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ARTICLE

ESIPT-Induced Fluorescent *o*-Hydroxycinnamate: Self-Monitoring Phototrigger for Prompt Image-Guided Uncaging of Alcohols

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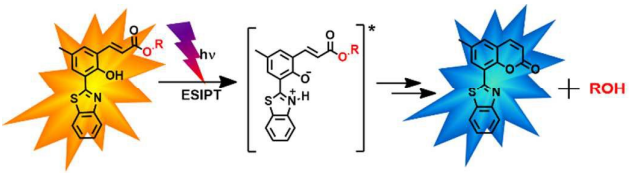
The *o*-hydroxycinnamate derivatives are well known phototriggers for fast and direct release of alcohols and amines without proceeding through the cleavage of carbonate or carbamate linkages. Despite these unique features, the *o*-hydroxycinnamates lack extensive applications in the biological systems mainly because of its non-fluorescent nature. To overcome this limitation, we have attached 2-(2'-hydroxyphenyl) benzothiazole (HBT) moiety, capable of rapid excited-state intramolecular proton transfer (ESIPT) to the *o*-hydroxycinnamate group. The ESIPT effect induced two major advantages to the o-hydroxycinnamate group: i) large Stokes' shifted fluorescence (orange colour) property, ii) distinct fluorescence colour change upon photorelease. *In vitro* studies exhibited image guided, photoregulated release of bioactive molecule by *o*-hydroxycinnamate-benzothiazole-methyl salicylate conjugate and real time monitoring of the release action.

Introduction

In recent years, we have witnessed a sustained development and upgradation of phototriggers based on their photophysical and photochemical characteristics in order to ensure better prospects in the field of drug delivery.[1] The *o*-hydroxycinnamate is a well known phototrigger which was introduced by Porter et al. to cage alcohols and amines.[2-7] The salient features which attracted us towards this phototrigger are: (i) clean and rapid uncaging, (ii) structural simplicity, (iii) it belongs to the category of very few phototriggers which can directly cage alcohols and amines without carbonate or carbamate linkage that are prone to be labile under physiological condition,[8-10] (iv) the resulting photoproduct coumarin serve as a fluorescent reporter, and (v) sufficient sensitivity towards both one photon (1PE) and two-photon excitation (2PE).[11-13] Despite these interesting features, the major limitation of the cinnamate series is that they are intrinsically non-fluorescent. This impedes their extensive usage in the area of drug delivery, where real time tracking of the drug delivery system by the means of highly sensitive fluorescence imaging technique is always beneficial to attain higher spatial and temporal control over the uncaging. Therefore, if we could modify *o*-hydroxycinnamate as a fluorescent phototrigger, it might open a new dimension in its application. Firstly, it can be extensively utilized for image-guided delivery of active molecules in the biological systems. Secondly, it will be helpful in self-monitoring the release action and quantifying the released substrate.

Nowadays, excited-state intramolecular proton transfer (ESIPT) process have attracted much attention because of their many advantageous properties, such as a strong fluorescence with a large Stokes' shift,[14] a low inner-filter effect, and low self-quenching ability.[15-23] ESIPT is an ultrafast enol–keto phototautomerization process exhibited by intramolecularly H bonded molecules which occur in their excited-states.[24-29] Recently, our group has reported ESIPT assisted photorelease of carboxylic acids using *p*-hydroxyphenacyl phototrigger.[30] Now, we are interested to design a phototrigger which can exhibit ESIPT induced fluorescence and image-guided direct uncaging of hydroxyl groups with the ability of self-monitoring the course of uncaging in real-time. Since a large number of bioactive molecules contains hydroxyl as a terminal functional group.



Scheme 1. Photoinduced uncaging of alcohols from the ESIPT induced fluorescent o-hydroxycinnamate phototrigger. ROH= ethanol, methyl salicylate.

Herein, we present *o*-hydroxycinnamate appended with ESIPT moiety as a new fluorescent phototrigger for the direct

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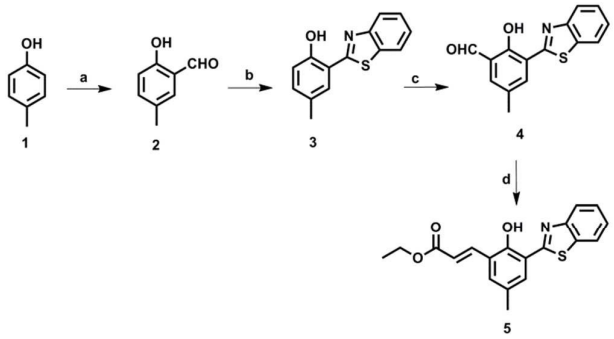
uncaging of alcohols (**Scheme 1**). For our investigation, we chose ethyl *o*-hydroxy-5-methylcinnamate-benzothiazole conjugate (**5**) as a model caged compound. Our designed phototrigger contains 2-(2'-hydroxyphenyl)benzothiazole (HBT) as a ESIPT moiety. ESIPT process from the hydroxyl group of *o*-hydroxycinnamate to the benzothiazole moiety in our newly designed phototrigger provided advantages like (i) induces large Stokes' shifted fluorescence (orange colour), (ii) causes distinct fluorescence colour change on photoinduced uncaging, which in turn leads to excellent self-monitoring the uncaging process and (ii) helps in quantification of the uncaged substrate. Finally, image-guided release of a biologically relevant molecule methyl salicylate and self-monitoring of the release by our phototrigger in real time was demonstrated *in vitro*. Biocompatibility of our caged compound before and after photolysis was also displayed by MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole) assay.

Results and discussion

Synthesis of caged compound 5

The ESIPT based fluorescent *o*-hydroxycinnamate phototrigger was synthesized following the sequence of reactions as shown in **Scheme 2.** Commercially available *p*-cresol was converted into compound **2** by Reimer-Tiemann reaction.[31] Treatment of **2** with 2-aminothiophenol in presence of iodine at room temperature furnished compound **3**.[32] Compound **3** was converted to **4** by Duff reaction using hexamethylenetetramine and trifluoroacetic acid as the solvent. [32] Compound **4** was condensed with ethyl triphenylphosphoranylideneacetate in toluene to provide caged compound **5** with the desired E configuration. Products obtained in each step were characterized by ^{1}HNMR, ^{13}CNMR (**Fig. S1–S4** in in the ESI†) and HRMS (**Fig. S7–S9,** ESI†).



Scheme 2. Synthesis of caged compounds **5**. Reagents and conditions: (a) NaOH, chloroform, reflux, 2 h; (b) 2-aminothiophenol, I_2, methanol, room temperature, 2 h; (c) hexamine, trifluoroacetic acid, reflux, overnight; (d) carboethoxymethylidenetriphenylphosphorane, toluene, 60 ^{0}C, 4 h.

Photophysical properties of caged compound 5

Photophysical properties of caged compound **5** (1×10^{-5} M) were studied in different solvent systems. It shows a strong absorption peak at 370 nm in all solvents (**Fig. 1a**). Interestingly, caged compound **5** exhibits a strong fluorescence (orange colour) with a large Stokes' shift due to the presence of HBT moiety which is known to exhibit an ESIPT process upon photoexcitation (**Fig. 1b**).[33] Its Fluorescence quantum yield was calculated as 14.6% (in acetonitrile), taking quinine sulphate as the standard (quinine sulphate quantum yield: 54%) as shown in **Table S1** in the ESI†.[34]

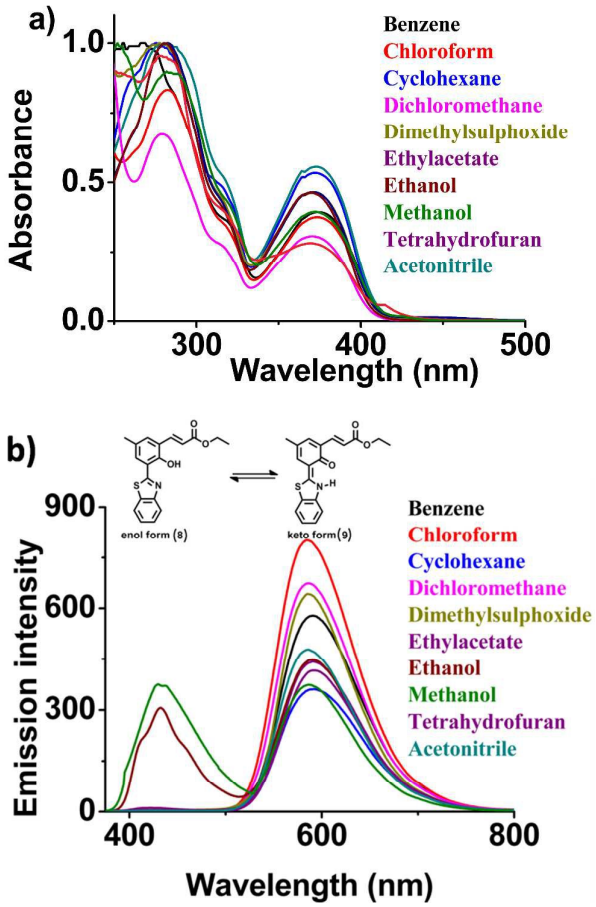


Fig. 1. (a) Absorption and (b) emission spectra of caged compound **5** in different solvents.

Further, due to ESIPT process, the caged compound **5** exhibited dual emission, one is normal Stokes' shifted emission maximum at 430 nm (enol form (**8**)) and another is a large Stokes' shifted emission maximum at 590 nm (keto form (**9**)) due to proton transfer tautomer. The dual emission process of caged compound **5** is strongly dependent on the solvent system. In non-hydrogen bonding solvents (cyclohexane, benzene, chloroform), only one emission maximum at 590 nm, corresponding to the keto form (**9**), was observed (**Fig. 1b**). In non-hydrogen bonding solvents there is an ultrafast proton transfer from the hydroxyl group of the *o*-hydroxycinnamate to the nitrogen atom of the benzothiazole moiety (ESIPT), leading to the existence of only keto form (**9**). On the contrary, in polar protic solvents (EtOH, MeOH), two emission peaks, one near 430 nm and another at 590 nm corresponding to the enol (**8**) and keto (**9**) form, respectively was observed. This is

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due to the presence of hydrogen bond between the solvent and the hydroxyl group of the caged compound **5** which restricts the ESIPT process.

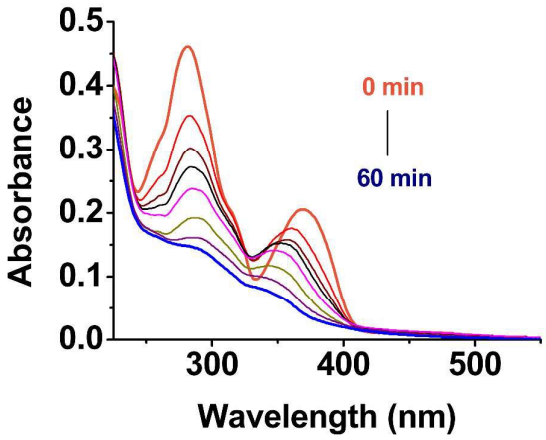


Fig. 2. Absorption spectra of the caged compound **5** as the irradiation time is gradually increased (0–60 min).

Photolysis of caged compound 5

The photoinduced uncaging of ethanol by our caged compound **5** was studied by irradiating the solution of **5** (1×10^{-4} M) in acetonitrile/H_2O (ACN/H_2O) (1:1 v/v) using 125 W medium pressure Hg lamp as the UV light source (λ ≥ 365 nm) and 1 M $CuSO_4$ solution as the UV cut–off filter. The photolysis of **5** was monitored by absorption spectroscopy (**Fig. 2)** ^{1}HNMR (**Fig. 3**), emission spectroscopy (**Fig. 6)**, and reversed-phase HPLC (**Fig. S12,** ESI†**)**. The ^{1}HNMR spectra of **5** were recorded at different irradiation time intervals in $CDCl_3$. At 0 min, signals at 4.29 ppm, 6.74 ppm and 13.16 ppm corresponding to the methylene protons (H_a) olefinic protons (H_b) and hydroxyl proton (H_c) of caged compound **5** were noted. With increase in irradiation time from 0 to 60 min, the intensity of the signals for H_a, H_b, and H_c decreases indicating the gradual photodecomposition of caged compound **5**. On the other hand, appearance and increase in the intensity of two new signals at 6.54 ppm (H_d) and 8.16 ppm (H_e) corresponding to two olefinic protons of the benzothiazole-coumarin (**14**) indicates the formation of the photoproduct. The uncaging of ethanol is indicated by the gradual appearance of quartet signal at 3.72 ppm corresponding to the methylene protons (H_f) of ethanol.

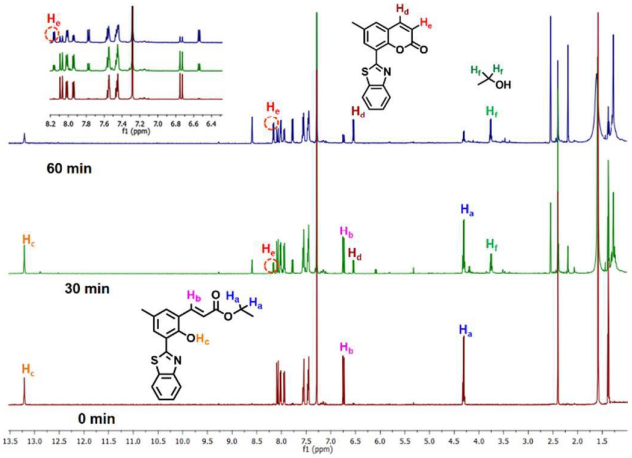
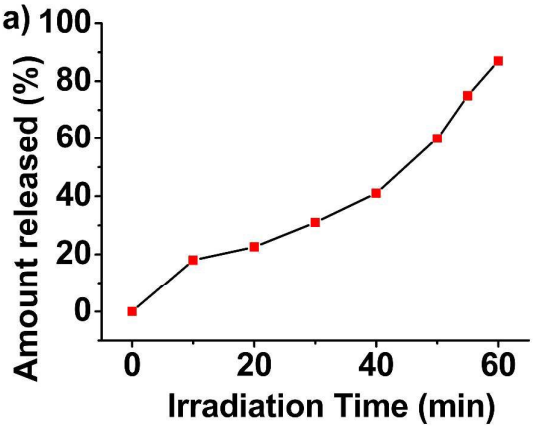


Fig. 3. Photolysis of caged compound **5** monitored by ^{1}H NMR in $CDCl_3$.

The NMR studies indicated clean photoconversion of *o*-hydroxycinnamate (**5**) to the corresponding benzothiazole-coumarin (**14**) photoproduct along with the uncaging of ethanol. The photochemical quantum yield for the uncaging reaction was calculated as 0.10 in acetonitrile/H_2O (ACN/H_2O) (1:1 v/v) solution (**Table S2,** ESI†). We observed that almost 87% of ethanol was released from caged compound **5** after irradiation with light (λ ≥ 365 nm) for 60 min (**Fig. 4a**). Furthermore, the precise control over the photoinduced uncaging was demonstrated by alternative exposure of our caged compound **5** to light and dark. It was observed that ethanol was uncaged only upon irradiation of light (**Fig. 4b**).[35]



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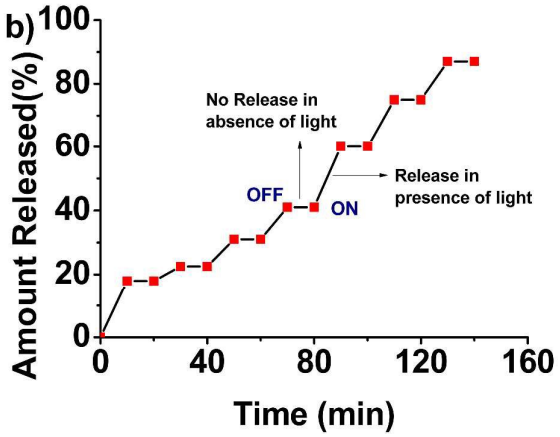
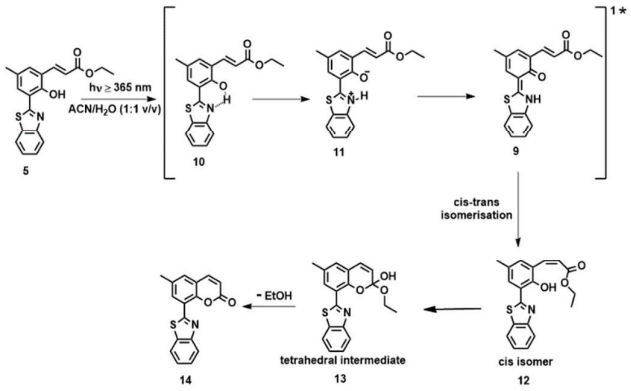


Fig. 4. (a) The amount of ethanol released from caged compound **5** on photolysis (≥ 365 nm) at different time intervals. (b) Release of ethanol under bright and dark conditions. "ON" and "OFF" implies the switching on and off of the light source, respectively.

Mechanism of the photolysis of caged compound 5

A possible mechanism for the photoinduced uncaging of ethanol by caged compound **5** in ACN/H_2O solution is described in **Scheme 3**. Upon exposure to light, caged compound **5** gets excited to the singlet state (supported by fluorescence quenching study in the presence of benzophenone in **Fig. S13** in ESI†. The average fluorescent lifetime (singlet state lifetime) of the caged compound **5** was 1.15 ns, obtained from a time-correlated single photon counting (TCSPC) experiment (**Fig. S14,** ESI†).[36] In the excited state, caged compound **5** undergoes rapid ESIPT from the hydroxyl group of *o*-hydroxycinnamate moiety to the benzothiazole moiety, resulting in deprotonation of the hydroxyl group to give a zwitterionic form (**11**) which immediately gets transformed to the more stable keto form (**9**). The keto form then undergoes rapid trans–cis photoisomerization to give **12**, followed by thermally driven lactonization through a tetrahedral intermediate (**13**) leading to quantitative release of ethanol[37] and 8-benzthiazole-6-methylcoumarin (**14**) as the photoproduct.



Scheme 3. Possible mechanism of ESIPT and photoinduced uncaging of ethanol from caged compound **5.**

Further to support the ESIPT mechanism computationally, a relaxed surface scan using density functional theory (See supporting information for details) was performed from **5** to **9** by decreasing the N—H bond length (from 1.67 to 0.97 Å). The potential energy surface (PES) of S_1 state was constructed from the vertical excitation energies calculated at each of the points on the ground-state PES. The PES of the S_1 state showed a monotonous decrease in energy on going from **5** towards **9**. Moreover, when the ground state geometry of **5** was optimized in the S_1 state, the geometry was converged to **9** which confirmed a barrierless ESIPT process. To understand the subsequent trans-cis isomerization, we have performed a rigid surface scan corresponding to one-bond flip (OBF) mechanism in the ground and photochemically excited states.[38] The PES (**Fig. 5**) indicates that the trans (**9**) to cis (**12**) photoisomerization proceeds through a conical intersection at a 90° torsion angle of the flipping bond. The vertical excitation energy ($\Delta E_{S1\text{-}S0}$) at the highest point of the S_0 surface was 1.13 eV at CASSCF(4,4)/cc-pVDZ[39] level of theory. The barrierless ESIPT and a low value of the $\Delta E_{S1\text{-}S0}$ confirmed that the two consecutive photochemical processes will be fast.

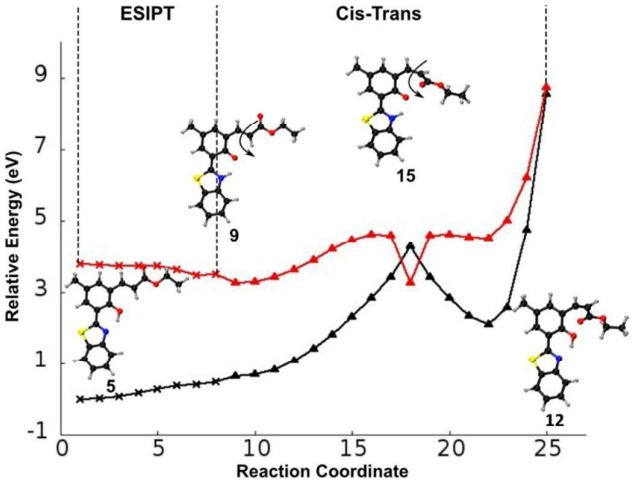


Fig. 5. Reaction profile for the ESIPT and the cis-trans isomerization at the CAM-B3LYP/cc-pVTZ//CAM-B3LYP/cc-pVTZ level of theory. The crossing point indicates the conical intersection. The black line indicates the PES in the S_0 state and the red line indicates the PES in the S_1 state. The OBF was performed by rotating the double bond. Here, the structure **15** corresponds to the conical intersection. The compound **12** is the cis isomer in the ground state.

Real time monitoring of the release

Another interesting feature of our designed system is that it shows distinct fluorescence colour change from orange to blue on photoinduced uncaging **(Fig. 6**). At 0 min the emission spectrum shows only an orange emission band corresponding to caged compound **5** (λ_{max} = 590 nm, **Fig. 6**). As the irradiation time gradually increased (0–60 min) the intensity of the orange emission band gradually decreased with concomitant appearance and increase in the intensity of a new blue emission band at 450 nm corresponding to the photoproduct benzothiazole-coumarin conjugate (**14**) was noted. The blue shift in the fluorescence spectrum of the

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photoproduct, benzothiazole-coumarin conjugate, is because of the absence of ESIPT process.

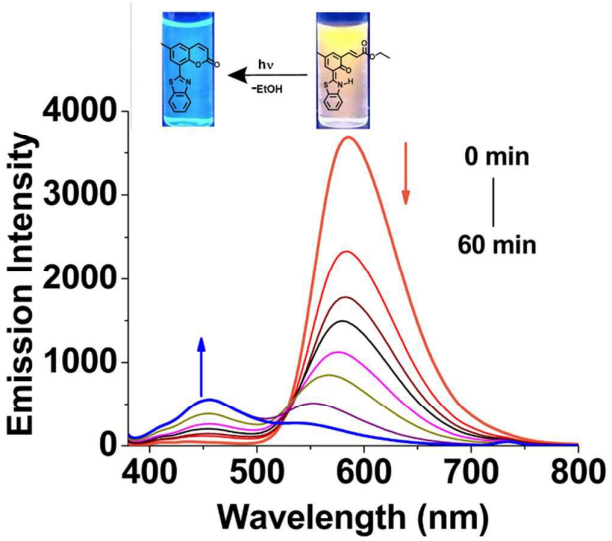
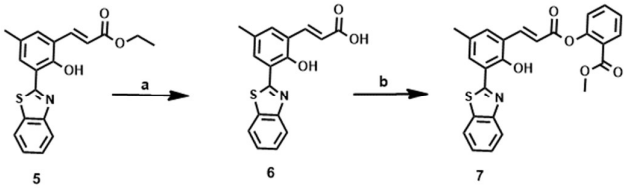


Fig. 6. Emission spectra of the caged compound **5** as the irradiation time is gradually increased (0–60 min).

Synthesis, photophysical, photochemical, cellular imaging and cytotoxic properties of caged methyl salicylate (7)

Synthesis of caged methyl salicylate. To explore the versatility of our fluorescent *o*-hydroxycinnamate phototrigger in the biological system, we caged methyl salicylate as depicted in **Scheme 3.** At first compound **5** was hydrolysed in presence of NaOH to produce compound **6**. **6** was converted into acid chloride and attached to methyl salicylate in the presence of trimethylamine to yield caged compound **7**. Products obtained in each step were characterized by ^{1}HNMR, ^{13}CNMR (**Fig. S5, S6,** ESI†**)** and HRMS (**Fig. S10, S11,** ESI†**).** Methyl salicylate was selected because of its excellent rubefacient, analgesic and antiseptic properties.



Scheme 3. Synthesis of caged methyl salicylate (**7**). Reagents and conditions: (a) NaOH, water, reflux, 6 h; (b) $SOCl_2$, methyl salicylate, triethylamine, dichloromethane, 10 h

Photophysical properties of caged methyl salicylate. Photophysical properties of caged compound **7** (1×10^{-5} M) was investigated in acetonitrile/HEPES buffer (1:9) solution and the results were similar to caged compound **5** (**Fig. 7**). The fluorescence quantum yield of caged compound **7** in acetonitrile/HEPES (1:9 v/v) buffer solution was calculated as 16.3%, taking quinine sulphate as the standard (quinine sulphate quantum yield: 54%) as shown in **Table S1** in the ESI†.

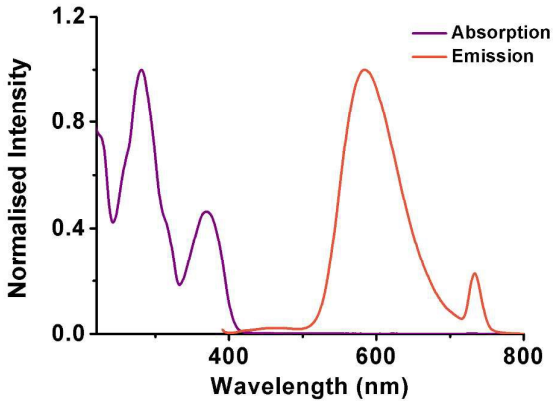
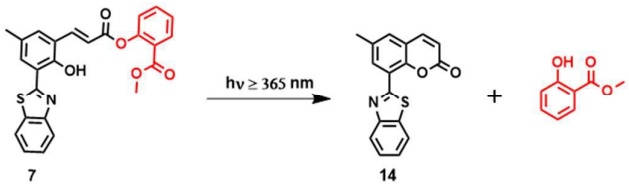


Fig. 7. Absorption and emission spectra of caged compound **7** in acetonitrile/HEPES buffer (1:9) solution.

Photolysis of caged methyl salicylate. Photolysis of caged compound **7** (1×10^{-4} M) was carried out in acetonitrile/HEPES (1:1) buffer (HEPES was used to resemble biological condition) using 125 W medium pressure Hg lamp as UV light source (λ ≥ 365 nm). The course of the photoinduced uncaging from caged compound **7** was monitored by fluorescence spectroscopy (**Fig. 8**). It was noted that the caged compound **7** efficiently released methylsalicylate (**Scheme 4**) in acetonitrile/HEPES buffer (1:1 v/v) solution with almost similar chemical (87 %) and quantum yield (0.10) (**Table S2,** ESI†).



Scheme 4. Photorelease of caged methyl salicylate from compound **7.**

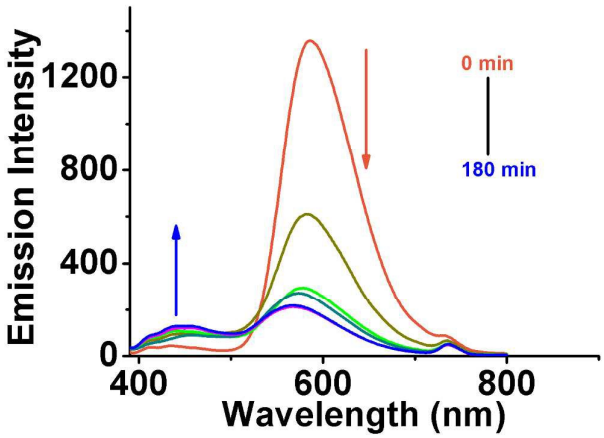


Fig. 8. Emission spectra of caged compound **7** during photolysis at different time intervals.

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***In vitro* cell imaging and real time monitoring studies.** The photoinduced release of methyl salicylate by caged compound **7** inside HeLa cell line was monitored in real time by confocal microscopy **(Fig. 9).** Initially, within the HeLa cells only orange fluorescence **(Fig. 9i)** was observed due to the internalization of caged methyl salicylate. Upon exposure to light of wavelength 365 nm for 30 min, both orange and blue fluorescence **(Fig. 9ii)** was observed which indicated partial release of methyl salicylate from the caged compound (7). Finally, after 60 min of irradiation, a complete change in fluorescence colour from orange to blue **(Fig. 9iii)** was noted, thus suggesting a greater extent of photorelease of methyl salicylate. The percentage of photorelease of methyl salicylate was calculated as a function of change in the fluorescence intensity (**Fig. 10**).[40]

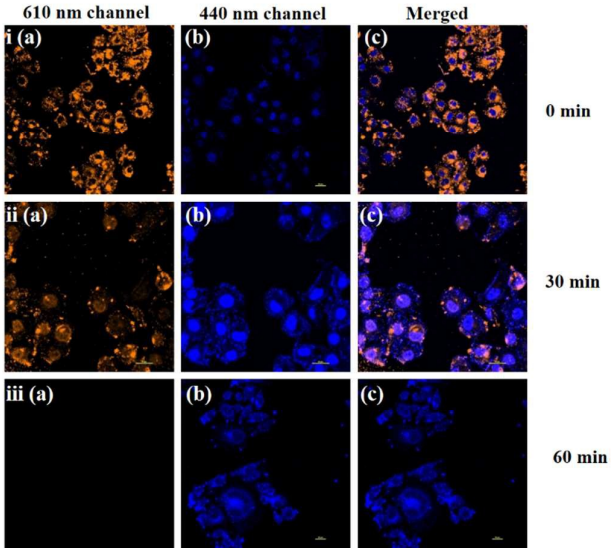

Fig. 9. Confocal fluorescence images of the cellular internalization of caged compound **7** (10 μM) in HeLa cell line and image-guided, self-monitored uncaging of methyl salicylate at different time intervals during photoirradiation: (i) 0 min, (ii) 30 min, and (iii) 60 min. (a) Blue emission: emission channel was 440 nm with band width of 40 nm, (b) Orange emission: emission channel was 610 nm with band width of 40 nm. In **Fig** (i) and (ii) HeLa cell nuclei were stained with 4',2-diamino-2-phenylindoline (DAPI). [Scale bar = 20 μm].

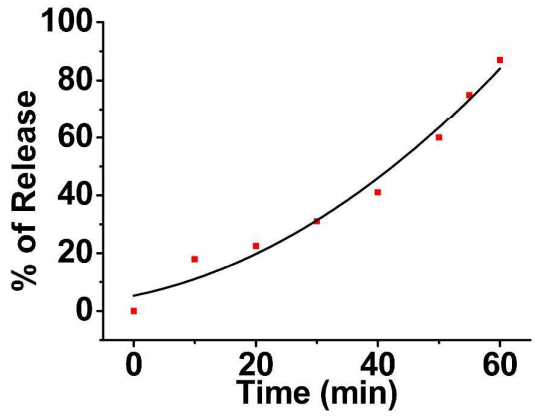

Fig. 10: Percentage of methyl salicylate released from caged methyl salicylate (**7**) as a function of fluorescence intensity change.

Cell cytotoxicity assay of caged methyl salicylate (7). *In vitro* cell viability of caged compound **7** was determined by performing MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole) assay in normal cells (NIH 3T3). The percentage of cell viability vs. concentration of caged compound **7** before and after photolysis is depicted in **Fig. 11** respectively. The figure indicates that caged compound **7** is biocompatible at any given concentration before and after irradiation.

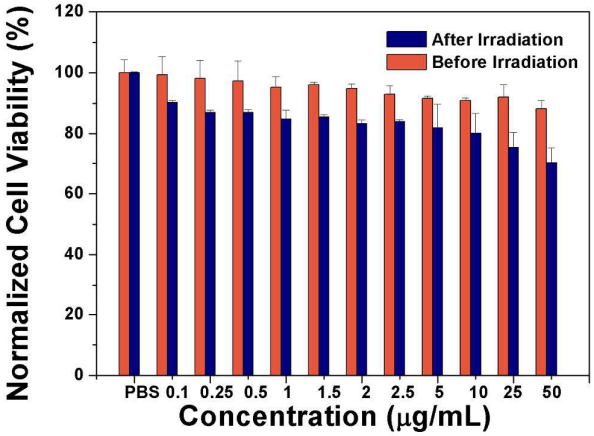

Fig. 11. Cell viability assay of caged compound **7** in NIH 3T3 cells in before and after irradiation. Values are presented as mean ± SD.

Conclusion

In conclusion, we have utilized the ESIPT process to upgrade the photophysical and photochemical properties of the well known *o*-hydroxycinnamate phototrigger for the photoinduced uncaging of alcohols. For the first time *o*-hydroxycinnamate has been converted to a fluorescent phototrigger by simple attachment of ESIPT moiety. The fluorescence (orange colour) property of the newly designed *o*-hydroxycinnamate phototrigger was utilized for the *in vitro* image-guided release of bioactive molecule. Quantification of the released active molecule by our designed phototrigger has been demonstrated by self-monitoring the release action in real time, owing to the distinct fluorescence colour change (from orange to blue) on photorelease. Induction of fluorescence property in the *o*-hydroxycinnamate has surely made it a better phototrigger encouraging a wider scope of applications. The limitation of our phototrigger is that it operates in UV region and the photoproduct (coumarin) is a strong chromophore which acts as optical filter. In future, we are interested to develop a system based on *o*-hydroxycinnamte overcoming the above mentioned limitations, to utilize it as a drug delivery vehicle.

Experimental Section

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Chemicals and starting materials

All reagents were purchased from Sigma Aldrich and were used without further purification. Dimethyl sulfoxide and dichloromethane were distilled from CaH_2 before use. All anhydrous reactions were performed under a dry nitrogen atmosphere.

Methods and techniques

^{1}H NMR spectra were recorded on a BRUKER-AC 400-MHz spectrophotometer. Chemical shifts are reported in ppm from tetramethylsilane with the solvent resonance as the internal standard (deuterochloroform: 7.26 ppm, DMSO-d_6: 3.313 ppm and 2.484 ppm). Data are reported as follows: chemical shifts, multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet), coupling constant (Hz). ^{13}C NMR (100 MHz) spectra were recorded on a BRUKER–AC 400-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, DMSO-d_6: 39.96 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; HRMS spectra were recorded on a JEOL–AccuTOF JMS–T100L mass spectrometer. Photolysis of the caged compounds was 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 recorded using acetonitrile in mobile phase, at a flow rate of 1 mL/min.

General Procedure for the preparation of caged compounds

Synthesis of 2-hydroxy-5-methylbenzaldehyde (2). A solution of the *p* cresol (5.15 g, 47.6 mmol) in 120 mL of 10 N NaOH (3 mol) was heated to 65 °C. Then 40 mL of $CHCl_3$ was added in three portions over 15 min. The mixture was heated at reflux in chloroform for 2 h. After cooling, the mixture was acidified to pH 1 with 12 N HCl, the organic layer collected and the aqueous layer extracted with chloroform. The combined chloroform solution was dried and evaporated to give a crude product which was distilled or recrystallized from an appropriate solvent to yield 2-hydroxy-5-methylbenzaldehyde as white solid, yield 95 %. ^{1}H NMR ($CDCl_3$, 400 MHz), δ = 2.37 (s, 3H), 6.90 (d, 1H, J = 8.60 Hz, 1H), 7.25-7.50 (m, 2H), 9.80 (s, 1H), 10.75 (s, 1H,). ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.6, 159.3, 138.2, 133.2, 129.9, 128.9, 117.4, 20.1.

Synthesis of 2-(benzo[d]thiazol-2-yl)-4-methylphenol (3). To a solution of 2-aminobenzenethiol (1.09 g, 8.7 mmol) and 2-hydroxy-5-methylbenzaldehyde (2 g, 14 mmol) in anhydrous CH_3OH (10 mL) was added I2 (1.89 g, 7 mmol). The mixture was stirred at room temperature. After about 5 min, yellow-green precipitate generated gradually. The reaction mixture was further stirred for another 2 h. The solid was collected on a filter and washed with cold CH_3OH. Further dried in a vacuum afforded **3** as a pale-yellow solid (1.5, yield 50.1%). ^{1} H NMR (400 MHz, $CDCl_3$) δ 12.31 (s, 1H), 7.98 (d, J = 8.2 Hz, 1H), 7.90 (d, J = 8.0 Hz, 1H), 7.54–7.45 (m, 2H), 7.40 (t, J = 7.2 Hz, 1H), 7.19 (d, J = 8.4 Hz, 1H), 7.01 (d, J = 8.4 Hz, 1H), 2.36 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.3, 155.7, 151.9, 133.7, 132.5, 128.6, 128.3, 126.5, 125.3, 122.0, 121.4, 117.6, 116.3, 20.4. HR-MS calc for $C_{14}H_{12}NOS$ [MH^+]: 242.0595, found: 242.0608.

Synthesis of 3-(benzo[d]thiazol-2-yl)-2-hydroxy-5-methylbenzaldehyde (4). Compound **3** (1.5 g, 6 mmol) was dissolved in 10 mL of trifluoroacetic acid and then hexamethylenetetramine (1.05 g, 7 mmol) was added. The mixture was refluxed over night until all the starting material was consumed. The reaction mixture was then cooled to room temperature and poured into 6 M HCl (30 mL) and extracted with CH_2Cl_2. The combined organic extracts were washed with saturated brine. Next purification was done by column chromatography (ethyl acetate: petroleum ether = 1:5) to afford the pure product as a yellow solid (1.16 g, yield 87%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.01 (s, 1H), 10.49 (s, 1H), 8.02 (d, J = 8.1 Hz, 1H), 7.94 (d, J = 7.8 Hz, 1H), 7.89 (s, 1H), 7.71 (s, 1H), 7.54 (t, J = 7.7 Hz, 1H), 7.45 (t, J = 7.6 Hz, 1H), 2.41 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.7, 158.4, 151.4, 135.0, 133.1, 132.4, 128.8, 126.8, 125.7, 123.7, 122.3, 121.5, 118.6, 115.8**,** 20.3. HR-MS calc. for $C_{15}H_{12}NO_2$ S [MH^+]: 270.0544, found 270.0552.

Synthesis of (E)-ethyl 3-(3-(benzo[d]thiazol-2-yl)-2-hydroxy-5-methylphenyl)acrylate (5). A mixture of **4** (500 mg, 2 mmol) and carboethoxymethylidenetriphenylphosphorane (970 mg, 3 mmol) in toluene (10 mL for 1 mmol of aldehyde) was heated at 60 °C under argon upon protecting from light. The course of the reaction was followed by TLC. After 4 h, the reaction was completed. After cooling to room temperature, toluene was removed in a vacuum. The crude residue was purified by flash chromatography on silica gel (ethyl acetate: petroleum ether = 1:9) to yield the desired cinnamate in high yield (90%; about 10% of the coumarin resulting from thermal trans-cis isomerization followed by lactonization was formed during the reaction). ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.16 (s, 1H), 8.05 (d, J = 16.2 Hz, 1H), 7.98 (d, J = 8.1 Hz, 1H), 7.91 (d, J = 7.9 Hz, 1H), 7.55 – 7.49 (m, 2H), 7.45 – 7.40 (m, 2H), 6.71 (d, J = 16.1 Hz, 1H), 4.28 (q, J = 7.1 Hz, 2H), 2.37 (s, 3H), 1.35 (t, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.1, 167.5, 155.2, 151.6, 139.5, 132.8, 132.6, 130.3, 128.4, 126.8, 125.7, 123.4, 122.2, 121.5, 119.6, 117.0, 60.4, 20.5, 14.4. HR-MS calc. for $C_{19}H_{18}NO_3S$ [MH+] : 340.0963, found: 340.0965.

Synthesis of (E)-3-(3-(benzo[d]thiazol-2-yl)-2-hydroxy-5-methylphenyl)acrylic acid (6). To the solution of **5** (500 mg, 1.5 mmol) in 10 ml ethanol 5 M sodium hydroxide (1 ml) and water (10 ml) was added and refluxed for 6 h. The dark solution obtained was cooled to room temperature and acidified with 12 N HCl to get white precipitate. The solid was filtered, washed with dichloromethane to yield 260 mg (73%) of the pure acid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.91 (s, 1H), 8.20 (d, J = 7.9 Hz, 1H), 8.11 (d, J = 8.0 Hz, 1H), 7.91 (d, J = 16.1

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Hz, 1H), 7.73 (d, J = 6.1 Hz, 2H), 7.59 (t, J = 7.6 Hz, 1H), 7.51 (t, J = 7.4 Hz, 1H), 6.65 (d, J = 16.1 Hz, 1H), 2.34 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.9, 168.2, 154.2, 151.3, 138.4, 132.8, 130.8, 129.4, 127.5, 126.4, 123.1, 122.8, 122.4, 120.5, 117.2, 20.3. HR-MS calc. for $C_{17}H_{14}NO_3S$ [MH^+]: 312.0650, found: 312.0651.

Synthesis of (E)-methyl 2-((3-(3-(benzo[d]thiazol-2-yl)-2-hydroxy-5-methylphenyl)acryloyl)oxy)benzoate (7). Methyl salicylate (132 mg, 0.87 mmol) was stirred with triethyamine (80 mg, 0.79 mmol) in 5 ml DCM at room temperature for 15 min. To this stirred solution, acid chloride of 6 was added and further stirred at room temperature for 10 h. Upon completion of reaction, monitored by TLC, the reaction mixture was poured into 1 N HCl an extracted with DCM. The combined organic extracts were washed with saturated brine. Purification was done by column chromatography (ethyl acetate: petroleum ether = 1:9) to afford the pure product as a yellow solid (60 mg, yield 50 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.28 (s, 1H), 8.27 (d, J = 16.1 Hz, 1H), 8.05 (d, J = 7.8 Hz, 1H), 8.00 (d, J = 8.1 Hz, 1H), 7.93 (d, J = 7.9 Hz, 1H), 7.60 (t, J = 7.8 Hz, 1H), 7.56 – 7.50 (m, 3H), 7.44 (t, J = 7.5 Hz, 1H), 7.34 (t, J = 7.7 Hz, 1H), 7.22 (d, J = 8.0 Hz, 1H), 6.98 (d, J = 16.1 Hz, 1H), 3.86 (s, 3H), 2.40 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.9, 165.9, 165.3, 155.5,151.6, 150.7, 141.8, 133.7, 133.2, 132.6, 131.8, 130.8, 128.5, 126.9,125.9, 125.8, 124.0, 123.7, 123.1, 122.2, 121.5, 118.3, 117.2, 52.3, 20.5. HR-MS calc. for $C_{25}H_{20}NO_5S$ [MH^+]: 446.1017, found: 446.1008.

Photolysis of caged compounds 5 and 7

A solution of 10^{-4} M of the caged compound **5** was prepared in acetonitrile/H_2O (1 : 1 v/v). Half of the solution was kept in dark and to the remaining half nitrogen was passed and irradiated under UV light (≥365 nm), 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 absorption spectroscopy, fluorescence spectroscopy and RP-HPLC using mobile phase acetonitrile , at a flow rate of 1 ml min^{-1} (detection: UV 254 nm). Peak areas were determined by RP-HPLC with the average of three runs, which indicated gradual decrease of the caged compound **5** with time. The reaction was followed until the consumption of the caged compound **5** 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. ^{1}H NMR analysis of the photolysis was also done by irradiating the solution of compound **5** in $CDCl_3$ and then taking the ^{1}H NMR spectra of the photolysis mixture at regular intervals of time.

Photochemical studies of the caged methyl salicylate was carried out following the similar procedure as described above.

***In vitro* application of compound 7**

Cellular internalisation and *in vitro* real-time monitoring of the release of active molecule by our phototrigger was studied in HeLa cell line.

Cellular Internalization Study of compound 7

The HeLa cells were grown in its log phase. Cells were seeded in 96-well plates in Dulbecco's modified Eagle's (DMEM) medium containing 10 % fetal bovine serum (FBS) for 8 h. Different concentrations of compound **7** in HEPES buffer containing 10 % ethanol were added and incubated at 37 °C in 5 % CO_2 for 4 h. Imaging was done using confocal laser scanning microscopy (CLSM) with suitable bandpass filters.

***In-vitro* self-monitoring of the release**

Self-monitoring of the release of methyl salicylate from our phototrigger **7** after UV light (≥ 365 nm) irradiation was also studied by fluorescence microscopy. HeLa cells were treated with 10 µM of compound **7** and put aside for 4 h at 37 °C. Then, compound-treated cells were irradiated with UV light (≥ 365 nm) for 0–60 min. The live imaging was done to observe image-guided release of methyl salicylate and self-monitoring of uncaging reactions inside the cells was visually observed by CLSM with suitable bandpass filters. For orange emission: emission channel was 610 nm with band width of 40 nm and for blue emission: emission channel was 440 nm with band width of 40 nm.

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