. Ramachandram, A. Samanta, 4-Aminophthalimide derivatives as environment sensitive probes, J. Fluoresc. 119 (1998) 405–410.
[2] G. Weber, F.J. Farris, Synthesis and spectral properties of a hydrophobic fluorescent probe: 2-dimethylamino-6-propionylnaphthalene, Biochemistry 18 (1979) 3075–3078.
[3] G. Loving, B. Imperiali, A versatile amino acid analogue of the solvatochromic fluorophore 4-N,N-dimethylamino-1,8-naphthalimide: a powerful tool for the study of dynamic protein interactions, J. Am. Chem. Soc. 130 (2008) 13630–13638.
[4] (a) R.K. Alan, N. Tamari, Fluorescent amino acids: advances in protein-extrinsic fluorophores, Org. Biomol. Chem. 7 (2009) 627–634;
(b) B. Imperiali, M.E. Vazquez, U.S. Pat. Appl. Publ. 2006, 25pp. US 2006234206 A1;
(c) M.E. Vazquez, J.B. Blanco, B. Imperiali, Photophysics and biological applications of the environment-sensitive fluorophore 6-N,N-dimethylamino-2,3-naphthalimide, J. Am. Chem. Soc. 127 (2005) 1300–1306.
[5] R.K. Alan, S. Ozcana, E. Todadze, Synthesis and fluorescence of the new environment-sensitive fluorophore 6-chloro-2,3-naphthalimide derivative, Org. Biomol. Chem. 8 (2010) 1296–1300.
[6] G. Loving, M. Sainlos, B. Imperiali, Monitoring protein interactions and dynamics with solvatochromic fluorophores, Trends Biotechnol. 28 (2010) 73–83.
[7] P. Venkatraman, T.T. Nguyen, M. Sainlos, O. Bilsel, S. Chitta, B. Imperiali, L.J. Stern, Fluorogenic probes for monitoring peptide binding to class II MHC proteins in living cells, Nat. Chem. Biol. 3 (2007) 222–228.
[8] E. Pazos, O. Vázquez, J.L. Mascarenas, M.E. Vazquez, Peptide-based fluorescent biosensors, Chem. Soc. Rev. 38 (2009) 3348–3359.
[9] M.E. Vazquez, D.M. Rothman, B. Imperiali, A new environment-sensitive fluorescent amino acid for Fmoc-based solid phase peptide synthesis, Org. Biomol. Chem. 2 (2004) 1965–1966.
[10] M. Nitz, A.R. Mezo, M.H. Ali, B. Imperiali, Enantioselective synthesis and application of the highly fluorescent and environment-sensitive amino acid 6-(2-dimethylamino-naphthoyl) alanine (DANA), Chem. Commun. (Camb.) (2002) 1912–1913.
[11] B.E. Cohen, et al., Probing protein electrostatics with a synthetic fluorescent amino acid, Science 296 (2002) 1700–1703.
[12] J.E. Rogers, L.A. Kelly, Nucleic acid oxidation mediated by naphthalene and benzophenone imide and diimide derivatives: consequences for DNA redox chemistry, J. Am. Chem. Soc. 121 (1999) 3854–3861.
[13] I. Saito, M. Takayama, S. Kawanishi, Photoactivatable DNA-cleaving amino acids: highly sequence-selective DNA photocleavage by novel l-lysine derivatives, J. Am. Chem. Soc. 117 (1995) 5590–5591.
[14] M.S. Alexiou, V. Tychopoulos, S. Ghorbanian, J.H.P. Tyman, R.G. Brown, P.I. Brittain, The UV–visible absorption and fluorescence of some substituted 1,8-naphthalimides and naphthalic anhydrides, J. Chem. Soc. Perkin Trans. 2 (1990) 837–842.
[15] (a) F. Cosnard, V. Wintgens, A new fluoroionophore derived from 4-amino-N-methyl-1,8-naphthalimide, Tetrahedron Lett. 39 (1998) 2751–2754;
(b) H.N. Lee, Z. Xu, S.K. Kim, K.M.K. Swamy, Y. Kim, S.J. Kim, J. Yoon, Pyrophosphate-selective fluorescent chemosensor at physiological pH: formation of a unique excimer upon addition of pyrophosphate, J Am. Chem. Soc. 129 (2007) 3828–3829.
[16] C.G. Niu, Z.Z. Li, X.B. Zhang, W.Q. Lin, G.L. Shen, R.Q. Yu, Covalently immobilized aminonaphthalimide as fluorescent carrier for the preparation of optical sensors, Anal. Bioanal. Chem. 372 (2002) 519–524.
[17] (a) A. Demeter, T. Berces, L. Biczok, V. Wintgens, P. Valat, J. Kossanyi, Comprehensive model of the photophysics of N-phenylnaphthalimides: the role of solvent and rotational relaxation, J. Phys. Chem. 100 (1996) 2001–2011;
(b) Z. Xu, K. Baek, H.N. Kim, J. Cui, X. Qian, D.R. Spring, I. Shin, J. Yoon, Zn^{2+} triggered amide tautomerization produces a highly Zn^{2+} selective, cell-permeable and ratiometric fluorescent sensor, J. Am. Chem. Soc. 132 (2010) 601–610.
[18] V. Wintgens, P. Valat, J. Kossanyi, A. Demeter, L. Biczok, T. Berces, Spectroscopic properties of aromatic dicarboximides part 3: substituent effect on the photophysical properties of N-phenyl-2,3-napthalimides, J. Photochem. Photobiol. A Chem. 93 (1996) 109–117.
[19] A. Demeter, T. Berces, L. Biczok, V. Wintgens, P. Valat, J. Kossanyi, Spectroscopic properties of aromatic dicarboximides. Part 2: substituent effect on the photophysical properties of N-phenyl-1,2-naphthalimide, J. Chem. Soc. Faraday Trans. 90 (1994) 2635–2641.
[20] H. Cao, V. Chang, R. Hernandez, M.D. Heagy, Matrix screening of substituted N-Aryl-1,8-naphthalimides reveals new dual fluorescent dyes and unusually bright pyridine derivatives, J. Org. Chem. 70 (2005) 4929–4934.

[21] Z. Huang, M.V. Lakshmikantham, M. Lyon, M.P. Cava, Synthesis and isolation of some benzo[c]tellurophenes, J. Org. Chem. 65 (2000) 5413–5415.
[22] V.M. Sena, R.M. Srivastava, R.O. Silva, Synthesis and hypolipidemic activity of N-substituted phthalimides part V, IL Farmaco 58 (2003) 1283–1288.
[23] E. Lippert, Z. Naturforsch, Dipole moment and electronic structure of excited molecules, Phys. Sci. A 10 (1955) 541–545.
[24] A. Morimoto, T. Yatsuhashi, T. Shimada, L. Biczok, D.A. Tryk, H. Inoue, Radiationless deactivation of an intramolecular charge transfer excited state through hydrogen bonding: effect of molecular structure and hard-soft anionic character in the excited state, J. Phys. Chem. A 105 (2001) 10488–10496.
[25] R. Wang, C. Hao, P. Li, N.N. Wei, J. Chen, J. Qiu, Time-dependent density functional theory study on the electronic excited-state hydrogen-bonding dynamics of 4-aminophthalimide (4AP) in aqueous solution: 4AP and 4AP-(H(2)O)(1,2) clusters, J. Comput. Chem. 31 (2010) 2157–2163.
[26] Y. Chen, M.R. Topp, Infrared-optical double-resonance measurements of hydrogen-bonded interactions in clusters involving aminophthalimides, Chem. Phys. 283 (2002) 249–268.
[27] J.R. Lakowicz, Principles of Fluorescence Spectroscopy, 3rd ed., Springer, Science, Business Media Publisher, New York, USA, 2006.
[28] R.G. Carlson, K. Srinivasachar, R.S. Givens, B.K. Matuszewski, New derivatizing agents for amino acids and peptides. I: facile synthesis of N-substituted 1-cyanobenz[f]isoindoles and their spectroscopic properties, J. Org. Chem. 51 (1986) 3978–3983.
[29] P. Valat, V. Wintgens, J. Kossanyi, L. Biczok, A. Demeter, T. Berces, Influence of geometry on the emitting properties of 2,3-naphthalimides, J. Am. Chem. Soc. 114 (1992) 946–953.