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PAPER

1-(Hydroxyacetyl)pyrene a new fluorescent phototrigger for cell imaging and caging of alcohols, phenol and adenosine†

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1-(Hydroxyacetyl)pyrene has been introduced as a new fluorescent phototrigger for alcohols and phenols. Alcohols and phenols were protected as their corresponding carbonate esters by coupling with fluorescent phototrigger, 1-(hydroxyacetyl)pyrene. Photophysical studies of caged carbonates showed that they all exhibited strong fluorescence properties. Irradiation of the caged carbonates by visible light (≥410 nm) in aqueous acetonitrile released the corresponding alcohols or phenols in high chemical (95–97%) and quantum (0.17–0.21) yields. The mechanism for the photorelease was proposed based on Stern–Volmer quenching experiments and solvent effect studies. Importantly, 1-(hydroxyacetyl)pyrene showed as a phototrigger for rapid photorelease of the biologically active molecule adenosine. *In vitro* biological studies revealed that 1-(hydroxyacetyl)pyrene has good biocompatibility, cellular uptake property and cell imaging ability.

1. Introduction

Recently, the development of new phototriggers has received great interest due to their ability to exert spatial and temporal control over the release.[1] Based on the above unique ability of the phototriggers, they have been utilized in several important applications, including the controlled release of bioactive molecules for studying complex and fast biological processes,[2] photolithography,[3] DNA synthesis,[4] studies of protein folding processes,[5] solid state synthesis,[6] microarray fabrication,[7] synthetic organic chemistry,[8] the controlled release of bioactive volatiles[9] and pesticides.[10] To date a variety of phototriggers such as 2-(dimethylamino)-5-nitrophenol,[11] α-carboxy nitrobenzyl,[12] 3-nitro-2-naphthalenemethanol,[13] *p*-hydroxyphenacyl,[14] α-keto amides,[15] 1-acyl-nitroindolines,[16] anthracene-9-methanol,[17] and derivatives of quinoline[18] as well as coumarin[19] have been reported.

For the last two decades, there has been tremendous interest in the fluorescent derivatisation of biologically important molecules by fluorophores in order to improve the sensitivity of detection as well as quantification of the active molecules involved in the physiological action.[20] Recently, tagging of the active molecules by light activated fluorophores has gained momentum, since, in addition to the quantification of the active molecules, it allows temporal and spatial controlled release of active molecules by externally regulated light stimuli.[21] So far, polycyclic aromatic compounds, namely 7-methoxycoumarin-4-yl,[19,22] anthracene-9-methanol,[16] pyren-1-ylmethyl,[23–27] perylene-3-ylmethyl,[28] and (5-dansyloxy-3-hydroxynaphthalen-2-yl)methyl[29] have been demonstrated as fluorescent phototriggers. Phototriggers which have improved fluorescence properties, good biocompatibility, fast release rates, high photochemical quantum yields and good absorption above 410 nm are still in demand. Recently, we have introduced a new environment sensitive fluorophore, namely 1-acetyl pyrene,[30] which acts as a phototrigger in the visible wavelength region (>410 nm) for carboxylic acids, including amino acids.

In continuation, here we report 1-(hydroxyacetyl)pyrene as a new fluorescent phototrigger for alcohols and phenol. The synthesis and the characterization of caged carbonates of 1-(hydroxyacetyl)pyrene were discussed. The absorption and emission properties of caged carbonates along with its phototrigger were investigated. The photorelease was studied by irradiating the caged carbonates by visible light (≥410 nm) in aqueous acetonitrile solution. We also demonstrated the phototrigger ability of the 1-(hydroxyacetyl)pyrene using the biologically relevant molecule adenosine. More importantly, we explored the fluorescence properties of the phototrigger, 1-(hydroxyacetyl)pyrene for *in vitro* cell imaging studies and also studied their cytotoxicity on the L929 cell line.

2. Results and discussion

2.1. Synthesis, photophysical and photochemical properties of carbonates (5a–f)

2.1.1. Synthesis of caged carbonates (5a–f). The fluorescent phototrigger **3** was initially synthesized from 1-acetyl pyrene (**1**)

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by α-bromination followed by treatment with sodium formate in ethanol under refluxing conditions.[31]

A series of alcohols and phenols were then protected, using the phototrigger 1-(hydroxyacetyl)pyrene **3**, as their corresponding carbonates, as depicted in Scheme 1. First, the chloroformates of the alcohols and phenols were synthesized following the standard procedure using triphosgene. Next, treatment of the freshly synthesized chloroformates of the alcohols and phenols (**4a–f**) with phototrigger **3** in the presence of DMAP in dry DCM at room temperature for a period of 8–10 h afforded the corresponding caged carbonates (**5a–f**) in good yields (Table 1).

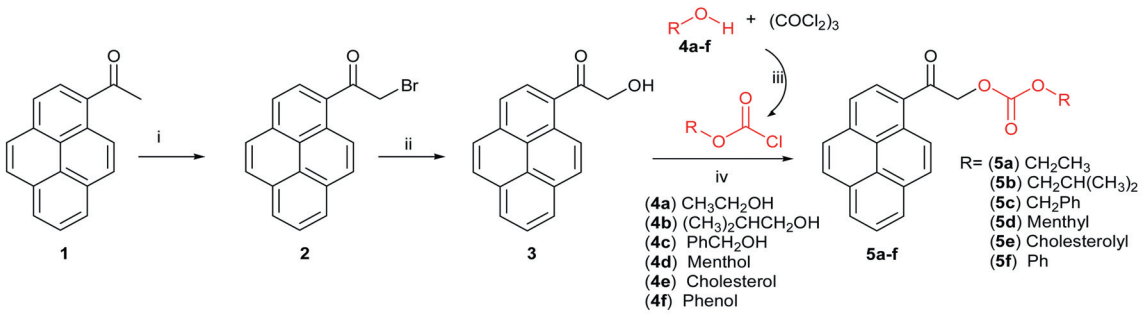
All the caged carbonates were characterized by IR, ^{1}H, ^{13}C NMR and mass spectral analysis. The IR spectra of the caged carbonates showed a new band at around 1740–1750 cm^{-1} due to the stretching vibration of the newly formed carbonate carbonyl group. The confirmation of the presence of the newly formed carbonate group was further supported by the ^{13}C NMR spectra, which showed the carbonate carbonyl at δ 155 in addition to the carbonyl signal of the 1-(hydroxyacetyl)pyrene at δ 196.

2.1.2. Photophysical properties of caged carbonates (5a–f). The photophysical properties of all caged carbonates (**5a–f**), and the phototrigger **3** were investigated. The UV/vis absorption and emission spectra of the degassed 2 × 10^{-6} M solution of the caged carbonates (**5a–f**), and the phototrigger **3** in absolute ethanol (EtOH) were recorded. The absorption and emission maxima, molar absorptivities and fluorescence quantum yield of the above compounds are summarized in Table 1. Fluorescence quantum yields were calculated using 9,10-diphenyl anthracene as standard (Φ = 0.95 in ethanol).[32] We noted that the fluorescence quantum yield of the caged carbonates of 1-(hydroxyacetyl)pyrene were lesser when compared to pyrene. The above fact can be attributed to the presence of ^{3}n–π* in the vicinity of lowest ^{1}π–π* excited state, which is similar to hepatonyl pyrene.[33] Hence, inter-system crossing occurs in competition with fluorescence, thereby resulting in lowering of the quantum efficiency.

We observed similar absorption and emission spectra for all the caged carbonates (see ESI, Fig. S2.a and S2.b†), which clearly suggests that the 1-(hydroxyacetyl)pyrene moiety only dictates the position of the absorption and emission maxima, ruling out the influence of its counterpart alcohols.

2.1.3. Photolysis of caged carbonates (5a–f). To explore the phototrigger ability of 1-(hydroxyacetyl)pyrene, we carried out photolysis of all carbonates in aqueous acetonitrile (50 : 50 v/v) at ≥410 nm by using a 125 W medium pressure Hg lamp. The course of the photocleavage reaction was monitored by UV/vis absorption spectroscopy, fluorescence spectroscopy, ^{1}H NMR spectroscopy, as well as reverse phase HPLC with an UV detector. We found the corresponding alcohols and phenols were released in high chemical (95–97%) and quantum (0.17–0.21) yields (Table 2).

In each case the photolysis was stopped when the conversion reached at least 95% (as indicated by HPLC/^{1}H NMR). For compounds indicated by "*d*" in Table 2, the photoproducts were



Reagents and condition: (i) $CuBr_2$, EtOAc, reflux, 6 h, (ii) HCO_2Na, EtOH, reflux, over night, (iii) Na_2CO_3, Toluene, 0° C, 6 h, (iv) DMAP, DCM, r.t, 8-10 h.

Scheme 1 Synthesis of 1-(hydroxyacetyl)pyrene caged carbonates (**5a–f**).

Table 1 Synthetic yields, UV/vis and fluorescence data for carbonates (**5a–f**), and the phototrigger **3** in absolute ethanol

	UV/vis			Fluorescence		
Compound	Synthetic yield[a] (%)	λ_{max}[b] (nm)	log ε[c]	λ_{max}[d] (nm)	Stocks' shift[e] (nm)	Φ_f[f]
5a	89	357	4.33	446	89	0.042
5b	90	357	4.32	449	92	0.045
5c	97	356	4.35	449	93	0.043
5d	97	356	4.33	448	92	0.040
5e	92	357	4.33	447	90	0.037
5f	89	355	4.33	445	90	0.040
3	—	359	4.34	439	90	0.043

[a] Based on isolated yield. [b] Maximum absorption wavelength. [c] Molar absorption coefficient at the maximum absorption wavelength. [d] Maximum emission wavelength. [e] Difference between maximum absorption wavelength and maximum emission wavelength. [f] Fluorescence quantum yield.

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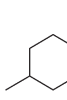
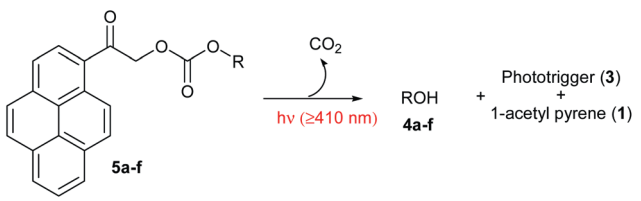
Table 2 Photorelease data of carbonates (**5a–f**) in acetonitrile–H_2O (50 : 50) solvent

		Photorelease data (≥410 nm)		
Caged carbonate	Alcohol/phenol	log ε^a	Yield^b (%)	Φ^c
5a		3.44	94	0.17
5b		3.43	94	0.17
5e[d]		3.33	95	0.19
5c[d]		3.38	97	0.19
5d[d]	Cholesterol	3.21	95	0.21
5f[d]		3.42	97	0.20

[a] Molar absorption coefficient at the irradiation wavelength. [b] Yield was calculated based on ^{1}H NMR/HPLC. [c] Photochemical quantum yield (error limit within ± 5%). [d] Carbonates for which the photoproducts were isolated and compared with authentic samples.



Scheme 2 Photorelease of alcohols and phenol involving loss of CO_2.

isolated and analyzed by spectroscopy and in each case we found three major photoproducts to be formed namely, released alcohols or phenol, phototrigger **3** and 1-acetyl pyrene **1** (Scheme 2).

As a representative example, in Fig. 1 we have shown the HPLC profile of the carbonate **5f** at regular intervals of irradiation. The HPLC chart shows a gradual decrease of the peak at a retention time 3.82 min with an increase in irradiation time, indicating the photodecomposition of the carbonate **5f**. On the other hand, we also noted a gradual increase of three new peaks at retention times 2.88, 3.57, and 4.35 min, corresponding to the photoproducts phenol, 1-(hydroxyacetyl) pyrene **3**, and 1-acetyl pyrene **1**, respectively.

2.1.4. Solvent effect on the photorelease. To understand the role of solvent on the rate of photorelease, we carried out photolysis of the carbonate **5f** in different solvents and the results are summarized in Table 3. Similar, to caged esters of 1-acetyl pyrene, caged carbonate **5f** also photocleaved efficiently in MeOH–H_2O (50 : 50) compared to other solvent systems. Further, we also noted an increase in the photocleavage efficiency of **5f** with increased amounts of water in acetonitrile. The above facts suggest the formation of a zwitterion-like intermediate during the photorelease mechanism.

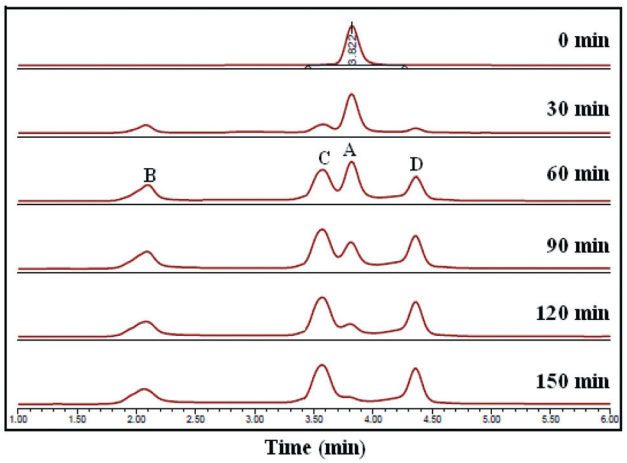


Fig. 1 HPLC profile for the photolysis of the carbonate **5f** (1×10^{-4} M) in ACN–H_2O (50 : 50 v/v) at regular interval of time (0–150 min, time interval = 30 min), (A = **5f**, B = **4f**, C = **1**, D = **1**).

Table 3 Photorelease data of carbonates **5f** in different solvents

	Photorelease data of **5f** (≥410 nm)		
Solvent	log ε^a	$K \times 10^{-3}$ [b] (M min^{-1})	Φ_p [c]
THF	3.43	1.20	0.02
MeOH	3.43	1.38	0.02
MeOH–H_2O (70 : 30)	3.42	10.12	0.13
MeOH–H_2O (50 : 50)	3.42	15.50	0.25
CAN	3.42	1.25	0.02
ACN–H_2O (90 : 10)	3.42	7.79	0.10
ACN–H_2O (70 : 30)	3.42	9.88	0.13
ACN–H_2O (50 : 50)	3.42	15.07	0.20
THF–H_2O (50 : 50)	3.43	14.52	0.19

[a] Molar absorption coefficient at the irradiation wavelength. [b] Specific photorelease rate. [c] Photochemical quantum yield (error limit within ± 5%).

2.1.5. Stern–Volmer quenching experiments. To determine whether the photorelease proceeds through a triplet or singlet excited state, we conducted Stern–Volmer quenching experiments on the carbonate ester **5f**. Photolysis of a 1×10^{-4} M solution of **5f** was carried out in presence of different (1×10^{-4}, 2×10^{-4}, and 3×10^{-4} M respectively) concentrations of a triplet quencher, potassium sorbate (PS) and the course of photolysis was monitored by HPLC and we plotted normalized peak area obtained from HPLC *versus* irradiation time (min). From Fig. 2 it can be seen that on addition of increasing amounts of PS, the rate of photorelease decreases drastically, indicating that photocleavage of **5f** proceeds *via* the triplet excited state. The triplet energy of potassium sorbate[34] and acetyl pyrene[35] are ≈58 and 45 kcal per mole respectively.

2.1.6. Mechanism of photorelease. Based on the literature precedence[14,17] on Stern–Volmer quenching experiments and solvent effect studies on photorelease, we suggest a possible mechanism for the photolysis of caged carbonates as shown in Scheme 3. Irradiation of the caged carbonates in aqueous media leads to a singlet excited state, which then undergoes intersystem

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crossing to the triplet state. Cleavage of the C–O bond in the caged carbonates proceeds from the triplet excited state either by heterolytic or homolytic cleavage followed by single electron transfer, to form an ion-pair intermediate **1a**. Trapping of the ion-pair intermediate by a polar solvent yields photoproduct **3** along with released alcohol. Further, formation of photoproduct 1-acetylpyrene **1** can be explained by the caged-escaped mechanism of intermediate **1b** followed by H-atom abstraction from the solvent. The formation of the 1-(acetylpyrenyl) carbocation in the given mechanism has already been demonstrated in the case of 1-(acetypyrene)-carboxylates.[30]

2.2. Synthesis, photophysical, photochemical, cellular imaging and cytotoxic properties of caged adenosine 9

2.2.1. Synthesis of caged adenosine. To explore the versatility of our fluorescent phototrigger **3**, we caged the biologically relevant molecule adenosine, as depicted in Scheme 4. For the protection of adenosine we followed a chemoselective protocol as reported by Suzuki *et al.*[36] The caged adenosine **9** was obtained, on treatment of 2′,3′-*O*-isopropylideneadenosine with compound **7**, which was *in situ* synthesized by the reaction of **3** with 4-nitrophenyl chloroformate **6** in the presence of DMAP in anhydrous chloroform at room temperature.

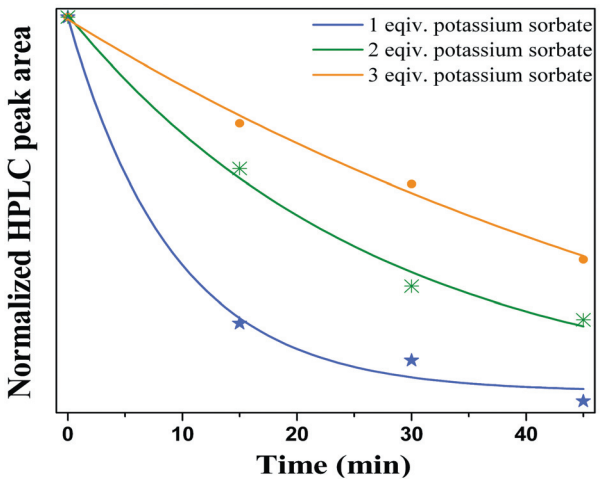


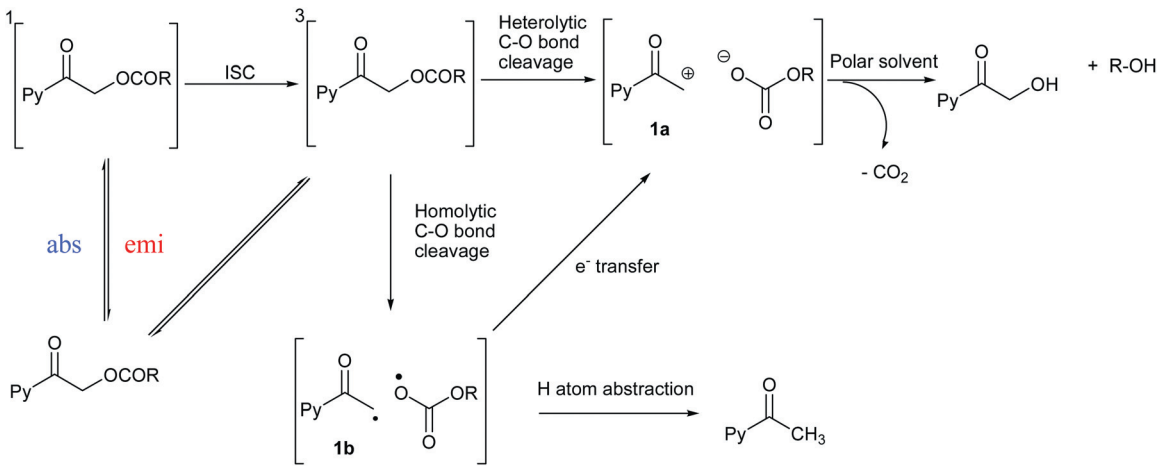
Fig. 2 Time course of photolysis for the carbonate **5f** in presence of different amount of triplet quencher PS.

2.2.2. Photophysical properties of caged adenosine. Photophysical properties of caged adenosine **9** were investigated in different solvent systems and the results are presented in Table 4. Fig. 3 shows the normalized absorption and the emission spectra of **9** in ethanol. The absorption spectrum of **9** shows an intense band centred at 343 nm with log ε 4.25, while in the emission spectrum the emission maxima was red shifted to about 430 nm.

In order to demonstrate the environment sensitive fluorescence properties of the caged adenosine, we recorded the emission spectra of **9** in acetonitrile–water binary mixtures. We observed that upon addition of increasing amounts of water the fluorescence spectra of **9** is red shifted with a concomitant increase in fluorescence intensity (Fig. 4), similar behaviour was also noted in other 1-acylpyrene derivatives.[33] The photophysical studies showed that caged adenosine is highly fluorescent with a large Stokes' shift and environment sensitive fluorescence behaviour.

2.2.3. Photolysis of caged adenosine 9. Photolysis of caged adenosine **9** was carried out both in ACN–H_2O (50 : 50) and ACN/4-(2-hydroxyethyl)piperazine-1-ethanesulfonicacid (HEPES) buffer solution (50 : 50) (HEPES was used to resemble biological condition) by visible light (≥410 nm) using a 125 W medium pressure Hg lamp filtered by 1 M $NaNO_2$ solution. The course of the photocleavage reaction was monitored by UV/vis absorption and fluorescence spectroscopy (see ESI, Fig. S.7a and S.7b respectively†). We noted that the phototrigger **3** efficiently released adenosine (Scheme 5) in both aqueous acetonitrile and buffer solution with almost similar chemical and quantum yields (Table 4).

2.2.4. *In vitro* cell imaging studies of caged adenosine 9 and phototrigger 3. To explore the fluorescence properties of the caged adenosine **9** and the phototrigger **3**, we carried out cell imaging studies using the L929 cell line obtained from the National Centre for Cell Sciences, Pune (NCCS) which was maintained in Dulbecco's Modified Eagle Medium (DMEM)



Scheme 3 Possible photorelease mechanism.

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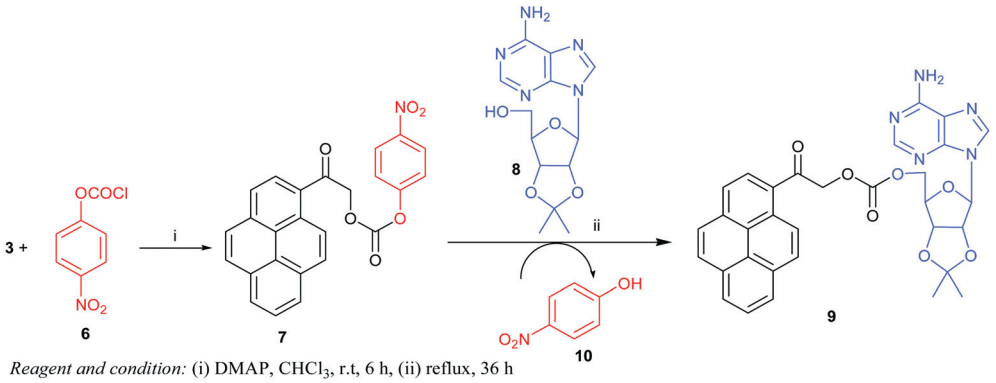

Reagent and condition: (i) DMAP, $CHCl_3$, r.t, 6 h, (ii) reflux, 36 h

Scheme 4 Synthesis of caged adenosine **9**.

Table 4 Photophysical and photorelease data of caged adenosine (**9**) in different solvent systems

	UV/fluorescence				Photorelease data (≥410 nm)	
Solvent	λ_{max} [a] (UV)(nm)	log ε λ_{max} (F)[b] (nm)	Stokes' shift[c] (nm)	Fluorescence quantum yield[d] (Φ_f)	log ε[e]	Φ_p[f]
EtOH	343	430	87	0.043	3.43	—
ACN–H_2O (50 : 50)	343	440	97	—	3.43	0.21
ACN–HEPES (50 : 50)	342	439	97	—	3.43	0.20

[a] Absorption maxima. [b] Emission maxima. [c] Difference between absorption maxima and emission maxima. [d] Fluorescence quantum yield. [e] Molar absorption coefficient at the irradiation wavelength. [f] Photochemical quantum yield (error limit within ± 5%).

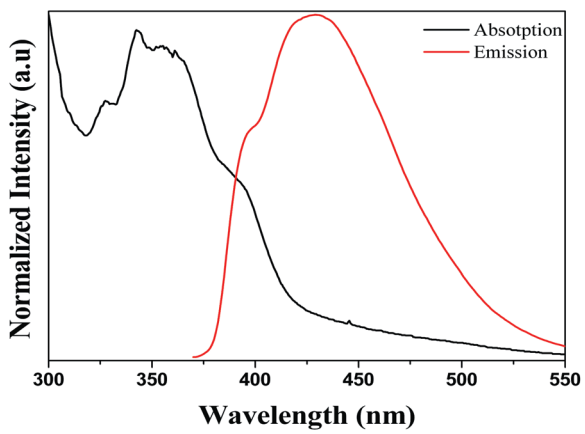

Fig. 3 Normalized absorption and emission spectra of caged adenosine **9**.

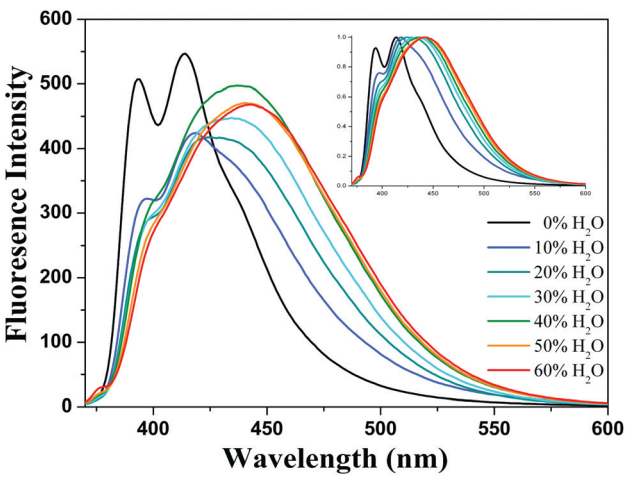

Fig. 4 Emission spectra of caged adenosine **9** in ACN–H_2O binary mixture (inset: normalized emission spectra of **9**).

containing 10% fetal bovine serum at 37 °C and 5% CO_2. To study the cellular uptake of caged adenosine **9** and phototrigger **3**, briefly L929 cells (6×10^3 cells per well) were plated on 12 well plates and allowed to adhere for 4–8 h. Cells were then incubated with 2×10^{-5} M of both compounds separately in the cell culture medium for 6 h at 37 °C and 5% CO_2.

Thereafter, cells were fixed for 15 min in paraformaldehyde, the media was discarded and the wells were washed three times with PBS. Imaging was done with an Olympus confocal microscope (FV1000, Olympus) using the respective filter. The cellular uptake study after 6 h incubation reveals that both the compound **3** and **9** are internalized by the cell membrane, leading to a uniform distribution of the sample inside the cell (Fig. 5).

2.2.5. Cell cytotoxicity assay of caged adenosine 9 and phototrigger 3. To determine the effect of caged adenosine **9** and phototrigger **3** on cell viability, the MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole) assay was performed.

The cytotoxic effect of each treatment was expressed as a percentage of cell viability relative to the untreated control cells.

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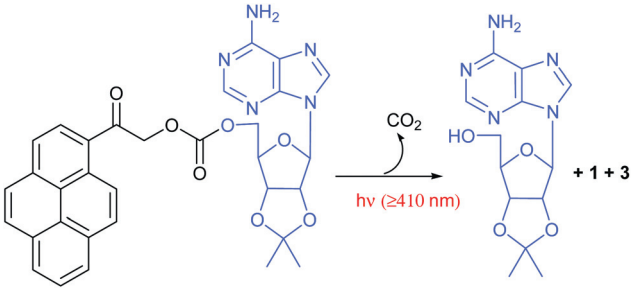

Scheme 5 Photorelease of caged adenosine **9**.

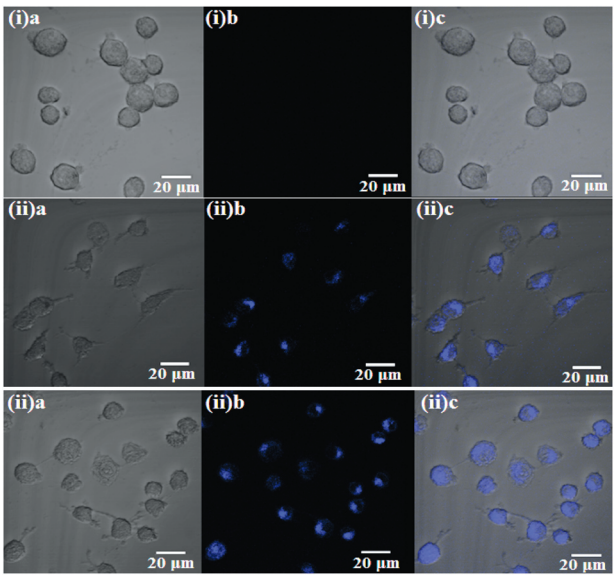

Fig. 5 Confocal fluorescence and brightfield images of L929 cells: (i) untreated cells, (ii) cells incubated with the phototrigger **3** (2×10^{-5} M), (iii) cells incubated with caged adenosine **9** (2×10^{-5} M). (a) brightfield, (b) fluorescence (λ_{ex} 410 nm), and (c) overlay image of a and b. Cells were incubated separately with compound **3** and **9** for 6 h.

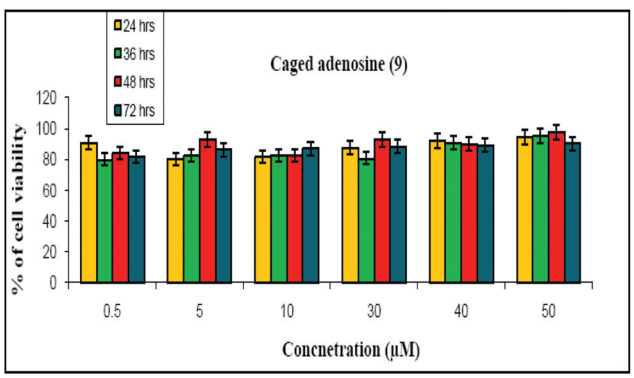

Fig. 6 Cell viability test for **9** against L929 cell line in different concentration at different incubation time.

The percentage of cell viability *vs.* concentration of caged adenosine **9** at different time intervals is plotted in Fig. 6. The cytotoxic effect of the caged adenosine was found to be insignificant and at least 80% of cells were viable after 72 h.

Further, we also studied the cytotoxicity effect of phototrigger **3** following the same procedure as explained for caged adenosine **9**, similar to caged adenosine the phototrigger **3** also did not show any significant cytotoxicity (see ESI, Fig. S8†).

3. Conclusion

In conclusion, alcohols and phenols were protected as their fluorescent carbonates by coupling with phototrigger, 1-(hydroxyacetyl)pyrene. Photolysis of the caged carbonates by visible light (≥410 nm) in aqueous acetonitrile released the corresponding alcohols or phenols in high chemical and quantum yields. Based on Stern–Volmer quenching experiments and solvent effect studies the mechanism for the photorelease was proposed. Effective caging and rapid release of adenosine by visible light and *in vitro* biocompatibility, cell uptake and imaging studies revealed that 1-(hydroxyacetyl)pyrene can be a promising phototrigger, which can perform dual functions such as cell imaging and release of biological effectors in cell and tissue cultures under visible light. Hence, in comparison to the already reported FPRPG,[17,23–27] 1-(hydroxyacetyl)-pyrene provides certain advantages, (i) it can perform dual functions, such as fluorophore for cellular imaging and phototrigger for rapid release of adenosine in visible light, (ii) has good cellular uptake property and (iii) showed good biocompatibility.

4. Experimental section

4.1. General experimental techniques

All reagents were purchased from Sigma Aldrich and used without further purification. Acetonitrile and dichloromethane were distilled from CaH_2 before use. ^{1}H NMR spectra were recorded on a BRUKER-AC 200 MHz spectrometer. Chemical shifts are reported in ppm from tetramethylsilane with the solvent resonance as the internal standard (deuterochloroform: 7.26 ppm). Data are reported as follows: chemical shifts, multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet), coupling constant (Hz). ^{13}C NMR (50 MHz) spectra were recorded on a BRUKER-AC 200 MHz Spectrometer with complete proton decoupling. Chemical shifts are reported in ppm from tetramethylsilane with the solvent resonance as the internal standard (deuterochloroform: 77.0 ppm). UV/vis absorption spectra were recorded on a Shimadzu UV-2450 UV/vis spectrophotometer, fluorescence emission spectra were recorded on a Hitachi F-7000 fluorescence spectrophotometer, FT-IR spectra were recorded on a Perkin Elmer RXI spectrometer and HRMS spectra were recorded on a JEOL-AccuTOF JMS-T100L mass spectrometer. Photolysis of all the caged carbonates were carried out using 125 W medium pressure Hg lamp supplied by SAIC (India). Chromatographic purification was done with 60–120 mesh silica gel (Merck). For reaction monitoring, precoated silica gel 60 F254 TLC sheets (Merck) were used. RP-HPLC was taken using mobile phase acetonitrile, at a flow rate of 1 mL min^{-1} (detection: UV 254 nm).

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4.2. General procedure for the synthesis of caged carbonates (5a–f)

1-(Hydroxyacetyl)pyrene (1 equiv) was dissolved in dry DCM (5 mL), and to the solution corresponding alcohol-chloroformate (1 equiv) was added followed by 1.2 equiv of *N*,*N*-dimethylpyridin-4-amine (DMAP). The reaction mixture was stirred at room temperature for 8–10 h. The solvent was removed by rotary evaporation under reduced pressure and the crude residue was purified by column chromatography with EtOAc in petroleum ether (boiling range 60–80 °C) as an eluant.

4.3. Characterisation data for caged carbonates (5a–f)

Ethyl 2-oxo-2-(pyren-3-yl)ethyl carbonate (5a). Yellow solid, mp: 104–107 °C; ^{1}H NMR ($CDCl_3$, 200 MHz): δ = 8.97 (d, J = 9.4 Hz, 1H), 8.17–7.90 (m, 8H), 5.48 (s, 2H), 4.39–4.28 (q, J = 7.2 Hz, 2H), 1.39 (t, J = 7.2 Hz, 3H) ppm; ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 196.0, 155.2, 134.5, 130.9, 130.5, 130.3, 130.1, 127.9, 127.0, 126.7, 126.6, 126.5, 125.8, 124.9, 124.5, 123.9, 70.2, 64.9, 14.4 ppm; $FTIR_{KBr}$ (cm^{-1}): 1750, 1683; HRMS cal. for $C_{21}H_{16}O_4$ = 332.1049, found = 332.1049.

Isobutyl 2-oxo-2-(pyren-3-yl)ethyl carbonate (5b). Yellow solid, mp: 135 °C; ^{1}H NMR ($CDCl_3$, 200 MHz): δ = 9.02 (d, J = 9.6 Hz, 1H), 8.29–8.04 (m, 8H), 5.51 (s, 2H), 4.04 (d, J = 6.6 Hz, 2H), 2.18–1.95 (m, 1H), 1.01 (s, 3H), 0.97 (s, 3H) ppm; ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 195.9, 155.3, 134.5, 131.0, 130.5, 130.3, 130.1, 128.2, 127.0, 126.6, 126.5, 125.7, 125.0, 124.5, 123.9, 74.8, 70.1, 27.9, 18.9 ppm; $FTIR_{KBr}$ (cm^{-1}): 1749, 1686; HRMS cal. for $C_{23}H_{20}O_4$ = 360.1362, found = 360.1362.

Benzyl 2-oxo-2-(pyren-3-yl)ethyl carbonate (5c). Yellow solid, mp: 135 °C; ^{1}H NMR ($CDCl_3$, 200 MHz): δ = 9.01 (d, J = 9.4 Hz, 1H), 8.29–8.045 (m, 9H), 7.38–7.31 (m, 4H), 5.51 (s, 2H), 5.27 (s, 2H) ppm; ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 195.7, 155.1, 134.9, 134.5, 131.0, 130.5, 130.3, 130.1, 128.6, 128.3, 128.0, 127.0, 126.6, 126.5, 125.7, 125.0, 124.5, 123.9, 70.4, 70.3 ppm; $FTIR_{KBr}$ (cm^{-1}): 1774, 1686; HRMS cal. for $C_{26}H_{18}O_4$ = 394.1205, found = 394.1205.

2-Isopropyl-5-methylcyclohexyl 2-oxo-2-(pyren-3-yl)ethyl carbonate (5d). Yellow solid, mp: 120–125 °C; ^{1}H NMR ($CDCl_3$, 200 MHz): δ = 8.94 (d, J = 9.4 Hz, 1H), 8.27–8.02 (m, 8H), 5.44 (s, 2H), 4.60–4.48 (m, 1H), 2.07–0.68 (m, 15H), 0.62 (d, 10.2 Hz, 3H) ppm; ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 196.6, 154.8, 134.3, 131.0, 130.5, 130.1, 129.9, 128.5, 127.0, 126.5, 126.4, 125.7, 125.0, 124.5, 124.1, 123.9, 79.2, 70.2, 46.9, 40.5, 34.1, 31.4, 26.0, 21.9, 20.6, 16.1 ppm; $FTIR_{KBr}$ (cm^{-1}): 1741, 1707; HRMS cal. for $C_{29}H_{30}O_4$ = 442.2144, found 442.2144.

(8*R*,9*R*,10*S*,13*S*,14*R*,17*S*)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-Tetradecahydro-10,13-dimethyl-17-((*S*)-6-methylheptan-2-yl)-1*H*-cyclopenta[*a*]phenanthren-3-yl 2-oxo-2-(pyren-3-yl)ethyl carbonate (5e). Yellow solid, mp: 155–158 °C; ^{1}H NMR ($CDCl_3$, 200 MHz): δ = 9.01 (d, J = 9.4 Hz, 1H), 8.29–8.04 (m, 8H), 5.49 (s, 2H), 5.38 (d, J = 4.0 Hz, 1H), 4.65–4.54 (m, 1H), 2.46 (d, J = 7.4 Hz, 2H), 2.04–0.85 (m, 38H), 0.68 (s, 3H) ppm; ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 195.9, 154.4, 139.3, 134.5, 131.0, 130.5, 130.2, 130.1, 128.2, 127.0, 126.6, 126.4, 125.7, 125.0, 124.5, 124.1, 123.9, 123.0, 78.8, 70.1, 56.7, 56.2, 50.0, 42.3, 39.7, 39.5, 37.9, 36.9, 36.6, 36.2, 35.8, 31.9, 28.2, 28.0, 27.6, 24.3, 23.9, 22.8, 22.6, 21.1, 19.3, 18.7 ppm; $FTIR_{KBr}$ (cm^{-1}): 1750, 1691; HRMS cal. for $C_{46}H_{56}O_4$ = 672.4179, found 672.4179.

2-Oxo-2-(pyren-3-yl)ethyl phenyl carbonate (5f). Yellow solid, mp: 110–115 °C; ^{1}H NMR ($CDCl_3$, 200 MHz): δ = 9.08 (d, J = 9.4 Hz, 1H), 8.34–8.08 (m, 9H), 7.45–7.34 (m, 4H), 5.66 (s, 2H) ppm; ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 195.0, 153.7, 151.2, 134.6, 131.0, 130.5, 130.4, 130.2, 129.5, 127.8, 127.0, 126.7, 126.6, 126.5, 126.2, 125.7, 125.1, 124.5, 124.0, 121.0, 70.7 ppm; $FTIR_{KBr}$ (cm^{-1}): 1774, 1691; HRMS cal. for $C_{25}H_{16}O_4$ = 380.1049, found 380.1049.

4.4. Photophysical properties of caged carbonates (5a–f)

The UV/vis absorption spectra of degassed 2 × 10^{-6} M solution of the caged carbonates (**5a–f**) in absolute ethanol were recorded on a Shimadzu UV-2450 UV/vis spectrophotometer, and the fluorescence emission spectra was recorded on a Hitachi F-7000 fluorescence spectrophotometer. Fluorescence quantum yield of the caged compounds was calculated using the eqn (1).

$$(\Phi_f)_{CG} = (\Phi_f)_{ST} \frac{(Grad_{CG})}{(Grad_{ST})} \frac{(\eta^2_{CG})}{(\eta^2_{ST})} \quad (1)$$

where, the subscript CG and ST denotes caged compound and standard respectively. 9,10-Diphenyl anthracene in ethanol was taken as standard. Φ_f is fluorescence quantum yield; Grad is the gradient from the plot of integrated fluorescence intensity *vs.* absorbance, and η the refractive index of the solvent.

4.5. Deprotection photolysis of caged carbonates (5a–f)

A solution of 10^{-4} M of the caged carbonates (**5a–f**) was prepared in acetonitrile–H_2O (50 : 50) individually. Half of the solution was kept in dark and to the remaining half nitrogen was passed and irradiated under visible light (≥410 nm) individually, using a 125 W medium pressure Hg lamp filtered by suitable filters with continuous stirring. At regular interval of time, 20 μl of the aliquots was taken and analyzed by ^{1}H NMR as well as RP-HPLC using mobile phase methanol, at a flow rate of 1 ml min^{-1} (detection: UV 254 nm). Peak areas were determined by RP-HPLC, which indicated gradual decrease of the caged compound with time, and the average of three runs. The reaction was followed until the consumption of the caged compound is less than 5% of the initial area. Based on HPLC data for each caged compounds, we plotted normalized [A] (HPLC peak area) *versus* irradiation time. We observed an exponential correlation for the disappearance of the caged compounds which suggested a first order reaction. Further, the quantum yield for the photolysis of caged compounds was calculated using eqn (2).

$$(\Phi_p)_{CG} = (\Phi_p)_{act} \frac{(k_p)_{CG}}{(k_p)_{act}} \frac{(Fact)}{(F_{CG})} \quad (2)$$

where, the subscript 'CG' and 'act' denotes caged compound and actinometer respectively. Potassium ferrioxalate was used as an actinometer.[37,38] Φ_p is the photolysis quantum yield, k_p is

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the photolysis rate constant and F is the fraction of light absorbed.

4.6. Preparative photolysis of caged carbonates (5a–f)

A solution of the caged carbonates (**5a–f**) (0.05 mmol) in acetonitrile–H_2O (50 : 50, pH = 7.5) were irradiated individually using a 125 W medium pressure Hg lamp filtered by suitable filter. The irradiation was monitored by TLC at regular interval of time. After completion of photolysis, solvent was removed under vacuum and photoproducts were isolated by column chromatography using EtOAc in hexane as an eluant to yield alcohols or phenol, phototrigger **3**, and 1-acetyl pyrene (**1**). The photoproducts were then weighed, analyzed by ^{1}H NMR and compared with the authentic sample.

4.7. General procedure for the synthesis of caged adenosine 9

1-(Hydroxyacetyl)pyrene (1 equiv) was dissolved in dry $CHCl_3$ (5 mL), and to the solution 4-nitro phenyl chloroformate (1 equiv) was added followed by 1.2 equiv of *N,N*-dimethylpyridin-4-amine (DMAP) and the reaction mixture was stirred at room temperature for 6 h. Then (4-(6-amino-9*H*-purin-9-yl)-tetrahydro-2,2-dimethylfuro[3,4-*d*][1,3]dioxol-6-yl)methanol (1 equiv) and 1.2 equiv of DMAP was added. The reaction mixture was refluxed for 36 h. The solvent was removed by rotary evaporation under reduced pressure and the crude residue was purified by column chromatography with $CHCl_3$–MeOH (70 : 30) as an eluant.

4.8. Characterisation data for the caged adenosine 9

(4-(6-Amino-9*H*-purin-9-yl)-tetrahydro-2,2-dimethylfuro[3,4-*d*]-[1,3]dioxol-6-yl)methyl 2-oxo-2-(pyren-3-yl)ethyl carbonate (9). Yellow solid, mp: 138 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 9.04 (d, J = 9.6 Hz, 1H), 8.34 (s, 1H), 8.30–8.24 (m, 3H), 8.23–8.19 (m, 3H), 8.13–8.08 (m, 3H), 6.42 (broad s, 2H, NH), 6.22 (s, 1H), 5.59–5.45 (m, 3H), 5.15 (d, J = 3.6 Hz, 1H), 4.61 (d, J = 3.2 Hz, 1H), 4.51 (d, J = 4.0 Hz, 2H), 1.65 (s, 3H), 1.42 (s, 3H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 195.3, 155.7, 154.9, 152.9, 134.9, 131.2130.7, 130.5, 130.4, 127.7, 127.2, 127.0, 126.9, 126.8, 126.0, 125.2, 124.6, 124.2, 114.9, 91.1, 84.8, 84.7, 81.6, 70.6, 68.0, 27.3, 25.5 ppm; $FTIR_{KBr}$ (cm^{-1}): 1749, 1653; HRMS cal. for $C_{32}H_{28}N_5O_7$ [MH^+] = 594.1989, found 672.4179.

4.9. Photophysical and photochemical properties of caged adenosine 9

Photophysical and photochemical properties of the caged adenosine **9** was carried out following the similar procedure as described under the section 4.4 and 4.5 respectively.

Preparative photolysis of the **9** was carried out following the same procedure as described under the section 4.6.

4.10. Cell imaging studies using compound 3 and 9

Cell imaging studies was carried out using the L929 cell line obtained from the National Centre for Cell Sciences, Pune (NCCS) which was maintained in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum at 37 °C and 5% CO_2. To study the cellular uptake of the compound **3** and **9**, briefly L929 cells (6×10^3 cells per well) were grown on 24 well plates for 24 h at 37 °C and 5% CO_2 followed by incubation with 2×10^{-5} M of both the compound separately in cell culture medium for 6 h at 37 °C and 5% CO_2. Cells were washed three times with PBS and subject to imaging by Olympus confocal microscope (FV1000, Olympus) using the respective filter. Cellular uptake study after 6 h incubation reveals that both the compound **3** and **9** is internalized by the cell membrane leading to a uniform distribution of the sample inside the cell.

4.11. Cell cytotoxycity assay

To determine the effect of the phototrigger 1 and the caged adenosine **9** on cell viability the MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole) cell proliferation assay was performed. Mouse fibroblast cell L929 were cultured in Dulbecco's Modified Eagle Media (DMEM) supplemented with 10% fetal bovine serum (FBS). Cells were trypsinezed and resuspended in fresh media. Aliquots of the cell suspension (200 µL) were seeded into 96 well microplates and allowed to grow for 24 h at 37 °C in a humidified 5% CO_2 environment. The spent media was aspirated carefully without disturbing the cell monolayer and replaced with 200 µL of fresh media containing different concentration of compound **3** and **9** (0.5, 5, 10, 30, 40, 50 µM respectively). Three replicates of cell population were taken for each concentration of both the compound **3** and **9**. For each concentration of compound **3** and **9**, cells were incubated for different time intervals (24 h, 36 h, 48 h, and 72 h respectively). After incubation for a desired time span the medium was discarded and 200 µL of 1 mg mL^{-1} MTT reagent in PBS was added to each well and incubated for 4 h at 37 °C in dark. The purple formazan crystals produced from reduced MTT by active mitochondria of viable cells was dissolved in 200 µL DMSO. The absorbance was measured spectrophotometrically using a benchtop microplate reader at 595 nm. The cytotoxic effect of each treatment was expressed as percentage of cell viability relative to the untreated control cells defined as: ([OD 570 nm treated cells]/[OD 570 nm control cells]) × 100.

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