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# Photophysical Studies on Drug Conjugates of Stavudine/ Zidovudine and 1,8-Naphthalimide in Different Solvent Systems

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A number of fluorescent conjugates of popular anti-HIV drugs 2′,3′-didehydro-2′,3′-dideoxythymidine (stavudine, $d_4T$) and 3′-azido-3′-deoxythymidine (zidovudine, AZT) with 1,8-naphthalimide were synthesized using the coupling reagent dicyclohexylcarbodiimide (DCC) in the presence of 4-dimethylaminopyridine (DMAP) and *N*,*N*-dimethylformamide (DMF) as solvent at room temperature. The steady-state fluorescence measurement studies on these conjugates showed solvatochromic effect. Further, the fluorescence of drug conjugates was recorded in the presence of ions like $Na^+$ and $K^+$ at body level concentration of 135-145 mmol $L^{-1}$ and 3.6-5.1 mmol $L^{-1}$, respectively, in phosphate buffer at pH 7.4 in aqueous media. It was observed that the drug conjugates did not show appreciable fluorescence quenching in presence of ions and buffer.

**Keywords: Fluorescence, Solvatochromism, Fluorescence lifetime, Stavudine, Zidovudine.**

## INTRODUCTION

A tremendous development in the field of molecular fluorescence spectroscopy has led to a fluorescent marker technique that has been widely appreciated and accepted as a non-radioactive marker technology. The fluorescent probes with the advantage of sensitivity and accuracy have attracted more attention broadly in medical science [1,2]. At present, this technique has been successfully applied in the identification of several diseases, like cancer, AIDS, *etc*. The fluorescent marker technology can also be used to determine varied kinds of substances, such as environmental pollutants, drugs, amino acids, chiral molecules, nucleotides and *in vitro*/*in vivo* study and thus, globally acknowledged as one of the convenient and effective methods for labeling. It is a process where a fluorescent probe is combined with other biomolecules by chemical methods to form a complex. Further, by detecting the fluorescence property, the study of the labeled biomolecule can be performed. The factors, like photo-stability, sensitivity, specificity and low cost based technology have underlined its importance in biological applications [3,4]. A complete study on synthesis and fluorescence properties of the fluorophores used in present study, have already been published [5].

Recently, efforts have been focused on design of prodrugs for tissue specific delivery to improve efficacy and safety of drugs. One of the prominent approaches pursued is fluorescent marker technique, relying on the development of targeted prodrugs that allow the drug release at the site of action and help in tracking drug molecule inside the cell. Thus, the technique addresses the problems associated with drug delivery.

The anti-HIV drugs, *viz*. stavudine ($d_4T$) and zidovudine (AZT), a kind of NRTIs, suffer from poor cellular uptake and their detection inside the cells [6-10]. These problems can be overcome by using lipophilic fluorescent markers attached to these drugs, provided the fluorescent markers have appreciable fluorescence for detection using flow cytometry techniques. Such fluorescent drug conjugates can, in turn, enhance the efficacy of drug molecules [11-14]. The ester linkage based fluorescent drug conjugates are expected to enhance the cellular uptake of the prodrug molecules and the drugs itself, in turn, after getting hydrolyzed by esterase enzyme *in vivo* [15-18]. A comprehensive study on fluorescence property of

drug conjugates of stavudine and zidovudine, the anti-HIV drugs, is being presented.

## EXPERIMENTAL

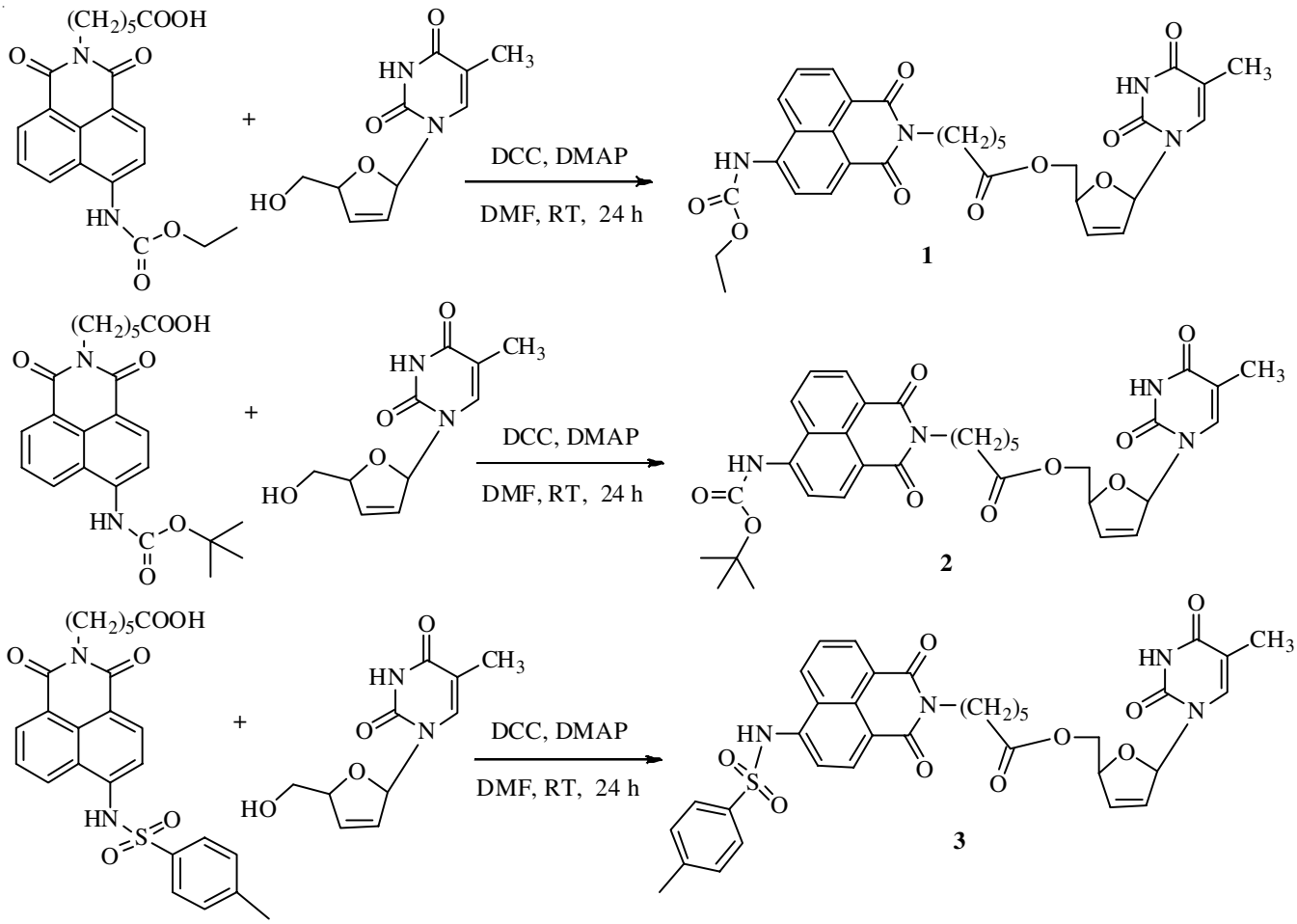
All reactions were monitored by TLC using Merck 60 $F_{254}$ precoated silica gel plates (0.25 mm thickness) and the products were visualized by UV detection. Flash chromatography was carried out with silica gel (200-300 mesh). Melting points were recorded on a Thomas Hoover capillary melting apparatus without correction. The solvents used were spectroscopic grade. $^1$H NMR spectra were recorded on a Bruker Avance (III) 400 MHz spectrometer.

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

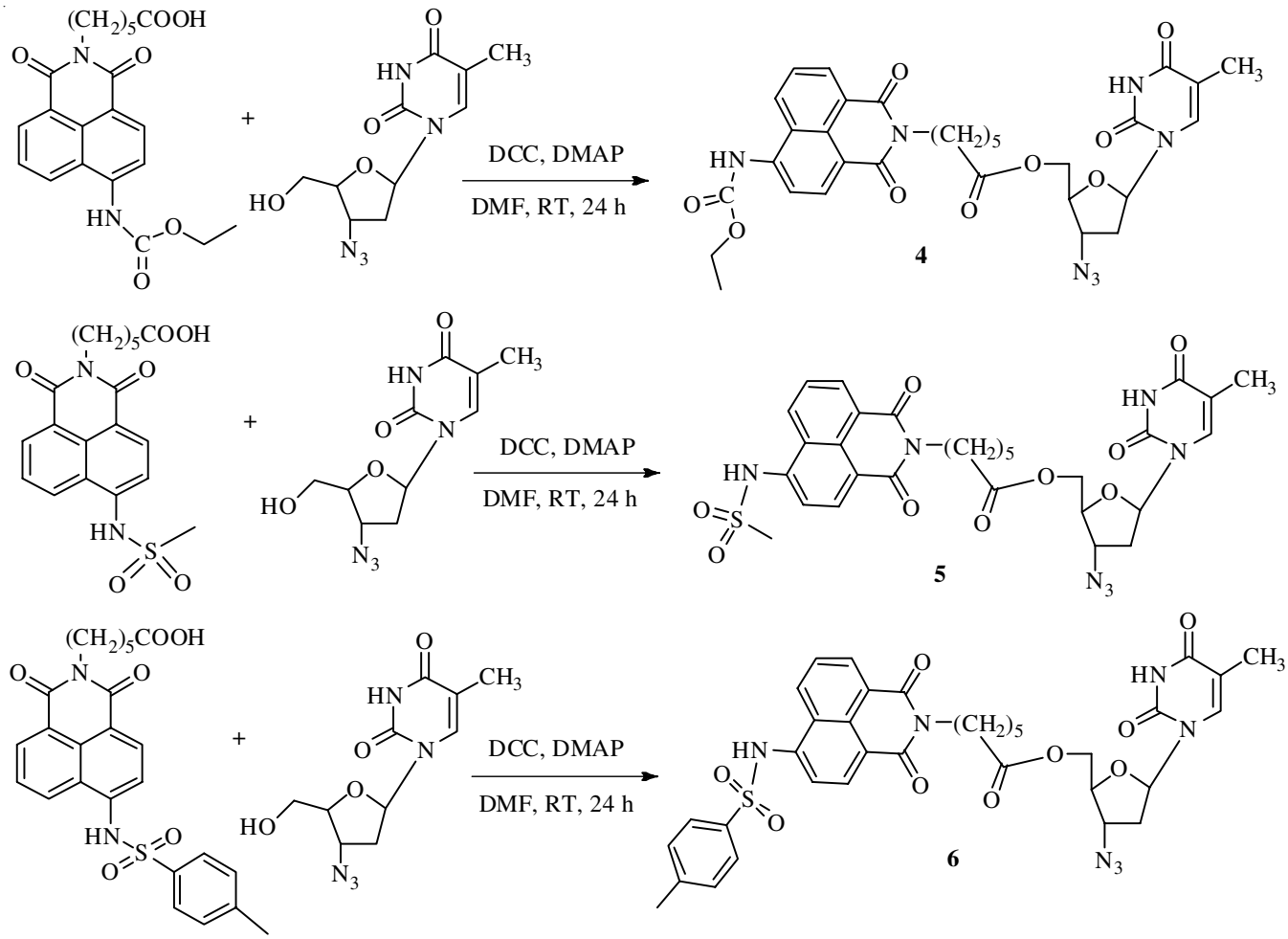
**General procedure:** The synthesis of drug conjugates bearing ester linkages has been shown in **Schemes I** and **II**. A general procedure was followed for ester bond formation between the $d_4$T/AZT and the fluorophore using dicyclohexyl carbodiimide (DCC) as coupling agent in the presence of dimethyl aminopyridine (DMAP) using *N*,*N*-dimethyl formamide (DMF) as solvent. The reaction was carried out at 0 ºC for 10-15 min and then at room temperature for 24 h.

**6-(6-Ethoxycarbonylamino-1,3-dioxo-1*H*,3*H*-benzo-[*d*,*e*]isoquinolin-2-yl)hexanoic acid 5-(5-methyl-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-2,5-dihydrofuran-2-yl-methyl ester (1):** To a solution of fluorophore (100 mg, 0.24 mmol) in DMF (4 mL), added DCC (1.1 equiv., 54.5 mg) and DMAP (10 mol %, 2.93 mg) at 0 ºC, stirred for 10-15 min and added stavudine (2 equiv., 108 mg). The reaction mixture was then stirred at room temperature for 24 h and monitored on TLC. After completion of reaction, the crude product was washed with brine and extracted with ethyl acetate. Evaporation of the solvent under vacuum left the crude product, which was purified by column chromatography using ethyl acetate and hexane to furnish the pure product (60 mg, 41% yield). m.p.: 180 ºC; $R_f$: 0.7 (EtOAc:hexane; 4:6); UV (MeOH): 420 nm. $^1$H NMR (DMSO-$d_6$): δ 8.67-8.69 (m, 1H), 8.42-8.44 (m, 1H), 8.17-8.19 (m, 1H), 7.68-7.77 (m, 2H), 6.86-6.88 (m, 1H), 5.54-5.56 (m, 4H), 1.69-1.72 (m, 10H), 1.58 (t, *J* = 4.0 Hz, 3H), 1.48-1.51 (m, 4H), 1.22 (S, 3H).

**6-(6-*tert*-Butoxycarbonylamino-1,3-dioxo-1*H*,3*H*-benzo[*d*,*e*]isoquinolin-2-yl)hexanoic acid 5-(5-methyl-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-2,5-dihydrofuran-2-ylmethyl ester (2):** To a solution of fluorophore (100 mg, 0.22 mmol) in DMF (4 mL), added DCC (1.1 equiv., 49.85 mg) and DMAP (10 mol %, 2.68 mg) at 0 ºC, stirred for 10-15 min and added stavudine (2 equiv., 100 mg). Further processing of the




**Scheme-I:** Synthesis of prodrugs **1-3**




**Scheme-II:** Synthesis of prodrugs **4**-**6**

reaction was carried out as described for compound **1**. Yield (42 mg, 30%); m.p.: 186 ºC; $R_f$: 0.7 (EtOAc:hexane; 4:6); UV (MeOH): 370 nm. $^1$H NMR (DMSO$d_6$): δ 8.67-8.69 (m, 1H), 8.42-8.44 (m, 1H), 8.17-8.19 (m, 1H), 7.65-7.72 (m, 2H), 6.82-6.90 (m, 1H), 5.54-5.56 (m,4H), 1.69-1.72 (m, 10H), 1.48-1.51 (m, 2H), 1.23 (s, 9H), 1.22 (S, 3H).

**6-[1,3-Dioxo-6-(toluene-4-sulfonylamino)-1*H*,3*H*-benzo[*d*,*e*]isoquinolin-2-yl]hexanoic acid 5-(5-methyl-2,4-dioxo-3,4-dihydro-2H-pyrimidin-1-yl)-2,5-dihydro-furan-2- ylmethyl ester (3):** To a solution of fluorophore (100 mg, 0.20 mmol) in DMF (4 mL), added DCC (1.1 equiv., 43.52 mg) and DMAP (10 mol %, 2.44 mg) at 0 ºC, stirred for 10-15 min and added stavudine (2 equiv., 90 mg). Further processing of the reaction was carried out as described for compound **1**. Yield (35 mg, 25%); m.p.: 192 ºC; $R_f$: 0.7 (EtOAc:hexane; 3:7); UV (MeOH): 420 nm. $^1$H NMR (DMSO-$d_6$): δ 8.67-8.69 (m, 1H), 8.42-8.44 (m, 1H), 8.17-8.19 (m, 1H), 7.63-7.77 (m, 3H), 7.36-7.38 (m, 1H), 6.81-6.88 (m, 1H), 6.37-6.39 (m, 1H), 5.9 (brs, 1H), 5.54-5.56 (m, 4H), 2.36 (s, 1.5H), 1.95 (s, 1.5H), 1.58-1.69 (m, 10H), 1.48-1.51 (m, 2H), 1.22-1.51 (s, 3H).

**6-(6-Ethoxycarbonylamino-1,3-dioxo-1*H*,3*H*-benzo-[*d*,*e*]isoquinolin-2-yl)hexanoic acid 3-azido-5-(5-methyl-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)tetrahydrofuran-2-ylmethyl ester (4):** To a solution of fluorophore (100 mg, 0.24 mmol) in DMF (4 mL), added DCC (1.5 equiv., 75 mg) and DMAP (20 mol %, 5.85 mg) at 0 ºC and stirred for 10-15 min followed by addition of zidovudine (1.5 equiv., 96 mg). Further processing of the reaction was carried out as described for compound **1**. Yield (50 mg, 32%); m.p.: 176 ºC; $R_f$: 0.7 (EtOAc:hexane; 4:6); UV ($CHCl_3$): 410 nm. $^1$H NMR (DMSO-$d_6$): δ 10.34 (brs, 1H), 6.74-6.86 (m, 6H), 5.16-5.14 (t, $J$ = 6.52 Hz, 1H), 4.32 (m, 3H), 3.46-3.49 (m, 3H), 2.87-2.90 (m, 2H), 2.68-2.70 (m, 10H), 1.57 (m, 3H), 1.15 (S, 3H).

**6-(6-Methanesulfonylamino-1,3-dioxo-1*H*,3*H*-benzo-[*d*,*e*]isoquinolin-2-yl)-hexanoic acid 3-azido-5-(5-methyl-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)tetrahydrofuran-2- ylmethyl ester (5):** To a solution of fluorophore (100 mg, 0.25 mmol) in DMF (4 mL), added DCC (1.5 equiv., 77.25 mg) and DMAP (20 mol %, 6.10 mg) at 0 ºC stirred for 10-15 min followed addition of zidovudine (1.5 equiv., 100 mg). Further processing of the reaction was carried out as described for compound **1**. Yield (37 mg, 25%); m.p.: 170 ºC; $R_f$: 0.7 (EtOAc: hexane; 4:7); UV ($CHCl_3$): 397 nm.

**6-[1,3-Dioxo-6-(toluene-4-sulfonylamino)-1*H*,3*H*-benzo-[*d*,*e*]isoquinolin-2-ylhexanoic acid 3-azido-5-(5-methyl-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-tetrahydrofuran-ylmethyl ester (6):** To a solution of fluorophore (100 mg, 0.20 mmol) in DMF (4 mL), added DCC (1.5 equiv., 62 mg)

and DMAP (20 mol %, 5 mg) at 0 ºC stirred for 10-15 min followed by addition of zidovudine (1.5 equiv., 80 mg). Yield (53 mg, 36%); m.p.: 189 ºC; $R_f$: 0.7 (EtOAc:hexane; 3:7); UV ($CHCl_3$): 400 nm. $^1$H NMR (DMSO-$d_6$): δ 10.38 (brs, 2H), 6.74-6.84 (m, 8H), 5.17-5.13 (t, $J$ = 7.43 Hz, 1H), 4.30 (s, 3H), 3.43-3.48 (m, 3H), 2.67-2.69 (m, 10H), 1.57 (m, 4H), 1.15 (s, 3H).

**Detection Method:** The absorption spectra were recorded on Varian UV-Vis spectrophotometer (model: Cary 100) and emission Spectra 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 all measurements. All measurements were done at 25 ºC. 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 had 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 smoothening and processing of graph at observed value of absorption and emission were performed using Origin Pro 8.1.

## RESULTS AND DISCUSSION

**Photophysical properties:** Nucleoside composed of a five-carbon based sugar moiety linked to nitrogenous heterocyclic purines/pyrimidines rings, are practically non-emissive or rarely emissive. These natural nucleobases/nucleosides are characterized by low fluorescence and associated with sub-pico-second excited state lifetimes [19]. These features pose a major challenge to the biophysical and extensive study for non-emissive or rarely emissive nucleoside analogs. In order to study the photophysical property of such molecules, a simple and appropriate approach is labeling of biomolecular core with established fluorophores through various linkages. The challenge of designing fluorophores for predicting the emissive properties of small organic molecules depends on their structure, conjugation system and their behavior towards electromagnetic irradiation. The most promising fluorescent probes have emitting parameters strongly dependent on the microenvironment. The fluorophores generally used undergo rapid excited state physico-chemical changes and show sensitivity towards nearby microenvironment. The possible electronic interactions and transitions lead the fluorophores to display wide range of emission wavelengths and show fluorescence. The photophysical properties depend on composition and proper substitution of the individual chromophore. The extension of the biomolecular core by electronically conjugating with additional aromatic moieties generates a unique and new photophysical characteristic, resulting from either locally excited or charge transfer states populated *via* a rather energetic absorption band. Naphthalimides are considerd as a strong chromophoric system, whose electronic transitions and emissions depend on the nature of the surrounding medium. The photophysical behaviour shown by 1,8-naphthalimide derivatives is dependent on C-4 substitution, which causes a variation in transition and the fluorescence emission. The substituents at position 4, being electron donors and carboxamide group being electron acceptor, favor the radiative deactivation of the excited state of the molecules. The extended aliphatic chain from imide nitrogen bearing reactive terminal moiety modifies the coplanarity of the chromophore and facilitates the electronic transition. The naphthalimide derivatives are well-known probes for medical and biological purposes, such as local anesthetics, as antitumor agents, potential HIV drugs and fluorescent cell markers [20-24]. With this rationale, various fluorescent conjugates of stavudine and zidovudine-thymidine based nucleoside analogs and considered as potent anti-HIV agents, are developed. Further, photophysical studies are performed to reveal the fluorescence maxima of various drug conjugates in different media.

**Fluorescence spectra:** The fluorescence spectra of compounds were studied in solvents of different polarity. All the compounds showed solvatochromism, as emission spectra of each compound revealed that emission occurred at higher wavelength with increasing polarity of solvents. This indicated the stabilization of excited state in moving from non-polar to polar solvents like DCM, THF, $CHCl_3$, $CH_3CN$:$H_2O$, MeOH, MeOH:$H_2O$. This behaviour of a fluorophore with different solvents is observed due to solvent effect and stabilization through hydrogen bonding [25,26].

The fluorescence spectra of compounds **1-3** was studied in solvents, like MeOH, MeOH:$H_2O$, $CH_3CN$, $CH_3CN$:$H_2O$ and $H_2O$ and the spectral data are given in Table-1. Compound **1** showed the highest degree of fluorescence in $CH_3CN$ at wavelength 531 nm. As the polarity of solvent increased, as in the case of methanol, a 20-25 nm red shift was observed with low intensity of fluorescence. In aqueous solvent, the compound showed low degree of fluorescence with blue shifted emission at 445 nm. As the polarity of the aqueous solvent system changed using binary mixture of water:methanol, there was an increase in the degree of fluorescence of compound with 20-40 nm red shift in emission spectrum (Fig. 1). Compound **2** showed the highest degree of fluorescence in acetonitrile at a wavelength of 468 nm. However, it showed very low degree of fluorescence in polar protic solvent as well as in binary aqueous solvent and aqueous solvent as compared to degree of fluorescence observed in compound 1 in the similar solvent systems. It showed maximum red shift emission spectrum in aqueous binary solvent system (Fig. 2). The observed fluorescence of compound **3** was found to be similar to that of compound **1**. Compound **3** showed the highest degree of fluorescence in $CH_3CN$ at a wavelength of 532 nm. As the polarity of solvent increased, as in the case of methanol, a 20-25 nm redshift was observed. In aqueous solvent, compound showed low degree of fluorescence with blue shifted emission at 430 nm. As the polarity of pure aqueous solvent system changed using binary mixture of water and methanol, there was an increase in the degree of fluorescence of compound with 5-10 nm blue shifted emission spectrum (Fig. 3). In order to study the effect of $Na^+$ and $K^+$ ions on the intensity of emission spectra, the fluorescence was recorded in the presence of ions, like $Na^+$ and $K^+$ at body level concentration of 135-145 mmol $L^{-1}$ and 3.6-5.1

TABLE-1
EMISSION MAXIMA $\lambda_{em}$ (nm) OF COMPOUNDS **1-3**
IN DIFFERENT SOLVENT SYSTEMS

| Compound | MeOH | MeOH:$H_2O$ | $CH_3CN$ | $CH_3CN$:$H_2O$ | $H_2O$ |
|---|---|---|---|---|---|
| **1** | 558 | 468 | 531 | 517 | 448 |
| **2** | 461 | 543 | 468 | 505 | 508 |
| **3** | 555 | 425 | 532 | 525 | 430 |

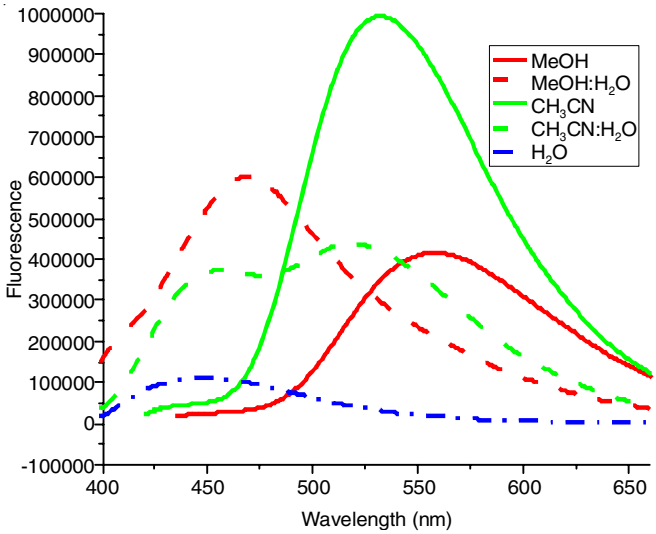



Fig. 1. Fluorescence spectra of compound **1**

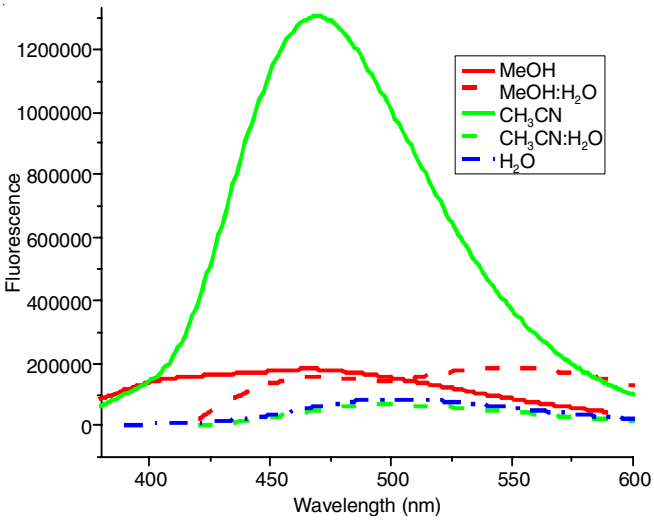



Fig. 2. Fluorescence spectra of compound **2**

mmol $L^{-1}$, respectively. It was also recorded using phosphate buffer at pH 7.4 in aqueous media. It was observed that upon gradual addition of metal ions, the fluorescence spectra showed gradual decrease in the intensity, however, the wavelength of emission spectra remained unchanged. The intensity of fluorescence of compound **1** substantially decreased in the presence of $K^+$ as compared to $Na^+$ at the same wavelength. Further, a gradual addition of buffer drastically decreased the intensity of fluorescence (Fig. 4).

The fluorescence of compounds **4-6** was studied in solvents, like DCM, THF, chloroform and toluene and the spectral data are given in Table-2. Compound **4** showed the highest degree of fluorescence in DCM at wavelength 497 nm (Fig. 5).

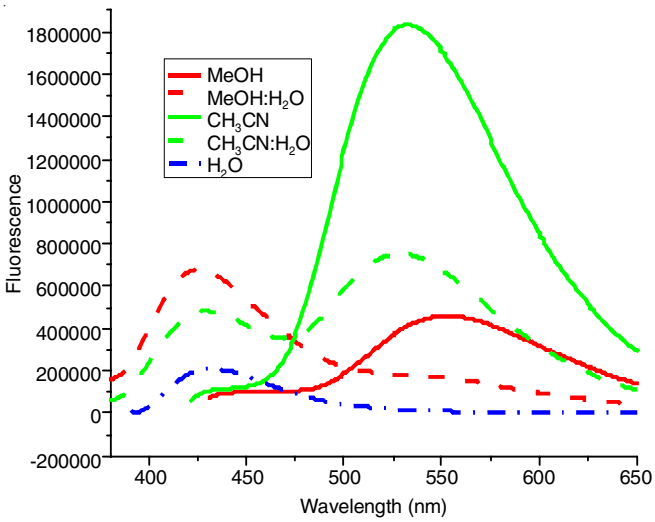



Fig. 3. Fluorescence spectra of compound **3**

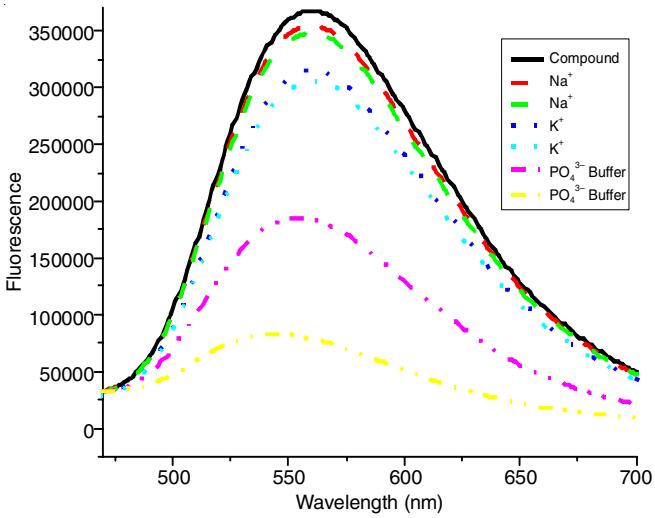



Fig. 4. Fluorescence spectra of compound **1** in the presence of ions and phosphate buffer

TABLE-2
EMISSION MAXIMA $\lambda_{em}$ (nm) OF COMPOUNDS **4-6**
IN DIFFERENT SOLVENT SYSTEMS

| Compound | DCM | THF | Toluene | Chloroform |
|---|---|---|---|---|
| **4** | 497 | 512 | 489 | 527 |
| **5** | 485 | 507 | 495 | 519 |
| **6** | 493 | 509 | 487 | 522 |

As the polarity of solvent increased, as in the case of THF and $CHCl_3$, a 15-20 nm redshift was observed with low intensity of fluorescence. Compound **5** showed the highest degree of fluorescence in DCM at wavelength 485 nm (Fig. 6). As the polarity of solvent increased, as in the case of THF and $CHCl_3$, a 10-15 nm redshift was observed with low intensity of fluorescence. Compound **6** showed the highest degree of fluorescence in DCM at wavelength 493 nm and showed similar spectrum, like compounds **4** and **5**, in other solvent systems (Fig. 7). In order to study the effect of $Na^+$ and $K^+$ ions on the intensity of emission spectra, the fluorescence was recorded in the presence of ions, like $Na^+$ and $K^+$ at body level concen-

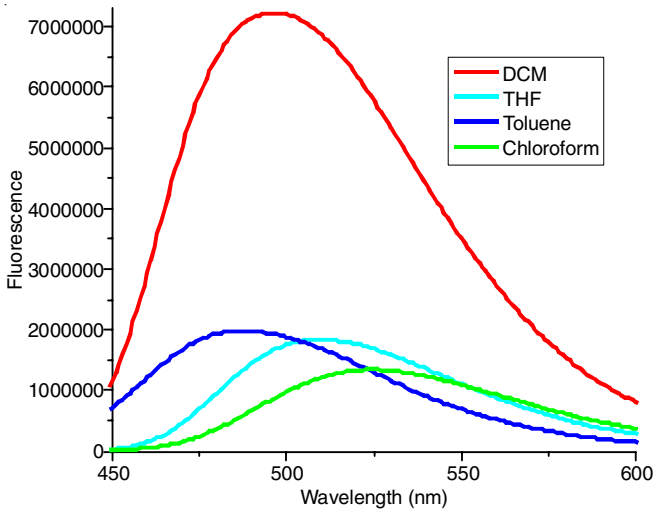



Fig. 5. Fluorescence spectra of compound **4**

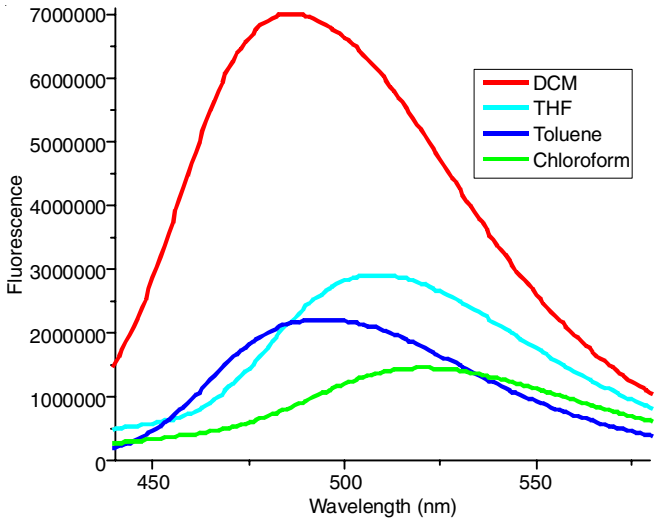



Fig. 6. Fluorescence spectra of compound **5**

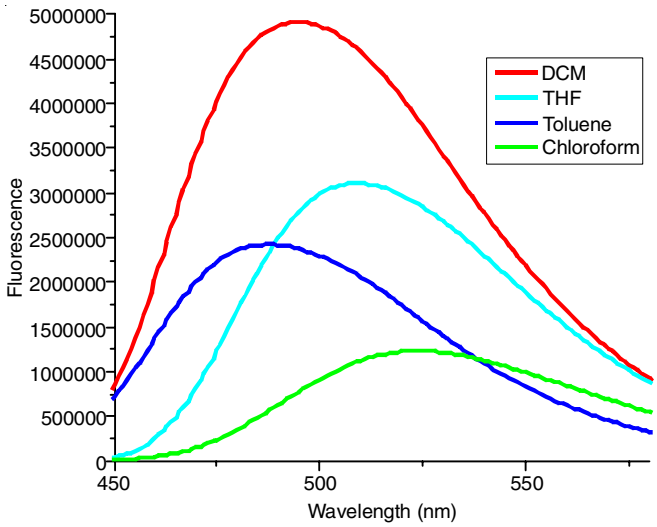



Fig. 7. Fluorescence spectra of compound **6**

tration of 135-145 mmol $L^{-1}$ and 3.6-5.1 mmol $L^{-1}$, respectively. It was also recorded using phosphate buffer at pH 7.4 in aqueous media and observed that upon gradual addition of metal ions, the fluorescence spectra showed gradual decrease in the intensity. However, the wavelength of emission spectra remained unchanged. The intensity of fluorescence of compound **4** substantially decreased in the presence of $K^+$ as compared to $Na^+$ at the same wavelength. Further, a gradual addition of buffer drastically decreased the intensity of fluorescence (Fig. 8). Compounds **5** and **6** showed a similar trend of fluorescence intensity in the presence of ions and phosphate buffer like compound **4** (Figs. 9 and 10).

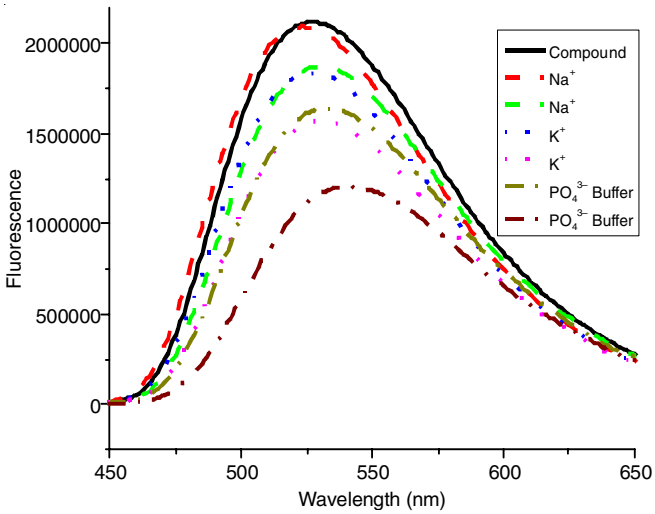



Fig. 8. Fluorescence spectra of compound **4** in the presence of ions and phosphate buffer

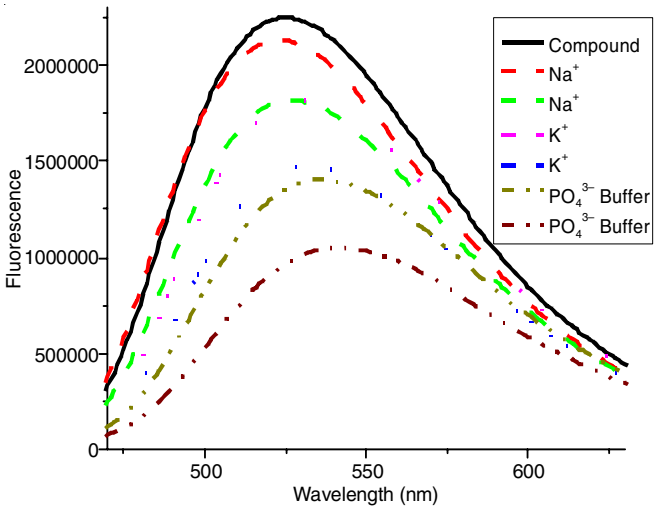



Fig. 9. Fluorescence spectra of compound **5** in the presence of ions and phosphate buffer

**Measurement of fluorescence lifetime:** The time correlated single photon counting (TCSPC) technique was used to analyze the nature of compounds **1-6** in the excited state and measure the fluorescence lifetime [27,28]. A comparative lifetime study on compounds **1-3** was done in $CH_3CN$ as shown in Fig. 11. Fluorescence decay was measured at the respective emission wavelength of the molecules **1-3** in $CH_3CN$. The larger and shorter components of fluorescence decay along with the respective amplitude and average lifetime of these molecules are given in Table-3.

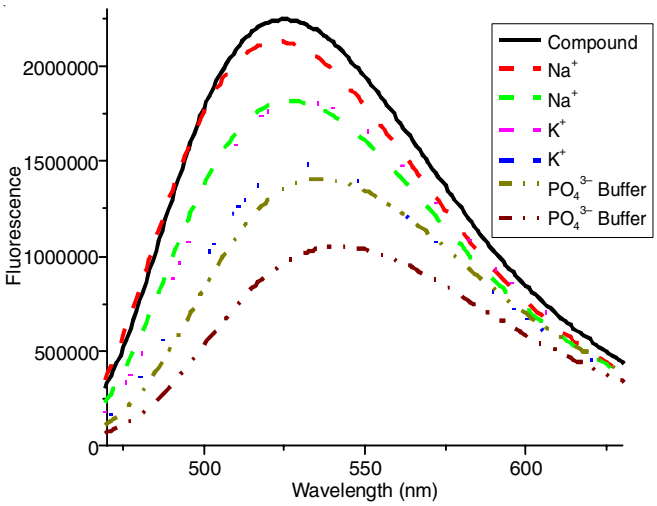



Fig. 10. Fluorescence spectra of compound **6**in the presence of ions and phosphate buffer

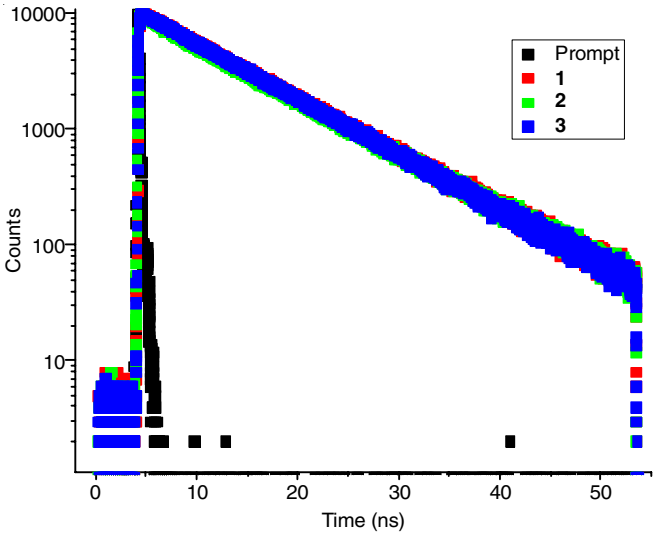



Fig. 11. Fluorescence lifetime of compounds **1-3** in acetonitrile

TABLE-3
AVERAGE FLUORESCENCE LIFETIME AND RELATIVE AMPLITUDE OF COMPOUNDS **1-3** IN ACETONITRILE

| Compd. | Solvent | Lifetime (ns) | | Relative amplitude | | Average lifetime (ns) |
|---|---|---|---|---|---|---|
| | | $T_1$ | $T_2$ | $A_1$ | $A_2$ | |
| **1** | $CH_3CN$ | 9.0 | - | 1 | - | 9.03 |
| **2** | $CH_3CN$ | 4.2 | 9.5 | 0.14 | 0.86 | 8.65 |
| **3** | $CH_3CN$ | 4.2 | 8.9 | 0.01 | 0.99 | 8.90 |

**Lifetime patterns:** Compound **1** showed monoexponential decay with the highest value of lifetime 9.00 ns at maximum relative amplitude 1.0 in $CH_3CN$, compound **2** showed biexponential decay at 9.5 ns at maximum relative amplitude 0.86 in $CH_3CN$ and compound **3** showed at 8.9 ns at relative amplitude 0.99 in $CH_3CN$. The order of average lifetime of compounds was found to be **2** < **3** < **1** in $CH_3CN$.

A comparative lifetime study on compounds **4-6** was also performed in DCM as shown in Fig. 12. Fluorescence decay was measured at the respective emission wavelength of the compounds **4-6** in DCM. The larger and shorter components

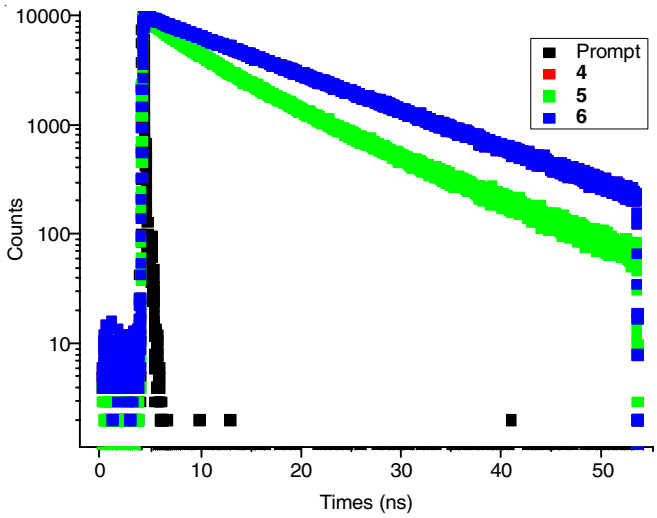



Fig. 12. Fluorescence lifetime of compounds **4-6** in dichloromethane

of fluorescence decay along with the respective amplitude and average lifetime of these molecules are given in Table-4.

TABLE-4
AVERAGE FLUORESCENCE LIFETIME AND RELATIVE AMPLITUDE OF COMPOUNDS **4-6** IN DICHLOROMETHANE

| Compd. | Solvent | Lifetime (ns) | | Relative amplitude | | Average lifetime (ns) |
|---|---|---|---|---|---|---|
| | | $T_1$ | $T_2$ | $A_1$ | $A_2$ | |
| **4** | DCM | 1.40 | 12.70 | 0.03 | 0.97 | 12.4 |
| **5** | DCM | 3.50 | 9.70 | 0.36 | 0.64 | 8.65 |
| **6** | DCM | 2.30 | 12.70 | 0.02 | 0.98 | 12.5 |

Compound **4** showed biexponential decay with the highest value of lifetime 12.70 ns at maximum relative amplitude 0.97 in DCM, compound **5** showed biexponential decay at 9.70 ns at maximum relative amplitude 0.64 in DCM and compound **6** showed at 12.70 ns at relative amplitude 0.98 in DCM. The order of average lifetime of compounds was found to be 5 < 4 ≤ 6 in DCM.

## Conclusion

On the basis of the fluorescence studies of the prodrug molecules, it can be concluded that the compounds **1-6** exhibited solvatochromic behaviour emission spectra at longer wavelength in the region 500-550 nm with increasing solvent polarity. Among the various solvent systems used, *viz*. MeOH, MeOH:$H_2O$, $CH_3CN$, $CH_3CN$:$H_2O$ and $H_2O$, compounds **1-3** showed the highest degree of fluorescence in $CH_3CN$ and the compounds **4-6** showed the highest degree of fluorescence in DCM. Time correlated single photon counting (TCSPC) system experiments revealed a good average lifetime (8.65-12.5 ns) for these compounds under excited state. The higher value of lifetime showed a radiative deactivation of the fluorescent state of the compounds. All compounds retained their stability and fluorescent property with negligible change in the intensity of fluorescence in the presence of ions, like $Na^+$, $K^+$ and phosphate buffer under aqueous conditions. The prodrugs thus showed easily detectable fluorescence, which can be utilized in tracking the drug molecules under *in vivo* experimental conditions and

the fluorophores can be developed as markers in molecular biology or diagnostics.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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