ORIGINAL ARTICLE



### Synthesis and Photophysical Studies on Naphthalimide Derived Fluorophores as Markers in Drug Delivery

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Abstract Derivatives of 4-amino-1,8-naphthalimide containing a free alkyl chain bearing carboxyl group as linker and different substituents at 4-amino function have been synthesized, characterized and studied for their photophysical properties. Steady state fluorescence studies showed quantum yield varied from 0.45 to 0.65 with Stokes shift in the range of 5824- $8558 \text{ cm}^{-1}$ . Spectroscopic and physicochemical parameters. like electronic absorption, emission, and extinction coefficient were investigated in order to explore the analytical potential of compounds. Solvatochromic studies demonstrated that all compounds were sensitive towards the polarity of different solvents showing the highest degree of fluorescence in acetonitrile. In addition, the compounds in the presence of ions, viz. Na<sup>+</sup>, K<sup>+</sup> and Mg<sup>2+</sup> at concentration of 0.1-2 equivalents, showed a decreasing trend in fluorescence with increasing ionic concentration. TCSPC set - up was used to measure the fluorescence lifetime of compounds, which was found to be bi-exponential with longer and shorter component at their respective amplitudes. The average lifetime of compounds was observed to be 5.76-9.96 ns indicating the possibility of their greater utilization in research and diagnosis.

**Keywords** Amino-naphthalimide derivative · Solvatochromism · Quantum yield · Steady state fluorescence · Average lifetime

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#### Introduction

The use of fluorescent molecules as reporter groups for labeling of biomolecules, like nucleic acids, proteins, etc. is a very vital and dynamic research field [1]. The synthesis of fluorophores possessing selectivity, sensitivity and desirable properties is always a great challenge. 1,8-Naphthalimide unit is a special class of widely accepted fluorescent probe [2, 3]. Owing to good properties, like strong fluorescence, good photostability and electroactivity, these compounds have found major applications in different areas, such as antitumor and anticancer research [4], fluorescent markers in biochemistry [5], high performance intramolecular charge transfer based electroluminescent materials [6, 7], light emitting diodes [8], analgesics in medicine [6], optical brighteners in detergent and textiles [9], fluorescence switchers [10], liquid crystal additives [11], photo induced electron transfer based sensors [12], laser dyes [13], etc. The 1, 8- naphthalimide shows a major variation in wavelength at emission when a polar group (electron donating or withdrawing) is present at C-4 position because these groups affect the delocalization of electron density inside the rings [14, 15]. 1, 8-Naphthalimide possessing yellow fluorescence has been reported as a fluorescent probe in hypoxic cells [16, 17]. Thus, design and synthesis of new compounds based on 1, 8-naphthalimide unit have gained importance in modern day researches in science and technology.

Here, we report some newly designed 4-amino-1,8naphthalimide derivatives, **3–6**, having different substituents at C-4 amino function and an alkyl chain bearing carboxylic group for attachment with any suitable molecule. The fluorescence of these molecules has been compared against our earlier reported molecule **2** used for oligonucleotide labelling [18–20]. Effect of different solvents and ions on fluorescence properties of these molecules along with their average

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fluorescence lifetime has been reported. Some other fluorescent molecules based on 4-amino-1,8-naphthalimide have been used previously in our laboratory for labelling and studying their effect on hybridization of oligodeoxyribonucleotides involving Tm measurement and gel shift assay [21–23].

#### Experimental

#### **Chemicals and Reagents**

Acenaphthene, aminocaproic acid, acetyl chloride, ptoluenesulphonyl chloride, di-*tert*-butyl-dicarbonate, ethyl chloroformate, methane sulphonyl chloride were purchased from Sigma Aldrich Chemicals Pvt. Ltd., New Delhi. Other reagents and solvents (AR grade) were used without further purification.

#### Apparatus

All reactions were monitored by TLC using Merck 60 F<sub>254</sub> precoated silica gel plates (0.25 mm thickness) and the products were visualized by UV detection. Flash chromatography was carried out with silica gel (200-300 mesh). FT-IR spectra were recorded on a Bruker Tensor-27 spectrometer. <sup>1</sup>H NMR spectra were recorded on a Bruker Avance (III) 400 MHz spectrometer. Data for <sup>1</sup>H NMR are reported as chemical shift  $(\delta \text{ ppm})$ , multiplicity (s = singlet, d = doublet, q = quartet, m = multiplet), coupling constant J (Hz), integration and assignment. Melting points were recorded on a Thomas Hoover capillary melting apparatus without correction. The solvents used were spectroscopic grade. The absorption spectra were taken on Varian UV-Vis spectrophotometer (model: Cary 100). Emission Spectra were taken on fluoromax-4p fluorimeter from Horiba Yovin (Model: FM-100). The fluorescence spectra were corrected for spectral sensitivity of the instrument. The excitation and emission slits were 2/2 nm for the emission measurements. All measurements were done at 25 °C. For the time resolved studies, a picosecond time correlated single photon counting (TCSPC) system from Horiba Yovin (Model: Fluorocube-01-NL) was used. The samples were excited at 375 nm using a picosecond diode laser (model: Pico Brite-375 L). The repletion rate was 5 MHz. The signals were collected at magic angle (54.70) polarization using a photomultiplier tube (TBX-07C) as the detector, which has a dark count of 20 cps. The instrument response function of the setup was ~140 ps. The data analysis was done using IBH DAS (version 6) decay analysis software.

The effect of ions on intensity of absorption and emission spectra were recorded in aqueous media. Since the compounds were insoluble in 100 % aqueous media, stock solutions of compounds were prepared in 10 mL of volumetric flask using acetonitrile/water (2:8,  $\nu/\nu$ ). The 20  $\mu$ L of prepared stock solution was further diluted to 2 mL by addition of the same solvent mixture. The prepared solutions of metal ions were 0.1 equivalent of the compound concentration.

All calculations, like the area under curve for calculation of quantum yield, calculation of molar absorptivity, smoothening and processing of graph at observed value of absorption and emission were performed using Origin Pro 8.1. IBH DAS (Version 6) decay analysis software was used to calculate fluorescence lifetime with pre-exponential factor throughout the present study.

#### Synthesis and Characterization

#### 6-(6-Amino-1,3-dioxo-1H,3H-benzo[de]isoquinolin-2-yl) -hexanoic acid (1)

Acenaphthene (64.9 mmol, 10 g) was dissolved in hot glacial acetic acid (80 mL) and cooled gradually upto 10 °C to form a crystalline magma. Nitric acid (6 mL) was added drop wise to this magma for 20–25 min. While maintaining the temperature at 10–15 °C. Now the temperature was allowed to rise to 30–35 °C and stirred the reaction mixture for another 30 min. The mixture was poured into crushed ice with constant stirring to make uniform mixture, filtered out the residue obtained and 5-nitroacenaphthene was purified through column chromatography using hexane and ethyl acetate as eluent. Yield 64.2 % (8.3 g); M.p.: 102 °C; R<sub>f</sub> 0.7 (EtOAc:Hexane; 2:8); UV (MeOH): 370 nm.

To a stirred solution of sodium dichromate (100 mmol, 6.2 g) and acetic acid (20 mL), 5-nitroacenaphthene (40 mmol, 8.0 g) was added gradually. The reaction mixture was then refluxed on oil-bath for 5 h. The reaction mixture was cooled and transferred to cold water followed by filtration to give the orange precipitate. The precipitate was purified by column chromatography to obtain 4-nitro-1,8-naphthalic anhydride using hexane and ethyl acetate as eluent. Yield 63 % (6.2 g); M.p.: 228 °C; R<sub>f</sub> 0.5(EtOAc:Hexane; 4:6); UV (MeOH): 340 nm; <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  8.93–8.95 (m, 1H), 8.73–8.79 (m, 2H), 8.45–8.46 (m, 1H), 8.05–8.09 (m, 1H).

To a stirred solution of 4-nitro-1,8-napthalic anhydride (16.4 mmol, 4.0 g) in ethanol (30 mL) was added the solution of  $SnCl_2.H_2O$  (49.2 mmol, 10 g) in concentrated HCl (10 mL) at room temperature. The reaction mixture was refluxed for 12 h. After ensuring complete reduction, the reaction mixture was cooled down to room temperature and aqueous solution of  $Na_2CO_3$  (10 %) was added to quench the reaction. The precipitate was collected by filtration, washed with water and dried in vacuo to afford the reddish orange crude product 4-amino-1,8-naphthalic anhydride, which was directly used





for further synthesis. Yield 50 % (2 g), M.p.: 358 °C; R<sub>f</sub> 0.6(DCM:MeOH; 9.5:0.5); UV (MeOH): 430 nm.

To a stirred solution of 6-aminocaproic acid (13.1 mmol, 1.75 g, 1.4 eq) in pyridine (6 mL) was added 4-amino-1,8naphthalic anhydride (9.4 mmol, 12 g). Reaction mixture was then stirred at 80-85 °C for 3-4 h and monitored by TLC. After completion of reaction, cooling the reaction mixture at room temperature, followed by evaporation of organic solvent, left the crude product, which was purified by column chromatography using DCM and MeOH as eluent. Yield 66 % (2 g); M.p.: 320 °C; R<sub>f</sub> 0.7(DCM:MeOH; 2:8); UV (MeOH): 430 nm, 260 nm.

6-(6-Acetylamino-1,3-dioxo-1H,3H-benzo[de] isoquinolin-2-yl)-hexanoic acid (2)

To a stirred solution of compound 1 (0.61 mmol, 200 mg) in small amount of pyridine/acetonitrile (1:1, v/v) acetyl chloride



# **Table 1** Absorption andemission maxima of compounds2-6 in different solvents

Compound	Absorbar	the $\lambda_{abs}$ (nm)			Emission $\lambda_{em}$ (nm)			
	CHCl <sub>3</sub>	CH <sub>3</sub> CN	EtOH	MeOH	CHCl <sub>3</sub>	CH <sub>3</sub> CN	EtOH	MeOH
2	345	347	346	346	436	454	461	465
3	339	405	428	424	511	530	548	565
4	338	335	339	340	429	430	470	440
5	402	410	422	425	516	533	551	555
6	406	413	432	433	499	514	531	532

(70 µL, 1.5 eq) was added drop wise. The reaction mixture was stirred for 6 h. After completion of the reaction (monitored by TLC), solvents were removed in vacuo and the crude product was purified by column chromatography using DCM and MeOH as eluent to give 125 mg of pure compound **2**. Yield 55 % (125 mg); M.p.: 163 °C; R<sub>f</sub> 0.7 (DCM:MeOH; 9.5:0.5); UV (MeOH): 345 nm; IR (KBr):  $\nu$  3077, 3033, 2942, 2864, 1856, 1703, 1660, 1624, 1583, 1520, 1496, 1464, 1437, 1409, 1386, 1340, 1261 cm<sup>-1</sup>; <sup>1</sup>HNMR(CDCl<sub>3</sub>):  $\delta$  11.14 (brs, 1H), 7.88–8.34 (m, 4H), 7.57 (s, 1H), 3.69 (s, 2H), 2.12–2.94 (m, 8H), 1.83 (s, 3H). MS (ESI): m/z (M<sup>+</sup>) 368.14.

## 6-(6-Ethoxycarbonylamino-1,3-dioxo-1H,3H-benzo[de] isoquinolin-2-yl)-hexanoicacid (3)

To an ice-cooled stirred solution of compound 1 (0.61 mmol, 200 mg) in small amount of DCM (3 mL) containing a trace of pyridine, added ethyl chloroformate (86  $\mu$ L, 1.5 eq) drop wise and the reaction mixture was allowed to stir for 2 h. After completion (monitored by TLC), evaporation of the organic solvent left the crude product, which was purified by column chromatography using DCM and MeOH as eluent. Yield 57 % (140 mg), M.p.: 185 °C; R<sub>f</sub> 0.64 (DCM:MeOH; 9.5:0.5), UV



**Fig. 1** Solvatochromism of compounds **2–6** in CH<sub>3</sub>CN

(MeOH): 422 nm; IR (KBr):  $\nu$  3431, 3342, 3238, 2923, 2853, 1732, 1695, 1620, 1579, 1524, 1478, 1386, 1360, 1320 cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-d<sub>6</sub> + Methanol-d<sub>4</sub>):  $\delta$  8.49–8.57 (m, 3H), 8.23–8.35 (m, 1H), 7.63–7.83 (m, 1H), 4.30 (q, *J* = 7.24 Hz, 2H), 4.11–4.17 (m, 2H), 1.66–1.75 (m, 4H), 1.45–1.48 (m, 2H), 1.38 (t, *J* = 7.2 Hz, 3H). MS (ESI): m/z (M<sup>+</sup>) 398.15.

### 6-(6-Tert-butoxycarbonylamino-1,3-dioxo-1H,3H-benzo[de] isoquinolin-2-yl)-hexanoic acid (4)

To an ice-cooled stirred solution of compound **1** (0.61 mmol, 200 mg) in small amount of DCM (3 mL) containing a trace of pyridine, added di-*tert*-butyl dicarbonate (210  $\mu$ L, 1.5 eq) dropwise and the reaction mixture was allowed to stir for 2 h. After completion (monitored by TLC), evaporation of the organic solvent left the crude product, which was purified by column chromatography using DCM and MeOH as eluent. Yield 52 % (135 mg), M.p.: 178 °C; R<sub>f</sub> 0.6 (DCM:MeOH; 9.5:0.5), UV(MeOH): 338 nm; IR (KBr):  $\nu$  3393, 2926, 2855, 1777, 1741, 1703, 1662, 1588, 1535, 1510, 1458, 1391, 1367 cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>):  $\delta$  8.53–8.79 (m, 2H), 8.16–8.37 (m, 1H), 7.78–7.94 (m, 2H), 4.01 (brs, 1H), 3.49–3.56 (m, 2H), 2.96–3.03 (m, 2H), 1.54–1.61 (m, 6H), 1.23 (s, 9H). MS (ESI): m/z (M<sup>+</sup>) 426.18.



$\lambda_{abs}(nm)$	$\lambda_{em}$ (nm)	$\epsilon (M^{-1} cm^{-1})$	φ	Stokes Shift (cm <sup>-1</sup> )	Fluorescence Lifetimes T <sub>i</sub> [ns]	Average Lifetime [ns]	
347	454	$1.1 \times 10^{4}$	0.015	6792	1.46 (0.23), 9.11 (0.77)	7.32	
405	530	$1.0  imes 10^4$	0.51	5824	3.96 (0.14), 9.29 (0.86)	8.54	
335	430	$1.0  imes 10^4$	0.62	6595	1.28 (0.37), 8.40 (0.63)	5.76	
410	533	$0.8  imes 10^4$	0.45	5629	4.10 (.06), 9.06 (0.94)	8.74	
413	514	$1.2 \times 10^4$	0.65	4758	1.39 (0.08), 10.7 (0.92)	9.96	
	λ <sub>abs</sub> (nm) 347 405 335 410 413	$\begin{array}{c c} \lambda_{abs} \mbox{ (nm)} & \lambda_{em} \mbox{ (nm)} \\ 347 & 454 \\ 405 & 530 \\ 335 & 430 \\ 410 & 533 \\ 413 & 514 \end{array}$	$\begin{array}{c ccc} \lambda_{abs} \ (nm) & \lambda_{em} \ (nm) & \varepsilon \ (M^{-1} \ cm^{-1}) \\ \hline 347 & 454 & 1.1 \times 10^4 \\ 405 & 530 & 1.0 \times 10^4 \\ 335 & 430 & 1.0 \times 10^4 \\ 410 & 533 & 0.8 \times 10^4 \\ 413 & 514 & 1.2 \times 10^4 \\ \hline \end{array}$	$\begin{array}{c cccc} \lambda_{abs} \ (nm) & \lambda_{em} \ (nm) & \varepsilon \ (M^{-1} \ cm^{-1}) & \varphi \\ \hline 347 & 454 & 1.1 \times 10^4 & 0.015 \\ 405 & 530 & 1.0 \times 10^4 & 0.51 \\ 335 & 430 & 1.0 \times 10^4 & 0.62 \\ 410 & 533 & 0.8 \times 10^4 & 0.45 \\ 413 & 514 & 1.2 \times 10^4 & 0.65 \\ \hline \end{array}$	$\begin{array}{c ccccc} \lambda_{abs} \ (nm) & \lambda_{em} \ (nm) & \varepsilon \ (M^{-1} \ cm^{-1}) & \varphi & \mbox{Stokes Shift} \ (cm^{-1}) \\ \hline 347 & 454 & 1.1 \times 10^4 & 0.015 & 6792 \\ 405 & 530 & 1.0 \times 10^4 & 0.51 & 5824 \\ 335 & 430 & 1.0 \times 10^4 & 0.62 & 6595 \\ 410 & 533 & 0.8 \times 10^4 & 0.45 & 5629 \\ 413 & 514 & 1.2 \times 10^4 & 0.65 & 4758 \\ \hline \end{array}$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	

 Table 2
 Photophysical properties of compounds 2–6 in CH<sub>3</sub>CN

\* Recorded at concentration of  $10^{-4}$  mol/L in CH<sub>3</sub>CN

6-(6-Methanesulphonylamino-1,3-dioxo-1H,3H-benzo[de] isoquinolin-2-yl)-hexanoic acid (5)

To an ice-cooled stirred solution of Compound **1** (0.61 mmol, 200 mg) in small amount of DCM (3 mL) containing a trace of pyridine, added methanesulfonyl chloride (75  $\mu$ L, 1.5 eq) drop wise and the reaction mixture was allowed to stir for 2 h. After completion (monitored by TLC), evaporation of the organic solvent left the crude product, which was purified by column chromatography using DCM and MeOH as eluent. Yield 52 % (130 mg), M.p.: 168 °C; R<sub>f</sub> 0.58 (DCM:MeOH; 95:05), UV (MeOH): 422 nm; IR (KBr):  $\nu$  3431, 3345, 3239, 2923, 2853, 1733, 1699, 1655, 1620, 1580, 1525, 1479, 1405, 1386, 1360, 1310, 1261 cm<sup>-1</sup>; <sup>1</sup>H NMR( Methanol-d<sub>4</sub>): 8 8.61–8.71 (m, 2H), 7.86–8.41 (m, 2H), 6.72–7.32 (m, 1H), 4.22 (brs, 1H), 3.70–3.73 (m, 2H), 3.41 (s, 3H), 2.54–2.56 (m, 4H), 2.35–2.43 (m,2H), 1.87–1.88 (m,2H). MS (ESI): m/z (M<sup>+</sup>) 404.10.

#### 6-[1,3-Dioxo-6-(toluene-4-sulfonylamino)-1H,3H-benzo[de] isoquinolin-2-yl]-hexanoic acid (6)

To an ice-cooled stirred solution of Compound 1 (0.61 mmol, 200 mg) in small amount of DCM (3 mL) containing a trace of pyridine, added p-toluene sulfonyl chloride (0.94 mmol,

180 mg, 1.5 eq.) and the reaction mixture was allowed to stir for 5 h. After completion (monitored by TLC), the crude product was extracted with DCM, washed with brine and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The evaporation of the organic solvent left the crude product, which was purified by column chromatography over silica gel using DCM and MeOH as eluent. Yield 52 %(162 mg), M.p.: 180 °C; R<sub>f</sub> 0.53 (DCM:MeOH; 9.5:0.5); UV (MeOH): 433 nm; IR (KBr):  $\nu$  3433, 3346, 3239, 2924, 2853, 1731, 1695, 1651, 1621, 1580, 1524, 1505, 1479, 1405, 1385, 1360 cm<sup>-1</sup>; <sup>1</sup>H NMR(DMSO-d<sub>6</sub>-Methanol-d<sub>4</sub>):  $\delta$  8.45–8.80 (m, 3H), 7.82–7.91 (m, 2H), 7.10–7.40 (m, 3H), 6.83–6.85 (m, 1H), 6.01 (brs, 1H), 3.29–3.38 (m, 2H), 2.79 (s, 3H), 2.61–2.63 (m, 2H), 2.22–2.24 (m, 3H), 1.89–1.95 (m, 3H). MS (ESI): m/z (M<sup>+</sup>) 480.14.

#### **Results and Discussion**

#### Synthesis

The base molecule 1 was prepared in several steps starting from acenaphthene (Scheme 1) and compounds 2-6 were prepared by substitution reaction at precursor 1 having free amine group. The compound 2 has already been synthesized and studied extensively in our laboratotry. In order to improve



Fig. 2 Effect of monovalent ionic concentration on fluorescence of compounds 2-6



Fig. 3 Effect of bivalent ionic concentration on fluorescence of compounds 2-6

and elaborate the fluorescence properties of compound 2, we synthesized the compounds 3, 4, 5, and 6 by replacing acetyl group at 4-amino by ethoxycarbonyl, tertiarybutoxycarbonyl, methane sulphonyl and p-toluene sulphonyl groups (Scheme 2). Compounds 2-6 were purified by silica gel column chromatography and characterized by <sup>1</sup>H NMR and FT-IR.

#### **Photophysical Properties**

In 1,8-naphthalimide having the naphthalene ring and a dicarboxamide (-CO-N-CO-) group in a six-membered ring, the excitation involves charge shift away from the electron donating nitrogen moiety at the 4-position to electron accepting carbonyl moiety and shows internal charge transfer



Fig. 4 Fluorescence decay and lifetime of compounds 2-6 in CH<sub>3</sub>CN

(ICT) state, resulting in large dipole moment and better stabilization through polarity of solvents in the excited state. The substituted compounds at position 4 show a clear electronic donor character for the carboxyimide group and favor the radiative deactivation of the excited state of the molecules. Any steric interaction destabilizes the excited state, and thus causes emission at lower wavelength with decreased radiative rate constant [24-27]. However, substituent groups at the amino function alter the fluorescence property of this molecule.

#### **Absorption and Fluorescence Spectra**

The UV-Vis absorption and emission spectra of compounds were studied in solvents, like CHCl<sub>3</sub>, CH<sub>3</sub>CN, EtOH, MeOH of different polarity [28, 29]. The absorption and emission

Table 3         Average fluorescence           lifetime and relative amplitude	compound	Solvent	Lifetime (ns)		Relative amplitude		Average Lifetime [ns]
of compounds $2-6$ in different solvents, viz. CHCl <sub>3</sub> , CH <sub>3</sub> CN			T <sub>1</sub>	T <sub>2</sub>	A <sub>1</sub>	A <sub>2</sub>	
and MeOH	2	CHCl <sub>3</sub>	1.39	8.84	0.51	0.49	5.03
		CH <sub>3</sub> CN	1.46	9.11	0.23	0.77	7.32
		MeOH	1.27	7.60	0.45	0.55	4.75
	3	CHCl <sub>3</sub>	4.70	10.05	0.11	0.89	9.46
		CH <sub>3</sub> CN	3.96	9.30	0.14	0.86	8.54
		MeOH	1.67	7.10	0.85	0.15	2.47
	4	CHCl <sub>3</sub>	0.80	5.98	0.96	.034	0.98
		CH <sub>3</sub> CN	1.28	8.40	0.37	0.63	5.76
		MeOH	0.97	6.75	0.73	0.27	2.53
	5	CHCl <sub>3</sub>	4.70	10.05	0.11	0.89	9.82
		CH <sub>3</sub> CN	4.10	10.00	0.06	0.94	8.74
		MeOH	2.05	5.96	0.62	0.38	3.53
	6	CHCl <sub>3</sub>	0.82	10.41	0.79	0.21	2.82
		CH <sub>3</sub> CN	1.39	10.71	0.08	0.92	9.96
		MeOH	0.94	7.56	0.44	0.56	4.64

studies on each compound in different solvents, like CHCl<sub>3</sub>, CH<sub>3</sub>CN, EtOH, MeOH have been shown in Table 1 (graphical representation can be found as supplementary material). Further, the absorption and emission spectra of these compounds in CH<sub>3</sub>CN have been shown in Fig. 1.

All compounds exhibited an intensive electronic absorption band in the region 345-435 nm. Compounds showed solvatochromism, meaning thereby a red shift was observed in the absorption and fluorescence maxima with increasing dielectric constant of solvents. This indicated that the molecules were better stabilized in their ground and excited states in more polar solvent, like EtOH, MeOH. Usually polar molecules have  $\pi$ - $\pi$ \* electronic transitions and higher dipole moments in the Frank Condon excited state S1 than ground state S<sub>0.</sub> The emission spectra of each compound also revealed that emission occurred at higher wavelength. This change in behaviour from non-polar to polar solvents was due to stabilization of excited state through polarity of solvent and solvent effect as all molecules had a capability to form hydrogen bond (through N and O atoms). The increasing order of wavelength in emission spectra of these compounds was  $4 < 2 < 6 < 3 \approx 5$ , which indicated that excited states of compounds 3 and 5 were more stabilized and hence, showed red shift. The electron donating nature of -NH group to naphthalimide ring depends on the nature of substituents attached and this is expressed in the degree of fluorescence shown by these compounds Thus the order of fluorescence of these compounds is  $6 > 4 > 3 \approx 5 > 2$ . The quantum yield of compounds 2–6 was measured using rhodamine 6G in ethanol as a reference and showed that electron releasing function at 4-amino group enhanced the fluorescence signal. Important characterstics of fluorescent compounds, viz. stokes shift (A-f), quantum yield, (Q), absorption ( $\lambda_{max}$ ), emission ( $\lambda_{max}$ ), fluorescence lifetime T<sub>i</sub> [ns], average lifetime [ns] have been studied for compounds 2-6 and summarized in Table 2.

#### **Fluorescence in Inorganic Media**

In order to study the effect of ions on intensity of absorption and emission spectra, the titration experiments were performed at a definite concentration of prepared stock solution of compounds and metal ions, like Na<sup>+</sup>, K<sup>+</sup> and Mg<sup>2+</sup>. Upon gradual addition of metal ions, the absorption and fluorescence spectra showed gradual decrease in the intensity, however, the wavelength of absorption and emission spectra remained unchanged. The fluorescence spectra of compounds **2–6** with univalent ion have been shown in Fig. 2 and with bivalent ion have been shown in Fig. 3 Univalent ions showed similar decrease in intensity for each compounds. In the presence of monovalent Na<sup>+</sup> and K<sup>+</sup> ions, compounds **4** and **6** showed about 20–22 % decrease whereas compounds **2**, **3** and **5** about 8 % decrease in fluorescence. This decrease in fluorescence in the presence of divalent Mg<sup>2+</sup> ion was very marginal. These observations reveal that molecules retained their fluorescence even in inorganic media and hence could be an excellent choice as fluorophores to be used for studying biological system.

#### **Measurement of Fluorescence Lifetime**

The time correlated single photon counting (TCSPC) technique was used to analyse the nature of compounds 2-6 in the excited state and measure the fluorescence lifetime. Analyses were performed in solvents of different polarity, like CHCl<sub>3</sub>, CH<sub>3</sub>CN and MeOH where each compound showed bi-exponential behaviour of lifetime in different solvents at relative amplitude in excited state (Table 2). Fluorescence decay was measured at the respective emission wavelength of the molecules 2-6 in CH<sub>3</sub>CN. The larger and shorter components of fluorescence decay along with the respective amplitude and average lifetime of these molecules have been shown in Table 3 (the fluorescence life time spectra of compounds in three different solvents can be found as supplementary material). The observation revealed that decrease in the value of lifetime is facilitated by increase in polarity of the solvent. In general, the solvent polarity affected the molecules in the excited state resulting in different structural and conformational changes in the molecule and thus generating other state for further deactivation [30-32]. Increase in polarity of the medium leads to a shortening of the lifetime because of increase in non-radiative decay as the energy gap between ground and relaxed excited state is reduced or sometimes hydrogen bonding becomes a dominating factor in excited state between molecules and polar solvent. Compound 2 showed longer component of lifetime 9.11 ns at highest pre-exponential factor 0.77, compound 4 showed longer component of lifetime 8.40 at pre-exponential factor 0.63, compound 5 showed longer component of lifetime 10.00 at highest pre-exponential factor 0.94, compound 6 showed longer component of lifetime 10.71 at highest pre-exponential factor 0.92 in CH<sub>3</sub>CN and compound 3 showed longer component of lifetime 10.05 ns at the highest pre-exponential factor 0.89 in chloroform. The average lifetime decay of compounds 2-6 has been shown in Fig. 4.

#### Conclusions

We have developed some new potential fluorescent compounds with high quantum yield. The compounds exhibited solvatochromic behavior and the fluorescence measurement studies on these molecules showed emission spectra at longer wavelength in the region 430–530 nm. TCSPC experiments revealed a good average lifetime of these molecules under excited state condition. Compounds retained their stability and fluorescence property with negligible change in intensity in the presence of ions, like  $Na^+$ ,  $K^+$  and  $Mg^{2+}$  under aqueous conditions and hence are supposed to be efficient and useful fluorescent reporter groups for drug delivery in biological systems.

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